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Comparison of Lipid Content and Fatty Acid Composition between *Tuber* Fermentation Mycelia and Natural Fruiting Bodies

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ABSTRACT: A comparison of lipid content and fatty acid (FA) composition between *Tuber* fermentation mycelia and natural fruiting bodies indicates that the lipid content in *Tuber* fermentation mycelia is higher than that in fruiting bodies. Unsaturated FAs (particularly linoleic acid and oleic acid) were the predominant constituents in total FAs in both *Tuber* fermentation mycelia and fruiting bodies. A total of 23 FAs, including arachidonic, eicosapentaenoic, docosahexaenoic, and γ-linolenic acids, were first identified in the *Tuber* species. A hierarchical clustering analysis showed that the FA profile of fermentation mycelia was quite similar, regardless of *Tuber* species. However, the FA profile of the fruiting bodies was significantly influenced by its species and habitat environments. Interestingly, the FA profile of the *Tuber indicum* and *Tuber aestivum* fruiting bodies was nearly identical to that of the *Tuber* fermentation mycelia, which partially confirms the similarity between the *Tuber* fermentation mycelia and the fruiting bodies.

KEYWORDS: truffle, *Tuber*, fermentation mycelia, fruiting bodies, lipid, fatty acid

INTRODUCTION

Truffles, the hypogeous fungus belonging to the *Tuber* genus with its characteristic aroma and delicious taste, are precious and expensive delicacies that are widely used in the famous cuisines of France and Italy. Because of the decrease in the natural production of truffles combined with the increase in worldwide demand, a new way to produce truffles on a large scale is urgently needed. By taking the Chinese truffle *Tuber sinense* as a typical example, our group recently studied the significance of its inoculation density, carbon source, and initial concentration in detail. We subsequently developed a novel submerged fermentation process for the production of mycelia and its bioactive metabolites for the first time.[1] This process is considered to be a potential alternative resource for truffles, and it may also be helpful for other mushroom fermentations for bioactive metabolite production. Furthermore, the chemical compositions of fermentation mycelia, that is, volatile organic compounds (VOCs),[2] androstenol,[3] and nucleoside,[4] were investigated, and the *Tuber* fermentation mycelia and fruiting bodies were confirmed to be similar in their VOC profiles.[5]

Lipids, a broad group of naturally occurring lipid-soluble molecules, include fatty acids (FAs), steroids, sphingolipids, and phospholipids. Lipids are the key energy source for metabolism and are the structural and functional components for biomembranes. However, the excessive intake of saturated fatty acids (SFAs) can cause a series of health problems, such as obesity,[6] cholesterol increase, and cardiovascular diseases.[7] However, the intake of unsaturated fatty acids (UFAs), particularly the polyunsaturated fatty acids (PUFAs) belonging to the omega-6 and omega-3 families (i.e., arachidonic acid (AA) and α-linolenic acid (ALA)), has many positive effects on health, including antioxidative,[8] colon cancer inhibitive,[9] and cardioprotective effects.[7] Therefore, a diet low in calories and high in UFAs is advocated for health, and the determination of lipid content and FA composition is essential for both routine food analysis and lipid research.

The nutritional components of truffles were first reported in 1892,[10] and recent studies show that truffles contain only 5−9% (by dry weight) total lipids and a relatively high amount of UFAs in their *Tuber* fruiting bodies.[11−13] Previous studies have mostly focused on the major FAs, such as linoleic, oleic, and palmitic acids. However, the minor FAs have not been adequately investigated. To the best of our knowledge, the lipid content and FA profile in *Tuber* fermentation mycelia have never been reported.

Because the truffle fermentation mycelium is viewed to be a potential alternative resource for natural fruiting bodies, a comparative study of lipid content and FA profile between natural fruiting bodies and fermentation mycelia would be highly anticipated. This work, then, includes the following four parts: (1) a comparison of the total lipid content between *Tuber* fermentation mycelia and fruiting bodies; (2) a qualitative and quantitative analysis of the FAs in *Tuber* fermentation mycelia and a indication of the similarity among fermentation mycelia by comparing the FA profiles of four *Tuber* species cultured under the same fermentation condition; (3) an elucidation of the differences in *Tuber* fruiting bodies by comparing the data from the qualitative and quantitative analysis of FAs; and (4) an elucidation of the relationship between *Tuber* fruiting

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bodies and fermentation mycelia from the viewpoint of FA profiles through direct comparison and a statistical study (hierarchical clustering analysis, HCA). This work will provide a useful database for the nutritional or nutraceutical evaluation of both *Tuber* fermentation mycelia and fruiting bodies.

MATERIALS AND METHODS

Chemicals and Reagents. All of the solvents and chemicals used were of analytical grade. Chloroform, methanol, potassium hydroxide, concentrated sulfuric acid, anhydrous sodium sulfate, and isooctane were purchased from Sinopharm Chemical Reagent Co., Ltd. (Shanghai, China). The following authentic standards were purchased from Sigma-Aldrich China Inc. (Beijing, China): caprylic (99%), nonanoic (99%, IS), capric (99%), lauric (99%), tridecylic (99%), myristic (99%), myristoleic (99%), pentadecylic (99%), palmitic (99%), palmitoleic (99%), margaric (99%), *cis*-10-heptadecenoic (99%), stearic (99%), oleic (99%), linoleic (LA, 99%), α-linolenic (ALA, 99%), γ-linolenic (GLA, 99%), arachidic (99%), gadoleic (99%), dihomo-linolenic (99%), dihomo-γ-linolenic (DGLA, 99%), arachidonic (AA, 99%), *cis*-5,8,11,14,17-eicosapentaenoic (EPA, 99%), heneicosanoic (99%), behenic (99%), erucic (99%), *cis*-4,7,10,13,16,19-docosahexaenoic (DHA, 99%), tricosaylic (99%), lignoceric (99%), and nervonic acid methyl esters (99%). The derivatized reagents, 10% H_2SO_4 methanolic solution and 11 g L^{-1} KOH methanolic solution, were freshly prepared before use.

