

Cross-Reactivity of Chili Peppers (*Capsicum* sp.) with the xMAP Food Allergen Detection Assay

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ABSTRACT: The xMAP food allergen detection assay (xMAP FADA) is an advanced multiplex immunoassay with multiple antibodies for each of 15 target food allergens and gluten, allowing for signal confirmation and antigenic profiling to occur in a single analysis. Botanicals used as spices are complex matrices for allergen analysis because they can exhibit inherent cross-reactivity with antibodies employed by the assays. Preliminary examinations of botanicals revealed chili peppers to have notably high levels of cross-reactivity with Brazil nut and hazelnut antibody bead sets in the xMAP FADA. This in-depth investigation of 29 pre-ground and whole chili peppers indicated Brazil nut and hazelnut cross-reactivity to be consistent among most members of genus *Capsicum*, although cross-reactive signals generated by chili peppers were distinguishable from signals indicative of target allergen detection. Using the requirements that complementary antibodies used in the assay generated positive responses and that the various secondary end points were characteristic of the target analytes, xMAP FADA reactivity to chilis of the genus *Capsicum* was categorized as cross-reactivity instead of confirmed detection of target allergenic foods.

KEYWORDS: food allergen, cross-reactivity, chili peppers, immunoassay

INTRODUCTION

The Food Allergen Labeling and Consumer Protection Act (FALCPA) of 2004 established new standards for the labeling of foods that contain or potentially contain food allergens in the United States.¹ As a result of FALCPA regulations, food products containing any of the eight major allergens must be clearly labeled.¹ The eight major allergens requiring labeling in the United States include milk, egg, soy, wheat, fish, peanut, tree nuts, and crustacean shellfish. At the time of enactment, the eight major food allergens defined in FALCPA represented 90% of all food allergic reactions. Additional legislation passed in 2021 included sesame as the ninth major food allergen requiring clear labeling on packaged foods.²

Independent laboratories and regulatory agencies have extensively relied on enzyme-linked immunosorbent assays (ELISAs) for detecting and quantifying food allergen residues on equipment and in foods. Although ELISAs are sensitive and specific, they also are limited to analysis of a single target allergen. As such, differentiating the signal generated by the target antigen or by cross-reactive proteins is not feasible. The xMAP food allergen detection assay (xMAP FADA) is a potential solution to challenges posed by single-analyte ELISAs. In addition to including at least two sandwich-based antibody assays for every target antigen, except for crustacean, it is currently capable of simultaneously detecting 15 food allergens plus gluten.^{3,4} Furthermore, the assay has two complementary extraction protocols to address specific needs. The buffered detergent extraction protocol employs UD (105 mM sodium phosphate, 75 mM sodium chloride, 2.5% non-fat dry milk, and 0.05% Tween 20) or PBST (phosphate-buffered saline with Tween 20) buffer, while the reduced denatured extraction protocol uses a sodium dodecyl

sulfate (SDS)- and β -mercaptoethanol-based buffer.⁵ The signal is generated upon streptavidin–phycoerythrin binding to the biotinylated detector antibody complexed with target antigen and magnetic-bead conjugated capture antibody. The xMAP assay has a modular plug-and-play capability, so that the assay may be tailored to meet the needs of the end-user, including expansion of the antibody repertoire, as was demonstrated with the recent addition of sesame bead sets.⁶ The customizable setup of the xMAP FADA gives the advantage over traditional ELISAs that the assay may be used to analyze samples for multiple food allergens or even unknown allergens;⁷ traditional ELISAs target only a single analyte and are generally used in applications where the identity of the potential contaminating antigen is known. Because the xMAP FADA is a multiplex assay, it greatly reduces the time, labor, and subsequent financial cost over traditional ELISA methods.

Botanicals, including spices that are ground prior to receipt by the end user, are sometimes subject to adulteration or unintentional cross-contact, as illustrated by previous work detailing the presence of undeclared food allergens in samples of cumin and garlic powders.^{8,9} Whole botanicals are thought to be less prone to cross-contact than commercially ground or otherwise processed botanicals.¹⁰ Beyond issues of cross-contact or adulteration, botanicals are sometimes inherently

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cross-reactive with major food allergens.¹¹ Cross-reactivity as a result of the presence of homologous proteins in botanicals can sometimes be the result of closeness of species.^{11,12} Through the use of secondary end points combined with the built-in redundancy of the assay, the xMAP FADA is far better suited than traditional ELISAs to assess cross-reactivity of samples containing homologous proteins and distinguish these signals from the detection of target antigen.

Documented chili allergens include Bet v 1 homologues, a profilin, and thaumatin-like pathogenesis-related protein P23. Cap a 1 and Cap a 2 are the major allergens derived from bell peppers (*Capsicum annuum* L.). Cap a 2 shares 77% sequence homology with Bet v 2, a birch pollen profilin.¹³ Interestingly, profilin Cor a 2 from hazelnut shares significant (77%) sequence homology with Bet v 2.¹³ Bet v 1 homologues and profilins present in chili peppers are largely destroyed through the drying and grinding processes used to prepare chili powders.^{14,15} However, the P23 thaumatin-like allergen, Cap a 1, is resistant to heat and proteolytic processing.¹⁶

The research presented here builds from research performed by Pedersen et al.,¹¹ in which researchers analyzed highly concentrated botanicals for cross-reactivity by xMAP FADA. Pedersen et al. drew attention to chili peppers, specifically cayenne red pepper, as a consistent and significant source of cross-reactivity in xMAP analyses with Brazil nut 14 and 15, hazelnut 29 and 30, and walnut 47 and 48 antibody bead sets. One sample of organic chili cayenne exhibited additional low-level cross-reactivity with several bead sets, although the prominent cross-reactive bead sets were present consistently across the cayenne samples evaluated.

In this research, 29 pre-ground and whole chili peppers sold in the U.S.A. were assessed for cross-reactivity with major food allergens detected by xMAP FADA. Cross-reactivities to chili peppers were most pronounced to Brazil nut 14 and 15, cashew 18 and 19, coconut 21, hazelnut 29 and 30, and walnut 48. Nearly all signals observed were distinguished as cross-reactivity using complementary antibody ratios and antigenic profiling. The approach of this research is not a survey for undeclared food allergens but rather an evaluation of the applicability of the xMAP FADA to detect food allergens in products containing these chili peppers.

