International Food Research Journal 16: 329-341 (2009)

Real-time PCR evaluation of seven DNA extraction methods for the purpose of GMO analysis

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Abstract: Successful DNA amplification is vital for the detection of specific DNA targets in feeds, and this in return depends on the ability of DNA extraction methods to produce good quality DNA. In this study, seven methods were compared for DNA extraction from feeds using quantitative polymerase chain reaction (PCR) of single copy maize (*Zea mays*) endogenous *hmg* (high mobility group) gene. Relative levels of *hmg* were used to evaluate the DNA quality. Spectrophotometer determination of DNA was also carried out to assess DNA yield and DNA purity, while electrophoretic analysis of genomic DNA extracts was carried out to investigate DNA integrity. The findings illustrate that the DNA extraction methods have a significant effect on DNA quality. Statistically, the Epicentre method extracted the highest DNA yield while the Wizard method had the lowest DNA yield with high DNA purity and integrity. However, the Wizard method recovered the most amplifiable DNA per reaction, indicating that template quality and integrity had greater influence over *hmg* amplification than DNA yield.

Keywords: DNA extraction, GMO, real-time PCR, DNA amplification, feeds

Introduction

Monitoring the presence of genetically modified organism (GMO) in a wide variety of food and feed matrices is important to countries with labeling laws for approved GMOs. In addition, countries may want to test for unapproved GM varieties. Much progress has been achieved in the development of genetic analysis methods in crops (Griffiths *et al.,* 2003). Analytical methods based on PCR technology are increasingly used for the detection of target DNA sequences in GMOs. PCR allows the selective amplification of specific segments of DNA in a mixture of other DNA sequences. Extraction of DNA would be the first step in such analytical methods. The aim of the extraction procedure is to isolate DNA of reasonable quantity, purity, integrity and quality to allow DNA amplification and is often the most time consuming step of a DNA-based detection method. The efficiency of the DNA extraction step can be critical for successful amplification since there are many compounds that inhibit DNA amplification that can be co-purified with the DNA, such as polysaccharides, lipids and polyphenols or extraction chemicals such as CTAB (Anklam *et al.,* 2002).

Virtually all GM crops to date have both food and feed use. This inadvertently will see more GMOs being used for

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Table 1. Summary of feed samples used in the study

Sample number	Sample type	Sample description
4, 9, 10	coarse mix	formed by 'cold' (milling, grinding, cracking, soaking) or 'hot' (steam rolling/flaking, extruding, pelleting) forms of processing (Tisch, 2006).
2, 3, 5, 6	pellet feed	processed hard cylinders of compressed feed ingredients and formed by grinding, blending and compression (Tisch, 2006).
1, 7, 8	expanded feed	undergone high operating temperature and drying stage. Also exposed to steam and forces of shearing and pressure (Tisch, 2006).

Table 2. Summary of DNA extraction methods used in this study

Methods	Basis & format	Starting material	Extraction buffer	Elution buffer	Reference
Epicentre	Solution-based; selective precipitation of DNA	5 – 9 mg	300 µL buffer[a]	50 µL TE buffer[b]	Master Pure Purification Kit.
Modified CTAB	Solution-based; selective precipitation of DNA	100 mg	1000 µL buffer (2% CTAB, 1.4 M NaCl, 20 mM EDTA, 100 mM Tris HCl pH 8.0)	150 µL TE buffer[b]	Tinker *et al.*, (1993)
NucleoSpin	Silica membrane binding; spin-column format	120 mg	550 µL buffer[a]	200 µL buffer[a]	Genomic DNA from food
Qiagen	Silica membrane binding; spin-column format	60 mg	400 µL buffer[a]	150 µL buffer[a]	DNeasy Plant Handbook
CTAB	Solution-based; selective precipitation of DNA	100 mg	1000 µL buffer (2% CTAB, 1.4 M NaCl, 20 mM EDTA, 100 mM Tris HCl pH 8.0)	150 µL TE buffer[b]	Gryson *et al.*, (2004)
Roche	Solution-based; magnetic glass particle technology	50 mg	800 µL buffer (10 mM Tris [pH 8.0], 100 mM NaCl, 2 mM EDTA, 1% SDS)	100 µL buffer[a]	Sakai *et al.*, (2002); MagNA Pure LC DNA Kit 1
Wizard	Silica resin binding; vacuum manifold format	250 mg	3.0 mL buffer (150 mM NaCl, 2 mM EDTA, 1% SDS, 10 mM Tris base pH 8.0)	100 µL TE buffer[b]	Spoth, and Strauss, (1998)

