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Analysis and Evaluation of Tasty Components in the Pileus and Stipe of *Lentinula edodes* at Different Growth Stages

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ABSTRACT: Tasty components in *Lentinula edodes* pileus and stipe at different growth stages were studied. Mannitol, trehalose, arabitol, and glucose were the main soluble polyols and sugars, whereas succinic acid, malic acid, and citric acid were the main organic acids. Mannitol contents were the highest in the pileus and increased at mature growth stages, although arabitol contents were the highest in the stipe and peaked at stage 5. Succinic acid contents peaked at stage 5 in the pileus and stipe during mature growth stages. Threonine (sweet taste) values were the highest among all the detected amino acids, followed by glutamic acid (MSG-like taste). MSG-like 5′-nucleotide contents could account for nearly 50% of the total 5′-nucleotides. Equivalent umami concentration (EUC) values of stage 5 exhibited higher levels during mature growth stages. Tasty components in the stipe were rich and EUC values were high, which might be useful for further processing and byproduct development of *L. edodes*.

KEYWORDS: *Lentinula edodes, tasty components, equivalent umami concentration, growth stages*

■ INTRODUCTION

Lentinula edodes (Berk.), usually called Xianggu in China, shiitake in Japan, is one of the most important traditional edible and medicinal fungus in China and other East Asian countries for its desirable tasty flavor and great medicinal properties.[1−6] The tasty components in edible mushrooms are primarily several water-soluble substances, including soluble sugars, free amino acids, 5′-nucleotides, and organic acids, which present a variety of taste, such as sweet, umami, sour, and so on. Soluble sugars and polyols, contained in mushrooms, contribute to a sweet taste but not to the typical mushroom taste.[7] Organic acids, related to the metabolic processes of synthetic phenols, amino acids, esters, and aromatic substances,[8] contribute an important part of edible mushroom flavor. Umami taste is recognized as a basic taste, typified by glutamic acid and its salt monosodium glutamate (MSG), which give a savory meat soup or rich meaty taste sensation.[9] Aspartic acid, glutamic acid, and some 5′-nucleotides were also considered as MSG-like components, and values of equivalent umami concentration (EUC) were used to evaluate umami of mushrooms.[10]

Currently, both the pileus and stipe of *L. edodes* were available for food flavor components in China. Recent literatures were just aimed at the tasty components in fruiting bodies of *L. edodes*.[11−13] However, there were few reports about the profiles of tasty components of *L. edodes* in different parts (pileus and stipe) or at different growth stages. Therefore, the aims of our study were to systematically evaluate the main tasty components in *L. edodes* pileus and stipe at different growth stages, in order to further elucidate the synergistic effect of umami tasty components and mechanism of flavor producing in the pileus and stipe during growth stages. The results would be beneficial for further processing in the flavors and fragrances industry.

■ MATERIALS AND METHODS

Mushroom Materials. *L. edodes* used in this study were cultivated in Xiufeng Edible Fungi Cooperative, Jinshan district, Shanghai, China. The strain, Shenxiang 16 ((2009) No. 002, was recognized by Edible Fungus of Shanghai Agricultural Commodities) and bred by the Improved and Standardized Spawn Breeding Center (ISSBC), Shanghai Academy of Agricultural Sciences, China. Fresh *L. edodes* were harvested at six different stages (Figure 1) according to their growth characteristics (Table 1). The stipe of *L. edodes* formed at stage 1 and was fully extended at stage 6. After stage 4, its inner membrane would crack naturally. Samples were dried by hot air (DHG-9145A, Hengke Technology Ltd., Shanghai, China) at 50 ± 2 °C with maximum air speed, and samples were ground using an ultracentrifugal mill and sieving machine to obtain powder (80 mesh).

Analysis of Soluble Sugars by HPAEC-PAD. Soluble sugars and polyols were extracted and analyzed according to Ajlouni et al.[14] Suspensions of powdered mushroom (0.5 g) in 50 mL of 80% ethanol were shaken at 150 rpm for 45 min at room temperature (26 °C). After filtration through Whatman No. 3 filter paper, the residue was washed five times with additional 25 mL volumes of 80% ethanol. The combined filtrate and rinsed water were evaporated to dryness at 50 °C on

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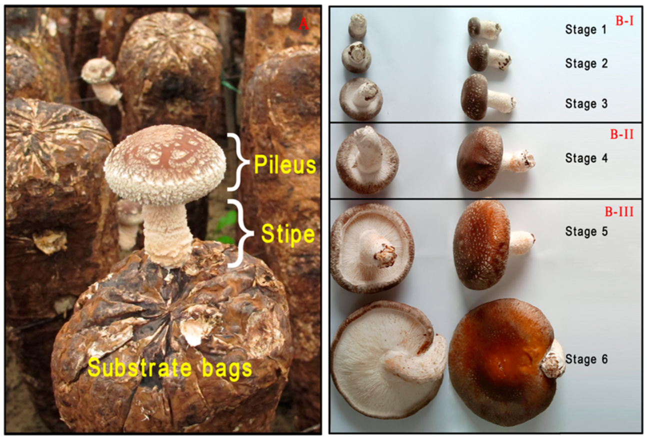

Figure 1. Photograph of *L. edodes* at different growth stages. **A** The fruit body consists of pileus (cap) and stipe (stalk) growing on substrate bags. Developmental processes were divided into three phases: **B−I**, primary developing stages; **B−II**, middle stage; and **B−III**, mature stages.

a rotary evaporator. Extracted solids were redissolved in deionized water to a final volume of 10 mL, and the solution was filtered (0.22 μm cellulose filter, Millipore) prior to analysis. Components were identified by high performance anion exchange chromatography with pulsed amperometric detection (HPAEC-PAD) using the Dionex ICS2500 HPAEC-PAD system (Thermo Fisher China Ltd., Shanghai). Corresponding condition of test method referenced the report by Li et al.[15] Each sugar or polyol was identified and quantified using authentic sugar or polyol standards (Sigma-Aldrich, St. Louis, United States).

