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RESEARCH NOTE

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Presence of Transgenic Genes and Proteins in Commercial Soybean Foods from Mexican Grocery Stores

Yendi Arely Cruz-Flores, Raúl Rodríguez-Herrera*, Cristóbal Noe Aguilar-González, Juan Carlos Contreras-Esquivel, and Maria de la Luz Reyes-Vega

School of Chemistry, Universidad Autónoma de Coahuila, V. Carranza e Ing. José Cárdenas s/n. C.P. 25000, Campus Saltillo, México

Abstract Commercial food products from major cities of Coahuila, Mexico were screened to identify residues of transgenic deoxyribonucleic acid (DNA) and/or proteins. After performed, an inventory on all products that contained a soybean-based ingredient in a commercial grocery store in the city of Saltillo, Coahuila, Mexico, 245 food products were identified and grouped in 15 classes according to the soybean ingredient as well as the manufacturing process used for their elaboration. Similar sampling was made for the different food classes in the cities of Monclova, Piedras Negras, and Torreon. A total of 88 samples were analyzed and DNA was extracted by the hexadecyltrimethyl-ammonium bromide (CTAB) technique with slight modification to obtain better DNA quality (1). In addition, segments of the transgenic genes one that codifies for 5-enolpyruvylshikimate-3-phosphate synthase (*epsps*), *cry* 1A, and the cauliflower mosaic virus (CaMV) promoter were amplified using polymerase chain reaction (PCR). The transgenic proteins 5-enolpyruvylshikimate-3-phosphate synthase (CP4 EPSPS) and insecticidal crystal protein (Cry 1Ab/Ac) were identified using double antibody sandwich-enzymatic linked immunoassay analysis (DAS-ELISA). Presence of transgenic genes and/or proteins was identified in 35.3% of the commercial products samples.

Keywords: *cry* 1AB, *epsps* gene, 35s cauliflower mosaic virus (CaMV) promoter, transgenic proteins 5-enolpyruvylshikimate-3-phosphate synthase (CP4 EPSPS), insecticidal crystal protein (Cry 1Ab/Ac)

Introduction

Recently, numerous advances have been made in the development of new transgenic crop varieties including soybean, maize, rapeseed, cotton, and potatoes with the aim to increase resistance to insects and diseases. Many governments around the world have authorized the marketing of genetically modified organisms (GMOs) as food and feed (1). Since GMOs were introduced to the food chain a scientific and public debate concerning their safety and the need for labeling information arose specially in Europe (2). Identification of GMO in foods is becoming an issue of great interest. The more GMO-derived products are launched into the food market, the more customers demanded for strict regulations and labeling of such foods (3).

The global area of genetically modified crops in 2004 was 81 million ha and the main planted genetically modified crop was herbicide tolerant soybean with 48.4 million ha, accounting for 60% of total area of worldwide transgenic crop production (4). The main transgenic soybean variety is 'Roundup Ready' (RR), which was developed by Monsanto Inc., USA and confers tolerance to the glyphosate-based herbicide Roundup (5).

Several techniques have been developed to identify GMOs, which could be detected by either directly using polymerase chain reaction (PCR) or indirectly using enzyme-linked immunoassay analysis (ELISA). PCR methods are preferable than ELISA for GMO detection due to its high sensibility and stability (6,7). Mexico imports soybeans from different countries. The soybean is utilized for elaboration of food and feed. Mexican soybean imports are originated mainly in the USA. In USA during the year 2001, more than 68% of the land grow with soybean was planted with different genetically modified soybean varieties. Soybean has been genetically modified by incorporation of some traits like insect resistance and herbicide tolerance which are based mainly on the insecticidal crystal proteins (*cry*) and 5-enolpyruvyl-shikimate-3-phosphate synthase (*epsps*) genes, respectively. To the best of our knowledge, the reports in the literature, regarding the presence of genetically modified genes and proteins in commercial foods at the Mexican grocery stores is scarce. The object of the present study was to detect the presence of transgenic genes and proteins in commercial soybean foods from grocery stores from the State of Coahuila using PCR and ELISA techniques.

