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THE EFFECTS OF GAMMA IRRADIATION ON SOYBEAN ISOFLAVONES CONTENTS

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ABSTRACT

Soybean (*Glycine max*) is the most common source of isoflavones in human feeding. It was suggested that there is a correlation among antioxidant activity of flavonoids and total phenolics content. Plants use isoflavones and their derivatives as part of the plant's defensive arsenal, to ward off disease-causing pathogenic fungi and other microbes. Highly processed foods made from legumes, such as tofu, retain most of their isoflavone content, with the exception of fermented miso, which has increased levels. Little is known about the influence of oxidative stress induced by radiation on the isoflavones contents. In the present paper, the effects of gamLma irradiation on soybean isoflavones contents are presented. Samples from several Brazilian soybean cultivars were gamma-irradiated with doses of 0, 1, 2, 5 e 10 kGy, dose rate about 3kGy/h in a ^{60}Co (Gammacell 220 – AECL). Isoflavones contents were determined after extraction with 70% ethanol containing 0.1% acetic acid by an HPLC method. The total isoflavone content remained almost unchanged with the increase of radiation dose up to 10kGy. Although a general correlation among total isoflavone content and radiation dose was not found, some data suggest that for a few of the isoflavones from specific cultivars, the increase in the radiation dose induced a decrease in their content as for glucosyl glucosides and malonyl isoflavones, as well as an increase in their aglycone content.

1. INTRODUCTION

There are twelve kinds of isoflavones contained in soybeans. They can be grouped in four forms: aglycons, malonylglucosides, acetylglucosides and glucosylglucosides. Isoflavones from soybean are known to have several biological properties such as estrogenic, antimicrobial and antitumoral activities, besides inhibiting the activity of enzymes linked to the cellular division. These properties are shown to be more accentuated in the aglycone than glycoside forms [1-3].

Studies have found a protective effect of soybean isoflavone against gamma-irradiation induced damages in mice [4]. According to Hyunki et al. [5] genistein, an aglycone, is a protein kinase inhibitor and able to block the invasion of pathogenic bacteria in mammalian epithelial cells. Furthermore, these authors suggested that the use of genistein in combination with probiotics may augment the effectiveness of antimicrobial therapies currently used in the management of infections.

Irradiation can be used to treat a wide variety of food stuffs for control of microorganisms and insects. Permissible energy sources include gamma rays, electrons and x-rays. As other food processes, irradiation produces physical and chemical changes. This paper reports the influence of gamma irradiation on the total and partial isoflavones contents of soybean.

2. MATERIAL AND METHODS

2.1. Soy cultivars

Seven varieties of soybean, crop year 2005, developed by the Genetic Improvement Program from Empresa Brasileira de Pesquisa Agropecuaria (EMBRAPA) were employed: BRS155, BRS156, BRS183, BRS216, BRS231, BRS233 and BRS267. The cultivars were grown in Paraná, Brazil (Londrina, 23°11′50″S).

2.2. Irradiation

The soybean cultivars were γ-irradiated with doses of 0, 1.0, 5.0 and 10.0 kGy, by using a ^{60}Co source (Gammacell 220 – AECL) at a dose rate about 3kGy/h.

2.3. Isoflavone determination by HPLC.

Isoflavones contents were determined after extraction with 70% ethanol containing 0.1% acetic acid following method described by Carrao-Panizzi et al. [6]. Quantification of the diverse isoflavones was made by High Performance Liquid Chromatography (HPLC) following methodology described by Berhow [7] and results were expressed in $mg.100g^{-1}$.

3. RESULTS AND DISCUSSION

Figure 1 shows the total isoflavones content of seven soy cultivars irradiated with doses of 0; 1; 5 and 10 kGy. The measurements were repeated at least twice and the mean error was no higher than 5%. There was no loss of isoflavones as a consequence of radiation treatment, within a significance level of 5% (t-Student). For most of the samples the isoflavones content remain almost unchanged, excepted for cultivar BRS231, where an increase was evident for the highest dose applied.

