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Review

Impact of food processing on the structural and allergenic properties of food allergens

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This article reviews recent studies that address one of the major unanswered questions in food allergy research: what attributes of food or food proteins contribute to or enhance food allergenicity?

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1 Introduction

Two of the major unanswered questions in food allergy research are (i) what makes one person, and not another, become allergic, and (ii) what are the attributes of some foods and food proteins that make them more allergenic than others. Seeking to answer, these questions are much more difficult than investigating the allergenic potency of inhalant or contact allergens since the proteins involved in sensitising or eliciting allergic reactions may have undergone extensive modification during food processing and be presented within complex structures within food. These physicochemical changes will alter the way in which they are broken down during digestion and may modify the form in which they are taken up across the gut mucosal barrier and presented to the immune system. Certainly, the structure of the food matrix can have a great impact on the elicitation of allergic reactions and fat-rich matrices may affect the kinetics of allergen release, potentiating the severity of allergic reactions [1]. However, because of its complex nature the impact of food processing and the food matrix on allergenicity of proteins has only recently become a subject of research. Such investigations are fraught with difficulties, not least the fact that food processing often renders food proteins insoluble in the simple salt solutions frequently employed in serological or clinical studies. As a consequence, our understanding of the impact of food processing on allergenicity is limited to the more soluble and extractable residues in foods and the allergenic potential of insoluble protein complexes is virtually unstudied despite representing the vast bulk of food proteins consumed.

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Abbreviations: β-Lg, β-lactoglobulin; **LPT,** lipid transfer protein

2 Proteins in fabricated food structures

Much of our understanding of the effects of food processing on food protein structure and the fabrication of different types of food structure has been gained from studying model food, notably the whey proteins from cow's milk [2]. Others include egg proteins and the 11S and 7S seed storage globulins, widely distributed abundant proteins found in many edible nuts and seeds and the major components in ingredients such as soya isolates. In addition to their role as a macronutrient, proteins play an important role in forming the structure of processed foods such as foams (for example whipped egg white in meringue) and gel networks (such as the white in boiled egg or protein gels found in cooked meat products). They can also play an important role in emulsifying oil in sauces such as mayonnaise, where they form an interconnected adsorbed layer coating the oil droplets, and together with other food ingredients, such as sugar, form glassy states in low water foods such as biscuits and pasta. The partially denatured and modified conformations they adopt in such processed foods are similar to those found in processed natural food matrices, where fruits, vegetables, nuts or seeds maybe wet-processed (*e.g.* boiled) or dry-heated (*e.g.* roasted or fried). In this case the interactions are more complex because of the ultrastructure of the natural food matrix where, e. g., plant seed proteins maybe compartmentalised in protein bodies and may breakdown during cooking to varying extents.

The types of modification that the food proteins may undergo during processing include protein unfolding and aggregation, in addition to chemical modifications (Fig. 1).

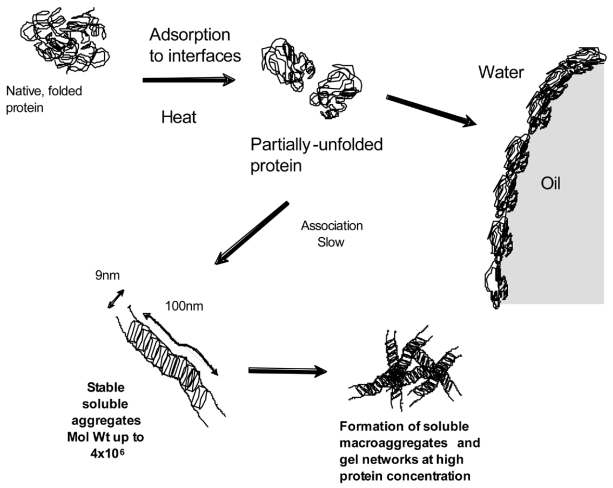

Figure 1. Ways in which proteins can unfold and denature following food processing.

