

**EFFECTS OF EXOGENOUS METHYL JASMONATE ON PHYTOCHEMICAL
PRODUCTION IN THE RADISH SPROUT**

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Abstract

Radish sprouts are recognized as health-promoting foods due to its high content of bioactive compounds. In this study it was shown that radish sprout treated with methyl jasmonate (MeJA) synergistically enhanced the accumulation of phytochemical production. The efficacy of spray of MeJA with various concentration (0.0 mM, 0.05 mM and 5.0 mM) and intensities during germination and growth period, on 7 days. The MeJA-treated sprouts exhibited significantly higher levels of antioxidant activity (AOA) and total glucosinolate content than control sprouts. There was a significant positive linear relationship between total glucosinolate content and antioxidant activity (AOA). The glucosinolate content obtained were correlated to AOA with $R^2 = 0.9958$. Spray application of 0.05 mM at growth was effective resulting in approximately 14.9% and 36.8% increase in antioxidant power and total glucosinolate content, respectively. Results indicated that stress-induced total glucosinolate content and AOA were dependent upon MeJA concentrations. Exposure to combined abiotic stresses could provide more AOA to radish sprout.

Key words: Methyl jasmonate, Radish sprouts, Phytochemical, Total glucosinolate

1. INTRODUCTION

Clinical evidence has shown that the consumption of plants and plant-derived foods is positively associated with the prevention of various chronic diseases and improvement of overall general health. Thus, interest in the use of plant-derived foods for health benefits is on the increase.

Radish (*Raphanus sativus* L.) sprouts have long been consumed globally due to high nutritional values that contain substantial amounts of antioxidants, vitamin C and healthy compounds such as glucosinolates and phenolic compounds (Barillari et al. 2006). Radish sprouts are an important source of glucosinolate having many interesting pharmacological effects such as detoxification of normal cells and anti-cancer activity (Martinez-Villaluenga et al. 2008).

Glucosinolates are beta-thioglycoside N-hydroxysulfates containing a side chain (R) and a beta-D-glucopyranose moiety. Glucosinolate are naturally occurring compounds in Brassica spp., including broccoli, Brussels sprouts, cauliflower, cabbage, mustard, turnip, and watercress (Fahey, Zalcmann & Talaly 2001). Sprouts have also shown to rapidly increase glucosinolates content in cruciferous seeds (Fahey, Zhang & Talalay 1997).

Methyl jasmonate (MeJA) is a substance used in plant defense and is also under early research for cancer treatment in humans. Plants produce methyl jasmonate in response to many biotic and abiotic stresses, which builds up in damaged parts of the plant (Flescher 2005). Methyl Jasmonate is a group of plant stress natural hormones. Although not subjected to any stress, healthy plants respond to exogenous MeJA and induce a self-defense system. Because of this particular role, exogenous MeJA is applied to stimulate the production of phytochemicals in plants.

Thus, if MeJA is supplemented during sprouting process, the synthesis of glucosinolate content in radish sprouts may increase, which in turn may increase the antioxidant activity of the sprouts. The objective of the study was to examine the effects of MeJA treatment at different concentrations on the total content of phenolic, FRAP, DPPH free radical scavenging activity, carotenoid content, and total glucosinolate content of radish sprouts.

2. MATERIAL AND METHOD

2.1. Materials

Methyl jasmonate was purchased from Sigma Co. (St. Louis, Mo., USA) and radish sprouts seeds were obtained from local market (Seongnam, Kyunggido, Korea) and stored at 4°C until used.

2.2. Methods

Radish seeds (200 g) were washed with water, drained, and soaked in 1,000 mL of distilled water for 4 h in the incubator at 25°C. Following this step, radish seeds were drained, divided into 3 equal parts on a wet weight basis, and placed in a commercial spout cultivator (MikroFarm, EasyGreen, USA) consisting of 4 separate trays, and cultivated in the dark at room temperature ($18 \pm 2^\circ\text{C}$) for 7 d. MeJA in 0.01% ethanol was sprayed on the sprouts by 100 mL of two-methyl jasmonate solutions (0.05 mM and, 5.0 mM). Ethanol 0.01% was also sprayed on the control sprouts. Water was automatically sprayed for 30 min on the radish seeds/sprouts every 8 h.

