Detection of Genetically Modified Soya, Maize, and Rice in Vegetarian and Healthy Food Products in Serbia

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Abstract

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The presence of genetic modifications was analysed in a total of 100 samples of non-labelled vegetarian and healthy food products. The basic raw materials in the samples tested comprised maize, soya, and/or rice. The screening of all samples was performed using the primers for CaMV35S promoter. The positive samples from this initial screening were further subjected to the analysis of specific transgenic material to determine the type of GMO present with subsequent quantification. Roundup Ready soya was found in eight samples, but its content was below the limit of 0.9%. None of the analysed samples of food products contained GM maize and GM rice. Considering that the investigated samples were imported mainly from EU countries, it can be concluded that the control of GMOs is carried out systematically and in accordance with the Serbian GMO Law.

Keywords: GMO; PCR; real time PCR; vegetarian food

The first genetically modified (GM) plant was introduced into commercial production in the USA in 1994. That was Flavr Savr, ripening-delayed tomato (Holst-Jensen 2009). Since then, GM plants have become an integral part of the agricultural production and more and more GM plant species are commercially available. GM soya continues to be the dominant GM crop occupying almost 50% of the global biotech area. The second most dominant GM crop is maize (30%), followed by cotton (14%) and canola (5%). Rice and sugar beet are grown in less than 1% of the area under GM crops (James 2011). GM plants have herbicide (glyphosate, gluphosinate and oxinyl) tolerance, insects tolerance and resistance (various forms of *Bacillus thuringiensis* Cry proteins), viral and fungal resistance, and modifications occur with altered nutrient composition (increased vitamin content, altered fatty acid composition)

Polymerase chain reaction (PCR) is one of the most commonly used method for routine analysis based on the detection of genetic elements common to most of the GMOs. CaMV35S promoter is used as a universal marker in the analysis of 95% of all GM plants (Holden *et al.* 2010). It can be found in RoundupReady soya (GTS 403-2), MON810, NK603, Bt11, Bt176, and T25 maize, LL62 rice etc. Since 0.9% is the threshold for labelling, it is necessary, in addition to determining the presence of GM, to define the exact percentage of GMOs in food. Real-time PCR is currently considered the most precise and the most widespread method for the quantification of specific nucleic acid sequences (Querci *et al.* 2010). Although the majority of food samples undergo numerous steps of industrial treatments (i.e. high temperature, pH, etc.), DNA as a molecule highly resistant to various physical processes can be found in traces enabling testing

for GM DNA presence (Bergerová *et al.* 2011). Also, it has been found that food processing has no practical consequences for the quantification of transgenic content in foods (Bergerová *et al.* 2010).

According to the Law on GMO, adopted in May 2009, Serbia strictly prohibits all imports, production, and commercial growing of GMO crops or products containing GMO (Law on Official Modified Organisms 2009). All shipments of soya, maize, rice, sugar beet, and rapeseed and their products entering Serbia must be tested for GMO content, and are allowed to be imported only if they are GMO-free. However, in the agricultural products of the plant origin, the contamination of 0.9% and 0.1% for seed is permitted.

*I*n order to detect and determine the validity of the labelling systems, *s*everal countries have conducted surveys to monitor the presence of GMO (Partridge & Murphy 2004; Brod & Arisi 2007; Kyrova *et al.* 2008; Ujhelyi *et al*. 2008; Ari & Cakir 2008; Zilio Dinon *et al.* 2010). In Serbia, over the past ten years have several studies dealt with the detection of GMOs in commercially available raw and processed foods (Maksimović *et al.* 2002; Nikolić *et al.* 2009; Taški-Ajduković *et al.* 2009; Matić *et al.* 2010; Miljuš-Djukić *et al.* 2010).

The aim of this work was to determine the presence of GM soya, maize, and rice, to identify the type of modifications, and to determinate the percentage of GM in non-labelled commercially available food products from the "health food" stores and supermarkets, in the section reserved for healthy, organic, and vegetarian food.

MATERIAL AND METHODS

Samples. The survey was conducted on a total of 100 non-labelled samples of healthy food and vegetarian products collected in the years 2009 and 2010. The samples came originally from countries with different GMO labelling legislations, such as the EU countries, USA, Argentina, Brazil, and Thailand. The basic raw materials in the samples that were tested comparised maize, soya, and/or rice (Table 1).

DNA extraction. For the DNA isolation, DNeasy Mini Plant Kit (Qiagen, Hilden, Germany) was used according to the manufacturer's manual, in duplicate. The quality and quantity of the extracted DNA was checked with a UV/VIS spectrophotometer (Evolution 100; Thermo Scientific, xxxxx, USA). The A_{260}/A_{280} of the extracted DNA ranged from 1.7 to 2.0.

