

A TaqMan Real-Time PCR Assay for Apricot (*Prunus armeniaca*) as an Authenticity Test for Detection of Traces of Persipan in Marzipan

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Abstract Almond (*Prunus dulcis*), typically used in marzipan, is becoming more valuable by increasing global demand. As a consequence, substitution with cheaper alternatives is a common practice. Marzipan can be adulterated with apricot (*Prunus armeniaca*) or peach (*Prunus persica*), because of similar characteristics. Both species are closely related to almond; however, small differences in non-specific lipid transfer protein (LTP), used as target sequence for a qPCR test, can be noticed. In this study, a TaqMan qPCR method for specific detection of apricot in marzipan was developed. After optimization, specificity and sensitivity tests were carried out on real samples and the limit of detection was determined. The TaqMan qPCR method showed high specificity for apricot and was able to detect as low as 1% persipan in marzipan.

Keywords Marzipan · Persipan · Real-time PCR · *Prunus armeniaca* · Apricot · Adulteration

Introduction

Marzipan is a valuable ingredient of confectionery, prepared exclusively from almond kernels and sugar, and has a long tradition as a source of almond paste (Luber et al. 2012). Almond paste or marzipan is a widely used product in the confectionery industry and is highly consumed in North-West

Europe. Possible alternatives to almonds, thanks to the similarity in texture and appearance, are apricot kernels (*Prunus armeniaca*) and peach kernels (*Prunus persica*), all belong to the *Rosaceae* family (Janick et al. 1974). They are not only members of the same family; a phylogenetic study has shown that the large *Prunus* genus can be split into two clades: *Amygdalus/Armeniaca/Prunus* (peach-almond/apricot/plum) and *Cerasus/Lauro-cerasus/Padus* (cherry/laurel-cherry) (Potter et al. 2007). Therefore, all the nuts in the first clade (almond, peach, and apricot) can be used to make persipan, or parzipan as it is sometimes referred to, without any difference in taste. Persipan is a confectionary product similar to marzipan, however with apricot or peach kernels and sugar. Interestingly, there is hardly any difference in taste, color, or smell between marzipan and persipan, which makes it almost impossible to distinguish between these two products. Mostly, almonds can be easily substituted by the cheaper seeds or kernels of apricot (Davidson 1999). For example, in 2012, the price for a kilogram of almonds (blanched broken USA almond kernels) was € 4.70/kg while a kilo of apricots (blanched sweet apricot kernels) costed € 2.85/kg in the European market (Van Gansbeke 2012). Thus, persipan is a less expensive marzipan surrogate in which the almonds are replaced mostly by apricot or, alternatively, by peach kernels (Bruning et al. 2011). This economic benefit, involved in the intentional addition of cheaper ingredients such as apricot or peach seeds in the production process of marzipan, is the main motivation for this study.

In the literature (Boukhchina et al. 2004; Bruning et al. 2011; Gurfinger and Letan 1973; Haase et al. 2013; Kirchhoff et al. 1998; Luber et al. 2012; Weber and Hauser 2007), several approaches are described to differentiate between an apricot kernel and an almond kernel in terms of their (bio)chemical and genetic composition. A simplified approach is the iodine-starch reaction which requires the