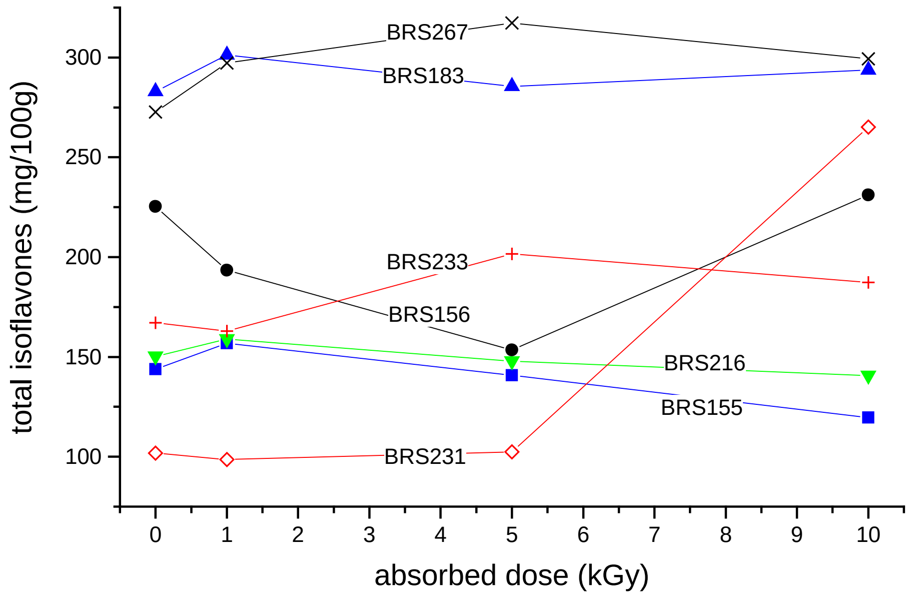

Figure 1.Total isoflavones content of seven soy cultivars, irradiated with doses of 0; 1; 2; 5 and 10kGy.

Moussaid et al. studied phenolic compounds and the color of oranges subjected to a combination treatment of waxing and irradiation [8]. Various studies demonstrated the loss of soy isoflavones during storage and the effect of processing conditions on specific modifications of soy isoflavones. Beyond apparent 1st-order degradation kinetics, there is a change in the isoflavones profile according to processing and storage conditions [9]. Irradiation treatment on the present work did not produce any decrease in total isoflavones content.

Variyar et al. found that antioxidant potential measured as percent 1,1-diphenyl-2-picrylhydrazyl scavenging activity showed an increasing trend with dose, indicating that radiation processing, as a method of food preservation, has a positive nutritional implication with soybeans [10].

It was also determined the contents of the total glucosyl-glucosides (daidzin, glicitin and genistin) as a function of irradiation absorbed dose (Figure 2).

