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Evaluation of the nutritional value, umami taste, and volatile organic compounds of *Hypsizygus marmoreus* by simulated salivary digestion in vitro

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ABSTRACT

Hypsizygus marmoreus is an edible medicinal mushroom species with a high dietary value. The main purpose of this study was to evaluate the nutritional value, umami taste, and volatile organic compounds (VOCs) of *H. marmoreus* treated with hot water combined with simulated salivary digestion in vitro. Seafood mushroom (Hm3) had the highest content of moisture, soluble polysaccharides, soluble proteins, and total flavonoids while white *Hypsizygus marmoreus* (Hm1) had the highest total phenolic content. Moreover, Hm1 had a more noticeable equivalent umami concentration (EUC) value, indicating the umami properties of Hm1 as a food or processing ingredient. Results from E-nose and HS-SPME-GC-MS revealed that the VOCs of Hm1 and brown *Hypsizygus marmoreus* (Hm2) were relatively similar, which differed substantially from Hm3. Among the 134 VOCs, 24 differential metabolites were identified by OPLS-DA analysis, characterized by VIP > 1, p-value < 0.05, and FC > 2 (pairwise comparisons). Furthermore, 10 biomarkers with VIP > 1 and p-value < 0.05 were identified by PLS-DA analysis based on the total differential metabolites to distinguish different strains of *H. marmoreus*. These results will benefit future research on the chemistry of *H. marmoreus* and serve as a guide for breeding, introducing, and using the species more effectively.

1. Introduction

Hypsizygus marmoreus (Peck) H.E. Bigelow are widely recognized as three different strains: white *Hypsizygus marmoreus* (Hm1), brown *Hypsizygus marmoreus* (Hm2), and "seafood mushroom" (Hm3). Hm1 and Hm3 are both white strains, but Hm3 has a longer pileus than Hm1; Hm2 is a brown strain. Very similar in appearance leading to their frequent confusion. *H. marmoreus* has been utilized as a common food in China, Japan and North America due to its excellent flavor, taste, and bioactive components. (Hu et al., 2021). *H. marmoreus* has a high concentration of bioactive components, including polysaccharides, proteins, phenolics, and flavonoids, which has been used as a nutritional product for maintaining health and preventing illness (Wang et al., 2022b). For instance, water-soluble extracts (*H. marmoreus*) have been shown by Bao et al. (2011) to suppress the proliferation of several cancer cell lines. From *H. marmoreus*, Oliveira et al. (2019) isolated a novel polysaccharide with potential uses in the treatment of melanoma.

In the modernization process of rapid economic development, consumers' demand for food nutritional value, umami taste, and VOCs cannot be overlooked. Nonvolatile taste elements, particularly free amino acids and 5′-nucleotides, are primarily responsible for the umami taste (Liu et al., 2022; Yang et al., 2022). VOCs are an important indicator of mushroom quality and play an influential role in consumer acceptance. The chemical composition of the mushrooms does not correspond to how the customer perceives. Cooking and salivary digestion altered the umami flavor and VOC levels (Li et al., 2018; Sun et al., 2020). After microwave drying, black tea contains more polyphenols and VOCs, while after far-infrared and hot air drying, it contains more amino acids (Qu et al., 2019). Furthermore, 8-carbon compounds, especially 8-carbon alcohols, are exceedingly sensitive to drying methods (Chen et al., 2017). Studies on mushrooms have often focused on evaluating the nutritional composition, umami taste, or VOCs of

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dried mushrooms (Ayimbila and Keawsompong, 2023; Dimopoulou et al., 2022; Jung et al., 2019). In daily cooking and industrial production, hot water treatment of edible mushrooms is more common. However, only a small amount of study on the hot water treatment of edible mushrooms has been reported. On the other hand, in vitro digestion has been used extensively to explore the variability of non-volatile and VOCs. Studies on in vitro digestion have found higher amounts of total amino acids in *Agaricus bisporus* mushroom (Reis et al., 2020).

Food flavors analysis techniques like headspace solid-phase microextraction-gas chromatography-mass spectrometry (HS-SPME-GC-MS) and electronic nose (E-nose) technologies are frequently utilized. The aroma properties of a variety of foods can be quickly, and objectively evaluated using E-nose (Lu et al., 2022). As comparison, HS-SPME-GC-MS takes a smaller sample than E-nose and is capable of detecting distinct aroma components (Wang et al., 2023).

Therefore, we used food flavors analysis techniques to evaluate the nutritional value, umami taste, and VOCs of different strains of *H. marmoreus* by hot water treatment combined with simulated salivary digestion in vitro. These results highlight a new research direction and support the growth of the *H. marmoreus* industry.

2. Materials and methods

2.1. Materials and sample preparation

The three different mushroom fruit bodys of *H. marmoreus* were obtained from the Freshippo supermarket, Chengdu, China. The hot water treatment methods were implemented as our previously reported (Zhao et al., 2023b). The mushroom fruit bodies were cut into tiny portions, and 30 g samples were crushed after being heated in 750 ml of hot water for 10 min at 90 °C. Subsequently, the solution was allowed to cool to room temperature, and the supernatant was collected for saliva digestion.

