

Determination of genetically modified corn and soy in processed food products

Ö. Ateş Sönmezoğlu^{1*}, H. Keskin²

¹Karamanoglu Mehmetbey University, Bioengineering Department, 70200 Karaman, Turkey.

²Karamanoglu Mehmetbey University, Biology Department, 70200 Karaman, Turkey.

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ABSTRACT

Genetically modified organisms (GMOs) and their raw materials used in processed foods should be analyzed and labeled. The aim of our study was to evaluate the present situation of the food products and to determine appropriate DNA isolation method in processed food products. Keeping in view, there is a need to determine different DNA extraction protocols specific to processed foods and these protocols should be modified and must be applied to test GMOs. In this study, six different DNA extraction protocols and two DNA kits were used for soybean or corn based 12 kinds of products with total of 27 processed food products including biscuit, cornflakes, cracker, corn chips, corn starch, corn flour, popcorn, sweet corn, baby food, cake, compressed popcorn, soy flour along with positive controls (GM soy and GM corn). Obtained results showed that DNA quantity and quality varied with type of food and processing. Investigation of genetically modified organisms in these products was determined by qualitative PCR analysis. Molecular screening of these food products were carried out by using 35S promoter and nos or tnos terminator sequences. As a result, some of the products containing corn/soy based raw material were found to be genetically modified.

1. INTRODUCTION

The global cultivation area of genetically modified crops is increasing every day. In recent years, a significant increase has been observed in the usage of foods containing genetically modified products. In order to protect biodiversity and to ensure conscious consumption, an analysis of genetically modified organisms (GMOs) and the processed foods in which GMOs are used as raw materials must be done and these products must be labeled. The importance of understanding the role of GMO products becomes clear when considering that corn is used in 700 kinds of foods, and soybeans are used in 900 kinds. Foods containing oil, flour, starch, glucose syrup, sucrose, and fructose, derived from corn and soybeans, are common elements of many daily consumed products. Primary products at risk of containing GMOs are: vegetable oils, flour, baby foods, candies, chocolate, waffles, ready-made soups, biscuits, cornflakes, crackers, chips, and foods made from chicken or similar animals that consumed corn and soybeans as feed. Isolation and purification of DNA comprise the first step in the process of analyzing and measuring GMO products. In order to apply qualitative and quantitative

PCR methods, sufficient measurements for purity, quality and amount of DNA must be determined. Physical and chemical processes applied to food samples during production can cause a reduction in the amount of DNA, due to amount by random fragmentation of DNA and distortion of DNA structure (Kakihara *et al.*, 2005). These factors complicate DNA isolation from processed food products (Gryson *et al.*, 2004; Holden *et al.*, 2003). In raw materials like corn, this process is performed easily; however, it becomes quite difficult with processed products, which are exposed to heat treatment causing DNA degradation. More DNA degradation occurs on the surface of cooked foods like cake and biscuits. For these reasons, specific DNA isolation methods must be developed for each processed food. Also, different DNA isolation techniques, those appropriate to the structure of the sample (acidic, oily, etc.), are applied. The aim of this study is to determine the most appropriate isolation method for each processed product containing corn and soybean by using different extraction protocols, modification types, and DNA extraction kits. The food products from different processing levels were obtained from various markets. Lectin-zein gene scanning of the food products was also done. In addition, it was determined with PCR analysis whether or not the food products contained GMO-based products. PCR analysis determining the regulatory sequences (35S promoter, nos and tNos terminator) in various food products made from corn and soybean, which come at the front of transgenic products, was performed with qualitative scanning.

* Corresponding Author

Dr. Özlem Ateş Sönmezoğlu, Karamanoglu Mehmetbey University,
Bioengineering Department, 70200 Karaman, Turkey.

