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Non-volatile taste components of Agaricus blazei, Antrodia camphorata and Cordyceps militaris mycelia

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Abstract

Three non-Ganoderma medicinal mushrooms are currently popular in Taiwan, including Brazilian mushroom (Agaricus blazei), chang-chih (Antrodia camphorata) and northern caterpillar fungus (Cordyceps militaris). The moisture contents of three dry mycelia ranged widely from 6.65 to 14.91%. All mycelia were high in carbohydrate content with chang-chih being the highest. The protein contents ranged from 9.49 to 29.1%. Soluble sugars found were arabitol, glucose and trehalose, and the contents exceeded 10%. Total free amino acid contents ranged from 7.01 to 11.1 mg g⁻¹ dry weight. Contents of monosodium glutamate-like components were relatively low and similar in Brazilian mushroom and chang-chih, but high in northern Cordyceps. Contents of bitter components were significantly high in Brazilian mushroom and northern Cordyceps. Contents of flavour 5'-nucleotides were similarly high in chang-chih and northern Cordyceps, and low in Brazilian mushroom. The three mushroom mycelia had different proximate compositions. However, northern Cordyceps and chang-chih might exhibit similar umami and sweet tastes. © 2001 Elsevier Science Ltd. All rights reserved.

Keywords: Mushroom mycelia; Agaricus blazei; Antrodia camphorata; Cordyceps militaris; Soluble sugars; Free amino acids; 5'-nucleotides

1. Introduction

Mushrooms have recently become attractive as functional foods, and a source of physiologically beneficial medicine. In addition to *Ganoderma* spp., three medicinal mushrooms are currently popular in Taiwan, including Brazilian mushroom, chang-chih and northern *Cordyceps*. Brazilian mushroom (*Agaricus blazei* Murill) was reported to possess antitumour and immunomodulating activities (Kawagishi, Katsumi, Sazawa, Mizuno, Hagiwara, & Nakamura, 1989). Its isolated polysaccharides could stimulate lymphocyte T-cells in mice (Mizuno, Morimoto, Minate, & Tsuchida, 1998).

Chang-chih or niu-chang-ku [Antrodia camphorata (Zang and Su)] in the Polyporaceae (Aphyllophorales) causes brown heart rot of Cinnamomum kanehirai Hay (Lauraceae) in Taiwan (Wu, Ryvarden, & Chang, 1997). "Niu-chang" is the Chinese common name for Cinnamomum kanehirai, which is one of the endangered species in Taiwan; "ku" in Chinese means mushroom; and

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"chih" means *Ganoderma*-like fungus. Chang-chih is well known in Taiwan as an expensive medicinal material, and is commonly used as an antidote, anticancer, antiitching and hepatoprotective drug.

Caterpillar fungus (*Cordyceps* sp.), a rare Chinese herbal medicine, looks like a worm in the winter and like a grass in the summer. Traditionally, *Cordyceps* is used for the treatment of general debility after sickness and for persons of advanced age. Several biologically active compounds have been isolated from northern *Cordyceps* [*Cordyceps militaris* (L.) Link] (Frederiksen, Malling, & Klenow, 1965; Furuya, Hirotani, & Matsuzawa, 1983).

Fruit bodies of chang-chih and northern *Cordyceps* are expensive and scarce, partially due to their rareness and difficulty in cultivation. Furthermore, Brazilian mushrooms are not cultivated in Taiwan. Thus, mycelia of these three mushrooms are mainly prepared from submerged culture for use in the formulation of nutraceuticals and functional foods. The chemical composition and taste components of these mycelia suggest good food product acceptability; however, these products are not available. Our objective was to examine the non-volatile taste component in the mycelia of three

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medicinal mushrooms, including proximate compositions, soluble sugars, free amino acids and 5'-nucleotides.

2. Materials and methods

2.1. Mushroom mycelia

Dried mycelia of *Agaricus blazei*, *Antrodia camphorata* and *Cordyceps militaris* were obtained from the Biotechnology Center, Grape King Inc., Chungli City, Taiwan.