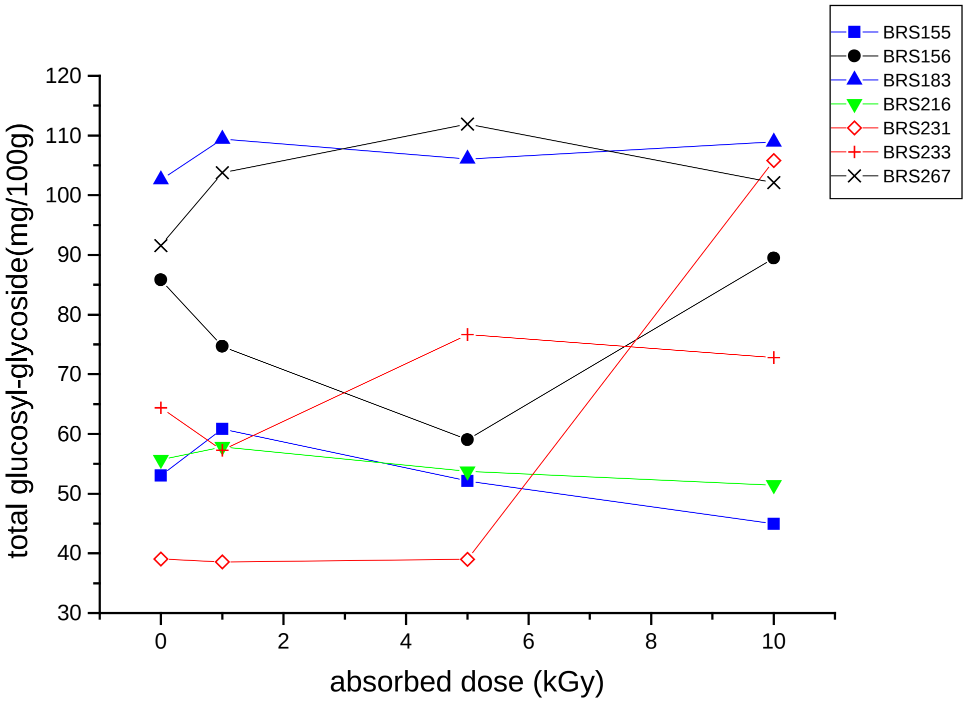

Figure 2. Total glucosyl-glucosides content for seven soy cultivars, irradiated with doses of 0; 1; 2; 5 and 10 kGy.

The behavior of glucosyl-glucosides isoflavone content from the different cultivars were no uniform. While BRS231 presented again an increase at the highest dose used, cultivars BRS216 and BRS155 had a slight decrease in their content as the radiation dose increases.

Figure 3 shows the dependence of malonyl isoflavones content and the radiation dose. Malonyl and glucosyl glucosides had a similar behavior. Cultivars BRS216 and BRS155 also presented a reduction in their isoflavone contents with increasing radiation doses.

Data presented in figures 1, 2 and 3 make evident that cultivars BRS183 and BRS267 presented the highest levels of total or partial (glucosyl-glucosides and malonyl-glucosides) isoflavones. Also, isoflavone content from cultivars BRS155, BRS183 and BRS267 resulted more stable against radiation treatment. On the other hand, isoflavone content from cultivar BRS231 seemed to be highly modified by irradiation when the applied dose was 10kGy.

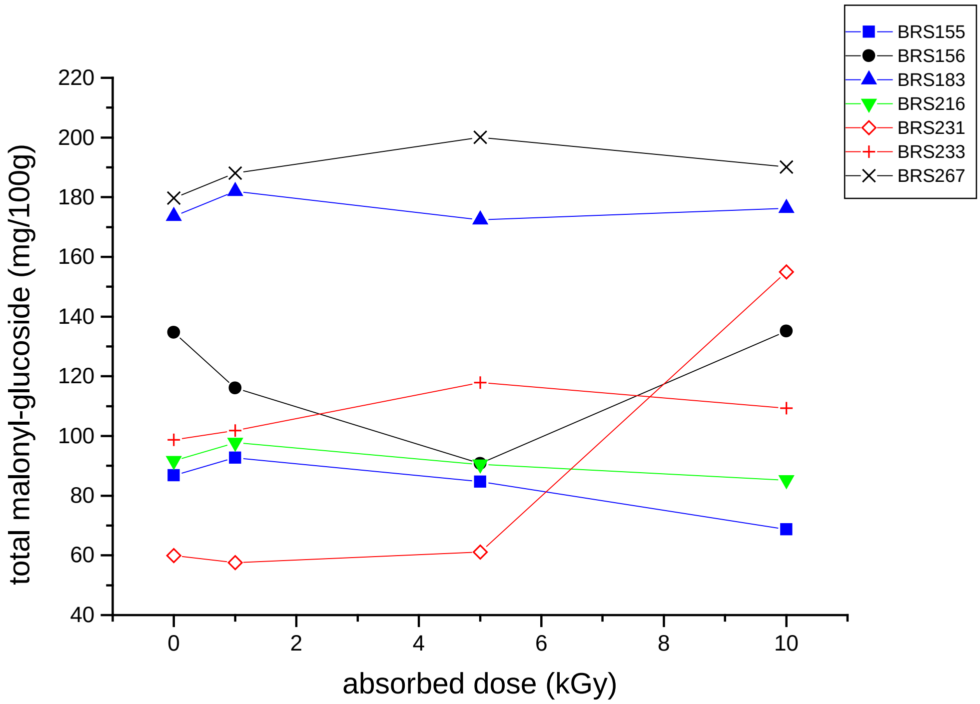

Figure 3. Total malonyl content for seven soy cultivars, irradiated with doses of 0; 1; 2; 5 and 10 kGy.

