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Effect of Mushroom Root Fermentation Broth on the Umami Taste and Nutrients of *Flammulina velutipes*

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Abstract:

As one of the most appealing edible mushrooms, the quality of *Flammulina velutipes* can be affected by the cultivation substrate, which has an impact on the mushroom umami taste and nutrients. In our study, the effect of mushroom root fermentation broth (MRFB) based substrate on umami taste and nutrients of *F. velutipes* were evaluated; four proportions of MRFB was conducted (CK 0%, E1 6.7%, E2 13.4%, E3 20.1%). Results indicated that MRFB was an effective nutrient supplement for mushroom cultivation substrate. E2 and E3 showed higher crude fiber, crude fat, soluble protein, and soluble sugar. Compared to CK, the content of monosodium glutamate-like (MSG-like) amino acids, essential amino acids, total amino acids and total 5'-nucleotides in E2 and E3 were higher. The equivalent umami concentration (EUC) values in E1, E2 and E3 were 1.70, 2.43 and 1.56 times of CK, respectively. Higher umami, saltness and sweetness taste values were found in E2. Thereby, it signified that better umami and richer nutrients were achieved by using substrate with proper volume of MRFB, especially, E2 with 13.4% of MRFB. Overall, better mushroom quality could be derived from the application of MRFB in cultivation. MRFB was a favorable nutrient supplement for *Flammulina velutipes* cultivation substrate.

Keywords: *Flammulina velutipes*; fermentation broth; umami taste; nutrients; electronic tongue; mushroom byproducts

1. Introduction

Flammulina velutipes, also known as enoki, winter mushroom or golden needle

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mushroom [1], is one of the most widely cultivated and consumed edible mushrooms in the world [2–4], and is known for its unique taste, edible and medicinal value [5]. As a terrific source of protein, dietary fiber, and bioactive compounds [6], *F. velutipes* has various medical functions, such as antioxidant, anti-cancer, immunomodulatory, and cholesterol-lowering activities [7–11]. Umami taste is a key factor in evaluating the quality of edible mushrooms and its products. Meanwhile, umami has an effect on consumers' choices of edible mushrooms [12]. The unique umami of edible mushrooms is mainly attributed to glutamic acid, aspartic acid, 5'-nucleotides and their synergistic efficiency [13,14]. Equivalent umami concentration (EUC) and electronic tongue were widely accepted as effective methods in gauging mushroom umami [15–17].

The umami taste of edible mushrooms varies depending on the variety, maturity, part, cultivation conditions, processing, and storage. The cultivation conditions of mushrooms, especially the cultivation substrate, makes a difference in umami taste [18,19]. The growth of mushroom can be affected by the substrate, due to the addition of supplements which contains carbohydrates, vitamins, carbon, and nitrogen [20,21]. The uplift of mushroom production has been achieved by using different substrates [22–25]. The content of 5'-nucleotides was relatively increased by adding soybean flour in the basal media of *Tuber* fermentation mycelia [26]. MSG-like amino acids content in oyster mushroom was higher when cultivated using bamboo sawdust substrate than those originated from conifer sawdust [27].

Similarly, in respect of nutrients quality, substrate types and nutrients in the

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substrate have an effect on the protein content of mushrooms [28,29]. The addition of moringa leaf powder in the cotton waste substrate of king oyster mushroom increased its crude fiber and protein [20]. The protein and fiber content was increased owing to the use of defatted pistachio meal as a nutrient supplement in the cultivation of *Agaricus bisporus* [30]. As crucial qualities of edible mushrooms, various cultivation conditions were adopted to improve their umami and nutrient. However, most researches about mushroom substrate that can improve mushroom quality focus on changing the formula of cultivation materials and adding solid nutrient supplements; a few studies in application of fermentation broth was reported.

Byproducts of *F. velutipes* production industry, including mycorrhizae, aged stipe, residues, spent mushroom substrate, were proven to contain plentiful nutrients such as polysaccharides and proteins [31]. These byproducts were used to prepare mushroom substrate [32], edible film [33,34], various polysaccharides which possess gut microbiota improving and antioxidant activities [35,36]. Waste water from synthesis of biodiesel and biogas digester liquid were utilized for edible mushrooms cultivation [37,38]. Waste fruits (orange, watermelon, apple, etc) were made into garbage enzyme to improve composting and soil remediation [39,40]. This organic solution with abundant enzymes can decompose the organic matter, thus providing better conditions for the microorganisms in composting. Therefore, this study used mushroom root and the fixing water from mushroom blanching procedure to prepare mushroom root fermentation broth (MRFB) by natural fermentation. Subsequently, added to the cultivation materials of *F. velutipes* to study its effect on the umami taste and nutrients.

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Moreover, this work offered a theoretical basis for the valorization of the byproducts of the edible mushroom industry.

2. Materials and Methods

2.1. Mushroom Materials

F. velutipes root and fixing water were mixed and allowed to ferment. Subsequently, the MRFB was blenched with the raw cultivation materials and water. The addition formula of MRFB was shown in the Table 1. The *F. velutipes* cultivation experiment was completed in Shenyang Hengsheng Biotechnology Development Co. Ltd. (Shenyang, China). The *F. velutipes* strain and culture materials used in this experiment were provided by the company uniformly. The cultivation process was carried out strictly in accordance with the technological process (50 d) of the company's factory production of *F. velutipes*. The *F. velutipes* was immediately transported to the laboratory within 2 h following harvest and stored at -40 °C.

