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Radiation Physics and Chemistry 71 (2004) 489–492
Radiation Physics and Chemistry
www.elsevier.com/locate/radphyschem

Irradiation influence on the detection of genetic-modified soybeans

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Abstract

Three soybean varieties were analyzed to evaluate the irradiation influence on the detection of genetic modification. Samples were treated in a ^{60}Co facility at dose levels of 0, 500, 800, and 1000 Gy. The seeds were at first analyzed by Comet Assay as a rapid screening irradiation detection method. Secondly, germination test was performed to detect the viability of irradiated soybeans. Finally, because of its high sensitivity, its specificity and rapidity the polimerase chain reaction was the method applied for genetic modified organism detection. The analysis of DNA by the single technique of microgel electrophoresis of single cells (DNA Comet Assay) showed that DNA damage increased with increasing radiation doses. No negative influence of irradiation on the genetic modification detection was found.

Keywords: Gamma radiation; Food irradiation detection; Soybean; PCR

1. Introduction

The discussion about the use of irradiation treatment is still very emotional as well as genetic engineering and until now a great part of the population in the different countries worldwide does not believe in the safety of those foods. In order to enforce labeling regulations, methods for detecting the irradiation treatment directly in the produce are required, and it is also necessary for the development of reliable and sensitive methods for GMO detection.

Soybeans are an important source of protein in many areas of the world. The extension of shelf-life and improvement of technological qualities are aims of radiation processing of foods (Ahmed, 1993).

As a phytosanitary treatment irradiation is adopted in many countries. For insect disinfestations in beans, irradiation offers an attractive alternative to chemicals (Delincée and Bognár, 1993; Villavicencio et al., 1998). Until now there is little information about the effect of irradiation alterations in the composition of the compounds after irradiation of GMO soy. Since radiation was employed for food disinfestations, half embryo test to identify irradiated foods and viability of seeds are utilized. We propose to check the germination results in roots after irradiation as a parameter to evaluate the effect of ionizing radiation and genetic modifications.

Because of its enormous potential, genetic engineering will have a tremendous implication for the food industry and all of agriculture in the near future. Since most genetically engineered foods will be altered only slightly in composition in comparison to the corresponding conventionally produced food they exhibit no new or

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doi:10.1016/j.radphyschem.2004.05.032

different risks, but to give the consumers the free choice to buy foods produced by genetic engineering or traditionally produced foods, a labeling of the foods derived from genetic engineering is necessary. Pressure from consumer groups and public demand has led several countries to require labeling for the presence of GMOs in foods (Matsuoka et al., 2002). Detection methods for food produced by means of genetic engineering mainly focus on the newly introduced genetic information (rDNA) (Hupfer et al., 1998; Zimmermann et al., 1998; Konietzny and Greiner, 1997; Gachet et al., 1999; Hübner et al., 1998; Pauli et al., 1998; Greiner et al., 1997, 2001a,b, 2004), but also the newly introduced protein or any other difference in composition such as the difference in fatty acid composition of a vegetable oil can be used for detection.

Because of its high sensitivity, high specificity and rapidity, the polymerase chain reaction (PCR) will be the method of choice for this purpose. The PCR is an in vitro method, which is used to amplify enzymatically a certain DNA sequence. The PCR is widely employed in a tremendous variety of situations to produce high yields of specific DNA target sequences.

2. Experimental

2.1. Samples

Two varieties of Argentinean soybean, named ST1 and ST2 and one Brazilian variety, named SC obtained from the local market in São Paulo, Brazil. Samples were packed in polyethylene bags, labeled and identified with its respective irradiation doses.

2.2. Irradiation

Irradiation was performed in Instituto de Pesquisas Energéticas e Nucleares (IPEN—CNEN/SP) at doses levels of 0, 500, 800 and 1000 Gy using a ^{60}Co gamma ray facility(Gammacell 220, A.E.C.L., dose rate: 4.79 kGy/h). Harwell Amber 3042 Dosimeters were used for the measurement of radiation dose.

2.3. Methodology

Samples were analyzed by germination test (Kawamura, 1992) to determine the influence of ionizing radiation in roots elongation after incubation and the DNA Comet Assay (Cerda et al., 1997) was used as an irradiation treatment method. Samples were analyzed by PCR (Koeppel et al., 1997) using primers P35S-F2/PETU-R1 to GMO detection. After electrophoresis (70 V), agarose gel (2%) was stained with 0.5 μg/ml ethidium bromide. The gel was photographed with a Vilber Lourmat Imager System.

3. Results and conclusions

The results of DNA Comet Assay to detect food irradiation show that the distance of DNA migration, "comet length", increases with radiation dose, for all samples (SC, ST1, ST2), as shown in Fig. 1. It is of most importance to be able to distinguish between non-irradiated and irradiated samples. In Fig. 2, it is possible to observe a huge amount of different types of comets in the sample SC non-irradiated, in comparison to Figs. 3 and 4. This fact could be explained because a large storage time or the use of previous chemical treatments.

