High-pressure and temperature autoclaving of peanuts reduces the proportion of intact allergenic proteins

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Abstract

BACKGROUND Peanut allergy is a particularly common cause of anaphylaxis and utilization of hospital emergency room resources. Peanut protein allergens do not appear to denature under normal cooking conditions. We evaluated the effects of thermal processing on the protein allergens Ara h 2, associated with a risk for anaphylaxis, and Ara h 8, a protein analogous to birch pollen associated with oral allergy symptoms. METHODS Raw, roasted and autoclaved peanuts were evaluated. Solution 1H NMR spectroscopy was used to obtain molecular profiles and identify chemical changes across processing conditions. Western blot and ELISA analyses were used to detect relative levels of specific peanut allergens. RESULTS NMR analysis of peanut-soaked solutions demonstrated an overall reduction of total intact protein in autoclaved peanuts as shown by the broadening of peaks in the spectral regions corresponding to peptide fragments when compared to raw. The results also showed that autoclaving reduces the amount of allergenic proteins Ara h 2 (50% reduction) and Ara h 8 (100% reduction). Upon skin prick testing of allergic subjects, this differential degradation demonstrated that the autoclaved peanut could be used to categorize patients into two groups: those at risk for anaphylaxis and those who only experience oral symptoms to peanut (predominantly Ara h 2- and Ara h 8-specific IgE, respectively). CONCLUSION The data reported in this study suggest that high-pressure and temperature autoclaving reduces the amount of intact protein in the peanut, including allergenic proteins. This could be further developed into an improved diagnostic test for peanut allergy.

Title:

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METHODS

Raw, roasted and autoclaved peanuts were evaluated. Solution^{1}H NMR spectroscopy was used to obtain molecular profiles and identify chemical changes across processing conditions. Western blot and ELISA analyses were used to detect relative levels of specific peanut allergens.

RESULTS

NMR analysis of peanut-soaked solutions demonstrated an overall reduction of total intact protein in autoclaved peanuts as shown by the broadening of peaks in the spectral regions corresponding to peptide fragments when compared to raw. The results also showed that autoclaving reduces the amount of allergenic proteins Ara h 2 (50% reduction) and Ara h 8 (100% reduction). Upon skin prick testing of allergic subjects, this differential degradation demonstrated that the autoclaved peanut could be used to categorize patients into two groups: those at risk for anaphylaxis and those who only experience oral symptoms to peanut (predominantly Ara h 2- and Ara h 8-specific IgE, respectively).

CONCLUSION

The data reported in this study suggest that high-pressure and temperature autoclaving reduces the amount of intact protein in the peanut, including allergenic proteins. This could be further developed into an improved diagnostic test for peanut allergy.

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Keywords: Autoclaving, Food Processing, IgE Binding, Peanut Allergy, Skin Prick Testing

Main text

BACKGROUND

Peanut allergy is extremely common, affecting approximately 1.5% of children in North America, Australia and the UK[1]. It is an important cause of anaphylaxis and utilization of hospital emergency room resources[2]. Most individuals with peanut allergy are not treated; rather, they strictly avoid peanut-containing foods and carry precautionary injected epinephrine in case of accidental ingestion.

In food allergies such as egg and milk, the rate of spontaneous resolution is considerably higher than for peanut. Indeed, children with egg or milk allergy can frequently introduce small amounts of well-cooked egg or milk into their diets safely as they grow[3-6]. Natural history studies of this practice have indicated that the patients are able to increase the cooked form of the allergen into their diets and ultimately a significant number evolve to complete tolerance[7-9]. Normal cooking processes denature or linearize egg or milk proteins, which may explain their decreased allergenicity, and frequent exposure for an extended period of time may act as a form of oral immunotherapy (OIT), albeit with more safety than conventional OIT[10].