In Figure 4 the behavior of the total aglycone contents (genistein, daidzein, and glycitein) as a function of radiation dose is illustrated. Most of the cultivars exhibited an increase in their isoflavone contents, with increasing radiation doses.

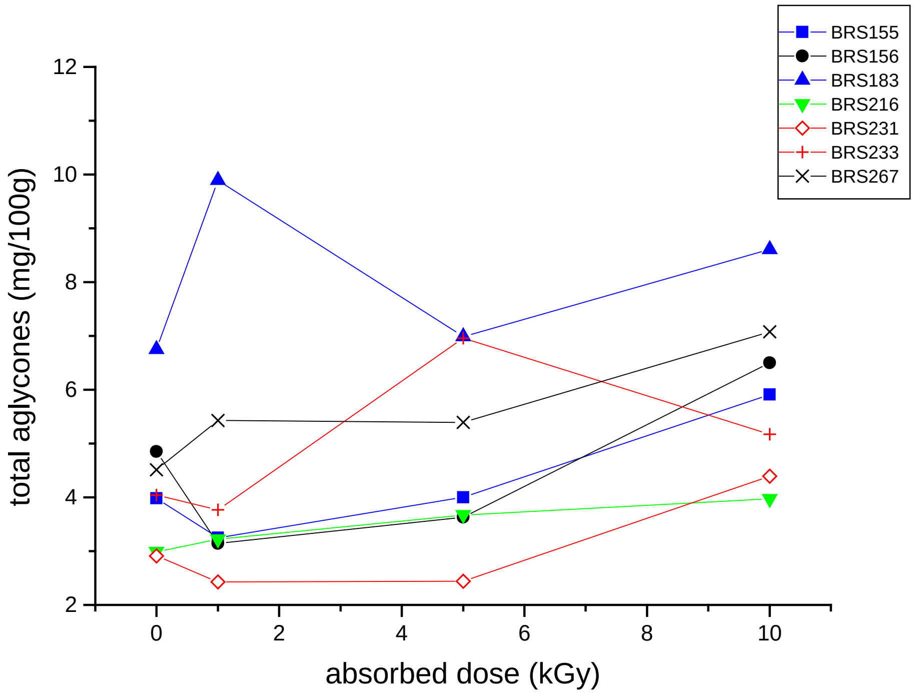

Figure 4. Total aglycone content for seven soy cultivars, irradiated with doses of 0; 1; 2; 5 and 10 kGy.

Similarly as present results, Kasuga et al. [11] analyzing six varieties of soy found differences in total content of isoflavones, higher than 100%. But they registered a decrease in glucosyl glucosides isoflavones and increase of malony glucosides isoflavones by heating.

According to Park et al. [12] malonyl are the most abundant in non processed soybeans. Our results also showed a high percentage of this partial isoflavone. When soy is submitted to processing, where high temperature and pressure are involved, these isoflavones are converted to acetyl forms and then to the glucosyl glucosides. On the other hand, fermentative processes can convert glucosides to the corresponding aglycones by the increase of the beta-glucosidase activity [13].

Total and partial isoflavone contents, as well as other phenolics from soybean, depend on the processing. The antioxidant capability of these products vary significantly [14]. Studies have found a high correlation between antioxidant activity and flavonoids content showing the importance of flavonoids as antioxidant agents with potential in reducing the risk of chronic diseases [14].