The method of simulating salivary digestion was modified from Wu et al. (2021) with some adjustments. Simulated saliva juice consists of potassium thiocyanate (1.45 g), sodium chloride (12.75 g), potassium chloride (6.52 g), sodium bicarbonate (6.16 g), sodium sulphate (4.15 g), sodium dihydrogen phosphate (6.46 g), urea (1.82 g), α-amylase (21.09 mg) and deionized water (100 mL). 20.0 mL supernatant treated with hot water and 20.0 mL 25% simulated salivary juice was incubated at 37 °C. After incubation (5 min) and deactivation (5 min), the solution with the digestion product was obtained. The supernatant of each sample was collected and stored in a −80 °C refrigerator for further analysis. A portion of the samples was utilized to determination the content of the soluble polysaccharides, soluble proteins, total phenolic, and total flavonoid strictly followed the instructions of the kit (Suzhou Grace Biotechnology CO., Ltd., Jiangsu, China).

2.2. Determination of free amino acid assay (FAA)

The amino acid content was determined after pre-column derivatization with o-phthalaldehyde (Hu et al., 2014). After being filtered, 80 μL of each *H. marmoreus* sample was added to 400 μL borate buffer (pH 10.4) and 160 μL derivatization solution. And then incubation at 25 °C (held for 5 min), the samples were instantaneously subjected to analysis on a Shimadzu i-Series Plus LC2030 HPLC system with a 5 μm Shim-pack GIST C18 column (150 * 4.6 mm) (Shimadzu Co., Kyoto, Japan). The extracts were separated at a flow rate of 1.0 ml/min when column chamber maintained at 35 °C. The content of each amino acid was calculated using the calibration curve using a UV detector at 254 nm.

2.3. Determination of 5′-nucleotide assay

The 5′-nucleotides of each *H. marmoreus* sample were separated by a 250 × 4.60 mm C18 column using an isocratic mobile phase of 5% methanol and 95% phosphoric acid solution (0.05%) for 40 min. The extracts were separated at a flow rate of 1.0 ml/min and the content of 5′-nucleotide was calculated using the calibration curve using a UV detector (Shimadzu Co., Kyoto, Japan) at 254 nm.

2.4. Equivalent umami concentration (EUC)

The umami intensity of each *H. marmoreus* sample was represented according to the parameters of the EUC, which was calculated using the concentration of umami amino acids and umami 5′-nucleotide in mushroom (Yang et al., 2022).

$$Y = \Sigma\, a_i b_i + 1218(\Sigma\, a_i b_i)\ (\Sigma\, a_j b_j)$$

where Y is the EUC (g MSG/100g); a_i is Asp or Glu concentration (g/100g); a_j is 5′-IMP, 5′-GMP, 5′-XMP or 5′-AMP concentration (g/100g); b_i is the relative umami concentration of Glu and Asp (Glu, 1; Asp, 0.077); b_j is the relative umami concentration of umami 5′-nucleotide (5′-IMP, 1; 5′-GMP, 2.3; 5′-XMP, 0.61; 5′-AMP, 0.18); and 1218 is a synergistic constant based on the concentration (g/100g) used.

2.5. E-nose analysis

Ten sensors based on various sensing materials have been embedded in the E-nose apparatus (AIRSENSE, Schwerin, Mecklenburg, Germany) (Liu et al., 2020). A sealed beaker (100 mL) containing 20 mL supernatants was used to equilibrate the mixture for 1 h. E-nose sensors were exposed to the VOCs for 80s at a flow rate of 400 ml/min. The data collected by each sensor was then used in more detailed analysis.

2.6. HS-SPME-GC-MS analysis

HS-SPME-GC-MS analysis was carried out following by the reference (Wang et al., 2022a) with some modifications. The supernatant samples (1000 μL) were taken into the 20 mL headspace bottles and internal standard (1 mg/L 1-Hexan-d13-ol) was added. A fused-silica fiber coated with 75 μm carboxen/polydimethylsiloxane (CAR/PDMS) was employed in order to absorb as much VOCs as possible from the sample. Following equilibration at 50 °C, the fibers were exposed to the headspace for 45 min (15 min of shaking time and 30 min of incubation time). Thereafter, the fiber was then taken out of the vial and put straight into the injector of the 7890B/5977B GC-MS for VOC analysis.

The 7890B/5977 B GC/MS (Novogene Co., Ltd, Beijing, China) was operated as follows: Helium was at a constant flow rate (1 ml/min), and temperature was set to 40 °C at the beginning. The temperature was raised from 40 °C to 120 °C at a rate of 4 °C/ min (held for 2 min), and then 5 °C /min to 135 °C (held for 2 min), then 10 °C /min to 180 °C (held for 3 min), and finally increased to 230 °C at a rate of 10 °C /min (held for 8 min). VOCs were separated by a DB-Wax capillary column (30 m × 0.25 mm i.d. × 0.25 μm film thickness). The data were collected in a full-scan acquisition mode (20–400 m/z).

