



Cross-reactivity by botanicals used in dietary supplements and spices using the multiplex xMAP food allergen detection assay (xMAP FADA)

Ronnie O. Pedersen¹ · William L. Nowatzke² · Chung Y. Cho¹ · Kerry G. Oliver² · Eric A. E. Garber¹

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Abstract

Food allergies affect some 15 million Americans. The only treatment for food allergies is a strict avoidance diet. To help ensure the reliability of food labels, analytical methods are employed; the most common being enzyme-linked immunosorbent assays (ELISAs). However, the commonly employed ELISAs are single analyte-specific and cannot distinguish between false positives due to cross-reactive homologous proteins; making the method of questionable utility for regulatory purposes when analyzing for unknown or multiple food allergens. Also, should the need arise to detect additional analytes, extensive research must be undertaken to develop new ELISAs. To address these and other limitations, a multiplex immunoassay, the xMAP® food allergen detection assay (xMAP FADA), was developed using 30 different antibodies against 14 different food allergens plus gluten. Besides incorporating two antibodies for the detection of most analytes, the xMAP FADA also relies on two different extraction protocols; providing multiple confirmatory end-points. Using the xMAP FADA, the cross-reactivities of 45 botanicals used in dietary supplements and spices commercially sold in the USA were assessed. Only a few displayed cross-reactivities with the antibodies in the xMAP FADA at levels exceeding 0.0001%. The utility of the xMAP FADA was exemplified by its ability to detect and distinguish between betel nut, saw palmetto, and acai which are in the same family as coconut. Other botanicals examined included allspice, amchur, anise seed, black pepper, caraway seed, cardamom, cayenne red pepper, sesame seed, poppy seed, white pepper, and wheat grass. The combination of direct antibody detection, multi-antibody profiling, high sensitivity, and a modular design made it possible for the xMAP FADA to distinguish between homologous antigens, provide multiple levels of built-in confirmatory analysis, and optimize the bead set cocktail to address specific needs.

Keywords Food allergen · Dietary supplements · Spices · Immunoassay · Cross-reactivity · xMAP

Introduction

Food allergies are an international problem affecting people worldwide with prevalence rates varying up to 10% depending on diet, cultural practices, and possibly genetic disposition [1–6]. Food allergies put tremendous stress on the allergic individual, their family and friends, and society, causing

approximately 200,000 emergency room visits in the USA each year [7, 8]. The Food Allergen Labeling and Consumer Protection Act of 2004 (FALCPA) was established to partially address this problem [9]. FALCPA requires the labeling of food products that contain any of the eight major allergenic food groups; crustacean shellfish, egg, fish, milk, peanut, soy, tree nuts, and wheat. In addition, the Gluten-free Regulation of 2013 was issued defining the use of the voluntary term gluten-free as indicating the presence of < 20 ppm intact gluten [10, 11]. Enforcement of FALCPA and the Gluten-free Regulation of 2013 depend in-part on the availability of analytical methods capable of measuring the specified allergens and gluten in widely different matrices. Commercial ELISA test kits are currently the primary methods used to analyze food samples suspected of containing undeclared food allergens or violating the Gluten-free Regulation of 2013. Despite being robust, sensitive, and easy to perform, ELISAs also have severe limitations that could result in misleading results [12]. A

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✉ Eric A. E. Garber
Eric.Garber@fda.hhs.gov

¹ Office of Regulatory Science, Center for Food Safety and Applied Nutrition (CFSAN), Food and Drug Administration, 5001 Campus Drive, College Park, MD 20740, USA

² Radix® BioSolutions, Georgetown, TX 78626, USA

major weakness of currently used single analyte ELISA technology is an inability to distinguish between target antigens and cross-reactive homologous proteins. As a result, single analyte-specific ELISAs are inappropriate for the accurate detection and quantification of unknown food allergens or multiple food allergens.

A potential solution to the problems encountered using classical ELISA technology entails the use of multiplexed methods. Numerous methods have been developed to reliably detect food allergens [13–15]. However, whatever method the FDA employs for regulatory enforcement must be available for others to use. Since antibodies are unique, this means that antibody-based methods used for regulatory analysis must be commercially available. To meet this restriction, the FDA worked with Radix BioSolutions to develop a commercial multiplex assay for food allergens using multi-analyte profiling (xMAP) technology. Critical to this endeavor were generous contributions by Morinaga Institute of Biological Science, Inc. (MIOBS), IEH Laboratories and Consulting Group, and Elution Technologies. xMAP-based methods have become popular for the detection of infectious agents and biomarkers in part due to high precision, sensitivity, and robustness [16–21]. Though xMAP technology has been used by other groups to detect food allergens, these assays employed either a competitive design or detected PCR-derived amplicons [22–25]. The xMAP® food allergen detection assay (xMAP FADA) developed by Radix BioSolutions employs a sandwich configuration based on established antibodies, two complementary extraction protocols, and a high level of redundancy to generate multiple built-in confirmatory endpoints. The two extraction protocols used by the xMAP FADA, a buffered-detergent (PBST or UD-Buffer) protocol and a reduced-denatured (SDS/ β -mercaptoethanol) protocol, mimic two popular extraction protocols employed by commercial ELISA test kits. The presence of the target antigen is indicated by formation of bead-capture antibody-antigen–detector antibody-biotin-streptavidin-phycoerythrin complex. The xMAP FADA is capable of simultaneously detecting 14 food allergens plus gluten, thereby reducing time, labor, and cost [26]. Redundant antibodies targeting different epitopes in the same allergenic food with sensitivity sufficient to measure cross-reactivity and perform multi-antibody profiling provide built-in confirmatory endpoints as was recently applied to the analysis of cumin, tree nuts, and legumes, and the detection of undeclared peanut in garlic powder [12, 27, 28].

Multi-antibody profiling, a form of antigenic finger printing, was recently used to distinguish between multiple tree nuts and legumes not specifically targeted by the antibodies in the xMAP FADA [28]. The approach was also successfully used to analyze regulatory samples suspected of containing pecan, despite the lack of pecan-specific antibodies [29]. These, along with the ability to add new antibody-bead set conjugates or selectively choose the bead sets included in a

cocktail, provide expandability and the ability to customize the assay to meet unanticipated analytical needs.

The growing use of botanicals, the global trade in herbs from novel cultivars, and new plant products make the possibility of allergic reactions due to cross-reactivity (allergenecity), agricultural co-mingling, and inadvertent cross-contact a serious threat to the allergic consumer. This becomes especially complex when dealing with pre-ground herbal products manufactured in facilities using shared equipment. Current analytical technologies (e.g., ELISAs) cannot address these problems without a substantial increase in resources (e.g., use of multiple test kits, manpower, finances, time, sample material). The problem is further complicated by the multiple uses of plant products, potential routes of exposure (e.g., oral and dermal), and dosage. For example, garlic, turmeric, and cinnamon are used as spices and dietary supplements [30–32]. The xMAP FADA provides a start in addressing these problems. The multiple possible endpoints make it possible to detect, identify, and characterize food allergens in botanicals used in dietary supplements and spices and distinguish between any homologous cross-reactive elements [27, 33]. Forty-five (45) different dietary supplements, spices, and botanicals sold in the USA were examined using the xMAP FADA for cross-reactivity, which is a necessary first step before applying the xMAP to determine the presence of undeclared food allergens in these products.

Materials and methods

Reagents Phosphate-buffered saline (PBS, cat# P5368) and Tween 20 (cat# P9416) were purchased from Sigma-Aldrich Inc. (St. Louis, MO). BD Difco skim milk powder and sodium dodecyl sulfate (SDS, cat# 28312) were purchased from Fisher Scientific (Waltham, MA). All other reagents were of the highest technical grade available.

Botanical products Botanical products were purchased from online vendors and local suppliers. Controls and duplicate samples from multiple vendors were acquired when possible to confirm the antigenicity of commercial products. *Hoodia gordonii*, Indian snakeroot, and betel (areca) nut were acquired from the National Center for Natural Product Research (NCNPR) at The University of Mississippi (University, MS). Cranberries were purchased both as fresh raw whole berries and as whole freeze-dried berries. Blueberries were purchased as whole freeze-dried berries. Black cohosh, cinnamon, saw palmetto berry, and eleuthero root were purchased as cut pieces. The products examined were not chosen to represent either a systematic survey of either commercial popularity or regulatory interest but instead are representative of products that were readily available and

to provide an initial exploration of commercial botanicals available locally.

xMAP food allergen detection assay The xMAP food allergen detection assay (xMAP FADA) (Radix BioSolutions, Georgetown, TX) was used as recommended by the manufacturer. Buffered detergent samples were extracted according to the UD Buffer protocol which entailed mixing 1 g of sample in 40 mL UD Buffer (105 mM sodium phosphate /75 mM NaCl/2.5% Difco skim milk powder/0.05% Tween 20) for 2 h with constant shaking in the dark, centrifuged, and 50 μ L of supernatant analyzed either as-is or following tenfold serial dilution with UD Buffer. The only exception to this was raw cranberries which were extracted by mixing 7.89 g in 33.1 mL UD buffer to compensate for the 87.3% water content [34]. Reduced-denatured extraction entailed suspending 1 g of sample in 10 mL PBST and mixing the sample 1:1 with 10 mL 0.5% SDS/2% β -mercaptoethanol for 30 min at 60 °C. After centrifugation, the supernatant was diluted 100-fold with PBST to reduce the SDS and β -mercaptoethanol content accordingly.

After extraction, 50 μ L of the extracted samples (and calibration standards) were mixed 1:1 with the appropriate (para)magnetic bead cocktail containing color-coded beads conjugated to different capture antibodies (Ab_c) for 30 min at 37 °C. The bead cocktail for the analysis of the buffered-detergent sample extracts contained 25 food allergen bead sets [almond (bead set-12, 13), Brazil nut (14, 15), cashew (18, 19), coconut (20, 21), crustacean (22), gluten (27, 28), hazelnut (29, 30), macadamia (33, 34), peanut (37, 38), pine nut (39, 42), pistachio (43, 44), soy (45, 46), walnut (47, 48)], 4 AssayChex® Process Control Panel bead sets (75, 76, 77, 78; Radix BioSolutions, Georgetown, TX) to detect operational failures, and for this work 4 additional experimental bead sets (data not shown) for the detection of egg (25, 26) and milk (35, 36). The bead cocktail used for the analysis of the reduced-denatured sample extracts consisted of five food allergen bead sets [egg (65), gluten (73), milk (66, 67), and peanut (72)] plus the four AssayChex® Process Control Panel bead sets.

