



Food allergens: Classification, molecular properties, characterization, and detection in food sources

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Abstract

Food allergy is a large and growing public health problem in many areas of the world. The prevalence of food allergy has increased in the last decades in a very significant way in many world regions, particularly in developed countries. In that respect, the research field of food allergy has experienced an extensive growth and very relevant progress has been made in recent years regarding the characterization of food allergens, the study of their immunological properties, and their detection in food sources. Furthermore, food labeling policies have also been improved decidedly in recent years. For that immense

progress made, it is about time to review the latest progress in the field of food allergy. In this review, we intend to carry out an extensive and profound overview regarding the latest scientific advances and knowledge in the field of food allergen detection, characterization, and in the study of the effects of food processing on the physico-chemical properties of food allergens. The advances in food labeling policies, and methodologies for the characterization of food allergens are also thoroughly reviewed in the present overview.



1. Food allergy, food allergens, and labeling policies

Food allergy is a hypersensitivity response characterized by adverse and reproducible reactions to foods mediated by the immune system. The most common type of food allergy is mediated by Immunoglobulin E (IgE), which is elicited rapidly after the ingestion of the offending food in sensitized individuals. Reactions can vary from mild to severe anaphylaxis which, in some cases, can have a life-threatening nature. Currently, food allergy is one of the leading causes of anaphylaxis treated in emergency rooms in developed countries, particularly in children (Sicherer & Sampson, 2018).

The true prevalence of food allergy is difficult to establish due to the heterogeneity in the methodology and design of the epidemiological studies carried out over the years. However, acknowledging these limitations, it seems well-established that the prevalence of food allergy has increased in developed countries over the past decades and is beginning to increase in developing countries (Nwaru et al., 2014; Sicherer & Sampson, 2018). In a meta-analysis carried out in 2007, the total prevalence of allergy to cow's milk, peanut, fish, or shellfish based only on the symptoms referred by the patients was well above the prevalence estimated based on objective measurements including skin prick tests (SPT), specific IgE in sera, or oral food challenges (Rona et al., 2007). The use of objective methods for food allergy diagnosis in non-selected populations seems therefore ideal for the study of prevalence of food allergy. A more recent meta-analysis which considered European studies, established the self-reported prevalence of food allergy to cow's milk, egg, wheat, soy, peanut, tree nuts, fish, and shellfish in adults and children between 0.4% and 6%. However, the prevalence of allergy defined by oral food challenges was 0.6% for cow's milk, 0.2% for egg, 0.1% for wheat, 0.3% for soy, 0.2% for peanut, 0.5% for tree nuts, 0.1% for fish, and 0.1% for shellfish (Nwaru et al., 2014). Important variations in the prevalence of food allergy have been also found when different

geographical regions or different areas in the same region (rural versus city) were considered (Tang & Mullins, 2017).

Food allergy can be divided into two phases. In the initial stage, the so-called sensitization phase, an immunological priming toward an allergic response takes place. In this phase, there is an exposition to the allergen through the gastrointestinal system, mucosa, or skin. On these sites dendritic cells, with several mechanisms and receptors for allergen uptake, are the specialized cells for the capture, processing, and presentation of allergens to T lymphocyte cells (T cells) and for the priming of these cells into different T helper (Th) cell subtypes, such as CD4 + Th2 cells, to initiate an adaptive immune response (Walsh & Mills, 2013). Allergy is driven by allergen-specific CD4 + Th2 cells, which produce IL-4, IL-5, and IL-13 cytokines. These cytokines have an important function on driving Th2 differentiation and induction of IgE production by allergen-specific B lymphocyte cells (B cells). IgE has a central role in food allergy. Once produced, IgE binds to the tetrameric form of the high-affinity receptor of IgE (FcεRI) on mast cells and basophils. In the elicitation phase, after a new exposure to the food allergen, there is a recognition of the food allergen by IgE bound to FcεRI. Subsequently, crosslinking of IgE molecules promotes a signaling cascade that triggers the release of histamine, prostaglandins, leukotrienes, and other factors which lead to the symptoms associated with allergy (Stone, Prussin, & Metcalfe, 2010).

Humans are constantly exposed to a large number of food proteins, however, only a very limited group acts as triggering factors, inducing sensitization that derives into IgE production and allergic reactions in certain individuals. Nowadays, eight foods are considered to be responsible for the most significant allergic reactions to food in the USA and Europe. These are peanut, tree nuts, milk, egg, fish, shellfish, soybean, and wheat. During childhood, the most prevalent foods that cause IgE-mediated allergy are cow's milk, egg, peanut, wheat, soybean, fish, and tree nuts, while in adulthood the most common allergenic foods are peanut, tree nuts, shellfish, and fish (Burks, Helm, Stanley, & Bannon, 2001). Such differences could be attributed to the differential exposure to certain group of foods, the immaturity of the immune system in the first years of life or a potential immature regulation of the cellular and molecular mechanisms that control different immune responses. Although allergy to cow's milk or egg is usually outgrown up to 60% of the pediatric patients by means of natural tolerance, others such as peanut, tree nut, or fish can persist for the whole lifetime of the patients (Burks & Ballmer-Weber, 2006). Different markers have

been proposed to act as predictive indicators of natural allergy resolution, such as elevated levels of specific IgG4, low IgE/IgG4 ratio, high frequency of regulatory T cells, low levels of mast cell protein-1, or low levels of macrophage inflammatory protein, among others (Ponce, Diesner, Szépfalusi, & Eiwegger, 2016).

Allergic reactions to food, unlike other adverse reactions, can be triggered by minimal amounts of allergen. Therefore, individuals with food allergy should carefully read the composition label before eating any food product. Moreover, they should be instructed by healthcare professionals to identify the various denominations of the food they are allergic to on the labels of food products. The declaration of food allergens on the labels of food products is regulated by law. In 1963, the Food and Agriculture Organization of the United Nations and the World Health Organization established the Codex Alimentarius Commission that updates the allergens that should be declared in the labels of food products. The Commission ensures that governments create the necessary regulations for food labeling, however, such regulations differ in different countries/continents (Fierro, Di Girolamo, Marzano, Dahdah, & Mennini, 2017). The main key allergens or group of allergens (milk, egg, fish, peanut, tree nuts, soybean, crustaceans, shellfish, wheat) contained in food products at any quantity must be declared on the labels in Europe, United States, Canada, Australia, New Zealand, China, South America, and South Africa. However, certain foods such as lupin, cereals, celery, mustard, sesame, peach, or tomato must be declared only in certain countries or continents (Fierro et al., 2017; Lopez, 2018; Taylor & Hefle, 2006). Recently, non-prepackaged foods in food retailers should also incorporate allergen labeling in certain countries such as the ones belonging to the European Union (Ross, Bremer, & Nielsen, 2018).

