Effects of cooking methods on peanut allergenicity

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Background: Allergy to peanut is a significant health problem. Interestingly, the prevalence of peanut allergy in China is much lower than that in the United States, despite a high rate of peanut consumption in China. In China, peanuts are commonly fried or boiled, whereas in the United States peanuts are typically dry roasted.

Objective: The aim of this study was to examine whether the method of preparing peanuts could be a factor in the disparity of allergy prevalence between the 2 countries.

Methods: Two varieties of peanuts grown in the United States were roasted, boiled, or fried. Proteins were analyzed by using SDS-PAGE and immunoblotting. Allergenicity was compared by using immunolabeling with sera from 8 patients with peanut allergy.

Results: The protein fractions of both varieties of peanuts were altered to a similar degree by frying or boiling. Compared with roasted peanuts, the relative amount of Ara h 1 was reduced in the fried and boiled preparations, resulting in a significant reduction of IgE-binding intensity. In addition, there was significantly less IgE binding to Ara h 2 and Ara h 3 in fried and boiled peanuts compared with that in roasted peanuts, even though the protein amounts were similar in all 3 preparations.

Conclusion: The methods of frying or boiling peanuts, as practiced in China, appear to reduce the allergenicity of peanuts compared with the method of dry roasting practiced widely in the United States. Roasting uses higher temperatures that apparently increase the allergenic property of peanut proteins and may help explain the difference in prevalence of peanut allergy observed in the 2 countries. (J Allergy Clin Immunol 2001;107:1077-81.)

Key words: *Peanut, allergens, allergenicity, cooking methods, SDS-PAGE, immunolabeling, Ara h 1, Ara h 2, Ara h 3, IgE*

The estimated prevalence of peanut allergy in the United States is about 0.6% of the general population and appears to be increasing,[1] with the prevalence of peanut allergy in some atopic populations considerably higher. About one third of American children with moderate-to-severe atopic dermatitis and food hypersensitivity are allergic to peanuts.[2]

In contrast, sensitization and reactivity to peanut is far less prevalent in China, despite the high rate of peanut consumption. The article by Hill et al[3] indicated that food allergy in China reportedly affects 3.4% to 5.0% of the residents in Beijing, Guangdong, and the Sheng-Li Oil Fields. However, the common food allergens in China are fish, shrimp, crab, and seaweed, but not peanut. In addition, of 29 children with diagnosed food allergy aged from 2 to 12 years, none was allergic to peanut, and of 130 children with atopic dermatitis, only 2% had a positive skin test response against peanut allergen.[3] In general, China has one of the lowest prevalence rates for certain atopic diseases in a worldwide comparison.[4]

In contrast, the Chinese-American population seems to have a similar prevalence of peanut allergy as the general US population (unpublished observation). The discrepancy between peanut allergy in the United States and China cannot therefore be a result of population genetics.

About 2.4 billion pounds of peanuts are consumed in the United States each year, about one half of it in the form of peanut butter.[5] The average American consumes more than 6 pounds of peanuts and peanut products per year.[6] In China, the domestic consumption of peanuts is estimated between 2.8 and 3.2 million metric tons per year.[7] This gives an average consumption rate of between 5.1 and 5.9 pounds per person.

There are several kinds of peanuts used in the United States, although the most common variety is Runner, including Florunner, which accounts for approximately 73% of total US production.[5,6] Runners are usually consumed in a roasted form, and a large proportion is further processed into peanut butter. A second variety, Valencia, accounts for much less of the US production but also is usually consumed in a roasted form.

In general, peanuts in the United States are roasted. Recently, it was shown that roasting may enhance the allergenic properties of peanuts compared with those in the raw form.[8] In contrast to the United States, peanuts in China are generally fried or boiled. Given this difference in methods of preparing peanuts for consumption, we wondered whether these different practices might play a role in the marked discrepancy in peanut allergy prevalence between the United States and China.

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In the present study we demonstrate that different methods of peanut preparation, such as frying (120°C) and boiling (100°C), reduced IgE-binding intensity to the major peanut allergens Ara h 1, Ara h 2, and Ara h 3 compared with roasted preparations (150°C-170°C). This decreased allergenicity may be a contributing factor in the lower prevalence of peanut allergy in China compared with that in the United States.

