

Food processing: effects on allergenicity

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Purpose of review

There has been an increase in the prevalence of food allergy, asthma and severe reactions to foods in the past decade. The reason for this increase is unknown and despite the potential for a lethal outcome, no treatments or therapies are available. Many areas are being explored to understand the increase in food allergy and the role of modern processing techniques is one of them.

Recent findings

The fact that processing influences allergenicity is not a new concept, but due to some definitive studies in the last few years the topic has been revitalized. Not only is the processed form of a particular allergen important in assessment of allergenicity, but the role of the food matrix in which an allergen is processed must also be evaluated. The issue of allergenicity of particular foods is therefore highly complex, and even with our existing knowledge we have just begun to understand some of the contributions of processing to allergenic properties of proteins and foods.

Summary

Researchers and medical personnel have begun to weigh the benefits and potential disadvantages of assessing the allergenicity of foods in unprocessed forms. In this review, the known effects of processing on the allergenicity of various foods will be discussed along with the implications of the most recent studies on the future direction of research in this area.

Keywords

allergenicity, processing, IgE, Maillard reaction

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Abbreviations

DBPCFC	double-blind, placebo-controlled food challenge
LTP	lipid transfer protein
RBL	rat basophilic leukemia

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Introduction

The prevalence of asthma, food, and environmental allergies seems to be increasing in industrialized countries. Prevalence studies of peanut and tree nut allergy in the UK, the US and Canada have reported an increased rate of sensitization [1–4] and a recent study confirmed previous reports that food allergy is a significant risk factor for life-threatening asthma in childhood [5]. Many reasons have been suggested for the rise in prevalence of food allergies. Among these reasons are consumption of novel and diversified foods, the advancement of food technology and the development of novel processing methods. Interestingly, the only successful treatments of food allergy have consisted of either avoidance or reduction of the allergenicity of a food through processing or genetic engineering [6–8]. Factors such as food composition, as well as the length, time, temperature and conditions of harvest, drying, storage, heating, and cooking can alter the properties of proteins in foods. Due to the large number of processes that different foods undergo before reaching consumers, and the limited number of studies in this area, the relationship between food antigenicity and processing is poorly understood. Thermal and nonthermal processing can cause the food proteins to become more or less antigenic or result in new allergens (neoallergens) being formed. Therefore, the study of processing is a necessary part of assessing the allergenicity of existing and newly introduced foods (reviewed in [9,10]).

Originally, it was thought that thermal processing would reduce allergenicity [11] due to the known disruptive effects of heat on most proteins. Interestingly, there have been cases of food allergy that either confer with or differ from this observation. For example, one of the very first documented allergic patients was found to react to proteins in cooked fish, but not raw fish [12]. Years later, Urisu *et al.* [13] demonstrated that some patients that were allergic to raw, freeze-dried egg did not have a reaction to cooked egg white. Cooke and Sampson [14] suggested that the IgE of patients, recognizing conformational (nonsequential) epitopes, reacted to raw egg, but not to cooked egg white, which was thought to be due to the destruction of the conformational epitope in cooked egg white. Similar findings were documented in the case of milk allergy in a more recent study [15]. There have also been studies that claim thermal processing or heating does not alter

the allergenic properties of proteins in foods (for example [16]). Although this is quite possible, these studies most often involve purified proteins, in the absence of sugars, lipids, oxidizing agents and other proteins, which normally exist in a food matrix during processing. Moreover, convincing evidence exists for the appearance of neoallergens due to processing. In one study, a patient showed a reaction to pecan nut after roasting or 2 weeks of storage, but not to fresh pecans [17]. Further evidence for the formation of neoallergens was provided in studies involving long-term storage of wheat flour extracts [18], heated soybean hulls and asthma [19], enhanced skin reactivity of β -lactoglobulin subjected to the Maillard reaction [20], and increased IgE binding to roasted peanut proteins [21].

