Analysis to support food allergen risk management: Which way to go?
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Analysis to support allergen risk management:

which way to go?

Tatiana Cucu, Liesbeth Jacxsens & Bruno De Meulenaer

NutriFOODchem Unit (member of Food2Know), Department of Food Safety and Food Quality, Ghent University, Coupure Links 653 B-9000 Gent, Belgium

* Corresponding author: Ghent University, Coupure Links 653, B-9000, Belgium.
Tel.:+32 92 64 61 66; fax: + 32 92 64 62 15. E-mail address: Bruno.DeMeulenaer@UGent.be
ABSTRACT

Food allergy represents an important food safety issue because of the potential lethal effects and the only effective treatment is the complete removal of the allergen involved from diet. However, due to the growing complexity of food formulations and food processing, foods may be unintentionally contaminated via allergen-containing ingredients or cross-contamination. This affects not only consumers’ wellbeing, but also food producers and competent authorities involved in inspecting and auditing food companies. To address these issues, food industry and control agencies rely on available analytical methods to quantify the amount of a particular allergic commodity in a food and thus to decide upon its safety. However, no “gold standard methods” exist for the quantitative detection of food allergens. Nowadays mostly receptor based methods and in particular commercial kits are used in routine analysis. However, upon evaluation of their performances, commercial assays proved often to be unreliable in processed foods, attributed to the chemical changes in proteins which affect the molecular recognition with the receptor used. Unfortunately, the analytical outcome of other methods among which chromatographic combined with mass spectrometric techniques, but also DNA based methods seem to be affected in a comparable way by food processing. Several strategies can be employed to improve the quantitative analysis of allergens in foods. Nevertheless, issues related to extractability and matrix effects remain a permanent challenge. In view of the presented results, it is clear that the food industry needs to continue to make extra efforts to provide accurate labeling and to reduce the contamination with allergens to an acceptable level through the use of allergen risk management on company level which needs to be supported inevitably by a tailor-validated extraction and detection method.

KEYWORDS: food allergens, risk management, detection, challenges, food processing, extraction, competent authority, food company
1. INTRODUCTION

Food allergies involve abnormal responses to specific foods (mostly proteins) which are normally harmless and are mediated by the immune system (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 in industrialized countries and its prevalence is reported to be higher in infants and children (6 – 8%) (3, 4). 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 almost always proteins or glycoproteins with molecular weights of 5-70 kDa (3). They mostly represent the major protein fraction a particular allergenic food commodity and are reported to be typically resistant to proteolysis and stable during food processing.

It is well reported that for very sensitive patients trace amounts of allergens can induce severe and even fatal reactions. For example, as little as 30 µg of hazelnut is able to elicit an allergic reaction (6) while the predicted threshold values giving a one-in-a-million response rate was reported to be of 0.07 µg milk, 0.003 µg egg, 0.5 µg peanut and 0.3 mg soybean (7). Therefore, the only effective treatment for food allergies is their complete avoidance from the diet (8). 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 not possible or desirable. Moreover, because of their functionality, several of these products or products thereof are frequently used as an ingredient in various composite foods. For individuals affected by severe, life-threatening food allergies this is a significant food safety issue and thus the protection of such allergic consumers is of concern for the food industry and public health authorities. Therefore, the
food industry is obliged to provide accurate labeling by clearly indicating the composition of each food product and to bring safe products on the market. The European Commission (Directive 2007/68/EC) set up a list of allergens which have to be labeled on foods regardless of the amount deliberately added as ingredient (9) (Table 1). In various countries, similar lists and labeling obligations are applicable (10-12). Unfortunately, despite these legislative frameworks, food allergens can still inadvertently be present in a product mainly due to the fact that several different food products are made within the same plant which can lead to an in-process and post-process 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 (13). This leads to the presence of the so called “hidden allergens”. Over 600 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 (14). The presence of such hidden allergens poses a serious threat to the allergic consumer and might lead to food recalls which are expensive for the industry (15). Moreover, in the European “General Food Law” it is stated that food manufacturers are responsible for the safety of food products, brought on the market (16). This means that food manufacturers need to take extra measures to prevent or control cross-contamination in order to protect the allergic consumers and their own reputation. Considering that no specific legislative framework is available for the labeling of food products possibly contaminated with allergens, it is not always clear for food producers how to manage this issue. Therefore, 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 often used. However, the extensive use of such warning messages can be confusing for the allergic consumers and parallel is leading to a narrowing of the food products available for them. Eating outdoors is another issue for the allergic consumers indicating that they are forced to rely mainly on the food prepared at home. In
order to offer some comfort to allergic consumers, warning messages should be accurately
applied. This implies that they are supported by a proper risk management on company level.
Pele et al. (17) 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
indicates that such extensive labeling practices run the risk of undervaluing the labels and as a
further consequence consumers lose their trust in them and in food producers applying them
in an ill-based manner.

