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Comparison of Chemical Compositions of Maitake (*Grifola frondosa* (Fr.) S. F. Gray) Cultivated on Logs and Sawdust Substrate

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Maitake mushroom was cultivated on logs and in sawdust substrate. Comparisons of proximate compositions, content of free amino acid, 5′-nucleotides and vitamin D_2 were conducted. Effects of the log and sawdust substrate compositions on the mushroom composition were also examined. Protein and ash in sawdust mushroom were significantly higher than that in the log mushroom. Protein content of fruit body cultivated on sawdust substrate was closely related to the content of the substrate. This fact well explained the difference in protein and ash contents between mushroom cultivated on log or sawdust substrate. Free amino acid content as MSG-like and sweetness components, and 5′-GMP were significantly higher in log mushroom. The content of vitamin D_2 was appreciably higher in sawdust mushroom than that in log mushroom.

Keywords: Maitake mushroom, log, sawdust substrate, proximate composition, free amino acid, 5′-GMP, vitamin D_2

Maitake mushroom, *G. frondosa* (Fr.) S. F. Gray, is a white-rot fungus found in the wild on dead stumps of broad leaf trees such as oak (*Quercus mongolica*) and chestnut (*Castanea crenata*) (Imazeki & Hongo, 1985). Almost 40,000 metric tons of the cultivated mushroom was consumed in 1999 in Japan being the fourth largest production (Ohashi, 2000).

Numerous investigations have been reported on the components of *G. frondosa* (Yokokawa 1980; Kurasawa *et al*., 1982; Sato *et al*., 1985; Takeuchi *et al*., 1985; Muratsubaki *et al*., 1986; Kawai *et al*., 1990; Sekizawa *et al*., 1992; Yoshida *et al*., 1996; Ohnishi *et al*., 1996; Shindo *et al*., 1999). It is interesting that consumption of the mushroom is a way to assimilate antitumor (Ohno *et al*., 1985; Zhuang *et al*., 1994; Kubo *et al*., 1994), antidiabetes and antihyperliposis (Kubo *et al*., 1994, 1997), blood pressure and body weight depressive substances (Ohtsuru *et al*., 1999). Furthermore, Seguchi *et al*. (2001) added *G. frondosa* powder to improve the quality of bread.

G. frondosa is mainly cultivated on the sawdust of broad leaf trees supplementing the source of nutrients such as rice or wheat bran. This method makes it possible to cultivate on a large scale and to harvest all year round. However, it has been said that sawdust *G. frondosa* is less tasty than log *G. frondosa*. A few investigations have been reported on the comparison of chemical compositions of mushroom cultivated on logs and sawdust substrates (Aoyagi *et al*., 1993; Kawai *et al*., 1994; Sasaki *et al*., 1995). However, no study is found on a comparison of the taste components between the sawdust mushroom and log mushroom.

Previously, we compared the chemical and free amino acid composition of *P. nameko* cultivated on log and in sawdust substrate beds (Yamasaki & Tabata, 2002).

In this paper, we compared the proximate compositions, free amino acid, 5′-nucleotides, vitamin D_2 of *G. frondosa* cultivated on logs and sawdust substrates and their relation to the composition of the substrates.

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Materials and Methods

The strain of *G. frondosa* was purchased from Onuki Kinjin Co., Utsunomiya-shi. The mushroom was cultivated at the farm of the Kobe Women's University Biotechnology Institute.

Sawdust-based cultivation of G. frondosa Hardwood sawdust, *Quercus acutissima* Carr., was made with a wood mill. The substrate was composed of 750 g (fresh weight) of hardwood sawdust, and 250 g of rice bran. Its moisture content was made up to 70% by the addition of tap water. Thereafter polypropylene bottles (approximate capacity: 900 ml) were filled and a cavity was made in the center for solid spawn inoculation, which was then sealed with a cap. Bottles with substrate were sterilized at 121°C (1.1 atm) for 2 h, and then allowed to cool overnight. Each substrate was inoculated with 10 g of solid sawdust spawn of *G. frondosa*. The bottles were incubated at 23°C in the dark.

When the spawn had grown well throughout the medium, the cultures were subjected to the Kinkaki treatment (scratching away and removal of surface mycelia in the culture bottle). Cultures were then transferred to culture rooms maintained at 15°C to initiate fruiting body formation. In the final step relative humidity was maintained at 75–80% to grow the fruiting bodies.

