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Biological and Chemical Stability of Garlic-Derived Allicin

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This study verifies the instability of garlic (*Allium sativum* L.)-derived allyl 2-propenylthiosulfinate (allicin) in various aqueous and ethanolic solutions as well as in vegetable oil through chemical and biological analyses performed simultaneously. Crushed fresh garlic cloves generated antibacterial activity and chemically detectable allicin, a major antibacterial principle, and both declined on a daily basis in aqueous and ethanolic solutions at room temperature, showing biological and chemical half-lives of about 6 and 11 days, respectively. Allicin was more stable in 20% alcohol than in water, but surprisingly unstable in vegetable oil, with an activity half-life 0.8 h, as estimated from its antibacterial activity toward *Escherichia coli*, and a chemical half-life of 3.1 h, based on chromatographic quantification. In alcoholic and aqueous extracts, the biological half-life of allicin tended to be longer than the chemical one, suggesting the occurrence of bioactive compounds other than allicin in the extracts.

KEYWORDS: Allicin; garlic; stability; antibacterial activity; *Staphylococcus aureus*; *Escherichia coli*

INTRODUCTION

Garlic (*Allium sativum* L.) has long been used as one of the representative vegetables possessing marked pharmacological potentials, such as antimicrobial activity (*1, 2*) and antiplatelet and antithrombotic activities (*3–7*) as well as anticancer activity (*8–10*). Studies on garlic have been reported in more than 3000 papers, which have disclosed that garlic produces many organosulfur compounds depending on the method of cooking, treatment, or preservation (*11–13*); these differences result in differences in its biological effects. Because crushed fresh garlic exhibits strong antimicrobial activity, people use garlic as an indispensable plant to preserve fresh meat as long as possible (*14, 15*). Allicin is the most classic of the effective substances found in the garlic; hence, the potential of this plant has been assumed to be due to the allicin (*16*). However, through much scientific research, allicin has taken second place to its daughter components, alk(en)yl sulfides, which have been accepted as substances exhibiting most of garlic's potential in vivo (*13, 17*). As one of the decisive shortcomings of allicin, upon ingestion, allicin changes from its reactive form into stable sulfides in an intrastomach acidic solution or into breakdown products involving allyl methyl sulfides and, finally, into mercaptan, in vivo (*18*).

Recent studies on the anticancer effects of garlic have revealed that allicin can induce apoptosis in cancer cells through oxidative modification of cellular sulfhydryl (thiol) groups (*19*). Arditti et al. (*20*) and Miron et al. (*21*) reported that characteristic properties of allicin, such as high membrane permeability and congenital high reactivity, worked favorably in the tumor. These researchers have tried to generate allicin in situ to induce apoptosis of cancer cells in vivo. For this purpose, they delivered alliinase onto the cell surface as a conjugate with a cancer cell-specific antibody, and then alliin was injected into the bloodstream to produce allicin at the target site (*20, 21*). In their studies, allicin's instability might be advantageous, minimizing damage to the surrounding normal cells, if any.

Therefore, for pertinent utilization and clinical application of allicin, determination of its instability under various conditions by systematic approaches is an urgent problem to be solved. Although there have been many papers describing the stability of allicin (*22, 23*), there is little information that has verified its stability from both chemical and biological viewpoints using ordinary prepared garlic extracts.

MATERIALS AND METHODS

Quantification of Allicin. Allicin in garlic extracts was quantified by chromatographic analysis using a C18, MG-II (5 μm) column with a size of 4.6 mm × 250 mm (Shiseido Co., Tokyo, Japan), an LC-10AT pump, and Chromatopac, C-R4A (Shimadzu Co., Kyoto, Japan) equipped with a UV monitor, 655A (Hitachi, Tokyo, Japan). The column was equilibrated with solvent containing 0.02 M phosphate buffer (pH 6.5), acetonitrile, and 1,4-dioxane in a ratio of 7:1:2. Allicin

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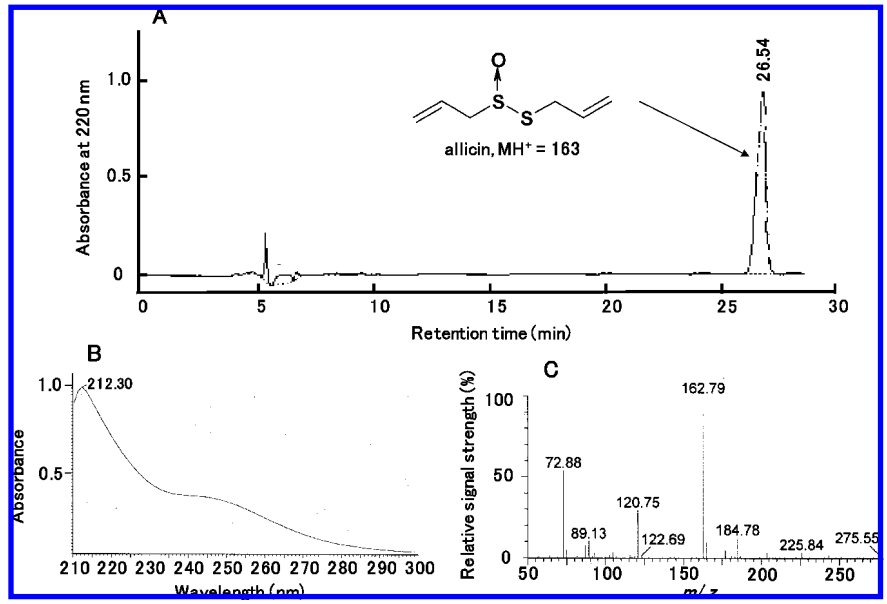

