SPECIAL GUEST EDITOR SECTION

Effects of Chemical, Physical, and Technological Processes on the Nature of Food Allergens

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A review is presented of studies of different processing techniques and their effect on the allergenicity and antigenicity of certain allergenic foods. An overview of investigated technologies is given with regard to their impact on the protein structure and their potential application in the production of hypoallergenic foods. The use of physical processes (such as heating, high pressure, microparticulation, ultrafiltration, and irradiation), chemical processes (such as proteolysis, fermentation, and refining by extraction), and biotechnological approaches, as well as the effects of these processes on individual allergenic foods, are included. Additionally, the implications of food processing for food allergen analysis with respect to food safety assessment and industrial quality control are briefly discussed.

According to several European and American authors, food allergies affect up to 2% of the adult population and up to 8% of children (1, 2). Food allergies are abnormal immunological reactions to a food or food component. Typically, allergic reactions are immediate hypersensitivity reactions mediated by allergen-specific immunglobulin E (IgE; 3, 4). Food allergens can be defined as those substances in foods that initiate and provoke the immunological reactions of allergy. In IgE mediated food allergy, the allergens are usually naturally occurring, often abundant, proteins or glycoproteins found in a particular food (5).

Over 160 food materials have been identified as allergenic. Only 8 of them account for more than 90% of all food allergies (6–8), including egg, milk, peanut, soya, tree-nuts, crustaceans, fish, and wheat. Numerous other food allergens have been identified, including fruits and vegetables (9). Polypeptide masses usually range between 5 and 70 kDa (5, 10); however, many allergens are oligomers with molecular masses >200 kDa (11).

Food allergens have several biochemical characteristics in common, including their glycosylation pattern and their resistance to proteases, heat, and denaturants. One of the more significant food allergen characteristics is that they are stable to the proteolytic and acidic conditions of the digestive tract, which imparts an increased probability of reaching the intestinal mucosa, where absorption can occur. Even though allergen stability has been demonstrated for a variety of food allergens, little is known about why these proteins have the ability to resist degradation (12).

Allergenic proteins are recognized by IgE because of their antigenic determinants or epitopes. Two different forms of epitopes have been identified: linear and conformational. Linear or continuous epitopes are composed of short peptide fragments (12–18 amino acids), which are believed to be more important for the heat-stable, classical food allergens (13). Conformational or discontinuous epitopes depend on the 3-dimensional structure of a protein and are displayed on the surface areas of the molecule. Segments of the polypeptide chain, which may be quite distant in amino acid sequence of a protein, are brought together spatially by the protein's 3-dimensional structure. Most epitopes are thought to be conformational in nature; hence, protein structure plays an important role in the stability of food allergens to resist food processing and digestion (14). One important issue is the presence of disulfide bonds, which typically occur between 2 residues (e.g., cysteine) along the same or of different polypeptide chains of the protein. Several enzymes, including pepsin or thioredoxin, can reduce these bonds. New epitopes may then be displayed on the protein surface (e.g., in the case of Ara h 2; 15), or allergenicity may be reduced by conformational changes (e.g., milk allergens; 16). Changes in protein structure have other consequences in addition to altering allergenicity. Most (bio)molecular recognition techniques rely on the specificity of antibody-antigen interactions. Changes in the target protein structure will inevitably impact the overall response (increasing, decreasing, or abolishing) and thus affect the sensitivity of the technique.

We present an overview of investigated processing technologies with regard to their impact on the protein structure and their (potential) application in the production of

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hypoallergenic foods. The use of physical processes (such as heating, high pressure, microparticulation, ultrafiltration, and irradiation), chemical processes (such as proteolysis, fermentation, and refining by extraction), and biotechnological approaches, as well as the effects of these processes on individual allergenic foods, are included. Special emphasis is also given to attempts to specifically alter the allergenic potential of various foods in the search for technologies to produce non- or hypoallergenic foods. Additionally, the implications of food processing for food allergen analysis with respect to food safety assessment and industrial quality control are briefly discussed.