MATERIALS AND METHODS

Reagents and xMAP Kits. Phosphate-buffered saline (PBS) and Tween 20 were purchased from Sigma-Aldrich, Inc. (St. Louis, MO, U.S.A.). Non-fat dry milk (BD Difco skim milk powder) was purchased from Fisher Scientific (Pittsburgh, PA, U.S.A.).

xMAP FADA kits were developed by United States Food and Drug Administration (FDA)'s Center for Food Safety and Applied Nutrition in collaboration with Radix BioSolutions (Georgetown, TX, U.S.A.) with antibodies produced by Morinaga Institute of Biological Science, Inc. (MioBS, Yokohama, Japan), IEH Laboratories and Consulting Group (Lake Forest Park, WA, U.S.A.), Elution Technologies (Colchester, VT, U.S.A.), and BioFront Technologies (Tallahassee, FL, U.S.A.).

Chili Peppers and Powders. Pre-ground (PG) chili peppers, including Kashmiri, California Sweet Paprika, Anaheim, Hatch New Mexico Red, Poblano, Guajillo, Jalapeno, Ancho, Chipotle Morita, Cayenne, Aji Amarillo, Thai Bird, Habanero, and Ghost Chili powders, were obtained from Spices, Inc. (Elysburg, PA, U.S.A.) and Mountain Rose Herbs (Eugene, OR, U.S.A.). Whole dried chilis were also obtained from Spices, Inc. and included Thai Bird, Guajillo, Ancho, Habanero, Aji Amarillo, Hatch New Mexico, Chilaca, Ghost, Anaheim, Chipotle Morita, Birdseye, and Mulato chilis. Whole dried chilis (LG) were ground to powder in the laboratory using a Kitchen-

Aid Coffee and Spice Grinder (St. Joseph, MI, U.S.A.). Between each sample, the grinder was disassembled and thoroughly washed and dried before reuse.

Except for California Sweet Paprika, none of the other pre-ground chili peppers listed any other ingredients in the formulation. California Sweet Paprika was described to contain anticaking and antioxidantizing agents.

xMAP FADA. Chili powders were analyzed by xMAP FADA using the UD buffered detergent protocol as previously described.¹¹ Briefly, 40 mL of UD buffer (105 mM sodium phosphate, 75 mM sodium chloride, 2.5% non-fat dry milk, and 0.05% Tween 20) was added to 1 g of chili powder in triplicate. Samples were extracted at room temperature for 2 h with shaking. The 10-fold, 100-fold, and 1000-fold dilutions were performed, corresponding to 400-, 4000-, and 40 000-fold final dilutions, including the 40-fold dilution associated with sample extraction.

Concentrated reference material was included in each kit by the manufacturer as a 200× solution. A calibration curve was developed by an initial 200× dilution of the reference material with UD buffer, followed by subsequent 1.8-fold dilutions to create calibration standards 1–7 (S1–S7). Reference material was diluted in triplicate series. The concentrations of calibration standards for each analyte are published elsewhere.¹² After dilution, each calibration standard and sample were added to the assay plate and mixed 1:1 (v/v) with the covalently bound magnetic bead-capture antibody cocktail and incubated at 37 °C in the dark for 30 min with continuous shaking. The capture bead cocktail consisted of 30 bead sets for the detection of 14 food allergens plus gluten: almond (12 and 13), Brazil nut (14 and 15), cashew (18 and 19), coconut (20 and 21), crustacean (22), egg (25 and 26), gluten (27 and 28), hazelnut (29 and 30), macadamia nut (33 and 34), peanut (37 and 38), pine nut (39 and 42), pistachio (43 and 44), sesame (66, 72, and 73), soy (45 and 46), and walnut (47 and 48). Because the UD buffer used to extract the samples contained non-fat dry milk, the milk bead sets (35 and 36) were not included in the analyses.

The detector bead cocktail consisted of biotinylated antibodies with specificity to the assayed antigens. A total of 50 µL of detector cocktail was added to each test well on the assay plate to bind antigen-capture antibody complexes and was incubated for 30 min at 37 °C with shaking. The streptavidin–phycoerythrin (SA–PE) reporter molecule was then added to the well, where the signal was generated upon binding the biotinylated detection antibody during the incubation (37 °C for 15 min). Between each step, washing steps were performed using a strong magnet held under the assay plate. Magnetic beads remained in the well during washing, while unbound material was washed away.

The kit-provided calibration standards are a composite of individually extracted representative materials that provide a useful first comparison of complementary antibody ratio consistency with evaluated samples. Samples of pure raw target analyte extracted and analyzed alongside unknown samples provide an improved comparison for determining consistency of complementary antibody ratios and antigenic profiles. In addition to chili powders, pure ground raw Brazil nut and hazelnut were evaluated by xMAP FADA to facilitate comparisons of reactivity. A total of 0.3 g of each respective ground tree nut was weighed into a 50 mL conical tube. A total of 30 mL of PBST was added to each tube and vortexed vigorously to suspend and distribute particles, creating a 10 000 ppm tree nut solution. The tree nut solutions were placed on a rocker for 2 h at a maximum speed at room temperature. After incubation, the stock solutions were vortexed thoroughly and diluted. A total of 1 mL of each dilution in triplicate was extracted according to the UD buffered detergent protocol. After extraction, additional dilutions were carried out to ensure that the concentration of soluble tree nut in solution would fall within the dynamic range of the assay.

Data Processing and Analysis. The median fluorescent intensity (MFI) of each well was measured after beads were resuspended in 100 µL of PBST using a Bio-Rad Bio-Plex 200 (Hercules, CA, U.S.A.). The background noise as measured by the average MFI of the “zero” calibration standard, S0, was subtracted from the response of

Table 1. Highest Dilution Level at Which a Quantifiable Signal Was Detected for Each Chili Sample and Bead Set

Grind	Chili	ALM ^a	BRAZ	CASH	COCO	CR	EGG	GLU	HAZ	MAC	PEAN	PINE	PIST	SOY	WALN	SESAME
		12 13	14 15	18 19	20 21	22	25 26	27 28	29 30	33 34	37 38	39 42	43 44	45 46	47 48	66 72 73
LG ^b	Aji Amarillo	c														
PG	Aji Amarillo															
LG	Anaheim															
PG	Anaheim															
LG	Ancho															
PG	Ancho															
LG	Birdseye															
PG	California Sweet Paprika															
PG	Cayenne A															
PG	Cayenne B															
PG	Cayenne C															
LG	Chilaca															
LG	Chipotle Morita															
PG	Chipotle Morita															
LG	Ghost															
PG	Ghost															
LG	Guajillo															
PG	Guajillo															
LG	Habanero															
PG	Habanero A															
PG	Habanero B															
LG	Hatch New Mexico															
PG	Hatch New Mexico															
PG	Jalapeno															
PG	Kashmiri															
LG	Mulato															
PG	Poblano															
LG	Thai Bird															
PG	Thai Bird															

^aALM, almond; BRAZ, Brazil nut; CASH, cashew; COCO, coconut; CR, crustacean; GLU, gluten; HAZ, hazelnut; MAC, macadamia nut; PEAN, peanut; PINE, pine nut; and WALN, walnut. ^bLG and PG indicate the type of grind of each sample. LG, lab ground; PG, pre-ground. ^cShading indicates the highest dilution level at which a signal was detected for a chili sample with each bead set. Gray, not detected; white, 40-fold dilution; yellow, 400-fold dilution; orange, 4000-fold dilution; and pink, 40 000-fold dilution.

each bead set. Calibration standards and samples were analyzed in triplicate, allowing for the calculation of a mean and standard deviation for each bead set response. Six kits were used on separate days to complete the analyses; all kits were from the same production lot. Cross-reactivity was calculated as previously described.¹¹ Complementary antibody ratios were also calculated using the dominant bead set for each target analyte as the denominator. Antigenic profiling was performed with both Brazil nut 14 and hazelnut 29 by normalizing the MFI responses of the most dominant bead sets to equal 100 and calculating the remaining responses as proportions of the dominant bead set.

