Food Biotechnology
Publication details, including instructions for authors and subscription information:
http://www.informaworld.com/smpp/title~content=t713597251

Effect of Ionizing Radiation on the Quantification of Genetically Modified Foods

Anthimia M. Batrinou [a]; Dora Koraki [b]; Vassilia J. Sinanoglou [a]; Amalia D. Karagouni [b]; Kostas Sflomos [a]; Vassiliki Pletsa [c]
[a] Food Technology Department, Technological Educational Institution of Athens, Egaleo, Greece [b] Faculty of Biology, Department of Botany, Group of Microbiology, National and Kapodistrian University of Athens, [c] Institute of Biological Research and Biotechnology, National Hellenic Research Foundation, Athens, Greece

Online Publication Date: 01 October 2008

To cite this Article Batrinou, Anthimia M., Koraki, Dora, Sinanoglou, Vassilia J., Karagouni, Amalia D., Sflomos, Kostas and Pletsa, Vassiliki(2008)'Effect of Ionizing Radiation on the Quantification of Genetically Modified Foods',Food Biotechnology,22:4,338 — 351
To link to this Article: DOI: 10.1080/08905430802458438
URL: http://dx.doi.org/10.1080/08905430802458438

Food Biotechnology, 22:338–351, 2008

ISSN: 0890-5436 print
DOI: 10.1080/08905430802458438

Effect of Ionizing Radiation on the Quantification of Genetically Modified Foods

Anthimia M. Batrinou[1], Dora Koraki[2], Vassilia J. Sinanoglou[1], Amalia D. Karagouni[2], Kostas Sflomos[1], and Vassiliki Pletsa[3]

[1]Food Technology Department, Technological Educational Institution of Athens, Egaleo, Greece
[2]Faculty of Biology, Department of Botany, Group of Microbiology, National and Kapodistrian University of Athens
[3]Institute of Biological Research and Biotechnology, National Hellenic Research Foundation, Athens, Greece

The rapid spread of Genetically Modified (GM) crops globally and the mandatory labeling of GM food and feed imposed by many countries has led to the development of relevant detection techniques. Polymerase Chain Reaction (PCR) – based methods are presently the most effective and reliable for GM detection even in processed food products. This study evaluated the effect of electron beam irradiation, used for food pasteurization, on the detection and quantification of dry GM soyabean and maize products; qualitative and quantitative (real-time TaqMan^{TM} probe) PCR analysis showed that electron beam irradiation treatment, even at a high dose (10 kGy), did not affect the traceability of transgenes.

Key Words: detection of genetically modified organisms; electron beam irradiation; real-time PCR

INTRODUCTION

Genetically modified (GM), or transgenic crops, have been continuously cultivated globally for over a decade, and hundreds of GM plants have been approved for commercialization worldwide. The global area dedicated to GM crops increased sixtyfold from 1996 to 2006, with an increasing proportion located in developing countries. The dominant genetically modified crop is herbicide-tolerant soybean that accounts for more than half (57%) of total

Address correspondence to Anthimia M. Batrinou, Biotechnology Laboratory, Department of Food Technology, Technological Educational Institution of Athens, Agiou Spyridonos St., 12210 Egaleo, Athens, Greece; Tel.: 30-210-5385175; Fax: 30-210-5385176; E-mail: bithrini@ath.forthnet.gr.

production of soybean worldwide (James, 2006). A large percentage of processed foods contain ingredients from GM plants such as flour, oil, lecithin, and protein. It is estimated that approximately 85% of processed foods in the United States contain genetically modified ingredients (NCBE, 2003; Chassy, 2002).

As more GM foods are introduced into the market, issues such as food safety, environmental risks, and ethical concerns are being discussed, and there are more than 40 countries and areas that have issued GM food labeling regulations to address them. The European Union (EU) has issued strict regulations, since all recent surveys indicate that the presence of GM ingredients in food products is of considerable concern to the majority of the consumers in the EU countries (Bonny 2003; Gaskell et al., 2003). Current EU legislation (EC Regulations 1829/2003 and EC 1830/2003) has set the labeling of all authorized GM food and feed mandatory and has introduced a labeling threshold of 0.9% (Europa, 2007a; GMO-Compass, 2007). Furthermore, tests are imposed on imported foods to check whether they contain genetically modified material not evaluated and authorized in line with the EU laws such as the Liberty Link Rice 601 (Agbios, 2007).

