

Evaluation of Extraction Buffers Using the Current Approach of Detecting Multiple Allergenic and Nonallergenic Proteins in Food

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The detection of food allergens has been a challenge because of the increasing need to ensure the absence of undeclared allergens in foods. The current trend in the detection of some food allergens, like peanuts, is based on the detection of multiple allergenic and nonallergenic proteins, and this is the approach that kit manufacturers have adopted. Because commercial kits differ in their ability to detect allergens, regulatory agencies, the food industry, and kit manufacturers are working together to standardize the detection methods. Three kits for the detection of peanuts have been evaluated for performance by the AOAC Research Institute. For this evaluation, a peanut butter suspension was used as a reference material. Several kit components contribute to between-kit analytical variation, even when the same sample is used. One component of commercial kits, which may be contributing to this variability, is the sample extraction buffer. In this study, differences in extractability of 3 allergenic foods were evaluated by using 4 different extraction buffers. The conclusion is that optimum allergen extractability was buffer-dependent, and no single buffer is appropriate for use as a universal extraction solution for all allergenic foods. Therefore, a thorough evaluation of sample preparation buffers needs to be performed for every individual allergenic food. In light of the results obtained, the current approach used for detection of peanut allergens based on the detection of multiple allergenic and nonallergenic proteins is being analyzed.

Proteins are complex organic compounds of important nutritional value in the human diet and are a major component of a wide variety of manufactured foods. Because of the diversity in structure of these molecules, proteins have many different physicochemical and technological properties. However, not all proteins are

beneficial to human health. Some proteins can elicit allergic reactions in sensitive individuals, which can be life-threatening in some cases. It is believed that about one in 25 Americans suffers from food allergies (1).

Prophylactic treatments are still under investigation, but currently there is no cure for food allergies. Consequently, the only solution possible to prevent allergic reactions is the total avoidance of the offending food. Food allergic individuals have to rely fully on the accuracy of the food ingredient list printed on labels of manufactured foods.

Immunoassays have been the method of choice for the detection of food allergens because antibodies can recognize specific proteins in a complex mixture of compounds of different nature. The development of this type of assay is not an easy task owing to the large number of factors to take into consideration. One of the major issues in the detection of food allergens is that allergen structure and/or its solubility in extraction buffers may be modified by different processing procedures, such as thermal and biochemical procedures, chemical preservation, and isolation and purification. As a consequence, detection of allergens by antibodies may be altered, modifying the final result of the test.

Other variables are inherent to the immunoassay. For example, several commercial kits differ in their ability to detect the same allergenic proteins (e.g., peanut). Most explanations for these differences are based on the idea that capture and detection antibodies vary from kit to kit because they were elicited with different immunogens. However, different calibrators and extraction buffers are used in the development of each kit, further contributing to analytical variation. Several studies have been conducted to standardize reference materials, such as the performance evaluation of commercial kits by the AOAC Research Institute using a peanut butter standard (National Institute of Standards and Technology, Standard Reference Material 2387).

Even though little attention has been paid to sample extraction buffers, the selection of an appropriate extraction solution can be as important as a selection of specific antibodies. The purpose of extraction solutions is to solubilize the compounds of interest from a sample for further use as calibrators or immunogens, or for analysis. Depending on their solubility, allergens can be classified into 3 main groups. Albumins are soluble in aqueous buffers, globulins are better extracted in saline solutions, and prolamines are soluble in a mixture of water and alcohol. Several factors might affect the

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Table 1. Protein content in 3 allergenic food extracts using 4 extraction buffers. Values are expressed as mean $\pm$ standard deviation. Mean within each column with different superscripts differs at $p < 0.05$

