

Rapid and accurate detection of genetically manipulated soybean using polymerase chain reaction

Abstract

Background: It is controversy over the GM products consumption around the world. However, the limitations of food supplies and population growth have forced the posterity to use transgenic products. Accurate strategies for consumption and use of proper diagnostic methods are crucial in this regard.

Materials and methods: The soybean commercial samples were purchased as a control from supermarkets located in Tehran. Considerable attention was paid to collecting samples from different brands. In this investigation, in order to identify the soybean oil in products, randomly, 25 raw oil and soybean oil samples were collected and numbered. An equivalent of 50 g of each sample was prepared for testing. In order to determine the transgenic soya samples, the specific primers for the 35S promoter region of mosaic virus CaMV35S and nephaline synthase (NOS) transcription terminator were designed from *Agrobacterium*. The sequence of the CaMV35S promoter and the NOS terminator were obtained according to that of Lipp and Brodmann investigation. The PCR product size for 35S and NOS primers was 195 and 180 bp, respectively.

Results: By PCR technique the specific Soybean (164bp), GMONOS180 (180bp) and GMO35S (195bp) genes were amplified. Among the 25 tested oils, 9 (36%) were GM, being a high percent and of these nine transgenic cases, two had CaV-35s, and also were nos-positive, indicating of their transfusion certainty (GM). This high percentage of transgenic oils is possibly due to the lack of awareness of the producers.

Conclusion: The microbiological and chemical methods alone cannot control the quality of food products, and detect the presence of transgenes. Therefore, the use of PCR technique is important in controlling the quality of this product. In cases where manufacturers and importers of products prepare and distribute transgenic products without labeling, they can be easily prevented from misuse and thus PCR as complementary and helpful to determine the composition and quality of the product.

Keywords: genetic manipulation, soya, olive, oil

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Introduction

The turning to genetic engineering has enabled the human to accelerate the process of evolution and make precise changes to the physical characteristics of the creatures to achieve useful and desirable features. The genetic manipulation (GM) began in 1973, when the first genetically manipulated gene was made, and then GM organisms (GMOs), whose genetic material (DNA) undergone alterations in abnormal routed such as recombinant DNA technology, have been developed.¹ GM foods (GMFs) or additives have also been derived from GMOs which are also genetically engineered foods (GEFs) produced by recombinant DNA technology (rDNA). The cloning and expression of a desired gene from a bacterium into a genome of a plant is comfortable and this issue could be potentially economically important.²

Most food changes are concentrated primarily on income-rich products such as soybeans, maize and canola. MaisGard™ was approved by the US government in 1996, and in 1997, approximately 1 million hectares of agricultural land was cultivated. Over a period of several years, from 1996 to 2010, global crop yields increased from 1.7 million hectares to 152 million hectares.³

By 2010, 29 countries had cultivated transgenic crops and had

passed the law of more than 31 countries for the introduction of GMOs.⁴ In 2011, the United States was licensed to GM with twenty-five GM products. In 2015, 92% of corn, 94% of soybeans and 94% of cotton produced in the United States were modified genetically.⁵ Since the Earth's population is expected to reach approximately 8.5 billion by 2025, to meet this challenge, it is necessary to double food production in 2025. Thus genetic engineering techniques have brought us the right solution. Genetic engineering on plants has been used to increase the content of ferritin (iron permeable protein) or to reduce phytase (an enzyme responsible for reducing the acidity of phytic acid that lowers iron).⁶ The soybean ferritin gene has been used to improve the storage of iron in lettuce, rice, corn, soybeans and wheat.⁶ In addition, popular aromatic rice varieties such as Jasmine and Basmati naturally contain a higher level of iron and zinc. GM's focus on carbohydrates is to optimize the content of useful carbohydrates or nutrients.⁷ Carbohydrates are useful to slowly metabolize and thus not absorbed in the small intestine, but are broken down by the natural flora of the intestine and converted into a short chain of saturated fatty acids enhancing the absorption of nutrients, which in turn reduce the low-density lipoproteins (LDL) cholesterol and colon cancer prevention by induction of apoptosis and increase of proper carbohydrates, suitable fatty acids.^{7,8} and to increase protein levels and essential amino acids in food products.