The volatile compounds were identified by comparing the mass spectra data with data from the mass spectral library (NIST 14), with a match of at least 85%. We use the peak area to obtain the relative quantitative value of the compound, and then standardise the relative quantitative result with the peak area of the internal standard, and use this data to represent the quantitative result.

2.7. Statistical analysis

The assays were all carried out in triplicate, and the mean and standard deviation of the data were reported. The SPSS Statistics 25 program (SPSS Inc., Chicago, USA) was used to assess differences with the ANOVA process utilizing Duncan's multiple range tests. For raw peak exacting, baseline filtering, baseline calibration, peak alignment, deconvolution analysis, peak identification, integration, and spectrum matching of the peak region, LECO Corporation's Chroma TOF 4.72.0.0

Table 1
Moisture, soluble polysaccharides, soluble proteins, total phenolic, and total flavonoid content of *Hypsizygus marmoreus* samples.

Component	Hm1	Hm2	Hm3
Moisture (g/100g)	90.12±0.19b	90.16±0.40b	93.20±0.07a
Soluble polysaccharides (mg/g)	288.04±10.51b	210.02±17.54c	344.33±35.33a
Soluble proteins (mg/g)	79.11±3.10b	70.75±4.89b	102.57±7.43a
Total phenolics (mg GAE/g)	12.95±0.09a	7.41±0.60c	9.32±0.95b
Total flavonoids (mg RE/g)	7.98±7.30a	4.14±0.17a	5.98±0.51a

Moisture content was based on fresh weight, while other indicators were based on the dry weight. Means followed by a different letter in the same row are significantly different ($p < 0.05$). Data were represented by the mean ± standard deviation of three independent measurements (n = 3). GAE, gallic acid equivalent; RE, rutin equivalent. Hm1, white *Hypsizygus marmoreus*; Hm2, brown *Hypsizygus marmoreus*; Hm3, seafood mushroom.

Table 2
The free amino acid content of *Hypsizygus marmoreus* samples.

Amino acids		Content (mg/g dry weight)		
		Hm1	Hm2	Hm3
Umami	Aspartic acid (Asp)	2.44±0.05b	6.94±0.07a	2.14±0.03c
	Glutamic acid (Glu)	7.89±0.21a	1.33±0.01c	3.73±0.10b
Sweet	Serine (Ser)	3.62±0.09a	2.74±0.03b	2.31±0.05c
	Glycine (Gly)	1.78±0.05a	0.36±0.00c	0.47±0.01b
	Threonine (Thr)	1.97±0.04b	3.24±0.03a	3.22±0.07a
	Alanine (Ala)	3.54±0.09a	1.25±0.01b	1.17±0.02b
	Proline (Pro)	6.43±0.18b	6.76±0.08b	8.93±0.25a
Bitter	Histidine (His)	1.00±0.02a	0.91±0.01b	0.86±0.01c
	Arginine (Arg)	6.14±0.17a	1.17±0.01b	0.93±0.03c
	Valine (Val)	2.09±0.05a	1.82±0.02b	0.95±0.01c
	Methionine (Met)	0.80±0.02a	0.66±0.00b	0.57±0.01c
	Isoleucine (Ile)	1.80±0.04a	1.69±0.02b	1.65±0.03b
	Leucine (Leu)	2.40±0.07a	1.63±0.02b	1.11±0.03c
	Phenylalanine (Phe)	31.83±0.86a	26.39±0.30c	28.56±0.76b
Tasteless	Cysteine (Cys)	0.58±0.00b	0.53±0.00c	0.99±0.01a
	Tyrosine (Tyr)	1.37±0.03b	1.15±0.01c	1.95±0.04a
	Lysine (Lys)	3.44±0.08a	2.94±0.03b	2.23±0.04c
Total umami		10.33±0.26a	8.27±0.08b	5.87±0.12c
Total sweet		17.34±0.17a	14.34±0.09c	16.1±0.39b
Total bitter		46.06±0.64a	34.27±0.26b	34.64±0.71b
Total tasteless		5.39±0.05a	4.62±0.02c	5.17±0.01b
Total		79.12±0.78a	61.51±0.12b	61.77±0.19b

Umami=Asp+Glu; Sweet=Ser+Gly+Thr+Ala+Pro; Bitter= His+Arg+Val+Met+Ile+Leu+Phe; Tasteless=Cys+Tyr+Lys. Means followed by a different letter in the same row are significantly different ($p < 0.05$). Data were represented by the mean ± standard deviation of three independent measurements (n = 3).

software and Nistdatabase were used. Furthermore, PCA, OPLS-DA, and cluster heatmap analysis were carried out using MetaboAnalyst 5.0 (https://genap.metaboanalyst.ca/).

3. Results

3.1. Proximate compositions

We measured the water content of fresh *H. marmoreus* in order to transform the content of soluble polysaccharide, soluble protein, total phenolic and total flavonoid in the supernatant into the dry weight of the mushrooms. According to Table 1, the moisture content of all three samples exceeded 90%, especially Hm3, which reached 93.2% and was significantly higher than Hm1 and Hm2. The result is consistent with prior research which found that mushrooms' moisture content ranged from 85.37 to 96.05 g/100g (Jacinto-Azevedo et al., 2021).

