

Review

Liquid Chromatography and Mass Spectrometry in Food Allergen Detection

CHRISTIANE KRUSE FÆSTE,^{1,2*} HELENE THORSEN RØNNING,² UWE CHRISTIANS,³ AND PER EINAR GRANUM²

¹Section of Chemistry, Department of Feed and Food Safety, National Veterinary Institute, P.O. Box 750 Sentrum, Oslo N-0106, Norway; ²Centre for Food Safety, Norwegian School of Veterinary Science, Oslo 0033, Norway; and ³Department of Anesthesiology, University of Colorado–Denver, Aurora, Colorado 80045, USA

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ABSTRACT

Food allergy is an important issue in the field of food safety because of the hazards for affected persons and the hygiene requirements and legal regulations imposed on the food industry. Consumer protection and law enforcement require suitable analytical techniques for the detection of allergens in foods. Immunological methods are currently preferred; however, confirmatory alternatives are needed. The determination of allergenic proteins by liquid chromatography and mass spectrometry has greatly advanced in recent years, and gel-free allergenomics is becoming a routinely used approach for the identification and quantitation of food allergens. The present review provides a brief overview of the principles of proteomic procedures, various chromatographic set ups, and mass spectrometry instrumentation used in allergenomics. A compendium of published liquid chromatography methods, proteomic analyses, typical marker peptides, and quantitative assays for 14 main allergy-causing foods is also included.

Food allergy is considered by the World Health Organization to be the fourth most important public health problem (161). Food allergies have implications for the general health status, economy, and legislation of a country if a considerable percentage of its population must deal with dietary restrictions and food-triggered immunoglobulin (Ig) E-mediated allergic reactions ranging from mild to life threatening. Therefore, national and international food labeling directives have been developed requiring food manufacturers to declare ingredients with known allergenic potential (272).

Correct labeling of food products allows allergic consumers to avoid the offending allergens, thus preventing reactions (240). Food allergens are common food proteins and peptides, e.g., plant seed storage proteins or animal muscle proteins that are recognized by the immune system of a sensitized patient even in small amounts (215, 280, 296). Threshold levels that would protect 99% of allergic consumers are under consideration, although it is uncertain whether levels at which there are no observable adverse effects can be identified (161). However, results from clinical studies indicate that 1 to 10 mg/kg concentrations of allergens in food is sufficient to elicit an allergic reaction. Allergenicity is caused by the food proteins either in their native forms or in commodities resulting from food processing (240). Thus, the implementation and control of product labeling guidelines depends on the availability of

reliable and sensitive analytical methods that specifically identify allergenic proteins in complex mixtures (283).

Various techniques have been applied to determine the presence of allergens in foods (161, 283). The methods are qualitative or quantitative and detect either the allergenic protein itself or a marker that typically signifies its presence. Protein-based methods usually are based on immunochemical allergen detection, involving either IgE from patient sera or polyclonal and monoclonal IgG antibodies generated by animal immunization. In contrast, PCR methods are commonly used indirect techniques that detect DNA that is representative for the allergenic food.

However, the enormous progress in mass spectrometry (MS) technology and methodology in the last decade has given a boost to the field of proteomics, including the identification, characterization, and determination of food allergens (179, 240). Proteomics refers to the large scale analysis of all proteins produced by a species (369). Whereas the genome of an organism is stable, the proteome undergoes constant changes depending on variations in protein expression rates due to extra- and intracellular conditions, co- and posttranslational modifications, splicing variants, covalent and noncovalent associations, and spatial and temporal differences in distributions (139).

Many MS techniques have been developed for different aspects of proteomic analysis (1, 96). MS-based proteomics can be used for the identification and quantitation of proteins and peptides, the determination of primary sequences, and the detection of protein interactions and modifications (30).

* Author for correspondence. Tel: +4723216232; Fax: +4723216201; E-mail: christiane.faste@vetinst.no.

Proteomic analysis usually involves the simultaneous measurement of thousands of proteins from a complex biological matrix. Separation of the proteins before MS analysis is therefore crucial to reduce sample complexity. This separation is commonly achieved electrophoretically on one-dimensional (1D) or 2D gels or by liquid chromatography (LC). Despite the many improvements made to address the technical limitations of gel electrophoresis, LC remains the superior separation method in terms of reproducibility, recovery, sensitivity, dynamic range, and quantifiability (315). LC is also more versatile because of a wide choice of stationary phases. Interfacing with commonly used MS instruments is not problematic.

The technical progress, decreasing costs of necessary instrumentation, and facilitated access to data processing tools have taken MS-based proteomics from being a method applied by specialists to a broadly used technique in protein analyses. Over the last decade, there has been an enormous increase in the number of publications describing the use of LC-MS methods in proteomics for the characterization of proteins in foods. Topics range from the characterization of single proteins in buffer to the identification by sequence determination of a protein in a complex mixture to the quantitative measurement of allergens in food matrices. Numerous review articles on the use of MS and LC for food peptides and proteins have been published (5, 63, 67, 179).

The present review is presented to provide a compendium of studies conducted as of April 2010 involving MS and LC for the identification and determination of 14 main allergy-causing foods. In four tables, published LC methods, MS methods, recognized marker peptides, and quantitative assays are listed for each food. Because of the speed of publication and the volume of new articles, the review cannot be considered complete. However, it is intended to provide a comprehensive overview over the current status.

MS IN PROTEOMICS

MS instruments used in proteomic analysis. Customized instruments have been built to serve specific purposes. Mass spectrometers are composed of three inline coupled components: an ion source, a mass analyzer, and a detector. Various designs and modes of action are available for each of the modules, adjusting them to different applications.

The most commonly used ion sources in proteomic analysis are electrospray ionization (ESI) and matrix-assisted laser desorption ionization (MALDI) (240, 360). With ESI, analytes are ionized and volatilized out of a solution by high electric voltage, and ESI instruments can be easily connected upstream to an LC system. With MALDI, samples are cocrystallized with a matrix compound on a plate and ionized with a laser. The coupling between MALDI and LC systems is more complicated and requires deposition of the mobile phase on the MALDI plate and mixing with the matrix (232). Until recently, quantitative analysis with MALDI was not considered practicable because of inhomogeneous crystallization on the target surface and resultant lack of reproducibility. However, these

technical problems can be overcome (101, 136). With MALDI, singly charged analyte ions are typically observed, whereas ESI generally induces multiply charged states. Surface-enhanced laser desorption ionization (SELDI) is a modification of the MALDI technology that introduces a further purification step on the probe surface before MS analysis (289). Fast atom bombardment is an older ionization technique in which the material to be analyzed is mixed with a nonvolatile matrix that is bombarded under vacuum with a high-energy beam of atoms (179).

Five types of mass analyzers are commonly used in proteomics: quadrupoles (Q), time-of-flight (TOF) analyzers, ion traps (IT), Orbitraps, and Fourier transform ion cyclotron resonance (FTICR) analyzers (1, 96, 139, 360). These types differ widely in their functionality and their sensitivity, mass resolution, mass accuracy, and mass range (240). The mass analyzers can be used individually or can be combined in tandem MS (MS-MS), which leads to combinations that are typically used for analysis in proteomic research such as Q-TOF, Q-IT, TOF-TOF, IT-FTICR, IT-Orbitrap, and QqQ (143, 201). All instruments separate the analyte ions by their mass-to-charge (m/z) ratios. Ion motion is controlled by electric and magnetic fields.

Quadrupoles are mass filters that use changing radio frequency and deflection voltage potentials to let pass ions of only selected m/z values. Reproducibility with this technique is good, but resolution and mass accuracy are limited. TOF measures the mass-dependent time it takes ions to move through the flight tube. Resolution and accuracy are moderate, and the mass range is high; however, the dynamic range can be limited. Ion traps operate by storing ions in deflection voltage- and radio frequency-controlled electric fields in carefully timed events. They are less suited for quantitative MS because only a limited number of ions can be held. In contrast, IT allows the generation of multiple-stage fragmentation spectra (MS^n) enabling analyte structure and sequence elucidation. The two most common IT types are Q-IT and IT-FTICR. Q-IT exists in linear (LIT) and 3D variants containing four parallel electrodes or three hyperbolic electrodes, respectively. FTICR instruments use a constant static magnetic field and a spatially nonhomogeneous static electric field to trap ions, which are forced into a circular cyclotron motion. The m/z values are calculated by Fourier transformation from the frequency of ion motion, resulting in excellent mass accuracy and resolution power (1, 96, 139). The more recently developed Orbitrap analyzers separate ions in an oscillating electric field. They thereby combine several advantages of other techniques, allowing mass determinations with high accuracy and resolution and quantitative analysis, and the LIT-Orbitrap hybrid can be used to perform accurate MS^n experiments (308).

Peptide mass fingerprinting and peptide sequence determination. The specific fragmentation of analyte precursor ions into definite product ions is an important resource for the identification of test compounds (360). The fragmentation is achieved in MS-MS instruments by

applying a collision gas (collision-induced dissociation) or by electronic destabilization (electron transfer dissociation, electron capture dissociation, or photo dissociation) of the analyte (201, 240) to break up the most labile molecular bonds. Spectra from single MS experiments are used for peptide mass fingerprinting to compare the acquired peptide masses to those in protein databases (250, 303), whereas MS-MS spectra can be used for either peptide fragmentation mass fingerprinting or to obtain peptide sequence information. Peptides generally split at the peptide bonds in the backbone, leading to six possible fragment ions: the a, b, and c ions containing charged amino terminals and the x, y, and z ions containing charged carboxyl terminals. The most common series of fragments are the b and y ions, which occur after cleavage of the CO-NH bond. Charge separation in multiply charged ions can produce complimentary b-y ion pairs in the MS-MS spectrum of a peptide, which facilitates peptide sequence interpretation.

Protein identification by top-down and bottom-up proteomics. There are two principle approaches to proteomic analysis: top down and bottom up (240). The top-down method is used when intact proteins are first separated and then presented to the mass spectrometer, either directly or after enzymatic digestion. The bottom-up method, also called “shot-gun” proteomics (43), is the more commonly used procedure and involves submitting complex protein mixtures for digestion into even more complex peptide mixtures, which then are chromatographically resolved and analyzed by MS. Spectra are collected for the greatest possible number of peptides and compared with spectra in databases for protein identification (139). Protein characterization is obtained by deriving peptide sequence information from multiple MS experiments (38, 67). Proteomics experiments generate enormous amounts of data, necessitating powerful bioinformatic tools for extraction of valuable information. The acquired peptide fragment masses are compared with data from databases, resulting in score list outputs. Multiple software products for subsequent data processing, e.g., for sequence alignment and protein structure elucidation, are available.

