1981

Allergenicity of Various Peanut Products as Determined by RAST Inhibition

Julie A. Nordlee

Steve L. Taylor

R. T. Jones

John W. Yunginger

Published in *Journal of Allergy and Clinical Immunology* 68:5 (1981), pp. 376–382; doi: 10.1016/0091-6749(81)90136-6

Submitted April 27, 1981; accepted July 31, 198l; published online November 22, 2004.

Allergenicity of Various Peanut Products as Determined by RAST Inhibition

Julie A. Nordlee, B.S., Steve L. Taylor, Ph.D., R. T. Jones, B.S.,

and John W. Yunginger, M.D.

Food Research Institute, Department of Food Microbiology and Toxicology, and the Department of Food Science, University of Wisconsin, Madison, and the Department of Pediatrics and Allergic Diseases Research Laboratory, Mayo Graduate School of Medicine, Mayo Clinic and Foundation, Rochester, Minnesota

Corresponding author – Steve L. Taylor, Food Research Institute, University of Wisconsin, Madison, WI 53706

Abstract
Extracts of 19 different peanut products and peanut oil were tested for their allergenicity by the radioallergosorbent test inhibition assay using a crude peanut extract from raw peanuts as the standard for comparison. Seventeen of the extracts were able to competitively inhibit the binding of serum IgE from peanut-sensitive patients with the solid-phase raw peanut extract. Peanut oil and the extract from hydrolyzed peanut protein did not inhibit binding, which suggests that these products are not allergenic. The peanut hull flour extract showed a slight ability to inhibit binding, suggesting that this product contains minor amounts of the peanut allergen.

Abbreviations used: RAST: radioallergosorbent test; HPP: hydrolyzed peanut protein

Peanuts are among the most allergenic foods in the American diet and in hypersensitive individuals may elicit a broad range of adverse reactions from abdominal discomfort to anaphylactic shock.[1–7] The incidence of severe anaphylactic reactions to peanuts is higher than for most other types of foods. Most peanut-allergic individuals avoid problems by eliminating peanuts from their diets. For the most part, avoidance of peanuts is not particularly difficult because of the limited uses for peanuts in the American food supply.

Peanuts appear in the American diet in easily recognized forms such as roasted peanuts and peanut butter. Peanuts are also used in snack food items such as candy, but this use is widely recognized and fairly easily avoided by peanut-allergic individuals. However, this situation may be changing.

In the future, peanuts may occupy an increasingly important role in the American diet because of their superior nutritional benefits. Peanuts can be a good source of protein in the diet. The trend toward the increasing use of peanuts in the diet has already begun with the development of new products made from peanuts, including peanut butter powder and syrup, deflavored peanuts, and a number of peanut flours.[8] New formulations are being developed to make use of these new products, e.g., peanut flour in bakery goods and snack foods.[9]

Although these new developments in the use of peanuts may have a positive nutritional effect on the American diet, the effect on individuals allergic to peanuts may be deleterious. As a result of the increased number of uses for peanuts in the diet, particularly in formulated foods, peanut-allergic individuals will find it increasingly difficult to identify products containing peanuts without close scrutiny of the labels. Part of the dilemma faced by these individuals is created by physicians who would advise peanut-allergic individuals to avoid all peanut products. This conservative advice has no substantial scientific basis in many cases because no definitive information exists on the allergenicity of these new peanut products.

Recently, a partial purification of peanut allergen was achieved by Sachs et al.,[10] revealing that the major allergen was a protein. Some commercially available peanut products, including HPP (made by acid hydrolysis of peanuts) and peanut oil,[11] do not contain any detectable "protein." Because the major allergen is a protein, one would predict that HPP and peanut oil would be nonallergenic and that there would be no need for the avoidance of these products by peanut-hypersensitive individuals: The protein allergen of peanuts is probably heat stable, since Gillespie et al.[2] showed that raw and roasted peanuts were equally allergenic, as determined by the RAST. However, the impact of various other processing techniques on the allergenicity of this protein is unknown. Consequently, we performed a systematic study of the allergenicity of various commercially available and feasible peanut products by the RAST inhibition procedure.

