



Identification of irradiated wheat by germination test, DNA comet assay and electron spin resonance

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Abstract

In several countries, there has been an increase in the use of radiation for food processing thus improving the quality and sanitary conditions, inhibiting pathogenic microorganisms, delaying the natural aging process and so extending product lifetime. The need to develop analytical methods to detect these irradiated products is also increasing. The goal of this research was to identify wheat irradiated using different radiation doses. Seeds were irradiated with a gamma ⁶⁰Co source (Gammacell 220 GC) in the Centro de Energia Nuclear na Agricultura and the Instituto de Pesquisas Energéticas e Nucleares. Dose rate used were 1.6 and 5.8 kGy/h. Applied doses were 0.0, 0.10, 0.25, 0.50, 0.75, 1.0, and 2.0 kGy. After irradiation, seeds were analysed over a 6 month period. Three different detection methods were employed to determine how irradiation had modified the samples. Screening methods consisted of a germination test measuring the inhibition of shooting and rooting and analysis of DNA fragmentation. The method of electron spin resonance spectroscopy allowed a better dosimetric evaluation. These techniques make the identification of irradiated wheat with different doses possible. © 2002 Elsevier Science Ltd. All rights reserved.

Keywords: Wheat; Germination test; Comet assay; Electron spin resonance

1. Introduction

In order to control irradiated food, it seems desirable to have analytical methods to detect the irradiation treatment directly in the food product itself (Cerda et al., 1997). The use of two or more techniques simultaneously and comparison between results is important to reduce uncertainty due to limitations in any one technique. Germination tests establish the radiobiological effects on inhibition shoot and root due to ionizing radiation. Inhibition of development has been related

with dose increases. Root or shoot elongation from germinating seeds has shown inhibition in the following irradiation wheat (Zhu et al., 1993; McMurray et al., 1996).

Since the large molecule of DNA is an easy target for ionizing radiation, changes in DNA offer potential as a detection method (McMurray et al., 1996). Irradiated products change in physical, chemical, biological and nutritional ways. However, the presence of free radicals as a radiolytic product is common in all these changes (Korkmaz and Polat, 1999). The electron spin resonance is a physical technique that can measure the concentration of free radicals produced following irradiation. Free radicals are a paramagnetic species and could be identified by an ESR spectrometer. Wheat is an important basic supply in the diet of Brazilian people which includes bread, macaroni, wheat flour, etc.

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2. Experimental

2.1. Germination test

Wheat variety IAC 289 (from Instituto Agronômico de Campinas) was irradiated in a Gammacell ^{60}Co source (model 220 Excell MDS Nordion) at Centro de Energia Nuclear na Agricultura. Dose rate was approximately 1.618 kGy/h with doses of 0.0, 0.10, 0.25, 0.50, 0.75, 1.0 and 2.0 kGy. Storage time was 1 month at ambient temperature following irradiation (between 20°C and 25°C). Seeds were germinated in Petri plates. Germination standard was > 80% and relative humidity of seeds were 13%. We used 3 replicates per dose and 10

seeds per plate. Germination occurred in a controlled-environment chamber (model 102F Electrolab) with temperature of $30 \pm 0.5^\circ\text{C}$ for 3 days (Kawamura et al., 1992). We estimated the shoot and root length for each dose.

2.2. DNA comet assay

Following irradiation of the same wheat cultivar under the conditions described above, seeds were stored for 6 months at ambient temperature (between 20°C and 25°C). The comet assay was carried out based on Cerda et al. (1997) and was modified for wheat.

