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ORIGINAL ARTICLE

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The smell and odorous components of dried shiitake mushroom, *Lentinula edodes* I: relationship between sensory evaluations and amounts of odorous components

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Abstract The smell of food is one of the most important factors in assessing its quality. Concerning the smell of dried shiitake mushrooms [*Lentinula edodes* (Berk.) Pegler], 1,2,3,5,6-pentathiepane, commonly known as lenthionine, has been reported as a key compound. However, other compounds have not been studied sufficiently in connection with smell. From the results of sensory intensity studies and sensory evaluations of dried shiitake mushrooms, a positive significant correlation at 1% risk was observed between sensory intensity and sulfur perception. This showed that the smell of dried shiitake mushrooms was characterized by a sulfurous smell. Also, comparing the sensory intensity with the amounts of volatile components showed positive significant correlations at 1% risk between sensory intensity and three compounds: 1,2,4-trithiolane, 1,2,4,6-tetrathiepane, and lenthionine. Furthermore, significant correlations at 5% risk were obtained between the amounts of these three compounds and sensory intensity by multiple regression analysis. This showed that the smell of dried shiitake mushrooms depended on these compounds. The partial regression coefficient of 1,2,4-trithiolane was larger than those of the others, and so it was proposed that 1,2,4-trithiolane could serve as an indicator to estimate the smell of dried shiitake mushroom.

Key words Sensory evaluation · 1,2,4-Trithiolane · 1,2,4,6-Tetrathiepane · Lenthionine · Dried shiitake mushroom

Introduction

The shiitake mushroom [*Lentinula edodes* (Berk.) Pegler] is one of the most popular edible mushrooms in Japan and other parts of the Far East. Dried shiitake has been especially prized since ancient times, because the dried mushroom has some characteristics that differ from the fresh shiitake mushroom. One example is that the amounts of nutritious compounds, such as 5′-guanylic acid and vitamin D_2 in dried shiitake mushroom, are higher than in fresh shiitake mushroom. Another is that dried shiitake mushroom has a characteristic smell, which is absent in the fresh mushroom. Many studies have been conducted on the volatile compounds of dried shiitake mushroom and lenthionine has been isolated as an odorous compound of dried shiitake.[1,2] For lenthionine, its stereochemical structure,[3] chemical properties,[2,4] and precursors[5–8] were analyzed, and the effects of pH,[9] temperature, and soaking time on exudation of lenthionine were studied to estimate food quality.[4,10,11] Besides lenthionine, it was reported that some cyclic sulfur compounds, such as 1,2,4,6-tetrathiepane and 1,2,3,4,5,6-hexathiepane, existed in dried shiitake,[12–15] and that eight-carbon alcohols were major components of the smell of fresh shiitake mushrooms.[15–18] However, these compounds were not reported as odorous compounds of dried shiitake mushrooms.

To assess the quality of food, smell is one of the most important factors, and no less than taste.[19–21] In addition, the preferences and criteria of consumers regarding smell vary according to fashion, sex, age, and other factors. Concerning the smell of dried shiitake mushrooms, consumers pay great attention, discuss widely, and desire the ability to select them according to their preference. However, past improvements of shiitake have been forced on the shapes of the fruiting body, the amounts of production per log, and others, because dried shiitake mushrooms are mostly evaluated in markets by their shape and not by qualities such as smell, taste, and texture. To provide dried shiitake mushrooms matching consumer preferences, improvements in qualities and indexes of qualities are needed. There have only been a few studies on the smell and taste of dried shiitake mushroom.[22,23]

For the analysis of volatile compounds, samples are usually prepared by steam distillation or by extraction, using organic solvents, from the fruiting body.[1,2,9,10,12–18] This

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means that the number of treated samples is limited because of complicated pretreatment and that samples are extracted excessively. To evaluate the product as a food, it is thought that not only the smell of the dried mushrooms, but also that of the resulting soup is an important factor. Because the amounts of volatile compounds in the soup are very small, Sakui et al.[24] and Miettinen et al.[25] reported simple, easy, and highly sensitive methods of analyzing flavor components by using a gas chromatography-mass spectrometer with a headspace sampler.

