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PCR Based Detection of Genetically Modified Soy in Processed Foods Commercially Available in Saudi Arabia

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الكشف باستخدام الـPCR عن الصويا المعدل وراثياً في منتجات الأغذية المتوفرة تجارياً في المملكة العربية والسعودية

ابراهيم بن عبدالله صالح العريض

خلاصة: في هذا البحث تم استخدام طريقة تفاعل سلسلة إنزيم البوليمريز (PCR) للكشف عن وجود الأغذية المعدلة وراثيا في أسواق المملكة العربية السعودية. تم تطبيق هذه الطريقة للكشف عن وجود الأغذية المعدلة وراثيا خصوصا وجود جين الـ Roundup Ready Soy (RRS). ومن ثم تم التأكد أن كل العينات تحتوي على فول الصويا من خلال وجود جين الليكتين (*lectin* gene) الموجود أصلاً في فول الصويا. تم فحص 80 عينة غذائية من المفترض أن تكون غير معدلة وراثيا. وجد أن عينتين منهما معدلتين وراثيا مما يثبت وجود فول الصويا المعدل وراثياً في منتجات الأغذية في أسواق المملكة العربية السعودية. هذا الاكتشاف يدعم أهمية تطوير طرق كمية ونوعية للتحليل الروتيني للكشف عن منتجات الأغذية في أسواق المملكة العربية السعودية. إن وجود الأغذية المعدلة وراثيا في أسواق المملكة قد يكون دليل عن إمكانية وجودها في دول الشرق الأوسط سواء تم توريد هذه المنتجات بقصد أو بدون قصد.

ABSTRACT: In this research, PCR (polymerase chain reaction) technique was applied to detect the presence of genetically modified soy (GMO) sold in the Saudi Arabian market. This method was applied to detect genetically modified soy (GM-soy), in particular the roundup ready soy (RRS). To confirm the presence of soy, samples were first tested for the existence of the soy specific *lectin* gene. A total of eighty samples were tested, out of which two samples tested positive as containing GM-soy. Not surprisingly, the findings showed the existence of GM-soy in food products in Saudi. This supports the necessity of developing precise quantitative and qualitative ways for routine analyses and detection of GMO products in the Saudi Arabian market. With the discovery of GM products in the Saudi Arabian market it would be of no surprise that other Middle Eastern nations also knowingly or unknowingly import GM crops.

KEYWORDS : RR soybean, Genetically modified organism, Processed foods, Soy, PCR.

1. Introduction

Nowadays, utilizing genetically modified soys (GMOs) as food and in food products has become common. Many transgenic plants have been widely grown and sold in the global market since 1996 (James, 2003). Maize and soy are the most cultivated GMOs. They constitute a basic component of many prepared foods (Gachet *et al.*, 1999). Particular labeling requisites have to be applied – in conformity with the European rules – to foodstuff which are not equivalent to a present food ingredient (Regulation 1139/98). The Saudi Authorities have issued a legislation that imported food should be labeled if contains genetically modified materials indicating that that the product is genetically modified (Regulation 1666). Therefore, it is essential to use analytical methods to detect GMOs in varied foods so as to investigate accordance with labeling requirements.

We can establish a way of detecting GMOs and GMO derived ingredients by examining molecules (DNAs, RNAs or proteins) which particularly targets the genes or sequences which were entered into the GMO. So far few ways have been developed for detecting proteins or RNAs, whereas most of the ways developed for detecting GMOs and derived ingredients concentrate on detecting DNA (Holst-Jensen, 2001). This is because DNA is a fairly stable molecule which allows its extraction from all types of tissue, and its analysis from processed and heat-treated food products (Abdullah *et al*., 2006; Anklam *et al*., 2002; Holst-Jensen, 2001; Gachet *et al*., 1999; Mathews & Holder, 1990), and because of the uniqueness of DNA in each kind of cell (Wolf *et al*., 2000). The genetic information content of DNA is greater than protein because of the degeneracy of the genetic code as one goes from DNA to protein (Wolf *et al.*, 2000). In addition, when introducing a foreign gene into the DNA of an organism it can be easily detected only at the DNA level (Heller, 2003).

