

Garlic Sprouting Is Associated with Increased Antioxidant Activity and Concomitant Changes in the Metabolite Profile

Alexandra Zakarova,^{†,‡} Ji Yeon Seo,^{†,‡} Hyang Yeon Kim,[§] Jeong Hwan Kim,^{||} Jung-Hye Shin,[⊥] Kye Man Cho,[#] Choong Hwan Lee,[§] and Jong-Sang Kim^{*,‡}

[†]School of Food Science and Biotechnology (BK21 plus), Kyungpook National University, Daegu 702-701, Republic of Korea

[§]Department of Bioscience and Biotechnology, Konkuk University, Seoul 143-701, Republic of Korea

^{||}Division of Applied Life Science (BK21 plus), Graduate School, Gyeongsang National University, Jinju 660-701, Republic of Korea

[⊥]Namhae Garlic Research Institute, Namhae 668-812, Republic of Korea

[#]Department of Food Science, Gyeongnam National University of Science and Technology, Jinju 660-758, Republic of Korea

ABSTRACT: Although garlic (*Allium sativum*) has been extensively studied for its health benefits, sprouted garlic has received little attention. We hypothesized that sprouting garlic would stimulate the production of various phytochemicals that improve health. Ethanolic extracts from garlic sprouted for different periods had variable antioxidant activities when assessed with *in vitro* assays, including the 2,2-diphenyl-1-picrylhydrazyl radical scavenging activity assay and the oxygen radical absorbance capacity assay. Extracts from garlic sprouted for 5 days had the highest antioxidant activity, whereas extracts from raw garlic had relatively low antioxidant activity. Furthermore, sprouting changed the metabolite profile of garlic: the metabolite profile of garlic sprouted for 5–6 days was distinct from the metabolite profile of garlic sprouted for 0–4 days, which is consistent with the finding that garlic sprouted for 5 days had the highest antioxidant activity. Therefore, sprouting may be a useful way to improve the antioxidant potential of garlic.

KEYWORDS: garlic, garlic sprout, antioxidant activity, metabolites

INTRODUCTION

Garlic, *Allium sativum*, is a member of the onion genus. For thousands of years, garlic has been used for medicinal purposes, such as the treatment of hypertension, infections, and snake bites. Today, garlic is used to reduce blood cholesterol levels and vascular disease risk, as well as for its anticancer and antimicrobial purposes.¹ Garlic's potent antioxidant activity,^{2,3} ability to stimulate immunological responsiveness,⁴ and modulation of prostanoid synthesis^{5,6} may underlie its health benefits.

Garlic contains distinctive organosulfur compounds,⁷ which impart its unique flavor and odor and most of its biological activity. The major sulfur-containing compounds in unprocessed garlic involve γ -glutamyl-S-allyl-L-cysteine and S-allyl-L-cysteine sulfoxide (alliin). Alliin was reported to have a protective effect in a myocardial infarction model.⁸ Alliin also reduces the level of tumor necrosis factor- α (TNF- α) in human umbilical vein endothelial cells and decreases serum levels of triglyceride, insulin, uric acid, and glucose, as well as insulin resistance.⁹

The sprouting of plant seeds usually promotes the *de novo* synthesis of bioactive compounds called phytoalexins that protect the plant from various exogenous insults.^{10,11} One study reported that the antioxidative activity of onion sprouts was similar to that of onions, suggesting that onion sprouts, like onions, can be used as a health-enhancing ingredient.¹² In addition, the total phenolic content and antioxidant activity of beans and grains increased with sprouting in the absence and presence of elicitors.^{13–15} The antioxidant activities of the polar fractions of mature garlic bulbs and immature plants were

partially attributable to the levels of phenolics and flavonoids.¹⁶ Garlic and its sulfur-containing compounds have been studied extensively to determine their health benefits and mechanism of action, but the potential biological activity of sprouted garlic has received little attention.^{7,17,18}

Therefore, in this study, we investigated whether sprouting enhanced the antioxidant activity of garlic. In addition, we identified metabolites whose concentrations were significantly altered during sprouting that might contribute to the bioactivity of sprouted garlic. Finally, the abilities of sprouted garlic extract (SGE) and garlic extract to protect against glutamate-induced neural damage were compared using the HT22 mouse hippocampal cell line, a model system used to study neuronal cell degeneration and screen for potential agents that prevent amyloid β - or glutamate-induced cell death.^{19–22}

MATERIALS AND METHODS

Chemicals and Reagents. Ferric chloride, butylated hydroxytoluene (BHT), 2,4,6-tripyridyl-s-triazine (TPTZ), 2',7'-dichlorofluorescein diacetate (DCFDA), and dihydroethidium (DHE) were from Sigma (St. Louis, MO). All cell culture reagents and fetal bovine serum (FBS) were obtained from HyClone (Logan, KS). All of the other reagents were of ACS grade.

Sample Preparation. A whitish yellow Namdo variety of garlic was provided by Namhae Garlic Research Institute (Namhae, GyeongNam, South Korea). The skin was removed, and the garlic was sprouted for 6 days in an incubator at 25 °C with 100% humidity.

