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Development of a Real-Time PCR Method To Detect Potentially Allergenic Sesame (*Sesamum indicum*) in Food

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Recent papers indicate that the prevalence of allergic reactions to sesame (*Sesamum indicum*) is increasing in European countries. This paper describes the development of a selective real-time PCR method for the detection of sesame in food. The assay did not show any cross-reactivity with 17 common food ingredients. The real-time PCR method was applied to determine sesame in several crackers, salty snacks, biscuits, tahina sesame paste and sesame oil. With the exception of sesame oil, in all of the samples where sesame was declared, sesame was detected by the real-time PCR assay (Ct value < 35). In the samples which might contain sesame or where sesame was not listed, sesame could not be detected (Ct value > 35).

KEYWORDS: Sesame; *Sesamum indicum*; allergen; real-time polymerase chain reaction (real-time PCR); molecular beacon; food

INTRODUCTION

Food allergies pose an increasing health problem, particularly in industrialized countries. The actual prevalence of allergic reactions to food has been estimated to be about 3% in the adult population and between 6 and 8% in young children (*1*). However, the prevalence of food allergy differs from country to country and is affected by genetic factors, by cultural and dietary habits, and, in particular, by the exposure date to the certain food product in life (*2*). In the past, sesame (*Sesamum indicum)* allergy was only common in Eastern countries, for example in Israel, where sesame containing foods are frequently consumed early in life (*3*). Recent papers, however, indicate that allergic reactions to sesame have become more frequent in European countries (*4*, *5*). The increasing prevalence of sesame allergy has been associated with an increasing consumption of sesame seeds and sesame oil in Western countries. These days, sesame seeds are contained in numerous food products, such as bakery products, fast-foods, and vegetarian and ethnic dishes whereas sesame oil is a common ingredient in oriental, Chinese, and South American cuisines.

Several allergic proteins have already been identified in sesame, such as a sulfur poor 2S albumin with a molecular weight of 10 kDa (Ses i 1) (*6*), a sulfur rich 2S albumin (Ses i 2, 7 kDa), and a 7S vicillin-like globulin (Ses i 3, 45 kDa) (*7*). Ses i 1 has been reported to show about 40% homology to allergens of sunflower seeds, Brazil nut, and castor bean, and Ses i 3 shows about 41% homology to the walnut allergen Jug r2 and 36% homology to the peanut allergen Ara h1.

Sesame seeds are known to be very potent allergens, causing particularly severe reactions in sensitized persons with a high risk of life threatening anaphylaxis (*4*, *5*, *8*). In oral challenge based studies, doses of 30 mg of sesame seed or 1–5 mL of sesame oil could already trigger allergic reactions in sesame allergic patients (*9*).

The only preventive strategy for sesame allergic patients is to strictly avoid sesame containing foods. In order to facilitate the identification of allergenic ingredients in foodstuffs, the European Union legislation has recently been modified. According to Directive 2006/142/EC, 14 specified allergenic foods must be indicated in the list of ingredients, including sesame seeds and products thereof (*10*). However, food may contain traces of sesame although it is not indicated on the list of ingredients. These undeclared, so-called "hidden" allergens originating from contamination during food processing pose a particular problem to sesame allergic persons.

Highly selective and sensitive analytical methods are necessary to verify if allergen containing products are labeled in compliance with the regulations and to enable the detection of hidden allergens. In general, the detection of allergens is a very difficult task, since they are often present in minute amounts in very complex, often highly processed food matrices.

The methods developed so far are based on either protein or DNA detection. Recent review articles on the detection of food allergens critically discuss the main benefits and problems of enzyme linked immunosorbent assays (ELISAs), the most common method used for detecting food allergens, and methods based on the polymerase chain reaction (PCR) (*11*–*13*). ELISAs have already been developed to detect food allergens such as peanut (*14*–*16*) or hazelnut (*16*–*19*). In spite of the controversial discussion of allergen detection by PCR, several papers have

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Table 1. Primers and Fluorescent Probe Used in PCR

primer/probe	sequence 5′→3′	T_m (°C)	product size (bp)
primer 1 forward	CACAGCAGGTTTACCAGAGG	55.2	143
primer 1 reverse	TTATACATTTCCTCGCACAACC	54.3	
primer 2 forward	TGAGGAACGTGGACGAGAG	55.5	117
primer 2 reverse	CCCTAGCCCTCTGGTAAACC	56.1	
molecular beacon (FAM)	FAM-CGCGATCGCAGTGAGGCAGCAGCAGCAGGGATCGCG-DABCYL	66.1	
primer 3 forward	GGTGAAGAGGATGAAGTTCTGG	55.9	111
primer 3 reverse	CTCACAGCGGCACCTCTC	56.6	

Table 2. Purity and Yield of DNA Extracted from Different Food Samples

sample	extraction method	A_{260}	A_{260}/A_{280}	DNA yield (μg)
crisp bread wheat	QIAmp DNA Mini Kit	0.348	1.8	17.4
salty snack	QIAmp DNA Mini Kit	0.395	1.9	19.8
salty snack with garlic	QIAmp DNA Mini Kit	0.505	1.9	25.3
cookies with sesame 1	QIAmp DNA Mini Kit	0.215	1.8	10.8
sesame oil	QIAmp DNA Mini Kit	0.147	1.2	4.4
tahina sesame paste	QIAmp DNA Mini Kit	0.843	1.3	42.2
bread with sesame	CTAB	0.791	1.9	31.6
crisp bread rye with sesame	CTAB	0.796	1.8	19.9
crisp bread rye	CTAB	0.694	1.8	3.5
crisp bread wheat and rye	CTAB	0.311	1.9	7.8
salty snack with sesame 1	CTAB	0.224	2.0	2.3
salty snack with sesame 2	CTAB	0.125	1.7	3.1
salty snack with herbs	CTAB	0.303	1.7	1.5
salty snack with sesame 3	CTAB	0.738	1.8	18.5
cracker with sesame	CTAB	0.475	1.9	2.4
cookies with sesame 2	CTAB	0.400	2.2	10.0
rice wafer with milk chocolate	CTAB	0.461	1.7	2.3

already demonstrated its applicability to detect peanut (*15*, *20*), hazelnut (*21*, *22*), or walnut (*23*) in food.

