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Review

Effects of food processing on food allergens

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Food allergies are on the rise in Western countries. With the food allergen labeling requirements in the US and EU, there is an interest in learning how food processing affects food allergens. Numerous foods are processed in different ways at home, in institutional settings, and in industry. Depending on the processing method and the food, partial or complete removal of the offending allergen may be possible as illustrated by reduction of peanut allergen *in vitro* IgE immunoreactivity upon soaking and blanching treatments. When the allergen is discretely located in a food, one may physically separate and remove it from the food. For example, lye peeling has been reported to produce hypoallergenic peach nectar. Protein denaturation and/or hydrolysis during food processing can be used to produce hypoallergenic products. This paper provides a short overview of basic principles of food processing followed by examples of their effects on food allergen stability. Reviewed literature suggests assessment of processing effects on clinically relevant reactivity of food allergens is warranted.

Keywords: Allergen / Epitope / Food / Processing / Proteins

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1 Introduction

Food allergies are receiving increased attention [1–19] and seem to be on the rise, especially in Western countries. Consumers are becoming more aware and educated about food sensitivities including allergies. Recent introduction of food allergen labeling requirements in the EU and the US has further heightened awareness of food allergies among consumers, food processors, and regulatory agencies. It is important to note that systematic, and statistically validated data on true food allergies are globally lacking. It is therefore possible that while food allergies seem to be on the rise in Western countries, they may also be underreported or simply go undiagnosed in underdeveloped and developing countries [9]. Statistical data based on household surveys and not clinical testing/assessment, while useful, may over- or under-estimate true allergies. With Asian countries accounting for a major portion of the global population, true incidence of food allergies may be significantly higher than estimated and/or documented.

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Abbreviations: LTP, lipid transfer protein; **Lyz,** lysozyme; **OTf,** ovotransferrin; **OVM,** ovomucoid; **PPO,** polyphenol oxidase; **RBL,** rat basophil leukemia

For the purpose of this paper, type I food allergy is defined as an IgE-mediated response to a protein (or proteins) in a food source. It is not known why a food protein that is innocuous and well-tolerated by most individuals triggers an allergic response in sensitive individuals. Although the exact mechanism of food allergies remains elusive, IgE cross-linking on the surface of mast cells by the allergen seems to be an obligatory step in triggering an allergenic response in a sensitive individual. The portion of the food protein recognized by IgE is called the epitope. Epitopes are generally categorized as linear or conformational, where linear epitope involves a contiguous stretch of amino acids, and a conformational epitope involves noncontiguous amino acids which form a three-dimensional/structural motif. Individual patients may differ significantly in their sensitivity toward an allergen; however, the basis of such differential sensitivities remains to be elucidated. In addition, not much is known about the threshold values (both clinical and analytical) and the absolute minimum requirements (amino acid residues and the three dimensional conformational characteristics) for a molecule to be allergenic. Added to this conundrum of uncertainties are the gaps in our understanding of protein–protein interactions that govern antigen (Ag)–antibody (Ab) interactions, effects of food matrices on Ag–Ab interactions, and the lack of detailed understanding of the cascade of biochemical pathways at the molecular level that typically follow basophil degranulation. Additionally, role of IgG, among other immune components, in food allergies remains poorly defined [20].

The literature review provided below is not meant to be comprehensive. It is intended to provide few select examples to illustrate the diversity and complexity involved when assessing effects of processing on food allergens.

2 Why are foods processed?

Globally, food processing industry has a significant economic impact. With increase in urban populations, demand for processed foods continues to increase. Consumers buy processed foods for a variety of reasons, convenience being perhaps the most important one. Additional reasons for buying processed foods include variety, out of season availability of particular food in processed form (*e.g.*, several fruits and vegetables), lack of time/skills/equipment needed for home food preparation of certain foods, and assured consistent quality and safety. Regardless of the type of food or the processing method to which a food is subjected to, whether at home, institutional, or industrial setting; the reasons why foods are processed include: (i) improvement of food qualities such as flavor, texture, taste, color; (ii) improve preservation and safety; (iii) enhance suitability for specific product applications; (iv) convenience, pleasure, and variety; (v) obtain or generate useful by-products; and (vi) increase marketability and/or revenue. In addition to industrial food processing, a significant quantity of food is processed by consumers at home and in institutional settings. The choice of processing method can be influenced by available infrastructure, product type, scale of processing, consumer preference, product sensory qualities, and economics. For these and other reasons, the same raw food is often processed differently in different markets.

