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Shyamali Jayasena

Mieke Smits

Daniëlle Fiechter

Aard de Jong

Julie Nordlee

See next page for additional authors

Authors

Shyamali Jayasena, Mieke Smits, Daniëlle Fiechter, Aard de Jong, Julie Nordlee, Joe Baumert, Steve L. Taylor, Raymond H. Pieters, and Stef J. Koppelman

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Comparison of Six Commercial ELISA Kits for Their Specificity and Sensitivity in Detecting Different Major Peanut Allergens

Shyamali Jayasena,[1] Mieke Smits,[2] Daniëlle Fiechter,[2] Aard de Jong,[3] Julie Nordlee,[1] Joe Baumert,[1] Steve L. Taylor,[1] Raymond H. Pieters,[2,4] and Stef J. Koppelman[1]

1. Food Allergy Research and Resource Program, Department of Food Science and Technology, University of Nebraska–Lincoln, Lincoln, Nebraska, USA
2. Institute for Life Sciences and Chemistry, Utrecht University of Applied Sciences, Utrecht, The Netherlands
3. TNO, Zeist, The Netherlands
4. Institute for Risk Assessment Sciences, Utrecht University, Utrecht, The Netherlands

Corresponding author – Joe Baumert, FARRP, Department of Food Science and Technology, University of Nebraska–Lincoln, 143 Food Industry Complex, Lincoln, NE 68583-0919, phone 402-472-3070, fax 402-472-5306, email jbaumert2@unl.edu

Abstract

Six commercial peanut enzyme-linked immunosorbent assay kits were assessed for their ability to recover peanut from the standard reference material 2387 peanut butter and also for their specificity in detecting four major peanut allergens, Ara h 1, Ara h 2, Ara h 3, and Ara h 6. The percentage recovery of peanut from peanut butter differed across different kits as well as at different sample concentrations. The highest recovery was observed with the Romer and R-Biopharm kits, while four other kits were found to underestimate the protein content of the reference peanut butter samples. Five of the kits were most sensitive in detecting Ara h 3 followed by Ara h 1, while hardly recognizing Ara h 2 and Ara h 6. The other kit showed the highest sensitivity to Ara h 2 and Ara h 6, while Ara h 1 and Ara h 3 were poorly recognized. Although Ara h 2 and Ara h 6 are known to be heat stable and

more potent allergens, antisera specific to any of these four peanut proteins/allergens may serve as good markers for the detection of peanut residues.

Keywords: peanut, allergens, Ara h 1, Ara h 2, Ara h 3, Ara h 6, immunoassay, ELISA, detection

Introduction

The prevalence of food allergy continues to increase in the USA as well as in most other industrialized nations, where currently 3−4% of the adult population and 4−8% of children in the USA are affected by some type of food allergy.[1] While almost any food has the potential to cause an allergic reaction, peanuts and tree nuts are responsible for most of the fatalities associated with food allergies in the USA.[2] Approximately 0.6% of adults and 1−2% of children/infants in the USA are affected by peanut allergy.[2,3] Moreover, peanut is the most common food allergen that affects children, with 25% of the food allergic children showing a reaction to peanuts.[3] In contrast to milk and egg allergies, peanut allergy is rarely outgrown and persists into adulthood.[4] Despite intensive clinical research within the past decade, a peanut avoidance diet remains the only established approach to the prevention of allergic reactions.[5]

However, the implementation of a safe and effective peanut avoidance diet can be quite challenging as undeclared peanut residues may be present in other foods, which pose a threat to sensitive individuals. Allergic individuals are at risk of being exposed to peanuts in many settings, including restaurants and other food service establishments such as school canteens, catering services, etc.[6] as well as from packaged foods. The packaged food industry has a responsibility to control the presence of undeclared peanuts in other foods that might arise from shared processing equipment or facilities. The validation of the effectiveness of industry allergen control plans has prompted the development of sensitive and specific analytical methods for the detection of peanut residues. Within the food industry, several methods are being used to detect and quantify peanut residues including DNA-based methods, such as polymerase chain reaction (PCR), and protein-based methods such as enzyme linked immunosorbent assay (ELISA).[7] Because of its high sensitivity, high specificity, ease of use, and high sample throughput, ELISA has become the method of choice in most food industry settings for allergen detection.[8] An additional advantage of ELISA over DNA-based methods is that the allergenic part, that is, protein, is detected rather than a component (i.e., DNA) that is not allergenic in itself. This is of particular importance for food ingredients that have been fractionated (i.e., use of casein or whey protein fractions in food manufacturing instead of whole milk).

