J Wood Sci (2016) 62:452–459
DOI 10.1007/s10086-016-1569-0

ORIGINAL ARTICLE

Shiitake (*Lentinula edodes*) cultivation in sawdust media consisting of kunugi (*Quercus acutissima*) mixed with sugi (*Cryptomeria japonica*): optimization of gaseous phase rate in media by three-phase-structure analysis

Takeshi Nitta[1] · **Ichiro Kamei**[2] · **Kazuhiro Sugamoto**[2] · **Sadatoshi Meguro**[2]

Received: 1 February 2016 / Accepted: 1 June 2016 / Published online: 30 June 2016

Abstract The effects of the gaseous phase rate of kunugi (*Quercus acutissima*) sawdust media mixed with sugi (*Cryptomeria japonica*) determined by a three-phase-structure analysis of the fruiting body yields of shiitake were investigated. The fruiting body yield on kunugi media was significantly lower than that on commercially available hardwood-sawdust-mixture (HSM) media with 64 % water content. Three-phase-structure analysis showed that the gaseous phase rate in kunugi media was lower than that in HSM media. When the gaseous phase rate in kunugi media was increased to the level in HSM media by decreasing the water content to 56 %, the fruiting body yield on kunugi media also increased. These results suggested that kunugi sawdust could be used for shiitake cultivation if the gaseous phase rate in the media was optimized. Because sugi has a lower specific gravity and higher porosity than kunugi, mixing sugi sawdust up to 30 % with kunugi media caused an increase in the gaseous phase rate, and the fruiting body yield reached the same level as that in HSM media. These results suggested that kunugi media could be used for shiitake cultivation by mixing with sugi sawdust.

Keywords *Lentinula edodes* · Mycelial block cultivation · Three-phase-structure · *Quercus acutissima* · *Cryptomeria japonica*

✉ Takeshi Nitta
nitta-takeshi@pref.miyazaki.lg.jp

[1] Miyazaki Prefectural Forestry Technology Center, Miyazaki 883-1101, Japan

[2] Interdisciplinary Graduate School of Agriculture and Engineering, University of Miyazaki, Miyazaki 889-2192, Japan

Introduction

Kunugi (*Quercus acutissima*) wood has been mainly used for the production of dried shiitake [*Lentinula edodes* (Berk.) Pegler] by bed-log cultivation in Kyushu, Shikoku, and west central Honshu, and has also been most commonly used in Miyazaki Prefecture. In the past, the nurturing of kunugi seedlings had been promoted by Miyazaki Prefecture to compensate for the increased demand for kunugi logs. However, dried shiitake production has recently decreased due to the decline in price and the aging of the growers. If the demand for kunugi-logs continues to drop, the current volume of growing kunugi forests would be too aged and they would eventually be left unused. The sprout growth function of kunugi is weakened in trees that are 30 or more years old as reported by Tanaka [1]. Therefore, acceleration of the effective use of overgrown kunugi logs is required to keep kunugi forest habitats sound in the future. Shiitake production by means of sawdust-based cultivation has recently increased to about 90 % of the total fresh shiitake production in Miyazaki. The increase of prices due to the increase of the cost of transportation of raw materials is becoming a problem, since most of the sawdust for shiitake production is imported from other prefectures in hardwood-sawdust-mixture (HSM) form. The possible use of kunugi wood instead of HSM as the substrate for sawdust media would benefit the growers in Miyazaki, but kunugi sawdust is not popular with the growers because shiitake cultivation takes longer on this medium than on HSM media.

On the other hand, Miyazaki Prefecture is one of the most prosperous areas in Japan for the production of sugi wood (*Cryptomeria japonica* D. Don). The demand for sugi sawdust has recently increased for supply of the woody biomass generation [2, 3]. However, the use of a large quantity of forest residue and lower sugi wood is still issue, and a large quantity of sugi sawdust is produced in sawmills as factory waste every year. Thus, the price of sugi sawdust for mushroom cultivation is estimated to be around one-third of that of HSM [4]. The cost of production of fresh shiitake would be reduced if the plentiful and inexpensive sugi sawdust could be used for the cultivation. However, Nakajima et al. [5] reported that ferruginol in methanol extracts of sugi inner bark inhibited shiitake mycelial growth. Matsui et al. [6] also showed that mycelial growth inhibition of shiitake is probably due to a synergistic effect of ferruginol and sandaracopimarinol, which are the major terpenoids in sugi wood. These results suggest that the poor shiitake mycelial growth on sugi wood meal compared with that on hardwood is caused by the inhibitory effects of extractives contained in sugi wood [7]. In addition, the mycelial growth of shiitake on sugi media was found to be only about 60 % of that on konara (*Q. serrata* Murray) media, even if extract-free sugi sawdust was used [7]. Therefore, shiitake cultivation on media using only sugi sawdust seems to be difficult.

