

Airborne concentrations of peanut protein

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ABSTRACT

Food allergy to peanut is a significant health problem, and there are reported allergic reactions to peanuts despite not eating or having physical contact with peanuts. It is presumed that an allergic reaction may have occurred from inhalation of airborne peanut allergens. The purpose of this study was to detect the possible concentrations of airborne peanut proteins for various preparations and during specific activities. Separate Ara h 1 and Ara h 2 monoclonal enzyme-linked immunosorbent assays and a polyclonal sandwich enzyme immunoassay for peanuts were used to detect the amount of airborne peanut protein collected using a Spincon Omni 3000 air collector (Sceptor Industries, Inc., Kansas City, MO) under different peanut preparation methods and situations. Air samples were measured for multiple peanut preparations and scenarios. Detectable amounts of airborne peanut protein were measured using a whole peanut immunoassay when removing the shells of roasted peanut. No airborne peanut allergen (Ara h 1 or Ara h 2) or whole peanut protein above the LLD was measured in any of the other peanut preparation collections. Ara h 1, Ara h 2, and polyclonal peanut proteins were detected from water used to boil peanuts. Small amounts of airborne peanut protein were detected in the scenario of removing shells from roasted peanuts; however, Ara h 1 and Ara h 2 proteins were unable to be consistently detected. Although airborne peanut proteins were detected, the concentration of airborne peanut protein that is necessary to elicit a clinical allergic reaction is unknown.

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Food allergy to peanuts remains a significant health problem, and its prevalence appears to be increasing.^{1–4} The potential for accidental exposure to peanuts can provoke significant anxiety for many patients and their families.⁵ There are reports of allergic reactions to peanuts despite no known ingestion or contact that are presumed to occur from inhalation exposure, but there are limited data to quantify the amount of airborne peanut proteins.^{6–10} To our knowledge, only one study has been able to detect the presence of airborne peanut protein; however, this was measured from the ventilation filters from two commercial airliners after 1 year of use.¹¹ There are surveys reporting subjective reactions to peanuts and other foods, particularly in the confined spaces of an airplane, although it is difficult to confidently exclude possible accidental contact or ingestion.^{12–14} One study failed to show a positive inhalation challenge to an open jar of peanut butter,¹⁵ and another was unable to detect Ara h 1 from participants wearing a personal air collector in the presence of peanut butter, opening shelled peanuts, and opening bags of unshelled peanuts.¹⁶ The specific peanut proteins Ara h 1, Ara h 2, and Ara h 3 have been more commonly associated with anaphylaxis compared with other pea-

nut proteins as exhibited by a Swedish study of individuals sensitized to peanuts in which anaphylaxis was reported among those sensitized to Ara h 1, Ara h 2, and Ara h 3 whereas specific IgE to rAra h 8, rAra h 9, profilin, or cross-reactive carbohydrate determinants were not associated with severe symptoms.¹⁷

There are reports and data of other foods causing allergic reactions *via* presumed airborne exposure or inhalation challenges,^{18–23} and it has been shown that an asthmatic response can be elicited in the presence of cooking food and increased levels of airborne soybean protein but not specifically to peanuts.^{24–30} Quantifications of airborne fish and shellfish allergen in low concentrations have been obtained,^{31,32} and there are studies showing subjective reactions to peanut protein as low as 100 µg after oral ingestion.^{33,34} Given multiple reports of allergic reactions attributable to inhalational exposure to peanut-containing foods, it seems plausible that airborne concentrations could become high enough to elicit a clinical allergic reaction in highly sensitized individuals. This seems especially true for confined spaces such as with commercial airplanes with recirculated air as there have been studies in regard to aircraft cabin quality and transport of human expiratory droplets in aircraft cabins.^{35–38} Therefore, we postulated that airborne peanut protein may be directly measured from the air.