The large number of analytes in complex mixtures makes automated data processing necessary. Differential MS is used to directly compare m/z ratios and intensities at each time point for the detection of quantitative differences in large MS profiles (227, 353).

Quantitative MS-based proteomics. The quantitative determination of specific proteins and peptides can be important in various fields of proteomics, such as translational and modificalomics (the study of posttranslational protein modifications). The main interest for food allergens is the measurement of the absolute quantity of an allergenic protein in a food matrix (161). LC-MS methods have been developed both on- and off-line (35). However, quantitation always depends on the proportionality between the amount of analyte in the sample and the recorded signal. In global experiments involving comparative relative quantitation, e.g., cellular protein synthesis and protein

abundance, measurements can be obtained by chemical, metabolic, or enzymatic labeling involving such techniques as isotope-coded affinity tagging (ICAT), isotope-coded protein (ICP) labeling, isobaric tagging for relative and absolute quantitation (iTRAQ), stable isotope-labeling by amino acids in cell culture (SILAC), or labeling with heavy water (^{18}O) (139, 161). Several different approaches for label-free relative quantitation also have been suggested (50, 276). Two samples can be compared by calculating the respective total or mean peptide precursor ion intensities, the intensity of a common peptide, the number of distinct peptides or merged MS-MS spectra, or the sequence coverage of a target protein.

By contrast, the absolute quantitation of distinct proteins and peptides is possible only by determining the correlation between the ion signal and a measured standard curve. Quantitation at the peptide level is preferable to quantitation at the protein level because the identification of intact proteins in complex matrixes can be substantially limited by ion suppression of coeluting proteins and peak superposition of the mass spectra (161). Unique marker peptides are selected as specific biomarkers for the food moiety for their stability in sample preparation and measurability by MS-MS with regard to selectivity, sensitivity, and reproducibility (161, 240). For food allergens, the marker peptide should be present in both native and processed foods.

The addition of an internal standard (ISTD), ideally an isotope-labeled analogue (labeled with D, ^{13}C , or ^{15}N) of the measured target protein or peptide that guarantees molecular characteristics similar to those of the analyte, ensures control over instrument performance and recovery from both sample extraction and the LC-MS process (43, 101). Optimally, the ISTD should already be incurred during sample preparation, although in most cases it will be mixed with the homogenized sample before the extraction procedure. Thus, there might occur differences in the conformations of the ISTD and the target protein that could have been degraded, cross-linked, or modified through processing and cooking of the food product. The intensities of the analyte and the ISTD are measured in the MS experiment, and method parameters such as limit of detection, working range, repeatability, and reproducibility are determined for the food matrices of interest and validated according to international standards (333).

Several protocols are available for the use of isotope-labeled ISTDs in quantitative analysis. These protocols differ in the enzymatic digestion of the ISTD during sample preparation and the direct or indirect determination of the target protein's concentration. In the absolute quantitation (AQUA) method and the quantitation by concatemers (QConCat) method, the ISTD is codigested with the analyte, and peptide concentrations are measured and used to generate the standard curve. The protein standard for absolute quantitation concept uses labeled proteins, obtaining the concentration of the protein directly (161).

The application of stable isotope label-free (SIF) techniques for absolute quantitation by MS-MS analysis is based on either the measurement of ion signal intensities or

spectral counting. Validation can be achieved by using a protein of constant quantity in the sample mixture as a “pseudo” ISTD (64, 65).

Various types of mass spectrometers can be employed for quantitative analysis. Triple quadrupole (QqQ) instruments running in multiple reaction monitoring (MRM) mode are particularly well suited for this task. Selected precursor ions are fragmented by collision-induced dissociation and the corresponding specific product ions are detected. However, the full scan mode, multiple ion monitoring (MIM), or selected ion monitoring (SIM) also have been used (152). Mass spectrometers regularly applied in semiquantitative proteomics include Q-TOF and Q-IT.

Sample preparation and cleanup. Proteome research is generally conducted pursuant to a well-proven pathway (169). Representative samples are collected from the test specimen and homogenized. Depending on whether a bottom-up or top-down strategy is followed, the proteins are either digested with proteases first and separated later or vice versa. Separation of the peptides or proteins is most often performed electrophoretically or chromatographically (189). Specific protocols for the enzymatic in-gel digestion (312, 313) and sample preparation from gels (133, 158) have been developed. For the processing of silver-stained bands (to remove the stain) or for the preparation of peptide sequencing experiments, a reduction-alkylation step using dithiothreitol and, e.g., iodoacetamide is performed before the start of the digestion procedure (313).

Following optional further cleanup steps, e.g., by solid-phase extraction (SPE) (132, 288), immunomagnetic beads (64), or adsorptive chromatography on restricted access material (RAM) (348), the samples are submitted to proteomic analysis.

LC FOR MS-BASED PROTEOMICS

The initial separation of proteins and peptides before the subsequent MS analysis has conventionally been performed with gel electrophoresis. However, this technique has its limitations regarding the detection of low abundance, very hydrophobic, acidic, or basic proteins. Other disadvantages of gel electrophoresis are the dispensability of gels, staining, and connected problems. High-performance LC (HPLC) is much more versatile because of a wide choice of stationary phases and may also be used to resolve small molecules at nonphysiological pHs; thus, it has become the preferred technology (315). Further benefits of HPLC are higher sensitivity, the option for miniaturization, the possibility to interface directly with various MS systems, and automation (169). Multidimensional identification (MultiID) (240) or multidimensional protein identification technology (MudPIT) (315) combines two or more of the HPLC methods that are commonly used in proteomic analysis and offers even higher separation power for the analysis of complex mixtures of enzymatically digested proteins. Typical combinations are size-exclusion LC (SEC) with reverse-phase LC (RPLC), ion-exchange LC (IEC or IEX) with RPLC, IEC with affinity LC (AC), and AC-RPLC.

Successful interfacing of LC with MS depends considerably on the selected mobile phase, which must allow sufficient analyte ionization in the MS source. LC eluents must be volatile, excluding high-salt buffers, and should not be associated with ion suppression, sensitivity loss, signal reduction, or spray instability. Protein conformation depends on LC eluent properties determining chromatographic retention and resolution (141). In RPLC-ESI-MS, the postcolumn addition of an organic sheath (e.g., weak acid in organic carrier solvent) has been successfully tested (121). The admixing of propionic acid in 2-propanol with the LC eluent succeeded in increasing 100-fold the signal-to-noise ratio for the detection of tryptic lysozyme peptides.

A challenge in coupling LC and MS is the efficient disposal of the LC eluent. Several approaches have been explored to solve this problem, e.g., split-flow systems or scaled steps of miniaturization, the latter of which resulted in increased chromatographic resolution, greater sensitivity, and less solvent consumption. Whereas normal HPLC (applied in preparative applications) uses columns with 4 to 5 mm inside diameter and flow rates between 1 and 10 ml/min, the column inside diameters and flows are 1 to 2.1 mm and 50 to 1,000 μ l/min, respectively, in micro-HPLC, 100 to 1,000 μ m and 0.4 to 200 μ l/min in capillary HPLC, and 25 to 100 μ m and 25 to 4,000 nl/min in nano-HPLC. The term nano-HPLC is used for both column-based LC and on-chip microfluidic devices, which are used both for the automated separation and detection of proteins and peptides and as a delivery system for the subsequent MS analysis (169). The size and volume reduction leads to taller peaks for fixed analyte amounts because of the inverse proportionality of peak height to cross sectional area of the column. Detection limits therefore decrease drastically, and separation and collection of picomole to femtomole amounts of proteins and peptides is possible. In addition to RPLC, miniaturization has also been applied to AC and IEC.

Because of the potential for hampering of intraparticle diffusion, large biomolecules can be difficult to transfer during separation on the stationary phase. Column fills with large-diameter perfusable, convective pores in combination with smaller diffusive pores can increase the particle permeability considerably. Hence, perfusion LC uses fluid dynamics to reduce the time needed to separate peptides and proteins, whereas loading capacity and resolution remain comparable to those in conventional LC (127). Perfusion LC stationary phases have been developed for RPLC, IEC, hydrophobic-interaction HPLC (HIC), and AC applications.

RPLC. RPLC is the most frequently used chromatography technique in proteomics. Peptides and proteins are positively charged by the addition of ion-pairing agents such as formic acid (in uncoupled RPLC, also trifluoroacetic acid) and separated in a water-acetonitrile or water-methanol mobile phase by hydrophobicity or hydrophilicity interaction with the stationary phase (67, 121). Elution gradients start with aqueous buffers and end with high concentrations of organic solvents. A peptide's retention time in RPLC is constant under stable chromatographic conditions because this time is directly related to the amino acid composition

TABLE 1. *Liquid chromatography methods for the separation, isolation, and detection of important allergy-causing foods*