It was found that the PCR is suitable for analyzing food (James, *et al*., 2003; Matsuoka *et al*., 2002; Meyer *et al.*, 1996; Allmann *et al*., 1993) as it is the method selected for detecting GMOs in food (Yamaguchi *et al.*, 2003; Anklam *et al*., 2002; Lipp *et al.*, 2000; Meyer, 1999; Vollenhofer *et al.*, 1999). This method includes a first amplification of certain soy sequences from plant DNA. This is needed to differentiate between negative and positive results because of the inhibition in the amplification (Forte *et al*., 2004). Secondly it is necessary to amplify GMO-specific sequences represented by 35S promoter and *nos* terminator, to sift for the existence of any transgenic material in samples. After that the samples that contain are analysed for specific transgenic substances to determine the strain of GMO in them (Lin *et al.*, 2000)

In this research, we characterize how a DNA extraction technique is used. It is a method of screening and is construct-specific for detecting GMO in various food products which are found in Saudi markets, supermarkets and grocery shops.

2. Materials and methods

2.1 Samples

Raw and processed products containing soy were purchased from supermarkets and grocery stores located in Riyadh, Saudi Arabia. The certified reference materials containing 0%, 1%, 2%, and 5% GM-Soy materials were obtained from the European Reference Materials (Belgium).

2.2 DNA extraction

The DNeasy Plant Kit (Qiagen, Hilden, Germany) was used for extraction of DNA from all samples of soy and its products due to its ease and rapid extraction methodology, and the CRM standard is applied.

2.3 DNA quantification

The concentration and purity of the extracted DNA was sampled using the Gene Quant spectrophotometer (Amersham, USA) at 260 and 280 nm wavelength absorbance.

2.4 Primers used

Three pairs of primers as listed in Table 1 were used in this study. These primers were the GM03/ GM04 primers for the amplification of a 118bp DNA fragment for soy specific lectin gene, 35S1F/35S1R primers for the amplification of a 101bp DNA fragment for 35S which is a marker for GMO content, and Pro1/Pro4 primers for the amplification of a 356bp DNA fragment that indicates the GMO specific Round-Up Ready soy.

Table 1 . Primers used in this study

Primers	Oligonucleotides primers	Amplicon (bp)	Reference
Lectin	5'-GCCCTCTACTCCACCCCCATCC-3' 5'-GCCCATCTGCAAGCCTTTTTGTG	118bp	Meyer *et al* ., 1996
35S promoter	5'-ATT GAT GTG ATA TCT CCA CTG ACG T-3' 5'-CCT CTC CAA ATG AAA TGA ACT TCC T-3'	101bp	Tengel *et al*., 2001
Roundup Ready	5'-TGG CGC CCA AAG CTT GCA TGG C-3' 5'-CCC CAA GTT CCT AAA TCT TCA AGT-3'	356bp	Tengel *et al*., 2001

2.5 PCR reaction and product analysis

Amplification by polymerase chain reaction (PCR) was carried out in reaction mixture (25µl). PCR amplification was carried out in Ready to Go PCR Beads (Amersham Bioscience, USA). Reaction consisted of 50 temperature cycles of denaturation for 45 seconds at 94^{o}C, annealing for 45 seconds at 60 ^{o}C, and extension for 25 seconds at 75 ^{o}C. Final extension was performed at 72 ^{o}C for 7 minutes. The PCR products were analyzed by gel electrophoresis on a 2.0% agarose gel stained with ethidium bromide and photographed using a gel documentation system. A 100bp ladder (Amersham, USA) was used as a size marker.

3. Results and discussion

In this study, analysis included three steps to define the nature of GMO samples. Firstly, genomic DNA extraction and amplification of certain soy sequences from plant DNA (*lectin* gene) were performed, which is important in order to differentiate between positive and negative results because of the inhibition in the amplification (Forte *et al*., 2004).

