

## EAACI/DSA Symposium Reviews

# How do we develop hypoallergenic foods, and is there a need for them?

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Food-allergic reactions may be life-threatening, and advising the severely allergic patient to avoid the offending food does not always guarantee safety. In fact, there have been reports of food-induced anaphylactic shock in subjects who had inadvertently eaten a food to which they were exquisitely allergic (1, 2). In most cases, the offending food was found to be a minor ingredient or contaminant in other foods.

What can be done to prevent severe food-induced anaphylaxis? First, labels could be more comprehensive on the content of prepackaged foods. Second, the severely allergic subject could choose foods from a list of “safe” foods in much the same way as the celiac patient does from a list of gluten-free foods. Third, food technology could be employed to develop hypoallergenic foods.

We will examine each of these three possibilities. Let us examine first the issue of food labels. Obviously, the “ideal” label should provide the consumer with a list of all the components of a food product, including substances found in trace amounts as contaminants or as additives. In fact, in 1995, the US Food and Drug Administration (FDA) “new food label” required the reporting of all the ingredients of standardized foods, as well as FDA-certified color additives and the source of protein hydrolysates (3). There are two narrow exemptions from this rule: first, spices, flavorings,

and colorings may be declared collectively; second, incidental additives present in a food at insignificant levels need not be declared. Moreover, the FDA is considering the fact that no quantity of a notoriously allergenic food can be considered insignificant.

Other countries have basically adhered to the Codex General Labeling Standard of the Codex Alimentarius\*. The rule of thumb for declaring ingredients by the Codex General standard on labeling, as contained in section 4.2.1.2, is that there shall be a complete and detailed declaration. However, less specific declaration of ingredients is permissible in certain cases, as in the so-called 25% rule and the use of class names. In fact, section 4.2.1.3 of the General Standards states that a compound ingredient that is not considered an additive and for which a name has been established in a Codex standard or in national legislation need not be mentioned on the label if it makes up less than 25% of the total food (4). In other words, the 25% rule establishes that an ingredient found in small quantities in a food must be declared, whereas the same ingredient found in large quantities as a

\*The Codex Alimentarius is a code of food standards for all nations, developed by an international commission established 1962 as a joint Food and Agriculture Organization (FAO) and World Health Organization (WHO) program to guide the world's growing food industry and to protect the health of consumers.

component need not be declared. Although the rule should be applied only to those component ingredients listed in the Codex standard or in national legislation, the rule is applied in practice with much leniency. It is clear that such permissive labeling represents a grave risk for exquisitely sensitized subjects.

At present, the Codex Committee on Food Labeling is considering the possibility of reducing the amount of a substance that can be excluded from labeling to 5% and of declaring some ingredients which can cause hypersensitivity (5). Moreover, a draft proposal for a European Parliament and Council Directive amending regulations pertaining to the labeling of foodstuff was prepared by the EU Directorate General III Industry (6). The intention of the proposal was to declare some ingredients, particularly cereals containing gluten, crustaceans, egg, fish, milk, peanut, soybean, nuts, sesame seed, and sulfites at concentrations of at least 10 mg/kg. However, there are reports of allergic reactions to more than 150 food items; thus, reducing the list to 10 foods seems illogical (7). Despite this undertaking, it is likely that current European labeling regulations will not adequately protect the patient who has severe food reactions to very small quantities of the offending food.

A second possibility is to provide the allergic subject with a list of foods that are sure to be free of certain food items. Although such lists could guide the food-allergic subjects in the purchase of "safe foods", the Codex Commission recently stated that the condition for claiming a food to be "free" of a certain nutrient did not actually ensure nutrient-free status (8). The inability to certify nutrient-free status is probably determined by the fact that contamination can occur at several steps of the production process, as during transportation of the raw material from the place of origin to the plant for processing; moreover, product characteristics may vary, as the substance purchased may be tainted with undeclared trace materials when furnishers of raw materials are not used on a permanent basis. Moreover, manufacturers of food products cannot guarantee continuity in product content, because food batches produced at a later time may undergo minor changes. Therefore, "safe" food lists appear to be ineffective at solving the problem.

In light of the above shortcomings, efforts are being made either to find hypoallergenic variants of fruits or vegetables, or to produce hypoallergenic foods; i.e., foods with very low or no allergenicity. Ideally, the allergic subject should be able to purchase hypoallergenic foods with the same ease that gluten-free foods can be obtained by the celiac patient.

