

Comparison of commercially available ELISA kits with human sera-based detection methods for peanut allergens in foods

P. Koch[†], G. F. Schäppi^{‡*}, R. E. Poms[§],
B. Wüthrich[¶], E. Anklam[§] and R. Battaglia[†]

[†]Swiss Quality Testing Services, CH-8953 Dietikon 1, Switzerland

[‡]Official Food Control Authority of the Canton of Zurich, CH-8030 Zurich, Switzerland

[§]European Commission, Joint Research Centre, Institute for Reference Materials and Measurements, B-2440 Geel, Belgium

[¶]Department of Dermatology, Allergy Unit, University Hospital, CH-8091 Zurich, Switzerland

(Received 2 December 2002; revised 4 June 2003; accepted 9 June 2003)

Undeclared peanut allergens as contaminants in foodstuffs represent a major health problem for sensitized persons. Various immunochemical techniques are employed to detect and quantify peanut allergens. There is an urgent need to compare and standardize those test systems to enable comparable allergen analyses of foodstuffs, comparable studies, and consequent and consistent measures against the presence of hidden peanut allergens. The present study compared commercially available peanut ELISA kits with human sera-based immunoassay techniques (dot blotting and Western blotting), enabling semiquantitative and quantitative detection, and identification of peanut contaminants in foodstuffs. Additionally, the effect of conventional roasting conditions on the detection and quantification of peanut with the selected methods was investigated.

Keywords: peanuts, allergens, quantification, ELISA, Western blotting, standardization

Introduction

Food allergies represent an important health problem in industrialized countries. According to several European studies, they are supposed to affect up to 2% of the adult population and up to 8% of children. In a sensitized individual, even the intake of minute amounts of allergens can provoke gastrointestinal (nausea, diarrhoea), respiratory (rhinitis, asthma) and skin reactions (acute urticaria) (Ortolani *et al.* 2001). For some allergic individuals, contact with a certain food allergen can even lead to severe systemic reactions (anaphylaxis) that are potentially fatal (Wüthrich 2000). Over the past few years, an increase of food allergen-induced life-threatening syndromes has been observed in hospital admissions (Sheikh and Alves 2000).

There are a number of food allergens of outstanding importance such as, for example, allergens from peanut, tree nuts, soybean, celery, milk, hen's eggs, fish and seafood (Wüthrich 1995, Hourihane 1998). The prevalence and severity of adverse reactions to those food allergens vary significantly with the dietary practices of different populations (Wüthrich 1995). Overall, peanut allergy is one of the most common and serious in terms of persistence and severity of reactions (Sampson and Burks 1996). Currently, the only effective treatment for food allergy is avoidance of the allergen-containing food. For peanut-allergic individuals, total avoidance is difficult since peanuts are increasingly used in the diet as an economical protein source in processed foods (Shin *et al.* 1998). Sensitive individuals may also be inadvertently exposed to peanut protein by consumption of supposedly peanut-free foods contaminated with peanut proteins. This can occur during shipping and storage, during processing, form carry-over due to inadequate cleaning of shared processing equipment used for non-peanut and peanut-containing products, or in reuse (rework) of peanut-containing products

*To whom correspondence should be addressed.
e-mail: georg.schaepi@klzh.ch

(Yunginger *et al.* 1983). Moreover, de flavoured peanuts have been used and sold as other types of nuts, such as almonds (Keating *et al.* 1990).

Peanut (*Arachis hypogaea*) seeds contain on average 25% (23–27%) protein (Souci 1994), of which approximately 20% can be attributed to the major allergen Ara h 1 (vicilin), with a molecular mass of 63.5 kD, and approximately 10% represent Ara h 2 (conglutin-like protein), with a molecular mass of 17 kD (Burks *et al.* 1998, Koppelman *et al.* 2001). Further relevant major allergens contained in peanuts are Ara h 3 and Ara h 4 (Bannon *et al.* 2000), which are glycinin proteins with a molecular mass of 14 kD. Those major allergens are seed storage proteins, well characterized concerning primary structure and major IgE binding epitopes and they are recognized by more than 50% of peanut allergic patients.

