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# Antimicrobial Activity of Garlic Heated under Different Conditions, Time of Heating, and pH

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**Abstract** Antimicrobial activity of garlic (pH 6.0) heated at 120°C reached its maximum at 45 min of heating and maintained the level for the rest of heating time (300 min) when tested against *Candida utilis* ATCC42416. The principal antimicrobial compound was allyl alcohol (AA), a highly volatile compound without sulfur in its molecule. The concentration of AA in heated garlic gradually increased to over 2,000 ppm for the first 90 min and stayed at the level without appreciable changes in spite of further heating. Other antimicrobial compounds secondary to AA were lowly volatile sulfur compounds including diallyl polysulfides (diallyl trisulfide, diallyl tetrasulfide, and diallyl pentasulfide) and heterocyclic sulfur compounds (4-methyl-1,2,3-trithiolane, 5-methyl-1,2,3,4-tetrathiane, and 6-methyl-1,2,3,4,5-pentathiepane). When the pH of the garlic extract was lowered before heating, considerably more secondary antimicrobial sulfur compounds were formed and the antimicrobial activity was stronger than the pH unadjusted garlic. Lowly volatile sulfur compounds contributed a significant part of antimicrobial activity of heated garlic only during the early period (45-120 min) of heating regardless of pH treatment.

**Keywords:** garlic, heating, pH, diallyl polysulfide, heterocyclic sulfur compound, antimicrobial activity

## Introduction

Allicin (*S*-allyl-2-propene thiosulfinate), found in garlic, is formed from alliin (*S*-allyl-L-cysteine sulfoxide) by allinase when the fresh tissue of garlic is injured. Allicin is representative compound responsible for the characteristic pungent flavor of garlic as well as the extremely potent antimicrobial activity of garlic. Since an enzyme is involved in this reaction it had been assumed that heated garlic was not antimicrobial (1). It has been only recently that heated garlic was found to be antimicrobial. Garlic heated at cooking temperatures was found to be antimicrobial because alliin in garlic is thermally degraded to simpler compounds without the action of alliinase enzyme (2). It was previously deduced that mild heating at around 100°C only inactivates alliinase enzyme, while severe heating at the cooking temperatures (about 120°C and up) for prolonged periods of time not only inactivates the enzyme but also thermally degrades alliin into compounds with antimicrobial activity (2).

It was recently found that the principal antimicrobial compound in heated garlic at 120°C was allyl alcohol (2-propene-1-ol), formed by thermal degradation of alliin (3). Allyl alcohol is different from all other known antimicrobial compounds found in garlic in that it does not contain a sulfur atom in the molecule. Although antimicrobial potency of allyl alcohol against *Candida utilis* is weaker than those of diallyl trisulfide (DATS) and diallyl tetrasulfide (DATTS), the quantity of allyl alcohol formed in garlic heated at 120°C was much greater than those of DATS and DATTS. Many reports have considered allyl alcohol as a flavor component of garlic but not as an antimicrobial (4-8).

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Allicin formed from alliin by the action of alliinase is known to be spontaneously decomposed to various sulfides including diallyl disulfide (DADS) and DATS which are the main components of garlic oil. Garlic oil is produced by heating the crushed garlic to boiling temperature and collecting the resulting vapor as a distillate (9). During the heating process, allicin in crushed garlic is converted to various types of sulfides (10,11) with DADS being the most abundant. Sulfides with more sulfur atoms, DATTS, and diallyl pentasulfide (DAPS) found in garlic oil are known to possess stronger antimicrobial activity than those with less sulfur atoms (12). Cavallito *et al.* (13) found that allicin decomposes to diallyl sulfides. They also reported that neither aqueous extract lacking allicin nor those with garlic oil were antimicrobial (14). All these sulfides commonly found in garlic were linear molecules (15). It was recently found in this lab that heterocyclic sulfur compounds (4-methyl-1,2,3-trithiolane, 5-methyl-1,2,3,4-tetrathiane, and 6-methyl-1,2,3,4,5-pentathiepane) were formed in garlic extract when heated at cooking temperatures (16). Heterocyclic sulfur compounds as well as linear sulfides were reported to be antimicrobial (16,17).

