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Electron paramagnetic resonance study of some varieties of gamma-irradiated soybean

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Abstract

Electron paramagnetic resonance (EPR) spectroscopy was used to investigate free radicals formed in gamma irradiated Brazilian soybean (*Glycine max*) cultivars. The beans were irradiated by a ^{60}Co source with doses ranging from 1 to 15 kGy. Before irradiation, the representative soybean EPR spectrum is composed of a sextet centered at $g = 2.0$ and a sharp singlet at the same g-value. The stability of the EPR signal of 10 kGy-irradiated samples was followed for over 7 months after irradiation. The results were compared with non-irradiated samples and showed a good possibility of detection of previous radiation treatment for higher doses. It was also analyzed the contribution to the EPR signal of different parts of the bean showing a prominent signal coming from the hilum.

Keywords: EPR; Food irradiation; Soybean; Isoflavones

1. Introduction

Epidemiological studies have been shown that populations with higher consumption of soy foods exhibit a lower incidence of cancer (colon, breast and prostate). Furthermore, soy products contribute for the reduction of cholesterol levels, menopausal symptoms and osteoporosis (Kuo, 1997; Barnes, 1998; Messina, 1997, 1999). Soybean and its processed products are considered health foods due to their high content of protein and essential amino acids, omega-3 fatty acids, fat soluble vitamins, polysaccharides and insoluble fibers (Watson et al., 2000). Besides these constituents, soybeans also contain isoflavones, biologically active phytochemical components which are thought to possess antioxidant effects (Setchell and Cassidy, 1999; Fritz et al., 2003). Among the components studied, isoflavones and proteins are the most likely responsible for those health benefits (Anderson et al., 1995; Barnes, 1998; Setchell and Cassidy, 1999; Messina 1997, 1999).

Gamma radiation treatment with doses up to 1 kGy has been recommended for quarantine treatment of legumes including soybeans, whereas exposure to higher doses (up to 5 kGy) resulted in improvement in quality such as reduction in cooking time and improvement in texture without production of off-flavor (Wilkinson and Gould, 1996; Byun et al., 1993). Current applications of food irradiation involving doses above 10 kGy include mainly high-quality shelf-stable convenience foods (WHO, 1999).

A number of techniques have been used to detect changes in irradiated food. Ionizing radiation generates free radicals in the matter which if stable for a given period of time are easily detectable by electron paramagnetic resonance spectroscopy (EPR) (Diehl, 1995; Haire et al., 1997).

The aim of the present work was to use EPR spectroscopy to study the behavior of radiation-induced free radicals over time in γ-irradiated Brazilian soybean cultivars and to assess the possibility of using it as a method of detection of previous radiation treatment.

2. Material and methods

Soybean samples were obtained from EMBRAPA Soya, Londrina, PR, Brazil. The seven soybean cultivars were ground in a coffee mill and sieved in particles no larger than 1.5 mm. For an especial assay, around 30 grains from a particular cultivar (named BRS 231) were separated in

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their parts: cotyledons (almost 90% of grain), coat, hilum and hypocotyl. From these parts the only one that required sieving was the cotyledons.

Irradiation was performed in a ^{60}Co gamma source (Gammacell 220 AECL), at a dose rate of 3.4 kGy/h, with doses ranging from 1 to 15 kGy. For the EPR measurements the samples were introduced in quartz tubes with internal and external diameters of 4.75 and 5.75 mm, respectively. The EPR spectroscopy was carried out in a Bruker X-band spectrometer (ER 041 XG Microwave Bridge, Bruker). Both irradiation and EPR readings took place at room temperature. When not in use the samples were kept at refrigerator temperature (5−10 °C). The parameters used in the EPR readings were: 6.32×10^4 (receiver gain), 2 G (modulation amplitude), 81.92 ms (conversion time), five scans and around 9.8 GHz (microwave frequency). Unless otherwise described, the microwave power was 10 mW. In order to test the saturation conditions we have tested powers in the range 0.5−100 mW. A sample of Diphenyl-picryl-hydrazyl (DPPH)—which g-factor is well known was used for calibration purposes.

3. Results and discussion

Radiation as several other processes induces the formation of reactive oxygen species (ROS). They are also formed by the decomposition and the inter-reactions of ROS. Hydroxy radical is the most reactive ROS, followed by singlet oxygen (Choe and Min, 2006). The origin of free radicals responsible for the EPR signal in irradiated dry plants is not clear. Up to now several suggestions have been made: semi-quinones, lignin or due to oxidation of fatty acids present (Yordanov et al., 2005) or melanin, which is a polymeric pigment containing condensed ring structures (Buchvarov and Gantcheff, 1984; Swartz et al., 1972).

