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Morphological features and dietary functional components in fruit bodies of two strains of *Pholiota adiposa* grown on artificial beds

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Abstract The morphological features and dietary functional components of two strains (FPF-13 and Oninome-B) of *Pholiota adiposa* (numerisugitake mushroom) grown on artificial bed blocks were examined. The components examined were chitin, mannitol, trehalose, guanosine 5′-monophosphate, ergosterol, and β-glucan. There was a significant difference in the external shape of the two strains. However, the differences in the contents of functional components between the two strains, as well as between the pilei and stipes of the strains, were small. In both strains the trehalose content was much higher than the mannitol content, in contrast to those of *Lentinula edodes*. From a commercial point of view, Oninome-B has a clear advantage over other strains of *P. adiposa* owing to its less removable scale.

Key words *Pholiota adiposa* · Strophariaceae · Basidiomycete · Functional components · Edible fungi

Introduction

For millennia mushrooms have been valued as flavorful foods and medicinal substances. They are widely sold as nutritional supplements and touted as beneficial for health. A number of bioactive molecules, including terpenoids, steroids, phenols, nucleotides and their derivatives, glycoproteins, and polysaccharides, have been identified in numerous mushroom species.[1,2] Edible mushrooms owe their taste primarily to the presence of small quantities of several water-soluble substances, including 5′-nucleotides, free amino acids, and soluble carbohydrates.[3,4] Therefore, it is important to clarify the contents of common functional components in edible mushrooms.

An edible mushroom, *Pholiota adiposa* (numerisugitake in Japanese), is cultivated not only in Japan but in several regions of Asia, Europe, and North America.[5] This fungus is one of the wood-rotting cosmopolitic wild, edible species that has been used as food for centuries in Japan. With the aim of commercially cultivating this fungus, Arita et al. investigated the possibility of artificial culture with rice-straw, chicken excrement,[6] and *Cryptomeria* wood[7] as the cultivation substrate. However, little information has been available about the functional components that have gastronomic value or the contents of those components in *P. adiposa*.

Recently, Kaneko cultivated two strains of *P. adiposa* (FPF-13 and oninome-B) on artificial bed blocks.[8] This research was designed to examine the morphological features and some common functional components [chitin, mannitol, trehalose, guanosine 5′-monophosphate (5′-GMP), ergosterol, and β-glucan] of these two strains of *P. adiposa* grown on artificial beds.

Materials and methods

The culture, which was grown previously on potato dextrose agar (PDA; Becton Dickinson, Frauklin Lakes, NJ, USA) at 22°C for 32 days was employed for the following experiments. Morphological characteristics of *P. adiposa* were examined by scanning electron microscopy (SEM).

Analysis of functional components

Pholiota adiposa

The fruit bodies were cultured in 850 ml polypropylene (PP) bottles containing 550 g of sawdust medium (*Cryptomeria*

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sawdust 12.5%, cotton hull 6.6%, corn-cob meal 6.7%, rice bran 7.2%, water 67.0%). The culture conditions for growing mycelia were 22.5°C and 65% relative humidity (RH) in the dark for 60 days. To induce fruit body formation, the surfaces of the cultures were scratched and sprinkled with water and then placed in the growing condition (16°C and >95% RH) under continuous illumination of about 700 lx from white fluorescent lamps for 30 days. The fruit bodies were divided into pilei and stipes, freeze-dried, ground to powder, and stored in a desiccator before use.

Lentinula edodes

The fruit bodies of *L. edodes* (Hokken 600) grown on artificial beds were a gift from Fukuoka Prefecture Forest Research and Extension Center (Fukuoka, Japan).