Received: October 24, 2013

Accepted: February 10, 2014

Garlic and sprouted garlic were minced, ground, and extracted with 10 volumes of 100% ethanol as a solvent. The extract was prepared by removing the solvent with rotary evaporation. The sample solution was prepared by redissolving the extract in ethanol at 40 mg/mL and diluting, if necessary, before use. The garlic extract was further fractionated with *n*-hexane, ethyl acetate, butanol, and water consecutively for use in the 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging assay.

Determination of the Total Phenolic Content. The total phenolic content was measured using the Folin–Ciocalteu reagent.²³ Briefly, 100 μ L of extract was mixed with 50 μ L of sodium bicarbonate (10%, w/v) solution; 15 μ L of the Folin–Ciocalteu reagent, which was diluted by 5-fold with distilled water, was then added. After 5 min at room temperature, the sample mixture was transferred to a 96-well microplate, followed by measuring the absorbance at 593 nm using a microplate reader (Sunrise, Tecan Retisoft, Inc., Mississauga, Ontario, Canada). The total phenolics were expressed as gallic acid equivalents (GAE).

DPPH Radical Scavenging Activity Assay. The DPPH radical scavenging activity assay was performed following a published method, with some modifications.²⁴ Briefly, 50 μ L of each sample was mixed with 200 μ L of 200 μ M DPPH methanolic solution in a 96-well plate, followed by gentle shaking. After incubation at 37 °C for 30 min, the absorbance was measured at 515 nm. The sample-dissolving solvent and Trolox were used as negative and positive controls, respectively.

Oxygen Radical Absorbance Capacity (ORAC) Assay. The peroxyl radical scavenging capacity of the sample extracts was evaluated by an ORAC assay system.²⁵ All data were expressed as micromoles of Trolox equivalents (TE), and 1 ORAC unit was equivalent to the net protection by 1 μ M Trolox.

Neuroprotective Effect of SGE. HT22 mouse hippocampal neuronal cells were routinely maintained in Dulbecco's modified Eagle's medium (DMEM) with 10% FBS and 100 units/mL penicillin and streptomycin in a humidified CO₂ incubator (MCO-19-AIC, Sanyo, Osaka, Japan) at 37 °C and 5/95% CO₂/air.

The cells were seeded in a 96-well culture dish in DMEM supplemented with 10% FBS at a density of 2×10^3 cells per well. The next day, various doses of SGE and 5 mM glutamate were added to the cells.²⁶ After cells were cultured for 20 h, the cell survival rate was determined using the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) cell proliferation assay. The cell viability was presented as the percentage of the untreated control.

Assessment of Intracellular Reactive Oxygen Species (ROS). The intracellular ROS level was determined by a previously reported method.²⁷ HT22 cells (2×10^3 cells/well) were seeded in a 96-well black culture plate and cultured for 24 h. After cells were attached on the culture plate, the cells were incubated in the presence of 5 mM glutamate and garlic samples for 6 h. The cells were then washed with phosphate-buffered saline (PBS), incubated for 30 min with DCFDA dissolved in DMEM (final concentration of 30 μ M), and then washed with PBS. The fluorescence generated by the interaction of the ROS with dichlorofluorescein (DCF) was observed and photographed under a fluorescence microscope (Eclipse 80i, Nikon, Tokyo, Japan).

Ultraperformance Liquid Chromatography Quadruple Time-of-Flight Mass Spectrometry (UPLC-Q-TOF-MS) Analysis of SGEs. To analyze the metabolites of garlic and sprouted garlic, ethanolic extracts from garlic and sprouted garlic were redissolved in ethanol. The metabolites were analyzed using an Acquity UPLC system (Waters, Milford, MA) equipped with a Waters Micromass Q-TOF Premier mass spectrometer and an Acquity UPLC BEH C18 column (100 \times 2.1 mm, 1.7 μ m, Waters, Milford, MA). Two solvents, 0.1% formic acid (v/v) and acetonitrile containing 0.1% (v/v) formic acid, were used as the mobile phase. The elution was initiated with the mobile phase containing 5% acetonitrile that was maintained for 1 min, gradually increased to 100% acetonitrile over 10 min, remained at 100% acetonitrile for 1 min, and decreased to 5% acetonitrile for 1 min. The sample (5 μ L) was injected, while the flow rate was maintained at 0.3 mL/min. Electron spray ionization was conducted in the negative and positive modes within a *m/z* range of 100–1000. The ion source and desolvation temperatures were 200 and 250 °C,

respectively. The capillary and cone voltages were 3.0 kV and 30 V, respectively. The cone gas flow was 50 L/h. Leucine enkephalin was used as the lock mass (*m/z* 554.2615) at a concentration of 0.5 ng/ μ L and a flow rate of 3.0 μ L/min.

Data Processing, Multivariate Analysis, and Tentative Identification of Discriminative Variables. The DataBridge program in the MassLynx software package (version 4.1, Waters) was used to export raw data sets to the netCDF file format, in which the netCDF files were automatically aligned using the XCMS package in R 3.0.1 (www.bioconductor.org). The result .txt file, which included the sample name, peak area, and retention time as a variable, was processed using SIMCA-P software 12.0 (Umetrics, Umea, Sweden) for multivariate statistical analysis. The data sets were autoscaled (unit variance scaling) and log-transformed before principal component analysis (PCA) modeling. To identify the different metabolites, we used partial least-squares discriminant analysis (PLS-DA, data not shown) models and created box-and-whisker plots with the mean data using STATISTICA (version 7.0, StatSoft, Inc., Tulsa, OK). We selected metabolites based on the variable important to projection (VIP) values (VIP > 1.0) and *p* values (*p* < 0.05) and tentatively identified them by comparing their molecular weights to those in the 2008 *Dictionary of Natural Products* (CRC Press, Taylor and Francis Group, Boca Raton, FL) and related references. In addition, the accurate mass from TOF-MS was matched with the elemental composition and the theoretical isotope distribution (i-FIT).

