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Calcium sulfate treatment enhances bioactive compounds and antioxidant capacity in broccoli sprouts during growth and storage

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ABSTRACT

The effect of preharvest $CaSO_4$ treatment on antioxidant enzyme activities, bioactive compounds and antioxidant capacity in broccoli sprouts during growth and storage was investigated in this study. Application of $CaSO_4$ increased the biomass and reduced electrolyte leakage of broccoli sprouts. Higher antioxidant enzyme activities and antioxidant capacity in sprouts were obtained in $CaSO_4$-treated sprouts during growth and storage. Total phenolic content in $CaSO_4$-treated sprouts was lower than that in control ones during growth; but was higher than in control sprouts during storage. Additionally, the decrease of ascorbic acid was suppressed by $CaSO_4$ treatment during storage. $CaSO_4$ treatment also dramatically enhanced glucosinolate content, especially glucoraphanin, in broccoli sprouts during growth and prevented its loss during storage. This was further supported by the up-regulation of glucoraphanin biosynthesis-related genes. During storage, $CaSO_4$-treated sprouts exhibited higher myrosinase activity and lower ESP activity, which resulted from higher expression of *MYR* and *ESM1*. Moreover, $CaSO_4$ treatment led to higher sulforaphane formation in sprouts than control during growth and storage.

1. Introduction

Nowadays, cruciferous sprouts have been widely consumed as functional foods, which are defined as those that lowering the risk of various diseases and/or exerting health promoting effects in addition to its nutritive value. Accumulated evidence indicates that broccoli sprouts have important physiological functions for maintaining and improving our health status (Baenas et al., 2017a; Bahadoran et al., 2011; Gawlik-Dziki et al., 2012; Medina et al., 2015). This could be attributed to their rich composition in bioactive compounds, such as phenolic compounds, flavonoids, ascorbic acid, anthocyanins, glucosinolates and sulforaphane (Ferruzza et al., 2016; Pereira et al., 2002). It has been reported that broccoli sprouts contain a great deal of glucoraphanin, an aliphatic glucosinolate, which content is 10–100 times of that in broccoli florets (Fahey et al., 1997). Intact glucoraphanin is biologically inactive, whereas the degradation product sulforaphane, exhibits multiple biological activities including decreasing the risk of cancer and cardiovascular diseases, anti-inflammatory, reducing blood glucose as well as inhibition of pathogenic fungal growth (Angelino and Jeffery, 2014; Annika Axelsson et al., 2017; Traka and Mithen, 2009). However, the action of the epithiospecifier protein (ESP) can drive the formation of sulforaphane nitrile at the expense of the potent anticancer agent sulforaphane (Matusheski et al., 2006).

Glucosinolates are a major class of sulphur-rich secondary metabolites involved in plant defense against pathogens. Hence, a number of studies applied sulphur-containing compounds to enhance the accumulation of glucosinolates in plants. The results of Pérez-Balibrea et al. (2010) showed that K_2SO_4 at 15, 30, and 60 mg/L enhanced glucosinolate content in 9- and 12-day old broccoli sprouts. Previous research indicated that application of methionine, the biosynthetic precursor of aliphatic glucosinolates, enhanced the level of glucosinolates, especially glucoraphanin in broccoli (Moreno et al., 2008). Besides, application of $ZnSO_4$ resulted in higher levels of glucoraphanin and sulforaphane in broccoli sprouts than K_2SO_4 and methionine treatments, but it significantly depressed the growth (Yang et al., 2015). To date, the main genes in the glucoraphanin pathway had been well described in *Brassica* plant (Li and Quiros, 2002; Zang et al., 2009). MYB28 is the most important transcription factor in aliphatic glucosinolate biosynthesis (Gigolashvili et al., 2007). The first step of the pathway is the chain elongation of methionine, which is catalyzed by the product of *Elong* (Li and Quiros, 2002). The genes of *CYP79F1*, *CYP83A1*, *UGT74B1* and *ST5b* take part in the development of core structure of aliphatic glucosinolates. Then, glucoraphanin is synthesized form glucoerucin by the product of $FMO_{GS\text{-}OX1}$, whereas AOP2 catalyzes glucoraphanin into gluconapin (Zang et al., 2009). The information is helpful to investigate the mechanism of glucoraphanin biosynthesis in broccoli sprouts.

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On the other hand, calcium plays an important role in regulation plant growth and signal transduction as well as the accumulation of secondary metabolites in plants (Kudla et al., 2010). Singh et al. (2012) reported that calcium treatment enhanced the contents of total phenolic acids and anthocyanins in carrot. Treatment with $CaCl_2$ was found to increase glucosinolate biosynthesis and isothiocyanate formation in broccoli sprouts (Yang et al., 2016). Nonetheless, to our knowledge, the effect of $CaSO_4$ treatment on bioactive compounds in broccoli sprouts has not been examined.

Although consumption of broccoli sprouts has nowadays increased, the industrialization is limited due to the rapid product deterioration and a very short shelf life. Calcium treatment was also found to extend the shelf life of fruits and vegetables (Martín-Diana et al., 2007). Previous research demonstrated that application of $CaCl_2$ enhanced productivity and improved postharvest quality and shelf-life of broccoli sprouts (Kou et al., 2014). Preharvest $CaSO_4$ application delayed postharvest softening and decrease weight loss of blueberry fruit (Angeletti et al., 2010). However, little information is available on the effect of preharvest $CaSO_4$ treatment on nutraceutical quality in broccoli sprouts during storage.

Therefore, the objective of the present study was to investigate the effect of preharvest $CaSO_4$ treatment on antioxidant enzyme activities, health-promoting compounds and antioxidant capacity in broccoli sprouts during growth and storage.

