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APPLICATION OF DIFFERENT TECHNIQUES TO IDENTIFY THE EFFECTS OF IRRADIATION ON BRAZILIAN BEANS AFTER SIX MONTHS STORAGE

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ABSTRACT

Four different techniques to detect the effect of irradiation in beans were investigated. Two types of Brazilian beans, ***Phaseolus vulgaris L.***, var. carioca and ***Vigna unguiculata (L.) Walp***, var. macaçar, were irradiated using a ^{60}Co source with doses ranging from 0 , 1.0 to 10.0 kGy. After 6 months storage at ambient temperature the detection tests were carried out. Firstly, germination tests showed markedly reduced root growth and almost totally retarded shoot elongation of irradiated beans as compared to non-irradiated beans. Secondly, DNA fragmentation was studied using a microgel electrophoresis. Irradiated cells produced typical comets with DNA fragments migrating towards the anode. DNA of non-irradiated cells exhibited a limited migration. Thirdly, electron spin resonance for detection of cellulose radicals was tested, since it was expected that these free radicals are quite stable in solid and dry foods. However, only in beans irradiated with 10 kGy a small signal could be detected. Fourtly, thermoluminescence, a method to analyze mineral debris adhering to food, turned out to be a good choice to detect irradiation effects in beans, even after 6 months of storage. The results indicate that three of these four techniques proposed, can be used to detect the effect of irradiation in these two varieties of Brazilian beans at a dose level useful for insect disinfestation (1 kGy).

KEYWORDS

Irradiation; Food Irradiation Detection; Food irradiation; Thermoluminescence; Electron Spin Resonance; DNA-migration; "Comet assay"; Germination test; Half-Embryo test; Beans Irradiation.

INTRODUCTION

Irradiation of food is recognized as a safe and effective technique for a range of specific applications, among them disinfestation of various food products, such as grains, dried fish, dried fruits and legumes (Loaharanu, 1994). Irradiation offers an attractive alternative to chemical treatments, for insect disinfestation. For the Latin-American people, beans are an important source of nutrients and energy (Pinn, Colli and Mancini-Filho, 1993). In Brazil, substantial quantities of beans produced annually, are affected by the insect infestation. Radiation processing of beans for the purpose of insect disinfestation with dosages up to 1 kGy is a promising technique for reducing storage losses of these nutritious foodstuffs (Delincée and Bognár, 1993). The World Health Organization recommends food irradiation as a technique for preserving and improving the safety of food, after more than thirty years of research on the toxicological, biological and nutritional quality of irradiated foods (Diehl,1993; WHO, 1994). Methods to identify irradiated foods are desirable for regulatory and surveillance purposes. Therefore, four methods for identification of irradiated beans were investigated.

Firstly, a half-embryo test to identify irradiated seeds or grains (Kawamura *et al.*, 1996a), was tested with our beans. Secondly, DNA fragmentation was studied using single cell gel electrophoresis.

Irradiated cells should easily be identified since extensive DNA damage will lead to migration of DNA fragments out of the cells, producing typical comet tails (Cerda *et al.*, 1993, 1997). Thirdly, electron spin resonance (ESR) to detect cellulose radicals in our beans was tested (according to EN-1787:1996). Fourtly, thermoluminescence (TL) measurements were applied. TL has been successfully tested in interlaboratory tests with herbs and spices and their mixtures from which silicate minerals could be isolated (Schreiber *et al.*, 1993, 1994; Delincée, 1992, 1993); it is also an established technique utilized in a variety of applied sciences (Mckeever, 1985; Bull, 1986). The principle of this method is based on mineral debris which keep energy by an imprisonment process as a result of exposition to ionizing radiation. The liberation of such energy is achieved by controlled heating of the isolated minerals. (IAEA-TECDOC-587,1991). To summarize, in this work, two methodologies used are based upon biological changes which occur in macaçar and carioca beans, another two methodologies are based on changes in physical properties.

EXPERIMENTAL

Phaseolus vulgaris L., var. carioca and *Vigna unguiculata (L.) Walp,* var. macaçar beans were bought in a local market in São Paulo. Irradiation was carried out in a Gammacell (AECL) ^{60}Co source (IPEN, São Paulo, dose-rate ~ 0.44 kGy/h) with doses of 0,1.0 and 10.0 kGy (nominal values ± 15%). The storage time was 6 months at ambient temperature (~24^0 C). For the germination test, ten half-embryo seeds were put into a black box and cultured at 35 ± 1^{0}C (Kawamura *et al.* 1996b). Based on the growth of half-embryos the shoots and roots were observed for 3 days of culturing period under the specified conditions. Microgel electrophoresis of bean DNA was made using the procedure modified by Cerda *et al.* 1993, 1997. Electron spin resonance was carried out with a Bruker EMS 104 A spectrometer, applying the settings as described in the European standard EN-1787:1996 for detection of irradiated food containing cellulose. The TL measurements were performed using an ELSEC model 7185 TL reader with heating rate of 10^{0}C/sec. and a final temperature of 500^{0}C. The heating chamber of TL reader was flushed with pure nitrogen (99.996%) and the system was checked with a ^{14}C light source. Mineral isolation and measurements were done according to EN-1788:1996.