3 Food processing basics

For readers unfamiliar or less familiar with food processing, a very short review of basic principles of food processing is provided in this section. Regardless of the product, the scale of processing, and desired end-product qualities, food processing is based on a few basic principles. For example, from a food preservation point of view, it is desirable to control water activity (Aw) of a food product. Typically, at Aw ≤ 0.6, microbial food spoilage is prevented. Alternatively, food products can be treated with methods or substances that inhibit growth and/or viability of microorganisms (and their spores). Food being a live matter contains a variety of chemical and biochemical components that affect product quality positively or negatively. Thus, careful control of interactions between food components is essential in managing food quality, and inactivation of microorganisms to protect food from microbial spoilage may not be sufficient to produce and preserve an acceptable final product. Additional measures may be required, such as use of food additives and preservatives, to prevent unwanted chemical and biochemical interactions that occur in the food product. For example, fruits and vegetables undergo browning due to enzyme-mediated food component interactions involving polyphenol oxidases (PPOs), which catalyze the initial oxidation of *o*-dihydroxyphenols to semiquinones (the rate limiting step) in enzymatic browning. PPOs, which require Cu^{2+} and oxygen for activity, are located on the outer side of the cell wall, while the enzyme substrate is inside the cell in intact plant tissues. Consequently, unless the tissue is bruised, PPOs are inactive, as substrate(s) remain inaccessible. Once generated, semiquinones may be further oxidized to quinones and polymerized quinones, which produce variable discoloration of the food product. Depending on the food, such discoloration may be undesirable (*e.g.*, browning of apples, bananas, lettuce, carrots, *etc.*) or desirable (*e.g.*, black tea, coffee, chocolate, black olives, *etc.*). A number of methods have been developed to inhibit food discoloration; these include physical methods (*e.g.*, blanching, steaming, cooking) and use of chemical additives such as SO_2 or sulfites, metal chelators (*e.g.*, EDTA or EGTA), or use food grade acids such as citric acid. Use of acidulants such as citric acid is advantageous as citric acid can also chelate Cu^{2+}-, an essential co-factor for PPOs. Metal chelation has an additional benefit of preventing metal catalyzed oxidative reactions (*e.g.*, lipid oxidation).

Most food processing methods involve energy input and/or mass transfer. During processing, the processed food may remain in a single phase (state) or may experience phase change. For example, during pasteurization, milk remains in one phase (liquid) while cheese manufacture involves phase change as milk (liquid phase) is converted to curd (milk protein curd in solid phase) and whey (liquid). Similarly, the heating medium (*e.g.*, steam) may experience a phase change (steam- vapor phase condensing to hot water- liquid phase). Such phase changes are often accompanied by large energy transfers. A variety of processes are used to tailor the demands of processed food product and economy. As a result, myriad of food processing methods are used. Despite the variety, food processing methods can be broadly classified as thermal (*e.g.*, blanching, canning, cooking, and steaming) and nonthermal (*e.g.*, washing, filtration, enzymatic modification, irradiation, high pressure processing). In some cases, both thermal and nonthermal processing methods are used to produce a single food product (*e.g.*, canned vegetables and fruits).

4 Food processing methods

4.1 Home processing

At home, food processing may involve a single step such as baking cookies from prepared frozen dough. Another example is microwave heating used to reheat a prepared meal to be served as a part of a multi-course festive/celebra-

tory meal. The festive/celebratory meals often involve preparation of multiple dishes using a variety of preparatory/processing techniques and ingredients. Among the numerous methods commonly used at home for processing foods are peeling, washing, soaking, blending, grinding, milling, pounding, cutting, chopping, rolling, purée, straining, filtration, blanching, cooking, pressure cooking, frying (pan and deep), sauté, roasting, toasting, baking, smoking, grilling, extrusion, shredding, dehydration, microwaving, cooling, freezing, canning, bottling, and crystallization.