Consumer concern over the safety of GM foodstuffs and changes in food-labeling legislation have necessitated the development and application of reliable and sensitive analytical methods for the detection of GM ingredients not only in raw materials but also in processed food products. The most preferred detection and quantification method due to its high sensitivity, high specificity, and rapidity is the DNA-based Polymerase Chain Reaction (PCR) that directly detects the transgenic DNA present in a food sample (Cankar et al., 2006; Miraglia et al., 2004; Lipp et al., 1999; Vaitilingom et al., 1999). Although successful quantification depends crucially on the quality of the sample DNA analyzed, the reliability of this method has been validated even when extreme conditions (e.g., high temperature, acidic pH) that lead to severe DNA degradation are applied to food processing (van den Eede et al., 2004; Lipp et al., 2001; Hupfer et al., 1998).

On the other hand, treatment of food by ionizing radiation such as γ, X-rays, or electron beam is a technological process considered to enhance food safety. Since it can damage and finally fragment DNA, ionizing irradiation can ultimately kill pathogens present in food samples (Mayer-Miebach et al., 2005; Smith and Pillai, 2004), thus contributing to the reduction of the incidence of illness caused by food-borne pathogens. In general, irradiation processing of food can extend shelf-life, minimize food losses, and can be used to eradicate insect pests. It is already applied as an effective safety measure for sterilization in 41 countries for more than 35 types of food products (Europa, 2007b; Black & Cumming, 2000; ICGFI, 1999). However, there is still a debate concerning the application of this technique (Ten Eyck and Deseran, 2001) in food processing due to lack of substantial evidence regarding food

safety upon irradiation. Therefore, many governments have been reluctant to authorize it (Ashley et al., 2004). The European Commission's Scientific Committee on Food (EC, 2003) has determined that food irradiated below 10 kGy is safe and that, according to the Codex Alimentarius Commission (CAC, 2003), the maximum dose delivered should not exceed 10 kGy except when necessary to achieve a legitimate technological purpose.

To evaluate the effect of electron beam irradiation on the detection of GM food ingredients by using DNA-based Polymerase Chain Reaction methods, food products containing genetically modified ingredients (glyphosate-resistant soybeans: Roundup Ready® and transgenic maize expressing *Bacillus thuringiensis* genes: Bt-176 event) were treated with medium to high doses of electron beam irradiation (up to 10 kGy). DNA of all treated and untreated samples was subjected to qualitative PCR analysis. Subsequently, samples containing Roundup Ready® soybean ingredients were further analyzed by quantitative Real-Time PCR-analysis (TaqMan probe).

MATERIALS AND METHODS

Samples

Samples tested were processed food products commercially available in the Greek market containing Roundup Ready® (RR) soya or Bt-176 maize, as shown in Table 1. The % GM content had been determined by real-time PCR (TaqMan probe) analysis before the onset of this study.

Irradiation

The samples were vacuum-packaged in low-density polyethylene (LDPE) bags (< 0.5 cm thickness). Irradiation was performed with electrons using a linear accelerator of the Institute of Process Engineering, Federal Research Center for Nutrition and Food, Karlsruhe, Germany (LINAC-CIRCLE III, Linac Technologies S.A.; Orsay, France, 10MeV, 10kW); instantaneous dose rate was 10^4 kGy/s. Dosimetry was performed using the dosimeter system alanin/ESR. The doses applied were 4 and 10 kGy. All samples were irradiated in triplicate.

DNA Extraction

For qualitative PCR analysis, DNA was extracted according to the GeneScan standard protocol (GMOScreen, Advanced Basic Screening System of GeneScan, Cat. No 5221102210, www.genescan.com, Berlin, Gemany). For quantitative PCR, the DNA extraction was performed with the CTAB method (Tinker et al., 1993). For each food sample (irradiated or control), two independent DNA extractions were performed in parallel with an additional

Table 1: Detection of the CaMV 35S promoter and NOS terminator sequences in nonirradiated and irradiated genetically modified food samples. The presence or absence of either the 35S or NOS amplicons in DNA electrophoresis is marked with +/–.

	Food samples	% GM (validated by Real-Time PCR TaqMan™ probe)	Type of genetic modification	Non-irradiated 0 kGy		Irradiated 4 kGy		Irradiated 10 kGy	
				35S	NOS	35S	NOS	35S	NOS
1	Soybean flour (S1)	90% ± 10%	Roundup Ready® (RR) soybean	+	+	+	+	+	+
2	Soybean flour (S2)	70% ± 10%	RR soybean	+	+	+	+	+	+
3	Soybean flour (S3)	60% ± 10%	RR soybean	+	+	+	+	+	+
4	Soybean flour (S4)	0.7% ± 0.3%	RR soybean	+	+	+	+	+	+
5	Dry cake mix (S5)	0.2% ± 0.02%	RR soybean	+	+	+	+	+	+
6	Soybean sausage	0.9% ± 0.1%	RR soyabean	+	+	+	+	+	+
7	Fish meal	12% ± 2%	mixture of maize Bt-176 and RR soya	+	+	+	+	+	+
8	Soybean flour	0	Negative control	–	–	–	–	–	–
9	Corn flour	0.3% ± 0.2%	maize Bt-176	+	*	+	*	+	*
10	Corn flour	0	Negative control	–	–	–	–	–	–

