

Research Note

Extension of xMAP Food Allergen Detection Assay To Include Sesame

CHUNG Y. CHO,^{1*} KATHERINE O. IVENS,¹ WILLIAM L. NOWATZKE,^{2†} JASON ROBOTHAM,³
MANSOUR SAMADPOUR,⁴ THOMAS GRACE,⁵ KERRY G. OLIVER,² AND ERIC A. E. GARBER¹

¹Office of Regulatory Science, Center for Food Safety and Applied Nutrition, U.S. Food and Drug Administration, 5001 Campus Drive, College Park, Maryland 20740; ²Radix BioSolutions, 111 Cooperative Way, Georgetown, Texas 78626; ³BioFront Technologies, 3000 Commonwealth Boulevard, Suite 2, Tallahassee, Florida 32303; ⁴IEH Laboratories and Consulting Group, Inc., 15300 Bothell Way N.E., Lake Forest Park, Washington 98155; and ⁵Bia Diagnostics, LLC, 480 Hercules Drive, Colchester, Vermont 05446, USA

MS 19-304: Received 1 July 2019/Accepted 25 September 2019/Published Online 19 December 2019

ABSTRACT

An estimated 0.1 to 0.2% of the North American population is allergic to sesame, and deaths due to anaphylactic shock have been reported. Detecting and quantifying sesame in various food samples is critical to safeguard the allergic population by ensuring accurate ingredient labeling. Because of the modular nature of the xMAP Food Allergen Detection Assay (FADA), it was possible through method extension to add sesame as a validated additional analyte. Because raw and toasted sesame are both commonly used and the two display significantly different antigenicity, three antibodies, one monoclonal and two polyclonal, were conjugated to bead sets to ensure reliable detection. The modified xMAP FADA successfully detected sesame incurred or spiked in baked muffins, spice mix, canola oil, and in both raw and toasted sesame oils with limit of quantitation values ≤ 1.3 ppm of sesame. Canola oil, sesame oil, toasted sesame oil, and olive oil inhibited sesame detection, as did the detection of sesame incurred in foods containing oil (e.g., hummus). Despite this inhibition, the xMAP FADA was still able to reliably detect sesame at levels throughout the dynamic range of the assay (22 to 750 ng of protein per mL) in all the foods examined. Further, the high signal-to-noise ratio of the lowest calibration standard and preliminary studies conjugating the antibodies at higher concentrations indicate an ability to increase the sensitivity of the assay should the need arise.

HIGHLIGHTS

- Sesame was added to the validated xMAP Food Allergen Detection Assay.
- Both raw and toasted sesame were successfully detected when incurred in foods and oils.
- Assay sensitivity can be increased by conjugating antibodies at a higher concentration as needed.

Key words: Detection; Food allergen; Method extension; Sesame; xMAP

An estimated 0.1 to 0.2% of North Americans are allergic to sesame (1, 24). However, sesame is a growing concern due to a continuous increase in the number of allergic incidents, including anaphylaxis (10). Currently, the Food Allergen Labeling and Consumer Protection Act of 2004 recognizes only eight major allergenic foods: milk, eggs, fish, crustacean shellfish, tree nuts, peanuts, wheat, and soybeans (9). Sesame is recognized as a major food allergen by Canada, Australia, New Zealand, member countries of the European Union, and Gulf Cooperation Council Standardization Organization member countries (i.e., Saudi Arabia, United Arab Emirates, Kuwait, Bahrain, Oman, Qatar, and Yemen) (13, 17, 21, 26, 27). In 2018, the U.S. Food and Drug Administration (FDA) issued a request

for comments regarding sesame as a food allergen (29). Rates of sesame-induced anaphylaxis vary greatly by region. In Canada, where the incidence of anaphylaxis among children is similar to that of the United States, only 2.8% of the recorded cases of anaphylaxis are caused by sesame, while 85% of all cases of anaphylaxis are caused by food (1). In contrast, in Israel and Saudi Arabia, sesame accounts for 43 and 29% of anaphylaxis caused by food, respectively (8, 23). The growing use of sesame, combined with the evidence that sesame can trigger immediate hypersensitivity reactions, creates a concern for allergic populations (2, 10, 14).

The FDA along with Radix BioSolutions, Ltd. (Georgetown, TX) developed the xMAP Food Allergen Detection Assay (FADA), capable of simultaneously detecting 14 food allergens plus gluten. The modular plug-and-play design provides the ability to adjust or expand the assay on the basis of user needs (4). Redundancy through the use of multiple antibodies targeting the same analyte, as well as

* Author for correspondence. Tel: 240-402-2044; Fax: 301-436-2332; E-mail: chung.cho@fda.hhs.gov.

