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DETECTION OF GENETICALLY MODIFIED MAIZE IN FOODS AND FEEDSTUFF BY PCR METHODS*

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ABSTRACT

In this study twenty six maize kernels and 64 processed maize food including maize flour, starch, corn flakes were collected from different markets located in Turkey, analyzed for genetic modification using the polymerase chain reaction. The samples were examined for the presence of genetic elements located in the majority of transgenic crops such as NOS terminator and CaMV 35S promoter using conventional PCR and then verified real time PCR, too. Additionally, Bt11, Bt176, Mon810, CBH351 and T25 events which have been enjoyed, limited data in Turkey, examined in the products using conventional PCR. Then, quantification of the all lines (except CBH351) was performed via real time PCR too. The results indicated that foreign genetic elements were found in analyzed 14 samples raw and processed materials and the quantity in one sample (maize flour) was greater than 0.9%, the limit at which labeling is required by Biosafety Law in Turkey.

Keywords: GM maize (corn), feedstuffs, PCR, real-time PCR

GIDA VE YEMLERDE GENETİK MODİFİYE MISIRIN PCR METOTLARI İLE TESPİTİ

ÖZ

Bu çalışmada Türkiye piyasasından toplanan ve mısır unu, mısır nişastası, mısır cipsi ve mısır gevreğini içeren 64 adet işlenmiş mısır ürünü ile 19 adet işlenmemiş yemlik tane mısırda PCR yöntemi ile genetik modifikasyon araştırılmıştır. Tüm numunelerde, birçok bitkide düzenleyici elementlerden olan 35S promotör ve NOS terminatör taranması önce konvensiyonel PCR ile yapılarak daha sonra real time PCR ile doğrulanmıştır. Ürünlerde, Türkiye' de kısıtlı veriye sahip olan Bt11, Bt176, Mon810, T25 ve CBH351 mısırların varlığı konvensiyonel PCR ile aranmış ve CBH351 hariç adı geçen tüm hatlarda real-time PCR kullanılarak miktar analizi yapılmıştır. Sonuçlar 14 örnekte yabancı genetik element bulunduğunu, bir örneğin (mısır unu) ise Türkiye "Biyogüvenlik Yasası" na göre %0.9 olan etiketleme değerinin üstünde olduğunu göstermektedir.

Anahtar kelimeler: GM mısır, yem, konvensiyonel PCR, real-time PCR

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INTRODUCTION

Maize is the most approved GMO crop (at 53 events) (CERA, 2018), followed by cotton (23 events) (James, 2010), canola (15 events), and soybean (11 events) (GM Crop Database, 2010). The United States (U.S.), followed by Argentina, Brazil, Canada, India, and China, are the principal adopters of GM crops (James, 2010). Consumers are concerned about genetically modified (GM) foods due to speculation about potential for danger to health and the environment. The monitoring of GM foods is necessary for public awareness and labeling of GM foods is a significant part of monitoring (IIA.MSU, 2007).

In recent years, many countries have adopted labeling policies for GM food and feed. The first labeling policies were introduced by the European Union (EU) in 1997 (EC., 1997), with marketing authorization for GM organisms controlled by Novel Food Regulation (EC) No. 2003/29 (EC., 2003, OCA, 2005). Since then many other countries have adopted different types of labeling policies for GM foods. The threshold level for labeling of GM ingredients ranges from 0.9% to 5% relative GM content (Guillaume et al., 2007). The U.S., Canada, Argentina and Iceland are among those countries where the authorities do not require GM foods to be labeled at all (Guillaume et al., 2007; Sanhoty et. al., 2002; Food Standart Agency 2008).

According to Biosafety Law (No.5977, Biosafety Law, 2010), feeds, foods and additives with more than %0.9 relative amount of genetically modified organisms must be labeled as GMO products and also GM crops can not be planted in Turkey. However there are only ten GM soybean events and 29 maize events are approved to import for feed, nowadays. The other GM events are under consulted to import Turkey (TBDDM, 2018). Ironically, in data from 2003, 81% of 1.8 million tons of maize imported to Turkey came from the U.S. and Argentina (TurkStat, 2010; Baran & Yilmaz, 2008), both among the biggest GM maize and soybean producers (James, 2010). Maize and soybean imported to Turkey have been suspected of being of GM origin. Because of many diverse issues such as potential long-term health problems, environmental risks, socio-economic concerns, GM products are an issue of some strong debate in Turkey (Baran & Yilmaz, 2008). The aim of this study was to determine using conventional PCR and real-time PCR techniques whether there is any known genetic modification in both raw and processed maize-derived food and feed samples imported into Turkey.

MATERIALS AND METHODS

Samples and reference material

Sixty-four samples of commercially available processed maize-based foods, including maize flour, starch, and corn flakes, were randomly collected from different markets located in Turkey. In addition, 7 maize kernels were provided by the Variety Registration and Feed Certification Centre, and 19 feedstuffs as maize kernels were collected by the Turkish Feed Manufacturers' Association from various parts of Turkey. Certified reference materials (CRMs), produced by ERM (European Reference Material), IRRM-Institute Reference Material and Measurements, in Geel, Belgium were used for negative and positive controls in the range of 0-5 %GMO: non-GMO; Bt176 ERM®-BF411a; Bt11 ERM®-BF412a; Mon810 ERM®-BF413a. Additionally, positive controls for T25 and CBH351 were provided from their own commercial kits.