*The NOS sequence is not present in genetically modified maize Bt-176, therefore it was not detected.

extraction control for each analysis (complete DNA extraction and PCR without sample material) to detect contamination of reagents or equipment with amplicons. The amount and the quality of extracted DNA was evaluated by DNA electrophoresis on 1.5% agarose gels stained with 0.5 μg/mL ethidium bromide and/or by measuring optical density at 260nm.

Qualitative PCR Analysis

For each DNA extraction, a control PCR reaction was first performed to test whether the DNA extracted was of sufficient quality and quantity for PCR amplification. The control PCR reaction amplified a specific sequence of 199 bp from the chloroplast gene present both in conventional and in genetically modified plant DNA (GMOScreen, Advanced Basic Screening System of GeneScan, Berlin, Germany). After ensuring that the control reaction was positive, specific PCR reactions were performed with the appropriate pairs of primers to detect (amplify) the specific sequences of the CaMV 35S promoter and NOS terminator. These two sequences are the most frequently used regulatory sequences in genetically modified plants and the detection of one or both indicates the presence of a genetic modification. Specific PCR amplifications of the CaMV 35S promoter and NOS terminator sequence were performed by using the GeneScan pair of primers amplifying a 123 bp fragment for both CaMV 35S and NOS sequence according to the GeneScan standard protocol. Since the successful application of PCR also relies on the length of the targeted DNA sequence to be amplified, an additional pair of primers was in parallel used especially for the CaMV 35S promoter sequence. These primers (35S1 and 35S2) are part of a standard EU method for the identification of genetically modified organisms in food (Lipp et al., 1999). They amplify a 195 bp fragment of the CaMV 35S promoter and have the following sequence: 35S1: *5′-GCTCCTACAAATGCCATCA-3′,* 35S2: *5′-GATAGTGGGATTGTGCGTCA-3′.*

In any case, the PCR reactions were performed in a total volume of 25–50 μL. The reaction mixture contained 25 ng DNA/reaction, 1.5 mM $MgCl_2$, 2 μg/mL BSA, 0.1 mM of each deoxynucleotide triphosphate (dNTP), 0.5 μM of each primer, 0.5 units/reaction Platinum® *Taq* DNA polymerase (Invitrogen, Paisley, UK). Reactions were thermally cycled in a Peltier Thermal Cycler, PTC-200 DNA Engine™ (MJ Research INC, Waltham, Mass., USA) as follows: a) initial denaturation at 94°C for 10 min, b) denaturation at 94°C, for 1 min, annealing at 62°C (or 54°C for specific 35S1/35S2 reaction only) for 1.5 min, extension at 72 °C for 2 min (50 cycles), c) final elongation at 72°C for 5 min, (1 cycle), and d) cooling at 4°C. Therefore, for every DNA extraction three individual PCR reactions (35S and NOS by GeneScan and 35S by EU method) were performed in duplicate together with the appropriate positive and negative control samples. Amplification products were run by electrophoresis on 2% agarose gels, stained with ethidium bromide and photographed under UV.

Quantitative (Real-Time PCR) PCR Analysis

For quantitative PCR, the TaqMan™ technique was used (Holland et al., 1991). Real-Time PCR reactions were run with ABI Prism 5700 sequence detection system (PE Applied Biosystems, Foster City, Calif., USA) with the following program: 10 min at 95°C, 15 s at 95°C, and 1 min at 60°C (45 cycles). Amplification reactions (25 μL) were performed with GeneScan Roundup Ready™ Soy DNA Quantification System (5700) reagents (Bremen, Germany). They contained 20 μL of Master Mix and 200ng DNA of samples. For the generation of standard curves 5 μL of the appropriate soya reference DNA standard and Roundup Ready reference DNA standard solution were included in the amplification reactions.

Statistical Analysis

Statistical analysis of the quantitative data was performed with the statistical program SPSS 13 (SYSTAT Inc.). Values were expressed as means and standard deviation of nine results. The significance of the difference between the RR content of irradiated and nonirradiated soy-processed food was evaluated by independent samples T-test ($p < 0.05$).