The presence of the target antigen (Ag) in the sample extract is indicated by the binding of biotinylated detector antibody (Ab_d -biotin) to the bead- Ab_c -Ag complex during the second incubation step (30 min at 37 °C with shaking). Streptavidin phycoerythrin (SAPE) is then added to visualize the bead- Ab_c -Ag- Ab_d -biotin complex (15 min at 37 °C with shaking). Between and after the final incubation step, the samples were washed with PBST.

The calibration standards were supplied as a 200X mixture of all 15 analytes, which was diluted in UD buffer for the buffered-detergent analyses or subjected to the same extraction and dilution procedure as the samples in the reduced-denatured analyses. The use of calibration standards that are

a mixture of multiple analytes may generate slightly distorted calibration curves, depending on the magnitude of any cross-reactivity. Such mixtures are fully suitable for normalizing data between multiple experiments or generated using reagents from different lots. However, for detailed quantification, the magnitude of any distortion should be ascertained as was done previously [26] and confirmed by including direct comparison controls (DCC) consisting of analyte-free samples that are spiked with a pre-defined amount of an individual analyte, usually at a target or critical threshold level (e.g., gluten at 20 ppm). Though the dynamic range of the food allergen analyses of the xMAP FADA are ascribed LoQ values comparable the lowest non-zero calibration standard, most display reproducible LoD values considerably less as indicated by the background and standard deviations measured empirically.

All food samples were extracted in triplicate and the median fluorescence intensities (MFI) measured of the washed beads suspended in 100 μ L PBST using a Bio-Plex 200 (Bio-Rad Laboratories, Inc. Hercules, CA) and the data subjected to statistical analysis as previously described [26, 27]. This typically entailed subtracting the average background MFI (S_0) for each bead set from the average MFI responses generated by the triplicate samples.

Inter-lot and experiment variability The bead sets and reagents are supplied in vials containing the equivalent of multiple test kits, typically five. This reduces variability between experiments, provided caution is taken not to inadvertently damage the unused portion of the reagents (e.g., unnecessary warming). However, since preparation of the xMAP FADA reagents entail multiple antibody purification and conjugation reactions, inter-lot variability may be greater than typical for ELISA test kits. Variability between different lots or experiments can be corrected-for by normalizing the results to standards analyzed alongside the samples. This is routinely accomplished using the calibration standards supplied with the xMAP FADA or by including other reference materials (e.g., DCC) in the analysis. Caution should be taken to assure that the reference materials match the samples or that any associated variances do not exceed acceptable limits.

Electronic supplementary material The electronic supplementary material (ESM) tabulates the label information of the botanicals that were supplied as containing multiple ingredients (ESM Table S1), the MFI responses generated by the calibration standards from the buffered-detergent analyses (ESM Table S2a), the MFI responses generated by the calibration standards from the reduced-denatured analyses (ESM Table S2b), and the concentrations of the analytes in the calibration standards (ESM Table S3). The xMAP FADA test kit calibration standards were ran on separate days using 16 different test kits from 2 production runs designated 1A–5A and

6B–16B. Lots A and B differ by more than the production runs used to produce the reagents (from scratch). The second lot (B) also employed a new peanut reference material at concentrations one-tenth that in lot A (ESM Table S3). As expected, the MFI results grouped together by lot (except 5A) with the ratios between the MFI generated by complementary antibodies (generated against the same food allergen) displayed the expected decrease in variance. The exception, 5A displayed considerably different MFI and ratios than the results generated in experiments using either reagents from the same lot (e.g., 1A, 2A, 3A, 4A) or lot B. This variation may reflect instrumentation problems that necessitated performance of a second verification prior to using the Bio-Plex 200 and/or possible damage to the reagents as may have resulted from the multiple times the stock solutions were used to prepare bead set and detection cocktails.

Results and discussion

Table 1 lists the botanical samples examined. Control reference materials are designated with a “C” with “B” to identify commercial botanical products. Though most of the products primarily contained the name ingredient, it was common for some to contain encapsulating, processing, anti-caking, and flow agents as itemized in ESM Table S1. Most of the botanicals purchased as spices did not list any other ingredients on the labels.

The average MFI generated by the various botanical products using the xMAP FADA are listed in Tables 2, 3, 4, and 5. Tables 2 and 3 present the buffered-detergent analyses of the samples derived from fruit/seeds and derived from other plant parts (e.g., flower, leaf, root, stem), respectively. Tables 4 and 5 present the reduced-denatured protocol analyses similar to Tables 2 and 3. MFI below the lowest calibration standard (S1, ran alongside the samples) are designated in the tables with a “<”; MFI within three standard deviations of background (S0) are designated as “<<.” Buffered-detergent extracts that generated MFI responses above the highest calibration standard (S7) were diluted and the MFI of the diluted sample listed alongside the undiluted sample with yellow, orange, or red highlight to indicate whether the dilution was 10-, 100-, or 1000-fold, respectively.

Alongside the MFI values are the ratios for the antibody pairs. If the MFI were due to the presence of the target analyte, the ratio between the paired antibodies would be the same as the ratios generated by the reference material (calibration standard) at the same concentration (see ESM Tables S2a and S2b), provided the MFI were within the dynamic range as defined by the calibration standards.

Relationship between MFI and cross-reactivity Figure 1 depicts the relationship between MFI and cross-reactivity on a

total mass-basis for 1 g samples extracted using the UD-Buffer protocol and diluted 100-fold following centrifugation (to generate a response within the dynamic range). The data used to generate the relationship curves are based on the average MFI generated by reagents from lot A, experiments 1A–4A (Fig. 1a–c) or reagents from lot B, experiments 6B–11B (Fig. 1d–f) as tabulated in ESM Table S2a. One bead set for each analyte is presented, except gluten which includes both bead sets 27 and 28. The percent cross-reactivity was calculated based on the concentration of protein in the calibration standards (ESM Table S3) converted to the amount of total analyte using the protein content as published in the National Nutrient Database [34] and in the certificates of analysis for the commercial reference materials used [35, 36]. For samples that were diluted 1000-fold, the interpolated percent cross-reactivity should be multiplied by 10. Similarly, samples analyzed as-is or after a tenfold dilution require that the interpolated cross-reactivity be divided by 100 or 10, respectively. For example, if a sample was extracted using the UD Buffer protocol, diluted 100-fold after centrifugation, and generated an MFI of 2000 with cashew-18 when analyzed using an assay kit from lot A, the MFI corresponds to a cross-reactivity of 0.006% (Fig. 1c). If the sample was diluted only tenfold, then the cross-reactivity would be 0.0006%, the value interpolated from the graph divided by ten. If the tenfold diluted sample also generated an MFI with cashew-19 that yielded a cashew-18/cashew-19 ratio comparable to the ratio generated by the comparable reference material, the interpolated result of 0.0006% would instead indicate the likely presence of 6 µg of cashew in the 1 g sample (0.0006%, 6×10^{-6} , or 6 ppm). Should it be necessary to be more precise than possible using the average MFI values derived from multiple analyses, the individual data sets for each experiment (ESM Table S2a and S2b) can be plotted to generate experiment-specific figures. Lastly, the protein content conversion factors are based on total protein (nitrogen) content, as determined by Dumas or Kjeldahl analysis, while the calibration standards are based on soluble protein and BCA analyses. As such, the calculated percent by mass (and derived cross-reactivity) is a lower limit.

Overview This combined use of multiple secondary endpoints, worse-case scenarios, and tests of reasonability make it possible to indirectly estimate allergen concentrations. The magnitude of the MFI responses measured for a subset of the dietary supplements and spices were significantly above background. While definitive, the MFI were mostly consistent with miniscule amounts of allergen or very low levels of cross-reactivity with only a few exceptions.

The fruit and seed samples displayed a higher prevalence of cross-reactivity in the xMAP FADA than extracts prepared from other plant parts (e.g., flower, leaf, root, or stem). This probably reflects multiple factors including

