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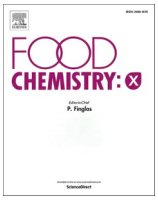

Comprehensive investigation on non-volatile and volatile flavor compounds in the *Morchella sextelata* and *Morchella importuna* by UPLC-MS/MS and GC × GC-TOF-MS

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ABSTRACT

Morchella sextelata and *Morchella importuna* are the main cultivars of morel. However, the key compounds affecting their flavors (taste and odor) are currently unknown. Here, an ultra performance tandem mass spectrometry combined with two-dimensional gas chromatography-time-of-flight mass spectrometry method was used to detect and relatively quantify the metabolites in both morel cultivars. A total of 631 non-volatile compounds and 242 volatile compounds were identified. The odor activity value was calculated to assess the contribution of key odor volatile. The results indicated that *M. importuna* had a sweeter flavor than *M. sextelata*. The former posed more prominent mushroom flavor than the latter based on the correlation analysis of the metabolites. The flavor differences of the two morel cultivars are highly relevant with the content of lipids, carbohydrates, amino acids and derivatives, alcohols and ketones. This study provides new insights into the theoretical basis for the flavor differences in both morel cultivars.

Introduction

The morel (*Morchella* sp.) belongs to the family *Morchellaceae*, which has attracted great interest due to its high nutritional value, unparalleled pharmaceutical benefits, economic importance, and unique culinary flavor (Tietel & Masaphy, 2018; Li, Chen, &Zhang, 2023). Morel is considered the second most delicious edible mushroom in Europe, and it is even considered the best edible mushrooms in the North America (Pilz, 2007). Morel is abundant in wild growth worldwide (Li, Chen et al., 2023). In addition, morel has also been successfully artificially cultivated since 2012 in China and is rapidly becoming a new edible mushroom industry with an annual output reached 91,081 tons by the end of 2021 (https://bigdata.cefa.org.cn/).Various varieties of morels have been artificially cultivated, including *Morchella sextelata*, *Morchella importuna*, *Morchella conica*, and *Morchella septimelata*, etc (Liu, Ma, Zhang, & Dong, 2018), among which the most commonly cultivated morels in China are *M. sextelata* and *M. importuna*, which have better commercial characteristics.

The mushroom flavor (odor and taste) is mainly originated from the volatile organic compounds (VOCs) and the non-volatile organic compounds (non-VOCs) (Aisala et al., 2020). Odorous compounds mainly include alcohols, aldehydes, ketones, esters, acids, and heterocycles, among which the eight-carbon-containing (C8) compounds, such as 1-octen-3-ol, 3-octanol, 1-octanol, 1-octen-3-one, and 3-octanone, are the major contributors to the mushroom odor (Zhang, Zhang, & Mujumdar, 2021). The taste substances of mushrooms are attributed to glutamic acid, aspartic acid, organic acid, and 5′-nucleotides, etc (Zhang et al., 2021). The enhanced flavor is not the result of a simple accumulation of these substances, but is rather due to the synergistic interactions between them. The flavor is not only one of the decisive factors of mushroom quality, but also an important factor influencing consumer preference (Zhang et al., 2021). Like the most edible mushrooms, the morel is rich in non-VOCs, including amino acids, organic acids, nucleotides, carbohydrates, etc (Deng et al., 2021; Dong et al.,

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2022; Yang et al., 2022). In addition, researchers have also been performed on the volatile composition in morel (Tietel & Masaphy, 2022; Li, Zhang et al., 2023). However, there are few studies that provide a comprehensive overview of the non-volatile metabolisms, aroma profiles, flavor differences of *M. sextelata* and *M. importuna*. Thus, to understand the contributions of different metabolite classes, there remains a need for a coherent investigation on the identification and quantification of the metabolites in two morel cultivars.

A qualitative and quantitative analysis of non-VOCs and VOCs can be achieved by metabolomics (Dong et al., 2022; Tietel & Masaphy, 2022; Zhao, E et al., 2022; Wu et al., 2022). Ultra performance tandem mass spectrometry (UPLC-MS/MS) based metabolomics analysis has been widely applied in non-VOCs analysis due to the advantages of high throughput, fast separation, high sensitivity, and wide coverage (Mei, He, & Zhang, 2022; Zou et al., 2020). Mei (Mei et al., 2022) identified a total of 938 metabolites in radish taproots by UPLC-MS/MS and analyzed the major flavor compounds including glucosinolates, polyphenols, carbohydrates, organic acids, amino acids, vitamins, and lipids. Zou (Zou et al., 2020) characterized the metabolite profiles of "Baiyu" (white-fleshed) and "Zaozhong No.6" (yellow-fleshed) loquats, and the results suggested that taste differences between the cultivars could be explained by variations in the composition and abundance of carbohydrates, organic acids, amino acids, and phenolics. Gas chromatography-mass spectrometry (GC–MS) based metabolomics analysis has been widely applied in VOCs analysis because it can identify specific chemical compounds corresponding to odor substances (Tietel & Masaphy, 2022; Yin et al., 2022; Li, Zhang et al., 2023). The aroma volatile compositions of *M. rufobrunnea*, *M. importuna*, and *M. dunalii* have been analyzed by using headspace GC coupled to MS (HS-GC–MS) (Tietel & Masaphy, 2022). A total of 16 VOCs could be used as the characteristic markers to distinguish low-grade from high-grade Yichang big-leaf green tea by HS-GC–MS combined with multivariate statistical analysis (Yin et al., 2022). The volatile profiles of *M. sextelata* are also investigated by GC × GC-TOF-MS, and the characteristic flavor substances of *M. sextelata* were 1-octen-3-ol, hexanal, and benzaldehyde as well as the unreported compounds such as 3-octanol, 3-octenone, 2-octenone, and 1-octen-3-one (Li, Zhang et al., 2023).

Currently, few information about the identification and comparative analysis of the non-VOCs and VOCs compounds in the morel varieties are available. Moreover, little is known about the flavor differences of the morel cultivars (Tietel & Masaphy, 2022). In the present study, *M. sextelata* and *M. importuna* were selected as the research materials, and their metabolites were identified and relatively quantified via UPLC-MS/MS and GC × GC-TOF-MS. Multivariate analysis was used to profile the VOCs and non-VOCs in two cultivars of morel, and the correlation between the flavor compounds and the morel species were discussed. To our knowledge, this study was the first time to comprehensively analyze the VOCs and non-VOCs in two cultivars of morel and study their flavor differences. The results could provide the useful data for clarifying the flavor differences in morel cultivars and further guide breeding research.

2. Materials and methods

2.1. Chemical reagents and materials

Methanol, acetonitrile, formic acid (HPLC grade) and SPME fiber (50/30 μm DVB/CAR/PDMS) were purchased from Merck (Darmstadt, Hesse, Germany). Ultrapure water was prepared on a Millipore purification system (Bedford, Massachusetts, UK). Standards, including 2-octanol (purity ≥ 99.5 %), *n*-alkanes (C7–C40) (chromatographic purity) and L-2-chlorophenylalanine (purity ≥ 98 %) were purchased from Sigma-Aldrich (St. Louis, MO, USA).

2.2. Morel cultivation experiment

From March 2021 to July 2021, the two morel varieties *M. sextelata* and *M. importuna* (Fig. 1) were cultivated under the same growth conditions and the cultivation base (32°57′10″ N, 100°44′9″ E, altitude 3486.7 m), which is located in Banma County, Tibetan Autonomous Prefecture of Golog, Qinghai, China. The standard field management was carried out during the morel growth. Exogenous nutrition bags (50 % of wheat, 25 % of sawdust, and 23.5 % of rice husk mixed with 1.5 % gesso powder) were uniformly prepared from the same batch of raw materials. The ripening date of each cultivar was recorded. A portion of 1 kg morel samples was collected from the four directions of the greenhouse, and pooled to form one biological sample. A minimum of three biological samples were created for each morel cultivar. The species identification of *M. sextelata* and *M. importuna* was performed by morphological characteristics and molecular sequence (ITS 1 and ITS 4 regions). The sequencing results were compared with the published sequences in the National Center for Biotechnology Information (NCBI). The strains of *M. sextelata* and *M. importuna* were identified with 99.71 % and 100 % identities compared with sequences MH468777.1 and MG121861.1, respectively.

2.3. Sample preparation

The morel samples were freeze-dried in vacuum freeze-dryer (Scientz-100F). Afterwards, the samples were crushed by using a mixer mill (MM 400, Retsch) with a zirconia bead for 1.5 min at 30 Hz, and then stored in a refrigerator at −80 °C until the sample extraction.

