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The Volatiles from Fermentation Product of *Tuber formosanum*

Menghao Du*, Sume Huang, Jingwen Wang

Research Institute of Subtropical Forestry, CAF, Fuyang, China
Email: *xiaoduchongcn@aliyun.com

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Abstract

The mycelium of *T. formosanum* (characterized by DNA analysis) grown in a sterile liquid medium produced some VOCs. The VOCs were analyzed by gas chromatography-mass spectrometry (GC-MS). A total of 23 compounds were identified and quantified. Among them, the main compounds were Dimethyl sulfide (19.82%), Isopropyl alcohol (9.84 ng/l), 2-Butanone (9.24%), Ethanol (7.84%), and 1, 3-Pentadiene (5.46%).

Keywords

***Tuber formosanum*, Truffle, Volatile Organic Compounds from the Mycelium, Dimethyl Sulfide**

1. Introduction

The genus Tuber includes a number of edible, economically valuable species of ectomycorrhizal fungi commonly known as truffles (Trappe, 1979). *T. formosanum* is an edible species of the genus Tuber. It is morphologically similar to *T. indica*, the one of finest truffle species.

Many volatile organic compounds (VOCs) from *T. formosanum* have been isolated: some of them derive from various degradation patterns while others are of bacterial origin (Talou et al., 1987; Fiecchi, 1988).

Fermented foodstuffs represent an important portion of the agro-industry production. They result from the action of a set of microorganisms that give these products their diversity, uniqueness, and quality. In this context, sulfur compounds play a key role. They are not only present in fermented foodstuffs but also in numerous fresh products, among others, fruits and vegetables (Berger 1995; Piloni et al., 2005; Rapior et al., 1997), which make such compounds of broader interest. Owing to their low detection thresholds and their strong reactivity, volatile sulfur compounds (VSC) significantly participate to the quality and the uniqueness of many foodstuffs.

Our study aims at the isolation and characterization of VOCs produced by the mycelium of *T. formosanum*

*Corresponding author.

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grown in sterile liquid medium.

2. Materials and Methods

2.1. Mycelium Growth

The mycelium of *T. formosanum* was cultured in YMT liquid medium modified as follows: Glucose 20 g/L, Yeast 2.0 g/L, malt extract 5 g/L, KH_2PO_4 1.0 g/L, $MgSO_4$ $7H_2O$ 0.5 g/L, pH = 6.5. The medium was autoclaved (121˚C; 1.2 bar; 20 min) and after cooling to room temperature, Sequestrene F 330 0.2 g/l and thiamine HCl 0.1 mg/L were added from filter sterilized stock solutions. Erlenmeyer flasks, each containing 150 ml of liquid medium, were inoculated with 0.2 g of fresh mycelium, incubated in table concentrator (23˚C ± 1˚C, 100 rpm/min) without agitation and subcultured at monthly intervals.

2.2. Molecular Characterization of the Mycelium

DNA was isolated from the mycelium and PCR amplified as described by Paolocci et al. (1999) using the universal ribosomal primers ITS1/ITS4 (White et al., 1990). The amplification product was purified through a G50 Sephadex column (Pharmacia Biotech) according to the suppliers' instructions and directly sequenced by using the Big Dye Terminator Kit (PE Biosystem). For sequencing, the following primers were used: ITS1, ITS4, 5.8 SB and 5.8 SF (Paolocci et al., 1997). The reaction products were run on an ABIPRISM 310 Genetic Analyser PE Biosystem automated sequencer. The Blasta similarity search program confirmed that the ITS sequence of the *in vitro* cultivated mycelium did belong to *T. formosanum*.

2.3. Extraction of Volatile Compounds

Extracting of volatile compounds was made by using a SPME at 60˚C for 1 h. After sample collection, the stopcocks were turned off; the traps were removed and transferred to the GC-MS system for analysis.