Currently, a number of commercial peanut ELISA kits are available on the market from several suppliers. Although all ELISA kits quantitatively detect peanut proteins, they differ widely from each other in sample extraction procedures, range of quantification, limits of detection and quantification, reference standards used, and expression of results.[9,10] This makes comparison between ELISA kits difficult and unreliable. Nevertheless, several studies have been conducted to compare the recovery of peanut protein when different commercial ELISA kits are used. These studies have compared the recovery and detection of peanut proteins with the use of different extraction buffers,[9,11] food matrices,[10,12] and

processing methods,[11,13–15] and the outcomes of these studies confirm that a wide variation in the recovery of peanut proteins exists among different kits.

Moreover, the reference material used to prepare the kit standards is not always known and is not standardized between ELISA kits, which is another source of variability between different kits. The National Institute of Standards and Technology (NIST) has developed a standard reference material (SRM) for peanut (2387), a peanut butter. However, a comparison of commercial peanut ELISA kits for their sensitivity of detection of peanut from SRM 2387 peanut butter is yet to be accomplished. Furthermore, although all peanut ELISA kits detect peanut proteins, no study has compared the specificity of recognition of individual peanut allergens by the antisera used in these kits.

Several peanut proteins have been identified as being major allergens including the seed storage proteins, Ara h 1, Ara h 2, Ara h 3, and Ara h 6. Both Ara h 1 and Ara h 3 belong to the cupin superfamily of proteins. Ara h 1 is a 7S vicilin-type protein, while Ara h 3 is an 11S legumin-type protein. Ara h 2 and Ara h 6 are both 2S albumins that show close homology to each other.[16,17] Although Ara h 1 and Ara h 3 are the more abundant peanut proteins, several studies have proven that Ara h 2 and Ara h 6 bind more strongly with IgE from peanut allergic patients and release mediators more efficiently from basophils, which confirm that these are also more potent allergens in in vitro systems[18–21] and in vivo.[20,22,23] Moreover, it has been observed that both Ara h 2 and Ara h 6 are recognized more strongly and more frequently than Ara h 1 and Ara h 3 by peanut allergic children with almost all of them showing IgE reactivity to these two allergens.[24] Other peanut allergens have been identified,[25,26] but their clinical significance remains to be firmly established.

Therefore, in the current study, we used the reference peanut butter SRM 2387 from NIST to compare the performance of six commercial peanut ELISA kits, and we applied purified peanut allergens Ara h 1, Ara h 2, Ara h 3, and Ara h 6 to assess the specificity of these ELISA kits.

Materials and Methods

Reference Material and Peanut Allergens

SRM 2387 peanut butter was purchased from NIST. The purified peanut allergens Ara h 1, Ara h 2, Ara h 3, and Ara h 6 were obtained from lyophilized stock preparations made as described earlier.[19,20,22]

Peanut ELISA Test Kits

Six commercial ELISA test kits were used in this study: Veratox for peanut allergen from Neogen, Lansing, Michigan, USA; BioKits peanut assay kit from Neogen, Lansing, Michigan, USA; AgraQuant peanut assay from Romer Laboratories UK Ltd.; RIDASCREEN fast peanut from R-Biopharm, Germany; Peanut protein ELISA kit from Morinaga Institute of Biological Science, Inc., Japan; and Peanut residue from ELISA Systems Pty Ltd., Australia.

Preparation of Reference Material and Peanut Allergens

Reference Material

An initial stock sample containing 50 000 ppm (mg/kg) peanut butter was prepared both as a suspension and an extract. Grinding or homogenization of the sample as instructed in the kit inserts was not required since SRM 2387 is of a creamy consistency. The suspension was prepared by mixing 1 g of SRM 2387 peanut butter with 20 mL of sample extraction buffer of each respective ELISA kit and vortexing to obtain a uniform suspension of 50 000 ppm peanut butter in extraction buffer. For the extract, 1 g of peanut butter was mixed with 20 mL of preheated (at RT with the Morinaga kit) extraction buffer and extracted at RT (Morinaga, BioKits) or at 60°C (Veratox, Romer, R-Biopharm, and ELISA Systems) for 10 min (R-Biopharm) or 15 min (Veratox, BioKits, Romer, Morinaga, and ELISA Systems). Following extraction, samples were centrifuged at 3000 g for 15 min at 25°C, and the resulting supernatant containing 50 000 ppm peanut butter was obtained. Any extraction additives supplied by the kit manufacturers (i.e., Veratox) were added to the initial extract but not to the initial suspension. A series of dilutions ranging from 10−500 ppm were prepared with both the initial suspension and extract for all six kits. Each of the initial stocks and the series of dilutions were prepared in duplicate. Protein extraction from each of these dilutions was carried out as per manufacturers' instructions for each kit. Following protein extraction, an aliquot of the extract was transferred to a 1.5 mL Eppendorf tube and centrifuged at 14 000 rpm for 5 min with the resulting supernatant used for subsequent testing by ELISA.

