

Impact of (bio)chemical and physical procedures on food allergen stability

A variety of methods have been used to attempt to decrease the allergenicity of food products, with highly variable success. Limited knowledge of allergen and epitope structures and the factors governing their stability may explain this, and the ensuing need for an empirical approach. A combined effort from a processing and genomics-based approach may open up new ways to improve the quality of foods from an allergenic perspective. Based on structural knowledge, a suitable approach can be chosen that is, for instance, heat stable but protease sensitive; protease resistant but not heat stable; easy to extract; easy to mask because the epitope is exactly on a crosslink site; a molecular probe design that allows screening of germplasm collections.

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What is food allergy?

Food allergy is a specific adverse reaction to the consumption of a food or a food ingredient, immunologically mediated through an IgE response (1). Food allergy manifests itself through a physiological response to the ingested food. The symptoms vary from respiratory, cutaneous, and gastrointestinal to systemic (2). The reaction requires prior sensitization, and occurs usually in only a small portion of the population and within minutes after consumption of the product to which allergy exists (3). Most allergies are caused by only a few foods (1). Regional differences may occur with respect to the occurrence of food allergy: in South Europe, for instance, peach allergy is more predominant than in other European regions (4). In this paper, we focus on allergens of plant origin.

Methods to assess allergenicity of food products

A variety of rapid assay methods are in use as analytical tools in research that is directed at the molecular structure, integrity and physiological activity of food allergens and their active structural elements, the epitopes (see below).

Jankiewicz et al. reported the use of an *in vitro* mediator-release assay based on rat basophil leukemia cells (5). Enzyme-linked immunosorbent assays (ELISA), radioallergosorbent tests (RAST) or radio-immunoassays (RIA), making use of IgE-containing sera from, for instance, sensitized patients, are accurate

and quantitative assay systems (7, 8). A powerful semiquantitative tool that allows insight into the integrity of allergens and their epitopes is Western blotting, the combination of protein electrophoresis and immunochemical detection (8, 9).

These assays are based on *in vitro* reactions, with limited relevance for clinical practice. Proof of improved safety of potentially less allergenic foods must be generated through human test studies, for instance via double-blind placebo-controlled food challenge (DBPCFC)-tests.

Structural basis of allergenicity

Allergic reactions to foods are in fact allergic reactions to food components – usually proteins with a molecular mass of 10–70 kDa, which may occur as multimers such as the peanut allergen Ara h1 (1, 3, 7). On such allergens, smaller structural elements, the so-called epitopes, can be found, which provoke the IgE-mediated allergic response (1, 2, 6, 8). An allergen of carbohydrate nature (possibly a hapten) has also been described from celery (5). Foods may contain multiple allergens (1, 2, 6).

T-cell epitopes are generally small, 12–18-residue, peptide fragments (1, 6). The structure of so-called conformational epitopes is also characterized by the tertiary structure of a protein, and their integrity therefore depends on the thermodynamics that determines tertiary protein structure (1).

Food allergens and their epitopes may be quite resistant and survive the effects of food processing and digestion (10).

Cross-reactivity between allergens and epitopes from various food products may occur, as described for allergens from crustacea (11) and legumes (10, 12).

Methods to reduce allergenicity of processed foods

Allergens and their epitopes may be quite resistant to digestive enzymes and to various forms of processing (1, 2). However, an increasing number of reports describing successful attempts to reduce allergenicity are appearing, and increased knowledge of the structural basis of allergenicity and of the factors that determine epitope integrity will lead to novel and more specific approaches to decrease allergenicity. Below, a variety of methods that have been tested to decrease allergenicity of foods will be reviewed.

Removal of epitopes

Recently, a method to decrease the allergenicity of peach juice and nectar was presented by Brenna et al. (9). As shown by SDS- and immunoblotting, the effective treatments were *chemical lye peeling* (a dip in 10% NaOH at 60 °C for 90 s) or *ultrafiltration* of the juice. Edible oils (such as from peanut, soybean, sunflower) can be extracted with solvents to remove all traces of protein (13-15), making the resultant oils safe for consumption by sensitive individuals.