RESULTS AND DISCUSSION

The germination test of these two varieties of beans revealed that for both roots and shoots the macaçar variety exhibited a better germination response as the carioca variety. Figure 1a and b show the roots length (mm) after 24h and 72 h incubation; whereas Fig. 2 a and b show the shoots length.

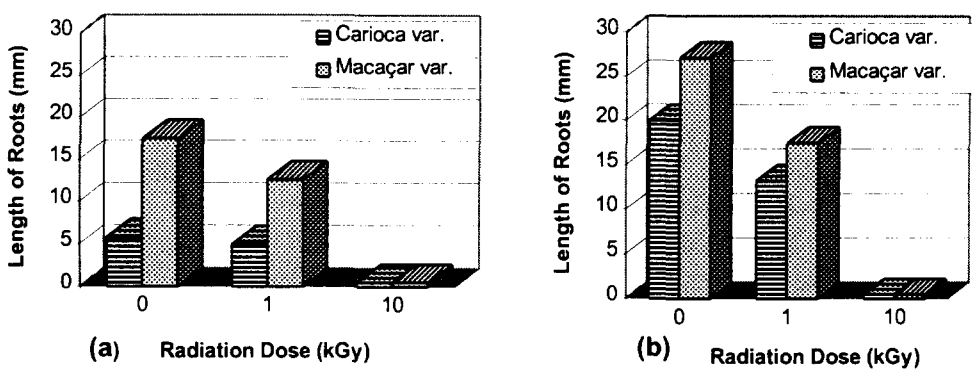

Figure 1. Roots 6 months after irradiation:(a) 24 hours; (b) 72 hours.

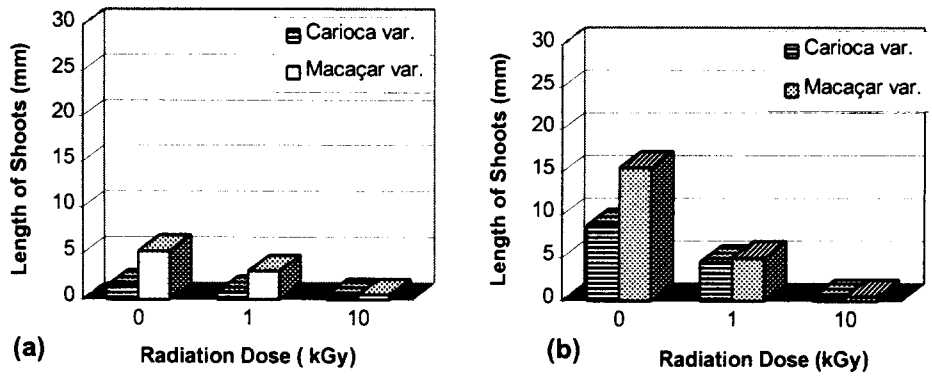

Figure 2. Shoots 6 months after irradiation: (a) 24 hours, (b) 72 hours.

Both varieties had an expressive response to irradiation already at doses up to 1 kGy. Kawamura *et al* 1992a, studied the germination of wheat and concluded that the critical dose that inhibits root growth varies from 0.15 to 0.5 kGy and also that storage periods of up to 12 months have little effects on radiation-induced reduction of root length. Furthermore, Fifield *et al.* 1967, showed that germination of wheat was unaffected by radiation dosages of 0.1 and 0.25 kGy, whereas, at 0.5 kGy or more germination was substantially reduced. In germination tests for rice, the critical dose that inhibits root growth was found to vary from 0.15 to 0.5 kGy (Kawamura *et al.*,1992b). The technique was able to discriminate between irradiated and non-irradiated rice for 12 months or more of storage. A half-embryo test to identify irradiated grapefruit (Kawamura *et al.*, 1989a) demonstrated that shoot elongation and root growth was totally retarded over 1.5 kGy and in this way a discrimination was achieved between irradiated and non-irradiated grapefruit. Half-embryo tests with lemon and orange seeds were also proposed by Kawamura *et al.*1989b, as an identification method for irradiated citrus. If the shooting was greater than 50% of the number of seeds within 3 days, the seeds are identified as "unirradiated", and if it is less than 50% after 3 days, the seeds are identified as "irradiated". Thus, the germination test is a valuable biological method for the identification of irradiated foods (Delincée, 1993; Stevenson and Stewart, 1995). In our case, differences between irradiated and non-irradiated half-embryos from beans could easily be observed. In extended experiments the shoots of half-embryos irradiated with more than 2.5 kGy did not undergo elongation, whereas the shoots of non-irradiated or those beans irradiated under 1.0 kGy elongated significantly within the 3 days test period (Villavicencio *et al.*, 1996). Further improvement of this methodology will be valuable for the identification of irradiated beans.

