



PCR detection of genetically modified maize and soy in mildly and highly processed foods

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ABSTRACT

Regulations for both the labeling and the traceability of GMOs in food and feed chains are coming into force worldwide. Turkey is one of the countries enforcing labeling of food products containing GMOs. For this purpose, reliable and accurate detection methods are a necessity. PCR-based methods are the most common and reliable methods developed thus far. The aim of our study was to evaluate the present situation of the food products that Turkish consumers eat. The effect of regulatory monitoring on the ratio of GMO-positive samples was also evaluated by screening products both before and after the regulation came into force. The screening of the products was based on detection of the CaMV 35S promoter and the *nos* terminator by PCR. According to our results, 25% of the samples tested were positive for GMOs. However, the ratio of positive samples decreased after the regulations came into force. These results demonstrate for the first time the screening for GMOs in foods sold in Turkey with a large time scale and wide product scope.

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1. Introduction

Genetically modified organisms (GMOs) are either microorganisms, plant or animal, in which their genetic compositions have been altered by a gene modification technique to gain new characteristics, such as herbicide tolerance, insect resistance, an enhanced shelf life or modified nutritional composition (Greiner & Konietzny, 2008; Lin, Chiueh, & Shih, 2000).

Crop plants are most widely used for this purpose and are more commercialized than other organisms. Soybean, maize, cotton and canola are the most cultivated genetically modified (GM) plants, occupying 47%, 32%, 15% and 5% of the global GM crop area, respectively (James, 2011). Soy and maize have a special importance because they represent the staple constituents of many foods (Forte et al., 2005; Meyer, Chardonens, Hübner, & Lüthy, 1996; Taski-Ajdukovic et al., 2009).

The first GM plant approved for consumption was produced in Canada in 1996. This plant was Roundup Ready (RUR) soy, developed by the Monsanto Company (Ujhelyi et al., 2008). The planting of GM crops worldwide has constantly increased (94-fold) since that first crop and has reached 160 million hectares in global area according to the final reports of 2011. The major producers of GM

crops are the United States, Brazil, Argentina, India, Canada and China (James, 2011).

Despite announced advances, GMOs have received a very strong consumer reaction since they were first commercialized (Baker & Burnham, 2002; Sieradzki, Walczak, & Kwiatek, 2006; Ujhelyi et al., 2008). Thus, numerous countries set up official regulations for the labeling of GMOs and GMO-derivative foods (Anonymous, 2003; Matsuoka et al., 2000; Miraglia et al., 2004; Vijayakumar, Martin, Gowda, & Prakash, 2009). Finally, regulations on the traceability and labeling of GM foods have come into force in Turkey. The regulations stipulate the labeling of foods that contain GM material above a threshold level (Anonymous, 2009, 2010a, 2010b).

To comply with the legislation requirements, it becomes necessary to identify whether a product is GM or contains any GM ingredient. For this purpose, the development of practical and reliable methods for the detection of these products is required worldwide (Birch, Archard, Parkes, & McDowell, 2001; Forte et al., 2005; Greiner & Konietzny, 2008). Most of the methods used so far include serological techniques, such as the enzyme-linked immunosorbent assay (ELISA), which is based on detecting the novel protein in raw transgenic products (Ahmed, 2002; Lin, Chiang, & Shih, 2001; Lipp, Anklam, & Stave, 2000; Miraglia et al., 2004), and PCR, which is based on detecting the novel DNA in both raw and processed foods (Greiner & Konietzny, 2008; Taski-Ajdukovic et al., 2009). PCR methods can be used for both qualitative and quantitative purposes (Lin et al., 2000). The qualitative methods are divided into two categories, depending on their

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specificity. Screening methods are less specific and detect common DNA elements, such as promoters and terminators, present in many different GMOs. Because most GM products contain either the cauliflower mosaic virus (CaMV) 35S promoter or the nopaline synthase (*nos*) terminator, or both, most of the PCR screening methods are based on detecting these sequences in the product (Ahmed, 2002; Forte et al., 2005; Kuiper, 1999; Miraglia et al., 2004). Event-specific PCR methods are more specific and they are used to identify the GMO event to allow for discrimination between approved and non approved traits. However, the screening method evaluation is generally the starting point of GMO detection, before either event identification or GMO quantification (Gryson, Dewettinck, & Messens, 2007).

The high production of GMOs worldwide is an important reason for concern about the current status of the foods we consume, which is fairly uncertain. The objective of this study was to determine the ratio of GM-containing maize and soy food products obtained from local retail shops in Turkey using a conventional PCR method. Maize and soy were chosen because they are major transgenic crops grown worldwide and are important ingredients in several foods.