2.2. Proximate analysis

The proximate compositions of the three species of mushroom mycelia, including moisture, ash, carbohydrate, crude fat, crude fibre and crude protein, were determined according to the methods of AOAC (1990). The nitrogen factor used for crude protein calculation was 4.38 (Crisan & Sands, 1978). Total reducing sugars were determined using the 3,5-dinitrosalicylic acid (DNS) method as described by James (1995). The absorbance of each sample solution was measured at 540 nm on a Hitachi 2001 spectrophotometer. Total reducing sugars were calculated, based on a calibration curve of glucose.

2.3. Soluble sugar assay

Soluble sugars were extracted and analysed as described by Ajlouni, Beelman, Thompson, and Mau (1995). Dried mushroom mycelia (600 mg) were extracted with 50 ml of 80% aqueous ethanol (95% pure, Taiwan Tobacco & Wine Monopoly Bureau, Taipei) and xylose (50 mg, Sigma Chemical Co., St. Louis, MO) was added as an internal standard. This suspension was shaken for 45 min at room temperature and filtered through Whatman No. 4 filter paper. The residue was washed five times with additional 25-ml portions of 80% ethanol. The combined filtrate was then rotary-evaporated at 40°C and redissolved in deionised water to a final volume of 10 ml. The aqueous extract was passed through a filter unit (13 mm, Lida, Corp., Kenosha, WI), and filtered using 0.45-µm CA non-sterile filter (Lida) prior to injection onto a high-performance liquid chromatograph (HPLC).

The HPLC system consisted of a Hitachi L-6000 pump, a Rheodyne 7161 injector, a 20-μm sample loop, a Hitachi D-2500 chromato-integrator, a Hitachi L-4000 UV detector, and a Phase Sep-NH₂ column (4.6×250 mm, 5 μm, Phase Separation Inc., Norwalk, CT). The mobile phase was acetonitrile (LC grade, Tedia Co., Fairfield, OH)/deionised water, 75:25 (v/v) at a flow rate of 2 ml min⁻¹ and UV detection at 190 nm. Each sugar was quantified by comparing the peak area of the sugar to that of the internal standard.

2.4. Free amino acid assay

Dried mushroom mycelia (500 mg) were shaken with 50 ml of 0.1 N HCl (Union Chemical Co., Hsinchu, Taiwan) for 45 min at ambient temperature and filtered through Whatman No. 4 filter paper. The filtrate was then passed through a filter unit (13 mm, Lida), and filtered using 0.45 μ m CA non-sterile filter (Lida). This filtrate was mixed with o-phthalaldehyde reagent (Sigma) in an Eppendorf tube, shaken to facilitate derivatisation and then immediately injected onto HPLC.

The HPLC system was the same as for sugar analysis but included a Hitachi F-1050 fluorescence detector with fluorescence excitation at 340 nm and emission at 450 nm, and a Prodigy 5 ODS-2 column (4.6×250 mm, 5 μ m, Phenomenex Inc., Torrance, CA). The mobile phases and gradient conditions were the same as described in Mau, Chyau, Li, and Tseng (1997). Each amino acid was quantified by the calibration curve of the authentic amino acid.

2.5. 5'-Nucleotide assay

5'-Nucleotides were extracted and analysed as described by Taylor, Hershey, Levine, Coy, and Olivelle (1981). Dried mushroom mycelia (500 mg) were extracted with 25 ml of deionised water. This suspension was heated to boiling for 1 min, cooled, and then centrifuged at 22,200 g for 15 min. The extraction was repeated once with 20 ml of deionised water. The combined filtrate was then evaporated, and filtered prior to HPLC injection in the same manner as in soluble sugar assay.

The HPLC system was the same as for sugar assay except for a Prodigy 5 ODS-2 column $(4.6\times250 \text{ mm}, 5 \text{ } \mu\text{m})$, Phenomenex). The mobile phase was 0.5 M KH₂PO₄/H₃PO₄ (pH 4.0, Wako Pure Chemical Co., Osaka, Japan) at a flow rate of 1 ml min⁻¹ and UV detection at 254 nm. Each 5'-nucleotide was quantified by the calibration curve of the authentic 5'-nucleotide.

