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Effects of strain and cultivation medium on the chemical composition of the taste components in fruit-body of *Hypsizygus marmoreus*

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Abstract

The effects of strain and cultivation medium on the chemical composition of the taste components, including soluble sugars and free amino acids, in the fruit-body of *Hypsizygus marmoreus* were investigated. Three strains, Hm 88-8, Hm 00-1 and Hm 00-5, commercially available in Japan, were cultivated. Fruit-bodies were picked from the cultivation media to determine soluble components by using HPLC. The fruit-bodies contained mannitol and trehalose as major soluble sugars. Difference of mannitol contents among the treatments was less than the trehalose contents. In contrast, trehalose contents were affected by both the strain and cultivation medium used. Aspartic acid, glutamic acid, glutamine and ornithine were identified as predominant amino acids. The yield of fruit-body and contents of MSG-like components, related to the taste of edible mushrooms, were improved by the addition of supplement to the cultivation medium.

Keywords: *Hypsizygus marmoreus*; Mushroom; Free amino acids; Soluble sugars; Fruit-body

1. Introduction

The production of *Hypsizygus marmoreus* (Peck) Bigelow has increased rapidly during the past decade in Japan, along with development of some commercial strains and various cultivation techniques for high productivity of the edible mushroom. Recently, this mushroom has been used as a popular food or a food-flavouring material in Japan, due to its delicious taste and unique texture. The typical flavour substances of edible mushrooms can be classified into volatile compounds and non-volatile components. The content of volatile compounds, especially 1-octen-3-ol, decreases greatly with prolonged storage time (Mau, Beelman, Ziegler, & Royse, 1991). Thus, non-volatile components are responsible for the taste of stored or processed mushrooms (Mau et al., 1991). The taste of edible mushrooms is primarily due to the presence of several water-soluble substances, including soluble sugars and free amino acids.

The free amino acids and soluble sugars in the cultivated mushrooms were investigated as biochemical changes during fruit-body development or after harvest (Gruen & Wong, 1982a, 1982b; Kitamoto & Gruen, 1976; Kitamoto, Kikuchi, Mori, & Ohga, 2000; Minamide Iwata, & Habu, 1985; Minamide, & Iwata, 1987) and as chemical comoponents affecting their taste (Kasuga, Fujiwara, & Aoyagi, 1999, 2000; Mau, Lin, Chen, Wu, & Peng, 1998; Mau & Tseng, 1998; Sugahara, Arai, Aoyagi, & Kunisaki, 1975; Terashita, Kono, Shishiyama, & Yamauchi, 1992; Yang, Lin, & Mau, 2001). Although tastes such as sweet, bitter and umami are important, only limited studies on the taste and flavour of commercial mushrooms have been reported. The relationships between the chemical composition of the taste components of *H. marmoreus* and factors affecting its taste, such as strains and cultivation media, are still unknown.

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In the present study, the effects of strain and cultivation medium on the chemical composition of the taste components, including soluble sugars and free amino acids, in fruit-body of *H. marmoreus* were investigated.

2. Materials and methods

2.1. Mushroom strains and culture conditions

The strains of *H. marmoreus* used in this study were Hm 88-8, Hm 00-1 and Hm 00-5, the stock cultures of Hokkaido Forest Products Research Institute. The stock cultures were maintained on PDA slants at 1.5 °C. Indivudual 850 ml plastic bottles, containing 600 g of sawdust-based medium, were used for cultivation. Basic medium was comprised of birch (*Betula ermanii* Cham) sawdust (137 g) and rice bran (85 g). The medium for high productivity of the mushroom was comprised of birch sawdust (109 g), rice bran (53 g), soybean shell (32 g), corncob meal (28 g) and $CaCO_3$ (3 g) (medium A). The other medium for the high productivity was treated with 15 g of the commercial supplement Oruga K-1 (Katsuragi Sangyo corp.) instead of $CaCO_3$ in medium A (medium B). Moisture content of the medium was adjusted to 63%, based on the fresh weight of the mixture of solid materials.