4.2 Institutional and restaurant processing

Many of the food processing methods are similar to those used at home with applicable adjustments related to production scale and desired outcome. For example, deep fat frying (*e.g.*, French fries, donuts, chicken nuggets, and others) in institutional and restaurant operations is typically done using large capacity fryers. Similarly brick ovens used in institutional and restaurant settings (*e.g.*, pizza baking) are specially fitted and are typically of higher capacity than those that may be used at home. Infra-red heating is used in food processing in institutional (*e.g.*, rotisserie chicken cooking) and industrial (*e.g.*, removing moisture after frying in potato chip manufacture) settings. Often the frequency and scale of use of these processing methods is higher than the ones used at home.

4.3 Industrial processing

In addition to methods listed above, mechanical harvesting and preparation (*e.g.*, washing, coring, peeling, cutting, chopping, dicing, slicing, splitting, *etc.*), various specialized drying methods (*e.g.*, spray drying, fluidized bed drying, drum drying, tray drying, tunnel drying, freeze-drying/lyophilization, *etc.*), ultrafiltration, reverse osmosis, γ-irradiation, freeze concentration, infra-red heating, high pressure processing, sonication, flash evaporation, electromotive force (*e.g.*, in Ohmic heating, electrical stimulation of carcass), chemical sterilization, ozone sterilization, ultra-high temperature (UHT) short time pasteurization/sterilization, high speed deep fat frying, high speed multi-temperature zone baking, lye peeling, various chilling, and freezing methods (*e.g.*, individual quick freezing- IQF of peas, chickens, *etc.*), and many others are also often employed.

5 Principles of thermal processing

In industry, thermal processing aimed at pasteurization or sterilization is perhaps the most widely used group of food processing methods. These methods are based on inactivation/destruction of a target bacteria (or spores) or a specific molecule (such as an enzyme or a toxin). Examples of target microorganisms used for process calculations and implementations are summarized in Table 1. If one were to start with the targeted microorganisms load at 10^6/g food, at the end of the 12D processing the microbial load would be reduced to 10^{-6}/g food. Therefore, typically 12D value is considered sufficient processing for most foods. It is important to stress here that starting microbial load in the food still must be controlled rigorously as high starting loads may increase survival rates and lead to food spoilage and/or presence of dangerous residual toxins. Since, food processing methods can be energy-intensive, every effort is made to save energy, while at the same time ensuring satisfactory processing of a specific food. Depending on the food and the microorganism/toxin/enzyme to be inactivated, suitable adjustments to processing conditions are often made. Acid content of food significantly influences the processing requirements as microbial resistance is partly dependent on food acidity. Typically, highly acidic foods (pH < 4.5) require less severe processing than moderately acidic (pH > 4.5 but <7.0) and neutral/alkali (pH ≥ 7.0) foods. Acidulants are therefore frequently used to reduce the processing costs.

Table 1. Resistance of spore-forming microorganisms as a basis of thermal processing

Microorganism	z value (°F)	$D_{121^\circ C}$ value (min)
Bacillus stearothermophilus	12.6	4.0
Bacillus subtilis	13.3–23.4	0.48–0.76
Bacillus cereus	17.5	0.0005
Bacillus megaterium	15.8	0.04
Clostridium perfringens	18.0	–
Clostridium sporegenes	23.4	0.15
Clostridium sporogenes (PA 3679)	19.1	0.48–1.4
Clostridium botulinum	17.8	0.21
Coxiella burnetti	8	–
C. thermosaccharolyticum	16–22	3.0–4.0

Source: Lund, D. B., Heat processing In Principles of Food Science, Part II. Karel, M., Fennema, O. R. Lund, D. B. (Eds.), *Physical Principles of Food Preservation*, Marcel Dekker, New York 1975, pp. 31–92, Chapter 3.
F value = time needed to reduce target population by a specified multiple of D value. D value refers to processing time to reduce the targeted population by a factor of 10 under the conditions used for processing the food.
z value = temperature change necessary to effect decimal reduction time by 90% (or one log cycle) of the target microorganism population

