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Apricot DNA as an Indicator for Persipan: Detection and Quantitation in Marzipan Using Ligation-Dependent Probe Amplification

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Supporting Information

ABSTRACT: The confectionery ingredient marzipan is exclusively prepared from almond kernels and sugar. The potential use of apricot kernels, so-called persipan, is an important issue for the quality assessment of marzipan. Therefore, a ligation-dependent probe amplification (LPA) assay was developed that enables a specific and sensitive detection of apricot DNA, as an indicator for the presence of persipan. The limit of detection was determined to be 0.1% persipan in marzipan. The suitability of the method was confirmed by the analysis of 20 commercially available food samples. The integration of a *Prunus*-specific probe in the LPA assay as a reference allowed for the relative quantitation of persipan in marzipan. The limit of quantitation was determined to be 0.5% persipan in marzipan. The analysis of two self-prepared mixtures of marzipan and persipan demonstrated the applicability of the quantitation method at concentration levels of practical relevance for quality control.

KEYWORDS: *Apricot DNA, marzipan, persipan, LPA, quantitation*

INTRODUCTION

Marzipan is a valuable ingredient of confectionery, prepared exclusively from almond kernels and sugar. According to German food guidelines, marzipan is "a mixture of marzipan paste and at most the same amount of sugar". Marzipan paste is a paste produced from blanched peeled almonds containing 17% moisture and 35% sugar. The amount of almond oil is at least 28%.[1] The addition of other oilseed ingredients, such as apricot, peach, or plum kernels, to marzipan paste does not comply with this standard. The addition of apricot kernels, the main components of so-called persipan, is of particular interest. Persipan is defined as "a mixture of persipan paste and of sugar not exceeding the 1.5-fold amount".[1] Persipan paste is a paste produced from blanched peeled debittered (as necessary) bitter almonds, apricot, or peach kernels.[1] From the beginning of the 20th century, the admixture of persipan to marzipan has been detected using an iodine starch reaction, which required the addition of starch to the persipan in quantities up to 0.5%.[1] Alternatives are the analysis of the tocopherol spectra of marzipan and persipan[2] or isoelectric focusing of an apricot-specific protein.[3]

The first end-point polymerase chain reaction (PCR) detection method was based on the amplification of a specific part of the apricot DNA and separation on an agarose gel.[3] This PCR method enabled the determination of 0.5% apricot kernels in marzipan, corresponding to a limit of detection (LOD) of 2.7% persipan (74% sugar and 8% moisture) in marzipan.[3] Recently, species-specific PCR detection systems of the plant species apricot, peach, bean, pea, soy, lupine, cashew, pistachio, and chick pea for the purity control of marzipan have been described;[4] for the detection of apricot, a sensitivity of 0.1% could be achieved in spiked materials.

In contrast to end-point PCR methods, real-time PCR assays allow for the quantitation of the respective analyte and exhibit higher specificity. The technique is employed to determine traces of plant materials, genetically modified organisms (GMO), allergens, and different animal species.[5−8] Weber and Hauser[9] described a real-time PCR method for the detection of DNA from apricot in marzipan, exhibiting a LOD of 0.1% apricot. For two commercially available real-time PCR assays, LODs of <5 copies/reaction[10] and <10 copies/reaction[11] have been reported.

Ligation-dependent probe amplification (LPA) is a variant of the PCR, allowing for a multiplex approach with sensitivity and specificity comparable to real-time PCR methods. The method, originally developed for clinical diagnostics,[12] is based on the use of bipartite sequence-specific probes. After hybridization to the specific target sequence, these probes are linked by a ligase and amplified in a subsequent PCR. During the amplification, the probes are labeled with a fluorescence dye. Subsequently, the probes are separated by capillary gel electrophoresis and detected and identified using size standards. The method has also been applied in food analysis, e.g., as a modular system for the screening and relative quantitation of DNA from GMOs[13,14] or for the simultaneous detection of DNA from food allergens.[15,16]

The objective of this study was the development of a LPA-based method for the analysis of apricot DNA as an indicator for persipan in marzipan. The approach should not only allow for a specific and sensitive detection but should also provide a basis for the quantitation of apricot DNA in marzipan.

