

Available online at www.sciencedirect.com

ScienceDirect

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

Enhancement of glucosinolate and sulforaphane formation of broccoli sprouts by zinc sulphate via its stress effect

Runqiang Yang^a, Liping Guo^{a,b}, Xiaolin Jin^a, Chang Shen^a,
Yulin Zhou^a, 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, Shandong 266109, China

ARTICLE INFO

Article history:

Received 8 June 2014

Received in revised form 9 January 2015

Accepted 13 January 2015

Available online 30 January 2015

Keywords:

Broccoli sprout

Glucosinolate

Sulforaphane

Myrosinase

Zinc sulphate

ABSTRACT

Broccoli sprouts have attracted much attention in recent years for their abundant phytochemicals such as glucosinolates and sulforaphane. In this study, zinc sulphate ($ZnSO_4$) as a sulphur (S)-source was compared with potassium sulphate (K_2SO_4) and methionine (Met), significantly increased glucosinolates content and stimulated sulforaphane formation by enhancing myrosinase activity and gene expressions related to glucoraphanin biosynthesis and sulforaphane formation in broccoli sprouts. However, it resulted in a stress effect on sprout growth. Thus, $ZnSO_4$ is a suitable S-source to improve sulforaphane formation in broccoli sprouts via its stress effect.

© 2015 Elsevier Ltd. All rights reserved.

1. Introduction

Epidemiological studies have revealed that dietary consumption of *Brassica* vegetables can potentially reduce chronic disease risk (Herr & Büchler, 2010). It is mainly attributed to the presence of glucosinolates, a group of thioglucosides with activated defensive biochemical compounds (Gu, Guo, & Gu, 2012). Upon disruption of plant tissues or organs, glucosinolates can be hydrolyzed by myrosinase (Thioglucosylhydrolase, E.C.3.2.1.147) into isothiocyanates, nitriles, thiocyanates, epithionitriles and oxazolidines (Angelino & Jeffery, 2013; Guo, Yang, Wang, Guo, & Gu, 2014). Isothiocyanates are one of the natural

anticarcinogenic group of compounds (Cartea & Velasco, 2007). Among them, sulforaphane has attracted increasing attention. Sulforaphane, hydrolyzed from glucoraphanin, is a natural inducer of phase II detoxication enzyme that detoxifies cancer-causing chemicals (Fahey, Zhang, & Talalay, 1997).

Broccoli is rich in glucosinolates which are sulphur (S)-rich compounds and represent up to 30% of S-compositions in its plant. Contents of glucosinolates depend largely on the S-status of the plants (Gu, Guo, & Gu, 2012). When S is deficient, glucosinolates are degraded for plant growth via biosynthesis of other S-compounds (Gu, Guo, & Gu, 2012). Consequently, S-chemicals application can enhance glucosinolate accumulation in plants (Kestwal, Lin, Bagal-Kestwal, & Chiang,

* Corresponding author. College of Food Science and Technology, Nanjing Agricultural University, Nanjing 210095, China. Tel.: +86 25 84396293; fax: +86 25 84396293.

E-mail address: guzx@njau.edu.cn (Z. Gu).

<http://dx.doi.org/10.1016/j.jff.2015.01.007>

1756-4646/© 2015 Elsevier Ltd. All rights reserved.

2011). Researchers focused mainly on the effect of only S or the interactive effect of nitrogen (N) and S on glucosinolate content in broccoli sprouts (Pérez-Balibrea, Moreno, & García-Viguera, 2010). Little information is available for that of different S-source chemicals such as zinc sulphate (ZnSO₄), potassium sulphate (K₂SO₄) and methionine (Met) application on glucosinolate biosynthesis and sulforaphane formation. Sulforaphane, as an anticarcinogen, is derived from glucoraphanin which can be biosynthesized by flavin-containing monooxygenase (FMO). Except for being hydrolyzed by myrosinase, glucoraphanin also serves as a precursor for biosynthesis of alkenyl-glucosinolate by AOP2 (Zang et al., 2009). In addition, sulforaphane formation depends on myrosinase and epithiospecifier protein (ESP) activity. High ESP activity could be beneficial for sulforaphane nitrile formation. Hence, it is necessary to investigate the effects of S-chemical application on gene expression of the enzymes and sulforaphane formation of broccoli sprouts.

The objectives of this study were to systematically investigate the effects of these S-source chemicals on glucosinolate content and sulforaphane formation of broccoli sprouts, and to scientifically explore relevant phytophysiological and biochemical mechanisms involved.

