Food Chemistry 121 (2010) 1014–1019

Contents lists available at ScienceDirect

Food Chemistry

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

Effect of salt stress on phenolic compounds, glucosinolates, myrosinase and antioxidant activity in radish sprouts

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ARTICLE INFO

Article history:
Received 3 August 2009
Received in revised form 10 November 2009
Accepted 21 January 2010

Keywords:
Glucosinolate
Myrosinase
Salt stress
Total phenolic content
Sprouts
Radish
Antioxidant activity

ABSTRACT

The germination, growth, total phenolics, glucosinolate, myrosinase and antioxidant activity of radish sprouts germinated under 0 (control), 10, 50 and 100 mM of NaCl were investigated. The glucoraphasatin (4-methylthio-3-butenyl-glucosinolate), total glucosinolate and total phenolic contents of 5- and 7-day-old sprouts treated with 10 and 50 mM of NaCl were significantly decreased. However, the antioxidant activity of sprouts treated with 10 and 50 mM of NaCl was not affected. The glucoraphasatin and total glucosinolate contents of 5- and 7-day-old sprouts, total phenolic contents of 3- and 5-day-old sprouts were significantly increased and myrosinase activities of 3- and 5-day-old sprouts were inhibited, although the germination was dramatically inhibited by 100 mM of NaCl treatment. These results indicated that salt stress (100 mM of NaCl treatment) could improve the nutritional value of radish sprouts, and germination of sprouts under adequate salt stress could be one useful way to enhance health-promoting compounds of plant food.

1. Introduction

Sprouts have long been consumed globally due to their high nutritional values. *Brassica* sprouts, in particular broccoli (*Brassica oleracea* var. *italica*) and radish (*Raphanus sativus* L.) sprouts, contain substantial amount of antioxidants, vitamin C and health-promoting compounds such as glucosinolates and phenolic compounds (Barillari et al., 2005; Ciska, Honke, & Kozlowska, 2008; Fahey, Zhang, & Talalay, 1997; Martinez-Villaluenga, Frias, Gulewicz, Gulewicz, & Vidal-Valverde, 2008). It has been reported that radish sprouts have anticancer and antioxidant activities both *in vivo* and *in vitro* (Barillari et al., 2008; Ippoushi, Takeuchi, Ito, Horie, & Azuma, 2007; Papi et al., 2008).

Glucosinolates, one of the main health-promoting secondary metabolites in *Brassica*, are a group of sulphur- and nitrogen-containing secondary metabolites. These compounds have gained much attention in recent years due to the remarkable anticarcinogenic activity of their major hydrolysis products, isothiocyanates. Glucosinolates are chemically stable until they come in contact with the enzyme myrosinase (β-thioglucoside glucohydrolase, EC 3.2.1.147), which is localised in idioblasts (myrosin cells) to separate from glucosinolates (Andreasson, Jorgensen, Hoglund, & Meijer, 2001). Upon tissue damage, glucosinolates are released from plant vacuoles and rapidly hydrolysed by myrosinase to glucose and other unstable thiohydroximate-*O*-sulphonate intermediates, which, as dictated by chemical conditions, spontaneously rearrange to isothiocyanates, thiocyanates, or nitriles. Usually, production of isothiocyanates is favoured in neutral conditions. Epidemiological studies have shown that isothiocyanates have a protective effect against cancer, particularly bladder, colon and lung cancers (Cartea & Velasco, 2008).

Growth conditions, for example nitrogen and sulphur fertilisation (Aires, Rosa, & Carvalho, 2006), and environmental stresses, for example temperature and light conditions (Ciska et al., 2008; Pereira et al., 2002; Perez-Balibrea, Moreno, & Garcia-Viguera, 2008a) have been reported to exert a significant influence on glucosinolate content. Recently, several studies showed that salt stress dramatically increased the total glucosinolate content in broccoli florets (Lopez-Berenguer, Martinez-Ballesta, Garcia-Viguera, & Carvajal, 2008; Lopez-Berenguer, Martinez-Ballesta Mdel, Moreno, Carvajal, & Garcia-Viguera, 2009). Salt affects the quality and nutritional value of strawberry fruit (Keutgen & Pawelzik, 2008) and romaine lettuce (Kim, Fonseca, Choi, Kubota, & Kwon, 2008), and polyphenols content and antioxidant activity in leaves of the halophyte *Cakile maritime* (Ksouri et al., 2007). However, limited information is available about the influence of salt stress on health-promoting compounds and nutrition value of *Brassica* sprouts (Perez-Balibrea, Moreno, & García-Viguera, 2008b). The objective of the present study was to investigate the effect of NaCl treatments at

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doi:10.1016/j.foodchem.2010.01.040

different concentrations on the content of glucosinolate and total phenolic, myrosinase activity, and ferric reducing antioxidant power (FRAP) in radish sprouts.