2.4. Analysis of non-VOCs in morel samples

2.4.1. Sample extraction

The three portions (100 mg homogenized, sieved and lyophilized powder for each) of *M. sextelata* (QH-M.s1, QH-M.s2, and QH-M.s3) and *M. importuna* (QH-M.i1, QH-M.i2, and QH-M.i3) were accurately weighed and dissolved in 1.2 mL of 70 % methanol solution containing an internal standard (L-2-chlorophenylalanine, 1 mg/mL). The samples were vortexed 30 s every 30 min for six times to facilitate the extraction and then stored at 4 °C. The mixture was then centrifuged at 10,000 g for 10 min. The non-VOC extracts were filtrated through a 0.22 μm microporous membrane (Anpel, Shanghai) and stored in amber autosamplers vial for UPLC-MS/MS analysis.

2.4.2. UPLC-QTOF-MS analysis

The qualitative analysis of non-VOCs in morel was performed using an UPLC-QTOF-MS system (UPLC-Triple TOF 6600, Sciex, Applied Biosystems, CA, USA). The samples were chromatographically separated on an Agilent SB-C18 column (100 mm × 2.1 mm, 1.8 μm). The flow rate

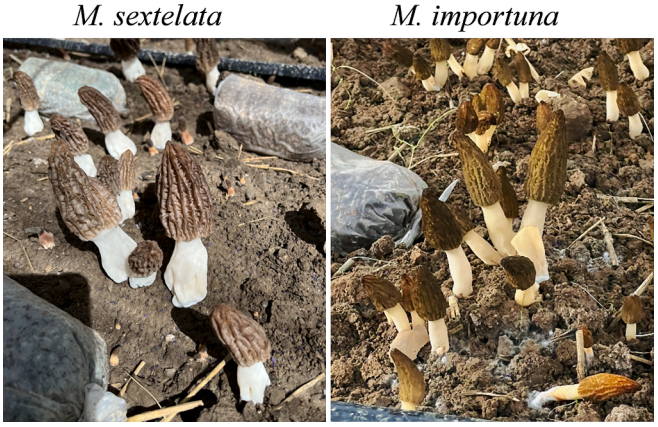

Fig.1. Morphological characteristic of *M. sextelata* and *M. importuna*.

was set as 0.35 mL/min, and the column was maintained at 40 °C. The mobile phase A was water containing 0.1 % (v/v) formic acid, and the mobile phase B was methanol containing 0.1 % (v/v) formic acid. The linear gradient program was as follows: 0–9 min, 5 %-95 % B; 9–10 min, 95 % B; 10–11.1 min, 95 %-5% B; 11.1–14 min, 5 % B. The sample volume injected was 4 μL. Data acquisition was performed in full scan mode (mass range from 50 to 1000 Da) with the information-dependent acquisition (IDA). The ESI source parameters were as follows: curtain gas (CUR), 25 psi; ion source temperature, 500 °C; gas 1 (GS1), 50 psi; GS2, 50 psi; ion spray voltage (IS), 5500 V (ESI^+)/-4500 V (ESI^-); declustering potential (DP), 60/-60 V; collision energy (CE), 30/–30 V; collision energy spread (CES), 15 V.

2.4.3. UPLC-QQQ-MS analysis

The quantitative analysis of non-VOCs was performed by the UPLC-QQQ-MS system consisting of SHIMADZU Nexera X2 UPLC system and AB Sciex 4500 QTRAP mass spectrometer. The chromatographic separation conditions were the same as those used for the qualitative analysis. The MS was operated in positive and negative ion modes and controlled by Analyst 1.6.3 software (AB Sciex, Singapore). The ESI source parameters were as follows: the source temperature was set at 550 °C; the IS was 5500 V (positive ion mode)/-4500 V (negative ion mode); the GS1, GS2 and CUR were set at 50, 60, and 25 psi, respectively; and the collision-activated dissociation (CAD) was set to high. The collision gas (nitrogen) of the QQQ scans was set to medium. DP and CE were optimized for individual MRM transitions. A specific set of MRM transitions was monitored for each period in accordance with the metabolites eluted within this period.

2.4.4. Quality control analysis

The mixed sample of the *M. sextelata* and *M. importuna* as a quality control (QC) sample was used to monitor the reproducibility of the assay. During the instrumental analysis, QC sample was analyzed every three test samples.

2.4.5. Qualitative and quantitative analysis of non-VOCs

Compound identification was performed based on the commercial self-built MWDB 2.0 database of Wuhan Metware Biotechnology Co., Ltd. (Wuhan, China), in accordance with the spectrum information including the retention time, accurate mass of precursor ion, product ion. The mass tolerance and retention time tolerance for the peak alignment were set to 20 ppm and 0.2 min, respectively. To ensure the accuracy of the metabolite annotations, the repeating signals containing K^+, Na^+ and NH_4^+, the isotopic signals and other fragment ions of large molecular weight substances were first removed. The metabolites were then quantitatively analyzed via the MRM of QQQ-MS. All the mass spectral peaks were subjected to the area integration, and the mass spectra peak of each metabolite in different samples were integrated and corrected using MultiaQuant software. Chromatographic peak area integrals were used to represent the relative contents of metabolites in each sample.

2.5. Analysis of VOCs in morel samples

2.5.1. Sample extraction

The VOCs of morel were determined by headspace solid-phase microextraction (HS-SPME) according to the articles published by our research group (Li, Zhang et al., 2023). Three portions (1.0 g) of *M. sextelata* and *M. importuna* were accurately weighed into 20 mL headspace bottles and dissolved with 3 mL of saturated sodium chloride solution. A portion of 10 μL 100 mg/L 2-octanol as the internal standard was spiked into the morel extract. After being sealed with a cap, the samples were equilibrated at 55 °C for 20 min and then extracted for 40 min at 55 °C by the preconditioned SPME fiber (50/30 μm DVB/CAR/PDMS) inserted into the headspace bottle. Finally, the fiber was inserted into the GC injection port at 250 °C for 5 min via splitless mode. The samples were used directly for analyzing VOCs by GC × GC-TOF-MS.

2.5.2. GC × GC-TOF/MS analysis

The qualitative and quantitative analysis of VOCs in morels was conducted following the method described by Li, Zhang et al., (2023). Specifically, the VOCs of morels were detected by the GC × GC-TOF-MS system containing an Agilent 7890 GC (Agilent Technologies, CA, USA), a cold-jet modulator, and a time-of-flight mass spectrometer (LECO Corp., St. Joseph, MI, USA). The column set consisted of a DB-WAX (30 m × 250 μm × 0.25 μm) in the first dimension and a lower polar DB-5 (1 m × 100 μm × 0.10 μm) in the second dimension. Helium (99.999 % purity) was used as carrier gas at a flow rate of 1 mL/min. The oven temperature of the first column was set as follows: maintained for 60 °C for 2 min, ramped at 2 °C/min to 170 °C, and finally increased at 5 °C/min to 240 °C and held for 4 min. The modulator was offset by 5–15 °C in relation to the secondary oven, and the modulation time was 4 s. The ion source temperature was set at 220 °C, and the ionization potential of MS was 70 eV. Spectra were collected in a mass range of 33–550 amu with an acquisition rate of 50 spectra/s.

2.5.3. Identification of VOCs and odor description

Data acquisition was performed using ChromaTOF™ Workstation (version 4.71, LECO Corp., St. Joseph, MI, USA). Compounds were tentatively identified coupled with the principle of greater than 75 % similarity to the National Institute of Standards and Technology (NIST) 17 database. The confirmation of identified compounds was done by comparison of the retention indices (RIs) for volatile metabolites determined after injection of the linear formula of *n*-alkanes (C7–C40), with the theoretical RIs from various published databases: Pherobase (https://www.pherobase.com/), Flavornet (https://www.flavornet.org/flavornet.html), NIST Chemistry WebBook (https://webbook.nist.gov/chemistry/) and the LRI database (https://www.odour.org.uk/lriindex.html). In addition, the relative contents of the volatile constituents were calculated on the internal standard method (Li, Zhang et al., 2023; Wang et al., 2023).

Odor description referred to the relevant literatures, or the Good Scent Company web database (https://www.thegoodscentscompany.com/) if no latest literature was available. The odor–activity values (OAVs) of the odor-active compounds were calculated as the ratio of the concentrations and odor threshold values (OTVs) in the water of these compounds obtained according to the LRI database (https://www.odour.org.uk/lriindex.html) or literatures.