We aimed to create dried shiitake mushrooms with a smell that matches consumer preferences. At first we examined the relationship between the evaluation of smell and the analytical results of volatile components in exuded soup, and clarified the key compounds of smell therein.

Materials and methods

Microorganisms

The strains of shiitake mushrooms used in this study were derived from stock cultures of the Mushroom Science Laboratory, Forestry and Forest Products Research Institute, Independent Administrative Institution, Japan. Among 246 shiitake mushroom strains, 52 strains were selected based on the result of a selective experiment that was conducted to obtain fruiting bodies on sawdust media with certainty. Finally, fruiting bodies of 15 strains, forestry mycology code (FMC) numbers 48, 126, 129, 140, 152, 158, 174, 180, 181, 194, 201, 208, 383, 386, and 392, were randomly selected and used in this study.

Cultivation conditions

The sawdust of Japanese beech (*Fagus crenata*) and rice bran were mixed in the ratio 3:1 (w/w) and the moisture content was adjusted to about 65% (w/w). The prepared 1-kg sawdust medium was packaged in a plastic bag (Hokken) for the fruiting body formation tests. These were autoclaved at 120°C for 60 min. Each strain was precultured in liquid medium containing 1% sucrose, 1% malt extract, and 0.4% yeast extract at 25°C for 2 weeks under darkness with intermittent shaking. The sterilized sawdust media was inoculated with homogenized mycelia. After incubation at 22°C under darkness for 4 months, the plastic bag was removed from the medium and the medium was placed in a room with the temperature at 15°C and the relative humidity at 95% under fluorescent illumination. Fruiting bodies were harvested when the cap was 80% open, and then weighed, and dried at 60°C for 24 h. The average yields of fruiting body of FMC 48, 126, 129, 140, 152, 158, 174, 180, 181, 194, 201, 208, 383, 386, and 392 were 118, 220, 127, 108, 144, 175, 243, 87, 196, 149, 129, 183, 190, 147, and 134 g (wet weight) per 1 kg sawdust medium, respectively.

Analysis of sensory intensity and sensory evaluation

The dried shiitake mushrooms were selected randomly and powdered. A suspension containing 0.3% (w/v) powder in water was left at 60°C for 30 min and then filtered. Sodium chloride was added to the filtrate at a final concentration of 0.4% (w/v). Eight flavorists conducted sensory intensity and sensory evaluations. The following four adjectives for sensory evaluations were prepared: sulfur perception, woody perception, fresh-shiitake perception, and soil-like perception. The ranking of the sensory intensity and sensory evaluations was as follows: 1 almost no smell, 2 very weak smell, 3 weak smell, 4 moderate smell, 5 strong smell, 6 very strong smell, and 7 extremely strong smell. All scores of sensory intensity and sensory evaluations were shown as the averaged values of the results from eight flavorists.

Analysis of odorous compounds

A part of the dried shiitake mushroom powder was dried at 105°C to obtain a constant weight. One gram of powder was suspended in 10 ml of water. The suspension was heated using a 600-W microwave oven for about 15 s, kept at 60°C for 30 min, and centrifuged at 39 800 *g* for 20 min. To a solution of the resulting 5-ml supernatant was added 1 μg benzophenone as an internal standard and 3 g of sodium chloride in a 20-ml glass headspace vial. Identification and quantification of the odorous compounds in the headspace gas were conducted with a headspace sampler (HS: HS 40XL, Perkin Elmer), gas chromatograph with cryofocusing system (GC: AutoSystem XL, Perkin Elmer) and mass spectrometer (MS: TurboMass, Perkin Elmer). The HS conditions were as follows: carrier gas pressure 140 kPa, oven temperature 100°C, and equivalent time 60 min. The GC conditions were as follows: column OV-1701 25 m $\times$ 0.25 mm i.d. (GL Sciences), inlet temperature 250°C, inlet pressure 55 kPa, precryofocusing time 5 min (the column temperature was finally down to −55°C), cryofocusing time 1 min and oven temperature held for 10 min at 40°C then elevated to 260°C, increasing at a rate of 6°C/min. The MS conditions were as follows: electron ionization, ionization voltage 70 eV. Some components were identified by comparisons with the retention times and mass spectra of authentic compounds.