Antioxidant activity (AOA): DPPH free radical scavenging activity and Ferric reducing antioxidant power (FRAP) of radish seeds/sprouts were determined by the method of Yamaguchi et al. (1998) and Benzie et al. (1999) with some modifications. Radish sprouts were washed with distilled water, freeze-dried (Ilsin Lab., Bondiro, Seoul, Korea), and ground with a blender (Kastech, Tokyo, Japan). The ground powder sample (0.1 g) was extracted with 1 mL of 80% ethanol using a vortex mixer at room temperature for 3 h. The extract was centrifuged (Model 5810 R, Eppendorf, Hamburg, Germany) at $10,000 \times g$ for 10 min, filtered through a $0.2 \mu\text{m}$ PTFE membrane filter paper (Alltech, Deerfield, Ill., USA), and then diluted 1,000 times with ethanol. An aliquot (0.2 mL) of the extract was added to 3 mL of 0.1 mM DPPH radical ethanolic solution. The reaction mixture was shaken vigorously, stored in the dark at room temperature for 30 min, and the absorbance measured at 517 nm using a spectrophotometer (V-570, Jasco Co., Japan). The free radical scavenging activity was calculated by the following equation: scavenging activity (%) = $[1 - (\text{absorbance sample}/\text{absorbance control})] \times 100$. The EC_{50} value represented the concentration of the sample leading to 50% reduction in the initial DPPH concentration. The antioxidant activity of sprouts was determined using the ferric reducing antioxidant power (FRAP) assay. In this assay, the Fe(III)/tripyridyltriazine complex is reduced to the blue ferrous form (Fe(II)/tripyridyltriazine) by reductants (antioxidants) in the sample, with the color change monitored at 593 nm. For the assay, a methanol extract diluted with distilled water was mixed with 3.6 mL of FRAP reagent containing 300 mM acetate buffer (pH 3.6), 10 mM 2,4,6-tripyridyl-s-triazine, and 20 mM $\text{FeCl}_3 \cdot 6\text{H}_2\text{O}$. After the reaction mixture was incubated at 37°C for 30 min, its absorbance was measured at 593 nm. A standard curve was prepared using $\text{FeSO}_4 \cdot 7\text{H}_2\text{O}$, and the reducing power was reported as millimoles of Fe^{2+} per gram of dried sample.

Total phenolic content in radish sprouts were estimated as gallic acid equivalents following the method described by Singleton et al. (1965). The extract of radish sprouts prepared, as described in the determination of DPPH radical scavenging activity, was centrifuged at $10,000 \times g$ for 10 min, filtered through a $0.2 \mu\text{m}$ PTFE membrane filter paper, and then diluted 100 times with water. An aliquot (1.0 mL) of the extract was added to 1.0 mL of 0.2 N Folin–Ciocalteu’s phenol reagent. After standing for 3 min at room temperature, 1.0 mL of saturated Na_2CO_3 (75 g/L) was added and kept in the dark for 1 h

at room temperature. Absorbance was measured at 765 nm using a spectrophotometer (V-570, Jasco Co., Japan) and the results expressed as mg of gallic acid/100 g.