† Present address: B2S Life Sciences, 97 East Monroe Street, Franklin, IN 46131, USA.

the ability to generate multiantibody profiles, provides built-in confirmation not possible by using single-analyte enzyme-linked immunosorbent assays. As such, the xMAP FADA is unique in its ability to recognize and distinguish between homologous proteins, which was critical in the recent analysis of legumes and tree nuts (3, 19, 25). The xMAP FADA also uses two different extraction protocols: buffered detergent and reduced denatured buffer (4). The performance of the xMAP FADA has been well illustrated in a single-laboratory validation study (18). The method has also been shown to be reliably effective in detecting food allergens in regulatory samples (11, 20). Finally, the xMAP FADA was able to detect and identify the presence of pecan in regulatory samples by using antigenic profiling (5). In this study, sesame was added to the already established xMAP FADA repertoire through method extension, and its performance was validated.

MATERIALS AND METHODS

Reagents. Phosphate-buffered saline (PBS; catalog P3813), Tween 20 (catalog P9416), and PBS with Tween 20 (PBST; catalog P3563) were purchased from MilliporeSigma (St. Louis, MO). Difco nonfat dried milk (catalog DF0032-17-3) was obtained from Thermo Fisher Scientific (Waltham, MA). Sesame bead sets were prepared by Radix BioSolutions, LLC conjugating three established commercial antibodies (one monoclonal and two polyclonal antibodies) obtained from BioFront Technologies (Tallahassee, FL), IEH Laboratories and Consulting Group (Lake Forest Park, WA), and 3M Food Safety (St. Paul, MN), respectively.

Preparation of samples. McCormick culinary white sesame seed was purchased in bulk and ground by using an IKA A11 Basic Analytical Mill (IKA Works, Inc., Wilmington, NC) to serve as a reference. Ground sesame was mixed with PBST for 2 h at room temperature on a shaker, and the resulting slurry, which contains both soluble and insoluble components of ground sesame, was used to spike into food and oil samples. Organic toasted sesame from McCormick (Baltimore, MD) from a separate lot of sesame seeds was obtained, and the slurry was prepared. Schwartz Thai 7 Spice Seasoning (spice mix) containing 14 different ingredients (chili powder, cumin, oregano, garlic, onion, coriander seed, ginger, lemon peel, cinnamon, star anise, white pepper, cayenne pepper, jalapeno chilies, and green peppercorn), 365 organic sesame seed oil, 365 toasted sesame seed oil, and 365 organic canola oil (products of Whole Foods Market, Austin, TX) were obtained and used in the assays. Nature's Promise canned organic garbanzo beans, 15.5 oz (ca. 439 g), Sicilia lemon juice, Bertolli organic extra-virgin olive oil, McCormick ground cumin, Morton iodized salt, fresh garlic cloves, Betty Crocker gluten-free cake mix, and Earth Balance soy-free butter were purchased from a local grocery store.

Incurred 1-g portion muffins were prepared according to the manufacturer's recipe (Betty Crocker) with ground raw sesame slurry spiked at 0, 1, 10, and 100 ppm, with a minor modification. Instead of butter, Earth Balance soy-free butter was substituted, and egg was not added to the muffin batter. Muffins were baked in mini-baking cups at 350°F for 9 min on a shiny metal pan. Hummus containing olive oil was prepared by grinding one can of Nature's Promise organic garbanzo beans with two garlic cloves, Sicilia lemon juice (29.6 mL), Bertolli organic extra virgin olive oil (44.4 mL), distilled water (44.4 mL), McCormick ground

cumin (2.85 g), and Morton iodized salt (1.42 g) combined by using Blixer 3 from Robot Coupe USA (Ridgeland, MS) following a published recipe (7). One-gram portions of hummus were weighed into 50-mL conical tubes, and ground raw sesame slurry was spiked at 0, 1, 10, and 100 ppm. The hummus samples were refrigerated at 4°C for up to a week to determine the effects of storage on sesame detection. One-gram portions of Thai 7 Spice Seasoning (spice mix) were aliquoted into 50-mL conical tubes, spiked with ground raw sesame slurry at 0, 1, 10, and 100 ppm, and stored at 4°C. Similarly, 1-mL samples of PBS were spiked with ground raw sesame slurry at 0, 1, 10, and 100 ppm. Canola, sesame, and roasted sesame oil samples were spiked with ground raw sesame slurry at 0, 1, 2.5, 10, 25, and 100 ppm. Olive oil was spiked with ground raw sesame slurry at 0, 1, 10, and 100 ppm. All oil samples were stored at room temperature for up to a week to determine the effects of storage on sesame detection.