The effects of processing on proteins

The ability of specific proteins to cause an allergic response in susceptible individuals is well established. Some widely accepted characteristics of allergenic proteins is that they bind to serum IgE, induce a Th2-type response, are relatively resistant to digestive enzymes, contain post-translational glycosylation and often have enzymatic activity such as proteolysis. Since processing can cause thousands of biochemical reactions to occur among food components, it is very important to determine which, if any, of these reactions influence the allergenicity of known allergens or other proteins within a food matrix. Previous studies with peanut allergens demonstrated that roasted peanut extracts bind serum IgE from allergic individuals at approximately 90-fold higher levels than raw peanuts, and the Maillard reaction contributes to the observed effect [21–24]. These studies unambiguously demonstrated that peanut protein extracts, Ara h 1 and Ara h 2 (two of the major allergens), from roasted peanuts are less soluble, less digestible, have higher IgE binding and undergo structural alterations that enhance their allergenic properties. Additionally, sensitization studies with roasted peanuts have shown that mice are more likely to become sensitized to roasted peanuts than to raw peanuts (K. Yamaki, S. Maleki, unpublished data). Interestingly, the mechanism thought to contribute to enhanced allergenicity of peanut proteins was found to be different for the two allergens. Reversible Ara h 1 trimers purified from raw peanut were found to become covalently cross-linked and modified by sugar molecules during roasting to form irreversible complexes. The Ara h 1 oligomer complex was shown to be less soluble and more resistant to digestive enzymes than the Ara h 1 monomer or reversible trimer [25]. Theoretically, inaccessible digestion sites permit passage of large fragments of Ara h 1 containing several intact IgE binding sites across the lumen of the small intestine, therefore contributing to its allergenicity [25]. The

majority of peanut proteins, modified via the Maillard reaction and other biochemical by-products of roasting, become less soluble, but bind higher levels of IgE. Ara h 2, with protein domain homology to trypsin inhibitors, was purified from raw peanuts and shown to have weak trypsin inhibitory activity [26•]. However, the trypsin inhibitory activity of Ara h 2 purified from roasted peanuts was enhanced.

Ara h 2 is a protein that consists mostly of α helices with eight cysteine residues. Partial reduction (reduction of some, but not all disulfide bonds) of Ara h 2 is possible without significant impact on the secondary structure, but results in a notable difference in trypsin inhibitory function. A full reduction of disulfide bonds, however, leads to significant alteration of structure and function. Based on secondary structure and functional analysis it was concluded that partially reduced Ara h 2 from raw peanuts mimicked the enhanced activity of roasted Ara h 2. Since the Maillard reaction can involve loss, disruption or rearrangement of sulfhydryl groups, it is reasonable to assume that one or more of the disulfide bonds of Ara h 2 are affected during roasting. In this study, not only was roasted Ara h 2 more resistant to digestion with trypsin, but it also protected Ara h 1 from digestion with trypsin. These results suggest a novel mechanism for synergistic enhancement of allergenic properties of different proteins in the same food or within a food matrix that can be further intensified due to processing.

A study by Beyer *et al.* [27] found that roasting peanuts resulted in increased IgE binding, confirming the previous work. By contrast, two recent studies analyzed the IgE binding properties of raw and roasted peanuts. One study used enzyme allegro sorbent test and serum IgE from 37 patients and found no difference in IgE binding between raw and roasted peanuts [28]. A second study [29] demonstrated the similarity in IgE binding to different raw and roasted nut extracts by direct ELISA. The discrepancy here is not surprising. In addition to the utilization of different assays, several parameters can contribute to divergent results. Primarily, the degree of processing such as roasting or cooking can affect the results of digestibility, solubility, IgE binding and other experimental parameters dramatically. The most significant consideration, however, is that roasting and frying or browning in general can cause large changes in the biochemical characteristics of proteins due to the Maillard reaction alone. Proteins can form oligomers, become denatured, degraded, aggregated, cross-linked, fragmented and re-assembled and these changes most often cause a reduction in solubility or can alter the binding capacity of proteins or peptides to ELISA plates. Furthermore, proteins become differentially soluble, therefore often one allergen, such as Ara h 1 in peanut, aggregates and falls out of solution while Ara h 2 remains

soluble. This does not automatically mean – although it can – that the extract is nonallergenic, reduced or equal in allergenicity, most often it just means that the soluble portion of the food assessed contains less allergenic proteins or products. In summary, if both the soluble and insoluble fractions of roasted peanuts or browned foods are assessed [22] or an equivalent amount of protein is in the soluble fraction, the IgE binding increases with the degree and time of browning.

