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RESEARCH ARTICLE

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Chemical Composition of Aromas and Lipophilic Extracts from Black Morel (*Morchella importuna*) Grown in China

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ABSTRACT

Morels (*Morchella* spp.) are valuable medicinal and edible mushrooms. In this study, chemical profiles of aromas and lipophilic extracts of black morel (*Morchella importuna*) grown in China were analyzed by gas chromatography/mass spectroscopy, along with the evaluation of antioxidant and antimicrobial activities for the lipophilic extracts. Sixty-five compounds in total were identified from the aromas, and 1-octen-3-ol was the main component for aromas of fresh (34.40%) and freeze-dried (68.61%) black morels, while the most abundant compound for the aroma of the oven-dried sample was 2(5*H*)-furanone (13.95%). From the lipophilic extracts, 29 compounds were identified with linoleic acid as the main compound for fresh (77.37%) and freeze-dried (56.46%) black morels and steroids (92.41%) as the main constituent for an oven-dried sample. All three lipophilic extracts showed moderate antioxidant activities against 2,2-diphenyl-1-picrylhydrazyl and 2,2′-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) with the IC_{50} values ranging 7.56 ~ 17.52 mg/mL and 5.75 ~ 9.73 mg/mL, respectively, and no obvious antimicrobial activity was observed for lipophilic extracts. The drying methods affect the chemical profile of black morel, and freeze-drying was favorable for retaining nutrients and morel smell. This is the first report on the aroma and lipophilic extracts of *M. importuna* grown in China.

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1. Introduction

Morels (species of *Morchella* Dill. ex Pers.) are a traditional food source and medicinal mushrooms in the northern hemisphere, especially in Asia, Europe, and North America [1]. As a food source, the morel is considered a nutritious food of high nutritional values as fish or meat in Asia. As a medicinal mushroom, the morel is recorded as "sweet, cold and non-toxic, beneficial to the stomach, reducing phlegm and promoting the circulation of qi" in "Ben-Cao-Gang-Mu," a Chinese outline treatise of medical herbs [2]. Currently, morels are mainly harvested from the wild in China, India, Turkey, Mexico, and the USA [3]. But, overharvesting of morels from the wild will cause the depletion of the wild resource. Excitingly, two species of *Morchella* mushrooms (*Morchella importuna* and *M. sextelata*) have been artificially cultivated in fields in China recently. Among the two, *M. importuna* (black morel) has been widely cultivated and accounted for more than 95% of the total cultivated area of morels [4].

Fresh mushrooms have high water content, high enzymatic activity, and hence are highly perishable [5]. Therefore, dehydration was required for their preservation. Most often drying treatments are applied for food processing. However, the drying processes change the composition and content of mushroom metabolites, which affect the nutrition, taste, and smell of mushrooms in turn [6,7].

In order to better understand the chemical profile of black morel (*M. importuna*) grown in China and the effect of drying method (oven and freeze) on chemical composition, aromas were studied by headspace solid-phase microextraction (HS-SPME), coupled with gas chromatography/mass spectroscopy (GC-MS). Meanwhile, lipophilic extracts were also prepared and analyzed by GC-MS, together with the evaluation of antioxidant and antimicrobial activities.

2. Materials and methods

2.1. Chemical compounds

1,1-Diphenyl-2-picrylhydrazyl (DPPH) and diammonium 2,2′-azino-bis(3-ethylbenzothiazoline-6-sulfonate)

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Supplemental data for this article can be accessed here.