xMAP FADA. The xMAP FADA analyses were performed according to the manufacturer's recommended instructions, as outlined in the product insert and in prior publications, with the inclusion of three new sesame bead sets (sesame-66, -72, and -73) and the sesame calibration standards (4). Sesame protein-containing calibration standards prepared in UD buffer at 750 ng/mL, for standard 7 (S7), was serially diluted 1.8-fold according to the established protocol (4, 12). All bead sets, including new sesame bead sets and new sesame-containing calibration standards, were prepared by Radix BioSolutions with their quality assurance and quality control protocols. The UD buffer protocol was used for most analyses, except for the calibration standards comparison analyses that used both PBST and UD buffers. Plates were washed manually by using the Invitrogen Magnetic-Ring stand (catalog 10331425) obtained from Thermo Fisher Scientific. All assays were analyzed by using the Bio-Plex 200 analyzer with Bio-Plex Manager version 6.2 software (Hercules, CA) with settings established by Radix BioSolutions.

Data analysis. Limit of detection (LoD) values were extrapolated from the background response (median fluorescence intensity [MFI]) plus 3 times the standard deviation (SD), and the limit of quantitation (LoQ) values were extrapolated from the background response plus 10 times the SD. The ppm of sesame in foods was calculated by using a stepwise linear regression extrapolated to 10 times the SD. Percent recovery values were calculated by dividing ppm of sesame in food by known spiked ppm of sesame value times 100. Ratio analyses were calculated on the basis of the MFI ratios between complimentary bead sets (e.g., almond-12 and -13). Calculated ratios in samples were compared with the ratios of complimentary bead sets in calibration standards.

RESULTS AND DISCUSSION

Calibration standards. The percentage of differences between the signal responses of bead sets to the calibration solution containing sesame and the calibration solution without sesame are depicted in Table 1. Among nonsesame bead sets, cashew bead set 19 (cashew-19) and coconut-21 consistently had the highest percentage of differences ranging from 18 to 22% and 18 to 27%, respectively, in the presence of sesame; no change in response was observed with either cashew-18 or coconut-20. Gluten bead sets (gluten-27 and -28) displayed a change in response at the upper limit of the calibration standards (S7, 750 ng of

TABLE 1. Percent difference in calibration standards with sesame compared with the respective calibration standard without sesame^a

Calibration standard	Almond		Brazil nut		Cashew		Coconut		Crust ^b		Egg		Gluten		Hazelnut		Macadamia		Peanut		Pine nut		Pistachio		Soy		Walnut		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
PBST	— ^c	—	—	< ^d	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
S1	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
S2	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
S3	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
S4	—	—	—	<	—	—	<	<	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
S5	—	—	—	<	—	—	<	<	-12	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
S6	—	—	5.9	<	—	—	27	-10	—	—	—	—	—	10	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
S7	—	—	7.6	<	—	—	25	-8	—	—	-38	-32	—	<	—	7.1	—	—	4.5	—	—	—	—	—	—	—	—	—	—	—
UD	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
S1	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
S2	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
S3	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
S4	—	—	—	—	—	22	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
S5	—	—	—	—	—	20	—	18	—	—	—	—	—	—	—	—	—	-6.3	-7	—	—	—	—	—	—	—	—	—	—	—
S6	—	—	—	—	—	19	—	<	—	—	—	—	—	—	—	—	—	-4.5	-3.7	—	—	—	—	—	-5.8	-5.9	—	—	—	—
S7	—	—	4.2	—	—	18	—	25	—	—	—	—	—	—	4.6	—	—	-2.4	—	—	—	—	—	—	-4.4	-5.6	—	—	—	—

^a The calibration standard with sesame (SS) and without sesame (SC) at calibration concentration X (where X represents 0 to 7 for the calibration concentration range) were run by using both PBST and UD buffer protocols. For each bead set, the percent difference is calculated by $[(SSX/SCX) - 1] \times 100$ for each calibration standard concentration X and depicted in the table. The negative number indicates that the compared calibration standard with sesame (SS) signal response is less than the calibration standard without sesame (SC) at the respective concentration. Positive number is vice versa.

^b Crust, crustacean shellfish.

^c —, SS – SC difference is less than or equal to S1 signal response of either PBST or UD.

^d <, SS – SC difference is less than or equal to $3 \times$ SD of the background of either PBST or UD.

^e >, all sesame bead set comparisons showed at least 1,500% signal difference.

