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# Nonvolatile taste components of *Agaricus bisporus* harvested at different stages of maturity

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**Abstract**

Common mushrooms [*Agaricus bisporus* (Lange) Imbach] were harvested at different stages of maturity and their nonvolatile taste components were studied. The moisture contents were in the range of 89.3–92.3% fresh weight. Based on dry weight, contents of other components were in the order: carbohydrate (38.3–48.9%) > crude protein (21.3–27.0%) > crude fibre (17.7–23.3%) > crude ash (7.77–11.0%) > crude fat (2.53–3.92%). Mannitol was the major soluble sugar and its content increased dramatically with maturation from 157 to 260 mg/g. The content of total free amino acids was in the range of 48.8–64.2 mg/g and peaked at stage 2 and then decreased significantly with maturation. The content of monosodium glutamate-like components was in the range of 10.6–13.5 mg/g and similar to those of sweet components (11.4–14.3 mg/g) but lower than those of bitter components (19.7–26.9 mg/g). Contents of total 5′-nucleotides fluctuated in the range of 6.59–8.14 mg/g. Contents of flavour 5′-nucleotides were higher in mushrooms harvested at stages 1 and 2 and decreased with maturation. Equivalent umami concentration values were 207–284 g MSG/100 g dry weight and higher at stages 2, 3 and 5. Based on the results obtained, *Agaricus* mushrooms possessed highly intense umami taste.


*Keywords:* *Agaricus bisporus*; Mushrooms; Soluble sugars; Free amino acids; 5′-Nucleotides; Equivalent umami concentration

## 1. Introduction

Flavour represents one of the most important quality attributes contributing to the widespread consumption of cultivated mushrooms. The typical flavour of mushrooms consists of nonvolatile components (Litchfield, 1967) 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 sugars and sugar alcohols (polyols) (Litchfield, 1967). The good taste provided by mushrooms is the umami taste, also called the palatable taste or the perception of satisfaction, which is an overall food flavour sensation induced or enhanced by monosodium glutamate (MSG) (Yamaguchi, 1979).

Because the second functionality of foods is their tasty properties, Mau (2005) calculated the equivalent umami concentrations (EUC) of mushrooms based on their contents of nonvolatile components. The EUC value is the concentration of MSG equivalent to the umami intensity given by a mixture of MSG and 5′-nucleotide (Yamaguchi, 1979). Among fruit bodies studied, common mushrooms and paddy straw mushrooms were two mushrooms with the most umami taste (Mau, 2005).

Common mushrooms [*Agaricus bisporus* (Lange) Imbach], also called button mushrooms, are cultivated throughout the world and usually harvested at veil intact stage. However, in Europe, mushrooms are harvested at veil-opened and gills-exposed stages. In addition to cultural diversity, the discrepancy might be due to the difference in flavour preference. In fact, no remarkable difference in the volatile components, especially 1-octen-3-ol, was found in mushrooms harvested at different stages of maturity (Mau, Beelman, & Ziegler, 1993). However, the profiles

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of nonvolatile components of mushrooms harvested at different stages of maturity are unknown. Accordingly, this research was designed to examine nonvolatile components of mushrooms, as influenced by the maturity of fruit bodies at harvest, including proximate compositions, soluble sugars and polyols, free amino acids and 5′-nucleotides. Also, EUC values of mushrooms harvested at different stages of maturity were evaluated.

## 2. Materials and methods

### 2.1. Mushrooms

Mushrooms (*A. bisporus* strain MS from France) were obtained from Hungyi Agricultural Co., Houli, Taichung County, Taiwan. At the second flush, some mushrooms were left growing until they were overmature (veil opened and gills exposed). Harvested mushrooms were sorted into five maturity categories as follows: stage 1, pin head; stage 2, veil intact (tight); stage 3, veil intact (stretched); stage 4, veil opened; and stage 5, gills exposed (Fig. 1). Mushrooms from each category were randomly divided into three samples (about 300 g each), freeze dried and ground using a mill (Retsch ultracentrifugal mill and sieving machine, Haan, Germany) to obtain coarse powder (60 mesh).

### 2.2. Proximate analysis

The proximate compositions of the mushrooms, including moisture, crude ash, 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). The carbohydrate content (%) was calculated by subtracting the contents of crude ash, fat, fibre and protein from 100% of dry matter. 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 against a blank on a Hitachi 2001 spectrophotometer. Total reducing sugars were calculated based on a calibration curve of glucose.