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Table 1. Oligonucleotides Used for Sequencing the LTPI Gene, Specific Detection of Apricot DNA, and Quantitation with *Prunus* DNA as a Reference Standard[a]

name	sequence	length (nucleotides)
	Primers Used for Sequencing of the LTPI Gene	
primer-LTPI-2010-F	5′-TTAGCCCCTCCACCAACTGC-3′	20
primer-LTPI-2010-2R	5′-CCCTAAGTGGATCACATAGCCACA-3′	24
	LPA Probes Used for Detection of Apricot and *Prunus* DNA	
apricot-L	5′-**GGGTTCCCTAAGGGTTGGA**gtgtgtgtgtgtgtTGCAGCGTGAAGTGAGCTTGATATCAA-3′	60
apricot-R	5′-TGCAATGGGACAAGTATTTTCGGCCGCtgtgtgtgtgtgtgtgtg**TCTAGATTGGATCTTGCTGGCAC**-3′	68
Prunus-L	5′-**GGGTTCCCTAAGGGTTGGA**gtgtgtgtgCGTTACAGAAATATTGTTTTGGGGTCATCTTATCTCATAA-3′	68
Prunus-R	5′-CAGAAAGATTCATCTGGTTTAATGTATATATTTGTGCAGGTtgtg**TCTAGATTGGATCTTGCTGGCAC**-3′	68
	Primers Used for Amplification of the Ligated Probes	
LPA-F	FAM-5′-**GGGTTCCCTAAGGGTTGGA**-3′	19
LPA-R	5′-**GTGCCAGCAAGATCCAATCTAGA**-3′	23

[a]Uppercase letters, plant DNA; lowercase letters, spacer sequence; and uppercase bold letters, primer binding sites.

MATERIALS AND METHODS

Materials and Samples. DNA samples of *Prunus* species (plum, cherry, and sour cherry) of 13 apricot cultivars (Bergarouge, Bergeron, Bergeval, Clarina, Goldrich, Hargrand, Hilde, Kuresia, Mino, Orangered, Pink Cat, Spring Blush, and Vertige) and other plant/food materials (apple, banana, barley, biscuit, brazil nut, cacao, cashew nut, chocolate, coconut, cow milk, hazelnut, kamut, lupine, macadamia, oat, orange, peanut, pear, pecan nut, pineapple, pistachio, rice, rye, sesame seeds, spelt, triticale, walnut, and wheat) were provided by the Bavarian Health and Food Safety Authority (Oberschleißheim, Germany). Peaches and the analyzed food samples were purchased from local supermarkets. Almond and apricot kernels were provided by Impetus Bioscience (Bremerhaven, Germany). Marzipan paste (content of almonds: 54%) and persipan paste (content of apricot kernels: 50%) were purchased from a local store. Marzipan was prepared by mixing the marzipan paste with the same amount of sugar. The persipan paste was mixed with the 1.5-fold amount of sugar to obtain persipan. Mixtures of 0.01, 0.1, 0.5, 1, 2.5, 5, 10, 15, 20, and 50% persipan in marzipan were prepared using a thermal mixer (type Thermomix 21/2-1, Vorwerk Elektrowerke GmbH and Co. KG, Wuppertal, Germany) for 5 min at 50 °C.

In addition, the following model materials were prepared: Mod 1, 0.1762 g of persipan and 20.1729 g of marzipan were mixed, resulting in a content of 0.87% persipan in marzipan; Mod 2, 0.8320 g of persipan and 20.0188 g of marzipan were mixed, resulting in a content of 4.16% persipan in marzipan.

