



Contents lists available at ScienceDirect

Food Science and Human Wellness

journal homepage: <http://www.keaipublishing.com/en/journals/food-science-and-human-wellness>

Aroma profile of two commercial truffle species from Yunnan and Sichuan, China: inter- and intraspecific variability and shared key compounds

Bin Lu^a, Jesús Perez-Moreno^b, Fengming Zhang^{a,c}, Andrea C. Rinaldi^{d,*}, Fuqiang Yu^{a,*}

^a Yunnan Key Laboratory for Fungal Diversity and Green Development, Kunming Institute of Botany, Chinese Academy of Science, Kunming, China

^b Colegio de Postgraduados, Campus Montecillo, Texcoco, México

^c Key Laboratory for Forest Resources Conservation and Utilisation in the Southwest Mountains of China, Ministry of Education, Southwest Forestry University, Kunming, China

^d Department of Biomedical Sciences, University of Cagliari, I-09042 Monserrato (CA), Italy

ARTICLE INFO

Article history:

Received 24 April 2020

Received in revised form 6 July 2020

Accepted 6 July 2020

Available online 1 March 2021

Keywords:

Edible mushrooms

HS-SPME

Tuber

Volatile organic compounds

ABSTRACT

Aroma is central to the worldwide success of truffles as gourmet food and the high prices paid for these edible mushrooms. In this study, volatile organic compounds (VOCs) from fruiting bodies of two Chinese truffles of commercial relevance, *Tuber indicum* and *Tuber pseudohimalayense*, were analyzed using headspace solid-phase microextraction (HS-SPME) coupled with gas chromatography-mass spectrometry (GC-MS). We aimed to characterize the aroma profile and determine whether it would be influenced by provenance and stage of maturation. We thus collected and analyzed young, middle mature and mature fruiting bodies of each species from different locations in Yunnan and Sichuan provinces, located in southwestern China. Overall, 76 VOCs were identified, belonging to different chemical classes, i.e. alcohols and phenols, aldehydes and ketones, benzenes and methoxy compounds, hydrocarbons and amines. A large number of volatiles identified in *T. indicum* and *T. pseudohimalayense* are reported here for the first time for these truffles. While more than 50% of identified VOCs were produced by both truffle species, considerable differences were present in the aroma profiles of fruiting bodies collected at various maturation stages, revealing a dynamic pattern in the biosynthesis of VOCs. Furthermore, truffles of different provenance had distinct proportions of volatile constituents, suggesting that, besides genetic factors, edaphic and microclimatic conditions influence the synthesis of VOCs in a complex manner.

© 2021 Beijing Academy of Food Sciences. Production and hosting by Elsevier B.V. on behalf of KeAi Communications Co., Ltd. This is an open access article under the CC BY-NC-ND license (<http://creativecommons.org/licenses/by-nc-nd/4.0/>).

1. Introduction

Truffles – hypogeous fungi belonging to the family Tuberaceae (Ascomycota, Pezizales) – are considered one of the most valuable gourmet foods in the world. *Tuber melanosporum* Vittad., the Périgord black truffle, and *T. magnatum* Pico, the delectable white Piedmont truffle, are the most prized amongst truffle species. The white truffle, in particular, can fetch surprisingly elevated prices, and

the species' industry turnover is estimated at some 495 million euros in Italy alone [1]. The global truffle market is growing rapidly in value, being expected to reach a CAGR (Compound Annual Growth Rate) of more than 19% during the period 2019–2023, according to the latest market research report [2]. Looking at the long-term, it is expected that within 20 years, the annual global trade in truffles will reach the staggering figure of 5.3 billion euros [1].

While just a couple of species of truffles hit the headlines – usually for the amount of money they are paid at special auctions – the variety of these prized mushrooms is much higher. Currently, the number of *Tuber* species worldwide is estimated to range between 180 and 220 [3], of which about 30 are commercially traded. The genus seems to have a prevalently Northern Hemisphere distribution, with the geographic origin being predicted to be either Europe or Asia, although much remains to be known on the diversification of *Tuber*, especially in Australasia [3,4]. Clearly, the evolution of the

* Corresponding author at: Kunming Institute Botany, Chinese Academy of Sciences, 132 Lanhei Road, Heilongtan, Kunming, Yunnan 650201, China; Department of Biomedical Sciences, University of Cagliari, I-09042 Monserrato (CA), Italy.

E-mail address: fqyu@mail.kib.ac.cn (F.Q. Yu); rinaldi@unica.it (A.C. Rinaldi)

Peer review under responsibility of KeAi Communications Co., Ltd



Production and hosting by Elsevier

genus has been significantly driven by the interaction with plant hosts that support the ectotrophic mode of nutrition of *Tuber* species [5,6], and with a variety of animals (insects and mammals) that accomplish spore dispersion [7]. The ecology of *Tuber*, however, might be even more complex than so far envisaged, as revealed by recent findings that hyphae of selected species can colonize the roots of nonectomycorrhizal plants as endophytes [8].

It is well known that what we call ‘taste’ is actually a mix of stimuli that our brain elaborates in a complex manner, somehow fusing food’s taste, sight, touch and smell in a single sensation [9]. Unquestionably, truffle aroma plays a pivotal role in qualifying the organoleptic properties of these mushrooms. As one might expect, however, volatiles released by *Tuber* have a broader purpose than pleasing our nose, making our mouth water. Indeed, the chemical ecology of these bioactive compounds is multifaceted, regulating the interactions of truffles with soil microorganisms, plants, and animals, including those responsible for spore dispersal [10,11]. Intriguingly, the bacterial community associated with *Tuber* is directly involved in the production of at least some of the volatiles typical of truffle aroma, such as the sulphur-containing thiophene derivatives [12,13].

China is currently an important arena for truffle studies. Forty species are present in the country, and new ones are being described [14]. Consumption of truffles is particularly important in the contiguous Sichuan and Yunnan provinces, located in southwestern China, and so are the relevant cultivation efforts [15,16]. The present work reports on the identification of volatiles from two commercial species of *Tuber* from Yunnan and Sichuan, namely *T. indicum*

Cooke & Massee [MycoBank #188017] and *T. pseudohimalayense* G. Moreno, Manjón, J. Díez & García-Mont. [MycoBank #437558] (= *T. pseudoexcavatum* Y. Wang, G. Moreno, L.J. Rioussset, Manjón & G. Rioussset) (Fig. 1). The variability of aroma composition depending on truffle maturity stage and provenance was also investigated, in an attempt to determine volatile profiles and how these might correlate with product commercialization and consumption. While most of previous studies on the aroma composition of Chinese truffles were aimed at the identification of volatile organic compounds (VOCs), comparison of aroma constituents on geographical/maturation basis is pivotal in order to eventually test authenticity and traceability.

2. Materials and Methods

2.1 Study sites and fungal material

Fresh specimens of *T. indicum* and *T. pseudohimalayense* were purchased from local markets (such as Yongping County and Yongren County, Yunnan Province, and Yumen Town and Huidong County, Sichuan Province) during the maturation period of fruit bodies from October to December, 2018. Truffle samples were brought to the laboratory, stored at -20°C until use, and identified by ITS sequence analysis. Samples used for VOCs analysis were from two provenances per truffle species as follows: *Tuber indicum* from Yongping and Yongren both in Yunnan province; and *T. pseudohimalayense* from Yumen and Huidong in Sichuan province (Fig. 2).

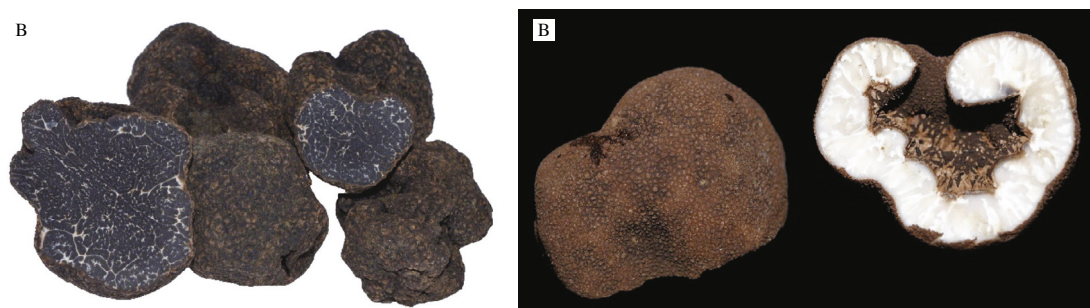


Fig. 1 Mature specimens of *T. indicum* (A) and *T. pseudohimalayense* (B) collected in Yunnan and Sichuan, respectively.

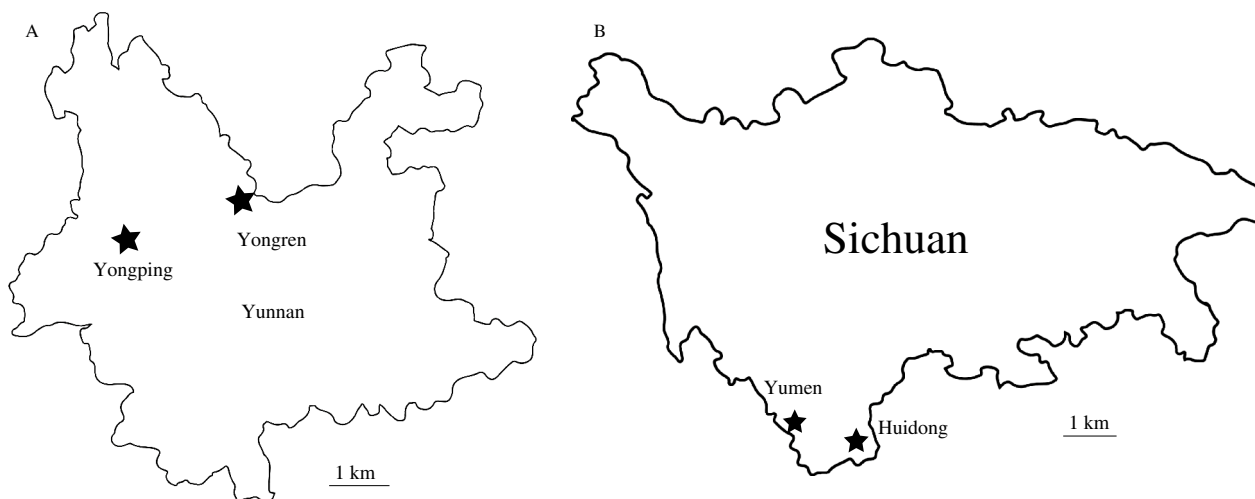


Fig. 2 Map of Yunnan and Sichuan provinces located in southwestern China, with indication of collection locations for *T. indicum* and *T. pseudohimalayense* examined in this study.

