

RESEARCH REVIEW

Antimicrobial Activity of Chemical Substances Derived from *S*-Alk(en)yl-L-Cysteine Sulfoxide (Alliin) in Garlic, *Allium sativum* L.

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Abstract Garlic (*Allium sativum* L.) contains a specific sulfur compound, the *S*-allyl derivative of L-cysteine sulfoxide, and has long been known for its antimicrobial activity against various microorganisms, including bacteria, fungi, and protozoa. The principal antimicrobial compound of garlic is *S*-allyl-L-propenethiosulfinate (allicin) which is generated by an enzyme, alliinase (L-cysteine sulfoxide lyase), from *S*-allyl-L-cysteine sulfoxide (alliin). This compound exists exclusively in *Allium* as a major non-protein sulfur-containing amino acid. *S*-Allyl-L-propenethiosulfinate belongs to the chemical group of thiosulfonates and is a highly potent antimicrobial. The potency of garlic extract is reduced during storage since thiosulfonates are unstable and are degraded to other compounds some of which do not have antimicrobial activity. Diallyl polysulfides and ajoene are sulfur compounds derived from allicin that do possess antimicrobial activity. It was recently found that garlic becomes antimicrobial on heating at cooking temperatures, and that the compound responsible for this is allyl alcohol, which is generated from alliin by thermal degradation.

Keywords: garlic, antimicrobial activity, sulfur compound, allyl alcohol, *S*-allyl-L-cysteine sulfoxide, alliin, diallyl polysulfide

Introduction

The antimicrobial activities of plant extracts, especially from *Allium* and *Brassica*, have been recognized for many years, since Walton *et al.* (1) and Sherman and Hodge (2) scientifically demonstrated the antimicrobial activity of garlic (*A. sativum*) and cabbage (*B. oleraceae*). The major antimicrobial activity of *Allium* (3, 4) and *Brassica* (5) vegetables is due to volatile sulfur compounds derived from *S*-alk(en)yl-L-cysteine sulfoxide existing in the vegetables as non-protein amino acids. *Brassica* contains glucosinolates as the precursors of another type of antimicrobial compounds (6-8) which differ from that of *Allium*. The presence of *S*-alk(en)yl-L-cysteine sulfoxide is essentially confined to two families; the *Cruciferae* and the *Lilliaceae* (9).

S-Methyl-L-cysteine sulfoxide, a non-protein sulfur-containing amino acid in cabbage (10-12), is structurally similar to alliin (Fig. 1) in garlic. *S*-Methyl-L-cysteine sulfoxide in cabbage and *S*-allyl-L-cysteine sulfoxide in garlic are simply methyl and allyl derivatives of L-cysteine sulfoxide, respectively. The two compounds generate methyl methanethiosulfinate and allyl 2-propenethiosulfinate,

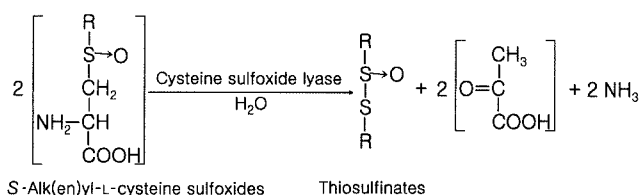


Fig. 1. Enzymatic cleavage of *S*-alk(en)yl-L-cysteine sulfoxides.

which are the principal antimicrobial compounds of *Brassica* and *Allium*, respectively.

Garlic is the vegetable that has been the most investigated for natural antimicrobials and its antimicrobial activity is much more potent than other vegetables, so this discussion will be focused only on the chemistry of *S*-allyl-L-cysteine sulfoxide in garlic and its antimicrobial activity.

Cavallito and Bailey (3) isolated the compound responsible for the antimicrobial activity of garlic, naming it allicin, and they correctly hypothesized its molecular structure. Allicin is absent in intact garlic but generated from its precursor alliin, through enzymatic hydrolysis when the tissue of garlic is damaged (3, 4, 13). Alliin was isolated by Stoll and Seebeck (14) in its crystalline form. Rundqvist (15) tried to do the same ahead of Stoll and Seebeck (14); however his preparation contained too much carbohydrate, which misled him to think that the precursor was carbohydrate in nature. Rundqvist (15) originally named the precursor compound alliin, a term which was adopted by Stoll and Seebeck (16).

