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ORIGINAL ARTICLE

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The smell and odorous components of dried shiitake mushroom, *Lentinula edodes* III: substances that increase the odorous compound content

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Abstract This study formed part of an effort to improve the quality of dried shiitake mushroom [*Lentinula edodes* (Berk.) Pegler], in accordance with consumer preferences, and deals with the search for substances that increase the odorous component content. From analysis of sulfur and sulfur-containing substances in the culture substrate, rice bran was found to be the main source of sulfur, and 75% of this was present as cysteine and methionine. The sulfur-containing substances were added to a sawdust medium containing only rice bran and sawdust as a substrate; shiitake mushrooms were cultivated in the medium, the fruiting bodies were dried, and the 1,2,4-trithiolane content in the fruiting bodies was measured as an indicator of the odorous compounds. Of the sulfur-containing substances, those that increased odorous compounds the most were cysteine and methionine. The efficiency of cysteine in this regard was higher than that of methionine. It was also noted that the amount of glutamic acid increased the odorous compounds in combination with cysteine and methionine. Furthermore, the addition of both amino acids and glutamic acid had no negative effect on the yield. These results showed that it is possible to produce dried shiitake mushrooms with a smell suitable for particular consumer preferences.

Key words Dried shiitake mushroom · 1,2,4-Trithiolane · Cysteine · Methionine · Glutamic acid

Introduction

Among many kinds of foods, shiitake mushrooms [*Lentinula edodes* (Berk.) Pegler] have been eaten since ancient times, and are one of the most popular edible mushrooms in Japan and other parts of the Far East. Dried shiitake mushrooms especially have a characteristic smell that is absent in fresh shiitake mushrooms and separates them clearly from other mushrooms. 1,2,4,5,6-Pentathiepane, commonly known as lenthionine, has been reported as an odorous compound of dried shiitake mushrooms,[1,2] and many other substances are also found in dried shiitake mushrooms, such as sulfur-containing substances and alcohols.[3–7] In an earlier report, it was found that 1,2,4-trithiolane and 1,2,4,6-tetrathiepane played important roles in the characteristic smell of shiitake mushrooms, as did lenthionine, and that 1,2,4-trithiolane could serve as an indicator to estimate the smell of dried shiitake mushrooms.[8]

In Japan, the consumption of dried shiitake mushrooms has been gradually decreasing in recent years. However, the value of the production business was over 10 billion yen, and the production of shiitake mushrooms is one of the biggest businesses in the mushroom industry. The proportion of the population that like dried shiitake mushroom is about 70%, those with neutral tastes about 16%, and about 14% for dislikers, showing that people generally like dried shiitake mushrooms.[9] The characteristic smell of dried shiitake mushrooms was suspected to be one of the main reasons for this, because their smell is an important factor in estimating the quality of food.[10–13] Preference for smells varies depending on the kind of food and people, and especially depends on factors such as age, sex, and region.[14–16] The previous article made it clear that hedonic preference for the smell of dried shiitake mushrooms was affected by the subject's original preference for dried shiitake mushrooms and the sensory intensity.[9] Hedonic preference was proportional to original preference, but the effect of sensory intensity was divided into two cases. For dried shiitake mushroom likers and neutralists, there was an optimal sensory intensity that gave the maximum hedonic preference. The sensory intensity was influenced by age class, the original preference grade, and the amount of dried shiitake mushroom; in other words the amount of 1,2,4-trithiolane content, and the amount producing the maximum hedonic preference was changed greatly by the other two factors. Adjusting the smell content using the amount of dried

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shiitake mushroom was difficult, because the amount to be consumed at each meal was defined. In contrast, the hedonic preference for dislikers was always "dislike," and it decreased in line with increases in sensory intensity. In other words, they wanted dried shiitake mushrooms with almost no smell. This indicates that it is necessary to adjust the smell of dried shiitake mushrooms, to make it suit the tastes of consumers, in order to increase the consumption of dried shiitake mushrooms.

Improvements in the quality of dried shiitake mushrooms must be based on consumer preference. However, past improvements of dried shiitake mushrooms have been made on the shape of the fruiting body, the amount of production per log and so on, because dried shiitake mushrooms are mostly evaluated by their shape and not by qualities such as smell, taste, and texture in Japanese markets. This study therefore aimed to clarify which substances increased the odorous compound content of dried shiitake mushrooms, to attempt to regulate this content.

