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Are Physicochemical Properties Shaping the Allergenic Potency of Plant Allergens?

Joana Costa[1] · Simona Lucia Bavaro[2,3] · Sara Benedé[4] · Araceli Diaz-Perales[5] · Cristina Bueno-Diaz[6] · Eva Gelencser[7] · Julia Klueber[8,9] · Colette Larré[10] · Daniel Lozano-Ojalvo[11] · Roberta Lupi[10] · Isabel Mafra[1] · Gabriel Mazzucchelli[12] · Elena Molina[4] · Linda Monaci[2] · Laura Martín-Pedraza[13] · Cristian Piras[14,15] · Pedro M. Rodrigues[16] · Paola Roncada[17] · Denise Schrama[16] · Tanja Cirkovic-Velickovic[18,19,20] · Kitty Verhoeckx[21] · Caterina Villa[1] · Annette Kuehn[8] · Karin Hoffmann-Sommergruber[22] · Thomas Holzhauser[23]

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Abstract

This review searched for published evidence that could explain how different physicochemical properties impact on the allergenicity of food proteins and if their effects would follow specific patterns among distinct protein families. Owing to the amount and complexity of the collected information, this literature overview was divided in two articles, the current one dedicated to protein families of plant allergens and a second one focused on animal allergens. Our extensive analysis of the available literature revealed that physicochemical characteristics had consistent effects on protein allergenicity for allergens belonging to the same protein family. For example, protein aggregation contributes to increased allergenicity of 2S albumins, while for legumins and cereal prolamins, the same phenomenon leads to a reduction. Molecular stability, related to structural resistance to heat and proteolysis, was identified as the most common feature promoting plant protein allergenicity, although it fails to explain the potency of some unstable allergens (e.g. pollen-related food allergens). Furthermore, data on physicochemical characteristics translating into clinical effects are limited, mainly because most studies are focused on in vitro IgE binding. Clinical data assessing how these parameters affect the development and clinical manifestation of allergies is minimal, with only few reports evaluating the sensitising capacity of modified proteins (addressing different physicochemical properties) in murine allergy models. In vivo testing of modified pure proteins by SPT or DBPCFC is scarce. At this stage, a systematic approach to link the physicochemical properties with clinical plant allergenicity in real-life scenarios is still missing.

Keywords Plant allergens · Protein families · Allergenicity · Food processing · Matrix effect

Abbreviations

ATI	α-Amylase trypsin inhibitors
BAT	Basophil activation test
DBPCFC	Double-blind placebo-controlled food challenge
EAST	Enzyme allergosorbent test
ELISA	Enzyme-linked immunosorbent assay
GMP	Good manufacture practices
HMW	High molecular weight
HPP	High-pressure processing
HHP	High hydrostatic pressure
IgE	Immunoglobulin E
LMW	Low molecular weight
MAT	Mast cell activation test
nsLTP	Non-specific lipid transfer proteins
OAS	Oral allergy syndrome
OFC	Open food challenge
PEF	Pulsed electric fields
PR-10	Pathogenesis-related 10 proteins
PTM	Post-translational modifications
PUV	Pulsed ultraviolet
RAST	Radioallergosorbent test
RBL	Rat basophilic leukaemia
S-poor	Sulphur-poor
SPT	Skin prick tests

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✉ Joana Costa
jbcosta@ff.up.pt

Extended author information available on the last page of the article

S-rich	Sulphur-rich
WDEIA	Wheat-dependent exercise-induced anaphylaxis
WHO/IUIS	World Health Organization/International Union of Immunological Societies

Introduction

What turns a food protein into an allergen? Why do some proteins act as allergens, while others do not [1]? What are the differences among proteins that increase their intrinsic allergenic potential? Which factors drive sustained tolerance to foods and food proteins? Which immunological events intervene in tolerance breakdown, leading to sensitisation, and most likely to subsequent food allergy [2]? The knowledge around the identity of food allergens and how they cluster into protein families has contributed to a better understanding of triggers and cross-reactivity in immunoglobulin E (IgE)-mediated food allergies [3]. However, despite these advances, there are still some pivotal questions that remain unanswered [2, 4].

In theory, any protein has the potential to elicit an allergic response, but this is not commonly the case [5]. Most allergens belong to a small set of protein families, and there are common patterns of biomolecular properties among them. This idea supports that allergens should possess special features and not every protein can become allergenic [6]. Still, not all proteins within a specific family are classified as allergens, and there are numerous allergenic proteins that do not present the typical properties associated with their allergenicity [4]. In the case of plants, there are several important groups of allergenic proteins, such as the prolamin and the cupin superfamilies.

The effects on physicochemical properties of allergens, such as thermal stability or resistance to proteolysis, have been addressed in order to correlate those characteristics with their impact on the allergenicity (as the ability to induce IgE production that may mediate clinical reactions) of certain proteins [3, 7, 8]. Post-translational modifications (PTM), such as glycosylation, hydroxylation or phosphorylation, alter the physicochemical properties of allergens. Protein post-translational glycosylation and process-related glycation seem to play a pivotal role in the allergenic potential of proteins [7–9]. Additionally, other characteristics (e.g. the ability of some proteins to disrupt and cross the intestinal epithelial barrier) have been emphasised as affecting the allergenicity of different proteins, since they are known to facilitate the presentation of food allergens to the immune system [4, 10].

Several studies reported on the effect of different physicochemical properties on protein allergenicity, but the overarching picture is still missing. This review is the first of two articles in a thematic compilation (plant and animal allergens), and it is focused on the allergenicity of plant food protein families as affected by different physicochemical parameters.

Plant Allergen Families

According to the AllFam (Database of Allergen Families) statistics in 2017, there are about 1042 proteins identified as allergens, with 88.4% (921 proteins) of them being included in the World Health Organization/International Union of Immunological Societies (WHO/IUIS) list of registered allergens [6, 11, 12]. From the total number of allergenic proteins, 959 allergens of all sources (animals, plants, fungi and bacteria) have been assigned to specific families (totalising 151 families of proteins), while 83 molecules have no family classification.

In the specific case of plants, there are about 467 allergens (95.7% included in the WHO/IUIS list of allergens, $n = 447$) and 436 of them being allocated to specific families of proteins [6, 11, 12]. Despite plant allergenic proteins being scattered over 65 families of proteins, more than 44% (that include most of the relevant plant allergens) belong to eight families: 2S albumins, non-specific lipid transfer proteins (nsLTP), cereal α-amylase trypsin inhibitors (ATI) and cereal prolamins (of the prolamin superfamily), legumins and vicilins (of the cupin superfamily), profilins and pathogenesis-related (PR)-10 proteins [6, 11]. In this review, besides considering the number and importance of the allergens, the criteria for the selection of plant protein families also included some cultural and geographic determinants. For example, it is well known that PR-10 proteins are very relevant and common allergens in regions such as Northern of Europe and Alpine regions, while nsLTP are very important allergens among the Mediterranean population (e.g. Spain, Italy) [13, 14].

Prolamin Superfamily

According to recent statistical data, the Prolamin superfamily ranks the first position in terms of total number of allergenic proteins (91 identified allergens) [6, 11]. It is composed by a diverse group of relevant seed-storage protein families, like the 2S albumins and the cereal prolamins (gliadins and glutenins), as well as the nsLTP and the ATI [15, 16]. The members of this superfamily have a large amount of proline and glutamine residues, which is typical for prolamins. Additionally, they share a highly conserved pattern of eight cysteine residues that stabilises their three-dimensional (3D) structure of four α-helices that form a right-handed super-helix. Apart from the cysteine skeleton and the α-helical

structures, these protein families share little sequence homology [8, 16, 17]. The cereal prolamins (gliadins and glutenins) are an exception in the prolamin superfamily concerning their 3D structure. Except for ω-gliadins, they contain α-helices and β-sheets and they are stabilised by disulphide bonds, but due to their low complexity sequence that mainly contain rich interspersed repeats, their final structures are considered to be disordered structures [18, 19].

2S Albumins

2S Albumins are one of the major groups of seed-storage proteins of the prolamin superfamily, comprising several allergens identified in peanut, tree nuts, legumes and cereals [12]. They are monomeric proteins between 10 and 18 kDa (Table 1), usually based on two polypeptide chains connected by disulphide bonds [20]. During their synthesis, they undergo proteolytic processing due to the presence of some cleavage points in their sequence (N-terminal signal sequence and connection peptide), leading to a final structure of two subunits, a large subunit of 8–10 kDa, and a small one of 3–4 kDa [21]. Contrarily to most allergenic 2S albumins, peanut Ara h 2 occurs as a single polypeptide chain stabilised by disulphide bonds and without a subunit structure [22]. 2S Albumins are encoded by a multigene family, leading to the presence of multiple isoforms. In addition, minor clipping at the N- and C-terminal of both subunits provides extra variants (e.g. Cor a 14, hazelnut) [23, 24].

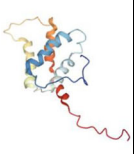
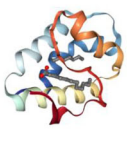
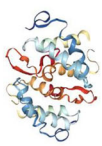
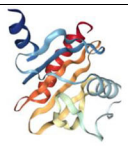
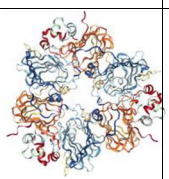
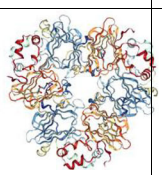
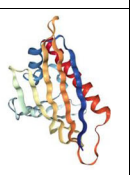
Allergenic 2S albumins show a relatively low-sequence identity among plant species, being more conserved among proteins from the same phylogenetic family [25]. Few exceptions of cross-reactivity have been reported, which is the case of Sin a 1 (mustard) with Pin p 1 (pine nut) [26], and Act d 13 (kiwi seed) with 2S albumins from walnut, peanut and almond [27]. 2S Albumins can present potent allergens, being responsible for triggering severe and systemic adverse immunological responses, as in the case of peanut Ara h 2/Ara h 6 [3].

Non-specific Lipid Transfer Proteins

The nsLTP are a large family of proteins that are profuse in all plants, representing as much as 4% of the total soluble protein fraction. The majority of its members are extracellular proteins associated with cell walls, being mainly accumulated/located at epidermal tissues surrounding the aerial organs (leaves, fruits, stems) [28–30]. The tissue-location of nsLTP has major clinical implications, a fact that is supported by the higher allergenic potency of the peels compared with the pulps of Rosaceae fruits [31]. nsLTP are a frequent cause of food allergy among the adult population, having a high sensitisation prevalence in the Mediterranean area [32, 33]. The reasons behind this geographical distribution of food allergy to nsLTP are still unknown, although it might result from primary sensitisation through the airways of homologous pollen allergens in LTP-endemic areas (e.g. olive tree Ole e 7 or oriental plane tree Pla or 3) [14]. Currently, nsLTP-sensitised allergies seems to be increasing in regions outside the Mediterranean area, with recent studies pointing out the importance of nsLTP as primary sensitisers in other European countries, namely in The Netherlands, Austria and the UK [34–37].

