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Comparison of DNA extraction efficiencies using various methods for the detection of genetically modified organisms (GMOs)

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Abstract: The ability to detect the presence of transgenes in crop-derived foods depends on the quantity and quality of DNA obtained from a product to be analyzed. The efficiency of DNA extraction protocols differs due to the nature of each food product. In this paper, we described two main DNA extraction protocols and their modifications that have been applied and evaluated for DNA extraction from raw and processed food as well as animal feed. The yield and quality for five categories of food and feed samples namely, raw soybean, raw maize, animal feed, smooth tofu and soymilk are discussed. The statistical interaction analyses showed that the cetyltrimethyl ammonium bromide (CTAB) method was proven to be the best method to extract DNA from raw soybean, maize and animal feed samples which not only obtained high DNA yield of 32.7, 28.4 and 33.4 ng DNA/mg sample respectively, but also produced high quality DNA with the absorbance A260/A280 ratio of 1.9, 1.9 and 2.0, respectively. These DNA were suitable for PCR amplification which produced a 164 bp DNA fragment of the lectin gene from soybean, and a 277 bp DNA fragment of the zein gene from maize. In the processed food category, the Wizard isolation method was found to be the best for the extraction of DNA from smooth tofu and soymilk with the yield of 13.2 and 3.4 ng DNA/mg sample, and the quality of the DNA at the absorbance A260/A280 ratio ranged from 1.9 to 1.7. These DNA were successfully amplified using primers specific to the lectin gene of soybean.

Keywords: DNA extraction, CTAB method, Wizard method, soybean, maize

Introduction

Genetically modified organisms (GMOs) contain specific traits which have been added to the organisms to improve their properties that have not occurred by mating or natural combination (Anklam *et al*., 2002; Taverniers *et al*., 2004). The addition of foreign genes has often been used in plants to produce novel protein that confer pest and disease tolerance and, more recently, to improve the chemical profile of process product, for example vegetable oils (Hemmer, 1997). In the European Community, foods and food ingredients derived from GMOs are strictly regulated and are labeled mandatory to keep under control the possible impact of GMOs both on public health and the environment (EC/258/97; EC/1139/98; EC/49/2000; EC/50/2000; EC/1829/2003). In most cases, the identification of GMOs is carried out based on the presence or absence of the introduced gene(s) at the DNA level in the sample matrix (Allmann *et al*., 1993; Meyer, 1995; 1996; 1999; 2003). Among DNA-based methods, polymerase chain reaction (PCR) technology is preferred by many analytical laboratories interested in detection of GMOs because of its high sensitivity, specificity and wide range of gene constructs (Ahmed, 2002; Anklam *et al*., 2002; Giovanini and Concilio, 2002; Holst-Jensen *et al*., 2003). In addition, any physical or chemical treatment of food samples, such as heat, pH or shear forces results in a decrease on the average size of genomic DNA due to random cleavage of these macro molecules (Hupfer *et al*., 1998; Kakihara *et al*.,

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2005). All of these factors make DNA isolation from foods difficult and challenging (Holden *et al*., 2003; Kakihara *et al*., 2005). DNA extraction protocol must be developed on a case-by-case basis for different food matrices, because of the differences on the type, composition and the level of processing of each food product (Gryson *et al*., 2004). In addition, Zimmermann *et al*. (1998) stated that although many DNA extraction protocols are available, they had been rarely compared in a comprehensive manner. The prerequisite to successfully identify GMO based on PCR depends on the ability to obtain enough DNA for amplification. Hence, there is a need to compare the efficiency of different extraction methods for each type of food matrix to ascertain which method is the most suitable to yield good and high quality DNA. In this study, two of main protocols based on the Wizard method (Hemmer, 1997) and CTAB method (Jankiewicz *et al*., 1999), and three sub-protocols are examined for DNA extraction. The qualities of the DNA are determined by using spectrophotometer and later subjected to PCR amplification. In addition, the role of beta-mercaptoethanol (BME) in the lysis buffer is elucidated. These methods are used to isolate DNA from food samples, namely raw soybean, raw maize, animal feed, smooth tofu and soymilk.

Materials and methods

Sample collection

Raw soybean and raw maize samples were obtained randomly from supermarkets in Kuala Lumpur, Malaysia while animal feed sample was collected from Seri Kembangan, Selangor, Malaysia. Smooth tofu and soymilk were purchased from Seri Serdang, Selangor, Malaysia.

