Characteristic Flavor Constituents in Water Extract of Garlic

Yoichi UEDA, Makoto SAKAGUCHI, Kazuo HIRAYAMA, Ryuichi MIYAJIMA and Akimitsu KIMIZUKA

Central Research Laboratories, Ajinomoto Co., Inc., 1–1 Suzuki-cho, Kawasaki-ku, Kawasaki 210, Japan

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The flavoring effects of a water extract of garlic (*Allium sativum* L.) being added to common soups (Chinese soup and curry soup) were examined by a sensory evaluation. When a small amount (0.1 or 0.4% w/v) of the extract was added to the soups, it showed characteristic *kokumi* flavors (continuity, mouthfulness, and thickness), and other tests revealed that this effect was clearly recognized in the *umami* solution composed of 0.05% w/v of monosodium glutamate and 0.05% w/v of disodium inosinate.

To find the key compounds which gave rise to the effect, the water extract was chromatographed on Duolite C-25 and the adsorbed fraction showed the activity. Further chromatographic studies of the fraction showed that the key compounds were sulfur-containing components, such as alliin, (+)-*S*-methyl-L-cysteine sulfoxide, and γ-L-glutamyl-*S*-allyl-L-cysteine.

Garlic (*Allium sativum* L.) is cultivated as an important seasoning material in many countries. It is used as a seasoning and also a healthful food on account of its virtue as medicine. The character of its odor, which comes up when its raw material is crushed, has been investigated for a long time. The developmental mechanism of the odor from sulfur-containing substrates, such as alliin ((+)-*S*-allyl-L-cysteine sulfoxide) and other cysteine derivatives, was shown by Stoll and Seebeck.[1,2]

Allicin (diallyl thiosufinate), the first product of alliin through the enzymatic conversion, has a pungent taste together with an antibiotic activity.[3] The thiosulfinate is known to be unstable and easily converted to various types of odorous compounds.[4] Other cysteine derivatives are also converted in the same way. The odor and pungent taste are important when it is used as a spice.

On the other hand we often use cooked garlic in various dishes. For instance, fried garlic is an important seasoning for dishes in the Chinese style. In addition, the powder and the water extract prepared from heated garlic are widely used in various kinds of food processing. The heating process changes the taste of raw garlic. The water extract itself has a weak aroma and sweetness. Addition of the extract to the dishes though in a small amount enhances their flavor characters, such as continuity, mouthfulness, and thickness. The nature of these flavors could not be explained by the customary sensory terms such as saltiness, sweetness, sourness, or bitterness. These flavor characters are often called *kokumi* flavors.

Monosodium glutamate (MSG) was reported by Yamaguchi and Kimizuka[5] not only to add the *umami* taste to various dishes but also to enhance their whole flavor. They reported that MSG strengthened the beef-like flavor when being added to a beef consommé soup. This suggests an interaction between MSG and other constituents in foods on their flavor.

In this work the authors investigated the characteristic *kokumi* flavor constituents in water extract of garlic.

Materials and Methods

Garlic. Garlic bulbs (white variety), which were harvested in July 1982 in Aomori Prefecture, were preserved at 5°C until used.

Addition of garlic extract to soup. Garlic extract was prepared as follows. One kilogram of intact garlic bulbs were heated with 4 l of distilled water at 95°C for 30 min and crushed by a homogenizer. The homogenate was heated again under the same conditions and centrifuged at 5000 rpm for 30 min. The supernatant (4.2 l) was concentrated to 600 ml by a rotary evaporator. For deodorization activated charcoal (6 g, Wako Pure Chem. Ind. Ltd.) was added to the solution and stirred moderately for 30 min. The charcoal was removed by filtration. Lyophilization of the odorless solution gave an almost white powder (60 g).

The effects of adding the extract to distilled water, Chinese soup, and curry soup (addition concentrations were 0.4% w/v, 0.1% w/v, and 0.4% w/v, respectively) were evaluated sensorially. Chinese soup was prepared from a commercial soup base (Ajinomoto Co., Inc.) and salt. Curry soup (pork curry) was prepared by the ordinary method used in common kitchens. The main materials of the soup were pork, flour, tomato, butter, onion, salt, and spices. The sensory evaluation tests were done by the method of Yamaguchi and Kimizuka.[5] A panel of 19 persons (10 men and 9 women) was asked to evaluated the samples by a five-point rating scale (-2–$+2$; apparently weak–apparently strong) for 9 paired terms. The result was statistically analyzed by Student's t-test.