Thus, the tertiary structure, and to some extent the secondary structure of native proteins will be altered as a consequence of either heating or through adsorption to interfaces, including air–water interfaces found in food foams like meringue, oil–water interfaces such as those found in oil-in-water emulsions in mayonnaise and salad dressings. Not all the structural elements of the protein are lost, with new NMR evidence from studies on the model whey protein α-lactalbumin indicating that proteins form partially folded forms following heating [3]. Such unfolding is frequently accompanied by changes in surface hydrophobicity, with residues normally buried in a native protein, becoming surface exposed. As a consequence, whilst the proteins may remain monomeric, and hence soluble, in dilute solution at the much higher protein concentrations found in foods the proteins will form large macromolecular aggregates and gel networks. An example of such a gel network is the formation of cold-setting gelatine gel.

Such alterations in protein folding have the potential to affect stability to digestion, and hence the form in which allergens are presented to the immune system with regards both sensitisation and elicitation. The extent to which proteins are affected by processing conditions is process dependent, since protein denaturation requires the presence of water, proteins becoming more thermostable in low-water systems [4]. Combinations of time and temperature, the presence of other ingredients, such as fats and sugars, also affect the patterns and kinetics of food proteins denaturation and aggregation.

One of the most widely studied food proteins is the globular protein β-lactoglobulin (β-Lg), one of the main components of the whey fraction of cow's milk and found in widely used food ingredients such as whey protein isolates and concentrates. β-Lg is a 18400 Da retinol-binding protein with a β-barrel structure characteristic of the lipocalin superfamily which is stabilised by two intra-molecular disulphide bonds (Cys^{106}–Cys^{119}, and Cys^{66}–Cys^{160}) together with a single free cysteine residue (Cys^{121}) [5]. It is thought that on heating β-Lg first dissociates into monomers which then partially unfold before associating into thread-like aggregates around 50 nm in diameter [6, 7] which at high protein concentrations will form string-like aggregates which associate with form gel networks [8]. Unfolding reveals the buried Cys^{121} which is then able to catalyse disulphide interchange to form a non-native monomer in which Cys^{119} is exposed [9] allowing the protein to become linked to other food proteins, such as caseins [10]. This ability to form intermolecular disulphide cross-links affects the mechanical stability of the gels, making them stronger [11].

Commonly used plant-derived ingredients, such as soya isolates, often comprise 11S and 7S seed storage globulins. In common with other members of the cupin superfamily these proteins are relatively thermostable. 7S globulins have their major thermal transition at around 70–75°C, whilst 11S globulins unfold at temperatures above 94°C, as determined by differential scanning calorimetry. However, even on heating to such temperatures they only appear to partially unfold, undergoing only minor conformational changes suggesting that the β-barrel motif characteristic of these proteins is a highly stable structure [12]. Like β-Lg, these unfolded forms also rapidly form aggregates on heating, which at high protein concentrations (2.5–10% by weight) assemble into string like aggregates similar to those formed by other food proteins, such as whey proteins which also associated to form heat-set gels [13].

Protein unfolding and aggregation may also be induced by mixing and shearing that occurs during other food processing, as well as adsorption processes involved in the stabilisation of air–water and oil–water interfaces found in food foams and emulsions. Thus, it appears that adsorbed proteins also undergo only limited unfolding at interfaces [14, 15] but do form aggregated structures at the interface akin to a semi-two-dimensional gel network [16], which may also be interconnected by disulphide bonds [17].

All these processes may impact on allergen structure in ways which are often pre-determined by the structure of the protein (Table 1) although the type of process and the addition of other ingredients (*e.g.* sugars) or pH may alter the effects.