PCR test kits for detecting allergenic foods are already commercially available. However, in most cases, the manufacturers do not reveal which gene is targeted. In addition, too little information is given concerning the applicability of the test kits to different food matrices. To our knowledge, a real-time PCR method allowing the detection of sesame in food has not been published in a peer reviewed journal up to now. The aim of the present study was to develop a real-time PCR method targeted at the gene coding for Ses i 1, one of the major allergenic proteins of sesame.

MATERIALS AND METHODS

Chemicals and Food Products. Cetyltrimethylammonium bromide (CTAB), tris(hydroxymethyl)aminomethane (Tris), isopropanol, and sodium chloride were purchased from Sigma (Vienna, Austria). Ethylenediaminetetraacetic acid (EDTA) and chloroform were obtained from VWR (Darmstadt, Germany). Agarose and ethidium bromide were obtained from BioRad (Hercules, United States). PCR reactions were carried out with water which had been bidistilled and subsequently autoclaved. Sesame samples from different producers (five white, three black, and one golden (roasted) sesame samples) and food products were bought in local food stores.

Extraction of Genomic DNA. About 5 g of the food samples were ground in a mechanical mortar (type MM 2000, Retsch, Haan, Germany) for 20 min and stored at 4 °C until DNA extraction.

Extraction Using the QIAmp DNA Mini Kit. The QIAmp DNA Mini Kit (Qiagen, Hilden, Germany) was used according to the instructions of the manufacturer. In brief, about 20–50 mg of the ground food sample were weighed in a microreaction tube and lysed with 180 μL of loading buffer 1 and 20 μL of proteinase K solution (reagents supplied with the kit). After incubating the mixture at 56 °C for either

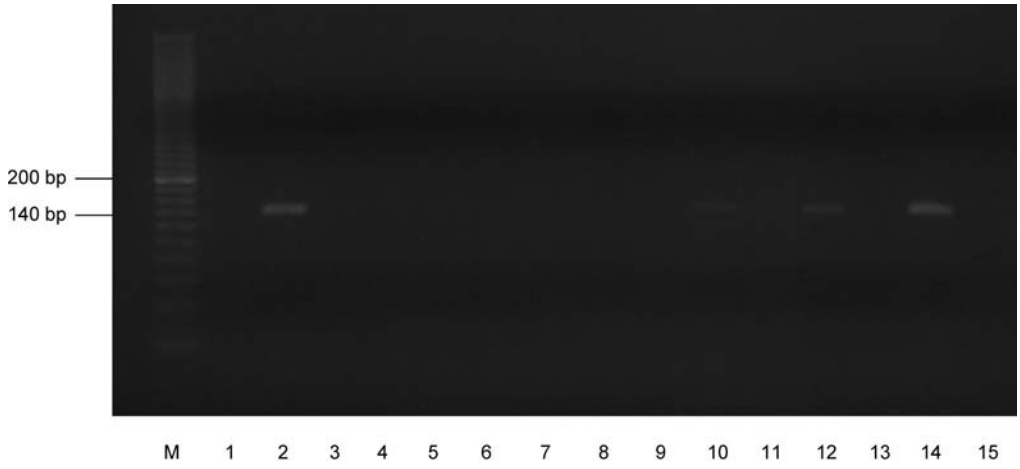

Figure 1. Agarose gel of PCR reaction mixtures obtained after Sybr Green PCR with primer pair 1: M, 20 bp marker; 1, negative control; 2, positive control; 3, brazil nut; 4, sunflower seeds; 5, peanut; 6, hazelnut; 7, walnut; 8, rice; 9, soy bean; 10, crisp bread; 11, salty snack 1; 12, sesame cookies; 13, black sesame; 14, tahina (sesame paste); 15. sesame oil.

