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Effects of Sulfur Fertilization on the Accumulation of Health-Promoting Phytochemicals in Radish Sprouts

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ABSTRACT: The effects of sulfur fertilization on the growth profile, the contents of glucosinolates, anthocyanins, vitamin C, carotenoids, chlorophylls, total phenolics, and the FRAP value in radish seeds and sprouts were investigated. The concentrations of glucosinolates and antioxidants in sprouts were strongly influenced by the process of germination. Sulfur fertilization induced significant increases in the contents of individual glucosinolates, carotenoids, chlorophylls, and total phenolics. The phenolic contents in sprouts cultivated using 20, 60, or 100 mg/L sulfate were 20.7%, 40.4%, and 40.8% higher, respectively, than those of 7-day-old control sprouts. No detectable effects were observed on the contents of 4-methoxy-glucobrassicin and vitamin C. In addition, the accumulation of anthocyanins in 7-day-old sprouts decreased by 14.8−39.3% upon sulfur fertilization. These findings indicated that the application of sulfur fertilization has the potential to enhance the levels of health-promoting compounds in radish sprouts.

KEYWORDS: *radish sprouts, germination, glucosinolates, phenolics, antioxidant activity*

INTRODUCTION

In recent years, cruciferous vegetables have received much attention due to their richness in phenolic compounds, vitamins, and anthocyanins, which possess strong antioxidant activity.[1] However, the most distinctive characteristic of the members of the Brassicaceae family is their high glucosinolate content. Glucosinolates, a group of sulfur-rich secondary metabolites, are usually classified into three types as aliphatic, indole, and aromatic glucosinolates, according to whether their amino acid precursor is methionine, tryptophan, or phenylalanine, respectively. Glucosinolates provide crucifers with a sulfur storage pool that is used to maintain normal metabolism under conditions of sulfur deficiency, and as such, their accumulation and the supply of sulfur are closely related.[2,3] Isothiocyanates, the major degradation products of glucosinolates, have a notable effect in inducing the activity of detoxification enzymes in normal cells and simultaneously inhibiting the growth of cancer cells.[4−6] Epidemiological evidence has shown that the consumption of cruciferous vegetables rich in glucosinolate (e.g., broccoli, radish, kale, cabbage, cauliflower) is associated with a significant reduction in cancer risk.[1] The edible sprouts of cruciferous vegetables are currently receiving a great deal of attention due to their much higher nutritional value compared to the mature plants. The levels of nutritional ingredients such as glucosinolates, carotenoids, and phenolics are approximately 15−50 times higher in edible sprouts than mature plants.[5,7,8]

The red radish (*Raphanus sativus* L.) is a traditionally and broadly consumed cruciferous vegetable in East Asian countries, especially China, Korea, and Japan. In recent years, hydroponically cultivated red radish sprouts appearing in Chinese markets and restaurants have gained high popularity because of their unique flavor and abundant nutrients. Due to the worldwide consumption of vegetables such as broccoli and kale, the effects of exogenous inducers, including sulfur fertilization, phytohormones, and light conditions, on the accumulation of health-promoting compounds in mature plants and sprouts have been widely studied.[2,8−10] However, few reports are available regarding the effects of such elicitors on the levels of nutrients in the mature red radishes and sprouts.

To improve our knowledge of the profiles of glucosinolates and other nutrients in the red radish sprouts, a widely cultivated Chinese red radish cultivar known as 'Mantanghong' was investigated in the present study with the aim of examining the effects of sulfur application on bioactive compounds and evaluating the possibility of improving the nutritional value of Chinese red radish sprouts during the sprouting process.

MATERIALS AND METHODS

Chemicals and Reagents. Sinigrin and sulfatase (E.C. 3.1.6.1, type H-1 from *Helix pomatia*) were purchased from Sigma-Aldrich (USA). Methanol, acetonitrile, and trifluoroacetic acid were of HPLC grade and purchased from Thermo Fisher Scientific Inc. (Shanghai, China). Other chemicals and solvents were of analytical grade and purchased from Sinopharm Chemical Reagent Co., Ltd. (Beijing, China).

Seed Germination and Sprout Cultivation. Radish (*Raphanus sativus* L. Mantanghon) seeds were purchased from the Beijing Academy of Agriculture and Forestry Sciences, China. The seeds were rinsed with deionized water and immersed in 5 mL/L sodium hypochlorite for 30 min. They were then drained, placed in deionized water, and soaked for 10 h, after which they were placed in culture trays on three pieces of wet filter paper. Each culture tray was irrigated with increasing concentrations of K_2SO_4 solution, at 0, 20, 60, and 100 mg of S/L; K was balanced through the application of KCl. After two days of germination in darkness at 23 °C, the sprouts were cultivated under a 16 h light−8 h dark photoperiod at 23 °C in a growth chamber (Life Apparatus Company, Ningbo, China) and were watered every 8 h. On each day for each treatment, the edible sprouts were

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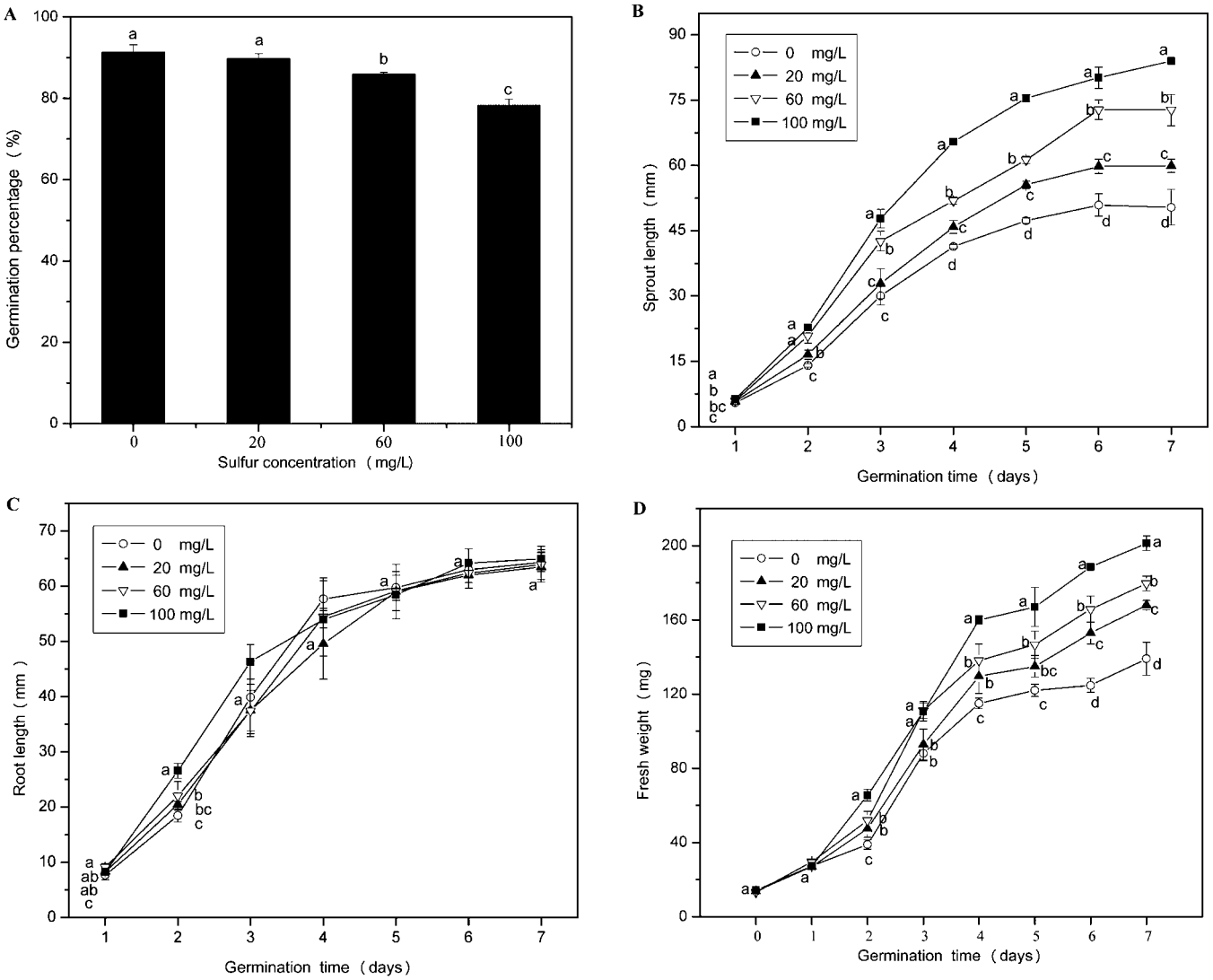

