Volatile Sulfur Compounds in Human Expiration after Eating Raw or Heat-Treated Garlic

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Summary Volatile sulfur compounds arising from grated raw or heat-treated garlic in both in-vitro and in-vivo tests were gas-chromatographically analyzed. In in-vitro tests, the head-space vapor gas from garlic in a vial was analyzed. It was clarified that allyl mercaptan arising from raw garlic decreased with the passage of time and other volatile low-molecular sulfur compounds (LMSC) did not show remarkable changes. The change of LMSC from heat-treated garlic was also studied. Methyl mercaptan and allyl mercaptan from heat-treated garlic gradually increased to some extent. On the other hand, the quantities of somewhat high-molecular sulfur compounds (HMSC) were much less in heat-treated garlic compared to those of raw garlic. These compounds increased till approx. 60 min and then decreased gradually. In in-vivo tests, human expiration after eating garlic was analyzed. Allyl mercaptan, methyl mercaptan and allyl methyl sulfide in LMSC were detected in significant amounts. The quantities of these compounds arising from heat-treated garlic were smaller than those from raw garlic. These compounds had the tendency of decreasing with the passage of time. On the other hand, almost no HMSC was detected in both raw and heat-treated garlic. By sensory testing, raw garlic showed a stronger smell than heat-treated garlic in both in-vitro and in-vivo tests. GC analysis exhibited higher values of volatile sulfur compounds in raw garlic. That is, the higher the volatile sulfur compound level, the stronger the garlic flavor or malodor.

Key Words raw garlic, heat-treated garlic, volatile sulfur compounds, oral malodor

Garlic (Allium sativum L.) has a specific odor. It contains allylcystein sulfoxide or alliiin, which is converted into various kinds of volatile organosulfur compounds.

According to extraction methods, the kinds of compounds and their quantity will be different. These have been separated by many researchers. Brodnitz et al (1) have reported diallyl thiosulfinate as the main odor component. Akashi et al (2) and Nishimura et al (3) have reported diallyl disulfide as one of the main odor...
components. On the other hand, Minami et al (4) have reported allyl mercaptan and diallyl disulfide as the main components of oral malodor after eating garlic.

Garlic has been cultivated and used for food since ancient times, primarily for its restorative properties. However, after eating garlic, a bad odor is generated in the mouth. For its characteristic as a material in food processing, we are interested in garlic, and specifically its functionality and odor. The present study deals with changes with time in volatile sulfur compounds arising from grated fresh or heat-treated garlic. Human expiration after eating garlic was also examined for its volatile sulfur compounds.

MATERIALS AND METHODS

Preparation of samples. Garlic cultivated in Aomori Prefecture was used for in-vitro and in-vivo tests. About 3 g of raw garlic was grated and 0.2 g was transferred into a 125 mL vial, which was then sealed tightly and allowed to stand at 23°C. On the other hand, about 3 g of garlic was heated for 1 min in a microwave oven and then crushed with a spoon. Immediately, 0.2 g was transferred into a vial, which was then sealed tightly and allowed to stand at 23°C.

In-vitro tests. One-milliliter of the head-space gas was withdrawn at regular intervals from a vial containing raw or heat-treated garlic. This gas was then subjected to gas chromatography (GC).

In-vivo tests. Each subject ate 1 g of grated raw or grated heat-treated garlic in a chewing fashion for 30 s. Her expiration after eating was trapped by the Aoki method (5) and submitted for GC analysis.

Chemicals. Methyl mercaptan, dimethyl sulfide and dimethyl disulfide were obtained from Wako Pure Chemical Industries (Osaka, Japan). Allyl mercaptan was obtained from Aldrich Chemical (USA). Allyl methyl sulfide was purchased from Tokyo Kasei Organic Chemicals (Tokyo, Japan).

Gas chromatographic conditions: apparatus, Shimadzu GC 14B; column temperature, programmed from 65°C for 3 min to 170°C for 14 min at 30°C/min; column, PPE 5 ring 10%, 3.2 mm × 3.1 m glass; detector, FPD (140°C); carrier gas, N₂ 55 mL/min.

GC-MS conditions: apparatus, Shimadzu GC-MS QP1000; column temperature, held at 60°C for 3 min, programmed from 60°C to 170°C at 30°C/min and held at 170°C for 2 min; column, fused-silica capillary column (Supelco SPB-1), 0.32 mm × 30 m; carrier gas, He (2 mL/min); EI mode, at 70 eV; source temperature, 180°C; GC-MS interface temperature, 250°C.

