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Effect of Heat Treatments on the Antimicrobial Activities of Garlic (*Allium sativum*)

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Abstract Aqueous extracts of garlic (*Allium sativum*) preparation were prepared after the samples were exposed to various heat treatments. A quantitative assessment of antimicrobial activities was carried out by determining the minimum inhibitory and microbicidal concentrations (MICs and MMCs) of the various extracts against some selected bacteria and fungi. The antimicrobial activity of garlic decreased as the heating temperature increased. This fact implies that alliinase may be the most critical rate-determinant to produce the activity when garlic is heated.

Key words: Antimicrobial activity, MIC and MMC, aqueous extracts of garlic, direct-heating and steam-heating, allicin and alliinase

Artificial food preservatives have been frequently used to inhibit the growth of food spoilage microorganisms. On the other hand, antimicrobials which inhibit microbial growth also exist in various plants [10-11]. Consequently, various studies have been conducted on the antimicrobial activities of plant extracts and their applications for food preservation [1, 3]. Among many plants used as spices or flavoring materials, garlic has been extensively studied for its antimicrobial properties. Allicin has been found to be the principal antimicrobial agent in the plants [9]. Earlier experimental data also suggest that the antimicrobial activity is highly dependent on the preparation used [9]. Recently, there has been growing public interest in Korea to incorporate garlic-based products into nutraceuticals, because of their health-promoting properties. Despite this interest, very little information is currently available concerning the manufacturing methods, i.e., heating preparations of garlic-based products and the stability of their antimicrobial action during preparation. Therefore, the objectives of this work were to (1) find optimal heating conditions to minimize the loss of antimicrobial activities and (2) describe the antibacterial and antifungal actions of garlic extracts prepared from garlic cloves treated under various heating conditions.

Unpeeled bulbs of a garlic ecotype used in this work (Hanjigye cultivated in Eusung, Korea) were purchased from a local wholesale market and stored at 4°C until use. All the strains used in this work are listed in Table 1. An initial study was carried out to find optimal heating conditions to keep the textural integrity properly maintained and the garlic odor maximally removed, but still retaining as much of the natural garlic-specific antimicrobial activity as possible (Table 2). The experimental data in Table 2 indicate the following two possibilities. The first possibility is that most of typical garlic odor was responsible for the major fraction of inhibitory activities, and all of the microbicidal activities of garlic were quite volatile or unstable at above 80°C, however, the remaining less volatile or more heat-stable garlic flavor compounds still possessed their weak inhibitory activities. The second possibility is that a considerable portion of alliinase might be inactivated during the heating processes at above 80°C.

Based on the sensory and antibacterial results obtained from the initial study, a set of the five differently treated garlic samples, including those unheated, were evaluated for their antimicrobial activities, remained after the corresponding treatments against a panel of common spoilage and pathogenic microorganisms (Tables 3 and 4). The crushed garlic sample, which had been steam-heated at 100°C for 20 min, was also included here to determine which of the above two possibilites contributed more to determining the antimicrobial activities of garlic.

A different spectrum of antibacterial activity of aqueous garlic extracts against the selected strains of bacteria has been obtained. MICs (Table 3) for garlic samples directly-

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Table 1. Strain list used in this study.

Microorganisms	Characteristics
Gram-positive bacteria	
Bacillus subtilis KCCM 11733	Food spoilage bacteria
Staphylococcus aureus KCCM 11764	Enterotoxin producing bacteria
Streptococcus mutans ATCC 27351	Dental-plaque causing bacteria
Gram-negative bacteria	
Escherichia coli KCCM 11750	Food spoilage bacteria
Escherichia coli O157:H7 ATCC 35150	Shiga-like toxin producing bacteria
Klebsiella pneumoniae ATCC 21204	Pneumonia causing bacteria
Salmonella typhimurium KCCM 40253	Enteric-fever causing bacteria
Yeasts	
Candida utilis KCCM 11750	Food spoilage yeast
Saccharomyces cerevisiae KCCM 11666	Food spoilage yeast
Molds	
Aspergillus flavus KCCM 60130	Aflatoxin producing mold
Aspergillus niger KCCM 11478	Food spoilage mold
Aspergillus parasiticus KCCM 12699	Aflatoxin producing mold
Penicillium citrinum KCCM 11663	Food spoilage mold

heated up to 85°C for 30 min inhibited growth of all the bacteria tested. No detectable inhibitory and bactericidal activities were found in the sample when an aqueous extract of whole garlic steam-heated for 20 min was used. But the growth of four bacteria strains was inhibited with an aqueous extract of crushed garlic steam-heated for 20 min. In general, the higher the heating temperature, the weaker was the antibacterial effect (Table 3). The lowest MIC value of 0.5% was found against the *S. mutans* strain with unheated garlic cloves or cloves heated at 75°C for 30 min. With Gram-positive bacteria, garlic steam-heated after crushing weakly inhibited the growth of all the bacteria tested; i.e., MICs of 10% against *B. subtilis* and *S. aureus* and 5% against *S. mutans*. But it resulted in loss of growth inhibition of all the Gram-negative bacteria tested, except for *K. pneumoniae*. On the other hand, it did not have any bactericidal activity against all the Gram-positive and negative bacteria tested.