[a] Buffers included with the kit
[b] TE elution buffer (10 mM Trizma base, 1 mM EDTA, pH 8.0)

improving animal diet and nutrition. The feed industry uses a range of raw materials of animal, cereal and vegetable origin. The cereals that are used for diets are maize, barley, oats and wheat while the main sources of plant protein are soybeans, canola and cottonseed. Maize meal is the major component in most complete feeds while soybean meal is the second most plentiful component of most complete feeds (Tisch, 2006).

While studies have already been conducted to evaluate the performance of various DNA extraction methods on food (Jaccaud *et al.,* 2003; Peano *et al.,* 2004; Tung *et al.,* 2009), there has been no study yet to compare the performance of these DNA extraction protocols on feeds in a comprehensive manner as is the main objective of this study. There was a study in Poland, which looked into the occurrence of transgenic maize and soybean in animal feeding stuffs, but the DNA extraction method used in this study was not mentioned (Sieradzki *et al.,* 2006).

In this study, seven DNA extraction protocols that were routinely used in our laboratory for analysis of genetically modified organisms in food were compared for the extraction of DNA from feeds. Commercial kits and methods utilizing reagents were evaluated. Initially, the DNA yield and purity were determined using spectrophotometric analysis. The integrity of genomic DNA was also assessed using gel electrophoresis. The extract quality was evaluated using real-time PCR. Comparison of DNA amplification among sample extracts remains a useful means of comparing DNA quality (Peano *et al.,* 2004; Holden *et al.,* 2003; Smith *et al.,* 2007). In this study, quantitative PCR of an endogenous maize gene, high mobility group (*hmg*, Data Bank accession number AJ131373) was used as a target for comparative quality assessment of DNA recovered from feeds using the different extraction methods. Pelleted feeds, expanded feeds and coarse mixes were used as samples. This study was designed to evaluate the influence of the extraction methods on the DNA amplification through real-time PCR. The results of this study are of considerable scientific use in providing guidance on DNA extraction conditions necessary for feed in order to obtain successful DNA amplification products.

Materials and Methods

Sample material

The experimental design used in this study is Completely Randomized Design with simple random sampling of mutually independent and homogenous feed samples purchased from local pet shops in various states throughout Malaysia in order to include as many different feed manufactures and different sources of raw crop material. The number of feed varieties is so great that it is not feasible to cover all varieties. For this study, three main forms of complete feeds were randomly selected, namely pelleted feeds, expanded feeds and coarse mixes. These feeds were chosen on the basis of their usage, easy availability in pet shops as well as levels of processing. The pelleted and expanded feeds were both highly processed compared to coarse mixes. The coarse mix samples contained a mixture of coarsely chopped maize grain and processed cereals such as barley, oats or wheat in various proportion.

Ten samples comprising three coarse mixes, four pellets and three expanded feeds were used (Table 1). To reduce matrix effect, the same 10 samples were utilized for all seven methods. For each method, each test sample was analyzed in triplicate. All equipments and instruments used in the study were calibrated to control the internal validity of the research. Replication of

treatments was carried out in order to obtain values close to the population mean. Relevant controls and blanks were used.

Genomic DNA extraction and purification

Seven methods were studied. The Roche method is optimized for the isolation of genomic DNA from mammalian whole blood or blood or cultured cells using the MagNA Pure LC instrument, while the other six methods are actually optimized for the isolation of DNA from various food samples of plant origin. Five different commercial kits were used: MagNA Pure LC DNA Isolation Kit I using the MagNA Pure LC Instrument (Roche), DNeasy® Plant Mini Kit (Qiagen), NucleoSpin® Food (Macherey-Nagel), Epicentre MasterPure™ Complete DNA and RNA Purification Kit (EPICENTRE Biotechnologies) and Wizard DNA Extraction and Cleanup Resin (Promega). Two in-house CTAB-based methods were also utilized, namely the standard CTAB (cetyltrimethylammonium bromide) precipitation of DNA protocol (Gryson *et al.,* 2004) and another CTAB protocol with ethanol precipitation of DNA (Tinker *et al.,*1993), which is referred to as 'modified CTAB' method in this study in order to distinguish it from the standard CTAB protocol. A brief summary of each DNA extraction method is outlined in Table 2.

