Food Sci. Biotechnol. Vol. 17, No. 5, pp. 1032 ~ 1037 (2008)

Food Science and Biotechnology



# Thermal Generation and Antimicrobial Activity of Unusual Heterocyclic Sulfur Compounds in Garlic

**Inshick Chung, Kyung Yun Chae[1], and Kyu Hang Kyung***

*Department of Food Science, Sejong University, Seoul 143-747, Korea*

*[1]Tourism and Food Service Business Department, Osan College, Osan, Gyeonggi 447-749, Korea*

**Abstract** Lowly volatile heterocyclic sulfur compounds generated in autoclaved garlic extract were isolated and identified, and their antimicrobial activity was determined. Two kinds of unusual volatile sulfur compounds were separated from heated garlic by preparative recycling high performance liquid chromatography (HPLC), and identified by gas chromatography (GC)-mass spectrometry (MS) and $^1$H-nuclear magnetic resonance (NMR). They had heterocyclic structures with 4 to 5 sulfur atoms in the molecules. 4-Methyl-1,2,3-trithiolane (MTTT) is highly volatile and was not able to be concentrated, and was identified by GC-MS only. MTTT and 6-methyl-1,2,3,4,5-pentathiepane (MPTP) are lowly volatile and were obtained in pure states to be positively identified for the first time. All 3 heterocyclic sulfur compounds began to appear by the time when the early-formed diallyl sulfides started to disappear. The minimum inhibitory concentration range of MTTT and MPTP was determined to be between 1 and 6 ppm against all yeasts tested. MTTT and MPTP were lowly volatile and sparingly soluble in water.

**Keywords:** garlic, heterocyclic, volatile 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 an 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 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 (minimum inhibitory concentration, MIC, 20 ppm) against *Candida utilis* is somewhat weaker than those of diallyl trisulfide (DATS; MIC 7 ppm) and diallyl tetrasulfide (DATTS; MIC 4 ppm), the quantity of allyl alcohol (1,500 ppm) formed in garlic heated at 120°C for 45 min was much greater than that (29 ppm) of DATS (unpublished data). The difference in quantity of production becomes even greater as time of heating increases. Many reports have considered allyl alcohol as a flavor component of garlic but not as an antimicrobial (4-8).

Allicin formed from alliin by the action of alliinase is known to be spontaneously decomposed to various sulfides including diallyl disulfide 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 diallyl disulfide being the most abundant. Sulfides with more sulfur atoms, DATTS and diallyl pentasulfide 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 are linear molecules (15).

The objectives of this investigation was to report the new finding of antimicrobial heterocyclic compounds containing sulfur atoms in their molecular structures in comparison to the so far known linear sulfur-containing molecules.

## Materials and Methods

**Materials** Garlic (*Allium sativum* L.) was purchased from a local market in Seoul, Korea. Garlic oil (GO) (Grupo Tecnaal Co., Zapopan, Mexico) was obtained from Hyangwon Spice Co. (Seongnam, Korea) as a gift. Diallyl monosulfide (DAMS), diallyl disulfide (DADS), and diallyl trisulfide (DATS) were purchased from LKT Laboratories Inc. (St. Paul, MN, USA).

**Microbial strains and culture conditions** *Staphylococcus aureus* B33, *Escherichia coli* B34, *Enterobacter aerogenes*

*Corresponding author: Tel: +82-2-3408-3225; Fax +82-2-3408-4319
E-mail: kyungkh@sejong.ac.kr
Received February 18, 2008; Revised March 31, 2008;
Accepted April 3, 2008

B146, *Leuconostoc mesenteroides* LA10, *Pediococcus pentosaceus* LA3, *Lactobacillus plantarum* LA97, *Pichia membranefaciens* Y20, *Saccharomyces cerevisiae* ATCC 4126, and *Candida utilis* ATCC 42416 were gifts from Dr. Henry P. Fleming (Food Fermentation Laboratories, USDA/ARS, North Carolina State University, Raleigh, NC, USA). *Candida albicans* KCTC 7121 and 7965 were purchased from the Korean Collection for Type Culture (KCTC; Daejeon, Korea). *C. albicans* HY1 was a clinical strain isolated from a child with oral candidiasis. *Zygosaccharomyces bisporus* KCCM 50168, *Zygosaccharomyces rouxii* (soya) KCCM 11300, *Z. rouxii* (*japonicus*) KCCM 11303, *Z. rouxii* (sake) KCCM 50523, and *Z. rouxii (gracilis*) KCCM 50546 were purchased from the Korea Culture Collection of Microorganisms (KCCM; Seoul, Korea).