Peanut Allergens

With each purified allergen, 1.5 mg was dissolved in either 1 mL of phosphate buffered saline (PBS), pH 7.4 containing 20 mM phosphate, 150 mM NaCl (Ara h 1 and Ara h 2), or distilled water (Ara h 3 and Ara h 6). Absorbance was measured at 280 nm (Thermo Scientific NanoDrop 2000c Wilmington, Delaware, USA), and the protein concentration (mg/mL) was determined by dividing the absorbance value by the absorbance coefficient for each allergen as can be found in the Uniprot database using ProtParam (0.657, 0.59, 0.77, and 0.21 for Ara h 1, Ara h 2, Ara h 3, and Ara h 6, respectively). Subsequently, the final protein concentration of each allergen was adjusted to 1 mg/mL by adding an appropriate volume of the primary dissolving fluid. Sample aliquots were put into screw-top Corning cryogenic tubes, frozen by snap-freezing in liquid nitrogen, and stored at −80°C until further use. Prior to testing by ELISA, further dilutions of the 1 mg/mL allergens were prepared in each kit's extraction buffer. On the basis of the sensitivity of the test kit and reactivity of the allergen, dilutions ranging from 0.001−100 μg/mL were prepared for each allergen. The extraction and dilution procedure outlined in each kit was followed for extraction of proteins from each of the prepared dilutions. An aliquot of the extracted sample was centrifuged at 14 000 rpm for 5 min, and the resulting supernatant was used for subsequent testing by ELISA.

ELISA Test Procedure

ELISA was performed in duplicate for each sample according to the instructions supplied by each kit manufacturer. Where appropriate, extracted sample dilutions of the SRM 2387 peanut butter were further diluted in extraction buffer prior to testing by ELISA in order for the sample to be within the range of the kits' quantification. The purified allergens were tested directly by ELISA without any further dilutions other than dilutions specified by the kit manufacturers (BioKits and Morinaga test kits). The absorbance of the samples as well as the kit standards was measured at the wavelength specified by each kit using a microtiter plate reader (BioTek Instruments Inc. USA). To allow for uniform comparison between test kits, the concentration of kit standards of each kit were converted to μg/mL peanut protein. For the SRM 2387 peanut butter, the peanut concentration in each sample dilution was interpolated from the standard curve using the software supplied by the kit manufacturers (Veratox, Romer Laboratories, and R-Biopharm) or the GraphPad Prism 4 software (BioKits, Morinaga, and ELISA Systems). By taking into account any dilutions and conversion factors, the total peanut protein recovered (in ppm) was calculated as a percentage relative to expected recovery for each dilution and test kit.

SDS-PAGE Analysis

The SRM 2387 peanut butter and the purified allergens Ara h 1, Ara h 2, Ara h 3, and Ara h 6 were analyzed under reducing conditions (β-mercapotoethanol) using Mini-Protean TGX Precast 4−20% gradient gels (Biorad, Veenendaal, The Netherlands). For the purified allergens, 5 ug per lane was loaded. For the SRM 2387 peanut butter, an amount of protein was loaded (20 μg) such that the multiple bands of the allergens could be visualized. Gels were run for 90 min at 120 V and stained with Coomassie Brilliant Blue R-250 (Sigma-Aldrich, Germany). Molecular weight (MW) markers were Precision Plus Protein (all blue standard, 10−250 kDa, Bio-Rad).

Statistical Analysis

SRM 2387 peanut butter samples and dilutions were prepared in duplicate and each dilution analyzed in duplicate ELISA wells. Tests on peanut allergens were repeated twice, and for each trial, all concentrations were tested in duplicate by ELISA. All statistical analyses were performed using the SAS 9.2 software package. Fisher's protected least significant difference (LSD) test and matched pairs t test were used to determine statistical significance, and the level of significance was set at $p \leq 0.05$.