For the Roche method, a pretreatment step was included before the utilization of the isolation kit (Sakai *et al.,* 2002). In a 2 mL tube, the sample was mixed with 800 µL extraction buffer (10 mM Tris [pH 8.0], 100 mM NaCl, 2 mM EDTA, 1% SDS). One hundred microliters of 5 M guanidine thiocyanate was added and incubated for 10 minutes at 60 ºC. Then 1 mL chloroform was added and shaken vigorously for about 20 seconds. The mixture was centrifuged for 5 min at 15000 rpm at room temperature. The upper phase was transferred into another tube before proceeding with the isolation kit, following manufacturer's instructions. Further, the modified CTAB extractions were performed in the same manner as the CTAB standard method, except that DNA precipitation with CTAB precipitation buffer was replaced with ethanol.

Genomic DNA quantification and purity measurement

DNA was quantified to measure total DNA concentration (nanograms of DNA per microliter extract) by measuring UV absorbance at 260 nm (Sambrook *et al.,* 1989). A calibrated Eppendorf spectrophotometer was used. Each quantification was repeated three times. Total DNA yield (nanograms of DNA per milligram of sample) was then calculated. The purity of genomic DNA was evaluated on the basis of UV absorption ratio at 260/280 nm.

Electrophoretic analysis of genomic DNA extracts

DNA extracts were analyzed on 0.8% agarose gels. The gels contained 0.5 µg/mL ethidium bromide and were run in 1 x TAE (diluted from 40x TAE, Promega) for 2 hours at 60V. A 10 kb DNA ladder (New England, Biolabs Inc.) which yielded 10 bands, was used as a ladder. Five microliters of the DNA extract were mixed with 1 µL of 6x Blue/Orange loading dye (Promega) prior to loading the mixture onto the gel. Digital images of the gels were viewed and captured using the AlphaImager™ 2200 imaging system (Alpha Innotech Corporation).

Real-time PCR

Real-time PCR was performed to estimate the amount of endogenous *hmg* gene in the DNA extracts. The PCR reactions were carried out on an ABI

7900HT Sequence Detection System using TaqMan chemistry. The product size was 79 bp. The real-time PCR mix contained 1x TaqMan Universal Mastermix (Applied Biosystems), 300 nM each primer, 160 nM probe and 200 ng of template DNA, making a final volume of 25 µL. The primer sequences were 5'-TTGGACTAGAAATCTCGTGCTGA-3' and 5'-GCTACATAGGGAGCCTTGTCCT-3'. The probe sequence was 5'-FAM-CAATCCACACAAACGCACGCGTA-TAMRA-3'. The reactions were run using the following program: 2 min at 50 ºC to allow uracil DNA glycosylase (UDG) to digest any amplicon carry-over, 10 min at 95 ºC, followed by 45 cycles of 15 s at 95 ºC and 1 min at 60 ºC (Hernandez *et al.,* 2004). All reactions were run in duplicate.

A range of standards was prepared by amplifying *hmg* from maize genomic DNA which was extracted with the Roche method. DNA dilution was carried out yielding 8 different amounts of DNA, assuming 37000 copies of *hmg*/100 ng DNA (Arumuganathan *et al.,* 1991). The amounts of DNA per reaction tube ranged from 88750 to 790 *hmg* copy numbers. Typically, slope values between -3.1 and -3.6 indicate excellent PCR efficiencies while correlation coefficients of $R^2 > 0.98$ indicate an excellent linear relationship with equally efficient PCR amplification over the measured dynamic range (Community Reference Laboratory GMO Methods Database). Concentrations of *hmg* in the sample extracts were determined relative to the standard curve generated.

Statistical analyses

Levels of amplifiable DNA (copies of *hmg* per reaction) were analyzed using one-way statistical between-groups analysis of variance (ANOVA) with multiple-comparison post-test to evaluate the influence of the various methods on DNA quality. DNA yield was also compared using ANOVA. All statistical analyses were performed using SPSS version 15 for Windows.

Results and Discussion

Genomic DNA assessment

The most common and fastest technique to determine DNA concentration and purity is spectrophotometer determination of DNA by measuring the absorbance. Table 3 summarizes the DNA yield and purity range obtained for all sample extracts using the seven extraction methods. These findings suggest that most of variations in the data can be attributed to the effects of the extraction methods used since matrix effect was reduced by using the same samples.