Flavor evaluation in umami solutions. Test samples were deodorized when necessary by the same method as described above and adjusted to pH 7.0 by addition of 6 N NAOH. Four *umami* test solutions composed of MSG and/or IMP (disodium inosinate) were used for the tests (0.05% w/v MSG (soln. (1)), 0.05% w/v IMP (soln. (2)), 3.1% w/v MSG (soln. (3)), and 0.05% w/v MSG and 0.05% w/v IMP (soln. (4)). *Umami* intensity of soln. (4) is as strong as that of soln. (3) as the result of the synergistic effect between MSG and IMP.[6] The samples were added to the solutions at the concentration of 0.1% or 0.2% (w/v).

Preparation of active fraction by column chromatography. Figure 1 shows the details of preparation procedures of Duolite C-25 adsorbed fraction. Duolite C-25 (Duolite International, SA) and Amberlite IRC-50 (Room & Haas Co.) were used for fractionation of the garlic extract. A reverse osmotic membrane (TL-198, Teijin Ltd.) was used for concentration, and charcoal (Shirasagi, Takeda Chem. Ind.) for deodorization.

After Moore *et al.*,[7] the Duolite C-25 adsorbed fraction (21 g) was put on a Dowex 50 × 8 column (100–200 mesh, Dow Chem. Co., 5 × 140 cm, pre-equilibrated with 0.2 N sodium citrate buffer (pH 3.0)) and chromatographed with four sodium citrate buffers ((1) 0.2 N (pH 3.0), 6.0 l (2) 0.2 N (pH 3.25), 3.0 l (3) 0.2 N (pH 4.25), 3.0 l (4) 0.35 N (pH 5.26), 6.5 l) and 2% ammonia (10 l). Each buffer was put on the column stepwise. Ninhydrin-positive components were detected with the ninhydrin reagent and sulfur-containing components were detected with an ICP (Induced Coupled Plasma Photometer, Seiko Instrument

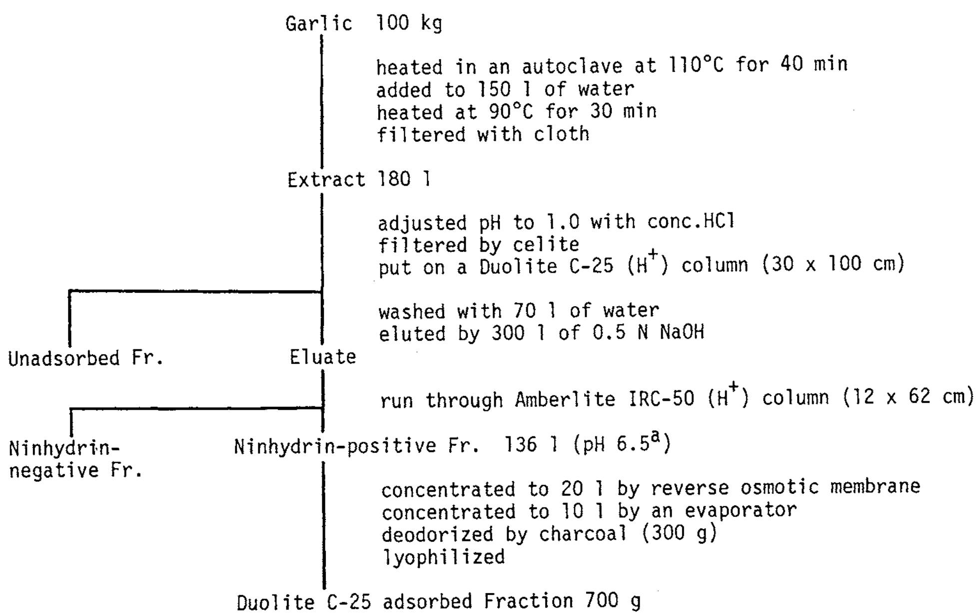

Fig. 1. Preparation of Duolite C-25 Adsorbed Fraction from Garlic.

