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Changes in contents of free amino acids and soluble carbohydrates during fruit-body development of *Hypsizygus marmoreus*

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Abstract

Changes in the contents of free amino acids and soluble carbohydrates in the fruit-body of *Hypsizygus marmoreus* during its development were investigated. At 25 days (stage A), 27 days (stage B) and 29 days (stage C) after fruiting initiation, fruit-bodies were picked from the cultivation media to determine their free amino acid and soluble carbohydrate contents by using HPLC. Glutamic acid, aspartic acid, asparagine and ornithine were predominant amino acids and their contents, except for ornithine, were higher in the pileus than in the stipe. In contrast, ornithine was much higher in the stipes than in the pilei. In whole fruit-body, the maximal contents of aspartic acid, asparagine and glutamic acid were observed at stage A, whereas ornithine content peaked at stage B. The fruit-bodies contained mannitol and trehalose as major soluble carbohydrates. Their contents were higher in the stipes than in the pilei, and showed a peak at stage B that was at morphological optimum for harvest.

Keywords: *Hypsizygus marmoreus*; Free amino acid; Soluble carbohydrate; Fruit-body development

1. Introduction

The production of *Hypsizygus marmoreus* (Peck) Bigelow has increased rapidly during the past decade in Japan, and some commercial strains and various cultivation techniques for high productivity have been developed. Although the tastes, such as sweet, bitter and umami, have become important, only limited studies on the taste and flavour of edible mushrooms have been reported. In general, the harvesting time of edible mushrooms has been judged by their morphological ripeness, and not by their chemical components, such as free amino acids and carbohydrates, that affect their taste.

The free amino acids and soluble carbohydrates in the cultivated mushrooms were investigated as biochemical changes during fruit-body development and after harvest (Gruen & Wong, 1982a, 1982b; Kitamoto, Kikuchi, Mori, & Ohga, 2000; Minamide & Hammond, 1985; Minamide & Iwata, 1987; Minamide, Iwata, & Habu, 1985) and also, composition of chemical components affecting taste (Kasuga, Fujiwara, & Aoyagi, 1999; Sugahara, Arai, Aoyagi, & Kunisaki, 1975; Terashita, Kono, Shishiyama, & Yamauchi, 1992). It has been reported that, for fruit-body of *Polyporus arcularius* Bastch.: Fr. grown on liquid media, the composition and content of free amino acids and soluble carbohydrates changed during fruit-body development (Kitamoto et al., 1978, 1980; Terashita et al., 1984). These results suggest that changes in the content of free amino acids and soluble carbohydrates would contribute to the taste of edible mushrooms at the final stage of cultivation.

In the present study, as a part of research on the components affecting the taste of *H. marmoreus*, changes in the contents of free amino acids and soluble carbohydrates of the fruit-body, during its development, were investigated.

2. Materials and methods

2.1. Mushroom strains and culture conditions

The strain of *H. marmoreus* used in this study was Hm 88-8, which was the stock culture of Hokkaido

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Forest Products Research Institute (HFPRI). The stock culture was maintained on a PDA slant at 1.5 °C. Each 850 ml plastic bottle, containing 600 g of sawdust-based medium was used for cultivation. The medium was comprised of birch (*Betula ermanii* Cham) sawdust (109 g), rice bran (53 g), soybean shell (32 g) and corncob meal (28 g). 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 HFPRI procedure, as outlined by Harada, Gisusi, Yoneyama, Nakaya, and Ito (2001). The spawn running process was carried out at 22 °C for 60 days. To induce fruiting, the cultures were treated, by the removal of both the spawn and the uppermost layer of medium, and grown 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 designated periods and divided into stipes and pilei to determine their oven dry weights. Three replications for each harvesting period were conducted.

2.2. Extraction and determination of free amino acids and soluble carbohydrates

The pilei and stipes were freeze-dried, powdered and stored at −30 °C until used. Extraction was carried out according to the method of Terashita et al. (1984, 1992). One gramme of stipe or pileus tissue was suspended in 40 ml of 80% ethanol and homogenized in a Waring blender for 3 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 with ethyl ether, successively. After concentration at 40 °C, the solid residues were dissolved in a lithium citrate buffer solution (pH 2.2) for amino acid determination, whereas water was used as the solvent for carbohydrate determination. Free amino acids and soluble carbohydrates were determined by using HPLC (Shimadzu, LC10A System).