To produce hypoallergenic foods, one must understand the main allergens one wishes to modify. Allergens are essentially proteins, and some plants may contain more than 1000 different proteins (9). However, only three to six of these proteins can be considered major allergens, and only 10–15 minor allergens. The number of allergens officially recognized by the WHO/IUIS Allergen Nomenclature Subcommittee is presently limited: 26 major allergens have been identified for about 17 food items<sup>†</sup>.

Although the identification of the major allergen(s) is a prerequisite in the endeavor to produce hypoallergenic foods, a better understanding of the major allergens also can lead to more reliable *in vivo* and *in vitro* diagnostic measures.

The sera of patients allergic to a specific food item are necessary to prepare an allergogram by SDS–PAGE immunoblotting for the identification of the major allergens of a food. Thus, it is clear that the role of the clinical allergologist is fundamental for allergen identification, inasmuch as the sera containing the specific IgE to foods must be obtained from clinically confirmed positive patients; and in these subjects, clinically confirmed positivity must be based on double-blind, placebo-controlled food challenge (DBPCFC) (10). An imbalance between laboratory and clinical investigation can lead to the identification of major allergens that do not correspond to symptomatology. The issue becomes evident if we analyze the allergens of cereal and grasses. Cereals and grasses both belong to the Poaceae family. The plants of this family account for celiac disease as well as for three manifestations of IgE-mediated allergic disorders: grass-pollen-induced respiratory allergy, baker's asthma, and food allergy to cereals. If patients with food allergy or baker's asthma are at the same time allergic to grass pollen, they may generate antibodies to pollen allergens that are also found in cereal flour; however, these pollen-induced antibodies have no clinical significance if we consider food allergy to cereals or baker's asthma. Therefore, if one does not consider cross-reacting antibodies in the process of allergen identification, the study results may be equivocal. For example, in a study by Jones et al. (11), 26 of the 126 subjects

<sup>†</sup>Official List of Allergens, IUIS Allergen Nomenclature Subcommittee (San Francisco, CA: 1997). 11. foods: *Gadus callarias* (cod), *Salmo salar* (Atlantic salmon), *Bos domesticus* (domestic cattle, milk), *Gallus domesticus* (chicken), *Metapenaeus ensis* (shrimp), *Penaeus aztecus* (shrimp), *Penaeus indicus* (shrimp), *Apium graveolens* (celery), *Brassica juncea* (oriental mustard), *Hordeum vulgare* (barley), *Malus domestica* (apple), *Oryza sativa* (rice), *Prunus avium* (sweet cherry), *Sinapis alba* (yellow mustard), *Glycine max* (soybean), *Arachis hypogaea* (peanut), *Actinidia chinensis* (kiwi).

with positive SPT for wheat had a positive DBPCFC to wheat, showing 69% false positivity for wheat SPT. The low accuracy of the SPT for wheat was due to the presence of allergens cross-reacting with grass pollen in the diagnostic extract. Similar results were reported for rye, barley, oats, rice, and corn (11).

The same study reported that antibodies to two allergens of wheat, a 20-kDa protein and a 47-kDa protein, were detected, respectively, in 100% and in 75% of nine patients with DBPCFC-confirmed food allergy to wheat but not sensitized to grass pollen. This study provides an appropriate example of how patients should be selected for the purpose of yielding sera to be used in the identification of food allergens. The following guidelines for food allergen identification have been proposed for a study protocol in progress with the EC<sup>‡</sup>. The aim of this study is the identification of the major allergen(s) of hazelnut, celery, and fruits of the Prunoideae family, as well as preparation of hypoallergenic foods. For each food in the study, 30–50 patients with a clinical history, positive diagnostic tests, and DBPCFC for the specific food item will be selected from Milan, Odense, and Zurich; SDS–PAGE immunoblotting will be performed with these sera, and the major allergens (found in >50% of the sera) will be recognized; the allergenic proteins will be purified and the amino-acid sequence determined; and, lastly, the functional characteristics of the allergens will be identified. Moreover, apart from providing technical details, the study of subjects from different European centers allergic to the same food item may help our understanding of the genetic and environmental factors that influence the allergic response.

At present food technology can prepare foods claimed to be hypoallergenic by three methods: agronomic, physicochemical, and transgenic.