DNA Extraction. The sample (2 g) was mixed with 10 mL of CTAB buffer [2% (w/v) cetyltrimethylammoniumbromide, 1.4 M NaCl, 0.1 M 2-amino-2-hydroxymethyl-propane-1,3-diol (TRIS), and 20 mM ethylenediaminetetraacetic acid (EDTA) at pH 8] and 30 μL of proteinase K solution (>600 mAU/mL) (Qiagen, Hilden, Germany) in a 50 mL Falcon tube and incubated for 120 min at 65 °C in a platform shaker with an incubator (Heidolph Instruments, Schwabach, Germany). After centrifugation at 7200*g* for 10 min, 700 μL of supernatant was added to 500 μL of ReadyRed chlorophorm/isoamylalcohol (MP Biomedicals, Illkirch, France) in a 2 mL tube and mixed for 30 s. The tube was centrifuged at 20000*g* for 15 min, and 500 μL of supernatant was added to 500 μL of cold isopropanol (≥99%). The solution was gently shaken and incubated for 30 min at room temperature (RT). After centrifugation for 15 min at 20000*g*, the supernatant was carefully removed and the residual pellet was washed with 500 μL of ethanol (98%). The sample was centrifuged at 20000*g* for 5 min, and the supernatant was discarded. After the pellet was dried for 30 min at 50 °C, it was solved in 100 μL of TE buffer (1×) [10 mM TRIS × HCl at pH ∼8.0 containing 1 mM EDTA].

The DNA extracts were purified using the QIAquick PCR purification kit (Qiagen, Hilden, Germany) and diluted in 50 μL of elution buffer. DNA concentrations were estimated using the fluorometrical intercalating dye Quant-iT PicoGreen dsDNA reagent (Invitrogen, Karlsruhe, Germany) on a GENious plus reader (Tecan, Männedorf, Swizerland). The measurement was carried out generating a λ-DNA (Invitrogen, Karlsruhe, Germany) calibration curve, and the DNA concentrations were calculated using a linear equation. Finally, the estimated DNA concentrations were normalized to 20 ng/μL. For every extraction series, a blank value was additionally performed to avoid false positive results because of inadvertent contamination.

Oligonucleotides. Primers used for sequencing analysis of the lipid transfer protein I (LTPI) gene of almond (accession number EU919665.1) were designed with National Center for Biotechnology Information (NCBI) primer-BLAST[17] and obtained from TIB MOLBIOL (Berlin, Germany). The amplification of LPA probes was carried out with the primers from the SALSA MLPA kit EK5 (MRC Holland, Amsterdam, The Netherlands). Apricot- and *Prunus*-specific LPA probes were designed in accordance with MRC Holland[18] and purchased from Biolegio BV (Nijmegen, The Netherlands). The probe mix was prepared mixing 0.8 μL of each 1 μM oligo solution (left probe oligo + right probe oligo) in a total volume of 600 μL of TE (1×). All oligonucleotides used in this study are listed in Table 1.

Ligation-Dependent Probe Amplification. The LPA reaction inclusive of the subsequent PCR was performed in a 96-Primus advanced thermocycler (Peqlab, Erlangen, Germany) using the SALSA MLPA reagents kit EK5 (MRC Holland, Amsterdam, The Netherlands). A total of 5 μL of sample DNA was pipetted into a 500 μL tube and heated for 5 min at 95 °C. After cooling to 25 °C, 3 μL of the hybridization mix (containing 1.5 μL of the probe mix and 1.5 μL of MLPA buffer) was added to the sample. The mixture was heated for 1 min at 95 °C and incubated subsequently at 60 °C for 16 h. For the ligation reaction, a ligation mix was prepared (containing 3 μL of ligation-65 buffer A, 3 μL of ligation-65 buffer B, 1 μL of ligase-65, and 25 μL of PCR-grade H_2O) and added to the sample after adjusting to 54 °C. The ligation reaction was continued for 12 min at 54 °C. Subsequently, the enzyme was inactivated by heating to 98 °C, and the samples were finally cooled to 4 °C.

The following PCR was started at 60 °C after adding 10 μL of PCR reaction mix containing 2 μL of SALSA PCR primer, 2 μL of SALSA enzyme dilution buffer, 5.5 μL of PCR-grade H_2O, and 0.5 μL of polymerase. A total of 35 amplification cycles were carried out at 95 °C for 30 s, 60 °C for 30 s, and 72 °C for 60 s, followed by a final step at 72 °C for 20 min. After cooling at 4 °C, the reaction mixture was stored.