Extraction buffers	Protein concentration, mg/g				
	Peanut		Soybean		White sesame
	Light roast, $n = 8$	Dark roast, $n = 8$	Raw $n = 4$	Roasted $n = 4$	Raw $n = 4$
PBS	34.7 $\pm$ 3.8 ^c	42.7 $\pm$ 2.1 ^{b,c}	207.4 $\pm$ 12.3 ^b	70.2 $\pm$ 8.1 ^c	56.4 $\pm$ 2.7 ^b
HSB	87.1 $\pm$ 7.9 ^b	47.7 $\pm$ 5.8 ^b	486.0 $\pm$ 34.7 ^a	73.6 $\pm$ 4.1 ^{b,c}	398.0 $\pm$ 56.9 ^a
Carb	126.4 $\pm$ 10.5 ^a	57.5 $\pm$ 4.6 ^a	483.0 $\pm$ 58.0 ^a	85.3 $\pm$ 3.0 ^{a,b}	46.9 $\pm$ 2.7 ^b
Tris	45.7 $\pm$ 8.9 ^c	40.4 $\pm$ 4.2 ^c	232.2 $\pm$ 27.9 ^b	93.7 $\pm$ 8.8 ^a	77.6 $\pm$ 4.2 ^b

extraction efficiency of allergens/proteins. Currently, most of the detection methods for food allergens are based on the detection of multiple proteins of diverse physical-chemical characteristics such as the presence of hydrophobic or hydrophilic amino acid residues. This fact makes the extraction and analysis of allergens more difficult than the detection of single analytes. Because of the structural diversity of proteins, the extraction solutions of specific pH and ionic strengths at a given temperature will be more efficient in the extraction of one type of protein than another.

In the present study, we show how different extraction buffers can affect qualitatively and quantitatively the proteins extracted from 3 allergenic foods and their effect on further analysis.

Experimental

Food Samples

(a) *Peanut flour*.—Light roast peanut flour, Cat. No. 521271, and dark roast peanut flour, Cat. No. 521261 (Golden Peanut Co., Alpharetta, GA).

(b) *Soybean flour*.—Nonroasted Cat. No. S9633, and roasted soybean flour, Cat. No. S9758 (Sigma-Aldrich Chemicals, St. Louis, MO).

(c) *White sesame seed*.—Acquired in an Asian market.

Apparatus

(a) *Blender*.—Variable speed blender (Waring Lab, Torrington, CT).

(b) *Mixer*.—Nutator (Becton Dickinson and Co., Sparks, MD).

(c) *Pipet and pipet tips*.—20, 100, and 1000 μ L, and 5 and 10 mL adjustable air displacement pipets with disposable tips (Gilson, Middleton, WI).

(d) *Multichannel pipet*.—12-Channel, 1000 mL (Biohit Inc., Neptune, NY).

(e) *Superspeed centrifuge*.—Sorvall RC-5B (Sorvall, Asheville, NC).

(f) *Microtiter plate*.—Maxisorp 96-well microtiter plate (Nalge Nunc International, Rochester, NY).

(g) *Electrophoresis power supply*.—PowerEase 500 (Invitrogen Corp., Carlsbad, CA).

(h) *Electrophoresis cuvette*.—Xcell Surelock mini-cell (Invitrogen Corp.).

(i) *Imaging system*.—ProXPRESS (Perkin Elmer Lifesciences, Boston, MA).

(j) *1-D electrophoresis analysis software*.—TotalLab (NonLinear Dynamics, Durham, NC).

(k) *ELISA plate reader*.—Microwell spectrophotometer with variable wavelength, Spectra Max 340 (Molecular Devices Corp., Sunnyvale, CA).

(l) *Software*.—SOFTmax PRO2.11 (Molecular Devices Corp.).

Reagents

(a) *Phosphate-buffered saline (PBS)*.—10mM, pH 7.4, Cat. No. P3813 (Sigma-Aldrich Chemicals).

(b) *High-salt extraction buffer (HSB)*.—20mM $\text{NaH}_2\text{PO}_4 \cdot \text{H}_2\text{O}$, 1M NaCl, pH 7.4.

(c) *Carbonate buffer*.—0.1M (Carb).

(d) *Tris buffer (Tris)*.—50mM Tris base; 0.22M NaCl, pH 8.2.

(e) *0.45 μ m PVDF filter*.—Cat. No. AV125UAQU (Whatman, Clifton, NY).

