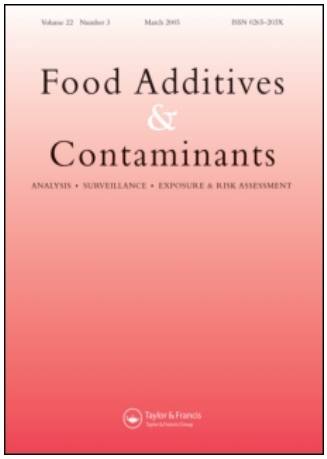

Food Additives & Contaminants
Publication details, including instructions for authors and subscription information:
http://www.informaworld.com/smpp/title~content=t713599661

Methods for allergen analysis in food: a review

R. E. Poms [a]; C. L. Klein [a]; E. Anklam [a]
[a] European Commission, DG Joint Research Centre, Institute for Reference Materials and Measurements, Retieseweg, B-2440 Geel, Belgium

Online Publication Date: 01 January 2004
To cite this Article: Poms, R. E., Klein, C. L. and Anklam, E. (2004) 'Methods for allergen analysis in food: a review', Food Additives & Contaminants, 21:1, 1 - 31
To link to this article: DOI: 10.1080/02652030310001620423
URL: http://dx.doi.org/10.1080/02652030310001620423

Food Additives and Contaminants, Vol. 21, No. 1 (January 2004), pp. 1–31

Taylor & Francis
Taylor & Francis Group

Methods for allergen analysis in food: a review

R. E. Poms, C. L. Klein and E. Anklam*
European Commission, DG Joint Research Centre, Institute for Reference Materials and Measurements, Retieseweg, B-2440 Geel, Belgium

(Received 9 April 2003; revised 27 August 2003; accepted 27 August 2003)

Food allergies represent an important health problem in industrialized countries. Undeclared allergens as contaminants in food products pose a major risk for sensitized persons. A proposal to amend the European Food Labelling Directive requires that all ingredients intentionally added to food products will have to be included on the label. Reliable detection and quantification methods for food allergens are necessary to ensure compliance with food labelling and to improve consumer protection. Methods available so far are based on protein or DNA detection. This review presents an up-to-date picture of the characteristics of the major food allergens and collects published methods for the determination of food allergens or the presence of potentially allergenic constituents in food products. A summary of the current availability of commercial allergen detection kits is given. One part of the paper describes various methods that have been generally employed in the detection of allergens in food; their advantages and drawbacks are discussed in brief. The main part of this review, however, focuses on specific food allergens and appropriate methods for their detection in food products. Special emphasis is given to allergenic foods explicitly mentioned in the Amendment to the European Food Labelling Directive that pose a potential risk for allergic individuals, namely celery, cereals containing gluten (including wheat, rye and barley) crustaceans, eggs, fish, peanuts, soybeans, milk and dairy products, mustard, tree-nuts, sesame seeds, and sulphite at concentrations of at least 10 mg kg^{-1}. Sulphites, however, are not discussed.

Keywords: allergen methods, RAST/EAST inhibition, SDS-PAGE immunoblotting, rocket electrophoresis, ELISA, dot immunoblotting, polymerase chain reaction (PCR), biosensors, celery, cereals, crustaceans, eggs, fish, peanuts, soybeans, milk and dairy products, mustard, tree-nuts, sesame seeds

* To whom correspondence should be addressed.
e-mail: elke.anklam@cec.eu.int

Background

Food allergy and food intolerances

Food allergies represent an important health problem in industrialized countries. According to several European and American authors (Sampson 1999, Wüthrich 2000, Ortolani *et al.* 2001, Sicherer *et al.* 2003), food allergies affect up to 2% of the adult population and up to 8% of children, although the perceived prevalence of food induced symptoms may be as high as 22% of the general population (Woods *et al.* 2002). In highly sensitized individuals, even the intake of minute amounts of allergens can provoke digestive disorders (emesis, diarrhoea), respiratory symptoms (rhinitis, asthma), circulatory symptoms (oedema, hypotension), and skin reactions (urticaria, atopic dermatitis/eczema). For some allergic individuals, contact with a specific food allergen can provoke life-threatening reactions (anaphylactic shock). Recent reports indicate an increase in food allergen induced life-threatening reactions (Sampson 1999, Wüthrich 2000, Ortolani *et al.* 2001).

Food allergies must be distinguished from food intolerances. Food allergies are abnormal immunological reactions to a food or food component. The involvement of the immune system distinguishes food allergies from other types of food sensitivities. According to the classification scheme by Gell *et al.* (1975) most allergic reactions to food can be classified as type I reactions (although types III and IV are also common). Type I reactions are often referred to as acute or immediate hypersensitivity reactions. Thus, allergic reactions are typically characterized by a rapid onset of symptoms and are mediated by allergen-specific immunglobulin E (IgE) (Taylor 1987, Holgate *et al.* 2001).

Food Additives and Contaminants ISSN 0265–203X print/ISSN 1464–5122 online
http://www.tandf.co.uk/journals
DOI: 10.1080/02652030310001620423

Most other food sensitivities do not involve the immune system and are not considered true food allergies. They may be caused by pharmaceutical reactions to food additives (e.g. benzoates, salicylates, sulphites, or infectious agents such as bacterial or viral products); by enzyme deficiencies (e.g. lactose intolerance); by diseases of the digestive system (e.g. obstructions); by idiosyncratic reactions (individualistic adverse reactions with unexplained mechanisms) to food or even psychological reasons. Non-immunological food sensitivities are characterized by the absence to produce allergen-specific IgE (or IgA and T cells) and the delayed onset of symptoms, thereby often masking the possible link between the cause (offensive substance) and the effect (symptoms).

A special mention should also be given to celiac disease, also known as celiac sprue or gluten sensitive enteropathy. This disease is characterized by malabsorption of nutrients from the intestine because of damage to the absorptive epithelial cells of the small intestine. This intestinal damage occurs in susceptible individuals after they consume the protein fraction of wheat, rye, barley (and rarely after the consumption of oats). The gliadin fraction of wheat protein and the equivalent prolamin fractions of barley and rye are responsible for the damage (Taylor 1987, Holgate *et al*. 2001). Investigators from several recent studies concluded that the consumption of oats was safe for adults with celiac disease (Janatuinen *et al*. 1995, Storsrud *et al*. 2003). However, one has to keep in mind that if oats themselves are safe, they nonetheless may be contaminated with wheat, rye, or barley. Although celiac disease is not classified as an allergy, an immunological aspect does exist, as celiac disease may be mediated at least partly by IgA and cytolytic T cells (Ciclitira 2002) There is also an important interaction leading to the induction of endogenous transglutaminase, which modifies glutamines of the glutens and of endogenous proteins in the patient.

Currently, the only effective treatment for food allergy, and for celiac disease, is avoidance of the allergen-containing food or those with the offending glutens. However, total avoidance is sometimes difficult for the allergic individual, since processed food products contain a large variety of ingredients including allergenic foods. Sensitive individuals may also be inadvertently exposed to allergenic proteins by consumption of food products that are supposed to be free of a certain allergen. Food products can be contaminated with 'foreign' food constituents during shipping and storage, during processing, from carry-over due to inadequate cleaning of shared processing equipment, or through rework of allergen-containing products (Huggett and Hischenhuber 1998). Moreover, refined ingredients are employed in food production as substitutes for expensive raw materials, which do not appear on the label, e.g. deflavoured peanuts have been used and sold as other types of nuts, such as almonds (Keating *et al*. 1990).

Allergen labelling — legal status

Over 160 food materials have been identified so far to be allergenic. Only eight of them report for more than 90% of all food allergies (FAO 1995, Hefle *et al*. 1996, Ellman *et al*. 2002). For the allergic consumer it is particularly important to have full information about potential allergens contained in a food product. Thus, the European Commission reacted to recommendations by the Codex Alimentarius Commission (1999) and issued a proposal to amend the food European Food Labelling Directive 2000/13/EC (European Commission 2000), which is intended to ensure that all consumers are informed of the complete contents of foodstuffs and to enable consumers with allergies to identify any allergenic ingredients that may be present. The proposal for amendment (European Commission 2002) will abolish the '25% rule' which currently means that for some products it is not obligatory to label the components of compound ingredients that make up less than 25% of the final food product. The new proposal however intends that all ingredients intentionally added will have to be included on the label. The proposal also contains a list of ingredients liable to cause allergies or intolerances and will include alcoholic beverages if they contain an ingredient on the allergen list. The foods or food ingredients listed are generally in accordance with the list of common allergenic foods adopted by the Codex Alimentarius Commission (1999) and the US Food and Drug Administration (FDA 2001). Only celery, mustard, and sesame were added to the list in the proposal due to recent evidence for concern. The list of ingredients to be labelled comprises the following:

- Celery and products thereof.
- Cereals containing gluten and products thereof.
- Crustaceans and products thereof.
- Eggs and products thereof.
- Fish and products thereof.
- Milk and dairy products (including lactose).

- Mustard and products thereof.
- Nuts and nut products.
- Peanuts and products thereof.
- Sesame seeds and products thereof.
- Soybeans and products thereof.
- Sulphite at concentrations of at least 10 mg kg^{-1}.

Numerous other food allergens have been identified (Sicherer *et al.* 2001), including fruits, vegetables and latex. However, the scope of this review is restricted to those allergenic foods listed in the amendment of the European Food Labelling Directive 2000/13/EC (European Commission 2002). Thus, all other potentially allergenic foods are not discussed here. Moreover, sulphites, which are also listed due to their association with asthmatic reactions in sulphite-sensitive subjects (Taylor *et al.* 1986, 1988), are not included in this article, since they are no allergens.

Methods/techniques for detection of allergens and potentially allergenic food or food constituents

Food allergens can be defined as those substances in foods that initiate and provoke the immunological reactions of allergy. In IgE-mediated food allergy, the allergens are usually naturally occurring — often abundant — proteins found in a particular food (Taylor 1992).

Reliable detection and quantification methods for food allergens are necessary in order to ensure compliance with food labelling and to improve consumer protection. However, the detection of allergens in food products can be very difficult, as they are often present only in trace amounts or are masked by the food matrix. Another question yet to be answered is how sensitive the detection methods need to be, as there are little data available about established threshold levels that have been determined by human oral challenge studies. Threshold levels for specific allergic reactions determined by double-blind placebo controlled food challenges (DBPCFC) range between less than 1 mg and more than 1 g of allergenic protein, depending on the food concerned and the sensitivity of selected allergic individuals (Taylor *et al.* 2002). There is general agreement that the detection limits for different food products need to be somewhere between 1 and 100 ppm (mg allergenic protein kg^{-1} food), depending on the respective food (Koppelman *et al.* 1996, Taylor and Nordlee 1996, Poms and Anklam 2003).

Nearly all allergens (antigens) are proteins or glycoproteins. Polypeptide masses usually range between 5 and 70 kDa (Taylor 1992, Bredehorst and David 2001), however, many allergens are oligomers with molecular masses greater than 200 kDa (Besler *et al.* 2000). The determination of the allergenicity of a food or a food product is generally based on human IgE binding from sera of sensitized individuals with a clear clinical disease associated with exposure. Once allergens are identified and purified, antibodies can be raised in animals like rabbit, rat, goat, sheep or chicken to be employed in immunological detection methods for routine food analysis.

Currently, there are several technical possibilities for the detection of potential allergens in food products. The methods employed are either targeting the allergen (protein) itself or a marker that indicates the presence of the offending food. While the ideal marker is the offending allergenic protein, at present detecting the allergen per se is not always feasible, as the chemical properties may not be well characterized or the detection limit of the used methodology is insufficient. Additionally, many allergenic foods contain multiple allergenic proteins that can vary in abundance. As markers for the presence of potentially allergenic food products or ingredients, specific proteins or DNA fragments are targeted. Protein-based methods usually involve immunochemical detection protocols such as the radio-allergosorbent test (RAST), enzyme allergosorbent test (EAST), rocket immuno-electrophoresis (RIE), immunoblotting, and enzyme-linked immunosorbent assay (ELISA). Whereas RIE and immunoblotting render only qualitative or semiquantitative results, RAST, EAST and ELISA are quantitative methods. Presently, only the ELISA technique is used in routine food analysis due to its high precision, simple handling and good potential for standardization. Methods operating on the DNA level are based on an amplification of a specific DNA fragment by the polymerase chain reaction (PCR). With real-time PCR highly accurate quantitative results can be obtained.

The choice of method is mainly dependent on the food concerned (availability of specific antibodies/DNA primers and the achievable detection limit) and on the history of processing involved during food production. Protein- and DNA-based methods, respectively, have their characteristic merits and drawbacks concerning their applicability in the detection and

quantification of allergens in various food products (Poms and Anklam 2003). However, the employment of DNA analysis in allergen detection is discussed controversially, since proteins are the allergenic component and processing may differentially affect nucleic acids and proteins.

In the following, several commonly used methods for the detection and quantification of food allergens are discussed in detail.

RAST/EAST inhibition

With RAST (radio-allergosorbent) or EAST (enzyme allergosorbent) assays allergen-specific IgE can be determined. RAST and EAST assays are *in-vitro* tests, which are mainly used in clinical diagnosis of food allergy (Holgate *et al.* 2001). However, RAST and EAST inhibition tests have been applied for qualitative allergen detection and for the assessment of potential allergenicity in a wide range of food (Nordlee *et al.* 1981, Oldaeus *et al.* 1991, Herian *et al.* 1993, Wigotzki *et al.* 2000, 2001). Only very few applications of RAST for quantitative allergen determination have been published (Fremont *et al.* 1996, Koppelman *et al.* 1999) with detection limits of $1\,mg\,kg^{-1}$. RAST and EAST inhibition represent competitive IgE binding assays. In principle, an antigen/allergen bound to a solid phase binds specific human IgE. Antigens in a sample solution inhibit IgE binding to the antigen immobilized on the solid phase. An anti-IgE antibody labelled with an isotope (RAST), e.g. ^{125}I, or an enzyme (EAST), e.g. horseradish peroxidase, followed by addition of a substrate that changes colour or emits light, is used to detect the bound human IgE antibodies. The bound IgE is quantitatively measured using a gamma counter, or a spectrophotometer as appropriate. Commercial applications of RAST and EAST for allergen quantification are limited due to the reliance on human sera from appropriately allergic subjects and difficulties in standardizing these assays (Nordlee and Taylor 1995).

SDS-PAGE/immunoblotting

One-dimensional sodium dodecyl sulphate (SDS) polyacryl gel electrophoresis (PAGE) followed by immunoblotting represents the standard procedure for protein/allergen separation and identification. The SDS denatures and coats the proteins, giving them a strong negative charge. The proteins are separated according to their molecular mass irrespective their original electrochemical charge (figure 1). Separated proteins are then transferred onto a membrane and detected with radio- or enzyme-labelled antibodies. Individual bands typically represent one protein, however, additional proteins may migrate at the same molecular weight. Human IgE blotting

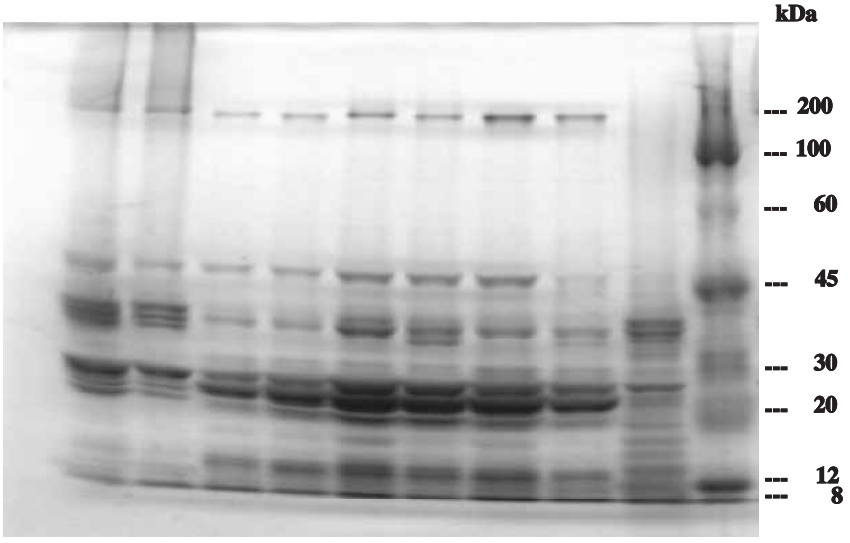

Figure 1. SDS-PAGE protein pattern of various hazelnut extracts (last lane on the right: molecular weight marker 8–200 kDa.

allows the detection and identification of individual allergens. SDS-PAGE and immunoblot with human IgE antibodies are used extensively to identify and characterize new allergens (Pastorello and Trambaioli 2001). However, the same method may be used for the qualitative determination of potentially allergenic food contaminants down to a detection limit of $5\,mg\,kg^{-1}$ (Scheibe *et al.* 2001). The major disadvantages of SDS-PAGE and immunoblotting with IgE for routine food analysis are the elaborate and time-consuming procedures and the reliance on appropriate human sera. However, once an allergen is characterized, antibodies may be raised in animals (e.g. rabbits or goats) for use in similar immunoblotting procedures, replacing the human sera.

Cell response factor release assays

The basophil histamine release assay is usually used for confirmation of the identification of allergens and for allergy screening. The assay is based on allergen induced, IgE-mediated histamine release from blood basophils (a type of white blood cell) from allergic individuals. As little as 20 µl freshly collected blood sample are added to an allergen extract and the histamine release is measured by fluorescence or radioactive immunoassay. For a given human sample, the histamine release is proportional to the concentration of a specific allergen in the extract, which offers the possibility to adapt the assay for allergen quantification. The test is as sensitive as the RAST and it takes only a few hours, but it is usually performed only by specialized laboratories (Holgate *et al.* 2001). However, as the assay is based on human donors and fresh samples, the method is difficult to standardize for allergen quantification and has therefore not been employed for routine analysis of food samples.

Assessing the release of β-N-acetylhexosaminidase in a mucosal mast cell line is another cell-based technique to measure the allergenic potency of food. This kind of assay has been employed for the quantitation of allergenic soy proteins in various food products, but depends on the production of IgE from rodent cells to the allergen of interest (Yamanishi *et al.* 1995, 1997).

Rocket immuno-electrophoresis (RIE)

Rocket immuno-electrophoresis employs an antibody containing gel. Antigens to be analysed migrate according to their electrophoretic mobility until antigen–antibody complexes precipitate in the gel. Rocket-shaped precipitates are formed at a constant antigen/antibody ratio. The height of the rockets is proportional to the amount of antigen applied (figure 2).

Rocket electrophoresis was applied to detect several allergens in various food products (Malmheden Yman *et al.* 1994, Holzhauser *et al.* 1998) with detection limits of 30 and $2.5\,mg\,kg^{-1}$, respectively. However, rocket immuno-electrophoresis is not widely used for allergen determination due to laborious gel preparation and immuno-staining procedures (Besler *et al.* 2002b).

Dot immunoblotting

Dot immunoblotting allows simple and inexpensive screening of food samples. Sample protein extracts are spotted onto a nitrocellulose or PVDF membrane and incubated with enzyme-labelled, protein specific antibodies, which bind to the target antigens. The dots are visualized by the formation of a coloured product after enzyme–substrate interaction (figure 3). Alternatively, it is also possible to use radioactively labelled antibodies and subsequently analyse by radiography. The intensity of the dots is proportional to the amount of antigen. This test is semiquantitative and allows detection of target proteins (e.g. peanut) in food down to $2.5\,mg\,kg^{-1}$ (Blais and Philippe 2000).

