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Detection of Potato DNA from Potato Tubers and Processed Food Products by Means of PCR

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Introduction

The polymerase chain reaction (PCR) (1) is widely used in many fields of analysis to detect even small amounts of DNA very specifically (2). Several PCR systems are described for various applications of food control such as species identification of fish (3–5) and meat (6–9), of food components (10–13) and several genetically modified organisms like e.g. Roundup Ready soya (14) and genetically modified maize (15, 16). Especially for the detection of GMO a species specific PCR-system like lectine- (soya) (17) or zein-PCR (maize) (16) is necessary for a parallel testing of suitability of the isolated DNA for PCR beside the GMO specific detection system. Physical and chemical parameters such as shearforces, heat and acidic pH may lead to degradation of DNA. The resulting decrease in length and quality of target DNA may hinder detection of any DNA (18). Beside, many ingredients such as salt and spices in high concentrations may inhibit the polymerase chain reaction. Recently it was reported that chlorogenic acid, one plant constituent in potato extracts, could inhibit polymerase chain reaction (19). Therefore the influence of processing and ingredients of potato food products must be investigated.

In this study we describe a highly specific nested PCR-system for amplification of a 146 bp fragment of the patatin gene. Patatin is a tuber specific storage protein of potatoes *(Solanum tuberosum)* (20). Several highly processed potato products such as instant mashed potatoes, potato crisps, potato crackers and instant potato soup and French fries were investigated for presence of DNA. Quality, amount and suitability for PCR were determined.

Materials and methods

Potato samples

Potatoes of six Swiss cultivars of *Solanum tuberosum* and eight processed potato food products were purchased at a local supermarket (table 1).

Table 1
Concentration of extracted total DNA of investigated potato cultivars and processed potato products

No.	*sample*	*DNA (µg/ml)*
154	potato cultivar Urgenta	17.4
155	potato cultivar Sirtima	n.d.
156	potato cultivar Stella	n.d.
157	potato cultivar Ostara	n.d.
158	potato cultivar Bintje	n.d.
159	potato cultivar Charlotte	n.d.
160	potato soup I	552.9
161	potato soup II	78.1
162	instant mashed potato I	20.6
163	instant mashed potato II	22.1
164	potato crisps	194.7
165	Pom-Teddies	47.9
166	potato cracker (with potato flour)	124.1
198	French fries (frozen)	30.1

n.d. = not determined

DNA extraction and purification

200 mg of homogenised samples (due to electrostatic charge instant samples were moistured with water to allow weighing out) were incubated in 1 ml extraction buffer (10 mM Tris-HCl [pH 8.0], 150 mM NaCl, 2 mM EDTA and 1% [w/v] sodium dodecyl sulfate [SDS]), 100 ml of 5 M guanidine hydrochloride and 50 µl of 20 mg/ml proteinase K (Merck, Darmstadt, Germany) on a thermomixer at 57° C over night. After digestion, the samples were centrifuged at 20000 x *g* for 15 min. Then 500 µl of the clear aqueous phase were incubated together with 5 µl RNase (10 mg/ml) at 57° C for 5 min and 1 ml Wizard® DNA purification Resin (Promega, Madison, WI) was added and mixed by gentle inversion. The mixture was transferred to a syringe plugged on a Wizard column, attached to a vacuum manifold. Vacuum was applied and the column was washed with 2 ml 80% isopropanol followed by centrifugation at 20000 x *g* for 1 min. After drying at 70° C for 10 min, the DNA was eluted with 50 µl of 70° C elution buffer (10 mM Tris-HCl [pH 9.0]) and stored at –20° C.

25 µl of the extracted DNA were loaded on a 0.8% agarose gel (Agarose LE, Promega) in 0.5x TBE buffer (0.045 M Tris-borate, 0.045 M boric acid, 0.001 M

EDTA, [pH8.0]) and stained by ethidium bromide. As size reference a 100 bp ladder (Gibco) was used.

DNA target sequence and primer selection

The *Solanum tuberosum* (potato) specific patatin genes were chosen from EMBL database as target for construction of the potato specific nested PCR system. A consensus sequence of the 288 bp Exon 5 of accession no. M18880, X03932 and X03956 was created and the outer (PAT 1/2) and inner (PAT 3/4) primer pairs were designed using Oligo Primer design software (table 2). Suitability of extracted DNA for PCR was tested with the chloroplast specific primer TAB03 and TAB04 (table 2). Primers were synthesised by Microsynth (Microsynth, Balgach, Switzerland). Aliquots of lyophilised primers were stored at –20° C.

