

Research Note

Detection of Sesame Seed DNA in Foods Using Real-Time PCR

JENNIFER L. BRZEZINSKI*

U.S. Food and Drug Administration, Forensic Chemistry Center, 6751 Steger Drive, Cincinnati, Ohio 45237-3097, USA

MS 06-430: Received 9 August 2006/Accepted 7 November 2006

ABSTRACT

The detection of potentially allergenic foods, such as sesame seeds, in food products is a major concern for the food-processing industry. A real-time PCR method was designed to determine if sesame seed DNA is present in food products. The PCR reaction amplifies a 66-bp fragment of the sesame seed 2S albumin gene, which is detected with a sesame-specific, dual-labeled TaqMan probe. This reaction will not amplify DNA derived from other seeds present in baked goods, such as pumpkin, poppy, and sunflower seeds. Additionally, this assay will not cross-react with DNA from several tree nut species, such as almond, Brazil nut, cashew, hazelnut, and walnut, as well as four varieties of peanut. This assay is sensitive enough to detect 5 pg of purified sesame seed DNA, as well as sesame seed DNA in a spiked wheat cracker sample.

The incidence of sesame seed allergy in children has been increasing for the past several decades (9) and has been documented in several countries, including Great Britain (7) and Australia (25). In Israel, sesame seed is a major cause of food allergy (5, 15); only egg and cow's milk allergy were found to be more prevalent in that country (6). Clinical presentation of sesame seed allergy can result in either a delayed-type hypersensitivity reaction, characterized by atopic dermatitis (23), or an immediate-type systemic immunoglobulin E-mediated response, resulting in anaphylactic shock in severe cases (1, 9, 19). Many sesame-allergic patients are also allergic to tree nuts or peanuts (7), which is not surprising given the high level of antigenic cross-reactivity observed between sesame seeds and tree nuts (28).

Sesame seeds may contain as many as 10 allergens (8, 13), including oleosins (14) and seed storage proteins such as the 7S vicilin-type globulin (2), and the β -globulin or 2S albumin proteins (21, 29, 30). The 2S albumin proteins are small water-soluble proteins that are rich in sulfur-containing amino acids, such as cysteine and methionine, and consist of two subunits held together via a disulfide linkage (3). The 2S albumins are highly resistant to proteolytic digestion, as well as thermal and chemical denaturation (16, 18). The 2S albumins have also been determined to be an allergen of Brazil nut (20), cashew (27), hazelnut (22), walnut (26), and mustard seed (10). Since sesame oil is cold pressed and therefore not heated during processing, sesame oil may also pose a risk for allergic individuals (4, 12).

The present study describes the development of a real-time PCR method designed to detect sesame seed DNA in food products. In this assay, amplification primers and an oligonucleotide probe are directed against the sesame seed 2S albumin gene sequence. The dual-labeled probe is

cleaved via the exonuclease activity of *Taq* polymerase, releasing a fluorescent molecule that is measured by the instrument in real time during the amplification process. The instrument records the amplification with the cycle threshold value (C_T), which is the amplification cycle number wherein the fluorescence reaches a specified threshold level; therefore, there is an inverse relationship between the amount of starting template and the C_T value (11).

MATERIALS AND METHODS

DNA sources. Locally purchased white and black sesame seeds, poppy seeds, pumpkin seeds, sunflower seeds, almonds, Brazil nuts, cashews, hazelnuts, walnuts, and several species of peanut (including Runner, Virginia, Valencia, and Spanish) were used in the preparation of genomic DNA templates.

DNA extraction. Genomic DNA was extracted from each of the above species (100 mg of ground material) with Genomic-tip 20/G columns (Qiagen, Valencia, Calif.), following the instructions for tissue samples. The protocol for the DNA extraction was modified to extend the proteinase K digestion from 2 to 16 h. After purification, DNA pellets were suspended in 100 μ l of 1 $\times$ TE (10 mM Tris and 1 mM EDTA [pH 8.0]). DNA concentrations were determined by measuring the ratio of the A_{260} and A_{280} of the sample. After quantification, the DNA samples were adjusted to a concentration of 10 ng/ μ l in 1 $\times$ TE.