In our previous study [8], three-phase-structure analysis was used to investigate the changes of the physical properties of the sawdust media that result from mixing them with volcanic ash. The results suggested that the change in the three-phase-structure of sawdust media by mixing it with ash or the decrease in volume of the gaseous phase caused the reduction in shiitake yields. Thus, the fruiting body yields of shiitake should be significantly affected by the physical properties of sawdust media illustrated as by their three-phase-structure. If the disadvantages of kunugi sawdust, which requires a longer incubation period than HSM, can be conquered by optimizing the three-phase-structure of the media, the growers could use kunugi sawdust instead of HSM as the substrate of media for shiitake cultivation.

In this study, the effects of the gaseous phase rate in kunugi sawdust media mixed with sugi, as determined by three-phase-structure analysis, on the fruiting body yields of shiitake were investigated.

Materials and methods

Strain and materials

A commercial dikaryotic strain of shiitake (*L. edodes*) No. 1791 (the variety registration number of Ministry of Agriculture, Forestry and Fisheries, Japan) was used in this study. This strain has been extensively used in the sawdust-based cultivation of shiitake in Miyazaki. The sawdust spawn was obtained from a commercial source and was used for the experiment involving the flushing of the fruiting body. A strain subcultured on potato dextrose agar media (Nissui Co., Ltd.) was used for the experiment in which mycelial growth was measured.

Commercially available sawdust, namely hardwood-sawdust-mixtures (abbreviated as HSM, *Castanopsis* spp.: *Quercus* spp. with the exception of *Q. acutissima* and *Q. serrata*: others = 5:3:2, wt/wt, dry weight), was used for the substrate of the media. HSM has been commonly used for the sawdust-based cultivation of shiitake in Miyazaki. Kunugi (*Q. acutissima*) and sugi (*C. japonica*) that were supplied from mushroom cultivator in Miyazaki and had been seasoned for over 3 months were also used. The particle size distribution of sawdust used for flushing the fruiting body was as follows: under 1.0 mm: 1.0–2.0 mm: over 2.0 mm = 2:7:1. The sawdust used for the measurement on mycelial growth and the analysis of the three-phase-structure was sieved to a particle size of 1.0–2.0 mm constituted with 70 % of sawdust used for flushing the fruiting body. Moreover, the particle size of the sawdust used in the experiment involving the measurement of physical properties was 0.25–1.00 mm, similar to that described by Hu et al. [9].

Wheat bran (Toku-Fusuma40, Nisshin Seifun Co., Ltd.) and rice bran were used as the nutrients in the media.

Preparation of the media

The control media were composed of 27 % (wt/wt, dry weight) HSM sawdust, 4.5 % (wt/wt, dry weight) wheat bran, 4.5 % (wt/wt, dry weight) rice bran and 64 % (wt/wt) tap water. In the experimental media, the kunugi sawdust was increased from 27 to 31 or 35 % (wt/wt, dry weight) and the water contents decreased from 64 to 60 or 56 % according to the mixing rate of the sawdust. In some of the experimental media, kunugi sawdust was replaced with sugi sawdust at rates of 10–50 % at a 64 % water content. The mixing rate of nutrients was the same as those in the control media.

Mycelial growth on sawdust media

Test tubes (internal diameter, 28 mm; length, 200 mm) were filled with 50 g (wet weight) media and were then autoclaved at 121 °C for 20 min. The strain mycelium was grown on PDA (potato dextrose agar, Nissui Co., Ltd.) agar media at 20 ± 1 °C with a relative humidity of 60 ± 3 % for 14 days. A disk of mycelia was cut out of the PDA agar media using a cork borer (internal diameter, 4 mm) and was then placed in the top center of the media for inoculation. The inoculated test tubes were incubated at 20 ± 1 °C with a relative humidity of 60 ± 3 %. The mycelial growth length was measured at predetermined periods on two points around the test tube using an electronic caliper. The results are given as the means of five replicates.