Materials and Methods

Peanut products

Raw Virginia peanuts, raw Florunner peanuts, oil-roasted Virginia peanuts, and dry-roasted Florunner peanuts were generously supplied by Standard Brands, New York, New York. Several types of peanut flour, including full-fat peanut flour, partially defatted peanut flour, and three types of toasted peanut flour (light, medium, and dark) were kindly provided by Flavored Nuts, Inc., Tyrone, Pennsylvania, a division of PERT, Inc., Edenton, North Carolina. The toasted flours were prepared essentially as described by McWatters and Cherry.[12] These peanut flours were prepared from blanched, ground peanuts in the cases of the full-fat flour and the toasted flours, or from raw, ground peanuts in the case of the partially defatted flour. Flavored Nuts, Inc., also provided the pressed or deflavored

peanuts. Another commercially available peanut flour, Gold Nut II flour (prepared from blanched, ground peanuts) was donated by Gold Kist, Inc., Atlanta, Georgia. Dr. Robert L. Ory, USDA Southern Regional Research Center, New Orleans, Louisiana, kindly supplied two different samples of raw, defatted white-skin peanut flour. Peanut butter syrup (a blend of corn syrup, peanut butter, water, dried whey, powdered cellulose, and lecithin) and peanut butter powder (a blend of peanut butter and dried whey) were generously provided by Home Brands, a division of Peavey, Minneapolis, Minnesota. A sample of peanut hull flour was obtained through the courtesy of Dr. J. L. Collins, University of Tennessee, Knoxville, Tennessee. The process for production of peanut hull flour has been reported.[13] The HPP, made by acid hydrolysis of peanuts, was a generous gift from Food Ingredient Specialties, Fribourg, Switzerland. Peanut butter, peanut butter flavored chips, and peanut oil were purchased from a local retail outlet in Madison, Wisconsin. The raw peanuts used as a standard in the RAST inhibition assay were obtained from a supermarket in Rochester, Minnesota.

Extraction procedure

The peanut product (100 gm) was ground in a blender (if necessary) and placed in a 500-ml Erlenmeyer flask to be defatted. In the defatting procedure, 250 ml of acetone was mixed with the peanut product. The suspension was allowed to settle, and the acetone was decanted and discarded. The peanut product was then mixed with 250 ml of ethyl ether and allowed to settle. The ether was decanted and discarded. The ether treatment was repeated five times. After the fifth ether extraction, the defatted peanut producte was separated from the ether by vacuum filration and was air dried overnight. An extract of the defatted peanut was prepared by adding 300 ml of 0.1 M ammonium bicarbonate, mixing, and adjusting the pH to 8.0. The mixture was stirred for 20 hr at 25°C. The extract was clarified by centrifugation for 30 min at 23,300 × g at 2°C. The protein concentration of the supernatant was determined by the method of Lowry et al.[14] The ahove procedure was used for all products except peanut oil and HPP. HPP was not defatted but was subjected to the rest of the procedure. Peanut oil was tested directly by RAST inhibition without defatting or extraction.

RAST inhibition assay

Comparative allergenicities of the peanut product extracts were determined by RAST inhibition according to the methods of Adolphson et al.[15] The IgE antibody pool comprised the combined sera obtained from five individuals highly sensitive to peanuts. These individuals' RAST scores ranged from 1500% to 2900% of negative control serum. The solid-phase peanut allergen was prepared by coupling crude raw peanut extract to cyanogen bromide–activated microcrystalline cellulose particles.[10] The RAST inhibitory activities of test peanut extracts were compared with the inhibition curve produced by standard reference peanut extract prepared in the Allergic Diseases Research Laboratory, Mayo Clinic. RAST inhihition results were evaluated by analysis of covariance[16] with a programmable Hewlett-Packard 9810A calculator.

