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Extraction and Amplification of DNA from 55 Foodstuffs

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Introduction

Labelling of genetically modified foodstuffs is mandatory in European countries. In order to analyse if a foodstuff contains ingredients derived from genetically modified organisms (GMO), many methods have been developed in the past (1–3). Most of these methods are based on the detection of the introduced genetic trait by the polymerase chain reaction (PCR). However, there is still a lack of knowledge concerning the extractability and the physical properties of DNA relevant for PCR isolated from different foodstuffs. The chosen foodstuffs either contain or are GMOs which are on the market today or alternatively they are at least at the stage of experimental evaluation as a GMO-variety. This work evaluates how much DNA can be extracted from these foodstuffs and if the DNA can be used as a template for PCR. For this reason, 55 foodstuffs representing different processing levels were analysed. The samples were chosen to represent a wide variety of products with different types of food matrices. In a first step, the amount of extractable DNA was determined. Then the quality of the extracted DNA was tested by amplification of the DNA with a eucaryote-specific PCR system (4). Positive results with the eucaryote-specific PCR system solely show the presence of amplifiable DNA, but at the same time allow a comparison of the amplificability between the analysed foodstuffs. The study was extended for three types of foodstuffs (soybean, corn and potato) to a species-specific amplification system, where the gene of interest is present in low/single copy to serve as a model system for genetically modified plants presently on the market.

Materials and methods

Homogenization of food samples, extraction and quantification of DNA

All food samples analysed in this study are available on the market to consumers (table 1). Foodstuffs were used directly (e.g. sugar, meal), gently homogenized in a mortar (e.g. flakes, bread) or ground in an electrical coffee mixer (e.g. grains). 860 µl of extraction buffer (10 mM Tris HCl pH 8.0, 150 mM NaCl, 2 mM EDTA, 1 % SDS), 100 µl of 5 M guanidinium hydrochloride and 40 µl of proteinase K (20 mg/ml) were added to 60–350 mg of the homogenate or of the original product. After incubation for at least 3 h at 58° C on a shaker, the tube was cooled to room temperature. It was centrifuged for 10 min at full speed on an microcentrifuge and 500 µl of the supernatant were transferred to a new tube containing 5 µl RNase (10 mg/ml). After incubation for 5 min at 58° C, the samples were extracted with the Wizard™ column extraction method according to the recommendations of the manufacturer (5). All samples were eluted in 50 µl H_2O. For raw potato the CTAB method was used (6), because of the higher yield of DNA. For each food sample extraction was repeated twice. Foodstuffs which were only concentrated, cut, heated, crushed or popped were considered as low processed. Foodstuffs which were precipitated, deep fried, roasted or steamed were considered as medium processed and foodstuffs which were fermented or extruded were considered as highly processed.

The DNA was quantified on a GeneQuant™ photometer with a micro-capillary (Pharmacia, Uppsala, Sweden). This equipment allows accurate measurements down to a concentration of 100 µg/ml. Concentrations below 100 µg/ml cannot be accurately measured but still allow to estimate the approximate concentration. Values <20 µg/ml are representing fluctuations of the background and do not allow any quantification. For these reasons the ratio A_{260}:A_{280} is not indicated in cases where the DNA concentration is below 100 µg/ml.

Amplification of extracted DNA, gel electrophoresis

The DNA concentration was adjusted to 20 µg/ml after extraction. If the DNA concentration was <20 µg/ml, 10 µl of the undiluted DNA were used for amplification. The PCR reaction was performed in 50 µl with the following mastermix: 1× concentrated PCR buffer (Promega, Madison, WI), 2 µg/ml BSA (except for the eucaryote-specific PCR, where no BSA was present), 0.2 µM dNTP, 0.5 µM of each primer, 1 U of Taq polymerase (Promega). $MgCl_2$ was used in the following concentrations: 1.25, 1.5, 2.0, 2.5, 3 mM (soybean, eucaryote, potato inner, potato outer and corn PCR, respectively). The DNA was subjected to a eucaryote-specific PCR with the following specifications: first denaturation for 4 min 30 sec at 95° C, 30 cycles with 46 sec at 95° C, 1 min 25 sec at 64° C, 1 min 25 sec at 72° C and final extension at 72° C for 3 min 15 sec (for primers and amplified genes see table 2). Samples containing corn, soybean or potato were further analysed with a species-specific PCR-

