Removing undesirable color and boosting biological activity in red beet extracts using gamma irradiation

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1. Introduction

Red beet (*Beta vulgaris* L.) is a traditional and popular vegetable distributed in many part of the world and has been used as a natural colorant in many dairy products, beverages, candies and cattle products. Red beet roots contain two groups of betalain pigments, red-violet betacyanins and yellow betaxanthins. Betalains possess several biological activities such as antioxidant, anti-inflammatory, hepatoprotective, and anticancer properities.

Recent trend of using natural products in industries tends toward multifunctional, high quality, and high-priced value foods and cosmetics. To meet the needs of consumers, cosmetics, medicine, and foods should contain the proper amount of natural products. Although the color removal processes such as filtration and absorption by clay are still useful, these procedures are difficult, time-consuming and costly [1]. To overcome this problem, the radiation technology has emerged as a new way. Radiation technology has been applied to the decomposition and decoloration of pigment and is an efficient technique for inactivating pathogens, removing undesirable color in biomaterial extracts and improving or maintaining biological activities [1,2]. Gamma-irradiation and electron beam-irradiation techniques in previous reports were applied in order to remove any undesirable color and to improve or maintain biological activities of various extracts such as green tea leaves, licorice root, and *S. chinensis* fruits [1,2,3]. Latorre et al. [4] reported that betacyanin concentration decreased with the irradiation dose and significantly, in 35%, after 2.0 kGy of gamma-ray, whereas betaxathin concentration increased (about 11%-ratio with respect to control) after 1 kGy but decreased (about 19%) after 2 kGy. However, they did not try to analysis for completed removal of red beet pigments. Therefore, it is necessary to find the optimum irradiation dose for entirely removing red pigments in red beet.

The aim of this work was to address the effects of the color removal and biological properties of red beet extracts by gamma irradiation.

2. Methods and Results

2.1 Gamma Irradiation

Gamma irradiation was carried out at ambient temperature using a high-level cobalt-60 irradiator (point source AECL, IR-79, MDS Nordion International Co., Ltd., Ottawa, ON, Canada) in the Advanced Radiation Technology Institute, Korea Atomic Energy Research Institute (Jeongeup, Korea). The source strength was approximately 320 kCi with a dose rate of 10 kGy/h. Sample solutions in capped vials were irradiated with 2.5-30 kGy (absorbed dose). The irradiated samples were immediately stored at 4°C in the dark.

2.2 Determination of Pigments

The contents of betacyanin and betaxanthin were monitored at 538 nm and at 476 nm, respectively, using high performance liquid chromatography (HPLC) equipped with a UV detector (Agilent Technologies, Palo Alto, CA, USA) and a Hydrosphere C18 column (5 μm, 250 × 4.6 mm) (YMC Co., Ltd., Kyoto, Japan) with a gradient elution system. Solvent A was a mixture of 100% methanol and 0.2% (v/v) formic acid in water with a ratio of 18: 82 (v/v). Solvent B was 100% methanol. The gradient programs were as follows; 0-6 min, 0% B; 6-12 min, 0-7% B; 12-17 min, 7-12% B; 17-21 min, 12-20% B; 21-35 min, 100% B; 35-40 min, 0% B. The flow rate was 1 ml/min and the injection volume was 20 μl. Authentic betanin (red beet extract diluted with dextrin) was purchased from TCI (Tokyo Chemical Industry Co., Ltd., Tokyo, Japan).

2.3 Enzyme Assay

DPPH (1,1-diphenyl-2-picrylhydrazyl) radical scavenging assay and tyrosinase inhibition assays were measured as previously described by Lee et al. [2].

2.4 Color Removal of Red Beet Extracts by Gamma Irradiation

Red beet roots contain two groups of betalain pigments such as red-violet betacyanins and yellow betaxanthins. Batanin and its isomer isobetanin were the main red component accounting for 88-93% of the total pigments of red beet [5].

The reddish extracts were exposed to gamma-ray ranged from 2.5 to 30 kGy. The colors of extracts after gamma irradiation ranging in dose from 2.5 to 30 kGy are shown in Fig. 1A. The red color of the red beet extracts disappeared in a dose-dependent manner following exposure to irradiation (Fig. 1A). The color of red beet extracts was significantly reduced major colorant of red beet and it was completely removed by doses greater than 10 kGy of gamma-ray.