3. Results and discussion

3.1. Changes in dry weights of fruit-bodies during fruit-body development

When vegetative mycelia grown at 22 °C for 60 days in the dark were transferred into a culture room at 16 °C under fluorescent lamps, a cluster of fruit-body primordia appeared within 7–10 days. At 25 days (stage A), 27 days (stage B), and 29 days (stage C) after fruiting initiation, fruit-bodies were picked from the media. Fig. 1 shows changes in the dry weights during fruit-body development. Dry weight of the fruit-body at stage C was about 1.7 times higher than that of stage A. The dry weights of pillei and stipes at stage C were 2.9 times and 1.2 times, respectively, higher than those of stage A. This result indicated that the pileus grew at a relatively late stage of the fruit-body development. Kitamoto et al. (1978, 1980) and Terashita et al. (1984) reported that large portions of free amino acids and soluble carbohydrates were translocated from the mycelium into the stipe and pileus at a relatively early stage of the fruit-body development. From these results, it was anticipated that the distribution of chemical components in *H. marmoreus* would also change during its development.

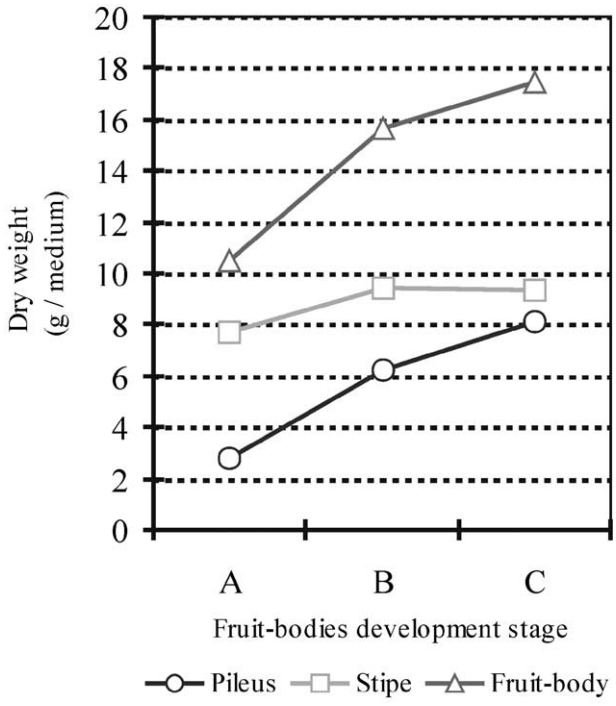

Fig. 1. Changes in the dry weights of fruit-body, pileus and stipe during fruit-body development: (A) fruit-bodies at 25 days after fruiting initiation, (B) fruit-bodies at 27 days after fruiting initiation, (C) fruit-bodies at 29 days after fruiting initiation.

3.2. Changes in free amino acid content of fruit-bodies during fruit-body development

As shown in Tables 1 and 2, the free amino acid pools in the pileus were qualitatively similar with those of the stipes. Their total contents were higher in the pilei than in the stipes. Among the free amino acids, aspartic acid, asparagine, glutamic acid and ornithine predominated. Their contents, except for ornithine, were much higher in the pilei than in the stipes. In contrast, the ornithine content was much higher in the stipes than in the pilei. In *Lentinus edodes* (Berk.) Sing., glutamic acid and alanine are predominant amino acids (Kasuga et al., 1999; Sugahara et al., 1975; Terashita et al., 1992), and their contents are higher in the pilei than in the stipes (Kasuga et al., 1999; Sugahara et al., 1975).