2.6. Statistical analysis

For each mushroom, three samples were used for the determination of every quality attribute. The experimental data were subjected to an analysis of variance for a completely random design as described by Steel, Torrie, and Dickey (1997), to determine the least significant difference among means at the level of 0.05.

3. Results and discussion

The moisture contents of the three dry mycelia ranged widely from 6.65 to 14.91% (Table 1). Based on dry weight, all mycelia were high in carbohydrate content, with chang-chih being the highest. This is in general

agreement with the finding in Hwang and Mau (1997). The fat contents (7.20–9.79% dry wt.) were surprisingly higher than that in mycelia of common mushroom Agaricus bisporus (1.73%) (Hwang & Mau, 1997). The fibre contents (19.2~26.4%) were consistently high and much higher than that in Agaricus bisporus mycelia (8.72%; Hwang & Mau, 1997). The protein contents ranged from 9.49 to 29.1% and in the ascending order of chang-chih, Brazilian mushroom and northern Cordyceps. Ash contents (3.92–6.97%) were not as high as those found in mycelia of Agaricus blazei (9.68%) (Huang, Huang, Chen, & Mau, 1999a) or chang-chih (16.03 and 8.92%) (Huang, Huang, Chen, & Mau, 1999b). The total reducing sugar contents were much lower than the carbohydrate contents for the three mycelia. Evidently, the difference between carbohydrate and total reducing sugar contents is the content of polysaccharides, which were thought to be the biologically active component of these mycelia, and contents were 30.8, 31.3 and 24.6% for Brazilian mushroom, chang-chih and northern *Cordyceps*, respectively.

Generally, common mushrooms are high in protein for both fruit bodies (43.6%) and mycelia (37.0%; Hwang & Mau, 1997). The pattern was consistent with the finding for fruit bodies of *Agaricus blazei* (35.86%; Huang, Huang, Chen, & Mau, 1999a). However, this pattern was not followed in this study for mycelia of *Agaricus blazei* (15.60%). The carbohydrate content in *Agaricus blazei* mycelia (42.41%) was lower than those in mycelia (52.9 and 59.4%), whereas the fibre content (26.4%) was much higher than those in mycelia (7.03 and 7.29%) (Huang, Huang, Chen, & Mau, 1999a). However, the fat and protein contents were between the findings in Huang, Huang, Chen, and Mau (1999a).

The carbohydrate content in chang-chih mycelia (56.66%) was similar to that in mycelia (53.5%) but higher than those in fruit bodies (31.4 and 36.9%; Huang, Huang, Chen, & Mau, 1999b). The fat content

Table 1 Proximate composition of *Agaricus blazei*, *Antrodia camphorata* and *Cordyceps militaris* mycelia

Component ^a	Content ^b (%)		
	A. bazei	A. camphorata	C. millitaris
Moisture	10.76 ± 0.22	6.65 ± 0.10	14.91±0.18
Dry matter	89.24 ± 0.22	93.35 ± 0.10	85.09 ± 0.18
Carbohydrate	42.4 ± 0.05 b	$56.7 \pm 0.49a$	$37.5 \pm 0.05c$
Total reducing sugar	$11.6 \pm 0.15b$	$25.4 \pm 1.25a$	13.0 ± 0.15 b
Crude ash	$5.90 \pm 0.18b$	$3.92 \pm 0.02c$	$6.97 \pm 0.18a$
Crude fat	$9.68 \pm 0.03a$	$9.79 \pm 0.06a$	$7.20 \pm 0.03b$
Crude fibre	$26.4 \pm 0.88a$	$20.1 \pm 0.99b$	$19.2 \pm 0.88b$
Crude protein	$15.6 \pm 0.35b$	$9.49 \pm 1.03c$	$29.1 \pm 0.35a$

^a Moisture and dry matter in mycelia are based on air-dried weight, others are based on dry weight.