6 Food allergens

Before understanding how a specific food processing may affect a food allergen, it is important to appreciate not only the diversity of food processing methods but also the molecular nature of food allergens. As stated earlier, type I food allergies are mediated by an allergen (a food protein) cross-linking IgE molecules on mast cell/basophil surface. Potentially, any food protein can trigger food allergy and a single

food may contain one or more allergens. The portions of the protein molecule responsible for IgE cross-linking are referred to as epitopes. More than one epitope or IgE binding site is required *per* fragment of an allergen to cause IgE cross-linking. Therefore a molecule with a single IgE binding site must be bound or cross-linked to another molecule with an IgE binding site in order to cause histamine release. Understanding molecular properties of the epitope(s) is therefore important in learning the nature of IgE–allergen interaction. Two types of epitopes, linear, and conformational, may occur on an allergen. In case of linear epitopes, amino acid residues that determine whether allergen would bind with IgE or not are known as critical amino acid residues. Any modification, deletion, or substitution of such critical amino acid residues may result in loss of IgE binding and may potentially result in reduction and or elimination of allergenicity. If the epitope is conformational in nature, change in epitope conformation may permit modulation of allergic activity. Food processing, under appropriate processing conditions, offers opportunities to alter nature of epitopes. For example epitope conformation may be modified as a result of protein denaturation treatments (*e.g.*, various thermal processing treatments) leading to reduction/elimination or in some cases, an increase, in IgE binding. Acid or enzyme hydrolysis of an allergenic protein may help delete critical amino acids of an epitope. Whether caused by protein denaturation or hydrolysis, loss of epitope and ensuing loss of IgE binding may help reduce/eliminate the bioactivity of an allergen. It should be emphasized here that processing, depending on the allergen and the processing method, may not affect the allergenic properties of all allergens. One such example is that of Ara h 1, a peanut vicilin protein [21]. Heating Ara h 1 to temperatures of up to 140°C has been reported to have no effect on affinity of Ara h 1 toward human sera IgE (specific for peanut Ara h 1).

7 Activation energy

Since, food allergens are proteins, any modification of epitopes must consider the energy needed to denature the targeted epitopes of the protein. Protein denaturation is an energy-intensive process while enzyme-catalyzed protein hydrolysis is energetically far more economical (Table 2). One can not predict, *a priori*, whether epitope denaturation may lead to *in vivo* loss of allergenicity. Therefore, attempts to inactivate food allergens by food processing should carefully consider the energy requirements of epitope denaturation and also the assessment of clinically relevant *in vivo* bioactivity of the targeted epitope(s).

7.1 Influence of product on the selection of processing method

The influence of food product on the selection of food processing method is often significant. For example, cereal grains are often cooked, steamed, flaked, roasted, toasted, fermented, autoclaved, milled, extruded. However, cereal grains are typically not germinated unless malt is being prepared to be used for fermentation. Fluid milk is processed in a variety of ways but is seldom subjected to fluidized bed drying. Edible nut seeds are usually not fermented or germinated prior to consumption. Meats are typically not freeze dried. As mentioned earlier, for the same raw food, the choice of processing method may differ significantly, depending on the consumer preference. For example, boiled peanuts are preferred in China while boiling in salt water may be preferred by some in the Southern USA. Soaking peanuts in salt water followed by roasting on dry sand is a popular snack food in many parts of India. Oil roasted peanuts with adequate seasoning (salt, sugar, spices, and others) are a widely enjoyed snack.

Table 2. Energy of activation (Ea)

Process	Ea (kJ/mol)
Enzyme catalyzed reactions	17–60
Heat transfer coefficients	8–40
Physical process rates (*e.g.*, drying, crystallization)	17–60
Water vapor pressure	40
Chemical hydrolysis	60–110
Maillard browning	100–200
Protein denaturation	350–700
Destruction of microorganisms	200–700

Source: Thijssen, H. A. C., Kekrkhof, D. J. A. M., Effect of temperature-moisture content history during processing on food quality, in: Hoyem, T., Kvale, O. (Eds.), *Physical, chemical, and Biological Changes in Foods, Caused by Thermal Processing*, Applied Science Publishing, London, UK 1977 pp. 10–30.

7.2 Select examples of influence of processing on food allergens

As stated earlier, foods are processed for a variety of reasons. Different food processing methods have different effects on food protein structure; thus, some methods may increase, decrease, or have no effect on allergenicity of specific food proteins. Such effects may be governed by the molecular properties of an allergen and its interactions with food components. If the offending protein is small, it may be possible to physically remove it from the food product. Thermal processing is one of the most commonly used methods in food processing and depending on the severity of the treatment, thermal processing may alter protein structure. Effects of thermal processing on food protein allergenicity have therefore been reviewed in several recent articles [15, 16, 22–24]. More often, protein denaturation and/or modification to inactivate IgE-reactive epitopes may be a more practical choice. Alternatively, one may use pro-

teolysis to destroy IgE-reactive epitopes and reduce allergenicity. It is imperative to maintain food quality when such processing techniques are to be used to attenuate or eliminate food allergens.