2.2. Free Amino Acids Assay

Free amino acids content of the mushrooms was determined by a modified method with some modifications [41]. Each group of fresh samples (5.0 g) was milled adequately, extracted with 30 mL of 0.1 M hydrochloric acid, and the samples were ultrasonic shaken for 30 min at 40 ℃ and 200 W, and then cooled to ambient temperature. The extract was centrifuged at 13,431 g for 15 min, the supernatant (5 mL) was added to sulfonyl salicylic acid (5 mL), placed for 30 min in the dark,

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centrifuged at 13,431 g and 4 ℃ for 15 min. The pH of the supernatant was adjusted to about 2.0 with 10 M sodium hydroxide, and then filtered through a 0.22 μm microporous membrane. The extracted solutions were analyzed by an automatic amino acid analyzer (L 8900; Hitachi Ltd, Japan).

2.3. Assay of 5′-Nucleotides

5′-Nucleotides content was measured using the modified method described earlier [17]. Samples (5.0 g) were ground sufficiently after being added 25 mL of distilled water, kept the suspension boiling for 1 min, cooled and centrifuged at 13,431 g for 30 min. The residues were extracted using 20 mL distilled water in accordance with the procedures above, the combined filtrates were redissolved in distilled water to a total volume of 50 mL, and filtered through a 0.45 μm filter membrane before analysis.

The 5′-nucleotide was analyzed by Waters 1525 HPLC system equipped with UV detector (Waters Corporation, Shanghai, China) and the analysis was performed on a LiChrospher RP-18 column (250mm×4.6 mm, 5 μm, Merck). The mobile phase was 10 mmol L^{-1} KH_2PO_4 (pH = 4.2, adjusted by H_3PO_4). The determinations were completed at a flow rate of 1.0 mL min^{-1}, 254 nm, oven temperature of 30 ℃, with injection volume of 20 μL. Each 5′-nucleotide was identified using the authentic 5′-nucleotide standards (Shanghai Yuanye Bio-Technology Co., Ltd, Shanghai, China) and quantified by the calibration curve of the authentic compound.

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2.4. Equivalent Umami Concentration (EUC)

The equivalent umami concentration signified the concentration of MSG (monosodium glutamate) equivalent to the umami intensity given by a admixture of MSG and 5′-nucleotides, and the calculation was conducted in the light of the following equation [42].

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

Where Y is the EUC of the admixture (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′-guanosine monophosphate (5′-GMP), 5′-inosine monophosphate (5′-IMP), 5′-xanthosine monophosphate (5′-XMP) or 5′-adenosine monophosphate (5′-AMP)]; b_i is the relative umami concentration (RUC) for each umami amino acid with respect to MSG (Glu, 1; Asp, 0.077); b_j is the RUC for umami 5′-nucelotide to 5′-IMP (5′-GMP, 2.3; 5′-IMP, 1.0; 5′-XMP, 0.61 and 5′-AMP, 0.18), and 1218 is a synergistic constant based on the concentration (g/100 g) used.

2.5. Electronic Tongue Measurement

Electronic tongue analysis was accomplished using the Taste-Sensing System SA 402B (Intelligent Sensor Technology, Inc., Kanagawa, Japan), comprising auto-sampler, reference electrodes, and multichannel lipid/polymer membrane electrodes. Mushroom (100.0 g) was sheared and mingled well, samples (5.0 g) were weighed and then blended finely with distilled water in the proportion of one to

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twenty, filtrated for subsequently tests after being placed for 30 min at ambient temperature. Taste values (sourness, astringency, umami, saltness, sweetness, bitterness) were measured after the electric potentials of all membranes had been balanced in standard solutions, each sample was measured for 120 seconds, and the sensors should be rinsed preliminary to each measurement. The averages of the three replicates performed for each sample were worked out [43].

2.6. Nutrients Compositions

Dry weighing bottle was weighed (m_1); 20.00 g of each of the four types of *F. velutipes* was weighed accurately, subsequently, weighed the bottles with the finely minced samples in them (m_2); dried them in an oven at 105°C and atmospheric pressure to constant weight (m_3). Three replicates were performed. Subsequently, moisture content was calculated using the formula below.

$$Moisture=1-(m_3-m_1)/(m_2-m_1)\times100\%$$

The content of crude fiber and crude fat was determined according to the standard AOAC method (2000) and (2002). Soluble protein content (N×4.38) was measured using Bradford method, modified from [44]. Soluble sugar content was quantified using the phenol-sulfuric acid method, with some modifications [45].

2.7. Statistical Analysis

Data were expressed as mean ± standard deviation (SD). The figures were drawn using Origin software 2021, and pearson correlation analysis was performed.

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One-way analysis of variance (ANOVA) followed by Duncan's test were performed using SPSS software (ver. 24.0) and Excel 2010. $P < 0.05$ was considered statistically.

