Latin American Section of the
American Nuclear Society, BsAs,
June 25-29, 2006

STUDY BY EPR OF GAMMA-IRRADIATED BRAZILIAN SOYBEAN

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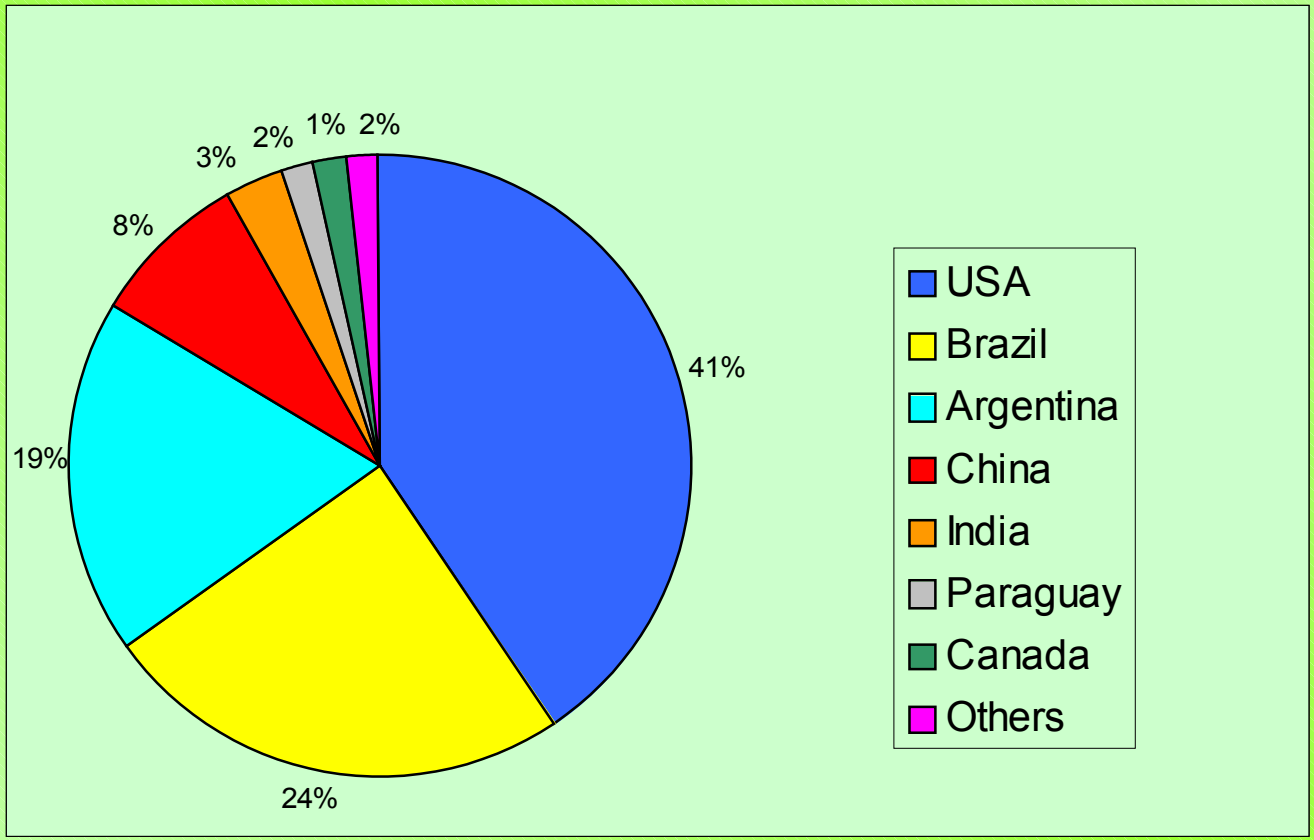
Soybean

- Increasing soybean production and utilization;
- Soybean has a unique chemical composition:
  Protein (40%) and oil (20%) contents.
- Soybean seeds contain also important phytochemicals: natural source of flavonoids - biologically active components which are thought to possess antioxidant effects in vivo and in vitro systems.