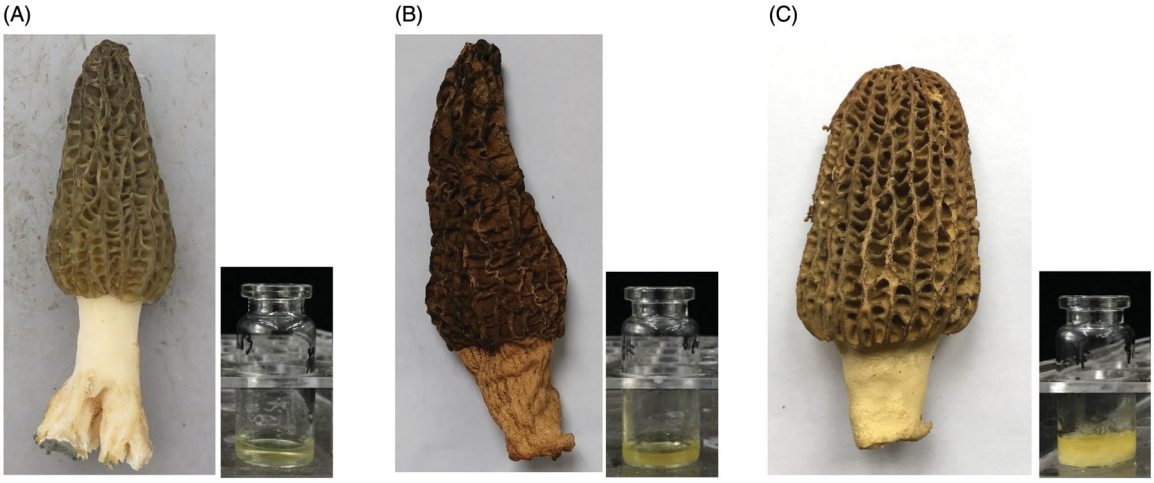

Figure 1. Fruit bodies of black morel (*M. importuna*) and their lipophilic extracts. (A) fresh black morel; (B) Oven-dried black morel; (C) Freeze-dried black morel.

(ABTS) were purchased from Sigma-Aldrich (St Louis, MO, USA). All other chemicals were purchased from Kelong Chemicals (Chengdu, China).

2.2. Mushroom samples

Mature fruit bodies of black morel (*M. importuna*) were collected from planting base in Xindu district, Chengdu, China, and identified by ribosomal DNA internal transcribed spacer (ITS), combined with morphological characteristics. Oven drying was carried out in an electro-thermostatic blast oven (Yiheng Scientific Instruments Co., Ltd., Shanghai, China). The cleaned fruit bodies were placed on a stainless steel bracket and then dried at 50 ± 2 °C until reaching a constant weight. The freeze-drying was carried out in a FreeZone 2.5 L freeze dryer (Labconco Corp., Kansas City, MO, USA). The cleaned fruit bodies were pre-frozen at −20 °C, and then dried under vacuum (<0.12 mbar), at −50 °C, until reaching a constant weight. The dried black morels (Figure 1) were further used for aromas and lipophilic extract extractions.

2.3. Extraction of aromas

The aromas extraction by HS-SPME was performed as previously reported [8]. Different samples (fresh, oven-dried, and freeze-dried fruit bodies of black morel) were ground adequately and weighed (2.5 g each sample) into a 20 mL glass vial with a silicon cap. The cap was perforated with a divinylbenzene/carboxen/polydimethylsiloxane (DVB/CAR/PDMS, 50/30 µm) fibers holder (Supelco Inc., Bellefonte, PA, USA). The vial perforated with fibers was incubated in a 50 °C water bath for 45 min. The fibers were desorbed for 1 min at 270 °C in the GC injector in splitless mode.

2.4. Extraction of lipophilic extracts

Lipophilic extracts were obtained according to the method reported in the literature [9]. Fresh, oven-dried, and freeze-dried fruit bodies of black morel were cut into small parts (1.0 ± 0.5 cm) and transferred into the flask with freshly distilled petroleum ether (30–60 °C). The flasks were placed in a dark and shady place for one week at room temperature. The petroleum ether layers were separated and dried by anhydrous Na_2SO_4, then recovered the solvents in a vacuum to afford lipophilic extracts. All three extracts were stored at 4 °C in the refrigerator for further use.