Enzyme-linked immunosorbent assay (ELISA)

Currently, the ELISA technique is the most commonly method used in laboratories of the food industry and official food control agencies to detect and quantify hidden allergens in food. With ELISA tests allergens or specific marker proteins can be detected by colorimetric reaction following binding with a specific enzyme-labelled antibody. The concentration of this antigen/antibody complex can be subsequently estimated based on a standard curve generated with purified reference standards (figure 4).

Two ELISA approaches are available for the quantification of allergens or proteins of potentially allergenic food: competitive ELISA and sandwich ELISA. The latter is the most common type of immunoassay for the detection of potential food allergens. This assay

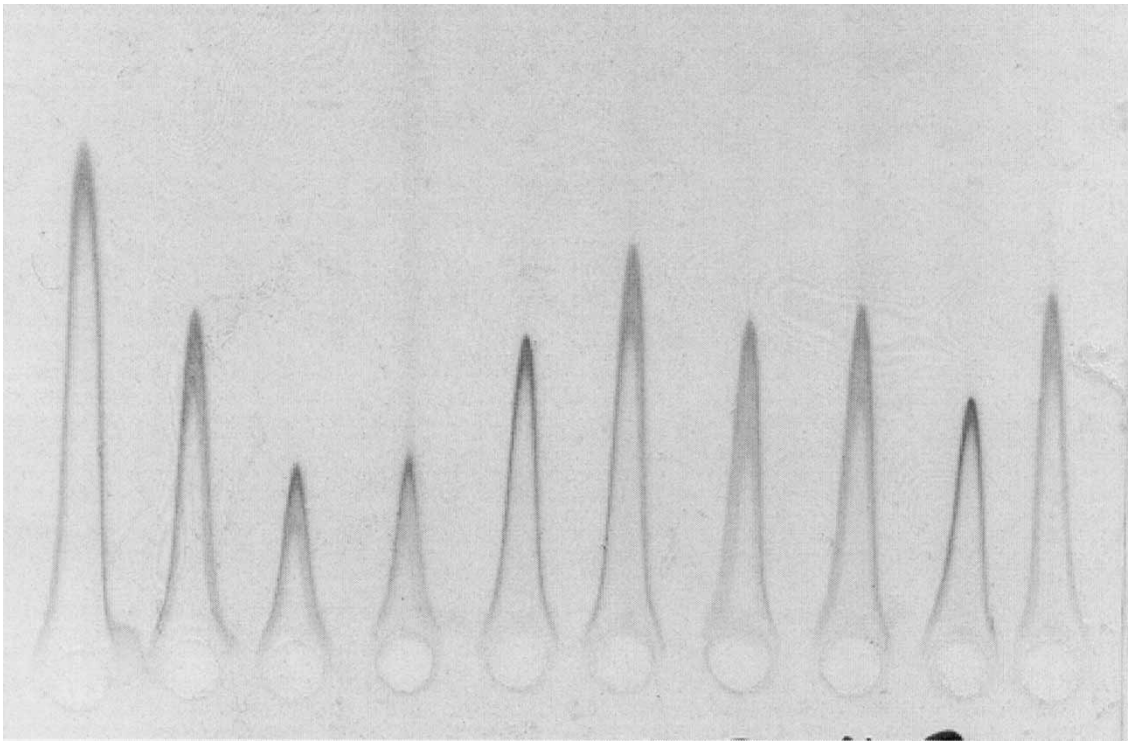

Figure 2. Rocket immuno-electrophoresis for the detection of egg protein in pasta samples (Malmheden Yman 2003).

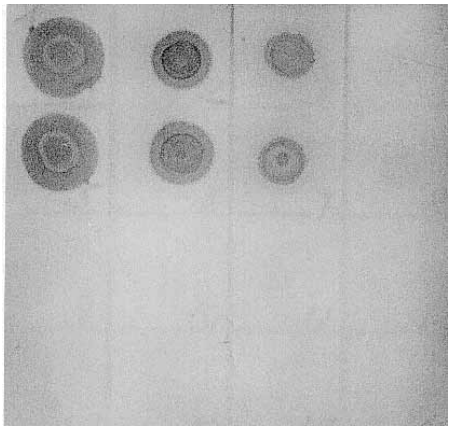

Figure 3. Dot blots of peanut extracts coupled with human IgE and detected by enzyme labelled, anti-IgE, goat IgG (Koch et al. *2003).*

involves a capture antibody immobilized on a solid phase, which is usually a microtitre plate or a multiple well strip. Specific proteins in the sample are captured by the first antibody, and detected by a second protein-specific, enzyme-labelled antibody, which binds to the analyte, forming a 'sandwich'. A particular substrate reacts with the enzyme tagged on the second antibody producing a coloured product. The measured absorption is directly proportional to the concentration of the analyte. Sandwich ELISA methods have been developed for several food allergens (Mäkinen-Kiljunen and Palosua 1992, Hefle *et al.* 1994, 2001, Tsuji *et al.* 1995, Bando *et al.* 1998, Holzhauser and Vieths 1999a, Koppelman *et al.* 1999, Hlywka *et al.* 2000) and numerous test kits have become commercially available in this format during the last decade (table 1).

The competitive ELISA is the preferred format for the detection of relatively small proteins. It involves immobilized antigens bound to a solid phase. The sera and appropriately diluted sample extract (inhibitor) are pre-incubated, then added to the solid-phase antigen. If no sample antigen is present in the inhibitor sample, the enzyme-labelled antibody shows maximal binding to the solid phase bound antigen, resulting in high absorption of the coloured product formed. Antigens in the sample inhibit the binding of the enzyme-labelled antibody to the immobilized antigen. The absorbance is inversely proportional to the concentration of antigen in the sample. Competitive ELISA methods have been described

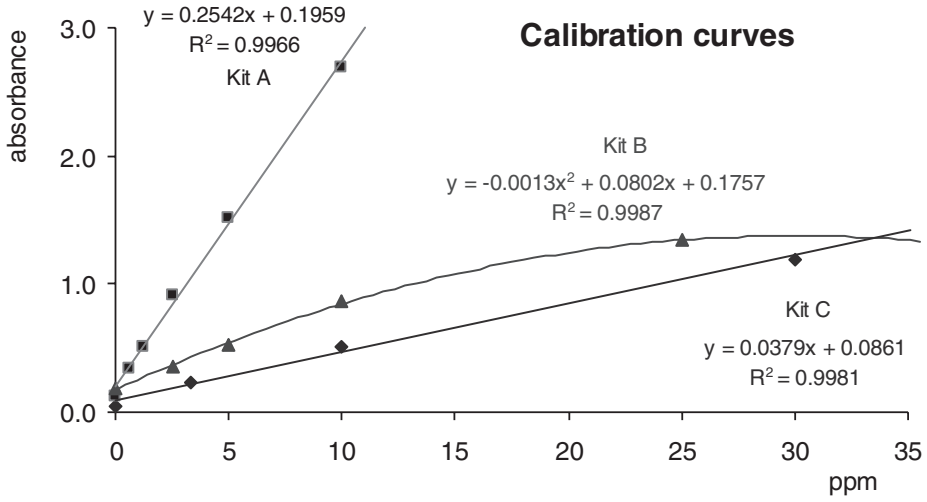

Figure 4. Examples of calibration curves of three commercial peanut ELISA test kits (Poms et al. *2003).*

for some food allergens (Mariager *et al.* 1994, Yeung and Collins 1996, Plebani *et al.* 1997, Holzhauser and Vieths 1999b, Koppelman *et al.* 1999, Roux *et al.* 2001) with sensitivities down to $0.4\,\mu g\,kg^{-1}$. For some allergens, competitive ELISAs are marketed in kit format (table 1).

An alternative to the ELISA format has earned increasing importance in recent years: dipstick assays (lateral flow test strips). Dipstick tests are very inexpensive, rapid, and portable, do not require instrumentation and are extremely simple to perform. Currently, dipstick tests are only qualitative. However, a recently developed dipstick assay for egg (Baumgartner *et al.* 2002) was shown to be highly specific and extremely sensitive with detection limits down to $0.02\,mg\,kg^{-1}$ food. Currently only one dipstick assay is marketed in kit format (table 1).

Polymerase chain reaction (PCR)

DNA-based methods are increasingly used for the detection of foreign food constituents, such as microbial pathogens (Allmann *et al.* 1995, Malorny *et al.* 2003), or the presence of genetically modified crop material (Hübner *et al.* 2001, Holst-Jensen *et al.* 2003). The methods are very specific and provide sensitive tools for the detection of specific allergenic components in food. However, they do not detect the allergen or any specific protein. Therefore, results cannot be tied to actual allergenic exposure. Moreover, food processing can affect proteins (allergens) and DNA rather differently and, moreover, protein and DNA could be separated during certain processing steps yielding erroneous results regarding the presence of allergens in the product. Despite these limitations, DNA-based methods offer many advantages over protein-based methodologies, primarily that the target DNA is efficiently extracted under harsh denaturing conditions and is less effected than the extraction of proteins from food matrices. Another advantage of analysing DNA is its stability against geographical and seasonal variations, which may vary protein composition.

In principle, a specific DNA fragment, flanked by two oligonucleotides serving as primers for the reaction, is amplified by a thermostable polymerase. The reaction consists of three functional steps per cycle of amplification, each determined by a different temperature to allow melting of the double stranded DNA, annealing of the primers, and extension of the primers by the polymerase. Typically, 25–45 cycles of this temperature profile are run to produce a detectable quantity of copies of the template DNA fragment. The amplified product is visualized by staining with a fluorescent dye (figure 5) or by southern blotting following electrophoresis in an agarose gel. Ordinary PCR results are only qualitative. However, by incorporating internal standards, the results provide semiquantitative measurements (Studer *et al.* 1998, Jankiewicz *et al.* 1999). Superior quantification can be achieved by employing real-time PCR or a PCR-ELISA.

Table 1. Commercially available ELISA test kits for allergen detection in food products — status by August 2003.

Allergenic food	Target	Format	LOD[1] (mg kg^{-1})	Interlaboratory validation	Supplier
Almond	almond protein	quantitative S-ELISA	< 2.5	no	Neogen
	almond protein	qualitative S-ELISA	< 5	no	Neogen
Crustaceans	tropomyosin	quantitative S-ELISA	0.05	no	ElisaSystems
Egg	ovomucoid and ovalbumin	quantitative S-ELISA	1	no	ElisaSystems
	egg protein	quantitative S-ELISA	0.3	no	Pro-Lab Diagnostics
	egg white protein	quantitative S-ELISA	2	no	R-Biopharm
	egg protein	quantitative S-ELISA	< 2.5	no	Neogen
	egg protein	qualitative S-ELISA	< 5	no	Neogen
Hazelnut	specific heatstable hazelnut protein	quantitative S-ELISA	1	no	ElisaSystems
	hazelnut protein	quantitative S-ELISA	10	no	R-Biopharm
Milk	β-lactoglobulin and casein	quantitative S-ELISA	1	no	ElisaSystems
	casein	quantitative S-ELISA	?	no	Announced for 2003 Pro-Lab Diagnostics
	casein	quantitative C-ELISA	< 5	no	Tepnel BioSystems
	BSA	quantitative C-ELISA	< 5	no	Tepnel BioSystems
	β-lactoglobulin	quantitative C-ELISA	< 5	no	Tepnel BioSystems
	β-lactoglobulin	quantitative C-ELISA	5	no	R-Biopharm
	casein	quantitative S-ELISA	< 2.5	no	Neogen
	casein	qualitative C-ELISA	< 5	no	Neogen
Peanut	Ara h 2	quantitative S-ELISA	1	no	ElisaSystems
	peanut protein	quantitative S-ELISA	1.6	no	Pro-Lab Diagnostics
	Ara h 1	quantitative S-ELISA	< 0.1	AOAC-RI 2003	Tepnel BioSystems
	peanut protein	quantitative S-ELISA	2	AOAC-RI 2003	R-Biopharm
	peanut protein	quantitative S-ELISA	< 2.5	AOAC-RI 2003	Neogen
	peanut protein	qualitative S-ELISA	< 5	AOAC-RI 2003	Neogen
	peanut protein	qualitative dip-stick ELISA	< 5	no	Announced for 2003 Neogen
Sesame	2S albumin	quantitative S-ELISA	1	no	ElisaSystems
	sesame protein	quantitative S-ELISA	< 1	no	Tepnel BioSystems
Soy	soy trypsin inhibitor	quantitative S-ELISA	1	no	ElisaSystems
	soy protein	quantitative C-ELISA	< 5000	no	Tepnel BioSystems
Wheat, rye, barley	gliadin	quantitative S-ELISA	< 2	no	Tepnel BioSystems
	gliadin	quantitative S-ELISA	1.5	PWG 2002	R-Biopharm
	gliadin	qualititative dip-sick ELISA	10	no	R-Biopharm

[1] LOD, limit of detection according to the manufacturer.
AOAC-RI, Association of Analytical Communities-Research Institute, first phase of validation completed.
PWG, Prolamine Working Group, laboratory validation completed, statistical validation ongoing.

Real-time PCR

Real-time PCR requires more expensive laboratory equipment, but it was shown to be extremely accurate and less labour-intensive than other DNA quantification methods (Desjardin *et al.* 1998). Real-time PCR allows gel free product detection 'in real time' by using a target-specific oligonucleotide probe with a reporter dye and a quencher dye attached. The probe anneals to the single-stranded amplified segment within the region flanked by the two oligonucleotides priming the enzyme reaction. Due to the proximity of the quencher to the reporter fluorescence is suppressed. During amplification the 5′exonuclease activity of the polymerase cleaves the hybridized probe and separates the dyes, which are displaced by the synthesized new copy strand and become soluble, with the intensity of the fluorescence of the free reporter dye producing a measurable signal. The resulting increase in fluorescence is proportional

to the amount of specific PCR product. The cycle number required until the fluorescence level exceeds a passive reference dye is used to calculate quantitative data (figure 6).

There are several real-time PCR kits for various allergens on the market (table 2).

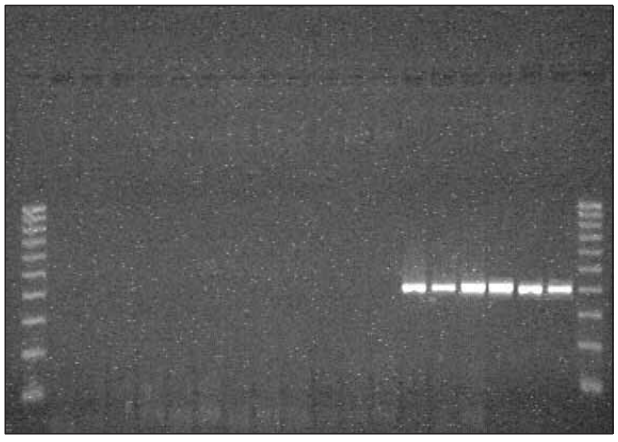

Figure 5. Agarose gel-electrophoresis after PCR amplification of a 414 bp fragment of the soy lectin gene (Poms 2001).

PCR-ELISA

PCR-ELISA combines the high specificity of a DNA-based methodology with the rather simple and economical ELISA assay for semiquantitative analysis. With PCR-ELISA, a specific DNA fragment of an allergenic food is amplified and the amplification product is then linked with a specific protein labelled DNA probe. This protein label is then coupled with a specific enzyme-labelled antibody. The concentration of the DNA can be quantified by the colour reaction resulting from an enzyme–substrate reaction.

Currently a few PCR-ELISA test kits are available on the market (table 2).

Biosensors

Another emerging technology that has not yet been commonly applied for food analysis is the use of biosensors. Biosensor instruments make it possible to measure specific molecular interaction in real-time. By immobilization of a target molecule, which may be an antibody (protein) or a single-stranded DNA

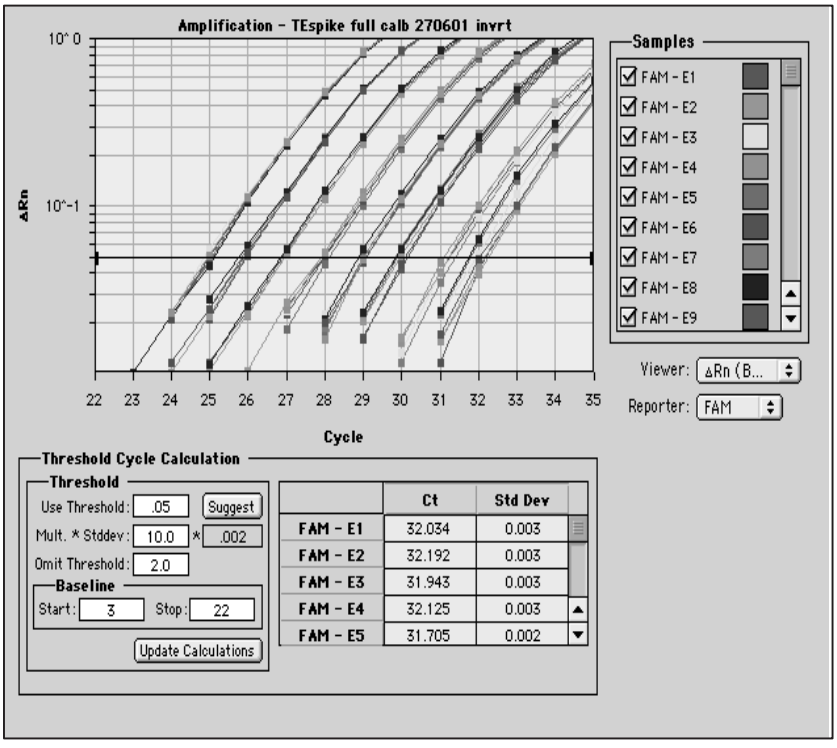

Figure 6. Result from a real-time PCR amplification of a fragment of the soy lectin gene (Poms 2003).

Table 2. Commercially available DNA-based test kits for allergen detection in food products — status by August 2003 (suppliers Congen GmbH and Tepnel BioSystems Ltd).

Allergenic food	Target	Method	Qualitative/quantitative	LOD[1] (ppm)	Interlaboratory validation
Almond	DNA[3]	DNA-ELISA	qualitative	< 10	no
	DNA[3]	real-time PCR	quantitative[4]	< 10	no
Celery	DNA[3]		market introduction by the end of 2003		
Gluten	DNA[3]		market introduction by the end of 2003		
Hazelnut	*Cor a 1.0401* gene	DNA-ELISA	qualitative	< 10	no
		real-time PCR	quantitative[4]	< 10	no
Milk	DNA[3]		market introduction by the end of 2003		
Peanut	DNA[3]	PCR + gel electrophoresis	qualitative	< 10	no
	DNA[3]	DNA-ELISA	qualitative	< 10	no
	DNA[3]	real-time PCR	quantitative[4]	< 10	no
Soya	lectin gene	DNA-ELISA	qualitative	< 10	only for GMO[5]
	lectin gene	real-time PCR	quantitative[4]	< 10	only for GMO[5]

[1] LOD, limit of detection according to the manufacturer.
[2] Total time of analysis, including incubation and hands on times.
[3] Specific DNA fragment not specified by the manufacturer.
[4] Real-time PCR may be quantitative when internal standards are available.
[5] GMO, genetically modified organism.

fragment to a sensor chip surface the binding interaction between one or more molecules can be measured quantitatively. There is no need to label molecules with fluorescent or radioactive tags, as the detection and quantification are determined by measuring the changes in refractive index. Attractive features of this technology are the short analysis time and a high degree of automation (Elliott 2002).

