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Comparison of Modified DNA Isolation Methods for the Detection of GMO in Processed Foods

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ABSTRACT

The highly degraded DNA content in processed food samples results in limited efficiency in detecting GMOs. Generally, conventional DNA isolation techniques from transgenic plant seeds or raw materials were available in the literature, whereas studies on DNA isolation techniques from processed food samples were more limited. Also, many processed food products contain genomic DNA from numerous complex plants or animal sources. In the present study, we proposed some beneficial modifications for high-quality DNA isolation of processed foods such as biscuits, cakes, crackers, corn chips, and flours. For this purpose, isolation protocols were investigated to obtain high molecular weight and quality DNA from food samples, the first step of GMO analysis in processed foods. To control the gene region of the target organism from the obtained DNA samples, PCR detection was performed with soybean and maize-specific primers. According to the statistical analysis, the A260/A280 ratios were the lowest in cake (1.58) and and highest in biscuit (1.83). The highest values of the total DNA presence belong to soy flour samples (211.80 ug/ul), and the lowest amount belongs to corn flour, cake, and corn chips samples Among the four isolation methods tested, the modified Wizard-CTAB method showed better results in most of the tested food products. Results showed that the modified Wizard-CTAB could be used in different food products for studies on corn and soybean specific genes and GMO detection.

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Introduction

According to the definition of the World Health Organization (WHO), genetically modified organisms (GMO) is defined as the unnatural modification of DNA. According to ISAAA (2021), Genetically modified (GM) seeds were sown in an area of 190.4 Mha in 29 countries in 2019. Soybean, corn, canola, and cotton are the main commercially grown genetically crops. GM soybeans are the most cultivated crop globally, accounting for 48.2% and 91.9 Mha of the global GM crop area. Corn has the most production area following soybeans. It accounts for 32% and 60.9 Mha of the international GMO crop area [1].

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In general, labeling GM products is mandatory or optional, up to a threshold GMO content level that varies between countries, although there is much debate [2]. The EU was one of the first regions to monitor and regulate the use of GMOs. Regulations on the use and law of GMOs in food, (EC) 1829/2003 and 1830/2003, have been in force since 2004. EU rules require that all ingredients in foodstuffs, including source materials, are well documented, and necessary precautions are taken for GMO traceability. Although according to the manufacturer food samples do not contain GMOs, GMO traces may be present in the products incidentally or unavoidably during transportation, storage, seed harvesting, planting, and processing of the food product. For this reason, according to EU law, the mandatory labeling requirement has been established at 0.9% per ingredient [3]. This labeling requirement applies to permitted GMOs. GMOs not authorized by the EU cannot be included in food samples. In Turkey, only accepted soybean and corn varieties are allowed to be used as animal feed within the framework of biosecurity law, but not for food consumption [4].

Food products have different polymerase chain reaction (PCR) inhibitors, such as polyphenols, proteins, and polysaccharides. Accurate detection of GMO presence depends on the specificity and sensitivity of the PCR, DNA isolation method, and the amount and quality of the obtained genomic DNA [5]. Proper sampling methods, inhibitors, biological factors, sample size, and matrix type in GMO detection affect the efficiency of DNA extracted from food, feed, and grain/seed samples. In the processed food industry, the addition of certain flavors and chemical components also changes the DNA quality and creates an inhibitor for amplification. Corn and soy content find wide use as food preservatives and additives. [6-7]. For this reason, it is important to use stadardized test methods applied in feed and food products. The use of these food components in the food industry and the fact that processed products have many contents simultaneously make detection difficult due to the process [8]. If the amount and purity of DNA obtained from the DNA extraction according to the GMO detection procedures are unsuitable, a plant DNA-specific detection method should be performed. If DNA cannot be detected, it should be appropriately reported in the relevant reports that the product does not contain DNA at a level that can be amplified in PCR [9].

Sönmezoğlu and Keskin [10] compared DNA extraction methods specific to processed foods in food products consisting of different varieties. Using six DNA isolation

methods and two commercial DNA extraction kits, Sönmezoğlu and Keskin [10] stated that the DNA yield varied according to the type of food and processing. Arun et al. evaluated the effect of different cooking temperatures on GMO detection in products by preparing cookies containing various amounts of GM soy and cooking them at different temperatures and at different times [11]. As a result, the heating process affected the sensitivity of the PCR screen of GM organisms and increased the detection limit. Three DNA extraction methods were compared to detect GMOs in 35 food products sold in the Equator. As a result of their PCR studies, they stated that DNA extraction with the DNeasy mericon food kit provided higher amplification efficiency and emphasized that other DNA extraction methods may be needed for PCR studies used in various food products [12].

Saadedin et al. [13] detected CaMV-35 promoter and T-nos terminator sequences that control gene expression in genetically engineered tomatoes using qualitative PCR. In the study, 78 tomato genotypes were collected from Iraqi institutions and markets, and DNA isolations were completed by the CTAB DNA isolation method [13]. Matthes et al. [14] focused on the efficiency of DNA extraction methods from corn gluten of protein-rich corn-containing feed samples. Ashrafi Dehkordi et al. [15] in their study for DNA extraction from soybean samples, compared phenol/chloroform methods, CTAB and modified CTAB method. Their results showed that the modified CTAB method is more promising than the other two DNA extraction methods [15].