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addition of starch to apricot (persipan) in quantities up to 0.5% (Leitsätze für Ölsamen und daraus hergestellte Massen und Süßwaren 2010). According to USDA's National Nutrient Database for Standard Reference (US Department of Agriculture 2014), the fatty acid composition is about 80% 16:0 total saturated fatty acids in almond oil as compared to 92% 16:0 in apricot kernel oil. Not only is the fatty acid composition important as a distinguishing feature, but also the presence of vitamin E (tocopherol) in almonds and its absence in apricot kernel. According to Gurfinger and Letan (1973), the amount of γ -tocopherol in almond oil is in excess (93% in $\mu\text{g/g}$ of oil) while in apricot oil, the amount of α -tocopherol was present in excess (96% in $\mu\text{g/g}$ of oil). This difference is important information to help distinguish an almond kernel from an apricot kernel. Boukhchina et al. (2004) described a different signature of glycerophospholipids (GPLs) present in oils that may provide an efficient means to differentiate between classes of membrane lipids in cells. According to them, the cell membrane of almonds consisted of different classes of GPL such as phosphatidyl ethanolamine, phosphatidyl choline, phosphatidyl glycerol, and phosphatidyl inositol in the ratio 45:45:8:2 (%), while the cell membrane of apricots has another ratio for the previous mentioned classes, i.e., 15:43:0:32 (Zlatanov and Janakieva 1998). Although food authentication based on ingredient detection routinely involves the detection of species-specific proteins, employing chemical or biochemical methods, genetically based methods have gained popularity in the recent past (Mafra et al. 2008). A DNA-based analysis is essentially a polymerase chain reaction (PCR) test to differentiate between marzipan and persipan and which can detect very low concentrations of persipan in marzipan (Bruning et al. 2011; Haase et al. 2013; Lubert et al. 2012). Although the extraction method is often laborious and time-consuming (Lubert et al. 2012), species-specific approaches to prove the contamination of marzipan have been published (Weber and Hauser 2007). These methods do not allow a quick and unambiguous distinction between the different species or have not been tested with a TaqMan probe to enhance specificity. Furthermore, Lubert et al. (2012) used a complex technique which is likely to be expensive. Simplicity and economic feasibility are two key novelties in this study. Kirchhoff et al. (1998) developed two independent test methods namely, an isoelectric focusing in polyacrylamide gel in an immobilized pH gradient and a molecular technique based on the detection of a certain apricot DNA fragment after amplification and agarose gel separation of the amplicons. The method is highly specific, and the representation of the protein band and the DNA section of the apricot kernels is independent of the manufacturing process and the country of origin. In addition, Weber and Hauser (2007) have designed a TaqMan probe assay for lipid transfer protein I in apricot with a detection limit below 0.1%. Unfortunately, no further information on

sequencing was available from their study. Almonds can give an allergic reaction, and for this purpose, tests are being developed. Brezna et al. (2015) compared and evaluated ten published methods for the detection of almond, in terms of their specificity. Costa et al. (2012) developed PCR tests for Pru 1 (11S globulin protein) as well as Pru du 5 allergen; however, cross-reactions with *P. armeniaca* and *P. persica* were noticed. Roder et al. (2011) described the differences between an ELISA and a new PCR test. They claim to have developed a specific test, however in some cases had to dilute the DNA template tenfold in order to have no cross reaction with, e.g., peach and apricot. Moreover, the authors' choice of primers is based on single-nucleotide differences between the DNA strands. López-Calleja et al. (2014) sequenced internal transcribed spacer 1 (ITS) between 18S and 5.8S rRNA of almond DNA which was the basis of making differences between the species. Not only is the DNA quality available for food authentication important, but also the cost of a given DNA-based analysis is significant and access to expensive instrumentation is required for sequencing or real-time PCR analysis (Lockley and Bardsley 2000). Nevertheless, despite all these biochemical techniques, adulteration can still occur by simply adding or removing a chemical compound. Therefore, a DNA-based approach will be the most conclusive method to detect different species: almond versus apricot. The objective of this study is to simplify the DNA extraction method and differentiate in particular between marzipan (almond-based paste), persipan (apricot-based paste), and their mixture, using a reliable and easy-to-implement qPCR method.

Materials and Methods

Sample Preparation

In this study, two types of samples were used: raw nuts or kernels and paste samples. Table 1 lists the used samples. Almond, apricot, and peach kernels as well as pastes were kindly provided by Euro-Mas NV (Brussels, Belgium), and the samples for the specificity test were bought in a local shop. The sour cherry kernels were kindly provided by the company SVZ Rijkevorsel NV (Belgium). The paste was already rolled during the manufacturing process; thus, further grinding was not needed. The kernels were frozen at $-80\text{ }^{\circ}\text{C}$ for at least 2 h. A longer refrigeration made it easier to grind the kernels. All kernels were ground with an ultracentrifugal mill (ZM 200, Retsch, Germany). The kernels were pulverized in two steps, initially with a sieve opening of 2 mm, followed by immediate storage at $-20\text{ }^{\circ}\text{C}$. Secondly, the sample was milled using a sieve with an opening of 0.75 mm. Remarkably, the apricot kernels directly gave a fine powder after milling with a 2-mm sieve.

