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Persistence of transgenic genes and proteins during soybean food processing

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ABSTRACT

The soybean is a typical legume which is used to elaborate several foods around the world. Ten imported soybean seed samples were collected and planted to identify possible genetic modifications. DNA was isolated from 20 days old seedlings. After that, PCR was carried out using the 35S, RR and *cry*1AB/1AS primer pairs. Nine soybean samples were identified as genetically modified. These soybeans were used to prepare six different soybean foods. During food processing, critical steps were identified; such as drastic changes in temperature and pH. A sample was taken from these critical points and from the final product for DNA extraction and PCR amplification. In most of the samples the presence of the CaMV 35S promoter and the gene *cry*1A was identified. In addition, presence of transgenic proteins was evaluated using ELISA-DAS assay. Presence of CP4 *EPSPS* proteins was detected in most of the studied soybean food samples except in yogurt and tofu. No *cry*1AB/1AC proteins were identified in any of the samples tested.

1. Introduction

During the last 20 years, development and commercial production of genetically modified (GM) crops has emerged. The GM crops have earned acceptance by farmers; that acceptance is seen in the increase in the soil used for GM crops cultivation, which has increased from 2 million hectares in 1996 to 134 million hectares in 2009 around the world (Clive, 2010). In 1994, Calgene released the first GM tomato crop utilized for human consumption, modified to have a greater shelf life (Uzogara, 2000).

Despite the agronomic advantages of GM crops, consumers have not totally accepted the products from these crops, because the suspicion that allergic problems might arise as a consequence of consumption and the lack of worldwide regulation on these crops. To assess the allergenicity of GM food, more research, including a selection of controlled sample materials and immunoassays of qualified sera, is needed (Hye-Yung, Soo-Young, Kyung-Eun, Myung-Hyun, & Kyu-Earn, 2005). In addition, the problem of contamination of non-GM foods with GM crops has made different countries to restrict the import of products made using these crops and

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prohibit the use of GM crops as an ingredient or the need for specific food labeling indicating the use of GM crops (Sasson, 2000). Analysis of food products consisting of, or produced from, GM organisms is required to verify compliance with labeling legislation and to detect any unauthorized transgenic crops (Branquinho, Ferreira, & Carderelli-Leite, 2010).

Mexico imports soybean from different countries. Soybeans are utilized for creation of food and feed. During 2009, more than 28 million hectares of the land cultivated with soybean in USA was planted with different GM varieties (Clive, 2010). A high percentage of soybeans is genetically modified for traits like insect resistance and herbicide tolerance which are based on the *cry* and *epsps* genes respectively (Rincon, Ruiz, & Serrato, 1999). Although some studies have been performed to detect the presence of GMO residues in raw material and food products (Abdullha, Radu, Hassan, & Hashim, 2006; Taški-Adjuković et al., 2009; Nikolić, Taški-Adjuković, Jevtić, & Marinković, 2009; Dinon, Tremi, Sousa-de-Mello, & Maisonnave-Arisi, 2010), at present there is a lack of knowledge about the persistence of GM genes and proteins during traditional soybean processing, and a need to determine in which processing step the GMO DNA fragment or protein is degraded. Current methodologies for the analysis of genetically modified organisms are focused on either one of two targets, the transgenic DNA inserted or the novel protein expressed in a GM product (Miraglia et al., 2004). The objectives of the present study were to make six different foods with transgenic soybeans and determine persistence of transgenic genes and proteins during food processing steps and in final product.