Fragment Length Analysis. Fragment length analysis was performed on an ABI PRISM 3130 genetic analyzer using capillaries (4 × 36 cm) and a POP-7 performance optimized polymer (Applied Biosystems, Foster City, CA). A total of 3 μL of the reaction mix was dissolved in 117 μL of high-performance liquid chromatography HPLC-grade H_2O to achieve a 1:40 dilution, so that obstructions of the capillaries are avoided. A total of 1 μL of the dilution was mixed with 15 μL of HiDi-formamide (Applied Biosystems, Foster City, CA)

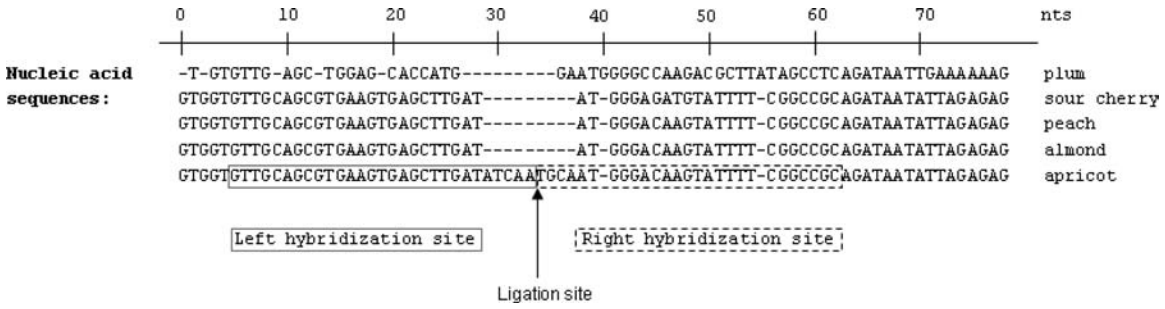

Figure 1. Position of the probe for the detection of apricot DNA with left and right hybridization sites and the ligation site at the nucleic acid sequences of parts of the LTPI genes of different *Prunus* species.

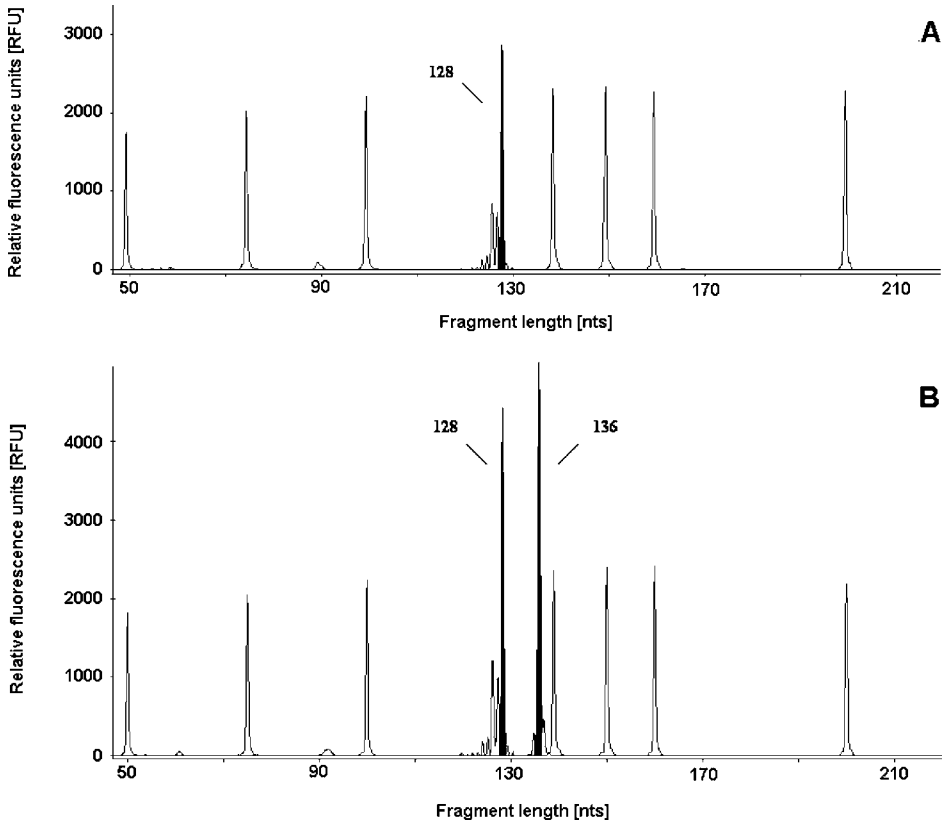

Figure 2. (A) Electropherogram of the LPA using the designed apricot-specific probe with apricot DNA as the template and (B) electropherogram of the LPA using the apricot- and *Prunus*-specific probe with DNA from pure persipan as the template. The apricot-specific signal (128.3 nucleotides) and the *Prunus*-specific signal (136 nucleotides) are shown in black. The size standards are shown in gray.