Materials and methods

Preparation of dried shiitake mushroom and analysis of odorous compounds

Cultivated shiitake mushrooms were dried and the odorous components analyzed using the same methods as in the previous report.[8] One strain of shiitake mushroom, Forestry Mycology Code 140, derived from stock cultures of the Mushroom Science Laboratory, Forestry and Forest Products Research Institute (FFPRI), was used.

Analysis of culture media

The protein content was calculated from the nitrogen content, which was obtained using a total nitrogen and carbon analyzer (CN Corder MT-600, Yanako). The total sulfur content was calculated from the $BaSO_4$ produced by burning the samples (Oxygen Combustion Bombs, Parr). The amino acid content was obtained using phenyl isothiocyanate derivatives after hydrolyzing the protein with hydrochloric acid.[17] For measuring the cysteine and methionine content, performic acid oxidation was performed before hydrolysis. The biotin and thiamin contents were measured using food analysis methods.[18] The sulfurous ion was extracted by applying 6N HCl three times, adding 5% $BaCl_2$ to the aqueous solution obtained, and calculating the content from the resulting $BaSO_4$.

Results and discussion

Sulfur content and sulfur-containing substance content

The odorous components of dried shiitake mushrooms are made up of the elements sulfur, carbon and hydrogen; sulfur is a relatively minor element for organisms compared with carbon and hydrogen. The origin of the sulfur was determined. Many kinds of additive are used in the real-world production of shiitake mushrooms, for example, to increase the amount produced in a given medium. However, to simplify the culture conditions, rice bran and sawdust were chosen as the basic culture media. The total sulfur in the rice bran was 46.27 μmol/g and the total sulfur in the sawdust was 0.68 μmol/g. The sawdust medium was assumed to consist only of rice bran and sawdust, in a ratio of 1:3 (w/w); the sulfur ratio originated from the rice bran was 95.8%, and the ratio from the wood 4.2%. This showed that almost all the sulfur used by shiitake mycelia, was derived from rice bran. The results of the analysis of the sulfur-containing substances in rice bran are shown in Fig. 1. The sulfur content derived from methionine was 18.77 μmol/g (40.55%), from cysteine 15.81 μmol/g (34.18%), from sulfurous ion 0.19 μmol/g (0.41%), from thiamine 0.08 μmol/g (0.18%), from biotin 0.02 μmol/g (0.04%), and from unknown sources 11.40 μmol/g (24.64%), showing that about 75% of the sulfur was attributable to amino acids.

Effect of sulfur-containing substances on the odorous compound content

A sawdust medium containing 5% rice bran (by dry weight) was chosen as a basic medium, and six sulfur-containing substances were added to the medium at three different levels. The additional amounts of the substances were equivalent to media with 5%, 15%, and 25% rice bran (by dry weight). The 1,2,4-trithiolane content of the resulting dried fruiting body was then measured (Fig. 2). The cysteine and methionine added gave the highest absolute level of 1,2,4-trithiolane content among the substances tested (Fig. 2a). The maximum levels of 1,2,4-trithiolane content were 80.7 μg/g for cysteine and 68.4 μg/g for methionine. There was significant correlation between the 1,2,4-trithiolane content and the additional amounts of both amino acids at 5% risk, with a correlation coefficient (r) of 0.95 for cysteine and 0.95 for methionine. To examine the influence of sulfurous ion, ammonium sulfate and sodium sulfate were used