2. Materials and methods

2.1. Samples and reference materials

One hundred soy- and maize-containing food samples, including a variety of processing steps (mild treated or highly processed), were purchased from random local retailers in Turkey. These samples consisted of the following types of items: maize flour, canned maize, soy flour, soy beans, bread, bread mix, infant formula, cake, biscuits, dehydrated soup, meat products (soy containing) and other goods containing soy (soy sprout, soy milk and soy sauce) and maize (maize kernels and maize starch).

Certified reference materials (CRMs) consisting of soybean powder (0, 0.1, 0.5 and 1% of RUR soybean powder) and maize powder (0, 0.1, 0.5, 1 and 5% of Mon 810, 5% of Bt 176 and 5% of Bt 11 maize powder), produced by the Institute for Reference Materials and Measurements (IRMM, Geel, Belgium), were used as the negative and positive controls in the study.

2.2. DNA extraction and purification

For DNA isolation from raw and mildly processed foods and CRMs, the Promega Wizard™ DNA isolation kit (Promega, Madison, USA) was used according to the manufacturer's instructions. Two hundred to three hundred milligrams of food material taken from a previously homogenized sample was mixed with 860 µl of extraction buffer (10 mM Tris–OH, 150 mM NaCl, 2 mM EDTA and 1% w/v sodium dodecyl sulfate), 100 µl of guanidine hydrochloride (5 M) and 40 µl of proteinase K (20 mg/ml), then incubated at 65 °C overnight. The samples were then centrifuged at 13,500 g for 10 min. After centrifugation, 500 µl of the supernatant was mixed with 1 ml of Wizard™ resin (Promega, Madison, USA) and pushed through a Wizard™ minicolumn (Promega, Madison, USA). The column was further washed with 2 ml of isopropanol. Following centrifugation of the column at 12,000 g for 5 min, the DNA was eluted with 50 µl of pre-warmed (65 °C) elution buffer (10 mM Tris–OH). The columns were incubated at room temperature for 1 min and centrifuged at 10,000 g for 2 min. The collected DNA was stored at –20 °C until used.

The DNA of the highly processed samples was extracted with the cetyltrimethyl ammonium bromide (CTAB) method, with some modifications, and was purified with the Promega Wizard™ DNA isolation kit (Anonymous, 2000). For this purpose, 2 g of

a homogenized sample was mixed with 5 ml of CTAB extraction buffer (0.1 M Tris–HCl, 20 g/l CTAB, 1.4 M NaCl and 20 mM Na₂EDTA, pH 8.0) and incubated for 1 h at 60 °C under constant shaking. Ten microliters of proteinase K (20 mg/ml) was added to 1 ml of this suspension and incubated at 60 °C for another hour. Following centrifugation at 15,000 rpm for 5 min, the supernatant was mixed with 600 µl of chloroform and again centrifuged at 15,000 rpm for 10 min. Then, 500 µl of isopropanol was added to 625 µl of the supernatant and incubated for at least 30 min at room temperature. The pellet was collected from the solution by centrifugation at 15,000 rpm for 15 min and washed with 75% ethanol. The pellet was dissolved in 200 µl of TE buffer (10 mM Tris–Cl and 1 mM EDTA, pH 8.3) and purified as described above.

DNA quantification was achieved by measuring the UV absorption at 260 nm using a T80 UV/VIS spectrometer (PG Ins. Ltd., UK).

2.3. PCR primers

Because nearly all of the GM maize and soy varieties contain the CaMV 35S promoter and the *nos* terminator, appropriate primers for amplifying those specific DNA sequences were used for the GMO screening of the products (Hemmer, 1997; Lipp, Brodmann, Pietsch, Pauwels, & Anklam, 1999; Lipp et al., 2001). The amplicability of extracted DNA was verified using plant-specific primers targeting the lectin gene for soy and the zein gene for maize. Primers for amplifying specific sequences present in RR soybean (GTS-40-3-4) and Bt 176 Maximizer maize, Mon 810 Yield Gard maize, T25 Liberty Link maize, Bt11 maize are used for specific GMO detection of samples which were positive in screening analyses. The names, sequences and origins of primers used in the study are summarized in Table 1.