2.2 Chemicals

A divinylbenzene/carboxen/polydimethylsiloxane (DVB/CAR/PDMS) Stable Flex fiber of 50/30 μm was purchased from Supelco, USA and used for headspace solid-phase microextraction (HS-SPME).

2.3 HS-SPME

For every SPME analysis, A PTFE silicon septum was used to close the vial and make it airtight. The vial was heated until the temperature was 55 $^{\circ}\text{C}$, and the equilibrium was reached after 6 min. The fiber was then exposed to the headspace of the sample for 20 min. Once the extraction time ended, the fiber was removed from the vial and placed in the injection port of the gas chromatogram. A desorption time of 6 min with the injection temperature of 270 $^{\circ}\text{C}$ was adequate to desorb most of the analytes from the fiber in all conditions. After desorption from the fiber, the extract was directly transferred to the analytical column. The fibers were cleaned daily to prevent contamination.

2.4 Gas chromatography-mass spectrometry (GC-MS) analysis

The analyses of volatile compounds were carried out using gas chromatography-mass spectrometry (Agilent Technologie, Agilent 7890A along with Agilent 5975C, USA). Chromatographic separation was performed on a DB-5 capillary column (50 m \times 0.25 mm ID, 0.25 μm film thickness; Agilent Technologie, USA). The following chromatographic program was used: the oven temperature was held for 1 min at 30 $^{\circ}\text{C}$, then increased to 100 $^{\circ}\text{C}$ at a rate of 2 $^{\circ}\text{C}/\text{min}$ (held for 5 min), then increased to 170 $^{\circ}\text{C}$ at a rate of 5 $^{\circ}\text{C}/\text{min}$ (held for 15 min). The carrier gas (He) flow was constant at 1 mL/min. The injection port was operated in the splitless mode at 270 $^{\circ}\text{C}$. The operating conditions for the MS system were as follows: the ion source temperature was 230 $^{\circ}\text{C}$, electron ionization mode at 70 eV. Full scan mode with a scan range of m/z 30–400 was used for data acquisition. Each component was subjected to NIST11 library search and data were analyzed by using MSD ChemStation software (Agilent, version G1701EA E.02.02.1431). For each analyte, its relative mass fraction was calculated by peak area normalization method.

2.5 Evaluation of main volatile aroma components

The relative odor activity value (ROAV) was used to evaluate the strong contribution of each volatile substance to the overall flavor of the sample [17–19]. The $ROAV_{\text{stan}} = 100$ is defined as the component that contributes the most to the flavor of the sample, and the ROAV of other volatile components, if present, is less than 100. The calculation formula was as the following:

$$ROAV_A \approx 100 \times \frac{C_A}{C_{\text{stan}}} \times \frac{T_{\text{stan}}}{T_A}$$

in which, C_A and T_A represent the relative content of each volatile compound percentage and odor threshold ($\mu\text{g}/\text{kg}$), respectively, and C_{stan} and T_{stan} represent the relative concentration of the component at which it makes the greatest contribution to the overall flavor and the corresponding odor threshold ($\mu\text{g}/\text{kg}$), respectively. Relevant available thresholds are from literature references. It is generally believed that

aromatic compounds with high ROAV are most likely to be the main contributors to the overall aroma.

2.6 Statistical analysis

Data were reported as mean \pm SD. There were three replicates for each treatment and P -values for differences between different treatments within the same species were calculated using the Student's t -test ($P \leq 0.05$).

3. Results

HS-SPME coupled with GC-MS is a popular approach for the analysis of volatile compounds in truffles species [10,20–24]. Our HS-SPME/GC-MS analysis of *T. indicum* and *T. pseudohimalayense* aromatic profiles revealed a large variety of compounds. Overall, 76 VOCs were identified, belonging to different chemical classes, i.e. alcohols and phenols, aldehydes and ketones, benzenes and methoxy compounds, hydrocarbons and amines (Tables 1 and 2, Fig. 3). When single truffle species are considered, 66 and 46 VOCs were detected in *T. indicum* and *T. pseudohimalayense*, respectively (Tables 1 and 2, Fig. 4), with 54 (*T. indicum*) and 40 (*T. pseudohimalayense*) substances being reported here for the first time for these particular truffle species. More than 50% of identified VOCs (40 over 76) were produced by both truffle species.

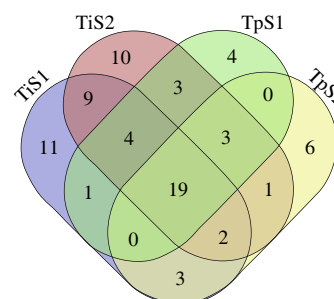


Fig. 3 Venn diagram showing the sets of specific and shared VOCs detected in *T. indicum* and *T. pseudohimalayense* from two different locations each. TiS1 = *T. indicum* from Yongping, Yunnan; TiS2 = *T. indicum* from Yongren, Yunnan; TpS1 = *T. pseudohimalayense* from Yumen, Sichuan; TpS2 = *T. pseudohimalayense* from Huidong, Sichuan.

Looking closely at the chemistry of identified VOCs and their relative abundance, when *T. indicum* is considered, the alcohols 1-octen-3-ol and phenylethyl alcohol and the aromatic 1-methoxy-3-methyl-benzene (3-methylanisole) were quantitatively important components of the aroma profile, at any stage of maturation (Table 1). 1-butanol, 2-methyl-, 3-octanol, 3,4-dimethoxytoluene, and 2,3-dimethoxytoluene were also relatively abundant, although their relative quantity varied significantly with maturation stages and provenance (Table 1). A comparable, but not identical aroma profile was recorded in the case of *T. pseudohimalayense* (Table 2). 1-octen-3-ol, phenylethyl alcohol, 3-octanol and above all 1-methoxy-3-methyl-benzene gave a relatively important contribution also to this truffle aroma; on the other hand, several volatiles such as (Z)-2-octen-1-ol, 1-octen-3-one, and 3-octanone were in average more concentrated in the aroma of *T. pseudohimalayense* than in *T. indicum* (Table 2). In different samples collected at the same location, there are significant changes in the content of some chemical components at different stages of maturity. Relevant examples include 2-methyl-

Table 1
Volatile compounds present in *T. indicum* at different maturation stages.