Carotenoid content was analyzed from the sprouts according to the procedure of Liu et al. (2007) with some modifications. The dried sample powder (0.2 g) was mixed with 6 mL of ethanol containing 0.1% BHT, and the sealed mixture solution was pre-incubated at 85 °C for 5 min. For saponification, 120 µL of 80% KOH were added to the preheated mixture solution, and the reaction solution was incubated at the same temperature for 10 min. The reaction solution was then immediately placed on ice, and 3 mL of distilled water and 3 mL of hexane were added. After centrifugation, the hexane layer containing the crude carotenoids was collected. The hexane separation step was repeated once and the resulting hexane layers were combined and dried with nitrogen gas. The dried residue was recovered with methanol/methyl *tert*-butyl ether (MTBE) (1:1) to produce a crude carotenoid extract of the sprouts. Individual carotenoids were carried out by HPLC using a Shimadzu LC-20 liquid chromatography system with automatic injector and Diode Array Detector. A column (250 mm × 4.6 mm × 3 µm), packed with 3 µm C₁₈ YMC carotenoid column, was used. Separation was performed using an elution gradient (flow-rate 1 mL min⁻¹) with the mobile phases (A) methanol/MTBE/water (81:15:4, v/v/v) and (B) methanol/MTBE (9:91, v/v). Detection was performed simultaneously at 450 nm. External standard calibration was used for quantitation.

Total glucosinolate content was estimated following the method of Thies (1982) after slight modifications. Fifty milligrams of lyophilized sample were extracted with 1.5 mL 70% (v/v) boiling ethanol in a water bath at 70°C for 5 min. After centrifugation (13,000 × g at 4°C for 20 min), the supernatant was collected in a test tube. The residue was extracted twice using the same procedure. The sprout extract was transferred to a 96-well microplate. Sodium tetrachloropalladate solution (300 µL, 2 mM) was added to each well of the 96-well plate. The plate was incubated at room temperature for 30 min. After incubation the color intensity was measured at 450 nm, using 96-well microplate reader, based on complex formation between glucosinolates and tetrachloropalladate (II). The absorbance of known concentrations of sinigrin was used as standard for quantification of total glucosinolates.

3. RESULT AND DISCUSSION

3.1. Total phenolics

The effects of methyl jasmonate concentration on the total phenolic content of radish sprouts, expressed as gallic acid equivalent, cultivated for 7 days at room temperature are shown in Fig. 1. The content of polyphenols in sprouts was similar in sprouts with methyl jasmonate treatment as compared with the control sprouts. As shown in Fig. 1. The control value was determined as 17.21 GAE mg/g and the 5 mM MeJA treatment was obtained as 17.22 GAE mg/g, and the difference was not significant.

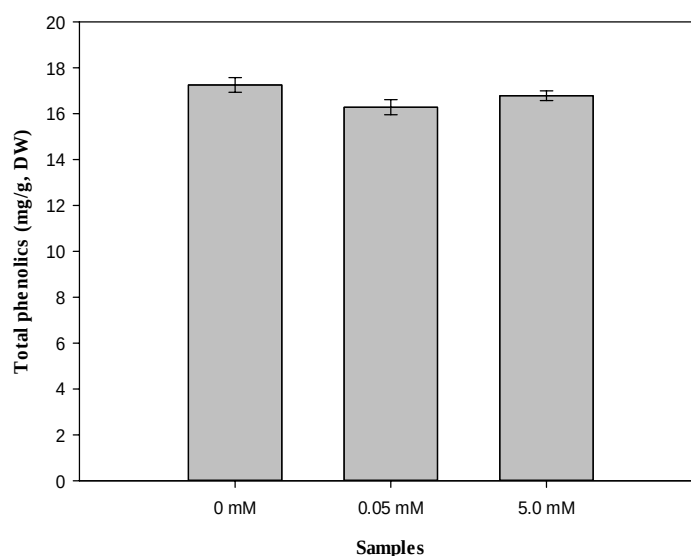


Fig. 1. Total phenolics of radish sprouts treated with various concentrations of MeJA. The absorbance of total phenolic extracts was measured at 765 nm, and the content was expressed as mg of gallic acid equivalent (GAE) per g of dry weight (DW). Different letters denote significant differences ($p < 0.05$).