Materials and Methods

Food samples In a commercial grocery store in the city of Saltillo, Coahuila, Mexico, an inventory was done on all products that contained a soybean-based ingredient. Two-hundred-and-forty-five products were identified and grouped in 15 classes according to the soybean ingredient as well as the process used for their elaboration. Once grouped (Table 1), the most representative products were chosen (at least 2 from each of the 15 classes). Forty-four soybean food products including oil, snack foods, condiments, soups,

*Corresponding author:
Tel: +52-844-416-9213, 416-1238
Fax: +52-844-415-9534, 439-0511
E-mail: rrh961@hotmail.com
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Table 1. Classification of products according to the ingredients of soybean and to the process submitted previous to its elaboration

Soybean ingredient type	Typical product	Product Characteristics	Commercial brand
Soybean oil	Soybean oil	Soybean 100%	Nutrioli™, Oltus™, Aurora™
Vegetal oil	Vegetal oil	Mixture of oils from soybean and other plants	Hogar™
Others oil with lecithin	Vegetal oil	Vegetal oil with soybean as additive	PAM™
Products with soybean oil and without heat treatment	Salad seasoning, mayonnaise	No use of temperature for food processing	Hellmann's™, Hellmann's™
Soybean oil with heat treatment	Cereal, bread, flour, cookies chocolate, cheese, French potatoes	Use of temperature for food processing	Quaker Instant™, Bimbo™, Fresqui Bon™, Smith Frams™ Gamesa™
Soybean lecithin without heat treatment	Frozen dessert	Soybean lecithin as emulsifier /anti oxidant and no use of temperature for food processing	Holanda™
Soybean lecithin with heat treatment	Chocolate, candies, bread, soup, cookies	Soybean lecithin as emulsifier /anti oxidant and use of temperature for food processing	Hershey's™, Bimbo™, Marinela™, Mead Johnson Nutry™, Nestle™, Nabisco™, Lara™
Hydrolyzed soybean protein/ soybean sauce without heat treatment	Sauces, seasoning.	No use of temperature for food processing and protein/sauce is used as flavoring	Hellmann's™, Kikkoman™
Hydrolyzed soybean protein/ soybean sauce with heat treatment	Chocolates, cereals, soup, peanut, chips	Use of temperature for food processing and protein/sauce is used as flavoring	Snickers™, Nestle™, Maruchan™, Sabritas™, Nipon™
Soybean flour	Bread, flour	Soybean flour as an ingredient.	Bimbo™, Wonder™, Pronto™
Isolated soybean protein	Cereal, bread, sausage, meat	Containing more than 90% of soybean proteins	Quaker Instant™, Bimbo™, Campbells™, Fud™, Ponderosa™, Chimex™, K.W. Foods™
Textured soybean	Imitation of bacon, tuna, sausage	Fibrillated proteins imitating beef tissue texture	McCormick™, Maz Atun™, Knorr™, Azteca™
Soybean without specifications	Cereal, chips, beverages	Soybean is only mentioned in the product label	Ades™, Encanto™
Active enzymes	Tortilla		Tia Rosa™
Soybean concentrate	Sausage	Containing about 70% proteins from soybean	Chimex™ , San Rafael™

breads, beverages, candies, desserts, and meats representing different levels of processing were chosen from Saltillo. Similar sampling was made at other cities of the State (Monclova, Piedras Negras, and Torreon) where 39 samples were chosen.

DNA isolation For the extraction of genomic DNA from the samples, the method reported by Graham *et al*. (8) was used. Also it was necessary to modify it according to the problems that showed up during the DNA extraction from the different matrices. The DNA quality was determined using agarose gel electrophoresis. The gel was prepared with 1% of agarose (Sigma-Aldrich, St. Louis, MO, USA) in Tris Borate EDTA (Sigma-Aldrich) (TBE 0.5%) with 0.5 µL/mL of ethidium bromide (EtBr) (Sigma-Aldrich). The running conditions were constant at 95 V for 40 min in TBE 0.5× buffer.

