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Comparison of DNA isolation methods for GMO detection from biscuit samples

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Bisküvi örneklerinden GDO tespiti için DNA izolasyon metotlarının karşılaştırılması

Abstract: The global increase in genetically modified organism (GMO) content in feed and food products has necessitated the development of precise detection methods to differentiate between biotechnologically derived foods and those without GMOs. Despite the various regulations in different countries, an internationally consistent approach to labeling GMO products is needed. For this reason, there is a widespread need to develop effective GMO detection methods to provide reliable and transparent food safety to consumers. The first experimental step in creating accurate and reliable detection methods for GMOs is effective DNA isolation. Determining DNA isolation methods specific to different processing levels of foods is very important. This study was aimed to compare different DNA extraction methods in biscuit samples. For this reason, DNA from different biscuit samples was isolated using three different protocols (CTAB-PVP, modified CTAB-1, and modified CTAB-2). DNA samples extracted from biscuits, were evaluated regarding DNA concentration and purity. According to the results obtained, the CTAB-2 procedure generally showed the best performance in terms of both DNA amount and purity rates for biscuit samples.

Key words: Biscuit, CTAB, DNA extraction, GMO, food safety

Özet: Genetiği değiştirilmiş organizmaların (GDO) kullanıldığı yem ve gıda ürünlerinin dünya çapında artış göstermesi, biyoteknolojik yollarla elde edilen bu gıdalar ile, GDO içermeyen gıdalar arasında ayrım yapabilen tespit yöntemlerinin geliştirilmesine yol açmıştır. Farklı ülkelerdeki çeşitli düzenlemelere rağmen, GDO'lu ürünlerin etiketlenmesi konusunda uluslararası düzeyde tutarlı bir yaklaşıma ihtiyaç vardır. Bu sebeple tüketicilere güvenilir ve şeffaf bir gıda güvenliği sağlamak açısından etkili GDO tespit yöntemlerinin geliştirilmesine dair ihtiyaç oluşmaktadır. GDO'ya yönelik doğru ve güvenilir tespit yöntemlerinin oluşturulmasında ilk deneysel işlem basamağı etkili bir DNA izolasyonudur. Bu konuda farklı işlenmişlik seviyesindeki gıdalara özgü DNA izolasyon yöntemlerinin belirlenmesi oldukça önemlidir. Bu çalışmada, bisküvi örnekleri için farklı DNA ekstraksiyon yöntemlerinin karşılaştırılması amaçlanmıştır. Bu sebeple farklı bisküvi örneklerine ait DNA'lar, seçilen üç faklı protokol (CTAB-PVP, modifiye CTAB-1 ve modifiye CTAB-2) kullanılarak izole edilmiştir. İşlenmiş gıda ürünleri olan bisküvilerden ekstrakte edilen DNA örnekleri, DNA konsantrasyonu ve saflığı bakımından değerlendirilmiştir. Elde edilen sonuçlara göre bisküvi örnekleri için hem DNA miktarı hem de saflık oranları bakımından genel olarak en iyi performansı CTAB-2 prosedürü göstermiştir.

Anahtar Kelimeler: Bisküvi, CTAB, DNA ekstraksiyonu, GDO, gıda güvenliği

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1. Introduction

The labeling of products containing genetically modified organisms (GMOs), which have been artificially altered to obtain desired traits, is subject to varying regulations in different countries (Arvas and Kocaçalışkan, 2020). For instance, the European Union (EU), Korea, and Japan have legal frameworks that mandate necessary measures for the traceability of GMOs. According to EU law, mandatory labeling procedures are applied for GMO content exceeding a certain threshold (0.9%) (Davison, 2010). However, in most African countries, labeling is still not obligated (Gbashi et al., 2021). In Türkiye, within the scope of the Biosafety Law, the use of plant products containing GMOs in food products and production processes is prohibited (Regulation, TR). Nevertheless, certain transgenic products are allowed to enter the country with the necessary permits, particularly for animal feed, and the likelihood of imported agricultural products being genetically modified increases daily (Arvas and Yılmaz, 2019). Therefore, the development and effective use of GMO detection methods are crucial to ensure the proper implementation of labeling and regulatory standards at an international level, providing consumers with more reliable and transparent food safety.

