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Romer Labs®

Spotlights

Volume 14

> EDITORIAL

Food allergies are of growing concern to the public, because the number of people believing to have a food allergy is increasing. As a result, more and more countries require labelling of allergens on packaged food in order to protect consumer health. This raises the need for test systems or test kits for food allergens to be available. Firstly, so that food manufacturers can provide reliable labelling information to the public by including allergen testing in their quality control programs. Secondly, to enable authorities to check for compliance with the respective legislation.

Responding to this emerging food safety risk is one of the greater challenges facing the Industry. Consequently, analysts will need reliable test systems and capability to cope with the new developments in this still very much evolving field.

Romer Labs® entry in to food allergen testing comes during this important formative period. Romer Labs® has 25 years experience in developing analytical methods and test systems for detection of low level, sporadic contamination (for example with mycotoxins) and consulting customers to find their optimal solutions. Romer Labs® is continuing to expand its' array of test systems to enable sampling, screening and confirmation (including standards and certified reference materials) and will do so in food allergy testing.

Richard Fielder

Food Allergy has become a topic of major consumer concern during the last few years. They are one of the potential causes of an adverse reaction to food. Underlying the rise in diagnosed cases of food allergy there is a increased awareness and the need to better inform sufferers on a restricted diet.

Food Allergens – Why they are problematic and how to detect them

Several mechanisms can lead to undesired reactions to food. The different possible causes are presented in *Figure 1*.

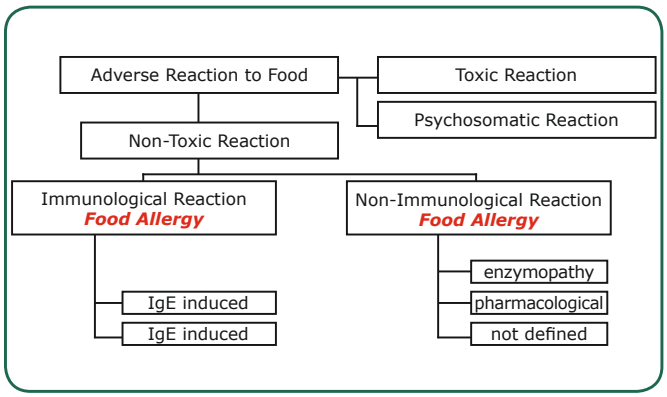

Figure 1. *Adverse Reactions to Food (Bruijnzeel-Koomen C, Ortolani C, Aas K, Bindslev-Jensen C, Bjorksten B, Moneret-Vautrin D, Wuthrich B. Adverse reactions to food. European Academy of Allergology and Clinical Immunology Subcommittee. Allergy 1995, 50:623-635).*

Toxic reactions, for example to aflatoxins in foods, will affect everybody and will occur with immediate exposure to the toxin. A special situation exists with psychosomatic reactions, as there can be no known objective mechanism or cause found. Non-toxic reactions will not affect everybody and the severity of reactions might differ strongly between individuals. Again, this category can be divided into reactions involving the immune system and others without, generally called food intolerances. Both are markedly different in cause, severity and spectrum of reactions. They may involve enzymes (e.g. lacking ß-galactosidase resulting in lactose intolerance) or can be caused by pharmacologically active substances. Food allergy itself is an immunological reaction, that can either be immunoglobulin E (IgE) mediated or be a non-IgE response. Most common food allergies, e.g. to peanut, milk, egg, etc., are caused by elevated levels of specific IgE in the patients. An example of non-IgE induced food allergy would be Coeliac disease, a hypersensitivity to gluten, a protein fraction in cereals.