Sample preparation. Samples of locally purchased prepared wheat crackers (containing enriched wheat flour, soybean oil, defatted wheat germ, sugar, cornstarch, high fructose corn syrup, salt, corn syrup monoglycerides, barley malt syrup, leavening, and vegetable color), fruit and nut bars, cookies, breakfast cereal, crackers, chocolate, and granola were each ground in a standard kitchen food processor for 30 to 60 s to obtain a fine powder. DNA was extracted from 200 mg of these preparations with the NucleoSpin Food kit (BD Biosciences, Palo Alto, Calif.). DNA was also extracted from hummus (soybean paste) without additional processing. After quantification, the DNA samples were adjusted to a concentration of 25 or 50 ng/ μ l in 1 $\times$ TE.

* Author for correspondence. Tel: 513-679-2700, Ext 217; Fax: 513-679-2761; E-mail: jennifer.brzezinski@fda.gov.

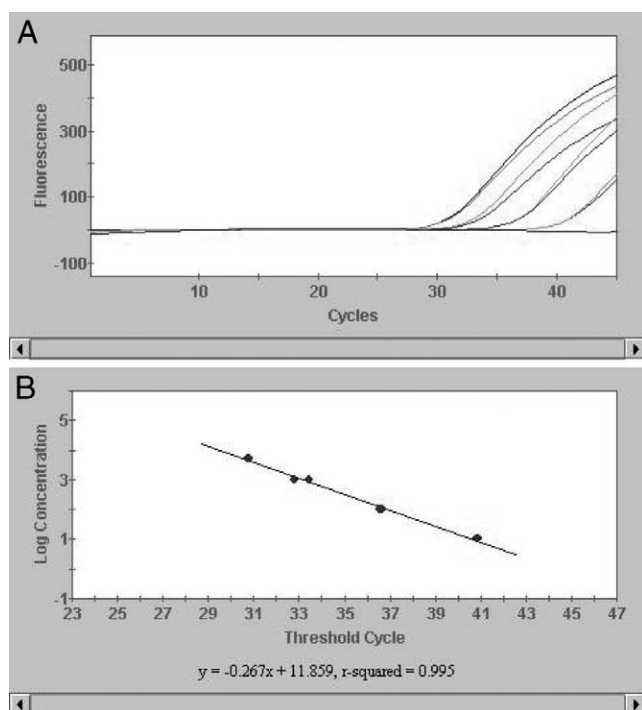


FIGURE 1. (A) Sensitivity of the sesame seed real-time PCR assay with various concentrations of purified white sesame seed genomic DNA. Amounts of DNA per reaction and C_T values appear in the figure from left to right as follows: 5 ng (30.73, 30.78); 1 ng (32.81, 33.43); 100 pg (36.58, 36.66); 10 pg (40.85, 40.88); and NTC (ND). (B) Standard curve generated from the data present in A. NTC, no template control; ND, not detected.

Sample spiking. For detection limit analysis, 20 g of ground wheat cracker was spiked with 20 mg of ground raw sesame seed (1,000 ppm) and combined in a food processor. Also, 20 g of ground wheat crackers was combined with 2 mg of ground raw sesame seed (100 ppm). DNA was extracted from 200 mg of each of these preparations with the NucleoSpin Food kit (BD Biosciences). After quantification, the DNA samples were adjusted to a concentration of 25 ng/ μ l in 1 $\times$ TE.