Besides belonging to the prolamin superfamily, nsLTP are also classified as PR-14 family, and their functions are related to in situ modulation of lipid composition, signal transduction, vesicular trafficking and lipid transfer [38, 39]. The nsLTP are

Table 1 Data on the composition and structure of proteins from the most important plant allergen families

	2S Albumins	nsLTP	ATI	Cereal prolamins	Profilins	Legumins	Vicilins	PR-10 proteins
Size (aa)	130-160	100-120	120-160 (subunit)	430-480 (gliadin) ~380 (LMW) ~850 (HMW)	~130	480-560	500-600	150-160
MW (kDa)	10-18	9.5-10.5	12-16 (subunit)	30-50 (gliadin) 40 (LMW) 85-90 (HMW)	12-15	360 ~60 (subunit)	150-190 40-80 (subunit)	15-17
Biological function (Abundance)	Seed storage proteins (20-60% depending on species)	Transport proteins. (highly expressed in pollens, leaves, fruit peels) (4% of total proteins)	Regulatory proteins. (4% of total proteins)	Seed storage proteins. (10-20% glutenins, 40-50% gliadins)	Structural proteins. (highly abundant in all cells, especially in pollen)	Seed storage proteins. (50-70% depending on species)	Seed storage proteins. (~20% depending on species)	Regulatory proteins. (highly expressed in case of biotic stress)
Protein structure	Tertiary Heterodimer	Tertiary Monomer	Tertiary/quaternary Homodimer	Tertiary Monomer (gliadin) Polymer (glutenin)	Tertiary Monomer	Quaternary Hexamer	Quaternary Trimer or homotrimer	Tertiary/quaternary Monomer
Crystal structures (Method: X-ray diffraction)				No crystal structures available for gliadins or glutenins				
Example of allergen (source)	Peanut Ara h 6	Peach Pru p 3	Wheat Tri a 28	Wheat	Birch pollen Bet v 2	Soybean Gly m 6	Peanut Ara h 1	Celery Api g 1
PDB accession number	1W2Q	2B5S	1HSS	NR	1CQA	2D5H	3SMH	2BK0

MW, molecular weight; *aa*, amino acid; *NR*, not reported; *LMW*, low molecular weight; *HMW*, high molecular weight; *PDB*, Protein Data Bank (https://www.rcsb.org/)

small proteins (9.5–10.5 kDa) with very compact and stable 3D structures (Table 1). The folding of the helices results in a tunnel-like hydrophobic cavity along the axis of the molecule, which makes them suitable for binding a wide variety of lipids [40, 41]. Differences in lipid-binding affinities of nsLTP might reflect in various immunomodulatory activities [42, 43], both by modifying their molecular structure and physicochemical properties, and/or by acting directly on the immune system [44].

nsLTP-induced allergic responses are most often described as severe and systemic (anaphylaxis), although mild symptoms can occur. Peach Pru p 3 is one of the most relevant nsLTP allergens and probably the best studied [3, 36, 45]. More than 42 allergenic nsLTP have been identified in several plant foods, including fruits, vegetables, nuts and cereals, as well as in latex [12]. Owing to their widespread distribution across the plant kingdom, allergenic nsLTP are commonly classified as panallergens, i.e. ubiquitous proteins accountable for the IgE cross-reactivity to a multiplicity of related and unrelated allergenic sources [46].

Cereal α-Amylase Trypsin Inhibitors

The ATI are proteins present in the endosperm of cereals (wheat, barley, rye, corn and rice seeds), with biological functions of plant defence against parasites, insects, mites and mammalians. Their inclusion in the prolamin superfamily is due to their high content in glutamine, asparagine and proline residues, as well as their sequence homology (ranging from 30 to 95%) with 2S albumins and cereal prolamins [47, 48]. They are composed by polypeptides of 12–16 kDa with four to five disulphide bonds that are essential for their inhibitory activity. Presenting one or more subunits with 120–160 amino acid residues, ATI exist as monomers, dimers or tetramers (Table 1) [17, 48].

In wheat, the monomeric α-amylase inhibitor 0.28 is named Tri a 15 (12 kDa), while the homodimers (24 kDa) are often referred as α-amylase inhibitors 0.19 and 0.53 (Tri a 28). The tetrameric proteins (60 kDa) are termed CM proteins due to their solubility in chloroform/methanol solvent, encompassing Tri a 29 (subunits CM1/CM2), Tri a 30 (CM3) and Tri a 40 (subunits CM16/CM17) [12, 49]. In wheat, the most abundant ATI are the Tri a 28, followed by Tri a 15 and the Tri a 30 [50]. ATI can sensitise individuals by inhalation or ingestion resulting in occupational allergies like bakers' asthma (wheat, barley and rye) or children atopic dermatitis [51], with clinical symptoms associated with gastrointestinal or cutaneous sensitisation (IgE-mediated food allergy) [52–54]. Tri a 30 (CM3) and Tri a 40 (subunit CM16) have also been reported to be involved in wheat-dependent exercise-induced anaphylaxis (WDEIA) [55].

Cereal Prolamins

The cereal prolamins are the major storage proteins found in the endosperm of cereal grains, being classified as glutenins and gliadins in wheat, secalins in rye and hordeins in barley [17]. They are usually divided in two groups according to their solubility in alcohol-water solutions: gliadins (soluble proteins) and glutenins (insoluble proteins) [56]. None of them was reported to be post-translationally modified. The glutenins are divided in high (HMW) and low molecular weight (LMW) subunits, while the gliadin fraction consists of three types of proteins, namely α/β-, γ- and ω-gliadins. These cereal prolamins differ in their methionine-cysteine contents and, accordingly, they are categorised in sulphur-poor (S-poor) or sulphur-rich (S-rich) groups [57]. Cereal prolamins from wheat are the best studied, being some of them registered as allergens, namely the gliadins Tri a 19, Tri a 20 and Tri a 21, and the glutenins Tri a 26 and Tri a 36 [12].

Glutenins have polymeric structures, while the gliadins are monomeric proteins, with α/β-type gliadins showing a compact globular 3D structure and γ-/ω-gliadins presenting extended rod-like structures (fibrous proteins) (Table 1) [58]. The S-rich prolamins encompass two types of gliadins (α- and γ-types) and LMW subunits of glutenins, which share similar structures with high α-helical and low β-sheet contents [59]. These proteins are organised in multi-domains with at least one repetitive proline-rich domain, consisting of blocks of residues. The S-poor prolamins are mostly constituted of ω-gliadins with a repetitive domain (poly-L-proline II and β-reverse-turn structures) close to the N-terminus of the sequence. When present, the non-repetitive domains are rich in α-helices stabilised by disulphide bridges [19, 60, 61]. In wheat-allergic patients, cereal prolamins are able to trigger clinical symptoms that include (among others) urticaria, angioedema, erythema, vomiting, persistent cough, respiratory distress, and in most severe case, anaphylaxis [3]. The ω-gliadins, and notably ω-5 (Tri a 19), are mainly associated with WDEIA (severe food allergy after ingestion plus cofactors such as exercise) in adults [62].

Profilins

The profilin family occupies the second position in terms of total number of allergenic proteins, with at least 50 plant profilins being identified as important allergens [6, 11, 12]. Profilins (12–15 kDa cytosolic proteins) are ubiquitous in all eukaryotic cells as highly conserved molecules, sharing sequence identities of 75–85% among members of distantly related organisms (Table 1) [46, 63, 64]. These proteins participate in the reorganisation of the cytoskeleton, acting as a critical control point in signal transduction from the outer to the inner cell membrane, regulating the intracellular calcium levels and the activity in the microfilament system [65]. Their structures comprise three α-helices, seven β-strands and ten turns that form two hydrophobic cores separated by a central six-stranded β-sheet [66].

Profilins can bind a variety of physiological ligands: (i) cytoskeletal components, like actin; (ii) polyphosphoinositides, like phosphatidylinositol-4,5-bisphosphate; and (iii) proline-rich

peptides, like formin-related proteins and vasodilator-stimulated phosphoprotein [67]. Plant profilins may be phosphorylated by MAP kinases for regulation proposes [68]. Profilins are also classified as important panallergens, although the extent of their allergenicity is still a matter of discussion, considering the extreme variability of their clinical expression, which is also dependent on the type of food [14]. Symptoms are, in most cases, mild and limited to oral allergy syndrome (OAS), but there are also reports of severe allergic responses to profilins [3].

Cupin Superfamily

The cupin superfamily is composed by a wide set of highly diverse protein families across all groups of organisms (including plants and animals), probably sharing a common prokaryotic ancestor [16]. It is the third most important superfamily of plant allergens, with 37 allergenic proteins identified so far [6, 11]. Members of this superfamily are named as cupins based on their common structural features, namely the presence of a β-barrel core domain (cupin core) and two short conserved consensus sequence motifs [17]. With two β-barrel core domains, the bicupins comprise the globulins, which are the major components of the protein fraction of most seeds, legumes and tree nuts. Based on their sedimentation coefficient, globulins are classified as 7/8S and 11/12S, also known as vicilins and legumins, respectively [17].

Globulins have a high clinical relevance, since they are responsible for inducing severe and life-threatening allergic reactions in individuals allergic to legumes (e.g. peanut, lupine, soybean) or to tree nuts (e.g. walnut, hazelnut, almond) [3].

Legumins

Legumins (11S globulins) represent a major portion of the seed storage proteins (50–70% of total protein fraction) in most plants, which aligned with other relevant characteristics contribute to their high importance as class I food allergens [69]. They are multimeric proteins with quaternary structures (360 kDa), occurring as hexamers or as a mix of trimers and hexamers, linked by non-covalent interactions (Table 1) [70, 71]. In their tertiary structures, legumins present regions of rigid conformation, as well as sections of mobile assemblies, most likely corresponding to extended loops or unresolved regions [70].

Different genes express each monomer as a single primary chain of approximately 60 kDa, which is post-translationally cleaved into one acidic and one basic polypeptide with molecular weights of 30–40 and 20 kDa, respectively (Table 1), that are held together by disulphide bonds [9, 69]. So far, several legumins have been registered as allergens in legumes (peanut Ara h 3, soybean Gly m 6), in tree nuts, such as cashew nut (Ana o 2), walnut (Jug r 4), hazelnut (Cor a 9) and almond (Pru du 6) [12] and more recently also identified in Goji berries [72].