Besides the regulation of the allergens that must be declared in the labels because they are part of a food product, a precautionary allergen labeling (PAL) promoted by some industries in certain countries also exists. PAL was introduced in order to improve the awareness and safety of consumers. Its purpose is to advise about the possible inadvertent presence of residues or traces of allergens due to the processes in the food industry. PAL was also created to decrease the possibilities of legal issues (Fierro et al., 2017; Yeung & Robert, 2018). Generally, PAL refers to the use of common manufacturing equipment for products with different formulations in the food industry, which can lead to contamination of the food product by other processed foods in the same place. In that way, some sentences used

in PAL include “this product may contain...,” “it may contain traces of...,” “manufactured on shared facilities/equipment with products containing (allergen)...” PAL is voluntary and, on many occasions, inconsistent and confusing for consumers and healthcare providers and a standardization is still lacking. A study found that 3.1% and 5.9% of a representative sample of products that did not declare egg and milk in their labels, actually contained egg and milk in the product. This means that allergic consumers could consider a product without precautionary allergen labeling as a safe product when it might not be the case (Ford et al., 2010). Therefore, it has been proposed that PAL should be subjected to regulation and standardization with clear and visible messages on food labels in order to improve the safety of allergic consumers (Fierro et al., 2017; Taylor & Hefle, 2006; Yeung & Robert, 2018).



2. Classification of food allergens

2.1 Classification of plant food allergens

Most plant food allergens can be grouped into a few highly conserved and ubiquitous families and superfamilies. Numerous plant food allergens belong to the superfamily of prolamines (2S albumins, nonspecific lipid transfer proteins, α -amylases/trypsin inhibitors, etc.) or the superfamily of cupins (such as 7S and 11S globulins). Furthermore, a large part of plant food allergens are homologous to defense proteins or pathogenesis-related (PR) proteins, which consists of a heterogeneous collection of families that are involved in the plant defense against external environmental dangers, biological aggressions and plant resistance (Breiteneder & Radauer, 2004). Finally, there are unrelated protein families such as profilins, oleosins or defensins that contain several plant food allergens.

2.1.1 Prolamin superfamily

The prolamin superfamily owes its name to the storage protein of cereals, prolamin. Allergens from plant foods that belong to the prolamin superfamily are characterized by a low molecular weight and several cysteine residues within their sequence. Usually, eight are the cysteines involved in the establishment of four intra-chain disulfide bonds on these proteins necessary for the 3D structure rich in α -helices. Most of the allergens belonging to the prolamin superfamily are highly resistant to heat, pH, and gastrointestinal enzymes due to their small and compact structures. Among the relevant allergens from plant foods that belong to this superfamily, we can find the

seed storage proteins 2S albumins, that normally have two protein chains (dimers) joined by disulfide bonds. Important allergens from tree nuts, legumes, or seeds are 2S albumins. Other seed storage proteins in the prolamin family are the cereal prolamins (glutenins and gliadins) that group important allergens from cereal grains. Further members of the prolamin superfamily are the nonspecific lipid transfer proteins (nsLTPs) that are monomeric proteins that can be found in the epidermis of different plant organs such as fruits. nsLTPs are important and potent allergens in several plant foods. α -amylases and protease inhibitors, described as allergens in certain plant foods, also belong to the prolamin superfamily and can be found as monomeric, dimeric, or tetrameric proteins. These proteins are involved in the plant defense by inhibiting stomach enzymes from different insects.

In peanut, 6 allergens belong to the prolamin superfamily: Ara h 2, Ara h 6, Ara h 7, Ara h 9, Ara h 16, and Ara h 17. The allergen Ara h 6, a 2S albumin, was found to be similar to Ara h 2 as well as Ara h 7, which are also members of the 2S albumins (Schmidt et al., 2010; Suhr, Wicklein, Lepp, & Becker, 2004). Ara h 2 has been actively characterized and its IgE-binding epitopes described (Chatel, Bernard, & Orson, 2003; Glaspole, de Leon, Rolland, & O'Hehir, 2005; Otsu, Guo, & Dreskin, 2015; Viquez, Summer, & Dodo, 2001). Ara h 2 has been widely associated with severe allergic reactions to peanut in several study populations (Kukkonen, Pelkonen, Mäkinen-Kiljunen, Voutilainen, & Mäkelä, 2015; Klemans, Broekman, et al., 2013; Klemans, Knol, et al., 2013). In that respect, IgE against Ara h 2 has been proposed as a predictor of clinical allergy to peanut. Currently double-blind, placebo-controlled food challenge (DBPCFC) is the gold standard for the diagnosis of food allergy, however, it is time-consuming and expensive. In that way, many studies have pointed out that IgE against whole peanut extract and against Ara h 2 could have an important predictive value for the outcome of oral challenges (Beyer et al., 2015; Dang et al., 2012). On the other hand, Ara h 9, Ara h 16, and Ara h 17 belong to the subgroup of ns-LTPs in peanut. Ara h 9 which was described in 2009 by two different research groups at the same time, was found to be especially relevant for the Mediterranean peanut-allergic population (Krause et al., 2009; Lauer et al., 2009). In Soybean, one allergen was described to belong to the prolamin superfamily. This allergy, Gly m 8, is a 2S albumin that has been proven to be an indicator for severe reactions to this legume not only in pediatric but also in adult soybean-allergic population (Ebisawa et al., 2013; Klemans, Broekman, et al., 2013; Klemans, Knol, et al., 2013). Further important allergens

belonging to the prolamin superfamily can be found in Brazil nut (the 2S albumin, Ber e 1), walnut (the 2S albumin, Jug r 1), hazelnut (the 2S albumin, Cor a 14), cashew (the 2S albumin, Ana o 3), pistachio (the 2S albumin, Pis v 1), peach (the nsLTP, Pru p 3), apple (the nsLTP, Mal d 3), apricot (the nsLTP, Pru ar 3), lentil (the ns-LTP, Len c 3), pea (the ns-LTP, Pis s 3), in green bean (the ns-LTP, Pha v 3), and in many other tree nuts, legumes, seeds and fruits.

2.1.2 Cupin superfamily

Cupin superfamily is constituted by proteins that share a core domain with a β -barrel structure that gives the name to this superfamily, since *cupa* in Latin means barrel. Allergens from plant foods that belong to the cupin superfamily include globulin seed storage proteins that can be classified according to their sedimentation coefficient in 7S globulins (or vicilin-type) and 11S globulins (or legumin-type).

7S globulins are normally trimeric proteins whose subunits have molecular weights of around 40–80 kDa. Their amino acidic sequences do not contain cysteines and for that reason, there is a lack of disulfide bonds. On the other hand, 11S globulins are trimeric proteins in their mature form. Each subunit is formed by an acidic subunit of a molecular weight between 30 and 40 kDa and a basic subunit of around 20 kDa. Both subunits are attached by a disulfide bond (Breiteneder & Radauer, 2004).

