Nonvolatile Taste Components of Three Strains of *Agrocybe cylindracea*

Jeng-Leun Mau* and Yu-Hsiu Tseng

Department of Food Science, National Chung-Hsing University, Taichung 40227, Taiwan, Republic of China

Three strains of the mushroom *Agrocybe cylindracea* (strains B, M, and W) are commercially available. Strain W contained higher moisture content (91.50%) than strains B and M (90.35 and 90.34%, respectively). *A. cylindracea* strains were low in fat (2.18−2.71% dry weight) and high in fiber (16.15−16.70%) and protein contents (34.17−44.94%). Fructose, mannitol, and trehalose were detected in all three strains, whereas glucose was not detected in strain B. Strain W contained the highest amount of total free amino acids (63.34 mg/g of dry weight), and strain M contained the lowest (39.30 mg/g). The three strains contained high amounts of glutamic acid, threonine, arginine, and phenylalanine, with glutamic acid being the most significant. The contents of monosodium glutamate-like components, including aspartic and glutamic acids, were similar in three strains. Strain B contained the highest amounts of total 5′-nucleotides and flavor 5′-guanosine monophosphate (1.51 and 0.63 mg/g), whereas strain W contained the lowest (0.67 and 0.21 mg/g, respectively). In this study, the three strains were considerably different in both their proximate compositions and taste components and their physical appearances.

Keywords: *Agrocybe cylindracea; mushrooms; proximate composition; soluble sugars; free amino acids; 5′-nucleotides*

INTRODUCTION

Due to their unique and subtle flavor properties, mushrooms have been used as a food or food-flavoring material in soups and sauces for centuries. The typical flavor substances of mushrooms can be classified into volatile compounds and nonvolatile components (Maga, 1981). However, the content of volatile compounds, especially 1-octen-3-ol, decreased dramatically over storage time (Wurzenberger and Grosch, 1983; Mau et al., 1991) and was reduced significantly during cooking and processing (Dijkstra, 1976; Sulkowska and Kaminski, 1977; MacLeod and Panchasara, 1983). Thus, nonvolatile components would be 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 small, water-soluble substances, including 5′-nucleotides, free amino acids, and soluble carbohydrates (Litchfield, 1967; Hammond and Nichols, 1975; Hammond, 1978; Chen, 1986; Lin, 1988).

Agrocybe cylindracea (DC: Fr.) Mre. [syn. *Agrocybe aegerita* (Briganti) Singer] is a newly cultivated edible mushroom in Taiwan. The mushroom is easily grown using poplar trunks, straw, or other vegetable remains, and under suitable conditions it will form its first fruiting bodies in 2−3 months (Leu, 1992). Currently, three strains with different appearances are commercially available in Taiwan, including strains B (brown color in caps, called liu-sung-ku, willow-pine mushroom), M (golden color, called pai-yang-ku, poplar mushroom), and W (white color, called hsueh-jung, snow mushroom). Willow-pine mushrooms were introduced from Japan into Taiwan by Lung-Kuo Mushroom Farm, Taichung, in 1986. The snow mushroom is a stable white mutant of strain B. The poplar mushroom is a strain (CCRC 36033) cultivated successfully by the Food Industry Research and Development Institute, Hsinchu, Taiwan.

Generally, commercialized products of *A. cylindracea* have long stipes and closed caps. The mushrooms have become increasingly popular in Taiwan recently, due to their delicious taste and unique texture. Also, the mushrooms are thought to have better bite and chew texture than oyster mushrooms (*Pleurotus* spp.) and are tastier than shiitake (*Lentinula edodes*). However, the taste components of *A. cylindracea* are unknown. Our objective was to examine the nonvolatile components in these three strains (strain B, M, and W) of *A. cylindracea*, including their proximate compositions, soluble sugars, free amino acids, and 5′-nucleotides.

MATERIALS AND METHODS

Mushrooms. Three strains of *A. cylindracea* (strains B, M, and W) were cultivated at Lung-Kuo Mushroom Farm, Taichung County, Taiwan. The three strains were cultivated in plastic jars using the same sawdust supplemented with rice bran but in separate production rooms under standard cropping conditions with temperature and humidity optimized for each strain. Mushrooms were harvested before the veils broke, freeze-dried, then ground to powder, and stored in a desiccator before use.

Proximate Analysis. The proximate compositions of *A. cylindracea* (strains B, M. and W), including moisture, ash, carbohydrate, crude fat, crude fiber, and crude protein, were determined according to the methods of the AOAC (1990). The nitrogen factor used for crude protein calculation was 4.38 (Crisan and Sands, 1978).

* Address correspondence to this author at the Department of Food Science, National Chung-Hsing University, 250 Kuokuang Road, Taichung 40227, Taiwan, ROC (telephone 886-4-285-4313; fax 886-4-287-6211; e-mail jlmau@dragon.nchu.edu.tw).

