Flavor Compounds in Straw Mushrooms *Volvariella volvacea* Harvested at Different Stages of Maturity

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Straw mushrooms were harvested at different stages of maturity. The volatile flavor compounds found in straw mushrooms were limonene, octa-1,5-dien-3-ol, 3-octanol, 1-octen-3-ol, 1-octanol, and 2-octen-1-ol, in which the major compound was 1-octen-3-ol, accounting for 71.6−83.1% of the total volatiles. More mature straw mushrooms possessed the most aroma. Straw mushrooms were high in trehalose (349.0−457.6 mg/g dry weight) and low in mannitol (0−25.5 mg/g). During the development of fruiting bodies, the contents of total free amino acids and monosodium glutamate components, including aspartic and glutamic acids, increased remarkably from 36.11 and 11.20 mg/g dry weight at stage 1 to 60.18 and 26.21 mg/g at stage 5, respectively, in which glutamic acid was the most significant, increasing from 7.72 at stage 1 to 21.00 mg/g dry weight at stage 5. The content of total 5′-nucleotides and flavor 5′-nucleotides (5′-IMP and 5′-GMP) accumulated steadily with maturation. In this study, more flavor compounds, including both aroma and taste compounds, occurred in straw mushrooms harvested at stages 4 and 5 (stipe elongated and cap opened).

Keywords: *Straw mushroom; Volvariella volvacea; flavor; volatile compounds; sugars; free amino acids; 5′-nucleotides*

INTRODUCTION

Mushrooms have long been used as a food or food-flavoring material because of their unique and subtle flavor. The typical flavor substances of mushrooms can be classified into nonvolatile components and volatile compounds (Maga, 1981). 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). The most important aroma components are a series of eight-carbon compounds, especially 1-octen-3-ol, which are common to most mushrooms. Differences arise from fluctuations in concentrations and particular compounds characteristic of specific genera, species, or strains (Mau et al., 1994).

Straw mushrooms (*Volvariella volvacea* (Bull. ex Fr.) Sing.), also called Chinese mushrooms, are widely cultivated in China and other regions of Southeast Asia. The fruiting body of straw mushrooms is egg shaped at first, then becomes bell shaped with a characteristic volva, and has a grayish-brown cap. Straw mushrooms are commonly harvested at egg-shaped and bell-shaped stages. Recently, in Southeast Asia, straw mushrooms have been harvested at stipe-elongated and cap-opened stages. The discrepancy might be due to the difference in flavor preference. In fact, straw mushrooms have a very slight earthy odor and insignificant aroma. However, the flavor profiles of straw mushrooms are unknown. This research was designed to examine the volatile and nonvolatile compounds in the straw mushrooms harvested at different stages of maturity.

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MATERIALS AND METHODS

Straw Mushrooms. Straw mushrooms were obtained from Taichung County, Taiwan. Some straw mushrooms were left growing on the bed until they were over mature (volva opened and gill exposed). Harvested mushrooms were sorted into five maturity categories as follows: stage 1, egg shaped; stage 2, bell shaped; stage 3, volva broken; stage 4, stipe elongated; and stage 5, cap opened. Straw mushrooms from each category were randomly selected into six trays without cover, about 100 g each. Immediately after sorting, three trays of samples from each category were used for the analysis of volatile compounds. The other three trays of straw mushrooms were freeze-dried, ground to powder, and stored in a desiccator before use.

Volatile Compound Extraction. Straw mushrooms (100 g) from a tray were cut into small cubes and blended with 300 mL 0.1 M sodium phosphate buffer (pH 6.5) containing 0.15% Tween 80 (Wako Pure Chemical Co., Osaka, Japan) and 1 mL methanol containing 1000 μg 1-nonanol (Sigma Chemical Co., St. Louis, MO) as an internal standard. After 1 min of blending, the homogenate was mixed with 50 mL *n*-hexane, and centrifuged at 10 000*g* for 10 min at 4 °C. The *n*-hexane layer was preconcentrated with a distillation apparatus at 40 °C and carefully reconcentrated to approximately 50 μL using a 0.2 mm i.d. × 10 cm Vigreux column (Tung Kawn Glass Co., Hsinchu, Taiwan) at 40 °C. Three samples from each category were examined.

