Determination of Volatile Organic Compounds from Truffles via SPME–GC–MS

Giacomo Mauriello[1], **Rosanna Marino**[2], **Maurizio D'Auria**[1,*], **Giuseppina Cerone**[2], and **Gian Luigi Rana**[2]

[1]Dipartimento di Chimica, Università della Basilicata, Via N. Sauro 85, 85100 Potenza, Italy, [2]Dipartimento di Biologia, Difesa e Biotecnologie Agro-Forestali, Università della Basilicata, C.da Macchia Romana, 85100 Potenza, Italy

Abstract

Volatile organic compounds (VOCs) of nine Tuber species and two corresponding forms are identified via solid-phase microextraction–gas chromatography–mass spectrometry analysis. Seventy-five compounds are identified. The most abundant are dimethylsulphide, 2- and 3-methylbutanal, 2-methylpropanol, and butanone.

Introduction

In the past few decades, volatile organic compounds (VOCs) in truffle aromas have been analyzed using several different methods (1–17). Black truffle (*Tuber melanosporum*) has been extensively studied, and several compounds have been identified in the odor volatiles. *Bis*(methylthio)methane was identified as the main component of white truffle (*Tuber magnatum Pico*) aroma (1). VOCs have also been determined in the mycelium and peridium of *Tuber borchii* (18,19).

In recent years, a noninvasive methodology known as solid-phase microextraction (SPME) was adopted to perform VOC analysis (20,21). This technique was applied to the analysis of odors (22–29). The use of SPME in the determination of sulfur components of black and white truffle flavor has hence been reported (30).

This paper presents VOC findings in truffles collected in the Southern Italy region of Basilicata (31,32). VOCs of nine truffle species and two forms were analyzed using SPME methods. The following results represent the most complete description of the VOCs produced by truffles, as determined via SPME.

Experimental

Samples of *T. melanosporum* were furnished by Prof. Mattia Bentivenga (Università di Perugia, Perugia, Italy). Carpophores of the other 10 truffle species and forms [*Tuber mesentericum Vitt.*; *Tuber borchii Vitt.*; *Tuber aestivum Vitt.* and its form *uncinatum* (Chatin); *Tuber magnatum Pico*; *Tuber brumale Vitt.* and its form *moschatum* (Ferry); *Tuber excavatum Vitt.*; *Tuber oligospermum* (Tul. & C. Tul.) *Trappe*; and *Tuber panniferum Tul.* and *C. Tul.*] were collected via well-trained "Epanieul Breton", "Lagotto", and "Pointer" dogs in the forty-woodland of the Basilicata region over the past 3 years. Collected truffles were carefully brushed, stored in plastic bags, and maintained at 4°C until the analysis could be performed, usually within 24–48 h.

A 100-µm PDMS-SPME modu (57300-U, Supelco, Milan, Italy) were used to determine truffle VOCs. The SPME fiber was maintained over a 1.0-g sample in a 20-mL vial at 36°C for 20 min. The analyses were performed with an HP 6890 Plus gas

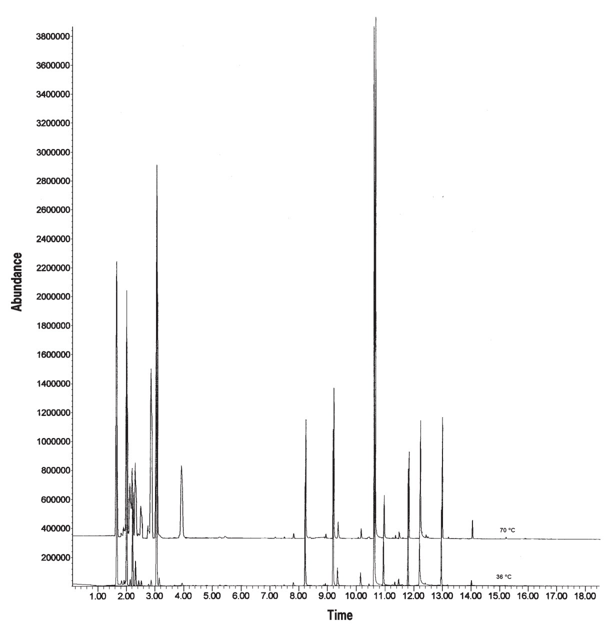

Figure 1. Chromatograms of a sample of *T. mesentericum* obtained after absorption of VOCs on SPME fiber at 36°C (bottom) and 70°C (top).

* Author to whom correspondence should be addressed: email dauria@pzuniv.unibas.it.

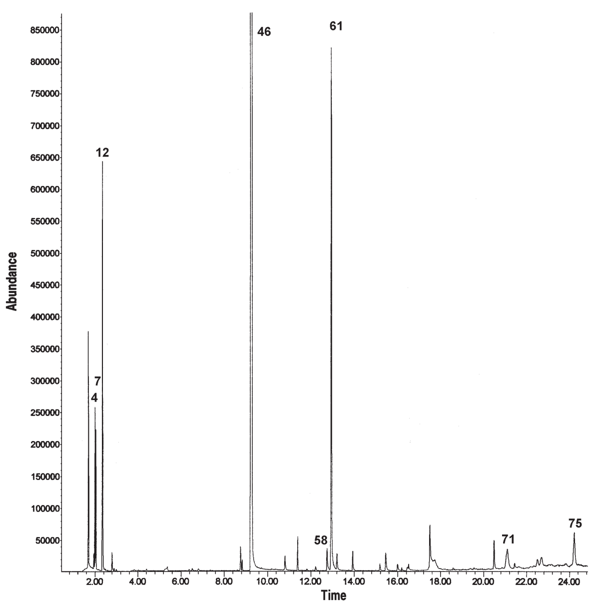

Figure 2. Chromatogram of a sample of *T. mesentericum*: (4) 1,2-pentadiene, (7) dimethylsulfide, (12) butanone, (46) 1-methoxy-3-methylbenzene, (58) 2,3-dimethoxytoluene, (61) 2,5-dimethoxytoluene, (71) hexacosane, and (75) heptacosane.

