Assessing Antioxidant Activity of some Varieties of Gamma-Irradiated Brazilian Soybean by ESR

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Abstract

Soybean production and utilization as food has increased during the last decades. The protein and oil contents are high in quantity and quality. Soy is natural source of flavonoids - biologically active components that are thought to possess antioxidant effects in vivo and in vitro systems. Alongside traditional methods of processing and preserving food, the technology of food irradiation is gaining more and more attention around the world. This study was undertaken to investigate by means of electron paramagnetic resonance (EPR) the stability of free radicals generated by radiation processing on three different soybean cultivars. The soybean cultivars investigated were gamma-irradiated with doses of 0, 2.0, 5.0, 10.0 and 15.0kGy. Both irradiation and EPR measurements were performed at room temperature. The EPR signal intensity correlated well with the ionizing radiation dose. EPR spectra were recorded 16h, 11days and 40 days after irradiation. The results showed that the EPR signal intensities remained almost constant up to 40 days after irradiation for all the varieties assayed. Further experiments are required in order to identify the species responsible for the EPR peaks and the proper antioxidant capability of these soybean cultivars against the radiation-induced oxidative shock.

1. INTRODUCTION

Soybean production and utilization as food has increased during the last decades. Soybean has a unique chemical composition. The protein and oil contents are high in quantity and quality. Soybean seeds contain also important phytochemicals that have been shown in recent years to offer important health benefits. They are natural source of flavonoids - biologically active components which are thought to possess antioxidant effects in vivo and in vitro systems.

Dietary flavonoids represent a family of polyphenol compounds found in common food items derived from plants. Depending on structural features, flavonoids can be further subdivided into flavones, flavonols, isoflavones, flavanes, and flavanols. The biological activities of flavonoids are structure dependent and epidemiological studies support their role in human cancer prevention. Several flavonoids inhibit cancer development in animal models of chemical and UV carcinogenesis [1]. Some authors described the in vivo antioxidant activity of soy isoflavones in human subjects determined by the urinary excretion of secondary lipid peroxidation products [2].

Most of the beneficial health effects of flavonoids are attributed to their antioxidant and chelating abilities. By virtue of their capacity to inhibit low-density lipoproteins (LDL) oxidation, flavonoids have demonstrated unique cardioprotective effects [3][4][5].

Alongside traditional methods of processing and preserving food, the technology of food irradiation is gaining more and more attention around the world [6]. The process involves exposing the food, either packaged or in bulk, to carefully controlled amounts of ionizing radiation for specific time to achieve certain desirable objectives. Chemically, radiation effects are induced through free radicals generated from the first radiation-matter interaction, mainly reactive oxygen species (ROS). The cytotoxic effect of free radicals is deleterious to mammalian cells and mediates the pathogenesis of many chronic diseases, but is responsible for the killing of pathogens by activated macrophages and other phagocytes in the immune system [7].

Electron spin resonance (ESR) provides a unique method for studying free radicals. It gives information not only about the concentration of free radicals but also about the structure of the radical center's surroundings. This method has gained acceptance in many scientific fields and has been successfully applied in biological sciences and food research. A difficulty in ESR applications is that the spectra obtained for foodstuffs are usually non-resolved singlets. However, to a certain expense, this difficulty can be overcome by an appropriate choice of frequency range and experimental methods [8].

Ionizing radiation produces, in irradiated materials, paramagnetic species of different kinds, *i.e.* radicals, radical-ions and paramagnetic centres, which can be measured by ESR but most of them are not stable enough to be used for the detection of irradiation [9][10].

Although scavenging and antioxidant activities of soy foods are attributed to the presence of flavonoids there is not a clear understanding about the ionizing radiation effect on whole soybeans neither their capability against free-radicals generated by radiation. Then, the aim of the present paper was to investigate the applicability of ESR spectroscopy in the study of the antioxidant activity of different cultivars of soybean against the radiation stress.