No.	Compound name	CAS No.	Molecular formula	Relative content (%)					
				Ti•S1•M	Ti•S1•MM	Ti•S1•Y	Ti•S2•M	Ti•S2•MM	Ti•S2•Y
Alcohols and Phenols									
1	(Z)-4-Decen-1-ol	57074-37-0	C ₁₀ H ₂₀ O	bd	bd	bd	bd	bd	0.30 ± 0.28
2	β-Ethylphenethyl alcohol	2035-94-1	C ₁₀ H ₁₄ O	bd	0.07 ± 0.03	bd	bd	bd	bd
3	2-methyl-1-Butanol	137-32-6	C ₅ H ₁₂ O	3.27 ± 0.12 ^a	2.90 ± 0.08 ^b	1.69 ± 0.30 ^c	8.97 ± 1.99 ^a	bd	6.18 ± 0.81 ^b
4	1-Hexanol	111-27-3	C ₆ H ₁₄ O	bd	bd	0.07 ± 0.01	bd	bd	bd
5	2-ethyl-1-Hexanol	104-76-7	C ₈ H ₁₈ O	bd	bd	bd	0.15 ± 0.03 ^a	0.14 ± 0.03 ^a	0.21 ± 0.10 ^a
6	1-Octanol	111-87-5	C ₈ H ₁₈ O	bd	bd	bd	bd	0.12 ± 0.03 ^a	0.19 ± 0.15 ^a
7	1-Octen-3-ol	3391-86-4	C ₈ H ₁₆ O	12.01 ± 8.08 ^a	8.08 ± 8.18 ^a	3.16 ± 2.86 ^a	5.28 ± 3.38 ^a	9.07 ± 7.43 ^{ab}	21.21 ± 8.38 ^b
8	(Z)-2-Octen-1-ol	26001-58-1	C ₈ H ₁₆ O	1.18 ± 1.20 ^a	bd	0.33 ± 0.36 ^a	0.63 ± 0.44 ^a	bd	2.59 ± 2.09 ^a
9	3-Methoxybenzyl alcohol	6971-51-3	C ₉ H ₁₀ O ₂	0.12 ± 0.09 ^a	0.10 ± 0.03 ^a	bd	bd	bd	bd
10	3-Octanol	589-98-0	C ₈ H ₁₈ O	bd	2.44 ± 0.44 ^a	0.92 ± 0.40 ^b	1.42 ± 0.02 ^a	2.46 ± 1.24 ^a	6.19 ± 0.67 ^b
11	3,4,5-trimethoxy-Benzenemethanol	3840-31-1	C ₁₀ H ₁₄ O ₄	bd	bd	bd	0.08 ± 0.03	bd	bd
12	Butylated Hydroxytoluene	128-37-0	C ₁₅ H ₂₄ O	0.55 ± 0.08 ^a	0.91 ± 0.22 ^b	0.82 ± 0.08 ^b	1.23 ± 0.21 ^a	1.76 ± 1.39 ^{ab}	1.55 ± 0.11 ^b
13	Creosol	93-51-6	C ₈ H ₁₀ O ₂	bd	bd	0.04 ± 0.01	bd	bd	bd
14	p-Cresol	106-44-5	C ₇ H ₈ O	bd	0.54 ± 0.30 ^a	0.41 ± 0.03 ^a	bd	bd	bd
15	3-methyl-6-propyl-Phenol	31143-55-2	C ₁₀ H ₁₄ O	bd	1.63 ± 0.01 ^a	0.83 ± 0.24 ^b	bd	bd	0.24 ± 0.12
16	3-propyl-Phenol	621-27-2	C ₉ H ₁₂ O	bd	0.16 ± 0.08 ^a	0.18 ± 0.14 ^a	bd	bd	bd
17	Phenylethyl Alcohol	60-12-8	C ₈ H ₁₀ O	3.46 ± 0.68 ^a	9.46 ± 1.92 ^b	6.76 ± 3.75 ^{ab}	10.51 ± 4.12 ^a	14.50 ± 4.40 ^a	8.80 ± 8.12 ^a
18	trans-(2-Ethylcyclopentyl)methanol	36258-08-9	C ₈ H ₁₆ O	bd	bd	0.07 ± 0.02	bd	bd	bd
Esters									
19	β-Phenylethyl butyrate	103-52-6	C ₁₂ H ₁₆ O ₂	bd	bd	0.05 ± 0.01	bd	bd	0.10 ± 0.07
20	γ-Butylbutyrolactone	18679-18-0	C ₁₂ H ₂₀ O ₂	bd	bd	bd	bd	bd	0.09 ± 0.06
21	γ-nonanoic lactone	104-61-0	C ₉ H ₁₆ O ₂	bd	bd	0.07 ± 0.06	bd	bd	bd
22	Tiglicacidisobutylester	61692-84-0	C ₉ H ₁₆ O ₂	bd	bd	bd	bd	bd	0.30 ± 0.15
23	Acetic acid, 2-phenylethyl ester	103-45-7	C ₁₀ H ₁₂ O ₂	bd	0.07 ± 0.03 ^a	0.09 ± 0.00 ^a	0.04 ± 0.01 ^a	0.10 ± 0.04 ^b	bd
24	Benzeneacetic acid, 2-methylpropyl ester	102-13-6	C ₁₂ H ₁₆ O ₂	bd	bd	bd	0.03 ± 0.01 ^a	0.06 ± 0.01 ^b	bd
25	Benzeneacetic acid, ethyl ester	101-97-3	C ₁₀ H ₁₂ O ₂	bd	bd	bd	0.03 ± 0.00	bd	bd
26	Butanoic acid, 2-methyl-, 2-methylpropyl ester	2445-67-2	C ₉ H ₁₈ O ₂	bd	bd	bd	bd	bd	0.29 ± 0.24
27	Butanoic acid, 2-methyl-, 3-methylbutyl ester	27625-35-0	C ₁₀ H ₂₀ O ₂	0.06 ± 0.01 ^a	0.09 ± 0.02 ^b	0.09 ± 0.06 ^{ab}	bd	bd	0.16 ± 0.11
28	Butanoic acid, 3-methyl-, 3-methylbutyl ester	659-70-1	C ₁₀ H ₂₀ O ₂	0.07 ± 0.02 ^a	0.12 ± 0.05 ^a	0.15 ± 0.11 ^a	bd	bd	0.08 ± 0.02
29	Dibutyl phthalate	84-74-2	C ₁₆ H ₂₂ O ₄	0.11 ± 0.01 ^a	0.13 ± 0.08 ^a	0.11 ± 0.02 ^a	0.21 ± 0.02 ^a	0.28 ± 0.08 ^a	bd
30	Hexanoic acid, 2-methylpropyl ester	105-79-3	C ₁₀ H ₂₀ O ₂	0.09 ± 0.01	bd	bd	bd	bd	0.26 ± 0.20
31	n-Amyl isovalerate	25415-62-7	C ₁₀ H ₂₀ O ₂	bd	0.06 ± 0.01	bd	bd	bd	bd
32	Propanoic acid, 2-methyl-, 2-methylpropyl ester	97-85-8	C ₈ H ₁₆ O ₂	0.41 ± 0.43 ^a	0.17 ± 0.13 ^a	bd	bd	bd	0.15 ± 0.04
Aldehydes and Ketones									
33	1-Octen-3-one	4312-99-6	C ₁₀ H ₁₈ O	bd	bd	bd	bd	1.42 ± 1.15 ^a	0.77 ± 0.66 ^a
34	(E,E)-2,4-Decadienal	25152-84-5	C ₁₀ H ₁₆ O	bd	bd	bd	bd	bd	0.06 ± 0.04
35	(E)-2-Octenal	2548-87-0	C ₈ H ₁₄ O	0.28 ± 0.27	bd	bd	1.25 ± 1.38 ^a	bd	0.63 ± 0.36 ^a
36	2-Undecanone	112-12-9	C ₁₁ H ₂₂ O	bd	0.05 ± 0.01 ^a	0.03 ± 0.01 ^b	bd	bd	0.07 ± 0.01
37	3-Octanone	106-68-3	C ₈ H ₁₆ O	3.11 ± 1.17 ^a	2.28 ± 0.09 ^a	0.69 ± 0.15 ^b	0.88 ± 0.19 ^a	1.44 ± 0.26 ^b	4.06 ± 0.71 ^c
38	(Z)-6,10-dimethyl-5,9-Undecadien-2-one	3879-26-3	C ₁₃ H ₂₂ O	bd	0.06 ± 0.03	bd	bd	bd	bd
39	Benzaldehyde	100-52-7	C ₇ H ₆ O	0.09 ± 0.03 ^a	0.22 ± 0.03 ^b	0.22 ± 0.01 ^b	bd	0.27 ± 0.14 ^a	0.29 ± 0.05 ^a
40	Benzaldehyde, 3-methoxy-	591-31-1	C ₈ H ₈ O ₂	0.13 ± 0.03 ^a	0.08 ± 0.06 ^a	bd	bd	bd	bd
41	Benzeneacetaldehyde	122-78-1	C ₈ H ₈ O	0.15 ± 0.06	bd	bd	0.98 ± 0.10 ^a	0.50 ± 0.01 ^b	0.90 ± 0.88 ^{ab}
42	α-ethylidene-Benzeneacetaldehyde	4411-89-6	C ₁₀ H ₁₀ O	bd	0.11 ± 0.10	bd	0.14 ± 0.05 ^a	0.17 ± 0.11 ^a	0.20 ± 0.11 ^a
43	Decanal	112-31-2	C ₁₀ H ₂₀ O	bd	bd	bd	0.05 ± 0.01 ^a	0.10 ± 0.03 ^b	bd
44	Hexanal	66-25-1	C ₆ H ₁₂ O	bd	bd	bd	0.75 ± 0.71	bd	bd
45	Nonanal	124-19-6	C ₉ H ₁₈ O	bd	bd	bd	0.15 ± 0.05 ^a	0.17 ± 0.06 ^a	bd
Benzene and Methoxy compounds									
46	Estragole	140-67-0	C ₁₀ H ₁₂ O	0.08 ± 0.05 ^a	bd	0.53 ± 0.65 ^a	0.18 ± 0.06	bd	bd
47	1-methoxy-3-methyl-Benzene	100-84-5	C ₈ H ₁₀ O	63.75 ± 6.48 ^{ab}	50.51 ± 11.37 ^a	65.91 ± 3.65 ^b	61.26 ± 4.20 ^a	61.04 ± 1.66 ^a	40.57 ± 3.13 ^b
48	1,4-dimethoxy-2-methyl-Benzene	24599-58-4	C ₉ H ₁₂ O ₂	1.93 ± 1.53 ^{ab}	2.30 ± 1.03 ^a	0.58 ± 0.02 ^b	1.13 ± 0.60 ^a	0.83 ± 0.31 ^a	0.55 ± 0.33 ^a
49	1,3-dimethoxy-Benzene	151-10-0	C ₈ H ₁₀ O ₂	0.92 ± 0.28 ^a	0.71 ± 0.13 ^{ab}	0.51 ± 0.16 ^b	0.34 ± 0.02 ^a	0.37 ± 0.14 ^a	0.18 ± 0.06 ^b
50	1,2-dimethoxy-Benzene	91-16-7	C ₈ H ₁₀ O ₂	bd	0.03 ± 0.00	bd	0.16 ± 0.00 ^a	0.40 ± 0.11 ^b	0.16 ± 0.01 ^a
51	1,2,3-trimethoxy-5-methyl-Benzene	6443-69-2	C ₁₀ H ₁₄ O ₃	0.33 ± 0.20 ^a	0.81 ± 0.43 ^a	0.68 ± 0.74 ^a	0.20 ± 0.05 ^a	0.48 ± 0.54 ^a	0.12 ± 0.13 ^a
52	(2-methoxyethyl)-Benzene	3558-60-9	C ₉ H ₁₂ O	bd	bd	bd	0.24 ± 0.20	bd	bd
53	3-Methoxybenzyl alcohol, methyl ether	1000365-13-0	C ₉ H ₁₂ O ₂	0.12 ± 0.06 ^a	0.09 ± 0.02 ^a	0.03 ± 0.00 ^b	bd	bd	bd

Table 1 (Continued)