3. CONCLUSIONS

The total isoflavone content remained almost unchanged with the increase of radiation dose up to 10kGy. That stability was more evident in cultivars BRS 155, BRS183 and BRS267. The last two presented also the highest isoflavone content. A general correlation among total isoflavone contents and radiation doses was not found. Data from some cultivars suggest that increasing radiation doses induced a decrease in the glucosides contents, followed by and increase in their aglycon forms. Those variations could be ascribe to a conversion from the glucoside forms into aglycones, which are particularly more bioavailable for humans.

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REFERENCES

1. Tikkanen, M.; Adlercreutz, H. Dietary soy-derived isoflavone phytoestrogens. *Biochemical Pharmacology*, Vol. 60, pp.1-5, (2000).
2. Tsukamoto, C.; Kudou, S.; Kikuchi, A. et al. Isoflavones in soybean products: composition, concentration and physiological effects. *Documentos da Embrapa*, Vol. 169, n.1, pp.9-14 (2001).
3. Watanabe, S.; Uesugi, S., Kikuchi, Y. Isoflavones for prevention of cancer, cardiovascular diseases, gynecological problems and possible immune potentiation. *Biomedicine & Pharmacotherapy,* Vol. 56, n.6, pp. 302-312 (2002).
4. Song, L.H, Yan HL, Cai DL. Protective effects of soybean isoflavone against gamma-irradiation induced damages in mice. *Journal of Radiation Research*, Vol. 47, n.2, pp. 157-165 (2006).
5. Hyunki, H.,Landauer,M.R. Foriska,M.A. Ledney.G.D. Antibacterial activity of the soy isoflavone genistein. *Journal of Basic Microbiology*, Vol. 46, n. 4, pp. 329-335 (2006)
6. Carrao-Panizzi, M.C.; Favoni, S.P.G.; Kikuchi, A. Extraction time for isoflavone determination. Brazilian Archives of Biology and Technology, Curitiba, v.45, n.4, p.515-518, (2002).
7. Berhow, M. A. Modern analytical techniques for flavonoid determination. In: BUSLIG, B. S.; MANTHEY, J. A. (ed.). Flavonoids in the living cell. New York: Klusher Academic, 2002. p.61-76. (Adv. Exp. Méd. Biol. v. 505).
8. Moussaid M, Lacroix M, Nketsia-Tabiri J, Boubekri C. Phenolic compounds and the colour of oranges subjected to a combination treatment of waxing and irradiation. *Radiat. Phys. Chem*., Vol. 57, n.3-6, pp. 273-275 (2000).
9. Shimoni E. Stability and Shelf Life of Bioactive Compounds during Food Processing and Storage: Soy Isoflavones. *J. Food Sci.,* Vol. 69, n. 6, pp. R160-166 (2004).
10. Variyar, P.S.; Limaye, A.; Sharma, A. Radiation-induced enhancement of antioxidant contents of soybean (*Glycine max* Merrill). *Journal of Agricultural and Food Chemistry*, Vol. 52, n. 11, pp. 3385-3388 (2004).

11. Kasuga, A.; Ogiwara, E.; Aoyagi, Y.; Kimura, H. Changes in isoflavone content of soybeans during heating process. *J. Japanese Soc. Food Sci. Technol.*, Vol. 53, n. 7., pp. 365-372 (2006).
12. Park, Y.K.; Aguiar, L.C.; Alencar, S.M.; Mascarenhas, H.A.A.; Scamparini, A.R.P. Avaliação do teor de isoflavonas em soja brasileira. *Ciência e Tecnologia de Alimentos*, Campinas, Vol. 3, n.3, pp. 156-160 (2001).
13. Carrão-Panizzi, M.C.; Bordignon, J.R. Activity of beta-glucosidase and levels of isoflavone glucosides in soybean cultivars affected by the environment. *Pesquisa Agropecuária Brasileira*, Brasília, Vol. 35, n.5, pp. 873-878 (2000).
14. Barbosa, A.C.L.;Hassimotto, N.M.A.;Lajolo, F.M.; Genovese, M.I. Teores de Isoflavonas e Capacidade Antioxidante da Soja e Produtos Derivados. *Ciênc. Tecnol. Aliment., Campinas*, Vol. 26, n. 4, p. 921-926 (2006).