Enzyme linked immunosorbent assay (ELISA) is the most commonly used technology to detect and quantify the allergen content in various foods. Several ELISA tests have been described for peanut quantification (Hefle *et al.* 1994, Yeung and Collins 1996, Holzhauser and Vieths 1999, Keck-Gassenmeier *et al.* 1999); some are marketed as commercial ELISA kits. The commercially available antibodies, which are included in ELISA tests, are polyclonal antibodies from animal origin, e.g. rabbit or goat. Up to now, comparisons between commercially available antibodies against food allergens and antibodies recovered from human sera are scarce.

The aim was to compare various commercially available ELISA kits for peanut quantification with human sera-based immunochemical techniques. The obtained results would provide important information for the standardization of test systems to achieve comparable allergen analyses of foodstuffs, comparable studies, and consequent and consistent measures against the presence of hidden peanut allergens. This would therefore be the basis for the implementation of a reliable and sensitive allergen-monitoring system that can be routinely employed in a food control authority setting.

Materials and methods

Samples

Raw and oil roasted (153°C, 5 min) peanuts (Runner Jumbos), and peanut-free cookies (Marie Croccant

1101.022, Midor, Meilen, Switzerland) were obtained from Migros, Switzerland. The oil-roasted peanuts and cookies used represented retail products as marketed by Migros. Marie Croccant cookies had the following approximate composition: 44% wheat flour, 21% sugar, 16.5% hazelnut croccant (sugar, hazelnut), 14% margarine (soya-oil, canola, mono- and diglycerides, lecithin), 3.5% condensed milk (sweetened with sugar), 0.2% egg-white powder, 0.2% ammonium bicarbonate and 0.2% salt.

To produce materials containing various concentrations of raw or roasted peanuts, spiked samples were prepared as follows: 1 g ground peanuts was homogenized with 99 g peanut-free cookies by employing a household blender (Grindomix GM 200, Retsch, Haan, Germany). In the same manner, the resulting fine powder with a peanut concentration of 10 000 mg kg⁻¹ was further 'diluted' with an appropriate amount of peanut-free cookies to yield peanut concentrations of 500, 1000, 1500, 2000 and 2500 mg kg⁻¹.

Protein extraction

To prepare whole extracts of soluble protein, 40 ml 6 M urea buffer were added to 20 g spiked food sample. The employed extraction buffer produced the strongest and most reproducible signals in Western blotting experiments. The mixture was shaken overnight at 4°C and then centrifuged for 10 min at 6000 rpm. The clear supernatant was carefully removed, aliquoted and stored at -20°C until analysis.

Standards were produced accordingly, using roasted peanuts at a concentration of 0.5 g ml⁻¹. The standards were then serially diluted in 6 M urea buffer for use in both dot blotting and gel electrophoresis.

As negative controls, peanut-free cookies were extracted as above.

Human sera-based assays

Sera. Sera from patients with a history of severe allergic reactions to peanuts were provided by the Allergy Unit of the University Hospital of Zurich, Switzerland. The selected patients showed a high level of sensitization to peanut allergens with a

positive CAP[®]-result of at least Class 4 (Pharmacia & Upjohn Diagnostics, Uppsala, Sweden). Both single and pooled sera were applied in the assays. Before use, the sera were diluted 1:4 with 0.5% bovine serum albumin (BSA) in phosphate-buffered saline (PBS).

Dot blots. Dot blots of protein extracts were analysed as described by Sch ppi *et al.* (2001). In brief, 4 µl samples, standards and negative controls (among others extracts from peanut-free cookies and urea buffer), respectively, were applied onto a nitrocellulose membrane (NCM; Protran BA84, Schleicher & Schuell, Riehen, Germany) and developed as described by Sch ppi *et al.* (1997, 2001). Briefly, the NCM was incubated in PBS containing 10% (w/v) skimmed milk powder for 1 h at room temperature (RT). Then, the NCM was washed for 5 min with gentle horizontal shaking in 0.1% Tween 20 in PBS (TPBS). This step was repeated twice in PBS. The blots were then incubated at RT for 1 h with the diluted human sera. After washing with TPBS and PBS as above, bound immunoglobulin (Ig) E was linked with peroxidase-conjugated goat IgG specific for the ε-chain of the human IgE (Sigma (Fluka), Buchs, Switzerland) by incubation for 1 h at RT. Again, the blots were washed with TPBS and PBS, respectively, as above, and developed in chromogenic 4-chloro-1-naphthol substrate (Sigma) in methanol. Semi-quantitative estimation of the allergen content was performed by visually comparing the intensities of the sample dots with the dot intensities of the standard dilution series, which was included in each individual assay to ensure that the series of assays were comparable (figure 1). The chosen standard ranges always