Figure 1. HPLC pattern and MS spectrum of authentic allicin: (**A**) isocratic elution of allicin produced a single peak with a retention time of 26.5 min (inset: structure of allicin); (**B**) optical absorption spectrum of authentic allicin peaked at 212 nm (for the detection of allicin and its breakdown products, we used 220 nm); (**C**) MS spectrum of the substance that eluted at 26.5 min (see **A**) showed its protonated mass to be 163, allicin (see the inset structure in **A**).

or allicin-containing extracts were applied onto the column and eluted by the isocratic solvent application at a flow rate of 0.5 mL/min. The allicin in effluents was detected by optical absorption at 220 nm. Quantification of allicin was performed by comparing the peak area (V•s) produced by crushed fresh garlic extracts with that of authentic allicin. The authentic allicin (allyl 2-propenylthiosulfinate) was purchased from LKT Laboratories, Inc., as a preparation with 99.39% purity and kept at −70 °C until used.

Preparation of Allicin-Containing Extracts. Garlic (*A. sativum* L.) grown in Aomori, Japan, was obtained at a market, stored at 4 °C, and used for analysis within 1 month. For the extraction of allicin, 10 g of garlic cloves was crushed with a utility garlic crusher by hand, and the juice and debris of the garlic were collected in a centrifuge tube by pouring 10 mL of water or solvent onto the crusher. After having been shaken 10 times, the tube was allowed to stand for 10 min at room temperature and then centrifuged twice at 5500 rpm for 5 min each time. The supernatants were combined and then analyzed for antibacterial activity and amount of allicin, as described below. The vegetable oil used as a solvent was a salad oil containing rapeseed and soybean oils (Nisshin Oillio Group, Co., Tokyo, Japan). The experiments were performed at room temperature (23−25 °C) unless otherwise stated.

Assay of Antibacterial Activity. The bacteria used were Gram-negative *Escherichia coli* C600, kindly provided by Dr. R. Takahashi of Nihon University, and Gram-positive *Staphylococcus aureus* (Rosenback 1884 NBRC 12732). These bacteria were cultured on a nutrient broth (NB) agar plate for 24 h or more at room temperature, and the colonies that formed were picked up twice with a platinum loop and inoculated with a sterilized cotton stick onto the surface of a new NB agar plate. Then 50 μL of garlic extract was put on an ethanol-sterilized paper disk (8 mm diam., EB #101, Advantech-Toyoroshi, Co., Tokyo, Japan) and the disk placed on the plate. After incubation at 37 °C for 24 h, the zone of inhibition was measured.

LC-MS/MS. MS was performed by using a Waters Acquity Ultra Performance (UP) LC system and Quattro Premier XE MS system (Waters Corp.) with a 50 mm × 2.1 mm column of Acquity BEH C18 (1.7 μm). A sample volume of 5 μL was injected with an autosampler. The mobile phase was composed of solvent A (0.1% formic acid in water) and solvent B (acetonitrile/1,4-dioxane = 1:2) and introduced at a constant flow rate of 0.2 mL/min. The gradient was programmed to increase the amount of B from an initial 30% B to a final 100% B in 7 min. The MS/MS was performed with a positive ion mode electrospray interface, loading cone voltage at 20 V, and capillary voltage at 2.5 kV.

RESULTS

Quantification of Allicin. Because allicin is unstable and reactive, it is known to change rapidly under the usual gas chromatographic conditions into smaller molecules, such as 2-propenesulfenic acid and thioacrolein, and these short-lived intermediates form larger molecules such as diallyl disulfide and dithiins (*24*). The allicin is also known to form ajoene, if it is in warm oil (*12*). Therefore, we used an HPLC to separate and quantify allicin in the extracts of freshly crushed garlic. The authentic allicin could be detected as a single peak by measuring its optical absorbance at 220 nm (**Figure 1A**,**B**), and the peak was identified as allicin having a molecular mass of $MH^+ = 163$ (**Figure 1C**). The inset in **Figure 1A** shows the structure of allyl 2-propenethiosulfinate (allicin).

The quantification of allicin performed by both chromatographic and biological activity analyses was successful (**Figure 2**). The authentic allicin produced its peak area in good proportion ($r^2 = 0.9894$) to the quantity loaded onto the column over the range of 0.1−2.0 mg/mL (**Figure 2A**). Similarly, the allicin exhibited dose-dependent antibacterial potential against both Gram-positive *S. aureus* (**Figure 2B**) and Gram-negative *E. coli* (**Figure 2C**), with correlation coefficients of $r^2 = 0.8893$ and 0.8712, respectively. Because a 50 μL aliquot of allicin solution was applied onto a paper disk, and 20 μL of the same solution onto a column, the detection sensitivity was about 2−3 times higher by the chemical assay than by the biological assays. Of the two types of bacteria, *E. coli* was less sensitive to allicin than was *S. aureus*.