Flushing conditions of fruiting body

The prepared media (2.7 kg wet weight) were filled in a polypropylene bag with one filter (Sun-bag SS37, 37 mm diameter, Santomi Sangyo Co., Ltd.) and autoclaved at 121 °C for 50 min. The spawn (approximately 12 g) was inoculated onto the media in the bag. The inoculated media were incubated at 21 °C with a relative humidity of 70 % for 100 days under dark conditions. After incubation, only the upper side of the bag was removed. The water was poured between the mycelial block and the bag, and then a rubber band (O-band#370, Kyowa Co., Ltd.) was set at the side of the bag for fixing the mycelial block. The fruiting bodies were flushed 7–8 times for 180–200 days at temperatures 13 °C for 16 h and 22 °C for 8 h of a day with a relative humidity above 80 %. The illuminance was 500–900 lx at 22 °C, and was dark conditions at 13 °C. The harvest and recuperation period in each flush were approximately 25 days under the conditions described above. All fruiting bodies were harvested when 70–80 % of their gills were exposed, and the fruiting bodies were sorted for each the diameter (2L: 8 cm or more, L: 8–6 cm, M: 6–4 cm, S: 4–3 cm, 2S: less than 3 cm) of the pileus, and then the number and the weight of fresh fruiting bodies were recorded. Just after each flush, the mycelial blocks were inverted in a plastic container that was 6 cm in depth. Water was supplied for 4 h by soaking the flushing surface of the inverted block.

Physical properties of wood chips and sawdust

Specific gravity and porosity of wood chips

The specific gravity was determined using wood chips (approximately 1 cm^3) according to the methods mentioned by Hu et al. [9]. Wood chips were prepared using kunugi or *Castanopsis* spp., which was contained primarily in HSM instead of HSM.

Water retention of sawdust

Sawdust (approximately 1 g) made of HSM or kunugi was soaked in water for 1 h with evacuation. Saturated sawdust was put into a 1G1 grass filter and centrifuged at $60 \times g$ for 1 min. The water retention of the sawdust was calculated using the following equation [10].

$$\text{Water retention } (\%) = (\text{Total weight after centrifugation} - \text{Total dry weight}) / \text{Dry weight of sawdust} \times 100.$$

Three-phase-structure analysis of sawdust media

The sawdust media (2.7 kg wet weight) were prepared under the same conditions used in the flushing experiment. The test samples were hollowed out from the block using a 100-mL core cylinder. The actual volume (a %), which is the sum of the volumes of the liquid and solid phases, was measured by a three-phase meter (DIK-1120, Daiki Rika Kogyo Co., Ltd.). The water content of the actual volume was gravimetrically measured by oven-drying (temperature, 105 °C), and the value obtained represents the liquid phase (c %). The gaseous phase, solid phase, total porosity and degree of saturation were calculated using the following equations [11]:

$$\text{Gaseous phase } (b\%) = 100 - a;$$
$$\text{Solid phase } (d\%) = a - c;$$
$$\text{Total porosity } (\%) = b + c;$$
$$\text{Degree of saturation } (\%) = c/(b + c).$$

The results were given as the means of three replicates.

Statistical analysis

All data collected were represented as mean ± standard deviation (SD). Statistical analyses were performed using the statistical analysis tool R (version 3.0.2). Differences in the data between the two groups were analyzed using a two-sided Student's *t*-test. In addition, differences in the data among three or more groups were analyzed using Tukey's HSD (honestly significant difference) test.

Results and discussion

Mycelial growth and fruiting body yield in HSM and kunugi media at 64 % water content

Test tubes or bags were filled with the HSM and kunugi sawdust media adjusted to have a water content of 64 %, which has been commonly used for the sawdust-based cultivation of shiitake (abbreviated as 64-HSM or 64-kunugi, respectively). The mycelial growth length of shiitake in the test tubes are shown in Fig. 1. The fruiting body yields of shiitake in 2.7 kg mycelial blocks are shown in Fig. 2. This test was repeated twice. The mycelial growth of 64-kunugi was significantly higher than that of 64-HSM at each incubation period, but the mycelial growth of 64-kunugi seemed almost the same as that of 64-HSM. On the other hand, the total fruiting body yields of 64-kunugi were lower, only 37-67 % of those in 64-HSM ($P < 0.001$). There are many kinds of extracts that include antimicrobial components in wood [12]. However, the

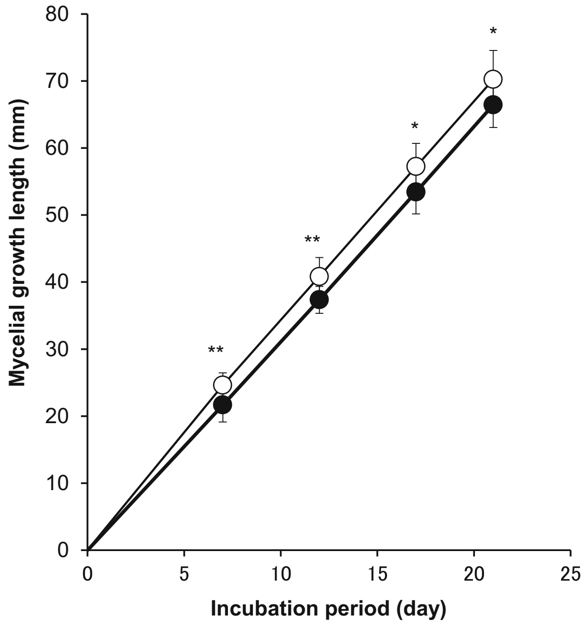

Fig. 1 Effects of mycelial growth length of shiitake in HSM or kunugi media at 64 % water content. *Closed circle*: 64-HSM, *open circle* 64-kunugi. *Vertical bars* indicate the standard deviation. HSM is an abbreviation of hardwood-sawdust- mixtures. *Asterisks* indicate a statistically significant difference between 64-HSM and 64-kunugi as determined by two-sided Student's *t*-test. $n = 10$: **$P < 0.01$, *$P < 0.05$