Clinical studies

The allergenicity of heat-processed celery, maize and roasted hazelnut was assessed by in-vitro IgE-binding assays and double-blind, placebo-controlled food challenge (DBPCFC) [30,31,32•]. In the study with celery [30] no patients were found that reacted only to cooked and not to raw celery, which argues against the formation of neoallergens in cooked celery. When rat basophilic leukemia (RBL) cells were loaded with serum IgE from mice sensitized to cooked celery, however, the raw celery had the weakest mediator release capacity. The reverse of this was true when RBL cells were loaded with IgE from mice sensitized to raw celery. This result argues for either the formation of a neoallergen in cooked celery or the possibility that novel IgE molecules are formed in the rats sensitized with cooked celery that do not recognize raw celery allergens as well. In addition, RBL cells loaded with mouse serum containing IgE against raw celery were all reactive (caused mediator release) to Api g 1, a major celery allergen, but no mediator release was obtained when serum from mice sensitized to cooked celery was used, which indicates that the allergenicity of Api g 1 is eliminated or highly reduced with cooking. This finding is also confirmed in enzyme allegro sorbent test inhibition experiments using serum from an individual that exclusively recognizes Api g 1 and cooked celery as inhibitor. Comparison of the clinical and in-vitro data, however, does not allow the authors to predict which patients will be reactive to cooked celery.

Another study that compares allergenicity of maize proteins in raw and thermally processed forms [31] suggests that the lipid transfer protein (LTP) is heat resistant and most likely does not contain conformational epitopes. While this study makes an important contribution to the field by demonstrating that cooking does not eliminate the allergenicity of maize, it is possible that reduced solubility of maize allergens should be considered in addition to enhanced stability. Specifically, when urea and SDS extracted whole proteins (soluble and insoluble proteins) are assessed for IgE binding, the LTP is mostly in the soluble fraction. When patient's serum, that is not exclusively LTP reactive, is used many other IgE reactive proteins and smears of proteins are seen in both soluble and whole protein fractions.

Indeed, the proteins in the different fractions are not all the same, which is not unexpected due to differential solubility. Reduced solubility of higher molecular weight proteins (possibly due to aggregation, denaturation, modification or degradation) is observed when the SDS-PAGE profiles of the soluble portions of raw and time-dependent, heated maize are compared. The authors in this study argue that the working epitopes on the LTP are linear rather than conformational due to the strong IgE binding to LTP in the heated samples in a Western blot analysis. The fact that LTP is soluble and binds IgE in a Western blot analysis does not argue for heat or structural stability. Also in this paper, purified LTP was heated to different temperatures; the secondary structure was assessed by circular dichroism and found to be partially stable, even at 100°C. It is important to note that structural conformation of thermally treated, purified LTP may not be representative of LTP heated within the context of other maize constituents. LTP is most likely more stable within the context of the maize than in purified form.

A third study [32•], by the same group, examining roasted hazelnut had a result that differed from the findings with peanut, maize and celery. In this study, the IgE binding, RBL mediator release, skin prick test (SPT) and DBPCFC to hazelnut was reduced with roasting. This was an interesting study because it was carried out on a population that reacts to birch pollen and the pollen related allergen, Cor a 1.04, which is homologous to and exhibits cross reactivity with Bet v 1. Some of the patients in this study also recognized recombinant Cor a 2, a profolin, but none of the individuals reacted to Cor a 8, a lipid transfer protein, or the seed storage proteins (i.e. Cor a 9). Previous reports indicate that heat treatment reduces the reactivity of Cor a 1 with both birch pollen and hazelnut specific IgE. Therefore, these results are not surprising, but they show that a specific process or treatment, such as roasting, can eliminate or reduce the allergenicity of a particular food for at least a certain population. Also, it indicates that studies with homologous, cross-reactive antigens can be useful in determining epitope or allergen inactivation due to certain treatments such as heat. Another interesting finding was that approximately 30% of the population tested still recognized and had a mild reaction to roasted hazelnut. One possibility is that some of the Cor a 1 remains partially intact in the insoluble fraction and is not detected with IgE binding assays in which only the soluble fraction of the defatted hazelnut is used. Also, the formation of a neoallergen (for the individuals tested) or IgE to a known allergen, other than Cor a 1, in the insoluble fraction of the roasted hazelnut cannot be discounted. Unfortunately, the in-vitro assays were not predictive of patients that would react to an oral challenge with roasted nuts. Considering