At 95% confidence level, the ANOVA test revealed that there was a statistically significant difference in the DNA yield between the seven groups $F(6, 203)=134.55$, $p=0.0001$. This suggested that the methods were each capable of producing significantly different DNA yields. The mean DNA yield for the Epicentre method was statistically the highest compared to the other six methods while the Wizard method produced the lowest mean DNA yield than all the other six methods.

The data in Table 3 revealed that there was some difference in DNA extracts' purity obtained with the different methods. DNA purity can be severely affected by various contaminants in sample matrices such as polysaccharides, lipids and polyphenols or extraction chemicals such as CTAB (Anklam *et al.,* 2002). The Roche and Wizard methods produced purity ratios in the range of 1.7-1.9. The other methods had some purity ratio readings outside of this range. These differences could be explained by the ability of some of the protocols in eliminating contaminating

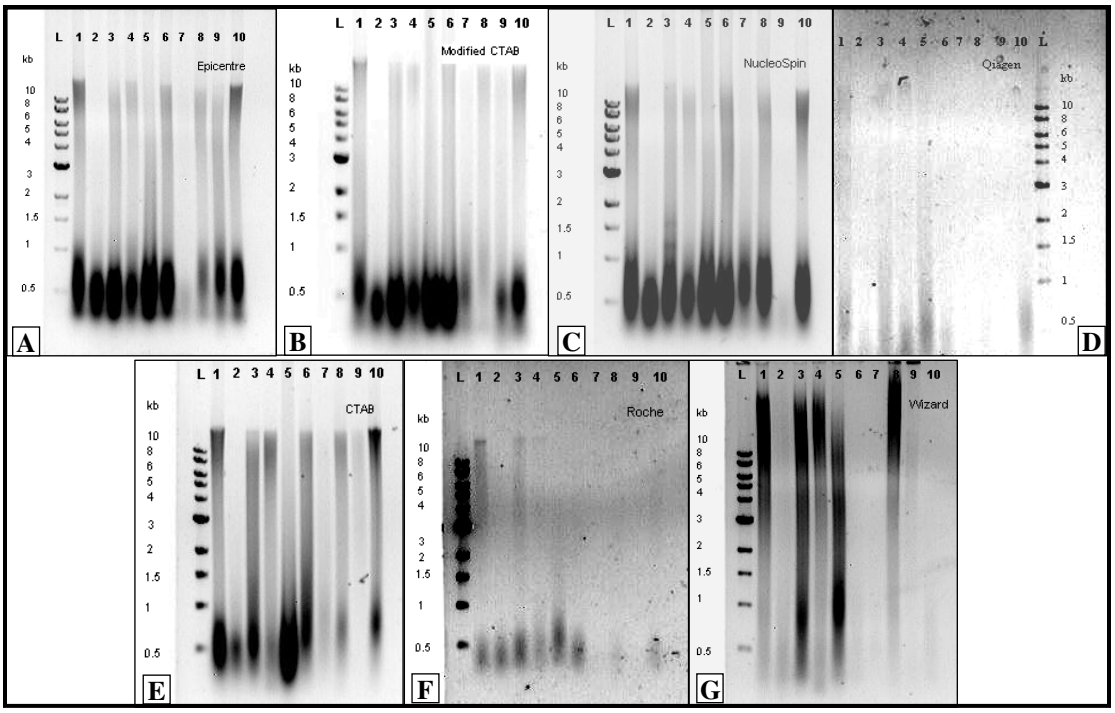

Figure 1. Agarose gel electrophoresis of genomic DNA using the Epicentre (A), Modified CTAB (B), NucleoSpin (C), Qiagen (D), CTAB (E), Roche (F) and Wizard (G) methods. L indicates DNA ladder. Lane number indicates sample number. Samples 1, 7, 8 were expanded feeds; samples 2, 3, 5, 6 were pellets and samples 4, 9, 10 were coarse mixes