There is also a huge economic impact of GM foods. Billions of dollars in losses are due to the lack of food production or the loss of crops due to pests and plant diseases. For example, damage to maize in the EU's land caused a huge financial loss to these countries. In addition, chemical insecticides are losing their effects due to the onset of resistance to pests. From 2006 to 2012, global revenues from GMFs reached \$ 116 billion, almost three times the past 10 years.⁹ It is estimated that approximately 42% of the economic benefits of increasing production are due to genetic progression and resistance to pests and weeds.

In 2014, approximately 18.5 million hectares of genetically modified crops have been planted in 28 countries, half of which being for soybeans. These soybeans were resistant to herbicides or insects. 90 % of the soybean cultivars are available to 11 countries, mostly such as the USA, Argentina and Brazil.¹⁰

Most concerns of GM foods can be divided into three categories: environmental hazards, human health risks, and economic concerns.¹¹ Raw materials and processed products derived from GM products can be identified by testing the presence of DNA or by detecting the expression of the protein encoded by genetic material.¹² The objective of this study was to analyze and evaluate the initial samples of processed soybean oil in Iran's food market using Polymerase Chain Reaction (PCR) technique.

Materials and methods

Collection of samples

The soybean commercial samples were purchased as a control from supermarkets located in Tehran. Considerable attention was paid

to collecting samples from different brands. In this investigation, in order to identify the soybean oil in products, randomly, 25 raw oil and soybean oil samples were collected and numbered. An equivalent of 50 g of each sample was prepared for testing. Samples were converted into powder using a mill. Then, they were mixed thoroughly to become homogeneous. During the combination, sterile equipment was used. In order to prevent contamination during sampling, specimens without any genetic modification were prepared in a place where GM materials had not previously been present. After homogenization, they were labeled for further studies and stored at 4 °C. The CRM Samples were also provided by the Food and Drug Administration. Samples of GM soybeans were used as homogeneous powder.

DNA extraction from raw soybeans

Extraction of DNA from powdered raw soybean was performed using DNA extraction kit from the Pishgaman gene transfer company according to proposed protocol. The quality and quantity of extracted DNA from each sample were examined using Nanodrop in 260/280 optical density to be >1.8µg/ml.

Primer design for polymerase chain reactions

The conserved sequence of external genes that were transmitted to GM products was used to design the specific primer. The BLAST and ClustalW applications were used to find the sequence specificity of the primers. Primer sequences were designed and sent to Macrogen (Korea) for synthesis (Table 1). A part of the specific soybean gene was targeted to PCR. Soybean specific primers (related to soybean lectin gene) were used to confirm the presence and quality of DNA extracted from raw soybeans and processed soy foods. The size of the PCR product with these primers was 164 bp.

Table 1 The primers used in this study, in which the annealing temperature for multiplex PCR of soybean and GMO35S primers was adjusted at 51 °C

Primer	Sequence: 5'-3'	Annealing T (°C)	Product (bp)
Soybean	F: GTGCTACTGACCAGCAAGGCAAACCTCAGCG R: GAGGGTTTTGGGGTGCCGTTTTTCGTC AAC	51	164
GMONOS180	F: GAATCCTGTTGCCGGTCTTG R: TTATCCTAGTTTGCGCGCTA	54	180
GMO35S	F: GCTCCTACAAATGCCATCA R: GATAGTGGGATTGTGCGTCA	55	195

Soybean transgenic diagnostic primers

In order to determine the transgenic soya samples, the specific primers for the 35S promoter region of mosaic virus CaMV35S and nephaline synthase (NOS) transcription terminator were designed from *Agrobacterium*. The sequence of the CaMV35S promoter and the NOS terminator were obtained according to that of Lipp and Brodmann investigation.^{13,14} The PCR product size for 35S and NOS primers was 195 and 180 bp, respectively (Table 1). The PCR reactions were performed by application of Thermo cycler Peqlab device (Germany) (Table1). The primers used in this study, in which the annealing temperature for multiplex PCR of soybean and GMO35S primers was adjusted at 51 °C.