Table 1 Botanicals used in dietary supplements and spices

Common name	No. ^a	Order	Family	Genus species	PP ^b	Type ^c
Acai	i,ii	Arecales	Arecaceae	<i>Euterpe oleacea</i> Engel	FS	B ₂
African mango	i,ii	Malpighiales	Irvingiaceae	<i>Irvingia gabonensis</i> Baill.	FS	B ₂
Allspice	i, ii	Myrtales	Myrtaceae	<i>Pimenta dioica</i> (L.) Merr.	FS	B ₂
Amchur	i, ii	Sapindales	Anacardiaceae	<i>Mangifera indica</i> L.	FS	B ₂
Anise seed	i ^d , ii	Apiales	Apiaceae	<i>Pimpinella anisum</i> L.	FS	B ₂
Bay leaves	i ^d , ii ^d	Laurales	Lauraceae	<i>Laurus nobilis</i> L.	L	B ₂
Bee pollen	i,ii	—	—	—	u	B ₂
Betel nut/Areca	i,ii	Arecales	Arecaceae	<i>Areca catechu</i> L.	FS	C,B
Black cohosh	i,ii	Ranunculales	Ranunculaceae	<i>Cimicifuga racemosa</i> Nutt.	R	C,B
Black pepper	i ^d , ii ^d	Piperales	Piperaceae	<i>Piper nigrum</i> L.	FS	B ₂
Blueberry	i,ii	Ericales	Ericaceae	<i>Vaccinium corymbosum</i> L.	FS	C,B
Caraway seed	i ^d , ii	Apiales	Apiaceae	<i>Carum carvi</i> L.	FS	B ₂
Cardamom	i ^d , ii	Zingiberales	Zingiberaceae	<i>Elettaria cardamomum</i> L.	FS	B ₂
Chili, red pepper ^c	i,ii,iii	Solanaceae	Solanaceae	<i>Capsicum annuum</i> L.	FS	B ₃
Cinnamon	i,ii,iii	Laurales	Lauraceae	<i>Cinnamomum cassia</i> (L.) J. Presl.	S	C,B ₂
Cloves	i,ii	Myrtales	Myrtaceae	<i>Syzygium aromaticum</i> L.	F	B ₂
Cranberry ^f	i–vi	Ericales	Ericaceae	<i>Vaccinium macrocarpon</i> Aiton	FS	C ₃ ,B ₃
Cumin	i,ii	Apiales	Apiaceae	<i>Cumin cyminum</i> L.	FS	B ₂
Dill weed	i	Apiales	Apiaceae	<i>Anethum graveolens</i> L.	L	B
Eleuthero root	i	Apiales	Araliaceae	<i>Eleutherococcus senticosus</i> Maxim.	R	C
Fennel seed	i ^d ,ii	Apiales	Apiaceae	<i>Foeniculum vulgare</i> Mill.	FS	B
Garlic	i	Asparagales	Amaryllidaceae	<i>Allium sativum</i> L.	S/L	B ₂
Ginger	i,ii	Zingiberales	Zingiberaceae	<i>Zingiber officinale</i> Roscoe	R	B ₂
Ginseng	i,ii	Apiales	Areliaaceae	<i>Panax quinquefolius</i> L.	R	B ₂
Grapeseed extract	i,ii,iii	Vitales	Vitaceae	<i>Vitis vinifera</i> L.	FS	B ₃
<i>Hoodia gordonii</i> ^g	i–vi	Gentianales	Apocynaceae	<i>Hoodia gordonii</i> Sweet	S	C,B ₃
Indian snakeroot	i,ii	Gentianales	Apocynaceae	<i>Rauvolfia serpentina</i> (L.) Benth. ex Kurz	R	C,B
Kelp ^h	i,ii	Fucales	Fucaceae	<i>Ascophyllum nodosum</i>	u	B ₂
Mace	i,ii	Magnoliales	Myristicaceae	<i>Myristica fragrans</i> Houtt	FS	B ₂
Marjoram ^h	i,ii	Lamiales	Lamiaceae	<i>Origanum majorana</i> L.	L	B ₂
Mustard	i,ii	Brassicales	Brassicaceae	<i>Sinapis alba</i> L.	FS	B ₂
Nutmeg	i	Magnoliales	Myristicaceae	<i>Myristica fragrans</i> Houtt	FS	B
Oregano	i,ii	Lamiales	Lamiaceae	<i>Origanum vulgare</i> L.	L	B ₂
Parsley flakes	i ^d ,ii ^d	Apiales	Apiaceae	<i>Petroselinum crispum</i> (Mill.) Fuss	L	B ₂
Poppy seed	i ^d ,ii	Ranunculales	Papaveraceae	<i>Papaver somniferum</i> L.	FS	B ₂
Rosemary	i ^d ,ii ^d	Lamiales	Lamiaceae	<i>Rosmarinus officinalis</i> L.	L	B ₂
Sage	i,ii	Lamiales	Lamiaceae	<i>Salvia officinalis</i> L.	L	B ₂
Saw palmetto	i,ii,iii	Arecales	Arecaceae	<i>Serenoa serrulata</i> (Michx.) G. Nicholson	FS	C,B ₂
Sesame seed	i ^d ,ii ^d	Lamiales	Pedaliaceae	<i>Sesamum indicum</i> L.	FS	B ₂
Spirulina ^h	i,ii,iii	Oscillatoriales	Phormidiaceae	<i>Arthrospira platensis</i>	u	B ₃
Thyme	i,ii	Lamiales	Lamiaceae	<i>Thymus vulgaris</i> L.	L	B ₂
Turmeric	i	Zingiberales	Zingiberaceae	<i>Curcuma longa</i> L.	R	B
Wheat grass	i,ii	Poales	Poaceae	<i>Triticum aestivum</i> L.	L	B ₂
White pepper	i,ii	Piperales	Piperaceae	<i>Piper nigrum</i> L.	FS	B ₂

^a Designations to distinguish between different suppliers of the same or similar samples (e.g., i, ii, iii, iv)^b Plant part: *F* flower, *FS* fruit/seed, *L* leaf, *R* root, *S* stem, *u* not designated^c *C* control, reference material, *B* botanical product. C,B₂ indicates i–control reference material, ii and iii–commercial botanicals^d Supplied as either flower, fruit/seed (FS), leaf, root, or stem and ground in the laboratory^e Red pepper marketed as i–cayenne red pepper; ii–chili cayenne; iii–chipotle chili pepper^f Cranberries i—fresh, whole, raw; ii—whole freeze dried; iii—fresh whole, raw freeze dried in lab,^g *Hoodia gordonii*—iii contained 50% “glucomannan root” possibly *Amorphophallus konjac*^h Kelp–ii listed based on a similar product (Kelp–i); i—sweet Marjoram or Majoram, ii—Marjoram; classification based on common *Spirulina* species

Table 2 Median fluorescence intensity generated by buffered-detergent extracts of fruit and seed samples

	No.	Exp. ^c	Almond ^d		Brazil nut		Cashew		Coconut		Crust.		Gluten		Hazelnut		Macadamia		Peanut		Pine nut		Pistachio		Soy		Walnut														
			12	13	12/13	14	15	14/15	18	19	18/19	20	21	20/21	22	27	28	29	30	27/28	33	34	27/34	37	38	37/38	39	42	39/42	43	44	43/44	45	46	45/46	47	48	47/48			
Acai	i	4A	<	<	<	<	<	<	<	<	995			<	<	<	<	<	<	<	<	<	<	<	<	<	<	<	<	<	<	<	<	<	<	<	<	<			
Acai	ii	3A	<	<	<	<	<	<	<	<	4714	50	94	<	<	<	<	<	<	<	<	<	<	<	<	<	<	<	<	<	<	<	<	<	<	<	<	<			
African Mango	i	10B	<	<	<	<	<	<	<	<	<	<	<	<	<	<	<	<	<	<	<	<	<	<	<	<	<	<	<	<	<	<	<	<	<	<	<	<			
African Mango	ii	10B	<	<	<	<	<	<	<	<	<	<	<	<	<	<	<	<	<	<	<	<	<	<	<	<	<	<	<	<	<	<	<	<	<	<	<	<			
Almonds	i	5A	<	<	<	<	<	<	<	<	<	<	<	<	91	<	<	<	<	<	<	<	<	<	<	<	<	<	<	<	<	<	<	<	<	<	<	<			
Almonds	ii	8B	<	<	<	<	<	<	<	<	<	<	<	<	512	<	<	<	<	<	<	<	<	<	<	<	<	<	<	<	<	<	<	<	<	<	<	<			
Amchur	i	10B	<	<	<	2654	<	<	9733	3640 ^f	6371	1199	13	1925	66	29	<	565	3375	617	1620	<	<	<	<	<	<	<	<	10101	1194	63	<	<	<	2393	2254	13			
Amchur	ii	10B	<	<	<	<	<	<	1185	366	<	<	<	<	<	<	<	122	692	618	<	<	<	<	<	<	<	<	<	3299	<	<	<	<	430	<	<	<			
Anise Seed	i	5A	<	<	<	102	<	<	<	105	<	<	21	<	<	<	297	182	18	<	<	<	<	<	<	<	<	<	<	23	<	<	321	<	110	<	<				
Anise Seed	ii	8B	<	<	<	<	<	<	274	<	<	<	211	<	<	<	253	1868	614	2193	989	22	<	<	<	<	<	<	<	<	<	<	1741	<	647	<	<				
Betel Nut	i	1A	<	<	<	<	221	<	<	<	<	<	24833	5082 ^g	5016	347	63	16	<	<	<	<	<	<	<	<	<	<	<	95	<	<	<	<	1749	2101	68				
Betel Nut	ii	7B	<	<	<	<	<	<	<	<	<	<	24004	6729	3718	378	63	16	<	<	<	<	<	<	<	<	<	<	<	<	<	<	<	1614	1462	13					
Black Pepper	i	5A	<	<	<	<	<	<	<	<	<	<	<	<	80	<	<	<	<	<	<	<	<	<	<	<	<	<	<	<	<	<	<	<	<	<	<	<			
Black Pepper	ii	8B	<	<	<	<	<	<	<	<	<	<	<	<	670	<	<	<	<	<	<	<	<	<	<	<	<	<	<	<	<	<	<	<	<	<	<	<			
Blueberry (freeze dry)	i	3A	<	<	<	1532	<	<	1000	302	33	<	39	<	<	<	1028	856	104	82	<	<	<	<	<	<	<	<	967	<	<	61	<	<	<	1280	310	43			
Blueberry Extract	i	4A	2959	927	32	2545	79	32	1559	563	24	<	46	<	<	<	147	1126	271	42	<	<	<	<	<	<	<	<	<	<	150	<	283	<	1684	604	19				
Caraway Seed	i	5A	<	<	<	<	<	<	<	<	<	<	<	<	<	<	<	<	<	<	<	<	<	<	<	<	<	<	<	<	<	<	<	<	<	<	<	<			
Caraway Seed	ii	8B	<	<	<	<	<	<	<	<	<	<	79	<	<	<	107	925	612	821	<	<	<	<	<	<	<	<	<	<	<	<	<	<	<	105	<				
Cardamom	i	9B	<	<	<	<	<	<	<	<	<	<	4595	10961 ^h	759	257	61	13	<	515	2019	<	<	<	<	<	<	<	280	29	87	<	<	<	1361	673	19				
Cardamom	ii	9B	<	<	<	<	<	<	201	<	<	<	3694	2237	700	570	5	18	<	948	1948	<	<	<	<	<	<	<	348	<	<	<	<	<	1149	594	19				
Cayenne, Red Pepper	i	5A	<	<	<	706	98	12	245	341	67	<	17	<	<	52	413	207	22	<	<	<	<	<	<	<	<	<	<	55	<	<	<	195	347	68					
Chili Cayenne	ii	11B	<	559	<	8180	1843	44	3501	1625	5405	2490	68	67	3035	172	18	<	100	2013	636	4743	2785	3066	1448	13	13	4326	155	28	1635	<	<	1117	<	2781	311	4617	578	68	63
Chipotle Chili Pepper	iii	5A	<	<	<	578	79	73	169	238	67	<	10	<	<	<	119	284	189	12	<	<	<	<	<	<	<	<	<	<	33	<	<	<	<	269	<	<			
Cranberry	i	1A	<	<	<	<	<	<	<	<	<	<	<	<	<	<	<	<	<	<	<	<	<	<	<	<	<	<	<	<	<	<	<	<	<	<	<	<	<		
Cranberry	ii	6B	<	713	<	12066	758	16	4707	4990	63	1967	100	20	<	<	90	<	4146	2162	19	4164	594	70	<	<	<	<	<	562	<	<	2892	339	4008	1350	17	13			
Cranberry	iii	1A	<	<	<	<	<	<	<	<	<	<	<	<	<	<	<	<	<	<	<	<	<	<	<	<	<	<	<	<	<	<	<	<	<	<	<	<	<		
Cranberry	iv	1A	<	<	<	3316	<	<	1805	608	30	<	<	<	<	<	809	291	28	<	<	<	<	<	<	<	<	<	<	<	103	<	<	<	843	673	13				
Cranberry	v	2A	<	<	<	2783	<	<	1738	508	34	<	<	<	<	<	861	243	27	<	<	<	<	<	<	<	<	<	<	<	87	<	<	871	582	13					
Cranberry	vi	6B	<	535	<	8942	368	34	3870	2942	13	<	59	<	<	99	<	2574	1335	19	<	361	<	<	<	<	<	<	344	<	<	382	<	<	2595	2442	13				
Cumin	i	5A	<	<	<	<	<	<	<	<	<	<	<	<	<	66	<	<	<	<	<	<	<	<	<	<	<	<	<	<	<	<	<	<	<	<	<	<	<		
Cumin	ii	8B	<	<	<	529	<	<	867	326	17	<	55	<	<	94	744	611	700	309	12	<	<	<	<	<	<	<	92	<	<	<	<	378	222	17					
Fennel Seed	i	5A	<	<	<	<	<	<	<	<	<	<	<	<	<	<	<	<	<	<	<	<	<	<	<	<	<	<	<	<	<	<	<	<	<	<	<	<			
Fennel Seed	ii	8B	<	<	<	<	<	<	<	<	<	<	<	<	<	50	<	<	462	3058	63	778	452	12	<	<	<	<	6561	5151	13	<	<	<	<	<	181	<			
Grassseed Extract	i	9B	<	<	<	<	<	<	<	<	<	<	<	<	<	<	<	<	<	<	<	<	<	<	<	<	<	<	<	<	<	<	<	<	<	<	<	<	<		
Grassseed Extract	ii	9B	<	<	<	<	<	<	<	<	<	<	<	<	<	<	<	<	<	<	<	<	<	<	<	<	<	<	<	<	<	<	<	<	<	<	<	<	<		
Grassseed Extract	iii	9B	<	<	<	<	<	<	<	<	<	<	<	<	<	<	<	<	<	<	<	<	<	<	<	<	<	<	<	<	<	<	<	<	<	<	<	<	<		
Mace	i	5A	<	<	<	<	<	<	<	<	<	<	<	<	<	122	<	<	<	<	<	<	<	<	<	<	<	<	783	<	<	<	<	<	<	<	<	<	<		
Mace	ii	8B	<	<	<	<	<	<	<	<	<	<	<	<	<	534	<	<	<	<	<	<	<	<	<	<	<	<	<	<	<	<	<	<	<	<	<	<	<		
Mustard	i	5A	<	<	<	<	<	<	<	<	<	<	<	<	<	16	92	62	<	80	<	<	<	<	<	<	<	<	<	<	<	<	<	<	<	<	<	<	<		
Mustard	ii	8B	<	<	<	<	<	<	<	<	<	<	<	<	<	<	256	540	63	<	<	<	<	<	<	<	<	<	<	<	<	<	<	<	<	678	<	<			
Nutmeg	i	4A	<	<	<	546	<	<	182	<	<	<	<	<	<	<	173	<	374	126	33	<	<	<	<	<	<	<	<	<	36	<	<	<	<	<	167	<	<		
Poppy Seed	i	7B	<	391	<	6033	619	67	3545	4590	63	3753	290	403	177	63	18	<	3144	2977	781	552	61	64	18799	11267	302	11325	82	10	1699	<	<	<	466	<	2886	2226	13		
Poppy Seed	ii	9B	4833	696	63	7337	613	67	7338	1373	15766	1950	63	67	6565	2803	397	323	17	87	<	32	299	63	6644	1512	2074	617	12	13	24693	7913	942	252	28	31	3095	<	<		

