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Characterisation of LC-MS-based low molecular weight compounds and fatty acids of four wild edible mushrooms

[1,2]Xun, W., [1,2]*Wang, G. Y., [3]Zhang, Y. J., [2]Ge, C. R. and [2]Liao, G. Z.

[1]*College of Food Science and Technology, Yunnan Agricultural University, Kunming, 650201, China*
[2]*Livestock Product Processing and Engineering Technology Research Centre of Yunnan Province, Yunnan Agricultural University, Kunming, 650201, China*
[3]*Henan Engineering Technology Research Centre of Mushroom Foods, Nanyang Normal University, Nanyang, 473061, China*

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Abstract

The total fatty acids and low molecular weight components were determined from four wild edible mushrooms (*Tuber aetivum, Cantharellus cibarius, Boletus edulis*, and *Tricholoma matsutake*). The fatty acids were investigated by gas chromatography-mass spectrometry (GC-MS), and the low molecular weight compounds were identified by liquid chromatograph-mass spectrometry (LC-MS) combined with multivariate statistical analysis. A total of 32 fatty acids were detected. The total fatty acids content was the highest in *Tub. aetivum*. Linoleic acid was the highest in the four wild edible mushrooms. Besides, a total of 144 and 91 differential with 40 mutual low molecular weight compounds were identified in positive and negative ion modes, respectively, mainly including amino acids, organic acids, sugars, and vitamins. Comprehensive multivariate statistics showed that betaine, choline, and 13 other compounds were differential low molecular weight compounds of the four wild edible mushrooms. These results expand our knowledge of the nutrition and flavour precursors in wild edible mushrooms.

Introduction

Wild edible mushrooms are widely consumed in many countries, with an increasing popularity due to their nutritional, organoleptic, and pharmacological characteristics. Mushrooms are excellent food components of low caloric diets because they are rich in digestible proteins, vitamins, and dietary fibres, have a good amino acid composition, and are low in fats (Wang *et al.,* 2014). Many wild edible mushrooms have high antioxidant activity, which may be due to their large number of phenolic compounds (Guo *et al.,* 2012). Wild edible mushrooms have a unique natural aroma and umami taste. The special aroma comes from the combination of several aroma compounds including alcohols, aldehydes, and ketones; and the umami taste results from the synergistic interactions of non-volatile components including umami amino acids, 5'-nucleotides, and umami peptides (Sun *et al.,* 2020). Different wild mushrooms can have different flavours due to differences in maturity, quality, and storage time, as well as species-specific differences in the flavour precursors and substances that develop with cooking. During cooking, mushroom flavour arises from a series of chemical reactions that occur during the heating of flavour precursors including the Maillard reaction, pyrolysis of amino acids and peptides, fat oxidation, and the degradation of carbohydrates and nucleotides. Volatile substances in cooked mushrooms exist as flavour substance precursors in raw mushrooms, and can be mainly divided into two categories; low molecular weight components and lipids. The low molecular weight components includes amino acids, sugars, organic acids, and nucleic acids, and are important for mushroom flavour formation and taste. Eight-carbon volatiles are important for mushroom aroma, and this aroma is produced by the oxidation of linoleic acid catalysed by lipoxygenase (Combet *et al.,* 2006). Fresh wild edible mushrooms have relatively higher water content; approximately 90% (Beluhan and Ranogaiec, 2011; Liu *et al.,* 2012). Water content may be affected by maturity, growing environment, and post-harvest evaporation. Therefore, the chemical composition of wild edible mushrooms should be normalised based on their dry weight (Krzysztof and Jerzy, 2008).

Yunnan province, located in Southwest China, has the most abundant wild mushroom

*Corresponding author.
Email: ynkmwgy@ynau.edu.cn (Wang, G. Y.) ; liaoguozhou@ynau.edu.cn (Liao, G. Z.)

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resources in China (Wu *et al.,* 2010). Yunnan has unique geographical and climatic conditions, with an area of 390,000 km^2, and an elevation of 76~6740 m. In the present work, we investigated four species of wild edible mushrooms found in Yunnan, namely *Tuber aetivum, Cantharellus cibarius, Boletus edulis*, and *Tricholoma matsutake*. Most studies on wild edible mushrooms have focused on the determination of the compounds present at conventional high concentrations. However, the characterisation of different mushrooms and their chemical composition information are scarce. In the present work, gas chromatography-mass spectrometry (GC-MS) and liquid chromatograph-mass spectrometry (LC-MS) were combined with multivariate statistics to analyse the composition profiles of fatty acids and low molecular weight compounds in the four wild edible mushrooms.

Materials and methods

Sample preparation

The four wild edible mushroom (*Tub. aetivum, C. cibarius, B. edulis*, and *Tri. matsutake*) samples were provided by Yimen Kangyuan Fungal Industry Co., Ltd. (Yuxi, China), and the identification of the mushroom samples was performed by All-China Federation of Supply and Marketing Cooperatives, Kunming Institute of Edible Fungi. Following dirt removal, all samples were freeze-dried using a SCINTZ-18N vacuum freeze dryer (Ningbo Scientz Biotechnology Co., Ltd., Ningbo, China). Freeze-dried samples (50 g) were powdered (40 mesh) with six samples (n = 6) for each kind of mushroom. The dried powder was stored in hermetically sealed bags, and stored at -80°C until analysis.

Chemicals

All chemicals used were of HPLC-grade. As standard, a mixture of 37 fatty acid methyl esters (FAMEs) was purchased from Sigma-Aldrich Co., Ltd. (Shanghai, China). DL-o-Chlorophenylalanine was purchased from GL Biochem. Ltd. (Shanghai, China). Methanol, acetonitrile, and *n*-alkane were purchased from Merck (Darmstadt, Germany).

