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ORIGINAL ARTICLE

Genetically modified Roundup Ready soybean in processed meat products in the Kingdom of Saudi Arabia

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Food composition

Abstract This work was conducted to monitor the presence of Roundup Ready (RR) soybean in processed meat products in the Kingdom of Saudi Arabia (KSA). Seventy-two samples of processed meat products were collected from the Kingdom of Saudi Arabia (KSA) food market. DNA was extracted and analyzed by polymerase chain reaction (PCR) to amplify the soybean lectin gene, and screened by GMOScreen 35S/NOS test kit for qualitative detection of GMO varieties on food, feed, and seed to amplify both 35S promoter and NOS terminators that present in Roundup Ready soybean by PCR. The positive samples for Roundup Ready soybean (GMO) were subjected to real-time quantification of GMO using TaqMan real-time PCR. The results showed that all collected samples were positive for lectin gene and 45 samples out 72 samples were positive for Roundup Ready soybean. Twenty-three samples contained more than 10 g kg^{-1} GMO and 22 samples contained less than 10 g kg^{-1} GMO.

Introduction

The promotion of a high level of food safety is a major policy priority worldwide. The innocuousness of foodstuffs is a concept inherent in food safety and is related to many aspects of agrarian technologies as well as to food production and processing. Food borne diseases are among the most serious public health concerns worldwide, being a major cause of morbidity (Wallace et al., 2000). Due to great advances in agricultural biotechnology, scientists are able to use artificial genetic manipulations to successfully transfer genes for herbicide tolerance and insect resistance into traditional crops or other advances. Many genetically modified (GM) plant cultivars have been registered worldwide. The most cultivated of genetically modified (GM) is the Roundup Ready soybean, which represents the staple

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constituents of many foods (Elsanhoty et al., 2006). Foods made from soybeans are among the most traditional and familiar foods in some countries around the world (Elsanhoty et al., 2011). Many of transgenic crops such as Soybeans A5547, A2704, MON89788, 356043, and MON87701 are approved for the EU market. A number of methods have been developed for the detection of Roundup Ready soybean. These include protein-based methods for the detection of the EPSPS gene product in transgenic raw or unprocessed soy products (Lipp et al., 2000; Meyer et al., 1996). Polymerase chain reaction (PCR) based methods, both qualitative and quantitative, are also used for more highly processed soy-based food products (Moriuchi et al., 2007; Wu et al., 2010). Moreover, DNA-based methods are the most widely used techniques for detection of genetically modified (GM) in food products. PCR methods have been proved to be the most sensitive and reliable means of detecting and quantifying genetically engineered traits in crops and foods (Holst-Jensen et al., 2006).

Soybean has many functional properties such as soybean proteins help to improve technological processes used in the manufacture of meat products and reduce their formulation costs (Criado et al., 2005). In addition, the growing demands of consumers for healthy and safer safe products have also promoted the use of soybean proteins in processed meat products as fat replacer (Castro-Rubio et al., 2005). Soy proteins are also widely used in emulsified meat products due to their unique functional properties such as water binding, fat binding, texture and emulsifying capability, and organoleptic features such as appearance, firmness, and slicing characteristics (Belloque et al., 2002).

Current EU regulations as well as those of the KSA stipulate that products containing an ingredient of more than 0.9% originates from a GMO product must be labelled. Although Kingdom of Saudi Arabia mainly depends on imported (ca. 70–80%) crops, feeds and foods, the control and evaluation of these crops, feeds, 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. There is a little information about the detection of genetically modified soybean in meat products in KSA. Therefore, this work was planned to detect and determine the percentage of Roundup Ready soybean in meat products using a conventional qualitative PCR assay to detect the presence of Roundup Ready soybean and a real-time PCR to quantify the amount of Roundup Ready soybean present in positive samples. Table 1 containing the meat products samples that collected from KSA food market.