Vicilins

Like legumins, the vicilins (7S globulins) are seed storage proteins particularly abundant in legumes and tree nuts (representing about 20% of their protein content depending on the species) (Table 1). They are typically trimeric proteins, though reversible aggregation into hexamers can occur, depending on their ionic strength [70]. Vicilins are large proteins (150–190 kDa), having two subunits in the range of 40–80 kDa and with a typical subunit of ~ 50 kDa (Table 1). These subunits are frequently glycosylated at one or two N-linked glycosylation sites, positioned at the C-terminal domain [69]. Vicilins present two β-barrel core domains, but contrarily to legumins, they lack disulphide bonds, being stabilised by non-covalent hydrophobic interactions, hydrogen bonds and van der Waals interactions. Several vicilins have been registered as allergens in legumes, such as peanut (Ara h 1), lupine (Lup an 1) and pea (Pis s 1), in tree nuts (e.g. hazelnut, Cor a 11; walnut, Jug r 2; pistachio, Pis v 3) [12] and more recently also identified in Goji berries [72].

PR-10 Proteins

The pathogenesis-related (PR)-10 proteins are an important group of allergens in fruits and vegetables, related to the birch pollen-associated (class II) food allergy. Presently, 29 proteins have been classified as allergenic molecules, 25 of those being registered in the WHO/IUIS list of allergens [6, 11, 12]. PR-10 proteins have a molecular weight of 15–17 kDa (Table 1), are slightly acidic, resistant to proteases and structurally not related to other PR proteins [73–75]. The crystal structure characterisation of a few PR-10 proteins showed that these proteins have a conserved, highly curved, seven-stranded antiparallel β-sheet surrounding a long 25-amino acid α-helix (α3) at the C-terminus [76]. Two additional N-terminal short α-helices (α1 and α2), located between the β1 and β2 strands, complete the scaffold creating a hydrophobic core, which works as a ligand-binding site [76, 77].

Like others, the PR-10 proteins participate in the defence mechanism of plants, namely in the response to biotic/abiotic stress and in the transport of amphiphilic compounds (fatty acids, cytokines, flavonoids and sterols) through the cellular barrier [77, 78]. However, some of these proteins are constitutively expressed, indicating a key biological role in plant development. The clinical relevance of these proteins encompass a multitude of different symptoms (ranging from mild to potentially life threatening) with a quick onset (up to few minutes) after the consumption of raw plant foods [3]. In the recent years, a variety of PR-10 proteins and their food homologues causing allergy in humans have been isolated and characterised [79–82]. Bet v 1 is the most representative member of PR-10 proteins, which exists in at least 18 different isoallergens and isoforms [83].

Physicochemical Properties Affecting Allergenicity

In the attempt to address the question 'what turns a food protein into an allergen?,' great attention has been devoted to the physicochemical characterisation of food allergens. By now, it is already well established that some physicochemical properties seem to play a major role in the allergenic potential of a protein.

In this review, the selected parameters concerned post-translational modifications, such as glycosylation, phosphorylation and hydroxylation, as well as protein structure and organisational level. Considering that, the majority of foods are commonly consumed after some kind of processing, it was also important to understand the protein characteristics which are influenced by processing, such as stability to heat, light/radiation and pressure, as well as, to mechanical and chemical activities.

Accordingly, the parameter of heat stability reflects the influence of different types of food processing with the application of thermal treatments, namely autoclaving, frying, boiling, dry or wet roasting, blanching and baking. Similarly, the effect of glycation and aggregation, which are associated with the use of thermal treatments during food processing were also considered. For the parameter of pressure stability, the effects of high-pressure processing (HPP) and high-pressure (HP) microfluidisation treatments were analysed, while for light/radiation stability, the information was retrieved from food processes involving gamma-radiation, high-voltage impulses, pulsed electric fields (PEF), pulsed ultraviolet (PUV) light and microwave treatments. For mechanical or chemical stabilities, data were collected from the application of sonication and ultrasound treatments or fermentation, alkylation/reduction and enzymatic hydrolysis, respectively. The influence of ligand binding on the allergenic potential of a protein as well as its potential lipid interactions and the resistance to digestion complete the set of physicochemical parameters that were analysed in this review.

For each plant protein family, literature review on their allergenic members was extensively performed and further described, which can be consulted in detail in the excel file of the Electronic supplementary material section.

Measuring the Effect on Allergenicity

Food allergy comprises several immunological mechanisms, although its most common form regards an immediate-type hypersensitivity in which specific IgE is bound by high-affinity Fcε-receptor on the mast cells and basophils of the allergic individuals. Cross-linking of allergen-specific IgE by the allergen starts a cascade of events, including the release of physiologically active mediators (e.g. histamine) that rapidly lead to biological responses in a number of target tissues [84]. The capacity to bind and cross-link specific IgE is an intrinsic immunological property of the allergenic proteins, which can be monitored by several in vivo and in vitro assays (Table 2).

The presence of IgE in the sera of food-sensitised individuals is not always accompanied by clinical symptoms, therefore assays screening for specific serum IgE are commonly used as complementary tools for food allergy diagnosis, after proper recording of anamnesis. The immunoblotting is normally used to assess the overall IgE-binding profile of allergens at a qualitative level, while ELISA enables their quantification [85]. Similarly, the radioallergosorbent test (RAST)/enzyme allergosorbent test (EAST)/ImmunoCap allow the quantification of allergen-specific IgE levels within human serum/plasma. IgE-binding and functional cross-linking can be tested by ex vivo or in vitro cellular assays, such as human basophil activation test (BAT), mast cell activation test (MAT) and rat basophilic leukaemia (RBL) mediator release assay. In these tests, effector cells are stimulated with allergens (native/recombinant) or extracts and their activation is measured either by mediator release (e.g. histamine, cytokines) or upregulation of cellular surface molecules (e.g. CD63, CD203c) [85, 86].

The skin-prick tests (SPT) and the food challenges are used for in vivo testing. The first induces specific skin mast cell degranulation after cross-linking of allergen-specific IgE, although its correlation to clinical symptoms is usually restricted to a good negative predictive value [87]. Food challenges, in open (OFC), in closed or in double-blind placebo-controlled (DBPCFC) formats, are the best way to confirm allergy. However, these food challenges are burdensome for patients, time consuming, expensive and they can only be performed in specialised medical facilities due to the high risk of severe and systemic allergic reactions during the trial. Moreover, if single allergen components (e.g. peanut Ara h 2) are used for in vivo challenges, those need to be prepared under good manufacture practices (GMP).

Alternatively, animal models have been proposed to assess the allergenicity (sensitisation and elicitation capacities) of different proteins/foods, namely murine IgE response and anaphylaxis.

Most of these assays have been used as excellent tools to evaluate the effect of different physicochemical properties on the IgE-binding capacity of plant food allergens (Table 2). Based on their simplicity and relative low cost, immunoblotting, ELISA and RAST/EAST/immunoCAP, using the serum/plasma from sensitised/allergic subjects, are the first-line assays, being applied to allergens from almost all plant families [88–99]. They are followed by

Table 2 Summary of the assays used to assess the effect of physicochemical parameters on the allergenicity of proteins from plant food families

Families	Specific serum screening			Cellular in vitro or ex vivo assays			In vivo assays			
	Immunoblot/ dot blot	ELISA	RAST/ EAST/ immunoCAP	Basophil activation test	RBL mediator release assay	T cell proliferation	Murine IgE response	Murine anaphylaxis	Human skin prick tests[a]	Human food challenges[a]
2S albumins	√	√	√	√	√	√	√	√	√	√
nsLTP	√	√	√	√	√	NR	NR	NR	√	√
ATI	√	√	NR	√	NR	√	√	NR	√	NR
Cereal prolamins	√	√	√	√	√	√	√	√	√	√
Profilins	√	√	√	NR	√	NR	NR	NR	√	√
Legumins	√	√	NR	NR	√	√	NR	NR	√	NR
Vicilins	√	√	√	√	√	√	NR	NR	√	√
PR-10	√	√	√	√	√	√	NR	NR	√	√

IL, interleukins; *IFN*, interferons; *RAST*, radioallergosorbent test; *EAST*, enzyme allergosorbent test; *RBL*, rat basophilic leukaemia; *ELISA*, enzyme-linked immunosorbent assay; '√' confirmation of tests performed as reported on literature; *NR*, no evidence found in literature

[a] Human SPT and food challenges are normally performed using pure-food extracts or entire food (either alone or hidden within a prepared matrix), respectively

the cellular ex vivo or in vitro cellular assays, which have also been widely employed to study the influence of physicochemical characteristics on protein allergenicity in most plant families [45, 91, 100–125]. Although used at less extent, the SPT and food challenges have been carried out to characterise the allergenicity of proteins as affected by different properties (e.g. denaturation, digestion) [90, 104, 107, 108, 110, 111, 117, 120, 121, 123, 124, 126–132]. The in vivo food challenges, SPT, and even more important, oral food challenges (OFC, DBPCFC) are of high value because of the true human clinical read-out. However, these procedures are difficult to perform, and the availability of test subjects and ethical considerations limit their general application [107, 120, 121, 124, 126–130]. Other 'functional' tests (BAT, RBL) are considered instead, which seem to have quiet good correlation to the clinical phenotype.

Finally, specific IgE testing is simpler but limited to the conclusion of 'sensitisation' IgE-binding in most cases. Nonetheless, there are some examples, such as the IgE-binding sensitisation to Ara h 2/6 in peanut allergy or to ω5-gliadin in WDEIA, which seem to have a good predictive value for clinical reactivity [133]. In most cases, in vitro IgE-binding properties are investigated for the ease of testing, although they usually do not allow for extrapolation to clinical reactivity. Likewise, the in vivo assays using animal models are also less applied to evaluate the allergenic potential of proteins [109, 115, 123, 134–138].

Owing to the complexity of this topic and the heterogeneity of the data collected, it was also important to provide some general definitions and terminology to avoid unnecessary misunderstandings. Herein, some terms were employed following the concepts defined by Verhoeckx et al. [2]. Thus, allergenicity/allergenic potential was used with the meaning of 'the potential of a material to cause sensitisation and allergic reactions, frequently associated with IgE', while immunoreactivity refers to 'the ability of a material to elicit an immune response' and IgE-binding capacity is 'an altered ability of IgE (also allergenic integrity) to bind to epitopes' [2].

In practice, the terminology of IgE-binding capacity was used for information retrieved from immunoblotting, ELISA and RAST/EAST/immunoCAP assays with the sera of food allergic/sensitised patients, while immunoreactivity was predominantly used to classify data from immunoassays with animal IgG. The terms allergenicity/allergenic potential were mostly used to classified data from assays where an elicitation of an allergic response is induced, namely in mediator release assays (RBL, BAT), in vivo assays (SPT, OFC and DBPCFC) and animal allergy models (mice physiological responses, mice anaphylaxis).