Analysis of fatty acids

For the preparation of extracts and FAMEs, we used previously described methods (Moreira *et al.,* 2018), with slight modification. Briefly, 0.2 g of mushroom sample was transferred into a 10 mL glass centrifuge tube, and then 2 mL of methanol and 4 mL of chloroform were added, and the mixture vortexed for 20 min. Next, 2 mL of deionised water was added, and the mixture centrifuged at 1,000 rpm for 10 min. The supernatant fraction was transferred into a new centrifuge tube, and dried with nitrogen. Next, 1 mL of *n*-hexane was added, followed by the addition of 25 µL of methyl nonadecanoate as an internal standard. The mixture was vortexed for 2 min, added with 1 mL of potassium hydroxide-methanol solution (0.4 mol/L), and vortexed again for 1 min. Following incubation for 30 min at 37°C, the solution was centrifuged at 2,000 rpm for 5 min, and the supernatant was used for GC-MS analysis.

The analysis of fatty acids was carried out using a 7890B-5977B GC-MS instrument (Agilent, Palo Alto, USA) and a DB-225 capillary column (Agilent, 30 m × 0.25 mm × 0.25 µm). The initial temperature of 70°C was maintained for 5 min, and then increased to 200°C at the rate of 25°C/min. Then, the temperature was raised to 240°C at the rate of 2°C/min, and then kept at 240°C for 5 min. The split ratio was 20:1, and helium was used as a carrier gas with a flow rate of 1 mL/min. The injector and detector temperatures were 280 and 230°C, respectively. The mass spectrometer was operated in electron impact (EI) mode at 70 eV in the scan range of 33~500 aum. A mixture of 37 FAMEs was used, and fatty acid identification was made by comparing the relative retention times of the FAME peaks of samples with those corresponding to the standards.

Analysis of low molecular weight compounds

For the sample preparation of low molecular weight components analysis, we used the methods in previous studies (Jin *et al.,* 2019), with slight modification. Briefly, 50 mg of sample was extracted with 800 µL of 80% methanol and 10 µL of internal standard (2.8 mg/mL, DL-o-Chlorophenylalanine), and all samples were ground for 90 s. The samples were then ultrasonicated for 30 min, and then incubated for 1 h at -20°C. The samples were then centrifuged at 12,000 rpm and 4°C for 15 min, and the resulting supernatant was subjected to LC-MS analysis.

LC-MS analysis was carried out using an Ultimate 3000LC-Q-Exactive-MS instrument (Thermo, California, USA) with a Hyper Gold C_{18} column (Thermo; 100 × 2.1 mm, 1.9 µm). The column was maintained at 40°C, the flow rate was 0.35 mL/min, and the mobile phase was water with 5% acetonitrile and 0.1% formic acid (A), and acetonitrile with 0.1% formic acid (B). The sample injection volume was 10 µL at 4°C. The parameters of mass detection were as follows: heater temperature, 300°C; sheath gas flow rate, 45 arb; aux

gas flow rate, 15 arb; sweep gas flow rate, 1 arb; capillary temperature, 350°C; spray voltage, 3.0 kV in positive ion mode (ESI+) and 3.2 kV in negative ion mode (ESI-); and s-lens rf level, 30 and 60% in ESI+ and ESI-, respectively.

Statistical analysis

The fatty acid data were extracted from the GC-MS data using Data Analysis software (Agilent, Palo Alto, USA), then the data were sorted according to the NIST database and edited in Microsoft Excel 2010 software (Microsoft, Washington, USA). The results were expressed as mean values ± standard deviations. The low molecular weight compounds data from LC-MS were extracted and pre-processed using the Compound Discoverer software (Thermo, California, USA). The data were normalised and edited in Microsoft Excel 2010, and organised into a two-dimensional data matrix containing information of retention time (RT), molecular weight, sample name, number of extractable substances, and peak intensity. The low molecular weight compounds were identified by full spectrum based on the network database Metlin, with differences between the actual molecular weight of compound and the theoretical molecular weight in the database of less than 10 ppm. Multivariate statistical analysis was performed using SIMCA14.1 (Umetrics, Malmo, Sweden). All statistical analyses were performed by one-way analysis of variance (ANOVA) followed by Duncan's multiple range test using SPSS 25.0 (IBM, Armonk, NY, USA), and a difference of $p < 0.05$ was considered to be significant.

Results and discussion

Fatty acids

The results for fatty acids composition, total saturated fatty acids (SFA), monounsaturated fatty acids (MUFA), and polyunsaturated fatty acids (PUFA) of the four wild edible mushrooms are shown in Table 1. A total of 32 fatty acids were identified from the four wild edible mushrooms, with significantly varied compositions among the four species. The fatty acid compositions have been effective in distinguishing mushroom species from each other (Çayan *et al.,* 2020). Thirty-one fatty acids were detected in *Tub. aetivum, Tri. matsutake*, and *B. edulis*, while another fatty acid, 8,11,14-eicosatrienoic acid, was detected in *C. cibarius* at low concentration (1.10 mg/kg), but not detected in the other three wild edible mushrooms. The fatty acid contents in the samples ranged from 4,545.54 to 16,555.87 mg/kg. *Tub. aetivum* had the highest fatty acids amounts, and *Tri. matsutake* had the lowest.