In peanut, the major allergens Ara h 1 and Ara h 3 belong to the cupin superfamily. Ara h 1, a 7S globulin, has been linked with severe allergic reactions in certain study populations. The allergenic potency of Ara h 1 has been suggested being dependable on its interaction with molecules such as lipid components. Such interaction with lipids confers Ara h 1 resistance to digestive enzymes and an interaction with dendritic cells that would favor the skewing to a Th2 response, contributing in that way to the allergic potency of Ara h 1 (Angelina et al., 2016). Ara h 3, a 11S globulin, is composed by an acidic and basic subunit that contains important and highly resistant IgE-binding epitopes (Rabjohn et al., 1999). In recent studies, it was shown that Ara h 3 purified from roasted peanut was uptaken and internalized more efficiently by monocyte-derived dendritic cells than Ara h 3 from raw peanut through the mannose receptor, which may translate in a higher antigen presentation of roasted Ara h 3 to T cells (Cabanillas, Maleki, Cheng, & Novak, 2018). In soybean, two allergens belong to the cupin superfamily: Gly m 5, a 7S globulin, and Gly m 6, an 11S globulin. Both allergens have been linked with severe allergic reactions in soybean-allergic populations

from Europe or Japan (Holzhauser et al., 2009; Ito et al., 2011). However, such association seems to be specific for pediatric patients rather than adult patients (Holzhauser et al., 2009; Ito et al., 2011; Klemans, Broekman, et al., 2013; Klemans, Knol, et al., 2013; Vissers, Jansen, Ruinemans-Koerts, Wichers, & Savelkoul, 2011).

Other relevant allergens that belong to the cupin superfamily are found in lentil (the 7S globulin, Len c 1), walnut (the 7S globulin, Jug r 2, and the 11S globulin Jug r 4), cashew (the 7S globulin, Ana o 1, and the 11S globulin Ana o 2), sesame seeds (the 7S globulin, Ses i 3 and the 11S globulins Ses i 6 and Ses i 7), hazelnut (the 7S globulin Cor a 11 and the 11S globulin Cor a 9), etc.

2.1.3 Pathogenesis related (PR) proteins

PR proteins do not form a superfamily per se, but a group of non-related protein families that have the function of plant defense. PR proteins are synthesized in response to different aggressions or dangers such as pathogen infections, wounds, chemicals such as phytohormones, metals, ultraviolet radiation or unfavorable growth conditions. There are 14 groups of PR proteins and among them the groups 1, 2, 3, 4, 5, 8, 10, and 14 contain allergens (Sinha et al., 2014).

PR-1 proteins have antifungal properties and the first allergen described belonging to this group, Cuc m 3 from muskmelon, was described in 2004 (Asensio et al., 2004). *PR-2 proteins* are β -1,3-glucanases involved in pathogenic defense and different physiological plant functions. Allergens from the PR-2 protein family have been described, for instance, in latex as Hev b 2. This allergen shares high sequence identities with other allergenic β -1,3-glucanases from fruits such as tomato, banana, etc. (Receveur-Bréchet et al., 2006). Class I chitinases form part of the *PR-3 and PR-4 families*. Allergens that are class I chitinases, such as Mus a 2 from banana, Pers a 1 from avocado or Cas s 5 from chestnut share a N-terminal hevein-like domain that has a high identity with the latex allergen hevein (Hev b 6.02). Such identities found in class I chitinases (PR-3) and β -1,3-glucanases (PR-2) in latex and fruits explains the IgE cross-reactivity between them and configures a syndrome called latex-fruit syndrome (Wagner & Breiteneder, 2002). This syndrome was described in 1994 based on the observation of the high frequency of IgE-mediated allergy to fruits in a group of 25 patients allergic to latex (Blanco, Carrillo, Castillo, Quiralte, & Cuevas, 1994).

Thaumatococcus-like proteins (TLPs) are included in the *PR-5 family*. TLPs can be grouped in two classes depending on their molecular weight.

Allergens belonging to the TLPs can be found in apple (Mal d 2), cherry (Pru av. 2), peach (Pru p 2), kiwi (Act d 2), etc. (Breiteneder, 2004). *PR-8 proteins* are composed of class III chitinases that also have lysozyme properties. The coffee allergen Cof a 1 is a class III chitinase (Manavski et al., 2012). Allergens from plant foods that belong to the *PR-10 family* have high sequence similarity with the major birch pollen allergen, Bet v 1, one of the best-known plant allergens. Such allergens are denominated Bet v 1 homologs. They are proteins of about 16–18 kDa that have been described as allergens in numerous species belonging to *Rosaceae* (peach, apricot), *Apiaceae* (carrot, celery), *Leguminosae* (peanut, soybean) families or tree nuts (hazelnut, chestnut) (Katelaris, 2010). These allergens have been implicated in the IgE cross-reactivity between plant foods and pollen (Hofmann & Burks, 2008). Finally, *PR-14 proteins* are nsLTPs belonging to the prolamin superfamily and described in Section 2.1.1.

2.1.4 Other families: Profilins, defensins, oleosins

Profilin family contains proteins that regulate the polymerization of plant microfilaments during the growth of pollen tubes, root hairs or cell elongation. Allergenic profilins in plant foods are normally associated with pollen allergy. Profilins are usually labile allergens that denature when are subjected to gastrointestinal enzymes or heat. Therefore, their allergenicity is maximized when the plant foods that contain profilins are consumed in raw form, being the allergenic symptoms usually restricted to the oral cavity. Allergy to birch or grass pollen due to IgE reactivity to profilins is frequently associated with IgE sensitization to profilins in fruits, vegetables or nuts, such as hazelnut (Cor a 2), celery (Api g 4), peach (Pru p 4), melon (Cuc m 2), etc. (Breiteneder & Radauer, 2004; Santos & Van Ree, 2011).

Further protein families that contain allergens in plant foods are oleosins and defensins. Up to date, four oleosins have been identified in peanut (Ara h 10, Ara h 11, Ara h 14, and Ara h 15). Oleosins were discovered during the investigation of peanut allergens associated with lipids and it prompted the development of laboratory strategies for the purification of allergens with presence only in the lipid fraction (Schwager et al., 2015). The role of oleosins in severe allergic reactions to peanut has been recently demonstrated (Schwager et al., 2017). Further allergens isolated from lipophilic peanut extracts involved in severe reactions were allergens that belong to the defensin family (Ara h 12 and Ara h 13). Such allergens are low-molecular-weight proteins and possess anti-fungal activity as part of the plant defense (Petersen et al., 2015).

2.2 Classification of animal food allergens

The most relevant food allergens of animal origin can be found in milk, egg, crustaceans, molluscs, and fish. The allergenic proteins of mammalian milk belong mostly to three families: α -lactalbumin, β -lactoglobulin, and casein. α -lactalbumin belongs to C-type lysozyme/ α -lactalbumin family and is a calcium-binding protein implicated in the synthesis of lactose. β -lactoglobulin is a member of the lipocalin family that acts as a transporter for lipids, hormones, steroids, etc. Casein is also a calcium-binding protein that binds IgE that frequently cross-reacts with caseins present in different mammalian milk such as cow, goat or sheep milk (Restani, Ballabio, Di Lorenzo, Tripodi, & Fiocchi, 2009). The most important allergens in the egg are ovomucoid and ovalbumin that are located in the egg white. Ovomucoid has been described as heat-resistant while ovalbumin is more sensitive to heat (Mine & Yang, 2008). In the case of crustaceans and molluscs, tropomyosins are proteins that play a key biological role in the regulation of muscle contraction and are important allergens resistant to heat and gastrointestinal enzymes. Tropomyosin is a ubiquitous allergen in invertebrates. Allergenic tropomyosins can be found in shrimp, crab, lobster, crawfish or prawn. Tropomyosins were not classically considered allergenic in vertebrates until its description as an allergen in tilapia in 2013 and in other species of fishes in a later study (González-Fernández, Alguacil-Guillén, Cuéllar, & Daschner, 2018; Liu, Holck, Yang, Liu, & Xue, 2013). Interestingly, it was found that the homology between the tropomyosin from invertebrates and vertebrates is about 57%, which is a low percentage for potential clinical allergenic cross-reactivity. Moreover, it has been proposed that tropomyosin from vertebrates is enzymatically digested into smaller peptides than tropomyosin from invertebrates with a reduced capacity to bind IgE and lower probability to act as allergens (Kourani, Corazza, Michel, & Doyen, 2019). Parvalbumins, on the other hand, are important allergens in several fish species that have the capacity to bind calcium and their biological function is involved in the relaxation of fish muscles (Hoffmann-Sommergruber & Mills, 2009).