Results

Comparison of the Sensitivity of the ELISA Kits

The six peanut ELISA test kits used in this study differed from one to another in detection range, detection limit, and unit of quantification as well as on the sample to extraction buffer ratio and the dilutions used. In four of the kits, results are expressed as ppm of total peanut (Veratox, BioKits, Romer, and R-Biopharm). In the ELISA Systems test kit, results are expressed in ppm peanut protein, while in the Morinaga assay, the results are given in ng/mL of peanut protein. To allow for a comparison of the sensitivity between different

kits, we calculated the range of quantification of each of the test kit standards in μg peanut protein/mL (Table 1). Since peanut kernels contain ~25% protein,[27] we used a conversion factor of 0.25 to calculate for peanut protein from total peanut for five of the test kits. For the R-Biopharm kit, a conversion ratio of 0.1 was used since the kit manufacturer states that a peanut raw material containing 10% protein was used for the preparation of their standards. As can be seen from Table 1, the six ELISAs were different in sensitivity. In particular, the sensitivity of one of them (Peanut Residue from ELISA Systems) seemed to be about 10-fold lower than that of the other kits. By applying different dilutions, as specified in the kit, this ELISA also reached a sensitivity range (relative to the unextracted food products) similar to that of the other kits, that is, 4−60 ppm.

Table 1. Comparison of Assay Characteristics and Sensitivity of the Kit Standards in the Detection of Peanut Proteins for Six Commercially Available Peanut ELISA Kits

					Range of quantification	
ELISA kit	Sample extraction ratio (w/v)	Sample dilution (1/fold)	Conversion weight to protein (1/fold)	Total dilution	Total peanut in unextracted product (ppm)	Peanut protein in the ELISA well (μg/mL)
Veratox (Neogen)	1/25	None	1/4	100	2.5−25	0.025−0.25
BioKits (Neogen)	1/10	1/10	1/4	400	1−20	0.0025−0.05
AgraQuant (Romer Laboratories)	1/20	None	1/4	80	1−40	0.0125−0.50
Ridascreen (R-Biopharm)	1/20	None	1/10	200	2.5−20	0.0125−0.1
Peanut Protein (Morinaga)	1/20	1/20	1/4	1600	1.25−80	0.00078−0.05
Peanut Residue (ELISA Systems)	1/10	None	1/4	40	4−60	0.1−1.5

Efficacy of Recovery of Peanut Protein from Peanut Butter

The percentage of peanut protein recovered from both the peanut butter suspension and extract at sample dilutions ranging from 10−500 ppm was widely variable with the different kits, as displayed in Figure 1. Of the six ELISA kits compared, the highest percentages of recovery of peanut proteins were obtained with the Romer and R-Biopharm kits with both methods of sample preparation. With the Veratox and Romer kits, a higher percentage of peanut was recovered when the sample was prepared as an extract rather than a suspension ($p < 0.05$). However, recovery was comparable with both methods of sample preparation with the BioKits, RBiopharm, and ELISA Systems kits ($p > 0.05$). Recovery of peanut differed widely within the same test kit at different sample dilutions especially with the Veratox kit (11−70%) and to a lesser extent with the BioKits kit (55−85%) when the initial sample was prepared as a suspension. This effect of sample concentration on peanut recovery was not evident with the remaining test kits with either method of sample preparation.

