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# Prevalence of Genetically Modified Rice, Maize, and Soy in Saudi Food Products

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**Abstract** Qualitative and quantitative DNA-based methods were applied to detect genetically modified foods in samples from markets in the Kingdom of Saudi Arabia. Two hundred samples were collected from Al-Qassim, Riyadh, and Mahdina in 2009 and 2010. GMOScreen 35S and NOS test kits for the detection of genetically modified organism varieties in samples were used. The positive results obtained from GMOScreen 35S and NOS were identified using specific primer pairs. The results indicated that all rice samples gave negative results for the presence of 35S and NOS terminator. About 26 % of samples containing soybean were positive for 35S and NOS terminator and 44 % of samples containing maize were positive for the presence of 35S and/or NOS terminator. The results showed that 20.4 % of samples was positive for maize line Bt176, 8.8 % was positive for maize line Bt11, 8.8 % was positive for maize line T25, 5.9 % was positive for maize line MON 810, and 5.9 % was positive for StarLink maize. Twelve samples were shown to contain <3 % of genetically modified (GM) soy and 6 samples >10 % of GM soy. Four samples containing GM maize were shown to contain >5 % of GM maize MON 810. Four samples containing GM maize were shown to contain

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>1 % of StarLink maize. Establishing strong regulations and certified laboratories to monitor GM foods or crops in Saudi market is recommended.

**Keywords** Genetically modified foods · GMO · DNA extraction · Real-time PCR · Saudi market

## Introduction

Genetically modified (GM) crops were developed and commercialized in 1996. Twenty-three countries planted commercialized GM crops, with the total cultivation area reaching 114.3 million hectares [19]. The aims of developing transgenic crops are to improve food quality and to solve some of the questions associated with commercial agriculture, including disease and weed management [20]. Until now, there has been a dramatic and steady increase in the surface area planted with transgenic crops. The most important GM plants are maize, soybean, oilseed rape (canola), and cotton seeds [4].

The application of recombinant DNA technology (genetic engineering) in modern plant breeding has resulted in the development of plants with improved agronomic and nutritional properties. Crops have been modified through the introduction of new agronomic traits or suppression of constituent genes, which code for disease, pest resistance, herbicide tolerance, inhibition of ripening, or increase of nutritional value, reduce toxins, and improve desirable characteristics. The potential risks of GM technology include the indirect effects of GM crops on the environment, changing of biodiversity, and possibility of the development of resistant insects and tolerant weeds. Therefore, the use of genetically modified organisms (GMO) raises several ethical issues. There are increased consumer concerns over the use of GMO in food products, mainly in European Union (EU) countries, while in North America, the technology is more accepted [7]. The EU has been regulating the labeling of novel foods [30]. EU Regulations (1829/2003/EC and 1830/2003/EC) stipulate that the ingredient contains, consists of, or produced from GMO products that contain more than 0.9 % GMO must be labeled.

The detection of GMO crops became necessary to allow consumers to choose products and to comply with labeling regulations. Methods for detecting genetically modified foods (GMF) are divided into three categories. The first includes nucleotide-based amplification methods, such as polymerase chain reaction (PCR); the second includes protein-based methods, such as enzyme-linked immunosorbent assay; and the third category is the detection of enzymatic activities [2, 5]. The DNA-based PCR methods are most widely applicable and could be applied for unprocessed and highly processed foods. Two types of PCR could be used for the examination: conventional PCR, which is able to confirm the presence of GMO with the help of gel electrophoresis, and real-time PCR, which is able not only to detect but also to quantify the GMO content. Several PCR-based methods have been developed to detect and quantify GMO in food and feed [7, 10, 11]. Real-time PCR was applied for the characterization of corn events such as Bt176 [21], Bt11 [21], MON 810 [17, 21], NK603 [17], CBH-351 [35], T25 [6], GA21 [21], and GM potato Spunta [10, 11, 29]. In addition, Elsanhoty et al. [12] compare six different methods for extracting DNA from raw maize and its derived products.

Detection of GMO in raw and processed foods in different countries was reported. In Brazil, Cardarelli et al. [4] analyzed food items for the presence of CaMV 35S promoter and NOS terminator and GMO positive samples were detected. Elsanhoty et al. [13] analyzed samples from the Egyptian food market containing soybean and maize. The results clearly demonstrated the incidence of GM maize and soybean in the Egyptian market. Rott et al. [30] found soy samples positive for GMO from the Canadian supermarkets. Greiner et al. [15] applied qualitative and quantitative PCR-based methods to detect GM soy (Roundup Ready™ soybean [RRS]) and maize (Bt176 Maximizer maize, Bt11 maize, MON 810 YieldGard corn, and T25 LibertyLink® maize) in

Brazilian processed foods. Abdullah et al. [1] found 80 positive food samples for lectin in the Malaysian food market, wherein the 35S promoter and the NOS terminator were present in 18 samples.

Although the Kingdom of Saudi Arabia (KSA) mainly depends on imported (approximately 60–70 %) crops and foods, the control of these crops and foods only depends on its nutrient content and the acceptable level of mycotoxins without paying any attention to genetic manipulation. Consequently, there is no report on the presence of GM crops for both human and/or animal consumption in KSA. Therefore, the objective of this work was to investigate the presence of GM maize, rice, and soy in different food products collected from traditional markets, supermarkets, and grocery stores in KSA using the construct-specific method for GMO detection.

## Materials and Methods

### Materials

Certified reference materials (CRMs), produced by the Institute for Reference Material and Measurements (Geel, Belgium), were used as negative and positive controls for soy and maize lines (Bt176 and Bt11). CRMs were purchased from Fluka (Table 1).