Figure 1. (A) Percentage of germination in radish seeds under sulfur treatment. The data represent the mean ± SD (n = 3). Values that do not share a common letter are significantly different ($p \leq 0.05$). (B) Sprout length under different sulfur concentrations. The data represent the mean ± standard deviation (n = 3). Means with different letters at each time point differed significantly ($p \leq 0.05$). (C) Root length under different sulfur concentrations. The data represent the mean ± standard deviation (n = 3). Means with different letters at each time point differed significantly ($p \leq 0.05$). (D) Fresh weight under different sulfur concentrations. The data represent the mean ± standard deviation (n = 3). Means with different letters at each time point differed significantly ($p \leq 0.05$).

rapidly and gently collected from the filter paper and flash frozen in liquid nitrogen, then kept in polyethylene bags at −80 °C for further analysis. Samples were collected every day until the seventh day after sowing. Three independent replicates were performed for each treatment.

Determination of Growth. The rate of germination was determined by counting fifty seeds that germinated after the second day. Any seed with a root or shoot length greater than 2 mm was regarded as germinated. Starting from the second day of germination, fifty sprouts were randomly selected from each culture tray, and the fresh weight, sprout length, and root length were recorded. All experiments were repeated three times.

Glucosinolate Analysis. *Sample Preparation.* The extraction of glucosinolates was performed as described by Zimmermann et al.[11] with slight modification. 100 mg of freeze-dried samples was extracted with 5 mL of boiling 70% methanol for 10 min at 85 °C. 200 μL of 500 mM barium acetate was added to precipitate proteins and free sulfate ions. 500 μL of 20 mM Sinigrin (Sigma-Aldrich, Germany) was added as internal standard before extraction. Following extraction, the suspension was centrifuged at 4000*g* at 4 °C for 20 min, and the supernatant was applied to a DEAE-Sephadex A-25 ion exchange (40 mg) column that had been washed with 3 mL of 0.02 M pyridine acetate and 3 mL of deionized water. The glucosinolates were converted into their desulfated derivatives through overnight treatment with 200 μL of purified sulfatase (E.C. 3.1.6.1, type H-1 from *Helix pomatia*) (Sigma) solution; the sulfatase was purified according to the method reported by Malik et al.[12] Finally, the desulfo-glucosinolates were eluted off the column with 3 mL of distilled water and filtered through a 0.45 μm membrane before analysis.

LC–DAD–ESI/MS Qualitative and Quantitative Analysis of Desulfo-Glucosinolates. All analysis were performed in an Agilent 1260 series HPLC coupled to an Agilent 6460 triple quadrupole mass spectrometer. An Agilent C18 column (3.5 μm, 2.1 × 150 mm) was employed at a flow rate of 0.3 mL/min. The mobile phase was a mixture of 0.1% trifluoroacetic acid (TFA) (solvent A) and acetonitrile–TFA (v/v 99.9:0.1) (solvent B) in a linear gradient starting with 0% B for 1 min, followed by a gradient of 0–20% B for 20 min and a linear gradient to 0% B for 5 min and, finally, 0% B for 5 min. Absorbance was detected at 229 nm with an injection volume of 10 μL. The concentration was quantified on the basis of the internal standard and relevant relative response factors.[13] The content of desulfo-glucosinolates was expressed as μmol/g dry weight (DW). The

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Table 1. Individual and Total Glucosinolate Contents (μmol/g dry weight) in Radish Sprouts under Sulfur Treatment