2.6. Statistical analysis

Peak areas of identified non-VOCs and VOCs were introduced into a free online platform named Metware Cloud (https://cloud.metware.cn) for multivariate analyses, including clustering heat map, orthogonal partial least squares-discrimination analysis (OPLS-DA) and volcano plot. Metabolites with a fold change (FC) ≥ 2 (upregulated) or ≤ 0.5 (downregulated), a p-value < 0.05, and a variable importance in projection (VIP) value ≥ 1 were defined as significantly different metabolites. All quantitative analyses were performed in triplicate.

3.Results and discussion

3.1. Analysis of non-VOCs in morel samples

3.1.1. Widely targeted metabolic profiling of morel based on UPLC-MS/MS

Widely targeted metabolic profiling based on UPLC-MS/MS was performed to analyze the comprehensive metabolic profiles and investigate the components between morel cultivars. Overlay analysis of the total ion chromatograms (TIC) for three QC samples was applied to assess the repeatability of the metabolite extractions and detections, indicating that the TIC plots of metabolites had a perfect overlap (Fig. S1A and S1B). Furthermore, the Pearson's correlation coefficients

of each sample were greater than 0.98, indicating that the replicates within the group had strong correlation, good repeatability and good sample homogeneity, and thus could be used for subsequent different metabolites analysis (Fig. S2).

After data collation, 631 non-volatile compounds were identified in morel samples, including 152 lipids, 113 amino acids and derivatives, 75 organic acids, 69 nucleotides and derivatives, 65 carbohydrates, 63 phenolic acids, 59 alkaloids, 16 vitamins, 5 triterpenes, 3 flavones, 3 coumarins, 8 others (Fig. 2A and Table S1).

3.1.2. Multivariate analysis of M. Sextelata and M. Importuna

Based on the OPLS-DA score plot (Fig. 2B), the samples in each group were clearly separated, suggesting significant difference in the metabolic phenotypes of the two varieties. In this study, the OPLS-DA model (Fig. 2C) was observed for comparisons between the two varieties of morel ($Q^2 = 0.989$, $R^2X = 0.731$, $R^2Y = 1$). The values of R^2X and R^2Y were greater than 0.5, and the Q^2 value of the comparison groups exceeded 0.9, demonstrating that these models were stable and reliable and they could be used to further screen for different metabolites. The OPLS-DA model was validated with a permutation test (n = 200) to avoid model overfitting.

3.1.3. Identification and classification of different non-VOCs

OPLS-DA was performed to determine significant differences between non-VOCs of *M. importuna* and *M. sextelata*, of which FC $\geq$ 2 or $\leq$ 0.5, VIP $\geq$ 1 and $p < 0.05$ indicated significant differences between non-VOCs. A total of 253 significantly different non-VOCs was screened out (Fig. 2D and Table S2). The different non-VOCs could be divided into 10 different categories, mainly including 79 lipids, 31 amino acids and derivatives, 34 nucleotides and derivatives, 28 alkaloids, 24 phenolic acids, 27 carbohydrates, 9 vitamins, 15 organic acids, 2 flavones, 2 triterpene, 2 lignans and coumarins. Among these, 97 metabolites were up-regulated and 156 metabolites were down-regulated in *M. sextelata* compared with those in *M. importuna* (Fig. 2D). Mushrooms are popular due to their unique culinary flavor, valuable culinary sensation, and richness of health-beneficial bioactive metabolites. In this study, we focused on some non-VOCs, such as lipids, amino acids and derivatives, and carbohydrates, which may be vital contributors to the taste of morels. To further elucidate the significant changes in these non-VOCs, clustering heat maps of these categories are illustrated in Fig. 3A-3C, and the detailed results are presented below.

3.1.4. Different lipids in morel varieties

Mushrooms are nutritionally well-balanced sources with lipids, especially unsaturated fatty acids including linoleic acid, making the mushrooms even healthier (Sande et al., 2019). Lipids are major factors that contribute to food flavor via interactions with other components due to their own degradation during food processing, cooking, and storage (Fereidoon Shahidi, 2020).

As shown in Table S1, morels contain a wide variety of nutritionally important lipids such as unsaturated fatty acids and oleic acid, palmitoleic acid, α-linolenic acid, palmitic acid, stearic acid and myristic acid, which are consistent with the published literatures (Taşkın et al., 2021). Specifically, Table S2 shows that lipids had the greatest differential number (31 % of all different metabolic components), 79 of which were upregulated or downregulated between *M. sextelata* and *M. importuna*, including 27 free fatty acids, 25 LysoPC, 23 LysoPE, 2 glycerol ester, 1 phosphatidyl choline, and 1 sphingolipid (Fig. 3A).

Meanwhile, a total of 76 lipids were upregulated in *M. importuna* compared with *M. sextelata*, except for choline alfoscerate, octadeca-11E,13E,15Z-trienoic acid, and 1-(9Z-octadecenoyl)-2-(9-oxo-nonanoyl)-*sn-glycero*-3-phosphocholine. Unsaturated fatty acids, the healthy type of fat presenting the anti-oxidative abilities, are main fatty acid in morel (Li, Chen et al., 2023). Punicic acid (PA) is referred as a "super

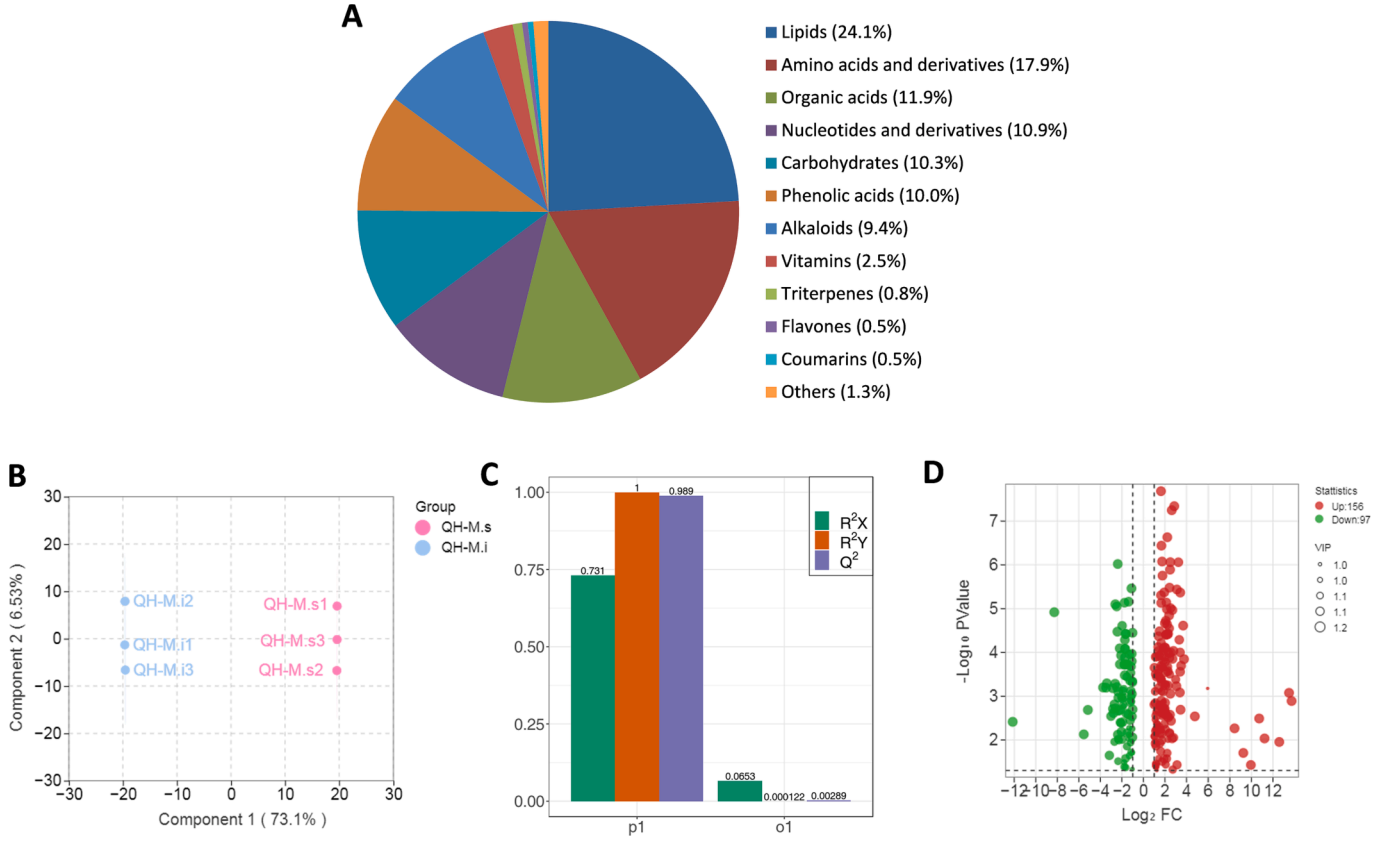

Fig.2. Non-VOCs in *M. sextelata* and *M. importuna*. (A) Pie chart of the biochemical categories of the 631 non-VOCs. (B) Score plot generated from OPLS-DA. (C) OPLS-DA model. (D) The volcano plot of the different non-VOCs in *M. sextelata* and *M. importuna*.

