

Detection of celery (*Apium graveolens*), mustard (*Sinapis alba*, *Brassica juncea*, *Brassica nigra*) and sesame (*Sesamum indicum*) in food by real-time PCR

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Abstract Legislation requires labelling of foods containing allergic ingredients, amongst them celery, mustard and sesame. Here we present robust quantitative and sensitive methods for real-time PCR detection of celery, mustard (*Sinapis alba* and *Brassica* sp.) and sesame in food. The development of the DNA-based assays was part of an effort to generate alternative detection methods for allergens for which effective protein-based assays are lacking. The celery and sesame methods were specific for the celery mannitol dehydrogenase gene and the sesame allergen encoding 2S albumin gene, respectively, when tested against a range of plant materials. The mustard method was specific for the allergen encoding *sinA* gene and its homologues present in different *Brassica* sp. All primer probe pairs gave high amplification efficiency and sensitivities below approximately ten molecules of purified template DNA. These DNA-based detection methods will constitute supplementary and complementary methods to the traditional protein-based methods. Laboratories may choose different analysis formats depending on the food matrix, the availability of specific tests and the performance characteristics of the tests.

Keywords Allergen detection · Real-time PCR · Taqman · Celery · Mustard · Sesame

Introduction

Food allergies are a major health concern in industrialised countries and may affect up to 2% of the adult population and up to 8% of children in Europe [1, 2]. The number of food-allergen-induced life-threatening syndromes is increasing. Symptoms after ingestion of allergens range from digestive disorders, respiratory symptoms such as rhinitis and asthma, skin reactions such as urticaria and atopic dermatitis to life-threatening anaphylaxis. The level of exposure to provoke a reaction varies from food to food and from person to person, most often between 1 and 100 ppm of the allergen. Sometimes, however, only minute amounts may elicit a response. Treatment of food allergy is difficult and avoiding the allergen containing food is often the only option. This may sometimes be difficult for processed foods, which may contain allergens either added deliberately, for example when using spices containing celery powder, or unintentionally when foods are contaminated during shipping and storage and from food production lines, etc. To enable consumers with allergies to identify allergenic ingredients in foods, a list of potential allergenic ingredients to be labelled has been established by the European Commission and comprises cereals containing gluten, crustaceans, eggs, fish, peanuts, soybeans, milk and dairy products (including lactose), tree nuts, celery, mustard, sesame seeds and sulfites of at least 10 mg/kg (Directive 2003/89/EG, <http://www.eur-lex.europa.eu/LexUriServ/LexUriServ.do?uri=CELEX:32003L0089:EN:HTML>).

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This list is in accordance with the recommendations of Codex Alimentarius Commission, except that the EU list in addition contains celery, mustard and sesame.

Traditionally, in vitro allergen testing has consisted of detecting the presence of allergenic proteins. However, the detection of allergens in food can be very difficult, as they are often present in trace amounts and are masked by the food matrix [3]. In many such cases, DNA-based methods constitute an alternative. The choice of method is mainly dependent on the food and the allergen concerned (i.e. the availability of specific antibodies/DNA primers and the achievable detection limit) and on the history of processing during food production [4]. The benefits and disadvantages of the protein- and DNA-based methods have been reviewed and involve considerations in conjunction with detectability, specificity, limit of detection, variability of target, matrix effects, existence of reference materials, sample preparation and the required equipment and costs [4, 5].

For some allergens, good immunological methods are not readily available. One option is then to develop DNA-based assays. DNA withstands many food treatments and will constitute an alternative and a supplementary method for analysis of both unintentionally and intentionally abused food. Real-time PCR assays

have been described for peanut [6, 7], pea [8], walnut [9] and celery [10, 11].

Here, we present robust quantitative and sensitive methods for real-time PCR detection of celery, mustard (*Brassica*) and sesame in food. The development of the DNA-based assays is part of an effort to generate alternative detection methods for allergens for which good protein-based assays are lacking.