Materials and methods

Certified reference materials and samples

The certified reference materials (CRM) used as positive controls were produced by European Reference Materials (BF410 set, ERM). They consisted of dried soybean powder containing 1, 5, 10, 20, and 50 g kg^{-1} Roundup Ready™ soybean acquired from Fluka (Sigma–Aldrich). As negative control non-genetically modified soybean (0% GMO) was obtained from Fluka Chemie GmbH (Buchs, Switzerland). Seventy-two processed meat products, 17 sausages, 14 of each chicken-fried steak and 14 luncheon meat, 12 mortadella and 15 luncheon chicken, were collected randomly from Al-Qassim, Riyadh, and Mahdina regions throughout the years 2009/2010. No sample was labelled as containing GM ingredients.

DNA extraction

Tested samples (200 mg each) and certified reference materials CRMs (100 mg) were used for the extraction of the genomic DNA by CTAB method according to Pietsch et al. (1997). All investigated samples were extracted twice in independent procedures. Furthermore, a blank sample consisting of 200°l autoclaved bi-distilled water was used to control the reagents. Further, DNA was purified from 1 ml of homogenate following the CTAB precipitation method (Querci et al., 2004). Two independent extractions were carried out from each sample. The DNA pellet was air dried and resuspended in 100°l sterile bi-distilled and deionized water purchased from Roche (Germany). The extracted DNA was stored at −20 °C until subsequent steps.

DNA yield and quality

The concentration and purity of the isolated DNA were measured by the NanoDrop™ Wilninggto, DE, USA, and spectrophotometer 2000 according to producer's instructions. Spectrophotometer optical densities of 260 nm and 280 nm were used to investigate the DNA quantity. DNA purity was measured using the appropriate ratio of OD260/OD280 (1.65–1.85). Concentrations (cng/μl) and A260/A280 readings were recorded. The extracted DNA was adjusted by dilution to conc. 25 ng/μl prior to PCR, using bi-distilled sterile water. The quality of nucleic acid extraction was controlled by agarose gel electrophoresis 0.5% (w/v).

Table 1 Samples of processed meat products analysis for the presence of lectin gene, 35S promoter, nos terminator, and structural gene (RR).

Samples	Number of samples	Presence of lectin gene		Screening (+ve)		Gene specific (+ve) (EPSPS/RR)
		No	%	35S promoter	Nos terminator	
Sausages	17	17	100	8	8	8
Chicken-fried steak	14	14	100	7	7	7
Luncheon Meat	14	14	100	11	11	11
Mortadella	12	12	100	9	9	9
Luncheon chicken	15	15	100	10	10	10
Total samples	72	72	100	45	45	45

GMOScreen CaMV35S promoter and NOS terminator

The extracted DNA screened for the presence of 35S promoter and NOS terminator by using of GMOScreen 35S/NOS test kit for qualitative detection of GM varieties in food and feeds (Eurofins-GeneScan-Germany Cat NO.: 5221102210) according to producer's instructions.

Oligonucleotide primers

Primers used in this study together with their target specific part of the investigated DNA are listed in Table 2. All primers were synthesized by Biosynthesis, Inc., USA, and obtained in a lyophilized state. All primers were solved before use to obtain a final concentration of 20 pmol/l for each.

DNA amplification and PCR condition

PCR was carried out on thermo cycler (Biometra, T1) using a prepared master mix. Each PCR mix had 25°l total volume and contained 2.5°l Reddy Mix buffer (10× concentrate, Thermo Scientific), 2°l $MgCl_2$ solution (25 mM), 1°l dNTPs solution (0.2 mM each of dATP, dCTP, dGTP, and dTTP), 0.5°M of each primer, 0.625 Unit Thermoprime Taq polymerase (Thermo Scientific), 2 l of template extracted DNA (50 ng DNA per PCR reaction), and water was to reach 25 l.

Table 3 explains the time/temperature profiles used in PCR for each primer pair including the conditions. All amplicons were stored at 4 °C until performing gel electrophoresis technique.

Detection of the Roundup Ready soybean

The samples that give positive results for the presence of 35S promoter and NOS terminators were detected and identified by using of PCR analysis with the specific primer pairs. These primer pairs were used to detect transition site from the CaMV35S promoter sequence to the petunia hybrida chloroplast-transit-signal sequence in Roundup Ready soybean (RRS). Tables 2 and 3 indicate the primer, their target, and the PCR condition.