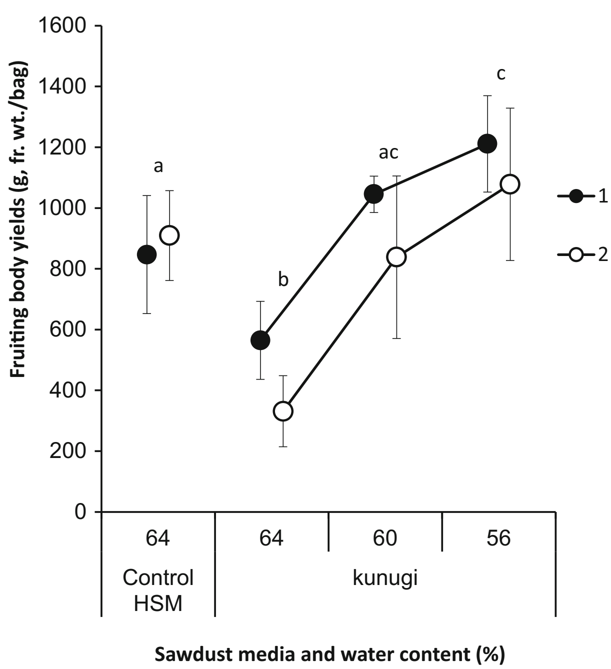

Fig. 2 Effects of fruiting body yields of shiitake in media prepared at different water contents using HSM or kunugi sawdust. *Number* (*1*, *2*) indicates the number of repeated experiments. *Vertical bars* indicate the standard deviation. HSM is an abbreviation of hardwood-sawdust-mixtures. *Different letters* represent a statistically significant difference as determined by Tukey's HSD test. $n = 6$ or 17: $P < 0.05$

extract components in kunugi sawdust seemed not to be the main cause of the decrease of the fruiting body yields judging from the results of mycelial growth (Fig. 1).

The physical properties of wood chips and sawdust, and the three-phase-structure analysis of sawdust media

The specific gravity and porosity of the wood chips and the water retention of the sawdust are shown in Table 1. The results of the three-phase-structure analysis of HSM and kunugi sawdust media with 64 % water content are shown in Table 2. Wood chips prepared in the experiment of specific gravity and porosity were used *Castanopsis* spp. instead of HSM since HSM was contained primarily *Castanopsis* spp. The physical properties were significantly different between the different species of wood. The specific gravity of kunugi was 1.23 times ($P < 0.001$) and the porosity was 0.88 times ($P < 0.001$) those of *Castanopsis* spp. Furthermore, the water retention of kunugi sawdust was 0.78 times as much as that of HSM sawdust ($P = 0.007$).

The indexes of three-phase-structure were also significantly different between the two wood species. The solid phase of 64-kunugi media was 1.33 times ($P < 0.001$) and the liquid phase was 1.25 times ($P < 0.001$) those of 64-HSM media, but the gaseous phase was 0.63 times ($P < 0.001$) that of 64-HSM media. The total porosity of 64-kunugi media was 0.94 times that of 64-HSM media ($P < 0.001$). Therefore, despite the media having the same water content, the degree of saturation of 64-kunugi media was 1.34 times higher than that of 64-HSM media ($P < 0.001$).

These results suggested that the causes of the decrease of the fruiting body yields in kunugi media at 64 % water

Table 1 Physical properties of HSM, kunugi and sugi

Physical property	HSM[a]	kunugi	sugi[b]
Specific gravity[c]	0.52 ± 0.02	0.64 ± 0.02***	0.33 ± 0.03
Porosity (%)[d]	65.3 ± 1.26	57.2 ± 1.27***	77.8 ± 2.12
Water retention	314 ± 12.7	246 ± 41.0**	389 ± 31.6

Data indicate the mean ± standard deviation

HSM hardwood-sawdust-mixtures

Asterisks indicate a statistically significant difference between HSM and kunugi as determined by two-sided Student's *t*-test. $n = 5$–10: *** $P < 0.001$, ** $P < 0.01$

[a] *Castanopsis* spp. contained primarily in HSM was used in the experiment of specific gravity and porosity