3.2. Carotenoid content

Individual values and averages are as shown in Table 1. The total carotenoid content (the sum of alpha-carotene, beta-carotene and lutein) of radish sprouts ranged from 32.20 mg/g, DW to 37.88 mg/g, DW. The total level of carotenoid compounds was higher in the control radish sprouts. 0.05 mM treatment showed that alpha-carotene and beta-carotene is similar to the control ones. In contrast, 5 mM treatment showed extremely decreased alpha-carotene. In the total carotenoid content, a significant difference ($p < 0.05$) was observed that differed from the 5 mM treatment. Thus, in 0.05 mM-treatment and 5 mM-treatment showed the amount of carotenoid was respectively 2.13% and 15.00% lower than the control sprouts.

Table 1. Carotenoid content (mg/g) of radish sprouts treated with various concentrations of MeJA

Sample	Alpha-carotene	Beta-carotene	Lutein	Total carotenoids
0 mM	11.00 $\pm$ 0.01	12.74 $\pm$ 0.03	14.14 $\pm$ 0.05	37.88 $\pm$ 0.09a ¹⁾
0.05 mM	11.00 $\pm$ 0.01	12.67 $\pm$ 0.01	13.44 $\pm$ 0.06	37.11 $\pm$ 0.08a
5 mM	0.00	12.19 $\pm$ 0.02	13.50 $\pm$ 0.05	32.20 $\pm$ 0.07b

¹⁾Different letters denote significant differences ($p < 0.05$).

3.3. DPPH radical scavenging activity

DPPH is a stable organic free radical and is widely used for evaluations of antioxidant activity of various samples. In the DPPH test, antioxidants reduced the DPPH radical to a yellow-colored compound, diphenylpicrylhydrazine. The reduction capability of this DPPH radical is determined by a decrease in absorbance at 517 nm induced by the antioxidant, that can donate an electron or hydrogen atom. The DPPH radical scavenging activity of different concentrations of extracts of radish sprouts was measured and the EC_{50} of each radish sprout was determined (Fig. 2). EC_{50} is known to be the half maximal effective concentration and in this case, it is defined as the concentration of the radish sprouts that causes a decrease of 50% of the initial DPPH concentration (Prior et al. 2005). EC_{50} of DPPH radical scavenging activity in sprouts was lower in sprouts subjected to methyl jasmonate treatment as compared with control sprouts. The control value was determined as 3.15 mg/g and the 5 mM treatment was obtained as 2.22 mg/g, and this difference was significant. The DPPH radical scavenging activity in sprouts treated with 0.05 and 5 mM was 1.32 fold and 1.42 fold higher than the control sprouts, respectively.

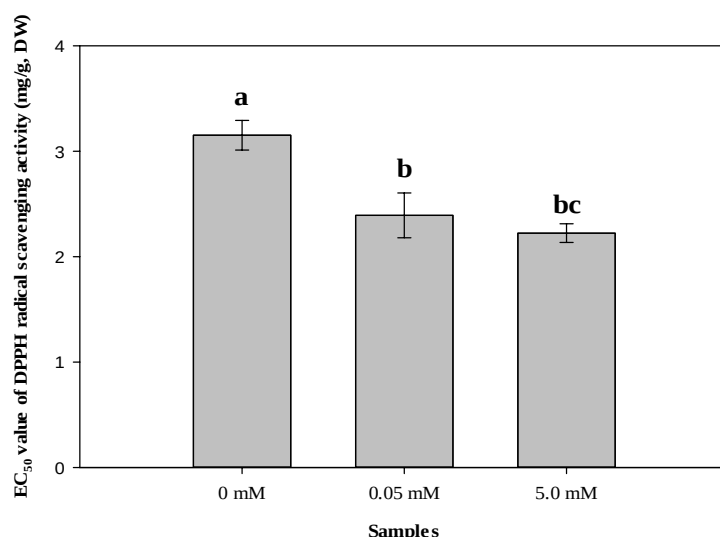


Fig. 2. EC_{50} value of DPPH radical scavenging activity of ethanol extracts of radish sprouts treated with various concentrations of MeJA. The scavenging activity was measured at 517 nm with a spectrophotometer. Different letters denote significant differences ($p < 0.05$).