Food Processing and Allergen Integrity

Many foods are processed for various reasons: to increase food quality, improve taste and flavor, alter appearance and texture, prepare for mixing with other foods, or extend shelf life by inactivation of microbes and toxins. Various techniques are used to achieve these goals: milling or squashing, heating or chilling, fermentation, and irradiation, just to name a few. Many of these processes have a profound influence on the protein structure and, hence, on the allergenicity and antigenicity (e.g., detectability by immunological tests). Processing can elicit an unintentional effect on allergenic foods, but it may also be a tool to produce nonallergenic or hypoallergenic foods. Examples of such products are fermented or hydrolyzed dairy products, genetically modified foods, or ultra-high heat-treated food products.

Depending on the food and the applied process, conventional processing can enhance or reduce the allergenicity of a particular food. Moreover, several chemical and technological approaches have been used to produce non- or hypoallergenic foods by removal, destruction, proteolytic modification, or masking of epitopes (13). These techniques comprise various heat treatments, changing pH, enzymatic digestion/fermentation, ultrafiltration, microparticulation, high pressure, irradiation, and genetic engineering. The allergenicity of highly refined food products (e.g., oils from tree nuts, peanut, soya, or soya lecithin) was investigated to assess the potential health risks for allergic individuals. On the other hand, processing might hamper the detectability of allergenic foods (e.g., when they are present as contaminants, or as misformulated or unlabeled ingredients), while at the same time, the allergenicity of the product is retained and thus a potential health risk remains (17).

Determination of Allergenicity

Methods to determine allergenicity of a food are directed at the molecular structure, integrity, and physiological activity of food allergens and their structural elements, the epitopes. In vitro techniques to assess allergenicity, such as an IgE enzyme-linked immunosorbent assay (ELISA), radio- or enzymeallergosorbent assay (RAST or EAST), and Western or dot blotting, require human sera from allergic individuals with a known history of allergic reactions (18). Cell mediator-release assays can also be used to determine allergenic activity and are thought to give a good correlation to potential in vivo reactions in allergic individuals. These assays are based on blood basophils (a type of white blood cell) from allergic individuals or on rat basophil leukemia cells. The test is as sensitive as the RAST, but is usually performed only by specialized laboratories (4).

Clinical relevance of results obtained via in vitro assays needs to be confirmed by human in vivo tests like the skin prick test (SPT) and/or oral challenge studies. The double-blind placebo-controlled food challenge (DBPCFC) is the gold standard procedure and the most powerful tool to assess allergenicity of any kind of food (19). However, DBPCFC studies are scarce because of ethical and technical limitations and cannot be used as routine tests. Hence, several in vitro (and in vivo) assays are applied in combination to determine allergenicity of a certain food.

Detection of Allergenic Foods

At present, the only effective treatment for food allergy is avoidance of the allergen-containing food or those with the offending glutens. However, total avoidance is sometimes difficult for the allergic individual, because processed food products contain a large variety of ingredients and may contain contaminants, including allergenic foods. To ensure food safety for allergic individuals, stringent labeling regulations and quality assurance procedures are enforced. Both industry and regulatory bodies need reliable methods to detect allergenic foods at relevant levels in complex food products (20).

Currently, there are several technical possibilities for the detection of potential allergens in food products. The methods target either the allergen (protein) itself or a marker that indicates the presence of the offending food. As markers for the presence of potentially allergenic food products or ingredients, specific proteins or DNA fragments are targeted. Protein-based methods usually involve immunochemical detection protocols such as rocket immuno-electrophoresis (RIE) and immunoblotting, which render only qualitative or semi-quantitative results, and fully quantitative methods such as ELISA, RAST, and EAST (21). Presently, only the ELISA technique is used in routine food analysis because of its high precision, simple handling, and good potential for standardization. Methods operating on the DNA level include polymerase chain reaction (PCR), real-time PCR, and PCR-ELISA (21). However, the use of DNA analysis in allergen detection is discussed controversially, because proteins are the allergenic component and processing may differentially affect nucleic acids and proteins.

Heat Processing

For many foods, thermal processing is necessary and unavoidable and may include drying, baking, roasting, frying, boiling, or microwave treatment. It is often thought that thermal processing should decrease allergenicity because

heating or cooking causes such a catastrophic effect on protein structure. This may be true for proteins that rely upon their quaternary structure for their function (e.g., enzymes) but is not necessarily true for their allergenic properties (22).

