Flavor Compounds in King Oyster Mushrooms *Pleurotus eryngii*

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King oyster mushrooms (*Pleurotus eryngii*) were divided into three parts: large fruiting bodies (LFB), small fruiting bodies (SFB), and the base. LFB comprised 79.90% of total weight, whereas the base comprised 15.47%. Volatile compounds found were 3-octanone, 1-octen-3-one, 3-octanol, 1-octen-3-ol, benzaldehyde, 1-octanol, and 2-octen-1-ol; the major compound in LFB and SFB was benzaldehyde. Both LFB and SFB contained high amounts of total free amino acids. Sweet and bitter components were comparable in the three parts, whereas monosodium glutamate-like components were high in LFB and SFB. Six 5′-nucleotides were found in three parts, of which 5′-cytosine monophosphate was the highest. Flavor 5′-nucleotide contents in LFB and SFB were comparable and higher than those in the base. In this study, LFB and SFB were similar in their proximate compositions, volatile compounds, and taste components.

Keywords: *Pleurotus eryngii; king oyster mushrooms; flavor; volatile compounds; soluble sugars; free amino acids; 5′-nucleotides*

INTRODUCTION

The king oyster mushroom [*Pleurotus eryngii* (DC.: Fr.) Quel.], also called almond oyster mushroom, umbel oyster mushroom, scallop mushroom, and Boletus of the steppes, is a typical fungus of the flora of the subtropics and steppes. It is widespread in southern Europe and the areas of central Asia and North Africa. It is easily recognized by its peculiar habitat; in Europe, it attacks the roots of *Eryngium campestre* L. (field eryngo) (Laessoe et al., 1996). In addition to *Pleurotus cystidiosus*, *Pleurotus ostreatus* and *Pleurotus sajor-caju*, the cultivation technique of this newly developed species has reached industrial scale by the Taiwan Agriculture Research Institute, Taichung, Taiwan (Peng, 1995).

Its better consistency, especially of the stipe, pleasant aroma, and culinary qualities would probably make it preferable to other species of *Pleurotus* (Stamets, 1993). However, the flavor profiles of this mushroom are unknown. Our objective was to examine the volatile flavor compounds and taste components in the king oyster mushrooms.

MATERIALS AND METHODS

Mushrooms. The king oyster mushrooms were cultivated in plastic jars using moist sawdust supplemented with rice bran under the standard cropping procedure of the Tai Mushroom Corp., Taichung County, Taiwan. At harvest, fresh mushrooms were pulled out of the jar and cut into large fruiting bodies (LFB), small fruiting bodies (SFB), and the remaining base as shown in Figure 1. Mushrooms from each part were weighed and randomly placed into six trays without cover, ∼100 g each. Immediately after sorting, three trays of fresh samples from each part were used for the analysis of volatile compounds. The other three trays from each part were freeze-dried, ground to powder, and stored in a desiccator before use.

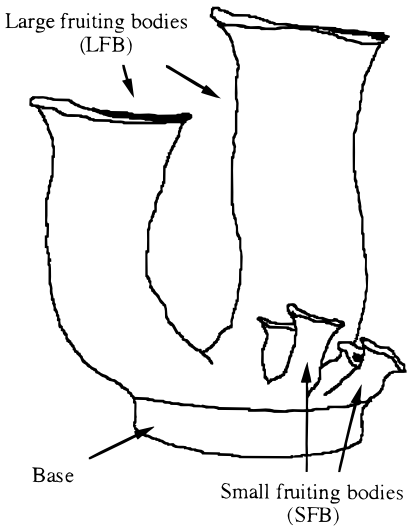

Figure 1. Drawing of the three parts of the king oyster mushroom (*P. eryngii*) harvested from a jar.