In this study, standard DNA isolation methods previously mentioned in the literature for food products were used, and these methods were modified for use in some processed food samples. In addition, a new modified isolation protocol (modified Wizard-CTAB) in which Wizard and CTAB methods are used as a hybrid has been tried. In the modified protocol, soybean and corn gene content in packaged food products belonging to different brands was determined to verify the DNA quality and determine the qualitative PCR amplification efficiency in GMO detection. It aims to obtain preliminary information on the isolation efficiency of the methods applied in general isolation protocols on processed foods and provide preliminary data for molecular studies on this subject.

Packaged food products containing soy or corn, such as biscuits, crackers, cakes, corn chips, corn and soy flour were used for DNA extraction. The products were obtained

from local markets between 2019-2020. The products used in DNA extraction consist of 20 different products with different brands and different processing levels (Table 1).

Material and Methods

Food materials

GM maize and GM soybean seed residues used as positive controls were obtained by request from Tubitak-MAM Biotechnology Institute. Wheat (Gediz-75) DNA was used as a negative control in PCR. Food samples were ground into flour by grinding in a mortar, and the experiments were carried out in three repetitions for each food product.

Table 1 Sample samples used in GMO analysis

Sample No	Food Product	Sample No	Food Product
1	Biscuit (brand 1)	**12**	Cracker (brand 2)
2	Biscuit (brand 2)	**13**	Cracker (brand 3)
3	Biscuit (brand 3)	**14**	Corn chips (brand 1)
4	Biscuit (brand 4)	**15**	Corn chips (brand 2)
5	Biscuit (brand 5)	**16**	Corn flour (brand 1)
6	Biscuit (brand 6)	**17**	Corn flour (brand 2)
7	Cake (brand 1)	**18**	Soy flour (brand 1)
8	Cake (brand 2)	**19**	Soy flour (brand 2)
9	Cake (brand 3)	**20**	GM Soy
10	Cake (brand 4)	**21**	GM Corn
11	Cracker (brand 1)	**22**	Negative control

Reagents

Preparation of 200 ml TNE Buffer: After adding 150 mM NaCl, 0.315 g Tris-HCL, 2 mM EDTA, 1% SDS, the total volume was made up to 200 ml with ddH_2O (pH: 8) and then the prepared buffer was autoclaved an than. After autoclaved the 1% β-mercaptoethanol (BME) was added to this solution. Chloroform-isoamyl alcohol (24:1 v/v), NaCl (1.2 M), cold 100% isopropyl alcohol, 70% ethanol, agarose (molecular grade) were used in DNA isolations. CTAB precipitation solution was prepared with NaCl (40 mM) and CTAB (0.5%) and the pH was adjusted by to 8.0. For the CTAB Lysis buffer, Tris/HCl (100 mM), Na2EDTA (20 mM), NaCl (1.4 M), CTAB (2% w/v) was used, then the pH was adjusted to 8.0.

DNA extraction methods

Method-1; Modified Wizard method [16, 10] method-2; CTAB isolation method [14], method-3; modified Wizard-CTAB and the method-4; modified classical CTAB method [17] are used for this study.

Food samples were prepared as homogenized samples, 50-100 mg each, in equal proportions. The reason why this amount is relatively low is to ensure homogeneous distribution in buffer solutions. For example, samples such as corn chips over-absorb the buffer solution and limit the amount of supernatant after centrifugation. To overcome this situation, the amount of buffer solution can be increased if needed.

For the repeatability of the extraction, the DNA isolation methods were applied in three repetitions. Distilled water was put in place one sample in each set during the experiment against possible contamination risks caused by the environment.

The Modified wizard method (method-1) [16, 10], CTAB isolation method (method-2) [14], and modified classical CTAB method (method 4) [17] used in DNA extraction of the food products examined in the study were applied based on the procedures specified in the source articles. Modified Wizard-CTAB method is explained in this study.

DNA isolation method-3 (modified Wizard-CTAB)

50-100 mg sample was weighed and mixed with 1000 µl TNE buffer and 30 µl Proteinase K (20 mg/ml). This mixture was kept in a 65 °C water bath for three hours and stirred every 15 minutes. Samples were incubated at 65 °C for an additional one hour by adding 5 µl (10 mg/ml) of RNase to the mixture. After centrifuging at 15 000 rpm for 15 minutes, the supernatant was taken into a new sterile Eppendorf tube. The same volume of Chloroform/isoamyl alcohol (24: 1) was added to it. After 10 minutes of centrifugation at 13 000 rpm, the supernatant was taken into new tubes. 2/3 isopropanol was added. Further sedimentation at 13 000 rpm for 20 minutes, the pellet was dissolved in 400 µl TE buffer and incubated overnight at 4°C. The next day, sample tubes were dissolved in a 60 °C water bath, and Chloroform/isoamyl alcohol (24:1) was added again to the tubes. After 15 minutes of centrifugation at 13 000 rpm, the supernatant was taken into new tubes. Nine µl of 3M sodium acetate (pH 5.2) and 30 µl of absolute ethanol solution were added to the supernatants taken for the precipitation step and mixed. The mixture is incubated on ice for 15 min to precipitate the DNA. Afterward, it is centrifuged at 13 000 rpm for 10 minutes. The supernatant was taken

into a new tube. Three µl of sodium acetate and 500 µl of pure ethanol solution were added and incubated on ice for 15 minutes again. The samples were precipitated by centrifugation at 13 000 rpm for 10 minutes, and the pellet was washed with 70% alcohol. Samples were re-centrifuged, dried thoroughly free from alcohol, and dissolved in 100 µl ddH_2O and used.

