Collaboration

Presence of genetically modified soya in products on the market

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Abstract

In recent years, the number of events of Genetically Modified Organisms (GMO) authorised in the European Union to be used as food or feed has notably increased, particularly in the case of genetically modified soya. The number has grown from September 2008, when there was only one authorisation, Monsanto Company's Roundup Ready® soya, to seven different events currently authorised: 40-3-2 soya, MON89788 soya, MON87701 soya, A2704-12 soya, A5547-127 soya, 356043 soya and MON87701 x MON89788 soya.

In order to know the possible presence of these varieties of soya in products on the market and to test the degree of compliance with the regulations in force, the National Food Centre (CNA) under the Spanish Agency for Consumer Affairs, Food Safety and Nutrition (AECOSAN), has conducted a study on 28 product samples acquired on the Spanish market. The complete GMO analysis process consisted of the homogenisation of the sample, the extraction and purification of DNA by means of the CTAB method (cetrimonium bromide), in order to subsequently carry out the detection, identification and quantification of the genetically modified soya content by means of the polymerase chain reaction (PCR) technique and real time PCR (RT-PCR).

The results obtained showed the presence of transgenic sequences in 25 % of the samples analysed. The presence of 40-3-2 soya was identified in three of them, and one simultaneously contained 40-3-2 soya and MON89788 soya. The results have shown that no type of infringement has been revealed since in no case were the levels of genetically modified soya found to be above the legal limit of 0.9 %.

Keywords

Genetically Modified Organisms, soya, DNA, PCR, real time PCR.

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1. Introduction

Today, Genetically Modified Organisms (GMOs) are a fact that represents an important component in the production of food and feed on the global market (Clive, 2013).

In the majority of countries, the introduction of GMO on the market is controlled by government policies that support a strong commitment to consumer protection and the freedom of choice. In the European Union (EU) in particular, the presence of GMO in food and feed is regulated by Regulations (EC) No 1829/2003 (EU, 2003a) and (EC) No 1830/2003 (EU, 2003b) which allow the adoption of the necessary measures to control traceability and/or labelling of the products that contain GMO along the food chain. European legislation requires, under the said regulations, the labelling of those products that consist of or contain more than 0.9 % GMO.

Compliance with legislation demands the availability of methods that allow the detection and identification of the presence of a given GMO in food or feed.

For each GMO authorised in the EU, it is an essential requirement to have an event-specific method of quantification using real-time PCR that has been checked and validated by the European Reference Laboratory for Genetically Modified Food and Feed (EURL GMFF), with the support of the European Network of GMO Laboratories (ENGL).

The GMOs authorised for commercialisation in the EU have increased notably in recent years. From the first authorisation in 1994 to present day, approximately 150 genetically modified organism crops have been authorised for use as food and feed.

In the case of genetically modified soya, there are currently seven different varieties authorised for use in the production of food and feed, one of them being a stacked event.

The first authorisation of genetically modified soya that cleared the way for the birth of the GMO on the global market took place in 1996 with the authorising of Roundup Ready® soya from Monsanto Company that contains resistance genes to the glyphosate herbicide.

In 2008 the use of two new soya crops was authorised, into which resistance genes to other herbicides had been introduced, such as Monsanto's new line MON89788 (MON-89788-1) soya and A2704-12 (ACS-GMØØ5-3) soya from Bayer Company into which the *pat* gene had been introduced. This gene confers resistance to the glufosinate-ammonium herbicide.

In 2012, in addition to the extension of the authorisation of Monsanto's RR soya (MON40-3-2 soya), four new GM soya crops were authorised for use as food and feed: Monsanto's MON87701 (MON-877Ø1-2) soya, in which the objective of the modification consists of conferring resistance to lepidopterans through the use of the *cry1Ac* gene; and two new soyas with resistance genes to herbicides, Pioneer's 356043 (DP-356Ø43-5) soya and Bayer's A5547-127 (ACS-GMØØ6-4) soya.

Additionally, in 2012, the MON87701 x MON89788 (MON-877Ø1-2 x MON-89788-1) soya stacked event from Monsanto was authorised. This was obtained by crossing two of the simple varieties modified genetically in order to obtain resistance to herbicides and resistance to lepidopteran pests in one single crop.