METHODS

Preparation of peanut extracts

Two varieties of raw peanuts, Florunner (Bridsong Peanuts, Gorman, Tex) and Valencia (Garden Spot Distributors, New Holland, Pa), were obtained for this study. Both varieties were roasted, fried, or boiled. The roasting was carried out at 170°C for 20 minutes, and frying was done in pure vegetable oil (Crisco; Canadian oil, Cincinnati, Ohio) for 5 minutes (Valencia) or 10 minutes (Florunner) to account for the difference in the size of the peanuts. Boiled peanuts were cooked for 20 minutes in water (100°C). In addition, the cooking water was collected and lyophilized for later analysis. Commercially roasted peanuts and peanut butter (Jif, Procter & Gamble Company, Cincinnati, Ohio) were also evaluated.

By using a coffee grinder (Krups, Peoria, Ill) followed by mortar and pestle, peanuts were ground until a smooth paste was achieved. The paste was defatted by washing with at least 20 volumes (wt/vol) of cold acetone and dried overnight at 4°C. Protein was extracted from dried powder by agitating with PBS and a protease-inhibitor cocktail without EDTA (Roche, Indianapolis, Ind) overnight at 4°C. After centrifugation at 2500*g* for 20 minutes at 4°C, supernatant was collected, filtered, and centrifuged at 12,000*g* for 3 minutes. Protein concentrations were determined by using spectrophotometry (SmartSpec; Biorad, Hercules, Calif) with a Coomassie Plus Protein Assay (Pierce, Rockford, Ill). All extracts were stored at –80ºC.

SDS-PAGE analysis

Proteins were separated by using SDS-PAGE (Tricine System; Invitrogen, Carlsbad, Calif), according to the protocol from the manufacturer. Ten micrograms of protein was loaded into each well. The resolved proteins were subsequently transferred to Immobilon-P membrane (Millipore, Bedford, Mass) and then stained with 2.2% Coomassie Blue for total protein analysis. Two different kinds of molecular weight markers were used: MultiMark Multi-Colored Standard and See Blue Pre-stained Standards (Invitrogen).

Probing immunoblots with sera from patients with peanut allergy

For detection of IgE binding to the different peanut preparations, immunolabeling was performed with patient sera from 8 individuals with peanut allergy. All patients with peanut allergy were children between 2 and 14 years of age. After ingestion, their clinical symptoms included vomiting, urticaria, wheezing, and angioedema. At the time of assessment, 7 patients had a peanut-specific IgE level greater than 100 kU/L, and 1 patient had a peanut-specific IgE level of 51.6 kU/L, as measured by using the Pharmacia CAP System. None of these patients underwent blinded food challenges because of their positive clinical histories and high levels of peanut-specific IgE. In previous studies the cutoff level of 15 kU_A/L for peanut was identified to predict clinical reactivity with greater than 95% certainty.[9] Normal human serum (Pharmacia & Upjohn Diagnostics, Upsalla, Sweden) was used as a negative control. Patient and control sera were diluted 1:10 in PBS-Tween plus 1% BSA and 10% normal goat serum and incubated with immunoblots for 1 hour with gentle agitation at room temperature. After rinsing with PBS, phosphatase-labeled goat anti-human IgE (KPL, Gaithersburg, Md) diluted 1:1000 in PBS-Tween plus 1% BSA and 10% normal goat serum was applied. After agitation for 45 minutes at room temperature and washing with PBS, immunoblots were developed with phosphatase substrate (KPL) and compared with a reflective densitometer (BioRad, Hercules, Calif) and Quantity One software (BioRad).

Statistical analysis

All analyses of data were performed with nonparametric tests (Mann-Whitney *U* test) by using Prism 2.01 software. Differences associated with *P* values of less than .05 were considered significant.

RESULTS

Comparison of dry-roasted peanuts with commercially available peanuts and peanut butter

The protein content of peanuts roasted in our laboratory was compared with that of commercially roasted peanuts or peanut butter by means of gel electrophoresis to confirm that our roasting procedure reflected the protein content of commercially available peanut preparations. Both American peanuts (Florunner and Valencia) roasted in our laboratory (Fig 1, lanes 5 and 9) showed the same protein pattern as the commercially available preparations (Fig 1, lanes 11 and 12).