[b] Based on the report of Hu et al. [9]

[c] Specific gravity was measured with wood chip

[d] Porosity was calculated from specific gravity

Table 2 Three-phase-structure in HSM, kunugi and sugi sawdust media at 64 % water content

Indexes of three-phase-structure	HSM	kunugi	sugi
Solid phase rate (%)	19.4 ± 0.8[a]	22.8 ± 0.3[b]	10.4 ± 0.5[c]
Liquid phase rate (%)	42.4 ± 1.3[a]	51.6 ± 1.6[b]	30.4 ± 1.0[c]
Gaseous phase rate (%)	38.1 ± 1.9[a]	25.6 ± 1.6[b]	59.2 ± 1.5[c]
Total porosity (%)	80.6 ± 0.8[a]	77.2 ± 0.3[b]	89.6 ± 0.5[c]
Degree of saturation (%)	52.6 ± 2.0[a]	66.9 ± 2.0[b]	33.9 ± 1.3[c]

Data indicate the mean ± standard deviation

HSM hardwood-sawdust-mixtures

Different letters represent a statistically significant difference as determined by Tukey's HSD test. $n = 3$: $P < 0.001$

content could be attributed to the higher liquid phase and lower gaseous phase in kunugi media than in HSM media.

Three-phase-structure of the media with lower water content than the control media

The results of three-phase-structure analysis of the media which were adjusted to 60 or 56 % water content are shown in Fig. 3 (abbreviated as 60- or 56-kunugi, respectively). The solid phase rates in kunugi media were maintained at about 20 % but the liquid phase rates were decreased to 40.7 or 37.9 % from 52.2 % in 64-kunugi media by decreasing the water content of kunugi media to 60 or 56 %, respectively. On the other hand, the gaseous phase rates of kunugi media increased to 38.1 or 41.3 % from 27.3 % in 64-kunugi media with the decrease in water content to 60 or 56 %, respectively. Total porosity was kept constant at about 80 % due to the absence of change in the solid phase, but the degree of saturation decreased to 51.7 or 47.8 % from 65.8 % in 64-kunugi media. Therefore, the gaseous phase rate in the total porosity increased to 48.3 or 52.2 % from 34.2 % in 64-kunugi media, respectively.

The above results suggested that the gaseous phase rate in kunugi media could be increased to the same level as that in 64-HSM media (control media) by decreasing the water content to 60 or 56 %.

Effects of decreasing the water content on fruiting body yields in kunugi media

The effects of decreasing the water content on fruiting body yields in kunugi media were investigated (Fig. 2). As mentioned earlier, the fruiting body yields on kunugi media at a 64 % water content (64-kunugi) were significantly lower than those on HSM media (64-HSM). However, the yields on kunugi media at a 60 % water content (60-kunugi) were almost the same as those on 64-HSM media, and those at a 56 % water content (56-kunugi) increased to 1.19–1.43 times those on 64-HSM media. The results of a

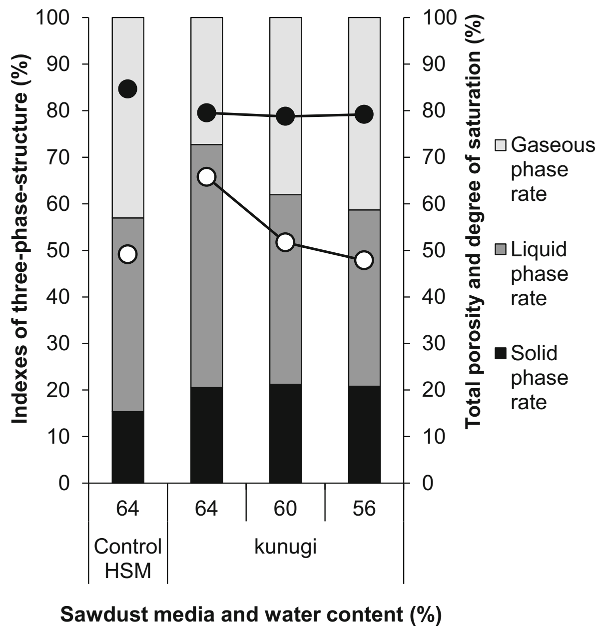

Fig. 3 Effects of three-phase-structure of the media prepared at different water contents using HSM or kunugi sawdust. *Closed circle* total porosity, *open circle* degree of saturation. HSM is an abbreviation of hardwood-sawdust-mixtures

multiple comparisons test showed that no significant differences in the yields were found between 60-kunugi media and 64-HSM media ($P = 0.985$), but they were found between 56-kunugi media and 64-HSM media ($P = 0.018$). In addition, the effects on the size of fruiting body in kunugi media were investigated. The numbers of fruiting bodies of M size or over and the flushing rates of fruiting bodies of M size or over to the total numbers of fruiting bodies were compared with those in HSM media as a base of 100. The numbers of fruiting bodies of M size or over in 64-kunugi media was 45, but those in 60-kunugi or 56-kunugi media were 108 or 122, respectively. The flushing rates of fruiting bodies of M size or over to the total numbers of fruiting bodies in 64-kunugi media was 86, but those in 60-kunugi or 56-kunugi media were 97 or