Abundance

It has been very difficult to establish a correlation between the abundance of an individual allergen in the plant with the risk of sensitisation, mostly due to the scarcity of data on quantitative thresholds for sensitised individuals [139]. Additionally, the few available data in literature can be conflicting. In fact, a recent report stated that no clear correlation could be found between legume protein consumption/allergen concentration and the prevalence of legume sensitisation, as demonstrated for the case study of peanut and soybean allergens [140]. Controversially, it has also been shown that by altering the expression of some allergenic proteins (suppressed by RNA interference), their IgE-binding capacity is

greatly reduced, as described for three major allergens in rice [141, 142]. Likewise, the mRNA silencing of Mal d 1 gene leads to a great decrease in the expression of Mal d 1 in the genetically modified apple lines, which enable a drastic reduction, and even complete elimination, of the clinical symptoms in apple-allergic patients, as demonstrated by a blind-sensory privation oral food challenge [143]. Both perspectives suggest that the amount of allergenic proteins may determine the overall IgE-binding capacity of a food, whereas it seems to be no link between the extent of sensitisation in relation to the amount of allergenic proteins in the diet.

When considering primary sensitisation via ingestion, it is expected that allergen expression at higher levels in a certain food (e.g. cereals) increases the potential for eliciting an allergic reaction in food-allergic individuals. This is the case for 2S albumins (20–60% of total protein fraction), legumins (50–70% of total protein fraction) and vicilins (~ 20% of total protein fraction) (Table 1). As they are major storage proteins in most nuts and other seeds, they make a significant contribution to the human diet, being widely correlated with their high incidence in terms of allergenic molecules capable of inducing adverse immunological responses [69, 144]. In cereals, prolamins (gliadins and glutenins) are the major fraction of storage proteins, consequently their high content in cereals (e.g. wheat) increases the risk of an allergic reaction.

The contribution of allergenic cross-reactivity to pollen allergens (e.g. Bet v 1) (primary sensitisation by inhalation) with proteins expressed at moderate or low quantities (e.g. soybean Gly m 4, hazelnut Cor a 1 or celery Api g 1) should not be neglected, because it also increases the risk for inducing allergic responses. Profilins are very relevant allergens, not only due to their high abundance but also because of their great potential for multiple sensitisation across different plants [145]. The nsLTP are tissue-specific proteins [31], and their allergenic potential is well correlated with their abundance, since the removal of nsLTP-containing tissues significantly decreases the allergenic potential of this family. Additionally, Mal d 3 expression is highly variable among apple cultivars, enabling different allergic responses. Cultivars with low amount of Mal d 3 are less allergenic (as assessed by SPT and DBPCFC) than the ones expressing higher amounts of this allergen [120]. The expression of ATI, nsLTP and the PR-10 protein families is highly dependent on plant cultivar and on environmental conditions [146].

Concluding remarks:

- The high content of 2S albumins, legumins, vicilins and cereal prolamins (gliadins and glutenins) in relation to total protein is correlated to increased allergic elicitation risk.
- The abundance of nsLTP is related to increased allergic elicitation risk.
- The abundance (low/high) of the profilins, ATI and PR-10 cannot be correlated to an increased allergenic risk.

Protein Structure

Conformational and/or linear IgE epitopes of proteins play a crucial role in the elicitation of an allergic response [147]. In general, most monomeric allergens are relatively small, stable and with very well organised structures, but some allergenic proteins may form large high-ordered structures (Table 1). There are also some exceptions of unstructured proteins, such as caseins, that act as allergens [148].

Regarding plant food allergens, most molecules have complex structures with a high level of organisation, namely presenting tertiary and quaternary conformations. This is the case of ATI, legumins, vicilins and PR-10 proteins, which present quaternary structures (Table 1) [48, 70, 76]. The loss of high-ordered structure contributes to a decrease in the overall allergenicity of most allergens (Table 3). In contrast, most members from legumins preserve immunogenic subunits (monomers) with IgE-binding capacity (e.g. peanut Ara h 3 and soybean Gly m 6) [71].

2S Albumins, nsLTP, gliadins and profilins are proteins with compact globular structures [41, 58, 66, 149]. The loss of protein 3D structures of 2S albumin and nsLTP families does not affect their allergenicity [23, 126, 150], while for gliadins and profilins, their allergenic potential is reduced [122, 149]. As expected, when the conformational changes occur at the secondary structure level, allergens from legumins, ATI and gliadins exhibit a decrease in their IgE-binding capacity [136, 151]. Likewise, the alkylation/reduction of 2S albumins and gliadins is another factor that reduces their allergenic potential [23, 122, 152, 153], which emphasises the importance of an intact structure that defines a major portion of allergenicity, although in some cases linear epitopes may also impact on allergenicity.

Concluding remarks:

- Destruction of conformation usually leads to:
- Complete loss of allergenicity in food allergens related to inhalant pollen allergen sensitisation (e.g. apple Mal d 1, hazelnut Cor a 1, cherry Pru av. 1).
- Partial loss of allergenicity in 2S albumins and LTP as primary sensitisers.
- Partial or minor loss of allergenicity in vicilins and legumins (because of partially intact secondary structured domains and linear epitopes) as primary sensitisers.
- Reduction/alkylation reduces the allergenic capacity of 2S albumins and gliadins caused by the destruction of disulphide bonds that are strong stabilisers of protein structure.

Table 3 Summary of the physicochemical parameters and their effect on the allergenicity of different plant protein families

Physicochemical parameters	2S albumins	nsLTP	ATI	Cereal prolamins	Profilins	Legumins	Vicilins	PR-10 proteins
PTM	Glycosylation (↑ Pin p 1), hydroxylation (↑ Ara h 2)	NR	Glycosylation (↑ Tri a 40 subunit CM16)	NR	NR	NR	↑ (glycosylation)	NR
Protein structure	→ (loss of 3D structure), ↓ (reduction/alkylation)	→ (loss of 3D)	↓ (loss of 3D or 4D), ↓ (rupture of S-S)	↓ (loss of 2D), ↓ (rupture of S-S)	↓ (loss of 3D)	↓ (loss of 4D), ↓ (rupture of S-S)	→ (loss of 4D)	↓ (loss of 3D or 4D)
Glycation	↑↓	→	NR	↓	NR	↓	→	↓, →
Aggregation	↑	No aggregation	NR	→	NR	↓	→	→ (Ara h 8)
Heat stability	Heat stable: ↑↓ (roasting), ↓ (boiling), ↓ (frying), ↓ (heat + pressure)	Heat stable: → (boiling), → (baking), ↓ (extreme heat)	Heat stable or heat labile?: ↓ (boiling); ↓ (steaming); ↓ (extreme heat)	Heat stable: ↓ (heat treatments)	Heat labile: ↓ (heat treatments)	Heat stable: → (frying), → (dry roasting), → (blanching), ↓ (autoclaving), ↓ (extreme heat)	Heat stable: ↑ (roasting), ↓ (boiling), ↓ (extreme heat)	Heat labile: ↓, → (roasting)
Pressure stability	Pressure stable: → (HPP), ↓ (HP microfluidisation)	Pressure stable: → (HPP), ↓ (HPP + heat)	NR	NR	Pressure stable: → (HPP)	Pressure stable: ↓, → (HPP), ↓ (HPP + heat)	Pressure stable: → (HPP), ↓ (pressure + heat)	Pressure stable: → (HPP)
Light/radiation stability	Light stable: → (microwave), ↓ (PUV light)	Light-stable: → (PEF), → (microwave + ultrasound)	NR	Light stable: ↑ (microwave)	Light stable: → (γ-radiation), → (high-voltage impulses), ↓ (microwave)	Light stable: → (γ-radiation), → (microwave), ↓ (PUV light)	Light stable: → (γ-radiation), → (microwave), ↓ (PUV light)	Light stable: ↓ (microwave)
Mechanical/chemical stability (protein integrity)	↓ (alkylation/reduction), → (enzymatic hydrolysis)	→ (ultrasound)	↓ (enzymatic hydrolysis)	↑↓ (enzymatic hydrolysis), ↑↓ (deamidation of gliadins)	→ (enzymatic hydrolysis with papain + heat)	↓ (fermentation; reducing agents + heat; or enzymatic hydrolysis + sonication + heat)	↓ (fermentation)	↓ (enzymatic hydrolysis)
Digestibility	→ (partial pepsin/trypsin); ↗ (presence of lipids)	→ (partial degradation, most peptides remain reactive)	→ (resistant to digestion)	→ (partial degradation, most peptides remain reactive)	↓ (after pepsin)	↓ (after pepsin), → (after trypsin), ↓ (after pepsin + trypsin), →, ↗ (presence of lipids)	→ (after digestion), →, ↗ (presence of lipids), →, ↗ (presence of glycation aggregates)	↓ (after pepsin), →, ↗ (presence of lipids)
Epithelial transport	↑	↑	NR	↑	NR	NR	↑	NR
Lipid binding/lipid interaction	↗	↑	NR	NR	NR	→, ↗	→, ↗	→

2D, secondary structure; *3D*, tertiary structure; *4D*, quaternary structure; *HPP*, high-pressure processing; *S-S*, disulphide bond; '→' maintain IgE-binding capacity; '↑' increase IgE-binding capacity; '↓' decrease IgE-binding capacity; '↑↓' contradictory data about the effect on IgE-binding capacity; '↗' slightly increase IgE-binding capacity; *N*R, not reported; *PEF*, pulsed electric fields; *PUV*, pulsed ultraviolet

- Stability of the protein structure is an important physical chemical characteristic of some allergenic proteins, but it is not a generic allergen characteristic.

Post-translational Modifications

PTM occur after translation in the cell and may affect the allergenic potential of proteins (Table 3). Among PTM, protein glycosylation is one of the most relevant and complex processes, consisting of a covalent interaction between a sugar and the side chains of serine and threonine (*O*-glycosylation) or asparagine (*N*-glycosylation) [154]. Glycosylated proteins are considered important mediators in different biological processes, such as protein folding, cell signalling, fertilisation and embryogenesis, but they are also involved in immune activation processes [155]. Hydroxylation and phosphorylation are also PTM of high importance for cellular processes. The first consists of a covalent process involving the addition of a hydroxyl group to the non-nucleophilic functional group of proline, lysine and asparagine, while the second implicates the covalent addition of a phosphate group to the side chain of an arginine, lysine, histidine, tyrosine, serine or threonine residue [154, 156].

