

Food from genetically modified organisms and potential for food allergy

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Abstract

Crops produced through genetic modification are beginning to reach the market and many genetically-modified crops are under development. Since genetic modification results in the introduction of new proteins into the food plant the safety of the newly introduced proteins must be assessed. The potential allergenicity of the newly introduced protein is a major consideration in that safety assessment. All allergens are proteins but only a few of the many proteins found in foods are allergenic. The assessment of the allergenicity of the newly introduced proteins should focus on the source of the gene, the sequence homology of the newly introduced protein to known allergens, the immunochemical reactivity of the newly introduced protein with IgE from the blood serum of individuals with known allergies to the source of the transferred genetic material, and the physicochemical properties of the newly introduced protein. © 1997 Elsevier Science B.V.

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1. Food from genetically modified organisms

The genetic modification of foods will have tremendous implications for the food industry and all of agriculture in the coming years. The use of biotechnology to genetically modify foods will likely be ranked with irrigation, mechanized farming, the use of agri-chemicals, and integrated pest management as a major revolution in agriculture worldwide. Food processors will certainly be impacted by the introduction of genetically modified crops and livestock on farms and ranches. In addition, other advances resulting from the genetic modification of food-producing organisms will be targeted specifically to food processors rather than production agriculture.

Biotechnology can be defined as any technique that changes the heritable traits of an organism including recombinant DNA (rDNA) technology. Obviously, traditional plant and animal breeding results in changes to the heritable traits of organisms. While this definition is broad, the ability to transfer genetic information across

a wide range of species, through rDNA technology, has dramatically improved in recent years. Thus, it is possible to conceive of the transfer of virtually any trait from one organism to another.

Food biotechnology is simply a more accurate and precise method for enhancing the beneficial traits of plants, animals, and microorganisms. While traditional plant breeding, for example, results in the transfer of hundreds of genes and their proteins, modern genetic modification approaches allow the selection of single genes and their products. Food biotechnology has the potential to assist the food industry in achieving its key objectives including improvements in nutritional characteristics, improved taste, elimination of undesirable traits, extension of shelf life, decreases in the use of additives, reduced processing costs, reduced energy consumption, and lessening of the environmental impact from food processing.

The first products of biotechnology are just entering the marketplace. Chymosin from *E. coli* was introduced in 1988. The FlavrSavr tomato from Calgene was introduced into limited markets in 1994. Genetically modified soybeans are due on the market in 1996.

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Many other products are under active development (Table 1), and the rate of introduction of new genetically modified products is likely to accelerate considerably in the next few years. The agricultural biotechnology industry has succeeded in genetically modifying most of the important agronomic crops and many of the minor ones as well. The genetic modification of microorganisms of importance to the food industry is widely practised. Animal biotechnology, other than the development of bovine somatotropin, is proceeding more slowly.

Plant biotechnology will likely have the greatest impact on the food industry in the next few years. Some of the traits being introduced into transgenic plants are primarily of benefit to producers. These traits would include enhanced insect-, disease-, and herbicide resistance and improved climatic tolerance. Such traits are being introduced into many commercially important crops including soybeans, potatoes, corn, and wheat. Thus, the food industry will likely be processing large quantities of genetically modified crops into foods within the coming decade. The food products emanating from these genetically modified crops will not necessarily have unique features, but will have slightly altered compositions.

Other traits being introduced into plants via genetic modification will lead to crops with improved characteristics that will directly benefit food processors. Examples that are likely to be introduced in the next few years would include improved taste and longer shelf life for certain fruits and vegetables especially tomatoes, healthier cooking oils, and improved nutritional qualities for soybeans. Many other changes are under consideration and development. The food products emanating from these genetically modified crops will have unique features and altered compositions in some cases.

Table 1
Examples of products of plant biotechnology

Crop	Trait	Primary gene(s)	Organization
Tomato	Delayed softening	Anti-polygalacturonase	Calgene
Soybean	Glyphosate resistance	EPSPS ^a	Monsanto
Squash	Virus resistance	Viral coat proteins	Asgrow
Potato	Insect resistance	cryIIIA ^b	Monsanto
Corn	Insect resistance	cryIA(c) ^c	Monsanto
Rapeseed	High laurate	Anti-12:0 thioesterase	Calgene

^a EPSPS, 5-enolpyruvylshikimate-3-phosphate synthase.

^b CryIIIA, insecticidal gene from *Bacillus thuringiensis tenebrionis*.

^c cryIAB(c), insecticidal gene from *B. thuringiensis Kurstaki* strain HD-1.