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Soluble Sugar Assay. Soluble sugars were extracted and analyzed as described by Ajlouni et al. (1995). Freeze-dried mushroom powder (600 mg) was 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 deionized 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 a 0.45 μm CA Non-ste 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 μL sample loop, a Hitachi D-2500 chromatointegrator, a Bischoff RI 8110 detector, and a Phase Sep-NH_2 column (4.6 × 250 mm, 5 μm, Phase Separation Inc., Norwalk, CT). The mobile phase was acetonitrile (LC grade, Tedia Co., Fairfield, OH)/deionized water, 90:10 (v/v), at a flow rate of 1 mL/min. Each sugar was quantified by comparing the peak area of the sugar to that of the internal standard.

Free Amino Acid Assay. Freeze-dried mushroom powder (500 mg) was 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 a 0.45 μm CA Non-ste filter (Lida). The purified filtrate was mixed with *o*-phthalaldehyde (OPA) reagent (Sigma) in an Eppendorf tube, shaken to facilitate derivatization, and then immediately injected onto the 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 μm, Phenomenex Inc., Torrance, CA). The mobile phases were (A) 50 mM sodium acetate (pH 5.7, Wako Pure Chemical Co., Osaka, Japan) containing 0.5% tetrahydrofuran (LC grade, Tedia), (B) deionized water, and (C) methanol (LC grade, Alps Chem Co., Hsinchu, Taiwan). The gradient was A/B/C 83:0:17 (v/v/v) to 33:0:67 for 0−37 min, 0:33:67 for 37−40 min, and 0:100:0 for 40−43 min. The flow rate was 1.2 mL/min. Each amino acid was quantified by the calibration curve of the authentic amino acid.

5′-Nucleotide Assay. 5′-Nucleotides were extracted and analyzed as described by Taylor et al. (1981). Freeze-dried mushroom powder (500 mg) was extracted with 25 mL of deionized water. This suspension was heated to boiling for 1 min, cooled, and then centrifuged at 22200*g* for 15 min. The extraction was repeated once with 20 mL of deionized 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 Hitachi L-4000 UV detector and a Prodigy 5 ODS-2 column (4.6 × 250 mm, 5 μm, Phenomenex). The mobile phase was 0.5 M KH_2PO_4/H_3PO_4 (pH 4.0, Wako) at a flow rate of 1 mL/min and UV detection at 254 nm. Each 5′-nucleotide was quantified by the calibration curve of the authentic 5′-nucleotide.

Statistical Analysis. For each strain of *A. cylindracea*, three samples of mushrooms 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 et al. (1997), to determine the least significant difference (LSD) among means at the level of 0.05.

RESULTS AND DISCUSSION

Strain W of *A. cylindracea* contained significantly higher moisture content (91.50%) than strains B and M (90.35 and 90.34%, respectively) (Table 1). Crisan and Sands (1978) reported that most fresh mushrooms contained ≈90% moisture. The nitrogen factor used for crude protein calculation was 4.38 instead of 6.25 (Crisan and Sands, 1978), because mushrooms usually contain a high amount of chitin, a biopolymer of *N*-acetylglucosamine, which interferes in the total nitrogen determination. Generally, mushrooms are a good source of protein, and their protein contents range from 19 to 35% dry weight (Crisan and Sands, 1978). However, strains B and W contained >40% protein (Table 1), higher than those mentioned above.

Table 1. Proximate Composition in the Three Strains of *A. cylindracea*

component[a]	content[b] (%) strain B	strain M	strain W
moisture	90.35b	90.34b	91.50a
dry matter	9.65a	9.66a	8.50b
ash	8.19b	7.66c	8.59a
carbohydrate	29.02b	39.54a	27.06c
crude fat	2.18c	2.48b	2.71a
crude fiber	16.37b	16.15c	16.70a
crude protein	44.24b	34.17c	44.94a

[a] Moisture and dry matter are presented based on fresh weight; others are presented based on dry weight. [b] Means with different letters within a row are significantly different ($P < 0.05$, LSD test).

Table 2. Content of Soluble Sugars in the Three Strains of *A. cylindracea*

sugar	content[a] (mg/g of dry weight) strain B	strain M	strain W
fructose	5.04c	36.72a	16.64b
glucose	ND[b]	4.84	3.36
mannitol	12.32c	18.87b	31.01a
trehalose	38.64a	25.71b	21.32c
total	56.00b	86.14a	72.33a

[a] Means with different letters within a row are significantly different ($P < 0.05$, LSD test). [b] ND, not detected.