E-mail: [ozlemsonmezoglu1\[at\]gmail.com](mailto:ozlemsonmezoglu1[at]gmail.com)

2. MATERIALS AND METHODS

2.1 Sample collection

In this study, total of 27 food products containing 12 different food products (biscuit (4), cornflakes (2), cracker (4), corn chips (5), corn starch (2), corn flour (2), popcorn, sweet corn, baby food (2), cake (2), compressed popcorn, soy flour) from different marks in different processing levels containing soybean and/or corn obtained from various markets in Turkey were chosen as materials. Standard materials used for positive control (GM soy and GM corn) were obtained from TUBITAK-MAM Biotechnology Institute. Wheat grain was used as negative control in PCR analysis.

2.2 DNA extraction methods

In this study, six extraction protocols called as Wizard method [protocol 1] (Hemmer, 1997), the modified Wizard method [protocol 2] by an addition of 1% BME (β -mercaptoethanol) in lysis buffer (Tung Nguyen *et al.*, 2009), the combination method [protocol 3] based on the pre-incubation of samples with TNE buffer (Tung Nguyen *et al.*, 2009), the CTAB method [protocol 4] (Jankiewicz *et al.*, 1999), the modified CTAB method [protocol 5] by an addition of 1% BME (β -mercaptoethanol) in lysis (Tung Nguyen *et al.*, 2009), and the CTAB method [protocol 6] (Rogers and Bendich, 1985), and two extraction kits known as DNeasy® mericon™ Food Kit [protocol 7], Qiagen and GENESpin, Eurofins GeneScan Kit [protocol 8] were used for DNA isolation. The most suitable extraction method for each processed food product was determined by using DNA isolation protocols and kits.

Food samples (350 grams from each food product) were homogenized in muller. In order to test the reliability and reproducibility of the process, the DNA isolation method was repeated three times; in addition, water instead of the sample was used in one tube in each set, in case of possible contamination risks that may be caused by the environment during the process. Processing steps were equally applied.

2.3 DNA quantification

The amount and purity of the DNA samples were determined by spectrophotometric and electrophoretic methods. All DNA samples were quantified using a spectrophotometer (Thermo Scientific Multiskan GO) at an absorbance of 260 nm. The purity of genomic DNA was estimated by measuring the A260/A280 absorbance ratio. The quality was also examined by running the extracted DNA samples from the food products on 1% agarose gel stained with 10 mg/ml ethidium bromide in 5 x TBE buffer. The gels were visualized under a UV transilluminator (Bio-Rad, ChemiDoc™ MP Imaging System).

2.4 PCR

In order to determine whether soybean and/or corn are present in a processed or unprocessed food product, lec-zein gene

scanning was performed in isolated DNAs with specific primers. Lec1/Lec2 (164 bp) and Zein03/Zein04 (277 bp) primer pairs were used for the lectin gene and the zein gene, respectively (Pauli *et al.*, 2000; Vollenhofer *et al.*, 1999).

GMO scanning of the investigated food products was also performed to determine whether or not they contained GMO products. For this purpose, the existence of 35s promoter, nos and tNos terminator sequences was investigated with qualitative PCR analysis using 35s-PF/PR (143 bp), Nos-1/3 (180 bp) and tNos2-3/5 (151 bp) primers (Oraby *et al.*, 2005; Wang and Fang, 2005). PCRs were performed under the conditions given in the source articles for each primer with some modifications. The PCR amplification was carried out in a 40 μ l reaction mix on a Bioneer (MyGenie 96) thermal cycler.

The PCR reactions contained 50-100 ng of genomic DNA, 250 nM each of the primers, 0.2 mM each of the nucleotides, 1.5 mM MgCl₂, 10 x PCR buffer and 0.5 units of *Taq* DNA polymerase (Promega). Obtained PCR products were electrophoresed in 2% agarose gel (in 5 x TBE buffer) and the picture of gels were taken in Bio Rad imaging device (Bio Rad, ChemiDoc™ MP Imaging System).

2.5 Statistical analysis

A two-way analysis of variance (ANOVA) was applied, using SPSS 15.0 software, to the spectrophotometer measurement data obtained as double replications according to randomized blocks design. The Duncan test was used to compare the mean values.