(9.79%) was higher than that in mycelia (5.81%) but much lower than those in fruit bodies (32.23 and 37.44%; Huang, Huang, Chen, & Mau, 1999b). The fibre content (20.1%) was much higher than that in mycelia (7.21%) and similar to those in fruit bodies (22.5 and 22.8%) (Huang, Huang, Chen, & Mau, 1999b). The composition of culture medium had great impact on the proximate composition of mycelia (Hwang & Mau, 1997). Hence, by changing the composition of medium, the profile of proximate composition in mycelia could be made more similar to that in fruit bodies.

The three mycelia contained arabitol, glucose and trehalose, and the contents exceeded 10% (Table 2). The profile of soluble sugars was consistent in the three mycelia and the contents of each component were in the descending order: glucose > arabitol > trehalose. Mannitol and trehalose were present in fruit bodies of common mushrooms (Hammond & Nichols, 1976) and other mushrooms (Bano & Rajarathnam, 1988; Mau, Chyau, Li, & Tseng, 1997). Chen (1986) found that mannitol was the taste-active component in mushroom sugars. However, mannitol was not found in mycelia of common mushrooms (Hwang & Mau, 1997) or in this study. Soluble sugars, contained in the mushrooms, contributed a sweet taste (Litchfield, 1967). Therefore, the high contents of soluble sugars would give rise to a moderately sweet perception, and not to the typical mushroom taste.

The total free amino acid contents in three mycelia ranged from 7.01 to 11.1 mg g⁻¹ dry weight and were in the ascending order: Brazilian mushroom < changchih < northern *Cordyceps* (Table 3). The amino acids with contents of more than 1 mg g⁻¹ were alanine and valine for Brazilian mushrooms, alanine and lysine for chang-chih, and alanine, aspartic acid, tyrosine and valine for northern *Cordyceps*. Table 4 divides the free amino acids into several classes based on their taste characteristics, as described by Komata (1969). Aspartic and glutamic acids were monosodium glutamate-like (MSG-like) components, which gave the most typical mushroom taste, the umami taste (or palatable taste)

Table 2 Content of arabitol and soluble sugars of *Agaricus blazei*, *Antrodia camphorata* and *Cordyceps militaris* mycelia

Sugar	Content ^a (mg g ⁻¹ dry wt.)			
	A. blazei	A. camphorata	C. militaris	
Arabitol	31.4±0.68b	$40.7 \pm 0.05a$	41.2±0.07a	
Glucose	$45.3 \pm 0.27b$	$51.8 \pm 0.55a$	$51.2 \pm 0.56a$	
Trehalose	$23.9 \pm 0.24a$	$22.4 \pm 0.69a$	$21.7 \pm 0.24b$	
Total	$101 \pm 1.48b$	115±1.59a	114±1.39a	

^a Each value is expressed as mean \pm S.E. (n=3). Means with different letters within a row are significantly different (P < 0.05).

^b Each value is expressed as mean \pm S.E. (n=3). Means with different letters within a row are significantly different (P < 0.05).

that is characteristic of MSG and 5'-nucleotides (Yamaguchi, 1979). Contents of MSG-like components were relatively low and similar in Brazilian mushroom and chang-chih, but significantly higher in northern *Cordyceps*. Contents of sweet components ranged from 1.67 to 2.57 mg g⁻¹ and were in the ascending order: < Brazilian mushroom < northern *Cordyceps* < changchih. Contents of bitter components were significantly high in the total free amino acid contents of Brazilian mushroom and northern *Cordyceps*.

Chen (1986) found that alanine, glycine and threonine (sweet), and aspartic and glutamic acids (MSG-like) were taste-active amino acids in common mushrooms, whereas none of the bitter components was found to be taste-active. The bitterness from bitter components in the three mycelia could probably be masked by the sweetness from sweet components and mainly the high amount of total sugars. Therefore, MSG-like and sweet components would be responsible for the natural taste of the three mushroom mycelia. Yang, Lin, and Mau (2001) reported that contents of MSG-like components could be divided into three ranges: low ($\leq 5 \text{ mg g}^{-1}$), middle (5 \sim 20 mg g⁻¹) and high (>20 mg g⁻¹). However, contents of MSG-like substances in these three mushroom mycelia were in the low range (0.47–2.97 mg g^{-1}).