Chitin analysis

The chitin content was determined according to the method of Yanase[9] with modification. Each freeze-dried sample (200–500 mg) was treated with 20 ml acetone and 20 ml diethyl ether to remove fat. The organic solvent-insoluble residue (150 mg) was treated with 30 ml of 2N NaOH for 2 h in a boiling waterbath. After centrifugation the sedimented residue was washed with 30 ml deionized water three times and left with 30 ml of HCl for 1 h at ambient temperature. The residue was washed with deionized water and treated with 30 ml of 2N NaOH three times. After being washed with deionized water, the residue was hydrolyzed in 30 ml of 6N HCl for 10 h in a boiling waterbath. After cooling to ambient temperature, each hydrolysate was filtered, and the filtrate was diluted to 40 ml by adding deionized water. The concentration of glucosamine hydrochloride in the hydrolysates was determined colorimetrically according to Boas's[10] modification of the method of Elson and Morgan.[11] The diluted hydrolysate solution (1.0 ml) was added to 1.0 ml of 2% acetyl acetone solution and heated at 90°C for 45 min. After cooling to ambient temperature, 2 ml of ethanol was added while shaking to dissolve precipitates. Next, 1.0 ml of 2.67% *N*,*N*-dimethyl-*p*-aminobenzaldehyde was added. The color formed in the solution was measured at 530 nm with a Beckman DU 640 spectrophotometer. Blanks consisted of the same reaction cocktail for chromogen development but with deionized water instead of the sample. Comparison with a standard value of D-(+)-glucosamine hydrochloride (Tokyo Kasei Kogyo, Tokyo, Japan) was used to calculate the mycelium chitin content, as a monomer equivalent to *N*-acetyl-β-D-glucosamine.

Ergosterol analysis

Each freeze-dried sample (1 g) was homogenized with 30 ml of chloroform/methanol (2:1) in polytron (Kinematica, Lucerne, Switzerland) and left for 1 h at 4°C; the supernatant solution was recovered by decantation. The chloroform/methanol treatment was performed twice. The combined supernatants were filtered through a glass fiber paper (GA200; Advantec MFS, Japan), after which the filtrate was washed with 15 ml of water and the chloroform phase was evaporated to dryness in vacuo. The residue was saponified in 10 ml of 0.5N potassium hydroxide/ethanol solution at 80°C for 30 min. The cooled saponified mixture was diluted with 10 ml deionized water and extracted with 50 ml diethyl ether twice. The combined diethyl ether fraction was dried with Na_2SO_4 prior to evaporation to dryness below 35°C. The residue was dissolved in diethyl ether, and 1 μl of the solution obtained served as the test material for determining ergosterol by gas chromatograph–mass spectrometry (GC-MS) using methyltestosterone (Tokyo Kasei Kogyo) as an internal standard. GC-MS analyses were conducted on an HP 5973 gas chromatograph (Hewlett Packard, Palo Alto, CA, USA) equipped with a DB-5MS column (30 m $\times$ 0.25 mm; film thickness 0.25 μm) (J&W Scientific, USA) and coupled to an HP 6890 mass selective detector (Hewlett Packard) operated in the electron impact mode at 70 eV. Helium was used as the carrier gas with a flow rate of 0.5 ml/min. The injection temperature was 300°C, and the oven temperature was programmed from 150° to 250°C at a rate of 25°C/min and then from 250° to 300°C at a rate of 10°C/min.

β-Glucan analysis

Each freeze-dried sample (1 g), phosphate buffer (80 mM, pH 6.5, 50 ml), and thermostable α-amylase solution (100 μl) (Termamyl 120L; Novo Nordisk A/S, Bagsvaerd, Denmark) were mixed in a beaker capped tightly with aluminum foil. Immediately after mixing, the samples were incubated for 30 min in a boiling waterbath, during which time the beakers were shaken intermittently by hand three times. After cooling to 30°C, the mixtures ware adjusted to pH 7.5 with 0.275 M NaOH and then incubated for 30 min at 60°C after adding a suspension of protease from *Bacillus licheniformis* (485 U/ml, 100 μl) Type VIII; Sigma, USA. After being cooled to 30°C, the mixtures were adjusted to pH 4.3 by adding HCl (0.325 M). Then a suspension of amyloglucosidase solution (100 μl) (Sigma, from *Aspergillus niger*) was added, and the beakers were shaken and incubated for 30 min at 60°C. Soluble polymers were precipitated with 95% ethanol (280 ml), and the samples were placed at room temperature. The ethanol-insoluble residue recovered by filtration (quantitative filter paper No.5B, Advantec MFS) was washed several times with 80% methanol solution until the filtrate showed no phenol-sulfuric acid reaction.[12] It then was washed twice with acetone (10 ml). The insoluble residue was dried and subjected to sequential acid hydrolysis by adding 72% (w/w) H_2SO_4 (10 ml) for 4 h at room temperature and then diluted by adding deionized water (140 ml) heated for 2 h at 95°C by autoclaving. After cooling, the hydrolysate solution was neutralized using 5 ml of NaOH, and the volume was adjusted to 250 ml and filtered (0.45 μm) (Millipore, USA). The released glucose in the filtrate was then specifically estimated using a glucose oxidase/peroxidase reagent according to the supplier's manual (Glucose CII test Wako; Wako Pure Chemical