Garlic has also been reported to have other biological activities, such as cancer-preventive (12, 17-19), serum lipid lowering (20), antiviral (21), antithrombotic (22), and enzyme inhibitory activities (23-27). This review, however, does not cover characteristics other than the chemistry of *S*-allyl-L-cysteine sulfoxide as it relates to the antimicrobial activity of garlic. The antimicrobial activity of garlic at cooking temperatures is included here since this phenomenon was found very recently and large amounts of cooked garlic are consumed worldwide.

Antimicrobial activity of fresh garlic: precursor, enzyme, and antimicrobial products The antimicrobial activity of garlic extract has been recognized for many years. It has been reported that 1-2% garlic extract inhibits microbial growth, and higher concentrations are germicidal (21, 28-40). The growth of *Salmonella typhimurium* (32),

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Bacillus cereus (33), *Clostridium botulinum* (34), *Clostridium perfringens* (35), *Candida utilis* (21, 36) and many other bacteria (21, 37, 38), and fungi (21, 39, 40) has been inhibited by garlic extract. Garlic extract has been shown to be more strongly inhibitory against yeasts (31) than bacteria (41).

Staphylococcus aureus, *Escherichia coli*, *Proteus mirabilis*, and *Pseudomonas aeruginosa*, which are resistant to multiple antibiotics including penicillin, streptomycin, doxycycline, and cephalexin, were inhibited by garlic extract (42). A multiply resistant *Klebsiella* sp. was not inhibited by garlic extract in the same study.

S-Allyl-L-cysteine sulfoxide, the precursor *Brassica* is taxonomically far apart from the genus *Allium*, which includes garlic and onion. However, S-alk(en)yl-L-cysteine sulfoxides as major non-protein amino acids are present in both genii (43). S-Alk(en)yl-L-cysteine sulfoxides were suggested to be important in sulfur metabolism, acting as a soluble pool for organic sulfur (44). The amounts vary widely depending on plant species and part of the plant (11, 45). The general structure of the S-alk(en)yl-L-cysteine sulfoxides is shown in Fig. 1. Two S-alk(en)yl-L-cysteine sulfoxides differing in R-side group have been described; both (methyl- and allyl-) in *Allium* and one (methyl-) in *Brassica*.

Rundqvist (15) made the first attempt to isolate the precursor compound from which volatile sulfur compounds are generated in garlic. Owing to the fact that his preparation contained considerable quantities of carbohydrate, he thought that the compound he was seeking was a glucoside, which he named 'alliin'. Later, pure alliin in crystalline form was isolated by Stoll and Seebeck (14) and identified as an amino acid. Stoll and Seebeck (14) used the term alliin coined by Rundqvist (15). Alliin was chemically synthesized in 1951 by the same group (4).

When S-alk(en)yl-L-cysteine sulfoxides of garlic were analyzed by reverse phase HPLC, S-methyl- and S-allyl-L-cysteine sulfoxides were the only compounds that were identified with certainty (46). Other sulfur amino acids were not found. Ziegler and Sticher (46) opined that the minor derivatives of L-cysteine sulfoxide were absent or were below the detection limits of the chromatographic conditions. GC analysis of thiosulfinates of crushed garlic by Freeman and Whenham (47) showed that the L-cysteine sulfoxide fraction of garlic consists of 85% alliin, along with 2% S-propyl cysteine sulfoxide and 13% S-methyl-L-cysteine sulfoxide. Therefore, it can be safely assumed that the concentration of minor L-cysteine sulfoxides could have been too low to be detected by HPLC by Ziegler and Sticher (46). Block *et al.* (13) described the HPLC analysis of thiosulfinates of garlic and showed allyl/methyl ratios that were similar to those of Freeman and Whenham (47). The allyl/methyl ratio of garlic differs according to where it is grown. Growth conditions are known to modify the profile of S-alk(en)yl-L-cysteine sulfoxides of garlic. Some garlic varieties grown in cooler climates show a higher allyl to methyl ratio than garlic grown in warmer climates. For example, New York grown garlic was seen to have low levels of S-methyl-L-cysteine sulfoxide (0.08-0.25 mg/g of garlic compared to 1-1.6 mg/g in California garlic) but normal levels of other

derivatives (13). Block *et al.* (13) suggested that garlic grown in colder climates is subject to stress and that this stress causes reduced synthesis of S-methyl-L-cysteine sulfoxide. Cruciferous vegetable species like kale, however, have been known to accumulate more S-methyl-L-cysteine sulfoxide when grown during periods of frost (48, 49).