Purity and concentration of DNA

Gel electrophoresis method and spectrophotometric techniques were used for the amount and purity determination of the isolated DNA. For this purpose, the samples were measured with NanoDrop (Denovix, DS-11 Spectrophotometer) at 260-280 nm wavelengths, and quantitative determinations were made [18]. Genomic DNAs obtained were run in 1% agarose gels with 1 X TBE buffer. Gels were stained with ethidium bromide (20 mg/ml) for visualization. After running, the gels were visualized using a UV transilluminator (BioRad, ChemiDocTMMP Imaging System).

Molecular screening of soy and corn gene

PCR processes were carried out using the Bio-RAD C1000 Touch Thermal Cycler. All primers used in the study were synthesized by Iontek company according to the base sequence in the reference articles. LEC1 / LEC2 primer pairs (164 bp) [19] were used to screening the presence of the lectin gene in the determination of soy content, and the ZEIN03 / ZEIN04 (277 bp)[20] primer pairs were used for corn.

Mixture solution prepared for PCR amplification for soy and corn determination of food samples; 10x Taq Buffer (Thermo Fisher Scientific), 25 mM $MgCl_2$ (Thermo Fisher Scientific), 3.2 µl of dNTP mix solution (Sigma Aldrich), 1 µl of 10 mM forward and reverse primer (Iontek Company), 0.5 U of Taq DNA polymerase (Thermo Fisher Scientific), 100 ng of DNA template. The mixing volume of the reaction was 40 µl. PCR cycles for the Lec1/Lec2 primers are as follows; Initial denaturation at 95 °C for 12 min followed by 95 °C for 60 s, 72 °C for 30 s and 72 °C for 30 s; in the last step, it is completed by extension at 72 °C for 10 minutes. PCR cycle for primer pair Zein03/Zein04, incubation at 95 °C for 10 minutes, followed by 60 seconds at 96 °C, 60 seconds at 60 °C, and 60 seconds at 72 °C, final extension of 72 °C was applied at for 10 minutes. The number of cycles for both primer pairs is planned as 40 cycles.

GMO screening

For the amplification of the 35S promoter region, primers P35s-cf3/cr4 [21-22] were used. The band sizes expected to be seen in a positive control due to PCR using these primers are expected to be 123 base pairs for the 35SP primer.

The PCR were based on the conditions specified in the source articles. The total reaction volume for the PCR mix was set to 25 µL and included 10x Taq Buffer, 0.24 µM of each reverse and forward primers, 160 µM of each dNTP, 1.5 mM $MgCl_2$, 0.5 U of Taq DNA polymerase, 100 ng of template DNA and ddH_2O.

Statistical analysis

Two-way analysis of variance (ANOVA) was used to evaluate the spectrophotometric data. Measurements were taken in duplicate, using SPSS 15.0 software according to random block design, and the Duncan test was used to compare the mean data.

Results and Discussion

High-density DNA fragments were visualized using gels with 1% agarose concentrations from samples obtained from various food products. Different DNA isolation protocols were applied for DNA isolation from the food products examined. It was determined that the high-density distinct band profiles for the Wizard method (a) and the modified Wizard-CTAB method (c) (this study) belonged to the soy flour samples (Fig. 1). The classical CTAB DNA isolation method is primarily suitable for DNA extraction from green plants or seeds. For this reason, its effectiveness on processed food samples is relatively low compared to other isolation methods on agarose gel images. According to the agarose gel images, the classical CTAB isolation method (Fig 1d) yielded clean band profiles for the GM corn sample (lane 21). However, according to the other three DNA isolation methods, the GM corn sample (lane 21) did not show a clean and dense band profile only the GM soy sample. This result may be because the GM corn sample was obtained with unprocessed cornmeal. The quantity and quality of DNA isolated from food, seed/cereal, or feed samples are affected by sample size, optimal sampling method, biological factors, inhibitors, and matrix type [23, 24, 8].

There are drifts towards the gel's lower molecular weight portions for the biscuit, cake, and cracker samples (Fig 1). In addition, the presence of a higher molecular weight and thick band in the gel means that genomic DNA is intact and minimally contaminated

[25]. For the corn chips sample (lane 14), a band profile was not obtained in all the DNA extraction methods. Corn chips sample (lane 15) showed a slight band presence only for the Wizard isolation method. It is reported that baking affect negatively the DNA isolation yield and PCR test results [8, 11, 26, 27].

