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Detection of genetically modified soy in processed foods sold commercially in Malaysia by PCR-based method

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Abstract

Regulations for the use and labeling of genetically modified organism (GMO) products and derived ingredients are being implemented worldwide, that demands reliable and accurate methods to detect GMO in raw materials and food products. In this study, polymerase chain reaction (PCR) method was established for monitoring products derived from GMO that are sold in the markets in Malaysia, which specifically amplify the 35*S* promoter, *nos* (nopaline synthase-terminator), *EPSPS* (5-enolpyruvylshikimate-3-phosphate synthase) and *RRS* (CTP/CP4EPSPS). Using this method, we investigated the incidence of genetically modified soy (GM-soy) and specifically the presence of roundup ready soy (RRS). All the soybean samples were evidenced by presence of the *lectin* gene. Out of 85 samples examined, the 18 positive GM samples were raw bean (9), tofu (8) and tempe (1) (a traditional Malay food). The results demonstrate for the first time the presence of GM-soy in Malaysian food products, reinforcing the need for the development of accurate quantitative methods for routine analyses.

Keywords: Genetically modified organisms; Food products; Detection; PCR; Soy

1. Introduction

Research involving genetically modified organisms (GMOs) in Malaysia is currently conducted on small scales mainly in government-funded research institutions and universities. As a developing country, Malaysia foresees much activity involving GMOs in the future and has therefore established a Genetic Modification Advisory Committee (GMAC), which is under the Ministry of Science, Technology and Innovation, Malaysia. The role of the GMAC includes identification, safety management of risks associated with the use of GMOs and products containing or consisting of GMOs, monitor, implement guidelines, handling inquiries, proposal and approvals concerning the use, release and introduction of GMOs.

The use of GMOs as food and in food products is becoming more and more widespread. The two most cultivated GMOs are maize and soy, which represent the staple constituents of many foods (Gachet, Martin, Vigneau, & Meyer, 1999). According to the European regulation, specific labeling requirements must be applied to foodstuffs that are considered to be no longer equivalent to an existing food ingredient (Reg. 1139/98). Thus, the analytical methods to detect GMOs in various foods are necessary in order to verify compliance with labeling requirement.

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A detection method of GMOs or derivatives of a GMO can be constructed from detection of molecules (DNAs, RNAs or proteins) that specifically targets for the specific sequences or genes that have been inserted to the GMO. The majority of the method hitherto developed for detection of GMOs and GMO-derivatives focuses on detecting DNA, while only a few methods have been developed for detecting proteins or RNAs (Holst-Jensen, 2001). DNA is a relatively stable molecule allowing its extraction from all kinds of tissue due to uniquity of DNA in every type of cell (Wolf, Burgener, Hübner, & Lüthy, 2000) and its analysis from processed and heat-treated food products (Anklam, Gadani, Heinze, Pijnenpurg, & Eede, 2002; Gachet et al., 1999; Holst-Jensen, 2001; Mathews & Holder, 1990). DNA carries an organism's genetic information, and the information content of DNA is greater than protein due to the degeneracy of the genetic code as one goes from DNA to protein (Wolf et al., 2000). Furthermore, the introduction of a foreign gene into the DNA of an organism can be unambiguously detected only at the DNA level (Heller, 2003)

The PCR has been found to be appropriate for the analysis of food (Allmann, Candrian, Höfelein, & Lvthy, 1993; Matsuoka et al., 2002; Meyer, Chardonnens, Hubner, & Luthy, 1996) and also seems to be the method of choice for the detection of GMOs in food (Anklam et al., 2002; Lipp, Anklam, & Stave, 2000; Meyer, 1999; Vollenhofer, Burg, Schmidt, & Kroath, 1999; Yamaguchi, Sasaki, Umetsu, & Kamada, 2003). This method involves a first amplification of specific soy sequence from plant DNA, necessary to discriminate between negative results and positive results due to inhibition in the amplification (Forte et al., 2004). The second step entails amplification of GMO-specific sequence, represent by 35*S* promoter and *nos* terminator, to screen for the presence of transgenic material in samples. Then GMO-containing samples are subjected to analysis of specific transgenic to determine the strain of GMO present (Lin, Chiueh, & Shih, 2000).