Peanut does not appear to denature under normal cooking conditions. Structural biology analyses have focused on the three-dimensional structure of the major peanut protein allergens and recent reports have thoroughly described their X-ray structures[11-13]. This class of proteins is rich in disulfide bridges, which explains their resistance to denaturation at high temperature[14]. In fact, glycation at high temperature

is proposed to be a primary mechanism of enhancement of allergenic responses to peanut, as shown by quantification of IgE-binding[15]. Glycation primarily results from the Maillard reaction, an addition of amines on reducing sugars to provide Schiff bases that rearrange to form a wide range of products, of which the advanced glycation end-products (AGE) are believed to be of relevance to allergenicity[16-19]. Importantly, although the molecular composition of the peanut is now well known (i.e. proteins, amino acids, metal ion, sugar content)[20, 21], the specific contribution of free sugars and amino acids to the enhancement of allergenicity of peanuts at high temperatures has yet to be defined[22].

Previous studies suggest a decrease in IgE-binding in boiled and fried peanuts when compared with raw[23, 24]. It has been reported that low-molecular-weight proteins are transferred from the peanuts into the cooking water throughout boiling, particularly the 2S albumins Ara h 2, Ara h 6 and Ara h 7, potentially explaining a decrease in IgE-binding[23]. Moreover, it has also been found that autoclaving roasted peanuts produces a significant decrease of IgE-binding capacity of peanut allergens and in wheal size by skin prick test, as well as the unfolding of proteins and reduction in overall secondary structure[25].

The objective of this study was to evaluate the effects of thermal processing, particularly roasting and autoclaving, on the resulting small molecule profiles, the major protein allergens, and thus, on peanut allergenicity.

METHODS

Physical Peanut Processing

Commercially available peanuts (Montreal Food Store, Canada) were purchased raw and shelled. Peanuts were roasted in a convection oven at 150°C for 30 minutes or were autoclaved in a tabletop autoclave at 136°C (2.5 atm) for 30 minutes. Additionally, roasted peanuts were autoclaved (Roast-Auto) and autoclaved peanuts were roasted (Auto-Roast). Analyses were performed in comparison with raw peanut (unprocessed).

^{1}H Nuclear Magnetic Resonance (NMR) Spectroscopy

Six whole peanuts of each condition were placed in 10 mL of double distilled water and soaked at room temperature for 48 hours. Three samples per condition of the resulting solutions (1 mL each) were evaporated under vacuum at 45°C for 1.5 hours and the resulting residue was reconstituted in 0.6 mL of double distilled water. The three samples were combined together to give a total volume of 1.8 mL, 450 µL of which was collected for analysis.

^{1}H NMR spectra were run on a Bruker 400 MHz NMR spectrometer for analysis using the water suppression pulse sequence, zgpr (Bruker standard sequence; Bruker, MA, USA). Thirty-two scans were taken with an acquisition time of 3 s and a spectral width of 12 kHz. The ^{1}H chemical shifts were internally referenced by adding 0.5 mM of deuterated 3-(trimethylsilyl)propionic-2,2,3,3,-d$_4$ acid sodium salt (TSP-d $_4$; Sigma-Aldrich, ON, Canada) set to 0.0 ppm.

Defatting into flour

Raw, roasted and autoclaved peanuts (6 to 12 of each) were ground into a smooth paste using a coffee grinder (Hamilton Beach/Proctor-Silex, ON, Canada). The paste was then suspended in hexanes and the peanut flour was collected by filtration under vacuum.

Preparation of Protein Extracts

Dry peanut flours were processed into whole protein extracts using a 20 mM Tris Buffer (pH 8.5), following the protocol optimized by Walczyk et al.[26] Extract concentrations were determined by Bradford Assay[27] using bovine serum albumin (BSA, Sigma-Aldrich) as a standard. Concentrations were adjusted to equal values across processing conditions accordingly.