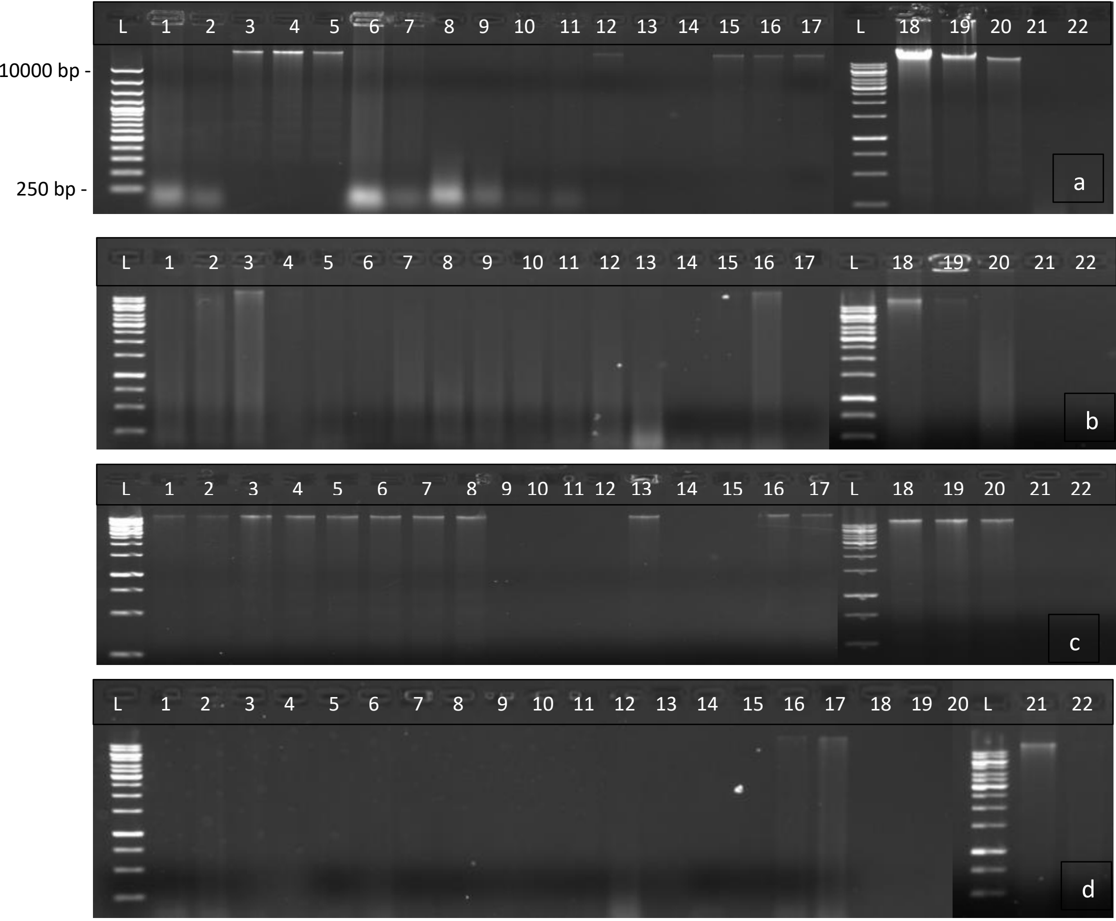

Fig 1 Agarose gel images of different DNA isolation protocols a) DNA Isolation Method-1, b) DNA Isolation Method-2, c) DNA Isolation Method-3 and d) DNA Isolation Method-4, respectively. Lane L, The GeneRuler™ 1 kb DNA Ladder (Thermo scientific). Lane 1-6 biscuit, 7-10 cake, 11-13 cracker, 14-15 corn chips, 16-17 corn flour, 18-19 soy flour, 20 GM soy, 21 GM corn, and the 22 is negative control (dH_2O)

The DNA concentrations, 260/280 ratios obtained due to the DNA isolation methods examined in this study vary according to product type (Table 2). The 260/280 ratio provides information on the purity and quality of the DNA. According to this ratio, values of 1.7 or greater can be considered acceptable [28]. The 1.8 ratio is a DNA grade with high purity in the absence of protein and phenolic compounds. As this ratio rises above 2, RNA contamination can be mentioned [29]. The range of calculated DNA

yields was between 8.27 ug/ul (Method-2, Cake) and 593.58 ug/ml (Method-1, Soy flour). According to the statistical analysis, the A260/A280 ratios were the lowest in cake (1.58) and and highest in biscuit (1.83). The highest values of the total DNA presence belong to soy flour samples (211.80 ug/ul), and the lowest amount belongs to corn flour, cake, and corn chips samples (Tablo 2). In addition to ingredients such as chocolate and sauces, it is difficult to obtain quality and intact whole genomic DNA from samples containing starch and lecithin [30]. For this reason, it is supported that besides the negative effect of the product processing level, the differences arising from the product composition may cause different results in terms of sample type. Turkec et al., [31] also pointed out that although the DNA yield among the products they examined differed according to the isolation method examined, generally lower purity values were obtained in products with medium and high processed corn content [32].

Subsequently, as expected, the lowest DNA yield was in the samples of cake (52.55 ug/ul) and corn chips (58.16 ug/ul), which are food samples with high processed levels. This result confirms a decrease in the amount of DNA depending on the processing levels in foods.

A purity ratio of> 1.9 indicates RNA, while a ratio of <1.7 in the extracted DNA sample indicates the presence of proteins in these samples [33]. According to all modified isolation methods examined in this study, the average 260/280 ratio of cake (1.58) and corn chips (1.68) in food samples was less than 1.7. The average ratio of 260/280 for other food samples examined were among 1.7 and 2.0, and these results indicate insignificant contamination levels by protein and polysaccharides in genomic DNA extracted [34]. The mean values of the 260/280 ratio for all samples were below 2.0, and this result indicates the minimal nucleic acid contamination in food samples in terms of the isolation protocols examined.

