Determination of apricot kernel components in marzipan and semi-finished marzipan products with realtime (probe) PCR

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Summary

A molecular biological realtime PCR test using hybridization probes for the detection and determination of apricot kernels (*Prunus armeniaca*) in marzipan and semi-finished marzipan products is presented. The detection limit is below 0.1 %. The method detects the percentage of apricot kernel DNA in the total amount of extracted DNA. The target sequence for this is the gene for the lipid transfer protein I in apricots with 119 bp. The method is 100 % apricot-specific and permits semi-quantitative statements.

Initial situation

Based on the principles of the German Codex Alimentarius for oilseed and mixtures and confectionary[1)] manufactured from this, marzipan raw mixture mainly consists of sweet almonds. Bitter almonds are added in small quantities for the sake of taste. The addition of apricot kernels and mountain almonds is not permitted. For the confectionary industry, product purity and identity of marzipan are of great significance. In the past, attempts were repeatedly made to identify apricot kernel mixtures with tocopherol spectra. This however is unsuccessful with mixtures under 10 percent, or the statement is not clear. Tocopherol spectra can only be used to distinguish between the raw ingredients almond and apricot. Immunological procedures were as yet unable to be established on the basis of the high affinity between almond and apricot and the resulting cross reactivity of extracted antiserums. As a method of choice, the polymerase chain reaction (PCR) is available[2–4)].

Extraction of samples

The DNA is isolated from the marzipan samples to be examined via classical CTAB extraction[5)]. For a representative sample initial weight, a 2 g sample is mixed twice with 10 ml lysis buffer and the DNA is released with 10 ml proteinase K. After precipitation of the DNA and purification, the DNA content of the sample is set to a uniform DNA content (100 ng total DNA per PCR reaction).

Calibration

A 1:10 dilution sequence of an apricot DNA serves as a calibrator. The first calibrator is set to 100 ng DNA per PCR reaction (corresponds to 100% apricot DNA).

PCR conditions

The implementation of the PCR occurs in realtime with hybridization probes under the following conditions:
The primers Apr-LF1 (5'-TTATCTGCGTCAAGCTCACA-3') and Apr-LR1 (5'-GATCATTGAAATTTTGGTCTAGC-3') are used with a concentration of 750 nM; the probe Apr-LS1 (5'-Fam-TGTTGACAATTAAATGCGGAATATTTAMRA-3') is used with a concentration of 220 nM in the 25 µl PCR batch.
The TaqMan® Universal PCR MasterMix of *Applied Biosystems* is used as a MasterMix and the amplification takes place in the *Applied Biosystems* 7500 Realtime Cycler with the following temperature profile:
95 °C for 10 min, 45 cycles with 95 °C for 15 sec and 60 °C
for 1 min.
The following figures display the amplification plots of the standards as well as the standard curve.