2. MATERIAL AND METHODS

The soybean cultivars investigated were obtained from the National Soybean Research Center (Embrapa-Soja), Londrina, Brazil, and have the composition described in Table I [11].

Table I. Protein and oil composition of the employed cultivars

Cultivars	Protein (%)	Oil (%)
BRS 184	39.0	24.2
BRS 212	38.0	19.9
BRS 216	43.6	17.6

Soybean grains were grounded in a coffee grinder and then irradiated with gamma radiation from a ^{60}Co Gammacell 220 (AECL) irradiator. The radiation doses employed were 0, 2.0, 5.0, 10.0 and 15.0 kGy at a dose rate of 4.7 kGy/h. Irradiation was carried out just after the grinding process. The gamma irradiator employed is located at the Center of Radiation Technology of the Institute of Nuclear and Energy Research, Brazilian Nuclear Energy Commission, Sao Paulo, Brazil. After irradiation the samples were put into EPR tubes (fused quartz tubes, about 1 mm diameter). The measurements were performed in a X-band spectrometer (ER 041 XG Microwave Bridge, Bruker) at the Biophysics Laboratory from the Physics Institute, University of Sao Paulo. The microwave frequency was around 9.8 GHz, 100 kHz modulation frequency, 2.00 G modulation amplitude, 6.32×10^4 gain and 10 mW microwave power except when otherwise indicated. The EPR measurements were performed 16h, 11days and 40 days after irradiation. Both irradiation and the EPR measurements were performed at room temperature.

3. RESULTS AND DISCUSSION

Radiation interactions in the condensed matter are still the subject of numerous works. In the present study we wanted to find out whether the radiation-produced radicals were able to surmount the antioxidant behavior of flavonoids contained in soybeans. The intensity of the EPR signal of the non-irradiated samples was practically null when compared with the irradiated ones. This fact suggests that the free radicals created by the grinding process are distinct from those created by gamma-irradiation. In Figure 1 the pattern of the EPR signals of the irradiated and non-irradiated cultivars can be seen. Free radicals generated in cultivar BRS 212 seemed to be in less proportion than in the other samples.