3. Molecular and immunological properties of food allergens

Food allergens have been traditionally described as proteins or glycoproteins with a molecular weight between 5 and 100 kDa. The solubility

in water is a common property of food allergens, however, there are food allergens that have been described as hydrophobic, such as oleosins. Food allergens can also be found beyond their traditional classification as proteins or glycoproteins and that is the case of purely carbohydrate determinants that can act as allergens, such as galacto-oligosaccharide (Soh, Huang, & Lee, 2015). Other common characteristics of food allergens rely on their stability to different food processing conditions involving heat, and their resistance to enzymes from the gastrointestinal system during the digestion process. Other factors such as high abundance in the food source, specific protein structures, or a large number of IgE-binding epitopes within the protein structure have also been traditionally associated with the typical properties of an allergen (Bannon, 2004). However, many exceptions exist to these common characteristics that an allergen must-have. Moreover, not all proteins or glycoproteins that have the aforementioned properties are allergens. For that reason, although there are characteristics common to a large number of allergens, such as the ones mentioned before, at the same time, there is great variability that makes it difficult to establish cohesive properties to define a protein/glycoprotein/carbohydrate as an allergen. Furthermore, novel characteristics linked to the allergenic potential of certain proteins have been proposed in recent years based on the fact that certain allergens can act as adjuvants or enhancers of the innate (and adaptive) immune system. In this context, it has been demonstrated that certain food allergens, such as the peanut allergen Ara h 1 have Th2-enhancing properties since they are able to stimulate dendritic cells to induce the differentiation of Th2 cells from naïve T cells. That property is based on the glycosylated status of Ara h 1, in a way that its carbohydrate structures are recognized by specific receptors on dendritic cells, acting as adjuvants and promoting a Th2-enhancing effect. Ara h 1 loses this property when it is deglycosylated *in vitro*, which proves that the carbohydrates within its structure, contribute to the mentioned effect (Shreffler et al., 2006). In line with this assumption, studies carried out by Hilmenyuk and collaborators have demonstrated that some allergens from egg become glycated during heating, a fact that increases their recognition by specific receptors on dendritic cells in contrast to non-heated, non-glycated allergens from egg. Glycated egg allergens also induced a Th2 response, with a higher potential than non-glycated allergens to induce IgE production and an allergic response (Hilmenyuk et al., 2010).



4. Effects of thermal processing on food allergenicity: Autoclaving as an example of treatment for allergy reduction

Different strategies have been investigated over the years and applied to certain categories of foods in order to decrease their allergenic potential reducing the risk for the end sensitive consumers. The types of processing typically fall in two distinct groups, thermal and non-thermal treatments, depending if heating (moist or dry) is applied or not. It is well known that these treatments may either reduce or enhance the allergenic potential of food proteins or sometimes also have a negligible effect. Irrespective of the specific processing applied, the food protein structure can be altered, and this might impair its digestibility and the levels of allergens absorbed across the mucosa thereby modulating the allergenic potential (Watanabe et al., 2014). Alongside traditional practices, novel processing approaches such as high-pressure processing, pulsed ultraviolet light, high-intensity ultrasound, irradiation, and pulsed electric field have been studied for some allergenic foods in terms of immunoreactivity reduction.

Thermal treatments based on dry or moist heating encompass boiling, baking, roasting, frying, autoclaving, microwave heating, blanching, steaming and are briefly schematized in Fig. 1 with the relative implications on food proteins structure. The structural changes induced on food proteins

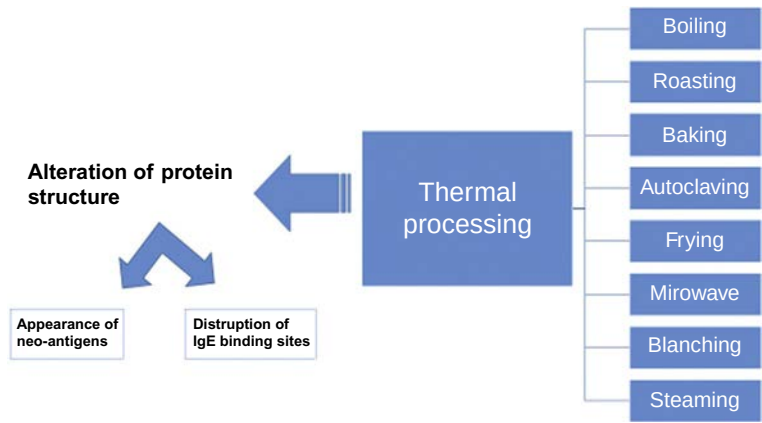


Fig. 1 Summary of the thermal treatments investigated and the relevant implications on food proteins.

by heating have been extensively investigated (Rahaman, Vasiljevic, & Ramchandran, 2015a; Verma, Kumar, Das, & Dwivedi, 2012). The phenomena accounting for such behavior employ protein unfolding, followed by loss of secondary and tertiary structure and formation of intra and/or inter-molecular covalent and non-covalent interactions. All these changes might mask or destroy conformational epitopes spread along the protein moiety thus influencing its final allergenicity.

Conditions such as pH and ionic strength are important factors for heat mediated denaturation of some proteins such as β -lactoglobulin (Schmitt et al., 2009), and are known to influence changes in its allergenicity. As an example, when β -lactoglobulin is heated at acidic pH (3) it leads to the unfolding of the protein molecule, partial acid hydrolysis, thus contributing to the appearance of some new epitopes with antigenicity enhancement (Rahaman, Vasiljevic, & Ramchandran, 2015b). By contrary, the decrease in the immunoreactivity of egg ovomucoid (OM) was reported to be higher at an alkaline pH (9.5) than at pH 7.5 (Lee, Lee, Yook, et al., 2002) and was attributed to the irreversible denaturation of OM with alteration of allergenic epitopes. In general, whether many food allergens are sensitive to heat and their allergenicity changes accordingly, others proved to be rather resistant and stable to heat denaturation and digestion (Mills, Sancho, Rigby, Jenkins, & Mackie, 2009) and consequently, their allergenicity remains unaltered. In particular, proteins more resistant have as common feature the presence of cysteines with intra or inter molecular disulphuric bridges that determine their structural integrity as well as antigenic stability (Maeno, Matsuo, & Akasaka, 2013). Because of the temperature applied, different effects on the final allergenicity can be displayed. An example is provided by β -lactoglobulin. Heating it up to 90 °C caused protein unfolding, exposure of conformational epitopes and enhanced susceptibility to proteolysis resulting in increased allergenicity. By contrast, raising the temperature to 100 and 120 °C led to irreversible aggregation with covalent and hydrophobic interactions, consequently masking and/or destroying conformational epitopes and causing a reduction of allergenicity. Similarly, also for egg white proteins it was reported allergenicity reduction upon heating with special regard to ovalbumin (Watanabe et al., 2014). As for the antigenicity of the wet-heated soy protein isolate (SPI) it was reported that it decreased much more than that of the dry-heated SPI probably due to the different processing conditions and the protein/sugar ratios between these two methods (van de Lagemaat, Manuel Silván, Javier Moreno, Olano, & Dolores del Castillo, 2007).