[a] High pH fractions (pH > 10) were not combined.

Ltd., detection wavelength; 184.1 nm). Each fraction was run through a Dowex 50 × 8 (H^+) column (5 × 140 cm) and the column was washed with water to remove citrate salt. Then the adsorbed fraction was eluted with 0.5 N ammonia, concentrated by an evaporator, and lyophilized.

Preparation of sulfur compounds. Alliin, cycloalliin (3-(*S*)-methyl-1,4-thiazane-5-(*R*)-carboxylic acid 1-oxide), MeCSO ((+)-*S*-methyl-L-cysteine sulfoxide), GAC (γ-L-glutamyl-*S*-allyl-L-cysteine), and GACSO (γ-L-glutamyl-*S*-allyl-L-cysteine sulfoxide) were prepared by various types of ion exchange column chromatography.

Alliin was prepared by repeated chromatographies on a column of Dowex 50 × 8 (100–200 mesh, 2.5 × 30 cm, eluent; 0.2 N sodium citrate (pH 3.0)). The works of Akashi *et al.*[8] and Carson *et al.*[9] were consulted. Alliin was finally isolated by crystallization from ethanol–water.

MeCSO and cycloalliin were prepared by chromatographies on the Dowex 50 column and a MWA-1 column (200–400 mesh, 1.0 × 40 cm, Dow Chem. Co., eluent; 0.01 N ammonia). The method of Virtanen and Matikkala[10] was used. MeCSO and cycloalliin were finally crystallized from ethanol-water and from acetone–water, respectively.

GAC was prepared by chromatographies on the Dowex 50 column and a Dowex 1 column (100–200 mesh, 2.5 × 30 cm, eluent; 1 N acetic acid). The method of Sugii *et al.*[11] was used. GACSO was prepared by chromatography on the Dowex 1 column. GAC and GACSO were finally prepared by lyophilization.

The work of Matikkala and Virtanen,[12] using an amino acid analyzer, was also consulted for all preparations. For detection of sulfur the ICP was used. TLC was done on a cellulose plate (0.25 mm, Merck & Co. Inc.) with the solvent system of butanol-pyridine–acetic acid–water (15 : 12 : 3 : 10, v/v). Sulfur compounds except for cycloalliin were detected with the ninhydrin reagent.

NMR and MS analyses. ^{1}H-NMR spectra (400 MHz) were measured by a JEOL GX-400. Ten milligrams of each isolate was dissolved in 0.3 ml of D_2O. ^{13}C-NMR spectra (100 MHz) of GAC and GACSO were also measured by the same spectrometer. ^{13}C-NMR spectra (25 MHz) of alliin, cycloalliin, and MeCSO were measured by a JEOL FX-100. Fifty milligrams of each isolate was dissolved in 2.0 ml of D_2O. DSS was used as an internal standard for all measurements. Mass spectra (FDMS) were measured by a JEOL DX-300.

Quantitative analysis of each sulfur compound in Duolite C-25 fraction. Alliin and MeCSO were measured by an amino acid (A.A.) analyzer (Hitachi 835). Cycloalliin was measured by HPLC using a Finepak SIL NH_2 (4 × 250 mm, JASCO Co.) with an eluting solvent of 0.05 M potassium phosphate (pH 3.5) and acetonitrile (3 : 7, v/v). Cycloalliin was detected by UV (210 nm) at a flow rate of 1.5 ml/min. GAC and GACSO were measured by chromatography on a MWA-1 column (OH^-, 1.0 × 40 cm). The eluent was 0.01 N ammonia (0.05 ml/min). The eluate was collected at 1 ml/one fraction and the two peptides were detected as sulfur compounds using the ICP. Methionine (Met) and cysteine (Cys) were measured by the A. A. analyzer. Glutathione (GSH) was measured by the HPLC method of Takahashi *et al.*[13] after electrical reduction of oxidized glutathione (GS-SG) in the fraction by the method of Weitzman.[14]

Results

Flavor characters of garlic extract in soups

To ascertain the flavor characters of the water extract of garlic, addition tests on two soups (Chinese soup and curry soup) were done. The prepared extract, a slightly yellowish powder, had no aroma or basic taste in distilled water at the concentration of 0.4% or less.