Food	Matrix	Analyte	Chromatography ^a	Reference(s)
Cereals (wheat)	Wheat	Glutenin subunits	SEC (filtration)	275
	Durum wheat	Gliadins	RPLC	56
	Wheat flour	High-molecular-mass gluten	IEC	314
	Wheat seeds	Aspartic proteinase	IEC	34
	Wheat	Storage proteins	RPLC	211
	Wheat	Storage proteins	RPLC	210
	Wheat	Low-molecular-mass glutenin subunits	RPLC	182
	Wheat	Gliadins	IEC	319
	Wheat flour	Glutenin subunits	RPLC	214
	Wheat	Gluten subfractions	SEC	81
	Wheat	Gliadins	RPLC	355
	Durum wheat	Low-molecular-mass glutenin subunits	RPLC	220
	Wheat endosperm	α - and γ -Gliadins	RPLC	294
	Wheat flour	Gluten	RPLC	354
	Wheat	Low-molecular-mass glutenin subunits	IEC, RPLC	318
	Wheat flour	Glutenins	RPLC	129
	Wheat proteins	Gliadins	RPLC	270
	Cell culture	Glutenin	RPLC	115
	Wheat flour	Gliadin	RPLC, SEC	343
	α -Gliadin	α -Gliadin peptides	RPLC	200
	Wheat	Gluten proteins	RPLC	285
Crustaceans	Horseshoe crab	Major proteins	RPLC	193
	Spiny lobster	Molt-inhibiting peptide	RPLC	212
Egg	Egg	Lysozyme, ovotransferrin major proteins	IEC (anion)	22
	Egg	Major proteins	IEC (anion)	341
	Egg white	Ovomucin, lysozyme	SEC (permeation), RPLC, IEC (anion)	20
	Egg	Lysozyme	RPLC	301
	Egg fractions	Major proteins	IEC, RPLC	21
	Mixed proteins	Major proteins	RPLC	153
	Protein mixture	Ovalbumin, ovotransferrin, lysozyme	IEC-RPLC	328
	Egg	Egg white proteins	RPLC	137
	Eggshell membrane	Major proteins	IEC, RPLC	206
	Egg yolk	Phosvitin (Gal d 6)	RPLC	10
	Egg white	Egg white proteins	IEC	331
	Egg white	Egg white proteins	IEC	262
Fish	Fish species (7)	Sarcoplasmic protein	RPLC	17
	Fish species (31)	Sarcoplasmic protein	RPLC	267
	Fish species (15)	Sarcoplasmic protein	RPLC	16
	Frozen cod	Actomyosin	SEC	332
	Gadoid fish	Water-soluble protein	RPLC	281
	Fish species (16)	Sarcoplasmic protein	RPLC	164
	Frozen cod fillet	Total proteins	RPLC	174
Milk	Bovine milk	Major proteins	RPLC	347
	Wheys	Major proteins	RPLC	92
	Comté cheese	Phosphopeptides	SEC (permeation), RPLC	297
	Bovine whey	β -Lactoglobulin	RPLC	290
	Bovine milk	κ -Casein	RPLC	230
	Bovine milk, whey	β -Lactoglobulin	RPLC	180
	Soybean milk	Bovine whey proteins	Perfusion RPLC	124
	Bovine serum albumin sample	Bovine serum albumin, α -lactalbumin	RPLC	93
	Mixed proteins	Whey proteins	RPLC	153
	Bovine whey	Major proteins	RPLC	104
	Various milks	All major proteins	RPLC (ion pair)	48

TABLE 1. *Continued*

Food	Matrix	Analyte	Chromatography ^a	Reference(s)
Nuts	Cheddar cheese	α_{s1} -Casein	RPLC	51
	Various milks	Bovine caseins	RPLC	344
	Milk products	α -, β -, κ -Casein	HIC	49
	Cheeses	β -Lactoglobulin	RPLC	114
	Caprine milk	β -Casein	RPLC	117
	Bovine milk	Major proteins	RPLC	44
	Bovine milk	Bovine caseins	RPLC	45
	Brazil nut	Albumins	RPLC	11
	Almond	Amandin-1	RPLC	300
	Hazelnut	Vicilin	IEC	173
	Hazelnut	Cor a 8	IEC	305
	Walnut	LTP	SEC (filtration)	273
	Brazil nut	2S albumins	IEC (anion)	244
	Brazil nut	Allergen ber e 2	SEC (filtration)	140
	Almond	Amandin	IEC (anion)	130
	Hazelnut	Major allergens	FPLC	172
	Hazelnut	Major allergens	SEC (filtration), SEC (cation), AC	292
Peanut	Peanut	Allergenic glycoprotein	AC, IEC, SEC (permeation)	27
	Mixed feed	Protein hydrolysate	RPLC	105
	Peanut kernel	Major proteins	RPLC	251
	Peanut seeds	Globulins	IEC (anion)	116
	Peanut	Major allergens	SEC, IEC	165
	Peanut	Major allergens	SEC (filtration), IEC (anion FPLC), RPLC	216
Soybean	Peanut	Conglutins	IEC (anion FPLC)	97
	Meat products	Soybean proteins	HPLC	183
	Soybean	Globulins	IEC	361
	Meat	Soy protein	RPLC	18
	Soybean	Major proteins	RPLC	277
	Soybean	Proteins	RPLC	54, 55
	Milk	Soy milk	FPLC	148
	Soybean	Globulins	RPLC	278
	Soybean globulins	Glycinin subunits	RPLC	356
	Dairy products	Soya proteins	SEC (permeation)	73
	Soybean	Major proteins	SEC	79
	Soybean	Proteins	RPLC, SEC	263
	Soybean seeds	β -Glycinin	SEC (permeation)	249
	Soybean	Soybean globulins	RPLC	125
	Commercial products	Eight soybean proteins	Perfusion RPLC	128
	Soy flakes	Globulins	SEC (filtration)	357
	Soybean seeds	Globulins	SEC (permeation)	116
	Commercial products	Soybean proteins	Perfusion RPLC	126
	Milk	Soybean proteins	RPLC	168
	Milks	Soybean proteins	Perfusion RPLC	106
	Cheeses	Soybean proteins	Perfusion RPLC	123
	Cereal products	Soybean proteins	Perfusion RPLC	71
	Commercial products	Soybean proteins	Perfusion RPLC	70
	Soybean	Proteins	Perfusion IEC	146
	Soybean cultivars	Soybean globulins	Perfusion RPLC	122
Celery	Celeriac	Major allergen Api g 5	AC (ConA), SEC (anion)	53
	Celeriac	Major allergen Api g 1	HIC, SEC	52
	Celeriac	Major allergens	AC (ConA), SEC (anion)	52
Lupin	Lupin seeds	Major proteins	IEC, SEC (filtration)	74
	Lupin seeds	Lupin globulins	IEC (anion FPLC), SEC (filtration FPLC)	223
	Lupin seeds	Globulins	IEC (anion)	116
	Lupin cell culture	Vacuole proteins	IEC (anion)	307
	Lupin seeds	γ -Conglutin	AC (insulin)	195

TABLE 1. *Continued*

Food	Matrix	Analyte	Chromatography ^a	Reference(s)
Molluscs	Lupin beverage	Major proteins	IEC (anion)	184
	Lupin seeds	α -, δ -Conglutin	AC (ConA)	102
	Lupin seeds	Conglutins	IEC (anion FPLC)	97
	Deep sea clam	Hemolymph	SEC (filtration)	365
	Blue mussel	Digestive gland	IEC (anion)	9
Mustard	Yellow mustard	Seed storage protein, Sin a 1	SEC (filtration)	226
	Yellow mustard	Sin a 1	SEC (filtration), RPLC	225
	Oriental mustard	Bra j 1	SEC (filtration)	135
	Oriental mustard	Bra j 1	IEC (cation), RPLC	241
	White mustard	Trypsin inhibitors	RPLC	298
	Yellow mustard	Sin a 2 11S globulin	SEC, IEC	268
	Yellow mustard	Sin a 1 2S albumin	SEC, IEC (cation), HIC	316
	Yellow mustard	Sin a 2	SEC	269
	Yellow mustard	Sin a 3, Sin a 4	SEC, RPLC	317
	Yellow mustard	Chloroplast DNA-binding protein	AC (heparin)	306
Sesame	Sesame seeds	Globulins	SEC (filtration)	287
	Sesame seeds	2S albumin	SEC (filtration), RPLC	274
	Sesame seeds	Major allergens	SEC (filtration)	246
Buckwheat	Buckwheat seeds	Trypsin inhibitor proteins	AC, IEC	155
	Buckwheat seeds	Cysteine proteinase	SEC (filtration), IEC	98
	Buckwheat seeds	Allergenic proteins	IEC, RPLC	362
	Buckwheat seeds	Metalloproteinase	IEC (anion FPLC), SEC (filtration)	33
	Buckwheat flour	α -Amylase inhibitor	SEC (filtration)	156
	Buckwheat seeds	Protease inhibitor BWI-1	IEC (anion FPLC), RPLC, SEC (filtration), AC	31
	Buckwheat seeds	BWI-1, -2, -4	SEC (filtration)	100
	Buckwheat seeds	Trypsin inhibitor BWI-2a, BWI-2b	RPLC	271
	Buckwheat seeds	Proteinase inhibitors	IEC	99
	Buckwheat seeds	Protease inhibitor BWI-4a	SEC (filtration), RPLC	32
	Buckwheat seeds	Major allergens	RPLC	363
	Buckwheat seeds	Serine proteases BWI-1c, BWI-2c, BWI-3c, BWI-4c	AC, IEC (anion FPLC)	339
	Buckwheat seeds	Pepsin-resistant 16 kDa-protein	IEC (anion), SEC (filtration)	330
	Buckwheat seeds	Protease inhibitors BWI-1c, BWI-2c, BWI-3c, BWI-4c	AC, IEC (anion FPLC)	338
	Buckwheat seeds	Globulins	IEC (anion)	78
	Buckwheat seeds	Globulins	SEC	77
	Buckwheat flour	Protein isolate	SEC (filtration)	192

^a FPLC, fast protein liquid chromatography.

and contingent on the peptide's physicochemical characteristics (25, 335). Quantitative analysis by RPLC is possible because ISTD-corrected relative peak areas can be directly correlated with the peptide and protein concentrations (233). Chromatographic resolution, and thus the speed of analysis, can be considerably increased by applying ultrahigh-pressure LC (UPLC or "TurboFlow") or elution-modified displacement LC (EMDC) (315). The development of new stationary phases, e.g., monolithic columns consisting of macroporous copolymers, provides enhanced column fill material stability, adjustable pore diameters, and lower back pressure, leading to improved peptide separation (67).

IEC. IEC achieves separation by making use of the electric interaction between the analyte ions and the charged

material of the stationary phase, which can be categorized into weak or strong cation exchangers and weak or strong anion exchangers. The composition of the mobile phase has great influence on binding capacity and resolution. Proteins and peptides usually are analyzed as protonated cations and eluted by salt gradients. Unfortunately, the high salt concentrations used in the buffers inhibit direct IEC-ESI-MS because of suppressed analyte ionization. However, this problem can be overcome by adding organic modifiers and volatile reagents to the mobile phase, by off-line removal of the salts, by changing the eluent pH (chromatofocusing), or by including a secondary RPLC step for multidimensional LC (121, 315).