Today, most of the corn and soybean crops grown are genetically modified. In the United States, most processed foods on the market contain GMOs (Abrams et al., 2024). Among GMOs, soybeans, corn, and canola are the most commonly grown plant sources found in many food products (Erkan and Destan, 2017; Soylu et al., 2020; Ashrafi-Dehkordi et al., 2021; Abrams et al., 2024). Genetically modified soybeans are the most widely cultivated crop globally, while corn, following soybeans, is the second most extensively produced plant product (ISAAA, 2019). The agricultural sector heavily utilizes GMO products for animal feed, and GMO components are frequently encountered in processed food products. These products include processed corn starch, soybean-based oils,

and high-fructose corn syrup (Abrams et al., 2024). Approximately 77% of global soybean production comes from genetically modified soybeans. In leading soybean-producing countries such as Brazil, the United States, and Argentina, most of these 'biotech crops' are glyphosate-tolerant varieties, with adoption rates ranging from 94% to 100% (Bøhn and Millstone, 2019).

The global increase in GMO-containing soybean feed and food products is driving the development of GMO detection methods worldwide. These methods must detect, identify, and quantify added DNA or expressed proteins. However, using processed and highly refined components can make DNA and protein detection challenging or even impossible when soybean or corn-derived components are present in low concentrations (Gryson et al., 2002; Aksoy and Ateş Sönmezoğlu, 2022).

GMO-containing foods can be identified through various biomolecules such as specific proteins, RNA, DNA, and metabolites. Among these targets, DNA is the only molecule that is stable, abundant, and easily amplifiable (Lin and Pan, 2016). Heat transfer processes, such as cooking, baking, drying, sterilizing, or freezing, are integral to almost every food processing operation (Vijayakumar et al., 2009). Therefore, the detection of specific DNA sequences using a PCR-based approach remains the most effective strategy (Lin and Pan, 2016; Singh et al., 2021). However, DNA quality in food products can deteriorate due to processing procedures. A validated extraction method is a prerequisite for obtaining detectable quantities of DNA with acceptable purity, not only for DNA-based food authentication (Ramos-Gómez et al., 2014) but also for GMO testing. In this context, an effective DNA isolation process is the first step in accurately and reliably detecting GMOs. However, specific flavors or chemical contents in processed foods can alter DNA quality and act as inhibitors for amplification (Ramos-Gómez et al., 2014; Singh et al., 2021). The presence of multiple components in processed foods, especially in the food industry, complicates the process by potentially providing insufficient quality and quantity of DNA for PCR amplification (Aksoy and Ateş Sönmezoğlu, 2022). Complex matrices, such as chocolate and biscuits, contain a range of PCR inhibitors like polysaccharides and polyphenols that can hinder DNA amplification.

Additionally, challenges in amplifying the lectin gene in contents like chocolate, biscuits, and cakes arise due to the low concentrations of soybean components found in sweetener industry products (Gryson et al., 2004). Thus, an efficient extraction procedure is crucial. To address these challenges, research in the literature has focused on using DNA extraction methods from processed foods for effective GMO analysis. Di Pinto et al. (2007) compared two different commercial kits (Wizard® Magnetic DNA Purification Kit and DNeasy® Tissue Kit) for DNA extraction from various food matrices, finding that the Wizard® Magnetic DNA Purification Kit was suitable for some vegetable matrices, while the DNeasy® Tissue Kit was more appropriate for other complex and processed matrices. Mafra et al. (2008) compared the CTAB (Cetyltrimethylammonium Bromide) method with three different commercial kit procedures, performing DNA extraction from various food products derived from soybeans. They demonstrated that the Wizard method was suitable for highly processed foods. Turkec et al. (2015) applied DNA extraction methods to corn products, including flour, starch, bread, cereal, chips, biscuits, diet breakfast cereals, canned corn seeds, and feed samples, and recommended the Wizard, Genespin, or CTAB methods for the highest DNA content. Singh et al. (2021) reported on the use of CTAB methods and modifications of the DNeasy Mericon Food Kit for a wide range of food products, from oils such as canola, cotton, mustard, and soybean to other products like apple juice, green apple, corn, potatoes, soy, and tomatoes, emphasizing that DNA extraction is a critical step in GMO detection tests for food derivatives.