Secondly, the GMO-specific sequence represented by the 35S promoter was amplified. This was to sieve for the existence of any transgenic substance in the sample. Thirdly, the samples that contained GMO were exposed to analysis of specific transgenic material (Roundup Ready^{TM} Soy specific gene) to define the kind of GMO present (Lin *et al*., 2000).

Electrophoresis through a 2% agarose gel (promega) was used to examine the PCR products. Highly intensive DNA bands were observed in the gel. It turned out that a high yield of genomic DNA had been extracted from the samples. This was enough to be used as a template for PCR amplification of the concerned gene. 2 samples out of 80 soy samples (2.5%) were positive for the two introduced genetic elements, the promoter (P35S) and structural gene (RRO) genes as shown in figures 1, 2 and 3. The classifications of the 2 positive GM-soy samples were as follows; a baby biscuit, and a cracker biscuit. In this research, all the positive samples were imported from abroad. The products were not labeled as containing positive GM-soy. A *lectin* gene primer targeting the endogenous gene of soybean was also used to confirm the presence of soy.

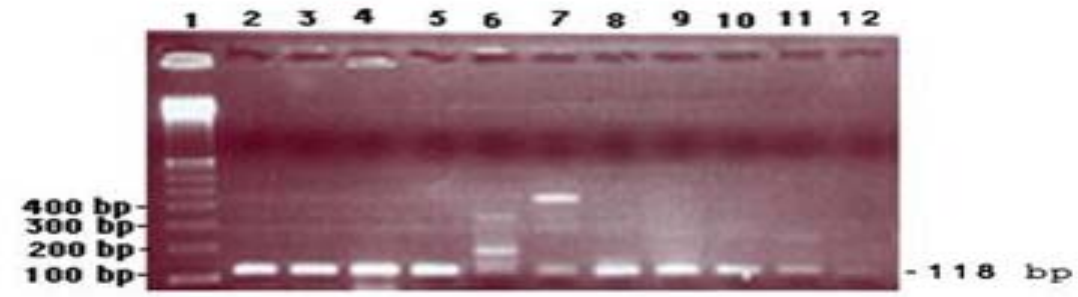

Figure 1. electrophoresis showing the detection of the specific bp *lectin* gene fragments in soy and soy products. Lane 1: 100bp molecular marker, Lane 2:Chocolate, Lane 3: Baby Biscuit, Lane 4: Milk Powder, Lane 5: Spice Powder, Lane 6:Biscuits, Lane 7: Toffee Crunch, Lane 8: Crackers, Lane 9: Cake Flour, Lane 10: Raw Soy, Lane 11: Soy Flour, Lane 12: Baby Rusk .

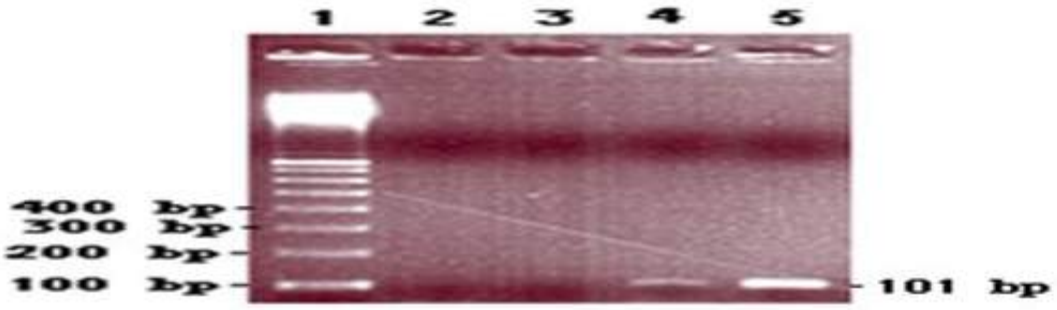

Figure 2. Gel electrophoresis showing the detection of the specific 101 bp 35S Promoter in soy and soy products. Lane 1: 100bp molecular marker, Lane 2: Negative Control, Lane 3: Soy Flour, Lane 4: Baby Biscuit, Lane 5: Cracker.

