Chemical Compositions and Volatile Compounds of *Tricholoma matsutake* from Different Geographical Areas at Different Stages of Maturity

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Abstract *Tricholoma matsutake* is a commercially important edible fungus. Volatile compounds, chemical compositions, and nutritional values of fruiting bodies at different stages of maturity from different geographical areas were analyzed. The main volatile compounds in *T. matsutake* fruiting bodies were (E)-2-octenal, phenylacetaldehyde, 3-octanone, methyl cinnamate, benzaldehyde, and 1-octen-3-ol. Kinds and levels of volatile compounds from different geographical areas varied. As the fruiting bodies aged, levels of methyl cinnamate and 1-octen-3-ol gradually declined. Potassium was the most abundant element in *T. matsutake* fruiting bodies. Of 17 amino acids detected in fruiting bodies, glutamate was the most abundant. Volatile compounds, chemical compositions, and nutritional values of *T. matsutake* varied with age and geographical origin and can serve as chemical indicators for classification of *T. matsutake* from different geographical areas and at different stages of maturity.

Keywords: *Tricholoma matsutake*, volatile, GC-MS, geographical area, maturity

Introduction

Tricholoma matsutake is an ectomycorrhizal fungus that grows in a symbiotic relationship with *Pinus densata* (1–2). The *T. matsutake* fruiting body is commercially important in Asia and northern Europe due to medicinal effects and an attractive flavor (3–6). Young *T. matsutake* fruiting bodies with an unopened pileus are especially valuable in Asia. Natural production of *T. matsutake* is limited. Growth requirements of symbiotic fungi are complicated and artificial cultivation of *T. matsutake* has not been successful (7–9).

The main production area of *T. matsutake* in China is Sichuan Province where the mushroom grows in the Hengduan Mountains at altitudes above 2,500 meters. Geographical isolation is linked with genetic variation and differences in nutritive value and fragrance (1). Volatile fungal compounds have been used as chemical indicators for specific identification and taxonomic classification (10,11). Qualities and quantities of volatile compounds affect sensory characteristics of analyzed fungal species (12–13). Characteristic aroma-active compounds in raw and cooked *T. matsutake* and differences between the pileus and stipe of fruiting bodies have been investigated (14,15). Volatile compounds from *T. matsutake* of a young age with an unopened

pileus that are preferred as food differ from characteristics of mature fruiting bodies with opened pilei. Also, assessment of volatile compounds of *T. matsutake* from different geographical origins can help in understanding effects of environmental conditions on *T. matsutake*, and provide a scientific basis for industrial production.

Materials and Methods

Mushroom collection Three thousand g of fruiting bodies were collected from Dechang, Daofu, and Maerkang Counties in Sichuan, China in July of 2014 (Appendix 1). Fruiting bodies were sorted into 3 maturity categories of stage 1, egg and bell shaped; stage 2, volva broken or stipe elongated; and stage 3, cap opened (Fig. 1). Fruiting bodies from the same geographical area at the same stage of maturity were mixed and analyzed together. Mushroom samples were cleaned of forest debris, wrapped in aluminum foil, and maintained at 4°C prior to analysis. Mushroom samples were analyzed within 24 h. Three carpophores of each species were analyzed. Representative voucher specimens were deposited in the herbarium of the Sichuan Academy of Agricultural Sciences (DC1-3,

Appendix 1. *Tricholoma matsutake* sampling sites in Sichuan, China

	Dechang	Daofu	Maerkang
Longitude (E)	102°07'21"	101°39'15"	101°48'36"
Latitude (N)	27°23'48"	30°28'53"	32°07'53"
Altitude (m)	2490	3740	3390
Vegetation	Pine and oak	Alpine oak	Alpine oak

Fig. 1. Fruiting bodies of *T. matsutake* at different stages of maturity. 1: stage 1, egg shaped or bell shaped; 2: stage 2, volva broken or stipe elongated; 3: stage 3, cap opened.

DF1-3, MEK1-3) (Chengdu, Sichuan, China).