2S Albumin real-time PCR. Sesame seed DNA was amplified in a 25- μ l reaction containing 1 $\times$ TaqMan Universal PCR Master Mix (Applied Biosystems, Foster City, Calif.), 120 nM of each of the forward and reverse primers (5'-TTACCAGAGGGC-TAGGGACCTT-3', melting [or midpoint temperature] [T_m] = 60.1°C and 5'-AACTCGGAATTGGCATTGCT-3', T_m = 54.6°C, respectively), and 200 nM probe (5'-FAM-CTCGCAGGTGCAACATGCGACC-TAMRA-3', T_m = 64.2°C) (all from Synthesgen, LLC, Houston, Tex.). GenBank accession number AF240005 was used in the design of primers and probes. Thermal cycling conditions were as follows: 50°C for 2 min, 95°C for 10 min, and then 50 cycles at 95°C for 15 s and at 56°C for 1 min. The master mix contained an enzyme that degrades previously amplified PCR products during the 50°C soak prior to polymerase activation (therefore reducing PCR product carryover contamination). All data were acquired with the Smart Cycler II (Cepheid, Sunnyvale, Calif.).

RESULTS AND DISCUSSION

Assay sensitivity. Figure 1A shows a typical amplification curve from purified sesame seed DNA. A total of 10 pg of sesame seed DNA can be detected with this assay in

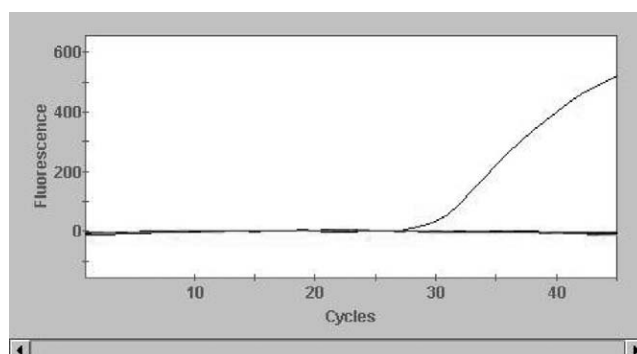


FIGURE 2. Specificity of the sesame seed real-time PCR. Data represent the amplification of 10 ng each of DNA from the following species: white sesame seed (C_T = 29.78); poppy seed (ND); pumpkin seed (ND); sunflower seed (ND); almond (ND); Brazil nut (ND); cashew (ND); hazelnut (ND); runner peanut (ND); Spanish peanut (ND); Valencia peanut (ND); Virginia peanut (ND); walnut (ND); and NTC (ND). NTC, no template control; ND, not detected.

a concentration-dependent manner. This reaction also demonstrates sufficient linearity and reproducibility, as shown in Figure 1B, to generate a standard curve for the purpose of sample quantification. Although 5 pg of sesame seed DNA can be consistently detected with this assay, quantification at that level is unreliable (data not shown). Attempts to amplify 1 pg of sesame seed DNA were unsuccessful (data not shown).

Assay specificity. The DNA of several species of tree nuts (almond, Brazil nut, hazelnut, and walnut), several types of peanut (Runner, Virginia, Valencia, and Spanish), and several varieties of edible seeds (poppy, pumpkin, and sunflower) was used to verify the specificity of the amplification. As shown in Figure 2, only the sesame seed DNA was amplified with this assay. Since sesame seeds are commonly found in baked goods—and therefore where likely contamination would occur—several baked goods, including crackers, cereals, granolas, and nut-containing chocolate bars, were tested for the presence of sesame seed DNA. Additionally, sesame seed-containing processed foods were used as positive controls. Only the products labeled as containing sesame were positive with this assay when 50 ng of DNA was amplified for 50 cycles. However, sesame seed DNA was not detected from a breakfast cereal sample that had been labeled to contain sesame seeds (Table 1). Interestingly, DNA was extracted from several samples of breakfast cereal, and in each case, the total DNA yield from an equivalent amount of starting material was generally 10 to 20% of other food matrices. It may be that the processing of breakfast cereal results in significant DNA degradation, which may hamper molecular allergen analysis.