Results

PCR amplification of Soybean, GMONOS180 and GMO35S (NOS) genes. Using PCR technique the specific Soybean (164bp),

GMONOS180 (180bp) and GMO35S (195bp) genes exhibiting GM product were amplified. The simplex and multiplex conditions were adjusted for amplification purposes. Among the 25 tested oils, 9 (36%) were GM, being a high percent and of these nine transgenic cases, two had CaV-35s, and also were nos-positive, indicating of their transfection certainty. This high percentage of transgenic oils is possibly due to the lack of awareness of the producers (Figures 1 & 2).

Discussion

It is controversy over the GM products consumption around the world. However, in any condition, the shortage of food supplies and population growth will force the posterity to use transgenic products.^{10,15} Compared to alfalfa, little attention has been given to soybean as a forage crop, and hence there are few studies of genetic dissection of forage traits. In Iran, soy beans are one of the most important foods widely used not only as raw material but also as an ingredient for food processing. Many soybeans come from South

Africa, Brazil, Argentina and the United States. So far, there has been no relevant information on the use of GM soybean protein in Iran.

In this study, the GMO samples collected from edible oils in the Iranian food market were detected using conventional PCR technique. It is noteworthy that of the 25 tested oils, 9 (36%) were GM, being a

high percent and of these nine transgenic cases, two had CaV-35s, and also responded to nos, indicating their transfection certainty. This high percentage of transgenic oils is possibly due to the lack of awareness of the producers. It is worth mentioning that more than half of GM soybeans are on the global market. The lack of regulatory mechanisms to import these soyas has led to such mistakes.

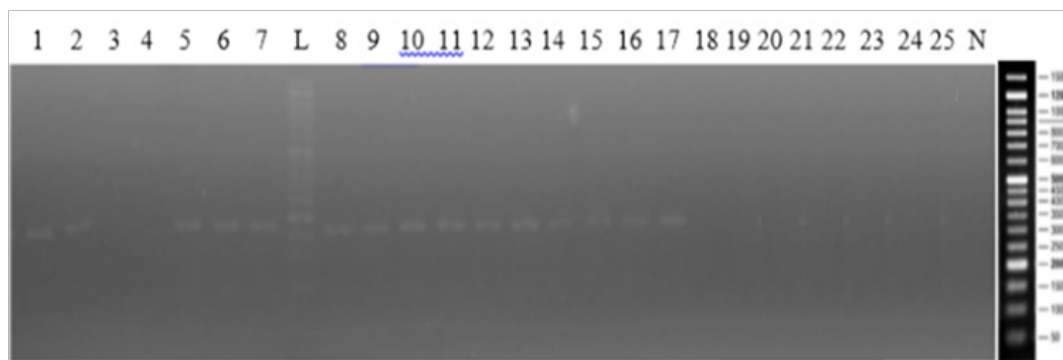


Figure 1 Gel electrophoresis of soybean PCR products with 164bp, well N: negative control, wells 1-25: positive oil samples, Lad: 100bp DNA marker.