Extraction of genomic DNA

Three different DNA isolation methods were carried out for DNA extraction using CTAB (Roger & Bendich, 1985), the High Pure DNA Isolation Kit (Roche, USA), and the Prep Plant X DNA Isolation Kit (Sure food, Germany). Maize kernels and other solid samples were ground in an electric mill as part of the sample extraction (GM200, Retsch). Duplicate extractions were done for each sample at least. The quality of nucleic acid extraction was assessed by agarose gel electrophoresis (Gene Genius, Syngene). The concentration of DNA in solution was measured spectrophotometrically with a Lambda EZ-201 system (Perkin-Elmer, USA).

Primer pairs and PCR conditions

The primer pairs to detect CaMV 35S promoter region (353 and 35S6, Berben, 2001) NOS terminator region (HA-nos-118-f and HA-nos-

118-r) and the conventional PCR conditions used in this study have been already described (Lipp et al., 2001). NOS terminator and CaMV 35S promoter were determined by real-time PCR using commercial kits (Lightcycler GMO Screening Kit, Roche) too.

In addition, plant gene was detected in all samples using GMO Screening kit to prove that DNA extraction was successfully carried out with screening kit. The zein gene was also identified to confirm the presence of maize derivatives in all samples. PCR conditions and the primer pairs specific for probing the zein gene have been described elsewhere **(**Querci et al., 2002). Commercial kits were purchased from Tepnel Biosystems (GMO Selection Module, U.K.) to detect Bt176, Bt11, CBH351, T25, Mon810 events.

GM variety testing for Bt176, Bt11, Mon810, T25 and CBH351 maize lines

GM variety testing was performed on those samples in which a positive, or trace GM signal was detected. This PCR tests identified specific genes used in GM maize.

Qualitative analysis of positive samples containing Bt176, Bt11, Mon810, T25 and CBH351 maize events were carried out with seperate multiplex PCR kits (Tepnel Biosystems, GMO Selection Module, and U.K). Two genes were detected by all test kits; one of them is a specific gene (e.g Bt176, Bt11, etc.) and the other is the zein gene as a positive control indication of maize in the samples.

Quantitative analyses of Bt176, Bt11, Mon810, T25 maize lines

Positive samples containing Bt176 maize were quantified with absolute and relative quantification methods using commercial kits from Congen Biotechnologie GmbH (Surefood GMO Bt176 Corn Kit-for absolute quantification) and Roche (Bt176 Quantification Kit-for relative quantification), respectively. Quantification of positive samples containing the other maize lines was performed with the absolute quantification method using commercial kits which were purchased from Congen Biotechnologie GmbH (Surefood GMO Bt111, Mon810, T25). For GMO DNA absolute quantitation in a sample, the Ct or Cp values (Cycle threshold or crossing point) are measured and converted to a corresponding copy number by comparison with a calibration curve generated with standard DNA.

All methods were performed according to manufacturer's instruction on Light Cycler 2.0 (Roche, USA).

Statistical analyses of the methods

Determination of GM percentages of samples (such as maize kernels, maize flours, etc.) was calculated by the average results of replicates. The samples were studied at least duplicate but some processed samples were done multiple. The paired *t* test method (Sheskin, 2000) was used to determine if there was or not significant difference between relative quantification and absolute quantification values for Bt176 ($p>0.05$).

As described above, both conventional and real-time PCR methods were used to determine 35S promoter and NOS terminator regions for the qualitative assessments. The Cohen's Kappa statistic was applied to the values which were present or absent to evaluate agreement between the methods. Additionally, the Kappa method was applied to the results which were obtained from the qualitative and quantitative test methods to determine agreement between the methods. The results of the Kappa method were evaluated by the kappa scale (Sim & Wright, 2005).

RESULTS AND DISCUSSION

Genomic DNA Extraction

No significant difference was detected between the results obtained by the commercial kits. The High Pure DNA isolation and Sure Food DNA isolation kits were superior to the use of the CTAB method DNA and were more rapidly completed. While Sure Food DNA isolation kit was used high processed samples (e.g. maize chips and maize cornflakes), The High Pure DNA isolation kit was used the others (e.g. maize granules, maize flours ect.). Other studies also showed that selection of DNA extraction method

was crucial because quantitative PCR results were affected by the DNA extraction method and purification methods used (Demeke & Ratmayaka, 2008; Minegishi et al., 2008; Papazova et al., 2008)

Sensitivity and spesifity

Collected samples had different processing steps varying from relatively mildly treated ground maize kernels to highly processed products, such as chips and cornflakes. The sensitivity and spesifity of the methods was summarized in Table 1. The sensitivity of the applied qualitative PCR method by conventional PCR was determined as 0.5% and 0.1% for the determination of 35S promoter and NOS terminator, respectively. On the other hand, the sensitivity of the applied qualitative PCR method by real-time PCR was 0.1% for both 35S promoter and NOS terminator (Table 1). CRM was available as a GM material.