Allergens, which are mainly proteins, typically cause a reaction in the immune system. On contact with an allergen, specific white blood cells, B-lymphocytes, produce allergen specific antibodies (IgE). These antibodies will be presented to mast cells which contain histamine. On second contact with the same allergen the proteins bind to the antibodies and in order to protect the body, mast cells will release histamine. This mechanism explains, why on a first contact with an allergen no allergic reaction will occur, but any subsequent exposure will lead to allergic symptoms. Symptoms can include reactions in the skin (itching, swelling, urticaria, etc.), eyes, the respiratory tract (from running nose to asthma bronchiale), gastrointestinal tract (vomiting, diarrhoea, etc.) to the cardiovascular system (anaphylactic shock). Definitive diagnosis of a food allergy is quite difficult and mainly done by anamnesis, skin tests, IgE determination or double blind placebo controlled food challenges (DBPCFC). As symptoms are not readily identifiable, not everything that looks like an allergy is really an allergy. Food intolerances, featuring non-immunological reactions and showing comparable symptoms, are more frequently found than food allergies.
So how do food allergens find their way into food products? Food allergens are regular food components and so part of many recipes and formulations. They are often added as an ingredient or as part of a premixed ingredient e.g. a herb mixture. In this case, manufacturers are aware of the presence of an allergen and can react accordingly by labelling the ingredient on the ingredients list. However, more problematic are cross contaminations of food with allergens. For example, this can happen during storage of ingredients when allergen containing foods are located next to ingredients without the respective allergens. Also, during food production itself there are several ways of transferring traces of one allergen to another product, e.g. when sharing utensils for mixing or weighing ingredients or when using an inadequately cleaned production line or piece of equipment. Inadequate cleaning can also cause so-called carryover, transmitting allergens via cleaning water or cleaning procedures. Even production staff can cross contaminate food by dirty clothing. Another possible cause could be the use of re-work materials. Cross contamination often leads to uneven distribution of an allergen in the food product, making finding this small or singular contamination a real challenge, e.g. one small piece of peanut in a chocolate bar amongst a large batch of chocolate bars.

An extremely important part of allergen management and control practices is the documentation of every aspect of the food manufacturing process. Such documentation serves the need for traceability in the event of future problems and potential product recalls. However, documentation cannot be relied upon alone without audits, inspection and testing to demonstrate that the control systems are working. Any agreed specifications require validation and this may include manufacturing, cleaning and analytical testing.

To protect consumers it became mandatory in many countries around the world to label the presence of certain food allergens in packaged food. European legislation following Commission Directive 2000/13/EC on the labelling, presentation and advertising of foodstuffs was amended to include Commission Directive 2003/89/EC indicating ingredients present in foodstuffs. It was further amended by Commission Directive 2005/26/EC establishing a list of food ingredients or substances provisionally excluded from Annex IIIa and corrected by Commission Directive 2005/63/EC concerning the list of food ingredients or substances provisionally excluded from Annex IIIa. In addition the Europe Union published Commission Regulation (EC) No 41/2009 concerning the composition and labelling of foodstuffs suitable for people intolerant to gluten.

To Date, April 2010, there are 13 food categories* (cereals containing gluten, crustaceans, eggs, fish, peanuts, soybeans, milk, nuts, celery, mustard, sesame, lupines, molluscs) and one chemical (sulphur dioxide and sulphite), which are included in these labelling directives.

Table 1. *Allergens regulated in different countries, Europe, USA and Japan*

Allergen	Europe	USA	Japan
Abalone			O
Apple			O
Beef			O
Buckwheat			X
Celery	X*		
Cereals containing gluten	X*		
Chicken			O
Crab			O
Crustaceans	X*	X	
Eggs	X*	X	X
Fish	X*	X	
Gelatine			O
Kiwifruit			O
Lupin	X*		
Mackerel			O
Milk	X*	X	X
Molluscs	X*		
Mushrooms			O
Mustard	X*		
(Tree)Nuts	X*	X	O
Oranges			O
Peaches			O
Peanuts	X*	X	X
Pork			O
Salmon			O
Salmon roe			O
Sesame	X*		
Shrimp/Prawn			O
Soybean	X*	X	O
Squid			O
Wheat		X	X
Yams			O

** …and products thereof"*
X … mandatory labelling
O … recommended labelling

Romer Labs® provides several antibody based test kits for different allergens. Table: Food Allergen ELISA Test kits