Calculations

All statistical treatments were conducted using SPSS for Windows version 11.0 J (SPSS Inc.).

Synthesis of cyclic sulfur compounds

1,2,4-Trithiolane and 1,2,4,6-tetrathiepane were synthesized by the modified published method.[1,12,26,27] A solution of Na_4S_5 was prepared from 30 g sodium sulfide nonahydrate and 6 g sulfur in 100 ml of water, through which was bubbled a stream of hydrogen sulfide to adjust the pH of the solution to 8.5. To the solution was added 20 g of 37% formaldehyde solution and it was then covered by 100 ml of chloroform. The mixture was vigorously stirred at room temperature for several hours. The chloroform layer was separated, washed, and dried over sodium sulfate. Excess chloroform was removed by rotary evaporator and the remaining oily liquid was separated on silica gel (Silica Gel 60 F_{254}; Merck) by elution with tetrachloromethane. Lenthionine was synthesized according to the methods of Morita and Kobayashi.[1] The synthesized compounds were analyzed by gas chromatography–mass spectrometry (GC-MS: DX-303, JEOL). The GC and MS conditions were as follows: column OV-1701 25 m × 0.25 mm i.d. (GL Sciences), inlet temperature 200°C, inlet pressure 114 kPa, oven temperature started at 80°C and elevated to 230°C by increasing at a rate of 10°C/min, electron ionization, and ionization voltage 70 eV. As the result of high-resolution mass spectrometric analysis, molecular ion peaks of 1,2,4-trithiolane, 1,2,4,6-tetrathiepane, and lenthionine were found at *m/z* 123.9473, 169.9352, and 187.9086, respectively. The calculated values for $C_2H_4S_3$, $C_3H_6S_4$, and $C_2H_4S_5$ were *m/z* 123.9475, 169.9352, and 187.8916, respectively.

Results and discussion

Characteristics of dried shiitake mushroom

The scores of the sensory intensity and sensory evaluations of dried shiitake mushrooms are shown in Table 1. The correlation between sensory intensity and sulfur perception was 0.97, woody perception 0.33, fresh-shiitake perception 0.51, and soil-like perception −0.65. A significant correlation was seen only between the sensory intensity and the sulfur perception at 1% risk. Subsequently, ten randomly selected dried shiitake mushrooms were assessed by two factors: sensory intensity and sulfur perception. The correlation coefficient between sensory intensity and sulfur perception was 0.919 and a significant correlation was observed at 1% risk (Fig. 1).

The results showed that the sensory intensity was characterized by sulfur perception. It is generally thought that sulfur perception is caused by hydrogen sulfide and sulfur dioxide, and other odorous sulfur compounds, and causes a negative impression of food. However, it is also known that very small amounts of methyl β-methyl-thiopropionate and *p*-menthen-8-thiol generate flavors of pineapple and grapefruit, respectively.[28] Concerning dried shiitake mushroom, lenthionine was reported as an odorous compound,[1,2,10] and related compounds, such as 1,2,4,6-tetrathiepane, were suggested as odorous compounds.[8,12,13] However, the relationship between sulfur-containing compounds and the smell of dried shiitake mushroom have not been confirmed except for the known effects of lenthionine.[3,10,11]

Key components of smell in dried shiitake mushroom

Before the analysis of volatile compounds, analytical conditions were investigated. When the HS conditions were set at oven temperatures from 60°C to 100°C at 20°C intervals and corresponding times from 30 min to 60 min at 15-min intervals, only one weak peak was observed at 100°C and 60 min. Sakui et al.[24] reported that adding sodium chloride was effective for analyzing headspace gas. After the addition of sodium chloride to samples, over 300 peaks were observed.