Western Blot Analysis

Whole protein extracts were diluted to concentrations of 1 mg/mL and separated by SDS-PAGE under reducing conditions (2.5% β-mercaptoethanol). Membranes were incubated with rabbit anti-Ara h 1, Ara h 2 or Ara h 8 polyclonal antibody (1:1,000, 4°C overnight; Indoor Biotechnologies, VA, USA). Bound antibodies were visualized using horseradish peroxidase (HRP)-conjugated donkey anti-rabbit IgG monoclonal antibody (1:1,000, 1h room temperature; BioLegend, CA, USA) and Clarity/Clarity Max enhanced chemiluminescence (ECL) substrates (Bio-Rad Laboratories, Canada). Products were visualized with the ChemiDoc XRS+ Imaging System (Bio-Rad Laboratories).

Relative Quantification of Allergens

Relative levels of Ara h 1, Ara h 2 and Ara h 8 in peanut extracts were quantified using the enzyme-linked immunosorbent assay (ELISA). Polystyrene plates (96-well) were coated overnight at 4°C with the different protein extracts at a range of concentrations (maximum 1 µg/mL for Ara h 1 and Ara h 2, maximum 1 mg/mL for Ara h 8). Following blocking with 1% BSA, rabbit anti-Ara h 1, Ara h 2 or Ara h 8 polyclonal antibody (1:1000, 50 µL/well, 2h room temperature; Indoor Biotechnologies) was used as the primary antibody and HRP-conjugated donkey anti-rabbit IgG monoclonal antibody (1:1,000, 50 µL/well, 1h room temperature; BioLegend) was used for detection. Following incubation with 3,3',5,5'-Tetramethylbenzidine (TMB) substrate (BioLegend), optical density (OD) values were measured at 450 nm with reference at 570 nm.

Determination of Specific IgE Responses

The specific IgE-binding capacity of the proteins of the various processing conditions was analyzed using the ELISA protocol described above. The serum of 4 patients highly allergic to peanuts diluted 1:1000 in 1% BSA were each used as the primary antibody (50 µL/well, 2h room temperature). Biotinylated polyclonal goat anti-human IgE antibody (1:20,000, 50 µL/well, 1h room temperature; Bethyl Laboratories Inc., TX, USA) followed by incubation with HRP-streptavidin (1:3,000, 50 µL/well, 1h room temperature; BioLegend) were used for detection.

A serial dilution of recombinant human IgE antibody at 50 ng/ml (ELISA Ready-SET-Go! Kit, Thermo Fisher Scientific, ON, Canada) binding to goat anti-human IgE capture antibody (1:1,000; Bethyl Laboratories Inc.) coated to the plate was used to construct a standard curve by plotting known concentrations versus OD values at 450 nm with reference at 570 nm.

Skin Prick Testing

Nine peanut-allergic subjects and three non-allergic healthy controls aged 19 to 29 years old were recruited to the Montreal Children's Hospital for Skin Prick Testing (SPT) after obtaining informed consent. Subjects were considered allergic based on previous history suggestive of immediate allergy to peanut and the presence of previous positive SPT to peanut (wheal diameter greater than 7 mm), detection of serum-specific IgE to peanut proteins (>0.35 kU/L) and/or a positive oral food challenge to peanut.

Peanuts processed by roasting, autoclaving, roasting then autoclaving, and autoclaving then roasting, along with raw peanuts, were further processed into protein extracts as described above. Extracts of each condition were diluted to equal concentrations equivalent to the commercial standard peanut extract (Allergy Canada Limited, Thornhill, ON, LOT No: 3467710) as measured by Bradford Assay. The SPTs were conducted by placing a drop of each extract, as well as the standard commercial extract, on the forearm and making a small scratch on the arm using a solid bore needle. Saline diluent and Histamine (1mg/mL, ALK-Abello Pharm., Inc., ON, Canada) were used as negative and positive controls, respectively. After 10 minutes, the size of the wheal diameter was measured (in mm). All skin tests were performed by an experienced allergy nurse. Research Ethics Board (REB) approval was obtained from the McGill University Health Centre (MUHC-REB 2020-5745).

Statistical Analyses

All results were statistically analyzed using GraphPad Prism Version 5.00 (GraphPad Software, San Diego,

CA, USA). Analysis of Variance (ANOVA) was used to determine significant differences between normalized IgE binding values of each processing condition. A two-tailed, paired Student's t-test was used to determine significant differences between wheal diameters using raw and autoclaved peanut protein extracts. The level of significance was set at $p < 0.05$ in both cases.