The objective of this investigation is to report the effect of heating time and pH adjustment on the antimicrobial activity of garlic extract and formation of antimicrobial sulfur compounds.

## Materials and Methods

**Materials** Garlic (*Allium sativum* L.) was purchased from a local market in Seoul, Korea. Allyl alcohol was purchased from Sigma-Aldrich (St. Louis, MO, USA).

**Microbial strain and culture conditions** *Candida utilis* ATCC 42416 was stored at –64°C in basal media containing 16% glycerol. The basal media was YMPG broth (0.3% yeast extract, 0.3% malt extract, 0.5% peptone, and 1%

glucose). The frozen cultures were revived by streaking onto agar growth media plates, and an isolated colony was picked and cultivated at least twice in growth medium prior to 24 hr culture for final inoculation. The yeast for seed culture was grown aerobically by shaking at 150 rpm (KSI-200L Shaker; Korea Environmental Control Co., Ltd., Gyeonggi, Korea). One-hundred μL of a 10× diluted aliquot of seed culture were inoculated into 10 mL of the appropriate broth in 16×150-mm glass culture tubes and statically incubated. The numbers of viable cells were estimated as colony forming units (CFU)/mL by spiral-plating (Spiral Autoplate System, Spiral Biotech Inc., Bethesda, MD, USA) onto plate count agar (Difco Laboratories, Grand Island, NY, USA) and incubating for 48 hr. All growth studies were performed at 30°C.

**Preparing heated garlic extract** Peeled and trimmed garlic cloves were blanched by boiling in water for 10 min to inactivate alliinase. The boiled garlic was cooled with flowing tap water, blended using a Waring blender (Dynamics Co., New Hartford, CT, USA) with an equal amount of sterilized distilled water, and centrifuged (HMR-2001V; Hanil Industrial Co., Incheon, Korea) at 17,600×g for 20 min to remove insolubles. The supernatant was dispensed into screw-capped glass tubes and heated for 15, 30, 45, 60, 75, 90, 120, 180, and 300 min at 120°C. In order to examine the pH effect, garlic extract was adjusted to pH 2 and 4 with 5 N HCl before heating. An original pH of garlic was 6±0.2.

**Estimation of antimicrobial activity of volatile sulfur compounds in heated garlic extract** Volatile sulfur compounds were removed with hexane from heated garlic extract by vortexing the equal volume of hexane with heated garlic extract for 10 min. After removal of volatiles, water layers were tested for the antimicrobial activity. Hexane was chosen over methylene chloride because the former did not extract AA from the heated garlic extract. The minimum inhibitory concentration (MIC) difference between garlic extract before (BHE) and after hexane extraction (AHE) is regarded as the antimicrobial activity contributed by total volatile sulfur compounds, including diallyl polysulfides and heterocyclic sulfur compounds extractable with hexane.

**High performance liquid chromatography (HPLC) analysis of allyl alcohol and volatile sulfur compounds** Allyl alcohol content was measured by HPLC (recycling preparative HPLC, JAI-LC908; Japan Analytical Industry Co., Ltd., Tokyo, Japan), equipped with JAI RI-5 detector and gel filtration chromatography (GFC) column (Jaigel GS-310 column, 50×2 cm, i.d., Japan Analytical Industry Co.). Water was used as the eluting solvent at a flow rate of 3 mL/min.