The occurrence of ethyl (50, 51), propyl (47), and butyl (51) derivatives in garlic has been suggested, but has never been positively confirmed (46).

Enzymes Alliin and alliinase are located in different compartments in garlic cloves (52). The substrates are found in the mesophyll storage cells and the enzyme in the vascular bundle sheath cells (52). This is the reason that uncut intact garlic is not antimicrobial and is odorless. The enzyme and substrate come into contact only when the garlic tissues are disrupted. The alliinase reaction (Fig. 1) takes place extremely rapidly, a fact that is in agreement with the instantaneous appearance of the typical odor when garlic is crushed. More than 80% of the alliin is hydrolysed by the enzyme within 2 min (4).

Bacteria are also known to have enzyme(s) that catalyze the hydrolysis of S-alk(en)yl-L-cysteine sulfoxides. Stoll and Seebeck (4) reported the development of garlic odor when *E. coli* was cultured in nutrient medium with 0.2% alliin. Bacterial enzymes of *Pseudomonas cruciviae* (53) and *Bacillus subtilis* (54) catalyzed the stoichiometric conversion of S-methyl-L-cysteine sulfoxide to methyl methane thiosulfinate, pyruvate, and ammonia and required pyridoxal phosphate as a coenzyme (54), as do other alliinases and L-cysteine sulfoxide lyases. S-Methyl-L-cysteine sulfoxide in feed kale is converted to dimethyl disulfide by unspecified rumen microorganisms and cause hemolytic anemia in cattle and sheep, known as kale poisoning (55).

Garlic products and their chemistry Cavallito and Bailey (3) succeeded in isolating a water-soluble antimicrobial substance from an aqueous ethanolic extract of garlic by steam distillation under reduced pressure and named it 'allicin', and the Cavallito group (56) correctly described the structure as allyl-S (O)-S-allyl (Fig. 1).

The HPLC analysis of thiosulfinates in garlic made by Block *et al.* (13) found that the major thiosulfinate from garlic was allicin (allyl 2-propenethiosulfinate). Other kinds of thiosulfinates were identified from the extracts of garlic, including allyl methanethiosulfinate, methyl 2-propenethiosulfinate, and methyl methanethiosulfinate, since it contains two different substrates; methyl and allyl derivatives of L-cysteine sulfoxide.

Block *et al.* (13) and Block (57) urged the use of non-thermal methods of analysis such as HPLC when volatile compounds of garlic are of interest because of artifact formation due to the very high injection port temperature which apparently induces chemical modification of less stable compounds during the chromatography procedure. Allicin is not stable, even at 3°C, and it loses its activity within 14 days (4). Brodnitz *et al.* (58) observed that allicin underwent complete decomposition at 20°C after 20 hr, resulting in sulfides including diallyl disulfide, diallyl trisulfide, diallyl monosulfide, and sulfur dioxide. Allicin

in garlic juice underwent complete decomposition at 40°C only after 144 hr (59). The authors postulated that alliin was more stable in garlic juice than in the pure state. The particular instability of the allyl compound appears to be associated with the double bond (60). Accordingly, the antimicrobial activity of garlic extract decreases very rapidly during storage. The minimum inhibitory concentrations (MIC) of garlic extract increased from less than 0.1% to higher than 16% after 30 days of storage at 37°C (61).

Antimicrobial compounds derived from alliin Before the antimicrobial substance of garlic was known to be alliin from the work by Cavallito and Bailey (3), some researchers ascribed the antimicrobial activity of garlic to other compounds, including acrolein and related aldehydes (62). In fact, the antimicrobial activity is not due to alliin alone, but is a general property of alk(en)yl esters of alka(e)nethiosulfinate (4).

The action of alliin, the first known natural thiosulfinate, is considerably more bacteriostatic than bactericidal (3). It is similarly effective against Gram-positive and Gram-negative bacteria. Several observations were made by Small *et al.* (60) regarding the chemical structure and antimicrobial activity of thiosulfinates. In general, it requires about the same quantities of the lower molecular weight thiosulfinates to inhibit both Gram-positive and Gram-negative bacteria, but as the carbon chain length increases, activity against Gram-negative organisms decreases, while that against Gram-positive bacteria increases. Branching results in lowered activity.