Gas Chromatography. A Hewlett-Packard 5890A gas chromatograph equipped with a flame ionization detector and a Hewlett-Packard 3396A integrator were used to analyze the volatiles of straw mushrooms. A fused silica column (0.53 mm × 30 m, J&W, Folsom, CA) coated with DB-Wax (0.25 μm thickness) was used. The oven temperature was programmed from 40 to 200 °C at 2 °C/min, 80 min total time per run. The injector and detector temperatures were 250 °C. The carrier gas was nitrogen at a flow rate of 1.2 mL/min. The linear retention indices of the volatile components were calculated with *n*-paraffin (C_5−C_{25}) as references (Schomberg and Dielmann, 1973). The amount of each component was determined

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using an internal standard method and calculated by each peak area of gas chromatograms.

Gas Chromatography−Mass Spectrometry. A Hewlett-Packard 5890A II gas chromatograph coupled to a Hewlett-Packard 5972A MSD mass spectrometer was used. The column and temperature program were the same as those used for gas chromatography. The operating conditions were as follows: injector temperature, 250 °C; GC−MS interface temperature, 265 °C; helium carrier flow rate, 1.0 mL/min. A split ratio of 60:1 was used. Mass spectra were obtained from electron multiplier voltage and electron ionization energy at 1500 V and 70 eV, respectively. Volatile compounds were identified by comparing the mass spectral data with those spectra available from the Wiley and the NIST libraries, and GC retention times of the components with those of authentic compounds.

Sugar Assay. Soluble sugars were extracted and analyzed as described by Ajlouni et al. (1995). Freeze-dried straw mushroom tissue (300 mg) from each tray was extracted with 50 mL of 80% aqueous ethanol (95% pure, Taiwan Tobacco & Wine Monopoly Bureau, Taipei) and xylose (50 mg, Sigma) 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 5-mL portions of 80% ethanol. The combined filtrate was then rotary evaporated and redissolved in deionized water to a final volume of 10 mL. An aliquot of the aqueous extract was passed through a filter unit (13 mm, Lida Corp., Kenosha, WI), and filtered using 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 chromato-integrator, 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:water, 90:10 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.

Amino Acid Assay. Freeze-dried straw mushroom tissue (500 mg) from each tray was shaken with 50 mL of 0.1 N HCl for 45 min at ambient temperature and filtered through Whatman No. 4 filter paper. An aliquot of the filtrate was then passed through a filter unit (13 mm, Lida), and filtered using 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 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) containing 0.5% tetrahydrofuran; B, deionized water; and C, methanol. The gradient was A:B:C 83:0:17 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 straw mushroom tissue (500 mg) from each tray was extracted with 25 mL deionized water. This suspension was heated to boiling for 1 min, cooled, and centrifuged at 22 200*g* for 15 min. The extraction was repeated once with 20 mL deionized water. The combined filtrate was rotary evaporated to a final volume of 10 mL. An aliquot of the aqueous extract was passed through a filter unit (13 mm, Lida), and filtered using a 0.45 μm CA Non-ste filter (Lida) prior to injection onto HPLC.

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) 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. Straw mushrooms from each category were examined in triplicate. 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.