Volatile sulfur compounds were extracted with methylene chloride from heated garlic and the solvent was evaporated by $N_2$ gas before the residue was dissolved in a mixture of acetonitrile/methylene chloride (80:20, v/v) for HPLC analysis. Individual sulfur compounds were separated by HPLC (Semi-Prep. LC-6AD; Shimadzu Corp., Kyoto, Japan) with an analytical octadecyl saline (ODS)-H column (4.6×250 mm, Shimadzu Corp.). HPLC analysis was run with a linear gradient starting with a mixture of acetonitrile/water (70:30, v/v) for the first 15 min and changing to 100% acetonitrile in 20 min. The total flow rate was 1 mL/min. The elution was monitored at 240 nm (SPD-10A UV-vis detector; Shimadzu Corp.).

**MIC determination** Heated garlic extracts were diluted with heat-sterilized YMPG broth to give the desired final concentrations. Broths containing appropriate levels of heated garlic extract were inoculated with *C. utilis* ATCC42416 to give initial numbers between $10^4$ and $10^5$ CFU/mL, and incubated at 30°C for 48 hr. The sensitivity of the test organism was expressed as the MIC in %. Experiments were performed in duplicate, and the higher value was taken as the MIC; a complete absence of growth based on the viable count after incubation was regarded as non-growth.

## Results and Discussion

**Effect of heating time on the formation of antimicrobial compounds** The formation of allyl alcohol (AA), the most abundant non-sulfur antimicrobial compound in heated garlic reached the maximum early in the heating process and the quantity stayed unchanged as the time of heating was increased up to 5 hr (Fig. 1). The concentration of AA was in the range of 2,100-2,200 ppm in heated garlic extract of which the pH was unadjusted (pH 6.0). The garlic extract of which pH was adjusted to 2.0 showed highest antimicrobial activity at the beginning of heating, up to 120 min, but the activity was progressively weakened as time of heating progressed. The AA concentration of pH 2.0 garlic extract was highest at the very beginning (15 min) of heating process and decreased gradually for further heating. Therefore the weakening of antimicrobial activity, i.e., the increased number of MIC, must be due to the reduction of the AA concentration.

Since the MIC of AA for *C. utilis*, an indicator yeast, was 20 ppm (3,18), the calculated MIC of heated garlic on the basis of AA concentration alone will be approximately 0.9%. However, the actual MIC of heated garlic was 0.6% or lower throughout the whole experimental period in all 3 pH treatments (Fig. 1). Therefore it was deduced that additional compounds were responsible for the rest of antimicrobial activity of heated garlic. It was recently reported that various diallyl polysulfides (DADS, DATS, and DATTS) and heterocyclic sulfur compounds [4-methyl-1,2,3-trithiolane (MTTL), 5-methyl-1,2,3,4-tetrathiane (MTTT), and 6-methyl-1,2,3,4,5-pentathiepane (MPTP)] were formed along with AA as secondary antimicrobial compounds (16). The various volatile sulfur compounds are formed most in the time range between 45 and 120 min of heating, and their concentrations abruptly decreased to very low concentration at 180 min of heating (Fig. 1), and the concentrations further decreased to minimal levels on further heating.

**Effect of pH on the formation of antimicrobial compounds** As the pH of the garlic extract was lowered before heating, the antimicrobial potency was more enhanced (Table 1) compared with pH unadjusted garlic extract (pH 6.0). The MIC of pH 6.0 garlic extract (BHE)