Table 1
List of analysed foodstuffs

Product category	*Samples*	*Processing*
Soybean		
flour	1	low
drink	2	↓
tofu	2	
sausage	2	
sauce	1	
bread with soy sauce	1	high
Corn		
flour	1	low
polenta grits	2	↓
popped popcorn	2	
tortilla chips	2	
starch	2	
extruded chips	2	
refined oil	1	high
Potato		
cooked slices	2	low
french fries	1	↓
chips	1	
mashed	1	
extruded chips	7	high
Tomato		
fresh	1	low
peeled	1	↓
concentrate	1	
puree	1	
ketchup	1	high
Wheat		
grains	1	low
flour	1	↓
toast bread	2	
extruded flakes	2	high
Rice		
grains	1	low
waffles	1	high
Sugar		
powdered	1	high
caramel	2	↓
rock	1	
cube	1	
refined	1	
cane refined/brown	2	
caramelized	1	high
Total samples	*55*	

Table 2
Primers used

Primer	*Gene*	*Size (PCR)*	*Sequence*	*Reference*
TR03	18S rDNA		TCT GCC CTA TCA ACT TTC GAT GGT A	(4)
TR04	18S rDNA	137 bp	AAT TTG CGC GCC TGC TGC CTT CCT T	(4)
Zein3	zein		AGT GCG ACC CAT ATT CCA G	(7)
Zein4	zein	277 bp	GAC ATT GTG GCA TCA TCA TTT	(7)
GM03	lectin		GCC CTC TAC TCC ACC CCC ATC C	(8)
GM04	lectin	118 bp	GCC CAT CTG CAA GCC TTT TTG TG	(8)
PAT1	patatin		GTT ATT ATC CCT TAG CGT TGC	(9)
PAT2	patatin	272 bp	AAT TGT TTT GTG AAT GAC GAG	(9)
PAT3	patatin		GGA TCC AGC ATT TTC TTC A	(9)
PAT4	patatin	146 bp	TAG CTA ACA TCC ATC GTA GAG G	(9)

system. For corn-specific PCR the conditions were: first denaturation for 4 min 30 sec at 95° C, 40 cycles with 1 min 45 sec at 96° C, 2 min at 60° C, 1 min 50 sec at 72° C and a final extension at 72° C for 4 min 15 sec. Soy-specific PCR was performed according to the following scheme: first denaturation for 4 min 30 sec at 95° C, 40 cycles with 50 sec at 97° C, 1 min 55 sec at 65° C, 1 min 55 sec at 72° C and a final extension at 72° C for 4 min 15 sec and for potato-specific nested PCR, the conditions for amplification were as follows: first denaturation for 4 min 30 sec at 96° C, 25 cycles with 1 min 15 sec at 96° C, 2 min at 60° C and a final extension at 72° C for 4 min 15 sec. The conditions for inner PCR (or single PCR with CTAB extracted DNA) were: 4 min 30 sec at 96° C, 35 cycles (40 cycles with CTAB extracted DNA) with 1 min 15 sec at 96° C, 2 min at 60° C and a final extension at 72° C for 4 min 15 sec.

The amplified fragments were electrophoresed in high resolution agarose gels (MS-agarose, Roche Diagnostics, Mannheim, Germany) and the bands were visualized and photographed under UV (302 nm) after staining with ethidium bromide (1 µg/ml).

Results and Discussion

Foodstuffs from major (GMO) crops available for consumers were analysed and most of them were shown to yield enough DNA with a quality to be amplified with the eucaryote-specific PCR system (table 3). In contrast, no amplification could be observed for refined oil, one soy drink, most sugar products and tomato concentrate. With the species-specific PCR systems, which are designed to detect single or low copy number genes, some foodstuffs do not show a positive amplification signal, indicating that it might not be possible to detect a genetically modified trait on the basis of DNA analysis. The evaluation of the different food categories are described and discussed below.