PCR reaction and product analysis A 23 µL reaction contained 1× PCR buffer (20 nm Tris, pH 8.4, 50 mM KCl) (Invitrogen, Carlsbad, CA, USA), 0.2 Mm dNTPs (Invitrogen), 2.0 mM $MgCl_2$ (Invitrogen), 0.5 µM of each primer (Invitrogen), and 0.1 U/µL of Taq DNA polymerase (Bioline, London, UK), PCR reaction was performed by spiking 2 µL of DNA extracts into a centrifugation tube and then the PCR reagent was added. The DNA in centrifugation tube was incubated in a PCR thermocycler model Px2 (Thermo Electron Corporation, Milford, MA, USA) under the following program: 95ºC for 5 min; 35 cycles of 94ºC for 1 min, an annealing temperature of 62ºC for *epsps* and *cry,* 60ºC for *35S* for 1 min, 72ºC for 1 min and a final extension at 72*°C* for 5 min. The 35s primer pair (35S1 5'GCTCCTACAAATGCCATCA3' and 35S2 5'GATAGTGGGATTGTGCGTCA3') was used to identify the CAMV promoter. Then RR primer pair (RR01 5'TGG CGCCCAAAGCTTGCATGGC3' and R04 5'CCCCAAGT TCCTAAATCTTCAAGT3') was used to identify the *epsps* gene, and the Cry1AB/1AS primer pair (5'ACCATCAA CAGCCGCTACAACGACC3' and Cry 1As 5'TGGGGA ACAGGCTCACGATGTCCAG3') was used to identify the *cry* 1AB gene. Oligonucleotide primers were used as purified and as desalted specimen from Invitrogen, then

diluted to a final concentration of 10 μM with distilled water and stored at −20°C until use. Products were passing through electrophoresis on a 2% agarose (gel containing 3 μL ethidium bromide. A 100 bp ladder was used for size control of amplified fragments. Detection of transgenic DNA was considered positive if the size of the amplified band was 356 bp with the epsps primers, 256 bp with CaMV primers and 186 bp with cry 1AB/1AS primers, these band sizes are similar to those reported in the literature for PCR amplifications with these primers, in addition some bands were sequenced to confirm results.

Presence of transgenic proteins in commercial soybean foods from Mexican grocery stores The immunoassay kit used in the ELISA-double antibody sandwich test (DAS) was the PathoScreen kit of Agdia®. (Agdia, Elkhart, IN, USA). The kit was used to detect the genetically modified proteins CP4 EPSPS and Cry1AB/1AC. The procedures for sample treatment and detection are described in the operation manual of the immunoassay kit (9).

Results and Discussion

Modifications done to the DNA extraction method Initially a method reported for DNA extraction was used (1), however due to the poor quality of DNA in the analyzed samples, some modifications were done. The modifications consisted of the amount of the sample, freezing time, temperature, and time of incubation. These modifications allowed obtaining a DNA with better quality. In spite of the modifications, DNA from some samples had poor quality, this let to more modifications. The modifications were in sample size, weighed around 0.3-0.5 g of sample and 48-72 hr the sample was left in liquid nitrogen, this allowed the sample to be frozen and the ground was easier. The samples were incubated at 60°C for 30-40 min. Following a 10 min of centrifugation at 10,625 ×g at room temperature. The time during the precipitation was increased from 1 hr to 48-72 hr.

These modifications allowed obtaining a DNA of better quality in most of the analyzed products. In the case of oils it was not possible to extract DNA because during their processing many steps take place which could degradate the DNA. As the levels of food processing increase, the amount and quality of soybean DNA decrease (6).

In the case of products with soybean oil, as a secondary ingredient, DNA extraction was obtained with a very good quality in the following foods: salted cookies, hot cakes flour, and powder drinks. This does not indicate that necessarily it is DNA from soybean is present but could come from others ingredients. The portion of soybean and other ingredients changes from one product to another, because in these products the concentration of soybean is very low and the DNA was degraded so most of the extracted DNA may comes from other ingredients.

DNA from food products containing chocolate requires additional purification steps to remove inhibitors of PCR (6,10-12). Many extractions kits have been designed to effectively remove compounds that may inhibit PCR in cocoa products (6,12). However, in the present study DNA was extracted using a method (1) with some modifications; For products with high content of cocoa, it was required a larger sample (0.5 g) in order to obtain sufficient DNA for analysis, in addition, incubation times and temperatures were extended.