In order to provide accurate information for allergic consumers, food industry must
have access to reliable extraction and detection methods meaning tailored and validated to the
production and product characteristics, and insight in possible contamination routes during
processing and manufacturing. Such extraction and detection methods are needed to screen
the incoming materials for the absence of undeclared residues of allergens, to evaluate the
efficiency of the implemented 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. Moreover, availability of robust analytical
methods would allow to decide whether a product should be recalled or not in case of
calamities. An allergen risk management program at company level to control the presence of
hidden allergens, backed up by reliable extraction and detection methods, validated for the
company specific production processes and product matrix, should be part of a food safety
management system in a food company.

2. QUANTIFICATION OF FOOD ALLERGENS

Currently there are several analytical approaches applied for the detection and
quantification of allergen traces in food products (3, 18-20). These can either target the
allergen itself (one or several proteins) or a marker that indicates the presence of the
allergenic commodity. Among the methods targeting the allergen (protein based methods), the
most used are the receptor-based methods (e.g., antibody based: enzyme-linked immunosorbent assays (ELISA) and biosensors (21-27) and non-antibody based such as DNA aptamers (28, 29)) and chromatographic and mass spectrometric methods. DNA based methods such as polymerase chain reaction (PCR) with real-time PCR providing quantitative results are also available for food allergen detection, but are an indirect indicator of the potential presence of the targeted allergen.

There are a number of requirements for the methods used for allergen determination in food. The used analytical methods need to be specific for the targeting compound, highly sensitive so that preferably the lowest amount able to trigger an allergic reaction can be detected, must be specific and should not be influenced by the presence of matrix components so that false positive and false negative results are avoided. Only recently, protocols on the validation of commercial available allergen detection systems became available by the European Committee for Standardization (CEN) (30-32).

2.1. Protein based methods

2.1.1. Receptor based methods

Receptor based methods are widely used for allergen detection and are based on the interaction between specific antibodies and an antigen (food allergen). They can be developed to detect one or several proteins (allergenic or not) from the allergenic food commodity. Considering that foods contain several proteins, the choice of the appropriate analyte is crucial for the development of reliable methods. Another important aspect for the receptor based methods is the choice of the primary antibodies (monoclonal or polyclonal). Nowadays, ELISAs are the method of choice by the food producers and control agencies for routine analysis of food allergen contaminations and commercially available kits are mostly used. ELISA is used because it is relatively cheap, easy to perform and has a high sensitivity. Generally two formats of ELISA are available: sandwich and competitive. In the case of the
sandwich ELISA the primary antibody immobilized on the plate captures the food allergens which are further detected by a secondary allergen specific antibody labeled with enzyme. In the case of competitive ELISA food allergens immobilized on the plate are competing with the allergens from the sample to bind with the primary antibody labeled with enzyme. Unfortunately no studies are available where comparison of the robustness of these two ELISA formats for food allergen detection is evaluated.

2.1.2. Chromatographic techniques

Chromatographic techniques, where direct detection of one or several allergenic proteins is possible, are also frequently used. For example, high performance liquid chromatography (HPLC) methods with fluorometric detectors were developed for the detection of lysozyme, a known food allergen from hen’s egg, in dairy products (33, 34). More complex protein mixtures, such as soybean proteins in adulterated meat, bakery and dairy products could also be analyzed by HPLC (35-38). A more extensive review of liquid chromatographic (LC) methods developed for especially major food allergen detection is available elsewhere (39). Nevertheless, the use of chromatographic methods coupled solely to UV or fluorescent detection can be troublesome in foods because the identification of the allergenic proteins can be hampered by the presence of other matrix proteins which can co-elute and might lead to false positive results. Therefore, coupling liquid chromatographic equipment to mass spectrometric analyzers enables to unambiguously confirm the presence of the allergens.