TABLE 2. *LoD and LoQ of sesame in foods^a*

Sample	Values for each sesame bead set					
	LoD			LoQ		
	-66	-72	-73	-66	-72	-73
PBS	0.02	0.01	0.01	0.07	0.04	0.03
Baked muffin	0.03	0.04	0.02	0.09	0.1	0.07
Spice mix ^b	0.4	0.3	0.05	0.6	1.3	0.1
Hummus	0.03	0.002	0.03	0.06	0.002	0.09

^a Limit of detection (LoD) values (in ppm) were extrapolated from the background response (MFI) plus 3 times the SD and limit of quantitation (LoQ) values (in ppm) were extrapolated from the background response plus 10 times the SD.

^b Higher LoD and LoQ values resulted from the higher background.

sesame protein per mL) by using the PBST protocol of 38 and 32%, respectively; however, these differences were not observed when following the UD buffer protocol. Similarly, the small differences of 2 to 7% were only observed with peanut and soy bead sets at higher concentrations of the calibration standard (S5 to S7) in the UD buffer. Other bead sets, such as Brazil nut-14, hazelnut-30, and crustacean shellfish-22 were also observed to display small differences with the higher concentrations of sesame standard. Overall, the responses of the bead sets were not significantly impacted by the addition of sesame to the calibration standards, especially at the lower concentrations (S1 to S4). Accordingly, ratio analysis using complementary bead sets was consistent with prior results, with only a slight change affecting cashew and coconut due to the increase in the MFI generated by cashew-19 and coconut-21 (18, 19).

As expected, there was minimal cross-reactivity of sesame bead sets sesame-66, -72, and -73 with the calibration standards mixture lacking sesame; observed cross-reactivity was 0.1, 2.5, and 0.3% in PBST buffer and 0.1, 1.2, and 0.1% in UD buffer, respectively.

Detection in food. The xMAP FADA was used to detect sesame in spiked or incurred foods, including a spice mixture with 14 spices and herbs, baked muffins, and hummus made with olive oil. The detection of sesame in foods was compared with that of sesame detected in PBS samples. The signal responses generated by the food samples in the xMAP FADA are listed in Supplemental Table S1 of the supplemental data. Cross-reactivity was observed with almond-13, Brazil nut-14, cashew-18 and -19, coconut-21, hazelnut-30, pistachio-44, and walnut-48 bead sets, but the most prominent cross-reactivity was observed with Brazil nut-14 and both cashew bead sets. For all bead sets other than Brazil nut and cashew, little or no cross-reactivity was observed. Cashew was the only analyte that exhibited cross-reactivity with both of the complimentary bead sets. The ratios between the complimentary cashew antibodies (i.e., cashew-18 and -19) due to sesame cross-reactivity (0.5 to 0.6) were different from the ratios

TABLE 3. *Percent recovery^a*

Sample	Sesame (ppm)	Recovery by sesame bead set		
		-66	-72	-73
PBS ^b	1	67 ± 3	94 ± 5	215 ± 27
	10	41 ± 1	87 ± 7	206 ± 7
	100	46 ± 8	121 ± 1	> ^c
Baked muffin ^d	1	23 ± 2	42 ± 4	83 ± 14
	10	37 ± 4	62 ± 8	92 ± 13
	100	78 ± 1	88 ± 2	>
Spice mix ^d	1	65 ± 9	49 ± 21	119 ± 9
	10	84 ± 7	82 ± 7	132 ± 9
	100	89 ± 1	86 ± 2	>
Hummus ^d	1	89 ± 2	72 ± 4	31 ± 1
	10	97 ± 1	78 ± 5	49 ± 6
	100	96 ± 3	83 ± 1	75 ± 2

^a Percent recovery of spiked ground sesame in PBS relative to sesame calibration standards calculated on the basis of interpolated ppm. Samples extracted (*n* = 3) according to UD buffer, buffered-detergent protocol. Extracts analyzed without subsequent dilution. Values are average % recovery of true triplicates ± 1 × SD of the % recovery.

^b Percent recovery of ground raw sesame in PBS relative to sesame calibration standards.

^c >, MFI exceeded dynamic range (>S7, 750 ng of protein per mL).

^d Apparent percent recovery of ground raw sesame in specified food relative to ground sesame spiked into PBS on a per MFI basis.

observed with cashew (1.6 to 3.1) and allows us to distinguish sesame detection from cashew (18, 19).

On the basis of the data in Table S1, the LoD and LoQ values were calculated for each of the sesame bead sets (Table 2). In all cases, the LoD and LoQ values were less than the lower limit of the dynamic range (22 ng of protein per mL), as defined by the sesame calibration standards. Although indicating a potential to reliably detect extremely low levels of sesame, from a practical perspective, the quantitation is restricted by the calibration standards (22 to 750 ng of protein). The decreased sensitivity in sesame detection observed in the spice mix was not unexpected owing to the higher background signal compared with other food matrices.