No.	Compound name	CAS No.	Molecular formula	Relative content (%)					
				Ti•S1•M	Ti•S1•MM	Ti•S1•Y	Ti•S2•M	Ti•S2•MM	Ti•S2•Y
54	3-Ethylphenol, methyl ether	1000333-41-0	C ₉ H ₁₂ O	bd	3.75 ± 0.66 ^a	3.64 ± 2.21 ^a	1.08 ± 0.04 ^a	1.15 ± 0.10 ^a	0.68 ± 0.08 ^b
55	3,5-Dimethoxytoluene	4179-19-5	C ₉ H ₁₂ O ₂	0.12 ± 0.09 ^a	0.17 ± 0.09 ^a	0.16 ± 0.13 ^a	0.05 ± 0.00 ^a	0.13 ± 0.09 ^a	bd
56	3,4-Dimethoxytoluene	494-99-5	C ₉ H ₁₂ O ₂	6.82 ± 1.60 ^a	10.41 ± 1.21 ^b	bd	1.70 ± 0.06 ^a	2.80 ± 1.51 ^a	0.97 ± 0.03 ^b
57	2,5-Dimethoxyethylbenzene	1199-08-2	C ₁₀ H ₁₄ O ₂	bd	0.07 ± 0.06	bd	bd	bd	bd
58	2,3-Dimethoxytoluene	4463-33-6	C ₉ H ₁₂ O ₂	0.68 ± 0.04 ^a	1.20 ± 0.27 ^b	10.83 ± 1.24 ^c	0.33 ± 0.04 ^a	bd	0.30 ± 0.02 ^a
59	1,2,4-Trimethoxybenzene	135-77-3	C ₉ H ₁₂ O ₃	0.18 ± 0.18 ^a	0.14 ± 0.02 ^a	bd	0.09 ± 0.06 ^a	0.07 ± 0.04 ^a	bd
60	1,2,3-Trimethoxybenzene	634-36-6	C ₉ H ₁₂ O ₃	bd	0.01 ± 0.00	bd	bd	bd	bd
61	1,2,3,4-Tetramethoxybenzene	21450-56-6	C ₁₀ H ₁₄ O ₄	0.02 ± 0.01 ^a	0.01 ± 0.00 ^a	0.16 ± 0.16 ^a	0.03 ± 0.01 ^a	0.19 ± 0.09 ^b	bd
Hydrocarbons									
62	3-ethyl-2-methyl-1,3-Hexadiene	61142-36-7	C ₉ H ₁₆	bd	bd	bd	0.21 ± 0.04 ^a	bd	0.12 ± 0.01 ^a
63	Tetradecane	629-59-4	C ₁₄ H ₃₀	bd	0.05 ± 0.01 ^a	0.05 ± 0.01 ^a	0.06 ± 0.01	bd	bd
64	Tridecane	629-50-5	C ₁₃ H ₂₈	bd	bd	bd	0.07 ± 0.01	bd	bd
N-containing compounds									
65	3-methyl-N-(3-methylbutylidene)-1-Butanamine	35448-31-8	C ₁₀ H ₂₁ N	bd	bd	0.15 ± 0.03	0.15 ± 0.08 ^a	bd	0.44 ± 0.23 ^b
66	3-methyl-N-(2-phenylethylidene)-1-Butanamine	139183-86-1	C ₁₃ H ₁₉ N	bd	0.04 ± 0.01 ^a	0.02 ± 0.01 ^b	bd	bd	bd

Note: bd = below detection limit; Y = young; MM = middle mature; M = mature; S1 = Yongping, Yunnan; S2 = Yongren, Yunnan. Relative content mean values ± standard deviations are shown ($n = 3$). For each provenance, different superscript letters indicate significant differences according to Student's *t*-test ($P < 0.05$).

Table 2

Volatile compounds present in *T. pseudohimalayense* at different maturation stages.

No.	Compound name	CAS No.	Molecular formula	Relative content (%)					
				Tp•S1•M	Tp•S1•MM	Tp•S1•Y	Tp•S2•M	Tp•S2•MM	Tp•S2•Y
Alcohols and Phenols									
1	β -Ethylphenethyl alcohol	2035-94-1	C ₁₀ H ₁₄ O	Bd	Bd	bd	0.21 \pm 0.03 ^a	0.62 \pm 0.49 ^a	bd
2	1-Hexanol	111-27-3	C ₆ H ₁₄ O	Bd	bd	bd	bd	0.31 \pm 0.13	bd
3	2-ethyl-1-Hexanol	104-76-7	C ₈ H ₁₈ O	Bd	bd	0.35 \pm 0.17	0.33 \pm 0.00 ^a	0.33 \pm 0.02 ^a	1.74 \pm 1.82 ^a
4	1-Octanol	111-87-5	C ₈ H ₁₈ O	Bd	0.28 \pm 0.06 ^a	0.59 \pm 0.30 ^a	bd	bd	bd
5	1-Octen-3-ol	3391-86-4	C ₈ H ₁₆ O	Bd	14.03 \pm 13.85	bd	6.33 \pm 4.27 ^a	3.61 \pm 0.79 ^a	16.52 \pm 18.31 ^a
6	1-Pentanol	71-41-0	C ₅ H ₁₂ O	Bd	bd	0.70 \pm 0.43	bd	bd	bd
7	(Z)-2-Octen-1-ol	26001-58-1	C ₈ H ₁₆ O	7.02 \pm 1.38 ^a	5.97 \pm 1.17 ^a	7.01 \pm 7.35 ^a	0.65 \pm 0.46 ^a	0.33 \pm 0.07 ^a	bd
8	3-Octanol	589-98-0	C ₈ H ₁₈ O	2.36 \pm 1.68 ^a	1.25 \pm 0.18 ^a	15.20 \pm 0.84 ^b	3.96 \pm 0.91 ^a	0.78 \pm 0.31 ^b	2.78 \pm 1.83 ^{ab}
9	Butylated Hydroxytoluene	128-37-0	C ₁₅ H ₂₄ O	1.65 \pm 0.27 ^a	1.21 \pm 0.51 ^a	1.95 \pm 0.62 ^a	1.63 \pm 0.73 ^a	2.93 \pm 0.47 ^b	6.74 \pm 0.95 ^c
10	3-methyl-6-propyl-Phenol	31143-55-2	C ₁₀ H ₁₄ O	0.09 \pm 0.06 ^a	bd	0.11 \pm 0.01 ^a	bd	0.35 \pm 0.27	Bd
11	Phenylethyl Alcohol	60-12-8	C ₈ H ₁₀ O	0.61 \pm 0.22 ^a	0.60 \pm 0.04 ^a	0.88 \pm 0.51 ^a	8.50 \pm 10.87 ^a	18.42 \pm 7.65 ^a	14.74 \pm 11.45 ^a
Esters									
12	Dibutyl phthalate	84-74-2	C ₁₆ H ₂₂ O ₄	0.16 \pm 0.00 ^a	0.15 \pm 0.06 ^a	bd	bd	bd	bd
13	Methyl salicylate	119-36-8	C ₈ H ₈ O ₃	bd	bd	bd	bd	0.44 \pm 0.13	bd
Aldehydes and Ketones									
14	1-Octen-3-one	4312-99-6	C ₁₀ H ₁₈ O	26.82 \pm 1.62 ^a	20.15 \pm 3.56 ^b	9.06 \pm 5.66 ^c	bd	bd	bd
15	(E)-2-Octenal	2548-87-0	C ₈ H ₁₄ O	4.51 \pm 1.11 ^a	2.95 \pm 0.54 ^b	3.59 \pm 0.94 ^{ab}	bd	bd	bd
16	2-Undecanone	112-12-9	C ₁₁ H ₂₂ O	bd	bd	0.12 \pm 0.01	bd	bd	0.45 \pm 0.14
17	3-Octanone	106-68-3	C ₈ H ₁₆ O	14.10 \pm 4.64 ^a	12.06 \pm 2.00 ^a	39.54 \pm 9.78 ^b	5.21 \pm 1.70 ^a	1.92 \pm 0.34 ^b	20.20 \pm 12.51 ^c
18	(E)-6,10-dimethyl-5,9-Undecadien-2-one	3796-70-1	C ₁₃ H ₂₂ O	bd	bd	bd	bd	0.12 \pm 0.06	bd
19	Benzaldehyde	100-52-7	C ₇ H ₆ O	bd	bd	bd	bd	bd	1.69 \pm 0.33
20	Benzeneacetaldehyde	122-78-1	C ₈ H ₈ O	0.20 \pm 0.16 ^a	0.24 \pm 0.10 ^a	0.36 \pm 0.14 ^a	bd	bd	6.54 \pm 6.01
21	α -ethylidene-Benzeneacetaldehyde	4411-89-6	C ₁₀ H ₁₀ O	bd	bd	bd	0.12 \pm 0.09 ^a	0.53 \pm 0.44 ^a	1.58 \pm 0.57 ^b
22	Decanal	112-31-2	C ₁₀ H ₂₀ O	bd	bd	0.16 \pm 0.15	bd	bd	bd
23	Nonanal	124-19-6	C ₉ H ₁₈ O	0.05 \pm 0.00 ^a	0.07 \pm 0.03 ^a	0.25 \pm 0.23 ^a	bd	bd	0.74 \pm 0.49
24	Octanal	124-13-0	C ₈ H ₁₆ O	0.29 \pm 0.21	bd	bd	bd	bd	bd
Benzene and Methoxy compounds									
25	1-methoxy-4-propyl-Benzene	104-45-0	C ₁₀ H ₁₄ O	bd	0.10 \pm 0.00	bd	bd	bd	bd
26	1-methoxy-3-methyl-Benzene	100-84-5	C ₈ H ₁₀ O	34.00 \pm 7.00 ^a	32.36 \pm 1.58 ^a	16.09 \pm 13.75 ^b	65.36 \pm 11.95 ^a	61.12 \pm 11.41 ^a	13.72 \pm 3.78 ^b
27	1,4-dimethoxy-2-methyl-Benzene	24599-58-4	C ₉ H ₁₂ O ₂	2.28 \pm 0.11 ^a	3.33 \pm 2.68 ^{ab}	0.86 \pm 0.59 ^b	0.28 \pm 0.13 ^a	0.51 \pm 0.11 ^b	bd
28	1,3-dimethoxy-Benzene	151-10-0	C ₈ H ₁₀ O ₂	2.77 \pm 0.56 ^a	1.77 \pm 1.30 ^a	0.19 \pm 0.02 ^b	0.23 \pm 0.02	bd	bd
29	1,2-dimethoxy-Benzene	91-16-7	C ₈ H ₁₀ O ₂	0.12 \pm 0.06 ^{ab}	0.06 \pm 0.02 ^a	0.15 \pm 0.02 ^b	bd	bd	bd
30	1,2,3-trimethoxy-5-methyl-Benzene	6443-69-2	C ₁₀ H ₁₄ O ₃	0.21 \pm 0.05 ^a	0.57 \pm 0.37 ^a	0.24 \pm 0.00 ^a	0.11 \pm 0.01 ^a	0.16 \pm 0.00 ^b	bd
31	3-Ethylphenol, methyl ether	1000333-41-0	C ₉ H ₁₂ O	0.51 \pm 0.18 ^a	0.50 \pm 0.05 ^a	bd	1.06 \pm 0.35 ^a	1.20 \pm 0.84 ^a	bd
32	3,5-Dimethoxytoluene	4179-19-5	C ₉ H ₁₂ O ₂	0.07 \pm 0.02 ^a	0.59 \pm 0.65 ^a	bd	0.20 \pm 0.09 ^a	0.14 \pm 0.03 ^a	bd

Table 2 (Continued)

