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ORIGINAL ARTICLE

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The smell and odorous components of dried shiitake mushroom, *Lentinula edodes* VI: increase in odorous compounds of dried shiitake mushroom cultivated on bed logs

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Abstract Odor is one of the most important characteristics affecting consumer preference for dried shiitake mushrooms [*Lentinula edodes* (Berk.) Pegler]. In our previous studies, we found that the odor content of commercial dried products was too weak for most people, and that the odorous compound content could be increased by adding amino acids to sawdust media. Currently, however, bed-log cultivation is used to produce fruiting bodies for dried products. The purpose of this study was to find a method to increase the content of odorous compounds in dried products cultivated on bed logs. Pressure injection of amino acids from the side of the bed log was the most efficient method, but it had some problems. Hence, a simpler and less troublesome method was developed, i.e., injecting amino acid solution from small bottles set in deep holes bored in the sides of the bed logs. In fruiting bodies cultivated on bed logs injected with amino acid solution by the improved method, the mean contents of lentinic acid, a precursor of the odorous compound lenthionine, approximately doubled compared to that in the untreated logs, although the infiltration area of the solution injected by the improved method was smaller than that by the former method.

Key words Dried shiitake mushroom · Lentinic acid · Bed-log cultivation

Introduction

The amount of fresh shiitake mushroom [*Lentinula edodes* (Berk.) Pegler] produced in Japan in 2006 was 66349 ton and that of dried shiitake mushroom was 3861 ton, generating sales of ¥73 billion.[1] The total accounted for 17% of the revenue from all forestry and forest products (¥432 billion) and 35% of the total mushroom output including matsutake mushrooms (¥209 billion). This confirms that the shiitake mushroom is one of the most important products in the industry. However, consumption of fresh shiitake mushroom has been decreasing since 2000, and that of dried shiitake has been decreasing since 1993.

As both fresh and dried shiitake mushroom are evaluated mainly by shape and size in Japanese markets, research efforts have concentrated on improving shape and increasing production amounts. To raise consumer interest and put the brakes on decreasing consumption, attractive products are required. Odor is an important factor in evaluating foods,[2–5] and dried shiitake mushrooms in particular are known for their characteristic smell upon rehydration. We previously reported that the smell of commercial products was evaluated as weak by most people.[6] In the case of sawdust-medium cultivation, the odorous compound content of dried shiitake mushrooms could be increased by adding cysteine (Cys, or methionine) and glutamic acid (Glu) to the medium.[7] In bed-log cultivation also, the odorous compound content in fruiting bodies should be increased as they are used to produce dried products in Japan. Increasing this content in shiitake mushrooms cultivated on bed logs requires a method such as the infiltration of amino acids. Thus, this study investigated appropriate methods for adding amino acids to the bed logs.

Materials and methods

Methods for adding amino acids to sawdust medium

We used one strain of *L. edodes*, Forestry Mycology Code 140, derived from stock cultures of the Mushroom Science Lab., Forestry and Forest Products Research Institute (FFPRI). As a control, 1-kg samples of sawdust medium with a ratio of rice bran to beech sawdust set at 5:95 (w/w) and a moisture content of 65% (w/w) were used. Amino acids, dissolved in 160 ml water, were added to media as

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follows: Conc11 (Cys 0.4 g/kg (w/w) and Glu 3.0 g/kg), Conc12 (Cys 0.52 and Glu 3.9), Conc13 (Cys 0.68, Glu 5.1), and Conc14 (Cys 0.8, Glu 6.0).[7] Two addition methods were used: a portion of the solution was poured into a plastic bag for infiltration from the surface of the medium, and another portion of the solution was directly injected into the medium at four positions using syringes. The media were settled in a developing room after 4 months of cultivation; therefore, the test using the infiltration method for a 4-month period was not carried out. The fruiting bodies obtained were dried at 60°C for 24 h.[8]

Development of fruiting bodies on bed logs

Bed logs (*Quercus serrata*, approximately 12 cm in diameter and 90 cm long) cultivating *L. edodes* hyphae for approximately 15 months outdoors were purchased from a producer in Tsukuba, Japan. The bed logs were submerged overnight, transferred indoors, and suspended from a rod inserted through a hole in each log, which was bored in the radial direction below 30 cm from the top face.