3. Results and Discussion

3.1. Free Amino Acids

Edible mushrooms are considered favorable source of free amino acids, which confer intense umami and pleased sweetness [46]. As indicated in Table 2, 16 sorts of amino acids were identified from all the four groups of mushroom samples. Aspartic and glutamic acids were monosodium glutamate-like (MSG-like) components, which conferred the most quintessential mushroom umami taste [47,48]. Compared with the other amino acids, glutamic acid was higher, significant higher ($p<0.05$) content was observed in E1 and E2 (1.275 mg/g, 1.264 mg/g, respectively, fresh weight) than that in the control group, the concentration in the E3 group decreased gradually to 1 mg/g. Meanwhile, E1 had the lowest aspartic acid content. MSG-like amino acids increased at first and then decreased, had highest content (1.287 mg/g) in E1, which was 1.22 times of CK. Sweet amino acids in our study ranged from 0.934 mg/g to 1.707 mg/g, bitter amino acids (1.002-1.327 mg/g) was higher than MSG-like amino acids (1.015-1.287 mg/g) and tasteless amino acids (0.583-0.699 mg/g). Ala, Gly, Thr (sweet), Asp and Glu (MSG-like) were found to be taste-active amino acids [49], and no bitter components were found to be taste-active in overall taste perception. Hence, the concentration of MSG-like, sweet components and total soluble sugar is high enough to curb and conceal the bitterness arising from bitter substance.

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With regard to total free amino acids and essential amino acids, both of them peaked in E2. The decrease may be attributed to the amino acid catabolism when the need of energy or synthesis of protein remains high. Asp and Glu are synthesized in the transamination reaction, in which Asn and Gln react with oxaloacetate and α-ketoglutarate, and were then hydrolyzed [50]. The addition of MRFB might be beneficial to this synthesis of amino acids. The extension of MSG-like amino acids was also achieved by using different substrate or supplements in previous study [27,51,52].

3.2. 5'-Nucleotides

The umami taste of mushroom is predominantly driven by the 5'-nucleotides. As demonstrated in Figure 1, we detected the 5'-nucleotides. The 5'-nucleotides content of *F. velutipes* declined in the sequence of XMP > GMP > AMP > IMP, which was consistent with the finding of [53]. So far as 5'-GMP, endows edible mushrooms with meaty aroma, a flavor enhancer stronger even than MSG [54], the concentration of three treatment groups was all markedly higher ($p<0.05$) than that in the control group (9.71 mg/kg, fresh weight), and their concentration increased at first and then decreased along with the addition of MRFB increasing (13.14 mg/kg, 19.96 mg/kg, 12.51 mg/kg, respectively). The content of 5'-IMP and 5'-XMP increased constantly along with the addition of MRFB increasing, and had significant ($p<0.05$) differences with each other. However, as an effectual inhibitor of bitterness [55], the concentration of 5'-AMP in the treatment groups was all higher than that of the

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control group. The highest total content of 5'-nucleotides was similarly found in the E2 group, which was 1.88 times of the control group (0.033 mg/g), the treatment groups were markedly higher ($p<0.05$) than that in the control group (0.046 mg/g, 0.062 mg/g, 0.059 mg/g, respectively).

3.3. Equivalent Umami Concentration (EUC)

The synergistic effect between MSG ingredients and flavor 5'-nucleotides may promote mushroom umami taste [42]. EUC was graded into four levels: first level, > 1000 g MSG/100 g (dry weight); second level, 100-1000 g MSG/100 g; third level, 10-100 g MSG/100 g; fourth level, < 10 g MSG/100 g [56]. As shown in Table 3, EUC values significantly ($p<0.05$) increased at first and then decreased along with the addition of MRFB increasing, E2, E1, E3 was 2.43, 1.70, 1.56 times of the lowest value in the control group, respectively. EUC values in our study ranged from 4.48 to 10.90 g MSG/100g (fresh weight), which was 5.09-12.49 g MSG/100g when converted to dry weight. It was classified into the third and fourth level, which was in line with the result of [14]. Therefore, in this study, stronger umami could be presented in the *F. velutipes* cultivated with MRFB.

3.4. Electronic Tongue

Electronic tongue could comprehensively measure the taste parameters of mushrooms, which relatively correlates with human sensory evaluation. As shown in Table 3, six taste values were gauged, including sourness, astringency, umami,

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saltness, sweetness, and bitterness (sourness, astringency, saltness were minus, and the others were positive numbers). In this study, we compared the taste values in the treatment groups with those in the control group, which were the benchmarks. The differences in taste values between all groups of *F. velutipes* samples could be perceived directly (Figure 2). With regard to umami, it was found that E2 had significantly higher ($p<0.05$) umami than that in other groups and 1.22 times of the control group, which was the lowest. Umami increased at first and then decreased, ranging from 9.56 to 11.65, which noteworthy coincided with the tendency of the previously EUC value and the 5'-nucleotides result, consequently, demonstrating that EUC value can finely reflect the variation of the umami in the samples. Corresponding to umami, saltness, and sweetness had the same tendency, peaked at E2. However, E2 had the lowest sourness, astringency, and bitterness, which could be attributed to the suppressive effect of umami on sourness and bitterness. What's more, umami could enhance the saltness and modulate the sweetness [13]. Nevertheless, sweet amino acids were found to show a synergistic effect of umami between sweet amino and 5'-IMP [57]. Generally speaking, E1 and E2 had higher umami, saltness, and sweetness, but lower sourness, astringency, and bitterness were presented. However, the E3 group presented a higher number of all the six taste values than that in the control group. The results of the electronic tongue indicated that better umami taste was conferred by adding MRFB.