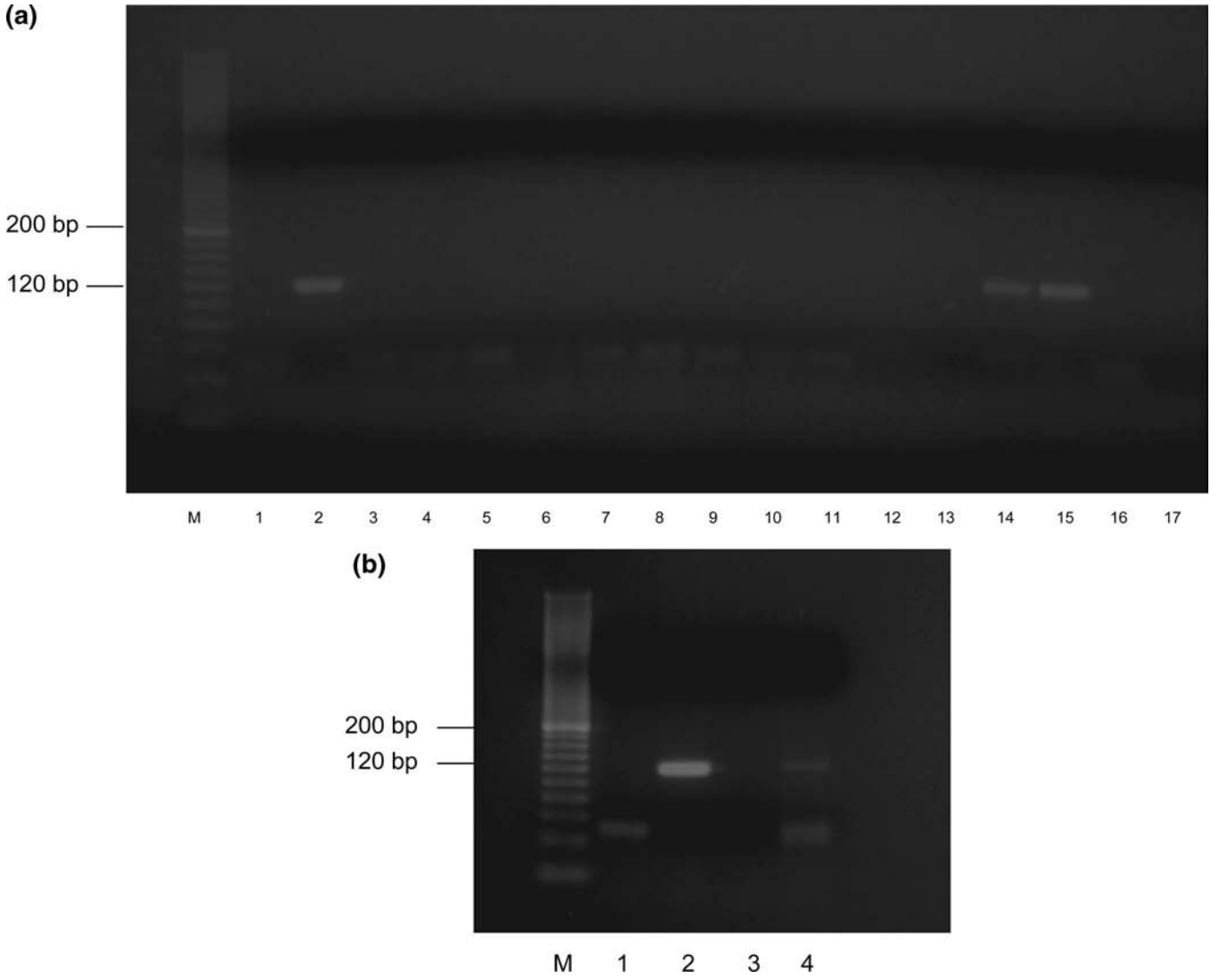

Figure 2. Agarose gel of PCR reaction mixtures obtained after Sybr Green PCR with primer pair 2: (**a**) M, 20 bp marker; 1, negative control; 2, positive control; 3, brazil nut; 4, sunflower seeds; 5, peanut; 6, hazelnut; 7, walnut; 8, rice; 9, soy bean; 10, honey; 11, poppy seeds; 12, oat; 13, rye; 14, sesame cookies; 15, tahina (sesame paste); 16, sesame oil; 17, black sesame. (**b**) M, 20 bp marker; 1, negative control; 2, positive control; 3, chocolate; 4, wheat.

3–5 h or overnight, 20 μL of RNase solution (20 μg/mL) were added. After incubating the mixture at room temperature for 2 min, 200 μL of loading buffer 2 (supplied with the kit) were added. After incubation at 70 °C for 10 min, 200 μL of ethanol were added and the mixture was applied to a spin column delivered with the kit. After centrifugation (model 5413, Laborpartner, Vienna, Austria) at 8400*g* for 1 min, the column was washed with 500 μL of washing buffer 1 and washing buffer 2 (supplied with the kit). Elution was carried out with 200 μL of elution buffer (supplied with the kit). The eluate was reapplied to the column, and the elution step was repeated. The purified DNA was stored at 4 °C until analysis.

Extraction Using the CTAB Method. 100 mg of the ground food sample were weighed in and mixed with 500 μL of CTAB extraction solution (20 mg/mL CTAB, 1.4 M NaCl, 0.1 M Tris, 0.02 M EDTA, adjusted to pH 8 with concentrated HCl) and incubated at 65 °C for 30 min. After centrifugation for 10 min at 8400*g*, the supernatant was transferred into a new 1.5 mL microreaction tube. After adding 200 μL of chloroform, the mixture was vortexed for 1 min and centrifuged for 5 min. The aqueous phase was transferred into a new tube, mixed with 2 parts by volume of CTAB precipitation solution (5 g/L CTAB, 0.04 M NaCl), and incubated at room temperature for 60 min. After centrifugation for 5 min, the precipitate was dissolved in 350 μL of 1.2 M sodium chloride solution. 350 μL of chloroform were added, and the mixture was vortexed for 1 min. The aqueous phase was transferred into a new tube, and 0.6 parts by volume of isopropanol were added. After centrifugation for 10 min, the pellet was mixed with 500 μL of ice cold ethanol. After a further centrifugation step (10 min), the supernatant was removed with a pipet. The remaining ethanol was dried in an oven (UE 500, Memmert, Schwabach, Germany) at 37 °C. The pellet was dissolved in 100 μL of H_2O_{dd}. The extracted DNA was stored at 4 °C until analysis.