METHODS

Separate Ara h 1 and Ara h 2 monoclonal enzyme-linked immunosorbent assays (ELISAs; Indoor Biotechnologies, Inc., Charlottesville, VA) and a polyclonal sandwich enzyme immunoassay (RIDASCREEN) for pea-

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nuts (R-Biopharm AG, Darmstadt, Germany) were used to detect the amount of airborne peanut protein collected using a Spincon Omni 3000 (Sceptor Industries, Inc., Kansas City, MO) air collector using different peanut preparation methods and situations. Protein content of peanut flour extract was determined using a bicinchoninic acid protein determination kit (Sigma-Aldrich, St. Louis, MO). Peanut flour containing 50% peanut protein per manufacturer (Byrd Mill, Ashland, VA), refined and unrefined peanut oil (refined peanut oil, Crisco, Orrville, OH; unrefined peanut oil, Loriva, Edison, NJ), peanut butter, and raw and roasted peanuts were obtained from local and specialty food stores. Single-serving peanut bags from a variety of airlines were generously provided by numerous colleagues.

The different peanut preparations included shelled and unshelled raw peanuts, shelled and unshelled roasted peanuts, an open jar of peanut butter, refined peanut oil, and unrefined peanut oil. In addition, air was sampled under various situations to simulate real-life circumstances that included boiling peanuts, removing raw and roasted peanuts from their shells, spreading peanut butter, pouring peanut flour, and opening actual airline peanut bags. Two different scenarios were performed with the opening of airplane peanut bags. The first consisted of opening a single airplane peanut bag adjacent to the collector. The second included an airplane scenario comprised of having six participants seated in three rows of two chairs, similar to what someone may experience on a commercial airline. Each participant opened three airplane peanut bags for each sample. The air collector was located at approximately shoulder level adjacent to the middle row. In addition, the water used for boiling peanuts was also sampled before and after boiling. All air samples were taken adjacent to the peanut source except as noted previously for the airplane scenario.

The air collector (Spincon Omni 3000) was placed in a well-ventilated room with a volume of 27.3 m³ and a constant supply vent flow rate of 6.2 m³/min, resulting in 7 air changes/hr when using an airflow correction factor of 0.5. This particular room was chosen mainly for convenience and to show proof of concept; however, most commercial airline cabins use ~50% recirculated air.^{35–38} The Spincon Omni 3000 was operated at a mean rate of 300 L/min for 10 minutes resulting in an air sample volume of 3 m³ of air. Three collections of each sample were performed and each collection was assayed in triplicate. Control air collections were performed before each sample. If consecutive samples were performed on the same day, a minimum of 1 hour was allowed between each sample to ensure adequate time for ventilation and reduce the possibility of air contamination between samples. A complete air collector line cleaning was performed per manufacturer's

specifications and all surrounding surfaces were cleaned with diluted bleach between each sample set to also prevent possible contamination.

Samples included an open jar of peanut butter; 3 tsp of refined and unrefined peanut oil in a small laboratory plate (surface area of 113 cm²); one airplane peanut bag; three airplane peanut bags for each of the six airplane scenario participants; and 1 C of raw shelled, dry roasted shelled, and dry roasted unshelled peanuts. Air samples were also collected after spreading 3 tsp of peanut butter onto a small laboratory plate, pouring 1 C of roasted peanut flour from a height of 6 in. onto a laboratory plate, manually removing the shells from 1 C of dry roasted shelled peanuts, and boiling 1 C of raw shelled peanuts in 800 mL of tap water. The peanuts were allowed to soak in water for 10 minutes and then boil for 5 minutes before beginning the air collection. The water was sampled after 10 minutes of soaking peanuts and after completing the boiling peanut air sample collection, which totaled 15 minutes of boiling. Although these two samples are nonairborne, this was performed primarily for further validation of the assay and because of results from our prior study.⁴⁰

The polyclonal assay was performed on the following samples: removing raw and roasted peanuts from their shells, roasted peanut, pouring peanut flour, an open jar of peanut butter, opening of an airplane peanut bag, the airplane scenario, boiling raw peanuts, and the water from soaked and boiled raw peanuts. Not all samples for all scenarios were assayed using the polyclonal method. Samples were excluded because the scenario did not produce detectable airborne amounts in a prior study.⁴⁰