Table 2
Primer sequences

primer	*sequence*	*reference*
PAT1	GTT ATT ATC CCT TAG CGT TGC	this work
PAT2	AAT TGT TTT GTG AAT GAC GAG	this work
PAT3	GGA TCC AGC ATT TTC TTC A	this work
PAT4	TAG CTA ACA TCC ATC GTA GAG G	this work
TAB03	CGA AAT CGG TAG ACG CTA CG	(23)
TAB04	GGG GAT AGA GGG ACT TGA AC	(23

PCR and DNA analysis

DNA amplification was carried out in a final volume of 50 µl in 0.5 ml tubes containing PCR buffer (20 mM Tris-HCl [pH8.4], 50 mM KCl), 2 µg/ml BSA, 2.5 mM $MgCl_2$ (PAT 1/2) and 2.0 mM $MgCl_2$ (PAT 3/4), 200 µM each dNTP, 0.5 µM each primer, 1 unit of *Taq* DNA polymerase (Life Technologies) and 100 ng DNA. For the nested PCR (PAT 3/4) 1 µl of the first PCR (PAT 1/2) was used as template. The cycling conditions on a Progene thermal cycler (Techne, Cambridge, UK) were as follows: 96° C for 3 min for initial denaturation, 25 (PAT 1/2) and 35 (nested PAT 3/4) cycles of amplification (96° C for 30 s, 60° C for 1 min) and final extension at 72° C for 3 min. PCR products were examined by electrophoresis through a 1.5 % agarose gel in 0.5x TBE buffer (0.045 M Tris-borate, 0.045 M boric acid, 0.001 M EDTA, [pH8.0]) and stained by ethidium bromide. As size reference a 100 bp ladder (Life Technologies) was used.

Results and discussion

Quality and quantity of extracted DNA

For detection of genetic elements in food products by means of PCR (polymerase chain reaction) processing of raw materials and the resulting decrease in

quality as well as in the amount of extracted genomic DNA are very important. From several potato products available on the market eight samples were selected, representing food products undergoing severe heat and mechanical treatment during production like: grading, deep frying or baking (table 1).

After DNA extraction applying the Wizard protocol (see also (9)), the quality of pure total DNA was determined on a 0.8% agarose gel (fig. 1). As expected, high molecular DNA was extracted from the six cultivars. Potato soups of both manufacturers as well as "Pom-Teddies" yielded high molecular DNA that was partly degraded. For potato crisps and crackers only highly degraded DNA of 100–2000 bp could be visualised on the gel. No DNA was detectable at the lanes of the two samples of instant mashed potatoes and French fries. Concentrations of DNA were determined measuring absorption at 260 nm. The amount of DNA per ml varied from 17.4 µg from potato tubers to 552.9 µg for one instant potato soup (table 1). The quality of DNA was not correlating to the amount of extracted DNA. The concentration of crisps for example was relatively high (194.7 µg/ml) but the average length of nucleic acids was very short (approx. 100 bp). For potato tubers a very small amount of high molecular DNA could be obtained. These results show that availability of extracted DNA for PCR can not only be assessed by gel electrophoresis or by measuring OD_{260} because a high concentration of very small

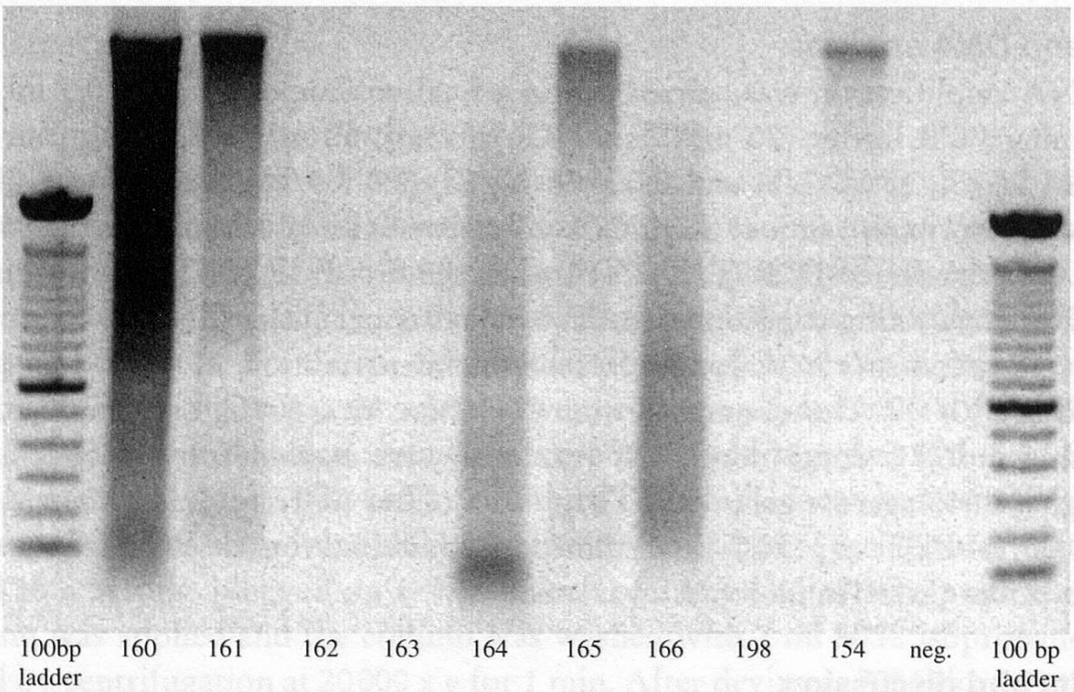

Figure 1 **Agarose gel electrophoresis (0.8% agarose) of DNA extracted from eight food samples (160–198) and one potato cultivar (154),** neg. = Extraction protocol without food sample