(f) *Whatman No. 1 paper*.

(g) *Hexane*.—HPLC grade.

(h) *Bicinchoninic (BCA) protein assay kit*.—Cat. No. 23227 (Pierce, Rockford, IL).

(i) *NuPAGE LDS sample buffer*.—Cat. No. NP0007 (Invitrogen Corp.).

(j) *NuPAGE reducing agent*.—Cat. No. NP0009 (Invitrogen Corp.).

(k) *NuPAGE antioxidant*.—Cat. No. NP0005 (Invitrogen Corp.).

(l) *NuPAGE 12% Bis-Tris precast gels*.—Cat. No. NP0341BOX (Invitrogen Corp.).

(m) *NuPAGE MES SDS running buffer*.—Cat. No. NP0002 (Invitrogen Corp.).

(n) *NuPAGE transfer buffer*.—Cat. No. NP0006-1 (Invitrogen Corp.).

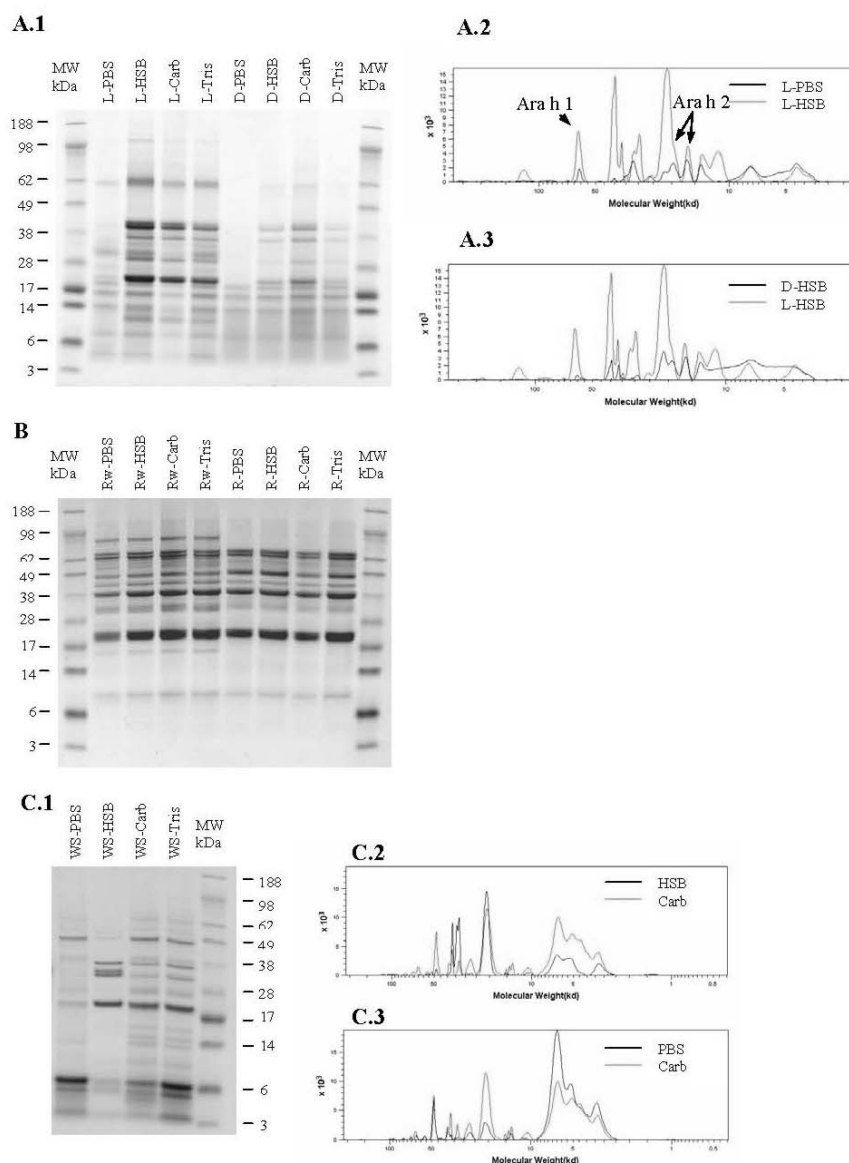


Figure 1. SDS-PAGE of allergenic extracts. (A.1.) Light roasted (L) and dark roasted (D) peanut extracts. (A.2.) Protein profile comparison between light peanut flour extracted with PBS and HSB. (A.3.) Protein profile comparison between dark and light peanut flours extracted with HSB. (B) Roasted (R) and not-roasted (Rw) soybean flour extracts. (C.1.) White sesame seed extracts. (C.2.) Protein profile comparison between white sesame seed extracted with HSB and Carb. (C.3.) Protein profile comparison between white sesame seed extracted with PBS and Carb.

(o) *SeeBlue Plus2 prestained molecular weight standard.*—Cat. No. LC5925 (Invitrogen Corp.).

(p) *Colloidal Blue staining kit.*—Cat. No. LC6025 (Invitrogen Corp.).

(q) *Bio-Kit peanut protein assay kit.*—Tepnel Life Sciences (Wythenshawe, Manchester, UK).

(r) *Veratox peanut allergen test.*—Neogen Corp. (Lansing, MI).

(s) *Ridascreen peanut.*—R-Biopharm GmbH (South Marshall, MI).

(t) *Enhancer nitrocellulose membrane.*—Geno Technology Inc. (St. Louis, MO).

(u) *Carnation skimmed powder milk.*—Nestle (Vevey, Switzerland).

METHODS

Sample Defatting

Because of the high fat content of sesame seeds, a sample was blended and defatted as follows: A 100 g portion of ground white sesame seeds was defatted with 700 mL hexane.

The mixture was stirred for 30 min, decanted for an additional 30 min, and vacuum-filtered through Whatman No. 1 paper. This operation was repeated 2 more times. The remaining defatted sesame sediment was dried overnight at room temperature (RT) in a hood.

Protein Extraction