Table I shows the result of addition tests on the two soups. When the extract was added to Chinese soup at the concentration of 0.1%, it didn't influence the intensities of the whole aroma or basic tastes such as saltiness, sweetness, sourness, bitterness, and *umami*. However, the result indicated statistically that the extract significantly strengthened the intensities of the *kokumi* flavors such as continuity, mouthfulness, and thickness. The addition effects on curry soup (0.4% of the

Table I. Additional Effects of Garlic Extract on Flavor Profiles of Soups

	Chinese soup	Curry soup
AROMA		
Whole Aroma	—	—
BASIC TASTE		
Saltiness	—	—
Sweetness	—	—
Sourness	—	—
Bitterness	—	—
Umami	—	—
FLAVOR CHARACTER		
Continuity	(0.84[a])*	(0.84)**
Mouthfulness	(0.90)*	(0.78)*
Thickness	(0.84)*	(0.74)*

*,** Stronger than control significantly. *$p<0.01$ and **$p<0.001$ *versus* control (Student's *t*-test). —, not significant.

[a] Average score.

extract was added) were the same as those on Chinese soup.

Flavor of garlic extract on umami solutions

The flavors of the extract with *umami* solutions were tested since MSG or IMP is supposed to enhance garlic's *kokumi* flavors.

Table II. *Kokumi* Flavors of Garlic Extract and Duolite C-25 Adsorbed Fraction in *Umami* Solution

Test solution	*Kokumi* flavors[a]	
	Garlic extract[b]	Duolite C-25 fraction[c]
Water	−	−
0.05% MSG[d] soln. (1)	+	+
0.05% IMP[e] soln. (2)	+	+
3.1% MSG soln. (3)	++	++
0.05% MSG & 0.05% IMP soln. (4)	++	++

[a] Continuity, mouthfulness, and thickness. −, unrecognized; +, recognized; ++, apparently recognized.
[b] 0.2% (w/v) of the extract was added.
[c] 0.1% (w/v) of the fraction was added.
[d] Monosodium glutamate monohydrate was used.
[e] Disodium inosinate containing 7.5 mol H_2O per 1 mol was used.

The extract and Duolite C-25 adsorbed fraction (described later) were added to four *umami* solutions.

The extract (0.2%) and the fraction (0.1%) had no taste or aroma in distilled water, however they gave *kokumi* flavors in solns. (1) and (2) as shown in Table II. The extract and the fraction showed no influence on the intensities of *umami* of the solutions but added the kokumi flavors to them. Addition tests to more concentrated test solutions were done as the *kokumi* flavors were definitely recognized in MSG or IMP solution. The result shows that the *kokumi* flavors were more clearly recognized by the panel in solns. (3) and (4) than in solns. (1) or (2). All the panelists said that the impressed intensities of the flavors of the two samples in solns. (3) and (4) were stronger than those in solns. (1) or (2). These screening methods were very simple and seemed to be useful, so we used soln. (4) as the screening solution for *kokumi* flavors in a series of following studies.

Preparation of Duolite C-25 fraction

In the first step of this study the Duolite C-25 adsorbed fraction was prepared to survey

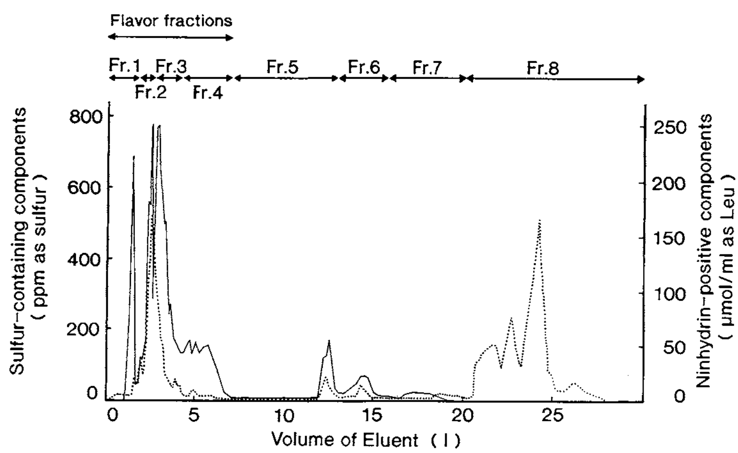

Fig. 2. Chromatogram of Duolite C-25 Fraction on Dowex 50 Column.