Table 3
Results of the evaluation of the different foodstuffs

Food	*Product*	*(mg)*	*eucaryot. PCR*	*species-specific PCR*	*Concentration (µg/ml)*	*Absorption Ratio A_{260}:A_{280}*	*Yield (µg/100 mg)*
Soybean	flour	300	+/+	+/+	625/485	1.45/1.50	10.4/8.1
	drink 1	300	+/+	+/+	60/58		< 1
	drink 2	300	–/–	–/–	47/44		< 1
	tofu 1	300	+/+	+/+	324/315	1.80/1.81	5.4/5.3
	tofu 2	300	+/+	+/+	281/307	1.76/1.75	4.7/5.1
	sausage 1	300	+/+	+/+	396/392	1.74/1.75	6.6/6.5
	sausage 2	300	+/+	+/+	345/339	1.68/1.75	5.8/5.7
	sauce	300	+/–	–/–	out/out		< 1
	bread with soy sauce	300	+/+	–/–	624/532	1.77/1.76	10.4/8.9
Corn	flour	300	+/+	+/+	289/388	1.78/1.79	4.8/6.5
	polenta grits 1	300	+/+	+/+	312/272	1.71/1.68	5.2/4.5
	polenta grits 2	300	+/+	+/+	124/90	1.61/	2.1/1.5
	popped popcorn 1	60	+/+	+/–	63/47		5.3/3.9
	popped popcorn 2	60	+/+	+/+	61/97		5.1/8.1
	tortilla chips 1	300	+/+	+/+	418/448	1.80/1.79	7.0/7.5
	tortilla chips 2	300	+/+	+/+	35/24		< 1
	starch 1	300	+/–	–/–	out/out		< 1
	starch 2	300	+/+	–/–	out/out		< 1
	extruded chips 1	300	+/+	+/+	562/597	1.80/1.78	9.4/10.0
	extruded chips 2	300	+/+	+/–	517/446	1.75/1.75	8.6/7.4
	refined oil	300	–/–	–/–	out/out		< 1

Food	*Product*	*(mg)*	*eucaryot. PCR*	*species-specific PCR*	*Concentration (µg/ml)*	*Absorption Ratio A_{260}:A_{280}*	*Yield (µg/100 mg)*
Potato WIZARD™	cooked sliced 1	300	+/+	+/–	out/23		< 1
	cooked slices 2	300	+/+	+/+	46/out		< 1
	french fries	300	+/+	+/+	out/out		< 1
	chips	300	+/+	+/+	180/171	1.83/1.98	3.0/2.9
	dried/mashed	200	+/+	+/+	23/27		< 1
	extruded chips 1	300	+/+	+/+	76/61		1.3/< 1
	extruded chips 2	300	+/+	+/+	62/88		< 1/1.5
	extruded chips 3	300	+/+	+/+	23/31		< 1
	extruded chips 4	300	+/+	+/+	out/43		< 1
	extruded chips 5	300	+/+	+/+	20/23		< 1
	extruded chips 6	300	+/+	+/+	out/23		< 1
	extruded chips 7	300	+/+	+/+	36/25		< 1
Potato CTAB	cooked slices 1	350	+/+	+/–	64/67		< 1
	cooked slices 2	350	+/+	+/–	86/74		1.2/1.1
	french fries	350	+/+	+/+	302/186	2.16/2.06	4.3/2.7
	chips	100	+/+	+/+	473/456	2.16/2.17	23.7/22.8
	dried/mashed	100	+/+	+/+	113/103	2.20/2.32	5.7/5.2
	extruded chips 1	100	+/+	+/–	264/246	2.23/2.15	13.2/12.3
	extruded chips 2	100	+/+	+/–	211/308	2.15/2.13	10.6 /15.4
	extruded chips 3	100	+/+	+/+	107/117	2.26/2.18	5.4/5.9
	extruded chips 4	100	+/+	+/+	50/51		2.5/2.6
	extruded chips 5	100	+/+	+/+	43/43		2.2/2.2
	extruded chips 6	100	+/+	+/+	44/54		2.2/2.7
	extruded chips 7	100	+/+	+/+	56/72		2.8/3.6
Tomato	fresh	300	+/+	nd	out/out		< 1
	peeled (pelati)	300	+/+	nd	out/out		< 1
	concentrate	300	–/–	nd	49/57		< 1
	puree	300	+/+	nd	out/out		< 1
	ketchup	300	+/–	nd	out/out		< 1

Food	*Product*	*(mg)*	*eucaryot. PCR*	*species-specific PCR*	*Concentration (µg/ml)*	*Absorption Ratio A_{260}:A_{280}*	*Yield (µg/100 mg)*
Wheat	whole grains	300	+/+	nd	33/39		< 1
	flour	300	+/+	nd	678/879	1.76/1.80	11.3/14.7
	toast bread 1	300	+/+	nd	642/633	1.74/1.75	10.7/10.6
	toast bread 2	300	+/+	nd	518/434	1.77/1.77	8.6/7.2
	extruded flakes 1	300	+/+	nd	414/436	1.77/1.76	6.9/7.3
	extruded flakes 2	300	+/+	nd	477/643	1.74/1.75	8.0/10.7
Rice	grains	300	+/+	nd	101/122	2.04/1.89	1.7/2.0
	waffles	200	+/+	nd	72/101	/1.70	1.8/2.5
Sugar	cane	300	+/+	nd	29/36		< 1
	cane raw	300	–/–	nd	out/20		< 1
	caramel 1	300	–/–	nd	out/out		< 1
	caramel 2	300	–/–	nd	out/out		< 1
	caramelized	300	–/–	nd	out/out		< 1
	powdered	300	+/–	nd	out/out		< 1
	rock	300	+/–	nd	28/30		< 1
	refined	300	–/–	nd	out/out		< 1
	cube	300	–/–	nd	out/out		< 1