Because there is no CRM available for maize lines MON 810 and T25, samples containing 1 % GMO were prepared in the laboratory from these lines and used as positive controls, whereas the negative control used was the normal non-GMO maize. GMOScreen 35S/NOS kit was obtained from GeneScan Germany (cat. no. 5221102210). The extracted DNA from the samples was screened for the presence of both 35S promoter and NOS terminator using the test kit for the qualitative detection of GMO varieties in food according to the producer's instructions. For the StarLink maize, the positive control and the negative control were provided with the commercial detection kit used for the detection of this maize line (Commercial GMO/dent StarLink™ kit; Europe Gene Scan, Bremen, Germany; cat. no. 5221102810). For quantities detection, GMOQuant Bt11 corn DNA quantification kit, GMOQuant MON 810 YieldGard corn DNA quantification kit, GMOQuant T25 LibertyLink corn DNA quantification kit, GMOQuant Bt176 corn DNA quantification kit, and GMOQuant Roundup Ready™ DNA quantification kit were from GeneScan (Freiburg, Germany).

### Sampling

Two hundred samples from commercially available soybean, maize, and rice were collected randomly from Saudi markets from different regions (Al-Qassim, Riyadh, and Mahdina) throughout the years 2009 and 2010. Samples included raw and relatively mild treated

**Table 1** CRMs ordering numbers (Fluka)

| CRM | 0 % GMO | 0.5 % GMO | 1 % GMO | 2 % GMO | 5 % GMO |
|---|---|---|---|---|---|
| Soy RRS | 85474 | 85477 | – | 85478 | – |
| Maize Bt176 | 63195 | 63197 | – | – | 17111 |
| Maize Bt11 | 09754 | – | 17947 | 09757 | – |

**Table 2** Soybean products screened by 35S promoter and NOS terminator and analyzed for the presence of Roundup Ready™ specific DNA

| Tested raw materials and processed products | Number of samples | Number of samples positive for both 35S and NOS | Number of RRS positive samples |
|---|---|---|---|
| Soybean granules from USA | 24 | 4 | 4 |
| Soybean granules from Brazil | 8 | 0 | 0 |
| Soybean flour | 12 | 2 | 2 |
| Cake with soybean flour (KFC) | 12 | 12 | 12 |
| Soybean mix for hamburger | 8 | 0 | 0 |
| Infants formula contained soybean | 4 | 0 | 0 |
| Total number of samples | 68 | 18 | 18 |

ground soybeans, soybean products, maize, maize products, and rice to highly processed products and snacks. The samples are listed in Tables 2, 3, and 4.

## Samples Pretreatment

The samples that contain lipids were defatted. Samples were soaked in chloroform/methanol/water (1:2:0.8, *v*/*v*/*v*) overnight. The defatted samples were recovered by filtration and stored frozen until DNA was extracted.

## Extraction of Genomic DNA

Soybean, rice, and maize samples were ground in an electric grinder. Frozen products were placed at room temperature until thawed. Two hundred milligrams of samples and from the CRMs were used for the extraction of the DNA according to the official German methods for soybean and maize [24, 25] by the cetyltrimethylammonium bromide (CTAB) method. DNA from CRMs and from all samples was extracted twice in independent procedures. A blank sample consisting of 200 μL autoclaved bidistilled water was used to control the reagents. About 200 mg of homogenized sample were weighed in 2 mL reaction vessels; 1,000 μL of CTAB extraction buffer [CTAB (20 g/L), NaCl (1.4 mol/L), Tris base/HCl (0.1 mol/L), and $Na_2$-EDTA (20 mmol/L) adjusted with HCl, pH 8] was added and mixed well (20 μL proteinase K and 20 μL RNAse were also added). The DNA pellet was air-dried and resuspended in 100 μL sterile bidistilled and deionized water (Roche, Penzberg, Germany). The extracted DNA was stored at −20 °C until used in subsequent steps.

**Table 3** Rice products screened by 35S promoter and NOS terminator and analyzed for the presence of LL601 rice, LL62 rice, and Bt63 rice

| Tested raw materials and processed products | Number of samples | Number of samples positive for both 35S and NOS |
|---|---|---|
| Egyptian rice | 10 | 0 |
| Indiana rice | 10 | 0 |
| Thailand rice | 11 | 0 |
| Rice starch | 6 | 0 |
| American rice | 18 | 0 |
| Rice imported from China | 9 | 0 |
| Total number of samples | 64 | 0 |

**Table 4** Maize products screened by 35S promoter and NOS terminator and analyzed for the presence of Bt176, Bt 11, T25, MON 810, and StarLink™ specific DNA

| Tested raw materials and processed products | Number of samples | Presence of 35S promoter | Presence of NOS terminator | Number of samples positive for both 35S and NOS | Number of samples positive for Bt176 | Number of samples positive for Bt11 | Number of samples positive for T25 | Number of samples positive for Mon 810 | Number of samples positive for StarLink™ |
|---|---|---|---|---|---|---|---|---|---|
| Whole maize kernel (golden sweet maize, Gody canned maize) | 12 | 7 | 1 | 5 | 2 | 3 | 2 | 1 | 2 |
| Maize kernel (yellow maize; USA) | 5 | 3 | 3 | 2 | 4 | 2 | 2 | | 1 |
| Yellow popcorn seeds (USA) | 6 | 0 | 3 | 0 | 3 | – | – | – | – |
| Corn flour (Riyadh Food) | 6 | 2 | 2 | 0 | 2 | – | – | – | – |
| Frozen maize (sweet corn) | 6 | 4 | 5 | 2 | 2 | 2 | 2 | 2 | 1 |
| Maize starch | 6 | 0 | 5 | 0 | – | 0 | 0 | | 0 |
| White popcorn | 5 | 0 | 5 | 0 | – | 0 | 0 | | 0 |
| Corn flakes with different flavors | 5 | 0 | 5 | 0 | 1 | 0 | 0 | | 0 |
| Crispy corn snacks with different flavors | 12 | 0 | – | 0 | – | 0 | 0 | 1 | 0 |
| White maize | 5 | 0 | 0 | 0 | 0 | 0 | 0 | | 0 |
| Total samples | 68 | | 28 | 9 | 14 | 7 | 6 | 4 | 4 |

### Extraction of DNA from Processed Food Samples

Because of the low quality and quantity of extracted DNA, DNA was obtained from processed food samples using the CTAB method. The DNA extraction kit Vivantis (cat. no. GF-FE-025: 25 preps) was used to extract DNA from processed food samples according to the instructions of the supplier. The extracted DNA was stored at −20 °C until further use.