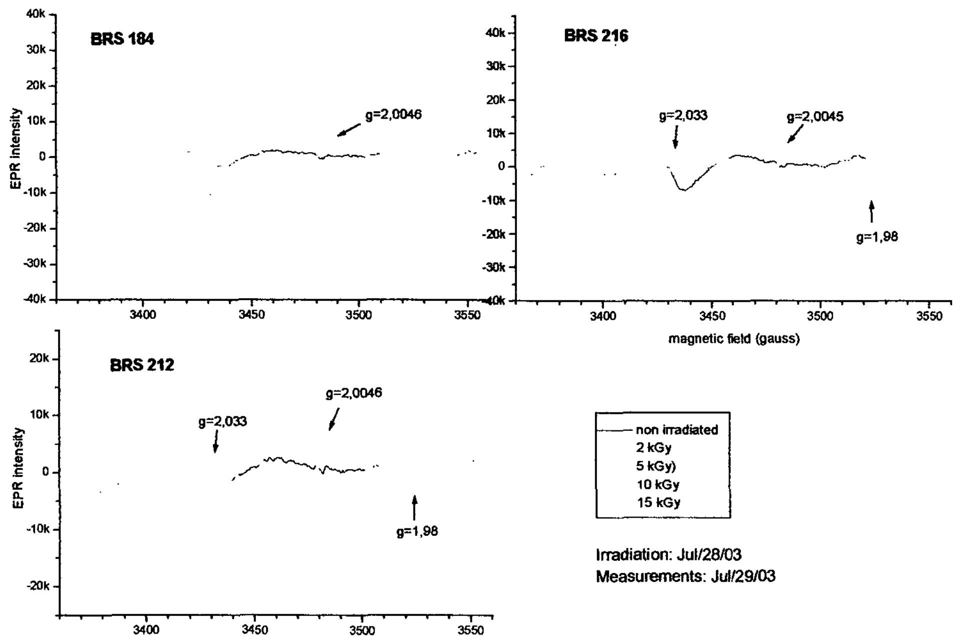

Figure 1. EPR spectra obtained 16h after irradiation of the three samples

The gamma-irradiation of soybean with doses from 2 to 15 kGy in the range of radiation decontamination, resulted in a dose-dependent increased of a signal at g=2.0045, as was described previously for other grains [12]. Irradiated samples exhibited a singlet as a major peak at the free electron position and a doublet with intensity of one quarter of it. The EPR intensity correlated well with the ionizing radiation dose. That relation can be observed in Figure 2, where a sub-linear relationship suggests a tendency to saturation of free radicals generation at higher doses. The BRS 212 cultivar for all doses presented a much lower free radical production if compared with the two other cultivars. It suggested a higher concentration of antioxidants in that cultivar. It is also noted a tendency of a negative correlation between protein percentage (Table I) and the antioxidant capability of the cultivars employed.

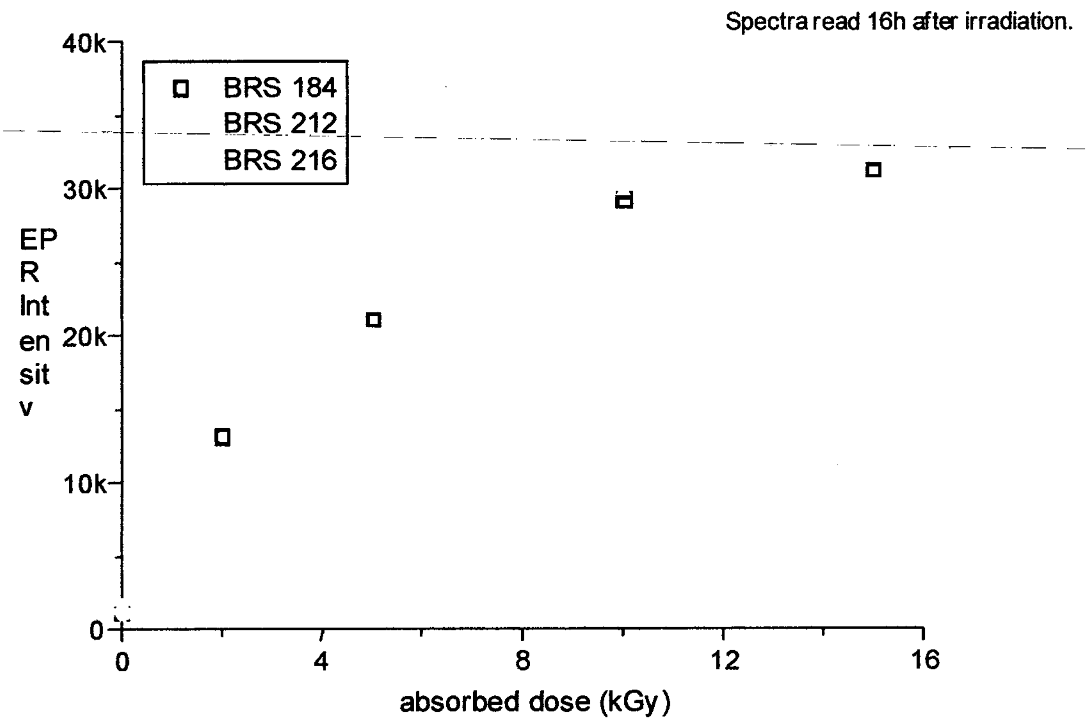

Figure 2. Main peak EPR intensity versus dose for the assayed samples 16 h after irradiation

EPR spectra were recorded 16 h, 11 days and 40 days after irradiation. The results (Figure 3) showed that the EPR signals remained almost constant up to 40 days after irradiation for all the varieties assayed.