Mushroom polysaccharides have a variety of biological functions, including maintaining intestinal microecological balance, and anti-inflammatory, antimicrobial, antioxidant, and immunomodulatory activity (Maity et al., 2021; Patel et al., 2021; Roszczyk et al., 2022; Zhao et al., 2023a). All three mushroom species showed significant differences in soluble polysaccharide content, with Hm3 having the highest content (344.33 mg/g), followed by Hm1 (288.04 mg/g) and Hm2 (210.02 mg/g). In the fruit bodies, the total polysaccharide content was 164.84–215.31 mg/g in White *Hypsizygus marmoreus* treated with different drying methods (Wu et al., 2014). Our result showed more soluble polysaccharides than those reported by previous authors because hot water treatment is more likely to lead to polysaccharide solubilisation compared to drying.

Edible mushrooms are a viable option for obtaining high-quality protein, as their production is often faster and cheaper than that of animal and plant proteins. Edible mushrooms contain high amounts of protein (from 19% to 37% DW), have a complete range of essential amino acids, and usually meet the needs of adults (Bach et al., 2017). The soluble protein content ranged from 70.75 mg/g DW (Hm2) to 102.57 mg/g DW (Hm3). In comparison to earlier findings, the soluble protein was lower, for example, the fruit bodies of normal and white *Hypsizigus marmoreus* (196.0 and 210.6 mg/g DW, respectively). A possible reason for this discrepancy could be the protein degradation by the simulated digestion or by the boiling process. (Liu et al., 2021).

Phenolics and flavonoids are considered beneficial antioxidants (Zhong et al., 2022). The total phenolic content, which was represented as milligrams of gallic acid equivalent per gram of dry weight (mg GAE/g DW), was 7.41 for Hm2, 12.95 for Hm1, and 9.32 for Hm3. Previous studies have described similar levels of total phenolic content in edible mushrooms (Bach et al., 2019), while the phenolic content varied from 5.66 to 13.16 mg GAE/g in dried mushrooms. Flavonoids are a class of yellow pigments that contain a C6–C3–C6 backbone (Lewandowska et al., 2016). Numerous studies have reported the presence of flavonoids in mushroom extracts from a variety of species (Pukalski and Latowski, 2022; Wang et al., 2022a). It is noteworthy that the total flavonoid content is nearly fifty percent of the total phenolic content, and that their distribution patterns remain the same, although no significant difference was observed between these three mushrooms.

Table 3
5′-Nucleotides content of *Hypsizygus marmoreus* samples.

5′-Nucleotide	Content (mg/g dry weight)		
	Hm1	Hm2	Hm3
5′-CMP	24.11±0.67b	21.41±0.59c	30.33±0.84a
5′-XMP	2.54±0.07b	2.92±0.09a	2.98±0.08a
5′-UMP	0.42±0.01b	0.06±0.00c	0.49±0.02a
5′-GMP	1.20±0.03b	1.06±0.03c	1.53±0.04a
5′-IMP	0.34±0.01b	0.24±0.01c	0.39±0.01a
5′-AMP	0.44±0.02b	0.45±0.01b	0.86±0.02a
Flavor 5′-nucleotides	4.52±0.13b	4.67±0.06b	5.76±0.06a
total	29.04±0.55b	26.14±0.65c	36.58±0.77a

5′-CMP, 5′-cytosine monophosphate; 5′-XMP, 5′-xanthosine monophosphate; 5′-UMP, 5′-uridine monophosphate; 5′-GMP, 5′-guanosine monophosphate; 5′-IMP, 5′-inosine monophosphate; 5′-AMP, 5′-adenosine monophosphate; Flavor 5′-nucleotides = 5′-GMP + 5′-IMP + 5′-XMP+5′-AMP. Means followed by a different letter in the same row are significantly different ($p < 0.05$). Data were represented by the mean ± standard deviation of three independent measurements (n = 3).

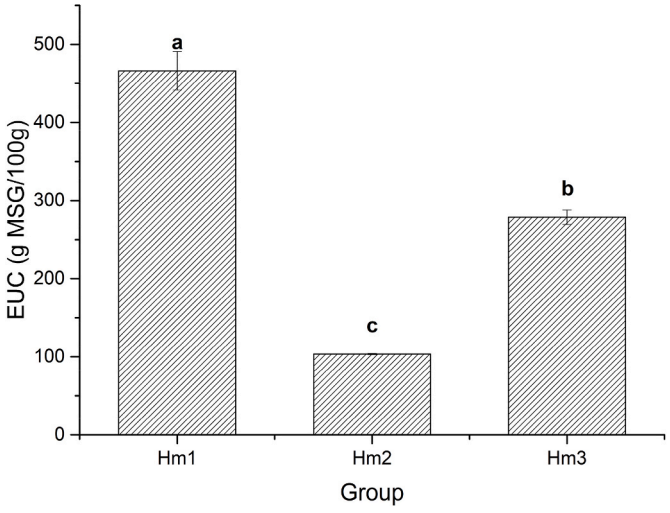

Fig. 1. Equivalent umami concentration (EUC) of *Hypsizygus marmoreus* samples. MSG, monosodium glutamate. Means followed by a different letter are significantly different ($p < 0.05$); MSG, monosodium glutamate.