Analysis of Organic Acids by HPLC. The extraction of organic acids was modified from a method described by Li et al.[16] Mushroom powder (1.0 g) diluted with 50 mL of 0.1 N HCl (analytical reagent, Sinopharm) was shaken for 60 min at 60 °C for analysis of organic acids. The suspension was centrifuged at 10 000 rpm (15 000*g*) for 20 min after cooling. Then the extraction was filtered through 0.22 μm mixed cellulose esters filter (Welch) to inject into HPLC. The HPLC conditions were carried by Li et al.[16] Each organic acid was identified and quantified using authentic standards (Sinopharm Chemical Reagent Co. Ltd., Shanghai, China) based on their retention time; samples were further quantified by comparing their peak area with the relevant calibration curves of the standard compounds.

Analysis of Free Amino Acids by Amino Acid Analyzer. Free amino acids were extracted and analyzed according to Li et al.[16] Dried *L. edodes* powder sample (0.5 g) was mixed with 30 mL of 0.1 mol/L HCl (GR) solution and then placed into an ultrasonic bath for 30 min at 40 °C. The sample was left to stand for 30 min and then centrifuged at 6000 rpm (4000*g*) for 3 min. The supernatant and 10% sulfosalicylic acid solution were mixed according to the proportion of 1:1 (v/v). The mixture was left at 4 °C for 1 h and then centrifuged at 12 000 rpm (18 000*g*) for 30 min at 4 °C, immediately transferred the supernatant to the tube, and adjusted pH value to 2.0 with 10 mol/L NaOH/HCl. The final supernatant liquid was filtered with Millipore 0.45 μm PES filters (Milford, U.S.A.). The filtrate was loaded onto L-8800 high-speed amino acid analyzer (Hitachi, Japan). The standard amino acid solutions, type ANII and type B, were obtained from Wako (Wako-shi, Japan).

Analysis of 5′-Nucleotides by HPLC. 5′-Nucleotides were extracted and analyzed as described by Taylor et al.[17] A suspension of *L. edodes* powder (1.0 g) in 25 mL of deionized water was boiled for 1 min, cooled, and then centrifuged at 12 000 rpm (18 000*g*) for 15 min. The sediment was repeated with 20 mL of deionized water, and the combined extraction was filtered through a 0.22 μm cellulose membrane (Millipore) prior to analysis by high-performance liquid chromatography (HPLC). The HPLC conditions were carried by Li et al.[15] Each 5′-nucleotide was identified and quantified using 5′-nucleotide standards (Sigma-Aldrich, St. Louis, United States).

Formula of Equivalent Umami Concentration (EUC). The equivalent umami concentration [EUC, g monosodium glutamate (MSG) per 100 g dry raw material weight] is the concentration of MSG equivalent to the umami intensity of that given by the mixture of MSG and a 5′-nucleotide and is represented by the following addition equation:[18]

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

where Y is the EUC of the mixture in terms of g MSG per 100 g dry raw material weight; a_i is the concentration (g per 100 g dry raw material weight) of each umami amino acid [aspartic acid (Asp) or glutamic acid (Glu)]; a_j is the concentration (g per 100 g dry raw material weight) 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 of g per 100 g dry raw material weight used.

Statistical Analysis. All experiments were performed three times. Tukey's Honestly Significant Difference (Tukey HSD) test was conducted when the samples exhibited a significance

Table 1. Growth Characteristics of *L.edodes* Fruiting Body at Different Stages of Maturity

	stipe		pileus		
stage	diameter (cm)	thickness (cm)	diameter (cm)	length (cm)	mass (g)
1	1.93 ± 0.23f	1.22 ± 0.20e	1.48 ± 0.26c	2.13 ± 0.32d	3.73 ± 1.04e
2	2.56 ± 0.16e	1.62 ± 0.11d	1.59 ± 0.23c	2.50 ± 0.40d	7.17 ± 1.52e
3	4.03 ± 0.22d	2.16 ± 0.14c	1.56 ± 0.32c	3.27 ± 0.49c	15.06 ± 1.96d
4	5.13 ± 0.45c	2.42 ± 0.37b	1.90 ± 0.21b	3.74 ± 0.48b	28.86 ± 5.24c
5	7.73 ± 0.57b	2.82 ± 0.16a	2.48 ± 0.46a	4.70 ± 0.43a	69.95 ± 11.45b
6	11.70 ± 0.57a	2.83 ± 0.21a	2.71 ± 0.22a	5.26 ± 0.65a	99.26 ± 13.31a