DNA extraction methods

Two of main protocols, and three sub-protocols for DNA extraction were evaluated, the Wizard method (protocol 1), the modified Wizard method by an addition of 1% BME in lysis buffer (protocol 2), the combination method based on the pre-incubation of samples with TNE buffer (protocol 3), the CTAB method (protocol 4) and the modified CTAB method by an addition of 1% BME in lysis buffer (protocol 5).

The Wizard method (Hemmer, 1997) – protocol 1

Homogenized food sample (350 mg) was mixed with 860 µl TNE buffer [10 mM Tris-HCl (pH 8), 150 mM NaCl, 2 mM EDTA, 1% SDS] and 40 µl proteinase K (20 mg/ml). The sample was incubated for 3h at 55°C in a water bath. After centrifugation at 13,000 rpm for 10 min, 500 µl of supernatant was transferred in a new 1.5 ml tube and added with the same volume of chloroform. The mixture was centrifuged at 13,000 rpm at 10 min and the upper phase was transferred into a new 1.5 ml tube. The chloroform extraction was repeated twice to get a clear interface. 500 µl of the supernatant was added with 15 µl of 3M sodium acetate (pH 5.2) and 50 µl of absolute ethanol to precipitate the remaining starch and polysaccharides. The mixture was kept on ice for 15 min and centrifuge at 13,000 rpm for 7 min. The supernatant were transferred into a new 1.5 ml tube, and 5 µl of 3M sodium acetate (pH 5.2) and 500 µl of absolute ethanol were added. The mixture was incubated on ice for 15 min to allow the DNA to precipitate, and later centrifuged for 7 min at 12,000 rpm. The DNA pellet was washed again with 500 µl of 70% ethanol, centrifuged at 12,000 rpm for 10 min and air-dried. The pellet was dissolved in 100 µl of distilled water and stored at -18°C until use.

The modified Wizard method – protocol 2

The protocol was similar to the Wizard method except that 1% BME was added to the TNE buffer.

The combination method – protocol 3

Homogenized sample (350 mg) was mixed with 860 µl of TNE buffer [10 mM Tris-HCl (pH 8), 150 mM NaCl, 2 mM EDTA, 1% SDS], 40 µl proteinase K (20 mg/ml) and vortexed vigorously. The mixture was incubated for 1 h and 30 min at 55°C in a water bath. Then 500 µl of CTAB buffer [20 g/l CTAB, 1.4 M NaCl, 100 mM Tris-HCl (pH 8), 20 mM EDTA] was added in the mixture and further incubated at 65°C for 30 min. After centrifugation at 13,000 rpm for 10 minutes, 650 µl of supernatant was transferred into a new 1.5 ml tube, and gently mixed with the same volume of chloroform. The mixture was centrifuged at 13,000 rpm at 10 min and the upper phase was transferred in a new 1.5 ml tube. The chloroform extraction was repeated twice to get a clear interface. Then 500 µl of the supernatant was added with 15 µl of 3M sodium acetate (pH 5.2) and 50 µl of absolute ethanol to precipitate the remaining starch and polysaccharides. The

Table 1. Sequences of oligonucleotides used in this study

Primer	Sequence	Gene specificity	Amplicon (bp)	References
LEC1	5'-GTG CTA CTG ACC AGC AAG GCA AAC TCA GCG-3'	Soybean lectin	164	Vollenhofer et al., 1999
LEC2	5'-GAG GGT TTT GGG GTG CCG TTT TCG TCA AC-3'			
ZE03	5'-AGT GCG ACC CAT ATT CCA		277	Pauli et al., 2000
ZE04	G-3' 5'-GAC ATT GTG GCA TCA TCA TTT-3'	Maize zein		

Table 2. PCR amplification conditions

Step	LEC1/LEC2	ZE03/ZE04
Pre-denaturation	12 min, 95°C	4 min 30 sec, 95°C
Denaturation	1 min, 95°C	1 min 45sec, 96°C
Annealing	30 sec, 72°C	2 min, 60°C
Extension	30 sec, 72°C	1 min 50 sec, 72°C
Final extension	10 min, 72°C	4 min 50 sec, 72°C

mixture was kept on ice for 15 min and centrifuge at 13,000 rpm for 7 min. Then the supernatant were transferred into a new 1.5 ml tube and was added with 5 µl of 3M sodium acetate (pH 5.2) and 500 µl of absolute ethanol. The mixture was incubated on ice for 15 min to allow the DNA to precipitate, and later centrifuged for 7 min at 12,000 rpm. The DNA pellet was washed again with 500 µl of 70% ethanol, centrifuged at 12,000 rpm for 10 min and air-dried. The pellet was dissolved in 100 µl of distilled water and stored at -18°C until use.