Table 1. Method sensitivity

Methods	LOD of method	LOQ of method	Spesifity testing
[a]Zein Gene	-	-	+
[a]35S	[g]0.5	-	+
[a]NOS	[g]0.1	-	+
[b]35S	[g]0.1	-	+
[b]NOS	[g]0.1	-	+
[a]Bt176	[f]0.1	-	+
[b]Bt176	[f]0.1	[f]0.1	+
[d]Bt176	[g]0.07	[g]0.1	+
[a]Bt11	[f]0.1	-	+
[c]Bt11	[f]0.01	[f]0.1	+
[a]Mon810	[f]0.1	-	+
[c]Mon810	[f]0.01	[f]0.1	+
[a]T25	[f]0.1	-	+
[c]T25	[f]0.01	[f]0.1	+
[a]CBH351	[f]0.1	-	+

[a] Qualitative analysis by conventional PCR
[b]Qualitative analysis by real-time PCR
[c]Absolute quantification by real-time PCR
[d] Relative quantification by real-time PCR
f as the kit supplier (but they were verified too)
g it was determined in raw and semi-processed matrixes (e.g. maize granules and maize flour)
'+' method spesifity was determined by different matrix.
'-' it could not be calculated

Trace (TR) indicates presence of trace amounts of GM DNA, i.e. a very weak signal represents a small amount presenting in some of the PCR reactions. It should be noted that, although some of the signals for the samples marked as '+', they were quite weak. Positive signals were detected for each of the three PCR tests performed, confirming the presence of GM DNA in the samples. DNA was detected in trace positive samples, so the percentage is simply recorded as trace.

For the transgenic maize lines Bt11, CBH351, Mon810, Bt176 and T25, reference materials were provided with the detection kits and were used for the qualitative methods. The kit supplier indicated a detection limit of 0.1%. For the transgenic maize lines Bt176, LOD (Limit of Detection) and

LOQ (Limit of Quantification) were determined 0.07% and 0.1% by relative quantification, respectively.

Also the LOQ (for the absolute quantification) in the determination of quantitative levels of Bt176, Bt11, Mon810 and T25 was also sensitivity as being 0.1%. Additionally, the quantitation limits were set to 50 copies of target sequence, meaning that target sequences under 50 copies were showed as trace (TR).

Impact of processing

Sufficient DNA of appropriate quality could be isolated from collected samples using the described extraction procedures. The concentration of extracted DNA among the processed samples varied significantly. As a result of various treatments during food processing, the quantity and quality of extracted DNA is much lower in processed food samples than in raw samples. It has been reported that the heating (Pauli et al., 2000; Bergerova et al., 2008), high pressure and the other physical actions involved in processing cause degradation of high molecular weight DNA (Pauli et al., 2000; Badulescu et al., 2008).

The presence of maize in Turkey maize products

The presence of maize was checked with the maize zein specific primer pair Zein3/Zein4 using conventional PCR. Additionally, the zein gene was determined by real-time PCR too. An amplicon of the expected size in raw samples (maize kernels) and flour samples was observed using conventional PCR. The Zein Gene in other samples was not identified by conventional PCR except for two chips samples. In contrast, the Zein Gene was detected (not quantified) using real-time PCR in all samples during the quantitative analyses. No amplification was observed in an extraction negative control, or with a PCR control not containing target DNA. Results are compiled in Table 2.

Table 2. Zein Gene and GM screening test results by conventional and real-time PCR

Samples	Number of Samples	Plant Specific PCR (Positive sample)			GM Screening Test (Positive sample)			
		Zein[a]	*Zein*[a]	*Plant gene* [b]	*35S*[a]	*NOS*[a]	*35S* [b]	*NOS* [b]
Maize flour	16	16	16	16	1	1	5	4
Maize starch	16	-	16	16	-	-	-	1
Imported feed containing maize granules	5	5	5	5	-	-	1	1
Local feed containing maize granules	14	14	14	14	1	1	1	1
Corn flakes	16	-	16	16	-	-	-	3
Corn chips	16	2	16	16	-	-	3	3
Local food products containing maize granules	7	7	7	7	-	-	-	-
Total number of samples	90	44	90	90	2	2	10	13

[a]Conventional PCR
[b]Real-time PCR

The presence of 35S promoter and NOS terminator in Turkish food and feed samples

Most of the commercialized GM crops contain either a CaMV 35S promoter or a NOS terminator (Tozzini et al., 2000; Kok et al., 2000; BATS, 2003; Abdel-Mawgood et al., 2003). Commonly used screening assays for GM plants are based on detecting CaMV 35S promoter and/or NOS terminator sequences using either conventional or real-time PCR (Ahmed, 2002; Holst-Jensen, 2003). The suitability of the DNA solutions for PCR reactions was examined with a plant gene according to the manufacturer's instructions.

The results indicated that transgenic sequences were found in analyzed raw and processed materials. The CaMV 35S promoter and/or NOS terminator were seen in two samples (2.2%) by conventional PCR and in 14 samples (15.5 %) by real-time PCR (Table 2).