A 1 g portion of defatted sample was extracted with 10 mL extraction buffer. The mixture was incubated for 1 h at RT in a mixer and then centrifuged at $20\,000 \times g$ for 30 min at 4°C, and the supernatant was filtered through a 0.45 µm PVDF filter.

Determination of Protein Content

BCA protein assay was used to determine protein concentration in sample extracts. Procedure followed manufacturer's instructions, using the microtiter plate method.

Sodium Dodecyl Sulfate-Polyacrylamide Gel Electrophoresis (SDS-PAGE)

Extracts were diluted in LDS sample buffer in the presence of reducing agent and heated for 70°C for 10 min. Proteins were separated in 12% Bis-Tris precast gels under denaturing conditions at 125 V for 90 min using MES as a running buffer. Proteins were further stained with colloidal blue or Western blotted onto a nitrocellulose membrane.

Enzyme-Linked Immunosorbent Assay (ELISA)

Samples to be analyzed were initially diluted to 10 ppm with PBS or the extraction buffer with which they were

extracted (HSB, Carb, or Tris). This preparation was treated as a typical sample to be analyzed with 3 different ELISA kits for peanuts. Either PBS or extraction buffers prepared in-house were used for sample preparation instead of using the extraction and dilution buffers included in the kits. The rest of the ELISA test was performed by following each manufacturer's protocol.

Western Blot

Light and dark peanut flour were separated by electrophoresis as described previously and then transferred to a nitrocellulose membrane at 25 V for 60 min following Invitrogen's manual. Membranes were blocked with 5% skimmed dry milk. They were incubated with the anti-peanut enzyme conjugates from each of the 3 kits used for ELISA determination of peanuts for 1 h at RT, and washed with 0.05% Tween 20 in PBS; color was developed by peroxidase reaction with CN-DAB substrate (Pierce, Rockford, IL).

Statistical Analysis

Tukey's Studentized Range (HSD) test was used following one-way analysis of variance (ANOVA) to compare the effect of the different extraction buffers in the total protein content of 3 allergenic foods. SAS system v.8.02 (SAS Institute Inc., Cary, NC) was used for statistical data processing.