The CTAB method (Jankiewicz et al.*, 1999) – protocol 4*

Homogenized samples of up to 350 mg were mixed with 500 µl CTAB buffer [20 g/l CTAB, 1.4 M NaCl, 100 mM Tris-HCl (pH 8), 20 mM EDTA] and incubated at 65°C for 30 min. The samples were then centrifuged for 10 min at 13,000 rpm. The supernatant was transferred to a new 1.5 ml tube, extracted with 200 µl chloroform and centrifuged for 10 min at 13,000 rpm. The upper phase was transferred into a new 1.5 ml tube, precipitated with 1 volume of isopropanol and centrifuged for 10 min at 13,000 rpm. The supernatant was discarded and the pellet was washed once with 500 µl of 70% ethanol and air-dried for approximately 45 min. The pellet was dissolved in 100 µl distilled water and stored at -18°C until use.

The modified CTAB method – protocol 5

The protocol was similar to the CTAB method except that 1% BME was added to the lysis buffer. Methods for DNA Quantification DNA concentration from all DNA stocks were determined by using a spectrophotometer at the absorbance of 260 nm (A260) and 280 nm (A280) in an Eppendorf Biophotometer 6131 spectrophotometer. The purity of extracted DNA was determined by using A260/A280 ratio and later tested by PCR amplification.

Polymerase chain reaction (PCR)

PCR amplification was carried out in a PCR mix of 25 µl on a PTC-200 thermal cycler (MJ Research, Watertown, MA). The final concentrations of each PCR reaction were as follows: 2.5 µl of 10 x PCR buffer (Finnzymes, Finland); 100 ng of genomic DNA; 0.5 M of each primers; 200 M of dNTPs mix; 0.625 unit/reaction of DyNAzyme II DNA polymerase.

Oligonucleotide primers

Oligonucleotide primers were synthesized by Research Biolabs Sdn Bhd. (Malaysia) at the final concentration of 100 mM. All oligonucleotide primers were diluted to working concentration of 10 pmol/l with sterilized deionized water and stored at -18°C until use. The sequences and amplification conditions are presented in Tables 1 and 2.

Agarose gel electrophoresis

Amplicons were analyzed using 1.8% agarose gel electrophoreses in a 1 x TBE [10 mM Tris-base (pH 8); 2.75 g/l Boric acid; 1mM EDTA (pH 8)] and were made visible under UV transilluminator after staining with 0.5 (g/ml of ethidium bromide).

Statistical analysis

The data of DNA yield and quality (A260/A280 ratio) were subjected to an analysis of variance for Completely Randomized Design (CRD) using the MSTAT-C program version 1.2 (Michigan State University, 1986). Duncan's Multiple Range Test was used to compare the mean values among the treatments at 95% probability.

Results and discussion

Evaluation on DNA yield (ng DNA/mg sample) of two plant DNA extraction protocols and their modification on five categories of samples

The efficiency of two main DNA extraction protocols of plant and their modifications involving SDS and CTAB were used as the main detergents in this work. The DNA produced was quantified using a spectrophotometer at the absorbance of 260 and 280 nm. The result in Table 3 shows that the CTAB-based protocols gave good DNA yield. Among CTAB-derived protocols, the CTAB method (protocol 4) gave the best result with a DNA yield of 19.7 ng DNA/mg sample (Table 3) and produced a clear DNA band on the agarose gel (Figure 1). This finding was in accordance with the result of Chen and Ronald (1999). They have used the CTAB protocol for extracting total DNA from grains of rice and maize, and leaves of other species yielding 2.3 – 5.2 g DNA / 25 – 50 mg fresh leaf tissue. In contrast, the SDS-based or CTAB-based protocols (protocols 2 and 5) containing BME (Table 3) produced the lowest yield of DNA. BME retards the oxidation of biological compounds in solution by breaking disulfide bonds in protein molecules and is also a potential health hazard. Both the highest and the lowest treatments were significantly different ($P<0.05$) from the other treatments. In addition, it was obvious that the trend of DNA yield dramatically decreased in the treatments using BME. The low recovery rate of DNA with BME addition in lysis buffer may be explained by the loss of DNA during the choloroform extraction. In the solution, BME could bind to polyphenolic compounds. This complex of BME and polyphenolic compounds continued to form a cross-link to high molecular weight DNA and resulted in