RESULTS AND DISCUSSION

Tables and Figures. Table 1 displays a simplified version of the numerical data, indicating the highest dilution level at which detectable MFI was recorded for each sample and target analyte. This table is particularly beneficial in making comparisons among samples. Numerical data, adjusted for background, is shown in Tables S1 and S2 of the Supporting Information.

Table 2A contains the complementary antibody ratios for the antibody bead sets in response to the calibration standards. Ratios generated by calibration standards represent the ratio between the antibody bead sets in response to the presence of the target analyte. Table 2B displays the complementary antibody ratios generated by chili samples; if similar in value to the ratios generated by the calibration standards, the data may indicate the presence of the target analyte in the sample. If, however, the signal is generated by a sample for an analyte in which the complementary antibody ratio is not similar, it indicates the presence of cross-reactive homologous proteins.

Table 3 displays the percent cross-reactivity profile of extracts of whole raw hazelnut and Brazil nut analyzed with xMAP FADA. The cross-reactivities are normalized to the dominant bead set for each sample. The dominant bead sets were hazelnut 29 when raw hazelnut was analyzed and Brazil nut 14 when raw Brazil nut was analyzed.

Overview. The antibody binding patterns of various chili peppers from family Solanaceae and genus *Capsicum* were assessed. The overarching goal of the study was to assess any positive antibody bead set responses in the analysis of chilis with xMAP FADA for consistency with the responses of antibody bead sets when exposed to target allergenic foods. Inconsistent responses are often indicative of sample cross-reactivity with the antibodies used in the assay.

Generally, chili peppers analyzed by xMAP FADA gave a strong signal from the following antibody bead sets: Brazil nut 14 and 15, cashew 18 and 19, coconut 21, hazelnut 29 and 30, pistachio 44, and walnut 48 (Table 1). In terms of intensity, chilis typically displayed reactivity with these antibody bead sets at a 4000-fold total dilution and even 40 000-fold total dilution with some chilis. Most chili samples exhibited very limited signal with the almond 12, and 13, coconut 20, crustacean 22, egg 25 and 26, gluten 27 and 28, macadamia nut 33 and 34, peanut 37 and 38, pine nut 39 and 42, pistachio 43, soy 45 and 46, and sesame 66 and 73 bead sets (Table 1). Samples exhibiting these characteristic chili patterns were Aji Amarillo, Ancho, Birdseye, California Sweet Paprika, Guajillo, Jalapeno, Kashmiri, Poblano, and Thai Bird chilis. Complementary antibody ratio analysis indicated that none of the reactivities observed were consistent with detection of

Table 2. (A) Complementary Antibody Ratios Generated by xMAP FADA Calibration Standards and (B) Complementary Antibody Ratios Generated by Chili Samples When Analyzed by xMAP FADA

A

Calibration Standard	Almond		Brazil Nut		Cashew	Coconut	Crust.	Egg	Gluten	Hazelnut	Mac Nut	Peanut	Pine Nut	Pistachio	Soy	Walnut	Sesame	
	13/12	15/14	19/18	21/20	22	25/26	27/28	30/29	34/33	38/37	42/39	44/43	45/46	48/47	73/72	73/66	72/66	
S1	0.20	0.15	0.13	0.01	-	0.59	0.17	0.09	0.04	1.01	0.09	0.02	0.13	0.12	0.11	0.02	0.18	
S2	0.20	0.16	0.13	0.01	-	0.58	0.17	0.09	0.03	0.98	0.11	0.02	0.13	0.12	0.17	0.03	0.19	
S3	0.20	0.15	0.14	0.01	-	0.57	0.16	0.08	0.03	0.91	0.12	0.02	0.14	0.11	0.24	0.05	0.20	
S4	0.21	0.15	0.15	0.01	-	0.56	0.15	0.08	0.03	0.79	0.14	0.02	0.15	0.11	0.39	0.09	0.22	
S5	0.22	0.15	0.16	0.01	-	0.55	0.14	0.08	0.04	0.67	0.17	0.02	0.16	0.11	0.54	0.13	0.24	
S6	0.23	0.15	0.18	0.01	-	0.56	0.13	0.08	0.05	0.59	0.21	0.03	0.17	0.11	0.80	0.21	0.26	
S7	0.25	0.15	0.22	0.02	-	0.57	0.12	0.10	0.06	0.60	0.25	0.04	0.18	0.12	1.04	0.31	0.30	