Bacterial and yeast cultures were stored at −64°C in basal media containing 16% glycerol. Basal media was MRS broth (Difco Laboratories, Detroit, MI, USA) for lactic acid bacteria (LAB), tryptic soy broth (TSB; Difco) for non-LAB, and YMPG broth (0.3% yeast extract, 0.3% malt extract, 0.5% peptone, and 1% glucose) for yeasts. Sodium chloride (10%) was added to YMPG broth to grow *Zygosaccharomyces* sp. For resuscitation, frozen cultures were streaked onto agar medium of the same composition used for growth and an isolated colony was picked and cultivated at least 2 times in growth medium before using a 24 hr culture for final inoculation for bacteria and non-xerotolerant yeasts and a 48 hr culture for xerotolerant yeasts. Yeasts were grown aerobically by shaking at 150 rpm (KSI-200L Shaker; Korea Environmental Control Co., Ltd., Gyeonggi, Korea). Ten μL of a 10× diluted aliquot of bacterial seed culture were inoculated into 10 mL of the appropriate broth in 16×150-mm glass culture tubes and statically incubated. One-hundred μL of a 10× diluted aliquot of yeast seed culture were inoculated into 20 mL of YMPG broth with or without NaCl in Erlenmeyer flasks. 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) and incubating for 24 to 48 hr. All growth studies were performed at 30°C.

**Preparing heated garlic extract** Heated garlic extract was prepared as previously described (15). 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 with an equal weight of sterilized distilled water, and centrifuged at 17,600×g for 20 min (HMR-2001V; Hanil Industrial Co., Incheon, Korea) to remove insolubles. The supernatant was dispensed into screw-capped glass tubes and autoclaved (120°C) for up to 120 min at 15-min intervals.

**Isolation and identification of sulfur compounds from heated garlic extract** Volatile compounds were extracted with hexane from heated garlic extract by vortexing for 10 min. Hexane was removed by vacuum rotary evaporator and the residue was dissolved in acetonitrile. Fractions of volatile compounds of heated garlic were obtained by using preparative high performance liquid chromatography (HPLC, JAI-LC-908; Japan Analytical Industry Co., Ltd., Tokyo, Japan) with a JAI RI-5 RI detector. A commercially available gel permeation column (Jaigel W252 column, 50×2 cm, i.d., Japan Analytical Industry Co., Ltd.) was employed, and a mixture of acetonitrile 70% and water 30% was used as the eluting solvent at a flow rate of 3 mL/min. The injection volume was 10 mL of heated garlic extract. Two major fractions obtained were tested for antiyeast activity after vacuum concentration.

The structures of the heterocyclic compounds were analyzed by gas chromatography-mass spectrometer (GC-MS) and $^{1}$H-nuclear magnetic resonance ($^{1}$H-NMR) spectrometer. The mass spectrum of the 2 isolated active compound was obtained by combination GC/MS (6890N GC; Agilent Technologies, Palo Alto, CA, USA). A 2 μL portion of the fractions obtained by preparative HPLC was injected for GC/MS analysis. The GC column (30 m×0.25 mm capillary; J & W Scientific Inc., Folsom, CA, USA) was coated with DB-5 (0.32 mm thickness). The oven temperature was programmed for 40 to 200°C at 30°C/min, with the initial temperature and final temperature held for 6 and 0 min, respectively. The carrier gas was He (1.5 mL/min) and the split ratio was 20:1. The injector port and detector temperatures were 150 and 185°C. Electron impact ionization (70 eV potential) was used and the mass range scanned was 40-200 Da.