Contents of total 5'-nucleotides were high in northern *Cordyceps* (38.0 mg g⁻¹) and chang-chih (29.2 mg g⁻¹), and low in Brazilian mushroom (7.00 mg g⁻¹; Table 5). Flavour 5'-nucleotides, which also gave the umami or

Table 3 Content of free amino acids of *Agaricus blazei*, *Antrodia camphorata* and *Cordyceps militaris* mycelia

Amino acid	Content ^a (mg g ⁻¹ dry wt.)			
	A. blazei	A. camphorata	C. militaris	
L-Alanine	1.05±0.09b	2.02±0.07a	1.19±0.11b	
L-Arginine	$0.45 \pm 0.01b$	$0.68 \pm 0.07a$	$0.04 \pm < 0.01c$	
L-Aspartic acid	$0.50 \pm 0.06b$	$0.42 \pm 0.08b$	$2.66 \pm 0.17a$	
L-Glutamic acid	nd^b	$0.05 \pm < 0.01b$	$0.31 \pm 0.02a$	
Glycine	nd	$0.18 \pm 0.08b$	$0.61 \pm 0.26a$	
L-Histidine ^c	$0.66 \pm 0.28a$	$0.27 \pm 0.05b$	$0.38 \pm < 0.01b$	
L-Isoleucine ^c	$0.21 \pm < 0.01a$	$0.18 \pm < 0.01b$	$0.13 \pm < 0.01c$	
L-Leucine ^c	$0.31 \pm < 0.01b$	$0.62 \pm 0.02a$	$0.08 \pm < 0.01c$	
L-Lysine ^c	$0.61 \pm < 0.01b$	$3.25 \pm 0.16a$	$0.58 \pm < 0.01b$	
L-Methionine ^c	$0.67 \pm 0.27a$	nd	$0.11 \pm < 0.01b$	
L-Phenylalanine ^c	$0.17 \pm < 0.01b$	nd	$0.76 \pm 0.02a$	
L-Serine	$0.09 \pm < 0.01a$	$0.04 \pm < 0.01b$	$0.11 \pm < 0.01a$	
L-Threonine ^c	$0.53 \pm 0.03a$	$0.33 \pm 0.02b$	$0.31 \pm 0.03b$	
L-Tyrosine	nd	$0.12 \pm < 0.01b$	$1.57 \pm 0.08a$	
L-Valine ^c	$1.76 \pm 0.07b$	nd	$2.21 \pm < 0.01a$	
Total	$7.01 \pm 0.28c$	8.16±0.25b	11.1±0.21a	

^a Each value is expressed as mean \pm S.E. (n=3). Means with different letters within a row are significantly different (P < 0.05).

palatable taste, were found to be 5'-guanosine monophosphate (5'-GMP), 5'-inosine monophosphate (5'-IMP), and 5'-xanthosine monophosphate (5'-XMP; Chen, 1986). However, contents of flavour 5'-nucleotides were similarly high in chang-chih (27.3 mg g⁻¹) and northern *Cordyceps* (23.9 mg g⁻¹), and low in Brazilian mushroom (5.51 mg g⁻¹). Yang, Lin, and Mau (2001) reported that contents of flavour 5'-nucleotides could be divided into three ranges: low (<1 mg g⁻¹), middle (1–5 mg g⁻¹) and high (>5 mg g⁻¹). Contents of flavour 5'-nucleotides in these three mushroom mycelia were in the high range (5.51–27.3 mg g⁻¹).