The extent of the physico-chemical impact on protein structure and functionality depends largely on the intrinsic characteristics of the protein, the temperature applied, the duration of the heat treatment, and the environment (e.g., pH, other reactive ingredients, etc.). The loss of tertiary structure can create new allergenic epitopes, e.g., by unfolding and exposing formerly hidden sites, as well as destroying existing epitopes (15, 23, 24). Typically, loss of secondary structure occurs at temperatures between 55° and 70°C, cleavage of disulfide bonds at 70°–80°C, formation of new intra-/intermolecular interactions and rearrangements of disulfide bonds at 80°–90°C, and the formation of aggregates at 90°–100°C. Besides those physical transformations, chemical modifications of the protein may also occur at high temperatures (100°–125°C; 23). One of the most important of these reactions is through reaction of protein amino groups with sugars, leading to an impressive cocktail of advanced glycation end products, such as Maillard reaction products. Some are antigenic, and many of the important neoantigens found in cooked or stored foods are probably such products (22).

Other covalent modifications of proteins caused by heating or storage can contribute to changes in antigenicity. These include reactions with oxidized lipids, direct oxidation, through reactive oxygen intermediates, disulfide bond scrambling, and deamination of asparagine. Reactions with polyphenols in many plant-derived foods can also cause substantial and unpredictable changes in protein structure (25).

Thermal processing will also reduce the solubility of the target protein, which can reduce allergenicity and antigenicity of a certain food, as well as the extractability of soluble protein, which is the basis for the detectability of allergenic food constituents in food products. As an example, roasted peanuts are widely used by food businesses because of their enhanced flavor characteristics over the raw ingredient, yet the allergenic protein is less soluble in the aqueous solutions required for detection (26). In addition, antigen recognition by immunological detection methods used in food control may be adversely affected.

Some allergenic foods are described as heat-stable (milk, egg, fish, peanuts, and products thereof), while others are considered partially stable (soya, cereals, celery, tree nuts, and their products) or labile (fruits, carrots; 27).

Milk

Pasteurization (75°C, 15 s) was not enough to reduce allergenic activity of milk, even when a homogenization step (60°C, 175 kg/cm^2) was included (28). Only boiling at 100°C for at least 10 min reduced the allergenic potential significantly (29).

Egg

Soft boiled and hard boiled eggs exhibited decreased, but still significant, allergenicity (30). However, the antigenic and allergenic potentials of ovomucoid by heating of egg white in the presence of durum wheat flour was significantly reduced in soft and hard wheat flour, respectively (31). The authors attributed these changes in immunoreactivity to heat-induced polymerization through intermolecular disulfide bonds among ovomucoid and wheat. This study shows that matrix effects may play an important role in the revelation of antigenicity and allergenicity of food products containing several ingredients.

Soybean

Heating of soybeans at 100°C by boiling or microwave decreased allergenicity but did not abolish the potential risk for allergic individuals (32, 33).

Peanut

Peanuts are usually not consumed raw; however, the roasting process increased the allergenicity of peanuts by approximately 100-fold compared with that of the native protein. This increase was linked with the formation of advanced glycation end adducts, the so-called Maillard products (34, 35) and depended also on the maturation and curing conditions before roasting (36). Apparently, the allergenicity of peanuts is unequally affected by different cooking methods. Beyer et al. (37) found that frying and boiling of peanuts, as used in China, reduced the allergenicity of peanuts compared with dry roasting practiced widely in the United States.

Tree Nuts

Almond.—Venkatachalam et al. (38) investigated the effects of roasting, blanching, autoclaving, and microwave heating on the antigenicity of almond proteins. They found a strong decrease in extractable soluble protein (0–85%); however, ELISA results from normalized extracts indicated antigenic stability of almond proteins. A significant change in antigenicity compared with unprocessed proteins was observed only at certain extremes of prolonged roasting and microwave conditions.

Hazelnut.—IgE binding to hazelnuts was unaltered after different heat treatments at 100°C (dry heat, boiling, microwave), but decreased after 15 min of conventional dry heating above 100°C and was abolished after heating to >170°C (39).

Wheat

Wheat allergens showed decreased IgE binding activity after heating wheat flour at 80°–120°C for 10–60 min, and the allergenic potential of heated dough was clearly lower than that of heated flour. No processing conditions entirely abolished IgE binding (40). However, the baking process apparently increased the proteolytic resistance of wheat

allergens, allowing them to reach the gastrointestinal tract, to elicit the immunological response (41).