3 Effect of processing on allergen structure and properties

3.1 Processing-labile proteins

The best example of a group of allergens which are frequently labile to common food processing technologies is the cross-reactive Bet v 1 family of plant food allergens. It appears that the cross-reactive IgE responses involved in the pollen-fruit/vegetable cross-reactivity syndrome are pri-

Table 1. Impact of food processing on different types of food allergens

Type of food allergen	Effect of thermal processing
Bet v 1 homologues from fruits such as Mal d 1, Pru av 1	Processing-labile allergens: Protein unfolding, modification by Maillard adducts in sugar-rich foods, modification by polyphenols
Cupins allergens, such as Ara h 1 from peanut. Lipocalins such as β-Lg and α-lactalbumin from milk	Partially denatured allergens: Partial unfolding of proteins, aggregation to form networks as emulsifiers around lipid or gelled systems. Maillard modifications may potentiate allergenicity.
Prolamin superfamily members belonging to the ns LTP, and 2S albumin sub-families such as Mal d 3; tropomyosins and parvalbumins.	Allergens able to refold: Proteins unfold to a limited extent during heating but can re-fold on cooling. Maillard modification may potentiate allergenicity
Caseins, seed storage prolamins of wheat (gluten), ovomucoid	Mobile rheomorphic proteins: Proteins do not adopt a rigid conformation but are very mobile and consequently do not denature following thermal treatment.

marily directed towards conformation epitopes. For example, two of the cross-reactive IgE epitopes on Bet v 1 homologues of cherry, Pru av 1, appear to be conformational in nature [18, 19]. The Bet v 1 scaffold contains no intramolecular disulphide bonds and appears to be less stable than many other structural types of food allergen, unfolding on heating with a consequential loss of conformational epitopes. Thus, as a general rule both the IgE-reactivity and ability of Bet v 1 homologues to trigger a reaction in sensitised individuals is reduced by food processing, but may depend on the form that processing takes. Whilst cooking of fruits such as cherry, which has a high water content, reduces their allergenicity in birch-pollen allergic individuals, the application of dry-heat, as in roasting of hazelnuts whilst reducing their allergenicity for many did not abolish it for all patients [20]. This is maybe because the application of dry heat does not denature Bet v 1 homologue of hazelnuts, Cor a 1, as effectively as wet processing would.

It is also emerging that the thermostability of all Bet v 1 homologues is not equivalent, the homologues from celery (Api g 10 and soya (Gly m 4) being more thermostable than Mal d 1 from apple. Thus, Ma l d 1 unfolds on heating to 90°C, and does not significantly re-fold on cooling to 20°C, whilst Api g 1 does not begin to unfold until heated above 80°C and regained more of its native structure on cooling (Mills E. N., unpublished observations). Its greater thermostability may contribute to the ability of Api g 1 to retain more of its allergenicity in cooked celery [21] and may underlie the observation that Gly m 4 can trigger allergic reactions even in a highly processed soya-based food supplement [22, 23].

3.2 Processing-stable allergens

An allergen family, which possess an inherently stable protein scaffold, is the prolamin superfamily. These proteins are characterised by a conserved pattern of cysteine residues, with either six or eight such residues forming either three or four intra-chain disulphide bonds which constrain the folded structure of the proteins. With the exception of the prolamin seed storage proteins of cereals, it is these disulphide bonds which play an important role in determining the stability of these proteins to a variety of chemical and physical denaturants, including low pH, chaotropes, high temperature and pressure.

Allergens belonging to the prolamin superfamily which exhibit such stability include the 2S albumin allergens such as the Brazil nut allergen Ber e 1 [24, 25], sesame allergen Ses i 1 [26], together with the non-specific lipid transfer protein (ns LTP) allergens from apple Mal d 3 and grape, Vit v 1 [27, 28]. As they show some limited unfolding at 90°C, the nsLTPs may be intrinsically slightly less stable than the 2S albumins, probably as a consequence of the lipid-binding tunnel, although the protein does completely refold on cooling [25, 28]. Ligand binding certainly increases the thermostability of ns LTPs [29]. Severe heat-treatment, such as heating to 100°C for 2 h, has been found to significantly reduce the IgE reactivity of the nsLTP allergen from apple, Mal d 3, possibly as a result of one of the disulphides being oxidised [28].