Determination of the Yield and Quality of DNA Extracts. DNA was quantified by measuring the absorbance at 260 nm (A_{260}) in either elution buffer (supplied with the Qiagen QIAmp DNA Mini Kit) or water (CTAB method) with a spectrophotometer (Spectronic Genesys 10UV, Thermo Scientific, Waltham, MA). The DNA concentration in the extracts was determined according to the equation c [μg/mL] = $A_{260} \times 50 \times$ dilution factor. The quality of the isolated DNA was determined by calculating the A_{260}/A_{280} ratio.

PCR Analysis. *Design of Primers and the Molecular Beacon Probe.* Oligonucleotide primers and the molecular beacon probe were designed using the Beacon Designer 6.0 software from Premier Biosoft International (Palo Alto, CA). In order to allow the detection of sesame, the gene coding for the Ses i 1 protein, one of the major allergenic proteins of sesame (NCBI accession No. AF240005), was selected.

The primers were designed to meet the following requirements: to produce an amplicon 100–150 bp long, to have a melting temperature between 50 and 60 °C, and to have a length between 18 and 22 bases. The probe was designed to have a melting temperature ~5 °C higher than that of the primers and a length between 18 and 30 bp.

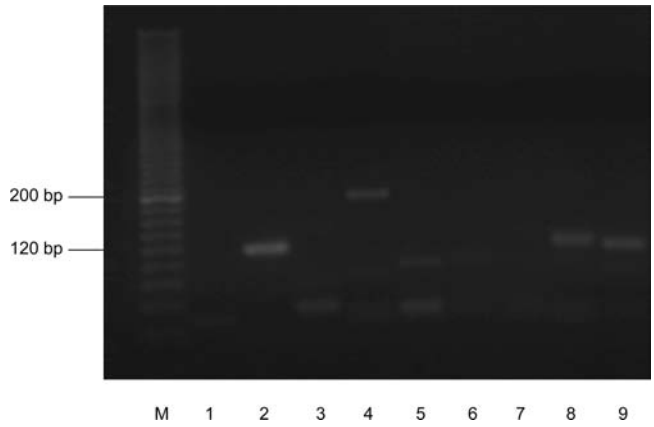

Figure 3. Agarose gel of PCR reaction mixtures obtained after Sybr Green PCR with primer pair 3: M, 20 bp marker; 1, negative control; 2, positive control; 3, brazil nut; 4, sunflower seeds; 5, peanut; 6, hazelnut; 7, walnut; 8, rice; 9, soy bean.

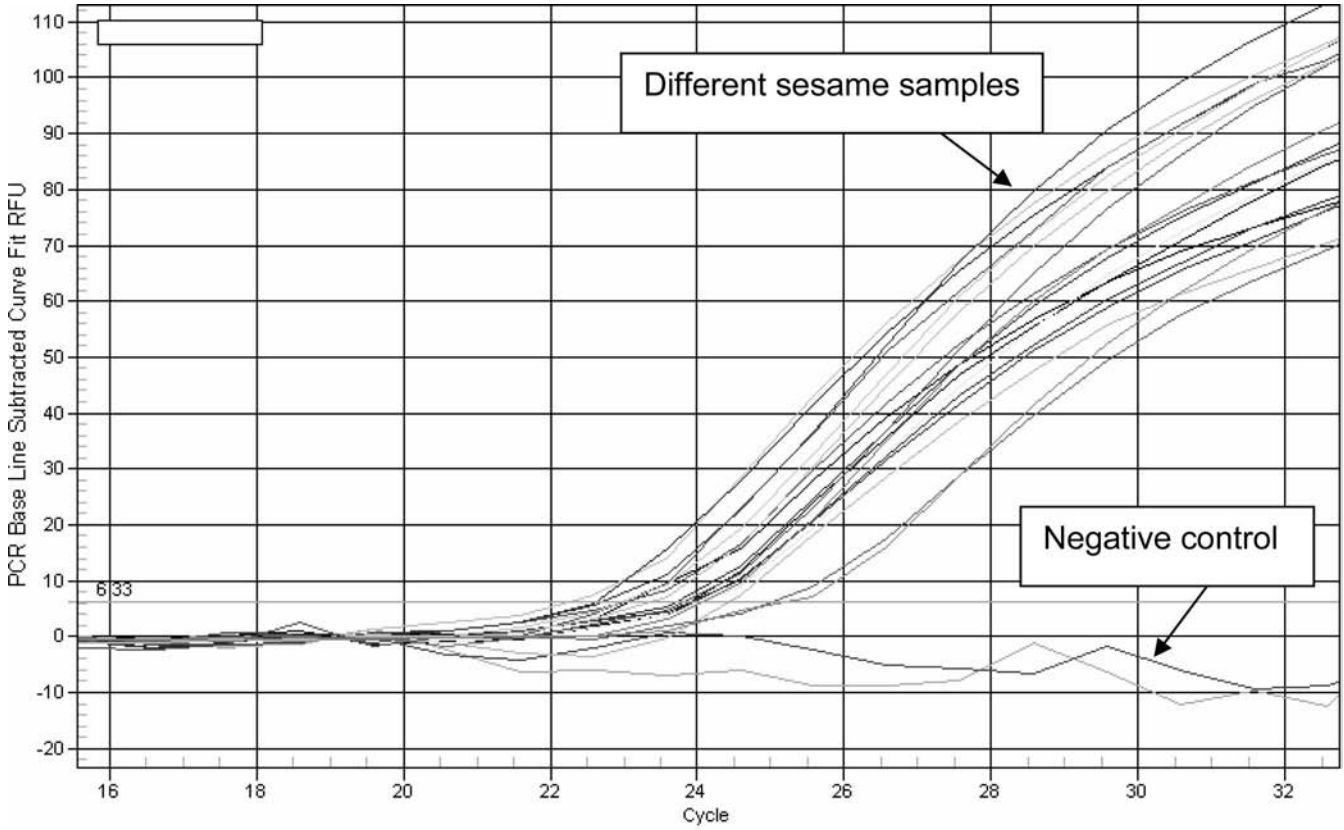

Figure 4. Real-time PCR analysis (using the molecular beacon) of DNA isolated from sesame samples from different producers (five white, three black, and one golden sesame samples).