Table 2. Sensory quality of garlic samples, and MIC and MMC values of garlic extracts against *S. aureus* and *E. coli* O157:H7.

Heating temp. (°C)	Heating time (min)	Firmness and garlic odor[a]	*S. aureus*		*E. coli* O157:H7	
			MIC (%)	MMC (%)	MIC (%)	MMC (%)
60 (direct)	30	+++++				
	60	++++				
65 (direct)	30	+++++	2.0	10	2.0	10
	60	++++				
70 (direct)	10	+++++				
	20	++++				
	30	+++				
75 (direct)	30	+++	2.0	10	2.0	10
80 (direct)	20	++				
	30	++	5.0	NM[c]	10	NM
85 (direct)	30	++	10	NM	10	NM
90 (direct)	30	+	NI[b]	NM	NI	NM
100 (steam)	20	+	NI	NM	NI	NM

[a] +++++, with natural garlic odor and no visual damage on firmness; ++++, showing (about 30%) partial decrease in garlic odor with partial damage on firmness; +++, obvious loss (about 70% reduction) in garlic odor but showing good firmness; ++, noticeable signs of odor loss and fair firmness; +, extensive or complete losses in firmness and odor. Average of triplicate samples.
[b] No inhibitory effect observed up to 10% garlic extracts.
[c] No microbicidal effect observed up to 10% garlic extracts.

Table 3. MIC and MMC values of garlic extracts against spoilage and pathogenic bacteria.

Gram staining	Strain names	Antibacterial activity	Heating conditions				
			No heat	Direct (75°C/30 min)	Direct (85°C/30 min)	Steam (whole)	Steam (crushed)
Gram (+)	*B. subtilis*	MIC (%)	1	1	5	NI[a]	10
		MMC (%)	2	2	NM[b]	NM	NM
	S. aureus	MIC (%)	2	2	10	NI	10
		MMC (%)	10	10	NM	NM	NM
	S. mutans	MIC (%)	0.5	0.5	2	NI	5
		MMC (%)	10	10	NM	NM	NM
Gram (-)	*E. coli*	MIC (%)	2	2	10	NI	NI
		MMC (%)	10	10	NM	NM	NM
	E. coli O157:H7	MIC (%)	2	2	10	NI	NI
		MMC (%)	10	10	NM	NM	NM
	K. pneumoniae	MIC (%)	1	1	5	NI	10
		MMC (%)	1	1	10	NM	NM
	S. typhimurium	MIC (%)	5	5	10	NI	NI
		MMC (%)	5	5	NM	NM	NM

[a]No inhibitory effect observed up to 10% garlic extracts.
[b]No microbicidal effect observed up to 10% garlic extracts.

Growth and survival of two yeast and four mold strains in the presence of aqueous garlic extracts treated with five different heating conditions were compared, and the results are shown in Table 4. An interesting observation in Table 4 was that steaming after crushing could keep antifungal activity against the yeast and mold strains more than steaming before crushing.

Unique compositional features of garlic are its low moisture content, high contents of fructans and common free amino acids, and very low contents of lipids and other oil-soluble compounds. Garlic is also one of the highest selenium-containing foods on a per gram basis. However, its most unique feature is its high content of organosulfur compounds, 99.5% of which consist of sulfur amino acid cysteine, even though free cysteine itself is absent. The sulfur content of garlic is four times greater than that of other high sulfur-containing vegetables and fruits, such as onions, broccoli, cauliflower, and apricots [6]. Nearly all (95%) of the sulfur in intact garlic cloves is in two classes of compounds in equal abundance; i.e., *S*-alkylcysteine

Table 4. MIC and MMC values of garlic extracts against spoilage and pathogenic fungi.

Fungi	Strain names	Antifungal activity	Heating conditions				
			No heat	Direct (75°C/30 min)	Direct (85°C/30 min)	Steam (whole)	Steam (crushed)
Yeasts	*C. utilis*	MIC (%)	0.5	0.5	2	NI[a]	5
		MMC (%)	0.5	0.5	2	NM[b]	5
	S. cerevisiae	MIC (%)	0.5	0.5	5	NI	5
		MMC (%)	0.5	0.5	5	NM	5
Molds	*P. citrinum*	MIC (%)	0.3	0.3	>0.5	>5	5
		MMC (%)	2	2	10	NM	NM
	A. niger	MIC (%)	0.2	0.2	1	NI	NI
		MMC (%)	1	1	5	NM	NM
	A. flavus	MIC (%)	0.5	0.5	>0.8	NI	4
		MMC (%)	1	1	10	NM	10
	A. parasiticus	MIC (%)	0.3	0.5	>0.5	NI	4
		MMC (%)	0.5	0.5	2	NM	5

[a]No inhibitory effect observed up to 10% garlic extracts.
[b]No microbicidal effect observed up to 10% garlic extracts.