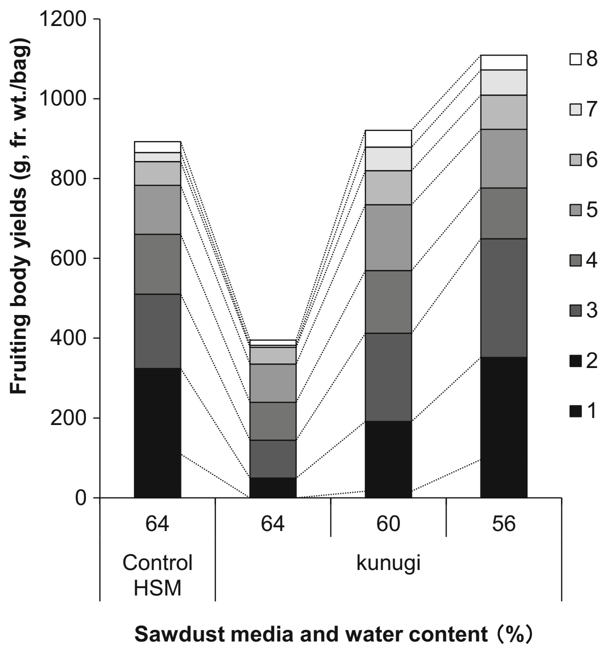

Fig. 4 Effects of fruiting body yields in each harvest stage in media prepared at different water contents using HSM or kunugi sawdust. *Numbers* (*1–8*) indicate flushes of fruiting bodies. HSM is an abbreviation of hardwood-sawdust-mixtures

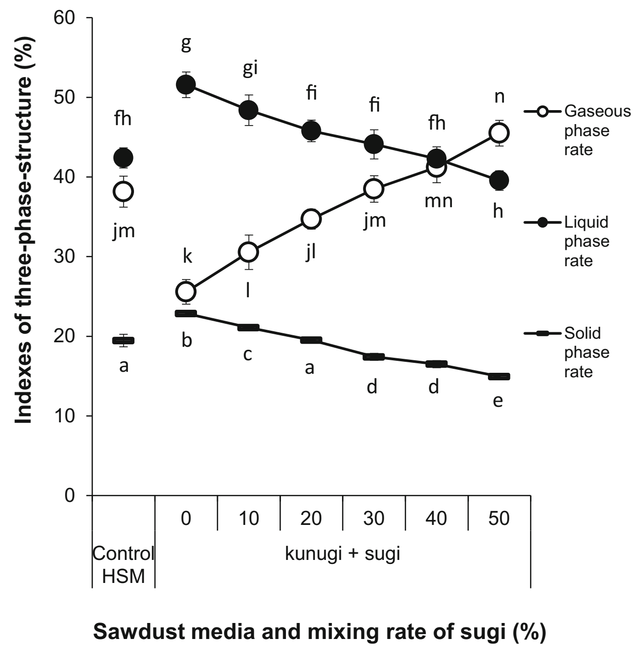

Fig. 5 Effects of three-phase-structure of kunugi media mixed with sugi sawdust. *Vertical bars* indicate the standard deviation. HSM is an abbreviation of hardwood-sawdust-mixtures. *Different letters* (*a–e* solid phase rate, *f–h* liquid phase rate, *j–n* gaseous phase rate) represent a statistically significant difference as determined by Tukey's HSD test. $n = 3$: $P < 0.05$

96, respectively. The decrease of the water content on kunugi media did not affect the lowering of the quality of the fruiting body. The effects of the water content of the media on fruiting body yields in each harvest stage are shown Fig. 4. The shiitake yields on kunugi media at the earlier harvest stages (first–second time) were lower than those on 64-HSM media. To the contrary, the yields in the later stages after the seventh harvest on 60-kunugi media or after the fifth harvest on 56-kunugi media were higher than those on 64-kunugi media and 64-HSM media. These results suggested that kunugi wood could be used as a substrate for shiitake cultivation since the yields in the later stages were increased if the gaseous phase were increased in the media.