SEC. In SEC, separation is based on differences in the hydrodynamic volumes of the analytes. The column fills

consist of porous materials with cavities of definite sizes through which molecules with smaller diameters can pass. Consequently, these small molecules are retained in the column, whereas larger molecules elute unhindered. SEC in aqueous mobile phase also is referred to as gel filtration, in contrast to gel permeation, which is carried out with organic eluents. SEC has limited resolution power and loading capacity but offers high reproducibility and stability. Peptides and proteins are generally not denatured by gel-filtration SEC because the buffers used as eluents have pHs close to 7. Direct coupling to ESI-MS is difficult because of the high load of nonvolatile buffer salts. However, SEC can be used off-line as a separation method or on-line in combination with RPLC in proteomic analysis (121, 315).

AC. AC takes advantage of specific biological interactions for the separation and purification of biomolecules by reversible binding to ligand-bearing stationary phases (176). Because of the high selectivity of this technique, low-abundance proteins can be effectively detected, and posttranslational modifications such as proteolytic processing, phosphorylation, and glycosylation become traceable. Regarding the respective application area, AC column fills can be uniquely tailored by introducing ligands with high analyte specificity. Common AC methods include immunoaffinity LC based on antibody-antigen interactions, immobilized metal affinity LC (IMAC) based on the binding affinity of histidine-containing or phosphorylated amino acids such as serine, threonine, and tyrosine to certain transition metals, and lectin affinity LC based on both the interaction of lectins such as concanavalin A (ConA) and the sugar moieties in glycosylated analyte molecules. Despite having problems similar to those associated with the other LC applications that depend on salt-containing eluents, direct AC-ESI-MS is possible (260). AC coupling to RPLC has been advantageous (121, 315).

HIC. Separation by HIC is based on interaction of the hydrophobic functional groups on the surface of an analyte molecule with the lipophilic stationary phase (67, 121). In contrast to RPLC, which basically works on the same principle, HIC elution gradients start at high salt concentrations to reduce sample solvation and proliferate hydrophobic interaction and are modulated toward lower ion loads and a certain fraction of organic solvent (e.g., isopropanol). The more hydrophobic the molecule is, the less salt is needed to promote binding. In the analysis of food proteins thus far, HIC has been used only as an off-line separation technique (49).

HILIC. Hydrophilic-interaction HPLC (HILIC) can be considered a variant of normal-phase LC because it uses water-miscible eluents such as acetonitrile, tetrahydrofuran, or dioxane exclusively (121). The analyte passes through a mainly organic mobile phase and a neutral hydrophilic stationary phase. The most hydrophilic compounds are thus retained in the column for the longest time. Usually, ion strength and pH of the mobile phase are controlled by the addition of low amounts of salt (e.g.,

tetraethylammonium). This addition has the requisite effect on ion suppression in the analysis of highly charged analytes. HILIC is superior to RPLC for the separation of polar peptides. Retention is proportional to the peptide's hydrophilicity and, at low pHs, is proportional to the number of basic residues, making it possible to manipulate selectivity by changing the pH in the mobile phase. Because the salt load in the eluent is rather low, ion suppression during analyte ionization in ESI-MS is not a relevant problem. HILIC-MS is not used often in proteomics, but a number of both off-line and on-line applications are currently under development.

ALLERGENOMICS

The application of proteomic methods for the analysis of allergenic proteins has been termed allergenomics. The allergenome comprises many different putative proteinous allergens, and allergenomic information is continuously accumulating and available in on-line directories like such as the Allergome database (215).

Food contains an enormous variety of proteins, but only a small fraction of these proteins are allergens, and these can be either abundant or minor ingredients. The known allergens belong predominantly to a limited number of protein families with biological functions such as storage, hydrolysis, metal and lipid binding, and cytoskeleton association. Molecular masses range generally from 10 to 70 kDa. Many allergens occur in multiple isoallergenic forms with 67% or more identity in their amino acid sequences. Posttranslational modifications and glycosylations contribute further to allergen diversity.

Food allergen legislation. Among the identified allergens, only a few can be considered major allergens that occur in common foods and affect a large proportion of allergic individuals. Variations in dietary habits of consumers and regulatory management by the food authorities among countries have resulted in national differences in food allergen legislation. In the United States, eight foods have been identified as causing the majority of food allergenic reactions: crustaceans, egg, fish, milk, nuts, peanut, soybean, and wheat. Regulations require that the presence of these foods be declared on prepacked food products. In addition to these eight major allergens, sesame must be labeled in Australia and New Zealand, and sesame and mustard must be labeled in Canada. The European Union has extended this list of 10 allergens further by changing "wheat" to "cereals containing gluten" and by adding celery, lupin, and molluscs to a total of 13 foodstuffs and their products that must be declared on labels (240). In Japan and Korea, allergy to buckwheat is of considerable importance in addition to the eight major allergens.

Implementation of food allergen legislation depends on reliable analytical methods that allow food surveys and product testing to be conducted in response to consumer complaints. MS-based proteomic techniques have been applied extensively for the characterization of food

TABLE 2. Mass spectrometry applications for the identification of important allergy-causing foods

Food	Sample ^a	MS instruments ^b	Reference(s)
Cereals (wheat)	α -Gliadins	MALDI-TOF MS	340
	Glutenin	MALDI-TOF MS	149
	Gliadin	MALDI-TOF MS	224
	Gliadins	MALDI-TOF MS	59
	Wheat proteins	MALDI-TOF MS	103
	Gluten gliadins	MALDI-TOF-MS	61, 62
	Gliadin	MALDI-TOF MS	41
	Glutenin mixtures	MALDI-TOF-MS	129
	Glutenin	MALDI-TOF-MS	115
	Gluten proteins	RPLC-ESI-Q-MS	199
	Gluten subunits 1Ax2, 1Bx7, 1Dy10	MALDI-TOF-MS	82, 83
	Glutenin subunit 1Dx2	MALDI-TOF-MS	86
	Gliadins in wheat varieties	MALDI-TOF-MS	324
	Wheat endosperm	MALDI-TOF-MS	345
		NanoRPLC-ESI-Q-IT-MS-MS	
	Glutenin	MALDI-TOF-MS	87
		MicroRPLC-ESI-Q-IT-MS-MS	
	Wheat endosperm heat shock protein	MALDI-TOF-MS	196
		NanoRPLC-ESI-Q-IT-MS-MS	
	Wheat endosperm prolamins	MALDI-TOF-MS	197
	Glutenin subunits 1Bx7, 1Bx20	MALDI-TOF-MS	85
		MicroRPLC-ESI-Q-IT-MS-MS	
	Wheat proteins	MALDI-TOF-MS	198
		NanoRPLC-ESI-Q-TOF MS-MS	
	B- and C-type glutenin subunits	MALDI-TOF-MS	252
		MicroRPLC-ESI-IT-MS	
	Wheat endosperm	MALDI-TOF-MS	346
		NanoRPLC-ESI-Q-TOF-MS-MS	
	Glutenin subunit 1Bx7	MALDI-TOF-MS	2
	Wheat gliadin	MALDI-TOF-MS	343
		RPLC-ESI-Q-MS	
	α -Gliadin	MALDI-TOF-TOF-MS-MS	200
	Gluten proteins	MALDI-Q-TOF MS-MS	285
	Glutenin subunits	MALDI-TOF-MS	367
	Wheat proteins	MALDI-TOF-MS	325
		NanoRPLC-ESI-Q-TOF-MS-MS	
		NanoRPLC-ESI-Q-IT-MS-MS	
	Wheat proteins	NanoRPLC-ESI-Q-TOF MS-MS	350
	Wheat grain	Collection tray ESI-Q-TOF-MS-MS	6
	Wheat endosperm	NanoRPLC-ESI-Q-TOF-MS-MS	154
	Glutenin subunits	MALDI-TOF-MS	120
Crustaceans	Crayfish sarcoplasmic protein	FAB-Q-MS	19
	Crayfish sarcoplasmic protein Scp-1	FAB-Q-MS	157
	Lobster tropomyosin	MALDI-TOF-MS	254
	Horseshoe crab	RPLC-ESI-Q-MS	193
	Black tiger shrimp	MALDI-TOF-MS (with PSD)	364
	Pacific white shrimp	MALDI-TOF-MS	368
		NanoRPLC-nanoESI-Q-TOF	
	Spider crab (<i>Hyas araneus</i>), shore crab (<i>Carcinus maenas</i>)	SELDI-TOF-MS	134
	Pacific white shrimp Lit v 3	MALDI-TOF-MS	23
	<i>Penaeus aztecus</i> Pen a 1	MALDI-TOF-MS	3
	Six shrimp species	MALDI-TOF-MS	265
	Seven shrimp species	MALDI-TOF-MS	266
		NanoRPLC-nanoESI-Q-IT-MS-MS	
	Black tiger shrimp, banana white prawn	MALDI-TOF-MS	264
	Pacific white shrimp Lit v 4	RPLC-nanoESI-Q-IT-MS-MS	24
Egg	Egg white	RPLC-ESI-Q-MS	94
	Ovalbumin	ICAT-labeled MALDI-Q-TOF-MS-MS	321
	Tempera paint	MALDI-TOF-MS	334
		NanoRPLC-ESI-Q-TOF-MS-MS	