Results and Discussion

Quantitative Effect of Extraction Buffers

Protein content in allergenic extracts.—Three different allergenic foods—peanut, soybean, and sesame seed—were

Table 2. ELISA quantitation of peanut content of buffer extracts containing 10 ppm peanut protein using 3 commercial kits^a

			Peanut, ppm		
Peanut flour	Extraction buffer	Dilution buffer	Kit 1	Kit 2	Kit 3
Light	PBS	PBS	18.9	19.5	5.7
	HSB	HSB	76.3	87.2	22.6
		PBS	82.9	83.7	37.8
	Carb	Carb	28.7	44.5	9.4
		PBS	89.6	83.8	33.8
	Tris	Tris	40.6	46.5	18.0
PBS		65.4	63.3	22.2	
Dark	PBS	PBS	10.0	<2.5	<1
	HSB	HSB	15.1	15.8	5.7
		PBS	20.7	19.0	11.0
	Carb	Carb	12.1	13.8	2.6
		PBS	19.1	19.2	10.6
	Tris	Tris	15.9	13.8	4.6
PBS		19.0	15.5	6.4	

^a Bold values correspond to extracts diluted in PBS.

chosen for this study. Peanuts are known to elicit severe symptoms such as systemic reactions (anaphylaxis), which can be followed by death in many cases (2). Soybean and soybean products are a very common source of protein widely used as food ingredients (3, 4). The incidence of allergy to soy is quite high, mainly among children (3, 5). These 2 allergenic foods are among the "big eight" regulated by many countries. However, some countries, including Canada, the European Union, and Australia, are expanding the "big eight" list to include other allergens, such as sesame seed.

The 3 allergenic foods were extracted with buffers of different pH and ionic strengths. PBS, HSB, and Tris buffers are commonly used in the extraction of samples to be analyzed by immunoassays; Carb was also used to evaluate the effect of high pH in the extractability of proteins from food. Table 1 summarizes the protein content found in the different extracts.

The type of extraction buffer used has a significant effect on the amount of protein extracted from light roast peanut flour. It was surprising to find that Carb buffer extracted significantly more protein than any of the other buffers. HSB also extracted more protein than PBS and Tris, but no significant differences were found between the protein amounts extracted by these last 2 solutions. Differences among extracts of dark roasted peanuts were not as marked as with light roast extracts, despite the fact that Carb extract contained significantly more protein than the other 3. In the case of soybean flour, HSB and Carb extracts from raw soybean flour contained double the amount of protein of the Tris and PBS extracts.

The ability of extraction buffers to solubilize proteins from food seems to have an important impact mainly in the analysis of the less-processed samples, where proteins retain most of their native structure. However, these data correspond only to a small percentage of allergenic foods and to one type of processing (roasting). A more extensive evaluation of extraction buffers is required, taking into consideration

different food processing procedures in a wider range of allergenic foods.

From the quantitative point of view, HSB extracts a greater amount of protein from white sesame seeds than the other buffers. It is possible that sesame seeds contain a high amount of globulins thus requiring a high ionic strength buffer to be solubilized.

Total protein assays provide only a quantitative measurement of the total protein extracted by buffers. Additional information regarding the qualitative aspects of the extraction is needed in order to better characterize the ability of different buffers to extract proteins from food. Do the extraction buffers extract the same proteins but in different proportions? Does the protein profile vary from sample to sample? Can different extraction buffers extract specific food allergens?

Qualitative Effect of Extraction Buffers: Electrophoresis of Allergenic Extracts

To answer these questions, proteins present in different sample extracts were separated by SDS-PAGE, and the profiles were compared by image analysis software.

