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GENETICALLY MODIFIED ORGANISM PRESENCE IN FOOD FROM ARAD COUNTY DURING 2007-2008

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ABSTRACT. During the last decades, crops for food are increasingly developed based on genetic engineering, where foreign DNA fragments with specific characteristics were inserted into the genome. The safety of these genetically modified organisms should be assessed for both livestock and human nutrition. The detection, the identification and the quantification of the genetically modified organism presence in crops and along the feed/food production is essential to properly fulfil legal requirements. It requires labelling of food and feed products containing authorized GMOs or GM derived material except if, on an ingredient-basis, the level of 0.9% is not exceeded and provided that this presence was the result of an accidental contamination. The results show the presence of GMO's in 22% of the samples but not a single sample exceeded the legal level for labelled non GMO products. Existence of valid detection methods and strategies are necessary for the enforcement of the European legislation that was progressively set up in this field.

Keywords: food, genetically modified organisms, food safety

INTRODUCTION

The first GM food (delayed-ripening tomato) was introduced on the US market in the mid-1990s. Since then, GM strains of maize, soybean, rape and cotton have been adopted by a number of countries and marketed internationally. In addition, GM varieties of papaya, potato, rice, squash and sugar beet have been trialed or released. It is estimated that GM crops cover almost 4% of total global arable land.

All over the world, authorities responsible for the assessment and surveillance of foods and feeds derived using gene technology and the environmental impacts of genetically modified organisms (GMO) have chosen specific strategies to assess their safety. Although different regulatory frameworks are in place, almost all adopted risk assessment strategies are based on a common set of principles and guidelines.

The main goal of this study was to establish to what extent Arad county consumers are exposed to consuming genetically modified foods.

MATERIAL AND METHOD

141 food samples were collected from the Romanian food business operators and examined by the Bucharest Veterinary Public Health Institute and Arad Sanitary Veterinary and for Food Safety Laboratory using PCR screening and Real Time PCR. The GMO screening was based on the detection of 35S (soy), respectively CaMV35S (maize) promoter and *nos* terminator sequences. The GM positive samples were subjected to event specific detection of RR soy with quantitative real-time PCR. The PCR is performed with suitable primers specific for the GMO under study and the amplification capacity of the extracted DNA is determined by an internal control. The amplicons are examined by agarose gel electrophoresis and their identity is confirmed by Southern blot.

RESULTS AND DISCUSSIONS

In the EU the presence of genetically modified organisms (GMO) in food is controlled by the Member States official laboratories within their national inspection and monitoring programs.

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GMO food surveillance in Arad county is taken by National Sanitary Veterinary and for Food Safety Authority National Strategic Plan, and consist of inspection and food with soy or maize non GMO labeled sample (Regulation (EC) No 1829/2003 of the European Parliament and of the Council; Regulation (EC) No. 1830/2003 of the European Parliament and of the Council; Commission Recommendation 2004/787/EC of October 4th, 2004).

Table 1

Market control for food containing soy in Arad county in 2007-2008

Crt. Nr.	Food busines operator	Inspections (Nr.)	Sample/products from		
			Indigen production	EU trade	Imports
1	Soy storehause	45	6	0	0
2	Meat processing plant	41	16	10	14
3	Groceries	55	32	12	0

Table 2

Market control for maize in Arad county in 2007-2008

Crt. Nr.	Food busines operator	Inspections (Nr.)	Sample/products from		
			Indigen production	EU trade	Imports
1	Maize storehause	45	32	0	0
2	Groceries	55	30	10	0