Statistical Analysis. Data were assessed by analysis of variance, followed by Duncan's multiple range test, using SPSS software (SPSS, Inc., Chicago, IL). The significance level was set at *p* < 0.05.

RESULTS

Total Phenolic Contents. Plant polyphenols are multifunctional; they can act as reducing agents, hydrogen-donating antioxidants, and singlet oxygen quenchers. As shown in Figure 1, the total phenolic content in garlic increased slightly during sprouting but there was no significant difference when garlic was sprouted for different periods.

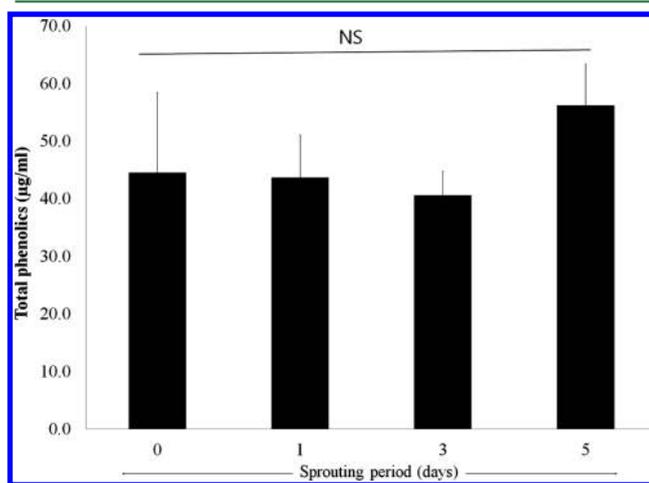


Figure 1. Total phenolic content of SGE. Data are expressed as the mean \pm standard deviation (SD) of four independent experiments. NS = no significant difference between the groups.

DPPH Radical Scavenging Activity of Sprouted Garlic.

The antioxidant activities of garlic and sprouted garlic are shown in Figure 2 as the percentage of DPPH radical scavenging activity in the control. The DPPH radical scavenging activity of sprouted garlic was higher than that of unsprouted garlic. Of the extracts from samples sprouted 0, 1, 3, or 5 days, the ethanol extract from garlic sprouted for 5 days showed the highest DPPH radical scavenging activity; the

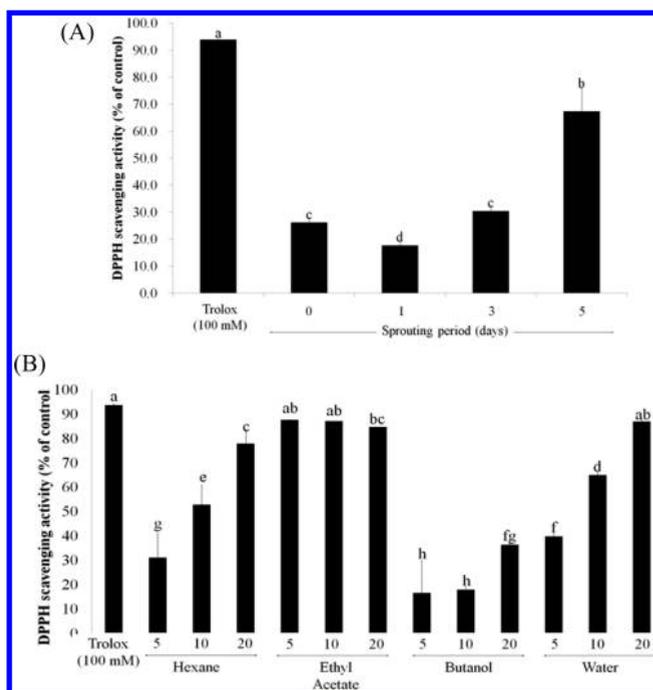


Figure 2. DPPH radical scavenging activity of (A) SGE and (B) solvent fractions. Data are expressed as the mean \pm SD of four independent experiments. Values not sharing a common letter differ significantly from each other ($p < 0.05$).

scavenging activity of the 10 mg/mL extract was 80–100% of the control (Figure 2).