## Agronomic method

There have been reports of fruit or vegetable strains that contain a reduced quantity of allergen. Vieths et al. found that EAST (RAST) scores for 16 apple varieties, measured with individual and pooled sera from patients allergic to apple, varied from 0.2 to 4.0 in relation to the relative quantity of the 18-kDa allergen (Mal d 1) present in each variety of apple (12). This finding has been confirmed by EAST inhibition data: the Jamba variety weakly inhibits EAST with Golden Delicious extract much

like ovalbumin, up to a concentration of 1000 nl of allergen extract; i.e., insignificant inhibition. This study shows that there are several strains of apple that contain either low (Boskop strain) or very low (Jamba, Hammerstein, etc.) concentrations of (Mal d 1) allergen. In fact, challenge with 150 g of the Jamba variety was negative in two subjects with allergy to apple. Given the vast variety of naturally existing strains of fruits and vegetables, knowledge of the physical and functional characteristics of the major allergens can lead to very interesting results in agronomic studies.

Another possibility would be to breed selected strains of fruits or vegetables that lack a protein known to produce disease. This has been carried out for strains of wheat deficient in gliadin for celiac disease patients. Although the etiopathogenesis of celiac disease differs from that of food allergy, the objective of modifying the concentration of a protein remains the same. Gliadins are encoded in the loci *gli 1* ( $A_1 - B_1 - D_1$ ) and *gli 2* ( $A_2 - B_2 - D_2$ ), found on the short arm of chromosomes 1 and 6 of wheat. The loci for *gli 1* code for gliadin  $\gamma$  and  $\alpha$  and the loci for *gli 2* code for gliadin  $\alpha$  and  $\beta$ . The possibility of wheat lacking some gliadin types has been known for some time: however, breeds of wheat that lack chromosome 6A are counterbalanced by two extra chromosomes, either 6B or 6D (nullitetra wheats) (13). These strains of wheat are of little use because the absence of gliadin  $\alpha$  and  $\beta$  is counterbalanced by greater gliadin synthesis by loci *gli B<sub>2</sub>* and *gli D<sub>2</sub>*, and they were found to be toxic. Wheat strains that lack the genes that code for gliadin  $\alpha$  and  $\beta$  which have the normal chromosome set and strains that lack gliadin  $\gamma$  and  $\omega$  have been bred to yield strains that lack several gliadin components (14). In this study, the investigators cultured (at 37°C) normal small intestine obtained from patients with celiac disease with normal wheat extract for 24 h. The result was the typical mucosal pattern of celiac disease as well as altered morphometry. On the other hand, tissue cultures that were incubated with wheat extracts that lacked gliadin were not altered, and morphometry was normal. These studies are promising, at least *in vitro*; however, we await *in vivo* verification.

## Thermal processing

Many allergens are heat stable (Table 1). Fruits can lose their allergenicity by canning, as in the preparation of preserves or jam. The allergen of apple loses its allergenicity upon heating (15). Air exposure has also been shown to denature apple allergen, because polyphenols, which are the constituents of many plants, are oxidized to quinones,

<sup>‡</sup>European Commission – Directorate General XII – Science, Research and Development: Shared Cost Contract No. FAIR-97-3224.

Table 1. Response to heat of some common allergens

| Heat stable            | Heat labile     |
|------------------------|-----------------|
| $\beta$ -Lactoglobulin | Mal d 1 (apple) |
| Casein                 | Api g 1         |
| Gad c 1                | Several fruits  |
| Shrimp allergens       | Coffee          |
| Ara h 1                | Cocoa           |
| Ara h 2                |                 |
| Ovomucoid              |                 |

and these can polymerize to form brown pigments or react with amino-acid residues (lysine, cysteine, and tryptophan), resulting in reduced allergenicity (16). Jankiewicz et al. reported that the major celery allergen, Api g 1, was heat labile, but was stable upon exposure to high voltage, high pressure, gamma rays, drying, and powdering (17). The remaining two celery allergens, "celery profilin" and "CCD", were more resistant to heat than Api g 1.

Tuna fish has also been shown to lose allergenicity upon boiling and canning (18). Eighteen patients with allergy to tuna and two allergic to salmon had negative DBPCFC with canned tuna or salmon. SDS-PAGE immunoblotting of salmon and tuna showed that many of the proteins present in the raw fish extracts were denatured upon cooking and formed high-molecular-weight conglomerates. In particular, the heat-labile proteins were found in the 40- and 90-kDa bands. In contrast, several investigators studying the major allergens of cod, mackerel, herring, and plaice found that allergen denaturation did not occur upon heating (19). Therefore, for fish, the question of heat lability of the allergen must be considered individually for each species.