Table 1
Contents of free amino acids in the pilei of fruit-bodies of *Hypsizygus marmoreus* (mg/100 g dry matter)

Amino acids	Stage A	Stage B	Stage C
o-Phosphoserine	3.4±0.19	3.0±0.86	1.9±0.25
Taurine	2.4±0.63	1.6±0.34	2.1±0.30
o-Phosphoethanol-amine	4.0±0.14	3.9±0.39	2.0±0.23
Aspartic acid	300±40.0	261±41.4	284±35.4
OH-proline	15.3±3.78	14.8±0.87	15.7±1.04
Threonine	16.2±0.79	16.1±2.47	24.3±2.98
Serine	35.7±1.19	34.6±3.94	39.2±2.75
Asparagine	270±46.2	194±16.8	236±24.0
Glutamic acid	292±34.7	190±20.8	195±23.1
Sarcosine	9.1±1.37	4.6±1.59	3.2±0.42
α-Aminoadipic acid	12.4±2.04	7.3±1.18	8.0±1.02
Proline	20.0±2.81	14.4±1.36	16.1±1.53
Glycine	10.8±0.66	9.8±1.00	13.0±0.97
Alanine	48.1±5.76	31.4±4.64	32.5±4.12
Citruline	ND	ND	ND
α-Amino-*n*-butyric acid	1.1±0.12	0.6±0.05	0.6±0.07
Valine	17.4±1.45	21.0±2.87	22.2±1.56
Methionine	72.6±5.88	64.7±6.53	86.1±6.08
Cystine	5.7±1.07	3.3±0.81	1.9±0.51
Isoleucine	12.9±1.13	16.9±2.06	18.4±2.56
Cystathionine	ND	ND	ND
Leucine	18.9±1.52	26.7±3.72	30.1±2.75
Tyrosine	8.0±0.21	7.2±5.41	13.5±5.08
Phenylalanine	15.9±0.57	19.6±2.91	22.5±2.03
β-Alanine	ND	ND	ND
β-Amino-iso-butyric acid	9.7±1.31	6.6±0.83	6.4±0.99
γ-Aminobutyric acid	15.5±1.49	13.4±2.04	16.1±1.63
Histidine	15.1±0.84	19.9±2.62	22.9±2.21
3-Methylhistidine	0.7±0.52	1.0±0.20	1.0±0.17
1-Methylhistidine	0.8±0.58	ND	ND
Carnosine	ND	ND	ND
Anserine	17.4±5.70	11.9±4.80	11.9±4.56
OH-Lysine	0.7±0.35	0.7±0.21	0.5±0.12
Ornithine	58.5±6.93	76.2±8.50	64.8±8.01
Lysine	8.3±1.24	10.2±1.23	11.7±1.40
Arginine	17.2±1.72	20.3±2.03	32.6±3.30
Total	1335±154	1108±166	1237±138

Results are means of three replicates. ND, not detected.

Table 2
Contents of free amino acids in the stipes of fruit-bodies of *Hypsizygus marmoreus* (mg/100 g dry matter)

Amino acids	Stage A	Stage B	Stage C
o-Phosphoserine	1.7±0.10	1.7±0.10	1.2±0.13
Taurine	2.0±0.48	4.8±0.39	5.3±0.51
o-Phosphoethanol-amine	4.0±0.71	3.2±0.19	2.6±0.20
Aspartic acid	158±21.5	198±15.3	198±13.3
OH-Proline	ND	ND	ND
Threonine	12.8±1.45	12.7±1.91	13.9±1.87
Serine	20.4±2.36	15.8±1.43	12.6±0.98
Asparagine	139±13.8	118±10.1	130±12.1
Glutamic acid	121±16.5	95.7±7.00	85.2±5.31
Sarcosine	ND	ND	ND
α-Aminoadipic acid	10.4±1.09	11.3±0.35	20.3±1.55
Proline	ND	ND	ND
Glycine	8.6±0.69	7.1±0.48	8.2±0.56
Alanine	31.1±6.95	23.1±2.45	23.5±2.98
Citruline	ND	ND	ND
α-Amino-*n*-butyric acid	ND	ND	ND
Valine	7.8±0.40	6.9±0.53	6.4±0.65
Methionine	ND	ND	ND
Cystine	3.2±0.22	3.5±0.45	4.0±0.56
Isoleucine	5.0±0.07	4.1±0.57	4.6±0.95
Cystathionine	ND	ND	ND
Leucine	21.3±0.22	16.4±1.68	12.3±1.16
Tyrosine	5.1±0.30	6.3±0.46	6.3±0.43
Phenylalanine	10.7±0.40	11.6±0.90	11.0±0.85
β-Alanine	ND	ND	ND
β-Amino-iso-butyric acid	5.2±0.30	5.0±0.24	4.4±0.21
γ-Aminobutyric acid	6.6±1.11	4.2±0.35	2.6±0.24
Histidine	17.8±0.55	20.2±1.09	16.7±0.95
3-Methylhistidine	ND	ND	ND
1-Methylhistidine	ND	ND	ND
Carnosine	ND	ND	ND
Anserine	7.2±2.63	6.3±1.67	ND
OH-Lysine	ND	ND	ND
Ornithine	157.8±7.69	160±3.48	131±3.86
Lysine	12.1±0.63	12.7±4.39	16.7±2.08
Arginine	85.3±9.02	74.2±4.72	64.2±5.11
Total	853±63.6	822±52.9	781±50.2