Gel electrophoresis

Agarose gel preparation 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, 100 bp, 1 K bp DNA marker, Roche Germany) together with the different amplicons were separated on 2% w/v agarose gel (LE, Roche) TBE buffer stained with 0.01% ethidium bromide solution (0.5 mg/l). 10 l of all amplicons and DNA marker were stained before gel electrophoresis by 2°l xylene cyanol dye solution (1 mg xylene cyanol, 400 mg sucrose and water was added to reach 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 Syngene UK.

Quantitative detection by real-time PCR

The TaqMan genetically modified organism (GMO) soy 35S detection Kit part number 4327692 (Applied Biosystems) was used on an ABI PRISMTM 7300 Detection System according to the instructions of the supplier (Applied Biosystems). Reactions were carried out in 96-well plates in a final volume of 25 µl, containing kit reagents and 50 ng of template DNA per reaction. For each plate, CRM standards (1, 5, 10, 20, and 50 g kg^{-1} RR) and each sample were analyzed in triplicates negative controls (negative control provide with kit,

Table 2 Oligonucleotide primer pairs sequence and their target elements.

Primer	Sequence	Fragment length	Target element	References
GM03/GM04	5′-gCC CTC TAC TCC ACC CCC ATC C-3′ 5′-gCC CAT CTg CAA gCC TTT TTg Tg-3′	118 bp	Soy bean lectin gene	Meyer et al. (1996)
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 bp	Transition site from the CaMV35S promoter sequence to the petunia hybrida chloroplast-transit-signal sequence in RRS	Wurz et al. (1999)

Table 3 Time/temperature profiles for qualitative PCR with DNA extracted from meat samples using the primer pairs described in Table 2.

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	40	10 min at 72 °C

non-GM DNA, and water) and a positive kit control were analyzed. The Ct values obtained for the samples were compared to the calibration curve, enabling the GMO content for each sample to be calculated (Brod and Arisi, 2008).

Results and discussion

DNA isolation

Three step analyses were employed in this work to determine the identity of GMO samples. The first step involves genomic DNA extraction and amplification of specific soy sequence from plant DNA (lectin gene), necessary to discriminate between negative and positive results due to inhibition in the amplification (Forte et al., 2004). The second step entails amplification of GMO-specific sequence, represented by the 35S promoter and NOS terminator, to screen for the presence of transgenic materials in the samples. In the third step, GMO-containing samples were subjected to analysis of specific transgenic material in Roundup Ready Soybean to determine the type of GMO (Lin et al., 2000). The quality of the extracted DNA from 200 mg samples using CTAB method was examined by electrophoresis through a 0.8% agarose gel (Fluka). DNA bands of high intensity appeared in the lanes (Figs. 1A and 1B), showing high yield of genomic DNA. The results presented in this study confirmed that the CTAB protocol can be used for DNA extraction and purification from meat products samples as a first step of GMO analysis. After agarose gel electrophoresis, the DNA was present as a high molecular weight band in all of the samples.

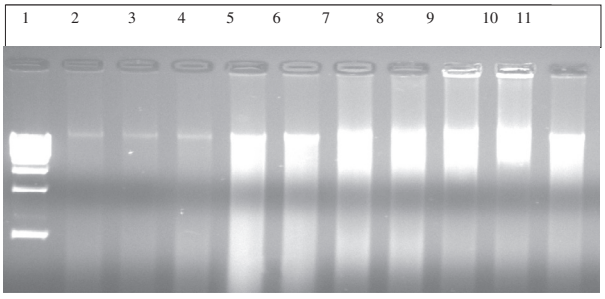

Fig. 1A Example of DNA electrophoresis on 0.5 agarose gel of DNA extracted from processed meat products. Lanes 1: M 1 K bp, lanes 2 and 3: DNA from some luncheon meat sample, lane 4 and 5: DNA from some chicken-fried steak, lanes 6 and 7: DNA from some sausages, lanes 8 and 9 DNA from mortadella, lanes 10 and 11: DNA from luncheon chicken.