Cultivation of G. frondosa using felled logs In March 1999 logs of *Quercus acutissima* Carr., a broad leaf tree, which had been cut in the fall of the previous year were cut into 15 cm lengths. Two segments were superposed to use as a substrate for the cultivation. Solid sawdust spawn of *G. frondosa* cultured on sawdust medium were inserted between paired segments. The pairs were piled up outside of a building, covered with PVC sheets, and left until the first of July (Karibuse). Attention was given to attaining a good air flow through the piles during the period of Karibuse. When the log segments were sufficiently per-

meated with the spawn, they were transferred to a Fusekomi. The paired segments were divided into two, and the sides that were permeated with the mycelium was embedded in soil. Each culture bed was occasionally sprinkled with water to keep it moist until the fruiting of *G. frondosa* took place. In the fall one year after the mushroom seeds were inoculated, the fruiting bodies of the mushroom were developed on almost the entire bed-log. Fruiting bodies harvested from the log or the sawdust cultivation were dried in a forced air-oven at 50–60°C for 70 h. Dried samples were ground in a stainless steel mill to obtain a homogeneous sample.

Proximate composition The moisture, protein, fat and ash were determined according to the conventional method (Sugahara, 1995).

The nitrogen-protein conversion factor of 6.25 and 4.33 was employed to calculate the protein contents for the substrate bed and mushroom, respectively. The use of common factor 6.25 in protein analysis for mushroom containing more nonprotein may result in overestimation of the protein content. Therefore, the later value of 4.33 was derived from analytical total nitrogen and amino acid residue data of *G. frondosa* (Fujihara *et al*., 1995). The amount of carbohydrate was estimated by subtracting the amount of protein, fat and ash from 100%.

Free amino acid assay Dried mushroom powder (1000 mg) was shaken with 25 ml of 10% (w/v) sulfosalicylic acid (Wako Pure Chemical Industries Co., Osaka) for 20 min at ambient temperature and filtered through Whatman No. 4 filter paper. The filtrate was adjusted to pH 2.2 with 3 N NaOH and made up to 100 ml with sodium citrate buffer (Wako reagent as described above). One milliliter of that extract was diluted to 5 ml and filtered through a Millipore LH nonsterile filter (pore size 0.45 μm). The diluted filtrate was mixed with *o*-phthalaldehyde reagent (Wako) in an Eppendorf tube, shaken to facilitate derivatization, and then immediately injected onto a high-performance liquid chromatograph (HPLC). A Shimadzu LC-6A equipped with fluorescence detector, with fluorescence excitation at 348 nm and emission at 450 nm, and a Shim-pack ISC-07/S 1504 Na column (Shimadzu) were used. Each amino acid was quantified by a calibration curve of the authentic amino acid.

5′-Nucleotide assay 5′-Nucleotides were extracted and analyzed as described by Kiribuchi and Kawashima (1992). Dried mushroom powder (1000 mg) was suspended in 50 ml of deionized water. It was treated for 25 min: 4 min until the extract reached a boil, 15 min of boiling, and 6 min of cooling. The extract was adjusted to 50 ml by adding deionized water, then was centrifuged at 3000 rpm for 10 min. The supernatant was filtered using 0.45 μm LH nonsterile filter (Millipore) prior to injection onto the HPLC.

The HPLC system was the same as for amino acid assay except for a UV detector and a Shim-pack CLC-ODS column (5×150 mm, Shimadzu). The temperature of the column was 40°C, the mobile phase was 24 mM 2-diethylaminoethanol-16 mM citric acid, the flow rate was 0.9 ml/min and UV detection at 250 nm. Each 5′-nucleotide was quantified by the calibration curve of the authentic 5′-nucleotide.

Vitamin D_2 assay The determination of vitamin D_2 in the fruiting bodies was carried out by the method of Kawazoe and Yuasa (1995). About 2 g of sample was accurately weighed and saponified. The extracted unsaponifiable matter was applied to the HPLC to determine vitamin D_2. The chromatography was performed using a TOSOH ODS-80, TM column (reversed-phase type, 4.6×150 mm i.d.) with methanol and water (95/5 v/v) as the mobile phase. The flow rate was 0.8 ml/min, and the UV detector was set at 265 nm. The content of vitamin D_2 in the fruit body was determined using pure vitamin D_2 (Wako).

Results and Discussion

The proximate compositions in these substrates are shown in Table 1. Protein, fat and ash were significantly higher in sawdust substrate than those in logs. On the contrary, carbohydrate was notably higher in logs. The carbohydrate content of the sawdust medium was reduced to less than that of the log by the supplementation of the rice bran which contained less carbohydrate than the log. Additionally, the high protein, fat, and ash content might be the result of the rice bran which was added to the sawdust.