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

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Table 2. Contents of Soluble Sugars and Polyols in the Pileus and Stipe of *L. edodes* at Different Stages of Maturity

	content (mg/g dry weight)								
	arabitol	mannitol	trehalose	arabinose	fructose	fucose	glucose	rhamnose	total
in the pileus									
stage 1	27.10 ± 0.15e[a]	37.65 ± 0.41f	10.85 ± 0.16b	nd[b]	nd	0.88 ± 0.02d	2.10 ± 0.03d	nd	78.65 ± 0.06a
stage 2	24.15 ± 0.23d	44.49 ± 0.54h	22.19 ± 0.23d	nd	0.52 ± 0.04 cd	0.81 ± 0.01d	2.99 ± 0.01e	nd	95.24 ± 0.58bc
stage 3	17.06 ± 0.06b	54.79 ± 0.60i	32.37 ± 0.12i	0.08 ± 0.03b	0.35 ± 0.03bc	0.86 ± 0.02d	3.30 ± 0.03f	nd	108.9 ± 0.57f
stage 4	19.21 ± 0.29c	34.22 ± 0.32e	35.35 ± 0.43j	0.00 ± 0.00a	0.82 ± 0.08d	0.97 ± 0.01e	5.60 ± 0.04i	nd	96.35 ± 0.29c
stage 5	18.58 ± 0.18c	41.88 ± 0.47g	27.63 ± 0.17g	0.00 ± 0.00a	0.48 ± 0.04de	0.84 ± 0.03d	5.03 ± 0.02h	nd	94.58 ± 0.22bc
stage 6	12.34 ± 0.09a	56.65 ± 0.31j	36.58 ± 0.28j	0.03 ± 0.00b	0.67 ± 0.05e	0.85 ± 0.02d	4.34 ± 0.03j	nd	111.6 ± 0.08g
in the stipe									
stage 1	60.75 ± 0.25i	22.86 ± 0.32a	7.32 ± 0.14a	0.50 ± 0.03d	0.21 ± 0.03a	0.14 ± 0.03bc	1.11 ± 0.04b	0.05 ± 0.01a	92.98 ± 0.32b
stage 2	64.46 ± 0.67j	25.44 ± 0.22b	13.11 ± 0.22c	0.36 ± 0.07c	0.33 ± 0.05b	0.16 ± 0.03c	0.65 ± 0.08a	0.11 ± 0.03b	104.7 ± 0.25d
stage 3	54.11 ± 0.25h	27.09 ± 0.20c	25.33 ± 0.09f	0.41 ± 0.01 cd	0.38 ± 0.01bc	0.02 ± 0.01a	0.82 ± 0.05a	0.11 ± 0.02b	108.9 ± 0.22ef
stage 4	51.60 ± 0.25g	23.07 ± 0.18a	30.85 ± 0.13h	0.50 ± 0.05d	0.27 ± 0.02ab	0.06 ± 0.01ab	0.59 ± 0.02a	0.02 ± 0.00a	107.0 ± 0.19e
stage 5	71.70 ± 0.54k	29.36 ± 0.34d	23.14 ± 0.13e	0.47 ± 0.04d	0.31 ± 0.03ab	0.08 ± 0.01b	0.99 ± 0.12b	nd	126.1 ± 1.06i
stage 6	47.83 ± 0.42f	34.40 ± 0.26e	40.10 ± 0.15k	0.39 ± 0.08c	0.43 ± 0.06c	0.09 ± 0.02bc	1.57 ± 0.08c	nd	125.0 ± 1.01h

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

Table 3. Contents of Organic Acids in the Pileus and Stipe of *L. edodes* at Different Stages of Maturity

	content (mg/g dry weight)							
	acetic acid	ascorbic acid	citric acid	fumaric acid	malic acid	succinic acid	tartaric acid	total
in the pileus								
stage 1	6.39 ± 0.63d	nd[b]	67.64 ± 3.21e	3.36 ± 0.06c	21.60 ± 0.94c	122.9 ± 4.76c	4.33 ± 0.22b[a]	226.1 ± 3.91e
stage 2	10.54 ± 0.85e	nd	77.60 ± 2.76f	4.41 ± 0.04d	79.93 ± 1.24e	197.1 ± 5.32h	5.21 ± 0.38c	374.7 ± 4.88h
stage 3	3.82 ± 0.45b	nd	53.22 ± 1.88d	3.10 ± 0.05bc	52.65 ± 0.75d	155.8 ± 3.55d	4.39 ± 0.26b	272.9 ± 3.27f
stage 4	5.60 ± 0.68c	nd	65.54 ± 2.07e	3.03 ± 0.03bc	57.52 ± 1.33d	184.3 ± 5.89g	4.43 ± 0.27b	320.3 ± 4.84g
stage 5	5.29 ± 0.87bc	nd	59.20 ± 2.14d	2.40 ± 0.04b	53.60 ± 1.28d	196.9 ± 5.12h	4.22 ± 0.29b	321.5 ± 4.68g
stage 6	4.81 ± 0.96bc	nd	59.11 ± 2.41d	3.19 ± 0.03bc	26.57 ± 1.17c	174.1 ± 7.83e	1.76 ± 0.11a	269.5 ± 5.93f
in the stipe								
stage 1	5.03 ± 0.13	0.50 ± 0.06c	40.6 ± 1.21c	2.36 ± 0.04b	16.8 ± 0.97b	60.0 ± 2.65ab	7.91 ± 0.53e	133.2 ± 1.87d
stage 2	2.16 ± 0.21a	0.34 ± 0.07b	22.5 ± 0.67ab	1.30 ± 0.01a	16.1 ± 0.81b	47.2 ± 3.11a	6.30 ± 0.29d	95.84 ± 2.34b
stage 3	2.62 ± 0.28a	0.42 ± 0.01bc	29.8 ± 0.74b	2.02 ± 0.03b	16.1 ± 0.87b	69.5 ± 3.43b	6.23 ± 0.42d	126.7 ± 1.98d
stage 4	nd	0.18 ± 0.03a	19.8 ± 0.97a	1.00 ± 0.05a	16.5 ± 0.77b	43.5 ± 2.96a	5.24 ± 0.27c	85.40 ± 2.04a
stage 5	2.56 ± 0.34a	0.35 ± 0.04b	27.1 ± 1.03b	0.94 ± 0.05a	10.1 ± 0.92ab	70.5 ± 3.04b	5.00 ± 0.45c	116.5 ± 2.65 cd
stage 6	nd	0.35 ± 0.05b	27.6 ± 1.11b	1.54 ± 0.03ab	7.71 ± 0.79a	68.4 ± 2.11b	5.01 ± 0.37c	109.8 ± 1.72c