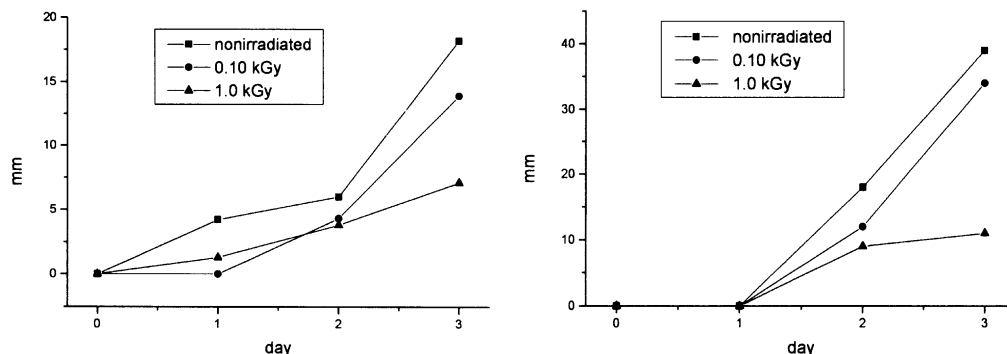


Fig. 1. Effect of gamma-radiation on shoot length (left) and root length (right) for different doses.

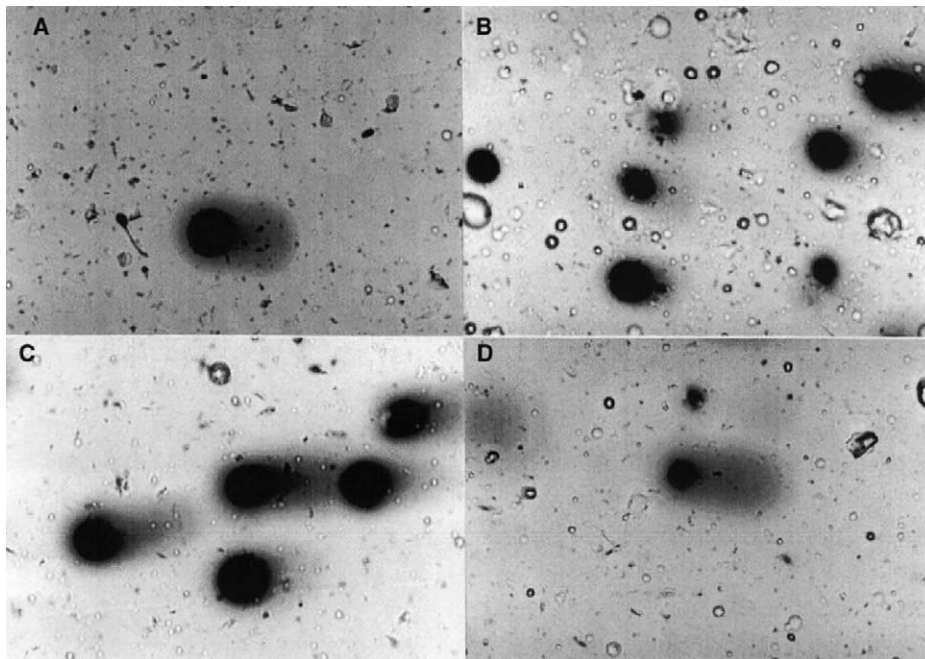


Fig. 2. Photomicrographs of DNA comets from nonirradiated and irradiated wheat seeds (microscope objective X40): (A) and (B) nonirradiated; (C) dose of 0.10 Gy; (D) dose of 1.0 Gy.

Cells were arbitrarily classified into four categories. Type A cells showed a large nucleus with defined contours between 9 and 11 μm and had a few or no DNA tail. Type B cells had a nucleus between 7 and 9 μm , lost their defined contour and had a dense DNA tail. Type C cells had a nucleus between 4 and 7 μm with a dense DNA tail. Type D cells had the smallest nucleus with 4 μm or less and a dense DNA tail.

2.3. Electron spin resonance

Husked wheat variety IAC 355 (from Instituto Agronômico de Campinas) was irradiated in a Gamma-cell ^{60}Co source at Instituto de Pesquisas Energéticas e Nucleares, São Paulo. Dose rate was approximately 5.8 kGy/h with doses of 0.0, 0.10, 0.25, 0.50, 0.75, 1.0 and 2.0 kGy. Irradiation was finished 1 h before the first ESR measure. Husked wheat with panicle was triturated in a electric grinder and placed in capillary quartz tube for measurement using an X-band EMX Bruker spectrometer at Instituto de Física da Universidade de São Paulo.