The proximate composition of fruiting bodies of *G. frondosa* is shown in Table 2. It has been reported that the fruiting bodies contain 4.4–6.5% ash, 1.5–4.5% fat, 13.1–18.4% protein and 70.6–80.8% carbohydrate based on dry weight (the originally reported values in protein and carbohydrate were reevaluated using nitrogen-to-protein conversion factor 4.33) (Kurasawa *et al*., 1982; Muratsubaki *et al*., 1986). The protein and ash contents in sawdust mushroom were slightly above this range, whereas that in log mushroom was within this range.

Ash and protein in the sawdust mushroom were higher than those in the log mushroom but carbohydrate was higher in the log mushroom. Therefore, the observed high carbohydrate content in log mushroom is partly responsible for the lower protein, for it was estimated by subtracting the protein and others from the sample. Our analytical data are in good agreement with the results of Shiitake (*Lentinus edodes*) by Aoyagi *et al*. (1993) that sawdust mushroom contained more nitrogen and ash than log

Table 1. Proximate composition in the log and sawdust media.

	Content (%)[a]	
	Sawdust substrate[b]	Log substrate
Moisture	68.40±0.87	47.60±0.22
Protein[c]	1.66±0.04 (5.25)	0.86±0.01 (1.64)
Fat	2.13±0.10 (6.74)	0.44±0.08 (0.84)
Ash	1.15±0.08 (3.64)	0.81±0.04 (1.55)
Carbohydrate	26.66±0.27(84.37)	50.29±0.09 (95.97)

[a] Mean±standard deviation (n=3). Numbers in parenthesis indicate the percentage on a dry basis. [b] The sawdust medium was composed of sawdust from *Quercus acutissima* Carr. and rice bran (3 : 1 w/w). [c] The nitrogen factor used for protein calculation was 6.25.

Table 2. Proximate composition in the sawdust mushroom and log mushroom.

	Content (%)[a]	
	Sawdust mushroom	Log mushroom
Moisture	90.41±1.09	92.47±0.87
Protein[b]	1.78±0.05 (18.56)	1.02±0.02 (13.55)
Fat	0.23±0.09 (2.40)	0.11±0.05 (1.46)
Ash	0.68±0.06 (7.08)	0.34±0.05 (4.52)
Carbohydrate	6.90±0.32 (71.95)	6.06±0.25 (80.47)

[a] Refer to Table 1.
[b] The nitrogen factor used for protein calculation was 4.33.

Table 3. Content of free amino acids in the sawdust mushroom and log mushroom.

Amino acid	Content (mg/g, dry weight)[a]	
	Sawdust mushroom	Log mushroom
L-alanine	2.15±0.06	3.13±0.08
L-arginine	3.02±0.06	3.21±0.09
L-aspartic acid	1.61±0.03	1.25±0.04
L-glutamic acid	8.01±0.08	9.10±0.12
Glycine	1.53±0.04	1.53±0.03
L-histidine	1.53±0.02	0.94±0.03
L-isoleucine	0.12±0.007	0.12±0.007
L-leucine	0.05±0.007	0.09±0.007
L-lysine	1.56±0.06	1.28±0.05
L-proline	2.35±0.04	2.55±0.03
L-phenylalanine	0.26±0.01	0.28±0.01
L-serine	2.91±0.11	2.82±0.05
L-threonine	1.43±0.03	1.44±0.04
L-tyrosine	1.77±0.02	0.73±0.02
L-valine	0.96±0.01	0.91±0.01
Total	29.26±0.58	29.38±0.61

[a] Mean±standard deviation (n=3).

Table 4. Taste characteristics of free amino acids in the sawdust mushroom and log mushroom.

Taste characteristic	Content (mg/g, dry weight)[a]			
	Sawdust mushroom	Ratio (%)	Log mushroom	Ratio (%)
Bitterness[b]	5.94±0.11	20.3	5.55±0.15	18.9
MSG-like[c]	9.62±0.11	32.9	10.35±0.16	35.2
Sweetness[d]	10.37±0.28	35.4	11.47±0.23	39.0
Tasteless[e]	3.33±0.08	11.4	2.01±0.07	6.9
Total	29.26±0.58	100	29.38±0.61	100

[a] Mean±standard deviation (n=3).
[b] Val+Ile+Leu+Phe+His+Arg. [c] Monosodium glutamate-like, Asp+Glu. [d] Thr+Ser+Pro+Gly+Ala. [e] Tyr+Lys.

mushroom, whereas the content of carbohydrate was lower than log mushroom.