***Tuber* Fruiting Body Collection and Mycelia Culture.** The strains of *T. melanosporum*, *T. sinense*, and *T. indicum* were provided by Mianyang Institute of Edible Fungi (Sichuan, China), and the strain of *T. aestivum* was provided by the Huazhong University of Agriculture. The mycelia were cultured under the following medium: 35 g L^{-1} sucrose, 5 g L^{-1} peptone, 5 g L^{-1} yeast extract, 0.5 g L^{-1} $MgSO_4 \cdot 7H_2O$, 1 g L^{-1} KH_2PO_4, and 0.05 g L^{-1} vitamin B_1; details of the culture procedure have been reported.[1] The freeze-dried samples were pulverized and then subjected to pass through a 250 μm stainless sieve.

The *Tuber* fruiting bodies of *T. aestivum*, *T. indicum*, *T. himalayense*, and *T. borchii* var. *sphaerospermum* were provided by the Kunming Rare Truffle Co. Ltd. (Yunan province, China). After harvest, the truffle fruiting bodies were immediately stored in a refrigerator at −20 °C. After freeze-drying, the dried fruiting bodies were pulverized and then passed through a 250 μm stainless sieve.

Lipid Extraction. According to the methods described by León-Guzmán et al.[14] and Ribeiro et al.,[15] an accurately weighed *Tuber* powder sample (recorded as W_s, ca. 1 g) was extracted by chloroform/methanol (2:1, v/v) on a Soxhlet apparatus at 65 °C for 4 h. The extracts were then concentrated to dryness under vacuum at 35 °C until the final weight was kept constant (recorded as W_f). The lipid content was calculated as $W_f/W_s \times 100\%$. The residue was redissolved in 5 mL of methanol for derivatization in the following step.

FA Derivatization. To transform the esterified FAs to free FAs in the samples, the aforementioned lipid extracts were hydrolyzed with 1 mL of KOH methanolic solution (11 g L^{-1}) at 90 °C for 10 min. Then, the total FAs, including the original free FAs and those resulting from the alkaline hydrolysis, were derivatized to their methyl ester forms by 2 mL of H_2SO_4 methanolic solution (10%, v/v) at 90 °C for 20 min. After a 250 μL internal standard (1 g L^{-1} nonanoic acid methyl ester methanolic solution) was spiked, the fatty acid methyl ester (FAME) solution was extract by 2 × 6 mL isooctane with occasional shaking. The anhydrous sodium sulfate was added to remove all water from the collected isooctane layer. Finally, the isooctane extract was evaporated under a nitrogen stream to a final volume of 1 mL, and it was stored at −20 °C for analysis. All of the assays were performed in triplicate for each sample.

GC-MS/FID Analysis of FA Composition. A Shimadzu 2010 plus GC system equipped with a quadrupole mass spectrometer (MS) detector (Shimadzu Inc., Tokyo, Japan) was used. The separation was performed on an HP-5 ms capillary column (30 m × 0.25 mm i.d., 0.25 μm film thickness, Agilent Technologies Inc., Palo Alto, CA). The system was operated in the mode of constant flow (1.0 mL min^{-1}) using helium as a carrier gas, and a 1 μL sample was injected in the split mode with a split radio of 40:1. The GC oven temperature was started at 40 °C and then programmed to 120 °C at a rate of 20 °C min^{-1}. The temperature was held for 1 min, then increased to 180 °C at a rate of 8 °C min^{-1}, held for 8 min, then increased to 240 °C at a rate of 5 °C min^{-1}, held for 18 min, then increased to 280 °C at a rate of 5 °C min^{-1}, held for 8 min, and finally increased to 300 at 5 °C min^{-1} and held for 4 min. The injection port, transfer line, and ion source temperature were maintained at 300, 300, and 200 °C, respectively. The MS was run in the electron impact (EI) mode with an electron energy of 70 eV.

The identification of FAs in the *Tuber* samples was based on a comparison of their mass spectra and the Kovats retention index with those of the authentic FAME standard and the mass spectra database (NIST 05).

A quantitative analysis of the identified FAs was performed on a Shimadzu 2010 GC-FID system with an HP-5 capillary column (30 m × 0.32 mm i.d., 0.25 μm film thickness). The column flow rate was 1 mL min^{-1}, and a 1 μL sample was injected into the gas chromatograph. The GC-FID conditions are described as follows: the injector temperature and detector temperature were both 300 °C, and the oven temperature program was the same as for the GC-MS operation. All samples were analyzed in triplicate, and the standard deviation was calculated.