that the Maillard reaction, known to enhance IgE binding, occurs during roasting of hazelnut it would be very interesting to repeat these studies with patients with sera directed against the non-pollen related hazelnut allergens, particularly the seed storage proteins, known to be affected by the Maillard reaction in peanuts. While, it is important to note that the specific effects of processing and the Maillard reaction on the allergenicity of different foods is not fully understood, it is also intriguing to find that a major hazelnut allergen may be mostly degraded or denatured during the roasting process and rendered less allergenic. This suggests that roasting can cause either an increase or decrease in allergenicity, depending on the particular allergenic protein or maybe even the IgE binding capacity of a particular allergic population. These studies are proof of the complexity of the effects of processing on allergenicity.

Conclusion

The studies addressed here clearly indicate that thermal processing as well as other processing events can dramatically alter the structure, function, and allergenicity of foods and that these alterations can be identified, characterized and manipulated through processing. The incomplete knowledge of the allergens in processed foods and commercially available diagnostic extracts increases the complexity of diagnosing food allergies [32]. To date, with the exception of genetic manipulation of plants, which have been equally successful, the only approach to reducing the allergenicity of foods has been through processing [6–8]. Processing methods have been and can be developed to reduce or even eliminate the allergenicity of certain foods. These hypoallergenic foods can then be utilized as desensitizing tools for allergic individuals as seen in the case of rice and wheat allergy.

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References and recommended reading