2. Material and methods

2.1. Plant material and cultivation conditions

Radish seeds were purchased from a sprout company in Guangdong, China. 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 placed in distilled water and soaked overnight. Control sprouts were grown on 0.5% agar without added nutrients in culture flasks. The treatment sprouts were grown on 0.5% agar with added 10, 50, or 100 mM of NaCl in culture flasks. All sprouts were grown under a 16-h light and 8-h dark photoperiod and a constant 23 °C in a growth chamber (Safe Experimental Instrument Company, Ningbo, China). Sprout samples were collected at different time points (3, 5 and 7 days postsowing). For each day and treatment, sprouts were rapidly and gently collected from the surface of the agar. Germination percentage and fresh weight were recorded for each treatment, then the sprouts were frozen in liquid nitrogen immediately and kept in polyethylene bags at −70 °C for the analysis of FRAP value, total phenolic content and glucosinolate, as well as myrosinase activity. For each treatment, three replicates were taken for analysis.

2.2. Glucosinolate assay

Glucosinolates were extracted and analysed as previously described with minor modifications (Jia et al., 2009; Yuan, Sun, Yuan, & Wang, 2010). Samples (500 mg) were boiled in 4 ml water for 10 min. After recovery of the liquid, the residues were washed with water (4 ml), and the combined aqueous extract was applied to a DEAE-Sephadex A-25 (40 mg) column (pyridine acetate form). 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 an 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 onto 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, Tokuhisa, Reichelt, and Gershenzon (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 of radish sprouts.

2.3. Myrosinase activity determination

Myrosinase activity was determined as described previous by Burow et al. (2009). Radish sprouts (0.5 g) were homogenised with 1.8 ml of 50 mM MES buffer (pH 6.0) in an ice bath, incubated at room temperature for 5 min, and centrifuged at 10,000*g* and 4 °C for 10 min. The supernatants were collected and used for measurements. The assays were conducted with 1 mM sinigrin and 20 μl of supernatants in a total volume of 100 μl. After incubation at 37 °C for 15 min, the reaction was stopped by boiling (100 °C for 5 min). The amount of glucose formed by myrosinase was measured using Glucose GOD/PAP Kit (Shanghai Rongsheng Biotech Inc., Shanghai, China). The myrosinase activity was expressed as nmol glucose formed per minute and mg total protein. The protein content of the supernatant was determined according to the method of Bradford (1976), using bovine serum albumin as standard.

2.4. Total phenolic content assay

The total phenolic compounds of radish sprouts were extracted with 50% ethanol and the samples were 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 were expressed as milligrams of gallic acid equivalent (GAE)/100 g of fresh weight.

2.5. FRAP value determination

FRAP assay was determined according to the method of Benzie and Strain (1996). The working FRAP reagent was prepared daily by mixing 300 mM acetate buffer (pH 3.6), 20 mM ferric chloride, and 10 mM 2,4,6-tripyridyl-S-triazine in 40 mM HCl in the ratio of 10:1:1 (v/v/v). The extracted samples (20 μl) were added to 2.8 ml of the FRAP working solution incubated at 37 °C and vortexed. The absorbance was then recorded at 593 nm using a UV–Vis spectrophotometer (UV-2500, Shimadzu Corp., Kyoto, Japan) after the mixture had been incubated in at 37 °C for 10 min. FRAP values were calculated from $FeSO_4{\cdot}7H_2O$ standard curves and expressed as mmol/100 g of fresh weight.

2.6. Statistical analyses

Statistical analysis was performed using the SPSS package program version 11.5 (SPSS Inc., Chicago, IL, USA). Data was analysed by one-way ANOVA, followed by Turkey's HSD multiple comparison test. The values are reported as means with their standard error for all results. Differences were considered significant at $p < 0.05$.

3. Results

3.1. Effect of salt stress on germination and fresh weight

The seed germination was not significantly affected by the 10 mM of NaCl treatment, while it was inhibited with the increase of NaCl concentration. The germination percentages in radish seeds treated with 50 mM and 100 mM of NaCl were reduced by 23% and 35%, respectively (Fig. 1).

In general, the fresh weights of all radish sprouts were significantly increased during the germination period. The fresh weight of 3-day-old sprouts was significantly increased after application of 10 mM of NaCl, while it was decreased after treatment with 100 mM of NaCl in comparison with the control. The fresh weights of 5- and 7-day-old sprouts were increased by 50% and 64% after