RESULTS

Qualitative PCR analysis

All samples, irradiated or not, were analyzed by qualitative PCR with three different sets of primers (35S and NOS-GeneScan kit, 35S1/35S2) as mentioned above. The results are summarized in Table 1. In all samples containing genetically modified material the CaMV 35S promoter and the NOS terminator sequence were effectively amplified irrespectively of the irradiation dose applied (0, 4 and 10 kGy). Representative results of the qualitative PCR analysis using the GeneScan kit are shown in Figures 1 and 2. Figure 1 shows the qualitative PCR analysis with GeneScan 35S primers of cake mix which contained the lowest content in GM ingredients (0.2% ± 0.02%) and was one of the most processed samples tested. Irradiation dose did not affect the detection of the 123 bp DNA band (lanes 1–6) indicating that this sample contained the sequence of the CaMV 35S promoter present in most GM plants. Detection of the CaMV promoter sequence was also carried out by using the 35S1/S2 pair of primers (EU method). In this case the amplicon indicating the presence of the CaMV 35S promoter sequence was longer (195 bp), and therefore theoretically more susceptible to degradation under stress conditions such as ionizing irradiation. However, the 35S sequence was again detected irrespectively of the irradiation dose applied (data not shown). Figure 2 shows the qualitative PCR analysis with GeneScan NOS primers of several samples irradiated at 10 kGy.

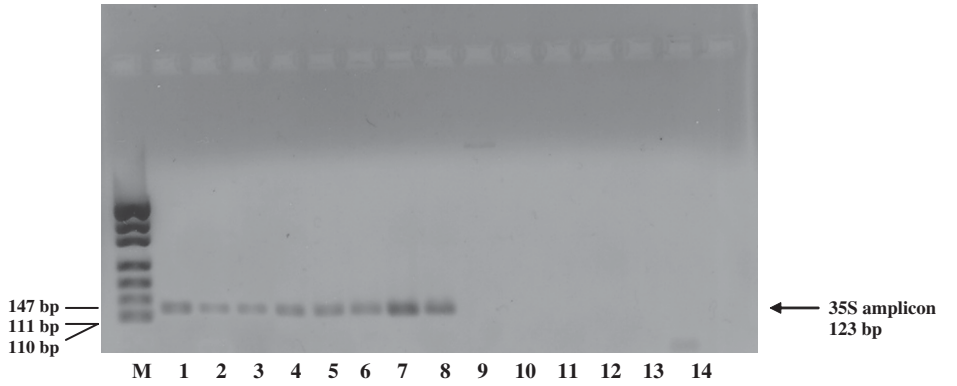

Figure 1: PCR qualitative analysis of Dry cake mix (sample S5 with 0.2% GM content) performed with the GeneScan kit 35S pair of primers, length of amplified DNA sequence is 123 bp. M = molecular weight marker (pUC19/MspI), 1–2 = dry cake mix 0 kGy (0.2% GM content), 3–4 = dry cake mix 4 kGy, 5–6 = dry cake mix 10 kGy, 7–8 = RR soybean (positive control), 9–10 = conventional soybean (negative control), 11–12 = control extraction, 13–14 = ddH_20.

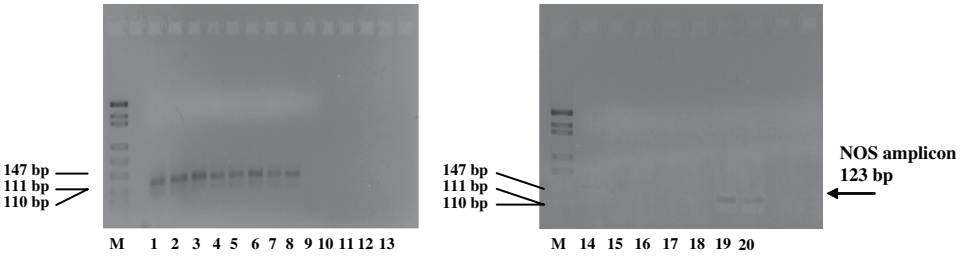

Figure 2: PCR qualitative analysis of samples irradiated at high dose (10 kGy) with the GeneScan kit NOS pair of primers, length of amplified DNA sequence is 123 bp. M = molecular weight marker, (pUC19/MspI), 1–2 = soybean flour (sample S1, 90% GM content), 3–4 = fish meal (12% GM content), 5–6 = soybean sausage (0.9% GM content), 7–8 = dry cake mix (sample S5, 0.2%, GM content) 9–10 = corn flour nongenetically modified, (negative control), 11–12 = corn flour (Bt-176, 0.3% GM content), 13–14 = control extraction, 15–16 = ddH_2O, 17–18 = conventional soya (negative control), 19–20 = RR soybean (positive control).