		days after germination							
glucosinolates	treatment (mg/L)	0	1	2	3	4	5	6	7
glucoraphenin	0	94.74 ± 0.72 a	75.44 ± 1.32 a	59.63 ± 1.66 a	42.67 ± 0.97 a	32.10 ± 0.38 a	19.1 ± 0.60 a	12.95 ± 0.59 a	10.20 ± 0.30 a
	20	94.62 ± 0.84 a	76.79 ± 2.06 a	62.02 ± 1.79 a	47.54 ± 1.41 b	39.34 ± 0.87 b	26.94 ± 0.83 b	20.62 ± 1.83 b	15.10 ± 0.81 b
	60	94.91 ± 0.73 a	76.55 ± 0.93 a	67.09 ± 2.25 b	48.59 ± 1.64 b	44.77 ± 0.88 c	28.22 ± 0.41 b	24.76 ± 1.11 c	18.05 ± 0.66 c
	100	95.04 ± 0.80 a	76.07 ± 1.39 a	70.76 ± 1.38 c	63.27 ± 0.57 c	52.64 ± 1.05 d	37.84 ± 1.21 c	29.58 ± 0.65 d	24.81 ± 0.67 d
glucobrassicin	0	4.60 ± 0.49 a	3.74 ± 0.22 a	3.03 ± 0.19 a	1.94 ± 0.09 a	1.04 ± 0.09 a	0.66 ± 0.06 a	0.47 ± 0.06 a	0.36 ± 0.05 a
	20	4.58 ± 0.32 a	3.66 ± 0.30 a	3.15 ± 0.08 a	2.60 ± 0.23 b	1.84 ± 0.11 b	1.44 ± 0.10 b	1.13 ± 0.15 b	0.87 ± 0.15 b
	60	4.78 ± 0.20 a	3.79 ± 0.22 a	3.53 ± 0.18 b	2.82 ± 0.37 bc	2.11 ± 0.12 c	1.64 ± 0.18 b	1.27 ± 0.09 b	1.01 ± 0.09 b
	100	5.04 ± 0.10 a	3.93 ± 0.20 a	3.68 ± 0.16 b	3.05 ± 0.10 c	2.66 ± 0.08 d	2.04 ± 0.17 c	1.52 ± 0.05 c	1.26 ± 0.10 c
4-methoxy- glucobrassicin	0	2.63 ± 0.11 a	2.02 ± 0.10 a	1.89 ± 0.12 b	1.18 ± 0.05 a	0.72 ± 0.10 a	0.65 ± 0.18 a	0.45 ± 0.11 a	0.23 ± 0.06 a
	20	2.63 ± 0.03 a	2.07 ± 0.03 a	1.59 ± 0.09 a	1.06 ± 0.17 a	0.91 ± 0.08 ab	0.65 ± 0.06 a	0.51 ± 0.10 a	0.23 ± 0.04 a
	60	2.58 ± 0.23 a	2.06 ± 0.17 a	1.68 ± 0.17 ab	1.07 ± 0.16 a	1.02 ± 0.11 b	0.69 ± 0.14 a	0.53 ± 0.05 a	0.25 ± 0.08 a
	100	2.60 ± 0.19 a	2.05 ± 0.13 a	1.70 ± 0.14 ab	1.13 ± 0.21 a	1.02 ± 0.18 b	0.7 ± 0.03 a	0.56 ± 0.09 a	0.30 ± 0.08 a
total glucosinolates	0	101.98 ± 0.98 a	81.20 ± 1.56 a	64.54 ± 1.44 a	45.78 ± 1.09 a	33.85 ± 0.37 a	20.41 ± 0.76 a	13.87 ± 0.73 a	10.79 ± 0.39 a
	20	101.83 ± 1.11 a	82.53 ± 1.82 a	66.76 ± 1.80 a	51.20 ± 1.41 b	42.09 ± 1.01 b	29.03 ± 0.75 b	22.27 ± 2.04 b	16.20 ± 0.77 b
	60	102.27 ± 1.04 a	82.40 ± 0.69 a	72.30 ± 1.92 b	52.49 ± 2.17 b	47.90 ± 1.09 c	30.55 ± 0.18 b	26.56 ± 1.19 c	19.32 ± 0.71 c
	100	102.68 ± 0.58 a	82.04 ± 1.47 a	76.14 ± 1.28 c	67.45 ± 0.73 c	56.33 ± 1.06 d	40.58 ± 1.27 c	31.65 ± 0.60 d	26.37 ± 0.58 d

[a]The total glucosinolate content was calculated as the sum of individual glucosinolates. [b]The data represent the mean ± standard deviation (n = 3). Means with different letters within the same column differed significantly ($p \leq 0.05$).

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desulfo-glucosinolates were identified according to previous work[11] from $[M + H]^+$ and $[M + H - glucose]^+$ protonated fragmentations.

Anthocyanin Content Determination. Frozen sprouts (200 mg) were extracted at 4 °C in 2 mL of 1% (v/v) hydrochloric acid in methanol for 1 day. The suspension was centrifuged at 12000*g* for 20 min at 4 °C. The supernatant was then collected, and the absorbance was detected at 530 and 657 nm. Anthocyanin content was calculated with the formula as previously described.[14]

Vitamin C Content Determination. Determination of vitamin C levels was performed according to the method reported by Pugliese et al.[15] with slight modifications. Briefly, 500 mg of the frozen samples was extracted with 5 mL of 1.0% (w/v) oxalic acid solution. The mixture was vortexed briefly and centrifuged at 12000*g* for 10 min. The supernatant was then filtered using a 0.45 μm membrane. HPLC analysis of vitamin C was carried out using an HPLC (Agilent 1200 series) equipped with an Agilent C18 column (5 μm, 4.6 × 250 mm). Oxalic acid (0.1%) was used as the mobile phase. The flow rate was 1.0 mL/min, and absorbance was detected at 245 nm with an injection volume of 20 μL. The vitamin C contents were expressed as mg 100 g^{-1} FW.

Total Carotenoids and Chlorophyll Content Determination. Freeze-dried samples (200 mg) were extracted with 5 mL of 80% (v/v) acetone for 24 h at 4 °C in the dark. The extracts were vortexed briefly and centrifuged at 6000*g* for 10 min. The supernatant was subsequently collected, and the absorbance was detected at 470, 646.8, and 663.2 nm. The carotenoid and chlorophyll concentrations were determined according to the formula described by Juszczuk et al.[16] and were calculated and expressed as mg 100 g^{-1} dry weight (DW).

Total Phenolic Content Determination. The total phenolic content in radish seeds and sprouts was determined as described previously[17] with minor modifications. Frozen sprouts (300 mg) were extracted with 95% ethanol and incubated at room temperature for 48 h in the dark. The suspension was then centrifuged at 12000*g* for 5 min, and the supernatant was collected. A 100 μL aliquot of the extract was combined with 100 μL of Folin–Ciocalteu reagent, 1 mL of distilled water, and 300 μL of 0.7 M Na_2CO_3. After incubation at room temperature for 2 h, the absorbance was measured at 765 nm. Gallic acid was used as a standard, and the results were expressed as milligrams of gallic acid equivalent (GAE) /100 g fresh weight (FW).

Determination of Ferric Reducing Antioxidant Power (FRAP). FRAP assay was conducted as described by Benzie et al.[18] The FRAP working 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 at a ratio of 10:1:1 (v/v/v). Extracts were prepared as described in the protocol for the phenolic content assay above (2.6), and a 300 μL aliquot of each extract was combined with 1.5 mL of the FRAP working solution and 3 mL of distilled water. The absorbance was detected at 593 nm after the mixture was vortexed briefly and incubated at 37 °C for 30 min. FRAP values were quantified by comparison with a standard curve of $FeSO_4 \cdot 7H_2O$. FRAP values were calculated and expressed as mmol/100 g fresh weight (FW).

Statistical Analysis. All experiments were conducted in three biological replicates, and the results are expressed as mean ± standard deviation. Statistical significance was assessed via one-way analysis of variance (ANOVA) followed by Duncan's multiple range tests using SPSS 16.0 (SPSS Inc., Chicago, IL, USA) ($P < 0.05$).

RESULTS AND DISCUSSION

Effects of Sulfur Fertilization on Growth. In the present study, the seed germination and growth of radish sprouts were both affected by the level of sulfur fertilization. There were slight decreases in the seed germination percentage under treatment with 60 mg/L and 100 mg/L sulfate, by 6% and 14%, respectively, compared with the control (Figure 1A). Similar inhibition was observed when the seeds were subjected to NaCl stress,[19] primarily due to an osmotic effect.