2.4. PCR conditions

All PCR reactions were performed with a CG Palm-Cycler (CG 1-96 Genetix Biotech, Australia & Asia). Amplification reactions contained 5 µl of genomic DNA and 20 µl of the appropriate PCR reaction mixture. PCR reaction mixture varied: for CaMV 35S and *nos* amplifications, it consisted of 1× buffer (Fermentas), 1.5 mM MgCl₂ (Fermentas), 0.6 µM of primers for 35S or *nos*, 0.16 mM of each dNTP (Fermentas) and 0.8 U of Maxima™ Hot Start *Taq* polymerase (Fermentas); for soy-specific lectin amplifications, it consisted of 1× buffer (Fermentas), 2 mM MgCl₂ (Fermentas), 0.5 µM of primers for lectin, 0.2 mM of each dNTP (Fermentas) and 2 U of Maxima™ Hot Start *Taq* polymerase (Fermentas); for maize-specific zein amplifications, it consisted of 1× buffer (Fermentas), 2 mM MgCl₂ (Fermentas), 0.3 µM of primers for zein, 0.2 mM of each dNTP (Fermentas) and 1.25 U of Maxima™ Hot Start *Taq* polymerase (Fermentas); for event specific detection of RUR soy, it consisted of 1× buffer (Fermentas), 1.5 mM MgCl₂ (Fermentas), 0.2 µM of each primer for RUR soy amplifications, 0.8 mM of each dNTP (Fermentas) and 0.5 U of Maxima™ Hot Start *Taq* polymerase (Fermentas), for event-specific detection of maize (duplex PCR with Bt 176 Maximizer maize and Mon 810 Yield Gard maize or T25 Liberty Link maize and Bt11 maize), it consisted of 1× buffer (Fermentas), 1.5 mM MgCl₂ (Fermentas), 0.5 µM of each primer of Bt11 maize and T25 liberty Link maize or Mon 810 maize and Bt 176 Maximizer maize, 0.2 mM of each dNTP (Fermentas) and 1.25 U of Maxima™ Hot Start *Taq* polymerase (Fermentas);

The amplification profiles used for these mixtures were as follows:

- For CaMV 35 S and *nos*: denaturation for 10 min at 95 °C; amplification for 25 s at 95 °C, for 30 s at 62 °C and for 45 s at 72 °C; number of cycles 50; final extension for 7 min at 72 °C.

Table 1
The summary of the primer pairs used in the study.

| Target gene | Primer | Sequences (5'...3') | Length of PCR product | Reference |
|-------------|--|--|-----------------------|---------------------------|
| CaMV 35S | p35S-cf3 P35S-cf4 | CCACgTCITCAAAGCAAgTgg TCCTCTCAAATgAAATgAACTTCC | 123 bp | Lip et al. 2001 |
| <i>nos</i> | HA- <i>nos</i> -118f HA- <i>nos</i> -118r | gCATgACgTTATTTATgAgATggg gACACCgCgCgATAATTTATCC | 118 bp | Lip et al. 2001 |
| Soya lectin | Lectin1 Lectin6 | gACgCTATTgTgACCTCCTC gAAAgTgTCAAAGCTAACAgCgACg | 318 bp | Tengel et al., 2001 |
| Maize zein | Zein-68f Zein-68r | TgTTAggCgTCATCATCTgTgg TgCAGCAACTgTTggCCTTAC | 68 bp | Arun-Ozgen & Garrett 2009 |
| RUR soya | 35s-f2: petu-r1 | TgATgTgATATCTCCACTgACg TgTATCCCTTgAgCCATgTTgT | 172 bp | Anonymous, 2005 |
| Bt11 | Bt 11 Cry 1A | CCATTTTTCAGCTAGGAAGTTC TCgTTgATgTTKgggTTgTTgTCC | 110 bp | Matsuoka et al., 2001 |
| Bt 176 | E176 1 Cry 1A | gTAGCAGACACCCCTCTCCACA TCgTTgATgTTKgggTTgTTgTCC | 343 bp | Matsuoka et al., 2001 |
| MON 810 | MON 810 Cry 1A | gAgTTTCCTTTTgTTgCTCTC TCgTTgATgTTKgggTTgTTgTCC | 199 bp | Matsuoka et al., 2001 |
| T25 | T25 1 sense T25 1 Anti sense | gCCAgtTAggCCAgTTACCCA TgAgCgAAACCCTATAgAACCTC | 149 bp | Matsuoka et al., 2001 |

- For lectin: denaturation for 3 min at 94 °C; amplification for 45 s at 94 °C, for 45 s at 60 °C, for 25 s at 72 °C; number of cycles 50; final extension for 7 min at 72 °C.
- For zein: denaturation for 10 min at 96 °C; amplification for 30 s at 94 °C, for 30 s at 60 °C, for 25 s at 72 °C; number of cycles 40; final extension for 7 min at 72 °C.
- For duplex PCRs: denaturation for 10 min at 95 °C; amplification for 30 s at 95 °C, for 60 s at 63 °C, for 60 s at 72 °C; number of cycles 10 and amplification for 30 s at 95 °C, for 60 s at 60 °C, for 60 s at 72 °C; number of cycles 30; final extension for 7 min at 72 °C.
- For RUR soy: denaturation for 10 min at 95 °C; amplification for 30 s at 95 °C, for 30 s at 60 °C, for 25 s at 72 °C; number of cycles 40; final extension for 3 min at 72 °C.