Figure 1 (A.1) shows the protein profile of peanut extracted with various buffers. For each sample, 1 μ L extract (equivalent to 100 ng peanut flour) was loaded on the gel, with the exception of the lane loaded with light roast peanut flour extracted with carbonate buffer [L-Carb in Figure 1 (A.1)]. In this case, 0.3 μ L extract (equivalent to 30 ng peanut flour) was used to avoid overloading the gel. Protein profiles from HSB, Carb, and Tris extracts from light and dark roast peanut flour are almost identical regardless of the protein concentration. However, PBS extracts not only show less amount of protein but also a change in the proportion of proteins extracted [Figure 1 (A.2)]. Bands of about 41, 40, and 21 kilodaltons (kDa) that are very abundant in HSB, Carb, and Tris are barely present in PBS extracts.

Regarding the presence of peanut allergens in the extracts, extracts were compared to purified Ara h 1 and Ara h 2 standards. A doublet corresponding to Ara h 2 (16.5 and 19.5 kDa) and the putative Ara h 3 fragment (14 kDa; 6) are present in all of the samples. However, the 61 kDa band corresponding to the molecular weight of Ara h 1 is clearly present in all of the light peanut flour extract, whereas only a very faint band can be seen in some of the dark peanut flour extracts.

As observed previously, the roasting of peanuts results in a generalized reduction in protein content reflected in the gel scan [Figure 1 (A.3)], whereas there is an increased band density below 20 kDa in dark roast extracts with respect to light ones. This increased band density in this region can be explained by occurrence of protein breakdown or degradation caused by roasting, resulting in the formation of small peptide fragments. This fact can also explain the reduction in intensity of larger protein bands in the SDS-PAGE profile of dark roasted extracts as compared with that of light roasted extracts. However, not all of the reduction in band intensity is necessarily a consequence of protein degradation. Processing,

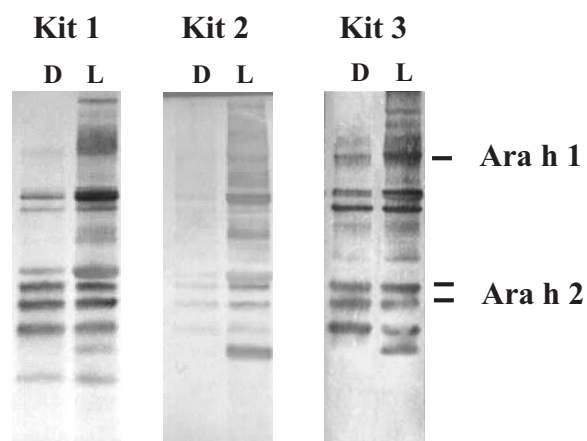


Figure 2. Western blot of peanut proteins in dark roasted (D) and light roasted (L) peanut flour extracts. Detection of proteins by peroxidase-conjugated antibodies from commercial kits.

in this case roasting procedures, may reduce protein solubility, as has been suggested for Ara h 1 (7).

About 0.25 μ L raw soybean flour extract (equivalent to 25 ng raw soybean flour) and 1 μ L roasted soybean extract (equivalent to 100 ng roasted soybean flour) were loaded into the gel. Figure 1 (B) shows that protein profiles were almost identical among all of the extracts from raw soybean flour. Protein profiles were also identical among raw extracts, but some minor proteins were missing in roasted when compared to raw samples. Therefore, roasting procedures seem to affect protein stability in processed soybean flour but less than in peanut.

Figure 1 (C.1) shows the SDS-PAGE profile of 1 μ L white sesame seed extract (equivalent to 100 ng defatted sesame seed). In this case, the HSB extract is diluted by a factor of 10 in order to avoid gel overload. The most relevant information related to white sesame seed is that the extraction of 21, 33, 34, and 37 kDa proteins is optimum when HSB is used as the extraction buffer Figure 1 (C.2). These proteins account for the big difference in protein content found between HSB and the other 3 buffers. PBS, Carb, and Tris extracts contain the same protein profile, but identical proteins are extracted in different proportions Figure 1 (C.3).