2. Materials and methods

2.1. Plant materials and treatment

Broccoli seeds were obtained from Hangzhou Sanxiong Seed Co., (Hangzhou, China). The seeds were sterilized, then rinsed and steeped with distilled water at 30 °C for 4 h. Next, they were sown evenly on trays filled with vermiculite and kept in a growth chamber with a 16 h light/8 h dark cycle at 25 °C. According to our preliminary experiment result (data were not shown), 10 mL of 10 mM $CaSO_4$ containing 0.01% tween 20 was used to treat broccoli sprouts every 12 h. The control sprouts were sprayed with 10 mL of distilled water. The sprouts were collected at day 3, day 6 and day 9 after sowing and labeled as "preharvest". On the other hand, harvested broccoli sprouts (day 9 after sowing) were labeled as 0 day postharvest and packaged in sealed bags prepared with polyethylene films of 16.6 pmol s^{-1} m^{-2} Pa^{-1} oxygen transmission rate. Packaged sprouts were stored at 4 °C and collected on postharvest day 5, 10, and 15. All samples were frozen in liquid nitrogen immediately and kept in polyethylene bags at −80 °C for later analysis. Each treatment was replicated three times.

2.2. Sprout length, fresh weight and electrolyte leakage assays

Sprout length, fresh weight and electrolyte leakage of broccoli sprouts were assayed as previous described (Guo et al., 2016a).

2.3. Determination of antioxidant enzyme activities

The sprouts (0.5 g) were homogenized with 5 mL of phosphate buffer (50 mM, pH 7.0) containing 1 mM EDTA and 2% PVP in a precooled mortar. The homogenate was centrifuged at 12,000*g* for 20 min at 4 °C, and the supernatant obtained was used for the further enzyme activity assays. The superoxide dismutase (SOD, EC 1.15.1.1), catalase (CAT, EC 1.11.1.6), and peroxidase (POD, EC 1.11.1.7) activities were determined using a SOD Assay Kit, a CAT Assay Kit and a POD Assay Kit, respectively (A084-3, Nanjing Jiancheng Bioengineering Institute, China). The glutathione peroxidase (GPX, EC 1.11.1.9) activity was measured as the decrease of absorbance at 340 nm of NADPH according to the method of Flohé and Gűnzler (1984). The reaction mixture consisted of 1.49 mL 50 mM phosphate buffer (pH 7.0), 0.1 mL of 1 mM EDTA, 0.1 mL of enzyme sample, 0.1 mL of 1 mM glutathione, 0.1 mL glutathione reductase, 0.1 mL of 0.2 mM NADPH and 0.01 mL of 0.25 mM H_2O_2. The reaction was started by the addition of H_2O_2 and the decrease in absorption at 340 nm is monitored for about 5 min.

2.4. Determination of total phenolic and ascorbic acid content, ferric reducing/antioxidant power (FRAP) value and DPPH· scavenging capacity

The contents of total phenolic and ascorbic acid were measured according to the method of Guo et al. (2014a). Total phenolic content was expressed as milligrams of gallic acid equivalent (GAE) per kilogram of fresh weight. Ascorbic acid was quantified by external calibration and results were recorded as milligrams per kilogram of fresh weight.

FRAP was measured using the experimental protocol described by Benzie and Strain (1996). Results were expressed as mmol Fe^{2+} per kilogram of fresh weigh. DPPH· scavenging capacity was determined following the method of (Brand-Williams et al., 1995). The result was calculated according to the following formula: DPPH· scavenging activity (%) = [1 − (absorbance of sample/absorbance of control)] × 100.

2.5. Determination of glucosinolate, myrosinase and ESP activities, as well as sulforaphane

Glucosinolates, myrosinase activity and sulforaphane were measured as previously described (Guo et al., 2014b). ESP activity was determined according to Guo et al. (2016a).

2.6. Quantitative real-time PCR analysis (qRT-PCR)

Total RNA was isolated from frozen tissue using the using a E.Z.N.A.™ Plant RNA Kit (OMEGA, R6827-01). Two micrograms of total RNA was used to synthesize cDNA following the instructions of the PrimeScript™ RT Master Mix Kit (RR036A, Takara, Dalian, China). qRT-PCR analysis of cDNA was performed on an ABI 7500 sequence detection system (Applied Biosystems, Foster City, CA, USA) using SYBRR *Premix Ex-Taq*™ (RR420A, Takara, Dalian, China). The sequence-specific primers for genes related glucosinolate metabolism used listed in Supplementary Table S1 according to previous studies (Guo et al., 2016b; Ku et al., 2013).

2.7. Statistical analyses

Experimental data were expressed as the mean ± standard deviation (SD) of three biological replicates. The SPSS 19.0 software package (LEAD Technologies, Inc., Chicago, IL, USA) was used for the significant difference test. One way analysis of variance (ANOVA) with Tukey's test was conducted on the data, and $p < 0.05$ was considered significant.

3. Results

3.1. Effect of $CaSO_4$ treatment on sprout length, fresh weight and electrolyte leakage of broccoli sprouts

Application of $CaSO_4$ did not affect the growth sprouts after 3 days, but significantly promoted it after 6 and 9 days (Fig. 1A and B). The sprout length and fresh weight of 9-day-old sprouts treated with $CaSO_4$ was increased by 23.3% and 27.6% compared with the control, respectively. Electrolyte leakage of sprouts firstly decreased, and then increased during storage. No significant difference was found in electrolyte leakage of preharvest sprouts between $CaSO_4$ treatment and the control. Nevertheless, $CaSO_4$ treatment significantly decreased electrolyte leakage of 5, 10, 15-day postharvest sprous by 27.4%, 36.1% and 25.1%, respectively (Fig. 1C).