In the face of this increase in the number of authorised varieties of soya in the EU for use as food and feed, the conducting of an analytical study was suggested in order to know the possible presence of these varieties of soya in products on the market and to check the degree of compliance with the regulations in force.

2. Materials and Methods

2.1 Samples

The study for detecting the presence of genetically modified soya in products on the market was carried out using 28 samples of processed foods. They were acquired from different establishments in the Region of Madrid and were selected taking into account the fact that one of their main ingredients was soya.

The samples were selected from among frequently consumed foods such as pancakes, biscuits, yoghurts, vegetarian meat substitutes, tofu and bread. Out of all them, 23 were products produced in Spain, while five were imported products.

The majority of the samples included in the study showed normal labelling. However, eight were products whose labelling indicated that their vegetative ingredients came from organic farming and six were specifically labelled as "GMO free" (Table 1).

Table 1. List of samples used in the study

Sample	Ingredients	Origin	Labelling
Soya bread	Soya flour and rapeseed oil	England	-
Soya biscuit	Soya, caramelised soya, vegetable fat, rice and malt flour and oat fibre	Spain	-
Corn and soya pancake	Soya and flax seeds, puffed corn and corn semolina	Spain	"GMO free"
Bread	Soya flour and brewer's yeast	Italy	-
Soya biscuit	Soya flakes, vegetable oil, corn starch and wheat starch	Spain	Organic agriculture
Soya bar	Soya flour, rice flour and vegetable oils	Japan	-
Soya yoghurt	Soya beans, soya protein and modified tapioca starch	Spain	"GMO free"
Fresh tofu	100 % soya, GDL coagulant	Spain	"GMO free"
Soya biscuit	Wheat flour, whole wheat flour, soya seeds and sunflower oil	Spain	-
Fried soya	Soya and sunflower oil	Spain	-
Soya chocolate yoghurt	Soya drink (soya beans and water), sugar, modified starch and cocoa	Spain	"GMO free"
Soya biscuit	Soya beans, whole wheat flour, sunflower oil and glucose	Spain	-
Vegetarian meat substitute	Wheat gluten, tofu (soya beans and water), algae (xanthan gum and carrageenates), tapioca starch and sunflower oil	Spain	Organic agriculture
Vegetarian lunchmeat substitute	Soya, wheat flower, sunflower oil and oats	Spain	Organic agriculture
Tofu	Fresh tofu (soya beans and water), olive oil and algae extract	Spain	Organic agriculture
Soya meat	Soya and rice flour	Portugal	-
Chocolate biscuits	Soya lecithin, wheat flour and vegetable fat	Spain	-
Cream biscuits	Soya lecithin, sunflower lecithin, wheat starch and vegetable fat	Spain	-
Soya dessert	Blended soya, corn starch	Spain	-
Soya biscuit	Wheat flour, vegetable oil, soya flakes and corn starch	Spain	-
Soya biscuit	Soya and vegetable fat	Spain	-
Soya juice	Soya seeds	Spain	-
Soya drink	Soya beans, agave and seaweed	Spain	Organic agriculture
Soya dessert	Soya beans and corn starch	Spain	"GMO free"
Soya cream	Sunflower oil, soya beans and wheat syrup	Belgium	Organic agriculture
Tofu	Fresh tofu (100 % soya), olive oil and algae	Spain	Organic agriculture
Tofu croquettes	Tofu, wheat flour, soya bean fibre and soya	Spain	Organic agriculture
Soya yogurt	Soya beans and soya protein	Spain	"GMO free"

2.2 Reference Materials

In the preparation of the positive and negative controls necessary to ensure the quality of the test performance, as well as in the preparation of the calibration curves used in the GMO quantification processes, Certified Reference Materials (CRM) originating from the *Institute for Reference Materials and Measurements* (IRMM) and from the *American Oil Chemist´s Society* (AOCS) have been used. The CRM used in this study are outlined in Table 2.