Spiked sample analysis. To determine the limit of detection of the assay in a complex food matrix, wheat crackers were spiked with ground sesame seed at concentrations of 0.1 and 0.01% (100 mg/kg). To measure the homogeneity of the mixtures, three 200-mg samples were taken from each spike preparation for DNA extraction. Each DNA sample was then assayed in triplicate. Table 2 shows

TABLE 1. Analysis of processed food samples for sesame seed DNA^a

DNA (ng)	Food	Average C_T
50	Fruit and nut bar 1	ND
50	Fruit and nut bar 2	ND
50	Fruit and nut bar 3	30.9 ± 0.33 ^b
50	Breakfast cereal 1	ND
50	Breakfast cereal 2	ND ^b
50	Chocolate w/almonds 1	ND
50	Chocolate w/almonds 2	ND
50	Snack cracker 1	30.8 ± 0.24 ^b
50	Snack cracker 2	ND
50	Granola 1	ND
50	Granola 2	ND
50	Granola 3	ND
50	Hummus	29.3 ± 0.24 ^b
50	Tahini cookie	26.2 ± 0.13 ^b
	No template control	ND

^a C_T , cycle threshold ± standard deviation; ND, not detected.

^b Labeled as containing sesame.

the average C_T values for each spike preparation, as well as the average recovery of sesame seed DNA from each sample. When compared with the predicted amount of sesame seed DNA that should be recovered in the assay based on the spike concentration (e.g., 100 ng of DNA from a 0.1% [1:1,000] sesame seed mixture should contain 100 pg of sesame seed DNA), the values obtained were similar to the quantity of sesame seed DNA present in each reaction vessel and demonstrated that sesame seed DNA can be detected in a sample containing as little as 100 mg of sesame seed per kg. DNA from control snack crackers to which no sesame had been added did not amplify with the sesame seed primers (Table 1). Because 5 pg of purified sesame seed DNA can be detected with this assay, the limit of detection for a wheat cracker sample is likely to be 50 mg/kg.

Care is necessary to reduce pipetting errors, which, given the sensitivity of the assay, can result in poor quantification of target DNA. Also, the sensitivity of the assay may be improved by analyzing more DNA per reaction or by increasing the probe concentration. Careful consideration of the food matrix to be analyzed should therefore be undertaken, and appropriate measures should be taken to minimize the effects of the high protein, salt, and fat concentrations that may be present in various food matrices.

DNA-based methods used for allergen testing do not detect the allergen in question, and therefore, may not coincide with allergen exposure (24). The lowest reactive threshold of sesame seed in sensitive individuals was determined to be less than 30 mg on the basis of oral challenges in a recent study (17). Therefore, the presence of sesame seed DNA may indicate that a food product has the potential to be a health hazard. This assay is likely to be most useful in the rapid identification of a potential contaminant and to be used with a secondary, more sensitive method, such as the commercially available enzyme-linked immunosorbent assay (which has a purported sensitivity of

TABLE 2. Quantification of sesame seed DNA from crackers spiked with various amounts of sesame seed^a

Quantity of DNA analyzed from sample	Mean C_T	Predicted pg/reaction	Mean pg/reaction	Relative SD (%)
100 ng/0.1% sesame	36.8 ± 0.4	100	113.4 ± 25.6	22.4
100 ng/0.01% sesame	41.3 ± 0.7	10	7.5 ± 3.1	41.3
Crackers only (no spike)	ND			

^a C_T , cycle threshold ± standard deviation; ND, not detected.

1 ppm). The identification of undeclared allergen DNA in a food product represents a violation of U.S. and European Union food labeling laws and may alert a food manufacturer to potential cross-contamination issues.