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

difference between samples, with the level of significance set at $P < 0.05$. Both ANOVA and Tukey HSD were performed with IBM SPSS Statistics 20.

RESULTS AND DISCUSSION

Analysis of Tasty Components in *Lentinula edodes* at Different Stages and Parts. The tasty components of fruit body (pileus and stipe) and different developmental stages of *L. edodes* were investigated. Results were compared with other findings in the literature.

Soluble Sugars and Polyols. Mannitol, trehalose, arabitol, and glucose, considered as the main mushroom polyols or soluble sugars in former reports,[11,12,15,19] were found in the pileus and stipe of *L. edodes* at different growth stages in this research (Table 2). The contents of mannitol were the highest in the pileus, whereas arabitol values were highest in the stipe. In the pileus, the contents of mannitol appeared as an "N" shape developing curve during all the growth stages. It had increased profiles at primary developing stages (stages 1−3) and stages 4−6, whereas it decreased from stage 3 to stage 4. Both mannitol and trehalose increased profiles in the pileus and stipe during mature growth stages. Arabitol showed a high peak at stage 5 in the stipe and had different profiles compared with the results reported by Yang et al.[11] Fucose, arabinose, and frutocose were detected both in the pileus and stipe, but rhamnose was just detected in the stipes. Kim et al.[12] found that freeze-dried *L. edodes* (Strain No. KKU-05, Hwago) fruiting body contained ribose, xylcose, manose, and sucrose (2.40, 7.98, 23.12, and 2.33 mg/g, respectively). Yang et al.[11] found that *L. edodes* (271) and *L. edodes* (Tainung 1) contained manninol, glucose, and trehalose (83.8 and 134 mg/g, 28.6 and 14.2 mg/g, 29.2 and 3.74 mg/g, respectively). The discrepancy in varieties and contents of main soluble sugars and polyols might be due to the difference in strains and cultivation media of fungus, and fucose, rhamnose, and fructose in *L. edodes* have not been reported or detected in the previous studies.

Organic Acids. As main organic acids, succinic acid, citric acid, and malic acid were detected both in the pileus and stipes of *L. edodes*, with descending order of succinic acid > citric acid > malic acid at each growth stage (Table 3). Hiroshi[20] reported that malic acid, fumaric acid, and citric acid were the main organic acids in shiitake (*L. edodes*), whereas in this research,

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Table 4. Contents of Free Amino Acids, 5′-Nucleotides and Equivalent Umami Concentration (EUC) in the Pileus of *L. edodes* at Different Stages of Maturity

