Food Chemistry 141 (2013) 552–556

Contents lists available at SciVerse ScienceDirect

Food Chemistry

journal homepage: www.elsevier.com/locate/foodchem

Effects of UV-B irradiation on the levels of anthocyanin, rutin and radical scavenging activity of buckwheat sprouts

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ARTICLE INFO

Article history:
Received 18 September 2012
Received in revised form 1 February 2013
Accepted 11 March 2013
Available online 20 March 2013

Keywords:
Buckwheat sprout
Antioxidant
Anthocyanin
Rutin
Light composition
UV-B

ABSTRACT

The effects of various light compositions on the levels of anthocyanin, rutin and 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical scavenging activity in buckwheat (Fagopyrum esculentum Moench) sprouts were evaluated. Dark-grown 6-day-old buckwheat sprouts were irradiated with different sources of visible and ultraviolet (UV) light. Particularly, we examined the effect of UV-B at wavelengths of 260–320 nm, 280–320 nm, and 300–320 nm on the production of flavonoid compounds, using multiple fluorescent lights and cylinders that filter out certain portions of the UV-B. The results showed that irradiation with UV-B > 300 nm increased the levels of anthocyanin and rutin, as well as the DPPH radical scavenging activity. When sprouts were irradiated with UV-B light at wavelengths of 260–300 nm, yellowing or withering occurred within 24 h of irradiation, indicating that wavelengths in this range are detrimental to the growth of buckwheat sprouts.

1. Introduction

Sprouts have received increasing attention in recent years because of their potential dietary value and vitamin and mineral content (Manchali, Chidambara Murthy & Patil, 2012). Broccoli, red cabbage, alfalfa, and similar sprouts have been recognized as health foods. Buckwheat (*Fagopyrum esculentum* Moench.) sprouts have received considerable attention because of their high levels of antioxidants, including rutin (Kim, Kim, & Park, 2004; Watanabe, 2007) and anthocyanin (Watanabe, 2007). Kim, Park, and Lim (2011) reported that buckwheat sprouts contain chlorogenic acid, catechin, isoorientin, orientin, rutin, vitexin, and quercitrin, which have various health effects. The sprouts are also frequently used as decoration because of the beautiful pink or red color of the hypocotyls. Anthocyanin is the main component giving the red color to buckwheat sprouts. Pigments like anthocyanin are not only functional components, but also stimulate the consumption of sprouts. Due to their health benefits and commercial potential, the efficient production of buckwheat sprouts with high levels of flavonoids and radical scavenging activity is desirable.

Flavonoids are produced as protective substances against UV-B radiation. There have been many studies on the production of flavonoids by buckwheat sprouts in response to UV-B irradiation (280–320 nm), but the part of the UV-B wavelength responsible for flavonoid production has not been reported. In addition, fluorescent lamps used for UV-B irradiation experiments often operate in the 260–280 nm wavelengths. The effect of UV-B on the flavonoid content of sprouts is still controversial. In studies investigating the rutin contents of buckwheat seedlings grown under three levels of UV-B irradiation (reduced, ambient, and enhanced), the rutin concentrations in seedlings grown under high UV-B levels were higher than for the other levels (Kreft, Strukenlj, Gaberscik & Kreft, 2002). It was also reported that both the rutin content and the rutin glucosidase activity in tartary buckwheat increased after UV-B irradiation (Suzuki et al., 2005). Hence, we examined the effects of UV-B at wavelengths of 260–320 nm, 280–320 nm, and 300–320 nm on the production of anthocyanin and flavonoid compounds, using multiple fluorescent lights and cylinders that filter out certain portions of the UV-B. Moreover, we also compared the effects of visible light and UV-A to those of UV-B.

2. Materials and methods

2.1. Materials

Buckwheat seeds were purchased from Nakahara Seed, Ltd (Fukuoka, Japan). Rutin, 1,1-diphenyl-2-picrylhydrazyl (DPPH),

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http://dx.doi.org/10.1016/j.foodchem.2013.03.032

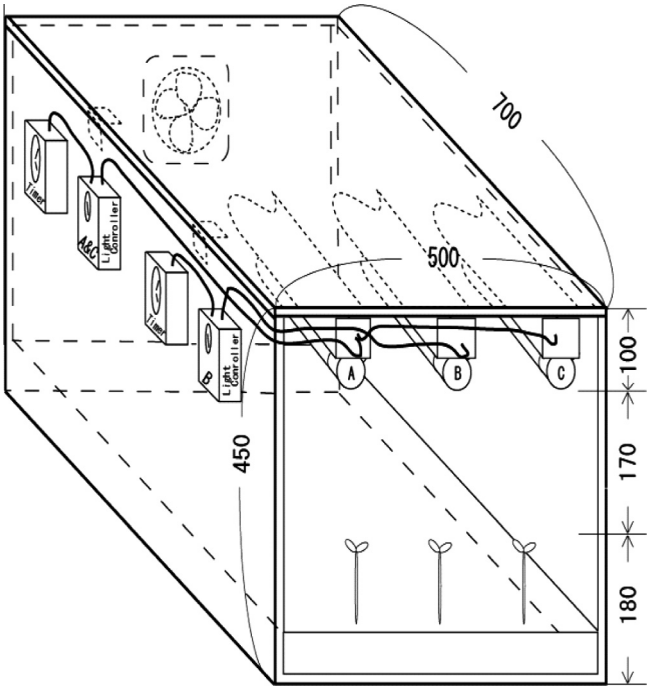

Fig. 1. Equipment for irradiation of buckwheat sprouts (units in mm).

Table 1
Intensity of irradiation from different sources.