3.4. Ferric reducing antioxidant power

FRAP assay has been widely used to directly test several extracts of foods and plants and is based on the ability to analyze the reduction of Fe^{3+}/Fe^{2+} coupling in the presence of antioxidants or reductant in a sample (Benzie & Strain 1996). Even though both the FRAP and DPPH assays were used to determine the antioxidant activity of radish sprouts, results were obtained due to the nature of each antioxidant assay. Each assay is based on a different reaction mechanism, where the FRAP assay measures only antioxidants that transfers a single electron, while DPPH measure antioxidants that transfer both a single electron and a hydrogen atom. Hence, it is difficult to obtain good agreements between the results obtained from both assays (Prior et al. 2005). The ferric-reducing antioxidant power (FRAP) of radish

sprouts analyzed is as shown in Fig. 3. The content of FRAP in sprouts was higher in sprouts subjected to methyl jasmonate treatment as compared to the control sprouts, and the difference was significant. The results indicated that methyl jasmonate treatment increased the FRAP (by 14.97% to 34.26% compared with that of the control ones).

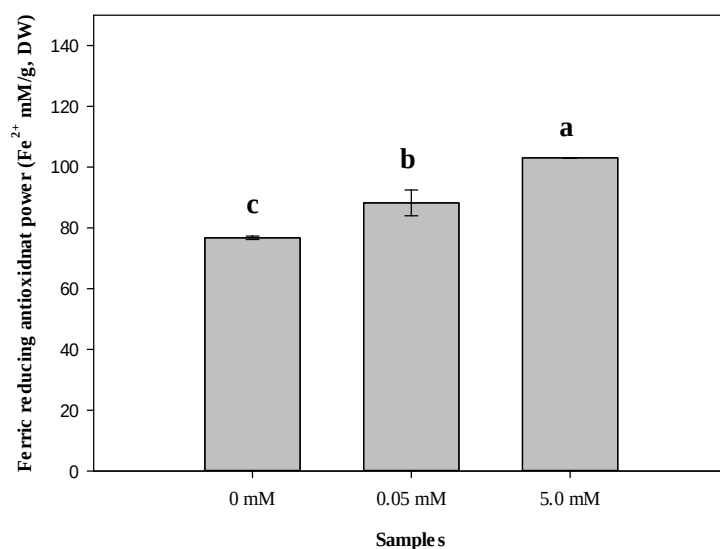


Fig. 3. Ferric reducing antioxidant power of ethanol extracts of radish sprouts treated with various concentrations of MeJA. The ferric reducing antioxidant power was measured at 593 nm with a spectrophotometer. Different letters denote significant differences ($p < 0.05$).

3.5. Total glucosinolate content

The effects of methyl jasmonate concentration on the total glucosinolate content of radish sprouts cultivated for 7 days at room temperature are shown in Fig. 4. The content of glucosinolate in sprouts was higher in those subjected to methyl jasmonate treatment as compared to the control sprouts, and the difference was significant. The results indicated that methyl jasmonate treatment increased the total phenolic content (by 36.82% to 71.48% compared with that of the control ones). In the present study, 5.0 mM methyl jasmonate treatment was the most effective for increasing the total glucosinolate.

Glucosinolate concentrations have been shown to vary not only between plant species but also between cultivars and individual parts of the same plant (Bellostas, Sorensen & Sorensen 2007). The concentration of total and individual glucosinolates for a given species has been found to be greater in seeds than in mature plants.

According to previous reports (Perez-Balibrea et al. 2011) glucoraphanin, glucoiberin and 4-hydroxy glucobrassicin were the main glucosinolate determined in broccoli sprouts, and these individual glucosinolates respond differently to the elicitor treatments. Scheuner et al. (2005a) reported that the total glucosinolate concentration increased by 25% in broccoli heads through methionine treatment, and the methionine treatment increased the aliphatic glucosinolate concentration in the broccoli plant (Scheuner et al. 2005b). Similar induction of glucosinolates by exogenous SA and MeJA application has been previously observed in several Brassica species (Smetanska et al. 2007).