The 2 compounds with molecular weight 170 and 202 were individually dissolved in chloroform ($CDCl_3$) before $^{1}$H-NMR analysis (Ultrashield, Avance 500; Bruker Co., Rheinstteten, Germany).

**Minimum inhibitory concentration (MIC) determination** The MICs of heterocyclic compounds were determined alongside with garlic oil (GO), DAMS, DADS, and DATS for the purpose of comparison. GO, DATS, and the 2 heterocyclic sulfur compounds were diluted with heat-sterilized culture broth to the desired final concentrations. Medium at the desired test concentration was inoculated with yeasts to yield initial numbers between $10^4$ and $10^5$ cells/mL and incubated at 30°C for 24 hr for bacteria and for 48 hr for yeasts. The sensitivity of the test organisms was expressed as the MIC (in ppm) of each compound (16). Experiments were performed in duplicate, and the highest value obtained was recorded as the MIC; a complete absence of growth based on the viable count (Spiral Autoplate system) after the incubation period was regarded as non-growth.

**Effect of cysteine on antiyeast activity of heterocyclic sulfur compounds** Individual heterocyclic sulfur compounds were dissolved in YMPG broth with 0.05%(w/v) Tween 80 as a surfactant to make a 500 ppm(w/v) stock solution and filter-sterilized. Stock solutions were diluted with YMPG broth containing cysteine (0 to 2 mM) to give the desired final concentrations. After 3 hr at room temperature, YMPG broth containing cysteine with heterocyclic sulfur compounds at the desired test concentration were inoculated with *C. utilis* ATCC42416 to give initial numbers 2.2-2.4×$10^4$ CFU/mL and incubated at 30°C for 48 hr. The sensitivity of the test organism was expressed as the MIC.

## Results and Discussion

**Volatile sulfur compounds in heated garlic extract** Common linear volatile sulfur compounds, DATS and DATTS, had the highest peak areas when garlic was heated for 45 min (Fig. 1) and the relative peak areas of the compounds were decreasing as time of heating further increased. The other common linear sulfide, DADS, had the highest peak area when garlic extract was heated for 75 to 90 min. Another sulfide, DAMS, which is commonly found in heated garlic is not included in the result even though it is formed about 4-5 times more than other sulfides (Fig. 2, Table 1), because DAMS has a very insignificant antimicrobial activity (18). DAMS reached maximum peak area when garlic extract was heated for 90 min (data not shown). DADS, DATS, and DATTS are the major sulfide components of commercial GO (1,10,11) and the individual sulfides are known to be strongly antimicrobial (10,12,18). Once the peak area was reached, the peak area of all 3 linear sulfides began to decrease as heating time further increased.

As the common linear sulfides began to disappear, other unusual sulfur compounds began to appear and reached maximum peak area at about 90 to 120 min of heating. When garlic extract was heated for 150 min or longer, the relative peak areas of all the sulfur compounds decreased to very low levels and the heated garlic extract were highly browned and much precipitation was noticed. It, however, was not sure whether those sulfur compounds turned into insoluble materials or non-volatile polymerized compounds. It was previously reported that allyl alcohol, the principal antimicrobial compound of heated garlic, is stable during heating and stayed at the same level during the heating under the condition for up to 2 hr (3). Therefore antimicrobial activity of the heated garlic extract for up to 2 hr did not change noticeably.

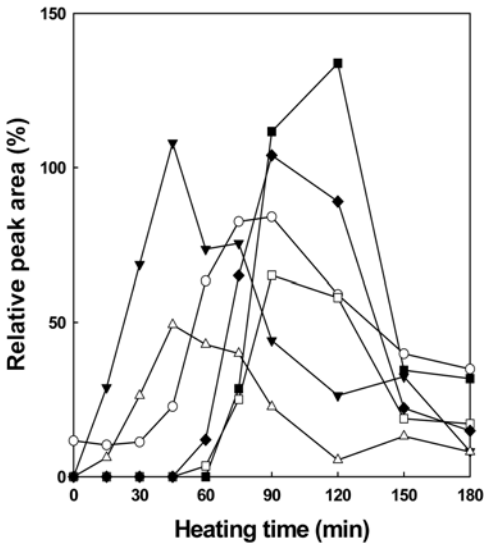



**Fig. 1. Volatile sulfur compounds in heated garlic (pH 6) depending on heating time at 120°C.** (○) DADS, (▼) DATS, (△) DATTS, (■) MTTL, (□) MTTT, (◆) MPTP.