Materials and methods

DNA isolation

The materials analysed are listed in Tables 1 and 2. The samples were homogenised in a food processor and thereafter ground in a mortar. DNA was isolated by adsorption to columns using the DNeasy plant mini kit (Qiagen, Germany), as described by the manufacturer with the following modifications. The initial buffer volume was doubled and lysis was carried out for 30 min at 65 °C in a shaking incubator at 350 rpm. When eluting DNA bound to the column, 50 µl of preheated buffer was used. Chromosomal DNA was analysed by agarose gel electrophoresis. DNA concentration was measured using NanoDrop (ND-1000 Spectrophotometer, Saveen Werner, USA).

Table 1 Foods analysed using real-time PCRs

Species	Ingredient	Real-time PCR ^a		
		Celery	Mustard	Sesame
Celery (<i>Apium graveolens</i>)	Celery sticks	+	ND	ND
	Celeriac	+	ND	ND
	Celery powder	+	ND	ND
	Celery seeds	+	ND	ND
Yellow Mustard (<i>Sinapis alba</i>)	Mustard seeds	ND	+	ND
Brown mustard (<i>Brassica juncea</i>)	Mustard seeds	ND	+	ND
Black mustard (<i>Brassica nigra</i>)	Mustard seeds	ND	+	ND
Oriental mustard (<i>Brassica juncea</i>)	Mustard seeds	ND	+	ND
Dijon mustard	Processed product	ND	+	ND
Sesame (<i>Sesamum indicum</i>)	White sesame seeds	ND	ND	+
	Black sesame seeds	ND	ND	+
Carrot		—	—	—
Curled parsley		—	—	—
Maize		—	—	—
Potato		—	—	—
Chive		—	—	—
Paprika		—	—	—
Leek		—	—	—
Garlic		—	—	—
Wheat		—	—	—
Radish		—	+	—
Broccoli		—	+	—
Swede		—	+	—
Chinese cabbage		—	+	—
Cabbage		—	+	—

ND Not determined

^a Analysed using undiluted and tenfold diluted template DNA

Table 2 Detection of genes in spiked food products and swabs

Food product*	PCR	Spiking level (%)**	C _T -values
Vegetable burger mix ¹	Celery	0.01	37.5 ± 0.6
Vegetable burger mix ¹	Celery	0.001	40.0 ± 1.4
Barbeque spice ¹	Celery	0.005	39.3 ± 1.5
Barbeque spice ¹	Celery	0.001	41.2 ± 2.4
Wok vegetables ¹	Celery	0.01	38.0 ± 1.2
Wok vegetables ¹	Celery	0.001	40.1 ± 0.4
Raw minced meat	Celery	0.01	38.9 ± 0.6
Raw minced meat	Celery	0.05	41.2 ± 2.3
Raw minced meat	Celery	0.001	41.6 ± 0.2
Swab test ¹	Celery	2	29.2 ± 0.1
Swab test ¹	Celery	0.2	32.7 ± 0.2
Swab test ¹	Celery	0.02	35.5 ± 0.5
Swab test ¹	Celery	0.002	39.3 ± 0.3
Wheat flour ²	Celery	0.005	38.8 ± 0.9
Maize flour ²	Celery	0.005	37.6 ± 0.4
Barbeque spice ²	Mustard	0.005	34.8 ± 0.9
Wheat flour ²	Mustard	0.005	35.3 ± 0.4
Barbeque spice ²	Sesame	0.005	35.5 ± 0.7
Wheat flour ²	Sesame	0.005	35.9 ± 0.5

* Samples labelled ¹ were analysed with two biological replicates and two technical parallels or with four technical parallels. Samples labelled ² were analysed with eight (celery) or five (mustard and sesame) biological parallels and three or two technical replicates. C_T values between different spiked compounds are not directly comparable due to some differences in dilution of the template DNA prior to PCR

** (w/w) Samples were spiked with celeriac powder, an equal mixture of brown and white mustard seeds crushed in a mortar or crushed white sesame seeds. In the swab tests, 150 µl of tenfold dilutions of 20 mg/ml celeriac powder in water was used