Spectrophotometric measurement results (Table 2) obtained from Method-4 showed lower or non-optimal values compared to the other three isolation methods. These results are consistent with the unclear band profiles obtained from the agarose gel electrophoresis images (Fig. 1d). Method-1, on the other hand, gave very high values in terms of DNA yield compared to other DNA isolation methods. Still, it should be considered that these high rates may be due to contamination when compared with agarose gel photographs (Fig 1a).

Turkec et al. [31] examined DNA extraction methods to evaluate GMO detection in Turkey's commercially available food and feed products containing corn. According to their observations, the CTAB method was the most suitable for raw soy and corn and highly processed food samples than commercial kits. The purity of the CTAB method was found to be above 1.5 for the samples examined, excluding the cornbread, indicating the suitability of the extracted DNA for amplification analysis [31, 35]. For the samples examined in this study, the mean was above 1.7 in all samples except soy flour and cake.

Mathess et al. [14] proposed a modified CTAB protocol for DNA extraction from protein-rich corn feeds. This DNA extraction protocol was used in this study with minor modifications (DNA isolation method-2). Accordingly, while the DNA yields were between 20.67 and 82.14 ng/ml in method-2, the 260/280 ratio was found between 1.45 and 1.88 (Table 2).

Table 2 Concentration and purity genomic DNA extracted various protocols

	DNA Isolation Method-1		DNA Isolation Method-2		DNA Isolation Method-3		DNA Isolation Method-4			
Food Product	**ng/ul**	**260/280**	**ng/ul**	**260/280**	**ng/ul**	**260/280**	**ng/ul**	**260/280**	**Mean* ng/ul**	**Mean* 260/280**
Biscuit	125.22ab	1.80a	52.19ab	1.88c	68.43ab	1.77b	35.05b	1.86c	70.22	1.83
Cake	118.87ab	1.72a	45.26ab	1.61ab	37.81ab	1.58ab	8.27a	1.4a	52.55	1.58
Cracker	274.58bc	1.80a	26.08ab	1.76c	57.83ab	1.58ab	68.02b	1.79bc	106.62	1.73
Corn chips	81.80ab	1.67a	45.24b	1.76c	58.98ab	1.72ab	46.65ab	1.57a	58.16	1.68
Corn flour	36.16a	1.75a	25.40ab	1.76c	25.03ab	2.05c	16.86a	1.67ab	32.86	1.81
Soy flour	593.58d	1.89a	82.14b	1.75abc	107.12cd	1.71bc	63.98ab	1.47a	211.80	1.71
GM Soy	380.77c	1.83a	78.53b	1.81bc	159.47e	1.75a	12.65a	2.14d	157.86	1.81
GM Corn	150.84ab	1.81a	20.67a	1.45a	134.61d	1.94c	13.66a	1.74abc	79.85	1.74

DNAs of the samples extracted by DNA extraction method-3 were used as template DNA for PCR studies (Fig 2). Most processed food products contain different genomic DNA content from various animal and plant sources. Within this complex matrix, only a small fraction of the genomic DNA used as a template for PCR contains the appropriate target for amplification. For this reason, in this research, the lectin gene's presence to determine the content of soy products and the presence of the zein gene to determine the content of corn products were performed (Fig. 2).

The samples containing soy content were biscuit, cake, crackers, corn chips, and soy flour (Fig. 2a). Soy flour and biscuit samples showed more apparent band profiles, showing the correct band profile in the expected base pair range. On the other hand, cake samples did not show significant band profiles in the agarose gel in soy content. Compared to average of the four isolation protocols, DNA quality has the lowest for cake samples (1.58). The DNA quality results showed that the DNA quality ratios of biscuit and soy flour samples, which are the sample groups with the cleanest band profile in determining the soy content, were 1.83 and 1.81, respectively (Table 2). These results suggest that DNA quality may be adequate in PCR results, a sensitive detection method depending on the product type and processing level.

According to the agarose gel image of the PCR results (Fig. 2b), the presence of the Zein gene was detected in the biscuit, corn chips and the cornflour. A band profile of the corn content was not obtained among cake and cracker. Although these results are expected for the cornflour samples, the corn content compared to the soybean content was less.

Arun et al. [36] analyzed soy-specific lectin and corn-specific zein sequences found in GM and non-GMO soy and corn, respectively, in CaMV 35S and nos negative samples to eliminate false-negative results. The distribution of positive products in soy and corn content from the screened products were, 14 (32.6%) of 43 corn samples and 11 (19.3%) of 57 soybeans. Transgenes of food products such as sugar, vegetable oils, and highly processed carbohydrates exposed to mechanical, high temperature, and chemical factors are degraded and damaged [37, 38]. Although this situation can be overcome with the efficiency of the isolation protocol, phenolic acid residues or polysaccharides cannot be removed entirely from the genomic DNA during DNA isolation. These contaminants affect and even inhibit the activation of DNA polymerase during PCR amplification [36, 39, 40]. However, small amounts of DNA can be amplified by PCR, but there may be differences in DNA quality and yield in band profiles according to the agarose gel results.

