Increased quercetin formation by combination treatment of gamma ray and H_2O_2 from cyanidin-3-*O*-xylosylrutinoside

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1. Introduction

The cyanidin-3-*O*-xylosylrutinoside (cya-3-*O*-xylrut) is one of the most abundant anthocyanin, which is responsible for the red, purple, and blue colors of most fruits and vesetable, in the fruit of *Schisandra chinensis* Baillon [1] and raspberries [2,3]. Especially the cya-3-*O*-xylrut is a major anthocyanin accounting for about 94% in the fruits of *S. chinensis*, which is a unique source of highly pure cya-3-*O*-xylrut, and exhibits high antioxidant activity [1,4]. Previously, we reported that the red color of the *S. chinensis* fruit extract dramatically disappeared in a dose-dependent manner of gamma irradiation [5]. The color of *S. chinensis* was effectively removed at 2 kGy of gamma irradiation, even at 1 kGy resulted in a 50% reduction. Interestingly, the destruction of cya-3-*O*-xylrut by gamma irradiation led to generate flavonoids such as quercetin and unknown compounds [5]. Quercetin, a typical flavonol-type flavonoid, is one of the most prominent dietary antioxidants. It is ubiquitously present in fruits and vegetables. Quercetin exhibits various biological activities such as anti-oxidative, anti-inflammatory, anti-allergic, vasodilating, and anti-cancer effects [6].

In the present study our purpose is to develop a promising tool for bio-conversion of organic compounds by combination treatment of H_2O_2 and gamma ray.

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 215 kCi with a dose rate of 10 kGy/h. Sample solutions in capped vials were irradiated with 2 kGy (absorbed dose). The irradiated samples were immediately stored at 4°C in the dark until used.

2.2 Determination of Pigment and Flavonoid

To analyze pigment (cya-3-*O*-xylrut) and flavonoid contents, samples were analyzed using an Agilent Technologies 1200 series system 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). The mobile phase was composed of 1% (v/v) formic acid (Sol A) and 100% (v/v) methanol (Sol B). A gradient program was performed with a linear gradient form 0% to 100% Sol B for 25 min. The detection wavelength was set at 520 nm for pigment and at 360 nm for flavonoid. The flow rate was 1 ml/min, and the injection volume was 20 μl.

2.3 Color Removal of Cya-3-O-xylrut by Combination Treatment of Gamma-ray and H_2O_2

The cya-3-*O*-xylrut is a major anthocyanin that is in charge of red colorant in the fruit of the *S. chinensis* Baillon. Previously, we reported [5] that the red color of the *S. chinensis* fruit extracts disappeared in a dose-dependent manner after gamma irradiation ranging in dose from 0.5 to 10 kGy. The gamma irradiation at 2 kGy effectively removed the major reddish colorant in *S. chinensis* fruit extracts, cya-3-*O*-xylrut, by 80-90%, whereas quercetin was generated by degradation of cya-3-*O*-xylrut (Fig. 1A).

To investigate the effect of the pretreatment of hydrogen peroxide (H_2O_2) on the color removal and generation of quercetin in cya-3-*O*-xylrut, we performed high-performance liquid chromatography (HPLC). The reddish samples (0.5 mg/ml of cya-3- *O*-xylrut in 100% methanol) were pretreated with 0.2% (v/v) H_2O_2 and then exposed to gamma-ray of 2 kGy. The colors of samples after gamma irradiation or combination treatment (gamma-ray and H_2O_2) were imaged (Fig. 1B) and the relative levels of cya-3-*O*-xylrut were determined by HPLC analysis (Fig. 2). The reddish color of cya-3-*O*-xylrut exposed to 2 kGy of gamma-ray only was significantly reduced about 92.5% compare to non-irradiated samples (Fig. 1 and Fig. 2). When the samples were treated with combination with gamm-ray and H_2O_2, the color was clearly disappeared

due to perfect degradation of the cya-3-*O*-xylrut (Fig. 2).

