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Nanotechnology and Food Allergy

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13.1
Introduction

Food allergies are one of several different types of reproducible adverse reactions to foods that have been described, which also include enzyme deficiencies, such as lactose intolerance, and pharmacological reactions to foods rich in compounds such as histamine. Food allergies share a common characteristic, namely an immunological basis, and so far two different forms have been recognized [1]. One of them involves the humoral arm of the immune system with the development of food-specific immunoglobulin E (IgE) responses that can trigger a host of reactions usually classified as type I hypersensitivity reactions. The other type involves activation of immune cells in the gut, and is manifested as the gluten intolerance syndrome known as celiac disease.

With regard to IgE-mediated food allergies, during normal healthy functioning, the immune system produces a type of immunoglobulin known as IgE, the role of which is to defend the body from parasitic infections, such as malaria. For reasons not fully understood, some individuals begin to make IgE in response to various environmental agents, including dust, pollens, and foods, which can lead to the development of allergic reactions. Such IgE-mediated allergies develop in two phases: (i) sensitization when IgE production is stimulated, and (ii) elicitation when an individual experiences an adverse reaction, mediated by IgE, upon re-exposure to an allergen. Both stages are triggered by allergens, which are almost always proteins. In an allergic reaction, allergen is recognized by IgE bound to the surface of histamine containing mast cells, cross-linking the IgE in the process and triggering the release of inflammatory mediators such as histamine. These mediators cause the acute inflammatory reactions that become manifested as respiratory (asthma, rhinitis), cutaneous (eczema, urticaria) or gastrointestinal (vomiting, diarrhea) symptoms, which may occur alone or in combination in an allergic reaction. A rare but very severe reaction is anaphylactic shock characterized by respiratory symptoms, fainting, itching, urticaria, swelling of the throat or other mucous membranes, and a dramatic loss of blood pressure.

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In contrast to the rapid onset characteristic of type I hypersensitivity reactions, the gluten intolerance syndrome, celiac disease, can take between hours and days to manifest itself after consumption of gluten-containing food. It is thought that around 1% of the population suffers from this gluten intolerance syndrome, and it seems to affect women more than men. It is caused by the recognition of gluten peptides that result from digestion, which have first been deamidated by the action of gut mucosal transglutaminase. These deamidated peptides can bind to receptors, known as class II human histocompatibility leukocyte antigen receptors, of certain types, namely DQ2 and DQ8. This then triggers an abnormal cell-mediated immune response, which results in an inflammatory reaction in the gastrointestinal mucosa, which causes the loss of the normal villous architecture that is characteristic of celiac disease [2].

There is no proactive treatment available for either IgE-mediated food allergies or celiac disease. Consequently, individuals who suffer from these conditions have to practice food avoidance and, in the case of IgE-mediated allergies, are provided with medication (such as adrenalin pens) to be used in case of accidental consumption of a problem food. In practice, it can be difficult to avoid some problem foods, especially widely used ingredients such as wheat, cows' milk or hens' egg. It is generally held that the vast majority of food allergies are caused by a limited number of foods [3], although a large number of foods have been documented as causing food allergies, reflecting the diversity of food species that humans consume. In order to help allergic consumers avoid problem foods, legislation has been brought in around the world that makes it mandatory to label certain allergenic foods and derived ingredients, irrespective of the level to which they are added to a foodstuff [4].

13.2
Molecules in Foods Involved in Triggering Allergies

The molecules that trigger both types of immunological reactions to foods are known as allergens and to date those responsible for almost all food allergies are proteins. Those involved in triggering celiac disease are confined to the prolamin seed storage proteins of cereals (wheat, rye, and barley). In contrast, the proteins involved in triggering IgE-mediated food allergies are still being identified and characterized, but they originate from a diverse range of foods of both plant and animal origin. Food allergens triggering IgE-mediated reactions appear to be restricted to certain structural types or protein families [4], and this has led to a classification based on protein family membership [5]. Thus, an analysis of plant food allergen families has shown that they belong to only 27 protein families [6], with four protein families (prolamin, cupin, Bet v 1, and profilin families) accounting for more than 65% of all plant food allergens. The distribution of animal food allergens was similar [7], with three protein families (tropomyosin, parvalbumin, and casein families) dominating. These observations suggest that conserved structures and biological activities play a role in determining or promoting allergenic properties of proteins, and are in part explained by the conservation of surface

structures in certain families, such as the Bet v 1 and parvalbumin superfamilies, which promote IgE cross-reactivity [6, 7].

