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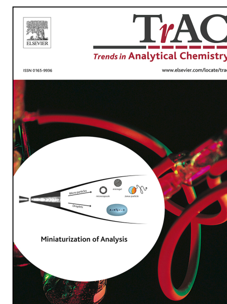

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**Omics methods as a tool for investigation of food allergies**

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**Abstract**

Use of foodomics, mostly proteomic and genomic based methods, for study of allergens in food is presented. Immunological methods and nucleic acid-based methods are still most frequently used for diagnosis of allergies and for qualitative and quantitative determination of food allergens. They are sensitive, and can be used for the determination of allergens in trace concentrations. However, lack of specificity and cross-reaction of some antibodies can still be a relevant source of bias. The epitopes of protein allergens with posttranslational modifications and their changes originated during food processing cannot be traced by use of nucleic acid-based strategies. Recent developments of both antibody and nucleic acid-based biosensors, their miniaturization and increasing application of nanotechnology, significantly supported further use of both strategies. Regarding accuracy, reliability and sensitivity, mass spectrometry-based methods bring important advantage over both above presented strategies. Furthermore, the increasing use of mass spectrometry (MS) is discussed. Combined with proper sample preparation, liquid chromatography (LC) and/or different electrophoretic methods, targeted approach in mass spectrometry-based allergen analysis brings an additional strategic advance. However, MS is still rarely used for high-throughput analyses and detection and quantification of allergens for the reasons of price and relatively long time necessary for analysis. Recent developments of new high-resolution instruments are encouraging and enable development in the direction of a high-throughput strategy. Consequently, fast, very sensitive, reliable and accurate detection and quantification of allergens in highly complex samples such as food matrices, and the use of MS in routine determination of allergens can be reached in near future.

**Keywords:** proteomics, allergen, food allergy, mass spectrometry

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**Abbreviations**

| | |
|---|---|
| BAT | Basophils activation test |
| CRD | Component-resolved diagnostic testing |
| ELISA | Enzyme-linked immunosorbent assay |
| FA | Food allergen |
| IgE | Immunoglobulin E |
| LC-MS/MS | Liquid chromatography coupled to tandem mass spectrometry |
| MRM | Multiple reaction monitoring |
| MS | Mass spectrometry |
| OFC | Oral food challenge |
| PCR | Polymerase chain reaction |
| PRM | Parallel reaction monitoring |
| SPR | Surface plasmon resonance |
| SPT | Skin prick test |
| SRM | Selected reaction monitoring |

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**1. Introduction**

Recent rise of food allergies is a well documented observation. This situation is not improving, and it is even getting worse in the first two decades of this century [1,2]. There are numerous studies, some of them ongoing, but it is still very difficult to get exact epidemiological data on this topic [3]. The moderate estimation by Sicherer & Sampson [4] assumes, on the basis of numerous studies, that food allergy alone affects nearly 5% of adults and 8% of children. These data are valid for the developed Western World, and the situation in developing countries is much less known [5]. List of allergens officially named by the World Health Organization according to their phyla, number and presence in food can be found in Table 1. The causes of the increase of allergic diseases are still not fully clarified and they seem to be the result of a combination of different factors. Genetics and lifestyle play an additional role in allergy development [1,3]. Food allergies that are the predominant topic of this review are affecting the gut as a primary organ [2,4]. As a source of uncertainty and stress for both the affected individuals and their environment, allergies are not only a growing problem for public health; they are also a social problem. The obligation of labeling potentially allergenic foods (*e.g.* European directive 2007/68/EC) is an important regulatory step which helps individuals at risk to make the right nutritional choice [6,7]. However, some accidents can still occur. The reasons are the possibility of cross-contamination due to insufficient and/or improper sanitation or waste management. Worldwide moving of food, use of unusual raw materials, intentional fraud, as well as materials for packaging caused by worldwide globalization process, are further unpredictable factors. Consequences, such as unexpected outbreaks of food allergies, can be very serious [6,7].

Modern studies of mechanisms of actions of allergens and detections of allergenic substances, allergology, started more than 50 years ago with the discovery of IgE by Ishizaka et al. [8]. By definition, food allergy is mediated by the immune system, and can be classified as (i) so-called type I hypersensitivity that is IgE mediated, (ii) non-IgE mediated hypersensitivity such as celiac disease, and (iii) cell mediated

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hypersensitivity such as allergic contact dermatitis [4,6]. Still valid hypothesis is that the gastrointestinal tract as the largest immunological organ is no more able to develop oral tolerance to a food allergen, or this tolerance has broken down as a result of influence of different factors. The most important of these factors are (i) biochemical properties of the allergen such as protein structure and posttranslational modifications, (ii) the level of mucin oligosaccharide containing layer, (iii) the concentration of secretory IgA and IgG4, structural integrity of the gut barrier and its intercellular junctions, and (iv) other factors from gut epithelium. Additionally, the allergen dose and timing (and time frame) of exposure and the enteric gut microbiome play an important role [6,9]. Interaction of these factors and the additional influence of non-oral routes such as respiratory tract, skin and cardiovascular system as well as other individual factors have also to be taken into consideration [10].