Biosensors can be used to detect either a specific allergen or protein, or a specific DNA fragment. Biosensors have been applied for the detection of a few potentially allergenic food like hazelnut, egg and milk (Jonsson 2002).

Detection and quantification of selected allergens in food products

Celery and products thereof

Allergy characteristics. IgE-mediated reactions to celery are common in food allergic adults in Europe (not so in the USA, Nowak-Wegrzyn *et al.* 2001), with about 30% of patients with oral allergy syndrome (OAS) being allergic to celery (Andre *et al.* 1994, Ballmer-Weber *et al.* 2000a). Sensitization to celery is frequently associated with birch (Central Europe) and/or mugwort (Southern Europe) pollinosis (Wüthrich *et al.* 1990), hence the terms 'birch–mugwort–celery syndrome' and 'celery–mugwort–spice syndrome' have been established.

Ballmer-Weber *et al.* (2000b) found the lowest provoking dose at 700 mg celery.

Allergenic properties. The major allergen from celery (*Apium graveolens*) is Api g 1, a 16 kDa homologous to Bet-v-1 (Breiteneder *et al.* 1995). Several additional allergens have been identified in the range of 30–70 kDa (Jankiewicz *et al.* 1998), including two heat stabile profilins (Api g 4, 15 kDa, Vallier *et al.* 1992, and Api g 5, 55/58 kDa, Ganglberger *et al.* 2000).

Celery tuber and stick are consumed as a raw or cooked vegetable and as a spice, which is a common hidden allergen in various processed foods, such as soups, salads, or sausages (Rueff *et al.* 2001).

Detection methods. To date no methods for the specific detection of celery in food products have been published. However, a celery specific DNA-based test

kit in the format of a PCR-ELISA and a real-time PCR are announced to be on the market by the end of 2003 (table 2).

Cereals containing gluten and products thereof

Allergy characteristics. Cereals like wheat, rye, and barley can cause IgE-mediated allergic reactions after ingestion or inhalation (Walsh *et al.* 1987), however, they have earned major attention due to their association with celiac disease. According to the international World Congress of Gastronterology, celiac disease or gluten-sensitive enteropathy affects one in every 200 people of the European population (Stern *et al.* 2001), and about one in every 250 in the USA (Neuhausen *et al.* 2002). For some sensitive subjects even 100 mg gliadin caused clinical symptoms (Codex Alimentarius Commission 2000).

Allergenic properties. Numerous distinct storage proteins are present in the gluten fraction of wheat, which consists of glutenins and gliadins (Stern *et al.* 2001). Different varieties of wheat often show subtle differences in the prevalence of specific gliadin or glutenin protein patterns. Celiac disease is apparently triggered mostly by specific toxic peptides of the gliadin fraction of wheat gluten (Devery *et al.* 1991, Stern *et al.* 2001). The different subgroups of gliadins, α-, γ-, ω_{12}- and ω_5-gliadins and their subtypes are characterized by their different molecular weight, amino acid composition, binding motifs, and N-terminal sequences and apparent contribution to celiac disease. Recent studies by Stern *et al.* (2001) describe a number of identified amino acid sequence motifs in gliadins tested for celiac toxicity.

Detection methods. Currently, analyses of gluten in food products face several challenges: (1) a clear definition of the relevant analyte; (2) the heterogeneity of the analyte (gliadin, peptides); (3) effects of processing on the molecular structure and biological activity of the analyte; (4) the cultivar-dependent variations in the composition of subgroups and subtypes of gliadins; and (5) the heterogeneity of the patient responses concerning the determination of relevant epitopes capable of inducing adverse reactions.

For the detection of gluten and gliadin in food a wide array of methods has been employed. Published procedures include the determination of wheat specific DNA by PCR (Allmann *et al.* 1993, Köppel *et al.* 1998), and detecting gluten and gliadin or fractions thereof by SDS-PAGE in combination with immunoblotting, counter immuno-electrophoresis, or mass spectroscopy and MALDI-TOF analysis (Mendez *et al.* 2000, Hernando *et al.* 2002). The most frequently used method is based on ELISA (Skerritt and Hill 1991, Troncone *et al.* 1986, Chirdo *et al.* 1995, Ellis *et al.* 1998).

High-performance liquid chromatography and capillary electrophoresis were employed to analyse wheat proteins in food (Scanlon *et al.* 1989, Wieser 1994). Gel permeation–high-performance liquid chromatography (GP-HPLC) has been described for the quantitative determination of both, gluten and gliadin. For starch samples a detection limit of about 10 mg kg^{-1} was achieved and sensitivities of at least 20 mg kg^{-1} with high repeatability were demonstrated for a variety of other matrices (Wieser and Antes 2002). Most recently a reference material for 'Gliadin from European wheat' was produced by the Institute for Reference Materials and Measurements of the European Commission's Joint Research Centre and undergoes at present a certification for its gliadin content, pattern and composition (Klein and Franchini 2003). A reference measurement system is currently under development in cooperation with the Codex Alimentarius Working Group on prolamine analysis and toxicity and EC-IRMM (van Eckert 2002, Klein and Franchini 2003).

The ELISA approach seems to be the method of choice for the determination of gluten in the mg kg^{-1} range. A number of protocols were developed and published (Denery-Papini *et al.* 1999). Skerritt and Hill (1991) first developed an ELISA method based on monoclonal antibodies that was used to detect trace quantities of gluten in various food products. Assays using several polyclonal antibodies specific to different binding sites have been used to overcome the problem of cultivars-dependent variation of obtained analytical results (Troncone *et al.* 1986, Chirdo *et al.* 1995). The ω-gliadin fraction is the most heat stable gliadin and therefore analyses employing antibodies specific to this fraction are least affected by heat processing. Unfortunately, these analyses are highly specific, discriminating between different cultivars, and therefore no single test or protocol is applicable for a large number of cultivars (Skerrit and Hill 1991). Prolamine from barley (and oats) is detected poorly by these immunoassays. However, a modified version of the method developed

by Skerrit and Hill (1991) was adopted by the Association of Official Analytical Chemists (AOAC) International. The accuracy of the method was tested only at relatively high concentrations of gluten and not at levels stipulated by the Codex Alimentarius Commission (2000) as relevant concentrations ($20\,mg\,kg^{-1}$) to guarantee food safety for sensitive individuals. Another approach to circumvent the problem of excluding various cultivars from the analytical scope is the employment of antibodies specifically detecting the toxic motifs of gliadins as described by Ellis *et al.* 1998.

Presently, there are several sandwich ELISA test kits commercially available. All of them are targeting the gliadin fraction and the tests show sensitivities between 1 and $2\,mg\,kg^{-1}$ (table 1). However, these assays show only weak detection of contaminations from barley (and oat). Monoclonal antibodies with a wider specificity are currently investigated in a collaborative study to overcome this problem (Klein *et al.* 2002). Additionally, a qualitative dipstick assay is commercially available with a detection limit of $10\,mg\,kg^{-1}$ (table 1).

A PCR assay for the detection of residues from gluten containing cereals will be introduced later in 2003 (table 2).

Crustaceans and products thereof

Allergy characteristics. As seafood has gained in popularity, there has been an increase of reported adverse reactions to these foods (O'Neil and Lehrer 1995).

Shrimp represents the most common allergen amongst other crustaceans such as prawn, crab, crawfish and lobster. It is estimated that 0.6–2.8% of allergic individuals suffer from shrimp allergy (Besler *et al.* 2001). Threshold doses for shrimp allergic individuals were 16 g in Daul *et al.* (1988).

Allergenic properties. At least 13 IgE binding proteins have been detected in shrimp meat, however, muscle tropomyosin has been identified to be the only major allergen in shrimp (Lehrer *et al.* 2002). Depending on the shrimp species the molecular weight ranges between 34 and 39 kDa and the proteins are named as Pen a 1 from *Penaeus aztecus* (Daul *et al.* 1994), Pen I 1 from *Penaeus indicus* (Shanti *et al.* 1993), Pen o 1 from *Penaeus orientalis* (Besler *et al.* 2001b), and Met e 1 from *Metapenaeus ensis* (Leung *et al.* 1994). Moreover, crabs may contain unique allergens in the ranges of 5–14 and 25–45 kDa molecular mass (Hefle *et al.* 1995).

Seafood proteins may be carried over during food processing and therefore may not always be labelled appropriately.

Detection methods. An ELISA assay using shrimp tropomyosin specific antibodies was recently developed by Ben Rejeb *et al.* (2002). The detection limit of the test was around $2.5\,mg\,kg^{-1}$. The assay showed significant cross-reactivity to other crustaceans like scampi, lobster, and crab, but demonstrated no cross-reactivity to vertebrate tropomyosin from chicken and pork, even though they are approximately 55% identical to shrimp tropomyosin (Reese *et al.* 1997). Applicability of this immunoassay to determine traces of crustaceans in various food preparations (e.g. soup, frozen prepared meals) was demonstrated (Ben Rejeb *et al.* 2002). Another ELISA assay determining Pen a 1, a major shrimp allergen, was developed by Jeoung *et al.* (1997), to standardize allergen concentrations of shrimp extracts used for clinical in vivo testing. The detectable levels for Pen a 1 ranged between 4 and 125 ng Pen a $1\,ml^{-1}$ extract.

Currently there is only one ELISA test kit commercially available for the detection of crustacean residues in food products. This assay is a sandwich ELISA targeting tropomyosin with a sensitivity of $0.05\,mg\,kg^{-1}$ tropomyosin (table 1).

Eggs and products thereof

Allergy characteristics. Hen's egg is one of the most frequent causes of adverse reactions to foods in children. The prevalence of egg allergy is about 35% in food allergic children and children with atopic dermatitis, respectively (Crespo *et al.* 1995, Resano *et al.* 1998), whereas the frequency of egg allergy in food allergic adults is about 12% (Wüthrich 1993).

Threshold doses between 1 and 200 mg egg (0.13–20 mg egg protein) were determined by several oral challenge studies with egg allergic individuals (Taylor *et al.* 2002). More recently, Hefle *et al.* (2003) described symptoms in an allergic individual after ingestion of 0.03 mg spray dried whole egg.

Allergenic properties. Several allergens have been identified in egg (Langeland 1982, 1983, Hoffman 1983, Anet *et al.* 1985, Holen and Elsayed 1990). The major allergens in egg white are ovomucoid Gal d 1 (28 kDa), ovalbumin Gal d 2 (44 kDa), ovotransferrin Gal d 3 (77 kDa), and lysozyme Gal d 4 (14 kDa). The major allergen in egg yolk is α-livetin, also called chicken albumin, with a molecular mass of 70 kDa. Egg white was shown to be more frequently responsible for allergic reactions in egg-allergic individuals than egg yolk (Wüthrich 1981, Anet *et al.* 1985) with the predominant allergen being ovomucoid (Bernhisel-Broadbent *et al.* 1994, Urisu *et al.* 1997).

Egg or egg components that are potentially allergenic are used in many different food products and may not be easily identified as such, especially when they are listed according to their functions e.g. as binder, emulsifier, or coagulant. Egg may be an ingredient in noodles, or it is used to give pretzels, bagels, and other baked goods their shiny appearance. Other foods that can contain egg are creams, soups, dressings, sauces, processed meat products, breakfast cereals or drinks. Egg derived products such as lecithin (emulsifier), provitamin A (colorant), or lysozyme (preservative) — an allergen per se — are sources of potential allergens for egg-sensitive individuals.

Detection methods. Several methods based on gel-electrophoresis or ELISA have been developed for the detection of egg in food products.

Egg was detected by rocket immuno-electrophoresis in various food products including processed meat products, noodles, chocolate and cake, with a detection limit of $30\,mg\,kg^{-1}$ (Malmheden *et al.* 1994). Leduc *et al.* (1999) compared isoelectric focusing (IEF) gel elelctrophoresis/immunoblotting with a newly developed ELISA involving rabbit anti-hen's egg white antibodies or human IgE sera for the detection of egg residues in meat products. Immunoblotting was able to detect 2% egg-white ingredients in pork meat, while the ELISA assays showed sensitivities between 0.03% ($300\,mg\,kg^{-1}$) for raw and pasteurized egg product and 0.125% ($1.25\,g\,kg^{-1}$) for sterilized egg product.

Recently, a highly sensitive ELISA test for the detection of egg in food products was described by Yeung *et al.* (2000). This assay involved polyclonal antibodies specific to whole egg proteins. The ELISA was applied to ice creams, noodles, pasta, and breads, and showed a detection limit of $0.2\,mg\,kg^{-1}$. Another sensitive sandwich ELISA employing a capture antibody raised against egg white and a detection antibody specific for ovalbumin was described by Hefle *et al.* (2001). The detection limit of this assay was $1\,mg\,kg^{-1}$ when tested on various pasta products.

Sato *et al.* (2001) developed a fully automated chemiluminescence sandwich enzyme immunoassay for lysozyme detection in food using antibody-conjugated bacterial magnetic particles (BMPs). BMPs are extracted from magnetic bacteria that synthesize intracellular magnetic particles (50–100 nm in size), which can be coupled with antibodies that specifically recognize an antigen. The BMP antibody–antigen complexes are separated magnetically and subsequently quantified by an enzyme immunoassay. This system allowed sensitive detection of lysozyme within 20 min.

Most recently, the development of a dipstick immunoassay for the detection of trace amounts of egg proteins in food was published by Baumgartner *et al.* (2002). The assay involved polyclonal antibodies against egg white protein. The dipstick assay rendered qualitative results with a detection limit of $0.02\,mg\,kg^{-1}$.

Additionally, a biosensor-based protocol for the quantitative determination of ovomucoid in food products was presented recently, but to date this test is not available in a kit format (Jonsson 2002).

Several ELISA test kits have become available during recent years (table 1). The quantitative tests are based on sandwich ELISAs involving polyclonal antibodies recognizing total egg protein, egg white protein, or a specific allergen (ovalbumin and/or ovomucoid), respectively, with detection limits ranging between < 1 and $5\,mg\,kg^{-1}$ for the respective test kit. Semiquantitative test devices for rapid screening of food products for the presence of egg are also on the market based on competitive ELISA with a detection limit of $< 2.5\,mg\,kg^{-1}$.

Fish and products thereof

Allergy characteristics. Together with peanuts, tree nuts, and shellfish, fish accounts for the vast majority of severe food anaphylactic reactions in the US (Sampson 2000). In a Spanish study evaluating food allergy in children, fish was the third common food

allergen after eggs and cow's milk (Boyano *et al.* 1987). However, symptoms of fish allergy usually diminish with age (Kayosaari 1982). Skin reactivity seemed to be more evident than symptoms associated with oral consumption. Individuals who are allergic to fish may be able to consume one or more kinds of fish other than the offending species without showing any adverse allergic reactions (Bernhisel-Broadbent *et al.* 1992), however, most fish-allergic people cannot eat other fish (Poulsen *et al.* 2001, Sicherer 2001).

Amounts of a few mg of codfish were found able to provoke allergic reactions (Besler *et al.* 2000). Various other studies determined threshold doses for fish in fish allergic subjects between 5 and 6000 mg (Taylor *et al.* 2002).

Allergenic properties. In fish, the dominating allergen is the homologues of Gad c 1 (12 kDa, Elsayed and Bennich 1975, Elsayed and Apold 1983), also known as allergen M, a parvalbumin from cod fish (*Gadus morhua*). Several other fish species (e.g. salmon, pike, carp, hake and whiting) show strong cross-reactivities, only tuna does not cross react extensively with other species (Bernhisel-Broadbent *et al.* 1992). So far, more than 15 additional allergens have been identified in codfish with molecular weights ranging between 15 and 200 kDa (Besler *et al.* 2000).

Although food processing, such as cooking and canning, can change the allergenic potency of fish allergens, some degree of allergenicity of the product may remain (Besler *et al.* 2001c).

Detection methods. Although several protocols are available to diagnose codfish allergy in sensitized individuals (e.g. skin prick test, RAST), to date there are no specific methods described to quantify fish allergens in food products. However, a radioimmunoassay involving pooled human sera from fish allergic individuals was developed by Taylor *et al.* (2000) to determine air-borne fish allergens in an open-air fish market.

Several competitive ELISA test kit are commercially available which determine fish histamine, which can cause human scombroid poisoning and serve as an indicator for microbial breakdown and elevated temperatures and time abuse of harvested fish. To date no ELISA assays are available for the detection of fish allergens in foods.

Milk and dairy products (including lactose) and products thereof

Allergy characteristics. The prevalence of cow's milk allergy (*Bos domesticus*) ranges from 1.6 to 2.8% in children younger than 2 years of age. Oral tolerance is frequently acquired in about 50–90% of children with cow's milk allergy within the first 6 years of life. However, severe allergy may persist into adulthood. Due to the high homology of protein composition sheep's (*Ovis* ssp.) and goat's milk (*Capra* ssp.) are cross-reactive in approximately 80% of subjects with cow's milk allergy (Besler *et al.* 2002a).

Allergenic properties. The major cow's milk allergens are caseins (Bos d 8, 20–30 kDa), and whey proteins β-lactoglobulin (Bos d 5, 18 kDa), and α-lactalbumin (Bos d 4, 14 kDa), and bovine serum albumin (Bos d 6, 67 kDa) and bovine immunoglobulins (Bleumink and Young 1968, Docena *et al.* 1996). Apparently, cow's milk allergens are rather stable and they retain their allergenicity after common industrial treatments (Host and Samuelsson 1988, Jedrychowski 1999).

Cow's milk allergens could be present in breast milk, infant formulas, milk and dairy products like cheese and yoghurt, as well as in 'non-dairy' food occurring as contaminants or unlabelled additives. Milk and products thereof may be found in a large variety of processed food, including confections, margarine, pies, cookies, pudding, sausage, sauces and soups. Extracted milk proteins are used as emulsifiers or foreign protein source.

Detection methods. Several methods for the determination of milk in food products have been published, most of them were developed primarily to distinguish cow's milk from sheep's or goat's milk (Anguita *et al.* 1997, Plath *et al.* 1997, Negroni *et al.* 1988), or to determine residual allergens in hypoallergenic infant formulae or milk substitutes (Niggemann *et al.* 1999, Docena *et al.* 2002). Moreover, RAST (Fremont *et al.* 1996), HPLC (Garcia *et al.* 1998), SDS-PAGE (Malmheden Yman *et al.* 1994, Molina *et al.* 1998), and ELISA (Mäkinen-Kiljunen and Palosuo 1992, Mariager *et al.* 1994, Plebani *et al.* 1997) have been employed to detect milk in food products.

A competitive ELISA developed for the detection of bovine milk in ovine and caprine milk and cheese

allowed the detection of 0.5–25% substitution (Anguita *et al*. 1997). This assay used monoclonal antibodies against bovine β-casein. Negroni *et al*. (1988) described two sandwich immunoassays employing pairs of monoclonal antibodies specific against native and denatured β-lactoglobulin, respectively. The detection limits were $0.03\,\mu g\,l^{-1}$ for native and $0.2\,\mu g\,l^{-1}$ for reduced β-lactoglobulin.

Niggemann *et al*. (1999) evaluated various in vivo and *in vitro* methods for determination of residual allergenicity of partially hydrolysed infant formulae, including oral provocation tests, skin prick test, EAST and RAST inhibition. All of these tested methods were found suitable for the quantification of residual allergenicity. A most recent study by Docena *et al*. (2002) employed *in vitro* methods including ELISA, EAST and immunoblotting to quantify residual allergenicity in a hypoallergenic infant formula.