The fresh weight and sprout length increased significantly ($p \leq 0.05$) with the increased sulfur dose, whereas no differences were detected in root length (Figures 1B, 1C, and 1D). These findings are similar to those of previous studies on other vegetables such as broccoli,[9] spinach, and pepper,[20,21] which have shown a positive effect of sulfur fertilization on marketable yield and quality.

Effects of Sulfur Fertilization on Individual and Total Glucosinolate Contents. The influence of sulfur and nitrogen fertilization on the glucosinolate content in broccoli, cabbage, watercress, and their sprouts is well documented, whereas there has been relatively less research on the radish and its sprouts.

Three individual glucosinolates, glucoraphenin, glucobrassicin, and 4-methoxy-glucobrassicin, were identified and quantified via LC–DAD–ESI/MS in raw seeds and sprouts (Table 1). As the reproductive organ, the seeds were found to exhibit the highest contents of both total and individual glucosinolates, which may reflect the need to maximize the defensive potential against insects.[22] The predominant glucosinolate was glucoraphenin, which accounted for more than 92% of the total glucosinolate content during the sprouting period. This finding was similar to the results reported by Baenas et al.[5] and Ciska et al.[23] However, potentially because of the different varieties used, no 4-hydroxy-glucobrassicin or napoleiferin was detected in the present study.

Both the total and individual glucosinolate contents in sprouts tended to decline gradually during germination (Table 1), in accord with previous results.[5,19,24] Compared with the raw seeds, the contents of glucoraphenin, glucobrassicin, and 4-methoxy-glucobrassicin in control sprouts cultivated for 7 days decreased significantly, by 89.2%, 92.2%, and 90.5%, respectively. However, despite an obvious dilution effect on the glucosinolate content due to tissue expansion and growth,[10] sprouts are still an excellent source of glucosinolates compared to mature plants.[25]

Sulfur treatment had a positive impact on the accumulation of glucoraphenin, as an increase was observed starting from the second day of cultivation (Table 1). When 20 mg/L of sulfur was applied during cultivation, the glucoraphenin contents were increased by 22.6% and 48.1% in 4- and 7-day-old sprouts, respectively, compared with the control. Moreover, higher concentrations of sulfur (60 and 100 mg/L) caused more significant increases, leading to glucoraphenin contents that were 1.39 and 1.64 times higher than the control, respectively, in 4-day-old sprouts. After 7 days of cultivation, the glucoraphenin contents in these sprouts were markedly increased, by 76.9% and 143.1%, respectively. In agreement with the results obtained in this study, Pérez-Balibrea et al.[10] observed a decline in glucobrassicin contents with a reduced sulfur supply in broccoli sprouts. An increase in glucosinolate concentrations associated with an enhanced S supply has also been observed in other brassica species.[26–28]

Sulfur fertilization affected the accumulation of specific glucosinolates differentially.[29] In the present study, with sulfur application ranging from 0 to 100 mg/L during cultivation, the synthesis of methionine (essential precursor for aliphatic glucosinolates), a sulfur-containing amino acid, is enhanced in radish sprouts through biosynthetic pathway. [2] As a result, the accumulation of glucoraphenin (belongs to aliphatic glucosinolates), which are derived from methionine, increased significantly. In contrast, biosynthesis of sulfur-free amino

acid tryptophan, the precursor for indole glucosinolates, was almost unaffected by varied levels of sulfur application during sprouting. This could be the reason why no detectable effect on 4-methoxy-glucobrassicin (belongs to indole glucosinolates) contents was observed in response to treatment with different concentrations of sulfur. Interestingly, the accumulation of glucobrassicin, which is an indole glucosinolate, was significantly affected by sulfur application. The results of some previous studies are consistent with the current findings[27] while some are not.[30] This discrepancy may be caused by differences in species varieties, growing stages, and the level of nitrogen application during cultivation.

Effects of Sulfur Fertilization on Anthocyanin Content. A group of natural pigments known as anthocyanins that exist in various plants contribute to potential antioxidant effects, reducing the risk of coronary heart disease and the prevention of some chronic diseases.[31] In the present study, the first two days of germination in darkness led to a sharp decrease in anthocyanin contents, with the concentration in 2-day-old control seedlings being reduced by 45.4% compared to raw seeds (Figure 2). It was previously reported that plants grown

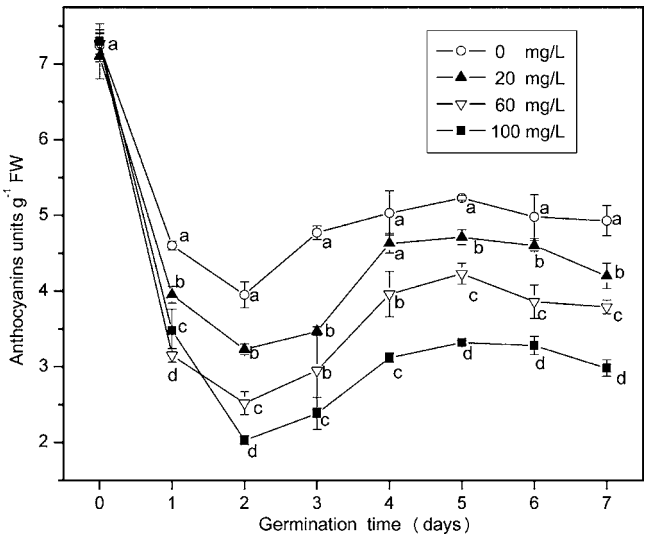

Figure 2. The effect of different concentrations of sulfur fertilization on anthocyanin content. The data represent the mean ± standard deviation (n = 3). Means with different letters at each time point differed significantly ($p \leq 0.05$).

in darkness accumulate lower levels of anthocyanin compared to those grown under light cultivation, and this effect is controlled by multiple regulatory genes.[32−34] A gradual rebound followed when sprouts were cultivated under a 16 h light−8 h dark cycle. Overall, compared to the raw seeds, there was a noticeable decrease in the anthocyanin content of both control and sulfur-treated sprouts after 7 days of cultivation.

In addition, a decrease in anthocyanin content was observed in sulfur-treated sprouts compared with the control (Figure 2), with the level of anthocyanin accumulation in 7-day-old sprouts treated with 20, 60, and 100 mg/L sulfur being 14.8%, 23.1%, and 39.6% lower, respectively. The results indicated that increased doses of sulfur resulted in a more obvious reduction of anthocyanin contents in radish sprouts. Previous reports have demonstrated that both biosynthetic and regulatory anthocyanin genes are stimulated by biotic and abiotic stress.[32,35,36] Under conditions of a decreased thylakoid membrane capacity caused by sulfur deficiency, plants produce more anthocyanins via the flavonoid biosynthesis pathway to neutralize high light radiation.[3] Our data indicate that a moderate level of sulfur fertilization (20 mg/L), without leading to a serious growth inhibition compared with sufficient sulfur supply, is better for accumulation of anthocyanins in radish sprouts than 60 mg/L and 100 mg/L sulfur doses.