### 2.3. Soluble sugar and polyol assay

Soluble sugars and polyols were extracted and analysed as described by Ajlouni, Beelman, Thompson, and Mau (1995). Mushroom power (600 mg) was extracted with 100 ml of 80% aqueous ethanol (95% pure, Taiwan Tobacco and Wine Monopoly Bureau, Taipei). 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 filtrates were 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 Millex-HV filter unit (13 mm, Millipore, Billerica, MA), and filtered using a 0.45-μm PVDF filter (Millipore) prior to injection onto a high-performance liquid chromatograph (HPLC).

The HPLC system consisted of a Shimadzu LC-10AT *VP* pump, a Rheodyne 7725i injector, a 20-μl sample loop, a Shimadzu RID-10A 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)/deionised water, 80:20 (v/v) at a flow rate of 1.0 ml/min. Each sugar or polyol was identified using the authentic sugar (Sigma Chemical Co., St. Louis, MO) and quantified by the calibration curve of the authentic compound.

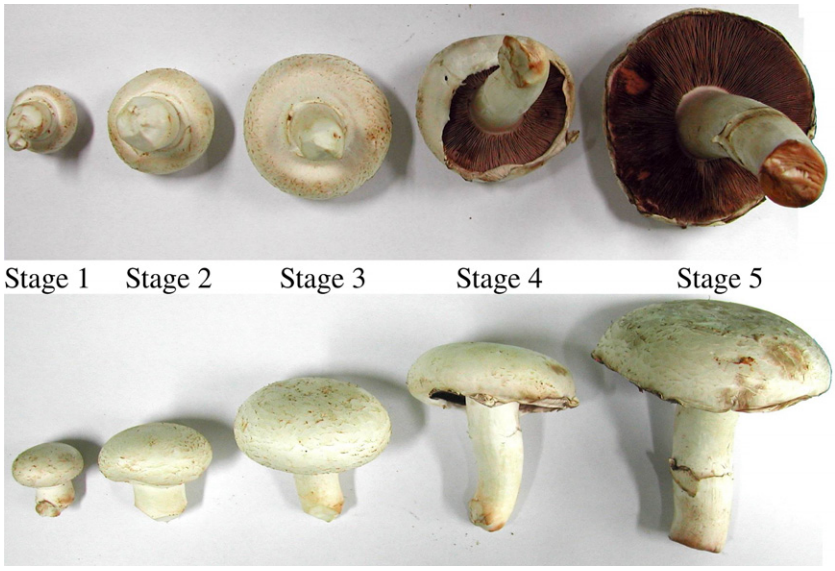


Fig. 1. Photographs of *Agaricus bisporus* harvested at different stages of maturity.

### 2.4. Free amino acid assay

Mushroom powder (600 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 Millex-HV filter unit (13 mm), and filtered using a 0.45-μm PVDF filter. 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 included a Shimadzu LC-10AT *VP* pump, a Rheodyne 7725i injector, a 20-μl sample loop, Hitachi L-7485 fluorescence detector with fluorescence excitation at 340 nm and emission at 450 nm, and a Synergi 4$\mu$ Fusion-RP 80 column (4.6 × 250 mm, 4 μm, Phase Separation Inc.). The mobile phases were A, 50 mM sodium acetate (pH 5.7) containing 0.5% tetrahydofuran; B, deionised water; and C, methanol. The gradient was A:B:C 76:0:24 (v/v/v) to 33:0:67 from 0 to 38 min, to 0:33:67 after 40 min to 0:100:0 at 43 min. The flow rate was 1.0 ml/min. Each amino acid was identified using the authentic amino acid (Sigma) and quantified by the calibration curve of the authentic compound.

### 2.5. 5′-Nucleotide assay

5′-Nucleotides were extracted and analysed, as described by Taylor, Hershey, Levine, Coy, and Olivelle (1981). Mushroom power (600 mg) was extracted with 25 ml of deionised water. This suspension was heated to boiling for 1 min, cooled, and then centrifuged at 11,800*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 that used for soluble sugar assay.

The HPLC system consisted of a Hitachi L-6000 pump, a Rheodyne 7161 injector, a 20-μl sample loop, a Hitachi D-2500 integrator, Shimadzu UV detector and a LiChrospher 100 RP-18 column (4.6 × 250 mm, 5 μm, Merck). The mobile phase was 0.5 M $KH_2PO_4/H_3PO_4$ (pH 4.3, Wako Pure Chemical Co., Osaka, Japan) at a flow rate of 1 ml/min and UV detection at 254 nm. Each 5′-nucleotide was identified using the authentic 5′-nucleotide (Sigma) and quantified by the calibration curve of the authentic compound.