Linoleic, oleic, palmitic, and stearic acids were the main fatty acids in the four wild edible mushrooms, accounting for 58~33, 12~28, 7~14, and 3~26%, respectively, and the four fatty acids showed significant variations among the four wild edible mushrooms ($p < 0.05$). Similar observations have been reported in other mushrooms (Barros *et al.,* 2007; Ribeiro *et al.,* 2009). Linoleic acid is a precursor of eight-carbon volatiles in mushrooms. Although there are many volatile flavours in wild edible mushrooms, eight-carbon volatiles are the principal contributors to mushroom flavour, especially 1-octen-3-ol, 1-octanol, 3-octanol, and 3-octanone (Maga, 1981; Combet *et al.,* 2006; Venkateshwarlu *et al.,* 2015). The four wild edible mushrooms analysed in the present work varied in linoleic acid contents: *Tub. aetivum* (5,534.36 mg/kg), *C. cibarius* (5,279.02 mg/kg), *B. edulis* (3,136.46 mg/kg), and *Tri. matsutake* (2893.18 mg/kg). This difference may explain the different flavours of the four wild edible mushrooms. Linoleic acid is an essential fatty acid that the human body cannot synthesise due to the absence of desaturase enzymes required for its production; so, it must be obtained from foods (Simopoulos, 1999). Oleic acid is not an essential fatty acid, but is known for its ability to lower cholesterol levels and promote the decrease of cardiovascular and cerebrovascular diseases (Tomás *et al.,* 2001). Stearic and palmitic acids are both SFA which are heart priority use of fatty acids (Lawson and Kummerow, 1979). Palmitic acid can reduce the increase of cholesterol in serum caused by lauric and myristic acids in the diet, which may help to treat thrombosis (Wang *et al.,* 2003). Small amounts of fatty acids with odd carbon atoms were detected in the four wild edible mushrooms as reported for other edible mushrooms (Barros *et al.,* 2007; Ribeiro *et al.,* 2009; Ergönül *et al.,* 2012). In the present work, the main fatty acids contents of *C. cibarius* were linoleic, oleic, palmitic, and stearic acids, in descending order. Similar observation has been reported for Turkish *C. cibarius* (Ayaz *et al.,* 2011); but, Kavishree *et al.* (2008) reported differently for Indian *C. cibarius* which was found to have the highest content of oleic acid instead. The linoleic acid content of *B. edulis* was the highest, which agrees with previous reports on Turkish *B. edulis* (Ayaz *et al.,* 2011) and Israeli *B. edulis* (Hanus *et al.,* 2008). However, the descending order of stearic, oleic, and palmitic acid contents of *B. edulis* in the present work is different from the content of reported Turkish and Israeli *B. edulis*. Liu *et al.* (2018) analysed the fatty acids in *Tri. matsutake* from

Table 1. Fatty acid composition of four wild edible mushrooms (mg/kg dry matter).[1]

	Mushroom			
	Tub. aetivum	***C. cibarius***	***B. edulis***	***Tri. matsutake***
C6:0[2]	0.29 ± 0.05[b]	0.39 ± 0.10[ab]	0.54 ± 0.12[a]	0.41 ± 0.04[ab]
C8:0	0.55 ± 0.01[c]	1.03 ± 0.12[b]	1.07 ± 0.29[b]	2.7 ± 0.40[a]
C10:0	0.28 ± 0.04[a]	0.15 ± 0.05[b]	0.20 ± 0.06[ab]	0.14 ± 0.04[b]
C11:0	2.19 ± 0.34	2.11 ± 0.13	2.42 ± 0.70	2.62 ± 0.31
C12:0	1.71 ± 0.10[a]	1.19 ± 0.16[b]	0.84 ± 0.11[c]	1.30 ± 0.05[b]
C13:0	0.20 ± 0.02[b]	0.52 ± 0.27[a]	0.26 ± 0.05[ab]	0.32 ± 0.01[ab]
C14:0	12.61 ± 0.91[a]	9.23 ± 2.11[b]	5.81 ± 1.11[bc]	9.95 ± 1.26[a]
C14:1	0.13 ± 0.03[c]	0.23 ± 0.05[b]	0.20 ± 0.05[bc]	0.32 ± 0.03[a]
C15:0	2.03 ± 0.14[c]	17.47 ± 2.36[b]	85.34 ± 8.61[a]	14.31 ± 2.21[b]
C16:0	1,156.63 ± 33.50[b]	1,445.9 ± 82.38[a]	516.77 ± 58.24[c]	619.61 ± 85.87[c]
C16:1	19.14 ± 2.02[b]	37.27 ± 6.13[a]	20.12 ± 5.32[b]	32.58 ± 6.61[c]
C17:0	16.72 ± 0.61[c]	23.24 ± 3.25[b]	44.11 ± 4.74[a]	12.58 ± 2.31[c]
C17:1	1.42 ± 0.50[c]	6.99 ± 0.66[a]	2.84 ± 0.38[b]	2.52 ± 0.26[b]
C18:0	4,236.19 ± 69.37[a]	661.19 ± 53.12[b]	709.67 ± 68.69[b]	117.51 ± 17.36[c]
C18:1	4,713.84 ± 163.54[a]	2,432.8 ± 46.44[b]	636.52 ± 72.96[c]	694.89 ± 96.45[c]
C18:2	5,534.36 ± 149.48[a]	5,279.02 ± 160.31[a]	3,136.46 ± 229.28[b]	2,893.18 ± 539.45[b]
C18:3	10.14 ± 0.77[c]	25.05 ± 3.31[b]	4.35 ± 0.32[c]	34.85 ± 7.38[a]
C20:0	122.55 ± 5.64[a]	34.06 ± 2.88[b]	16.45 ± 2.66[c]	6.1 ± 0.62[d]
C20:1	162.03 ± 7.64[a]	50.27 ± 6.38[b]	3.04 ± 0.61[c]	9.09 ± 1.45[c]
C20:2	308.43 ± 15.31[a]	36.05 ± 4.36[b]	4.62 ± 1.16[c]	14.28 ± 2.91[c]
C20:3n6	ND	1.10 ± 0.18	ND	ND
C21:0	15.48 ± 0.04[a]	2.35 ± 0.07[c]	3.6 ± 0.45[b]	2.97 ± 0.64[bc]
C20:4	2.78 ± 0.21[ab]	14.45 ± 10.12[a]	1.10 ± 0.15[b]	12.58 ± 6.58[ab]
C20:3n3	2.86 ± 0.23[d]	7.75 ± 0.27[a]	4.19 ± 0.24[c]	6.62 ± 0.42[b]
C20:5	1.70 ± 0.33[b]	4.10 ± 0.87[a]	1.41 ± 0.13[b]	3.97 ± 1.15[a]
C22:0	136.99 ± 6.75[a]	26.78 ± 1.86[c]	54.59 ± 6.20[b]	14.34 ± 2.85[d]
C22:1	9.45 ± 1.21	7.20 ± 0.71	7.58 ± 1.58	8.00 ± 1.22
C22:2	4.12 ± 0.95[b]	6.58 ± 1.31[a]	ND	2.56 ± 0.67[b]
C23:0	20.01 ± 2.00[a]	4.34 ± 0.51[c]	13.73 ± 1.64[b]	1.96 ± 0.23[c]
C24:0	7.26 ± 1.05[d]	76.71 ± 7.42[b]	117.58 ± 12.14[a]	9.88 ± 0.77[b]
C22:6	48.39 ± 4.00[a]	5.69 ± 0.19[b]	1.26 ± 0.09[c]	1.74 ± 0.21[c]
C24:1	5.38 ± 0.71[c]	16.6 ± 1.36[a]	9.09 ± 1.72[b]	11.65 ± 1.53[b]
SFA	5,731.70 ± 117.56[a]	2,306.65 ± 151.30[b]	1,572.96 ± 162.00[c]	816.71 ± 94.08[d]
PUFA	4,911.39 ± 169.67[a]	2,551.36 ± 47.98[b]	679.38 ± 78.96[c]	759.05 ± 105.03[c]
MUFA	5,912.78 ± 170.38[a]	5,379.80 ± 183.28[a]	3,153.38 ± 231.80[b]	2,969.78 ± 150.84[b]
Total	16,555.87 ± 467.95[a]	10,237.81 ± 403.48[b]	5,405.73 ± 481.93[c]	4,545.54 ± 281.82[c]
UFA/SFA	1.9	3.4	2.4	4.6