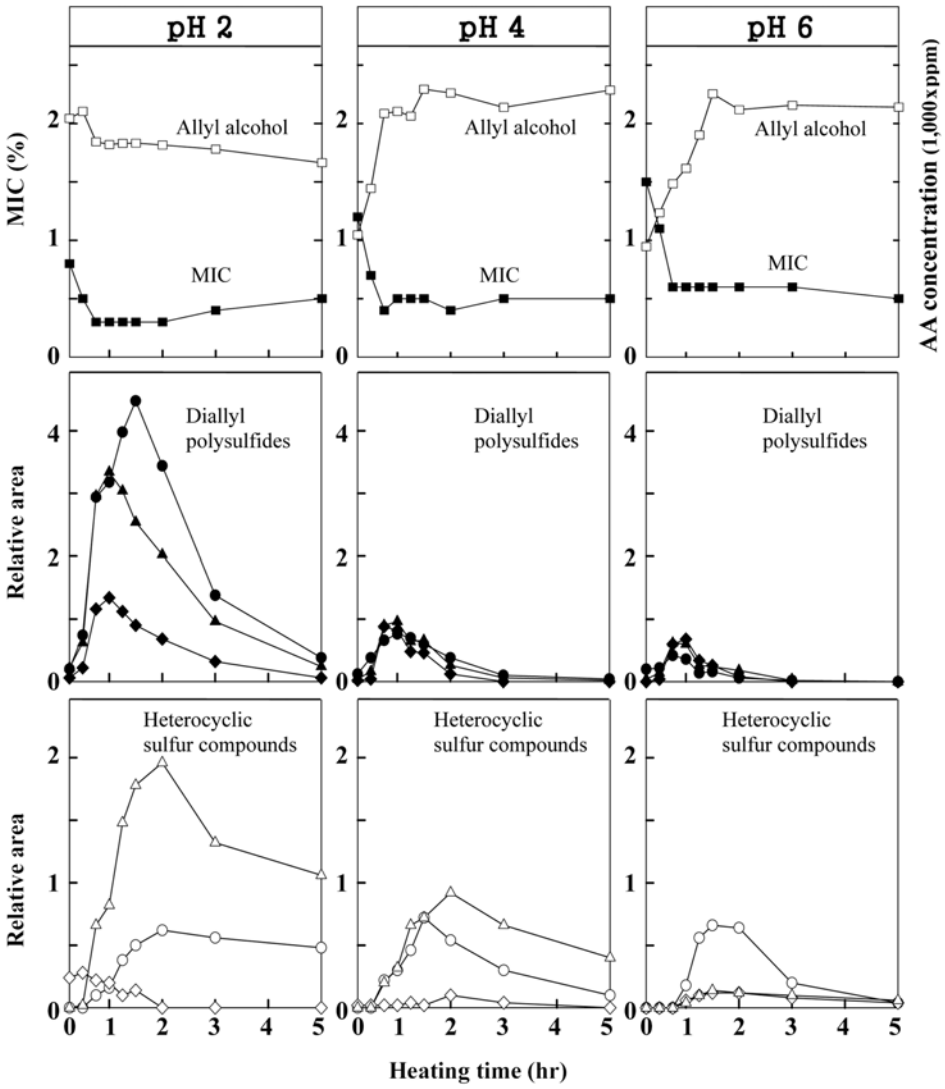


**Fig. 1. Change of allyl alcohol content and minimum inhibitory concentration (MIC) and major volatile sulfur compounds in heated garlic depending on different pH and heating time at 120°C against *C. utilis* ATCC 42416.** (●) DATS; (▲) DATTS; (◆) DAPS; (◇) MW 138; (△) MW 170; (○) Mw 202

was 0.6-0.7% against *C. utilis*, while those of garlic extract (BHE) with pHs adjusted to 2.0 and 4.0 were 0.3-0.4 and 0.4-0.5%, respectively throughout the experimental period (300 min) of heating.