	content (mg/g dry weight)					
	stage 1	stage 2	stage 3	stage 4	stage 5	stage 6
amino acids						
alanine	0.91 ± 0.04b	0.94 ± 0.06b	1.40 ± 0.05c	0.64 ± 0.02a	1.54 ± 0.09c	1.01 ± 0.11b
arginine	2.75 ± 0.05h	2.01 ± 0.04g	1.46 ± 0.04e	1.33 ± 0.08d	1.48 ± 0.11e	1.39 ± 0.12d
aspartic acid	0.63 ± 0.04c[a]	0.47 ± 0.03b	0.25 ± 0.01a	0.74 ± 0.03d	0.26 ± 0.03a	0.24 ± 0.02a
cysteine	0.21 ± 0.03c	0.10 ± 0.02b	0.45 ± 0.03d	0.23 ± 0.03c	0.09 ± 0.01b	0.40 ± 0.04d
GABA	0.31 ± 0.02bc	0.35 ± 0.02c	0.27 ± 0.04b	0.27 ± 0.03b	0.35 ± 0.03c	0.17 ± 0.02a
glutamic acid	4.02 ± 0.03f	3.49 ± 0.08e	2.66 ± 0.06 cd	2.28 ± 0.05c	2.96 ± 0.14d	2.10 ± 0.08c
glycine	0.66 ± 0.01d	0.34 ± 0.03b	0.76 ± 0.04e	0.24 ± 0.02ab	0.26 ± 0.01ab	0.65 ± 0.03d
histidine	0.68 ± 0.04f	0.51 ± 0.02e	0.37 ± 0.05c	0.40 ± 0.02 cd	0.33 ± 0.03c	0.45 ± 0.01d
hydroxyproline	0.08 ± 0.01b	0.07 ± 0.02ab	0.11 ± 0.02c	0.12 ± 0.02c	0.07 ± 0.01ab	0.13 ± 0.02c
isoleucine	0.28 ± 0.03c	0.20 ± 0.01c	0.29 ± 0.04c	0.09 ± 0.02b	0.10 ± 0.01b	0.24 ± 0.01c
leucine	0.47 ± 0.05d	0.32 ± 0.04c	0.41 ± 0.06d	0.14 ± 0.08b	0.16 ± 0.02b	0.35 ± 0.03c
lysine	1.44 ± 0.07e	1.36 ± 0.04d	1.08 ± 0.08c	0.92 ± 0.03c	0.92 ± 0.07c	1.02 ± 0.03c
methionine	0.08 ± 0.02e	0.06 ± 0.02d	0.06 ± 0.08d	0.06 ± 0.02d	0.04 ± 0.01b	0.06 ± 0.01d
ornithine	0.69 ± 0.06a	1.82 ± 0.06e	1.86 ± 0.06e	1.86 ± 0.09e	2.79 ± 0.09g	2.40 ± 0.04f
phenylalanine	0.90 ± 0.05e	0.89 ± 0.05c	0.89 ± 0.08c	0.73 ± 0.05c	0.70 ± 0.05c	0.90 ± 0.03e
proline	0.00 ± 0.00a	0.00 ± 0.00a	0.11 ± 0.04e	0.00 ± 0.00a	0.00 ± 0.00a	0.08 ± 0.03d
serine	1.36 ± 0.02g	0.97 ± 0.05f	0.74 ± 0.05e	0.43 ± 0.04c	0.71 ± 0.03d	0.73 ± 0.04d
tyrosine	0.33 ± 0.03ab	0.45 ± 0.07b	0.64 ± 0.05c	0.40 ± 0.06b	0.48 ± 0.01b	0.68 ± 0.05c
tryptophan	0.00 ± 0.00a	0.11 ± 0.03c	0.13 ± 0.01c	0.05 ± 0.02b	0.02 ± 0.01ab	0.26 ± 0.03d
threonine	8.04 ± 0.03c	11.9 ± 0.07g	9.61 ± 0.10ef	10.1 ± 0.14f	8.67 ± 0.07 cd	9.20 ± 0.05d
valine	1.81 ± 0.05h	1.37 ± 0.05g	1.22 ± 0.05g	0.99 ± 0.08e	0.99 ± 0.04e	1.12 ± 0.08f
taste characteristics[b]						
bitter	6.97 ± 0.08h	5.47 ± 0.03g	4.83 ± 0.07f	3.79 ± 0.11e	3.82 ± 0.10e	4.77 ± 0.04f
MSG-like	4.65 ± 0.05g	3.96 ± 0.06f	2.91 ± 0.02d	3.02 ± 0.05d	3.22 ± 0.07de	2.34 ± 0.13bc
sweet	11.0 ± 0.07f	14.1 ± 0.09h	12.6 ± 0.03g	11.4 ± 0.09f	11.2 ± 0.13f	11.7 ± 0.08f
tasteless	1.98 ± 0.03d	1.91 ± 0.04d	2.17 ± 0.05e	1.55 ± 0.08c	1.49 ± 0.05c	2.10 ± 0.11e
total	25.7 ± 0.11g	27.7 ± 0.09h	24.8 ± 0.15f	22.0 ± 0.22d	22.9 ± 0.17d	23.6 ± 0.27e
5′-nucleotides[c]						
5′-AMP	2.47 ± 0.13e	1.11 ± 0.11b	1.77 ± 0.14d	1.34 ± 0.12b	1.53 ± 0.09c	1.88 ± 0.11d
5′-CMP	3.34 ± 0.14e	1.51 ± 0.10a	2.59 ± 0.15	2.29 ± 0.12c	2.24 ± 0.11c	2.46 ± 0.12d
5′-GMP	2.81 ± 0.12g	1.34 ± 0.11c	2.12 ± 0.15f	1.53 ± 0.12d	1.67 ± 0.09e	2.16 ± 0.11f
5′-UMP	2.81 ± 0.13f	1.05 ± 0.09c	1.70 ± 0.13e	1.51 ± 0.13d	1.56 ± 0.10d	1.61 ± 0.10d
MSG-like 5′-nucleotides[d]	5.28 ± 0.12h	2.45 ± 0.09c	3.89 ± 0.13f	2.87 ± 0.11d	3.21 ± 0.10e	4.04 ± 0.11g
flavor 5′-nucleotides[e]	2.81 ± 0.12g	1.34 ± 0.11c	2.12 ± 0.15f	1.53 ± 0.12d	1.67 ± 0.09e	2.16 ± 0.11f