2.5. GC-MS analysis

GC-MS analysis of the aromas and extracts was performed on the QP2010 Plus GC-MS instrument (SHIMAZHU Corp., Kyoto, Japan), fitted with an SH-Rxi-5MS capillary column (30 m × 0.25 mm i.d., 0.25 µm film thickness) (SHIMAZHU Corp.).

For the GC analysis of aromas, the temperature was programmed at 40 °C for the first 5 min, increased at a rate of 5 °C/min to 160 °C, held isothermal at 160 °C for 2 min, then increased at a rate of 10 °C/min to 280 °C, and held isothermal at 280 °C for the next 5 min. The injector temperature was 270 °C. Helium was used as carrier gas at a flow rate of 1 mL/min with a split ratio of 5:1.

For the GC analysis of lipophilic extracts, the temperature was programmed at 40 °C for the first 5 min, increased at a rate of 10 °C/min to 220 °C, held isothermal at 220 °C for 2 min, increased at a rate of 5 °C/min to 280 °C, held isothermal at 280 °C for 5 min, increased at a rate of 5 °C/min to 290 °C, and held isothermal at 290 °C for the next 20 min; The injector temperature was 290 °C and the injected volume was 1 µL. Helium was used as

carrier gas at a flow rate of 1 mL/min with a split ratio of 5:1.

Mass spectrometry conditions were EI ionization mode, 70 eV, scan range 33–600 amu, and ion source temperature was 200 °C. Individual components were identified by matching their mass spectra with those of the spectrometer database (NIST08.LIB). For quantification purposes, the relative area (%) was used without the use of correction factors.

2.6. DPPH radical scavenging assay of lipophilic extracts

The DPPH radical scavenging activity was assessed according to the literature [10]. Ascorbic acid was used as a positive control. The test samples were prepared by 2-fold serial dilution method in ethanol, and their final concentrations were ranged from 0.625 to 40 mg/mL. Absorbance measurements of 50 µL of tested samples and 100 µL DPPH solutions (0.1 mg/mL) were read at 517 nm, after 25 min of incubation time in a dark place, at room temperature. Absorption of a blank sample containing the same amount of ethanol and DPPH solution was used as the negative control. The percentage inhibition of the DPPH radical by the samples was calculated according to the formula: Inhibition percentage (%) = $[(A_{Control} - (A_{Sample} - A_{Blank}))/A_{Control}] \times 100$, where $A_{Control}$ is the absorption of the negative control, A_{Sample} is the absorption of the DPPH solution containing tested samples and A_{Blank} is the absorption of the sample solution without DPPH. All analyses were carried out in triplicate. The effective concentration of the sample required to scavenge DPPH radical by 50% (IC_{50} value) was calculated by SPSS.

2.7. ABTS radical scavenging assay of lipophilic extracts

The ABTS radical cation decolorization assay was performed according to the method of Re et al. [11]. ABTS radical cation ($ABTS^{\cdot+}$) produced by reacting 7 mM ABTS solution with 2.45 mM potassium persulphate was diluted with ethanol to an absorbance of 0.6 ~ 0.7 at 734 nm. The test samples were prepared by 2-fold serial dilution method in ethanol, and their final concentrations were ranged from 0.625 to 40 mg/mL. After the addition of 50 µL of sample solution to 100 µL of $ABTS^{+}$ solution, the absorbance was recorded after 5 min at 734 nm. Tests were carried out in triplicate and ascorbic acid was used as a positive control. Inhibitions of ABTS radical cation, as well as IC_{50} value, were determined as reported for the DPPH test.