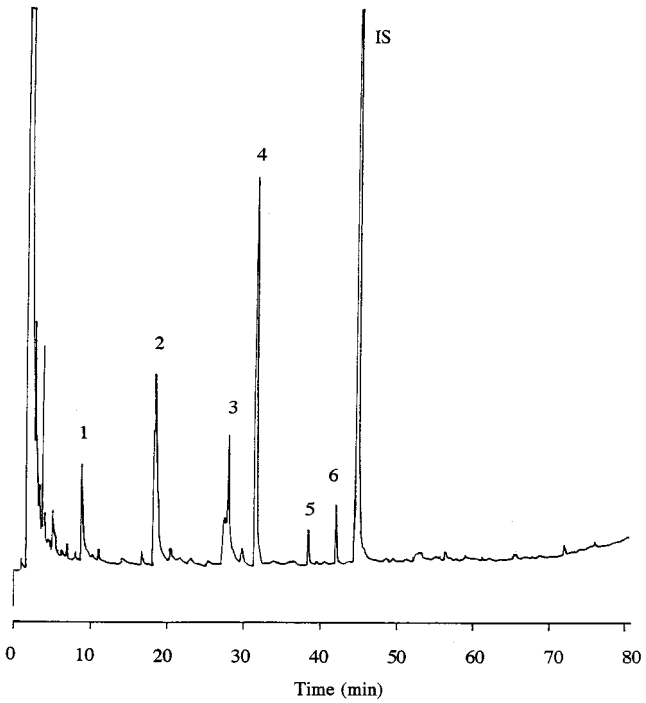

Figure 1. Qualitative gas chromatogram of volatile compounds of fresh straw mushroom (*V. volvacea*) harvested at stage 2. The peak numbers correspond to compounds in Table 1; IS, internal standard, 1-nonanol.

RESULTS AND DISCUSSION

The volatile flavor compounds in straw mushrooms were found to be limonene, octa-1,5-dien-3-ol, 3-octanol, 1-octen-3-ol, 1-octanol, and 2-octen-1-ol (Figure 1 and Table 1). The total volatiles content at all stages of

Table 1. Contents of Volatile Flavor Compounds in *V. volvacea* at Different Stages of Maturity

peak no.[a]	compound	RI[b]	content[c] (μg/g fresh weight)				
			stage 1	stage 2	stage 3	stage 4	stage 5
1	limonene	1190	0.76A	0.52BC	0.59BC	0.62B	0.48C
2	octa-1,5-dien-3-ol	1357	1.52A	0.84B	1.03B	1.02B	0.92B
3	3-octanol	1406	0.30A	0.11A	0.21A	0.06A	0.28A
4	1-octen-3-ol	1472	9.07C	6.23D	7.13D	12.58B	15.53A
5	1-octanol	1579	0.25B	0.18C	0.18C	0.27AB	0.29A
6	2-octen-1-ol	1637	0.77C	0.47E	0.59D	0.93B	1.19A
	total		12.67C	8.35D	9.73D	15.48B	18.68A

[a] The peak numbers correspond to Figure 1. [b] Linear retention index determined on DB-Wax column using *n*-paraffins (C_8−C_{25}) as reference standards. [c] Means with the same letter within a row are not significantly different ($p < 0.05$, LSD test).

Table 2. Content of Soluble Sugars in *V. volvacea* at Different Stages of Maturity

	content[a] (mg/g dry weight)				
sugar	stage 1	stage 2	stage 3	stage 4	stage 5
mannitol	15.0B	25.5A	17.0B	16.3B	−[b]
trehalose	357.8B	403.8AB	440.6A	374.2AB	349.0B
total	372.8BC	429.3AB	457.6A	390.5BC	349.0C

[a] Means with the same letter within a row are not significantly different ($p < 0.05$, LSD test). [b] Not detected.

maturity ranged from 8.35 to 18.68 μg/g fresh weight. Compared to the total volatiles in common mushrooms, *Agaricus bisporus* (53.03 μg/g fresh weight) (Mau and Hwang, 1997), the aroma of straw mushrooms was less intense. However, like most mushrooms, the major compound found in straw mushrooms was 1-octen-3-ol, accounting for 71.6, 74.6, 73.3, 81.3, and 83.1% of the total volatiles for stages 1, 2, 3, 4, and 5, respectively.

The total volatiles content and 1-octen-3-ol content in straw mushrooms at stages 1, 2, and 3 (egg shaped, bell shaped, and volva broken) were significantly less than those at stages 4 and 5 (stipe elongated and cap opened) (Table 1). Mau et al. (1993) found that immature common mushrooms with closed veils had higher 1-octen-3-ol content than more mature common mushrooms with open veils. However, these results were not observed in straw mushrooms. Straw mushrooms harvested at stipe-elongated and cap-opened stages possessed the most aroma.

Trehalose was the major soluble sugar found in straw mushrooms at all stages of maturity (Table 2). Mannitol, the major sugar in common mushrooms (Hammond and Nichols, 1975), also was found in straw mushrooms, but only those with closed caps. Total sugar content increased from 37.3% at stage 1 to 45.8% at stage 3, and then decreased to 34.9% at stage 5. Apparently, total sugars, especially trehalose, accumulated as straw mushroom fruiting bodies developed to the broken volva stage, then rapidly decreased due to stipe elongation and cap expansion.