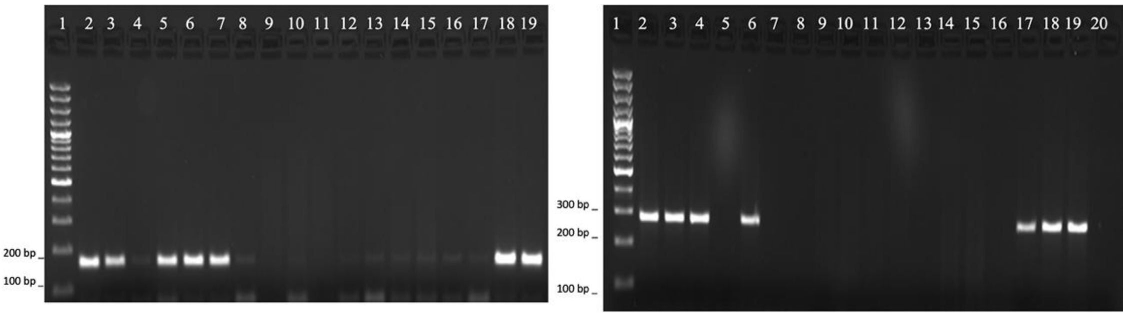

Fig 2 Agarose gel image of PCR samples with lectin (a) and zein (b) primer a)- 1) Thermo 100 bp DNA Ladder 2) GM Soy (positive control); 3-8) biscuit; 9-12) cake; 13-15) cracker; 16-17) corn chips; 18-19) soy flour. b)- 1) Thermo 100 bp DNA Ladder 2) GM Corn (positive control); 3-8) biscuit; 9-12) cake; 13-15) cracker; 16-17) Corn chips; 18-19) Corn flour; 20) Wheat DNA (negative control)

As a result of the GMO screening for the 35S promoter gene region, the expected band size was determined was only the corn flour (Fig 3). There are strict legal measures on using GMO in food products in Turkey [4]. In this study, GMO screening of genomic DNA samples obtained according to the Method-3 isolation protocol was evaluated using the amplification results of the 35S promoter region (Fig. 3).

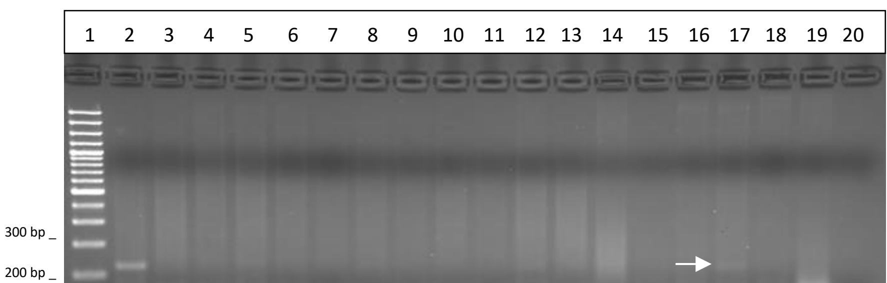

Fig 3 Agarose gel image of PCR samples with 35s cf3/cf4 primer 1) Thermo 100 bp DNA Ladder 2) GM Soy positive control); 3-8) biscuit; 9-11) cake; 12-14) cracker; 15) Corn chips; 16-17) Corn flour; 18-19) Soy flour; 20) Wheat DNA (negative control)

GMO content was not found in any tested food samples, except for one corn sample (sample 17). CTAB and commercial kit isolation methods were applied to the samples examined in a study in which GMO detection was performed in chips and breakfast products offered for sale in Turkey. A sufficient quality and amount of DNA could not be obtained in any of the samples examined so this study results support that product processing processes cause DNA damage [41]. Artuvan and Aksay, in which GMO content was screened in baby formulas and baby continue milk offered for sale in markets in Turkey, p35S, tNOS and pFMV transgenic contents were not found [42]. In other studies, on GMOs in food products in Turkey, no GM content was found in terms

of soy and corn content [10, 11, 31, 45]. It is recommended to examine the low amount of GMOs determined by qualitative and quantitative studies by sequence analysis in the next step. It should be noted that the band 17th sample (Fig 3) in this study may be positive for GMO content at low concentrations, which can be attributed to accidental contamination in the transfer or the same production line. However, the complex zygotic structure of corn is a limiting factor for GMO content because not all tissue types have the same GMO content. In the production of corn starch, maize flour, seed coat and embryo are separated. In snacks containing corn, the endosperm is milling and used as a raw material [43]. Embryo is diploid, endosperm is triploid and pericarp is haploid [44]. Therefore, the GMO content of the corn sample may be higher than in the other processed food samples studied. It is recommended to perform quantitative analyses to determine the limit threshold value determined by the relevant laws in GMO analysis studies conducted with the traditional PCR method and sequence analyzes for the result [36, 45].

Conclusion

In the present study we investigated the most suitable DNA isolation protocols for different types and brands samples at different processing levels. Although the most suitable isolation method for different sample types varies, it was determined that the appropriate isolation protocol was the modified Wizard-CTAB method among the four isolation methods tested. It was determined that the highest DNA content was obtained from the biscuit samples, and the lowest DNA content was obtained from the cake samples among the biscuit, cake, cracker, and chips samples examined.