### DNA Yield and Quality

The concentration and purity of extracted DNA were measured at 260 and 280 nm using an Ultraspec 2000 spectrophotometer (Pharmacia Biotech, Piscataway, NJ, USA) in relation to a DNA standard of known concentrations (calf thymus final concentration of 25 ng/μL). Concentrations (in nanograms per microliter) and A260/A280 readings were recorded for each sample. The extracted DNA concentration was measured and adjusted by dilution to 20–25 ng/μL prior to PCR using bidistilled and deionized water.

### PCR Detection of Lectin Gene and Invertase Gene

To confirm that extracted DNA is free from any inhibitors, DNA solutions from soybean samples were checked by the amplification of the soy-specific endogenous lectin gene. The primer pair GM03/GM04 is specific for the single-copy lectin gene LE1 and yields a PCR product of 118 bp size [22]. It is detectable in transgenic and in conventional soybean (soy specific primer pair). Specific primer pairs served as a control for the amplification of the isolated DNA and PCR procedure (PCR quality control). On the other hand, maize and maize products were checked using the invertase gene as a control for the presence of maize, wherein the PCR system was checked using the maize invertase specific primers pair IVR1-F/IVR1-R (Table 5). The primer pair IVR1-F/IVR1-R is specific for the invertase gene and flanks part of exon number 3 of this gene. It gives rise to a 226-bp amplicon [8].

### GMOScreen 35S/NOS

The extracted DNA was screened for the presence of the 35S promoter and NOS terminator using the test kit for the qualitative detection of GMO varieties (GeneScan Germany, cat no. 5221102210) according to the producer's instructions.

### *Identification of GMO Food by Qualitative PCR*

The primers used in this study, together with their target specific part of the investigated DNA, are listed in Table 5. All primers were synthesized by Bio-Synthesis Inc. (Lewisville, TX, USA) and obtained in a lyophilized state. All primers were dissolved before use to obtain a final concentration of 20 pmol/μL of each. For StarLink maize, the primer pair and the complete master mix without the polymerase enzyme were provided with the commercial detection kit used. PCR was carried out on a thermocycler (Biometra T1, Göttingen, Germany) using a master mix. Each PCR mix had 25 μL total volume which contained 2.5 μL Reddy Mix buffer (10× concentrate; Thermo Scientific), 2 μL $MgCl_2$ solution (25 mM), 1 μL dNTPs solution (0.2 mM each of dATP, dCTP, dGTP, and dTTP), 0.5 μM of each primer, 0.625 U ThermoPrime Taq polymerase (Thermo Scientific), and 2 μL of template-extracted DNA. For StarLink detection, 1 U AmpliTaq Gold polymerase (Perkin Elmer) was added to the master mix obtained with the commercial kit prior to PCR. Table 6 shows the time and temperature profiles used in PCR for

**Table 5** Oligonucleotide primer pairs sequence and their target element

| Primer | Sequence | Fragment length (bp) | Target element | Reference |
|---|---|---|---|---|
| GM03/GM04 | 5′-gCC CTC TAC TCC ACC CCC ATC C-3′; 5′-gCC CAT CTg CAA gCC TTT TTg Tg-3′ | 118 | Soy lectin gene | Mayer et al. [22] |
| p35s-f2/petu-r1 | 5′-TgA TgT gAT ATC TCC ACT gAC g-3′; 5′-TgT ATC CCT TgA gCC ATg TTg T-3′ | 172 | Transition site from the CaMV35S promoter sequence to the *Petunia hybrida* chloroplast–transit–signal sequence in RRS | Wurz et al. [36] |
| IVR1-F/IVR1-R | 5′-CCg CTg TAT CAC AAg ggC Tgg TAC C-3′; 5′-ggA gCC CgT gTA gAg CAT gAC gAT C-3′ | 226 | Maize invertase gene | Ehlers et al. [8] |
| Cry03/Cry04 | 5′-CTC TCg CCg TTC ATg TCC gT-3′; 5′-ggT CAg gCT CAg gCT gAT gT-3′ | 211 | Transition site from the CCDPK-promoter into the amino terminal sequence of synthetic cry1A(b) gene in Bt176 maize | Hupfer et al. [18] |
| IVS2-2/PAT-B | 5′-CTg ggA ggC CAA ggT ATC TAA T-3′; 5′-gCT gCT gTA gCT ggC CTA ATC T-3′ | 189 | Transition site from the intron IVS2 into the PAT gene in Bt11 maize | Anonymous [3] |
| T25-F7/T25-R3 | 5′-ATg gTg gAT ggC ATg ATg TTg-3′; 5′-TgA gCg AAA CCC TAT AAg AAC CC-3′ | 209 | Transition site from the CaMV terminator into the PAT gene in T25 maize | Anonymous [3] |
| VW01/VW03 | 5′-TCg AAg gAC gAA ggA CTC TAA Cg-3′; 5′-TCC ATC TTT ggg ACC ACT gTC g-3′ | 170 | Transition site from the genomic maize DNA into the CaMV promoter in MON 810 maize | Anonymous [3] |

each primer pair, including the conditions for the detection of StarLink. All amplicons were stored at 4 °C until gel electrophoresis. Agarose gel preparations and electrophoresis were carried out using Tris base/borate (TBE) buffer solution (pH 8.0), containing 45 mmol/L Tris base/boric acid and 1 mmol/L EDTA adjusted with hydrochloric acid. To determine the size of the DNA fragments, DNA of known size (50 and 100 bp DNA marker; Roche) together with different amplicons was separated on 2 % *w/v* agarose gel (LE, Roche) and stained with 0.01 % ethidium bromide solution (0.5 mg/L). Ten microliters of all amplicons and DNA marker were stained before gel electrophoresis by 2 μL xylene cyanol dye solution (1 mg xylene cyanol, 400 mg sucrose completed to 1 mL with water) and then subjected to electrophoresis for 45 min. The amplicons were made visible by ethidium bromide staining and documented using UV transillumination and Dolphin-View Wealtec.