A few select examples are presented below, to illustrate the opportunities and challenges in using food processing methods to reduce or eliminate food allergens.

(i) Peanuts: Beyer *et al.* [25] reported that cooking peanuts in boiling water (100°C for 20 min) or frying in vegetable oil (5 min for Valencia peanuts and 10 min for Florunner) reduced IgE binding intensity of Ara h 1 more than roasting (170°C, 20 min). In addition, the authors reported significantly less IgE binding to Ara h 2 and Ara h 3 in fried and boiled peanuts than in roasted peanuts. These studies indicate that both the type of processing and the allergen molecule are important when assessing the effects of processing on allergenicity. Mondoulet *et al*. [26] investigated the effects of boiling Virginia peanuts in water (100°C, 30 min) and found that the median IgE reactivity was1.5- to 2-fold lower in boiled than in raw or roasted peanuts. This decrease was attributed to the loss of soluble proteins in the water used for boiling the peanuts. The proteins recovered from the cooking water retained IgE reactivity, as assessed by Western blotting. The same study reported that roasting (commercially processed sample) increased the IgE binding capacity of Ara h 1 (7S globulin) and Ara h 2 (2S albumin). The investigators suggested that wet heat processing may denature some peanut allergens but such protein denaturation may not be adequate for the management and prevention of the peanut allergy risk. Chung *et al*. [27] and Chung *et al*. [28] reported that peroxidase treatment of peanuts reduced allergenicity of Ara h 1 and Ara h 2 in roasted but not in raw peanuts. The authors suggested that the oxidation products of PPO-catalyzed reactions lead to protein–protein cross-linking with subsequent formation of large cross-linked protein aggregates resulting in reduced accessibility of immunoreactive epitopes. The investigators used Western blotting and ELISA (IC_{50}, μg/mL) values for IgE binding to assess immunoreactivity. Compared to the control (5 μg/mL), the IC_{50} values for PPO/caffeic acid; caffeic acid, pH 10.5; and PPO; were 33, 20, and 14 μg/mL, respectively. While these IC_{50} values suggest an IgE reactivity reduction by 6.6-, 4.0-, and 2.8-fold (assuming linearity), they do not indicate elimination of the IgE reactivity. Such residual IgE reactivity in the processed product may be clinically relevant if it is within the threshold sensitivity range of an allergic patient. The study did not assess immunoreactivity of several other known allergenic proteins in peanuts. A recent study on peanut 2S albumin-type proteins (Ara h 2 and Ara h 6) by Lehmann *et al*. [29] reported the two allergens to contain a highly stable core structure that was resistant to proteolytic digestion as well as heating (up to 100°C). These investigators found that even though the IgE binding capacity was reduced by protease treatment, such reduction did not translate into reduction of the allergenic potential as assessed by mediator release from a functional equivalent of a mast cell or basophil, the humanized rat basophil leukemia (RBL) cell. Maleki *et al.* [30] demonstrated that trypsin inhibitory activity of purified Ara h 2 from roasted peanuts was four-fold higher than Ara h 2 from raw peanuts suggesting heat processing *increased* the bioactivity of Ara h 2. Mechanism for such an increase in trypsin inhibitory activity of Ara h 2 remains to be elucidated. Gruber *et al.* [31] compared the effect of thermal processing and nonenzymatic browning on IgE binding activities of recombinant Ara h 2 (rAra h 2) and native Ara h 2 (nAra h 2) (Maillard browning). The results suggested that IgE reactivity of rAra h 2 increased, thus confirming the earlier observations of Maleki *et al*. [30]. Roychaudhuri *et al.* [32] have shown that soybean Kunitz trypsin inhibitor is a 21.5 kDa allergenic protein that belongs to a family of all antiparallel β-sheet proteins that are highly resistant to thermal and chemical denaturation.