On the one hand, the extension of umami and nutrients boils down to the nutrient supplements of MRFB. The nutrients uptake and fruiting body development can be

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promoted by the organic supplements in the substrate [20]. The metabolite products or trace elements in MRFB may also be conducive to improving mushroom quality. On the other hand, the vegetative growth and mushroom formation could be affected by bacteria in the substrate, which can stimulate profitable microbes and inhibit pathogens, thus modulating the microbiota communities [58]. The lettuce growth was promoted by adding microbial agents to the spent mushroom substrate, the bacterial and fungal communities were also enriched [59]. The underlying microbiota communities in MRFB and their metabolism function could be presumed to contribute to the accumulation of umami and nutrients of *F. velutipes*.

However, the excessive addition of MRFB decreased the content of MSG-like amino acids, 5'-nucleotides, EUC value, and umami taste value in E3, which might be attributed to the lower levels of degradation and deficient utilization of excess nutrients in the substrate. Furthermore, the balance of substrate C/N ratio was probably broken by the excess MRFB. Dramatically, the fruiting body induction can be curbed by high nitrogen and carbon substance in the substrate [60]. Thus, the apt addition of MRFB has received better umami and nutrients profiles.

3.5. Nutrients Composition

The results of the nutrients compositions analysis were shown in Figure 3. The moisture in the treatment groups was lower than that in the control group. The crude fiber, crude fat, and soluble protein increased along with the addition of MRFB. However, the soluble sugar content increased first and decreased markedly ($p<0.05$)

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later. Significant ($p<0.05$) difference of crude fiber, soluble protein, and soluble sugar was found in all groups. Consequently, the addition of MRFB can result in an extension to the crude fiber, crude fat, and soluble protein derived from the *F. velutipes* sample, whilst excessive MRFB will reduce the content of soluble sugar, a familiar decrease of sugar content was also found when adding excessive supplements in the mushroom substrate [20]. The nutrients content in our study (moisture, 85.81-88.01%, fresh wight, crude fiber, 6.7-9.6%, crude fat, 2.66-2.96%, soluble protein, 6.77-8.99%, soluble sugar, 5.61-6.51%, converted to dry weight) were lower than that in *F. velutipes* (moisture, 89.06%, crude fiber, 15.99%, crude fat, 8.89%, crude protein, 20.0%, total sugar, 32.5%) [53]. Lower fat (2.1%) and higher protein (23.4%) than our results were reported [61]

3.6. Correlation Analysis

The correlation heat map indicated that the addition of MRFB was positively correlated with crude fiber, soluble protein, sweet amino acids, 5'-IMP, and 5'-XMP (Figure 4). The crude fiber was positively correlated with sweet amino acids and 5'-IMP. 5'-IMP and 5'-XMP showed a positive correlation with soluble protein. Umami taste value was positively correlated with glutamic acid, essential amino acids, and 5'-AMP, which was in line with the previous finding that umami taste value was correlated with 5'-AMP level [17]. A positive correlation was also found between 5'-IMP and sweet amino acids, 5'-AMP and essential amino acids, total 5'-nucleotides and 5'-XMP. The EUC value was markedly correlated with 5'-GMP, which coincided

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with that EUC values reflected the synergistic effect between the MSG-like ingredients and flavor 5'-nucleotides [18]. Nevertheless, 5'-GMP and 5'-IMP are the chief contributors to umami taste [12]. Thus, the utilization of MRFB led to better umami taste.

3.7. PCA Analysis

Principal components analysis was performed using the above umami and nutrients content of four groups with different additions of MRFB. As shown in Figure 5, 49.8% and 33.2% variation were explained by PC1 and PC2, respectively. Apparently, E1, E2, E3, and CK were located in different quadrants (Figure 5A), most of the umami and nutrients indicators were located on the right of the PCA map (Figure 5B). Thus, the four groups were finely characterized by umami and nutrients. The addition of MRFB can ameliorate the quality of *F. velutipes*.

4. Conclusions

MRFB provided more nutrients for mushroom cultivation. E2 and E3 showed higher crude fiber, crude fat, soluble protein, and soluble sugar. Higher content of MSG-like amino acids, essential amino acids, total amino acids, and total 5'-nucleotides in E2 and E3 were found. The treatment groups applying MRFB had higher EUC values than CK. The peak of umami, saltness, and sweetness taste values were detected in E2. Thus, the results indicated that substrate with MRFB conferred stronger umami and higher content of nutrients. In our study, the application of

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MRFB, especial E2 with 13.4% of MRFB, could enhance mushroom quality profiles. Mushroom byproducts provided better conditions for the cultivation of *F. velutipes* as a favorable nutrients supplement.