B

Grnd ^a	Chili Sample	15/12		15/14		19/18		21/20		25/26		27/28		30/29		34/33		38/37		42/39		44/43		45/46		48/47
		1:40	1:400	1:400	1:4000	1:40000	1:400	1:4000	1:40000	1:400000	1:4000000	1:40000000	1:400000000	1:4000000000	1:40000000000	1:400000000000	1:4000000000000	1:40000000000000	1:400000000000000	1:4000000000000000	1:40000000000000000	1:400000000000000000	1:4000000000000000000	1:40000000000000000000		
PG	Aji Amarillo				0.08	0.08	1.29		0.09						0.52											
PG	Aji Anarillo				0.08	0.05	1.28		0.07						0.51											
LG	Anheim		0.12	0.08			1.28	0.08		0.07																
PG	Anheim		0.09												0.30											
LG	Ancho		0.09	0.07											0.10 ^d											
PG	Ancho		0.10	0.07			1.10	0.12						0.20	0.11											
LG	Birdseye		0.08	0.06			1.05															0.24				
PG	Birdseye		0.11	0.07			0.98	0.07														0.23				
PG	California Sweet Paprika																									
PG	Cayenne A				0.11	0.09																				
PG	Cayenne B				0.12	0.09																				
PG	Cayenne C				0.08			0.43																		
PG	Chilaca				0.10			0.06																		
PG	Chilapote Morita				0.10	0.07		0.09																		
PG	Chipotle Morita				0.10	0.07		1.19																		
PG	Chipotle Morita				0.11	0.07		1.30	0.07						0.34	0.23										
LG	Ghost				0.08			1.30							0.31											
PG	Ghost					0.10	0.07								0.42											
PG	Ghost							1.51																		
LG	Guajillo		0.10	0.08			1.17								0.39											
PG	Guajillo																									
LG	Guajillo							0.07																		
PG	Habanero				0.08		1.29																			
PG	Habanero A				0.09	0.07																				
PG	Habanero A						1.24	0.13							0.26	0.18										
PG	Habanero B		0.05												0.24											
LG	Hatch New Mexico				0.08																					
PG	Hatch New Mexico				0.11		1.36																			
PG	Hatch New Mexico Red				0.08	0.06	1.06	0.14							0.45											
PG	Jalapeno		0.08					0.14	0.02						0.28	0.21										
PG	Kashmiri				0.11	0.09		0.05							0.66											
PG	Kashmiri				0.07	0.04		1.43	0.05																	
PG	Mildato				0.07		0.90																			
PG	Pobkano				0.10		1.30	0.13																		
LG	Thai Bird				0.10	0.07																				
PG	Thai Bird				0.10	0.07	1.20								0.40	0.45										

^aIn part A, no complementary antibody ratio could be calculated for crustacean, because only one crustacean-specific bead set is used in xMAP FADA. In part B, bead sets for crustacean, pine nut, and sesame are not shown as a result of the lack of generating complementary antibody ratios for any chili sample. ^bCOCO indicates coconut analyte. ^cLG indicates that the sample was lab-ground, while PG indicates that the sample was commercially pre-ground. ^dYellow highlight indicates similarity between the complementary antibody ratios generated by the standards and the samples.

Table 3. Percent Cross-Reactivity Profile of Raw Hazelnut and Brazil Nut^a

Analyte	ug/mL	Almond	Brazil n.	Cashew	Coconut	Crust.	Egg	Gluten	Hazelnut	Mac. Nut	Peanut	Pine nut	Pistachio	Soy	Walnut	Sesame
		12	13	14	15	16	17	18	19	20	21	22	23	24	25	26
Hazelnut	0.02	<	<	<	<	<	<	<	100	<	<	<	<	<	<	<
	0.025	<	<	<	<	<	<	<	100	<	<	<	<	<	<	<
	0.1	<	<	<	<	<	<	<	100	<	<	<	<	<	<	<
	0.2	<	<	<	<	<	<	<	100	<	<	<	<	<	<	<
Brazil Nut	0.02	<	100	37	<	<	<	<	<	<	<	<	<	<	<	<
	0.025	<	100	37	<	<	<	<	<	<	<	<	<	<	<	<
	0.1	<	100	35	<	<	<	<	<	<	<	<	<	<	<	<
	0.2	<	100	33	<	<	<	<	<	<	<	<	<	<	<	<
	0.25	<	100	30	<	<	<	<	<	<	<	<	<	<	<	<

^aValues are averages of triplicate samples extracted from ground nuts. Responses of ground raw nuts were evaluated using the buffered detergent UD buffer protocol minus background and normalized to the most responsive bead set for the analyte (Brazil nut 14 and hazelnut 29). < indicates a cross-reactivity of less than 1%. Responses greater than 2% are listed. Responses in black text are below the dynamic range of the bead set. Responses highlighted in blue had MFI values above the dynamic range of the bead set.

comparable target food allergens but rather were indicative of cross-reactivity (parts A and B of Table 2).

A few chilis, including the Anaheim chili, did not exhibit this characteristic chili pattern when analyzed by xMAP FADA. Only four chilis gave a signal for Brazil nut 14 and 15 within the standard curve at the 40-fold dilution level: Anaheim pre-ground, Anaheim lab ground, Ancho lab ground, and Guajillo lab ground. At higher dilution levels, other chilis gave responses within the standard curves, indicative of increased cross-reactivity with the assay.

Generally, the signals observed for each chili were easily identified as cross-reactivity and distinguished from detection of target analyte using secondary end points, including complementary antibody ratios and antigenic profiling. Although some of the chilis showed responses consistent with the presence of macadamia nut residues, because the reactivity was observed in seven varieties, the signal was likely due to the presence of homologous proteins or otherwise cross-reactive epitopes and not cross-contact of the samples with macadamia nut.

Anaheim Chili. Pre-ground Anaheim chili exhibited low levels of cross-reactivity at the 400-fold dilution level with coconut 21, gluten 28, hazelnut 29, and walnut 48 (Table 1). Additionally, pre-ground Anaheim chili only displayed reactivity with Brazil nut 14 and 15 at the 40-fold dilution level. Surprisingly, the lab-ground (whole) Anaheim chili sample showed a slightly different cross-reactivity pattern with a notable signal from Brazil nut 14 and 15, cashew 19, coconut 21, hazelnut 29 and 30, and walnut 48 at the 400-fold dilution level. Ratio analysis revealed that pre-ground Anaheim chili exhibited a hazelnut 30/29 ratio at the 40-fold dilution level that was similar to the ratios generated by the calibration standards (0.10 and 0.08–0.10, respectively; parts A and B of Table 2). If hazelnut was indeed present in the sample of pre-ground Anaheim chili, the estimated concentration was 1.6–1.9 ppm hazelnut (as interpolated from hazelnut 29 and 30 standard curves, respectively; data not shown).

Lab-ground Anaheim chili exhibited a hazelnut 30/29 ratio of 0.30, significantly different from the complementary antibody ratios of the pre-ground Anaheim chili and the calibration standards. Ratios for the other antibody bead sets generated by both pre- and lab-ground Anaheim chilis were distinctly different from the ratios of the calibration standards, indicating that none of the other target allergenic foods were present in the samples at concentrations above the limit of quantitation as well as the absence of homologous proteins that could complicate allergen detection using the xMAP FADA.

Cayenne Chili. Three samples of pre-ground Cayenne chili sourced from India were purchased. Cayenne A was strongly cross-reactive at the 4000-fold dilution level and beyond with Brazil nut 14 and 15, cashew 19, coconut 21, hazelnut 30, pistachio 44, and walnut 48 (Table 1). Cayenne B displayed high levels of cross-reactivity at the 4000-fold dilution level with Brazil nut 14 and 15, cashew 18 and 19, coconut 21, hazelnut 29 and 30, pistachio 44, and walnut 48 (Table 1). Cayenne A gave a soy 45/46 ratio of 0.155 at 40-fold total dilution, while the calibration standards had soy 45/46 ratios ranging from 0.13 to 0.18, dependent upon the concentration (parts A and B of Table 2). The associated potential positive result of cayenne A with soy 45/46 corresponds to an estimated concentration of 2 ppm of soy in the sample (data not shown).