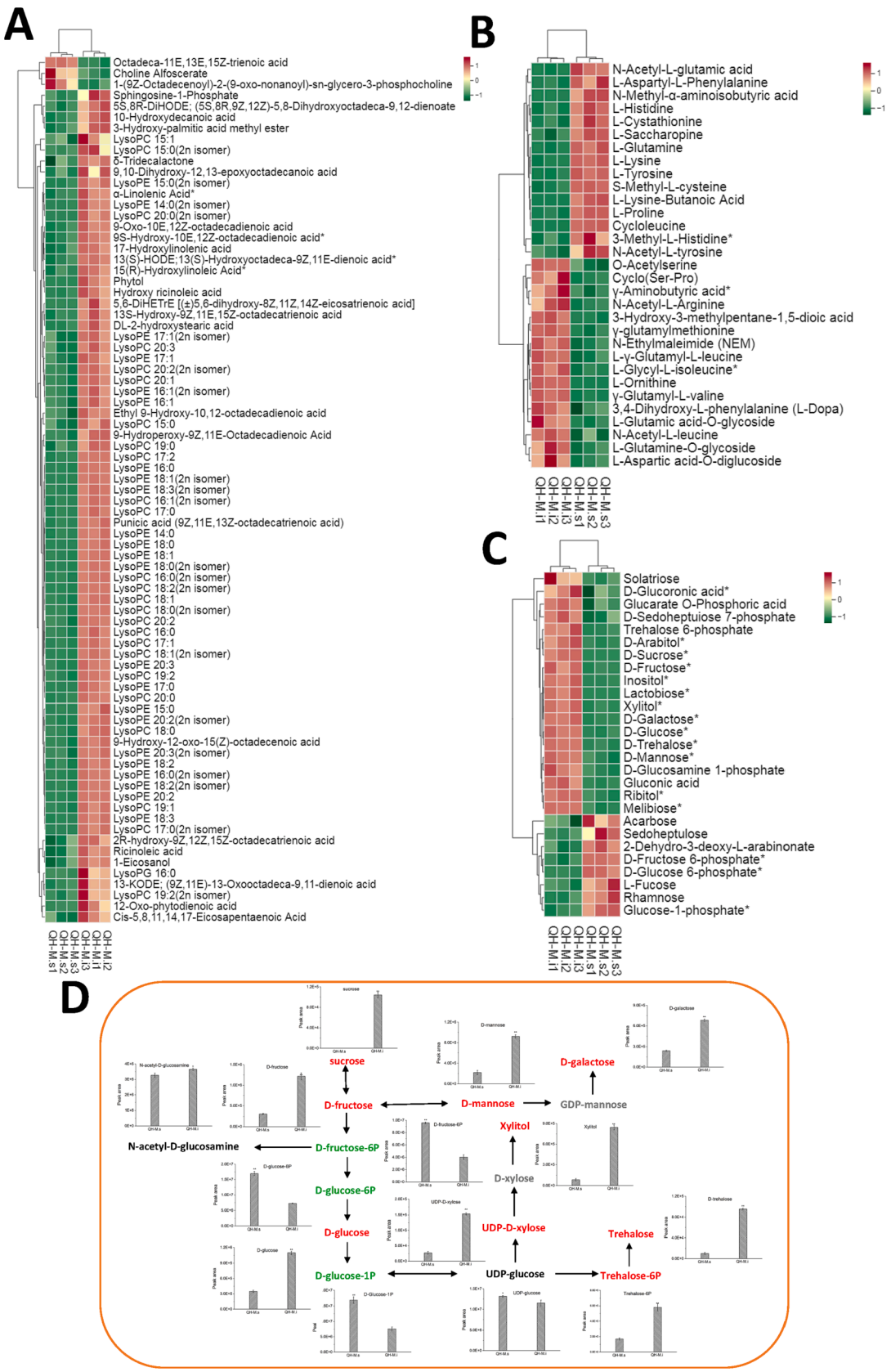

Fig.3. Clustering heat maps of the contents of significantly different lipids (A), amino acids and derivatives (B), and carbohydrates (C) in *M. sextelata* and *M. importuna*. The color indicates the level of accumulation of each metabolite, from low (green) to high (red). (D) Proposed pathway of the main sugars metabolism in morel. The red and green words indicate that these compounds upregulated and downregulated in *M. importuna* compared to *M. sextelata*, respectively. The black words indicate that these compounds have no significant difference. The gray words indicate that these compounds have not been detected in this study. *, $p < 0.05$; **, $p < 0.01$. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

conjugated linolenic acid", which is an ω-5 long-chain polyunsaturated fatty acid (PUFA) possessing a wide array of biological properties, including antidiabetic, anti-obesity, anti-proliferative, and anti-carcinogenic activities against various forms of cancer (Guerra-Vázquez et al., 2022). α-Linoleic acid, one of the two PUFAs, is associated with improved intelligence, antithrombotic effect, and liver protection (Burdge, 2006). PUFAs are the precursors of some short-chain fatty acids which are known to contribute to a wide range of health benefits in fungi including anti-inflammation, anti-cancer, and maintaining gut health (Li, Chen et al., 2023). Those two lipids are generally higher in *M. importuna* compared to *M. sextelata*, indicating the potential health-promoting effects of *M. importuna*.

In addition, the previous studies have shown that phosphatidylcholine and phosphatidylethanolamine can induce lipid degradation under heating to form the carbonyl metabolites such as hexanal, 2,4-dienal, and 1-octen-3-one (Lin, & Blank, 2003). Fatty acids, such as linoleic acid as flavor precursors in mushrooms could form 1-octen-3-ol and 1-octen-3-one (Tasaki et al., 2019). These indicated that fatty acid and phospholipid degradation may be closely related to the aroma formation in morel. The difference in the accumulation of lipids may also contribute to flavor difference between the two varieties of morel, i.e., and *M. importuna* may have better mushroom flavor because of its higher abundance of lipids.

3.1.5. Different amino acids and derivatives in morel varieties

Amino acids are chiefly divided into umami, sweet, bitter, and tasteless categories according to their taste characteristics (Davila, Muniz, & Du, 2022). Amino acids are one of the most important taste active compounds in mushrooms; for example, MSG-like amino acids (Glu and Asp) could induce umami taste (Davila et al., 2022). The composition, content, and degradation of amino acids could affect the flavor quality (Davila et al., 2022). The complicated sour and mouth-drying taste of morels are also partially ascribed to their abundant amino acid content (Li, Chen et al., 2023). As shown in Table S2 and Fig. 3B, 31 amino acids and derivatives were differentially accumulated, and all were significantly different in the two cultivars (Student's *t* test, $p < 0.05$).

In this class, 15 kinds of amino acids and derivatives were significantly greater in *M. sextelata* than that in *M. importuna*. Of these, the different metabolites in *M. sextelata* were mainly free amino acids (Table S2), including L-proline, L-lysine, L-histidine, L-tyrosine and L-homocysteine. L-lysine is the major essential amino acid in most mushroom (Li, Chen et al., 2023). The content of L-lysine in *M. sextelata* was 5.3 times of the content of *M. importuna*. L-histidine and L-tyrosine with bitter flavor, were significantly higher in *M. sextelata* than that in *M. importuna*. L-glutamine is the dominant different metabolite in the *M. sextelata*. It has been used as a nutritional supplement and flavor enhancer in the food industry, and it plays a fundamental role in the cardiovascular physiology and pathology (Durante, 2019).

A total of 16 kinds of amino acids and derivatives were significantly greater in *M. importuna* than that in *M. sextelata*. The contents of L-ornithine and γ-aminobutyric acid (GABA) in *M. importuna*, which are two unusual non-protein composition amino acids, were 6.5 times and 3.0 times higher than that in *M. sextelata*. GABA exhibited a slightly sour taste except for the typical mouth-drying and mouth-coating sensation. It has been demonstrated that a threshold concentration of 0.02 mmol/L GABA can result in the mouth-drying effect (Rotzoll, Dunkel, & Hofmann, 2005). So, the mouth-drying sensation of *M. importuna* is stronger compared with *M. sextelata*. L-ornithine is a kokumi substance exhibiting a slightly sweet taste (Mizuta, Kumamoto, Ugawa, & Yamamoto, 2021). Kokumi described a complicated taste sensation by thickness, richness, mouthfulness, and continuity (Li, Zhang, & Lametsch, 2022). In addition, the contents of γ-glutamyl peptides, including γ-glutamine-L-valine, γ-glutamine-L-leucine, and γ-glutamine methionine, were 4.7, 2.6, and 2.7 times higher in *M. importuna* than that in *M. sextelata*, respectively. Thus, the small peptides in *M. importuna* may produce better kokumi taste compared with *M. sextelata*, which can improve the nutritional value and sensory quality.