ORAC of Sprouted Garlic. The ORAC assay measures the loss of fluorescence caused by the oxidative degradation of fluorescein, a fluorescent molecule, when mixed with 2,2'-azobis(2-amidinopropane) dihydrochloride (AAPH) (final concentration of 11.4 mM), a peroxy radical generator (Figure 3). The presence of an antioxidant suppresses or slows the loss of fluorescence, resulting in a high ORAC value. Extracts from garlic sprouted for 5 days suppressed the generation of the peroxy radical more than extracts from raw garlic and garlic

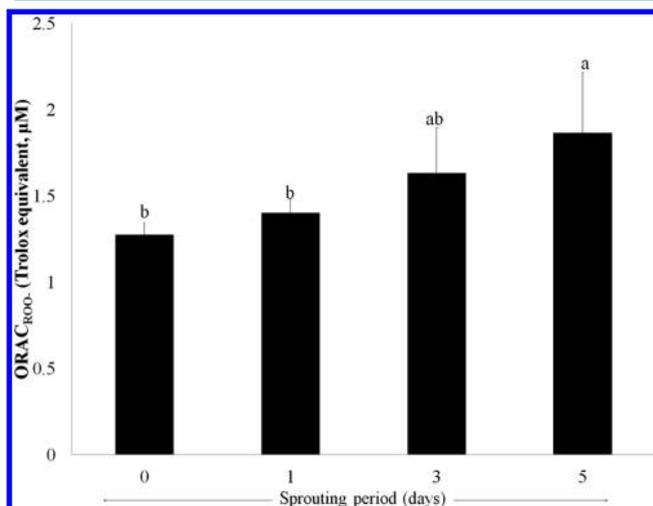


Figure 3. Oxygen radical absorbance activity of SGE. All data are expressed as micromoles of TE. One ORAC unit is equivalent to the net protection area provided by 1 μ M Trolox. Values not sharing a common letter differ significantly from each other ($p < 0.05$).

sprouted for shorter times, consistent with the results of the other antioxidant assays.

Suppression of Intracellular ROS Generation by SGE in Mouse Hippocampus Cells. We investigated the inhibitory effect of SGE on ROS generation using the oxidant-reactive fluorescent dye DCFDA. SGE prevented glutamate-induced cytotoxicity in the HT22 mouse hippocampal neuronal cell line (Figure 4), whereas raw garlic extract

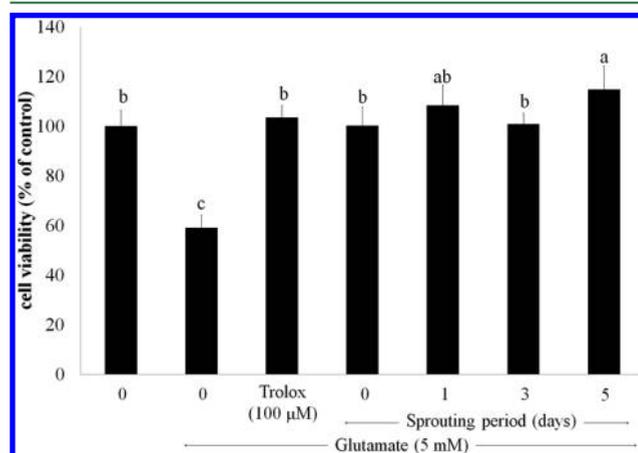


Figure 4. Inhibition of glutamate-induced cell death by SGE in HT22 cells. Cells were treated with 5 mM glutamate in the absence and presence of sprouted garlic samples for 24 h. Cell viability was measured with the MTT assay. The cell survival rate is presented as the percentage of the untreated control. Values not sharing a common letter differ significantly from each other ($p < 0.05$).

had a limited protective effect. Furthermore, ROS generation mediated by glutamate treatment in HT22 cells was significantly suppressed by the ethanolic extract from garlic sprouted for 4–5 days. These results confirm that sprouted garlic has higher antioxidant potential than non-sprouted garlic (Figure 5).

Multivariate Analysis of Raw and Sprouted Garlic Metabolites. After aligning the UPLC-Q-TOF-MS spectral data, we obtained 1098 and 1904 variables with the negative and positive detection modes, respectively. The aligned data were presented as two-dimensional PCA (Figure 6) score plots: vector t[1] explained 37.99 and 40.62% of the variation, and vector t[2] explained 16.55 and 22.77% of the variation in the positive and negative modes, respectively. The PCA score plots demonstrated that the metabolite profiles of garlic differed depending upon the sprouting period. In particular, in PC1 of the positive and negative analyses, the metabolite profile of garlic that sprouted for 5 and 6 days (group 1) differed from the metabolite profile of garlic that sprouted for <5 days (group 2). The metabolites that discriminated between the two groups were identified on the basis of the criteria of $p < 0.05$ and VIP > 1.0 (Table 1). Among these metabolites, we identified isoguanosine (or 1-aminoinosine) (2), γ -glutamyl-S-trans-1-propenyl cysteine, glucoside (3), 8,11,12-trihydroxy-9-octadecenoic acid (5), and spirostane-3,6-diol, 6-O-D-glucopyranoside (7). These metabolites increased during the sprouting period (Figure 7).

DISCUSSION

Garlic (*Allium sativum*) has long been used for medicinal purposes, owing to its immune-enhancing functions and