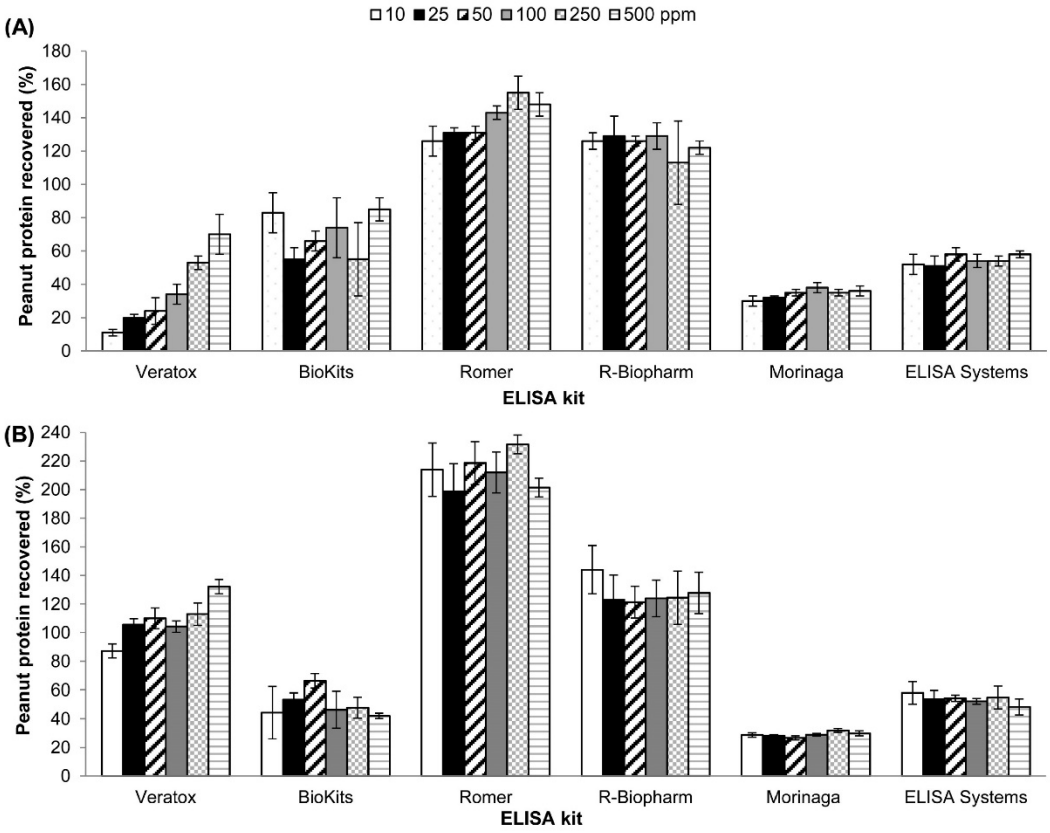

Figure 1. Comparison of percentage peanut protein recovered from an initial suspension (A) and extract (B) of SRM 2387 peanut butter by six different commercial peanut ELISA kits at sample concentrations ranging from 10−500 ppm. Protein extraction for each sample concentration was performed twice, and each extract was analyzed in duplicate by ELISA. Data are expressed as mean ± standard deviation (n = 4).

Recognition of Individual Peanut Allergens by ELISA

The polypeptide profiles obtained with the individual peanut proteins (Ara h 1, Ara h 2, Ara h 3, and Ara h 6) and SRM 2387 peanut butter are shown in Figure 2. The gel profile and molecular sizes of the allergens Ara h 1 (63 kDa), Ara h 2 (17−20 kDa doublet), Ara h 3 (a series of polypeptides ranging from 14−45 kDa), and Ara h 6 (15 kDa) are in line with previously published findings.[20,22] Furthermore, the gel profile confirms the purity of allergens and that none of the allergens contain other allergen types except perhaps as minor contaminants. The protein profile of SRM 2387 peanut butter indicates that Ara h 3 is the most abundant protein and that the other three allergens are also present but in lower amounts.

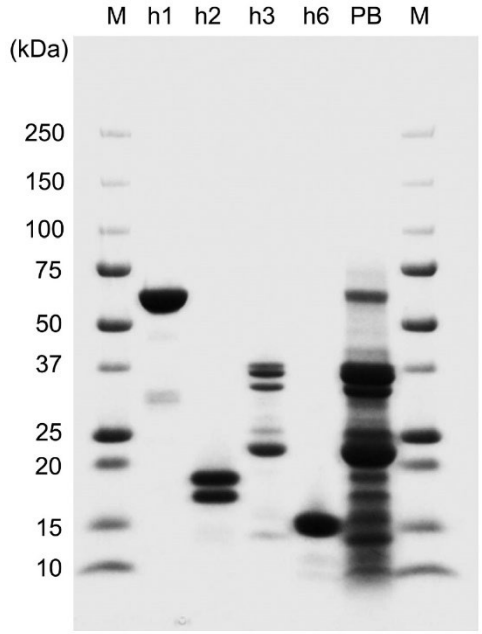

Figure 2. SDS-PAGE analysis of purified peanut allergens and SRM 2387 peanut butter under reducing conditions. M, molecular weight marker (indicated in kDa in left margin); h 1, Ara h 1; h2, Ara h 2; h3, Ara h 3; h6, Ara h 6; PB, SRM 2387 peanut butter from NIST. The protein load in each lane was 5 μg for the purified proteins and 20 μg for SRM 2387 peanut butter.