The characteristics of major allergen families are summarized below. More detailed reviews of food allergen structure and properties, including both major and minor allergen families, can be found in references [8] and [9].

13.2.1 Plant Food Allergens

13.2.1.1 Prolamin Superfamily

The prolamin superfamily comprises the seed storage prolamins of cereals, 2S albumins, non-specific lipid transfer proteins, and α-amylase/trypsin inhibitors of cereals [4, 5]. Apart from the seed storage prolamins, these are all low-molecular-weight cysteine-rich proteins that share the same three-dimensional fold, are rich in α-helices, and are generally stable to thermal processing and proteolysis. The 2S albumins are a major group of storage proteins present in many dicotyledonous plants. They include major allergens from tree nuts and seeds such as Brazil nut, walnut, sesame, and mustard. The non-specific lipid transfer proteins play an important role in plant defense against fungi and bacteria. They are found in a diverse range of plant foods, including fruits, nuts, seeds, and vegetables, and are an important group of allergens in the Mediterranean area [10]. The family of cereal α-amylase and protease inhibitors mediates a certain degree of resistance to insect pests that feed on plant tissues, allergenic members having been identified in wheat, barley, rice, and corn. Like the 2S albumins and the non-specific lipid transfer proteins, these allergens are able to sensitize susceptible individuals through either ingestion or inhalation.

The prolamin seed storage proteins appear to have evolved through insertion of a highly repetitive domain within the cysteine skeleton. They are involved in triggering some IgE-mediated allergies and, more importantly, trigger the gluten intolerance syndrome, celiac disease, involving the homologous proteins from wheat, rye, and barley. Many celiacs can tolerate oats, which contain much lower levels of prolamin storage proteins (known as avenins). But while they have a slightly different structure compared to the prolamins from the Triticeae, there are still some concerns about the safety of oats for celiacs in general.

It appears that, while all prolamin fractions appear to trigger the condition, the most potent appears to be α-gliadin, which can trigger more severe reactions [11]. The key feature appears to involve recognition of prolamin-derived peptides by receptors such as histocompatibility leukocyte antigen DQ2 (and some to DQ8), which results in stimulation of T-cell responses that initiate inflammatory reactions. One particular peptide that appears to stimulate the majority of T-cells in untreated celiacs corresponds to a 33-amino-acid peptide derived from α-gliadin. This peptide is not completely digested by enzymes in the gastrointestinal tract lumen or the brush border enzymes of the mucosa and includes epitopes corresponding to amino acid sequences such as Pro-Phe-Pro-Gln-Pro-Gln-Leu-Pro-Tyr, Pro-Gln-Pro-Gln-Leu-Pro-Tyr-Pro-Gln, and Pro-Tyr-Pro-Gln-Pro-Gln-Leu-Pro-Tyr [12].

13.2.1.2 Cupin Superfamily

The cupins are a functionally diverse superfamily of proteins that share a β-barrel structural core domain to which the term "cupin" (Latin *cupa*, meaning "barrel") was given. The cupin superfamily comprises the major globulin storage proteins mainly from legumes and nuts. The globulins are divided into the 7S vicilin-like globulins and the 11S legumin-like globulins. Globulins have been found to be highly relevant allergens in plant foods including peanuts, soybean, lentils, walnut, hazelnut, and sesame [4, 5]. Despite having very low levels of sequence identity, members of the cupin superfamily have highly conserved structures. In contrast to the Bet v 1 family of plant food allergens, there is little evidence of IgE cross-reactivity between cupin allergens, with an overall sequence identity of less than 40%. This results in very limited cross-reactivity between cupins from even closely related species such as peanut and pea [13].

