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Effect of sucrose and mannitol on the accumulation of health-promoting compounds and the activity of metabolic enzymes in broccoli sprouts

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ABSTRACT

The contents of ascorbic acid, glucosinolates, sulforaphane, anthocyanins, total phenolics, the activity of myrosinase and phenylalanine ammonialyase (PAL) and the antioxidant activity of broccoli sprouts grown under 88 mM and 176 mM of sucrose and mannitol were investigated. The results showed that the contents of sulforaphane, ascorbic acid and anthocyanins in broccoli sprouts were significantly increased after treatment with 88 mM of sucrose compared with the control. The contents of glucosinolates and total phenolics, the activity of PAL and the antioxidant activity in broccoli sprouts treated with 176 mM sucrose were also significantly increased, whereas the activity of myrosinase was significantly reduced. On the other hand, the contents of glucosinolate, sulforaphane and total phenolics in broccoli sprouts were also significantly increased after treatment with 176 mM of mannitol, although the contents of sulforaphane were markedly reduced compared to those treated with 176 mM of sucrose. Sucrose might induce the production of health-promoting compounds through its role of signaling, generating osmotic pressure or serving as a substrate. These results indicate that sucrose treatment could improve the nutritional value of broccoli, and the sprouts growing under adequate concentration of sucrose could benefit our diet by producing more health-promoting compounds.

1. Introduction

Sprouts have long been consumed globally due to their high nutritional values. *Brassica* sprouts, in particular broccoli (*Brassica oleracea* var. *italica*) sprouts, contain substantial amount of antioxidants, vitamin C and health-promoting compounds such as glucosinolates and phenolic compounds (Fahey et al., 1997; Martínez-Villaluenga et al., 2008; Yuan et al., 2010).

Glucosinolates as an important class of secondary metabolites have gained particular interest as potential cancer and chemo-preventing agents. Glucosinolates, localized in plant vacuoles, are chemically stable until they come into contact with myrosinase (EC 3.2.1.147), which is localized in idioblasts to separate from glucosinolates (Andréasson et al., 2001). Upon tissue damage, glucosinolates are released from vacuoles and rapidly hydrolyzed by myrosinase to isothiocyanates, thiocyanates or nitriles. Isothiocyanates have been shown to have protective effect against cancer, particularly bladder, colon and lung cancers (Cartea et al., 2008). Cruciferous vegetables, such as broccoli florets are a major source of glucosinolates in the human diet. However, recently an immense interest is found in sprouts that may constitute an important source of glucosinolates. It has been reported that the levels of 4-methylsulphinylbutyl glucosinolate (glucoraphanin), an abundant glucosinolate in broccoli sprouts, is 15 times higher than that in the mature plants (Aires et al., 2006; Fahey et al., 1997).

Polyphenols, ascorbic acid and anthocyanins are other health-promoting metabolites in broccoli sprouts. Polyphenols have been extensively investigated for its ability to lower the risk of cardiovascular diseases (Knekt et al., 1996; Volden et al., 2009). Ascorbic acid is closely linked to human health and, as it is not formed within the human organism and has to be administered on a regular basis (Davey et al., 2000). Anthocyanins are a large group of water-soluble pigments that are widely distributed in higher plants. They are not only responsible for red, blue and purple color of many fruits, vegetables, flowers and seeds, but also protect plants against various biotic and abiotic stresses (Harborne and Williams, 2000). In addition, growing evidence suggests anthocyanin-rich foods could offer protection against cardiovascular disease, cancer, and some other chronic diseases (Hou, 2003; Lila, 2004; Yuan et al., 2010). The purple stem of broccoli sprouts is potentially a source of anthocyanins.

Environmental conditions could affect the contents of secondary metabolites in plants. Growth conditions (e.g., nitrogen and sulfur fertilization) and environmental stresses (e.g., temperature and

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light conditions) have been reported to exert a significant influence on glucosinolate content (Ciska et al., 2008; Pereira et al., 2002; Perez-Balibrea et al., 2008). Sucrose induced the biosynthesis of anthocyanins in *Arabidopsis* and had a positive effect on ascorbic acid accumulation of harvested broccoli florets (Nishikawa et al., 2005; Solfanelli et al., 2006). However, little information is available about the influence of sucrose on the health-promoting compounds and nutrition value of *Brassica* sprouts. The objective of this study was to investigate the effect of sucrose on the contents of glucosinolates, sulforaphane, polyphenols, ascorbic acid and anthocyanins, ferric reducing antioxidant power (FRAP) as well as the activity of phenylalnine ammonialyase (PAL) and myrosinase in broccoli sprouts.

2. Materials and methods

2.1. Plant material and cultivation conditions

Seeds of broccoli (*B. oleracea* var. *italica* cv. Youxiu) were purchased from Sakata Seed Corporation (Japan). The seeds were immersed in 7 ml/l sodium hypochlorite for 30 min, then drained and washed with distilled water until they reached neutral pH. They were then placed in distilled water and soaked overnight. The broccoli sprouts were grown in petri dishes with wet filter papers. The treatment sprouts were watered with 88 mM and 176 mM sucrose and mannitol respectively after 5 days, and the control sprouts are watered with distilled water. All sprouts were grown under a 16-h light and 8-h dark photoperiod and a constant 23 °C in a culture room. Finally, 7-day-old sprouts were collected for measurements. Sprout samples were rapidly and gently collected from the surface of the filter paper. At the same time the biomass of the broccoli sprouts was weighed. Then the sprouts were immediately frozen in liquid nitrogen and kept in polyethylene bags at −80 °C for analysis of ascorbic acid, FRAP value, total phenolic content, glucosinolates and sulforaphane, as well as myrosinase and PAL activity. For each treatment, three replicates were taken for analysis.