When the specificity and sensitivity of recognition of purified peanut allergens, Ara h 1, Ara h 2, Ara h 3, and Ara h 6, at concentrations ranging from 0.001−100 μg/mL by the six commercial peanut ELISA test kits were compared, five of the kits were most sensitive in the recognition of Ara h 3, while their reactivity for the other allergens was substantially lower (Figure 3). Initially, each allergen was screened against each of the test kits in order to identify the range of sensitivity for each allergen−test kit combination. On the basis of this preliminary data, the range of dilutions tested was adjusted accordingly to span the range from nonreactivity to maximum reactivity. In four of these five kits (the exception being ELISA Systems), Ara h 3 was more reactive than the respective kit standards provided by each kit. The Morinaga kit was significantly different from the other five kits in that Ara h 3 was the least recognized allergen by this kit. Moreover, while the other five kits were least sensitive in the recognition of Ara h 2 and Ara h 6, the Morinaga kit showed the highest sensitivity of recognition for these two allergens. Thus, the Morinaga kit stands out from the other five kits in being the most sensitive in detecting Ara h 2 and Ara h 6, the two allergens that were least recognized by the other kits.

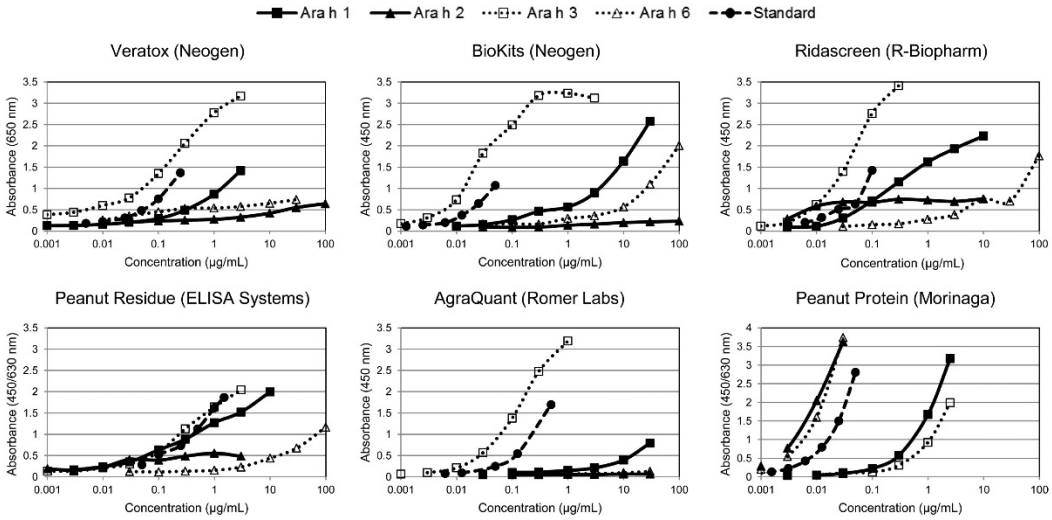

Figure 3. Recognition of the peanut allergens Ara h 1, Ara h 2, Ara h 3, and Ara h 6 by six ELISA kits at sample (peanut allergen) concentrations ranging from 0.001−100 μg/mL. The x-axis in logarithmic scale indicates sample concentration in the ELISA well. Standard refers to the calibration standards included in each respective ELISA kit.

Except for Morinaga, in the other five kits, Ara h 1 was the second most reactive allergen, and in all five, reactivity of this allergen was less than the kit standard. However, the reactivity of Ara h 1 did show differences between these kits (Figure 3), most notably that the reactivity between Ara h 3 and Ara h 1 was approximately two-fold for the ELISA Systems kit, while it was about 300-fold in the Romer kit.

When Ara h 2 was considered, the signal was found to increase at very low allergen concentrations (0.003−0.03 μg/ mL) but then level off at a low plateau with the R-Biopharm and ELISA Systems kits. This observation of being recognized at low concentrations and at the same time showing very low reactivity could most probably be due to having a low concentration of highly reactive antibodies against Ara h 2. However, with the Veratox and especially the Morinaga kits, the signal of Ara h 2 continued to increase with increasing concentrations of the allergen. In contrast, for both the BioKits and Romer kits, the signal remained at a low plateau for the entire range of concentrations where Ara h 2 seemed to show a low binding affinity for the antibodies.