13.2.1.3 Bet v 1 Family

Individuals with pollen allergy frequently suffer from allergic symptoms after eating certain plant foods. The majority of these reactions are caused by allergens of Rosaceae fruits like apple, peach, cherry, and apricot, and certain vegetables such as celery root (celeriac) and carrot, which cross-react with allergens that are present in birch pollen, particularly the major birch pollen allergen Bet v 1 [5]. Bet v 1 was the first of many allergens published that showed homology to family 10 of the pathogenesis-related proteins. Bet v 1-type allergens are rather unstable to heating and digestion. Consequently, symptoms are mostly restricted to the oral cavity. In general, Bet v 1 from birch pollen is thought to act as the primary sensitizing agent, with allergies to foods developing subsequently [14]. The overall high levels of conserved surface residues between the members of the Bet v 1 family plays an important role in conservation of IgE binding sites and underlies the fruit–vegetable–pollen cross-reactive syndromes [6].

13.2.1.4 Profilins

Being cytosolic proteins, profilins are ubiquitous proteins found in all eukaryotic cells, which are thought to play a role in regulating the polymerization and depolymerization of actin during a variety of cellular processes including cell movement [15]. Like members of the Bet v 1 family, profilins are involved in cross-reactive allergies, where sensitization to pollens results in IgE responses toward homologs found in fresh fruits and vegetables [16]. However, the clinical relevance of plant food profilin-specific IgE is still under debate [17].

13.2.2 Animal Food Allergens

13.2.2.1 Tropomyosins

Tropomyosins are cytoskeletal proteins and, together with other contractile proteins, such as actin and myosin, play a key role in regulation of muscle contraction [18]. Together with actin and myosin, tropomyosins play a key regulatory role in

muscle contraction. Being two-stranded α-helical coiled proteins, tropomyosins form head-to-tail polymers along the actin filaments. Tropomyosins have been described as allergens in Crustacea (such as shrimps, crab, and lobster) and Molluscs (such as abalone) and are recognized as invertebrate pan-allergens [19]. The proteins are heat-stable and, because of the extensive homologies between invertebrates, tend to show IgE cross-reactivity between crustacean and molluscs [20, 21].

13.2.2.2 **Parvalbumins**

The second largest animal food allergen family are the fish β-parvalbumins, a calcium-binding protein found in the white muscle with a characteristic EF-hand structure [22]. They have been characterized as allergens in many different fish species and are considered as pan-allergens in fish [23], their conservations of surface structures explaining the IgE cross-reactivity that is frequently observed between fish species [7]. The proteins show considerable thermal stability when calcium is bound [24], but changes in conformation when calcium is lost is associated with a loss of IgE reactivity [25, 26].

13.2.2.3 **Caseins**

The major proteins found in milk, caseins are structurally mobile proteins that bind calcium through clusters of phosphoserine and/or phosphothreonine residues. The casein fraction of cows' milk comprises α_{s1}-, α_{s2}- and β-caseins, which assemble into micelles stabilized by κ-casein [27]. They are the major food allergens in cows' milk allergy, which is primarily an allergy of infancy. There is considerable sequence similarity between caseins from different species, with sequence identities of over 90% between cows' milk and goats' milk caseins, explaining the cross-reactive allergies between cows' milk and goats' milk [28].

13.3
Food Structure, Processing, and Food Allergy

While we are gaining an extensive knowledge of the molecules in foods that trigger food allergies, they are not consumed as individual purified molecules, but rather as part of foods. Indeed, many allergens are abundant in foods and make an important contribution to forming the food structure itself. During food processing procedures, allergens may undergo complex physical and chemical changes, altering their three-dimensional structure, and promoting interactions (both covalent and non-covalent) with other food constituents, including proteins, lipids, and sugars. These changes, coupled with the effects of the food matrix itself, may affect the release and stability of allergens, and all have the potential to either reduce or enhance the allergenic potential of food allergens by modifying the way in which they are presented to the immune system. Such effects may be mediated at both a molecular and a macroscopic level.

