Radiation Physics and Chemistry 81 (2012) 1516–1519

Contents lists available at SciVerse ScienceDirect

Radiation Physics and Chemistry

journal homepage: www.elsevier.com/locate/radphyschem

Radiation-induced electron paramagnetic resonance signal and soybean isoflavones content

Marcos R.R. de Oliveira [a], José M.G. Mandarino [b], Nelida L. del Mastro [a,*]

[a] Nuclear and Energy Research Institute, IPEN—CNEN/SP, São Paulo, SP, Brazil
[b] Embrapa Soybean, Londrina, PR, Brazil

ARTICLE INFO

Article history:
Received 23 August 2011
Accepted 12 December 2011
Available online 20 January 2012

Keywords:
Soybean
Isoflavone
Electron Paramagnetic Resonance (EPR)
Ionizing radiation

ABSTRACT

Electron Paramagnetic Resonance (EPR) is a well-known spectroscopic technique that detects paramagnetic centers and can detect free radicals with high sensitivity. In food, free radicals can be generated by several commonly used industrial processes, such as radiosterilization or heat treatment. EPR spectroscopy is used to detect radioinduced free radicals in food. In this work the relation between EPR signal induced by gamma irradiation treatment and soybean isoflavones content was investigated. Present results did not show correlation between total isoflavones content and the EPR signal. Nevertheless, some isoflavone contents had a negative correlation with the radiation-induced EPR signal.

1. Introduction

The human consumption of soybean, *Glycine max* (L.) Merrill, has increased considerably in the last years. With a chemical composition rich in two macronutrients, protein and oil, it also contains flavonoids as minor components, with soybean being the most common source of isoflavones in human food.

It is evident from epidemiological studies and numerous experimental works the health benefits inherent to soy foods (Kuo, 1997; Barnes, 1998; Riesco et al., 2010). Among the components studied, isoflavones and proteins are the most likely responsible for those benefits (Tikkanen and Adlercreutz, 2000; Watanabe et al., 2002; Hillman and Singh-Gupta, 2011). Isoflavones from soybeans are known to have several biological properties such as estrogenic, antimicrobial and antitumoral activities, besides inhibiting the activity of enzymes linked to cellular division (Hyunki et al., 2006; Velazquez et al., 2010).

Flavonoids are found in all higher plants, many display strong absorption in the UVB spectral region. It has been suggested that they play a role in the protection of plants by screening vital cellular components from damaging UV radiation. Plants use isoflavones and their derivatives as part of the plant's defensive arsenal, to ward off disease-causing pathogenic fungi and other microbes. In addition, soybean uses isoflavones to stimulate soil-microbe rhizobium to form nitrogen-fixing root nodules and could act as antioxidant i.e. quench oxidation by transferring hydrogen atoms to free radicals. It was suggested that there is a correlation among antioxidant activity and the content of the flavonoids and total phenolics Barbosa et al., 2006.

There are 12 different forms of isoflavones in soybeans: the malonyl glucosides, the acetyl glucosides, the glucosyl glucosides or beta glucosides (daidzin, genistin and glycitin) and the aglycons named diadzein, genistein and glycitein.

Permissible energy sources for foodstuffs treatment include gamma rays, electrons and x-rays. As other food processes, irradiation produces physical and chemical changes. In this work an investigation of the relation between electron paramagnetic resonance (EPR) signal induced by gamma irradiation treatment and soybean isoflavones content has been performed.

2. Material and methods

Seven samples of Brazilian soybean cultivars were obtained from the Embrapa Soybean, Londrina, PR, Brazil.

The soybean samples were gamma-irradiated with doses of 0, 2.5, 5.0, 10.0 and 15.0 kGy, dose rate about 3 kGy/h in a ^{60}Co Gammacell 220 (AECL).

Isoflavones contents were determined after extraction with 70% ethanol containing 0.1% acetic acid by High Performance Liquid Chromatography (HPLC) following the methodology described by Berhow (2002).