In another study were done by Gurakan et. all in Turkey (Gurakan et al., 2011). Out of 31 samples tested, 11 were CaMV 35S promoter positive. Of these 11 samples, 7 sample were both CaMV 35S promoter and NOS terminator positive. However they could not found any CaMV 35S promoter and NOS terminator positive sample in highly processed maize products such as; maize starch and corn flakes ect. In a study done in maize samples obtained in Poland, 61% of 87 samples were GM-positive using as reference genetic elements the CaMV 35S promoter and NOS terminator (Sieradzki et al., 2006). In another study, three products from 24 food samples were positive with 35S promoter in Egypt (Oraby et al., 2005). Similarly, in a study conducted in Saudi Arabia about detection of GM foods on the market 202 samples were investigated and according to the results, 9% of total samples and %6 of corn and corn products were GM positive (Abdel-Mawgood et al., 2010).

The kappa ($\varkappa$) value is a statistically determined indicator of the agreement between methods for the results from the same sample for both the application of conventional PCR and of real-time PCR in the determination of CaMV 35S promoter and NOS terminator genetic elements. Results are presented in Table 3. For flour samples, according to the NOS terminator results, there is moderate agreement ($\varkappa$ =0.600) between the two methods for this genetic element, but with the CaMV 35S promoter results shows only fair agreement ($\varkappa$=0.256). According to 35S promoter results, there is substantial agreement ($\varkappa$ =0.642) between the methods when analyzing maize kernels. Because of the inadequate number of positive samples seen with conventional PCR, this statistical analysis could not be applied to other product groups such as chips and cornflakes.

Table 3. Kappa test results to determine method agreements between conventional PCR and real-time PCR methods for 35S promoter and NOS terminator regions (*n= 16; maize samples, n= 16; mazie flour; n=19; maize kernels*) and kappa test results to determine method agreements between quantitative and qualitative results for Bt176, Bt11, Mon810, T25 lines (n= 14)

Samples	Kappa Value ($\varkappa$ [a])	Approx. Sig.
Maize Flour (NOS)	0.600	0.009
Maize Flour (35S)	0.256	0.126
Maize Kernels (35S)	0.642	0.003
Bt176-1	0.169	0.255
Bt176-2	0.169	0.255
Bt11	0.176	0.469
Mon810	0.553	0.036

$\varkappa$ [a] (≤0.10; no agreement, 0.11-0.20; slight agreement, 0.21-0.40; fair agreement, 0.41-0.60; moderate agreement, 0.61-0.80; substantial agreement, 0.81-1; almost perfect agreement)

The presence of maize lines Bt176, Bt11, Mon810, T25 and CBH351 in Turkish food and feed samples

Using specific kits (Tepnel Biosystems, GMO Selection Module) for conventional PCR in the detection of Bt176, Bt11, Mon810, T25 and CBH351, 14 in 90 samples obtained from different locations in Turkey were found to be GM-positive. The results of the qualitative

analysis for all GM-positive samples are given in Table 2. GM variety testing was performed on those samples in which a positive or trace GM signal was detected. This PCR tests identified specific genes used in GM maize crops.

The amplified sequence of 211 bp in length for Bt176 was observed in two maize flour samples (2.2 %); one of the samples had trace amounts of Bt176 maize as well. Amplicons of size 186 bp for Bt11 was seen in three maize samples, all of which had trace amount of Bt11 as well. For Mon810, an amplicon of size 203 bp was found in five samples, three of which were also found to have trace amounts of Mon810. The amplified sequences of 243 and 250 bp for T25 and CBH351, respectively, were not observed in any samples (but were observed in positive controls). Of the varieties listed in the categories in Table 4, two (Bt11, Mon810) are approved for food and feed use within the EU (James, 2010; Aguilera et al., 2008; Goerlich et al., 2008, CERA, 2018) and T25 is approved for feed use in the U.S (CERA, 2018).

Table 4. Screening test results by conventional PCR for GM maize variety

Tested raw materials and processed products	Number of samples	Bt176	Bt11	CBH351 (Star-LinkTM)	Mon810	T25
Maize flour	16	1^{D},1^{TR}	-	-	$1^{D)}$,1^{TR}	-
Maize starch	16	-	-	-	-	-
Imported feed containing maize granules	5	-	-		1^{TR}	
Corn flakes	16	-	1^{TR}	-	-	-
Corn chips	16		2^{TR}		1^{TR}	
Local feed containing maize granules	14	-	-	-	1^{D}	-
Local food products containing maize granules	7	-	-	-	-	-
Total number of samples	90	1^{D},1^{TR}	3^{TR}	-	2^{D},3^{TR}	-

D= GM material detected
TR= Trace amounts of GM material detected
(-) = No GM material detected

Gurakan et. al. (2011) 31 samples analyzed for Bt11 maize using ivs/pat region specific primer in Turkey and 8 of them showed an amplicon of 189 bp, confirming the presence of Bt11 maize in Turkey. Sanhoty et al. (2002) reported that 15% of 40 maize samples tested positive for Bt176, and 12.5% were positive for Bt11 maize; additionally, it was found that Bt176, Bt11, and Star- LinkTM were present as mixtures in four samples in Egypt market. However, the maize lines T25 and Mon810 were not identified. Several countries used survey studies to monitor the presence of event specific GMO by qualitative analysis. For instance, Treml and Arisi (2008) detected three Roundup Ready soybeans of 47 meat samples in Brazilian market by nested PCR method. In Singapore, three Bt rice and four LLrice601 were found among 267 imported rice product samples (Wang et al., 2008).