Commercial kits are more expensive compared to traditional CTAB-based DNA extraction methods and are suitable for only a limited number of samples. In contrast, conventional CTAB-based DNA extraction methods are more cost-effective, with the necessary chemicals and materials often being readily available, providing researchers with affordable options for large-scale studies. These procedures can be modified according to laboratory conditions, sample types, or experimental requirements. Ateş Sönmezoğlu and Keskin (2015) compared eight different DNA extraction protocols, including two commercial kits, for 27 processed food products. They reported that extraction kits (protocols 7 and 8) did not yield high DNA outputs but provided good DNA quality regarding A260/A280 ratios (1.67 and 1.64). Among the two CTAB procedures used in the study, the protocol-4 CTAB method produced higher DNA yields than the protocol-6 CTAB method, and the Wizard methods (protocols 1 and 2) were identified as the most suitable for extracting DNA from highly processed foods such as breakfast cereals, corn chips, biscuits, and cakes. Ashrafi-Dehkordi et al. (2021) compared three different CTAB-based methods and found that the modified CTAB method yielded promising results due to higher concentrations compared to the standard CTAB and phenol/chloroform methods. Different food products may require different DNA extraction protocols because DNA purity and concentration are significantly influenced by food processing, contaminants in sample matrices such as polysaccharides, lipids, and polyphenols, and physical parameters or extraction chemicals like CTAB (Xia et al., 2019). While the CTAB method is suitable for extracting DNA from complex food matrices or more challenging samples, SDS-based methods are more appropriate for extracting DNA from less processed foods (seeds, powder, pulp) (Wang et al., 2012; Ashrafi-Dehkordi et al., 2021). PVP (Polyvinylpyrrolidone) (1–2% w/v) is used in CTAB-based extraction methods to isolate DNA from plant species by absorbing polyphenols and preventing their oxidation, thus preventing DNA degradation (Sahu et al., 2012). Further comprehensive data on such extraction methods require similar studies on different food materials.

This study compared various CTAB-based DNA extraction methods to determine GMO content in biscuit samples. DNA extraction was performed on different biscuit samples using three different protocols (CTAB-PVP, modified CTAB-1, and modified CTAB-2). The DNA samples obtained from processed food products like biscuits were analyzed spectrophotometrically for DNA concentration and purity.

2. Materials and Method

2.1 Food materials

In this study, four different brands of biscuits containing soybean ingredients were used as food materials. The biscuit samples were sourced from local markets between 2023 and 2024. The GMO-positive control, GM soy (soybean meal), was obtained from the TÜBİTAK-MAM Biotechnology Institute. The soybean meal used in the experiment is a protein-rich feed product derived from the residual part of soybean seeds after oil extraction (Ergin and Aydemir, 2018). The biscuit and soybean meal samples were ground into flour using a mortar and pestle, and the experiments were conducted in triplicate for each food product.