Table 3 Median fluorescence intensity generated by buffered-detergent extracts of flower, leaf, root, and stem samples

Part ^a	No. ^c	Exp. ^d	Almond ^e	Brazil nut	Cashew	Coconut	Crust.	Gluten	Hazelnut	Macadamia	Peanut	Pine nut	Pistachio	Soy	Walnut
			12 13	14 15	18 19 18/19	20 21 20/21	22 27	28 27/28	29 30 29/30	33 34	37 38 37/38	39 42 39/42	43 44	45 46	47 48 47/48
Bay Leaves	L i	5A	< <	< <	< <	< <	< <	< <	< <	< <	< <	< <	< <	< <	< <
Bay Leaves	L ii	8B	< <	< <	< <	< <	< <	< <	< <	< <	< <	< <	< <	< <	< <
Bee Pollen	u i	4A	< <	< <	< <	< <	< <	< <	< <	< <	< <	< <	< <	< <	< <
Bee Pollen	u ii	4A	< <	< <	< <	< <	< <	< <	< <	< <	< <	< <	< <	< <	< <
Black Cohosh	R i	1A	< <	< <	< <	< <	< <	553	< <	< <	< <	< <	43	< <	< <
Black Cohosh	R ii	1A	< <	< <	< <	< <	< <	592	< <	< <	< <	< <	< <	< <	< <
Cinnamon	S i	1A	< <	< <	< <	< <	< <	279	< <	< <	< <	< <	< <	< <	< <
Cinnamon	S ii	1A	< <	< <	< <	< <	< <	426	< <	< <	< <	< <	< <	< <	< <
Cinnamon	S iii	4A	< <	< <	< <	1015 56 18	< <	< <	< <	< <	< <	< <	< <	< <	< <
Cloves	F i	5A	< <	< <	< <	< <	< <	< <	< <	< <	< <	< <	< <	< <	< <
Cloves	F ii	8B	< <	< <	< <	< <	< <	781	< <	< <	< <	543	< <	< <	< <
Dill weed	L i	5A	< <	< <	< <	< <	< <	75	< <	< <	< <	< <	< <	< <	< <
Elaeuthero Root	R i	4A	< <	< <	< <	< <	29 750	0.04	< <	< <	< <	250	< <	33	< <
Garlic	U/S i	2A	< <	< <	< <	< <	< <	< <	< <	< <	< <	403	< <	< <	< <
Ginger	R i	5A	< <	< <	< <	< <	< <	127	< <	< <	< <	< <	< <	< <	< <
Ginger	R ii	8B	< <	< <	< <	< <	29 195	2475 0.08	< <	< <	2388 2337 1.0	443	< <	87	< <
Ginseng, Am.	R i	4A	< <	959	781 368 2.1	< <	< <	314	547 257 2.1	< <	< <	299	< <	148	353 186 1.8
Ginseng, Panax	R ii	4A	< <	< <	< <	< <	< <	< <	< <	< <	< <	< <	< <	< <	< <
Hoodia gordonii	S i	1A	< <	< <	< <	< <	< <	< <	< <	< <	< <	< <	< <	< <	< <
Hoodia gordonii	S ii	1A	< <	< <	< <	< <	< <	< <	< <	< <	< <	< <	< <	< <	< <
Hoodia gordonii	S iii	1A	< <	< <	< <	< <	84 1214	0.07	< <	< <	< <	523	< <	63	< <
Hoodia gordonii	S iv	1A	< <	< <	< <	< <	290 2217	0.13	< <	< <	< <	325	< <	46	< <
Indian Snakeroot	R i	4A	< <	< <	< <	2108 37	< <	332	< <	< <	< <	< <	< <	< <	< <
Indian Snakeroot	R ii	11B	< <	321 832	743 587 1.3	< <	2518 219 ^h 13522 1386 0.19 0.18	< <	< <	< <	1561	< <	502	< <	245 < 1483 373 263 1.4
Kelp	u i	1A	< <	< <	< <	< <	< <	< <	< <	< <	< <	< <	< <	< <	< <
Kelp	u ii	1A	< <	< <	< <	< <	< <	< <	< <	< <	< <	< <	< <	< <	< <
Marjoram	L i	8B	< <	< <	< <	< <	291 1834	0.16	< <	< <	< <	< <	< <	< <	< <
Marjoram	L ii	5A	< <	< <	< <	< <	23 132	0.17	< <	< <	< <	< <	< <	< <	< <
Oregano	L i	5A	< <	107	< <	< <	49 111	0.44	< <	< <	< <	< <	11	< <	80 <
Oregano	L ii	8B	< <	747	< <	368	251 2239	0.11	< <	< <	< <	1085 93 12	< <	94	< <
Parsley Flakes	L i	5A	< <	< <	< <	< <	< <	< <	< <	< <	457	< <	< <	< <	< <
Parsley Flakes	L ii	8A	< <	< <	< <	< <	< <	< <	< <	< <	< <	< <	< <	< <	< <
Rosemary	L i	5A	< <	< <	< <	< <	9	< <	< <	< <	< <	< <	< <	< <	< <
Rosemary	L ii	8B	< <	< <	< <	< <	< <	< <	< <	< <	< <	< <	< <	< <	< <
Sage	L i	5A	< <	< <	< <	< <	35 138	0.25	< <	< <	< <	< <	< <	< <	< <
Sage	L ii	9B	< <	< <	< <	< <	45 948 288 ^h 3070 708 0.31 ⁱ 0.41	< <	< <	< <	< <	< <	< <	< <	153
Spirulina	u i	1A	< <	< <	< <	< <	< <	< <	< <	< <	< <	< <	103	< <	< <
Spirulina	u ii	1A	< <	< <	< <	< <	< <	< <	< <	< <	< <	< <	75	< <	23 <
Spirulina	u iii	3A	< <	< <	< <	< <	< <	< <	< <	< <	< <	< <	78	< <	< <
Thyme	L i	5A	< <	< <	< <	< <	34 134	0.3	< <	< <	< <	< <	< <	< <	< <
Thyme	L ii	8B	< <	< <	< <	< <	461 2296	0.2	459 139 3.3	< <	< <	941	< <	< <	194
Turmeric	R i	5A	< <	< <	< <	< <	< <	163	< <	< <	< <	< <	< <	< <	< <
Turmeric	R ii	8B	< <	< <	< <	< <	< <	< <	< <	< <	< <	< <	< <	< <	< <
Wheat Grass	L i	3A	< <	< <	< <	< <	< <	< <	< <	< <	< <	< <	< <	< <	< <
Wheat Grass	L ii	4A	< <	< <	< <	< <	606 ⁱ 262 ⁱ 4842 1884 0.14 0.14	< <	< <	< <	< <	< <	< <	< <	< <