The quantitative analysis of maize lines Bt176, Bt11, Mon810 and T25

Positive samples were also subjected to quantitative analysis to determine the amount of GM maize DNA present. These results (Table 5 and Table 6) represent the percentage of GM maize DNA relative to non-GM maize DNA in each sample tested. However, due to the limitations of the technology with respect to low levels of target DNA, it was not possible to quantify the amount of GM maize DNA in trace positive samples, so the percentage was simply recorded as trace (TR). For example; the quantitation limits were set to 50 coppies of target sequence, meaning that target sequences under 50 coppies were showed as trace (TR).

Table 5. General evaluation of the results-1

Samples	[a]Zein Gene	[a]Plant gene	[a]35S	[a]NOS	[b]35S	[b]NOS
MF5	+	+	+	+	+	+
MF6	+	+	-	-	+	-
MF8	+	+	-	-	+	+
MF11	+	+	-	-	+	+
MF16	+	+	-	-	+	+
MK16	+	+	+	+	+	+
MK18	+	+	-	-	+	+
MS1	-	+	-	-	-	+
MC2	-	+	-	-	+	+
MC4	-	+	-	-	+	+
MC10	-	+	-	-	+	+
MCo1	-	+	-	-	-	+
MCo5	-	+	-	-	-	+
MCo7	-	+	-	-	-	+

[a] Qualitative analysis by conventional PCR
[b]Qualitative analysis by real-time PCR
[c]Absolute quantification by real-time PCR
[d]Relative quantification by real-time PCR
'+' GM material detected
'-' no GM material detected
MF=Maize Flour, MK=Maize Kernel, MS=Maize Starch, MC=Maize Chips, MCo=Maize Cornflakes

Table 6. General evaluation of the results-2

Samples	[a]Bt176	[c]Bt176	[d]Bt176	[a]Bt11	[c]Bt11	[a]Mon810	[c]Mon810	[a]T25	[c]T25	[a]CBH351
MF5	+	0.14	0.05	+	1.48	+	2.83	-	0.16	-
MF6	-	-	TR	-	-	-	-	-	-	-
MF8	-	-	TR	-	-	TR	-	-	TR	-
MF11	TR	TR	0.01	-	TR	-	TR	-	-	-
MF16	-	-	TR	-	-	-	-	-	-	-
MK16	-	TR	0.04	-	-	+	TR	-	-	-
MK18	-	TR	TR	-	-	TR	TR	-	-	-
MS1	-	ND	TR	-	-	-	-	-	-	-
MC2	-	TR	ND	TR	-	TR	TR	-	ND	-
MC4	-	ND	ND	-	-	-	-	-	-	-
MC10	-	ND	ND	TR	-	-	ND	-	ND	-
MCo1	-	ND	ND	TR	-	-	ND	-	ND	-
MCo5	-	-	TR	-	-	-	ND	-	ND	-
MCo7	-	ND	ND	-	-	-	ND	-	ND	-

[a] Qualitative analysis by conventional PCR
[b]Qualitative analysis by real-time PCR
[c]Absolute quantification by real-time PCR
[d]Relative quantification by real-time PCR
'+' GM material detected
'-' no GM material detected
'TR' trace amounts
ND: Not detected (It is not appropriate result or inadequate copy number)
MF=Maize Flour, MK=Maize Kernel, MS=Maize Starch, MC=Maize Chips, MCo=Maize Cornflakes

Two samples of maize kernel for feed (10.5%) were tested positive for Bt176 and Mon810. One of the samples was imported from Ukraine, and the sample had trace amounts of both maize lines. The other sample has Bt176 (0.04% <LOD and LOQ) and a trace amount of Mon810; this sample was found to be domestic maize obtained from Adana, Turkey, which is a region designated for he trial productions of GM maize, cotton, and other crops. The GM maize lines tested were not observed with the other maize kernels.

Five maize flours were tested positive for Bt176 (31.3 % of samples) but the results of the relative and absolute PCR quantification were not similar. For instance, a relative result of 0.14% (>LOQ) and an absolute result of 0.05% (an average being 0.09%,<LOQ) for Bt176 were seen in the same maize flour sample (MF5). Similarly, another maize flour sample (MF8) had a 0.01% (<LOD and LOQ) relative quantification for Bt176, but a trace amount for the absolute quantification. The results were expected to be similar because relative GM content of the samples was obtained by both of the methods. Thus, results for Bt176 absolute and relative quantifications levels were tested using a paired *t* test to assess whether there was any significant difference between methods (the null hypothesis being there is no difference). The paired t test results indicate that there is no statistical difference between mean values of relative quantification and the mean values of absolute quantification of the samples (flours, feeds, chips and cornflakes).