2. Food safety and assessment of allergenic potential

Safety issues have also been raised with regard to agricultural biotechnology. However, a recent FAO/WHO consultation concluded that the 'food safety considerations regarding organisms produced by techniques that change the heritable traits of an organism, such as rDNA technology, are basically of the same nature as those that might arise from other ways of altering the genome of an organism, such as conventional breeding' (FAO/WHO, 1996). One particular focus of concern is the possible transfer or introduction of allergens.

Assessment of the allergenic potential of a genetically modified food is but one component of the overall food safety evaluation process. However, the allergenicity of the newly introduced protein is a source of some concern. Virtually all allergens are proteins. However, only a very few of the many proteins in nature are allergens so the inherent risk of allergenicity from any randomly selected protein is rather small. Allergens are those proteins which can trigger the production of allergen-specific immunoglobulin E (IgE) in susceptible individuals (Taylor et al., 1992). Even in commonly allergenic foods, only a few of the many proteins have the capability of inducing allergic sensitization in susceptible individuals. In nature, allergens can be found in many foods, pollens, mold spores, insect venoms, and other sources. Obviously, the risk of transfer of an allergenic protein is much greater when the DNA source is derived from a known allergenic donor, although it is quite possible to transfer a non-allergenic protein from a known allergenic source. Appropriately, the US FDA has focused their concern on the presence of DNA from known, especially commonly, allergenic sources in the transgenic crop (FDA, 1992). Thus, biotechnologists can begin to assess the allergenicity of the transferred protein by considering the source of the gene, the allergenic potential of the protein, and any effects on the endogenous allergens of the host.

3. Sources of genetic material

Recently, the International Food Biotechnology Council (IFBC) in collaboration with the Allergy and Immunology Institute of the International Life Sciences Institute (ILSI) developed an approach to the assessment of the potential allergenicity of genetically modified foods (Fig. 1, Metcalfe et al., 1996). This decision-tree strategy focuses on the source of the gene, the sequence homology of the newly introduced protein to known allergens, the immunological reactivity of the newly introduced protein with IgE from the blood serum of individuals with known allergies to the source of the transferred genetic material, and the physicochemical properties of the newly introduced protein.

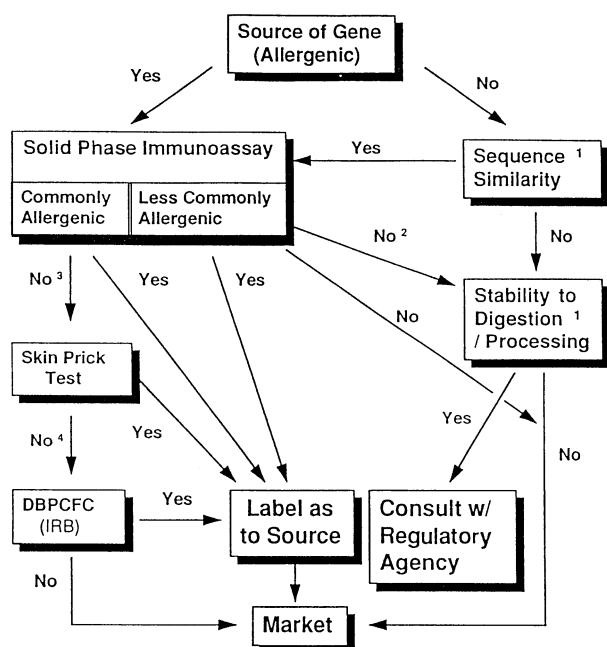


Fig. 1. Assessment of the allergenic potential of foods derived from genetically engineered crops. Adapted from Metcalfe et al. (1996).

The sources of genetic material can be classified as commonly allergenic, less commonly allergenic, or unknown allergenic potential. Commonly allergenic foods include milk, eggs, peanuts, soybeans, tree nuts, fish, crustacea, and wheat (FAO, 1995). These few foods probably account for more than 90% of all food allergies on a worldwide basis. The IFBC–ILSI report contains an extensive list of more than 160 foods and food-related substances that have been associated with allergic reactions in individuals (Hefle et al., 1996). While some of these foods would appropriately be classified as less commonly allergenic foods, some judgment is required because these reactions were not always well investigated.

If a gene is obtained from a known allergenic source and the encoded protein is expressed in the edible portion of the genetically modified organism, then data should be generated to assure that the gene does not encode an allergen. Expression of a gene from a known allergenic source in a part of the organism that is not ordinarily consumed, such as potato leaves, should not be a source of concern. While genes obtained from any known allergenic source raise some concerns, the greatest concern would be raised if the genes were obtained from a commonly allergenic food.