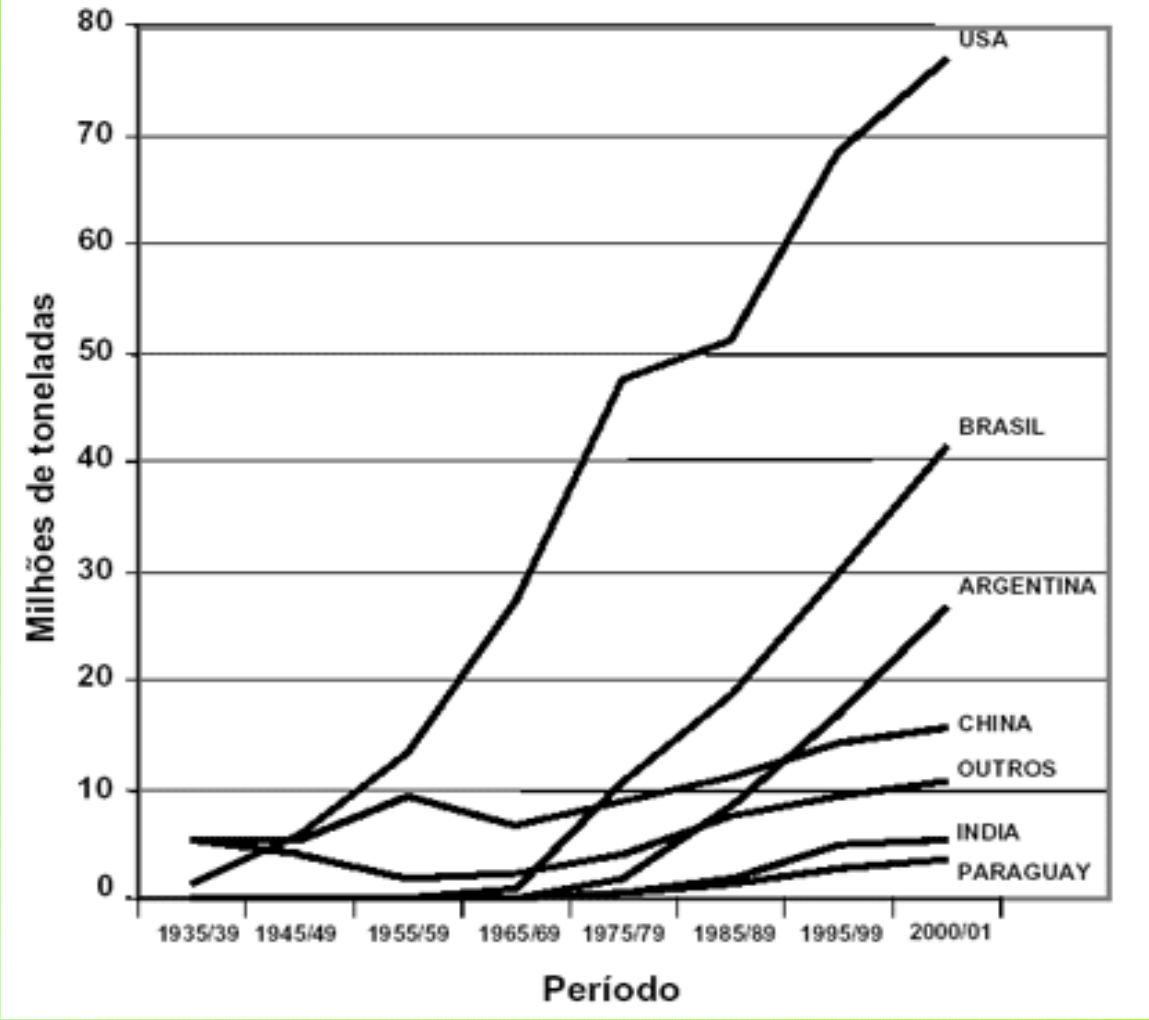
World Soybean Production, 2005



Total Production: 204 milions of tons

Source: FAO, United Nations

Evolution of Soybean Production



Source: USDA

The Brazilian Soybean Development is in great part due to the role played by The National Soybean Research Company (EMBRAPA):

- Constant Development of new cultivars to different regions;
- Development of cultivars with special features.