TABLE 2. *Continued*

Food	Sample ^a	MS instruments ^b	Reference(s)
Fish	Egg white	LC-ESI-MS-MS	138
	Eggshell membrane	NanoRPLC-Q-IT-FT-MS-MS	206
	Egg white	NanoRPLC-Q-IT-FT-IT-FTICR-MS-MS	204
	Egg white	SELDI-TOF-MS	91
		RPLC-ESI-IT-MS-MS	
	Egg yolk	NanoRPLC-Q-IT-FT-MS-MS	207
	Egg proteins	NanoRPLC-Q-IT-FT-MS-MS	208
	Egg vitelline	NanoRPLC-Q-IT-FT-MS-MS	205
	Egg yolk	SELDI-TOF-MS	108
		CapRPLC-nanoESI-Q-Orbitrap-MS-MS	
	Egg proteins	MALDI-TOF-MS	175
		RPLC-ESI-Q-TOF-MS-MS	
	Hake proteins	MALDI-TOF-MS	282
		MicroRPLC-nanoESI-Q-IT-MS-MS	
	Rock cod plasma	ESI-QqQ-MS-MS	359
	Rainbow trout	MALDI-TOF-MS	217
	Rainbow trout skin	MALDI-TOF-MS	110
	Carp parvalbumin	MALDI-TOF-MS	328
	Rainbow trout	SELDI-TOF-MS	151
	Infected salmon	NanoRPLC-ESI-Q-TOF-MS-MS	46
	Cod muscle protein	NanoRPLC-ESI-Q-TOF-MS-MS	162
	Cod plasma protein	SELDI-TOF-MS	171
	Salmon proteins	SELDI-TOF-MS	284
	Bonito muscle	MALDI-TOF-TOF-MS-MS	160
	Hake parvalbumin	MALDI-TOF-MS	68
	Hake NDKB	MALDI-TOF-MS	69
Milk		NanoRPLC-ESI-Q-IT-MS-MS	
	Zebrafish	NanoRP-TiO ₂ -RPLC-ESI-Orbitrap-MS-MS	178
	Fish species	MALDI-TOF-MS	221
	Parvalbumins	NanoRPLC-ESI-Q-TOF-MS-MS	191
	Sea bream muscle	MALDI-TOF-MS	304
	Bovine α -casein	MicroRPLC-ESI-QqQ-MS-MS	185
	Phosphopeptides	MALDI-TOF-MS	297
	Bovine β -casein	MALDI-TOF-MS	260
	Bovine milk	RPLC-ESI-Q-MS	181
	Bovine κ -casein	RPLC-ESI-Q-MS	235
	Bovine milk	MALDI-TOF-MS	72
	Bovine milk	RPLC-ESI-Q-MS	57
	Grana Padano	FPLC-HPLC, FAB-MS	112
	Milk, whey, β -lactoglobulin	MicroRPLC-ESI-QqQ-MS-MS	180
	BSA, α -lactalbumin	MALDI-TOF-MS	93
	Cheddar cheese	RPLC, microESI-QqQ-MS	4
	Buffalo cheese	MALDI-TOF-MS	13
	Cow's milk, whey proteins	MALDI-TOF-MS	366
	Infant formulae	MALDI-TOF-MS	299
	α -Lactalbumin	RPLC-ESI-Q-MS	320
	Milk powder	MALDI-TOF-MS	118
	Goat whey	RPLC-MALDI-TOF-MS	47
	Ovine casein	Anion exchange FPLC-Q-MS	247
	Ovine milk	CapLC-ESI-Q-MS	337
	Caprine milk	CapLC-ESI-Q-MS	336
	Bovine milk	MALDI-TOF-MS	119
	Bovine whey	RPLC-ESI-TOF MS	144
	BSA	RPLC-ESI-ion mobility-TOF-MS	342
	Caprine κ -casein	RPLC-ESI-Q-MS	248
	Ovine caseins	LC-ESI-MS; MALDI-TOF-MS	111

TABLE 2. *Continued*

Food	Sample ^a	MS instruments ^b	Reference(s)
	β -Lactoglobulin, α -lactalbumin, BSA, ovine casein	ICAT-labeled MALDI-Q-TOF-MS-MS	321
	Caprine casein, β -lactoglobulin	RPLC-ESI-Q-MS	245
	Bovine caseins	RPLC-ESI-QqQ-MS-MS	145
	Bovine milk	RPLC-MALDI-TOF-MS	231
	Caprine β -casein	MicroRPLC-ESI-Q-IT-MS-MS MALDI-TOF-MS	117
	β -Lactoglobulin	MALDI-TOF-MS	255
	Milk proteins	NanoRPLC-MALDI-TOF-MS	358
	BSA	CapRPLC-MALDI-Q-IT-MS-MS CapRPLC-MALDI-TOF-MS CapRPLC-ESI-Q-TOF-MS-MS	84
	Bovine milk	MicroRPLC-ESI-QqQ-MS-MS	238
	Bovine milk	2D (cation exchange-RP)-nanoLC-ESI-Q-TOF-MS-MS 2D (cation exchange-RP)-nanoLC-MALDI-Q-TOF-MS-MS	234
Nuts	Hazelnut	MALDI-TOF-MS	253
	Hazelnut globulin	MALDI-TOF-MS	37
	Hazelnut vicilin	MALDI-TOF-MS	173
	Brazil nut	MALDI-TOF-MS	244
		RPLC-ESI-QqQ-MS-MS	
	Cashew nut	MALDI-TOF-MS	293
	Brazil nut selenoproteins	MALDI-TOF-MS NanoRPLC-ESI-Q-TOF-MS-MS	95
	Hazelnut	MALDI-TOF-MS	172
	Hazelnut, walnut, cashew	MALDI-TOF-MS	28
	Hazelnut allergens	MALDI-TOF-MS RPLC-ESI-QqQ-MS-MS	292
	Hazelnut proteins	MALDI-TOF-MS NanoRPLC-ESI-Q-TOF MS-MS	351
Peanut	Peanut seeds	CapLC-nanoESI-Q-TOF-MS-MS	75
	Peanut	MALDI-TOF-MS	28
	Peanut seeds	CapLC-nanoESI-Q-TOF-MS-MS	76
	Peanut seeds	NanoRPLC-ESI-Q-IT-MS-MS	326
Soybean	Trypsin inhibitor	MicroRPLC-ESI-QqQ-MS-MS	185
	Soy agglutinin	MicroRPLC-ESI-QqQ-MS-MS	202
	Dietary soy proteins	MALDI-TOF MS	131
	Soybean seeds	MALDI-TOF MS	243
	Soybean protein	MALDI-TOF-MS	26
	Soybean seeds	MALDI-TOF MS	142
	Soybean seeds	MALDI-TOF MS	256
		NanoRPLC-ESI-Q-IT-MS-MS	
	Meat samples	NanoRPLC-ESI-Q-IT-MS-MS	177
	Soybeans	MALDI-TOF MS RPLC-ESI-Q-IT-MS-MS	258, 259
	Soybean flour	CapRPLC-ESI-Q-Orbitrap-MS-MS	29
	Soybean	MALDI-TOF-MS	28
	Industrial soy protein isolates	MALDI-TOF MS	152
	Soybean seeds	MALDI-TOF MS	166, 167
	Soybean seeds	MALDI-TOF MS	257
		NanoRPLC-ESI-Q-IT-MS-MS	
	Soybean seeds	MALDI-TOF MS	291
	Soybean in skimmed milk	NanoRPLC-nanoESI-Q-TOF-MS-MS	80
Celery	Api g 5	MALDI-TOF-MS	53
	Api g 1	NanoRPLC-ESI-Q-TOF MS-MS	42
	Api g 1, Api g 4, Api g 5	CapRPLC-nanoESI-Q-TOF-MS-MS	52
	Mannitol dehydrogenase	NanoRPLC-nanoESI-Q-TOF-MS-MS	40
	Celery tuber proteins	NanoRPLC-nanoESI-Q-IT-MS-MS	107
		Infusion-ESI-QqQ-MS-MS	

TABLE 2. Continued

Food	Sample ^a	MS instruments ^b	Reference(s)
Lupin	Lupin phloem proteins	MALDI-TOF-MS	213
	Lupin phloem peptides	MALDI-TOF-MS	150
	Lupin seeds	MicroRPLC-nanoESI-Q-TOF-MS-MS	307
	Defatted lupin flakes	MicroRPLC-nanoESI-Q-TOF-MS-MS	349
	Lupin beverage	MicroRPLC-nanoESI-Q-IT-MS-MS	184
	Defatted lupin flour	MicroRPLC-nanoESI-Q-IT-MS-MS	194
Molluscs	Zebra mussel (<i>Dreissena polymorpha</i>)	MALDI-TOF-MS	12
	Deep sea clam (<i>Calyptogena magnifica</i>)	Infusion-ESI-QqQ-MS-MS	365
	Blue mussel (<i>Mytilus edulis</i>)	MALDI-TOF-MS	58
	<i>Mytilus</i> mussels	MALDI-TOF-MS	187, 188
		MicroRPLC-nanoESI-Q-IT-MS-MS	
	Striped Venus (<i>Chamalea gallina</i>)	MALDI-TOF-MS	295
		RPLC-ESI-Q-TOF-MS-MS	
	Blue mussel (<i>M. edulis</i>)	SELDI-TOF-MS	163
	Blue mussel (<i>M. edulis</i>)	MALDI-TOF-MS	203
		NanoRPLC-ESI-Q-IT-MS-MS	
	Bay mussel (<i>Mytilus galloprovincialis</i>)	ESI-Q-TOF-MS-MS	229
	Blue mussel (<i>M. edulis</i>)	MALDI-TOF-MS	15
		NanoRPLC-nanoESI-Q-TOF-MS-MS	
	Blue mussel (<i>M. edulis</i>)	SELDI-TOF-MS	39
	Blue mussel (<i>M. edulis</i>)	SELDI-TOF-MS	242
	Razor clam (<i>Sinonovacula constricta</i>)	MALDI-TOF-MS	322
	Blue mussel (<i>M. edulis</i>)	MALDI-TOF-MS	228
	Blue mussel (<i>M. edulis</i>)	RPLC-ESI-Q-IT-MS-MS	9
	Rough periwinkle (<i>Littorina saxatilis</i>)	MALDI-TOF-TOF-MS-MS	218
		MicroRPLC-nanoESI-Q-TOF-MS-MS	
Mustard	Asian clam (<i>Corbicula fluminea</i>)	MALDI-TOF-MS	219
	Bay mussel (<i>M. galloprovincialis</i>)	MALDI-TOF-MS	14
	White mustard (<i>Sinapis alba</i>) trypsin inhibitors	MALDI-TOF-MS	298
	Yellow mustard (<i>S. alba</i>) Ca-soluble protein	Direct injection-ESI-QqQ-MS-MS	7
	Yellow mustard (<i>S. alba</i>) Sin a 2	MALDI-TOF-MS	268
		MALDI-TOF-TOF-MS-MS	
	Yellow mustard (<i>S. alba</i>) Sin a 2	MALDI-TOF-TOF-MS-MS	269
	Yellow mustard (<i>S. alba</i>) Sin a 1	MALDI-TOF-MS	316
	Yellow mustard (<i>S. alba</i>) Sin a 3, Sin a 4	MALDI-TOF-MS	317
		MALDI-TOF-TOF-MS-MS	
	Indian mustard (<i>Brassica juncea</i>) roots	NanoRPLC-nanoESI-Q-TOF-MS-MS	8
	Indian mustard (<i>B. juncea</i>) protein concentrate	NanoRPLC-nanoESI-Q-IT-MS-MS	286
Sesame	Sesame (<i>Sesamum indicum</i>) allergens	MALDI-TOF-MS	36
	Sesame (<i>S. indicum</i>) allergens	MALDI-TOF-MS	246
		MicroRPLC-nanoESI-QqQ-MS-MS	
Buckwheat	Buckwheat (<i>Fagopyrum esculentum</i>) BWI-2a, -2b	MALDI-TOF-MS	271
	Buckwheat (<i>F. esculentum</i>) BWI-4a	MALDI-TOF-MS	32
	Buckwheat (<i>F. esculentum</i>) BWI-1c,-2c,-3c,-4c	MALDI-TOF-MS	339
	Buckwheat (<i>F. esculentum</i>) proteome	NanoRPLC-nanoESI-Q-TOF-MS-MS	329
	Buckwheat (<i>F. esculentum</i>) globulin	MALDI-TOF-MS	323
	Buckwheat (<i>F. esculentum</i>) peptides	NanoRPLC-nanoESI-Q-TOF-MS-MS	192

^a NDKB, nucleoside diphosphate kinase B; BSA, bovine serum albumin.