4. Comparison of amino acid sequences

With proteins derived from commonly allergenic and less commonly allergenic sources as well as

sources of unknown allergenic potential, a comparison of the amino acid sequence of an introduced protein with the amino acid sequence of known allergens is a useful initial approach in the determination of allergenic potential (Metcalf et al., 1996). The amino acid sequences of many major allergens including food allergens are known. In some cases, the IgE, T-cell, and B-cell epitopes of these allergens are known. The IFBC–ILSI report contains a list of 198 sequences of food and non-food proteins that are reported to be allergens (Metcalf et al., 1996).

An immunologically significant sequence similarity would require a match of at least 8 contiguous identical amino acids (Fuchs and Astwood, 1996; Metcalfe et al., 1996). In general, the optimal peptide length for binding to T cells appears to be between 8 and 12 amino acids; even longer peptide sequences appear to be involved in B cell binding (Rothbard and Gefter, 1991). Thus, a match based upon the identity of 8 contiguous, identical amino acids seems to be a conservative approach. The presence of 8 contiguous, identical amino acids is well below the level of homology expected between biologically related, homologous proteins (Doolittle, 1990). Since this approach assesses the entire protein sequence, it is not based upon the identity of amino acid sequences just to known epitopes of known allergens. Because insufficient information is available on the amino acid sequences of allergen epitopes, the more appropriate approach is to look for matches along the entire length of the polypeptide. In fact, this approach is likely to identify conserved sequences that are unrelated to the allergenic potential of the proteins. Certainly, as more information becomes available on the molecular structure of allergens and, in particular, on the IgE, T cell, and B cell epitopes of food allergens, the matching requirement of 8 amino acids anywhere along the entire length of the polypeptide, that is currently proposed by IFBC–ILSI, might change.

Without a match of 8 contiguous, identical amino acids, there is little probability that the introduced protein would possess a shared linear epitope with a known allergen. The exact conservation of epitope sequences in homologous pollen allergens of disparate species has been observed, and can explain IgE cross-reactivity among allergens (Mohapatra et al., 1994; Astwood et al., 1995). Conservative amino acid substitutions introduced into synthetic epitopes (Jorgensen et al., 1992) or introduced by site-directed mutagenesis (Smith and Chapman, 1995) have been shown to reduce epitope efficacy. Clearly, this approach is limited in that it cannot identify discontinuous conformational epitopes which depend on the tertiary structure of the protein (Metcalf et al., 1996).

5. Assessment of immunoreactivity

The next step in the evaluation of the allergenic potential of foods derived from genetically modified plants involves an assessment of the immunoreactivity of the newly introduced proteins with IgE antibodies from the sera of individuals allergic to the donor plant. With proteins derived from known allergenic sources, blood serum containing allergen-specific IgE can be obtained from individuals with allergies to the donor plant. The blood serum can be tested for reactivity with the newly introduced protein or extracts of the transgenic food using immunoassays such as the radioallergen sorbent test or RAST (Yunginger and Adolphson, 1992). A positive test certainly raises concerns about the allergenicity of the newly introduced protein. Unless these concerns can be convincingly discounted by additional *in vivo* testing, foods containing the newly introduced gene should be considered to be allergenic.

In the case of negative or equivocal results in the *in vitro* immunoassays, the transgenic food should be investigated further using *in vivo* skin-prick tests with an appropriate number of allergic test subjects (Bock et al., 1977). The skin-prick test is an *in vivo* indication of the allergenicity of the transgenic food. The allergenicity of the transgenic product could ultimately be confirmed with double-blind, placebo-controlled food challenges with allergic individuals (Bock et al., 1988). A positive result in either of these *in vivo* tests would be sufficient evidence of the allergenicity of the transgenic food.

6. Proteins from less commonly allergenic food and non-food sources

Since it is more difficult to identify allergic individuals for the less commonly allergenic foods, the approach for transgenic products containing proteins from such sources is a combination of immunoreactivity and physiochemical stability. Allergens tend to be proteins which are stable to digestion (Taylor et al., 1987; Fuchs and Astwood, 1996). Thus, if evidence is found that a protein is easily digested, then the protein is much less likely to be capable of inducing allergic sensitization. The enzyme transferred into soybeans to make them tolerant of the herbicide, glyphosate, is rapidly digested *in vitro* (Harrison et al., 1996) and is therefore unlikely to induce allergic sensitization.