Mineral elements assay Potassium (K), Magnesium (Mg), Iron (Fe), Zinc (Zn), Copper (Cu), Manganese (Mn), Calcium (Ca), Cadmium (Cd), and Lead (Pb) contents of mushroom samples were determined using an atomic absorption spectrophotometer (PerkinElmer Analyst 800; PerkinElmer, Waltham, MA, USA) with a deuterium background corrector (16). Operating parameters for working elements were set as recommended by the manufacturer. All plastic and glassware was cleaned by soaking in a 10% nitric acid solution overnight and rinsing with distilled water prior to use. Elemental standard solutions and HNO_3 used for digestion were supplied by Merck. Lead and cadmium were analyzed using an HGA graphite furnace with argon as an inert gas. The temperature program for analysis of lead and cadmium was drying at 110°C, a second drying at 130°C, pyrolysis at 450°C, atomization at 2,400°C, and cleaning-out at 2,500°C. Fe, Cu, Zn, Ca, and K contents were determined using a flame technique (17). After determining the ash content using the oven method (18), ash was dissolved in 100 mL of nitric acid and kept in a refrigerator (BCD-321WDJ; Haier, Qindao, China) at 4°C prior to analysis. All analyses were carried out in triplicate. Data were analyzed using Duncan's test with the statistical program MINITAB, Release 13.20 (TechMax Information Technical Co. Ltd., Shanghai, China). Significance was defined as $p < 0.05$.

Major nutrients and amino acid assay Five hundred mg of freeze-dried mushroom tissue was shaken (HNY-100B; Honour Instrument Co. Ltd., Tianjin, China) with 50 mL of 0.1 N HCl for 45 min at 25°C and filtered through Whatman No. 4 filter paper (Whatman,

Maidstone, UK). An aliquot of the filtrate was filtered using a 0.45 μm CA non-sterile filter (Lida), then the purified filtrate was mixed with o-phthalaldehyde (OPA) reagent (Sigma-Aldrich, St. Louis, MO, USA), shaken up and down to facilitate derivatization, and immediately injected into an HPLC apparatus. The HPLC system included an Hitachi F-1050 fluorescence detector with fluorescence excitation at 340 nm and emission at 450 nm, and a 5 ODS-2 column (4.6×250 mm, 5 μm , Phenomenex Inc., Torrance, CA, USA). Mobile phases A, B, and C were 50 mM sodium acetate (pH 5.7) containing 0.5% tetrahydrofuran, deionized water, and methanol, respectively. Mushroom samples were eluted at 1.2 mL min⁻¹ with A:B:C ratios of 83:0:17 to 33:0:67 for 37 min, 0:33:67 for 3 min, and 0:100:0 for 3 min. Each amino acid was quantified based on a calibration curve of an authentic amino acid.

Protein, crude oil, and ash contents were determined according to AOAC procedures on a dry basis (18). The protein content was analyzed following the Kjeldahl method using a conversion factor of 6.25 (19). The crude oil content was determined using Soxhlet extraction with hexane (20). The ash content was analyzed based on incineration of mushroom samples at 550°C. The fiber content was calculated as a residual difference after subtraction of protein, ash, and crude oil contents.

Extraction of volatiles One hundred g of mushroom samples consisting of fruiting bodies from the same geographical area of the same age crushed and mixed together were extracted using 200 mL of dichloromethane, which was redistilled (Beijing Glassware Factory, Beijing, China) before use. After 0.1 mL of 100 ppm dodecanoic acid methyl ester (v/v, in dichloromethane) was added as an internal standard, the solution was agitated using a magnetic stirrer at 400 rpm for 30 min, then filtered through filter paper (No. 41; Whatman) under a vacuum. Volatile components were separated from non-volatiles using high-vacuum sublimation at an operating vacuum less than 2×10^{-5} Torr (4). Extracts were dehydrated over anhydrous sodium sulfate, evaporated on a Vigreux column (50 cm length, 3 cm i.d.) in a water bath at 45°C, then concentrated under a slow stream of nitrogen gas to obtain a final volume of 0.1 mL (21).