Analyzing the viability of seeds by germination test, it was observed the differences between non-irradiated and irradiated samples. It was observed that radiation treatment did not influence negatively in root grown. In some cases the highest dose applied even stimulates germination. It was found that Brazilian soybean germinated until 48 h of incubation, after this period there was not a significative grown. Similar results were obtained in Argentinean soybean 1. On the other hand, Argentinean soybean 2 had an increasing germination and root elongation. It was observed that most of seeds of Brazilian and Argentinean soybean 1 after 96 h of incubation became infeasible (Fig. 5).

To identify a food as being derived from genetic engineering by PCR, it has to be shown first, that purity and yield of the extracted DNA are sufficient for PCR. The presence of amplifiable DNA can be determined by performing a PCR using a target sequence always present in the product to be analyzed. Since most of transgenic crops approved for food use contain the 35 S promoter of Cauliflower mosaic virus, this genetic element were used as target sequences for soybean (screening method). It was found that the Argentinean soybean samples when compared to RoundupReady™ (positive control) were similar. On the other hand, Brazilian soybean sample when compared to the positive control did not show any similarity. We could observe

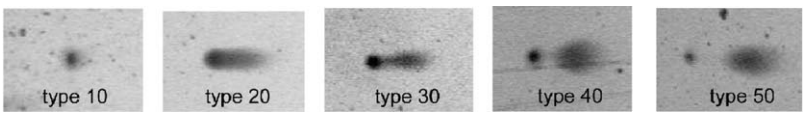

Fig. 1. Photomicrographs of different comet types after irradiation processing.

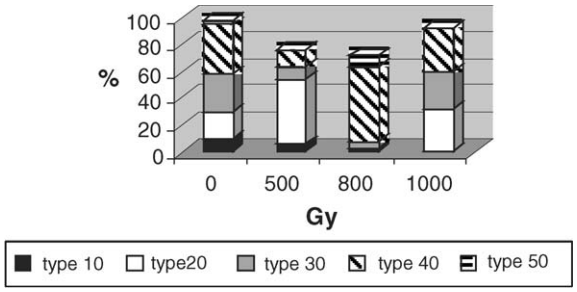

Fig. 2. Sample SC—percentage of comets types after irradiation treatment.

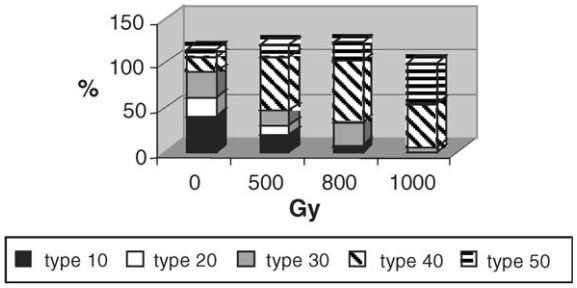

Fig. 3. Sample ST1—percentage of comets types after irradiation treatment.

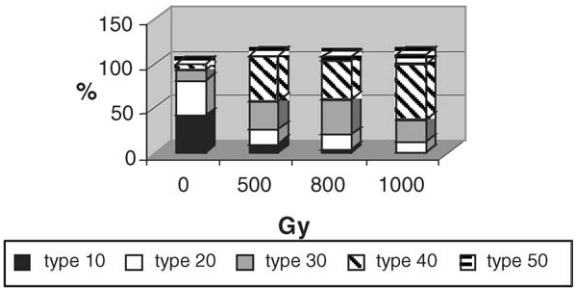

Fig. 4. Sample ST2—percentage of comets types after irradiation treatment.

that after the different radiation processing doses, we can detect also transgenic varieties (Fig. 6).

In conclusion, the DNA Comet Assay and Germination test showed satisfactory results as a rapid screening test for qualitative detection of irradiation treatment of foods. Irradiation treatment does not affect the GMO detection. PCR is very sensitive, a single genetically modified soybean maybe found among one million traditional ones.

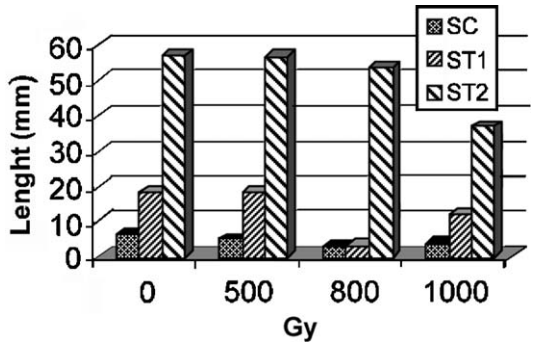

Fig. 5. Effect of gamma radiation on the root growth, 96 h.