2.4. Gas Chromatography

GC-MS analysis were performed on a HP6890 gas chromatograph equipped with a HP5973 mass selective detector using a 30 m × 0.25 mm id., 0.25 μm film thickness HP-5 capillary column (5% Phenyl Methyl Siloxane, Agilent 19091 J-413) with helium as carrier gas. Column temperature program was 50˚C for 5 min and then programmed to 85˚C for 2 min at 5˚C/min, then increased to 280˚C for 10 min at 3˚C/min. Volatile compounds were identified by comparing their mass spectra with the mass spectra from MS database (NIST 05, WILEY 7). The MSD unit was operated in the scan mode by collecting all ions ranging from m/z 20 to 700. When available, MS identifications were confirmed by comparing GC retention times of the analysts with those from pure standards. The identification was confirmed by using retention indices (RI) of the value compared with those reported in the literature (Jordán et al., 2002). Linear retention indices of the compounds were calculated using a series of n-alkanes (C7-C30, Sigma-Aldrich, Germany) injected in the same conditions. When standard chemicals were not available, tentative identification was carried out by matching the mass spectra. The results are given in **Table 1**.

3. Results and Discussion

Sterile mycelia of *T. formosanum* were inoculated in fresh liquid medium and 15 days later the volatiles were collected by SPME. And its chemical composition was analyzed for chemical composition by using GC-FID and GC-MS showed in **Figure 1**. In total, 23 compounds were detected in the volatiles from the mycelium cultures, but not in the volatiles from the control cultures, categories and quantities of volatile components were identified and their percentage was listed in order of their elution on the HP-5 column (**Table 1**). The volatiles were mainly constituted of ester (10%, 34.90%), sulfocompound (1%, 19.92%), alcohols (6%, 25.57%), ketone (3%, 12.89%), alkenes (1%, 5.46%) and aldehyde (1%, 2.63%) (**Figure 2**). Among them, most predominant compounds were Dimethyl sulfide (19.82%), Isopropyl alcohol (9.84 ng/l), 2-Butanone (9.24%), Ethanol (7.84%), and 1, 3-Pentadiene (5.46%) (**Table 1**). The predominant components were also found to be the major components in the other truffle species, such as *T. melanosporum*, *T. miesentericum*, *T. rufum*, and *T. simonea* (Raymond et al., 2006).

Table 1. Volatile organic compounds emission from the sterile mycelium of *Tuber formosanum*.

	Components	Cas#	Peak area (%)	Identification method
1	1, 3-Pentadiene	504-60-9	5.46	GC-MSa
2	Acetaldehyde	75-07-0	2.63	GC-MS
3	Dimethyl sulfide	75-18-3	19.92	GC-MS, RTb
4	Acetone	67-64-1	1.3	GC-MS, RT
5	Acetic acid, methyl ester	79-20-9	2.75	GC-MS
6	Acetic acid, ethyl ester	141-78-6	3.76	GC-MS, RT
7	2-Butanone	78-93-3	9.24	GC-MS, RT
8	Isopropyl alcohol	67-63-0	9.84	GC-MS
9	Ethanol	64-17-5	7.84	GC-MS, RT
10	Propanoic acid, ethyl ester	105-37-3	2.48	GC-MS
11	4-Hydroxy-3-methyl-2-butanone	9006-26-2	2.35	GC-MS
12	Butanoic acid, methyl ester	623-42-7	16.6	GC-MS
13	Butanoic acid, 2-methyl-, methyl ester	868-57-5	1.23	GC-MS
14	2-Butanol	78-92-2	4.52	GC-MS, RT
15	Butanoic acid, ethyl ester	105-54-4	1.38	GC-MS
16	Propanoic acid, propyl ester	106-36-5	0.22	GC-MS
17	Butanoic acid, 2-methyl-, ethyl ester	7452-79-1	0.27	GC-MS
18	1-Propanol, 2-methyl-	78-83-1	0.62	GC-MS
19	Butanoic acid, propyl ester	105-66-8	3.46	GC-MS
20	Butanoic acid, 1-methylpropyl ester	819-97-6	2.75	GC-MS
21	1-Butanol	71-36-3	0.35	GC-MS, RT
22	2-Pentene, 3-ethyl-2-methyl-	19780-67-7	0.21	GC-MS
23	1-Butanol, 2-methyl-	137-32-6	0.27	GC-MS

[a]Indentified by good match of mass spectrometer; [b]Identified by retention time of standard compounds.