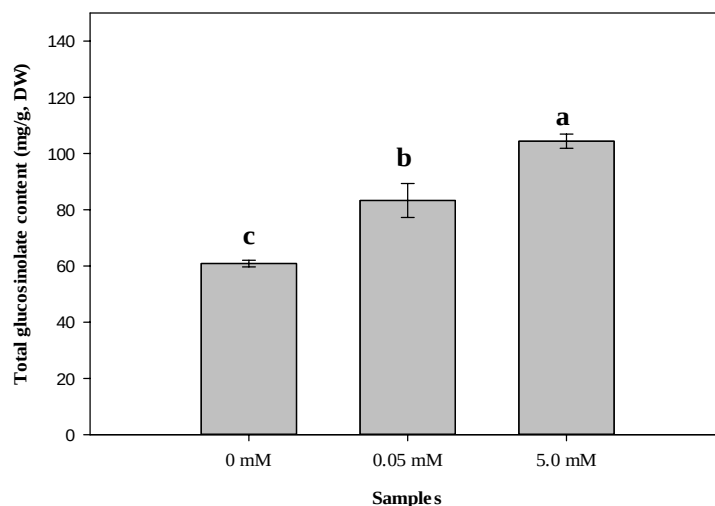


Fig. 4. Total glucosinolate content (mg/g, DW) of ethanol extracts of radish sprouts treated with various concentrations of MeJA. The glucosinolate was measured at 450 nm with a spectrophotometer. Different letters denote significant differences ($p < 0.05$).

3.6. Correlation coefficients between assays

The analysis of correlation between FRAP of radish sprouts and total glucosinolate content is fundamental for the evaluation of MeJA treatment effects on antioxidant activity. In this study, the overall increase in FRAP and DPPH radical scavenging activity, previously observed in methyl jasmonate treated sprouts would be related to the increases observed in glucosinolate content. The FRAP obtained were linearly correlated to glucosinolate content as $R^2=0.9958$ (Table 2). Therefore, a high FRAP value of MeJA-treated radish sprouts in this study was related to the high total glucosinolate content on top of the MeJA treatment.

Table 2. Correlation among antioxidant activity (DPPH radical scavenging activity and FRAP), total phenolic content, total glucosinolate content, total carotenoid content

	Total phenolic content	FRAP	DPPH radical scavenging activity	Total glucosinolate content	Total carotenoid content
Total phenolic content	1				
FRAP	-0.4207*** ¹⁾	1			
DPPH radical scavenging activity	0.7571***	-0.9112***	1		
Total glucosinolate content	-0.5011**	0.9958*	-0.9447**	1	
Total carotenoid content	0.1086***	-0.9475**	0.7316***	-0.9147*	1

¹⁾*** $p < 0.001$, ** $p < 0.005$, * $p < 0.05$

To increase the nutritional value, the radish sprouts were sprayed with exogenous methyl jasmonate, which is well-known as a plant hormone, on the 7 day. Methyl Jasmonate treatments were effective for increasing the total glucosinolate content, FRAP value and DPPH radical scavenging activity of a plant, but the efficacy of treatment was not shown in the total phenolics and total carotenoid. The results in increased antioxidant activity confirmed previous studies and showed that glucosinolate compounds produced by elicitor treatment function as antioxidants (Perez-Balibrea et al. 2011). In this study, the MeJA treatment showed a meaningful increase in AOA (FRAP value and DPPH radical scavenging activity) compared to that of the control ones. Thus, MeJA treatment can improve the health benefits of radish sprouts by producing abundant antioxidant activities, and also allow for various applications to sprouts. Further studies are required to determine the effects of various secondary metabolic processes of radish sprouts owing to MeJA treatments.

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