Product name	Item No.	Standard Range	LOD
AgraQuant® Peanut	COKAL0148	1-40 ppm	0.1 ppm
AgraQuant® Gluten	COKAL0248	4-120 ppm	0.6 ppm
AgraQuant® Hazelnut	COKAL0348	1-40 ppm	0.3 ppm
AgraQuant® Soy	COKAL0448	40-1000 ppb	16 ppb
AgraQuant® Almond	COKAL 0748	0.4-10 ppm	0.2 ppm
AgraQuant® Egg White	COKAL0848	0.4-10 ppm	0.05 ppm
AgraQuant® Walnut	COKAL0948	2-60 ppm	0.35 ppm
AgraQuant® Beta-Lacto-globulin	COKAL1048	10-400 ppb	1.5 ppb

All substances listed in Annex IIIa must be labelled for food and beverages. Substances which are not additives but are used in the same way and with the same purpose as processing aids and are still present in the finished product, even if in altered form shall be labelled. Any ingredients used in production of a food stuff and still present in the finished product, even if in altered form, and listed in Annex IIIa or originating from an ingredient listed in Annex IIIa shall be indicated on the label. Annex IIIa will be systematically re-examined and, where necessary, updated on the basis of the most recent scientific knowledge.

US Legislation is laid down in the Food Allergen Labeling and Consumer Protection Act. There the term "major food allergens" means any of the following: milk, egg, fish, crustacean shellfish, tree nuts, wheat, peanuts and soybeans. Also included is any food containing protein from a food specified as an allergen, except any highly refined oil and ingredient derived from such highly refined oil.

Japanese Legislation lists potentially allergenic ingredients which require labelling. Where labelling is mandatory those ingredients are eggs, milk, wheat, buckwheat and peanuts. In addition a list of ingredients where labelling is recommended is also published, containing abalone, squid, salmon roe, shrimp/prawn, oranges, crab, kiwi fruit, beef, tree nuts, salmon, mackerel, soybeans, chicken (poultry), pork, mushrooms, peaches, yams, apples and gelatine. The five products subject to mandatory labelling must be labelled even in the case of carry-over or processing aids. The main difference of Japanese to European and US labelling regulations is that indicating "May contain (name of allergen)" on the food label is not allowed.

In Australia, a new way of labelling allergens in food products is being promoted by the Allergen Bureau, organised by Australian Food & Grocery Council Allergen Forum. They promote a concept called VITAL (Voluntary Incidental Trace Allergen Labelling) as an essential standardised allergen risk assessment tool for food producers. VITAL allows food producers to assess the impact of allergen cross contact and provide appropriate precautionary allergen labelling on their products using different action levels relying on concentration ranges for different allergens.

In general, besides gluten and sulphite in the European legislation, there are no threshold levels defined in the regulations, creating major challenges for analysts. Ideally, specific thresholds for different allergens should be defined which would help development of methods and give analysts guidelines for results to comply with regulations. The absence of limits makes it impossible to identify foods with regard to a „safe" residual allergen content, a problem especially for highly sensitive allergy sufferers .

Another situation exists with gluten. Where the Codex Alimentarius recommends limits for labelling food „gluten-free". This threshold concentration is 20 ppm gluten in the finished food. The European Union adopted the recommendation in the Commission Regulation 2009/41/EC. This legislation is completely separate and cannot be applied to any labelling requirements under Commission Directive 2003/89/EC.

Since there are many regulations on allergen labelling in place there is a need for appropriate detection methods. Several technologies like specific antibody based tests e.g. enzyme linked immunosorbent assays (ELISA) or lateral flow assays, polymerase chain reaction (PCR) methods and mass spectrometry are available, all with varying degrees of commercialisation, giving both qualitative and quantitative results.

Antibody based tests (e.g. ELISA) will directly detect proteins from the foodstuff, whereas the DNA methods (e.g. PCR) will give an indirect result since they detect DNA specific to the foodstuff rather than the protein. Both methods are commercially available and used in routine analysis. Detection and quantification limits of those methods vary greatly depending on the test, allergen and food matrix. Decisions on which method should be used for the determination of the allergen are dependant on when and where the testing has to be performed. Cross contamination of production equipment might need a fast and easy method, like lateral flow tests, whereas analysing a final food product in a quality control lab or governmental lab will use more advanced and quantitative methods. Mass spectrometry methods are not currently used in routine testing due to prohibitive costs of equipment and the complexity of the analysis and interpretation.