The volatile compounds of the same samples used for sensory intensity were analyzed. Some peaks were observed within 5 min, the major components of which were identified as acetaldehyde, hydrogen sulfide, ethanol,

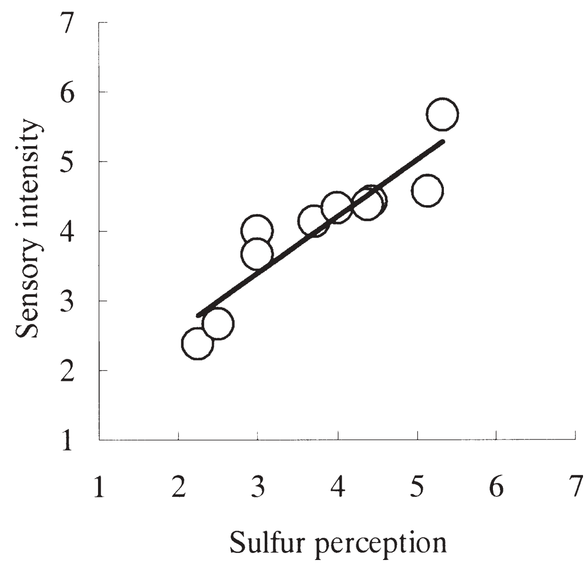

Fig. 1. The relation between sensory intensity (SI) and sulfur perception (SP) of dried shiitake mushroom. The correlation coefficient was 0.919 and the regression equation was SI = 0.814 SP + 0.951

Table 1. Relations between scores of sensory intensity and sensory evaluations with dried shiitake mushroom

Strain no.	Sensory intensity	Sulfur perception*	Woody perception	Fresh-shiitake perception	Soil-like perception
181	2.88	3.13	3.13	3.75	3.00
386	3.38	3.38	3.75	3.38	3.38
174	4.17	3.83	4.17	2.83	2.83
180	4.33	4.33	3.17	4.33	2.67
201	4.67	4.33	3.67	5.33	2.83

* Significant correlation between sensory intensity and sulfur perception at 1% risk

isovaleraldehyde, and 2-methylbutyraldehyde. Because these compounds did not have the smell of dried shiitake mushroom, the peaks that appeared after 5 min were also examined. An example of the resulting GC-MS analyses is shown in Fig. 2. Two hundred and ninety-nine peaks were observed from 5 min to 40 min and 65 major peaks were selected because they had sufficient peak area for analysis and were found in more than half of the samples. Each peak area ratio was obtained as follows.

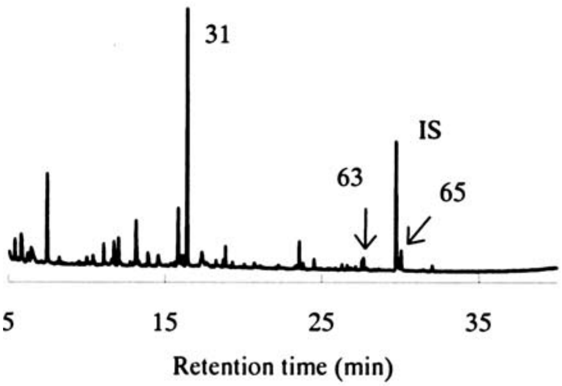

Fig. 2. An example of a total ion chromatogram of the volatile components in dried shiitake mushroom. The numbers on the figure show peak no. and *IS* indicates internal standard

$$\text{peak area ratio} = \frac{\text{peak area of compound}}{\text{peak area of internal satndard} \times \text{sample weight}} \quad (1)$$

It is known that a linear regression exists between sensory intensity and the logarithm of compound concentration, as given by Weber-Fechner's law. Therefore, it was assumed that a linear regression existed between scores of sensory intensity and the logarithm of the peak area ratios. Correlation coefficients were calculated using data of ten samples estimated by eight flavorists (Table 2). Among the 65 peaks, 1 peak (no. 10) showed a significant negative correlation at 5% risk, with a correlation coefficient of −0.735. On the other hand, 3 peaks (nos. 31, 63, and 65) showed a significant positive correlation at 1% risk, with correlation coeffi-

Table 2. Correlation coefficients between the peak area ratios and scores for sensory intensity