3. RESULTS AND DISCUSSION

3.1 Evaluation of DNA concentration (μ g/ μ l) of eight DNA extraction protocols on food samples

In this study, different DNA extraction protocols, modification types, and extraction kits were used to isolate DNA from 27 food products with different processing levels. Amount and quality tests of the obtained DNA samples were performed with agarose gel electrophoresis and spectrophotometric measurements.

When the average DNA concentrations obtained from 14 different food products in various processing levels were examined, the highest average DNA amounts were obtained from soy flour (2.19 μ g/ μ l), soy (2.11 μ g/ μ l), popcorn (1.53 μ g/ μ l), and genetically modified (GM) corn (0.56 μ g/ μ l). Cracker, compressed popcorn, biscuit, corn flakes, cake, corn chips, and corn flour followed those products.

As a result of the statistical analysis performed by considering all protocols, products giving the lowest values in terms of average DNA yield were cornstarch (0.08 μ g/ μ l), sweet corn (0.17 μ g/ μ l), and baby food (0.20 μ g/ μ l) (Table 1). The mean in Table 1 shows that soy flour and GM soy was produced the highest DNA yield within the analyzed food samples because they were less processed products. In addition, DNA yields of raw corn samples (popcorn and GM corn) were high.

Table 1: Comparison of amounts of DNA ($\mu\text{g}/\mu\text{l}$) isolated by using different DNA extraction methods in food products.

Food Product	Protocols (Mean Standard Error)								Mean*
	Pro 1	Pro 2	Pro 3	Pro 4	Pro 5	Pro 6	Pro 7	Pro 8	
Biscuit	0.63 b	0.89 a	0.58 b	0.31 c	0.28 cd	0.07 e	0.22 cde	0.13 de	0.39 cd
Cornflakes	1.02 a	0.70 b	0.55 c	0.11 e	0.31 d	0.07 e	0.06 e	0.06 e	0.36 cd
Cracker	0.75 a	0.86 a	0.70 a	0.23bcd	0.42 b	0.06 d	0.26 bc	0.14 cd	0.43 cd
Corn chips	0.67 a	0.65 a	0.65 a	0.21 b	0.26 b	0.07 b	0.10 b	0.08 b	0.34 cd
Corn starch	0.14 a	0.13 a	0.07 b	0.07 b	0.07 b	0.07 b	0.07 b	0.06 b	0.08 d
Corn flour	0.61 a	0.63 a	0.55 a	0.27 b	0.31 b	0.07 c	0.21 bc	0.09 c	0.34 cd
Popcorn	1.08 b	3.05 a	2.38 a	0.42 b	2.77 a	0.10 b	2.32 a	0.10 b	1.53 b
Sweet corn	0.22 bc	0.39 a	0.26 ab	0.13 bc	0.17 bc	0.06 c	0.10 bc	0.07 c	0.17 d
Baby Food	0.28 c	0.23 d	0.33 b	0.10 e	0.41 a	0.07 e	0.09 e	0.08 e	0.20 cd
Cake	0.77 a	0.96 a	0.47 b	0.14 c	0.26 bc	0.08 c	0.15 c	0.08 c	0.36 cd
Compress p.	0.69 a	0.72 a	0.59 a	0.42 ab	0.59 a	0.08 b	0.12 b	0.08 b	0.41 cd
Soy flour	3.1 ab	3.3 a	2.54 b	3.17 ab	3.13 ab	0.38 d	1.8 c	0.1 d	2.19 a
GM soy	3.09 a	3.17 a	3.14 a	2.97 a	2.81 a	0.32 c	1.24 b	0.14 c	2.11 a
GM corn	1.13 ab	0.94 bc	0.75 c	0.16 d	1.24 a	0.06 d	0.13 d	0.11 d	0.56 c
Mean	1.01 a	1.19 a	0.97 a	0.62 bc	0.93 ab	0.11 d	0.49 c	0.09 d	

*Means in the same row followed by different letters differed significantly ($P < 0.05$).

Table 2. Comparison of the DNA quality (A_{260}/A_{280}) isolated by using different DNA extraction methods in food products containing soy and corn.