RAST inhibition test was employed by Fremont *et al*. (1996) to identify masked α-lactalbumin in baby-food cereal flour at concentrations between 1 and $5\,mg\,kg^{-1}$.

A perfusion reversed-phase high-performance liquid chromatographic method was developed by Garcia *et al*. (1998) simultaneously to separate soybean and bovine whey proteins (α-lactalbumin and β-lactoglobulin), which achieved results in a very short analysis time. However, the method was not able to detect milk residues lower than 1%.

Malmheden Yman *et al*. (1994) detected milk proteins in various food products by rocket immuno-electrophoresis with a sensitivity of $30\,mg\,kg^{-1}$. Molina *et al*. (1998) found bovine milk proteins down to levels of 0.1% in soy protein by employing SDS-PAGE and immunoblotting (identifying α-lactalbumin and β-lactoglobulin).

Mäkinen-Kiljunen and Palosuo (1992) developed a sandwich ELISA involving anti-β-lactoglobulin sera from rabbits. This assay showed a limit of detection of $0.002\,\mu g\,l^{-1}$. A competitive ELISA was developed by Mariager *et al*. (1994). A polyclonal rabbit antibody against heat treated was compared with a monoclonal mouse antibody against native β-lactoglobulin. Sensitivities were 0.08 and $3.2\,\mu g\,l^{-1}$ and measuring ranges were 0.1–1000 and $4–50\,ng\,ml^{-1}$ for the polyclonal and the monoclonal antibodies, respectively. This study gave clear preference to the employment of polyclonal antibodies for competitive ELISAs to be used for allergen detection in food products. These findings were confirmed by Plebani *et al*. (1997), who evaluated the use of monoclonal and polyclonal antibodies against casein components of cow milk for determination of residual antigenic activity in hypoallergenic infant formulas.

Biosensors have also been employed to determine β-lactoglobulin in food with a detection limit of $< 10\,mg\,kg^{-1}$ (Jonsson 2002).

Methods for the detection of cow's DNA in food products and for the discrimination of milk from various animal sources have been published (Plath *et al*. 1997), however, their applicability for the detection of the presence of potential milk allergens is questionable, as many products contain only concentrated or purified milk fractions such as milk protein or milk fat.

Several methods for the detection of milk residues in food products have become commercially available in kit formats during the past few years (table 1). Most test kits for the determination of milk residues in food are based on competitive ELISA. They detect either bovine serum albumin, casein, β-lactoglobulin, or unspecified whey proteins, respectively, with detection limits between < 2.5 and $< 5\,mg\,kg^{-1}$. In addition, sandwich-type ELISA test kits for the determination of milk residues (targeting β-lactoglobulin) in food products are on the market with sensitivities down to $1\,mg\,kg^{-1}$. Another qualitative rapid screening test detects casein with a sensitivity of $< 2.5\,mg\,kg^{-1}$.

One PCR-based test kit for the detection of bovine milk DNA was announced to be placed on the market in 2003 with a detection limit < 10 ppm (table 2).

Mustard and products thereof

Allergy characteristics. Although mustard is frequently consumed, only isolated cases of mustard allergy have been reported (Caballero *et al*. 2002). However, the prevalence of mustard allergy is increasing (Monsalve *et al*. 2001). According to a French study, mustard accounts for 3% of all food induced anaphylactic reactions (Andre *et al*. 1994). A threshold dose of 1 mg mustard (0.3 mg mustard protein) was sufficient to provoke adverse reactions in mustard allergic subjects (Rance 2003).

Allergenic properties. The major allergens of mustard, named Sin a 1 (Menendez-Arias *et al*. 1988) in

yellow mustard and Bra j 1 (Gonzales de la Pena *et al.* 1991) in oriental mustard, have a molecular weight of about 14 and 16 kDa, respectively, and belong to the 2S albumin class of seed storage proteins.

Table mustard is usually a blend of flour obtained from the seeds of two different species: yellow (*Sinapis alba*) and oriental (*Brassica juncea*) mustard. Mustard is mainly used in the meat packing industry as an aid to flavour, emulsification, water binding, and seasoning of processed meats. It is also used for pickles and as an essential ingredient in mayonnaise, salad dressings, relishes and many sauces. Some mustard is refined to oil and spices (Golz 1993).

Detection methods. To date no methods for the specific detection of mustard in food products have been published.

Nuts and nut products

The terms 'nuts' or 'tree nuts' refer to shell (nut) fruits of various botanical families including almond, brazil nut, cashew nut, hazelnut, pecan nut, pistachio, and walnut. Peanut and/or tree-nut allergy affects approximately 1.1% of the general population in the USA (Sicherer *et al.* 1999), with walnut being the most frequently reported tree-nut allergy in 34% of respondents, followed by cashew (20%), almond (15%), pecan (9%), pistachio (7%), and others at less than 5% each (Sicherer *et al.* 2001). In Europe, hazelnut has earned most attention as trigger for atopic reactions in tree-nut allergic individuals (Groot *et al.* 1996). However, a recent UK study found allergy to Brazil nuts most prevalent with 15% of peanut and tree-nut allergic individuals, followed by hazelnut (6%), almond (4%) and walnut (4%) (Ewan and Clark 2001).

Almond (Prunus dulcis)

Allergenic properties. Almond major protein (AMP or amandin) is the major allergen recognized by almond-allergic patients (Sathe *et al.* 2001). Moreover, the plant-pan allergens profilin and a lipid-transfer protein are also present in almonds (Besler *et al.* 2001b, Poltronieri *et al.* 2002).

Almonds are used in various forms (whole, chopped, sliced, or paste) predominantly in bakery products such as cookies, cakes, and pies as well as in confectionery products. Moreover, almonds are a source of gourmet edible oils that potentially contain residual protein.

Detection methods. A very sensitive protocol for the detection of almond proteins in chocolates was described using SDS-PAGE/immunoblot with a chemiluminescence detection method (Scheibe *et al.* 2001). The limit of detection for the procedure was $5\,mg\,kg^{-1}$.

Hlywka *et al.* (2000) developed a sandwich ELISA involving antisera from rabbit and sheep as capture and secondary antibodies, respectively. The assay had a detection limit of less than $1\,mg\,kg^{-1}$, however, it showed also significant cross-reactivity to several nuts and sesame seed. More recently, a competitive almond ELISA was developed by Roux *et al.* (2001). This assay involved polyclonal rabbit antisera recognizing amandin. The ELISA showed minor cross-reactivity to some globulins and albumins from other nuts and legumes, and the limit of detection was less than $5\,mg\,kg^{-1}$ almond in various food products.

Only recently an almond ELISA test kit was placed on the market. The kit is available in two formats: a quantitative assay with a sensitivity of $< 2.5\,mg\,kg^{-1}$, and a qualitative screening test including a reference at $5\,mg\,kg^{-1}$ (table 1). Another almond detection kit based on a PCR protocol is offered either as PCR-ELISA or as real-time PCR with a sensitivity of less than $10\,mg\,kg^{-1}$ (table 2).

Brazil nut (Bertholletia excelsa)

Allergenic properties. The 2S albumin was identified as the major allergen from Brazil nut (Nordlee *et al.* 1996, Pastorello *et al.* 1998) and it constitutes about 30% of the total protein content (Sun *et al.* 1987).

Brazil nut is used as a minor ingredient in processed foods, such as cookies, cakes, and confectionary products (Clemente *et al.* 2003).

Detection methods. Most recently, an indirect competitive ELISA was developed by Clemente *et al.* (2003). The assay was based upon the detection of the abundant 2S protein and showed a detection limit of $1\,mg\,kg^{-1}$ for both raw and roasted Brazil nuts in a range of food matrices.

Cashew nut (Anacardium occidentale)

Allergenic properties. The major allergens in cashew nuts are anacardein (cashew major protein, CMP, a 13S legumin-like protein), which accounts for about 50% of the total soluble seed protein, and 2S albumin (Teuber *et al*. 2002). Another minor allergen of about 50 kDa was designated Ana o 1 (Wang *et al*. 2002).

Cashew nuts are widely used in snack foods and as an ingredient in a variety of processed foods, such as bakery and confectionary products (Wang *et al*. 2002).

Detection methods. Recently Wei *et al*. (2003) published the development of a sandwich enzyme-linked immunosorbent (ELISA) to detect the predominant cashew protein fraction (anacardein or cashew major protein, CMP). Protein G-purified goat antiwhole cashew extract IgG and rabbit anti-CMP IgG were used as capture and secondary antibodies, respectively. The assay was optimized to detect as little as $0.02\,mg\,kg^{-1}$ of CMP and was successfully used to quantify cashew in various food matrixes.

Hazelnut (Corylus avellana)

Allergy characteristics. In Europe hazelnut allergy has earned major interest due to its high prevalence 0.1–0.5% of the general population in Europe was estimated (Groot *et al*. 1996). Hazelnut allergy is often associated with allergy to pollens from trees like birch, alder, hazel, hornbeam, and oak, often causing oral allergy syndrome (OAS). Prevalence of IgE-mediated adverse reactions to hazelnut in tree pollen allergic patients is approximately 50–70% (Groot *et al*. 1996). There is also important non-pollen related hazelnut allergy and anaphylaxis has been reported (Ewan 1996, Schocker *et al*. 2000, Pastorello *et al*. 2002).

In a recent study by Wensing *et al*. (2002), threshold doses for eliciting adverse reactions varied from a dose of 1–1000 mg hazelnut protein (equivalent to 6.4–6400 mg hazelnut meal).

Allergenic properties. The major hazelnut allergen is Cor a 1 (18 kDa), which was found to be cross-reactive with Bet v 1 from birch pollen. Up to now four isoforms of Cor a 1 (Schenk *et al*. 1994) were identified in hazel pollen (Cor a 1.01 to Cor a 1.03) and hazelnuts (Cor a 1.04). A 14 kDa hazelnut allergen showed cross-reactivity to birch profilin (Bet v 2). Other allergens not related to pollens were recently identified as Cor a 9 (40 kDa) and a 9 kDa lipid transfer protein (Schocker *et al*. 1999, Beyer *et al*. 2002b, Pastorello *et al*. 2002).

Hazelnut is often used as a food ingredient in pastry, confectionary products and ice cream. Hazelnuts are also processed to oils. Undeclared hazelnut might be present as cross-contaminant in cookies, muesli bars, or nut-free chocolate products (Holzhauser and Vieths 1999b).

Detection methods. Various techniques have been described to detect and quantify hazelnut in food products, including methods based on gel electrophoresis, ELISA, and PCR.

Rocket immuno-electrophoresis was used by Malmheden Yman *et al*. (1994) to determine the hazelnut content in various food products. The detection limit of this method was $30\,mg\,kg^{-1}$. Koppelman *et al*. (1999) performed an SDS-PAGE/immunoblot with human IgE sera and with polyclonal antisera from rabbits, respectively. Hazelnut concentrations between 0.3 and 10% depending on the food product were detectable. More recently, Scheibe *et al*. (2001) achieved a sensitivity of $5\,mg\,kg^{-1}$ by employing an SDS-PAGE/immunoblot and a chemiluminescence detection system for the qualitative detection of almond and hazelnut proteins in chocolates.

A competitive ELISA involving human IgE sera was performed by Koppelman *et al*. (1999) with a sensitivity of $1\,mg\,kg^{-1}$. Recoveries from chocolate, cookies, and cake ranged from 67 to 132%, however, significant cross-reactivity for several nuts and peanut were observed. While retaining the same sensitivity of $1\,mg\,kg^{-1}$, the specificity of the hazelnut ELISA could be increased showing hardly any cross-reactivity by designing a sandwich ELISA using polyclonal antisera from rabbits (Koppelman *et al*. 1999). Holzhauser and Vieths (1999b) published another sandwich ELISA involving polyclonal antisera from rabbit and sheep as capture and secondary antibodies, respectively. This assay was tested on chocolate and products thereof and on muesli; it performed well down to a detection limit of $2\,mg\,kg^{-1}$. A very sensitive hazelnut ELISA involving egg yolk antibodies was published by Blais and Phillippe (2001). Polyclonal egg yolk derived IgY antisera were used for both capture and secondary antibodies and specific detection of hazelnut proteins in a large variety

of food products was feasible with detection limits ranging from 0.12 to 1 mg kg^{-1}, depending on the food product analysed. The use of antibodies isolated from egg yolk represents an inexpensive and convenient alternative to the commonly used immuno-reagents raised in animals and harvested from blood.

Most recently, a competitive hazelnut ELISA was developed by Ben Rejeb *et al.* (2003) by using polyclonal antibodies generated against a protein extract of roasted hazelnut. No cross-reactivity was observed in tests against 39 commodities, including many common allergens, tree nuts, and legumes. An extraction and quantification method was developed and optimized for chocolate, cookies, breakfast cereals, and ice cream, which allowed detection of 1 mg kg^{-1} hazelnut protein.

A dipstick immunoassay for the determination of hazelnut in processed food was recently developed by Stephan *et al.* (2002). Ten mg kg^{-1} hazelnut were reliably detectable in milk chocolate and semisweet chocolate. Even lower quantities of hazelnut were detectable in various commercial food products including chocolate products, cereals, and cookies, when the performance was compared with a previously developed hazelnut ELISA (Holzhauser and Vieths 1999b).

A Biosensor-based protocol was presented recently that detected the major hazelnut allergen corylin in food products at a detection limit of less than 10 mg kg^{-1} (Jonsson 2002).

A DNA-based method for the detection of hazelnut residues in food was developed based on a PCR assay amplifying a 182 bp fragment of the major hazelnut allergen Cor a 1.0401 that was able to detect < 10 mg kg^{-1} hazelnut in various complex food matrices (Holzhauser *et al.* 2000). When compared with positive results obtained by employing a hazelnut ELISA, the PCR assay could also detect hazelnut quantities < 2 mg kg^{-1}, therefore being in the range of the limit of quantification of the ELISA. More recently, Holzhauser *et al.* (2002) improved the hazelnut PCR assay and developed a PCR-ELISA system that detects a 152 bp fragment of the Cor a 1.0401 gene. The modified assay avoided the formation of artefacts during PCR amplification and thus increased the sensitivity of the system. The DNA-based PCR-ELISA was shown able to detect as little as 4 mg kg^{-1} of hazelnut in various food products.

Currently, there is one sandwich ELISA and one PCR-based method in a kit format commercially available (tables 1 and 2). The ELISA involves antibodies recognizing hazelnut proteins in various food matrices with a limit of detection of 10 mg kg^{-1}. A slightly better sensitivity is achieved by the PCR method detecting a 152 bp fragment of the Cor a 1.0401 gene. The PCR assay is offered in two different formats: as real-time PCR and as PCR-ELISA.

Walnut (Juglans regia) *and other nuts*

Allergenic properties. Major walnut allergens are 2S albumin Jug r 1 (15–16 kDa) and vicilin Jug r 2 (44 kDa) (Besler *et al.* 2001c).

Detection methods. A sandwich ELISA for the detection of undeclared walnut residues in food was recently presented by Niemann and Hefle (2003). The walnut ELISA had a detection limit of 1 mg kg^{-1} walnut. The assay showed some cross-reactivity with hazelnut, pecan, and sesame seed, but it did not cross-react with any of the other 50 food and food ingredients tested.

Peanuts and products thereof

Allergy characteristics. Among all other food allergies peanut allergy has earned the greatest attention in the medical and food production communities because of the high frequency and the severity of adverse reactions in sensitized individuals and because of being responsible for the greatest number of deaths the ubiquity of peanut products. Epidemiological studies of the general population estimate a prevalence rate of 0.5% in the UK (Emmett *et al.* 1999) and 0.6% in the USA (Sicherer *et al.* 1999); peanut allergy accounts for 10–47% of food-induced anaphylactic reactions and for more than 50% of food allergy fatalities (Bock *et al.* 2001).

Threshold doses for peanut allergic reactions have been found to range from as low as 100 μg up to 1 g of peanut protein (Hourihane *et al.* 1997, Wensing *et al.* 2002), which equal about 400 μg to 4 g peanut meal.

Although peanut shares cross-reacting proteins with other legumes (e.g. soybean, pea), clinical cross-reactivity is not common. Allergy to peanut most commonly occurs in atopic individuals who may have

other food allergies (e.g. egg, milk, tree nut), but there are no known clinically relevant cross-reacting proteins with tree nuts (e.g. hazelnut, walnut). In most cases peanut allergy persists through lifetime and may increase over time (Sicherer 2002).

Allergenic properties. Allergens from peanut (*Arachis hypogaea*) are seed storage proteins composed of two major globulin families, arachin (legumin) and conarachin (vicilin) (Jones and Horn 1930, Krishna *et al*. 1986). The major allergens in peanut (Burks *et al*. 1998, Koppelman *et al*. 2001) are Ara h 1 (vicilin, 63.5 kDa) and Ara h 2 (17 kDa). Further relevant allergens contained in peanuts are Ara h 3 and Ara h 4, which are glycinin proteins with a molecular mass of about 60 kDa and 14 kDa, respectively, and the minor allergens Ara h 6 and Ara h 7 (both conglutin-homologue proteins) as well as the pan-allergen profilin Ara h 5 (Kleber-Janke *et al*. 1999).

Some of the most popular commercially grown peanut types include Runner, Virginia, Spanish, Valencia, and Chinese (Sholar *et al*. 1995, personal communications). The major part of the global peanut production enters the food market as salted peanuts, as peanut butter, in confectionery (chocolates, biscuits, etc.), or further processed for oil. Other peanut containing food products include breakfast cereals, ice cream, peanut flour and peanut milk. Peanuts are also used as supplemental protein source in a wide variety of food and may be an ingredient in highly processed, refined products, such as syrups, sauces, soups, etc. (Singh and Singh 1991). However, due to improper labelling or contamination during food processing traces of peanut may also be present in food supposed to be free of peanuts, which can pose a potential risk for peanut allergic individuals (Koppelmann *et al*. 1996).

It was shown that a level of 100 µg orally administered peanut protein were capable of triggering mild subjective reactions in peanut allergic individuals (Hourihane *et al*. 1997, Wensing *et al*. 2002). Thus, the consumption of 100 g of a food product containing 1 mg kg^{-1} peanut protein (about 4 mg peanut meal) could cause an allergic reaction in a sensitized person.

Detection methods. Several analytical techniques have been employed for the detection of hidden peanut proteins/allergens in food including immunoblotting, rocket immuno-electrophoresis (RIE), radioimmunoassay (RIA), radioallergosorbent assay (RAST), and enzyme-linked immunosorbent ELISA. Furthermore, several peanut kits based on ELISA or PCR are commercially available (tables 1 and 2).

RAST was employed by Yunginger *et al*. (1983) to determine peanut butter contamination in sunflower butter ranging from 0.3 to 3.3%.

Recently, several dot immunoblotting applications were described for the detection of peanut in various food including chocolate products, cookies and ice cream (Blais and Philippe 2000, Schäppi *et al*. 2002) with a detection limit down to 2.5 mg kg^{-1}.

Rocket immuno-electrophoresis was employed by Malmheden Yman *et al*. (1994) to detect the presence of undeclared peanuts in various food products with a detection limit of 30 mg kg^{-1}. The sensitivity could be improved to 2.5 mg kg^{-1} by using an enzyme-labelled IgG antibody based system rather than Coomassie brilliant blue for staining the gel (Holzhauser *et al*. 1998).

Keating *et al*. (1990) established a solid-phase radioimmuno inhibition assay (RIA) using pooled sera from five peanut-sensitive patients, which provided the first method for monitoring finished products for potential peanut allergens. The assay was highly specific for peanut allergens with a sensitivity of 0.00875% (w/w), which translated to 87.5 mg peanut kg^{-1} food. The use of radioisotopes and the requirement of human blood sera limited the applicability of RIA test formats and they were not useful for most food industries.