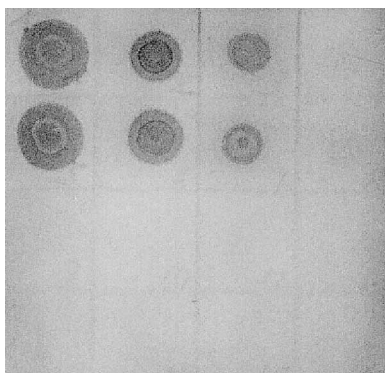


Figure 1. Dilution series of peanut standard extract; dots correlate to 1000, 500, 100 and 50 mg kg⁻¹ peanut (from left to right, samples in duplicates; lower two lines contain negative controls).

included the measured sample values, so there was no need for extrapolations. Usually, ranges between 0.1 and 10 g kg⁻¹ were chosen. To exclude reactions with other food allergens, sera from patients with a clear history of severe allergic reactions to peanuts were employed. The selected patients showed a high level of sensitization to peanut allergen with a positive CAP result of at least Class 4 and negligible reactivity to other food allergens. Other food allergens were included in our assays as negative controls in order to avoid false-positive signals. Furthermore, sodium dodecyl sulphate polyacrylamide gel electrophoresis (SDS-PAGE)/Western blotting was employed to determine the molecular mass of the reacting proteins.

Gel electrophoresis and immunoblot. Proteins of food extracts, standards and negative controls were separated by SDS-PAGE according to Laemmli (1970). In each run, gel electrophoresis of two identical polyacrylamide gels (10%, with a 4% stacking gel) was carried out on a PROTEAN 3 Mini Cell (BioRad, Hercules, CA, USA) at 200 V constant voltage. Immediately after electrophoresis, the separated proteins of one of the gels were transferred to a NCM according to Towbin *et al.* (1979) using a Mini Trans-Blot Module (BioRad) at 100 V constant voltage. The other identical gel was developed in Coomassie Brilliant Blue solution (BioRad) to stain the protein bands. Molecular masses of the protein bands were calculated in relation to pre-stained broad range protein standards (Bio-Rad), which had been included in every run.

Blotted membranes of SDS-PAGE gels were incubated with the diluted patient sera and peroxidase-labelled goat IgG specific for the ε-chain of human IgE, consecutively, and developed in the same manner as the dot blots described above.

Non-human sera-based assays

ELISA kits. Three commercially available ELISA kits for peanut protein quantification were used: (A) Prolisa Peanut PAK (Pro-Lab Diagnostics, Inc., Neston, UK), (B) Ridascreen Peanut (R-Biopharm, Darmstadt, Germany), purchased from Dispolab (Dielsdorf, Switzerland), and (C) Veratox Peanut Protein Test Kit (Neogen, Lansing, MI, USA), purchased from BAG Med AG (Greppen, Switzerland).

For protein extraction and analysis, the manufacturers' instructions were followed.

In principle, all the tests employed were based on a non-competitive, sandwich-type, enzyme immunoassay. Peanut protein was extracted from homogenized food samples. The extracts were diluted and incubated in plastic microwells coated with peanut protein-specific antibodies. Peanut protein present in the sample extracts and standard solutions would bind to the specific capture antibodies. Unbound components were removed by a washing step. Bound peanut protein was determined by antibody-sera (from rabbit) conjugated to peroxidase (enzyme conjugate), which binds to the captured peanut protein. Adding substrate to the bound enzyme conjugate converts the colourless chromogen into a blue product. The addition of the stop reagent leads to a colour change from blue to yellow. Measurement was done photometrically at 450 nm. The colour development was directly proportional to the peanut concentration in the extract. The concentration of peanut material in the food sample ($\text{mg kg}^{-1} = \text{ppm}$) was determined by interpolating the corresponding concentration of the diluted extract from the calibration curve.

The tests, however, used different extraction buffers and different peanut-specific antibodies. In addition, the working ranges of the kits were between 0 and 25 mg kg^{-1} for the Veratox, between 0 and 24 mg kg^{-1} for the Prolisa PAK, and between 0 and 90 mg kg^{-1} for the Ridascreen Peanut kit. According to the respective manufacturers, the detection limits were at 0.875, 1.6 and $< 2.5 \text{ mg kg}^{-1}$ for the Veratox, Prolisa PAK and Ridascreen Peanut kits, respectively.