Results are means of three replicates. ND, not detected.

Both in the pileus and stipe, the contents of asparagine and glutamic acid at stage A were highest. Glutamic acid content decreased by about 65% in the pilei and 70% in the stipes during fruit-body development. Similar results have been reported in fruit-bodies of *P. arcularius* (Terashita et al., 1984) and *Flammulina velutipes* (Curt.: Fr.) Sing. (Gruen & Wong, 1982a, 1982b). Changes in glutamic acid content in fruit-body may depend on glutamate dehydrogenase activities (Ikegaya, Goto, & Hayashi, 1994; Kawamura & Goto, 1978). At stage A, aspartic acid content in the pileus was highest among the three stages whereas, in the stipe it was lowest. Ornithine showed a peak at stage B, both in the pileus and stipe. These results suggest that changes of the amino acid contents depend both on mushroom species and cultivation conditions.

Aspartic acid, asparagine and glutamic acid in whole fruit-body peaked at stage A, although the ornithine content reached maximum at stage B (Table 3). Predominant amino acids except for ornithine are related to sourness, while sodium aspartate monohydrate and sodium glutamate monohydrate are related to umami (Ninomiya, Ikeda, Yamaguchi, & Yoshikawa, 1966). Ornithine provides a bitter taste (Ninomiya et al., 1966). The contents of predominant amino acids, except for ornithine, were maximal at stage A. Hydrophobic amino acids (Valine + Isoleucine + Leucine + Tyrosine + Phenylalanine + Arginine + Proline), relating to bitterness (Sasaki et al., 1989), were maximal at stage C. Changes in chemical components during fruit-body development apparently affected the taste of edible mushrooms. The results showed that the fruit-body at stage A might be tasty.

Table 3
Contents of free amino acids in the fruit-bodies of *Hypsizygus marmoreus* (mg/100 g dry matter)

Amino acids	Stage A	Stage B	Stage C
o-Phosphoserine	2.9	2.5	1.5
Taurine	2.3	2.9	3.6
o-Phosphoethanol-amine	4.0	3.6	2.3
Aspartic acid	263	236	244
OH-Proline	11.3	9.0	8.4
Threonine	15.3	14.8	19.5
Serine	31.7	27.1	26.8
Asparagine	235	164	187
Glutamic acid	247	153	144
Sarcosine	6.7	2.8	1.7
α-Aminoadipic acid	11.8	8.8	13.8
Proline	14.7	8.7	8.6
Glycine	9.1	8.7	10.8
Alanine	35.6	28.1	28.3
Citruline	ND	ND	ND
α-Amino-*n*-butyric acid	0.8	0.3	0.3
Valine	14.9	15.4	14.8
Methionine	53.4	39.1	46.1
Cystine	5.0	3.3	2.9
Isoleucine	10.8	11.8	12.0
Cystathionine	ND	ND	ND
Leucine	19.5	22.6	22.1
Tyrosine	7.2	7.1	10.2
Phenylalanine	14.5	16.4	17.2
β-Alanine	ND	ND	ND
β-Amino-iso-butyric acid	8.5	6.0	5.5
γ-Aminobutyric acid	13.1	9.8	16.4
Histidine	15.8	20.0	20.0
3-Methylhistidine	0.5	0.6	0.6
1-Methylhistidine	ND	ND	ND
Carnosine	ND	ND	ND
Anserine	12.8	9.7	6.3
OH-Lysine	0.5	0.4	0.3
Ornithine	84.7	109.3	95.8
Lysine	9.3	11.2	14.0
Arginine	35.2	41.7	47.3
Total	1196	994	1032

