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Tracking and tracing for allergen-free food production chains

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Abstract

Tracking and tracing for allergen-free food production chains has become important due to consumer-safety concerns and new international labelling regulations. An overview of the technical possibilities and commercially available kits for the detection of residual allergenic foods in the food production chain and in the final food product are given, including the discussion of some pros and cons when considering the right choice of method for certain applications.
Keywords: food production chain; allergen; labelling; detection method; commercial ELISA kits

Introduction

Food allergies represent an important health problem in industrialized countries (Sicherer et al. 2003). In a sensitized individual, even the intake of minute amounts of allergens can provoke digestive disorders, respiratory and skin reactions. For some allergic individuals, the contact with a certain food allergen can even provoke life-threatening reactions (anaphylaxis).

Since no cure for allergic patients is available to-date, allergic individuals must strictly avoid the offending allergens in their diet. Total avoidance is sometimes difficult, as processed food usually contains a wide variety of ingredients including potential allergens. Sensitive individuals may also be inadvertently exposed to allergenic proteins by consumption of food products supposed to be free of a certain allergen. Food products can be contaminated with 'foreign' food constituents during shipping and storage, during processing, e.g. by carry-over due to inadequate cleaning of shared processing equipment, or by reuse (rework) of allergen-containing products (Huggett and Hischenhuber 1998).

Allergen labelling

To provide full information to consumers about potential allergens contained in a food and thus assure food safety for allergic individuals, stringent labelling regulations and quality-assurance procedures are enforced. In fact, the European Parliament and the Council adopted the Directive 2003/89/EC in November 2003, amending the Directive 2000/13/EC in regard of the indication of ingredients present

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in foodstuffs (EU 2003). The Directive also established a list of ingredients liable to cause atopic reactions due to food allergies and food intolerances.
The list of ingredients to be labelled comprises:

- Cereals containing gluten and products thereof (i.e. wheat, rye, barley and oat)
- Crustaceans and products thereof
- Eggs and products thereof
- Fish and products thereof
- Peanuts and products thereof
- Soybeans and products thereof
- Milk and dairy products (including lactose)
- Nuts and nut products (i.e. almond, hazelnut, walnut, cashew, pecan nut, brazil nut, pistachio nut, macademia nut and Queensland nut)
- Celery and products thereof
- Mustard and products thereof
- Sesame seeds and products thereof
- Sulphur dioxide and sulphites at concentrations of more than 10 mg/kg or 10 mg/l expressed as sulphur dioxide.

Exemptions from these labelling regulations may be granted for highly processed food products. However, until 2007 such products have to be proven to be safe for allergic individuals.

Allergen detection in foods

Both industry and law-enforcement institutions need reliable methods to detect allergenic foods at relevant levels in complex food products. 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. As markers for the presence of potentially allergenic food products or ingredients, specific proteins or DNA fragments are targeted (Poms, Klein and Anklam 2004).

Protein-based methods usually involve immunochemical detection protocols such as rocket immuno-electrophoresis (RIE) and immunoblotting, which render only qualitative or semi-quantitative results, and fully quantitative methods such as enzyme-linked immunosorbent assay (ELISA), the radio-allergosorbent test (RAST) and the enzyme allergosorbent test (EAST). RAST, EAST and some other assays rely on human serum IgE. The latter is difficult to standardize and can only be handled in specialized (clinical) laboratories. Human-sera-based methods are therefore not suitable for routine food analyses (Poms, Klein and Anklam 2004).

ELISA-based methods

Presently, only the ELISA technique is routinely used in food analysis due to its high precision, simple handling, and good potential for standardization. Sandwich and competitive ELISA methods, and recently also dip-stick assays or lateral-flow devices (LFD), have been developed for several food allergens, and numerous test kits have become commercially available in this format during the last decade. The ELISA-based assays are highly specific for the respective food and depend largely on the molecular recognition of the employed food-specific antibodies. Any changes in the protein structure in a food due to processing will inevitably affect the performance of

the assay. Presently, not many studies on the effects of processing on the detectability of allergenic foods in food products have been published. However, the influence of heat-processing on the detectability and quantification of peanut protein is probably the most investigated (Poms 2003). Conventional heat-processing has shown to decrease the recovery of peanut material from processed food products, particularly when dry-roasted peanuts were tested. This effect can be attributed partly to reduced solubility of heat-denatured peanut proteins and partly to impaired antigen recognition by the employed antibodies.