Average percent recovery values for the food samples are listed in Table 3. Because the incurred samples were prepared by using a different source of raw sesame than the raw sesame used to prepare the calibration samples, it is not surprising that the recoveries deviated from 100% for the PBS samples. To estimate the effects of the foods and processing on recoveries, the responses generated by the food samples were normalized relative to the MFI of the PBS samples as an alternate approach to calculate the recoveries. The normalized recovery on the basis of the MFI response provides only an apparent recovery, likely different than a true recovery calculated on a per mole basis. However, to exclusively interpolate concentrations on the basis of the calibration standards could be potentially misleading, as indicated by the PBS results (Table 3). Using

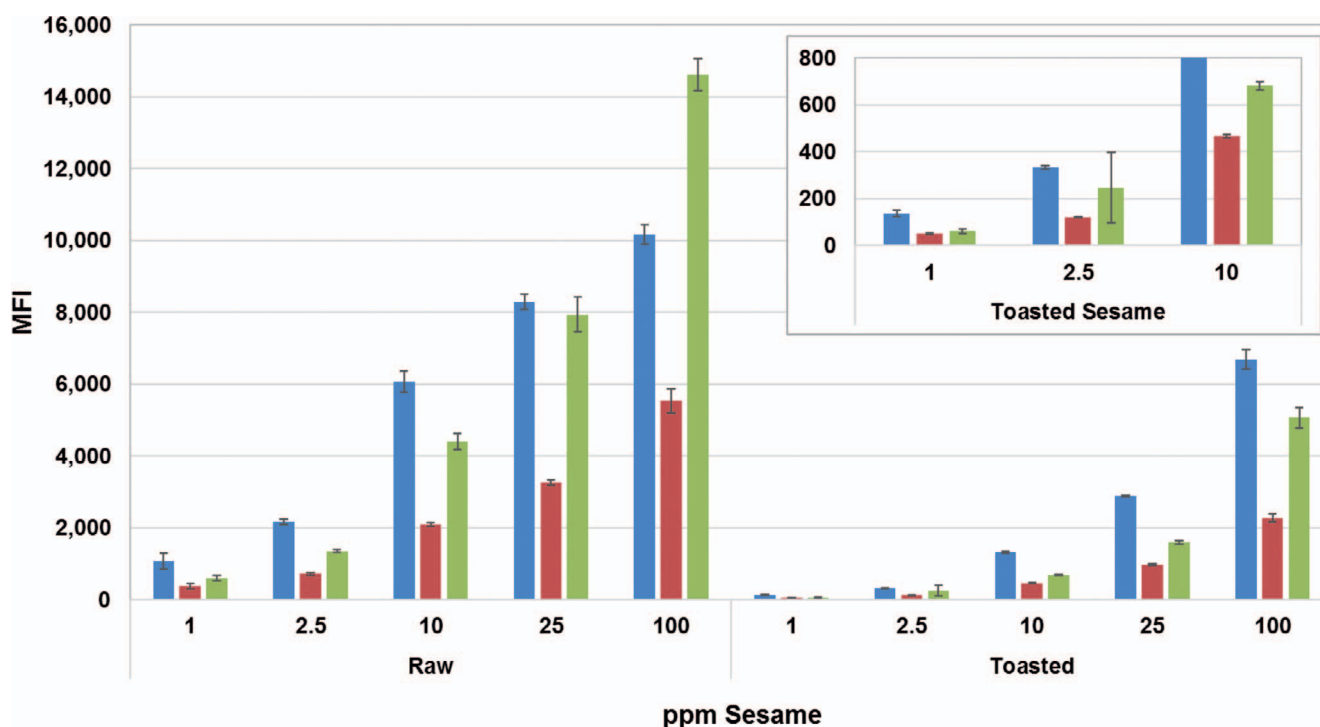


FIGURE 1. Detection of raw and toasted sesame ($n = 3$) at 1, 2.5, 10, 25, and 100 ppm in PBST buffer analyzed by using the UD buffer extraction protocol. Three bead sets sesame-66 (blue bars), sesame-72 (red bars), and sesame-73 (green bars) were included in the analysis. Error bars indicate $\pm 1 \times SD$. The UD buffer background for each bead set, sesame-66 (background = 70 MFI), sesame-72 (background = 82 MFI), and sesame-73 (background = 31 MFI), was subtracted from the signal response.