2.5. Agarose gel electrophoresis

PCR products were electrophoresed through a 2% agarose gel containing ethidium bromide. As a size reference, a 50 bp DNA ladder (Fermentas) was used. Visualization of the gels was performed with a UV transilluminator, and the gels were captured with the Dolphin-DOC system and Dolphin 1D Gel analyzing software (Wealtec, Nevada, USA).

3. Results and discussion

The presence of GMOs in processed foodstuffs sold in retail shops in Turkey is currently screened by the PCR method. One hundred processed food products, both local and imported, were analyzed during this project. The production dates of the samples were between October 2008 and June 2011. Soy-containing products were grouped into soy milk, soy sprouts, soy flour, bread, biscuits, meat products, infant formula, meat patties, bread mix, cake, soy beans, soy meat, and dehydrated soup; maize-containing products were grouped into maize flour, canned maize, maize starch and maize kernels (Table 2).

3.1. Quality control

Appropriate quality controls were run during the tests to avoid both false negative and false positive results (Anonymous, 2011; Hübner et al., 1999).

To eliminate false negative results, the CaMV 35S and *nos* negative samples were further analyzed for soy-specific lectin and maize-specific zein sequences which are present in both GM and non GM soya and maize, respectively. Additionally, lectin and zein negative samples were spiked with DNA extracts of GM soy and maize CRMs and analyzed to eliminate any false negative results related to PCR inhibitors (Cardarelli, Branquinho, Ferreira, Da Cruz, & Gemal, 2005; Greiner & Konietzny, 2008; Taski-Ajdukovic et al., 2009; Wurz, Bluth, Zeltz, Pfeifer, & Willmund, 1999).

For elimination of any false positive results related to contamination during DNA sampling and extraction, sterile MILLI Q water was processed in parallel to the samples at each step of the extraction. A false positive result due to contamination during PCR was eliminated by running a no template control (sterile MILLI Q water) in each PCR (Hübner et al., 1999).

The sensitivity of the test method was determined by testing 0.1, 0.5 and 1% soy and maize CRMs in parallel with the samples in each PCR. Positive detection of both 0.1% soy and maize CRMs proved that the detection limit of the method is not above 0.1% (Figs. 1 and 2). However, PCR screening of the *nos* terminator sequence from the 0.1% soy CRM was possible from many but not all of the PCR trials; this finding is in agreement with previous results that the sensitivity of *nos* screening is 0.5% (Cardarelli et al., 2005; Lipp, Brodmann, et al., 1999).

Table 2
General summary of the results.

| Sample type | Number of samples | CaMV 35S positive | <i>nos</i> positive | Negative | Lectin or Zein negative |
|---------------------------------|-------------------|-------------------|---------------------|-----------|-------------------------|
| Meat products (soya containing) | 18 | 2 | 2 | 16 | |
| Dehydrated soup | 5 | 4 | 2 | 1 | |
| Bread & Bread mix | 13 | 2 | 0 | 7 | 4 |
| Canned maize | 13 | 1 | 0 | 10 | 2 |
| Maize flour | 28 | 13 | 5 | 15 | |
| Soya bean & flour | 7 | 3 | 1 | 4 | |
| Infant formula | 5 | | | 1 | 4 |
| Cake & biscuit | 4 | | | 3 | 1 |
| Other soya containing products | 5 | | | 4 | 1 |
| Other maize containing products | 2 | | | 2 | |
| Total | 100 | 25 | 10 | 63 | 12 |

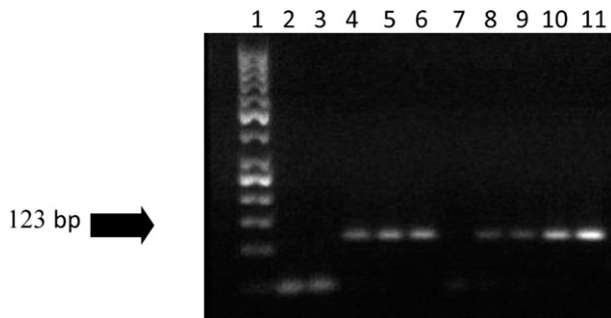


Fig. 1. CaMV 35 S PCR: Lane-1: 50 bp DNA ladder, Lane 2: PCR milli q water, Lanes 3–6: CRM soy 0%, 0.1%, 0.5%, 1% and Lane 7–11: CRM maize 0%, 0.1%, 0.5%, 1%, 5%.