3.1.6. Different carbohydrates in morel varieties

The carbohydrates present in mushroom fruit bodies could produce sweetness, and they are the main ingredients that determine the taste of edible fungi (Liu et al., 2022). As shown in Table S1, 65 carbohydrates were identified in morel cultivars. Based on fold changes, VIP values and p-values, 27 carbohydrates were identified as differentially accumulated (Fig. 3C). Among them, 12 important different compounds related to the biosynthesis and degradation of these carbohydrates were identified in this study (Fig. 3D).

The eight upregulated carbohydrates in *M. sextelata*, including 2-dehydro-3-deoxy-L-arabinonate, rhamnose, L-fucose, sedoheptulose, D-fructose 6-phosphate, glucose-1-phosphate, D-glucose 6-phosphate, and acarbose, are the mainly phosphorylated six-carbon sugars. Among them, it was proved that acarbose can reduce risk of cardiovascular incidents (Bhatnagar, & Mishra, 2022). The results maight support the use of morel as natural antidiabetic agents. So, morels, especially *M. sextelata*, could be beneficial to human health. Nineteen other carbohydrates were upregulated in *M. importuna* compared with *M. sextelata*, most of which are monosaccharide and disaccharide. In particular, the contents of xylitol, D-fructose, D-mannose, gluconic acid, D-glucose and inositol in *M. importuna*, were 10, 4, 4.2, 4.1, 3.1 and 3.6 times of the contents of *M. sextelata*, respectively (Table S2, Fig. 3C and 3D). Sucrose was more prevalent in *M. importuna* than in *M. sextelata*, with a FC value of 13.5. D-glucose and D-fructose have higher sweetness. Gluconic acid and xylitol possess the equal sweetness as sucrose (Zhao, Duan, Hou, Liu, & Sun, 2022). D-mannose and inositol achieve more than half the sweetness of sucrose (Zhao, Duan et al., 2022). These carbohydrates provide a material basis for rich-sweet flavor in *M. importuna*. Thus, the results denoted that difference in the accumulation of the composition and content of carbohydrates could contribute to the taste difference between the two varieties of morel.

3.2. Identification of characteristic VOCs in morels

3.2.1. Multivariate analysis revealed VOCs differences between M. Sextelata and M. Importuna

The VOCs in morels analyzed by GC × GC-TOF-MS were visualized in the form of three-dimensional chromatograms (Fig. 4). The GC × GC-TOF-MS data was compared with the NIST 2017 mass spectral database and the LECO/Fiehn Metabolite mass spectral library. By using a match threshold > 650 and a RI deviation < 15 % followed by the manual supervision, a total of 242 VOCs were identified, mainly small-polar compounds including 42 heterocycles, 36 ketones, 31 alcohols, 28 aromatic compounds, 24 aldehydes, 17 acids, 16 hydrocarbons, 14 esters, 11 S-containing compounds, and 24 other compounds (Table S3).

According to the OPLS-DA score plot (Fig. 5A), *M. sextelata* and *M. importuna* were distinguished and divided into two uncorrelated sections, suggesting that there were significant differences in the two varieties. The R^2X and Q^2 values of the OPLS-DA model (Fig. S3) established with VOCs in *M. sextelata* and *M. importuna* were 0.797 and 0.992, respectively. This result indicated the model had good stability and prediction ability and that it could be used to identify and distinguish *M. sextelata* and *M. importuna*. With VIP ≥ 1, $p < 0.05$ and FC ≥ 2 or ≤ 0.5 as the screening condition, 87 different VOCs (37 upregulated and 50 downregulated) were found in *M. importuna* compared with *M. sextelata* (Table S4), as shown in the volcano plot (Fig. 5B) and clustering heat map (Fig. 5C).

3.2.2. Key odor compounds of M. Sextelata and M. Importuna

The contribution of VOCs to the aroma characteristics of *M. sextelata* and *M. importuna* cannot be determined only by the content of VOCs, and the contribution of the volatiles to the overall aroma of the morel depends on the OAVs. The OAVs of the volatile substances are calculated

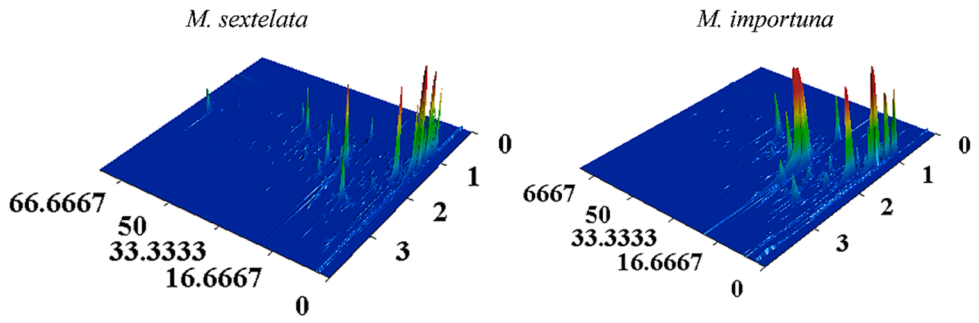

Fig.4. Profiles of VOCs isolated from *M. sextelata* and *M. importuna* via GC × GC-TOF-MS. Column I represented the retention time of the compounds, and column II referred to the chemical polarity. Each colored peak represented a VOC. Different color indicated varying intensity, with red representing high intensity and blue indicating not present. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