The first enzyme-linked immunosorbent assay (ELISA) for the detection of peanut proteins in food was developed by Hefle *et al*. (1994). It was a sandwich ELISA with immobilized monoclonal antibodies against several selected peanut proteins with molecular weights in the range between 14 and 44 kDa as capture antibody and a polyclonal rabbit antiserum, which was raised against a crude peanut extract, as detector. This method was able to detect peanut protein in various food with a detection limit $\leqslant 40$ mg kg^{-1}, which was well above the level of a positive skin prick test result determined by Hefle *et al*. (1994) in a parallel study with seven peanut-sensitive adults. The obtained results were also compared with radio-allergosorbent inhibition assay (RAST), which appeared to be more sensitive. However, ELISA and RAST results were proportional and showed a correlation coefficient $r^2 = 0.85$. This newly introduced method opened the door for the development of several other ELISA formats with increased sensitivity

for peanut protein/allergen detection in food by using amplification systems, improved monoclonal and/or polyclonal antibodies, and optimized extraction protocols to reduce food matrix interference problems.

During the following years several immunochemical assays for peanut detection in food were published: Yeung and Collins (1996) developed a competitive ELISA with polyclonal antibodies against a crude protein extract of roasted peanuts. When testing various food matrices recoveries of peanut protein ranged from 68 to 90% depending on the commodity and the detection limit was 0.4 mg peanut protein kg^{-1} food product. Holzhauser and Vieths (1999a) developed an indirect competitive ELISA with a commercially available peanut-specific polyclonal antiserum, which had been raised against native peanut protein. This assay allowed detection of hidden peanut residues down to 2 mg kg^{-1}, recognizing both native and roasted peanuts. In addition, Holzhauser and Vieths provided extensive validation data for the introduced assay. Koppelman *et al.* (1996) developed a direct sandwich ELISA using polyclonal antibodies raised against partially purified Ara h 1. This assay could measure different peanut species, either fried or raw, with recoveries between 35 and 75% and a detection limit of about 0.1 mg peanut kg^{-1} processed food. Pomes *et al.* (2003) published another two-site antibody-based ELISA employing monoclonal antibodies against purified Ara h 1. This very specific assay showed a comparable detection limit, however, Ara h 1 could not always be recovered in spiked chocolate samples.

Another approach by Newsome and Abbott (1999) combined the use of immunoaffinity chromatography and ELISA to improve sensitivity for the detection of peanut proteins in chocolate. Recoveries ranged between 72–84% with a detection limit of 0.1 mg kg^{-1}.

Dipstick assays are also based on the ELISA technology, but they are only semiquantitative and they allow fast and cost-effective screening of food. Several dipstick assays for peanut detection in food have been described in the literature. Mills *et al.* (1997) used polyclonal rabbit sera against purified conarachin, the 7S globulin of peanut to develop a rapid dipstick immunoassay. The dipstick sandwich ELISA was able to detect peanut in various food such as marzipan and chocolate at concentration levels of 100 and 1000 mg kg^{-1}, respectively. Although this dipstick ELISA offered a rapid and easy-to-use format, it lacked sufficient sensitivity for allergen control in the food industry. Moreover, Stephan *et al.* (2002) published the development and validation of a dipstick sandwich immunoassay for peanut determination with a sensitivity of 1 mg kg^{-1}. This assay was validated against a peanut ELISA that had been developed by Holzhauser and Vieths (1999a) and showed similar detectability of peanut in various food products. Currently, there are no dip-stick assays for peanut commercially available, however at least one such assay was announced to be placed on the market shortly (table 1).

In recent years, several ELISA kits for peanut determination in food have been placed on the market (table 1). Depending on the offered format, they yield semiquantitative or quantitative results. Main differences between the offered kits are the target (Ara h 1, Ara h 2, selected proteins, or a crude peanut protein extract), the extraction procedure (sample preparation, extraction buffer, incubation time and temperature), the detection limit (between < 0.1 and < 2.5 mg kg^{-1}), the time of analysis (between 30 min and 3.5 h), and the costs (Poms and Anklam 2003). Drawbacks in the performance of the currently available commercial peanut ELISA kits are the impaired recovery of peanut from highly processed material and the comparability of results between kits. Several recently performed studies showed that the commercial ELISA kits tested were suitable for qualitative screening for most products, but yielded significantly varying results in quantitative assays (Hurst *et al.* 2002, Koch *et al.* 2003, Poms *et al.* 2003, Poms and Anklam unpublished). These findings may be due to the fact that the detection and in particular the quantification of peanut allergens in food products can be impaired by food matrix interactions (e.g. tannins in chocolate), reduced solubility of heat denatured proteins (e.g. variable temperature/time profiles for oil or dry roasting of peanuts), differences in antibody affinity/recognition of peanut proteins form different species and geographical origin (Keck-Gassenmeier *et al.* 1999, Hischenhuber 2001, Hurst *et al.* 2002, Poms and Anklam unpublished). Sensitivity of the currently available test kits is around 1 mg kg^{-1}. Taking into account that as little as 100 μg of orally administered peanut protein (equals about 400 μg whole peanut) were found to trigger a mild reaction in a peanut allergic person (Hourihane *et al.* 1997, Wensing *et al.* 2002), this seems to be a useful detection limit. However, data about established threshold levels that have been determined in human oral challenge studies are scarce, and it may become necessary to revise the required

sensitivities of peanut ELISA kits, when more data on threshold levels will be available.

In addition to immunochemical methods to detect peanut antigens, DNA-based techniques have been established for determination of the presence of peanut in food products. Only recently, Hird *et al.* (2003) published a real-time PCR protocol for the detection of peanut traces in food products. The method amplifies a specific fragment of the *Ara h 2* gene, which encodes a major peanut allergen. The method was in-house validated against a large variety of food matrices and showed a detection limit of 2 ppm in spiked cookies.

PCR-based test kits for peanut detection in food are becoming increasingly available. There are two test formats on the market: DNA-ELISA and real-time PCR, which are offered as qualitative kits with detection limits below $10\,mg\,kg^{-1}$ (table 2).

Currently, there is no information available on the specific DNA fragments that are targeted in the offered assays.

Multiplex assays for peanut and tree-nuts

Ben Rejeb *et al.* (2002) presented a semiquantitative multiscreening immunoassay for the detection of peanut, hazelnut, almond, cashew and brazil nuts in chocolate in one single run. The cut off level detected with this assay was $2\,mg\,kg^{-1}$ of protein for each ingredient.

Another very sensitive multiplex enzyme immunoassay system was recently published by Blais *et al.* (2003). This assay used a reverse dot blot format in which egg yolk antibodies (IgY) specific for peanut, hazelnut, and Brazil nut, respectively, were immobilized in discrete spots on a strip of polyester cloth. The detection limits for the individual allergenic foods in various food matrices ranged between 0.1 and $1\,mg\,kg^{-1}$ (hazelnut: 0.1–$1\,mg\,kg^{-1}$, Brazil nut: 0.1–$0.5\,mg\,kg^{-1}$, peanut $0.1\,mg\,kg^{-1}$).

Sesame seeds and products thereof

Allergy characteristics. Representing less than 1% of all food allergy cases sesame seed (*Sesamum indicum*) and sesame seed oil have been thought of as rare causes of food allergy, however, anaphylactic reactions after inadvertent consumption of sesame have been reported frequently (Malish *et al.* 1981, Chiu and Haydik 1991, Kagi and Wüthrich 1993, Asero *et al.* 1999, Pajno *et al.* 2000) and severe allergic reactions to sesame are increasing in frequency, which is probably due to the growing use of sesame seeds or sesame oil in food (Kanny *et al.* 1996, Fremont *et al.* 2002).

Allergenic properties. White sesame seeds contain at least 10 allergenic proteins (Beyer *et al.* 2002a, Fremont *et al.* 2002), one of them has been identified as the major sesame allergen: 2 S albumin (Pastorello *et al.* 2001, Beyer *et al.* 2002a, Wolff *et al.* 2003).

In the food industry, sesame seeds are used as whole seeds or for the production of sesame paste and oil. Sesame containing products include salad dressings, confections and various fast food bakery items. Sesame is also used increasingly in vegetarian food (vegetarian burgers) and gluten-free foods (e.g. bread, cakes, pastries, and biscuits) used to treat celiac disease (Kagi and Wüthrich 1993, Pajno *et al.* 2000).

Detection methods. An immunoassay for sesame proteins was developed by Brett *et al.* (1998), but there was no validation data available.

Currently, there are only a few sandwich ELISA test kits for the detection of sesame in food products commercially available (table 1). The assays either target total soluble sesame protein or a 2S albumin, they have sensitivities of <0.1 and $<1\,mg\,kg^{-1}$, respectively.

Soybeans and products thereof

Allergy characteristics. Soybean allergy is less prevalent than peanut allergy in the food allergic population (Bock and Atkins 1990, Sicherer *et al.* 2000), however, reactions may occur to very small quantities of soy protein, and anaphylaxis to soybean has been reported (Sampson *et al.* 1992, Foucard and Malmheden Yman 1999, Sicherer *et al.* 2000).

Allergenic properties. Major allergens from soybean include the seed storage proteins Gly m Bd 30K (30 kDa, also called P34 or oil body-associated protein), the glycinin acidic chain (40 kDa), and the 70 kDa α-subunit of β-conglycinin (Ogawa *et al.* 1993, 1995, Zeece *et al.* 1999, Helm *et al.* 2000). Additional allergens are the 14 kDa profiling (Gly m 3)

and the 20 kDa Kunitz-trypsin inhibitor (Rihs *et al.* 1999). Moreover, several inhalative soybean shell allergens exist like the Gly m 1 and Gly m 2 with molecular masses of 7 and 8 kDa, respectively (Gonzalez *et al.* 1992, Codina *et al.* 1997).

Because of the almost unlimited uses of soy (e.g. as a texturizer, emulsifier, or protein filler), it is a particularly insidious hidden allergen (e.g. in pastries, bakery products, infant food, sausages, processed meats, and hamburgers). Soy is widely used in Asian food products, it is also used as substitute for meat or milk protein in various food products. In addition, many food products contain soy lecithin and soy oil, which may exhibit allergenicity (Awazuhara *et al.* 1998, Paschke *et al.* 2001).

Detection methods. Various methods have been applied to detect soybean in food products. Several methods based on electrophoretic separation coupled with immunological detection have been developed primarily to identify soy proteins in meat products (Catsimpoolas and Leuthner 1969). Sensitivities of these procedures were usually in the $g\,kg^{-1}$ range. Janssen *et al.* (1986) presented a more sensitive protocol to determine soy in meat products by SDS-PAGE/immunoblotting and dot blotting. This procedure achieved a detection limit of 0.02% ($200\,mg\,kg^{-1}$) soy in meat.

SDS-capillary electrophoresis was applied to detect soy protein in milk powder, however, a detection limit lower than 10% corresponding to $100\,g\,kg^{-1}$ could not be achieved (Lopez-Tapia *et al.* 1999).

Several immunochemical assays have been developed for the detection of soy allergens in food products. Most of them were originally designed to determine adulteration of meat products with soy protein (Hitchcock *et al.* 1981, Griffiths *et al.* 1984, Rittenburg *et al.* 1987, Yasumoto *et al.* 1990, Macedo-Silva *et al.* 2001). These protocols are lacking sufficient sensitivity for the control of soy traces in food products below the $g\,kg^{-1}$ range. Porras *et al.* (1985) developed an ELISA for the detection of soy protein in soy lecithin, margarine and soy oil, with a sensitivity between 100 and $200\,mg\,kg^{-1}$.

To evaluate (residual) allergenicity of soy products several ELISA methods based on the detection of various immunogenic soy proteins were employed. Heppell *et al.* (1987) selected glycinin and β-conglycinin to determine allergenicity in soybean-based infant formulae. However, levels of these proteins below the detection limit of the employed ELISA could still evoke an immune response in various animal models. Brandon *et al.* (1991) developed ELISA approaches using monoclonal antibodies against the Kunitz and the Bowman-Birk trypsin inhibitors. This assay was able to measure low levels of these proteins in processed food. Tsuji *et al.* (1995) and Bando *et al.* (1998) measured two soy allergens by sandwich ELISA (Gly m Bd 30K and Gly m Bd 28K, respectively) with monoclonal antibodies for both the immobilized and the capturing antibodies. These assays were developed for soy products and processed foods that contain soybean protein isolates. Soy protein was detected in various food products within the range $140–700\,mg\,kg^{-1}$, but could not be detected in fermented products.

Only a few ELISA test kits for soya detection in food are commercially available at the moment (table 1). However, the sensitivities are usually not much lower than $0.1–1\,g\,kg^{-1}$, which is not sufficient for insuring safety of food products for soy sensitive individuals (Meyer *et al.* 1996). However, most recently a new ELISA kit was placed on the market with an attested detection limit of $< 1\,mg\,kg^{-1}$ (table 1). This assay is based on a sandwich ELISA determining the soy trypsin inhibitor in the food sample.

Moreover, sensitivities of DNA-based methods generally show lower detection limits in the $mg\,kg^{-1}$ range. Meyer *et al.* (1996) presented a PCR assay for the detection of soy in processed meat products by designing a nested PCR protocol for the amplification of specific 414 and 118 bp fragments, respectively, of the soy lectin gene. The PCR protocol detected less than 1% soy hydrolysate and 0.01% soy suspension in meat ($100\,mg\,kg^{-1}$). A recent study showed that the detection limit for soy DNA in milk and some dairy products could be increased a 100-fold by analysing a larger sample size (Poms *et al.* 2001). Furthermore, several protocols for quantitative detection of soy DNA in food by real-time PCR were published, mainly with the aim to improve methodology for determining genetically modified soy constituents in food. Detection limits for PCR-based methods were found to be between 0.0001 and 0.1% ($1–1000\,mg\,kg^{-1}$) depending on the food matrix and the DNA extraction procedure employed (Hörtner 1999, Terry *et al.* 2002). Currently, two test kit formats based on the amplification of a specific soy DNA fragment (detailed information not available) are on the market: a qualitative PCR-ELISA and a semiquantitative real-time PCR kit. Both offer highly

specific detection of soy in a variety of food products with detection limits below 10 mg kg^{-1} (table 2).

Soy DNA was also detected by employing biospecific interaction analysis based on biosensor technologies (Feriotto *et al.* 2002). This procedure used single stranded biotinylated oligonucleotides containing soybean lectin gene sequences, which were immobilized on a chip.

Conclusion

Food allergy is an important health problem and the prevalence of allergic reactions to foods has been on the rise in recent years. Currently the only effective treatment is total avoidance of the offending food. International and national Organizations have reacted to growing consumer concerns and have stipulated a comprehensive labelling policy for processed foods, including an explicit declaration of allergenic ingredients. Major attention needs to be paid to the problem of hidden allergens, which make their way into the food product via unintended routes, such as misformulation, improper clean up, and cross-contamination by dust or by pieces of an allergen remaining in the processing system (Deibel *et al.* 1997). Only in a joint effort of the food industry and governmental food safety control agencies the safety of food products for all — including the allergic — consumers can be assured.

This review shows that many methods have been published to date to detect most of the allergenic foods accounting for the majority of adverse reactions in food allergic individuals. Some methods have become commercially available in kit formats, which make an easy and rapid quality/safety check feasible. However, analytical methods that can be applied for detection of potential allergens in food products do not exist for all food allergens, such as for mustard and celery. Sensitivity and specificity of the existing methods are another important issues. Detection limits need to be at least in the range of established allergic threshold levels determined by oral food challenge studies — or maybe even a magnitude lower (Koppelman *et al.* 1996) — taking into account the quantity of food product typically consumed. In the case of peanut, with a very low threshold dose potentially leading to severe symptomatic reactions in highly allergic individuals, a test sensitivity of lower than 1 mg kg^{-1} may be required to assure food safety.

Currently the method of choice for allergen detection in food is the ELISA. ELISA assays are sensitive and specific tests, able to yield fully quantitative results. In some instances sensitivity and specificty of the currently available immunological tests may not be satisfactory and a PCR-based test might be preferred (e.g. soya detection in food products). However, the employment of DNA analysis in allergen detection as an attractive alternative to immunological methods is discussed controversially, since proteins are the allergenic component and processing may differentially affect nucleic acids and proteins. On the other hand (quantitative) results obtained with protein-based methods are subject to biological variations (e.g. botanical variety, seasonal and geographical impacts) and are sometimes strongly affected by food processing (e.g. heat). Several studies — mainly done on peanut detection — assessed significant variations in quantitative results due to various processing conditions, botanical variety, employed method or kit (i.e. specificity and antigen recognition potency of antibodies employed in immunological assays), and food matrix (Keck-Gassenmeier *et al.* 1999, Hischenhuber 2001, Hurst *et al.* 2002, Koch *et al.* 2003, Poms *et al.* 2003).

There is an urgent need for method validation in order to sustain significance of obtained results. To date none of the methods developed and none of the offered test kits has been fully validated internationally in an interlaboratory study for real food matrices. Currently, the allergens with highest priority are gluten (from wheat, rye, and barley) and peanut. In both cases, comparability of results is often difficult, due to a big variability of the raw material, matrix effects during extraction and detection procedures, and a big variance of results obtained with different test kits or methods.

Moreover, several validation studies for peanut detection in various food products are underway. The first phase of an interlaboratory validation has been completed by the AOAC Research Institute in 2003 involving four selected laboratories. Three commercial peanut detection kits (based on ELISA) were approved as *Performance Tested Methods* (Douglas L. Park, FDA, personal communications) on the bases that they were able to detect a peanut butter reference standard at a level of 5 mg kg^{-1} in four different food matrices (breakfast cereals, cookies, ice cream, and milk chocolate). In addition, the

analytical quantitation performance of five different peanut ELISA kits is being validated in an international collaborative study coordinated by the Joint Research Centre of the European Commission. This study involves processed samples (cookies and dark chocolate) with various concentrations of peanut in the low mg kg^{-1} range.

Moreover, the European Commission has started a work programme to investigate food allergens in more detail. As food allergy is a global problem, this work will be carried out in collaboration with other international research organizations and validation bodies. The European Committee for Standardization (CEN) has recently established a new working group on food allergens in order to standardize analytical methods available so far. As more knowledge will be gained on the structure and composition of allergens, it can be expected that in a few years from now there will be more analytical approaches available to detect hidden food allergens. These efforts and achievements will be for the benefit of allergic individuals. Rapid screening methods (e.g. dipstick assays) will certainly be an asset.

References

Allmann, M., Candrian, U., Höfelein, C., and Lüthy, J., 1993, Polymerase chain reaction (PCR): a possible alternative to immunochemical methods assuring safety and quality of food. *Zeitschrift fuer Lebensmittel-Untersuchung und -Forschung*, **196,** 248–251.

Allmann, M., Höfelein, C., Koppel, E., Lüthy, J., Meyer, R., Niederhauser, C., Wegmüller, B., and Candrian, U., 1995, Polymerase chain reaction (PCR) for detection of pathogenic microorganisms in bacteriological monitoring of dairy products. *Research in Microbiology*, **146,** 85–97.

Andre, F., Andre, C., Colin, L., Cacaraci, F., and Cavagna, S., 1994, Role of new allergens and of allergens consumption in the increased incidence of food sensitizations in France. *Toxicology*, **93,** 77–83.