Effects of Sulfur Fertilization on Vitamin C Content. Acting as one of the main indices in the nutritional evaluation for vegetables and fruits, vitamin C is mainly involved in chelating metal ions and scavenging harmful free radicals. In the present study, vitamin C was found to be absent in seeds (Figure 3), similar to the findings reported by Cristina et al.[24]

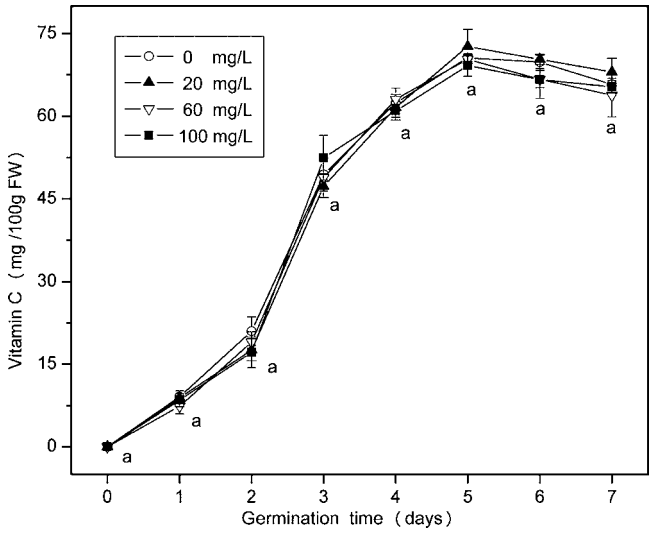

Figure 3. The effect of different concentrations of sulfur fertilization on vitamin C content. The data represent the mean ± standard deviation (n = 3). Means with different letters at each time point differed significantly ($p \leq 0.05$).

and Pérez et al.[37] Previous research has indicated that the biosynthesis of vitamin C in broccoli sprouts responds sensitively to elicitors such as light[37] and salicylic acid.[38] However, the vitamin C contents in the control and sulfur-treated radish sprouts showed no differences in the current study. Elwan et al.[9] reported that the growing season and genotype affect the accumulation of vitamin C in broccoli, whereas the sulfur supply does not. The vitamin C content in the radish sprouts increased sharply with the progress of germination, reaching a peak on day 5 at a concentration of 70.63 ± 0.7 mg/100 g (Figure 3), which was 8 and 3.5 times higher than that in 1- and 2-day-old seedlings, respectively. Our results confirmed those of Martinez et al.[24] and Guo et al.,[39] who observed an increase of vitamin C contents in edible sprouts in a time-dependent manner during germination. Compared with the 5-day-old sprouts, the vitamin C content in sprouts cultivated for 7 days was reduced slightly by 7.2%, similar to what has been observed in broccoli sprouts[37] and mung bean sprouts,[39] in which a slight decrease of the vitamin C concentration occurs after the peak is reached during seed germination. The increased activity of L-galactono-1,4-lactone dehydrogenase (GLDH, EC 1.3.2.3) in soybean sprouts observed by Xu et al. demonstrated that the enhanced accumulation of vitamin C during sprouts cultivation could be linked to the reactivation of ascorbic acid biosynthesis

during germination, in which GLDH plays an important role as one of the key enzymes involved in catalyzing the oxidation of L-galactono-1,4-lactone to ascorbic acid.[40,41]

Effects of Sulfur Fertilization on Chlorophyll and Total Carotenoid Contents. The response of the chlorophyll content to sulfur fertilization is presented in Figure 4A. The process of germination led to a 14-fold increase in the chlorophyll content in control sprouts from the second to the fifth day, with values ranging from 0.86 ± 0.01 to 12.17 ± 0.33 mg/100 g (DW). Subsequently, the chlorophyll content remained at a relatively stable level. Moreover, the supply of sulfur caused a remarkable increase in the chlorophyll content starting on the second day. After 7 days of cultivation, the chlorophyll contents in sprouts treated with sulfur at concentrations of 20, 60, and 100 mg/L were 14.6%, 48.7%, and 88.5% higher, respectively, than that in control sprouts, indicating that sulfur fertilization had a positive effect on chlorophyll accumulation.

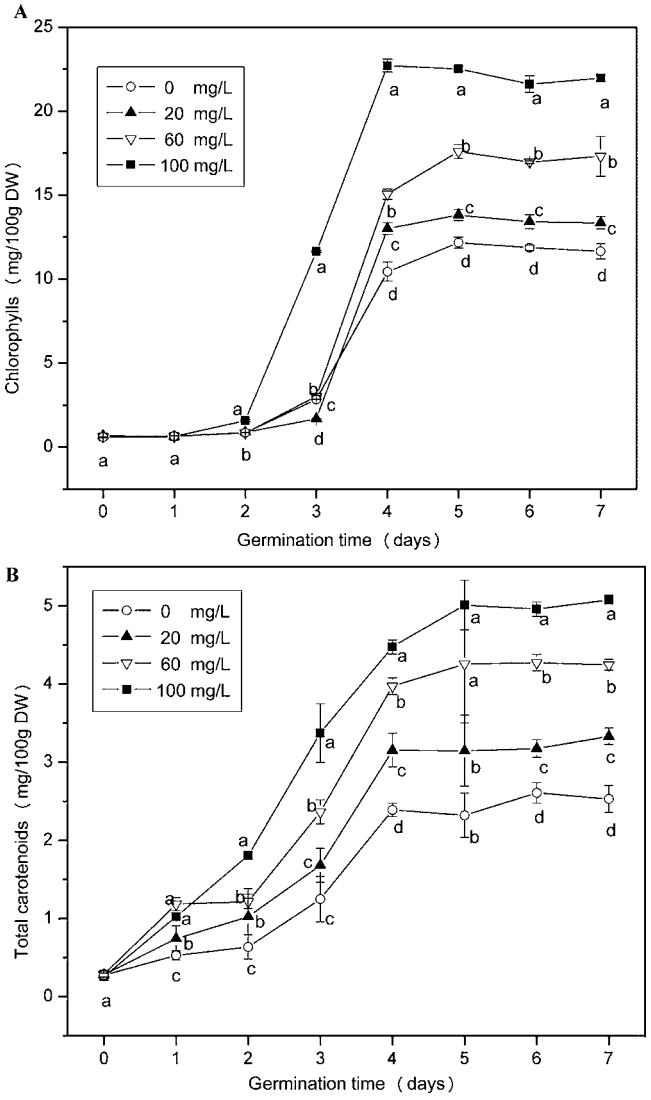

Figure 4. (A) The effect of different concentrations of sulfur fertilization on chlorophyll content. The data represent the mean ± standard deviation (n = 3). Means with different letters at each time point differed significantly ($p \leq 0.05$). (B) The effect of different concentrations of sulfur fertilization on the total carotenoid content. The data represent the mean ± standard deviation (n = 3). Means with different letters at each time point differed significantly ($p \leq 0.05$).