Quantitative PCR Analysis

The real-time PCR method for GM quantification relies on the amplification of transgenic DNA sequences and their quantification by comparison to an amplified reference sequence. Analysis was carried out using the ABI Prism 5700 sequence detection system (TaqMan™ technique). Selected samples containing Roundup Ready® (RR) Soybean DNA, irradiated at the high dose (10 kGy), were analysed with the double standard curve method. For relative quantification of the RR Soybean DNA sequence (glyphosate-resistant soya line GTS 40–3–2, Monsanto Inc.) two separate standard curves were generated, each with a different set of primers/probe: a) one curve was constructed with known quantities of conventional soya DNA (in which case the lectin

gene found in all soybean plant cells was used as reference gene) and b) one with known quantities of RR-soya DNA. The parameter calculated was the threshold-cycle (Ct) where each reaction trespasses a certain fluorescence level and is inversely correlated to the initial concentration of the specific target DNA sequence. To construct the calibration curves, triplicate of four dilution steps of the calibration standard DNA solutions (STND1 to STND2) were included in the analysis with both reaction systems. The double standard curve method yielded two equations of the regression line $Ct = f$ (initial concentration of the specific target DNA), the first was used to calculate the amount of soybean DNA of the samples ($Y = -1.5990\text{Ln}(x) + 41.063$, $R^2 = 0.9938$) and the second was used to calculate the amount of Roundup Ready® soybean ($Y = -1.4679\text{Ln}(x) + 38.987$, $R^2 = 0.9963$). The correlation coefficient was within acceptable values (values of correlation coefficient R^2 should be ≥0.99).

As already mentioned, samples possessing a wide range of % RR content (ranging from 0.2–90%, Table 1) were analyzed before and after treatment in order to evaluate the effect of ionizing irradiation on the percentage of genetically modified material. Results of the quantitative analysis are summarized in Table 2 and Figure 3. As shown in Figure 3, concerning the nonirradiated samples, the results summarized in Table 2 were in full agreement with those

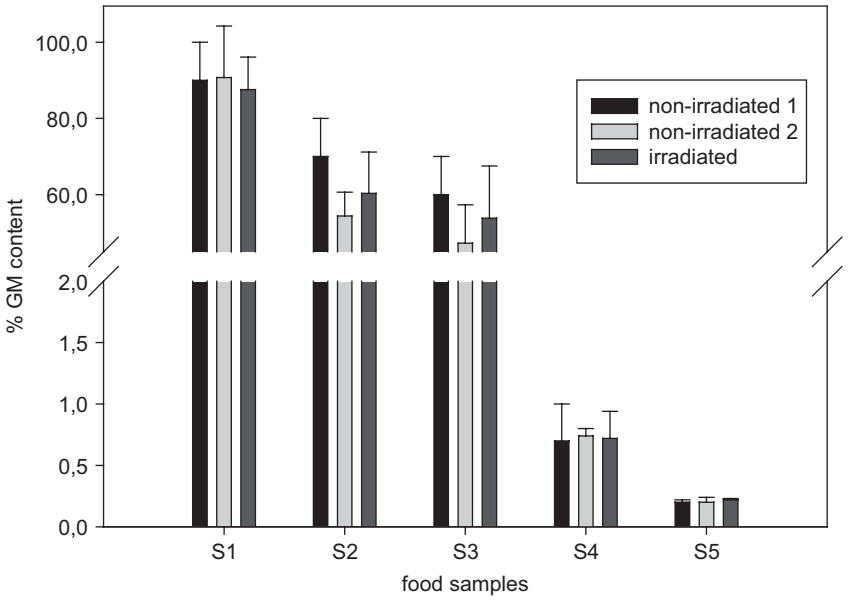

Figure 3: GM quantification (% GM content) of food samples before and after electron beam irradiation (10 kGy); S1 = soybean flour GM content 90 ± 10%, S2 = soybean flour 70 ± 10%, S3 = soybean flour 60 ± 10%, S4 = soybean flour 0.7 ± 0.3%, S5 = dry cake mix 0.2 ± 0.02%; **nonirradiated 1** represents values determined before the onset of the study, **nonirradiated 2** represents values presented in Table 2 corresponding to 0 kGy, **irradiated** represents values presented in Table 2 corresponding to 10 kGy.

Table 2: Results[a] of quantitative real-time PCR in nonirradiated and irradiated food samples containing RR soybean. Qty Lec = quantity (gene copy number) of the lectin gene that corresponds to the amount of total soybean DNA, Qty RR = quantity (gene copy number) of the RR DNA that corresponds to the amount of genetically modified soybean, %QtyRR/QtyLec = % of RR soya DNA in relation to the amount of total soybean DNA in a sample that corresponds to the % GM content of the sample.