### 2.6. Equivalent umami concentration

EUC (g MSG/100 g) is the concentration of MSG equivalent to the umami intensity given by a mixture of MSG and a 5′-nucleotide and is represented by the following addition equation (Yamaguchi, Yoshikawa, Ikeda, & Ninomiya, 1971):

$$Y = \sum a_i b_i + 1218\left(\sum a_i b_i\right)\left(\sum a_j b_j\right),$$

where $Y$ is the EUC of the mixture in terms of g MSG/100 g; $a_i$ is the concentration (g/100 g) of each umami amino acid [aspartic acid (Asp) or glutamic acid (Glu)]; $a_j$ is the concentration (g/100 g) of each umami 5′-nucleotide [5′-inosine monophosphate (5′-IMP), 5′-guanosine monophosphate (5′-GMP), 5′-xanthosine monophosphate (5′-XMP) or 5′-adenosine monophosphate (5′-AMP)]; $b_i$ is the relative umami concentration (RUC) for each umami amino acid to MSG (Glu, 1 and Asp, 0.077); $b_j$ is the RUC for each umami 5′-nucleotide to 5′-IMP (5′-IMP, 1; 5′-GMP, 2.3; 5′-XMP, 0.61 and 5′-AMP, 0.18); and 1218 is a synergistic constant based on the concentration (g/100 g) used.

### 2.7. Statistical analysis

For mushrooms from each category, ten fruit bodies were used for the determination of their characters, and 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 to determine the least significant difference among means at the level of 0.05.

## 3. Results and discussion

### 3.1. Mushroom characters

Due to the rapid development of fruit bodies growing on bed, the mushrooms harvested were sorted into five stages of maturity based on their distinguishing features. At stage 1 (pin head), a fruit body weighed 3.38 g and its cap diameter and stipe length were 20.1 and 12.3 mm, respectively (Table 1). Along with the development of the fruit body, its weight gained, cap expanded and stipe elongated continuously. However, when mature on bed, the stipe elongated significantly at stage 4 (veil opened). Normally, in Taiwan the mushrooms were harvested before the veil was broken (stage 3).

### 3.2. Proximate composition

The moisture contents of mushrooms were in the range of 89.3–92.3% on the basis of fresh weight (Table 2). The lowest moisture contents and correspondingly the highest dry matter content of mushrooms at stage 5 might be

Table 1
Characteristic of the fruit body of *Agaricus bisporus* harvested at different stages of maturity

| Stage | Weight (g/fruit body) | Cap diameter (mm) | Stipe length (mm) |
|---|---|---|---|
| 1 | 3.38 ± 0.12a[a] | 20.09 ± 0.27a | 12.28 ± 0.76a |
| 2 | 15.74 ± 1.29b | 36.82 ± 1.27b | 18.86 ± 1.34b |
| 3 | 27.82 ± 0.24c | 50.69 ± 0.53c | 29.99 ± 0.71c |
| 4 | 29.00 ± 0.43d | 54.71 ± 0.65d | 52.64 ± 0.55d |
| 5 | 29.97 ± 0.38d | 61.05 ± 0.45e | 58.35 ± 0.96e |

[a] Each value is expressed as mean ± SD ($n = 10$). Means with different letters within a column are significantly different ($P < 0.05$).

Table 2
Proximate composition of *Agaricus bisporus* harvested at different stages of maturity

| Component | Content[a] (%) | | | | |
|---|---|---|---|---|---|
| | Stage 1 | Stage 2 | Stage 3 | Stage 4 | Stage 5 |
| Moisture | 91.2 ± 0.45b[b] | 92.0 ± 0.16bc | 91.5 ± 0.15bc | 92.3 ± 0.61c | 89.3 ± 0.77a |
| Dry matter | 8.76 ± 0.45b | 7.96 ± 0.16ab | 8.52 ± 0.15ab | 7.70 ± 0.61a | 10.7 ± 0.77c |
| Carbohydrate | 38.3 ± 0.45a | 41.8 ± 0.57b | 42.1 ± 0.30b | 46.6 ± 0.21c | 48.9 ± 0.51d |
| Reducing sugar | 14.9 ± 0.22ab | 13.9 ± 0.22a | 15.9 ± 1.84b | 15.1 ± 0.31ab | 15.4 ± 0.78ab |
| Crude ash | 11.0 ± 0.34d | 8.78 ± 0.65b | 8.51 ± 0.18b | 10.1 ± 0.14c | 7.77 ± 0.21a |
| Crude fat | 2.95 ± 0.18ab | 2.53 ± 0.01a | 3.53 ± 0.41bc | 3.91 ± 0.51c | 3.92 ± 0.71c |
| Crude fibre | 20.7 ± 0.92b | 20.4 ± 0.53b | 23.3 ± 0.30c | 18.2 ± 0.03a | 17.7 ± 0.71a |
| Crude protein | 27.0 ± 0.42c | 26.5 ± 1.09c | 22.5 ± 0.31b | 21.3 ± 0.14a | 21.7 ± 0.40ab |

