JOURNAL OF FUNCTIONAL FOODS 9 (2014) 70–77

Available at www.sciencedirect.com

ScienceDirect

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

Glucoraphanin, sulforaphane and myrosinase activity in germinating broccoli sprouts as affected by growth temperature and plant organs

Liping Guo [a,b], *Runqiang Yang* [a], *Zhiying Wang* [a], *Qianghui Guo* [a] *and Zhenxin Gu* [a,*]

[a] *College of Food Science and Technology, Nanjing Agricultural University, Nanjing, 210095, China*
[b] *College of Food Science and Engineering, Qingdao Agricultural University, Qingdao, 266109, China*

ARTICLE INFO

Article history:
Received 2 March 2014
Received in revised form 8 April 2014
Accepted 10 April 2014
Available online 3 May 2014

Keywords:
Glucoraphanin
Sulforaphane
Myrosinase
Broccoli sprouts
Organ
Germination

ABSTRACT

The effect of growth temperature on glucoraphanin, sulforaphane and myrosinase activity in germinating broccoli sprouts was firstly evaluated, and then their distributions in the cotyledon, hypocotyl and root at the optimum temperature were investigated. The profiles of isothiocyanates and nitrile in seeds, sprouts and different organs were also analyzed. Glucoraphanin content and sulforaphane formation declined with sprouts growth. Myrosinase activity in sprouts, stable for the first 3 days of germination, slowly increased threefold by day 7. The highest levels of glucoraphanin and sulforaphane were recorded in non-germinated seeds. Sprouts grown at 25 °C had higher glucoraphanin content and sulforaphane formation than that grown at 20 and 30 °C. In addition, cotyledon had significantly higher glucoraphanin content and sulforaphane formation than hypocotyl and root. Glucoraphanin and sulforaphane were barely detectable in root, despite its high myrosinase activity. The most abundant hydrolysis product in seeds and sprouts, cotyledon and hypocotyl was sulforaphane.

1. Introduction

It has been shown that consumption of cruciferous vegetables such as broccoli and cabbage can reduce the risk of cancer and cardiovascular diseases. These beneficial effects are attributed to an important class of sulfur-containing secondary metabolites called glucosinolates (Herr & Büchler, 2010). Glucosinolates can be hydrolyzed by the enzyme myrosinase into various products including isothiocyanates (ITCs), thiocyanates, nitriles, epithionitriles and oxazolidines (Bones & Rossiter, 2006; Tang, Paonessa, Zhang, Ambrosone, & McCann, 2013). ITCs have been demonstrated to present strong anti-cancer activities (Traka & Mithen, 2009). Sulforaphane, hydrolyzed from glucoraphanin, is one kind of ITC. It is known as one naturally induced compound showing protective effects against a number of cancers, such as breast, lung, stomach, and leukemia (Losso & Truax, 2009; Traka & Mithen, 2009; Yeh & Yen, 2009). The chemoprevention properties of sulforaphane against cancer are exhibited mainly through dual actions. One is inhibiting the phase I enzymes that convert procarcinogens to carcinogens, the other is inducing the phase II enzymes that increase detoxification (Juge, Mithen, & Traka, 2007). In broccoli, the most abundant glucosinolate is glucoraphanin which accounts for over 50% of the total glucosinolates content (Brown et al., 2002; Martinez-Villaluenga et al., 2010; Winkler, Faragher,

* *Corresponding author.* Tel.: +86 025 84396293; fax: +86 025 84396293.
E-mail address: guzx@njau.edu.cn (Z. Gu).
http://dx.doi.org/10.1016/j.jff.2014.04.015
1756-4646/

Franz, Imsic, & Jones, 2007). Moreover, the content of glucoraphanin in broccoli sprouts is much higher than their mature counterparts (Fahey, Zhang, & Talalay, 1997). Considering the above issues, broccoli sprouts as natural and health-promoting food has received much attention around the world.

Climate and environmental factors (e.g., temperature, light conditions) have significant influence on glucosinolate contents (Charron, Saxton, & Sams, 2005; Gu, Guo, & Gu, 2012a; Pereira et al., 2002; Rosa & Rodrigues, 1998). The content of glucosinolates in broccoli sprouts cultivated at 30/15 °C (day/night) was higher than those at lower temperatures (22/15 and 18/12 °C) (Pereira et al., 2002). The diurnal variation in glucosinolates of cabbage sprouts at 20 °C was smaller than that at 30 °C. The reason suggested was that 20 °C was close to the optimum for cabbage sprouts growth (Rosa & Rodrigues, 1998). Plant organs can also significantly influence glucosinolate levels. In broccoli sprouts, the content of glucosinolates in cotyledons was higher than that in stems and roots (Pérez-Balibrea, Moreno, & García-Viguera, 2008), while it was higher in roots than in leaves of cabbage sprouts (Rosa & Rodrigues, 1998). However, the aforementioned studies have only focused on glucosinolates, but did not involve sulforaphane and myrosinase.