Destruction of epitopes

Physical processing. As a rule, the allergenicity of fresh fruits can be reduced rather easily by heat processing, allowing the production of hypoallergenic jams and juices (16). Apple slices already lose allergenicity by storage at room temperature. Whether this reflects physical instability of the epitopes or the effect of, for instance, a protease is not quite clear (17, 18).

Heat treatment has been very successful in decreasing celery allergenicity, as shown by immunoblotting (5). In contrast, non-thermal processing only mildly reduces allergenicity. Both microwave cooking and conventional cooking have turned out to be effective. Other physical processing methods, such as ultra high pressure treatment (600 MPa, 20 °C), high voltage treatment (10 kV at 50 min⁻¹) or γ -irradiation (10 kGy), however, did not result in destruction of the Pru p 1 and the profilin epitope structure. The heat stability was found to be: carbohydrate epitopes > profilin > Pru p 1 (5).

Heat treatment is not always successful: at 121 °C for 30 min it was insufficient to remove or destroy the Pru p 1 epitope during peach processing (9). This somewhat surprising result (considering the relative vulnerability

of Pru p 1 analogues such as Mal d 1 and Api g 1) of fairly harsh heat treatment suggests that the Pru p 1 epitope is rather stable.

The peanut Ara h 1 epitope is also quite heat resistant (19).

Proteolytic modification of epitopes. An example, although not concerning an allergen, that proves it is possible to specifically destroy the structure of an epitope by protease treatment, without destroying the function of a protein, can be found in the proteolytic activation of the browning enzymes polyphenol oxidases (PPOs). PPO from mushrooms is initially formed as a 67 kDa latent isoform. The enzyme can be proteolytically activated by a variety of proteases (20). Although activation with both trypsin and subtilisin leads to a fully active enzyme, the immunological properties of the activated enzymes differ strongly (Fig. 1). Trypsin treatment converts this 67 kDa form into 58 and 43 kDa forms. Subtilisin treatment destroys epitope structure, after which the enzyme can no longer be detected in immunoblots (Fig. 1). These observations suggest that it is possible to dramatically alter an enzyme's epitope structure without altering too much of its catalytic properties.

Proteolytic processing has been used to reduce soy allergenicity. Of the tested proteases, two out of eight were effective, also suggesting that the combination of protease and epitope is important (21).

Another clear example of the potential of the application of a specific protease based on knowledge of epitope structure is presented by the decreased allergenicity of wheat flour gluten following treatment with the protease bromelain (22, 23). The wheat glutenin IgE-binding epitope has the structure Gln-Gln-Gln-Pro-Pro (23), which makes it susceptible to cleavage by bromelain, a peptide that cleaves near Pro-residues. The IgE-ELISA used in the experiments suggests that the treatment may well have resulted in hypoallergenic flour. A similar result has been achieved with the (non-food grade) enzyme collagenase from *Clostridium*. This suggests that epitopes with the structure Gln-X-Y-Pro-Pro, where X and Y represent any amino acid, may be sensitive to proteolytic modification. An undesirable side effect of this type of proteolytic treatment may be that the proteases will not restrict themselves to allergen epitopes, but will change the structure of the bulk of the available protein, thereby affecting the baking quality of the flour. Indeed, many proteins were degraded; yet Tanabe et al. produced a kind of muffin with the hypoallergenic flour (22).

Another example is the production of hypoallergenic rice by a two-stage enzymatic process, making use of actinase. Treated rice grains retained acceptable textural properties (24, 25).