Thermal treatments can also influence the interaction of allergens with other food ingredients or promoting the formation of new products or neoantigens. Protein glycation can be a consequence of the application of food-processing techniques. It has been reported that glycation can improve food protein physicochemical properties and functionality (Liu, Ru, & Ding, 2012), but at the same time immunoreactivity of glycated proteins may decrease, remain unchanged, or even increase depending on the specific food and heating conditions applied. Protein glycation is capable of generating new epitopes (linear or conformational) and/or conformational changes of the existing epitopes recognized by IgE and/or IgG antibodies. Depending on the method used and other factors such as sources of protein and sugar, protein/sugar ratio and processing conditions (dry or wet methods, temperature, and incubation time), the final effect on protein immunoreactivity can vary considerably.

As an example, heat-induced glycation can influence β -lactoglobulin allergenicity due to the Maillard reaction occurring at the Lys residues causing the modification of linear epitopes (Taheri-Kafrani et al., 2009) with a consequent reduction of IgE reactivity. In addition, the high level of glycation reduced the susceptibility of β -lactoglobulin to proteolysis resulting in an increased IgE reactivity of the hydrolysates (Corzo-Martínez, Soria, Belloque, Villamiel, & Moreno, 2010). Roasted peanut extracts bound IgE from patients with peanut allergy at approximately 90-fold higher levels than raw peanuts, and the protein modifications induced by Maillard reaction contributed to the observed effect (Chung & Champagne, 2001; Maleki, Chung, Champagne, & Raufman, 2000).

Boiling, proved to alter the allergenicity of legumes and peanuts, due to the transfer of low-molecular-weight peanut allergens, such as Ara h 2, Ara h 6, or Ara h 7, from the seeds to the boiling water (Schmitt, Nesbit, Hurlburt, Cheng, & Maleki, 2010). Heat treatment applied to soybean affected allergenicity differently (Lee et al., 2009). Cabanillas et al., 2018 reported that IgE reactivity of soybean was not affected by boiling for up to 30 min, but it was partially decreased by boiling it for 60 min. Pressure-cooking at specific conditions has also been found to impact on the IgE-binding properties of legumes (Cabanillas, Cuadrado, et al., 2018).

Microwave heating has been investigated and applied to many food commodities for allergenicity reduction thanks to the potential of this kind of heating (propagating through electromagnetic energy across water) to impact the native structure of allergic proteins thus altering their

recognition sites for IgE binding of sensitized individuals (Jiménez-Saiz, Benedé, Molina, & López-Expósito, 2015). In particular, it was reported that exposing lupines to microwave heating in water-induced minimal change in the final immunoreactivity (Alvarez-Alvarez et al., 2005). In other cases, like wheat and several types of nuts, microwave treatment did not produce any immunogenicity decrease under the applied conditions (Cabanillas & Novak, 2019).

While for certain nuts and legumes it was proved that specific thermal treatments showed to decrease the IgE binding capacity in vitro (Cabanillas et al., 2012; Clemente, Vioque, Sanchez-Vioque, Pedroche, & Millán, 1999), the reduction of IgE binding of processed food sometimes does not correlate with a decreased capacity of treated food to cross-link IgE on effector cells, such as basophils or mast cells (Shi et al., 2013). As a consequence, an extensive protein fragmentation seems to be necessary to ensure a reduced allergenicity.

Autoclaving has been recently explored by some authors as an alternative thermal processing to reduce allergenicity and has been applied to several allergenic commodities with promising results (Bavaro et al., 2018; Cabanillas et al., 2014). This treatment combines pressure and temperature and proved to significantly reduce the IgE binding as assessed in immunoblot analysis by using sera of allergic patients. Autoclaving at 2.56 atm, for 30 min, produced a relevant decrease in the IgE-binding capacity of lentil and chickpea allergens, although several immunoreactive proteins still remained in these legumes upon harsh autoclaving (Cuadrado et al., 2009). Autoclaving soybeans led to a higher decrease of soybean IgE reactivity, due to a potential protein fragmentation, although some soybean allergens (such as 7S globulins) remained after the treatment. Likewise, other studies proved that heat/pressure treatments were able to decrease the IgE-binding properties of cashew and pistachio protein extracts assessed both by IgE-ELISA and IgE-western blot (Sanchiz et al., 2018).

Autoclaving was reported to alter peanuts immunoreactivity. Conditions such as the temperature and the duration of the treatment play a pivotal role on allergenicity modulation (Cabanillas & Novak, 2015). In particular, Bavaro et al., 2018 reported that hydration prior to autoclaving proved to increase the efficacy of the autoclaving contributing to the disappearance of the main allergenic protein bands and altering significantly the final immunoreactivity as assessed by immunoblot experiments. According to their findings, only a protein banding at MW 25 kDa showed to retain some

residual reactivity, giving rise to exclude any aggregation phenomena whereas a probable fragmentation of proteins might have occurred because of the prolonged exposition to water before the thermal treatment.

In summary, according to the data shown the type and conditions of the thermal treatment proved to have a remarked effect on the modulation of the final allergenicity. In turn, the selection of appropriate parameters during processing turns out to be the critical step. As far as boiling and autoclaving are concerned, they proved to reduce immunogenicity at different extensions. In general, the type of food processing capable of fragmenting the proteins in short peptides endowed with a decreased IgE binding capacity might turn to be a good alternative and a promising approach for inducing oral tolerance in sensitive consumers and reduce the risk of severe allergic reactions in future.



5. Methodology for the characterization of food allergens

One of the first steps in the characterization of food allergens should be the identification of a study population of allergic patients with a good clinical characterization of food allergy based on a positive double-blind placebo-controlled food challenge or a history of anaphylaxis documented with the specific food. Positive levels in blood serum of specific IgE against the offending food or/and positive skin prick test results are also part of the diagnosis of food allergy (Lucas & Atkinson, 2008). The serum IgE and certain cells of the immune system of these patients are not only essential in the evaluation of food allergy but also they are used in the experiments for the identification of specific food allergens. In order to carry out the identification of an allergen, it is necessary to analyze a number of characteristics responsible for its allergenicity, such as resistance to digestion and processing, relationship with known allergens, etc. However, these characteristics are not unique to all allergenic proteins and therefore they do not identify them completely. Thus, there are no established rules that can predict the potential allergenicity of a food protein, only a combination of different methodologies *in vivo*, *in vitro*, and *in silico* that can characterize the allergenic properties of a given food protein (Sanchez-Monge & Salcedo, 2005).