(ii) Hen Egg: hen egg white proteins ovalbumin (OA), ovomucoid (OVM), ovotransferrin (OTf), and lysozyme (Lyz) are known allergens (see [33] and references therein). Mine and Zhang [34] showed that reduction and carboxymethylation resulted in 22.6, 18.6, and 23.8% decrease in patient sera IgE binding of OTf, OVM, and Lyz; respectively. These investigators also noted that heat treatment (protein denaturation in 20 mM phosphate buffer, pH 7.2 at 95°C for 15 min) caused a 17.8 and 18.2% reduction in IgE binding capacity of OA and OVM, respectively. Interestingly, urea denaturation (6 M urea exposure at 37°C for 12 h) significantly increased, 63.1 and 51.00%, respectively, the IgE binding activity of OTf and Lyz. Although these studies were done *in vitro* the data do suggest that protein conformation and its interaction with the environmental factors are of consequence when assessing the allergenic potential of the targeted proteins.

(iii) Cow's Milk: cow's milk contains various milk caseins and whey proteins, many of which are known allergens [35]. Milk allergies are outgrown by many but not all infants [36]. Ehn *et al*. [37] showed that *Lactobacilli* fermentation of milk caused proteolysis of β-lactoglobulin, one of the whey proteins known to be an allergen, but did not decrease the IgE binding when compared with its counterpart from nonfermented pasteurized milk. Trypsin hydrolysis of β-lactoglobulin, however, seemed to significantly decrease IgE binding. Sélo *et al*. [38] also showed that some of the allergenic epitopes of β-lactoglobulin survive proteolysis. Conjugating acidic oligosaccharides (alginic acid and phosphoryl oligosaccharides) to bovine β-lactoglobulin has been shown to reduce T cell responses in experimental animals [39, 40]. The possible mechanisms suggested for this reduced response were: (i) steric hindrance for the reactive epitopes by the bulky oligosaccharides used for conjugation, and (ii) interruption/disruption of phagocytosis by the acidic polysaccharides used for conjugation during the antigen pickup by the antigen presenting

cells. Whether such an approach would be useful for human allergy management remains to be determined.

(iv) Barley lipid transfer protein (LTP) 1: LTPs are ubiquitous lipid binding proteins found in plants, originally thought to be responsible for transfer of lipids across membranes *in vitro*. The *in vivo* role of LTPs remains unknown [41]. Garcia-Casado *et al*. [42] purified barley LTP 1 (a 9 kDa protein), using sera from four patients known to be allergic to beer. Immunoblotting, and skin prick testing, demonstrated the LTP 1 to be an allergen. Perrocheau *et al*. [43] have subsequently shown that barley LTP 1, an abundant soluble protein (~5% of soluble barley proteins) in aleurone layers from barley endosperm, is stable toward malting, glycation, and brewing processes.

(v) Wheat proteins: Simonato *et al*. [44] systematically investigated the stability of wheat proteins during bread making using patient sera IgE binding as well as Western blotting experiments to demonstrate that although some allergenic proteins (*e.g*., a 16 kDa allergen) were destroyed by baking, many wheat allergenic proteins remain stable, in bread crust and crumb. Further, these investigators also found that bread making process (especially baking) made some proteins more resistant to *in vitro* pepsin digestion. The crust experiencing higher temperature (>180 – 200°C) than the crumb (<100°C) registered significant protein aggregation as evidenced by the presence of smeared protein bands at the top of the separating gels and that such aggregates contained carbohydrates (as shown by carbohydrate staining of the gels). The authors further noted that IgE binding proteins in the bread crumb had similar electrophoretic mobility before and after *in vitro* pepsin digestion while those in the crust failed to be efficiently extracted by SDS and β-mercaptoethanol. These data indicate that bread baking produced protein aggregates in crumb and crust that had differing solubility. The authors suggested the crumb aggregates were stabilized mainly by hydrophobic bonds and disulfide linkages while in case of crust Maillard browning reactions seemed to have produced insoluble protein aggregates.

(vi) Sesame 2S Albumin (Ses i 1): Moreno *et al*. [45] using proteomics identified and purified a sesame allergen Ses i 1, a 12.062 kDa protein, to homogeneity and found the protein to be stable to heat up to 90°C at acidic and neutral pH. The protein was also highly resistant to digestion under physiologically relevant conditions. Heating or inclusion of a surfactant phosphatidylcholine prior to gastric and duodenal digestion did not facilitate hydrolysis of the protein. The authors indicated that these findings were consistent with the literature reporting stability of Brazil nut 2S albumin allergen Ber e 1.

