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Research Article

Effects of gamma irradiation at different combinations of dose-rate and time of exposure on the isoflavone contents of soybean

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Abstract

Even though the effect of irradiation on isoflavone is already known, study on effect of dose-rate on isoflavone content is still lacking. The purpose of this research was to study the influence of dose-rate on the rate of change of isoflavone concentrations. Research on the effect of gamma irradiation at different combinations of dose-rates and times of exposure on the isoflavone content of soybean has been carried out. Soybean samples were irradiated at room temperature (28 ± 2°C) by gamma rays at the dose-rates of 1.30; 3.17; 5.71 and 8.82 kGy/h with time of exposure varied from 0.5 to 55 h. The results showed that gamma irradiation at different dose-rates caused changes in the concentration of the isoflavones. Irradiation at lower dose-rates appears to produce more free daidzein and free genistein than that of irradiation with higher dose-rates. These findings suggest that radiation process at same dose level may potentially be optimized by selecting the most appropriate combination of dose-rate and time of exposure, depending on the objective of irradiation.

Keywords: daidzein, genistein, *Glycine max* L, Indonesia.

Introduction

Legumes such as soybean (*Glycine max* L.) are the main source of isoflavone. Research on the potential health benefits of soybean isoflavones has been widely published, especially associated with its anti-carcinogenicity, anti-oxidant activity, as well as its ability to prevent or inhibit heart attack, osteoporosis and menopause symptoms [1, 2, 3].

In soy beans, isoflavone is mainly in the form of β-glucoside and its aglycone (free from glucose molecule). Isoflavon mainly consists of daidzein, genistein and glycitein [1, 4, 5]. Isoflavone content of soybean varies depending on the degree of processing and also on the type (variety) of soybean [6, 7, 8].

Application of ionizing radiation to cereal products has been applied in the past to reduce toxic and anti-nutritional compounds [9, 10, 11, 12] and to achieve sanitary and phytosanitary purposes [13, 14], for example, of legumes including soybeans. Gamma radiation at dose level of 1 kGy (low dose) has been applied for quarantine treatment of legumes including soybeans. Higher dosages of irradiation have been applied for fresh meat products, dry or frozen fresh vegetables and ready to eat food products to increase safety, maintain the quality and prolong the shelf life of the food [15, 16].

Variyar *et al.*, [17] reported that isoflavone of soybean irradiated with gamma irradiation at the dose level of 0.5-5 kGy is more in the form of aglicones. This suggests that irradiation may attack the glycosidic bond of soy isoflavone glycosides (genistin, daidzin and glycitin) to produce aglycones (genistein, daidzein and glycitein). Gamma irradiation at the dose level of up to 10 kGy, however, did not affect total isoflavone content of soybean [18]. Previous research reported that gamma irradiation at the same radiation dose showed different effects on the decrease of anti-nutritional concentration and the changes of colour of soybean as influenced by the dose-rate used [19]. At the irradiation dose; the higher the dose-rate (the lower the irradiation time) is more effective in decreasing concentration of anti-nutritional compounds, but less effective in reducing the quality of soybean colour.

Specifically, this research will evaluate the influence of the dose-rate selection on the rate of change of isoflavones of soybean during gamma irradiation.

Materials and Methods

Raw materials

Soybeans of the Mitani variety were obtained from plant breeding section of CAIR. Isoflavone standard reagents (daidzein and genistein) were purchased from Sigma Chemical Co, methanol HPLC, chloride acid, ammonium acetate from Merck, acetonitril and water solvent from J.T. Baker, respectively.

Equipment

A gamma source radiation facility of Natural Rubber Irradiator (NRI), was used to irradiate sample materials at 122.9 kCi activity. NRI is at CAIR, Pasar Jumat, Jakarta. A Shimadzu liquid chromatograph equipped with LC 20 AD type pump, SPD 20A type UV detector and RF 10AXL type flourescense, water-bath (Napco model 220A, USA), centrifuge (IEC Centra 8 Centrifuge, USA), magnetic stirrer (Velp Scientifica type ate, Italy) and the other supporting equipment.

Sample preparation

Soybean was used as the sample material. The samples were weighed into 100 g, packed in polietilen plastic bags for each treatment. The samples were prepared in triplicate.