Table 1 Samples used for specificity assessments

Sample name	Scientific name	Sight or type	Origin
Nonpareil almond	<i>Prunus dulcis</i>	Unpeeled, whole	Chile
Faro almond	<i>Prunus dulcis</i>	Unpeeled, whole	Portugal
PG almond	<i>Prunus dulcis</i>	Unpeeled, whole	Sicily
C132 almonds	<i>Prunus dulcis</i>	Pealed, broken	USA
C133 almonds	<i>Prunus dulcis</i>	Pealed, broken	Valencia
Sweet apricot kernel	<i>Prunus armeniaca</i>	Pealed, broken	Turkey
Bitter apricot kernel	<i>Prunus armeniaca</i>	Pealed, broken	China
Sweet cherry kernel	<i>Prunus avium</i>	Pealed, whole	Bought in local shop
Sour cherry kernel	<i>Prunus cerasus</i>	Pealed, whole	SVZ Rijkvorschel NV
Plum kernel	<i>Prunus domestica</i>	Pealed, whole	Bought in local shop
Ground coconut	<i>Cocos nucifera</i>	Pure flour	Bought in local shop
Hazelnut	<i>Corylus avellana</i>	Whole nuts	Bought in local shop
Pine nuts	<i>Pinus koraiensis</i>	Whole and unpeeled	Bought in local shop
Pistachio nuts	<i>Pistacia vera</i>	Whole and unpeeled	Bought in local shop
Walnuts	<i>Juglans regia</i>	Whole and unpeeled	Bought in local shop
Green lentils	<i>Lens culinaris</i>	Whole fruits	Bought in local shop
Wheat flour	<i>Triticum species</i>	Pure flour	Bought in local shop

DNA Extraction and Quality Check

In general, DNA was isolated by lysing the cells with chaotropic salts, followed by binding the DNA on a column; the column was washed and finally eluted. More specifically, the DNA extraction was done using DNeasy Plant Mini kit (Qiagen, Hilden, Germany). A test portion of 200 mg was weighed from all ground samples. Each sample was extracted in duplicate. The standard protocol was followed with some changes as explained further. Approximately 200 mg of ground material was mixed with 500 µl preheated (65 °C) AP1 buffer and 4 µl RNase (100 mg/ml). The sample was vortexed gently and incubated for at least 30 min at 65 °C. It was recommended to shake gently by reversing every 10 min. After the incubation, 162.5 µl P3 buffer was added and tubes were gently agitated and incubated for 5 min on ice. Further, the whole sample was transmitted on the purple column and centrifuged for 2 min at 20,000g. The flow-through was measured and transferred to a new tube, without disturbing the pellet and 1.5 volumes of AE/E buffer were added. The sample was mixed thoroughly. Next, 650 µl was loaded on the colorless column and centrifuged 2 min at 6000g. The last step was repeated with the remaining sample. The column was washed twice with 500 µl wash buffer and centrifuged respectively 1 min at 6000 g and 2 min at maximum speed. The column was replaced on a new tube and eluted two times with 50 µl preheated elution buffer AE (65 °C) and each time incubated for 5 min at room temperature. After the incubation, the sample was centrifuged for at least 1 min at 6000g. DNA quality and yield were checked with a Nanodrop spectrophotometer ND1000 (Thermo Scientific, Wilmington, MA,

USA), using Nanodrop 1000 V3.8.1 software and the optical densities (OD) of 230, 260, and 280 nm were used for further calculations. DNA samples were measured in triplicate.