Results and discussion

The performances of the three different commercially available peanut ELISA kits were tested and compared with results obtained by dot blotting and Western blotting. Thus, the correlation of human sera-based and non-human sera-based 'quantification' methods was investigated.

Dot blotting was used to estimate the amount of peanut protein in the samples, whereas Western blotting was used to analyse the extracts further and identify specific peanut proteins. Figure 1 shows the results obtained from a dot blot analysis of peanut

standard extracts with concentrations correlating with $50\text{--}1000 \text{ mg peanut kg}^{-1}$ food product. Although dot blotting and Western blotting only offered a rough semiquantitative evaluation of the peanut content in the extract, the results obtained were reliable for the range of the original quantity of peanut spike (tables 1 and 2). The results from dot blotting and Western blotting were consistent. In addition, no difference in response intensities was remarkable between raw and roasted peanuts by dot blotting.

These tests involved antibodies from patients with a well-defined allergy. The use of human sera in analytical methods offered the possibility of interpreting results with respect to their *in vivo* reactivity in the human organism. Thus, potentially they can provide answers to questions that might not be answered when mono- or polyclonal animal antibody-based methods are used (e.g. allergenicity of a product, contents of allergens rather than marker proteins). However, in comparison with methods involving antibodies from animal origin, our tests might be limited by the availability of sera from correctly diagnosed patients. In addition, it might

Table 1. Results for roasted peanuts in cookies obtained with three different commercial ELISA kits and human sera-based immunoblotting techniques (dot blot).

Added (mg kg^{-1})	Found by ELISA (mg kg^{-1})			Immunoblotting (mg kg^{-1})
	Kit A	Kit B	Kit C	
500	286	234	459	500–1000
1000	717	304	789	500–1000
1500	1285	613	1219	1000–2000
2000	1754	801	1236	1000–2000
2500	2033	895	1409	> 2000

Table 2. Results for raw peanuts in cookies obtained with two different commercial ELISA kits and human sera-based immunoblotting techniques (dot blot).

Added (mg kg^{-1})	Found by ELISA (mg kg^{-1})			Immunoblotting (mg kg^{-1})
	Kit A	Kit B	Kit C	
500	1723	594	n.d.	500–1000
1000	3223	1142	n.d.	500–1000
1500	5022	1583	n.d.	1000–2000
2000	6268	2490	n.d.	1000–2000
2500	7843	3392	n.d.	> 2000

n.d., not determined.

be difficult to standardize the method completely and certain analytical measures are necessary to achieve satisfying specificity. In this study, new sera to be employed in the assays were thoroughly tested and compared with the previously used sera by immunoblotting and SDS-PAGE/Western blotting. For each serum, a panel of allergens was included in the assays to assess cross-reactivities. The results are based on the employment of single, not-pooled, human sera.

When the performance of the commercial ELISA test kits was investigated, most reproducible quantitative results were obtained in the central region of the linear range of the kits' standard curves, which was around 10 mg kg^{-1} . Thus, for the intercomparison of the different test kits, the extracts of the spiked samples were diluted with the corresponding extraction buffer to yield final concentrations of about 10 mg kg^{-1} , in order to achieve best accuracy and precision. The actual concentrations were then calculated based on the respective dilution factors. As shown in tables 1 and 2, the different peanut ELISA brands gave considerably varying results. Generally, depending on the respective test kit, raw peanuts showed three to four times higher responses when compared with the equivalent content of roasted peanuts in the sample. An explanation for this phenomenon could be that the peanut antibodies provided in the ELISA kits were raised against an extract of raw

peanuts and that the denaturing of proteins during roasting modified or eliminated epitopes necessary for antibody binding. On the other hand, it is known that the solubility of denatured proteins is decreased and the yield of protein by aqueous extraction might therefore be impaired (Poms, unpublished data). However, the extraction efficiency of processed peanuts with 6 M urea buffer in connection with the human sera-based quantification methods did not seem to be affected negatively in this study. Hence, the recovery rates for roasted peanuts from cookies were between 52.2 and 87.7% with kit A, between 30.4 and 46.8% with kit B, and between 56.4 and 91.8% with kit C (figure 2), in each case depending on the various concentrations of peanut material in the spiked samples ($500\text{--}2500 \text{ mg kg}^{-1}$). For raw peanuts only, test kits A and B were tested. The recovery rate of raw peanut protein at the various concentration levels in the spiked samples was between 313.4 and 344.6% with kit A, and between 105.5 and 135.7% with kit B. The significant over-reporting of the tested ELISA kits for raw peanuts can be explained by considering the use of extracts from raw peanut material for production of the primary antibody as apart of the ELISA methodology and additionally — in the case of kit A, by the fact that the standard solutions (included in the test kits) to propagate a standard curve were also produced from raw peanuts.