[a] Moisture and dry matter were presented based on fresh weight; other data were presented based on dry weight.
[b] Each value is expressed as mean ± SD ($n = 3$). Means with different letters within a row are significantly different ($P < 0.05$).

due to higher water vaporisation occurring when gills were well exposed. Based on dry weight, contents of other components were in the order: carbohydrate (38.3–48.9%) > crude protein (21.3–27.0%) > crude fibre (17.7–23.3%) > crude ash (7.77–11.0%) > crude fat (2.53–3.92%). Obviously, mushrooms were high in carbohydrate, protein and fibre contents but low in ash and fat.

Interestingly, carbohydrate and fat contents increased with maturation while protein content decreased. Crude fibre content peaked at stage 3 and greatly decreased when mature. However, the content of reducing sugar was in the range of 13.9–15.9%. The difference between carbohydrate and reducing sugar contents is the content of soluble polysaccharides, which were thought to be biologically active substances in mushrooms (Wasser & Weis, 1999), and contents were 23.4%, 27.9%, 26.2%, 31.5% and 33.6% for mushrooms harvested at stages 1, 2, 3, 4 and 5, respectively. It seems that higher contents of soluble polysaccharides were found in mushrooms harvested at stages 4 and 5.

### 3.3. Soluble sugars and polyols

Mannitol was the major soluble sugar in fresh mushrooms and its content increased dramatically with maturation from 157 to 260 mg/g (Table 3). Glucose was the second highest and its contents were in the range of 17.6–28.1 mg/g and almost absent at stages 1 and 5. Like mannitol, contents of total sugars and polyols increased with maturation. Chen (1986) found that mannitol was a taste-active component in mushroom sugars and polyols. Soluble sugars and polyols, contained in mushrooms, contributed a sweet taste (Litchfield, 1967). Accordingly, the high amount of sugars and polyols, especially mannitol, would give rise to a sweet perception, and not to the typical mushroom taste.

Tseng and Mau (1999) found that total sugar and polyol content of *A. bisporus* Tainung 3 was 319 mg/g dry weight, higher than that of *A. bisporus* MS at stage 3 (286 mg/g, Table 3). The discrepancy in the total sugar and polyol contents might be due to different strains used. In addition, contents of total sugars and polyols were found to be 349–458 mg/g in *Volvariella volvacea* (Mau, Chyau, Li, & Tseng, 1997), 98.7–316 mg/g in *Auricularia* spp. and *Tremella fuciformis* (Mau, Wu, Wu, & Lin, 1998), 56.0–86.1 mg/g in *Agrocybe cylindracea* (Mau & Tseng, 1998), and 6.96–20.8 mg/g in *Pleurotus eryngii* (Mau, Lin, Chen, Wu, & Peng, 1998). However, Mau, Lin, and Chen (2001) found that *Ganoderma* spp. contained low amounts of total soluble sugars, ranged from 16.8 to 83.7 mg/kg. It seems that total sugar and polyol content of *A. bisporus* was lower than that of *V. volvacea* but higher than that of the other mushrooms mentioned above.

Table 3
Content of soluble sugars and polyols of *Agaricus bisporus* harvested at different stages of maturity