Fish

Boiling did not have a significant impact on the allergenicity of 10 fish species (42); however, IgE binding activity was reduced 100- to 200-fold in canned fish (43).

Fruit

During cutting or heating, the allergenicity of fruits is commonly destroyed (27); however, technological processing like heat and protease treatments failed to decrease IgE binding activity in sodium dodecyl sulfate–polyacrylamide gel electrophoresis (SDS-PAGE) immunoblot for peach allergens (44).

Vegetables

Celery.—The allergenic potential of celery was slightly reduced but not abolished by cooking (45). In another study, allergenic reactivity was observed in some allergic individuals even after extended (76 min) heat treatment at 100°C (46).

Carrot.—Raw carrots have been involved in severe allergic reactions, whereas cooked carrots were tolerated by the affected individuals (47, 48).

Proteolytic Processing

Proteolysis has been investigated for use in reducing allergenicity of foods. Proteolytic processing is based on enzymatic digestion of proteins, which may cause the destruction of allergenic epitopes. Provided that epitope structure of an allergenic food is known and a specific protease is available to selectively attack the epitope, proteolysis can be applied to reduce or destroy the allergenic potential of a certain food. Challenges associated with this technique are the correct combination of epitope and protease, the existence of multiple epitopes, the destruction of other protein structures, which are important for qualitative characteristics of the food, and the accessibility of epitopes in or on the protein.

Milk

Hypoallergenic infant formulas are produced from caseins or whey proteins by means of heat denaturation and enzymatic hydrolyses, sometimes in combination with ultrafiltration (27). The commercial products are either partially or extensively hydrolyzed. Although both kinds of products have decreased allergenic potential, only the extensively hydrolyzed formulas are tolerated by most individuals who are allergic to cow's milk (49).

Soy

Yamanishi et al. (50) tested 8 different proteases, 2 of which were selected to reduce soy allergenicity.

Wheat

Hypoallergenic wheat flour was produced by using bromelain, a protease which cleaves near prolin residues and breaks down the wheat glutenin IgE-binding epitope Gln-Gln-Gln-Pro-Pro (51). Another approach to reduce the allergenicity of wheat flour was investigated by Watanabe et al. (52), who used a 2-stage enzymatic process with celulase and actinase, respectively, which rendered a hypoallergenic wheat flour.

Rice

Watanabe et al. (53) produced hypoallergenic rice with acceptable textural properties by applying a 2-stage enzymatic process.

Fruit

In some cases, proteolytic treatment is insufficient to abolish or reduce allergenicity, as shown with the major allergenic epitope from peach (44).

Fermentation

Typically, fermentation is achieved by microorganisms and occurs under anaerobic conditions. Technological fermentation processes are accomplished in tanks (fermenters) under controlled conditions (temperature, pH, substrate/product concentrations, etc.). A large variety of different microbes are able to metabolize organic compounds to various products. Typical substrates include sugars (such as glucose) and amino acids. Major products are volatile fatty acids, alcohol, carbon dioxide, and organic acids, such as lactic acid. The generated products depend largely on the selected microorganism and the process conditions.

Lactic acid fermentation is used to produce a large variety of dairy products mainly derived from cow's, goat's, and sheep's milk. Fermentations with lactic acid bacteria and/or molds are also applied to make products from soy, cabbage, and other vegetables. To generate a certain taste and texture, specific microbial starter cultures are exploited which, during fermentation metabolize and thus alter the properties of the source material via a complex system of enzymatic reactions.

Milk

Fermentation of sterilized cow's milk using a mixture of meso- and thermophile lactic acid bacteria hardly affected the allergenicity determined by skin test (54).

Legumes

Fermented soy products such as tempeh, miso, and mold-fermented soy sauce lost a large part of their IgE binding ability, although allergenic activity was not entirely abolished. However, fermentation appeared to be the most effective conventional treatment in reducing allergens present in soy products (55).

For peas, Barkholt et al. (56) showed that detectable amounts of antibody-binding material from all analyzed

components remained after fermentation with 3 different lactic acid bacteria, and 2 molds, respectively, typically used in food fermentation processes.