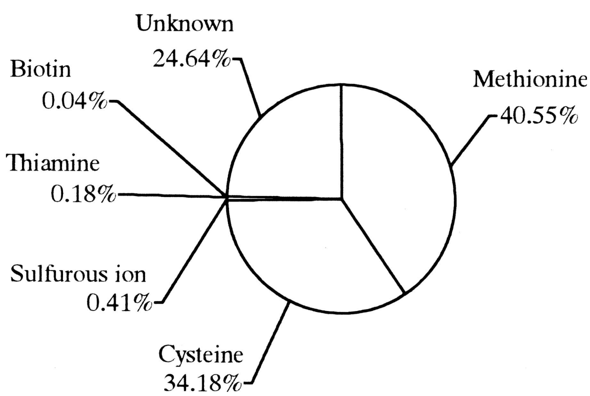

Fig. 1. Breakdown of the sulfur content of rice bran

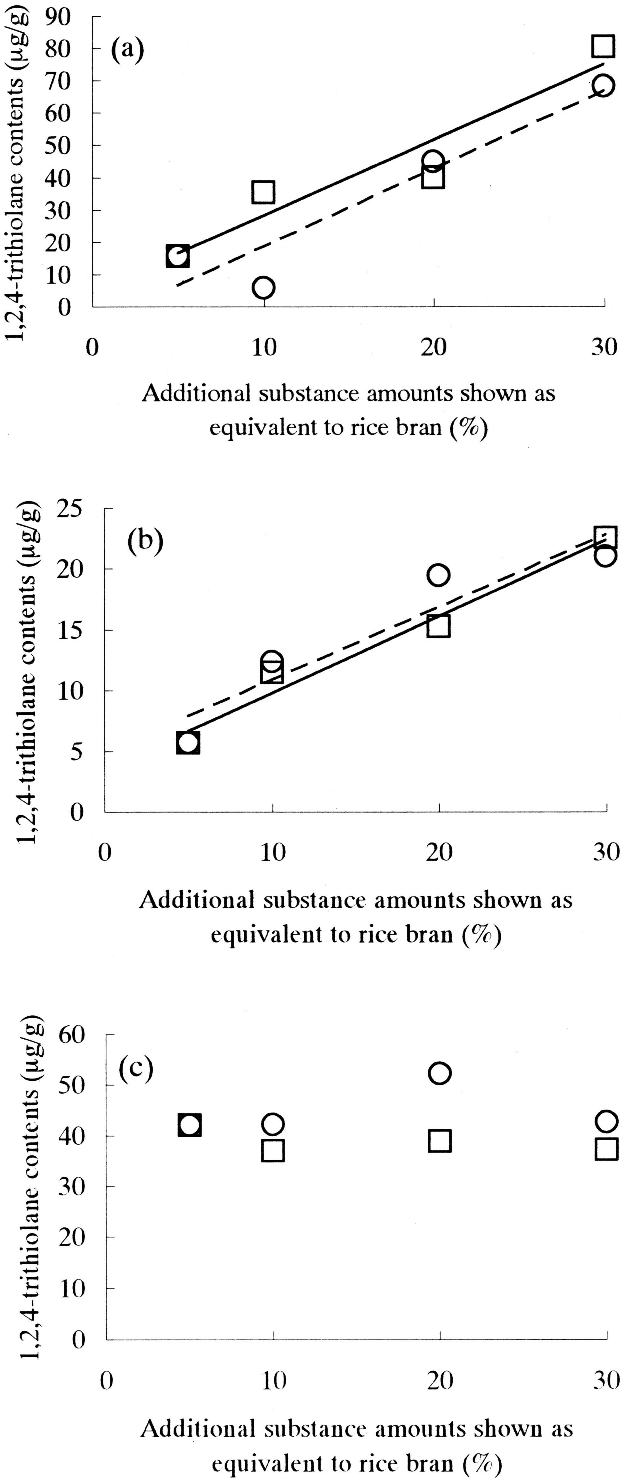

Fig. 2a–c. Effects of cystein (Cys), methionine (Met), ammonium sulfate (AS), sodium sulfate (SS), biotin (Bio), and thiamin (Thi) on 1,2,4-trithiolane content. The basic medium consisted of 95% sawdust and 5% rice bran (by dry weight). **a** Sulfur-containing amino acids; *squares*, Cys; *circles*, Met; the continuous regression line shows the content with Cys added ($r = 0.95$) and the broken regression line shows the content with Met added ($r = 0.95$). **b** Sulfurous ion; *squares*, AS; *circles*, SS; the continuous regression line shows the content with AS added ($r = 0.99$) and the broken regression line shows the content with SS added ($r = 0.94$). **c** Vitamins; *squares*, Bio; *circles*, Thi. All correlations indicated were significant at 5% risk

as sulfur sources, considering the effect of counter ions (Fig. 2b). Both sulfur sources produced an increase in 1,2,4-trithiolane content, and the maximum levels of 1,2,4-trithiolane content were 22.6 μg/g for ammonium sulfate and 21.1 μg/g for sodium sulfate. Significant correlation between the 1,2,4-trithiolane content and additional amounts of sulfur source were also found at 5% risk, with $r = 0.99$ for ammonium sulfate and $r = 0.94$ for sodium sulfate. The slopes of the regression equations were 0.63 for ammonium sulfate and 0.60 for sodium sulfate, with intercepts at 3.56 for ammonium sulfate and 4.92 for sodium sulfate. There was almost no difference between counter ions. Additionally, the slopes of the regression equations of cysteine and methionine were 2.34 and 2.42, respectively, and the influence of sulfurous ions on 1,2,4-trithiolane content was weaker than that of sulfur-containing amino acids. There was no significant correlation between 1,2,4-trithiolane contents and additional amounts of either vitamin (Fig. 2c). Adding biotin and thiamine made no difference to the 1,2,4-trithiolane content. These results showed that the substances containing sulfur that had most effect on 1,2,4-trithiolane content were cysteine and methionine.