Methods for adding acid fuchsin solution to sound logs and bed logs

To visualize the solution-infiltration area, 0.1% acid fuchsin solution was used.[9] On the top face of sound logs and bed logs, holes 1.2 cmϕ were bored vertically to a depth of 3 cm. Additionally, two deep holes were drilled diagonally through the heartwood to just inside the bark on the opposite side at 10 and 50 cm from the top face. Two injection methods were used. In one, 1-l plastic bottles hanging 20 cm above the top face were connected to the holes by tubes. In the other, the solution was poured into a 200-ml plastic bottle. The bottle was equipped with a conical cap, of which the top was cut off, then the bottle was inverted and the cap part was inserted into the hole. After injection, the bed logs were transversally sliced approximately 2 cm from the top or base face, photos of the disks were taken, and the infiltration areas were measured using the GNU Image Manipulation Program (GIMP, http://www.gimp.org/index.html) and ImageJ (an image processing and analysis program, http://rsb.info.nih.gov/ij/).

Methods for adding amino acid solution to bed logs

Three deep holes were bored at 10, 40 and 70 cm from the top face on the bed-log side and one of two different 600-ml amino acid solutions was injected into each bed log: Conc21 (Cys 2 mg/ml and Glu 15 mg/ml) or Conc22 (Cys 3 and Glu 22.5).

Analysis of 1,2,4-trithiolane and lentinic acid

The 1,2,4-trithiolane content was analyzed using the same method as described in previous reports.[8] The lentinic acid content was analyzed as follows.[10] Lentinic acid was extracted from crushed samples of fruiting bodies with 0.1 N HCl. After protein was denatured using 75% acetonitrile (ACN), lentinic acid derivatized with 9-fluorenylmethyloxycarbonyl chloride was analyzed consecutively by an HPLC system equipped with an ODS column (Kanto Chemical, Mightysil RP-18 GP 4.6 mmϕ × 15 cm) and a fluorescent detector. Analytical conditions were as follows: flow rate 1 ml/min; eluant, 0.1 M CH_3COOH/Na buffer (pH 3.8)/ACN = 78/22(0 min) → 69/31(16) → 58/42(23) → 55/45(37) → 20/80(45) → 10/90(55); column temperature 38°C; excitation wavelength 263 nm, emission wavelength 313 nm.

Results and discussion

Timing of addition

The timing of addition was tested using sawdust media, because the addition methods to bed log were still uncertain. The timing of addition to media was assumed as follows: at inoculation, during cultivation, and just before development of fruiting bodies. In the case of bed-log cultivation, amino acid addition at inoculation and early-stage cultivation would cause contamination, because it is performed outdoors; therefore, the timing of addition to sawdust media was set at the end stage, namely, the shiitake mycelia spread through the whole media. On the other hand, the addition methods to the media were assumed as follows: infiltration from the surface of the media or injected into the media. Then, the timing of addition was assayed by both addition methods.