Source	PPFD ($\mu mol\ m^{-2}s^{-1}$)	UV-B (Wm^{-2})
Dark	0	0
FL	110	12
FL-FR	63	3
FL-R	70	1
FL-G	64	2
FL-BL	70	15
UV-A	10	250
UV-B (>300 nm)	10	890
UV-B (>280 nm)	7	410
UV-B (>260 nm)	8	550

The intensity was measured at a distance of 17 cm from each light source.
Dark indicates buckwheat sprouts received no light. FL = FL 20SS·EX-N/18 lamp, FL-FR = FL20S·FR·P lamp, FL-R = FL20S·R lamp, FL-G = FL20S·G lamp, FL-BL = FL20S·BL lamp, UV-A = FL20S·BL-K. UV-B (>300 nm) = TL20 W/01RS lamp. UV-B (>280 nm) = GL20SE lamp and cylinders that absorb wavelengths shorter than 280 nm. UV-B (>260 nm) was used GL20SE.

Folin–Ciocalteu reagent, Trolox and ethanol were obtained from Wako Chemicals, Ltd. (Osaka, Japan). Acetonitrile (analytical grade) was purchased from Sigma–Aldrich Japan K. K. (Tokyo, Japan).

2.2. Cultivation and irradiation of buckwheat sprouts

Seeds were soaked in tap water at 25 °C for 16 h. Afterward, the seeds were spread evenly on a tray (30 cm × 40 cm), and cultured using equipment for temperature control (25 °C) and water addition by spraying at regular intervals. Sprouts were grown in the dark for 6 days to allow them to germinate and elongate. The sprouts were then transferred to a custom-made controlled environment chamber (Panasonic Electric Works Co., Osaka, Japan) at 25 °C (Fig. 1) and irradiated with different types of light (white, red, green, blue, UV-A, and UV-B). An additional sample was kept in the dark. Two fluorescent lamps (20 W, FL 20SS·EX-N/18 and FL20S·FR·P; National, Tokyo, Japan) were used as the white light source; the former is generally used for illumination, while the latter is used to promote plant growth. Red (FL20S·R), green (FL20S·G) and blue (FL20S·BL) fluorescent lamps, as well as UV-A (FL20S·BL-K) fluorescent lamps, were obtained from Panasonic. UV-B fluorescent lamps were obtained from Sankyo (GL20SE; Tokyo, Japan) and Philips (TL20W/01RS; Lahore, Pakistan). TL20W/01RS lamps do not irradiate at wavelengths less than 300 nm. UV-B lamps (GL20SE) and cylinders that absorb wavelengths shorter than 280 nm were used. Photon flux density was measured with a quantum sensor (Li-Cor, Lincoln, NE, USA). UV-B was measured with an ultraviolet meter (310 nm, Topcon, Tokyo, Japan). The irradiation intensity of the lighting sources is shown in Table 1. Whole buckwheat sprouts were picked 24 h after irradiation treatment. All samples were freeze-dried and powdered for subsequent analyses.

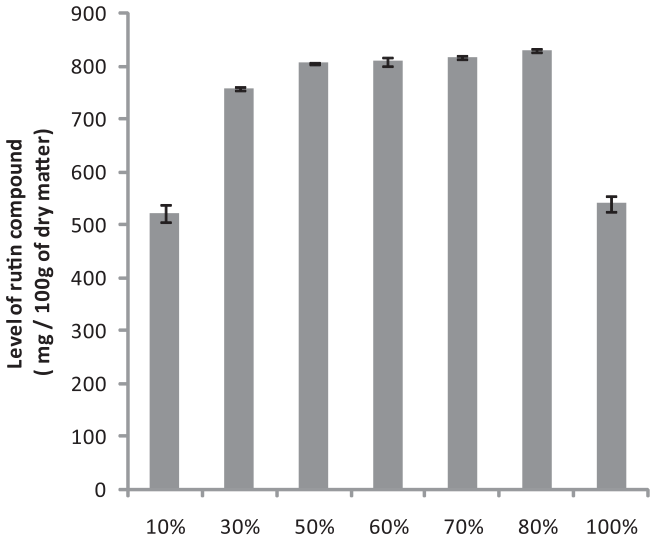

Fig. 2. Effect of the ethanol concentration on the rutin levels in buckwheat sprouts. Means are shown, with vertical bars indicating standard deviation ($n = 3$).

2.3. Anthocyanin assay

The anthocyanine content was determined by the depth of the color, which was obtained from its absorbance at 540 nm (Higashio, Hirono, Sato, Tokuda, & Uragami, 2009). To extract anthocyanin, freeze-dried samples (200 mg) were suspended in 20 ml of 5% (v/v) formic acid, and kept at 5 °C for 48 h. The volume was adjusted to 25 ml using 5% formic acid. After centrifugation at 8000×*g* for 10 min, 200 μl of the extract was pipetted into a 96-well plate. Absorption was measured at 540 nm, using a Sunrise Thermo microplate reader (Tecan, Salzburg, Austria). Cyanidin 3-glucoside was used as a standard reagent, and the measured values are expressed as cyanidin 3-glucoside equivalents (mg cyanidin 3-glucoside eq./100 g of dry matter).

2.4. Preparation of samples for determination of rutin and DPPH radical scavenging activity

Generally, aqueous solutions of ethanol or methanol, particularly those ranging from 40% to 80% alcohol, are more efficient for polyphenol extraction than pure water, ethanol or methanol. We confirm that an 80% ethanol solution was the most efficient extractant for rutin from buckwheat sprouts (Fig. 2). Therefore, we used 80% ethanol solution in the present study. To extract