No.	Compound name	CAS No.	Molecular formula	Relative content (%)					
				Tp•S1•M	Tp•S1•MM	Tp•S1•Y	Tp•S2•M	Tp•S2•MM	Tp•S2•Y
33	3,4-Dimethoxytoluene	494-99-5	C ₉ H ₁₂ O ₂	1.28 ± 0.21 ^a	1.08 ± 0.40 ^a	1.06 ± 0.04 ^a	0.27 ± 0.04 ^a	0.70 ± 0.05 ^b	0.23 ± 0.02 ^a
34	2-Propylphenol, methyl ether	1000333-48-4	C ₁₀ H ₁₄ O	bd	bd	bd	0.13 ± 0.01	bd	bd
35	2,3-Dimethoxytoluene	4463-33-6	C ₉ H ₁₂ O ₂	0.42 ± 0.12 ^a	0.32 ± 0.02 ^a	0.41 ± 0.03 ^a	0.26 ± 0.06	bd	bd
36	1,2,4-Trimethoxybenzene	135-77-3	C ₉ H ₁₂ O ₃	0.34 ± 0.10 ^a	0.25 ± 0.21 ^{ab}	0.13 ± 0.08 ^b	bd	bd	bd
37	1,2,3-Trimethoxybenzene	634-36-6	C ₉ H ₁₂ O ₃	0.04 ± 0.01 ^a	0.03 ± 0.00 ^b	0.05 ± 0.02 ^a	bd	bd	bd
Hydrocarbons									
38	3-ethyl-2-methyl-1,3-Hexadiene	61142-36-7	C ₉ H ₁₆	bd	bd	bd	bd	0.37 ± 0.18	bd
39	Dodecane	112-40-3	C ₁₂ H ₂₆	bd	bd	bd	bd	bd	0.26 ± 0.04
40	Styrene	100-42-5	C ₈ H ₈	bd	bd	0.15 ± 0.04	bd	bd	bd
41	Tetradecane	629-59-4	C ₁₄ H ₃₀	0.05 ± 0.01 ^a	0.04 ± 0.01 ^a	0.08 ± 0.01 ^b	0.07 ± 0.02 ^a	0.10 ± 0.02 ^{ab}	0.35 ± 0.11 ^b
42	Tridecane	629-50-5	C ₁₃ H ₂₈	0.06 ± 0.00 ^a	0.05 ± 0.01 ^b	0.12 ± 0.02 ^c	bd	bd	0.35 ± 0.11
N-containing compounds									
43	2,2-diethyl-3-methyl-Oxazolidine	161500-43-2	C ₈ H ₁₇ NO	bd	bd	bd	0.25 ± 0.09	bd	bd
44	3-Acetamido-1-phenylpyrazole	2753-56-2	C ₁₁ H ₁₁ N ₃ O	bd	bd	bd	0.07 ± 0.00	bd	bd
45	3-methyl-N-(3-methylbutylidene)-1-Butanamine	35448-31-8	C ₁₀ H ₂₁ N	bd	bd	0.61 ± 0.12	4.70 ± 0.21 ^a	4.85 ± 2.67 ^a	11.39 ± 6.22 ^a
46	3-methyl-N-(2-phenylethylidene)-1-Butanamine	139183-86-1	C ₁₃ H ₁₉ N	bd	bd	bd	0.08 ± 0.02 ^a	0.17 ± 0.13 ^a	bd

Note: bd = below detection limit; Y = young; MM = middle mature; M = mature; S1 = Yumen, Sichuan; S2= Huidong, Sichuan. Relative content mean values ± standard deviations are shown (n = 3). For each provenance, different superscript letters indicate significant differences according to Student's *t*-test (*P* < 0.05).

1-butanol, 3-octanol, 3-octanone, 1-methoxy-3-methyl-benzene, 1-octen-3-one, (*E*)-2-octenal, 1,4-dimethoxy-2-methyl-benzene, 1,3-dimethoxy-benzene. Among these, 1-methoxy-3-methyl-benzene, 1,4-dimethoxy-2-methyl-benzene, and 1,3-dimethoxy-benzene displayed the largest statistical variability (Table 1 and Table 2).

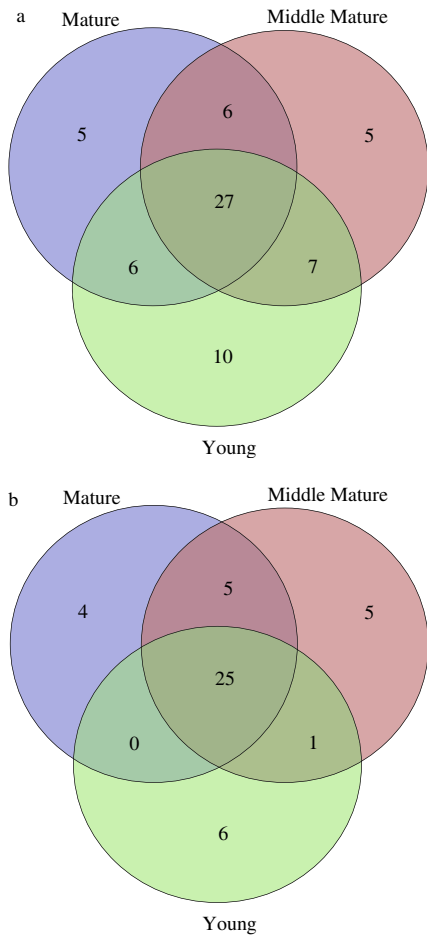


Fig. 4 Venn diagrams of the VOC's produced by *T. indicum* (TiS1 + TiS2, a) and *T. pseudohimalayense* (TpS1 + TpS2, b) at different stages of maturation. See Fig. 3 for abbreviations of truffle names and provenances.

In general, only a small proportion of volatile compounds contribute significantly to the overall flavor of any truffles, and some other ingredients may only assist the overall aroma. The contribution of volatile compounds to the aroma of truffles is determined by their content and odor threshold. Some compounds with low content but low odor threshold may also play an important role in the overall flavor of truffles. The ROAV values of the aroma components of truffles at different maturity stages according to this test are shown in Table 3 and Table 4 for *T. indicum* and *T. pseudohimalayense*, respectively. According to Table 3, the key aroma compounds (ROAV ≥ 1) at different maturation stages in *T. indicum* were 1-octen-3-ol, 3-octanone, 1-methoxy-3-methyl-benzene, 1-octen-3-one; in addition, (*E*)-2-octenal, phenethyl alcohol, 1-octanol, nonanal, 3-octanol, 2-undecone, and hexanal play an important role in modifying the overall aroma (0.1 ≤ ROAV < 1). As shown in Table 4, the key aroma compounds (ROAV ≥ 1) at different maturation stages in *T. pseudohimalayense* are 1-octen-3-ol, 1-octen-3-one, 3-octanone, phenylacetaldehyde, 1-methoxy-3-methyl-benzene, (*E*)-2-octenal; furthermore, 1-octanol, 3-octanol, phenethyl alcohol, 2-undecone, hexanal, and nonanal, play an important role in overall aroma modification (0.1 ≤ ROAV < 1).

The compound 1-octen-3-ol, also known as mushroom alcohol, is an extremely potent olfactory attractant for many insect species [25]; it has been isolated from a number of mushrooms and from the essential oils of aromatic plants, and has also been identified in mature fruiting bodies of *T. borchii* [26,27]. Anisole homologues were reported for the first time in black truffles thanks to the study of *T. melanosporum* aroma, with 3-methylanisole being described as characterized by “an earthy and flowery note, with an undertone of chocolate,” while 1,3-dimethoxybenzene (resorcinol dimethyl ether), also present (although in low amount) in both *T. indicum* and *T. pseudohimalayense*, is reported to possess “a sweet and earthy odor, with a powerful hazelnut note” [28].

A specific aim of our research was to investigate the influence exerted by two key factors, i.e. truffle provenance and maturation stage, on the composition of aroma in two of the most important wild Chinese truffles. To this end, we selected two different Yunnan or Sichuan locations for both *T. indicum* and *T. pseudohimalayense*,

Table 3Relative contribution of volatiles to aroma in *T. indicum* at different maturation stages.

Compound name	Odor threshold ($\mu\text{g/kg}$)	ROAV					
		Ti•S1•M	Ti•S1•MM	Ti•S1•Y	Ti•S2•M	Ti•S2•MM	Ti•S2•Y
1-Hexanol	100 ^[1]	-	-	< 0.01	-	-	-
1-Octanol	37 ^[1]	-	-	-	-	0.01	0.03
1-Octen-3-ol	1 ^[1]	39.56	33.59	10.07	18.10	31.20	109.79
3-Octanol	22 ^[1]	-	0.46	0.13	0.22	0.39	1.46
Phenylethyl Alcohol	80 ^[1]	0.14	0.49	0.27	0.45	0.62	0.57
1-Octen-3-one	0.8 ^[1]	-	-	-	-	6.11	4.98
(E)-2-Octenal	3 ^[2]	0.31	-	-	1.43	-	1.09
2-Undecanone	7 ^[2]	-	0.03	0.01	-	-	0.05
3-Octanone	1.3 ^[1]	7.88	7.29	1.69	2.32	3.81	16.17
Benzaldehyde	320 ^[1]	< 0.01	< 0.01	< 0.01	-	< 0.01	< 0.01
Benzeneacetaldehyde	0.7 ^[1]	0.71	-	-	4.80	2.46	6.66
Hexanal	9 ^[1]	-	-	-	0.29	-	-
Nonanal	40 ^[1]	-	-	-	0.01	0.02	-
1-methoxy-3-methyl-Benzene	2.1	100	100	100	100	100	100

Note: “-” means that this compound was not detected, so that sensory threshold analysis of the compound could not be performed. Reference data are from: 1 = [40], 2 = [19]. Abbreviations of provenances and maturation stages are described at the foot of the Table 1.

Table 4Relative contribution of volatiles to aroma in *T. pseudohimalayense* at different maturation stages.