Results

The results of the RAST inhibition assays of the peanut product extracts are displayed in Figures l to 5. The eight peanut flours prepared from the cotyledons of the peanut were allergenic as demonstrated by their abilities to inhibit the binding of serum IgE to the solid-phase peanut allergen (Figs. l and 2). The slopes of the RAST inhibition lines for five of the peanut flour extracts (white-skin peanut flour PI288160, toasted peanut flour–light, toasted peanut flour–medium, toasted peanut flour–dark, and full-fat peanut flour) did not vary significantly from the slope of the RAST inhibition line of the standard raw peanut extract. The similarities in the slopes of these lines suggest that these products contain a similar allergen or allergens. The slopes of the RAST inhibition lines for three of the peanut flour extracts (white skin peanut flour C32W, Gold Nut II flour, and PERT partially defatted peanut flour) were significantly different from the slope of the RAST inhibition line of the standard raw peanut extract. The reasons for this variance are unclear but may be related to the impact of processing on certain allergenic detenninants or to some difference between the allergenic detenninants of distinct source varieties of peanuts.

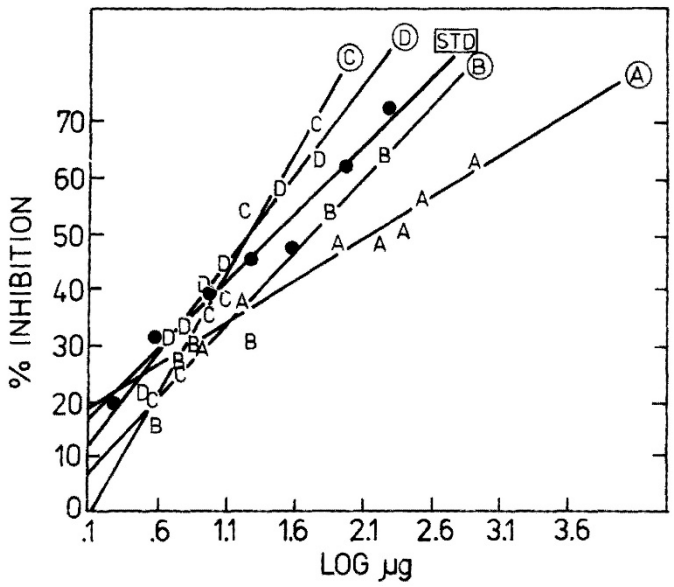

Figure 1. RAST inhibition by four peanut flour extracts. In this and subsequent figures, the percent inhibition of the binding of serum IgE to solid-phase raw peanut extract produced by the various extracts is plotted as a function of the log of the protein concentration (μg), and the standard (STD) line represents inhibition by fluid-phase raw peanut extract. *Extract A*, white-skin peanut flour C32W; *extract B*, white-skin peanut flour PI288160; *extract C*, Gold Nut II peanut flour; *extract D*, PERT partially defatted peanut flour.

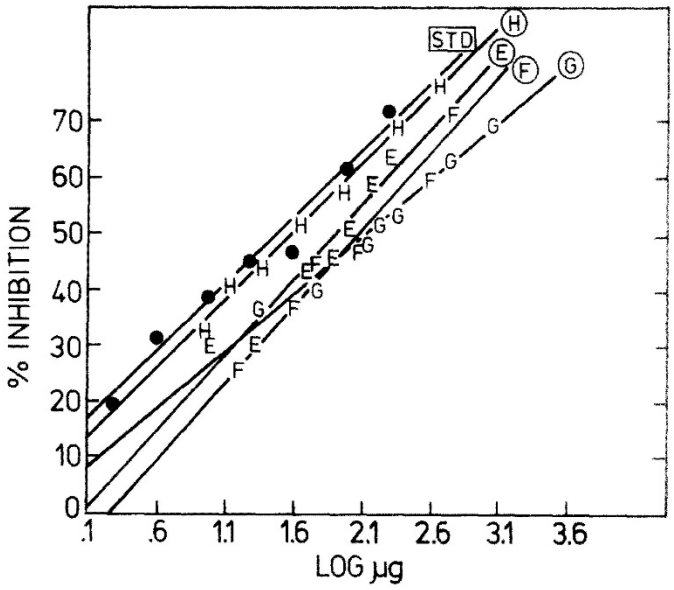

Figure 2. RAST inhibition by four peanut flour extracts. *Extract E*, toasted peanut flour–light; *extract F*, toasted peanut flour–medium; *extract G*, toasted peanut flour–dark; *extract H*, full-fat peanut flour.