2.1.3. Mass spectrometry

Mass spectrometric (MS) techniques are extensively used to study proteins and peptides. Since food allergens are mostly proteins, many mass spectrometric methods for their identification and detection are reported as well. Most of the reported methods are subjected
to separation via SDS-PAGE, 2D-PAGE or LC prior analysis by MS of the either intact protein or peptides obtained after proteolytic digestion. Detection of intact allergenic proteins, especially derived from milk, was previously reported (40-42). Technique in which detection of food allergens is based on the finding of specific markers resulting from the proteolytic digestion of native and/or modified proteins are also often employed. When using such approaches it is important to identify stable peptide markers especially when developing quantitative methods. Targeted detection of such selected molecules (selected reaction monitoring) or multireaction monitoring enables high selectivity and thus accurate quantification. So far, peptide markers for peanut were identified and used for development of quantitative MS techniques (43-46). A more extensive review of mass spectrometric techniques used for identification and detection of food allergen detection is available elsewhere (5, 18, 39). The mass spectrometric methods represent a valuable tool for confirmation of the presence of food allergens in different food matrices if contradictory results are obtained using other methods. Unfortunately, due to the fact that the equipment used is rather expensive and highly qualified personnel is needed to operate such systems, the MS techniques are not appropriate to be used in routine analysis in a food company based setting, contrasting typically with immunological based methods.

2.2. DNA-based methods

The principle of the DNA-based methods involves targeting a segment of the gene coding for the allergenic or other proteins of interest and amplifying only this DNA fragment to make it detectable (18). Unfortunately, DNA-based methods do not detect the allergen itself, and the results are difficult to be correlated to actual allergen quantities and thus cannot be used in a proper risk assessment and management. Despite these disadvantages, DNA
methods are extensively used for allergen detection because of their ease in application (47-52).

2.3. Challenges related to allergen detection in food

The main disadvantage of the receptor based method are the fact that small variations in the analytical target lead to great variability of the analytical outcome. De Meulenaer et al. (53) showed that antibodies developed towards roasted Virginia peanuts had a cross reactivity of below 50% with other roasted peanuts varieties. This indicates that underestimations of the actual contamination levels might occur. A certain level of cross reactivity especially when different varieties of allergenic commodities are analyzed is advisable. On the other hand, cross reactivity with other food components (especially bulk proteins) might lead to false positive results or overestimation of the contamination levels. For example, if antibodies raised against hazelnut proteins have 1% cross reactivity with milk proteins this would lead to an analytical result of 100 ppm hazelnut in a cookie containing 1% milk proteins when extraction is performed on 1 g cookie/10 mL extraction buffer. Additionally, major issues related to extraction, matrix effects and impact of processing can be expected during food allergen analysis. Therefore they are further discussed more in details.

2.3.1. Incomparable results with commercial kits

Unfortunately there is no general agreement on the expression of reporting units which makes it difficult to compare the results of allergen detection kits. Some kits use standards reported as “amount of allergenic commodity” while other report “amount allergenic protein”. Considering that the protein content in different commodities (soybean, tree nuts, lupine seeds, etc) might depend on the variety, this hampers comparison of the results between kits. Remarkably, a similar issue plays also with respect to the interpretation of LC-MS based methods. Indeed typically one or several particular indicator peptides are used to quantify the
content of a particular allergenic commodity in the analyzed food, based on the concentration of the indicator peptide in a kind of reference allergenic commodity. Again, the concentration of such an indicator peptide may depend upon several factors however and cannot be regarded as absolutely constant (54). Coming back to immune assays, we have shown in a previous study that detection of native hazelnut and soybean proteins depends highly on the type of commercial kit (55, 56) (Table 2 & 3). When testing 4 commercial kits for hazelnut, only one kit proved to give accurate results with untreated hazelnut proteins, 2 kits underestimated the actual protein content with on average 20% and another kit underestimated the actual content with over 90%. On the contrary, one of the commercial kits for soybean highly overestimated the actual protein content, another underestimated with about 20%. When analyzing heat treated samples either in the presence or absence of glucose, the obtained results were also kit dependent although mostly an underestimation was observed. It should be noted that these results were obtained using relative simply protein solutions, not real food matrices. The difficulties related to quantitative comparisons between kits. Further, the performance of commercial kits depends highly on the type of food matrix analyzed as well. Whitaker et al. (57) for instance showed that the performance of 4 commercial peanut kits was especially poor in cookies.