Typical Radiation Doses used in Radiation Technology

Growth Stimulation	1-10Gy
Insect Control	100Gy
Sprouting Control	150Gy
Irradiation of aq.solutions	200Gy
Production of New vegetal varieties	1.000Gy
Microbiological Spoilage Control	1.000Gy
Polimerization	5.000Gy
Sterilization of medical products	25.000Gy
Effluent Gases Treatment	40.000Gy
Soil Sterilization	50.000Gy
Polymer crosslinking	100.000Gy

Food irradiation

Food	Main Objective	By Means of	Dose (kGy)
Low-Dose treatment (up to 1kGy)			
Potatoes, yams, onions, garlic, shallots	Extension of storage life	Inhibition of sprouting	0,05-0,15
Certain fruits and vegetables	Improved of losses caused by insects	Delay in maturation and senescence	0,25-1,00
Cereal grain, flour, dried fruits, nuts, pulses	Prevenção de perdas causadas por insetos	Killing or sexual sterelization of insects	0,20-0,70
Fruits	Prevention of spreading of pests. Quarantine treatment.	Killing or sexual sterilization of insects	0,20-0,70
Meat	Prevention of parasitic disease transmitted through food	Destruction of parasities such as *Trichinella spiralis* and *Taenia saginata*	0,30-0,50

Food	Main Objective	By Means of	Dose (kGy)
Medium dose treatments (1-10 kGy)			
Certain frutis and vegetables, sliced bread	Improved keeping properties	Reduction of populations of bacteria, molds and yeasts	1-3
Meat, poultry, fish	improved refrigerated storage	Reduction of population of microorganism capable of growth at refrig. Temperature	1-5
Meat, poultry, eggs, egg powder, froglegs, frozen seafood, and other foods carrying pathogenic microorganisms	Prevention of poisoning	Destruction of Samonella, Shigella, Campylobacter, Vibrio, Yersinia, and other non-spore-forming pathogens	3-10
Spices, dried vegetables, and other food ingredients	Prevention of contamination of food to which the ingredients are added	Reduction of population of microorganism in the ingredient	3-10

Os objetivos da irradiação de alimentos.

Food irradiation

Food	Main objective	By Means of	Dose (kGy)
High dose treatment (10-45 kGy)			
Meat, poultry	Sage long-term storage without refrigeration	Destruction of spoilage organisms and pathogens, includeing spore-formers	25-45
Complete hostipal meals or constituents of such meals	To supply patients with sterile meals	Same as above	25-45

Source: Safety of Irradiated Food, by J.F. Diehl, 1990

Electron paramagnetic resonance

- **Electron spin resonance (ESR) spectroscopy has been used for over 50 years to study a variety of paramagnetic species.**

- **The electron spin resonance spectrum of a free radical or coordination complex with one unpaired electron is the simplest of all forms of spectroscopy.**

Electron paramagnetic resonance

From a classical point of view an electron may be considered as a negatively charged rotating sphere. The angular momentum due to this rotation along its own axis is named spin.