^a Median fluorescence intensity (MFI), all samples extracted in triplicate and the average MFI presented after subtraction of the buffer background (S0)

^b Plant part; *F* flower, *L* leaf, *R* root, *S* stem, *L/S* mixture of leaves and stem, *u* not specified

^c Numbers (i, ii, iii, iv) to distinguish between different suppliers of the same sample

^d Experiment number associated with each sample analysis. 1A–5A entailed reagents from the same lot using peanut calibration standards at 15–525 ng/mL. 6B–17B from a separate lot which included peanut calibration standards at 1.5–52.5 ng/mL

^e Primary antigenic target (e.g., almond) and associated bead set(s) (e.g., -12 and -13) and ratio between MFI responses of complementary bead sets (e.g., gluten-27/gluten-28). Ratios between identically diluted samples for comparisons to reference materials at comparable MFI. Crust stands for Crustacean

^f “<” indicates a response below the lower limit of quantitation (S1). “<<” indicates a response below background (S0) plus 3X standard deviation

^g MFI of extracts diluted tenfold following centrifugation (yellow highlight)

^h MFI of extracts diluted 100-fold following centrifugation (orange highlight)

ⁱ MFI of extracted wheat grass samples diluted after centrifugation either twofold (gray-yellow highlight) or tenfold (yellow highlight) extracts diluted tenfold following centrifugation applied to plate 4, plate 3 samples not diluted (dark yellow highlight)

uniquely different from the ratios displayed by coconut, betel nut, and saw palmetto.

Betel nut Betel nut (Areca nut) is popular in many Asian countries and often chewed (e.g., betel quid) despite its being linked with various adverse health effects [39–41]. Betel nut in the xMAP FADA displayed exceptional cross-reactivity with coconut-20 and -21, walnut-47 and -48, macadamia-33, and hazelnut-29. The cross-reactivity of the betel nut samples with the coconut antibodies required an additional 1000-fold dilution following centrifugation to generate MFI responses within the dynamic range of the assay. The coconut-20/coconut-21 ratios generated by the two betel nut samples analyzed using reagents from two different lots (1A and 7B) were 18

and 15. In contrast, the calibration standard (S4) that generated comparable MFI with coconut-20, ran alongside the two betel nut samples displayed ratios of 49 and 55. This considerable difference in antigenicity toward the two bead sets by the betel nut samples and the calibration standards may be due to either extensive modification of one or multiple epitopes in undeclared coconut present in the samples, as may occur during food processing, or the presence of homologous cross-reactive proteins. Since the betel nuts were not processed, the differences in the bead set ratios rule-out the presence of coconut and indicate the presence of 0.4% cross-reactive homologs (interpolated from Fig. 1c, f and corrected for dilution). This is not surprising since betel nuts and coconuts are both members of the Arecaceae family.

Table 4 MFI of reduced-denatured extracts of fruit and seed samples

	No ^a	Exp. ^b	Egg ^c	Milk			Peanut	Gluten
			65	66	67	66/67	72	73
Acai	i	13B	<< ^d	<<	<<	—	<<	<<
Acai	ii	13B	<<	< ^{b, d}	<<	—	<<	<<
African mango	i	16B	<<	<<	<<	—	<	<
African mango	ii	16B	360	<	<	—	455	<
Allspice	i	12B	3171	2888	2770	1.0	2778	<
Allspice	ii	15B	2343	1792	1650	1.1	1922	<
Amchur	i	16B	<<	<<	<<	—	<<	<
Amchur	ii	16B	978	666	780	0.85	612	<
Anise seed	i	12B	<<	<<	<<	—	<<	<
Anise seed	ii	15B	<<	<<	<	—	<<	<
Betel nut	i	14B	<<	<<	<<	—	<<	<<
Betel nut	ii	14B	<<	<<	<<	—	<<	<<
Black pepper	i	12B	<	<<	<	—	<	<
Black pepper	ii	15B	<	<<	<<	—	<	<
Blueberry (freeze dried)	i	14B	<<	<<	<<	—	<	<<
Blueberry extract	i	13B	<<	<<	<<	—	<<	<<
Caraway seed	i	12B	<<	<<	<	—	<	<
Caraway seed	ii	15B	<<	<<	<<	—	<<	<
Cardamom	i	15B	<<	<<	<<	—	<<	<<
Cardamom	ii	15B	<	<<	<<	—	2	<
Cayenne, red pepper	i	12B	<<	<<	<<	—	<<	<
Chili cayenne	ii	15B	<	<<	<<	—	263	<
Chipotle chili pepper	iii	12B	<	<<	<	—	135	<
Cranberry	ii	13B	<<	<<	<<	—	<<	< ^b
Cranberry	iii	13B	<<	<<	<<	—	<<	<<
Cranberry	iv	14B	<<	<<	<<	—	<<	<<
Cranberry	v	14B	<<	<<	<<	—	<<	<<
Cranberry	vi	13B	<<	<<	<<	—	<<	<<
Cumin	i	12B	<	<<	<	—	156	<
Cumin	ii	15B	<<	<<	<<	—	<<	<
Fennel seed	i	12B	<<	<<	<<	—	<<	<
Fennel seed	ii	15B	<<	<<	<<	—	<<	<
Grapeseed extract	i	16B	<<	<<	<<	—	<<	<<
Grapeseed extract	ii	16B	<<	<<	<<	—	<<	<<
Grapeseed extract	iii	16B	<	<<	<<	—	<<	<
Mace	i	12B	<<	<<	<<	—	<<	<
Mace	ii	15B	<<	<<	<<	—	<<	<
Mustard	i	12B	<<	<<	<<	—	<	<
Mustard	ii	15B	<	<<	<<	—	720	<
Nutmeg	i	14B	<<	<<	<<	—	<<	<<
Poppy seed	i	12B	1853	1259	1358	0.93	1837	<
Poppy seed	ii	15B	339	<<	<<	—	734	<
Saw palmetto	i	14B	<<	1885	<<	—	<<	<<
Saw palmetto	ii	14B	<<	<<	<<	—	<<	<<
Saw palmetto	iii	13B	<<	< ^b	<<	—	<<	< ^b
Sesame seed	i	12B	1431	566	<	—	1512	<
Sesame seed	ii	15B	652	319	<	—	784	<

Table 4 (continued)

	No ^a	Exp. ^b	Egg ^c	Milk			Peanut	Gluten
			65	66	67	66/67	72	73
White pepper	i	12B	<<	<<	<<	–	<	<
White pepper	ii	15B	<	<<	<<	–	<	<

Median fluorescence intensity (MFI), all samples extracted in triplicate and average MFI presented after subtraction of SDS/β-mercaptoethanol diluent background (S0)

^a Numbers (i, ii, iii, iv, v, vi) to distinguish between different suppliers of the same sample

^b Experiment number associated with sample analysis. 6B–16B were from a lot which included peanut calibration standards at 1.5–52.5 ng/mL. For 13B, the two lowest calibration standards backfitted outside 85–115% and were therefore omitted, making S3 the lower limit of quantitation for data derived from 13B

^c Primary antigen and associated bead set(s) (e.g., -65). Ratios between complementary bead sets (e.g., milk-66/milk-67) for comparisons to reference materials at comparable MFI

^d “<” indicates a response below the lower limit of quantitation (S1). “<<” indicates a response below background (S0) plus 3X standard deviation

Betel nut also displayed cross-reactivity with walnut (family Juglandaceae) and macadamia (family Proteaceae) antibodies that did not fit either allergen being present. Specifically, Betel nut-i and Betel nut-ii displayed walnut-47/walnut-48 ratios of 0.8 and 1.1 while the comparable calibration standards were 4.5 and 3.7, respectively. An MFI of 15,000 with macadamia-33 and no measurable MFI with macadamia-34 is inconsistent with the macadamia-33/macadamia-34 ratio of 5 established using comparable calibration standards which would have resulted in a measurable MFI of 3000 with macadamia-34. The conclusion that betel nut cross-reacts with walnut-47 and macadamia-33 antibodies (0.001 and 0.12% for betel nut-i; 0.0008 and 0.01% for betel nut-ii; Table 2 and Fig. 1) is consistent with coconut IgE studies. Specifically, though clinical studies did not observe a correlation between coconut and tree nut allergies [42], the IgE from individuals allergic to coconut strongly recognized walnut [43] and serum-specific IgE (sIgE) cosensitization patterns indicated a strong recognition by coconut IgEs of macadamia [44].

Unexpected was the cross-reactivity between betel nut-i and betel nut-ii with hazelnut-29 at 0.0015% (MFI 5700) and 0.003% (MFI 3600), respectively, and no cross-reactivity with hazelnut-30 (MFI < 130). Whether the quantitative differences between betel nut-i, a pure seed control, and betel nut-ii, a pre-ground mixture, represent production or plant-based differences requires further research.