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

- of special interest
- of outstanding interest

- 1 Sicherer SH, Munoz-Furlong A, Sampson HA. Prevalence of peanut and tree nut allergy in the United States determined by means of a random digital dial telephone survey: a 5 year follow up. *J Allergy Clin Immunol* 2003; 112:1203–1207.
 - 2 Kagan RS, Joseph L, Dufresne C, et al. Prevalence of peanut allergy in primary school children in Montreal, Canada. *J Allergy Clin Immunol* 2003; 112:1223–1228.
 - 3 Tariq SM, Stevens M, Matthews S, et al. Cohort study of peanut and tree nut sensitization by age of 4 years. *BMJ* 1996; 313:514–517.
 - 4 Grundy J, Matthews S, Bateman B, et al. Rising prevalence of allergy to peanut in children: data from 2 sequential cohorts. *J Allergy Clin Immunol* 2001; 110:784–789.
 - 5 Roberts G, Patel N, Levi-Schaffer F, et al. Food allergy as a risk factor for life-threatening asthma in childhood: a case controlled study. *J Allergy Clin Immunol* 2003; 112:168–174.
 - 6 Ikezawa Z, Ikebe T, Ogura H, et al. Mass trial of hypoallergenic rice (HRS-1) produced by enzymatic digestion in atopic dermatitis with suspected rice allergy. HRS-1 Research Group. *Acta Derm Venereol Suppl (Stockh)* 1992; 176:108–112.
 - 7 Ikezawa Z, Tsubaki K, Yokota S. Effect of hypoallergenic wheat (HAW_A1) on atopic dermatitis (AD) with wheat allergy, and its antigenic analysis using sera from patients with AD. *Alerugi* 1994; 43:679–688.
 - 8 Nakamura R, Matsuda T. Rice allergenic protein and molecular-genetic approach for a hypoallergenic rice. *Biosci Biotechnol Biochem* 1999; 60:1215–1221.
 - 9 Wal J-M. Thermal processing and allergenicity of foods. *Allergy* 2003; 58:727–729.
 - 10 Sanchez C, Fremont S. Consequences of heat treatment and processing of food on the structure and allergenicity of component proteins. *Rev Fr Allergol Immunol Clin* 2003; 43:13–20.
 - 11 Malanin K, Lunberg M, Johansson SGO. Anaphylactic reaction caused by neoallergens in heated pecan nut. *Allergy* 1995; 50:833–836.
 - 12 Prausnitz C, Kustner H. Studies on supersensitivity. *Centralbl Bakteriell Abt Orig* 1921; 86:160–169.
 - 13 Urisu A, Ando H, Morita Y, et al. Allergenic activity of heated ovomucoid-depleted egg white. *J Allergy Clin Immunol* 1997; 100:171–176.
 - 14 Cooke SK, Sampson HA. Allergenic properties of ovomucoid in man. *J Immunol* 1997; 159:2026–2032.
 - 15 Vila L, Beyer K, Jarvinen KM, et al. Role of conformational and linear epitopes in the achievement of tolerance in cow's milk allergy. *Clin Exp Allergy* 2001; 31:1599–1606.
 - 16 Koppelman SJ, Bruijnzeel-Koomen CADM, Hessing M, et al. Heat induced conformational changes of Ara h 1, a major peanut allergen, do not affect its allergenic properties. *J Biol Chem* 1999; 274:4770–4777.
 - 17 Berrens L. Neoallergens in heated pecan nut: products of Maillard-type degradation? *Allergy* 1996; 51:277–278.
 - 18 Berrens L. Standardization of allergens by complement consumption. *Ann Med Nancy (Symposium IgE)* 1977; 17:69–73.
 - 19 Rosen JP, Selcow JE, Mendelson LM, et al. Skin testing with natural foods in patients suspected of having food allergies: is it necessary? *J Allergy Clin Immunol* 1994; 93:1068–1070.
 - 20 Bleumink E, Berrens L. Synthetic approaches to the biological activity of beta-lactoglobulin in human allergy to cow's milk. *Nature* 1966; 212:541–543.
 - 21 Maleki SJ, Chung SY, Champagne ET, et al. The effects of roasting on the allergenic properties of peanut proteins. *J Allergy Clin Immunol* 2000; 106:763–768.
 - 22 Maleki SJ, Chung SY, Champagne ET, et al. Allergic and biophysical properties of peanut proteins before and after roasting. *Food Allergy Intolerance* 2001; 2:211–221.
 - 23 Chung SY, Champagne ET. Association of end-product adducts with increased IgE binding of roasted peanuts. *J Agric Food Chem* 2001; 49:3911–3916.
 - 24 Chung SY, Butts CL, Maleki SJ, et al. Linking peanut allergenicity to the processes of maturation, curing and roasting. *J Agric Food Chem* 2003; 51:4273–4277.
 - 25 Maleki SJ, Kopper RA, Shin DS, et al. Structure of the major peanut allergen Ara h 1 may protect IgE-binding epitopes from degradation. *J Immunol* 2000; 164:5844–5849.
 - 26 Maleki SJ, Viquez O, Jacks T, et al. The major peanut allergen, Ara h 2, functions as a trypsin inhibitor and roasting enhances this function. *J Allergy Clin Immunol* 2003; 112:190–195.
- This study is one of very few that describes a molecular mechanism for the enhanced allergenic properties of an allergen due to processing. In addition, it provides evidence that proteins or components within a food matrix can influence the allergenicity of other proteins, which in turn can be affected by processing.
- 27 Beyer K, Morrow E, Li X-M. Effects of cooking methods on peanut allergenicity. *J Allergy Clin Immunol* 2001; 107:763–768.
 - 28 Mondoulet L, Drumare M-F, Ah-Leung S. Influence of thermal processing on the IgE binding capacity of peanut allergens. *Rev Fr Allergol Immunol Clin* 2003; 43:486–491.

- 29 de Leon MP, Glaspole IN, Drew AC, *et al.* Immunological analysis of allergic cross-reactivity between peanut and tree nuts. *Clin Exp Allergy* 2003; 33:1273–1280.
- 30 Ballmer-Weber BK, Hoffmann A, Wuthrich B, *et al.* Influence of food processing on the allergenicity of celery: DBPCFC with celery spice, cooked celery in patients with celery allergy. *Allergy* 2002; 57:228–235.
- 31 Pastorello EA, Pompei C, Pravettoni V, *et al.* Lipid-transfer protein is the major maize allergen maintaining IgE-binding activity after cooking at 100°C, as demonstrated in anaphylactic patients and patients with positive double-blind placebo controlled food challenge results. *J Allergy Clin Immunol* 2003; 112:775–783.
- 32 Skamstrup Hansen K, Ballmer Webber BK, Kuttkopf D, *et al.* Roasted hazelnuts-allergic activity evaluated by double-blind placebo-controlled food challenge. *Allergy* 2003; 58:132–138.

The work in this paper very comprehensively addresses the issue of roasting and allergenicity of hazelnut. IgE binding studies and histamine release assays are followed up by DBPCFC. Clinical reduction in the allergenicity of hazelnut through roasting confirms that processing can be used to alter the allergenicity and sensitization to particular foods. It also indicates that the same processing method can affect similar foods differently.