S-Adenosylmethionine (SAM) is a sulfur-containing metabolite required in a vital step of chlorophyll biosynthesis.[42] In our research, the rise in chlorophyll concentration was mainly due to the increased availability of SAM in sprout tissues under increased concentration of sulfur fertilization.

Carotenoids, a hydrophobic class of pigments that have been shown to display chemopreventative properties,[43] were almost undetectable in the seeds (Figure 4B). Subsequently, during the sprouting process, the content of total carotenoids in control sprouts displayed a remarkable increase until the fifth day after cultivation, when the concentration of total carotenoids (2.32 ± 0.29 mg/100 g DW) was 4.4 times higher than that in 1-day-old seedlings (0.53 ± 0.06 mg/100 g DW). We observed that the variation of total carotenoid content in sprouts under sulfur treatment was similar to that of the chlorophyll content, reflecting a positive correlation between carotenoid and chlorophyll concentrations reported by Reif et al.[44] Genomic data have also confirmed the close relationship between chlorophyll and carotenoid biosynthetic pathways.[27] In other words, our results confirmed that the carotenoid content increased under sulfur fertilization to a certain degree in radish sprouts.

Effects of Sulfur Fertilization on the Total Phenolic Content and FRAP Value. The germination process caused a significant decrease of the total phenolic content, with values ranging from 380.5 ± 3.0 to 132.31 ± 8.0 mg per 100 g FW (Figure 5A). This finding is in agreement with the results reported by Yuan et al.[19] and Pérez et al.,[38] who observed a gradual decline of total phenolic contents in radish and broccoli sprouts, possibly due to the sprouts' rapid growth and the consumption of some phenolic compounds for the biosynthesis of lignin, which is involved in plant defense systems.[45] Although a decrease was observed during sprouting, the 7-day-old control sprouts showed a 5−10 times higher total phenolic content compared to commercial, mature cruciferous vegetables.[7] In addition, an increase in phenolic content was observed in the sulfur-treated sprouts compared with the control (Figure 5A), although the enhancement caused by the application of 20 mg/L sulfur was less robust than that caused by treatment with 60 mg/L and 100 mg/L sulfur. These results are similar to findings indicating that sulfur application is an effective method of enhancing phenolic contents in cruciferous sprouts.[26] Exposure to abiotic stresses, such as a high sulfur fertilization concentration, may inhibit the activity of both peroxidase (POD) and the phenolic enzyme polyphenol oxidase (PPO), thus reducing the consumption rate of phenolics and then resulting in an increase in the total phenolic accumulation, as previously reported by Vallejo et al.[46]

Because Brassicaceae sprouts are rich sources of phenolics and exhibit considerable antioxidant activity, we assessed the ferric reducing antioxidant power (FRAP) of the radish sprouts (Figure 5B). Similar to the change in the total phenolic content, the FRAP value of the sprouts decreased during the germination period. Meanwhile, an increase in the response to increasing sulfur concentrations was also observed. As the major natural antioxidants present in cruciferous vegetables, phenolic compounds account for 80−95% of the total antioxidant capacity.[5] Our results showed a significant and positive correlation between the total phenolic content and the FRAP values (R^2 = 0.9635) (Figure 5C). A similar correlation between the total phenolic content and antioxidant activity has

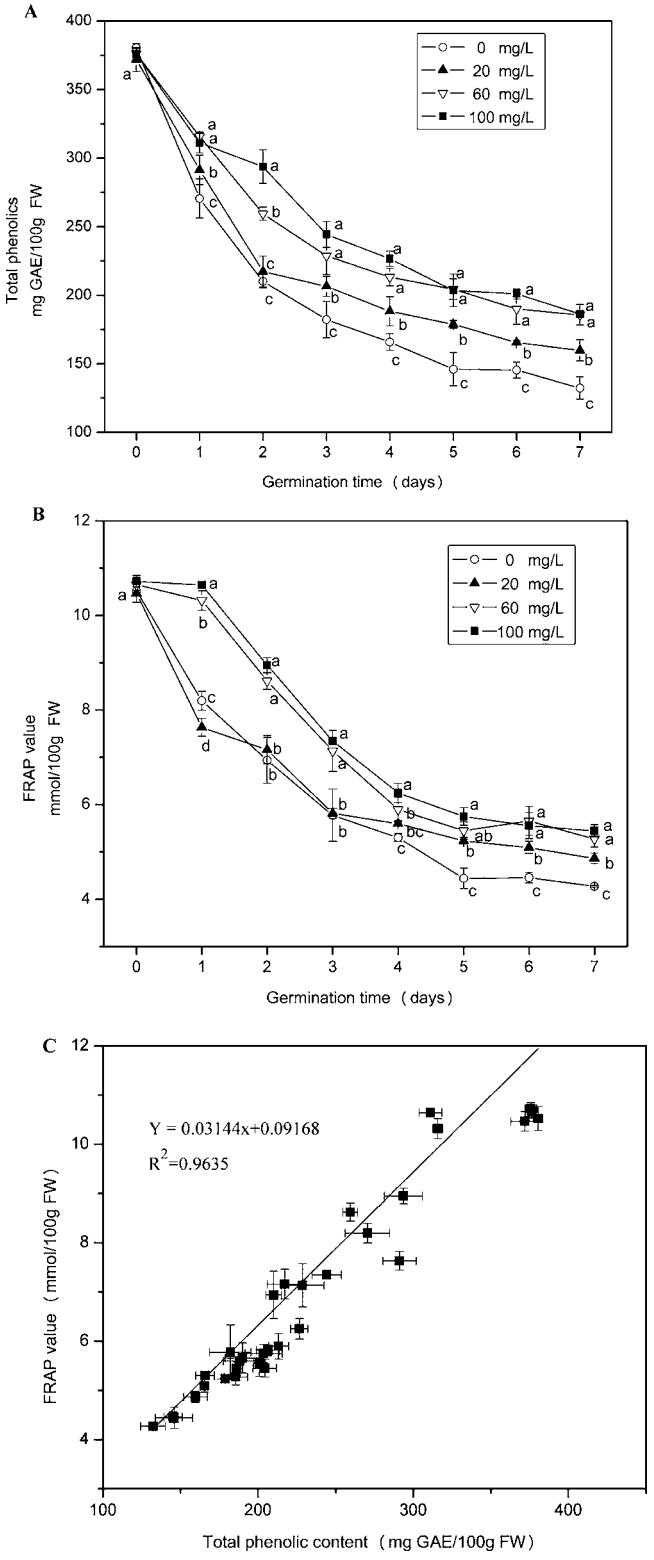

Figure 5. (A) The effect of different concentrations of sulfur fertilization on the total phenolic content. The data represent the mean ± standard deviation (n = 3). Means with different letters at each time point differed significantly ($p \leq 0.05$). (B) The effect of different concentrations of sulfur fertilization on the FRAP value. The data represent the mean ± standard deviation (n = 3). Means with different letters at each time point differed significantly ($p \leq 0.05$). (C) The correlation between total phenolic contents and FRAP values.

been reported in buckwheat sprouts[47] and other Brassicaceae species.[5]

In conclusion, the germination process leads to a remarkable increase in the vitamin C, chlorophyll, and total carotenoid contents of Chinese red radish sprouts. Sulfur fertilization not only increases yield but also has a positive effect on the accumulation of bioactive compounds such as glucosinolates, carotenoids, and phenolic compounds. Chinese red radish sprouts are an ideal source of health-promoting nutrients with strong antioxidant activity. The data obtained in this study confirmed that the application of a moderate level of sulfur fertilization represents an efficient and economic strategy for enhancing the nutritional value of red radish sprouts.