This study describes the use of a DNA extraction method, a screening and a construct-specific method for GMO detection in different food products collected from traditional markets, supermarkets and grocery stores in Malaysia.

2. Materials and methods

2.1. Samples

Raw soy and its processed produced were purchased from traditional markets, supermarkets and grocery stores in Meru, Sungai Buloh and Serdang in Malaysia. The Certified Reference Materials (CRM) standards consisting of dried soybean powder with 2% and 0% GM-soy materials produced by the Institute for Research Materials and Measurements (IRMM – http://irmm.jrc.cec.eu.int/) and commercialized by Fluka were used as positive and negative controls.

2.2. DNA extraction

The DNeasy® Protocol for plant provided with the DNeasy® Plant Kit (Qiagen, Hilden, Germany) was used for the extraction of genomic DNA from samples of soy and its product, and the CRM standards.

2.3. DNA quantification

The concentration and purity of the extracted DNA were measured by absorbance at 260 and 280 nm using a spectrophotometer Bio Mate™ 3 series, Thermo Spectronic, Cambridge, UK.

2.4. Oligonucleotide primers

The primer pairs used were: p35S1-5(5′-ATT GAT GTG ATA TCT CCA CTG ACG T-3′)/p35S1-3(5′-CCT CTC CAA ATG AAA TGA ACT TCC T-3′) for detection of *CaMV 35S* promoter (Tengel, Schvssler, Setzke, Balles, & Sprenger-Haussels, 2001), HA-*nos* 118-r(5′-GAC ACC GCG CGC GAT AAT TTA TCC-3′)/HA-*nos*118-f(5′-GCA TGA CGT TAT TTA TGA GAT GGG-3′) for detection of *nos*-terminator (Lipp, Brodmann, Pietsch, Pauwels, & Anklam, 1999; Lipp et al., 2001), and RRO1 (5′-TGG CGC CCA AAG CTT GCA TGG C-3′)/RRO4 (5′-CCC CAA GTT CCT AAA TCT TCA AGT-3′) for detection of Roundup Ready Soy (Tengel et al., 2001). The amplificability of DNA extracted was verified using plant-specific primer pair Lectin 1/Lectin 6 targeting the *lectin gene*, specific to soy (Tengel et al., 2001, Tengel, Schvssler, Setzke, Balles, & Sprenger-Haussels, 2002).

2.5. Detection of gene sequences by PCR

Amplifications by polymerase chain reaction (PCR) were carried out in reaction mixtures (25 μl) containing 2.5 μl of 10 × PCR buffer, 2.5 μl of 0.2 mM dNTP, 3 μl 3 mM $MgCl_2$, 1 μl primers with 0.2 μM each and 0.125 μl AmpliTaq Gold (Applied Biosystem, Inc., New Jersey, USA). Amplification was performed with a Perkin–Elmer (Gene Amp PCR system 9700) thermal cycler according to the following PCR step-cycle program for all primer pairs used: pre-denaturation of 94 °C for 3 min, followed by 50 cycles of denaturation at 94 °C for 45 s, annealing at 60 °C for 45 s and extension at 75 °C for 25 s. A final extension at 72 °C for 7 min followed the final cycle for complete synthesis of elongated DNA molecules. The PCR products were analyzed by electrophoresis on a 2.0% agarose gel

(Promega, Madison, USA), stained with ethidium bromide and photographed using the Syngene gel documentation system. A 100 bp DNA ladder (Promega, Madison, USA) was used as size reference.

3. Results and discussion

We employed a three step analysis to determine the identity of GMO samples. The first step involves genomic DNA extraction and amplification of specific soy sequence from plant DNA (*lectin gene*), necessary to discriminate between negative and positive results due to inhibition in the amplification (Forte et al., 2004). The second step entails amplification of GMO-specific sequence, represented by the 35*S* promoter and *nos* terminator, to screen for the presence of transgenic material in the samples. Then, GMO-containing samples were subjected to analysis of specific transgenic material (Roundup Ready™ Soy specific gene) to determine the type of GMO present (Lin et al., 2000).