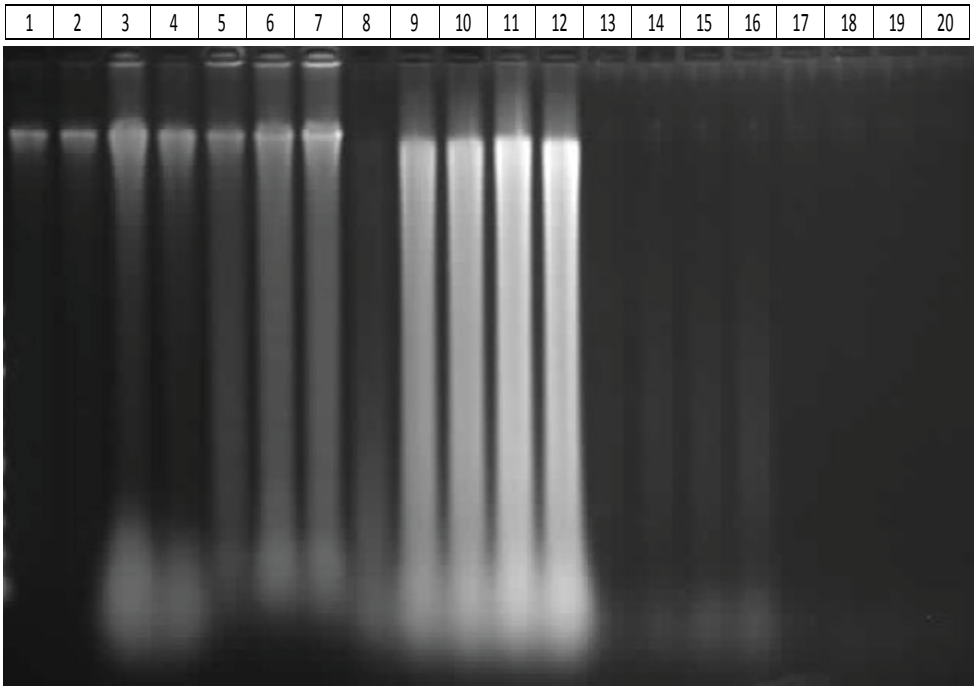

Figure 1. Agarose gel electrophoresis (1%) of genomic DNA from five categories of samples extracted by CTAB method with four replications; Lanes 1-4; 5-8; 9-12; 13-16 and 17-20: raw soybean; raw maize; animal feed; smooth tofu and soymilk, respectively

Table 3. Comparison of DNA yield (ng DNA/mg sample) obtained by five DNA extraction methods on five categories of samples

Treatments	Raw soybean	Raw maize	Animal feed	Smooth tofu	Soy milk	Mean
Protocol 1	4.6^{fgh}	8.1^{ef}	25.6^{b}	13.2^{d}	3.4^{fgh}	11.0N
Protocol 2	20.7^{c}	3.6^{fgh}	5.4^{fgh}	1.8^{h}	0.9^{h}	6.5Q
Protocol 3	17.8^{c}	10.3^{de}	10.4^{de}	5.6^{fgh}	1.1^{h}	9.0P
Protocol 4	32.7^{a}	28.4^{b}	33.4^{a}	2.6^{gh}	1.1^{h}	19.7M
Protocol 5	1.1^{h}	20.2^{c}	7.3^{efg}	2.4^{gh}	2.3^{h}	6.7Q
Mean	15.4^{AB}	14.1^{B}	16.4^{A}	5.1^{C}	1.7^{D}	
Effects	Sample (P<0.05)	(P<0.05)	Method	(P<0.05)	Sample*	Method

^{a,b,c,d,e,f,g,h} Means with different superscripts across columns and rows denoted interaction differed significantly (P<0.05)
^{A,B,C,D} Means with different superscripts in the same row differed significantly among samples (P<0.05)
^{M,N,P,Q} Mean with different superscripts in the same column differed significantly among methods (P<0.05)

DNA loss due to the co-precipitation phenomena. Zimmermann *et al*. (1998) also observed the low recovery rate of DNA about 70% in ROSE method with 1% of polyvinylpolypyrolidone. For this reason, the protocols 2 and 5 with the presence of 1% BME produced the lowest yields of DNA of 6.5 and 6.7 ng DNA/mg sample, respectively. In addition, they were similar at P<0.05. The combination method (protocol 3) originated from the research of Kang *et al*. (1998) resulted in a medium yield of DNA of 9 ng DNA/mg sample. The Wizard method (protocol 1) and the combination method (protocol 3) were in the mid-point of DNA yield of 11 and 9 ng DNA/mg sample, respectively.