Qualitative PCR. The screening of all samples was performed using primers for CaMV35S promoter (Metabion, Martinsried, Germany), which gives the product size of 123 bp (Lipp *et al.* 2000). As a quality control of DNA and PCR, the efficiency reference genes were used specific for soy and maize (lectin, zein) (Meyer *et al.* 1996; Studer *et al.* 1996) and universal primers for plant chloroplast DNA for rice (Taberlet *et al.* 1991). The CRM consisting of 0, 0.1, 0.5, 1, 2, and 5% of dried RR soya and MON 810 maize powder (IRMM, Geel, Belgium) and 100% LL62 rice (AOCS, Urbana, USA) were used as a positive control. The sensitivity of PCR reaction was 0.1%. The samples showing the presence of GMOs were analysed using the primers for RR soya (Jankiewicz *et al.* 1999).

Quantitative Real-time PCR. Quantitative analysis of GM was performed on 7500 Real Time PCR System (Applied Biosystems, Carlsbad, USA). The CRM consisting of dried RR soy powder in the amount of 0, 0.1, 0.5, 1, 2, and 5%, produced by IRMM (Geel, Belgium) was used. A commercial kit for the detection of 35S promoter was used for the analysis (GMO Soy 35S TaqMan Detection Kit; Applied Biosystems, Carlsbad, USA).

RESULTS AND DISCUSSION

The presence of GM was analysed in a total of 100 samples of non-labelled vegetarian and healthy food products. The basic raw materials in the samples tested were maize, soy, and/or rice. CaMV35S promoter was detected in 8 samples (soya cream, isolated soya protein, soya flakes, soya flour, soya pate). The positive samples were subjected to analysis for specific transgenic material to determine the type of GMO present. The amplification of the samples showed the presence of segments of the length of 172 bp, indicating the presence of RR soya (Figure 1). Quantification of the samples showed that the percentage of GM soya ranged between 0.1% and 0.62%, and that in all samples of GM soya was present below the limit of 0.9%. (Table 1). None of the analysed samples of food products contained GM maize and GM rice.

The screening methods are of a great importance for routine analyses. CaMV35S promoter

Table 1. GMO analysis of non-labeled samples of healthy food and vegetarian products collected in the territory of Vojvodina in the year 2009 and 2010

Type of sample	No of samples	Qualitative analysis		Quantitative analysis	
		positive	negative	< 0.9%	> 0.9%
Soya					
Wafers (contains soya lecithin)	3	0	3	0	0
Soya milk	3	1	2	1	0
Soya flakes	4	1	3	1	0
Isolated soya protein	11	3	8	3	0
Tofu	5	0	5	0	0
Soya flour	4	2	2	2	0
Soya pate	2	1	1	1	0
Total	32	8	24	8	0
Maize					
Pop corn	11	0	11	0	0
Corn flakes	20	0	20	0	0
Maize flour	7	0	7	0	0
Grits	10	0	10	0	0
Total	48	0	48	0	0
Rice					
Rice cakes	3	0	3	0	0
Rice milk	3	0	3	0	0
Rice noodles	2	0	2	0	0
Extruded rice	4	0	4	0	0
Rice	8	0	8	0	0
Total	20	0	20	0	0
Total	100	8	92	8	0

is used as a universal molecular marker for the analysis of 95% of currently commercialised GMO plants in EU (Holden *et al.* 2010). Host specific internal target gene has been tested in all assays as a control to evaluate the DNA quality and PCR efficacy, reducing the risk of false negatives and thereby increasing the reliability. The use of internal control, as the reference gene, is necessary to exclude the possibility of false negative results due to the possible inhibitor presence or inappropriate DNA quality. However, the positive test for the presence of CaMV35S promoter

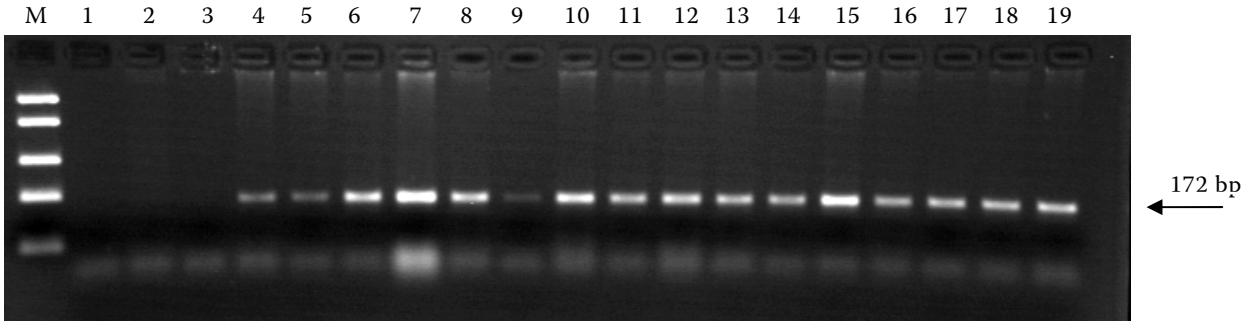

Figure 1. Agarose gel electrophoresis of PCR products from soya samples for analysis of the RoundupReady (RR) soya

M – low range DNA ladder, 50–1500 bp; 1 – Blank – no template control; 2 – negative control; 3 – CRM RR soya 0%; 4 – CRM RR soya 0.5%; 5 – CRM RR soya 0.1%; 6 – CRM RR soya 1%; 7 – CRM RR soya 2%; 8–19 – samples positive for RR

is not always sufficient (Ovesná *et al.* 2010). This sequence also occurs naturally in plants and plants may be naturally infected with the *Cauliflower mosaic virus*. Therefore, a positive result of CaMV35S will suggest the probability of transgene sequence being present. In such cases, further PCR tests should be performed with primers designed to amplify the specific transgenic DNA (Jinxia *et al.* 2011).