13.3.1 Molecular Effects of Food Processing on Allergenicity

The impact of processing on allergenic potential, especially in terms of eliciting an allergic reaction, can be considered in terms of the effect that processing-induced changes in allergen structures have on IgE binding, particularly in relation to IgE epitopes. One type of epitope comprises linear stretches of contiguous amino acids and is generally known as a linear or continuous epitope. IgE binding to such epitopes is usually unaffected by the folded state of the protein. A second type of epitope (often known as a conformational epitope) is formed from different segments of a polypeptide chain that are brought together in space as a consequence of the way in which a protein is folded. Such epitopes can be disrupted as a consequence of protein denaturation, reducing or even abolishing antibody recognition [29]. It has been suggested that an antibody developed toward a highly disordered state (such as a denatured protein) is able to recognize the more highly ordered states found in native, folded proteins possibly because it recognizes linear epitopes [30]. This is especially so if an epitope is located on the surface of the folded form of the protein. However, antibodies directed toward a folded protein (conformational epitopes) tend to be directed to conformational epitopes and hence often recognize denatured forms poorly, if at all.

Lastly, small molecules (such as sugars) attached to proteins can form part of an epitope. Such molecules are unable to elicit an immune response alone but can stimulate humoral immune responses when linked to a carrier molecule, such as a protein. Known as haptens, such small molecular substituents on proteins have been shown to stimulate IgE responses, particularly in relation to cross-reactive carbohydrate determinants. However, for cross-reactive carbohydrate determinants at least, such haptens are often unable to stimulate a biological response, possibly because their sparse distribution on a protein limits their ability to cross-link IgE on mast cells and hence stimulate histamine release [31, 32].

Thus, food processing has the ability to destroy IgE epitopes, but may also introduce novel (neo)epitopes into a protein, either by changing protein conformation, by linking proteins together in aggregated states, or as a consequence of introducing novel haptens, through Maillard modification, by conjugation with lipid oxidation products, or via a host of other chemical changes that may result from the thermal treatments frequently used in food production. The effect of thermal processing on allergen structure depends on many factors such as time–temperature combinations, protein concentration, water activity, and whether a protein is heated alone or in combination with other proteins or food ingredients such as sugars. These types of interaction can be illustrated by processing-induced changes that have been described for the major whey proteins (see Figure 13.1), β-lactoglobulin (β-Lg) and α-lactalbumin (α-La), which have been structurally well defined using techniques such as nuclear magnetic resonance spectroscopy and X-ray diffraction, and are both important cows' milk allergens [33]. Other food allergens that have been shown to undergo similar types of interactions, unfolding and forming aggregates, are the allergenic 7S and 11S seed storage globulins from