**Table 1. Volatile compounds found in heated garlic extract**

| No. | Retention time | Compounds | Molecular weight |
|---|---|---|---|
| 1 | 4.15 | Diallyl monosulfide | 114 |
| 2 | 7.65 | Diallyl disulfide | 146 |
| 3 | 8.85 | 4-Methyl-1,2,3-trithiolane | 138 |
| 4 | 11.10 | Diallyl trisulfide | 178 |
| 5 | 12.13 | Unknown | 170 |
| 6 | 14.50 | Diallyl tetrasulfide | 210 |
| 7 | 15.91 | Unknown | 202 |

A GC chromatogram of heated garlic volatiles is shown in Fig. 2 and the volatile compounds separated are listed in Table 1. Many of the sulfur compounds identified (Table 1) including diallyl monosulfide, diallyl disulfide, diallyl trisulfide, and diallyl tetrasulfide are degradation products (7,8) of allicin (allyl-2-propene thiosulfinate) and are expected to have varying degrees of antimicrobial activity. Diallyl sulfides mentioned above are major components of commercial garlic oil (1,10,11) except diallyl monosulfide which is highly volatile and insignificantly antimicrobial. The principal antimicrobial compound in garlic heated at 120°C for 30 min or longer was found to be allyl alcohol (2-propen-1-ol) thermally generated from alliin, is not seen because of its high volatility. Even though allyl alcohol is generated in high quantities in heated garlic, it has not been detected in GC chromatograms until recently (3) because it is removed during the concentration process of the extract or because it is eluted with solvents.

Diallyl sulfides, especially diallyl trisulfide which is potently antimicrobial and present in high quantities in garlic oil, are postulated to be the major antimicrobial compounds in heated garlic in early studies of heated garlic (19), it was corrected by ensuing work (3) that allyl alcohol is the single most important antimicrobial compound of heated garlic and that sulfides were only secondary to allyl alcohol. When volatile sulfur compounds were extracted from heated garlic by hexane, the heated garlic retained most of its antimicrobial activity. The concentration of allyl alcohol in heated garlic was not affected by hexane extraction.

**Isolation and identification of heterocyclic sulfur compounds from heated garlic extract** Three unusual sulfur compounds, found to have molecular weight (Mw)

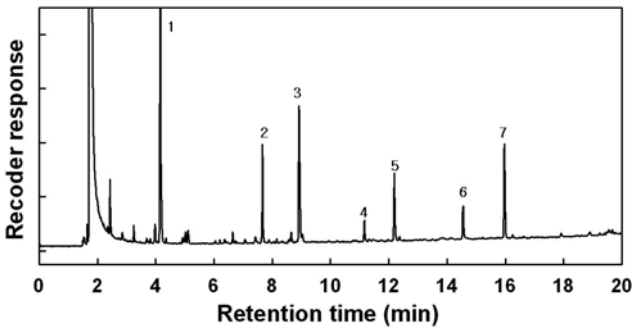
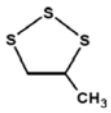
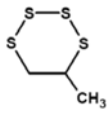
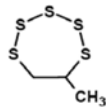



**Fig. 2. A GC chromatogram of volatile compounds of garlic extract heated at 120°C for 90 min.** 1, DAMS; 2, DADS; 3, MTTL; 4, DATS; 5, MTTT; 6, DATTS; 7, MPTP.