Author Contributions:

Conceptualization, G.X. and H.X.; Methodology, R.X., Z.H. and S.P.; Validation, X.B.; Formal Analysis, Z.W., Y.L. and Y.F.; Investigation, X.B., R.X. and Z.H.; Resources, H.X.; Data Curation, Z.W. and X.B.; Writing – Original Draft Preparation, Z.W. and H.X.; Writing – Review & Editing, H.X.; Visualization, Z.W., Y.L. and Y.F.; Supervision, G.X. and S.P.; Project Administration, G.X.; Funding Acquisition, G.X. All authors have read and agreed to the published version of the manuscript.

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The authors declare no conflict of interest.

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Figure captions:

Figure 1. Four types of 5'-nucleotides content, total 5'-nucleotides content in four groups of *Flammulina velutipes* samples. Bars with different superscript (a, b, c, d) are significantly different ($p < 0.05$). 5'-GMP, 5'-guanosine monophosphate; 5'-IMP, 5'-inosine monophosphate; 5'-XMP, 5'-xanthosine monophosphate; 5'-AMP, 5'-adenosine monophosphate. Total 5'-nucleotides = GMP + IMP + XMP + AMP.

Figure 2. The relative content of six taste values in four groups of *F. velutipes* samples.

Figure 3. Effect of mushroom root fermentation broth (MRFB) on the nutrients content of *F. velutipes*. Bars with different superscript (a, b, c, d) are significantly different ($p < 0.05$).

Figure 4. Pearson correlation coefficient (*r*) matrix for umami and nutrients quality of *F. velutipes*. * or ** indicate statistically significant correlations at $p < 0.05$ or 0.01, respectively.

Figure 5. Score plot (**A**) and loading plot (**B**) of umami, nutrients and different additions of MRFB.

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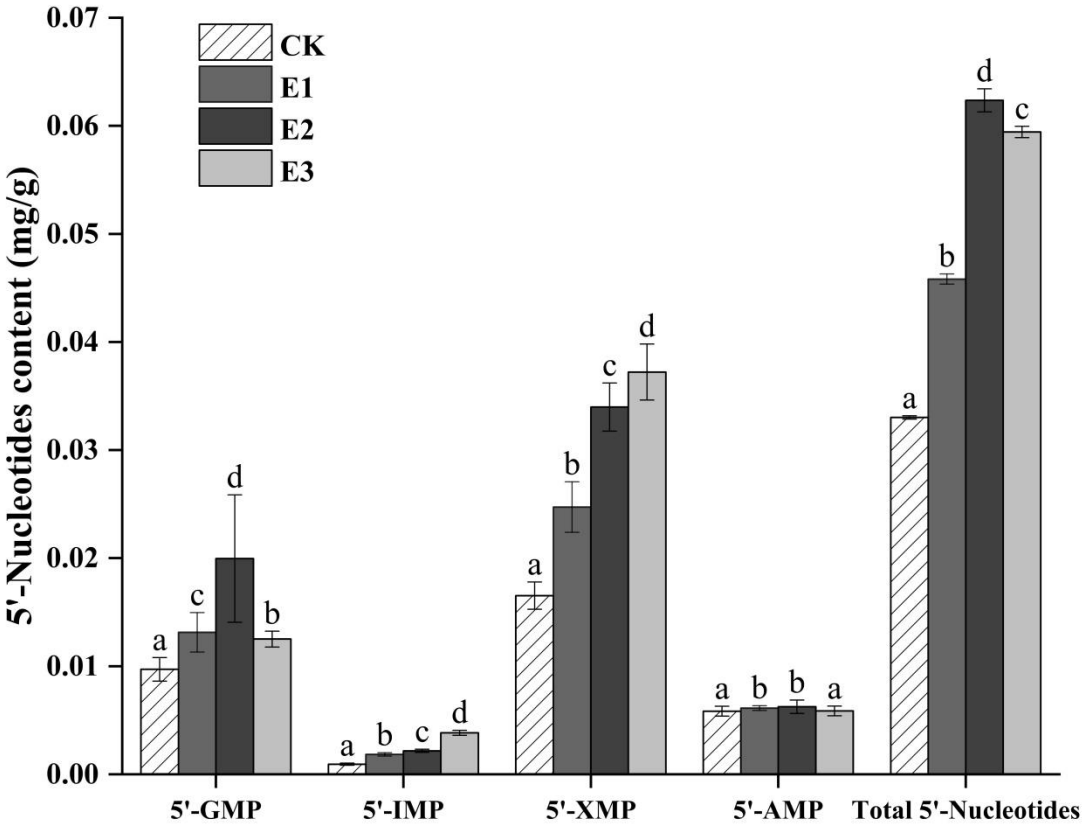