Gradient PCR

Primers and probes for gradient PCR and 5'-nuclease PCR were from MWG-Biotech, Germany (Table 3). Primers and probes were designed using the Primer Express™ (version 1.0) software (Applied Biosystems, USA). For gradient PCR, DNA was amplified in a Gene Amp PCR system 9700, Applied Biosystems, using 1 µl template DNA (0.2–6 ng/µl), 2.5 µl Dynazyme II polymerase 10× buffer (Finnzymes, Finland), 0.2 mmol/l dNTPs (each), 0.2 µmol/l of specific primers and 2 U Dynazyme II DNA polymerase (total volume 25 µl). Amplification conditions were 40 cycles of 95 °C for 30 s, 50–70 °C annealing for 30 s and 72 °C elongation for 30 s.

5'-nuclease PCR

Amplification reactions (25 µl) contained a DNA sample (2.5 µl, 0.5–15 ng/µl), 2.5 µl TaqMan™ 10× buffer A, 5 mmol/l MgCl₂, 200 µmol/l dATP, dCTP, dGTP and 400 µmol/l dUTP, 0.2 µmol/l of each specific primer, 0.1 µmol/l of specific probes, 0.2 U AmpErase™

Uracil N-glycosylase (UNG) and 2.5 U AmpliTaq Gold™ DNA polymerase (Applied Biosystems, USA). Before amplification, the PCR mixture was heated to 50 °C for 2 min to let the UNG destroy possibly contaminating PCR products, and 95 °C for 10 min to denature the template DNA and to activate the polymerase. Amplification profile: 40 cycles of 95 °C for 15 s and 60 °C for 1 min. The reactions were performed in the ABI Prism® 7900HT Sequence Detection System (Applied Biosystems, USA). The TaqMan probes consisted of oligo nucleotides with a 5'-FAM reporter dye and a 3'-TAMRA quencher dye. Specificity analyses were performed in duplicates on undiluted and tenfold diluted DNA to dilute possible inhibitory compounds. For calibration curves, the samples were analysed in triplicate. The C_T-values were plotted against log₁₀ input DNA and gave an estimation of the amplification efficiency and the sensitivity of the reactions (Applied Biosystems 1997). The PCR products were verified using ethidium bromide-stained agarose gels. The DNA was visualised using a Typhoon 8600 fluorescence scanner (Amersham Biotech Pharmacia, USA).

Results

Celery

APIg1 is the major allergen for celery (*Apium graveolens*). Primer-probe sets for this gene have been described previously [10, 11]. FASTA searches with the APIg1 gene revealed homology to carrot genes. To avoid possible cross reactions between celery and carrot allergens, we chose to design a real-time primer probe system from the celery mannitol dehydrogenase gene (Fig. 1). This gene has previously been described as celery specific and primers for qualitative detection of the gene have been developed [12]. Sequence comparisons with mannitol dehydrogenase indicated only weak homology to a gene in curled parsley. The robustness of the primer set (Cell2F/Cell2R) was confirmed by gradient PCR giving one specific band of the expected 151 bp. The band was observed in the range 50–67 °C annealing temperature. No other bands were visible. The amplicon obtained at 60 °C annealing is shown in Fig. 2a. The specificity of the primer-probe set was shown by real-time PCR against undiluted and tenfold diluted DNA from a range of other species (Table 1). The primer-probe set gave positive signals for celery with C_T values of 24 and 27 for the undiluted and diluted samples, respectively. All other DNAs were negative. Celery DNA was serially diluted and