Within the analyzed samples in Table 3, the animal feed sample produced the highest yield at 16.4 ng DNA/mg sample because of the fine texture and the presence of various plant materials. The differences observed in DNA recovery might be caused by the surface and interfacial tensions between detergent and different compositions of the particles size fractions of the samples. In fact, Holden *et al*. (2003) found that genomic DNA recovery increased as a function of decreasing particle size of sample. In contrast, the soymilk sample being highly processed gave the lowest yield of 1.7 ng DNA/mg sample with the most degradation of DNA molecules. They were significantly different at 95% confidence level. In addition, the DNA yield of raw soybean and raw maize samples were similar and ranged from 15.4 to 14.1 ng DNA/mg sample.

The interaction analysis was carried out to identify the optimum combination between the various DNA extraction methods and the different kinds of samples. The analysis of variance showed a significant variation on DNA yield for each combination (P<0.05). The result denoted that the CTAB method (protocol 4) was the most favorable method for extracting DNA from raw soybean and raw maize as well as animal feed with a DNA yield of 32.7, 28.4 and 33.4 ng DNA/mg sample, respectively (Table 3). The yields of raw soybean (32.7 ng DNA/mg sample) and animal feed (33.4 ng DNA/mg sample) were highest. It demonstrated that the CTAB method is highly applicable for extracting total DNA from raw soybean, raw maize and animal feed samples. Similarly, a very good result of DNA yield was obtained from maize leaves, corncob (Chen and Ronald, 1999; Schneerman *et al*., 2002) and soybean seed (Kang *et al*., 1998). In contrast, the Wizard method was found to be most suitable in isolating DNA from highly processed foods such as smooth tofu and soymilk with the yield of 13.2 and 3.4 ng DNA/mg sample respectively, in comparison with the other methods. In fact, Zimmermann *et al*. (1998) demonstrated that the Wizard method could be used to extract total nucleic acid from tofu with relatively low yield (2.5 g nucleic acid/mg sample) to obtain a DNA quality that is pure and suitable for downstream analyses such as PCR amplification.

Evaluation on DNA quality (A260/A280 Ratio) of five plant DNA extraction methods on five categories of samples

The detection or identification of GMO by using PCR method depends on the ability to extract intact DNA from raw or processed food. Extraction of DNA