Allergenic proteins can be initially analyzed if they are physically separated by electrophoretic methods and transferred to synthetic membranes and subsequently incubated with serum from allergic patients for IgE-binding detection, in what is called IgE-western blot (Sanchez-Monge & Salcedo, 2005). The deeper characterization of food allergens requires their

purification by chromatographic methods, ensuring their purity throughout the process by different techniques such as mass spectrometry, or other methods that analyze the purity of the sample containing the purified allergen. After the purification process, the characterization of food allergens requires further immunoassays that evaluate their allergenic potential. In an expert report from FAO/WHO regarding the allergenicity of foods modified by biotechnology, it is recommended in the immunoassays to avoid the use of combined pools of sera in a number greater than five since the specific antibodies could be diluted in that pool. Instead, the report recommends the use of a selection of 25 individual serum samples with high levels of IgE that recognizes the specific food protein (FAO/WHO, 2001). In an IgE-western blot, the individual bands typically represent a single protein, however, if several proteins have the same molecular weight, they will migrate in a similar way and they will form a single band (Poms, Klein, & Anklam, 2004). This problem can be solved using two-dimensional electrophoresis, which allows the separation of samples with heterogeneous proteins based on their isoelectric points and molecular weights (Larbi & Jefferies, 2009). As explained in Section 4, ELISA has been the most widely used method in food industry laboratories to detect and quantify food allergens (Poms et al., 2004). ELISA is also the method of choice when evaluating the allergenicity of food proteins and protein hydrolysates due to the sensitivity and the relative simplicity of the technique (Clemente, Olías, & Olías, 2000). Another system based on the so-called fluorimetric enzyme immunoassay (FEIA) is the method most commonly used in the clinical practice for the determination of specific serum IgE. In that respect, a dose-response curve of total IgE is used to interpolate the data of specific IgE. The units used are kilounits of IgE per liter (kU/L), where 1 unit equals 2.4 ng of IgE (Sanchez-Monge & Salcedo, 2005). Although this technique is mainly used in clinical practice, CAP (trade name of a type of FEIA) inhibition assays can be used in competition experiments in order to evaluate cross-reactivity between allergens. These assays are based on the analysis of the higher or lower inhibition of IgE binding to the allergens immobilized in the solid phase due to the binding of IgE to allergens in solution (Poms et al., 2004).

Confirmation of the allergenicity of a protein can be determined by skin prick tests, histamine release assays or quantification of degranulation markers from effector cells of allergy (Lucas & Atkinson, 2008). These methods evaluate the ability of a protein to activate effector cells such as basophils or mast cells. Histamine release assays are performed by incubating heparinized blood with increasing concentrations of the allergen and

quantifying, after centrifugation of the cells, histamine using techniques such as ELISA, fluorimetry, etc. In the case of quantification of degranulation markers, two membrane proteins that are found in activated basophils, CD63 and CD203c, are used. These markers are quantified by flow cytometry using monoclonal antibodies conjugated with fluorochromes. CD203c is expressed only in basophils and mast cells, while CD63 is also expressed in other cells like monocytes, having to use a second basophil specific reagent in the test (Sanchez-Monge & Salcedo, 2005).



6. Detection of allergens in foods

Tracing accidental contamination of allergenic ingredients in food commodities is an important goal to guarantee the safeguard of consumers' health and ensure compliance with food labeling legislation. In the last decades, multitudes of methodological approaches coping with this aim have been proposed, differing in the analytical target, the applied technology, and the analytical performance provided. Detection methods may target either the allergenic protein itself (protein-based method), or DNA sequences tracing for the presence of the allergenic ingredient (DNA-based method). Starting from these two main categories, the allergen detection methods can be further classified into (i) immunoassay-based, (ii) DNA-based and (iii) mass spectrometry (MS) based methods (Monaci & Visconti, 2010; Pilolli, Monaci, & Visconti, 2013; Monaci, De Angelis, Montemurro, & Pilolli, 2018). Each category achieved until now features pro and cons and achieved a different level of diffusion and acceptability. As a general overview, we reported in Fig. 2 the publication trend in the food allergen detection field of the last 15 years, identifying according to specific keywords the over-time establishment of immunoassay-, DNA- and MS-based methods. Immunoassay and DNA based method found the first application in the food allergen detection field, offering several advantages that contributed to the wide commercialization of commercial kits targeting single allergens for routine analysis (see Fig. 3).

However, these approaches encounter also several drawbacks that decrease the confidence in the results obtained, among them, cross-reactivity phenomena, susceptibility to both false positive and false negative. In view of this, the development and validation of high-throughput, reliable and sensitive confirmatory methods based on MS represents the current challenge for researchers.

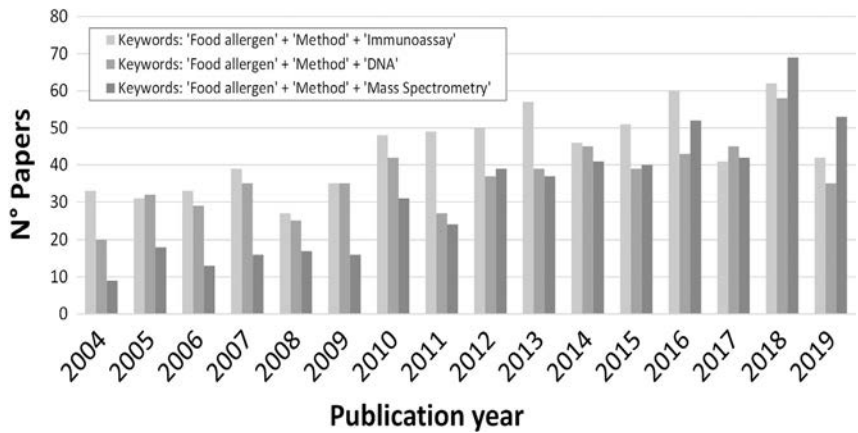


Fig. 2 Overview of the publication trend of the last 15 years according to the on-line database Scopus®. The results counting was limited to “articles” and “conference papers” as document types. Accessed on January 24, 2020 (Copyright © Elsevier B.V.).

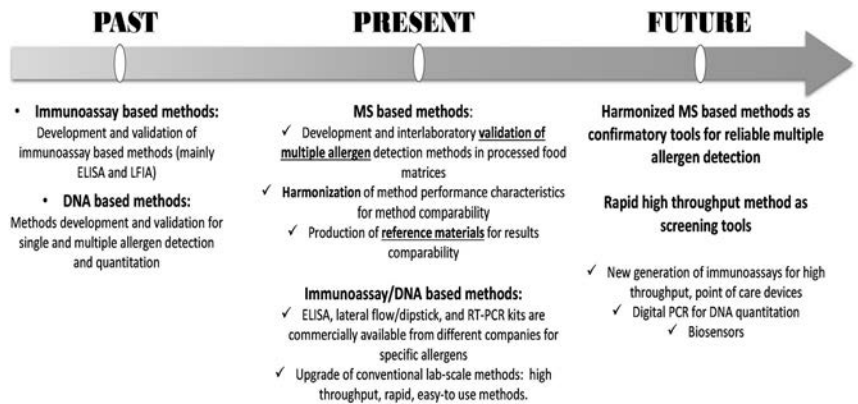


Fig. 3 Past establishment and future perspective in food allergen detection methods.