Table 2. List of certified reference materials

Event	Code	Concentration		Origin
		Nominal value (%)	m/m	
40-3-2 soya	ERM-BF 410ak	0 %	< 0.7 g/kg	IRMM
	ERM-BF 410b	0.1 %	1.0 g/kg ± 0.5 g/kg	
	ERM-BF 410dk	1 %	10.0 g/kg ± 1 g/kg	
	ERM-BF 410gk	10 %	100 g/kg ± 7 g/kg	
DP-356043-5 soya	ERM-BF 425a	0 %	< 0.5 g/kg	IRMM
	ERM-BF 425b	0.1 %	1.0 g/kg ± 0.4 g/kg	
	ERM-BF 425c	1 %	10.0 g/kg ± 1.1 g/kg	
	ERM-BF 425d	10 %	100 g/kg ± 9 g/kg	
A2704-12 soya	AOCS 0707-A3	0 %	<0.1 ng/µl	AOCS
	AOCS 0707 B2	100 %	>999.9 ng/µl	
MON89788 soya	AOCS 0906-A	0 %	>999.0 g/kg	AOCS
	AOCS 0906-B	100 %	>994.0 g/kg	
MON87701 soya	AOCS 0906-A	0 %	>999.0 g/kg	AOCS
	AOCS 0809-A	100 %	>999.4 g/kg	
A-5547-127 soya	AOCS 0707-A3	0 %	<0.1 ng/µl	AOCS
	AOCS 0707-C3	100 %	>999.9 ng/µl	AOCS

2.3 Experimental design

The detection, identification and quantification of GMO in food and feed consists of an analytical process that comprises a series of steps from the DNA extraction of the sample to the detection and identification of the different genetic sequences introduced by the polymerase chain reaction (PCR) and finally the quantification of the content of GMO in the sample by real time PCR.

2.3.1 DNA extraction and purification

A representative amount from each of the samples was milled and homogenized in order to achieve the correct distribution of all the components in the sample

The DNA extraction and purification was carried out on 250 mg of the homogenised sample, following the extraction procedure based on the CTAB method (Murray and Thompson, 1980).

Each of the samples was extracted twice and the purified DNA was diluted with 100 µl of TE buffer solution (10 mM Tris-ClH, 1 mM EDTA, pH 8.0).

The quantification and the purity of the DNA obtained was performed spectrophometrically by measuring the absorbance at 280 nm and the A260/A280 ratio using a NanoDrop 2000 spectrophotometer.

In the majority of the samples, the results obtained for concentration and purity were considered satisfactory to continue with the analytical process and to proceed to the amplification of target sequences PCR.

In only two of the samples analysed, vegetarian lunchmeat substitutes and vegetarian meat substitutes, the results obtained for concentration and purity in the DNA extraction by the CTAB method were not suitable enough to continue the assay. Therefore, after having carried out prior degreasing treatment with hexane, it was necessary to use another extraction method: commercial kit SureFood® PREP Plant X (R-Biopharm), especially recommended for DNA extraction in highly processed food.

The CRM used were extracted simultaneously with the samples by the CTAB method.

The DNA solution was diluted with the TE buffer solution when it was needed in order to obtain the concentration of the 40 ng/μl working solution.

2.3.2 Detection of genetically modified soya

In order to detect the presence of transgenic sequences in the samples analysed, the detection was carried out by conventional PCR to detect genetic sequences present in the majority of authorised GMOs and therefore indicative of the possible presence of one of the six GM soya events currently authorised in the EU.

As screening sequences were chosen: the 35S Promoter from the Cauliflower Mosaic Virus (Lipp et al., 2001), the NOS terminator from *Agrobacterium tumefaciens* (Lipp et al., 2001), the CP4-EPSPS gene to express tolerance to glyphosate herbicide (Köppel et al., 1997) and the p34S gene originating from the Figwort Mosaic Virus (Pan et al., 2007) that is used as a promoter in the MON89788 soya.

The presence of soya DNA and the amplification of the DNA extracted were verified by the amplification of the lectin (le1) gene specific to soya by conventional PCR. The chosen target sequence and the conditions of the reaction are those described by Meyer et al. (1996).

The sequences used as well as the expected size of the amplicon are described in Table 3.