this approach, the baked muffins displayed apparent low recoveries, as were typically observed with incurred samples in other validation studies. Unexpectedly, hummus displayed exceptionally low recovery by sesame-73. On day 0, approximately up to a threefold decrease in recovery by sesame-73 with hummus (relative to the PBS samples) was observed and by day 7, no measurable sesame was detected, which was not observed with the other sesame bead sets or other foods. A similar pattern of inability to detect sesame by sesame-73 was observed upon storage of ground sesame for 1 week in olive oil, an ingredient of the hummus in all concentration ranges tested. In contrast, sesame-66 and sesame-72 displayed an average reduction of 64 to 70% in MFI in olive oil and 3 to 36% reduction in hummus after 1 week of storage. Storage in the presence of either canola or sesame oil did not have such a dramatic effect on sesame-73 (see the following).

Detection of toasted sesame. The detection of 1, 2.5, 10, 25, and 100 ppm of raw and toasted sesame is depicted in Figure 1. Both raw and toasted sesame were detected by all sesame bead sets above the LoQ. The MFI generated by toasted sesame were, on average, 74% less than the comparable amount of raw sesame with all bead sets. It is not known if the lower responses with toasted sesame are because of a reduction in the protein extractability and the antigenicity due to heat processing or the detection of residual raw sesame in the toasted portion (22).

Detection in oil samples. The detection of raw sesame spiked at 0, 1, 2.5, 10, 25, and 100 ppm in raw sesame oil, toasted sesame oil, and canola oil and is illustrated in Figure S1. In raw sesame oil at 0 ppm (nonspiked samples), the presence of residual sesame proteins was observed by the xMAP FADA 1.2- to 2-fold above the LoQ for all bead sets. In toasted sesame oil, a response equal to the LoD was observed only with sesame-72. These results may indicate that very few sesame proteins are present in these oils, labeled as unrefined, and caution must be taken before deciding whether the residual amount of protein present poses a health risk to allergic consumers (2, 6, 14). In canola oil, labeled as refined, no responses indicative of residual sesame proteins were detected.

Prolonged storage of ground raw sesame in raw sesame oil, toasted sesame oil, canola oil, olive oil, and hummus for 7 days resulted in a significant decrease in detectable sesame (see Fig. S1). Storage in raw sesame oil for 7 days at room temperature caused an average reduction in MFI, ranging from 37% to approximately 70% for all bead sets. Storage in toasted sesame seed oil displayed a greater effect, with an average reduction in MFI of 66 to 84%. The greatest decrease in detectable sesame was observed upon storage for 7 days at either 4°C (not shown) or room temperature in olive oil, with almost no measurable sesame detected with sesame-73. Sesame-66 was also extremely sensitive to storage in olive oil, while sesame-72 displayed only approximately a 60% reduction in MFI. Storage in hummus, which contains olive oil, was similar to the effects of olive oil on sesame-73, but less of an effect was observed on

sesame-66 or sesame-72. In contrast, storage in canola oil only induced approximately a 40% reduction in detectable sesame with all three bead sets.

Olive oil, compared with canola and sesame oil, is higher in phenolic compounds such as tyrosols, which may be the basis from the loss of detectable sesame by sesame-73 bead set (15). In addition, the use of canned garbanzo beans and the addition of lemon juice may have contributed to lowering the pH of the prepared hummus, which could have potentially affected the detectability of sesame protein, as seen in other studies (16, 28). It is possible that the combination of the acidity of ingredients and the presence of olive oil may have contributed to the complete reduction in detectability of sesame-73 in hummus after 7 days. Despite the complete reduction of sesame-73, the other two sesame bead sets were able to detect sesame above the detection limit at the lowest concentration tested.

Adaptability to meet future needs. Bead sets prepared by conjugating twice the amount of antibody displayed similar cross-reactivity patterns, with increased responses by the sesame bead sets. The sesame calibration standards ranged from 22 to 750 ng/mL, with the lowest calibration standard (S1) displaying signal-to-noise ratios in excess of 15 for all three sesame beads sets. Thus, although the sesame bead sets define a dynamic range of 22 to 750 ng of protein per mL, if necessary, it should be possible to extend the dynamic range by extending the calibration standards to a concentration that generates a signal-to-noise ratio of 5 and, if necessary, doubling the concentration of the antibodies conjugated.