The lipid contents in the mushrooms ranged from 1.1 to 8.3% dry weight, with the mean being ∼4.0% (Crisan and Sands, 1978). However, three strains contained 2.18−2.71% lipid (Table 1). The carbohydrate contents in the mushrooms ranged from 44.0 to 74.3% dry weight (Crisan and Sands, 1978). Surprisingly, the three strains contained 27.06−39.54% carbohydrate. The crude fiber contents in three strains were 16.15−16.70%, which were higher than those of common mushrooms *Agaricus bisporus* (8.1%), shiitake *Lentinula edodes* (7.3−8.0%), enokitake *Flammulina velutipes* (3.7%), oyster mushrooms *Pleurotus* sp. (11.5%), and straw mushrooms *Volvariella volvacea* (9.3%) (Crisan and Sands, 1978). The major component of crude fiber in the mushrooms was chitin, which is an important structural polysaccharide found in the cell wall (Michalenko et al., 1976). The consistency of high protein and low carbohydrate contents found in strains B and W could be explained by the fact that strain W is a stable white mutant originated from strain B. Summarily, from the proximate composition shown in Table 1, like other edible mushrooms, these three strains could be recommended as a valuable healthy food.

Soluble sugars contained in the mushrooms contributed a sweet taste (Litchfield, 1967). Fructose, mannitol, and trehalose were detected in all three strains, while glucose was not detected in strain B (Table 2). Mannitol contents varied among the three strains, with the highest being found in strain W, which was consis-

Table 3. Content of Free Amino Acids in the Three Strains of *A. cylindracea*

amino acid	content[b] (mg/g of dry weight)		
	strain B	strain M	strain W
L-alanine	3.02b	2.94b	3.93a
L-arginine	4.02b	3.07b	6.07a
L-aspartic acid	1.96a	2.21a	2.33a
L-glutamic acid	8.89a	9.68a	10.72a
glycine	2.07ab	1.09b	2.40a
L-histidine[a]	2.35b	2.41b	3.98a
L-isoleucine[a]	2.11ab	1.37b	2.78a
L-leucine[a]	2.15ab	1.55b	2.88a
L-lysine[a]	2.18ab	1.37b	2.89a
L-methionine[a]	0.81b	0.71b	1.44a
L-phenylalanine[a]	4.67a	3.62a	5.40a
L-serine	2.43b	2.00b	3.99a
L-threonine[a]	4.85b	3.62b	7.96a
L-tryptophan[a]	0.52a	0.36b	0.53a
L-tyrosine	2.17ab	1.42b	3.04a
L-valine[a]	2.54a	1.88b	3.00a
total	46.74ab	39.30b	63.34a

[a] Essential amino acid. [b] Means with different letters within a row are significantly different ($P < 0.05$, LSD test).

Table 4. Content of Taste Characteristics of Free Amino Acids in the Three Strains of *A. cylindracea*

taste characteristic[a]	content[b] (mg/g of dry weight)		
	strain B	strain M	strain W
MSG-like	10.85a	11.89a	13.05a
sweet	12.37b	9.65b	18.28a
bitter	19.17ab	14.97b	26.08a
tasteless	4.35ab	2.79b	5.93a
total	46.74ab	39.30b	63.34a

[a] MSG-like, monosodium glutamate-like: Asp + Glu. Sweet: Ala + Gly + Ser + Thr. Bitter: Arg + His + Ile + Leu + Met + Phe + Try + Val. Tasteless: Lys + Tyr. [b] Means with different letters within a row are significantly different ($P < 0.05$, LSD test).

tent with its high moisture content. These findings might be explained by the role of mannitol to act as an osmotic solute in increasing turgidity of fruiting body hyphae to help support the structure (Hammond and Nichols, 1976). Mannitol was the major soluble sugar alcohol in common mushrooms, while trehalose, a nonreducing sugar, was the second soluble sugar component (Hammond and Nichols, 1976). In *A. cylindracea*, the highest content of trehalose was observed in strain B. However, trehalose contents in these three strains (21.32−38.64 mg/g of dry weight) were much higher than that in common mushrooms (6.5 mg/g) (Hwang and Mau, 1997) and much lower than those in straw mushrooms (349.0−457.6 mg/g) (Mau et al., 1997). Due to its highest fructose content, strain B might taste sweeter than the other two strains. Generally, these three strains contained from 5.6 to 8.6% dry weight of soluble sugars.

Strain W contained the highest amount of total free amino acids (63.34 mg/g of dry weight), and strain M contained the lowest (39.30 mg/g) (Table 3). These three strains of *A. cylindracea* contained high amounts of glutamic acid, threonine, arginine, and phenylalanine, with glutamic acid being the most significant. Table 4 divides the free amino acids into several classes on the basis of 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 or palatable taste that was the characteristic taste of MSG and 5′-nucleotides (Yamaguchi, 1979). The contents of MSG-like components were similar in the three strains, whereas the contents of sweet and bitter components were higher in strain W. Chen (1986) conducted a series of sensory evaluations on synthetic mushroom extracts prepared by omitting and adding soluble components and 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 were found to be taste-active. Therefore, MSG-like and sweet components would be responsible for the natural taste of *A. cylindracea*. The bitterness from bitter components in *A. cylindracea* could probably be masked by the sweet components and also a high amount of soluble sugars. The contents of MSG-like and sweet components in strains B, M, and W were 23.22, 21.54, and 31.33 mg/g of dry weight, respectively.