The accuracy of commercial kits depends upon the chemical modifications induced in the untreated food but also depends upon the actual protein concentration present (56-59). As a consequence, care should be taken when using such kits for quantitative analysis because erroneous decisions related to risk assessment can be made.

2.3.2. Matrix effects

Another important issue related to detection of food allergens, by any of the above mentioned methods, is that they are present in trace amounts and their presence which might
be masked by the matrix. Especially the receptor based methods tend to perform differently depending on the type of matrix analyzed. This is mostly caused by: (i) interaction of the analyte 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 hindered extractability due to interaction of analyte with the matrix can affect the detectability of food allergens by MS methods as well (60, 61). Interaction with matrix components, such as polyphenols and tannins from chocolate, might impair the extractability of the analyte (62).

Husain et al (63) showed that extracts from foods can influence the IC50 value of the calibration curves at different degrees. In order to take into account such matrix effects, it is possible to work with calibration curves prepared in extracts of particular allergen free foods (63-65). Unfortunately, this means that the developed methods should be validated for each particular food product separately.

2.3.3. Processing

Food allergens (proteins) have a very complex structure and upon processing they can be affected through numerous ways. They can be 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 proteins). However, due to processing the interaction between the antibodies and the modified allergens can be affected which can lead to erroneous results. Unfortunately a decreased detectability due to allergen modifications does not necessarily mean a decrease in allergenicity with in some cases even an increase in allergenicity being observed (55, 66-70). It is therefore of outmost importance
to have reliable methods able to detect not only the native allergens but also the allergens modified through reactions typically occurring during processing and storage.

Previously it was shown that detection of proteins modified through Maillard reaction, protein oxidation and partial hydrolysis is severely affected if commercial kits are used \((58, 59, 71-77)\). Complete loss of detectability of an in-house developed method using antibodies against Kunitz Trypsin Inhibitors was observed in cookies prepared with lactose \((64)\) while Ecker et al. \((78)\) observed a decrease in detectability of lupine in cookies upon baking. Additionally, losses of detectability using LC-MS in strongly processed dairy products were also reported \((40)\), indicating that MS-based proteomic approaches are prone to protein modifications as well. Such loss in detectability might be either due to the fact that the targeted proteins and/or peptides are modified during processing resulting in mass shifts and poor ionization but also because of reduced proteolysis \((79)\). The integrity of DNA can also be modified during processing which might lead to erroneous results as well \((73, 80, 81)\).

### 2.3.4. Extractability

Extraction represents another important cause of erroneous results obtained by all of the analytical methods used. Any of the analytical methods mentioned above will only detected 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 the extraction methods should be also validated for specific products and processing conditions to help evaluate their applicability. Thermal processing is impairing the solubility of the allergens \((82)\) and this can directly affect the robustness of the developed methods most often leading to false negative results. Fu et al. \((74)\) showed that dry or moist heating of whole egg powder decreased with over 75 % the yield of extractable protein content. Similarly, Monaci et al. \((75)\) reported over 80 % decrease in the yield of the extractable proteins from cookies.
baked for 9 min at 180°C. It is therefore important to make sure that the maximum amount of the targeted analyte is extracted.

Furthermore, when developing new analytical methods for food allergen detection, the robustness is often evaluated based on the determination of the recovery after spiking allergen free products shortly before the extraction step (63, 83-88). Even in such cases the time when the spiking is performed is critical. A comparison of the recovery assessed on samples spiked 24h before extraction revealed that the recovery is significantly lowered probably due to interactions with the matrix compared to spiking shortly before extraction even if no processing is applied (33). Similarly, good recoveries were be obtained when spiking blank cookies with hazelnut shortly before extraction (73 – 107 %) (65). On the contrary, spiking 2 h before extraction led to an approximate 60 % decrease in recovery, again without the application of any processing step as such. However, contamination of food products with allergens is most likely caused by the use of contaminated primary materials or due to the use of shared equipment and this before any food processing conditions are applied. Therefore, determination of the recovery in samples incurred before applying any processing is more accurate and should be applied in a proper validation process of any analytical method. Several studies where evaluation of the robustness by incurring samples before processing was done are published (64, 89-96). We have shown that when determining the robustness in cookies incurred before processing was applied only about 10 % recovery were obtained (65).However, the recovery of the DNA seems to also be affected by processing mainly because of its degradation and/or hindered extractability (51).