Additionally, more than one GM maize line was observed in three samples of maize flours. For example; an average of 0.01% Bt176 (<LOD and LOQ), 1.48% Bt11, 2.83% Mon810 and 0.16% T25, for a total of 4.67% GM maize lines, were tested in the same maize flour sample (MF5). The quantity of the sample was greater than 0.9%, which is the limit at which labeling is required, according to The GM Food and Feed Regulation (EC) No. 1829/2003. Trace amounts of Bt176 (or 0.01% <LOD and LOQ), Bt11, Mon810 and T25 maize line were found in another maize flour sample (MF8). Trace amounts of Bt176 were detected (while the other lines were not) in a starch sample. One sample of chips had trace amounts of Mon810 and Bt176, while the other samples of chips and corn flakes were negative for GM lines (either the results were not inconclusive and/or adequate copy numbers were not found).

A commonly occurring phenomenon is the occurrence of more than one transformation in the same plant. This is the case, e.g., for several GM maize hybrid currently awaiting marketing approval in the European Union (TemaNord., 2004; GMO-Compass, 2012). Although there are publications about hybrid formation Mon810 and Bt11 with the other events, no hybrid forming between BT11 and Mon810 was recorded (GMO-Compass, 2012). Additionally no hybrid forming was recorded about CBH351, T25 and Bt176 too. Unless a specific marker is introduced in the hybrid between two GM plants, it is not possible to determine whether a given sample contains the hybrid or a mixture between the two plants (TemaNord., 2004).

Aside from the results presented here, the presence of GM elements in sweet corn and popcorn (FSAI, 2004), and tortilla chips and taco shells (FSAI, 2001) were reported for samples found in Ireland: of 26 samples, 19 were positive for presence of GM maize, and trace levels of GM DNA were detected in eight samples. Sanhoty et. al. (2002) found levels of 0.1%, 0.3%, 0.6% and 0.8% of StarLink in four samples of 40 maize products in Egypt. In 2007, the Bavarian Health and Food Safety Authority detected transgenes sequences of 63 corn and 19 rape-seed samples below 0.1% (Goerlich et al., 2008). In Romania and Serbia, surveys were conducted especially for RR soybean. In Romania, Zaulet et al. (2008) investigated 118 soybean and foods derived soybean samples by qualitative and quantitative PCR methods. They detected more than 0.9% RRS in 53 samples. In contrast, 12 products of 50 processed meat products gave positive results with 35S promoter but all of them contained RR soya below 0.1% in Serbia (Taski-Ajdukovic et al., 2008).

Turkey, there have only been a few studies to detect GM foods and/or feeds in market-supplied

products. The results indicated that in almost all soy bean products (Aril & Çakır, 2008; Ertugrul et al., 2008; Cetiner, et al., 2009), and some maize products (Ertugrul et al. 2008) were GM positive.

Because there were differences between the quantitative and qualitative results for the methods used to evaluate Bt176, Bt11, Mon810, T25, the agreement between these methods was statistically investigated using the Kappa statistic method (Table 3). According to the Bt176 results, there was slight agreement ($\varkappa$ =0.169) between conventional PCR results and both relative and absolute quantification methods. Similarly slight agreement ($\varkappa$ = 0.176) was found in the methods used to evaluate Bt11. A moderate agreement ($\varkappa$ =0.553) was observed between conventional and real-time methods to determine Mon810. Because of inadequate count of positive samples in conventional PCR for evaluating the T25 region, statistical analysis were not done to evaluate method agreement.

CONCLUSION

In conclusion, the health, socio-economic and environmental issues related to the existence of GM materials in food and feedstuff is discussed major concern of bio-safety in many nations. Labeling of GM products is essential to provide information to consumers where there is no wide consensus about the (long-term) safety of GM products. Maize is important in human nutrition in Turkey, and our results clearly demonstrate the presence of GM maize in the Turkish food market and in feed industry. The strict application of EU directives would have required the labeling of one corn flour sample (MF5) as GM-derived. The others were under the limit required for labeling (0.9%) although GM genetic elements were clearly detected.

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REFERENCES

Abdel-Mawgood, A.; Gassem, M. A.; Alsadon, A. A.; Alghamdi, S. S. & Al-Doss, A. A. (2010). Monitoring of genetically modified food in Saudi Arabia. *African Journal of Food Science, 4*(8) pp. 536 -540.

Aguilera, M.; Querci, M.; Balla, B.; Pastor, S.; Ermolli, M. & Van den Eede, G. (2008). Genetic and phenotypic stability of the Mon810 trait in commercial seed maize varieties: qualitative and quantitative approaches. *Abstract Book of 1st Global Conference on GMO Analysis*. (Organized by the Joint Research Centre of the European Commission) 24-27 June. Villa Erba, Como, Italy.

Ahmed, F.E. (2002). Detection of genetically modified organisms in food. *Trends in Biotechnol. 20*, 215-223.

Aril, S. & Çakir, O. (2008). Detection of genetically modified soy in processed foods sold commercially in Turkey by PCR based method. *Abstract Book of 1st Global Conference on GMO Analysis.* (Organized by the Joint Research Centre of the European Commission) 24-27 June. Villa Erba, Como, Italy.

Badulescu, L.; Dobrescu, A.; Ispas, D.; Delian, E. & Burzo, I. (2008). The evaluation of test portion and sample soybean matrix influences on the DNA quantification by real-Time PCR. *Abstract Book of 1st Global Conference on GMO Analysis*. (Organized by the Joint Research Centre of the European Commission) 24-27 June, Villa Erba, Como, Italy.

