

Immunoassay of peanut allergens in food-processing materials and finished foods

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To quantitate trace amounts of peanut allergens in food-processing materials and finished foods, we established a solid-phase radioimmunoassay inhibition using pooled sera from five peanut-sensitive subjects and a roasted peanut-meal extract covalently linked to polyacrylamide beads. Test samples were extracted, dialyzed, lyophilized, and reconstituted at 10 to 225 mg of dry weight per milliliter concentrations. The peanut-allergen content of test samples was expressed relative to a reference extract of roasted peanut meal that was assigned an arbitrary potency of 100,000 U/ml. In confectionary products spiked with varying quantities of peanut (0.7% to 0.0175%), the recovery of peanut allergen ranged from 31% to 94%. The sensitivity of the assay was 2.5 U/mg of dry weight from these samples (0.00875% peanut). Peanut allergens were undetectable in virgin vegetable oil used to roast peanuts, but 600 to 7600 U/mg of dry weight were present in oil after varying periods of use. The allergen content of used oil was reduced to 8 U/mg of dry weight by filtration and steam cleaning. The availability of such assays provides a means of monitoring finished food products for potential allergens. This monitoring in turn will provide the means to correlate symptoms experienced by sensitive patients with the exact quantity of allergen ingested. At the present time, there is little information on the quantities of ingested peanut allergen that pose risks to peanut-sensitive subjects. (J ALLERGY CLIN IMMUNOL 1990;86:41-4.)

The peanut continues to be a frequent cause of allergic reactions after food ingestion. Symptoms vary from mild abdominal discomfort to severe anaphylactic shock.¹⁻⁸ In a study by Yunginger et al.,⁷ four of seven patients who experienced fatal food-induced anaphylaxis died after peanut ingestion. All four patients unknowingly ingested peanut in food prepared and consumed away from home. It is highly desirable for peanut-sensitive individuals to have a means of recognizing and avoiding peanut-containing products. However, peanut allergens have been identified in nonpeanut foodstuffs manufactured on common pro-

Abbreviation used

RIA: Radioimmunoassay

cessing equipment⁹; this cross-contamination occurred as a result of inadequate cleaning. Therefore, this research was undertaken to evaluate an RIA inhibition as a means of quantitating peanut allergens in food-processing materials and finished foods.

MATERIAL AND METHODS

Reference peanut-meal extract

Roasted peanut meal was ground with mortar and pestle and defatted repetitively with petroleum ether. The defatted peanut meal was separated from the ether by vacuum filtration and air-dried overnight.

Defatted peanut-meal extract was prepared by mixing 10 gm of defatted peanut meal with 100 ml of phosphate-buffered saline. The mixture was stirred for 20 hours at 25° C. The supernatant was removed and stored at 4° C.

Test-sample extracts

Our test samples included experimental candy samples prepared especially for this project. These samples were

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TABLE I. Allergenic activity of several peanut-containing samples as measured by RIA inhibition

Sample ID	% Peanut solids	Sample wt (gm)	Total extractable solids (gm)	Test concentration (mg dry wt/ml)	Roast peanut RIA inhibition	
					Protein concentration mg/ml by ninhydrin	Allergen content (U/ml)
Peanut cream alone	34	73.6	3.4	200	63	931,097
Experimental candy spiked with varying quantities of peanut cream						
2%	0.7	30.1	2.2	200	71	8907
1%	0.35	30.1	2.1	200	83	5787
0.5%	0.175	30.1	1.8	200	93	2142
0.25%	0.088	29.4	1.7	200	143	1269
0.1%	0.035	30.1	3.9	200	53	305
0.05%	0.0175	30.1	3.3	200	44	59
M&M's plain chocolate	Unknown	182	4.6	250	ND	7135

ID, Identification; ND, not done.