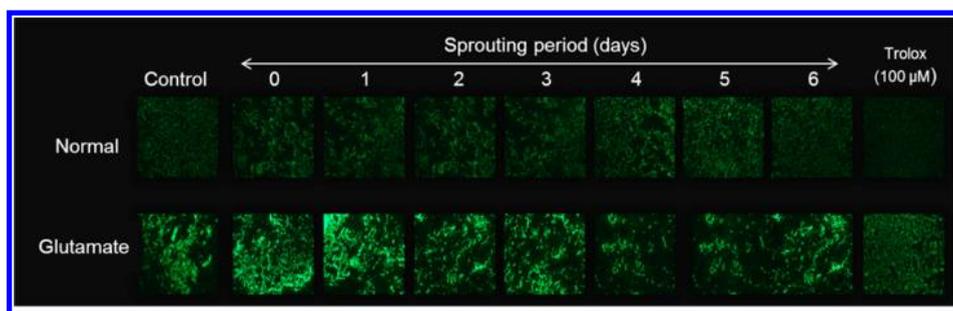


Figure 5. Inhibition of glutamate-induced ROS production by SGE in HT22 cells. The cells (2×10^3 cells/well) were seeded in a 96-well black-bottom plate and cultured for 24 h. After cell attachment, the cells were incubated in the presence of 5 mM glutamate and garlic samples for 6 h. The treated cells were washed with PBS, incubated for 30 min with DCFDA dissolved in DMEM (final concentration of $30 \mu\text{M}$), and then washed with PBS. The fluorescence generated by the interaction of ROS with DCF was visualized under a fluorescence microscope.

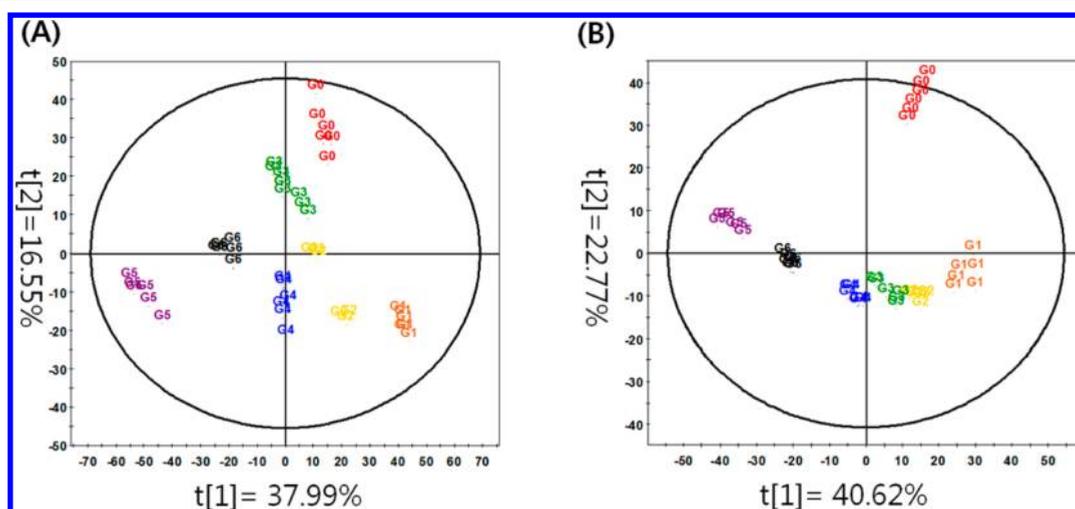


Figure 6. PCA score scatter plots of the first two components for UPLC-Q-TOF-MS analysis in (A) positive and (B) negative modes. Sprouting time: red, 0 days; orange, 1 day; yellow, 2 days; green, 3 days; blue, 4 days; purple, 5 days; and black, 6 days.

Table 1. Tentative Identification of Significant Variables Distinguished by Groups 1 and 2 in UPLC-Q-TOF-MS

number	t_R^a (min)	UPLC-Q-TOF-MS				MW	tentative compound	p value ^b	reference
		negative	error (ppm)	positive	error (ppm)				
1	0.79	131.0827				NI ^c	1.03×10^{-7}		
2	1.31	282.0851	4.6	284.1007	4.2	isoguanosine or 1-aminoinosine	2.92×10^{-12}		
3	3.05	451.1385	-0.2	453.1532	1.1	γ -glutamyl-S-trans-1-propenylcysteine, glucoside	5.84×10^{-5}	29	
4	5.59			325.2287		NI	2.51×10^{-7}		
5	5.90	329.2328	0.0	353.2299	-8.2	8,11,12-trihydroxy-9-octadecenoic acid	4.11×10^{-12}	31	
6	6.19			257.0114		NI	1.23×10^{-8}		
7	6.84	593.3707	2.9	595.3843	-0.5	spirostane-3,6-diol, 6-O-D-glucopyranoside	7.15×10^{-10}	30	
8	8.47	614.3359		616.3417		NI	1.55×10^{-12}		
9	8.51	311.1681				NI	3.41×10^{-5}		
10	8.78			496.3370		NI	5.01×10^{-13}		

^a t_R = retention time. ^bSignificance was determined by the t test. Components with a p value of ≤ 0.05 indicate moderate evidence of a statistically significant effect. ^cNI = not identified.

antibacterial, antifungal, antiviral, and anticancer activities. Garlic prevents platelet aggregation, and it has hypotensive and cholesterol- and triglyceride-lowering effects.²⁸

We found that sprouting enhanced the DPPH radical scavenging activity and ORAC of garlic. Sprouting also increased the capacity of garlic to suppress intracellular ROS induced by hydrogen peroxide in HT22 cells. The HT22 mouse hippocampal cell line, a subclone of the original HT4 clone, is sensitive to amyloid- β ($A\beta$) and particularly vulnerable

to glutamate; thus, HT22 cells provide a good model system with which to study neuronal cell degeneration and screen for potential agents, such as antioxidants, that prevent $A\beta$ - or glutamate-induced cell death.^{19,26,29,30} Glutamate reduced the viability of HT22 cells, but viability was restored by treatment with an extract from garlic sprouted for 5 days. Furthermore, the same effect was observed with extracts prepared from garlic at an early stage of sprouting, suggesting that certain