Cultivation was conducted using the standard procedure reported earlier (Harada, Gisusi, Yoneyama, Nakaya, & Ito, 2001). The spawn running process for the strain Hm 88-8 was carried out at 22 °C for 60 days. For the other two strains, it was performed at 22 °C for 90 days. To induce fruiting, the cultures were treated by the removal of both the spawn and the uppermost layer of medium, and then maintained at 16 °C. After fruiting initiation, those cultures were exposed to 350 lx from white fluorescent lamps for 12 h per day. Fruit-bodies were harvested after the veil had broken. The harvested fruit-bodies were divided into stipes and pilei to determine their oven-dry weights. Three replications for each strain and medium were conducted.

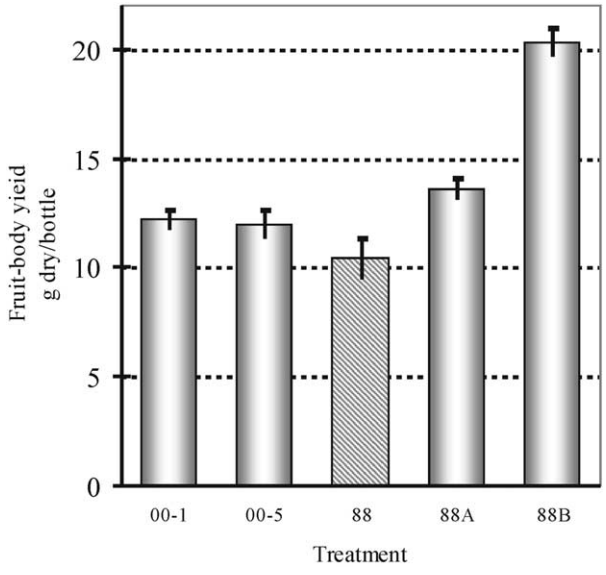

Fig. 1. Influence of strains and cultivation media on fruit-body yields of *Hypsizygus marmoreus*. 00-1 (Hm 00-1), 00-5 (Hm 00-5) and 88 (Hm 88-8): the stock cultures of Hokkaido Forest Products Research Institute, A: medium A for high productivity of mushroom, B: medium B for high productivity of mushroom.

2.2. Extraction and determination of soluble sugar

The pilei and stipes were freeze-dried, powdered and stored at −30 °C until used. Extraction with aqueous ethanol was carried out according to the method of Sugahara and Maekawa (2000). Freeze-dried powder (1.0 g) was extracted with 40 ml of 80% aqueous ethanol at 80 °C for 30 min. The resulting suspension was centrifuged at 15,000 *g* for 10 min. The supernatant was concentrated at 60 °C under reduced pressure and defatted three times with 10 ml of ethyl ether, successively. After concentration at 40 °C, the solid residues were dissolved in water to a final volume of 5 ml. Soluble sugars were determined by using HPLC (SHIMADZU, LC10A System) at 25 °C.

The HPLC system was equipped with a SHIMADZU RID-6A RI detector and with a Shim-pack CLC-NH_2 column (6.0×150 mm, 5 μm, SHIMADZU). The mobile phase was acetonitrile/deionized water, 7:3 (v/v) at a flow rate of 1.2 ml/min.

2.3. Extraction and determination of free amino acids

Extraction of free amino acids was carried out according to the method of Terashita et al. (1992). Freeze-dried powder (1.0 g) was suspended in 40 ml of 80% aqueous ethanol and homogenized in a Warring blender for 3 min. Successively, the same method as for sugar analysis was performed. The resulting aqueous ethanol extracts were dissolved in a lithium citrate buffer solution (pH 2.2). Free amino acids were determined

Table 1
Predominant soluble sugar contents of fruit-bodies of *H. marmoreus*

Sugar	Treatment[a]				
	00-1	00-5	88	88A	88B
Mannitol	5.3±0.27	3.9±0.30	5.3±0.82	5.9±0.12	4.8±0.09
Trehalose	9.1±0.20	11.8±0.38	6.0±0.87	9.1±1.51	9.4±0.36
Total	14.4±0.47	15.8±0.61	11.3±1.69	15.0±2.69	14.2±0.31

[a] Refer to Fig. 1.

by using HPLC (SHIMADZU, LC10A System) at 39 °C.

The HPLC system was equipped with a SHIMADZU RF-10A fluorescence detector with fluorescence excitation at 350 nm and emission at 450 nm, and with a Shim-pack ISC-07/S1504 Li column (4.0×150 mm, 7 μm, SHIMADZU). For the mobile phases and reaction mixture, Li type kits for amino acid analysis (SHIMADZU) were used.