EPR spectrum of untreated soybean is composed of an equally spaced sextet due to Mn^{2+} ion (Fig. 1) and a singlet resonance line originating from a natural free radical both appearing at $g = 2.0039$.

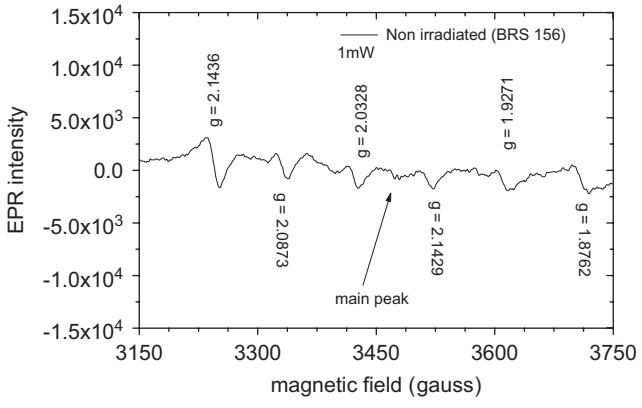

Fig. 1. EPR spectra exhibiting equally spaced sextet and a singlet resonance line for a non-irradiated soybean sample.

Radiation was observed to cause an exponential increase in the intensity of the singlet resonance line but not creating any effect on Mn^{2+} ion resonance line intensities in the study range of 1–15 kGy. The results showed in Fig. 2 indicate an under-saturation condition for all soybean samples.

In order to investigate the stability of the EPR signal, resonance measurements were performed from 3 days up to 7 months after irradiation, considering just the peak-to-peak intensity of the singlet signal. Despite the appreciable decrease with time, the intensities of the main peak are still much higher than those observed in non-irradiated samples. Fig. 3 shows the stability of EPR signal as a function of time for three representative cultivars.

If considered the weakest signal after 7 months, for doses of 10 kGy, the ratio between EPR intensities of irradiated

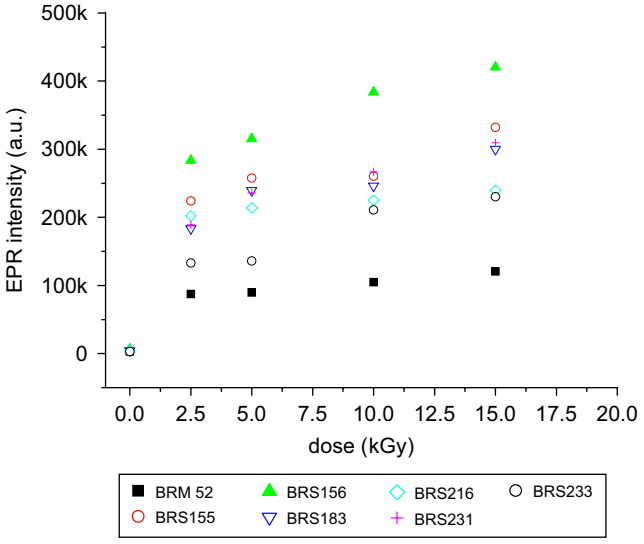

Fig. 2. EPR intensity of singlet peak for all soybean cultivars assayed versus dose.

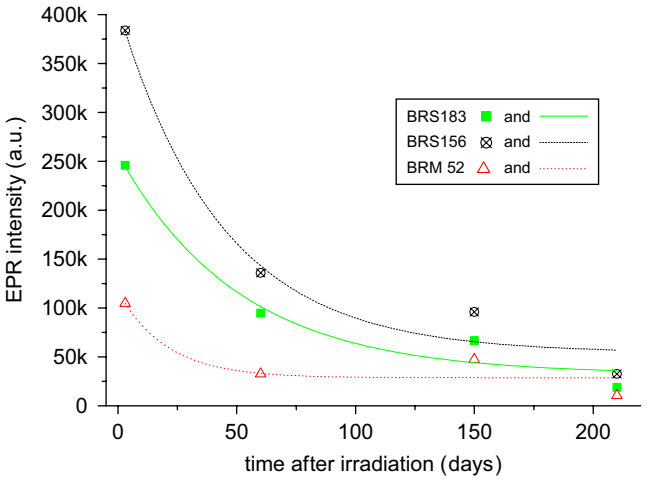

Fig. 3. Time dependence of EPR signals for three soybean cultivars irradiated after grinding with 10 kGy (experimental values and exponential fittings).