Regarding these PTM (Table 3), glycosylation is commonly referred to as one of the most important physicochemical properties of several allergens. However, besides the glycosylated allergens belonging to the vicilin family [69], only few other glycosylated allergens could be found within plant protein families, namely in legumins (lupine Lup a alpha-conglutin), in 2S albumins (stone pine Pin p 1) and in ATI (wheat Tri a 40). In general, the glycosylation of vicilins and wheat Tri a 40 (subunit CM16), has been considered to increase the allergenic potential of these proteins.

The presence of N-glycans in glycoproteins has been positively correlated with increasing human IgE responses, although their clinical relevance is still a matter of debate. Shreffler et al. [157] demonstrated that peanut Ara h 1, but not its deglycosylated form, activated monocyte-derived dendritic cells as a measure of sensitisation, and by their ability to drive T cell proliferation. Contrarily, Lauer et al. [158] reported that the presence of N-glycans in hazelnut Cor a 11 did not affect its IgE-binding capacity at the site of elicitation. By now, in the case of vicilins, there is no conclusive evidence that the presence of N-glycans may be associated with increased allergenic responsiveness.

Hydroxylation has been described to increase the elicitation potential of peanut Ara h 2 (2S albumin family) [113]. Phosphorylation can occur in members of the profilin family [68], but no correlation with their allergenic potency has been described so far.

Concluding remarks:

- Glycosylation can increase the efficiency in the sensitisation capacity of vicilins. No effects for other allergens were found.
- Hydroxylation increased the IgE-binding capacity of peanut Ara h 2 at the site of elicitation. No information was found for other allergens.
- There is no hard evidence for the relation between a PTM (especially glycosylation) and the allergenic potential of a protein. At least for the plant allergens, glycosylation is not a prerequisite for a protein to have a high probability of being an allergen.

Glycation and Aggregation

Glycation, also referred as Maillard reaction, is a common effect of processing that allows modifying proteins to improve the technological properties of foods, such as solubility, gelling capacity, heat stability, water retention capacity, texture and flavour. The glycation process is a complex form of a non-enzymatic reaction between an available amino group (from proteins) and a carbonyl-containing moiety (usually from a reducing sugar), which normally occurs under mild and safe processing conditions [159]. Besides altering the functional/technological properties of proteins, glycation is also known to affect some biological parameters of food proteins, such as their bioavailability, digestibility and allergenicity. The conformational and biochemical alterations of proteins as a result of glycation may contribute not only to mask existing epitopes but also to create neo-epitopes which are able to activate an IgE response [160]. A very typical consequence of conformational changes of proteins, during glycation, is related to the formation of aggregates (complex macrostructures).

For plant families (Table 3), glycation can differently affect proteins at the site of elicitation, although glycated products may also decrease the threshold for allergen sensitisation, comparing with their native counterparts [161]. For example, it has been described to have contradictory effect on the IgE-binding capacity of 2S albumins. In the case of peanut Ara h 2, there are reports stating the increased IgE-binding capacity of Ara h 2 glycated products [162], while others describe that the formation of dimers and tetramers of Ara h 2 (aggregated structures) leads to a decrease in its degranulation capacity of mast cells/basophils [100, 103, 116]. Additionally, heat-processing of peanut seems to be needed for the sensitisation of mice to native Ara h 6, suggesting that complex structures of high molecular weight (between Ara h 6 and Ara h 1) formed during peanut roasting induced the production of IgE-specific to native Ara h 6 [163].

In the case of vicilins and nsLTP, Maillard reactions do not seem to affect their elicitation capacity. For vicilins, this fact is also well correlated with the presence of large insoluble aggregates that contribute to maintain their allergenicity. This is

the case of hazelnut Cor a 11 and peanut Ara h 1, whose glycation contributed to slightly decrease their IgE/IgG-binding properties but not the degranulation capacity of basophils. In fact, glycated Cor a 11 and Ara h 1 products increased basophil degranulation capacity [116, 164].

Glycation of nsLTP seems to protect the IgE-binding capacity of these proteins by stabilising their conformational structures, as reported for Mal d 3 [119], whose glycation led to the addition of up to four glucose residues attached to Mal d 3 and to a minor reduction (two- to tenfold) in Mal d 3 potency to induce basophil histamine release compared with its native counterpart. A similar outcome was reported for cereal prolamins, whose Maillard products tend to form large aggregates, contributing to a maintain their IgE-binding capacity [89].

Legumins form insoluble aggregates due to glycation, which decreases their IgE-binding capacity, as it was demonstrated for soybean Gly m 6, almond Pru du 6 and tartarian buckwheat Fag t 3 [151, 165–167]. The IgE-binding capacity of the majority of the proteins from the PR-10 family decreases in glycated products (cherry Pru av. 1) [168]. However, when PR-10 members (peanut Ara h 8) tend to form aggregates due to glycation, their IgE-binding capacity is maintained or even slightly enhanced [169].

Some members of the ATI family are naturally aggregated (e.g. wheat Tri a 28), a fact that might be strongly correlated with their high IgE-binding frequency among wheat-allergic patients [170, 171] and high sensitising capacity in murine food allergy model [172]. Regarding profilins, no data describing the effect of glycation and/or formation of aggregates could be found in literature.

Concluding remarks:

- Glycation and aggregation have, in most protein families, similar effects on protein allergenicity.
- Glycation and aggregation decrease the IgE-binding capacity of gliadins, legumins and PR-10 proteins (except for Ara h 8).
- Glycation and aggregation do not affect the IgE-binding capacity of nsLTP and vicilins.
- Glycation has contradictory effects on the IgE-binding of 2S albumins, while the formation of aggregates increases their IgE-binding capacity.

Heat Stability

The high stability of allergens towards food processing has been proposed as one of their most pertinent physicochemical characteristics [8]. Heat stability is a parameter of major importance and to evaluate its influence, all conventional thermal treatments (autoclaving, frying, boiling, dry or wet roasting, blanching and baking) applied to allergens of all plant protein families were extensively reviewed. Overall, the heat stability of allergens is well correlated with their allergenicity (Table 3).

Members from 2S albumins, nsLTP, cereal prolamins, legumins and vicilins are classified as heat-stable allergens. This fact is in good agreement with their high tendency to return to their native state, after being submitted to heat variations, as well as with their high content in conformational epitopes [23, 71, 89, 95]. However, if pH is changed during heat treatments, proteins might not revert to their native state, such is the case of nsLTP which returns to native state under acidic conditions, but not under neutral ones [150].

In contrast, proteins from PR-10 family are defined as heat-labile, since they are not likely to return to their original folding, highlighting the importance of the conformational epitopes in these allergens. This is the case of celery Api g 1, carrot Dau c 1, apple Mal d 1 and hazelnut Cor a 1, whose allergenicity is significantly reduced or even abolished upon submitted to different heat treatments [107, 124, 173, 174].

Profilins have been considered heat labile, since their presence is commonly associated with raw or minimal processed foods (fruit, vegetables). However, there are also reports of profilins that preserve some IgE-binding capacity after being thermally processed, which is the case of celery Api g 4, tomato Sola l 1 and mustard Sin a 4 [129, 149, 175]. ATI proteins seem to be heat-labile [88, 176], although they have also shown evidences of thermal stability [108].

Different heat treatments have distinct effects on the allergenicity of each plant protein family. Boiling/steaming are processes commonly contributing to maintain or reduce the IgE-binding capacity of most allergens (2S albumins, nsLTP, ATI and vicilins) [100, 114, 176, 177], while roasting has contradictory effects depending on the allergen or allergen family (vicilins and 2S albumins). In the specific case of peanut 2S albumins (Ara h 2/Ara h 6), roasting has been reported to increase, maintain or even slightly reduce their IgE-binding capacity and ability to elicit histamine release [103, 116, 134, 178]. Additionally, most of the severe thermal treatments (e.g. autoclaving) are able to reduce the IgE-binding capacity of several heat-stable allergens, such as nsLTP, legumins and vicilins, as are the examples of apple Mal d 3, cashew nut Ana o 2 and peanut Ara h 3, and peanut Ara h 1, respectively [117, 119, 179, 180].

Concluding remarks:

- 2S Albumins, nsLTP, cereal prolamins, legumins and vicilins are heat stable (high tendency to return to native conformation, depending on the pH environment).
- Profilins and PR-10 proteins are heat labile (tendency to suffer irreversible unfolding). ATI have dual behaviour.
- IgE-binding capacity of allergens of most protein families decreased at high temperatures (100 °C) in the presence of water (boiling/steaming), except for nsLTP (no effect).

- IgE-binding capacity of 2S albumins, legumins, vicilins and PR-10 proteins increased/decreased (dual behaviour) at very high temperatures (e.g. above 180 °C without the presence of water—roasting).
- IgE-binding capacity decreased for nsLTP, legumins and vicilins upon extreme thermal conditions (e.g. autoclave).

Pressure Stability

Non-thermal treatments, such as those involving the use of high hydrostatic pressure (HHP) or HPP, are faced as innovative food preservation techniques, alternative to conventional thermal treatments [181]. Initially used to inactivate the growth of microorganisms in foods, thereby increasing product shelf life without affecting their quality and flavour, the application of high-pressure (HP) treatments has different effects depending on the food components. In proteins, HP techniques are known to disrupt non-covalent interactions (hydrogen, ionic and hydrophobic bonds), thus affecting their secondary and tertiary structures [182, 183]. In the recent years, the application of high-pressure technology has gained special attention with respect to the potential effect on the allergenicity of food proteins.

In general, allergens from plant families are typically pressure stable, since the application of HP treatments has no (or very limited) effect on the allergenic potential of nsLTP, profilins, vicilins and PR-10 proteins (Table 3). The application of HP treatments is known to alter the conformation of proteins (secondary and tertiary structures), although there are no evidences of significant modifications on the native state of referred proteins [91, 127, 184, 185].

2S Albumins and legumins are also classified as pressure-stable proteins, although some contradictory effects have been reported for both families (Table 3). Within 2S albumins, the immunoreactivity of peanut Ara h 2 is decreased by HP microfluidisation [186], while the immunoreactivity of Ara h 2 and Ara h 6 is not affected by HPP [184]. Likewise, the immunoreactivity of legumins, namely Ses i 6 and Ses i 7 (sesame) is decreased by the application of HP treatments [187], while the IgE-binding capacity of walnut Jug r 4 is not affected by HPP [104]. However, the combination of pressure with other types of treatment (e.g. HPP coupled with heat) has been reported to decrease the allergenic potential of nsLTP, legumins and vicilins, as described for apple Mal d 3, walnut Jug r 4 and peanut Ara h 1, respectively [104, 117, 188]. Additionally, the combination of autoclave (pressure + heat) with enzymatic hydrolysis also contribute in decreasing the IgE-binding capacity of legumins, as reported by cashew nut Ana o 2 and pistachio Pis v 2 and Pis v 5 [96]. Regarding ATI and cereal prolamins, no effect on the application of HP treatments has been reported so far.