Such studies are important to determine the market situation of food products in terms of food safety and legal control. In this study, the traditional PCR method, which is one of the methods used for routine control analysis, was used. It is desired to draw attention to DNA isolation yield or quality on PCR yield in screening corn and soybean assets, which is essential for GMO analysis in food samples with complex matrix content.

Abbreviations

ANOVA: Analysis of variance; BME: Beta-mercaptoethanol; CaMV: Cauliflower mosaic virus; CTAB: Cetyltrimethylammonium Bromide; DNA: Deoxyribonucleic acid; dNTP: Deoxynucleotide triphosphates; EC: European commission; EDTA: Ethylenediamine tetraacetic acid; EU: European union; GMO: Genetically modified organisms; ISAAA: International service for the acquisition of agri-biotech applications; Mha: million hectares; NaCl: sodium chloride; PCR: Polimerase chain reaction; RNA: Ribonucleic acid; RNAse: Ribonuclease; rpm: revolutions per minute; SDS: Sodium dodecyl sulfate;

SPSS: Statistical package for the social sciences; TBE: Tris-Boric acid EDTA; UV: Ultraviolet; WHO: World health organization.

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Availability of data and material
Please contact the corresponding author for any data request.

References

1. ISAAA, ISAAA Brief 55-2019: Executive Summary Biotech Crops Drive Socio-Economic Development and Sustainable Environment in the New Frontier. 2019. In: https://www.isaaa.org/resources/publications/briefs/55/executivesummary/default.asp.
2. Mahgoub, S.E., Regulation of GMOs. Testing and Analysis of GMO-containing Foods and Feed, S.E. Mahgoub, and L.M., Nollet, Editors. 2019, CRC Press. Boca Raton: Florida. p. 22-25.
3. Davison, J., GM plants: Science, politics and EC regulations. Plant Science, 2010. 178(2): p. 94-98.
4. Regulation (TR). Turkish regulation on genetically modified organisms and their products; Official Gazette No: 27671. 2010. Accessed June 20, 2020. http://www.resmigazete.gov.tr/eskiler/2010/08/20100813-4.htm.
5. Singh, M., et al. Development and Utilization of Analytical Methods for Rapid GM Detection in Processed Food Products: A Case Study for Regulatory Requirement. Research Square (in press, 2022), 2022.
6. Ramos-Gómez, S., et al. Development of a method to recovery and amplification DNA by real-time PCR from commercial vegetable oils. Food Chemistry, 2014. 158, p. 374-383.
7. Singh, M., et al. Efficient DNA Extraction Procedures for Processed Food Derivatives—a Critical Step to Ensure Quality for GMO Analysis. Food Analytical Methods, 2021. 14(11): p. 2249-2261.
8. Gryson, N., Effect of food processing on plant DNA degradation and PCR-based GMO analysis: a review. Analytical and Bioanalytical Chemistry, 2010. 396(6): p. 2003-2022.
9. Linnhoff, S., et al., An examination of millennials' attitudes toward genetically modified organism (GMO) foods: is it Franken-food or super-food? International Journal of Agricultural Resources, Governance and Ecology, 2017. 13(4): p. 371.
10. Sönmezoğlu, Ö. and H. Keskin, Determination of genetically modified corn and soy in processed food products. Journal of Applied Biology & Biotechnology, 2015. (3): p. 32-37.
11. Arun, Ö.Ö., The effect of heat processing on PCR detection of genetically modified soy in bakery products. Journal of Food and Health Science, 2016. p. 130-139.
12. Pacheco Coello, R., et al. Comparison of three DNA extraction methods for the detection and quantification of GMO in Ecuadorian manufactured food. BMC Research Notes, 2017. 10(1): p. 758.
13. Saadedin, S., M. Abbas and A. Suleiman, Detection of CaMV-35S Promoter and NOS Terminator in Genetically Modified Tomato Seed in Iraqi Markets. Iraqi Journal of Biotechnology, 2019. 18(2): p. 160-168.
14. Matthes, N., et al. Validation of a modified CTAB method for DNA extraction from protein-rich maize feedstuffs. Journal of Consumer Protection and Food Safety, 2020. 15(4): p. 331-340.