3.2. Free amino acid

The total free amino acid contents (Table 2) in the three types of *H. marmoreus* ranged from 61.51 to 79.12 mg/g, with significantly higher levels observed in Hm1 compared to Hm2 and Hm3. In this investigation, the total free amino acid content was a little bit greater than the similar values published by Gao et al. (2021), whose research demonstrated that different drying methods (hot air drying, vacuum drying, microwave-vacuum drying, and vacuum freeze-drying) impacted the free amino acid levels. Serine, glycine, threonine, alanine, histidine, valine, methionine, isoleucine, leucine, cysteine, tyrosine, and lysine did not surpass 4 mg/g, while phenylalanine exceeded 26.39 mg/g (reaching up to 31.83 mg/g in Hm1) in all the samples.

Based on their flavor properties, the amino acids in edible mushrooms are divided into numerous classes. Only aspartic acid and glutamic acid, two free amino acids, are involved in the MSG-like compounds that give mushrooms their most well-known flavor, the umami or pleasant taste. Prior research by Yang et al. (2001) classified MSG-like amino acids into three levels: low (5 mg/g), intermediate (5–20 mg/g), and high (>20 mg/g). The medium level was represented by the MSG-like concentrations in this research (5.87–10.33 mg/g). However, the sweet component content (Hm1 > Hm3 > Hm2) ranged from 14.34 to 17.34 mg/g. The sweet component concentration (16.10 mg/g), in particular for Hm3, was nearly three times more than the overall umami (5.87 mg/g). The MSG-like and sweet ingredients are also in charge of giving these three strains of *H. marmoreus* their typical taste.

3.3. 5′-Nucleotides

According to a study by Davila et al. (2022), four of the six 5′-nucleotides often present in mushrooms, 5′-AMP, 5′-IMP, 5′-GMP and 5′-XMP, contribute to the umami taste. According to Table 3, Hm2 had the lowest concentrations of 5′-CMP, 5′-UMP, 5′-GMP, and 5′-IMP, whereas Hm3 had the greatest concentrations. According to Yang et al. (2001), the flavor nucleotides were divided into three ranges: low (1 mg/g), medium (1–5 mg/g), and high (>5 mg/g). Table 3 shows that the flavor 5′-nucleotide concentrations in these three *H. marmoreus* strains ranged from 4.52 to 5.76 mg/g (Hm3 > Hm2 > Hm1). Hm1 and Hm2 had moderate amounts of flavor 5′-nucleotides, but Hm3 had a high amount. This result was in line with research that found mushrooms umami-enhancing 5′-nucleotides (0.38–13.88 mg/g DW) (Chen et al., 2015). Following are the total nucleotide levels: Hm3 < Hm1 < Hm2. A comparatively considerable quantity of 5′-nucleotides was discovered when comparing this study to others that examined the effects of different drying procedures on umami components (Liu et al., 2022). This appears to be because nucleotides are more likely to breakdown during heat processing (Hu et al., 2020).

3.4. Equivalent umami concentration (EUC)

The EUC values ranged from 103.48 to 466.06 mg/g in descending order of Hm1 > Hm3 > Hm2 (Fig. 1). Compared with other mushroom fruit bodies, the EUC values of *Cordyceps flower*, *Lentinus edodes*, *Agrocybe aegerita*, *Pleurotus eryngii*, and *Hericium erinaceus* were 1317.72, 891.42, 314.19, 156.84, and 37.7 g MSG/100 g dry weight, respectively (Yang et al., 2022). Therefore, the EUC value of Hm1 was higher than that of *Agrocybe aegerita*, *Pleurotus eryngii*, and *Hericium erinaceus*. In addition, Mau (2005) grouped the EUC values into four levels: first level

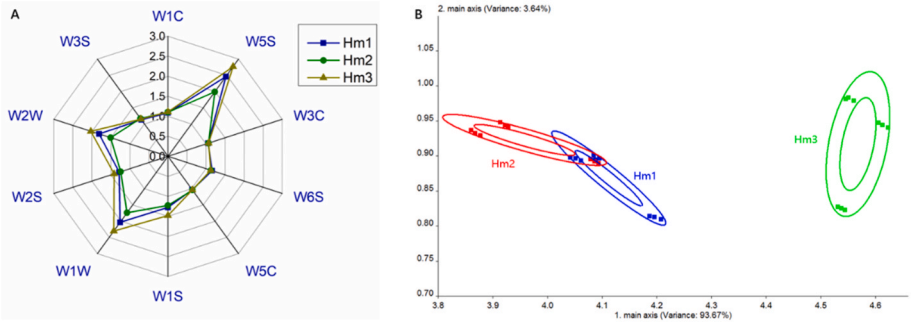

Fig. 2. Radar chart (A) and PCA score plots (B) of the E-nose response data of *Hypsizygus marmoreus* samples.

