



The role of incurred materials in method development and validation to account for food processing effects in food allergen analysis

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Received: 21 November 2018 / Revised: 22 January 2019 / Accepted: 24 January 2019
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Abstract

The issue of undeclared allergens represents a matter of great concern, being the subject of many alert notifications by the Rapid Alert System for Food and Feed portal of the European Commission, often leading to food recalls. The availability of reliable analytical approaches able to detect and quantify hidden allergens in processed foods is increasingly requested by the food industry, food safety authorities and regulatory bodies to protect sensitive consumers' health. The present review discusses the fundamental role of incurred materials for method development and analytical performance assessment in a metrology perspective on testing for undeclared allergens in processed foodstuffs. Due to the nature of the analytes and their susceptibility to various processing effects, reliability and comparability of results have posed a great challenge. In this context, the use of incurred samples as reference materials permits simulation of the effects of food processing on target analyte structure affecting analyte extractability and detectability.

Keywords Food allergens · Incurred materials · Method validation · Reference materials · Food processing · Food safety

Introduction

The most recent epidemiology studies agree on the continuous increasing prevalence of food allergy worldwide and highlight global disparities of the incidence proportion, influenced by numerous genetic and environmental factors, as well as by gene-environment interactions [1, 2]. Consequently, food labelling regulations vary considerably around the world [3], also in accordance with local dietary habits.

The only treatment for sensitive individuals is the lifelong dietary avoidance of food allergens that requires a very complex and careful management involving, as main actors, not only the patients but also the food industries, the government

agencies, and the public health authorities for proper education, legislation, and constant food safety surveillance.

Although hundreds of different foods, including legumes and some fruits and vegetables as emerging food allergens, may have the potential to elicit allergic reactions even at trace levels [4, 5], current labelling requirements are focused on some “priority food allergens” accounting for the vast majority of severe reactions. In particular, European Union Regulation No. 1169/2011 establishes a list of 14 food allergens that must be declared on labels or indicated as being present in foods, namely, gluten and gluten-containing cereals, fish, crustaceans, molluscs, milk, eggs, mustard, sesame, celery, lupine, sulfite, soybeans, peanuts, and other nuts [6]. However, current legislation does not address the unintentional inclusion of allergens due to cross-contamination along the entire food chain from production to point-of-sale. The European Commission Rapid Alert System for Food and Feed (RASFF) portal notifies every week alerts on the presence of undeclared allergens in food. A very recent and comprehensive survey by Pádúa and co-workers shows how the entry into force of EU Regulation 1169/2011 in December 2014 has resulted in a significant increase of the number of allergen-related RASFF notifications from 2015, probably due to the intensification of controls by industry and producers [7]. The 2017 RASFF Annual Report states 114 notifications concerning undeclared food allergens,

Published in the topical collection *Young Investigators in (Bio-)Analytical Chemistry* with guest editors Erin Baker, Kerstin Leopold, Francesco Ricci, and Wei Wang.

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being milk, gluten, soya, and nuts the most commonly detected allergens, whereas cereals and bakery products the most often notified foodstuffs [8].

In the last years, the issue of hidden allergens and the lack of regulated threshold levels have caused the uncontrolled proliferation of different types of unregulated precautionary allergen labels (PAL), such as “it may contain”, “produced in a facility that also processes”, that food industry voluntarily adopts to alert consumers about the possible inadvertent presence of undeclared allergens. Interestingly, very recent analytical surveys proved the inconsistent application of PAL, since many food products with PAL statements were found to not contain any amount of detectable allergens [9], or even the allergen warned for was not necessarily related to the undeclared ingredients detected [10]. This excessive precautionary behavior of food producers leads not only to the unnecessary restriction of food choices by sensitive consumers, but also to confusion and to self-interpretation of risk [2, 3, 11]. In addition, undeclared allergens may even result from food adulteration and fraud [12], mainly aimed at economic gains.

According to the ISO 22000 international standard, in food industry the Food Safety Management System has to consider the hidden allergen risk through the use of reliable and properly validated analytical methods to ensure safe food supply throughout the chain [13, 14]. The determination of allergens in food is a challenging analytical issue, since food allergens are in most cases a complex mixture of proteins, which differ greatly in their physicochemical properties and relative abundance; in addition, their amount and composition is prone to vary due to the origin, variety, growing and harvesting conditions, transport, storage, and processing [15–17]. In particular, uncertainties in the effects of processing on the physical and chemical properties of a protein pose a significant challenge in the development of reliable methods for allergen detection and determination.

Except for gluten (limit value of 20 mg/kg [18]) and sulfur dioxide/sulfite (concentration limit 10 mg/kg or 10 mg/l in terms of the total SO₂ [6]), lack of regulatory limits for compliance assessment requires the development of analytical methods sensitive enough to detect and quantify allergens at trace levels in complex food matrices. In addition, considering also limited availability of suitable certified reference materials, there is no general agreement on the result expression units [19], e.g., “amount of allergenic commodity” or “amount of allergenic protein”, making difficult comparison of data obtained using different analytical methods/assays, thus hampering metrological traceability of the measurement results [19–23]. Parker et al. [24] devised a novel targeted multi-allergen liquid chromatography (LC)-tandem mass spectrometry (MS/MS) method using isotopically labeled peptides as chemical surrogates to the native marker allergen peptides for peptide identification, confirmation, and quantitation purposes. Quantitative results were achieved by translating the LC-MS/MS peak area associated to a monitored

peptide into parts per million of dry allergen ingredient through the application of a series of conversion factors including nominal protein molecular weight, relative allergen protein content in total protein composition, total extractable protein, moisture content, and sample preparation recovery.

If mandatory allergen labelling has improved the safety of food for allergic consumers, on the other hand, it should be stressed that food safety management and food compliance assessment demand the development of reference harmonized methodologies for the detection and quantification of allergens in foods. In this context, in the last years, European Union funding programs financed projects dealing with assessment and management of food allergen risk. In 2011, MoniQA (Monitoring and Quality Assurance in the Food Supply Chain) non-profit-distributing association was launched as EU-funded Network of Excellence (MoniQA, 2007–2012) involving international and interdisciplinary competences (more than 500 experts from over 40 countries on 5 continents) with the final aim of harmonizing worldwide strategies and guidelines for food safety and quality monitoring [25], focusing the attention also to food allergen analysis issues affecting data reliability [21]. MoniQA has also liaised with the EU-funded project iFAAM (Integrated Approaches to Food Allergen and Allergy Risk Management; 2013–2017), aimed to the development of evidence-based approaches and tools for the management of allergens in food [26]. Recently, EFSA launched the call GP/EFSA/AFSCO/2017/03 “Detection and quantification of allergens in foods and minimum eliciting doses in food allergic individuals”, the project partnership being formed from five organizations distributed in four different member states (UK, IT, BE, and FR).

The present review paper aims to gather evidence from literature about the fundamental role of incurred food materials as suitable reference materials in analytical method development and validation to assure the quality, reliability, and comparability of results obtained with different allergen detection methods. Incurred control samples allow accounting for food processing effects on analytical performance in food allergen analysis. Since incurred reference material is produced to mimic as closely as possible the processing through which an allergen-containing food goes during production, there is a distinct advantage as it more closely reflects the real-world foods that are routinely tested in analytical laboratories across the world and gives a good indication of the suitability of the analytical methods used to detect processed allergens.

Analytical strategies for food allergen analysis

Since legislation lists allergens in terms of whole food ingredients, the analytical target definition is anything but trivial, so that different factors such as the allergenic ingredient and the

final food product types, the required sensitivity, the food processing effects, the biological variations, and the presence of regulated limits for food compliance assessment have to be taken into account in analytical method development [27, 28]. In this context, it is worth to point out the great challenge concerning compliance assessment of “gluten-free” and “very low gluten” foods, starting from the definition of gluten as “protein fraction from wheat, rye, barley, oats, or their cross-bred varieties and derivatives thereof, to which some persons are intolerant and which is insoluble in water and 0.5 M sodium chloride solution”, according to the European Union Regulation and the Codex Alimentarius [18, 29]. Gluten is a complex mixture of hundred storage proteins, the expression of which depends on biological factors and growing conditions. These proteins have high level of homology and are characterized by different physico-chemical properties; owing to different solubility in aqueous alcohol solutions (40–70% (v/v) ethanol), they can be roughly separated into two fractions: the soluble prolamins and the insoluble glutelins, according to Osborne’s solubility-based fractionation. As a consequence, different measurands giving different response factors, sensitivity, and extractability can be operationally defined. In addition, when only a fraction is determined, a conversion factor has to be applied to calculate gluten concentration in order to comply with the action level units established by current legislation; however, this value was assessed only approximately (e.g., generally a multiplying factor 2 is recommended to convert prolamins into gluten concentration [29]) and is variable among different gluten sources and growing conditions [30].

In this respect, the analytical strategies available for food allergen determination can be classified as protein-based or DNA-based methods according to the analytical target of the allergenic food, i.e., characteristic proteins or specific DNA sequences, respectively. Although the direct monitoring of the proteins responsible for the allergic reaction would be desirable, sometimes the selection of alternative proteins or DNA-sequence targets, which are indicative of the presence of the allergenic ingredient, is needed.

The growing and actual interest of analytical research on food allergen analysis is proved by recent extensive reviews and book chapters focused on methodological aspects [22, 27, 31–43], to which the reader is referred for technical details; in particular, in the last years, emphasis is put on advances in mass spectrometry-based proteomics [22, 35–37] and on future trends towards biosensors and consumer-friendly devices [38–43].