Volatile Compound Extraction. A tray of each part (100 g) was cut into small cubes and blended with 300 mL of 0.1 M sodium phosphate buffer (pH 6.5) containing 0.15% Tween 80 (Wako Pure Chemical Co., Osaka, Japan) and 1 mL of methanol containing 1000 μg of 1-nonanol (Sigma Chemical Co., St. Louis, MO) as an internal standard. After 1 min of blending, the homogenate was placed into a modified Likens−Nickerson apparatus and extracted with 25 mL of diethyl ether (Merck, Darmstadt, Germany; glass distilled) and 25 mL of *n*-pentane (Merck, glass distilled). The steam distillation−solvent extraction was allowed to proceed for 2 h, and the extract thus obtained was dried over anhydrous sodium sulfate (Merck) and filtered. The filtrate was preconcentrated with a distillation apparatus at 40 °C and carefully reconcentrated

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to ~50 μL using a 10 cm × 0.2 mm i.d. Vigreux column (Tung Kawn Glass Co., Hsinchu, Taiwan). Three samples from each part were examined.

Gas Chromatography. A Hewlett-Packard 5890A Series II gas chromatograph (GC) equipped with a flame ionization detector and an HP 3396A integrator were used to analyze the volatiles. A fused silica column (0.32 mm × 60 m, J&W, Folsom, CA) coated with DB-Wax (0.25 μm thickness) was used. The operating conditions were the same as described in Mau et al. (1997). 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 using an internal standard method and calculated by each peak area of gas chromatograms.

Gas Chromatography/Mass Spectrometry. An HP 5890A Series II GC coupled to an HP 5972A MSD mass spectrometer was used. The column was the same as those used for gas chromatography. The operating conditions were the same as described in Mau et al. (1997). Volatile compounds were identified by comparing the mass spectral data with those spectra available from the Wiley and NIST libraries (McLafferty and Stauffer, 1989) and by comparing the GC retention times of the components with those of authentic compounds.

Proximate Analysis. The proximate compositions of three parts of king oyster mushrooms, 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).

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 and 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 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 0.45 μm CA nonsterile filter (Lida) prior to injection onto the 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. The sugars and sugar alcohols sought were arabinose, fructose, galactose, glucose, maltose, mannose, ribose, trehalose, arabitol, mannitol, and sorbitol.

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 nonsterile 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 and gradient conditions were the same as described in Mau et al. (1997). 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 the soluble sugar assay.

The HPLC system was the same as for the 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 part of king oyster mushrooms, 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 et al. (1997), to determine the least significant difference among means at the level of 0.05. After multiple comparisons, the means in the following tables were followed with different capital letters A−D on the basis of their values and statistical differences. In the case that a mean is followed with AB, this mean was not significantly different from a mean with A and was not significantly different from another mean with B. However, means with different letters were significantly different at the level of 0.05.

Table 1. Proximate Composition in the Three Parts of *P. eryngii*

component[b]	content[a] (%)		
	LFB[c]	SFB[c]	base
weight ratio	79.90A	4.63C	15.47B
moisture	87.64A	87.46A	88.08A
dry matter	12.36A	12.54A	11.92A
ash	5.76B	7.21A	5.15C
carbohydrate	64.55B	61.12C	77.33A
crude fat	1.57B	1.81A	0.81C
crude fiber	5.97C	9.15A	7.59B
crude protein	22.15A	20.71A	9.12B

[a] Means with different letters within a row are significantly different ($p < 0.05$). [b] Weight ratio, moisture, and dry matter were presented on the basis of fresh weight; others were presented on the basis of dry weight. [c] LFB, large fruiting body; SFB, small fruiting body.

RESULTS AND DISCUSSION

No difference was found in the moisture content among the three parts of *P. eryngii* (Table 1). Crisan and Sands (1978) reported that most fresh mushrooms contained ~90% moisture. LFB comprised 79.90% of total weight, whereas the base, which was usually discarded, comprised 15.47%. In their proximate compositions, the base was high in carbohydrate (77.33% dry weight) and low in crude protein contents (9.12%). However, SFB contained the highest amounts of ash and crude fiber (7.21 and 9.15%, respectively). Generally, mushrooms are a good source of protein, and their protein contents range from 10 to 35% of dry weight (Crisan and Sands, 1978). Only the protein content in the base was below this range. The protein contents were 25% in *P. eous*, 27% in *P. florida*, 10.5−30.4% in *P. ostreatus*, and 26.6% in *P. sajor-caju* (Chang and Miles, 1989). On the contrary, the protein contents were 17.5% in *P. eous*, 21.6% in *P. flabellatus*, 18.9% in *P. florida*, and 10.5% in *P. ostreatus* (Bano and Rajarathnam, 1988). However, the protein contents in two fruiting bodies of *P. eryngii* (LFB and SFB) were similar to those of Bano and Rajarathnam (1988).