Calibration Curve. A methyl ester mixture of FAs (28 kinds of FAMEs except palmitic, stearic, oleic, and linoleic acid methyl esters) standard solution was prepared by dilution in cold CH_2Cl_2 with a concentration series of 200, 100, 50, 20, 10, 5, and 2 μg mL^{-1}. The mixture standard solution of four other kinds of FA methyl esters was prepared in a concentration series of 10^5, 5×10^4, 10^4, 5000, 1000, 500, and 100 μg mL^{-1}. The calibration curves were obtained by plotting the peak area ratios of analyte/IS to the spiked concentrations (250 μg mL^{-1}).

Statistical Analysis. The statistical data were processed, and a one-way ANOVA was performed using the SPSS 16.0 software (Chicago, IL). To evaluate the difference of fatty acid content in the *Tuber* samples, a post hoc analysis was performed using Tukey's test. Differences were considered to be significant when $p < 0.05$. The HCA was calculated on the bais of the data matrix in Table 2.

Precision and Repeatability. The injection precision was assessed by repetitive injections of the same sample solution six times in one day. The RSD of the relative peak area was <1.36%.

The repeatability of analysis method was determined by analyzing six independently prepared samples of the fermentation mycelia using the same method, and it was found that the RSD of the relative peak area was <4.84%.

The repeatability of the fermentation method was determined by analyzing six mycelial samples fermented under the same medium in parallel, and it was found that the RSD of the relative peak area was ≤6.00%.

RESULTS AND DISCUSSION

Total Lipid Content of *Tuber* Fermentation Mycelia and Fruiting Bodies. The total lipid contents of the *Tuber* fermentation mycelia and the natural fruiting bodies were measured to compare their nutritional qualities. Table 1 indicates that the total lipid content of the *Tuber* fermentation mycelia varied from 10.95 to 16.32%, whereas it varied from 5.62 to 9.47% in the fruiting bodies. The lipid contents of the fruiting bodies reported in this work were similar to those of *T. texense*,[11] *T. melanosporum*,[12,13] and the desert truffle *Terfezia claveryi Chatin*,[16] as well as some popular edible mushrooms (i.e., *Amanita rubescens*, *Lepista nuda*).[17] Obviously, the lipid content of the fermentation mycelia was higher than that of the fruiting bodies (Table 1). This result may be due to the harvest time of the fermentation mycelia being in the vegetative stage, which is

Table 1. Lipid Content of *Tuber* Samples

sample	lipid content (% dry wt)
fermentation mycelia	
T. melanosporum	16.32 ± 1.17[a]
T. sinense	13.21 ± 0.76
T. aestivum	10.95 ± 0.84
T. indicum	11.59 ± 0.84
natural fruiting bodies	
T. aestivum	5.93 ± 0.44
T. indicum	9.47 ± 0.89
T. himalayense	5.62 ± 0.37
T. borchii var. *sphaerospermum*	7.39 ± 0.74

[a] The standard deviation was calculated from three samples.

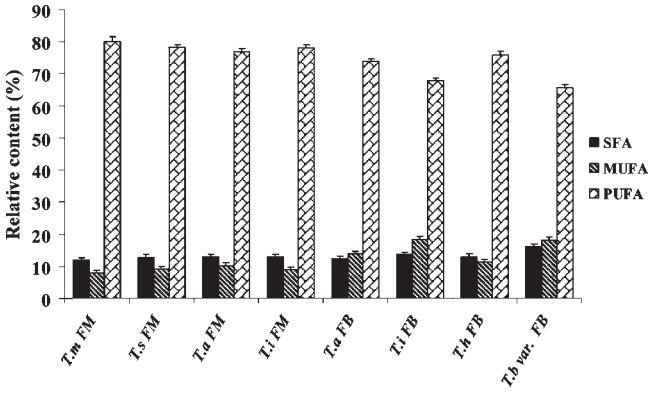

Figure 1. Relative content of SFA, MUFA, and PUFA in *Tuber* samples. *T. m* = *T. melanosporum*, *T. s* = *T. sinense*, *T. a* = *T. aestivum*, *T. i* = *T. indicum*, *T. h* = *T. himalayense*, *T. b* var. = *T. borchii* var. *sphaerospermum*, FM = fermentation mycelia, FB = natural fruiting bodies. The relative content was expressed as a percentage of dry matter, and the value shows the mean from experiments performed in triplicate (±SD < 5%).

speculated as the energy storage status for maturation.[18] Table 1 indicates that all of the lipid contents of the *Tuber* fermentation mycelia and the fruiting bodies did not exceed 20% (by dry weight). This percentage was much lower than the lowest value for the definition of a "high in fat" food according to the Coronary Prevention Group (CPG) and the Food Standards Agency (FSA), whose values are ca. 30 and 20%, respectively.[19]

FA Profiles of *Tuber* Fermentation Mycelia. The FA profiles of the *Tuber* fermentation mycelia were studied. The total amount of FAs in the fermentation mycelia ranged from ca. 71 to 84 mg g^{-1} (Table 2). The UFAs (i.e., the sum of MUFAs and PUFAs), which had levels approximately 8 times higher than that of SFAs, comprised >80% of the total FAs (Figure 1). These results are consistent with those regarding mushroom *H. agathosmus* and *Cantharellus cibarius*.[15] Interestingly, the relative content of PUFA in the *Tuber* fermentation mycelia was 8 times higher than that of MUFA (Figure 1). Except for *C. cibarius*,[15] such a high ratio of PUFA is rarely found in edible mushrooms.