In conclusion, all the analytical methods mentioned above are prone to erroneous results especially if the extractability of the analyte is reduced. Obviously the results obtained by all methods applied should always be considered with outmost care. False negative results can present a potentially fatal risk for allergic consumers, while false positive results may lead
to unnecessary product withdrawal. It is therefore advisable to be very critical when using such methods because the results obtained are mostly semi-quantitative or qualitative, but in general unreliable. Ideally these methods should be validated via a case to case approach (individual allergens and as many matrixes as possible), but considering the number of possible products produced within one single plant, this is not realistic.

3. NEW TRENDS IN QUANTIFICATION OF FOOD ALLERGENS

In order to overcome the above mentioned issues related to the detection of food allergens in processed foods, new strategies for the development of more robust methods can be employed.

3.1. Use of antibodies raised against modified proteins

Most of the ELISA methods developed for food allergen detection are using antibodies raised against one or several non-modified proteins (protein extracts) (63, 78, 86, 88, 92, 94, 97-102). However, as above mentioned, decreases in detectability of food allergens due to hampered recognition of the modified analytical target by the used antibodies can occur. Therefore, development of antibodies against proteins modified through reactions typically occurring during processing might help improve the detectability. We have previously shown that the detectability of soybean proteins could be improved by using antibodies against modified soybean protein extract compared to antibodies raised against Kunitz trypsin inhibitor in especially highly processed foods (64). In general antibodies can be raised either against thermally treated protein extracts (64, 65) or protein extracts from processed foods (53, 83, 87, 91, 103-105) but also against partially hydrolyzed proteins (106). Gaskin et al. (91) developed an ELISA method where antibodies raised against protein extracts from raw and roasted cashew were used. Using antibodies with good affinity to the native as well as modified proteins can help to improve the detectability.
3.2. Use of antibodies raised against stable proteins

Using antibodies raised against one stable protein could also be a potential strategy to improve the robustness of analytical assays. In such cases it is crucial to investigate how stable the proteins are under different processing conditions. In a previous study we have shown that Cor a 9 from hazelnut is rather stable upon incubation with carbohydrates and lipids (58). Moreover, several stable peptides derived from Cor a 9 could be detected in modified hazelnut protein extract as well. An additional advantage of choosing the Cor a 9 as analytical target is its abundance in hazelnut thus, it can be easier detected in foodstuffs contaminated by hazelnuts. Therefore, Cor a 9 was evaluated as a promising target for the detection of hazelnut traces in processed foods. When using anti-Cor a 9 antibodies in a sandwich ELISA, the robustness of the receptor based analytical methodology was improved (26). Similarly, antibodies against one single allergen such as the stable Gly m Bd 30K allergen were used for the detection of soybean (93, 95) and antibodies against the 2S albumin for the detection of walnut in processed foods (96).

3.3. Use of stable peptides as analytical targets

A relatively new strategy employed to improve the detectability of food allergens via receptor based methods is by using antibodies raised against a single peptide. Earlier Akkerdaas et al. (106) showed that using an ELISA with antibodies raised against pepsin digested hazelnut proteins can improve detectability of hazelnut residues in chocolate. You et al. (107) and Liu et al. (108) developed antibodies against specific epitopes from β-conglycinin. When using such approaches it is of utmost importance to select peptides which are stable during processing ensuring that the affinity of the antibody towards the selected peptide is not affected. On the other hand, stable peptides are also needed for the development of accurate quantitative MS techniques. As mentioned previously, marker peptides were
identified only for a limited number of food allergens. In a previous study, we have identified stable peptides derived from whey, soybean and hazelnut allergens (109-111).

3.4. Multi-allergen methods

Multi-allergen detection methods are often used for diagnosis of allergy (112, 113). However, the interest for developing receptor based methods as well as mass spectrometric techniques for the detection of several allergens in foods is growing rapidly. Rejeb et al. (104) developed a multi-allergen immunoassay for the detection of several nuts. Multi-allergen mass spectrometric methods were developed as well. Heick et al. (114) developed a LC-MS method which allowed to detect simultaneously seven allergens (milk, egg, soybean, hazelnut, peanut, walnut and almond). Bignardi et al. (115) developed a multi-allergen MS method for the detection of several tree nuts (cashew, hazelnut, almond, peanut and walnut) with detection limits of as low as 10 mg/kg. Tortajada-Genaro et al. (116) developed a DNA microarray method for the simultaneous detection of hazelnut, peanut and soybean in foods while Ehler et al. (117) developed a PCR method for the simultaneous detection of 10 allergens (peanuts, cashews, pecans, pistachios, hazelnuts, sesame seeds, macadamia nuts, almonds, walnuts and brazil nuts). Development of such multi-allergen detection methods is interesting and can be especially useful for the screening of incoming ingredients from different suppliers but also for the fast screening of the final products.

