Food allergies

A public health problem

Food allergy is a significant public health problem that has increased in recent years. It affects 2 to 3% of the worldwide population and its prevalence has reached 6 to 8% in children. Furthermore, an increase in the frequency and severity of allergic reactions has been observed over the last decade (Superior Health Council, publication N°8513). According to the World Health Organisation, food allergies are the 4th largest health problem.

Food allergies should be distinguished from food intolerances such as lactose intolerance or celiac disease. Allergies are abnormal IgE-dependent immunologic reactions to a particular foodstuff. Intolerances are either non-immunological reactions dependent on individual susceptibility such as lactose (with individuals that have a lactase deficiency), or immunological reactions that are not IgE-dependant such as celiac disease or gluten intolerance.

The symptoms vary (urticaria, diarrhoea, constipation, etc.) and can be severe (respiratory or cardio-vascular problems and anaphylactic shock). In serious cases, an allergic reaction can result in death. Minute amounts of allergens, in units of mg of protein, may cause reactions in sensitive patients. Only complete avoidance of the allergenic food can prevent an allergic reaction in such patients. The full and accurate labelling of all foodstuffs is therefore essential for these patients. The European directive 2007/68/EC has made the labelling of 14 allergenic ingredients for pre-packaged products mandatory:

- cereals containing gluten (namely wheat, rye, barley, oats, spelt, kamut or their hybridised varieties)
- seafood
- eggs
- fish
- peanuts
- soja
- milk
- nuts (namely almonds (Amygdalus communis L.), hazelnuts (Corylus avellana), walnuts (Juglans regia), cashew nuts (Anacardium occidentale), pecan nuts [Carya illinoiesis (Wangenh.) K. Koch], Brazil nuts (Bertholletia excelsa), pistachio nuts (Pistacia vera), Macadamia nuts and Queensland nuts (Macadamia ternifolia)
- celery
- mustard
- sesame
- molluscs
- white lupine
- sulphites

and products derived from these foodstuffs (with some exceptions).

This legislation relates to allergens intentionally added to products. However, allergens can be added non-intentionally as a result of cross-contamination between production lines, for example through raw materials or via staff. These hidden allergens constitute a risk for the allergic consumer. In order to evaluate the presence of these allergens, it is essential to have reliable and approved analytical tools at hand.
The methods of analysis

There are two main techniques currently used for detecting allergens: PCR (Polymerase Chain Reaction) and immunochemical methods such as ELISA (Enzyme-Linked ImmunoSorbent Assay) or LFD (Lateral Flow Device). Table 1 shows a comparison of both techniques. The PCR is based on the detection of allergen-specific DNA in the product tested. The advantage of this technique is its high specificity. However, highly refined products which still contain DNA but are protein free will be detected positive without posing a risk to allergic consumers. In addition, certain foods such as milk, eggs and food extracts contain a very low level of DNA in relation to the amount of protein. As a result they will not be detected by the PCR.

The immunochemical methods are based on the interaction between the antibodies and their antigens, or in this particular case, the food allergen proteins. ELISA is currently the most frequently used method for allergen detection. This method is simple, quick, sensitive (in units of mg/kg) and specific. Various approaches have been chosen in the development of ELISA methods for allergens. The antibodies are either directed against specific proteins with or without an allergenic potential, or against soluble protein extracts. Unlike DNA detection, protein detection can be interpreted as a potential risk for allergic consumers. However, certain proteins are sensitive to processing during the manufacturing stage (cooking, sterilisation, fermentation) and can be partially degraded or modified. As a result, they cannot be detected by the antibody test although their allergenic potential may be unaffected or even increased. Neo-allergens may also be produced during food processing as described in the case of the groundnut roasting process. Another problem of immunochemical methods is the capacity of the antibody to recognise protein motifs similar to the target protein. This is known as cross-reactivity, which may lead to false positive results. This may be the case for example with the nut family that has homologous proteins.

LFDs are very quick tests (5 - 10 min). If an allergen is present in a sample, a coloured line appears on the test strip. Currently, these tests pose problems in the control of allergens in foodstuffs following numerous false positive results and due to a lack of reproducibility.

Mass spectrometric analysis (MS) is increasingly described in literature as a means of addressing the disadvantages of the two other methods: specificity, the targeting of allergenic proteins, the possibility of analysing several allergens at the same time, etc. Following extraction, the proteins are directed by an enzyme (mainly trypsine), thereby forming peptides. Next, these peptides are separated by chromatography and identified by the mass spectrometer. The extraction efficiency and matrix interference are two parameters which limit the sensitivity of this technique. Moreover, this technique requires costly materials and highly qualified staff. As a result, it is not used routinely.

The lack of data

Currently, there is very little validation data available to compare the results obtained with these methods. This is no doubt due to the lack of internationally harmonised validation protocols and recognised reference materials. In fact, the standards are developed by research teams and are specific to the test used. A harmonised validation protocol has been published for the ELISA allergen detection methods (Abbott et al., 2010). It recommends the use of reference standards and supplemented reference materials. However, fully representative reference material is scarce. The protein profile of allergenic foods varies depending on the type of food, climatic conditions, crop location, production processes, etc. In addition, depending on the matrix, the allergen can be found in a raw state (milk powders in a soya milk powder) or in a processed state (milk powder in bread dough before cooking). Several international projects (MoniQA, Europrevall) aim to develop such model materials that are representative of the cross-contamination that can occur (Dumont et al., 2010). The results of interlaboratory validations based on the harmonised protocol should soon be published in order to ensure the comparability of the ELISA methods used.
Findings

Significant advances have been made over recent years in the area of food allergies, both on a clinical basis and on an assessment basis, using analytical tools and cross-contamination risk management in the agri-food industry. Much more extensive research is still required in terms of, among other things, determining the analytical method detection limits based on the clinical sensitivity thresholds and interpreting and correlating the results of the different methods and techniques. The FASFC has therefore designated the CER Group and ILVO-T&V as the National Reference Laboratory whose main task will be to answer such questions.

Table 1: Comparison of the two main allergen analysis techniques. (Kerbach et al., 2009)

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<tr>
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<th>Immunochemical</th>
<th>PCR</th>
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<tr>
<td>Detection</td>
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<td></td>
<td>Climatic variability</td>
<td>Extraction efficiency</td>
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<td></td>
<td>Extraction efficiency</td>
<td>Stable DNA at high temperatures but may fragment at low pH levels.</td>
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<td>Modification (glycosylation, phosphorylation, etc.) following processing during the production stage</td>
<td>Matrix effect - PCR inhibitors in the matrix</td>
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<td>Matrix effect</td>
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References:


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