3.2. DNA extraction and purification

Extracting a sufficient amount and quality of DNA is the first and most important step in reliable GMO screening (Ahmed, 2002; Tengler, Schüßler, Setzke, Balles, & Sprenger-Haußles, 2001). In our study, an appropriate quality and quantity of DNA could be extracted from the highly processed and raw/mildly processed samples using the CTAB and Promega Wizard™ methods, respectively. The initial sample size used for extraction was approximately 200 mg for raw or mildly processed materials and 2 g for highly processed materials. Other researchers have found that increasing the sample weight allows for extraction of a sufficient amount of DNA (Vijayakumar et al., 2009). The absorbance readings of the DNA extracts at 260 nm ranged from 0.03 to 0.69, and the DNA amounts calculated according to these readings ranged from 37 ng/μl to 863 ng/μl. Similarly, many other researchers have stated the possibility of extracting a sufficient amount of DNA even from processed foods by using these extraction procedures (Cardarelli et al., 2005; Greiner & Konietzny, 2008; Lipp, Anklam, Brodmann, Pietsch, & Pauwels, 1999). Successful species-specific PCR testing (zein for maize and lectin for soy) was achieved with sample extracts giving low absorbance values (absorbances between 0.03 and 0.04 at 260 nm), which confirmed that these extracts still contain a sufficient amount of amplifiable DNA.

DNA extraction was not possible from samples of 2 maize starches and 1 soy sauce with the extraction methods used, despite repeated attempts (data not shown). Similarly, Kuiper (1999) showed the difficulty of extracting DNA from starch derivatives. Additionally, Ujhelyi et al. (2008) and Greiner, Konietzny, and Villavicencio (2005) reported that they could not isolate DNA from soy sauce. In fact, several other researchers mentioned the difficulty of extracting a sufficient amount of amplifiable DNA from

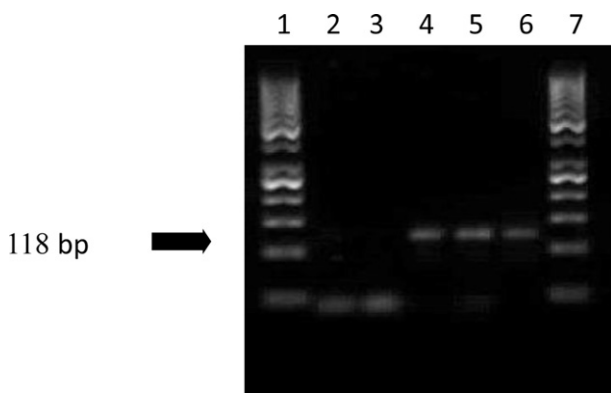


Fig. 2. Nos PCR: Lane-1: 50 bp DNA ladder, Lane 2: PCR milli q water, Lanes 3–6: CRM soy 0%, 0.1%, 0.5%, 1% Lane-7: 50 bp DNA ladder.

processed foodstuffs because of the effect that processing techniques, such as heat, enzymatic activity and low pH, have on the quantity and integrity of DNA (Lin et al., 2000; Lipp et al., 2001; Peano, Samson, Palmieri, Gulli, & Marmiroli, 2004; Tengler et al., 2001; Vijayakumar et al., 2009; Wurzel et al., 1999). However, as opposed to the results of Cardarelli et al. (2005), who stated that they could extract a sufficient amount and quality DNA from only 1 of 4 canned samples (maize, sausages and soup), we could successfully extract DNA from 11 of 13 total canned maize samples by means of the CTAB method combined with the Promega Wizard™ resin purification method. Other researchers also indicated that combining these two methods improves DNA yields (Kuiper, 1999).

3.3. Screening and specific detection of GMOs

Of all 100 products screened, 25 (25%) were determined to be positive for either or both of the novel sequences which indicates the presence of GMOs. The dispersion of these positive products within soy and maize were as follows: 14 of the 43 total (32.6%) tested maize samples, and 11 of the 57 total (19.3%) tested soy samples (Fig. 3).