*Detection and Identification of GMF*

The samples positive for the presence of both 35S promoter and/or NOS terminator were detected and identified using of PCR analysis. Tables 5 and 6 present the primer and their target and the PCR condition.

*Quantitative PCR*

For quantitative PCR, GMOQuant Bt176 corn DNA quantification kit, GMOQuant Bt11 corn DNA quantification kit, GMOQuant MON 810 YieldGard corn DNA quantification kit, GMOQuant T25 LibertyLink corn DNA quantification kit, and GMOQuant Roundup Ready™ DNA quantification kit were used on Applied Biosystems 7300 real-time PCR (Applied Biosystems, Foster City, CA, USA) according to the instructions of the supplier.

## Results and Discussion

DNA Extraction, Concentration, and Purity

DNA was extracted from foodstuffs based on the complexity of their composition and technology process. Foodstuffs used in this study were classified and described in Tables 2, 3, and 4. The samples usually contain ingredients able to inhibit PCR such as fatty acid, polysaccharides, polyphenols, and others compounds that could interfere with the isolation

**Table 6** Time/temperature profiles for qualitative PCR with DNA extracted from maize and soybean samples using the primer pairs

| Primer pair | Initial denaturation | Denaturation | Annealing | Extension | Cycles | Final elongation |
|---|---|---|---|---|---|---|
| GM03/GM04 | 10 min at 95 °C | 30 s at 95 °C | 30 s at 60 °C | 1 min at 72 °C | 35 | 3 min at 72 °C |
| p35s-f2/petu-r1 | 10 min at 95 °C | 30 s at 95 °C | 30 s at 62 °C | 25 s at 72 °C | 35–40 | 10 min at 72 °C |
| IVR1-F/IVR1-R | 12 min at 95 °C | 30 s at 95 °C | 30 s at 64 °C | 30 s at 72 °C | 42 | 10 min at 72 °C |
| Cry03/Cry04 | 12 min at 95 °C | 30 s at 95 °C | 30 s at 63 °C | 30 s at 72 °C | 38 | 10 min at 72 °C |
| IVS2-2/PAT-B | 12 min at 95 °C | 30 s at 95 °C | 30 s at 64 °C | 30 s at 72 °C | 38 | 10 min at 72 °C |
| T25-F7/T25-R3 | 12 min at 95 °C | 30 s at 95 °C | 30 s at 64 °C | 30 s at 72 °C | 40 | 10 min at 72 °C |
| VW01/VW03 | 12 min at 95 °C | 30 s at 95 °C | 30 s at 64 °C | 30 s at 72 °C | 40 | 10 min at 72 °C |
| StarLink kit | 10 min at 94 °C | 25 s at 94 °C | 30 s at 62 °C | 45 s at 72 °C | 50 | 3 min at 72 °C |

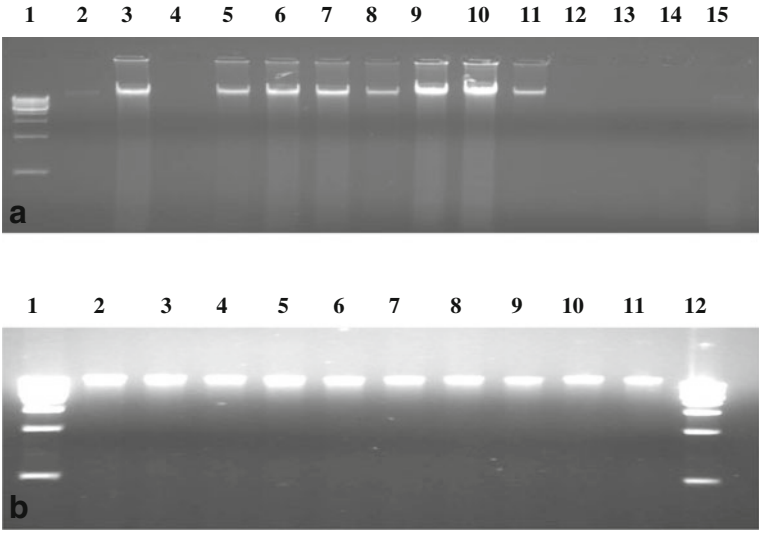


**Fig. 1** Agarose **a** gel electrophoresis of total DNA extracted from different food samples extracted by the CTAB method. *Lanes 1* and *15* 1 kbp DNA ladder, *lane 2* DNA extracted from cake with soy flour, *lane 3* DNA extracted from maize granules, *lane 4* DNA from maize starch, *lane 5* DNA from maize flour, *lane 6* DNA from canned maize, *lane 7* DNA from frozen maize (sweet corn), *lane 8* DNA from soy flour, *lane 9* DNA from rice, *lane 10* DNA from popcorn, *lane 11* DNA from rice starch, *lane 12* DNA extracted from corn flour, *lane 13* DNA extracted from soybean mix for hamburger, *lanes 14* and *15* DNAs extracted from corn flakes and crispy corn snacks. **b** Agarose gel electrophoresis of total DNA extracted from different food samples extracted by Vivantis kit. *Lanes 1* and *12* 1 kbp DNA ladder, *lane 2* DNA extracted from cake with soy flour, *lane 3* DNA extracted from maize granules, *lane 4* DNA from maize starch, *lane 5* DNA from maize flour, *lane 6* DNA from canned maize, *lane 7* DNA from frozen maize (sweet corn), *lane 8* DNA from soy flour, *lane 9* DNA from soybean mix for hamburger, *lane 10* DNA from corn flakes, *lane 11* DNA from crispy corn snacks

of the DNA or even degrade it [16, 28]. The quantification of DNA on agarose was achieved for all samples and different DNA extractions to detect the band corresponding to the genomic DNA (Fig. 1a, b).