Table 3 Primers and probes for real-time PCR

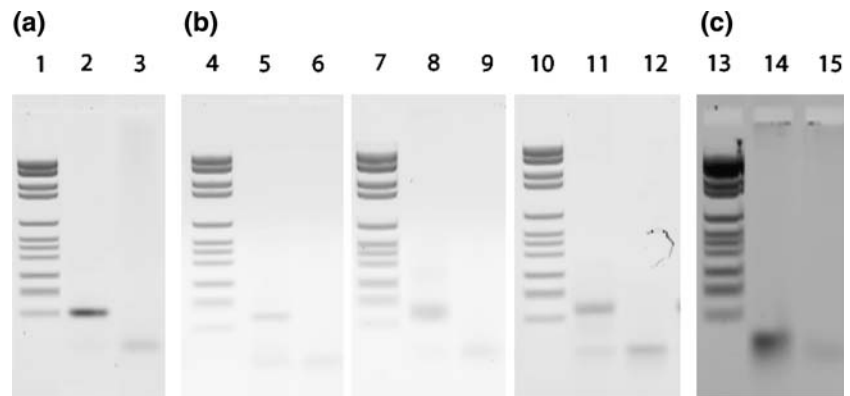
Gene ^a	Primers and probes ^b	Sequence ^c
Celery		
<i>Apium graveolens</i>	Cell2F	5'-TTT GAT CCA CCG ACT TAC AGC C-3'
Mannitol	Cell2R	5'-ATA AAA ACA GAT AAC GCT GAC TCA TCA C-3'
Dehydrogenase	Cell2pr	5' FAM-ATT ACA TGC TGA GTC ACG ATG AGC GTG TAC TG-TAMRA 3'
AF067082		
Mustard		
<i>Sinapsis alba</i>	SennF2	5'-CC CAA CYT TGA AAG GAG CWT CCA AAG C-3'
SinA1	SennR5	5'-C ATG GTC TTC TKG AAG GGA CAA ACA CTA ACT TG -3'
S54101	SennR6	5'-C ATG GTC TTC TTG AAG GGA CAA ACR CTW ACT TG -3'
	SennR7	5'-C ATG GTC TTC TGG AAG GGR CAA ATG CTA ACT TG-3'
	Sennpr1	5' FAM-TGC AGC AWG TRA TTA GCC GTA TCT ACC AGA CYK C-TAMRA 3'
	Sennpr2	5' FAM-AGC AGC AAA TGG TGA GCC GTA TCT ACC AGA CCG C-TAMRA 3'
Sesame		
<i>Sesamum indicum</i>	SesF	5'-CCA GAG GGC TAG GGA CCT TC-3'
2S albumin	SesR	5'-CTC GGA ATT GGC ATT GCT G-3'
AF240005	Sespr	5' FAM-TCG CAG GTG CAA CAT GCG ACC-TAMRA 3'

^a The Genbank accession numbers are given^b Primers and probes were from MWG Biotech (Germany)^c Degenerate bases, W = A,T; R = A,G; Y = T,C; K = T,G

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2601 TTAGTCGGAT CACCTAAATT TGATCCACCG ACTTACAGC TGTTCCTCGT ACGAGATATA
2661 TTTTGTCTG GTTTGAGATA TATATTACAT GCTGAGTCAC GATGAGCGTG TACTGAGTCA
2721 GTGTTATGTT TGGATTACGG TGTGATGAGT CAGCGTTATC TGTTTTATA TGTTCGTAT
2781 GATTAATGTT AGTTCCTATT ACCTAACCGG TTGCATCACC

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Fig. 1 Primers and probe for the celery-specific mannitol dehydrogenase gene (Genbank Acc. no. AF067082). The forward and the reverse primers are shown by *arrows*, while the position of the probe is *underlined***Fig. 2** Specificity of primers by gradient PCR and agarose gel electrophoresis. Results after annealing temperature of 60 °C are shown. **a** Celery, **b** mustard, **c** sesame. 1, 4, 7, 10, 13 Mw standard type VI (Boehringer Mannheim, Germany), 2 celery PCR product, 5 white mustard, 8 brown mustard, 11 black mustard, 14 sesame, 3, 6, 9, 12, 15 no template controls

used to generate a DNA standard curve (Fig. 3a). The curve based on the dilutions showed a linear relationship between the $\log_{10}$ input DNA and Ct values. The slope of the curve was -3.49 and the square regression coefficient (R^2) after the linear regression was 0.999. The slope indicated an amplification efficiency of 93 % (<http://www.gmo-crl.jrc.it>). The linearity showed that the PCR operated with a constant efficiency and is therefore suitable for quantitative measurements. The presence of celery DNA was easily detected when food products were spiked with celery powder in the

0.01–0.001 % range (w/w) (Table 2). No rigorous determination of LOD (limit of detection, i.e. the lowest number of target copies that must be present prior to the commencement of the PCR to be detected with 95% probability) and LOQ (Limit of quantification) was performed. However, using the values of 2.9×10^9 base pairs of the haploid celery genome [13] and knowledge of the DNA input in the highest dilution in the standard curve, it was estimated that the method easily detected approximately four template molecules.