In the DNA comet assay non-irradiated cells of the two varieties of beans exhibited only limited DNA migration out of the cells, whereas irradiation with 10 kGy caused extensive DNA fragmentation. The DNA fragments migrated towards the anode and produced typical comets with long tails. At dose levels for insect disinfestation, here represented by 1 kGy, already changes in the migration pattern could be observed (fig. 3a,3b).

Previous experiments with irradiated food using the DNA comet assay have shown that this method is a rapid and simple technique to detect the radiation treatment in both animal food (Delincée and Marchioni, 1993; Delincée, 1995) as well as in plant material (Delincée, 1996).

Since free radicals are quite stable in solid and dry foods, it was expected that electron spin resonance had potential for detection of the radiation processing in beans (Raffi and Agnel, 1989; Desrosiers et al., 1996). Using the ESR mode for food containing cellulose (EN-1787:1996), only at a dose level of 10 kGy a small signal composed of a pair of lines with a spacing of about 60 gauss around the central signal could be observed in our beans. Probably, the long storage time of 6 months has led to a decay in signal strength. This decay of the ESR cellulose signal with storage time has also been observed in aromatic herbs and paprika powder (Raffi et al., 1994; Kispéter *et al.*, 1996).

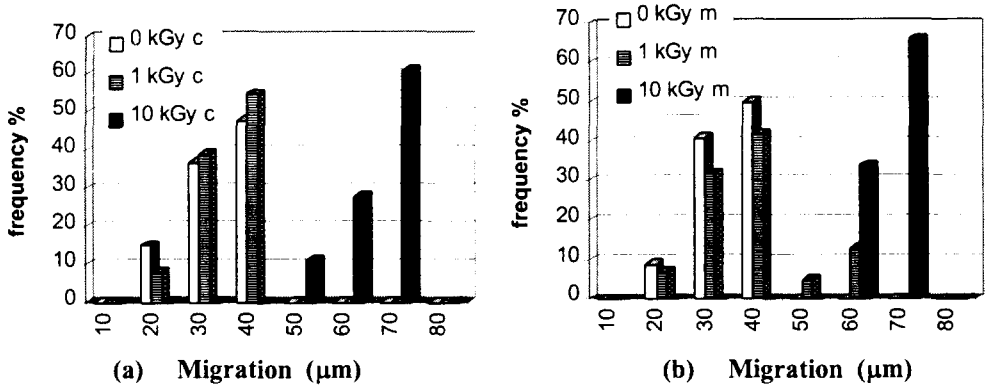

Figure 3. Frequency histograms of DNA comets in beans: (a) Carioca var.; (b) Macaçar var.

Thermoluminescence measurements of the isolated mineral debris from the beans enable an easy identification of the radiation treatment both at 1.0 and 10 kGy, also after 6 months of storage (Table 1). TL glow ratios from the irradiated samples are mostly higher than 0.5, whereas those from unirradiated samples are lower or around 0.1. Interpretation of the shape of the glow curves showed that the curves of the irradiated beans exhibited maxima between 150°C up to 250°C, whereas in the non-irradiated beans the effects of low levels of natural radioactivity can be seen in the deep traps above 300°C. Our TL results confirm the results obtained by many other authors, e.g. Sanderson et al.(1989,1996), Pinnioja et al. (1993), Schreiber et al. (1995), Khan and Delincée (1995a,b), that TL is a sensitive method to detect whether foods have been irradiated or not.

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Table 1. TL glow ratios over the whole temperature interval (70-500°C) and the recommended temperature interval (170-246°C) of irradiated carioca and macaçar beans.

Temperature interval		70 - 500^{0}C		170 - 246^{0}C	
Dose (kGy)	Ratio	Carioca	Macaçar	Carioca	Macaçar
0	1/2*	0.11	0.071	0.072	0.040
	1/3**	0.16	0.10	0.12	0.073
1.0	1/2	0.35	0.44	0.41	0.57
	1/3	0.47	0.59	0.73	0.99
10	1/2	1.26	1.26	2.40	2.54
	1/3	1.32	1.47	4.42	4.88

- * 1/2 is the ratio of integrated TL intensities of glow curve 1 (recorded from the minerals of the sample as received), and glow curve 2 (recorded from the minerals of the sample after the first measurement and then subsequent exposed to a dose of 1.0 kGy for the purpose of normalization).
- **1/3 is the ratio of integrated TL intensities of glow 1 to glow 3 (normalization dose 0.5 kGy).

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