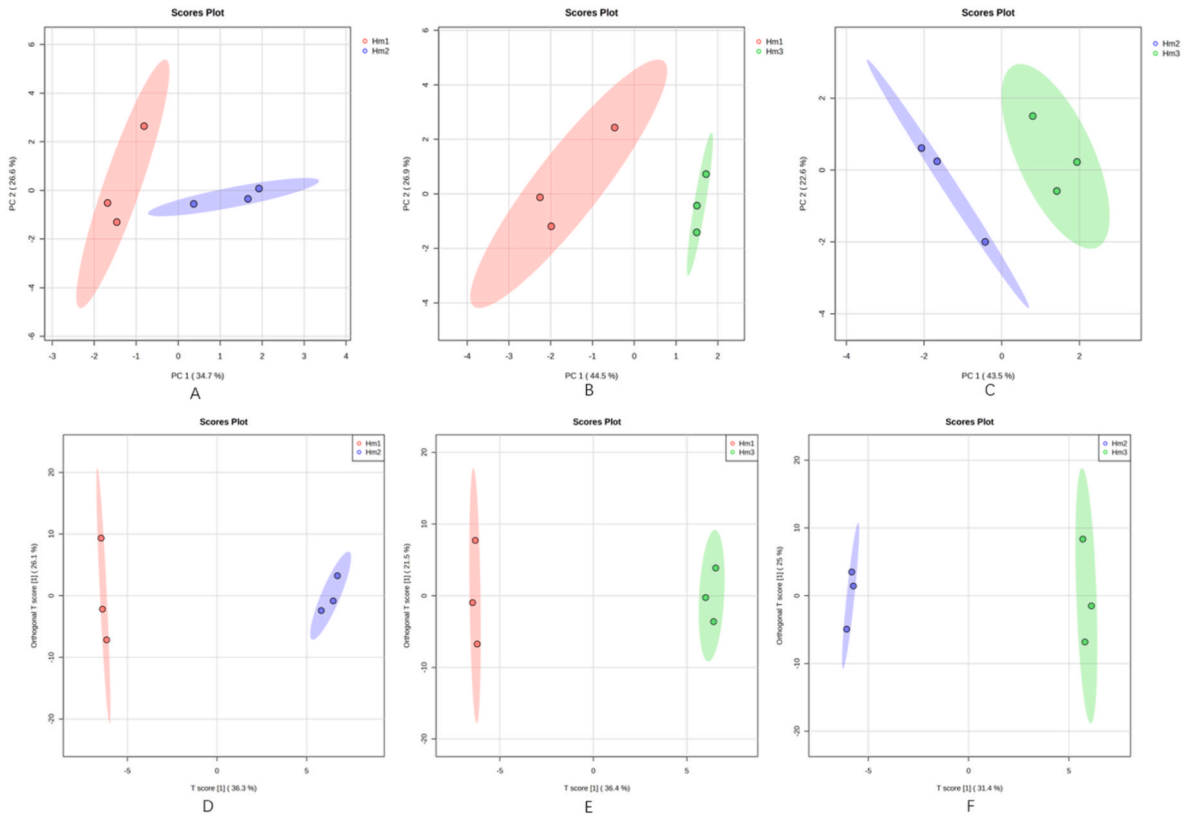

Fig. 3. PCA (A–C) and OPLS-DA (D–F) score plots of *Hypsizygus marmoreus* samples (based on total volatile metabolites identified by GC-MS date).

Table 4
Statistical table of specific differential metabolites.

Group	Volatiles compounds	P-value	VIP	FC	Up. Down
Hm1 vs. Hm2	3-methylnonane	0.00	1.48	0.44	down
	4-methyl-2-pentanol	0.01	1.40	2.20	up
	hexanal	0.02	1.44	2.18	up
Hm1 vs. Hm3	styrene	0.00	1.16	0.47	down
	2-methyl-1-propanol	0.03	1.46	0.40	down
	tetrahydro-2,2,5,5-tetramethylfuran	0.02	3.01	7.62	up
	phenylethyl alcohol	0.04	1.68	0.35	down
Hm2 vs. Hm3	2,2,4-trimethyl-1,3-dioxolane	0.03	1.60	0.40	down
	undecanal	0.01	1.35	0.45	down

Hm1 vs. Hm2 indicates that Hm1 is the experimental group and Hm2 is the control group. Up means that the expression of the corresponding volatile substance in the experimental group is greater than in the control group. Down means that the expression of the corresponding volatile substance in the experimental group is less than in the control group.

(>1000 g MSG/100 g), second level (100–1000 g MSG/100 g), third level (10–100 g MSG/100 g), and fourth level (<10 g MSG/100 g). The three *H. marmoreus* all had second-level EUC values and may be employed as food or food flavoring components since they had a delectable umami flavor.

3.5. Electronic nose

A sensitive strategy for analyzing odor data is provided by the E-nose. For each sensor, the 72s values during the 80s sampling period were noted and utilized for result visualization.