Column, 5 × 140 cm; flow rate, 28 ml/hr.

Elution buffer: 1) 0.2 N sodium citrate (pH 3.0) 6.0 l; 2) 0.2 N sodium citrate (pH 3.25) 3.0 l; 3) 0.2 N sodium citrate (pH 4.25) 3.0 l; 4) 0.35 N sodium citrate (pH 5.26) 6.5 l; 5) 2% ammonia 10 l.

The eluate was collected by 50 ml/one fraction.

------, ninhydrin-positive components (570 nm, μmol/ml as Leu); ——, sulfur-containing components (detected by ICP (184.1 nm), ppm as sulfur).

the key compounds which exhibited garlic's *kokumi* flavors. Figure 1 shows the result of the preparation. One hundred kilograms of garlic bulbs gave 700 g of the adsorbed fraction powder. The flavors of the fraction were tested in the *umami* solution composed of MSG and IMP described above.

The fraction had no aroma or basic taste in distilled water at the concentration of 0.2% or less but it had apparent *kokumi* flavors in the *umami* solution (see Table II). The flavors were recognized at the concentration of 0.05% or over in the solution by the panel. The unadsorbed fraction as added to the *umami* solution in the same manner. The fraction had no *kokumi* flavors but a weak sweetness.

Chromatography of Duolite C-25 fraction

As the Duolite C-25 fraction was responsible for the *kokumi* flavors and was assumed to contain amino acids and other nitrogenous components, chromatography was done on a Dowex 50 column at 5°C. Fifty-ml fractions were collected and assayed by the ninhydrin test and ICP (sulfur).

The elution profiles of ninhydrin-positive components and sulfur-containing ones were different from each other as shown in Fig. 2. The eluate was divided into Frs. 1–8 according to the peaks of sulfur compounds. About 90% of the total sulfur compounds were recovered and the sum of Frs. 1–4 accounted for 82% of the total recovery. The fractions desalted through a Dowex 50 column were evaluated by the panel in the *umami* solution. The panel tests revealed that Frs. 1–4 were responsible for *kokumi* flavors which coincided well with the distribution of sulfur compounds.

Preparation of sulfur compounds

Each sulfur-containing component from the flavor fractions was prepared by various types of ion exchange column chromatography and identified by FDMS, ^{13}C-NMR, and ^{1}H-NMR. Alliin and cycloalliin were prepared from Fr. 3 (1000 mg) in the yield of 300 mg and 147 mg, respectively. MeCSO and GAC were prepared from Fr. 2 (1000 mg) in the yield of 57 mg and 50 mg, respectively. GACSO was prepared from Fr. 1(150 mg) in the yield of 50 mg. Prepared alliin, cycloalliin, MeCSO, and GAC were confirmed to be almost pure by TLC and NMR. Prepared GACSO was contaminated with a small amount of an unknown compound (from NMR). The procedures were repeated twice or more for the preparation of the components in the following sensory tests.

Quantitative analysis of each sulfur compound in the Duolite C-25 fraction

Table III shows the composition of the sulfur-containing components in the Duolite C-25 adsorbed fraction. Alliin was the major constituent of the fraction and its content was 16.0% (weight %). The sulfur in alliin occupied 37% to the total sulfur of the fraction. Cycloalliin was the second abundant sulfur-containing component and GAC the third. Total sulfur of the components, including MeCSO, GACSO, Cys, Met, and glutathione, accounted for 88.7% of the sulfur in the fraction.