Our results of soy-containing products were similar to the results of Cardarelli et al. (2005), who found that 20% of their samples were positive for RUR soy. Similarly, the results of Taski-Ajdukovic et al. (2009) proved that 12 of the 50 (24%) processed meat products they screened contained GM soy. The results of Ujhelyi et al. (2008) showed that 38% of the soy-containing foods they tested were GM positive, while 6% of them were above the threshold level introduced in EU legislation. However, another study performed in Poland showed that 44 of 45 (92.6%) soy-containing feed and feed-supplement samples were genetically modified, although most of them were below the threshold level (Sieradzki et al., 2006).

Our results of maize-containing products were close to those of Gürakan, Aydın, and Yılmaz (2011), who also tested for CaMV 35S in maize products sold in Turkey and determined that 11 of the total 31 samples (35%) they tested were positive. Our results were also consistent with the results of our previous study, performed with maize samples collected in 2001. In that study, a limited number of maize products were screened with the same strategy and 5 (35.7%) of all 14 samples were determined to be GM positive (Arun & Garrett, 2009). However, the results of all of these studies performed on maize products sold in Turkey were significantly higher than the results of Greiner & Konietzny (2008), who reported that the ratio of GM-positive maize products varied between 6% and 8%, depending on the year. In contrast, the results of Di Pinto et al. (2008) were significantly higher, with 9 of 10 ready-to-cook breaded food samples testing positive for GM. The ratio of positive soy-containing products seems to be lower than that of the maize products, which was a different result than was expected. The distribution of the positive samples among the product types is given in Table 2.

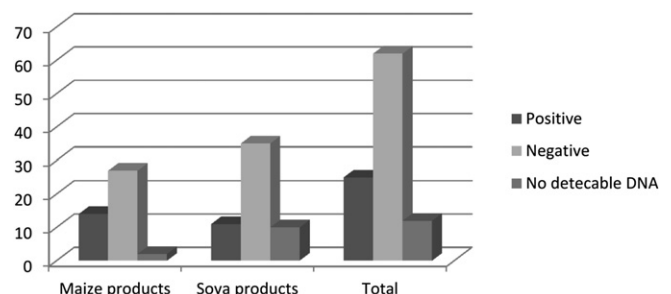


Fig. 3. Distribution of positive, negative and no detectable DNA samples.

Amplification of the maize-specific zein sequence in 27 negative maize samples and amplification of the soy-specific lectin sequence in 36 negative soy samples confirmed that these samples were true negatives. Other researchers also reported that successful detection of lectin in processed products by means of conventional PCR is possible (Brod & Arisi, 2007; Cardarelli et al., 2005; Taski-Ajdukovic et al., 2009). However, 12 of the negative samples were negative for both lectin and zein, which indicated that these products either contain very little detectable maize or soy DNA or present DNA was severely fragmented, possibly due to processing. The absorbance values of the DNA extracts of these samples ranged from 0.06 to 0.69, which indicated that it was not related to low DNA concentration but was instead possibly due to breaks in DNA strands. Peano et al. (2004) also proved that chemical and physical treatments, such as heat, can cause fragmentation of high molecular weight DNA strands and thus reduce the average fragment size. Likewise, Gryson et al. (2007) showed that heat processing reduces the overall DNA fragment length without reducing the DNA concentration. Similarly, Lipp et al. (2001) showed that DNA degradation might result in a significant decrease in the amount of DNA fragments that are sufficiently long to allow for the detection of GMOs in foods. In our study, 5 of the lectin-negative soy-containing products used soy lecithin as the soy source; the difficulty of extracting a sufficient amount of DNA from lecithin has been reported by other researchers as well (Lockley & Bardsley, 2000). These species-specific PCR-negative food products were also analyzed after spiking with a known DNA solution, to eliminate any false negative results related to PCR inhibitors; none of the samples seem to contain any PCR inhibitors.

The above mentioned 11 CaMV 35S and/or the *nos* positive soy-containing samples were analyzed for RUR soy and all of them gave positive amplification signal (Fig. 4). The other 14 positive maize-containing samples were screened for the presence of specific GM maize events. For this purpose duplex PCR detections of specific sequences of either Mon 810 Yield Gard maize and Bt 176 Maximizer maize or T25 Liberty Link maize and Bt11 maize were performed (Figs. 5 and 6). Of those 14 positive samples, four samples were identified as T25 Liberty Link maize and one sample as Bt 11 maize while three samples were positive for both Bt11 maize and T25 Liberty Link maize. Similar with our results, Greiner et al. (2005) also informed that in four of the 17 GM DNA positive