(vii) Lychee Fruit: Hoppe *et al*. [46] investigated various processing parameters pertinent to industrial processing, notably thermal processing encountered during canning, as well as postprocessing storage at 4°C for 8 months on lychee fruit allergen stability. They found that only under severe processing conditions (sterilization) several bands (24, 28, 32, 35 kDa) lost their IgE binding ability. The sterilization parameters used were: F-value = 12.4 ± 0.23, maximum internal temperature = 121.5°C, maximum autoclave temperature = 120 – 121°C, and holding time at the maximum autoclave temperature = 12 min. However, the 55 kDa polypeptide remained intact and did not lose its ability to bind with the patient sera IgE. These data suggested that while certain allergens in lychee fruit may be heat labile others were not sensitive enough to heat processing to be eliminated or rendered inactive. It is important to note that under the processing conditions where several allergens were denatured, the fruit quality was also adversely affected resulting in a product with unacceptable sensory, especially color, and texture, qualities.

(viii) Mango: using pooled sera from nine mango allergic patients and inhibition enzyme allergosorbent tests (EAST-inhibition), SDS-PAGE, and immunoblotting; Dube *et al*. [47] assessed effects of various processing parameters in mango fruits (originating in Costa Rica) purée and nectar manufacturing and found that processing decreased EAST-inhibition activity in processed samples but did not eliminate mango allergenicity.

(ix) Apple nsLTP: nonspecific lipid transfer protein (nsLTP) in apples (Mal d 3) was reported to be heat stable (90°C, 20 min heating) in presence/absence of glucose. However, severe heat treatment (100°C, 2 h) did decrease IgE binding (30-fold) and biological activity (100- to 1000-fold). Adding glucose increased the stability of Mal d 3 in processing [48]. These results suggest that appropriate processing conditions may help reduce (but not eliminate) Mal d 3 allergenicity.

(x) Lupine seed proteins: extrusion cooking, boiling (100°C, up to 60 min), autoclaving (121°C, 1.18 atm., up to 20 min and 138°C, 2.56 atm, up to 30 min), and microwave heating (30 min) were evaluated for possible effects on IgE binding proteins. The findings included disappearance of two IgE reactive bands (23 and 29 kDa) upon autoclaving at 138°C for 20 min with a simultaneous generation of previously undetected 70 kDa band (in the unprocessed control) suggesting formation of a neoallergen. The appearance of the 70 kDa band, upon autoclaving, may be due to aggregation of smaller polypeptides or breakdown of a large protein. The authors opined that autoclaving was a promising treatment to consider [49].

(xi) Peach allergen: Brenna *et al*. [50] used Western blotting and immunoblotting inhibition as the assessment tools and noted peach allergens to be thermostable (121°C for 10 and 30 min). Ultrafiltration (membrane nominal molecular mass cut off of 10 kDa) could remove the low molecular mass allergenic polypeptides (molecular mass < 14 kDa). The authors noted that lye peeling was also effective producing a hypoallergenic nectar.

(xii) Tree nut seed allergens: stability of almond, cashew, and walnut allergens toward a variety of processing methods including γ-irradiation alone as well as γ-irradiation

followed by blanching, autoclaving, roasting, frying, and microwave heating has been reported [51, 52]. Similarly, hazelnut allergens have been shown to be heat stable [53]. Koppelman *et al*. [54] noted Brazil nut 2S allergen (Ber e 1) to be stable toward thermal treatment as well as proteolysis. More recently Venkatachalam *et al*. [55] found pecan allergens to be thermostable as well as stable toward *in vitro* gastric and pancreatic digestions. Sze-Tao *et al*. [56] showed that walnut proteins are stable during storage at -70°C for 10 months or -0°C for 7 months.

Pollen contamination of foods may complicate assessment of food allergens. A recent study on birch pollen allergen Bet v 1 (Schimek *et al*. 2005) [57] is particularly instructive in this regard. The study found that gastrointestinal digestion of Bet v 1 eliminated the histamine releasing but not T-cell activating property of Bet v 1. The RBL cell sub line transfected with human FceRI (RBL-30/25) was used in the study. The study demonstrated presence of birch pollen specific T-cells and that the exposure of hazelnut to gastrointestinal proteases did not completely abolish the ability of hazelnut allergens to cross-react with Bet v 1-specific T-cells.