Food Product	Protocols (Mean Standard Error)								Mean*
	Pro 1	Pro 2	Pro 3	Pro 4	Pro 5	Pro 6	Pro 7	Pro 8	
Biscuit	1.29 c	1.44 bc	1.51 abc	1.44 bc	1.58 ab	1.49 abc	1.69 ab	1.73 a	1.52 cde
Cornflakes	1.14 b	1.19 b	1.22 b	1.22 b	1.44 a	1.18 b	1.18 b	1.52 a	1.26 g
Cracker	1.57 bc	1.67 ab	1.64 ab	1.51 bc	1.64 ab	1.41 c	1.79 a	1.78 a	1.63 bc
Corn chips	1.56 ab	1.63 ab	1.57 ab	1.36 b	1.52 ab	1.44 ab	1.59 ab	1.67 a	1.54 cd
Corn starch	1.17 c	1.18 c	1.27 abc	1.22 bc	1.42 ab	1.07 c	1.19 bc	1.49 a	1.25 g
Corn flour	1.70 b	1.76 ab	1.91 a	1.67 b	1.84 ab	1.48 c	1.85 ab	1.72 ab	1.74 ab
Popcorn	1.88 ab	1.48 b	1.69 ab	1.53 b	1.59 ab	1.50 b	1.96 a	1.85 ab	1.68 ab
Sweet corn	1.18 a	1.42 a	1.6 a	1.26 a	1.52 a	1.32 a	1.57 a	1.56 a	1.43 def
Baby Food	1.28 b	1.51 a	1.25 bc	1.06 c	1.52 a	1.29 b	1.60 a	1.67 a	1.40 ef
Cake	1.22 b	1.21 b	1.34 ab	1.49 a	1.51 a	1.4 ab	1.42 ab	1.42 ab	1.38 f
Compress. p.	1.53 ab	1.70 ab	1.59 ab	1.63 ab	1.71 ab	1.70 ab	1.72 a	1.45 b	1.63 bc
Soy flour	1.09 d	1.15 d	1.10 d	1.16 d	1.13 d	1.93 b	2.04 a	1.59 c	1.40 ef
GM soy	1.24 d	1.39 c	1.23 d	1.22 d	1.09 d	1.97 a	2.04 a	1.81 b	1.50 def
GM corn	1.86 ab	1.80 ab	1.84 ab	1.81 ab	1.95 a	1.48 c	1.73 b	1.77 ab	1.78 a
Mean	1.41 c	1.47 bc	1.48 bc	1.40 c	1.53 b	1.47 bc	1.67 a	1.64 a	

*Means in the same row followed by different letters differed significantly ($P < 0.05$).