GC-MS analysis GC-MS analysis was performed using a 6890N gas chromatography-5975 mass selective detector (Agilent Technologies Inc., Palo Alto, CA, USA), equipped with a DB-5 ms column (30 m length × 0.25 mm i.d. × 0.25 mm film thickness) (J&W Scientific, Folsom, CA, USA) and a nHP 5890 series II GC/5972 MSD (Hewlett-Packard, Palo Alto, CA, USA) equipped with a DB-wax column (30 m length, 0.25 mm i.d., 0.25 mm film thickness). The carrier gas was helium at a constant flow rate of 0.8 mL/min. One μL of mushroom extract was injected in splitless mode. The oven temperature was held at 40°C for 1 min, then increased to 200°C at 4°C min⁻¹, and held at 200°C for 10 min. The injector and detector temperatures were 200 and 250°C, respectively. The mass detector was operated in electron impact mode with an ionization energy of 70 eV and a 33-550 amu scanning

range (22).

Identification of volatiles Volatile components were identified based on comparison of mass spectra and linear retention indices (RIs) with authentic compounds. When standards were not available, compounds were tentatively identified using the Wiley 275 mass spectral database or by manual interpretation. All key odorants were identified based on comparison of mass spectra, RIs, and aroma properties perceived at the sniffing port in comparison with authentic standards. Compound RIs were calculated using the n-alkanes C7–C22 as external references (23). Semi-quantitative analysis of volatile components was performed based on comparison of peak areas to an internal standard compound of 0.1 mL of 100 ppm dodecanoic acid methyl ester in dichloromethane (v/v) on a GC-MS total ion chromatogram.

Statistical analysis All data were presented as means±standard deviation (SD) ($n=3$). An analysis of variance (ANOVA) was performed using the general linear model (GLM) procedure in SPSS (version 10.1, SPSS, Chicago, IL, USA) for evaluation of significant differences at $p<0.05$.

Results and Discussion

Main volatiles in *T. matsutake* Sixty six volatile compounds were identified in *T. matsutake* from 3 geographical areas and 3 stages of maturity (Table 1). Levels of 1-octen-3-ol, methyl cinnamate, 3-octanone, and benzaldehyde were highest. The C8 components 1-octene, (2Z)-2-octene-1-ol, 3-methyl-1-heptene, (E)-2-octenal, 1,3,5,7-cyclooctatetraene, octanal, and phenylacetaldehyde were dominant. Other volatile components, including 3,6-dodecadienoic acid methyl ester, D-limonene, 1,2-benzofulvene, 1-nonanal, and hexaldehyde, were also abundant in *T. matsutake*. C8 components of volatiles are formed in enzymatic reactions involving linoleic acid or linolenic acid (24). Octanal is used commercially as a component in perfumes and in flavor production for the food industry. 3-octanone is a flavor and fragrance ingredient, and phenylacetaldehyde has an antibiotic activity (25). 1-octen-3-ol and methyl cinnamate can repel mycophagous *Proisotoma minuta* to protect the fruiting bodies of *T. matsutake*, serve as a raw material in the pharmaceutical sector, and as a mosquito attractant in mosquito killing products (26). D-limonene has a pharmacological activity as a dietary chemotherapeutic agent and provides a novel therapeutic option for some CNS neoplasms and other solid tumors, particularly for treatment of gliomas (27).

Volatiles in *T. matsutake* different based on geographical origin

Average values of 14, 14, and 16 volatile compounds from a total of 66 different compounds were identified in *T. matsutake* from Dechang, Daofu, and Maerkang Counties, respectively (Table 1). Contents of volatile components of *T. matsutake* from different geographical

areas were different. Bicyclo[4.1.0]hept-2-ene, 2-dodecenal, and 1-hepten-3-one were detected only in *T. matsutake* from Dechang County. 2-isopropyl-2-methyloxirane, 2,6-di-tert-butyl-4-sec-butylphenol, 2,4-nonadienal, (E,E)-2,4-nonadienal, and phenylethylene were detected only from Daofu County. (2Z)-2-octene-1-ol, b-chamigrene, (–)-b-cadinene, and 2(10)-pinene were unique volatiles in *T. matsutake* from Maerkang County. Site specific volatiles can be used as chemical indicators for classification of *T. matsutake* from different geographical areas in southwestern China.