For the resonance measurements the soybean grains were grounded in a coffee grind mill, and wrapped in plastic packs of 6 g each. The EPR measurements were performed on an X-band spectrometer (ER 041 XG Microwave Bridge, Bruker). Although

* Corresponding author. Tel.: +55 11 3133 9829; fax: +55 11 3133 9765.
E-mail address: nlmastro@ipen.br (N.L. del Mastro).

0969-806X/$ - see front matter © 2012 Elsevier Ltd. All rights reserved.
doi:10.1016/j.radphyschem.2011.12.010

radiation and EPR readings were carried out at room temperature, when not being used, the samples were kept at temperatures of around 5 °C. The parameters used were receiver gain: 6.32×10^4; modulation frequency: 100 kHz; modulation amplitude: 2 G; conversion: 81.92 ms; time constant: 327.68 ms; sweep time: 83.886; number of scans: 5; microwave: 10 mW and around 9, 8 GHz; sweep field: 200 G; being the spectra showed in the derivative form. A DPPH sample with well known g was also used, in order to correct some possible field deviations of the measured field.

Linear correlations among isoflavones content and EPR intensity signals were done through statistic calculation with the t-Sudent parameter distribution. For a measured value of r (quotient of covariance among the variables and variables standard deviations), the respective t-value is calculated using the expression

$$t_{n-2} = r\sqrt{n-2/1-r^2}$$

$n-2$ being the number of freedom degrees.

3. Results and discussion

Table 1 presents the protein and oil composition of the cultivars evaluated.

The total and partial contents of isoflavones are listed in Table 2. Although particularly rich in daidzein and genistein as other authors mentioned too (Couto et al., 2011), there are great differences in partial and total isoflavone content among the different cultivars coming from the same sowing local and growing season.

Kasuga et al. (2006) analyzing six varieties of soy also found differences in total content of isoflavones, higher than 100%. They also registered a decrease in glucosyl glucosides isoflavones and increase of malonyl glucosides isoflavones by heating. Barbosa et al. (2006) had already mentioned that total and partial isoflavones' contents as well as other phenolics from soybean depend on the processing and the antioxidant capability of these products that varies significantly. They also found a high correlation between antioxidant activity and flavonoids content, a fact that can explain the importance of flavonoids as antioxidant agents with potential in reducing the risk of chronic diseases. Recently, Chung et al. (2011) and Silva et al. (2011) corroborated that processing, fermentation and aging treatment affected content and profile of phenolic compounds in soybean.

Britz et al. (2008) found a similar discrepancy when analyzing tocopherols, another important antioxidants present in soybean. They also found that α-tocopherol in soybean seeds increased several folds as a result of warmer temperature or drought during seed maturation, but total tocopherols stays approximately constant.

It was already established that upon gamma-ray irradiation, a new EPR signal was detectable in the vicinity of $g=2.0$ region that can be used as indicator in the identification of irradiated soy samples (Oliveira and Mastro, 2007).

In Fig. 1 the EPR spectra of the seven irradiated soybean cultivars are presented. Non-irradiated samples from all cultivars always presented a very weak signal compared with those irradiated.

In order to investigate the possible relationship between isoflavone contents and EPR signal intensity, linear correlations for all doses were calculated.

As can be seen in Table 3 there are no correlation between total isoflavones contents and the radiation-induced EPR signal. The strongest correlations were observed for the glycitin (β-glycoside form). In that case a negative correlation was observed that can indicate a free radicals scavenging behavior. The reduction of the radiation-induced oxidative stress suggests that this isoflavone is acting as antioxidant, i.e., quenching oxidation by transferring hydrogen atoms to free radicals. Malonyl glicitin glucosides, on the other hand, presented a slight positive correlation than could suggest a prooxidant activity. Free isoflavones (aglycone forms) are believed to be closely associated to antioxidant activity. Nam et al. (2011) hypothesized that the conversion of isoflavone glycosides—contained in soybean—into aglycones enhances the antioxidant capacity. In the present study no correlation among aglycone forms contained in soybean seeds and EPR radiation-induced free radicals was possible to notice. Variyar et al. (2004) described some radiation-induced enhancement of antioxidant contents of soybean, while others (Ademiluyi et al., 2010) found that fermentation increased the phenolic content and antioxidant capacity of fermented soybean. Weiss and Landauer (2003) had also described that antioxidant activity of phytochemicals like genistein resulted in protection against radiation effects. Other records from the literature relate irradiation treatment and antioxidant behavior of soybean flavonoids. Song et al. (2006) found protective effects of soybean isoflavones against gamma-irradiation induced oxidative damages in mice. As Heim et al. (2002) had already pointed out, the structural heterogeneity of flavonoids, their multiple mechanisms of action and the diverse methods used to evaluate their antioxidant activity pose challenges in assembling a collective hierarchy of structure-activity relationships.