^b FAB, fast atom bombardment; PSD, postsource decay; RP, reverse phase; Cap, capillary.

proteomes and peptidomes, delivering information on food quality, food authenticity, and food functionality (201). These techniques also can be used to address food safety issues if quantitative methods are applied.

Allergenic proteins in the main food allergens. Allergenicity in foods can be caused by a variety of proteins with comparable allergenic capacity or by a single dominating allergen. Leguminous plants such as peanut,

TABLE 3. Characteristic marker peptides and biomarkers (as detected by MS-MS analysis) for important allergy-causing foods

Food	Analyte ^a	Marker peptide	Mass (m/z)	Reference	
Cereals (wheat)					
<i>Triticum</i> spp.	Dy glutenin	AQQPATQLPTVJR	1,518.0 ⁺	87	
		QQPGQGQHPEQ GK	1,418.6 ⁺		
		ELQESSLEAJR	1,370.1 ⁺		
	α -Gliadin	GSVQPQQLPFEEIR	1,581.8 ⁺	196	
	Glutenin subunit 1Bx20	QQAGQGQSGQGQQR	1,586.6 ⁺	88	
	α -Gliadin	LQLQFPFPQLPY	784.9 ²⁺	198	
	γ -Gliadin	VPPECSIMRAPF	1,396.8 ⁺	200	
	ω -Gliadin	KELQSPQQSF	1,190.6 ⁺		
		LQSEPQPQQPQQPS	1,590.8 ⁺		
	α -Gliadin	LGQQQPFPPQQPYQPQPF PSQQPY	2,922.4 ⁺	285	
	Ax glutenin	QQPGQGQQLR	1,138 ⁺	325	
		YYPTSPQQPGQEQQPR	1,902 ⁺		
		α -Amylase inhibitor	DCCQQLAHISEWCR		
	α -Amylase trypsin inhibitor	SGNVGESGLIDLPGCPR			
	β -Amylase	SAPEELVQQVLSAGWR			
	Profilin	YMQIQGEGPVVIR			
Crustaceans					
<i>Penaeus monodon</i>	Pen m 2 (arginine kinase)	IPFSHHDR	1,008.5 ⁺	364	
<i>Litopenaeus vannamei</i>	Hemocyanin	ADAAVIEK	858.4 ⁺	368	
		FTFDEGR	436.2 ²⁺		
<i>Penaeus</i> spp.	Arginine kinase	YALPPGVLEHFETA	601.0 ³⁺	266	
		FLQAANAC ^{Meth} R	525.6 ²⁺		
		TFLVWVNEEDHLR	829.3 ²⁺		
<i>Pandalus borealis</i>	Lit v 2	ADAAVIEKLEAGFK-(N-Acyl)	752.3 ²⁺	23	
<i>Pleoticus muelleri</i>		SFLVWVNEEDQLR	817.8 ²⁺		
<i>L. vannamei</i>		TFLVWC ^{Meth} NEEDHLR	860.1 ²⁺		
<i>Fenneropenaeus indicus</i>	Pen m 2	YLSKDIFDKLK	1,369.8 ⁺	23	
<i>P. monodon</i>		GTYFPLTGMSK	601.2 ²⁺		
<i>F. merguensis</i>		YLSKEVFDQLK	1,369.8 ⁺		
<i>L. vannamei</i>	Lit v 4 (SCP) α -chain	YMYDIDDDGFLDKNDFECLAVR NLWNEIAELADFNKDGEVT VDEFK VFIANQFKAIDVNGDGK SAFAEVKEIDDAYNK	539.2 ²⁺		
	Lit v 4 (SCP) β -chain	YMYDIDNDGFLDKNDFECLAVR YQELYAQFISNEDEK	539.2 ²⁺		
Egg					
<i>Gallus gallus domesticus</i>	Ovotransferrin	TAGWVIPMGLIHNR	522.3 ³⁺	334	
		TDERPASYFAVAVAR	551.6 ³⁺		
		SAGWNIPIGTLLHR	512.3 ³⁺		
		AQSDFGVDTK	534.3 ²⁺		
		KDSNVNWN NLK	444.6 ³⁺		
	Lysozyme	FESNFNTQATNR	714.8 ²⁺	334	
	Vitellogenin II	AGVR	402.3 ⁺		
	Ovalbumin	AFKDEDTQAMPFR	519.2 ³⁺		
			ISQAVHAAHAEINEAGR		887.5 ²⁺
Fish					
<i>Merluccius</i> spp.	Group A polypeptide (NDK-like)	VAIKPDGVQR	541.3 ²⁺	282	
		MMIGETNPADSKPGSIR	1,805 ⁺		
		NIHGSDDTVENAK	1,398 ⁺		
<i>Salmo salar</i>	NKEF liver protein	CVFVIGPDKK		46	
	Viral resistance protein	VRPCIDLIDSLR			

TABLE 3. *Continued*

Food	Analyte ^a	Marker peptide	Mass (<i>m/z</i>)	Reference
<i>Merluccius</i> spp.	NDKB	GDFCINIGR NIIHGSDTVENAK TFVAIKPDGVQR (N-Acyl)-MEQTFIAIKPDGVQR	526.1 ²⁺ 699.3 ²⁺ 665.8 ²⁺ 887.9 ²⁺	69
<i>Danio rerio</i>	β-Catenin Pumilo-1 Nucks GEF 7 MAPK3	RLpSVELTSSLFR RPGQGFHGVGEAGSVLpSPR KVVNYSQFNEpSDDADEDYGRK MpSGFIYQ GK IADPEHDHTGFLpTEpYVATR	178	
Milk				
<i>Bos taurus</i>	β-Casein	RELEELNVPGEIVEpSLpSpSpS EESITR	1,042.0 ³⁺	260
<i>Capra aegagrus hircus</i>	Caprine β-lactoglobulin	FQpSEEQQQTEDELQDK EGQCHV	1,023.0 ²⁺ 671.3 ⁺	145
<i>Bos taurus</i>	Caprine κ-casein	WQVLPDTPPAKSCQDQPTTL	1,114.5 ²⁺	
<i>Bos taurus</i>	β-Casein	DMPIQAFLLYQQPVLGPVR	2,188.1 ⁺	231
<i>Bos taurus</i>	κ-Casein macropeptide	VQVTSTAV	805 ⁺ /228 ⁺ (MRM)	236
<i>Bos taurus</i>	BSA	KVPQVSTPTLVEVSR LKPDPNTLCDEFK VPQVSTPTLVEVSR RHPEYAVSVLLR QTALVELLK EYEATLEECCA DDPHACYSTVFDK HLVDEPQNLIK LVNELTEFAK YLGYLEQLLR FFVAPFPEVFGK	1,640.0 ⁺ 1,576.8 ⁺ 1,511.9 ⁺ 1,439.9 ⁺	84
<i>Bos taurus</i>	α _{s1} -Casein		634.2 ²⁺ 692.8 ²⁺	352
Nuts				
<i>Bertholletia excelsa</i>	2S albumin	GMEPHM ^{Se} SECCEQLEGMDSCR MMQQQEM ^{Se} QPR	2,505.7 ⁺ 1,354.5 ⁺	95
Peanut				
<i>Arachis hypogaea</i>	Ara h 1	NNPFYFPSR SFNLDEGHALR NTLEAAFNAEFNEIR IFLAGDKDNVIDQIEK	571.3 ⁺ 629.8 ⁺ 869.9 ⁺ 606.6 ⁺	311
	Ara h 1	VLLEENAGGEQEER DLAFPGSGEQVEK	786.9 ²⁺ /804.4 ⁺ (MRM) 688.8 ²⁺ /930.5 ⁺ (MRM)	310
	Ara h 2	CCNELNEFENNQR CMCEALQQIMENQSDR	807.2 ²⁺ /1,050 ⁺ (MRM) 949.9 ²⁺ /1,120 ⁺ (MRM)	64
	Ara h 3/4	AHYQVVD SNGDR SPDIYNPQAGSLK AQSENYEYLAFK	648.8 ²⁺ /1,089 ⁺ (MRM) 695.3 ²⁺ /700 ⁺ (MRM) 732.8 ²⁺	
	Ara h 1	QFQNLQNH NTLEAAFNAEFNEIR VLLEENAGGEQEER LFEVKPDDK SRKVR	592.8 ⁺ 869.9 ⁺ 786.9 ²⁺ 552.3 ⁺ 330.2 ⁺	76
	Ara h 2	ANLRPCEQ NEFENNQR	494.2 ⁺ 448.2 ⁺	
	Ara h 3/4	QIVQNLR NLMILQ	435.8 ⁺ 759.4 ⁺	
Soybean				
<i>Glycine max</i>	β-Conglycinin B	NFLAGEKDNVVR	681.4 ²⁺	26
	Alcohol dehydrogenase	FGVTDFVNPK	820.3 ⁺	256
	Glycinin G2, A2	KPQQEEDDDDEEQPQCVETD KGCQR	797.8 ⁴⁺	177

