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

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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 albums are highly resistant to proteolytic digestion, as well as thermal and chemical denaturation (16, 18). The 2S albums 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 μL of 1× 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/μL in 1× TE.

Sample preparation. Samples of locally purchased prepared wheat crackers (containing enriched wheat flower, 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/μL in 1× TE.

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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/µl in 1× TE.

2S Albumin real-time PCR. Sesame seed DNA was amplified in a 25-µl reaction containing 1× TaqMan Universal PCR Master Mix (Applied Biosystems, Foster City, Calif.), 120 nM of each of the forward and reverse primers (5′-TTACCAGGGGCTAGGGACCTT-3′, melting [or midpoint temperature] [Tm] = 60.1°C and 5′-AACTCGGAATTGGCATTGCT-3′, Tm = 54.6°C, respectively), and 200 nM probe (5′-FAM-CCTGCAAGTGCAACTGCGACC-TAMRA-3′, Tm = 64.2°C) (all from Synthegen, 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 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 DNAa

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

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 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