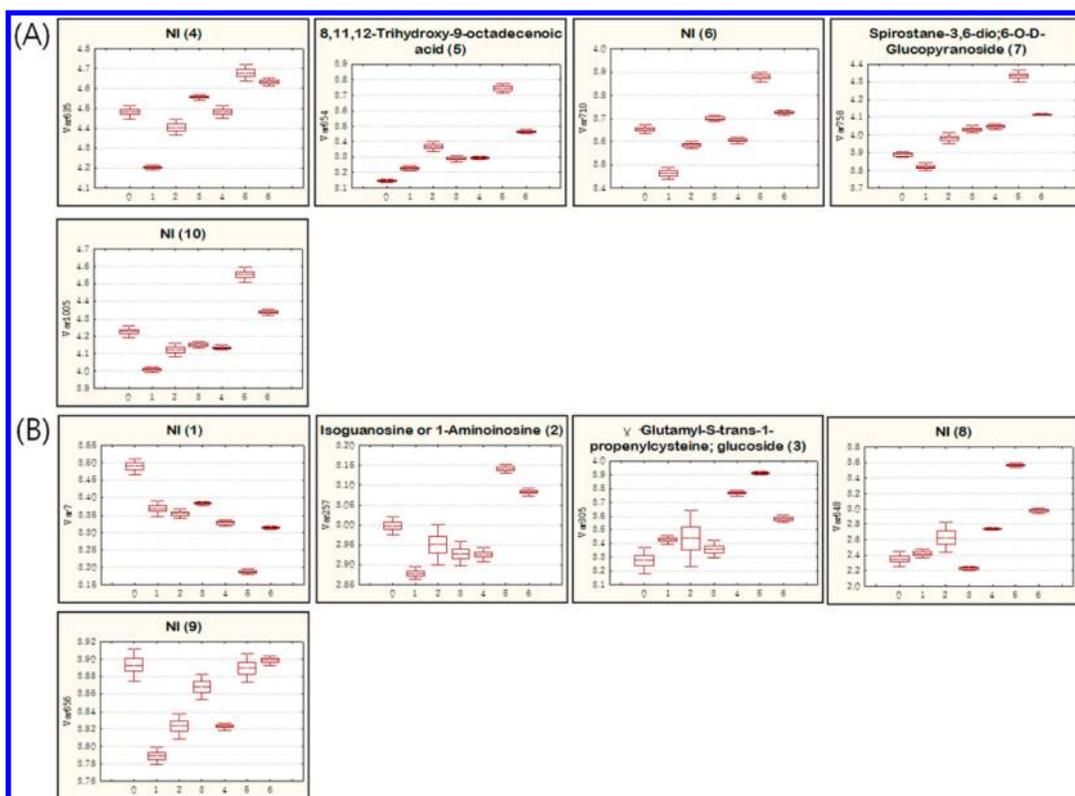


Figure 7. Box-and-whisker plots of changes in metabolite levels with sprouting time when mass spectrometry was run in (A) positive and (B) negative modes.

antioxidants or neuroprotective compounds like flavonoids are produced during the early stage of sprouting.

Although the increased neuroprotective effect could be ascribed to the enhanced antioxidant capacity of sprouted garlic, it is not clear which components in sprouted garlic are primarily responsible for the increased antioxidant capacity. Furthermore, sprouting did not increase the total phenolic content of garlic, suggesting that phenolics are not associated with the increased antioxidant activity and neuroprotective effect of SGE.

The health benefits of garlic are attributed to organosulfur compounds that mainly derive from alliin. Alliin is quickly converted into allicin through extremely reactive intermediates, sulfenic acids (R-SOH), by the enzyme alliinase (alliin lyase EC.4.4.1.4) when garlic is physically damaged. Alliin, allyl disulfide, and allyl cysteine scavenge hydroxyl radicals, whereas allicin does not.¹⁸ In our multivariate analysis, the metabolite patterns differed depending upon the sprouting period and correlated closely with the antioxidant activities. Moreover, the PCA score plots were different before and after 4 sprouting days. Among the 10 metabolites that influenced the multivariate analysis, γ -glutamyl-S-trans-1-propenyl cysteine; glucoside (3) and spirostane-3,6-diol, 6-O-D-glucopyranoside (7) were previously detected in their derivative forms, γ -glutamyl-S-trans-1-propenyl cysteine (GSPC)³¹ and steroidal oligoglycoside,³² respectively, in *Allium* species. GSPC is a major metabolite³³ in garlic and a precursor of isoalliin, which has antioxidant activity. Spirostane-3,6-diol, 6-O-D-glucopyranoside is a steroidal oligoglycoside and an antineoplastic agent. Although there is no information on the antioxidant activities of these two compounds in their glycosidic forms, the glycosidic forms of flavonoids have a similar level of antioxidant

activity as the aglycone forms.³⁴ Furthermore, it has been reported that certain glycosidic forms could be hydrolyzed into aglycones by intracellular glucosidases in neuronal tissues.³⁵ However, further studies are required to define the antioxidative potential of the compounds that increased in sprouted garlic and to characterize the components responsible for the antioxidative and neuroprotective activities.