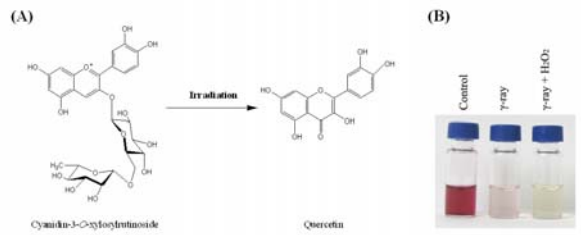

Fig. 1. The generation of quercetin by degradation of cya-3-*O*-xylrut after gamma irradiation (A). Effects of the combination treatment with gamma irradiation and H_2O_2 on the color removal of cya-3-*O*-xylrut (B). Samples (0.5 mg/ml of cya-3-*O*-xylrut in 100% methanol) were pretreatment without or with 0.2% H_2O_2 and then exposed to gamma irradiation at 2 kGy.

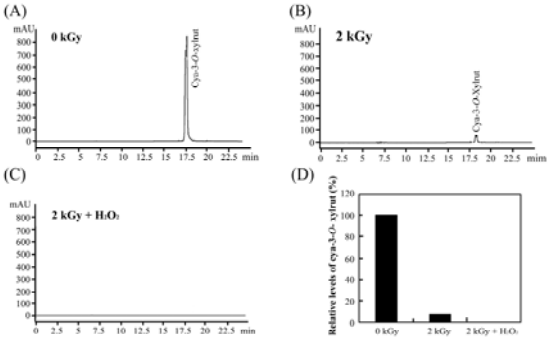

Fig. 2. Fig. 2. Effects of the combination treatment with gamma irradiation and H_2O_2 on the degradation of cya-3-*O*-xylrut. Samples (A) were pretreatment without (B) or with 0.2% H_2O_2 (C) and then exposed to gamma irradiation at 2 kGy. HPLC chromatograms for detection of cya-3-*O*-xylrut were monitored at 520 nm. (D) The relative levels of cya-3-*O*-xylrut.

2.4 Enhancement of Quercetin Content by Combination Treatment

To determine the amount of the quercetin generated by gamma irradiation or combination treatment, we prepared a calibration curve by HPLC based on peak areas using 25 to 100 μg/ml of authentic quercetin in methanol. A plot of mean peak area against concentration gave a linear relationship. Using the regression analysis, the linear equation, y = 265.4x – 1736, was obtained and the correlation coefficient (R^2) = 0.999, where y was the mean peak area and x was the quercetin concentration (μg/ml). Quercetin corresponding to a peak at 21.9 min was newly generated about 3.2 μg/ml at 2 kGy of gamma ray (Fig. 3B and 3D), concomitant with disappearance of cya-3-*O*-xylrut corresponding to a peak at 18.4 min (Fig. 3A and 3B). This result implies that quercetin formation would be derived from cya-3-*O*-xylrut pigment. In addition, the formation of quercetin was considerably increased to 7.7 μg/ml by the combination treatment of H_2O_2-gamma ray (Fig. 3C and 3D).

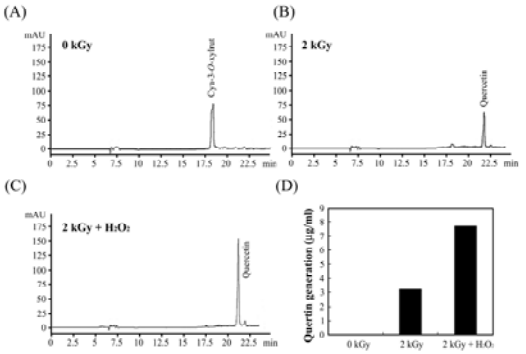

Fig. 3. Effects of combination treatment of gamma irradiation and H_2O_2 on the formation of the quercetin. Samples (A) were pretreatment without (B) or with 0.2% H_2O_2 (C) and then exposed to gamma irradiation at 2 kGy. HPLC chromatograms for detection of flavonoids were monitored at 360 nm. (D) The amount of generated quercetin.

3. Conclusions

Cya-3-*O*-xylrut was significantly degraded (~93%) by gamma irradiation at 2 kGy and it was completely removed by a combination treatment (0.2% H_2O_2 and 2 kGy gamma ray). The formation of quercetin was significantly appeared at 2 kGy of gamma ray, together with disappearance of cya-3-*O*-xylrut. The combination treatment is more effective to convert cya-3-*O*-xylrut into quercetin than gamma irradiation only. In addition, H_2O_2 would act as an accelerator for breaking chemical bond of cya-3-*O*-xylrut, resulting in a bleaching effect. In conclusion, gamma ray combined with H_2O_2 would be a promising tool for bio-conversion of organic compounds.

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