Peak no.	Retention time (min)	Correlation coefficient	*n*	Peak no.	Retention time (min)	Correlation coefficient	*n*
1	5.876	0.382	10	34	17.630	0.474	10
2	6.277	0.222	7	35	18.045	−0.593	8
3	6.606	0.558	10	36	18.285	−0.207	9
4	7.450	0.601	10	37	18.721	0.221	7
5	7.701	−0.805	6	38	18.881	0.577	7
6	8.115	−0.402	7	39	19.331	0.103	6
7	8.221	0.335	8	40	19.794	−0.2	7
8	8.596	−0.452	7	41	20.076	−0.2	9
9	9.055	−0.002	8	42	20.391	−0.271	8
10	9.498	−0.735**	8	43	20.729	0.093	8
11	10.058	−0.133	10	44	20.939	−0.16	8
12	10.297	0.480	8	45	21.079	−0.312	7
13	10.845	−0.412	8	46	21.468	0.056	7
14	11.076	0.418	9	47	21.648	−0.444	8
15	11.721	0.465	10	48	22.071	0.121	8
16	12.016	0.563	10	49	22.263	0.147	6
17	12.166	−0.404	9	50	22.566	0.166	8
18	12.767	−0.242	8	51	23.273	0.433	6
19	12.973	0.128	8	52	23.591	0.469	10
20	13.151	0.390	10	53	23.834	0.506	8
21	13.318	−0.137	7	54	24.519	0.508	6
22	13.735	−0.186	8	55	24.984	−0.467	9
23	13.954	−0.198	10	56	25.414	−0.344	8
24	14.345	0.105	8	57	25.621	−0.454	6
25	14.881	−0.046	7	58	25.997	−0.366	6
26	15.064	−0.260	7	59	26.290	0.588	7
27	15.325	0.425	8	60	26.624	−0.124	10
28	15.500	0.075	8	61	26.994	0.096	8
29	15.842	0.044	10	62	27.436	0.66	7
30	16.097	0.353	9	63	27.664	0.831*	10
31	16.413	0.878*	10	64	29.147	−0.267	9
32	16.981	0.003	7	65	30.067	0.822*	10
33	17.376	0.482	10				

The sensory intensity was obtained as described in Materials and methods. The peak areas of the volatile compounds in dried shiitake mushrooms and that of the internal standard were obtained by GC-MS analysis. The peaks were numbered from 5 min. Each peak area ratio was calculated from the peak area divided by that of the internal standard and dried sample weight. Each correlation coefficient was obtained from peak area ratio and the sensory intensity