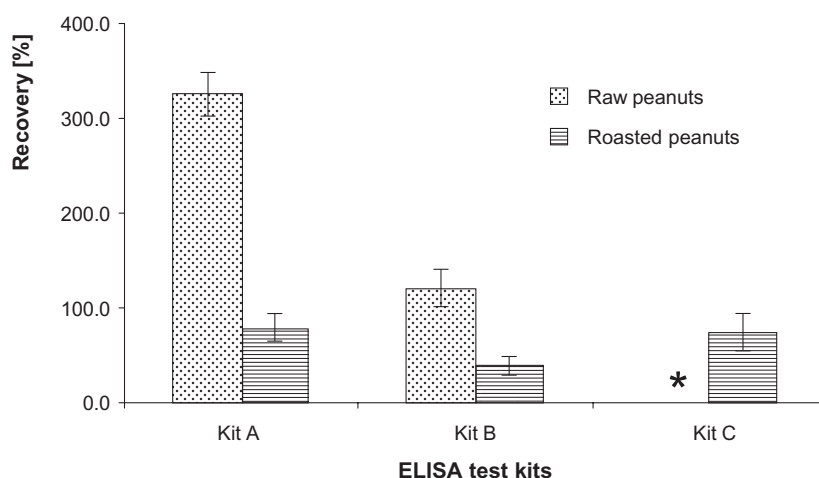


Figure 2. Recovery (%) of raw and roasted peanuts from cookies by using three different commercial ELISA test kits (A–C). The recovery at the different spiking levels ($500, 1000, 1500, 2000, 2500 \text{ mg kg}^{-1}$) was averaged for each kit; error bars show the maximum and minimum recoveries (%) achieved at various spiking levels. *Not performed for raw peanuts.

All ELISA kits tested were suitable for qualitative analysis of peanut content in cookies. No false-positives or false-negatives were obtained. However, the large variation of the data resulting from the employment of the three different kits indicate that the tested ELISA kits were not well adapted for precise quantitative analysis of the food matrix investigated. Hurst *et al.* (2002) reported similar variations in recoveries of peanut from dark and milk chocolate between four different commercial ELISA kits. They speculated that besides potential inhomogeneities of the samples, the use of different peanut standards might contribute to the variability of the results. Considering the intrinsic variability of biological material — due to variety/type of peanut, geographic and seasonal variations, etc. — and adding the impact of various processing techniques and conditions (chosen by the food manufacturers according to the desired product characteristics), the standards included in the various kits were most likely derived from different materials and thus tend to yield varying results.

In addition, the effect of the roasting process on the ELISA results was investigated. Peanuts were roasted at 153°C for 1–5 min before analysis. The results (figure 3) were obtained by employing kit B. A drastic drop of the ELISA response from 16 mg kg⁻¹ of the raw peanut material to 2.5 mg kg⁻¹ of the peanuts roasted for 5 min was observed. These results are of

significance, as the main portion of contamination with peanut material originates from processed (i.e. heat-treated) material. Raw peanuts are rather unlikely to enter the food chain, except via transporting vessels, which were not cleaned thoroughly. In the case of roasted peanuts, it is also important to note that detection and quantification were impaired when commercial ELISA test kits were used, but remained unaffected when human sera-based methods were employed. Apparently, human IgE antibodies of sensitized individuals recognize raw and roasted peanuts with an equal quantitative response, which implies that IgE binding to peanuts was not significantly affected by conventional processing conditions. This result is in accordance with findings by Keating *et al.* (1990) and Maleki *et al.* (2000), who described that peanut roasting did not destroy the allergenic components. Moreover, Maleki *et al.* (2000) postulated that the allergenicity of peanut protein increased by the heat treatment owing to the exposure of previously unavailable IgE-binding sites and the creation of novel sites. No increase in IgE-binding affinity to processed peanuts was determined in the present study. However, if allergenicity remains unaffected or is even enhanced during processing, food samples containing amounts of peanut that could be potentially hazardous to sensitized individuals might be found acceptable by industrial quality control, when commercially available ELISA kits were employed for food safety control.