Presence of 35s CaMV promoter, *epsps* and *cry* 1AB genes in commercial soybean foods Annealing temperature for the 35s, RR, and *cry* pair of primers was standardized using a PCR Thermocycler model Px2. After that, DNA was amplified using the follow primers RR01, RR04, 35S1, 35S2, Cry 1Ab, and Cry 1As the optimum annealing temperature was 62ºC for *epsps* and *cry,* 60ºC for 35 sec. The results showed that from the 83 samples analyzed, 7 samples were positive for *epsps* gene, 21 for 35S CaMV promoter, and 10 for *cry* 1AB gene.

During the analysis, several problems with the PCR method were found. First, processed foods made the detection difficult because of their high level of processing, presence of many contaminants from DNA purification, and PCR performance. In the case of soybean it is especially difficult due that is manipulated in bulk, in great amounts, and many farmers mix the different crop varieties after harvest. In addition, contamination can also take place during transportation of raw material to the industry or during the processing of the products. Some companies elaborate foods with raw materials with or without GMO, as the case of the foods that use soybean like main ingredient or as a derived ingredient including: oil, bran, flour, sauce, isolated and concentrated of protein, textured protein, lecithin (13). Also, considering that the analyzed foods were not transgenic, reason why it was not possible to detect GMO residues.

In this study 38 out of 83 analyzed samples were positive to transgenic segments detection by PCR. Transgenic positive samples were detected in samples from 4 different cities (Table 2) which indicate that most of the people in the State of Coahuila are eating transgenic food. In some cases, products from the same brand, but obtained in different cities were identified as positive to GM residues. Also, products from the same brand but from different batch lot were identified in some cases positive but in the others negative. These results suggest that food companies do not analyze for transgenic segment presence in raw material or final products. Figure 1 shows the *epsps* amplified band (350 bp) from hot cakes flour, sausage, oat cereal, chocolate cake flour, and cookies. Figure 2 shows the amplified 35S band (238 bp). Not all *epsps* or *cry* positive samples were also positive to the CaMV 35S promoter presence, which may suggests that other promoters different to 35S have been incorporated to the *epsps* or *cry* events. It was observed a differential distribution of transgenic foods in the cities because the highest percentage of transgenic residues were found in Monclova foods (44%), while the lowest percentage of transgenic samples was found in Torreon (21%), Similar percentages of genetically modified samples were obtained from in samples from Saltillo and Piedras Negras (31%). Monclova is the city with the lowest academic and lab facilities in comparison to the other cities where this work took place. This fact may influence the decision of transgenic food distribution. Samples that were classified with the lowest food processing were detected to have more percentage of transgenic

Table 2. Identification of the gene *epsps*, *35S*, and *cry*, and the proteins CP4 EPSPS and Cry 1Ab/1Ac

Product[1)]	epsps	35S	*cry*1A	Protein CP4 EPSPS	Protein Cry 1Ab/ 1Ac
Hot cakes flour 1	-	+	+	+	-
Hot cakes flour 2	+	+	-	+	-
Salad seasoning	-	-	+	+	-
Salad cookies	-	-	+	-	-
Chocolate bar	-	+	-	+	-
Imitation of bacon	-	+	-	+	-
Sausage	+	-	-	+	-
Tortillas	-	+	-	+	-
Dust for drink	-	+	+	+	-
Oat cereal	+	-	-	+	-
Dust chocolate	-	+	+	+	-
Flour for chocolate cake	+	+	-	+	-
Turkey sausage	-	+	-	+	-
Peanut snacks	-	-	-	+	-
Seasoning with chesse	-	-	-	+	-
Bread	-	-	-	+	-
Cookies	-	-	-	+	-
Instantaneous soup	-	+	-	-	-
Bread (*M*)	-	+	+	-	-
Salad cookies (*M*)	-	+	-	-	-
Cookies (*M*)	+	-	-	-	-
Turkey sausage (*M*)	-	+	+	-	-
Peanut snacks (*T*)	-	+	-	-	-
Hot cakes flour (*T*)	+	+	-	+	-
Dust chocolate (*T*)	-	+	+	-	-
Salad cookies (*P*)	-	+	+	-	-
Peanut snacks (*P*)	-	+	-	-	-
Bread (*P*)	-	+	+	-	-
Instantaneous soup (*P*)	-	+	-	-	-
Hot cakes flour (*P*)	+	+	-	+	-

[1)]M=Monclova, T=Torreon, P=Piedras Negras; (+) detection of transgenic residues in food samples, (-) no detection of transgenic residues in food samples.