Solvent Efficiency for the Extraction of Allicin from Crushed Garlic. The amounts of allicin in the garlic extracts prepared with water and other solvents were compared immediately after the extraction. As can be seen in **Figure 3**, water was not the best extractant for allicin, as ethanolic solvents, from 20 to 100%, were significantly superior to it. On the other hand, the vegetable oil and *n*-hexane were incompetent to extract allicin. Although the instability of allicin in *n*-hexane could be explained by the lower polarity of hexane (*22*), the worst stability of allicin in the vegetable oil could not be explained only in terms of the polarity of the solvent.

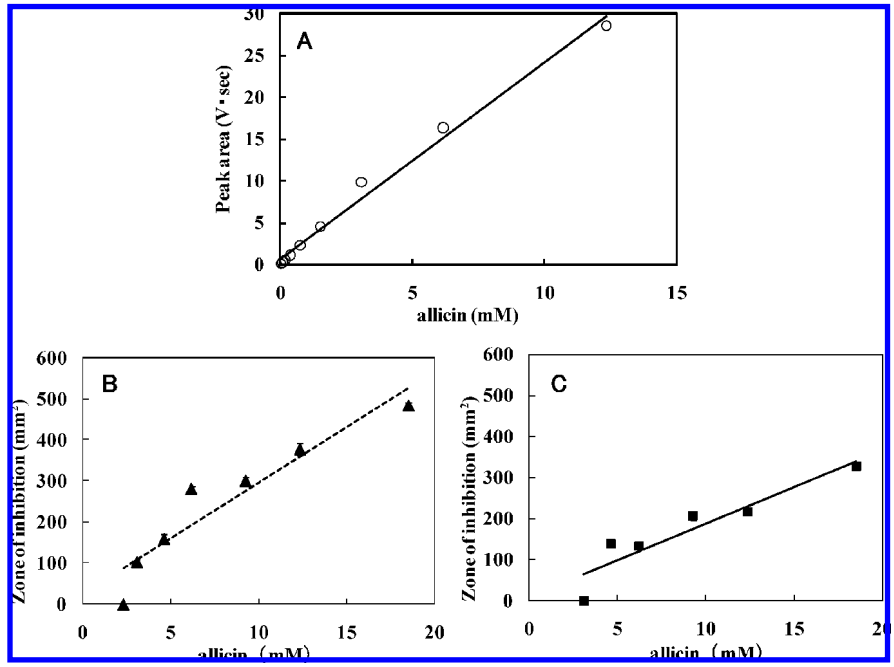

Figure 2. Standard curves of authentic allicin drawn from data from both chemical and biological assays. (**A**) Various amounts of allicin produced their comparable peak areas ($r^2 = 0.9894$). Each plot was derived from three independent experiments involving three or four runs of chromatography, in which a volume of 20 μL or less of allicin was loaded. (**B**, **C**) Allicin exhibited antibacterial activity depending on its amount loaded onto a paper disk in a 50 μL aliquot. Each datum point is presented as an average value from six disks. The correlation coefficient from the experiment with *S. aureus* was $r^2 = 0.8893$ and that with *E. coli* was $r^2 = 0.8712$.

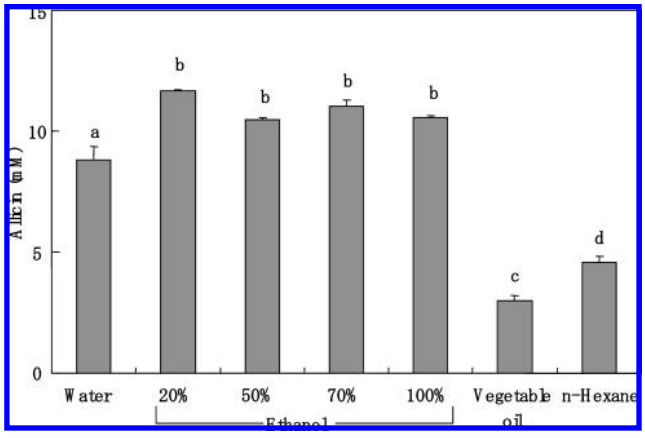

Figure 3. Amounts of allicin in freshly prepared garlic extracts with several solvents. The quantification was performed by HPLC five times for every extract immediately after the extraction. The letters on the bars show the statistical relationships; if different from each other, they differed at $p < 0.05$, according to Student's t test.

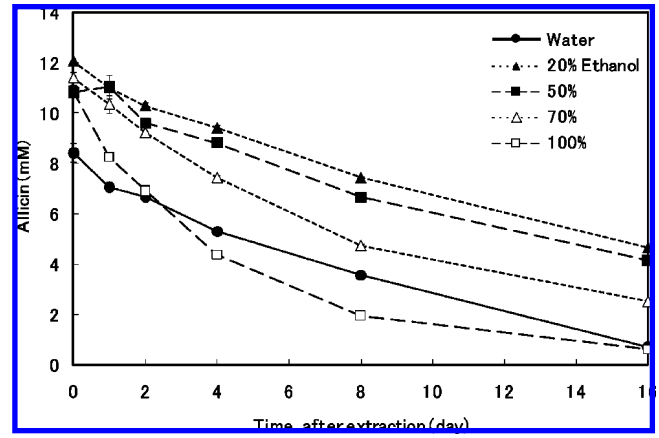

Figure 4. Stability of allicin in garlic extracts prepared with various concentrations of ethanol. Each plot is the average obtained from five runs of chromatography performed on the freshly extracted solution from a single bulb or the same lot of garlic.