Small *et al.* (63) compared the antimicrobial activity of thiosulfinate and thiosulfonate (synthetic ethyl ethanethiosulfinate and ethyl ethanethiosulfonate, which are not found naturally). These two thiol esters were of comparable antimicrobial activity, with thiosulfonate being slightly more effective against *S. aureus* and *Klebsiella pneumoniae*.

A derivative of alliin, ajoene (Fig. 3), which originally had its potent antithrombotic activity described (22), exhibited a strong antifungal activity toward *Aspergillus niger* and *Clostridium albicans* at <20 µg/mL (64). Yoshida *et al.* (64) concluded that ajoene had stronger antifungal activity than alliin and that it damages the cell wall of fungi. They therefore maintained that bacterial growth inhibition by ajoene would not be expected except for a specific strain. Later Naganawa *et al.* (65) described a different result concerning the antimicrobial activity of ajoene. They reported that ajoene was strongly inhibitory against Gram-positive bacteria and yeasts and had various degrees of inhibition against Gram-negative bacteria like *E. coli* and *P. aeruginosa*.

Diallyl disulfide, one of the degradation products of alliin, has been shown to possess antituberculosis activity (66).

Cysteine inhibits the antimicrobial activity of alliin, which may be reactivated by hydrogen peroxide (67). Antimicrobial activity of garlic is known to be stabilized by hydrogen peroxide (68). However, this is not in agreement with the report (21) that catalase positive bacteria were sensitive to garlic while catalase negative bacteria (e.g., lactic acid bacteria) were less sensitive.

Mode of action of thiosulfinates The principal antimicrobial compounds of garlic are those belonging to a group known as thiosulfinates. The antimicrobial activity of thiosulfinates has been explained as a general reaction between thiosulfinates and -SH groups of essential cellular proteins (36, 56, 60, 63). Small *et al.* (60) mentioned that -S(O)-S- was responsible for the antimicrobial activity and reacted readily with cysteine to yield mixed disulfides. Fujiwara *et al.* (50) showed essentially the same reaction between alliin and thiamine. The general reaction (Fig. 2) as proposed by Small *et al.* (60) could apply whenever thiosulfinates are involved and the reaction is believed to be the common mechanism of antimicrobial activity of thiosulfinates.

Since a general inhibitory mechanism of thiosulfinates was proposed, some researchers (23-27) have reported target processes and enzymes specific to thiosulfinates. Ghannoum (25) and Feldberg *et al.* (26) reported that alliin inhibited lipid biosynthesis and RNA synthesis, but did not suggest any target enzymes. Wills (23) reported that alliin inhibited the activity of many -SH enzymes. Among them the most strongly inhibited were xanthine oxidase, succinic dehydrogenase, and triose phosphate dehydrogenase. He confirmed the results of Small *et al.* (60) that the -S(O)-S-group was essential for the inhibition of -SH enzymes, while -S-S-, -S-, and -SO-groups were not effective.

Focke *et al.* (27) found that alliin inhibited the incorporation of acetate, but not of acetyl CoA or malonate, into fatty acids and concluded that only acetyl CoA synthetase for fatty acid synthesis was inhibited by alliin. They explained that the inhibition of acetyl CoA synthetase by alliin was a specific non-sulphydryl effect.

Potent antimicrobial activity has also been described for ajoene. Yoshida *et al.* (64) maintained that ajoene was an even more potent antifungal agent than alliin. They assumed that ajoene damages the cell walls of fungi, thus did not expect significant antibacterial activity, except against *S. aureus*. Later, however, the findings of Naganawa *et al.* (65) concerning the antimicrobial activity of ajoene were different. They reported that ajoene was strongly

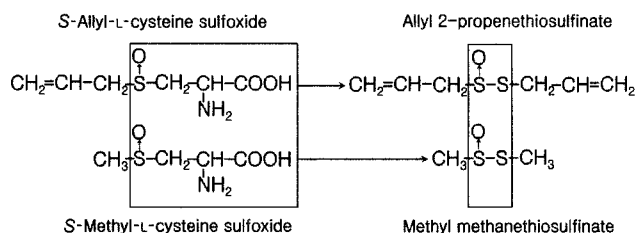


Fig. 2. Generation of antimicrobial compounds from S-alk(en)yl-L-cysteine sulfoxides.