3. Results and discussion

3.1. Influence of strains and cultivation media on fruit-body development

When vegetative mycelia, after the spawn running process, were transferred into a culture room at 16 °C, a cluster of fruit-body primordia appeared within 7–10 days. At 25–26 days (Hm 88-8), and 20–21 days

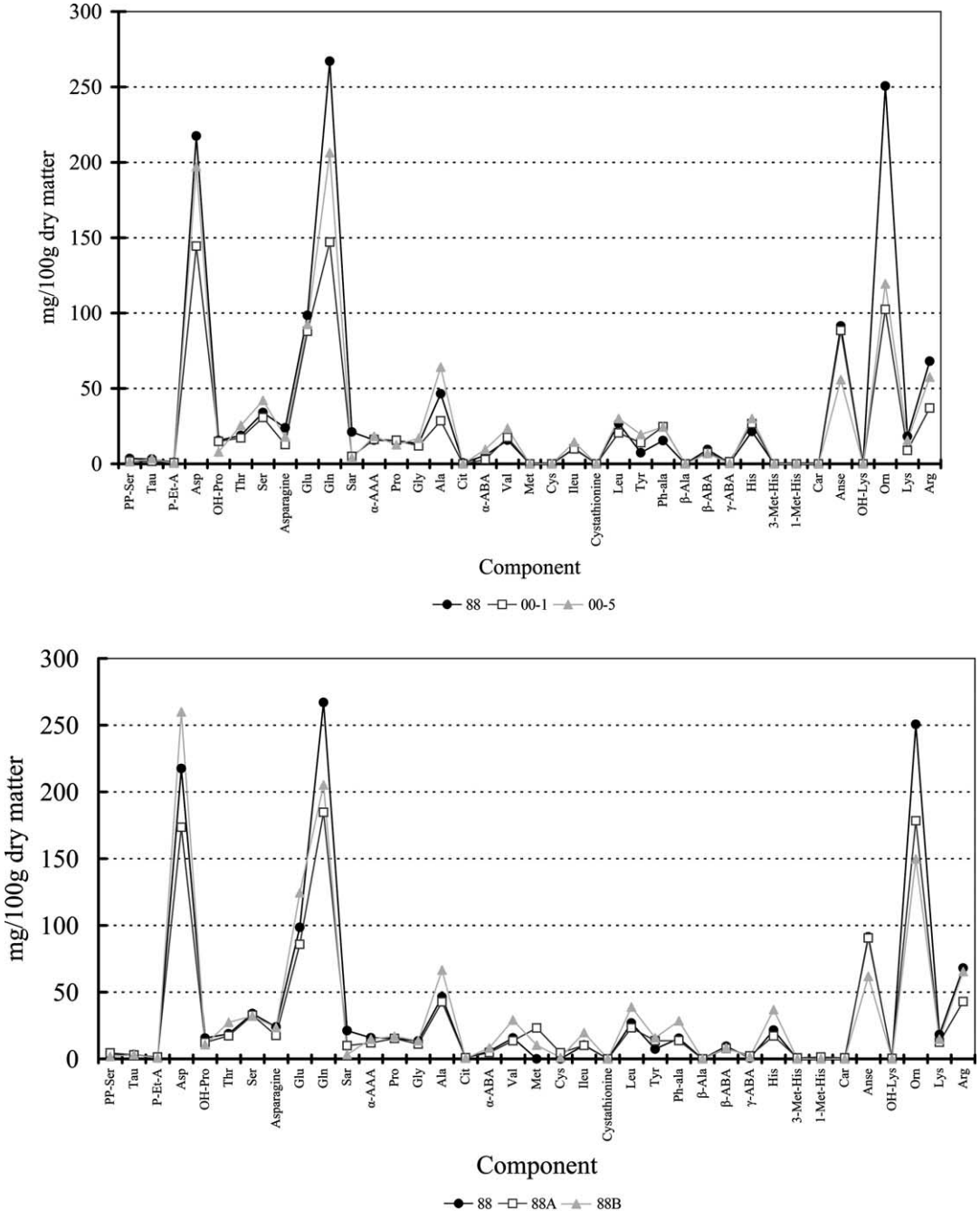

Fig. 2. Patterns of free amino acid content in fruit-bodies of *H. marmoreus*. 00-1, 00-5, 88, 88A and 88B: Refer to Fig. 1.