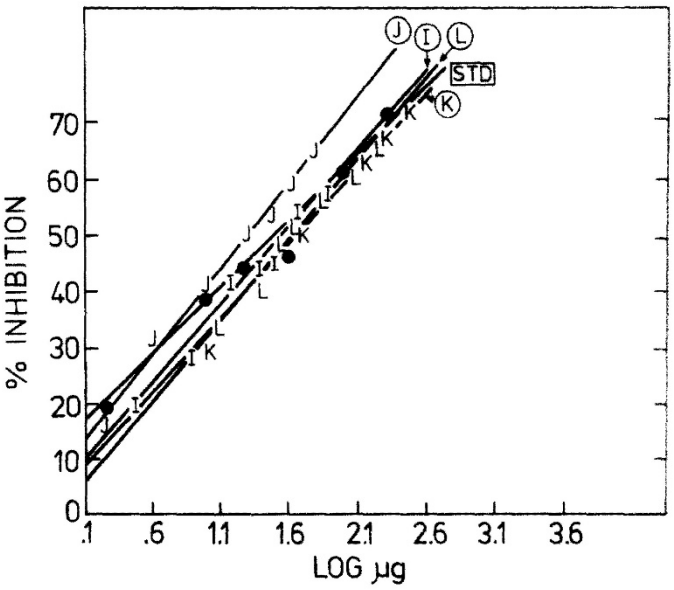

Figure 3. RAST inhibition by peanut butter product extracts. *Extract I*, peanut butter powder; *extract J*, peanut butter; *extract K*, peanut butter syrup; *extract L*, peanut butter–flavored chips.

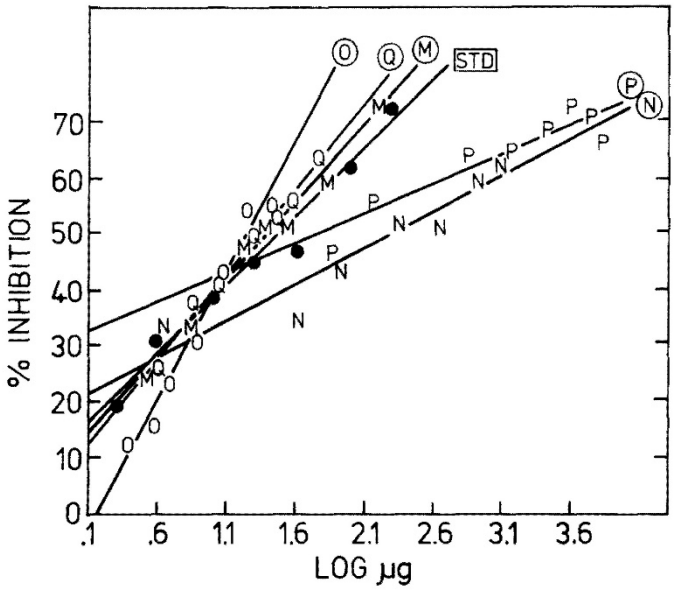

Figure 4. RAST inhibition by raw and roasted peanut extracts. *Extract M,* deflavored peanuts; *extract N,* raw Virginia peanuts; *extract O,* oil-roasted Virginia peanuts; *extract P,* raw Florunner peanuts; *extract Q,* dry-roasted Florunner peanuts.