The most difficult assessment involves proteins obtained from sources of unknown allergenic potential such as viruses, bacteria, insects, non-food plants, and other non-food sources. Although such substances are sometimes found in foods at very low levels, they are not considered food sources. The likelihood that such proteins will be allergens is not very high since most

proteins in nature are not allergens. Additionally, many of these proteins will be expressed in the transgenic food at very low levels, while allergenic sensitization is more likely to occur to the major proteins that exist in foods. The assessment of the allergenicity of proteins obtained from sources of unknown allergenic potential would include evaluations of the amino acid sequence homology to known allergens and the physicochemical stability of the protein (Metcalf et al., 1996).

7. Physiochemical stability

The digestive stability of the newly introduced protein will be a critical factor in the assessment of the protein's allergenic potential. The ability of food allergens to reach and cross the mucosal membrane of the intestinal tract is a likely prerequisite to allergenicity. Simulated gastric and intestinal digestive models of mammalian digestion have been used to compare the relative stability of known allergenic food proteins with several proteins introduced into foods by genetic modification. As expected, the allergenic protein exhibited much greater proteolytic stability compared to the genetically introduced proteins which were rapidly digested (Fuchs and Astwood, 1996). The comparative digestibility of these newly introduced proteins should also be assessed in model systems which mimic humans having limited gastric acid secretion and other gastrointestinal maladies. The relative stability of these proteins to digestion should also be assessed in food matrices where the presence of other food proteins might slow the proteolysis of the newly introduced protein.

8. Assessment of the allergenicity of soybeans with a protein from brazil nuts

Such a comprehensive approach has already proven useful in the assessment of the allergenicity of a transgenic food product. Pioneer Hi-Bred International succeeded in cloning a high-methionine protein from brazil nuts into soybeans to correct the inherent methionine deficiency of soybeans. The high-methionine protein from brazil nuts represented a significant fraction of the total protein in the transgenic soybean seed. The transgenic soybeans would have enhanced value for animal feeding purposes due to the elevated methionine content. However, since Brazil nuts are known to be allergenic (Gillespie et al., 1976; Arshad et al., 1991), the possible allergenicity of the transgenic soybeans and the high-methionine protein was evaluated (Nordlee et al., 1996). RAST inhibition employing

blood serum from individuals with documented brazil nut allergy was used to determine if an IgE-binding protein had been transferred from the brazil nut to the transgenic soybean. The RAST inhibition results showed that the gene obtained from the brazil nut likely encoded an allergen. Using SDS-polyacrylamide gel electrophoresis and immunoblotting with the sera of brazil nut-allergic individuals, it was demonstrated that the high-methionine protein from Brazil nuts was indeed the major allergen of brazil nuts recognized by the sera of 8 of the 9 patients. This protein was also identified in the transgenic soybeans. The potential allergenicity of the transgenic soybeans was confirmed by positive skin-prick tests on three brazil nut-allergic individuals. As a result, Pioneer Hi-Bred International decided not to commercialize these transgenic soybeans. This example demonstrates that the decision-tree approach described above works in the assessment of the allergenicity of transgenic foods.

9. FAO/WHO recommendations

The recent FAO/WHO Consultation on Biotechnology and Food Safety held this autumn in Rome reached several conclusions regarding the allergenicity of genetically modified organisms (FAO/WHO, 1996):

(a) The transfer of genes from commonly allergenic foods should be discouraged unless it can be documented that the gene transferred does not code for an allergen.

(b) Foods found to contain an allergen transferred from the organism which provided the DNA should not be considered for marketing approval unless such products can be clearly identified in the marketplace and this identity will not be lost through distribution and processing. Further, the labelling approaches may not be practical in these situations, and that particular problems exist for consumers who cannot read, or who may not be provided with labels.

(c) Involved organizations should consider the appropriateness of, and/or actions to take, in respect to foods containing new protein(s) that are determined to have the characteristics of an allergen, even though no patient population is known to exist which has an allergy to this gene product.

(d) The identification of food allergens and the characteristics of these allergens that define their immunogenicity be encouraged.

Biotechnology also offers the potential to allow the elimination of allergens from known allergenic foods. Japanese investigators have succeeded in dramatically decreasing the level of allergens present in rice through genetic modification (Matsuda et al., 1993).

10. Conclusion

Biotechnology will ultimately affect many of the products currently in commercial markets. These transgenic products must be evaluated for their safety. However, most transgenic foods will be altered only slightly in composition from traditional foods. Thus, the safety evaluation should be focused on the compositional differences especially the safety, including the potential allergenicity, of any newly introduced proteins. Few of these newly introduced proteins are likely to become food allergens. A decision-tree strategy has recently been developed for the assessment of the allergenicity of genetically-modified foods and has been shown to be useful in the evaluation of transgenic soybeans.

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