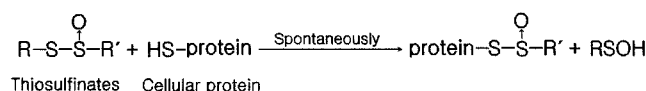


Fig. 3. Proposed reaction between thiosulfinates and SH group of cellular proteins.

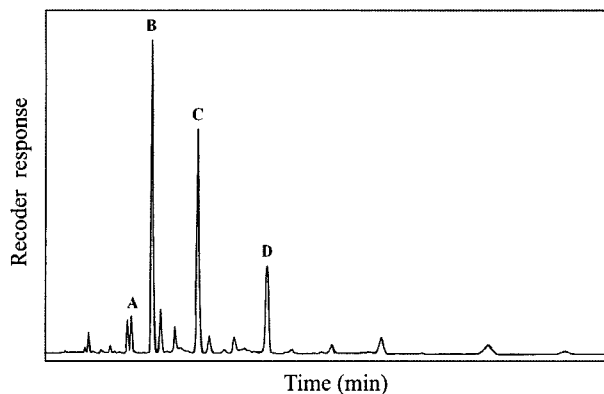


Fig. 4. HPLC chromatogram of garlic oil. A, diallyl monosulfide; B, diallyl disulfide; C, diallyl trisulfide; D, diallyl tetrasulfide.

inhibitory against the growth of Gram-positive bacteria and yeasts and had various degrees of inhibition of Gram-negative bacteria like *E. coli* and *P. aeruginosa*. They postulated that the disulfide group in ajoene appears to be necessary for the antibacterial activity, since reduction by cysteine abolished its antimicrobial activity. Ajoene does not possess a thiosulfinyl group.

Antimicrobial activity of heated garlic; precursor and antimicrobial products Originally, heated garlic was reported to be non-antimicrobial (1) on the basis of experimental results. Since an enzyme is involved in the generation of antimicrobial activity in fresh garlic, it was natural to assume that heated garlic is not antimicrobial. Indeed, there have been no reports on the antimicrobial activity of garlic generated by heating in the absence of alliinase activity. Lawson (69) concluded that prolonged heating at high temperatures (not specified) caused a loss of the antimicrobial activity of garlic because alliinase is inactivated by heat.

However, it was recently found that garlic heated at autoclaving temperature was antimicrobial (41). Kyung *et al.* (41) reported that autoclaved garlic extract had antibacterial activity against *S. aureus* B33, though this activity was much reduced compared to unheated fresh garlic extract. The MIC (with complete bacteriostatic activity) of heated garlic against *S. aureus* was 15%. In contrast, the MIC of fresh garlic extract against the same bacterium was 1.5%, 10 times less than heated garlic (61).

It has recently been reported that autoclaved garlic inhibited yeast growth was much more strongly than that of bacteria (61). The MICs of autoclaved garlic for all the tested bacteria were more than 20%, while those for yeasts were in the range of 0.5 to 3.0%. Inhibition of common yeasts, including *Candida*, *Saccharomyces*, and *Pichia* was the greatest. The MICs for these yeasts were equal to or lower than 1.0%. Xerotolerant yeasts belonging to the genus *Zygosaccharomyces* were comparatively resistant to the antimicrobial activity of autoclaved garlic, with MICs being between 1.5 and 3.0%.

The noteworthy differences in the antimicrobial characteristics of fresh and heated garlic extracts are that fresh garlic is about 10 times more potent than heated garlic and that the activity of heated garlic is stable while

that of fresh garlic is extremely unstable. The MIC of fresh garlic against *C. utilis* ATCC 42416 changed from 0.075% on day 0 to 16% after 30 days of storage at 37°C (61). This was a more than 200-fold reduction in antimicrobial potency. In contrast, the antimicrobial activity of heated garlic did not change at all during the same period under the same storage conditions.

Precursor and antimicrobial products An autoclaved aqueous alliin solution (1.0%) showed exactly the same pattern of growth inhibition as that of autoclaved garlic with an alliin content of 1.0% (61). Garlic heated for 45 min had the greatest antibacterial activity and the relative peak areas of 4-heptenal, methyl allyl disulfide, diallyl disulfide, 2-vinyl-4H-1,3-dithiin, and diallyl trisulfide were highest after 45 min of heating. Apart from 4-heptenal, all these compounds are known to possess varying degrees of antibacterial activity. Diallyl trisulfide was thought to be the primary volatile antibacterial compound in the heated garlic extract (61). Diallyl trisulfide was shown to have MICs of 7 and 40 ppm against *C. utilis* ATCC 42416 and *S. aureus* B33 respectively (70).