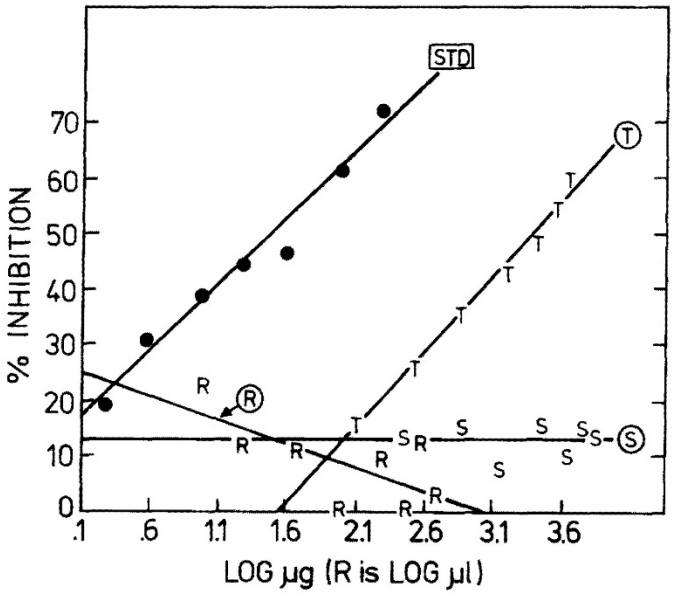

Figure 5. RAST inhibition by other peanut product extracts and peanut oil. The percent inhibition of the binding of serum IgE to solid-phase raw peanut extract that is induced by the various extracts is plotted as a function of the log of the protein concentration (μg) or the volume of extract (μl) in the case of peanut oil. *R,* peanut oil; *extract S,* HPP; *extract T,* peanut hull flour.

The four products containing peanut butter were allergenic as determined by the RAST inhibition relationships (Fig. 3). The slopes of the RAST inhibition lines for the peanut butter product extracts were very similar to the RAST inhibition slope produced by the standard raw peanut extract, although the slope of the peanut butter extract differed significantly from that of the standard peanut extract ($F_{1,10} = 7.03$; $p < 0.025$). The peanut butter extract was somewhat more allergenic, as indicated by the degree of inhibition obtained by a given amount of this extract. Since the peanut butter extract contained a higher proportion of peanut protein, this result was expected. The other peanut butter products contained whey protein in addition to peanut protein.

The RAST inhibition results obtained with the raw and roasted peanut products are shown in Figure 4. These products were allergenic, although extracts of both raw Virginia and raw Florunner were somewhat less allergenic than the standard raw peanut extract. This finding might be explained by varietal differences in the nature of the allergenic determinants. However, other factors must also be involved because the extracts of oil-roasted Virginia peanuts and dry-roasted Florunner peanuts had approximately the same degree of allergenicity as the standard raw peanut extract. The slopes of the RAST inhibition lines obtained with some of these extracts (raw Virginia peanuts, oil-roasted Virginia peanuts, and raw Florunner peanuts) were significantly different ($p < 0.005$) from the RAST inhibition slope obtained with the standard raw peanut extract. The slopes for the extracts of the deflavored peanuts and the dry-roasted Florunner peanuts did not differ significantly from the slope obtained with the standard raw peanut extract.

As shown in Figure 5, peanut oil and the extract of HPP apparently do not contain the peanut allergen, whereas the peanut hull flour extract contains only small amounts of the peanut allergen. Some technical difficulties were encountered in incorporating peanut oil into the RAST inhibition assay, which may explain the negative slope of the RAST inhibition line. Possibly, the physical properties of the oil altered the nonspecific adsorptive properties of the discs. However, 10 μL of undiluted peanut oil effected only a 22% inhibition of the binding of serum IgE to solid-phase raw peanut extract; this level of inhibition does not indicate appreciable allergenic activity. The lack of allergenicity associated with the extract of HPP is clearly demonstrated. Even 4000 μg of this extract elicited only a 13% inhibition of binding. The peanut hull flour extract exhibited allergenic activity but at a far lower level than that obtained with the extracts of the peanut flours, the peanut butter products, or raw and roasted peanuts (Figs. 1 to 4). The slope of the RAST inhibition line obtained with the extract of peanut hull flour differed significantly from the slope obtained with the standard raw peanut extract ($F_{1,10} = 11.78$; $p < 0.01$).

Discussion

Seventeen of the 20 peanut products were allergenic as determined by the RAST inhibition assay. The standard extract and two of the allergenic test peanut products were raw peanuts of different varieties. The other 15 allergenic peanut products were all processed in some way. Apparently, the processing treatments used in preparing these products had a negligible effect on the allergenicity of the final product. The processing treatments used

with these 15 products included shelling, blanching, dry roasting, oil roasting, toasting, grinding, defatting, extracting, and combining with other ingredients.