The lack of reference methods as well as reference materials for food allergens makes comparison of different analytical methods extremely difficult, if not impossible. The fact that every method uses different calibrants and may target different components adds to this complexity. Although there are certain allergenic proteins which are well characterised the labelling regulations do not demand analysing specific allergens instead stating that the presence of the food itself which can cause the allergy needs to be labelled. In addition, proteins can change their structure during processing (e.g. by heat and pressure treatment) which can therefore lead to issues with detection. A similar problem is found with DNA which can be broken down during processing into small pieces and therefore may not be detected any more. This situation might be overcome to some extent by amplifying only very small fragments of the target gene. It cannot be stated that protein is more stable than DNA or vice versa, but combining these two techniques can be useful as a confirmatory approach, because they detect different molecular targets.

ELISA methods are at the moment the most widely used technology in quality control and government labs as a quantitative screening tool for on-site control and has become more popular in the manufacturing environment.
Until the time when new technologies, such as gene cloning or immunotherapy, will end food intolerance and allergy, patients have to rely on manufacturers' allergen control practices. However, it would seem that enforcement of allergen labelling legislation globally is still not as effective as it could be and is still absent in many places. Therefore, there is the danger that this ignorance of the importance of testing could lead to a higher risk of undeclared contaminated food and further product recalls, not to mention the risk to the allergic consumer. Food allergy must be taken seriously since it can be life threatening!

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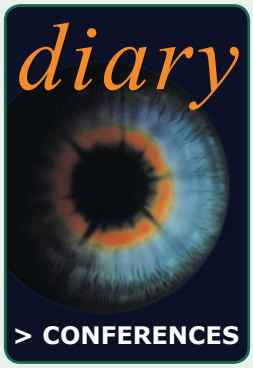

> CONFERENCE DIARY (FOOD SAFETY, ALLERGENS) 2010

Eurofins International Seminar Food Safety Solutions
April 21– 22, 2010, Paris, France

Food Allergen thresholds: implications for the Industry
April 29, 2010, Campden, UK

6th Workshop on Food Allergen Methodologies
May 09-12, 2010, Toronto, CND

Unravelling Allergens
May 20, 2010, Fartinghoe, UK

Symposium on Gluten-free Cereal Products and Beverages
June 8-11, 2010, Tampere, FIN

MoniQA Conference: Emerging and persisting food scares: Analytical challenges and socio-economic impact
June 8-10, 2010, Krakow, PL

AOAC Canada
June 10, 2010, Winnipeg, CND

AOAC-Midwest
June, 2010, Minneapolis, MN, US

IAFP
August 01-04, 2010, Anaheim, CA, US

AOAC International Conference
September 26-29, 2010, Orlando, FL, US

24th Meeting Working Group on Prolamin Analysis and Toxicity
October 1-2, Ancona, Italy

AACC Annual Conference
October 24-27, 2010, Savannah, GA, US

> Bibliography:

1) Bruijnzeel-Koomen C, Ortolani C, Aas K, Bindslev-Jensen C, Bjorksten B, Moneret-Vautrin D, Wuthrich B. Adverse reactions to food. European Academy of Allergology and Clinical Immunology Subcommittee. Allergy 1995, 50:623-35.

2) Management of Food Allergens (2009), eds. Coutts & Fielder, published by Wiley-Blackwell

3) **Commission Directive 2000/13/EC** on the approximation of the laws of the Member States relating to the labelling, presentation and advertising of foodstuffs

4) **Commission Directive 2003/89/EC** amending Directive 2000/13/EC as regards indication of the ingredients present in foodstuffs

5) **Commission Directive 2005/26/EC** establishing a list of food ingredients or substances provisionally excluded from Annex IIIa of Directive 2000/13/EC of the European Parliament and of the Council

6) **Commission Directive 2005/63/EC** - correcting Directive 2005/26/EC concerning the list of food ingredients or substances provisionally excluded from Annex IIIa of Directive 2000/13/EC of the European Parliament and of the Council

7) **Commission Directive 2007/68/EC** amending Annex IIIa to Directive 2000/13/EC of the European Parliament and of the Council as regards certain food ingredients

8) **Commission Regulation(EC) No 41/2009** concerning the composition and labelling of foodstuffs suitable for people intolerant to gluten

9) Labelling and consumer protection act

> IMPRINT

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