Selection of Target Sequences and Oligonucleotide (Primers, Probe) Design

Selection of Target Sequences

The selected genomic region for molecular detection of almond and apricot is the non-specific lipid transfer protein (LTP). The appropriate accession numbers of the selected region of interest for the respective species are listed in Table 2. The non-specific LTP gene encodes for an extracellular protein which can bind to a broad variety of lipids. Researchers have found that LTP could be involved in plant defense mechanisms and plays an important role in food allergies (Salcedo et al. 2007). The LTP gene was used because Lubert et al. (2012) described the possibility to distinguish the species with this gene. All sequences were retrieved from the website of the National Center for Biotechnology Information (NCBI).

Table 2 Selected regions and accession numbers for alignments in the used LTP region

Species	LTP region
<i>Prunus dulcis</i>	HM234053.1
<i>Prunus armeniaca</i>	HM234047.1
<i>Prunus persica</i>	HM234040.1

Sequence Alignments and Design of Primers and Probes

Before primers and probes could be designed, an alignment of the LTP sequences was needed. Figure 1 shows the alignment of the selected regions for the three different species (almond, apricot, and peach). The primers are set around the gaps for a species-specific result. More specifically, the LTP reverse primer is bridging a gap of several nucleotides, showing theoretical specificity for apricot (Fig. 1). Theoretically, primer LTP-RW (apricot, Fig. 1) is a species-specific primer, as mentioned in Table 3. An apricot-specific TaqMan probe was designed to increase the specificity of the test. This probe is targeting the middle of the amplicon (Fig. 1). The primers were designed with the open-source software Primer3 version 4.0.0 and the probe with version 0.4.0. The designed primers and probe are listed in Table 3.

Real-Time PCR Optimization

The use of the TaqMan probes makes the test more accurate and species specific. In this case, the TaqMan probe was labeled dually with FAM at the 5' end and with TAMRA at the 3' end (Eurogentec, Belgium). Further, the reaction mixture contained 5 µl template DNA, 5 µl 5× HOT FIREPol Probe qPCR Mix Plus (Solis BioDyne, Estonia), 200 nM of the probe (LTP - probe), 100 nM of the specific primer (LTP-RW), and 200 nM of the non-specific primer (LTP-FW) and the mixture was diluted to a total reaction volume of 25 µl.

The qPCR Mix Plus contained the following components: DNA polymerase, probe qPCR buffer, 15 mM MgCl₂, dNTPs, and no ROX dye. PCR reactions were conducted with the LightCycler 480 (Roche, Germany), and the PCR program was conducted in the following way. Firstly, a denaturation step of 5 min at 95 °C was performed. Secondly, 45 cycles which consisted of two steps: a denaturation step of 10 s at 95 °C and an annealing and elongation step of 1 min at 58 °C were performed. After the elongation step, a single measurement was done. Finally, the plate was cooled to 40 °C.

Real-Time PCR Validation

LOD Assessment

After selecting the optimum PCR program, the next step was to determine the limit of detection (LOD) for the TaqMan qPCR. For this, firstly, a pool of PG almond and sweet apricot DNA was made and the concentration was measured as explained in the DNA extraction protocol and quality check. Equation 1 was used to convert the concentration of the DNA into a number of sequence target copies used per reaction:

$$\text{number of copies} = C \times \frac{1}{C_p} \times V_{\text{PCR}} \times 10^3 \quad (1)$$

where C is the DNA concentration of the sample (ng/µl), C_p is the mass of 1 haploid copy DNA (pg), and V_{PCR} is the volume

361-420	
Pr. dulcis	CTTACCAGATTAGCCCCTCCACCAACTGCGCCAAGTACGTATTAATAAATAAAGAAGCTC
Pr. persica	CTTACAAGATTAGCGCCTCCACCAACTGCGCCACGTACGTATTAATAAATAAAGAAGCTC
Pr. armeniaca	CTTACAAGATTAGCATCTCCACCAACTGCGCCGCGTACGTATTAATAAATAAAGAAGCTC