2.8. Antimicrobial active assay of lipophilic extracts

Escherichia coli (ATCC 25922), *Staphylococcus aureus* (ATCC 25923), *Bacillus subtilis* (ATCC 6633), and *Candida albicans* (ATCC 10231) were used for the evaluation of the antimicrobial activity. Antimicrobial activity assessments were performed in 96-well sterilized microplates using a microdilution method described previously [12,13]. The 18-h old bacterial cultures from *E. coli*, *S. aureus*, and *B. subtilis* were added to LB broth medium (1 L water, 10 g tryptone, 5 g yeast extract, and 10 g NaCl) to reach 1×10^5 CFU/mL, and the 4-day-old spores from *C. albicans* were added to PDB medium (potato extract 20%, glucose 2%) to reach 1×10^3 spores/mL. The test samples were dissolved in DMSO, and their final concentrations were ranged from 0.5 to 512 µg/mL, which were determined by 2-fold serial dilution method. The wells containing test strains and diluted samples were incubated at 37 °C (24 h) for bacteria and 28 °C (4 days) for fungi. The wells containing a culture suspension and DMSO were run as negative controls. Kanamycin (for bacteria) and nystatin (for fungi) were used as positive controls. All experiments were repeated twice. The minimal inhibitory concentration (MIC) was defined as the lowest antibiotic concentration that produced complete growth inhibition of the tested microorganisms.

3. Results and discussion

3.1. Physical characteristics of the lipophilic extracts

The lipophilic extracts (Figure 1) of black morel (*M. importuna*) were light yellowish oil with a yield of 0.148% (*w*/*w*, wet weight) for fresh sample, yellowish oil with a yield of 0.11% (*w*/*w*, dry weight) for the oven-dried sample and a yield of 0.132% (*w*/*w*, dry weight) for the freeze-dried sample.

3.2. Chemical composition of aromas and lipophilic extracts from black morel

The chemical composition of aromas and lipophilic extracts extracted from black morel are listed in Table 1.

3.2.1. Fresh black morel

Twenty-seven compounds were identified from the aroma of fresh black morel, comprised 98.82% of the total aromas, and 3-methyl-butanal (11.49%), benzene acetaldehyde (24.31%), and 1-octen-3-ol (34.40%) were the main components (Figure 2(A)). Nineteen compounds were identified from the lipophilic extracts of fresh black morel, comprised