Cayenne B did not exhibit the same soy 45/46 complementary antibody ratio as cayenne A (ratio of 0.9) or the calibration standards.

Like cayenne A and B, cayenne C was highly cross-reactive at the 4000-fold dilution level with Brazil nut 14 and 15, cashew 18, coconut 21, hazelnut 29 and 30, pistachio 44, and walnut 48. Additionally, cayenne C sample was strongly cross-reactive with walnut 47 at the 4000-fold dilution level (Table 1). Comparatively, cayenne C displayed qualitatively similar cross-reactivity profiles to cayenne A and B, although the intensity of reactivity was less for cayenne C than for the two other samples for several of the bead sets (Table 1).

Cayenne C displayed reactivity with macadamia 33 and 34 at the 40-fold dilution level, with a resulting macadamia nut 34/33 complementary antibody ratio of 0.06 for the sample and 0.03–0.06 for the macadamia nut calibration standards (parts A and B of Table 2). Calculations indicated that, if macadamia nut was indeed present in this sample of cayenne, the approximate concentration suggested by the data would be between 6.1 and 6.5 ppm of macadamia nut (data not shown).

In addition, peanut 37 and 38 bead sets were reactive with cayenne C at the 40-fold dilution and gave a resulting peanut 38/37 complementary antibody ratio that was consistent with the ratio generated by the peanut calibration standards (0.71 versus 0.60–1.01; parts A and B of Table 2). According to calculations, the reactivity observed with the peanut bead sets was indicative of the potential presence of approximately 0.3–0.4 ppm of peanut in the original sample (data not shown).

Chilaca Chili. Whole Chilaca chili was analyzed, and cross-reactivity was observed with Brazil nut 14 and 15, cashew 19, coconut 21, hazelnut 29 and 30, and walnut 48 at the 4000-fold dilution level and beyond. At the 40-fold dilution level, reactivity was observed for both macadamia nut 33 and 34. The resulting macadamia nut 34/33 complementary antibody ratio for Chilaca chili was 0.03, consistent with the ratio of the calibration standards, which ranged from 0.03 to 0.06, dependent upon the concentration (parts A and B of Table 2). According to calculations, if macadamia nut was indeed present in the sample of Chilaca chili, the estimated concentration would fall between 6 and 17 ppm of macadamia nut (data not shown). However, the observed low-level response to macadamia nut is likely due to inherent cross-reactivities present in a number of chili varieties. The Chilaca chili sample did not exhibit any other consistencies between complementary antibody ratios and the calibration standards.

Chipotle Morita Chili. Chipotle Morita chilis are dried and smoked jalapeno peppers. Samples of pre-ground and lab-ground Chipotle Morita chili, both originating in Mexico, were analyzed. Pre-ground Chipotle Morita chili was cross-reactive at the 4000-fold dilution level with Brazil nut 14 and 15, cashew 18 and 19, coconut 21, hazelnut 29 and 30, pistachio 44, and walnut 48 (Table 1). Lab-ground Chipotle Morita chili exhibited largely identical cross-reactivity profiles. At lower dilution levels (40- and 400-fold), ratio analysis indicated that pre-ground Chipotle Morita chilis had peanut 38/37 similar to the ratios generated by the peanut calibration standards (parts A and B of Table 2). Interpolation revealed that the concentration of peanut potentially present in the pre-ground sample was approximately 2.7–3 ppm of peanut (data not shown). The macadamia nut 34/33 ratios for lab-ground Chipotle Morita chili were consistent with the complementary antibody ratios exhibited by the calibration standards at the 40- and 400-fold dilution levels (parts A and B of Table 2). The

calculated concentration of macadamia nut potentially present in the sample of lab-ground Chipotle Morita chili was between 10 and 40 ppm of macadamia nut (data not shown). Because the incidence of macadamia nut cross-contact is relatively rare, macadamia nut bead set reactivity in chili samples is likely due to homologous proteins rather than the presence of actual macadamia nut residues.

Ghost Chili (Bhut Jolokia). Ghost chilis (*Capsicum chinense* L.) were analyzed as both pre-ground and lab-ground samples. Pre-ground peppers were strongly reactive with Brazil nut 14, and 15, cashew 18 and 19, hazelnut 29 and 30, pistachio 44, and walnut 48 at the 4000 and/or 40 000-fold dilution levels (Table 1). Complementary antibody ratio analysis indicated that pre-ground Ghost chili did not exhibit any ratios comparable to those of the calibration standards. Lab-ground Ghost chili was highly cross-reactive with Brazil nut 14, cashew 19, hazelnut 30, and walnut 48 at the 4000-fold dilution level (Table 1). Ratio analysis suggested that, as a result of the similarity in value to the calibration standard ratio, the lab-ground Ghost chili may harbor some macadamia nut residues (parts A and B of Table 2). According to the appropriate calculations, the lab-ground Ghost chilis may contain approximately 3.2–3.8 ppm of macadamia nut (data not shown).

Habanero Chili. Two samples of pre-ground and one sample of lab-ground Habanero (*Capsicum chinense* L.) chilis were obtained. Habanero A was cross-reactive with Brazil nut 14 and 15, cashew 19, coconut 21, hazelnut 29 and 30, and walnut 48 at the 4000-fold dilution level (Table 1). Lab-ground habanero chili was cross-reactive with Brazil nut 14 and 15, cashew 18 and 19, coconut 21, hazelnut 29 and 30, and walnut 48 at the 4000-fold dilution level (Table 1). Complementary antibody ratio analysis indicated that lab-ground Habanero had similar macadamia nut 34/33 ratios to the calibration standards at the 40- and 400-fold dilution levels (parts A and B of Table 2). According to calculations, the estimated concentration of macadamia nut in the lab-ground Habanero chili was approximately 40–50 ppm of macadamia nut (data not shown). However, the observed response to macadamia nut is likely due to inherent cross-reactivities noted in many chili varieties.

Hatch New Mexico Chili. Pre-ground Hatch chilis showed a cross-reactivity pattern similar to other chilis at the 4000-fold dilution level: Brazil nut 14, and 15, cashew 19, hazelnut 30, pine nut 39, walnut 48, and sesame 72 (Table 1). Lab-ground chilis were cross-reactive to Brazil nut 14 and 15, cashew 19, coconut 21, gluten 28, hazelnut 30, and walnut 48 at the 4000-fold dilution level (Table 1). At the 400-fold dilution level, pre-ground Hatch New Mexico chilis exhibited similar ratios to calibration standards for Brazil nut 15/14 (0.14 versus 0.15, respectively; parts A and B of Table 2). Interpolation of the standard curve indicated that the sample potentially contains between 160 and 175 ppm of Brazil nut (data not shown). However, when normalized to Brazil nut 14, antigenic profiling reveals that the signal observed from Brazil nut 15 in the analysis of pre-ground Hatch New Mexico chili was not comparable to that which would be observed if raw Brazil nut was present in the sample.