[1]Each value is expressed as mean ± SD (n = 6). [2](C6:0) caproic acid, (C8:0) caprylic acid, (C10:0) capric acid, (C11:0) undecanoic acid, (C12:0) lauric acid, (C13:0) tridecanoic acid, (C14:0) myristic acid, (C14:1) myristoleic acid, (C15:0) pentadecanoic acid, (C16:0) palmitic acid, (C16:1) palmitoleic acid, (C17:0) heptadecanoic acid, (C17:1) heptadecenoic acid, (C18:0) stearic acid, (C18:1) oleic acid, (C18:2) linoleic acid, (C18:3) linolenic acid, (C20:0) arachidic acid, (C20:1) eicosenoic acid, (C20:2) eicosadienoic acid, (C20:3n6) 8,11,14-eicosatrienoic acid, (C21:0) heneicosanoic acid, (C20:4) arachidonic acid, (C20:3n3) 11,14,17-eicosatrienoic acid, (C20:5) eicosapentaenoic acid, (C22:0) behenic acid, (C22:1) erucic acid, (C22:2) docosadienoic acid, (C23:0) tricosanoic acid, (C24:0) lignoceric acid, (C22:6) docosahexaenoic acid, and (C24:1) nervonic acid. Different lowercase superscripts within a row indicate significant difference ($p < 0.05$). ND: not detected.

Tibet, China, and reported that the content of oleic acid was the highest, followed by linoleic, palmitic, and stearic acids, which disagrees with the results in the present work (linoleic acid being the highest). The fatty acid composition of *Tub. aetivum* has not been reported by others. For the four wild edible mushrooms investigated, their fatty acids profiles were different to some extent from those described in the literature, which could be explained by the differences in sample drying or fatty acid analysis methods, localities, and harvesting stages.

The contents of SFA, PUFA, and MUFA were significantly different among the four wild edible mushrooms ($p < 0.05$). All species exhibited high concentrations of unsaturated fatty acids (UFA). During cooking, UFA oxidises to produce hydroperoxides, which continue to react to produce flavour substances of mushrooms such as alcohols, ketones, and aldehydes (Zeng *et al.,* 2013). Therefore, from the perspective of fatty acid evaluation, *Tub. aetivum* had the best flavour quality. MUFA was the main group in the four wild edible mushrooms, which accounted for 36~65%. This result is in agreement with previous reports from different regions (Yilmaz *et al.,* 2006; Ergönül *et al.,* 2012; Ogwok *et al.,* 2017), which indicates that the composition of fatty acids in mushrooms has a certain regularity and relative stability. The UFA/SFA ratio is important for evaluating the nutritional values of mushrooms, with daily diets rich in SFA being associated with an increase in coronary heart disease and atherosclerosis (Zhang and Ran, 2005). The UFA/SFA ratios of the four wild edible mushrooms ranged from 1.9 to 4.6, almost in line with the standard as healthy food.