Sensory test. The panelists were 20 students of a junior women's college, and tests were conducted in pairs. All subjects signed a written consent form. The panelists were selected by olfactometer and triangle test.

Olfactometer. A difference test by standard odors was conducted for the selection of panel members with the concentration of β-phenylethyl alcohol 100 ppm, methyl cyclopentenolone 30 ppm, isovaleric acid 10 ppm, γ-undeca-
lactone 30 ppm, and skatole 10 ppm.

**Triangle test.** A difference test between raw and heated garlic was conducted for both the in-vitro (the weight of garlic, 0.2 g) and in-vivo (the weight of garlic, 1 g) tests.

**In-vitro tests.** About 3 g of raw garlic was grated and 0.2 g was transferred into a 125 mL vial, while about 3 g of garlic was heated for 1 min in a microwave oven and then grated. Immediately following, 0.2 g was transferred into a vial of the same size. Panelists were asked which odor was stronger.

**In-vivo tests.** Four students who had almost no oral malodor were divided into two groups: One group ingested 1 g of grated raw garlic, and the other ingested 1 g of grated heated garlic with chewing for 30 s. After 5 min, panelists were asked which oral malodor was stronger.

### RESULTS AND DISCUSSION

**In-vitro experiment**

The enzyme “alliinase” in raw garlic is activated by grating or cutting and the formation of allicin from alliin is brought about. By chemical reactions following the enzymatic reaction, somewhat high-molecular sulfur compounds (HMSC), such as allyl methyl disulfide, diallyl disulfide and so on, which have specific odor and sharp taste, are finally produced (5). What kind of volatile sulfur compounds arise from grated or cut garlic?

In in-vitro tests, volatile sulfur compounds (VSC) arising from grated raw garlic and heat-treated garlic were analyzed by gas chromatography (Fig. 1). Nine peaks were found and peak numbers 1, 2, 3, 4, 5, 6, 7, 8, and 9 corresponded to methyl mercaptan, dimethyl sulfide, allyl mercaptan, allyl methyl sulfide, dimethyl disulfide, allyl methyl disulfide, methyl propyl sulfide, diallyl disulfide, and 3-(allylthio)propionic acid, respectively.

A study was conducted on the difference between VSC in raw garlic and heated garlic immediately after garlic is grated. The results of the in-vitro test showed the presence of methyl mercaptan, dimethyl sulfide and allyl mercaptan in slightly larger quantities than allyl methyl sulfide and dimethyl disulfide in both raw and heat-treated garlic (Fig. 2). The production of methyl mercaptan was conspicuous in both grated raw and heat-treated garlic. The production of this substance by the cutting of heated garlic had been reported by Yu et al (6), and was ascertained in the present study. The production of allyl mercaptan in grating raw and heated garlic has already been reported in literature (6–8). Our observation was in agreement with previous studies. The presence of a trace amount of dimethyl disulfide in raw and heated garlic has been reported in literature (9, 10). The presence of allyl methyl sulfide in both raw and heat-treated garlic has been reported in literature (10, 11), and similar results were also obtained in this study. As a result of this experiment, it can be concluded that there is little difference in low-molecular sulfur compounds (LMSC) relative to HMSC between raw and heat-treated garlic after grating.
Fig. 1. Gas chromatograms of the head-space vapors sampled just after grating raw garlic (left) and heat-treated garlic (right). Analytical conditions are described in the text. Peak components were as follows: 1, methyl mercaptan; 2, dimethyl sulfide; 3, allyl mercaptan; 4, allyl methyl sulfide; 5, dimethyl disulfide; 6, allyl methyl disulfide; 7, methyl propyl disulfide; 8, diallyl disulfide; and 9, 3-(allylthio)propionic acid.

Allyl mercaptan in grated raw garlic was detected at 0 min, which showed that grating induced a rapid conversion from alliin to allyl mercaptan. Further, allyl mercaptan readily converts to diallyl disulfide. Although the amount of dimethyl sulfide began to increase after 60 min, other LMSC did not show remarkable changes. Methyl mercaptan and allyl mercaptan amounts from heat-treated garlic increased somewhat with the passage of time. The tendency of the mercaptan quantities to increase with time was considerably greater for heated garlic as compared to raw garlic. Therefore, it can be assumed that the accelerated production of allyl mercaptan was attributed to physicochemical conditions such as heating and its accompanying reactions rather than enzymatic reactions. Dimethyl sulfide and dimethyl disulfide did not show any remarkable change with time.