Three-phase-structure of kunugi media mixed with sugi sawdust

The results of three-phase-structure analysis of the media in which sugi sawdust was mixed with kunugi media at rates of 10–50 % are shown in Fig. 5 (abbreviated as kunugi + sugi 10–50 %, respectively). The indexes of the three-phase-structure were significantly different among the different mixing rates of sugi sawdust. The solid phase and the liquid phase in kunugi media decreased according to the increase in the mixing rate of sugi sawdust. The solid phase in kunugi + sugi media decreased to 15.0 % from 22.9 % in the original kunugi media (kunugi + sugi 0 %) as a result of increasing the mixing rate of sugi sawdust to 50 %, and the liquid phase similarly decreased to 39.5 % from 51.6 %. In contrast, the gaseous phase of kunugi media increased to 45.5 % from 25.6 % in kunugi + sugi 0 % media. These results suggested that the three-phase-structure of the media in which 30 % of the kunugi sawdust was replaced with sugi (kunugi + sugi 30 %) could be similar to that of HSM media.

Effects of mixing sugi sawdust on fruiting body yields in kunugi media

The effects of the mixing rate of sugi sawdust with kunugi media on fruiting body yields were investigated. The results of a multiple comparisons test showed that no difference in the yields were found between HSM and kunugi + sugi 10 % and kunugi + sugi 30 %, but differences were found between kunugi + sugi 0 % and kunugi + sugi 50 % and other experiment groups. As shown in Fig. 6, the fruiting body yields on the media containing only kunugi sawdust (kunugi + sugi 0 %) were significantly lower than those on HSM media, much like the results shown in Fig. 2. However, on the media (kunugi + sugi 10 or 30 %) in which 10 or 30 % of kunugi sawdust was replaced with sugi, the fruiting body yields increased to be almost the same as those on HSM media. In addition, the effects on the size of fruiting body in kunugi media mixed with sugi sawdust were investigated. The

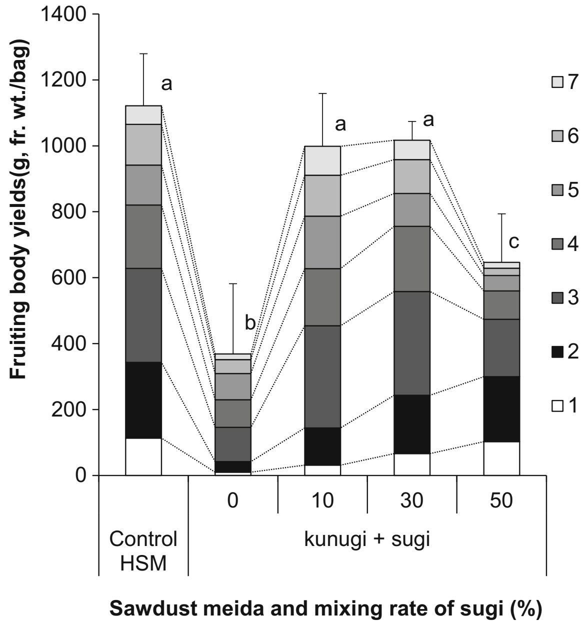

Fig. 6 Effects of mixing sugi sawdust on fruiting body yields in kunugi media. *Numbers* (*1–7*) indicate flushes of fruiting bodies. *Vertical bars* indicate the standard deviation. HSM is an abbreviation of hardwood-sawdust-mixtures. *Different letters* represent a statistically significant difference as determined by Tukey's HSD test. $n = 16$–21: $P < 0.05$

numbers of fruiting bodies of M size or over and the flushing rates of fruiting bodies of M size or over to the total numbers of fruiting bodies were compared with those in HSM media as a base of 100. The numbers of fruiting bodies of M size or over in kunugi + sugi 0 % was 35, but those in kunugi + sugi 10 %, kunugi + sugi 30 % or kunugi + sugi 50 % were 91, 95 or 61, respectively. The flushing rates of fruiting bodies of M size or over to the total numbers of fruiting bodies in kunugi + sugi 0 % was 90, but those in kunugi + sugi 10 %, kunugi + sugi 30 % or kunugi + sugi 50 % were 93, 105 or 112, respectively. The mixing of sugi sawdust with kunugi media did not affect the lowering of the quality of the fruiting body. As shown in Fig. 5, the gaseous phase rates in kunugi + sugi 10 and 30 % were found to be 30.5 and 38.5 %, respectively. Keeping the gaseous phase rate in the media at 30–40 % seemed to be essential when using kunugi sawdust as a substrate for the sawdust-based cultivation of shiitake.