Sensory evaluation of each sulfur-containing component

The prepared sulfur-containing components and pure L-Cys, L-Met, and glutathione (reduced form, Sigma) were used for sensory tests as shown in Table IV. All these compounds had no aroma or basic taste in distilled water

Table III. Content of Each Sulfur-containing Component in Duolite C-25 Adsorbed Fraction

Component	Gravimetrical content (%)	Sulfur content (%)[a]
Alliin	16.0	37.0
Cycloalliin	9.2	21.4
MeCSO	2.1	5.8
GAC	5.3	7.5
GACSO	4.7	6.3
Glutathione	0.2	0.3
Cys	3.0	10.2
Met	0.1	0.2
Total	40.6	88.7

[a] Ratio of sulfur in each compound to the total sulfur of the fraction.

Table IV. *Kokumi* FLAVORS OF EACH SULFUR-CONTAINING COMPONENT

Component[a]	*Kokumi* flavors[b]	Other flavor
Alliin	+++	Garlic-like
Cycloalliin	+	
MeCSO	++	Leek-like
GAC	++	Garlic-like
GACSO	+	Garlic-like
Glutathione	+++	
Cys	+	
Met	+	

[a] 0.2% (w/v) of each compound was added to the *umami* solution.

[b] Continuity, mouthfulness, and thickness in the solution. +++, strongly recognized; ++, apparently recognized; +, recognized.

at low concentrations (0.2% or less) but they had *kokumi* flavors when dissolved in the *umami* solution.

Although they all had *kokumi* flavors, their whole flavors had some varieties. Alliin, GAC, and GACSO had garlic-like flavor in addition to *kokumi* flavors in the *umami* solution. MeCSO had a leek-like flavor. The impressed intensities of the *kokumi* flavors of alliin and glutathione were stronger than those of others when all the compounds were added at the same concentration (0.2%). The absolute threshold of alliin in the *umami* solution was 0.005%. The addition effect of alliin (0.05%) on the flavor profiles of the soups described was tested in the same way. The amino acid had *kokumi* flavors in the soups.

Discussion

Flavors of sulfur-containing components of garlic extract

In this work new types of flavor constituents in foodtuffs were found. Sulfur-containing compounds, in which we have found *kokumi* flavors, had no basic taste themselves but had the flavors when dissolved in soups or the *umami* solution. Hayashi *et al.*[15] described how L-arginine (Arg) showed a characteristic crab-like flavor in the completely reconstructed extract of sea crab, which contained L-glutamic acid. The flavor character of the sulfur-containing compounds in garlic differed from that of Arg. Although Arg is classified as a bitter amino acid, the compounds doesn't have any basic tastes. The sulfur compounds in garlic are thought to be a new type of flavor constituents.

Garlic and its extract are often used as seasonings in various dishes. In most cases, glutamate (or inosinate) might coexist with the sulfur-containing flavor constituents in garlic. The *umami* materials were speculated to enhance *kokumi* flavors of the sulfur-containing constituents. This study showed a new screening method to investigate for new types of flavors in foodstuffs.

In the results of sensory tests of each sulfur compound, alliin, GAC, and GACSO showed a garlic-like flavor and MeCSO showed a leek-like flavor in the *umami* solution (the flavors were not recognized in distilled water). It remained a possibility that some volatile compounds derived from the compounds influenced the sensory evaluation, although the tested materials were deodorized with caution before the tests.

Sulfur-containing constituents of garlic

This study revealed the composition of sulfur-containing flavor components in garlic. Alliin, MeCSO, and GAC are well known as major sulfur-containing constituents in the plant,[16] but the presence of GACSO was supposed to be first reported in this paper. However, it was not clear that this peptide existed in the intact organism or was converted from GAC by oxidation in the purification process. More studies are needed on the peptide. We detected cycloalliin as one of the major sulfur-containing components in garlic. Cycloalliin is not thought to be a precursor of the characteristic odor of garlic, but is well known to be formed from *trans*-(+)-*S*-propenyl-L-cysteine sulfoxide, a major sulfur-containing component in onion (*Allium cepa* L.), by alkaline treatment.[17] The role of cycloalliin is a very interesting subject that should be studied.

It is concluded that the characteristic flavor constituents in garlic extract are constructed of alliin and other sulfur-containing compounds.

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