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Food Control 16 (2005) 753–759
FOOD CONTROL
www.elsevier.com/locate/foodcont

Qualitative and quantitative detection of genetically modified maize and soy in processed foods sold commercially in Brazil by PCR-based methods

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Abstract

Qualitative and quantitative polymerase-chain-reaction-based methods to detect genetically modified (gm) soy (Roundup-Ready™ soy) and maize (Bt176 Maximizer maize; Bt11 maize, MON810 Yield Gard corn, T25 Liberty® Link maize) were applied to processed foods sold commercially in Brazil. In total 100 foods containing maize and 100 foods containing soy were analysed in 2000 and again in 2001. In 2000, 13% of the soy containing products and 8% of those containing maize were shown to consist of material derived from the corresponding genetically modified organisms. In five samples of the products containing gm soy <4% and in eight samples >4% of the soy derived material was identified as coming from gm soybeans, whereas in the case of products containing gm maize five samples of the maize derived material were found to contain <4% and three samples >4% of gm maize. In 2001, gm soy was found in 21% of the soy containing foods, and gm maize was found in 9% of the maize containing products. Eight samples of the products containing gm soy were shown to contain <4% and 13 samples >4% of gm soy, the corresponding values for gm maize were five samples <4% and four samples >4%.

Keywords: Detection method; gm maize; gm soy; Polymerase-chain-reaction; Transgenic food

1. Introduction

In the nineties, genetic engineering came to food. In May 1994, the Flavr Savr™ tomato developed by Calgene was approved for sale in the United States. Until now, the number of approved genetically modified plants has continuously grown. In addition, a wide range of additives and processing aids are produced using genetic engineering. In the course of commercialisation of foods derived from genetic engineering an intensive discussion on their detection and labelling emerged. Detection methods for these foods mainly focus on the newly introduced DNA (Busch, Mühlbauer, Schulze, & Zagon, 1999; Greiner, Konietzny, & Jany, 1997; Hupfer, Hotzel, Sachse, & Engel, 1998; Jankiewicz, Broll, & Zagon, 1999; Koeppel, Stadler, Lüthy, & Hübner, 1997; Meyer, 1995; Waiblinger, Wurz, Freyer, & Pietsch, 1999). Because of its high sensitivity, its specificity and rapidity the polymerase-chain-reaction (PCR) will be the method of choice for this purpose. The first official method for the detection of a food product derived from genetic engineering was published in 1993 in the Collection of Official Methods Under Article 35 of the German Federal Foodstuffs Act (Official Collection of Test Methods, 1997a). Since this time several official PCR-based methods for different

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0956-7135/$ - see front matter © 2004 Elsevier Ltd. All rights reserved.
doi:10.1016/j.foodcont.2004.06.015

genetically modified organisms used for food production have been published, mainly in Germany (Official Collection of Test Methods, 1997a, 1997b, 1997c, 1998, 1999, 2001, 2002; Swiss Food Manual, 1998, Chap. 52B). So far, these PCR methods have been strictly qualitative. It is hence not possible by qualitative PCR to distinguish a product derived from genetic engineering from any contamination non-intentionally brought in by carry-over effects during breeding, storage, processing and transport. Therefore, quantitative PCR systems are needed. For this purpose, competitive PCR (Huebner, Studer, & Lüthy, 1999a, 1999b; Pietsch, Bluth, Wurz, & Waiblinger, 1999; Studer, Rhyner, Lüthy, & Hübner, 1998) and real-time PCR-systems (Huebner, Waiblinger, Pietsch, & Brodmann, 2001; Wurz, Bluth, Zeltz, Pfeiffer, & Willmund, 1999) have been developed.

Although qualitative and quantitative PCR-based systems to identify a food as produced by means of genetic engineering have been developed, only one report is available in the literature on the distribution and relative concentration of material derived from genetically modified organisms in foods sold commercially (Busch, Mühlbauer, Böhm, & Liebl, 2001). Therefore, the objective of this work was to determine qualitatively and quantitatively the occurrence of products derived from genetically modified soy and maize in foods sold commercially in Brazil. Soy and maize were chosen, because these are the major transgenic crops grown world-wide. In 1999 soy was grown on 53% and maize on 31% of the total gm plant area under cultivation. The corresponding values in 2000 have been 63% for soy and 19% for maize and in 2001 68% for soy and 16% for maize (http://www.transgen.de). Furthermore, quantitative PCR-based detection systems are commercially available for the major transgenic soy and maize events (RoundupReady™ soy, Bt176 Maximizer maize; Bt11 maize, MON810 Yield Gard corn, T25 Liberty® Link maize) cultivated.