containing 0.15 μL of size standard GeneScan 500-LIZ (Applied Biosystems, Foster City, CA) and injected for fragment length analysis. The settings were as follows: oven temperature, 60 °C; prerun voltage, 15.0 kV; prerun time, 180 s; injection voltage, 1.2 kV; injection time, 16 s; run voltage, 15.0 kV; and run time, 1200 s. The peak heights were calculated from the electropherograms using the GeneMapper3 software (Applied Biosystems, Foster City, CA). Only signals greater than or equal to 100 relative fluorescence units (RFUs) were considered as positive.

Sequencing. For sequencing analysis, an end-point PCR was performed on a MWG-Biotech primus 96 plus thermocycler (MWG, Ebersberg, Germany) using 12.5 μL of HotStart Mastermix (Qiagen, Hilden, Germany), 6.5 μL of PCR-grade H_2O, 0.5 μL of each primer (Table 1), and 5.0 μL of sample DNA for one reaction mix (25 μL). Cycling conditions were set to an initial denaturation step at 95 °C for 8 min, followed by 40 cycles at 95 °C for 30 s, 61 °C for 30 s, and 72 °C for 30 s, and a final step at 72 °C for 2 min. The amplification products were purified with a QIAquick PCR purification kit in accordance with the advice of the manufacturer and used as a template for sequencing PCR.

The sequencing reaction was performed using the BigDye Terminator version 1.1 cycle sequencing kit (Applied Biosystems, Warrington, U.K.). One reaction mix (10 μL) for sequencing of the almond LTPI gene contained 2 μL of RR mix, 2 μL of PCR-grade H_2O, 1 μL of primer, 1 μL of 5× buffer, and 4 μL of template. For sequencing of apricot, plum, sour cherry, and peach, the amounts of all components were doubled, resulting in a reaction mix volume of 20 μL. Cycling conditions were set to an initial denaturation step at 95 °C for 1 min, followed by 30 cycles at 95 °C for 10 s, 57 °C for 5 s, and 72 °C for 4 min.

Prior to sequencing, the amplified products were purified. A total of 16 μL of double-distilled water (ddH_2O), 4 μL of 3 M Na−acetate, and 50 μL of ethanol (98%) were mixed with PCR products (10 or 20 μL) in a 1.5 mL tube and centrifuged at 20000*g* for 15 min. After careful removal of the ethanol, 50 μL of ethanol (70%) was added again and centrifuged at 20000*g* for 5 min. The ethanol was carefully removed for the second time, and the residual pellet was dried at 50

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°C for at least 30 min. The pellet was dissolved in 20 μL of ddH_2O, and an aliquot of 5 μL was mixed with 10 μL of HiDi-formamide (Applied Biosystems, Foster City, CA).

The sequencing was performed using an ABI PRISM 3130 genetic analyzer with four capillaries (4 × 36 cm) and a POP-7 performance optimized polymer (Applied Biosystems, Foster City, CA). The settings were as follows: oven temperature, 60 °C; prerun voltage, 15.0 kV; prerun time, 180 s; injection voltage, 1.2 kV; injection time, 12 s; run voltage, 8.5 kV; and run time, 2780 s.

RESULTS AND DISCUSSION

Detection of Apricot DNA as an Indicator for Persipan. *Design of an Apricot-Specific LPA Probe.* The study by Weber and Hauser[9] indicated differences in the LTPI genes of almond (EU919665.1) and apricot (sequence not published). Therefore, the primer pair LTPI-2010-F and LTPI-2010-2R was designed on the basis of this part of the almond DNA sequence. The forward primer sequence is complementary to nucleotide numbers 386−405 of the LTPI gene sequence. The reverse primer matches nucleotide numbers 695−672. Using the primer pair in an end-point PCR with almond DNA as the template, the expected amplicon (310 bp) was obtained.