DNA from sample was extracted according to protocols (ISO/DIS 21569 (2004) Foodstuffs – Methods of analysis for the detection of genetically modified organisms and derived products – Qualitative nucleic acid based methods. CEN/TC 275/WG 11, ISO/DIS 21570 (2003) Foodstuffs – Methods of analysis for the detection of genetically modified organisms and derived products – Quantitative nucleic acid based methods; CEN/TC 275/WG 11, ISO/FDIS 21571 (2004) Foodstuffs – Methods of analysis for the detection of genetically modified organisms and derived products – Nucleic acid extraction. CEN/TC 275/WG 11, ISO, under development; ISO/DIS 24276 (2003) Foodstuffs – Methods of analysis for the detection of genetically modified organisms and derived products – General requirements and definitions. CEN/TC 275/WG 11). DNA was amplified using a primer corresponding to the CaMV 35S promoter, a fragment of DNA found in most commercial transgenic maize and not known to exist naturally in the maize genome (sequence available upon request). DNA was extracted from individual plants as a bulk (15 individuals per population). DNA isolated from a known transformed plant containing the CaMV 35S promoter was run as a positive control. To further ensure that the reactions were working correctly, all DNA samples were amplified using a primer corresponding to a fragment of DNA known to exist naturally in the maize genome. All positive controls amplified correctly. No bulk of an accession amplified the CaMV 35S promoter sequence, thus clearly indicating that the CaMV 35S promoter sequence was not present in any of the samples tested. The methods used are the following quantification limits:

- Real time PCR 0,1%
- PCR – for soy 0.04% (using ERM-BF410a and ERM-BF410b reference material)
- PCR – for maize 0.1% (using ERM-BF416a and ERM-BF416b reference material)

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Table 3

Sequences of the primers used for the PCR amplification

Primer name	Orientation	Sequence	Predicted band(bp)
Plant chloroplast	Sense, Anti-sense,	CP3 5-CGAAATCGGTAGACGCTACG-3 CP4 5-GGGGATAGAGGGACTTGAAC-3	500
Soy lectin	Sense, Anti-sense,	LEC 1 5-GCCCTCTACTCCACCCCCATCC-3 LEC 2 5-GCCCATCTGCAAGCCTTTTTGTG-3	118
35 S Promoter	Sense, Anti-sense,	CaMV1 5-GAAGGTGGCTCCTACAAATGCC-3 CaMV2 5-GTGGGATTGTGCGTCATCCC-3	199
NOS terminator	Sense, Anti-sense,	NOS A 5-GAATCCTGTTGCCGGTCTTGCG-3 NOS D 5-GCGGGACTCTAATCATAAAAACCC-3	127

According to the results, 22% of the examined samples indicated presence of RR soy (Roundup Ready GTS 40-3-2) in sample of soy/ soy-containing foods but none of these samples contained RR soy above the 0.9% threshold.

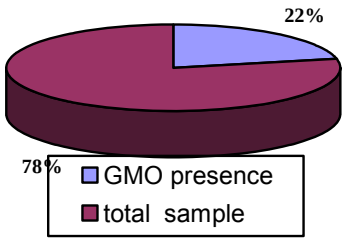

Fig. 1 Detectable DNA from GTS 40-3-2 soy in Arad county food sample in 2007-2008

Successful quantification depends crucially on the quality of the sample DNA analyzed. Methods for GMO detection are generally validated on certified reference materials that are in the form of powdered grain material, while detection in routine laboratories must be performed on a wide variety of sample matrixes. Due to food processing, the DNA in sample matrixes can be present in low amounts and also degraded. In addition, molecules of plant origin or from other sources that affect PCR amplification of samples will influence the reliability of the quantification. Further, the wide variety of sample matrixes presents a challenge for detection laboratories (G. Berben). Existing sampling plans and needs for the development of novel sampling approaches for Genetically Modified Organism (GMO) evaluation.

The DNA isolation procedure can substantially influence the quantification, since different methods differ in their effectiveness in removing substances that interfere with the PCR reaction. In addition, components of the DNA isolation solutions can themselves influence PCR reactions (W. Moens, Tracing and authentication of GMOs and derived products in the food-processing area, Final report, Belspo, Brussels, 2005).

Therefore the appropriate choice of an extraction method suitable for a particular sample matrix is a prerequisite for successful downstream analysis. As a sample matrixes are highly variable and difficult to define exactly, optimization of DNA isolation procedures for each matrix is impossible. Appropriate controls must be included in PCR quantification to evaluate the suitability of the isolated DNA for quantitative analysis (L. Bonfini, P. Heinze, S. Kay, G. Van den Eede, Review of GMO detection and quantification techniques, European Commission, JRC-Ispra, Italy, 2001).