**Table 2. Identification of heterocyclic sulfur compounds from heated garlic volatiles**

| Compound | | Spectrum | Structure |
|---|---|---|---|
| 4-Methyl-1,2,3-trithiolane[1)] | MS | 138 (100, $M^+$), 96 (12), 74 (43), 73 (42), 64 (15), 59 (14), 45 (16), 42 (4), 41 (19), 32 (3) |  |
| 5-Methy-1,2,3,4-tetrathiane[2,3)] | MS | 170 (100, $M^+$), 138 (3), 128 (38), 106 (33), 96 (2), 74 (5), 73 (6), 64 (24), 59 (9), 45 (9), 4 2(3), 41 (21), 32 (1) |  |
| | NMR | 1.22 (d, 3H, J=6.5 HZ), 2.83 (d, 1H, J=14.5 HZ), 3.29-3.39 (m, 1H), 3.38-3.5 (m, 1H) | |
| 6-Methyl-1,2,3,4,5-pentathiepane[3,4)] | MS | 202 (20, $M^+$), 170 (9), 160 (6), 138 (100), 128 (5), 106 (9), 96 (12), 74 (43), 73 (46), 64 (49), 59 (12), 45 (17), 42 (4), 41 (31), 32 (7) |  |
| | NMR | 1.39 (d, 3H, J=7.0 HZ), 3.65 (dd, 1H, J=15.0, 6.5 HZ), 3.83 (dd, 1H, J=15.0, 4.0 HZ), 3.90-3.94 (m, 1H) | |

[1)]Positively identified by Block *et al.* (1988) by GC-MS and $^1$H-NMR.
[2)]Temporarily identified by Block *et al.* (1988) and Yu *et al.* (1994).
[3)]Structures newly positively confirmed by GC-MS and $^1$H-NMR in this work.
[4)]Temporarily identified by Kubec *et al.* (1997) by GC-MS.

of 138, 170, and 202 from GC-MS analysis, were isolated from heated garlic as well as the common volatile sulfides, DAMS, DADS, DATS, and DATTS. The latter 3 sulfides are the major constituents of GO (1,10,11). Molecular weight 170 and 202 showed the highest peak area when garlic extract was heated for 90 min, and Mw 138 was highest when garlic extract was heated for 120 min. The 3 unusual compounds began to show up after 60 and 75 min of heating, by the time DATS and DATTS passed their maximum peak area and began to decrease in their peak area (Fig. 1). It was postulated that the unusual sulfur compounds were generated from the common sulfides by further heating.

Molecular weight of 138, 170, and 202 compounds were separated from heated garlic and were identified as heterocyclic sulfur compounds which are 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), respectively, GC-MS and $^1$H-NMR (Table 2). It was not possible to obtain Mw 138 to certain amount because of its volatility. Molecular weight 170 and 202 were obtained with high purity. It was found that the 2 compounds were relatively unstable at room temperature and interconverted to each other in aqueous solution.

All 3 of the heterocyclic compounds have been previously reported to be the flavor components of heated garlic (8, 20,21). MTTL (Mw 138) was positively identified by GC-MS and $^1$H-NMR by (20). The other 2 were temporarily identified only by GC-MS. MTTT and MPTP were obtained in purity and analyzed for GC-MS and $^1$H-NMR in this work. It was positively confirmed by GC-MS and $^1$H-NMR that MTTT and MPTP have a methyl group attached (Table 2), excluding the possibility of linear structures, like diallyl tetrasulfide and diallyl pentasulfide, respectively. Heterocyclic sulfur compounds are not just flavor components of heated garlic, but the major volatile sulfur components.

**Antimicrobial activity of heterocyclic sulfur compounds**

The antimicrobial activity of the 2 heterocyclic sulfur compounds (MTTT and MPTP) were compared with other antimicrobial materials originated from garlic, such as garlic oil (GO), diallyl sulfides (DAMS, DADS, DATS, and DATTS). The antimicrobial potency of the 2 heterocyclic sulfur compounds was similar to DATS, showing MIC range of 1-6 ppm. MIC of MTTT against *S. aureus* B33 was 150 ppm (Table 3).