| Sugar or polyol | Content (mg/g dry weight) | | | | |
|---|---|---|---|---|---|
| | Stage 1 | Stage 2 | Stage 3 | Stage 4 | Stage 5 |
| Fructose | 6.02 ± 0.52c[a] | 3.95 ± 1.18b | 0.62 ± 0.03a | 0.95 ± 0.25a | 2.30 ± 0.83ab |
| Glucose | 18.3 ± 0.02b | 25.1 ± 0.36b | 26.7 ± 1.48b | 28.1 ± 2.36b | 17.6 ± 0.25a |
| Lactose | nd[b] | 2.86 ± 0.24b | 2.60 ± 0.13b | 3.80 ± 0.52c | 1.32 ± 0.26a |
| Mannitol | 157 ± 5.40a | 189 ± 6.19b | 245 ± 2.70cd | 230 ± 6.18c | 260 ± 9.05d |
| Myo-inositol | 1.89 ± 0.12a | 2.20 ± 0.37a | 2.30 ± 0.16a | 2.40 ± 0.29a | 1.88 ± 0.10a |
| Ribose | 9.42 ± 1.79b | 9.32 ± 2.56b | 4.63 ± 0.78a | 3.48 ± 1.47a | 7.24 ± 1.93ab |
| Sucrose | 1.48 ± 0.01d | 0.76 ± 0.04b | 1.19 ± 0.15cd | 1.08 ± 0.16bc | 0.41 ± 0.01a |
| Trehalose | 5.31 ± 0.44d | 4.38 ± 0.27c | 2.98 ± 0.46b | 2.75 ± 0.08b | 0.88 ± 0.02a |
| Total | 199.89 ± 4.58a | 237.18 ± 3.43b | 286.17 ± 1.01d | 272.28 ± 8.62c | 292.10 ± 11.63d |

[a] Each value is expressed as mean ± SD ($n = 3$). Means with different letters within a row are significantly different ($P < 0.05$).
[b] Not detected.

### 3.4. Free amino acids

The content of total free amino acids was in the range of 48.8–64.2 mg/g and peaked at stage 2 and then decreased significantly with maturation (Table 4). The amino acids with contents more than 5 mg/g were: histidine (14.6–18.1 mg/g), glutamic acid (7.28–10.6 mg/g) and alanine (6.46–8.19 mg/g). Not surprisingly, γ-aminobutyric acid (GABA), a hypotensive agent (Kohama et al., 1987; Kushiro et al., 1996), was found in the range of 2.16–5.79 mg/g. Similarly, GABA content peaked at stage 2 and then decreased with maturation. Tsai (2004) found that the contents of GABA in *Agaricus blazei*, *A. cylindracea*, *Boletus edulis* and *Coprinus comatus* were 0.11–0.63 mg/g, much lower than those shown in Table 4. Since GABA is a biologically active compound, the presence of GABA in *Agaricus* mushrooms would be beneficial to humans.

Table 5 tabulated the free amino acids into several classes on the basis of their taste characteristics, as described by Komata (1969). Aspartic and glutamic acids were MSG-like components, which gave the most mushroom taste, the umami taste that was the characteristic taste of MSG and 5′-nucleotide (Yamaguchi, 1979). The content of MSG-like components were in the range of 10.6–13.5 mg/g and similar to those of sweet components (11.4–14.3 mg/g) but lower than those of bitter components (19.7–26.9 mg/g).

Tseng and Mau (1999) found that the content of MSG-like components in *A. bisporus* Tainung 3 were 22.7 mg/g dry weight, much higher than that of *A. bisporus* MS at stage 3 (13.3 mg/g, Table 5). Contents of MSG-like components were found to be 11.2–26.2 mg/g in *V. volvacea* (Mau et al., 1997), 10.9–11.9 mg/g in *A. cylindracea* (Mau & Tseng, 1998), 3.75–9.06 mg/g in *Lentinula edodes* (Lin, 1988), 0.05–0.34 mg/g in *Auricularia* spp. and *T. fuciformis* (Mau, Wu, et al., 1998), and 1.01–1.77 mg/g in *P. eryngii* (Mau, Lin, et al., 1998). Furthermore, Mau, Lin, Ma, and Song (2001) found that contents of MSG-like components in four speciality mushrooms, including *Dictyophora indusiata*, *Hericium erinaceus* and *Tricholoma giganteum* ranged from 0.68 to 1.09 mg/g. In addition, Yang, Lin, and Mau (2001) found that contents of MSG-like components in several commercial mushrooms, including