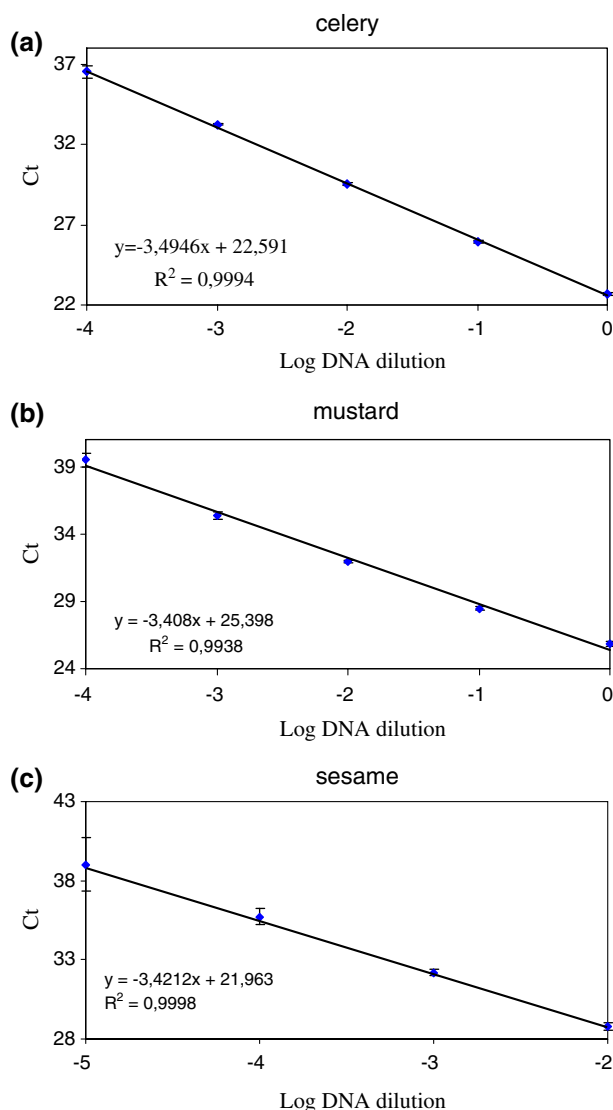


Fig. 3 C_t values after 5'-nuclease PCR of tenfold dilutions of **a** celery DNA, **b** brown mustard DNA and **c** sesame DNA. The linear regression lines are shown. In **c** the regression line is calculated for the DNA dilutions where no amplification inhibition is observed

Mustard

Mustard belongs to the family Brassicaceae. *Brassica* is a large and complicated interrelated genus and contains many different plants such as mustard, rape, swede and broccoli. Commercial mustard is often a mixture of *Sinapis alba* (white or yellow mustard) and *Brassica juncea* (brown mustard). Also black mustard (*Brassica nigra*), oriental mustard (*Brassica juncea*) and English mustard (a mixture) are being used. The major allergen in *Sinapis alba*, SinA, exists in many variants with small differences in sequence. The *sinA* gene is homologous to genes in the other mustard