Lab-ground Hatch New Mexico chilis exhibited bead set reactivity at the 4000-fold dilution level to Brazil nut 14 and 15, cashew 19, coconut 21, hazelnut 29 and 30, and walnut 48 (Table 1). Lab-ground Hatch New Mexico chili displayed complementary antibody ratios similar to those generated by

the calibration standards for macadamia 34/33. If macadamia nut was indeed present in the sample of lab-ground Hatch New Mexico chili, the expected concentration would be approximately 3–10 ppm of macadamia nut. As discussed previously with several other chili samples, this is likely low-level cross-reactivity from homologous proteins, which may be confirmed in the future with orthogonal methods.

Mulato Chili. During examination of cross-reactivity patterns of Mulato chilis, a reactivity pattern typical of other chilis is observed at the 4000-fold dilution level: Brazil nut 14 and 15, cashew 19, coconut 21, hazelnut 29 and 30, and walnut 48 (Table 1). Analysis of the macadamia nut 34/33 complementary antibody ratio indicated that the Mulato chili may also contain protein moieties cross-reactive with macadamia nut bead sets (parts A and B of Table 2). Conversion of macadamia nut reactivity to the estimated concentration indicated that there may be between 5 and 40 ppm of macadamia nut present in the sample. As discussed previously, the low-level responses to macadamia observed in multiple pepper varieties is likely due to cross-reactivity from homologous proteins.

In this research, Brazil nut and hazelnut bead sets were substantially more reactive to chilis compared to other bead set responses. To eliminate the possibility that residues of Brazil nut or hazelnut were present in the chilis, raw Brazil nut and hazelnut samples were ground, extracted, and analyzed by xMAP FADA at several concentrations within the dynamic range of the assay. Data were normalized to the dominant bead set for both Brazil nut and hazelnut to illustrate characteristic antigenic profiles and appropriate bead set responses given the presence of the target analyte extracted and analyzed in the same manner as the chili samples (Table 3). In addition to reactivity with Brazil nut 14 and 15, the profile generated by raw Brazil nut showed reactivity with cashew 18 and 19, hazelnut 30, pistachio 43, and walnut 48. Antibody reactivity profiles were inconsistent in terms of both pattern and intensity between chilis and raw Brazil nut, indicating that any observed assay reactivity with chilis was not due to the presence of Brazil nut but rather that reactivity is likely due to an inherent property of chilis (Table 3). Similarly, the antigenic profile observed when pure hazelnut was analyzed was inconsistent with the antigenic profiles of the chili samples, indicating that any chili reactivity observed with the xMAP FADA is not a result of the presence of raw hazelnut in the samples. In addition to Brazil nut 14 and 15 cross-reactivity, chilis consistently exhibited cross-reactivity with cashew 18 and 19 and walnut 48 bead sets that were not consistent with the raw hazelnut antigenic profile (Table 3). These data further support the conclusion that the antigenic profiles displayed by the chilis are inconsistent with the profiles expected if raw Brazil nut or hazelnut were present in the samples. Seven chilis showed complementary antibody ratios for macadamia 34/33 that were indistinguishable from the ratios generated by the calibration standards (parts A and B of Table 2). Potential detection of macadamia nut residues in whole and commercially ground chili peppers was unexpected and quite unusual. These chilis were qualitatively evaluated for trends that would explain or refute the presence of these residues. Of the seven chilis with possible macadamia nut residues, six were sold as whole chilis from the same distributor and were packaged at the distributor level on the same date. The seventh chili was a pre-ground sample purchased from a second distributor. The chilis had three different source countries: four

from Mexico, two from India, and one from the U.S.A. The six whole chilis were ground in the lab on three separate occasions, while the seventh was pre-ground at a commercial level. Finally, these seven chilis were analyzed by xMAP FADA on three different dates. Perhaps of additional importance, there was not an apparently consistent factor that would explain the possibility of macadamia nut residues in these seven chilis, with the absence of these residues in all other chilis assessed with the same trends in mind (distributor, pack date, commercial ground versus lab ground, source country, date of lab grind, and date of xMAP FADA analysis). While macadamia nut bead sets indicated the potential presence of macadamia nut in several samples, the observed low-level response to macadamia nut is likely due to inherent cross-reactivities present in a number of chili varieties. Because the incidence of macadamia nut cross-contact is relatively rare, consistent low-level responses across different grinds and chilis are likely due to homologous proteins rather than the presence of actual macadamia. Further research is necessary to confirm results by evaluating chilis exhibiting antigenic profiles consistent with macadamia nut and employing confirmatory orthogonal methods.

While chili peppers displayed some prominent cross-reactivities with antibodies employed by the xMAP FADA, these signals were able to be distinguished from signals indicative of target analyte detection. The cross-reactivities observed at advanced dilution levels with Brazil nut, cashew, and hazelnut bead sets did not exhibit analytical characteristics consistent with the presence of these target analytes. Low levels of underlying cross-reactivity were observed in different varieties of peppers, consistent with the presence of homologous proteins and not targeted allergens. Future research directions include exploring how the xMAP FADA performs when trace amounts of target analyte are present in a sample that displays high levels of inherent cross-reactivity with the same antibodies used to detect the target, investigating the potential additive nature of cross-reactivity and establishing antigenic profiles for target analytes that have undergone varying degrees of commercially relevant food processing. The combination of secondary end points, including ratio analysis and antigenic profiling, allowed for the signal as a result of target analyte detection to be distinguished from the signal generated by cross-reactive homologous proteins when concentrated chili samples were analyzed using xMAP FADA.

■ ASSOCIATED CONTENT

SI Supporting Information

The Supporting Information is available free of charge at <https://pubs.acs.org/doi/10.1021/acs.jafc.1c02156>.

Common names and taxonomic classifications of the chili peppers evaluated in this study, with most peppers evaluated classified as *Capsicum annuum* L., although members of *Capsicum baccatum* L., *Capsicum chinense* L., and *Capsicum frutescens* Jacq. are also represented (Table S1), and MFI adjusted for background and averaged across replicates that was generated by the various chili peppers evaluated with xMAP FADA, with the displayed MFI values falling in the dynamic ranges of the assays (Table S2) (PDF)

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Notes

The authors declare no competing financial interest.