In conclusion, sprouting could be a useful way to increase the antioxidative potential of garlic and thereby expand its use.

AUTHOR INFORMATION

Corresponding Author

*Telephone: 82-53-950-5752. Fax: 82-53-950-6750. E-mail: vision@knu.ac.kr.

Author Contributions

†Alexandra Zakarova and Ji Yeon Seo contributed equally to this work.

Funding

This work was supported by a grant from Korea Institute of Planning and Evaluation for Technology in Food, Agriculture, Forestry and Fisheries (IPET) (High Value-Added Food Technology Development Program, 2012, 112066-3), Ministry for Food, Agriculture, Forestry and Fisheries, Republic of Korea.

Notes

The authors declare no competing financial interest.

REFERENCES

- (1) Tattelman, E. Health effects of garlic. *Am. Fam. Physician* **2005**, *72*, 103–106.
- (2) Weik, R.; Francky, A.; Striedner, G.; Raspor, P.; Bayer, K.; Mattanovich, D. Recombinant expression of alliin lyase from garlic

- (*Allium sativum*) in bacteria and yeasts. *Planta Med.* **1998**, *64*, 387–388.
- (3) Borek, C. Antioxidant health effects of aged garlic extract. *J. Nutr.* **2001**, *131*, 1010S–1015S.
- (4) Reeve, V. E.; Bosnic, M.; Rozinova, E.; Boehm-Wilcox, C. A garlic extract protects from ultraviolet B (280–320 nm) radiation-induced suppression of contact hypersensitivity. *Photochem. Photobiol.* **1993**, *58*, 813–817.
- (5) Chang, H. S.; Yamato, O.; Yamasaki, M.; Maede, Y. Modulatory influence of sodium 2-propenyl thiosulfate from garlic on cyclooxygenase activity in canine platelets: Possible mechanism for the anti-aggregatory effect. *Prostaglandins, Leukotrienes Essent. Fatty Acids* **2005**, *72*, 351–355.
- (6) Ali, M. Mechanism by which garlic (*Allium sativum*) inhibits cyclooxygenase activity. Effect of raw versus boiled garlic extract on the synthesis of prostanoids. *Prostaglandins, Leukotrienes Essent. Fatty Acids* **1995**, *53*, 397–400.
- (7) Block, E. The chemistry of garlic and onions. *Sci. Am.* **1985**, *252*, 114–119.
- (8) Sangeetha, T.; Quine, S. D. Protective effect of S-allyl cysteine sulphoxide (alliin) on glycoproteins and hematology in isoproterenol induced myocardial infarction in male Wistar rats. *J. Appl. Toxicol.* **2008**, *28*, 710–716.
- (9) Ortuno Sahagun, D.; Marquez-Aguirre, A. L.; Quintero-Fabian, S.; Lopez-Roa, R. I.; Rojas-Mayorquin, A. E. Modulation of PPAR- γ by nutraceuticals as complementary treatment for obesity-related disorders and inflammatory diseases. *PPAR Res.* **2012**, *2012*, 318613.
- (10) Moriguchi, T.; Matsuura, H.; Itakura, Y.; Katsuki, H.; Saito, H.; Nishiyama, N. Allixin, a phytoalexin produced by garlic, and its analogues as novel exogenous substances with neurotrophic activity. *Life Sci.* **1997**, *61*, 1413–1420.
- (11) Kim, H. J.; Suh, H. J.; Lee, C. H.; Kim, J. H.; Kang, S. C.; Park, S.; Kim, J. S. Antifungal activity of glyceollins isolated from soybean elicited with *Aspergillus sojae*. *J. Agric. Food Chem.* **2010**, *58*, 9483–9487.
- (12) Takahashi, M.; Shibamoto, T. Chemical compositions and antioxidant/anti-inflammatory activities of steam distillate from freeze-dried onion (*Allium cepa* L.) sprout. *J. Agric. Food Chem.* **2008**, *56*, 10462–10467.
- (13) Alvarez-Jubete, L.; Wijngaard, H.; Arendt, E. K.; Gallagher, E. Polyphenol composition and in vitro antioxidant activity of amaranth, quinoa buckwheat and wheat as affected by sprouting and baking. *Food Chem.* **2010**, *119*, 770–778.
- (14) Randhir, R.; Lin, Y. T.; Shetty, K. Stimulation of phenolics, antioxidant and antimicrobial activities in dark germinated mung bean sprouts in response to peptide and phytochemical elicitors. *Process Biochem.* **2004**, *39*, 637–646.
- (15) Kim, H. J.; Suh, H. J.; Kim, J. H.; Park, S.; Joo, Y. C.; Kim, J. S. Antioxidant activity of glyceollins derived from soybean elicited with *Aspergillus sojae*. *J. Agric. Food Chem.* **2012**, *58*, 11633–11638.
- (16) Bozin, B.; Mimica-Dukic, N.; Samojlik, I.; Goran, A.; Igc, R. Phenolics as antioxidants in garlic (*Allium sativum* L., Alliaceae). *Food Chem.* **2008**, *111*, 925–929.