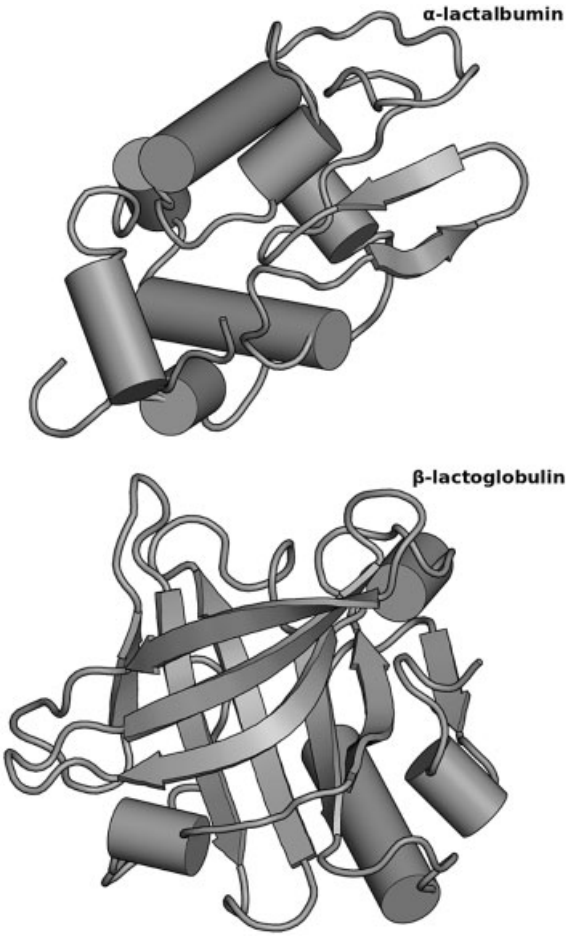

Figure 13.1 Structures of the important whey proteins in cows' milk: α-lactalbumin (Protein Data Bank number 1HFX) and β-lactoglobulin (Protein Data Bank number 1BSY) at pH 7.1. The α-helices are shown as cylinders. The β-pleated sheet and loops are shown as broad flat arrows and as strings/wires, respectively. (The pictures were generated using the open-source molecular visualization system PyMOL.)

foods such as peanut and soybean [34] and the allergenic potato tuber protein patatin [35].

A 18400 dalton retinol binding protein, β-Lg is a β-barrel protein belonging to the lipocalin superfamily and is stabilized by two intramolecular disulfide bonds (Cys^{106}–Cys^{119} and Cys^{66}–Cys^{160}), together with a single free cysteine residue (Cys^{121}) [36]. It is present as a mixture of monomers and dimers at neutral pH, dissociating on heating to 70 °C [37] and appears to adopt a partially folded state following thermal denaturation [38], before forming thread-like aggregates around 50 nm in diameter [39]. Heat-induced unfolding of β-Lg reveals the buried Cys^{121}, which is

then able to catalyze disulfide interchange with other disulfides in β-Lg to form a non-native monomer in which Cys^{119} is exposed [40].

Also being a low-molecular-weight 14 200 dalton disulfide-bond-stabilized calcium binding protein, α-La has a role in regulating lactose synthase [41]. Primarily an α-helical protein, it exists at low pH or moderately elevated temperatures in a partially folded or "molten globule" state [42]. Thus α-La expands on formation of the low-pH-induced molten globule from 19.4 to 21.6 nm, while after heating it is further expanded to 23.8 nm. The heat-induced partially folded form is kinetically trapped as a consequence of intermolecular disulfide interchange and retains much of the secondary structure of the native protein.

While thermally induced changes in the structure of these important whey allergens are well defined, little is known about their impact on IgE reactivity. Some limited studies have shown that the IgE binding capacity of β-Lg (variants A and B) is reduced following thermal treatments able to denature the protein, some trace of IgE binding remaining [43]. Such studies are also consistent with clinical observations that the allergenicity of extensively baked milk products (muffins, heated to 180 °C for 30 min) is substantially reduced, especially in children whose cows' milk allergy is beginning to resolve [44]. Investigations into the effect of processing on sensitization potential are more difficult to undertake and rely on the use of animal models. Nevertheless there are indications that heat-induced aggregation of whey proteins affected the path of uptake across the mucosal barrier and that soluble proteins were endocytosed by epithelial cells. But after pasteurization the resulting aggregates were preferentially taken up by the Peyer's patches and this was associated with a shift toward a Th2-associated antibody and cytokine pattern. However, the soluble proteins were much more effective in triggering an anaphylactic reaction [45].

13.3.2
Macroscopic Effects of Food Processing on Allergenicity

Both naturally occurring food structures, and those formed in fabricated foods, may act to trap allergens, preventing their becoming solubilized in fluids such as saliva, gastric or duodenal secretions, and possibly protecting them from degradation by intestinal proteases. This can affect their allergenic potential, in terms of both sensitization and elicitation.

13.3.2.1 Natural Cellular Structures

Allergens are contained within the natural cellular and tissue structures of fruits, vegetables, and seeds, and in the cellular and fibrous structures of meats. For example, the non-specific lipid transfer protein allergens of fruits are largely confined to the skins of fruits such as apple and peach, reflecting the greater allergenicity of peel with respect to flesh for patients suffering from fruit allergies involving non-specific lipid transfer proteins [46]. In contrast, Bet v 1 allergens are largely confined to the flesh [47]. It may be that differences in the structure and components in different fruits and vegetables account for the different allergenic

properties of homologous allergens. For example, the allergenic Bet v 1 homolog of celery root (celeriac) has been shown to be stable to processing, retaining its ability to elicit an allergic reaction after cooking [48], but the Mal d 1 homolog found in apple is lost after processing [49].