Relations between 1,2,4-trithiolane contents and the content of amino acids containing sulfur

With the sawdust media containing 5% rice bran (dry weight) chosen as the basic medium, sawdust media containing cysteine 33.3 mg/kg and methionine 44.0 mg/kg (wet weight), respectively, were used. Preparations containing both amino acids in quantities from 100 to 500 mg/kg (wet weight) were then produced. The average yield of fruiting bodies in the basic medium was 82 g/kg (wet weight). Those in the medium with both amino acids added gradually increased and reached their maximum yields at 500 mg/kg, 113 g/kg on cysteine and 92 g/kg on methionine (wet weight), respectively. It was suspected that the increase in yield was due to nutrient deficiency being eliminated, because additives were usually mixed in a ration of between 10% and 25% of the medium (by either dry weight or dry volume).

The 1,2,4-trithiolane content of dried shiitake mushroom on the basic media was 14 μg/g, and increased in accordance with both amino acids addition, but decreased when both amino acids were added at 500 mg/kg (Fig. 3). When cysteine was added to the media, the maximum content of 1,2,4-trithiolane was 237 μg/g at 300 mg/kg addition, and the elevation rate was 0.83 in the range from 33 to 300 mg/kg content. Adding methionine, the maximum content was 248 μg/g at 400 mg/kg addition, and the elevation rate was 0.67 in the range from 49 to 400 mg/kg content. These results showed that both amino acids gave rise to 1,2,4-trithiolane content. However, the amounts of cysteine, which produced the maximum 1,2,4-trithiolane content, were smaller than the amounts of methionine, and the elevation rate when adding cysteine was larger than adding methionine, suggesting that the effect of cysteine in increas-

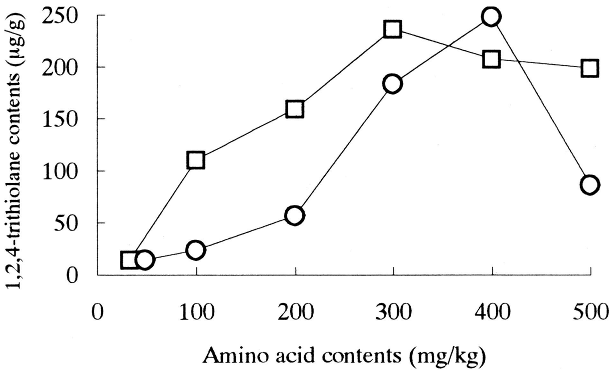

Fig. 3. Relations between the 1,2,4-trithiolane content and the content of added sulfur-containing amino acids. *Squares*, 1,2,4-trithiolane content in the medium with Cys added; *circles*, 1,2,4-trithiolane content with Met added

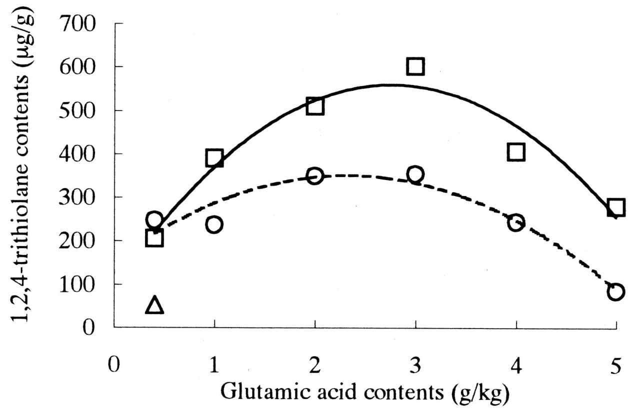

Fig. 4. Relations between the 1,2,4-trithiolane content and the amount of added glutamic acid. The *squares* show the 1,2,4-trithiolane content in a medium with Cys added; *circles* show the 1,2,4-trithiolane content with Met added; the *triangle* shows the 1,2,4-trithiolane content on basic medium. The continuous regression curve is for added Cys [$y = -60.7x^2 + 335.5x - 97.1$ ($R = 0.97$, $P < 0.05$)] and the broken regression curve is for added Met [$y = -36.3x^2 + 168.5x - 155.0$ ($R = 0.96$, $P < 0.05$)]. All media contained 400 mg/kg (wet weight) Cys or Met except for the basic medium

ing 1,2,4-trithiolane content was higher than that of methionine.