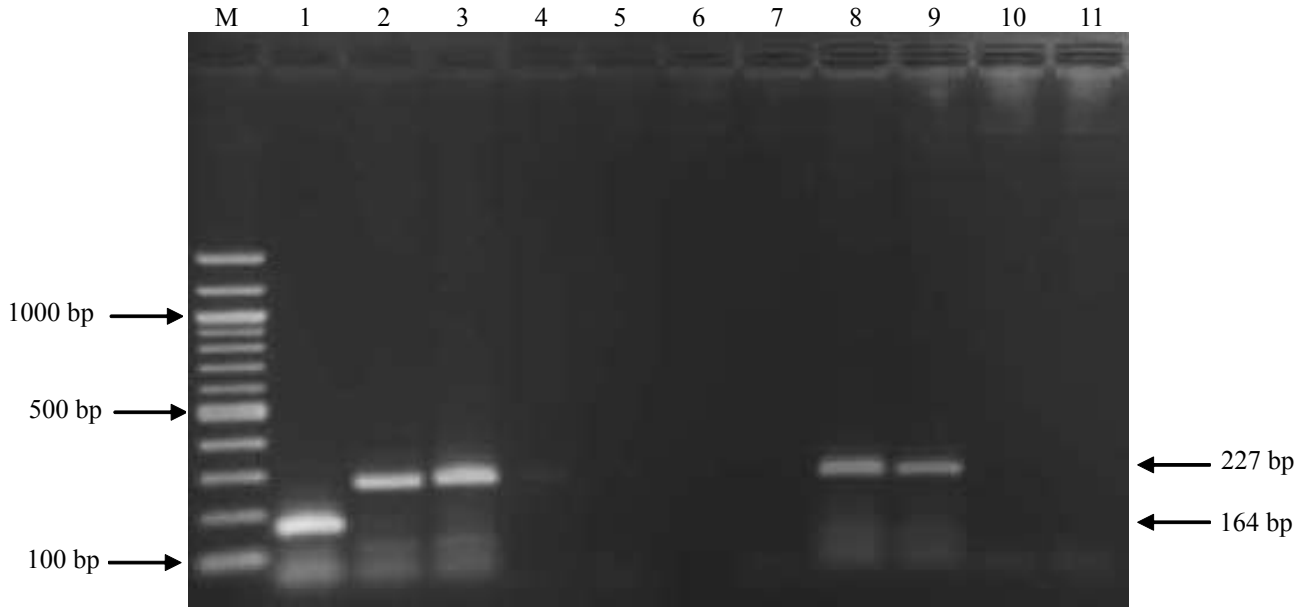

Figure 2. Agarose gel electrophoresis of PCR amplicons using DNA templates extracted by the following methods; CTAB (lanes 1 to 5), the CTAB with 1% BME (lanes 7 to 11). Lane M: 100 bp DNA ladder (New England Biolabs); Lanes 1-5; 7-11: raw soybean, raw maize, animal feed, smooth tofu and soymilk samples, respectively; Lane 6: empty

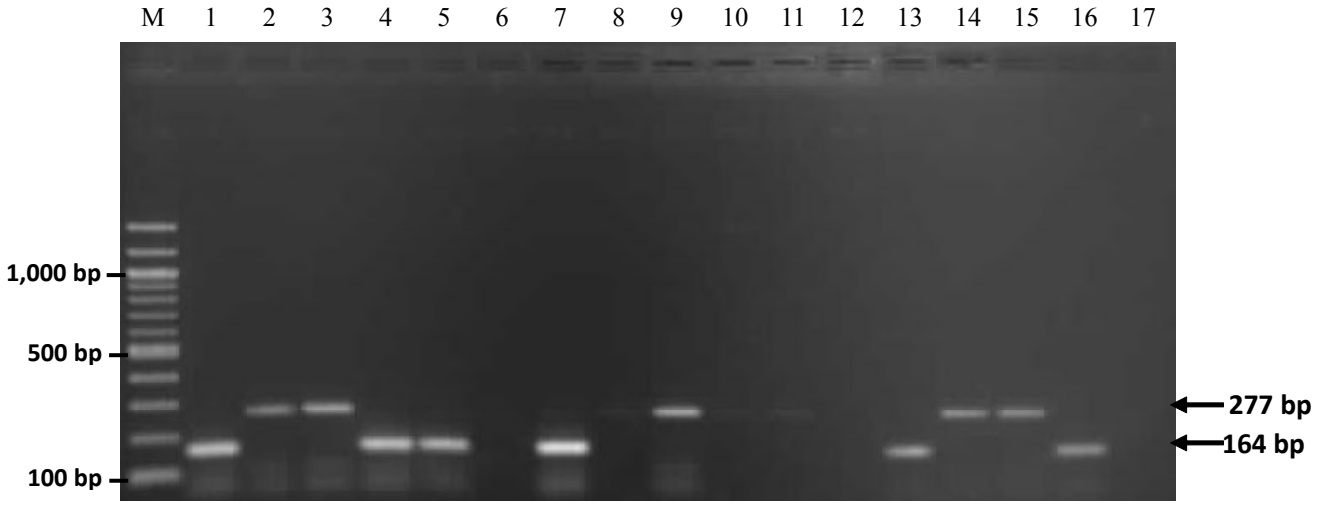

Figure 3. Agarose gel electrophoresis of PCR amplicons using DNA templates extracted by the following methods; Wizard (lanes 1 to 5), the Wizard with 1% BME (lanes 7 to 11) and the combination method (lanes 13 to 17). Lane M: 100 bp DNA ladder (New England Biolabs); Lanes 1-5; 7-11; 13-17: raw soybean, raw maize, animal feed, smooth tofu and soymilk samples, respectively; Lanes 6 and 12: empty

from raw materials is much easier when compared to processed food such as tofu and soy milk where the DNA is degraded. Nevertheless, if the appropriate method is used then the efficiency to recover DNA can be maximized even from highly processed food. The DNA that is obtained for analysis must be pure and intact to make any meaningful analysis for the presence of GMO. The usual contaminant during DNA purification is protein. The A260/A280 ratio is usually used to determine the purity of isolated DNA. This ratio for pure double-stranded DNA is customarily taken to be between 1.8 and 1.9 (Sambrook *et al*., 1998). A good DNA extraction method should give not only high DNA yield but also high DNA purity.