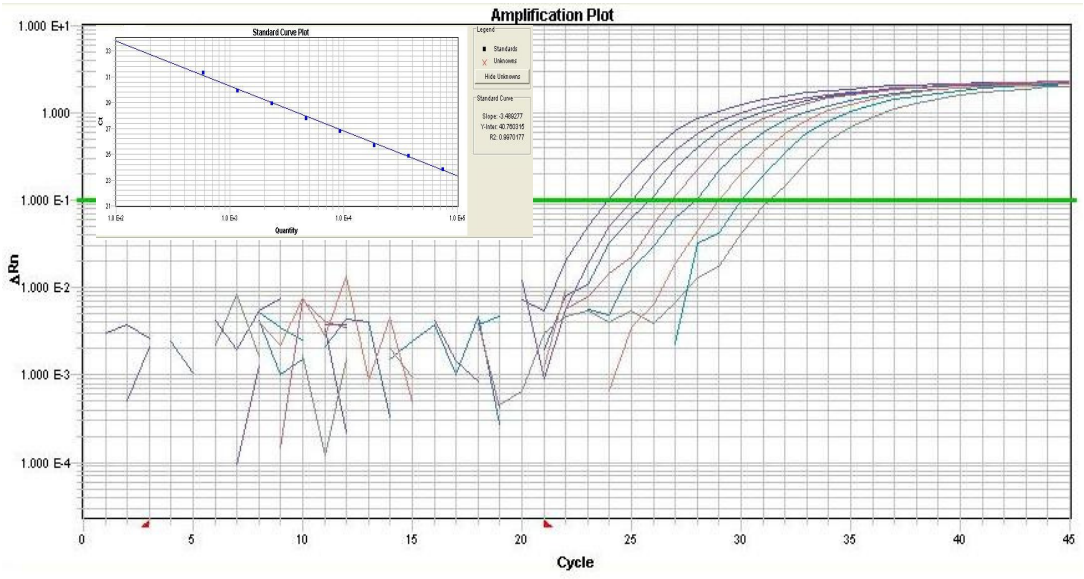

Figure 2. Amplification plots generated by dilution of maize DNA. The standard curve generated from the amplification data is also given

molecules. The methods with purity ratios above 1.9 may indicate some presence of ribonucleic acid (RNA). The modified CTAB and CTAB methods have a few readings below 1.7 probably indicating some presence of protein. However, all extracts

Table 3. Summary of DNA yield and purity for all samples using different DNA extraction methods

DNA extraction methods	DNA yield (ng DNA/mg sample)	DNA purity A_{260nm}/A_{280nm} ratio
Epicentre	> 1000	1.95 - 2.07
Modified CTAB	> 1000	1.54 -1.97
NucleoSpin	> 1000	1.99 - 2.05
Qiagen	200 - 1000	1.80 - 1.95
CTAB	200 - 1000	1.61 - 2.00
Roche	< 200	1.77 - 1.97
Wizard	< 200	1.73 - 1.96

had positive amplification. As demonstrated by Holden *et al.* (2003), sufficient purity does not guarantee successful amplification of a gene. There are other factors that come into consideration.

Further in the study, the integrity of genomic DNA was examined by agarose gel electrophoresis with ethidium bromide staining (Figure 1). The technique has been routinely used for checking the integrity and size of genomic DNA (Zimmermann *et al.,* 1998; Smith *et al.,* 2005; Smith *et al.,* 2007). At a glance, it was observed that none of the lanes displayed intact bands. DNA was smeared and highly degraded with very little high molecular weight DNA observed in almost all samples, except in the Wizard method (Figure 1G). The Epicentre (Figure 1A), modified CTAB (Figure 1B), NucleoSpin (Figure 1C), Qiagen (Figure 1D) and CTAB (Figure 1E) methods all had highly fragmented DNA with low molecular weight (< 1 kb). Among these five methods, CTAB (Figure 1E) seemed to have some fragments between 10 kb-1 kb. As for the Roche method (Figure 1F), there were comparatively faint smears (< 1 kb) in some samples.

Overall, it is evident that method of extraction can have a great influence on integrity of the extracted DNA since the same samples were used, but Wizard produced larger fragments than the other methods. In the Wizard method which had the lowest DNA yield, a mixture of large size fragments (> 10 kb) and average size fragments (10 kb-1 kb) were observed indicating good DNA integrity. From the gel images it is observed that the DNA in feed samples were highly degraded and/or fragmented due to the effects of processing. This observation was consistent with other studies which have indicated that high fragmentation of DNA is expected with the extent to which a crop is processed (Forbes *et al.,* 1998; Gawienowski *et al.,* 1999). Damaged DNA may impair the amplification process and effectively reduce the sensitivity of the test.