species and with storage proteins in other *Brassica* species (Fig. 4). This is reflected in an extensive cross-reactivity between mustard and other *Brassica* species in persons with mustard allergy [14]. It appears therefore difficult to design mustard-specific primers and probes. Perhaps more appropriate is designing *Brassica* sp. specific primers and probe sets that will detect as many of the *sinA* homologous genes as possible, since the proteins from all these genes have allergenic potential. Because of the microheterogeneity, we designed from the known *sinA* genes a system with one forward primer (SennF2), 3 reverse primers (SennR5, SennR6, SennR7) and 2 probes (Sennpr1, Sennpr2), most of which carried some degeneracy (Table 3). Different mustard templates were analysed by gradient PCR. All gave expected bands of about 170–180 bp from 50 to 64 or 67 °C. Results from annealing at 60 °C are shown in Fig. 2b. When analysed by real-time PCR, the *Brassica*-specific primer probe set gave positive signals with all *Brassica* species tested and radish (*Raphanus sativus*), as expected from the sequence alignments (Fig. 4). The other analysed species were negative. DNA from brown mustard was serially diluted and a DNA standard curve established (Fig. 3b). The slope of the curve was -3.41 and the square regression coefficient (R^2) after the linear regression was 0.994. This gave an amplification efficiency of 96%. Again, the linearity showed a constant PCR efficiency and the assay is therefore suitable for quantitative measurements. Using the values of 1.1×10^9 base pairs of the haploid brown mustard (*Brassica juncea*) genome [15], it was estimated that the method detected approximately four molecules at the highest dilution. The method easily detected mustard at 0.005% (w/w) level in spiked barbeque spice and wheat flour (Table 2).

Sesame

For sesame (*Sesamum indicum*), several allergens have been described, for example the 11S globulin, the 2S albumin and the 7S globulin. These are important storage proteins. The 2S albumin is described as the major allergen and two 2S albumins have been published [16, 17], Acc. No. AF240005 and AF091841). The two genes show only limited sequence homology, and the amino acid sequences deduced from the genes are also quite different. FASTA searches in GenBank using the two genes as query sequences did not reveal any homologous genes. Primers and probe sets were designed for both 2S albumin genes. The set for the AF240005 entry is shown in Fig. 5. Upon gradient PCR using DNA from white sesame, the SesF/SesR primer pair for the AF240005 entry 2S albumin gene gave a