There were a total of 26 FAs identified from the *Tuber* fermentation mycelia, the carbon chains of which ranged from 8 to 24 and double bond numbers ranged from 0 to 6 (Table 2). Briefly, linoleic acid (LA) (ca. 53.7−65.6 mg g^{-1}), palmitic acid (ca. 6.7−7.3 mg g^{-1}), oleic acid (ca. 5.3−6.5 mg g^{-1}), and stearic acid (ca. 1.3−1.7 mg g^{-1}) were the main FAs, which

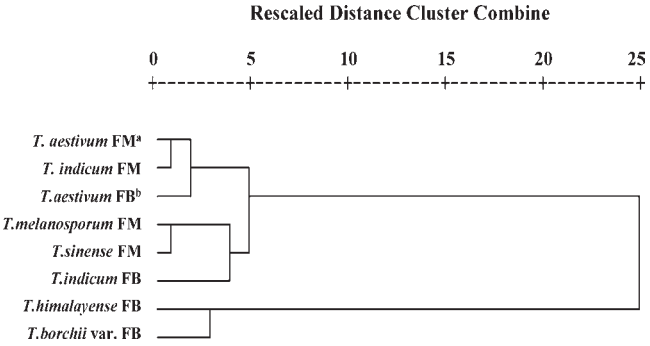

Figure 2. Diagram of hierarchical cluster analysis on the content of FAs from *Tuber* samples. Options set were as follows: method, between-groups linkage; measure of distance, squared Euclidean distance. FM = fermentation mycelia, FB = fruiting bodies.

occupied approximately 95% of the total FAs in the *Tuber* fermentation mycelia. LA, which comprised approximately 78% (average value of the investigated mycelia samples) of the total FAs, is considered to be the most significant contributor to the overall high percentage of PUFA in fermentation mycelia (Figure 1). Such a high relative percentage of LA is extremely rare in edible mushrooms.[15] From the viewpoint of bioactivity, LA, the essential fatty acid (EFA) belonging to the n-6 family, is a significant precursor for a wide variety of health benefits from synthesized metabolites,[7] such as GLA, AA, triacylglycerols (TGs), prostaglandins (PGs), leukotrienes (LTs), and lipoxins (LXs). Moreover, the appropriate intake of LA could reduce the risk of cardiovascular diseases, such as coronary heart disease,[20] hypertension,[21] hypercholesterolemia,[22] and atherosclerosis,[23] as well as incidences of diabetes[24] and cancer.[25] Oleic acid is another important FA found in the fermentation mycelia, the content of which was 8−12 times lower than that of LA (Table 2). This result is not consistent with most mushrooms, in which oleic acid is always of the highest content.[15,26] Although oleic acid is not the EFA, its bioactivity is known to reduce LDL-cholesterol levels,[27] thus decreasing the risk of cardiovascular diseases. In addition to linoleic and oleic acid, other bioactive FAs belonging to the n-3, n-6, and n-9 families were identified in the *Tuber* fermentation mycelia, such as ALA, GLA, gadoleic acid, DHA, and nervonic acid (Table 2). Although these FA concentrations were not high, their nutritional value cannot be neglected. The most worthy FA of DHA is essential for optimizing neuronal and retinal functions in the nervous system, and DHA is the vital molecule influencing the signaling events in neuronal survival and differentiation.[28] The amount of DHA in the *Tuber* fermentation mycelia varied from 48.9 to 108.9 $\mu g\ g^{-1}$ (Table 2), which accounted for approximately 1% of the total FAs. For the first time, DHA was identified in the *Tuber* genus. As far as we know, DHA is always abundant in marine fish oil but not in higher fungi.[15,26] From the viewpoint of abundant UFA, the *Tuber* fermentation mycelium is a potential functional food resource.

The FA profiles for the fermentation mycelia of *T. melanosporum*, *T. sinense*, *T. aestivum*, and *T. indicum* are compared in Table 2. Both the type and amount of FAs among the aforementioned four *Tuber* species were quite similar. However, myristoleic acid existed only in the *T. aestivum* fermentation mycelia, in which only GLA was absent. HCA indicated that there were two groups for the FA profiles of the *Tuber* fermentation mycelia: *T. aestivum* and *T. indicum* fermentation mycelia and *T. melanosporum* and *T. sinense* fermentation mycelia (Figure 2). Although a distinction

Table 2. Fatty Acid Composition of *Tuber* Fermentation Mycelia and Natural Fruiting Bodies[a]