Volatile sulfur compounds including diallyl polysulfides and heterocyclic sulfur compounds were removed by hexane from heated garlic extracts to figure the antimicrobial activity contributed by them. Hexane was the solvent of choice because it removes almost all volatile sulfur compounds, but not AA. The antimicrobial potency of heated garlic extract decreased as sulfur compounds were removed by extraction with hexane. Antimicrobial activity of water layer of garlic extract after hexane extraction (AHE) was not much different depending on the different pH treatment (Table 1). The MIC of garlic extract (AHE) with pH 4.0 and 6.0 were in the range of 0.7-0.9%, while those of garlic extract (AHE) with pH 2.0 were in the range of 0.8-1.0%. The garlic extract adjusted to pH 2.0 showed the greatest difference in potency compared before and after hexane extraction, showing that pH 2.0 garlic extract had most antimicrobial activity reduction by hexane extraction, suggesting that those sulfur compounds exhibiting secondary antimicrobial activity were most produced at the pH. The activity difference was least with the garlic extract without pH adjustment. The difference in MIC between BHE and AHE of the garlic extract with pH 4.0 was on the middle of the 2 other treatments. As the pH of the garlic extract before heating was lower, the portion of antimicrobial activity contributed by volatile sulfur compounds was larger. The differences in MIC are the antimicrobial activity portions contributed by volatile sulfur compounds (Table 1). MIC differences of 0.5-0.6, 0.3-0.5, and 0.1-0.3% of garlic extracts with pH 2.0, 4.0, and 6.0, respectively, are antimicrobial activity portions contributed by volatile sulfur compounds. The MIC of the pH 2.0 extract was lower than those of pH 4.0 and 6.0 even though the AA concentration was lower than the other 2 preparations. This can be explained that the very high concentration of various sulfur compounds more than compensated the lower concentration of AA (1,700-

**Table 1. Minimum inhibitory concentrations (MIC) of heated garlic before and after extracting with hexane tested against *C. utilis* ATCC 42416**

| Heating time (min) at 120°C | MIC[1] (%) | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | pH 2 | | | pH 4 | | | pH 6 | | |
| | BHE | AHE | Difference | BHE | AHE | Difference | BHE | AHE | Difference |
| 45 | 0.4 | 1.0 | 0.6 | 0.4 | 0.9 | 0.5 | 0.7 | 0.9 | 0.2 |
| 60 | 0.3 | 0.9 | 0.6 | 0.5 | 0.9 | 0.4 | 0.6 | 0.8 | 0.2 |
| 75 | 0.3 | 0.9 | 0.6 | 0.5 | 0.8 | 0.3 | 0.7 | 0.9 | 0.2 |
| 90 | 0.3 | 0.8 | 0.5 | 0.5 | 0.9 | 0.4 | 0.7 | 0.9 | 0.2 |
| 120 | 0.3 | 0.9 | 0.6 | 0.4 | 0.7 | 0.3 | 0.6 | 0.7 | 0.1 |
| 180 | 0.4 | 1.0 | 0.6 | 0.5 | 0.8 | 0.3 | 0.6 | 0.9 | 0.3 |

[1]BHE, before hexane extraction; AHE, after hexane extraction.

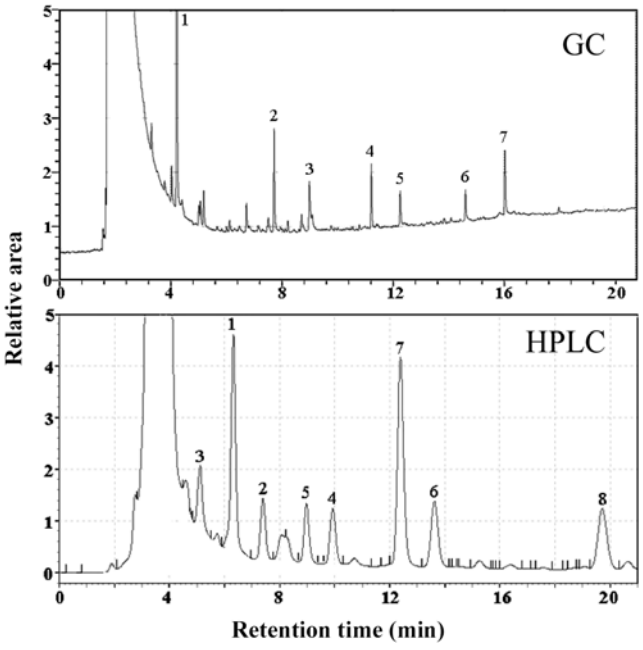


**Fig. 2. Comparison of GC [Chung *et al.* (16)] chromatogram with HPLC chromatogram of volatile sulfur compounds in heated garlic extract heated at 120°C for 90 min.**

1,800 ppm) in pH 2.0 garlic extract during the early period of heating (45 to 120 min).