CONCLUSIONS

Regulations on the labeling of GMOs largely are observed. Infringements are limited to single exceptions, the number of which is declining. Primarily, foods containing soy or maize are analyzed. When GMOs are detected, the amount generally lies far below the labeling threshold of 0.9 percent. When GMO portions above 0.9 percent are measured, this occurs usually in a product containing soy. Mostly, these are imported products sold outside the major food store chains

22 percent of the analyzed foods containing soy contain GMO but the consumer don't have the choice because is under labeling limit. For foods containing maize none of the sample was GM-positive. The appropriate choice of an extraction method suitable for a particular sample matrix is a prerequisite for successful downstream analysis

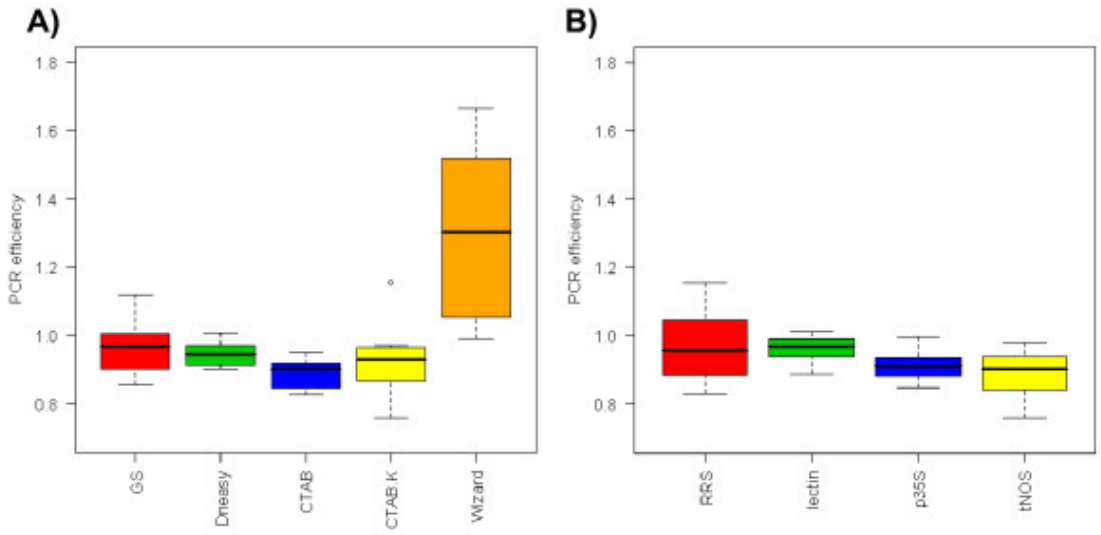

Fig. 2 Influence of the DNA extraction method on PCR efficiency: (A) Variability of PCR efficiency for different DNA isolation methods. Outlier for the CTAB procedure with proteinase K and RNase A treatment is shown as circle above the boxplot. (GS = GENESpin, CTAB.K = CTAB procedure with proteinase K and RNase A treatment); (B) The distribution of PCR efficiencies of 4 tested amplicons on different DNA extracts is presented in boxplots (efficiency data for DNA isolated with Wizard method was excluded because of high variability of results), Cankar et al., 2006