systematic name (shorthand notation)	trivial name	fermentation mycelia				fruiting bodies			
		T. melanosporum	*T. sinense*	*T. aestivum*	*T. indicum*	*T. aestivum*	*T. indicum*	*T. himalayense*	*T. borchii*[b]
octanoic (C8:0)	caprylic[c]	tr[d]	1.6 ± 0.5 A	1.9 ± 0.3 A	1.5 ± 0.4 A	tr	6.2 ± 0.6 B	tr	tr
decanoic (C10:0)	capric[c]	1.7 ± 0.5 A	tr	4.9 ± 0.4 B	4.9 ± 0.7 B	2.3 ± 0.4 AC	3.0 ± 0.2 C	0.3 ± 0.0 D	4.3 ± 0.5 B
dodecanoic (C12:0)	lauric[c]	5.9 ± 0.7 A	10.4 ± 0.8 B	7.2 ± 0.5 A	6.8 ± 0.8 A	248.6 ± 12.0 C	12.0 ± 0.7 B	91.0 ± 7.0 D	10.4 ± 0.7 B
tridecanoic (C13:0)	tridecylic[c]	3.8 ± 0.4 A	4.0 ± 0.3 A	3.4 ± 0.7 A	3.5 ± 0.3 A	tr	3.6 ± 0.1 A	3.8 ± 0.2 A	3.8 ± 0.3 A
tetradecanoic (C14:0)	myristic	111.2 ± 6.3 A	131.0 ± 7.9 B	107.0 ± 3.7 AC	159.1 ± 6.2 D	67.9 ± 5.1 E	93.2 ± 2.7 C	75.7 ± 5.5 E	72.0 ± 4.0 E
cis-9-tetradecenoic (C14:1)	myristoleic[c]	nd[e]	nd	4.3 ± 0.1 A	nd	nd	nd	nd	nd
pentadecanoic (C15:0)	pentadecylic[c]	237.8 ± 5.0 A	259.0 ± 7.2 B	271.2 ± 3.4 B	308.4 ± 4.0 C	35.4 ± 2.6 D	40.3 ± 6.9 D	44.9 ± 2.6 D	37.6 ± 2.1 D
hexadecanoic (C16:0)	palmitic	7039.5 ± 85.1 A	7332.2 ± 70.4 A	6662.7 ± 40.0 A	6684.3 ± 73.6 A	4374.3 ± 363.6 B	5298.0 ± 787.3 B	3118.4 ± 199.0 C	2779.5 ± 235.5 C
cis-9-hexadecenoic (C16:1)	palmitoleic[c]	611.0 ± 40.2 A	715.7 ± 79.7 AB	682.3 ± 68.7 AB	738.6 ± 37.2 B	201.6 ± 18.5 C	108.8 ± 21.7 C	186.9 ± 30.7 C	167.9 ± 18.7 C
heptadecanoic (C17:0)	margaric[c]	246.2 ± 56.2 A	261.3 ± 58.4 A	212.6 ± 33.6 A	241.4 ± 32.7 A	53.8 ± 4.2 B	63.4 ± 4.7 B	55.4 ± 7.4 B	56.1 ± 1.6 B
cis-10-heptadecenoic[c] (C17:1)	*f*	285.1 ± 39.5 A	282.6 ± 19.4 A	198.7 ± 61.7 B	249.1 ± 25.8 AB	37.1 ± 8.2 C	43.3 ± 5.6 C	30.9 ± 5.0 C	29.7 ± 4.3 C
octadecanoic (C18:0)	stearic	1425.5 ± 74.5 A	1720.4 ± 41.4 A	1340.1 ± 37.3 A	1301.6 ± 60.4 A	2466.6 ± 99.4 B	7759.8 ± 484.6 C	1366.8 ± 94.9 A	1278.5 ± 81.2 A
cis-9-octadecenoic (C18:1n9)	oleic	5263.6 ± 188.2 ADE	6528.2 ± 170.2 A	5940.7 ± 191.4 AE	5296.9 ± 161.4 ADE	8289.6 ± 694.7 B	17777.4 ± 1233.0 C	4109.6 ± 337.0 D	4718.2 ± 91.7DE
cis-9,12- octadecadienoic (C18:2n6)	linoleic	63944.7 ± 2466.2 A	65573.8 ± 2067.1 A	53654.1 ± 1878.8 B	55317.5 ± 1791.7 B	46177.0 ± 1115.8 C	67166.1 ± 2649.9 A	29532.2 ± 2617.7 D	17679.7 ± 1378.6 E
cis-9,12,15- octadecatrienoic (C18:3n3)	α-linolenic	5.2 ± 0.8 A	15.5 ± 0.8 B	1.3 ± 0.1 A	10.7 ± 1.0 C	9.7 ± 0.8 C	nd	197.4 ± 17.8 D	296.6 ± 16.3 E
cis-6,9,12- octadecatrienoic (C18:3n6)	γ-linolenic[c]	3.0 ± 0.4 A	tr	nd	1.8 ± 0.4 A	41.6 ± 3.2 B	25.9 ± 3.0 C	3.1 ± 0.1 A	14.8 ± 2.2 D