Moreover, Simplesse, a microparticulate protein obtained from heat and high-shear treatment of egg and milk, does not lose allergenicity after heat or high shear treatment; therefore, it may still pose a risk for the subject sensitized to egg or milk (20).

## Enzymatic proteolysis

Enzymatic proteolysis has been employed extensively in the preparation of hypoallergenic cow's milk formulas. This topic is reviewed by Halken & Høst in this Symposium series. Nevertheless, hypoallergenic milk preparations have shown some limitations, because, first, hypoallergenic formulas can be obtained only after extensive hydrolysis, and, second, the extensive hydrolysis has, in rare cases, been ineffective at avoiding severe reactions (21–23).

Two hypoallergenic types of wheat flour have been obtained by enzymatic proteolysis. Watanabe

et al. (24) and Tanabe et al. (25) have prepared hypoallergenic wheat flour by treating the flour with actinase, collagenase, and transglutaminase, and, in a second study, also with bromelain. They reported that the epitope of gluten has the following structure: glu-X-Y-pro-pro, where X and Y are the replaceable amino-acid residues. The investigators also showed that bromelain had an elevated capacity for hydrolysis of the essential prolin residues. The product thus obtained does not contain the above amino-acid sequence, and can be used to produce flour that has valid organoleptic characteristics for the preparation of a good bread substitute. Unfortunately, tolerability studies in subjects allergic to wheat have not been reported.

Hypoallergenic rice has been obtained by treating rice with proteolytic enzymes and surfactant. SDS-PAGE immunoblotting studies showed that the 16-kDa major allergen of rice had been eliminated, and RAST of the hypoallergenic rice tested with sera of rice-allergic patients was negative (26). Hypoallergenic rice was shown to be well tolerated by rice-allergic subjects and is now available on the market (27).

A procedure for preparing hypoallergenic flour has been patented (28). It consists of the removal of the protein fractions below 30 kDa, and, subsequently, also the removal of the protein fractions of 30–50 kDa. In the hypoallergenic product, no more than 100  $\mu$ g/ml of the 30-kDa fraction was detectable, and no more than 60  $\mu$ g/ml of the 30–50-kDa fraction. Here, as well, testing on allergic subjects is lacking.

## Genetic engineering

Genetic engineering methods can be used to prepare hypoallergenic foods. Nakamura & Matsuda were able to produce transgenic rice that contained less major allergen (29). Many water-soluble rice proteins of 15–16 kDa, separated by HPLC, were shown to react with IgE, but the 16-kDa protein showed greatest reactivity. The authors used antisense strategy in rice; i.e., genes that produced antisense RNA were introduced in rice, and the result was an 80% reduction in the 16-kDa protein. Although hypoallergenic rice does not appear to have been used in DBPCFC in rice-allergic subjects, the method allows us to suppress the expression of an allergenic protein in a botanic species and its offspring.

As we have seen, present technology offers several methods with which hypoallergenic foods can be produced or identified. However, it appears that food technology studies have often been performed without carrying out appropriate clinical controls. In fact, investigation in the field of food technology

has often proceeded without proper identification of the major allergen(s) of the food one intends to make hypoallergenic. Moreover, the hypoallergenicity of the hypoallergenic foods that have been produced to date has rarely been subject to thorough *in vitro* and *in vivo* trials. It is in the latter phase of a study that the clinical allergologist has a fundamental role. A food presumed to be hypoallergenic can be confirmed so if it meets the requirements of each of the following consecutive steps:

- 1) preparation of the hypoallergenic food
- 2) *in vitro* verification of claimed hypoallergenicity (SDS-PAGE immunoblotting)
- 3) *in vivo* testing of the hypoallergenic food in sensitized animals
- 4) SPT with the extract of the hypoallergenic food in sensitized subjects
- 5) *in vivo* verification by administering the hypoallergenic food openly and then by DBPCFC of sensitized subjects (30, 31).

Is there a need for hypoallergenic foods? I believe the answer is "yes". However, the development of hypoallergenic foods is still in its first stages, and future research in this area needs to be directed at producing foods in which the allergen is absent, and not only reduced.

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