Amplification of maize endogenous gene by real-time PCR

Amplifiable *hmg* per reaction was used to estimate the overall quality of the DNA extracted from feeds. DNA quality is generally influenced by the presence of PCR inhibitors, the extent of DNA damage and the length of the extracted DNA fragment. Hernandez *et al.* (2004) described four detection systems for the specific detection and quantification of maize, and the *hmg* gene was the smallest amplicon (79 bp) studied with the lowest limit of detection (LOD) and limit of quantification (LOQ). Therefore for highly processed feeds in this study, this gene was an ideal choice for

DNA amplification. In this study, the LOD for *hmg* gene was < 10 copies and the LOQ was < 30 copies.

For each reaction there was successful *hmg* amplification. This indicated that all the methods had sufficient DNA quantity and quality for the detection and quantification of the *hmg* gene. Amplifiable *hmg* was determined relative to the standard curve which had linear correlation coefficients R^2 of 0.99 and a slope of -3.4 (Figure 2). The copy number values for *hmg* were intended to express a comparative measure of overall quality of the extract.

Comparison of amplified DNA for the three feed types (coarse mix, expanded and pelleted feed) among the various methods would have been interesting to determine which feed type could yield highest amplifiable DNA. However, any direct comparison would not be entirely accurate because in reality the genomic maize DNA integrity is influenced by many factors, such as the quality of starting material, processing nature, storage condition, storage period and the matrix itself. For example sample no. 10 was a coarse mix and contained raw chopped maize grain which theoretically would not pose any extraction problems. However, compared to the other methods where sample no. 10 amplified well, the Wizard method had the lowest amount of amplified DNA for this sample despite the repetition of the extraction process. There was also just a faint smear in lane 10 in the Wizard gel image (Figure 1G). One possible explanation could be that DNA was trapped in the mini column together with the cell debris resulting is only small amounts of DNA in the eluate. This particular problem with the Wizard method was not encountered with the other samples. It should be pointed out that each sample differs in its composition, resulting in unique extraction and purification problems. For the analyst, this problem is easily overcome by selecting other extraction protocols. As demonstrated in this study other methods did work well with sample no. 10. In summary, no single extraction method could produce consistently high amounts of amplifiable DNA in all the 10 samples.

At 95% confidence level, the one-way ANOVA test revealed that there was a statistically significant difference in the levels of *hmg* copy number per-reaction between the seven groups $F(6, 69)=6.45$, $p=0.0001$. This suggested that the methods are each capable of producing significantly different levels of *hmg*. The Wizard method recovered the highest amplifiable *hmg* per reaction from most samples (Figure 3). The Post Hoc multiple comparisons test further revealed that the levels of *hmg* for the Wizard method were significantly different from the other five methods but not significantly different from the CTAB method. The modified CTAB method had the lowest *hmg* levels than all the other six methods but this difference was not significant when compared to the Epicentre, NucleoSpin, Qiagen and Roche methods.

Even though the Epicentre method yielded the highest mean levels of DNA from the feeds, it did not recover the highest quantities of amplifiable DNA in the samples (Figure 3). The same goes for the modified CTAB and NucleoSpin methods which had high DNA yield (Table 3). Another study (Di Bernardo *et al.,* 2007) also demonstrated that while the Epicentre and the CTAB/PTB methods yielded the highest DNA yield in the majority of foodstuffs, both the methods had low level of template quality. One possible reason could be over-estimation of DNA because the smears in the gel in Figure 1 may indicate presence of RNA. Proteins, RNA and salts, all of which are contaminants of

DNA extracted from various biological sources, can increase the spectrophotometric estimation of DNA concentration (Haque *et al.,* 2003). Therefore it is advisable that contaminating RNA is eliminated by digestion with RNase, even though this step is optional in most of the methods. However, RNA will not be amplified in the PCR reaction (Zimmermann *et al.,* 1998). The second possible reason could be that the DNA recovered using these methods was more damaged resulting in much smaller fragments than 79 bp. The third possible reason could be that DNA amplification in these methods was inhibited by PCR contaminants.