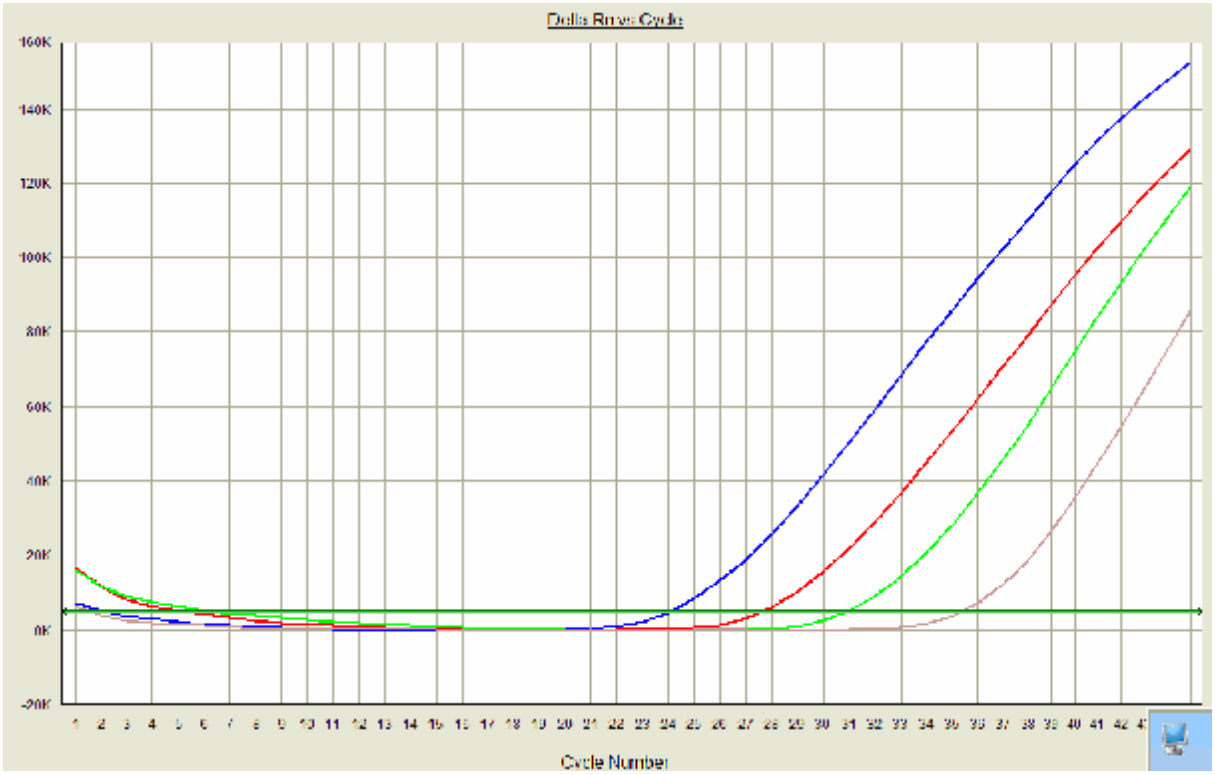

Fig. 1 Amplification blots Apricot standards 100% (blue), 10% (red), 1% (green), 0.1 % (violet) Apricot DNA