Owing to the homology in the nucleic acid sequences of *Prunus* species, these primers also hybridize to the corresponding part of the nucleotide sequence of apricot (*Prunus armeniaca*), plum (*Prunus domestica*), peach (*Prunus persica*), and sour cherry (*Prunus avium*). After amplification and sequencing, the sequences were aligned for each species using the DNA-Star software (DNASTAR, Inc., Madison, WI). As shown in Figure 1, this alignment revealed an apricot-specific insert of eight nucleotides compared to the other *Prunus* species, thus offering an appropriate location for designing a probe for the detection of apricot DNA.

The LPA probe was designed in accordance with the protocol provided by MRC Holland.[18] It is composed of two oligonucleotides [left probe oligonucleotide (LPO) and right probe oligonucleotide (RPO)]; each of them consists of a primer binding site, a spacer sequence, and a specific hybridization site [left hybridization site (LHS) or right hybridization site (RHS), respectively] (Figure 1). The length of the probe was adjusted to 128 nucleotides using a spacer sequence composed of GT repeats. This specific length was chosen because it provides the option to include the probe in the LPA mix developed for the detection of nuts, peanuts, and sesame.[15]

With the performance of a LPA with apricot DNA as the template, the designed probe resulted in a specific signal with a nucleotide length of 128.3 (Figure 2A). The slight shift of 0.3 nucleotide (standard deviation of 0.05) is in agreement with previously described observations.[15] Additionally, the LPA was performed using DNA from 13 apricot cultivars (see the Materials and Samples section), resulting in an apricot-specific signal for all samples.

For the determination of the specificity of the developed apricot-specific probe, the combined left and right hybridization site sequence was aligned with all online available nucleic acid sequences within the NCBI GenBank using BLAST software. No theoretical cross-reactivity was observed. The specificity was also tested using DNA from the *Prunus* genus, cereals, and various other foods (see the Materials and Samples section) as the template for LPA. The reactions were carried out in duplicate. Except for plum DNA, no cross-reactivity with the tested DNA extracts was observed. Owing to the lack of sequencing data on the complete genome of *Prunus domestica*, the reasons for the observed cross-reactivity with plum remain speculative.

LOD. The LOD was determined by LPA analysis of marzipan spiked with persipan. Three extracts were prepared per concentration level. Each of them was analyzed twice, resulting in a total of six reactions. The LOD was defined as the lowest amount of persipan in marzipan, which could be detected in all performed reactions. As shown in Table 2, this criterion resulted in a LOD of 0.1% persipan in marzipan. No apricot-specific signal was detected using DNA from self-made marzipan as the template in the LPA by 6-fold analysis.

Table 2. Determination of the LOD by LPA Analysis of Marzipan Spiked with Persipan[a]

persipan content in marzipan (%)	extract	1		2		3	
	replicate	A	B	A	B	A	B
20		+	+	+	+	+	+
5		+	+	+	+	+	+
1		+	+	+	+	+	+
0.1		+	+	+	+	+	+
0.01		−	+	−	−	−	−
0		−	−	−	−	−	−

[a] −, <100 RFUs; +, ≥100 RFUs.

Analysis of Commercial Samples. The detection system was applied to commercially available food samples. DNA extracts of the samples were prepared and analyzed twice by LPA (Table 3). Two products, for which apricots or persipan had been declared in the list of ingredients (persipan paste and

Table 3. Results Obtained by LPA Analysis of Commercial Samples[a]

samples	results	
	replicate	
	A	B
Samples with Declaration of Apricots in the Ingredients List		
persipan paste	+	+
Amaretti biscuits	+	+
Samples without Declaration of Apricots or Persipan in the Ingredients List		
Lübeck fine marzipan mocca	+	+
modeling marzipan paste (red, green, and yellow)	+	+
Alpenmilch chocolate pralines	+	+
Christmas baubles pralines with marzipan filling	+	+
chocolate bar marzipan	−	−
marzipan eggs	−	−
marzipan egg with chocolate	−	−
fine marzipan praline	−	−
fine marzipan zartherb	−	−
nougat and fine marzipan tree trunk	−	−
fine marzipan with dark chocolate	−	−
mini marzipan butter stollen	−	−
marzipan with chocolate	+	−
fine marzipan stollen	+	−
Lübeck fine marzipan rum	+	−
marzipan paste	+	−
marzipan potatoes	+	−
marzipan praline eggs in dark chocolate	+	−

[a] −, <100 RFUs; +, ≥100 RFUs.