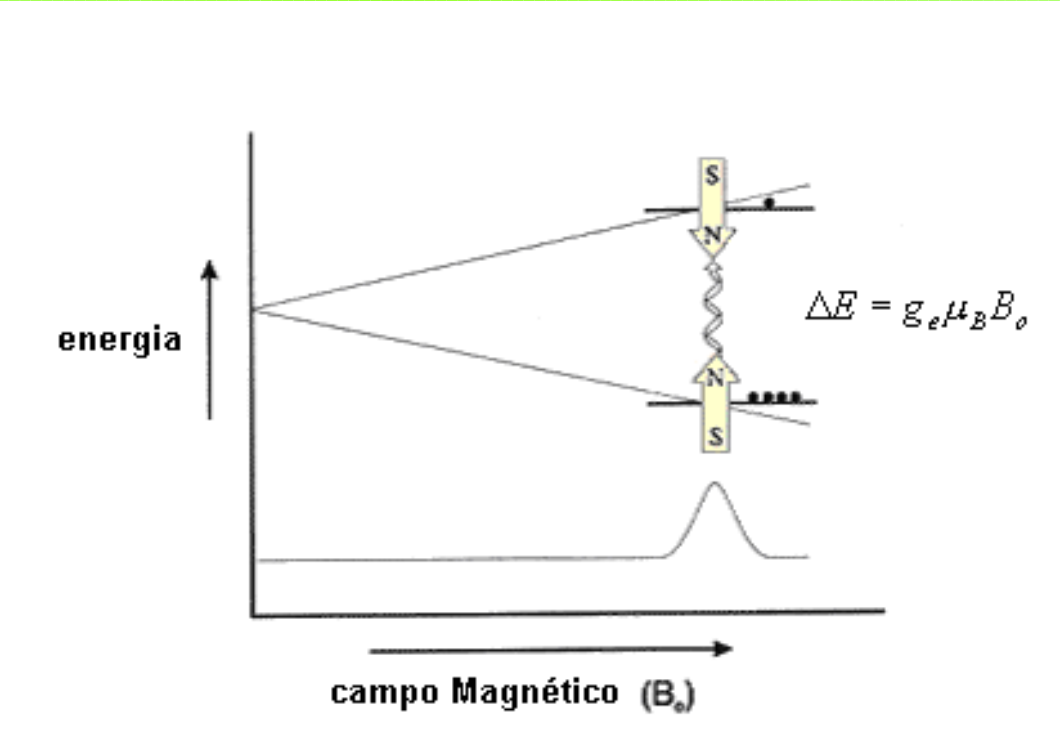
This rotation produces a magnetic moment. Therefore the electron may be considered equivalent to a tiny magnet.

Electron paramagnetic resonance

As the electrons are usually paired in atoms, with opposite spins, there is no net magnetic moment. However, if the atoms are ionized the electrons can be unpaired, forming the free radicals.

Without an external magnetic field these spin states are degenerated, i.e., they have the same energy.

In an external magnetic field these energy states are separated and if the sample is submitted to an appropriate microwave, the spins can change from one state to another. The ESR measurement is the detection of such resonance.



Some ESR Applications

- Dating
- Dosimetry
- Microscopy (imaging)

European Norms for the detection of irradiated Foods

- EN 1786:1996 Detection of irradiated food containing bone - Method by ESR spectroscopy
- EN 1787:2000 Detection of irradiated food containing cellulose by ESR spectroscopy
- EN 13708:2001 Detection of irradiated food containing crystalline sugar by ESR spectroscopy

Objectives

The aim of the present study was to investigate the stability of free radicals generated by radiation processing on three different soybean cultivars and to verify any correlation among their constituents and the EPR intensity. Scavenging and antioxidant activities of soybean can be involved on the response of the free radicals generated by radiation.

Material and Methods

Material.

Three soybean cultivars from National Soybean Research Center (EMBRAPA-SOJA), Londrina, Brazil, were investigated.

The samples were sieved and gamma-irradiated.

Composition of varieties employed

Isoflavone, protein and oil content of the cultivars evaluated.

Cultivar	Isoflavones (mg/100g)	Protein (%)	Oil (%)
BRS 94	**210**	**39.1**	**20.6**
BRS 155	**174**	***41.0***	**22.6**
BRS 231	**124**	**40.2**	**21.2**

Irradiation

- Co-60 γ-irradiation
- **0, 2.0, 5.0, 10.0 and 15.0 kGy**
- dose rate of 3.5kGy/h
- dose uniformity factor 1.13.