n, Numbers of samples in which a corresponding peak was observed

* Significant correlation at 1% risk

** Significant correlation at 5% risk

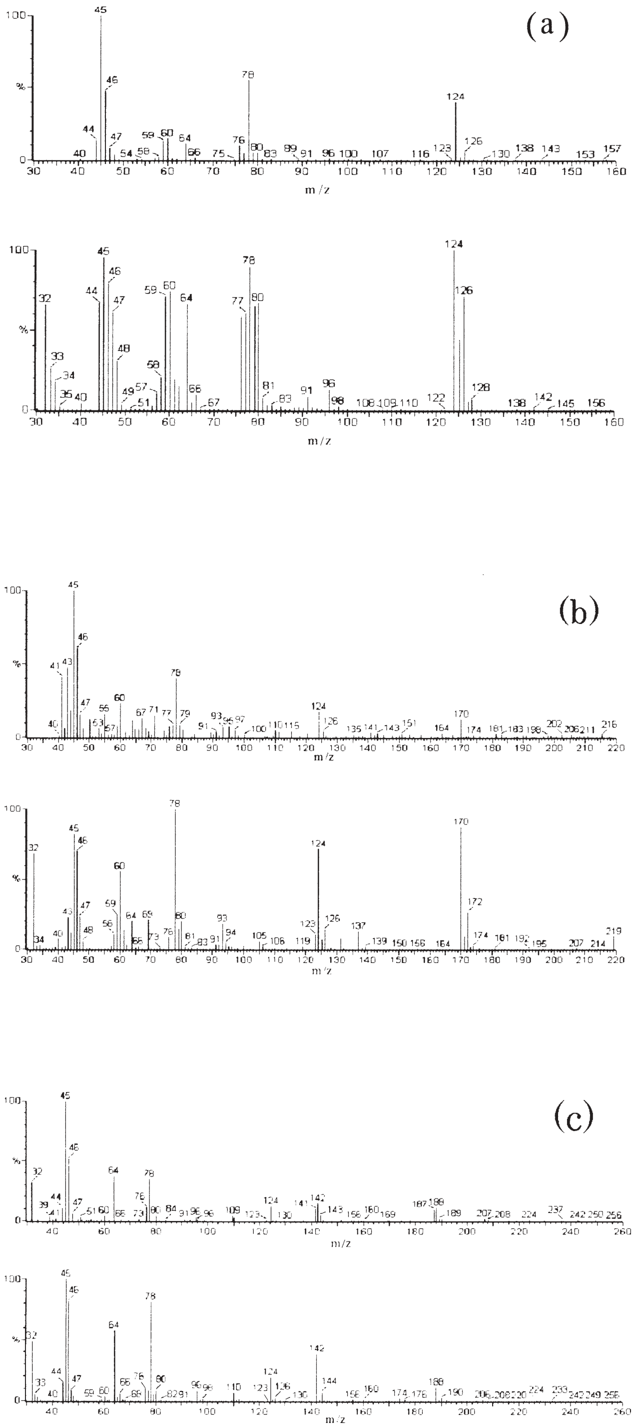

Fig. 3. Mass spectra of **a** 1,2,4-trithiolane, **b** 1,2,4,6-tetrathiepane, and **c** lenthionine. For each figure section, the *upper* mass spectrum shows the detected compounds in the volatile components and *lower* shows the related authentic compounds

cients of 0.878, 0.831, and 0.822, respectively. The compounds that showed negative correlation or no correlation did not contribute a positive effect to sensory intensity, so the authors focused on the compounds that showed a positive correlation. By comparing mass spectral data and retention times of peak nos. 31, 63, and 65 with published data[12,13,15,29] and authentic compounds, peak no. 31 was assigned to 1,2,4-trithiolane (Fig. 3a), no. 63 to 1,2,4,6-tetrathiepane (Fig. 3b), and no. 65 to lenthionine (Fig. 3c). It was reported that lenthionine had completely decomposed under analytical conditions similar to our study,[4] however, the cyclic sulfur compounds involving lenthionine were detected in our experiments. Not only lenthionine, but also 1,2,4-trithiolane and 1,2,4,6-tetrathiepane generally gave a smell of dried shiitake mushroom.

The correlation coefficients between the actual amounts of the three compounds and sensory intensity were recalculated and the relations are shown in Fig. 4. The correlation coefficients between the amounts of 1,2,4-trithiolane, 1,2,4,6-tetrathiepane, and lenthionine and sensory intensity were 0.871, 0.806, and 0.765, respectively. All correlations were significant at 1% risk.

Sulfur-containing substances were also reported to exist in dried shiitake as volatile compounds,[12–15] and only 1,2,4,5-tetrathiane was observed during our GC-MS analysis (peak no. 54) in addition to the above three compounds and hydrogen sulfide. However, it was obtained in only 6 of 10 strains, its peak area ratio was smaller than that of lenthionine and others, and it showed no significant correlation with sensory intensity. 1,2,4-Trithiolane, which has a characteristic "egg aroma" and gave a garlic odor in the synthesis process,[27] had not been reported as a characteristic flavor compound in shiitake. No reports regarding the smell of 1,2,4,6-tetrathiepane have been found. Because 1,2,4-trithiolane, 1,2,4,6-tetrathiepane, and lenthionine had significant correlations with sensory intensity tests of dried shiitake mushroom, our results showed that not only lenthionine, but also 1,2,4-trithiolane and 1,2,4,6-tetrathiepane are characteristic flavor compounds of dried shiitake mushrooms.