PCR inhibition

To investigate PCR inhibition, DNA amplification was performed on a serial dilution of two DNA preparations. Sample no. 8 (expanded feed) from the Epicentre and Wizard methods was randomly selected for this purpose. A two-fold serial dilution of the extracted DNA was prepared (1:1 and 1:2) for both the methods and all four extracts were analyzed by the same real-time PCR as described above. The Ct difference between the two amplifications (1:1 and 1:2) should be one (CRL GMO Methods Database). Deviation from this relationship may indicate that the extracted DNA contains PCR inhibitors. In this experiment, the Ct difference between the two amplifications for the Epicentre method was 1.68, indicating the likely presence of inhibitors. The Ct difference between the two amplifications for the Wizard method was 0.97 indicating absence of PCR inhibitors. This may explain why the Wizard method was able to produce higher amplifiable DNA. Therefore some DNA extracts may have experienced PCR inhibition resulting in lower *hmg* copies even though the method(s) recovered high quantities of DNA. Samples with PCR inhibition may require an additional clean-up step which could remove PCR inhibitors and produce higher amounts of amplification products as demonstrated in a study by Gryson *et al.* (2004). To test if this was true for sample no. 8 from the Epicentre method, the DNA extract was purified using the Wizard DNA Cleanup Resin (Promega). A two-fold serial dilution of the cleaned-up DNA was prepared (1:1 and 1:2) and analyzed by the same real-time PCR as described above. Now the Ct difference between the two amplifications (1:1 and 1:2) was 1.25 which was a marked reduction compared to the Ct difference of the DNA without clean-up. Furthermore, the average *hmg* copy number increased by 12.7% compared to DNA without clean-up. In this particular case, even though there was no big increase in the amount of amplification product after DNA clean-up, the experiment still demonstrated that certain methods can do with an additional DNA clean-up to remove some inhibitors. This may come in helpful in the detection of genetically modified organisms, in particular for samples that contain low levels of the genetically modified gene.

When comparing the in-house methods, even though the modified CTAB method produced significantly higher DNA yield than the CTAB method (Table 3), the latter resulted in higher *hmg* copy number (Figure 3). Statistically, the modified CTAB method had the lowest mean levels of *hmg*. The modified method had some extracts with low purity (Table 3) indicating perhaps presence of protein. Since feeds are rich in protein, the CTAB method seem to be a better choice among the two methods because the CTAB precipitation step in the CTAB method is necessary for protein-rich matrices (ISO 21571:2005(E)).

The Roche method uses proprietary glass magnetic particles to bind DNA to their surface. Despite having low DNA yield