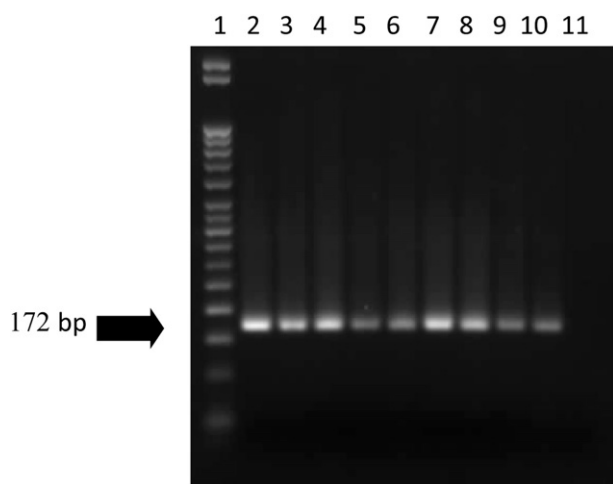


Fig. 4. Roundup ready soy PCR: Lane-1: 50 bp DNA ladder, Lane 2–4: % 1 RUR soy CRM, Lanes 5–6: RUR soy positive sample (dehydrated soup) Lane 7–8: RUR soya positive sample (meat product), Lane 9–10: RUR soya positive sample (soya flour), Lane 11: PCR milli q water.

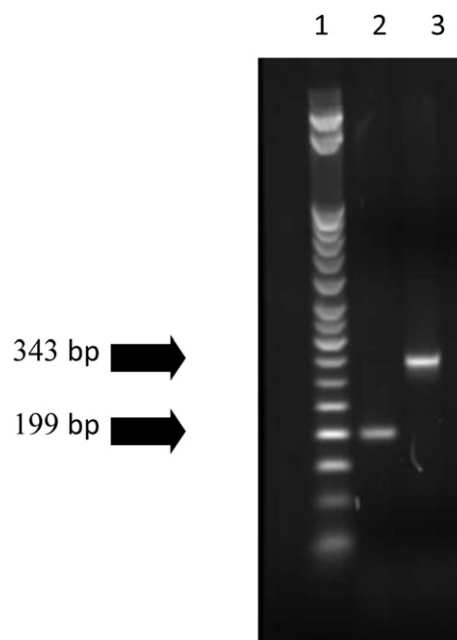


Fig. 5. Mon 810 Yield Gard and Bt 176 Maximizer maize PCR: Lane-1: 50 bp DNA ladder, Lane 2: Mon 810 Yield Gard maize CRM, Lane 3: Bt 176 Maximizer maize.

maize products they screened, two different GM events were found, while in the rest of the samples only one GM event was detected. In another study performed in Turkey, eight of the 11 CaMV 35S positive maize samples were also positive for T 25 and Bt 11 maize specific sequence. However because other events are not screened in that study the rest of the positive maize samples could not be identified (Gürakan et al., 2011). The other five CaMV 35S and/or *nos* positive maize samples in our study did not give any positive amplification signal for the events we screened.

The results of our study showed that; out of 25 positive soy and maize products, 15 were negative for the *nos* terminator while

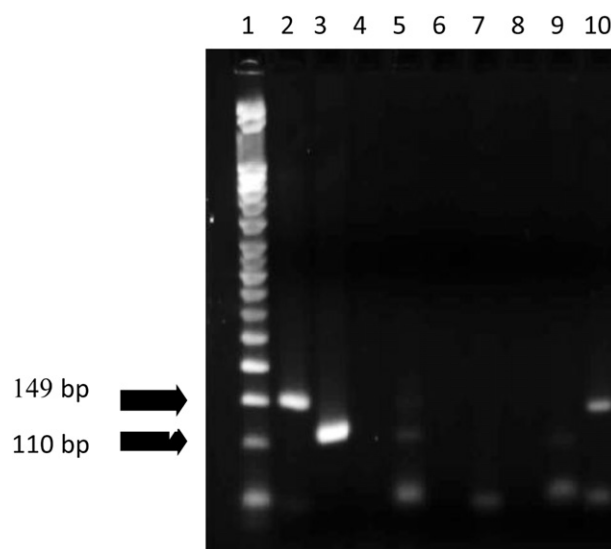


Fig. 6. T25 liberty Link maize and Bt11 maize PCR: Lane-1: 50 bp DNA ladder, Lane 2: T25 Liberty link positive sample (maize flour), Lane 3: Bt11 maize CRM, Lane 4: negative sample (maize flour), Lane 5: Bt11 and Liberty link positive sample (maize flour), Lane 6–8: negative samples (maize flour), Lane 9: Bt 11 positive sample (maize flour), Lane 10: T25 positive sample (maize flour).