Most of the kits reacted at higher concentrations of Ara h 6 (≥ 0.03 μg/mL), although Veratox did show some reactivity at 0.01 μg/mL. The exception was the Morinaga kit where the kit reacted to the allergen at a concentration as low as 0.001 μg/mL. At the same time, other than for the Romer and Veratox kits where reactivity plateaus off, in all of the other kits, reactivity of Ara h 6 showed an increase with increasing allergen concentrations. However, compared to the reactivity of Ara h 3, reactivity of Ara h 6 was about 1000−10 000-fold lower in the R-Biopharm, BioKits, and Romer kits. Moreover, in comparison to the other kits, the kit from Romer Laboratories was found to be the least sensitive in the detection of Ara h 1, Ara h 2, and Ara h 6.

Some variations in kit specificity occurred among the kits. The Veratox, Romer, and Morinaga kit manufacturers do not indicate that their peanut ELISAs are targeted against specific peanut proteins/allergens. For the BioKits ELISA, it is stated that this kit specifically detects the peanut allergen Ara h 1. Both the R-Biopharm and the ELISA Systems kits specify that their antibodies target peanut proteins including the allergens Ara h 1 and Ara h 2. As this study indicates, although these three kits do show reactivity against the specified allergens, they are most sensitive in the detection of Ara h 3. Importantly, the quantification of individual allergens within the same kit as well as between kits is not easy for several reasons. First, other than the BioKits and R-Biopharm kits, in which the standards are calibrated with SRM 2387 peanut butter, none of the other kits specify the source or type of peanut used in the preparation of the kit standards, and as such this may vary between kits. Second, the reactivity curves of the individual allergens are not completely parallel with the standard curve or with each other.

Discussion

Several ELISA kits for the detection and quantification of peanut residues in food products are commercially available. In this study, we compared the recovery of standard peanut butter (NIST SRM 2387) with six commercial sandwich ELISA kits and tested their ability to recognize individual peanut allergens.

All six test kits gave reasonably good recoveries with SRM 2387 peanut butter even though percentage recoveries varied widely between test kits. Given the differences in extraction buffers and the likely differences in the sources of peanut used to raise antibodies, which would translate into differences in the amount of protein extracted and differences in the antigens recognized by the antibodies, respectively, this is not unduly surprising. Overall, a low recovery of peanut was observed with the Morinaga kit with both methods of sample preparation (Figure 1). The kit manufacturers of Morinaga indicate that their kit gives 100% reactivity with dry peanuts and 60.3% reactivity with roasted peanuts. Since roasted peanuts are used in the manufacture of SRM 2387 peanut butter, this could at least partially explain the low recovery observed with the Morinaga kit. Poms et al.[11] suggest that antibodies raised against raw peanuts would have a lower affinity to heat-treated antigens, although none of the manufacturers of the six kits provide information about the sources of peanut used to raise antibodies.

Two kits, namely BioKits and R-Biopharm, indicate that their standards have been calibrated with SRM 2387 peanut butter. The high recovery observed with the R-Biopharm kit could be largely because it has already been standardized using SRM 2387 peanut butter. Nevertheless, as demonstrated by this study, the method of preparation of the peanut extracts influences the protein content in the extract. Thus, use of the same source to prepare the standard material does not necessarily guarantee uniform recoveries (compare BioKits vs R-Biopharm in Figure 1).

The underestimation of protein content in heat-treated peanut samples by ELISA is well documented.[13–15,28] Since the SRM 2387 peanut butter is heat-treated, the underestimation of peanut content from this peanut butter with some of the kits is supported by those previous observations. Accordingly, the kit manufacturers have optimized extraction

procedures used with their kits, provided instructions intended to optimize extraction, and in some cases included an extraction additive to increase the recovery. Indeed, these steps are helpful for some of the kits (Figure 1).

The Morinaga kit differs significantly from the rest in containing the reducing agent, β-mercaptoethanol (2%), in its extraction buffer. This same manufacturer has developed a similar ELISA to detect egg albumin where they use an extraction buffer containing both a surfactant (SDS) and a reducing agent (β-mercaptoethanol), and the antibodies are also raised against denatured proteins.[29] Such an ELISA has the advantage of detecting denatured proteins in heat-processed samples as the reducing agent helps to redissolve aggregated proteins as was shown for an ELISA for soy earlier.[30]