PCR-based methods

Methods operating on the DNA level include PCR (polymerase chain reaction), Real-Time PCR and PCR-ELISA. The major advantages of DNA-based methods are the high specificity and the relatively high stability against environmental and technological influences. However, DNA is sensitive to low pH and shearing forces employed in some processes. Moreover, the employment of DNA analysis in allergen detection is discussed controversially, since proteins are the allergenic component and PCR results cannot be linked to any allergen/protein content. Additionally, processing may differentially affect nucleic acids and proteins (Poms, Anklam and Kuhn submitted).

Biosensors

The use of surface plasmon resonance biosensors has not yet been commonly applied for food analysis. Attractive features of this technology are short analysis time and a high degree of automation. Biosensor instruments make it possible to measure specific molecular interaction in real-time. 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 foods such as hazelnut, egg and milk, but to-date no kits have become commercially available (Jonsson 2002).

Fluorescence-Polarisation-based methods

Another approach for the detection of allergenic food residues on food production equipment or in food products is the employment of Fluorescence Polarisation (FP) (Poms unpublished). In a Fluorescence Polarisation ImmunoAssay (FPIA) the specificity of an antibody-based method is combined with a spectrophotometric detection system employing a fluorescence-tagged molecule. It is a competitive binding assay, similar to the competitive ELISA and radioimmunoassay (RIA). However, the reaction takes place in a cuvette (no immobilized molecules) and renders fast and highly quantitative results. The FP protocol combines easy handling with a highly robust analytical system, which has the potential for automation or can be applied for on-site testing. So far no kits have become commercially available.

Method considerations for allergen detection in foods

Sensitivity

Methods must be sensitive enough to specifically detect the allergens or allergenic foods in those amounts that might trigger allergic reactions in sensitized individuals. Unfortunately, data about established threshold levels that have been determined in human studies are scarce. However, it was shown that a level of 100 microgram of peanut proteins triggered a mild reaction in a peanut-allergic person. This could be caused by the consumption of 100 g chocolate or biscuits containing 1 ppm (mg/kg)

peanuts. Therefore, experts are of the opinion that lower detection limits (LOD) for allergens in different food products need to be in the low ppm (mg/kg) range, depending on the respective allergen and food product.

Specificity

Methods need to specifically detect the allergenic food of concern, but not necessarily only one specific allergenic protein (or epitope) of a particular food, as different proteins may be affected differently by varied processing steps and even allergic consumers might react differently to various allergens of the same food. However, a highly stable allergen may serve as a marker substance in the analysis of the respective allergenic food.

Matrix and processing effects

The matrix of a particular food can mask the allergen and likewise antibody-binding sites (epitopes) can be hidden or exposed after food-processing steps. These effects can impair the detection/quantification of food allergens by at the same time retaining the original allergenicity (Poms and Anklam submitted).

Method validation

Validation of the available methods is important to show that they are fit for the purpose to assure food safety for allergic individuals and to determine the absence/presence of certain ingredients/contaminants in food products. Method validation establishes performance criteria such as detection limit, recovery, accuracy and precision of the method. So far only five commercially available peanut ELISA test kits have been validated in an international collaborative trial (Poms et al. submitted).

Reference materials

To compare results and standardize/calibrate test systems, internationally recognized reference materials are needed. The Joint Research Centre of the European Commission is in the progress to produce such materials for peanut and gliadin. However, so far no certified reference materials for allergen analysis in foods have become commercially available.

The right choice

The choice of the right 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. Various test systems offered for the same allergenic food may be suitable for a particular food product but not for another. Moreover, protein-based 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.

Commercially available allergen detection/quantification assays

ELISA

ELISA test kits (see Table 1) are offered in two formats – as sandwich or as competitive ELISA, respectively. Sandwich ELISAs are available for the detection and quantification of almond, crustaceans, egg, hazelnut, milk, peanut, sesame, soy and gluten-containing cereals (wheat, rye, barley). Competitive ELISAs are available

Table 1. Commercially available ELISA test kits for the detection/quantification of food allergens. Status January 2004