Compound name	Odor threshold ($\mu\text{g/kg}$)	ROAV					
		Tp•S1•M	Tp•S1•MM	Tp•S1•Y	Tp•S2•M	Tp•S2•MM	Tp•S2•Y
1-Hexanol	100 ^[1]	-	-	-	-	0.01	-
1-Octanol	37 ^[1]	-	0.05	0.21	-	-	-
1-Octen-3-ol	1 ^[1]	-	91.05	-	20.34	12.40	106.32
1-Pentanol	4 000 ^[1]	-	-	< 0.01	-	-	-
3-Octanol	22 ^[1]	0.66	0.37	2.27	0.58	0.12	0.81
Phenylethyl Alcohol	80 ^[1]	0.05	0.05	0.04	0.34	0.79	1.19
1-Octen-3-one	0.8 ^[1]	207.07	163.45	37.23	-	-	-
(E)-2-Octenal	3 ^[2]	9.29	6.38	3.93	-	-	-
2-Undecanone	7 ^[2]	-	-	0.06	-	-	0.41
3-Octanone	1.3 ^[1]	66.99	60.20	100	12.88	5.08	100
Benzaldehyde	320 ^[1]	-	-	-	-	-	0.03
Benzeneacetaldehyde	0.7 ^[1]	1.77	2.23	1.69	-	-	60.13
Nonanal	9 ^[1]	0.03	0.05	0.09	-	-	0.53
Octanal	0.7 ^[1]	2.56	-	-	-	-	-
1-methoxy-3-methyl-Benzene	2.1	100	100	25.19	100	100	42.05

Note: “-” means that this compound was not detected, so that sensory threshold analysis of the compound could not be performed. Reference data are from: 1 = [40], 2 = [19]. Abbreviations of provenances and maturation stages are described at the foot of the Table 2.

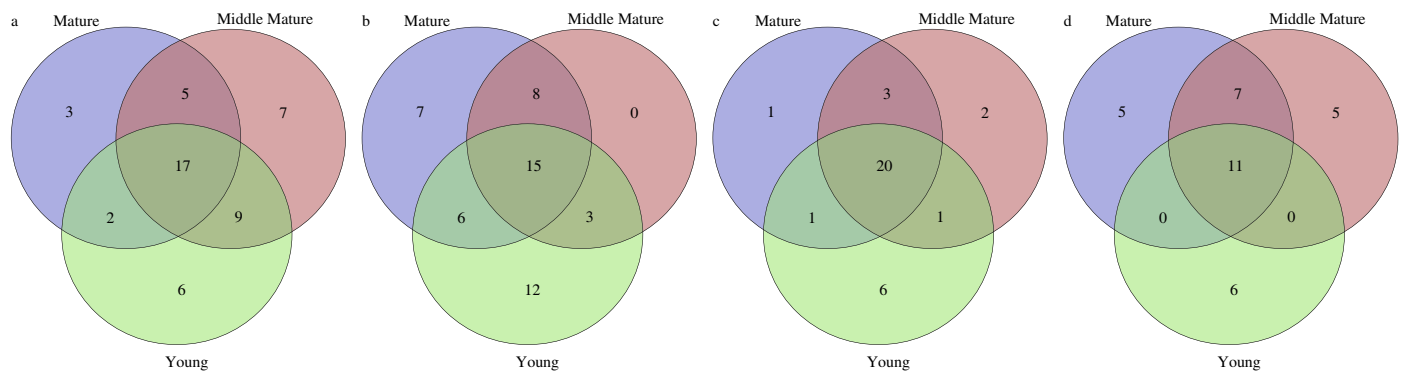


Fig. 5 Different composition of aroma from fruit bodies of *T. indicum* (a,b) and *T. pseudohimalayense* (c,d) collected in two distinct locations at three stages of maturation (see Fig. 6 for further details). Abbreviations of the truffle species and provenances are described in the legend of Fig. 3.

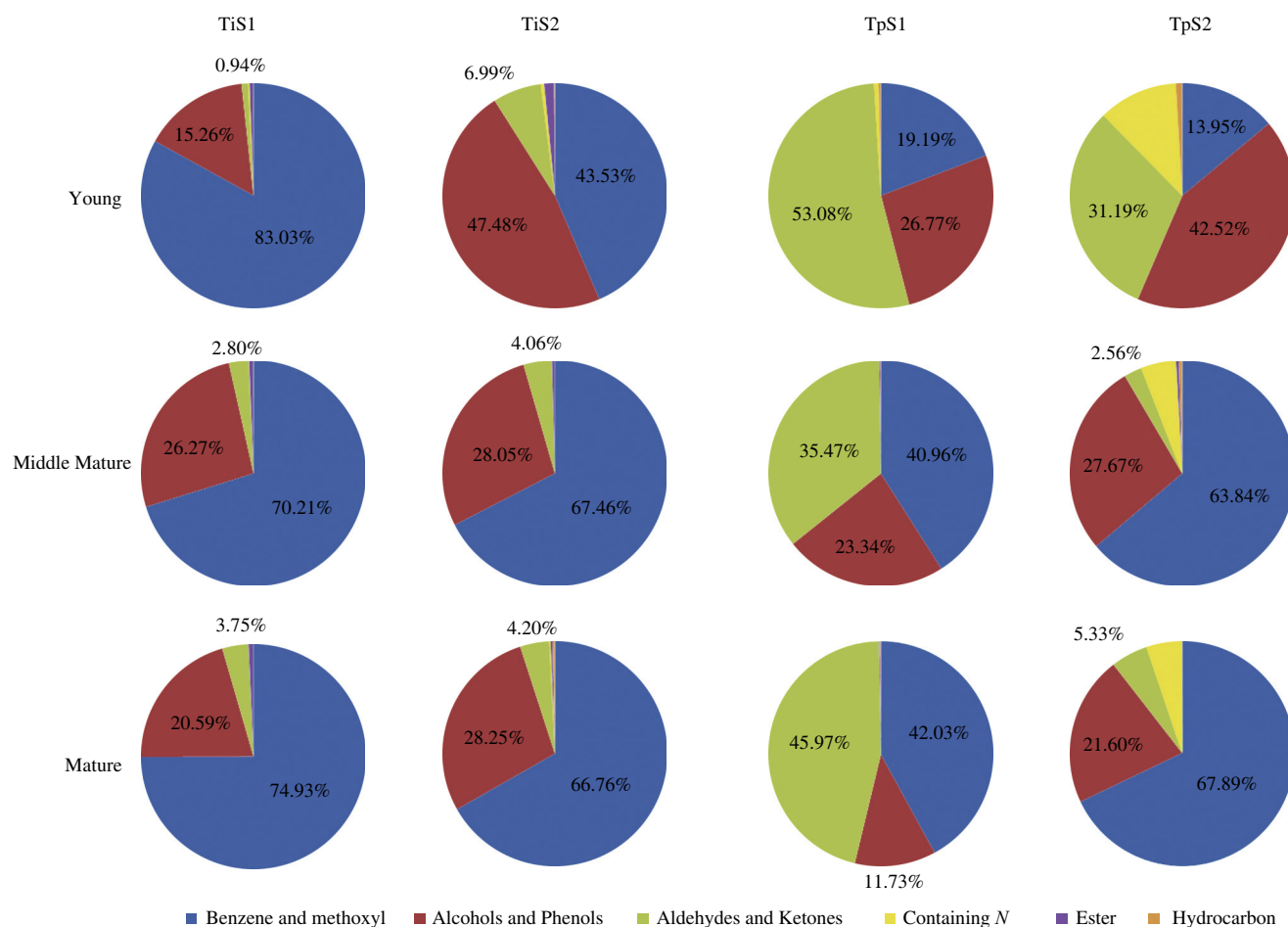


Fig. 6 Chemical classes of VOCs and their relative amount from fruit bodies of *T. indicum* and *T. pseudohimalayense* collected in two distinct locations at three stages of maturation (see Fig. 5 for further details). Abbreviations of the truffle species and provenances are described in the legend of Fig. 3.

respectively (Fig. 2), and in each location collected young, middle mature and mature fruiting bodies of the given truffle. Looking at the resulting Venn diagrams shown in Fig. 4, it is clear that a large number of VOCs in both species are shared among all stages of maturations. However, each single stage has its own VOCs profile, and some other volatiles are shared among any pairs of maturation stages (with the single exception of the young-mature pair in *T. pseudohimalayense*) (Fig. 4). These findings reveal a dynamic pattern in the biosynthesis of VOCs, with some compounds that most likely play their role only in a specific moment in the part of the fungal life cycle that culminates with the production of fruiting bodies. When looking at the VOCs of truffles originating from different locations, other interesting data emerge. While for both *T. indicum* and *T. pseudohimalayense* there was little or no variation in the total number of VOCs detected in fruiting bodies of different provenance, the composition and distribution of VOCs varied significantly among populations, particularly in the case of *T. pseudohimalayense* (Table 1, Table 2, Fig. 5 and Fig. 6). For example, *N*-containing compounds 3-methyl-*N*-(3-methylbutylidene)-1-butanamine and 3-methyl-*N*-(2-phenylethylidene)-1-butanamine were present almost exclusively in *T. pseudohimalayense* from a single origin (S2 in Table 2 and Fig. 6). This indicates that aroma maturation is a complex process, likely influenced by the genotype of the fungus and of symbiotic bacteria (and possibly also of host plant) but also by a number of

edaphic factors and microclimatic conditions that our limited survey cannot disclose at this stage.

4. Discussion

T. indicum and *T. pseudohimalayense* are truffles of great commercial interest, in China and beyond (Fig. 7). The truffles are regularly offered on sale in the markets and are collected in the wild, and the two are sold together occasionally. At the moment, there are no certified data on wild truffle yields – that are strongly influenced by the growing environment, natural climate, and other conditions – while cultivation with professional standards has started relatively recently and the output is still limited [15]. Based on the information we gathered, we can safely estimate that the annual output of *T. indicum* in China is about 1 000 t, while no plausible data can be currently provided for *T. pseudohimalayense*.

The Asian black truffle, *T. indicum*, is widely distributed in Himalayan India and China and it looks quite similar to *T. melanosporum*, although it is not endowed with the same aroma and taste. Starting from the 1990s, *T. indicum* has progressively invaded European markets as a cheap alternative to the Périgord black truffle, when not misbranded for it. Some data on the production of *T. indicum* in China show a steep decline in the last few years: exportation, for example, dropped from 200–300 t annually from



Fig. 7 Both *T. indicum* and *T. pseudohimalayense* are regularly offered on sale in large and small markets in Yunnan and Sichuan, and eagerly bought by locals. (A) *T. pseudohimalayense*, at the Mushuihua market in Kunming, Yunnan; (B–C) Whole and sliced *T. indicum*, at the Yongfu farmers market in Panzhihua, Sichuan.