Samples (with predetermined % RR w/w)	Irradiation dose	Ct values Lec	Qty Lec (copies of the Lectin gene)	Ct values RR	Qty RR (copies of the RR gene)	% Average[b] Qty RR/Qty Lec	SD
S1 (90 ± 10%)	0 kGy	25.86	13462	25.19	12107	90.72	13.52
	10 kGy	24.91	24323	24.37	21356	87.55	8.54
S2 (70 ± 10%)	0 kGy	29.07	1806	28.88	986	54.41	6.24
	10 kGy	28.79	2172	28.48	1300	60.33	10.86
S3 (60 ± 15%)	0 kGy	30.32	833	30.25	388	47.26	10.06
	10 kGy	30.73	659	30.45	341	53.81	13.70
S4 (0.7 ± 0.3%)	0 kGy	22.93	84375	29.55	622	0.74	0.06
	10 kGy	23.17	80534	29.88	516	0.72	0.22
S5 (0.2 ± 0.02%)	0 kGy	27.39	5172	35.57	10	0.20	0.04
	10 kGy	26.66	8159	34.72	18	0.22	0.01

[a]Values are the average of nine reactions (samples were irradiated in triplicate and each irradiated sample was analyzed by three PCR reactions).
[b]Means within the same sample for different doses are not statistically different ($p > 0.05$)

obtained from previous analysis. All samples of irradiated soybean flour at the high dose (10 kGy) yielded the same % amount of RR DNA as the nonirradiated samples (differences were not found to be statistically significant at $p < 0.05$). Therefore, irradiation did not seem to affect the quantification of genetically modified DNA in the samples tested. Note that irradiation at a high dose did not seem to affect the GM quantification even in low GM content and highly processed samples (S4 and S5).

DISCUSSION

In order to execute the labeling requirements imposed by many countries worldwide, qualitative and quantitative polymerase-chain-reaction-based methods are commonly applied to detect genetically modified organisms in food and feed. Real-time PCR is the most effective and important method for GM quantification since it can detect very low amounts of specific DNA sequences that have been inserted into a plant's naturally occurring DNA. The method is highly sensitive and reliable, but successful quantification depends crucially on the quality of DNA template. Food processing (physical and/or chemical treatment) is known to have an effect on the amount and size of the DNA extracted, which could lead to the reduction of the sensitivity of the method (Hurst et al., 1999; Spiegelhalter et al., 2001). The appropriate length of the targeted DNA sequence is a crucial parameter for successful PCR amplification, with the shortest sequences having more probabilities of remaining intact after processing (Moreano et al., 2005; Hupfer et al., 1998). Another critical factor is the DNA extraction method. It should ensure high yield and quality of the DNA obtained, so it must be carefully selected since even components of DNA extraction solutions can influence PCR reactions (Cankar et al., 2006).

This study is an attempt to evaluate the effect of ionizing radiation (electron beam) on GM detection in dry products by applying PCR-based methods. Irradiation is one of the processing technologies used for food safety that employs controlled amounts of ionizing radiation to destroy bacteria, pathogens, and pests in food and agricultural products. Ionizing radiation is an effective DNA-damaging agent, producing a range of lesions in cellular DNA, among them single-strand breaks or double-strand breaks leading to cell death. It is estimated that ~40 double-strand breaks are induced per 1 Gy in a typical cell (Prise et al., 2005). The present study showed that electron beam irradiation, used for food sterilization at doses 4–10 kGy, although expected to fragment DNA, does not fragment it to such a degree that target sequence detection is affected.

During qualitative analysis, in an effort to test the amplicon length effect, two pairs of primers were used in parallel for the CaMV 35S promoter sequence, the first amplifying a 123 bp sequence (GeneScan kit) and the second

a 195 bp sequence (35S1/35S2). In both cases there was no irradiation effect on the detection, possibly because both amplicons were of short length (< 200 bp). Plant cells may have relatively large genome sizes with considerable differences within and between plant species. Maize (*Zea mays*) has a large genome of 2,500 million bp (about the same size as the human genome, Chandler and Brendler, 2002) and soybean (*Glycine max*) a genome of 1,100 million bp (Lin et al., 2005). Furthermore some plants may be tetraploid or polyploid (having four or more sets of chromosomes), thus containing the same genes in more than two copies. An event-specific target sequence amplified by PCR that has a length between 100–200 base pairs is a very small fragment ($1/10^7$) in such a large genome pool and the possibility that it is fragmented by the processing method is unlikely. Briefly, through qualitative analysis 35S and NOS sequences were reliably detected in all irradiated GM samples tested. These results are in agreement with a previous study having shown that a low dose of gamma irradiation (up to 1 kGy) applied in genetically modified soybeans (whole seeds) does not affect the detection of the genetic modification with qualitative PCR (Villavicencio et al., 2004).