Concluding remarks:

- Most plant allergens are pressure stable (minor changes to protein conformational structure) since pressure-processing methods (e.g. HPP) normally contribute to maintain the protein in its native-like state when compared with temperature processing. No data available for members of ATI and cereal prolamin families.
- The IgE-binding capacity of nsLTP, profilins, vicilins and PR-10 is not affected by the application of high pressures, while for 2S albumins and legumins, it can be slightly reduced.
- Combination of pressure-heat and pressure-heat-enzymatic hydrolysis treatments is more efficient in reducing the IgE-binding capacity of nsLTP, legumins and vicilins, because pressure changes protein at conformational level (3D and 4D structures) making it more susceptible to enzyme activity and temperature.

Light/Radiation Stability

Besides HP treatments, other novel non-thermal technologies have been used by the food industry to increase the safety and quality of foods, which include the application of gamma-radiation (γ-radiation), PEF and PUV light [182, 189]. Treatments, like gamma-radiation and UV radiation, are frequently used to increase the storage duration by destroying the surface pathogens present in foods, either by the application of an ionising radiation (2–7 kGy, medium dosage level) or UV rays (ranging 100–400 nm), respectively [182].

The PEF technology uses short pulses of electricity to inactivate microorganisms, preserving the organoleptic features of foods [190]. The application of microwave radiation (electromagnetic wave) has been widely used for food processing (e.g. thawing of frozen foods, pasteurisation, drying and pre-cooking). Although being normally classified as a thermal processing technique, microwave radiation has also a non-thermal effect on food proteins [182].

Like in the previous cases, protein stability towards light or radiation treatments also constitutes an important physico-chemical parameter of allergens, although the knowledge about their effects is still very limited (Table 3). The application of pulsed UV light significantly reduces the IgE-binding capacity of 2S albumins (peanut Ara h 2), legumins (Ara h 3 and soybean Gly m 6) and vicilins (Ara h 1) [191–193]. The effect of gamma radiation was exploited for legumins, vicilins and profilins, but in all cases, this technology did not induce any alteration in the IgE-binding capacity of sesame Ses i 6 and Ses i 7, cashew nut Ana o 1 or celery Api g 4, respectively [91, 179, 194].

The allergenic potential of profilins and PR-10 proteins is significantly reduced or even abolished by microwave (100 °C, 30 min), as assessed by SPT, mediator release assays and/or EAST, namely in the case of celery Api g 4 and Api g

1, respectively [90, 91]. For cereal prolamins (gliadins), their immunoreactivity varies according to the energy applied, increasing to a maximum around 40 kJ and then gradually returning to its initial level [195]. Microwave heating has no effect on the IgG-binding capacity of 2S albumins (cashew nut Ana o 3), legumins (almond Pru du 6 and cashew nut Ana o 2) and vicilins (Ana o 1) [167, 179, 196]. Likewise, microwave heating in combination with ultrasound does not affect the IgE-binding capacity of nsLTP (e.g. peach Pru p 3) [188]. Data on the effect of light/radiation on the allergenicity of ATI is not yet available in the literature.

Concluding remarks:

- The IgE-binding capacity of most plant families is not affected by treatments with radiation (only minor exceptions).
- Exceptions: the IgE-binding capacity is increased in cereal prolamins and decreased in profilins and PR-10 towards microwave radiation.
- 2S Albumins, legumins and vicilins are less stable towards light treatments, since their IgE-binding capacity is decreased (by conformational alterations in protein native structure).

Mechanical/Chemical Stability

Another non-thermal process commonly used by the food industry is the ultrasound or sonication treatment. This type of food processing applies mechanical waves (20–100 kHz) to promote the formation/collapse of bubbles, due to compression and rarefaction phenomena. When the bubbles reach a critical size, they collapse generating local regions of high temperatures and pressures, which subsequently induce protein conformational changes [182].

In general, most allergens present stability towards mechanical processes (e.g. sonication), thus maintaining their allergenicity after being treated with the mechanical processes (Table 3). Considering the referred food-processing methods, the ultrasound treatment can be applied in combination with other processes (Table 3). However, the information on the effect of ultrasound on the allergenicity of plant protein families is very limited, contributing to maintain or to slightly decrease the IgE-binding capacity of nsLTP (when combined with microwave heating) [188] or legumins (when combined with enzymatic hydrolysis and heat), respectively [96].

The application of chemical and enzymatic processes often reduces the allergenicity of several proteins [197]. Among those, the fermentation and enzymatic hydrolysis using different enzymes are considered traditional methods to process foods. The first is a microbial process based on the production of enzymes by the microorganisms, which alters the organoleptic characteristics of the food, contributing to increase its stability and duration [198]. The second uses specific enzymes to disrupt protein structure, thus increasing the added value of food proteins by altering the sensory quality of proteins (modification of food texture and flavour). Additionally, this process is also known to improve the digestibility and nutrient bioavailability of food proteins [199].

The enzymatic hydrolysis can be considered one of the most effective methods of modifying the allergenicity of food proteins (depending on the type of enzymes used) because it is able to induce the collapse of conformational epitopes and the cleavage of linear ones [182]. Deamidation is one of this processes, which enables the conversion of glutamine and asparagine into glutamic acid and aspartic acid residues, respectively, to increase the solubility of gliadins, by means of chemical processes or enzymatic ones (e.g. transglutaminases) [200].

In general, the enzymatic hydrolysis of proteins from ATI and PR-10 families reduce their IgE-binding capacity (Table 3), as described for brown rice Ory s aA_TI and cherry Pru av 1 [168, 201]. For 2S albumins, enzymatic hydrolysis has been described to maintain or decrease their allergenicity [98, 102]. In the case of cereal prolamins, their enzymatic hydrolysis with transglutaminase has been reported to increase the IgE-binding capacity of gliadins [132].

The deamidation of gliadins has been reported to present contradictory effects on their allergenicity. On one side, deamidation decreases the IgE-binding capacity of gliadins towards the sera of wheat allergic patients [136], but on the other side, it contributes to an increased severity of the clinical symptoms in patients allergic to deamidated gluten [121, 202, 203]. When allied to other processes (heat, sonication), the enzymatic hydrolysis contributes to the reduction or maintenance of IgE-binding capacity of legumins or profilins, respectively [96, 175].

The process of microbial fermentation has only been tested for legumins and vicilins, in the specific case of soybean, which is often consumed in the form of tempeh, miso and yogurt. Microbial fermentation enabled a drastic reduction in the IgE-binding capacity of both Gly m 6 (legumin) and Gly m 5 (vicilin), as well as other allergenic proteins from soybean [204]. Acid fermentation has been tested for profilins and PR-10 proteins, namely for Api g 4 and Api g 1, respectively, contributing to a reduce the IgE-binding capacity of both celery allergens [91].

The use of reducing agents (e.g. sodium sulphite) for food processing is also commonly applied. The treatment of legumins (cashew nut Ana o 2) and 2S albumins (peanut Ara h 2, cashew nut Ana o 3) with reducing agents seems to contribute to a significant decrease in their IgE-binding capacity, which might be related to the destruction of allergen conformational epitopes [97, 153].

Concluding remarks:

- The stability of proteins is affected by different processing techniques, whereof hydrolysis influences the intactness (integrity) of the proteins and mechanical, heat and pressure change the protein structure (e.g. unfolding).
- Changes in protein structure are seen for nsLTP and legumins (when ultrasound and heat are applied), maintaining or lowering their IgE-binding capacity.
- Changes in protein size (resulting in protein fragmentation, as consequence of fermentation, enzymatic hydrolysis or treatments with reducing agents) normally contribute to maintain or decrease the IgE-binding capacity of 2S albumins, ATI, legumins, vicilins, profilins and PR-10.
- Changes in protein size by formation of large aggregates or cross-linked proteins (oxidases), maintain or enhance the IgE-binding capacity of cereal prolamins and 2S albumins, respectively.
- Deamidation can result in decreased allergenicity of gliadins in wheat-allergic patients but increased allergenic potential in deaminated-gluten-allergic patients (increased severity of clinical symptoms).

Digestibility and Epithelial Transport

Resistance to gastrointestinal digestion may not be a conclusive factor for a protein to be an allergen, since it does not predict whether a protein is likely to be or to become an allergen [205, 206]. Additionally, in vitro digestion may not be representative of physiological conditions (in vivo digestion), when considering enzyme-to-protein ratios (e.g. pepsin), optimal pH, absence of pancreatin digestion or the presence of co-factors (such as surfactants) and other food components (matrix effect) [205, 206]. In order to comprehend the allergic response, it is essential to evaluate the influence of digestion on allergens, with special concern to their structural integrity and subsequent capacity to prompt T cell differentiation and IgE-mediated activation of effector cells upon gastrointestinal uptake [85].

Regarding plant food allergens (Table 3), most of the studies evaluate the effect of digestion on allergens using pure proteins or extracts, whereas only very few include or use the whole food [109, 207–209]. The profilins and PR-10 proteins are critically affected by the gastrointestinal digestion, being totally degraded during the process, which can lead to a severe reduction, or even to the elimination of their IgE-binding capacity. This is the case of profilins, such as mustard Sin a 4, apple Mal d 4, melon Cuc m 2 and cherry Pru av. 4 [118, 149, 210–212]. Likewise, the simulated gastrointestinal degradation of PR-10 proteins, such as apple Mal d 1, celery Api g 1 and hazelnut Cor a 1, revealed that these proteins were completely fragmented after a few minutes of exposure to pepsin [106, 213, 214]. This is well correlated to the fact that both families of proteins are classified as class 2 food allergens [215], which are normally responsible for triggering mild clinical symptoms, often limited to the OAS.

Members of the 2S albumins (Brazil nut Ber e 1, peanut Ara h 2, mustard Sin a 1 and hazelnut Cor a 14), ATI and cereal prolamins are highly resistant to proteolysis, allowing only partial degradation, thus contributing to the preservation of their IgE-binding capacity [23, 89, 93, 109, 112, 149, 152, 216]. Proteins of the nsLTP family are also able to preserve their allergenicity since they are only partially digested, although their resistance to proteolysis is highly influenced by the type of lipid-ligand associated with each allergen [42, 110].

2S Albumins, nsLTP, cereal prolamins and vicilins partially maintain some structural integrity after digestion, thus facilitating their involvement in transcellular mechanisms that allow them to cross the epithelium barrier in their native state, which greatly contribute to increase their allergenic potential [10, 45, 102, 112, 217–219]. This is well related to the fact that these protein families are classified as potent class 1 food allergens, being capable of primary sensitisation and induction of moderate to severe clinical symptoms in allergic individuals.