Low molecular weight compounds

The low molecular weight compounds were well separated by LC-MS in only 18 min. In the four wild edible mushrooms, a total of 144 and 91 differential with 40 mutual substances were recognised in positive and negative ion modes, respectively. The low molecular weight compounds identified mainly included amino acids, nucleic acids, sugars, organic acids, lipids, and their derivatives. The low molecular weight compounds are important in human life activities, and have many physiological functions. Besides that, they, as flavour precursors and compounds, play an important role in the taste formation in mushrooms.

Principal components analysis (PCA) and partial least-squares discriminant analysis (PLS-DA) were applied to visualise the differences in the different wild edible mushrooms. The data obtained by LC-MS in positive and negative ion modes were analysed using PCA. PCA is an unsupervised multivariate statistical method, which is mainly used for data dimensionality reduction, including clustering and outliers among the samples. The PCA results are shown in Figure 1a (positive ion mode data) and Figure 1b (negative ion mode data), and the colour and shape of scatter points represent the experimental groups of samples. All the samples were within the 95% confidence interval, with a significant trend of separation among them, thus indicating different profiles of metabolites. Quality control (QC) samples were also plotted and indicated the stable operation of the instrument throughout the detection process. PLS-DA is a supervised multivariate statistical method that can separate samples to obtain improved discrimination. Complete separation was observed for the four wild edible mushrooms (Figure 1c, positive ion mode data; Figure 1d, negative ion mode data), and all the samples were within 95% confidence intervals. R2Y represents the interpretability of the Y matrix information percentage. Q2 (cum) represents the (cumulative) predictability of models. These two values should be greater than 0.5, and the closer to 1 the better. In the present work, R2Y (cum) and Q2 (cum) values were 0.983 and 0.972 in positive ion mode, and 0.881 and 0.947 in negative ion mode, thus indicating that both models were valid. PLS-DA model verification was performed next between the four wild edible mushrooms (Figure 1e, positive ion mode data; Figure 1f, negative ion mode data). The horizontal axis represents the correlation between the permuted Y-vectors and the original Y-vector for the selected Y. The green dots represent the R2 values obtained by the substitution test, and the blue squares represent the Q2 values obtained by the substitution test. The original Y value correlates 1.0 with itself, defining the high point on the horizontal axis. The R2 intercept and Q2 intercept represent the R2 and Q2 regression lines and the Y-axis. The R2 intercept obtained in the present work was greater than 0, and the Q2 intercept was less than 0.5, thus indicating that the models had good predictability, and there was no over-fitting.

Variables with variable importance for projection (VIP) values > 1 of the PLS-DA models are considered discriminating metabolites. A higher VIP value indicates a higher contribution of the corresponding metabolite for the discrimination of sample. Figure 1g and Figure 1h show clearly that the VIP values of betaine, L-valine, phenylethylamine, choline, isopentenyladenine, and other 24 substances; and citric acid, D-mannitol, 9(S)-HOTrE,