2.2. Glucosinolate assay

Glucosinolates were extracted and analyzed as previously described with minor modifications (Jia et al., 2009; Yuan et al., 2010). Samples (500 mg) were boiled in 3 ml water for 10 min. After transferring the supernatant to a new tube, the residues were washed with water (3 ml), and the combined aqueous extract was applied to a DEAE-Sephadex A-25 (30 mg) column (pyridine acetate form) (GE Healthcare, Piscataway, NJ). The column was washed three times with 20 mM pyridine acetate and twice with water. The glucosinolates were converted into their desulpho analogues by overnight treatment with 100 μl of 0.1% (1.4 units) aryl sulphatase, and the desulphoglucosinolates were eluted with 2× 0.5 ml water. HPLC analysis was performed using a HPLC system consisting of a Waters 2695 separations module and a Waters 2996 photodiode array detector (Waters Corp., Milford, MA, USA). The HPLC system was connected to a computer with Empower Pro software. A Hypersil C18 column (5 μm particle size, 4.6 mm × 250 mm; Elite Analytical Instruments Co., Ltd., Dalian, China) was used with a mobile phase of acetonitrile and water at a flow rate of 1.0 ml/min. The procedure employed isocratic elution with 1.5% acetonitrile for the first 5 min; a linear gradient to 20% acetonitrile over the next 15 min followed by isocratic elution with 20% acetonitrile for the final 10 min. A 40-μl sample was injected into the column by an autosampler. Absorbance was detected at 226 nm. Sinigrin (Sigma St. Louis, MO, USA) was used as an internal standard for HPLC analysis. Desulphoglucosinolates were identified by comparison of retention time and quantified by peak area. For calculation of molar concentrations of individual glucosinolates, the relative response factors reported by Brown et al. (2003) were used to correct for absorbance differences between the standard and the other glucosinolates. The glucosinolate concentration was expressed as μmol/g fresh weight (FW) of broccoli sprouts.

2.3. Sulforaphane measurement

Sulforaphane content was determined according to the method of Brader et al. (2006). Glucosinolate degradation products were extracted from 300 mg broccoli sprouts as described by Lambrix et al. (2002). The samples were ground in 2 ml 50 mM MES buffer solution (pH = 5.8). After 5 min at room temperature, the mixture was centrifuged at 8000 rpm for 5 min. The supernatant (400 μl) was added to 50 μL benzonitrile as internal standard and then to 2× 750 μl dichloromethane. The dichloromethane layer was recovered, dried over Na_2SO_4, and concentrated to about 100 μl in a nitrogen stream, Samples were analyzed by gas chromatography with flame ionization detection (FID) using an Shimadzu 2014 series gas chromatograph with an Rtx-5 column (30 m × 0.25 mm × 0.25 μm), split injection at 200 °C, and a temperature program of 35 °C for 3 min, a 12 °C/min ramp to 96 °C, a 20 °C/min ramp to 240 °C, and a 20 °C/min ramp to 300 °C. The sulforaphane concentration was expressed as μmol/g FW of broccoli sprouts.

2.4. Ascorbic acid determination

Determination of ascorbic acid was performed according to the protocol of Volden et al. (2009). Briefly, 500 mg of fresh broccoli material was extracted with 8 ml of 1.0% (w/v) oxalic acid. The mixture was centrifuged at 13,000 rpm for 10 min and 1 ml supernatant was filtered using a 0.45 μm Millipore. Separation and detection were performed using a Waters 2996 photodiode array detector (Waters Corp., Milford, MA, USA) with a Hypersil C18 column (5 μm particle size, 4.6 mm × 250 mm; Elite Analytical Instruments Co., Ltd., Dalian, China) at 254 nm. Ascorbic acid was quantified by external calibration and results are reported as mg ascorbic acid per 100 g FW.

2.5. Anthocyanin measurement

Anthocyanin content of sprouts was determined according to the method of Mita et al. (1997a) and Teng et al. (2005). Frozen, homogenized seedlings (200 mg) were extracted at 4 °C in 1 ml of 1% (v/v) hydrochloric acid in methanol. The mixture was centrifuged at 13,000 rpm for 15 min and the absorbance of the supernatant was measured at 530 and 657 nm. Relative anthocyanin concentrations were calculated with the formula $[A_{530} - (1/4 \times A_{657})]$. The relative anthocyanin amount was defined as the product of relative anthocyanin concentration and extraction solution volume. One anthocyanin unit equals one absorbance unit $[A_{530} - (1/4 \times A_{657})]$ in 1 ml of extraction solution.

2.6. Total phenolic content assay

The total phenolic compounds of broccoli sprouts were extracted with 50% ethanol and incubated at room temperature for 24 h in the dark. The suspension was centrifuged at 10,000 rpm for 10 min at room temperature and the supernatant was collected. Phenolic compounds were determined using Folin–Ciocalteu reagent method by reading the absorbance at 765 nm with a UV–Vis spectrophotometer (UV-2500, Shimadzu Corp., Kyoto, Japan) according to the method of Ainsworth and Gillespie (2007). Gallic acid was used as a standard and the results