Baran, M. & Yilmaz, R. (2008). Guest Editorial.. The biosafety policy on genetically modified organisms in Turkey. Environ. Biosafety Res., 7, 57-59.

BATS (2003). BATS (Centre for biosafety and sustainability) report. Genetically modified (GM) crops: Molecular and regulatory details. *2*, pp.192.

Berben, G. & Dardenne, P. (2001). Traçage et authentification des produits à base d'organismes génétiquement modifiés, Recherches financées par les SSTC dans le cadre du programme de "Recherche pré-nominative dans le secteur alimentaire", CRA-W, rapport finale contract NP/42/026.

Bergerova, E.; Hrncirova, Z.; Stankovska, M.; Laposovska, M. & Siekel, P. (2008). Effect of heat on DNA degredation in selected food plant matrices. *Abstract Book of 1st Global Conference on GMO Analysis*. (Organized by the Joint Research Centre of the European Commission) 24-27 June, Villa Erba, Como, Italy.

Biosafety Commission. (2011). Official Journal. 27827. http://www.resmigazete.gov.tr/eskiler/2011/01/20110126-9.htm

Biosafety Law. (2010). No. 5977. Official Journal. No. 27533. (26.03.2010). In force 26 September 2010.

CERA (2018). GM Crop Database; Global status of approved genetically modified plants. *International Life Sciences Institute*. http://cera-gmc.org.

Cetiner, S.; Budak, H. & Ertugrul, A. (2009). A comprehensive survey of genetically modified products in the Turkish market using DNA based qualitative and quantitative techniques. *Abstract Book of 3rd International Congress on Food and Nutrition*. 22-25 April. p.56 Antalya, Turkey.

Demeke, T. & Ratmayaka, I. (2008). Effect of DNA extraction and purification methods on Real-Time quantitative PCR analysis of Roundup Ready soybean. *Abstract Book of 1st Global Conference on GMO Analysis*. (Organized by the Joint Research Centre of the European Commission) 24-27 June .Villa Erba, Como, Italy.

EC. (1997). Regulation (EC) No. 258/97 of the European Parliament and of the Council, concerning the novel foods and novel food ingredients. 27 January 1997.

EC. (2003). Regulation (EC) No. 1830/2003., of the European Parliament and of the Council, concerning the Traceability and Labeling of Genetically Modified Organisms and the Traceability of Food and Feed Products Produced from Genetically Modified Organisms and Amending Directive 2001/18/EC. 15798/1/ 02 REV1. European Council, Brussels. In force 18 April 2004.

Ertugrul, A.; Budak, H. & Cetiner, S. (2008). The DNA based qualitative and quantitative analysis of genetically modified products in Turkish market. *Abstract Book of 1st Global Conference on GMO Analysis*. (Organized by the Joint Research Centre of the European Commission) 24-27 June.Villa Erba, Como, Italy.

Food Standard Agency. (2004). GM Labeling. http://www.food.gov.uk

FSAI (2001). Survey of Tortilla Chips and Taco Shells for Genetically Modified Ingredients. FSAI (The Food Safety Authority of Ireland). Ireland. http://www.fsai.ie

FSAI (2004). GM Maize Survey. Sweet corn and Popcorn. FSAI (The Food Safety Authority of Ireland). Ireland. http://www.fsai.ie

GMO-Compass (2012). GMO database. Genetically modified food and feed: Authorization in the EU. . http://www.gmo-compass.org/eng/gmo/db/.

Goerlich, O.; Müler, A.; Hilen, M. & Busch, U. (2008). Analysis of conventional seed for genetically modified components in Bavaria. Abstract Book of 1st Global Conference on GMO Analysis. (Organized by the Joint Research Centre of the European Commission) 24-27 June. Villa Erba, Como, Italy.

Guillaume, P. ; Gruère, S. & Rao, R. (2007). A review of international labeling policies of genetically modified food to evaluate India's proposed rule. *AgBioForum*, *10*(1), 51-64.

Gurakan, C.G., Aydin, G. & Yilmaz, R. (2011) Qualitative detection of GM maize (Bt11) in food and feed sold commercially in Turkey by PCR based methods. *Indian Journal of Biotechnology. 10,* 143-146.

Holst-Jensen A.; Rønning S. B.; Løvseth, A. & Berdal, K. G. (2003). PCR technology for screening and quantification of genetically modified organisms (GMOs). *Anal. Bioanal. Chem*., *375*, 985-993.

IIA.MSU (2007). Institute of International Agriculture. Michigan State University. Biosafety and risk assessment in agricultural biotechnology. *A workbook for technical training*. Part 5: Monitoring. http://www.iia.msu.edu.

James, C. (2010). Brief 42: Global status of commercialized Biotech/GM crops: ISAAA Briefs. Ithaca, NY. (http://www.isaaa.org).

Kok J.E.; Scholtens, I.M.J.; Van Hoef, A.M.A. & Aarts, H.J.M. (2000). Detection of genetically modified DNA in food. 1-3. www. ifis.org.