All samples, which showed the presence of CaMV35S segments, were analysed using primers for the presence of specific transgenic elements. It was found that these contained RR modification of soya, which is tolerant to the herbicide glyphosate. These results are in agreement with those presented by Berdal *et al.* (2001) and Ujhelyi *et al.* (2008) according to which RR soya is the most commonly used GM crop. RR soya is currently the only transgene soya line approved for the EU market, but its cultivation is not allowed. There are many different kinds of soya-derived products (protein additives, meat substitutes, diet foods, imitation milk products, lecithin for desserts, baked goods, etc.) which are common ingredients in many processed foods. It is estimated that as much as 60% of the processed food inventory of a typical supermarket contains material from soya (Nikolić *et al.* 2009). The demands of consumers for healthier and safer products have promoted the use of soya proteins in processed meat products as fat replacers (Castro-Rubio *et al.* 2005). Soya products are also present in many products that are sold as vegetarian foods in health food stores and, increasingly, in major supermarkets. There is a wide range of soya products ranging from whole soya and unrefined soya flour to textured soya proteins and soya milk (Partridge & Murphy 2004).

GMO detection conducted in the Czech Republic in 1164 samples over a period of five years indicated that most of the positive samples were mainly RR soya, 3 varieties of GM maize, and one of GM rice (Kyrova *et al.* 2008). On the ground of the PCR measurements, 38% of the analysed foods, coming from the Hungarian food market, proved to be GMO positive and 6% of these samples contained RR soya above 0.9% (Ujhelyi *et al.* 2008). In Ireland, it was found that 12 out of 75 samples tested were positive for GM ingredients and several soya products contained between 0.1% and 0.7% of GM soya (Partridge & Murphy 2004).

In previous studies on GMO detection in products derived from soya and coming from the Serbian market, similar results were obtained. In 2006 and 2007, in a total of 347 samples Nikolić *et al.* (2009) found RR soya in 29 samples in the range of 0.1–0.9%, while 10 samples (additives for food industry and supplements for human consumption) contained RR soybean above 0.9%. Taški-Ajduković *et al.* (2009) analysed 50 processed meat products commercially available: 12 were positive for RR soya and all samples contained GMO in the amount below 0.1%, while Matić *et al.* (2010) determined less than 0.9% of GM soya in soya pate for vegetarian diet.

0.9% of GMO is the threshold for labelling in the EU, and also the value defined in our Law on GMO. It is necessary to define the exact percentage of GMO in food, which is achieved by analysing the samples with real time PCR. In our case, all of the samples contained less than 0.9% GM soya, and, according to our law on GMOs, can be used for human consumption. The results were expected since the majority of the analysed samples originated from the EU, where the labelling is mandatory. The samples that were positive for the presence of GM soya originated from Brazil which commercially produces GM food and feed crop (GM soya) and has mandatory labelling, but has yet to implement actually these laws (Gruere & Rao 2007). Given the increased demand for soya products and the very small level of soya production in the EU, increasing import exists of soya from the main crop producing areas, i.e. USA, Brazil, and Argentina. The challenge facing the importers and food manufacturers who wish to produce only non-GM labelled foods is to ensure that they receive non-GM soya from their suppliers (Partridge & Murphy 2004).

In practice, the only GM crop currently available to EU farmers for cultivation is the GM maize resistant to insects, commonly known as Bt maize (James 2011). On the teritory of Serbia, the number of positive maize samples varied from 12% in 2006 year, to 14.5% in 2008, with the amount of GM mostly below 0.9%, except for two samples with the content above 0.9%, in 2006 (Nikolić & Vujaković 2011). In 2009, we continued to test maize and products derived from maize. The samples analysed did not contain CaMV35s promoter and, therefore, did not contain any of the GM maize authorised in the EU.

No GM rice variety has been listed as authorised or risk-evaluated in Europe but a risk exists that the imports of rice into the EU are contaminated

with non authorised GM rice (Vogel 2008). In this paper, we analysed samples of rice mostly originating from EU countries: Italy and Bulgaria, as well as from Thailand, where labelling the products containing more then 5% for is mandatory. None of the analysed samples of rice and rice products contained GM rice.

EU member states are responsible for the import controls at its borders and for the prevention of placing on the market shipments contaminated by genetic modification. In addition, they should implement the control of the products on the market, to ensure that they are GM-free. Considering that the investigated samples were imported mainly from EU countries, it can be concluded that the control of GMOs is carried out systematically and in accordance with the Law.

Based on our results, in order to protect the consumers and their concern about the quality of food that they consume, it would be advisable to continue with the control of all of the imported ingredients and food products by the authorities responsible for the food chain.

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