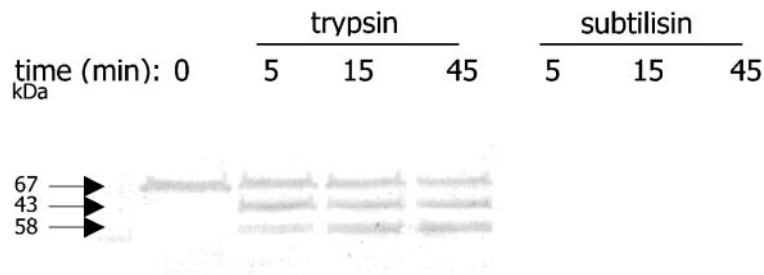


Figure 1. Destruction of epitopes on latent mushroom polyphenol oxidase during proteolytic activation. Protein detection by SDS-and Western blotting as described in (17).

Proteolytic treatment was insufficient to remove or destroy the Pru p1 epitope from peach (9). This lack of success for the proteolytic treatment is perhaps related to the specificity of the tested proteases (*Rhizopus* and *Aspergillus* proteases) or to inaccessibility of the epitopes for the proteases in the rather intact peach tissue.

Considering the vast number of known enzymes able to hydrolyze carbohydrates, there might also be a possibility to use enzymes to decrease the allergenicity of celery (5).

The given examples demonstrate the outlook for lowering the allergenicity of foods proteolytically, provided a sufficiently specific combination of protease and epitope can be identified. Apart from the correct combination of epitope and protease, factors such as accessibility of epitopes are very likely to determine potential success.

Masking of epitopes

Various reports describe the successful reduction of allergenicity of foods, for instance of wheat proteins, by using crosslinking enzymes, such as transglutaminase (7, 26). Transglutaminase treatment results in the appearance of high molecular weight proteinaceous components, which indicates that, as for protease treatment, other processing properties may be affected (7).

The most likely explanation for the decreased allergenicity of crosslinked protein preparations is “masking” of the epitopes, which may become unavailable to the immune system. Enzymatic crosslinking, as with transglutaminase, is preferred to chemical crosslinking, which may achieve similar effects.

Genetic modification to design foods with decreased allergenicity. Genetic modification does hold promise for quality improvement of foods, also from an allergenicity perspective. Although genetic modification is not a direct approach to reduce allergenicity as suggested in the title of this review,

two examples of attempts with impact on allergenicity, again with variable success, are given here.

Genetic modification was successfully applied to reduce allergenicity of rice, by knocking out an α -amylase (27). Less successful, however, was the attempt to increase the S-content of soy by introducing a gene from Brazil nut. A strong allergic response to the introduced protein was observed (1).

The recent rapid development of genomics, and its relation to proteomics, will lead to the introduction of powerful techniques that will also be applicable to reduce allergenicity. Genomics will, for instance, lead to effective screening systems to screen vast germplasm collections for modified or absent epitope structure. More will also become known of epitope structures, creating chances to induce mutations, some of which will result in reduced allergenicity. Knowledge from the processing research domain on the (bio)chemical and physical stability of epitopes will further contribute to the development of an effective strategy.

Conclusions

A variety of methods have been attempted to decrease the allergenicity of food products. The most general conclusion that can be drawn is that the success of the methods is highly variable. As a consequence, empiric approaches have been used. Likely, limited knowledge of allergen and epitope structures and the factors governing their stability explains the variable success and the need for an empirical approach. Growing insight, however, also through the development of bioinformatics, will increase our understanding of critical factors for reduction of allergenicity of food products.

Knowledge of the epitope structure, cross-reactivities and the factors that determine chemical and physical stability may lead to the design of both generic and specific approaches to decrease food allergenicity.

In conclusion, a combined effort from a processing and a genomics-based approach may open up novel

ways to improve the quality of foods, from an allergenic perspective as well. Based on structural knowledge, a suitable approach can be chosen that is, for instance

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Protease resistant, but not heat stable

Easy to extract

Easy to mask because the epitope is exactly on a crosslink site

A molecular probe design that allows screening of germplasm collections.

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