Immunoassays, based on the specificity of antigen-antibody interaction, are traditionally used for routine analysis, in particular in the format of sandwich or competitive enzyme-linked immunosorbent assay (ELISA). Commercially available ELISA kits are particularly suited to be implemented both for qualitative and quantitative analysis

in food industry laboratories for allergen risk management thanks to their operational ease, low costs, good sensitivity as well as automatable execution. However, there are several critical factors in the quality and reliability of the immunoassays, leading to false positive or false negative results and high discrepancies among the results obtained from different ELISA kit assays [22, 30, 44–47]. These limitations are mainly related to (i) antibody cross-reactivity; (ii) partial or complete loss of immunoreactive epitopes caused by food processing; (iii) extraction conditions affecting antigen or antibody reactivity; and (iv) absence of harmonized results between kits of different manufacturers due to differences in extraction procedures, analytical targets, calibrants, and antibodies.

Advances in protein-based methods for food allergen analysis involve the use of LC-MS-based techniques as confirmatory strategies for unambiguous identification and accurate quantification of proteins due to intrinsic high specificity and sensitivity. For this purpose, a bottom-up proteomic approach based on the monitoring of characteristic proteolytic peptides as analytical targets is usually applied [26, 48]. Technology improvements and advanced signal acquisition modes permit to overcome the issue of antigen-antibody recognition process in immunoassays, by providing information on the primary structures of proteins, and to perform effective multiplex high-throughput investigations in a single chromatographic run [22, 49]. For all these reasons, in the last years analytical research efforts aimed at allergen analysis mainly focused on the development and validation of a variety of innovative MS-based proteomic methods [50–61]. In this context, Careri and coworkers [62–64] and Gu et al. [65] developed bottom-up MS-based proteomics approaches paying particular attention to protein extraction, pre-concentration, and clean-up procedures for enhanced sensitivity in LC-MS-based multi-allergen determination in complex food matrices.

DNA-based methods represent an indirect approach for food allergen analysis measuring allergen coding genes, in which the analytical target is not the protein itself but the gene sequence encoding for it. Selectivity of DNA-based strategy relies on careful selection of DNA target sequences indicative of allergenic species, often coupled with nucleic acid amplification by traditional polymerase chain reaction (PCR)-based methods for enhanced sensitivity [66–70]. Recently, an alternative nucleic acid amplification method termed loop-mediated isothermal amplification (LAMP) proved successful for the specific, sensitive and rapid identification of peanut DNA [71]. DNA-based techniques present advantages over methods based on protein detection owing to the efficient extraction of DNA in denaturing conditions as in processed food analysis, and DNA stability over geographical and seasonal changes; however, it has to be highlighted that the indirect nature of the approach does not permit to achieve a quantitative result on a particular allergenic protein or ingredient,

and is prone to false-negative or false-positive results for the presence of allergens in food [34].

Biosensors have recently emerged as powerful alternatives to traditional immunoassay methods for the detection and determination of allergens, offering suitable sensitivities and selectivity while allowing for on-site detection. Biosensors combine a transducer with a bio-recognition element, such as an antibody, an antigen, a single-stranded DNA/RNA sequence, an aptamer, etc., able to achieve the required selectivity for target analyte determination. High versatility, also in terms of sensing platform materials, high speed, ease of use, and system miniaturization are among the main advantages offered by biosensing devices, resulting suitable also for hidden allergen analysis in food safety assessment [72–77]. These features make them suitable also for on-line screening along the production chain and on-site real-time analysis, consistent with the concept of “point of care” (POC) testing, i.e., performed at the time and place of the patient care. In this context, as reported in a very recent review article [43], the latest trends in the technological advancements in food allergen detection concern novel POC devices, being developed in combination of immunosensors and genosensors. However, one of the biggest challenges for food allergen detection is the lack of commercially available biosensors. In addition, immunosensing and genosensing devices do not solve the above discussed intrinsic limitations associated with classical immunoassays and DNA-based methods. For this reason, a proper analytical validation process is required and parallel development of a MS-based method for confirmatory purposes is encouraged.

Food processing: an issue for allergen analysis

Food processing techniques are used to transform raw food materials into final consumable products. In the last years, significant efforts have been focused on the study of traditional and novel processing techniques for the production of hypoallergenic foods: both thermal and non-thermal techniques have been shown to alter allergen reactivity by modifying allergen structure via changes in structural epitopes [78–80], with applications also in infant formula [81].

The effects on protein structure depend on the processing technology, on the food constituents, and on moisture content: the principal modifications include protein denaturation, conformational change, aggregation, interaction, and reaction with other matrix constituents (i.e., lipids, carbohydrates, polyphenols, tannins), intra- and inter-molecular bond formation, chemical modifications, and protein hydrolysis [82]. If modifications of structural integrity of proteins by food processing could improve the quality of life for allergic individuals, on the other hand, they considerably complicate food allergen detection and control for safety assessment, making

important the evaluation of food processing impact at the molecular level [83, 84]. The modification of epitope structure affecting antigen-antibody affinity by food processing is critical in any immunoassay, leading to underestimation or even false-negative results, whereas different solubility/extractability of proteins in processed food is known to affect target analyte recovery [45, 53, 55, 85]. Using ELISA and flow cytometry analysis, the influence of baking time, cookie dimension, and temperature profile was studied [86]: these parameters were found to affect not only accuracy of quantitative results of casein, egg, gluten, and soy allergens in cookies, but also to hinder detectability of some of them (soy and egg) when the strongest processing conditions were applied; in particular, the authors observed a decrease of allergen recovery with increasing processing time and decreasing cookie size, that are conditions corresponding to the highest temperature at the center of the cookie during the baking process. These results can be explained by structural changes of allergenic epitopes promoted by heat application and by formation of insoluble aggregates that drastically reduce protein extractability. Also, Monaci et al. [85] observed a decrease in the extraction efficiency of the total protein fraction as baking time increases: choosing baked cookies as a model food system, protein extractability by phosphate-buffered saline buffer was found to drop by almost 80% within the first 10 min of baking. The authors investigated also the influence of baking time on milk allergen detection by four commercial ELISA kits for analyses of different classes of milk allergens, i.e., total milk proteins, caseins, and lactoglobulins. As shown in Fig. 1, a decrease of measured milk allergen content with increasing baking time is observed in all cases; however, the analytical targets exhibited a different behavior ascribable both to differences in their heat stability, affecting solubility/extractability

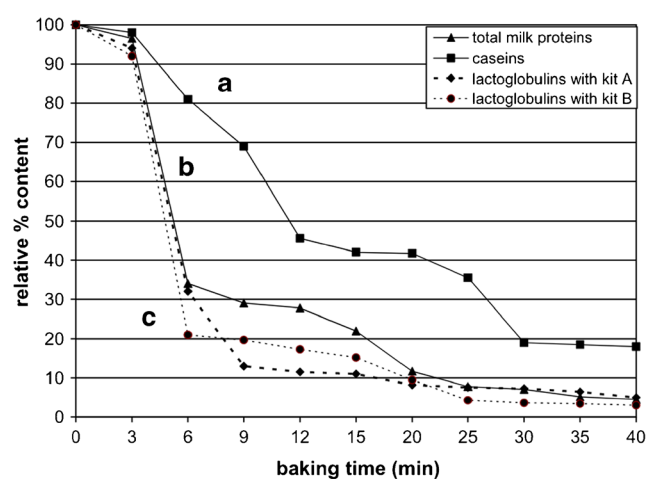


Fig. 1 Effect of the baking time on the results from four different ELISA kits: (line a) kit for total milk detection; (line b) kit for caseins; (lines c) kits (A and B) for lactoglobulins. The relative ELISA signal is expressed as a percentage of the value obtained from the dough. (Reproduced with permission from reference [85]. Copyright © 2011 Elsevier Ltd.)

and epitope structure maintenance, and to different experimental conditions between the ELISA kits tested (i.e., antibody, extraction protocol, calibrator, ELISA format).

In the study of food processing impact on protein structure and detectability, it is interesting also to decompose the whole food processing procedure in order to evaluate, separately, the contribution effects of the main subset events [21, 24]; in this context, Bugyi et al. [21] performed ELISA assays for milk and egg allergens on samples from powder mixture, raw dough, and cookies, observing a slight decrease in measurable protein amount due to kneading, whereas baking process caused a severe drop (36% and 2% for cookies with milk and egg powder, respectively).

Another analytical issue for accurate food allergen analysis is related to a partial degradation of proteins to peptides and amino acids during enzymatic fermentation or chemical hydrolysis. Li and coworkers [87] focused attention on fermented condiments, as soy sauce and vinegar, that may contain gluten from wheat and/or barley. In this case, despite the great advantage of MS-based approach regarding the possibility to identify and detect hydrolyzed proteins, gluten peptide markers were not detected due to an extensive hydrolyzation even though wheat was reported among ingredient in the soy sauce sample.

All these findings evidence the urgent need of appropriate extraction protocols involving the use of proper detergents, reducing and denaturing agents to solubilize target analyte from processed foods for food allergen analysis [88–90]; at the same time, sample preparation procedures have to be compatible with the analytical technique involved, in terms of extract purification especially for LC-MS-based analysis, and in terms of immuno-recognition process that should occur also in the case of denatured antigens in immunoassay strategies. The sample treatment procedure devised should be validated for specific sample matrix types and food processing conditions.

For DNA-based methods, the effect of food processing is lighter than protein-based approaches, since target DNA molecules can be efficiently extracted under harsh denaturing conditions and are characterized by higher stability to food processing than proteins; on the other hand, it has been proved that DNA molecules can be negatively affected by thermal treatments as well in terms of molecular integrity and quality, with a reduction in DNA yield and amplificability [70, 91].