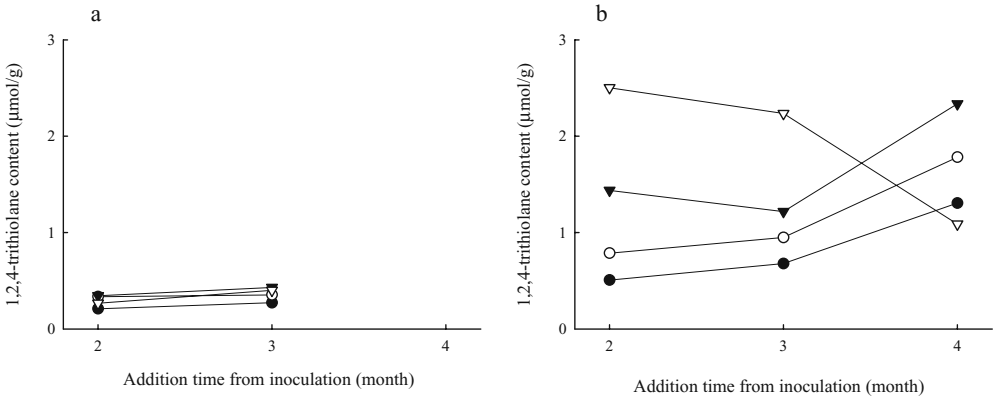

Fig. 1a,b. Relation between 1,2,4-trithiolane content in dried shiitake mushrooms and amino acid addition time. **a** Amino acid solutions infiltrated from the surface of sawdust medium, and **b** directly injected into the sawdust medium. The average contents were calculated by three repetitions. *Solid circles*, Conc11; *open circles*, Conc12; *solid triangles*, Conc13; *open triangles*, Conc14

The content of the odor indicator for dried fruiting bodies (1,2,4-trithiolane) was measured in dried fruiting bodies from sawdust medium and was found to maintain the same level regardless of the infiltration time (Fig. 1a). The absence of any positive effect by the infiltration method was probably due to the inhibition of amino acid infiltration caused by the thick coating of mycelia on the medium. The infiltration method was assumed as a model for spraying amino acid solution on bed logs or submerging bed logs in the solution. In the case of bed logs, there is no thick coating such as that found on the sawdust medium; therefore, the solution might be able to infiltrate into the logs at any time. The infiltration method was considered a potentially efficient addition method because an increased yield of fruiting bodies was reported by submergence in nutrient solution containing nitrogen and carbon sources.[11] However, the method required washing away of excess amino acids from the surface of the bed logs in order to prevent contamination and treating waste water. Consequently, applying this method at production sites would be labor intensive.

The 1,2,4-trithiolane content of fruiting bodies cultivated on sawdust medium using the injection method increased in close correlation to the fruiting body development and amino acid concentration, except for Conc14 (Fig. 1b). Decreases in mycelial growth and 1,2,4-trithiolane content at high cysteine concentrations in the medium have also been seen in previous studies.[7] The Conc14 concentration by injection after the 4-month inoculation period seemed to correspond to this case. However, the addition effect of Conc14 after 2- and 3-month periods showed an increase in 1,2,4-trithiolane content. The cysteine concentration was considered excessive for odorous compound production and growth; however, the amino acids were gradually consumed and happened to coincide with an appropriate concentration during fruiting body development. Developing fruiting bodies require abundant nutrients, which are gathered not only from the hyphae but also from the medium.[12,13] Even with a short interval between nutrient addition and fruiting body development, such as by submergence in nutrient solution instead of water, the effects were seen in the increased yield of fruiting bodies.[11] An increase in 1,2,4-trithiolane content closely correlated to fruiting body development was expected due to the assumption that the added amino acids would be gradually consumed and the residue preferentially used during the development of fruiting bodies. Effective timing for amino acid addition might be just prior to fruiting body development. The submerging of bed logs in water is usually performed in indoor cultivation for fruiting body development, with the addition timing set to just before submergence.

Methods for injecting solution into bed logs

Holes were bored in the sapwood at the top face of sound logs and bed logs because heartwood parts are difficult to infiltrate. Regarding the infiltration area of the acid fuchsin solution, most sound logs showed approximately 10 cm depth and a very small area on the transection within 4 weeks, although one log showed infiltration 57 cm from the top face and a maximal 9 cm^2 on the transection within 1 week. Injecting the solution was essentially difficult due to the very low permeability of *Quercus serrata* wood.[14] On the other hand, the acid fuchsin solution in bed logs reached a maximal 30 cm from the top face and an expanded maximal 23 cm^2 on the transection within 4 weeks (Fig. 2). Detailed studies on the patterns of wood decay by *L. edodes* show that vessels, tyloses, and parenchyma cells are resistant to fungal decay but wood fiber walls start thinning from an early stage.[15,16] The increase in infiltration area for bed logs compared to that for sound logs might be caused by the enlargement of the wood fiber lumina due to degradation of the wood fiber wall. The infiltration depth of the solution could not be increased, although the infiltration area on the transection could be enlarged by increasing the number of holes or injecting the solution under pressure.