Lee and Chang (1975) studied the nutritional composition of straw mushrooms at egg stage (stage 1) and reported total soluble sugars to be 33.8% dry weight, similar to our results (Table 2). Chen (1986) found that mannitol was the major taste-active component in common mushrooms. Unfortunately, trehalose was not evaluated in that study. However, high trehalose content and low amounts of mannitol would most likely contribute to a sweet taste, and not to the typical flavor of straw mushrooms.

During the development of fruiting bodies, the content of total free amino acids remained level at 36.11 mg/g dry weight at stage 1 to 38.13 mg/g at stage 3, and significantly increased to 43.48 mg/g at stage 4 and to 60.18 mg/g at stage 5 (Table 3). Obviously, the elongation of stipe and further expansion of cap stimulated the accumulation of free amino acids. Among 13 amino acids assayed, glutamic acid was the most significant and its content increased slowly from 7.72 mg/g at stage 1 to 9.86 mg/g at stage 4, and then dramatically increased to 21.00 mg/g at stage 5.

Table 4 tabulates the free amino acids into several classes on the basis of their taste characteristics as described by Komata (1969). During the development of fruiting bodies, the contents of monosodium glutamate (MSG), sweet (SWE), and bitter (BIT) components were similar at stage 1 and slowly increased at the same rate to stage 4. However, along with cap expansion from stages 4−5, the content of MSG components increased more remarkably than that of SWE or BIT components. The bitterness from BIT components in straw mushrooms could probably be masked by the sweetness from SWE components and also a high amount of soluble sugars, including trehalose and mannitol. Chen (1986) found that alanine, glycine, and threonine (SWE) and aspartic and glutamic acids (MSG) were taste-active amino acids in common mushrooms, whereas none of BIT components were found to be taste-active. Furthermore, MSG components produced the most typical mushroom flavor, the umami or palatable taste which is the characteristic taste of MSG and 5′-nucleotides (Yamaguchi, 1979). MSG and SWE components were higher in more mature straw mushrooms.

Table 3. Content of Free Amino Acids in *V. volvacea* at Different Stages of Maturity

	content[a] (mg/g dry weight)				
amino acid	stage 1	stage 2	stage 3	stage 4	stage 5
L-alanine	4.84B	4.64BC	4.43CD	4.20D	5.77A
L-arginine	2.05BC	1.92C	1.89C	2.18B	4.56A
L-aspartic acid	3.48C	4.44B	4.33B	4.97A	5.21A
L-glutamic acid	7.72D	7.91CD	8.52C	9.86B	21.00A
glycine	1.15D	1.22CD	1.35C	1.56B	2.34A
L-histidine[b]	3.08C	3.42B	3.30BC	3.94A	4.25A
L-isoleucine[b]	1.07AB	0.87A	1.28AB	1.50AB	1.64A
L-leucine[b]	0.82B	0.71B	0.75B	1.12A	1.13A
L-methionine[b]	0.44B	0.40B	0.45B	0.51B	0.63A
L-phenylalanine[b]	1.77AB	1.84AB	2.78A	3.10A	1.03B
L-serine	2.77D	2.85CD	3.12C	3.54B	4.47A
L-threonine[b]	4.46BC	4.14BC	3.96C	4.74B	5.57A
L-valine[b]	2.46A	2.00C	1.97C	2.26B	2.58A
total	36.11C	36.36C	38.13C	43.48B	60.18A

[a] Means with the same letter within a row are not significantly different ($p < 0.05$, LSD test). [b] Essential amino acid.

Table 4. Content of Taste Characteristics of Free Amino Acids in *V. volvacea* at Different Stages of Maturity

	content[a] (mg/g dry weight)				
compound	stage 1	stage 2	stage 3	stage 4	stage 5
MSG[b]	11.20D	12.35C	12.85C	14.83B	26.21A
SWE[c]	13.22BC	12.85C	12.86C	14.04B	18.15A
BIT[d]	11.69C	11.16C	12.42BC	14.61AB	15.82A
total	36.11C	36.36C	38.13C	43.48B	60.18A

[a] Means with the same letter within a row are not significantly different ($p < 0.05$, LSD test). [b] MSG: taste like monosodium glutamate; Asp + Glu. [c] SWE: sweetness; Ala + Gly + Thr + Ser. [d] BIT: bitterness; Arg + His + Ile + Leu + Met + Phe + Val.