TABLE 3. Continued

Food	Analyte ^a	Marker peptide	Mass (<i>m/z</i>)	Reference
	β-Conglutin precursor	LSEGDIFVIPAGYPISINASSNLR NTLEATFNTR DQQSYFSGFSR QAYNLEYYGDALR	845.3 ³⁺	184
	Vicilin-like protein	NFLAGESEDNVIR		
	Legumin-like protein	EGGQGGQQEGGNVLSFDDE FLEEALSVN GKNDDREGGIVEVK		
	γ-Conglutin	AGIALGTHQLEENLVVFDLAR		
	α-Conglutin	QQQENECQFQR LNALPDNTVQSEAGTIETWNP RPFYTNAPQEIIYQQGR RFYLSGNQEFLQYQEK LRHNIGESTSPDAYNPQAGR LTSIDFPILGWLGLAAEHGSIYK TNDIPQIATLAGANSEISALPLE YYAHAFNLNR ATPAEVLANAFGLR		194
	γ-Conglutin acidic subunit	PNLLVLPQQDASTK LHWGNILK RTPLMQVPVLLDLNGK ANTHCFTCTDSTTSR IPQFLFSCAPTFLTOK GLPNNVOGALGLGHAPISL PNQLFSHFFGLK QGEYFIQVSAIR		
	γ-Conglutin small subunit	HSIFEVFTQVFANNVPK AVGPFGLCYDTK ISGGVPSVDLIMDK ISGENLMVQAQDGVSCCLGF VDGGVHTR AGIALGTHQLEENLVVFDLAR VGFNTNSLK SCSNLFDLNNP		
	δ-Conglutin large subunit	ALQQIYENQSEQCQGR QEEQLLEQELNLPR TCGFGPLR		
Molluscs				
<i>Mytilus edulis</i>	Tropomyosin	YTAAASKLEEASKAADESER	2,156.5 ⁺	187
<i>M. galloprovincialis</i>		YTAAASKLEEASKAADESER	2,156.3 ⁺	
<i>M. trossulus</i>		YDTAASKLEEASKAADESER	2,168.9 ⁺	
<i>M. edulis</i>		EVDRLDELLTEKEKYK	2,137.0 ⁺	
<i>M. galloprovincialis</i>		EVDRLDELLTEKEKYK	2,136.9 ⁺	
<i>M. trossulus</i>		EVDRLDELLTEKEKYK	2,137.1 ⁺	
<i>Chamalea gallina</i>	Tropomyosin	KNALDKAEQ ESLSKLEEASK KLAITEVDLER LTEELSEELAVVGNN		295
<i>M. galloprovincialis</i>	Epoxide hydrolase	PFAFFEYVYNSK LEAMFRGSK		229
	Peroxisomal antioxidation enzyme	GCSKTHLPGFV EEWGRAHQAEQKVR		
<i>M. edulis</i>	Glutathione-S-transferase	GANEDNIRSR		15
	Carbonic anhydrase	YSSLEAAASKGGPFSDDYR		
	Mn superoxide dismutase	QNVKADYFK		
	ATP synthase beta subunit	IPVGPATLGRISLTDDTSK		
	Peroxin 12	QSNPALGLSSRT		
	Enoyl-CoA hydratase	SPVAVQGSK		
	Catalase	LSSTDYPDIALR		

TABLE 3. Continued

Food	Analyte ^a	Marker peptide	Mass (m/z)	Reference
<i>M. edulis</i>	Cystein proteinase	DECGIESGVVGIPK		9
	AMP-binding protein	GTNVCVRRVTAAG		
	Acyl-CoA dehydrogenase	LATDAVQVFG		
	Glutathione-S-transferase	RGLTXPIRM		
	Phosphoglycerate kinase	TGGVPGFQK		
	HSC70	PFTIINDGTPK		
		KSTSGDTHLGGEDFDNRM		
	HSC71	KTGPAIGIDLGTYSVGVFQ		
		HGKVEIANDQGNN		
		KETAEAYLGKLVNNSVITVPA		
		YFNDSQRQ		
		KDAGTISGMNVLRINEPTAAA		
		IAYGLDKK		
		RMVNHFIQEFK		
	Aldehyde dehydrogenase	RYYAGWADK		
<i>Littorina saxatilis</i>	Arginine kinase	KVAFTGSTEVGKL		218
		FGGTLADCIR		
		FLVWVNEEDHLR		
	Fructose-bisphosphate aldolase	LQGIGVENTEENR		
	Predicted protein	GILAADESTGTMG		
Mustard				
<i>Sinapis alba</i>	Sin a 2 (11S globulin)	VKGPFQVVRPPLR	1,492 ⁺	268
		TSVNSYTLPIQYIR	1,867 ⁺	
		ALPLEVITNAFQISLEEAR	2,130 ⁺	
<i>S. alba</i>	nsLTP Sin a 3, Sin a 4	TTPDRQQACR	1,232.6 ⁺	317
		ACGVNIPYKISK	1,349.7 ⁺	
		GCCTGVTNLNNMAR	1,567.7 ⁺	
Sesame	Ses i 1	QQSQQCRQQQLQGR	1,570.7 ⁺	246
		QFRSCQR	924.5 ⁺	
		YLSQGRSPY	1,070.5 ⁺	
		DCCQQL	979.4 ⁺	
		CRCEAIR	907.4 ⁺	
		QQQEGGYQEGSQQVYQR	2,269.0 ⁺	
		RCNMRPQQCAFR	1,566.5 ⁺	
Buckwheat	Major allergenic storage protein	VQVVGDER		329
		SVFDDNVGR		
		GQILVVPQGFAVVLK		
		NDDNAITSPIAGLK		
		AIPVEVLANSYDISTK		

^a NDK, nucleoside diphosphate kinase; NKEF, natural killer enhancing factor; BSA, bovine serum albumin; GEF, guanine nucleotide exchange factor; MAPK, mitogen-activated protein kinase; FAD, flavin adenine dinucleotide; HSC, heat shock cognate.

soybean, and lupin are typical examples of foods containing a large number of potent allergenic proteins (296). In contrast, crustacean, fish, and molluscan allergies are caused predominantly by allergic reactions to a single prevailing muscle protein of the respective animal (279, 280). Many food allergens are characterized by their high stability in the presence of heat, low pH, and enzyme digestion and their ability to survive food processing. Detection methods are based on the determination of one or several of the allergenic proteins in food.

Cereals containing gluten. In wheat (*Triticum aestivum*), the storage protein gluten with its ethanol-

soluble gliadin is a causative agent of non-IgE-mediated celiac disease, which is an autoimmune disorder. Other cereals such as rye, barley, and durum wheat contain gliadin homologues. The control of gluten-free products is important because of the relatively high prevalence of this disease in the total population. In contrast, cereal-related cases of food allergy, most frequently wheat allergy, occur predominantly in children. Wheat grain proteins can be classified into water-soluble albumins, salt-soluble globulins, ethanol-soluble prolamins, and acid- or alkali-soluble glutenins. A number of different allergens have been identified, e.g., the prolamins gliadin (Tri a 19), glutenin (Tri a 26), and LTP (Tri a 14) and the pollen-related profilin (Tri a 12) (215).

Crustaceans. In aquatic arthropod species such as shrimp, crab, lobster, and crayfish, the dominant allergen is the muscle protein tropomyosin (e.g., Pen a 1 in Brown shrimp, *Penaeus aztecus*), which has been identified in 21 different crustaceans to date and is responsible for about 80% of all shrimp-related allergic incidents. A second potential cross-reactive allergen, arginine kinase, has been identified in Tiger shrimp (*Penaeus monodon*) (Pen m 2) and Pacific white shrimp (*Litopenaeus vannamei*) (Lit v 2). Myosin (Lit v 3) and sarcoplasmic Ca^{2+} -binding protein (Lit v 4) are considered minor allergens (215).

Eggs. The most important allergens in hen (*Gallus domesticus*) egg white are ovomucoid (Gal d 1), ovalbumin (Gal d 2), ovotransferrin (Gal d 3), lysozyme (Gal d 4), and serum albumin (Gal d 5). In hen egg yolk, the allergens are phosvitin (Gal d 6) and apovitellin VI (Gal d Apo VI) (215).

Fish. The muscle protein parvalbumin (e.g., Gad m 1 from Atlantic cod, *Gadus morhua*), which has been identified in 50 fish species thus far, is the dominant allergen responsible for approximately 95% of the allergic incidents associated with fish (215).

Milk. The allergens α -lactalbumin (Bos d 4), β -lactoglobulin (Bos d 5), serum albumin (Bos d 6), and immunoglobulin (Bos d 7) have been found in bovine (*Bos domesticus*) whey. Allergenic caseins (α S1, α S2, β , and κ) have been identified in milk from cows (Bos d 8), goats (*Capra aegagrus hircus*) (Cap h casein), sheep (*Ovis aries*) (Ovi a casein), and the domestic water buffalo (*Bubalus bubalis*) (Bub b casein) (215).

Nuts. Several nut-producing plants, including hazelnut (*Corylus avellana*), walnut (*Juglans regia*), almond (*Prunus dulcis*), Brazil nut (*Bertholletia excelsa*), Cashew nut (*Anacardium occidentale*), Macadamia nut (*Macadamia integrifolia*), and Pecan nut (*Carya illinoensis*), are combined by this collective term. The main nut allergens belong mostly to the non-pollen-related seed storage proteins such as the 2S albumins (Jug r 1, Ber e 1, Ana o 3, Cor a 14, and Pru du 2S albumin). The 11S globulins (Cor a 9 and Ana o 2), 7S globulins (Cor a 11, Jug r 2, Ana o 1, and Pru du 6), and lipid transfer proteins (Cor a 8, Jug r 3, Pru du 3, and Pru du 4) also are important allergen families in nuts. Pollen-related nut allergens such as the pollinosis-associated proteins (Cor a 1 and Pru du 1) and the profilins (Cor a 2, Jug r 5, and Pru du 4) are heat sensitive and lose allergenicity after roasting (215).

Peanut. The majority of the identified allergens in peanut (*Arachis hypogaea*) belong to the same protein families as those found in nuts. Major peanut allergens are the 11S globulin (Ara h 3/4), the 7S globulin (Ara h 1), and the 2S albumins (Ara h 2, Ara h 6, Ara h 7, and Ara h 9). Pollen-related allergens are the peanut pollinosis-associated protein (Ara h 8) and the profilin (Ara h 5) (215).

Soybean. The most important allergens in soybean (*Glycine max*) belong to the same allergen families as do their homologues in the other allergenic legumes, peanut and lupin. Soybean 11S globulin (Gly m 6), 7S globulin (Gly m 5), 2S albumin (Gly m 2S albumin) and lipid transfer protein (Gly m LTP) are relatively stable seed storage allergens, whereas soybean profilin (Gly m 3) is degraded more easily (215).