Industries, Japan). All β-glucan values are expressed on a dry-weight basis. The following equation was applied.

$$\beta\text{-Glucan}(\%) = \text{glucose}(\text{g}) \times 100 \times 162/180$$

where the value 162/180 denotes calibration from free glucose to anhydroglucose as in glucan.

5′-GMP assay

Each 0.5-g freeze-dried sample was suspended in 40 ml deionized water. The suspension was heated for 12 min in a boiling waterbath, adjusted to 40 ml by adding deionized water, and allowed to stand for 1 h at room temperature. The supernatant was analyzed by high-performance liquid chromatography (HPLC) after filtration with a 0.45 μm Millipore filter. The HPLC system consisted of a Jasco PU-1580 pump, Rheodyne 7725 injector, 20-μl sample loop, Jasco 807-IT integrator, and Jasco UV-970 UV/VIS detector, connected with an the Inertsil ODS-3 (4.6 × 250 mm, 5 μm; GL Sciences, Japan). The mobile phase was 0.1% trifluoroacetic acid (TFA) solution at a flow rate of 1 ml/min and ultraviolet (UV) detection at 254 nm. Under these conditions the retention time of the 5′-GMP was 13.4–13.5 min. The 5′-GMP was quantified by the calibration curve of an authentic sample.

Mannitol and trehalose assays

Soluble sugars (mannitol and trehalose) were extracted and analyzed as described by Yoshida et al.[13] with modification. The freeze-dried sample (5.0 g) was refluxed with ethanol for 1 h. The extraction procedure was repeated three times. The combined ethanol extracts were filtered and adjusted to 50 ml by addition of ethanol. Aliquots of ethanol extracts (20 ml) were subjected to Dowex 50W × 8 (20–50 mesh; Muromachi Kagaku Kogyo, Japan) with deionized water as eluent. The eluted fraction (120 ml) was evaporated and adjusted to 100 ml. An aliquot of the eluted fraction (10 ml) was evaporated to dryness and dissolved in 1 ml of pyridine. Sucrose (1 mg) (Sigma) was added as an internal standard. TMS derivatives of sugars were formed by the addition of 0.2 ml of hexamethyldisilazane and 0.1 ml of trimethylchlorosilane. After silylation 5 ml of diethyl ether and 5 ml of deionized water were added, and the mixture was shaken.

The diethyl ether layer was analyzed by GC-MS. The GC-MS analyses were conducted on a Shimadzu (Japan) GC-17A gas chromatograph equipped with a Neutra Bond-5 (30 m × 0.25 mm; film thickness 0.4 μm) (GL Science) and coupled to a QP-5000 quadruple mass spectrometer injector. The mass spectrometer was operated in the electron impact mode at 70 eV. Helium was used as the carrier gas with a flow rate of 0.8 ml/min. The first oven temperature was 200°C, with the temperature then increased to 320°C at a rate of 10°C/min. The sample (1 μl) was injected into the GC apparatus at an injector temperature of 300°C.

Results and discussion

There were significantly different macroscopic features in the fruit bodies (Fig. 1) and the microscopic features (Fig. 2) of the spores of FPF-13 and Oninome-B. The FPF-13 spore had a distinct structure, whereas the Oninome-B spores had an imperfect form. It should be noted that Oninome-B had many fewer scales on the pileus than FPF-13. Generally, the market value of *P. adiposa* is low because of its easily removed scale. Therefore, from a commercial point of view, Oninome-B has a clear advantage over FPF-13 and other strains of *P. adiposa*.

All of the functional components assayed (β-glucan, chitin, 5′-GMP, trehalose, mannitol, ergosterol) were detected in both strains (FPF-13 and Oninome-B) of *P. adiposa* (Table 1). The differences in the contents of functional components between the two strains, as well as between the pilei and stipes, were small (Table 1).