Qualitative detection of genetically modified Roundup Ready soybean on meat products

The presence of soybean DNA and its amplifying ability were confirmed using the soy-specific primer pair GM03/GM04 for the lectin gene. A total of 72 food samples were analyzed, and all samples showed the positive signal, a fragment size of 118 bp for soybean lectin gene (Table 1 and Fig. 2), demonstrating that DNA of sufficient integrity for PCR analysis can be isolated using this CTAB protocol. The difficulties in extracting DNA from food matrices may be due to the binding of DNA to food ingredients and thus interfering with the release of nucleic acid (Greiner et al., 2004). However, the CTAB method proved to be useful for DNA isolation from processed food products (Mafra et al., 2008; Moriuchi et al., 2007; Gryson et al., 2004; Olexova et al., 2004; Elsanhoty et al., 2011). All 72 samples with positive signal for lectin gene detection were analyzed for 35SCaMV promoter and NOS terminator as specific detection of RR soybean using GMOScreen35S/NOS kit. Forty-five samples gave a positive signal for CaMV promoter and NOS terminator, and the PCR products appear at 123 bp (Figs. 3A and 3B). To confirm the specific amplification of RR fragment, PCR was performed with primers

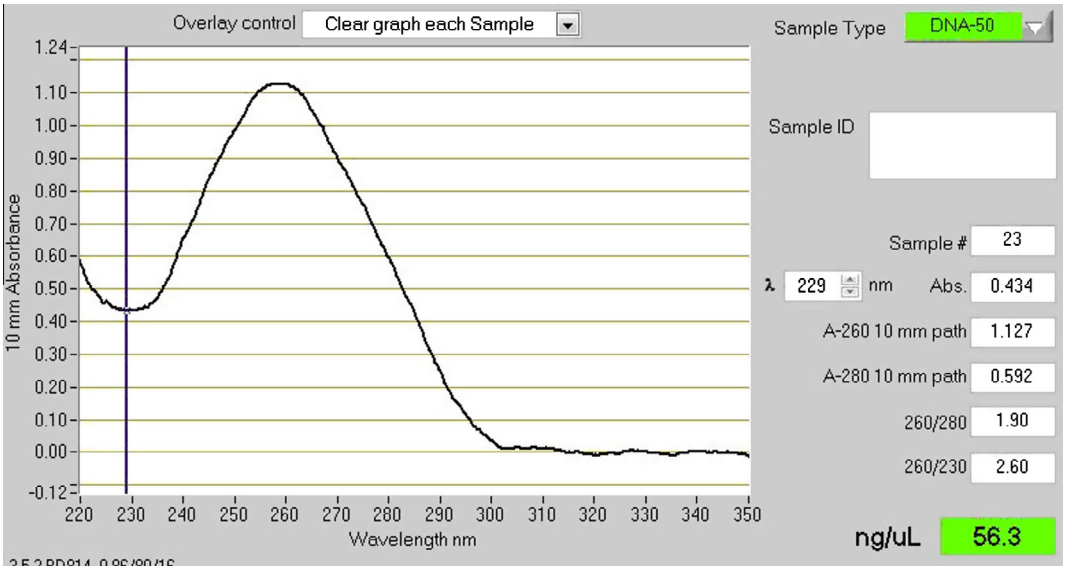

Fig. 1B Examples of the results obtained by NANODROP 2000 spectrophotometer for DNA amount of extracted DNA from some meat products samples by using of CTAB method.