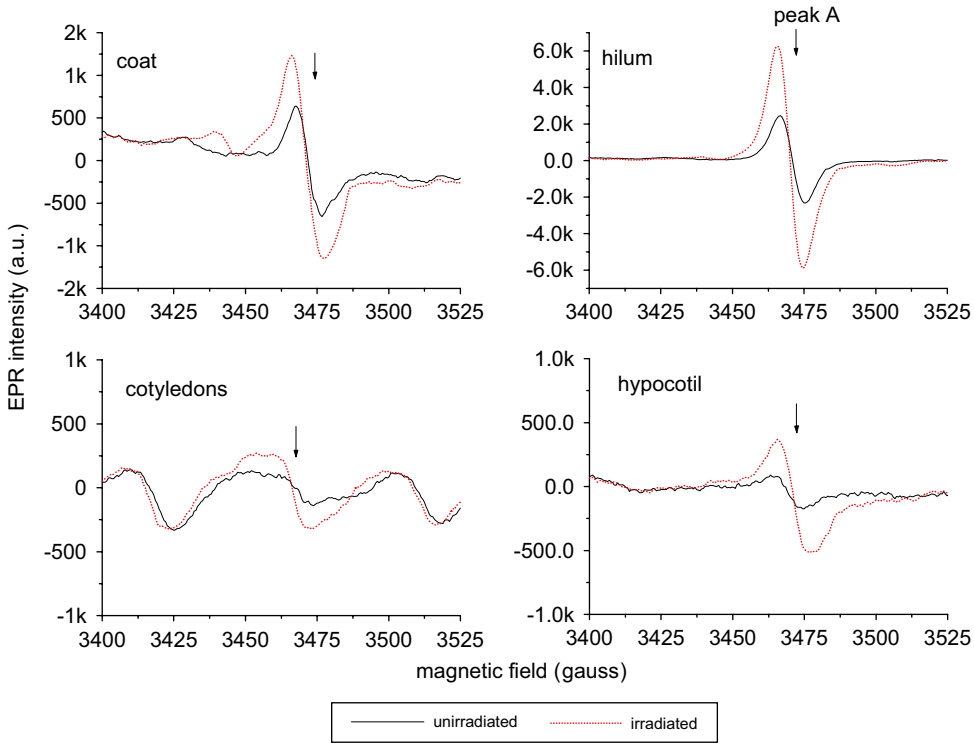

Fig. 4. EPR signals for different parts of soybean, before (solid) and after irradiation with 2 kGy (dash). Singlet peak (↓).

and unirradiated samples are around three times higher. Hence, the EPR signal could still be used to identify an irradiated sample. The diverse cultivars employed presented a similar behavior. It was also observed that samples irradiated before grinding presented signal intensities slightly lower when compared with those irradiated after grinding. However, for low doses and depending upon the time elapsed after irradiation the occurrence of previous radiation treatment cannot be assumed.

Preliminary tests were performed to establish the contribution of different part of the bean to the EPR signal and whether some parts of the grain could be more effective in assessing previous radiation treatment for lower doses. Fig. 4 presents the EPR spectra from different parts of cultivar BRS 231. When measurements are performed with the ground grain, the EPR signal comes from the cotyledons which are the main constitutive of the bean. However, when isolated, the coat and the hilum presented signals higher and easier to be analyzed as the sextet, almost radiation independent, has a negligible contribution in their spectra.

4. Conclusions

EPR spectrum of untreated soybean is composed of an equally spaced sextet due to Mn^{2+} ion. Before irradiation, the representative EPR spectrum of soybean is composed of a sextet centered at $g = 2.0$ and a sharp singlet at the same g-value. Radiation was observed to cause an exponential increase in the intensity of the singlet resonance line but not creating any effect on Mn^{2+} ion resonance line intensities in the study range of 1–15 kGy. Its EPR intensities, for higher doses, remained considerably higher than those observed in non-irradiated samples even after several months after irradiation. Then, EPR measurement could enable irradiated samples to be distinguished from the non-irradiated ones. When considered separate parts of the soybean, instead of the whole seed, the results seemed to be more promising for detection purposes. Following irradiation with a much lower dose (2 kGy) the coat and hilum presented a more prominent signal than that of the whole seed.

The main EPR signal obtained for soybeans were qualitatively similar to those described for other legumes (Polat and Korkmaz, 2001) and even for ginseng (Nakamura et al., 2006) corresponding to an organic free radical. The free radical observed in the present experiment can be considered similar also to that of melanin mentioned before.

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