Three of the peanut products (peanut oil, HPP, and peanut hull flour) had little or no allergenic activity in the RAST inhibition assay. The processes used in preparing these products must remove or destroy the peanut allergen. Peanut oil contains only the lipid fraction of the peanut. The major peanut allergen, which Sachs et al.[10] have determined to be a protein, is likely not extracted with the oil during the pressing and filtration treatments. Peanut oil contains no protein,[11] and its lack of allergenicity was recently confirmed by a double-blind challenge test with 10 peanut-hypersensitive patients.[17] HPP is made by the acid hydrolysis of defatted peanuts; the final product is a mixture of amino acids supplemented with salt. Hydrolysis of the peanut allergen to its constituent amino acids would be expected to destroy the allergenic activity, and apparently this was the case. However, a double-blind challenge test with peanut-hypersensitive individuals will be necessary to confirm beyond question that HPP is not allergenic. Peanut hull flour had only a slight amount of allergenic activity, much lower than that for flours made from the kernel portion of the peanut (Figs. 1 and 2). Peanut hull flour has been developed as a possible source of dietary fiber that utilizes the normally wasted peanut hulls.[13] The final product contains 7% protein,[13] and the allergenic activity may arise from residual cotyledon material in the product. The majority of the allergen is probably removed with the cotyledons before the product is prepared, but the flour may very likely contain enough residual allergenic activity to elicit adverse reactions in peanut-hypersensitive individuals.

Although only three peanut products had little or no allergenic activity, quantitative differences were observed in the comparative allergenicities of the other 17 peanut products. Comparisons can be made on the basis of the amount of protein required to achieve 50% inhibition in comparison with the standard raw peanut extract. With some products (Gold Nut II flour, PERT partially defatted peanut flour, peanut butter, deflavored peanuts, oil-roasted Virginia peanuts, and dry-roasted Florunner peanuts), lower amounts of protein were necessary to achiew 50% inhibition than those required with the standard raw peanut extract. This finding suggests that these products were more allergenic than the standard. Since heat treatment is involved in the processing of four of these products (Gold Nut II flour, peanut butter, oil-roasted Virginia peanuts, and dry-roasted Florunner peanuts), the suggestion might be made that heat treatment increases the allergenicity of peanuts perhaps by increasing the availability of allergenic binding sites on the protein. However, it is widely recognized that such comparisons may be misleading when the slopes of the RAST inhibition lines are significantly different from the standard, as they were in many of these cases. Sachs et al.[10] obtained evidence for multiple allergens in peanuts. It is likely that the various peanut extracts contain different allergenic determinants or different proportions of the allergenic determinants, which would affect their abilities to inhibit binding with serum IgE.

Peanuts contain two principal storage proteins, arachin and conarachin.[18] Arachin and conarachin and various subfractions obtained from these two principal classes have similar amino acid compositions.[19] Consequently, it is very likely that arachin, conarachin, and the subfractions contain similar amino acid sequences. This would explain the association of allergenicity with many of the protein fractions.[10] The proportions of arachin, conarachin,

and their subfractions can be influenced by ionic strength, type of ions, pH, and temperature.[19–23] Also, the amino acid composition and electrophoretic mobilities of the peanut proteins are different for different varieties of peanuts.[24,25] Within the same variety of peanuts, the amino acid composition and electrophoretic mobilities of the peanut proteins can change as a function of growing conditions, soil conditions, climate, and moisture.[26] Consequently, the number and proportion of allergenic determinants in the various peanut product extracts would be expected to change.

However, such changes in the nature of the allergenic determinants do not have any practical influence on the risk of these peanut products to peanut-hypersensitive individuals. These individuals must avoid all peanut products with the exception of peanut oil and possibly HPP, in which the peanut allergen(s) is destroyed by acid hydrolysis. Other processes may be developed eventually that will allow the destruction of the peanut allergen(s).

Funding – Supported in part by contributions from the food industry and by the College of Agricultural and Life Sciences, University of Wisconsin.