The sequences of both the selected primer pairs and the molecular beacon probe are given in **Table 1**. The molecular beacon probe was labeled with the reporter dye FAM (6-carboxyfluorescein) on the 5′ end and the fluorescent quencher dye DABCYL (4-(4-dimethylaminophenylazo)benzoic acid) on the 3′ end. Primers and probe were synthesized by Sigma-Genosys (Steinheim, Germany).

Real-Time PCR Conditions. The PCR was performed in the iCycler thermocycler equipped with the IQ5 multicolor real time PCR detection system (BioRad). PCR reactions were carried out in 96 well PCR plates (BioRad) in a total volume of 25 μL. All DNA samples were analyzed in duplicate. On each 96 well plate, two nontemplate controls (NTCs) and two positive controls were used to check the PCR performance. Missing fluorescence signals and fluorescence signals at Ct > 35 were both interpreted as negative results, and fluorescence signals at Ct ≤ 35 were interpreted as positive results. This threshold value was chosen since with Sybr Green primer dimers were detected at Ct > 35.

Real-Time PCR with Sybr Green. Each reaction consisted of 12.5 μL of IQ Sybr Green Supermix (containing 100 mM KCl, 40 mM Tris-HCl, pH 8.4, 1.6 mM dNTPs, iTaq DNA polymerase, 50 units/mL, 6 mM $MgCl_2$, SYBR Green I, 20 nM fluorescein) from BioRad, 800 nM primer forward, 400 nM primer reverse, 5 μL of template DNA (20 ng/μL), and H_2O_{dd}. The PCR program was as follows: initial denaturation at 95 °C for 3 min followed by 45 cycles of denaturation at 95 °C for 30 s and primer annealing and elongation at 58 °C for 40 s. A final elongation step was carried out at 72 °C for 3 min.

Real-Time PCR with a Molecular Beacon. Each reaction consisted of 12.5 μL of IQ Supermix (containing 100 mM KCl, 40 mM Tris-HCl, pH 8.4, 1.6 mM dNTPs, iTaq DNA polymerase, 50 units/mL, 6 mM $MgCl_2$) from BioRad, 800 nM primer forward, 400 nM primer reverse, 100 nM molecular beacon probe, 5 μL of template DNA (20 ng/μL), and H_2O_{dd}.

The PCR program was as follows: initial denaturation at 95 °C for 3 min followed by 45 cycles of denaturation at 95 °C for 30 s and primer annealing and elongation at 62 °C for 50 s. A final elongation step was carried out at 72 °C for 3 min.