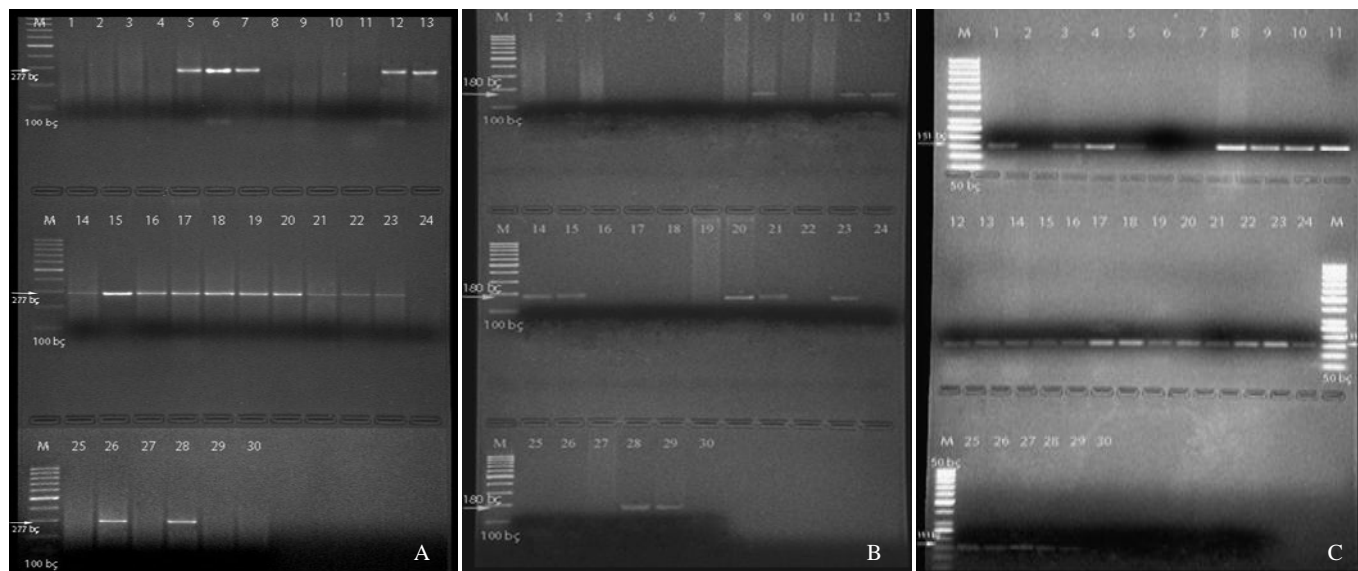


Fig 1. Gel image amplification with zein (A), nos (B) and tNos (C) primers in food samples (lanes 1-4 biscuit; lane 5 corn chips; lanes 6-7 cornflakes; lanes 8-11 cracker; lanes 12-15 corn chips; lanes 16-17 corn starch; lanes 18-19 corn flour; lane 20 popcorn; lane 21 sweet corn; lane 22-23 baby food; lanes 24-25 cake; lane 26 compressed popcorn; lane 27 soy flour; lane 28 GM corn; lane 29 GM soy; Lane 30 wheat).

Of the eight DNA isolation protocols, the highest DNA amounts were obtained from protocol 2 (1.19 µg/µl), protocol 1 (1.01 µg/µl), and protocol 3 (0.97 µg/µl). Protocols 5, 4, and 7 followed them in order. Statistical analysis on all food products showed that the methods resulting in the lowest values in terms of average DNA yield were protocol 6 (0.11 µg/µl) and protocol 8 (0.09 µg/µl) (Table 1).

The mean in Table 1 shows that Wizard protocols (protocols 1 and 2) resulted in a good DNA yield. These findings were in accordance with the results of Marcelino *et al.*, (2008) and Smith *et al.*, (2005). For popcorn, protocols containing BME (2 and 5) produced higher DNA yields than protocols 1 and 4. For GM corn, the modified CTAB method (protocol 5) produced higher DNA yield than the CTAB method (protocol 4). DNA yield did not change significantly for other products in modified protocols.

Of the two CTAB procedures used in this study, the CTAB method by Jankiewicz *et al.*, (1999) produced higher DNA yields than the CTAB method by Rogers and Bendich (1985). The modified CTAB method (protocol 5) is highly applicable for extracting DNA from raw corn such as popcorn and GM corn. Similarly, Tung Nguyen *et al.*, (2009) and Schneerman *et al.*, (2002) determined that good DNA yields are obtained from maize leaves and corn cobs. The Wizard methods (protocols 1 and 2) were found to be the most suitable for DNA extraction from highly processed foods such as cornflakes, corn chips, biscuits, and cakes. Tung Nguyen *et al.*, (2009) demonstrated that the Wizard methods could be used successfully to extract DNA from smooth tofu and soy milk.

The interaction analysis was carried out to identify the optimum combination of DNA extraction protocols and the different food samples. The ANOVA showed significant variation in DNA concentration for each combination ($P < 0.05$). The results indicated that protocols 1, 2, 3, 4, and 5 were the most favorable methods for extracting DNA from soy flour and GM soy. The Wizard protocols (protocols 1 and 2) and the combination method (protocol 3) were the most favorable extraction methods for corn flour, crackers, and corn chips. Similarly, the Wizard methods were suitable for DNA isolation from cakes.