1995 to 2005 to just 30 t in 2014 [29]. It is generally believed that this species is being over-harvested due to its commercial value, with the concurrent use of destructive harvesting methods and the collection of immature specimens, which in turn leads to habitat damage and production decline, to the point that the conservation status of *T. indicum* is currently under assessment as ‘near threatened’ (see The Global Fungal Red List Initiative, <http://iucn.ekoo.se/en/iucn/welcome>). *T. pseudohimalayense* is an excavated (i.e., produces a distinct cavity at the center of their ascoma) truffle that was originally described on the basis of specimens found in Spanish markets [30] and was later detected in Chinese truffle shipments to Italian markets [31]. Extensive morphological and molecular work has confirmed that *T. pseudohimalayense* is a good species, distinct from *T. indicum* and conspecific with *T. pseudoexcavatum* [32–35], with a wide distribution in Yunnan and Sichuan provinces of southwestern China [36].

Previous work on the volatiles of both *T. indicum* and *T. pseudohimalayense* exists, allowing direct comparison. Some of the volatiles identified in *T. indicum* in early works, like 3-methylbutanal and 2-methylbutanal, were shown to be common to several other *Tuber* species [11]. Nevertheless, the definition of the aroma profile of a given species that could be used as a fingerprint for recognition has been a much sought-after goal in this field of research. Given the morphological similarity between *T. indicum* and the more valuable *T. melanosporum*, considerable efforts have been devoted to the development of identification methods that could avoid commercial frauds. DNA barcoding has been a much-favored approach to this end [37,38], but the differences between the aromatic compounds of both species have also been investigated. As for *T. indicum*, eight main odorants were identified: 1-octen-3-one, 1-octen-3-ol, ethyl isobutyrate, ethyl 2-methylbutyrate, 3-methyl-1-butanol, isopropyl acetate, and the two sulfides, dimethyl sulfide and dimethyl disulfide [39]. This study indicated the important role played by the family of C_8 compounds in the aroma of *T. indicum* species, demonstrating the importance in the aromatic profile of this truffle of 1-octen-3-one and 1-octen-3-ol, in particular, both reported as having a characteristic mushroom aroma; intriguingly, these compounds are either absent or of scarce aromatic significance in *T. melanosporum* [39]. Recently, Feng and colleagues [40] analyzed the volatile constituents of three truffle species from Yunnan by flavoromics approach through SPME extraction combined with several GC techniques and aroma recombination. One of the species considered, *T. sinense* should be treated as a synonym of *T. indicum* [35]. The study detected a total of 44 substances and 38 aroma-active compounds

in this species, with dimethyl sulfide, dimethyl disulfide, octanal and 1-octen-3-one having relatively higher aroma intensities [40]. The only work on *T. pseudohimalayense* aroma components that we could spot in the literature is that performed by Li et al. [19], naming the species as *T. pseudoexcavatum*. In this study, ten volatiles were identified in the species’ aroma (1-octen-3-ol, phenylethyl alcohol, 3-octanol, 3-octanone, benzene acetaldehyde, (*E*)-2-octenal, nonanal, tricyclo[5.4.0.0(2,8)]undec-9-ene, 2,6,6,9-tetramethyl-, (1R,2S,7R,8R)-, cis-thujopsene, 1-butanamine, 3-methyl-*N*-(3-methylbutylidene-)), with 1-octen-3-ol and phenyl acetaldehyde (benzene acetaldehyde) as key aroma components [19].

The overall number of volatiles identified in the present study, many of which present at very low level, is very high compared to previous studies, for both *T. indicum* and *T. pseudohimalayense*. However, *per se* this is not unprecedented nor surprising. As back as the early 1990s, 125 volatile constituents were identified by headspace analysis of fresh *T. melanosporum* [28]. More recently, a Proton Transfer Reaction-Mass Spectrometry (PTR-MS) system was applied to the analysis of VOCs from *T. magnatum* fruiting bodies, leading to the identification of 111 compounds, some of which were only present in traces [41]. Our data and these examples clearly show how complex VOCs profiles of *Tuber* spp. are.

During our study, we did not detect any sulfur-containing VOCs. This might be interpreted as a somewhat surprising finding, given the fact that such compounds, like dimethyl sulfide, dimethyl disulfide and bis(methylthio)methane, are characteristic constituents of the aroma of many truffle species, and in some instances have been reported also for *T. indicum* (see above). However, other meta-analyses revealed that sulfur-containing compounds are not abundant, when present, in *T. indicum* [11], suggesting that their absence among our results might be due to methodological reasons. In particular, it has been reported before that the DVB/CAR/PDMS extraction fiber used in this study has a lower sensitivity and reproducibility for the extraction of volatile organic sulfur compounds [42]. Also, given the preeminent role played by the truffle-associated microbiome in the synthesis (either direct or mixed with the fungus) of aroma components [13], it might well be that microbiome composition and therefore its contribution to aroma formation in *T. indicum* (and possibly *T. pseudohimalayense*) could largely depend on the provenance and maturation stage of the fruit bodies.

In this study, for the first time, we provide clear evidence that the VOCs production of *T. indicum* and *T. pseudohimalayense*, two of the most commercially important Chinese truffles, is dependent

on both their maturation stage and provenance. Since the aroma of truffles, besides its biological function, is closely related to the organoleptic properties of the mushroom and hence its economic value, understanding the influence of these factors on the truffle quality under natural conditions (and looking forward, eventually in truffle orchards), is key to the development of a modern and environmentally sustainable truffle industry in China. Our results show that aroma details can distinguish Chinese truffles of different origin, even within the same region, probably because of a mix of genetic polymorphism, identity and metabolic activity of associated microorganisms, and different pedoclimatic conditions, although determining the contribution of each factor is not straightforward [43]. Not surprisingly, the relationship between chemical composition of truffle fruiting bodies and their geographic distributions is not limited to VOCs. Investigating the composition of *T. indicum* from five geographical regions of China, Li and colleagues [44] reported that truffles collected in Yunnan possessed the highest amount of free sugars, total flavonoids, total phenolics and the highest antioxidant activity. An elevated antioxidant activity, coupled to a high concentration of phenolic compounds, was also reported for *T. pseudohimalayense* [45].

Other studies have addressed the not trivial matter of using VOCs to distinguish truffles of different origin and maturation stage. Zeppa and colleagues [27] identified and characterized 49 VOCs released during the different stages of *T. borchii* fruit body maturation. Solid-phase microextraction coupled to gas chromatography/mass spectrometry (SPME-GC/MS) was the technique of choice by Gioacchini and colleagues [46] to characterize the volatile profile of *T. magnatum* produced in seven geographical areas of Italy, detecting “significant differences in the proportion of volatile constituents from truffles of different geographical areas”. More recently, Vita and colleagues [41] applied PTR-TOF-MS technology to VOCs analysis of *T. magnatum*, being able to distinguish aroma profiles between fruiting bodies originating from two Italian regions (Piedmont and Tuscany) and also summer and fall/winter growth period.

It will certainly take more time and considerable effort to disclose the secrets of the aromas produced by Chinese truffles. It would be particularly important to address the key issue of the truffle-associated microbiome in the formation of volatiles, and how this correlates with the maturation- and geographic-related changes in the aromatic properties of *T. indicum* and *T. pseudohimalayense* described in the present work. Our team is currently conducting research along these lines.

Declaration of Competing Interest

The authors declare no conflicts of interests.

Acknowledgement

This work was supported by the National Key Research and Development Program of China under grant No. 2018YFD0400200 and Guizhou Science and Technology Program under grant No. 4002 (2018). The authors are very grateful to Jianwei Liu, Ran Wang for providing specimens and figures for this study. The authors thank Dr. Mariana Herrera for helping making a geographical map. We are also grateful to Dr. Fei Li (Service Center for Experimental Biotechnology) for his assistance in GC-MS analysis.