Allergenic food	Target	Format	LOD [mg/kg]	Inter-laboratory 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	1	no	ElisaSystems
Egg	Ovomucoid and ovalbumin	Quantitative S-ELISA	1	no	ElisaSystems
	Egg protein	Quantitative S-ELISA	< 2.5	no	Neogen
	Egg protein	Qualitative S-ELISA	< 5	no	Neogen
	Egg protein	Quantitative S-ELISA	0.3	no	Pro-Lab Diagnostics
	Egg-white protein	Quantitative S-ELISA	2	no	R-Biopharm
	Ovomucoid	Quantitative S-ELISA	0.3	no	Tepnel BioSystems
Hazelnut	Specific heat-stable hazelnut protein	Quantitative S-ELISA	1	no	ElisaSystems
	Hazelnut protein	Quantitative S-ELISA	10	no	R-Biopharm
Milk	β-Lactoglobulin	Quantitative S-ELISA	1	no	ElisaSystems
	Casein	Quantitative S-ELISA			Announced 2003 Pro-Lab Diagnostics
	Casein	Quantitative S-ELISA	< 2.5	no	Neogen
	Casein	Qualitative C-ELISA	< 5	no	Neogen
	β-Lactoglobulin	Quantitative C-ELISA	5	no	R-Biopharm
	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
Peanut	Ara h 2	Quantitative S-ELISA	1	no	ElisaSystems
	Peanut protein	Quantitative S-ELISA	< 2.5	AOAC-RI 2003	Neogen
	Peanut protein	Qualitative S-ELISA	< 5	AOAC-RI 2003	Neogen
	Peanut protein	Quantitative S-ELISA	1.6	no	Pro-Lab Diagnostics
	Peanut protein	Quantitative S-ELISA	2	AOAC-RI 2003	R-Biopharm
	Ara h 1	Quantitative S-ELISA	< 0.1	AOAC-RI 2003	Tepnel BioSystems
Sesame	2S albumin	Quantitative S-ELISA	1	no	ElisaSystems
	Sesame protein	Quantitative S-ELISA	< 0.1	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	1.5	PWG 2002	R-Biopharm
	Gliadin	Quantitative S-ELISA	< 2	no	Tepnel BioSystems

only for various milk proteins and soya. Sensitivities of the respective ELISA test kits for the detection of allergenic foods are between 0.1 and 10 ppm (mg/kg) with the exception of a soya test kit with a limit of detection of 5000 ppm (0.5 %).

Dipstick assay/Lateral-Flow Device (LFD)

Currently, there are dipstick assays (see Table 2) available for peanut and gliadin. However, dipstick assays for egg and milk have been announced for 2004. The LOD of the offered dipstick assays for the detection of allergenic foods is between 5 and 10 ppm (mg/kg).

Table 2. Commercially available dipstick assays/Lateral-Flow Devices for the detection of food allergens. Status January 2004

Allergenic food	Target	Format	LOD [mg/kg]	Inter-laboratory validation	Supplier
Egg	Ovomucoid	Qualitative dipstick ELISA	< 10	no	Announced 2004 Tepnel BioSystems
Milk	Casein	Qualitative dipstick ELISA	< 10	no	Announced 2004 Tepnel BioSystems
Peanut	Peanut protein	Qualitative dipstick ELISA	< 5	no	Neogen
	Ara h 1	Qualitative dipstick ELISA	< 10	no	Tepnel BioSystems
Wheat, rye, barley	Gliadin	Qualitative dipstick ELISA	10	no	R-Biopharm
	Gliadin	Qualitative dipstick ELISA	< 10	no	Tepnel BioSystems

PCR-based methods

PCR-based detection kits for allergenic foods (see Table 3) are available in various formats. In fact, these different methods use either end-point detection of the amplified target DNA by DNA-ELISA or Agarose-gel electrophoresis (qualitative methods) or Real-time PCR (quantitative method). PCR-based test kits are available for almond, hazelnut, milk, peanut and soya. Additional kits will be introduced in 2004 for celery and gluten-containing cereals.

Table 3. Commercially available PCR test kits for the detection of food allergens. Status January 2004

Allergenic food	Target	Method	Qualitative/ quantitative	LOD[1] [ppm]	Inter-laboratory validation
Almond	DNA[3]	DNA-ELISA	qualitative	< 10	no
	DNA[3]	Real-time PCR	quantitative[4]	< 10	no
Celery	DNA[3]	Real-time PCR	Announced for 2004		
Gluten	DNA[3]	PCR + gel electrophoresis	Announced for 2004		
	DNA[3]	Real-time PCR	Announced for 2004		
Hazelnut	*Cor a 1.0401* gene	DNA-ELISA	qualitative	< 10	no
		Real-time PCR	quantitative[4]	< 10	no
Milk/Casein	DNA[3]	PCR + gel electrophoresis	qualitative	< 10	no
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	PCR + gel electrophoresis	qualitative	< 10	no
	Lectin gene	DNA-ELISA	qualitative	< 10	only for GMO[5]
	Lectin gene	Real-time PCR	quantitative[4]	< 10	only for GMO[5]

Conclusion

Several methods have been developed for the detection of food allergens in food products; however for some allergenic foods liable to the amended EU Labelling Directive no routinely applicable assays are available. Most of the currently available test systems are yet to be tested for their fitness for purpose by inter-laboratory validation studies, particularly in the light of adequate sensitivities (e.g. allergen contaminants in processed foods) to ascertain food safety for allergic individuals.

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