The data indicated that there were differences in the DNA obtained from foodstuffs that were mechanically or thermally treated using different extraction methods. It was impossible to perform genomic quantification on agarose gel or by spectrophotometer, except for the samples that used the Vivantis kit which gave the highest yield of DNA. The grade of DNA damage (e.g., depurination), the presence of PCR inhibitors in food matrices, and the average fragment length of the extracted DNA are the factors that generally influence the quality of the extracted DNA from food items. These factors are dependent on the samples itself, the processes carried out during the production of the food, and the physicochemical parameters of the extraction method [12, 27].

The exposure to heat causes fragmentation of high-molecular-weight DNA. On the other hand, physical and chemical treatments cause random breaks in DNA strands, thus reducing the average DNA fragment size [9, 18, 33, 34]. Many foods are characterized by their acidity, thus accelerating the acid-catalyzed reactions during thermal treatments [2, 37]. Good results were obtained when the Vivantis DNA extraction kit was applied for processed foods. DNA extracted with the Vivantis kit gave higher concentrations and purities. Similar results was obtained by Smith et al. [31], Yohimitsu and Hori [38] and Sisea and Pamfil [32], who compared methods for DNA extraction from potato-derived products and found that the

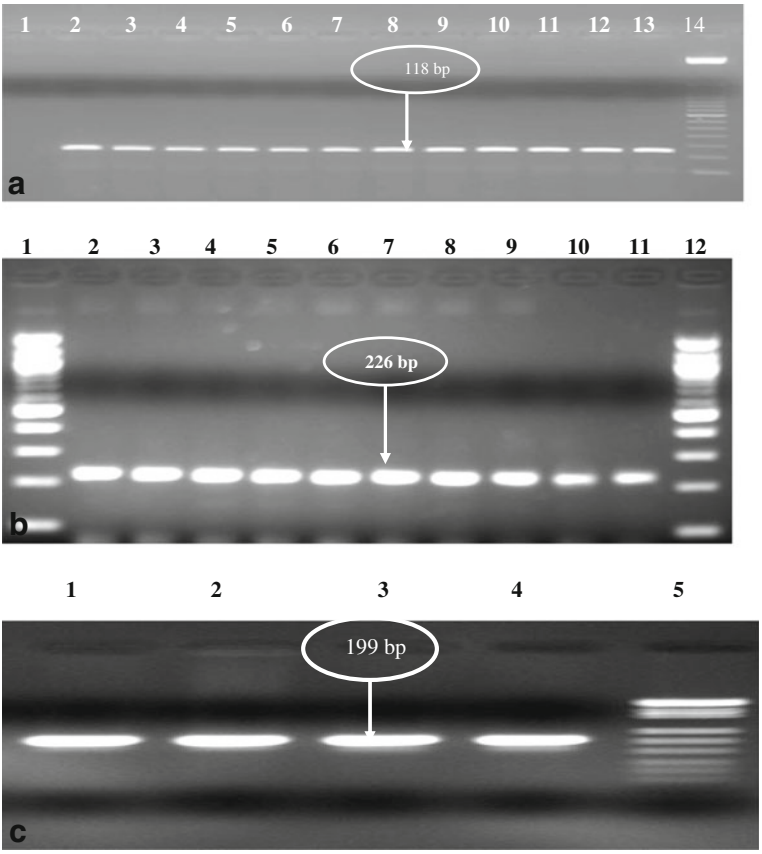


**Fig. 2** **a** Detection of the soybean lectin gene in the DNA from different food samples. DNA was extracted and examined by PCR analysis using the primer pair GMO3/GMO4. The size and location of the expected amplification product is indicated. *Lane 14* 50 bp molecular weight ladder, *lane 1* PCR control without template DNA, *lanes 2* and *12* DNA from different food samples that contained soybean, *lane 13* DNA from 2 % RRS as positive control. **b** Example for the detection of the maize invertase gene in different maize samples. Analysis was performed as described in the "Materials and Methods" section, except that the primer pair IVR1-F/IVR1-R was used for PCR analysis. *Lanes 1* and *12* 100 bp DNA ladder, *lane 2* DNA from 0.5 % GM 176 maize, *lanes 3–11* DNA from different maize samples. **c** Example for the detection of chloroplast DNA in different rice samples. Analysis was performed using the GMOScren kit to detect 35S promoter and/or NOS terminator. *Lanes 1* 100 bp DNA from positive control included in kit, *lanes 2–4* DNA from different rice samples, *lane 5* DNA ladder included in the kit as size reference

yield and quality of DNA were influenced by cooking and processing. The obtained results were not in agreement with those obtained by Milia et al. [23] who used three extraction methods to detect RRS in processed food and found that all used methods (CTAB, Kit PrepMan™ Ultra, and ABI PRISM 6100) were suitable to isolate DNA.

## Detection of Lectin and Invertase Genes

The presence of soybean was checked using primers for the soy-specific gene (lectin gene). On the other hand, the presence of maize in food samples' DNA was checked for the presence of the invertase gene. The lectin gene was detected in all samples that contained soy ingredients (Fig. 2a).