The quality of the extracted DNA from 200 mg of samples (soy and its product) using the DNeasy® Protocol for plant provided with the DNeasy® Plant Kit (Qiagen) was examined by electrophoresis through a 1.7% agarose gel (Promega). DNA bands of high intensity appeared in the lanes (Fig. 1), showing high yield of genomic DNA extracted from the samples and were sufficient to be used as template for PCR amplification of the gene of interest.

Eighty-five samples were analyzed (Table 1) and the results showed that 18 out of 85 soy samples (21%) were positive for the three introduced genetic elements, the promoter (*P35S*), terminator (*NOS*) and structural gene (RRO) genes. Of the 18 positive GM-soy samples, nine of them were raw bean, eight were tofu and one was tempe (a traditional Malay food). All of the positive samples in this study came from unclear source and domestic processed products (traditional market and supermarket). The test samples were also configured to be soybean products by using a *lectin gene* primer targeting the endogenous gene of soybean (Lin, Chiang, & Shih, 2001; Lin et al., 2000). Elsewhere, Lin et al. (2001) reported that 22 out of 28 commercial GM crops examined were found to contain the 35S-promoter or NOS-terminator genes.

All samples analyzed produced sufficient amount of DNA with a quality to be amplified with the eucaryote-specific PCR. Agarose gel electrophoresis of the PCR amplified products (Fig. 2) from the samples resolved a band of approximately 318 bp for the detection of *lectin gene*. However, no amplification could be observed for soy sauce. This could be expected since soy sauce is a highly fermented product and in some cases, soy sauce does not contain soy proteins but proteins

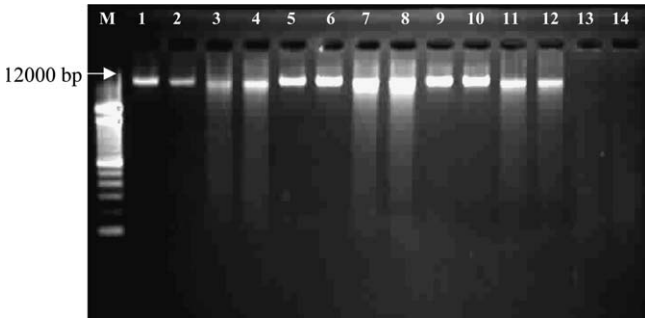

Fig. 1. Representative agarose gel electrophoresis for DNA extracted from soy and soy derived raw materials. Lanes: M, 1 kb plus DNA ladder; 1 and 2, CRM 2% Roundup Ready™ soy; lanes 3 and 4, raw soy; 5 and 6, soy flour; 7 and 8, tofu; 9 and 10, fucuk; 11 and 12, tempe; 13 and 14, soy sauce.

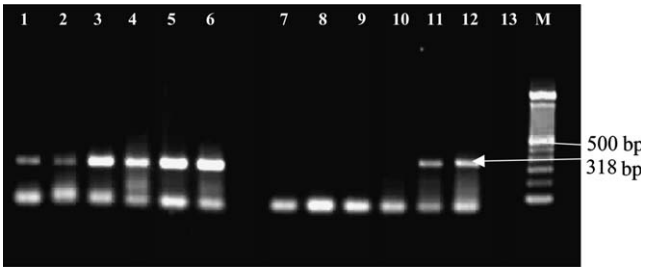

Fig. 2. Representative agarose gel electrophoresis of PCR products from soy and soy derived raw materials for analysis of positive *lectin gene*. Lanes: 1, raw soy; 2, soy flour; 3, tofu; 4, fucuk; 5, tempe; 6, 0% RRS; 7, 8, 9 and 10, soy sauce; 11, 0% RRS; 12, 2% RRS; 13, negative control (without DNA); and M, 100 bp DNA ladder.