eicosanoic (C20:0)	arachidic[c]	91.4 ± 11.0 A	129.9 ± 9.3 B	83.5 ± 3.0 A	68.0 ± 7.9 A	126.0 ± 21.0 B	165.8 ± 9.4 C	72.5 ± 6.4 A	65.7 ± 9.3 A
cis-11-eicosenoic (C20:1n9)	gadoleic[c]	92.1 ± 7.8 AB	125.4 ± 6.2 AB	tr	77.1 ± 9.2 A	146.8 ± 9.4 B	434.1 ± 51.5 C	101.6 ± 8.7 AB	98.9 ± 15.1 AB
cis-11,14- eicosadienoic (C20:2)	dihomo- linolenic[c]	100.1 ± 6.1 A	149.8 ± 12.0 B	53.5 ± 7.2 C	56.2 ± 6.3 C	203.4 ± 5.8 D	649.4 ± 28.6 E	131.1 ± 8.1 AB	107.3 ± 6.6 A
cis-8,11,14- eicosatrienoic (C20:3n6)	dihomo-γ- linolenic[c]	nd	nd	nd	nd	9.8 ± 0.7 A	nd	nd	nd
cis-5,8,11,14- eicosatetraenoic (C20:4n6)	arachidonic[c]	nd	nd	nd	nd	73.8 ± 3.3 A	40.4 ± 2.2 B	nd	13.3 ± 0.9 C
cis-5,8,11,14,17- eicosapentaenoic (C20:5n3)	EPA[c]	nd	nd	nd	nd	27.8 ± 1.7 A	tr	nd	nd
heneicosanoic (C21:0)	heneicosylic[c]	20.3 ± 3.5 AC	34.6 ± 5.5 B	27.4 ± 2.7 BC	15.9 ± 1.3 A	17.9 ± 3.9 AC	30.3 ± 4.3 B	14.4 ± 1.3 A	18.1 ± 3.6 AC
docosanoic (C22:0)	behenic[c]	135.7 ± 8.1 AD	200.3 ± 3.0 B	159.3 ± 4.9 AC	145.4 ± 5.9 A	191.0 ± 22.4 BC	142.2 ± 27.1 A	101.7 ± 8.7 D	100.3 ± 6.1 D
cis-13-docosenoic (C22:1n9)	erucic[c]	23.7 ± 2.0 AB	29.3 ± 0.9 AC	18.6 ± 2.5 B	tr	34.2 ± 2.1 C	50.5 ± 3.1 D	34.8 ± 6.6 C	9.3 ± 0.3 E
cis-4,7,10,13,16,19- docosahexaenoic (C22:6n3)	DHA[c]	62.1 ± 7.2 AC	67.0 ± 8.1 A	108.9 ± 6.3 B	48.9 ± 6.4 C	8.5 ± 0.6 D	nd	tr	nd
tricosanoic (C23:0)	tricosylic[c]	61.9 ± 4.6 A	118.6 ± 3.0 B	83.6 ± 5.5 C	62.9 ± 9.2 A	43.8 ± 5.0 D	31.6 ± 3.9 DE	29.0 ± 2.2 EF	16.2 ± 1.2 F
tetracosanoic (C24:0)	lignoceric[c]	263.7 ± 9.1 A	386.4 ± 6.5 B	49.4 ± 1.4 C	213.9 ± 9.5 D	132.6 ± 4.6 E	68.0 ± 8.0 F	68.3 ± 5.0 F	49.2 ± 3.2 C
cis-15-tetracosenoic (C24:1n9)	nervonic[c]	28.2 ± 3.7 A	67.0 ± 4.2 B	306.1 ± 16.6 C	37.4 ± 2.8 A	27.3 ± 3.4 A	21.8 ± 2.3 AD	5.3 ± 0.4 D	8.4 ± 0.9 D
SFA		9644.6 ± 272.0 AB	10589.7 ± 202.2 A	9014.2 ± 263.4 B	9217.6 ± 271.9 B	7760.2 ± 631.0 C	13717.4 ± 456.3 D	5042.2 ± 451.0 E	4491.7 ± 211.6 E
MUFA		6303.7 ± 192.4 A	7748.2 ± 233.6 AB	7150.7 ± 172.2 A	6399.1 ± 131.4 A	8736.6 ± 704.0 B	18435.9 ± 2514.0 C	4469.1 ± 290.7 D	5032.4 ± 183.7 D
PUFA		64115.1 ± 2365.7 A	65806.1 ± 2076.0 A	53817.8 ± 2276.6 B	55435.1 ± 1893.8 B	46551.6 ± 2361.2 C	67881.8 ± 3216.6 A	29863.8 ± 2462.0 D	18111.7 ± 1388.6 E
total		80063.4 ± 3013.1 A	84144.0 ± 2892.8 A	69982.7 ± 2554.7 B	71051.8 ± 2563.1 B	63048.4 ± 3405.0 B	100035.1 ± 5236.0 C	39375.1 ± 2147.0 D	27635.8 ± 1234.0 E