Quantitative analysis with Real-Time PCR (TaqMan™ probe) of the samples containing Roundup Ready® (RR) Soybean DNA and irradiated at the high dose (10 kGy) revealed that the quantification of the transgene detected was not affected by electron beam irradiation (Fig. 3). The DNA isolation method used for quantitative analysis was the CTAB method (Tinker et al., 1993) which is considered among the most appropriate ones for accurate gene quantification (Cankar et al., 2006). As shown in Table 2, even highly processed commercial samples such as the dry cake mix (sample S5) with a very low GM content (~0.2%), well below the EU threshold level, were reliably quantified following irradiation. All samples of dry cake mix yielded quantities within the range of $0.20 \pm 0.02\%$ verifying the reliability of this quantification system.

In conclusion, the application of reliable techniques to detect and quantify genetic modifications in food and feed is an important issue since labeling is mandatory in more than 40 countries worldwide. Therefore, it is important to know that the sensitivity of the most reliable tool to detect and quantify genetically modified organisms in dry food samples (DNA-based PCR methods) is not affected by controversial food technologies such as irradiation even in highly processed, low GM content food samples.

ACKNOWLEDGMENTS

This study was co-funded by the European Social Fund and National Resources- (EPEAEK II) ARCHIMEDES. We would like to thank Dr. M.R. Stahl (Federal Research Center for Nutrition and Food, Karlsruhe, Germany) and Dr. K. Riganakos (University of Ioannina, Greece) for their valuable help in irradiating the samples.

REFERENCES

Agbios (2007). EU votes to test U.S. rice *Agbios News*. Retrieved from http://www.agbios.com/static/news/NEWSID_7930.php, accessed February 1, 2007.

Ashley, B.C., Birchfield, P.T., Chamberlain, B.V., Kotwal, R.S., McClellan, S.F., Moynihan, S., Patni, S.B., Salmon, S.A., Au, W.W. (2004). Health concerns regarding consumption of irradiated food. *International Journal of Hygiene and Environmental Health* 207:493–504.

Black, G., Cumming, R. (2000). Irradiation, In: Food shelf life stability: chemical, biochemical and microbiological changes. N.A.M. Eskin and D.S. Robinson, eds. Boca Raton, FL: CRC Press, pp. 87–128.

Bonny, S. (2003). Why are most Europeans opposed to GMOs? Factors explaining rejection in France and Europe. *Electronic Journal of Biotechnology* 6(1). Retrieved from http://www.ejbiotechnology.info/content/vol6/issue1/full/4/, accessed October 15, 2006.

CAC. (2003). Revised codex general standard for irradiated foods. CODEX STAN 106–1983, Rev. 1–2003. Rome, Italy: Codex Alimentarius Commission, Retrieved from http://www.fao.org/english/newsroom/news/2003/20363-en.html, accessed October 15, 2006.

Cankar, K., Stebih, D., Dreo, T., Zel, J., Gruden, K. (2006). Critical points of DNA quantification by real-time PCR-effects of DNA extraction method and sample matrix on quantification of genetically modified organisms. *BMC Biotechnology* 6:37–52.

Chandler, V.L., Brendel, V. (2002). The maize genome sequencing project. *Plant Physiology* 130:1594–1597.

Chassy, B.M. (2002). Food safety evaluation of crops produced through biotechnology. *Journal of the American College of Nutrition* 21(3):166S–173S.

European Commission. (2003). Revision of the opinion of the Scientific Committee on Food on the irradiation of food. Brusells: European Commission. Retrieved from http://europa.eu.int/comm/food/fs/sc/scf/out193_en.pdf, accessed October 15, 2006.

Europa. (2007a). http://europa.eu.int/comm/food/index_en.html

Europa. (2007b). Annual report from the Commission on Food Irradiation for 2004. Retrieved from http://eur-Elex.europa.eu/LexUriServ/site/en/oj/2006/c_230/c_23020060923en00280045.pdf, accessed February 5, 2007.

Gaskell, G., Allum, N., Stares, S. (2003, March). Europeans and biotechnology in 2002, Eurobarometer 58.0. *A report to the EC Directorate General for Research from the project "Life Sciences in European Society," QLG7-CT-1999-00286*.

GMO-Compass. (2007). *GMO Labeling Guidelines*. Retrieved from http://www.gmo-compass.org/eng/regulation/labelling/89.gmo_labelling_guidelines_threshold.html, accessed February 5, 2007.

Holland, P.M., Abramson, R.D., Watson, R., Gelfand, D.H. (1991). Detection of specific polymerase chain reaction product by utilizing the 5'-3' exonuclease activity of *Thermus aquaticus* DNA polymerase. *Proceedings of the National Academy of Sciences USA*. 88:7276–7280.