Comparison of protein fractions in dry-roasted, fried, and boiled peanuts by means of SDS-PAGE

Fried and boiled peanut extracts from both American peanut varieties were compared with the dry-roasted preparations by means of SDS-trycine PAGE. Frying and boiling both varieties of peanuts altered them in a similar way (Fig 1). Some protein bands seem to be clearly reduced compared with those of the roasted form, whereas others were enhanced. The monomeric form of Ara h 1 (63 kd) was markedly reduced in fried peanuts compared with roasted peanuts (Fig 1, lanes 4, 5, 8, and 9, and Fig 2, *A*), whereas changes in boiled peanuts were much less pronounced (Fig 1, lanes 3 and 7, and Fig 2, *B*). The Ara h 1 trimer was only present in the roasted preparation and completely undetectable in the fried and boiled peanut preparations (Figs 1 and 2). In contrast, the Ara h 2 (17 and 19 kd) and Ara h 3 fragment (14 kd) appeared to be similar in all 3 methods of preparation (Figs 1 and 2). Although quantitative analyses of peanut proteins in fried and boiled peanuts indicated a reduction only in Ara h 1, only unidentifiable low-molecular-weight protein fragments could be found in the water used to boil the peanuts. The apparent loss of Ara h 1 could not be accounted for by the minimal amounts of Ara h 1 found in the cooking water of Florunner and Valencia peanuts, respectively (data not shown).

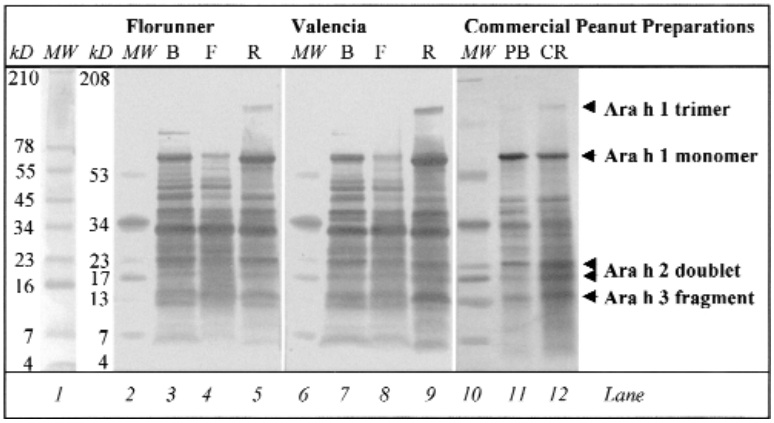


FIG 1. SDS-PAGE analysis for 2 varieties of peanuts, Florunner and Valencia, which were either roasted (*R*; *lanes 5* and *9*), fried (*F*; *lanes 4* and *8*), or boiled (*B*; *lanes 3* and *7*). In addition, peanut butter *(PB)* and commercially roasted peanuts *(CR)* are shown in *lanes 11* and *12*. Two different kinds of molecular weight standards are shown in *lanes 1, 2, 6*, and *10*, respectively. *Arrows* indicate the location of the Ara h 1 trimer and monomer, the Ara h 2 doublet, and an Ara h 3 fragment.