Disregarding these new strategies that can be used to improve the detectability of allergens, the number of variables that can affect their quantitative detection is enormous. Due to this, it is difficult to say with certitude whether a certain food product is free of allergens or not. In the absence of a reliable and validated extraction and/or detection method, food producers should take the responsibility for producing allergen free products through a proper allergen risk management.

4. ALLERGEN RISK MANAGEMENT
The three important quality assurance systems for a food company to assure food safety and hygiene are (118): GMP (Good Manufacturing Practice), PRP (Prerequisite Programs) and HACCP (Hazard Analysis Critical Control Points). Within this approach, a PRP is foreseen for allergen risk management on company level. Allergen risk management does not have the intention to make a whole new food safety management system, but it should be included as a basic condition in a food company to control and manage direct allergens and hidden allergens due to cross-contaminations (119). The main objective of the allergen risk management must be to prevent adverse reactions in food-allergic consumers without unnecessarily limiting their food choices. Extraction and analytical methods are necessary to back up the allergen risk management in validation and verification activities e.g. screening of raw materials and ingredients and validation of the impact of preventive measures such as cleaning or separated storage activities.

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 (16). Allergen risk management means that the chance for inadvertent presence of allergens in the end product through for example cross-contamination is thoroughly evaluated. 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). Many of them are obviously very generic and a further tailoring to the company specific setting and validation is therefore necessary. In general food industry should evaluate particular available methods (either commercial or in-house developed) for their reliability within the company and when these prove to be so then the allergen management program can be validated. This especially because some tests can prove to be extremely accurate with
particular food matrix and processing conditions while not with others as earlier also discussed.

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). Figure 1 is illustrating the concept of an allergen risk management on company level. Ideally, the complete physical separation of allergen-free and allergen containing production lines or areas should solve the cross-contamination issues. However, this approach is mostly not economically feasible for the producer and above this, allergens could still be introduced in the plants when contaminated raw materials or ingredients are purchased. The supplier must provide accurate information to the producer concerning the presence of allergens in the raw materials or ingredients. Also, information about possible cross-contamination should be transferred to the producer. Forms or checklists can be used in order to give the correct information (120). When developing a new food product, it is important to determine whether the allergens used in the product can be replaced by an non-allergic component (120). When using new ingredients food business operators should always determine whether they contain allergens or not. A change of the production process could also affect the final presence of allergens in a food product (121).

Unfortunately, because “zero tolerance” level is difficult to achieve, preventive measures should be taken to avoid cross-contamination. Little is known about the frequency or amount of cross-contamination of allergens due to shared materials or infrastructures. 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 (122). An important preventive measure is cleaning. Validation of the cleaning is necessary in order to estimate the risk of cross-contamination correctly and to adjust the cleaning methods (123).
Roder et al. (13) investigated the cleaning efficiency for the reduction of cross-contamination of hazelnuts in the industrial production of cookies. Kerkaert et al. (122) used lysozyme as an indicator for the protein carry-over in fresh cut vegetables through washing water. They reported that allergenic proteins can be transferred via wash water to the fresh-cut vegetables in the next batch to quantities which are able to pose a risk for the allergic consumers. This innovative approach could potentially be extended to validate e.g. cleaning or other typical processes. By using one or several indicator proteins of which the analytics are properly validated with respect to the actual process in which they are applied, their carry over can be monitored in a quantitative way, thus enabling to extrapolate their behavior to that of a particular allergen present in the product as well. The rework of products represents another significant potential risk. Using this knowledge in combination with the accurate control of the critical points in production lines, prevention of contamination of allergen free foods might be facilitated. Further, it is important that products containing allergens are clearly labeled and kept separately if they are later reused in other products (120).

Unfortunately, because of the complexity of the food allergen issue, a single solution will probably not be realistic. A multiple strategy to support a validated allergen management on company or even production line level seems to be the only valid approach to enable the food industry to produce safe foods for allergic patients.