Table 3. Primers used in the qualitative detection of GMO screening sequences

Target	*Primer*	**Sequence**	**Amplicon (bp)**	**Reference**
35S promoter Cauliflower Mosaic Virus (CaMV P35S)	35S-cf3 35S-cr4	CCACGTCTTCAAAGCAAGTGG TCCTCTCCAAATGAAATGAACTTCC	123 bp	(Lipp et al., 2001)
T-Nos (Nopaline synthase terminator) *A. tumefaciens*	HA-nos118-f HA-nos118-r	GCATGACGTTATTTATGAGATGGG GACACCGCGCGCGATAATTTATCC	118 bp	(Lipp et al., 2001)
CP4-EPSPS	CTP-EPSPS CP4-EPSPS	CCCCAAGTTCCTAAATCTTCAAGT TGCGGGCCGGCTGCTTGCA	180 bp	(Köppel et al., 1997)
FMV 34-S Figworth Mosaic Virus (P-FMV)	FMV-1 FMV-2	AAGCCTCAACAAGGTCAG CTGCTCGATGTTGACAAG	196 bp	(Pan et al., 2007)
Lectin (Le1) gene	GM03 GM04	GCCCTCTACTCCACCCCCATCC GCCCATCTGCAAGCCTTTTTGTG	118 bp	(Meyer et al., 1996)

In each PCR reaction the corresponding positive and negative controls prepared from the CRMs have been analysed simultaneously with the samples. The limit of detection in the assay was established at 0.025 % by the corresponding validation of each one of the sequences over certified reference material.

2.3.3 Amplified Product Detection

The amplified PCR products were analysed by electrophoresis in a 2 % (w/v) agarose gel (Roche) in 1X TBE buffer solution containing 0.89 M Tris, 0.98 M Boric acid, 0.02 M EDTA, pH 8.0 and later visualised by staining with ethidium bromide in a UV transilluminator (Bio-Rad). As molecular weight markers PCR 100 bp Low Ladder Sigma was used.

2.3.4 Identification of the different GM Soya Events

In the samples in which amplification in the screening sequences was detected, the identification of the GM soya events was conducted by real time PCR. As the reaction target, event specific sequences described in the methods validated by the European Reference Laboratory (EURL-GMFF) for determining RR soya (soya 40-3-2), A2704-12 soya, A5547-127 soya, MON89788 soya, MON87701 soya and 356043 soya were used. In order to standardise the analytical process, the reaction conditions were modified with respect to those recommended in the methods validated by the EURL-GMFF.

The PCR reaction was prepared in a final volume of 25 µl for which 12.5 µl of 2X TaqMan® Universal Master Mix (Applied Biosystems), 5µl of the DNA extracted in a concentration of 40 ng/µl 250 nM of each of the primers and 150 nM of probe. The PCR reactions were conducted in the Applied Biosystems 7 500 device, using the following programme: 2 minutes at 50 ºC, 10 minutes at 95 ºC and 45 cycles of 15 seconds at 95 ºC and 1 minute at 60 ºC. Each one of the PCR targets was analysed three times.

The detection system was previously verified for the recommended reaction conditions by using three independent assays, analysing 10 PCR replicates, with each one of the CRM of each of the GM soya events prepared in the limit of detection concentration. The results showed that 0.025 % of the GM soya content could be detected with a 95 % confidence interval at least.

The presence of some of the varieties of GM soya was considered positive when signals of amplification were observed before the 40 cycle. The analytical process was considered acceptable so long as the results obtained for the positive and negative controls complied with the established acceptance criteria. As a positive control, CRM prepared at the limit of detection 0.025 % was used and as a negative control, CRM negative for the desired target.

The oligonucleotide sequences used as primers and the probes labelled with FAM and TAMRA were synthesized by the Metabion GMBH trade house.

2.3.5 Determining the MG Soya Content

In the samples in which the presence of any of the soya events under study were identified, the quantification was carried out by real time PCR strictly following the methods validated by the EURL-GMFF (2007a, 2007b, 2009, 2011, 2013a, 2013b).

The calibration curves to carry out the quantification processes were prepared from the DNA extracted from the corresponding reference material with a certified value of 10 % (m/m) in GMO content, by conducting serial dilutions containing (200, 40, 8, 1.6 y 0.32) ng of DNA by reaction, the transgenic gene content was estimated on the basis of the proportional value of certified transgenic content in the reference material.

By means of the graphic representation of the values obtained from the amplification cycle Ct and the logarithm of the DNA concentration, the corresponding calibration curves for endogenous genes and transgenic genes were obtained. The estimation of the endogenous gene and transgenic gene content in the unknown samples was determined by interpolating the results obtained in the standard curves. The relative quantification, in percentages

(%), of the content of a given transgenic event in an unknown sample was obtained by dividing the transgenic content by the endogenous gene content and multiplying it by 100.