Incurred materials for development and validation of food allergen methods

The effective implementation of analytical methods in food safety control management, especially in case of legislative compliance assessment, requires the demonstration that they “fit for purpose” through method validation [92]. Providing

test reliability, method suitability assessment is the key aspect to ensure method applicability to real samples, especially when challenging analytical issues have to be addressed, as in the case of hidden allergen determination in complex processed food matrices. In fact, the strategies proposed for food allergen determination have to take into account also issues related to sampling and sample treatment processes, since the complexity of the food matrix, its physical form and heterogeneity as well as food processing technology have a significant effect on the analytical results [46, 93]. In this context, the extraction of trace amounts of food allergens from the matrix can be a key issue in recovery tests for accuracy assessment. In fact, in a method validation protocol, it is essential to specify the strategy of fortification with food allergen ingredients, since analytical method performance considering spiked samples, i.e., reference samples artificially prepared in the laboratory by adding analyte to processed blank matrix, can vary compared with incurred food samples. In fact, spiked materials are produced from the addition of allergenic ingredients to processed blank food, whereas incurred materials derives from their inclusion in blank raw materials before food processing. It is clear that the latter approach permits to reproduce conventional formulation, realistic incorporation of allergens inside the food microstructure and allergen modifications due to food processing, thus allowing more rigorous assessment of quantitative method performance in terms of extraction yield, accuracy, and sensitivity when processed foods are analyzed [53–55].

Incurred materials can be prepared in a food processing pilot plant or test kitchen facility, paying attention to the homogeneous distribution of food allergen ingredients and to cross-contamination avoidance. In addition, the shelf-life of the incurred control samples must be ascertained if they are to be stored for a certain period of time, since further reactions between the proteins of the allergen and the components of the food matrix into which it is incorporated could occur during storage. The choice of allergenic ingredients to be used for fortification is a crucial aspect during method development and validation, with the preference towards model components typically used in industrial food manufacturing (e.g., peanut butter or defatted peanut instead of raw peanuts or even allergenic protein standard solution). Sharma et al. [94] observed that the recovery values of four different commercial ELISA kits for gluten determination varied with the allergenic ingredient used for incurred cornbread preparation, i.e., gluten or wheat flour, depending also on the kit used. In addition, in the study of Parker et al. [24] nonglutinous cereal bars and muffins were compared for recovery of milk, egg, or peanut before and after baking using commercial ELISA kits, evidencing reductions in recovery for all allergens analyzed after baking; in particular, the authors observed that analytical accuracy might be further impaired when dark roast peanut flour is used as ingredient for incurred baked samples. In fact,

often roasted tree nuts are used as ingredients in food formulation, thanks to improved flavor and taste ascribable to Maillard reaction between proteins and reducing sugars, having however an impact on solubility and detectability of the allergenic proteins [95].

An overview of published studies involving the use of incurred materials for method development and validation is illustrated in Table 1. At present, owing to the few commercially available reference materials, a lack of harmonization in allergen ingredients used for incurred material preparation is observed. On the other hand, it has to be considered that the preparation of incurred materials might be time-consuming and expensive, not only when National Institute of Standards and Technology (NIST) or the Joint Research Centre of the European Commission (EC-JRC) reference materials specifically intended for use in food allergen testing are utilized for the preparation of incurred materials at different allergen levels, but also when different matrix types and food processing have to be investigated. Among the different approaches proposed, incurred samples were prepared from a highly concentrated incurred dough and the further dilutions, before heating process, were performed by mixing it with blank dough [98]. In other approaches, incurred materials, prepared at high concentration levels, were dry diluted with processed blank matrices to obtain incurred samples with intermediate food allergen concentrations [50, 55, 58, 107]. Another strategy involved the preparation of highly concentrated incurred samples and serial dilutions of incurred sample extracts with appropriate volumes of blank sample extract were performed in such a way to cover a proper concentration range in incurred samples for calibration purposes [55]. However, it should be noted that in this way, no investigation can be undertaken on the possible variability on the extraction efficiency and on the impact of the whole food processing due to different concentration levels of allergen ingredients in the incurred material. In the study of Khuda and coworkers [108], impact of food processing on recovery and variability of multiple allergens analysis in a single matrix when using immunochemical methods was investigated. For this purpose, incurred cookies samples were rigorously prepared starting from a highly concentrated flour premix of nonfat dry milk, egg powder, and peanut flour, then added at appropriate level to the remaining dry ingredients before kneading and baking processes. Using different commercial ELISA kits for quantitative purposes, the authors observed not only a negative effect of baking process on percent recovery and precision for all the investigated allergens, but also a certain variability on the recovery values at the different incurred concentrations and higher analytical coefficients of variation at the lowest incurred levels.

The overview reported in Table 1 clearly points out how the very recent research efforts are focused on the development of new LC-MS-methods, as the most promising non-

immunological strategy for accurate and reliable food allergen analysis. In fact, despite ELISA assay still represents the currently applied approaches for routine food allergen controls due to its operative simplicity and high-throughput capability, there is an urgent need to strengthen confidence both in raw materials and in processed products. As a consequence, MS-based methods are characterized by high versatility in terms of instrumental technology, data acquisition modes, target or untarget analysis, selection of species to monitor, multi-target analysis, and calibration approaches to such an extent that a certain method can be properly tailored for the specific application, as discussed further below.

In this context, Careri and coworkers developed a multiplex LC-MS/MS method for the analysis of celiototoxic cereals, namely, wheat, oats, barley, and rye, in raw and processed food products, to assess the different behavior of spiked and incurred samples with regard to analytical recovery; unlike ELISA assays, the proposed MS-based approach was able to discriminate among the investigated cereals through unique marker peptides monitoring [53]. Method performance assessed in spiked rice flour evidenced satisfactory recovery values. However, when self-made incurred bread was investigated, poor trueness in terms of recovery was achieved, i.e., between 3 and 30% depending on the peptide, probably due to a reduced gluten extractability due to the baking process. These findings evidenced the important role of an efficient sample extraction and of the use of proper reference materials for protein calibration purposes for accurate determination of gluten.

A very recent study of Pilolli et al. [55] deals with the development of a high resolution mass spectrometry-based method for multi-allergen, i.e., egg, milk, soy, peanut and hazelnut, determination in cookies; three sample sets, i.e., mixture of allergen ingredients, multi-allergen spiked cookies, and multi-allergen incurred cookies, were used to construct three different calibration curves. By comparison of the calibration slopes, matrix effects and food processing effects were separately assessed, since the former takes into account interference of the matrix components, whereas the latter permits to isolate the contribution of thermal processing on method sensitivity. As expected, Fig. 2 shows how the three sample sets provided a significant difference in method sensitivity, i.e., up to 2–3 orders of magnitude, the lowest performance being exhibited for incurred cookie samples. In addition, matrix and processing effects were found to differ not only between allergen ingredients but also to depend on the monitored peptide.

A recent work of Parker et al. [24] was aimed at investigating the effects of thermal treatment on allergen detection in industry-processed model foods incurred with egg, milk, and peanut allergens. Analytical performances of commercial ELISA kits for allergen determination in thermally processed bakery products were studied. In particular, method

Table 1 Overview of the published articles involving food incurred materials for method development and performance assessment in food allergen analysis

Food/protein allergens	Incurred processed food materials	Allergenic ingredients	Analytical techniques	Protein/DNA extraction from incurred materials	Reference
Egg; milk	Cookies	Whole milk powder (IRMM BCR-380R) (from EC-JCR); whole egg powder (NIST RM 8415) (from NIST)	Commercial ELISA kits	According to kit producer's instructions	[21]
Egg; milk; peanut	Cereal bars; muffins	Whole egg powder (from Rembrandt Foods, USA)	Commercial ELISA kits; nanoUHPLC-QTRAP-MS/MS (MRM mode)	ELISAs: according to kit producer's instructions; LC-MS/MS: defatting and extraction with TBS buffer containing urea and dithiothreitol, vortexing, sonication, end-over-end rotation	[24]
Milk; egg; soybean; peanut	Tomato sauce; chocolate; cookies; ice cream	Lyophilized milk powder (27% protein), soy flour (36% protein) (from local store); egg yolk (16% protein) isolated from egg (from local store); egg white powder (79% protein) (from Barry Farm Wapakoneta, USA); peanut butter (NIST 2387 22.2% protein) (from NIST, USA)	UHPLC-QqQ-MS/MS (MRM mode)	Extraction with Tris-HCl buffer containing urea, shaking, sonication	[50]
Milk; egg; soy; peanut; tree nuts (pistachio, cashew, hazelnut, almond, walnut, pecan)	Tomato sauce; chocolate; cookies; ice cream	Peanut butter (NIST 2387 22.2% protein), milk powder (NIST 1549a 25.64% protein), whole egg (NIST 8445 48% protein), soy flour (NIST 2324 53.37% protein) (from NIST); tree nuts (from local store)	UHPLC-QqQ-MS/MS (MRM mode)	Extraction with Tris-HCl buffer containing urea, shaking, sonication	[51]
Peanut	Chocolate dessert	Not specified; produced in the framework of the iFAAM project	UHPLC-MS/MS (MRM-mode)	Extraction with Tris-HCl buffer containing urea, shaking, sonication	[52]
Gladians (<i>gluten /wheat</i>); barley; rye; oats	Bread	Gladiin from wheat (powder) (from Sigma-Aldrich, Italy); oat, barley, and rye seeds (from local supermarket)	HPLC-LTO-MS/MS (pseudo-SRM)	Defatting, extraction with ammonium bicarbonate buffer containing 2-mercaptoethanol and guanidine hydrochloride, heating, intermittent vortexing, addition of ethanol 80% (v/v), agitation	[53]
Egg; milk; soy; peanut; hazelnut	Cookies	Skimmed milk powder; egg powder; pre-cooked soy flour; ground roasted peanut, ground roasted hazelnut (from Besana s.p.a., Italy)	UHPLC-LIT-MS/MS (SRM mode)	Extraction with Tris-HCl buffer, probe-type sonication	[54]
Egg; milk; soy; peanut; hazelnut	Cookies	Egg powder, skimmed milk powder (from Sigma-Aldrich, Italy); pre-cooked soy flour (from local retailer); roasted peanuts and hazelnuts (from Besana s.p.a., Italy)	UHPLC-Q-Orbitrap (t-SIM/dd2 mode)	Extraction with Tris-HCl buffer, probe-type sonication	[55]
Egg white; milk; peanut; soy; tree nuts (almond, Brazil nut, cashew, hazelnut, pecan, pine nut, pistachio, walnut)	Bread; cookies	For quantitative analysis: whole-egg powder (SRM 8445), whole milk powder (SRM 1549a), peanut butter (SRM 2387) (NIST, USA); in-house prepared baked hazelnut. For screening analysis: egg-white powder, skim-milk powder (from local baking supplier store); raw peanuts (shelled), soy, tree nuts (shelled) (from local wholesaler)	HPLC-QTRAP-MS/MS (MRM mode)	Defatting and extraction with Trizma base buffer containing urea and β -D-glucopyranoside, vortexing	[56]
Egg; milk; peanut	Cookies	Spray-dried whole-egg powder (NIST 8445) reference material, nonfat dry milk (NIST 1549) standard reference material (from NIST, USA); partially defatted light-roast peanut flour (from Golden Peanut Co., USA)	nanoUHPLC-QTRAP-MS/MS (MRM mode)	Defatting and extraction with TBS buffer containing urea and dithiothreitol, agitation, sonication, end-over-end rotation	[57]