Alcohols, ketones, and aldehydes are reported to be present in dried shiitake mushrooms[17,19] and some peaks observed in our study were caused by such compounds. However, these compounds did not show significant correlations with sensory intensity in our experiments. It was thought that these compounds were not sufficiently accumulated to affect sensory intensity, or inherently had no effect on the sensory intensity of the unique smell of dried shiitake mushroom.

Relation with sulfur-containing compounds

The correlation coefficients between the amount of hydrogen sulfide, 1,2,4-trithiolane, 1,2,4,6-tetrathiepane and lenthionine (Table 3) showed significant positive correlation at 1 % risk, reciprocally. Lentinic acid, 2-(γ-glutamylamino)-4,6,8,10,10-pentaoxo-4,6,8,10-tetraundecanoic acid, had been reported as a precursor of lenthionine[5,6,8] and it was proposed that the pathway of lenthionine synthesis is roughly divided into two stages.[15] In the first stage, lentinic acid is converted to methyl disulfide with the help of γ-glutamyltransferase and *S*-alkyl-L-cysteine sulfoxide lyase.[30–32] In the second stage, cyclic and linear sulfur compounds are synthesized as the result of polymerization of the methyl disulfide.[13] Our results supported that 1,2,4-

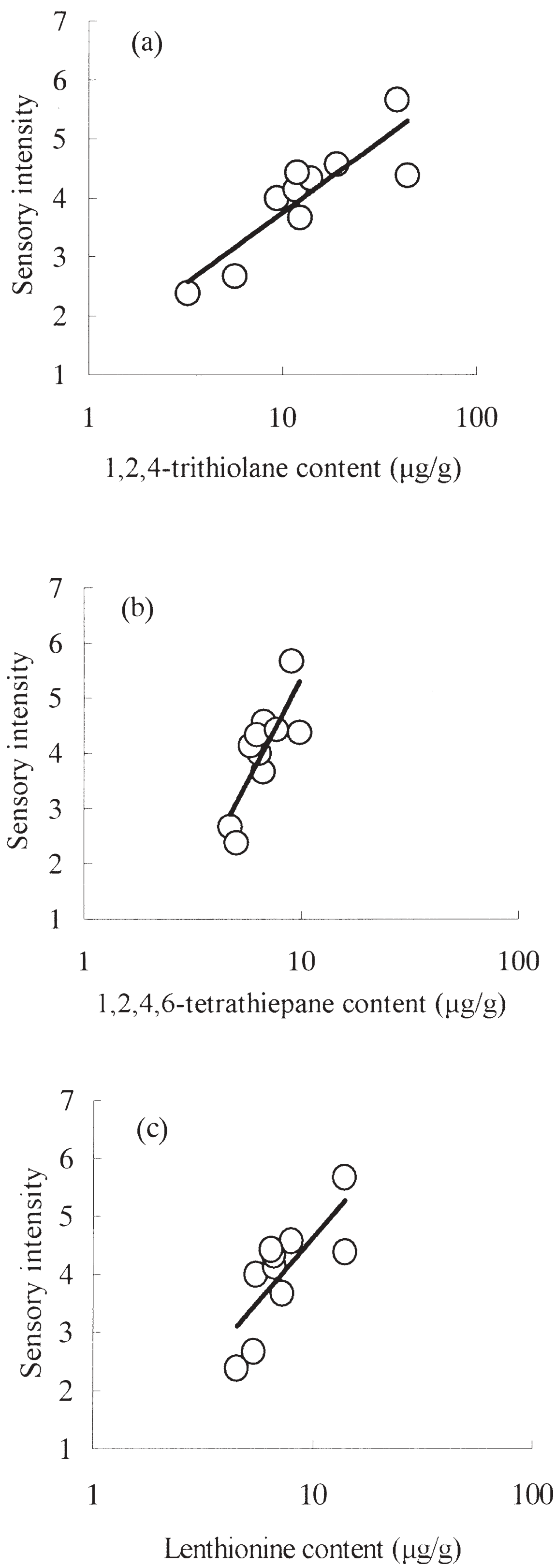

Fig. 4a–c. Relations between the amounts of compounds and average sensory intensity as evaluated by eight flavorists using ten dried shiitake mushroom samples. **a** 1,2,4-trithiolane (TL) [sensory intensity = 1.048 ln(TL) + 1.341, $R = 0.871$, $P < 1\%$], **b** 1,2,4,6-tetrathiepane (TP) [sensory intensity = 3.285 ln(TP) −2.201, $R = 0.806$, $P < 1\%$], **c** lenthionine (LE) [sensory intensity = 1.916 ln(LE) + 0.217, $R = 0.765$, $P < 1\%$]

trithiolane, 1,2,4,6-tetrathiepane, and lenthionine are synthesized from the same precursor, because the amount of 1,2,4-trithiolane was usually larger than that of the others, and the amount of lenthionine was usually a little larger than that of 1,2,4,6-tetrathiepane (Fig. 5). Shieh and Sumimoto[15] reported that 1,2,4-trithiolane was the largest compound among sulfurous compounds in dried shiitake mushrooms. It was also reported that pH affected the composition of sulfurous compounds during chemical synthesis.[9] It was thought that 1,2,4-trithiolane was synthesized preferentially over other cyclic sulfurous compounds during the second stage.