Table 4. Comparison of DNA quality (A260/A280) obtained by five DNA extraction methods on five categories of samples

Treatments	Raw soybean	Raw maize	Animal feed	Smooth tofu	Soy milk	Mean
Protocol 1	1.9^{bcdef}	1.8^{cdefg}	1.8^{defgh}	1.9^{bcd}	1.7^{ghi}	1.8^{P}
Protocol 2	1.8^{bcdef}	1.6^{hi}	1.6^{hi}	1.6^{i}	1.4^{j}	1.6^{R}
Protocol 3	1.8^{bcdefg}	1.7^{efghi}	1.7^{fghi}	1.9^{bcde}	1.4^{j}	1.7^{Q}
Protocol 4	1.9^{abc}	1.9^{abcd}	2.0^{ab}	2.0^{ab}	2.0^{a}	2.0^{M}
Protocol 5	1.9^{abc}	1.8^{bcdef}	1.8^{cdefg}	1.8^{bcdef}	1.9^{abc}	1.9^{N}
Mean	1.9^{A}	1.8^{B}	1.8^{B}	1.8^{B}	1.7^{C}	
Effects	Sample (P<0.05)	(P<0.05)	Method	(P<0.05)	Sample*	Method

^{a,b,c,d,e,f,g,h,i,j} Mean with different superscripts across columns and rows denoted interaction differed significantly (P<0.05)
^{A,B,C} Mean with different superscripts in the same row differed significantly among samples (P<0.05)
^{M,N,P,Q,R} Mean with different superscripts in the same column differed significantly among methods (P<0.05)

The results in Table 4 showed that the quality of DNA produced by the CTAB method, modified CTAB method with addition of 1% BME in lysis buffer, and the Wizard method with means A260/A280 ratio at 2.0, 1.9 and 1.8, respectively. According to Meyer (2003), a higher A260/A280 is indicative of an RNA contamination, whereas a lower A260/A280 ratio is encountered when a contamination with proteins occurred. This was true because the CTAB protocol did not include any treatment of RNase; nevertheless, this did not significantly affect the DNA quality. The DNA qualities from these DNA extraction methods were relatively free of contaminants and good enough for any downstream processes. For example, a clear amplicon of 1.4 kb from the Xa21 transgene conferring bacterial blight resistance was amplified from the total DNA of transgenic rice extracted by the CTAB based protocol (Chen and Ronald, 1999). A similar application of CTAB method for extraction of total DNA from another transgenic rice line, either the RFLP amplification using random primer URP-6 or the specific detection of the transformed bar gene using BF/BR primer were obtained successfully (Kang *et al*., 1998). DNA extracted from half seeds of transgenic rice using the CTAB method was suitable for both PCR amplification (Yamaguchi *et al*., 2003) and Southern blot analysis with RG220 as the DNA probe (Kang *et al*., 1998). On the other hand, the modified Wizard method and the combination method produced moderate qualities of DNA with the presence of traces of proteins with A260/A280 ratio of 1.6 and 1.7, respectively. However, according to Pich and Schubert (1993), the A260/A280 was 1.6-1.7, indicating the absence of contaminants. At this ratio of A260/A280, a positive signal in the range of 6 kb for the FKBP-77 gene was obtained in Southern hybridization. This analysis required high-quality intact DNA free of polysaccharides, proteins and other inhibitors (Sharma *et al*., 2002).

The addition of BME, an agent for retarding oxidation of biological compounds and a potential health hazard, to lysis buffer of both CTAB protocol (Schneerman *et al*., 2002) and Wizard protocol did not give any significant positive effect on DNA yield or in preventing contamination from the final DNA stock.

The interaction result of A260/A280 in Table 4 is in agreement with the selected combination of DNA yield analysis. The CTAB method gave not only high DNA yield but also good DNA quality with the A260/A280 in the range of 1.9 to 2.0 for raw materials and animal feeds, respectively. This purity of DNA was good enough for PCR amplification and other molecular applications (Yamaguchi *et al*., 2003). This is in agreement with Song *et al*. (1995), Wang *et al*. (1998), and Chen and Ronald (1999) who have shown that the Xa21 and bar transgenes (Kang *et al*., 1998) can be successfully detected from the genomic of transgenic rice lines extracted by the CTAB protocol. In contrast to CTAB method, the Wizard method resulted in the A260/A280 ratio of 1.9 and 1.7 for smooth tofu and soymilk, respectively. Despite this, very good amplification products were obtained from both DNA sources (Figure 3). This indicates that the PCR is very versatile and may tolerate slight contamination of DNA extracted using either the Wizard and CTAB methods (Surzycki, 2000).