2. Materials and methods

2.1. Materials and reagents

Broccoli (*Brassica oleracea* var. *italica*, cv. Lvlingxiang) seeds were purchased from Jinshengda Seed Co. Ltd. (Nanjing, Jiangsu, China). Standard samples of sulforaphane, sinigrin and glucotropaeolin were obtained from Sigma-Aldrich Chemical Co. (St. Louis, MO, USA). Other analytical grade chemicals and reagents were from Shanghai Institute of Biochemistry (Shanghai, China).

2.2. Seed germination and S-source treatment

Dry seeds were immersed in 1.5% sodium hypochlorite for 15 min. They were drained and washed with distilled water until reaching a neutral pH. After soaking in distilled water at 30 °C for 4 h, they were put on a filter paper in petri dishes (15 cm in diameter, filled with sterilized quartz sand), and put into dark incubators at 30 °C for 4-day germination. Seeds in each petri dish were sprayed with 40 mL of distilled water on the 1st day of germination. From the 2nd day they were divided into four groups with three replicates, and sprayed with 20 mL of 2 mmol/L ZnSO₄, K₂SO₄, and Met every 12 h. The control was sprayed with 20 mL of distilled water only. Twenty of 4-day-old sprouts were randomly sampled from each group. After weighing fresh weight (FW), they were immediately frozen by liquid nitrogen and kept in polyethylene bags at -80 °C until biochemical analyses.

2.3. Sulforaphane determination

Sulforaphane hydrolyzed from glucoraphanin in broccoli sprouts was measured according to Gu, Guo, Zhang et al. (2012).

2.4. Glucosinolates assay

Glucosinolates were analyzed as Guo, Yuan, and Wang (2011) with minor modifications. Glucosinolates were extracted using boiled methanol and purified with DEAE-Sephadex A-25 column (Pyridine acetate form, GE Healthcare, Piscataway, NJ, USA). After desulphation, desulphoglucosinolates were eluted twice with distilled water to filter through a 0.45 µm membrane before analyzed by HPLC (Agilent 1200 HPLC system equipped with a Eclipse XDB-C18 column (5 µm particle size, 4.6 × 150 mm), Agilent Technologies Co. Ltd., Santa Clara, CA, USA).

2.5. Myrosinase activity determination

Myrosinase was extracted using the method of Kim, Chen, Wang, and Choi (2006). One myrosinase unit corresponded to 1.0 nmol sinigrin transformed per minute. The specific activity is expressed as the units per mg of protein.

2.6. Gene expression assay (Quantitative Real-Time PCR, QRT-PCR)

Total RNA from broccoli sprouts was isolated by a E.Z.N.A.TM Plant RNA Kit (OMEGA, R6827-01). Two micrograms of its template were used for the first strand cDNA synthesis based on Li, Xin, Sun, Shen, and Xu (2006), which was performed with a RT-PCR Kit (TaKaRa: DR027S). The PCR amplification was performed using TaKaRa Ex-TaqTM polymerase for target genes and Actin. Table 1 listed sequence-specific primers for QRT-PCR analysis. Triplicate quantitative assays were performed on each cDNA with the SYBR[®]Premix Ex TaqTM (TAKARA: RR420A) with ABI 7500 sequence detection system (Applied Biosystems, Foster City, CA, USA). The PCR process included 1 cycle of 95 °C for 30 s, followed by 40 cycles of 95 °C for 3 s, 60 °C for 30 s for 1 cycle.

2.7. Sprout length and thiobarbituric acid reactive substances (TBARS) determination

Broccoli sprouts length was determined using a vernier caliper (Shanghai Precision Instruments Co., Ltd., Shanghai, China).

TBARS were measured by the method of 2-thiobarbituric acid reaction. Fresh sprouts (0.5 g) were homogenized with 5 mL of 5% trichloroacetic acid, and centrifuged at 4 °C at 11,200 g for 15 min. The supernatants were used to determine TBARS

Table 1 – Sequence-specific primers used in this study.

Gene name	Primer name	Primer sequences (5'→3')
FMO _{GS-ox}	Sense	TCGCTCTGGTGTGTCGGTCTC
	Ant-sense	ATCCTCATAAGCCTCCGCAAT
AOP2	Sense	GAGTAACGGAAAGAAAGAAGACAAGG
	Ant-sense	ATAAGCGTGAAGAGTAGAACGAGGT
MYR	Sense	AAGGTCATCAGGGAGAAGGGTG
	Ant-sense	TGTTTGGCAGGGTCTTAGTGG
ESP	Sense	ACATTGGGACCAGGGACG
	Ant-sense	TTTCATACAGGTGGCAGTC
Actin	Sense	CTGTTCCAATCTACGAGGGTTTCT
	Ant-sense	GCTCGGCTGTGGTGGTAA

Download English Version:

<https://daneshyari.com/en/article/1219811>

Download Persian Version:

<https://daneshyari.com/article/1219811>

[Daneshyari.com](https://daneshyari.com)