Because the three-phase-structure in the media was markedly influenced by the physical properties of the wood, the specific gravity and water retention of the wood are important factors in selecting a substrate for the media for the sawdust-based cultivation of mushrooms. HSM would have a higher porosity compared with rates of kunugi sawdust since HSM was contained primarily *Castanopsis* spp., and so the water retention of kunugi sawdust must have been lower than that in HSM. In addition, kunugi media with a 64 % water content had a higher liquid phase rate and a lower gaseous phase rate due to the lower water retention of kunugi sawdust compared with the rates of HSM sawdust, and so ventilation through the media should be prevented. Shiitake mycelia seemed to be difficult to grow in the center part of the media due to a lack of oxygen. As shown in Fig. 4, the fruiting body yields on HSM media were highest in the second flush and gradually decreased as the number of flushes increased, but the yields on kunugi media with 60 or 56 % water content increased in the later flushes due to the improvement of ventilation in the media resulting from decay and evaporation. To increase the fruiting body yields in the earlier flush stages on kunugi media as occurs with HSM media, decreasing the initial water content to 60 or 56 % from the standard 64 % is important, as it promotes mycelial growth in the center part of the media.

The rate of the gaseous phase in kunugi media could also increase as a result of mixing with sugi sawdust because sugi wood has lower specific gravity and higher porosity as shown in Tables 1 and 2. Therefore, on the media in which 30 % of kunugi sawdust was replaced with sugi, the fruiting body yields were almost the same as on HSM media because the ventilation in kunugi media was improved through the mixing with sugi sawdust. Since sugi sawdust used to this experiment had been seasoned for over 3 months, it seemed that the inhibitory effects of extractives contained in sugi sawdust for the mycelial growth of shiitake is not almost. Physical properties of kunugi media would had been improved by the mixing of sugi sawdust which does not contained the inhibitory components.

As described above, the cost of sugi sawdust is relatively low, approximately one-third the cost of hardwood sawdust. Thus, the use of sugi sawdust for the sawdust-based cultivation of shiitake has been repeatedly attempted, but those attempts were not successful. If the method prepared by the mixing of sugi sawdust with kunugi media is used, it seems to be expected enough use of them in the sawdust-based cultivation of shiitake. Our results showed that the fruiting body yields in earlier flush stages in kunugi media partially mixed with sugi sawdust were better rather than those in the media only composed of kunugi sawdust. The effective utilization of unused overgrown kunugi wood and the cost reduction in the sawdust-based cultivation of shiitake could be achieved through its combination with inexpensive sugi sawdust, an industrial waste product.

References

1. Tanaka K (1983) Bassai-jurei to hassei-hoga no seicyo. In: Kunugi no zorin (in Japanese). Kuroda Tosyado, Miyazaki, p 197
2. Mibayashi T, Yoshida S, Mizoue N (2011) Study on supply potentiality of logging residues in biomass energy utilization (in Japanese). Kyushu J For Res 64:132–134

3. Yokota Y (2015) Supply system of woody biomass to feed-in tariff (FIT) power plant: a case study of “Forest residue” in Miyazaki (in Japanese). Kyushu J For Res 68:15–19
4. Terashima Y (2009) Present conditions of distribution of wood powder for mushroom cultivation and utilization of waste substrates in Chiba prefecture (in Japanese). CAFRC Res Bull 1:1–12
5. Nakajima K, Yoshimoto T, Fukuzumi T (1980) Substances inhibiting growth of shiitake mycelium in sugi wood (in Japanese). Mokuzai Gakkaishi 26:698–702
6. Matsui T, Matsushita Y, Sugamoto K, Ogawa K, Komiyama A, Muta S (2001) Mycelial growth inhibition of shiitake by several terpenoids isolated from sugi wood (in Japanese). Mokuzai Gakkaishi 47:58–62
7. Meguro S, Ishii E, Kawachi S (2002) Cultivation of shiitake in sugi wood meal II: effects of seasoning treatment for wood meal on mycelial growth. J Wood Sci 48:516–520
8. Nitta T, Meguro S (2013) Effects of volcanic ash from eruption of Shinmoedake on shiitake (*Lentinula edodes*) cultivation in sawdust media—effects of ash mixed sawdust on mycelial growth and fruit-body formation (in Japanese). Mushroom Sci Biotechnol 21:92–97
9. Hu C, Meguro S, Kawachi S (2003) Cultivation of shiitake (*Lentinula edodes*) of wood-meal of *Cryptomeria japonica* III. Effect of water content of media on mycelial growth (in Japanese). Mokuzai Gakkaishi 49:47–52
10. J TAPPI test methods No. 26-78 (1978) Determination of water retention value of pulp. J TAPPI, Tokyo
11. Hatano R (2005) Dojo no butsurisei. Saishin dojo-gaku (in Japanese). Asakurashokan, Tokyo, pp 96–98
12. Takahashi M (1989) Mokuzai no fukyu. Kinoko no seibutsu-gaku series 6 kinoko to mokuzai (in Japanese). Tsukijishokan, Tokyo, pp 70–73