The contents of functional components in *P. adiposa* were compared with those of a common edible mushroom, *L. edodes*. Interestingly, the trehalose contents were much higher than the contents of mannitol in both strains of *P. adiposa*, in contrast to those of *L. edodes* (Table 1). This tendency was similar to that for *P. nameko*, which belongs to the same genus.[13] It was reported that the distribution patterns and contents of free sugars and free sugar alcohols were similar among closely related species of edible mushrooms.[13] Soluble sugars contained in the mushrooms contribute to their sweet taste.[3] Mannitol was the major soluble sugar alcohol in common mushrooms, and trehalose, a nonreducing sugar, was the second most soluble sugar component.[14] Moreover, trehalose was reported to enhance the hair growth effects of water-soluble polymers in C3H mice.[15]

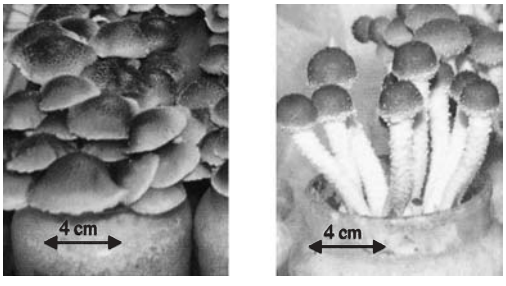

Fig. 1. Fruit bodies of two strains of *Pholiota adiposa* grown on artificial beds

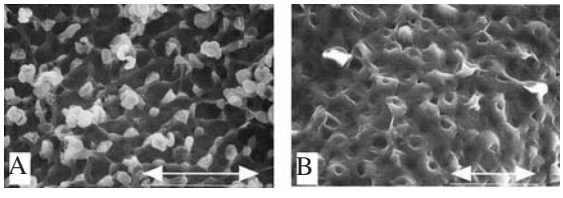

Fig. 2 *Pholiota adiposa.* **A** FPF-13. Bar 40 μm. **B** Oninome-B. Bar 20 μm

Table 1. Contents of functional components of two strains (FPF-13 and Oninome-B) *of Pholiota adiposa* and of *Lentinula edodes*

Component	FPF-13		Oninome-B		*Lentinula edodes*	
	Pileus	Stipe	Pileus	Stipe	Pileus	Stipe
β-Glucan ($n = 3$)	11.1 ± 0.5	10.4 ± 0.9	14.9 ± 0.6	12.4 ± 1.2	22.8 ± 1.2	49.5 ± 0.1
Chitin ($n = 2$)	1.16 ± 0.04	2.53 ± 0.05	2.44 ± 0.07	2.65 ± 0.12	2.09 ± 0.07	2.54 ± 0.13
5′-GMP ($n = 3$)	0.0556 ± 0.0206	0.0425 ± 0.0212	0.0533 ± 0.0058	0.0323 ± 0.0293	0.2374 ± 0.0240	0.0760 ± 0.0084
Trehalose ($n = 2$)	4.06 ± 1.75	5.97 ± 0.73	3.27 ± 0.06	6.65 ± 0.04	1.07 ± 0.31	0.68 ± 0.33
Mannitol ($n = 2$)	0.21 ± 0.24	0.21 ± 0.21	0.21 ± 0.25	0.32 ± 0.21	3.24 ± 0.80	1.64 ± 0.23
Ergosterol ($n = 2$)	0.52 ± 0.07	0.38 ± 0.04	0.41 ± 0.01	0.47 ± 0.06	0.32 ± 0.05	0.29 ± 0.04

Results are given as the percent in dry matter and are means ± SD
5′-GMP, 5′-glucose monophosphate

Mushrooms contain β-glucans, chitin, and heteropolysaccharides (e.g., pectinous substances, hemicelluloses, polyuronides) belonging to dietary fibers, comprising as much as 10%–50% of the weight of the dried matter. β-Glucans and chitinous substances with carcinostatic activity are primarily contained in the dietary fiber of mushrooms, and they adsorb such hazardous materials as carcinogenic substances, promoting their excretion (laxative action) by physiochemical interactions. These substances may thus work effectively to prevent cancer of the colon and rectum.[2] The β-glucan content of *P. adiposa* was less than that of *L. edodes*.