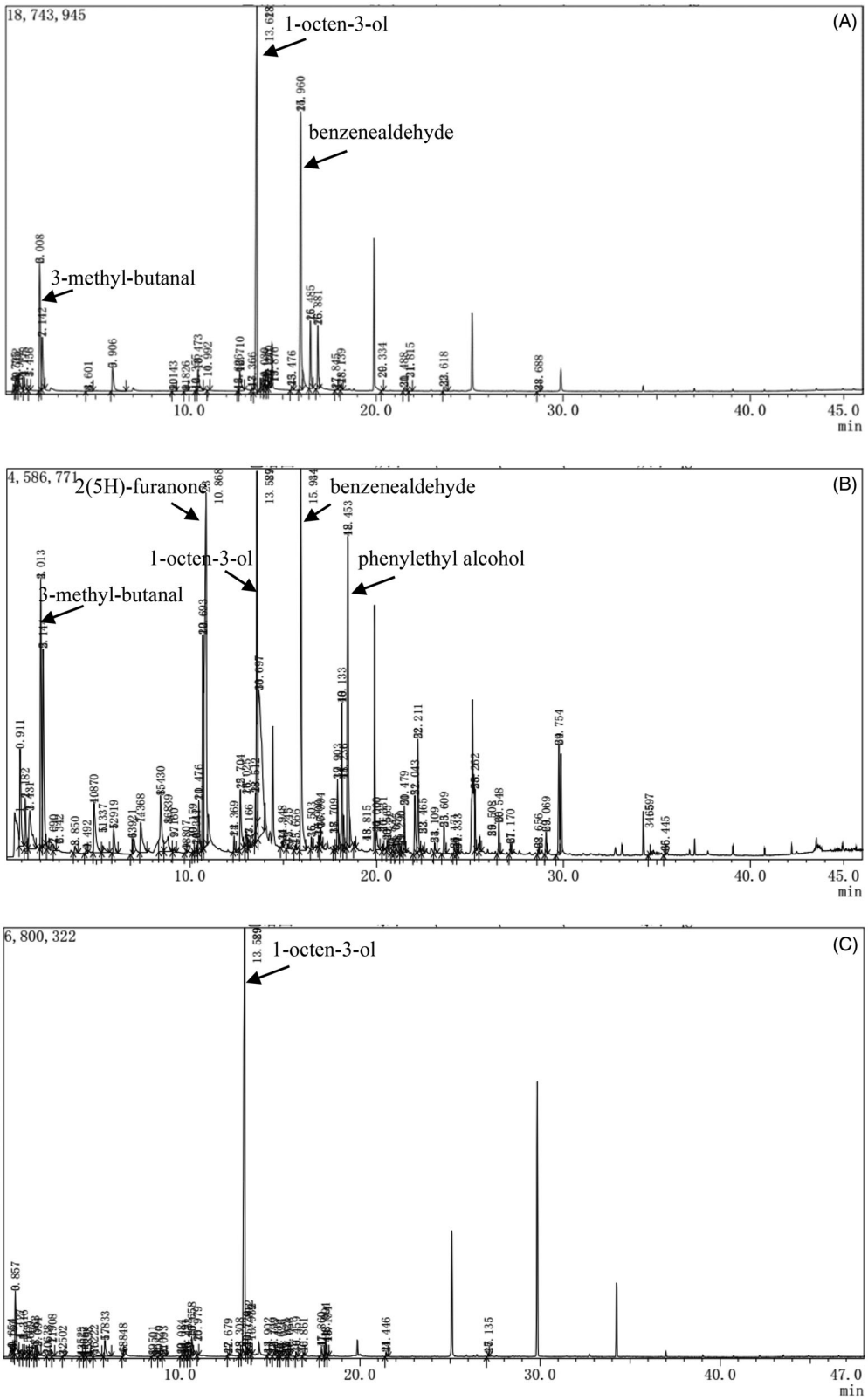

Figure 2. GC-MS total ion chromatogram (TIC) for aromas of black morel (*M. importuna*). (A) Fresh black morel; (B) Oven-dried black morel; (C) Freeze-dried black morel.

97.58% of the total extracts, and linoleic acid was the main component (77.37%).

3.2.2. Oven-dried black morel

Forty-three compounds were identified from the aroma of oven-dried black morel, comprised 91.09% of the total aromas, and benzene acetaldehyde (8.63%), 5-hydroxy-2-methyl-3-hexanone (10.43%), and 2(5*H*)-furanone (13.95%) were the main components (Figure 2(B)). Thirteen compounds were identified from the lipophilic extracts of oven-dried black morel, comprised 96.59% of the total extracts, and steroids were the main components (92.41%).

Table 1. Chemical composition of the aromas and lipophilic extracts from black morel (*M. importuna*).