The principal anti-yeast compound of heated garlic was isolated and identified by high-performance liquid chromatography (HPLC) and gas chromatography-mass spectrometry (GC-MS) as allyl alcohol (2-propen-1-ol) (71). The generation of allyl alcohol was observed in heated garlic as well as in heated pure alliin solution. The pattern of growth inhibition of *C. utilis* ATCC 42416 by allyl alcohol was essentially the same as that seen with heated garlic and heated alliin solution. The mode of action of allyl alcohol is fungicidal. The MICs of heated garlic, with an alliin content of 1.5%(w/v), and allyl alcohol were 0.6%(v/v) and 0.002%(v/v), respectively, against *C. utilis* ATCC 42416. Yeasts are extremely sensitive to allyl alcohol, with MICs ranging from 0.002 to 0.014%(v/v), while bacteria were not very sensitive to allyl alcohol, with MICs ranging from 4 to 7%. Among yeasts, xerotolerant strains, including *Zygosaccharomyces rouxii*, were significantly less sensitive to allyl alcohol and heated garlic extract than other strains. Allyl alcohol is different from all other known antimicrobial compounds found in garlic in that it does not contain sulfur (Fig. 3).

Another garlic material known to possess antimicrobial activity is garlic oil. Garlic oil is not used in Korea as commonly as it is in Western countries. Garlic oil is produced by heating the crushed garlic to boiling temperature and collecting the resulting vapor as a distillate (72). During the heating process, alliin in crushed garlic is converted into various types of sulfides (73-75) with diallyl disulfide being the most abundant. Garlic oil and its diallyl constituents were recently found to be antibacterial (73, 74, 76). Sulfides (diallyl trisulfide, diallyl tetrasulfide, diallyl pentasulfide) with more sulfur atoms than are found in garlic oil are known to possess stronger antimicrobial activity (76) than those with less sulfur atoms such as diallyl disulfide. Cavallito *et al.* (3, 56) found that alliin decomposed into diallyl sulfides. They also reported that neither aqueous garlic extracts lacking alliin nor those with garlic oil (or diallyl sulfides) were antimicrobial (3). There have been few investigations concerning the antimicrobial activity of garlic oil and its

constituting disulfides, because the early pioneers of garlic research concluded that garlic oil and its constituents were not antimicrobial.

Mode of action of allyl alcohol All alcohols (methyl, ethyl, propyl, isopropyl, amyl, isoamyl, and hexyl) tested showed essentially the same growth inhibitory activity against bacteria and yeasts except allyl alcohol and 2-buten-1-ol, which were approximately three and one orders of magnitude respectively more potent against yeasts than bacteria. The average MICs of allyl alcohol for bacteria and yeasts were 5.0 and 0.0056%, respectively (77).

The extraordinarily potent anti-yeast activity of both allyl alcohol and 2-buten-1-ol can be explained by the fact that the unsaturated alcohols are oxidized by cellular alcohol dehydrogenase (ADH) in yeasts to corresponding aldehydes (78). The aldehyde keto groups are conjugated with double bonds, which are known to be potent protein alkylating agents (79). Rando (80) reported that yeast ADH oxidizes allyl alcohol into the highly cytotoxic agent acrolein. Secondary alcohols including 3-buten-2-ol (77) are not expected to be potent anti-yeasts since they are metabolized to ketones which are excreted without doing much harm to cellular proteins (80).

An ADH⁺ yeast strain, *Saccharomyces cerevisiae* ATCC 4126, was very sensitive to allyl alcohol, the MIC being 0.004%, while an ADH⁻ strain of the species, *S. cerevisiae* ATCC 64456, was not as sensitive, with a MIC of 5%, which was at the top of the high end of the allyl alcohol MIC range for all bacteria tested (77). This indicates that allyl alcohol itself inhibits yeasts at high concentrations, whereas acrolein, the oxidized product of allyl alcohol, inhibits yeasts at a much lower concentration. Therefore the potent selective growth inhibitory activity of allyl alcohol involves the catalyzing activity of yeast ADH.

E. coli was highly resistant to allyl alcohol when grown aerobically, but was very sensitive to allyl alcohol when grown anaerobically (81). ADH defective mutants of *E. coli* were resistant to allyl alcohol when they were grown both aerobically and anaerobically (78).