[a] Means of three analyses ± standard deviation; each value is expressed as $\mu g\ g^{-1}$ of fatty acids in dry matter. Values in the same row bearing different capital letters were significantly different ($p < 0.05$). SFA, saturated fatty acid; MUFA, monounsaturated fatty acid; PUFA, polyunsaturated fatty acid. [b] var. *sphaerospermum*. [c] This FA was reported in *Tuber* species for the first time. [d] tr, content was found at a trace level. [e] nd, not detected. [f] This FA does not have trivial name at present.

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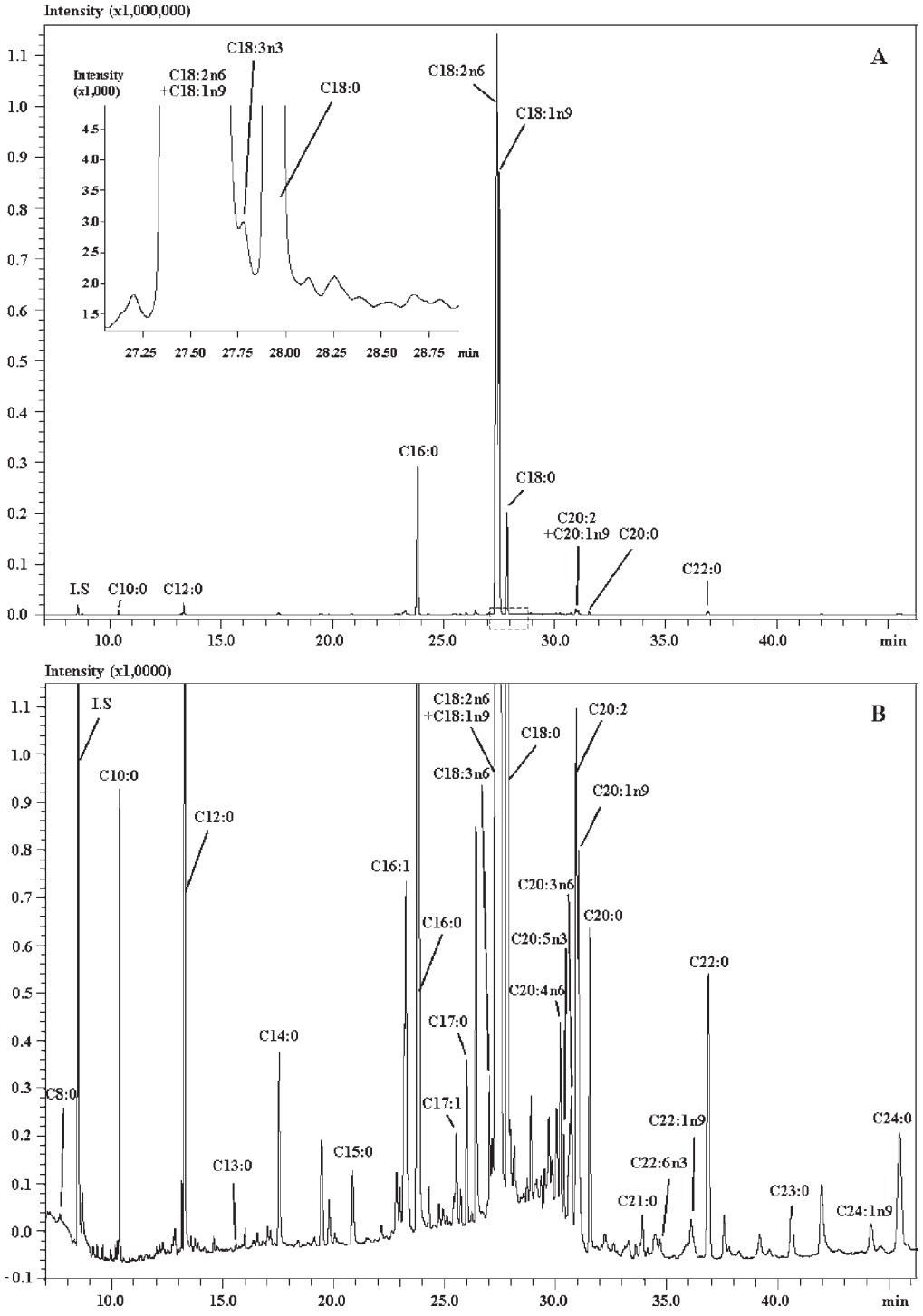

Figure 3. Representative GC-FID chromatogram of the FA profile of *T. aestivum* fruiting bodies (IS = C9:0): (A) chromatogram of overall FA profiles (local enlarged area surrounded with dashed line); (B) global enlarged chromatogram of (A).

existed between these two groups, the maximal distance was not more than 5 in the HCA diagram, which indicates that the FA profile of the *Tuber* mycelia cultured under the same conditions was quite similar regardless of *Tuber* species.

FA Profiles of *Tuber* Fruiting Bodies. Figure 3 shows a typical GC-FID chromatogram for the FA profile of the *T. aestivum* fruiting bodies. The total FA content in the *Tuber* fruiting bodies of *T. brochii* var. *sphaerospermum*, *T. himalayense*, *T. aestivum*, and *T. indicum* varied from ca. 28 to 100 mg g^{-1} (Table 2). The percentages of SFAs, MUFAs, and PUFAs were ca. 12−16, 11−18, and 66−73%, respectively. The data were approximately at the same level as the aforementioned *Tuber* fermentation mycelia (Figure 1) and the previously reported results for *T. texense*[11] and *T. melanosporum*.[12,13]

There were a total of 28 FAs identified in the fruiting bodies of the aforementioned four *Tuber* species, and 25 existed in both the

fermentation mycelia and fruiting bodies (Table 2). Twenty-three FAs were first identified in the *Tuber* species.[11−13] Similar to the FAs identified in the fermentation mycelia and in the literature,[11−13] linoleic, palmitic, oleic, and stearic acids were also the main FAs in the *Tuber* fruiting bodies, whereas some minor FAs were still specific to the fermentation mycelia or fruiting bodies. For example, in the species *T. aestivum*, myristoleic acid and GLA were unique to the *T. aestivum* mycelia and fruiting bodies, respectively (Table 2). In addition to GLA, the characteristic FAs of DGLA, AA, and EPA were also specific to the *T. aestivum* fruiting bodies. In *T. indicum*, the characteristic FAs of AA and EPA were specific to the fruiting bodies, whereas ALA and DHA were identified only in the fermentation mycelia. One of the characteristic FAs in *Tuber* fruiting bodies is AA. AA is an important n-6 FA for infant brain development and is a direct precursor for eicosanoids, which function to regulate lipoprotein metabolism, blood rheology, leukocyte function, and platelet activation.[29] As another characteristic bioactive FA in fruiting bodies, EPA is useful for preventing diseases such as atherosclerosis, hyperlipemia, and certain cancers.[29]