Table 1 (continued)

Food/protein allergens	Incurred processed food materials	Allergenic ingredients	Analytical techniques	Protein/DNA extraction from incurred materials	Reference
Milk; egg; soy; hazelnut; peanut; walnut; almond	Bread	Skim milk powder, soy flakes, hazelnut, peanut, walnut, almond (from local retailer); egg white powder (from Ovobest, Germany)	HPLC-QTRAP-MS/MS (MRM mode)	Extraction with Tris-HCl buffer, heating	[58]
Peanuts; nuts (almonds, cashews, hazelnuts, pistachios, walnuts)	Bread; cookies	Peanuts, almonds, cashews, hazelnuts, pistachios, walnuts from local store	HPLC-QTRAP-MS/MS (MRM mode) and MRM ³ mode	Extraction with Tris base buffer containing urea and thiourea, homogenization	[60]
Egg	Mayonnase (with and without vinegar); chocolate cake batter; sponge cake	Raw liquid whole egg (12.49% protein), pasteurized liquid whole egg (12.49% protein)(from chicken farms, Belgium); then freeze-dried upon arrival and vacuum packed	UHPLC-UHR-TOF-MS/MS (DDA analysis)	Extraction with TRIS-HCl buffer containing urea, shaking, sonication	[61]
Walnut	Cake batter; bread	Walnut (from market)	Single-tube nested real time-PCR	Commercial extraction kit	[67]
Lupin	Bread	Lupine flour (<i>L. albus</i>) (from Biosagesse, France)	Real-time PCR	Commercial extraction kit. Extraction with buffer TNE, guanidine hydrochloride and proteinase K, DNA purification resin	[68]
Egg	Bread	Lyophilized egg powder	Magnetic beads-based sandwich amperometric immunosensor	Extraction with PBS buffer, heating, continuous stirring	[76]
Milk	Cookies	Skimmed milk powder (from Sigma-Aldrich, USA)	Commercial ELISA kits	According to kit producer's instructions	[85]
Gladiin (intact or hydrolyzed) (<i>gluten/wheat</i>)	Bread	PWG (Prolamin Working Group) gliadin standard (from Working Group on Prolamin Analysis and Toxicity)	Commercial sandwich ELISA kit; new competitive ELISA assay	Sandwich ELISA: according to kit producer's instructions; competitive ELISA: extraction with UPEX solution, vortexing, heating, addition of ethanol 80% (v/v), vortexing, head-over-head shaking	[90]
Gluten	Bread	Gluten (from Sigma Chemical Co., USA); wheat flour (SRM 1567a) (from NIST, USA)	Commercial ELISA assays; commercial lateral flow devices	According to kit producer's instructions	[94]
Egg	Pasta	Egg white, whole egg (from Sigma Aldrich, Canada)	Commercial ELISA kits; nanoUHPLC-Q-TOF-MS/MS (MS ^E mode)	ELISAs: according to kit producer's instructions; LC-MS/MS: extraction with ammonium bicarbonate buffer	[96]
Egg; milk; wheat; peanut; buckwheat	Juice; jam; jelly; retorted tomato stew; retorted vegetable soup	Wheat, peanut and buckwheat reference materials (from Oriental Yeast Co., Ltd., Japan); egg and milk (from Nippon Gene Co., Ltd., Japan)	Commercial ELISA kits; commercial lateral flow tests	Extraction with sodium dodecyl sulfate/sodium sulfite solution, shaking	[97]
Milk	Cookies	Certified non-fat milk powder material (SRM-159) (from LCG standard, UK)	Commercial ELISA kits; HPLC-IT-MS/MS (MRM mode)	ELISAs: according to kit producer's instructions; LC-MS/MS: extraction with ammonium bicarbonate buffer containing sodium dodecyl sulfate, stirring	[98]
Peanut Milk	Biscuits	Peanut butter (from Chocolates Lacasa, Spain)	ELISA assays	Extraction with sodium borate buffer, shaking	[99]
	Cookies	Skimmed milk powder (from Sigma-Aldrich, USA)	Capillary LC-Q-TOF-MS/MS (DDA mode)	Extraction with Trizma-base buffer containing tween, shaking	[100]
Ovalbumin (<i>egg</i>)	Orange juice; salad dressing; soy milk	Ovalbumin (for immunochemistry) (from Wako Pure Chemical Industries Ltd., Japan)	ELISA assay; UPLC-QqQ-MS/MS (SRM mode)	Extraction with sodium dodecyl sulfate/sodium sulfite solution, shaking	[101]

Table 1 (continued)

Food/protein allergens	Incurred processed food materials	Allergenic ingredients	Analytical techniques	Protein/DNA extraction from incurred materials	Reference
Peanut	Chocolate dessert	Lightly roasted defatted peanut flour manufactured by the Golden Peanut Company (GPC) (from Byrd Mill Co., USA)	Capillary LC-QqQ-MS/MS (MRM mode)	Extraction with Tris-HCl buffer containing dithiothreitol, sonication, vortexing	[102]
Soy	Sausages; bread; pâté	Soy protein isolates; raw soy seeds (from local supermarket)	ELISA assays	Extraction with PBS buffer, heating	[103]
Brazil nut	Cookie dough; cookies	Brazil nuts, peanut squish (from local supermarket)	Real-time PCR	Cell lysis with CTAB buffer, proteinase K and ceramic beads action, precipitation with ice-cold isopropanol	[104]
Gladiin (<i>gluten/wheat</i>)	Cookies	PWG (Prolamin Working Group) gliadin isolate (from Prolamine Working Group)	Commercial ELISA kits	According to kit producer's instructions	[105]
Fish	Creamy croquette; chicken meat-ball; pork meatball dumpling; vegetable and chicken soup; rice gruel	Horse mackerel muscle (from local store) powder	ELISA assay	Extraction with TRIS-HCl buffer containing bovine serum albumin, tween, sodium dodecyl sulfate and β -mercaptoethanol, shaking	[106]
Milk; egg	Cookies	Non-fat milk powder (RM 1549), egg spray-dried whole egg (RM 8445) (from NIST, USA)	Commercial ELISA assays	According to kit producer's instructions	[107]
Peanut; milk; egg	Cookies	Non-fat dry milk (SRM 1549), spray-dried whole egg powder (RM 8445) (from NIST, USA); light-roasted peanut flour (from Golden Peanut Co., USA)	Commercial ELISA assays	According to kit producer's instructions	[108]
Casein (<i>milk</i>); soy protein; gluten	Cookies	Casein (90% protein), gluten (80% protein) (from Sigma-Aldrich, USA); soy protein concentrate (81.5% protein) prepared in-house from press-cake (from La Ferme Jirah, Canada)	Commercial ELISA kits; flow cytometry; nanoUHPLC-Q-TOF-MS/MS (MS^E mode)	ELISAs: according to kit producer's instructions; Flow cytometry and LC-MS/MS: extraction with PBS buffer containing 2-mercaptoethanol and guanidine hydrochloride, heating, shaking, sonication	[109]

Abbreviations: *SRM* standard reference material, *RM* reference material, *NIST* National Institute of Standards and Technology, *EC-JRC* European Commission, Joint Research Centre, *TBS* Tris-buffered saline, *PBS* phosphate-buffered saline, *TRIS-HCl* tris(hydroxymethyl)aminomethane hydrochloride buffer, *TNE* extraction buffer (Tris, NaCl, EDTA, SDS), *CTAB* cetyltrimethylammonium bromide buffer, *UPEX* extraction buffer (PBS, tris(2-carboxyethyl)-phosphine, N-lauroylsarcosine), *ELISA* enzyme-linked immunosorbent assay, *HPLC* high-performance liquid chromatography, *UHPLC* ultra-high performance liquid chromatography, *MS/MS* tandem mass spectrometry, *Q* quadrupole, *QqQ* triple quadrupole, *IT* ion trap, *LIT* linear ion trap, *Q-TOF* quadrupole time of flight, *QTRAP* quadrupole-linear ion trap, *UHR-TOF* ultra-high resolution time of flight, *MRM* multiple reaction monitoring, *SRM* selected reaction monitoring, *MRM*² multiple reaction monitoring with multistage fragmentation, *DDA* data dependent acquisition, *MS^E* data independent acquisition, *t-SIM/dd2* timed-selection ion monitoring and data-dependent acquisition, *PCR* polymerase chain reaction, *iFAAM* "Integrated Approaches to Food Allergen and Allergy Management" project