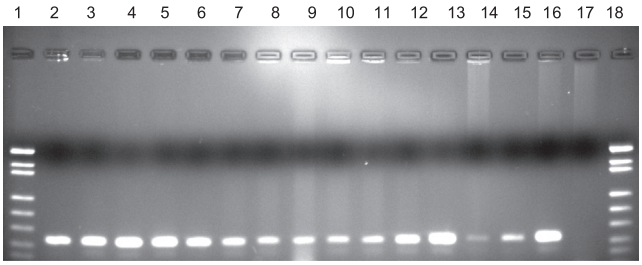

Fig. 2 Example of agarose gel electrophoresis of DNA extracted from processed meat products and examined by PCR analysis using primer GM03 and GM04. Lane 1: DNA ladder 50 bp, lanes 2–4: PCR products of DNA extracted from certified references materials RR soybean, 0.5%, 1%, and 5% respectively as positive control, Lanes 5–17: DNA from processed meat samples, lane 18: PCR control from negative control (0% GM) non-genetically modified Roundup Ready soybean DNA.

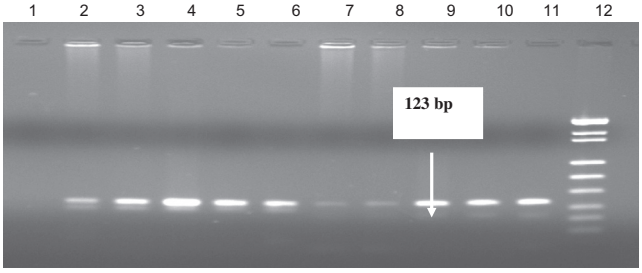

Fig. 3A Example for detection of the 35S Promoter in meat samples collected from Kingdom of Saudi Arabia food market. Analysis was performed and documented as described in materials and methods, GMOScreen 35S/NOS test kit was used for PCR analysis for qualitative detection of GMO varieties. Lane 1: PCR without DNA, lanes 2 and 3: DNA from some luncheon meat sample, lane 4: PCR control with DNA positive provide with kit, lanes 5 and 6: DNA from some chicken-fried steak, lanes 7 and 8: DNA from some sausages samples, lanes 9 and 10: DNA from some mortadella samples, lane 11: PCR product from some luncheon chicken, lane 12: marker provide with GMOScreen kits.

P35s-f2 petu-r1. The primer pair attaches to the CaMV35S promoter sequence and the petunia hybrida chloroplast transit-signal sequence (Wurz et al., 1999). The same samples that gave positive results for the presence of CaMV35S promoter and NOS terminator by GMOScreen35S/NOS kit gave positive results also with the P35s-f2/petu-r1. PCR products that showed positive signal at 172 bp (Fig. 4 lines 4–13 and Table 1) confirm the presence of GM soybean as it was demonstrated in 45 cases: eight sausages, nine mortadella, eleven luncheon meat, seven chicken-fried steak, and ten luncheon chicken.

Quantitative real-time PCR

The GM positive samples containing Roundup Ready Soybean were analyzed by the TaqMan® genetically modified organism (GMO) 35S Soy Detection Kit (Applied Biosystems). Data indicated that 22 positive samples contained RR

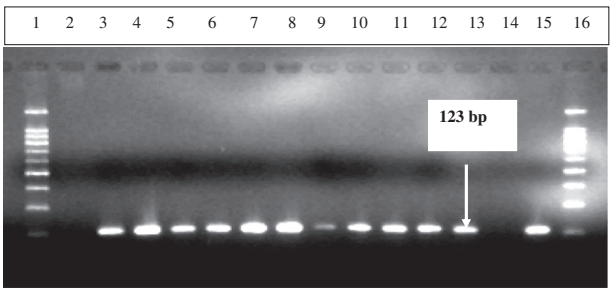

Fig. 3B Example for detection of the NOS Terminator in meat samples collected from Kingdom of Saudi Arabia food market. Analysis was performed and documented as described in materials and methods, GMOScreen 35S/NOS test kit was used for PCR analysis for qualitative detection of GMO varieties. Lane 1 and 16: DNA marker 100 bp, lane 2: PCR control PCR without DNA, lanes 3 and 4: DNA from some luncheon meat sample, lanes: 5 and 6: DNA from some chicken-fried steak, lanes 7 and 8: DNA from some sausages samples, lanes 9–11: DNA from some mortadella samples, lanes 12 and 13: DNA from some luncheon chicken products, lane: 14 DNA from negative control soybean DNA, lane 15: PCR control with DNA positive provide with GMOscreen 35S/NOS kit.