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Notes

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REFERENCES

(1) Manchali, S.; Chidambara Murthy, K. N.; Patil, B. S. Crucial facts about health benefits of popular cruciferous vegetables. *J. Funct. Foods* **2012**, *4*, 94−106.

(2) Schonhof, I.; Blankenburg, D.; Müller, S.; Krumbein, A. Sulfur and nitrogen supply influence growth, product appearance, and glucosinolate concentration of broccoli. *J. Plant Nutr. Soil Sci.* **2007**, *170*, 65−72.

(3) Nikiforova, V.; Freitag, J.; Kempa, S.; Adamik, M.; Hesse, H.; Hoefgen, R. Transcriptome analysis of sulfur depletion in Arabidopsis thaliana: interlacing of biosynthetic pathways provides response specificity. *Plant J.* **2003**, *33*, 633−650.

(4) Yan, X.; Chen, S. Regulation of plant glucosinolate metabolism. *Planta* **2007**, *226*, 1343−1352.

(5) Baenas, N.; Moreno, D. A.; García-Viguera, C. Selecting Sprouts of Brassicaceae for Optimum Phytochemical Composition. *J. Agric. Food Chem.* **2012**, *60*, 11409−11420.

(6) Singh, S. V.; Singh, K. Cancer chemoprevention with dietary isothiocyanates mature for clinical translational research. *Carcinogenesis* **2012**, *33*, 1833−1842.

(7) Singh, J.; Upadhyay, A. K.; Prasad, K.; Bahadur, A.; Rai, M. Variability of carotenes, vitamin C, E and phenolics in Brassica vegetables. *J. Food Compos. Anal.* **2007**, *20*, 106−112.

(8) Kopsell, D. E.; Kopsell, D. A.; Randle, W. M.; Coolong, T. W.; Sams, C. E.; Curran-Celentano, J. Kale Carotenoids Remain Stable while Flavor Compounds Respond to Changes in Sulfur Fertility. *J. Agric. Food Chem.* **2003**, *51*, 5319−5325.

(9) Elwan, M. W. M.; Abd El-Hamed, K. E. Influence of nitrogen form, growing season and sulfur fertilization on yield and the content of nitrate and vitamin C of broccoli. *Sci. Hortic. (Amsterdam, Neth.)* **2011**, *127*, 181−187.

(10) Pérez-Balibrea, S.; Moreno, D. A.; García-Viguera, C. Glucosinolates in Broccoli Sprouts (Brassica oleracea var. italica) as Conditioned by Sulphate Supply during Germination. *J. Food Sci.* **2010**, *75*, C673−C677.

(11) Zimmermann, N. S.; Gerendás, J.; Krumbein, A. Identification of desulphoglucosinolates in Brassicaceae by LC/MS/MS: Comparison of ESI and atmospheric pressure chemical ionisation-MS. *Mol. Nutr. Food Res.* **2007**, *51*, 1537−1546.

(12) Malik, M. S.; Riley, M. B.; Norsworthy, J. K.; Bridges, W. Glucosinolate Profile Variation of Growth Stages of Wild Radish (Raphanus raphanistrum). *J. Agric. Food Chem.* **2010**, *58*, 3309−3315.