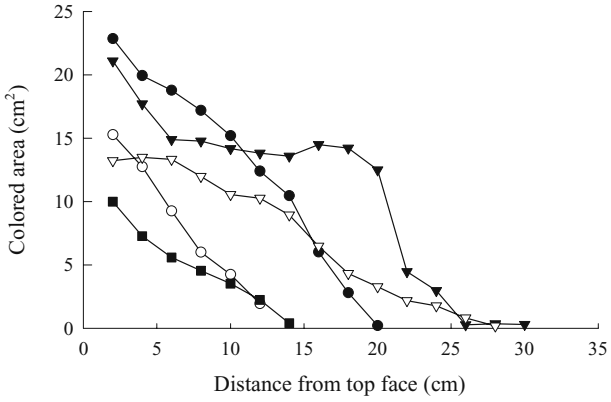

Fig. 2. Infiltration areas on transect of 5 bed logs infiltrated from top face. *Solid circles*, bed log 1; *open circles*, 2; *solid triangles*, 3; *open triangles*, 4; *solid squares*, 5

To increase the infiltration area in the axial direction of the bed logs, two deep side-holes were bored at 10 and 50 cm from the top face and the acid fuchsine solution was injected under pressure. As a result, entire bed logs were stained by the solution within 2 weeks. If the infiltration area for the amino acid solution could be improved to the same level as that of the acid fuchsin solution, the purpose of this study would be achieved. However, applying the method at production sites would be difficult because special equipment is required and there are technical problems to resolve, such as leaking around the holes. For a simpler and less troublesome method, a 200-ml bottle filled with 200 ml of solution was set at each hole. Within 1 week, the infiltration area had spread out from the injection holes to an average area of approximately 30 cm^2 on the injected transection, approximately 20 cm upward and approximately 30 cm downward (Fig. 3). Nutrient absorption from the bed log would have occurred far from the fruiting body position because increased cellulase and xylanase activity was observed 17 cm from just under the fruiting bodies in the axial direction of the bed logs, coinciding with fruiting body development.[17] Then, the number of bottles for the

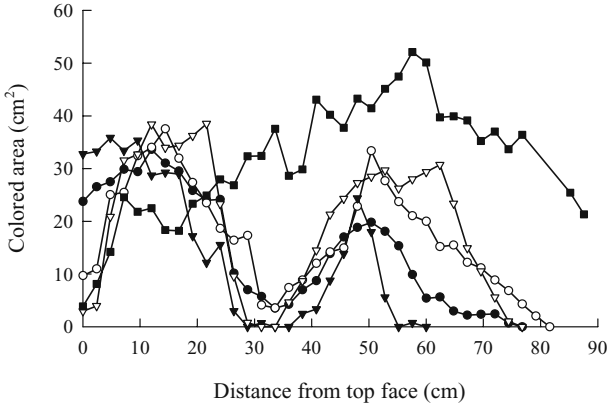

Fig. 3. Infiltration areas on transect of 5 bed logs infiltrated from side face. Acid fuchsin solutions were injected at 10 and 50 cm from top face. *Solid circles*, bed log 1; *open circles*, 2; *solid triangles*, 3; *open triangles*, 4; *solid squares*, 5