Table 4
Content of free amino acids of *Agaricus bisporus* harvested at different stages of maturity

| Amino acid | Content (mg/g dry weight) | | | | |
|---|---|---|---|---|---|
| | Stage 1 | Stage 2 | Stage 3 | Stage 4 | Stage 5 |
| L-Alanine | 6.46 ± 0.09a[b] | 8.19 ± 0.11c | 7.54 ± 0.06bc | 7.19 ± 0.06ab | 8.01 ± 0.73c |
| L-Arginine | 0.99 ± 0.03a | 1.54 ± 0.33b | 0.86 ± 0.05a | 0.84 ± 0.03a | 0.79 ± 0.04a |
| L-Aspartic acid | 3.35 ± 0.19ab | 3.79 ± 0.23b | 3.65 ± 0.41b | 3.25 ± 0.11ab | 2.91 ± 0.27a |
| GABA[a] | 4.85 ± 0.02c | 5.79 ± 0.08d | 3.04 ± 0.16b | 2.40 ± 0.27a | 2.16 ± 0.02a |
| L-Glutamic acid | 7.28 ± 0.12a | 8.77 ± 0.07b | 9.61 ± 0.27c | 9.73 ± 0.15c | 10.59 ± 0.03d |
| Glycine + L-threonine | 1.27 ± 0.05c | 1.62 ± 0.15d | 1.35 ± 0.02c | 1.06 ± 0.04b | 0.83 ± 0.01a |
| L-Histidine | 14.62 ± 0.32a | 18.06 ± 0.16c | 15.83 ± 0.16b | 14.94 ± 0.16a | 16.18 ± 0.55b |
| L-Isoleucine | 0.91 ± 0.05c | 1.24 ± 0.10d | 0.90 ± 0.06c | 0.61 ± 0.01b | 0.47 ± 0.01a |
| L-Leucine | 1.49 ± 0.11c | 1.94 ± 0.08d | 1.58 ± 0.08c | 1.04 ± 0.03b | 0.75 ± 0.05a |
| L-Lysine | 2.27 ± 0.10c | 3.06 ± 0.18d | 2.20 ± 0.13c | 1.41 ± 0.06b | 0.96 ± 0.02a |
| L-Methionine + L-tryptophan | 0.46 ± 0.03d | 0.56 ± 0.03e | 0.41 ± 0.01c | 0.30 ± 0.01b | 0.24 ± 0.01a |
| L-Phenylalanine | 1.44 ± 0.05c | 1.96 ± 0.08d | 1.53 ± 0.05c | 1.16 ± 0.04b | 0.82 ± 0.02a |
| L-Serine | 3.99 ± 0.25b | 4.51 ± 0.36c | 3.65 ± 0.04b | 3.16 ± 0.10a | 2.82 ± 0.06a |
| L-Tyrosine | 1.23 ± 0.08b | 1.62 ± 0.05c | 1.30 ± 0.04b | 0.88 ± 0.04a | 1.28 ± 0.01b |
| L-Valine | 1.18 ± 0.05c | 1.56 ± 0.07d | 1.18 ± 0.06c | 0.81 ± 0.02b | 0.61 ± 0.01a |
| Total | 51.79 ± 1.05b | 64.21 ± 1.76d | 54.63 ± 1.67c | 48.80 ± 0.38a | 49.42 ± 1.13a |

[a] GABA, γ-aminobutyric acid.
[b] Each value is expressed as mean ± SD ($n = 3$). Means with different letters within a row are significantly different ($P < 0.05$).

Table 5
Content of taste characteristics of free amino acids in *Agaricus bisporus* harvested at different stages of maturity

| Taste characteristic[a] | Content (mg/g dry weight) | | | | |
|---|---|---|---|---|---|
| | Stage 1 | Stage 2 | Stage 3 | Stage 4 | Stage 5 |
| Bitter | 21.09 ± 0.04b[b] | 26.86 ± 0.35d | 22.29 ± 0.33c | 19.72 ± 0.23a | 19.86 ± 0.06a |
| MSG-like | 10.63 ± 0.08a | 12.56 ± 0.18b | 13.26 ± 0.67c | 12.98 ± 0.25bc | 13.50 ± 0.25c |
| Sweet | 11.72 ± 0.37a | 14.32 ± 0.59c | 12.54 ± 0.05b | 11.41 ± 0.16a | 11.66 ± 0.68a |
| Tasteless | 8.35 ± 0.20c | 10.47 ± 0.19d | 6.54 ± 0.02b | 4.69 ± 0.26a | 4.40 ± 0.05a |
| Total | 51.79 ± 1.05b | 64.21 ± 1.76d | 54.63 ± 1.67c | 48.80 ± 0.38a | 49.42 ± 1.13a |

[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 + GABA.
[b] Each value is expressed as mean ± SD ($n = 3$). Means with different letters within a row are significantly different ($P < 0.05$).