Dosimetry

Radiation dose measurement was done using Harwell amber 3042 and Harwell red 4034 dosimeter (Harwell Dosimeters Co. Ltd., Oxfordshire, UK) by using spectronic spectrophotometer [20].

Irradiation

The sample was placed in the irradiation area at different locations to get irradiation treatment with specific dose-rates. Dose-rates used were previously determined during a preliminary study. Four (4) locations of irradiation areas have been identified to have dose-rates of 1.30; 3.17; 5.71 and 8.82 kGy/h. Irradiation treatments were done at room temperature (28 ± 2°C) for each of the samples with exposure time ranging from 0.5-55 h; depending on dose-rate.

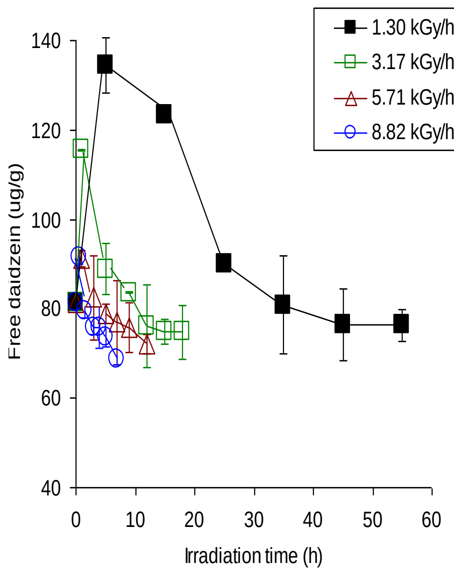
Determination of isoflavone

Isoflavone concentration was determined according to the method of Wang *et.al*. [21]. The two grams of ground sample was extracted with hexane for 6 h to remove fat. The isoflavone content of non-fat sample was analyzed using a high performance liquid chromatographer equipped with a UV detector at wavelength of 254 nm and each flourescense *o*f excitation and emission wavelength was 365 nm and 418 nm. Agilent XDB-C18 column (5 µm), 4.6 x 15 cm length was used. A mobile phase was a mixture of (metanol : 1 mM ammonium asetat, 6:4) at a flow rate of 1.0 ml/min.

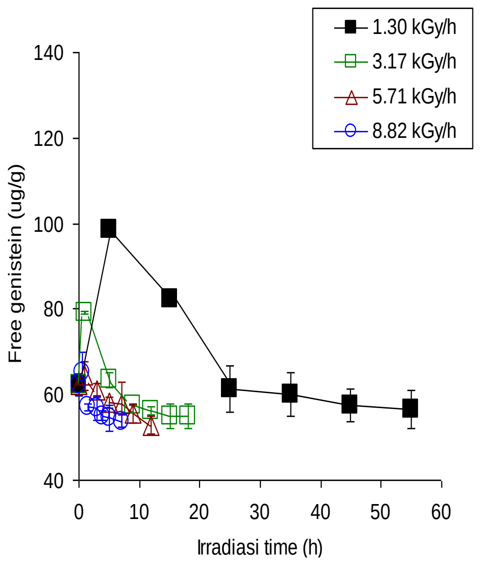
Results and Discussion

The initial concentration of isoflavones in soybean sample used in this study was 81.39 µg/g and 367.42 µg/g (for free and total daidzein) and 62.27 µg/g and 232.55 µg/g (for free and total genistein), respectively. Wang *et al*., [21] reported that free and total daidzein contents in soybean were 25.6 µg/g and 330.6 µg/g, and for free and total genistein were 28.4 µg/g and 461.6 µg/g. The difference in isoflavone concentration among soybeans used could probably be due to the varieties, planting location and harvesting time [22].

Changes of isoflavone content of soybean during the irradiation are presented in Figure 1. The results showed that gamma irradiation at different dose-rates of 1.30; 3.17; 5.71 dan 8.82 kGy/h provide different patterns of change for isoflavone content. In general, there is an increase of free daidzein and genistein concentration (Figures 1a and 1b); especially during the beginning of the radiation process. Furthermore, higher increase of free daidzein and genistein concentration occurs during the radiation process with lower dose-rate. Figures 1c and 1d, however, show that irradiation with dose-rates of 1.30; 3.17; 5.71 and 8.82 kGy/h used in this study did not show any significant difference in both total of daidzein and genistein.