Almost all allergy testing methods are based on the detection and measurement of specific IgE against a tested allergen and on the IgE-mediated patient's reaction after the contact with tested substance. Either (i) raw food as assumed allergen source, (ii) extracted food proteins, or (iii) individual purified allergens, were used [1]. After the cloning of the first allergen in 1988, first skin tests with these recombinant proteins were performed. This development goes further in direction of use of recombinant allergens for high-throughput detection of food allergens and for specific immunotherapy [2,4,10,11]. Commercially available raw protein extracts derived from potentially allergenic foods are still most commonly used for testing of allergies. These preparations can be used for *in vitro* serological tests as well as *in vivo* skin tests. The main disadvantage of these tests is that both the allergen composition and concentration in raw extract are variable and standardization is difficult, sometimes even impossible [12]. It was the reason that purified preparation of individual allergens is now introduced in clinics for both *in vivo* and *in vitro* tests by use of different kits containing standardized reagents. According to Ciardiello et al. [1], in an ideal case, a reagent for the diagnosis of allergy in a specific food should contain an exact mixture of all potential allergens, and "nothing more",

and "to achieve this aims following two conditions shall be fulfilled: (i) the entire profile of allergenic molecules contained in the allergenic sources should be known, and (ii) reliable protocols and methodologies, useful to asses the pattern of allergic components really contained in the reagents used by the allergy test systems, should be available." These conditions are challenging, but also highly necessary in order to avoid false (both positive and negative) responses, and to reach an exact diagnosis. Hence, the result of one method used for diagnosis of allergy should be validated by another, independent method, especially in the case when false positive or negative results are assumed [6]. The use of high-throughput, highly reproducible and simply-to validate methods is now preferred. For this sake, enzyme-linked immunosorbent assays (ELISA) is still a leading method in clinical laboratories [13], but surface plasmon resonance (SPR) immunoassays [13], DNA-based methods [6] as well as mass spectrometry-based approaches [1,3,6], are progressively used. A list of methods of characterization for major allergenic proteins and their corresponding allergen sources is represented in Table 2. An overview and discussion about the use of these strategies for the detection and quantification of allergens will be presented in this review.

## 2. Methods for allergen determination

### 2.1. Immunological methods

#### 2.1.1. Diagnostic approaches in food allergies

Food allergy has been defined as an "adverse health effect arising from a specific immune response that occurs reproducibly on exposure to a given food" [14]. Food allergens are proteins or glycoproteins (usually of 10-80 kDa) basically resistant to digestive enzymes in the gastrointestinal tract (GIT) and heat treatment. In genetically predisposed (*i.e.* atopic) individuals IgE - mediated food allergy develops in two stages. In the *sensitization* phase, which usually occurs via the GIT, allergen specific IgE antibodies are produced by plasma cells after exposure to the source of

food allergen. In the second contact with the food allergen, cross-linking of specific IgE bound for high affinity receptors (FcεRI) on effectors cells (mast cells and basophils) induce release of mediators (histamine, leukotriens and cytokines) which induce clinical symptoms of allergy in the *elicitation* phase. Some food allergens from fruits and vegetables cause allergic reactions eaten raw, while most food allergens cause clinical reactions after thermal treatment (cooking) or digestion in the gastrointestinal tract. Cross-reactivity in food allergy occurs when a food allergen shares structural (conformational epitopes) or sequence (linear epitopes) similarity with a different food allergen or aeroallergen, which may then trigger an adverse reaction similar to that triggered by the original food allergen [14].

*In vivo* tests for detection of food allergy includes skin prick test (SPT) and oral food challenges (OFCs). In food allergy diagnosis by SPT commercial food extracts are most often employed in clinical practice [15]. However, because of the complexity of the starting material it is extremely difficult to standardize crude food allergen extracts which may vary in allergen content because of various factors. Indeed, commercial food allergen extracts did not show satisfactory sensitivity in diagnostic procedures [16], sometimes because of the lability of certain allergens. Therefore, instead of commercial food extracts culprit food has been employed in prick-to-prick testing particularly for plant-derived foods (fruits, vegetables). However, different plant-derived extracts may differ in allergenicity (difference in qualitative and quantitative content of allergens) and therefore influence food allergy diagnosis [17]. Besides commercial food extracts, SPT has been performed with various well-defined natural or recombinant food allergens in clinical trials [18]. The only definitive diagnostic method for food allergy is the oral food challenge. [1,19,20].

**2.1.2. Assays for quantification of food allergen-specific IgE**

Commercially available crude allergen extract-based tests for food allergy, particularly fruit allergy, frequently lack high sensitivity and specificity [16,19].

Allergen component-resolved diagnostic testing (CRD) is a new methodology in clinical food allergy diagnosis, which improves the ability to identify specific clinical phenotypes [21]. Instead of the crude allergen extracts used in standard allergy diagnostics, CRD utilizes purified natural or recombinant allergens for identification of specific IgE. The application of the recombinant DNA technology in the field of molecular allergology has allowed the production and characterization of a number of food allergens including those which are low abundant in the natural allergen source. For instance, in a model of allergy to kiwifruit the use of 6 structurally well-defined allergens (Act d 1, Act d 2, Act d 5, Act d 8, Act d 9, and Act d 10) improved the diagnostic performance in comparison with fruit extract [18]. Moreover, evaluated CDR of kiwifruit allergy with purified natural and recombinant allergens revealed that Act d 1 (cysteine protease) is important in monoallergy to kiwifruit, in which symptoms are often more severe [16].

In addition, CDR provides the possibility to perform cross-reactivity analysis among food allergens and to monitor specific IgE. Advances in detailed structural characterization of food allergens, together with the development of new technologies of producing high-capacity solid-phase matrices such as microarrays, the diagnosis of food allergy has become more precise. Microarray analysis can also be used to explain the different molecular sensitizations, including cross-reactivity phenomena [22]. The already available multiplex test systems based on microarray technology, such as ISAC system, allows investigation of the IgE binding profile for a panel of allergen proteins in a single test with minute amounts of patient's sera [1].

Assessment of biological reactivity of food allergen in terms of cross-linking of high affinity IgE receptors *ex vivo* is performed in basophils activation test (BAT). BAT provides accurate diagnosis of food allergies, but it is currently used primarily in research settings [15].

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**2.1.2. Detection and measurement of allergens in food**

Immunoanalytical methods have been the most widely employed for detection of allergens in foods. They have been designed in different formats, with the most conventional enzyme-linked immunosorbent assays (ELISAs) and strip tests. Strip tests are rapid, inexpensive, and do not require instrumentation. The limitation is that they are only qualitative tests; however, it is expected that in the future suppliers will develop simple handheld readers with which semi-quantitative results will be obtained [23].