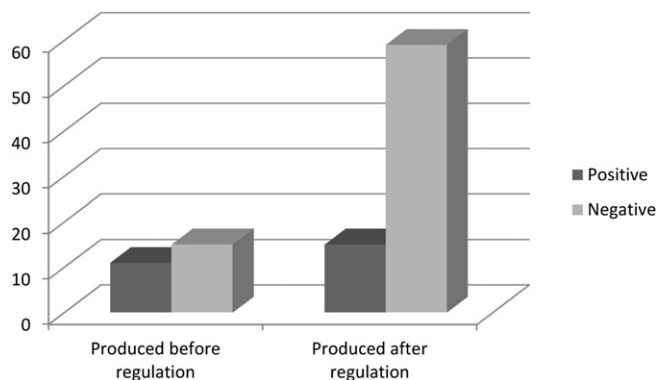


Fig. 7. The results of the samples in respect with the production date.

positive for the CaMV 35S promoter (six soy containing, nine maize containing products). As mentioned by many researchers the CaMV 35 S promoter derives from cauliflower mosaic virus and so plants from the *Criciferae* group (cauliflower, cabbage, broccoli oilseed rape, mustard and other criciferous plants) can be infected. Thus a CaMV 35 S positive result in processed foods containing these vegetables in the formulation will not confirm the presence of a GM material, but indicates a probability (Anonymous, 2005; Cardarelli et al., 2005; Ujhelyi et al., 2008). In our study, all six of these *nos* negative soy samples (bread, flour and dehydrated soup) were determined to be RUR soy and thus confirmed that these samples are true positive. Similarly, Ujhelyi et al. (2008), also informed that seven of the 80 food products they screened were determined to be RUR soy although they did not give any amplification signal with the *nos*. In the case of maize containing products eight of the 14 positive maize samples (flour, canned maize) were determined to be Bt 11 maize and/or T25 Liberty Link maize which confirmed the presence of GM material. Similarly, detection of both the CaMV 35 S and the *nos* sequences in another sample confirmed the GM maize presence. There are only five maize samples present that the *nos* could not be detected parallel to the CaMV 35S and the result could not be confirmed with another test. However, because these samples are maize flour and canned maize they are very unlikely to be contaminated cauliflower mosaic virus. According to many other researchers, further explanation for the presence of the CaMV 35 S alone is that the detection of the *nos* terminator is less sensitive than that of the 35 S promoter (Cardarelli et al., 2005; Lipp, Brodmann, et al., 1999; Ujhelyi et al., 2008). In our study, positive amplification of RUR soy in six *nos* negative samples supported this finding. In case of maize it could also be related to the lack of the *nos* terminator which is common for several maize events.

Evaluation of these results with respect to the first release of the GM food regulations shows that the ratio of GM-positive products dramatically dropped after the first announcement of the regulation in October 2009 in Turkey (Anonymous, 2009). Although this regulation was later canceled and a revised version introduced in 2010, after the Turkish biosafety law came into force on March 18, 2010, it generated important public and food-producer attention for the subject and induced significant monitoring of imports (Anonymous, 2010a, 2010b). In our study, 26 samples produced before the end of 2009 were screened and 11 of them (42.3%) were GM-positive, while only 15 (20.3%) of the 74 samples produced after 2009 were genetically modified (Fig. 7).

4. Conclusions

Because of consumer concern, genetically modified foods are subjected to hefty regulations in many countries. Since late 2009,

Turkey is one of the countries that enforce the labeling of both food and feed containing GMOs above a certain threshold level. After those regulations were introduced, a great demand occurred for methods of detection and quantification of GMOs in food and feed products.

Based on the results of this study, we can conclude that conventional PCR is a satisfactory method for the detection of GMOs, even in highly processed food products; the screening method can also be used successfully as a starting point for trait-specific identification and quantification methods. However, the extraction methods we used are still not sufficient for certain products, such as some starch, oil and lecithin samples. Additionally, CaMV 35S and *nos* screening methods can detect many but not all of the GM maize varieties.

Our results clearly demonstrated that many maize- and soy-containing food products sold commercially in Turkey contain GMOs. The significant reduction in the ratio of GM-positive food products after the regulation came into force proves the benefit of monitoring for GMOs at the point of import. However, no products containing GMOs had any such indication on their label, in spite of the present regulations stating that the consumer has the right to know what they are consuming. This reality proved that the control of GMOs within the country cannot be performed only by monitoring during importation, but it has to be monitored at the point of sale as well. The traceability of the GM materials in the food chain is also a very important tool for controlling its inclusion and ensuring correct labeling. For this purpose, the implementation of effective traceability systems and the strict monitoring of corresponding documentation by a competent authority are advised based on our results.

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