Amaretti), were included as positive controls; both gave positive results.

A total of 18 samples without declaration of apricot or persipan in the list of ingredients were analyzed. A total of 4 samples showed positive results for both replicates; the absence of persipan traces above the LOD of the LPA method was verified by consistently negative results for 8 samples. For 6 samples, ambiguous results were obtained, indicating that these products contain traces of persipan below or around the LOD. These results were compared to a previously published real-time PCR method.[9] The cycle threshold (Ct) values determined for selected samples from the set shown in Table 3 were in good agreement with the results obtained using the LPA method. Persipan paste showed a Ct value of 24.58. A "LPA-positive" sample (Lübeck fine marzipan mocca) showed a Ct value of 35.17. A "LPA-ambiguous" sample (marzipan praline eggs in dark chocolate) showed a Ct value of 38.35. A "LPA-negative" sample showed a Ct value of 41.83.

Quantitation of Persipan in Marzipan. The suitability of the LPA system for the relative quantitation of GMO has previously been shown.[13] The approach was based on the generation of a calibration curve by plotting the ratios of peak heights of the recombinant genes and the reference genes against the GMO content of the respective standards. This resulted in a good linear correlation between the normalized peak heights and the increasing GMO contents in the reference materials in the range of 0−5% GMO content ($R^2 > 0.98$).

This principle was applied to the quantitation of persipan in marzipan. In analogy to the normalization of the signals of the recombinant gene with the species-specific gene, the apricot-specific signal was correlated to a *Prunus*-specific signal. Normalization was achieved by dividing the apricot-specific peak area by the total peak area, i.e., the sum of the apricot peak and the *Prunus* peak areas. This normalized signal was correlated to the persipan content in marzipan using samples containing 0−100% persipan.

Design of a Prunus-Specific Probe. The Pru du 1.04 gene of almond (EU424245.1) served as a basis for the design of a *Prunus*-specific probe. Performing the LPA with DNA from either apricot or almond led to a signal in the electropherogram corresponding to the expected probe length of 136 nucleotides. Using DNA from plum, peach, cherry, or sour cherry as the template in the LPA, the *Prunus*-specific signal was also obtained in the respective electropherograms.

Optimization of Probe Concentrations. Performing the LPA with DNA from pure persipan and employing the same concentrations of the apricot- and *Prunus*-specific probes resulted in a 3-fold smaller area for the apricot-specific signal than for the *Prunus*-specific signal. To ensure maximum sensitivity of the quantitation method, the concentrations of the probes were adjusted to obtain similar intensities of the two signals when analyzing DNA from pure persipan. The use of 2.0 fmol of apricot-specific probe and 0.095 fmol of *Prunus*-specific probe resulted in approximately equal intensities of the apricot- and *Prunus*-specific signals in the electropherogram (Figure 2B).

Limit of Quantitation (LOQ). The correlation between the persipan contents and the measured peak areas was determined by analysis of pure marzipan, pure persipan, and mixtures containing 50, 20, 15, 10, 5, 2.5, 1, 0.5, and 0.1% persipan in marzipan. Each sample was extracted 3-fold and analyzed in duplicate. The observed logarithmic correlation between the normalized peak areas and the persipan contents is expressed by the equation $y = 0.071 \ln(x) + 0.1022$. The intersection of the graph with the x axis, corresponding to a content of 0.24% persipan in marzipan, represents the theoretical LOQ of the method. This is in agreement with inconsistent results obtained for a content of 0.1% persipan in marzipan (only three of the six replicates were positive). On the other hand, for the content of 0.5% persipan in marzipan, consistently positive results were obtained; therefore, this concentration is considered as the practical LOQ of the method.

Analysis of Model Material. Because of the lack of commercially available reference materials, two mixtures of marzipan and persipan (Mod 1, 0.87% persipan in marzipan; Mod 2, 4.16% persipan in marzipan) were self-prepared. Mod 1 should represent a material containing persipan as inadvertent contamination, whereas Mod 2 was selected as an example for a material containing persipan as intentional admixture.