The thermostability of the 2S albumin and ns LTP allergens explains the observation that these proteins retain their allergenic properties in processed foods. Thus, even severe thermal processing such as sterilisation at 121°C for 30 min does not remove the allergenicity of peach juice [30] nor is it destroyed following boiling or baking (180°C) of apple peel [31]. Similarly, the allergenicity of maize was retained even after cooking polenta at 100°C [32]. Furthermore, the IgE reactivity of ns LTPs can even survive fermentation during production of wine [33] or beer [34], despite the fact that the ns LTP in beer is essentially an unfolded highly modified protein [35]. These observations may also reflect the fact that many individuals reacting to ns LTPs suffer severe fruit allergy which may influence the way in which their IgE is able to recognise both folded and extensively denatured forms of the protein.

In contrast, in cow's milk allergic individuals, thermal treatments that would cause β-Lg (variants A or B) to denature reduced, but did not abolish, IgE binding [36]. This may reflect the fact that, depending on the severity of the thermal treatment, a proportion of the protein molecules may remain in the native state, or that the unfolded forms of β-Lg are still able to bind IgE, albeit with a much reduced capacity. Such subtle differences can only be distinguished by using well-characterised denatured proteins. They are worth defining since if serum IgE from cows' milk allergic individuals can still bind to denatured protein, no matter how much the sample is heated, its IgE-binding capacity will not be further reduced until the heating is sufficiently intense to induce chemical modification of amino acids and hydrolysis of the polypeptide chain through β-elimination reactions.

In addition to proteins which possess well-defined three-dimensional structures, there are regions of some proteins which instead comprise ill-defined, disordered, mobile structures [37]. Such structures are dynamic and comprise an ensemble of interchanging conformations which do not show the same co-operative transition on heating from a folded to an unfolded or partially folded structure observed in more ordered proteins. Two notable examples of food allergens that fall into this category of proteins are caseins from milk and the prolamin seed storage proteins from wheat which form gluten. There are four structurally distinct components found in the casein fraction of cow's milk, α_{s1}, α_{s2}, β and κ-casein which assemble into casein micelles in milk. The absence of a co-operative transition as determined by differential scanning calorimetry and their lack of secondary structure has led to them being termed rheomorphic, rheo meaning to flow, and morphe meaning shape, a property shared by seed storage prolamins [38, 39]. As a consequence of their dynamic nature, these proteins possess many linear, and hence potentially thermo-stable, IgE epitopes and is probably the reason why the IgE binding capacity of caseins and wheat prolamins is largely unaltered by thermal processing [40, 41].

Stability to processing is also a function of the process itself. Thus, many seed storage globulins will denature to some extent and form aggregated networks following heating in a highly hydrated state [42] and those from many legumes, such as soybean and lentils, are consumed in a boiled form. However, peanuts and many tree nuts are often consumed after being thermally processed at low water levels, in a roasted or fried form. Studies on the seed storage globulin allergen from peanuts are difficult to undertake as much of the peanut protein becomes insoluble. Nevertheless, they indicate that the protein only becomes unfolded on roasting peanuts to 140°C for 15 min [43]. IgE binding to wet or dry-heat denatured protein was essentially unchanged leading to the suggestion that either Ara h 1 does not contain any conformational epitopes, or that they are restricted to the thermostable regions of the structure.

4 Processing-induced chemical modification of allergens

One of the main chemical modifications occurring in foods during processing is the reaction between free amino groups on proteins and the aldehyde or ketone groups of sugars known as Maillard's reaction. Such non-enzymatic glycation reactions can subsequently undergo a range of further rearrangements giving rise to a range of structurally diverse compounds known as Amadori products or advanced glycation end products (AGE_S). The formation of these adducts is affected by types of non-reducing sugars, pH, water activity, temperature and is important as the volatile compounds contribute to the aromas and flavours associated with many cooked foods. The rearrangement products can result in the cross-linking of food proteins, and studies on the IgE-reactivity of bread in a panel of wheat allergic individuals suggested that some of the IgE-reactive protein was extensively cross-linked by Maillard adducts [40].