2.2 DNA extraction methods

2.2.1. Procedure 1 (CTAB-PVP Method)

This procedure, described by Costa et al. (2015) with some minor modifications, was used in this study. According to this protocol, 200 mg of ground biscuit sample was placed into 2 mL sterile tubes. To this, 1 mL of CTAB extraction buffer (%2 CTAB (w/v), 0.1 mol/L Tris, 1.4 mol/L NaCl, %1 PVP-40 (w/v), 0.02 mol/L EDTA, pH 8.0) (Sigma Aldrich, USA) was added. The buffer was preheated to 65 ± 0.5°C, and 20 µL of β-mercaptoethanol (Sigma Aldrich) was added. After incubation with continuous mixing (900 rpm) at 65°C for 1 hour, the mixture was centrifuged (15 minutes, 18,500 × g, 4°C). The upper phase (700 µL) was collected into a separate Eppendorf tube and centrifuged again for 5 minutes under the same conditions. The supernatant was mixed vigorously with 500 µL of chloroform (Sigma Aldrich) for 20 seconds and then centrifuged (10 minutes, 12,000 × g, 4°C). The supernatant (approximately 500 µL) was separated and transferred to a new Eppendorf tube. To this solution, CTAB precipitation solution (%0.5 CTAB (w/v), 0.04 mol/L NaCl) (Sigma Aldrich) was added in double volume (1000 µL) and incubated at room temperature for 1 hour. The mixture was centrifuged again (10 minutes, 12,000 × g, 4°C), and the supernatant was discarded. The precipitated DNA was dissolved in a 350 µL solution containing 1.2 mol/L NaCl. This solution was subjected to liquid-liquid extraction with 350 µL of chloroform and centrifuged under the same conditions. The upper phase (300 µL) was mixed with 80% isopropanol (v/v) (Sigma Aldrich) at -20°C. The mixture was centrifuged again, and the supernatant was discarded. The pellet was washed with 70% ethanol (Merck, USA) solution at -20°C and dried at 50°C for 30 minutes. DNA was dissolved overnight at 4°C in 100 µL of Tris-EDTA buffer (1 mmol/L Tris, 0.1 mmol/L EDTA).

2.2.2. Procedure 2 (Modified CTAB-1 Method)

According to the modified CTAB protocol proposed by Gryson et al. (2004), 300 µL of sterile deionized water was added to a microcentrifuge tube containing 100 mg of homogenized sample, and the mixture was homogenized with a Petri stick. Subsequently, 500 µL of CTAB buffer (20 g/L CTAB, 1.4 M NaCl, 0.1 M Tris-HCl, 20 mM Na2EDTA) (Sigma Aldrich) was added and the mixture was mixed again. Then, 20 µL of Proteinase K (20 mg/mL) (Sigma Aldrich) was added, the tube was vortexed, and incubated at 65°C for 90 minutes. Following this, 20 µL of RNase A (10 mg/mL) (Thermo Fisher Scientific) was added and incubated at 65°C for 5-10 minutes. The tube was then centrifuged at approximately 16,000 g for 10 minutes. The upper phase was transferred to a new microcentrifuge tube containing 500 µL of chloroform, and the tube was vortexed for 30 seconds. It was centrifuged at 16,000 g for 10 minutes and left until phase separation occurred. The upper phase was transferred to a new microcentrifuge tube. A double volume of CTAB precipitation solution (5 g/L CTAB, 0.04 M NaCl) was added as the precipitation solution. The solution was pipetted to mix, incubated at room temperature for 60 minutes, and then centrifuged at 16,000 g for 5 minutes. The supernatant was discarded, and the pellet in the solution was dissolved in 350 µL of 1.2 M NaCl. 350 µL of chloroform was added, vortexed for 30 seconds, and centrifuged again at 16,000 g for 10 minutes. The upper phase was transferred to a new microcentrifuge tube, and 0.6 volume of isopropanol was added. The tube was vortexed and centrifuged at 16,000 g for 10 minutes, and the supernatant was discarded. The pellet was washed in 500 µL of 70% ethanol, vortexed gently, and centrifuged at 16,000 g for 10 minutes. Finally, the supernatant was discarded, the pellet was dried, and the DNA was dissolved in 100 µL of sterile deionized water (Sisea and Pamfil, 2007; Ashrafi-Dehkordi et al., 2021).