421-480	
Pr. dulcis	CAAGTTTTAATTTT-GTAGGTTTGATCAAAATTCAACGGTTCATATTATCTCAAAACCTA
Pr. persica	CAAGTTTTAATTTT-GTAGGTTTGATCAAAATTCAACGGTTCATATTATCTCAAAACCTA
Pr. armeniaca	CAAGCTTTAATTTTATAGGTTTGATCAAAATTCAACGGTTCATATCATCTCAAAACCTA

481-540	
Pr. dulcis	ACTTTTAAATGTGGATCGTTGAAATTTTGGTCTAGCTTGTTGAGAATTAATGCGGAAC
Pr. persica	ACTTTTAAATGTGGATCGTTGAAATTTTGGTCTAGCTTGTTGAGAATTAATGCGGAAC
Pr. armeniaca	<u>ACTTTTAAATGTGGATCGTTGAAATTTTGGTCTAGCTTGTTGACAATT</u> -----

541-600	
Pr. dulcis	ATTACATAACTGACTGACTTTTGGGTTTGTGTTGTTGGTGTGTCAGCGTGAAGTGAGC
Pr. persica	ATTACATAACTGACTGACTTTTGGGTTTGTGTTGTTGGTGTGTCAGCGTGAAGTGAGC
Pr. armeniaca	----- <u>GACTGACTTTTGGGTTGTGTTTGTGTTGGTGTGTCAGCGTGAAGTGAGC</u>

601-660	
Pr. dulcis	TTGATATGGGACAAGTATTTTCGGCCGCAGATAATATTAGAGAGTCGGAATAAAATGTG
Pr. persica	TTGATATGGGACAAGTATTTTCGGCCGCAGATAATATTAGAGAGTCGGAATAAAATGTG
Pr. armeniaca	TTGATATGGGAGATGTGTTTTCGGCCGCAGATAATATTAGAGAGTGAGAATAAAATGTG

Fig. 1 Sequence alignments and selected oligonucleotide sequences for apricot (LTP PCR) CLUSTAL X (1.81) multiple sequence alignment for the LTP gene for the species *Pr. dulcis* (HM234053.1), *Pr. persica*

(HM234040.1), and *Pr. armeniaca* (HM234047.1). It shows the result between bp 361 and 660. In **bold** are the forward respectively reverse primers and **bold and underlined** presents the TaqMan probe

Table 3 Designed primers and probes used in this study

Species	Primer name	Primer sequence	Length amplicon
Apricot (<i>Prunus armeniaca</i>)	LTP-FW 1	CCAAGCTTTAATTTTATAGGTTTGAT	112 bp
	LTP-RW 1 ^a	TCAATTGTCAACAAGCTAGACCA	
	LTP-probe	TTCAACGGTTCATATCATCTCAAAACCTAAC	

^a Species-specific primer

of DNA in PCR reaction (μl). The C_p values were found in the literature. Arumuganathan and Earle (1991) reported a C_p value of 0.30 pg for 1C or one haploid genome of apricot. For almond, Baird et al. (1994) reported a C_p of 0.33 pg. When the number of copies was known, the pool was diluted to a concentration of 100,000 copies per 5 μl. Furthermore, a tenfold serial dilution was made from 100,000 copies down to 0.1 copies in 5 μl. The test was done for the primers with SYBR Green as well as the primers together with the probe. Both PCR tests were performed in duplicate, thus two independent replicates of the whole experiment.

Specificity Assessment

False-positive results were needed to be avoided; thus, a set of samples which could give false-positive results were chosen. The list of chosen products can be seen in Table 1. All the samples were analyzed in the same way: this means the same DNA extraction and TaqMan qPCR.

Sensitivity Assessment

A sensitivity test of the primers was conducted for the TaqMan qPCR. To this end, persipan was spiked into

marzipan in different relative concentrations as shown in Table 4. The total amounts of spiked material (persipan) and spiked matrix (marzipan) are also shown. It is noteworthy to remind that the overall purpose of this study is to detect adulteration; thus, higher concentrations are more likely to be present in a product. The sensitivity was only tested with the primers and respective probes.