Table 1
Protein and oil composition of the soybean cultivars

Cultivars	Protein (%)	Oil (%)
BRS 155	41.0	22.6
BRS 156	38.6	21.6
BRS 183	40.6	20.6
BRS 216	43.6	17.6
BRS 231	40.2	21.2
BRS 233	40.9	19.5
BRS 267	39.1	20.6

Table 2
Total and partial contents of soy isoflavones in mg/100 g of dry material.

Cultivar	Beta			Malonyl			Aglicons		Total
	daidzin	glicitin	genistin	daidzin	glicitin	genistin	daidzein	genistein	
BRS267	23.8	49.2	14.9	2.6	2.7	26.3	5.1	4.1	128.7
BRS155	19.4	6.7	26.1	17.9	5.6	19.7	3.9	2.7	101.8
BRS156	13.7	17.5	28.0	8.4	8.4	32.5	2.9	1.9	113.2
BRS183	9.2	16.1	13.5	6.5	6.6	25,2	0.0	0.0	77.0
BRS216	10.3	18.2	12.8	8.0	8.1	19.2	1.6	1.2	79.4
BRS231	9.6	6.8	7.4	19.9	9.9	14.5	0.0	0.0	68.1
BRS233	12.9	31.9	19.8	8.3	8.4	40.2	2.0	1.4	124.9