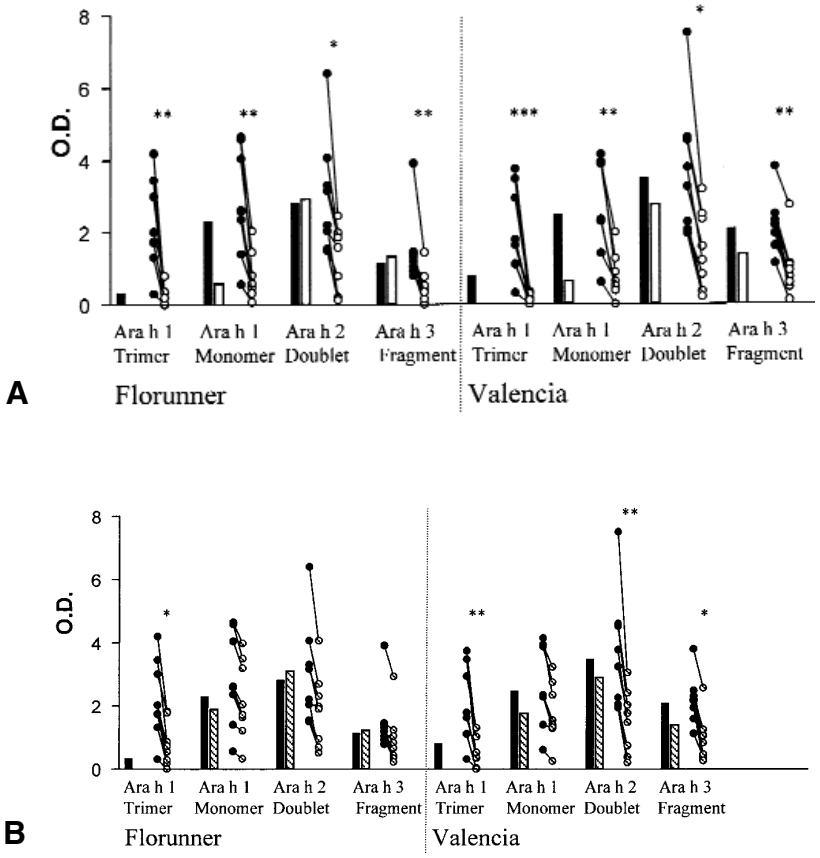


FIG 2. Comparison of the protein content and allergenicity between roasted and fried (**A**), as well as roasted and boiled (**B**), peanut preparations. *Black bars* represent the OD for protein of roasted peanuts, *open bars* represent the OD of fried peanuts, and *hatched bars* represent the OD of boiled peanuts after protein separation by SDS-PAGE. *Circles* represent the corresponding OD of immunoblotted membranes for anti–peanut-specific IgE by using serum from 8 patients with peanut allergy. *Black circles* represent binding to roasted peanuts, *open circles* represent binding to fried peanuts, and *hatched circles* represent binding to boiled peanuts. For Ara h 2, the cumulative OD of both bands is shown. $*P < .05$, $**P < .01$, $***P < .001$.

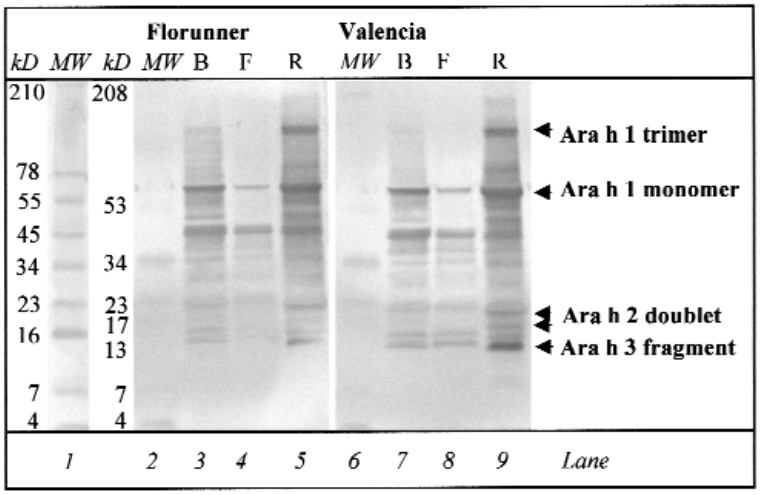


FIG 3. Immunoblot for anti–peanut-specific IgE against 2 varieties of peanuts, Florunner and Valencia, which were either roasted (*R*; *lanes 5* and *9*), fried (*F*; *lanes 4* and *8*), or boiled (*B*; *lanes 3* and *7*). Shown is a representative example from 8 patients with peanut allergy (see Fig 2). Two different kinds of molecular weight standards are shown in *lanes 1, 2,* and *6,* respectively. *Arrows* indicate the location of the Ara h 1 trimer and monomer, the Ara h 2 doublet, and the Ara h 3 fragment.

Comparison of IgE binding to roasted, fried, and boiled peanuts

In parallel with the reduced quantities of Ara h 1 protein, IgE-binding intensities to the Ara h 1 monomer and trimer were also significantly reduced by frying compared with roasting in both peanut varieties (Florunner: $P < .005$ for both; Valencia: $P < .005$ for the Ara h 1 monomer and $P < .0005$ for the Ara h 1 trimer; Fig 2 and Fig 3, lanes 4, 5, 8, and 9). For boiled peanuts, the Ara h 1 trimer showed a similar loss of allergenic protein and IgE-binding intensities compared with that of roasted peanut protein ($P < .05$ for Florunner and $P < .01$ for Valencia; Fig 2 and Fig 3, lanes 3 and 7). The Ara h 1 monomer showed a similar trend. Interestingly, the IgE-binding intensities to Ara h 2 and Ara h 3 fragments were also reduced in fried and boiled peanuts, although the total protein quantities were mainly unaltered by the cooking methods (comparison roasted vs fried: $P < .05$ for Ara h 2 and $P < .01$ for Ara h 3 in both Florunner and Valencia; comparison roasted vs boiled: $P < .01$ for Ara h 2 and $P < .05$ for Ara h 3 in Valencia; Figs 2 and 3).

DISCUSSION

In the present study we were able to show that frying and boiling, methods of preparing peanuts used primarily in China, produce fewer allergenic peanut products in comparison with roasting, the usual method of cooking peanuts in the United States. The apparent amount of IgE binding to Ara h 1, Ara h 2, and Ara h 3 was lower in fried and boiled peanuts compared with that found in roasted peanuts. The decrease of allergenicity for Ara h 1 was paralleled by a decrease in the quantity of protein.

