SCREENING FOR GENETICALLY MODIFIED SOYFOOD AVAILABLE AT SELECTED SUPERMARKETS IN SRI LANKA

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Introduction

In recent years, foods produced by genetic engineering technology have been in the world food market. The biosafety aspects, regulations, and labeling of these foods are still contentious issues in most countries including Sri Lanka. Although regulations have been prepared to control the entry of GMOs/F, due to the non-compliance with the laws by many importers, GMF still freely enter the country and are available in the local market without labeling. In addition, mixing European Union approved GMF with non-GMF is common in the 'black market' and this issue is more prominent with soy products (Gillam, 2008). Thus, detection GMOs play crucial role for developing regulations on GM foods in Sri Lanka (Food Act 2007). Mixing of biotech crop products with conventional non-GM crop products can also occur during many phases of harvest, storage and shipment (Gillam, 2008). Hence, it is essential to identify the detection limit of the GMF after mixing of GM and non-GM products. Therefore, the objective of this study is to screen for GM soya products, which are available in selected supermarkets in Sri Lanka and determine the PCR detection limit of GM soy in flour mixtures.

Methodology

Twenty soya food samples were randomly obtained from a super market in Colombo, Sri Lanka, during the period from December 2008 to January 2009. Out of the 20 products collected, there were 8TVP, 5 instant soya mixes, 1 soya milk powder (1), 3 soya sauces, 2 processed soya flour and 1 soya animal feed samples. The DNA was isolated from 0.1 g of grounded samples using a CTAB protocol method, as described by Kqueen (2006). Thereafter, PCR (Stratagene *RoboCycler*®) was performed with internationally validated programs (Kqueen, 2006) for screening the presence of regulatory elements (i.e. NOS terminator and CaMV 35S promoter) and structural genes [i.e. Cry (ab) gene and EPSPS gene) with primers that are given in Table 1. The PCR amplification products were analyzed using 1% agarose gels (Promega, USA). Gels were digitized using a camera-based documentation system (IBM PC Camera, USA) and the respective Multi Analyst software (IBM Odyssey multimedia, USA). The above procedure was repeated thrice to confirm the results. Soya flour mixtures were prepared by mixing flour from EU tested + GM mixed soya flour and conventional non GM soya flour in the ratios of 1.0, 0:1, 1:1,

1:2, 1:10, 1:100 and 1:100. The mixtures were then vortexed for 1 min and test samples were taken using a statistical sampling method (quartering techniques). The DNA was isolated from the above mixtures separately and PCR was conducted for the identification GM detection end point of flour mixers with P35s Primers and HA-NOS primers. An approximate quantification of DNA was carried out by comparing of PCR end points of certified reference Roundup® Ready soya (IRMM, Geel, Belgium, distributed by Fluka Chemie CP4-EPSPS structural gene. They did not yield PCR amplifications for the AG Buchs, Switzerland) and + GM mixed soya flour.

Table 1- Primers for the detection of 35S promoter, NOS terminator, EPSPS gene andry gene.

Primer	Sequence Amplicon (3'-5')	Length	Identification Region	Amplicon length
P35S-cf3 5'	CCACGTCTTCAAAGCAAGTGG	21	CaMV promote	118bp
P35S-cr4 5'	TCCTCTCCAAATGAAATGAACTTCC	25	CaMV promote	
HA-NOS 118-f	GCATGACGTTATTTATGAGATGGG	24	NOS terminator	123bp
HA-NOS 118-r	GACACCGCGCGCGATAATTTATCC	24	NOS terminator	
EPSPG169F 5'	ATCCCACTATCCTTCGCAAGA	21	EPSPS gene	169 bp
EPSPG169R 5'	TGGGGTTTATGGAAATTGGAA	21	EPSPS gene	
Cry1Ab184F	ACCATCAACAGCCGCTACAACGACC	25	Cry gene	129 bp
Cry1Ab184R	TGGGGAACAGGCTCACGATGTCCAG	25	Cry gene	

Results

Based on the PCR, out of 20 tested samples, either the 35S promoter (123bp) or the NOS terminator (118bp) sequences were detected in four samples (1 TVP, 1 Soya milk, 1 animal feed, and 1 instant soya mixture) while neither the 35S promoter (123bp) nor the NOS terminator (118bp) was detected in the other 16 samples. All the GM positive specimens were also positive for the Cry1 ab gene. Based on the PCR analysis with NOS 118-f/p35s primers up to 10:1 w/w (conventional flour: GM mixed flour) were detected (CAMV 123bp and NOS 118bp) in the gel electrophoresis. According to the quantification assay, GM-mixed soy flour sample contained approximately 1.7% GM content.

Discussion

In this market screening, out of 20 tested soy samples, four soy samples were GM positive for CAMV promoter and NOS terminator in the PCR assay. Testing was based on the detection of these two regulatory elements as more than 95% of the presently available GM crops have either the CaMV 35S promoter or the NOS terminator or both (Kqueen, 2006).

Further, the GM positive samples yielding a positive result for the CP4-EPSPS structural gene indicated the presence of the EPSPS gene, which is found in the Roundup® Ready biotech soy category. Since about 8% of the total biotech crops contain both herbicide-tolerant and insect-resistant ("stacked genes") structural genes (James 2007), further analyses was carried out to determine the presence of the insect resistance gene (Bt – Crops) in the specimens that were positive for GM content. Although the Food Act (2007) gives provisions for labeling of food that contains GM products, none of the products that tested positive in the present study had been labeled as containing GM. Instead, one of these products was labeled as "non-GM", while the other three did not have any label about their GM status.

Based on the PCR analysis up to 10:1 w/w (conventional flour: GM mixed flour) gives positive results in the PCR assay. As the GM-mixed flour used in this study had 1.7% of GM content, 1 g of GM flour will have only 0.017g of GM content. This implies that a ratio of 116:1 w/w of conventional non-GM flour: GM flour can be detected using this PCR assay with a detection level of about 0.1% GM content.

Conclusion

Although legislation is available to control GMF in Sri Lanka, these products are available in the local market without any reference to their GM status. The PCR-based GM detection method used in this study can detect up to 116:1 w/w ratio of non-GM soya flour: GM mixed soya flour. This study addresses important safety issues required for the enforcement of legislative guidelines on labeling rules, and appropriate traceability systems in order to guarantee public health and consumer choice with respect to GMOs/Fs.

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