Volatiles in *T. matsutake* of different ages Differences between *T. matsutake* from different areas were mainly based on volatile component contents, whereas differences between *T. matsutake* of different ages were mainly based on levels of volatiles (Table 1). Levels of the main volatiles 1-octen-3-ol and methyl cinnamate, which help *T. matsutake* repel mycophagous *Proisotoma minuta* and play an important role in *T. matsutake* metabolism, were significantly ($p<0.05$) lower in older fruiting bodies than in young bodies. 1-octen-3-ol is known as mushroom alcohol and methyl cinnamate has a strong, aromatic odor, and decreases in concentrations will influence the odor characteristics of *T. matsutake*. Whether this weakens the ability of *T. matsutake* to repel insect pests needs verification. Differences in concentrations of attractive flavor substances are probably why young *T. matsutake* is preferred as a food.

Mineral composition of *T. matsutake* Mineral elemental contents of *T. matsutake* from different geographical areas were different (Table 2). The average content of potassium, the most abundant element in *Tricholoma matsutake* fruiting bodies, was 30,143.94 mg kg⁻¹ of dry weight (DW). Iron was the second most abundant element with an average content of 1,877.65 mg kg⁻¹ of DW. The calcium content in *T. matsutake* fruiting bodies varied from 128.81 to 372.75 mg kg⁻¹ of DW. The cadmium content ranged from 0.75 to 3.54 mg kg⁻¹ of DW. Iron, calcium, and manganese contents were higher and cadmium was lower in fruiting bodies from Maerkang County than in fruiting bodies from other areas. The iron content in fruiting bodies from Maerkang County was 6x higher than in fruiting bodies from Daofu County, indicating that mineral concentrations can be affected by soil and environmental conditions.

Mineral contents of *T. matsutake*, except for Cu and Pb, were generally in agreement with previous reports (28-31). Cu and Pb contents in *T. matsutake* were lower than in other mushrooms. European Commission regulations require a maximum level of Cd of 10 µg g⁻¹ of DW (32). Mean values of Cd concentrations in fruiting bodies in this study were less than 10 µg g⁻¹ of DE for all sites. The tolerable weekly intake (PTWI) for Cd has been reported as 2.5 µg kg⁻¹ of body weight (33). Therefore, daily consumption of 200 to 400 g of fruiting bodies during the mushroom season is recommended. The acceptable daily intake of Pb for adults is between 210 and 250 µg per day (33), so consumption of fresh fruiting bodies would be no more than 2,500 g per day.

Table 1. Volatile components of *Tricholoma matsutake* at different stages of maturity from 3 geographical areas