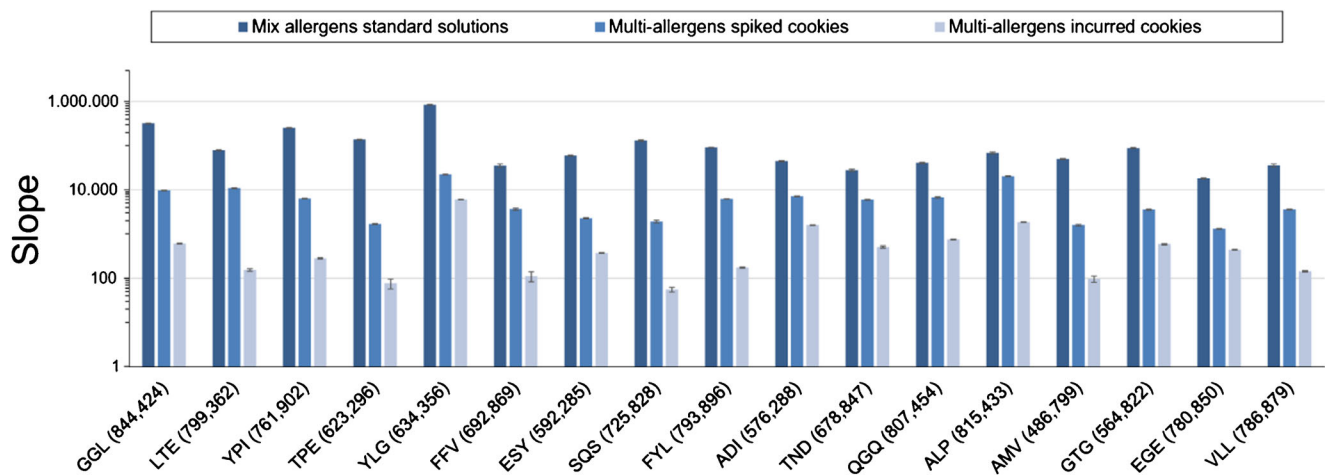


Fig. 2 Evaluation of matrix and food processing effects on the detection sensitivity by direct comparison of the calibration curves slopes referred to allergen standard solutions, allergen spiked cookies, and allergens incurred cookies. Peptide denomination and corresponding allergenic food (in brackets): GGL, LTE, YPI (egg); TPE, YLG, FFV (milk);

ESY, SQS, FYL (soybean); ADI, TND, QGQ, ALP (hazelnut); AMV, GTG, EGE, VLL (peanut). (Reproduced with permission from reference [55]. Copyright © 2018 Springer-Verlag GmbH Germany, part of Springer Nature)

performance as a function of baking process was assessed for each allergen by comparing ELISA results in terms of concentrations in the pre- and post-processed matrix. The authors isolated and expressed the influence of baking process on ELISA results as the ratio of allergen protein concentration calculated for incurred cereal bars and muffin samples collected at the final processing step (final product) and at the mixer step (dough/batter). Heating was determined to negatively influence the recovery and quantitation of egg, milk, and peanut to different extents depending on the allergen, matrix, and analytical test method.

The preparation of incurred food materials is useful not only for the assessment of analytical performances but also for the development of sample treatment procedures and target analyte monitoring strategy.

For this purpose, Planque et al. [50] investigated the use of incurred chocolate, ice cream, cookie, and tomato sauce samples to guide the selection of possible marker peptides to be monitored by LC-MS/MS technique. Marker peptides were selected in different subsequent steps: uniprot database query, Skyline in-silico digestion, analysis of raw ingredients, and finally analysis of incurred samples, reporting for each step the number of peptides and transitions suitable for MS/MS-based monitoring. Interestingly, a drastic reduction from in-silico digestion to incurred cookies is observed, identifying a list of peptides with high signal intensity resistant to thermal process; a further decrease occurs when only peptides in common to the four matrices were considered.

In a recent investigation, Montserrat and coworkers exploited the use of incurred biscuits for the development of an ELISA assay, evidencing that the selection of assay format (direct competitive vs sandwich) and of analytical target protein (Ara h1 vs Ara h2) influences test performance for the

detection of peanut allergens in processed foods [99]. In particular, determination of Ara h2 protein allowed detecting lower peanut concentration with respect to the Ara h1 protein, owing to a greater stability of Ara h2 to baking process, leading to higher extractability and epitope structure maintenance for recognition by specific antibodies. In addition, direct competitive format achieved the best sensitivity, probably because only one epitope is required for antigen recognition. For the same reason, competitive format is suggested for hydrolyzed foods for gluten analysis [90], since in hydrolyzed foodstuffs, gluten proteins are fragmented during food processing and converted into peptides in which only one toxic epitope may appear. Consequently, the gluten content is likely to be underestimated if a sandwich system is used to quantitatively assess gluten of a product containing hydrolyzed gluten, whereas a competitive ELISA is proved more suitable to accurately determine both intact and fragmented gluten.

Reference materials

Confidence in analytical results is strictly related to the availability of reference materials (RMs), which play an essential role in method calibration and validation, measurement uncertainty assessment, proficiency testing and quality assurance in order to guarantee accuracy and reliability of the analytical data, as well as comparability of results from different laboratories. In this respect, the quality of RMs is critical for accuracy and comparability of analysis results: to this aim, homogeneity and traceability to appropriate and stated references is strictly required. Analysis of reference materials is one of the approaches used to evaluate bias [92]. It is also recommended that a RM used for the determination of bias shall not also be

used for calibration purposes, i.e., during a validation study RM should only be used for one purpose [92].

Many efforts of the scientific community are focused on the development of novel RMs and traceable certified reference materials (CRMs), involving a very rigorous process for their preparation, extensive characterization, testing for stability and homogeneity, and property value assignment [110]. Unlike reference materials for single analyte molecule or well-defined classes of compounds, the preparation and certification of RMs for food allergens are very challenging issues and a matter of great concern due to variable nature of the allergenic commodities themselves, characterized by complex and different protein profiles, and due to discrepancies in the results among different analytical approaches. As a consequence, at now certification of food allergen reference materials according to International Standard Organization (ISO) requirements is not possible; however, the research community and in particular MoniQA association are focusing many efforts on the discussion of appropriate certification procedures in accordance with ISO standards. In addition, taking into account the drastic effects of food processing on food protein structures, also depending on the food matrix components, the final aim should be the development of incurred “matrix reference materials”, being characteristic of real-life foods relevant to the application. The possible allergen/matrix combinations are quite unlimited, so the scientific community should attribute an order of priority which could be based mainly on the probability of contamination of a food product with a particular allergen, which is partly reflected in the most recently notified allergen/matrix combinations, e.g., milk, egg, gluten, soy, peanuts and nuts in cereals, and bakery products and confectionery according to EU RASFF portal or those most frequently involved in incidents (milk, eggs, nuts, and peanuts) [7, 8]. Another criterion for allergen selection for RMs preparation could be their prevalence in sensitive population, with milk and egg being two of the most common food allergens in most countries, especially in children, even if diverse patterns of food allergy occur in individual geographic regions [111]. From data reported in Table 1, it is interesting to note as the previously mentioned allergen/matrix combinations actually correspond to those mostly investigated in the current literature.

As for the preparation of incurred RM, the best strategy should involve the use, if available, of specific allergen commodity RMs: in fact, this might guarantee a common starting point for the different incurred matrices and food processing and a better reproducibility, thanks to their extensive characterization in terms of protein, fat, carbohydrates, ash content, protein profiles, stability, solubility, homogeneity, and particle size/mesh. Since the incurred materials have to mimic real food products, the allergenic food RMs and food processing should respectively represent ingredients and recipe commonly used in the food industry. In this context, as shown in

Table 1, the most exploited RMs for milk, egg, and peanut are NIST RM-1549 (whole milk powder), NIST RM-1549a (non-fat milk powder), NIST RM-8445 (spray-dried whole egg, specified for allergen detection), and NIST RM-2387 (peanut butter) that are also suggested by AOAC International association [112, 113]. Also, it is worth mentioning the MoniQA Reference Materials for milk allergen analysis, recently included in AOAC’s SMPR (Standard Method Performance Requirements); they comprise positive control (characterized dried skim milk powder), negative control (based on a gluten-free cookie), and two incurred materials (dried skim milk powder incurred in gluten-free cookies) at two concentration levels of skim milk powder, thus of milk proteins. Other reference materials and quality control materials are available from FAPAS (Food Analysis Performance Assessment Scheme), LGC Standards (Teddington, Middlesex, UK) and EC-JRC.

As for gluten analysis, the study published by Bugyi and coworkers [114] represents an attempt to develop incurred RM (cookie model product) for gluten quantitation, with particular attention to achieve a good homogenization and to investigate the impact of using different gliadin sources among the ingredients. In that investigation, in order to avoid poor recovery, the Prolamin Working Group (PWG) gliadin material extracted from a mixture of 28 European wheat cultivars was chosen. Even if it is a consensus material, PWG-gliadin did not gain the status of reference material; in addition, since PWG is the prolamin fraction of wheat, it is not representative of gluten, which contains also glutelins and can derive also from other cereals. For these reasons, MoniQA association took the initiative to prepare a gluten reference material starting from selected flours of all the celiotoxic cereals and gluten-free reference materials. However, it should be noted that in gluten analysis, the development and application of LC-MS-based method offer the advantage, contrary to ELISA assays, to detect and quantify individually the celiotoxic cereals [53]: this implies the possibility to design a reference material for each cereal, thus improving the varieties representativeness and accuracy of quantitative results.

As for incurred allergen levels, they should meet the concentration range of test samples, but also blank RMs are needed to assess method performances below or near the detection limits. If a regulated limit is available for food product compliance assessment, the preparation of negative and positive RM controls close to this threshold value should be recommended.

Correct use of reference materials is essential to appropriate management; therefore, for aspects such as quality control, precision studies, the checking of the variability between operators, where the results are compared relatively, any suitable reference material including in-house reference materials can be used. Internally produced matrix RMs have to be

sufficiently homogeneous and stable for the intended use, but the requirements for them are less demanding than those for a certified reference material. In addition, when a certain allergen/matrix combination is not available, the preparation and the use of these types of materials is strictly required.

Homogeneity is an important requirement for all RMs, especially for heterogeneous materials such as food products, and refers to both within- and between-unit variation of the property of interest [115]. Milling, sieving, and packaging processes contribute to homogeneity property. Also inter-batch variability is important to assess [115]: reference ingredients, a standard recipe and fixed processed food item size and shape contribute to preparation reproducibility.