2. Materials and methods

2.1. Material

Food samples were purchased from local supermarkets in São Paulo and Rio de Janeiro, Brazil. GMOQuant Bt11 Corn DNA Quantification kit, GMOQuant MON810 YieldGard Corn DNA Quantification kit, GMOQuant T25 LibertyLink Corn DNA Quantification kit, GMOQuant Bt176 Corn DNA Quantification kit, and GMOQuant RoundupReady™ DNA Quantification kit were from GeneScan Europe (Freiburg, Germany). PCR-primers were synthesised by Invitrogen (São Paulo, Brazil). The Wizard™ DNA isolation kit was purchased from Promega (São Paulo, Brazil).

2.2. DNA isolation

For DNA isolation the Wizard™ DNA isolation kit was used according to the instructions of the supplier. 300–500 mg of food material were carefully mixed with 1000 μL 10 mM Tris–OH, 150 mM NaCl, 2 mM EDTA, 1% (w/v) sodium dodecyl sulfate; pH 8.0, 116.3 μL guanidinium hydrochloride (5 mol/100 mL) and 46.5 μL proteinase K (20 mg/mL) and incubated at 65 °C over night. The samples were centrifuged at 13,500*g* for 10 min and the supernatants were transferred into a new tube. After centrifugation at 13,500*g* for 10 min 500 μL of the supernatants were mixed well with 1 mL of the Wizard®-resin and the slurry was pushed gently into a Wizard®-mini column. The column was washed first with 2 mL isopropanol. After centrifugation at 12,000*g* for 2 min, the DNA was eluted with 50 μL of pre-warmed (65 °C) 10 mM Tris–OH pH 9.0. After 1 min of incubation at room temperature, the DNA was collected by centrifugation for 2 min at 10,000*g*. The DNA solutions were stored at −20 °C until further use.

2.3. Qualitative PCR

The sequences of the oligonucleotide primers are given in Table 1. Amplification was performed in 0.2 ml tubes using 25 μL total reaction volumes containing 1.0 μL of the DNA preparations. The reaction mixture consisted of 10 mM Tris–HCl pH 8.3, 50 mM KCl, 1.5 mM $MgCl_2$, 0.001% (w/v) gelatine, 1.0 μM of each primer, 200 μM of each deoxynucleotide triphosphate and 0.5 units of a Hot-Start DNA polymerase in a Perkin-Elmer GeneAmp PCR System 2400. The PCR programmes are summarised in Table 2. Amplification products were held at 4 °C until analysed by standard agarose gel electrophoresis followed by ethidium bromide staining.

2.4. Quantitative PCR

For quantitative PCR the GMOQuant Bt11 Corn DNA Quantification kit, the GMOQuant MON810 YieldGard Corn DNA Quantification kit, the GMOQuant T25 LibertyLink Corn DNA Quantification kit, the GMOQuant Bt176 Corn DNA Quantification kit, and the GMOQuant RoundupReady™ DNA Quantification kit were used on a ABI PRISM™ 5700 Detection System(Applied Biosystems, Weiterstadt, Germany) according to the instructions of the supplier.

3. Results

Qualitative and quantitative polymerase-chain-reaction-based methods to detect genetically modified soy (RoundupReady™ soy) and maize (Bt176 Maximizer

Table 1
Oligonucleotide primers used to detect species-specific and transgenic DNA sequences in foods by PCR

Target	Primer	Primer sequence	Amplicon length [bp]	Ref.
Control:				
Maize	IVR1-F	5′-CCG CTG TAT CAC AAG GGC TGG TAC C-3′	226	Official Collection of Test Methods (2002)
	IVR1-R	5′-GGA GCC CGT GTA GAG CAT GAC GAT C-3′		
Soy	GMO3	5′-GCC CTC TAC TCC ACC CCC ATC C-3′	118	Official Collection of Test Methods (1998)
	GMO4	5′-GCC CAT CTG CAA GCC TTT TTG TG-3′		
Specific:				
Bt176 maize	Cry03	5′-CTC TCG CCG TTC ATG TCC GT-3′	211	Official Collection of Test Methods (2002)
	Cry04	5′-GGT CAG GCT CAG GCT GAT GT-3′		
Bt11 maize	IVS2-2	5′-CTG GGA GGC CAA GGT ATC TAA T-3′	189	Official Collection of Test Methods (2002)
	PAT-B	5′-GCT GCT GTA GCT GGC CTA ATC T-3′		
MON810 maize	VW01	5′-TCG AAG GAC GAA GGA CTC TAA CG-3′	178	Official Collection of Test Methods (2002)
	VW03	5′-TCC ATC TTT GGG ACC ACT GTC G-3′		
T25 maize	T25-F7	5′-ATG GTG GAT GGC ATG ATG TTG-3′	209	Official Collection of Test Methods (2002)
	T25-R3	5′-TGA GCG AAA CCC TAT AAG AAC CC-3′		
RoundupReady™ soy	p35s-f2	5′-TGA TGT GAT ATC TCC ACT GAC G-3′	172	Official Collection of Test Methods (1998)
	petu-r1	5′-TGT ATC CCT TGA GCC ATG TTG T-3′		