ELISA is the most popular methodology for the routine monitoring of allergens because of its suitable sensitivity and precision [1,6]. ELISA has become the method of choice for food producers and control agencies performing routine analysis of food allergen contaminations [6]. Two formats of ELISA can be developed: competitive (direct) and sandwich test. While ELISA methods are appropriate for the detection of low levels of allergens (usually expressed as parts per million, ppm; μg/g of allergen) in complex matrices, discrepancies in quantitative results can arise due to limitations in protein extraction lack of standard reference materials, variations in batch and cultivar sampling, or epitope modifications due to food processing [1,6]. The majority of commercially available allergen detection methods are single-allergen based, which contributes to labor costs in evaluations of multiple-analyte food matrices. Demand for analytical strategies that can be used outside the laboratory environment to assess the safety and quality of foods is high, resulting in the development of a low-cost, rapid, miniaturized, and highly sensitive micro fluidic ELISA device for the detection of food allergens [24]. It has to be taken in consideration that immunoassays are antibody based, and consequently, different epitopes will be recognized, especially when monoclonal antibodies are used. As a consequence, variable results may be obtained when different systems are used. Additionally, ELISA can be time-consuming and expensive, especially if small numbers of samples are tested, that frequently happens in research laboratories [1,6]. Application of novel technologies provides innovative approaches in allergen

detection such as a flow-cytometry-based method for simultaneous detection of several allergens in a complex food matrix.

**2.2. Nucleic acid based strategies**

The DNA-based methods offer an alternative to immunological methods. The DNA-based test involves the extraction of a specific protein (allergen) encoding fragment that is followed by amplification by polymerase chain reaction (PCR). Alves et al. [25] summarize the most frequently used methods for allergen detection in food that are DNA-based, (i) PCR-ELISA; (ii) real-time PCR; (iii) PCR-peptide nucleic acid HPLC; (iv) duplex PCR; and multiplex real-time PCR. They stress the advantage of the multiplex approach that enables a simultaneous amplification of several DNA fragments by application of several pairs of primers. The absolute sensitivity of the method was very high, and was reported to be between 0.5 and 5pg for several types of nuts. In a recent comprehensive review, Du and Dong [26] report about recent advances of nucleic acid-based biosensors for protein (and allergen) detection. Most of methods that are used are still as listed above (i) DNA-based; (ii) aptamer-based (aptasensors); and (iii) DNAzyme-based biosensors. DNAzyme biosensors are based on catalytic nucleic acids, and until now, they are not used for allergen detection. The basic reactions of aptamer-based sensors that are used for allergen detection will be shortly described; the detection methods will be listed later.

Aptamers are single-stranded oligonucleotides or peptides that are able to bind a wide range of ligands with high affinity and specificity [25]. These molecules are used for in vitro selection or systematic evolution of ligands by exponential enrichment, so called SELEX. They are the sensing elements in so-called aptasensors, and can be used for binding of inorganic and small organic molecules, peptides and proteins, and even whole organisms and nanoparticles. The aptamers have a broad use in different sensors with almost all kinds of detection.

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As already mentioned above, although very sensitive, DNA-based methods for allergen detection can sometimes yield in false negative results. The reason is that the food processing can differently affect nucleic acids and proteins, and the levels of allergen encoding DNA is not always correlated with the presence of the allergen [24]. Moreover, heating or other food processing techniques might lead to changes in the structure of the target protein or target DNA, and it significantly influences the final detection. In this case, alternative methods for allergen detection and quantification shall be applied [24,26].

**2.3 Biosensors – Development, use and strategies**

Use of biosensors in medicine has a long tradition. Since 1962 the initial concept of glucose enzyme electrode was proposed, the fulminant start for development of biosensors for real time measurement of blood glucose was initiated [27]. The above shortly discussed blood glucose-measuring device clearly demonstrates the advantage of biosensors over other methods, especially for "every day's", routine use. It is the possibility of miniaturization (down to development of nano-devices, see Ref. 28) and high-throughput analysis [24]. These concepts are still not fully filled by other high-performance techniques [1,6,26]. However, several authors stressed the importance of proper sample preparation that is usually time consuming [6,29,30]. According to Alves et al. "…although the analytical measurement is immediate (and the sensor is considered as a "high-throughput device", Authors' comment), the time spent in the preparation of the sample is often not considered. Moreover, biosensors are sometimes developed with a purified allergenic protein as a standard, but the final device has not been applied to real samples" [24]. It means that the influence of food matrix is frequently neglected. Additional difference between the standard and the allergenic protein can be in posttranslational modifications [6], and possible modifications during food processing [6,24]. Sample preparation is topic of several recent reviews [24,29], and will be here discussed only shortly.

A biosensor is a device that contains a biological recognition component and a signal transduction (and signal amplification) device that is connected to a computer for both data acquisition and processing. The reaction between the target and sensing molecule can be further sensed and amplified (see Figure 1 A-D). Sensing molecules are most frequently monoclonal or polyclonal antibodies (for immunoassay-based biosensors), or allergen (or marker protein) encoding-DNA fragment that is in next step amplified by polymerase chain (PCR reaction), see Ref. [6] and Ref. [30]. Regarding the electrochemical immunosensors, reference can be made to recent comprehensive review by Wen et al. [31]. The most frequently used detection techniques are based on: (i) voltametry and amperometry; (ii) electrochemiluminiscence; (iii) photoelectrochemistry; and (iv) impedance. The mostly used amplification methods are nanomaterial-enhanced amplification, enzyme-based amplification and DNA-based amplification. Regarding additional detection techniques that are used for nucleic acid-based biosensor, Du and Dong [31] listed following ones: (i) fluorescent; (ii) electrochemiluminiscent; (iii) chemiluminiscent; (iv) colorimetric; (v) surface plasmon resonance; (vi) surface-enhanced Raman scattering; and (vi) gravimetric detection.