Effect of glutamic acid

The 1,2,4-trithiolane content in the basic medium ranged from 5.7 to 42.1 μg/g in each batch. It is thought that some substances other than cysteine and methionine were related to the 1,2,4-trithiolane content, which was present in the rice bran. Lentinic acid, which is known to be a precursor of lenthionine, contains cysteine and glutamic acid in its molecular structure.[19–22] The protein, free amino acid, and lentinic acid contents in the fruiting bodies were related to nitrogen content in the media.[23] Glutamic acid is known to be one of the key compounds in nitrogen metabolism, especially amino acid synthesis. It was therefore thought likely that there was an interaction between amino acids containing sulfur and glutamic acid.

The protein content calculated from the nitrogen content in rice bran was 163.1 mg/g, and the protein content in wood was 7.7 mg/g (dry weight). When the same calculation was performed for protein content using the sulfur content, the ratio of protein originating from rice bran was 87.6% and that from wood was 12.4%. This showed that most of the resources for protein used by shiitake mycelia came from rice bran. The glutamic acid content of rice bran was 19.7 mg/g (by dry weight), and the basic medium (5% rice bran) contained 344.8 mg/kg (by wet weight). Accordingly, preparations containing between 1 and 5 g/kg of glutamic acid (by wet weight) were added to the sawdust medium. The cysteine and methionine contents were set to 400 mg/kg. The average yield of fruiting bodies in the basic medium was 75 g/kg (by wet weight). The amounts of fruiting bodies in the medium with added cysteine increased up to the level where 3 g/kg glutamic acid was added and reached 94 g/kg (by wet weight), but decreased after that. In contrast, those in the medium with methionine added gradually increased and reached 94 g/kg (by wet weight) with 5 g/kg added. The addition of glutamic acid had no negative effects in this range.

The 1,2,4-trithiolane content increased in proportion to the quantity of glutamic acid added up to 3 g/kg, which produced 602.8 μg/g for cysteine and 355.7 μg/g for methionine (Fig. 4). These decreased with the addition of more glutamic acid. The patterns for the lenthionine content and 1,2,4,6-tetrathiepane content were also similar to those for 1,2,4-trithiolane. Convex quadratic curves were extrapolated from the relation between the 1,2,4-trithiolane content and additional amounts of glutamic acid on media with both amino acids added; the curves were significant at 5% risk and the correlation coefficients were 0.97 for cysteine and 0.96 for methionine. These results made it clear that the content of odorous compounds increased with the addition of both amino acids, and rose further if glutamic acid was mixed in. The 1,2,4-trithiolane content at the peak of the curve for cysteine was 561 μg/g at 2.8 g/kg glutamic acid addition and that for methionine was 351 μg/g at 2.3 g/kg addition. It is known that methionine and cysteine transformed each other in vivo. It was thought that cysteine and glutamic acid were much closer to lentinic acid in the biosynthetic pathway than methionine, and that methionine had to transform into cysteine to synthesize lentinic acid, or that it transformed other substances when the glutamic acid content increased.

Domestic dried shiitake mushrooms are only produced on logs in Japan. However, only 32% of the consumption was from domestic production and 68% was imported as of 2003.[24] Almost all imported shiitake mushrooms are cultivated in sawdust medium, and it is thought that these are mainly for commercial use, but some of them are distributed for household use. This shows that ordinary people were beginning to accept dried shiitake mushrooms cultivated in a sawdust medium, even though the main reason for this was price, with the price of the domestic product being three times higher than that of the imported product.

The most effective method for increasing domestic production is a decrease in price. However, the percentage of the production cost of raw materials when producing shiitake mushroom on logs was 64% in 2003.[25] This shows that reducing costs is very difficult. For production in sawdust medium, the cost of raw materials is 43%. However, an additional energy bill, 13% of the total cost, is needed, and efforts are being made to improve the media.[26–29] If a decrease in price is to be achieved and dried shiitake mushroom produced in a sawdust medium in the Japanese industry, a method for improving quality is needed. The results of this study afford the methods of controlling the contents of aromatic compounds in dried shiitake mushroom cultivated on sawdust media.

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