Hydrogen sulfide showed correlations with 1,2,4-trithiolane, 1,2,4,6-tetrathiepane, and lenthionine. Because lenthionine decomposes and is converted to hydrogen sulfide and thiocarbonyl compound,[4] it was assumed that the correlation was due to the decomposition of cyclic sulfur compounds during the analytical process.

To determine the effect of each compound on the sensory intensity, multiple regression analysis was conducted using the data for ten samples that were assessed by eight flavorists. The regression equation was

$$y = 1.52\ln(a) + 0.17\ln(b) - 0.84\ln(c) \qquad R = 0.905 \qquad (2)$$

where y is sensory intensity, and a, b, and c are the amounts of 1,2,4-trithiolane, 1,2,4,6-tetrathiepane, and lenthionine, respectively, with all data standardized. The multiple correlation coefficient was 0.905 and there was a significant correlation at 5% risk. The partial regression coefficients of 1,2,4-trithiolane, 1,2,4,6-tetrathiepane, and lenthionine were 1.52, 0.17, and −0.84, respectively. The result showed that the most effective compound toward the sensory intensity was 1,2,4-trithiolane.

The authors aimed to create dried shiitake mushrooms with a smell that is able to match consumer preferences and it was proposed that 1,2,4-trithiolane could serve as an indicator to estimate the smell of dried shiitake mushroom.

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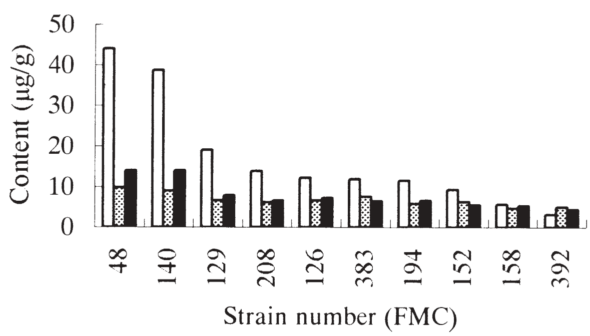

Fig. 5. Amounts of 1,2,4-trithiolane (*open bars*), 1,2,4,6-tetrathiepane (*gray bars*), and lenthionine (*filled bars*) in ten different dried shiitake mushrooms. *FMC*, forestry mycology code

Table 3. Correlation coefficients among sulfur-containing components in dried shiitake mushroom

Component	Hydrogen sulfide	1,2,4-Trithiolane	1,2,4,6-Tetrathiepane	Lenthionine
Hydrogen sulfide	1	0.972*	0.845*	0.918*
1,2,4-Trithiolane	–	1	0.894*	0.958*
1,2,4,6-Tetrathiepane	–	–	1	0.874*
Lenthionine	–	–		1

Correlation coefficients were calculated from the compound contents
* Significant correlation at 1% risk

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