Table 2
PCR programmes used to detect species-specific and transgenic DNA sequences in foods

	IVR1-F/IVR1-R	GMO3/GMO4	Cry03/Cry04	IVS2-2/PAT-B	VW01/VW03	T25-F7/T25-R3	p35s-f2/petu-r1
Initial denaturation	10 min, 95 °C	10 min, 95 °C	10 min, 95 °C	10 min, 95 °C	10 min, 95 °C	10 min, 95 °C	10 min, 95 °C
No. of cycles	40	35	40	40	40	40	35
Denaturation	30 s, 95 °C	30 s, 95 °C	30 s, 95 °C	30 s, 95 °C	30 s, 95 °C	30 s, 95 °C	30 s, 95 °C
Annealing	30 s, 64 °C	30 s, 60 °C	30 s, 64 °C	30 s, 64 °C	30 s, 64 °C	30 s, 64 °C	30 s, 62 °C
Extension	30 s, 72 °C	25 s, 72 °C	30 s, 72 °C	30 s, 72 °C	30 s, 72 °C	30 s, 72 °C	25 s, 72 °C
Final extension	7 min, 72 °C	7 min, 72 °C	7 min, 72 °C	7 min, 72 °C	7 min, 72 °C	7 min, 72 °C	7 min, 72 °C

maize; Bt11 maize, MON810 Yield Gard corn, T25 Liberty^{R} Link maize) were applied to processed foods sold commercially in Brazil. The RoundupReady™ soy developed by Monsanto, USA harbours a genetic construct conferring tolerance to the herbicide glyphosate (Roundup®). Bt176 Maximizer maize (Novartis, Switzerland), Bt11 maize (Novartis, Switzerland), and MON810 Yield Gard corn (Monsanto, USA) contain genes from the bacterium *Bacillus thuringiensis* so that the plant will produce proteins to protect itself against insect pests such as the European corn borer, whereas T25 Liberty^{R} Link maize (AgrEvo, Germany) has a modified version of a *pat* gene introduced, which encodes the enzyme phosphinothricin acetyl transferase (PAT). PAT can detoxify glufosinate-ammonium herbicides and thereby confer resistance or tolerance.

In total 100 foods containing maize and 100 foods containing soy were analysed in 2000 and again in 2001. In 2000, 13 of the 100 soy containing products (13%) and 8 of those containing maize (8%) were shown to consist of material derived from the corresponding genetically modified organisms (Tables 3 and 4). Of the foods containing soy only soy flour (40%), tofu-based products (37.5%), and bakery products (16%) have been shown to contain RoundupReady™ soy (Table 3), whereas gm maize was detected in tortilla chips (35.7%), polenta (11%), and maize flour (5.5%) (Table 4). In the gm maize positive foods, Bt11, Bt176 and

Table 3
Detection of RoundupReady™ soy in soy containing products

Products	Number of samples analysed	Number of samples in which RR-DNA was detected	
		2000	2001
Soy flour	15	6	7
Bakery products	24	4	6
Pasta	10	0	0
Vegetarian foods	5	0	1
Infant foods	7	0	0
Desserts	11	0	1
Sweets	2	0	0
Soy-based drinks	6	0	0
Soy protein isolates	3	0	1
Tofu-based foods	8	3	2
Instant soups	9	0	3
Total	100	13	21

Table 4
Detection of genetically modified maize in maize containing products

Products	Number of samples analysed	Number of samples in which gm maize DNA was detected	
		2000	2001
Maize flour	18	1	2
Bakery products	12	0	2
Cornflakes	14	0	0
Tortilla chips	14	5	1
Polenta	18	2	4
Maize starch	6	0	0
Infant foods	3	0	0
Desserts	3	0	0
Instant soups	4	0	0
Maize cob	8	0	0
Total	100	8	9

MON810 were detected, whereas none of the food products analysed was identified to contain T25. Bt11 and MON810 were identified as the predominant events in the products containing gm maize. In six of the products only one gm maize event was detected, whereas in two products 2 gm maize events were found (Table 5). Quantitative analysis revealed, that in two samples of the products containing gm soy <1% of the soy derived material came from gm soybeans, in three samples 1–4%, in four samples 4–10%, and in four sample >10%, whereas in the case of products containing gm maize two samples of the maize derived material were found to contain <1% of gm maize, three samples 1–4%, two samples 4–10%, and 1 sample >10%. None of these foods has been appropriately labelled.