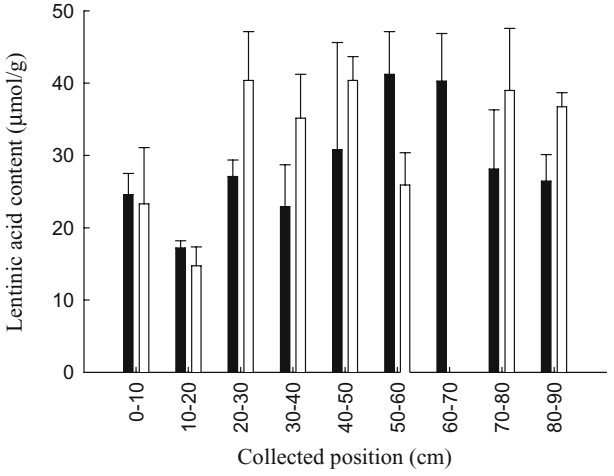

Fig. 4. Lentinic acid content in fruiting bodies by collected position on bed logs. *Solid bars*, Cys 2 and Glu 15 mg/ml; *open bars*, Cys 3 and Glu 22.5 mg/ml

injection method without pressure was adjusted and the solution was moderately spread in the bed log. The injection effect would appear even if the solution did not spread in the whole bed tog. The injection method without pressure was superior to that with pressure, because the former method needed no special equipment.

Effect of amino acid injection to bed logs

The quality of fruiting bodies cultivated on a bed log can differ even on the same bed log, and the differences would be likely to increase after injecting amino acid. Thus, the odor content of each fruiting body had to be measured. Lentinic acid is known as a precursor of odorous compounds,[18] and there is a positive correlation between lentinic acid content and odorous compound content.[10] The lentinic acid content could be analyzed using small samples of approximately 200 mg wet weight; however, measuring 1,2,4-trithiolane requires at least 3 g of dried fruiting body. Therefore, in this section, lentinic acid content was used as an indicator for the smell of dried shiitake mushrooms. In preliminary tests, injecting solutions of Cys 2.3 mg/ml and Glu 17 mg/ml from the top face of the bed logs resulted in fruiting bodies that contained approximately threefold the lentinic acid compared to those from untreated bed logs. Consequently, the amino acid content was set as follows: Conc21 (Cys 2 mg/ml and Glu 15 mg/ml) and Conc22 (Cys 3 and Glu 22.5).

Almost all the amino acid solutions were introduced to the bed logs within a 4-day period. The bed logs were submerged the following day and fruiting bodies developed. The lentinic acid content of fruiting bodies from untreated bed logs was 13.7 ± 2.9 μmol/g (dry weight), and those from bed logs injected with Conc21 and Conc22 were 29.1 ± 10.0 and 31.1 ± 13.4 μmol/g, respectively. Moreover, the glutamic acid content of fruiting bodies from untreated bed logs was 27.4 ± 3.0 μmol/g (dry weight), and those from bed logs injected with Conc21 and Conc22 were 47.9 ± 16.6 and 46.4 ± 17.3 μmol/g, respectively. As no special treatment was applied to the holes after injection, contamination appeared around the holes, but it decreased and disappeared afterwards. When the amino acid solution was injected from the top face of the bed logs, an increase in lentinic acid content was seen within 30 cm from the top face, and the value varied widely among fruiting bodies and bed logs. Lentinic acid content in fruiting bodies by collected position on the bed logs is shown in Fig. 4. The P values of analysis of variance for the lentinic acid contents by position were 0.223 and 0.221 for the Conc21 and Conc22, respectively. Excessive imbalance of amino acid infiltration was not observed, because a significant difference for position was not detected. The mean lentinic acid contents of fruiting bodies obtained from treated bed logs were approximately twofold higher compared with those from untreated bed logs; however, significant differences in terms of concentration were not seen ($P = 0.456$), although they were seen in the "timing of addition" section and a previous study. Lentinic acid production requires the absorption of substances (e.g., amino acids) and their transportation and biosynthesis. These functions worked well when the amino acid solution was injected into the bed log, although it was thought that any one of those functions was saturated, it was not certain which function was. One more reason might be related to some shortage of the functions and to which content in the bed log was less than that in the sawdust medium.

This study focused on the odorous compound content, so other qualities were not taken into consideration. However, the glutamic acid content of fruiting bodies was also increased by amino acid injection, indicating that this method could be applied to other types of quality improvement by using the appropriate agent.

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