Stability of Allicin in Ethanolic Solvents. As shown in **Figure 4**, the allicin could be extracted more efficiently with ethanolic solutions than with water, but these levels decreased gradually at room temperature, and most of the allicin disappeared within a half-month, especially in 100% ethanol or water. It is of interest that the 20 and 50% aqueous ethanol solutions were the most suitable solvents to maintain allicin for a couple of weeks at room temperature.

Biological stability of allicin was judged by assaying the loss of its antibacterial activity against Gram-positive *S. aureus* and Gram-negative *E. coli* during incubation at room temperature for several days. The reduction in the activity of garlic extracts toward both bacterial species proceeded largely coincident with that of the quantity of allicin in the extracts (**Figures 4**−**6**). In 20% alcohol, allicin kept its biological activity longer than in other ethanolic or aqueous solutions. The faster loss of activity in 100% ethanol or water than in the other solvents can be explained by the chemical instability of allicin in these solutions; however, the mechanisms by which allicin is degraded in ethanol and in water would be quite different. With respect to the stability in water, there was discrepancy between the fastest decline in antibacterial activity (see **Figure 5**) and modest reduction in allicin level (see **Figure 4**). These phenomena can partly be explained by the hydrophobicity of allicin. In the 50 and 70% ethanolic solutions, the antibacterial activity against *E. coli* was kept longer than in water or similar to that in water against *S. aureus* (**Figures 5** and **6**). Because the zone of inhibition exhibited by ethanolic extracts for *S. aureus* was about 3 times larger than that for *E. coli*, allicin seemed to be more effective against the Gram-positive bacterium than against the Gram-negative one. As the daily decrease in the biological activity of the aqueous and ethanolic extracts of garlic paralleled

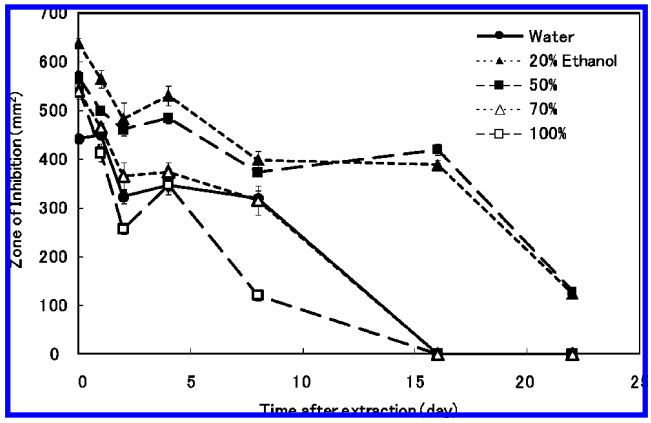

Figure 5. Antibacterial activity of ethanolic extracts of garlic assayed with *E. coli*. An aliquot of 30 μL of the freshly extracted garlic solution was applied onto a paper disk to form a zone of inhibition against the bacteria growth, as described under Materials and Methods. Each plot was obtained from five measurements of the extract prepared from a single bulb of garlic and presented as the mean ± SE.

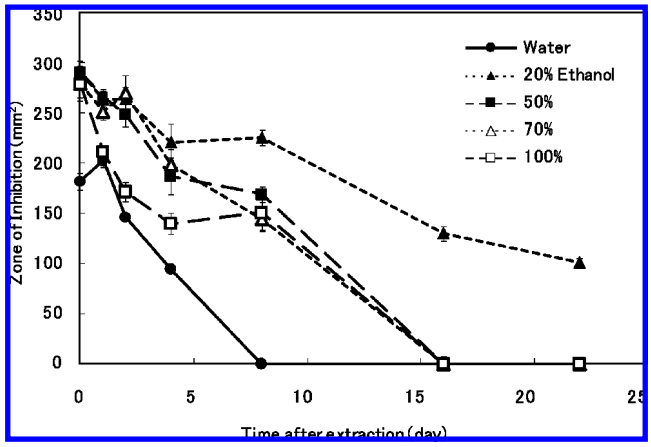

Figure 6. Antibacterial activity of ethanolic extracts of garlic assayed with *S. aureus*. The experimental procedures and calculation of results were similar to those for *E. coli*, as mentioned in the legend of **Figure 5**.

the chemical breakdown of allicin (see **Figure 4**), the antibacterial activity of the extracts may be attributed to the most abundant sulfinyl compound, allyl 2-propenylthiosulfinate (allicin), in the extracts.

In both *n*-hexane and the vegetable oil, the amount of allicin and its biological activity decreased more rapidly than those in ethanolic aqueous solutions (**Figure 7**). Surprisingly, allicin was more unstable in vegetable oil than in *n*-hexane.