2. Materials and methods

Soybean seed samples were taken from lots imported to Mexico through Laredo City and sown in polystyrene boxes packed with agricultural soil. Subsequently the boxes were maintained in field conditions and irrigated once every 2 days. Twenty days after planting, leaves of seedling were cut. Leaves and food samples were treated as follows: 200 mg of each sample was ground in liquid nitrogen and crushed. DNA was extracted by the method reported by Graham, Mayers, and Henry (1995). The quality of DNA was determined using agarose gel (1% w/v) electrophoresis (95 V, 40 min) in TAE buffer (Tris-Acid Acetic-EDTA) 0.5 × with 0.5 μl/mL of ethidium bromide. PCR was performed in a final concentration 25 μL with the following reagent concentrations: genomic DNA (150 ng/μL), PCR buffer (1 ×), $MgCl_2$ (3.5 mM) dNTPs (0.4 mM) and Invitrogen® Taq DNA polymerase (0.15 U/μL). PCR protocol was performed as follow; pre-incubation step at 95 °C for 5 min; 35 cycles consisting of dsDNA denaturation at 94 °C for 1 min; primer annealing at 60 °C for all *cry*'s primers, 35S and *epsps* primer pairs for 1 min and primer extension at 72 °C for 1 min. Final elongation was performed at 72 °C for 5 min. The 35S primer pair (35S1 5′-GCT CCT ACA AAT GCC ATC A-3′ and 35S2 5′-GAT AGT GGG ATT GTG CGT CA-3′) was used to identify of CaMV (Cauliflower Mosaic Virus) 35S promoter; the RR primer pair (RR01 5′-TGG CGC CCA AAG CTT GCA TGG C-3′ and RR04 5′-CCC CAA GTT CCT AAA TCT TCA AGT-3′) was used to identify the *epsps* gene, and the *cry*1AB/1AS primer pair (*cry*1AB 5′-ACC ATC AAC AGC CGC TAC AAC GAC C-3′ and *cry*1AS 5′-TGG GGA ACA GGC TCA CGA TGT CCA G-3′) was used to identify the *cry*1A gene. The PCR products were 238, 356 and 184 bp for 35S, *epsps* and *cry* primers respectively. Purified and desalted oligonucleotide primers were synthesized at Invitrogen®, before respectively diluted to a final concentration of 10 μM with double distilled water and stored at −20 °C, until further use.

All nine soybean samples identified as genetically modified were mixed and used to make six different soybean foods: tofu, soy milk, yogurt, sausages, flour and soy sprouts. During food processing, critical steps involving drastic changes of temperature and pH were identified. Following were some of the critical steps involved, soy milk (before soak, before boiling and pasteurization), yogurt (inoculation and incubation), tofu (before boiling and milk with lemon), soy sprouts (humid seeds, start of germination, and on germination at 3rd and 9th day) and soy flour (first grinding, second grinding and after dried). In the case of sausages, there was no critical step as only ingredients were mixed. A sample was taken from every critical step and also from the final product for DNA extraction and PCR amplification.

For ELISA-DAS test (Enzyme Linked Immunosorbent Assay-Double Antibody Sandwich), PathoScreen Kit of Agdia® was used to detect the GM proteins CP4 *EPSPS* (Agdia, Catalog PSP 74000 1-7) and *cry*1AB/1AC (Agdia, Catalog PSP 06200 1-5). For these tests the samples from processed foods and from each critical points were analyzed twice. The results of each sample were done in a visual form; in addition, its optical density was determined in an ELISA plate reader from Dynatech Laboratories at 630 nm.

3. Results and discussion

3.1. *Identification of genetically modified soybean*

The optimum annealing temperature for primers RR01 and RR02 which were used for amplification of part of the *epsps* gene, was determined by testing different temperatures among 50 and 62 °C; the best annealing temperature was 62 °C. The primer pair cry1AB and cry1AS which was used to identify the *cry*1A gene, also in this case a range of annealing temperatures among 55–62 °C was tested, the optimum annealing temperature for the cry primer pair was 60 °C. Results showed that the pair of primers amplified a segment of the *epsps* gene of 356 bp; and for the *cry* primer pair, the amplified segment had a size of 184 bp. Identification of the *epsps* and *cry*1A genes and CaMV 35S promoter was confirmed with sequencing of the fragment. The size of the PCR product plays a key role in the detection and quantification of GM organisms in processed foods (Yoshimura et al., 2005).

After DNA extraction and amplification, 90% of the soybean samples tested were GM and only 10% of the soybean samples were not transgenic. The *epsps* gene was detected in 80% of the tested samples, while *cry*1A gene was identified only in 40% of the imported soybean samples. Further it was observed that 40% of the imported soybean samples contained two or more genetically modified genes.