sulfoxides and γ-glutamyl-*S*-alkylcysteines [4-6]. The most abundant sulfur compound in garlic is alliin (*S*-allylcysteine sulfoxides), which is typically present at 10 mg/g of wet weight and accounts for about 80% of the total cysteine sulfoxides. However, it is less abundant in several other *Allium* species and absent in onions, and rarely occurs in only small amounts in other plants. When garlic cloves are cut, crushed, or chewed, the cysteine sulfoxides which are odorless and insoluble in organic solvents are very rapidly converted to a new class of compounds, thiosulfinates. The thiosulfinates contain two sulfur atoms, are more soluble in organic solvents than in water, and are somewhat volatile with a typical freshly chopped garlic odor. The thiosulfinates are produced when cysteine sulfoxides in the clove mesophyll storage cells come in contact with the enzyme alliinase from the vascular bundle sheath cells. Due to very large abundance of alliinase (10 mg/g fresh garlic), the rate of thiosulfinates formation is extremely rapid, being completed upon crushing in less than 10 sec for alliin and isoalliin and about 60 sec for methiin, an alliin derivative [6]. The main thiosulfinate formed upon crushing garlic is allicin, because of its abundance, which ranges from 60 to 90% of the total thiosulfinates, the typical being 75% [7, 9].

The thiosulfinates are self-reactive compounds that can be quite unstable, depending upon solvent, temperature, and concentration. Since they undergo self-reaction through both monomolecular and bimolecular mechanisms, both dilution and the presence of solvents, in which hydrogen bonds with the oxygen atom (water, and alcohols to a lesser extent), greatly improve their stability. The odor of fresh-cut garlic is mainly due to allicin, while the odor of cooked garlic is due mostly to diallyl, allyl methyl trisulfides, and to a less degree their disulfides [6, 9, 11]. None of the main sulfur compounds present in processed garlic products, such as oil products and aged extracts, are found significantly in whole or crushed garlic. The compounds to which the thiosulfinates are converted depend upon solvent and temperature. In the presence of water, diallyl trisulfide, diallyl disulfide, and allyl methyl trisulfide are the principal products. Upon steam-distillation of crushed cloves, a commercial essential oil is produced, in which as many as 30 different sulfides have been found [8, 11]. When incubated at room temperature in organic solvents, such as hexane, ether, or triglyceride oils (oil-macerated products), three additional types of compounds such as ring-structured vinyldithiins (70–80%), ajoene (12–16%), and other allyl sulfides (4–18%), are formed [2, 8, 12].

Antimicrobial activity in garlic samples is produced by a reaction, where an enzyme and substrates are involved. The enzyme (alliinase) is a glycoprotein of MW 55 kDa and is one of the two most abundant proteins in garlic. The substrates (cysteine sulfoxides) are odorless and insoluble in organic solvents, but their products (thiosulfinates) are volatile and unstably self-reactive. Although all, if not most of the antimicrobial activity of garlic samples comes from allicin (a typical garlic thiosulfinate) and its self-reacted products, allicin and other thiosulfinates are not produced from alliin and other cysteine sulfoxides by the action of alliinase, unless garlic cloves are physically damaged. As indicated by our data, the allicin production catalyzed by alliinase in the garlic samples is temperature-dependant. The higher the temperatures the at which garlic samples were direct-heated before being crushed, the less the antimicrobial activity they had. As heating temperatures increased, the garlic samples increasingly lost garlic odor, indicating gradually less production of thiosulfinates from cysteine sulfoxides due to heat-inactivation of the enzyme or gradual loss of those products due to their volatility upon heating. As for the steam-heated garlic samples, ones crushed before steaming retained higher antimicrobial activity than those crushed after steaming. In fact, our data demonstrated that the garlic samples lost virtually all of their antimicrobial activity upon steaming before crushing, however, they retained some of the activity when steamed after crushing. This could be due to the fact that some of the antimicrobial reaction products were produced and retained when steamed after crushing, but the same antimicrobial reaction products were lost when steamed before crushing. This implies that alliinase can be heat-inactivated and can not convert alliin into allicin, especially at above 80°C, because it is denatured by heating. Collectively, therefore, alliinase seems to be the most critical rate-determinant to produce antimicrobial activity, when garlic is heat-treated.

Garlic and numerous spices have been proposed as natural food preservatives. Recently, use of garlic in the health conscious population has been increasing. The potential for more extensive usage of garlic-originated antimicrobials or garlic extract which contains their antimicrobials would appear to be good, in light of the consumers' desire for so-called 'natural' foods; i.e., foods to which man-made preservatives are not added. The challenge is to isolate, purify, stabilize, and incorporate these natural antimicrobials into foods without adversely affecting sensory, nutritional, and safety characteristics. All of this, of course, should be done without increased costs for formulation, processing, or marketing. Therefore, the first step toward such goals has already been taken here; quantitative estimation of the antimicrobial activity of the plant material or extract was made, and changes in the activity during various heat treatments were monitored, employing a panel of common spoilage and pathogenic microorganisms. Obviously, however, more extensive research is necessary to meet all the above challenges before garlic or its antimicrobials can be applied as a popular natural food preservative.

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