6.1 Immunoassay-based methods

Among the analytical approaches based on the antibody recognition, Enzyme-Linked Immunosorbent assay (ELISA) has been the methodology most widely used for routine allergen management due to its precision, sensitivity (1–25 ppm), simple handling, and good potential for standardization (Jayasena et al., 2015). The affinity and specificity of the integrated antibodies toward the selected analyte deeply affect the detection performance of the method in terms of sensitivity, specificity, and robustness.

The allergenic ingredients tackled in the current legislation contain a complex proteome including both allergenic and non-allergenic proteins. For some of them, the main allergenic proteins are well characterized therefore both monoclonal and polyclonal antibodies have been selected for inclusion in ELISA assays (Croote & Quake, 2016). Despite monoclonal antibodies recognizing a specific epitope, polyclonal antibodies are more widely used in allergen detection featuring some benefits in terms of applicability. Indeed, polyclonal antibody can bind different epitopes on the same allergenic protein, and as such can tolerate small changes in the nature of antigens.

The crucial points in the application of ELISA are the extraction method and the standard solution used as calibrants both lacking standardization and impairing the comparability of the results among different kits. The protein solubility might differ significantly in the native state of raw ingredients or after food processing due to protein aggregation, denaturation or chemical modification (Verhoeckx et al., 2015). As a result, the effects of the processing applied to food should also be taken into account for selecting the most suitable kit and stronger buffers should be preferred especially when dealing with hard to analyze commodities (Azarnia, Boye, Mongeon, & Sabik, 2013; Mattarozzi et al., 2014). In addition, the processing can affect the efficiency of the antibody recognition due to the masking effect of the targeted epitopes or to their chemical modification, impairing the final detection sensitivity and reliability (Verhoeckx et al., 2015). In this regard, several reports have shown that thermal processing during food manufacture can markedly affect the performance of commercial ELISA kits, resulting in reduced food allergen recovery (Gomaa & Boye, 2013; Khuda et al., 2012a; Khuda et al., 2012b; Monaci, Brohee, Tregoeat, & van Hengel, 2011; Parker et al., 2015; Sharma et al., 2013). The most relevant ELISA methods tailored to allergen detection in different food commodities with the LODs and methods performances have been recently reviewed by De Angelis, Bavaro, Pilolli, & Monaci, 2019. The last years have seen increasing efforts in developing sensitive ELISA kits for food allergens analysis in processed food matrices (Ecker & Cichna-Markl, 2012), also using new eco-friendly strategies for the efficient and simultaneous extraction of different allergenic proteins (Ito et al., 2016).

Alternative immunoassay-based methods are represented by Lateral Flow immunoassay (LFIA). These latter were first commercially launched in 1984 and have attracted attention as an alternative to ELISA kits for their time- and cost-effectiveness (Anfossi et al., 2013; Anfossi, Baggiani, Giovannoli, D'Arco, & Giraudi, 2013). The LFIA is present in two formats,

(i) direct format (sandwich assay) or (ii) competitive assay. The direct assay features the detection of high molecular weight analytes, characterized by multiple antigenic sites. In this case, the complex analyte/antibody (conjugated with latex or colloidal metal) migrates along a test strip reacting with an immobilized analyte-specific capture antibody (test zone) and an immobilized secondary antibody (control zone). The specific biological recognition produces in the test zone a colorimetric reaction, proportional to the analyte content (Singh, Sharma, & Nara, 2015; Wong, 2009). Differently, in the competitive format, the analyte is characterized by a low molecular weight and displays a single epitope. With this assay, the analytical response is inversely proportional to the analyte concentration. In direct and competitive assays, the presence of a control zone whose activation is independent from the presence of targeted analytes, is important to confirm the correct functionality of the lateral flow test (Ngom, Guo, Wang, & Bi, 2010; Singh et al., 2015).

The main application for LFIA to allergen management is tailored to the detection of allergen traces on surfaces to assess the risk of cross-contamination along shared production lines. However, the feasibility to detect allergen contamination in food matrices has been also proved, boasting a shorter time of execution and simpler protocols than ELISA kits. The first version of LFIA applied to the food allergen field, was devised by Mills, Potts, Plumb, Lambert, and Morgan (1997) to detect peanut proteins. Since then, numerous LFIAs have been developed and commercialized for the detection of allergens not only in raw but also in processed goods (Koizumi, Shiota, Akita, Oda, & Akiyama, 2014). Similarly, to ELISA, the performances of LFIAs are affected by both processing and matrix effects and great efforts are being devoted to the validation of these kits also in interlaboratory studies promoted by AOAC International.

6.2 DNA-based methods

The DNA-based methods provided an alternative to immunological methods, still with the main limitation of an indirect detection. These tests based on the polymerase-chain reaction (PCR) for the detection of specific DNA sequences encoding for allergenic proteins. The specificity and sensitivity of the method depend on the purity and integrity of the DNA extracted, as well as on the specificity of the primers and probes designed for the PCR.

Most of the published DNA-based methods for the detection of allergenic ingredients are based on PCR-ELISA, multiplex PCR, quantitative

real-time PCR (qPCR), and, more recently, a qPCR combined with high-resolution melting analysis (Prado et al., 2016). The qPCR allows real-time monitoring of DNA amplification providing a quantitative estimation of the target analyte (Higuchi, Dollinger, Walsh, & Griffith, 1992). However, as an indirect detection method, quantification results are reported as DNA copy number or DNA concentration, therefore, conversion factors to protein content should be designed to provide useful information about the actual level of contamination from allergenic ingredients. Moreover, their accuracy in tracing allergenic ingredients in foods is also affected by the food processing that impairs DNA stability (Sharma, Khuda, Parker, Eischeid, & Pereira, 2017). Indeed, the thermal treatment has a strong impact on the recovery of DNA, due to DNA fragmentation and this can lead to an underestimation of the actual allergen concentration, up to false-negative results (López-Andreo, Aldegue, Guillén, Gabaldón, & Puyet, 2012). Finally, also the composition of the food matrix poses challenges to the DNA-based detection because interfering components such as fat, sugar, proteins and secondary plant materials can inhibit the PCR reaction (Kenk, Panter, Engler-Blum, & Bergemann, 2012; Poms et al., 2004).

Recently, big efforts have been devoted to overcoming some of the drawbacks of such methodology. As for the absolute quantification of DNA and its conversion to allergenic protein content, the use of a DNA calibration curve obtained by spiking food samples with different percentages of the allergenic ingredient was proposed to simulate contaminated samples at known concentration levels (i.e., Waiblinger, Boernsen, Näumann, & Koepfel, 2014; Waiblinger et al., 2017). In addition, due to the severe impact of food processing on DNA stability with relevant limitation on method performance, several authors have proposed the use of incurred food as starting material for method development, proving promising results for different allergens (López-Calleja et al., 2017; Eischeid, 2016). However, more investigations should address the use of internal standards to evaluate the influence of food components on the PCR reaction.