When the samples were analyzed, they generated enough DNA of a quality to be amplified with the eucaryote-specific PCR. For the detection of *lectin* gene, agarose gel electrophoresis of the PCR amplified products from the samples resolved a band of 118 bp as observed in Figure 1. However, no amplification occurred in soy sauce as it is very difficult to extract DNA from oils and sauces. In addition, soy sauce is a fermented product and sometimes contains proteins from other sources for fermentation and not soy protein. Consequently, we can say that detecting soy endogenous DNA is difficult. Other researchers have supported our observations. Greiner *et al.,* (2004) stated that out of soy margarine, soy sauce, soy and maize oil, no DNA can be extracted.

Two kinds of PCR application using different sets of primers can be differentiated; the screening system and the specific system. As for the screening systems, they are not specific for one certain GMO. They detect commonly used elements in genetic engineering, for example promoters or terminators that contain the *35S promoter of CaMV (Cauliflower Mosaic Virus)* (Figure 2), the (*nos*) terminator of *Agrobacterium tumefaciens* or the kanamycin-resistance maker genes (nptII) (Ahmed, 2002; Shirai *et al.*, 1998). Only one certain GMO can be detected by very specific systems, such as the detection of the Roundup Ready soy (RRS) where the CP4EPSPS gene (5-enolpyrunylshikimate-3-phosphatesynthase from *Agrobacterium tumefaciens* strain CP4) that is- a

structure gene of inserted recombination in RRS) is detected as indicated by the primers Pro1/Pro4. They can allow quantification by using an emulative PCR method (Studiers *et al.*, 1999).

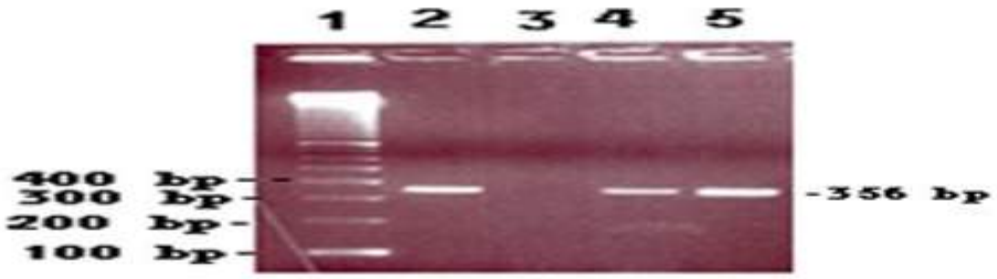

Figure 3. Gel electrophoresis showing the detection of the specific 118 bp Roundup Ready soy gene fragments in soy and soy products. Lane 1: 100bp molecular marker, Lane 2: CRM 2% Roundup Ready, Lane 3: Negative Control, Lane 4: Baby Biscuit, Lane 5: Cracker.

4. Conclusion

The PCR method and thermocycle has become a basic technique in life science laboratories. This method is capable of detecting target regions in different test samples using different conditions and primers. Nowadays, detecting genetically modified organism (GMOs) in food has an increasing importance.

Nonetheless, governments face many challenges, particularly in the fields of food labeling, international policy, regulation and safety testing. The importance of human health has to be taken more seriously in Saudi Arabia. As GMOs are constantly produced, an urgent need of cooperation between the public, commerce and the government has to be addressed. Thus a thorough national policy and food testing facility with the latest scientific instruments and trained personnel has to be established for this purpose, not only for Saudi Arabia but also as a centre for the whole gulf region. This facility need not be only for GMOs, but should also be a testing and research facility that monitors all foodstuffs for meat contamination in terms of non-halal contamination and toxicity with general compliance by food manufacturers with internationally recognized food safety standards. Moreover this facility or center should also produce and seek the advice of qualified and recognized individuals in terms of Islamic law and legislation regarding food, and eventually guide the direction of Food Science in Islamic countries.

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