However, a clear difference in HMSC between raw and heated garlic was observed immediately after grating, as shown in Table 1. In raw garlic after grating, four components were detected (Table 1). On the other hand, in heat-treated garlic, the presence of diallyl disulfide (the main decomposition product from alliin) and allyl methyl disulfide were conspicuous in HMSC. Their quantities were found to be much less in heated garlic as compared to raw garlic. Further, these compounds were not detected when heating time was prolonged (data not shown). The formation of these HMSC would be stopped probably by thermal inactivation of the enzyme.
Fig. 2. Time course of LMSC formation in raw garlic and heat-treated garlic (in-vitro test). Analytical conditions were the same as in Fig. 1, except for sampling at indicated times: ○●, methyl mercaptan; △▲, dimethyl sulfide; ■■, allyl mercaptan; ◆◆, allyl methyl sulfide; and *, dimethyl disulfide. Solid and broken lines represent grated heat-treated and raw garlic, respectively.

The changes of HMSO with time were investigated in the same way as above. In the case of raw garlic, almost all compounds showed a tendency to increase until 60 min after grating and then to decrease gradually. When raw garlic was grated, the change of alliin into allicin occurred immediately, followed by conversion into HMSO. It took about 60 min to reach the maximum of HMSC formation.

In the case of heat-treated garlic, allyl methyl disulfide and methyl propyl disulfide increased until 30 min and then decreased gradually. However, other components were scarcely detected. Comparing the time courses of HMSC between raw and heated garlic, the value in the former remained higher than that in the latter.

**In-vivo experiment**

Oral malodor, a general term including all breath air and bad odor generated in the mouth, is classified into physiological breath air and pathological oral malodor. There have been many studies regarding the latter, but not the former. Methyl mercaptan has been recognized as one of the main components of pathological oral malodor or breath air of healthy people (12, 13).
Table 1. Time course of HMSC formation in raw garlic and heat-treated garlic (in-vitro test).

<table>
<thead>
<tr>
<th>Compound</th>
<th>Time (min)</th>
</tr>
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<tbody>
<tr>
<td></td>
<td>0</td>
</tr>
<tr>
<td>Raw garlic</td>
<td></td>
</tr>
<tr>
<td>Allyl methyl disulfide</td>
<td>31.5</td>
</tr>
<tr>
<td>Methyl propyl disulfide</td>
<td>8.80</td>
</tr>
<tr>
<td>Diallyl disulfide</td>
<td>142</td>
</tr>
<tr>
<td>3-(Allylthio)propionic acid</td>
<td>171</td>
</tr>
<tr>
<td>Heat-treated garlic</td>
<td></td>
</tr>
<tr>
<td>Allyl methyl disulfide</td>
<td>4.24</td>
</tr>
<tr>
<td>Methyl propyl disulfide</td>
<td>tr.</td>
</tr>
<tr>
<td>Diallyl disulfide</td>
<td>2.65</td>
</tr>
<tr>
<td>3-(Allylthio)propionic acid</td>
<td>nd.</td>
</tr>
</tbody>
</table>

These high-molecular sulfur compounds (HMSC) were characterized and quantified by GC-MS under the analytical conditions described in the text. Values are expressed in $\mu V \cdot S \times 10^{-3}$ as FPD response; tr. and nd. represent ‘trace’ and ‘not detected,’ respectively.

Fig. 3. Gas chromatograms of the expirations of women after eating raw garlic (left) and heat-treated garlic (right). Experimental conditions were the same as in Fig. 1, except for sampling of human expiration instead of the head-space vapor. Peak components were the same as in Fig. 1.
It has been reported that a large quantity of allyl mercaptan is immediately generated in the mouth when raw garlic is eaten (9, 10), but there is little information on other LMSC. For this reason, VSC in human expiration after eating grated raw garlic or heat-treated garlic was analyzed.

Typical gas chromatograms of raw and heat-treated garlic in the in-vivo test are shown in Fig. 3. Eight components were detected from the breath air after eating raw garlic. However, the breath air from a subject who took heat-treated garlic did not contain allyl methyl disulfide, methyl propyl disulfide, diallyl disulfide or 3-(allylthio)propionic acid. It is also worth noting that methyl mercaptan has been shown to be a major cause of oral malodor (11, 12). In the present experiment, the amount of methyl mercaptan in the breath air of a subject before eating garlic proved to be negligibly small according to gas chromatography (Fig. 4).