	Lipophilic extracts			Aromas		
Compounds	F* (%)	OD** (%)	FD*** (%)	F* (%)	OD** (%)	FD*** (%)
Aldehydes						
Acetaldehyde				0.48		
3-Methyl-butanal				11.49	6.21	1.48
2-Methyl-propanal				1.26	1.23	0.64
2-Methyl-butanal				5.00	4.89	1.31
Hexanal	0.19			2.86	0.68	2.73
Furfural					1.44	
Heptanal				0.07	0.15	0.20
(Z)-2-Heptenal				0.09		
Benzaldehyde				2.65	1.73	0.34
Benzeneacetaldehyde				24.31	8.63	
(*E*)-2-Octenal				4.93	0.25	0.52
Nonanal				0.39	2.43	1.81
(*E*)-2-Nonenal					0.53	
Decanal				0.25	0.82	0.43
(*E*,*E*)-2,4-Nonadienal				0.04		
(*E*,*E*)-2,4-Decadienal	0.09					
2-Methyl-3-phenyl-2-propenal				0.45	0.63	
α-(2-Methylpropylidene) benzene acetaldehyde					0.64	
5-Methyl-2-phenyl-2-hexenal					2.33	
Ketones						
2,3-Butanedione						0.41
2-Butanone				0.04		
4-Hydroxy-3-hexanone						0.20
2,3-Octanedione					0.27	
3,4-Dimethyl-3-penten-2-one					0.22	
5-Hydroxy-2-methyl-3-hexanone					10.43	
5-Methyl-3-heptanone				0.37		
4-Methyl-2,4,6-cycloheptatrien-1-one	0.19					0.23
2-Nonanone						1.23
trans-Geranylacetone				0.06	0.11	
1-Phenyl-1-pentanone	0.05					
Alcohols						
Ethanol						0.19
1-Methoxy-2-propanol					0.11	
1-Pentanol						0.06
(*R*)-2-Hexanol	0.02	0.05	0.07			
2-Furanmethanol					2.12	
1-Octen-3-ol	0.29			34.40	6.76	68.61
2-Ethyl-1-hexanol				0.32		
(*E*)-2-Octen-1-ol	0.01			4.37	0.19	0.69
Phenylethyl alcohol					6.68	
(*E*)-2-Tridecen-1-ol					0.13	
(*R*)-(-)-14-Methyl-8-hexadecyn-1-ol	0.60	3.23				
(*Z*,*Z*)-2-(9,12-Octadecadienyloxy)-ethanol			2.50			
Geranylgeraniol	0.21	0.22	0.30			
Carboxylic acids/carboxylic acid derivatives						
Acetic acid					2.29	
2-Methyl-propanoic acid					0.13	
2-Methylbutanoic acid					0.98	
Octanoic acid	0.07					
Pentadecanoic acid	4.50		0.74			
17-Octadecynoic acid		0.09				
Linoleic acid	77.37		56.46			
1-(Acetyloxy)-2-propanone					0.08	
Formic acid 2-phenylethyl ester					0.21	
Acetic acid 2-phenylethyl ester					0.25	
10-Methyl-undecanoic acid methyl ester			0.10			
14-Methyl-pentadecanoic acid methyl ester		0.07				
(*Z*,*Z*)-9,12-Octadecadienoic acid methyl ester	0.25	0.27	0.72			
(*Z*)-7-Hexadecenoic acid methyl ester		0.10	0.10			
6,9-Octadecadienoic acid methyl ester	0.12					
Trilinolein	1.63		5.59			
N,*N*-Dimethyl formamide					0.37	1.01
Butanimidamide					1.38	
(*Z*)-9-Octadecenamide	0.15					
Heterocycles/sulfurs						
Methanethiol				1.20		
3-(Methylthio)-propanal				2.43	1.30	0.38
Dimethyl trisulfide					0.38	
2,2-Dimethyl-oxetane						0.65
Pyrazine						0.19
2-Methyl-pyrimidine					0.29	0.38
2(5*H*)-Furanone				0.04	13.95	
2,5-Dimethyl-pyrazine					5.27	

(*continued*)

Table 1. Continued.

Compounds	Lipophilic extracts			Aromas		
	F* (%)	OD** (%)	FD*** (%)	F* (%)	OD** (%)	FD*** (%)
Dihydro-2(3*H*)-furanone						2.91
2-Pentylfuran				0.33		
Steroids						
(3β, 22*E*)-Ergosta-5,22-dien-3-ol	6.48	35.48	16.47			
Ergosterol		32.58	9.24			
(3β)-Stigmasta-5,24(28)-dien-3-ol	5.05	9.93				
(3β)-Ergost-5-en-3-ol		7.12				
Campesterol			1.91			
(3β)-Ergosta-5,8-dien-3-ol		7.30				
Others						
Methyl isocyanide					2.64	
1-Nitro-propane						9.22
1,3,5-Cycloheptatriene				0.18		
1,3,5,7-Cyclooctatetraene					0.07	
3,5,5-Trimethyl-2-hexene				0.17		0.25
1,5-Dimethyl-1,5-cyclooctadiene		0.15	0.07			
3-Ethyl-2-methyl-1-pentene				0.64		
p-Mentha-1,4(8)-diene					0.11	
o-Cymene						0.28
Dodecane					0.29	0.40
2-Naphthalenol					1.49	
2,2′-Methylenebis(4-methyl-6-tert-butylphenol)	0.31					
In total (%)	97.58	96.59	94.27	98.82	91.09	96.75