6.3 Mass spectrometry-based methods

Mass spectrometry-based methods represent the most recent alternative to conventional antibody-based approaches. In particular, the coupling between MS and liquid chromatography proved to be successful in food allergen detection and quantification, by applying the classical bottom-up proteomic approach. The experimental workflow for MS-based analysis

includes the proteins extraction/purification from potentially contaminated food, their tryptic digestion, and the LC-MS detection of proteotypic peptides under proper instrumental conditions. Each step of this general workflow needs an appropriate optimization during method development in order to guarantee the best performance in terms of sensitivity, specificity and robustness. As a fact, markers identification is one of the most important steps, because the overall analytical method will be strictly affected by the reliability of the signature peptides (uniqueness, specificity, stability) tracing for the target proteins. Different routes have been proposed, suggesting the need for general selection criteria for marker peptides, in light of the desired harmonization of method development strategies. Most of the issues besetting the marker selection have been recently reviewed by several authors (Downs & Johnson, 2018; Pilolli et al., 2020) including, differences in ingredients formulations, extraction yield in thermally processed foods, incomplete genomic and proteomic sequence information, as well as all common issues associated with MS-based protein quantitation. The main critical point was that the target selection cannot be accounted for the *in-silico* only approach, because methodological details, such as matrix composition, protein extraction, and digestion, could affect both the specificity and sensitivity of the method. In addition, attention should be paid to the optimization of protein extraction and purification, especially if the method is tailored to monitor contamination in complex and processed food matrices due to impaired extraction yield and/or amino acids modifications (Campbell, Raikos, & Euston, 2003; Davis & Williams, 1998; Mueller et al., 2013; Sayers et al., 2016).

The allergen detection and quantitation at the peptide level can be achieved with specific pros and cons by several MS platforms and analysis modes. Low resolution MS based on selected reaction monitoring (SRM) acquisition mode, also called multiple reactions monitoring (MRM), has been the most common choice for accurate and reproducible quantification (Monaci et al., 2018). Specific precursor/transitions of the selected marker peptides are monitored providing high-throughput capability. Initially developed on triple quadrupole instruments (QqQ), this approach has been successfully transferred to other analyzers like linear ion traps with comparable or improved performances for multi-allergen detection (De Angelis, Pilolli, & Monaci, 2017; Monaci, Pilolli, De Angelis, Godula, & Visconti, 2014; Pilolli, De Angelis, & Monaci, 2017).

The absolute quantitation by SRM relies on the principles of stable isotope dilution (SID) methods. Stable isotope-labeled standards such as AQUA

peptides, QconCAT (concatenated peptide constructs) and PSAQ (protein standard absolute quantification) are commonly utilized as a reference, providing a robust protein quantification, with LOD in sub-ppm range (Sharma et al., 2017). An alternative approach for relative allergen quantification grounds on the label-free quantification, which uses MS1 ion current or MS2 spectral counting to disclose peptide different abundances. In this case, external calibration (Monaci & van Hengel, 2008; Monaci et al., 2014; Mattarozzi, Bignardi, Elviri, & Careri, 2012), modified synthetic peptides (Zhang et al., 2012) and standard addition (Posada-Ayala et al., 2015) are the main strategies used for allergen quantification.

In addition, HR-MS approach based on Orbitrap™ technology has been proposed for high throughput screening of allergens in different food commodities (Korte, Lepski, & Brockmeyer, 2016; Monaci, Losito, De Angelis, Pilolli, & Visconti, 2013). The proposed approach featured specific advantages based on the possibility to simultaneously identify numerous peptide markers, even retrospectively without preliminary information/selection required, as well as to elucidate alternative information about food components due to its non-targeted character (Kaufmann, 2012; Kaufmann, Widmer, & Maden, 2010; Makarov & Scigelova, 2010). Besides, the combination of HR-MS Full scan and HCD All Ion Fragmentation acquisition modes provides confirmative and quantitative features at the highest resolving power and mass accuracy within a single chromatographic run. Recently, also targeted analysis modes on hybrid quadrupole/Orbitrap based platforms have been tested and successfully applied to the food allergen detection in incurred food matrices (Pilolli, De Angelis, & Monaci, 2018), coupling the selectivity/specificity provided by the targeted mode (isolation of selected precursor ions) and by the high mass accuracy supplied by the Orbitrap technology.

Notwithstanding, the undeniable advances provided by the MS-based multiplex detection methods compared to both immunoassay- and DNA-based methods, some criticisms still need to be tackled, mainly concerning the results comparability among independent investigations. The current opinion is to focus all international efforts into the harmonization of MS-methods development and validation, reaching consensus on the evaluation of method performance characteristics, the reporting units, and the general acceptance criteria, as well as promoting the production of proper reference materials. The achievement of these goals would pave

the way toward the establishment of harmonized reference MS-based methods as a confirmatory tool for the quantitative detection of multiple allergen contaminations in food.



7. Conclusions

Food allergy is an important concern since it is well-established that the prevalence of food allergy has dramatically increased in developed countries in the past decades and is beginning to increase in developing countries. Scientific advances made in recent years have shown that although some food allergies can be overcome through natural tolerance, others can persist and be a serious problem throughout the life of allergic individuals. In that respect, the establishment of thorough and relevant laws for the declaration of food allergens on the labels of food products are of critical importance for society. Although food labeling policies have been improved in the last decades in many countries, a thorough regulation and standardization of the precautionary allergen labeling is still needed in order to improve the safety of consumers with food allergy.

Important advances have also been made during the last decades in the molecular aspects of food allergy, including the description of new food allergens and their IgE epitopes that have been carried out diligently by many research groups. Although it has been traditionally thought that most of the food allergens can be grouped into a few families and superfamilies, the description of novel allergens belonging to new families are challenging this idea. Considerable progress has also been made in the description of new molecular and immunological properties of food allergens that are expanding the already described traditional characteristics of food allergens. Novel properties of certain food proteins linked to their ability to act as adjuvants or enhancers of the innate (and adaptive) immune system are opening a new research path in relation to the interaction of food allergens with different molecular and cellular components involved in the sensitization phase of food allergy.

Important progress has also been made in the last years in the study of the effects of certain food processing, such as thermal treatments, on the allergenicity of foods. Such progress is an important asset and very relevant scientific information for consumers with food allergy since a high proportion of foodstuff is consumed after processing. In that respect, the accurate

detection of food allergens in foodstuff able to trace accidental contamination of allergenic ingredients is a relevant benefit for scientists, consumers, and industry. In that respect, a great scientific effort has been made in the description of multiple methods for such detection, each with its advantages and disadvantages, but all of them seeking the common objective of detecting the smallest amounts of food allergens in order to ensure the maximum protection of consumers with food allergy.

The scientific progress made in the research field of food allergy is producing a decisive expansion of the molecular and cellular knowledge of this disease, of the components that trigger it, and the procedures and methods that, to a greater or lesser extent, have a significant impact on food allergy. In a very promising way, we will witness even greater progress in this area of research in the coming decades.

Acknowledgments

This work was supported by a grant from the research program Talento Investigador of the Community of Madrid (Regional Ministry of Science, Universities, and Innovation, Madrid, Spain). B.C. is funded in the senior modality of the research program Talento Investigador of the Community of Madrid (2019-T1/BIO-12690).

Conflict of interest

The authors declare no conflict of interest.

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