(a)



(b)

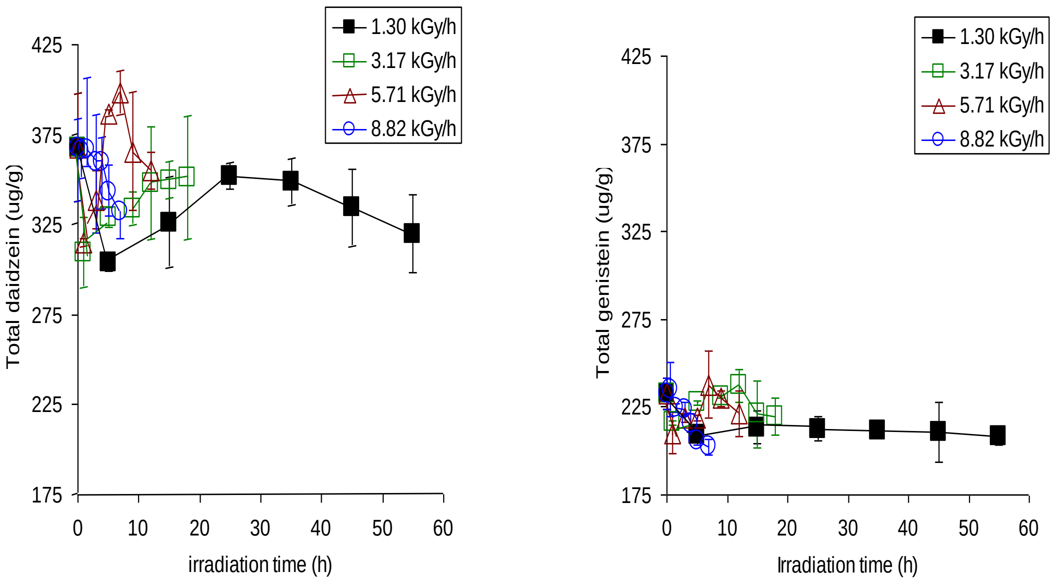


Figure 1. Changes in isoflavones of soybean after radiation processing at different dose-rates (a) free daidzein; (b) free genistein; (c) total daidzein and (d) total genistein.

Figure 2 shows how isoflavone changes as a function of radiation dose. Soybean irradiated at the absorbed dose will have higher concentration of free daidzein and free genistein as compared to those irradiated at higher dose-rate. Soybean irradiated at 1.30 kGy/h show a marked increase in free daidzein as compared to those irradiated at higher dose-rate of 8.82 kGy/h (Figure 2a). Similar pattern was also observed for changes of free genistein (Figure 2b). In general, there is strong indication, especially at the beginning of radiation up to the dose level of 20 kGy, that irradiation treatment with lower dose-rate (and longer time) results in the higher free daidzein and genistein as compared to that of irradiation process at higher dose-rate (shorter time). Figures 2c and 2d show patterns of concentration change of total daidzein and total genistein as a function of radiation dose. No significant reduction of total isoflavone was obseved, but in general, there is a tendency of slight decrease in the total isoflavone as the dose of irradiation was increased. This is in agreement with Yun *et al*. [18] who reported that gamma irradiation at the doses up to 10 kGy did not increase total isoflavone content.

These results (Figure 1) suggest that irradiation at different dose-rates decomposes glycoside isoflavone into aglycone at different rates. Similar phenomenon was a reported by Kao *et al*. [23] in which soaking of soybean at higher temperature would cause more decomposition of acetyl-glucoside and glucose- and malonil-glucoside isoflavone into its aglycone. In general, Figure 2 also shows that the increase of free isoflavone concentration due to irradiation at a certain dose level was also influenced by the dose-rate applied. The phenomenon of isoflavone increase was also reported by Variayar *et al*. [17], who showed that gamma irradiation in 0.5-5 kGy on soybean would potentially increase aglycone isoflavone.