RESULTS

^{1}H NMR Analysis of Peanut-Soaked Solutions

Under various processing conditions, the molecular composition of the peanut is expected to change due to chemical reactions such as the Maillard reaction, macromolecule hydrolysis, protein denaturation and the generation of small molecules such as monosaccharides and free amino acids. Thus, we sought to monitor differences between solutions derived from soaking the peanut under different conditions. In order to track these modifications, we chose ^{1}H Nuclear Magnetic Resonance (NMR) spectroscopy as the most suitable method.^{1}H NMR spectroscopy provides a characteristic spectrum dependent on the chemical environments of individual hydrogen nuclei in the sample. We used this technique to obtain molecular profiles and to identify the chemical changes across different processing conditions. Raw, roasted and autoclaved peanuts were each soaked in distilled water for 48 hours and the resulting solution was analyzed by^{1}H NMR spectroscopy (Figure 1).

The spectra of the raw and roasted peanut-soaked solutions did not differ greatly in peak distribution or intensity (Figure 1A & 1B). However, that of the autoclaved peanut-soaked solution was strikingly different (Figure 1C). Particularly, in the regions between 0.5 ppm and 2.0 ppm as well as between 6.5 ppm and 8.5 ppm, the autoclaved peanut-soaked spectrum had a different peak distribution with a series of broad peaks, indicating the presence of more soluble molecules, likely amino acids and peptides leaching out into solution. The higher-field region in the spectrum (0.5 ppm – 2.0 ppm) corresponds to^{1}H atoms of alkyl groups, while the lower-field region (6.5 ppm – 8.5 ppm) corresponds to ^{1}H atoms of amide bonds and aromatic groups, which likely represent free amino acids and peptide side chains.

Anti-Ara h Western Blots

In order to determine whether the autoclaving process denatured specific allergenic proteins, following gel electrophoresis, we performed Western blot analyses on protein extracts of each processing condition using antibodies specific for peanut allergens.

Figure 2A shows a Western blot using an antibody selective for Ara h 1, a 64-kilodalton (kDa) 7S globulin. Both the raw and roasted peanut extracts appear to have the highest proportion of Ara h 1 as demonstrated by the greatest band intensity. The autoclaved extracts have very little to no detection of Ara h 1 via Western blot.

Ara h 2 is a 2S albumin protein of size 17 kDa and has been established as the most potent peanut allergen, being recognized by the serum IgE of over 90% of peanut-allergic patients[28, 29]. Moreover, recent literature suggests that Ara h 2 exists as two distinct isoforms in mature peanuts, differing by a stretch of 12 amino acids, and that the larger of the two isoforms may be a more potent allergen[30, 31]. In Figure 2B, we observe the presence of two distinct and intense bands in the roasted extract and to a lesser degree in the raw extract. In all autoclaved extracts, no distinct bands were observed for Ara h 2 but rather a general smear throughout the lanes.

In the case of Ara h 8, a plant panallergen of size 17 kDa, we observed a similarly high band intensity for both the raw and roasted peanut extracts, and very little to no detection in the autoclaved extracts (Figure 2C).

Roasting peanuts either before or after autoclaving (lanes 5 and 4, respectively) did not substantially alter the levels of allergen detection.

Anti-Ara h ELISAs

The ELISA was used to quantify the relative amounts of allergens present in the protein extracts of each condition. In the case of Ara h 2, raw and roasted protein extracts showed similarly high levels of detection at a protein concentration of 1 µg/mL coated on the plate. However, in the case of the autoclaved extract, detection was reduced by approximately 50% for the same concentration (Figure 3A).

Figure 3B shows similar detection levels of Ara h 8 for raw and roasted peanut proteins at high extract concentrations coated on the plate (1 mg/mL). Interestingly, the autoclaved peanut extracts detected no Ara h 8, independent of coated extract concentration.