GammaCell 220 and soybeans prepared for irradiation

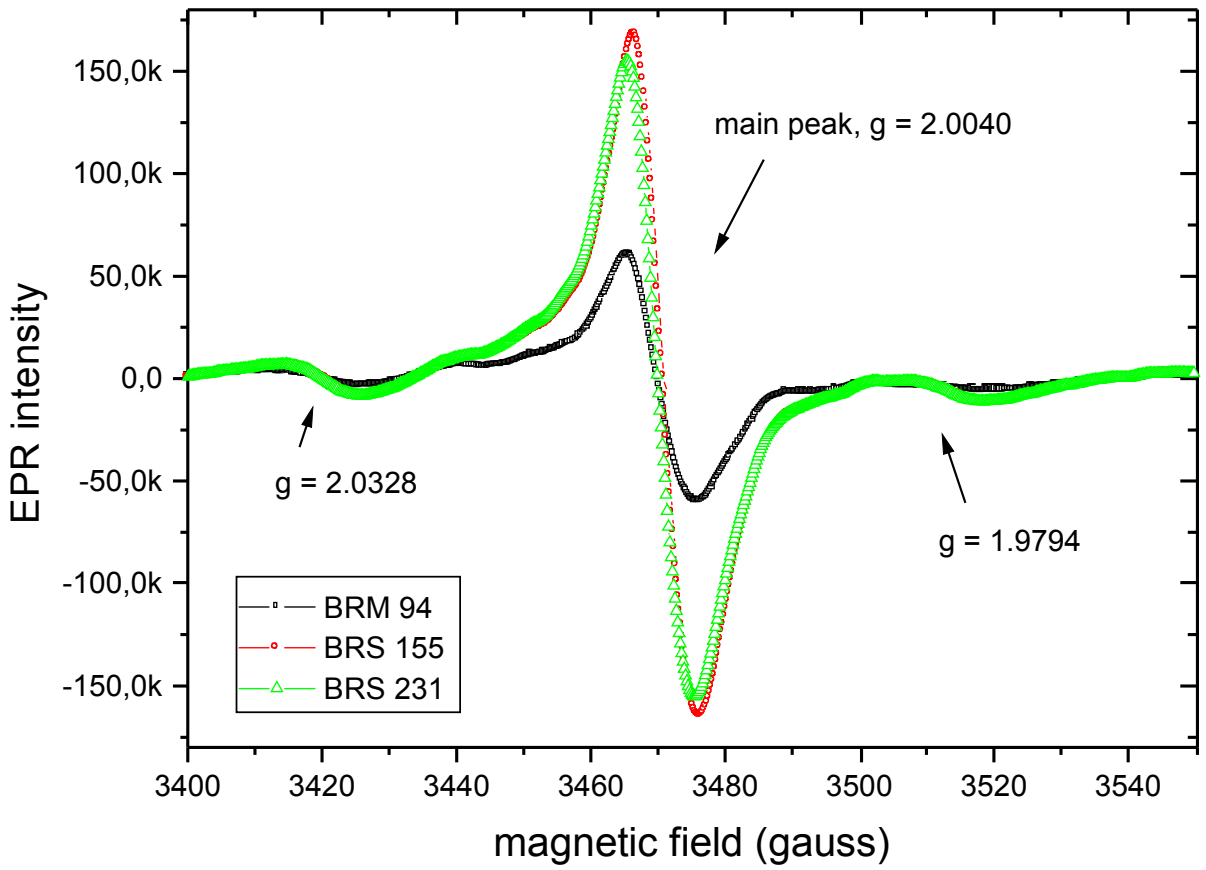
EPR Determination

- **X-band spectrometer (ER 041 XG Microwave Bridge, Bruker) from "Laboratório de Biofísica do Instituto de Física da Universidade de São Paulo", with five scans and most of the time with the following parameters:**
  Receiver: 6.32.104(gain), 7 deg.(phase), 100 kHz (mod. freq.) and 2G (mod. amp.);
  Field: 3450G (center), 200 G (width), 1024 pts (resolution);Signal Channel: 81.92 ms (conv.), 327.68 ms (t. const.), 83.886 s (sweep t.);
- **Microwave: around 9.77 GHz (freq.) and 10.1 mW (power).**