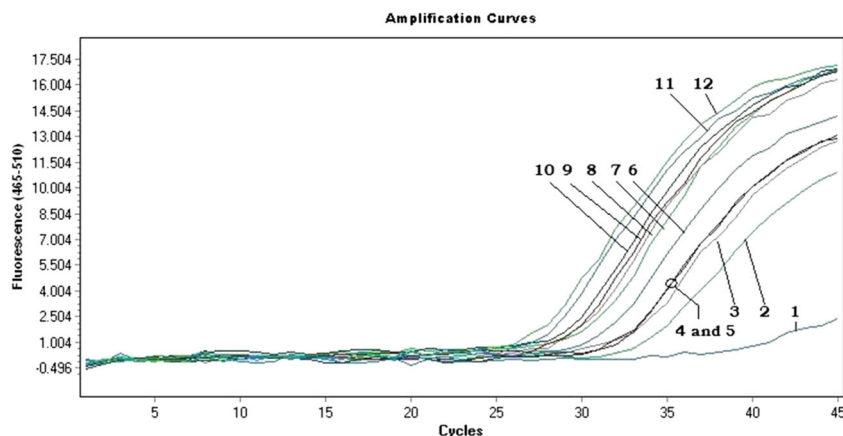
Results and Discussion

DNA concentrations as well as purity ratios for all DNA extracts listed in Table 1 were measured. Table 1 is divided into two parts—the upper part is the samples which were used during the optimization of the PCR reaction and the lower part contains those for the specificity test. DNA concentrations for the upper part were between 6.53 and 37.63 ng/μl (data not shown). A significant difference can clearly be seen between peeled and unpeeled almonds: DNA concentrations were almost five times higher for unpeeled almonds. Additionally, the peeled apricot kernels deliver more DNA with respect to the peeled almonds. Lastly, the samples of the lower part were tested and their concentration varied between 5.36 and 161.38 ng/μl (data not shown). In addition to the measurement

Table 4 Percentage spike of persipan in several samples of marzipan used for the sensitivity test

Sample number	Amount persipan (mg)	Amount marzipan (mg)	Spiked mixture (%)	Number in Fig. 2	C_T value in Fig. 2	Sample number	Amount persipan (mg)	Amount marzipan (mg)	Spiked mixture (%)	Number in Fig. 2	C_T value in Fig. 2
1A	0	209	0.00	1	—	7A	43	163	20.87	7	30.62
1B	0	203	0.00			7B	43	162	20.98		
2A	2	198	1.00	2	33.37	8A	63	139	31.19	8	29.64
2B	3	199	1.49			8B	62	142	30.39		
3A	5	196	2.49	3	32.88	9A	79	120	39.70	9	29.20
3B	5	197	2.48			9B	80	119	40.20		
4A	8	192	4.00	4	32.12	10A	102	101	50.25	10	29.16
4B	8	192	4.00			10B	101	102	49.75		
5A	10	189	5.03	5	32.21	11A	150	53	73.89	11	27.75
5B	11	189	5.50			11B	156	50	75.73		
6A	22	181	10.84	6	31.06	12A	207	0	100.00	12	27.47
6B	20	180	10.00			12B	199	0	100.00		

Fig. 2 Detection of persipan concentrations in marzipan mixtures with LTP probe. See also Table 4 for the legend of the numbers



of the concentration, the quality of the DNA was also tested. The expected ratio A_{260}/A_{280} must be between 1.8 and 2.0, but most of the values ranged between 1.6 and 1.8 and thus were suboptimal (data not shown). The ratio A_{260}/A_{230} is a backup method to exclude other contaminations. Here, the optimal ratio is between 2 and 2.2. In the results presented in this study, this optimal ratio was not achieved.