Figure 1

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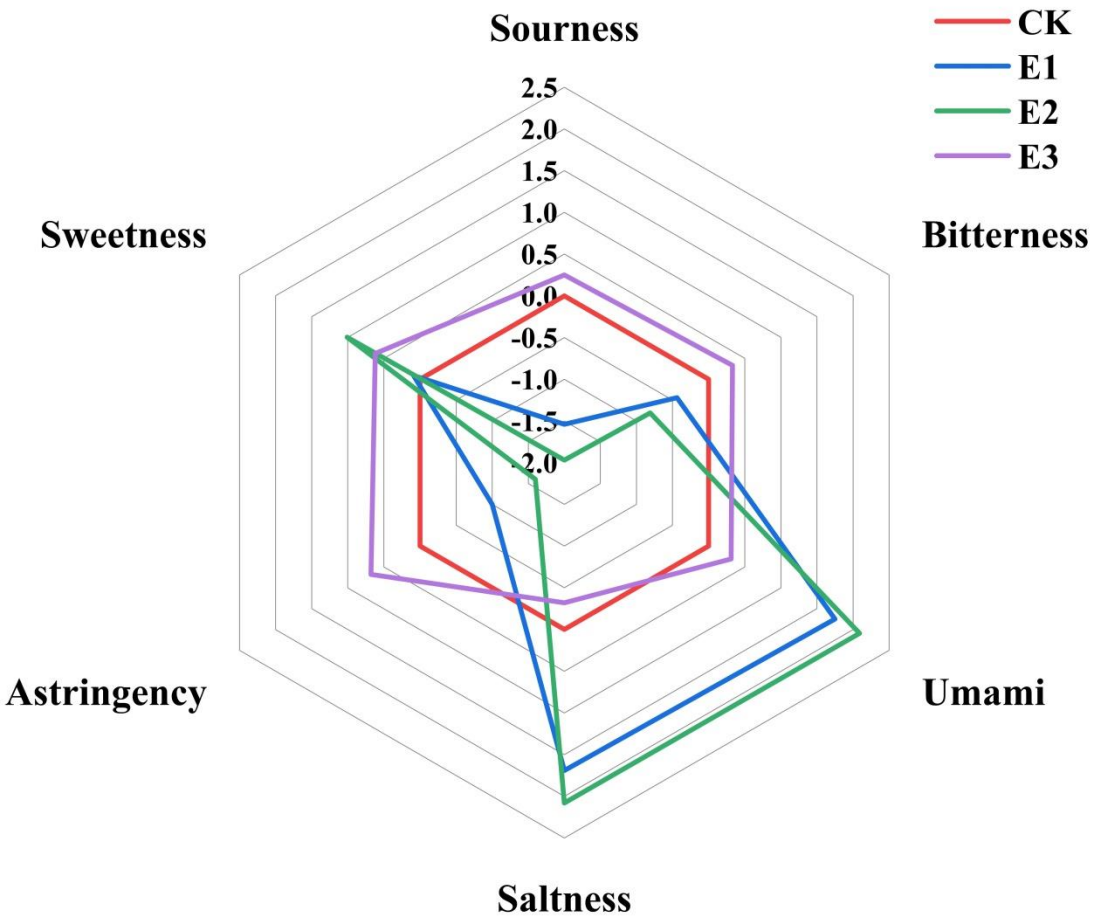

Figure 2

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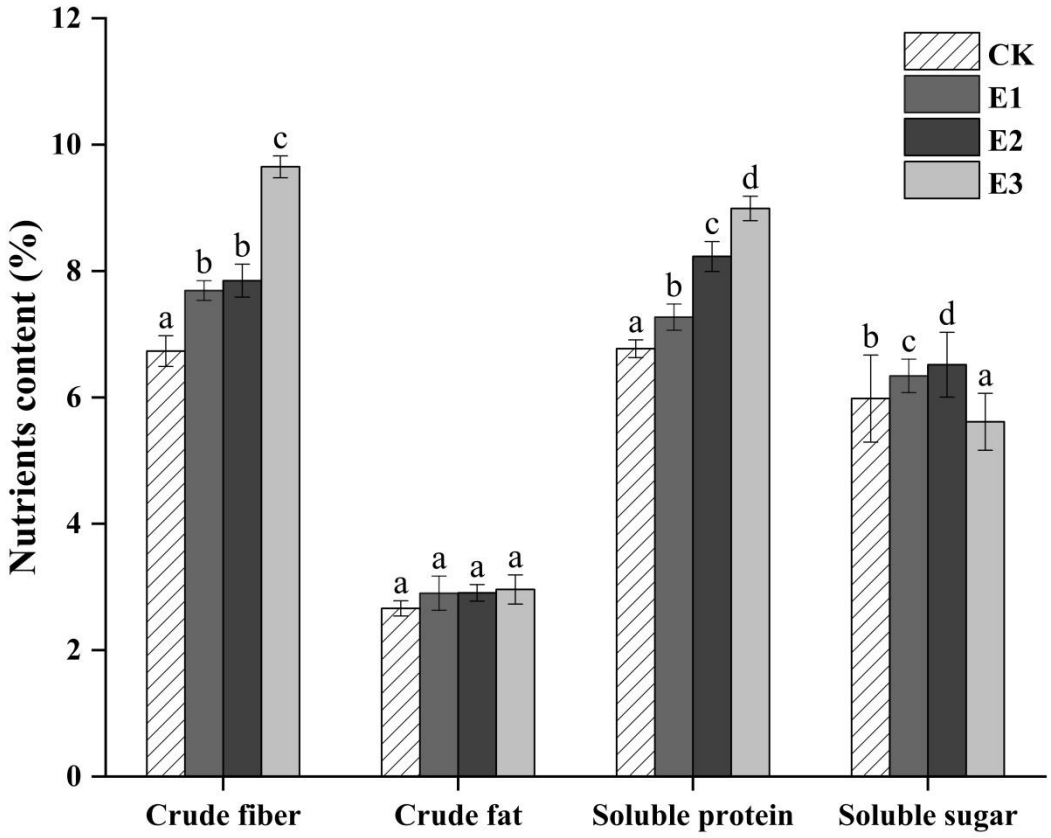