The difference in expiratory VSC immediately after eating both raw and heated garlic was studied. The results of the LMSC content of the breath air after eating raw or heated garlic are shown in Fig. 5. Methyl mercaptan and allyl methyl sulfide, in addition to allyl mercaptan, were detected in both cases. On the other hand, dimethyl sulfide and dimethyl disulfide were not detected. Furthermore, methyl mercaptan and allyl mercaptan from heat-treated garlic were about 0.05 ppm, which is not so large as the value for raw garlic.

In in-vivo tests, LMSC were found in large amounts immediately after eating raw garlic and then decreased rapidly. However, dimethyl sulfide and dimethyl
disulfide were not detected. In LMSC from heat-treated garlic, methyl mercaptan, allyl mercaptan and allyl methyl sulfide showed a significant decrease within 30 min and from that time continued to decrease gradually (Fig. 5).

Diallyl disulfide, allyl methyl disulfide and 3-(allylthio)propionic acid in HMSC were detected at 0 min immediately after eating raw garlic. No HMSC were detected except for 0 min. HMSC from heat-treated garlic were not detected at all, suggesting that HMSC would not be derived enzymatically within the body (Table 2).

**Relationship between sensory test and GC analysis**

A sensory test was conducted and the relation between sensory test results (Table 3) and GC analysis was studied. In the sensory test, the strength of garlic odor was evaluated. Raw garlic showed a stronger smell than heat-treated garlic for both in-vivo and in-vitro tests.

Regarding the relation between the sensory test (Table 3) and GC analysis, eating raw garlic provided a stronger smell than eating heat-treated garlic. In in-vitro tests, GC analysis revealed higher values of HMSC in raw garlic, while there was not such a large difference in LMSC between raw and heated garlic.

In in-vivo tests, GC analysis showed especially higher values of LMSC after
Table 2. Time course of HMSC formation in raw garlic and heat-treated garlic (in-vivo test).

<table>
<thead>
<tr>
<th>Compound</th>
<th>Time (min)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0</td>
</tr>
<tr>
<td>Raw garlic</td>
<td></td>
</tr>
<tr>
<td>Allyl methyl disulfide</td>
<td>184</td>
</tr>
<tr>
<td>Methyl propyl disulfide</td>
<td>nd.</td>
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<tr>
<td>Diallyl disulfide</td>
<td>340</td>
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<tr>
<td>3-(Allylthio)propionic acid</td>
<td>113</td>
</tr>
<tr>
<td>Heat-treated garlic</td>
<td></td>
</tr>
<tr>
<td>Allyl methyl disulfide</td>
<td>nd.</td>
</tr>
<tr>
<td>Methyl propyl disulfide</td>
<td>nd.</td>
</tr>
<tr>
<td>Diallyl disulfide</td>
<td>nd.</td>
</tr>
<tr>
<td>3-(Allylthio)propionic acid</td>
<td>nd.</td>
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</tbody>
</table>

Analytical conditions were the same as in Table 1. Values are expressed in µV·S as FPD response; nd. represents 'not detected.'

Table 3. Sensory test for the perception of garlic odor in head-space vapor or human expiration.

<table>
<thead>
<tr>
<th></th>
<th>Raw garlic</th>
<th>Heat-treated garlic</th>
</tr>
</thead>
<tbody>
<tr>
<td>Head-space vapor</td>
<td>20*</td>
<td>0</td>
</tr>
<tr>
<td>Expiration after eating</td>
<td>20*</td>
<td>0</td>
</tr>
</tbody>
</table>

*All the panelists (students) perceived the head-space vapor from raw garlic or the expiration of women who ate raw garlic to be stronger of garlic odor in either case.

eating raw garlic than in the case of eating heated garlic. HMSC from both raw and heated garlic were almost not detectable. Nevertheless, it may well be said that a stronger smell originated from VSC as a whole rather than in the minority.

What is the difference in VSC between oral malodor from eating garlic and pathological breath? In in-vivo tests, methyl mercaptan, allyl mercaptan and allyl methyl sulfide were detected in human expiration after eating garlic. Tonzetich (11) has detected hydrogen sulfide, methyl mercaptan, and dimethyl sulfide in pathological breath air, and Tsunoda (12) also has confirmed the presence of these VSC. The presence of allyl compounds are caused by eating garlic, and these compounds are not present in pathological breath air. In many cases, there exists a significant difference in quality between oral malodor after eating garlic and pathological breath air.
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