The relationship between the amounts of allicin assayed by chromatography and the antibacterial activities assayed with these two bacteria was then determined for every extract of garlic, involving those samples unincubated and incubated for up to about 1 month. Plotting of the data thus obtained revealed an obvious correlation between the amount of allicin and antibacterial activity, with $r^2 = 0.7280$ for *S. aureus* (**Figure 8A**) and $r^2 = 0.8214$ for *E. coli* (**Figure 8B**). Although allicin was unstable in *n*-hexane, if it were active, it would have tended to exhibit stronger antibacterial activity toward both *E. coli* and *S. aureus* than that in other aqueous and ethanolic solutions, because the highly volatile hexane would have facilitated the radial diffusion of allicin in the agar plates.

Determination of Half-Life of Allicin in Several Solutions. Finally, we determined the half-life of allicin in the garlic extracts through graphical analysis of each declining curve as

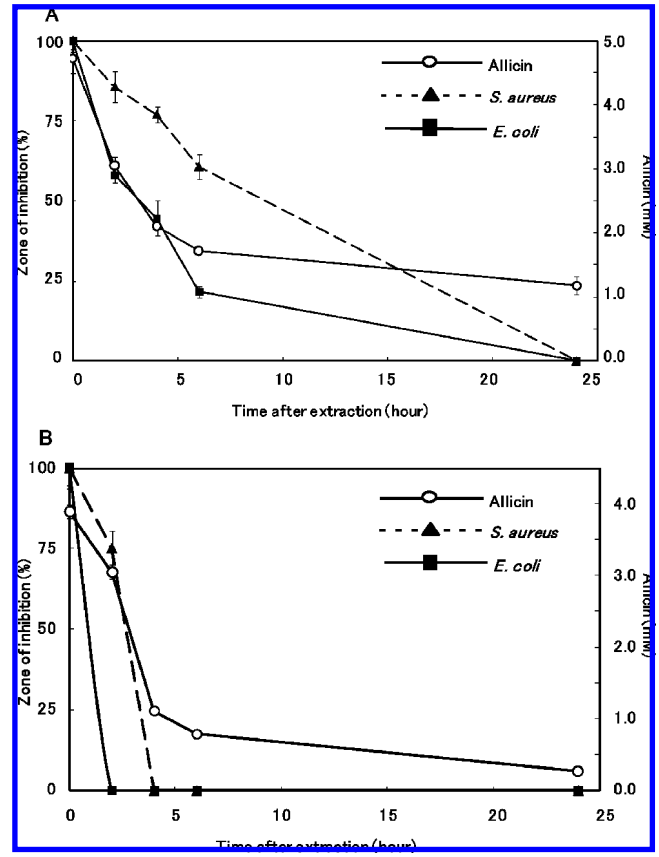

Figure 7. Biological and chemical stabilities of allicin in the *n*-hexane extract (**A**) and vegetable oil extract (**B**) of garlic. The inhibitions by these extracts were different between *E. coli* (■) and *S. aureus* (▲), and inhibition was found to be strong toward *S. aureus*. The decrease in the amounts of allicin (○) was largely in parallel with that of the antibacterial activities. In both extracts, the inhibitory activity disappeared more quickly than allicin. Each plot is presented as the mean ± SE ($n = 5$).

drawn above (**Figures 4–8**). **Table 1** shows the results of the calculation, which disclosed that allicin was rather stable even in the ethanolic extracts. The biological half-life of allicin measured with *E. coli* was shorter than that with *S. aureus*. This difference would be due to the lower sensitivity of *E. coli*; thus, allicin becomes ineffective toward *E. coli* in a shorter time than toward *S. aureus*. One of the half-life-diminishing factors, if any, would be solvent polarity, and in low-polar hexane and triglyceride, allicin would first be inactivated. In the vegetable oil, the chemically measured half-life of allicin (3.1 h) was clearly longer than its biologically measured half-lives (2.4 h for *S. aureus* or 0.8 h for *E. coli*), demonstrating the presence of some kind of allicin-neutralizing or -reducing factor in the oil.

DISCUSSION

Allicin, a general name for alk(en)yl thiosulfinates, is well-known as an unstable volatile organosulfur compound produced by garlic and other plants of the genus *Allium* when they are damaged by worms, fungi, bacteria, or by physical forces involved in cooking (*13, 25, 26*). Owing to the toxic and stimulative activities of allicin, it seems that this compound should not be present long at the damaged site of the host plant of garlic because if it is generated accidentally inside its tissues, it would be harmful to the host. As the most plausible explanation of the defense mechanism operative in the plant, allicin modifies the alliinase molecule at