Taking all these results into account, the potent anti-

yeast activity of allyl alcohol seems to be via the oxidation of allyl alcohol by cellular ADH to acrolein, a potent protein alkylating agent. It was deduced that a very efficient NAD(P)-dependent ADH is expressed in yeasts, but not in bacteria under the test conditions. Antimicrobial actions of allyl alcohol and heated garlic are fungicidal, while that of garlic oil is fungistatic (unpublished data).

Use in foods as a preservative Among the common vegetables, garlic has been studied the most extensively as far as beneficial biological activities are concerned. Since garlic is used as a flavoring agent or food material and is also known to have various beneficial biological functions, garlic has been mentioned as a good candidate for a natural food preservative. Although the antimicrobial activity of *Allium* including garlic represents a promising area of research, use of the vegetable as natural food preservatives has not been common. There are only a few known examples of testing garlic as a food preservative.

When ground camel meat was treated with 5% or more of ground garlic, the storage shelf life of the meat at any given storage temperature was extended (82). Fifteen% or more garlic was found to act as a strong bactericide, since the initial microbial population of ground camel meat was completely destroyed and no further growth of any type of microorganism was observed.

In another report, antimicrobial materials including garlic oil, heated garlic, and allyl alcohol were investigated for the use as alternative natural food preservatives to inhibit the growth of film-forming yeasts in fermented vegetables (83). Garlic oil, heated garlic, and allyl alcohol were effective in preventing film formation at concentrations of 0.006, 3.0, and 0.02%, respectively, in fermenting cucumber pickles used as a model system. The effectiveness of heated garlic in preventing the growth of a film yeast, *Hansenula anomala*, was not influenced by pH, while that of potassium sorbate, a typical anti-yeast food preservative, was highly influenced. All tested materials could be added at the beginning of the fermentation because of their negligible inhibitory activity toward lactic acid bacteria.

Such a high level of garlic added to foods would be fine with some segments of population of the world, but not with others. There are difficult problems to be solved before garlic products are used as natural food additives, such as the strong garlic flavor and the instability of the functional compounds in garlic. Moon *et al.* (85) hypothesized that garlic may increase the resistance of bacteria to antibiotics.

Perspectives Levels of garlic normally used as food-flavoring materials may not be sufficient to obtain the desired preservative effects. Garlic showed an acceptable preservative effect only when substantial levels were added to food, which may not be acceptable by many people because of the strong flavor.

The relative instability of the activity further discourages the use of them as food preservatives. We have to acquire ways to enhance the stability of antimicrobial compounds and to decrease the objectionable sulfurous odor before finding more use of this vegetable, which possesses potent natural antimicrobial activities, as food preservatives.

Table 1. Antimicrobial compounds derived from alliin in garlic

Sulfur-containing antimicrobial compounds	
Allicin	$\text{CH}_2=\text{CH}-\text{CH}_2-\overset{\text{O}}{\underset{ }{\text{S}}}-\text{S}-\text{CH}_2-\text{CH}=\text{CH}_2$
Ajoene	$\text{CH}_2=\text{CH}-\text{CH}_2-\overset{\text{O}}{\underset{ }{\text{S}}}-\text{CH}_2-\text{CH}=\text{CH}-\text{S}-\text{S}-\text{CH}_2-\text{CH}=\text{CH}_2$
Diallyl disulfide	$\text{CH}_2=\text{CH}-\text{CH}_2-\text{S}-\text{S}-\text{CH}_2-\text{CH}=\text{CH}_2$
Diallyl trisulfide	$\text{CH}_2=\text{CH}-\text{CH}_2-\text{S}-\text{S}-\text{S}-\text{CH}_2-\text{CH}=\text{CH}_2$
Diallyl tetrasulfide	$\text{CH}_2=\text{CH}-\text{CH}_2-\text{S}-\text{S}-\text{S}-\text{S}-\text{CH}_2-\text{CH}=\text{CH}_2$
Diallyl pentasulfide	$\text{CH}_2=\text{CH}-\text{CH}_2-\text{S}-\text{S}-\text{S}-\text{S}-\text{S}-\text{CH}_2-\text{CH}=\text{CH}_2$
Nonsulfur-containing antimicrobial compound	
Allyl alcohol	$\text{CH}_2=\text{CH}-\text{CH}_2-\text{OH}$

Acknowledgments

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