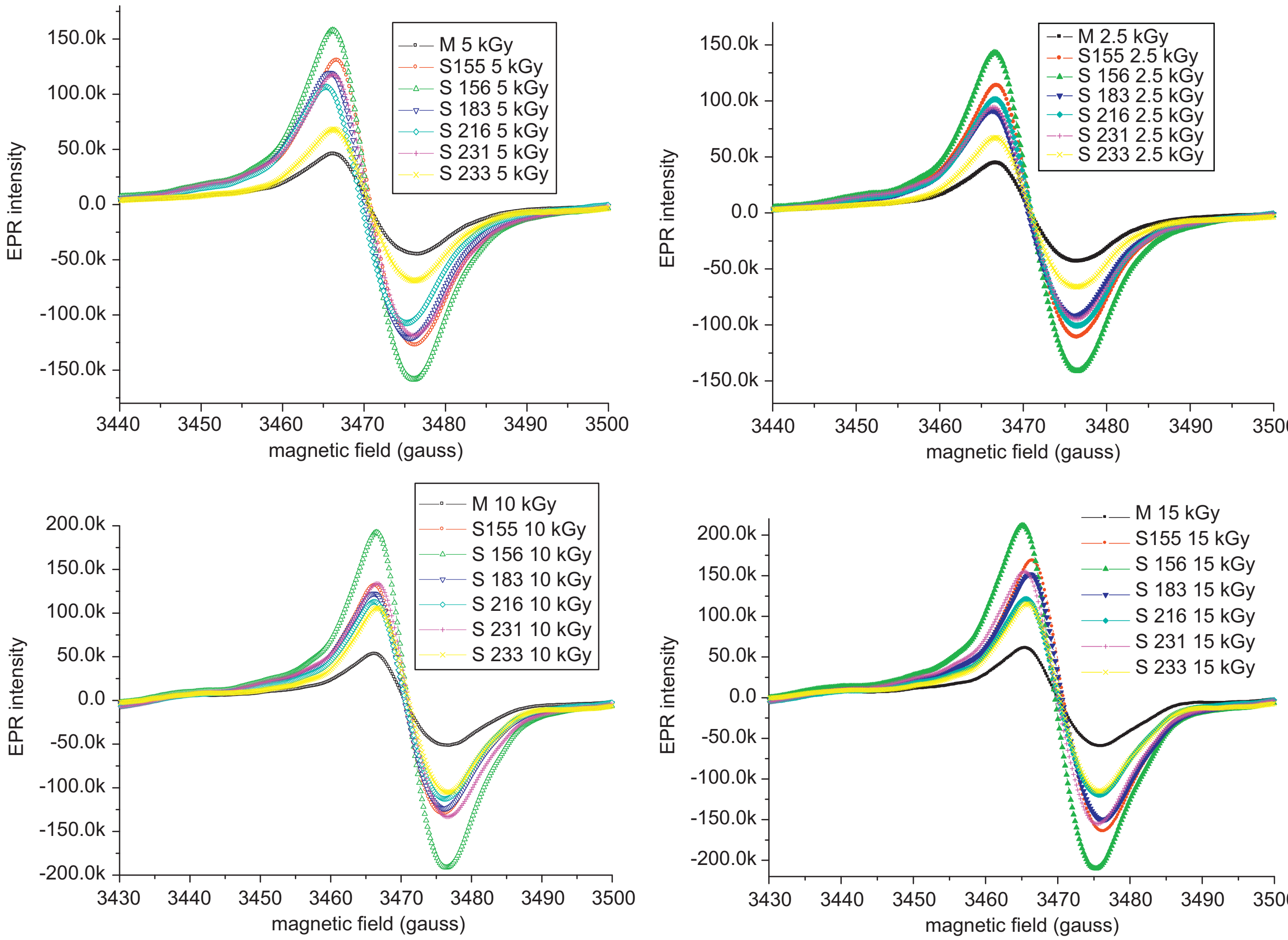

Fig. 1. EPR spectra for different cultivars irradiated with doses of (top right counterclockwise): 2.5 kGy, 5.0 kGy, 10.0 kGy and 15.0 kGy.

Table 3
Linear correlation among main peak EPR intensity and partial and total isoflavones contents, for each radiation dose employed.

Dose	Beta			Malonyl			Aglicons		Total
(kGy)	daidzin	glicitin	genistin	daidzin	glicitin	genistin	daidzein	genistein	
2.5	−0.38	−0.76	0.49	0.41	0.51	−0.16	−0.22	−0.32	−0.34
5.0	−0.46	−0.84	0.37	0.48	0.51	−0.26	−0.35	−0.44	−0.48
10.0	−0.48	−0.71	0.47	0.40	0.65	−0.04	−0.34	−0.44	−0.27
15.0	−0.43	−0.78	0.48	0.49	0.57	−0.05	−0.33	−0.43	−0.32

4. Conclusion

Present results did not show linear correlation between total isoflavones contents and the EPR signal produced by ionizing radiation. Nevertheless, some glucosyl glucosides or beta glucosides isoflavones presented a negative linear correlation with their corresponding EPR intensity suggesting antioxidant behavior.

References

Ademiluyi, A.O., Oboh, G., Ademosun, A.O., 2010. Antioxidant properties of soy-daddawa: a condiment produced from fermented soybean. Riv. Ital. Sostanze Grasse 87, 226–235. (the).

Barbosa, A.C.L., Hassimotto, N.M.A., Lajolo, F.M., Genovese, M.I., 2006. Teores de isoflavonas e capacidade antioxidante da soja e produtos derivados. Ciênc. Tecnol. Aliment. (Campinas) 26, 921–926.

Barnes, S., 1998. Evolution of the health benefits of soy isoflavones. Proc. Soc. Exp. Biol. Med. 217, 386–392.