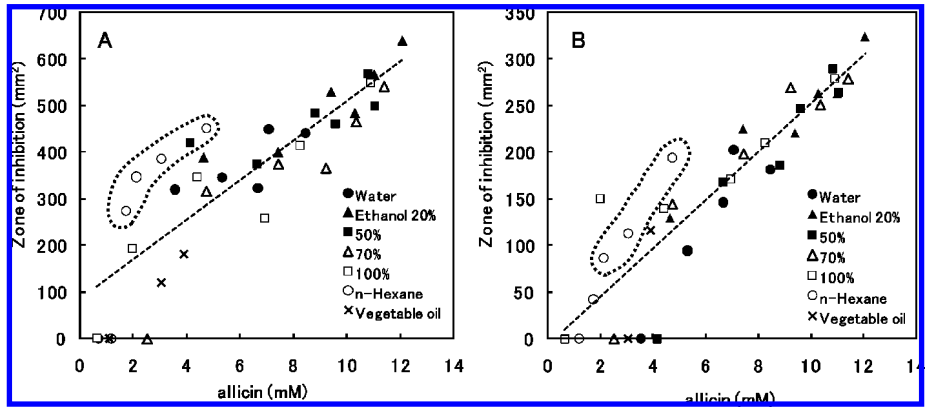

Figure 8. Relationship between the amount of allicin and antibacterial activity of garlic extracts. The data plotted were those from assays of allicin between the two parameters in various solutions: ●, in water; ▲, 20%; ■, 50%; △, 70%; and □, 100% ethanol; ○, *n*-hexane; ×, vegetable oil. Each plot was derived from inhibitory activities toward *E. coli* (**A**) and *S. aureus* (**B**) and amounts of allicin in all garlic extracts. The relationship between the allicin concentration in the extracts and inhibitory activity was well correlated, with $r^2 = 0.7280$ (**A**) and $r^2 = 0.8214$ (**B**). The encircled plots represent those from the garlic extracts with *n*-hexane.

Table 1. Biological and Chemical Half-Lives of Allicin in Garlic Extracts Made with Various Solvents at 23 °C

		biological half-life	
extract	chemical half-life	*S. aureus*	*E. coli*
water	6.5 ± 0.09 days	10.3 ± 0.3 days	3.7 ± 0.2 days
ethanol			
20%	12.0 ± 0.14 days	17.6 ± 0.3 days	14.7 ± 0.8 days
50%	11.9 ± 0.30 days	17.7 ± 0.2 days	8.3 ± 0.3 days
70%	6.6 ± 0.07 days	8.7 ± 0.9 days	7.8 ± 0.6 days
100%	3.2 ± 0.06 days	6.5 ± 0.3 days	7.2 ± 1.6 days
n-hexane	3.4 ± 0.13 h	8.8 ± 1.0 h	3.3 ± 0.5 h
vegetable oil	3.1 ± 0.05 h	2.4 ± 0.2 h	0.8 ± 0.1 h

its −SH groups, leading to inactivation of this allicin-producing enzyme (*27*). As to the utilization of allicin, Lawson et al. reported that allicin is rather stable in water (half-life = 30−40 days) and more so in diluted aqueous solution than in concentrated extracts (*13*). They also revealed that the instability of allicin is remarkable in low-polar solvents, such as hexane, in which its half-life was as short as 2 h. However, there have been few works on the stability of allicin evaluated by both chemical and biological analyses. It is of great importance now to know the detailed nature of allicin to manage this reactive tool appropriately, especially for its clinical use. To utilize allicin as a chemical knife to kill microbes and cancer cells, we performed a basic study on allicin.

Our method of HPLC using the isocratic elution was successful for the quantification of allicin (allyl 2-propenylthiosulfinate, a representative allicin). There was a good correlation ($r^2 = 0.9894$) between the amount of authentic allicin and its peak area produced, indicating a high credibility of this method. The antibacterial potency measured as an index of the biological activity of allicin was proportional to the amount of allicin (allyl 2-propenethiosulfinate). In any garlic extract, these two parameters were well correlated, demonstrating that allicin determines the activity of the extract. According to Lawson et al., this kind of allicin is contained at 70−75% of total thiosulfinate compounds in freshly crushed garlic (*13*). Therefore, our observation was correctly derived from such large amounts of allicin.

In extracting the allicin from garlic, ethanolic solutions were better than the aqueous solution. This may be due to the hydrophobicity of allicin, which can be dissolved more in the diluted alcohol than in water (*13*). In addition, ethanol has an allicin-stabilizing hydroxyl group in its molecule. Reciprocally, the lower activity in water than in alcohol might be due to low solubility of allicin. Because we extracted garlic components with an equal volume of solution to the garlic weight (1:1, v/w), allicin in the aqueous extracts would be decreased by some water soluble substances involving sulfhydryl groups in garlic's proteins (*13*). The extremely low extraction rates obtained with the vegetable oil and hexane could partly be explained by their hydrophobicity and low polarity; in addition, the vegetable oil can break down allicin to ajoene (*5*).

Our data on allicin's stability demonstrated that in different concentrations of ethanolic solutions the antibacterial potential declined time dependently, correlating well with the chemical breakdown of allicin. Such relationships between biological activity and substantial quantity of allicin were also seen in either solutions of hexane or vegetable oil. Hence, we are able to conclude again that allicin is the major substance responsible for the antibacterial potential of garlic extracts.

Whenever biological activity was detected, the allicin in *n*-hexane exhibited relatively higher activity than that in other solutions. This would not be due to its instability, because allicin in vegetable oil exhibited always lower activity than that in other solutions; instead, it would be due to a methodological reason that highly volatile *n*-hexane disappears from the paper disk and facilitates the diffusion of allicin into the agar plate bearing the inoculated bacteria.