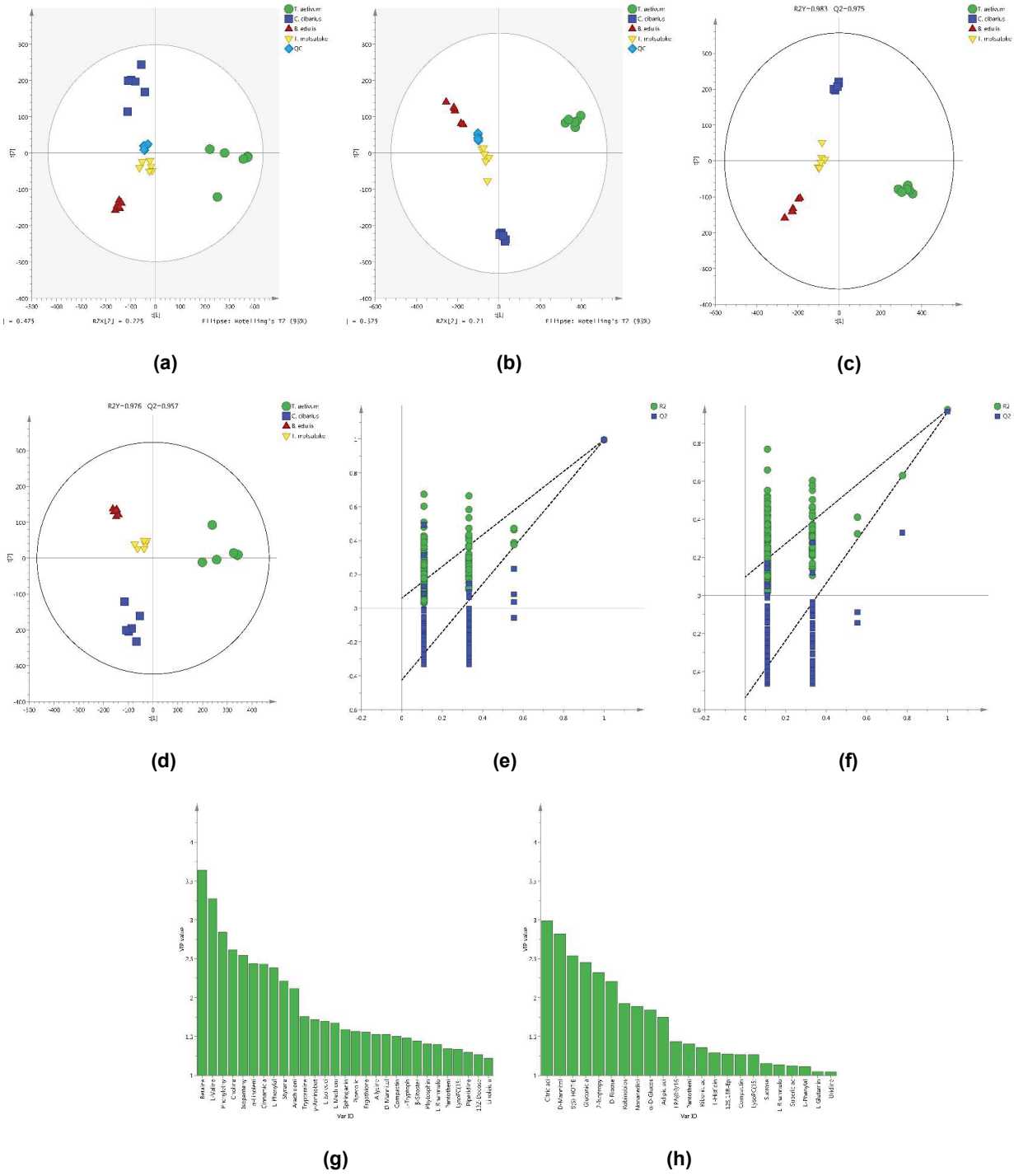

Figure 1. PCA score plots (a, b), PLS-DA score plots (c, d), Permutations plots (e, f), and VIP predictor plots (g, h) of four wild edible mushrooms (a, c, e, g are positive ion mode data, and b, d, f, h are negative ion mode data).

gluconic acid, 2-isopropylmalic acid, and other 19 substances were greater than 1 in positive and negative ion modes, respectively. To further investigate the difference of low molecular weight compounds in the four wild edible mushrooms, coupled comparative p-value < 0.05 established from the LC-MS extracted data of the four wild edible mushrooms was performed. Comprehensive multivariate data analysis showed that a total of 15 metabolites in the four wild edible mushrooms contributed significantly to the difference among groups. The 15 differential low molecular weight compounds consisted of three amino acids (L-isoleucine, pipecolic acid, and L-glutamine), one sugar (α-D-glucose), three organic acids (nonanedioic acid, adipic acid, and suberic acid), three lipids (α-linolenic acid, LPA (0:0/16:0), and LysoPC (15:0), one vitamin (pantothenic acid), and four other

Table 2. Identification of significantly different metabolites in four wild edible mushrooms.

No.	Mode	Name	RT (min)	Molecular weight	VIP
1	ESI+	Betaine	0.94	117.08	3.64
2	ESI+	Choline	0.88	103.10	2.62
3	ESI+	Isopentenyladenine	0.91	203.12	2.55
5	ESI+	α-Linolenic acid	8.03	278.22	2.44
5	ESI+	L-Isoleucine	0.96	131.09	1.69
6	ESI+	Sphinganine	8.07	301.30	1.59
7	ESI+	Pipecolic acid	0.89	129.08	1.57
8	ESI-	Nonanedioic acid	3.98	188.10	1.89
9	ESI-	α-D-Glucose	0.88	180.06	1.86
10	ESI-	Adipic acid	1.42	146.06	1.75
11	ESI-	LPA (0:0/16:0)	8.95	410.24	1.44
12	ESI-	Pantothenic acid	2.06	219.11	1.41
13	ESI-	LysoPC (15:0)	9.33	481.32	1.27
14	ESI-	Suberic acid	3.52	174.09	1.12
15	ESI-	L-Glutamine	0.85	146.07	1.05

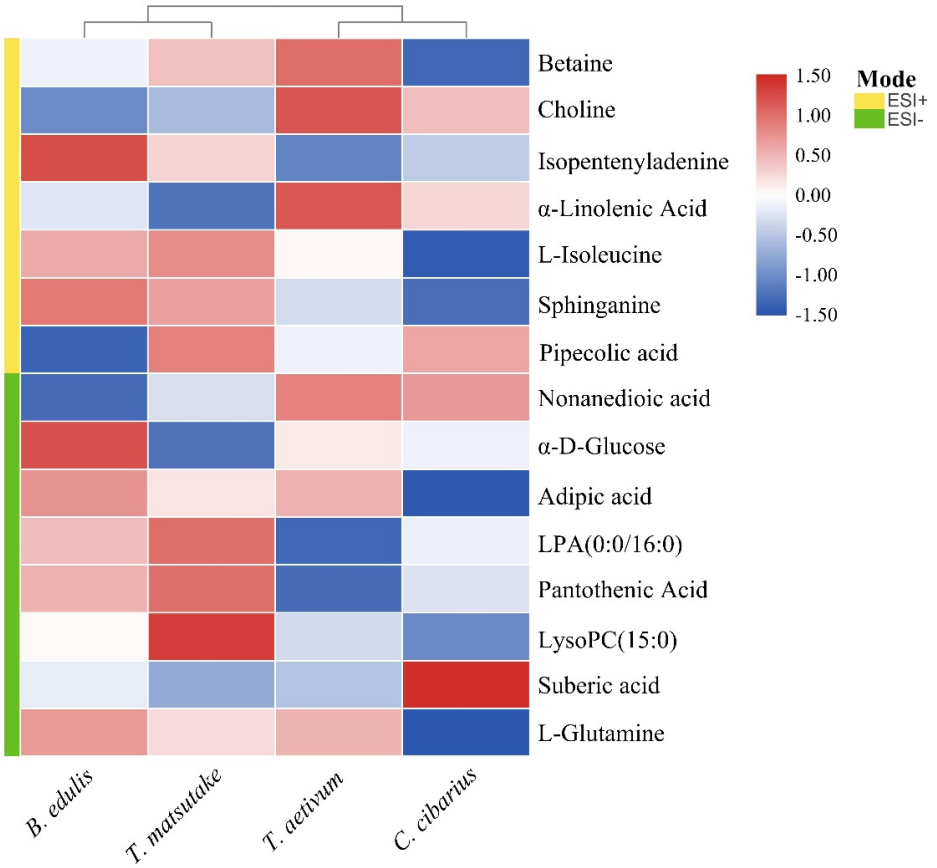

Figure 2. Heat map visualisation of the differential metabolites in the four wild edible mushrooms.