References

1. May CD, Bock SA: A modern clinical approach to food hypersensitivity. Allergy 33:166, 1978.
2. Gillespie DN, Nakajima S, Gleich GJ: Detection of allergy to nuts by the radioallergosorbent test. J Allergy Clin Immunol 57:302, 1976.
3. Bock SA, Lee WY, Remigio LK, May CD: Studies of hypersensitivity reactions to foods in infants and children. J Allergy Clin Immunol 62:327, 1978.
4. May CD: Objective clinical and laboratory studies of immediate hypersensitivity reactions to foods in asthmatic children. J Allergy Clin Immunol 58:500, 1976.
5. Haddad ZH, Korotzer JL: Immediate hypersensitivity reactions to food antigens. J Allergy Clin Immunol 49:210, 1972.
6. Chua YY, Brrner K, Llobet JL, Kokubu HL, Collins-Williams C: Diagnosis of food allergy by the radioallergosorbent test. J Allergy Clin Immunol 58:477, 1976.
7. Aas K: The diagnosis of hypersensitivity to ingested foods: reliability of skin prick testing and the radioallergosorbent test with different materials. Clin Allergy 8:39, 1978.
8. Lusas EW: Food uses of peanut proteins. J Am Oil Chem Soc 56:245, 1979.
9. McWatters KH: Cookie baking properties of defatted peanut, soybean, and field pea flours. Cereal Chem 55:853, 1978.
10. Sachs MI, Jones RT, Yunginger JW: Isolation and partial characterization of a major peanut allergen. J Allergy Clin Immunol 67:27, 1981.
11. Tattrie NH, Yaguchi M: Protein content of various processed edible oils. J Inst Can Sci Technol Aliment 6:289, 1973.
12. McWatters KH, Cherry JP: Influence of defatting and toasting on binding and processing characteristics of peanut flours utilized in snack type peanut chips. J Food Sci 45:831, 1980.
13. Collins JL, Post AR: Peanut hull flour as a potential source of dietary fiber. J Food Sci 46:445, 1981.

14. Lowry OH, Rosebrough NJ, Farr AL, Randall RJ: Protein measurement with the Folin phenol reagent. J Biol Chem 193:265, 1951.
15. Adolphson CA, Yunginger JW, Gleich GJ: Standardization of allergens, *in* Rose NR, Friedman H, editors: Manual of clinical immunology, ed. 2. Washington, DC, 1980, American Society for Microbiology, p. 778.
16. Dixon WJ, Massey FJ Jr: Introduction to statistical analysis, ed. 3. New York, 1969, McGraw Hill Book Co., Inc., p. 222.
17. Taylor SL, Busse WW, Sachs MI, Parker JL, Yunginger JW: Peanut oil is not allergenic to peanut-sensitive individuals. J Allergy Clin Immunol 68:372, 1981.
18. Johns CO, Jones DB: The proteins of the peanut, *Arachis hypogaea.* l. The globulins arachin and conarachin. J Biol Chem 28:77, 1916.
19. Dawson R: Comparison of fractionation of ground nut proteins by two different methods. Anal Biochem 41:305, 1971.
20. Tombs MP: An electrophoretic investigation of ground nut proteins—the structure of arachins A and B. Biochem J 96:119, 1965.
21. Johnson P, Shooter EM: The globulins of the ground nut (*Arachis hypogaea*). I. Investigation of arachin as a dissociation system. Biochim Biophys Acta 5:375, 1950.
22. Yamada T, Aibara S, Morita Y: Dissociation-association behavior of arachin between dimeric and monomeric forms. Agric Biol Chem 43:2549, 1979.
23. Cherry JP, Ory RL: Gel electrophoretic analysis of peanut proteins and enzymes. II. Effects of thiol reagents and frozen storage. J Agric Food Chem 21:656, 1973.
24. Basha SMM: Identification of cultivar differences in seed polypeptide composition of peanuts (*Arachis hypogaea* L) by two-dimensional polyacrylamide gel electrophoresis. Plant Physiol 63:301, 1979.
25. Cherry JP: Comparative studies of seed proteins and enzymes of species and collections of *Arachis* by gel electrophoresis. Peanut Sci 2:57, 1975.
26. Cherry JP, Neucere NJ, Ory RL: Comparison of proteins of peanuts grown in different areas. I. Disc electrophoretic analysis of qualitative and quantitative variations. J Am Peanut Res Educ Admin 3:63, 1971.