The lipid contents in oyster mushrooms mentioned earlier range from 1.1 to 2.2% of dry weight (Chang and

Table 2. Content of Volatile Compounds in the Three Parts of *P. eryngii*

compound	RI[b]	content[a] (μg/g of fresh weight) LFB[c]	SFB[c]	base
3-octanone	1270	0.27A	0.29A	0.03B
1-octen-3-one	1316	0.20A	0.07B	0.17A
3-octanol	1406	0.10B	0.14A	0.03C
1-octen-3-ol	1472	0.03B	0.03B	0.89A
benzaldehyde	1534	26.75A	15.61B	0.03C
1-octanol	1579	1.05A	0.83A	0.01B
2-octen-1-ol	1637	0.70A	0.81A	0.05B
total		29.10A	17.78B	1.21C

[a] Means with different letters within a row are significantly different ($p < 0.05$). [b] Linear retention index determined on DB-Wax column using *n*-paraffins (C_8−C_{25}) as reference standards. [c] LFB, large fruiting body; SFB, small fruiting body.

Miles, 1989) and from 1.0 to 2.4% (Bano and Rajarathnam, 1988). The lipid contents in two fruiting bodies of *P. eryngii* were within this range, whereas that in the base was below this range (Table 1). The carbohydrate contents in oyster mushrooms range from 50.7 to 81.8% of dry weight (Chang and Miles, 1989) and from 46.6 to 81.8% (Bano and Rajarathnam, 1988). The carbohydrate contents in three parts of *P. eryngii* were within this range. The high carbohydrate content in the base might be related to its role in fruiting as an energy reservoir. The fiber contents in oyster mushrooms range from 7.5 to 13.3% of dry weight (Chang and Miles, 1989) and from 7.5 to 12.0% (Bano and Rajarathnam, 1988). However, the fiber contents in the three parts were slightly below this range. Summarily, the proximate compositions of two fruiting bodies (LFB and SFB) of *P. eryngii* were almost comparable to those of *Pleurotus* spp.

The volatile flavor compounds found in king oyster mushrooms were 3-octanone, 1-octen-3-one, 3-octanol, 1-octen-3-ol, benzaldehyde, 1-octanol, and 2-octen-1-ol (Table 2). The major compound in the two fruiting bodies was benzaldehyde. This result was in general agreement with its trivial name of the almond oyster mushroom. However, benzaldehyde was insignificant in the base. The contents of eight-carbon compounds in *P. eryngii* were 2.35, 2.17, and 1.18 μg/g of fresh weight for LFB, SFB, and the base, respectively. Surprisingly, 1-octen-3-ol was not significant in king oyster mushrooms as compared to other mushrooms, such as common (*Agaricus bisporus*) and shiitake mushrooms (*Lentinula edodes*). On the contrary, 1-octen-3-ol and other eight-carbon compounds were the major volatiles found in other oyster mushrooms, including *P. florida*, *P. ostreatus*, and *P. sajor-caju* (Jung and Hong, 1991). Nevertheless, benzaldehyde was produced in *P. ostreatus* in response to the stress of carbon tetrachloride (Beltran-Garcia et al., 1997) but was not produced during normal sample preparation and steam distillation extraction (Jung and Hong, 1991). This revealed that the king oyster mushrooms could not be perceived as other oyster mushrooms and other mushrooms. However, the king oyster mushroom was unique due to its different volatile profile.