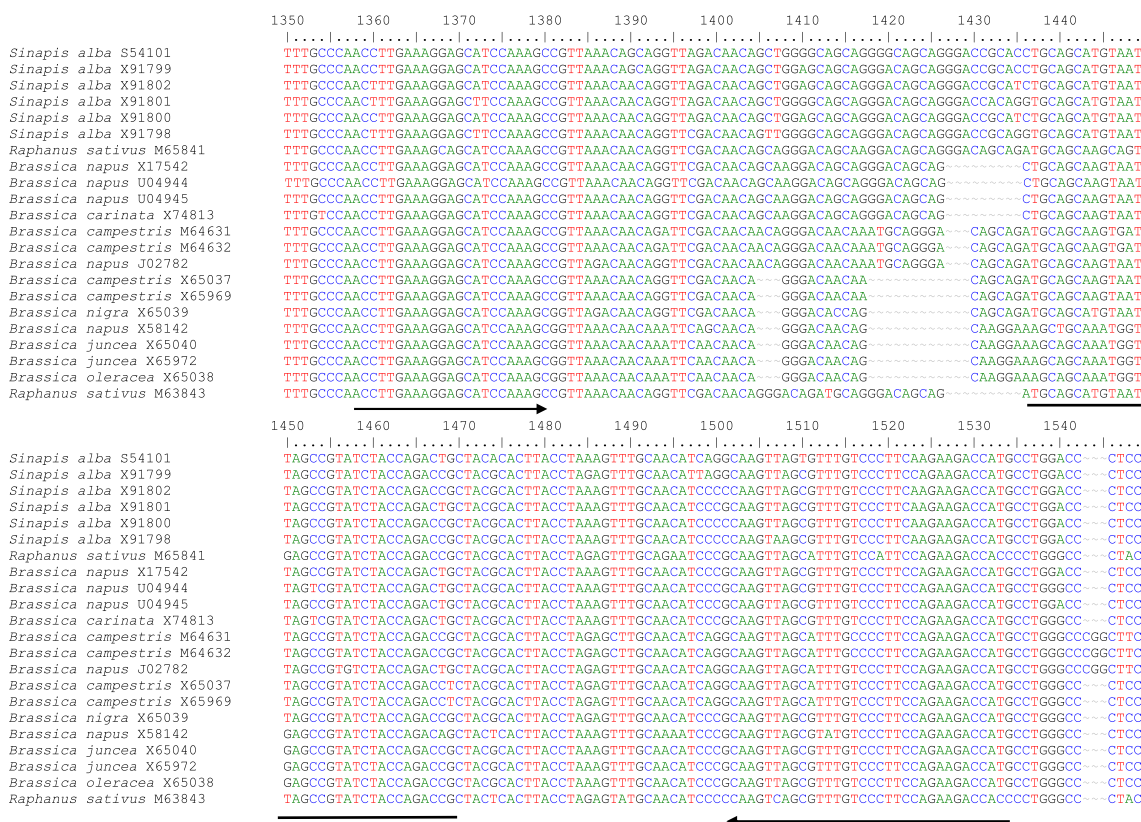


Fig. 4 Alignment of the gene for the major allergen in mustard, the *sinA* gene, with homologous genes in other *Brassica* species. *Sinapis alba* (white or yellow mustard), *Raphanus sativus* (radish), *Brassica napus* (rape), *Brassica napus* var. *rapifera* (swede), *Brassica carinata* (Ethiopian mustard), *Brassica campestris*

(turnip, chinese cabbage, wild turnip), *Brassica nigra* (black mustard), *Brassica juncea* (brown mustard), *Brassica oleracea* (cabbage). The Genbank accession numbers are given. The positions of the forward and reverse primers and probe are indicated as horizontal arrows and bar, respectively

strong 64 bp band as expected in the whole 50–70 °C annealing temperature range tested. No unspecific amplification occurred above 55 °C. The amplicon obtained at 60 °C annealing temperature is shown in Fig. 2c. When the primer probe pairs were tested in real-time PCR with DNA from white and black sesame, the SesF/SesR primers produced good signals, whereas only weak or absent signals were obtained with the primer probe set for the AF091841 albumin 2S gene (not shown). Further work was therefore carried out using the SesF/SesR primer set. The SesF/SesR Sespr primer probe set gave a negative result for all other plant DNA (Table 1). DNA from white sesame was serially diluted to establish the DNA standard curve (Fig. 3c). Some inhibition of the amplification was evident for the higher DNA concentrations. This was probably caused by compounds inhibiting the DNA amplification. At higher dilutions, the slope of the curve was -3.42 and the square regression coefficient (R^2) 1.000. This gave an amplification efficiency of 96%. At the highest dilution, specific signals were obtained with 0.5 pg sesame DNA. A value of 1×10^9 base pairs of the haploid sesame genome (size

unknown) would correspond to detection of around one molecule. Again, the method easily detected sesame at 0.005% (w/w) level in spiked barbeque spice and wheat flour (Table 2).

Discussion

Results from real-time PCR analysis must be interpreted with caution. The knowledge of the food that is analysed and the processing it has been subjected to is important. Depending on the food and the processing, DNA may be partly degraded. Sometimes DNA can be totally degraded leading to no PCR product and consequently give false negative results, while the food still may contain the allergen. In addition, food matrix contaminants may interfere with the PCR and lower the amplification efficiency. This problem is usually circumvented by diluting the template DNA and thereby concomitantly diluting the inhibitors. An example of this was seen when sesame DNA was diluted 100-fold to give acceptable amplification efficiency (Fig. 3c). The DNeasy plant mini kit DNA purification method

Fig. 5 Primers and probe for the gene encoding the sesame (*Sesamum indicum*) 2S albumin (Acc. no. AF240005). The forward and the reverse primers are shown by *arrows*, while the position of the probe is *underlined*