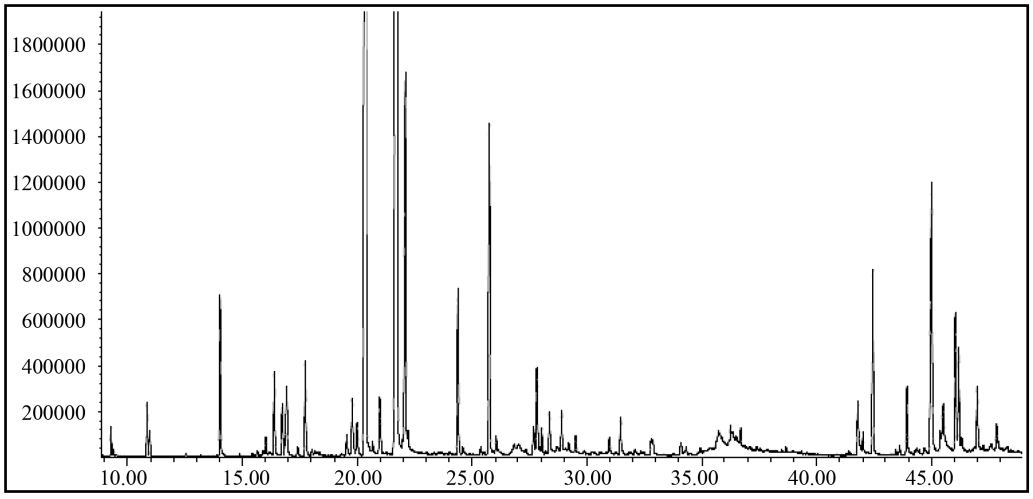

Figure 1. GC profile of the volatile components extracted from mycelia of *T. formosanum* by SPME.

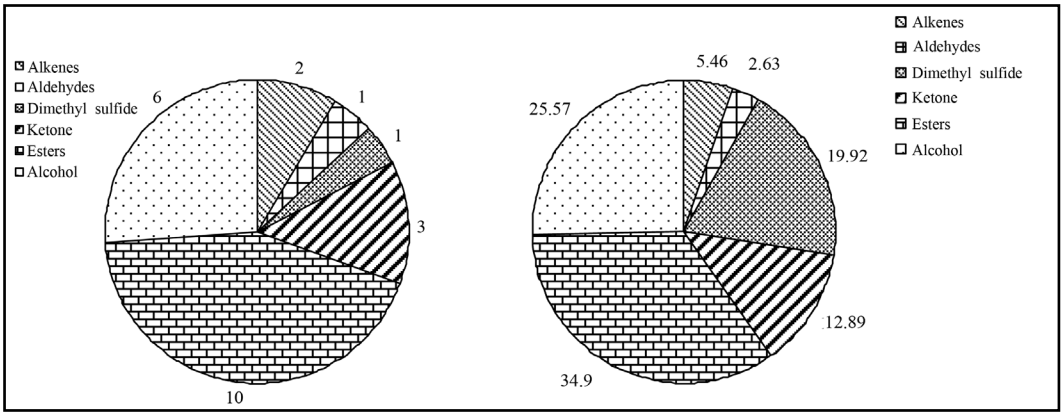

Figure 2. Categories and quantities of volatile components extracted from mycelia of *T. formosanum*.

Truffles (Tuber spp.) are symbiotic fungi that develop underground in association with plant roots. Food connoisseurs describe their scent as sensual, seductive and unique. SPME/GC-MS has permitted rapidly identifying compounds of the truffles species investigated here. The technique is therefore a powerful tool to identify new VOCs. A list of compounds of mycelial origin could be distinguished from those VOCs of mixed origin. Such an approach could be of special interest to the food industry.

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