Refining

Refining often involves physical and/or chemical processing of a basic food material in order to extract and/or purify certain compounds from the food (e.g., oils from plants or lecithin from soya or sunflower seeds). The refining process renders a food product free or almost free of the originally accompanying compounds. In the case of plant oils or derived products, the refining process rids the products from the naturally accompanying protein, carbohydrate, water, ash, fiber, and most minerals. Depending on the degree of refining, the product may or may not contain any residual substances.

Plant Oils

Refined plant oils are produced either by chemical or physical refining. Both processes typically include heating to 95°C, an acid treatment, neutralization, bleaching, and finally deodorization at temperatures between 230° and 250°C (57). Full refining of plant-derived oils results in the almost complete removal of protein (which is responsible for allergic reactions). Available data suggest that the major refined oils (peanut, soy, maize, sunflower, palm) do not provoke allergic reactions in the overwhelming majority of susceptible people and are considered safe for consumption by allergic individuals. However, unrefined and partially refined oils can provoke allergic reactions in sensitive individuals, and anaphylactic reactions to seed oils have occurred (57–59).

Soy Lecithin

Lecithin is used extensively as emulsifier and stabilizer in the food and pharmaceutical industry. It is mainly produced from soybean by a relatively easy process involving distillation, water extraction, and centrifugation. Several studies have pointed out that the residual protein content present in many commercially available soy lecithins may exhibit an allergenic potential (59, 60). Again, the safety of lecithin, as well as seed oils, is mainly dependent on the completeness of protein removal during the refining process.

High-Pressure Processing

High hydrostatic pressure can be applied to alter structural properties of foods in order to improve textural qualities or modify the cellular integrity. When high hydrostatic pressure is applied, cell walls and membranes become porous and permeable for small molecules. Depending on the magnitude of the applied pressure, the integrity of the food may persist, and at the same time allergenic proteins may be released into the surrounding solution.

Rice

High-pressure processing has been successfully used to alter allergen conformation and hence decrease allergenic activity in rice. When high pressure (100–400 MPa) was applied to rice grains immersed in water, low molecular weight proteins, including the major rice allergens, were preferentially released and no apparent structural changes in protein bodies were detected. The removal of allergens by pressurization alone was insufficient to abolish allergenic activity. However, allergenic activity of the product was almost completely eliminated by pressurization in the presence of proteolytic enzymes (61).

Irradiation

Gamma irradiation has been applied widely for food preservation, especially for herbs, spices, and tea. Microbes and enzymes can be inactivated by the application of variable doses of gamma irradiation. Proteins which have been exposed to irradiation present distinct structural modification caused by aggregation, fragmentation, and amino acids modification, which affect the solubility of proteins, their tertiary and secondary structure, and their immunoreactivity. It was found that the main part of the conformation-dependent antigenic structure of protein is lost by irradiation, but that some antigenicity caused by sequence-dependent epitopes remains even at higher radiation doses (62). In some cases, irradiation enhanced allergenicity, which was probably due to the exposure of linear epitopes (33, 63).

Celery

Gamma irradiation was tested on celery by Vieths et al. (33) to determine if this technology could be used to reduce or abolish allergenicity. IgE binding activity of irradiated celery tuber was not decreased; moreover, a neo-allergen was detected by SDS-PAGE after the treatment.

Egg

The combination of irradiation and heating was very effective in reducing the amount of intact ovomucoid, a major allergen of hen's egg, regardless of the pH condition (64).

Milk

Lee et al. (65) evaluated the usability of gamma irradiation for the production of hypoallergenic milk by reducing allergenicity and antigenicity of the major milk allergens, alpha-casein and beta-lactoglobulin. They concluded that epitopes on milk allergens are structurally altered and that the allergenicity of milk allergens can be reduced by gamma irradiation.

Wheat

Leszczynska et al. (63) investigated the influence of gamma irradiation on the immunoreactivity of gliadin and wheat flour. They found that irradiated gliadin samples showed increased allergenicity measured by ELISA.

Moreover, the immunoreactivity of gliadin extracted from irradiated wheat flour was higher than the immune response of pure gliadin irradiated with the same dose.

Shrimp

The allergenic activity of heat-stable protein from shrimp was reduced significantly after irradiation at doses >7 kGy (66). These results indicate the possible use of irradiation technology to reduce or eliminate immunoreactivity of shrimp allergens with an adequate radiation dose, which is permitted in food irradiation (≤10 kGy).