Figure 3

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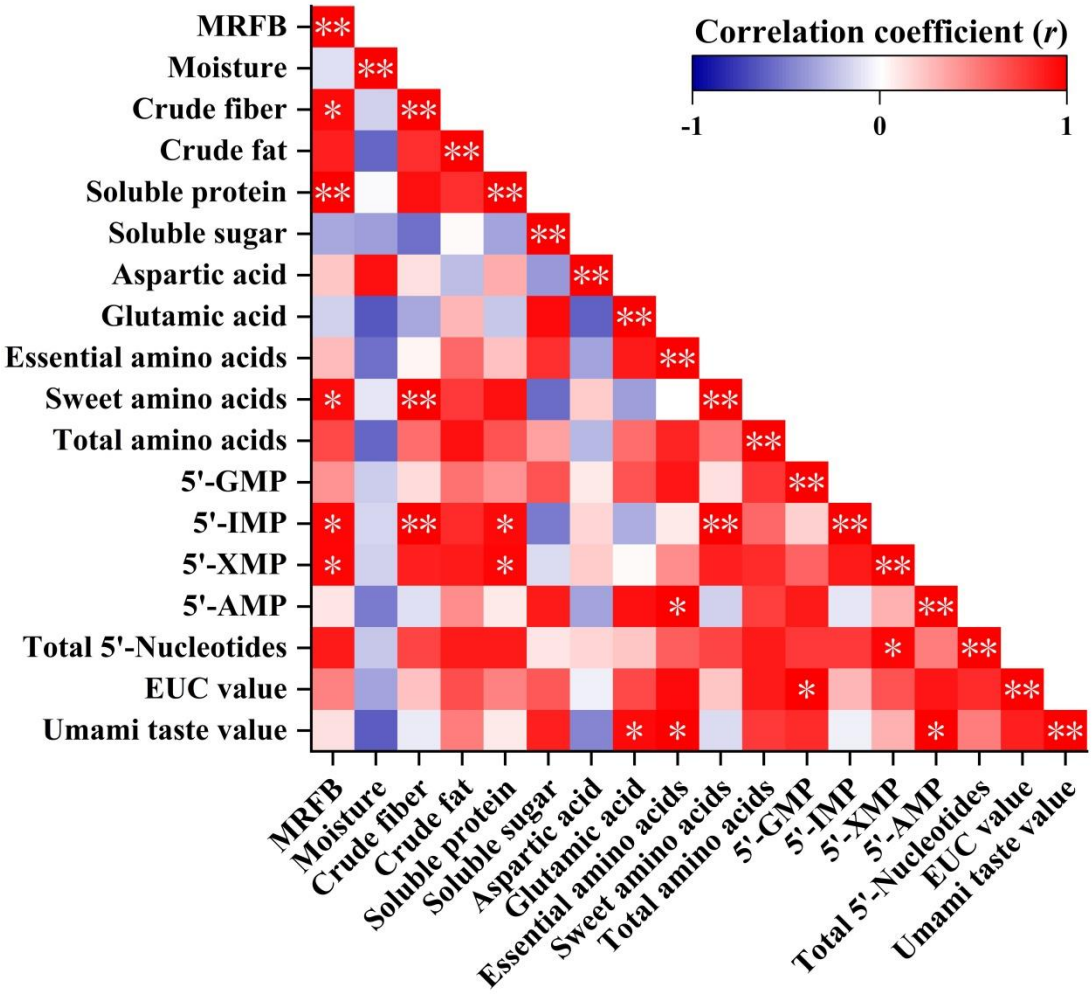

Figure 4

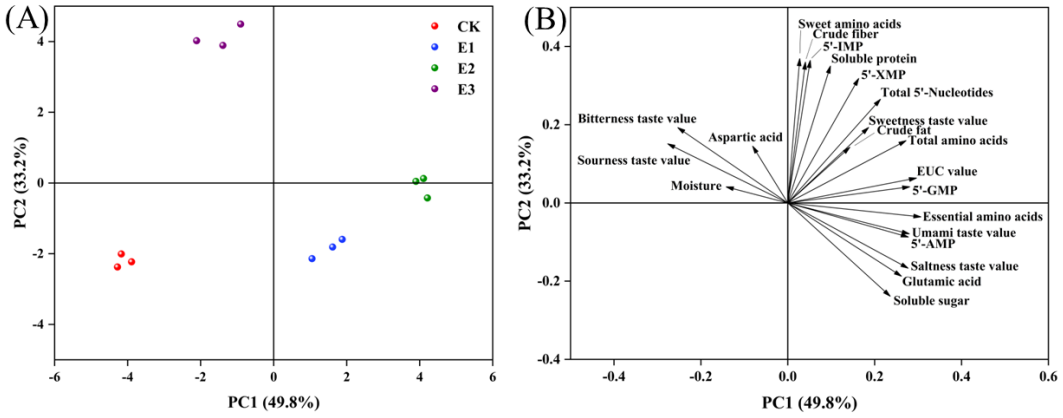

Figure 5

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Table 1. MRFB addition of cultivation substrate formula.