2.2.1. Procedure 3 (Modified CTAB-2 Method)

This procedure, used in this study with minor modifications, was proposed by Lipp et al. (1999). Initially, 100 mg of the homogenized sample was transferred to a sterile reaction tube, and 500 µL of CTAB buffer (20 g/L CTAB, 1.4 M NaCl, 0.1 M Tris/HCl, 20 mM EDTA) was added to the solution. The mixture was then incubated at 65°C for 30 minutes. After centrifugation at 12,000 x g for 10 minutes, the upper phase was transferred to a tube containing 200 µL of chloroform. The mixture was vortexed for 30 seconds and then centrifuged at 11,500 x g for 10 minutes. The supernatant was transferred to a new tube. A double volume of CTAB precipitation solution (5 g/L CTAB, 0.04 M NaCl) was added. The mixture was incubated at room temperature for 60 minutes and then centrifuged at 12,000 x g for 5 minutes. The pellet was dissolved in 350 µL of NaCl (1.2 M), and 350 µL of chloroform was added and mixed. The mixture was vortexed for 30 seconds and then centrifuged at 12,000 x g for 10 minutes. The upper phase (aqueous phase) was transferred to a new reaction tube, and isopropanol (0.6 volume) was added. The mixture was then centrifuged at 11,500 x g for 10 minutes. Ethanol solution (70%; 500 µL) was added to the pellet tube and centrifuged at 11,500 x g for 10 minutes. The supernatant was discarded, the separated pellet was dried, and the DNA was dissolved in 100 µL of sterile deionized water (Lipp et al., 2001; Leão-Buchir et al., 2022).

2.3 DNA concentration and purity measurements

The quantity and purity of DNA isolated from biscuit samples were determined using spectrophotometric methods. For this purpose, the samples were quantitatively assessed at 260-280 nm wavelengths using a NanoDrop (Denovix, DS-11 Spectrophotometer) (Wilfinger et al., 2006).

2.4 Statistical analysis

To evaluate the spectrophotometric values of DNA yield and purity obtained from the extraction procedures used in this study, a one-way ANOVA test was conducted using the

SPSS software package (IBM SPSS Statistics, version 22, New York, USA). Before analysis, all data were assessed for normality and homogeneity of variances. Individual differences were compared using Tukey's test. Differences were considered statistically significant at $p < 0.05$ (Costa et al., 2015).

3. Results and Discussion

The DNA concentrations and purity of biscuit samples subjected to DNA isolation methods were examined both across different brand sample groups (Table 1) and in terms of average values according to the extraction procedure (Table 2).

The A_{260}/A_{280} ratio provides important information about the purity and quality of a DNA sample (Vahdani et al., 2024). It indicates the degree of contamination of DNA with proteins and other organic components. Ideally, values between 1.8 and 2.0 indicate that the DNA is free from unwanted proteins and has been isolated with high quality. Additionally, values of 1.7 and above are considered acceptable in the literature (Sambrook and Russell, 2001). While a ratio of 1.8 signifies high purity of DNA, values above 2 suggest RNA contamination in the sample (Ateş Sönmezoğlu and Terzi, 2018).

Previous studies, including those by Arun et al. (2013) and Li et al. (2011), have emphasized that food processing methods such as temperature and pH changes can affect nucleic acid integrity. In this study, the observation that A_{260}/A_{280} ratios in biscuit samples did not exceed 2.0 suggests that the integrity of nucleic acids may have been compromised (Table 1). Consequently, this indicates that protein contamination is likely higher than anticipated. However, for the GM soy samples used as controls, the A_{260}/A_{280} ratio was above 2.0 for both protocol-1 (2.47) and protocol-3 (2.10) (Table 1). In protocol-2, this ratio was lower (1.67). Among all extraction protocols, only protocol-2 included RNAse A (10 mg ml^{-1}). Thus, using RNAse A was observed to be effective in preventing RNA contamination.