*L. edodes* shiitake, *Flammulina velutipes* strain white, *Pleurotus cystidiosus* and *Pleurotus ostreatus*, ranged from 0.84 to 1.93 mg/g and in *F. velutipes* strain yellow was 7.06 mg/g. However, Mau, Lin, Chen, et al. (2001) found that contents of MSG-like components in medicinal mushrooms, including *Ganoderma lucidum*, *Ganoderma tsugae* and *Coriolus versicolor* were in the range of 0.17–0.50 mg/g. Yang et al. (2001) reported that contents of MSG-like components could be divided into three ranges: low (<5 mg/g), middle (5–20 mg/g) and high ranges (>20 mg/g). Based on the previous results, the contents of MSG-like components in *A. bisporus* (10.6–13.5 mg/g) were in the middle range.

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 in the overall taste perception. Therefore, MSG-like and sweet components would be responsible for the natural taste of mushrooms. However, contents of MSG-like and sweet components and total soluble sugars and polyols were considerately higher in mushrooms and might be sufficient to suppress and cover the bitter taste arising from the contents of bitter components.

### 3.5. 5′-Nucleotides

Contents of total 5′-nucleotides fluctuated in the range of 6.59–8.14 mg/g (Table 6). Generally, mushrooms contained higher amounts of 5′-CMP (2.96–3.88 mg/g) and 5′-XMP (1.89–3.23 mg/g). Flavour 5′-nucleotides were found to be 5′-GMP, 5′-IMP and 5′-XMP (Chen, 1986). Contents of flavour 5′-nucleotides were higher in mushrooms harvested at stages 1 and 2 and deceased with maturation.

Tseng and Mau (1999) found that contents of total and flavour 5′-nucleotides in *A. bisporus* Tainung 3 were 11.3 and 4.19 mg/g dry weight, respectively, much higher than those of *A. bisporus* MS at stage 3 (7.57 and 3.00 mg/g, Table 6). Contents of total and flavour 5′-nucleotides were found to be 27.0–44.7 and 4.42–9.00 mg/g in *V. volvacea* (Mau et al., 1997), 0.67–1.51 and 0.21–0.63 mg/g in *A. cylindracea* (Mau & Tseng, 1998), 0.69–5.39 and 0.39–2.17 mg/g in *Auricularia* spp. and *T. fuciformis* (Mau, Wu, et al., 1998) and 15.7–639 and 1.63–4.89 mg/g in *P. eryngii* (Mau, Lin, et al., 1998), respectively.

Furthermore, Mau, Lin, Ma, et al. (2001) found that contents of total and flavour 5′-nucleotides in four speciality mushrooms ranged from 7.43 to 31.9 mg/g and 0.62–13.6 mg/g, respectively. Yang et al. (2001) found that contents of total and flavour 5′-nucleotides in several commercial mushrooms ranged from 9.51 to 24.2 mg/g and 1.60 to 11.6 mg/g, respectively. However, Mau, Lin, Chen, et al. (2001) found that contents of total and flavour 5′-nucleotides in medicinal mushrooms were in the range of 1.61–16.97 mg/g and 1.18–5.65 mg/g, respectively. Yang et al. (2001) reported that contents of flavour 5′-nucleotides could be divided into three ranges: low (<1 mg/g), middle (1–5 mg/g) and high ranges (>5 mg/g). Based on the previous results, contents of flavour 5′-nucleotides in *A. bisporus* (2.25–3.54 mg/g) were in the middle range.

5′-GMP contributes to meaty flavour, and is a much stronger flavour enhancer than MSG (Litchfield, 1967). The synergistic effect of flavour 5′-nucleotides with MSG-like components might greatly increase the umami taste of mushrooms (Yamaguchi et al., 1971). Based on the contents of MSG-like components and flavour 5′-nucleotides, the umami intensities of *Agaricus* mushrooms were expected to be in the range of 14.1–16.3 mg/g (the sum of contents of MSG-like components and flavour 5′-nucleotides).

### 3.6. Equivalent umami concentration

Using the equation derived from sensory evaluation (Yamaguchi et al., 1971), EUC values of mushrooms were in the range of 207–284 g MSG/100 g dry weight and higher at stages 2, 3 and 5 (Table 7). Usually, mushrooms picked at stage 3 exhibited higher EUC value. After gills exposed, EUC value of mushrooms at stage 5 was also