Standard curve for apricot

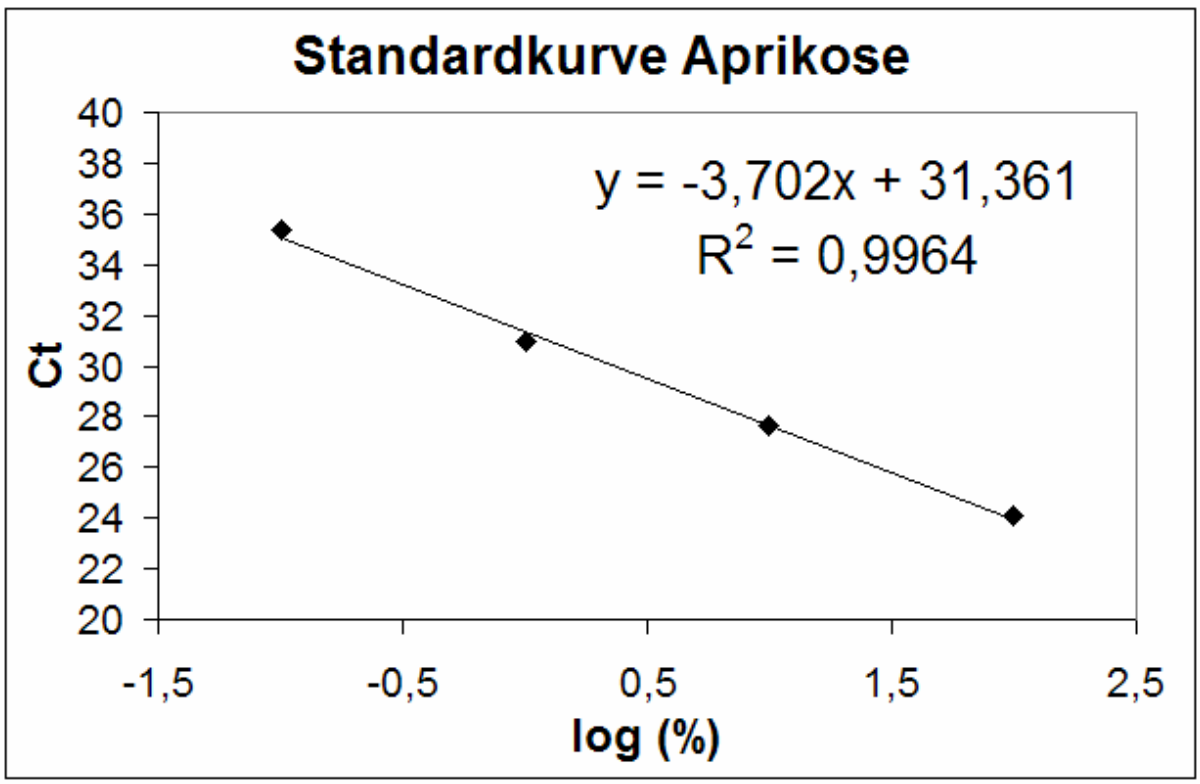

Fig. 2 Linear equation of the standards from four individual stipulations

Sensitivity
The method permits a determination of the apricot proportion of less than 0.1%.

Specificity
The test is 100% apricot-specific. The species in table 1 was tested with 100 ng DNA for cross reactivity.

Species		Species		Species		Species	
Apricots							
South Africa, bitter	+	China, bitter 1	+	Turkey, bitter 1	+	Iran, bitter	+
Turkey, sweet 1	+	China, bitter 2	+	Turkey, bitter 2	+	Greece, bitter	+
Turkey, sweet 2	+	China, bitter 3	+	Syria, bitter	+	China, sweet	+
Almonds							
Italy, sweet	–	Caifornia, sweet	–	Iran, bitter	–	Morocco, bitter	–
Afghanistan	–	Greece	–	Spain, bitter	–	Syria, bitter 1	–
Turkey, bitter	–	California	–	Almond, bitter	–	Syria, bitter 2	–
Spain 1	–	Spain 2	–				
mountain almond	–	cherry	–	cashew	–	plum	–
pecan nut	–	peanut	–	hazelnut	–	walnut	–
buckwheat	–	pistachio	–	corn	–	soybean	–

Tab. 1 Specificity PCRFast® apricot kernel realtime (+: amplification; –: no amplification) (von links nach rechts)

Analysis of reference samples and samples
Table 2 records the measurement results of marzipan samples with known apricot kernel contents (2 % and 0.5 %), 5 % mixtures of various ground apricot kernels with ground almonds, 10 ng total DNA of apricot kernels and 4 unknown marzipan samples.
The following figures display the amplification plots of various apricot DNAs (sweet and bitter), as well as the amplification plots of 5 % mixtures. It could be shown that the selected DNA section has the same amplification efficiency in all apricots that serve as a reference and can thus be used for the quantification (Fig. 3).

Sample	Ct value	Quantification
Sample 2 % A	30.17	approx. 2 %
Sample 2 % B	30.26	approx. 2 %
Sample 0.5 % A	32.63	approx. 0.5 %
Sample 0.5 % B	32.04	approx. 0.6 %
Mixture of 5 % Syrian, bitter apricot	28.7	approx. 5 %
Mixture 5 % Chinese, bitter apricot	28.07	approx. 7 %
Mixture 5 % Turkish, sweet apricot	28.47	approx. 6 %
10 ng Syrian, bitter apricot	27.53	approx. 11 %
10 ng Chinese, bitter apricot	27.63	approx. 11 %
10 ng Turkish, sweet apricot	27.8	approx. 10 %
Sample 1 A	37.22	< 0.1 %
Sample 1 B	37.84	< 0.1 %
Sample 2 A	37.75	< 0.1 %
Sample 2 B	37.2	< 0.1 %
Sample 3 A	29.76	approx. 3 %
Sample 3 B	29.49	approx. 3 %
Sample 4 A	30.71	approx. 2 %
Sample 4 B	30.25	approx. 2 %