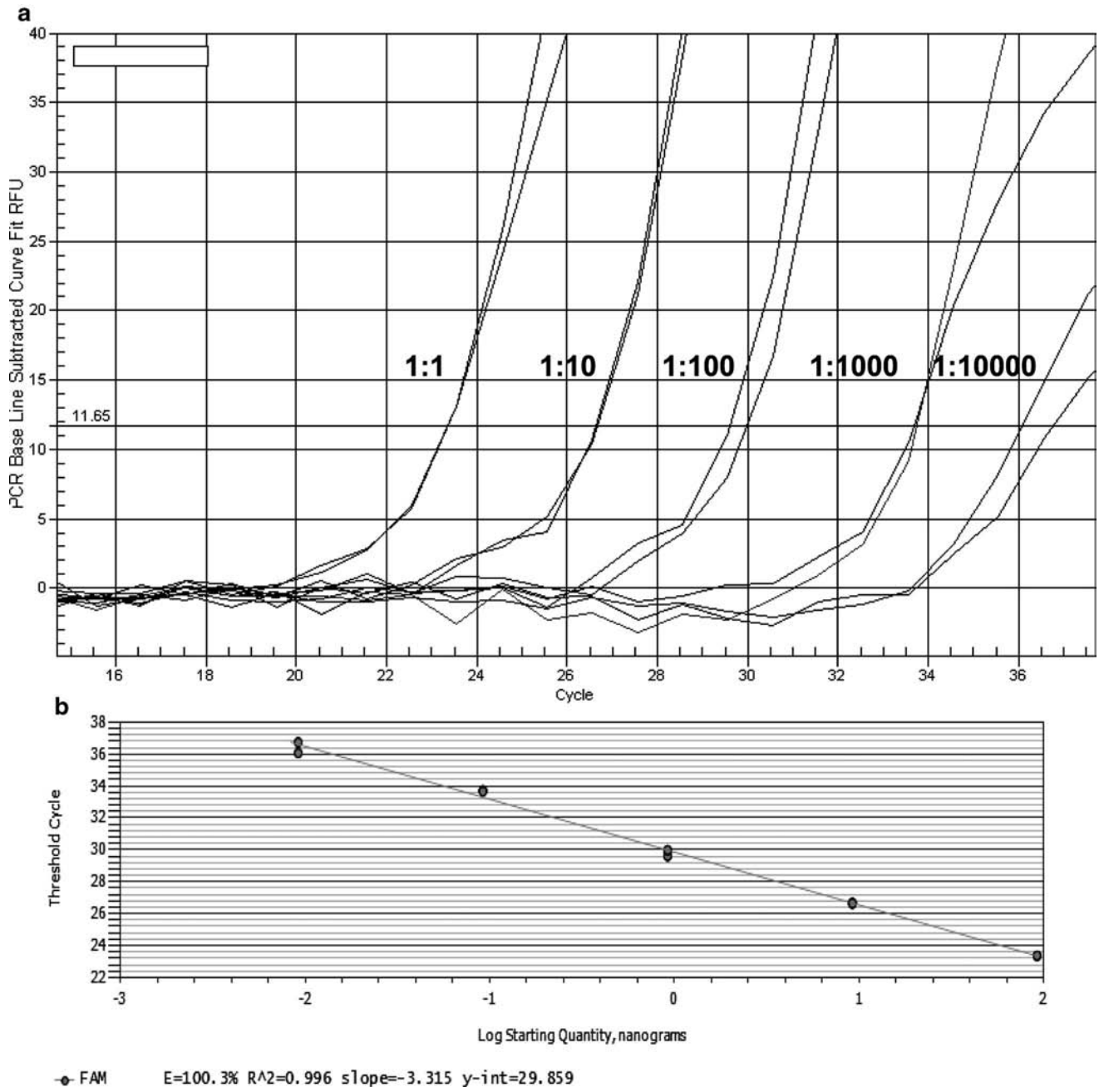

Figure 5. (**a**) Real-time PCR analysis (using the molecular beacon) of sesame DNA which was serially diluted from 1:1 to 1:10000. (**b**) Standard curve obtained by amplifying the sesame genomic DNA at different dilutions with real-time PCR using the molecular beacon.

Amplification Efficiency and Limit of Detection (LOD). The amplification efficiency and the limit of detection (LOD) were determined both in pure sesame and in crisp bread.

In the case of pure sesame, PCR reactions were carried out with serially diluted (from 1:1 to 1:1000000) DNA extracted from sesame seeds.

In order to determine the influence of matrix components on the amplification efficiency and the LOD, crisp bread with different sesame concentrations was prepared by spiking known amounts of sesame to crisp bread which had been shown to be free of sesame (Ct > 35) in a previously carried out PCR reaction.

After spiking ground crisp bread with 5% (w/w) sesame seeds, the mixture was ground in the mill for 10 min. The crisp bread containing 5% sesame was mixed with pure crisp bread to obtain concentrations of 2.5, 1, 0.5, 0.1, 0.05, 0.01, and 0.001% sesame. After extracting the DNA with the CTAB method, the real time PCR was carried out.

Inhibition Control. One microliter of sesame DNA extract (corresponding to 140 ng of DNA) was applied to a well of a PCR plate and allowed to desiccate at room temperature overnight. The PCR plate was stored at 4 °C until use. After adding DNA from negative tested food samples, the molecular beacon PCR reaction was carried out as described above. The inhibition control was carried out in duplicate.

Agarose Gel Electrophoresis. In order to analyze the PCR products by gel electrophoresis, 10 μL of the PCR product were mixed with 10 μL of a loading buffer which was prepared by diluting the nucleic acid sample loading buffer, 5× from BioRad 1:5 with water. The products were loaded on a 3% (w/v) agarose gel containing 10 μg/μL ethidium bromide. The size of the amplicons was determined by comparison with a 20 bp ladder (BioRad). Electrophoresis was carried out with 1xTAE buffer at 150 V. The gels were visualized on a UVT-20 M transilluminator (Herolab, Wiesloch, Germany).

RESULTS AND DISCUSSION

DNA Extraction. Each food sample was initially extracted using the commercially available QIAmp DNA Mini Kit. However, when loading certain samples, in particular crisp bread and salty

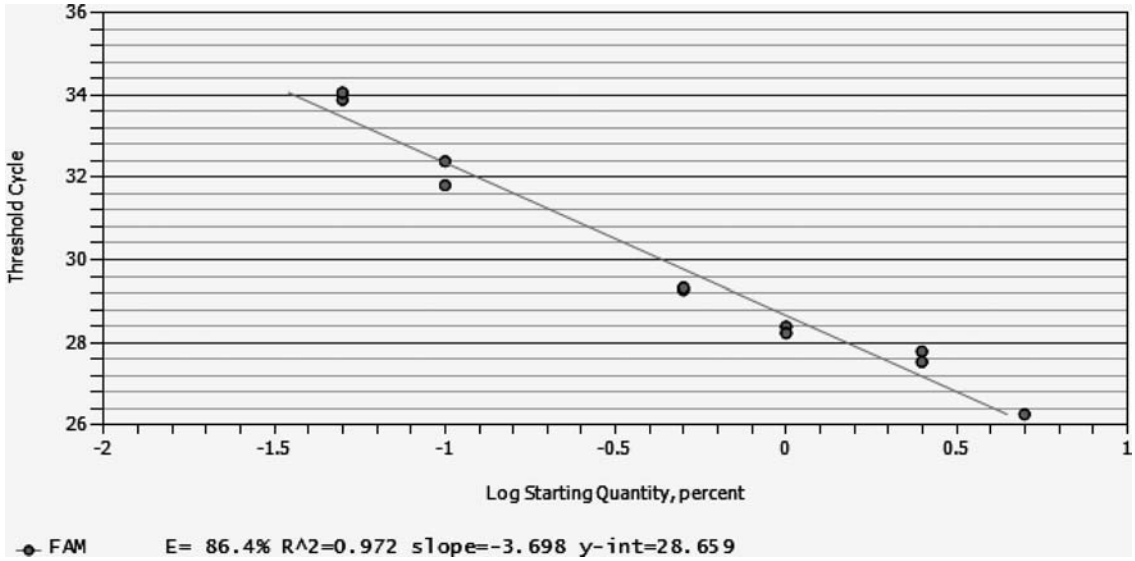

Figure 6. Standard curve obtained by amplifying DNA extracted from crisp bread which had previously been spiked with different concentrations of sesame.