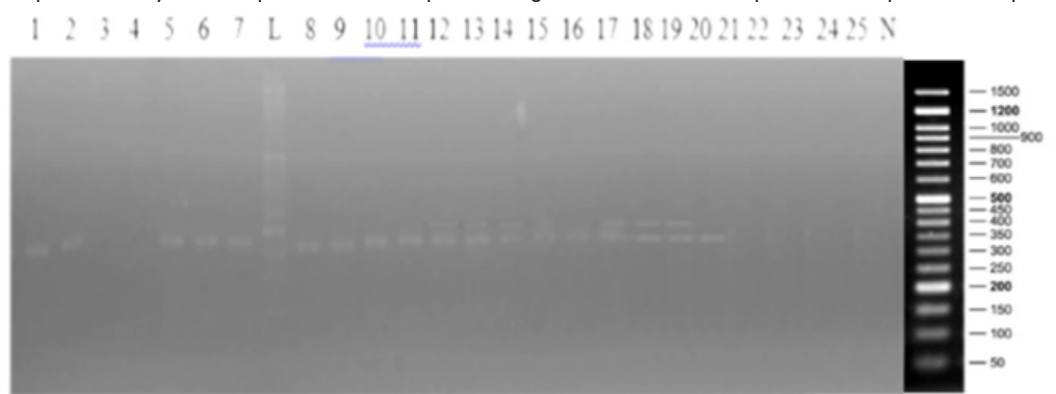


Figure 2 Gel electrophoresis of products of multiplex PCR including GMONOS180 and GMO35S (NOS) genes with 180bp and 195bp, respectively, well N: negative control, wells 12-15 and 17-20: positive samples for both genes, Lad: 100bp DNA ladder.

Therefore, in light of these results, it is recommended and emphasized that soy oil samples should be collected and be further investigated in a valid GMO diagnostic laboratory.¹⁶ Due to the importance of the issue and to prevent the misuse of some traders and mediators, it is necessary to develop precise laws to prevent and control this problem. For example, coercive labeling of GM soybeans is one of the first steps to respect consumer rights so that they have the right to choose GM products.

This investigation provides a simple and reliable PCR method for detecting genetically engineered products. According to PCR results, 5 of the 25 tested specimens revealed a positive response to the CaMV35S primer, suggesting that they are GM products. The use of PCR to detect GM has been fully proven, however, using different methods for DNA extraction according to the type of product depending on the amount of processing, the appropriate method for DNA extraction should be used. Although GM plants have potential benefits to consumption, there is concern regarding the safety of GMO use as food. According to the law in the EU and several other countries, GMO-containing products must be certified and labeled, which require a reliable and accurate method for the detection of GMOs in raw materials and food products.¹⁷

In this study, we tried to introduce a precise, fast, reliable, and reproducible method for the identification of soybeans used in

products. GM foods are improved in the aspects of larger production and effective utilization. Recent climate change which has influenced on food production and overcrowded population and food limitations needs its more consumption. Various methods such as DNA based and protein based methods have been employed to determine GM foods.

Most studies have been performed on olive oil. Nicholas Garrison and colleagues first succeeded in extracting DNA from soybean oil.¹⁸ A remarkable point of their research was the success of DNA extraction from oils that had undergone a degree of refinement and purification. Amplification reactions confirmed their extraction method. Nicholas Grayson later used the same method to extract high amounts of DNA from soybean oils, and finally, using specific genes and PCR reactions, they succeeded in tracing soybean oil. Pauli et al. Used Nested PCR responses to identify genetically manipulated materials in edible oils.¹⁹

Bogani and colleagues firstly employed biosensors to detect GMOs in 2009.²⁰ They tested the reactions on eight different transgenic soybean products, including grain, soybean flour and soybean oil. They ultimately used DNA fragments with a length of 500 bp to bind to a particular sensor. In 2007, Dewey and Lee successfully combined PCR-based DNA extraction protocols on sunflower, olive, and soybeans.²¹

Costa and colleagues managed to detect genetically modified soybeans in edible oils while co-optimizing the appropriate method for extracting DNA from refined oils. They used a variety of methods such as the CTAB method and different kits for proper DNA extraction. Finally, they used PCR and Real Time PCR methods to detect transgenic soybeans.²²

Conclusion

The microbiological and chemical methods alone cannot control the quality of food products, and detect the presence of transgenes. Therefore, the use of PCR technique is important in controlling the quality of this product. In cases where manufacturers and importers of products prepare and distribute transgenic products without labeling, they can be easily prevented from misuse and thus PCR as complementary and helpful to determine the composition and quality of the product. Given the ever-increasing development of factories and manufacturers of food products and GM products, as well as increasing the size of the target market of these factories, the likelihood of increased fraud in the production of these products is predictable. Therefore, in order to avoid this issue, it is suggested to apply more accurate monitoring and surveillance based on accurate and rapid molecular tests by healthcare organizations and regulatory agencies.

Acknowledgments

None.

Conflicts of interest

The authors declare no conflicts of interest in this work.

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