Hupfer, C., Hotzel, H., Sachse, K., Engel, K.H. (1998). Detection of the genetic modification in heat-treated products of Bt maize by polymerase chain reaction. *European Food Research and Technology* 206:203–207.

Hurst, C.D., Knight, A., Bruce, I. (1999). PCR detection of genetically modified soya and maize in foodstuffs. *Molecular Breeding* 5:579–586.

ICGFI. (1999). Facts about food irradiation, international consultative group on food irradiation (ICGFI). Retrieved from http://www.iaea.org/icgfi/, accessed October 15, 2006.

James, C. (2006). Preview: Global status of commercialized biotech/GM crops: 2006. ISAAA Briefs No.35. ISAAA: Ithaca, NY. Retrieved from http://www.isaaa.org, accessed February 5, 2007.

Lin, J.Y., Jacobus, B.H., SanMiguel, P., Walling, J.G., Yuan, Y., Shoemaker, R.C., Young, N.D., Jackson, S.A. (2005). Pericentromeric regions of soybean (*Glycine max* L. Merr.) chromosomes consist of retro-elements and tandemly repeated DNA and are structurally and evolutionary labile. *Genetics* 170:1221–1230.

Lipp, M., Bluth, A., Eyquem, F., Kruse, L., Schimmel, H., Van den Eede, G., Anklam, E. (2001). Validation of a method based on polymerase chain reaction for the detection of genetically modified organisms in various processed foodstuffs. *European Food Research and Technology* 212:497–504.

Lipp, M., Brodmann, P., Pietsch, K., Pauwels, J., Anklam, E. (1999). IUPAC collaborative trial study of a method soy beans and maize in dried powers. *Journal of AOAC International* 82: 923–928.

Mayer-Miebach, E., Stahl, M.R., Eschrig, U., Deniaud, L., Ehlermann, D.A.E., Schuchmann, H.P. (2005). Inactivation of non-pathogenic strain of *E.coli* by ionizing radiation. *Food Control* 16:701–705.

Miraglia, M., Berdal, K.G., Brera, C., Corbisier, P., Holst-Jensen, A., Kok, E.J., Marvin, H.J.P., Schimmel, H., Rentsch, J., van Rie, J.P.P.F., Jagon, J. (2004). Detection and traceability of genetically modified organisms in the food production chain. *Food and Chemical Toxicology* 42(7):1157–1180.

Moreano, F., Busch, U., Engel, K.H. (2005). Distortion of genetically modified organism quantification in processed foods: influence of particle size compositions and heat-induced DNA degradation. *Journal of Agricultural and Food Chemistry* 53:9971–9979.

National Center for Biotechnology Education. (2003). Soya products. Available at: http://www.ncbe.reading.ac.uk/NCBE/GMFOOD/soya.html.

Prise, K.M., Schettino, G., Folkard, M., Held, K.D. (2005). New insights on cell death from radiation exposure. *The Lancet Oncology* 6:520–528.

Smith, J.S., Pillai, S. (2004). Irradiation and food safety. *Food Technology* 58:48–55.

Spiegelhalter, F., Lauter, F.R., Russel, J.M. (2001). Detection of genetically modified food products in a commercial laboratory. *Journal of Food Science* 66:634–640.

Ten Eyck, T.A., Deseran, F.A. (2001). In the words of experts: The interpretive process of the food irradiation debate. *International Journal of Food Science and Technology* 36:821–831.

Tinker, N.A., Fortin, M.G., Mather, D.E. (1993). Random amplified polymorphic DNA and pedigree relationship in spring barley. *Therapeutic Applied Genetics* 85:976–984.

Vaitilingom, M., Pijnenburg, H., Gendre, F., Brignon, P. (1999). Real-time quantitative PCR detection of genetically modified maximizer maize and roundup ready soybean in some representative foods. *Journal of Agricultural and Food Chemistry* 47:5261–5266.

van den Eede, G., Aarts, H., Buhk, H.-J., Corthier, G., Flint, H.J., Hammes, W., Jacobsen, B., Midtvedt T., van der Vossen, J., von Wright, A., Wackernagel, W., Wilcks, A. (2004). The relevance of gene transfer to the safety of food and feed derived from GM plants. *Food and Chemical Toxicology* 42(7):1127–1156.

Villavicencio, A.L.C.H., Araujo, M.M., Baldasso, J.G., Aquino, S., Konietzny, U., Greiner, R. (2004). Irradiation influence on the detection of genetic-modified soybeans. *Radiation and Physics Chemistry* 71:489–492.