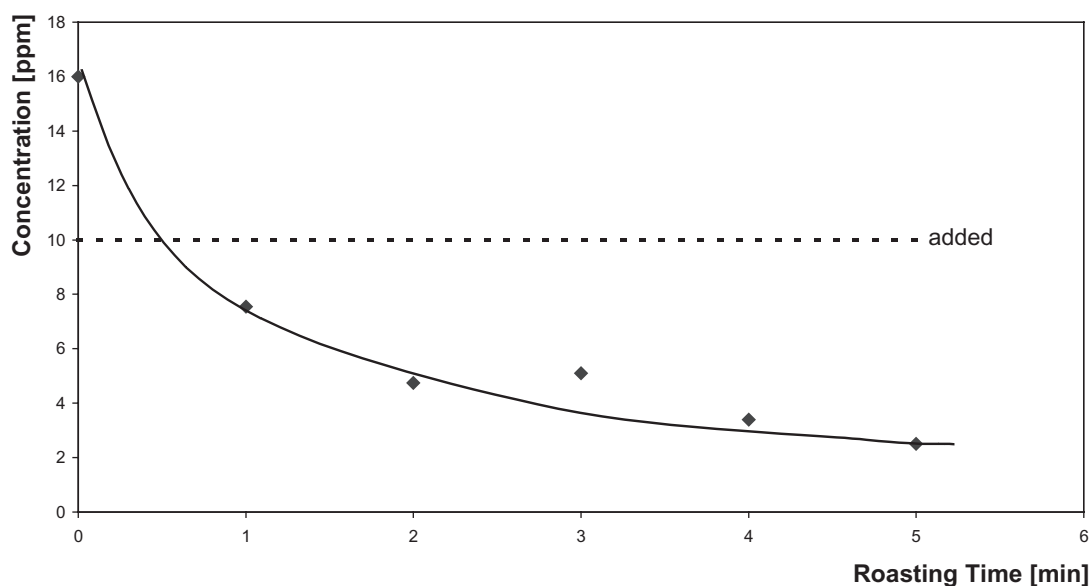


Figure 3. Effect of roasting time on the ELISA response for peanut material at a concentration of 10 mg kg⁻¹.

Conclusions

Currently, ELISA methodology is the method of choice for rapid and sensitive protein-based food analysis for routine laboratories in both industry and food safety control institutions. Several ELISA test kits for peanut (allergen) detection and quantification are currently commercially available. This comparative study showed that quantitative results differed significantly between the various test kits investigated. Moreover, the detection of peanut material in foods in the lower mg kg^{-1} range and its quantification might be impaired significantly by the degree of industrial processing of the raw material. Compared with raw peanuts, the responses to roasted peanuts were dramatically reduced, but showed no remarkable difference when human sera-based immunological methods were employed. To guarantee the best possible safety of food products and make comparisons of results between different commercial detection systems feasible, there is need for standardization of ELISA kits used for allergen detection and quantification.

Acknowledgements

The authors thank Ms Weber and Ms Cuhat, Allergy Unit, Department of Dermatology, University Hospital of Zurich, for valuable assistance.

