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Gamma rays as an effective tool for removing undesirable color without adverse changes in biological activities of red beet extracts

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ABSTRACT

The ethanolic extracts of red beet (*Beta vulgaris* L.) hairy root were used to investigate the removal of color and improvement of biological activity for enhanced industrial applications. The extracts were exposed to gamma rays ranging from 2.5 to 30 kGy. The red beet hairy root is composed of two major red-colorants, betanin and isobetanin. Gamma ray radiation at 5 kGy remarkably reduced the levels of the major colorants by 94% and the reddish color was eliminated by doses greater than 10 kGy. Color removal was likely due to the gamma ray radiolysis of ethanol. Although details on the mechanism responsible for the decay of the chromophore have not been entirely determined, our results suggest that the free radicals that are produced during this process are capable of destroying the chromophore group in isobetanin, thus bleaching the substrate solution. In spite of the degradation of the major colorants, the biological activities of constituents of the extract such as DPPH radical scavenging and tyrosinase inhibition were negligibly affected by the gamma ray radiation up to 20 kGy. The antioxidant activity was 92.7% in control samples and 90.0–92.0% in irradiated samples (2.5–20 kGy), and a slight decrease to 87.5% was observed for gamma ray radiation at 30 kGy. In addition, tyrosinase inhibition activity has also the same pattern; the activity is slightly increased from 50.7% of control to 49.1–52.8% of irradiated samples (2.5–20 kGy) with a 46.8% at 30 kGy.

1. Introduction

The red beet (*Beta vulgaris* L.) is a traditional and popular vegetable in many parts of the world and is used as a natural colorant in dairy products, beverages, candies, and beef. Red beet roots contain two groups of betalain pigments, red–violet betacyanins (betanin and isobetanin) and yellow betaxanthins. Betalains possess antioxidant, anti-inflammatory, hepatoprotective, and anticancer properties (Escribano et al., 1998; Georgiev et al., 2010; Gliszczyńska-Świgo, 2006; Kanner et al., 2001; Kapadia et al., 2003; Mukainake et al., 2003; Winkler et al., 2005). In addition, they exhibit radioprotective effects in mice that have been irradiated with gamma rays (Lu et al., 2009).

Recent trends of using natural products in industry have tended toward producing multifunctional, high quality, and high-priced value foods and cosmetics. To meet the needs of consumers, cosmetics, medicine, and foods should contain proper amounts of natural products. Although color removal processes such as filtration and absorption by clay are useful, these procedures are difficult, time-consuming, and costly to carry out (Jo et al., 2003a). Radiation technology has emerged as a solution to overcome these problems. Radiation technology, an advanced oxidation process, has been applied to the decomposition and decoloration of dyes (Ting and Jamaludin, 2008; Vahdat et al., 2010; Wang et al., 2006). It is also an efficient method for inactivating pathogens, removing undesirable colors in biomaterial extracts, and improving and maintaining biological activities (Jo et al., 2003a; Kim et al., 2005; Lee et al., 2011).

Although irradiated natural products have attracted considerable attention regarding their industrial applications, there is limited information on the radiation sensitivity of natural products that are extracts derived from *Lithospermum erythrorhizon*, green tea leaves, and *Schizandra chinensis* (Jo et al., 2003a; Lee et al., 2010, 2011) in terms of their color characteristics. Therefore, more information is needed urgently about the relationship between radiation sensitivity and biological activity concerning natural pigments and metabolites that are obtained from various plants. The aim of this work was to examine the effects of color removal and the biological properties of red beet extracts that are exposed to gamma ray radiation.

2. Materials and methods

2.1. Gamma ray radiation

Gamma ray radiation 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

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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. Extraction of red beet pigments from hairy roots

Hairy roots were grown for two weeks in MS medium. They were collected and samples were stored at −80 °C until use. The harvested samples were dried using a freeze-dryer at −80 °C for at least 48 h. The samples were ground into a fine powder using a mortar and pestle. The samples (5 g) were extracted with one liter of 50% ethanol for 24 h with constant stirring at ambient temperature in the dark according to a method by Kim et al. (2007). The solution was filtered using 0.45 μm Omnipore membrane filters (Millipore, Bedford, MA, USA) and stored in glass vials at 4 °C in the dark until further analyses.

2.3. Determination of pigments

The composition of pigments in red beet extracts were analyzed according to a modified method by Kim et al. (2009). Betacyanin and betaxanthin were monitored at 538 nm and at 476 nm, respectively, using high performance liquid chromatography (HPLC) in conjunction 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).