DNA fragments may not result in a successful PCR reaction due to degradation of DNA during processing. The amount and quality of DNA is shown in table 1 and figure 1. Due to further ingredients, the extracted nucleic acid does not just represent potato DNA but total DNA in each product.

Selection of target gene and primer design

The potato specific patatin gene, encoding a family of glycoproteines representing 40 % of the total soluble protein in potato tubers, was chosen as target for the species specific nested PCR system. Due to partly high degradation of DNA, the outer PCR amplicon should be as short as possible to allow detection of DNA even in highly processed food products and long enough to enable construction of a sensitive, specific inner, nested primer pair. For obvious differentiation between expected PCR amplicon and short artifact products the inner (nested) amplicon should be about 150 bp in length or longer. By the help of the OLIGO primer design software four primers were defined on the 288 bp exon 5 of the patatin gene resulting in a 272 bp outer fragment and a 146 bp inner fragment.

Specificity of detection

For all six selected potato cultivars (Urgenta, Stella, Sirtima, Charlotte, Bintje and Ostara) the expected amplicon at 146 bp could be generated by nested PCR (fig. 3: 154–159). In contrast to other reports (19) no inhibition of PCR due to special potato constituents was found neither with potato tuber nor with potato product DNA extracts. Specificity of the new PCR system was tested for several foodstuffs (tomato, broccoli, cauliflower, maize, soya, rice, wheat, oat, barley, rye,

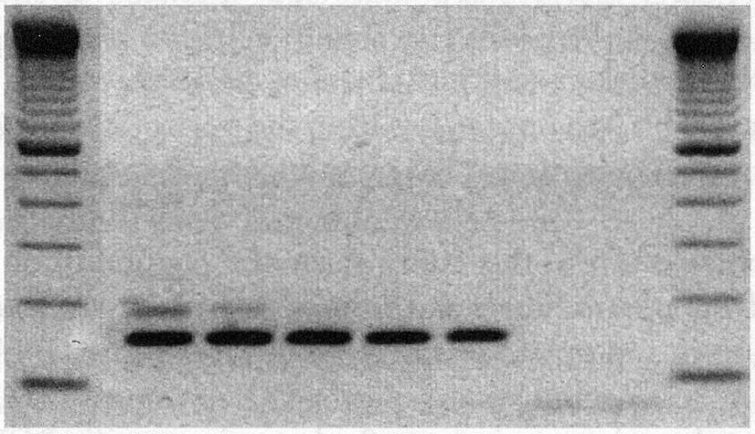

Figure 2 **Agarose gel electrophoresis of nested PCR of serial dilutions of DNA extracted from potato tuber (urgenta cultivar, 100 ng–1 pg).** neg. = PCR amplification without template DNA

lentils and meat of pig, perch and chicken) but no amplification product could be detected (data not shown). The chloroplast (TAB 03/04) (23) and the cytochrome *b* (L14841/H15149) (21) specific PCR served as control for suitability of isolated DNA of the above control foodstuffs for PCR (data not shown).

At higher concentrations of DNA a weak additional band was visible at ca. 180 bp after nested PCR (fig. 2). For quantitative PCR the conditions (number of cycles) have to be adapted in the way that no additional bands occur on the agarose gel which may result in a decline of sensitivity of the nested PCR system.

Detection limits

The detection limit of each single primer system was 1 ng of purified DNA isolated from potato tuber. The limit of the nested system was of a factor 100 lower at 10 pg corresponding to ca. 1–10 genome copies (table 3, fig. 2).

Table 3
Sensitivity of single and nested PCR-systems; DNA was extracted from Urgenta cultivar

PCR-system		*cycles*	*sensitivity*	*amplicon*
PAT 1/PAT 2	single	40	1ng	272 bp
PAT 3/PAT 4	single	40	1ng	146 bp
PAT 1/PAT 2		25		
PAT 3/PAT 4	nested	35	10 pg	146 bp

Food products

For all potato food products the 146 bp PCR fragment could be amplified (fig. 3: 160–198). Even those samples showing no or highly degraded DNA on the 0.8 % agarose gel contained sufficient template of appropriate length to allow a nested PCR.