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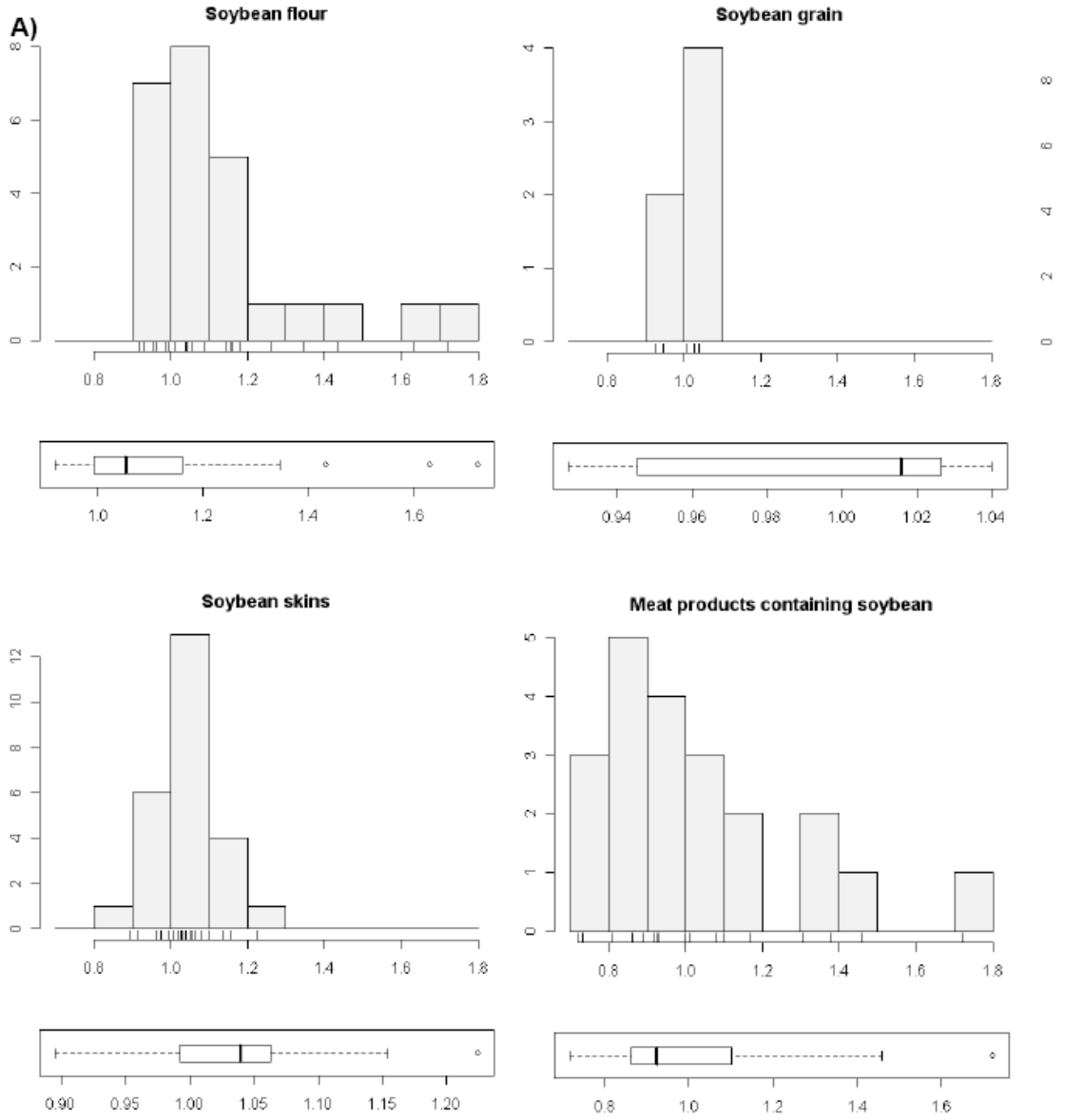

Fig. 3A Variability of PCR efficiency within matrixes. PCR efficiencies for soybean matrixes were determined for the plant specific genes, lectin and invertase, respectively. The dispersions of PCR efficiencies is shown in a histogram with PCR efficiency on the x axis and number of samples on the y axis. For a scaled view and detection of outliers, box plots of efficiency data for each matrix are presented below histogram (1 each)