Anet, J., Back, J. F., Baker, R. S., Barnett, D., Burley, R. W., and Howden, M. E. H., 1985, Allergens in the white and yolk of hen's egg. A study of IgE binding by egg proteins. *International Archives of Allergy Immunology*, **77,** 364–371.

Anguita, G., Martin, R., Garcia, T., Morales, P., Haza, A. I., Gonzales, I., Danz, B., and Hernandez, P. E., 1997, A competitive enzyme-linked immunosorbent assay for the detection of bovine milk in ovine and caprine milk and cheese using a monoclonal antibody against bovine β-casein. *Journal of Food Protection*, **60,** 64–66.

Asero, R., Mistrello, G., Roncarolo, D., Antoniotti, P. L., and Falagiani, P., 1999, A case of sesame seed-induced anaphylaxis. *Allergy*, **54,** 526–527.

Awazuhara, H., Kawai, H., Baba, M., Matsui T., and Komiyama, A., 1998, Antigenicity of the proteins in soy lecithin and soy oil in soybean allergy. *Clinical Experiments in Allergy*, **28,** 1559–1564.

Ballmer-Weber, B., Besler, M., Hoffmann-Sommergruber, K., Vieths, S., and Wüthrich, B., 2000a, Allergen Data Collection: Celery (*Apium graveolens*), [http://www.food-allergens.de]. *Internet Symposium on Food Allergens*, **2,** 145–167.

Ballmer-Weber, B. K., Vieths, S., Lüttkopf, D., Heuschmann, P., and Wüthrich, B., 2000b, Celery allergy confirmed by DBPCFC. A clinical study in 32 subjects with a history of adverse reactions to celery root. *Journal of Allergy and Clinical Immunology*, **106,** 373–378.

Bando, N., Tsuji, H., Hiemori, M., Yoshizumi, K., Yamanishi, R., Kimoto, M., and Ogawa, T., 1998, Quantitative analysis of Gly m Bd 28K in soybean products by a sandwich enzyme-linked immunosorbent assay. *Journal of Nutrition Science and Vitaminology*, **44,** 655–664.

Baumgartner, S., Steiner, I., Kloiber, S., Hirmann, D., Krska, R., and Yeung, J., 2002, Towards the development of a dipstick immunoassay for the detection of trace amounts of egg proteins in food. *European Food Research and Technology*, **214,** 168–170.

Ben Rejeb, S., Abbott, M., Davies, D., Querry, J., Cleroux, C., Streng, C., Delahaut, P., and Yeung, J. M., 2003, Immunochemical-based method for detection of hazelnut proteins in processed foods. *Journal of the Association of Official Analytical Chemists International*, **86,** 557–563.

Ben Rejeb, S., Davies, D., Cleroux, C., Langlois, D., and Delahaut, P., 2002, Enzyme immunoassay for the detection of crustacean proteins in foods. Association of Official Analytical Chemists International, Annual Meeting, Los Angeles, CA, pp. 102.

Ben-Rejeb, S., Abbot, M., Davies, D., Cleroux, C., and Streng, C., 2002, Multi-screening immunoassay for the detection of nut proteins in chocolate. Proceedings TNO International Food Allergy Forum, Noordwijkerhout, The Netherlands, pp. 58.

Bernhisel-Broadbent, J., Dintzis, H. M., Dintzis, R. Z., and Sampson, H. A., 1994, Allergenicity and antigenicity of chicken egg ovomucoid (Gal d III) compared with ovalbumin (Gal d I) in children with egg allergy and in mice. *Journal of Allergy and Clinical Immunology*, **93,** 1047–1059.

Bernhisel-Broadbent, J., Scanlon, S. M., and Sampson, H. A., 1992, Fish hypersensitivity. I. *In vitro* and oral challenge results in fish-allergic patients. *Journal of Allergy and Clinical Immunology*, **89**, 730–737.

Besler, M., Daul, C. B., and Leung, P. S. B., 2001a, Shrimp allergy [http://www.food-allergens.de]. *Internet Symposium on Food Allergens*, **3,** 37–53.

Besler, M., Eigenmann, P., and Schwartz, R. H., 2002a, Allergen Data Collection — Update: cow's milk (*Bos domesticus*) [http://www.food-allergens.de]. *Internet Symposium on Food Allergens*, **4,** 19–106.

Besler, M., Elsayed, S., and Helbling, A., 2000, Allergen Data Collection — Update: codfish [http://www.food-allergens.de]. *Internet Symposium on Food Allergens*, **2(Suppl. 6),** 1–18.

Besler, M., Kasel, U., and Wichmann, G., 2002b, Determination of hidden allergens in foods by immunoassays [http://www.food-allergens.de]. *Internet Symposium on Food Allergens*, **4,** 1–18.

Besler, M., Rodriguez, J., and Taylor, S. L., 2001b, Allergen Data Collection — Almond (*Prunus dulcis*) [http://www.food-allergens.de]. *Internet Symposium on Food Allergens*, **3,** 71–85.

Besler, M., Steinhart, H., and Paschke, A., 2001c, Stability of food allergens and allergenicity of processed foods. *Journal of Chromatography B: Biomedical Sciences and Applications*, **756,** 207–228.

Beyer, K., Bardina, L., Grishina, G., and Sampson, H. A., 2002a, Identification of sesame seed allergens by 2-dimensional proteomics and Edman sequencing: seed storage proteins as common

food allergens. *Journal of Allergy and Clinical Immunology*, **110,** 154–159.

Beyer, K., Grishina, G., Bardina, L., Grishin, A., and Sampson, H. A., 2002b, Identification of an 11S globulin as a major hazelnut food allergen in hazelnut-induced systemic reactions. *Journal of Allergy and Clinical Immunology*, **110,** 517–523.

Blais, B. W., and Phillippe, L., 2001, Detection of hazelnut proteins in foods by enzyme immunoassay using egg yolk antibodies. *Journal of Food Protection*, **64,** 895–898.

Blais, B. W., and Phillippe, L. M., 2000, A Cloth-based enzyme immunoassay for detection of peanut proteins in foods. *Food Agricultural Immunology*, **12,** 243–248.

Blais, B. W., Gaudreault, M., and Phillippe, L. M., 2003, Multiplex enzyme immunoassay system for the simultaneous detection of multiple allergens in foods, *Food Control*, **14,** 43–47.

Bleumink, E., and Young, E., 1968, Identification of the atopic allergen in cow's milk. *International Archives of Allergy and Applied Immunology*, **34,** 521–543.

Bock, S. A., and Atkins, F. M., 1990, Patterns of food hypersensitivity during sixteen years of double-blind, placebo-controlled food challenges. *Journal of Pediatrics*, **117,** 561–567.

Bock, S. A., Munoz-Furlong, A., and Sampson, H. A., 2001, Fatalities due to anaphylactic reactions to foods. *Journal of Allergy and Clinical Immunology*, **107,** 191–193.

Boyano, M. T., Martin Estaban, M., Diaz Pena, J. M., and Ojeda, J. A., 1987, Alergia a alimentos en al nino. Clinica y diagnostico. *Anales Esperimentos Pediatres*, **26,** 235–240.

Brandon, D. L., Bates, A. H., and Friedman, M., 1991, ELISA analysis of soybean trypsin inhibitors in processed foods. *Advanced Experiments in Medicine and Biology*, **289,** 321–337.

Breiteneder, H., Hoffmann-Sommergruber, K., O'Riordain, G., Susani, M., Ahorn, H., Ebner, C., Kraft, D., and Scheiner, O., 1995, Molecular characterization of Api g 1, the major allergen of celery (*Apium graveolens*), and its immunological and structural relationships to a group of 17-kDa tree pollen allergens. *European Journal of Biochemistry*, **233,** 484–489.

Brett, G. M., Bull, V. J., and Morgan, M. R., 1998, Identification of hidden allergens within foods. *Allergy*, **53(46 Suppl.),** 109–110.

Burks, A. W., Sampson, H. A., and Bannon, G. A., 1998, Review article series II: Peanut allergens. *Allergy*, **53,** 725–730.

Caballero, T., San-Martin, M. S., Padial, M. A., Contreras, J., Cabanas, R., Barranco, P., and Lopez-Serrano, M. C., 2002, Clinical characteristics of patients with mustard hypersensitivity. *Annals of Allergy, Asthma, and Immunology*, **89,** 166–171.

Catsimpoolas, N., and Leuthner, E., 1969, Immunological methods for detection and quantitation of Kunitz soybean trypsin inhibitor. *Analytical Chemistry*, **31,** 437–447.

Chirdo, F. G., Fossati, C. A., and Anon, M. C., 1995, Optimization of a competitive ELISA with polyclonal antibodies for quantitation of prolamines in food. *Food Agricultural Immunology*, **7,** 333–343.

Chiu, J. T., and Haydik, I. B., 1991, Sesame seed oil anaphylaxis. *Journal of Allergy and Clinical Immunology*, **88,** 414–415.

Ciclitira, P. J., 2002, Coeliac disease: foreword. *Digestive and Liver Disease*, **34,** 214–215.

Clemente, A., Chambers, S. J., Lodi, F., Nicoletti, C., and Brett, G. M., 2003, Use of the indirect competitive ELISA for the detection of Brazil nut in food products. *Food Control* (in press) (published online 4 March 2003).

Codex Alimentarius Commission, 1999, Food labeling — complete texts. Joint FDA/WHO Food Standards Programme (Rome: FAO/WHO).

Codex Alimentarius Commission, 2000, Draft revised standard for gluten-free foods, Codex document CX/NFSDU 00/1.

Codina, R., Lockey, R. F., Fernandez-Caldas, E., and Rama, R., 1997, Purification and characterization of a soybean hull allergen responsible for the Barcelona asthma outbreaks. II. Purification and sequencing of the Gly m 2 allergen. *Clinical Experiments in Allergy*, **27,** 424–430.

Crespo, J. F., Pascual, C., Burks, A. W., Helm, R. M., and Esteban, M. M., 1995, Frequency of food allergy in a pediatric population from Spain. *Pediatry and Allergy Immunology*, **6,** 39–43.

Daul, C. B., Morgan, J. E., Hughes, J., and Lehrer, S. B., 1988, Provocation-challenge studies in shrimp-sensitive individuals. *Journal of Allergy and Clinical Immunology*, **81,** 1180–1186.

Daul, C. B., Slattery, M., Reese, G., and Lehrer, S. B., 1994, Identification of the major brown shrimp (*Penaeus aztecus*) allergen as the muscle protein tropomyosin. *International Archives of Allergy and Immunology*, **105,** 49–55.

Deibel, K., Trautman, T., DeBoom, T., Sveum, W. H., Dunaif, G., Scott, V. N., and Bernard, D. T., 1997, A comprehensive approach to reducing the risk of allergens in foods. *Journal of Food Protection*, **60,** 436–441.

Denery-Papini, S., Nicolas, Y., and Popineau, Y., 1999, Efficiency and limitations of immunochemical assays for the testing of gluten-free foods. *Journal of Cereal Science*, **30,** 121–123.

Desjardin, L. E., Chen, Y., Perkins, M. D., Teixeira, L., Cave, M. D., and Eisenach, K. D., 1998, Comparison of the ABI 7700 system (TaqMan) and competitive PCR for quantification of IS6110 DNA in sputum during treatment of tuberculosis. *Journal of Clinical Microbiology*, **36,** 1964–1968.

Devery, J. M., Bender, V., Penttila, I., and Skerritt, J. H., 1991, Identification of reactive synthetic gliadin peptides specific for coeliac disease. *International Archives of Allergy and Applied Immunology*, **95,** 356–362.

Docena, A., Rozenfeld, G., Fernandez, R., and Fossati, C. A., 2002, Evaluation of the residual antigenicity and allergenicity of cow's milk substitutes by *in vitro* tests. *Allergy*, **57,** 83–91.

Docena, G. H., Fernandez, R., Chirdo, F. G., and Fossati, C. A., 1996, Identification of casein as the major allergenic and antigenic protein of cow's milk. *Allergy*, **51,** 412–416.

Elliot, Ch., 2002, Optical Biosensors: from a research idea to an analytical necessity. *Proceedings of the 3rd Joint CSL/JIFSAN Symposium on food Safety and Nutrition*, York, UK.

Ellis, H. J., Doyle, A. P., Day, P., and Ciclitira, P. J., 1998, Coeliac disease: measurement of gluten using a monoclonal antibody to a coeliac toxic peptide of A-gliadin. *Gut*, **43,** 190–195.

Ellman, L. K., Chatchatee, P., Sicherer, S. H., and Sampson, H. A., 2002, Food hypersensitivity in two groups of children and young adults with atopic dermatitis evaluated a decade apart. *Pediatric Allergy and Immunology*, **13,** 295–298.

Elsayed, S., and Apold, J., 1983, Immunochemical analysis of cod fish allergen M: locations of the immunoglobulin binding sites as demonstrated by the native and synthetic peptides. *Allergy*, **38,** 449–459.

Elsayed, S., and Bennich, H., 1975, The primary structure of Allergen M from cod. *Scandinavian Journal of Immunology*, **4**, 203–208.

Emmett, S. E., Angus, F. J., Fry, J. S., and Lee, P. N., 1999, Perceived prevalence of peanut allergy in Great Britain and its association with other atopic conditions and with peanut allergy in other household members. *Allergy*, **54,** 380–385.

European Commission, 2000, Food Labelling Directive, *Official Journal*, **L 109**.

European Commission, 2002, Food without fear: amended food labelling Directive allows consumers to discover details on allergens. Press release IP/02/1680, 14 November 2002.

Ewan, P. W., 1996, Clinical study of peanut and nut allergy in 62 consecutive patients: new features and associations. *British Medical Journal*, **312,** 1074–1078.

Ewan, P. W., and Clark, A. T., 2001, Long-term prospective observational study of patients with peanut and nut allergy after participation in a management plan. *Lancet*, **357,** 111–115.

FAO (Food and Agricultural Organization) of the United Nations, 1995, *Report of the FAO Technical Committee on Food Allergies* (Rome: FAO).

FDA (Food and Drug Administration), 2001, *Compliance Policy Guide, Sec. 555. 250 Statement of Policy for Labeling and Preventing Cross-contact of Common Food Allergens* (Washington, DC: FDA).

Feriotto, G., Borgatti, M., Mischiati, C., Bianchi, N., and Gambari, R., 2002, Biosensor technology and surface plasmon resonance for real-time detection of genetically modified Roundup Ready soybean gene sequences. *Journal of Agriculture and Food Chemistry*, **50,** 955–962.

Foucard, T., and Malmheden Yman, I., 1999, A study on severe food reactions in Sweden — is soy protein an underestimated cause of food anaphylaxis? *Allergy*, **54,** 261–265.

Fremont, S., Kanny, G., Bieber, S., Nicolas, J. P., and Moneret-Vautrin, D. A., 1996, Identification of a masked allergen, alpha-lactalbumin, in baby-food cereal flour guaranteed free of cow's milk protein. *Allergy*, **51,** 749–754.

Fremont, S., Zitouni, N., Kanny, G., Veneri, V., Metche, M., Moneret-Vautrin, D. A., and Nicolas, J. P., 2002, Allergenicity of some isoforms of white sesame proteins. *Clinical and Experimental Allergy*, **32,** 1211–1125.

Ganglberger, E., Radauer, C., Grimm, R., Hoffmann-Sommergruber, K., Breiteneder, H., Scheiner, O., and Jensen-Jarolim, E., 2000, N-terminal sequences of high molecular weight allergens from celery tuber. *Clinical and Experimental Allergy*, **30,** 566–570.

Garcia, M. C., Marina, M. L., and Torre, M., 1998, Ultrarapid detection of bovine whey proteins in powdered soybean milk by perfusion reversed-phase high-performance liquid chromatography. *Journal of Chromatography A*, **822,** 225–232.

Gell, P. G. H., Coombs, R. R. A., and Lachmann, D. J., 1975, *Clinical Aspects of Immunology*, 3rd edn (Philadelphia: Lippincott).

Golz, T., 1993, Mustard. North Dakota State University (NDSU) Extension Service, NDSU, Institute for Business and Industry Development [http://www.ext.nodak.edu/extpubs/alt-ag/mustard.htm], Alternative Agriculture Series, Number 3.

Gonzalez, de la Pena, M. A., Menendez-Arias, L., Monsalve, R. I., and Rodriguez, R., 1991, Isolation and characterization of a major allergen from oriental mustard seeds, BrajI. *International Archives of Allergy and Applied Immunology*, **96,** 263–270.

Gonzalez, R., Polo, F., Zapatero, L., Caravaca, F., and Carreira, J., 1992, Purification and characterization of major inhalant allergens from soybean hulls. *Clinical Experiments in Allergy*, **22,** 748–755.

Griffiths, N. M., Billington, M. J., Crimes, A. A., and Hitchcock, C. H. S., 1984, An assessment of commercially available reagents for an enzyme-linked immunosorbent assay (ELISA) of soya protein in meat products. *Journal of the Science of Food and Agriculture*, **35,** 1255–1260.

Groot, H. D., de Jong, N. W., Vuijk, M. H., and Gerth van Wijk, R., 1996, Birch pollinosis and atopy caused by apple, peach, and hazelnut; comparison of three extraction procedures with two apple strains. *Allergy*, **51,** 712–718.

Hefle, S. L., Bush, R. K., Yunginger, J. W., and Chu, F. S., 1994, A sandwich enzyme-linked immunosorbent assay (ELISA) for the quantification of selected peanut proteins in foods. *Journal of Food Protection*, **57(Suppl. 5),** 419–423.

Hefle, S. L., Nordlee, J. A., and Taylor, S. L., 1996, Allergenic foods. *Critical Reviews in Food Science and Nutrition*, **36 (Suppl.),** 69–89.

Hefle, S. L., Bush, R. K., Lehrer, S. B., Malo, J.-L., and Cartier, A., 1995, Snow crab allergy: Identification of IgE binding proteins. *Journal of Allergy and Clinical Immunology*, **95,** 332–337.

Hefle, S. L., Christie, L., Sicherer, S. H., Althage, K., Burks, A. W., Sampson, H. A., Mofidi, S., Noone, S. A., Michaelis, L., Strobel, S., Hourihane, J., Eigenmann, P., Nordlee, J. A., and Taylor, S. L., 2003, Threshold dose for egg allergy determined by oral challenge. *Proceedings of the Institute of Food Technology Annual Meeting*, Chicago, IL.

Hefle, S. L., Jeanniton, E., and Taylor, S. L., 2001, Development of a sandwich enzyme-linked immunosorbent assay for the detection of egg residues in processed foods. *Journal of Food Protection*, **64,** 1812–1816.

Helm, R. M., Cockrell, G., Connaughton, C., West, C. M., Herman, E., Sampson, H. A., Bannon, G. A., and Burks, A. W., 2000, Mutational analysis of the IgE-binding epitopes of P34/Gly m Bd 30K. *Journal of Allergy Clinical Immunology*, **105,** 378–384.

Heppell, L. M., Sissons, J. W., and Pedersen, H. E., 1987, A comparison of the antigenicity of soya-bean-based infant formulas. *British Journal of Nutrition*, **58,** 393–403.

Herian, A. M., Taylor, S. L., and Bush, R. K., 1993, Allergenic reactivity of various soybean products as determined by RAST inhibition. *Journal of Food Science*, **58,** 385–388.

Hernando, A., Valdes, I., Garcia, E., and Mendez, E., 2002, New strategy for the analysis of gliadins by MALDI-TOF/MS in maize or rice based foods. *Proceedings of the 16th meeting of the working group on prolamine analysis and toxicity of the Codex Alimentarius*, Sitges, Spain, 2001 (Skripten).