ABBREVIATIONS USED

xMAP FADA, xMAP food allergen detection assay; PG, commercially pre-ground chili; LG, laboratory-ground chili

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Supplementary Table Captions

Table S1. Taxonomy of chili peppers (Family Solanaceae) evaluated using the xMAP Food Allergen Detection Assay

Table S2. Average median fluorescent intensity (MFI) generated by chili peppers when evaluated by xMAP FADA

Table S1. Taxonomy of chili peppers (Family Solanaceae) evaluated using the xMAP Food Allergen Detection Assay

Chili Common Name	Grind ^a	Genus species	Country of Origin
Anaheim	PG, LG	<i>Capsicum annuum</i> L.	USA
Ancho	PG, LG	<i>Capsicum annuum</i> L.	Mexico, Peru
Birdseye	LG	<i>Capsicum annuum</i> L.	Uganda
California Sweet Paprika	PG	<i>Capsicum annuum</i> L.	USA
Chilaca	LG	<i>Capsicum annuum</i> L.	Mexico
Chipotle Morita	PG, LG	<i>Capsicum annuum</i> L.	Mexico
Guajillo	PG, LG	<i>Capsicum annuum</i> L.	Mexico
Hatch New Mexico	PG, LG	<i>Capsicum annuum</i> L.	USA
Jalapeno, Green	PG	<i>Capsicum annuum</i> L.	USA
Kashmiri	PG	<i>Capsicum annuum</i> L.	India
Mulato	LG	<i>Capsicum annuum</i> L.	Mexico
Poblano	PG	<i>Capsicum annuum</i> L.	Mexico
Aji Amarillo	PG, LG	<i>Capsicum baccatum</i> L.	Peru
Ghost	PG, LG	<i>Capsicum chinense</i> L.	India
Habanero	PG ² , LG	<i>Capsicum chinense</i> L.	Mexico, USA
Thai Bird	PG, LG	<i>Capsicum frutescens</i> Jacq.	Thailand
Cayenne A	PG	<i>Capsicum</i> L. ^b	India
Cayenne B	PG	<i>Capsicum</i> L. ^b	India
Cayenne C	PG	<i>Capsicum</i> L. ^b	India

^aPG and LG indicate the grind of the sample: PG indicates sample was pre-ground, while LG indicates sample was lab-ground.

^bCayenne pepper often contains several species of genus *Capsicum*, including, *C. annuum*, *C. baccatum*, and *C. chinense*. Source: U.S. National Plant Germplasm System.

Table S2. Average median fluorescent intensity (MFI) generated by chili peppers when evaluated by xMAP FADA.

Table 32. Average median fluorescent intensity (SMI) generated by chili peppers when evaluated by SMI-217 (FAD-A)																											
Grind ^b	Chili	ALMOND ^a			BRAZIL NUT						CASHEW						COCONUT						Egg				
		12	13		14		15				18		19				20		21				25	26			
LG	Aji Amarillo	< ^e	788	225 ^d	>> ^e	>>	674	81	>>	>>	56		>>	1354	258	>>	>>	333	34	<	>>	180	81	<	<		
PG	Aji Amarillo	<	725	208	>>	>>	991	151	>>	>>	84	7		3188	1347	358	>>	>>	460	59	949	80	61	29	<	<	
LG	Anaheim	<	<			1098	198			134	16			407			443	63			<	186	50		<	<	
PG	Anaheim	<	<			184				17				<			58				<	43	13		<	<	
LG	Ancho	<	<			536	68			50	5			<			199				<	210	54		<	<	
PG	Ancho	<	134		>>	1257	266		>>	129	19			1584	472		>>	517	89		1078	126	84	29	<	<	
LG	Birdseye	<	241		>>	1521	274		>>	128	17			1932	638		>>	671	100		<	>>	182	98	17	707	1661
PG	California Sweet Paprika	<	170		>>	1419	316		>>	152	23			2061	634		>>	624	106		2275	156	120	66	12	<	<
PG	Cayenne A	<	257		>>	>>	568	81	>>	>>	61	7		2123	915		>>	299	37	<	91	76	46	12	<	<	
PG	Cayenne B	<	554	163	>>	>>	935	126	>>	>>	111	11		3111	1404	340	>>	>>	505	64	1535	99	84	52	15	<	<
PG	Cayenne C	<	286		>>	>>	721		>>	>>	58			1913	733		>>	>>	281		935	85	50	26		<	<
LG	Chilaca	<	402		>>	>>	514	61	>>	>>	50			2606	879		>>	>>	183		1199	>>	>>	94	13	<	<
LG	Chipotle Morita	<	575	169	>>	>>	933	134	>>	>>	90	9		>>	1589	362	>>	>>	431	53	2216	>>	>>	147	24	<	<
PG	Chipotle Morita	<	442	137	>>	>>	807	114	>>	>>	85	8		2848	1308	328	>>	>>	425	52	1973	128	112	69	18	<	<
LG	Ghost	<	174		>>	514	60		>>	43				1027			>>	290	32		<	156	35			<	<
PG	Ghost	<	656	249	>>	>>	1005	155	>>	>>	104	11		2834	1527	389	>>	>>	587	79	<	100	61	24		<	<
LG	Guajillo	<	<		656	77			65	6				260			306	32			<	114	19			<	<
PG	Guajillo	1156	514		>>	>>	453	58	>>	>>	47			2527	815		>>	>>	195		1888	132	98	52		<	<
LG	Habanero	<	643	205	>>	>>	674	91	>>	>>	56			3171	1334	258	>>	>>	333	44	933	>>	>>	81		<	<
PG	Habanero A	<	171		>>	826	125		>>	72	9			1367	330		>>	409	51		924	118	65	14		<	<
PG	Habanero B	<	<		362	67			18					<			120				<	50	13			<	<
LG	Hatch New Mexico	<	265		>>	>>	315		>>	195	25			2180	683		>>	>>	125		779	>>	>>	67		<	<
PG	Hatch New Mexico	<	187		>>	918	138		>>	127	15			1569	316		>>	430	53		<	103	54	14		<	<
PG	Jalapeno	<	<		>>	842	166		>>	71	10			1311	368		>>	391	65		822	119	71	21		<	<
PG	Kashmiri	<	705	177	>>	>>	1119	167	>>	>>	128	15		>>	1551	404	>>	>>	576	74	2117	116	88	57	18	<	<
LG	Mulato	<	175		>>	1357	251		>>	90	10			2354	648		>>	581	86		1711	>>	>>	95	12	<	<
PG	Poblano	<	322		>>	>>	662	90	>>	>>	63			2679	1083	240	>>	>>	311	36	842	113	81	43		<	<
LG	Thai Bird	<	<		>>	690	115		>>	69	8			908	302		>>	334	46		<	>>	171	88	13	<	<
PG	Thai Bird	<	<		>>	537	98		>>	223	54	7		801	259		>>	311	46		<	80	57	19		<	<
S ₀ kg _{std} Average		10	16		26				11					49			10				38	11				2314	4388

^a Crustacean-22, Pine nut-42, Sesame-66 and Sesame-73 bead sets did not detect any signal within the dynamic ranges of the assays for any of the chilis and are excluded from the table.^b Grind indicates the type of grind of the chili: PG indicates sample was commercially pre-ground, while LG indicates the sample was received as intact chilis and was ground in the laboratory.^c "<" represents the sample MFI is less than that of the lower limit of detection (LoD).^d White, yellow, orange, and pink fill indicates the sample MFI at the 40-, 400-, 4,000-, and 40,000-fold dilution levels, respectively.^e ">" represents the sample MFI is greater than that of the highest calibration standard (S7).