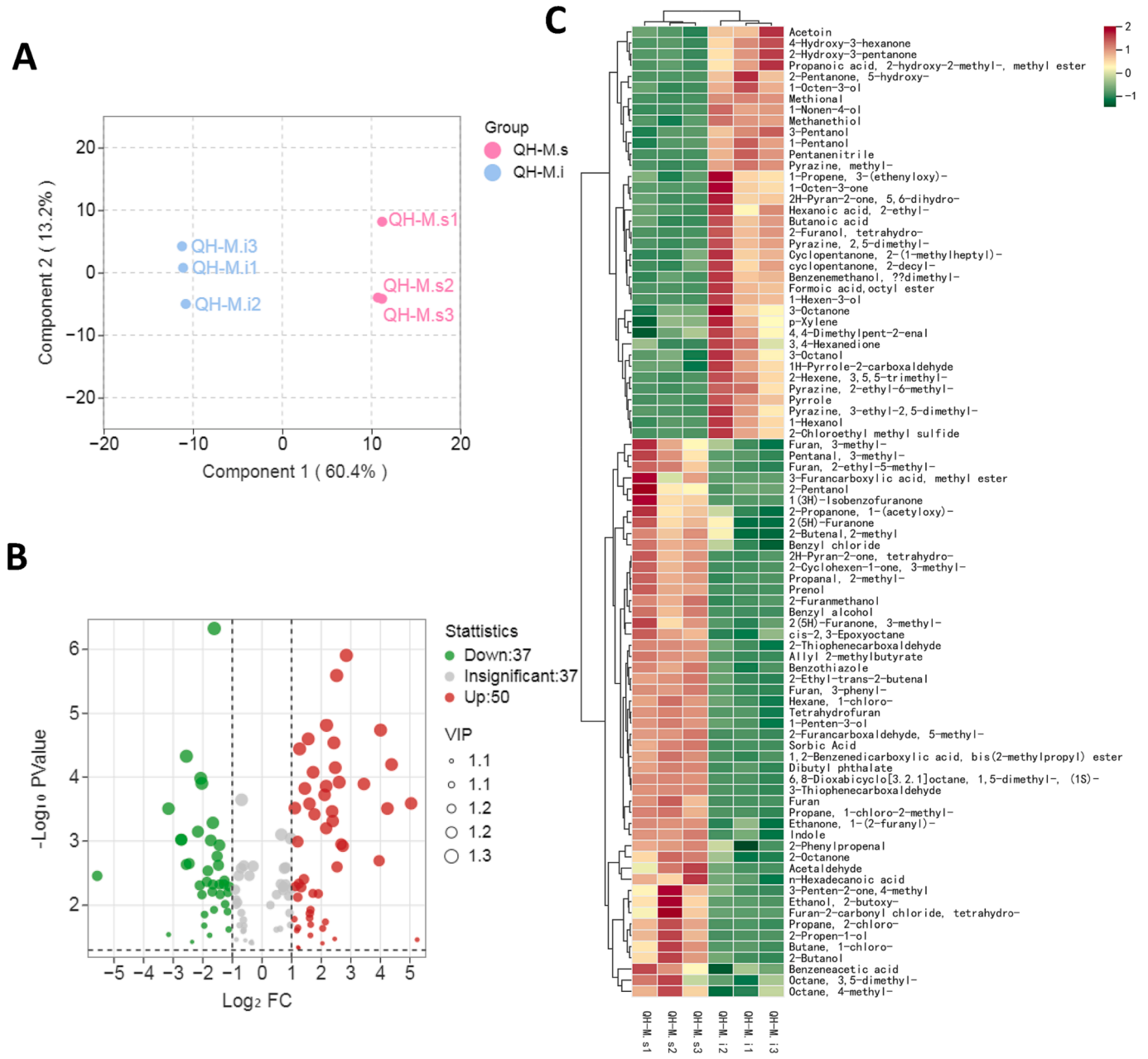

Fig.5. Multivariate analysis of VOCs in *M. sextelata* and *M. importuna*. (A) Score plot generated from OPLS-DA. (B) The volcano plot of the different VOCs. (C) Clustering heat map of different VOCs. The color indicates the level of accumulation of each metabolite, from low (green) to high (red). (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

as the ratio of the concentrations and OTVs, reflecting a tendency for the contribution of volatiles to mushroom flavor (Fan et al., 2021). Therefore, the OAVs were introduced to further explore the key VOCs of *M. sextelata* and *M. importuna*. VOCs are generally regarded as active aroma compounds at OAVs > 1, and VOCs with high OAV are considered the major contributors to the aromatic characteristics of food (Zhuang et al., 2020). Among them, 1-octen-3-one, 1-octen-3-ol, 2-octanone, 3-octanone, methanethiol, methional, acetaldehyde, isobutyraldehyde and 2-methylpyrazine were the volatiles with OAV > 1 in all samples. Thus, these nine volatile compounds with the highest OAVs (OAV > 1) were selected as the key odors contributing to the flavor profile of morel (Table 1).

The main VOCs with higher abundance in *M. sextelata* were acetaldehyde (OTV = 15 μg/L), isobutyraldehyde (OTV = 34.69 μg/L), and 2-octanone (OTV = 50 μg/L), whose OAVs were above 1. Acetaldehyde was an important contributor to the pungent, fresh, aldehydic, refreshing and green odor of *M. sextelata*. Isobutyraldehyde, which was significantly higher in abundance in the *M. sextelata*, had pungent-, malt-, and green-like odors. 2-Octanone was likely to be a key contributor to the odor characteristic (earthy, weedy, natural, woody, herbal) of *M. sextelata* due to its lower odor threshold.

The distinct different VOCs in *M. importuna* contained alcohols, ketones, S-containing compounds and pyrazines. 1-Octen-3-ol, known as mushroom alcohol for its characteristic mushroom flavor, was the most abundant VOC in morel. In addition, some VOCs identified in morel, such as 3-octanone and 1-octen-3-one, were critical contributors to the aroma profile of morel because of their low odor thresholds, and they were more abundant in *M. importuna* than that in *M. sextelata*. These also make the sensory score of mushroom notes of *M. importuna* samples higher than *M. sextelata* samples. In particular, the OAV of 1-octen-3-ol (OTV = 1 μg/L) was extremely higher than 1 in all morel samples, followed by methanethiol (OTV = 0.59 μg/L), methional (OTV = 7.1 μg/L) and 1-octen-3-one (OTV = 0.016 μg/L). Meanwhile, the OAV of 3-octanone (OTV = 28 μg/L) was above 1, and it was characteristic VOC in *M. importuna* sample. 2-Methylpyrazine, as meat-flavored pyrazine, was the typical VOC in *M. importuna* sample (Table 1).

3.3. Correlations between non-VOCs and VOCs in morels

Various fatty acids, amino acids and carbohydrates in food as aroma precursors were catalyzed to form numerous volatile compounds under the action of some enzymes (Zhang, Zhang, & Mujumdar, 2021). As mentioned above, C8 compounds, namely, 1-octen-3-ol, 1-octen-3-one, and 3-octanone, as the characteristic volatile substances of mushroom (Zhuang et al., 2020), were found to be critical contributors to the mushroom aroma of morel, especially *M. importuna*. They are usually produced by the lipids catalyzed through lipoxygenase and peroxide lyase within mushrooms. Unsaturated fatty acids are easy to oxidize due to their double bonds and forming of peroxides, which are further decomposed into ketones, aldehydes, acids, and other volatile carbonyl compounds, thus producing a unique aroma (Domínguez et al., 2019). 1-Octen-3-ol and 1-octen-3-one are proceeded through the oxidation of linoleic acid by lipoxygenase or dioxygenase and subsequent cleavage by hydroperoxide lyase (Tasaki et al., 2019). This result (Fig. 6) was consistent with the finding in section 3.1.4 that the lipids contents were significantly higher in *M. importuna* than that in *M. sextelata*. It indicated that lipid metabolites might be a good indicator to assess the varieties of morel samples and an important cause of flavor difference between the two morels.

Importantly, we identified that *M. importuna* contained a lower concentration of bitter flavor amino acids (L-histidine and L-tyrosine) and a higher concentration of monosaccharides such as sucrose, glucose, and fructose, which may partially reveal the fact that *M. importuna* has a rich-sweet flavor. In addition to the contribution to taste, the combination of amino acids and sugars was also considered important precursors to the formation of aroma compounds (Tu et al., 2023). The higher sugar content (sucrose, glucose, and fructose, etc) in Fig. 3C-3D could also increase the levels of pyrazines, furans, ketones, and heterocyclic nitrogen-containing compounds. Carbonyl derivatives produced by amino acids interacting with carbohydrates participate in

Table 1
Identified VOCs in *M. importuna and M. sextelata* detected by GC × GC-TOF-MS.

No.	Classification	Compounds	RIs (exp.) [a]	RIs (ref.) [b]	Concentrations (μg/kg, mean ± SD, n = 3)		Odor [c]	OTVs (μg/L)	OAVs	
					M. importuna	*M. sextelata*			*M. importuna*	*M. sextelata*
1	heterocycles	2-methylpyrazine	1256	1256	128.3 ± 9.2	21.8 ± 3.4	nutty cocoa roasted chocolate peanut green	60[d]	2.1 ± 0.2	0.4 ± 0.06
2	ketones	1-octen-3-one	1291	1317	16.6 ± 5.1	4.3 ± 0.2	herbal mushroom earthy musty dirty	0.016[e]	1037.5 ± 318.8	268.8 ± 12.5
3	ketones	3-octanone	1241	1240	29.5 ± 12.8	5.7 ± 4.1	fresh herbal lavender sweet mushroom	28[d]	1.1 ± 0.5	0.2 ± 0.1
4	ketones	2-octanone	1274	1297	50.5 ± 34.0	188.8 ± 31.5	earthy weedy natural woody herbal	50[d]	1.0 ± 0.7	3.8 ± 0.6
5	alcohols	1-octen-3-ol	1430	1430	2478.3 ± 306.5	914.9 ± 113.2	mushroom earthy green oily fungal raw chicken	1[d]	2478.3 ± 306.5	914.9 ± 113.2
6	S-containing compounds	methional	1434	1480	363.9 ± 6.6	119.0 ± 2.7	musty potato tomato earthy vegetable cream	7.1[f]	51.3 ± 0.9	16.8 ± 0.4
7	S-containing compounds	methanethiol	772	696	23.5 ± 1.5	5.7 ± 1.5	–	0.59[e]	39.8 ± 2.5	9.7 ± 2.5
8	aldehydes	acetaldehyde	648	677	30.1 ± 2.6	64.1 ± 14.6	pungent ethereal aldehydic fruity	15[d]	2.0 ± 0.2	4.3 ± 1
9	aldehydes	isobutyraldehyde	749	808	71.1 ± 3.8	153.6 ± 11.6	fresh aldehydic floral green	34.69[h]	2.0 ± 0.2	4.4 ± 1

[a]RI values are obtained according to determine after injection of the linear formula of *n*-alkanes (C7–C40).
[b]RI values are obtained according to the NIST Chemistry WebBook (http://webbook.nist.gov/chemistry/).
[c] Odor is obtained according to the Good Scent Company web database (http://www.thegoodscentscompany.com/).
[d] Odor threshold values in water are obtained according to the LRI database (http://www.odour.org.uk/lriindex.html).
[e] Odor threshold value in water is obtained in water according to Wagner, Granvogl, & Schieberle (2016).
[f] Odor threshold value in water is obtained according to Song et al (2021).
[g] Odor threshold value in water is obtained according to Li, Schieberle, & Steinhaus (2017).
[h] Odor threshold value in water is obtained in water according to Guan et al (2022).
-, no odor description information was found in the literature.
Values (mean ± standard deviation) of determinations in three replicates.