Assay for DNA quality using PCR amplification

Another crucial parameter for the detection of GMO is whether the DNA extracted using various method is good enough in terms of quantity and quality for PCR amplification. In this study, one representative of four replications was subjected to PCR amplification with primer pair LEC1/LEC2 targeting the lectin gene (Vollenhofer *et al*., 1999) for products derived from soybean and primer pair ZE03/ ZE04 targeting the zein gene (Pauli *et al*., 2000) for the products derived from maize. Lectin gene is soy-specific while the zein gene is maize-specific. The amplification results using LEC1/LEC2 primer for samples derived from soybean and ZE03/04 primer for samples derived from maize were presented in Figures 2 and 3. The lectin and zein genes-derived amplicons were 164 bp and 277 bp in sizes respectively for the DNA extracted from raw soybean and maize samples extracted by the CTAB protocol and Wizard protocol (Figures 2 and 3). In processed foods, an amplicon of 164 bp from lectin gene was successfully produced by DNA derived from smooth tofu and soymilk extracted by the Wizard extraction method (Figure 3). Amplicons from either from the Xa21 (Chen and Ronald, 1999; Song *et al*., 1995; Wang *et al*., 1998) or bar genes transformed crops (Kang *et al*., 1998) were successfully amplified from the genomic DNA of transgenic rice lines extracted by the CTAB protocol. Zimmermann *et al*. (1998) were able to detect the lectin gene in both tofu and soy flour samples using PCR when 1 ng DNA extracted using the Wizard method was used as templates.

Conclusions

PCR-based technique for GMO detection appears to be the method of choice because of their high sensitivity and specificity (Ahmed, 2002; Anklam *et al*., 2002; Forte *et al*., 2005; Giovanini and Concilio, 2002; Holst-Jensen *et al*., 2003). However, an essential prerequisite for the application of food labeling directives is the availability of genetic materials for analysis and GMO detection. The development and optimization of protocols for extraction of DNA for the detection GMOs in foods are getting more and more critical as the numbers of GM crops that reach the market are increasing rapidly. Hence, this work was designed to determine the best combination between the five DNA extraction protocols, and the five categories of samples with the consideration on the composition and processing level. Two critical factors that have been taken into account in the comparative analysis among DNA extraction protocols are the DNA yield and the DNA quality (Csaikl *et al*., 1998). A good and suitable DNA isolation method for any plant materials should fulfill these two requirements. Based on the statistical and PCR amplification analysis, the CTAB method proved to be the best for the extraction of DNA from raw soybean, raw maize and animal feed samples with DNA yield at 32.7, 28.4 and 33.4 ng DNA/mg sample, respectively and DNA quality at the A260/ A280 ratios of 1.9, 1.9 and 2.0, respectively. With the same consideration, the performance of CTAB-based method was the best protocol among five methods investigated for the isolation of DNA from chocolate and biscuits although this method was time-consuming (Gryson *et al*., 2004). In addition, the 164 bp and 277 bp amplicons were amplified from soybean and maize derived DNA sources respectively using DNA templates extracted by the CTAB protocol. This showed that the quality and quantity of the DNA were good enough for PCR amplification. The CTAB method was successful to produce the amplifiable DNA stocks by PCR. In the case of smooth tofu and soymilk, the Wizard method was the most favorable choice for extraction of DNA with the yield of 13.2 and 3.4 ng DNA/mg sample respectively, and the A260/A280 ratio ranged from 1.9 to 1.7. In addition, amplifiable DNA (A260/A280 ratio from 1.6 to 1.9) was also obtained from raw soybean, raw maize and animal feed using the Wizard method with presence of proteinase K in lysis buffer. In all cases, the DNA has been successfully amplified using the lectin gene specific primers. The DNA yield and quality from the Wizard method deduced that the solvent-based precipitation worked well as the resin-based system in the original Wizard method. Finally, the addition of BME, a potential health hazard, to the lysis buffer did not give any significant increase in yield or prevention of contamination of the DNA and therefore it can be omitted. The obtained results clearly indicate that the methods of choice depend on the food and feed types. Moreover, DNA quality is more important than DNA quantity in determination of optimal extraction method. Hence, it is hoped that the findings in this study can be used as a guide to select the appropriate method for the extraction of DNA from a specific type of food. Additionally, the presence or absence of transgenes can be done properly and accurately.

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