Table S2 (continued).

		GLUTEN						HAZELNUT						MACADAMIA						PEANUT						PINE NUT	
Grind ^b	Chili	27			28			29			30			33			34			37			38			39	
LG	Aji Amarillo	125			1346	276		4790	2437	617		>>	>>	318	37	2735		546	148	1050		<			450		
PG	Aji Amarillo	49			744	382		4641	2527	916		>>	>>	469	72	<		617	165	1052		<			<		
LG	Anaheim	<			<			1581	359			>>	109			<		<		<		<			<		
PG	Anaheim	<			353	226		977	235			94				<		<		<		<			<		
LG	Ancho	<			<			1236	275			247	31			<		<		<		<			<		
PG	Ancho	<			328	171		3967	1839	558		>>	>>	139		<		62		<		<			<		
LG	Birdseye	<			560	300		3152	1461	370		>>	>>	147		1546		230	60	<		<			<		
PG	California Sweet Paprika	<			488	260		>>	2666	991		>>	>>	166	20	<		78		<		<			353		
PG	Cayenne A	52			893	369		3292	1927	707		>>	>>	276	39	<		54		2085		1302			465		
PG	Cayenne B	45			953	421		4534	2647	987		>>	>>	433	61	<		111		3291		2383			558	246	
PG	Cayenne C	<			635	318		3277	1701	643		>>	>>	277		1745		112		1705		1218			576	231	
LG	Chilaca	<			293			>>	2652	734		>>	>>	265	31	3442		106		<		<			400		
LG	Chipotle Morita	<			445			>>	3538	1318		>>	>>	446	62	5082	1648	186	46	<		<			373		
PG	Chipotle Morita	74			890	267		>>	3070	1221	243	>>	>>	381	55	<		137	46	10249	1400	6461	1173		467		
LG	Ghost	<			364			2173	516			>>	219	23		1049		59		<		<			<		
PG	Ghost	49			489			4456	2708	955		>>	>>	482	73	<		309	88	935		<			<		
LG	Guajillo	<			168			730				287	31			<		<		<		<			<		
PG	Guajillo	>>	100		>>	1004	278	>>	2642	1029		>>	>>	226	27	<		111		1138		<			892	364	
LG	Habanero	<			142			>>	3054	617		>>	>>	318	39	4394	1360	299	70	1474		<			266		
PG	Habanero A	<			387	206		3743	1542	336		>>	403	61		<		95		1032		<			290		
PG	Habanero B	<			229			557				135	25			<		<		<		<			<		
LG	Hatch New Mexico	<			216			4468	2052	476		>>	>>	163		2452		56		<		<			369		
PG	Hatch New Mexico	25			1030	622	190	3207	1039			>>	470	73		<		70		<		<			441		
PG	Jalapeno	<			300	139		3521	1432	404		>>	398	83		<		51		<		<			392		
PG	Kashmiri	60	<	28	1120	506	234	>>	2991	1234	229	>>	>>	>>	81	<		154		6553		4330			784	366	
LG	Mulato	<			<			>>	2611	665		>>	>>	95		5187	1393	82		<		<			<		
PG	Poblano	<			700	346		4422	2282	779		>>	>>	312	41	<		89		<		<			612		
LG	Thai Bird	<			<			1873	875			>>	353	64		<		<		<		<			<		
PG	Thai Bird	<			205			1318	630			>>	281	52		<		<		<		<			<		
S ₀ kgd Average		34			248			42				15				32		10		30		32			57		

Table S2 (continued).

		SOY		WALNUT						SESAME		
Grind ^b	Chili	45	46	47		48				72		
LG	Aji Amarillo	<	<	2154	820		>>	>>	310	37	>>	1584
PG	Aji Amarillo	<	<	2031	755		>>	>>	480	74	3122	1555
LG	Anaheim	<	<	<			752	153			<	
PG	Anaheim	<	<	<			280	78			<	
LG	Ancho	<	<	<			461	94			<	
PG	Ancho	<	<	816			>>	>>	234		1964	
LG	Birdseye	<	<	1470	515		>>	651	147		1991	
PG	California Sweet Paprika	<	<	2374	539		>>	>>	390	71	2236	
PG	Cayenne A	161	1028	1272	529		>>	>>	363	61	2372	1346
PG	Cayenne B	<	<	1845	837		>>	>>	564	92	>>	1908
PG	Cayenne C	<	<	2132	811	233	>>	>>	308		-	
LG	Chilaca	<	<	1792	430		>>	>>	346	44	3080	1425
LG	Chipotle Morita	<	<	2245	499		>>	>>	633	93	>>	1979
PG	Chipotle Morita	599	701	2377	914		>>	>>	592	101	3119	1720
LG	Ghost	<	<	<			>>	328	40		1384	
PG	Ghost	<	<	935	284		>>	>>	617	90	>>	1899
LG	Guajillo	<	<	<			376	56			<	
PG	Guajillo	<	<	4020	1802	528	>>	>>	353	56	2832	1224
LG	Habanero	<	<	2082	544		>>	>>	310	60	>>	1697
PG	Habanero A	<	<	1056			>>	679	132		1623	
PG	Habanero B	<	<	<			212	55			-	
LG	Hatch New Mexico	<	<	1132			>>	>>	221		2432	
PG	Hatch New Mexico	<	<	1088			>>	584	105		2340	
PG	Jalapeno	<	<	991			>>	635	161		1661	
PG	Kashmiri	<	663	2749	1170	299	>>	>>	654	119	>>	1963
LG	Mulato	<	<	1408			>>	>>	247		2489	
PG	Poblano	<	<	2118	600		>>	>>	395	62	2900	1380
LG	Thai Bird	<	<	312			>>	409	93		1240	
PG	Thai Bird	<	<	<			>>	344	81		<	
	SO _{hkgd} Average	20	14	74			17				40	