When compared with vicilins, legumins are slightly less resistant to proteolysis, suffering partial degradation after the gastrointestinal digestion. Legumins are more degraded by pepsin activity than by trypsin, leading to a small decrease in their IgE-binding capacity [71, 105, 180, 220, 221]. However, legumins are highly organised structures, presenting immunogenic subunits even after digestion, thus contributing to partially retain their allergenic potential.

Concluding remarks:

- Profilins and PR-10 proteins are rapidly degraded by pepsin during gastric digestion, drastically reducing or even eliminating their IgE-binding capacity.
- 2S Albumins, nsLTP, ATI, cereal prolamins, legumins and vicilins are strongly resistant to proteolysis, with only partial degradation after complete gastrointestinal digestion. All these proteins tend to preserve their allergenicity after passing the digestion process.
- 2S Albumins, nsLTP, cereal prolamins and vicilins basically preserve their structural integrity after digestion, allowing them to cross the epithelium barrier (by transcellular mechanisms) in a native state and increasing their allergenic potential.

Ligand/Lipid Binding and Interactions

Among their physicochemical properties, several food allergens are also capable of binding ligands, such as metal ions and lipids, which are known to enhance their thermal and proteolytic stability [9]. Some plant food allergens occur

naturally as lipid-protein complexes, such as the nsLTP that are structurally prepared to transport lipids in their hydrophobic cavity. Owing to this characteristic, nsLTP are more resistant to thermal processing and to proteolytic activity, thus contributing to preserve their allergenicity (Table 3) [110].

Besides nsLTP, there are two proteins from the 2S albumin and PR-10 families, namely the Brazil nut Ber e 1 and peanut Ara h 8, respectively, which present hydrophobic regions capable of binding lipids [135, 169]. Like for the nsLTP, lipid binding allows these two allergens to maintain some IgE-binding capacity upon processing and digestion. Additionally, the interaction between Ber e 1 and its lipid-ligand increases the sensitisation capacity of this allergen [135].

Along with lipid-binding capacity of some allergens, the interactions between lipids and proteins have also been highlighted as very relevant factors in the elicitation of an allergic response. Lipid-protein interactions can modify the digestion of the allergen within the gastrointestinal tract, aiding their passage through the intestinal epithelial barrier, thus influencing the allergenic potential of a protein [43].

The interaction of legumins and vicilins with lipids protect these proteins from enzymatic degradation, enabling to preserve their structural integrity and subsequently contributing to maintain their allergenicity (Table 3), which has been described for Sin a 2 (mustard legumin) and Ara h 1 (peanut vicilin) [105]. Similarly, in the case of 2S albumins (mustard Sin a 1) and PR-10 proteins (apple Mal d 1 and birch pollen Bet v 1), the presence of lipids seems to preserve the integrity of the molecules upon digestion, thus conserving or even slightly increasing their allergenicity [149, 222]. These data are well correlated with some recent studies reporting that lipids can act as adjuvants, stimulating the innate immunity followed by improved allergen-specific immune responses when used in combination with a specific allergen [43].

Concluding remarks:

- Lipid-binding propensities of nsLTP and two proteins: Brazil nut Ber e 1 (2S albumin) and peanut Ara h 8 (PR-10).
- Allergens (nsLTP, Ber e 1 and Ara h 8) that interact with their lipid ligands show molecular stability against food processing and (duodenal) digestion.
- Increased stability towards food-technological processing and digestion leads to preservation of the IgE-binding capacity of nsLTP, Ber e 1 (2S albumin) and Ara h 8 (PR-10).
- The presence of lipids during protein digestion has a protecting effect on the proteolysis of proteins, contributing to maintain or even increase the IgE-binding capacity of 2S albumins, legumins, vicilins and PR-10 proteins.

Can Physicochemical Properties Shape Allergenicity?

Food allergens were previously defined as small in size (10–70 kDa), with globular conformation, often glycosylated and resistant to heat, low pH and enzymatic activity [223, 224]. However, despite the commonly accepted concept that allergens conserve a certain pattern of specific physicochemical properties, this might not always be true. In fact, with the increasing number of proteins that has been identified and classified as food allergens, there are several important allergens that do not fit into this general classification (Tables 2, 3 and 4). So, at this stage, is it possible to establish straightforward correlations between specific physicochemical properties and their impact on protein allergenicity? Or should this concept be carefully revised?

According to the tendencies observed in our analysis, the impact that each physicochemical property has on protein allergenicity is summarised in Table 4. In most cases, independent effects of distinct physicochemical proteins often result in a common pattern, which relates to the preservation of protein structural integrity.

In the universe of food allergenic proteins, some are glycosylated, but this PTM is not always synonym of increased allergenicity. Among the most relevant families of plant proteins, glycosylation is not a common feature of allergenicity, since only vicilins and three other proteins (stone pine Pin p 1, lupine Lup a alpha-conglutin and wheat Tri a 40) are glycosylated. Nonetheless, in those cases, glycosylation contributes to enhance their IgE-binding capacity.

Small proteins with globular structure are more stable to external interactions, but there are plenty of examples of potent allergens that present high molecular weight (> 70 kDa) and high level of structural organisation (quaternary structure), such as vicilins and legumins. Still, the loss of secondary structures and the destruction of disulphide bonds normally contribute to reduce the allergenicity of most proteins, which means that protein structure is an important physicochemical parameter.

Protein stability towards heat could be considered a physicochemical parameter that potentially shapes the allergenicity of plant allergens, since most potent food allergens are heat stable (e.g. 2S albumins, vicilins). However, this property fails to explain why many heat-labile proteins are highly relevant food allergens (e.g. profilins, PR-10 proteins). In this context, it is also important to emphasise that profilins and PR-10 allergens in fruits and vegetables are usually allergenic because they are ingested as raw or weakly processed foods, which means that proteins are still in their native shape. This type of allergy is usually restricted to the oral cavity (OAS), although reports of symptoms with increased severity have also been described [94, 225].

Table 4 Main conclusions about the adequacy of each physicochemical property as potentially shaping allergenicity

	Impact on IgE-binding capacity	Supporting evidence/main concerns
Abundance (allergen content in relation to total protein)	High	Potent allergens are often highly abundant.
Biological function	High	Potent allergens display biological functions as capacity, transport and defence.
PTM		
Glycosylation	Limited	Increases allergenicity, most likely IgE-binding regions containing N-glycans. Information mostly limited to vicilins
Hydroxylation	Limited	Increase the allergenic potential of Ara h 2 (limited to 2S albumins)
Phosphorylation	–	Not reported
Lipid binding	High	Lipid binding stabilises protein structure, increasing resistance to proteolysis and processing.
Protein structure		
Loss of 2D	High	Decreases allergenicity of most plant allergens. Loss of structural integrity. Valid for conformational epitopes
Loss of S-S bonds	High	Decreases allergenicity of most plant allergens. Loss of structural integrity. Valid for conformational epitopes
Glycation	Low or inconclusive	Depending on the protein family, glycation decreases, maintain or increase allergenicity
Aggregation	Low or inconclusive	Depending on the protein family, aggregation decreases, maintain or increase allergenicity
Heat stability	High	Potent allergens are heat stable. Fails to explain potent heat-labile allergens (e.g. profilins, PR-10 proteins)
Pressure stability	Limited	Potent allergens are pressure stable, but in vivo evidence has hardly been studied. Maintain protein integrity.
Light/radiation stability	High	Potent allergens are light/radiation stable. Maintain protein integrity.
Mechanical stability	Low	Most allergens are stable to mechanical processing. Maintain protein integrity.
Chemical stability		
Changes in protein structure	High	Maintain or reduce the IgE-binding capacity. Limited information to nsLTP and legumins
Changes in protein size (fragmentation)	High	Maintain or reduce the IgE-binding capacity of 2S albumins, ATI, legumins, vicilins, profilins and PR-10 families. Fragmentation of protein into peptides. Loss of protein primary structure.
Changes in protein size/structure	High	Enhance and maintain the IgE-binding capacity. Limited information to cereal prolamins and 2S albumins
Digestibility		
Pepsin resistance	Low or inconclusive	Fails to explain potent pepsin-labile allergens (e.g. Ara h 3, Gly m 6)
Trypsin/chymotrypsin resistance	High	Most allergens are resistant to trypsin/chymotrypsin activities.
Lipid interaction	High	Presence of lipids protects allergens from proteolysis. Maintain protein integrity.

Protein resistance towards proteolytic activity (enzymatic hydrolysis, chemical hydrolysis or fermentation) could be positively correlated with allergenic potential, although with different expected outcomes. The breakdown of protein in small size peptides (strategy followed for the production of hypoallergenic foods) normally contributes to mitigate peptide allergenicity, while enzymatic cross-linking of proteins can contribute for the opposite effect (e.g. cereal prolamins) (Table 4) [132, 137].

Protein resistance to digestion process, especially pepsin resistance, cannot be considered a good predictor for allergenicity, once it fails to explain the existence of potent pepsin-

labile allergens, such as peanut Ara h 3 (legumin) and soybean Gly m 6 (legumin) [71]. The way allergenic proteins interact with lipids during the digestion process might contribute to conserve their allergenic potential, since lipids may stabilise proteins and thus preserve structure-related allergenicity.

It is also important to stress that it is very difficult to determine how protein changes affect the development or clinical manifestations of food allergies in real-life scenarios. Ethical reasons and limitations in monitoring the molecular changes of allergens in real-life processed food matrices, and subsequent processing in humans, highlight the currently unmet needs faced in the food allergy field. The few studies that evaluate the sensitising capacity of modified proteins are mostly performed in murine allergy models. For instance, Bellinghausen et al. [172] used a humanised murine allergy model to assess allergen-induced gut inflammation. The authors concluded that allergen-specific human IgE was greatly enhanced in mice on wheat ATI-containing diet than in mice on gluten-free diet. Accordingly, ATI were considered key sensitisers of wheat allergy and that these proteins can be used in nutritional therapeutic strategies to address allergen- and gluten-induced intestinal and extraintestinal inflammation [172]. Likewise, Denery-Papini et al. [121] and Gourbeyre et al. [123] reported that, despite different sensitisation paths (oral and intraperitoneal), deamidated gliadins were much more competent than native gliadins in inducing allergic sensitisation in mice, and subsequently, in triggering a more severe elicitation phase. Mirotti et al. [135] also described that lipids were necessary for the sensitisation of mice to Ber e 1 (Brazil nut).

Studies carrying out SPT or DBPCFC are rare, mostly due to all ethical issues associated with it. SPT are normally conducted using whole protein extracts [95, 104, 117, 124, 126–128], whose results require further verification by performing oral food provocation. In addition, appropriate analytical methods are required to understand the physicochemical changes in the food allergens. The combination of analytical and clinical characterisation may allow drawing conclusions about the impact of physicochemical parameters on protein allergenicity with regard to clinical manifestation.