In comparison to our recent short overview [6] and as expected, nanomaterial-based amplification is a rapidly growing application in this field. Due to both their large surfaces and electron-transfer abilities, they also have a very high catalytic activity, they are biocompatible, and can be used as biolabels with significant signal amplification [25,26,30,31,32]. The use of optical nanoprobes in immunoassays leads to enormous increase of sensitivity and selectivity for the detection of analytes, such as trace amounts of allergenic proteins. According to Fu *et al.* [30] the nanomaterials used (i) as supports for the loading pf numerous indicators (*e.g.* biomolecules or fluorescent dyes) in order to amplify the recognition through their high surface-to-volume ratio (see above) and/or (ii) as the indicator that is generated with the aid of biochemical reactions to achieve multiple signal amplification. In this highly actual review, the authors also introduced various novel

types of nanomaterial-based optical immunosensors that mostly use above listed signal detection strategies.

### 2.3.2. Current perspectives in biosensor development

The use of microarray technology for allergy diagnosis and monitoring has been reviewed by a group of scientists participating in the allergy EU Framework Research Program that introduced an optimized allergen chip for monitoring of IgE and IgG reactivity again 170 allergen molecules and sera (so-called MeDALL allergen chip, that was initially design in 2002, see Ref. 33). Huang et al. [34] present recent development of membrane-based lateral flow immunochromatographic strips (LFICS) that can be used for fast and inexpensive multiplex detection of molecules, and optimization of this method towards quantitative analysis. Some interesting approaches for integration of sample preparation into devices for allergen detection and quantification can be also emphasized. Huang et al. [35] introduce "cell-to-cell" electrochemical microfluidic chip for detection and quantification of food allergens that is able to detect changes in secreted inflammatory cytokines in cultivated cell grown in the presence of allergens. Zhong et al. [36] developed a mass-barcoded nanoparticles with immobilized antihuman IgE antibodies for immunomagnetic capture of allergens. The captured proteins were identified by MALDI ToF mass spectrometry. Comprehensive reviews about development of this field were recently published by Fu et al. [30], Du and Dong [25] and Wen at al. [31].

## 2.4. Mass spectrometry (MS) based proteomic methods

MS-based proteomics, a twenty-year –old field, is one of the main pillars of foodomics and omics in general [37]. Nowadays MS-based proteomics methods can be applied to obtain: 1) qualitative and quantitative information about thousands of

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components of proteome, including allergen proteins in food proteome, in a highly reproducible and accurate manner [38], 2) structure of a single protein (*e.g.* allergen) including its proteoforms (chemically distinct species arising from a single gene as a result of alternative RNA splicing, endogenous proteolysis, mutations, coding single nucleotide polymorphisms, post-translational modifications), 3) structural information about protein – protein interaction (*e.g.* allergen – antibody interaction, identification of binding epitopes, interaction of allergen with human and microbial proteins in the gastro intestinal tract, interaction of allergen with surface proteins of epithelial cells) and structure of macromolecular assembles.

#### 2.4.1. Use of MS for quantification of allergens

Food industry and regulatory agencies, as key players in allergen risk management, require reliable approaches for quantification of allergens in food and food production facilities [39, 40]. Every physical or chemical treatment during food processing which may change structure of allergen protein may change its allergenicity and immunoreactivity [6]. Also, food processing may change components of food matrix impairing or promoting its extractability e.i. effective allergen concentration [24, 40]. Allergenicity of food proteins may differ between different strains of the same species due to expression of different isoforms [39, 41]. Inadequate sample preparation procedure can introduce modifications of amino acids which will also compromise quantification, and, for example, every quantification method based on specific recognition between allergen protein and antibody will be compromised by structural change of epitope or by cross reactivity with components of food matrix or neoepitopes which may be formed during food processing [28, 40]. Building of databases, containing information about possible sources of variability, is very important for development of high quality standard procedures for sample preparation and allergen quantification [39]. Problem of reliable quantification is one of the reasons why the threshold levels of safety for food allergens are hard to define.

Quantification of allergens by MS offers advantage in selectivity over immunological based quantification since it is not based on a molecular recognition of an epitope, but on mass/charge values in MS and MS/MS spectra which are characteristic for particular allergen and that can be monitored in a whole amino acid sequence to provide exclusive selectivity. Lack of complete structural information, combined with high costs of MS equipment and deficit of MS specialist are obstacles for wider application of this method. Technical advances are providing environment to overcome obstacles making LC-MS/MS based methods the fastest growing analytical methodologies in food analysis [42]. The amount of available structural information about proteoforms of allergens rapidly increases mostly thanks to MS-based proteomic methods, Table 3. Availability of structural information and further advances in sample preparation will strongly promote further application of MS in quantification of food allergens [28].

Absolute quantification strategies in LC-MS/MS are based on technology of stable isotope dilution [43]. LC-MS/MS based proteomic quantification can be performed at the level of whole protein (top-down approach) or at the level of peptides generated by protease digestion of analysed sample (bottom-up approach) [44]. Currently most exploited for quantification of food allergens are bottom-up approaches [26]. In bottom-up absolute quantification the reference can be supplied to the sample using different strategies: AQUA (absolute quantification), QconCAT (quantification concata-mer) and PSAQ (Protein standard absolute quantification) [43]. Quantification of very low abundant proteins requires their enrichment [45]. Enrichment can also be performed at the level of peptides using SISCAPA (Stable Isotope Standards and Capture by Anti-Peptide Anti-bodies) strategy [45].