Saw palmetto A botanical that displayed exceptional cross-reactivity was Saw Palmetto. Saw Palmetto, like betel nut and acai, is a member of the palm tree family Arecaceae and could be distinguished from the other members based on its cross-reactivity with both cashew antibodies. Three saw palmetto samples were examined and all three generated similar MFI responses despite i being derived from a berry control. Specifically, the three saw palmetto samples generated

responses with coconut-20, coconut-21 (ratios 11, 9, 5; average 8.3), cashew-18, cashew-19 (ratios 0.9, 1.0, 1.1; average 1.0), walnut-47, walnut 48 (ratios 0.8, 2.2, 0.4; average 1.1) that yielded ratios inconsistent with the calibration standards which ranged from 42–56, 1.8–3.1, and 3.6–4.6 for the three allergens, respectively. Only the hazelnut-29 and hazelnut-30 bead sets generated ratios (3.9, 5.8, 3.8; average 4.5) similar to the ratio observed with the comparable calibration standard (S6 ratio of 4.4), which if due to the presence of hazelnut would indicate 6 ppm hazelnut (0.0006%). Saw palmetto also consistently displayed MFI of 2200 with Brazil nut-14 and 3800 with macadamia-33 which would be comparable to cross-reactivities of 0.00036 and 0.00045%.

Amchur Amchur, also spelled amchoor or aamchur, is made from dried green mango and often used in Indian foods. Amchur belongs to the cashew family, Anacardiaceae in the order of Sapindales. It was therefore not surprising that amchur-i displayed high cross-reactivity with cashew-18 and cashew-19 with a ratio of 3, close to the 2.5 interpolated from the two calibration standards bracketing the response with cashew-18. The similarity to cashew is supported by the decrease in the ratio to 1.5 for the undiluted samples, which was also comparable to the extrapolated ratio from the calibration standards. The lack of cross-reactivity by amchur-ii with cashew-19 is not understood since at a ratio of 3, a response at least twice the magnitude of the limit of quantitation (S1, 200 MFI) should have been observed. As observed with cashews, slight cross-reactivity was also observed with hazelnut, pistachio, and walnut antibodies [26, 28]. Unexpected were the cross-reactivity with gluten and peanut. Amchur-i and amchur-ii displayed with gluten-28 cross-reactivity of 0.0005 and 0.00006% and gluten-27/gluten-28 ratios of 0.17 and 0.18, respectively; comparable to the 0.12 and 0.14 of the comparable calibration standards. Similarly, amchur-i and amchur-ii displayed cross-reactivity of 0.00012 and

Table 5 MFI of reduced-denatured extracts of flower, leaf, root, and stem samples

	No. ^a	Exp. ^b	Egg ^c	Milk			Peanut	Gluten ^e
			65	66	67	66/67	72	73
Bay leaves	i	12B	<< ^d	<<	<<	—	<<	<<
Bay leaves	ii	15B	<<	<<	<<	—	<<	<<
Bee pollen	i	13B ^c	<<	<<	< ^{b,d}	—	<<	<
Bee pollen	ii	13B	712	2611	2020	1.3	668	<
Black cohosh	i	14B	<	<<	<<	—	<	<<
Black cohosh	ii	13B	1235	1009	<	—	595	<
Cinnamon	i	14B	<	<<	<<	—	<<	<<
Cinnamon	ii	13B	560	<	<	—	213	<
Cinnamon	iii	14B	497	<	<<	—	162	<
Cloves	i	12B	3241	2899	3265	0.9	2657	<
Dill Weed	i	12B	1521	3793	2969	1.3	1479	<
Eleuthero root	i	14B	2104	914	1017	0.9	884	<
Ginger	i	12B	<<	<<	<<	—	<	<<
Ginger	ii	15B	400	<<	<	—	126	<
Ginseng, Am.	i	13B	<<	<<	<<	—	<<	<
Ginseng, Panax	ii	13B	<<	<<	<<	—	<<	<<
Hoodia gordonii	i	13B	<	<	<<	—	<	<
Hoodia gordonii	ii	14B	<<	<<	<<	—	<<	<<
Hoodia gordonii	iii	13B	<	<	<	—	<	<
Hoodia gordonii	iv	13B	338	912	2494	0.37	315	<
Indian snakeroot	i	14B	<<	<<	<<	—	<<	<<
Indian snakeroot	ii	14B	<<	<	<<	—	<<	5186
Kelp	i	13B	3480	2866	3361	0.9	2663	<
Kelp	ii	13B	3376	2717	3225	0.8	2524	<
Marjoram	i	15B	3100	1634	1722	0.9	1963	<
Marjoram	ii	12B	2272	982	1239	0.8	1493	<
Oregano	i	12B	1847	612	<	—	1440	<
Oregano	ii	15B	1976	952	1518	0.6	1089	<
Parsley flakes	i	12B	<<	<<	<<	—	<<	<<
Parsley flakes	ii	15B	<<	<<	<<	—	<<	<<
Rosemary	i	12B	2821	1639	1991	0.8	1705	<
Rosemary	ii	15B	790	419	<	—	424	<
Sage	i	14B	5436	4125	3620	1.1	4161	3804
Sage	ii	16B	5153	3931	3940	1.0	3949	3347
Spirulina	i	13B	<<	<<	<<	—	<<	<<
Spirulina	ii	13B	<<	<<	<<	—	<	<<
Spirulina	iii	13B	<<	<<	<<	—	<	<<
Thyme	i	12B	2152	918	1244	0.7	1247	<
Thyme	ii	16B	4144	2610	2801	0.9	2890	1519
Turmeric	i	12B	624	342	1125	0.3	214	<
Turmeric	ii	15B	<<	<<	<<	—	<<	<
Wheat grass	i	13B	<<	<<	<<	—	<<	<<
Wheat grass	ii	13B	<	<<	<	—	<	10,721

Median fluorescence intensity (MFI), all samples extracted in triplicate and average MFI presented after subtraction of SDS/β-mercaptoethanol Diluent background (S0)

^a Numbers (i, ii, iii, iv) to distinguish between different suppliers of the same sample

^b Experiment number associated with sample analysis. 6B–16B were from a lot which included peanut calibration standards at 1.5–52.5 ng/mL. For 13B, the two lowest calibration standards backfitted outside 85–115% and were therefore omitted, making S3 the lower limit of quantitation for data derived from 13B

^c Primary antigen and associated bead set(s) (e.g., -65). Ratios between complementary bead sets (e.g., milk-66/milk-67) for comparisons to reference materials at comparable MFI

^d “<” indicates a response below the lower limit of quantitation (S1). “<<” indicates a response below background (S0) plus 3X standard deviation

^e The reduced cross-reactivity of gluten-73 may reflect its high S0 relative to the other bead sets

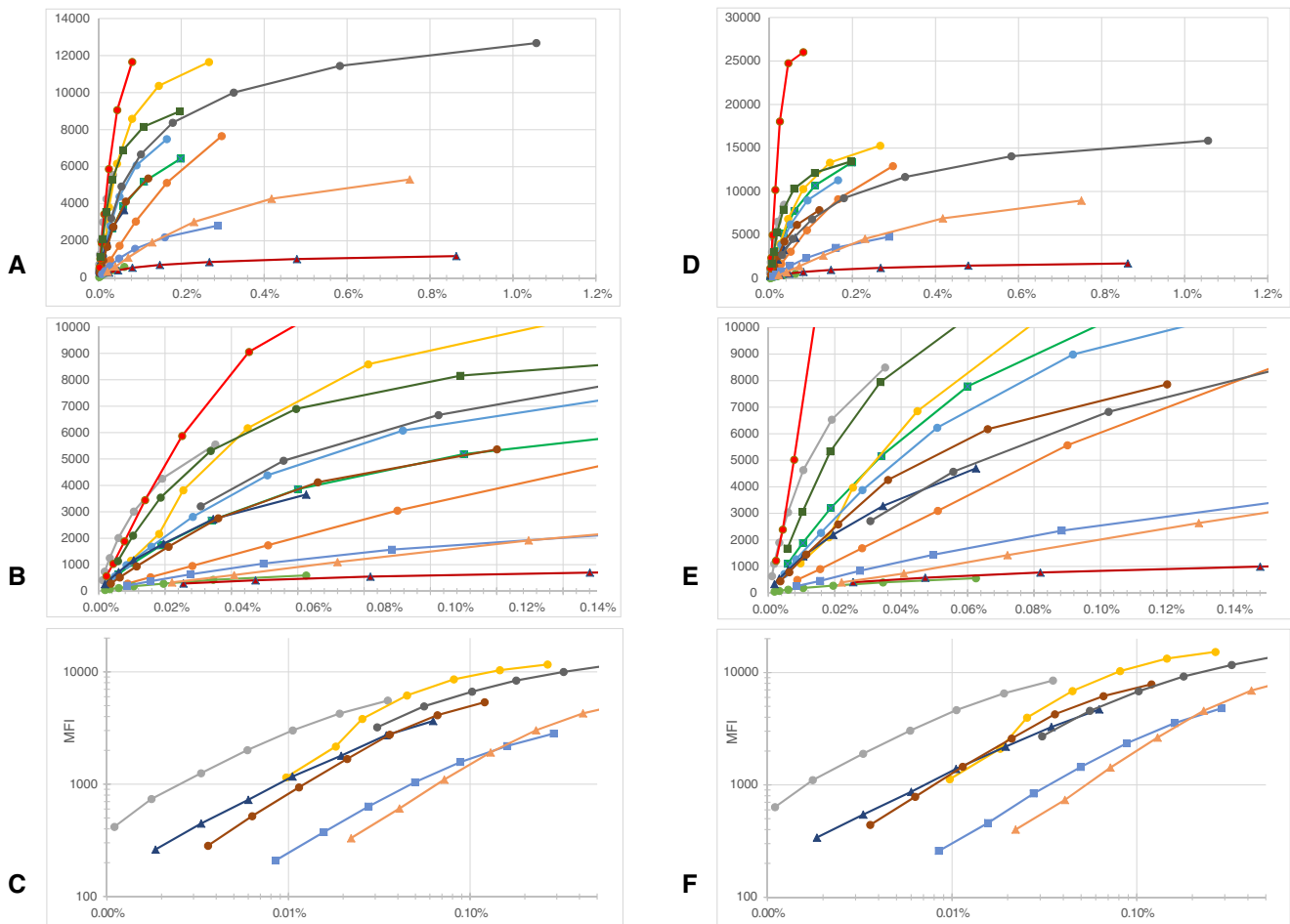


Fig. 1 Relationship between MFI and percent cross reactivity. The observed MFI of the various calibration standards (S1–S7) plotted against the calculated mass percentage if the calibrant was present in a sample of 10 mg (the same as if a 1 g sample was diluted 100-fold). Conversion from ng protein to ng mass based on protein content reported in the USDA National Nutrient Database and Certificates of Analysis provided for Standard Reference Materials [34–36]. The protein content conversion factors are based on total protein (nitrogen) content, as determined by Dumas or Kjeldahl analysis, while the calibration standards are based on soluble protein and BCA analyses. As such, the calculated percent by mass is a lower limit. Panels **a–c** are the data derived from the average MFI generated by the calibration standards