Ara h 1 is a well-defined major peanut allergen recognized by IgE antibodies from greater than 90% of peanut-sensitive patients.[10,11] Ara h 1 has a high degree of structure at the secondary level, with 31% α-helices, 36% β-structure, and 33% random coil, as well as a distinct tertiary structure. Heat treatment of purified Ara h 1 at 83°C to 87°C results in a complete and irreversible denaturation of the protein, which shows an immediate aggregation behavior. In peanuts, denaturation of Ara h 1 also can take place but requires higher temperatures (90°C-110ºC) compared with the isolated protein.[12]

In most proteins thermal modification ruptures the delicately balanced intramolecular forces and drastically reorganizes all levels of protein structure. Hydrophobic groups that had been inward oriented are turned outward, exposing them to unfavorable interactions with solvent water. Denaturation at temperatures above 80°C results in the loss of almost all secondary and tertiary structure, causing the protein to adopt a configuration that approaches a fully unfolded, random-coil conformation.[13] In contrast to this, denaturation of Ara h 1 leads to a secondary, more structured conformation of the protein, with an increased content of extended β-structures. This appearance of extended β-structures leads to the formation of large protein complexes. Despite these heat-induced conformational changes, the allergenic properties of Ara h 1 are not affected.[12] These findings confirm earlier reports on the heat stability of Ara h 1. By using ELISA inhibition analysis, Burks et al[14] showed that heat treatment of purified Ara h 1 for up to 1 hour at 100°C did not change IgE- or IgG-specific binding. However, these studies are based on purified Ara h 1. It was recently shown that the extraction yield of Ara h 1 progressively decreases at temperatures above 90°C, and no protein extraction was observed at 150°C,[9] possibly because of the aggregation of the protein. This reaction might explain the reduced protein amount of Ara h 1 in the boiled (100ºC) and fried (120ºC) peanuts in our study.

Heated Ara h 1 forms stable dimers, trimers, and larger complexes.[12] The Ara h 1 trimer is highly stable and possesses digestion-resistant fragments containing IgE-binding sites, indicating that the quaternary structure of Ara h 1 protects IgE-binding epitopes from degradation and may play a significant role in overall allergenicity.[15] In the present study we were able to show that the Ara h 1 trimer was almost completely absent in boiled and fried peanut extracts, highlighting the importance of the preparation methods on the allergenicity of peanut products.

In contrast to the small amounts of extractable Ara h 1 in boiled (100°C) and fried (120°C) peanuts, large amounts of Ara h 1 were extractable from self-roasted and commercially roasted peanuts (170°C), as well as peanut butter, in the present study. These findings are in agreement with those of others.[8,10] At higher temperatures, irreversible alterations of proteins occur, which include chemical modification of side chains. One of the most important chemical modifications is the Maillard reaction between reducing sugars and primary amine groups.[13] Peanuts undergo these thermal modifications when they are roasted. About 20 years ago, Nordlee et al[16] studied the allergenicity of various peanut products and showed that roasted products have an increased allergenicity. Recently, Maleki et al[8] showed that this enhancement of the allergenic properties of roasted peanuts is caused by the chemical modifications resulting from the Maillard reaction. In an in vitro model mimicking the Maillard reaction, they were able to show that Ara h 1 heated in the presence of sugars was visible on SDS-PAGE and showed enhanced allergenicity. In contrast, Ara h 1 heated in the absence of sugars became degraded and was undetectable.

In the present study the IgE binding to Ara h 2 and Ara h 3 was, in parallel with Ara h 1, significantly lower in boiled and fried peanuts in comparison with roasted preparations. These differences in allergenicity were observed, even though the quantity of protein was similar in all 3 peanut preparations in contrast to Ara h 1. Much less is known about heat-induced changes of Ara h 2 and Ara h 3. In contrast to Ara h 1, Ara h 2 and Ara h 3 do not aggregate on denaturation. This may explain why no differences in the amounts of protein were observed between the roasted, fried, and boiled peanuts in the present study. Maleki et al[8] showed that in parallel with Ara h 1, the allergenic properties of Ara h 2 are increased in roasted peanuts as a result of the protein modification brought about by the Maillard reaction. At high temperatures, this chemical modification of Ara h 2 may result in new IgE-binding epitopes responsible for the higher allergenicity.

With the present study, we were able to show that differences in methods of peanut preparation have an important effect on their final allergenicity. These findings may explain, at least in part, the differences in the prevalence of peanut allergy in the populations of the United States and Asian countries.

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