Microparticulation

Microparticulation is a process in which protein is shaped into microscopic round particles that roll easily over one another. Microparticulated proteins may develop naturally in some foods or arise secondary to processing techniques in the food industry, such as blending, mixing, pasteurization, pH alteration, or baking (67).

Egg/Milk

Simplesse is a commercial protein-based product used to substitute fat. It is produced from egg and milk. It consists of nanoparticles (0.1–3 mm), which are coagulated by heat and high shear-force processing. Despite this harsh treatment, the allergenic potential of the source materials (egg and milk) was not abolished and there was no difference in IgE binding activity between the Simplesse proteins and native egg and milk proteins (67).

Ultrafiltration

Ultrafiltration is a filtering process that involves membranes, which can selectively let pass or retain molecules below or above a certain molecular weight, respectively. These membranes are molecular sieves, which are available with different cut-off sizes (e.g., a 50 kDa cut-off membrane will retain molecules >50 kDa).

Peach

Ultrafiltration with suitable cut-off membranes (10 kDa) or chemical lye peeling (a dip in 10% NaOH at 60°C for 90 s) decreased the allergenic potency of peach juice (44).

Milk

Ultrafiltration is also used in combination with heat and proteolytic processing to produce hypoallergenic infant formulas (27).

Genetic Modification

Recombinant DNA techniques provide a unique opportunity to reduce the levels of specific allergens in the food supply. However, an unintentional consequence may be the introduction of new allergens into novel foods. In brief, genetic engineering offers the possibility to selectively control the expression of one or more specific proteins by insertion or deletion of DNA segments or specific mutation. A special mention should be given to the antisense RNA strategy, which uses the fact that antisense mRNA binds to its corresponding native mRNA and thus prevents the synthesis of the encoded polypeptide or protein. Another approach is a specific single site mutation, which causes a change in only one amino acid in the expressed protein. This modification may be sufficient to destroy a linear epitope without changing the characteristics of the protein or alter the structural integrity of a conformational epitope. Even though the majority of genetically modified foods are currently not aimed at the reduction or elimination of allergenic properties, testing for the allergenic potency of a novel food is mandatory in the series of risk assessment procedures before approval of its marketing.

The necessity for evaluation of allergenicity was demonstrated by a recent attempt to raise levels of sulfur-containing amino acids in soy and thus improve the quality of soybean meal for animals. The introduction of the 2S storage albumin gene from Brazil nut failed to get approval for commercialization because of the revelation of a strong allergic response to the novel protein in soy (68).

Rice

Genetic manipulation was successfully applied to reduce allergenicity of rice by introducing native genes encoding an mRNA for a 14–16 kDa allergenic protein in antisense orientation, resulting in the reduction of the corresponding allergenic proteins in the mature seeds (69, 70).

Soybean

An alternative approach studied in soybean was the modification of several immunodominant epitopes of a major allergenic protein. Two of the 5 IgE binding peptides of Gly m Bd 30K could be mutagenized by single-site amino acid sustitution to produce a hypoallergenic soybean plant (71).

Egg

Mine et al. (72) showed that the substitution of 2 amino acids within 6 peptides along the polypeptide chain of the third domain of ovomucoid had an important impact on the allergenicity and the antigenicity as well as the structural integrity of the major allergen in hen egg white.

Detectability of Processed Allergenic Foods

Currently, the ELISA technique is the most commonly method used in laboratories of the food industry and regulatory food control agencies to detect and quantify hidden allergens in food. An ELISA is based on an antigen-antibody binding reaction. Allergenic protein or marker protein binds to specific capture antibodies and can be detected by colorimetric reaction following binding with an antibody enzyme conjugate. The concentration of the allergen or the allergenic food can be determined by plotting the measured

optical densities obtained with the sample on a standard curve generated with reference standards.

Sandwich and competitive ELISA methods have been developed for several food allergens and numerous test kits have become commercially available in this format during the last decade (73). All of these test systems involve specific polyclonal animal IgG antibodies raised against one or more (paritally) purified allergen(s) or a crude extract of a specific allergenic food. The ELISA assays are highly specific for the respective food and depend largely on the molecular recognition of the food-specific antibodies. Any changes in the protein structure in a food as a result of processing will inevitably affect the performance of the assay.