Ergosterol was reported to inhibit 12-*O*-tetradecanoylphorbol-13-acetate (TPA)-induced tumor promotion in two-stage carcinogenesis in mouse skin and TPA-induced inflammatory ear edema.[16] Ergosterol is also well known as provitamin D.

The flavor nucleotide 5′-GMP is referred to as a *umami* compound in Japanese. In English it is termed a flavor potentiator or flavor enhancer. The *umami* compounds appear to be chemically related to substances that produce the four basic tastes (sweet, salty, sour, bitter), though their taste properties are independent of these four classes.[17] The 5′-GMP contents of *P. adiposa* were less than those of *L. edodes*.

The contents of several common functional components (β-glucan, chitin, 5′-GMP, trehalose, mannitol, ergosterol) in the fruit bodies of *P. adiposa* (FPF-13 and Oninome-B) were clarified. Furthermore, from a commercial point of view, Oninome-B (with less easily removable scale) has a clear advantage over other strains of *P. adiposa*.

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References

1. Mizuno T, Saito H, Nishitoba T, Kawagishi H (1995) Antitumor-active substances from mushrooms. Food Rev Int 11:23–61
2. Wasser SP, Weis AL (1999) Therapeutic effects of substances occurring in higher basidiomycetes mushrooms: a modern perspective. Crit Rev Immunol 19:65–96
3. Litchfield JH (1967) Morel mushroom mycelium as a food flavoring material. Biotechnol Bioeng 9:289–304
4. Hammond JBW, Nichols R (1975) Changes in respiration and soluble carbohydrates during the post-harvest storage of mushrooms *Agaricus bisporus*. J Sci Food Agric 26:835–842
5. Imazeki R, Hongo T (1957) In: Coloured illustrations of fungi of Japan (in Japanese). Hoikusha, Osaka Tokyo, Japan, p 63
6. Hashioka Y, Arita I (1979) Naturalization of several saprophytic mushrooms under rice-straw-culture. Mushroom Sci 11:127–135
7. Arita I, Teratani A, Shione Y (1980) The optimal and critical temperatures for growth of *Pholiota adiposa*. Rep Tottori Mycol Inst (Japan) 18:107–113
8. Kaneko S (2000) Cultivation of *Pholiota adiposa* sporeless strain. Transact Meeting Kyusyu Branch Jpn For Soc 53:157–158
9. Yanase M (1982) In: Nihon Syokuhin Kogyo Gakkai. Syokuhinbunnsekihou. Korin, Tokyo, pp 205–207
10. Boas NF (1953) Method for the determination of hexosamines in tissues. J Biol Chem 204:553–563
11. Elson LA, Morgan WTJ (1933) A colorimetric method for the determination of glucosamine and chondrosamine. Biochem J 27:1824–1828
12. Dubois M, Gilles KA, Hamilton JK, Rebers PA, Smith F (1956) Colorimetric method for determination of sugars and related substances. Anal Chem 28:350–356
13. Yoshida H, Sugahara T, Hayashi J (1982) Studies on free sugars, free sugar alcohols and organic acids of edible mushrooms. Nippon Shokuhin Kogyo Gakkaishi 29:451–459
14. Hammond JBW, Nichiols R (1976) Carbohydrate metabolism in *Agaricus bisporus* (Lange) Imbach: change on soluble carbohydrates during growth of mycelium and sporophore. J Gen Microbiol 93:309–320
15. Chiba T, Watanabe T, Ono T, Hatanaka M, Miura H (1999) Acceleration of hair regrowth in mice with topical water-soluble polymer. J Jpn Cos Sci Soc 23:69–76
16. Yasukawa K, Aoki T, Takido M, Ikekawa T, Saito H, Matsunaga T (1994) Inhibitory effects of ergosterol isolated from the edible mushroom *Hypsizygus marmoreus* on TPA-induced inflammatory ear oedema and tumor promotion in mice. Phytother Res 8:10–13
17. Bigelis R (1992) Flavor metabolites and enzymes from filamentous fungi. Food Technol Chicago 46:151–161