Compound	Relative amount %								
	Dechang Stage 1 ¹⁾	Dechang Stage 2	Dechang Stage 3	Daofu Stage 1	Daofu Stage 2	Daofu Stage 3	Maerkang Stage 1	Maerkang Stage 2	Maerkang Stage 3
2-Isopropyl-2-methyloxirane	nd ²⁾	nd	nd	0.31±0.03b	0.12±0.01c	0.43±0.02a	nd	nd	nd
1-Octene	0.28±0.16d ³⁾	nd	0.12±0.02e	0.58±0.32a	nd	nd	0.32±0.05c	0.44±0.11b	nd
3-Methyl-1-heptene	1.52±0.25b	nd	2.31±0.13a	nd	1.56±0.34b	nd	nd	1.4±0.75c	nd
Hexaldehyde	nd	1.49±0.11c	nd	1.64±0.09b	nd	0.91±0.04e	0.13±0.03f	1.83±0.22a	1.25±0.09d
1,3-Octadiene	nd	nd	nd	nd	nd	nd	nd	nd	0.51±0.15a
2-Furaldehyde	nd	nd	nd	nd	1.09±0.02a	nd	0.12±0.09b	nd	nd
3-Furaldehyde	nd	nd	nd	nd	nd	0.15±0.01b	nd	nd	0.81±0.02a
phenylethylene	nd	nd	nd	nd	0.92±0.33a	nd	nd	nd	nd
1,3,5,7-cyclooctatetraene	nd	nd	1.32±0.22e	2.30±0.13d	nd	3.44±0.24b	2.60±0.63c	2.30±0.34d	5.34±0.56a
3-Thujene	nd	0.39±0.07b	nd	nd	0.56±0.09a	nd	nd	0.33±0.12c	nd
Benzaldehyde	3.96±0.58d	17.38±0.46a	7.6±0.58b	1.83±0.27f	1.37±0.28i	5.4±0.35c	1.45±0.26h	1.71±0.26g	1.08±0.06j
2(10)-Pinene	nd	nd	nd	nd	nd	nd	1.23±0.08b	4.53±0.09a	nd
3,7,7-trimethyl-3-norcaren	0.58±0.11c	0.34±0.07d	0.32±0.06d	0.21±0.04e	nd	nd	nd	0.65±0.05b	0.73±0.03a
1-Isopropenyl-4-methylenecyclohexane	1.26±0.37c	2.73±0.83b	5.78±1.02a	nd	nd	nd	nd	nd	nd
L-Camphene	nd	nd	nd	nd	nd	nd	3.32±0.08b	nd	3.45±0.12a
Sabenene	1.29±0.43b	nd	nd	nd	nd	nd	0.19±0.06c	nd	1.44±0.45a
1-Hepten-3-one	2.07±1.10b	nd	5.83±1.25a	nd	nd	nd	nd	nd	nd
3-Octanone	0.51±0.11f	4.00±0.09d	13.76±0.55b	4.72±0.36c	13.78±0.96b	14.07±0.83a	2.39±0.13e	nd	nd
2-Pentylfuran	5.3±1.07a	nd	nd	nd	nd	nd	nd	nd	nd
octanal	1.62±0.25c	1.14±0.08e	1.12±0.02e	1.76±0.05b	1.10±0.01f	1.00±0.32g	0.98±0.10g	1.79±0.88a	1.22±0.30d
1-Octen-3-ol	14.89±0.37b	7.79±0.68e	2.32±0.13h	17.74±0.38a	8.76±0.57d	3.52±0.32f	9.98±0.12c	3.34±0.25g	1.07±0.26i
Bicyclo[4.1.0]hept-2-ene,4,7,7-trimethyl-	1.89±0.52b	1.66±0.07c	2.32±0.23a	nd	nd	nd	nd	nd	nd
Dipentene	nd	nd	nd	2.34±0.32b	nd	3.42±0.14a	nd	nd	3.42±0.15a
D-Limonene	10.23±0.21c	15.91±1.32a	9.32±1.67d	nd	nd	nd	1.68±0.92f	13.24±1.76b	5.43±0.34e
5-ethylcyclopentene-1-carbaldehyde	nd	0.77±0.24a	0.54±0.17c	0.32±0.11d	nd	nd	nd	nd	0.67±0.08b
3-Ethyl-2-methyl-1,3-hexadien	3.22±0.23c	nd	nd	nd	6.34±0.12a	nd	nd	3.76±0.33b	nd
Phenylacetaldehyde	1.53±0.42i	1.97±0.68h	6.22±0.37g	14.44±0.22b	14.85±0.67a	13.8±0.24d	14.34±0.65c	7.11±0.09f	11.07±0.26e
(E)-2-Octenal	8.84±0.19b	3.36±1.02f	2.14±0.97g	14.46±2.20a	8.79±1.19b	7.04±2.07c	5.9±1.96d	5.6±1.06e	7.01±1.32c
g-Terpinene	nd	0.53±0.13a	nd	nd	nd	nd	nd	nd	nd
2,6-Dimethylocta-2,7-dien-6-ol	0.35±0.02d	nd	nd	nd	2.9±0.02c	3.5±0.01b	nd	nd	4.42±0.04a
3,7-Dimethyl-1,3,6-octatriene	0.44±0.09b	0.32±0.01c	0.24±0.03d	nd	0.14±0.03e	nd	0.12±0.01f	0.57±0.12a	nd
1-Nonanal	1.46±0.66d	1.33±0.12e	7.99±0.11b	8.50±0.11a	7.40±0.97c	0.83±0.04f	0.25±0.01g	nd	nd
(Z)-2-Octene-1-ol	nd	nd	nd	nd	nd	nd	5.12±0.23a	2.98±0.35c	3.43±0.25b
2-Phenyl-2-propenal	nd	nd	nd	nd	nd	4.68±1.54a	1.32±0.55b	nd	nd