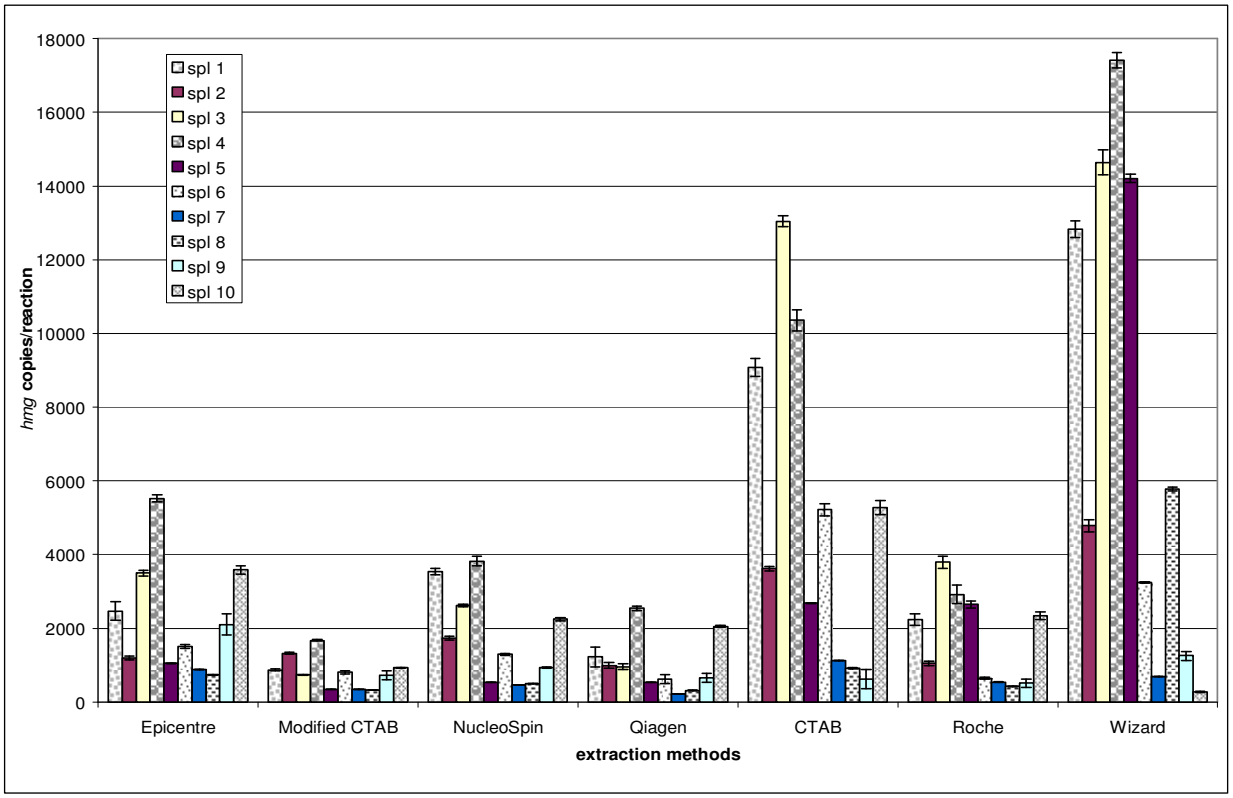

Figure 3. Comparison of different methods for DNA extraction from feed samples by assessing levels of *hmg* measured with real-time PCR. Samples 1, 7, 8 were expanded feeds; samples 2, 3, 5, 6 were pellets and samples 4, 9, 10 were coarse mixes. Error bars represent the standard deviation around the mean values

and displaying faint smears in the gel images but having good purity overall, the Roche method seem to have amplified well resulting in *hmg* copies that are statistically comparable to the Epicentre, modified CTAB, NucleoSpin and Qiagen methods (Figure 3). This is consistent with another study (Hahnen *et al.,* 2002) that also used the MagNA Pure LC purification system for DNA extraction from maize tissue and food samples where no DNA was visible in the gel image but samples were successfully amplified. This suggested that automated DNA preparation with Isolation Kit I which is actually optimized for the isolation of genomic DNA from mammalian whole blood and cultured cells allows extraction of good quality DNA from highly processed feeds.

These experiments have shown that the resin-based extraction method has resulted in comparatively low amounts of DNA but much higher quality for PCR amplification. In two studies by Smith *et al.* (2005, 2007) it was also observed that the Wizard method recovered the highest levels of amplifiable DNA from highly processed products and cornstarch respectively. This indicated that among commercial kit-based methods, the Wizard method does have a wide application range.

Economic evaluation of the extraction methods

In terms of simplicity and speed, the Epicentre, modified CTAB, NucleoSpin Food and Qiagen kits were easy to use compared to Wizard which used a vacuum manifold format. The CTAB method was the most laborious and time-consuming method. The modified CTAB protocol was the cheapest among the methods while the Roche method was the most expensive method due to the procurement of the MagNA Pure LC automation station. However, the use of an automation system for DNA extraction reduces manual labor and cross contamination due to human handling. The most expensive of the tested kits was the Roche Isolation kit I while the Epicentre kit was the least expensive.

Conclusion

The above findings illustrate that the various DNA extraction methods which have been traditionally used for food samples may be successfully employed for feeds samples as well. However no single method was found to produce high amounts of amplifiable DNA in all the samples. Therefore, it is extremely important to use the DNA extraction method that correlates best with subsequent DNA analysis such as real-time PCR, which is commonly used in GMO analysis. All the seven methods had a significant effect on DNA yield and the overall quantity of the amplifiable DNA. In this study, the amount of amplifiable *hmg* recovered from each of the extracts using the seven methods did not correlate to the respective DNA yield. While statistically, the Epicentre method produced the highest DNA yield with moderate DNA purity, the Wizard method which had the lowest DNA yield but high DNA integrity recovered the most amplifiable DNA per reaction. Amplification of DNA was more influenced by DNA quality and the overall structural integrity of the DNA compared to DNA yield. Quantification of the genomic DNA is critical and steps should be taken not to overestimate it. Finally, additional purification steps may be required for some DNA extraction methods prior to amplification using PCR.

Acknowledgement

The authors gratefully acknowledge funding provided by the Department of Chemistry, Ministry of Science, Technology and Innovation Malaysia.

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