Natural structures, in particular the plant cell walls found in a particular plant tissue, may affect the stability, release, and presentation of allergenic molecules to the immune system. For example, the mechanical break-up of plant tissues, either during food processing (such as cooking, or preparation of fruit purée) or during chewing, is determined by the plant cell wall properties and will both affect release of allergens into solution and generate a range of particulate structures made up of fragments of the original plant tissue structure. The cell wall structure and composition will also determine how intact cells, clusters of cells or larger fragments of plant tissue structures respond to the environment of the upper gastrointestinal tract, and hence may alter the ingress of degradative enzymes and biosurfactants, as well as the release of allergens into the gut lumen. Similarly, it may be that the cellular and fibrous structures found in the flesh of animals, such as fish, crustacean, and molluscs, may affect the way in which fish and shellfish allergens are released from cooked flesh.

13.3.2.2 **Processed Food Structures**

Structures formed in complex processed foodstuffs may also affect the stability and release of allergenic molecules. Many foods are in the form of dispersions, with one phase (such as oil, starch granules or other particulates) dispersed in a second immiscible phase in the form of droplets (like oil droplets in water found in sauces such as mayonnaise), air bubbles (like the air bubbles found in bread dough) or particulates (like starch granules in a sauce made using corn starch). These dispersions include the following.

- **Gels** These can be like either the low-pH-set gels of milk-based yogurts or the heat-set gels formed when boiling an egg.
- **Foams** In this group fall the whipped egg whites in meringues and mousse-style desserts. In some cases the foams become set by cooking, with either the protein or starch forming a solid network, which usually needs to rupture following baking to form a sponge network such as is found in cakes.
- **Emulsions** Either oil-in-water (salad dressings or cream) or water-in-oil (spreads and margarines) emulsions, these are unstable unless a surface-active agent is added, such as a protein or a low-molecular-weight surfactant such as lecithin.

In many cases, food structures are formed from the allergenic proteins – gels may be formed from milk or egg proteins, or set foams formed by gluten proteins in bread and cakes. Additionally, other allergens such as whey proteins may be used as emulsifiers. However, there is an almost complete lack of knowledge on how such classical food structures may affect the allergenic potential of foods. This is partly because many clinical investigations have utilized soluble extracts of foods and processed food systems rather than investigating the allergenic activity of the

insoluble matrix because of the technical difficulties in studying such insoluble systems. One of the few clinical studies undertaken in this difficult area of research showed that enhancing the fat content of a chocolate matrix containing peanuts affected the kinetics of allergen release and potentiated severe allergic reactions [50]. Such studies that have been published have often been restricted to investigations on the ability of processed foods to elicit reactions in individuals already suffering from a food allergy. We currently lack effective animal models for investigating the potential for allergens or foods to sensitize, and hence our knowledge base in this topic is almost non-existent.

13.3.3 Molecular and Macroscopic Effects of Processing on Allergenicity of Foods

The complex interplay between molecular and macroscopic effects of food processing in relation to allergenicity of foods can be illustrated by a couple of well-characterized allergen families, the Bet v 1 and prolamin superfamilies. One type of food allergy where the IgE binding is dominated by conformational epitopes is the pollen–fruit allergy syndrome involving the birch pollen allergen Bet v 1. In this condition, individuals become sensitized to native Bet v 1 through inhalation of birch pollen, and consequently the main IgE binding sites are primarily directed toward conformational epitopes on the native protein [51, 52]. Thus, it might be expected that processing could disrupt these conformational IgE epitopes, reducing the allergenicity of a cooked, compared with a fresh, food. However, the extent to which this happens will be determined by the inherent thermostability of the protein. Bet v 1 itself is relatively thermostable, the protein irreversibly unfolding only at temperatures above 68 °C [53], and in some foods, such as celeriac, this is expressed in the stability of the allergenic Bet v 1 homologue, Api g 1, to processing [10]. Similarly the Bet v 1 homolog from soybean, Gly m 4, retains its allergenicity even in a processed soya-based food supplement [54, 55].