Berhow, M.A., 2002. Modern analytical techniques for flavonoid determination. In: Buslig, B.S., Manthey, J.A. (Eds.), Flavonoids in the Living Cell. Klusher Academic, New York, pp. 61–76.

Britz, S.J., Kremer, D.F., Kenworthy, W.J., 2008. Tocopherols in soybean seeds: genetic variation and environmental effects in field-grown crops. J. Am. Oil Chem. Soc. 85, 931–936.

Chung, I.M., Seo, S.H., Ahn, J.K., Kim, S.H., 2011. Effects of processing, fermentation, and aging treatment to content and profile of phenolic compounds in soybean seed, soy curd and soy paste. Food Chem. 127, 960–967.

Couto, C., Silva, L.R., Valentao, P., et al., 2011. Effects induced by nodulation with *Bradyrhizobium japonicum* on *Glycine max* (soybean) metabolism and antioxidant potential. Food Chem. 127, 1487–1495.

Heim, K.E., Tagliaferro, A.R., Bobilya, D.J., 2002. Flavonoid antioxidants: chemistry, metabolism and structure-activity relationships. J. Nutr. Biochem. 13, 572–584.

Hillman, G.G., Singh-Gupta, V., 2011. Soyisoflavones sensitize cancer cells to radiotherapy. Free Radical Biol. Med. 51, 289–298.

Hyunki, H., Landauer, M.R., Foriska, M.A., Ledney, G.D., 2006. Antibacterial activity of the soy isoflavone genistein. J. Basic Microbiol. 46, 329–335.

Kasuga, A., Ogiwara, E., Aoyagi, Y., Kimura, H., 2006. Changes in isoflavone content of soybeans during heating process. J. Jpn. Soc. Food Sci. Technol. 53, 365–372.
Kuo, S.M., 1997. Dietary flavonoid and cancer prevention: evidence and potential mechanism. Crit. Rev. Oncogenesis 8, 47–69.
Nam, D.H., Kim, H.J., Lim, J.S., et al., 2011. Simultaneous enhancement of free isoflavone content and potential of soybean by fermentation with *Aspergillus oryzae*. J. Food Sci. 76, H194–H200.
Oliveira, M.R.R., Mastro, N.L.d., 2007. Electron paramagnetic resonance study of some varieties of gamma-irradiated soybean. Radiat. Phys. Chem. 76, 1459–1462.
Riesco, E., Aubertin-Leheudre, M., Maltais, M.L., et al., 2010. Synergic effect of phytoestrogens and exercise training on cardiovascular risk profile in exercise-responder postmenopausal women. A pilot study. Menopause 17, 1035–1039.
Silva, L.H., Celeghini, R.M.S., Chang, Y.K., 2011. Effect of the fermentation of whole soybean flour on the conversion of isoflavones from glycosides to aglycones. Food Chem. 128, 640–644.
Song, L.H., Yan, H.L., Cai, D.L., 2006. Protective effects of soybean isoflavone against gamma-irradiation induced damages in mice. J. Radiat. Res. 47, 157–165.
Tikkanen, M., Adlercreutz, H., 2000. Dietary soy-derived isoflavone phytoestrogens. Biochem. Pharmacol. 60, 1–5.
Variyar, P.S., Limaye, A., Sharma, A., 2004. Radiation-induced enhancement of antioxidant contents of soybean (*Glycine max Merrill*). J. Agric. Food Chem. 52, 3385–3388.
Velazquez, E., Silva, L.R., Alvarez-Martinez, E., Peix, A., 2010. Legumes: a healthy and ecological source od flavonoids 6, 109–144Curr. Nutr. Food Sci. 6, 109–144.
Weiss, J.F., Landauer, M.R., 2003. Protection against ionizing radiation by nutrients and phytochemicals. Toxicology 189, 1–20.
Watanabe, S., Uesugi, S., Kikuchi, Y., 2002. Isoflavones for prevention of cancer, cardiovascular diseases, gynecological problems and possible immune potentiation. Biomed. Pharmacotherapy 56, 302–312.