Hird, H., Lloyd, J., Goodier, R., Brown, J., and Reece, P., 2003, Detection of peanut using real-time polymerase chain reaction. *European Food Research and Technology*, **217** (published online 22 May 2003).

Hischenhuber, C., 2002, Analytical methods for the detection of hidden allergens—their use and limitations. *Minutes and Proceedings of an International Workshop on Food Allergy: Chemical and Technological Aspects*, Ispra, Italy, 2001, EUR 20241 EN 2002.

Hitchcock, C. H. S, Bailey, F. J., Crimes, A. A., Dean, D. A. G., and Davis, P. J., 1981, Determination of soya proteins in food using an enzyme-linked immunosorbent assay procedure. *Journal of Science of Food and Agriculture*, **32,** 157–165.

Hlywka, J. J., Hefle, S. L., and Taylor, S. L., 2000, A sandwich enzyme-linked immunosorbent assay for the detection of almonds in foods. *Journal of Food Protection*, **63,** 252–257.

Hoffman, D. R., 1983, Immunochemical identification of the allergens in egg white. *Journal of Allergy and Clinical Immunology*, **71,** 481–486.

Holen, E., and Elsayed, S., 1990, Characterization of four major allergens of hen-egg white by IEF/-SDS-PAGE combined with electrophoretic transfer and IgE-Immunoautoradiography. *International Archives of Allergy Immunology*, **91,** 136–141.

Holgate, S. T., Church, M. K., and Lichtenstein, L. M., 2001, *Allergy*, 2nd edn (St Louis: Mosby).

Holst-Jensen, A., Ronning, S. B., Lovseth, A., and Berdal, K. G., 2003, PCR technology for screening and quantification of genetically modified organisms (GMOs). *Analytical and Bioanalytical Chemistry*, **375,** 985–993.

Holzhauser, T., and Vieths, S., 1999a, Indirect competitive ELISA for determination of traces of peanut (*Arachis hypogaea* L.) protein in complex food matrices. *Journal of Agriculture and Food Chemistry*, **47(Suppl. 2),** 603–611.

Holzhauser, T., and Vieths, S., 1999b, Quantitative sandwich ELISA for determination of traces of hazelnut (*Corylus*

avellana) protein in complex food matrixes. *Journal of Agriculture and Food Chemistry*, **47,** 4209–4218.

HOLZHAUSER, T., DEHNE, L. I., HOFMANN, A., HAUSTEIN, D., and VIETHS, S., 1998, Rocket immunoelectrophoresis (RIE) for determination of potentially allergenic peanut proteins in processed foods as a simple means for quality assurance and food safety. *Zeitschrift fuer Lebensmittel-Untersuchung und -Forschung*, **206,** 1–8.

HOLZHAUSER, T., STEPHAN, O., and VIETHS, S., 2002, Detection of potentially allergenic hazelnut (*Corylus avellana*) residues in food: a comparative study with DNA PCR-ELISA and protein sandwich-ELISA. *Journal of Agriculture and Food Chemistry*, **50,** 5808–5815.

HÖRTNER, H., 1999, *Gentechnisch veränderte Lebens- und Futtermittel — Definition und Identifikation, in Gesundheitsrelevante Faktoren in Lebensmittel tierischer Herkunft* (Friedrich Bauer).

HOST, A., and SAMUELSSON, E. G., 1988, Allergic reactions to raw, pasteurized, and homogenized/pasteurized cow milk: a comparison. A double-blind placebo-controlled study in milk allergic children. *Allergy*, **43,** 113–118.

HOURIHANE, J. O'B., KILBURN, S. A., NORDLEE, J. A., HEFLE, S. L., TAYLOR, S. L., and WARNER, J. O., 1997, An evaluation of the sensitivity of subjects with peanut allergy to very low doses of peanut protein: a randomised, double-blind, placebo-controlled food challenge study. *Journal of Allergy and Clinical Immunology*, **100,** 596–600.

HÜBNER, P., WAIBLINGER, H. U., PIETSCH, K., and BRODMANN, P., 2001, Validation of PCR methods for quantitation of genetically modified plants in food. *Journal of the Association of Official Analytical Chemists International*, **84,** 1855–1864.

HUGGETT, A. C., and HISCHENHUBER, C., 1998, Food manufacturing initiatives to protect the allergic consumer. *Allergy*, **53(Suppl. 46),** 89–92.

HURST, W. J., KROUT, E. R., and BURKS, W. R., 2002, A comparison of commercially available peanut ELISA test kits on the analysis of samples of dark and milk chocolate. *Journal of Immunoassay and Immunochemistry*, **23,** 451–459.

JANATUINEN, E. K., PIKKARAINEN, P. H., KEMPPAINEN, T. A., KOSMA, V. M., JARVINEN, R. M., UUSITUPA, M. I., and JULKUNEN, R. J., 1995, A comparison of diets with and without oats in adults with celiac disease. *New England Journal of Medicine*, **333,** 1033–1037.

JANKIEWICZ, A., AULEPP, H., ALTMANN, F., FÖTISCH, K., and VIETHS, S., 1998, Serological investigation of 30 celery-allergic patients with particular consideration of the thermal stability of IgE binding celery allergens. *Allergo Journal*, **7,** 87–95.

JANKIEWICZ, A., BROLL, H., and ZAGON, J., 1999, The official method for the detection of genetically modified soybeans (German Food Act LMBG § 35): a semi-quantitative study of sensitivity limits with glyhpphosate-tolerant soybeans (Roundup Ready) and insect-resistant Bt maize (Maximizer). *European Food Research and Technology*, **209,** 77–82.

JANSSEN, F. W., VOORTMAN, G., and DE BAAIJ, J. A., 1986, Detection of soya proteins in heated meat products by 'blotting' and 'dot blot'. *Zeitschrift für Lebensmittel Untersuchung und Forschung*, **182,** 479–483.

JEDRYCHOWSKI, L., 1999, Reduction of the antigenicity of whey proteins by lactic acid fermentation. *Food and Agricultural Immunology*, **11,** 91–99.

JEOUNG, B. J., REESE, G., HAUCK, P., OLIVER, J. B., DAUL, C. B., and LEHRER, S. B., 1997, Quantification of the major brown shrimp allergen Pen a 1 (tropomyosin) by a monoclonal antibody-based sandwich ELISA. *Journal of Allergy and Clinical Immunology*, **100,** 229–234.

JONES, D. B., and HORN, M. J., 1930, The properties of arachin and conarachin and the proportionate occurrence of these proteins in the peanut. *Journal of Agricultural Research*, **40,** 673.

JONSSON, H., 2002, Sensitive and specific biosensor detection of hazelnut proteins and other allergens in food. *Proceedings TNO International Food Allergy Forum*, Noordwijkerhout, The Netherlands, pp. 61.

KAGI, M. K., and WÜTHRICH, B., 1993, Falafel burger anaphylaxis due to sesame seed allergy. *Annals of Allergy*, **71,** 127–129.

KANNY, G., DE HAUTECLOCQUE, C., and MONERET-VAUTRIN, D. A., 1996, Sesame seed and sesame seed oil contain masked allergens of growing importance. *Allergy*, **51,** 952–957.

KAYOSAARI, M., 1982, Food allergy in Finnish children aged 1 to 6 years. *Acta Paediatra Scandinavia*, **71,** 815–819.

KEATING, M. U., JONES, R. T., WORLEY, N. J., SHIVELY, C. A., and YUNGINGER, J. W., 1990, Immunoassay of peanut allergens in food processing materials and finished foods. *Journal of Allergy and Clinical Immunology*, **86,** 41–44.

KECK-GASSENMEIER, B., BENET, S., ROSA, C., and HISCHENHUBER, C., 1999, Determination of peanut traces in food by a commercially-available ELISA test. *Food Agricultural Immunology*, **11,** 243–250.

KLEBER-JANKE, T., CRAMERI, R., APPENZELLER, U., SCHLAAK, M., and BECKER, W. M., 1999, Selective cloning of peanut allergens, including profilin and 2S albumin, by phage display technology. *International Archives of Allergy and Immunology*, **119,** 265–274.

KLEIN, C. L., and FRANCHINI, F., 2002, Towards a certified reference material for gliadin from European wheat. *Proceedings of the 18th Meeting of the Working Group on Prolamine Analysis and Toxicity of the Codex Alimentarius*, London, UK (Skripten).

KLEIN, C. L., FRANCHINI, F., and SCHIMMEL, H., 2003, Towards a certified reference material for gliadin from European wheat: study design and methodological approaches. *Proceedings of the 17th Meeting of the Working group Prolamin Analysis and Toxicity*, London, UK, 2002, edited by M. Stern (Skripten), pp. 113–117.

KOCH, P., SCHÄPPI, G. F., POMS, R. E., WÜTHRICH, R. ANKLAM, E., and BATTAGLIA, R., 2003, Comparison of commercially available ELISA kits with human sera-based detection methods for peanut allergens in foods. *Food Additives and Contaminants* (in press).

KÖPPEL, E., STADLER, M., LÜTHY, J., and HÜBNER, P., 1998, Detection of wheat contamination in oats by polymerase chain reaction (PCR) and enzyme-linked immunosorbent assay (ELISA). *Zeitschrift fuer Lebensmittel-Untersuchung und -Forschung*, **206,** 300–403.

KOPPELMAN, J., BLEEKER-MARCELIS, H., DUIJN, G., and HESSING, M., 1996, Detecting peanut allergens. The development of an immunochemical assay for peanut proteins. *World of Ingredients*, **12,** 35–38.

KOPPELMAN, S. J., VLOOSWIJK, R. A. A., KNIPPELS, L. M. J., HESSING, M., KNOL, E. F., VAN REIJSEN, F. C., and BRUIJNZEEL-KOOMEN, C. A. F. M., 2001, Quantification of major peanut allergens Ara h 1 and Ara h 2 in the peanut varieties Runner, Spanish, Virginia, and Valencia, bred in different parts of the world. *Allergy*, **56,** 132–137.

KOPPELMAN, S. J., KNULST, A. C., KOERS, W. J., PENNINKS, A. H., PEPPELMAN, H., VLOOSWIJK, R., PIGMANS, I., VAN DUIJN, G., and HESSING, M., 1999, Comparison of different immunochemical methods for the detection and quantification of hazelnut proteins in food products. *Journal of Immunological Methods*, **229,** 107–120.

KRISHNA, T. G., PAWAR, S. E., and MITRA, R., 1986, Variation and inheritance of the arachin polypeptides of groundnut (*Arachis hypogaea* L.). *Theoretical Applied Genetics*, **73,** 82.

LANGELAND, T., 1982, A clinical and immunological study of allergy to hen's egg white. III. Allergens in hen's egg white studied by crossed radioimmunoelectrophoresis (CRIE). *Allergy*, **37,** 521–530.

Lehrer, S. B., Ayuso, R., and Reese, G., 2002, Current understanding of food allergens. *Annals of the New York Academy of Science*, **964,** 69–85.

Leung, P. S. C., Chu, J. H., Chow, W. K., Ansari, A., Bandea, C. I., Kwan, H. S., Nagy, S. M., and Gershwin, M. E., 1994, Cloning, expression, and primary structure of meatpenaeus ensis tropomyosin, the major heat-stable shrimp allergen. *Journal of Allergy and Clinical Immunology*, **94,** 882–890.

Lopez-Tapia, J., Garcia-Risco, M. R., Manso, M. A., and Lopez-Fandino, R., 1999, Detection of the presence of soya protein in milk powder by sodium dodecyl sulfate capillary electrophoresis. *Journal of Chromatography*, **A 836,** 153–160.

Macedo-Silva, A., Shimokomaki, M., Vaz, A. J., Yamamoto, Y. Y., and Tenuta-Filho, A., 2001, Textured soy protein quantification in commercial hamburger, *Journal of Food Composition and Analysis*, **14,** 469–478.

Mäkinen-Kiljunen, S., and Palosuo, T., 1992, A sensitive enzyme-linked immunosorbent assay for determination of bovine beta-lactoglobulin in infant formulars and in human milk. *Allergy*, **47,** 347–352.

Malish, D., Glovsky, M. M., Hoffman, D. R., Ghekiere, L., and Hawkins, J. M., 1981, Anaphylaxis after sesame seed ingestion. *Journal of Allergy and Clinical Immunology*, **67,** 35–38.

Malmheden Yman, I., Eriksson, A., Everitt, G., Yman, L., and Karlsson, T., 1994, Analysis of food proteins for verification of contamination or mislabelling. *Food and Agricultural Immunology*, **6,** 167–172.

Malorny, B., Tassios, P. T., Radstrom, P., Cook, N., Wagner, M., and Hoorfar, J., 2003, Standardization of diagnostic PCR for the detection of foodborne pathogens. *International Journal of Food Microbiology*, **83,** 39–48.

Mariager, B., Solve, M., Eriksen, H., and Brogen, C.-H., 1994, Bovine β-lactoglobulin in hypoallergenic and ordinary infant formulars measured by an indirect competitive ELISA using monoclonal and polyclonal antibodies. *Food and Agricultural Immunology*, **6,** 73–83.

Meier-Davis, S., Taylor, S. L., Nordlee, J., and Bush, R., 1987, Identification of peanut allergens by immunoblotting. *Journal of Allergy and Clinical Immunology*, **79,** 218.

Mendez, E., Valdez, I., and Camafeita, E., 2000, Analysis of gluten in foods by MALDI-TOF mass spectrometry. *Methods in Molecular Biology*, edited by M. D. Chapman Jr (Totowa: Humana), pp. 355–367.

Menendez-Arias, L., Moneo, I., Dominguez, J., and Rodriguez, R., 1988, Primary structure of the major allergen of yellow mustard (*Sinapis alba* L.) seed, Sin a I. *European Journal of Biochemistry*, **177,** 159–166.

Meyer, R., Candrian, U., and Lüthy, J., 1996, Polymerase chain reaction (PCR) in the quality and safety assurance of food: detection of soya in processed meat products. *Zeitschrift fuer Lebensmittel-Untersuchung und -Forschung*, **203,** 339–344.

Mills, E. N. C., Potts, A., Plumb, G. W., Lambert, N., and Morgan, M. R. A., 1997, Development of a rapid dipstick immunoassay for the detection of peanut contamination of food. *Food and Agricultural Immunology*, **9,** 37–50.

Molina, E., Amigo, L., and Ramos, M., 1998, Detection of bovine milk proteins in soymilk by Western blotting. *Journal of Food Protection*, **61,** 1691–1694.

Monsalve, R. I., Villalba, M., and Rodriguez, R., 2001, Allergy to mustard seeds: the importance of 2S albumins as food allergens [http://www.food-allergens.de]. *Internet Symposium on Food Allergens*, **3,** 57–69.

Negroni, L., Bernard, H., Clement, G., Chatel, J. M., Brune, P., Frobert, Y., Wal, J. M., and Grassi, J., 1998, Two-site enzyme immunometric assays for determination of native and denatured β-lactoglobulin. *Journal of Immunological Methods*, **220,** 25–37.

Neuhausen, S. L., Feolo, M., Camp, N. J., Farnham, J., Book, L., and Zone, J. J., 2002, Genome-wide linkage analysis for celiac disease in North American families. *American Journal of Medical Genetics*, **111,** 1–9.

Newsome, W. H., and Abbot, M., 1999, An immunoaffinity column for the determination of peanut protein in chocolate. *Journal of the Association of Official Analytical Chemists International*, **82,** 666–668.

Niemann, L., and Hefle, S. L., 2003, Detection of undeclared walnut residues in food. *Journal of Allergy and Clinical Immunology*, **111(Suppl.)**, 248.

Niggemann, B., Binder, C., Klettke, U., and Wahn, U., 1999, In vivo and *in vitro* studies on the residual allergenicity of partially hydrolysed infant formulae. *Acta Paediatrica*, **88,** 394–398.

Nordlee, J. A., and Taylor, S. L., 1995, Immunological analysis of food allergens and other food proteins. *Food Technology*, **2,** 129–132.

Nordlee, J. A., Taylor, S. L., Jones, R. T., and Yunginger, J. W., 1981, Allergenicity of various peanut products as determined by RAST inhibition. *Journal of Allergy and Clinical Immunology*, **68,** 376–382.

Nordlee, J. A., Taylor, S. L., Townsend, J. A., Thomas, L. A., and Bush, R. K., 1996, Identification of a Brazil-nut allergen in transgenic soybeans. *New England Journal of Medicine*, **334**, 688–692.

Nowak-Wegrzyn, A., Conover-Walker, M. K., and Wood, R. A., 2001, Food-allergic reactions in schools and preschools. *Archives of Pediatric and Adolescent Medicine*, **155,** 790–795.

O'Neil, C. E., and Lehrer, S. B., 1995, Seafood allergy and allergens: a review. *Food Technology*, **49,** 103–116.

Ogawa, T., Bando, N., Tsuji, H., Nishikawa, K., and Kitamura, K., 1995, α-subunit of β-conglycinin, an allergenic protein recognized by IgE antibodies of soybean-sensitive patients with atopic dermatitis. *Bioscience, Biotechnology and Biochemistry*, **59,** 831–833.

Ogawa, T., Tsuji, H., Bando, N., Kitamura, K., Zhu, Y. L., Hirano, H., and Nishikawa, K., 1993, Identification of the soybean allergenic protein Gly m Bd 30K, with soybean seed 34-kDa oil-body-associated protein. *Bioscience, Biotechnology and Biochemistry*, **57,** 1030–1033.

Oldaeus, G., Bjorksten, B., Einarsson, R., and Kjellman, N. I. M., 1991, Antigenicity and allergenicity of cow milk hydrolysates intended for infant feeding. *Pediatric Allergy and Immunology*, **2,** 156–164.

Ortolani, C., Ispano, M., Scibilia, J., and Pastorello, E. A., 2001, Introducing chemists to food allergy. *Allergy*, **56(Suppl. 67),** 5–8.

Pajno, G. B., Passalacqua, G., Magazzu, G., Barberio, G., Vita, D., and Canonica, G. W., 2000, Anaphylaxis to sesame. *Allergy*, **55,** 199–201.

Paschke, A., Zunker, K., Wigotzki, M., and Steinhart, H., 2001, Determination of the IgE-binding activity of soy lecithin and refined and non-refined soybean oils. *Journal of Chromatography B: Biomedical Science Applications*, **756,** 249–254.

Pastorello, E. A., and Trambaioli, C., 2001, Isolation of food allergens. *Journal of Chromatography B*, **756,** 71–84.

Pastorello, E. A., Farioli, L., Pravettoni, V., Ispano, M., Conti, A., Ansaloni, R., Rotondo, F., Incorvaia, C., Bengtsson, A., Rivolta, F., Trambaioli, C., Previdi, M., and Ortolani, C., 1998, Sensitization to the major allergen of Brazil nut is correlated with the clinical expression of allergy. *Journal of Allergy and Clinical Immunology*, **102,** 1021–1027.

Pastorello, E. A., Varin, E., Farioli, L., Pravettoni, V., Ortolani, C., Trambaioli, C., Fortunato, D., Giuffrida, M. G., Rivolta, F., Robino, A., Calamari, A. M., Lacava,

L., and Conti, A., 2001, The major allergen of sesame seeds (Sesamum indicum) is a 2S albumin. *Journal of Chromatography B: Biomedical Sciences and Applications*, **756,** 85–93.