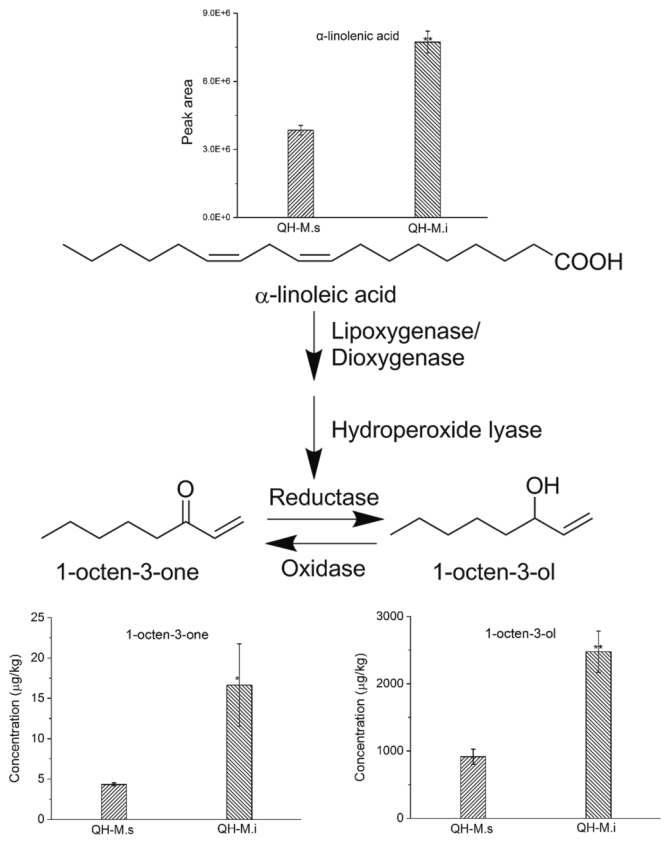

Fig.6. Proposed pathway for the biosynthesis of 1-octen-3-ol and 1-octen-3-one in morels. *, $p < 0.05$; **, $p < 0.01$.

Strecker degradation reaction and then produce the volatile flavor compounds (Zhang, et al., 2021), such as 2-methylpyrazine (screened in section 3.2.2 with significant difference in *M. importuna*).

Overall, the combination of lipids, carbohydrates, amino acids, C8 compounds, and heterocycle compounds works together to provide a special morel flavor. A lower concentration of bitter flavor amino acids and a higher concentration of monosaccharides make *M. importuna* sweeter than *M. sextelata*. *M. importuna* has more prominent mushroom flavor than *M. sextelata* based on the result of systematic comparison of the flavor differences in morel cultivars.

4. Conclusion

In this study, a combination of UPLC-MS/MS and GC × GC-TOF-MS as well as the multivariate statistical analysis was successfully constructed as a suitable platform for the flavor metabolites analysis in *M. sextelata* and *M. importuna*. A total of 631 non-VOCs and 242 VOCs was identified in both morel species. Among them, 253 non-VOCs and 87 VOCs were significant differences. In this study, 76 lipids were significantly enriched in *M. importuna*, and they produced C8 compounds, such as 1-octen-3-ol, 3-octanol, 1-octen-3-one, and 3-octanone, which contributed to the mushroom aroma of morel, especially *M. importuna*, due to their low odor threshold. *M. importuna* has a sweeter flavor than *M. sextelata* because of a lower concentration of bitter flavor amino acids and a higher concentration of monosaccharides. Comparative analysis revealed that the characteristic metabolites differences affected the odor and taste of morels, which were highly relevant with lipid, carbohydrate, amino acid, alcohol and ketone-related compounds. This study provides novel evidence of metabolic profiles underlying flavor differences between *M. sextelata* and *M. importuna*.

CRediT authorship contribution statement

Yanmei Zhang: Methodology, Writing – original draft, Validation, Visualization. **Xiaobei Li:** Methodology, Writing – original draft. **Zhiyong Zhao:** Validation. **Hengchao E:** Data curation. **Tingting Fan:** Validation. **Hui Dong:** Validation. **Xiangwei He:** Validation. **Xiaoyan Zhao:** Conceptualization, Supervision, Funding acquisition, Writing – review & editing. **Lihua Tang:** Supervision, Writing – review & editing. **Changyan Zhou:** Supervision, Writing – review & editing.

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

Additional Supplementary material 1 contains Figs. S1-S3 and Supplementary material 2 contains Table S1–S4. Supplementary data to this article can be found online at https://doi.org/10.1016/j.fochx.2023.100961.

References

Aisala, H., Manninen, H., Laaksonen, T., Linderborg, K. M., Myoda, T., Hopia, A., et al. (2020). Linking volatile and non-volatile compounds to sensory profiles and consumer liking of wild edible Nordic mushrooms. *Food Chemistry, 304*, Article 125403. https://doi.org/10.1016/j.foodchem.2019.125403

Bhatnagar, A., & Mishra, A. (2022). α-Glucosidase Inhibitors for Diabetes/Blood Sugar Regulation. In *Natural Products as Enzyme Inhibitors* (pp. 269–283). Springer.

Burdge, G. C. (2006). Metabolism of α-linolenic acid in humans. *Prostaglandins, Leukotrienes and Essential Fatty Acids, 75*(3), 161–168. https://doi.org/10.1016/j.plefa.2006.05.013

Davila, M., Muniz, A., & Du, X. F. (2022). The impact of roasting and steaming on savory flavors contributed by amino acids, 5′-nucleotides, and volatiles in Agaricus bisporus mushrooms. *International Journal of Gastronomy and Food Science, 30*, Article 100590. https://doi.org/10.1016/j.ijgfs.2022.100590

Deng, K. J., Lan, X. H., Fang, Q., Li, M. K., Xie, G. B., & Xie, L. Y. (2021). Untargeted Metabolomics Reveals Alterations in the Primary Metabolites and Potential Pathways in the Vegetative Growth of Morchella sextelata. *Frontiers in Molecular Biosciences, 8*. https://doi.org/10.3389/fmolb.2021.632341

Domínguez, R., Pateiro, M., Gagaoua, M., Barba, F. J., Zhang, W., & Lorenzo, J. M. (2019). A comprehensive review on lipid oxidation in meat and meat products. *Antioxidants, 8*(10), 429. https://doi.org/10.3390/antiox8100429

Dong, H., Zhao, X. Y., Cai, M., Gu, H. T. E. H. C., Li, X. B., et al. (2022). Metabolomics Analysis of Morchella sp. From Different Geographical Origins of China Using UPLC-Q-TOF-MS. *Frontiers in Nutrition, 9*, Article 865531. https://doi.org/10.3389/fnut.2022.865531

Durante, W. (2019). The Emerging Role of l-Glutamine in Cardiovascular Health and Disease. *Nutrients, 11*(9), 2092. https://doi.org/10.3390/nu11092092

Fan, X. J., Jiao, X., Liu, J. G., Jia, M., Blanchard, C., & Zhou, Z. K. (2021). Characterizing the volatile compounds of different sorghum cultivars by both GC-MS and HS-GC-IMS. *Food Research International, 140*, Article 109975. https://doi.org/10.1016/j.foodres.2020.109975

Fereidoon Shahidi, W. Y. O. (2020). Lipid-derived flavor and off-flavor of traditional and functional foods: An overview. *Journal of Food Bioactives, 10*, 20–31. https://doi.org/10.31665/JFB.2020.10224

Guan, Q. J., Meng, L. J., Mei, Z. L., Liu, Q. R., Chai, L. J., et al. (2022). Volatile compound abundance correlations provide a new insight into odor balances in sauce-aroma baijiu. *Foods, 11*, 3916. https://doi.org/10.3390/foods11233916

Guerra-Vázquez, C. M., Martínez-Ávila, M., Guajardo-Flores, D., & Antunes-Ricardo, M. (2022). Punicic acid and its role in the prevention of neurological disorders: A review. *Foods, 11*(3), 252. https://doi.org/10.3390/foods11030252

Li, Schieberle, & Steinhaus. (2017). Development of stable isotope dilution assays for the quantitation of the food odorants hydrogen sulphide, methanethiol, ethanethiol, and

propane-1-thiol. *European Food Research and Technology, 243*, 69–79. https://doi.org/10.1007/s00217-016-2723-z

Li, Q., Zhang, L. T., & Lametsch, R. (2022). Current progress in kokumi-active peptides, evaluation and preparation methods: A review. *Critical Reviews in Food Science and Nutrition, 62*(5), 1230–1241. https://doi.org/10.1080/10408398.2020.1837726

Li, X. B., Zhang, Y. M., E, H. C., He, X. W., Li, J. Y., Zhao, X. Y., et al. (2023). Characteristic fingerprints and comparison of volatile flavor compounds in *Morchella sextelata* under different drying methods. *Food Research International*, 172, 113103. Doi: 10.1016/j.foodres.2023.113103.