[a]Each value is expressed as mean ± standard deviation ($n = 3$). 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 + Try + Val; Tasteless: Lys + Tyr + GABA. [c]5′-CMP: 5′-sytosine monophosphate; 5′-UMP: 5′-uridine monophosphate; 5;-GMP: 5′-guanosine monophosphate; 5′-AMP: 5′-adenosine monophosphate. [d]MSG-like 5′-nucleotides: 5′-AMP + 5′-GMP + 5′-IMP + 5′-XMP, while 5′-IMP and 5′-XMP was not detected in this study. [e]Flavor 5′-nucleotides defined by Chen (1986): 5′-GMP + 5′-IMP + 5′-XMP, while only 5′-GMP was detected in this study.

the contents of succinic acid were significantly higher than those of other detected organic acids, peaked at stage 2 in the pileus and stage 3 in the stipe, respectively, during primary developing stages, and came to a relatively stable levels at mature growth stages. Succinic acid and its sodium salt were the characteristic umami components, rich in marine shellfishes, such as scallop (370 mg/g) and oyster (50 mg/g).[21] Those results would be benefit to a further research of the umami formation mechanism in *L. edodes*. The contents of succinic acid were from 122.9 mg/g to 197.1 mg/g in the pileus and 43.5 mg/g to70.5 mg/g in the stipe during the growth of *L. edodes* fruiting body, higher than the contents of succinic acid in several cultivated mushrooms reported by Li et al.[15] Citric acid, had a mild and refreshing acidity,[22] as the second highest organic acid in pileus and stipe ranged from 53.22 mg/g to 77.60 mg/g and 19.8 mg/g to 40.6 mg/g, respectively, and reached certain steady values at mature growth stages. It also got a top value at stage 2 in the pileus and stage 1 in the stipe, respectively. Malic acid, with a refreshing acidity and a slightly bitter taste,[23] ranked at the third level at mature growth stages in pileus and stipe. At mature stages, the contents of succinic acid in pelius were approximately 3-fold higher than those in stipe, whereas values of citric acid in pelius were 2-fold compared with those in stipe.

Free Amino Acids and 5′-Nucleotides. In this study, 21 amino acids were identified in the pileus and stipe of the growth stages of *L. edodes* (Table 4 and Table 5). The results showed that the contents of threonine were the highest among all amino acids, accounting for 90% of sweet amino acids, with higher values in pileus compared with those of stipe, and achieved relatively steady level at mature growth stages. Glutamic acid as the second highest free amino acid, also accounting for 90% of MSG-like amino acids, were higher in pileus than those of stipe and peaked at stage 5 during the

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Table 5. Contents of Free Amino Acids, 5′-Nucleotides and Equivalent Umami Concentration (EUC) in the Stipe of *L. edodes* at Different Stages of Maturity[a]