However, this is not so for all foods involved in the birch pollen–fruit allergy syndrome, and especially for fruits such as apple [49], while roasting hazelnuts reduced but did not abolish their allergenic properties in a group of patients with birch-pollen-associated allergy to hazelnuts [56], as has been shown more recently by others [57]. Therefore, it appears that other factors, such as the food matrix itself, as well as the inherent thermostability of a protein and the type of processing procedures employed, may be responsible for the apparent lability of Bet v 1 homologs in foods such as apple compared with celery root.

Another family of allergens that are inherently thermostable are the various members of the prolamin superfamily. With the exception of the prolamin seed storage proteins of cereals, the large number of intramolecular disulfide bonds present in these proteins play an important role in determining their thermostability. Both the 2S albumin allergens, such as Ber e 1 from Brazil nut and Ses i 1 from sesame seeds, have secondary structures that are almost unaltered by heating [58, 59], as well as the allergenic non-specific lipid transfer proteins from a variety of fruits such as apple [60]. However, despite such inherent thermostability, in

some instances the allergenicity of specific lipid transfer proteins is retained even after the extensive thermal treatments and fermentation involved in brewing and wine-making [61], as is the IgE binding capacity of wheat α-amylase inhibitors when a model cooking procedure involving preparation of a flour gel comparable to a porridge was used in a study of wheat allergy [62]. However, in the same study of wheat allergy, some patients lost their IgE binding capacity toward wheat-specific lipid transfer protein [62], while in a study of specific lipid transfer protein-mediated rice allergy, boiling abolished IgE binding [63].

In such complex food systems, there is an interplay between the stability of individual allergens, coupled with interactions with other components in the food matrix that could render proteins insoluble and hence no longer accessible and able to trigger a reaction. The ability of wheat prolamins to form disulfide bonds could alter the allergenic properties of other ingredients in baked goods, and it has been shown that the egg white allergen ovomucoid becomes disulfide-linked to the gluten proteins during baking, rendering it insoluble and hence reducing the allergenic activity of soluble extracts made from such baked goods [64]. Alternatively, this loss of IgE reactivity might be due to leaching of the allergen into the cooking water, as has been observed for another prolamin superfamily member, the peanut allergen Ara h 2 [65].

As well as physical changes induced in protein structure through denaturation and aggregation, processing may introduce the formation of complexes with other food components that may also alter protein stability and bioaccessibility. Thus, the plant polyphenol epigallocatechin has been shown to cause compaction of cows' milk caseins, with the casein molecules wrapping around the polyphenol, forming a complex held together by hydrophobic interactions [66]. Modification of peanut allergens Ara h 1 and Ara h 2 with phytic acid showed that this compound reduced both their solubility and their IgE reactivity, an effect mirrored by treatment of peanut butter with phytic acid [67].

13.4
Impact of Nanoscale Structures on Allergenic Potential of Foods

Our lack of knowledge about the impact that food processing and structure have on the allergenicity of foods makes it difficult to assess the potential impact that novel processes, including the use of nanoscale structures in foods, will have on allergenicity. However, as described above, the formation of protein aggregates and networks, complexes with lipids, and other food components, results in the formation of nanoscale structures, which we have been consuming probably ever since mankind began using heat to preserve and cook foods. There are no published data on the impact of nanotechnology in relation to food allergy, and studies in relation to the impact of fabricated nanoscale structures on allergy in general are in their infancy, particularly regarding their use in drug delivery. Thus, delivery of a deoxyribonucleic acid (DNA) vector expressing transforming growth factor beta (TGFβ) in chitosan nanoparticles via the gastrointestinal tract was able to

ameliorate the symptoms of food allergy in an animal model, using the egg allergen, ovalbumin, as a model food allergen [68]. Similar beneficial effects have been observed in using chitosan particles to deliver mite allergens for immunotherapy in the treatment of mite allergy [69] and with biodegradable poly(D,L-lactic-*co*-glycolic acid) nanospheres used to deliver Bet v 1 in immunotherapy [70].