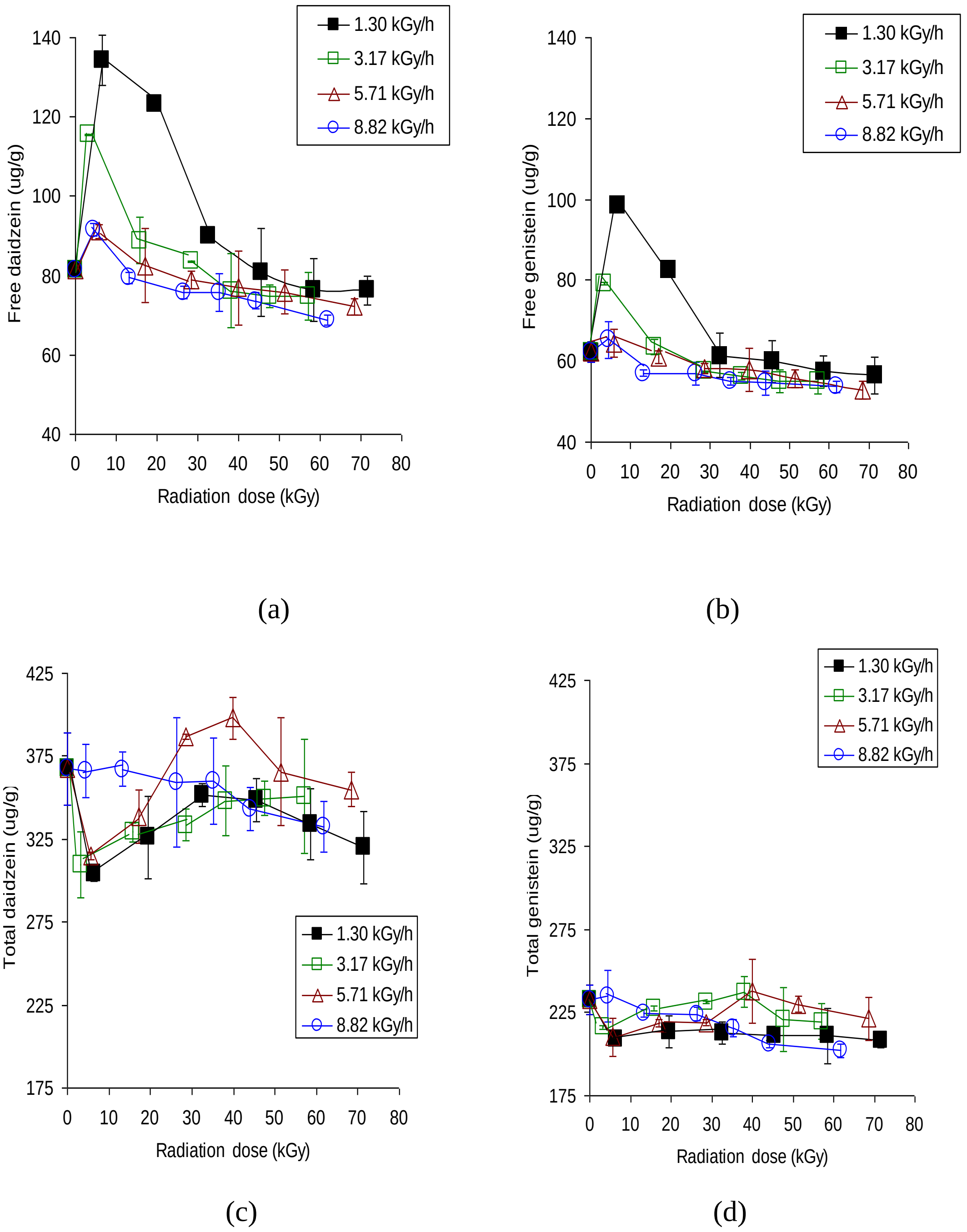


Figure 2. Changes of isoflavone concentrations of soybean as affected by irradiation dose absorbed at different dose-rates (a) free daidzein; (b) free genistein; (c) total daidzein and (d) total genistein.

Conclusion

In conclusion, results presented in this report indicate that dose-rate can be one significant factor in the effort to control and to optimize the radiation process. There is a good indication that irradiation with lower dose-rate (longer time) of soybean may potentially be used to promote free daidzein and genistein. Consequently, dose-rate selection may be used as a tool to control isoflavone content, especially with respect to free daidzein and genistein.

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