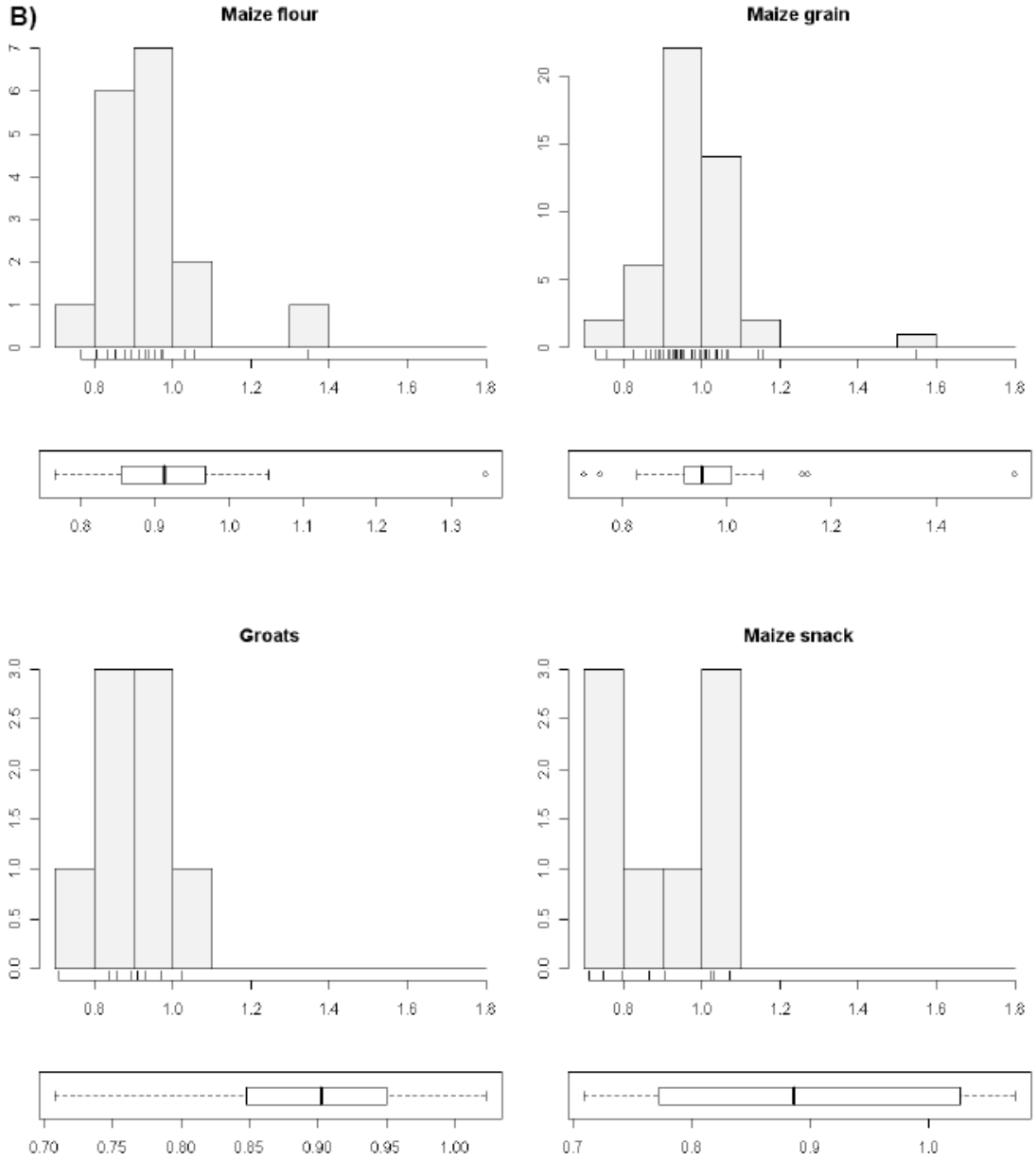

Fig. 3B Variability of PCR efficiency within matrixes. PCR efficiencies for maize matrixes were determined for the plant specific genes, lectin and invertase, respectively. The dispersions of PCR efficiencies is shown in a histogram with PCR efficiency on the x axis and number of samples on the y axis. For a scaled view and detection of outliers, box plots of efficiency data for each matrix are presented below histogram (1 each)

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