Table 1. Continued

Compound	Relative amount %								
	Dechang Stage 1 ¹⁾	Dechang Stage 2	Dechang Stage 3	Daofu Stage 1	Daofu Stage 2	Daofu Stage 3	Maerkang Stage 1	Maerkang Stage 2	Maerkang Stage 3
1-Octanol	nd ²⁾	nd	nd	2.23±0.14b	nd	3.34±0.31a	nd	nd	3.35±0.24a
1,2-Benzofulvene	nd	3.20±0.43d	1.21±0.12f	nd	4.30±0.73b	nd	2.34±0.45e	3.43±0.67c	5.76±1.11a
Naphthalene	nd	nd	0.78±0.12c	nd	nd	2.3±0.02a	1.5±0.08b	nd	nd
Cyclooctane	nd	0.43±0.04b	nd	nd	0.56±0.32a	nd	nd	0.22±0.07c	nd
4-Dodecene	nd	nd	nd	0.31±0.13d	1.47±0.74a	1.42±0.12b	nd	1.23±0.09c	nd
2-Decanone	nd	nd	nd	0.71±0.18a	nd	nd	nd	nd	nd
1-Methoxy-4-(2-propenyl)benzene	nd	nd	nd	nd	nd	nd	0.58±0.03b	0.30±0.03c	3.70±0.02a
n-Dodecane	0.51±0.26a ³⁾	0.12±0.03b	0.09±0.01c	nd	nd	nd	nd	nd	nd
1-Ethyl-2-methylcyclopentane	nd	0.22±0.04b	nd	nd	0.12±0.05c	nd	nd	nd	4.10±1.09a
(E,E)-2,4-Nonadienal	nd	nd	nd	0.93±0.09c	1.23±0.22a	1.09±0.32b	nd	nd	nd
2,4-nonadienal	nd	nd	nd	0.53±0.12a	0.22±0.02b	0.13±0.03c	nd	nd	nd
(E,E)-2,4-Nonadienal	5.3±0.03a	nd	nd	nd	nd	2.2±0.05b	nd	nd	1.3±0.02c
2-Dodecenal	1.78±0.48b	2.07±0.56a	1.50±0.67c	nd	nd	nd	nd	nd	nd
6-Undecanone	nd	nd	nd	0.51±0.13a	nd	nd	nd	nd	nd
1-Methoxy-4-[(Z)-prop-1-enyl]benzene	nd	nd	nd	nd	nd	nd	0.29±0.03c	2.09±0.05a	1.23±0.11b
2-Hendecanone	nd	nd	nd	0.67±0.11b	0.13±0.01c	nd	0.87±0.05a	nd	nd
4-Decenoic acid methyl ester	nd	nd	0.44±0.21d	nd	0.66±0.42b	nd	0.48±0.22c	0.32±0.09e	0.95±0.15a
2,4-Decadienal	nd	nd	nd	0.99±0.19a	0.78±0.23b	0.16±0.07e	0.14±0.03e	0.43±0.04d	0.55±0.15c
a-Cubebene	nd	0.36±0.11b	nd	0.46±0.32a	nd	nd	nd	nd	nd
d-Longifolene	nd	0.34±0.04c	nd	nd	nd	2.50±0.47a	1.97±0.73b	0.33±0.02c	nd
Methyl cinnamate	12.3±0.12d	7.89±0.06e	5.43±0.56g	14.3±0.32b	4.03±0.05h	2.97±0.04i	23.18±1.56a	13.28±0.39c	7.19±0.86f
Methyl trans-cinnamate	1.85±0.58a	nd	nd	nd	1.35±0.53b	nd	nd	nd	1.32±0.65c
Methyl 4-ethenylbenzoate	nd	nd	1.01±0.32c	nd	nd	3.45±0.54a	2.34±0.43b	nd	nd
Sesquichamene	0.33±0.01b	nd	nd	nd	4.20±0.22a	nd	0.15±0.02c	nd	nd
1,4-Dichloro-2,5-dimethoxybenzene	nd	nd	nd	nd	nd	0.20±0.01a	nd	nd	nd
(Z)-Ethyl cinnamate	0.32±0.11c	0.97±0.34b	nd	nd	nd	0.11±0.06d	nd	2.13±0.91a	nd
b-Chamigrene	nd	nd	nd	nd	nd	nd	0.62±0.06b	0.55±0.15c	0.73±0.22a
(+)-b-Himachalene	nd	2.4±0.42b	nd	nd	nd	3.6±0.09a	0.31±0.06d	nd	1.56±0.34c
(-)-b-Cadinene	nd	nd	nd	nd	nd	nd	0.28±0.10c	2.01±0.21a	1.54±0.36b
d-Cadinene	2.3±0.25a	nd	nd	0.38±0.01d	1.32±0.34c	nd	nd	1.90±0.21b	nd
3,6-Dodecadienoic acid methyl ester	nd	2.20±0.05b	1.23±0.11c	nd	nd	1.09±0.22d	3.02±0.13a	nd	1.21±0.11c
2,6-Di-tert-butyl-4-sec-butylphenol	nd	nd	nd	0.23±0.09b	0.43±0.06a	0.12±0.03c	nd	nd	nd