*F: fresh black morel; **OD: oven-dried black morel; ***FD: freeze-dried black morel.

3.2.3. Freeze-dried black morel

Twenty-seven compounds were identified from the aroma of freeze-dried black morel, comprised 96.75% of the total aromas, and 1-octen-3-ol (68.61%) was the main component (Figure 2(C)), which was similar with black morels grown in Israel [14]. Thirteen compounds were identified from the lipophilic extracts of freeze-dried black morel, comprised 94.27% of the total extracts, and linoleic acid (56.46%) were the main components.

3.3. Difference in chemical composition of aromas

The difference in chemical composition and content for aromas was found among fresh, oven-dried, and freeze-dried black morels. As shown in Table 1, a total of sixty-five constituents were identified from three aroma samples, which can be divided into six types: aldehydes, ketones, alcohols, carboxylic acids/carboxylic acid derivatives, heterocycles/sulfurs, and others (Table 2).

Aldehydes reveal fresh, floral, and fatty smell, and they were identified from aromas of many common edible mushrooms [7,15,16]. 3-Methyl-butanal (11.49%), a compound with fruity aroma [17], and benzene acetaldehyde (24.31%), which imparts floral and honey-like flavor [18], were found to be the major aldehydes in fresh black morel (*M. importuna*). Compared with fresh black morel, oven-dried and freeze-dried samples showed a decrease in content for these two compounds, especially freeze-dried samples, which will weaken the fruity and floral smell in turn.

1-Octen-3-ol, an alcohol with "mushroom-like" odor [19,20], was found to be the major component (34.4%) in fresh black morel (*M. importuna*). It was also found to be the major compound in freeze-dried black morel (68.61%). But, a sharp decrease of 1-octen-3-ol was found in the oven-dried sample (6.76%), which was associated with the loss of "mushroom-like" smell, and freeze-drying was proved to be more suitable for retaining morel smell.

Methyl esters, a group of compounds associated with waxy, fatty, fruity aromas of morels, were found to be the major esters of aroma volatiles extracted from black morels grown in Israel, comprising 10.9% of total volatiles [14]. But from black morels grown in China, no methyl ester was found in the aromas, and only trace amounts were found in lipophilic extracts. It is the major difference in aromas between black morels grown in Israel and China.

3.4. Difference in chemical composition of lipophilic extracts

Due to the failure in obtaining the essential oil from black morel by steam distillation, we turned to the extraction of lipophilic extracts by petroleum ether. A total of 29 constituents were identified from lipophilic extracts of fresh, oven-dried, and freeze-dried black morels, including two aldehydes, two ketones, six alcohols, eleven carboxylic acids, and carboxylic acid derivatives, six steroids, and two others (Table 1). The most obvious difference in the chemical composition of lipophilic extracts was the content of linoleic acid. From the fresh and freeze-dried black morels, linoleic

Table 2. Proportion of the different chemical families of aromas in black morel (*M. importuna*).