RIDASCREEN FAST Peanut (Art. No.: R6202) is a sandwich enzyme immunoassay for the quantitative analysis of peanuts and parts of peanuts in food. Breakfast cereals, cookies, ice cream, and milk chocolate have been approved by the Association of Analytical Communities Research Institute according to the Performance Tested Method Program with license no. 030404.⁴¹ The manufacturer-stated limit of detection is 1.5 ppm of peanuts corresponding to 1.5 µg/mL and the limit of quantification is 2.5 ppm of peanuts corresponding to 2.5 µg/mL. The polyclonal antibody specifically detects peanut proteins, including the peanut allergen Ara h 1 and Ara h 2. Extracts from oat, corn, wheat, barley, sesame, or soy do not interfere with the test. With this particular assay, different kinds of nuts such as hazelnut, almond, walnut, cashew, pistachio, and pecan showed no cross-reaction.⁴² However, other studies have shown some degree of cross-reactivity between peanuts and tree nuts and among certain tree nuts *via* immunoassay.^{43–45} The cross-reactivity to chickpea is 0.001% and to green pea is 0.2%.⁴² Ara h 1 and Ara h 2 immunoassay reagents and standards for

the quantitative analysis of peanut allergens were obtained from Indoor Biotechnology Laboratories. These are sandwich immunoassays constructed using purified monoclonal antibodies directed to specific epitopes on their respective allergen proteins. The Ara h 1 ELISA kit has a manufacturer-stated sensitivity of 4 ng/mL and limit of detection of 31.5 ng/mL.⁴⁶ The Ara h 2 ELISA kit has a manufacturer-stated sensitivity of 0.5 ng/mL and limit of detection of 2 ng/mL.⁴⁷

Assays were performed per manufacturer's protocol. A BioTek XL microtiter plate spectrophotometer (Biotek, Winooski, VT) was used to calculate the optical density using KC4 Version 3.4 data analysis software (KC4 Software, Biotek). This software was also used to calculate standard curves and interpolated protein concentrations. In addition, a particle count was performed on certain sample sets using an ART instruments, Inc., handheld particle counter, model HHPC6IAG (Art Instruments, Inc., Grants Pass, OR). Further calculations and statistical values including 95% CIs of the samples were obtained using Microsoft Excel 2010 (Microsoft, Inc., Redmond, WA). Lower level of detection values were approximated from control and low-level assays.⁴⁸

RESULTS

Results of many of the multiple experimental scenarios are listed in Table 1. Any scenarios listed in the Methods section but not appearing in Table 1 were below the lower limit of detection (LLD). Nearly all air samples for the multiple scenarios failed to collect measurable levels of peanut protein evaluated either by polyclonal assay for whole peanut or specific monoclonal assay for major peanut allergen proteins Ara h 1 and Ara h 2. Measureable amounts of airborne peanut protein were detected in the removing shells of roasted peanut scenario; however, only one collection of this scenario was consistently positive with an average value of 5.5 µg/mL (CI, 2.2–8.9; Fig 1). All other air samples using the polyclonal peanut assay were less than the LLD. All air samples with the Ara h 1 and Ara h 2 assays were less than the LLD. Water collections from the boiled peanut samples were positive in all three assays except for the Ara h 1 of the water from the soaked peanut sample.

All ambient air samples performed before and between peanut sample collections and all negative controls were less than the LLD. Negative controls for each assay were performed using 1% bovine serum albumin–PBS with 0.05% Tween-20 and 18 MΩ of distilled water for the Ara h 1 and Ara h 2 assays. For the polyclonal peanut assay, the negative assay controls included the manufacturer supplied controls and 18 MΩ of distilled water. The positive controls consisted of 500 mg (504 mg measured) of roasted peanut flour

Table 1 Airborne concentration of peanut protein results of multiple experimental scenarios