Li, Y. T., Chen, H. Y., & Zhang, X. (2023). Cultivation, nutritional value, bioactive compounds of morels, and their health benefits: A systematic review. *Frontiers in Nutrition, 10*, 1159029. https://doi.org/10.3389/fnut.2023.1159029

Lin, J. M., & Blank, I. (2003). Odorants Generated by Thermally Induced Degradation of Phospholipids. *Journal of Agricultural and Food Chemistry, 51*(15), 4364–4369. https://doi.org/10.1021/jf034300m

Liu, Q. Z., Ma, H. S., Zhang, Y., & Dong, C. H. (2018). Artificial cultivation of true morels: Current state, issues and perspectives. *Critical Reviews in Biotechnology, 38*(2), 259–271. https://doi.org/10.1080/07388551.2017.1333082

Liu, Y., Meng, F. B., Tang, P. Y., Huang, D. M., Li, Q. X., & Lin, M. (2022). Widely Targeted Metabolomics Analysis of the Changes to Key Non-volatile Taste Components in Stropharia rugosoannulata Under Different Drying Methods. *Frontiers in Nutrition, 9*. https://doi.org/10.3389/fnut.2022.884400

Mei, S. Y., He, Z. J., & Zhang, J. F. (2022). Identification and analysis of major flavor compounds in radish taproots by widely targeted metabolomics. *Frontiers in Nutrition, 1587*. https://doi.org/10.3389/fnut.2022.889407

Mizuta, H., Kumamoto, N., Ugawa, S., & Yamamoto, T. (2021). Additive effects of L-ornithine on preferences to basic taste solutions in mice. *Nutrients, 13*(11), 3749. https://doi.org/10.3390/nu13113749

Pilz, D. (2007). Ecology and management of morels harvested from the forests of western North America.

Rotzoll, N., Dunkel, A., & Hofmann, T. (2005). Activity-guided identification of (*S*)-malic acid 1-O-d-glucopyranoside (morelid) and γ-aminobutyric acid as contributors to umami taste and mouth-drying oral sensation of morel mushrooms (Morchella deliciosa Fr.). *Journal of Agricultural and Food Chemistry, 53*(10), 4149–4156. https://doi.org/10.1021/jf050056i

Sande, D., Oliveira, G. P. d., Moura, M. A. F. e., Martins, B. d. A., Lima, M. T. N. S., & Takahashi, J. A. (2019). Edible mushrooms as a ubiquitous source of essential fatty acids. *Food Research International, 125*, 108524. Doi: 10.1016/j.foodres.2019.108524.

Song, X. B., Wang, G. N., Zhu, L., Zheng, F. P., Ji, J., Sun, J. Y., et al. (2021). Comparison of two cooked vegetable aroma compounds, dimethyl disulfide and methional, in Chinese Baijiu by a sensory-guided approach and chemometrics. *LWT-Food Science and Technology, 146*, Article 111427. https://doi.org/10.1016/j.lwt.2021.111427

Tasaki, Y., Kobayashi, D., Sato, R., Hayashi, S., & Joh, T. (2019). Variations in 1-octen-3-ol and lipoxygenase gene expression in the oyster mushroom Pleurotus ostreatus according to fruiting body development, tissue specificity, maturity, and postharvest storage. *Mycoscience, 60*, 170–176. https://doi.org/10.1016/j.myc.2019.02.005

Taşkın, H., Süfer, Ö., Attar, Ş. H., Bozok, F., Baktemur, G., Büyükalaca, S., et al. (2021). Total phenolics, antioxidant activities and fatty acid profiles of six Morchella species. *Journal of Food Science and Technology, 58*(2), 692–700. https://doi.org/10.1007/s13197-020-04583-3

Tietel, Z., & Masaphy, S. (2018). True morels (Morchella)—nutritional and phytochemical composition, health benefits and flavor: A review. *Critical Reviews in Food Science and Nutrition, 58*(11), 1888–1901. https://doi.org/10.1080/10408398.2017.1285269

Tietel, Z., & Masaphy, S. (2022). Chemotyping of three Morchella species reveals species- and age-related aroma volatile biomarkers. LWT–Food. *Science and Technology, 154*, Article 112587. https://doi.org/10.1016/j.lwt.2021.112587

Tu, Z., Liu, Y. Y., Lin, J. Y., Lv, H. W., Zhou, W., Zhou, X. F., et al. (2023). Comparison of volatile and nonvolatile metabolites in green tea under hot-air drying and four heat-conduction drying patterns using widely targeted metabolomics. *Food Chemistry: X, 19*, Article 100767. https://doi.org/10.1016/j.fochx.2023.100767

Wagner, Granvogl, & Schieberle. (2016). Characterization of the key aroma compounds in raw licorice (Glycyrrhiza glabra L.) by means of molecular sensory science. *Journal of Agricultural and Food Chemistry, 64*, 8388–8396. https://doi.org/10.1021/acs.jafc.6b03676

Wang, H. J., Hua, J. J., Yu, Q. Y., Li, J., Wang, J. J., Deng, Y. L., et al. (2023). Widely targeted metabolomic analysis reveals dynamic changes in non-volatile and volatile metabolites during green tea processing. *Food Chemistry, 363*, Article 130130. https://doi.org/10.1016/j.foodchem.2021.130131

Wu, W. L., Lu, M. L., Peng, J. K., Lv, H. P., Shi, J., Zhang, S. G., et al. (2022). Nontargeted and targeted metabolomics analysis provides novel insight into nonvolatile metabolites in Jianghua Kucha tea germplasm (Camellia sinensis var. Assamica cv. Jianghua). *Food Chemistry X, 13*, Article 100270. https://doi.org/10.1016/j.fochx.2022.100270

Yang, Y. H., Yang, J., Wang, H. L., Jin, Y. S., Liu, J., Jia, R. R., et al. (2022). Analysis of primary metabolites of Morchella fruit bodies and mycelium based on widely targeted metabolomics. *Archives of Microbiology, 204*(1), 1–9. https://doi.org/10.1007/s00203-021-02612-z

Yin, X. L., Fu, W. J., Chen, Y., Zhou, R. F., Sun, W. Q., Ding, B. M., et al. (2022). GC-MS-based untargeted metabolomics reveals the key volatile organic compounds for discriminating grades of Yichang big-leaf green tea. *LWT-Food Science and Technology, 114148*. https://doi.org/10.1016/j.lwt.2022.114148

Zhang, L. H., Zhang, M., & Mujumdar, A. S. (2021). Development of flavor during drying and applications of edible mushrooms: A review. *Drying Technology, 39*(11), 1685–1703. https://doi.org/10.1080/07373937.2021.1875231

Zhao, L. Y., Duan, X. L., Hou, C., Liu, H. B., & Sun, H. (2022). Identification of key taste components in two aromatic rice varieties by widely targeted metabolomics in China. *Cereal Chemistry, 100*, 289–298. https://doi.org/10.1002/cche.10605

Zhao, X. Y., E, H. C., Dong, H., Zhang, Y. M., Qiu, J., Qian, Y. Z., et al. (2022). Combination of untargeted metabolomics approach and molecular networking analysis to identify unique natural components in wild Morchella sp. by UPLC-Q-TOF-MS. *Food Chemistry, 366*, Article 130642. https://doi.org/10.1016/j.foodchem.2021.130642

Zhuang, J. D., Xiao, Q., Feng, T., Huang, Q. R., Ho, C. T., & Song, S. Q. (2020). Comparative flavor profile analysis of four different varieties of Boletus mushrooms by instrumental and sensory techniques. *Food Research International, 136*, Article 109485. https://doi.org/10.1016/j.foodres.2020.109485

Zou, S. C., Wu, J. C., Shahid, M. Q., He, Y. H., Lin, S. Q., Liu, Z. H., et al. (2020). Identification of key taste components in loquat using widely targeted metabolomics. *Food Chemistry, 323*, Article 126822. https://doi.org/10.1016/j.foodchem.2020.126822