There was a perceptible shift of the spectra due to small variations of the microwave frequency, but even though g values remained the same. Instead of a decrease in the intensity of the last readings, a little increase seems to be present (Figure 3). It can be due to slight variation of the sample tubes employed. Free-radicals responsible for the EPR signal presented stability over the period investigated.

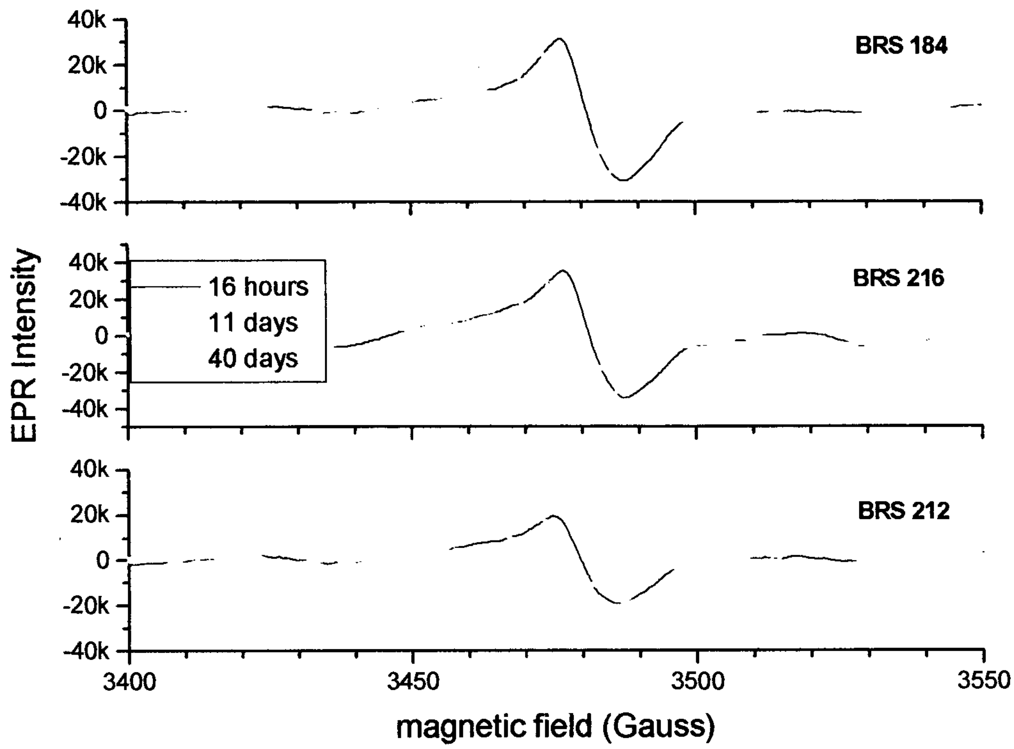

Figure 3. Main peak EPR intensity versus dose for the assayed samples 16h after irradiation

The identification of the transient species (mainly flavonoid phenoxyl radicals) originated in the scavenging reactions, and the characterization of their physico-chemical properties may help to elucidate better the mechanism of action involved and the proper antioxidant capability of these soybean cultivars against the radiation-induced oxidative shock.

4. CONCLUSIONS

The intensity of the EPR signal of the non-irradiated samples was insignificant if compared with the irradiated ones. Meanwhile the irradiated samples exhibited a singlet as a major peak at the free electron position and a doublet with intensity of one quarter of it. In all cases, the EPR intensity of the main peak correlated well with the ionizing radiation dose.

BRS 212 cultivar for all doses presented a much lower free radical production if compared with the two other cultivars. This fact could be associated with higher concentration of antioxidants in that cultivar. Characterization of the transient species, for instance, flavonoid phenoxyl radicals is required for a better understanding of the antioxidant capability of soy cultivars.

ACKNOWLEDGEMENTS

The author, N.L.d.M, is recipient of a Research Productivity Fellowship from the Brazilian National Research Council (CNPq).

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