5'-GMP gave the meaty flavour, and is a flavourenhancer which is much stronger than MSG (Litchfield, 1967). The synergistic effect of flavour 5'-nucleotides with MSG-like components might greatly increase the umami taste of mushrooms (Yamaguchi, Yoshikawa,

Table 4 Content of taste groups of free amino acids in *Agaricus blazei*, *Antro-dia camphorata* and *Cordyceps militaris* mycelia

Taste characteristic ^a	Content ^b (mg g ⁻¹ dry wt.)			
	A. blazei	A. camphorata	C. militaris	
Bitter	4.23±0.12b	1.75±0.15c	$3.71 \pm 0.25a$	
MSG-like	$0.50 \pm 0.06b$	$0.47 \pm 0.09b$	$2.97 \pm 0.17a$	
Sweet	$1.67 \pm 0.11c$	$2.57 \pm 0.17a$	2.22 ± 0.05 b	
Tasteless	$0.61 \pm 0.01c$	$3.37 \pm 0.16a$	$2.15 \pm 0.03b$	
Total	$7.01 \pm 0.28c$	$8.16 \pm 0.25b$	11.05±0.21a	

^a MSG-like, monosodium glutamate-like, Asp+Glu. Sweet, Ala+Gly+Ser+Thr. Bitter, Arg+His+Ile+Leu+Met+Phe+Val. Tasteless, Lys+Tyr.

Table 5 Content of 5'-nucleotides of *Agaricus blazei*, *Antrodia camphorata* and *Cordyceps militaris* mycelia

5'-Nucleotide ^a	Content ^b (mg g ⁻¹ dry wt.)			
	A. blazei	A. camphorata	C. militaris	
5'-CMP	1.49±0.15b	1.93±0.49b	12.8±3.01a	
5'-GMP	nd ^c	$2.63 \pm 0.68a$	$2.59 \pm 0.04a$	
5'-IMP	$1.86 \pm 0.15a$	nd	$1.84 \pm 0.05a$	
5'-UMP	nd	nd	1.33 ± 0.11	
5'-XMP	$3.65 \pm 1.20b$	$24.7 \pm 4.80a$	$19.5 \pm 3.16a$	
Flavour				
5'-nucleotides ^d	$5.51 \pm 1.35b$	$27.31 \pm 5.48a$	$23.93 \pm 6.25a$	
Total	$7.00 \pm 1.50c$	29.24 ± 5.97 b	$38.01 \pm 9.36a$	

^a 5'-AMP, 5'-adenosine monophosphate; 5'-CMP, 5'-cytosine monophosphate; 5'-GMP, 5'-guanosine monophosphate; 5'-IMP, 5'-inosine monophosphate; 5'-UMP, 5'-uridine monophosphate; 5'-XMP, 5'-xanthosine monophosphate.

b nd, not detected.

^c Essential amino acid.

^b Each value is expressed as mean \pm S.E. (n=3). Means with different letters within a row are significantly different (P < 0.05).

^b Each value is expressed as mean \pm S.E. (n=3). Means with different letters within a row are significantly different (P < 0.05).

c nd, not detected.

^d Flavour 5'-nucleotide: 5'-GMP + 5'-IMP + 5'-XMP.

Ikeda & Ninomiya, 1971). Due to their low contents of MSG-like components, the umami or palatable taste of these three mushroom mycelia mainly depends on contents of flavour 5'-nucleotides. Therefore, based on contents of MSG-like components and flavour 5'nucleotides, the umami intensities were expected to be in the ascending order: Brazilian mushroom < northern Cordyceps < chang-chih. However, the umami intensities might be similar for northern Cordyceps and chang-chih. Hence, further study on the umami intensity by sensory evaluation is needed to compare these two mycelia. In addition, based on their contents of total soluble sugars and sweet components, it anticipated that the sweet intensities would be similarly high in northern Cordyceps and chang-chih, and low in Brazilian mushroom. In this study, the three mushroom mycelia had different profiles of proximate composition. However, northern Cordyceps and chang-chih might exhibit similar umami and sweet tastes. To determine the relationship of these mycelia with their taste components, and determine the taste threshold of these components for use in the formulation of nutraceuticals and functional foods, further sensory evaluation is in progress.

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