The acceptance criteria for the calibration curves were established in accordance with the criteria established by the EURL-GMFF and the ENGL network (2008). The calibration curve was considered acceptable when the coefficient correlation was superior to 0.98 ($R^2 > 0.98$), the slope was between the range of values of (-3.1 ≥ slope ≥ -3.6) and the efficiency of the PCR reaction, calculated by the formula ((10^(-1/ slope)-1) x 100), was between 110-89 %

As a quality control the CRM was analysed on two different levels of concentration at the limit of quantification (LOQ) and in a concentration close to the level of specification (0.9 %). As a requirement for the acceptance of the assay, the results obtained with the CRM had to comply with the criteria of ± 25 % accuracy.

All the samples were analysed twice (two independent assays). The results of the quantification of the test portions were obtained from the average of two independent assays that complied with internal reproducibility criteria, CV ≤ 35 %.

Results and Discussion

The results obtained in the samples analysed showed that the method chosen for the extraction (CTAB method) can be considered an acceptable method for obtaining the DNA of highly processed products (Gryson, 2010). As can be observed in the results shown in Table 4, the concentration of DNA, obtained in the majority of cases by means of the extraction process used, was superior to the necessary working concentration (40 ng/µl) and the purity values were close to the optimum values (A260/A280= 1.8). However, it has been proven that the efficiency of the extraction considerably decreased in highly processed products like soya yoghurts or deserts in which the values of concentration obtained were below 40 ng/µl. The low efficiency is attributed to the fragmentation of the DNA as a consequence of exposure to heat and chemical and physical treatments of the processing that could be the cause of the random rupture of the strands of DNA (Peano et al., 2004), nevertheless, the quality of the DNA was considered acceptable for use in the amplification assays.

Table 4. Concentration and purity results from the DNA extracted using the CTAB method

Sample	CTAB Method	
	Concentration (ng/µl)	Purity (A_{260}/A_{280})
Soya bread	95	1.8
Soya biscuit	102	1.8
Corn and soya pancake	126	1.8
Soya bread	112	1.8
Soya biscuit	149	1.8
Soya bar	108	1.6
Soya yoghurt	19.2	2
Fresh tofu	49	1.9
Soya biscuit	64	1.8
Fried soya	84	1.8
Soya chocolate yoghurt	14	1.9
Soya biscuit	132	1.8
Vegetarian meat substitute	**3.5**	**2.5**
Vegetarian lunchmeat substitute	**6.2**	**3.1**
Tofu	44	1.8
Soya meat	75	1.9
Chocolate biscuits	45	1.8
Cream biscuits	60	1.9
Soya dessert	12.2	1.9
Soya biscuit	188	1.8
Soya biscuit	173	1.7
Soya juice	32	1.8
Soya drink	47	1.8
Soya dessert	25	1.8

Table 4. Concentration and purity results from the DNA extracted using the CTAB method

Sample	CTAB Method	
	Concentration (ng/µl)	Purity (A_{260}/A_{280})
Soya cream	65	1.8
Tofu	43	1.7
Tofu croquettes	43	1.8
Soya yogurt	10	1.8

In spite of the fact that it was pointed out in the study conducted by Gryson (2010) that the elevated increase in temperature and the chemical treatments which soya milk is subjected to in the creation of products like tofu, makes the extraction difficult, the results obtained in this study coincide with those shown in the study conducted by Zimmermann et al. (1998). The results of the said study considered the efficiency of the CTAB method to be suitable for the extraction of this type of product except for two samples, vegetarian lunchmeat substitute and vegetarian meat substitute, in which, in addition to obtaining a low efficiency they did not comply with the quality and purity criteria. In these two cases it was necessary to modify the extraction process, and after having subjected the samples to a prior degreasing treatment in order to avoid the high vegetable fat content of the said products inhibiting the extraction process, a commercial extraction and purification system was used. As can be seen in Table 5, the results improved considerably when they were extracted with a commercial system, SureFood® PREP Plant X, R-Biopharm, after being degreased.

Table 5. CTAB method and SureFood® PREP PlantX, R-Biopharm commercial kit results comparison

Sample	CTAB		Kit (R-Biopharm)	
	Concentration (ng/µl)	Purity (A260/A280)	Concentration (ng/µl)	Purity (A260/A280)
Vegetarian meat substitute replacement	3.5	2.5	43	1.7
Vegetarian lunchmeat substitute	6.2	3.1	38	1.6

The visualisation of the agarose gels in bands of 118 bp due to the amplification of the lectin (le1) gene in all the samples analysed, confirmed the availability of amplifiable DNA free from inhibitors and demonstrated the presence of soya DNA in the samples.