Presently, not many studies on the effects of processing on the detectability of allergenic foods in food products have been published. However, the influence of heat processing on the detectability and quantification of peanut protein is probably the best studied.

Peanut

Several ELISA test assays for peanut determination in food products have been developed. In recent years, several ELISA kits recognizing either Ara h 1, Ara h 2, selected peanut proteins, or a crude peanut protein extract have been placed on the market (21). Drawbacks in the performance of some of the currently available commercial peanut ELISA kits are the impaired recovery of peanut from highly processed material (17), particularly dry roasted peanuts (74, 75). Decreased recoveries can be attributed partly to reduced solubility of heat-denatured peanut proteins and partly to impaired antigen recognition by the antibodies used (75). Several kits use antibodies raised against raw or minimally processed peanuts, which may not recognize processed material. These findings are relevant to the attestation of safe products, because using an inferior kit for the determination of trace amounts of peanut contaminants in food products could result in false-negative results. Several researchers have pointed out that the allergenic activity of peanut proteins is not decreased by heat processing (17, 76), but may be enhanced by the formation of adducts, such as Maillard reaction products (34). If allergenicity remains unaffected or is even enhanced during processing, food samples containing amounts of peanut that could be potentially hazardous to sensitized individuals might be found acceptable by industrial quality control, when commercially available ELISA kits are used for food safety control. A careful selection of the marker protein(s) and the raised antibodies, paired with the development of more efficient protein extraction procedures, is necessary to guarantee the best possible safety of food products for allergenic individuals. For the analysis of some foods, the use of DNA-based techniques may be advantageous because of their specificity, sensitivity, and the relative ruggedness of DNA against heat treatments (77). However, at present the use of DNA analysis in allergen detection as an attractive alternative to immunological methods is discussed controversially, because proteins are the allergenic component, and processing may differentially affect nucleic acids and proteins.

Conclusions

Many of the conventionally used food processing technologies have an important impact on the allergenicity and antigenicity of allergenic foods. Recent studies have attempted to determine the effect of processing on certain food allergens in order to assess the risks involved in certain products for the allergic consumer and to find methods to reduce or abolish allergenic activity of food allergens as a prerequisite for the production of non- or hypoallergenic foods. In general, allergenic foods are resistant to processes commonly used in food manufacturing. Nearly all causative proteins (allergens) retain their allergenicity after treatment by heat and/or proteolysis. Notable exceptions exist; for example, the allergenicity of many fresh fruits and vegetables is decreased or removed by relatively mild processes such as gentle heating or mashing. Although several chemical and physical processes reduce allergenicity, they typically fail to entirely eliminate the immunoreactivity. In fact, processing of some foods may increase the allergenicity, as seen with roasted peanuts (34) and irradiated gluten and wheat dough (65). Apparently, the cooking method (37) and the surrounding matrix (31) have a significant impact on the allergenic potential of allergenic foods. Physical processing typically affects the 3-dimensional structure of proteins and, hence, conformational or discontinuous epitopes. Irradiation is a powerful tool to reduce the immunoreactivity of allergens dependent on conformational epitopes, but may enhance allergenicity because of the exposure of linear epitopes. Biochemical processing can likewise affect the 3-dimensional structure and the primary protein structure, and is successfully applied to produce hypoallergenic infant formulas based on milk proteins.

Any one process on its own is very unlikely to be sufficient to substantially reduce or entirely eliminate the allergenicity of an allergenic food, but combinations of various treatments have proven rather effective in producing hypoallergenic peach juice (44) and hypoallergenic rice (61). Processing can also result in the complete removal of the allergenic qualities of a food, such as the removal of proteins in oilseed processing, which renders refined oils hypoallergenic and safe for consumption by the majority of allergic individuals (57). Recombinant DNA techniques offer promising possibilities to benefit the allergic consumer; an example is the production of hypoallergenic rice (69, 70). However, this novel technology harbors potential risks through the unintended introduction of novel allergens.

Novel non- and hypoallergenic foods produced by the use of various biochemical and technological processes will become more readily available in the future, which will be a welcome benefit for allergic individuals. At the same time, more research is needed to increase the understanding of chemical and physical processing on the allergenic and antigenic properties of food allergens.

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