Table 3. Data on the Repeatability of the Method[a]

day 1	Ct	mean Ct	*S*	RSD (%)	day 2	Ct	mean Ct	*S*	RSD (%)
1. extraction					1. extraction				
1. replicate	28.47				1. replicate	28.96			
2. replicate	28.55				2. replicate	29.32			
3. replicate	nd				3. replicate	29.15			
		28.51	0.06	0.20			29.14	0.18	0.62
2. extraction					2. extraction				
1. replicate	29.15				1. replicate	28.81			
2. replicate	29.08				2. replicate	28.58			
3. replicate	28.88				3. replicate	29.05			
		29.04	0.14	0.48			28.81	0.24	0.82
3. extraction					3. extraction				
1. replicate	28.83				1. replicate	28.52			
2. replicate	28.59				2. replicate	28.16			
3. replicate	28.85				3. replicate	29.07			
		28.76	0.14	0.50			28.58	0.46	1.60
4. extraction					4. extraction				
1. replicate	28.45				1. replicate	28.84			
2. replicate	29.14				2. replicate	28.67			
3. replicate	28.02				3. replicate	28.48			
		28.54	0.57	1.98			28.66	0.18	0.63
all replicates		28.73	0.35	1.21	all replicates		28.80	0.33	1.15
					interday		28.77	0.33	1.16

[a] DNA was extracted four times from sesame cookies with the Qiagen QIAmp DNA Mini Kit. Real-time PCR assays with a molecular beacon were run in triplicate. The whole procedure was repeated on the next day. nd = not determined.

snacks, to the spin column, the column got clogged. DNA was therefore extracted from these samples using the CTAB method. **Table 2** summarizes the extraction method, A_{260}, A_{260}/A_{280}, and the DNA yield. For most samples, the A_{260}/A_{280} was within 1.7 and 1.9, which indicates a high DNA purity. $A_{260}/A_{280} < 1.7$ was only obtained for sesame oil and tahina (sesame paste). Extraction of DNA from salty snack with sesame 1 and cookies with sesame 2 resulted in $A_{260}/A_{280} > 1.9$. DNA yields > 20 μg were obtained for salty snack with garlic, tahina sesame paste, and bread with sesame, while low DNA yields (<5 μg) were obtained for sesame oil, crisp bread rye, salty snack with sesame 1, salty snack with sesame 2, salty snack with herbs, cracker with sesame, and rice wafer with milk chocolate.

Optimization of the Real-Time PCR Reaction with Sybr Green. In preliminary experiments, the PCR reaction with Sybr Green was used in order to optimize the primer concentrations and the annealing temperature. For this reason, with each of the three primer pairs (see **Table 1**), a primer matrix was carried out (400 nM, 800 nM, 1.2 μM), applying a temperature gradient from 52 to 62 °C. The experiments were carried out with a three-step thermal program: 3 min at 95 °C followed by 45 cycles of 95 °C for 30 s and a gradient with 52–62 °C for 30 s and 72 °C for 30 s. The optimal reaction conditions were defined as those where the lowest Ct value and the highest ΔRn (which represents the background subtracted reporter fluorescence signal) were obtained. The optimal reaction conditions were found to be as follows: (primer pair 1) 800 nM primer forward, 800 nM primer reverse, annealing temperature 56 °C; (primer pair 2) 800 nM primer forward, 400 nM primer reverse, annealing temperature 58 °C; (primer pair 3) 1200 nM primer forward, 400 nM primer reverse, annealing temperature 53 °C.

Species Specificity of the Primer Pairs. In order to investigate the species specificity of the primer pairs, the DNA of different food samples and ingredients which might be present

Table 4. PCR Results Obtained by Analyzing Different Food Products[a]

sample	product declaration	result	mean Ct value
bread with sesame	no declaration	+	30.1
crisp bread rye with sesame	+ (10% sesame)	+	32.4
salty snack with sesame 2	+ (9% sesame)	+	30.3
salty snack with sesame 1	+ (8% sesame)	+	29.6
salty snack with sesame 3	+	+	30.8
cracker with sesame	+	+	26.8
cookies with sesame 1	+	+	28.9
cookies with sesame 2	+	+	24.9
tahina sesame paste	+	+	26.7
sesame oil	+	−	n.a.
crisp bread wheat	±	−	n.a.
salty snack	±	−	n.a.
salty snack with garlic	±	−	n.a.
rice wafer with milk chocolate	±	−	n.a.
crisp bread rye	−	−	n.a.
crisp bread wheat and rye	−	−	n.a.
salty snack with herbs	−	−	n.a.

[a] Declaration: ±, may contain sesame seeds; −, sesame components not listed; +, sesame listed; n.a., no amplification within 45 cycles.

in sesame containing food was subjected to PCR assays with Sybr Green. DNA extracts of Brazil nut, sunflower seeds, peanuts, hazelnuts, walnuts, rice, black sesame, soybeans, honey, chocolate, poppy seeds, oat, rye, wheat, potatoes, almonds, and maize were tested.

Figure 1 shows the agarose gel obtained by loading PCR reaction mixtures obtained with primer pair 1. Primer pair 1 showed a high specificity for sesame. None of the other plant species was amplified with this primer pair.