Evaluation of Peanut Extracts in Commercial ELISA Kits

It is known that commercial kits differ in their ability to detect peanut proteins (8, 9). A common understanding is that the antibodies and calibrators used in the kits contribute to the differences found among them. However, little attention has been paid to the fact that these kits use different sample extraction buffers and, in some cases, dilution buffers. This study demonstrated that use of different extraction solutions quantitatively and qualitatively affects the protein extracted from food. In order to study the ability of the different kits to detect proteins under the previously defined conditions of extraction and assay, all of the extracts were diluted to 10 ppm with the extraction buffer with which they were extracted, or with PBS. This sample concentration was used in further analyses with the commercial ELISA kits.

Table 2 summarizes the results obtained for the 3 kits used in the study. Even though the antibodies from these 3 peanut kits detect peanut proteins, the final concentrations are expressed as ppm peanut material (not protein) as a result of an internal conversion factor used by every kit. As a consequence, recoveries of 10 ppm peanut protein samples cannot be calculated unless the conversion factors are known, which is not the case. This factor also makes kit comparison more difficult. As expected, PBS works better as a dilution buffer because its pH of 7.4 is compatible with antibody-antigen binding. The following comments refer to those samples diluted with PBS only.

In general, results obtained with kits 1 and 2 for the same sample do not differ, and they are higher than those values obtained with kit 3. Only kit 1 could detect peanut material extracted from dark flour using PBS. Peanut content in HSB- and Carb-extracted samples was similar when any of the 3 kits

were used. Because of the parallelism between the results of both buffers it is highly possible that proteins present in the normalized HSB and Carb are the same and are in the same proportion, as indicated in the analysis of the extracts by SDS-PAGE. The total protein content in the carbonate extract was significantly higher than the HSB extract. It is possible that part of the additional protein extracted with Carb buffer is not recognized by the antibody components of the commercial kit, because the antibodies were probably raised against an immunogen obtained in more neutral conditions.

Even though all extracts were normalized to 10 ppm, the results for dark peanut flour extracts are lower than those for light peanut flour. Changes in protein structure during food processing can affect antigenicity and, consequently, antibody recognition. Davis et al. (10) summarized how heating and storage can modify antigenicity. Some possible modifications of proteins result from reactions with oxidized lipids, formation of disulfide bonds, or reactions with polyphenols.

In the case of peanut, carbonate seems to be an appropriate sample extraction buffer because it can extract more proteins than other buffers. However, the main drawback is that it is not compatible with the immunoassay, as shown in Table 2. Extraction buffers must be compatible with the immunoassay, keeping intact the antibody properties as well as the antigenicity of the proteins. This is the main reason why saline extraction buffers of neutral pH are usually used in the immunochemical detection of food allergens. This does not mean that Carb buffer cannot be used in the analysis of food allergens. It can be used for the extraction of proteins from a particular sample and then be diluted, if possible, with a buffer compatible with the assay such as PBS. Another possibility is to do a buffer exchange between carbonate buffer and PBS, but this step is time-consuming.

Evaluation of Peanut Extracts in Western Blot Using Antibody-Enzyme Conjugate from Commercial ELISA Kits

Figure 2 shows a Western blot of dark and light flour peanut extracts that are recognized by the peroxidase-conjugated antibody from the 3 kits used in this study. Even though proteins are denatured in SDS-PAGE, Western blot provides an idea of which proteins are recognized by the antibodies, possibly explaining some of the differences observed in the ELISA results. Protein from dark and light peanut extracts loaded on membranes incubated with conjugates from kits 1 and 3 was normalized at the same concentration in order to show why 10 ppm extracts, in ELISA, showed reduced values for the dark extract solution. Degraded proteins recognized by the conjugates are very similar to those patterns shown in the SDS-PAGE. However, as mentioned above, modifications of immunoreactive sites of the proteins caused by roasting may be responsible for the lower levels of protein detected in the dark roasted extract. The difference in peanut content from dark to light extracts may also result from protein breakdown during processing. Protein breakdowns can still be measured by BCA protein

assay, whereas results from ELISA depend on the binding of intact epitopes by antibodies.