In summary, the xMAP FADA was expanded to include sesame as the 16th food target analyte in addition to egg, milk, gluten, peanut, soy, crustacean shellfish, and nine tree nuts (almond, Brazil nut, cashew, coconut, hazelnut, macadamia nut, pine nut, pistachio, and walnut). Of the bead sets for the original 14 allergens and gluten in the xMAP FADA, the addition of sesame into the calibration standards mixture showed the greatest impact on the cashew bead sets, which were observed to have the strongest cross-reactivity to sesame. All other nonsesame bead sets were not affected by the addition of sesame. As such, the ratios between complimentary antibody bead sets were consistent with previously known values. The detection of sesame in food samples displayed LoQ values ≤ 1.3 ppm of sesame, with very consistent results for the detection of sesame at 10 ppm or lower. The newly incorporated sesame bead sets were able to detect both raw and toasted sesame in complex foods. In olive oil and hummus, the loss of sesame detectability by the sesame-73 bead set after 7 days of storage may have been influenced by the type of oil used in the sample preparation and the low pH of hummus due to the addition of lemon juice. The addition of three sesame bead sets to the established xMAP FADA brings the combination of the unique properties of each antibody and enables the detection and confirmation of the presence of sesame in many complex food matrices, including oils.

ACKNOWLEDGMENTS

Our gratitude is expressed to Masahiro Shoji (Morinaga Institute of Biological Sciences, Inc.) and John Leslie (Bia Diagnostics, LLC; Elution Technologies—3M Food Safety).

SUPPLEMENTAL MATERIAL

Supplemental material associated with the article can be found online at: <https://doi.org/10.4315/0362-028X.JFP-19-304.s1>

REFERENCES

- Ben-Shoshan, M., S. La Vieille, H. Eisman, R. Alizadehfar, C. Mill, E. Perkins, L. Joseph, J. Morris, and A. Clarke. 2013. Anaphylaxis treated in a Canadian pediatric hospital: incidence, clinical characteristics, triggers, and management. *J. Allergy Clin. Immunol.* 132:739–741.
- Chiu, J. T., and I. B. Haydik. 1991. Sesame seed oil anaphylaxis. *J. Allergy Clin. Immunol.* 88:414–415.
- Cho, C. Y., O. Carolyn, W. Nowatzke, K. Oliver, and E. A. E. Garber. 2017. Cross-reactivity profiles of legumes and tree nuts using the xMAP® multiplex food allergen detection assay. *Anal. Bioanal. Chem.* 409:5999–6014.
- Cho, C. Y., W. Nowatzke, K. Oliver, and E. A. E. Garber. 2015. Multiplex detection of food allergens and gluten. *Anal. Bioanal. Chem.* 407:4195–4206.
- Cho, C. Y., C. Puente-Lelievre, G. D. Jones, S. R. Stadig, D. A. Taylor, A. C. Eischeid, and E. A. E. Garber. 2018. Application of multiantigen profiling to detect pecan. *J. Food Prot.* 81:700–704.
- Crevel, R., M. Kerkhoff, and M. Koning. 2000. Allergenicity of refined vegetable oils. *Food Chem. Toxicol.* 38:385–393.
- Crunch Creamy Sweet. 2018. Easy hummus without tahini. Available at: <https://www.crunchcreamsweet.com/2018/02/28/easy-hummus-without-tahini/>. Accessed 4 September 2018.
- Dalal, H., I. Binson, R. Reifen, A. Ballin, and E. Somekh. 2002. Sesame as a major cause of severe IgE-mediated food allergic reactions among infants and children in Israel. *J. Allergy Clin. Immunol.* 109:S217.
- Food Allergen Labeling and Consumer Protection Act of 2004 (FALCPA). 2004. Public Law 108-282. Title II. 21 USC § 343 note. Available at: <https://www.fda.gov/downloads/Food/GuidanceRegulation/UCM179394.pdf>. Accessed 13 June 2014.
- Gangur, V., C. Kelly, and L. Navuluri. 2005. Sesame allergy: a growing food allergy of global proportions? *Ann. Allergy Asthma Immunol.* 95:4–11.
- Garber, E. A., C. H. Parker, S. M. Handy, C. Y. Cho, R. Panda, M. Samadpour, D. H. Reynaud, and G. C. Ziobro. 2016. Presence of undeclared food allergens in cumin: the need for multiplex methods. *J. Agric. Food Chem.* 64:1202–1211.
- Garber, E. A., K. V. Venkateswaran, and T. W. O'Brien. 2010. Simultaneous multiplex detection and confirmation of the proteinaceous toxins abrin, ricin, botulinum toxins, and *Staphylococcus enterotoxins* A, B, and C in food. *J. Agric. Food Chem.* 58:6600–6607.
- Government of Canada. 2011. Regulations amending the food and drug regulations (1220—enhanced labelling for food allergen and gluten sources and added sulphites). Available at: <http://gazette.gc.ca/rp-pr/p2/2011/2011-02-16/html/sor-dors28-eng.html>. Accessed 4 April 2019.
- Kanny, G., C. De Hauteclouque, and D. Moneret-Vautrin. 1996. Sesame seed and sesame seed oil contain masked allergens of growing importance. *Allergy* 51:952–957.
- Koestel, C., C. Simonin, S. Belcher, and J. Rösti. 2016. Implementation of an enzyme linked immunosorbent assay for the quantification of allergenic egg residues in red wines using commercially available antibodies. *J. Food Sci.* 81:T2099–T2106.
- Lee, P. W., L. M. Niemann, D. M. Lambrecht, J. A. Nordlee, and S. L. Taylor. 2009. Detection of mustard, egg, milk, and gluten in salad dressing using enzyme-linked immunosorbent assays (ELISAs). *J. Food Sci.* 74:T46–T50.