These samples were analyzed in three series. In each series, the samples were extracted 3 times and each extract was analyzed twice. In addition, for each series, a three-point calibration curve (1, 5, and 15% persipan in marzipan) was established and used for the quantitation. As shown in Table 4,

Table 4. Contents (%) of Persipan in Marzipan Determined via LPA in Self-Prepared Model Mixtures

		Mod 1[a]		Mod 2[b]	
series	extract	$A/(A + P)$[c]	persipan in marzipan (%)	$A/(A + P)$[c]	persipan in marzipan (%)
1[d]	1	0.112	1.23	0.196	3.13
	2	0.118	1.33	0.178	2.56
	3	0.105	1.15	0.243	5.25
			1.24 ± 0.09		3.64 ± 1.42
2[d]	4	0.063	1.26	0.147	3.20
	5	0.044	1.02	0.151	3.35
	6	0.069	1.35	0.151	3.35
			1.21 ± 0.17		3.30 ± 0.08
3[d]	7	0.049	0.96	0.145	3.92
	8	0.060	1.13	0.145	3.92
	9	0.054	1.04	0.128	3.06
			1.04 ± 0.08		3.64 ± 0.50
RSD %[e]	intraseries		9.7		18.4
	interseries		12.0		21.9

[a]Mod 1 is 0.87% persipan in marzipan. [b]Mod 2 is 4.16% persipan in marzipan. [c]A is the peak area of the apricot-specific signal. P is the peak area of the *Prunus*-specific signal. Values are the mean of duplicate analysis. [d]Regressions according to three-point calibrations (1, 5, and 15% persipan in marzipan): $y = 0.0909 \ln(x) + 0.0925$, $R^2 = 0.9940$ (series 1); $y = 0902 \ln(x) + 0.0420$, $R^2 = 0.9988$ (series 2); and $y = 0.0684 \ln(x) + 0.0515$, $R^2 = 0.9906$ (series 3). [e]RSD = relative standard deviation.

the low concentration level (0.87% persipan in marzipan) was slightly overestimated (on average +33%) and the high concentration level (4.16% persipan in marzipan) was slightly underestimated (on average −15%). For both levels, the standard deviations within each series indicate good repeatability of the method. The comparison of the data from the three series demonstrates the reproducibility of the approach. The overestimation (+33%) of the persipan content at the 0.87% level is slightly above the range (±25%) recommended, for example, for the PCR-based analysis of GMOs.[19] However, it should be noted that the validation was only performed with one self-prepared model mixture. Tests with further mixtures

might confirm the suitability of the method to quantitate persipan in this low concentration range.

In conclusion, the developed LPA method enables a specific and sensitive detection of apricot DNA in marzipan. According to German food guidelines, persipan paste may not only be produced from apricot but also from peach kernels.[1] However, the use of apricot kernels is commercially the far more important approach; therefore, the developed method can be considered as a useful indicator for the presence of persipan, even if the detection of peach DNA is not included.

In addition, the use of a *Prunus*-specific sequence as a reference allows for the relative quantitation of persipan in marzipan at concentration levels of practical relevance for quality control. It has to be kept in mind that the employed quantitation approach resulted in the unit "content of persipan in marzipan (%)", because the compositions of the marzipan and persipan materials used to prepare the analyzed mixtures, that is the ratios of marzipan paste and persipan paste versus sugar, were known. Otherwise, the method only provides information on the proportion of apricot DNA in *Prunus* DNA. This proportion would be independent from the presence of *Prunus* DNA other than almond DNA. The value might, for example, be used for an estimation of the persipan content by assuming the maximum amount of sugar (1.5-fold amount of persipan paste) allowed according to the guidelines.[1]

The developed LPA approach is laborious; however, it offers the possibility to be extended to a multiplex LPA. Thus, the array of species-specific PCR detection systems developed for the purity control of marzipan[4] could be combined with the presented probe in a "one-tube" approach.

■ ASSOCIATED CONTENT

Supporting Information

Correlation between the content of persipan in marzipan (0.5−100%) and the ratios of the apricot- and *Prunus*-specific peak areas (Figure S1). This material is available free of charge via the Internet at http://pubs.acs.org.

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Notes

The authors declare no competing financial interest.

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