EPR Spectrometer

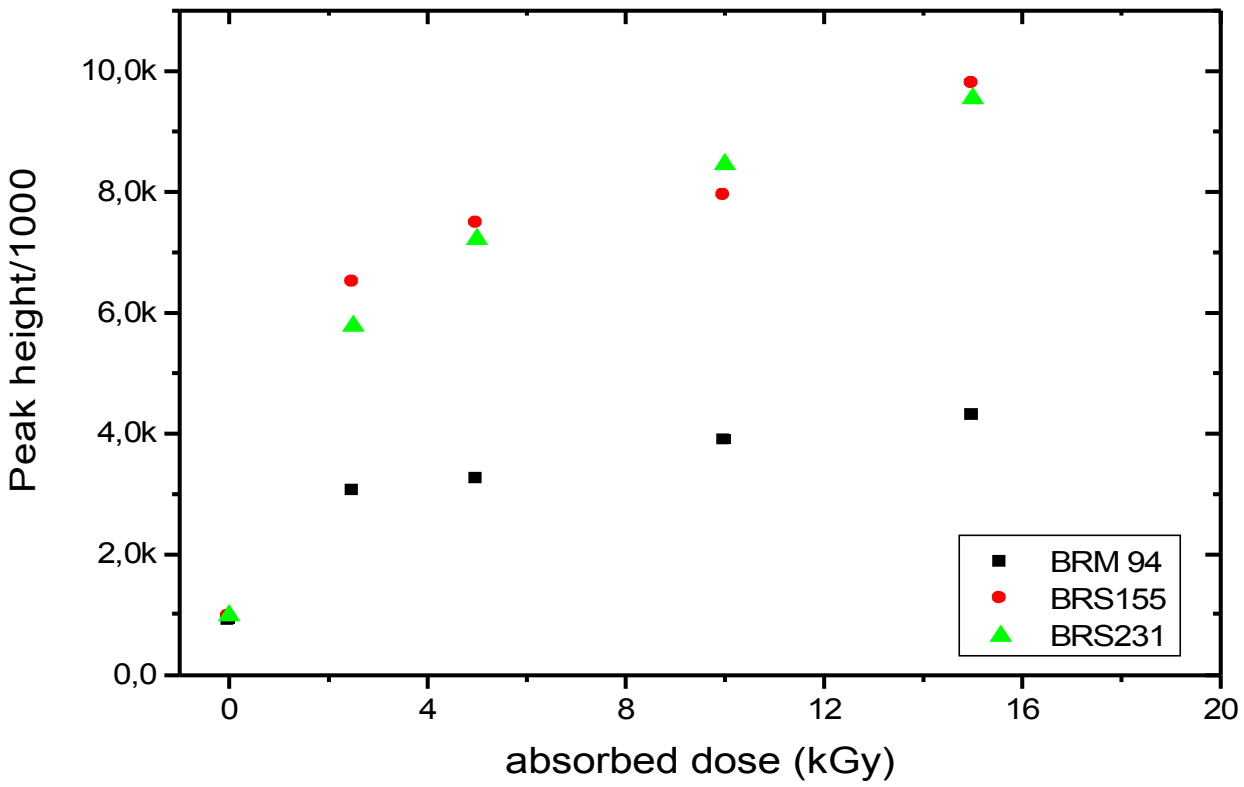
Results

- For the present work we have investigated just the interval containing the main peak and two central lines of allegedly Mn2+.
- The **unirradiated** samples presented an EPR spectra composed of six lines of nearly the same intensity, separated by around 93 gauss and with g values of 2.0328 and 1.9794 for the two central lines. This peak is probably due to Mn2+, often observed in organic materials
- The **irradiated** samples showed, besides the former six-line signal, **a main peak with intensity four times higher** than the last, and with g value of 2.0040. In some scans the main peak was also observed for non irradiated samples, but with practically null intensity, and then could be used to verify whether the sample were irradiated or not.