Other studies have focused on adverse effects of inorganic nanoparticles, such as titanium dioxide, on allergic reactions to personal products such as cosmetics, using conditions such as atopic dermatitis, a symptom often associated with food allergies, especially in infants, as a model system. Using a dust mite model system in mice, titanium dioxide nanoparticles irrespective of size (15, 50 or 100 nm in size) were found to aggravate immunological markers of the atopic dermatitis-like skin lesions in this model system, including serum IgE [71]. In contrast, nanocrystalline silver had beneficial effects in reducing inflammation in a guinea pig model of contact dermatitis to a similar extent as topical steroids [72].

The impact of nanoparticles, especially those resulting from atmospheric pollution and found in for example, diesel exhaust, has also been studied in relation to allergic disease, and relates to their ability to have a pro-inflammatory effect on the respiratory epithelium. The concerns are that they might have adjuvant effects on allergic sensitization, and this is reflected in reports of studies undertaken in animal models using ultra-fine carbon particles, which increased inflammation by increasing oxidative stress [73–76]. In contrast to such adverse effects, there are indications that novel carbon structures, such as fullerenes, may have beneficial effects, reducing mediator release involved in elicitation of allergic reactions, including a model of anaphylaxis [77].

The efficacy of such nanoscale structures for delivery of therapeutics is explained in part by the observations that biodegradable nanoparticles, such as poly(D,L-lactic-*co*-glycolic acid), can be taken up by cellular models of the respiratory and gut epithelium [78], although the cell models do not include the mucus layer, an important biophysical barrier that particulates must traverse before contacting the underlying epithelium. However, there is evidence that combinations of dextran and chitosan nanoparticles 500 nm in size were muco-adhesive, and were effective at rendering insulin bioavailable through the oral route [79]. Such effectiveness at overcoming the gut barrier has clear benefits in terms of delivery of bioactive molecules via the oral route. However, there is almost nothing known about the potential impact on allergenicity, especially of nanoparticles that may be included in foods to enhance delivery of important health-promoting micronutrients [80–82].

13.5 Conclusions

Food allergy is an emerging problem, and while our documentation of the molecules responsible for triggering allergic reactions is extensive, the way in which these molecules are altered by food processing conditions and how food structures

may alter their presentation to the immune system is very incomplete. Of particular relevance to considering the potential impact of nanotechnology, undoubtedly one of the most important aspects is the use of nanoparticles to deliver therapeutic agents, such as those involved in immunotherapy. Utilization of nanoparticles for oral delivery of other important therapeutics, such as insulin, is showing promise, and it is likely, as therapies are developed for food allergy, that such technology may play an important role in providing the effective cure for food allergy that is currently lacking. Such a therapy would undoubtedly improve the quality of life for food-allergic consumers, which can be acute [83, 84].

The broader utilization of nanoparticles in foods will, as for other types of novel technology, need to undergo an allergenicity risk assessment [85], although there can be difficulties in undertaking such assessments for other types of novel process or novel foods, including genetically modified organisms, partly because of our lack of effective animal models for food allergy. Biologically derived nanoparticles are probably produced during the digestion of foods, and nanoscale structures have been described in conventionally processed foods for many years. Any nanoparticle-containing ingredients derived from allergenic food that it is mandatory to label will need to be declared, and in this way allergic consumers will be able to avoid their consumption. However, novel types of bionanoparticles and inorganic nanoparticles based on carbon or silver for example, may have unintended effects, but there are no clear agreed experimental approaches or frameworks to develop data on which to base an effective risk assessment. Further research is required to address these gaps in our knowledge and hence ensure that the considerable benefits that may arise from this new technology are realized while minimizing the risks of potentiating existing allergic conditions or introducing new ones.

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