Pastorello, E. A., Vieths, S., Pravettoni, V., Farioli, L., Trambaioli, C., Fortunato, D., Lüttkopf, D., Calamari, M., Ansaloni, R., Scibilia, J., Ballmer-Weber, B. K., Poulsen, L. K., Wütrich, B., Hansen, K. S., Robino, A. M., Ortolani, C., and Conti, A., 2002, Identification of hazelnut major allergens in sensitive patients with positive double-blind, placebo-controlled food challenge results. *Journal of Allergy and Clinical Immunology*, **109,** 563–570.

Plath, A., Krause, I., and Einspanier, R., 1997, Species identification in dairy products by three different DNA-based techniques. *Zeitschrift fuer Lebensmittel-Untersuchung und Forschung A*, **205,** 437–441.

Plebani, A., Restani, P., Naselli, A., Galli, C. L., Meini, A., Cavagni, G., Ugazio, A. G., and Poiesi, C., 1997, Monoclonal and polyclonal antibodies against casein components of cow milk for evaluation of residual antigenic activity in 'hypoallergenic' infant formulas. *Clinical and Experimental Allergy*, **27,** 949–956.

Poltronieri, P., Cappello, M. S., Dohmae, N., Conti, A., Fortunato, D., Pastorello, E. A., Ortolani, C., and Zacheo, G., 2002, Identification and characterisation of the IgE-binding proteins 2S albumin and conglutin gamma in almond (*Prunus dulcis*) seeds. *International Archives of Allergy and Immunology*, **128,** 97–104.

Pomes, A., Helm, R. M., Bannon, G. A., Burks, A. W., Tsay, A., and Chapman, M. D., 2003, Monitoring peanut allergen in food products by measuring Ara h 1. *Journal of Allergy and Clinical Immunology*, **111,** 640–645.

Poms, R. E., and Anklam, E., 2003, Current trends in detecting food allergens. *GIT Laboratory Journal Europe* (in press).

Poms, R. E., Glössl, J., and Foissy, H., 2001, Increased sensitivity for detection of specific target DNA in milk by concentration in milk fat. *European Food Research and Technology*, **213,** 361–365.

Poms, R. E., Ulberth, F., Klein, C. L., and Anklam, E., 2003, Peanut allergens in food products. *GIT Laboratory Journal Europe*, **3,** 132–135.

Poms, R. E., Lisi, C., Summa, C., Stroka, J., and Anklam, E., 2003, In-house validation of commercially available ELISA test kits for peanut allergens in food. *Report from the Institute for Reference Materials and Measurements, Joint Research Centre, European Commission*, EUR 20767 EN.

Porras, O., Carlsson, B., Faellstroem, S. P., and Hanson, L. A., 1985, Detection of soy protein in soy lecithin, margarine, and, occasionally, soy oil. *International Archives of Allergy and Applied Immunology*, **78,** 30–32.

Poulsen, L. K., Hansen, T. K., Norgaard, A., Vestergaard, H., Stahl Skov, P., and Bindslev-Jensen, C., 2001, Allergens from fish and egg. *Allergy*, **56(Suppl. 67),** 39–42.

Rance, F., 2003, Mustard allergy as a new food allergy. *Allergy*, **58,** 287–288.

Reese, G., Jeoung, B. J., Daul, C. B., and Lehrer, S. B., 1997, Characterization of recombinant shrimp allergen Pen a 1 (tropomyosin). *International Archives of Allergy and Immunology*, **113,** 240–242.

Resano, A., Crespo, E., Fernandez Benitez, M., Sanz, M. L., and Oehling, A., 1998, Atopic dermatitis and food allergy. *Journal of Investigational Allergology and Clinical Immunology*, **8,** 271–276.

Rihs, H. P., Chen, Z., Rueff, F., Peterson, A., Rozynek, P., Heimann, H., and Baur, X., 1999, IgE binding of the recombinant allergen profilin (rGly m 3) is mediated by conformational epitopes. *Journal of Allergy and Clinical Immunology*, **104,** 1293–1301.

Rittenburg, J. H., Adams, A., Palmer, J., and Allen, J. C., 1987, Improved enzyme-linked immunosorbent assay for determination of soy protein in meat products. *Journal of the Association of Official Analytical Chemists International*, **70,** 582–587.

Roux, K. H., Teuber, S. S., Robotham, J. M., and Sathe, S. K., 2001, Detection and stability of the major almond allergen in foods. *Journal of Agriculture and Food Chemistry*, **49,** 2131–2136.

Rueff, F., Eberlein-Konig, B., and Przybilla, B., 2001, Oral hyposensitization with celery juice. *Allergy*, **56,** 82–83.

Sampson, H. A., 1999, Food allergy. Part 1: immunopathogenesis and clinical disorders. *Journal of Allergy and Clinical Immunology*, **103,** 717–728.

Sampson, H. A., 2000, Food anaphylaxis. *British Medical Bulletin*, **56,** 925–935.

Sampson, H. A., Mendelson, L., and Rosen, J. P., 1992, Fatal and near-fatal anaphylactic reactions to food in children and adolescents. *New England Journal of Medicine*, **327,** 380–384.

Sathe, S. K., Teuber, S. S., Gradziel, T. M., and Roux, K. H., 2001, Electrophoretic and immunological analyses of almond (*Prunusdulcis* L.) genotypes and hybrids. *Journal of Agriculture and Food Chemistry*, **49,** 2043–2052.

Sato, R., Takeyama, H., Tanaka, T., and Matsunaga, T., 2001, Development of high-performance and rapid immunoassay for model food allergen lysozyme using antibody-conjugated bacterial magnetic particles and fully automated system. *Applied Biochemistry and Biotechnology*, **91,** 109–116.

Scanlon, M. G., Sapirstein, H. D., and Bushuk, W., 1989, Evaluation of the precision of HPLC for wheat-cultivar identification. *Cereal Science*, **66,** 112–116.

Schäppi, G. F., Konrad, V., Imhof, D., Etter, R., and Wüthrich, B., 2001, Hidden peanut allergens detected in various foods: findings and legal measurements. *Allergy*, **56,** 1216–1220.

Scheibe, B., Weiss, W., Rueff, F., Przybilla, B., and Gorg, A., 2001, Detection of trace amounts of hidden allergens: hazelnut and almond proteins in chocolate. *Journal of Chromatography B Biomedical Science and Applications*, **756,** 229–237.

Schenk, S., Hoffmann-Sommergruber, K., Breiteneder, H., Ferreira, F., Fischer, G., Scheiner, O., Kraft, D., and Ebner, C., 1994, Four recombinant isoforms of Cor a 1, the major allergen of hazel pollen, show different reactivities with allergen-specific T-lymphocyte clones. *European Journal of Biochemistry*, **224,** 717–722.

Schocker, F., Lepp, U., Thomas, M., Schlaak, M., and Becker, W. M., 1999, Identifizierung und Charakterisierung nicht-pollenassoziierter Allergene der Haselnuss. *Allergology Journal*, **8,** 32–33.

Schocker, F., Lüttkopf, D., Müller, U., Thomas, P., Vieths, S., and Becker, W. M., 2000, IgE binding to unique hazelnut allergens: identification of non pollen-related and heat-stable hazelnut allergens eliciting severe allergic reactions. *European Journal of Nutrition*, **39,** 172–180.

Shanti, K. N., Martin, B. M., Nagpal, S., Metcalfe, D. D., and Subba Rao, P. V., 1993, Identification of tropomyosin as the major shrimp allergen and characterisation of its IgE-binding epitopes. *Journal of Immunology*, **151,** 5354–5363.

Sholar, J. R., Mozingo, W., and Beasley, P. J., 1995, Peanut cultural practices. *Advances in Peanut Sciences*, edited by E. H. Pattee and H. T. Stalker (Stillwater: American Peanut Research and Education Society, Inc.), pp. 354–382.

Sicherer, S. H., 2001, Clinical implications of cross-reactive food allergens. *Journal of Allergy and Clinical Immunology*, **108,** 881–890.

Sicherer, S. H., 2002, Clinical update on peanut allergy. *Annals of Allergy, Asthma and Immunology*, 350–360.

Sicherer, S. H., Furlong, T. J., Munoz-Furlong, A., Burks, A. W., and Sampson, H. A., 2001, A voluntary registry for peanut and tree nut allergy: characteristics of the first 5149 registrants. *Journal of Allergy and Clinical Immunology*, **108,** 128–132.

Sicherer, S. H., Munoz-Furlong, A., Burks, A. W., and Sampson, H. A., 1999, Prevalence of peanut and tree nut allergy in the US determined by a random digit dial telephone survey. *Journal of Allergy and Clinical Immunology*, **103,** 559–562.

Sicherer, S. H., Munoz-Furlong, A., Murphy, R., Wood, R. A., and Sampson, H. A., 2003, Symposium: Pediatric food allergy. *Pediatrics*, **111,** 1591–1594.

Sicherer, S. H., Sampson, H. A., and Burks, A. W., 2000, Peanut and soy allergy: a clinical and therapeutic dilemma. *Allergy*, **55,** 515–521.

Singh, B., and Singh, U., 1991, Peanut as a source of protein for human foods. *Plant Foods for Human Nutrition*, **41,** 165–177.

Skerritt, J. H., and Hill, A. S., 1991, Enzyme immunoassay for determination of gluten in foods: collaborative study. *Journal of the Association of Official Analytical Chemists*, **74,** 257–264.

Stephan, O., Möller, N., Lehmann, S., Holzhauser, T., and Vieths, S., 2002, Development and validation of two dipstick type immunoassays for determination of trace amounts of peanut and hazelnut in processed foods. *European Food Research and Technology*, **215,** 431–436.

Stern, M., Ciclitira, P. J., van Eckert, R., Feighery, C., Janssen, W., Mendez, E., Mothes, Th., Troncone, R., and Wieser, H., 2001, Analysis and clinical effects of gluten in coeliac disease. *European Journal of Gastroenterology and Hepatology*, **13,** 741–747.

Storsrud, S., Olsson, M., Arvidsson Lenner, R., Nilsson, L. A., Nilsson, O., and Kilander, A., 2003, Adult coeliac patients do tolerate large amounts of oats. *European Journal of Clinical Nutrition*, **57**, 163–169.

Studer, E., Rhyner, C., and Lüthy, L., 1998, Quantitative competitive PCR for the detection of genetically modified soybean and maize. *Zeitschrift fuer Lebensmittel-Untersuchung und - Forschung A*, **207,** 207–213.

Sun, S. S., Altenbach, S. B., and Leung, F. W., 1987, Properties, biosynthesis and processing of a sulfur-rich protein in Brazil nut (*Bertholletia excelsa* H. B. K.). *European Journal of Biochemistry*, **162,** 477–483.

Taylor, A. V., Swanson, M. C., Jones, R. T., Vives, R., Rodriguez, J., Yunginger, J. W., and Crespo, J. F., 1999, Detection and quantitation of raw fish aeroallergens from an open-air fish market. *Journal of Allergy and Clinical Immunology*, **105,** 166–169.

Taylor, S., 1992, Chemistry and detection of food allergens. *Food Technology*, **46,** 148–152.

Taylor, S. L., 1987, Allergic and sensitivity reactions to food components. *Nutritional Toxicology*, vol. II, pp. 173–197.

Taylor, S. L., Bush, R. K., Selner, J. C., Nordlee, J. A., Wiener, M. B., Holden, K., Koepke, J. W., and Busse, W. W., 1988, Sensitivity to sulfited foods among sulfite-sensitive subjects with asthma. *Journal of Allergy and Clinical Immunology*, **81,** 1159–1167.

Taylor, S. L., Hefle, S. L., Bindslev-Jensen, C., Bock, S. A., Burks, A. W. Jr., Christie, L., Hill, D. J., Host, A., Hourihane, J. O., Lack, G., Metcalfe, D. D., Moneret-Vautrin, D. A., Vadas, P. A., Rance, F., Skrypec, D. J., Trautman, T. A., Yman, I. M., and Zeiger, R. S., 2002, Factors affecting the determination of threshold doses for allergenic foods: how much is too much? *Journal of Allergy and Clinical Immunology*, **109,** 24–30.

Taylor, S. L., Higley, N. A., and Bush, R. K., 1986, Sufites in foods—uses, analytical methods, residues, fate, exposure assessment, metabolism, toxicity, and hypersensitivity. *Advances in Food Research*, **30,** 1.

Terry, C. F., Shanahan, D. J., Ballam, L. D., Harris, N., McDowell, D. G., and Parkes, H. C., 2002, Real-time detection of genetically modified soya using Lightcycler and ABI 7700 platforms with TaqMan, Scorpion, and SYBR Green I chemistries. *Journal of the Association of Analytical Chemists International*, **85,** 938–944.

Teuber, S. S., Sathe, S. K., Peterson, W. R., and Roux, K. H., 2002, Characterization of the soluble allergenic proteins of cashew nut (*Anacardium occidentale* L.). *Journal of Agriculture and Food Chemistry*, **50,** 6543–6549.

Troncone, R., Vitale, M., Donatiello, A., Farris, E., Rossi, G., and Auricchio, S., 1986, A sandwich enzyme immunoassay for wheat gluten. *Journal of Immunological Methods*, **92,** 21–23.

Tsuji, H., Okada, N., Yamanishi, R., Bando, N., Kimoto, M., and Ogawa, T., 1995, Measurement of Gly m Bd 30K, a major soybean allergen, in soybean products by a sandwich enzyme-linked immunosorbent assay. *Bioscience, Biotechnology and Biochemistry*, **59,** 150–151.

Urisu, A., Ando, H., Morita, Y., Wada, E., Yasaki, T., Yamada, K., Komada, K., Torii, S., Goto, M., and Wakamatsu, T., 1997, Allergenic activity of heated and ovomucoid-depleted egg white. *Journal of Allergy and Clinical Immunology*, **100,** 171–176.

Vallier, P., DeChamp, C., Valenta, R., Vial, O., and Deviller, P., 1992, Purification and characterization of an allergen from celery immunochemically related to an allergen present in several other plant species. Identification as a profilin. *Clinical and Experimental Allergy*, **22,** 774–782.

Van Eckert, R., 2002, The PWG gliadin, a new reference material. *Proceedings of the 16th Meeting of the Working Group on Prolamine Analysis and Toxicity of the Codex Alimentarius*, Sitges, Spain, 2001 (Skripten).

Walsh, B. J., Baldo, B. A., Bass, D. J., Clancy, R., Musk, A. W., and Wrigley, C. W., 1987, Insoluble and soluble allergens from wheat grain and wheat dust: detection of IgE binding in inhalant and ingestion allergy. *New England and Regional Allergy Proceedings*, **8,** 27–33.

Wang, F., Robotham, J. M., Teuber, S. S., Tawde, P., Sathe, S. K., and Roux, K. H., 2002, Ana o 1, a cashew (Anacardium occidental) allergen of the vicilin seed storage protein family. *Journal of Allergy and Clinical Immunology*, **110,** 160–166.

Wei, Y., Sathe, S. K., Teuber, S. S., and Roux, K. H., 2003, A sensitive sandwich ELISA for the detection of trace amounts of cashew (*Anacardium occidentale* L.) nut in foods. *Journal of Agriculture and Food Chemistry*, **51,** 3215–3221.

Wensing, M., Penninks, A. H., Hefle, S. L., Akkerdaas, J. H., van Ree, R., Koppelman, S. J., Bruijnzeel-Koomen, C. A., and Knulst, A. C., 2002a, The range of minimum provoking doses in hazelnut-allergic patients as determined by double-blind, placebo-controlled food challenges. *Clinical and Experimental Allergy*, **32,** 1757–1762.

Wensing, M., Penninks, A. H., Hefle, S. L., Koppelman, S. J., Bruijnzeel-Koomen, C. A., and Knulst, A. C., 2002b, The distribution of individual threshold doses eliciting allergic reactions in a population with peanut allergy. *Journal of Allergy and Clinical Immunology*, 110, 915–920.

Wieser, H., 1994, Cereal protein chemistry, edited by C. Feighery and C. O'Farrelly. Celiac Symposium, Dublin 1992 (Oak Tree Press), pp. 191–202.

Wieser, H., and Antes, S., 2002, Development of a non-immunological method for the quantitative determination of gluten in wheat starch. Proceedings of the 16th meeting of the working group on prolamine analysis and toxicity of the Codex Alimentarius, 8–11 November 2001, Sitges, Spain (Skripten).

Wigotzki, M., Steinhart, S., and Paschke, A., 2000, Influence of varieties, storage and heat treatment on IgE-binding proteins

in hazelnuts (*Corylus avellana*). *Food Agricultural Immunology*, **12,** 217–229.

WIGOTZKI, M., STEINHART, S., and PASCHKE, A., 2001, Determination of the allergenicity of various hazelnut products by immunoblotting and enzyme allergosorbent test inhibition. *Journal of Chromatography*, **B 756,** 239–248.

WOLFF, N., COGAN, U., ADMON, A., DALAL, I., KATZ, Y., HODOS, N., KARIN, N., and YANNAI, S., 2003, Allergy to sesame in humans is associated primarily with IgE antibody to a 14 kDa 2S albumin precursor. *Food and Chemical Toxicology*, **41,** 1165–1174.

WOODS, R. K., STONEY, R. M., RAVEN, J., WALTERS, E. H., ABRAMSON, M., and THIEN, F. C., 2002, Reported adverse food reactions overestimate true food allergy in the community. *European Journal of Clinical Nutrition*, **56,** 31–36.

WÜTHRICH, B., 1993, Nahrungsmittelallergie. *Allergologie*, **16,** 280–287.

WÜTHRICH, B., 2000, Lethal or life-threatening allergic reactions to food. *Journal of Investigational Allergology and Clinical Immunology*, **10**, 59–65.

WÜTHRICH, B., STÄGER, J., and JOHANSSON, S. G., 1990, Celery allergy associated with birch and mugwort pollinosis. *Allergy*, **45**, 566–571.

YAMANISHI, R., KONDO, K., TSUJI, H., and OGAWA, T., 1995, Micro-assay to measure the allergenicity of a Kunitz-type soybean trypsin inhibitor toward Balb/c mice by using RBL-2H3 cells. *Bioscience, Biotechnology and Biochemistry*, **59,** 1272–1275.

YAMANISHI, R., TSUJI, H., BANDO, N., YOSHIMOTO, I., and OGAWA, T., 1997, Micro-assay method for evaluating the allergenicity of the major soybean allergen, Gly m Bd 30K, with mouse antiserum and RBL-2H3 cells. *Bioscience, Biotechnology and Biochemistry*, **61,** 19–23.

YASUMOTO, K., SUDO, M., and SUZUKI, T., 1990, Quantitation of soya protein by enzyme linked immunosorbent assay of its characteristic peptide. *Journal of the Science of Food and Agriculture*, **50,** 377–389.

YEUNG, J. M., and COLLINS, P. G., 1996, Enzyme immunoassay for determination of peanut proteins in food products. *Journal of the Association of Official Analytical Chemists International*, **79(Suppl. 6),** 1411–1416.

YEUNG, J. M., NEWSOME, W. H., and ABBOTT, M. A., 2000, Determination of egg proteins in food products by enzyme immunoassay. *Journal of the Association of Official Analytical Chemists International*, **83,** 139–143.

YUNGINGER, J. W., GAUERKE, M. B., JONES, R. T., DAHLBERG, M. J. E., and ACKERMAN, S. J., 1983, Use of radioimmunoassay to determine the nature, quantity and source of allergenic contamination of sunflower butter. *Journal of Food Protection*, **46,** 625–628.

ZEECE, M. G., BEARDSLEE, T. A., MARKWELL, J. P., and SARATH, G., 1999, Identification of an IgE-binding region in soybean acidic glycinin G1. *Food and Agricultural Immunology*, **11,** 83–90.