In addition to these unique FAs of the specific *Tuber* samples, there was a significant difference in the amounts they had of some common FAs in the fruiting bodies and fermentation mycelia. Generally speaking, pentadecylic acid, palmitic acid, margaric acid, *cis*-10-heptadecenoic acid, DHA, and tricosylic acid showed remarkably lower contents in the *Tuber* fruiting bodies than in the fermentation mycelia, particularly for DHA. The content of DHA was 48.9−108.9 μg g^{-1} in the fermentation mycelia; however, DHA was extremely low and even undetectable in the investigated fruiting bodies (Table 2). In contrast, dihomo-γ-linolenic acid, arachidonic acid, and EPA were present only in the fruiting bodies. By comparing the FA profiles between *Tuber* fermentation mycelia and fruiting bodies, it seemed that the fermentation mycelia contained more FAs from C14 to C17, whereas the fruiting bodies contained more FAs from C18 to C22 (Table 2).

The effect of *Tuber* species on the FA profiles of the fruiting bodies (i.e., *T. aestivum*, *T. indicum*, *T. himalayense*, and *T. borchii* var. *sphaerospermum*) was investigated. Regardless of the species distinction, the relative contents of SFAs, MUFAs, and PUFAs in all *Tuber* species were almost the same (Figure 1). However, among the four investigated fruiting bodies, *T. indicum* contained the highest absolute contents of FA, SFA, MUFA, and PUFA, which were all triple those of *T. brochii* var. *sphaerospermum* (Table 2). The contents of some individual FAs in the fruiting bodies of different species also varied remarkably. For example, both the main FAs (i.e., stearic, oleic, and linoleic acids) and minor FAs (i.e., caprylic, myristic, arachidic, gadoleic, dihomo-linolenic, heneicosylic, and erucic acids) were more abundant in *T. indicum* than in the other species. In addition, the ALA content was 197.4 and 296.6 μg g^{-1} in *T. himalayense* and *T. brochii* var. *sphaerospermum*, respectively. However, the ALA content was only 9.7 μg g^{-1} in *T. aestivum*, and it was even undetectable in *T. indicum*. The concentrations of DGLA, EPA, and DHA in *T. aestivum* were 9.8, 27.8, and 8.5 μg g^{-1}, respectively, whereas DGLA was not present in other fruiting bodies. Moreover, EPA in *T. indicum* and DHA in *T. himalayense* were both at trace levels and AA was found in all *Tuber* fruiting bodies except *T. himalayense* (Table 2).

An analysis of HCA regarding the species and FA variables was adopted to elucidate the relationship among the investigated fruiting bodies. As shown in Figure 2, the four investigated species were separated into three groups: *T. brochii* var. *sphaerospermum* and *T. himalayense* fruiting bodies and the fruiting bodies of *T. indicum* and *T. aestivum* in two separate groups. The group containing the *T. brochii* var. *sphaerospermum* and *T. himalayense* fruiting bodies was remarkably different from the other two in the HCA diagram. This result indicates that the FA profiles of the *T. brochii* var. *sphaerospermum* and *T. himalayense* fruiting bodies were similar, whereas they were significantly different from those of the *T. indicum* and *T. aestivum* fruiting bodies. This remarkable difference was mainly due to species distinction and other complex factors (e.g., habitat and harvest time). Interestingly, on the basis of the HCA diagram (Figure 2) for all of the *Tuber* samples, including the fermentation mycelia and fruiting bodies, the *T. aestivum* fruiting body was clustered with the *T. aestivum* and *T. indicum* fermentation mycelia as one group, whereas the *T. indicum* fruiting bodies were clustered with the *T. melanosporum* and *T. sinense* fermentation mycelia as another group (Figure 2). Although there was a distinction between these two separate groups, the short distance (no more than 5) in the HCA diagram indicates that the fruiting bodies of *T. indicum* and *T. aestivum* are somewhat similar to the investigated *Tuber* fermentation mycelia in the FA profiles. This result also partially confirms the similarity between *Tuber* mycelia and fruiting bodies in some species from the viewpoint of FA profiles.

In conclusion, the lipid content of *Tuber* fermentation mycelia was relatively higher than that of the fruiting bodies. Within the range of investigation, the FA profiles of *Tuber* fermentation mycelia cultured under the same condition were quite similar, whereas the FA profiles of *Tuber* fruiting bodies were quite different. Interestingly, the FA profiles of the *T. indicum* and *T. aestivum* fruiting bodies were nearly identical to that of the *Tuber* fermentation mycelia, which partially confirms the similarity between the *Tuber* fermentation mycelia and the fruiting bodies.

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ABBREVIATIONS USED

FA, fatty acid; VOCs, volatile organic compounds; FAME, fatty acid methyl ester; UFA, unsaturated fatty acid; SFA, saturated fatty acid; MUFA, monounsaturated fatty acid; PUFA, polyunsaturated fatty acid; EFA, essential fatty acid; LA, linoleic acid; ALA, α-linolenic acid; GLA, γ-linolenic acid; DGLA, dihomo-γ-linolenic acid; AA, arachidonic acid; EPA, *cis*-5,8,11,14,17-eicosapentaenoic acid; DHA, *cis*-4,7,10,13,16,19-docosahexaenoic acid; TGs, triacylglycerols; PGs, prostaglandins; LTs, leukotrienes; LXs, lipoxins; HCA, hierarchical cluster analysis.

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