+ amplification band. – no amplification band.
nd = not determined. out = Absorption measurement too low.

Soybean products

The soy product samples yielded DNA in the range of <1 to 10 µg DNA/100 mg product with an absorption ratio A_{260}:A_{280} between 1.45 and 1.8. The lower ratio at 1.45 indicates the presence of proteins in the DNA fraction whereas the ratio at 1.8 indicates pure DNA. With the eucaryote PCR system one soy drink and the soy sauce showed no or only faint amplification bands, respectively. Further analysis with the soy-specific PCR system showed that, in addition to these two products, bread with the soy sauce did not yield amplification bands whereas the other products displayed clear amplification bands. It can be concluded that analysis of tofu, soy sausages and soy flour can generate a positive amplification signal, whereas soy drink, soy sauce and bread with soy sauce might not or not always be suitable for this type of analysis.

Corn products

The corn product samples yielded DNA in the range of <1 to 10 µg DNA/100 mg product with an absorption ratio A_{260}:A_{280} between 1.6 and 1.8, which still indicates a good purity of the DNA. However, although popped popcorn is only heated, very low amounts of DNA could be extracted. It seems that popping either is destroying the DNA by the applied pressure/heat or alternatively that the DNA cannot be extracted from the cells with the applied extraction method. On the other side, the extruded corn chips contain the highest amount of extractable DNA among the corn samples analysed. Interestingly tortilla chips from different manufacturers varied at least 10-fold with regard to yields of extracted DNA. With the exception of refined oil all investigated corn derived foodstuffs yielded a positive amplification signal with the eucaryote-specific PCR system indicating the presence of amplifiable plant DNA. A further corn-specific PCR showed that starch and oil did not yield amplification bands. It can be concluded that analysis of polenta grits, corn meal, extruded chips, tortilla flour and ready-to-eat popcorn can potentially generate a positive signal for low copy number genes, whereas refined oil and starch are probably not suitable for DNA analysis.

Potato products

The potato product samples yielded around 1 µg DNA/100 mg product with an absorption ratio A_{260}:A_{280} between 1.8 and 2.0 when the Wizard™ method was used for extraction. The value of 2.0 indicates the presence of RNA in the final extract. The amount of extracted DNA could considerably be increased in all types of potato products, when the CTAB method was used which yielded DNA (RNA) in the range of up to 23 µg DNA(RNA)/100 mg product with an absorption ratio A_{260}:A_{280} between 2.1 and 2.3. As with the Wizard™ method, the higher values of the ratio A_{260}:A_{280} indicate the presence of RNA. Since no RNase treatment was used in the procedure this was expected. For this reason, it is advisable to introduce an RNase treatment in the CTAB procedure.

The DNA of all potato product samples could be amplified with the eucaryote PCR system. When the potato-specific PCR system was used, both methods resulted in amplification bands of similar intensity. This indicates an overestimation of the DNA content for the CTAB samples due to the presence of RNA. Since DNA extraction and amplification from potato products were successful for all evaluated samples, the analysed potato products are suitable for the analysis of DNA.

Tomato products

The investigated tomato products yielded amounts of DNA which are lower than 1 µg DNA/100 mg product. In four out of five cases the DNA could be amplified with the eucaryote-specific PCR system. However, in the case of tomato concentrate where the highest amount of DNA could be extracted, no amplification could be observed. This indicates that the DNA may be fragmented, i.e. too small to be amplified. Due to the lack of a species-specific PCR system this type of experiment could not be performed for tomato and the following products.

Wheat products

The analysed wheat products yielded up to 14 µg DNA/100 mg product and could all be amplified with the eucaryote-specific PCR system. The ratio A_{260}:A_{280} between 1.75 and 1.8 indicates a good purity of the DNA. Interestingly, the least processed product (grains) yielded the lowest amount of DNA with the Wizard™ method. The reason for this observation is not yet clear.