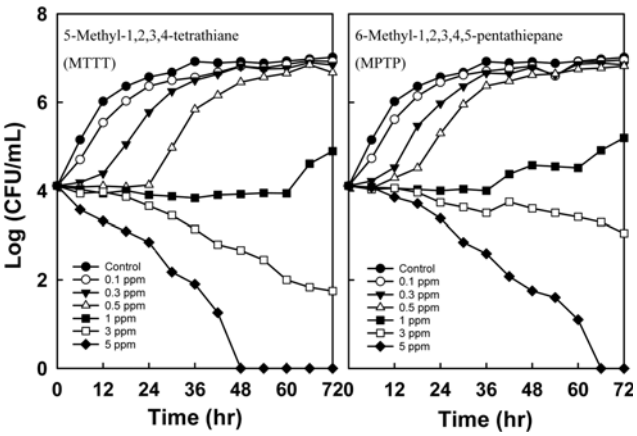



**Fig. 3. Pattern of growth inhibition of MTTT and MPTP against *Candida utilis* ATCC42416.**

The heterocyclic sulfur compounds showed static effect (inhibit growth) in lower concentrations compared to MIC, but it showed cidal effect (cell death) in higher concentrations (Fig 3). The pattern of growth inhibition was almost identical to those of heated garlic extract and heated alliin (2). From the Fig. 3, it can be judged that MTTT is somewhat stronger in antiyeast activity than MPTP since lag periods were longer at all levels of MTTT.

From Table 3 it is apparent again that the heterocyclic sulfur compounds are extremely potent antiyeast compounds. MTTT and MPTP were more inhibitory to yeasts compared with DADS and DATS. However, the inhibitory activity toward bacteria was not as potent. The growth inhibitory activities of MTTT and MPTP are almost identical, and the

**Table 3. Minimum inhibitory concentrations (MICs) of garlic oil and sulfur compounds found in garlic against various bacteria[1)] and yeasts[2)]**

| Microorganism | MIC (ppm) | | | | | |
|---|---|---|---|---|---|---|
| | GO[3)] | DAMS[3)] | DADS[3)] | DATS[3)] | MTTT | MPTP |
| *Staphylococcus aureus* B33 | 100 | >1,000 | 1,000 | 100 | 150 | - |
| *Escherichia coli* B34 | >1,000 | >1,000 | >1,000 | >1,000 | >200 | - |
| *Enterobacter aerogenes* B146 | >1,000 | >1,000 | >1,000 | >1,000 | >200 | - |
| *Leuconnostoc mesenteroides* LA10 | >1,000 | >1,000 | >1,000 | 500 | >200 | - |
| *Pediococcus pentosaceus* LA3 | >1,000 | >1,000 | >1,000 | 500 | >200 | - |
| *Lactobacillus plantarum* LA97 | >1,000 | >1,000 | >1,000 | 500 | >200 | - |
| *Candida albicans* (Clinical) | 20 | >1,000 | 100 | 7 | 3 | 3 |
| *Candida albicans* KCTC7121 | 25 | 1,000 | 100 | 9 | 4 | 5 |
| *Candida albicans* KCTC7965 | 30 | 1,000 | 120 | 10 | 4 | 5 |
| *Candida utilis* ATCC42416 | 25 | 1,000 | 110 | 4 | 1 | 2 |
| *Saccharomyces cerevisiae* ATCC4126 | 10 | >1,000 | 100 | 6 | 3 | 4 |
| *Pichia membranefaciens* Y20 | 10 | 1,000 | 80 | 3 | 1 | 2 |
| *Zygosaccharomyces rouxii* KCCM50523 | 40 | 1,000 | 140 | 12 | 3 | 6 |

[1)]MIC after 24 hr of incubation.
[2)]MIC after 48 hr of incubation.
[3)]Tested by Kim (16), -: not tested.

**Table 4. Change of minimum inhibitory concentration (MIC) of 5-methyl-1,2,3,4-tetrathiane (MTTT) and 6-methyl-1,2,3,4,5-pentathiepane (MPTP) depending on cysteine concentration against *Candida utilis* ATCC42416**

| Cysteine (mM) | MIC (ppm) | |
|---|---|---|
| | MTTT | MPTP |
| 0 | 1 | 1.5 |
| 0.5 | 2 | 3 |
| 1 | 3 | 5 |
| 3 | 5 | 7 |
| 5 | 10 | 12 |

2 compounds are the most potent among sulfur compounds derived from garlic.