Regarding the A_{260}/A_{280} ratio, protocols 1 and 2, with the exception of sample-1 in protocol-2, yielded ratios below 1.8. Protocol-3, on the other hand, showed ideal results with values between 1.8 and 2.0 for all samples. Protocol-3 demonstrated an ideal extraction performance in terms of being free from protein contamination (Table 1).

Among all the extraction procedures examined, the highest average DNA yield was 51.76 ng μl^{-1} in protocol-3. The lowest DNA yield was found in protocol-2, with an average of 13.95 ng μl^{-1} (Table 2). Al-Salameen et al. (2012) noted that DNA extracted from processed foods is generally of low quality, present in very low concentrations, and may even be severely damaged. Therefore, in terms of providing sufficient DNA content for molecular detection and further analysis, the modified CTAB-2 method (protocol-3) contains a higher amount of DNA compared to other procedures (Table 2).

Although proteins may denature during food processing, detectable or trace amounts of DNA fragments can remain in the products; however, ensuring DNA quality is crucial (Singh et al., 2021). Pacheco Coello et al. (2017) reported that DNA quality extracted from processed foods could be a limiting factor for GMO testing. In Table 2, the A_{260}/A_{280} ratios obtained from the extraction methods vary according to the extraction procedure ($p<0.05$). While protocol-1 yields higher DNA quantities than protocol-2, the DNA quality is significantly lower, as indicated by the A_{260}/A_{280} ratio. This suggests a high level of protein contamination in protocol-1. Residual impurities from the DNA extraction process, such as phenol or ethanol, have also been reported to lower the A_{260}/A_{280} ratio (Piskata et al., 2019). Protocol-1, unlike the other protocols, involves drying the pellet at 50°C for 30 minutes after washing with ethanol. This may suggest that ethanol was not completely removed from the pellet.

As a secondary measure of nucleic acid purity, polysaccharide contamination can also be assessed by obtaining the A_{260}/A_{230} absorbance ratio (Walker and Wilson, 2005). This ratio is used to evaluate the level of salt residues in pure DNA, and it is recommended that this ratio be greater than 1.5 and preferably close to 1.8 (Aboul-Maaty and Oraby, 2019). Although Procedure-3 exhibits the best average A_{260}/A_{230} ratio of 1.79 (Table 2), individual sample analysis (Table 1) reveals that the presence of values above or below the optimal ratio does not provide a reliable result. Additionally, the negative control, soybean meal, showed significantly lower A_{260}/A_{230} ratios across all extraction protocols compared to the biscuit samples (Table 1). Chemical reagents involved in the isolation procedure can affect the purity of the extracted DNA, potentially leading to contamination (Piskata et al., 2019). Therefore, careful attention should be given to the final steps of DNA extraction, particularly the purification and washing stages.

Analysis of the DNA quantity and quality ratios of the GM soy used as a control reveals a significant difference in DNA amount (ng/µl) (Table 1). Ateş Sönmezoğlu and Keskin (2015) demonstrated in their study of various processed

Table 1. DNA quantity and purity measurements of biscuit samples

	Procedure-1			**Procedure-2**			**Procedure-3**		
Sample name	ng μl^{-1}	$A_{260/280}$	$A_{260/230}$	ng μl^{-1}	$A_{260/280}$	$A_{260/230}$	ng μl^{-1}	$A_{260/280}$	$A_{260/230}$
Sample -1	69.38	1.38	2.05	15.22	1.91	1.18	51.0	1.90	1.93
Sample -2	22.13	0.44	1.49	14.35	1.35	1.45	56.23	1.84	1.59
Sample -3	30.17	0.60	2.69	11.72	1.31	1.09	76.82	1.89	2.06
Sample -4	20.87	0.54	1.45	14.52	1.39	1.18	23.0	1.95	1.04
PC	123.57	2.47	0.95	394.17	1.67	0.18	156.06	2.10	0.98