The results obtained in the detection of screening sequences showed that in 25 % of the samples analysed signals of amplification were detected for some of the sequences analysed. The results are detailed in Table 6.

As it can be seen, in three of the seven samples in which signals of amplification for the P35S promoter were detected, positive amplification results were obtained also for other analysed screening sequences: The T-NOS terminator and the CP4-EPSPS gene. Besides in one of them amplification for the P34S promoter was also detected (Table 6).

Table 6. Results obtained in the screening assays. Limit of detection 0.025 %, determined by CRM

Sample	P 35S	T-Nos	P34S	CP4-EPSPS
Soya bread	+*	ND**	ND	ND
Soya biscuit	+	+	ND	+
Corn and soya pancake	+	ND	ND	ND
Soya yoghurt	+	+	+	+
Soya meat	+	+	ND	+
Soya biscuit	+	ND	ND	ND
Soya biscuit	+	ND	ND	ND

*+: Detected. **ND: Not detected.

In the samples in which the amplification of the P35S promoter was the only sequence detected, it was considered that, when dealing with complex samples that contain different vegetables among their ingredients, mainly corn, the presence of these transgenic sequences could be due to the presence of one of the 17 corn events currently authorised in the EU. This was only after the possibility of it being due to an infection by the Cauliflower Mosaic Virus (CaMV) was dismissed by looking for the virus specific sequences (Cankar et al., 2005).

In the positive samples for the screening sequences the identification was conducted by real time PCR in the different GM soya events. Out of the seven samples with positive results, in three of them the presence of 40-3-2 soya was detected and in one of the samples, soya yoghurt, both 40-3-2 soya and MON89788 soya were detected (Table 7). In all the samples, the signals of amplification detected were in Ct values comprising of between the 36 Ct and the 38 Ct and the CRM at the limit of detection in the assay at 0.025 %, the quality controls included, complied with the acceptance criteria value of Ct ± 3SD of the values of Ct obtained in the validation.

Table 7. GM soya identification results. Limit of detection 0.025 % determined by CRM

Sample	40-3-2 soya	MON87701 soya	MON89788 soya	A2704-12 soya	A5547-127 soya	DP-356043-5 soya
Soya biscuit	+*	ND**	ND	ND	ND	ND
Soya yogurt	+	ND	+	ND	ND	ND
Soya meat	+	ND	ND	ND	ND	ND

*+: Detected. **ND: Not detected.

The quantification process of the 40-3-2 soya and MON89788 soya content was carried out by Real time PCR. In the 40-3-2 soya quantification process, the calibration curves were obtained with an average slope of -3.5, which implies a reaction efficiency of 93 % and with an R^2 of 0.99. In the MON89788 soya, the slope values were -3.6; the efficiency, 89 % and the R^2, 0.99. The efficiency was 100 % with a slope of -3.3 and an R2 of 1 in the endogenous gene curves in the two quantification processes, so the quantification processes' compliance with the requirements demanded in the methods validated by the EURL-GMFF can be assured. The 40-3-2 soya content was 0.49 % in the soya meat sample, 0.09 % in a soya yoghurt sample and less than the limit of quantification (LOQ 0.05 %) in a soya biscuit sample. The soya yoghurt sample simultaneously contained <0.1 % of MON89788 soya (Table 8).

Table 8. Results for the presence of GM soya in the samples. 40-3-2 soya (LOQ: 0.05 %). MON89788 soya (LOQ: 0.1 %)

Event	Soya biscuit	Soya yogurt	Soya meat
40-3-2 soya	< 0.05 %	0.09 %	0.49 % ± 0.16 %
MON89788 soya	ND*	< 0.1 %	ND

*ND: Not detected.

The study carried out shows that GM soya was detected in 25 % of the samples analysed. The results have shown that no type of infringement has been revealed since in no case were the levels of genetically modified soya found to be above the legal limit of 0.9 % Additionally, GM soya has not been detected in the samples labelled as "produce of organic farming" and, however, it has to be pointed out that one of the positive samples contained the writing “GMO free”.

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