AA stayed stable during the heating process at all times at the heating pH of 4.0 and 6.0. It showed a minimal instability at pH 2.0, AA content decreasing only very slowly and MIC increasing progressively as time of heating increased. This is a direct evidence that AA is stable at 120°C at a very wide pH (4-6) range for as long as 300 min, but rather slightly unstable at pH 2.0.

The composition of sulfur compounds in heated garlic extract was different from that of previous report (16) where the composition of sulfur compounds was analyzed by gas chromatography (GC). Volatile sulfur compounds were analyzed by HPLC in this report. More abundance of DATTS, MPTP, and MTTT and less abundance of MTTL were apparent by HPLC analysis (Fig. 2). DAPS which was not detected by GC analysis was found to be abundant when analyzed by HPLC. It was probable that DATTS, MPTP, MTTT, and DAPS were destroyed when they pass through high temperature injection port and detector of GC. Therefore it is recommended to use HPLC to analyze sulfur compounds in garlic.

**Formation of antimicrobial compounds** As the lower the pH of the garlic extract before heating, the earlier the formation of AA was (Fig. 1). AA was formed in pH 2.0 garlic extract right from the start of heating. AA formation reached maximum after 45 and 90 min of heating at pH 4.0 and 6.0, respectively. It can be deduced that the lower pH facilitates the thermal degradation of alliin. The maximum level of AA formed in pH 2.0, 4.0, and 6.0 garlic extract was 1,800, 2,200, and 2,200 ppm, respectively.

The formation of diallyl polysulfides reached maximum level after 45 and 60 min of heating under all pH treatments, except DATS in pH 2.0 garlic extract which reached

**Table 2. Volatile sulfur compounds found in heated garlic extract**

| Peak No. | Compound | Molecular weight | Identification | MIC[1] in ppm |
|---|---|---|---|---|
| 1 | Diallyl monosulfide | 114 | GC/MS | 1,000(12) |
| 2 | Diallyl disulfide | 146 | GC/MS | 110(12) |
| 3 | 4-Methyl-1,2,3-trithiolane | 138 | GC/MS, $^1$H-NMR | - |
| 4 | Diallyl trisulfide | 178 | GC/MS | 7(12) |
| 5 | 5-Methyl-1,2,3,4-tetrathiane | 170 | GC/MS, $^1$H-NMR | 1(11) |
| 6 | Diallyl tetrasulfide | 210 | GC/MS | 4(12) |
| 7 | 6-Methyl-1,2,3,4,5-pentathiepane | 202 | GC/MS, $^1$H-NMR | 2(11) |
| 8 | Diallyl pentasulfide | 242 | HPLC | - |

[1]Minimum inhibitory concentrations after 48 hr of incubation against *Candida utilis* ATCC42416 for which the MIC of AA was 20 ppm; -, data not available.

maximum level at 90 min heating. The total quantity of diallyl polysulfides produced in pH 2.0 and 4.0 garlic extract was about 6 and 1.5 times more than that produced in pH 6.0 garlic extract, respectively (Fig. 1). Diallyl polysulfides are known to be potent antimicrobial compounds (Table 2).

The maximum levels of heterocyclic sulfur compounds were reached at approximately 90-120 min of heating, when the level of diallyl polysulfides decreased. It is deduced that heterocyclic sulfur compounds are formed as the result of transformation of diallyl polysulfides. Actually the difference between diallyl polysulfides and heterocyclic sulfur compounds is only whether the compounds are linear or cyclized (16).

The production of maximum volatile sulfur compounds are achieved first 90 to 120 min of heating the garlic extracts. pH significantly affected the formation of the volatile sulfur compounds, pH 2.0 being the best condition for the maximum production of the compounds.