Targeted proteomic approaches were developed for accurate and reproducible quantification of any protein or a set of proteins in any biological sample [46]. First targeted approach was based on MS acquisition technique called selected reaction monitoring (SRM), or its multiplexed version know as multiple reactions monitoring (MRM) [46]. Initially developed on triple quadruple (QqQ) mass spectrometers,

these acquisition techniques may also be applied on other LC-ESI-MS/MS systems. Total number of peptides that can be reliably quantified with SRM/MRM in one complex sample during a 60 min LC run is about a 100. If two peptides are selected for highly selective identification and quantification of particular protein allergen total number of monitored allergens can reach 50. High sensitivity comparable to immunoassays can be achieved. With design of scheduled MRM experiment it is possible to increase number of quantified peptides in a single LC-MS/MS run. Most important factor in design of SRM/MRM method is selection of specific (signature) peptides with characteristic transitions that will be monitored by MS/MS. Thus, detail structural information about allergen proteoforms and information about possibly interfering transitions in particular food sample are crucial [47,48,49].

Parallel reaction monitoring (PRM) is a MS acquisition technique based on high resolution hybrid quadruple Orbitrap (Q-OT) mass spectrometer. High resolution of Orbitrap mass analyser increased selectivity leading to partially improved quantification performance compared to SRM/MRM. With internal standards and adjustment of acquisition parameters it is possible to quantify 600 peptides in complex samples in less than 70 min LC-MS/MS run [41]. Method design in PRM is substantially less demanding but detail structural information about allergen proteoforms is prerequisite for reliable quantification.

Data independent acquisition (DIA) techniques provide possibility to overcome limitations of S/MRM and PRM in absolute quantification: substantially increase number of proteins that can be simultaneously quantified, simplify experimental design and provide flexible postacquisition data analysis [50]. The SWATH MS is a combination of DIA and targeted data analysis, developed on quadruple –time of flight (QqTOF) mass spectrometer, and can be applied on other high resolution MS/MS systems. This method vastly extends the number of proteins that can be quantified in complex sample. Recent study employing SWATH MS demonstrated quantification of 2500 proteins in a 3-h LC-MS/MS run with reproducibility, precision and accuracy comparable to S/MRM [51].

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Once developed, optimized and validated MS based proteomic methods for quantification of allergen in a particular food sample can be deposited in data bank and easily transferred between laboratories. For MS based proteomic approaches for quantification it is possible to establish metrological traceability which enables meaningful comparison of quantitative results for allergens among laboratories [52].

## 2.4. Identification and characterisation of food allergen proteins by use of MS

Our knowledge of primary sequences of proteins as food allergens, especially their proteoforms, is far from the level which can enable us clear answer to the question which structural features makes a particular protein evoke food allergy. So, how many food allergens are there? The 3D structure is known only for about 12% of FA and this information is of importance for understanding of IgE binding structures and prediction of IgE binding epitopes of novel or modified proteins [53]. Identification of new allergens in food requires probing of particular food proteome with individual sera from a very large population of allergic subjects [1]. Sensitivity and specificity of MS based proteomic technology in combination with immunoaffinity separation and enrichment provides possibility to efficiently use blood banks for screening in high-throughput manner. Allergenicity of proteins detected by use of immunoaffinity due to possible cross-reactivity has to be confirmed with oral food challenge test or skin prick test [4]. A recent study demonstrated component resolved diagnostic of milk allergy directly from milk fractions using only 2 µl of blood serum [54]. MS based proteomics enable complete analysis of primary structure, including structure of glycans in a case of glycoproteins, of unknown allergen from 10-150 ug (MW<100kDa), depending on complexity of particular allergen. Software tools for *de novo* sequencing are shortening analysis time considerably but manual annotation is still required. Identification of changes in primary structure of allergen introduce by food

processing may require much more material. FA amino acid sequence can be confirmed much easier in regard to the distinction between isobaric amino acids (Ile/Leu) if analysis involves genomic or transcriptomic data. When genomic or transcriptomic data are available the amount of protein which is necessary for confirmation of particular FA amino acid sequence can be lower than 1 ug. However, since information about allergen proteoforms cannot be deduced from genomic information this analysis requires above mentioned amounts of protein material. MS based proteomic methods can be employed for identification of allergen IgE binding epitopes [55,56]. Significant group of food allergens exhibits hydrolytic activity (*e.g.* cysteine protease, glucosidase, chitinase). Native form of FA which exhibits enzyme activity can be analysed by MS based proteomic approaches using activity based probes [57].

Risk assessment strategies for introduction of novel food processing technologies, novel food sources and new varieties are required. MS based proteomic methods can be applied to study effect of food processing technologies [58,59,60,61]. Different *in silico, in vitro* and rodent models are developed for screening and prediction of allergenicity of novel food proteins [1, 39, 62]. MS based proteomic methods are powerful tools for identification of MHC I / II displayed peptides [63]. Arrays of different MHC molecules, covering over 90 % of population, are developed to assess the likelihood of allergic responses to novel proteins occurring *in vivo* [62].

**3. Conclusions**

Immunological methods are still most frequently used for diagnosis of allergies and for detection and quantification of food allergens. They are sensitive, and can be used for determination of allergens in trace concentrations, but the lack of specificity and cross-reaction of some antibodies can still be a relevant source of bias. Nucleic acid based methods are fast and reliable ways for detection of protein allergens. However, the epitopes of protein allergens with posttranslational modifications and their changes originated during food processing cannot be traced by use of this analytical strategy. Big advantage of both immunological and nucleic

based methods is relatively simple adaptation for high-throughput analysis and detection of allergens. Both strategies are significantly supported by rapid development of biosensors [25,30] and their miniaturization and increasing application of nanotechnology [30,34,35].

Regarding accuracy, reliability and sensitivity, MS brings important advantage over both antibody- and nucleic acid-based strategies. Targeted approached in MS-based allergen protein analysis introduced further advance in analysis of protein allergens. However, the disadvantage of mass spectrometry is, that it still cannot be used for high-throughput analyses for the reason of price and still long analysis time as well as the need for highly qualified MS specialists. Proper sample preparation is further frequently neglected aspect in detection and quantification of allergens. Recent results in direction of high-throughput strategy are encouraging, but further development is still necessary [6,24,29].