PC: positive control (GM soy)
ng μl^{-1}: DNA amount (yield)
260, absorbance at A_{260} nm; 280, absorbance at A_{280} nm

Table 2. Summary of DNA yield and purity for biscuit samples using DNA extraction methods

DNA extraction method	DNA yield (ng µl^{-1})±SD of medium	DNA purity (A_{260}/A_{280})	A_{260}/A_{230}
Procedure - 1	35.64±22.86ab	0.74±0.43a	1.92±0.58a
Procedure - 2	13.95±1.53a	1.49±0.28b	1.22±0.15a
Procedure - 3	51.76±22.17b	1.89±0.04b	1.79±0.65a
***P* value**	0.05	0.001	0.181

Mean ± SD: mean ± standard deviation
A_{260}, absorbance at 260 nm; A_{280}, absorbance at 280 nm
a,b Different letters in each column indicate significant differences between DNA concentration or purity values (p<0.05)

food samples, including different biscuit types, that soybean flour and soybean meal (GM soy) yielded the highest DNA content among the analyzed food samples, attributed to their lower degree of processing. In this study, GM soybean meal was chosen as a positive control for comparing DNA yields from biscuit samples, given its relatively lower processing. The results are consistent with Ateş Sönmezoğlu and Keskin (2015) findings, confirming that biscuit samples had significantly lower DNA yields compared to the GM soy control. For Procedure-2, the average DNA yield for biscuit samples was approximately 13.95 ng/µl, while the highest DNA yield of 394.17 ng/µl was obtained from GM soy. Similar results in terms of DNA quantity were observed between biscuit samples and GM soy across other extraction procedures. This difference is likely related to the physical grinding process and the high degree of food processing undergone by the biscuit samples before DNA extraction, resulting in lower DNA yields (Turkec et al., 2015; Pacheco Coello et al., 2017; Bitir et al., 2020; Leão-Buchir et al., 2022). Additionally, Ramos-Gómez et al. (2014) noted that the presence of specific components (including fats and carbohydrates) can significantly impact DNA yield and quality. Therefore, GM soy samples are expected to yield higher DNA results than biscuit samples.

The quality of DNA extracted from food samples is typically influenced by factors such as the degree of damage, the presence of PCR inhibitors in complex food matrices, and the average fragment length of the extracted DNA. These factors depend on both the samples themselves and the processes involved in food production, as well as the physical and chemical parameters of the extraction method (Peano et al., 2004; Elsanhoty et al., 2011). Abdel-Latif and Osman (2017) reported that in their study using a CTAB-based extraction method with 1% PVP added, they could not observe an absorbance peak at 260 nm when measuring with a NanoDrop device. In this study, the CTAB extraction procedure with added PVP (Procedure-1) provided spectroscopic measurements of considerably lower purity for biscuit samples compared to the other two extraction procedures. Thus, the addition of PVP to the CTAB extraction method did not result in improved DNA isolation in this study. Previous studies have confirmed that the CTAB method yielded better results for DNA extraction from raw soybeans, raw corn, animal feeds (Tung-Nguyen et al., 2009), dairy products (Pirondini et al., 2010), and chocolate and biscuits (Gryson et al., 2004; Mutlu et al., 2021). In this study, among the CTAB-based extraction procedures compared, Procedure-3 demonstrated sufficient DNA yield and quality for potential use in GMO detection analyses of biscuit samples, aligning with results from previous similar studies.

Based on the results obtained from the DNA extraction methods, it is evident that the quality and quantity of DNA significantly influence the accuracy and reliability of GMO detection in processed food samples, such as biscuits. For example, in a study by Arun et al. (2016) investigating the impact of heat treatment on GMO detection in baked, it was reported that DNA integrity in soybean samples baked at 220°C significantly decreased over time. The findings of this study suggest that the modified CTAB-2 method (Procedure-3) provides better DNA yields and purity, which are critical for subsequent GMO analysis. Given the inherent challenges posed by processed food matrices, such as the presence of PCR inhibitors and degraded DNA, the selection of the extraction method is of paramount importance.