low molecular weight compounds (betaine, choline, isopentenyladenine, and sphinganine). The details are presented in Table 2. The differential low molecular weight compounds among the four wild edible mushrooms were clearly divided into four groups combined with a heat map (Figure 2), thus indicating that the abundances of low molecular weight compounds differed substantially among the four wild edible mushrooms. *B. edulis* and *Tri. matsutake* were clustered together, and *Tub. aetivum* and *C. cibarius* were clustered together, thus indicating similarity for these two pairs. The PCA score graphs indicate relatively close distances between *B. edulis* and *Tri. Matsutake*, and between *Tub. aetivum* and *C. cibarius*. The result was consistent with the heatmap.

The differential low molecular weight compounds for the four wild edible mushrooms are compared in Figure 3. The composition and content of amino acids are important indicators of the nutrition and flavour of foods. As shown in Figure 4, the signal intensities of L-isoleucine and pipecolic acid were significantly higher in *Tri. matsutake* than in the other three wild edible mushrooms ($p < 0.05$), with the lowest level in *B. edulis*. In contrast, the signal intensity of L-glutamine was the highest in *B. edulis*, and this was significantly different from that of the other three wild edible mushrooms ($p < 0.05$). The amino acids were divided into four categories according to their different flavour characteristics: umami, sweet, bitter, and tasteless amino acids, among which, umami and sweet amino acids are the main contributors to the taste of wild edible mushrooms (Yang *et al.,* 2001). In addition, some authors opined that mushrooms could be good essential amino acid sources (Bárbara *et al.,* 2008; Beluhan and Ranogajec, 2011; Sun *et al.,* 2017). L-isoleucine is a bitter amino acid and also an essential amino acid (Manninen *et al.,* 2018). Pipecolic acid is a sweet amino acid and a critical regulator of inducible plant immunity (Návarová *et al.,* 2012). L-glutamine is a sweet amino acid and a precursor required for the synthesis of reduced glutathione, the source of amino acids in protein and nitrogen for synthesis of ammonia-containing biological substances. Reduced glutathione is closely related to tissue growth and repair, and plays an important role in the life activities of wild mushrooms (Lacey and Wilmore, 2010). Besides amino acids, sugars contribute greatly to the pleasantly sweet taste of wild edible mushrooms (Litchfield, 1967). There was a significant difference in the signal intensities of α-D-glucose among the four wild edible mushrooms ($p < 0.05$), and the descending order of the signal intensities was *B. edulis* > *C. cibarius* > *Tub. aetivum* > *Tri. matsutake*. Organic acids are non-volatile flavour substances in edible mushrooms, which could improve and synthesise their taste. In the present work, nonanedioic acid, adipic acid, and suberic acid were identified as differential low molecular weight compounds. Nonanedioic acid had the highest VIP score in negative ion mode, and its signal intensity in *Tub. aetivum* was significantly higher than that of the other three wild edible mushrooms ($p < 0.05$), with the lowest level in *B. edulis*. However, adipic acid had the highest signal intensity in *B. edulis*, followed by *Tub. aetivum, Tri. matsutake*, and *C. cibarius*. The signal intensity of suberic acid was the highest in C. cibarius and was significantly different from that of the other three wild edible mushrooms ($p < 0.05$). In addition, the signal intensity of pantothenic acid in *Tri. matsutake* was significantly higher than that of the other three wild edible mushrooms ($p < 0.05$). Pantothenic acid is a water-soluble vitamin and maintains normal physiology; as important precursor for the biosynthesis of coenzyme A and acyl carrier protein. It is also involved in the metabolism of carbohydrates, fatty acids, proteins, and energy in mushrooms, and synthesised by an enzymatic reaction in organism cells (Tahiliani and Beinlich, 1991). The VIP score of betaine among the four wild edible mushrooms was the highest in positive ion mode. *Tub. aetivum* had the highest signal intensity of betaine, followed by *Tri. matsutake*, and *B. edulis* had the lowest. Betaine is a quaternary ammonium alkaloid and can give mushrooms a refreshing sweetness. It has a long history in Japan as a flavour compound used to imitate seafood products. It is a quaternary ammonium alkaloid with important physiological functions widely present in mushrooms, and acts as an osmotic and methyl donor, which promotes the breakdown of cells, proteins, and enzymes from environmental pressures (Craig, 2004). Choline is a metabolic precursor of betaine. The signal intensity of choline was the highest in *Tub. aetivum*, and the lowest in *B. edulis*. Choline is a component of biofilm and has physiological functions by promoting fat metabolism and regulating apoptosis. In 1998, choline was officially recognised as an essential nutrient by the Institute of Medicine (IOM). Choline-deficiency is thought to have an impact on diseases such as liver disease, atherosclerosis, and neurological disorders (Zeisel and Kerry-Ann, 2010). The signal intensity of sphingosine in *B. edulis* was the highest when compared with the other three wild edible mushrooms. Sphingosine is a product of