In Fig. 2 (A), the radar chart for VOCs is displayed. All three samples received positive response values from the sensors W5S, W1S, W1W, W2S, and W2W, with the comparative data of Hm3 being substantially greater than those of Hm1 and Hm2. In these three samples, the reaction value of Hm1 and Hm2 are comparable.

PCA was employed to decrease the dimensionality of the data since the multidimensional data gathered by the E-nose could not visually observe the odor variations. The PCA score graphs are shown in Fig. 2 (B). The first and second principal components (PCs) had variance contribution rates of 93.67% and 3.64%, respectively, suggesting that they included the most VOC data. The PC1 values were Hm2, Hm1, and Hm3, in that order. The PCA plots were partially placed in the same region and the discriminating index between Hm1 and Hm2 was 51.3%, showing the closeness of the VOCs in Hm1 and Hm2. Meanwhile, the discrimination index between Hm3 and Hm1, Hm2 was very high (95.9% and 95.0%, respectively) and they were located in different areas, indicating a noticeable difference in VOCs.

3.6. Multivariate statistical analysis

Following HS-SPME-GC-MS analysis, 133 VOCs were recorded in the samples of *Hypsizygus marmoreus* by simulated salivary digestion in vitro. The comprehensive data on these discovered VOCs is condensed in Table S1. The processed metabolite assets were subjected to multivariate statistical analysis for additional analysis to reduce the complexity of the

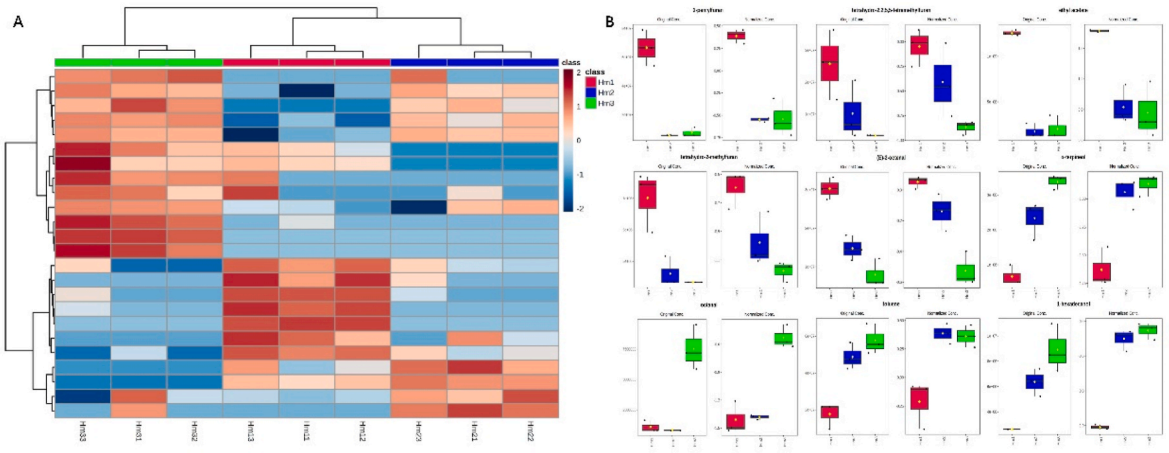

Fig. 4. Cluster heatmap analysis (A) and box plots (B) of aroma substances with VIP values > 1 obtained from the PLS-DA model (based on total differential VOCs).

data and enhance its interpretability and validity.

The PCA score plot (Fig. 3, A-C) displayed the distribution of the two main components, defining the overall variance contribution rate (Hm1-Hm2: 34.7% and 26.6%; Hm1-Hm3: 44.5% and 26.9%; Hm2-Hm3: 43.5% and 22.6%). Furthermore, the simulated salivary digestion samples of Hm1, Hm2 and Hm3 could be easily distinguished from one another, particularly using the first principal component.

In general, PCA is unable to explain the precise distinctions between samples (Ai et al., 2021). As a result, the OPLS-DA model was used to execute supervised classification in order to track metabolites. R^2Y and Q^2 in the OPLS-DA model can be used to assess the model's predictability and dependability, respectively. The values of these indices should ideally be as close to 1 as possible, however in reality, $Q^2 > 0.50$ is taken as a sign that the existing OPLS-DA models are capable of making accurate predictions (Becerra-Martinez et al., 2017). Fig. 3 (D-F) displays the score-plot result of the OPLS-DA, which met these desirable specifications (Hm1-Hm2: $R^2Y = 0.957$, $Q^2 = 0.76$; Hm1-Hm3: $R^2Y = 0.979$, $Q^2 = 0.774$; Hm2-Hm3: $R^2Y = 0.983$, $Q^2 = 0.643$). The high R^2Y values suggested that the model had been fitted, whereas the high Q^2 value indicated that the model has a high predictive potential. Hence, the OPLS-DA model was successful and accurate, implying that it can be utilized to investigate metabolic differences.