Gamma irradiation induced a dose-dependent decrease of betanin and isobetanin (Fig. 1B). Betacyanin levels were clearly disappeared greater than 10 kGy of gamma-ray, and even at 5 kGy of gamma-ray were decreased by 94% (0.26 mg/ml) compared with non-irradiated sample (3.68 mg/ml).

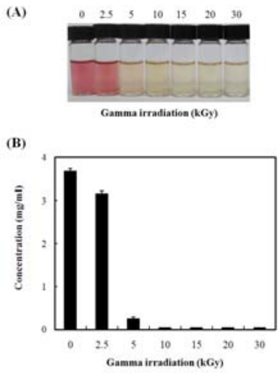

Fig. 1. The effect of gamma irradiation on the color of red beet extracts. (A) Clolor changes in red beet extracts following gamma irradiation. (B) The amounts of betanin and isobetanin in red beet extracts after gamma irradiation. Bars indicated the means ± S.E. (n=3).

2.5 DPPH Radical Scavenging Activity of Irradiated Red Beet Extracts

The radical scavenging activities of various concentrations of the red beet extracts (5, 25, 50, 100, 250, and 500 μg/ml) were measured to determine the optimal concentration for irradiation experiments (Fig. 2A). If free radicals have been scavenged, violet color of DPPH will converted into yellow color. Radical scavenging activity increased in a concentration-dependent with 92.7% inhibition observed at a concentration of 25 μg/ml. Samples (25 μg/ml) were exposed to gamma-ray from 2.5 to 30 kGy (Fig. 2B). Percent inhibition of DPPH radical scavenging activity was retained from 92.7% in control to 90.0-92.0% in irradiated samples (2.5 to 20 kGy), whereas it was slightly decreased as an 87.5% at 30 kGy.

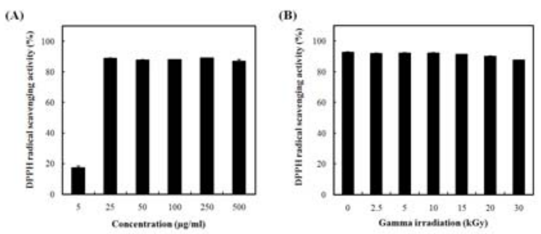

Fig. 2. DPPH radical scavenging activity of reed beet extracts according to concentration (A) and radiation dose (B). Bars indicated the means ± S.E. (n=3).

2.6 Tyrosinase Inhibition Activity of Irradiated Red Beet Extracts

Tyrosinase regulates melanin biosynthesis, and tyrosinase inhibition activity is closely related to skin-whitening. Tyrosinase inhibition activity in red beet extracts gradually increased with increasing extract concentration, peaking at 50.7% in extracts of 20 μg/ml (Fig. 3A). When exposed to gamma-rays, like a DPPH radical scavenging acitivity, the activities were not changed (49.1 to 52.8% in irradiated samples as compared to 50.7% in the non-irradiated sample) by 20 kGy, together with slight decrease as a 46.8% at 30 kGy (Fig. 3B).

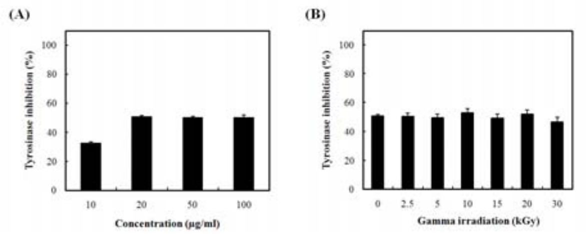

Fig. 3. Tyrosinase inhibition activity of reed beet extracts according to concentration (A) and radiation dose (B). Bars indicated the means ± S.E. (n=3).

3. Conclusions

The biological activities such as DPPH radical scavenging and tyrosinase inhibition were not little affected by gamma irradiation up to 30 kGy. Therefore, radiation technology can be applied to various useful products to remove undesirable color together with a minimum change of biological activity.

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