Galactose was the only soluble sugar found in the highest amount in the base, and it was also present in LFB and SFB (Table 3). In addition, sorbitol was present in LFB, whereas both sorbitol and maltose were found in SFB. Mannitol and trehalose were present in common mushrooms (Hammond and Nichols, 1976),

Table 3. Content of Soluble Sugars in the Three Parts of *P. eryngii*

sugar	content[a] (mg/g of dry weight) LFB[b]	SFB[b]	base
galactose	3.28B	2.03B	20.80A
maltose	ND[c]	2.84	ND
sorbitol	3.68	2.83	ND
total	6.96B	7.70B	20.80A

[a] Means with different letters within a row are significantly different ($p < 0.05$). [b] LFB, large fruiting body; SFB, small fruiting body. [c] ND, not detected.

Table 4. Content of Free Amino Acids in the Three Parts of *P. eryngii*

amino acid	content[a] (mg/g of dry weight) LFB[b]	SFB[b]	base
L-alanine	2.29A	1.85B	1.21C
L-arginine	0.94A	0.79B	0.20C
L-aspartic acid	0.24A	0.16B	0.39A
L-glutamic acid	1.53A	1.69A	0.62B
glycine	0.11A	0.03B	0.05B
L-histidine[c]	0.19A	0.15A	0.06B
L-isoleucine[c]	0.33A	0.24B	0.15C
L-lysine[c]	0.12A	0.16A	0.19A
L-methionine[c]	0.16C	0.67A	0.36B
L-phenylalanine[c]	0.23A	0.15B	0.22A
L-serine	0.15B	0.23A	0.06C
L-threonine[c]	0.40A	0.40A	0.10B
L-tryptophan[c]	0.03A	0.03A	0.02A
L-tyrosine	0.06A	0.05A	0.05A
L-valine[c]	0.30A	0.23B	0.22B
total	7.08A	6.83A	3.90B

[a] Means with different letters within a row are significantly different ($p < 0.05$). [b] LFB, large fruiting body; SFB, small fruiting body. [c] Essential amino acid.

straw mushrooms (*Volvariella volvacea*) (Mau et al., 1997), and other oyster mushrooms (*P. ostreatus* and *P. flabellatus*) (Bano and Rajarathnam, 1988). Mannitol, trehalose, and fructose were found in black poplar mushrooms (*Agrocybe cylindracea*) (Mau and Tseng, 1998). However, *P. eryngii* showed a different composition of soluble sugars. Furthermore, total soluble sugars were low in *P. eryngii*, especially LFB and SFB (6.96−7.70 mg/g of dry weight), as compared to those reported in common mushrooms (215.5 mg/g) (Hwang and Mau, 1997), straw mushrooms (372.8−457.6 mg/g) (Mau et al., 1997), and black poplar mushrooms (56.0−86.1 mg/g) (Mau and Tseng, 1998). Soluble sugars contained in mushrooms contributed a sweet taste (Litchfield, 1967). However, the low content of total soluble sugars in LFB and SFB revealed that the sweetness of this mushroom would not be comparable to other mushrooms mentioned above.

Both LFB and SFB contained high amounts of total free amino acids (Table 4). The three parts of *P. eryngii* contained high amounts of alanine and glutamic acid. Table 5 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). Sweet and bitter components were comparable in the three parts of *P. eryngii*, whereas MSG-like components were high in LFB and SFB. Chen (1986) conducted a series of sensory evaluations on synthetic mushroom extracts

Table 5. Content of Taste Characteristics of Free Amino Acids in the Three Parts of *P. eryngii*

taste characteristic[b]	content[a] (mg/g of dry weight)		
	LFB[c]	SFB[c]	base
MSG-like	1.77A	1.85A	1.01B
sweet	2.95A	2.51A	1.42B
bitter	2.18A	2.26A	1.23B
tasteless	0.18A	0.21A	0.24A
total	7.08A	6.83A	3.90B

[a] Means with different letters within a row are significantly different ($p < 0.05$). [b] MSG-like, monosodium glutamate-like, Asp + Glu; sweet, Ala + Gly + Ser + Thr; bitter, Arg + His + Ile + Leu + Met + Phe + Trp + Val; tasteless, Lys + Tyr. [c] LFB, large fruiting body; SFB, small fruiting body.