(13) ISO9167-1, Rapeseed-determination of glucosinolates content—Part 1: Method using gradient elution high performance liquid chromatography. 1992.
(14) Teng, S.; Keurentjes, J.; Bentsink, L.; Koornneef, M.; Smeekens, S. Sucrose-Specific Induction of Anthocyanin Biosynthesis in Arabidopsis Requires the MYB75/PAP1 Gene. *Plant Physiol.* **2005**, *139*, 1840−1852.
(15) Pugliese, A. G.; Tomas-Barberan, F. A.; Truchado, P.; Genovese, M. I. Flavonoids, Proanthocyanidins, Vitamin C, and Antioxidant Activity of Theobroma grandiflorum (Cupuassu) Pulp and Seeds. *J. Agric. Food Chem.* **2013**, *61*, 2720−2728.
(16) Juszczuk, I. M.; Ostaszewska, M. Respiratory activity, energy and redox status in sulphur-deficient bean plants. *Environ. Exp. Bot.* **2011**, *74*, 245−254.
(17) Ainsworth, E. A.; Gillespie, K. M. Estimation of total phenolic content and other oxidation substrates in plant tissues using Folin-Ciocalteu reagent. *Nat. Protoc.* **2007**, *2*, 875−877.
(18) Benzie, I. F. F.; Strain, J. J. The Ferric Reducing Ability of Plasma (FRAP) as a Measure of "Antioxidant Power": The FRAP Assay. *Anal. Biochem.* **1996**, *239*, 70−76.
(19) Yuan, G.; Wang, X.; Guo, R.; Wang, Q. Effect of salt stress on phenolic compounds, glucosinolates, myrosinase and antioxidant activity in radish sprouts. *Food Chem.* **2010**, *121*, 1014−1019.
(20) Smatanova, M.; Richter, R.; Hlusek, J. Spinach and pepper response to nitrogen and sulphur fertilization. *Plant Soil Environ.* **2004**, *50*, 303−308.
(21) De Pascale, S.; Maggio, A.; Pernice, R.; Fogliano, V.; Barbieri, G. Sulphur fertilization may improve the nutritional value of Brassica rapa L. subsp. sylvestris. *Eur. J. Agron.* **2007**, *26*, 418−424.
(22) Brown, P. D.; Tokuhisa, J. G.; Reichelt, M.; Gershenzon, J. Variation of glucosinolate accumulation among different organs and developmental stages of Arabidopsis thaliana. *Phytochemistry* **2003**, *62*, 471−481.
(23) Ciska, E.; Honke, J.; Kozłowska, H. Effect of Light Conditions on the Contents of Glucosinolates in Germinating Seeds of White Mustard, Red Radish, White Radish, and Rapeseed. *J. Agric. Food Chem.* **2008**, *56*, 9087−9093.
(24) Martinez-Villaluenga, C.; Peñas, E.; Ciska, E.; Piskula, M. K.; Kozlowska, H.; Vidal-Valverde, C.; Frias, J. Time dependence of bioactive compounds and antioxidant capacity during germination of different cultivars of broccoli and radish seeds. *Food Chem.* **2010**, *120*, 710−716.
(25) Jing, P.; Zhao, S.-J.; Ruan, S.-Y.; Xie, Z.-H.; Dong, Y.; Yu, L. Anthocyanin and glucosinolate occurrences in the roots of Chinese red radish (Raphanus sativus L.), and their stability to heat and pH. *Food Chem.* **2012**, *133*, 1569−1576.
(26) Kestwal, R. M.; Lin, J. C.; Bagal-Kestwal, D.; Chiang, B. H. Glucosinolates fortification of cruciferous sprouts by sulphur supplementation during cultivation to enhance anti-cancer activity. *Food Chem.* **2011**, *126*, 1164−1171.
(27) Kopsell, D. A.; Barickman, T. C.; Sams, C. E.; McElroy, J. S. Influence of Nitrogen and Sulfur on Biomass Production and Carotenoid and Glucosinolate Concentrations in Watercress (Nasturtium officinale R. Br.). *J. Agric. Food Chem.* **2007**, *55*, 10628−10634.
(28) Li, S. M.; Schonhof, I.; Krumbein, A.; Li, L.; Stutzel, H.; Schreiner, M. Glucosinolate concentration in turnip (Brassica rapa ssp rapifera L.) roots as affected by nitrogen and sulfur supply. *J. Agric. Food Chem.* **2007**, *55*, 8452−8457.
(29) Rangkadilok, N.; Nicolas, M. E.; Bennett, R. N.; Eagling, D. R.; Premier, R. R.; Taylor, P. W. J. The Effect of Sulfur Fertilizer on Glucoraphanin Levels in Broccoli (B. oleracea L. var. italica) at Different Growth Stages. *J. Agric. Food Chem.* **2004**, *52*, 2632−2639.
(30) Stavridou, E.; Kristensen, H. L.; Krumbein, A.; Schreiner, M.; Thorup-Kristensen, K. Effect of Differential N and S Competition in Inter- and Sole Cropping of Brassica Species and Lettuce on Glucosinolate Concentration. *J. Agric. Food Chem.* **2012**, *60*, 6268−6278.
(31) Li, H.; Deng, Z.; Zhu, H.; Hu, C.; Liu, R.; Young, J. C.; Tsao, R. Highly pigmented vegetables: Anthocyanin compositions and their role in antioxidant activities. *Food Res. Int.* **2012**, *46*, 250−259.
(32) Li, X.; Thwe, A. A.; Park, N. I.; Suzuki, T.; Kim, S. J.; Park, S. U. Accumulation of Phenylpropanoids and Correlated Gene Expression during the Development of Tartary Buckwheat Sprouts. *J. Agric. Food Chem.* **2012**, *60*, 5629−5635.
(33) Kim, S.-J.; Maeda, T.; Zaidul; Takigawa, S.; Matsuura-Endo, C.; Yamauchi, H.; Mukasa, Y.; Saito, K.; Hashimoto, N.; Noda, T.; Saito, T.; Suzuki, T. Identification of Anthocyanins in the Sprouts of Buckwheat. *J. Agric. Food Chem.* **2007**, *55*, 6314−6318.
(34) Paśko, P.; Bartoń, H.; Zagrodzki, P.; Gorinstein, S.; Fołta, M.; Zachwieja, Z. Anthocyanins, total polyphenols and antioxidant activity in amaranth and quinoa seeds and sprouts during their growth. *Food Chem.* **2009**, *115*, 994−998.
(35) Yuan, Y.; Chiu, L.-W.; Li, L. Transcriptional regulation of anthocyanin biosynthesis in red cabbage. *Planta* **2009**, *230*, 1141−1153.
(36) Guo, R.; Yuan, G.; Wang, Q. Sucrose enhances the accumulation of anthocyanins and glucosinolates in broccoli sprouts. *Food Chem.* **2011**, *129*, 1080−1087.
(37) Pérez-Balibrea, S.; Moreno, D. A.; García-Viguera, C. Influence of light on health-promoting phytochemicals of broccoli sprouts. *J. Sci. Food Agric.* **2008**, *88*, 904−910.
(38) Pérez-Balibrea, S.; Moreno, D. A.; García-Viguera, C. Improving the phytochemical composition of broccoli sprouts by elicitation. *Food Chem.* **2011**, *129*, 35−44.
(39) Guo, X.; Li, T.; Tang, K.; Liu, R. H. Effect of Germination on Phytochemical Profiles and Antioxidant Activity of Mung Bean Sprouts (Vigna radiata). *J. Agric. Food Chem.* **2012**, *60*, 11050−11055.
(40) Wheeler, G. L.; Jones, M. A.; Smirnoff, N. The biosynthetic pathway of vitamin C in higher plants. *Nature* **1998**, *393*, 365−369.
(41) Xu, M.-J.; Dong, J.-F.; Zhu, M.-Y. Effects of germination conditions on ascorbic acid level and yield of soybean sprouts. *J. Sci. Food Agric.* **2005**, *85*, 943−947.
(42) Nikiforova, V. J.; Kopka, J.; Tolstikov, V.; Fiehn, O.; Hopkins, L.; Hawkesford, M. J.; Hesse, H.; Hoefgen, R. Systems Rebalancing of Metabolism in Response to Sulfur Deprivation, as Revealed by Metabolome Analysis of Arabidopsis Plants. *Plant Physiol.* **2005**, *138*, 304−318.
(43) Palozza, P.; Torelli, C.; Boninsegna, A.; Simone, R.; Catalano, A.; Mele, M. C.; Picci, N. Growth-inhibitory effects of the astaxanthin-rich alga Haematococcus pluvialis in human colon cancer cells. *Cancer Lett.* **2009**, *283*, 108−117.
(44) Reif, C.; Arrigoni, E.; Schärer, H.; Nyström, L.; Hurrell, R. F. Carotenoid database of commonly eaten Swiss vegetables and their estimated contribution to carotenoid intake. *J. Food Compos. Anal.* **2013**, *29*, 64−72.
(45) Kim, H.-J.; Chen, F.; Wang, X.; Choi, J.-H. Effect of Methyl Jasmonate on Phenolics, Isothiocyanate, and Metabolic Enzymes in Radish Sprout (Raphanus sativus L.). *J. Agric. Food Chem.* **2006**, *54*, 7263−7269.
(46) Vallejo, F.; Tomás-Barberán, F. A.; García-Viguera, C. Effect of climatic and sulphur fertilisation conditions, on phenolic compounds and vitamin C, in the inflorescences of eight broccoli cultivars. *Eur. Food Res. Technol.* **2003**, *216*, 395−401.
(47) Lim, J.-H.; Park, K.-J.; Kim, B.-K.; Jeong, J.-W.; Kim, H.-J. Effect of salinity stress on phenolic compounds and carotenoids in buckwheat (Fagopyrum esculentum M.) sprout. *Food Chem.* **2012**, *135*, 1065−1070.