- (17) Ichikawa, M.; Ide, N.; Ono, K. Changes in organosulfur compounds in garlic cloves during storage. *J. Agric. Food Chem.* **2006**, *54*, 4849–4854.
- (18) Chung, L. Y. The antioxidant properties of garlic compounds: allyl cysteine, alliin, allicin, and allyl disulfide. *J. Med. Food* **2006**, *9*, 205–213.
- (19) Behl, C.; Skutella, T.; Lezoualc'h, F.; Post, A.; Widmann, M.; Newton, C. J.; Holsboer, F. Neuroprotection against oxidative stress by estrogens: Structure–activity relationship. *Mol. Pharmacol.* **1997**, *51*, 535–541.
- (20) Herrera, F.; Martin, V.; Garcia-Santos, G.; Rodriguez-Blanco, J.; Antolin, I.; Rodriguez, C. Melatonin prevents glutamate-induced oxytosis in the HT22 mouse hippocampal cell line through an antioxidant effect specifically targeting mitochondria. *J. Neurochem.* **2007**, *100*, 736–746.
- (21) Sucontphunt, A.; De-Eknamkul, W.; Nimmannit, U.; Dan Dimitrijevic, S.; Gracy, R. W. Protection of HT22 neuronal cells against glutamate toxicity mediated by the antioxidant activity of *Pueraria candollei* var. *mirifica* extracts. *J. Nat. Med.* **2011**, *65*, 1–8.
- (22) Huang, W. Y.; Chao, X. J.; Ouyang, Y.; Liu, A. M.; He, X. X.; Chen, M. H.; Wang, L. H.; Liu, J.; Yu, S. W.; Rapposelli, S.; Pi, R. B. Tacrine-6-ferulic acid, a novel multifunctional dimer against Alzheimer's disease, prevents oxidative stress-induced neuronal death through activating Nrf2/ARE/HO-1 pathway in HT22 cells. *CNS Neurosci. Ther.* **2012**, *18*, 950–951.
- (23) Singleton, V. L.; Rossi, J. A. Colorimetry of total phenolics with phosphomolybdic–phosphotungstic acid reagents. *Am. J. Enol. Vitic.* **1965**, *16*, 15.
- (24) Hatano, T.; Kagawa, H.; Yasuhara, T.; Okuda, T. Two new flavonoids and other constituents in licorice root: Their relative astringency and radical scavenging effects. *Chem. Pharm. Bull.* **1988**, *36*, 2090–2097.
- (25) Kurihara, H.; Fukami, H.; Asami, S.; Toyoda, Y.; Nakai, M.; Shibata, H.; Yao, X. S. Effects of oolong tea on plasma antioxidative capacity in mice loaded with restraint stress assessed using the oxygen radical absorbance capacity (ORAC) assay. *Biol. Pharm. Bull.* **2004**, *27*, 1093–1098.
- (26) Behl, C.; Widmann, M.; Trapp, T.; Holsboer, F. 17- β estradiol protects neurons from oxidative stress-induced cell death in vitro. *Biochem. Biophys. Res. Commun.* **1995**, *216*, 473–482.
- (27) Wang, H.; Joseph, J. A. Quantifying cellular oxidative stress by dichlorofluorescein assay using microplate reader. *Free Radical Biol. Med.* **1999**, *27*, 612–616.
- (28) Iciek, M.; Kwicien, I.; Wlodek, L. Biological properties of garlic and garlic-derived organosulfur compounds. *Environ. Mol. Mutagen.* **2009**, *50*, 247–265.
- (29) Li, B.; Jeong, G. S.; Kang, D. G.; Lee, H. S.; Kim, Y. C. Cytoprotective effects of lindenyl acetate isolated from *Lindera strychnifolia* on mouse hippocampal HT22 cells. *Eur. J. Pharmacol.* **2009**, *614*, 58–65.
- (30) Howes, M. J.; Perry, E. The role of phytochemicals in the treatment and prevention of dementia. *Drugs Aging* **2011**, *28*, 439–468.
- (31) Kamel, A.; Saleh, M. Recent studies on the chemistry and biological activities of the organosulfur compounds of garlic (*Allium sativum*). *Stud. Nat. Prod. Chem.* **2000**, *23*, 455–486.
- (32) Ikeda, T.; Tsumagari, H.; Nohara, T. Steroidal oligoglycosides from the seeds of *Allium tuberosum*. *Chem. Pharm. Bull.* **2000**, *48*, 362–365.
- (33) Xu, Q. M.; Liu, Y. L.; Li, X. R.; Li, X.; Yang, S. L. Three new fatty acids from the roots of *Boehmeria nivea* (L.) Gaudich and their antifungal activities. *Nat. Prod. Res.* **2011**, *25*, 640–647.
- (34) Lee, C. H.; Yang, L.; Xu, J. Z.; Yeung, S. Y. V.; Huang, Y.; Chen, Z. Y. Relative antioxidant activity of soybean isoflavones and their glycosides. *Food Chem.* **2005**, *90*, 735–741.
- (35) Lee, P.; Lee, J.; Choi, S. Y.; Lee, S. E.; Lee, S.; Son, D. Geniposide from *Gardenia jasminoides* attenuates neuronal cell death in oxygen and glucose deprivation-exposed rat hippocampal slice culture. *Biol. Pharm. Bull.* **2006**, *29*, 174–176.