Table 1
Detection of lectin gene, 35S promoter, nos terminator and structural gene (RR) in food samples containing soy product

Products	Number of samples	Positive sample for lectin	Screening (+ve)		Gene specific (+ve) (EPSPS/RR)
			Promoter	NOS	
Raw soybeans	20	20	9	9	9
Soy flour	5	5	0	0	0
Tofu	37	37	8	8	8
Fucuk	10	10	0	0	0
Tempe	8	8	1	1	1
Soy sauce	5	0	0	0	0
Total	85	80	18	18	18

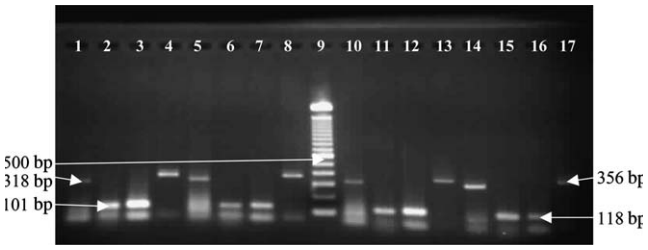

Fig. 3. Representative agarose gel electrophoresis of PCR products obtained from soy and soy derived raw materials for analysis of positive Roundup Ready™ soy (RRS). Lanes: 1–4, total DNA from soy flour samples corresponding to *lectin gene*, 35*S* promoter, *nos*-terminator and Roundup Ready™ Soy specific gene; 5–8, total DNA from tofu samples corresponding to *lectin gene*, 35*S* promoter, *nos*-terminator and Roundup Ready™ Soy specific gene; 10–13, total DNA from fucuk samples corresponding to *lectin gene*, 35*S* promoter, *nos*-terminator and Roundup Ready™ Soy specific gene; 14–17, total DNA from tempe samples corresponding to *lectin gene*, 35*S* promoter, *nos*-terminator and Roundup Ready™ Soy specific gene; and 9, 100 bp DNA ladder.

from other source for fermentation, thus, the detection of soy endogenous DNA is obviously difficult. Our observations were corroborated by Greiner, Konietzny, and Villavicencio (2004), who reported that no DNA could be extracted from soy margarine, soy sauce, soy and maize oil.

There are two different types of PCR that can be distinguished, the screening systems and the specific system. The screening systems, which are not specific for one particular GMO detects commonly used elements in genetic engineering, such as promoter or terminator which contain the *35S promoter* of *CaMV* (*Cauliflower Mosaic Virus*), the (*nos*) terminator of *Agrobacterium tumefaciens* or the kanamycin-resistance marker genes (*npt*II) (Ahmed, 2002; Shirai et al., 1998). The highly specific systems can detect only one particular GMO and allow quantification using a competitive PCR technique (Studer, Rhyner, Luthy, & Hubner, 1999). Fig. 3 showed the PCR amplified product from the samples with bands of approximately 318, 101, 118 and 356 bp for *lectin gene*, 35*S* promoter, *nos*-terminator and Roundup Ready™ Soy specific genes, respectively, that fulfilled the product-size requirement and completed the whole detection procedure of GM events for raw soybean

4. Conclusion

The importance of the detection of genetically modified organism (GMOs) in food is increasing dramatically. The regulatory body recently adopted an important legislation package on GMOs which establish a sound community system to trace and label GMOs and to regulate the placement on the market and label of food products derived from GMOs (Reg 258/97, Reg 1139/98, Reg 49/00, Reg 50/00). It requires that GMOs be traceable throughout the chain from farm to table and provide consumers with information by labeling all food consisting of; containing or produced from GMO (Forte et al., 2004; Regulation, 1997, 1998, 2003a, 2003b).

However, there are many challenges ahead for governments, especially in the areas of safety testing, regulation, international policy and food labeling. GMOs in Malaysia are in their infancy, but through co-operation between public health and commerce, the significance for human health can be recognized. GMO testing is needed as a precursor to promoting high standards of regulation, tracking developments, acting on new evidence and instituting population health surveillance in Malaysia.

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