analyzed with lot A reagents (experiments 1A–4A, each in triplicate per experiment) plotted on three scales to facilitate interpolation. Similarly, panels **d–f** are the average MFI generated by lot B reagents (experiments 6B–11B). Plotted are the results for the first of each antibody pair except gluten for which both antibodies are represented. **almond-12**; **Brazil nut-14**; **cashew-18**; **coconut-20**; **egg-25**; **gluten-27**; **gluten-28**; **hazelnut-29**; **macadamia-33**; **peanut-37**; **pine nut**; **pistachio-43**; **soy-45**; **walnut-47**. Panels **c, d** depict only cashew-18, coconut-20, gluten-28, hazelnut-29, macadamia-33, soy-45, and walnut-47 (symbols as above)

0.00005% with peanut-37 and ratios of 1.3 and 1.0 which were indistinguishable from the ratios determined with the calibration standards. Whether these cross-reactivity measurements can be used to reliably detect and distinguish amchur from cashew, peanut, or other allergens requires the testing of additional amchur samples, preferably certified reference materials. However, if the cross-reactivities were due to undeclared gluten and peanut, the concentrations would be only 5 and 0.6 ppm gluten and 1.2 and 0.5 ppm peanut in amchur-i and amchur-ii, respectively.

The analysis of reduced-denatured extracts prepared from amchur-i and amchur-ii are presented in Table 4. Amchur-i displayed no significant cross-reactivity with any of the

antibodies in the reduced-denatured aspect of the assay. The presence of undeclared gluten and peanut in the amchur samples is unlikely considering the reduced-denatured results; amchur-i displayed no cross-reactivity with either gluten-73 or peanut-72 despite displaying responses of 3400, 12,000, and 9100 with the buffered-detergent bead sets gluten-28, peanut-37, and peanut-38, respectively. Amchur-ii displayed a similar pattern, significant MFI with gluten-28 (690), peanut-37 (2900), and peanut-38 (2900) but no measurable response with gluten-73 and low cross-reactivity with peanut-72 (610). Indeed, the comparable low cross-reactivities exhibited by amchur-ii with egg-65, milk-66, and milk-67 indicate that the low response with peanut-72 may be

due to non-specific binding with the negative response associated with gluten-73 reflecting the considerably large MFI that defines the lower limit of the measurement (S1, 2850) for gluten-73 (background, S0 is 75 ± 2); the lower limits for measurement (S1) for egg-65, milk-66, milk-67, and peanut-72 were 114, 130, 560, and 63, respectively.

Reduced-denatured specificity The pattern of (low) cross-reactivity of comparable intensity with the egg-65, milk-66, -67, and peanut-72 but no measurable response with gluten-73 for several dietary supplements and spices as listed in Tables 4 and 5 may reflect low stringency by the 100-fold diluted SDS/ β -mercaptoethanol extracts combined with an exceptionally high limit of measurement (S1) for gluten-73. Consistent with this explanation are the milk-66/milk-67 ratios for all but two of the dietary supplements and spices listed in Tables 4 and 5. Ideally, non-specific binding should display no preference with bead set and thus a ratio of 1, provided the conjugation process did not substantially differentially change the non-specific binding properties of the bead sets. The calibration standards for milk displayed an average milk-66/milk-67 ratio of 0.31 ± 0.09 ($n = 33$) with values ranging from 0.18 to 0.50 (ESM Table S2b). In contrast, the milk-66/milk-67 ratios in Tables 4 and 5 averaged 0.98 ± 0.11 ($n = 4$) and 0.85 ± 0.27 ($n = 16$), respectively, with the result for Table 5 improving to 0.93 ± 0.19 ($n = 14$) upon removing the two results that were within the range defined by the calibration standards. Such non-specific cross-reactivity was not observed with the buffered-detergent extracts due to the use of UD Buffer, specifically designed to minimize lectin and other non-specific binding [45]. The occurrence of this non-specific binding associated with the analysis of pure plant products is not expected to be a problem with complex food samples which mimic the blocking potential of UD buffer and thereby improve the stringency of the assay. Further research into the use of SDS/ β -mercaptoethanol extraction and the need for subsequent dilution to facilitate antibody binding may help improve this problem.

African mango African Mango is a member of the order Malpighiales and not related to either the commonly consumed mangoes or amchur (order Sapindales). It was therefore not surprising that African Mango did not display cross-reactivity similar to unrelated amchur. The only cross-reactivities observed by African Mango-i were with gluten-28 (MFI 390, 0.00002%) and pistachio-43 (MFI 2700, 0.00007%), none by African Mango-ii.

Cardamom Cardamom, also known as cardamum, is a spice that is rarely associated with anaphylaxis and has been associated with positive health effects in-part due to its antioxidant and free-radical scavenging ability [46–48]. In the xMAP FADA, cardamom, prepared from two sources i-whole seeds

and ii-pre-ground were compared. Cardamom-i and Cardamom-ii displayed similar properties, cross-reactivity with coconut (0.01 and 0.002% with coconut-20) and walnut (0.0007% for both samples with walnut-47) that was not due to either the presence of coconut or walnut; the average coconut-20/coconut-21 ratio of 3.8 and walnut-47/walnut-48 ratio of 1.9, both differed from the 44–55 and 4.6 ratios of the calibration standards, respectively. The cross-reactivities with gluten-28 (0.00003%, 0.00007%), hazelnut-29 (0.00016%), macadamia-33 (0.0016%, 0.0012%), and pine nut-39 (0.0002%) were significantly greater than the limits of quantitation but, other than macadamia-33, relatively inconsequential.

Cranberry Cases of food allergies due to cranberry (*Vaccinium macrocarpon*) are rare with the only detailed study of a related food allergy being a recent report describing a case due to the closely related lingonberry (*Vaccinium vitis-idaea* L.) in a 25-year-old woman with no history of food allergies [49]. Six cranberry samples were examined using the xMAP FADA. Cranberry-i, -ii, and -iii were control reference samples while iv, v, and vi were dietary supplement products which contained various additives as listed in ESM Table S1. Cranberry-i, fresh raw cranberry and laboratory freeze-dried cranberries (Cranberry-iii), did not display any measurable cross-reactivity with any of the antibodies in the xMAP FADA. In contrast, all four industrial products, Cranberry-ii, Cranberry-iv, Cranberry-v, and Cranberry-vi, displayed cross-reactivity. It is not obvious why industrial processing resulted in the observed cross-reactivities, but the magnitude of the cross-reactivities were small ($< 0.01\%$, typically $< 0.002\%$) and only the cross-reactivity by Cranberry-iv and Cranberry-v with the cashew antibodies (bead sets -18 and -19) were characteristic of cashew. Specifically, all four industrially produced cranberry samples (Cranberry-ii, Cranberry-iv, Cranberry-v, and Cranberry-vi) displayed cross-reactivity with both cashew antibodies (ratios 0.9, 3, 3.4, 1.3; calibration stds 2.5, 3.1, 3.1, 2.9), both hazelnut antibodies (ratios 1.9–2.8, calibration stds 3.9–4.2), both walnut antibodies (ratios 0.3–1.5, calibration stds 3.6–4.6), pistachio-44, and Brazil nut-14. Cranberry-ii also displayed cross-reactivity with both coconut antibodies (ratio 20, calibration std. 47) both macadamia antibodies (ratio 7, calibration std. 14), almond-13, Brazil nut-15 (ratio 16, calibration std. 3.6), and gluten-27. Cranberry-vi displayed low cross-reactivity with almond-13, Brazil nut-15 (ratio 24, calibration std. 3.8), coconut-21, gluten-27, macadamia-34, and soy-45.

Blueberry Blueberry belongs to the same genus as cranberry, *Vaccinium*. Like cranberry allergies, blueberry allergies are rare with data implicating a 10 kDa lipid transfer protein (LTP) as a major allergen [50, 51]. It was therefore interesting to compare the cross-reactivity patterns to those observed with

the analogous cranberry samples. Blueberry-i and Blueberry-ii were commercial products. Blueberry-i, like cranberry-ii, consisted of commercially freeze-dried berries. Blueberry-ii, like cranberry-vi, contained added gelatin, silica, and magnesium stearate with the only notable difference being the addition of rice powder. Blueberry-i and blueberry-ii generated similar profiles to each other with the various antibodies and were similar to the profiles exhibited by cranberry-ii and cranberry-vi with the minor additions of cross-reactivity with almond-12, gluten-28, and pine nut-39. The cross-reactivities exhibited by the blueberry samples were typically < 0.0002%, except walnut-47 at 0.0007%. Whether fresh blueberries and laboratory-freeze-dried blueberries display the same lack of cross-reactivity as the cranberry (i and iii) samples needs to be explored along with whether in the commercial manufacturing process other agents were added that did not meet the criteria for listing on the ingredient label but could explain the low cross-reactivity pattern observed.