8 Conclusions

Processing of foods can influence the allergenicity of food proteins. The extent of the effects depends on several factors, including the allergen and its biochemical and immunological properties, food matrix, processing conditions, thermodynamics of allergen–IgE interaction, and patient sensitivity (threshold, tolerance, and permanency of allergenic reaction to a specific allergen). Small allergenic proteins may be physically removed from certain foods; in contrast, for larger proteins, enzymatic hydrolysis, chemical modification, or a combination of physical, chemical and biochemical processes are often required to reduce or eliminate food allergens. Protein oligomerization, as a result of processing, is one of the major contributing factors in how processing alter allergens. Labile oligomeric proteins on the other hand may undergo separation of constituent polypeptides. If the separated polypeptide is responsible for the allergenicity of the native molecule, subsequent removal of the polypeptide from the food containing the native oligomeric protein may help reduce/eliminate allergenicity of the food. Literature survey suggests investigations focused on assessing allergenic potential of industrially processed foods/food components are very limited. A recent investigation [58] illustrates one of the ways how such studies may be conducted. The study, which tested 108 commercially-produced Australian wines, used a double-blind placebo-controlled challenge *via* skin prick test for selected wines and a basophil activation assay for a separate group of wines. The selected wines were fined by different methods including egg white (24 wines), isinglass (23 wines), milk proteins (34-milk fined and 25-casein fined) or nongrape-derived tannins (21 wines). The study included one white and two red "control" wines that were made using nonfood protein-processing aids and or filtration. The patients used in the study had confirmed allergies to fish (ten patients), peanuts (ten), eggs (five), and milk (one). Eleven nonfood-allergic control subjects were also included in the study. Among the tested clarifying agents, egg whites and milk proteins are known food allergens. The study concluded that "the normal, highly regulated, and standardized wine-making process presents an extremely low risk of anaphylaxis" even when food proteins known to be allergens for adult consumers were used in the wine-making process. It is not apparent why these investigators used only one patient with confirmed milk allergies when testing 34 milk protein fined wines while including 10 peanut allergic patients when no wine sample fined with peanuts was included in the study. Protein hydrolysis, chemical and/or enzymatic, may provide a practical approach to inactivate allergenic protein. The rationale for such an approach is that hydrolysis may sufficiently modify or even destroy allergy relevant epitopes permitting production of safe hypoallergenic products. Indeed a number of hypoallergenic feeding formulae are globally advertized and sold. Such formulae may be useful for patients who do not exhibit high sensitivity toward offending agent(s). While hypoallergenic formulae may work for some individuals, caution is warranted in making declarations akin to (i) hypoallergenic formulae are safe for all patients and (ii) hypoallergenic formulae prevent allergies. A recent paper [59] concluded that currently available evidence on the efficacy of hypoallergenic formulae in preventing allergy is insufficient to justify blanket advice about preventing allergies. Simple economical home processing methods such as fermentation and germination remain largely underexplored as such processes may be useful in reducing allergens in certain seed samples (*e.g*., mung beans, chick peas, and others) where the end product can tolerate such processing. Depending on the patient immunological profile and the allergen, partial inactivation may be sufficient to mitigate/eliminate the allergic response in certain patients. However, in case of exquisitely sensitive patients, complete avoidance of the offending food may be the only practical option currently available to safeguard the patient from unwarranted exposure to the offending food allergen. While food processing may be able to inactivate/eliminate certain allergens either partially/completely in specific foods, unless clinically relevant thresholds, and no observable effect (*i.e*., symptoms) levels for the targeted food product (NOEL) and the population consuming such product are established, it may not be possible to predict or determine adequacy of food processing in inactivating targeted food allergens. In certain instances, food processing may be unable to affect allergens. Another emerging trend in the food industry is the role of "functional foods in human health" with special emphasis on various health benefits that may result from consuming specific foods/food components. One such area is the use of probiotics in allergy pre-

vention and treatment. A decline in exposure to different microbes during early childhood and its subsequent effect on less than optimal immune system development, the hygiene hypothesis, has prompted many to investigate the effects of probiotics on food allergies. Probiotics are thought to have immunomodulatory effects. However, a recent paper [60] suggested that there is insufficient evidence to indicate that probiotics are effective in preventing allergies.

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