Maillard modification may affect the allergenicity of food proteins. Thus, Maillard modifications can cross-link the peanut allergens Ara h 1 and Ara h 2 to form high Mr aggregates which bind IgE more effectively than unmodified allergens, and are also more resistant to gastric digestion [44], IgE binding to modified proteins being partially inhibited by antibodies to AGE adducts [45]. IgE from human peanut allergic sera binds peanut allergens Ara h 1, 2 and 3 more strongly from roasted compared with boiled or fried peanuts [46], indicating that certain types of thermal processing can introduce additional IgE binding sites. However, these observations may be complicated by the fact that peanut allergens leach out of peanuts during boiling, lowering the residual allergen content in the boiled nuts [47]. Maillard modification has also been found to increase the IgE-binding capacity of the allergenic shellfish tropomyosin [48]. Individuals may have become sensitised to glycated tropomyosin itself, through consumption of the dried fish products frequently used, especially in oriental cuisine.

However, glycation of fruit allergens does not appear to increase their allergenicity in the same way as the peanut or shellfish allergens. Thus, glycation of Pru av 1, the allergenic Bet v 1 homologue of cherry, with sugars, such as fructose and ribose, significantly reduced the proteins IgE reactivity, whilst modification with carbonyl compounds formed during carbohydrate breakdown, such as glyoxal and glycoaldehyde, almost completely abolished IgE binding [49]. Maillard modification of the nsLTP allergen from apple, Mal d 3, protected the IgE-binding capacity of the protein following harsh thermal treatment [28].

Other types of processing-induced modification which may affect allergenicity include interactions with oxidised lipids [50] and enzymatic modification with polyphenols catalysed by the polyphenol oxidase. Modification with typical plant polyphenols widely found in plants including

fresh fruits, epichatechin and caffeic acid, reduced the IgE-binding capacity of Pru av 1. However, the extent to which it was reduced was highly dependent on the polyphenol involved, with quercetin and quercetinglycoside, rutin, having a lesser effect [49]. Such enzymatic modifications may be responsible for the highly labile nature of many fruit Bet v 1 type allergens.

5 Conclusions

Understanding the impact of food processing and food structure on allergenic potential is central to managing allergen risks in the food chain. However, our current knowledge of the impact of food processing on allergen structure indicates that there are no clear rules regarding how different allergens respond to food processing. Thus for some, such as the Bet v 1 family of allergens found in fruits and vegetables, their allergenicity is destroyed by cooking, but for many others it is unaltered or may even be increased. What is emerging is that the impact that is related to the type of sensitivity (is it related to an inhalant allergy or does it involve oral sensitisation by ingestion of food), the structural attributes and inherent stability of the allergen scaffold, the type of processing the allergen is subjected to and the food structure in which the allergen becomes embedded.

Investigations into the impact of processing on allergen structure and allergenic potential are still in their infancy, but two areas that remain neglected are the impact that food processing procedures have on sensitisation potential and the way in which it may alter thresholds for elicitation of allergic reactions in sensitised individuals. A knowledge of how processing or food structure may alter threshold doses of allergens able to elicit an allergic reaction is highly relevant for managing allergens in a factory environment, particularly in relation to cross-contact allergens. In such instances allergens may find their way into foods otherwise free from them, through use of parallel or common processing lines for manufacture of allergen containing or allergen free foods. It may be that certain types of food structure, for example fat-continuous versus aqueous continuous matrices, may raise or lower the threshold doses for important allergens such as those from peanut. Such information is necessary to inform hazard control procedures as it may affect the extent to which it is necessary to clean-down common processing lines or the rigour with which ingredients are segregated within a factory environment. It is also acknowledged that food processing can affect the responsiveness of the immunoassay methods used to monitor allergens in foods and equipment clean-down [51]. A better understanding of how processing affects allergen structure, and hence allergen screening assays, would help support interpretation of immunoassay results especially when used to monitor highly processed ingredients.

Another aspect of allergen management in foods is the allergenic risk assessment process which forms part of the regulatory framework of Novel Foods and Processes. Our lack of understanding of the impact of conventional food processing procedures also makes the assessment of novel processes, such as high pressure, or novel thermal processing procedures, less certain than would otherwise be the case. The complexity of food processing makes managing allergens in foods difficult but demonstrates the importance of understanding its impact at the molecular level if risk assessors are to move towards knowledge-based ways of managing allergen risks.

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