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## Table and figure legends

**Table 1.** List of allergens officially named by the WHO/IUIS according to their phyla, number and presence in food. (www.allergen.org, accessed on 15.03.2017.)

**Table 2.** A list of major allergenic proteins and their corresponding allergen sources for which 5 or more allergens are known. (www.allergome.org, accessed on 17.03.2017.)

**Table 3.** Absolute and relative quantification of allergens, list of strategies and key References.

**Figure 1.** Examples of biosensor devices, A) General scheme of a biosensor, B) Voltameric genosensor, C) Surface Plasmon Resonance, D) Impedimetric biosensor

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| Kingdom | Total allergens | Phylum | Total allergens | Food allergens |
|---|---|---|---|---|
| **Animalia** | 355 (41%) | Arthropoda | 250 | 33 |
| | | Chordata | 82 | 43 |
| | | Cnidaria | 1 | 0 |
| | | Mollusca | 4 | 4 |
| | | Nematoda | 18 | 14 |
| **Plantae** | 404 (46%) | Liliopsida | 97 | 31 |
| | | Magnoliopsida | 289 | 180 |
| | | Pinopsida | 18 | 2 |
| **Fungi** | 111 (13%) | Ascomycota | 86 | 0 |
| | | Basidiomycota | 23 | 0 |
| | | Zygomycota | 2 | 0 |

Table 1. List of allergens officially named by the WHO/IUIS according to their phyla, number and presence in food. (allergen.org, accessed on 15.03.2017.)

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| | Fruit | | Vegetable | | Cereal | | Legume | |
|---|---|---|---|---|---|---|---|---|
| **Number of known allergens** | Kiwi | 48 | Olive | 32 | Wheat | 124 | Peanut | 52 |
| | Apple | 41 | Carrot | 15 | Corn | 32 | Soybean | 48 |
| | Tomato | 26 | Celery | 13 | Barley | 29 | Rice | 22 |
| | Peach | 19 | Potato | 12 | Rye | 20 | Pea | 11 |
| | Strawberry | 17 | Turnip | 9 | Sesame | 15 | Mung bean | 11 |
| | Banana | 16 | Bellpepper | 7 | Oat | 11 | Kidney bean | 10 |
| | Cherry | 14 | | | Amaranth | 7 | Chickpea | 7 |
| | Orange | 12 | | | Millet | 6 | Lentil | 6 |
| | Cantaloupe | 10 | | | | | | |
| | Pear | 10 | | | | | | |
| | Grape | 10 | | | | | | |
| | Canola | 6 | | | | | | |
| | Asparagus | 5 | | | | | | |
| | Pineapple | 5 | | | | | | |
| **Methods of characterization for major allergenic proteins** | **Profilin** – LC/MS-MS (Moya R, et al. Mol Immunol. 2017, 83:100-106)<br><br>**Chitinase** – LC/MS (Kabir SR, et al. Int J Biol Macromol. 2016, 84:62-8)<br><br>**Thaumatin** - MALDI–TOF MS (Hegde VL, et al. Mol Nutr Food Res. 2014, 58:894-902)<br><br>**Lipid transfer protein** – LC-MS/MS, MALDI-IMS (Cavatorta V, J Mass Spectrom. 2009, 44:891-7) | | **Profilin** – ELISA, immunoblot (Sankian M, et al. Rep Biochem Mol Biol. 2013, 1:49-63)<br><br>**Lipid transfer protein** – LC-MS/MS, MALDI-IMS (Bencivenni M, et al. J Mass Spectrom. 2014, 49:1264-71)<br><br>**Thaumatin** - LC-MS/MS, ELISA (Muñoz-García E, et al. Mol Nutr Food Res. 2013, 57:2245-52) | | **Globulin, Albumin** – circular dichroism, FTIR spectroscopy, fluorescence spectroscopy (Jing X, et al. J Food Sci. 2016, 81:C2337-C2343)<br><br>**Gliadin, Glutenin, α-amylase/trypsin inhibitors** – UPLC-Q-TOF (Uvackova L, et al. J Proteome Res. 2013, 12:4862-9) | | **Oleosin** – LC-MS/MS (Schwager C, et al. PLoS One. 2015, 10:e0123419)<br><br>**Defensin** – immunoblot, LC-MS/MS (Petersen A, et al. J Allergy Clin Immunol. 2015, 136:1295-301)<br><br>**Globulin** – MALDI-TOF MS (Nadal P, et al. J Agric Food Chem. 2011, 59:2752-8)<br><br>**Lipid transfer protein** - MALDI-TOF/TOF MS (Bogdanov IV, BMC Plant Biol. 2016, 30:107) | |