The objective of this study was to evaluate the influence of temperature on glucoraphanin, myrosinase activity and sulforaphane in broccoli sprouts and their distributions in different organs at the optimum temperature, as well as the profiles of ITCs and nitrile produced from seeds, sprouts and different organs.

2. Materials and methods

2.1. Plant material and cultivation conditions

Seeds of broccoli (*Brassica oleracea* var. *italica* cv. Lvxiong 5) were purchased from Hangzhou Sanxiong Seed Co., (Hangzhou, China). The seeds were immersed in 5 mL/L sodium hypochlorite for 15 min, then drained and washed with distilled water until they reached a neutral pH. Afterwards, they were soaked in distilled water for 4 h at 30 °C. After pouring off the soaking water, the seeds were weighed and sown evenly on trays (15 cm in diameter) filled with vermiculite. They were watered by an automatic spraying system providing a 5 s mist every 10 min. Germination of broccoli seeds was carried out at a constant 20, 25 and 30 °C, respectively, in photoperiod conditions (8 h dark and 16 h light). The sprouts were harvested on day 0, 1, 2, 3, 4, 5, 6 and 7 after sowing. The cotyledon, hypocotyl and root were dissected from 3-, 5- and 7-day-old sprouts which were grown at 25 °C. All samples were rapidly collected, weighted, and then flash frozen in liquid nitrogen and stored at –80 °C for further experiments.

2.2. Extraction and determination of glucoraphanin

Glucoraphanin was extracted and analyzed as previously reported by Font, Río-Celestino, Cartea, and de Haro-Bailón (2005) with minor modifications. Briefly, samples (500 mg) were extracted with 2 mL of 75% methanol at 80 °C for 15 min in a temperature-controlled water bath, with vortexing at regular intervals. The supernatant was collected after centrifugation (5 min, 10000 *g*), and the residues were extracted once again by using 2 mL of 75% methanol, centrifuged. One milliliter of the combined supernatant was applied to a 1 mL DEAE-Sephadex A-25 column (acetic acid activated) and rinsed with 2 mL of 0.02 mol/L sodium acetate. After addition of 200 μL of arylsulfatase solution and incubation for 16 h at 35 °C, the desulphoglucoraphanin was eluted with 4 mL of Milli-Q water and filtered through a 0.45 μm membrane filter. Separation and detection were performed on an Agilent 1200 HPLC system (Agilent Technologies Co. Ltd., Palo Alto, CA, USA) equipped with a G1314B UV detector and a G1311A quat pump, using a Eclipse XDB-C18 column (5 μm particle size, 4.6 × 150 mm; Agilent Technologies Co. Ltd.) at 226 nm. Determination was conducted at a flow-rate of 1.0 mL/min in a linear gradient starting with 0% acetonitrile for 1 min, reaching 20% acetonitrile at 21 min, 0% acetonitrile at 26 min. Sinigrin (2-propenyl glucosinolate) (Sigma, St. Louis, MO, USA) was added to each sample as an internal standard before the first extraction. The glucoraphanin content was expressed as μmol/g fresh weight of broccoli sprouts.

2.3. Myrosinase activity determination

Myrosinase activity determination was conducted as described previously by Kim, Chen, Wang, and Choi (2006) and Burow, Müller, Gershenzon, and Wittstock (2006) with minor modifications. A total of 500 mg broccoli sprouts were homogenized with 3 mL of 0.1 mol/L sodium phosphate buffer (pH 6.5) in an ice bath. After centrifugation at 10,000 *g* for 15 min at 4 °C, the supernatant was used as the crude enzyme. Its activity was assessed with 500 μL of 0.25 mmol/L sinigrin and 500 μL of the crude enzyme. After incubation at 37 °C for 15 min, the reaction was stopped by boiling for 5 min. The amount of glucose formed by myrosinase was measured using Glucose GOD/PAP Kit (Nanjing Jiancheng Biotech Inc., Jiangsu, China). The protein content of the supernatant was determined according to Bradford (1976) using bovine serum albumin as the standard. One myrosinase unit corresponded to 1 nmol glucose formed per minute. The specific activity is expressed as units per milligram of protein.

2.4. Sulforaphane determination

Sulforaphane formation was measured according to the method of Gu et al. (2012b) with slight modifications. A total of 500 mg broccoli sprouts were homogenized with 4 mL of distilled water, then hydrolyzed at 37 °C for 3 h. After hydrolyzing, it was extracted three times with 9 mL of dichloromethane. The dichloromethane fraction was dried at 35 °C under vacuum on a rotary evaporator. The residue was dissolved in 2 mL of 10% acetonitrile and filtered through a 0.45 μm membrane filter. The extracts were analyzed using an Agilent 1200 HPLC system (Agilent Technologies Co. Ltd.) with an Eclipse XDB-C18 column (5 μm particle size, 4.6 × 150 mm; Agilent Technologies Co. Ltd.). The flow rate was 0.6 mL/min in a linear gradient of 10–60% acetonitrile from 0 to 25 min, reaching 100% acetonitrile at 30 min. The injection volume was 20 μL and chromatogram was recorded at 254 nm. Sulforaphane (Sigma) was used as an external standard.