Table 6
Content of 5′-nucleotides of *Agaricus bisporus* harvested at different stages of maturity

| 5′-Nucleotide[a] | Content (mg/g dry weight) | | | | |
|---|---|---|---|---|---|
| | Stage 1 | Stage 2 | Stage 3 | Stage 4 | Stage 5 |
| 5′-AMP | 0.27 ± 0.03b[c] | 0.32 ± 0.01c | 0.25 ± 0.02b | 0.20 ± 0.02a | 0.26 ± 0.01b |
| 5′-CMP | 3.13 ± 0.18ab | 2.96 ± 0.23a | 3.42 ± 0.03b | 3.45 ± 0.16b | 3.88 ± 0.19c |
| 5′-GMP | 0.08 ± 0.01a | 0.11 ± 0.01b | 0.14 ± 0.02c | 0.11 ± 0.01b | 0.13 ± 0.01bc |
| 5′-IMP | 0.54 ± 0.04c | 0.20 ± 0.02a | 0.26 ± 0.05ab | 0.25 ± 0.03ab | 0.39 ± 0.13b |
| 5′-UMP | 0.91 ± 0.03ab | 0.77 ± 0.07a | 0.90 ± 0.16ab | 0.69 ± 0.02a | 1.17 ± 0.31b |
| 5′-XMP | 2.83 ± 0.14cd | 3.23 ± 0.24d | 2.60 ± 0.20bc | 1.89 ± 0.04a | 2.31 ± 0.33ab |
| Flavor 5′-nucleotide[b] | 3.45 ± 0.15cd | 3.54 ± 0.27d | 3.00 ± 0.27bc | 2.25 ± 0.04a | 2.83 ± 0.45b |
| Total | 7.76 ± 0.31b | 7.59 ± 0.56ab | 7.57 ± 0.39ab | 6.59 ± 0.18a | 8.14 ± 0.94b |

[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] Flavour 5′-nucleotide, 5′-GMP + 5′-IMP + 5′-XMP.
[c] Each value is expressed as mean ± SD ($n = 3$). Means with different letters within a row are significantly different ($P < 0.05$).

Table 7
Equivalent umami concentration (EUC) from five stages in the development of harvested mushrooms of *Agaricus bisporus*

| | EUC (g/100 g dry weight) |
|---|---|
| Stage 1 | 230 ± 4.0a[a] |
| Stage 2 | 275 ± 14.6b |
| Stage 3 | 268 ± 5.9b |
| Stage 4 | 207 ± 5.4a |
| Stage 5 | 284 ± 26.5b |

[a] Each value is expressed as mean ± SD ($n = 3$). Means with different letters within a column are significantly different ($P < 0.05$).

higher. It seems that with regard to the umami taste, mushrooms harvested at stages 2 and 3 in Taiwan and America were comparable to those harvested at stage 5 in Europe (Mau et al., 1993; Tseng & Mau, 1999).

Mau (2005) reported the EUC values from mushroom taste components using the equation of Yamaguchi et al. (1971) and grouped calculated EUC values into four levels: >1000% (>1000 g MSG/100 g dry weight), 100–1000%, 10–100% and <10%. An EUC value of 100% represents that the umami intensity per 1 g dry weight is equivalent to the umami intensity given by 1 g of MSG or in other words, 1 g MSG/g dry weight. Therefore, the umami intensity of 1 g *Agaricus* mushrooms harvested at different stages of maturity was equivalent to the umami intensity given by 2.07–2.84 g of MSG. However, *A. bisporus* Tainung 3 had an EUC value of 1144 g/100 g (Tseng & Mau, 1999), much higher than that found in this study. Apparently, the strain and cultivation conditions used in this study would give rise to a product with less intense umami taste.

With regard to EUC values, *A. bisporus* MS was less than *V. volvacea* at the first level (1048–4465 g/100 g) and *Pleurotus citrinopileatus* and *F. velutipes* strain yellow at the second level (511 g/100 g and 363 g/100 g, respectively), and higher than several other mushrooms at the second, third and fourth levels (Mau, 2005). However, EUC values of *C. versicolor*, *G. lucidum* and *G. tsugae* were at the fourth level (0.66–7.92 g/100 g) (Mau, 2005).

Tape (1961) showed that the flavour intensity of *Agaricus* mushrooms gradually increased from young "buttons" to old "flats." Mau et al. (1993) found no differences in the volatile components of mushrooms harvested at different stages of maturity. However, the results in this study do not support the findings of Tape (1961). Based on the results obtained, *Agaricus* mushrooms possessed highly intense umami taste. This finding might explain why mushrooms have long been used as a food or food-flavouring material. In addition, the sensory EUC values of *Agaricus* mushrooms might be used as a means for the selection of new strains of *Agaricus* mushroom with more intense umami taste.

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