Group	Raw cultivation materials (%)	Water (%)	MRFB (%)
CK	33.0	67.0	0.0
E1	33.0	60.3	6.7
E2	33.0	53.6	13.4
E3	33.0	46.9	20.1

Table 2. Free amino acids levels in four groups of *F. velutipes* samples.

Amino acids	Content (mg/g fresh weight)			
	CK	E1	E2	E3
Asp	0.015±0.001^{b}	0.012±0.001^{a}	0.015±0.001^{b}	0.015±0.000^{b}
Glu	1.044±0.008^{b}	1.275±0.006^{c}	1.264±0.011^{c}	1.000±0.011^{a}
Thr*	0.223±0.006^{a}	0.267±0.006^{b}	0.278 ±0.004^{c}	0.315 ±0.007^{d}
Ser	0.148±0.003^{a}	0.178±0.005^{b}	0.187 ±0.004^{c}	0.215 ±0.004^{d}
Pro	-	-	-	-
Gly	0.084±0.004^{a}	0.105±0.002^{b}	0.113±0.006^{b}	0.204±0.005^{c}
Ala	0.472±0.002^{a}	0.580±0.005^{b}	0.631±0.004^{b}	0.974±0.003^{c}
Val*	0.150±0.003^{a}	0.179±0.005^{b}	0.194±0.003^{c}	0.190±0.003^{c}
Met*	0.026±0.002^{b}	0.037±0.002^{c}	0.037±0.001^{c}	0.023±0.001^{a}
Ile*	0.073±0.002^{a}	0.093±0.001^{c}	0.107±0.003^{d}	0.082±0.002^{b}
Leu*	0.140±0.003^{a}	0.173±0.003^{c}	0.191±0.004^{d}	0.152±0.004^{b}
Phe*	0.333±0.004^{b}	0.389±0.003^{c}	0.447±0.005^{d}	0.298±0.003^{a}
His	0.198±0.004^{b}	0.219±0.001^{c}	0.216±0.003^{c}	0.179±0.005^{a}
Arg	0.095±0.001^{b}	0.135±0.001^{c}	0.135±0.002^{c}	0.079±0.007^{a}
Trp*	-	-	-	-
Tyr	0.203±0.002^{b}	0.242±0.010^{c}	0.251±0.003^{c}	0.168±0.003^{a}
Cys	0.161±0.003^{a}	0.184±0.005^{b}	0.204±0.004^{c}	0.209±0.003^{c}
Lys*	0.246±0.003^{b}	0.269±0.004^{c}	0.243±0.002^{b}	0.207±0.002^{a}
MSG-like	1.059±0.008^{b}	1.287±0.009^{c}	1.279±0.005^{c}	1.015±0.008^{a}
Sweet	0.934±0.007^{a}	1.129±0.009^{b}	1.209±0.002^{c}	1.707±0.016^{d}
Bitter	1.015±0.012^{a}	1.224±0.014^{b}	1.327±0.012^{c}	1.002±0.005^{a}
Tasteless	0.609±0.011^{b}	0.695±0.012^{c}	0.699±0.018^{c}	0.583±0.007^{a}
Necessary amino acids	1.190±0.006^{a}	1.407±0.008^{c}	1.498±0.015^{d}	1.267±0.013^{b}
Total	3.611±0.004^{a}	4.336±0.020^{c}	4.514±0.018^{d}	4.308±0.040^{b}

Means±standard error (n=3). Means in the same row with different superscript (a, b, c, d) are significantly different ($p < 0.05$). MSG-like = Asp + Glu, Sweet = Thr + Ser + Pro + Gly + Ala, Bitter = Val + Met + Ile + Leu + Phe + His + Arg + Trp, Tasteless = Tyr + Cys + Lys. * represents necessary amino acid. Necessary amino acids = Thr + Val + Met + Ile + Leu + Phe + Lys + Trp. - represents not detected.

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Table 3. EUC values and six taste values in four groups of *F. velutipes* samples.

Indicators	Treatments			
	CK	E1	E2	E3
EUC (g MSG/100g)				
EUC	4.48±0.09^{a}	7.62±0.14^{c}	10.90±0.31^{d}	6.97±0.04^{b}
Taste values				
Umami	9.56±0.30^{a}	11.31±0.50^{b}	11.65±0.62^{b}	9.87±0.43^{a}
Saltness	-11.43±0.40^{b}	-9.74±0.03^{c}	-9.35±0.10^{d}	-11.75±0.11^{a}
Sweetness	9.21±0.36^{a}	9.30±0.25^{a}	10.22±0.25^{c}	9.83±0.48^{b}
Sourness	-42.05±0.50^{b}	-43.59±0.20^{a}	-44.02±0.37^{a}	-41.80±0.50^{b}
Astringency	-1.66±0.40^{c}	-2.66±0.13^{b}	-3.26±0.03^{a}	-0.98±0.07^{d}
Bitterness	10.95±0.13^{c}	10.51±0.21^{b}	10.14±0.21^{a}	11.28±0.39^{d}

Means±standard error (n=3). Means in the same row with different superscript (a, b, c, d) are significantly different ($p < 0.05$).