TABLE II. Allergenic activity of vegetable oil during various stages of roasting peanuts

Sample ID	Sample wt (gm)	Extractable solids (gm)	Test concentration (mg dry wt/ml)	Roast peanut RIA inhibition	
				Allergen content (U/ml)	Specific activity (U/mg dry wt)
Vegetable oil					
Virgin	169	0.01	10	ND	ND
Virgin	216	0.02	100	ND	ND
Used	177	0.03	150	95,735	638
Used	212	0.07	100	769,250	7692
Cleaned	188	0.07	100	780	7.8

ID, Identification; ND, not detected.

spiked with varying quantities (0.05% to 2%) of peanut cream (34% peanut solids blended with other nonpeanut ingredients). We also tested M&M's plain chocolate candies (M&M/Mars, Hackettstown, N.J.), virgin vegetable oil, and the same vegetable oil that had been used to roast peanuts. Each sample was weighed, ground with mortar and pestle, and extracted with 1:5 (wt/vol) of warm (37° C) phosphate-buffered saline for 4 hours at 37° C with intermittent mixing. The extracts were then placed at 4° C overnight and recovered by centrifugation at 7500 g for 15 minutes at 4° C. The supernatants (aqueous layer) were filtered through Whatman No. 4 filter paper (Whatman, Inc., Clifton, N.J.) and recentrifuged at 7500 g for 15 minutes at 4° C. The filtered extracts were then dialyzed at 4° C against three changes of 200 mmol/L of ammonium bicarbonate with Spectrapor-3 dialysis casing (nominal 3500 molecular weight cutoff) and lyophilized.

RIA inhibition

The relative allergenicities of reconstituted test-sample extracts were determined by RIA inhibition according to the method of Adolphson et al.¹⁰ We used defatted roasted peanut-meal extract coupled to polyacrylamide beads¹¹ (Matrex Pel 102 [Amicon Corp., Danvers, Mass.]; 300 mg of ninhydrin protein per 1 gm of Matrex¹²) as the solid-phase allergen, pooled IgE containing sera from five peanut-sensitive individuals as the antibody, and the defatted roasted peanut-meal extract described previously as the standard. The peanut-allergen contents of test samples were expressed relative to the standard extract of roasted peanut meal that was assigned an arbitrary potency of 100,000 U/ml. RIA inhibition results were evaluated by a computerized version of the parallel-line assay developed by the Center for Biologics Evaluation and Research of the U.S. Food and Drug Administration.¹³ All assays met four criteria

Roast peanut RIA inhibition

Specific activity (U/mg dry wt)	Specific activity (U/mg protein)	% Recovery
4655	14,779	100
44.5	125.5	76
28.94	69.7	94
10.71	23	60
6.35	8.9	68
1.53	5.8	92
0.30	1.3	31
28.5	ND	—

for validity: (1) minimum of four data points on the inhibition curve, (2) data points bracketed the 50% inhibition point, (3) linear correlation coefficient of ≥ 0.90 for each inhibition curve, and (4) slopes of inhibition curves and standard curve were not statistically different by Student's paired *t* test at $p < 0.01$.

RESULTS

The results of the RIA inhibition of the test samples are presented in Tables I and II. The allergenic activity of several peanut-containing samples, as well as the percent recovery of peanut allergen from the experimental candies, which ranged from 31% to 94%, is presented in Table I. The calculations necessary to derive the percent recovery are

$$\text{Percent recovery} = \frac{\text{U out of sample}}{\text{U into sample}} \times 100$$
$$\text{U out of sample} = \text{SAS} \times \text{ESS}$$

where SAS is the specific activity of the sample (unit per milligram of dry weight) and ESS is the extractable solid in the sample (milligrams).

$$\text{U into sample} = \frac{\text{SAC} \times \text{ESC}}{\text{C}} \times \%C \times \text{S}$$

where SAC is the specific activity of peanut cream (units per milligrams of dry weight), ESC is the extractable solid in the peanut cream (milligrams), C is the weight of peanut cream (gram), %C is the percentage of peanut cream in the sample, and S is the weight of the sample (gram).