Chili pepper Three Cayenne red chili pepper (*Capsicum annuum* L.) products were examined and designated Cayenne red pepper-i; chili Cayenne-ii, and Chipotle chili pepper-iii. Cayenne-i and Chipotle chili-iii are produced by the same manufacturer and displayed cross-reactivity with 11 and 10 out of 25 antibodies, respectively, in the buffered-detergent component of the xMAP FADA. Chili Cayenne-ii was labeled organic and displayed cross-reactivity with 19 of the 25 antibodies. Most of the cross-reactivities observed with chili Cayenne-ii were low (< 0.001) except walnut-47 at 0.13% (ratio ≤ 0.7 , calib std. 4.8), Brazil nut at 0.006% (ratio 4.4, calib std. 4.2) and hazelnut-29 at 0.004% (ratio 1.9, calib std. 5.2); the rest were typified by cashew-18 at 0.0006%, macadamia at 0.0005%, coconut-20 at 0.0002%. The cross-reactivity with Brazil nut by chili Cayenne-ii displayed a Brazil nut-14/Brazil nut-15 ratio comparable to that of the calibration standards. To ascertain whether this was unique to this sample or a common property of Cayenne red chili pepper, the ratios generated by Cayenne-i and Chipotle chili-iii were compared. Cayenne-i and Chipotle chili-iii cross-reacted with Brazil nut-14 at 0.0002% with Brazil nut-14/Brazil nut-15 ratios of 7 and 7.3, respectively; considerably different from the ratios observed with the comparable calibration standards (4.2 and 4.5). Thus, the cross-reactivity observed with chili Cayenne-ii was unique to this specific sample. Whether this was due to the use of a different cultivar, crop, growth conditions (e.g., temperature, nutrients—"organic" versus "nonorganic"), or an effect of food processing cannot be determined from the current data.

Poppy seed Another spice/dietary supplement that displayed cross-reactivity with a majority of the antibodies in the buffered-detergent part of the xMAP FADA is poppy seed. Two poppy seed samples were examined; Poppy seed-i which

was acquired as whole seeds and ground in the laboratory and poppy seed-ii which was pre-ground by the manufacturer. Poppy seed-i cross-reacted with 16 antibodies and poppy seed-ii cross-reacted with 22 of the 25 antibodies. The cross-reactivity by the poppy seed samples, especially poppy seed-ii, was typical of non-specific binding. Indeed, though the MFI with crustacean-22 was below the limit of quantitation, it was considerably greater than what was observed with other spices and dietary supplements (e.g., 30 versus typically ≤ 5). Before over interpreting, more research is needed to determine whether the cross-reactivities were an artifact due to insufficient stringency in the binding conditions, inefficient sample extraction, or sample variability.

Sesame seed Two sesame seed samples, both prepared from whole seeds ground in the laboratory, displayed similar profiles cross-reacting with 11 of the 25 antibodies in the buffered-detergent part of the xMAP FADA. Specifically, low amounts of cross-reactivity were observed with both Brazil nut, both cashew, both hazelnut, and both walnut antibodies at 0.00019, 0.00002, 0.0001, and 0.0009% for cashew-18, hazelnut-29, and walnut-47, respectively. Additional cross-reactivity to coconut-21, gluten-27, and pistachio-44 were observed. Though the profiles were similar, the magnitudes were different, with sesame-ii usually greater.

Indian snakeroot Indian snakeroot-ii, after a 100-fold dilution, displayed a gluten-27/gluten-28 MFI ratio of 0.16, comparable to the 0.14 ratio exhibited by the calibration standards. The magnitude of the MFI response by gluten-28 of the 100-fold diluted Indian snakeroot-ii sample was 1390, which is equivalent to a cross-reactivity of 0.011% or a gluten content of 110 ppm. As previously indicated, since a voluntary "gluten-free" claim was not made on the label of the Indian snakeroot sample, the product is not subject to any of the requirements of the gluten-free regulation, including the requirement that it contain "less than 20 ppm gluten." In contrast, Indian snakeroot-i was a pure reference material obtained from NCNPR and accordingly displayed no cross-reactivity with gluten-27 and a MFI of 332 with gluten-28. If the MFI of 332 with gluten-28 was due to gluten (which is not supported by the inability to detect a measurable response with gluten-27), the maximum amount possible would be 0.2 ppm based on a cross-reactivity of 0.00002%. Indian snakeroot-ii also displayed low MFI (cross-reactivity) with 11 non-gluten antibodies. In contrast, Indian snakeroot-i displayed only additional cross-reactivity with coconut-20 and coconut-21, which was not observed with Indian snakeroot-ii. The conclusion that Indian snakeroot-ii contained gluten is supported by the reduced-denatured analyses of the Indian snakeroot samples. Indian snakeroot-i tested negative for all analytes in the reduced-denatured part of the xMAP FADA, while Indian snakeroot-ii tested positive for only gluten (MFI 5186).

Wheat grass Wheat grass from two sources was studied. Wheat grass-i was labeled with the voluntary declaration “gluten-free” and accordingly displayed no cross-reactivity with either gluten antibody or with any of the other antibodies associated with the buffered-detergent xMAP FADA. In contrast, wheat grass-ii, which did not make a gluten-free declaration, displayed cross-reactivity with gluten-27 and gluten-28, generating a 0.14 ratio comparable to the 0.16 observed with calibration standards. As supported by the ratio analysis, the gluten-28 MFI, after tenfold dilution, of 1884 is equivalent to a cross-reactivity of 0.002% or a gluten content of 20 ppm. The conclusion that wheat grass-ii contained gluten is supported by the reduced-denatured analyses of the wheat grass samples. Wheat grass-i tested negative for all analytes in the reduced-denatured part of the xMAP FADA, while wheat grass-ii tested positive for only gluten (MFI 10,721).

Sage Sage also displayed indications that gluten was present but the average MFI of sage-i was insignificant and the gluten-27/gluten-28 ratios for Sage-i and both concentrations of sage-ii (0.25, 0.31, and 0.41) were inconsistent with the ratios of the comparable calibration standards (0.14, 0.12, and 0.10). Though the two sage samples tested positive in the reduced-denatured analyses for gluten, both sage samples also tested positive for the other four analytes, indicating a possible lack of specificity/non-specific binding. A review of the analyses in Table 5 indicates a high number of samples displaying cross-reactivity with egg-65, milk-66, milk-67, and peanut-72 with some displaying no comparable positive responses with gluten-73. Gluten-73 displays a higher MFI defining the lowest limit for gluten measurement (S1, ESMTTable S2b; 25). Thus, it would be consistent with non-specific binding to not observe a response with gluten-73 until the amount of non-specific binding exceeded a threshold. Sage displayed the most non-specific binding as exemplified by the MFI responses with egg-65, milk-66, milk-67, and peanut-72; consistent with sage exceeding the higher threshold for generating a measurable response with gluten-73. Lastly, all of this is inconsequential from a health or regulatory perspective since the MFI of 3070 for sage-ii with gluten-28 (0.0003% cross-reactivity) would indicate a maximum of only 3 ppm if gluten was the cause of the measured response.

Bee pollen Bee pollen has been the focus of several studies regarding the cross-reactivity between pollen and food allergens [52–54]. Two bee pollen samples were examined. Neither displayed cross-reactivity with any of the buffered-detergent bead sets. Only bee pollen-ii displayed cross-reactivity with the reduced-detergent bead sets. However, the pattern of cross-reactivity with bead sets 65, 66, 67, 72, and not 73 was consistent with the possibility of the cross-reactivity due to non-specific binding as observed with amchur-ii and sage (see above). However, the MFI values

observed with the reduced-denatured bead sets were not perfectly characteristic of non-specific binding. The milk-66/milk-67 ratio was 1.3, significantly greater than observed with the other dietary supplements and spices (av 0.94 ± 0.17 , $n = 18$). In addition, the MFI observed with milk-66 and milk-67 were considerably greater than the MFI observed with egg-65 and peanut-72, a pattern also observed with dill weed. It would therefore be interesting to ascertain whether other “bee pollen” of different floral origins and dill weed products displayed similar properties and if cross-reactivity was displayed in other milk-specific antibody-based assays and the identity of the antigen (e.g., Western blot analysis and immuno-affinity purified mass spectral analysis of antigenic peptides).

Spirulina Spirulina is a blue green algae/cyanobacteria that is consumed as a dietary supplement due to its nutritional value [55]. A reported case of anaphylaxis attributed to spirulina ingestion has increased interest in understanding its allergenicity and any cross-reactivity with food allergens [56]. Three different spirulina products were analyzed and only a very weak cross-reactivity (75–103) was observed by all three products with pine nut-42 but no cross-reactivity with pine nut-39 or any of the other bead sets in either the buffered-detergent or the reduced-denatured parts of the assay. This was unusual inasmuch as pine nut typically displays a pine nut-39/pine nut-42 ratio of 6–7 for calibration standards at comparable intensities. As such, the lack of any measurable MFI with pine nut-39 is distinctly different from pine nut.

Miscellaneous The remaining botanicals (e.g., allspice, black pepper, caraway seed) only displayed slight cross-reactivity with few of the antibodies at levels reproducibly above the limits of quantification (S1) but typically < 1 ppm if the suspected allergen was present.

Conclusion Cosensitization across products (e.g., flowers, fruit/seed, leaves, roots, and stems) derived from different plants has been well established and observed in RAST and skin prick tests [57]. While clinical tests or biological activity-based assays are ideal approaches, this cross-reactivity is potentially detectable using antigenicity, antibody-based assays used to detect food allergens. The xMAP FADA, by virtue of its sensitivity, wide dynamic range, and ability to simultaneously detect cross-reactivity toward multiple antibodies provided a rare opportunity to study this cross-reactivity and ability to detect and distinguish between allergenic and potentially allergenic foods. Analyses of botanical products sold as dietary supplements and spices rarely displayed cross-reactivities in the xMAP FADA. Cross-reactivities, when observed, were of low intensity requiring the use of pure or almost pure samples for detection. As a result, the observed cross-reactivities were unlikely to cause analytical problems or pose a concern

when consumed in typical oral portions. Central to these comparisons was the use of antibodies with different affinity constants and specificity. The goal of using only highly specific, high affinity antibodies (as used in ELISAs) is potentially counter-productive in the xMAP FADA where cross-reactivity enables multi-antibody profiling and the detection of novel analytes. Ultimately, phylogenetic correlations should be possible when the number and type of reference products examined are expanded.

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Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

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