In 2001, gm soy was found in 21 of the soy containing foods (21%), and gm maize was found in nine of the maize containing products (9%) (Tables 3 and 4). Of the foods containing soy flour (46.7%), instant soups (33.3%), tofu-based products (25%), bakery products (25%), soy protein isolates (1 sample of 3), and vegetarian foods (1 sample of 5) have been shown to contain RoundupReady™ soy (Table 3), whereas gm maize was detected in polenta (22%), bakery products (16.7%), maize flour (11%), and tortilla chips (7%) (Table 4). In the gm maize positive foods, Bt11 and MON810 were detected, whereas none of the food products analysed was identified to contain Bt176 and T25. In six of the products only one gm maize event was detected, whereas in three products 2 gm maize events were found (Table 5). Quantitative analysis revealed, that three samples of the products containing gm soy contained <1% of gm soy, five samples 1–4%, six samples 4–10% and seven samples >10%, the corresponding values for gm maize were three samples <1%, two samples 1–4%, two samples 4–10% and two samples >10%. Again, no indication of the presence of material derived from genetically modified organisms was found on the label.

Table 5
Genetically modified maize events identified in the products which scored positive for the presence of genetically modified maize

Product	2000	2001
Bakery product	–	Mon810
Bakery product	–	Bt11
Maize flour	Bt11	Bt11, Mon810
Maize flour	–	Bt11
Polenta	Bt176	Bt11
Polenta	Mon810	Bt11, Mon810
Polenta	–	Mon810
Polenta	–	Mon810
Tortilla chips	Bt11	Bt11
Tortilla chips	Bt11, Bt176	–
Tortilla chips	Bt11	–
Tortilla chips	Bt11, Mon810	–
Tortilla chips	Bt11	–

4. Discussion

There are two different DNA extraction methods used in the official methods for the detection of a food product derived from genetic engineering. DNA extraction using a DNA-binding silica resin (Wizard™) is described in the Swiss Food Manual (1998, Chap. 52B) to purify DNA directly from a solution obtained after enzymatic and chemical treatment of the food sample. The CTAB method described in the official methods for the detection of genetically modified foods of the German Food Act LMBG §35 (Official Collection of Test Methods, 1997a, 1997b, 1997c, 1998, 1999, 2001, 2002) is based on a classical protocol for DNA isolation from plant tissue: Incubation of food samples in the presence of the detergent CTAB and treatment of the DNA extracts with chloroform followed by precipitation of the DNA with isopropanol. Compared with the Wizard™-system, the classical CTAB-based method

resulted in higher DNA-yields, but the purity of the isolated DNA is lower and the extraction is more time-consuming (Greiner & Jany, 2002). Since the crucial point in PCR analysis is the exclusion of PCR inhibitors (Dickinson, Kroll, & Grant, 1995; Greiner & Jany, 2002), the Wizard™-system was used for DNA isolation in this study. To check the DNA quality a PCR was performed using a target sequence always present in the product to be analysed. For foods containing maize, the maize invertase gene (Official Collection of Test Methods, 2002) and for foods containing soy, the soybean lectin gene (Official Collection of Test Methods, 1998), were used as target sequences for that purpose. Not only the quantity and purity of the extracted DNA is important if PCR is used as a tool in food control, but also its average molecular mass. The average molecular mass of the DNA depends on the processing and storage of the food. The limitation caused by a strong degradation of DNA by food processing could be circumvented by using a strategy in which small DNA fragments (100–250 bp) (Table 1) are amplified.

Since the suitability of the parameters of the PCR (primers, programme) to detect the target sequence is checked in a separate reaction using pure target DNA, a missing amplification product in the above mentioned control reactions points either to PCR inhibitors or poor DNA quantity in the DNA preparations. As shown by the control PCRs, DNA suitable for PCR could be isolated from all food products included in this investigation by using the Wizard™-system. In agreement with other reports, no DNA could be extracted from soy margarine, soy sauce, soy and maize oil (Busch et al., 2001).