We used peanut cream that contained 34% peanut

solids. The sensitivity of the assay was 2.5 U/mg of dry weight from these candy samples; this finding corresponds to a final peanut concentration of 0.00875% in the experimental candy studied. Peanut allergenic activity was easily measured in the M&M's plain chocolate candy, which contained approximately the same specific activity as the experimental candy spiked with 1% peanut cream (or 0.34% peanut solids).

The allergenic activity of the vegetable oils tested is presented in Table II. Peanut allergens were undetectable in virgin vegetable oil. After the oil was used for peanut roasting, peanut allergens were present at levels of ~ 600 to 7600 U/mg of dry weight. The allergen content of this used oil was reduced to ~ 8 U/mg of dry weight by filtration and steam cleaning. The sensitivity of the RIA inhibition was 0.16 U/mg of dry weight when it is used to assay the various oils.

DISCUSSION

Peanuts contain two major storage proteins, arachin and conarachin. The major peanut allergen, Peanut-I, as identified by Sachs et al.,¹⁴ is believed to represent a subfraction of either arachin or conarachin. The allergens are present in the protein portion of the kernel. Taylor et al.¹⁵ demonstrated that virgin peanut oil was not allergenic in 10 individuals with marked peanut sensitivity. Although some peanut protein may be present in crude pressed oil, finished peanut oil sold commercially contains no detectable protein.¹⁵ However, new food products containing peanut allergens are being introduced into the American diet that consumers may be unaware of. Deffavored peanuts are an example. These are peanuts that have been pressed, deffavored, reffavored, and sold as other nuts (walnuts, almonds, etc.). Nordlee et al.¹⁶ and our own unpublished data have demonstrated that these peanut products retain their allergenicity.

It is logical to expect that peanut oil (or any other kind of oil, for that matter) might be contaminated with peanut protein if the oil is used to roast peanuts. To our knowledge, this was demonstrated for the first time in our present study. The oil that was used to roast peanuts became contaminated with peanut protein after varying periods of use. This can also be the case with other allergens. Cooking oil contamination by fish allergen is the most likely cause of anaphylaxis and subsequent death for case 7 in the study by Yunginger et al.⁷ in which a known fish-sensitive individual consumed french fries that tasted like fish.

Peanut-sensitive individuals have IgE antibodies to

components in both raw and roasted peanuts,² despite the fact that most peanuts consumed by humans are roasted. Our study confirms previous studies that peanut roasting does not destroy the allergenic components.¹⁶⁻¹⁸ We observed an increase in allergenicity in the oil used to roast peanuts. We also documented a decrease in the allergenicity with filtration and steam cleaning of the used oil. This suggests that the peanut allergen may be preferentially soluble in the steam or may be localized to particulates suspended in the used oil. This assay may be used to determine if contamination of a food product by a suspected allergen has occurred within the analytical limits of detection. Manufacturers that produce a variety of potentially allergenic food products using common equipment can thus have a means of monitoring food products for possible equipment malfunction or inadequate cleaning.

Using this RIA, we were able to detect microgram quantities of peanut allergen in various experimental and commercial products. The M&M's plain candy was chosen for study because it is not widely recognized as containing peanuts, although peanut appears on the package-ingredient list. Although there are no published data on how frequently peanut-sensitive individuals have reacted to this or other peanut-containing candy, peanut-sensitive individuals should be cautioned to avoid eating any commercially available products in which minute amounts of peanuts are present. They should be encouraged to review the ingredient list of every product they consume.

At the present time, there are little data on the quantities of ingested peanut allergen that pose risks to peanut-sensitive individuals. There have been reports of peanut-sensitive individuals developing symptoms by inhalation of allergen when the lid is removed from a jar of peanut butter.⁸ A study by Atkins et al.¹⁹ demonstrated that quantities between 100 to 350 mg of cottonseed-meal protein produced symptoms in cottonseed meal-sensitive individuals. Symptoms experienced by sensitive individuals depend on both the degree of sensitivity and the quantity of contaminated food eaten. Assays, such as the assay described here, can provide a means to correlate quantities of ingested allergens with the development of clinical symptoms in food-sensitive individuals.

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