Rare, medium, or well done? The effect of heating and food matrix on food protein allergenicity
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Introduction
It has been long recognized that the allergenicity of food proteins can be altered by food processing [1–6]. Thermal processing may involve dry or moist heat, whereas nonthermal processing includes germination, fermentation, proteolysis, ultrafiltration, storage, mechanical and enzymatic issue disintegration, pulping, peeling, mashing, and pasteurization treatment [7,8]. The Maillard reaction, enzymatic browning or roasting, and all dry heat processes are most capable of modifying allergenicity of food proteins. Food processing can decrease protein allergenicity in several ways including destruction of predominantly conformational epitopes, with limited effect on sequential epitopes, and chemical reactions between proteins and fat and sugars in the food matrix that account for limited availability of protein to the immune system. Food processing might potentially increase protein allergenicity by formation of neoepitopes and by the effect of food matrix leading to decreased protein digestibility in the stomach and preservation of allergenic epitopes for interactions with the immune system in the intestine (Table 1). In general, the so-called classic, type 1 or complete food allergens (e.g. in cow’s milk, egg white, peanut, and soybean) that have the capacity to induce IgE sensitization via the mucosa of the gastrointestinal tract are heat-stable and acid-stable, water-soluble glycoproteins ranging in size from 10 to 70 kDa. The class 1 food allergens (e.g. ovomucoid, Gal d 1 in egg white, and Ara h 2 in peanut) are less readily affected by food processing, although recent findings underscore the importance of conformational epitope modification in cow’s milk and egg allergy. In contrast, type 2 (incomplete) food allergens are postulated to lack the capacity to induce IgE sensitization via the gastrointestinal tract exposures due to their susceptibility to thermal processing and gastric digestion. These proteins are believed to elicit symptoms only after primary sensitization with cross-reactive inhalant allergens and are referred to as ‘nonsensitizing elicitors’. The classic examples are birch tree pollen allergen Bet v 1 cross-reactive proteins in apple (Mal d 1) and carrot (Dau c) that...
in the uncooked form cause immediate oral symptoms but following heating are readily tolerated [5].

**IgE-binding epitopes: conformational versus sequential epitopes**

IgE antibodies produced by B cells may be directed at sequential epitopes comprising sequential amino acids, or conformational epitopes comprising amino acid residues from different regions of the allergen brought together by folding of the protein (Fig. 1). As food allergens are subjected to extensive chemical and proteolytic digestion prior to absorption and uptake by the cells of gut-associated lymphoid tissue, it has been assumed that in class 1 food allergy, immune responses are directed predominantly against sequential epitopes [9]. However, the growing body of evidence regarding allergy to beef, cow’s milk, egg white, and fish supports the importance of conformational epitopes in patients with class 1 food allergy (two, 10–12). Analysis of IgE-binding epitopes with the use of SPOTs membrane technology revealed that cow’s milk and egg-allergic patients who lacked IgE antibodies against certain sequential epitopes of the major allergens were more likely to achieve tolerance to these foods than those whose IgE antibodies were directed against those epitopes [10]. Cooke and Sampson [11] evaluated IgE binding to linearized versus native ovomucoid and reported that pooled sera from egg-allergic patients showed no significant differences in native ovomucoid binding, although sera from several individual patients with transient egg allergy showed a marked reduction in IgE binding to linearized ovomucoid. Jarvinen et al. [12] found that IgE antibodies of children with persistent egg allergy recognized more linear ovomucoid epitopes than children with transient egg allergy, suggesting that different phenotypes exist among egg-allergic children. These studies suggested that ovomucoid conformational epitopes might be important in some egg-allergic patients.

**Effect of heating on class 1 and class 2 food allergens**

High temperature reduces allergenicity, presumably by altering the conformation of heat-labile proteins that results in loss of conformational epitopes (Fig. 1). This paradigm has been generally accepted for the pollen—food allergy syndrome to Bet v 1 cross-reactive proteins in Rosaceae fruit (e.g., apple, peach, and cherry). However, Bet v 1 cross-reactive protein in soybean, Gly m 4 retains
allergenicity in heat-processed foods, suggesting that thermostability is highly variable and food-specific, even for the food allergens from the same protein family [7**].

Each food is a mixture of allergenic proteins that differ in their physicochemical properties, stability to heat and digestion, and the potential to induce IgE sensitization and IgE-mediated hypersensitivity reactions. For example, plant foods contain heat-stable proteins such as lipid-transfer proteins that are recognized as a cause of systemic reactions and primary sensitizers, independent of exposure to pollen [5,7**]. In cow’s milk, the caseins and serum albumin have higher heat stability than the whey proteins, α-lactalbumin, β-lactoglobulin, and lactoferrin. Casein bands were preserved in the SDS-PAGE gel even after 120 min of boiling at 100°C. Serum albumin band became progressively weaker after 10 min of boiling but was still visible at 120 min. In contrast, α-lactalbumin band disappeared after 30 min, β-lactoglobulin disappeared after 15 min, and lactoferrin disappeared after 10 min of boiling [13]. In egg white, ovomucoid is the dominant allergen. Although ovalbumin is the most abundant protein found in egg white, it is sensitive to thermal denaturation with resultant decrease in allergenicity. In contrast, ovomucoid is heat resistant and remains soluble after extensive heating; purified ovomucoid heated for 1 h at 100°C retained its antibody-binding activity.

**Matrix effect**
Protein interactions with other ingredients such as other proteins, fats and sugars in processed foods are also important, in general resulting in decreased availability of protein for interaction with the immune system. Heating of β-lactoglobulin results in the formation of intermolecular disulfide bonds and subsequent binding to other food proteins, making β-lactoglobulin less allergenic [7**]. A recent study by Kato et al. [14] demonstrated a marked decrease in the solubility of ovomucoid when egg white was mixed with wheat flour and wheat gluten and then heated at 180°C for 10 min, mimicking the process of bread making. Immunoblotting suggested that ovomucoid polymerizes and forms high-molecular weight complexes with gluten leading to aggregation and insolubilization of ovomucoid.

**Can heating enhance allergenicity?**
High temperature may enhance allergenicity of peanut and shrimp as a result of glycation, the Maillard reaction between free amino acids, and aldehyde or ketone groups of sugars (Table 1). The Maillard reaction induces the
different effects on food allergens, even those contained in the same complex food. Structural homology does not reliably predict the effect of processing on allergenicity, and individual foods have to be tested. Interactions with other proteins, fat, and carbohydrates in the food matrix are complex and poorly understood. Better characterization of these aspects of food allergy is critical for elucidation of food protein interactions with the gut-associated lymphoid tissue, the ability to induce IgE sensitization, the potential to trigger hypersensitivity reactions, and different clinical phenotypes of food allergy with regard to severity and persistence.

**References and recommended reading**

Papers of particular interest, published within the annual period of review, have been highlighted as:

* of special interest
** of outstanding interest