With regard to the half-life determined for biological activity and the chemical quantity of allicin, there was a little difference between biological and chemical half-lives. All of the extracts except for the vegetable oil gave a longer half-life in biological activity assayed with *S. aureus* than that in chemically assayed quantity, suggesting that there are either breakdown products, which still have bioactivity, or antibiotic compounds other than allicin (allyl 2-propenylthiosulfinate, which we assayed) present in lesser quantities but having longer half-lives than allicin. As the breakdown products, ajoenes and polysulfides are known to exhibit antibacterial activity (*28, 29*). On the other hand, alk(en)yl thiosulfinates other than allicin, such as allyl methylthiosulfinate, are also known to exhibit the effect (*28*), although their half-lives are not determined yet.

The half-life of allicin in water was determined to be <11.5 days, and this value was shorter than the 30−40 days reported by Lawson et al. (*13*), although their method of measurement

was unclear. Because a higher concentration of aqueous extract of garlic makes allicin more unstable, our extracts (1:1) would be higher in concentration than those of Lawson et al. (*13*). The shortest half-life measured with *E. coli* would solely be due to the lower susceptibility of this Gram-negative bacterium to allicin compared with that of the Gram-positive *S. aureus*. Such a difference can partly be explained by the structural differences of the two bacteria, especially in their cell membranes; that is, *E. coli* has a 10 times higher content of lipids than *S. aureus* (*30*). Thus, in *E. coli*, allicin may be trapped by these lipids, and, therefore, lose it potency to modify its major targets, proteins responsible for RNA synthesis, lipid biosynthetic acetyl-CoA synthetase, or other components essential to survival (*31*).

The chemical and biological decreases in allicin might be due to many reasons involving spontaneous change in its molecular form, as described by Block (*24*) and Lawson et al. (*13*). However, little evidence has been obtained for capturing the breakdown products of allicin from the "allicin waste." According to the *Allium* chemistry described by Block et al. (*24*), allicin is degraded rapidly into 2-propenesulfenic acid, thioacrolein, and allyl alcohol, and these short-lived intermediates quickly form large molecules, such as dithiins and ajoenes. Unfortunately, we could not separate the allicin-derived products clearly and identify these molecules by our LC-MS/MS operation.

Our present systematic study on allicin and garlic extracts revealed in detail the fragility of allicin and its fate in various extracts. Our data should be useful for developing novel applications of garlic and its active organosulfur components in our routine life, as well as for therapeutic purposes.

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LITERATURE CITED

(1) Feldberg, R. S.; Chang, S. C.; Kotik, A. N.; Nadler, M.; Neuwirth, Z.; Sundstrom, D. C.; Thompson, N. H. In vitro mechanism of inhibition of bacterial cell growth by allicin. *Antimicrob. Agents Chemother.* **1988**, *32*, 1763–1768.

(2) Hughes, B. G.; Lawson, L. D. Antimicrobial effects of *Allium sativum* L. (garlic), *Allium ampeloprasum* L. (elephant garlic), and *Allium cepa* L. (onion), garlic compounds and commercial garlic supplement products. *Phytother. Res.* **1991**, *5*, 154–158.

(3) Makheja, A. N.; Vanderhoek, J. Y.; Bailey, J. M. Inhibition of platelet aggregation and thromboxane synthesis by onion and garlic. *Lancet* **1979**, *1*, 781–781.

(4) Ariga, T.; Oshiba, S.; Tamada, T. Platelet aggregation inhibitor in garlic. *Lancet* **1981**, *1*, 150–151.

(5) Apitz-Castro, R.; Cabrera, S.; Cruz, M. R.; Ledezma, E.; Jain, M. K. Effects of garlic extract and of three pure components isolated from it on human platelet aggregation, arachidonate metabolism, release reaction and platelet ultrastructure. *Thromb. Res.* **1983**, *32*, 155–169.

(6) Block, E.; Ahmad, S.; Catalfamo, J. L.; Jain, M. K.; Apitz-Castro, R. Antithrombotic organosulfur compounds from garlic-structural, mechanistic, and synthetic studies. *J. Am. Chem. Soc.* **1986**, *108*, 7045–7055.

(7) Ariga, T.; Seki, T.; Ando, K.; Teramoto, S.; Nishimura, H. Antiplatelet principle found in the essential oil of garlic (*Allium sativum*) and its inhibition mechanism. In *Developments in Food Engineering*; Yano, T., Matsuno, R., Nakamura, K., Eds.; Blackie Academic and Professional: Tokyo, Japan, 1994; pp 1056−1058.

(8) Milner, J. A. Garlic: its anticarcinogenic and antitumorigenic properties. *Nutr. Rev.* **1996**, *54*, S82–S86.

(9) Hirsch, K.; Danilenko, M.; Giat, J.; Miron, T.; Rabinkov, A.; Wilchek, M.; Mirelman, D.; Levy, J.; Sharoni, Y. Effect of purified allicin, the major ingredient of freshly crushed garlic, on cancer cell proliferation. *Nutr. Cancer* **2000**, *38*, 245–254.

(10) Hosono, T.; Fukao, T.; Ogihara, J.; Ito, Y.; Shiba, H.; Ariga, T.; Seki, T. Diallyl trisulfide suppresses the proliferation and induces apoptosis of human colon cancer cells through oxidative modification of β-tubulin. *J. Biol. Chem.* **2005**, *280*, 41487–41493.