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1  CTACTCCTTT CCTACATATA AATCTCAGTT CAAGAACAAC CATGGCGAAG AAGCTCGCAC
61  TCGCAGCAGT CCTCCTGGTG GCCATGGTAG CGTTGGCCAG CGCCACGACC TACACCACCA
121 CTGTGACCAC CACAGCCATC GACGATGAAG CCAACCAGCA GAGCCAACAG TGCCGCCAGC
181 AGCTGCAGGG CCGGCAGTTC AGGTCTTGCC AGAGGTACTT GTCGCAAGGA CGCAGCCCAT
241 ATGGTGGTGA AGAGGATGAA GTTCTGGAAG TGAGCACTGG GAATCAGCAG TCCGAGCAGT
301 CCTTGAGAGA TTGCTGCCAG CAGCTGAGGA ACGTGGACGA GAGGTGCCGC TGTGAGGCCA
361 TTAGGCAAGC AGTGAGGCAG CAGCAGCAGG AGGGTGGCTA CCAGGAGGGC CAATCACAGC
421 AGGTTTACCA GAGGGCTAGG GACCTTCCTC GCAGGTGCAA CATGCGACCC CAGCAATGCC
481 AATTCGAGT TATCTTTGTG TAGAATCGCA ATCAACCAAG CGTGGTTTCT CATGAGGTTG
541 TGCAGGAAA TGTATAAATA AGGTTGTACG TGTGACTGTG TTTTGTGAAT AAGTATCTAT
601 AAGTAGTCTG TGTGTACATC CTCGAAATAA AAATGAAGAA CAGCCGTCTT TTGTTGTTTA
661 AAAAAAAAAA AAAAAAAAAA

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used in this work is a reliable method suitable for a large number of food matrices. For specific food samples, however, other special DNA purification methods may be required.

It is still an open question how sensitive a detection method needs to be. Threshold levels for specific allergic reactions determined by double blind placebo controlled food challenges range between less than 1 mg and more than 1 g of allergenic protein depending on the food in question and the sensitivity of the allergic individual [18]. Real-time PCR is a very sensitive technique detecting down to a few molecules of DNA. The sensitivity of the assay, however, is highly dependent on the sample preparation. This may vary from food product to food product. Sensitivities in the range of 5–50 mg/kg in heterogeneous matrices are generally achieved by using sample quantities between 500 mg and 2 g and by using a DNA extraction and purification method, which yields DNA pure enough for the subsequent PCR analysis [4]. Similar sensitivities were obtained when food was spiked with allergens (Table 2). An example of increased sensitivity is a PCR-based kit for soy, which offers a sensitivity of 1.5 mg/kg, which is approximately 500 times lower than the detection limit obtained using ELISA [4]. The assays developed in this work detected template DNA in the range of below approximately ten molecules and therefore meet the requirements for sensitivity, provided the DNA lends itself to amplification. Also high reproducibility was observed when spiking flour and spices with low amounts of allergenic material (Table 2).

Real-time PCR is a highly specific technique when employing primers and probes designed from unique template sequences, as was evident from the lack of cross-reactivity using the celery and sesame-specific primer-probe pairs. This may be a specific advantage over protein-based methods in cases where cross-reactivity must be prevented. Specificity of the DNA assay will depend on the primer probe design and may also be used to detect groups of closely related genes encoding

proteins with allergenic potential, as was the case with the mustard (*Brassica* sp.) primer probe sets. Again, this highlights the importance of knowing the history of the food so the results are interpreted correctly. If for example a food containing broccoli is analysed for mustard, false positive results may occur. In other cases, it may be beneficial to detect the allergen even though not present in mustard, since allergic persons might cross-react with homologous allergens from other sources.

Here, we have presented robust and efficient assays for real-time PCR-based detection of celery, mustard (*Brassica*) and sesame. Regarding real-time PCR-based genetically modified organism (GMO) detection, the European Network of GMO Laboratories (ENGL) has devised minimum performance requirements for analytical methods suitable for validation testing (<http://www.gmo-crl.jrc.it>). For the criteria analysed such as specificity, dynamic range, R^2 coefficient and amplification efficiency, the primer probe pairs developed in this work fulfil the stated criteria.

With real-time PCR methods, accurate quantitative results can be obtained. Though the DNA-based methods do not detect the allergens as such, an advantage is that DNA is efficiently extracted under harsh conditions and is less affected by extraction from food matrices compared to proteins. The DNA-based methods will not replace the protein-based methods, but exist as additional and complementary methods. Laboratories may choose different analysis formats depending on the food matrix, the availability of specific tests and the performance characteristics of the tests.

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