Samples	Aldehydes (%)	Ketones (%)	Alcohols (%)	Carboxylic acids/ carboxylic acid derivatives (%)	Heterocycles/sulfurs (%)	Others (%)
Fresh black morel	54.27	0.47	39.09		4.00	0.99
Oven-dried black morel	32.59	11.03	15.99	5.69	21.19	4.60
Freeze-dried black morel	10.15	2.07	69.55	1.01	4.00	10.15

Table 3. Antioxidant activity of the lipophilic extracts from black morel (*M. importuna*) (IC_{50}, mg/mL).

Method	F*	OD**	FD***	Ascorbic acid
DPPH radical	17.52 ± 0.45	7.56 ± 0.17	14.63 ± 0.68	0.41 ± 0.03
$ABTS^{+}$ radical	9.73 ± 0.17	5.75 ± 0.09	7.18 ± 0.28	0.17 ± 0.01

*F: fresh black morel; **OD: oven-dried black morel; ***FD: freeze-dried black morel.

acid was found to be the major component (77.37% and 56.46%, respectively), but no linoleic acid was found in oven-dried black morel. Linoleic acid, a member of polyunsaturated fatty acids, is a key nutrient throughout all human life [21]. Higher linoleic acid status was associated with reduced risk of heart diseases and type 2 diabetes mellitus, and it also improves insulin sensitivity and modulates body composition, which argues for the key role of linoleic acid-rich oils to promote cardiometabolic health [22]. Recently, linoleic acid was also found to bind the receptor-binding domains of SARS-CoV-2 spike protein, and this binding stabilizes a locked S conformation giving rise to reduce ACE2 interaction *in vitro* [23].

3.5. Bioactivities of lipophilic extracts

3.5.1. Antioxidant activity

The principle of antioxidant activity is based on the availability of electrons to neutralize any free radicals [24]. In this study, the antioxidant activities of lipophilic extracts of black morel were evaluated by the DPPH and ABTS radical scavenging activity assessment, and the results were shown in Table 3. All of the tested extracts were able to reduce the stable violet-colored DPPH radical to yellow-colored DPPH-H, and showed moderate antioxidant activities (IC_{50} 7.56 ~ 17.52 mg/mL), compared with ascorbic acid (IC_{50} 0.41 mg/mL). Moreover, the lipophilic extracts of oven-dried black morel (IC_{50} 7.56 mg/mL) were twice more active than that of fresh (IC_{50} 17.52 mg/mL) and freeze-dried (IC_{50} 14.63 mg/mL) black morel.

In ABTS assay, all the lipophilic extracts showed moderate antioxidant activities (IC_{50} 5.75 ~ 9.73 mg/mL), compared with ascorbic acid (IC_{50} 0.17 mg/mL), and the extracts of oven-dried black morel (IC_{50} 5.75 mg/mL) was also more active than that of fresh (IC_{50} 9.73 mg/mL) and freeze-dried (IC_{50} 7.18 mg/mL) black morel, which was similar with DPPH assay. The major components of oven-dried black morel were steroids, which may be the cause for its better antioxidant activity.

3.5.2. Antimicrobial activity

The antimicrobial activity of lipophilic extracts of black morel was evaluated using the microdilution method in a 96-well plate against a set of microorganisms (Gram-positive bacteria: *S. aureus*, *B. subtilis*; Gram-negative bacteria: *E. coli*; fungi: *C. albicans*). Compared with the positive control (Kanamycin, MIC 1 μg/mL for bacterial; Nystatin, MIC 1 μg/mL for fungi), no obvious antimicrobial activity was observed for lipophilic extracts (Table S1).

4. Conclusions

In this study, the chemical composition of aromas and lipophilic extracts of black morel (*M. importuna*) grown in China was studied for the first time. We discuss the effect of the drying method (oven and freeze) on the chemical profile of black morel and found that different drying methods would induce the difference in the chemical composition of the black morel. Freeze drying was found to be more favorable than oven drying in holding nutrients and morel smell. Regarding the antioxidant and antimicrobial potentials of lipophilic extracts, only moderate antioxidant activities were shown.

Disclosure statement

No potential conflict of interest was reported by the author(s).

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