3.2 Evaluation of DNA quality (A₂₆₀/A₂₈₀ ratio) of the extraction protocols on food samples

In order to measure the purity and quality of DNA, the ratio of 260 nm to 280 nm absorption values were used. The values of pure DNA and RNA are approximately 1.8 and 2.0, respectively. The A₂₆₀/A₂₈₀ ratios calculated in this study are shown in Table 2. The most suitable average ratios that can be said to be obtained from processed food products were GM corn (1.78), corn flour (1.74), popcorn (1.68), cracker (1.63), and compressed popcorn (1.63). These products were followed by corn chips, biscuits, soy, and sweet corn. As a result of statistical analysis performed by taking all protocols as basis, products with the lowest A₂₆₀/A₂₈₀ ratio in terms of average DNA purity were corn starch (1.25), cornflakes (1.26), and cake (1.38). Data related to

A₂₆₀/A₂₈₀ ratios of DNA isolated from 14 food products in different processing levels were compared by evaluating each protocol separately (Table 2). When the average of A₂₆₀/A₂₈₀ ratios is investigated, the highest DNA purities were obtained using protocol 7 (1.67) and protocol 8 (1.64). These were followed by protocols 5, 3, 6, and 2, with A₂₆₀/A₂₈₀ ratios changing between 1.47 and 1.53.

As a result of statistical analysis performed by considering all food products, in terms of A₂₆₀/A₂₈₀ ratios (DNA quality), methods giving the lowest values were protocol 1 (1.41) and protocol 4 (1.40).

The extraction kits (protocol 7 and 8) did not produce high DNA yield, but did result in good DNA quality in terms of the means of A₂₆₀/A₂₈₀ ratio at 1.67 and 1.64. According to Pich and Schubert (1993), an A₂₆₀/A₂₈₀ ratio of 1.6–1.7 signifies the absence of contaminants. For raw maize, Tung Nguyen *et al.*, (2009) determined that the Wizard methods and the combination method gave 1.8 and 1.6 ratios, respectively. This result was similar to the results we obtained.

3.3 PCR for GMO detection

After DNA isolation was performed in food products, soy-corn DNA scanning was done to determine which genes the samples contained. The zein gene is maize-specific while the lectin gene is soy-specific. To identify the zein gene, amplification was made with Zein03/Zein04 primer pairs, and the band expected for this gene (Figure 1A) was found in 18 samples (lane 5-7, 12-23, 26, 28). Accordingly, the samples containing the zein gene were determined to contain corn. In order to determine soy gene in the analyzed food products, Lec1/Lec2 primer pairs were used. In the gel image obtained as a result of amplification, band sizes expected for the lectin gene in four samples (soy flour, soy and corn chips) were obtained, and it was determined that soy was found in the content of food products in which lectin genes were detected. The amplicons were 164 bp (for lectin) and 277 bp (for zein), detected with the DNA extracted from food samples by the Wizard methods and modified CTAB method. The detection of zein and lectin genes accorded with the ingredient list on label of food products. In processed foods, amplification was performed successfully. Tung Nguyen *et al.*, (2009) and Zimmermann *et al.*, (1998) were able to detect the lectin gene in soy-based foods using DNA isolated by the Wizard methods. Scanning for 35S promoter, Nos and tNos terminators was performed to determine the existence of GMOs in food products (Figure 1 B, C). Firstly, 35S promoter, obtained from Cauliflower Mosaic Virus, in samples of 1-5 and 7-29, amplified as a result of PCR scanning by using 35sPF and 35sPR primers, returned positive results by indicating 143 bp expected band size. Therefore, these food products containing soy and corn sold in markets were found to contain GM products in terms of the existence of 35S promoter. Secondly, PCR scanning was performed in food products by using Nos1-Nos3 primer pair for Nos terminator. The 180 bp band size expected as a result of amplification was found in samples 9, 12-15, 20, 21, 23, 28, and 29. Since the Nos terminator was identified in these

samples, these analyzed food products were concluded to be genetically modified. In order to determine tNos terminator existence in food products, the 151 bp band size expected as a result of PCR amplification made by using tNos2-5/ tNos2-3 primer pairs was observed in all samples except 2, 6, 7, and 30. Thus, most of the examined food products were determined to be genetically modified. As a result of DNA isolations performed by using six different isolation methods and two DNA kits, the quality and amount of the obtained DNA shows variations depending on food type and processing level. With the performed analysis, most of the investigated food materials were determined to contain GM products. In parallel with the obtained results, it is concluded that more sensitive control is needed to determine and label GMOs in Turkey.