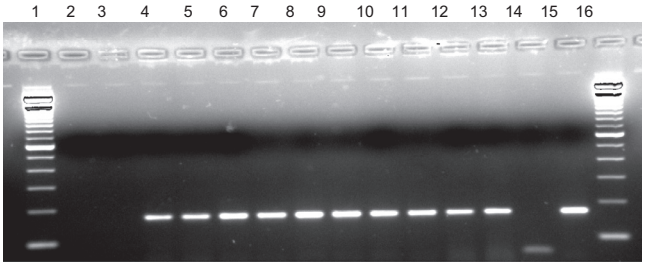

Fig. 4 Example of agarose gel electrophoresis of DNA extracted from processed meat products and examined by PCR analysis using primer P35s-f2/Petu-r1. Lanes 1 and 16: DNA ladder 100 bp, lane 2: PCR control (by deionized water), lane 3: PCR products from non-genetically modified soybean DNA negative control (0% genetically modified Roundup Ready soybean, lanes 4–14: DNA extracted from some processed meat samples, lane 15: PCR control with DNA positive control from genetically modified 5% Roundup Ready™ soybean from Fluka (Sigma–Aldrich).

soybean of more than 1%; 23 samples contained Roundup Ready soybean of less than 0.1% (Fig. 5). Similar results were obtained by Taski-Ajdukovic et al. (2009), Brod and Arisi (2008), Dinon et al. (2010), and Abdel-Mawgood et al., 2010) in Serbian, Brazilian, and Saudi Arabia food market. The obtained results indicated that 62.2% of the collected samples were positive for RR soybean. The product labels did not indicate the presence of GMO ingredients. It requires that GMOs are traceable throughout the chain from producers to table and provide consumers with information by labeling all food consisting of, containing or produced from GMO (Forte et al., 2004; Regulation, 1998, 2003a,b). However, there are many challenges ahead for governments, especially in the areas of safety testing, regulation, international policy, and food labeling. From the obtained results, it could be concluded

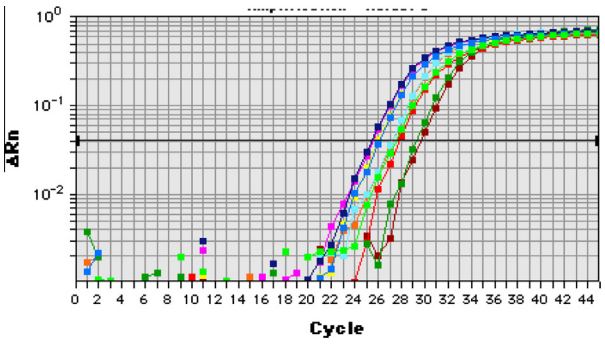

Fig. 5 Example of an amplification plot for analysis of DNA from meat samples using the TaqMan genetically modified organism (GMO) 35S soy detection kit (Applied Biosystems) by real-time PCR.

that the DNA extraction method used in this study allowed the extraction of amplifiable soybean DNA from meat samples. For all of the samples that tested positive by PCR by lectin gene, the results were then confirmed using the PCR method using GMO Screen kit and with primers P35s-f2/petu-r1 specific primer for RR soybean. The obtained results demonstrated the presence of Roundup Ready soybean in the commercial processed meat products in Kingdom of Saudi Arabia. Moreover, some of the samples contain RR soybean at more than 1% and need to labeling. The labeling of Saudi Arabian GMO requirement is set to be 1% maximum threshold limit for defining a GM foodstuff. If a product contains one or more GM ingredients, a triangle should be drawn and in it the text should read "Contains Genetically Modified Product(s)". The Saudi Arabian Ministry of Agriculture (MOA) banned imports of GM seeds in January 2004, and thus, no GM crop is grown in the country. Both the MOA and the (MOCI), respectively, allow imports of GM grain and plant/vegetable based processed foodstuffs as long as they are labeled (Mousa and Giles, 2005).

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