Peanut-Specific IgE ELISA

With the aim of understanding what effect autoclaving has on the peanut allergens in the context of IgE binding, the ELISA was used to quantify peanut-specific IgE binding using serum from highly allergic patients as the primary antibody. IgE binding decreased significantly upon peanut autoclaving (Figure 4, $p < 0.0001$). These results were unchanged with extracts from peanuts roasted either before or after autoclaving. Moreover, no significant change in IgE binding was observed when comparing roasted to raw peanuts.

Skin Prick Test

In an attempt to assess IgE binding *in vivo* , peanut-allergic subjects and non-allergic healthy controls were skin prick tested (SPT) with a panel of protein extracts created from raw, roasted and autoclaved peanuts and the resulting wheal diameters were measured (Table 1). Within the peanut-allergic group, a statistically significant reduction in mean wheal diameter was observed using the autoclaved extract when compared to raw ($p < 0.05$).

Additional information was revealed when the allergic group was further stratified into two groups: one group of patients who have previously experienced anaphylaxis to peanut or have demonstrated high likelihood of it based on past clinical tests, and the second group of patients known to experience only oral symptoms upon peanut consumption. A striking decrease in wheal size was observed when using autoclaved extracts compared to raw in the group that experiences oral symptoms. In contrast, this was not observed in the case of the group at risk for anaphylaxis. The roasted peanut extract showed a slight decrease in wheal size in the group at risk for anaphylaxis and an increase in the group of patients who experience oral symptoms.

DISCUSSION

In this study, we evaluated the effects of roasting and autoclaving on the major peanut allergens and on peanut allergenicity. We demonstrated that high-pressure and temperature autoclaving reduces the detection of the major allergens Ara h 1, Ara h 2, and Ara h 8 as well as peanut-specific IgE binding when compared to raw or roasted peanuts. Moreover, the effect of autoclaving was observed even when it was performed prior to or following roasting of the peanut, further indicating the strong denaturing effects of autoclaving. Indeed, NMR analyses showed that while the molecular profiles between raw and roasted peanuts were similar, those associated with autoclaving treatment were dramatically different, with evident peak-broadening in regions corresponding to amino acids associated with proteins. Larger molecules in solution move or "tumble" more slowly, resulting in a range of molecular orientations and thus broader peaks in the NMR spectrum[32]. Since these experiments were performed with peanut-soaked solutions using ^{1}H NMR, the results suggest that the autoclaved peanut sample contained a larger number and a wider range of sizes of soluble molecules than the raw and roasted solutions. Overall, the data indicate that of the processing methods evaluated, autoclaving produces conditions for the most significant denaturation, resulting in complete degradation of Ara h 8 and partial degradation of Ara h 1 and Ara h 2. In contrast, under raw and roasted conditions, these allergens remained largely intact.

Consistent with these findings, autoclaving was associated with the weakest IgE binding using serum samples of peanut-allergic patients. The decrease in IgE binding may be partly caused by the complete denaturation of Ara h 8 or major structural changes affecting the accessibility of its epitope regions. Likewise, the partial degradation of Ara h 2 may also affect epitope accessibility. However, due to its rigid structure maintained

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by a number of disulfide bonds, Ara h 2 is more resistant to denaturation[14]. Nevertheless, we cannot rule out the possibility that the results observed are the outcome of a dose-related phenomenon. It is known that Ara h 2 exists in high proportions relative to others (5.9%-9.3% of total protein content)[33], and Ara h 8 in much lower abundance, indicating that the remaining levels of Ara h 2 and absence of Ara h 8 could perhaps be proportional to their initial concentrations in the peanut.