¹⁾Stage 1, egg and bell shaped; Stage 2, volva broken or stipe elongated; Stage 3, cap opened.²⁾nd = not detected.³⁾Values in a row marked with the same letter are not significantly different ($p>0.05$) based on Duncan's multiple comparison test.

Table 2. Chemical Composition of *Tricholoma matsutake* from 3 geographical areas

	Dechang	Daofu	Maerkang
K (mg kg ⁻¹)	27895.63±31.20b ¹⁾	30322.40±23.87a	32213.79±30.29a
Mg (mg kg ⁻¹)	708.61±5.16b	756.64±3.22b	1107.19±5.29a
Zn (mg kg ⁻¹)	85.12±2.02a	80.80±2.02b	85.41±2.34a
Ga (mg kg ⁻¹)	128.81±5.92a	271.03±4.13c	372.75±3.27b
Fe (mg kg ⁻¹)	2254.19±12.15a	479.95±3.96b	2899.80±9.68a
Cu (mg kg ⁻¹)	26.73±1.02c	33.94±2.45a	29.63±1.12b
Mn (mg kg ⁻¹)	19.11±1.09b	14.62±1.02b	50.77±1.99a
Cd (mg kg ⁻¹)	3.54±0.03a	1.68±0.05b	0.75±0.06c
Pb (mg kg ⁻¹)	0.85±0.01b	0.43±0.02c	1.74±0.03a

¹⁾Values in a row marked with the same letter are not significantly different ($p>0.05$) based on Duncan's multiple comparison test.