Lipp, M.; Bluth, A.; Eyguem, F.; Kruse, L.; Schimmel, H.; Van Den Eede, G. & Anklam, E. (2001). Validation of a method based on polymerase chain reaction for the detection of genetically modified organisms in various processed foodstuffs. *European Food Research and Technology. 212*, 497-504

Minegishi, Y.; Kurosawa, Y.; Nishikawa, C.; Doi, N.; Kanayama, S.; Kodama, T.; Kasahara, M.; Matsuoka, T.; Watanabe, T.; Akiyama, H.; Teshima,R.; Mano, J.; Furui, S.; Hino, An. & Kitta, K. (2008). Evaluation of a new DNA extraction method for PCR detection of genetically modified soybean. *Abstract Book of 1st Global Conference on GMO Analysis*. (Organized by the Joint Research Centre of the European Commission) 24-27 June. Villa Erba, Como, Italy.

OCA. (2005). Organic Concumers Association. OrganicDetailed Description on New GMO Labeling Rules in EU. www.organicconsumers. org.

Oraby, H.A.S; Hassan, A.A. & Abou Mossallam, A. A. (2005). Screening food products for the presence of CaMV 35S promoter and NOS terminator. *J. Sci. Food Agric.*, *85*, 1974-1980.

Papazova, N.; Taverniers, I.; Van Houdth, L. & De Loose, M. (2008). Study on parameters influencing the quality of DNA for GMO quantification. *Abstract Book of 1st Global Conference on GMO Analysis*. (Organized by the Joint Research Centre of the European Commission) 24-27 June**.** Villa Erba, Como, Italy.

Pauli, U.; Liniger, M.; Zimmerman, A. & Schrott, (2000). M. Extraction and amplification of DNA from 55 foodstuffs. *Mitt. Lebensm. Hyg. 91*, 491-501.

Querci, M., Van den Eede, G. & Jermini, M. (2002). The analysis of food samples for the presence of genetically modified organisms. European Commission. *Joint Research Centre Manual.* Part *2*, 5-8.

Roger, S. O. & Bendich, A. J. (1985). Extraction of DNA from milligram amounts of fresh, herbarium and plant tissues. *Plant Mol. Biol. 5*, 69-76.

Sanhoty, R. E.; Broll, H.; Grohmann, L.; Linke, B.; Spiegelberg, A.;, K. W. & Zagon,. J. (2002). Genetically modified maize and soybean on the Egyptian food market. *Nahrung* Food, 46 (5), 360–363.

Sheskin, D.J. (2000). Handbook of parametric and nonparametric statistical procedures, 2nd ed. Chapman 8. Hall/CRC.

Sieradzki, Z.; Walczak, M. & Kwiatek, K. (2006). Occurrence of genetically modified maize and soybean in animal feedingstuffs. *Bull Vet Inst.* Pulawy. *51*, 567-570.

Sim, J. & Wright, C. C. (2005). The Kappa Statistic in reliability studies: Use, interpretation, and sample size requirements. Physical Therapy. 85, 257-268**.**

Taski-Ajdukovic, K.; Nicolic, Z.; Vujokovic, M., Milosevic, M.; Ingjatov, M. & Petrovic, D. (2008). Monitoring of Roundup Ready soybean in processed meat product on the Serbia food market. *Abstract Book of 1st Global Conference on GMO Analysis*. (Organized by the Joint Research Centre of the European Commission) 24-27 June. Villa Erba, Como, Italy.

TBDDM (2018). Türkiye Biyogüvenlik Bilgi Değişim Mekanizması. www.tbddm.gov.tr

TemaNord. (2004). Control of GMO Content in Seed and Feed- possibilities and limitations. TemaNord 2004:541. Nordic Council of Ministers, Copenhagen. 74p.

Tozzini, A.; Martinez, M. C.; Lucca, M. F.; Rovere, C. & Distefano, A. J. (2000). Semiquantative detection of genetically modified grain based on CaMV 35S promoter amplification. Technical Note. *Electronic Journal of Biotechnology, 3*, 149-153.

Treml, D. & Arisi, A. C. M. (2008). Monitoring of Roundup Ready soybean in processed meat

products sold in Brazilian markets from 2007 to 2008. Abstract Book of 1st Global Conference on GMO Analysis. (Organized by the Joint Research Centre of the European Commission) 24-27 June.Villa Erba, Como, Italy.

TurkStat. (2010). Turkish Statistical Institute. Foreign trade. No.151. www.turkstat. gov.tr.

Wang, Z. M.; Koh, M. C.,; Teo, E., Liau, S. H.; Chiew, K. T. & Chew, S. T. (2008). A practical analytical approach to screen for GM presence in rice products. Abstract Book of 1st Global Conference on GMO Analysis. (Organized by the Joint Research Centre of the European Commission) 24-27 June. Villa Erba, Como, Italy.

Zaulet, M.; Georgescu, S.; Petcu, C.; Popovici, A.; Tanasuica, R. & Rebedea, M. (2008). PCR technology for screening and quantification of genetically modified soybeans from foods. *Abstract Book of 1st Global Conference on GMO Analysis*. (Organized by the Joint Research Centre of the European Commission) 24-27 June. Villa Erba, Como, Italy.