The presence of soybean and the functioning of the PCR system were checked using the soybean lectin specific primer pair GMO3/GMO4 (Table 5). The primer pair GM03/GM04 is specific for the single-copy lectin gene LE1 and yields a PCR product of 118 bp size [22]. It is detectable in GM as well as in conventional soybean (soy specific primer pair). Specific primer pairs served as a control for the amplification of the isolated DNA and PCR procedure (PCR quality control). For all samples containing maize, an amplicon of the expected size has been obtained in PCR using the primer pair IVR1-F/IVR1-R. The results are shown in Fig. 2b as an example for the PCR analysis. The primer pair IVR1-F/IVR1-R is specific for invertase gene in GM maize and conventional maize (maize specific primer pair), wherein a PCR product was revealed at 226 bp. All extracted DNA from samples contained maize ingredients that gave good results with the invertase gene (Fig. 2b). The obtained results indicated that the extracted DNA free from any inhibitor compounds led to incorrect results. The extracted DNA from rice samples were screened using the GMOScreen 35S/NOS test kit for the detection of GMO. The control reaction (chloroplast DNA) was done as an indicator whether DNA of sufficient quantity and quality has been isolated from rice samples (Fig. 2c) as an example for the PCR analysis by GMOScreen 35S/NOS. Data in Fig. 2c indicated that the specific DNA sequence of 199 bp in length from the chloroplast gene was amplified from both conventional plant DNA and from GM plant DNA.

## Detection of GMO Specific Genetic Elements (35S Promoter or NOS Terminator)

All extracted DNAs were screened using the GMOScreen 35S/NOS test kit for the detection of GMO varieties in food, feed, and seeds. The amplicon was specific for GMO specific

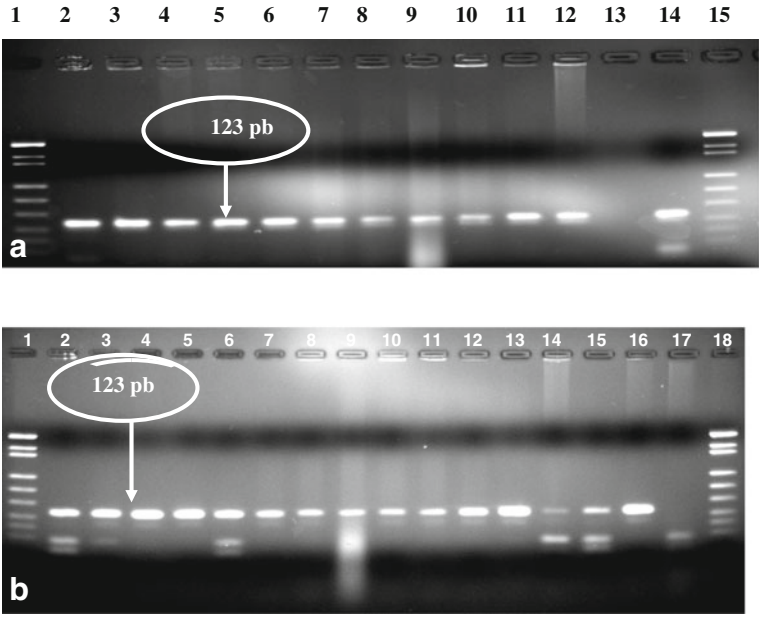


**Fig. 3** **a** Analysis of food samples for the presence of 35S promoter specific DNA. Analysis was performed as described in the "Materials and Methods" section, except that the GMOScreen kit was used for PCR analysis. **b** Analysis of food samples for the presence of 35S promoter specific DNA. Analysis was performed as described in the "Materials and Methods" section, except that the GMOScreen kit was used for PCR analysis

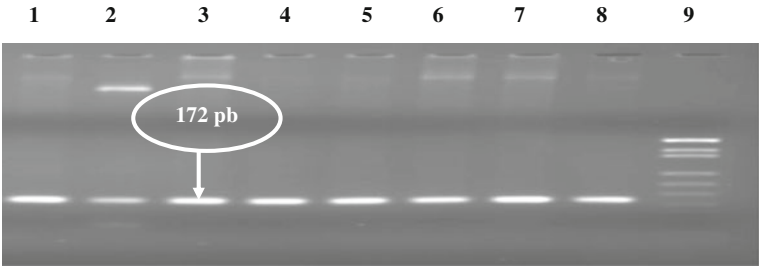


**Fig. 4** Analysis of food samples for the presence of RRS DNA. DNA was extracted from different samples and examined by PCR analysis using the primer pair p35S-f2/petu-r1 used for PCR analysis. *Lanes 1–7* food samples containing soybean, *lane 8* positive control 0.5 % RRS, *lane 9* 100 bp DNA ladder

genetic elements (35S promoter or NOS terminator). Soybean samples gave positive results from GMOScreen 35S/NOS and the amplicon rose at the expected size of 123 bp (Fig. 3a, b). All rice samples gave negative results for the presence of 35S promoter or NOS terminator. Maize samples gave positive results for the presence of 35S promoter or NOS terminator or positive results for both 35S promoter and NOS terminator (Fig. 2a, b).

## Specific Detection of Roundup Ready™ Soybean

All positive samples obtained from the screening by the GMOScreen kit were analyzed for the presence of RRS. PCR with RRS-positive samples resulted in an amplicon of 172 bp in length using the primer pair p35s-f2/petu-r1 (Fig. 4). The primer pair p35s-f2/petu-r1 is specific for the genetic modification in RRS and amplifies a 172-bp segment. The primer pair attaches to the CaMV35S promoter sequence and the *Petunia hybrida* chloroplast–transit–signal sequence [36]. The amplicon is detected only in transgenic samples and GMO-containing CRM. Eighteen out of 60 samples tested are shown in Table 2 and an example for the PCR analysis is given in Figs. 3 and 4.