With primer pair 2, a PCR product of 117 bp was obtained (**Figure 2**). The sesame specific PCR product was amplified in the positive control sample, the black sesame, and all sesame containing food samples except sesame oil. However, in the case of black sesame, the band was of very low intensity. The sesame specific PCR product was not amplified in the samples from other plants (**Figure 2a**). However, an amplicon of similar size was obtained in the wheat sample (**Figure 2b**).

Amplification with primer pair 3 resulted in a PCR product of 111 bp. **Figure 3** shows that primer pair 3 resulted in a lot of unspecific DNA fragments and a PCR product of about 111 bp in soy bean.

The following experiments were carried out with primer pair 2, since it was more specific than primer pair 3 and amplified a smaller DNA fragment than primer pair 1, which was an advantage in the analysis of processed food samples. However, after optimizing the concentration and annealing temperature of the sesame specific probe, we had to verify the sesame specificity of the PCR method again.

Real-Time PCR Reaction with a Molecular Beacon. The molecular beacon was designed in the middle of the sequence of the amplicon obtained with primer pair 2. For optimization of the molecular beacon PCR assay, a temperature gradient was performed from 58 to 68 °C in order to determine the annealing temperature. In order to find the optimum molecular beacon concentration, 50, 100, 200, and 300 nM of the fluorescent probe were tested.

The lowest Ct and highest Rn values were obtained with a molecular beacon concentration of 100 nM and an annealing temperature of 62 °C.

In order to shorten the PCR reaction time, we tried to replace the three-step protocol with a two-step protocol (see Materials and Methods). Since both the signal intensities and the Ct values did not differ from those of the three-step protocol, the following experiments were carried out with the two-step protocol.

Species Specificity. In order to assess the specificity of the real-time PCR with primer pair 2 and the molecular beacon, the food samples and ingredients were tested again. The real-time PCR assay proved to be specific for sesame. All sesame containing foods, with the exception of sesame oil, gave positive results, while all materials other than sesame gave either no amplification or an amplification signal at Ct > 35. In contrast to the PCR with Sybr Green, no amplification signal was obtained for wheat. In order to investigate which kinds of sesame can be detected, the method was applied to five white, three black, and one golden (roasted) sesame samples purchased from different producers. **Figure 4** indicates that each of the sesame samples gave a positive amplification signal.

Amplification Efficiency and LOD. The amplification efficiency and sensitivity were determined both with serially diluted sesame DNA and with crisp bread spiked with different amounts of sesame.

In the case of sesame DNA, 10-fold dilutions of a DNA extract from sesame (from 1:1 to 1:1000000) were amplified. In **Figure 5**, the real-time PCR profile and the standard curve are shown. A good linearity was obtained up to a dilution of 1:10000 (corresponding to 10 pg/μL); this corresponds to an absolute amount of 50 pg or—assuming a haploid sesame genome size of 0.97 pg (*24*)—52 genome copies.

The correlation coefficient, r^2, was 0.996, indicating a high correlation between the sesame DNA amounts and their corresponding Ct values. The *y*-intercept of the standard curve was 29.9, which indicates a high sensitivity. The slope of the calibration curve was 3.315, indicating an amplification efficiency of 100.3%.

Analysis of DNA extracted from crisp bread containing either 2.5, 1, 0.5, 0.1, 0.05, 0.01, or 0.001% sesame resulted in the standard curve shown in **Figure 6**. The correlation factor, r^2, was 0.972. The amplification efficiency was 86.4%. For crisp bread with a sesame concentration < 0.05%, sesame Ct values > 35 were obtained.

Reproducibility of the Real-Time PCR Assay. The reproducibility of the method was investigated by preparing four DNA extracts from sesame cookies using the Qiagen QIAmp DNA Mini Kit and running a PCR assay with each DNA extract in triplicate. The whole procedure was repeated on the next day. The Ct values listed in **Table 3** demonstrate the high reproducibility of both the extraction and the real-time PCR assay. All Ct values were in the range from 28.02 and 29.32, resulting in very low intraday and interday standard deviations.

Determination of Sesame in Food Samples. The applicability of the real-time PCR assay for determining sesame in food was investigated by analyzing several crackers, salty snacks, biscuits, tahina sesame paste, and sesame oil (**Table 4**). Some of the food products contained sesame, some of them had no labeling for sesame, and some of them had a note that sesame could be included in the product. All samples were tested in duplicate. With the exception of sesame oil, in all of the samples where sesame was declared, sesame was detected by the real-time PCR assay (Ct value < 35). Subjecting sesame oil to the inhibition control test indicated that the negative result in the PCR assay was not caused by enzyme inhibition. We assume that the negative result is caused by the low DNA content in sesame oil and/or by the low quality of the isolated DNA (indicated by the low ratio A_{260}/A_{280} in **Table 2**).

In the samples which might contain sesame or where sesame was not listed, sesame could not be detected (Ct value > 35). In order to ensure that the negative results were not caused by enzyme inhibitors, these food samples as well as pure milk chocolate were tested for inhibition control. In all samples, the sesame DNA was amplified successfully, even in matrices known to contain inhibitory compounds (e.g., chocolate).

CONCLUSION

The application of PCR in food allergen analysis is discussed controversially due to the fact that with PCR—instead of the allergenic protein itself—a specific DNA fragment is detected. The present paper demonstrates the applicability of a real-time PCR method to specifically detect traces of potentially allergenic sesame in food. Real-time PCR should play an important role in food allergen analysis as an independent method for validating/confirming results obtained by other methods.

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