17. New Zealand Government. 2018. Food labels explained. Available at: <https://www.mpi.govt.nz/food-safety/food-safety-for-consumers/food-labelling/food-labels-explained/>. Accessed 5 February 2019.
18. Nowatzke, W. L., K. G. Oliver, C. Y. Cho, P. Rallabhandi, and E. A. Garber. 2018. Single-laboratory validation of the multiplex xMAP food allergen detection assay with incurred food samples. *J. Agric. Food Chem.* 67:484–498.
19. Pedersen, R. O., W. L. Nowatzke, C. Y. Cho, K. G. Oliver, and E. A. Garber. 2018. Cross-reactivity by botanicals used in dietary supplements and spices using the multiplex xMAP food allergen detection assay (xMAP FADA). *Anal. Bioanal. Chem.* 410:5791–5806.
20. Pedersen, R. O., T. Peters, R. Panda, P. Wehling, and E. A. E. Garber. 2017. Detection and antigenic profiling of undeclared peanut in imported garlic using an xMAP multiplex immunoassay for food allergens. *J. Food Prot.* 80:1204–1213.
21. Popping, B., and C. Diaz-Amigo. 2018. European regulations for labeling requirements for food allergens and substances causing intolerances: history and future. *J. AOAC Int.* 101:2–7.
22. Redl, G., F. T. Husain, I. E. Bretbacher, A. Nemes, and M. Cichna-Markl. 2010. Development and validation of a sandwich ELISA for the determination of potentially allergenic sesame (*Sesamum indicum*) in food. *Anal. Bioanal. Chem.* 398:1735–1745.
23. Sheikh, F., R. Amin, A. M. Rehan Khaliq, T. Al Otaibi, S. Al Hashim, and S. Al Gazlan. 2015. First study of pattern of anaphylaxis in a large tertiary care hospital in Saudi Arabia. *Asia Pac. Allergy* 5:216–221.
24. Sicherer, S. H., A. Muñoz-Furlong, J. H. Godbold, and H. A. Sampson. 2010. US prevalence of self-reported peanut, tree nut, and sesame allergy: 11-year follow-up. *J. Allergy Clin. Immunol.* 125:1322–1326.
25. Speer, F. 1975. Multiple food allergy. *Ann. Allergy* 34:71–76.
26. University of Nebraska–Lincoln. 2018. Food allergens—international regulatory chart. Available at: <https://farrp.unl.edu/IRChart>. Accessed 5 February 2019.
27. U.S. Department of Agriculture, Foreign Agricultural Service. 2011. Saudi Arabia—Food and agricultural import regulations and standards—narrative. Available at: http://agriexchange.apeda.gov.in/IR_Standards/import_regulation_details.aspx?URL=Saudi_Arabia_Food_and_Agricultural_Import_Regulations_and_Standards_-_Narrative_Riyadh_Saudi_Arabia_12-27-2011.pdf. Accessed 4 April 2019.
28. U.S. Food and Drug Administration. 2008. Acidified and low-acid canned foods. Available at: <http://ucfoodsafety.ucdavis.edu/files/266402.pdf>. Accessed 6 March 2019.
29. U.S. Food and Drug Administration. 2018. Statement from FDA commissioner Scott Gottlieb, M.D., on the FDA's new consideration of labeling for sesame allergies. Available at: <https://www.fda.gov/NewsEvents/Newsroom/PressAnnouncements/ucm624484.htm>. Accessed 5 February 2019.

Copyright of Journal of Food Protection is the property of Allen Press Publishing Services Inc. and its content may not be copied or emailed to multiple sites or posted to a listserv without the copyright holder's express written permission. However, users may print, download, or email articles for individual use.