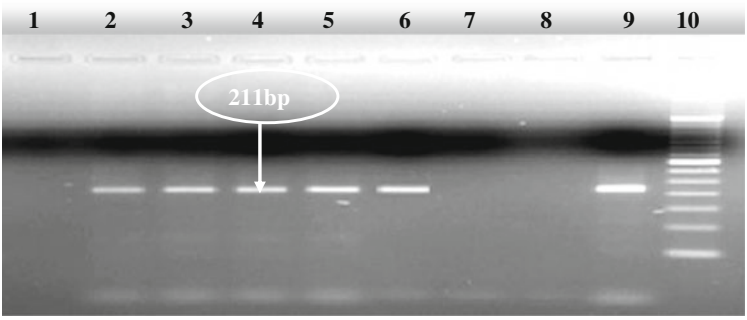


**Fig. 5** Detection of the transgenic from Bt176 maize in different food samples. Analysis was performed as described in the "Materials and Methods" section, except that the primer pair Cry03/Cry04 was used for PCR analysis. *Lane 1* PCR control without DNA; *lanes 2* and *3* DNA from maize granules (USA), *lane 4* DNA from golden sweet maize (canned maize), *lane 5* DNA from corn flour, *lane 6* DNA from corn flakes, *lane 7* DNA from maize starch, *lane 8* DNA from non-GMO maize, *lane 9* DNA from 5 % Bt176 maize, *lane 10* molecular weight marker 50 bp DNA ladder

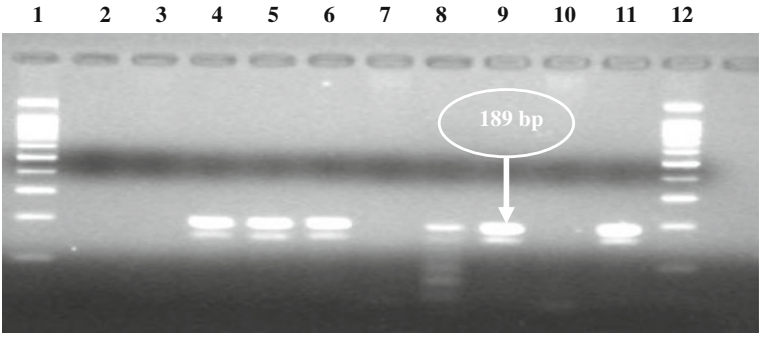


**Fig. 6** Detection of the transgene from Bt11 maize in different food samples. Analysis was performed as described in the "Materials and Methods" section, except that the primer pair IVS2-2/PAT-B was used for PCR analysis. *Lane 1* PCR control without DNA template, *lane 2* extraction control, *lane 3* DNA from some corn flakes sample, *lanes 4* and *5* DNA from maize granules (USA), *lane 6* DNA from maize flour (USA), *lane 7* DNA from white maize, *lanes 8* and *9* DNA from frozen maize (sweet corn), *lane 10* DNA from non-GMO maize, *lane 11* DNA from 2 % Bt11 maize, *lane 12* 100 bp DNA ladder

## Specific Detection of GM Maize Lines

To confirm the results obtained from GMOScreen 35S/NOS, positive maize samples (Table 4) were analyzed using specific primer pairs to detect Bt176, Bt11, MON 810, T25, and StarLink™ using PCR (Tables 5 and 6). Bt176 was identified using the primer pair Cry03/Cry04. The primer pair Cry03/Cry04 is specific for the identification of transgenic maize event Bt176. The resulting sequence of 211 bp size is amplified from a genomic region between two adjacent genetic elements, namely, the CDPK promoter and the N terminus of the synthetic cry1A(b) gene [18]. The expected 211-bp amplicon appeared only with the transgenic samples and GMO-containing CRM as shown in the example (Fig. 5, lanes 2, 3, 4, 5, 6, and 9).

By using primers specific for Bt11 maize (IVS2-2/PAT-B), an amplified sequence of 189-bp length was obtained from maize grain samples imported from the USA, frozen maize (sweet corn), and the positive control (Fig. 6, lanes 4, 5, 6, 8, 9, and 11).

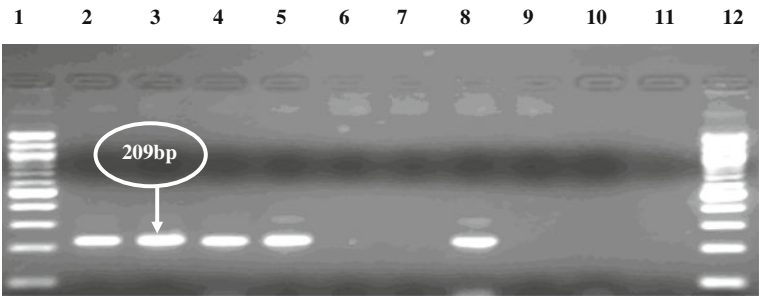


**Fig. 7** Detection of the transgene from maize T25 in food samples. Analysis was performed as described in the "Materials and Methods" section, except that the primer pair T25-7/T25-R was used for PCR analysis. *Lanes 1* and *12* 100 bp marker DNA ladder, *lane 2* DNA from maize kernel (USA), *lanes 3–5* DNA from maize (canned maize, frozen maize, and whole maize kernel), *lanes 6* and *7* DNA from maize flour, *lane 8* DNA from positive control, *lane 9* DNA from crispy corn snacks, *lane 10* PCR control, *lane 11* DNA from non-GMO maize T25