However, very few SPT have been performed with modified proteins, such as the case of the work described by Sancho et al. [119] and Vassilopoulou et al. [110], where purified native versus heat Mal d 3 (100 °C, 60 min) and purified native versus digested Vit v 1, respectively, were used to assess the effects of thermal processing or enzymatic hydrolysis on the allergenicity of tested proteins. Likewise, Denery-Papini et al. [121] and Palosuo et al. [132] tested natural versus deaminated gluten and native versus transglutaminase cross-linked Tri a 19, respectively, by SPT to evaluate the effect of chemical hydrolysis and enzymatic cross-linking on the allergenicity of specific wheat proteins.

DBPCFC is normally conducted using the whole allergenic food blinded within a complex food matrix, and the outcome of a food challenge is correlated with the specific allergenic food. As example, a DBPCFC was carried out in humans using raw wheat flour and deamidated gluten in a stewed apple, where authors concluded that deamidated gluten could induce sensitisation to deamidated gliadins in wheat-tolerant individuals [121].

Conclusions

The data collected from all the reported studies present a huge variability, being normally defined at a qualitative level, which increases the complexity of the analysis presented in this review. It became clear that our knowledge provided by the literature still presents numerous gaps.

One of these major gaps concerns the lack of harmonised protocols since each study is conducted in different conditions. The use of different immunoassays, different sources of patients' sera (affected by geographical differences, age, sex, presence of other diseases, genomic heritage, among others), sera or plasma and the use of pure protein, pure extracts or matrix, are just some examples among the numerous variables that must be considered when conducting this type of studies. Additionally, most information come from studies evaluating the IgE-binding capacity of allergenic proteins as affected by different parameters, rather than from functional assays, DBPCFC or sensitisation tests, thus hampering their correct correlation with real clinical outcomes.

Another important gap concerns the lack of studies evaluating simultaneously the impact of such physicochemical proteins in non-allergens and allergens of the same family or even of the same type [205, 226]. Studying the physicochemical properties of allergens can be faced as the fundamental background for better understanding food allergy and subsequently for developing a better allergenicity assessment of food proteins.

The exact role that each parameter has on the allergenicity of different types/families of plant food allergens is not yet fully understood. Within each protein family of plant foods, their allergenic members seem to follow the same tendency, although occasional exceptions can be observed. At individual basis, some parameters like heat stability, resistance to proteolytic activity and structural stability are considered of vital importance for protein allergenicity. However, since most of the methods used for allergenicity assessment are made indirectly, there is still a significant gap between the influence of each physicochemical parameter and their real clinical impact.

Ideally, the impact of processing should be assayed in vivo in humans using food-grade preparations. Therefore, in order to study the molecular characteristics in detail, the allergens

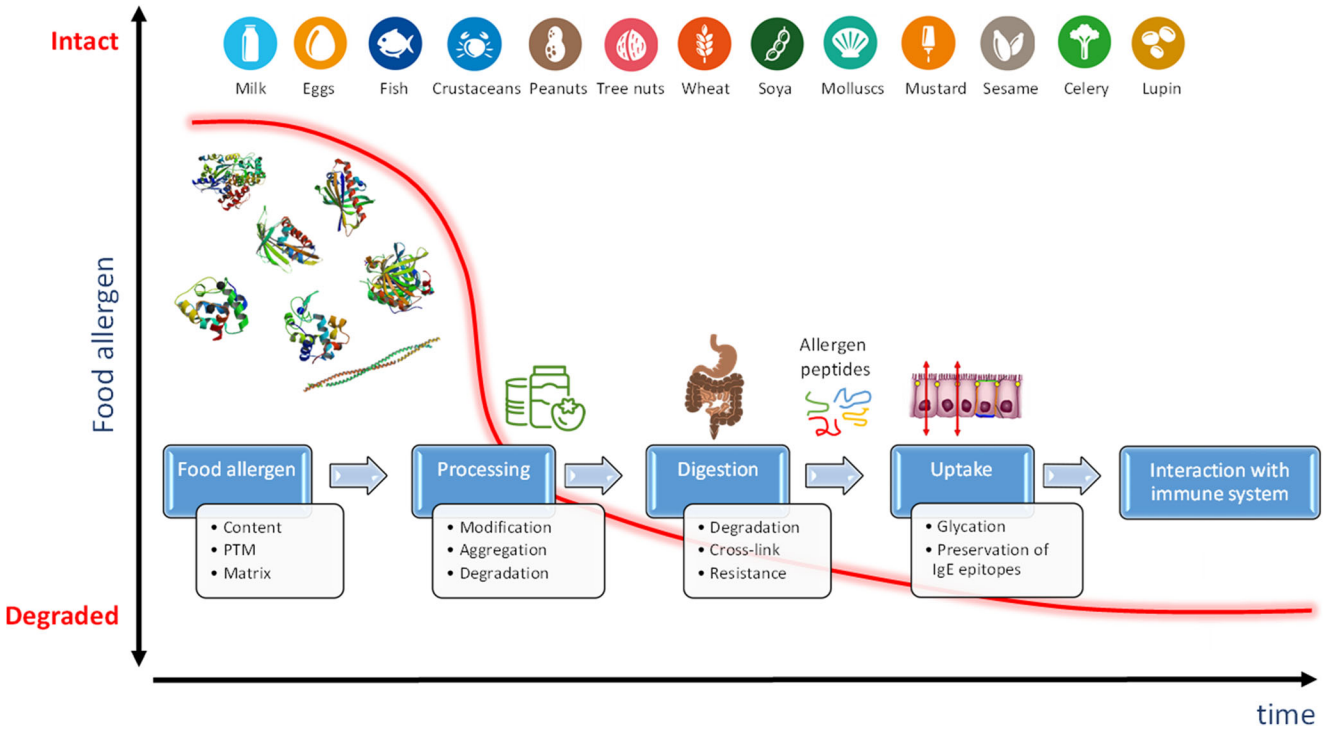


Fig. 1 Life cycle of food allergens: from intact source molecules to highly degraded peptides with immunological activity

would need to be prepared (extracted, purified) from the food-grade material, which can result in a hard task (difficult and prone to bias), since usually only native-like structures are extracted. Consequently, other immunoreactive and bioavailable structures might be neglected due to the fact of being insoluble under the conditions of experimental investigation. By contrast, one must consider that working on single allergens, processed under lab conditions might help to better characterise allergens at the molecular level, although this experimental setting might not fully reflect the in vivo reality. Moreover, for human in vivo studies, such food-grade material would need to be prepared under good manufacture practice conditions. There is also the debate about using animal models to predict the allergenicity, but there is still a certain degree of uncertainty to which extent the findings can be extrapolated to the human condition.

In summary, several physicochemical parameters have been described in the scientific literature that can explain their impact on plant protein allergenicity (Fig. 1). Especially, parameters that support protein structure integrity are of importance. Despite observed tendencies within conserved protein families of plant food allergens, several exemptions exist at the level of individual allergens. Hence, we are likely able to explain allergenicity for many of the identified plant food allergens, especially at the site of symptom elicitation. However, using this information for allergenicity prediction of novel proteins or in relation to food-processing parameters remains a future challenge. Moreover, the knowledge about physicochemical parameters that influence sensitisation is scarce and requires further attention in food allergen research.

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Compliance with Ethical Standards

Conflict of Interest The authors declare that they have no conflict of interests.

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Affiliations

Joana Costa[1] · Simona Lucia Bavaro[2,3] · Sara Benedé[4] · Araceli Diaz-Perales[5] · Cristina Bueno-Diaz[6] · Eva Gelencser[7] · Julia Klueber[8,9] · Colette Larré[10] · Daniel Lozano-Ojalvo[11] · Roberta Lupi[10] · Isabel Mafra[1] · Gabriel Mazzucchelli[12] · Elena Molina[4] · Linda Monaci[2] · Laura Martín-Pedraza[13] · Cristian Piras[14,15] · Pedro M. Rodrigues[16] · Paola Roncada[17] · Denise Schrama[16] · Tanja Cirkovic-Velickovic[18,19,20] · Kitty Verhoeckx[21] · Caterina Villa[1] · Annette Kuehn[8] · Karin Hoffmann-Sommergruber[22] · Thomas Holzhauser[23]

1 REQUIMTE-LAQV/Faculdade de Farmácia Universidade do Porto, Rua de Jorge Viterbo Ferreira 228, 4050-313 Porto, Portugal

2 Institute of Sciences of Food Production (ISPA) - National Research Council (CNR), Bari, Italy

3 Moorepark Food Research Centre, Teagasc, Fermoy, Co. Cork, Ireland

4 Instituto de Investigación en Ciencias de la Alimentación (CIAL), CSIC-UAM, Madrid, Spain

5 Centro de Biotecnologia y Genomica de Plantas (UPM-INIA), Universidad Politecnica de Madrid, Pozuelo de Alarcon, Madrid, Spain

6 Departmento de Bioquímica y Biología Molecular, Facultad de Ciencias Químicas de la Universidad Complutense de Madrid, Madrid, Spain

7 Department of Biology, Food Science Research Institute, National Agricultural Research and Innovation Centre, Budapest, Hungary

8 Department of Infection and Immunity, Luxembourg Institute of Health, Esch-sur-Alzette, Luxembourg

9 Department of Dermatology and Allergy Center, Odense Research Center for Anaphylaxis, University of Southern Denmark, Odense C, Denmark

10 INRAE, UR 1268, Biopolymers Interactions Assemblies, Nantes, France

11 Precision Immunology Institute, Jaffe Food Allergy Institute, Icahn School of Medicine at Mount Sinai, New York, NY, USA

12 Mass Spectrometry Laboratory, MolSys Research Unit, University of Liege, 4000 Liege, Belgium

13 Biochemistry and Molecular Biology department, Chemistry Faculty, Complutense University of Madrid, 28040 Madrid, Spain

14 Department of Veterinary Medicine, University of Milan, Milan, Italy

15 Department of Chemistry, University of Reading, Whiteknights, Reading RG6 6AD, UK

16 CCMAR, Universidade do Algarve, Campus de Gambelas, Faro, Portugal

17 Department of Health Sciences, University 'Magna Græcia', Catanzaro, Italy

18 University of Belgrade - Faculty of Chemistry, Belgrade, Serbia

19 Ghent University Global Campus, Yeonsu-gu, Incheon, South Korea

20 Faculty of Bioscience Engineering, Ghent University, Ghent, Belgium

21 Department of Dermatology/Allergology, University Medical Center Utrecht, Utrecht, The Netherlands

22 Department of Pathophysiology and Allergy Research, Medical University of Vienna, Vienna, Austria

23 Division of Allergology Paul-Ehrlich-Institut, Langen, Germany