(Hm 00-1 and Hm 00-5) after fruiting initiation, the fruit-bodies were picked from the media. Fig. 1 shows fruit-body yields from the different strains and cultivation media. Dry weights of the fruit-bodies of Hm 00-1 and Hm 00-5 were 1.2 times higher than that of Hm 88-8 in the basic medium. The dry weights of Hm 88-8 in medium A and medium B were 1.3 times and 2.0 times, respectively, higher than those in the basic medium.

The result indicated that the fruit-body development was affected by both the strains and the cultivation media used. It has been reported that the contents of free amino acids and soluble sugars varied significantly in the tissues during the fruit-body development of *Agaricus bisporus* Sing. (Minamide & Hammond, 1985; Minamide et al., 1985), *Flammulina velutipes* (Curt.: Fr.) Sing. (Gruen & Wong, 1982a, 1982b; Kitamoto et al.,

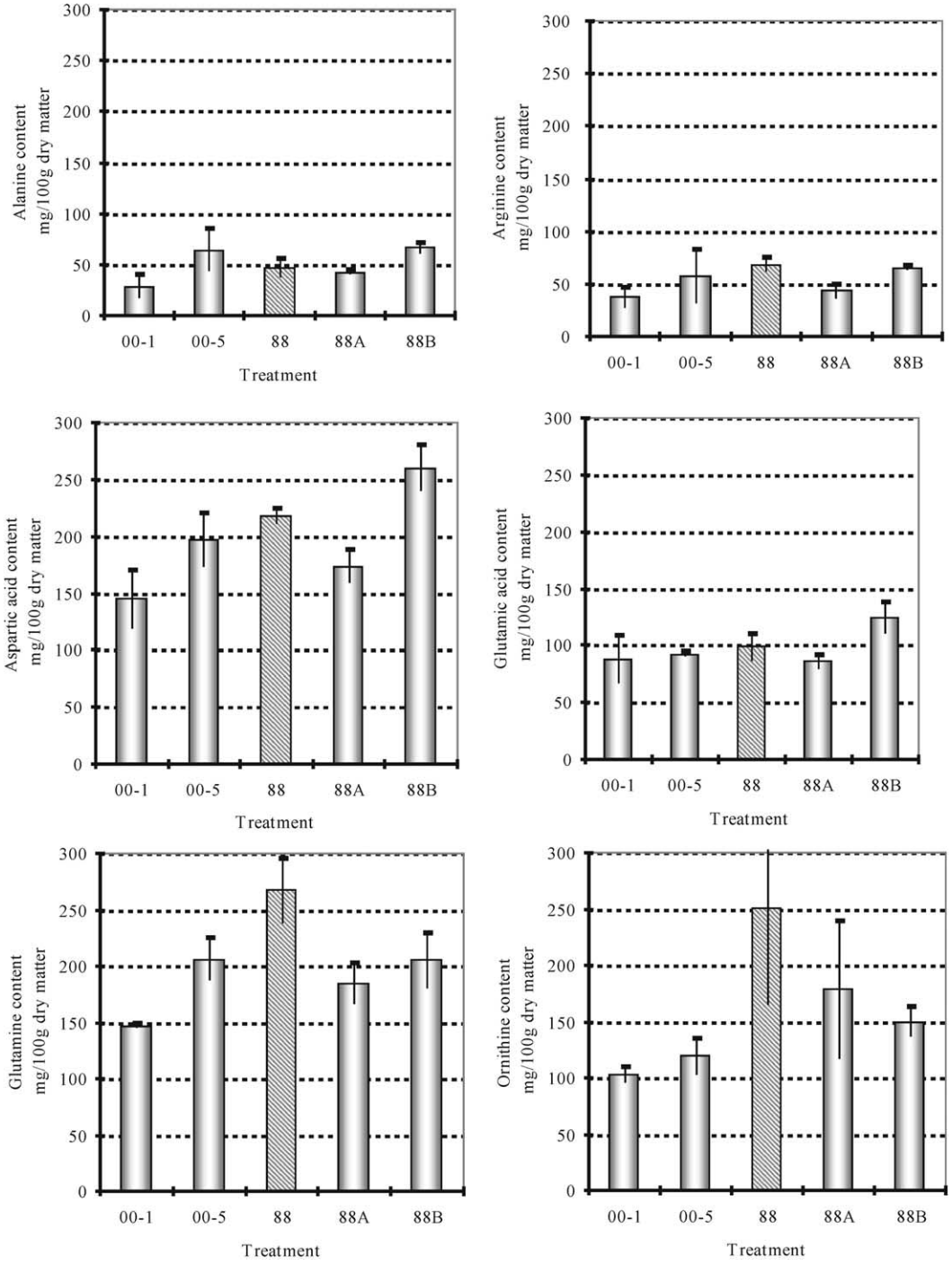

Fig. 3. Predominant free amino acid contents of fruit-bodies of *H. marmoreus*. 00-1, 00-5, 88, 88A and 88B: Refer to Fig. 1.

2000; Kitamoto & Gruen, 1976) and *Lentinus edodes* (Berk.) Sing. (Minamide & Iwata, 1987).

3.2. Influence of strains and cultivation media on soluble sugar content in fruit-body

The fruit-body of *H. marmoreus* contained trehalose and mannitol as major soluble sugars. The accumulation of trehalose and mannitol in the fruit-bodies of *A. bisporus* (Minamide & Hammond, 1985; Minamide et al., 1985), *F. velutipes* (Kitamoto & Gruen, 1976) and *L. edodes* (Minamide & Iwata, 1987; Yang et al., 2001) has been reported.

As shown in Table 1, differences of the mannitol contents among the treatments were less than those of the trehalose contents. Trehalose contents of Hm 00-1 and Hm 00-5 were 1.5 and 2.0 times, respectively, higher than in Hm 88-8. The contents of Hm 88-8 on medium A and medium B were 1.5 times and 1.6 times, respectively, higher than that on the basic medium. Differences in the sugar contents apparently affect the taste of *H. marmoreus*.

3.3. Influence of strains and cultivation media on free amino acid content in fruit-body