Also at governmental level and the competent authority, inspecting and monitoring the safety of the food chain, could progress towards other strategies to manage the problem of allergens. During inspections and audits performed in food companies, attention could be made towards the implementation of a risk management at company level, as previously described. Also during the monitoring of products available on the market, the reliability of used extraction/detection methods is questionable considering the issues raised earlier. A possible approach in the monitoring and selecting the best extraction and detection method
could be the evaluation of the impact of the process and matrix on the extraction and detection of the allergen from the food matrix. If a low impact is seen than the methods can be applied for the monitoring purposes. However, from the discussion above it became clear that this is not always the case. Therefore, it would be recommended to know the recovery of the extraction and to know the best performing detection method for a certain food matrix. The obtained result of the quantity of specific allergen could be recalculated for the loss of recovery towards the final food product. Instead of elaborating such an approach for each food product, a classification of food products could be made. This approach is in line with the current European legislation for migration of components from plastic food contact materials, where reduction and correction factors are introduced to recalculate the actual migration from the obtained laboratory results for specific food products (124).

**ACKNOWLEDGEMENTS**

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6. FIGURE CAPTIONS

Figure 1: Overview of the concept of allergen risk management

Figure 2: Table of Contents Graphic
7. TABLES

Table 1. Annex III a of Directive 2007/68/EC

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<thead>
<tr>
<th>Cereals containing gluten and products thereof</th>
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<td>Lupin and products thereof</td>
</tr>
<tr>
<td>Molluscs and products thereof</td>
</tr>
<tr>
<td>Sulfur dioxide and sulfites (concentrations of more than 10 mg/kg or 10 mg/liter)</td>
</tr>
</tbody>
</table>
Table 2 Ratio (%) of measured over actual hazelnut protein concentration after duplicate analysis of hazelnut proteins (40 ng/mL) either in native form or after 48h heat treatment in the absence or in the presence of glucose (55)

<table>
<thead>
<tr>
<th></th>
<th>Veratox</th>
<th>Ridascreen</th>
<th>HN residue</th>
<th>Biokits</th>
</tr>
</thead>
<tbody>
<tr>
<td>Native hazelnut</td>
<td>5.32</td>
<td>85.69</td>
<td>73.08</td>
<td>116.54</td>
</tr>
<tr>
<td>Heat treated hazelnut</td>
<td>4.33</td>
<td>79.76</td>
<td>61.13</td>
<td>85.23</td>
</tr>
<tr>
<td>Heat treated hazelnut with glucose</td>
<td>1.80</td>
<td>112.15</td>
<td>39.99</td>
<td>59.26</td>
</tr>
</tbody>
</table>
Table 3 Ratio (%) of measured over actual soybean protein concentration after duplicate analysis of soybean proteins (50 ng/mL) either in native form or after 48h heat treatment in the absence or in the presence of glucose (56)

<table>
<thead>
<tr>
<th></th>
<th>Veratox</th>
<th>Soy residue</th>
<th>Biokits</th>
</tr>
</thead>
<tbody>
<tr>
<td>Native soybean</td>
<td>374.67</td>
<td>84.81</td>
<td>*</td>
</tr>
<tr>
<td>Heat treated soybean</td>
<td>n/d</td>
<td>n/d</td>
<td>364.72</td>
</tr>
<tr>
<td>Heat treated soybean with glucose</td>
<td>n/d</td>
<td>n/d</td>
<td>176.67</td>
</tr>
</tbody>
</table>

* - not calculated, absorbance value obtained for the present concentration was above the calibration range; n/d – not detected
Level 1: research and development:
- product formulation
- impact of process on proteins?

Level 2: Selection raw materials/ingredients:
- specifications: presence of allergens
- allergen management of supplier: risk estimation of presence of hidden allergens
- database of (hidden) allergens per product

Level 3: Production:
- identification of possible cross-contamination routes
- preventive measures to control cross-contaminations e.g. segregation, cleaning procedures
- management of re-work

Level 4: Consumer awareness:
- legal direct allergens
- hidden allergens: ‘may contain’ labeling based on qualitative or quantitative data

Level 5: Validation and verification:
- sampling and analysis to demonstrate preventive measures and allergen risk management is effective (=validation)
- time to time sampling and analysis, internal auditing, people performance to demonstrate allergen risk management is working properly (=verification)

Figure 1
Figure 2