REFERENCES

1. Agne, P. S., E. Bidat, P. S. Agne, F. Rance, and E. Paty. 2004. Sesame seed allergy in children. *Allergy Immunol. (Paris)* 36:300–305.
2. Beyer, K., L. Bardina, G. Grishina, and H. A. Sampson. 2002. Identification of sesame seed allergens by 2-dimensional proteomics and Edman sequencing: seed storage proteins as common food allergens. *J. Allergy Clin. Immunol.* 110:154–159.
3. Breiteneder, H., and C. Ebner. 2000. Molecular and biochemical classification of plant-derived food allergens. *J. Allergy Clin. Immunol.* 106:27–36.
4. Chiu, J. T., and I. B. Haydik. 1991. Sesame seed oil anaphylaxis. *J. Allergy Clin. Immunol.* 88:414–415.
5. Dalal, I., I. Binson, A. Levine, E. Somekh, A. Ballin, and R. Reifen. 2003. The pattern of sesame sensitivity among infants and children. *Pediatr. Allergy Immunol.* 14:312–316.
6. Dalal, I., I. Binson, R. Reifen, Z. Amitai, T. Shohat, S. Rahmani, A. Levine, A. Ballin, and E. Somekh. 2002. Food allergy is a matter of geography after all: sesame as a major cause of severe IgE-mediated food allergic reactions among infants and young children in Israel. *Allergy* 57:362–365.
7. Derby, C. J., M. H. Gowland, and J. O. Hourihane. 2005. Sesame allergy in Britain: a questionnaire survey of members of the Anaphylaxis Campaign. *Pediatr. Allergy Immunol.* 16:171–175.
8. Fremont, S., N. Zitouni, G. Kanny, V. Veneri, M. Metche, D. A. Moneret-Vautrin, and J. P. Nicolas. 2002. Allergenicity of some isoforms of white sesame proteins. *Clin. Exp. Allergy* 32:1211–1215.
9. Gangur, V., C. Kelly, and L. Navuluri. 2005. Sesame allergy: a growing allergy of global proportions? *Ann. Allergy Asthma Immunol.* 95: 4–11.
10. Gonzalez De La Pena, M. A., R. I. Monslave, E. Batanero, M. Vilalba, and R. Rodriguez. 1996. Expression in *Escherichia coli* of Sin a 1, the major allergen from mustard. *Eur. J. Biochem.* 237:827–832.
11. Holland, P. M., R. D. Abramson, R. Watson, and D. H. Gelfand. 1991. Detection of specific polymerase chain reaction product by utilizing the 5′–3′ exonuclease activity of *Thermus aquaticus* DNA polymerase. *Proc. Natl. Acad. Sci. USA* 88:7276–7280.
12. Kanny, G., C. De Hauteclouque, and D. A. Moneret-Vautrin. 1996. Sesame seed and sesame seed oil contain masked allergens of growing importance. *Allergy* 51:952–957.
13. Kolopp-Sarda, M. N., D. A. Moneret-Vautrin, B. Gobert, G. Kanny, M. Brodschii, M. C. Bene, and G. C. Faure. 1997. Specific humoral immune responses in 12 cases of food sensitization to sesame seed. *Clin. Exp. Allergy* 27:1285–1291.
14. Leduc, V., D. A. Moneret-Vautrin, J. T. C. Tzen, M. Morisset, L. Guerin, and G. Kanny. 2006. Identification of oleosins as major allergens in sesame seed allergic patients. *Allergy* 61:349–356.