The growth inhibitory activity of heterocyclic sulfur compounds were reversed by cysteine (Table 4), suggesting that antimicrobial action of heterocyclic sulfur compounds is caused by reacting with sulfhydryl group of essential proteins of microorganisms. Biological activity of heterocyclic sulfur compounds has not reported previously. The antimicrobial activity of thiosulfinates (13,22,23) cabbage (24), and garlic (13) is inactivated by cysteine. Therefore, MTTT and MPTP work in the same manner as thiosulfinates, represented by allicin in garlic. It was not shown that MTTL (Mw 138) has antimicrobial activity or not, because quantity of it has not been obtained.

Theories as to how dimethyl disulfide (DMDS) inhibits the growth of microorganisms have been proposed. Stevens *et al.* (25) suggested that DMDS inactivates active papain by forming an inactive complex. Smith (26), in reporting kale poisoning of ruminant animals feeding on cruciferous vegetables, explained that DMDS may harm red blood cells by oxidizing reduced glutathione to oxidized one. The basic principles of the two theories are not very different, and are similar to the way in which thiosulfinates, represented by allicin in garlic, inhibit microorganisms by reacting with sulfhydryl groups of essential cellular proteins (22). Small *et al.* (22) postulated that *-S-S(O)-* was essential for the molecules to be inhibitory for microbial growth. Diallyl sulfides and heterocyclic sulfur compounds which do not have the functional group were later found to exhibit antimicrobial activity.

## Acknowledgments

This work was supported by the Brain Korea 21 project. Assistance in isolating the heterocyclic sulfur compounds by Mr. YH Woo is greatly appreciated.

## 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. Le Dinh H, Kyung KH. Inhibition of yeast film formation in fermented vegetables by materials derived from garlic using cucumber pickle fermentation as a model system. Food Sci. Biotechnol. 15: 469-473 (2006)
17. Kyung KH, Kim MH, Park MS, Kim YS. Alliinase-independent inhibition of *Staphylococcus aureus* B33 by heated garlic. J. Food Sci. 67: 780-785 (2002)
18. Kim JW, Kim YS, Kyung KH. Inhibitory activity of essential oils of garlic and onion against bacteria and yeasts. J. Food Protect. 67: 499-504 (2004)
19. Kyung KH, Kim MH, Park MS, Kim YS. Alliinase-independent inhibition of *Staphylococus aureus* B33 by heated garlic. J. Food Sci. 67: 780-785 (2002)
20. Block E, Iyer R, Grisoni S, Saha C, Belman S, Lossing FP. Lipoxygenase inhibitor from the essential oil of garlic: Markovnikov addition of the allyldithioradical to olefins. J. Am. Chem. Soc. 110: 7813-7827 (1988)
21. Kubec R, Velisec J, Kubelka V. Sulfur-containing volatiles arising by thermal degradation of alliin and deoxyalliin. J. Agr. Food Chem. 45: 3580-3585 (1997)
22. Small LD, Bailey JH, Cavallito CJ. Alkyl thiosulfinates. J. Am. Chem. Soc. 69: 1710-1713 (1947)
23. Small LD, Bailey JH, Cavallito CJ. Comparison of some properties of thiosulfonates and thiosulfinates. J. Am. Chem. Soc. 71: 3565-3566 (1949)
24. Kyung KH, Fleming HP. *S*-Methyl-L-cysteine sulfoxide as the precursor of methyl methanethiosulfinate, the principal antibacterial compound in cabbage. J. Food Sci. 59: 350-355 (1994)
25. Steven FS, Griffin MM, Smith RH. Disulfide exchange reactions in the control of enzymic activity. Eur. J. Biochem. 119: 75-78 (1981)
26. Smith RH. Kale poisoning: The *Brassica anaemia* factor. Vet. Rec. 107: 12-15 (1980)