	content (mg/g dry weight)					
	stage 1	stage 2	stage 3	stage 4	stage 5	stage 6
amino acids						
alanine	0.59 ± 0.01a	1.09 ± 0.01b	0.70 ± 0.01a	0.69 ± 0.04a	1.42 ± 0.02c	0.66 ± 0.03a
arginine	1.56 ± 0.01f	1.22 ± 0.02c	1.15 ± 0.01bc	0.80 ± 0.03a	1.01 ± 0.03b	0.89 ± 0.01a
aspartic acid	1.04 ± 0.01f	0.42 ± 0.01b	0.23 ± 0.02a	0.87 ± 0.01e	0.25 ± 0.01a	0.22 ± 0.02a
cysteine	0.25 ± 0.02c	0.26 ± 0.01c	0.24 ± 0.01c	0.19 ± 0.01c	0.00 ± 0.00a	0.22 ± 0.01c
GABA	0.16 ± 0.01a	0.35 ± 0.02c	0.17 ± 0.01a	0.25 ± 0.01b	0.32 ± 0.01c	0.17 ± 0.01a
glutamic acid	2.52 ± 0.01 cd	2.19 ± 0.04c	1.33 ± 0.02a	1.29 ± 0.01a	1.86 ± 0.02b	1.36 ± 0.02a
glycine	0.45 ± 0.01c	0.31 ± 0.03b	0.40 ± 0.01c	0.19 ± 0.01a	0.15 ± 0.01a	0.31 ± 0.02b
histidine	0.32 ± 0.01c	0.21 ± 0.01b	0.13 ± 0.01a	0.15 ± 0.01a	0.13 ± 0.02a	0.12 ± 0.03a
hydroxyproline	0.04 ± 0.01a	0.04 ± 0.01a	0.04 ± 0.01a	0.09 ± 0.01b	0.05 ± 0.01a	0.04 ± 0.02a
isoleucine	0.10 ± 0.01b	0.07 ± 0.02ab	0.10 ± 0.01b	0.02 ± 0.01a	0.03 ± 0.01a	0.08 ± 0.02b
leucine	0.16 ± 0.01b	0.11 ± 0.01b	0.13 ± 0.01b	0.04 ± 0.00a	0.05 ± 0.01a	0.11 ± 0.02b
lysine	0.66 ± 0.02b	0.96 ± 0.00c	0.56 ± 0.03ab	0.60 ± 0.02b	0.63 ± 0.01b	0.42 ± 0.01a
methionine	0.05 ± 0.01c	0.03 ± 0.01a	0.04 ± 0.01b	0.03 ± 0.01a	0.03 ± 0.01a	0.04 ± 0.01b
ornithine	0.67 ± 0.01a	0.97 ± 0.01c	0.79 ± 0.02b	0.83 ± 0.01b	1.13 ± 0.03d	1.12 ± 0.02d
phenylalanine	0.45 ± 0.01b	0.39 ± 0.01b	0.40 ± 0.02b	0.27 ± 0.02a	0.30 ± 0.01a	0.35 ± 0.03ab
proline	0.00 ± 0.00a	0.00 ± 0.00a	0.06 ± 0.01c	0.00 ± 0.00a	0.00 ± 0.00a	0.04 ± 0.01b
serine	0.65 ± 0.02d	0.48 ± 0.02c	0.33 ± 0.01b	0.19 ± 0.02a	0.20 ± 0.01a	0.24 ± 0.01a
tyrosine	0.22 ± 0.01a	0.20 ± 0.02a	0.20 ± 0.02a	0.35 ± 0.01ab	0.16 ± 0.02a	0.19 ± 0.01a
tryptophan	0.00 ± 0.00a	0.00 ± 0.00a	0.05 ± 0.01b	0.00 ± 0.00a	0.01 ± 0.00ab	0.01 ± 0.00ab
threonine	9.05 ± 0.03d	5.82 ± 0.02b	5.06 ± 0.02ab	4.52 ± 0.03a	4.36 ± 0.03a	5.05 ± 0.03ab
valine	0.87 ± 0.01d	0.68 ± 0.03c	0.53 ± 0.01b	0.40 ± 0.02a	0.35 ± 0.01a	0.42 ± 0.01a
taste characteristics[b]						
bitter	3.51 ± 0.01d	2.71 ± 0.04c	2.53 ± 0.02bc	1.71 ± 0.01a	1.91 ± 0.02ab	2.02 ± 0.01b
MSG-like	3.56 ± 0.02e	2.61 ± 0.04c	1.56 ± 0.00a	2.16 ± 0.03b	2.11 ± 0.01b	1.58 ± 0.02a
sweet	10.7 ± 0.06e	7.70 ± 0.03d	6.55 ± 0.01c	5.59 ± 0.04a	6.13 ± 0.01b	6.30 ± 0.02bc
tasteless	1.13 ± 0.03bc	1.42 ± 0.01c	1.00 ± 0.01b	1.14 ± 0.02bc	0.79 ± 0.01a	0.83 ± 0.04a
total	19.8 ± 0.12c	15.8 ± 0.03b	12.6 ± 0.04a	11.8 ± 0.03a	12.4 ± 0.03a	12.1 ± 0.05a
5′-nucleotides[c]						
5′-AMP	0.88 ± 0.09a	1.49 ± 0.10c	0.92 ± 0.07a	1.11 ± 0.07b	1.28 ± 0.09b	0.87 ± 0.07a
5′-CMP	1.48 ± 0.09a	2.49 ± 0.08d	1.54 ± 0.11a	1.82 ± 0.07b	1.96 ± 0.12b	1.44 ± 0.08a
5′-GMP	0.95 ± 0.09ab	1.41 ± 0.09c	0.92 ± 0.11a	1.03 ± 0.08b	1.14 ± 0.08bc	0.86 ± 0.12a
5′-UMP	0.92 ± 0.11bc	1.45 ± 0.09d	0.81 ± 0.10b	1.00 ± 0.07c	1.07 ± 0.12c	0.61 ± 0.06a
MSG-like 5′-nucleotides[d]	1.83 ± 0.10a	2.90 ± 0.09d	1.84 ± 0.10a	2.14 ± 0.08b	2.42 ± 0.11c	1.73 ± 0.08a
flavor 5′-nucleotides[e]	0.95 ± 0.09ab	1.41 ± 0.09c	0.92 ± 0.11a	1.03 ± 0.08b	1.14 ± 0.08bc	0.86 ± 0.12a

[a]Each value is expressed as mean ± standard deviation ($n = 3$). 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 + Try + Val; Tasteless: Lys + Tyr + GABA. [c]5′-CMP: 5′-sytosine monophosphate; 5′-UMP: 5′-uridine monophosphate; 5;-GMP: 5′-guanosine monophosphate; 5′-AMP: 5′-adenosine monophosphate. [d]MSG-like 5′-nucleotides: 5′-AMP + 5′-GMP + 5′-IMP + 5′-XMP, while 5′-IMP and 5′-XMP was not detected in this study. [e]Flavor 5′-nucleotides defined by Chen (1986): 5′-GMP + 5′-IMP + 5′-XMP, while only 5′-GMP was detected in this study.

mature growth stages. The contents of total free amino acids were in the range of 22.0−27.7 mg/g in the pileus and 11.8−19.8 mg/g in the stipe, which peaked at stage 2 in the former and stage 1 in the latter during the primary developing stages, and both reached steady levels at mature stages. Komata grouped the free amino acids into several classes on the basis of their taste characteristics (Table 4).[24] Asp and Glu acids were MSG-like components, which gave the most mushroom taste (the umami taste or palatable taste).[25] The contents of MSG-like amino acids were in the range of 2.34−4.65 mg/g in the pileus and 1.56−3.56 mg/g in the stipe. The values of sweet amino acids in the pileus (11.0−14.1 mg/g) were higher than those in the stipe (5.59−10.7 mg/g). The MSG-like amino acids and sweet amino acids were considered as the main amino acids in *L. edodes* fruiting body. γ−Aminobutyric acid (GABA) is a biologically active compound, and the presence of GABA in *L. edodes* would be beneficial to humans in addition to their palatable taste and other therapeutic effects.[26,27]