15. Levy, Y., and Y. L. Danon. 2001. Allergy to sesame seed in infants. *Allergy* 56:193–194.
16. Mills, E. N., J. A. Jenkins, M. J. Alcocer, and P. R. Shewry. 2004. Structural, biological, and evolutionary relationships of plant food allergens sensitizing via the gastrointestinal tract. *Crit. Rev. Food Sci. Nutr.* 44:379–407.
17. Morisset, M., D. A. Moneret-Vautrin, G. Kanny, L. Guenard, E. Beaudouin, J. Flabbee, and R. Hatahet. 2003. Thresholds of clinical reactivity in milk, egg, peanut and sesame in immunoglobulin E-dependent allergies: evaluation by double-blind or single-blind placebo-controlled oral challenges. *Clin. Exp. Allergy* 33:1046–1051.
18. Murtagh, G. J., D. B. Archer, M. Dumoulin, S. Ridout, S. Matthews, S. H. Arshad, and M. J. Alcocer. 2003. *In vitro* stability and immunoreactivity of the native and recombinant plant food 2S albumins Ber e 1 and SFA-8. *Clin. Exp. Allergy* 33:1147–1152.
19. Panizzolo, C., M. Tura, and A. Barbato. 2005. Anaphylaxis to sesame paste. *Allergy Immunol. (Paris)* 37:34–35.
20. Pastorello, E. A., L. Farioli, V. Pravettoni, M. Ispano, A. Conti, R. Ansaloni, F. Rotondo, C. Incorvaia, A. Bengtsson, F. Rivolta, C. Trambaioli, M. Previdi, and C. Ortolani. 1998. Sensitization of the major allergen of Brazil nut is correlated with the clinical expression of allergy. *J. Allergy Clin. Immunol.* 102:1021–1027.
21. Pastorello, E. A., E. Varin, L. Farioli, V. Pravettoni, C. Ortolani, C. Trambaioli, D. Fortunato, M. G. Giuffrida, F. Rivolta, A. Robino, A. M. Calamari, L. Lacava, and A. Conti. 2001. The major allergen of sesame seeds (*Sesamum indicum*) is a 2S albumin. *J. Chromatogr. B Biomed. Sci. Appl.* 756:85–93.
22. Pastorello, E. A., S. Vieths, V. Pravettoni, L. Farioli, C. Trambaioli, D. Fortunato, D. Luttkopf, M. Calamari, R. Ansaloni, J. Scibilia, B. K. Ballmer-Weber, L. K. Poulsen, B. Wutrich, K. S. Hansen, A. M. Robino, C. Ortolani, and A. Conti. 2002. Identification of hazelnut major allergens in sensitive patients with positive double-blind, placebo-controlled food challenge results. *J. Allergy Clin. Immunol.* 109:563–570.
23. Pecquet, C., F. Leynadier, and P. Saiag. 1998. Immediate hypersensitivity to sesame in foods and cosmetics. *Contact Dermatitis* 39: 313.
24. Poms, R. E., C. L. Klein, and E. Anklaam. 2004. Methods for allergen analysis in food: a review. *Food Addit. Contam.* 21:1–31.
25. Sporik, R., and D. Hill. 1996. Allergy to peanut, nuts and sesame seed in Australian children. *Br. Med. J.* 313:1477–1478.
26. Tueber, S. S., A. M. Dandekar, W. R. Peterson, and C. L. Sellers. 1998. Cloning and sequencing of a gene encoding a 2S albumin seed storage protein precursor from English walnut (*Juglans regia*), a major food allergen. *J. Allergy Clin. Immunol.* 101:807–814.
27. Tueber, S. S., S. K. Sathe, W. R. Peterson, and K. H. Roux. 2002. Characterization of the soluble allergenic proteins of cashew nut (*Anacardium occidentale* L.). *J. Agric. Food Chem.* 50:6543–6549.
28. Vocks, E., A. Borga, C. Szliska, H. U. Seifert, B. Seifert, G. Burrow, and S. Borelli. 1993. Common allergenic structures in hazelnut, rye grain, sesame seeds, kiwi, and poppy seeds. *Allergy* 48:168–172.
29. Wolff, N., U. Cogan, A. Admon, I. Dalal, Y. Katz, N. Hodos, N. Karin, and S. Yannai. 2003. Allergy to sesame in humans is associated primarily with IgE antibody to a 14 kDa 2S albumin precursor. *Food Chem. Toxicol.* 41:1165–1174.
30. Wolff, N., S. Yannai, N. Karin, Y. Levy, R. Reifen, I. Dalal, and U. Cogan. 2004. Identification and characterization of linear B-cell epitopes of β -globulin, a major allergen of sesame seeds. *J. Allergy Clin. Immunol.* 114:1151–1158.