Kawai *et al*. (1994) reported that the protein content of fruiting bodies of Hiratake (*Pleurotus ostreatus*) was significantly related to the nitrogen content of the sawdust medium. However, Sasaki *et al*. (1995) reported that the nitrogen content of Bunashimeji (*Hypsizigus marmoreus*), Nameko (*Pholiota nameko*) and Enokitake (*Flammulina velutipes*) was not significantly related to that of the sawdust substrate.

Table 3 shows that there is no significant difference in total amino acid contents between the sawdust mushroom and log mushroom; however, log mushroom contained more glutamic acid and alanine than sawdust mushroom. On the other hand, histidine and tyrosine were significantly higher in sawdust mushroom than in log mushroom. The two mushroom types had the same degree of the essential amino acids isoleucine, leucine, lysine, phenylalanine, threonine and valine. The absence of sulfur-containing amino acid could be due to their instability in the course of the experiment, although it is known that these are not very common amino acids in mushroom.

Table 4 divides the free amino acids into several classes on the basis of their taste characteristics as described by Mau *et al*. (1998a, 1998b, 1998c). Aspartic and glutamic acids are classified as monosodium glutamate-like (MSG-like) components that contribute to the most typical mushroom taste, MSG-like and sweetness components would mainly be responsible for the attractive taste of *G. frondosa*. Sweet tasting amino acids such as alanine were appreciably higher in the mushroom cultivated on logs than on sawdust substrate, while the bitter and tasteless amino acids valine, tyrosine, and histidine were low in total free amino acids in the mushroom cultivated on logs. The bitterness from bitter components in mushroom was probably masked by sweet components (Mau *et al*., 1998a). It is thought, therefore, that when the *G. frondosa* cultivated on logs were exposed to sun, the umami and sweet tasting amino acids increased, but bitter tasting ones decreased. Kiribuchi (1991) found that the free amino acids pertaining to umami and sweet taste in *L. edodes, P. ostreatus* and *F. velutipes* were increased by sun or ultraviolet light irradiation, but bitter tasting amino acids were decreased. These findings indicate that the log mushroom may taste better than sawdust mushroom.

Table 5. Content of 5′-nucleotides and vitamin D_2 in the sawdust mushroom and log mushroom.

Cultivation	Content (mg/g, dry weight)[a]		Content (IU/g, dry weight)[a]	
	5′-AMP	5′-GMP	5′-UMP	Vitamin D_2
Sawdust mushroom	0.35±0.02	0.42±0.02	0.53±0.02	15.46±0.14
Log mushroom	0.25±0.02	1.00±0.03	0.86±0.03	7.84±0.06

[a] Mean±standard deviation (n=3).

Free amino acids in the fruit body were not related to protein content in the substrates. The total free amino acids in the mushroom cultivated on the two substrates were of the same degree in spite of there being less protein in the log substrate. These different behaviors of total amino acids and protein could be caused by the temperature and moisture of the medium, and the nutrient integration in the formation stage of the fruiting bodies. Komatsu and Tokimoto (1982) observed that the primordium formation of *L. edodes* was influenced by the temperature and moisture content of the bed-log. Moreover, Matsumoto and Kitamoto (1987) found that the exposure to light of the cultures in the mycelial growth stage is indispensable for the fruit body formation of *L. edodes*.

The content of 5′-nucleotides and vitamin D_2 is shown in Table 5. Flavor 5′-GMP was significantly higher in the log mushroom than in sawdust mushroom. It is well known that 5′-nucleotides and MSG-like components greatly increases synergistically the umami or palatable taste (Yamaguchi *et al*., 1971; Yamaguchi, 1979).

The taste intensities (Ariyoshi, 1974) of sawdust and log mushroom are evaluated as 12 and 19, respectively, based on the mixture ratios of 5′-GMP to MSG-like components shown in Table 4 and Table 5.

The vitamin D_2 content in sawdust mushroom was significantly higher than that in the log mushroom. Kiribuchi (1990a, 1990b, 1992) found that the amount of vitamin D_2 in *F. velutipes* is significantly increased by sun light or ultraviolet irradiation. This fact contradicts our experimental result. However, Takeuchi and others (1985) reported that vitamin D_2 in *G. frondosa* was produced without solar radiation. It is left for a future study to understand the reason why the mushroom cultivated in the sawdust substrate contains more vitamin D_2.

Finally, according to the results of the taste components, we

believe that *G. frondosa* mushroom cultivated on logs are tastier and more natural tasting than those cultivated on sawdust substrate. However, a further sensory evaluation is needed to confirm the findings of chemical compositions.

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