For the Western blot used with conjugate of kit 2 the same volume of light and dark peanut extracts was applied to the gel/membrane. The purpose of showing this is to simulate a real situation in which the same volume of extract from a blind sample is analyzed by the kit. The proteins present in the dark extract are barely seen because they are present in the extract at lower concentrations than in light extracts. Moreover, the immunoreactivity of the protein has probably been reduced in the dark roasted peanut flour. For all of these reasons, in addition to the reduced solubility of proteins in processed foods, the result may lead to underestimation of the presence of peanut proteins in highly processed foods.

Another question concerning the results is the conversion factor internally used in the standard curve to convert peanut protein into peanut material. The conversion factor could be used for the analysis of raw or lightly processed peanuts or peanut-containing products, but in light of the results it may not be advisable to use it in the case of highly processed peanuts. The main reason for this is that the ratio between immunochemically stable proteins to peanut material has been reduced in processed peanut flour with respect to the less roasted flour. This apparent problem of the conversion factor can also explain why the peanut content determined by ELISA of 10 ppm of dark peanut flour extracts is significantly lower than 10 ppm of the light flour extracts. The situation could become more complicated in the presence of different matrixes. Because of all of the apparent problems rising from the analysis of peanut flour extracts, it may not be a bad approach to analyze a single protein that conserves its solubility and immunoreactivity along the food process. This protein could be used as a marker for the presence of peanut residues in manufactured foods in the same way that caseins or β -lactoglobulin are used to detect milk and ovalbumin, or that ovomucoid is used to detect egg residues. According to all of the tests performed in this study, peanut allergen Ara h 2 could be a good target candidate for the evaluation of the presence of peanut traces in food. SDS-PAGE shows that this protein is soluble because it is present in all of the extracts, showing almost the same band intensity in light and dark extracts. This also means that it is less affected by roasting procedures. Ara h 2 retains most of its immunoreactivity, as shown by the strong binding with conjugates from the kits in both light and dark peanut flour extracts.

Implication of the Use of Different Extraction Buffers in Method Standardization

The use of extraction buffers goes beyond sample preparation; they play a key role in the development of immunoassays for food allergens. The use of a well-characterized extract, containing the allergens/proteins of interest, is also important because (1) the extract may be used to develop antibodies to be used in immunoassay development. The quality of this immunogen will define the characteristics of the elicited antibodies. Because current methods for food allergens detect nonallergenic proteins as

well as allergens, antibodies used for the development of a kit should use sera from animals that have been exposed to the maximum number of different food allergens/proteins that may be present in commercial varieties of the allergenic food. Ideally, antibodies should be able to recognize all of the forms of a protein, native and processed. (2) Extracts are also used as standards and calibrators in immunoassays. Extraction buffers should be optimized so that the maximum number of specific proteins and the largest amount of these proteins are extracted from the food of interest.

The applicability of such extracts is not limited to kit development for the food industry. The same concepts are also relevant to the development of allergen extracts for clinical use. An appropriate extract would contain all of the different allergenic proteins from the food in order to be used for in vitro and in vivo studies, such as the skin prick test and double blind placebo controlled food challenge (DBPCFC). If specific allergenic proteins are missing from an extract used for patient diagnosis, the patient may receive a false-negative diagnosis.

Conclusions

These results show that there is no universal extraction buffer to be used in the analysis of food allergens. Therefore, extraction buffers need to be evaluated for every allergenic food. Because the antigenicity of proteins may be drastically affected by food processing, standardization should also include the characterization of processed and nonprocessed allergens or proteins present in a particular extract. This is the best method to determine which proteins are being analyzed, the protein content of calibrators, and recognition of these proteins by the antibodies used in their analysis.

This study examined 3 types of allergenic foods, but an extensive study of processed food containing these allergens as ingredients is required to evaluate extraction efficiency of these buffers and how they behave in order to reduce matrix effect and interference by other food components. Moreover, the current approach for the detection of peanut residues in food, based on the detection of multiple proteins, may not be adequate. Because the antigenicity and solubility of different proteins are affected in different manners by food processing, the use of a single protein, immunochemically stable throughout food manufacturing, may be more suitable as a marker for the presence of peanut traces in food. Peanut allergen Ara h 2 seems to be a good candidate for that purpose.

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