Arun *et al.*, (2013) determined the present situation of the food products that Turkish consumers eat. For this purpose they made PCR detection of genetically modified maize and soy in mildly and highly processed foods. The screening of the products was based on detection of the CaMV 35S promoter and the nos terminator by PCR. According to the results, 25% of the samples tested were positive for GMOs.

Ozturk (2011) made several scanning in order to determine the qualitative and quantitative contents of corns and foods made from corn. In 8 of 13 samples, the nos terminator sequence was identified, and 3 of those 8 products were found to be baby food samples. As a result of these analyses, it is stated parallel to our results that some of the food products, including baby foods sold in markets, contain GMO-derived products.

Turhan (2008) performed GMO analysis on soy and corn seeds and products such as flour, biscuits, chocolate, chips, cornflakes, starch, and baby foods containing soy or corn. In this study, where 35sPF, 35sPR, and Nos1, Nos3 primers are used for scanning, all imported soy and corn seeds were determined to be genetically modified.

4. CONCLUSIONS

PCR-based techniques for GMO detection have a high sensitivity and specificity (Ahmed, 2002; Anklam *et al.*, 2002; Forte *et al.*, 2005; Meriç *et al.*, 2014). The optimization and development of DNA extraction protocols for the detection of GMOs in foods is getting more and more urgent. Hence, this study was designed to determine the best combination between the eight DNA extraction protocols and the 27 food products in different processing levels. DNA yield and DNA quality are two critical factors in the detection of suitable DNA extraction protocol for each of the food samples. Based on the statistical analysis, the modified CTAB method (protocol 5) resulted in good DNA yield for the extraction of DNA from raw maize (popcorn and GM corn). The Wizard protocols (protocols 1 and 2) and CTAB protocols (protocols 4 and 5) were the best protocols for extracting DNA from GM soy and soy flour. In addition, PCR amplification using DNA templates extracted by the Wizard methods and modified CTAB method were performed successfully in processed

food. This showed that the quantity and quality of the extracted DNA were good enough for PCR amplification. The Wizard methods were the most favorable protocols for DNA extraction from most of the food samples examined in this study. In addition, the modified Wizard methods and CTAB method with addition of BME did not provide any significant increase or difference in DNA yield in all samples except popcorn and GM corn. BME is a potential health hazard and therefore it can be omitted.

The determination of GMO by using PCR amplification depends on the quality and quantity of extracted DNA from raw or processed food. DNA extraction from raw food samples is much easier when compared to processed food. If the appropriate method is used, then efficiency can be maximized when recovering DNA even from highly processed food. The DNA that is obtained must be pure for detection of GMO. A good DNA extraction method should not only give high DNA yield but also high DNA purity (Sambrook *et al.*, 1989; Tung Nguyen *et al.*, 2009).

As a result of DNA isolation performed by using six different DNA extraction methods and two kits, the quality and quantity of obtained DNA varied depending on the type of food and processing level. Finally, the obtained results clearly indicated that the extraction methods of choice depended on the food types. DNA quality was as important as DNA quantity in the determination of a suitable extraction method.

Most of the food materials, examined with performed analysis, were determined to contain GM products. Expected band size as a result of scanning for 35S promoter, Nos and tNos terminators was observed in positive controls (GM corn and GM soy). However the amplification was not found in negative control (wheat). In accordance with these findings, more precise control regarding the detection of GMOs and labeling is needed in Turkey.

In conclusion, we hope that the findings in this study can be used as a guide in selecting suitable DNA extraction methods for the examined food products.

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