References

- BANNON, G. A., BESLER, M., HEFLE, S. L., HOURIHANE, J. O., and SICHERER, S. H., 2000, Allergen data collection: peanut *Arachis hypogaea*. Internet Symposium on Food Allergens 2 [http://www.food-allergens.de], **2**, 87–123.
- BURKS, A. W., SAMPSON, H. A., and BANNON, G. A., 1998, Review article series II: Peanut allergens. *Allergy*, **53**, 725–730.
- HEFLE, S. L., BUSH, R. K., YUNGINGER, J. W., and CHU, F. S., 1994, A sandwich enzyme-linked immunosorbent assay (ELISA) for the quantification of selected peanut proteins in foods. *Journal of Food Protein*, **57** (suppl. 5), 419–423.
- HOLZHAUSER, T., and VIETHS, S., 1999, Indirect competitive ELISA for determination of traces of peanut (*Arachis hypogaea* L.) protein in complex food matrices. *Journal of Agriculture and Food Chemistry*, **47** (suppl. 2), 603–611.
- HOURLIHANE, J. O., 1998, Prevalence and severity of food allergy — need for control. *Allergy*, **53**, 84–88.
- KEATING, M. U., JONES, R. T., WORLEY, N. J., SHIVELY, C. A., and YUNGINGER, J. W., 1990, Immunoassay of peanut allergens in food processing materials and finished foods. *Journal of Allergy and Clinical Immunology*, **86**, 41–44.
- KECK-GASSENMEIER, B., BENET, S., ROSA, C., and HISCHENHUBER, C., 1999, Determination of peanut traces in food by a commercially-available ELISA test. *Food and Agriculture Immunology*, **11**, 243–250.
- KOPPELMAN, S. J., VLOOSWIJK, R. A. A., KNOL, E., and BRUIJNZEEL-KOOMEN, C. A. F. M., 2001, Quantification of major peanut allergens Ara h 1 and Ara h 2 in peanut varieties. Proceedings of the 8th International Symposium on Immunological, Chemical and Clinical Problems of Food Allergy, Venice, Italy.
- LAEMMLI, U. K., 1970, Cleavage of structural proteins during assembly of the head of the bacteriophage T4. *Nature*, **227**, 248–254.
- MALEKI, S. J., CHUNG, S.-Y., CHAMPAGNE, E. T., and RAUFMANN, J.-P., 2000, Effects of roasting on the allergenic properties of peanut proteins. *Journal of Allergy and Clinical Immunology*, **106** (suppl. 4), 763–768.
- ORTOLANI, C., ISPANO, M., SCIBILIA, J., and PASTORELLO, E. A., 2001, Introducing chemists to food allergy. *Allergy*, **56** (suppl. 67), 5–8.
- SAMPSON, H. A., and BURKS, A. W., 1996, Mechanisms of food allergy. *Annual Reviews in Nutrition*, **16**, 161–177.
- SCHÄPPI, G. F., KONRAD, V., IMHOF, D., ETTER, R., and WÜTHRICH, B., 2001, Hidden peanut allergens detected in various foods: findings and legal measures. *Allergy*, **56**, 1216–1220.
- SCHÄPPI, G. F., SUPHIOGLU, C., TAYLOR, P. E., and KNOX, R. B., 1997, Concentrations of the major birch tree allergen Bet v 1 in pollen and respirable fine particles in the atmosphere. *Journal of Allergy and Clinical Immunology*, **100**, 656–662.
- SHEIKH, A., and ALVES, B., 2000, Hospital admission for acute anaphylaxis: time trend study. *British Medical Journal*, **320**, 1441.
- SHIN, D. S., COMPADRE, C. M., MALEKI, S. J., KOPPER, R. A., SAMPSON, H., HUANG, S. K., WESLEY, BURKS, A., and BANNON, G. A., 1998, Biochemical and structural analysis of the IgE binding sites on Ara h1, an abundant and highly allergenic peanut protein. *Journal of Biological Chemistry*, **273** (suppl. 22), 13753–13759.
- SOUCI, S. W., 1994, *Food Composition and Nutrition Tables* (Stuttgart: Medpharm Scientific).
- TOWBIN, H., STAHELIN, T., and GORDON, J., 1979, Electrophoretic transfer of proteins from polyacrylamide gels to nitrocellulose sheets: procedure and some applications. *Proceedings of the National Academy of Sciences, USA*, **76**, 4350–4354.
- WÜTHRICH, B., 1995, Food allergy: definition, prevalence, social impact. Proceedings of the XVI European Congress of Allergology and Clinical Immunology, Madrid, Spain, pp. 859–867.
- WÜTHRICH, B., 2000, Lethal or life-threatening allergic reactions to food. *Investigations in Allergy and Clinical Immunology*, **10**, 59–65.
- YEUNG, J. M., and COLLINS, P. G., 1996, Enzyme immunoassay for determination of peanut proteins in food products. *Journal of AOAC International*, **79** (suppl. 6), 1411–1416.
- YUNGINGER, J. W., GAURERKE, M. B., JONES, R. T., DAHLBERG, M. E., and ACKERMAN, S. J., 1983, Use of radioimmunoassay to determine the nature, quantity, and source of allergenic contamination of sunflower butter. *Journal of Food Protein*, **46**, 625–629.