(11) Nakagawa, S.; Kasuga, S.; Matsuura, H. Prevention of liver damage by aged garlic extract and its components in mice. *Phytother. Res.* **1989**, *3*, 50–53.

(12) Lawson, L. D.; Wang, Z. Y. J. Changes in the organosulfur compounds released from garlic during aging in water, dilute alcohol, or dilute acetic acid. *J. Toxicol.* **1995**, *14*, 214.

(13) Lawson, L. D. The composition and chemistry of garlic cloves and processed garlic. In *Garlic the Science and Therapeutic Application of Allium sativum L. and Related Species*, 2nd ed.; Koch, H. P., Lawson, L. D., Eds.; Williams &and Wilkins: Baltimore, MD, 1996; pp 37−107.

(14) Ankri, S.; Mirelman, D. Antimicrobial properties of allicin from garlic. *Microbes Infect.* **1999**, *1*, 125–129.

(15) Cavallito, C. J.; Bailey, J. H. Allicin, the antibacterial principle of *Allium sativum*. 1. Isolation, physical properties, and antibacterial action. *J. Am. Chem. Soc.* **1944**, *66*, 1950–1951.

(16) Stoll, A.; Seebeck, E. Chemical investigation of alliin, the specific principle of garlic. *Adv. Enzymol.* **1951**, *11*, 377–400.

(17) Bordia, A.; Bansal, H. C. Letter: Essential oil of garlic in prevention of atherosclerosis. *Lancet* **1973**, *2*, 1491–1492.

(18) Egen-Schwind, C.; Eckard, R.; Kemper, F. H. Metabolism of garlic constituents in the isolated perfused rat liver. *Planta Med.* **1992**, *58*, 301–305.

(19) Weisberger, A. S.; Pensky, J. Tumor inhibition by a sulfhydryl-blocking agent related to an active principle of garlic (*Allium sativum*). *Cancer Res.* **1958**, *18*, 1301–1308.

(20) Arditti, F. D.; Rabinkov, A.; Miron, T.; Reisner, Y.; Berrebi, A.; Wilchek, M.; Mirelman, D. Apoptotic killing of B-chronic lymphocytic leukemia tumor cells by allicin generated in situ using a rituximab-alliinase conjugate. *Mol. Cancer Ther.* **2005**, *4*, 325–331.

(21) Miron, T.; Rabinkov, A.; Mirelman, D.; Wilchek, M.; Weiner, L. The mode of action of allicin: its ready permeability through phospholipid membranes may contribute to its biological activity. *Biochim. Biophys. Acta: Biomembranes* **2000**, *1463*, 20–30.

(22) Hunter, R.; Caira, M.; Stellemboom, N. Thiosulfinate allicin from garlic—inspiration for a new antimicrobial agent. *Ann. N. Y. Acad. Sci.* **2005**, *1056*, 234–241.

(23) Lawson, L. D.; Gardner, C. D. Composition, stability, and bioavailability of garlic products used in a clinical trial. *J. Agric. Food Chem.* **2005**, *53*, 6254–6261.

(24) Block, E. The organosulfur chemistry of the genus *Allium*—implications for the organic-chemistry of sulfur. *Angew. Chem. Intl. Ed. Engl.* **1992**, *31*, 1135–1178.

(25) Fenwick, G. R.; Hanley, A. B. XI. Antimicrobial effects. In *The Genus Allium, Part 3. CRC Critical Reviews in Food Science and Nutrition*; CRC Press: London, U.K., 1985; Vol. 23, Issue 1, pp 1−73.

(26) Ariga, T.; Seki, T. Functional foods from garlic and onion. In *Asian Functional Foods*; Shi, J., Ho, C.-T., Shahidi, F., Eds.; CRC Press/Taylor and Francis: Boca Raton, FL, 2005; pp 433−489.

(27) Ankri, S.; Miron, T.; Rabinkov, A.; Wilchek, M.; Mirelman, D. Allicin from garlic strongly inhibits cysteine proteinases and

cytopathic effects of *Entamoeba histolytica*. *Antimicrob. Agents Chemother.* **1997**, *41*, 2286–2288.
(28) Naganawa, R.; Iwata, N.; Ishikawa, K.; Fukuda, H.; Fujino, T.; Suzuki, A. Inhibition of microbial growth by ajoene, a sulfur-containing compound derived from garlic. *Appl. Environ. Microbiol.* **1996**, *62*, 4238–4242.
(29) Avato, P.; Tursil, E.; Vitali, C.; Miccolis, V.; Candido, V. Allylsulfide constituents of garlic volatile oil as antimicrobial agents. *Phytomedicine* **2000**, *7*, 239–243.
(30) Salton, M. R. J. *The Bacterial Cell Wall*; Elsevier: Amsterdam, The Netherlands, 1964.
(31) Focke, M.; Feld, A.; Lichtenthaler, H. K. Allicin, a naturally-occurring antibiotic from garlic, specifically inhibits acetyl-CoA synthetase. *FEBS Lett.* **1990**, *261*, 106–108.

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