The spectrometer used a microwave frequency of around 9.759 GHz with 2 G modulation amplitude, 100 kHz modulation frequency, 2 mW microwave power

and 100 G sweep width. The spectrum was analysed by Bruker WINEPR software.

3. Results and discussion

3.1. Germination test

The coefficient of variation (Pimentel, 1990) for 3 replicates per dose was $> 30\%$ indicating a low accuracy experiment. This result suggest increase replicates per dose (see Fig. 1).

Analysis of variance (Pimentel, 1990) was significant at the 5% level ($p < 0.05$) for shoot length at last date (F test).

3.2. DNA comet assay

For 0.0 kGy (control) the percentage of type A cells was 50% and of type C cells was 11%. For 0.25 kGy, type A cells were 24% and type C cells were 23% of the total. For 0.75 kGy, type A cells were 18% and type C cells were 29%. For 1.0 kGy, type A cells were 20% and type C cells were 37%. We observed a percentage change for each dose. This qualitative phenomenon enabled the identification of damaged cells (see Fig. 2).

3.3. Electron spin resonance

In this research, our goal was only to measure the signal amplitude variation with increasing dose of radiation. Table 1 shows signal intensity, in arbitrary units, by mass of material in milligrammes observed during 63 days.

We observed signal decay rapidly with time (Dadayli et al., 1997; Sunnetçioğlu et al., 1998). The irradiated samples could be differentiated only for the first three weeks of storage (see Fig. 3).

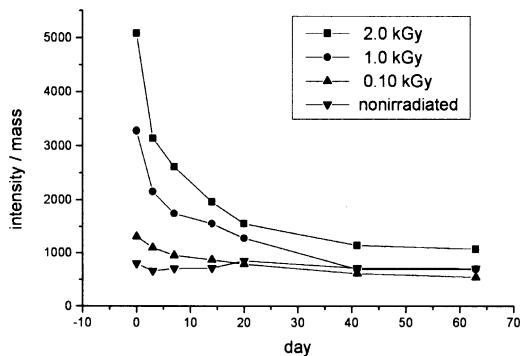


Fig. 3. ESR signal decay during 63 days for nonirradiated and irradiated husked wheat.

Table 1

ESR signal decay with time for different doses in irradiated husked wheat. Intensity is in arbitrary units (Δh) by mass of material in milligrammes (mg)

Dose (kGy)	$\Delta h/\text{mg}$ day 0	$\Delta h/\text{mg}$ day 3	$\Delta h/\text{mg}$ day 7	$\Delta h/\text{mg}$ day 14	$\Delta h/\text{mg}$ day 20	$\Delta h/\text{mg}$ day 41	$\Delta h/\text{mg}$ day 63
0.0	802 \pm 31	665 \pm 28	711 \pm 31	716 \pm 31	854 \pm 37	716 \pm 31	711 \pm 31
0.10	1309 \pm 59	1097 \pm 48	954 \pm 42	873 \pm 37	791 \pm 37	614 \pm 30	545 \pm 30
0.25	1536 \pm 66	1265 \pm 54	1120 \pm 47	1059 \pm 47	987 \pm 41	804 \pm 36	610 \pm 36
0.50	2675 \pm 105	1942 \pm 85	1721 \pm 70	1604 \pm 70	1234 \pm 56	636 \pm 28	883 \pm 28
0.75	3046 \pm 126	2254 \pm 93	1838 \pm 76	1538 \pm 67	1323 \pm 59	931 \pm 42	800 \pm 42
1.0	3278 \pm 280	2157 \pm 92	1747 \pm 74	1555 \pm 73	1279 \pm 55	694 \pm 36	694 \pm 36
2.0	5085 \pm 660	3145 \pm 140	2615 \pm 110	1968 \pm 134	1554 \pm 71	1141 \pm 50	1069 \pm 50

4. Conclusion

The germination test was a very simple and low precision test that makes it possible to identify the irradiated wheat using different doses. Comet assay was a qualitative test that we used to identify irradiated wheat at least 6 months after storage. It is possible to use ESR, a quantitative technique, to identify irradiated husked wheat until 3 weeks after the date of irradiation. After 3 weeks, the signal decays quickly with time.

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