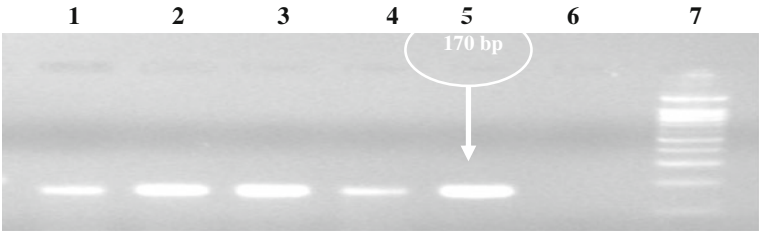


**Fig. 8** Detection of the transgene from maize MON 810 in food samples. Analysis was performed as described in the "Materials and Methods" section, except that the primer pair VW01/VW03 was used for PCR analysis. *Lane 1* DNA from whole maize kernel (USA), *lanes 2* and *3* DNA from frozen maize, *lane 4* DNA from crispy corn snacks, *lane 5* DNA from 1 % GMO maize MON 810, *lane 6* DNA from non-GMO maize, *lane 7* 100 bp DNA ladder

The primer pairs IVS2-2/PAT-B were used for the detection of the transition site from the intron IVS2 into the PAT gene in Bt11 maize. The bacterial PAT gene codes for the enzyme phosphinothricin *N*-acetyltransferase, giving rise to the resistance of Bt11 maize to the herbicide phosphinothricin [3]. The primer pair T25-F7/T25-R3 was used for the detection of the transition site between the CaMV terminator into the PAT gene in T25 maize and yields a PCR product of 209 bp. The amplicon was obtained from maize grain samples imported from the USA, frozen maize (sweet corn), the positive control yellow maize kernel (USA), frozen maize, canned maize (Gody), and GMO-containing CRM (Fig. 7, lanes 2, 3, 4, 5, and 8).

For the identification of maize MON 810, the primer pair VW01/VW03 flanks the transition site from the genomic maize DNA into the CaMV promoter in MON 810 maize [3]. The expected 170 bp appeared with maize kernel, frozen maize, crispy corn samples, and GMO-containing CRM as a positive control (Fig. 8, lanes 1, 2, 3, 4, and 5).

The identification of StarLink™ maize was carried out with the GMO*Ident* StarLink™ test kit (GeneScan Europe AG). The expected 133-bp fragment appeared only with the maize grain samples from the USA, frozen maize, and canned maize (Fig. 9, lanes 1, 4, 5, and 6).

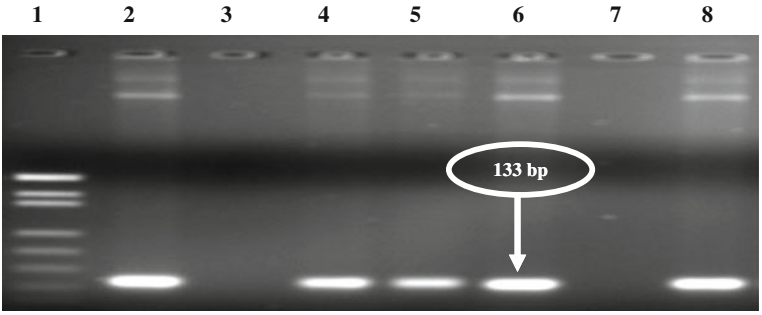


**Fig. 9** Detection of the transgene from StarLink™ maize in food samples. Analysis was performed as described in the "Materials and Methods" section, except that the primer pair from the GMO*Ident* StarLink test kit was used for PCR analysis. *Lane 1* molecular weight ladder with GMO*Ident* StarLink, *lane 2* DNA from whole maize (canned maize), *lane 3* DNA from corn flour, *lane 4* DNA from yellow maize kernel (USA), *lane 5* DNA from frozen sweet corn, *lane 6* DNA from whole maize kernel canned maize, *lane 7* DNA from maize non-GMO maize, *lane 8* positive DNA from GMO*Ident* StarLink test kit

Quantitative Detection of GM Soybean and Maize Lines

Quantitative analysis revealed that 12 samples of the products contained <3 % of GM soy and six samples contained >10 % of GM soy. Correspondingly, six samples contained <2 % of GM maize Bt176 and eight samples contained >10 % of GM maize Bt176. Five samples contained >7 % of GM maize Bt11 and one sample contained <1 % of GM maize Bt11. On the other hand, all positive samples (six) contained GM maize T25 (>20 %). Four samples of the products containing GM maize were shown to contain >5 % of GM maize MON 810. Four samples of the products containing GM maize were shown to contain >1 % of GM maize StarLink. The presence of material derived from GMO was found without label. These results were in agreement with previous data reported about the distribution of GMO in commercial foods [4, 13, 15, 26]. Greiner and Konietzny [14] analyzed 100 Brazilian foods containing maize for the presence of MON 810, Bt11, Bt176, and T25 events. They found 11 samples positive for GM maize, wherein 4 out of 18 maize flour and 3 out of 18 polenta samples contained GM maize. The majority of the GM maize positive products were not of Brazilian origin. The presence of GM maize was screened in 32 samples of foodstuffs sold commercially in Argentinean markets, and 8 out of 32 showed positive results (Margarit et al. 2006). Park et al. [26] found that most of the maize in the storage products from five Korean provinces were GM, wherein about 50 % of the grains were germinated.

## Conclusion

It could be concluded that the methods of DNA isolation were suitable for most food products. In all samples, DNA was successfully isolated using the CTAB method or Vivantis kits. The results clearly demonstrated the presence of GM maize and soybean in the KSA food market. The existence of StarLink™ maize in the food chain supplies is an evidence for the uncontrolled arrival of unauthorized GMOs for food use in KSA. The product labels did not indicate the presence of GMO ingredients to give the consumers the chance to select food products. Depending on the results, it would be advisable to control all of the imported raw materials and food products. Establishing strong regulations and certified laboratories to monitor GM foods or crops is recommended.

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