Table 5. Content of 5′-Nucleotides in the Three Strains of *A. cylindracea*

5′-nucleotide[a]	content[b] (mg/g of dry weight)		
	strain B	strain M	strain W
5′-AMP	0.16a	0.13b	0.12b
5′-GMP	0.63a	0.35b	0.21c
5′-UMP	0.72a	0.43b	0.34b
total	1.51a	0.91b	0.67c

[a] 5′-AMP, 5′-adenosine monophosphate; 5′-GMP, 5′-guanosine monophosphate; 5′-UMP, 5′-uridine monophosphate. [b] Means with different letters within a row are significantly different ($P < 0.05$, LSD test).

The contents of total free amino acids and MSG-like components in common mushrooms were 77.92 and 22.67 mg/g of dry weight, respectively (Tseng and Mau, 1997). The contents in shiitake mushrooms were 19.43−35.89 and 3.75−9.06 mg/g, respectively (Lin, 1988). Furthermore, the contents in straw mushrooms were 36.11−60.18 and 11.20−26.21 mg/g, respectively (Mau et al., 1997). Compared to the contents of total free amino acids and MSG-like components shown in Table 4 (39.30−63.34 and 10.85−13.05 mg/g, respectively), the taste components of *A. cylindracea* might be comparable to those of straw mushrooms, less intense than those of common mushrooms, but more intense than those of shiitake mushrooms.

Only three 5′-nucleotides were detected in the three strains (Table 5), in which 5′-guanosine monophosphate (5′-GMP) was a flavor 5′-nucleotide (Chen, 1986). 5′-GMP gave the meaty taste (Litchfield, 1967), and the synergistic effect of 5′-GMP with glutamic and aspartic acids might greatly increase the umami taste of *A. cylindracea* (Yamaguchi et al., 1971). Strain B contained the highest amounts of total 5′-nucleotides and flavor 5′-GMP (1.51 and 0.63 mg/g of dry weight), whereas strain W contained the lowest (0.67 and 0.21 mg/g). Due to the fact that no difference was found in the contents of MSG-like components in the three strains (Table 4), the synergistic effect of the umami taste essentially depended on the content of flavor 5′-nucleotide present (Yamaguchi et al., 1971). From the 5′-GMP contents shown in Table 5, the umami taste intensity of *A. cylindracea* was in the order of strains B, M, and W.

Total 5′-nucleotide content in *A. cylindracea* (0.67−1.51 mg/g of dry weight) was much lower than that in shiitake mushrooms (7.26−11.47 mg/g) (Lin, 1988), in common mushrooms (11.35 mg/g) (Tseng and Mau, 1997), and in straw mushrooms (27.01−44.71 mg/g)

(Mau et al., 1997). Furthermore, flavor 5′-GMP content in *A. cylindracea* (0.21−0.63 mg/g of dry weight) was much lower than that in shiitake mushrooms (1.73−3.67 mg/g) (Lin, 1988), in common mushrooms (4.19 mg/g) (Tseng and Mau, 1997), and in straw mushrooms (4.42−9.00 mg/g) (Mau et al., 1997). On the basis of previous results, these three strains of *A. cylindracea* contained the least amount of 5′-nucleotides, especially the flavor 5′-nucleotide, 5′-GMP.

The sweetness of *A. cylindracea* could be affected by the contents of soluble sugars along with sweet components, which were 68.37 mg/g of dry weight (56.00 ± 12.37), 95.79 mg/g (86.14 ± 9.65), and 90.61 mg/g (72.33 ± 18.28) for strains B, M, and W, respectively (Tables 2 and 4). Although the content of MSG-like components in *A. cylindracea* was lower than that of only common mushrooms, the umami taste of the three strains of *A. cylindracea* might be the least intense among mushrooms, including straw, shiitake, and common mushrooms, due to their lowest flavor 5′-nucleotide contents.

Among the three strains of *A. cylindracea*, strain W contained the highest moisture content and strains B and W contained >40% dry weight of protein. The contents of soluble sugars and sweet components in *A. cylindracea* were in the order of strains M, W, and B. The umami taste intensity of *A. cylindracea* could be in the order of strains B, M, and W. From the results shown above, it could be concluded that these three strains, in addition to their different physical appearances, were considerably different in both their proximate compositions and their taste components. However, to determine the relationship of the palatability of three strains of *A. cylindracea* with their soluble components, further sensory evaluation is needed.

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