Peanut Allergen: Arachis hypogaea 1

Monica Trejo

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A food allergy is an unfavorable immune response to a substance in food being digested. According to the Food Allergy Research and Education (FARE) group, roughly 15 million Americans have food allergies. In 2013, the Centers for Disease Control and Prevention estimated that the number of children with food allergies have increased by 50% between 1997 and 2011. The number of people affected with food allergies is increasing over time, and the exact reason for it is unknown. The mechanism of food allergy involves an antibody within the immune system called immunoglobulin E (IgE), which is found throughout the blood. In order for a reaction to occur, an individual is first "sensitized" to the food allergen. This process of "sensitivity" occurs the first time an individual is exposed to a food allergen, the exact cause for it is unknown. During the first exposure, the allergen signals lymphocytes to produce the IgE antibody that is specific for the food allergen. The IgE antibody is found attached to a mast cell or basophil. A food allergen will bind to the immunoglobulin, which will signal the mast cell or basophil to release histamine and other chemicals causing an allergic reaction to food. The allergic reaction to a food allergen can cause swelling, hives, shortness of breath or even anaphylaxis. The symptoms of anaphylaxis are quite extreme involving: drop in blood pressure, trouble breathing, dizziness, and death. The purpose of this report is to look specifically into the aggregation of *Arachis hypogaea* 1 (Ara h 1) fragments because of its connection to allergic response, and to suggest possible remedies for this food allergen.

*Arachis hypogaea* 1 (Ara h 1) is known to be the primary cause of food allergy in peanuts, and also serves as a seed storage protein. This protein is utilized as the
nitrogen and amino acid source during the development of a new peanut plant.\textsuperscript{4} Ara h 1 is notoriously known for its allergen capacity, making it one of the most common food allergens in North America. Although there are several types of \textit{Arachis hypogaea} proteins (Ara h 2, Ara h 3, and others), the binding capacity of IgE specifically to Ara h 1 occurs in more than 90\% of peanut-sensitive individuals, thus making Ara h 1 a popular food allergen to study.\textsuperscript{4} The global impact of peanut allergy has caused this issue to become a public health concern. In some cases, school and work institutions have gone as far as banning peanut-based products in their foods. However, trends do exist where roasted peanuts will cause a more severe allergic reaction than raw peanuts. In addition, there have been instances where refined peanut oil will not affect an individual, but crude peanut oil will.\textsuperscript{5} The exact reason for this difference is still undergoing investigation.

From the paper "Evaluating pH-Induced Gastrointestinal Aggregation of \textit{Arachis hypogaea} 1 Fragments as Potential Components of Peanut Allergy," the research group sought to study the effect of Ara h 1 fragments under various pH levels.\textsuperscript{6} In one of the experiments, size exclusion chromatography (SEC) was used to obtain elution profiles of Ara h 1 at specific points of digestion. The peanut samples were purified by double-distilled water extractions with sodium azide, containing a protease inhibitor tablet, for one hour at room temperature. The conditions used for this study contained samples of Ara h 1 after various digestion times under solely acidic conditions, and also under acidic-to-basic conditions. The acidic conditions were used to represent digestion in the stomach, and the acidic-to-basic condition was used to represent the digestion track from the stomach to the intestines. From this experiment, the researchers were
able to conclude that digestion of Ara h 1 forms aggregates in the acidic-to-basic environment. Aggregation in the gastro-intestines encourages an increase binding of IgE to Ara h 1 fragments. In addition, more studies were conducted on the effect of Ara h 1 through the digestive system. From the paper "Digested Ara h 1 Loses Sensitizing Capacity When Separated into Fractions," the research group focused on the effect of Ara h 1 as it underwent digestion. In one of the experiments gel permeation chromatography (GPC) was performed on the digested fragments of Ara h 1, and for the analysis of the aggregated segments. The first trial of GPC was used mainly to get a reference for the aggregates that form from Ara h 1. The GPC spectrum showed the overall digested Ara h 1, and broke it down into large and small complexes. The second trial ran on GPC showed the comparison chromatography profiles for the digested Ara h 1, along with fractions of the large and small complexes. With this data, the research group was able to conclude that Ara h 1 loses its sensitizing capacity once the peptides are separated into fractions. When the sensitizing capacity is lowered (or lost), it means that binding capacity of IgE to Ara h 1 is lowered rather than increased. It appears as if the sensitivity of Ara h 1 depends on the accumulation of the peptides combined with aggregation, rather than a specific peptide in the sequence. However, it is crucial to note that the research group was not confident in whether the sensitizing ability of Ara h 1 depended upon a variety of peptides, or simply on peptides in the aggregated state.