Experimental scenario	Whole Peanut Assay µg/mL (LLD = 2.0)			Ara h1 Allergen Assay ng/mL (LLD = 1.7)			Ara h2 Allergen Assay ng/mL (LLD = 11)		
	Trial 1	Trial 2	Trial 3	Trial 1	Trial 2	Trial 3	Trial 1	Trial 2	Trial 3
Air during removing shells from raw peanuts	<LLD	<LLD	<LLD	<LLD	<LLD	<LLD	<LLD	<LLD	<LLD
Air during removing shells from roasted peanuts	5.5 (3.0)	<LLD	<LLD	<LLD	<LLD	<LLD	<LLD	<LLD	<LLD
Air during pouring peanut flour	<LLD	<LLD	<LLD	<LLD	<LLD	<LLD	<LLD	<LLD	<LLD
Air during open jar of peanut butter	<LLD	<LLD	<LLD	<LLD	<LLD	<LLD	<LLD	<LLD	<LLD
Air during opening single-serving peanut bag	<LLD	<LLD	<LLD	<LLD	<LLD	<LLD	<LLD	<LLD	<LLD
Air during opening 18 single-serving bags (airplane scenario)	<LLD	<LLD	<LLD	<LLD	<LLD	<LLD	<LLD	<LLD	<LLD
Air during boiling raw shelled peanuts	<LLD	<LLD	<LLD	<LLD	<LLD	<LLD	<LLD	<LLD	<LLD
Water from soaked raw shelled peanuts	6.8 (1.0)	4.6 (3.9)	8.6 (0.6)	<LLD	<LLD	<LLD	84.3 (21.6)	63.9 (9.4)	64.4 (9.8)
Water after boiling raw soaked peanuts	2.9 (0.5)	2.4 (0.4)	2.7 (0.5)	2.6 (0.9)	2.1 (0.2)	2.0 (1.3)	19.2 (14.4)	23.0 (6.7)	23.0 (13.0)
1:20 Extract of peanut flour in extraction buffer	6450.2	6810.2	5988.1	1121.9	928.97	866.94	240.76	374.49	256.58

LLD = lower limit of detection.

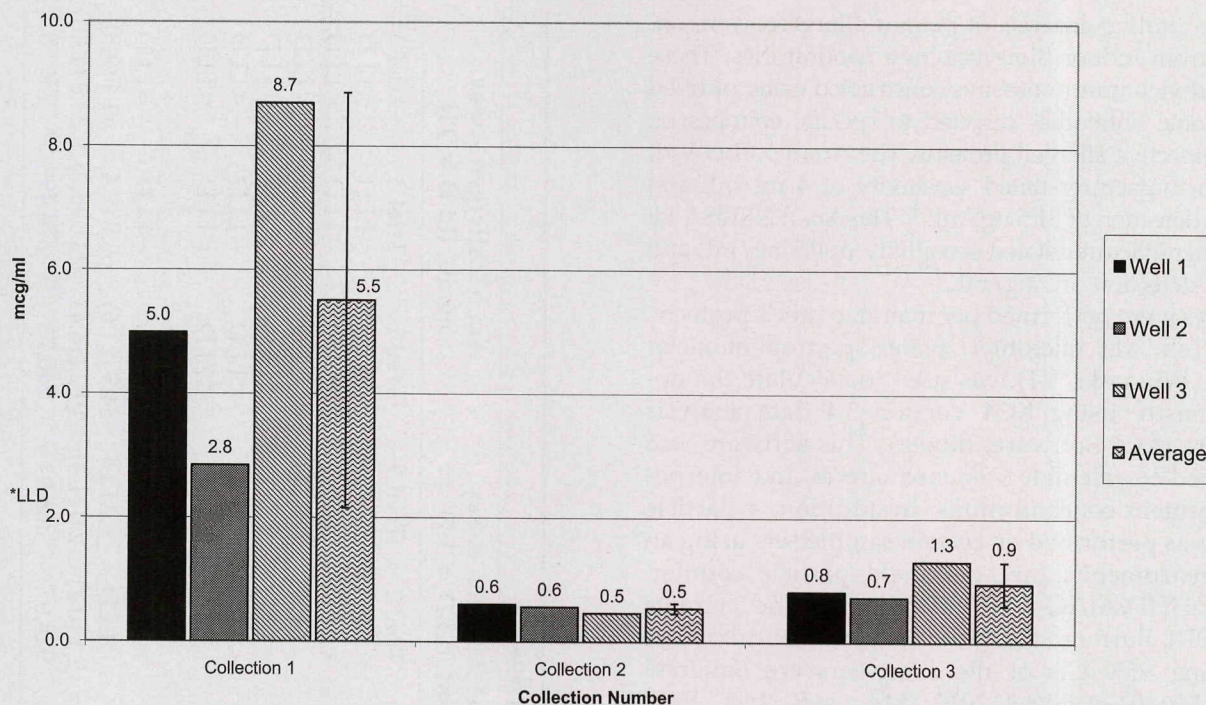


Figure 1. Polyclonal peanut protein concentrations of removing shells of roasted peanut sample.