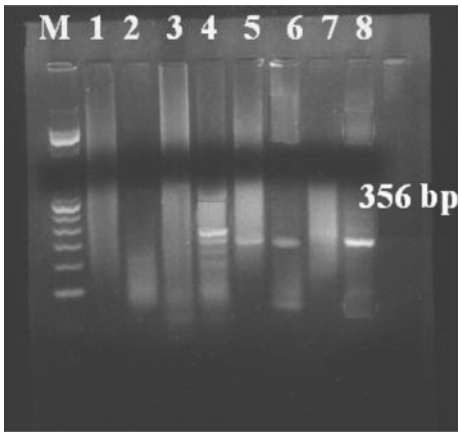

Fig. 1. PCR analysis result by epsps primers and genomic DNA of commercial products at Saltillo City. M, Ladder 100 bp; 1, bread; 2, peanut snacks; 3, sausage; 4, hot cakes flour 2; 5, oat cereal; 6, sausage; 7, chocolate bar; 8, flour for chocolate cake.

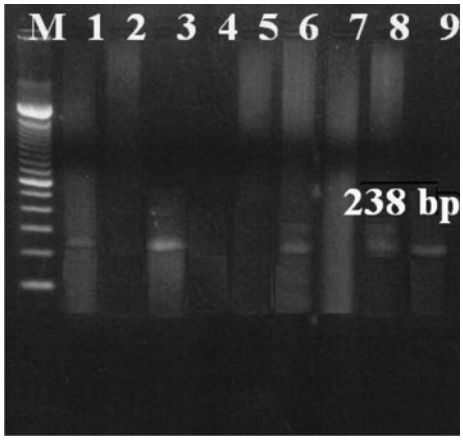

Fig. 2. PCR analysis result by CaMV promoter primers and genomic DNA of commercial products at Saltillo City. M, Ladder 100 bp; 1, chocolate bar; 2, bread with chocolate; 3, sausage; 4, juice; 5, tuna; 6, imitation of bacon; 7, bread; 8, hot cakes flour 2; 9, instantaneous soup.

sequences. In this class, products like hot cakes and cake flour, and ground bread where in this range of foods.

Presence of transgenic proteins in commercial soybean foods from Mexican grocery stores For the detection of transgenic proteins 'Roundup Ready' CP4 EPSPS and Bt-Cry 1Ab/1Ac the kit PathoScreen (Agdia) was used. In order to determine if the samples were positive plates, they were checked in visual form, where blue color is indicative of a positive and the absence of color a negative. Also the plates were analyzed with optical density in a reader for ELISA plates (Opsys MR^{TM}, Dynex Technologies Inc., Chantilly, VA, USA) using the filters 405 and 630 nm, samples with optical densities with values higher than 0.10 were taken as positive as much for proteins CP4 EPSPS as Cry 1Ab/1Ac taking like reference, the readings of the positive and negative control. Nineteen out of 83 samples analyzed were positive for the presence of the protein CP4 EPSPS (Table 2), while for the protein Cry 1Ab/1Ac all the samples were negative.

The fact of negative results indicates absence, does not assure that the protein was not present in soybean from which the product was elaborated. An explanation of this result is that the protein could have denatured during the elaboration process. With the results obtained, we cannot conclude that the proteins CP4 EPSPS are more resistant to the food elaboration conditions, that Cry 1AB/1AC nevertheless there is a report (14), in where proteins CP4 EPSPS are resistant to adverse conditions such as pH and temperature during soybean processing.

It is possible to mention that some samples like hot cakes flour, where the transgenic proteins values (1.300) were higher than those of positive controls (1.000-1.300) which indicated a very high concentration of the protein CP4 EPSPS in the food sample. Due to the great number of ingredients that each food product contains, it is not

possible to assured that the protein comes from transgenic soybean in each case. In a positive result we can assume that one or more of the ingredients are transgenic, this is due to the mixture of the ingredients. It is important to emphasize that during food preparation, mixtures of transgenic and non transgenic raw products are used, which makes the identification more difficult.

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