Tab. 2 Ct values and quantification of various samples

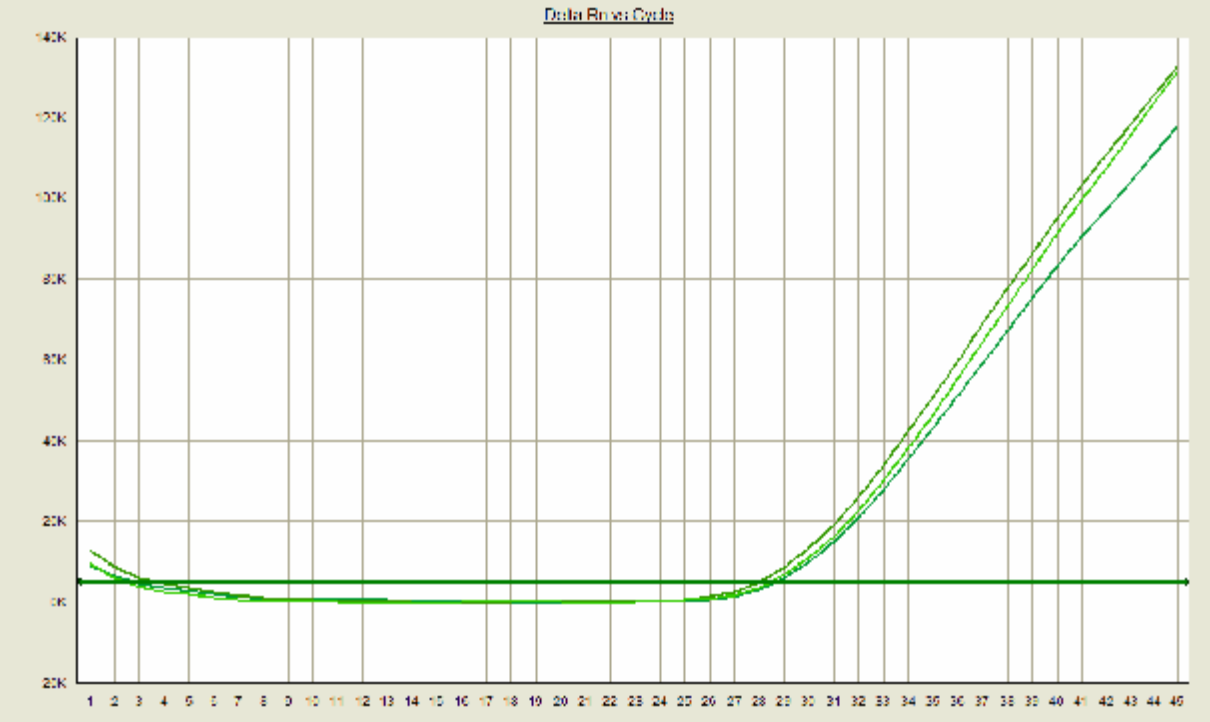

Fig. 3 Amplification plots of 10 ng apricot DNAs of various origin and types

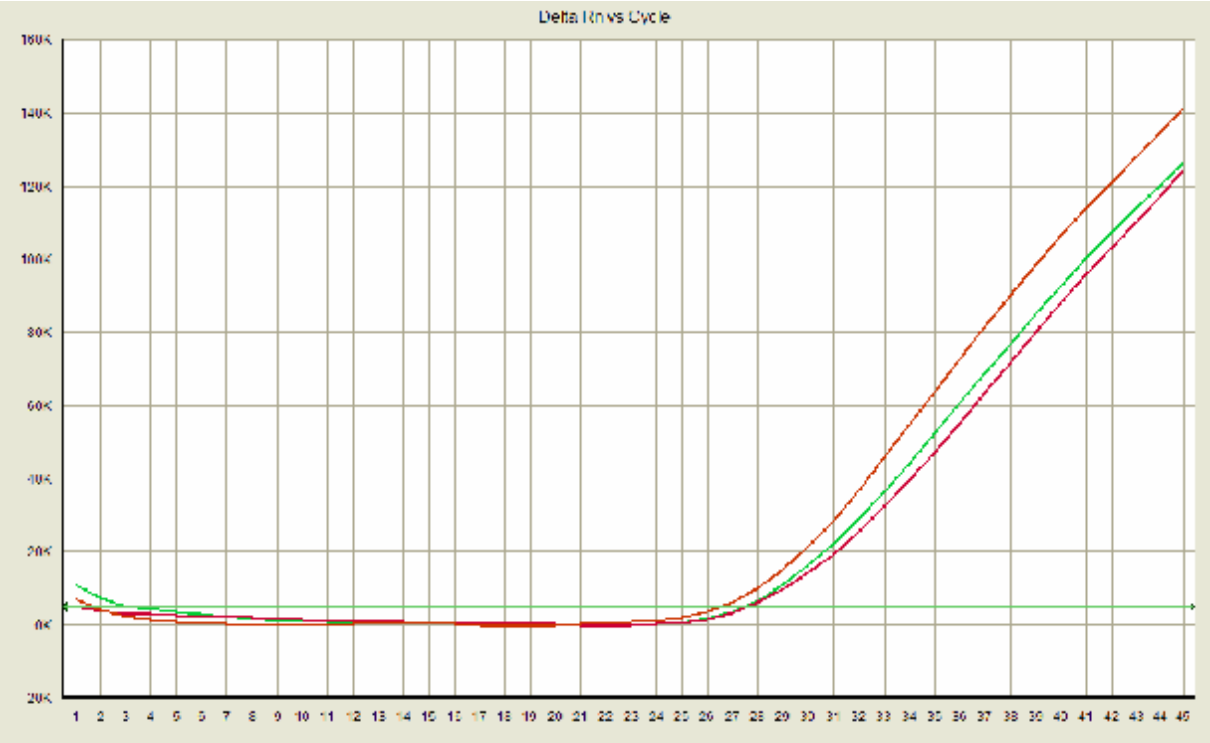

Fig. 4 Amplification plots of 5 % mixtures (curves from left: red-green-red)

Precision of methodology

Generally, in PCR the samples should be processed in double batches. A greater deviation than 30% between a double determination should not be accepted. The accuracy of the result is dependent on the precise setting of the total DNA per reaction. For this purpose, the DNA must exist in a purified form or not contain any inhibitory accompanying substances for the PCR reaction.

Discussion and results

The realtime PCR method presented with hybridization probes permits the reliable detection of apricot components in marzipan and semi-finished marzipan up to less than 0.1 %. The method was validated with all available bitter and sweet apricot kernels on the market from all countries of origin, as well as with all available sweet and bitter almonds on the market.
Cross reactions were unable to be determined; the method is thus classed as highly specific.
Experience shows that the quality of the DNA is highly significant for the semi-quantitative determination. Fluctuations in the quantitative evaluation are caused by the type of DNA extraction and quality of isolated DNA. The method presented can be used to make a semi-quantitative statement. This was able to be verified with specially produced reference materials.
This method can in future be used to clearly and reliably detect accidental contaminations and intended additions of apricot kernels. The procedure is thus suitable for quality control in the manufacture and inspection of imported goods. The ifp Institut für Produktqualität (Institute for Product Quality) has further molecular biological and immunological detection procedures for checking the purity of marzipan and oilseed products. The further detection portfolio includes
the PCR detection of mountain almonds, soybeans, cashews and lupines, as well as the immunological detection of chickpeas, white beans, lupines and cashews with specific antiserums extracted from sheep and rabbits.

Literature

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4) *Sambrook, J., E. F. Fritsch,* and *T. Maniatis*: Molecular cloning. A laboratory manual. 3rd ed., Cold Spring Harbor Laboratory Press, New York (2002).
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