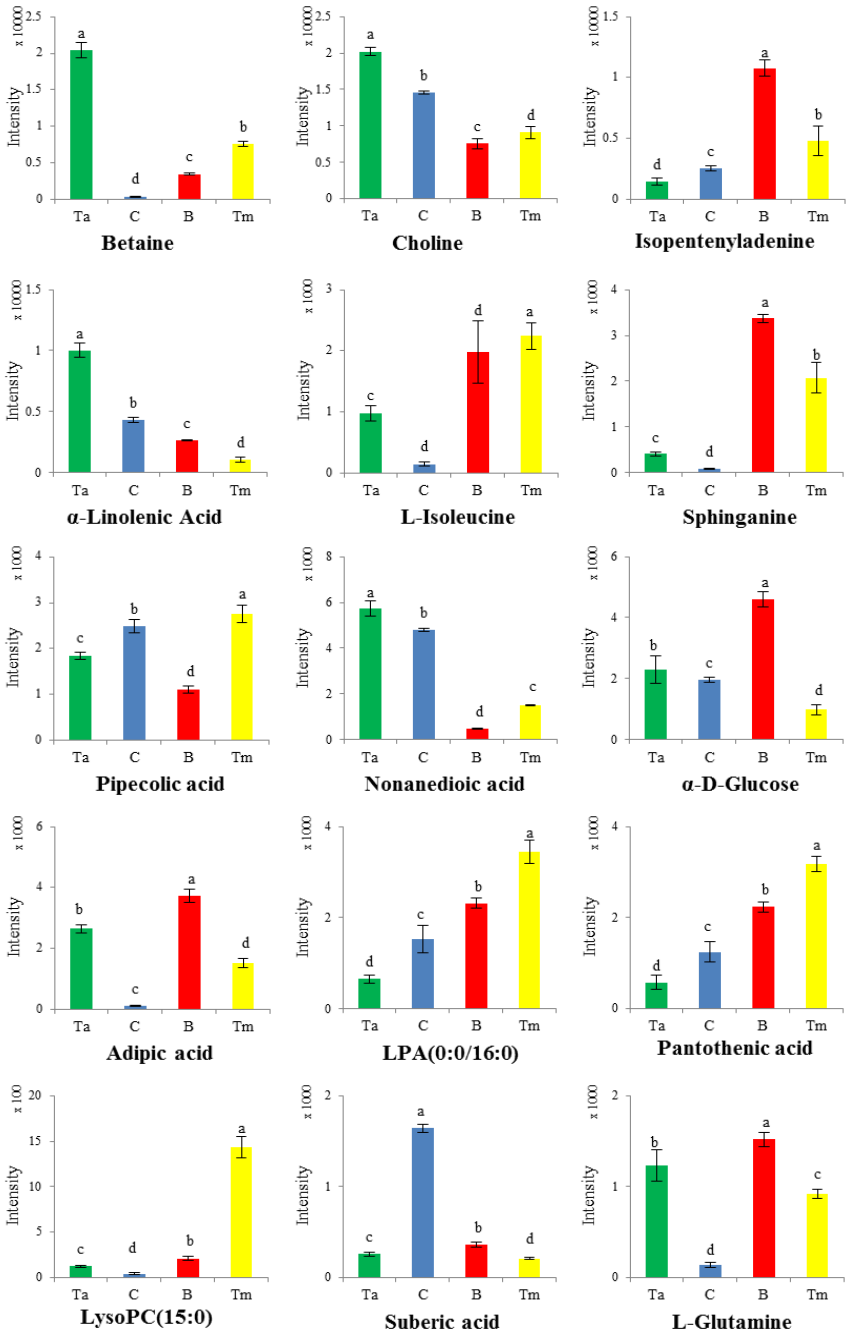

Figure 3. Differential low molecular weight compounds in four wild edible mushrooms (green bars and "Ta" represent *Tub. aetivum*, blue bars and "C" represent *C. cibarius*, red bars and "B" represent *B. edulis*, yellow bars and "Tm" represent *Tri. matsutake*).

sphingomyelinase hydrolysis of sphingomyelin, the parent compound that synthesises sphingolipids *in vivo*, and also one of the important components in biofilms. In the present work, components in mushrooms were detected using an LC-MS-based metabolomics approach. The identified components included amino acids, nucleic acids, sugars, organic acids, and some substances that are difficult to detect by general methods such as betaine, choline, and L-glutamine. These substances also have important effect to the edible quality of wild mushrooms. The use of metabolomics approaches to study mushrooms has been more applied in the determination of components of medicinal interest, with no report of compounds affecting the flavour. The present work has made an attempt in this respect.

Conclusions

In the present work, a total of 32 fatty acids were identified in four edible wild mushrooms. The fatty acid profiles were different, but linoleic, oleic,

and palmitic acids were the most abundant in the four edible wild mushrooms. Low molecular weight compounds of the four edible wild mushrooms mainly included amino acids, sugars, organic acids, nucleic acids, and vitamins. Multivariate statistical analysis revealed 15 compounds (betaine, choline, isopentenyladenine, α-linolenic acid, L-isoleucine, sphinganine, pipecolic acid, nonanedioic acid, nonanedioic acid, α-D-glucose, adipic acid, LPA (0:0/16:0), pantothenic acid, LysoPC (15:0), suberic acid, and L-glutamine) as the differential low molecular weight compounds among the four edible wild mushrooms. The present work suggests that similarities and differences in the compounds in these four edible wild mushroom species would affect their flavour and nutrition.

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