Celery. Five allergens have been characterized in celery (*Apium graveolens*), each belonging to a different protein family. The heat-labile pollinosis-associated protein (Api g 1) and profilin (Api g 4) are important for pollen-related cross-reactivity, whereas celery LTP (Api g 2) and glycoprotein (Api g 5) are more important in processed foods. The relevance of an allergenic chlorophyll-binding protein (Api g 3) is unclear (215).

Lupin. In lupin, seed storage proteins highly homologous to other leguminous allergens are primarily responsible for eliciting allergic incidents. From the 450 species in the genus *Lupinus*, only four are edible, the so-called sweet lupins: *Lupinus albus*, *Lupinus angustifolius*, *Lupinus luteus*, and *Lupinus mutabilis*. Lupin 11S globulin (α -conglutins: Lup a alpha conglutinin and Lup an alpha conglutinin), 7S globulin (β -conglutins: Lup a 1 and Lup an 1), 2S albumins (δ -conglutins: Lup a delta conglutinin and Lup an delta conglutinin), and basic globulin (γ -conglutins: Lup a gamma conglutinin and Lup an gamma conglutinin) all show considerable allergenicity (215).

Molluscs. This group of marine invertebrates includes a large number of different species of shells, clams, mussels, oysters, scallops, squids, octopus, abalones, and cuttlefish. The muscle protein tropomyosin (e.g., Myt e 1 in Blue mussel, *Mytilus edulis*) is the most important molluscan allergen and has been identified in 12 mollusc species so far (215).

Mustard. Seed storage proteins are the most important allergens in mustard. In the three commercially used mustard species, White mustard (*Sinapis alba*), Oriental mustard (*Brassica juncea*), and Black mustard (*Brassica nigra*), allergenic 2S albumins (Sin a 1, Bra j 1), 11S globulin (Sin a 2), LTP (Sin a 3), and profilins (Sin a 4 and Bra ni 8) have been identified.

Sesame. In sesame (*Sesamum indicum*) seeds, insoluble 11S globulins (Ses i 6 and Ses i 7) constitute about 70% of the total seed protein, and soluble 2S albumins (Ses i 1 and Ses i 2) account for 25%. Sesame 7S globulin (Ses i 3), LTP (Ses i LTP), oleosins (Ses i 4 and Ses i 5), and profilin (Ses i 8) also have been characterized (215).

Buckwheat. Buckwheat (*Fagopyrum esculentum*) is a crop plant commonly used in Asia. The seed storage 2S albumins (Fag e 2 and BWp16) that account for 30% of the total protein content and the 13S globulin (Fag e 1) have considerable allergenic potential. Tartary buckwheat (*Fa-*

TABLE 4. Mass spectrometry applications for the quantitative determination of important allergy-causing foods

Food	Matrix	Analyte	Quantitation ^a	Limit of detection (mg/kg)	Reference
Cereals (wheat)	Food samples	Gluten gliadins	MALDI-TOF MS	4	60
	Maize-, rice-based foods	Gliadin	MALDI-TOF MS	100	147
Crustaceans	—	—	—	—	—
Egg	Mixed proteins	Lysozyme	RPLC-ESI-QqQ-MS-MS	10	153
Fish	—	—	—	—	—
Milk	β-Casein	β-Casein peptides	MicroIMAC-ESI-QqQ-MS-MS	0.0007	260
	Hydrolysates	Lactolated peptides	LC-ESI-QqQ-MS-MS	0.0002	237
	Whey drink	α-Lactalbumin	RPLC-ESI-QqQ-MS-MS	685	153
		β-Lactoglobulin A	—	1,600	—
		β-Lactoglobulin B	—	1,840	—
	Dairy products	κ-Casein	RPLC-ESI-QqQ-MS-MS	1,000	236
	Cookies, ice cream, cereals	α _{s1} -Casein	NanoRPLC-ESI-Q-TOF-MS-MS	1.25	352
	Milk products	β-Lactoglobulins	MicroRPLC-ESI-QqQ-MS-MS	24	89
	Fruit juices	Whey proteins	MicroRPLC-ESI-QqQ-MS-MS	1	239
	Buffalo milk	Caseins	RPLC-ESI-Q-TOF	1,500	109
Nuts	—	—	—	—	—
Peanut	Ice cream	Ara h 1 tryptic peptide	MicroRPLC-ESI-Q-TOF-MS-MS	10	311
	Chocolate	Ara h 1 tryptic peptide	MicroRPLC-ESI-Q-TOF-MS-MS	2	310
	Chocolate snacks, cereals, rice crispies	Ara h3/4 + Ara h 2	LC-ESI-QqQ-MS-MS	>1/5	64
		Peanut	FIA-ICP-Q-MS	2.5	66
		Ara h 3/4 tryptic peptide	RPLC-ESI-Q-IT-MS-MS	3	65
Soybean	Meat	Polyphenols	RPLC-thermospray-Q-MS	1,000	222
	Plant proteins	ε(γ-glutamyl)lysine	RPLC-ESI-Q-IT-MS-MS	138	302
	Milk powder	Soy globulins	NanoRPLC-ESI-Q-TOF-MS-MS	10,000	190
Celery	—	—	—	—	—
Lupin	Meat	Polyphenols	RPLC-thermospray-Q-MS	1,000	222
	Plant proteins	ε(γ-glutamyl)lysine	RPLC-ESI-Q-IT-MS-MS	138	302
	Lupin flakes, lupin beverage	β-Conglutin precursor	MicroRPLC-nanoESI-Q-IT-MS-MS	50	184
Molluscs	—	—	—	—	—
Mustard	—	—	—	—	—
Sesame	—	—	—	—	—
Buckwheat	—	—	—	—	—

^a FIA, flow injection analysis.

gopyrum tataricum) or “bitter buckwheat,” a less common crop, contains an allergenic 11S globulin (Fag t 1) (215).

Compendium of LC and MS methods for identification of food allergens. An overview of the published LC and MS methods used for the analysis of 14 main allergy-causing foods, i.e., cereals containing gluten (wheat), crustaceans, egg, fish, milk, nuts, peanut, soybean, celery, lupin, molluscs, mustard, sesame, and buckwheat, is provided in Tables 1 through 4. Because crustaceans, fish, nuts, and molluscs are defined as collective allergens (food groups containing two or more species) and milks other than bovine milk can elicit allergenic reactions, the respective analyte and matrix have been added for each

entry listed in the tables. The references are listed in chronological order, starting with the oldest. A development from relatively basic methods detecting proteins in solution to more advanced analyses of allergens in food matrices can be observed.

Chromatographic methods for the identification of main food allergens. Chromatographic techniques are widely used for the separation and isolation of food allergens. For some allergenic foods such as cereals containing gluten (wheat), milk, and soybean, many identification methods have been developed, whereas other allergenic foods such as celery, sesame, and molluscs have received less attention. Table 1 is focused primarily, with

some exemptions for certain pioneering articles, on method publications that have been presented in the last 15 years. Articles from previous years have been summarized in dedicated review articles, such as that for milk (327).

MS methods for the identification of main food allergens. MS has become increasingly important in the analysis of nutritionally relevant proteins in general and allergenic proteins in foods in particular. Many reports on MS-based proteomic methods for individual food allergens have been published (Table 2).

Several reviews on MS-based proteomic studies of individual foods also are available. Proteomics involving proteins in cereals (wheat) (5, 88, 113, 159, 296), crustaceans (279, 280), egg (5, 90), fish (5, 279, 280), milk (5, 209, 261), peanut (159, 296), soybean (159, 296), lupin (296), molluscs (186, 279, 280, 309), mustard (296), and buckwheat (159) have been summarized. In recent years, the benefits of MS-based proteomics for nutritionally relevant proteins have been discussed in general reviews (35, 43, 67, 101, 136, 169, 179, 201, 240, 353).

Marker peptides in MS-based proteomics of main food allergens. The most stable, volatile, and signal-intense peptides of individual food proteins, generated by enzymatic digestion and detected by MSⁿ, are used for characterization and identification. These peptides also are potential biomarkers for quantitative MS analysis. Only peptide sequence information obtained by MS-MS experiments has been included in Table 3, leaving out results from peptide mass fingerprinting. The number of marker peptides published for each main food allergen differs considerably. For some foods, peptide sequences for several of the major proteins are available (e.g., peanut, soybean), whereas for other foods (e.g., sesame) only one protein has been characterized. The collective allergens crustaceans, fish, and molluscs are listed with entries for a number of species. MS sequence data for some important allergens such as fish parvalbumin and shrimp tropomyosin can be hard to find because of the thorough investigation of these proteins by genomic methodology.

MS for the quantitation of main food allergens. Quantitative MS-based proteomics for food allergens is becoming more important as a protein-specific confirmatory method (161). The field is still under development; however, for some important food allergens such as milk, peanut, soybean, and wheat gliadin, quantitative methods for the detection of the allergen in typical matrices have been published (Table 4). The limits of detection achieved, especially for peanut (66, 310) and wheat gliadin (60) allergens, are already low enough to ensure sufficient protection for food allergic consumers.

CONCLUSIONS

Gel-free proteomics of food allergens by MS analysis is widely used for the identification and characterization of allergenic proteins because of the high specificity and resolution power of this technique. MALDI-TOF-MS and

ESI-Q-IT-MS-MS are the most frequently used configurations for this task. However, Q-TOF and Orbitrap are rapidly becoming more popular. LC also is used extensively for the separation and isolation of protein analytes. RPLC is the preferred LC application and is especially suited for on-line coupling with ESI-MS systems.

The use of LC-MS-MS for the quantitative detection of food allergens in matrices is presently gaining acceptance as a protein-based confirmatory technique over the routinely performed enzyme-linked immunosorbent assays. Special challenges for these MS techniques are effective sample preparation, the reduction of matrix interferences, and the increase of method throughput. Quantitative MS assays generally rely on measuring marker peptides (biomarkers) using an ISTD and a calibration curve for the determination of the analyte's relative concentration. MS-MS is obligatory for quantitative analysis, and RPLC-ESI-QqQ and RPLC-ESI-Q-IT have been successfully used in many studies. Quantitative MS of food allergens is a field under development, and more methods will be developed in the near future, including multiallergen assays for the simultaneous determination of several major food allergens from complex processed food matrices (170).

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