2.4. Enzyme assay

A DPPH (1,1-diphenyl-2-picrylhydrazyl) radical scavenging assay and tyrosinase inhibition assays were performed as previously described by Lee et al. (2011). To monitor free radical scavenging activity, each sample (0.5 ml) was added to an equal volume of 0.2 mM DPPH. After an incubation period of 30 min at room temperature, absorbance was measured at 517 nm with a UV/vis spectrophotometer (Genesys 10 UV; Thermo Electron Co., Madison, WI, USA). The free radical scavenging activity of each sample was calculated as

$$\text{DPPH radical scavenging activity}(\%) = [1-(A/B)]100$$

where A is the absorbance of the test solution and B is the absorbance of the control reaction (containing all reagents except red beet extracts). All tests were carried out in triplicate.

For measurement of tyrosinase inhibition activity, each sample (0.1 ml) was added to a reaction mixture containing 10 mM L-3, 4-dihydroxyphenyl-alanine (Sigma, St. Louis, USA) and 0.2 ml of mushroom tyrosinase (110 unit/ml, Sigma, St. Louis, USA) in 0.175 M sodium phosphate buffer (pH 6.8). The reaction mixture was incubated at 25 °C for 2 min. Tyrosinase inhibition activity was monitored by observing dopachrome formation at 475 nm with a UV/vis spectrophotometer (Genesys 10 UV). Percent tyrosinase inhibition activity was calculated as

$$\text{Inhibition}(\%) = [1-(A/B)]100$$

where A is the absorbance of the test solution and B is the absorbance of the control solution.

3. Results and discussion

3.1. Color removal of red beet extracts by gamma ray radiation

Radiation has been applied to the decomposition and decoloration of pigments (Ting and Jamaludin, 2008; Vahdat et al., 2010; Wang et al., 2006) and is efficient at inactivating pathogens, removing undesirable colors in biomaterial extracts, and improving or maintaining biological activities (Jo et al., 2003a; Kim et al., 2005; Lee et al., 2011). Gamma ray radiation and electron beam irradiation techniques in previous reports were applied in order to remove undesirable colors and to improve or maintain the biological activities of various extracts such as green tea leaves, licorice root, and *S. chinensis* fruit (Jo et al., 2003a,b; Lee et al., 2011). Latorre et al. (2010) reported that the betacyanin concentration decreased significantly to 35% after applying 2.0 kGy of gamma rays, whereas the betaxathin concentration increased (about 11%-ratio with respect to control) after 1 kGy but decreased (about 19%) after being treated with 2 kGy of gamma rays. However, they did not try the analysis for completed removal of red beet pigments. Therefore, it is necessary to find the optimum radiation dose for entirely removing red pigments in red beets.

Red beet roots contain two groups of betalain pigments, red–violet betacyanins (betanin and isobetanin) and yellow betaxanthins. The reddish color is regarded as a major attribute in determining red beet product acceptability. Batanin and its isomer isobetanin (Fig. 1) are the main red components and account for 88–93% of the total pigments of red beets (Gasztonyi et al., 2001;

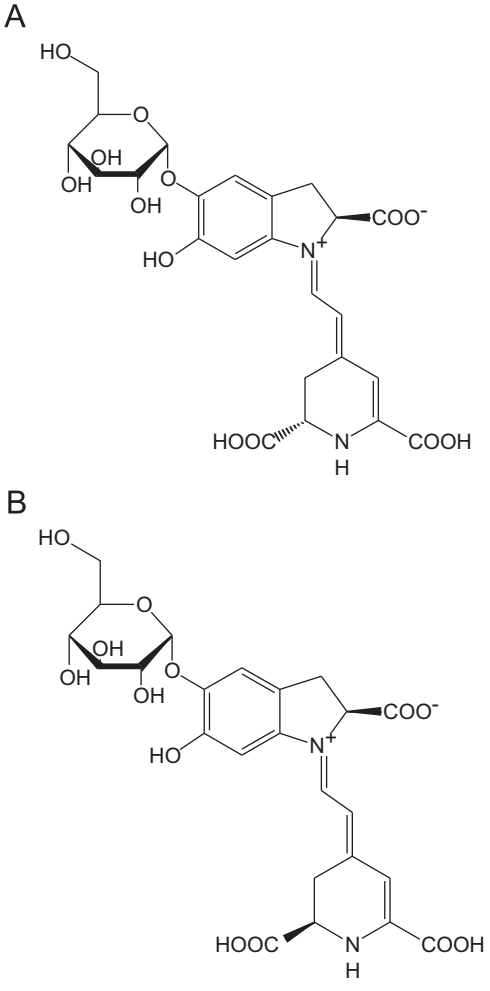

Fig. 1. Chemical structures of betanin (A) and isobetanin (B).