Some products yielded an additional band at ca. 180 bp which is also visible at the potato cultivars. This band originates from the potato DNA (see also fig. 2) and not from other food ingredients and has no influence on specificity of the PCR system.

The results obtained from this study show the possibility of amplification of DNA extracted from potato tubers and from highly processed food products such as instant potato soup and potato crisps. Even in case of low DNA concentrations or degraded DNA, a successful potato specific PCR was possible applying the new primer system based on the patatin gene. Such species specific PCR systems which already exist for detection of maize and soya (16, 17) are essential as control of availability of DNA for PCR and as one part of a double competitive PCR (22) for quantification of genetically modified organism in complex food products.

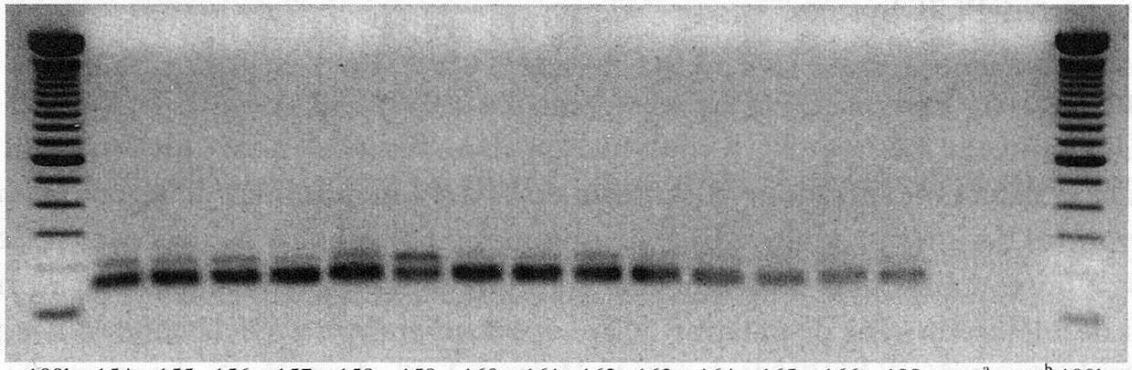

Figure 3 **Agarose gel electrophoresis of nested PCR of six potato cultivars (154–159) and eight potato food products (160–198).** neg[a] = PCR of extraction protocol without food sample, neg[b] = PCR amplification without template DNA

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Summary

A new method for specific and sensitive detection of potato DNA *(Solanum tuberosum)* in processed potato food products has been developed using polymerase chain reaction (PCR). A nested PCR system amplifying a 146 bp fragment of the patatin gene of *Solanum tuberosum* was evolved. Quality and amount of DNA in potato products were determined by gel-electrophoresis and absorption at 260 nm. Several potato cultivars authorised in Switzerland and highly processed food products like instant potato soup, instant mashed potatoes, potato crisps and crackers and pommes frites were investigated. For all samples the 146 bp fragment could be amplified with the new nested PCR system.

Zusammenfassung

Eine neue Methode wurde entwickelt zum spezifischen und sensitiven Nachweis von Kartoffel-DNA basierend auf dem Speicherproteingen Patatin von *Solanum tuberosum* unter Verwendung der Polymerasekettenreaktion (PCR). Eine nested-PCR, die ein 146 bp Fragment amplifiziert, wurde evaluiert. Die Qualität und Menge der extrahierten DNA aus verschiedenen Kartoffelprodukten wurde durch Absorptionsmessung bei 260 nm sowie durch Agarose-Gelelektrophorese bestimmt. Neben stark prozessierten Kartoffelprodukten wie Kartoffelbrei, Chips, Cräcker und Pommes frites wurden verschiedene, in der Schweiz zugelassene,

Kartoffelsorten untersucht. Alle Proben ergaben das 146 bp Fragment mit dem neuen nested-PCR-System.

Résumé

Une nouvelle méthode PCR (polymerase chain reaction) a été mise au point qui permet la détection spécifique et sensible de l'ADN de pommes de terre *(Solanum tuberosum)* dans les produits à base de pommes de terre issus de procédés de technologie alimentaires. Un nouveau système PCR (nested PCR) amplifiant un fragment de 146 bp du gène de patatine de *Solanum tuberosum* a été développé. La qualité et la quantité d'ADN dans les produits à base de pomme de terre ont été déterminés par électrophorèse sur gel et absorption à 260 nm. Différents cultivars de pomme de terre autorisés en Suisse et des produits à base de pomme de terre ayant subis des traitements technologiques complexes tels que soupe instantanée de pommes de terre, purée de pommes de terre instantanée, chips de pommes de terre, crackers et pommes frites ont été étudiés. Tous les échantillons produisirent le fragment de 146 bp avec le nouveau système PCR.

Key words

Potato DNA, Polymerase chain reaction, Specific PCR, Food products

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