Yang et al. reported that contents of MSG-like taste active amino acids could be divided into three ranges: low (<5 mg/g), middle (5−20 mg/g), and high ranges (>20 mg/g).[11] Our study found that the contents of MSG-like taste active amino acids in the growth of *L. edodes* (1.56−4.65 mg/g) were in the low range, but they were higher than some medicinal mushrooms (0.17−0.50 mg/g) reported by Mau et al.[28] and most commercial mushrooms reported by Yang et al.[11]

Yamaguchi et al. reported that 5′-nucleotides, detected in mushrooms, could contribute to MSG-like taste.[10] Our study identified four 5′-nucleotides (5′-CMP, 5′-AMP, 5′-GMP and 5′-UMP) in the pileus and stipe of *L. edodes* fruiting body (Table 4 and Table 5). As for the MSG-like taste active 5′-nucleotides, their contents were 2.45−5.28 mg/g in the pileus and 1.73−2.90 mg/g in the stipe, and they accounted for nearly

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50% of total 5′-nucleotides contents at each growth stage of pileus and stipe. The contents of 5′-AMP (0.87−2.47 mg/g) and 5′-GMP (0.86−2.81 mg/g) were similar during the whole stages in the pileus and stipe, and their contents were mostly higher in the pileus than those of stipe.

5′-GMP gave the meaty favor, and is also a flavor enhancer, which could induce much umami taste production, and these umami tastes were stronger than MSG.[7] The contents of flavor 5′-nucleotides were 1.34−2.81 mg/g in the pileus and 0.86−1.41 mg/g in the stipe during the growth of *L. edodes* fruiting body in this research (Table 4 and Table 5). It demonstrated a peak at stage 1 in the pileus and stage 2 in the stipe during the primary developing stages, with slightly increasing profiles in the pileus and stable levels in the stipe, respectively, at mature growth stages. Yang[11] reported that contents of flavor 5′-nucleotides could be divided into three ranges: low (<1 mg/g), middle (1−5 mg/g), and high (>5 mg/g), and found the contents of flavor 5′-nucleotides in *L. edodes* (271) and *L. edodes* (Tainung 1) were, respectively, in high range and middle range, whereas the contents of flavor 5′-nucleotides in the pileus of *L. edodes* during whole growth stages in this research were all in the middle range.

Evaluation of *Lentinula edodes* at Different Stages and Parts Using Equivalent Umami Concentration (EUC). Yamaguchi et al. reported that the combination of umami amino acids and umami 5′-nucleotides would synergistically increase the umami taste of mushrooms. Using the equation derived from sensory evaluation,[18] the EUC value of stage 1 in the pileus was the highest among all the *L. edodes* growth stages (Figure 2). However, *L. edodes* fruit bodies were

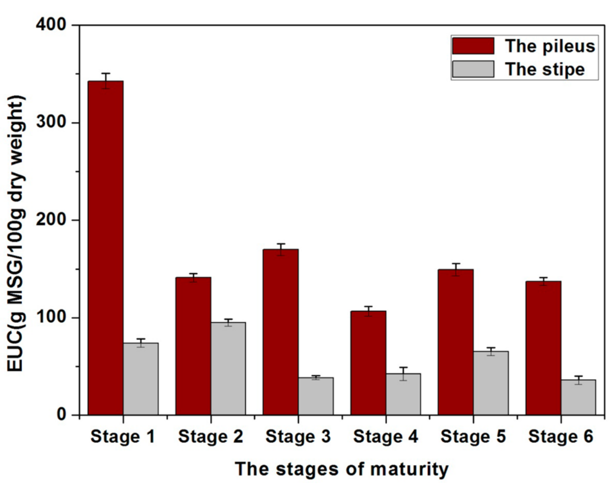

Figure 2. Changes in the equivalent uamami concentration (EUC) of *L. edodes* at different stages of maturity in the pileus and stipe. Each value was expressed as mean ± standard deviation (n = 3).

usually picked at stage 5, which exhibited higher EUC values at mature stage. At stage 6, when *L. edodes* fruit bodies pileus are exposed completely, the fruit bodies became overmatured, and their EUC values decreased. These results were different from those reported by Tsai et al.[29]

Mau reported that the EUC values of flavor components in mushroom were usually classified into four levels: first level of >1000 g MSG/100 g dry weight, second level of 100−1000 g MSG/100 g dry weight, third level of 10−100 g MSG/100 g dry weight, and fourth level of <10 g MSG/100 g dry weight.[30] EUC values of several cultivated mushrooms were at third level (11.19−88.37 g MSG/100g dry weight),[19] and those of several medicinal mushrooms were at the fourth level (0.66−7.92 g MSG/100g dry weight).[30] In this study, EUC values of the pileus (106.8−342.7 g MSG/100 g dry weight) were at the second level, and those of the stipe (35.93−95.11 g MSG/100g dry weight) were at the third level. The EUC values of the pileus were mostly higher than those of *L. edodes* (271), and significantly higher than those of *L. edodes* (Tainung 1).[11] Furthermore, tasty components in the stipe of *L. edodes* were rich and EUC values were higher than other several cultivated mushrooms[15] and medical mushrooms,[30] which might be useful for further processing and byproduct development of *L. edodes*.

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Author Contributions

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Notes

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