As shown in Fig. 2, patterns of free amino acid content were similar among different strains and media. Aspartic acid, glutamic acid, glutamine and ornithine were predominant amino acids of the fruit-body of *H. marmoreus*. High contents of aspartic acid, glutamine and ornithine are characteristic of this mushroom species.

Fig. 3 shows the effects of the strain and cultivation medium on the content of predominant amino acids, i.e., alanine, arginine, aspartic acid, glutamic acid, glutamine and ornithine. In these amino acids, except for glutamic acid, significant differences were observed between the strains. In Hm 88-8 on medium A, their contents were lower than those on basic medium. Moreover, contents of alanine, aspartic acid and glutamic acid increased on medium B. Aspartic and glutamic acids are monosodium glutamate-like (MSG-like) components, giving the most typical mushroom taste, i.e., the umami or palatable taste (Ninomiya, Ikeda, Yamaguchi, & Yoshikawa, 1966; Yamaguchi, Yoshikawa, Ikeda, & Ninomiya, 1971). Alanine and ornithine provide sweet and bitter taste, respectively (Ninomiya et al., 1966). Arginine, a hydrophobic amino acid, is known to be related to bitterness (Kasuga et al., 2000; Mau & Tseng, 1998, Mau et al., 1998, Sasaki et al., 1989, Yang et al., 2001).

Differences in the amino acid composition apparently would contribute to the taste of fruit-body. Based on the contents of MSG-like components, the umami taste is expected to be strongest in Hm 88-8 cultivated on medium B. From the contents of alanine and soluble sugars, it is anticipated that the sweetness is highest in Hm 00-5 (Table 1). Based on the contents of arginine and ornithine, the bitterness is expected to be highest in Hm 88-8 on the basic medium.

In conclusion, the content of free amino acids and soluble sugars in the fruit-body of *H. marmoreus* greatly varied with the strains and the cultivation media. Therefore, it is suggested that the taste of the mushroom depends upon the strains and cultivation media. Although the palatability of fruit-body is influenced by the strain used, the taste of the mushroom can be improved by using an appropriate cultivation medium.

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