extracted in 10 mL of buffer using the materials and protocol as supplied by the RIDASCREEN assay manufacturer and three 10-fold dilutions resulting in a final 1:1000 dilution. Based on controls and standard curves developed with each assay, the LLD for Ara h 1, Ara h 2, and polyclonal assays were 1.7 and 11.0 ng/mL and 2.0 $\mu\text{g/mL}$, respectively. All positive controls were positive including at the lowest 1:1000 dilution except for one collection for Ara h 1. All positive controls at the 1:1000 dilutions were near the LLD of each assay. The average level of the positive control with the polyclonal assay at the 1/1000 dilution was 6.4 $\mu\text{g/mL}$ (CI, 5.9–6.9). The special extraction buffer provided with the RIDASCREEN kit was used for all positive control extractions. An assay of a 1:5 water extract of peanut flour indicated a protein concentration of 6.5 mg/mL of protein or a yield of only 3.2% and a 4.4% yield when extracted with PBS. The particle count data were mostly unremarkable with the only major elevation noted after boiling peanuts (data not shown).

DISCUSSION

For the numerous scenarios and air collections performed, no amounts above the LLD of specific Ara h 1 or Ara h 2 allergens were measured. The additional measurement of Ara h 3 peanut proteins was preferred; however, a specific validated assay for Ara h 3 was not available for this study. The RIDASCREEN assay is a polyclonal assay and would detect the presence of Ara h 3 proteins, but it would not be able to differentiate this allergen from other peanut proteins.⁴⁹

In addition, other peanut proteins such as Ara h 6 and Ara h 8 would have been included in the study if such assays were available because studies have shown improved diagnosis of peanut allergy with component-resolved diagnostics.^{50,51}

Measurable concentrations of peanut protein were only detected in one air sample taken during the removing the shells of the roasted peanut scenario. Although this sample measurement was near the LLD, the average level of this one collection was in the range of the average value of the positive control at the 1/1000 dilution (6.4 $\mu\text{g/mL}$; CI, 5.9–6.9). A reasonable estimation of the detectable lower limit for airborne peanut protein using our methods is 2.7 μg of peanut protein/ m^3 of air. A similar lower detection limit could be calculated for Ara h 1 and Ara h 2 assays that would be much lower because of their sensitivity in the nanograms per milliliter range; however, they represent only a small percentage (1–0.1%) of the total peanut protein. Because we were not able to detect any of these specific allergen proteins in the air collections, we can not estimate a LLD for our method and this would represent weakness of this study.

The relatively low level of specific protein allergen measured in the water after boiling the peanuts was unexpected. However, it was consistent with the poor protein yield in the water and buffer extracts of peanut flour. Peanut protein is relatively insoluble in water at neutral pH.⁵² This solubility can be increased substantially through the use of heat, acid/base conditions or surfactants. The special extraction buffer provided

with the commercial peanut detection kit provided a much more efficient extraction with a 12.8% yield relative to total material and 25.6% yield relative to the amount of protein in the peanut flour as stated by the manufacturer.

The absence of detectable Ara h 1 protein in all of the airborne samples is in contrast to our prior data that showed detectable amounts of polyclonal peanut protein and Ara h 1 protein for the removing shells from roasted peanut sample.⁴⁰ In our prior study, the positive Ara h 1 results were from a single collection on two separate samples and were not performed in triplicate as in this current study. Because of the multiple histories of presumed allergic reactions to inhaled peanuts during airline flights, an ideal experiment scenario would have included multiple air samples on multiple commercial airlines where peanuts are being served. However, the logistics and commercial airline security measures currently in place made this scenario difficult to be performed.

Our experiments indicate that measureable airborne concentrations of polyclonal peanut proteins can be detected, however, not consistently. This inconsistency may be caused by the difficulty of uniform methods of removing the shells from the roasted peanuts and would suggest that a larger peanut particle or more peanut particles were released during one of the collections. In addition, many of the epitopes measured with the polyclonal peanut assay may not be allergenic. The measured concentrations in this study are low, and it is unknown if more or less inhaled airborne allergen is needed to elicit an allergic reaction than when ingested; however, these levels are near the range of prior studies that have reported subjective reactions at levels as low as 100 μg after oral ingestion.^{33,34} The question regarding the possibility that these concentrations are enough to produce clinical reactions as subjective histories and case reports suggest could be raised. Additional studies on possible eliciting doses of airborne concentrations of foods are needed.

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