# FOOD ALLERGENS – A CHALLENGE FOR THE FOOD INDUSTRY

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**Abstract:** Food allergy represents an important food safety issue and the only effective treatment is the complete removal of the allergen from diet. However, food formulation are becoming more complex which means that foods may be unintentionally contaminated via allergencontaining ingredients or cross contamination affecting consumers, food companies and competent authorities. To tackle food allergen issue, food industry and control agencies rely on analytical methods to quantify the amount of a particular allergic commodity in a food. Nowadays mostly receptor based methods and in particular commercial kits are used in routine analysis. However, during processing chemical changes in allergens/proteins occur which affect the analytical outcome with issues related to extractability and matrix effects being the biggest challenge. Therefore, food industry needs to make extra efforts to provide accurate labeling and to reduce the contamination with allergens.

Keywords: food allergens, detection, challenges, food processing, extraction

# Introduction

Food allergy, an abnormal immunological response due to sensitization to food proteins, has become an important food safety problem, especially in industrialized countries (1). Food allergens pose a risk only to a limited number of consumers while being harmless to most of the other consumers regardless of the amount ingested. When ingested by allergic consumers, the symptoms can range from mild to severe and life threatening (2). Food allergies are estimated to affect about 2 % of the adult population and its prevalence is reported to be higher in infants and children (6-8 %). Over 180 allergenic food proteins have been identified until now with a few major allergens occurring in common foods (e.g. egg, milk, fish, crustaceans, peanut, soybean, wheat and tree nuts) (5). Food allergens are proteins or glycoproteins representing the major protein fraction in food and are typically reported to be resistant to proteolysis and food processing.

### Legislation and labelling obligations

Food allergens represent a serious safety issue because many of the allergic food commodities are important nutrient sources (milk, eggs, wheat based products, etc.) and thus their complete exclusion from diet is, while possible, not desirable. Because of their functionality, several of these products are frequently used as a food ingredient. Therefore, food industry is obliged to provide accurate labeling by clearly indicating the composition of the produced foods. The European Commission (Directive 2007/68/EC) sets up a list of allergens which have to be labeled on foods regardless of the amount deliberately added as ingredient (Table 1) (4).

<b>Tuble 1.</b> Ingreatent included in the Annex III d of the Directive 2007/08/EC
Cereals containing gluten and products thereof
Crustaceans and products thereof
Eggs and products thereof
Fish and products thereof
Peanuts and products thereof
Soybeans and products thereof
Milk and products thereof
Nuts and products thereof
Celery and products thereof
Mustard and products thereof
Sesame seeds and products thereof
Lupin and products thereof
Molluscs and products thereof
Sulfur dioxide and sulfites (concentrations of more than 10 mg/kg or 10 mg/liter)

Table 1. Ingredient included in the Annex III a of the Directive 2007/68/EC

Unfortunately, food allergens can still inadvertently be present in a product due to the fact that several different food products are produced within the same plant which can lead to cross-contamination. Cross-contamination might be caused by improper equipment cleaning/sanitation procedures, in case of a change from one product to the next but also due to re-work (5). This leads to the presence of the so called "hidden allergens". Over 1168 alerts due to presence of undeclared allergens in foods have been reported by the Rapid Alert System for Food and Feed in the EU alone (6).

The European "General Food Law" states that food manufacturers are responsible for the safety of food products, brought on the market (7). This means that food manufacturers need to take extra measures to prevent and control cross-contamination in order to protect the allergic consumers and their own reputation (expensive recalls). Nowadays warning labeling messages such as "May contain ..." or "Present in the processing environment" or "This product is made on a line/in a factory that also handles.." are extensively and sometimes unnecessarily used which is confusing for the allergic consumers. Pele et al. (8) showed that food products free of warning messages are often contaminated with food allergens while some of the labeled foods were reported to be allergen free. This shows that extensive preventive labeling practices run the risk of undervaluing the labels and consumers might lose their trust in food producers applying them in an excessive manner. In order to provide accurate information for allergic consumers, food industry must therefore have access to reliable extraction and detection methods. Such methods are needed to screen the incoming materials for the absence of undeclared residues of allergens, to evaluate the efficiency of the preventives measures such as sanitation programs applied to remove residues of allergenic foods from shared equipment in the frame of risk management at company level and to control the end products.