3.3. Changes in soluble carbohydrate contents of fruit-bodies during fruit-body development

The fruit-body of *H. marmoreus* contained trehalose and mannitol as major soluble carbohydrates. Their contents were higher in the stipes than in the pilei (Figs. 2 and 3). At stage B, mannitol content of the pilei and the stipes were 1.5 and 1.4 times, respectively, higher than those of stage A. Trehalose content also significantly increased in the incipient stage of the fruit-body development and showed a peak at stage B. The accumulation of trehalose, in both the pilei and stipes, indicates that trehalose is a major soluble carbohydrate translocated from vegetative mycelium (Kitamoto et al., 1978). The accumulation of mannitol in the fruit-bodies of *Agaricus bisporus* Sing. (Minamide & Hammond, 1985; Minamide et al., 1985) and *Coprinus phlyctodosporus* (Fr.) Fr. (Kitamoto, Fujino, & Mori, 1999) has been reported. Mannnitol was mainly translocated from vegetative mycelium. Changes in the contents of sugar and sugar alcohol also would contribute to a sweet taste of *H. marmoreus*, although their intensity of sweetness is lower than sucrose.

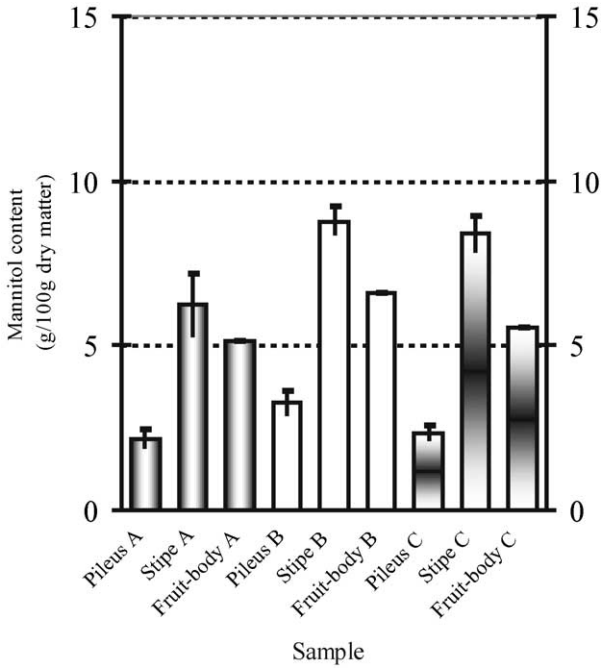

Fig. 2. Changes in the mannitol content during fruit-body development. A, B, C: refer to Fig. 1.

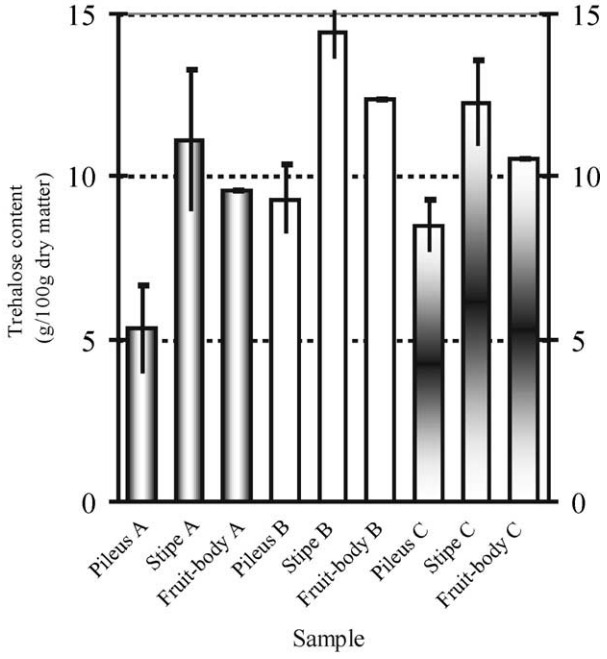

Fig. 3. Changes in the trehalose content during fruit-body development. A, B, C: refer to Fig. 1.

In conclusion, the contents of free amino acids and soluble carbohydrates in the fruit-body of *H. marmoreus* varied greatly during its development. Chemical

components affecting taste can be adjusted by the harvesting time of the fruit-body.

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