Finally, the short-term and long-term stabilities are other characteristics to assess: in particular, incurred materials are prone to modification of certain properties due to transport and storage conditions as well as temperature and humidity levels, especially when within-unit subsampling has to be performed from the same package in a certain period of time.

Conclusions and outlook

Incurred materials cover an essential role in food allergen analysis allowing increasing of knowledge on the influence of food processing on the chemical and structural properties of allergenic ingredients, which are embedded in the food matrix. Their use is also fundamental for quality assurance purposes in the analysis of food allergens. The preparation of incurred samples permits to assess the performance characteristics and reliability of methods developed for allergen detection, thus raising awareness of the main limitations associated to the available methods. Inter-laboratory comparisons would be very useful to improve confidence on the results from different laboratories [116], paving the way towards an effective allergenic risk assessment and management to protect sensitive consumers' health. However, only few certified reference materials are commercially available for allergenic food/ingredients and incurred matrices, thus hampering result comparability and method harmonization. Certified reference materials should be evaluated for their suitability for use in spike-and-recovery experiments and for assessing recovery from naturally incurred samples. In the last years, great effort towards method development, guideline harmonization, and reference material preparation and characterization was made, but there is still a long way to go. Even if measurement traceability in case of food allergens is a particular issue still far from being solved, a first step towards the harmonization of the analytical results and comparability could be an international consensus on the materials used at least for the purposes of whole method calibration. Another aspect that seems to be quite overlooked and that

deserves increasing attention in the future is the development and optimization of new sample treatment procedures, capable of efficiently extracting also processed proteins in order to improve analytical performances and ideally comparability of results.

Further challenges arise from globalization of food markets and dietary habits, not yet compatible with current regional differences of food labelling regulations; thus harmonization of legislative actions is also required, extending also the list of regulated allergen ingredients. In addition, food globalization, innovative food production technologies as well as demand for sustainable food sources led to the introduction of novel foods, defined and regulated within the European Union by Regulation (EC) No 2015/2283 [117]. Consequently, novel foods could represent novel allergenic sources, taking into account that new production processes could have a certain effect on allergen detectability. Among novel foods, emerging alternative sources of proteins in the Western World are insects; recent studies demonstrated a potential allergenic risk associated to them, also warning that the ingestion of insects by individuals allergic to some crustaceans can elicit allergic reactions [118, 119]. In this context, many efforts will be focused on the allergenicity assessment of novel foods, on the molecular identification and characterization of new allergenic proteins, and finally on the development and validation of methods for their detection [120]. Of course, strategic approach to method development and validation implies a very challenging issue: the availability of novel foods as reference ingredients for incurred samples preparation.

Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

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References

1. Dunlop JH, Keet CA. Epidemiology of food allergy. *Immunol Allergy Clin N Am*. 2018;38:13–25.
2. Sicherer SH, Sampson HA. Food allergy: a review and update on epidemiology, pathogenesis, diagnosis, prevention, and management. *J Allergy Clin Immunol*. 2018;141:41–58.
3. Allen KJ, Turner PJ, Pawankar R, Taylor S, Sicherer S, Lack G, et al. Precautionary labelling of foods for allergen content: are we ready for a global framework? *World Allergy Organ J*. 2014;7:10.
4. Babu BNH, Wilfred A, Venkatesh YP. Emerging food allergens: identification of polyphenol oxidase as an important allergen in eggplant (*Solanum melongena* L.). *Immunobiology*. 2017;222:155–63.

5. Nikolić J, Nešić A, Kull S, Schocker F, Jappe U, Gavrović-Jankulović M. Employment of proteomic and immunological based methods for the identification of catalase as novel allergen from banana. *J Proteome*. 2018;175:87–94.
6. European Commission. Regulation (EU) no 1169/2011 of the European Parliament and of the Council of 25 October 2011 on the provision of food information to consumers. *Off J Eur Union*. 2011;L304:18–63.
7. Pádua I, Moreira A, Moreira P, Melo de Vasconcelos F, Barros R. Impact of the regulation (EU) 1169/2011: Allergen-related recalls in the rapid alert system for food and feed (RASFF) portal. *Food Control*. 2019;98:389–98.
8. Rapid Alert System for Food and Feed (RASFF), European Union. 2017 Annual Report. 2018. https://ec.europa.eu/food/sites/food/files/safety/docs/rasff_annual_report_2017.pdf. Accessed 12 November 2018.
9. Allen KJ, Taylor SL. The consequences of precautionary allergen labeling: safe haven or unjustifiable burden? *J Allergy Clin Immunol Pract*. 2018;6:353–60.
10. Blom WM, Michelsen-Huisman AD, van Os-Medendorp H, van Duijn G, de Zeeuw-Brouwer ML, Versluis A, et al. Accidental food allergy reactions: products and undeclared ingredients. *J Allergy Clin Immunol*. 2018;142:865–75.
11. Versluis A, Knulst AC, Kruizinga AG, Michelsen A, Houben GF, Baumert JL, et al. Frequency, severity and causes of unexpected allergic reactions to food: a systematic literature review. *Clin Exp Allergy*. 2014;45:347–67.
12. Garber EAE, Parker CH, Handy SM, Cho CY, Rakhi Panda R, Samadpour M, et al. Presence of undeclared food allergens in cumin: the need for multiplex methods. *J Agric Food Chem*. 2016;64:1202–11.
13. Cucu T, Jaxsens L, De Meulenaer B. Analysis to support allergen risk management: which way to go? *J Agric Food Chem*. 2013;61:5624–33.
14. Walker MJ, Burns DT, Elliott CT, Gowlandc MH, Mills ENC. Is food allergen analysis flawed? Health and supply chain risks and a proposed framework to address urgent analytical needs. *Analyst*. 2016;141:24–35.
15. Lacorn M, Immer U. Standardization in allergen determination. *Accred Qual Assur*. 2010;15:207–16.
16. Hajas L, Scherf KA, Török K, Bugyi Z, Schall E, Poms RE, et al. Variation in protein composition among wheat (*Triticum aestivum* L.) cultivars to identify cultivars suitable as reference material for wheat gluten analysis. *Food Chem*. 2018;267:387–94.
17. Schopf M, Scherf KA. Wheat cultivar and species influence variability of gluten ELISA analyses based on polyclonal and monoclonal antibodies R5 and G12. *J Cereal Sci*. 2018;83:32–41.
18. European Commission. Commission implementing regulation (EU) no 828/2014 of 30 July 2014 on the requirements for the provision of information to consumers on the absence or reduced presence of gluten in food. *Off J Eur Union*. 2014;L228:5–8.
19. Lacorn M, Immer U. Allergen determination in food: reference materials and traceability of results. *Accred Qual Assur*. 2011;16:449–52.
20. Török K, Horváth V, Horváth A, Hajas L, Bugyi Z, Tömösközi S. Investigation of incurred single- and multi-component model food matrices for determination of food proteins triggering allergy and coeliac disease. *Eur Food Res Technol*. 2014;239:923–32.
21. Bugyi Z, Nagy J, Török K, Hajas L, Tömösközi S. Towards development of incurred materials for quality assurance purposes in the analysis of food allergens. *Anal Chim Acta*. 2010;672:25–9.
22. Monaci L, De Angelis E, Montemurro N, Pilolli R. Comprehensive overview and recent advances in proteomics MS based methods for food allergens analysis. *Trends Anal Chem*. 2018;106:21–36.
23. Bianchi F, Giannetto M, Careri M. Analytical systems and metrological traceability of measurement data in food control assessment. *Trends Anal Chem*. 2018;107:142–50.
24. Parker CH, Khuda SE, Pereira M, Ross MM, Fu T-J, Fan X, et al. Multi-allergen quantitation and the impact of thermal treatment in industry-processed baked goods by ELISA and liquid chromatography-tandem mass spectrometry. *J Agric Food Chem*. 2015;63:10669–80.
25. Poms RE. MoniQA's contribution towards a global harmonisation of foodstuff quality and safety assessment and monitoring strategies. *Qual Assur Saf Crop Foods*. 2013;5:3–6.
26. Nitride C, Lee V, Baricevic-Jones I, Adel-Patient K, Baumgartner S, Mills ENC. Allergen analysis within a risk assessment framework: approaches to development of targeted mass spectrometry methods for allergen detection and quantification in the iFAAM project. *J AOAC Int*. 2018;101:83–90.
27. Prado M, Ortea I, Vial S, Rivas J, Calo-Mata P, Barros-Velázquez J. Advanced DNA- and protein-based methods for the detection and investigation of food allergens. *Crit Rev Food Sci Nutr*. 2016;56:2511–42.
28. van Hengel AJ. Food allergen detection methods and the challenge to protect food-allergic consumers. *Anal Bioanal Chem*. 2007;389:111–8.
29. Codex Alimentarius Commission. Standard for foods for special dietary use for persons intolerant to gluten. CODEX STAN 118–1979 (Adopted in 1979. Amendment: 1983 and 2015. Revision: 2008).
30. Diaz-Amigo C, Popping B. Accuracy of ELISA detection methods for gluten and reference materials: a realistic assessment. *J Agric Food Chem*. 2013;61:5681–8.
31. Monaci L, Visconti A. Immunochemical and DNA-based methods in food allergen analysis and quality assurance perspectives. *Trends Food Sci Technol*. 2010;21:272–83.
32. Elvir L, Mattarozzi M (2012) Food Proteomics. In: Elsevier Inc. editor. *Chemical Analysis of Food: Techniques and Applications*. pp 519–37.
33. Andjelković U, Gavrović-Jankulović M, Martinović T, Josić D. Omics methods as a tool for investigation of food allergies. *Trends Anal Chem*. 2017;96:107–15.
34. Słowianek M, Majak I. Methods of allergen detection based on DNA analysis. *Biotechnol Food Sci*. 2011;75:39–44.
35. Koeberl M, Clarke D, Lopata AL. Next generation of food allergen quantification using mass spectrometric systems. *J Proteome Res*. 2014;13:3499–509.
36. Ahsan N, Shyama Prasad Rao R, Gruppuso PA, Ramratnam B, Salomon AR. Targeted proteomics: current status and future perspectives for quantification of food allergens. *J Proteome*. 2016;143:15–23.
37. Planque M, Arnould T, Gillard N. Food allergen analysis: detection, quantification and validation by mass spectrometry. *Allergen: IntechOpen publisher*; 2017. p. 8–41.
38. Ross GMS, Bremer MGE, Nielsen MWF. Consumer-friendly food allergen detection: moving towards smartphone-based immunoassays. *Anal Bioanal Chem*. 2018;410:5353–71.
39. Pilolli R, Monaci L, Visconti A. Advances in biosensor development based on integrating nanotechnology and applied to food-allergen management. *Trends Anal Chem*. 2013;47:12–26.
40. Hosu O, Selvolini G, Marrazza G. Recent advances of immunosensors for detecting food allergens. *Curr Opin Electrochem*. 2018;10:149–56.
41. Alves RC, Barroso MF, González-García MB, Oliveira MBPP, Delerue-Matos C. New trends in food allergens detection: toward biosensing strategies. *Crit Rev Food Sci Nutr*. 2016;56:2304–19.
42. Khedri M, Ramezani M, Rafatpanah H, Abnous K. Detection of food-born allergens with aptamer-based biosensors. *Trends Anal Chem*. 2018;103:126–36.