### Challenges related to food allergen detection in processed foods

Currently there are several analytical approaches applied for the detection and quantification of allergen traces in food products. These can either target the allergen itself (one or several allergenic or non allergenic proteins) or a marker that indicates the presence of the allergenic food. Among the methods targeting the allergen (protein based methods), the most used are the receptor-based methods (e.g. antibody based: enzymelinked immunosorbent assays (ELISA) and biosensors) and non-antibody based such as DNA-based and chromatographic and mass spectrometric methods. There are a number of requirements for the methods used for allergen determination in food namely they should be specific for the targeting compound, highly sensitive so that the lowest amount able to trigger an allergic reaction can be detected, must be specific and should not be influence by the presence of matrix components so that false positive and false negative results are avoided.

Food allergens/proteins have a very complex structure and upon processing they can be modified through numerous ways. They can be heat-denatured with disruption of the tertiary and secondary structure which might lead to modification of the conformational epitopes, they can be modified through Maillard reaction or partial hydrolysis which might modify the linear epitopes and they can aggregate and lose solubility. As previously mentioned, ELISA methods are based on the molecular recognition between the receptor (antibody) and the analytical target (the allergens/proteins). However, due to processing the interaction between the antibodies and the modified allergens/proteins can be affected which can lead to erroneous results (9-11).

Another important issue related to detection of food allergens is that they are present in trace amounts and their presence is often masked by the matrix compounds (12). This especially in case of the receptor-based methods (ELISA) which is caused by: (i) interaction of the target proteins/allergens with the matrix which hinders its extraction or (ii) co-extraction of matrix proteins which can non-specifically bind with antibodies therefore giving false positive results. But the most important is that there might be severe loss of extractability due to interaction of the target proteins/allergens with the matrix can which affect the detectability of food allergens. It is further known that for example interaction with matrix components, such as polyphenols and tannins from chocolate, might impair the extractability of the proteins/allergens as well.

Extraction represents another important cause of erroneous results obtained by all of the analytical methods used. One can only detect what is extracted. The yield of the extracted allergen depends on the type of allergen analyzed and the degree of modifications induced by processing. This means that preferably extraction methods should be also optimized and validated for specific products and processing conditions to help evaluate their applicability. Thermal processing is impairing the solubility of the allergens (13) and this can directly affect the robustness of the developed methods most often leading to false negative results. Fu et al. (14) showed that dry or moist heating of whole egg powder decreased with over 75 % the yield of extractable protein content. Similarly, Monaci et al. (15) reported over 80 % decrease in the yield of the extractable proteins from cookies. It is therefore important to make sure that the maximum amount of the targeted proteins/allergens is extracted.

In conclusion, all the analytical methods are prone to erroneous results especially if the extractability of the allergens/proteins is reduced. The results obtained by any analytical method should be evaluated with outmost care. False negative results can present a potentially fatal risk for allergic consumers, while false positive results may lead to unnecessary and costly product withdrawal.

#### What next?

Food companies are responsible for the safety of food products brought on the market and must implement a food safety management system based on good practices and Hazard Analysis Critical Control Point (HACCP) principles. Allergen risk management means that the chance for inadvertent presence of allergens in the end product through for example cross-contamination is thoroughly evaluated and minimized. Several guidelines are available for the food industry to set up an allergen management (e.g. on international level VITAL via http://www.allergenbureau.net/ or for Europe via http://www.eu-vital.org/en/home.html). Several key points of the production process need to be considered and managed (i.e. product design and formulation, raw material purchase, cross-contamination during production process, evaluation of the impact of rework, labeling and finally validation and verification). Unfortunately, "zero tolerance" level is difficult to achieve, therefore preventive measures should be taken to avoid cross-contamination. Cross-contamination can occur through several ways: air, contact materials, via personnel, carry-over from batches and through media such as oil and water during re-use. An important preventive measure is therefore cleaning. Validation of the cleaning is also necessary in order to estimate the risk of cross-contamination correctly and to adjust the cleaning methods if necessary (16).

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