3.7. Differential metabolite analysis

In OPLS-DA analysis, the variable importance projection (VIP) values are regarded as relevant parameters to assess the degree of intensity and ability to comprehend of the expression pattern between groups. A higher VIP score implies that the variable contributed more to the grouping. The T-test *p*-value is used to determine the likelihood of a difference between groups. Furthermore, the fold change (FC) value is regarded as an additional signal of the difference in the relative expression of metabolites between the two groups. Furthermore, differential metabolites were identified using an OPLS-DA model PC1 with a VIP value > 1, *p*-value < 0.05, and FC > 2 (or FC < 0.5). Overall, 12 differential metabolites (8 up-regulated and 4 downregulated) were identified between Hm1 and Hm2, with 16 differential metabolites (7 up-regulated and 9 down-regulated) between Hm1 and Hm3, and with 7 differential metabolites (1 upregulated and 6 down-regulated) between Hm2 and Hm3. In order to identify potential VOCs that could be used to discriminate *Hypsizygus marmoreus* by simulated salivary digestion in vitro, the specific differential metabolites were screened out of the three groups (pairwise comparison). Table 4 shows the specific differential metabolites of the samples by simulated salivary digestion in vitro. Between Hm1 and Hm2, three distinct differential metabolites were found (2 up-regulated: 4-methyl-2-pentanol, hexanal; 1 down-regulated: 3-methyl-nonane). In addition, four unique differential metabolites (1 up-regulated: tetrahydro-2,2,5,5-tetramethyl-furan; 3 downregulated: styrene, 2-methyl-1-propanol, phenylethyl alcohol) were also identified between Hm1 and Hm3, and two unique differential metabolites (2 downregulated: 2,2,4-trimethyl-1,3-dioxolane, undecanal) were identified between Hm2 and Hm3.

Meanwhile, a cluster heatmap analysis was carried out to examine the diversity of metabolite profiles in these three distinct samples using the complete differential VOCs data set (Table S2). Blue to red hues in Fig. 4A denote low to high expression of metabolites, respectively. Hm1 and Hm2 clustered together and were clearly separated from Hm3, indicating that the VOCs of Hm1 and Hm2 are relatively close, which is consistent with the E-nose PCA results. Fig. 4B displays the box plots of the 9 differential metabolites with VIP values >1 obtained from the PLS-DA model in the total differential volatile data set. There was a significant variation between these three samples for the 9 key biomarkers. The relative contents of 2-pentylfuran (odor: green bean, butter), tetrahydro-2,2,5,5-tetramethylfuran, ethyl acetate (odor: ethereal, fruity, sweet, weedy, green), tetrahydro-2-methylfuran, and (E)-2-octenal (odor: green, nut, fat) were higher in Hm1. Moreover, the relative contents of α-terpineol (odor: oil, anise, mint), octanal (odor: fat, soap, lemon, green), toluene (odor: sweet) and 1-hexadecanol (odor: wax, flower) were significantly higher in Hm3 than in other groups.

Hm1 is a white variant of Hm2 and has a lower bioavailability than Hm2. In most cases, Hm1 and Hm2 are cultivated in bottles, while Hm3 is cultivated in bags. Edible mushrooms cultivated in bags promote stipe elongation due to the high concentration of carbon dioxide that accumulates at the top of the bags. Differences in their own cultivation characteristics, cultivation modes, and cultivation environments lead to such a big difference in substantial volatile or non-volatile compounds.

4. Conclusion

This study was the first to explore the nutritional value, umami taste, and VOCs of *H. marmoreus* by applying hot water treatment combined with simulated salivary digestion in vitro. Among the three mushroom strains, Hm3 had the highest moisture, soluble polysaccharide, soluble protein, and total flavonoid content, while Hm1 had the highest total phenolic content. In contrast, Hm1 had a significantly higher EUC value, almost five times that of Hm2, indicating that the hot water treatment combined with simulated salivary digestion resulted in a higher umami content in Hm1. A total of 124 metabolites were identified, with 24 differential metabolites revealed by OPLS-DA (pairwise comparisons). Cluster heatmap analysis of the total differential metabolites revealed that Hm1 and Hm2 were clustered together, indicating that the VOCs of Hm1 and Hm2 were relatively close, which was consistent with the

results of PCA analysis of the E-nose. In addition, ten nine biomarkers (2-pentylfuran, tetrahydro-2,2,5,5-tetramethylfuran, ethyl acetate, tetrahydro-2-methylfuran, (E)-2-octenal, α-terpineol, octanal, 1-hexadecanol, and toluene) could be used to distinguish between these three *H. marmoreus* strains after hot water treatment combined with simulated salivary digestion in vitro. The present study revealed the differences in nutritional value, umami taste, and VOCs of different *H. marmoreus* species, which provides a theoretical basis for future research and offers new insights into the potential applications of *H. marmoreus* as a natural source for the food industry.

CRediT authorship contribution statement

Jin Zhao: Methodology, Writing – original draft. **Junbin Lin:** Investigation. **Junjie Yan:** Visualization, Conceptualization. **Chen Zhang:** Data curation. **Tao Wang:** Writing – review & editing. **Bingcheng Gan:** Project administration, Funding acquisition.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

Data will be made available on request.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.crfs.2023.100591.

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