EPR signal of the three cultivars irradiated with 15 kGy, at room temperature, four days after he irradiation. The intensity was obtained with five scans

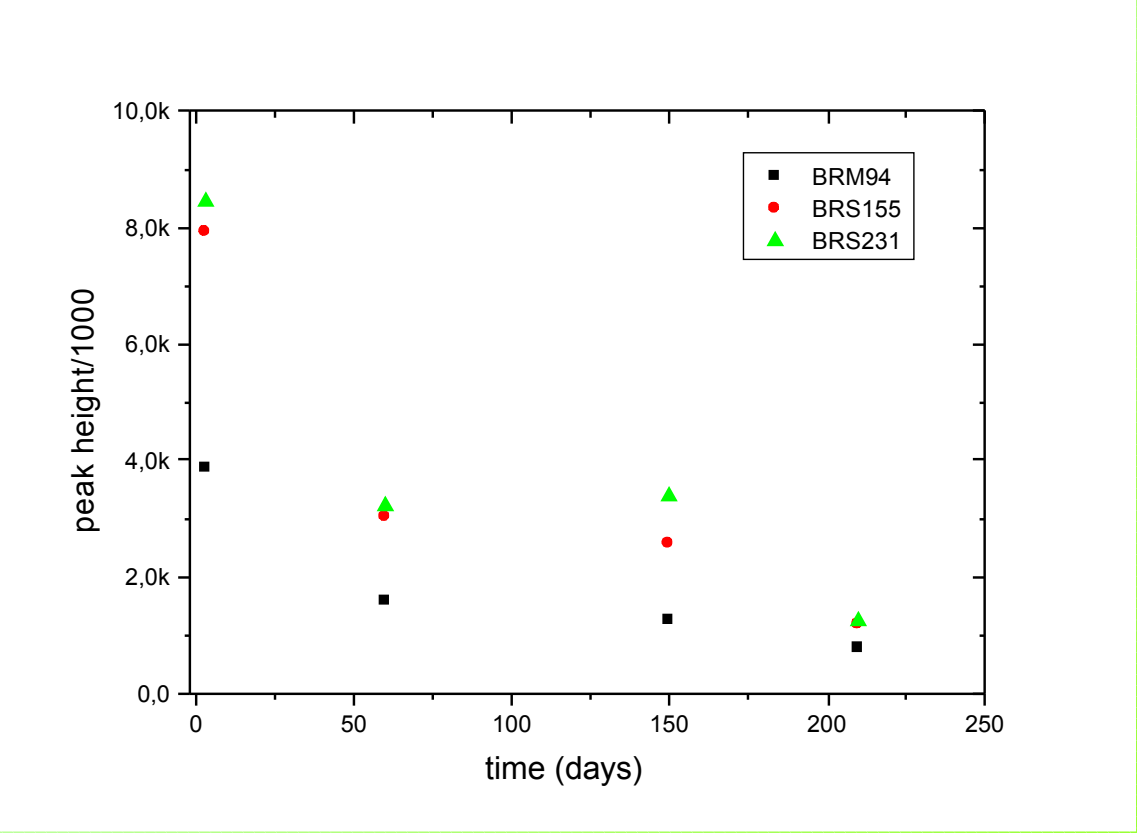
- The behavior of EPR signal intensity is linear with the ionizing radiation dose for the range assayed
- The sample with the highest total isoflavone content presented the smallest peak. However, this inverse relationship among isoflavone content and the number of free radicals formed, can not be sustained if we compare the two other cultivars. In that case, their intensities are almost the same .



EPR signal intensity (integrated peak) of the main peak as a function of the absorbed dose, 4 days after irradiation.

Stability of Signal

The irradiation carried out at room temperature just after the grinding process. The EPR measurements recorded at **4, 60, 150 and 210 days after irradiation**, soybean samples kept at 4-7°C.



Conclusion

- **The free-radicals responsible for the EPR signal presented a good stability especially in the interval between two and five months after irradiation.**
- **A good linear relationship was observed between EPR signal intensity and absorbed dose;**
- **As to the correlation between isoflavone content and the EPR intensity, it is only possible to suspect of an antioxidant action. A more representative group of cultivars, with important differences in their isoflavone contents as well as other constituents, should be investigated before attributing such a role to these phytochemicals.**

Acknowledgement

The authors are grateful to the Brazilian National Research Council (**CNPq**) for the Doctorate (OLIVEIRA) and the Research Productivity (MASTRO) fellowships. We also acknowledge Dr. Jose M. G. MANDARINO from **EMBRAPA**, for providing the soy samples and for his helpful communication and information exchanges. A special thanks for the **Biophysics Laboratory-University of São Paulo**, where the author performed the measurements.