Autoclaving is a condition where temperature, pressure and moisture play a significant role. There are currently few studies in the literature addressing the effect of autoclaving on peanut proteins. One major study to date has investigated the effect of heat and pressure treatments on peanut allergenicity[25]. Cabanillas et al. (2012) demonstrated that peanut-specific IgE binding, as well as the detection of major allergens Ara h 1, Ara h 2, and Ara h 3, can be reduced by autoclaving roasted peanuts[25]. This was explained by the observation that autoclaving resulted in a decrease of α-helix content and an increase in random coils and/or loops as a function of autoclave pressure and duration as shown by circular dichroism experiments[25]. Similar decreases in specific IgE binding have been observed when autoclaving other legumes such as lupine allergens[34] and green pea[35]. While our results are in agreement with these findings, it is important to note that our work is the first report on the complete absence of detection of Ara h 8 from autoclaved peanut extract. This is in line with the fact that Ara h 8 is an allergen deprived of disulfide bonds, thereby leaving its α-helices as the major barrier to denaturation under autoclaving conditions.

The literature is more extensive on the comparison between the allergenicity of raw versus roasted peanuts. Maleki et al. (2000) found that roasted peanut proteins bound to IgE from patients with peanut allergy at approximately 90-fold higher levels than the raw proteins[15]. The proposed explanation for this enhancement of IgE binding is the glycation of major allergens to form advanced glycation end-products (AGE) via the Maillard Reaction[17]. More recently, Rao et al. (2016) found that roasting the peanut at temperatures greater than 130°C resulted in a reduction of IgE binding to Ara h 1 and Ara h 3, but an increase in binding to Ara h 2 and Ara h 6, two major peanut allergens[36]. However, Blanc et al. (2011) found no difference in IgE binding between raw and roasted Ara h 1 protein[37]. In this study, our findings are more in agreement with this work as we did not observe a significant difference between the allergen detection and IgE binding responses of raw versus roasted peanut. We believe this may be due to the restriction of our analysis to only the soluble fractions of the peanut extracts.

Our discovery of complete and partial degradation of Ara h 8 and Ara h 2, respectively, under autoclaving may have significant clinical implications. In a preliminary study, we observed a striking decrease in wheal size in a group of patients that experiences oral symptoms to peanut upon exposure to the autoclaved extract when compared to raw and roasted extracts. These differential levels of detection of Ara h 2 and Ara h 8 may be part of the explanation of the results observed from the SPT. Currently, whole protein extracts created from raw or roasted peanuts are used routinely in SPTs for the diagnosis of peanut allergy in the clinic. Ara h 2 has proven to be one of the best predictors of anaphylaxis in allergic patients[38], while isolated Ara h 8 sensitization indicates only oral symptoms or tolerance to peanut in almost all cases[39]. Our results indicate that the use of an autoclaved peanut extract, in addition to the current whole protein extract (non-autoclaved), has the potential to serve as an improved diagnostic technique (patent applied[40]) distinguishing between two subsets of peanut-allergic patients: those at risk for anaphylaxis, and primarily have Ara h 2-specific IgE, versus those who will only experience oral symptoms to peanut, and predominantly have Ara h 8-specific IgE. As depicted in Figure 5, patients with a positive SPT result using both the whole peanut extract (raw or roasted) and the autoclaved peanut extract will be classified as at risk for anaphylaxis. Importantly, patients with a positive SPT result using the whole peanut extract, but a negative SPT result using the autoclaved extract, will experience only oral symptoms upon peanut consumption. Those who experience a negative SPT result using both extracts will be classified as tolerant to peanut.

Altogether, the data reported in this study suggest that high-pressure and temperature autoclaving lead to a significant denaturation of Ara h 8 and other allergenic proteins. This discovery is being further developed into an improved diagnostic test for peanut-allergic patient stratification. Further studies are required to optimize a degree of complete reduction of intact allergens by autoclaving.

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Tables:

Table 1 Skin Prick Test (SPT) results displaying the mean wheal diameter in millimetres.