43. Neethirajan S, Weng X, Tah A, Cordero JO, Ragavan KV. Nanobiosensor platforms for detecting food allergens – New trends. *Sens Bio-Sens Res*. 2018;18:13–30.
44. Rzychon M, Brohée M, Cordeiro F, Haraszi R, Ulberth F, O'Connor G. The feasibility of harmonizing gluten ELISA measurements. *Food Chem*. 2017;234:144–54.
45. Török K, Hajas L, Horváth V, Schall E, Bugyi Z, Kemény S, et al. Identification of the factors affecting the analytical results of food allergen ELISA methods. *Eur Food Res Technol*. 2015;241:127–36.
46. Taylor SL, Nordlee JA, Niemann LM, Lambrecht DM. Allergen immunoassays-considerations for use of naturally incurred standards. *Anal Bioanal Chem*. 2009;395:83–92.
47. Koeberl M, Sharp MF, Tian R, Buddhadasa S, Clarke D, Roberts J. Lupine allergen detecting capability and cross-reactivity of related legumes by ELISA. *Food Chem*. 2018;256:105–12.
48. Johnson PE, Baumgartner S, Aldick T, Bessant C, Giosafatto V, Heick J, et al. Current perspectives and recommendations for the development of mass spectrometry methods for the determination of allergens in foods. *J AOAC Int*. 2011;94:1026–33.
49. Brockmeyer J. Novel approaches for the MS-based detection of food allergens: high resolution, MS3, and beyond. *J AOAC Int*. 2018;101:124–31.
50. Planque M, Arnould T, Dieu M, Delahaut P, Renard P, Gillard N. Advances in ultra-high performance liquid chromatography coupled to tandem mass spectrometry for sensitive detection of several food allergens in complex and processed foodstuffs. *J Chromatogr A*. 2016;1464:115–23.
51. Planque M, Arnould T, Dieu M, Delahaut P, Renard P, Gillard N. Liquid chromatography coupled to tandem mass spectrometry for detecting ten allergens in complex and incurred foodstuffs. *J Chromatogr A*. 2017;1530:138–51.
52. Planque M, Arnould T, Delahaut P, Renard P, Dieu M, Gillard N. Development of a strategy for the quantification of food allergens in several food products by mass spectrometry in a routine laboratory. *Food Chem*. 2019;274:35–45.
53. Manfredi A, Mattarozzi M, Giannetto M, Careri M. Multiplex liquid chromatography-tandem mass spectrometry for the detection of wheat, oat, barley and rye prolamins towards the assessment of gluten-free product safety. *Anal Chim Acta*. 2015;895:62–70.
54. Pilolli R, De Angelis E, Monaci L. Streamlining the analytical workflow for multiplex MS/MS allergen detection in processed foods. *Food Chem*. 2017;221:1747–53.
55. Pilolli R, De Angelis E, Monaci L. In house validation of a high resolution mass spectrometry Orbitrap-based method for multiple allergen detection in a processed model food. *Anal Bioanal Chem*. 2018;410:5653–62.
56. New LS, Schreiber A, Stahl-Zeng J, Liu H-F. Simultaneous analysis of multiple allergens in food products by LC-MS/MS. *J AOAC Int*. 2018;101:132–45.
57. Boo CC, Parker CH, Jackson LS. A targeted LC-MS/MS method for the simultaneous detection and quantitation of egg, milk, and peanut allergens in sugar cookies. *J AOAC Int*. 2018;101:108–17.
58. Heick J, Fischer M, Pöpping B. First screening method for the simultaneous detection of seven allergens by liquid chromatography mass spectrometry. *J Chromatogr A*. 2011;1218:938–43.
59. Monaci L, Losito I, De Angelis E, Pilolli R, Visconti A. Multi-allergen quantification of fining-related egg and milk proteins in white wines by high-resolution mass spectrometry. *Rapid Commun Mass Spectrom*. 2013;27:2009–18.
60. Korte R, Oberleitner D, Brockmeyer J. Determination of food allergens by LC-MS: impacts of sample preparation, food matrix, and thermal processing on peptide detectability and quantification. *J Proteome*. in press. <https://doi.org/10.1016/j.jprot.2018.11.002>.
61. Gavage M, Van Vlierberghe K, Van Poucke C, De Loose M, Gevaert K, Dieu M, et al. Selection of egg peptide biomarkers in processed food products by high resolution mass spectrometry. *J Chromatogr A*. 2019;1584:115–25.
62. Careri M, Elviri L, Boquett Lagos J, Mangia A, Speroni F, Terenghi M. Selective and rapid immunomagnetic bead-based sample treatment for the liquid chromatography–electrospray ion-trap mass spectrometry detection of Ara h3/4 peanut protein in foods. *J Chromatogr A*. 2008;1206:89–94.
63. Bignardi C, Mattarozzi M, Penna A, Sidoli S, Elviri L, Careri M, et al. A rapid size-exclusion solid-phase extraction step for enhanced sensitivity in multi-allergen determination in dark chocolate and biscuits by liquid chromatography–tandem mass spectrometry. *Food Anal Methods*. 2013;6:1144–52.
64. Mattarozzi M, Milioli M, Bignardi C, Elviri L, Corradini C, Careri M. Investigation of different sample pre-treatment routes for liquid chromatography-tandem mass spectrometry detection of caseins and ovalbumin in fortified red wine. *Food Control*. 2014;38:82–7.
65. Gu S, Chen N, Zhou Y, Zhao C, Zhan L, Qu L, et al. A rapid solid-phase extraction combined with liquid chromatography-tandem mass spectrometry for simultaneous screening of multiple allergens in chocolates. *Food Control*. 2018;84:89–96.
66. Svobodova M, Mairal T, Nadal P, Bermudo MC, O'Sullivan CK. Ultrasensitive aptamer based detection of β -conglutinin food allergen. *Food Chem*. 2014;165:419–23.
67. Pierboni E, Rondini C, Torricelli M, Ciccone L, Tovo GR, Mercuri ML, et al. Digital PCR for analysis of peanut and soybean allergens in foods. *Food Control*. 2018;92:128–36.
68. Costa J, Oliveira MBPP, Mafra I. Effect of thermal processing on the performance of the novel single-tube nested real-time PCR for the detection of walnut allergens in sponge cakes. *Food Res Int*. 2013;54:1722–9.
69. Villa C, Costa J, Gondar C, Oliveira MBPP, Mafra I. Effect of food matrix and thermal processing on the performance of a normalised quantitative real-time PCR approach for lupine (*Lupinus albus*) detection as a potential allergenic food. *Food Chem*. 2018;262:251–9.
70. Linacero R, Ballesteros I, Sanchiz A, Prieto N, Iniesto E, Martinez Y, et al. Detection by real time PCR of walnut allergen coding sequences in processed foods. *Food Chem*. 2016;202:334–40.
71. Sheu S-C, Tsou P-C, Lien Y-Y, Lee M-S. Development of loop-mediated isothermal amplification (LAMP) assays for the rapid detection of allergic peanut in processed food. *Food Chem*. 2018;257:67–74.
72. Manfredi A, Giannetto M, Mattarozzi M, Costantini M, Mucchino C, Careri M. Competitive immunosensor based on gliadin immobilization on disposable carbon-nanogold screen-printed electrodes for rapid determination of celiotoxic prolamins. *Anal Bioanal Chem*. 2016;408:7289–98.
73. Hideshima S, Saito M, Fujita K, Harada Y, Tsuna M, Sekiguchi S, et al. Label-free detection of allergens in food via surfactant-induced signal amplification using a field effect transistor-based biosensor. *Sensors Actuators B*. 2018;254:1011–6.
74. Eissa S, Zourob M. In vitro selection of DNA aptamers targeting β -lactoglobulin and their integration in graphene-based biosensor for the detection of milk allergen. *Biosens Bioelectron*. 2017;91:169–74.
75. Angelopoulou M, Petrou PS, Makarona E, Haasnoot W, Moser I, Jobst G, et al. Ultrafast multiplexed-allergen detection through advanced fluidic design and monolithic interferometric silicon chips. *Anal Chem*. 2018;90:9559–67.
76. Lin H-Y, Huang C-H, Park J, Pathania D, Castro CM, Fasano A, et al. Integrated magneto-chemical sensor for on-site food allergen detection. *ACS Nano*. 2017;11:10062–9.
77. Benedé S, Ruiz-Valdepeñas Montiel V, Povedano E, Villalba M, Mata L, Galán-Malo P, et al. Fast amperometric immunoplateform for ovomucoid traces determination in fresh and baked foods. *Sensors Actuators B*. 2018;265:421–8.