The contents of total free amino acids and MSG components in common mushrooms were 77.92 and 22.67 mg/g dry weight, respectively (Tseng and Mau, 1997), whereas the contents in shiitake mushrooms, *Lentinula edodes*, were 19.43−35.89 and 3.75−9.06 mg/g, respectively (Lin, 1988). Compared to the contents of total free amino acids and MSG components shown in Table 4 (36.11−60.18 and 11.20−26.21 mg/g dry weight, respectively), the taste components of straw mushrooms might be less intense than that of common mushrooms, but more intense than that of shiitake mushrooms.

Total 5′-nucleotide content increased steadily from 27.01 mg/g dry weight at stage 1 to 44.71 mg/g at stage 5 (Table 5). Flavor 5′-nucleotides were found to be 5′-guanosine monophosphate (5′-GMP), 5′-inosine monophosphate (5′-IMP), and 5′-xanthosine monophosphate (5′-XMP) (Chen, 1986). However, 5′-XMP was not found in straw mushrooms. 5′-Cytosine monophosphate (5′-CMP) contents were the highest (12.82−28.49 mg/g), but

Table 5. Content of 5′-Nucleotides in *V. volvacea* at Different Stages of Maturity

	content[a] (mg/g dry weight)				
5′-nucleotide	stage 1	stage 2	stage 3	stage 4	stage 5
5′-AMP[b]	3.54A	2.64A	5.36A	5.39A	4.52A
5′-CMP[b]	15.84C	19.87B	12.82C	17.27B	28.49A
5′-GMP[b]	4.88B	4.25B	8.37A	8.31A	6.92AB
5′-IMP[b]	0.27A	0.17A	0.16A	0.69A	0.39A
5′-UMP[b]	2.48B	2.26B	5.01AB	6.89A	4.39AB
total	27.01B	29.19B	31.72AB	38.55AB	44.71A

[a] Means with the same letter within a row are not significantly different ($p < 0.05$, LSD test). [b] 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.

it is not a flavor component. Flavor 5′-GMP and 5′-IMP ranged from 4.25 to 8.37 and 2.64 to 5.39 mg/g, respectively. The flavor 5′-nucleotide contents were 5.15, 4.42, 8.53, 9.00, and 7.31 mg/g for stages 1, 2, 3, 4 and 5, respectively.

Total 5′-nucleotide contents in straw mushrooms at all stages (27.01−44.71 mg/g) were much higher than those in common mushrooms (11.35 mg/g) (Tseng and Mau, 1997) or shiitake mushrooms (7.26−11.47 mg/g) (Lin, 1988). Furthermore, flavor 5′-nucleotide contents at all stages (4.42−9.00 mg/g) were greater than in common mushrooms (4.19 mg/g) (Tseng and Mau, 1997) or shiitake mushrooms (1.73−3.67 mg/g) (Lin, 1988). Two flavor 5′-nucleotides gave the meaty taste (Litchfield, 1967), and the synergistic effect of these two 5′-nucleotides with glutamic and aspartic acids might greatly increase the umami flavor of straw mushrooms (Yamaguchi et al., 1971).

More mature straw mushrooms possessed more aroma than egg- and bell-shaped straw mushrooms. Along with the development of fruiting bodies, the contents of total free amino acids and MSG components remarkably increased. Moreover, the contents of total 5′-nucleotides and flavor 5′-nucleotides were accumulated over the course of maturation. In this study, more flavor compounds, including both aroma and taste compounds, occurred in straw mushrooms harvested at stages 4 and 5 (stipe elongated and cap opened). In addition, straw mushrooms harvested at stipe-elongated and cap-opened stages gave rise to higher yield, but had a faster rate of senescence and deterioration, and thereby shorter shelf life as compared to egg- and bell-shaped straw mushrooms. However, the climate in Southeast Asia is warm enough to stimulate the fast growth of straw mushrooms to more mature stages. This might be the main reason that more mature straw mushrooms are commonly found in these regions. To determine the relationship of the palatability of straw mushrooms at different maturities with volatile compounds and total soluble components, further sensory evaluation is needed.

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