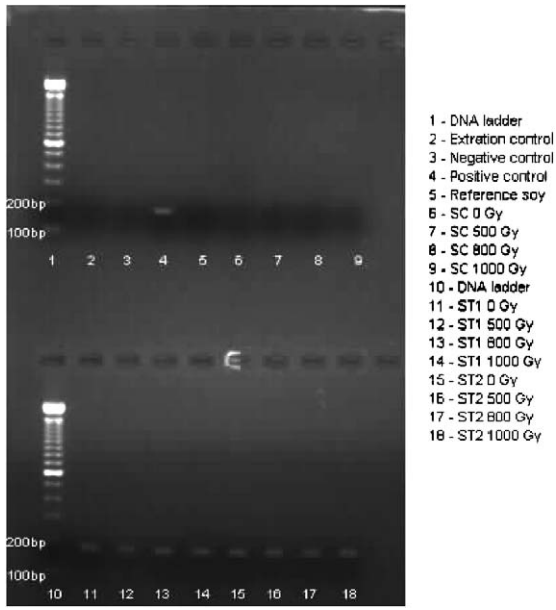

Fig. 6. Agarose gel electrophoresis analysis of irradiated soybeans amplified by PCR with P35S- F2/PETU.

Acknowledgements

We thanks to CNPq and FAPESP for financial support.

References

Ahmed, M., 1993. Up-to-date status of food irradiation. Radiat. Phys. Chem. 42 (1–3), 245–251.

Cerda, H., Delincée, H., Haine, H., Rupp, H., 1997. The DNA "comet assay" as a rapid screening technique to control irradiated food. Mutat. Res. 375, 167–181.

Delincée, H., Bognár, A., 1993. Effect of ionizing radiation on the nutritional value of legumes. In: Bioavailability'93. Nutritional, chemical and food processing implications of nutrient availability Karlsruhe. Proceedings, Karlsruhe, BFE, part. 2, pp. 367–371 (Berichte der Bundesforschungsanstalt für Ernärung BFE-R-93-01).

Gachet, E., Martin, G.G., Vigneau, F., Meyer, G., 1999. Detection of genetically modified organisms by PCR: a brief review of methodologies available. Trends Food Sci. Technol. 9, 380–388.

Greiner, R., Konietzny, U., Jany, K.-D., 1997. Is there any possibility of detecting the use of genetic engineering in processed foods? Z. Ernährungswiss. 36, 155–160.

Greiner, R., Konietzny, U., Villavicencio, A.L.C.H., 2001a. Detection of pathogenic micro-organism in food samples by Polymerase-Chain-Reaction (PCR). Poster at the XVII Congresso brasileiro de ciência e tecnologia de alimentos, Fortaleza.

Greiner, R., Konietzny, U., Villavicencio, A.L.C.H., 2001b. Detection of food produced by means of genetic engineering, Poster at the XVII Congresso brasileiro de ciência e tecnologia de alimentos, Fortaleza.

Greiner, R., Konietzny, U., Villavicencio, A.L.C.H., 2004. Qualitative and quantitative detection of genetically maize and soy in processed foods sold commercially in Brazil by PCR-based methods. Food Control, doi: 10.1016/j.foodcont.2004.06.015.

Hübner, P., Rhyner, C., Lüthy, J., Hübner, P., 1998. Quantitaive competitive PCR for the detection of genetically modified soybean and maize. Z. Lebensm. Unters. Forsch. A 207, 207–213.

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. Z. Lebensm. Unters. Forsch. A 206, 203–207.

Kawamura, Y., 1992. Germination test for identification of gamma-irradiated wheat. Radiat. Phys. Chem. 39 (2), 203–207.

Koeppel, E., Stadler, M., LÜthy, J., Hübner, P., 1997. Sensitive Nachweismethode für die gentechnisch veränderte sojabohne "Roundup readyTM". Mitt. Gebiete Lebensm. Hyg. 88, 164.

Konietzny, U., Greiner, R., 1997. Model systems for developing detection methods for foods derived from genetic engineering. J. Food Compos. Anal. 10, 28–35.

Matsuoka, T., Kuribara, H., Takubo, K., Akiyama, H., Miura, H., Goda, Y., Kusakabe, Y., Isshiki, K., Toyoda, M., Hino, A., 2002. Detection of recombinant DNA segments introduced to genetically modified maize (zea mays). J. Agric. Chem. 50, 2100–2109.

Pauli, A., Liniger, M., Zimmermann, A., 1998. Detection of DNA in soybean oil. Z. Lebensm. Unters. Forsch. A 207, 264–267.

Villavicencio, A.L.C.H., Mancini-Filho, J., Delincée, H., 1998. Application of different techniques to identify the effect of irradiation on Brazilian beans after six months storage. Radiat. Phys. Chem. 52 (1–6), 161–166.

Zimmermann, A., Lüthy, J., Pauli, U., 1998. Quantitative and qualitative evaluation of nine different extraction methods for nucleic acids on soya bean food samples. Z. Lebensm. Unters. Forsch. A 207, 81–90.