78. Vanga SK, Singh A, Raghavan V. Review of conventional and novel food processing methods on food allergens. *Crit Rev Food Sci Nutr*. 2017;57:2077–94.
79. Ekezie F-GC, Cheng J-H, Sun D-W. Effects of nonthermal food processing technologies on food allergens: a review of recent research advances. *Trends Food Sci Technol*. 2018;74:12–25.
80. Khan MU, Ahmed I, Lin H, Li Z, Costa J, Mafra I, et al. Potential efficacy of processing technologies for mitigating crustacean allergenicity. *Crit Rev Food Sci Nutr*. 2018. <https://doi.org/10.1080/10408398.2018.1471658>.
81. Golkar A, Milani JM, Vasiljevic T. Altering allergenicity of cow's milk by food processing for applications in infant formula. *Crit Rev Food Sci Nutr*. 2018. <https://doi.org/10.1080/10408398.2017.1363156>.
82. Rahaman T, Vasiljevic T, Ramchandran L. Effect of processing on conformational changes of food proteins related to allergenicity. *Trends Food Sci Technol*. 2016;49:24–34.
83. Gomaa A, Boye J. Impact of irradiation and thermal processing on the immunochemical detection of milk and egg allergens in foods. *Food Res Int*. 2015;74:275–83.
84. Guan A, Mei K, Lv M, Lu J, Lou Q, Yang W. The effect of electron beam irradiation on IgG binding capacity and conformation of tropomyosin in shrimp. *Food Chem*. 2018;264:250–4.
85. Monaci L, Brohée M, Tregoeat V, van Hengel A. Influence of baking time and matrix effects on the detection of milk allergens in cookie model food system by ELISA. *Food Chem*. 2011;127:669–75.
86. Gomaa A, Boye JI. Impact of thermal processing time and cookie size on the detection of casein, egg, gluten and soy allergens in food. *Food Res Int*. 2013;52:483–9.
87. Li H, Byrne K, Galimov R, Mendoza-Porras O, Bose U, Howitt CA, et al. Using LC-MS to examine the fermented food products vinegar and soy sauce for the presence of gluten. *Food Chem*. 2018;254:302–8.
88. Khuda SE, Jackson LS, Fu T-J, Williams KM. Effects of processing on the recovery of food allergens from a model dark chocolate matrix. *Food Chem*. 2015;168:580–7.
89. García E, Llorente M, Hernando A, Kieffer R, Wieser H, Méndez E. Development of a general procedure for complete extraction of gliadins for heat processed and unheated foods. *Eur J Gastroenterol Hepatol*. 2005;17:529–39.
90. Mena MC, Lombardía M, Hernando A, Méndez E, Albar JP. Comprehensive analysis of gluten in processed foods using a new extraction method and a competitive ELISA based on the R5 antibody. *Talanta*. 2012;91:33–40.
91. Iniesto E, Jiménez A, Prieto N, Cabanillas B, Burbano C, Pedrosa MM, et al. Real time PCR to detect hazelnut allergen coding sequences in processed foods. *Food Chem*. 2013;138:1976–81.
92. Eurachem. Eurachem Guide. “Eurachem Guide: The Fitness for Purpose of Analytical Methods - A Laboratory Guide to Method Validation and Related Topics. (2nd ed. 2014). B. Magnusson and U. Örnemark (eds.). Available from www.eurachem.org. Accessed 12 November 2018.
93. Trucksess MW, Whitaker TB, Slate AB, Williams KM, Brewer VA, Whittaker P, et al. Variation of analytical results for peanuts in energy bars and milk chocolate. *J AOAC Int*. 2004;87:943–9.
94. Sharma GM, Khuda SE, Pereira M, Slate A, Jackson LS, Pardo C, et al. Development of an incurred cornbread model for gluten detection by immunoassays. *J Agric Food Chem*. 2013;61:12146–54.
95. Downs ML, Baumert JL, Taylor SL, Mills ENC. Mass spectrometric analysis of allergens in roasted walnuts. *J Proteome*. 2016;142:62–9.
96. Azamia S, Boye JI, Mongeon V, Sabik H. Detection of ovalbumin in egg white, whole egg and incurred pasta using LC-ESI-MS/MS and ELISA. *Food Res Int*. 2013;52:526–34.
97. Ito K, Yamamoto T, Oyama Y, Tsuruma R, Saito E, Saito Y, et al. Food allergen analysis for processed food using a novel extraction method to eliminate harmful reagents for both ELISA and lateral-flow tests. *Anal Bioanal Chem*. 2016;408:5973–84.
98. Lamberti C, Acquadro E, Corpillo D, Giribaldi M, Decastelli L, Garino C, et al. Validation of a mass spectrometry-based method for milk traces detection in baked food. *Food Chem*. 2016;199:119–27.
99. Montserrat M, Sanz D, Juan T, Herrero A, Sánchez L, Calvo M, et al. Detection of peanut (*Arachis hypogaea*) allergens in processed foods by immunoassay: influence of selected target protein and ELISA format applied. *Food Control*. 2015;54:300–7.
100. Monaci L, Nørgaard JV, van Hengel AJ. Feasibility of a capillary LC/ESI-Q-TOF MS method for the detection of milk allergens in an incurred model food matrix. *Anal Methods*. 2010;2:967–72.
101. Nimata M, Okada H, Kurihara K, Sugimoto T, Honjoh T, Kuroda K, et al. A harmonized immunoassay with liquid chromatography-mass spectrometry analysis in egg allergen determination. *Anal Bioanal Chem*. 2018;410:325–35.
102. Sayers RL, Gethings LA, Lee V, Balasundaram A, Johnson PE, Marsh JA, et al. Microfluidic separation coupled to mass spectrometry for quantification of peanut allergens in a complex food matrix. *J Proteome Res*. 2018;17:647–55.
103. Segura-Gil I, Nicolau-Lapeña I, Galán-Malo P, Mata L, Calvo M, Sánchez L, et al. Development of two ELISA formats to determine glycinin. Application to detect soy in model and commercial processed food. *Food Control*. 2018;93:32–9.
104. Röder M, Filbert H, Holzhauser T. A novel, sensitive and specific real-time PCR for the detection of traces of allergenic Brazil nut (*Bertholletia excelsa*) in processed foods. *Anal Bioanal Chem*. 2010;398:2279–88.
105. Bugyi Z, Török K, Hajas L, Adonyi Z, Popping B, Tömösközi S. Comparative study of commercially available gluten ELISA kits using an incurred reference material. *Qual Assur Saf Crop Foods*. 2013;5:79–87.
106. Shibahara Y, Uesaka Y, Wang J, Yamada S, Shiomi K. A sensitive enzyme-linked immunosorbent assay for the determination of fish protein in processed foods. *Food Chem*. 2013;136:675–81.
107. Dumont V, Kerbach S, Poms R, Johnson P, Mills C, Popping B, et al. Development of milk and egg incurred reference materials for the validation of food allergen detection methods. *Qual Assur Saf Crop Foods*. 2010;2:208–15.
108. Khuda S, Slate A, Pereira M, Al-Tajer F, Jackson L, Diaz-Amigo C, et al. Effect of processing on recovery and variability associated with immunochemical analytical methods for multiple allergens in a single matrix: sugar cookies. *J Agric Food Chem*. 2012;60:4195–203.
109. Gomaa A, Boye J. Simultaneous detection of multi-allergens in an incurred food matrix using ELISA, multiplex flow cytometry and liquid chromatography mass spectrometry (LC-MS). *Food Chem*. 2015;175:585–92.
110. Wise SA. What is novel about certified reference materials? *Anal Bioanal Chem*. 2018;410:2045–9.
111. Loh W, Tang MLK. The epidemiology of food allergy in the global context. *Int J Environ Res Public Health*. 2018;15:2043.
112. Abbott M, Hayward S, Ross W, Godefroy SB, Ulberth F, Van Hengel AJ, et al. Validation procedures for quantitative food allergen ELISA methods: community guidance and best practices. *J AOAC Int*. 2010;93:442–50.
113. AOAC International. Standard Method Performance Requirements (SMPRs®) for Detection and Quantitation of Selected Food Allergens. 2016;AOAC SMPR 2016.002.
114. Bugyi Z, Török K, Hajas L, Adonyi Z, Poms RE, Popping B, et al. Development of incurred reference material for improving conditions of gluten quantification. *J AOAC Int*. 2012;95:382–7.

115. International Organization for Standardization. Reference materials - Guidance for the characterization and the assessment of the homogeneity and stability of the material. 2017; ISO/Guide 35:2017.
116. Johnson PE, Rigby NM, Dainty JR, Mackie AR, Immer UU, Rogers A, et al. A multi-laboratory evaluation of a clinically-validated incurred quality control material for analysis of allergens in food. *Food Chem.* 2014;148:30–6.
117. European Commission. Regulation (EU) no 2015/2283 of the European Parliament and of the Council of 25 November 2015 on novel foods. *Off J Eur Union.* 2015;L327:1–22.
118. de Gier S, Verhoeckx K. Insect (food) allergy and allergens. *Mol Immunol.* 2018;100:82–106.
119. Broekman H, Verhoeckx KC, den Hartog Jager CF, Kruizinga AG, Pronk-Kleinjan M, Remington BC, et al. Majority of shrimp-allergic patients are allergic to mealworm. *J Allergy Clin Immunol.* 2016;137:1261–3.
120. Pali-Schöll I, Verhoeckx K, Mafra I, Bavaro SL, Mills ENC, Monaci L. Allergenic and novel food proteins: state of the art and challenges in the allergenicity assessment. *Trends Food Sci Technol.* in press. <https://doi.org/10.1016/j.tifs.2018.03.007>.