15. Ashrafi-Dehkordi, E., S.M. Mazloomi, and F. Hemmati, A comparison of DNA extraction methods and PCR-based detection of GMO in textured soy protein. Journal of Consumer Protection and Food Safety, 2021. 16(1): p. 51-57.
16. Tung Nguyen, C., et al. Comparison of DNA Extraction Efficiencies Using Various Methods for the Detection of Genetically Modified Organisms (GMOs). International Food Research Journal, 2009. 16: p. 21-30.
17. Doyle, J.J. and J.L. Doyle, Isolation of Plant DNA From Fresh Tissue. Focus (Madison), 1990. 12: p. 13-15.
18. Wilfinger, W.W., K. Mackey, and P. Chomczynski, Assessing the Quantity; Purity and Integrity of RNA and DNA following Nucleic acid purification. DNA sequencing II optimising preparation and cleanup, 2006. p. 291-312.
19. Vollenhofer, S., et al. Genetically Modified Organisms in FoodScreening and Specific Detection by Polymerase Chain Reaction. Journal of Agricultural and Food Chemistry, 1999. 47(12): p. 5038-5043.
20. Pauli, U., et al. Extraction and Amplification of DNA From 55 Foodstuffs. Mitteilungen aus Lebensmitteluntersuchung und Hygiene, 2000. 91: p. 491-501.
21. Cardarelli, P., et al. Detection of GMO in food products in Brazil: the INCQS experience. Food Control, 2005. 16(10): p. 859-866.
22. Lipp, M., et al. 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, 2001. 212(4): p. 497-504.
23. Ahmed, F.E., Testing of Genetically Modified Organisms in Food. 2004, Binghamton, New York: Food Products Press.
24. Laffont, J.L., et al. Testing for adventitious presence of transgenic material in conventional seed or grain lots using quantitative laboratory methods: statistical procedures and their implementation. Seed Science Research, 2005. 15(3): p. 197-204.
25. Shokere, L.A., M.J. Holden, and G. Ronald Jenkins, Comparison of fluorometric and spectrophotometric DNA quantification for real-time quantitative PCR of degraded DNA. Food Control, 2009. 20(4): p. 391-401.
26. Bauer, T., The effect of processing parameters on DNA degradation in food. European Food Research and Technology, 2003. 217(4): p. 338-343.
27. Gryson, N., K. Dewettinck, and K. Messens, Detection of Genetically Modified Soy in Doughs and Cookies. Cereal Chemistry Journal, 2007. 84(2): p. 109-115.
28. Sambrook, J., and D. Russell, Molecular cloning: a laboratory manual, 3rd ed. 2001, New York, ABD: Cold Spring Harbor Laboratory Press.
29. Ateş Sönmezoğlu, Ö., and B. Terzi, Characterization of some bread wheat genotypes using molecular markers for drought tolerance. Physiology and Molecular Biology of Plants, 2018. 24(1): p. 159-166.
30. Greiner, R., U. Konietzny, and A.L.C.H. Villavicencio, Qualitative and quantitative detection of genetically modified maize and soy in processed foods sold commercially in Brazil by PCR-based methods. Food Control, 2005. 16(8): p. 753-759.
31. Turkec, A., et al. DNA extraction techniques compared for accurate detection of genetically modified organisms (GMOs) in maize food and feed products. Journal of Food Science and Technology, 2015. 52(8): p. 5164-5171.
32. DiBernardo, G., et al. Comparative Evaluation of Different DNA Extraction Procedures from Food Samples. Biotechnology Progress, 2007. 23(2): p. 297-301.
33. Abdel-Latif, A., and G. Osman, Comparison of three genomic DNA extraction methods to obtain high DNA quality from maize. Plant Methods, 2017. 13(1): p. 1.

34. Pervaiz, Z.H., et al. Methodology A modified method for high-quality DNA extraction for molecular analysis in cereal plants. Genetics and Molecular Research, 2011. 10(3): p. 1669-1673.
35. Querci, M., et al. From sampling to quantification: developments and harmonisation of procedures for GMO testing in the European Union. Collection of Biosafety Reviews, 2007. 3: p. 8-14.
36. Arun, Ö., F., Yılmaz, and K. Muratoğlu, PCR detection of genetically modified maize and soy in mildly and highly processed foods. Food Control, 2013. 32(2): p. 525-531.
37. Anklam, E., et al. Analytical methods for detection and determination of genetically modified organisms in agricultural crops and plant-derived food products. European Food Research and Technology, 2002. 214(1): p. 3-26.
38. Rastegar, H., et al. Investigation of transgenic elements in genetically modified maize germ (Zea mays) and maize germ oil distributed in local market by qualitative PCR method. Human, Health and Halal Metrics, 2021. 1(2): p.64-70.
39. Jia, R., et al. Microbiologically influenced corrosion and current mitigation strategies: A state of the art review. International Biodeterioration & Biodegradation, 2019. 137: p. 42-58.
40. Aboul-Maaty, N.A.F., and H.A.S. Oraby, Extraction of high-quality genomic DNA from different plant orders applying a modified CTAB-based method. Bull Natl Res Cent, 2019. 43(1): p. 25.
41. Mutlu, Ş., O., Şimşek, and Ö. Öksüz, Investigating The GMO Existence in Chips and Breakfast Cereals Marketed in Turkey. Tekirdağ Ziraat Fakültesi Dergisi, 2021. 18(3): p. 375-385.
42. Artuvan, E., and S., Aksay, Investigation of the Genetically Modified Organism (GMO) Existence in Baby Foods on the Market in Turkey. Turkish Journal of Agriculture-Food Science and Technology, 2021. 9(10): p. 1945-1952.
43. Yoshimura, T., et al. Applicability of the quantification of genetically modified organisms to foods processed from maize and soy. Journal of Agricultural and Food Chemistry, 2005. 53(6): p. 2052-2059.
44. Zhang, D., A., Corlet and S., Fouilloux, Impact of genetic structures on haploid genome-based quantification of genetically modified DNA: theoretical considerations, experimental data in MON 810 maize kernels (Zea mays L.) and some practical applications. Transgenic Research, 2008. 17(3): p. 393-402.
45. Avsar, B., et al. Identification and quantitation of genetically modified (GM) ingredients in maize, rice, soybean and wheat-containing retail foods and feeds in Turkey. Journal of Food Science and Technology, 2020. 57(2): p.787-793.