References

- [1] D. Midgley, How white truffles became a multi-billion pound business - and where to find the best of the best. The Telegraph, 16 September (2019). <https://www.telegraph.co.uk/luxury/drinking-and-dining/truffles-became-multi-billion-pound-business-find-best-best/>
- [2] Technavio Research, Global Truffles Market 2019-2023. Infiniti Research Limited, Toronto, Canada (2019).
- [3] G. Bonito, M.E. Smith, General systematic position of the truffles: evolutionary theories. In: Zambonelli A, Iotti M, Murat C (eds.), *True Truffle (Tuber spp.) in the World*. Soil Biology, 47 (2016). Springer, Cham, pp. 3-18.
- [4] G. Bonito, M.E. Smith, M. Nowak, et al., Historical biogeography and diversification of truffles in the Tuberaceae and their newly identified southern hemisphere sister lineage, PLoS One 8 (2013) e52765. <https://doi.org/10.1371/journal.pone.0052765>.
- [5] A.C. Rinaldi, O. Comandini, T.W. Kuyper, Ectomycorrhizal fungal diversity: separating the wheat from the chaff, Fungal Divers. 33 (2008) 1-45.
- [6] O. Comandini, A.C. Rinaldi, T.W. Kuyper, Measuring and estimating ectomycorrhizal fungal diversity: a continuous challenge. In: Mycorrhiza: occurrence in natural and restored environments (Pagano M, ed). (2012) 165-200. New York: Nova Science Publishers.
- [7] F. Ori, A. Zambonelli, I.R. Hall, Mycophagy and spore dispersal by vertebrates. In: Dighton J, White JF, eds., *The Fungal Community: Its Organization and Role in the Ecosystem*. (2017) 4th edition, CRC Press, Boca Raton, Florida, pp. 347-358.
- [8] L. Schneider-Maunoury, A. Deveau, M. Moreno, et al., Two ectomycorrhizal truffles, *Tuber melanosporum* and *T. aestivum*, endophytically colonise roots of non-ectomycorrhizal plants in natural environments, New Phytol. 225 (2020) 2542-2255. <https://doi.org/10.1111/nph.16321>.
- [9] J. Delwiche, The impact of perceptual interactions on perceived flavor, Food Qual. Prefer. 15 (2004) 137-146. [https://doi.org/10.1016/S0950-3293\(03\)00041-7](https://doi.org/10.1016/S0950-3293(03)00041-7).
- [10] L. Culleré, V. Ferreira, B. Chevret, et al., Characterisation of aroma active compounds in black truffles (*Tuber melanosporum*) and summer truffles (*Tuber aestivum*) by gas chromatography-olfactometry, Food Chem. 122 (2010) 300-306. <https://doi.org/10.1016/j.foodchem.2010.02.024>.
- [11] R. Splivallo, S. Ottonello, A. Mello, et al., Truffle volatiles: from chemical ecology to aroma biosynthesis, New Phytol. 189 (2011) 688-699. <https://doi.org/10.1111/j.1469-8137.2010.03523.x>.
- [12] R. Splivallo, A. Deveau, N. Valdez, et al., Bacteria associated with truffle-fruiting bodies contribute to truffle aroma, Environ. Microbiol. 17 (2015) 2647-2460. <https://doi.org/10.1111/1462-2920.12521>.
- [13] M. Vahdatzadeh, A. Deveau, R. Splivallo, The role of the microbiome of truffles in aroma formation: a meta-analysis approach, Appl. Environ. Microb. 81 (2015) 6946-6952. <https://doi.org/10.1128/AEM.01098-15>.
- [14] W. Xu, S. Wan, L. Huang, et al., *Tuber sinoniveum*, a new white Chinese truffle species from Yunnan, China, Phytotaxa 298 (2017) 253-260. <https://doi.org/10.11646/phytotaxa.298.3.4>.
- [15] X. Wang, Truffle cultivation in China. In: Zambonelli A, Bonito G (eds) *Edible Ectomycorrhizal Mushrooms*, Soil Biology, 34 (2012). Springer, Berlin, Heidelberg, pp. 227-240.
- [16] R. Wang, A. Guerin-Laguette, R. Butler, et al., The European delicacy *Tuber melanosporum* forms mycorrhizae with some indigenous Chinese *Quercus* species and promotes growth of the oak seedlings, Mycorrhiza 29 (2019) 649-661. <https://doi.org/10.1007/s00572-019-00925-y>.
- [17] D. Liu, G. Zhou, X. Xu, A novel analytical method for key odor compounds of Chinese sausage, Meat Research 25 (2011) 15-20.
- [18] S. Gu, N. Tao, N. Wu, A new method based on ROAV value to identify the characteristic key volatile compounds of crab flavor, J. Food Sci. Technol. 33 (2012) 410-416.
- [19] X.L. Li, C. Chen, Y. Qing, Analysis of volatile aroma components in different species of truffle in Huidong county by GC-MS, Food Sci. 36 (2015) 132-136. <https://doi.org/10.7506/spkx1002-6630-201518024>.
- [20] P. Diaz, E. Ibañez, F. Senorans, et al., Truffle aroma characterization by headspace solid-phase microextraction, J. Chromatogr. A 1017 (2003) 207-214. <https://doi.org/10.1016/j.chroma.2003.08.016>.

- [21] R.E. March, D.S. Richards, R.W. Ryan, Volatile compounds from six species of truffle–head-space analysis and vapor analysis at high mass resolution, *Int. J. Mass Spectrom.* 249 (2006) 60–67. <https://doi.org/10.1016/j.ijms.2005.12.038>.
- [22] R. Splivallo, N. Valdez, N. Kirchhoff, et al., Intraspecific genotypic variability determines concentrations of key truffle volatiles, *New Phytol.* 194 (2012) 823–835. <https://doi.org/10.1111/j.1469-8137.2012.04077.x>.
- [23] E. Torregiani, S. Lorier, G. Sagratini, Comparative analysis of the volatile profile of 20 commercial samples of truffles, truffle sauces, and truffle-flavored oils by using HS-SPME-GC-MS, *Food Anal. Method.* 10 (2017) 1857–1869. <https://doi.org/10.1007/s12161-016-0749-2>.
- [24] S. Fang, B. Pu, A. Chen, et al., A Box-behnken design for characterizing Chinese truffles (*Tuber indicum*) aroma by HS-SPME-GC-MS, *J. Food Res.* 1 (2012) 219–229. <https://doi.org/10.5539/jfr.v1n3p219>.
- [25] R. Ramoni, F. Vincent, S. Grolli, et al., The insect attractant 1-octen-3-ol is the natural ligand of bovine odorant-binding protein, *J. Biol. Chem.* 276 (2001) 7150–7155. <https://doi.org/10.1074/jbc.M010368200>.
- [26] R. Zawirska-Wojtaslak, Optical purity of (R)-(-)-1-octen-3-ol in the aroma of various species of edible mushrooms, *Food Chem.* 86 (2004) 113–118.
- [27] S. Zeppa, A.M. Gioacchini, C. Guidi, et al., Determination of specific volatile organic compounds synthesised during *Tuber borchii* fruit body development by solid-phase microextraction and gas chromatography/mass spectrometry, *Rapid Commun. Mass Sp.* 18 (2004) 199–205. <https://doi.org/10.1002/rcm.1313>.
- [28] I. Flament, R. Näf, Surfing on the scent waves in the food flavor sea. In: Teranishi R, Wick EL, Hornstein I (eds) *Flavor Chemistry*. Springer, Boston, MA, (1999) pp. 189–198.
- [29] J. Chen, C. Murat, P. Oviatt, et al., The black truffles *Tuber melanosporum* and *Tuber indicum*. In: Zambonelli A, Iotti M, Murat C (eds.), *True Truffle (Tuber spp.) in the World*, *Soil Biology*, 47 (2016). Springer, Cham, pp. 19–32.
- [30] G. Moreno, J.L. Manjón, J. Diéz, et al., *Tuber pseudohimalayense* sp. nov. An Asiatic species commercialized in Spain, similar to the “Perigord” truffle, *Mycotaxon* 63 (1997) 217–224.
- [31] G.D. Massimo, M. Bencivenga, E. Tedeschini, et al., Nuova specie di *Tuber* importata dall’oriente, *Micologia Italiana* 27 (1998) 13–18.
- [32] L.F. Zhang, Z.L. Yang, D.S. Song, A phylogenetic study of commercial Chinese truffles and their allies: taxonomic implications, *FEMS Microbiol. Lett.* 245 (2005) 85–92. <https://doi.org/10.1016/j.femsle.2005.02.028>.
- [33] J.L. Manjón, L.G. García-Montero, P. Alvarado, et al., *Tuber pseudoexcavatum* versus *T. pseudohimalayense*-new data on the molecular taxonomy and mycorrhizae of Chinese truffles, *Mycotaxon* 110 (2009) 399–412.
- [34] J. Chen, P.G. Liu, Delimitation of *Tuber pseudohimalayense* and *T. pseudoexcavatum* based on morphological and molecular data, *Cryptogamie Mycol.* 32 (2011) 83–93.
- [35] J. Chen, S.X. Guo, P.G. Liu, Species recognition and cryptic species in the *Tuber indicum* complex, *PLoS ONE* 6 (2011) e14625. <https://doi.org/10.1016/10.1371/journal.pone.0014625>.
- [36] L. Fan, J.Z. Cao, Y. Li, A reassessment of excavated *Tuber* species from China based on morphology and ITS rDNA sequence data, *Mycotaxon* 124 (2013) 155–163.
- [37] F. Paolocci, A. Rubini, B. Granetti, et al., Typing *Tuber melanosporum* and Chinese black truffle species by molecular markers, *FEMS Microbiol. Lett.* 153 (1997) 255–260. [https://doi.org/10.1016/S0378-1097\(97\)00226-7](https://doi.org/10.1016/S0378-1097(97)00226-7).
- [38] J.P. Douet, M. Castroviejo, D. Mabru, et al., Rapid molecular typing of *Tuber melanosporum*, *T. brumale* and *T. indicum* from tree seedlings and canned truffles, *Anal. Bioanal. Chem.* 379 (2004) 668–673. <https://doi.org/10.1007/s00216-004-2643-9>.
- [39] L. Culleré, V. Ferreira, M.E. Venturini, et al., Potential aromatic compounds as markers to differentiate between *Tuber melanosporum* and *Tuber indicum* truffles, *Food Chem.* 141 (2013) 105–110. <https://doi.org/10.1016/j.foodchem.2013.03.027>.
- [40] T. Feng, M. Shui, S. Song, et al., Characterization of the key aroma compounds in three truffle varieties from China by flavoromics approach, *Molecules* 24 (2019) 3305. <https://doi.org/10.3390/molecules24183305>.
- [41] F. Vita, C. Taiti, A. Pompeiano, et al., Volatile organic compounds in truffle (*Tuber magnatum* Pico): comparison of samples from different regions of Italy and from different seasons, *Sci. Rep.-UK* 5 (2015) 12629. <https://doi.org/10.1038/srep12629>.
- [42] R.S. Liu, D.C. Li, H.M. Li, et al., Evaluation of aroma active compounds in *Tuber* fruiting bodies by gas chromatographyolfactometry in combination with aroma reconstitution and omission test, *Appl. Microbiol. Biot.* 94 (2012) 353–363. <https://doi.org/10.1007/s00253-011-3837-7>.
- [43] A. Rubini, B. Belfiori, C. Riccioni, et al., Genomics of *Tuber melanosporum*: new knowledge concerning reproductive biology, symbiosis and aroma production. In: Zambonelli A, Bonito G (eds) *Edible Ectomycorrhizal Mushrooms*, *Soil Biology*, 34 (2012). Springer, Berlin, Heidelberg, pp. 57–72.
- [44] J.M. Li, H.Q. Liang, P. Qiao, et al., Chemical composition and antioxidant activity of *Tuber indicum* from different geographical regions of China, *Chem. Biodivers.* 16 (2019) e1800609. <https://doi.org/10.1002/cbdv.201800609>.
- [45] X. Yan, Y. Wang, X. Sang, et al., Nutritional value, chemical composition and antioxidant activity of three *Tuber* species from China, *AMB Express* 7 (2017) 136–144. <https://doi.org/10.1186/s13568-017-0431-0>.
- [46] A.M. Gioacchini, M. Menotta, M. Guescini, et al., Geographical traceability of Italian white truffle (*Tuber magnatum* Pico) by the analysis of volatile organic compounds, *Rapid Commun. Mass Sp.* 22 (2008) 3147–3153. <https://doi.org/10.1002/rcm.3714>.