In practical terms, this study underscores the importance of selecting the most appropriate DNA extraction protocol based on the specific characteristics of the food sample for applications in the food industry and regulatory bodies involved in GMO detection. The conventional CTAB method is a widely used technique for extracting DNA from food matrices and is manually performed using chloroform to separate DNA from contaminants (Verginelli et al., 2023). The EU Reference Laboratory for GM Food and Feed (EURL GMFF) frequently reports this method, often with potential modifications depending on the matrix (Sajali et al., 2018). Alternative methods exist that separate DNA from other cellular components using DNA-binding silica columns or magnetic beads (Ohmori et al., 2008; Krinitsina et al., 2015). While numerous articles have been published over time comparing different DNA extraction methods across various food matrices (Mafra et al., 2008; Tan and Yiap, 2009; Elsanhoty et al., 2011; Verginelli et al., 2023), the aim of this study is to validate to what extent the manually performed DNA extraction methods, commonly used in GMO detection of processed foods, meet the performance criteria for DNA quality and quantity in biscuit samples. For instance, in situations where high DNA purity is crucial, Procedure-3, which exhibited ideal A_{260}/A_{280} ratios and minimized protein contamination, would be the most appropriate choice. Conversely, if the primary concern is maximizing DNA yield, particularly from highly processed foods, Procedure-3 also appears to be the best method among the procedures tested, providing the highest average DNA yield.

Moreover, these findings emphasize the necessity of considering the impact of food processing on DNA integrity when designing GMO detection strategies.

Regulatory bodies may consider recommending specific extraction methods, such as Procedure-3, for routine analysis of processed foods to ensure reliable and consistent results. By adopting standardized and validated DNA extraction procedures, the food industry can improve the accuracy of GMO labeling, thereby enhancing consumer confidence and compliance with food safety regulations. Overall, this study provides valuable insights that can guide both the selection of extraction methods in research and their practical application in the food industry.

4. Conclusions

This study is based on a comparative analysis of DNA samples obtained from biscuit samples using different DNA extraction methods. Spectroscopic measurements were performed to obtain comparable information on the effectiveness and yield of routine DNA extraction procedures applied as an initial step for detecting GMO content. The results include an evaluation of DNA concentration and purity based on spectrophotometric measurements for DNA samples obtained using three different protocols (CTAB-PVP, modified CTAB-1, and modified CTAB-2).

The findings indicate that the yield and purity values of DNA samples extracted from processed food products, such as biscuits, varied depending on the extraction methods. The lowest DNA content was found in samples extracted using the modified CTAB-1 method. On the other hand, samples extracted with the modified CTAB-2 method exhibited the highest average DNA concentration and optimal purity in terms of the A_{260}/A_{280} ratio.

These results suggest that the modified CTAB-2 method (Procedure-3) could be preferred as an effective DNA isolation method for determining GMO content in biscuit samples. Furthermore, the study provides insights into the effects of routine chemicals and content used in DNA extraction protocols on the extraction of biscuit samples and evaluates the effectiveness of modified CTAB-based DNA extraction methods. The results from this study are expected to contribute to the development and improvement of CTAB-based methods as an alternative to commercial kits for routine analyses, addressing important food safety issues such as GMO detection and labeling. More comprehensive data on such extraction methods will require larger sampling and similar studies on different food materials with agarose gel imaging and PCR analyses. Such studies could enhance our understanding of the performance of DNA extraction methods across various food types and help achieve more reliable results.

Conflict of Interest

The authors of the articles declare that they have no conflict of interest.

Authors' Contributions

The data for this study were collected by Özlem Ateş Sönmezoğlu and Dr. Begüm Terzi Aksoy. Laboratory analyses for the study were conducted by Begüm Terzi Aksoy and Ahlem Sattuf. The text of the article was written by Begüm Terzi Aksoy.

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