Given that a number of people suffer food allergy from peanuts, research groups have strived to find methods in which peanut allergy can be lessened, or diminished. From the paper "Boiling Peanut Ara h 1 Results in Formation of Aggregates with
Reduced Allergenicity, the research group focused on the effect of boiling and roasting Ara h 1 protein in peanuts. The study included raw peanuts, roasted peanuts, peanuts that have been boiled at 100°C for about 15 minutes, and even glycated peanut samples. Sodium dodecyl-polyacrylamide gel electroporesis (SDS-PAGE) was used to analyze the Ara h 1 samples after thermal treatment. After the protein was denatured by SDS-PAGE, circular dichroism (CD) spectroscopy was used to compare the variants of the native and heat-treated Ara h 1 spectra. CD spectroscopy is used to study the adsorption of left and right circularly polarized light. In biochemistry, CD analysis can exhibit the absorption bands of optically active chiral molecules, which can be used to investigate protein structure. The data collected compared the samples that were only boiled and those that were boiled in glucose. The CD spectra showed that both these samples caused a partial loss of a secondary structure between 192-195 nm and the molar ellipticity at 190 nm. This result indicates that IgE binding occurred at a low rate for these samples. Atomic force microscopy (AFM) was also used to characterize Ara h 1 samples. The AFM demonstrated that the aggregated structures were either rod-like, simple rod-like, globular or shorter-like rods. In addition, the research group incorporated the use of enzyme allergosorbent test (EAST) to understand the IgE-binding capacities for the all of the Ara h 1 samples. The data from the EAST experiments found that both the boiled only and boiled in glucose samples showed a reduction for IgE-binding, further agreeing with the results from CD spectroscopy. From the work conducted in this paper, the authors were able to conclude that the boiled Ara h 1 aggregates possessed a lower allergenicity, and were varied from the other Ara h 1 samples studied. In addition, the results from the paper were able to
show that glycation had no influence on the sensitivity capacity of Ara h 1 compared to the samples ran as only boiled.\textsuperscript{8}

On the other hand, a different research group incorporated the use of ultrasound to reduce peanut allergenicity. In the paper "Reduction of Major Peanut Allergens Ara h 1 and Ara h 2 in Roasted Peanuts by Ultrasound Assisted Enzymatic Treatment," the research group was inspired by previous studies on the reduction in peanut allergenicity through the application of pulsed-UV and gamma irradiation.\textsuperscript{9} The focus of this paper is to study the three key influences on the Ara h 1 protein in peanuts. One is the effect of ultrasound technology on peanuts; second is to determine the best combination of enzyme concentration and treatment time on the allergens using trypsin and α-chymotrypsin; and third to study the efficiency of the overall experiment in hopes to find a methodology that could reduce allergenicity to peanuts. Bicinchoninic acid (BCA) assay method was used to determine the protein concentrations in the samples studied. Also, SDS-PAGE was used on both treated and untreated enzyme samples to obtain the purified allergen: Ara h 1. Enzyme-linked immunosorbent assay (ELISA) was used to determine the individual concentrations of Ara h 1 in the samples obtained. The incorporation of competitive inhibition enzyme-linked immunosorbent assay (ciELISA) was used to study the binding of IgE on the Ara h 1 allergen. The authors concluded that ultrasound technology significantly reduced allergenicity of peanuts. A figure illustrating the plot of IgE Binding against the concentration of proteins in extract samples was used to summarize the outcome of the data. The plot showed a significant trend in which the IgE binding decreased at higher concentrations of the protein (Ara h 1). The variables collected included untreated, ultrasound treated, trypsin treated,
ultrasound and trypsin treated, chymotriypsin treated, and ultrasound and chymotriypsin treated samples. The greatest change in showing a lowered IgE binding was found in samples treated with ultrasound only versus the combination of ultrasound and trypsin or chymotriypsin treated samples. The results show that applying ultrasound technology to peanuts removes the Ara h 1 and other allergens. Furthermore, the binding of IgE to the Ara h 1 allergen was greatly reduced because Ara h 1 was found in low concentrations, or not at all within the samples.9

Another study was conducted in the hopes of reducing the allergy capacity of peanuts. From the paper "Heat and Pressure Treatments Effects on Peanut Allergenicity," the research group studied the allergenicity of peanuts once they had undergone standard food processing protocol.10 Previous investigation on peanut allergens have determined that roasted peanuts contain a higher level of allergenicity than raw peanuts. This research paper focus primarily on the IgE binding capacity to Ara h 1 (and other peanut allergens) under thermal-processing methods. SDS-PAGE was used to prepare the peanut samples. Then the samples underwent Enzyme-linked immunosorbent assay (ELISA) to determine specific IgE binding capacity to the allergen: Ara h 1. In addition, CD spectroscopy was used to obtain spectra for both the raw and roasted peanut samples that underwent thermal processing and/or were autoclaved. In the end, the authors were able to conclude that IgE binding was significantly decreased in roasted peanuts under extreme autoclaving experiments. Also, the authors found a significant decrease in IgE binding of peanut allergens when the samples were autoclaved at 2.56 atm for about 30 minutes.10
In conclusion, the purpose of this report was to understand the aggregation of Ara h 1 fragments, and the methodologies used to reduced its allergenicity. Ara h 1 is of particular interest because it is the leading cause for food allergy in peanuts. The first half of this paper discussed the aggregation of Ara h 1 fragments in the digestive system under different pH environments, and the effect of fragment separation of the protein. Research demonstrates that Ara h 1 aggregation can be caused by pH changes in the gastro-intestines, and/or peptide fragments. Regardless of the cause of Ara h 1 aggregation in a specific situation, there is hope of reducing peanut allergenicity. Some possible remedies included boiling the peanuts at 100°C, exposing it to ultrasound assisted enzymatic treatment, and heat and pressure treatments. Research has concluded that the binding capacity of IgE to Ara h 1 and the aggregation of Ara h 1 can both be decreased by applying any of the methods discussed. With continuous research and applications, it appears as if there could be a commercialized treatment for individuals who suffer from peanut allergies in the near future.
References:


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