Extract used	Mean Wheal Diameter (mm) Allergic[+] (n = 9)	Mean Wheal Diameter (mm) Allergic[+] (n = 9) Anaphylaxis (n = 5)	Mean Wheal Diameter (mm) Allergic[+] (n = 9) Oral Symptoms (n = 4)	Mean Wheal Diameter (mm) Non-Allergic (n = 3)
Standard	10.2	12.8	7.0	1.3
Raw	**9.6****	**12.4**	**6.0***	1.0
Roast	9.9	9.0	11.0	1.3
Autoclave	**6.9****	**11.0**	**1.8***	1.0
Autoclave-Roasted	6.7	10.2	2.3	1.3
Roasted-Autoclave	6.9	10.6	2.3	1.0
(+) Control (Histamine)	4.2	4.2	4.3	4.3
(−) Control (Saline)	0.7	0.6	0.8	1.3

*: $p < 0.05$, **: $p < 0.01$, Student's t-test.

[+]Allergic subjects were divided into two sub-groups based on previous exposure to peanut: those at risk for anaphylaxis and those who experience only oral symptoms.

Figure legends:

Figure 1 [1]H Nuclear Magnetic Resonance (NMR) spectra of raw (A), roasted (B) and autoclaved (C) peanut-soaked solutions. Horizontal axis represents ^{1}H chemical shifts in parts per million (ppm) referenced using an internal standard (TSP-d_4) set to 0.0 ppm. Vertical axis represents relative intensity. Framed regions correspond to methyl region (right) and amide/aromatic regions (left) of peptide fragments and amino acid side chains.

Figure 2 Western blot following SDS PAGE using antibodies specific for Ara h 1 (A), Ara h 2 (B) and Ara h 8 (C). Lanes correspond to the processing conditions as follows: M = Molecular weight marker, 1 = Raw, 2 = Roast, 3 = Autoclave, 4 = Autoclave then Roasted, 5 = Roasted then Autoclaved.

Figure 3 Relative Ara h 2 (A) and Ara h 8 (B) quantification by ELISA. Optical density (OD) values were measured at 450 nm and referenced at 570 nm. Plates were coated with a maximum concentration of 1 µg/mL and 1 mg/mL peanut protein in A and B, respectively.

Figure 4 Peanut-Specific IgE ELISA using the serum of 4 highly allergic subjects. Optical density (OD) values measured at 450 nm, referenced at 570 nm, were normalized to corresponding raw values. Auto-Roast:

autoclaved, then roasted. Roast-Auto: roasted, then autoclaved. n = 4 patients. ****: p < 0.0001, one-way ANOVA, Tukey's multiple comparisons test. ns: not significant.

Figure 5 Summary figure of hypothesized effect of high-temperature and pressure autoclaving on peanut protein allergens Ara h 2 and Ara h 8 when compared to raw or roasted allergens and expected outcomes of proposed diagnostic method. 50% and 0% of intact Ara h 2 and Ara h 8, respectively, can be detected in autoclaved peanut extracts when compared to raw or roasted extracts. Highlighted areas in red represent epitope regions in protein structures. When using the whole protein extract made from raw or roasted peanuts for Skin Prick Testing (SPT) as currently done in the clinic, patients who have IgE specific for any combination of peanut allergens will experience a positive SPT result. However, when using the autoclaved extract, patients at risk for anaphylaxis (specific IgE primarily for Ara h 2) will experience a positive SPT result while patients who experience only oral symptoms to peanut (specific IgE primarily for Ara h 8) will experience a negative SPT result. Patients tolerant to peanut will experience a negative SPT result to both extracts.

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A

Anti-Ara h 2 ELISA

- RAW
- ROAST
- AUTOCLAVE

OD Value (450 nm)

Peanut Protein Bound to Plate (ng/mL)

B

Anti-Ara h 8 ELISA

- RAW
- ROAST
- AUTOCLAVE

OD Value (450 nm)

Peanut Protein Bound to Plate (ug/mL)

IgE Bound, Normalized to Raw

****

ns   ns

Raw   Roast   Autoclave   Auto-Roast   Roast-Auto

Ara h 2

100%

Ara h 8

100%

Expected SPT Result

Raw/Roast

(+) Risk for Anaphylaxis

(+) Oral Symptoms

(−) Tolerant

Autoclave

(+) Risk for Anaphylaxis

(−) Oral Symptoms

(−) Tolerant

50%   *0%*

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