In contrast to the Morinaga kit, the other five kits were highly sensitive in the recognition of Ara h 3 but recognized Ara h 2 and Ara h 6 only at high protein concentrations or at sensitivities about 1000-fold lower than that for Ara h 3. Both Ara h 2 and Ara h 6 are 2S albumins containing up to five disulfide bonds, which makes them resistant to heat and digestive enzymes.[31] Moreover, Ara h 2 and Ara h 6 have been identified as being major peanut allergens that also share homologous regions.[18−20,24,32] However, a study on immune responses to peanut allergens has shown that upon oral exposure, Ara h 1 and Ara h 3 induce a comparatively higher IgG response, while Ara h 2 and Ara h 6 induce much lower responses.[33] Thus, this study confirms that Ara h 1 and Ara h 3 would be better immunogens for raising an IgG antibody response than Ara h 2 and Ara h 6. Accordingly, this would support our observation that five of the kits showed the highest recognition for Ara h 3 followed by Ara h 1, while recognition of Ara h 2 and Ara h 6 was much lower. Additionally, of the total protein content of peanut kernels, Ara h 3 is the most abundant and Ara h 1 content is about 12−16%, while both Ara h 2 and Ara h 6 are present in approximately the same concentration, 6−9%.[20,34,35] Although none of the kit manufacturers provide information regarding the peanut extract/type used in the production of antibodies, the relative content of each allergen in peanut kernels could also contribute to the significantly lower sensitivity of recognition of Ara h 2 and Ara h 6 by five of the test kits.

Another reason that antibodies against both Ara h 2 and Ara h 6 seem under-represented in five out of the six kits may lie in the stable core structure of both Ara h 2 and Ara h 6. Processing of these two allergens by antigen presenting cells (APCs) in the laboratory animals used to raise antibodies would be less efficient. Thus, immunization to produce antibodies would lead to a response against other proteins, particularly Ara h 3. Reduction of the disulfide bonds of these two allergens will denature Ara h 2[36] as well as Ara h 6,[37] which would perhaps make these allergens more susceptible for processing by APC and consequently into better immunogens. Thus, the approach used with the Morinaga kit, that is, under conditions that reduce the disulfide bonds, would indeed increase detection of Ara h 2 and Ara h 6 especially since the antibodies are also produced against reduced peanut proteins.

The Veratox ELISA has been shown to have a low sensitivity in the detection of both Ara h 1 and Ara h 2.[38] Compared to a sensitivity of 30 ng/mL for a monoclonal antibody-based ELISA that specifically targets Ara h 1, Veratox showed a sensitivity of only 2−4 μg/mL for Ara h 1. This observation is in agreement with that of the present study where the Veratox ELISA is primarily specific for the detection of Ara h 3.

Additionally, the peptide profile of SRM 2387 peanut butter in Figure 2 shows that although all four allergens are represented, Ara h 3 appears the most intense, while Ara h 1 is much lower in intensity. Several previous studies have shown that heating/roasting of peanuts results in a decrease in Ara h 1 levels[28,39,40] presumably owing to a loss of solubility. The loss of Ara h 1 due to heating/roasting would be a disadvantage with a kit that was oriented to the specific detection of Ara h 1. However, the five test kits that predominantly recognized Ara h 3 showed a high recovery of peanut from SRM 2387 peanut butter. In contrast, the Morinaga kit, with a low reactivity to Ara h 3, showed low recovery of peanut protein from peanut butter in the present study.

Therefore, five of the commercial kits show the highest reactivity to the most abundant peanut protein, Ara h 3, a protein that also happens to be an excellent immunogen. In contrast, the Morinaga kit shows the highest reactivity to the more potent and therefore more relevant peanut allergens, Ara h 2 and Ara h 6. Given that Ara h 2 and Ara h 6 are less susceptible to denaturation and aggregation than other peanut proteins, these allergens may be better targets than Ara h 1 or Ara h 3 for quantification of peanut in processed food.

In conclusion, the present study proved that SRM 2387 peanut butter is recognized by all six commercial ELISA kits, but recovery was not uniform across different kits or at different sample concentrations. With regard to purified allergens, regardless of what is specified by some of the commercial kit manufacturers, five of the kits were most sensitive in the detection of Ara h 3, the most abundant protein in peanut kernels. The Morinaga kit was unique in that it showed the highest sensitivity in the detection of Ara h 2 and Ara h 6, the most relevant allergens in terms of allergenicity. Thus, while the detection of peanut residues in processed food products can likely be accomplished with any of these kits, comparable results should not be expected when analyzing the same sample across more than one kit. Surprisingly, this marked difference in specificity does not preclude kits recognizing heat labile proteins from quantifying peanut in peanut butter.

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