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| | Tree nut | | Dairy | | Fish | | Crustacean shellfish | | Mollusk | |
|---|---|---|---|---|---|---|---|---|---|---|
| **Number of known allergens** | Hazelnut<br>Walnut<br>Chestnut<br>Almond<br>Pistachio<br>Pecan<br>Pine | 34<br>23<br>15<br>14<br>11<br>6<br>6 | Cow<br>Chicken<br>Sheep | 53<br>32<br>10 | Cod<br>Salmon<br>Tuna<br>Mackerel<br>Crayfish<br>Carp<br>Herring | 32<br>26<br>17<br>14<br>11<br>10<br>5 | Shrimp<br>Crab<br>Lobster<br>Prawn | 95<br>54<br>27<br>22 | Clam<br>Squid<br>Octopus<br>Cuttlefish<br>Mussle | 14<br>13<br>8<br>6<br>4 |
| **Methods of characterization for major allergenic proteins** | **2S albumin, 7S globulin, 11S globulin** – MALDI-TOF/TOF, LC/PDA/ESI-MS (Reitsma M, et al. J Agric Food Chem. 2016, 64:1191-201)<br><br>**Lipid transfer protein** – MALDI-TOF MS, circular dichroism (Offermann LR, et al. J Agric Food Chem. 2015, 63:9150-8)<br><br>**Bet V 1-like** - ESI-QTOF-MS (Hauser M, et al. Clin Exp Allergy. 2011, 41:1804-14) | | **Casein, Lactalbumin** – LC-MS/MS (Madende M, et al. J Dairy Sci. 2015, 98:8308-18)<br><br>**Lysozyme, Ovalbumin, Ovotransferrin, Ovomucoid** – MALDI-TOD/TOF MS, circular dichroism, NMR, ELISA (Jacobsen B, et al. Mol Nutr Food Res. 2008, 52 Suppl 2:S176-85) | | **Parvalbumin** – LC-MS/MS (Li Z, et al. J Agric Food Chem. 2014, 62:6212-8)<br><br>**Enolase** – MALDI-TOF MS (Liu R, et al. J Agric Food Chem. 2011, 59:458-63)<br><br>**Aldolase** – MALDI-TOF/TOF MS (Liu R, et al. Int J Food Sci Nutr. 2012, 63:259-66) | | **Tropomyosin** – circular dichroism, immunoblot, ELISA (Kumjim S, et al. Asian Pac J Allergy Immunol. 2016, 34:229-235)<br><br>**Arginine kinase** – MALDI-TOF/TOF MS (Chen HL, et al. Food Chem Toxicol. 2013, 62:475-84) | | **Tropomyosin** - MALDI-TOF MS (Mohamad Yadzir ZH, et al. Biomed Res Int. 2015) | |

Table 2. A list of methods of characterization for major allergenic proteins and their corresponding allergen sources for which 5 or more allergens are known. (www.allergome.org, accessed on 17.03.2017.)

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Table 3. Absolute and relative quantification of allergens

| MS method | MS system | Food allergen source | Reference |
|---|---|---|---|
| Targeted SRM | UPLC(C18)-ESI-QqQ | Wheat, rye, barley and oats, flours (corn, soy and rice) | Martínez-Esteso MJ, et al. Defining the wheat gluten peptide fingerprint via a discovery and targeted proteomics approach. J. Proteomics 147 (2016) 156 |
| Targeted MRM | UHPLC(C18)-ESI-QqQ | Soybean Seed | Hill RC, et al. Development, Validation, and Interlaboratory Evaluation of a Quantitative Multiplexing Method To Assess Levels of Ten Endogenous Allergens in Soybean Seed and Its Application to Field Trials Spanning Three Growing Seasons. J. Agric. Food Chem. (2017) doi: 10.1021/acs.jafc.7b01018 |
| Targeted | nanoHPLC(C18)-ESI-LTQ-OT Velos | Wheat | Rogniaux H, et al. Allergen relative abundance in several wheat varieties as revealed via a targeted quantitative approach using MS. Proteomics. 15 (2015) 1736 |
| Targeted MRM | HPLC(C18)-ESI-Q-TOF | Shrimp | Abdel Rahman AM, Kamath SD, Gagné S, Lopata AL, Helleur R. Comprehensive Proteomics Approach in Characterizing and Quantifying Allergenic Proteins from Northern Shrimp: Toward Better Occupational Asthma Prevention. J. Proteome Res. 12 (2013) 647 |
| Targeted MRM and $MRM^3$ | HPLC(C18)-ESI-QqQ | Shrimp | Korte R, et al. New High-Performance Liquid Chromatography Coupled Mass Spectrometry Method for the Detection of Lobster and Shrimp Allergens in Food Samples via Multiple Reaction Monitoring and Multiple Reaction Monitoring Cubed. J. Agric. Food Chem. 64 (2016) 6219 |
| Targeted MRM | UHPLC(C18)-ESI-Q-TOF | Nuts | Sealey-Voyksner J, Zweigenbaum J, Voyksner R. Discovery of highly conserved unique peanut and tree nut peptides by LC–MS/MS for multi-allergen detection. Food Chem. 194 (2016) 201 |
| Targeted MRM | HPLC(C18)-ESI-IT | Baked food | Cristina L, et al. Validation of a mass spectrometry-based method for milk traces detection in baked food. Food Chem. 199 (2016) 119 |
| Targeted SMIM | HPLC-nanoESI-LTQ | Fish | Carrera M, Cañas B, Gallardo JM. Rapid direct detection of the major fish allergen, parvalbumin, by selected MS/MS ion monitoring mass spectrometry. J. Proteomics 75 (2012) 3211 |
| Targeted PRM | nanoHPLC(C18)-ESI-LTQ-OT Velos | Anisakid (fish-borne parasites) | Carrera M, et al. Protein biomarker discovery and fast monitoring for the identification and detection of Anisakids by parallel reaction monitoring (PRM) mass spectrometry. J. Proteomics 142 (2016) 130 |