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## References

1. Lawson LD. The composition and chemistry of garlic cloves and processed garlic. pp. 37-107. In: Garlic, the Science and Therapeutic Applications of *Allium sativum* L. and Related Species. 2nd ed. Koch HP, Lawson LD (eds). Williams & Wilkins, Baltimore, MD, USA (1996)
2. Kim JW, Kyung KH. Antiyeast activity of heated garlic in the absence of alliinase enzyme action. J. Food Sci. 68: 1766-1770 (2003)
3. Choi JH, Kyung KH. Allyl alcohol is the sole antiyeast compound in heated garlic extract. J. Food Sci. 70: M305-M309 (2005)
4. Brodnitz MH, Pascale JV, Derslice LV. Flavor components of garlic extract. J. Agr. Food Chem. 19: 273-275 (1971)
5. Block E, Ahmed S, Jain MK, Creely RW, Aptiz-Castro R, Cruz MR. (E,Z)-Ajoene: A potent antithrombotic agent from garlic. J. Am. Chem. Soc. 106: 8295-8296 (1984)
6. Yu T-H, Wu C-M, Liou Y-C. Volatile compounds from garlic. J. Agr. Food Chem. 37: 725-730 (1989)
7. Yu T-H, Wu C-M, Ho C-T. Volatile compounds of deep-oil fried, microwave heated, and oven-baked garlic slice. J. Agr. Food Chem. 41: 800-805 (1993)
8. Yu T-H, Wu C-M, Rosen RT, Hartman TG, Ho C-T. Volatile compounds generated from thermal degradation of alliin and deoxyalliin in an aqueous solution. J. Agr. Food Chem. 42: 146-153 (1994)
9. Pentz R, Siegers CP. Garlic preparations: Methods for quantitative and qualitative assessment of their ingredient. pp. 109-134. In: Garlic, the Science and Therapeutic Application of *Allium sativum* L. and Related Species. 2nd ed. Koch HP, Lawson LD (eds). Williams & Wilkins, Baltimore, MD, USA (1996)
10. O'Gara EA, Hill DJ, Maslin DJ. Activities of garlic oil, garlic powder, and their diallyl constituents against *Helicobacter pylori*. Appl. Environ. Microb. 66: 2269-2273 (2000)
11. Lawson LD, Wang ZJ, Hughes BG. Identification and HPLC analysis of sulfides and dialk(en)yl thiosulfinates in commercial garlic products. Planta Med. 57: 363-370 (1991)
12. Ross ZM, O'Gara EA, Hill DJ, Sleighttholme HV, Maslin DJ. Antimicrobial properties of garlic oil against human enteric bacteria: Evaluation of methodologies and comparisons with garlic oil sulfides and garlic powder. Appl. Environ. Microb. 67: 475-480 (2001)
13. Cavallito CJ, Buck JS, Suter CM. Allicin, the antibacterial principles of *Allium sativum*. II: Determination of the chemical structure. J. Am. Chem. Soc. 66: 1952-1954 (1944)
14. Cavallito CJ, Bailey JH. Allicin, the antibacterial principle of *Allium sativum*. I: Isolation, physical properties, and antimicrobial action. J. Am. Chem. Soc. 66: 1950-1951 (1944)
15. Choi MK, Chae KY, Lee JY, Kyung KH. Antimicrobial activity of chemical substances derived from *S*-alk(en)yl-L-cysteine sulfoxide (alliin) in garlic, *Allium sativum* L. Food Sci Biotechnol. 16: 1-7 (2007)
16. Chung I, Chae KY, Kyung KH. Thermal generation and antimicrobial activity of unusual heterocyclic sulfur compounds in garlic. Food Sci. Biotechnol. 17: 1032-1037 (2008)
17. Kim JW, Huh JE, Kyung SH, Kyung KH. Antimicrobial activity of alk(en)yl sulfides found in essential oils of garlic and onion. Food Sci. Biotechnol. 13: 235-239 (2004)
18. Lee S, Woo YH, Kyung KH. Allyl alcohol found in heated garlic is a potent selective inhibitor of yeasts. J. Microbiol. Biotechn. 16: 1236-1239 (2006)