In addition to control PCRs, specific detection system is needed to identify product as derived from genetic engineering. Since most of the transgenic crops approved contain the ^{35}S promotor of *Cauliflower Mosaic Virus* and/or the NOS terminator of *Agrobacterium*, these genetic elements are used as target sequences for a general screening (Official Collection of Test Methods, 2001). This has the advantage of getting a hint on the presence of transgenic sequences from a wide number of different GMO. The absence of an amplification product points to the absence of transgenic sequences, whereas the presence of an amplification product points to the presence of a GMO or a contamination by Cauliflower Mosaic Virus and/or *Agrobacterium*, respectively. Since it is not possible to identify the GMO itself by using the screening approach, a unique DNA sequence for the GMO to be identified has to be used as a target sequence in the PCR. Unique DNA sequences are part of the newly introduced DNA spanning the boundary of at least two adjacent genetic elements. In this study, RoundupReady™ soy as well as the transgenic maize events Bt176, bT11, Mon810 and T25 were specifically identified using the detection systems described in the German Food Act LMBG §35 (Official Collection of Test Methods, 1998, 2002).

In Brazil as well as in Bavaria a broad spectrum of different food products was identified to contain material derived from RoundupReady™ soy. With 13% in 2000 and 21% in 2001, the percentage of food products consisting of material derived from RoundupReady™ soy was higher in Brazil compared to Bavaria, Germany. Busch et al. analysed 488 food products containing soy commercially sold in Bavaria in 1999 (Busch et al., 2001) and 1131 in 2000 (http://www.luas.bayern.de/mlmgen.htm). 9.6% and 9.5% of these products were found to contain material derived from RoundupReady™ soy.

The percentage of food products tested positive for transgenic maize was comparable in Brazil and Bavaria. Of 436 food products containing maize sold commercially in Bavaria which were analysed in 1999, 5.7% were identified to consist of material derived from transgenic maize (Busch et al., 2001) and in 2000, 9% of 980 food products were found to contain material from transgenic maize (http://www.luas.bayern.de/mlmgen.htm). The corresponding values from Brazil were 8% in 2000 and 9% in 2001. In Brazil as well as in Bavaria material derived from transgenic maize was mainly found in tortilla chips and none of the products tested positive for the presence of material from transgenic maize contained the event T25; only the events Bt176, Bt11 and Mon810 were identified.

For quantitative PCR, the so called TaqMan™ technique was used. This technique makes use of the 5′–3′ exonuclease activity of the polymerase to generate a template-specific fluorescent signal after hydrolysing an internal probe during each step of the PCR. The parameter measured is the threshold-cycle (CT) where each reaction trespasses a certain fluorescence level. This quantitative PCR method is sufficiently robust to be effective for virtually all DNA-containing matrices and has sufficient sensitivity to distinguish between a portion of a product derived from genetic engineering below and above the threshold level of 1% legal in the European Union (Regulation (EC) No. 49/2000, 2000). The method is well suited for automation and high throughput of samples, and can be used for raw, processed, and even mixed products. In 2000, 75% of the food products tested positive for the presence of material from RoundupReady™ soy contained less than 1% of this transgenic variety and 61% of the food products tested positive for the presence of material from transgenic maize contained less than 1% of transgenic maize (http://www.luas.bayern.de/mlmgen.htm). In Brazil, the majority of food products tested positive for transgenic soy or maize contained more than 1%. In 2000, only 15% of the soy and 25% of the maize containing products and in 2001, only 14% of the soy and 33% of the maize containing products contained less than 1%.

The higher percentage of soy products containing transgenic soy and the higher concentration might be due to the cultivation of RoundupReady™ soy in Brazil and the missing segregation of transgenic and conventionally grown soy. RoundupReady™ soy is cultivated illegally on 3.6 Mio ha, which represent 50% of the area soybeans are cultivated (http://www.transgen.de). This, and the higher concentration of transgenic maize in the food products might be also due to difference in legislation between Brazil and Europe and the low prevalence of controls in Brazil compared to Germany.

In Brazil as well as in Bavaria a broad spectrum of different food products was identified to contain material derived from RoundupReady™ soy and/or gm maize. Some of this food products are demonstrated to contain gm material above the threshold levels for labelling of 1% legal in the European Union and 4% legal in Brazil, respectively, but none of these food products has been appropriately labelled. It has to be kept in mind, that the described detection methods are only capable to identify the product under investigation as derived from genetic engineering. The safety of the product or risk related to the product has to be addressed separately.

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