Rice products

The two analysed rice products yielded extractable DNA around 2 µg/100 mg of product and showed positive results for amplification with the eucaryote PCR-system.

Sugar products

All the analysed sugar products can be considered as highly processed. Therefore it is clear that only traces of DNA can be extracted. Still, clear amplification results with the eucaryote PCR system can be observed for cane sugar.

This study, which is far from showing a complete overview on all possible foodstuffs, shows how much DNA can be extracted from a certain food stuff and if it can be amplified with a eucaryote- or a species-specific PCR system. Much more information on the best extraction methods and the yield/quality of the DNA has to be accumulated during the next years in order to apply the optimal condition for analysis of a specific foodstuff.

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Summary

With the enforcement of mandatory labelling of genetically modified foodstuffs in Europe, methods for their analysis are getting more and more important. Because of the nature of the genetic modifications, methods based on DNA analysis were developed first. In order to evaluate the extractability and the amplificability of DNA, 55 food products derived from soybean, corn, potato, rice, sugar beet, tomato and wheat were analysed for their DNA content. DNA fragments were subsequently amplified with PCR. In most cases where DNA was extractable, amplification was feasible with a eucaryote-specific PCR system. In addition, DNA of foodstuffs derived from corn, soybean and potato were amplified with a species-specific PCR for the detection of low copy number genes. No DNA could be extracted from refined sugar and oil, whereas all other food samples yielded DNA. Furthermore, potato samples were extracted with the CTAB extraction method since the Wizard™ column method yielded only very low amounts of DNA. These results show that DNA can be extracted from a wide variety of foodstuffs and processing stages.

Zusammenfassung

Durch den Vollzug der Deklaration gentechnisch veränderter Lebensmittel in Europa, werden Analysemethoden für diese immer wichtiger. Wegen der Art der gentechnischen Veränderung wurden zuerst Methoden auf der Basis der Nukleinsäuren-Detektion (DNA) entwickelt. Um die Extrahier- und die Amplifizierbarkeit der DNA aus Lebensmitteln zu evaluieren, wurde aus 55 Lebensmitteln (hergestellt aus Sojabohnen, Mais, Kartoffeln, Zuckerrüben, Tomaten und Weizen) die DNA extrahiert und mit der Polymerase-Kettenreaktion (PCR) amplifiziert. In den meisten Fällen, wo DNA extrahiert werden konnte, war eine Amplifikation mit einem eukaryontenspezifischen PCR System erfolgreich. Zusätzlich wurden die Soja-, Mais- und Kartoffelprodukte mit einem artspezifischen PCR System amplifiziert, welches eine Detektion von wenigen Kopien erlaubt. Es konnte keine DNA aus raffiniertem Öl und Zucker gewonnen werden. Bei allen anderen Lebensmitteln konnte DNA extrahiert werden. Die Kartoffelprodukte wurden zusätzlich mit der CTAB-Methode extrahiert, weil die Wizard™ Methode nur wenig DNA lieferte. Die vorliegenden Resultate zeigen, dass aus einer grossen Anzahl von verschieden prozessierten Lebensmitteln amplifizierbare DNA extrahiert werden kann.

Résumé

En Europe, l'obligation de déclarer les denrées alimentaires fabriquées à partir d'organismes génétiquement modifiés demande l'application de méthodes analytiques appropriées. De par la nature des modifications génétiques apportées, les

méthodes ont d'abord été développées pour détecter les acides nucléiques (ADN). 55 aliments (fabriqués à partir de soja, de pommes de terre, de betteraves à sucre, de tomates et de blé) ont été examinés dans le but d'évaluer l'extraction et l'amplification de l'ADN, amplification réalisée au moyen de la méthode PCR. Pour presque chaque échantillon où l'ADN a pu être extrait, l'analyse PCR spécifique à l'eucaryonte a été réalisée avec succès. De plus, l'ADN des produits faits à base de soja, de maïs et de pommes de terre a été amplifié avec un système PCR spécifique à la plante qui détectait un faible nombre de copies. Aucun ADN n'a été détecté dans l'huile raffinée et le sucre. Tous les autres aliments examinés contenaient de l'ADN. Les produits à base de pommes de terre ont été extraits avec la méthode CTAB car la méthode Wizard™ ne permettait pas une extraction suffisante d'ADN. Les résultats démontrent que l'ADN peut être extrait et amplifié à partir d'une grande variété de denrées alimentaires transformées.

Key words

Genetically modified organsim, Qualitative PCR, Extraction methods

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