Table 3. Amino acid contents of fresh *T. matsutake* from 3 geographical areas

	Dechang	Daofu	Maerkang
Asp (%)	1.14±0.02b ¹⁾	1.32±0.02a	1.38±0.02a
Thr (%)	0.60±0.02b	0.59±0.01b	0.64±0.02a
Ser (%)	0.70±0.01a	0.67±0.01b	0.68±0.01b
Glu (%)	3.24±0.02a	2.85±0.03c	3.10±0.03b
Gly (%)	0.58±0.03c	0.63±0.01b	0.70±0.02a
Ala (%)	0.91±0.04b	0.88±0.01c	0.96±0.02a
Cys (%)	0.08±0.01b	0.12±0.01a	0.13±0.02a
Val (%)	0.61±0.01b	0.65±0.01b	0.71±0.01a
Met (%)	0.11±0.01c	0.19±0.01a	0.16±0.02b
Ile (%)	0.47±0.02b	0.53±0.03a	0.56±0.01a
Leu (%)	0.89±0.03c	0.94±0.02b	0.99±0.02a
Tyr (%)	0.26±0.01b	0.31±0.01a	0.34±0.01a
Phe (%)	0.55±0.01c	0.59±0.01b	0.63±0.01a
Lys (%)	0.72±0.02b	0.77±0.02a	0.75±0.02a
His (%)	1.28±0.03a	0.77±0.02b	0.82±0.01b
Arg (%)	0.90±0.02a	0.78±0.01b	0.81±0.02b
Pro (%)	0.47±0.02b	0.60±0.02a	0.66±0.01a
TAA ²⁾ (%)	13.53±0.05c	13.83±0.06b	14.02±0.05a

¹⁾Values in a row marked with the same letter are not significantly different ($p>0.05$) based on Duncan's multiple comparison test.

²⁾TAA, total amino acids.

Nutritional value of *T. matsutake* Fruiting bodies from Dechang County contained greater amounts of fiber with lower crude oil contents than fruiting bodies from the other two areas (Table 4). The protein content of mushrooms is affected by a number of factors (34), including location. In this study, the average fruiting body protein content was 19.40%. Fiber contents of fruiting bodies from Dechang, Maerkang, and Daofu Counties were 22.3, 20.1, and 18.8% on a DW basis, respectively, and may be considered low. The ash content of fruiting bodies from Maerkang County was higher than for the other two areas. The average vitamin B₂ content in fruiting bodies was high at 49.4 mg kg⁻¹.

Amino acids are important nutrients for humans and 17 amino acids were detected in *T. matsutake* fruiting bodies, including 7 essential amino acids, showing that *T. matsutake* is a rich source of amino acids (Table 3). Essential amino acids accounted for 29.2 to 31.7% of the total amino acid content. Glutamate was the most

Table 4. Nutritional composition of *Tricholoma matsutake* from 3 geographical areas

	Dechang	Daofu	Maerkang
Protein (%)	19.86±0.1a ¹⁾	18.63±0.1b	19.72±0.0a
Crude oil (%)	1.9±0.0b	2.3±0.1a	2.2±0.0a
Fiber(%)	22.3±1.1a	18.8±0.4c	20.1±0.6b
Ash (%)	8.65±0.9b	8.74±0.8b	15.82±1.2a
Vitamin B ₁ (mg/kg)	5.6±0.1a	5.1±0.2b	5.2±0.1b
Vitamin B ₂ (mg/kg)	41.5±0.8c	58.2±0.9a	48.4±0.4d
Vitamin C (mg/kg)	24.80±1.5b	40.23±1.9a	24.36±1.3b

¹⁾Values in a row marked with the same letter are not significantly different ($p>0.05$) based on Duncan's multiple comparison test.

abundant, with an average value of 3.06%, followed by asparagine at 1.28%. The average amino acid content of *T. matsutake* fruiting bodies from all geographical areas was high at 13.8%.

Sixty six volatile compounds were identified in *T. matsutake* fruiting bodies from 3 geographical origins and 3 stages of maturity. 1-octen-3-ol, methyl cinnamate, 3-octanone, benzaldehyde, and some C8 components were dominant in all mushroom samples. Compositions of volatile components of *T. matsutake* from different geographical origin were different, which could be considered as chemical indicators for classification of *T. matsutake* from different geographical areas in southwestern China. Levels of the main volatiles 1-octen-3-ol and methyl cinnamate were lower in older fruiting bodies. *T. matsutake* is rich in protein, fiber, amino acids, vitamins, and low in crude oil, leading to commercial valuation in many countries. Minerals analyzed in *T. matsutake* were, at most, present at a general level, compared with other mushrooms. Consumption of *T. matsutake* fresh fruiting bodies is recommended at no more than 2,500 g per day in consideration of Cd and Pb contents.

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