During the development of the test, different approaches were used to obtain the most specific result. The specific primer (LTP-RW) was reduced to 100 nM to enhance apricot-specific binding of the primer. Different types of almonds and apricots were further tested, as mentioned in Table 1. The same results were obtained for the different samples of the same species. More cycles, i.e., 45 cycles, are needed to ensure a true negative result for the non-targeted species. The probe is designed with a FAM label at the 5'-side and a TAMRA label at the 3'-side. The exonuclease activity of the DNA polymerase led to a measurable fluorescent signal. The specificity between almond and apricot increased for primers LTP by combining with a specific TaqMan probe. The mean C_T value is 29.23 for a probe concentration of 200 nM when using LTP probes in apricot samples. A signal is positive when an amplification is visible and a related C_T value is calculated. When all reactions for a certain amount of copies give a positive result, this is called the LOD. The LOD for the LTP probe test is 1000 copies per 5 μ l. The appropriate C_T value is 36.64. A high specificity was obtained when the different species were tested with the TaqMan qPCR. All samples gave a negative result for the depicted species in the lower part of Table 1.

The specificity of the primers was tested by a BLAST in the NCBI. No other alignment was found than for the respective target. The specificity was checked with other possible nuts which can be used as adulteration, and no co-reactivity of the apricot qPCR assay with any other species was obtained.

The sensitivity test was conducted for the TaqMan detection method. The tested samples were mixtures of persipan and marzipan (samples 2–11, Table 4), in addition to pure

marzipan (sample 1, Table 4) and pure persipan (sample 12, Table 4). Although the tests detected marzipan correctly, making a difference between the different high concentrations of marzipan, based on the C_T number, was not possible. Figure 2 shows that the smallest adulteration of 1% persipan was detected, which means an addition of 10-g persipan in 1 kg of marzipan. All adulterations were detected from 1 to 100% (samples 2–12, Fig. 2). Moreover, the content of almonds was also shown in all the samples from 0 to 75% (samples 1–11, Table 4).

It is not easy to answer why there is such a big difference in yield between the unpeeled and peeled almonds. Probably, the skin of a kernel provides more DNA in comparison to the pure kernel itself. The concentrations below 10 ng/ μ l were initially not used, because at first, these low concentrations were thought to represent cross-contamination of DNA extraction impurities. Later, it was seen from the real-time PCR results that concentrations below 10 ng/ μ l were also sufficient. The absorbance spectra showing different peaks (data not shown) indicated that other compounds have very likely been co-extracted and are present in the final DNA extracts from almonds. Just like for the ratio A_{260}/A_{280} , also for the ratio A_{260}/A_{230} , optimal values were never achieved probably because of the contaminants present in the DNA extracts. Nevertheless, in comparison to the extraction method of Bruning et al. (2011), the purity was lower but DNA was certainly present. This extraction method is also easier and quicker than the method outlined in their study. The optimization of the PCR was done in two parts. Firstly, the best annealing temperature had to be found. Here, an annealing temperature of 58 $^{\circ}$ C provided a better separation than when using the standard annealing temperature of 60 $^{\circ}$ C. Therefore, the program with the lower annealing temperature was chosen. Secondly, an adapted primer mix was used to get a better separation. A lower concentration of the primers and a competition for the specific region usually give better results. Nevertheless, the specificity increased due to a better separation based on the melting curves.

Conclusions

An efficient and easy method was applied to extract DNA from almonds, apricot kernels, and kernels from other closely related species, as well as from marzipan and persipan. Primers were developed for apricot (*P. armeniaca*), in addition to a dual-labeled TaqMan probe. After optimization of the SYBR Green I detection as well as the TaqMan real-time detection assays, both methods were compared. The theoretically expected specificity was experimentally proven and thus confirmed for both methods. The apricot-specific TaqMan assay showed a LOD of 1000 target copies per 5 µl DNA. The results of the persipan-in-marzipan concentration matrix experiment clearly showed that with the persipan-specific TaqMan probe, a sensitivity as low as 1% of persipan in marzipan could be obtained. A 1 % adulteration, corresponding to 10 g of persipan in 1 kg marzipan, can thus be retrieved. In conclusion, the presented method is a reliable and easy-to-implement PCR method to detect economically motivated adulteration in marzipan.

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Compliance with Ethical Standards

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Ethical Approval This article does not contain any studies with human participants or animals performed by any of the authors.

Informed Consent Not applicable.

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