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|---|---|---|---|
| Targeted MRM and DIA SWATH | nanoHPLC(C18)-ESI-Q-TOF | Barley | Colgrave ML, et al. Comparing Multiple Reaction Monitoring and Sequential Window Acquisition of All Theoretical Mass Spectra for the Relative Quantification of Barley Gluten in Selectively Bred Barley Lines. Anal. Chem. 88 (2016) 9127 |
| Bottom-up Top6 DDA LFQ | UHPLC(C18)-nanoESI-LIT-OT Elite | Walnuts | Downs ML, Baumert JL, Taylor SL, Mills EN. Mass spectrometric analysis of allergens in roasted walnuts. J. Proteomics 142 (2016) 62 |
| Bottom-up DIA $MS^E$ | nanoHPLC(C18)-ESI-Q-TOF | Wheat | Uvackova L, Skultety L, Bekesova S, McClain S, Hajduch M. $MS^E$ Based Multiplex Protein Analysis Quantified Important Allergenic Proteins and Detected Relevant Peptides Carrying Known Epitopes in Wheat Grain Extracts. J. Proteome Res. 12 (2013) 4862 |
| Determination of allergen structure and identification of new allergens | | | |
| Proteomic shotgun, Top2 DDA | HPLC(C18)-ESI-IT | Lotus japonicas seeds | Dam S, Thaysen-Andersen M, Stenkjær E, Lorentzen A, Roepstorff P, Packer NH, Stougaard J. Combined N-glycome and N-glycoproteome analysis of the Lotus japonicus seed globulin fraction shows conservation of protein structure and glycosylation in legumes. J. Proteome Res. 12 (2013) 3383. |
| Glycomic | HPLC(PGC)-ESI-IT | | |
| Glyco-proteomic | MALDI-TOF/TOF | | |
| Shotgun Top5 DDA | UHPLC(C18)-ESI-LTQ-OT XL | Mustard seeds | Hummel M, Wigger T, Brockmeyer J. Characterization of mustard 2S albumin allergens by bottom-up, middle-down and top-down proteomics: A consensus set of isoforms of Sin a 1. J. Proteome Res. 14 (2015) 1547 |
| Top-down | UHPLC(C4)-ESI-LTQ-OT XL | | |
| Shotgun Top5 DDA | UHPLC(C18)-ESI-LTQ-OT XL | Hazelnut seeds | Korte R, Happe J, Brümmer I, Brockmeyer J. Structural Characterization of the Allergenic 2S Albumin Cor a 14: Comparing Proteoform Patterns across Hazelnut Cultivars. J. Proteome Res. 16 (2017) 988 |
| Top-down | UHPLC(C4)-ESI-LTQ-OT XL | | |
| Shotgun Top8 DDA | nanoHPLC(C18)-ESI-LTQ-OT XL | Peanut kernels | Hebling CM, Ross MM, Callahan JH, McFarland MA. Size-Selective Fractionation and Visual Mapping of Allergen Protein Chemistry in Arachis hypogaea. J. Proteome Res. 11 (2012) 5384 |
| Bottom-up IM assisted DIA | nanoHPLC(C18)-ESI-Q-IM-TOF | Wheat gluten | Bromilow S, et al. A curated gluten protein sequence database to support development of proteomics methods for determination of gluten in gluten-free foods. J. Proteomics 163 (2017) 67 |
| Shotgun Top3 | nanoHPLC(C1 | Wheat | García-Molina MD., et al. Comparative proteomic analysis of two transgenic low-gliadin wheat lines and non-transgenic wheat |

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|---|---|---|---|
| DDA | 8)-ESI-LIT | gluten | control. J. Proteomics (2017) doi: 10.1016/j.jprot.2017.06.010 |
| Shotgun Top3 DDA | nanoHPLC(C18)-ESI-LIT | Wheat | Arena S, et al. Differential representation of albumins and globulins during grain development in durum wheat and its possible functional consequences. J. Proteomics (2017) 86 |
| Shotgun DDA | UHPLC(C18)-nanoESI-Q-OT-LIT Fusion | Ovine milk | Cunsolo V, et al. Polyphemus, Odysseus and the ovine milk proteome. J. Proteomics 152 (2017) 58 |
| Shotgun Top20 DDA | UHPLC(C18)-nanoESI-LIT-OT Elite | Beer | Grochalová M, et al. Deep coverage of the beer proteome. J. Proteomics 162 (2017) 119 |
| Shotgun Top10 DDA | UHPLC(C18)-ESI-Q-TOF | Beer | Picariello G, et al. Proteomics, peptidomics, and immunogenic potential of wheat beer (Weissbier). J. Agric. Food Chem. 63 (2015) 3579 |

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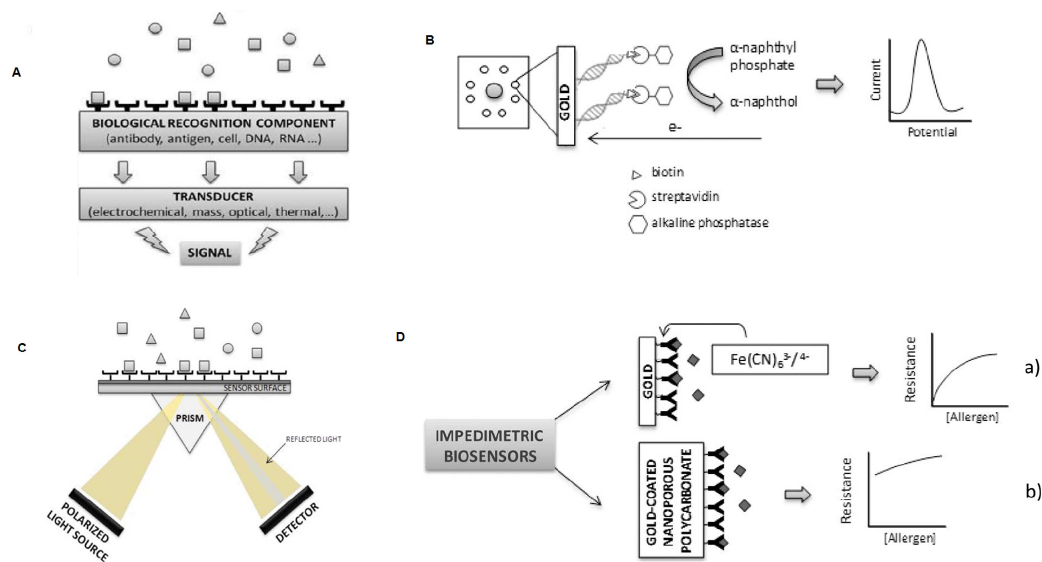


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**Highlights**

- Use of omics methods with main focus on proteomics for study food allergies
- Immunoanalytical and nucleic acid-based methods for detection of allergens
- Biosensors as tools for food allergen high-throughput detection and quantification
- Mass spectrometry provides qualitative and quantitative data on food allergens

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