Table 6. Content of 5′-Nucleotides in the Three Parts of *P. eryngii*

5′-nucleotide[b]	content[a] (mg/g of dry weight)		
	LFB[c]	SFB[c]	base
5′-AMP	2.40A	3.05A	ND[d]
5′-CMP	23.00B	31.63A	9.25C
5′-GMP	1.21A	1.81B	0.63C
5′-IMP	0.42	ND	ND
5′-UMP	1.77B	2.58A	1.51B
5′-XMP	ND	ND	4.26
flavor 5′-nucleotides[e]	1.63B	1.81B	4.89A
total	28.80B	39.07A	15.65C

[a] Means with different letters within a row are significantly different ($p < 0.05$). [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; 5′-XMP, 5′-xanthosine monophosphate. [c] LFB, large fruiting body; SFB, small fruiting body. [d] ND, not detected. [e] Flavor 5′-nucleotides, 5′-IMP + 5′-GMP + 5′-XMP.

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 *P. eryngii*. The bitterness from bitter components in *P. eryngii* could probably be masked by sweet components and also soluble sugars.

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). Those in straw mushrooms were 36.11−60.18 and 11.20−26.21 mg/g, respectively (Mau et al., 1997). Those in black poplar mushrooms were 39.30−63.34 and 10.85−13.05 mg/g, respectively (Mau and Tseng, 1998). Furthermore, those in *P. ostreatus* were 27.22 and 7.49 mg/g, respectively (Bano and Rajarathnam, 1988). Compared to the contents of total free amino acids and MSG-like components shown in Table 5 (3.90−7.08 and 1.01−1.85 mg/g, respectively), the taste components of *P. eryngii* might be less intense than those mentioned above.

Six 5′-nucleotides were found in the three parts of *P. eryngii*, in which 5′-cytosine monophosphate was the highest (Table 6). 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). Flavor 5′-nucleotides were high in LFB and SFB. 5′-GMP gave the meaty taste (Litchfield, 1967), and the synergistic effect of flavor 5′-nucleotides with MSG-like components might greatly increase the umami taste of king oyster mushrooms (Yamaguchi et al., 1971). The contents of MSG-like components and flavor 5′-nucleotides in LFB and SFB were both comparable and higher than those in the base (Tables 5 and 6). This revealed that the umami taste intensities of LFB and SFB were similar and stronger than that of the base.

Total 5′-nucleotide content in LFB and SFB of king oyster mushrooms (27.51−39.07 mg/g of dry weight) was higher than that in black poplar mushrooms (0.67−1.51 mg/g) (Mau and Tseng, 1998), in shiitake mushrooms (7.26−11.47 mg/g) (Lin, 1988), and in common mushrooms (11.35 mg/g) (Tseng and Mau, 1997) and comparable to that in straw mushrooms (27.01−44.71 mg/g) (Mau et al., 1997). Furthermore, flavor 5′-nucleotide content in both LFB and SFB (4.03−4.86 mg/g of dry weight) was higher than that in black poplar mushrooms (0.21−0.63 mg/g) (Mau and Tseng, 1998) and in shiitake mushrooms (1.73−3.67 mg/g) (Lin, 1988) and comparable to that in common mushrooms (4.19 mg/g) (Tseng and Mau, 1997) and straw mushrooms (4.42−9.00 mg/g) (Mau et al., 1997). On the basis of the previous results, LFB and SFB contained relatively low amounts of MSG-like components but relatively high amounts of flavor 5′-nucleotides. The synergistic effect of the high amount of flavor 5′-nucleotides with the low amount of MSG-like components might give rise to the umami taste of king oyster mushrooms, different from those mentioned above. However, further sensory evaluation is needed.

Among the three parts of king oyster mushrooms, LFB and SFB were similar in their proximate compositions and their compositions of volatile compounds and nonvolatile taste components. However, the base was remarkably different in its flavor profile. Although the base contained lower amounts of flavor compounds and taste components, it could be processed or prepared as a flavoring base to increase its further utilization as food. To determine the relationship of the palatability of the three parts of *P. eryngii* with their flavor compounds, further sensory evaluation is needed.

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