chromatograph equipped with a Phenomenex Zebron ZB-5 MS capillary column (30-m × 0.25-mm i.d. × 0.25-µm FT) (Agilent, Milan, Italy). An HP 5973 mass selective detector (Agilent) was utilized with helium at 0.8 mL/min as the carrier gas. A splitless injector was maintained at 250ºC and the detector at 230ºC. The oven was held at 40ºC for 2 min, then warmed 8ºC/min until 250°C was reached, and held for 10 min. Tentative identification of aroma components was based on mass spectra and Wiley 6 and NIST 98 library comparison. The peak was considered as identified when experimental spectrum matched that in the library with a score over 90%.

Results and Discussion

The aim of this work is to find a mild analytical method able to determine the actual composition of truffle aromas. In order to achieve this result, SPME offers the possibility to avoid chemical manipulations of the samples. However, temperature can be a possible source of artifacts. In Figure 1, two chromatograms of the same sample of *T. mesentericum* treated at 36ºC and 70ºC, respectively, are shown. At 70ºC, no additional compounds were found; however, the composition changed and more volatile compounds were present in larger

Table I. VOCs Identified in *T. mesentericum* and Corresponding Area Percent

	Compound	t_R* (min)	Sample 1	2	3	4	5	6	7	8	9	10	11	12	13	14
4	1,2-Pentadiene	1.95		1.1	0.4											1.3
5	2-Methyl-1,3-butadiene	1.99	0.8			0.2		2.6		2.4						
6	Thiourea	2.01					0.2									
7	Dimethylsulfide	2.03	3.4	0.1	1.9	4.5	1.8	5.4	20.0	0.3	3.3	4.1	1.3	1.3	2.9	10.2
8	1-Propanol	2.05					1.8									
11	2-Butanol	2.26					2.5									
12	Butanone	2.35	3.5	2.2								8.0			3.0	13.4
17	2-Methylbutanal	2.74									6.4			3.4	0.4	1.1
18	3-Methylbutanal	2.76			2.8						29.6		1.5	22.7	8.2	4.8
27	Hexanal	4.77													0.3	0.1
30	1,2-Propandiol	5.28					0.8									
32	2,3-Butandiol	5.80					2.5									
35	Heptanal	6.76													0.1	
39	3-Octanone	7.91											6.7		0.8	
40	Benzaldehyde	8.00													0.3	0.6
46	1-Methoxy-3-methylbenzene	9.23	1.4	80.1	7.7	72.8	29.3	65.0	21.6	83.0			6.4		0.9	0.7
55	1-Undecene	11.24					0.7									
56	2-Ethylanisole	11.64				0.7				0.2						
57	2-Phenylethanol	11.88					0.3		0.8							
58	2,3-Dimethoxytoluene	12.75		0.3		0.1	0.2			0.3						
59	Nonanol	12.84					0.2									
61	2,5-Dimethoxytoluene	12.94		6.1		2.4	1.5		0.5	8.1			12.4		0.3	1.0
65	3,4-Dimethoxytoluene	14.11						0.3							0.3	
67	Methyldiethyldithiocarbamide	16.71				0.1										
70	2,3,5-Trimethoxytoluene	17.44				0.1										1.0
71	Hexacosane	21.10		0.8												
75	Heptacosane	24.21		0.9												

* t_R = retention time.

amount. Furthermore, the chromatogram obtained with the sample treated at 70°C is less resolved. Treatment at 36°C was able to identify the VOCs present in the truffle.

Table I lists the 27 VOCs identified in samples of *T. mesentericum* (Figure 2). The percent area for each identified component is also given. Compounds found with high frequency were dimethylsulfide, which was found on 100% of the samples; 3-methylanisole on 79% of samples; 2,5-dimethoxytoluene on 57%, 3-methylbutanal on 43%; and butanone on 36%.

It should be noted that SPME adsorbs each VOC to a different degree; care must be taken to correctly interpret the data. However, comparison across samples is permissible because the influence of differential absorptions is the same in all the samples.

VOCs found in *T. excavatum* are listed in Table II. A large degree of variability was observed in samples analyzed. Dimethylsulfide and 3-octanone were present in three of the four ascocarps subjected to analysis. Other VOCs such 3-octanol, ethanol, acetic acid, and decane were found in only two ascocarps. Remaining VOCs were encountered in single carpophores only. The large variability could be because of sample maturity or carpophore location differences.

Samples of *T. borchii* also showed high variability in the aroma composition, as evident in Table III. All samples lacked dimethylsulfide. Two VOCs, 2-methyl-1,3-butadiene and 1,2-pentadiene, produced area percentages in two ascocarps, which also contained other volatile substances in lesser amounts.

VOC number per sample varied from 6–16. 1,2-Pentadiene was sometimes present in lower percentages and accompanied by discrete amounts of 1-methylpropyl formate, tetradecanal, and tetradecane or by 3-octanone and 15 additional VOCs.

Tirillini et al. analyzed VOCs in mycelium of *T. borchii* (18). Little superposition can be reported between our results and those given in the Tirillini work. In fact, only 3-octanone, decane, and ethynylbenzene were found in both studies. Bellesia et al. recently studied the VOCs in *T. borchii*, showing that 1-octen-3-ol was the main component in the flavor (19) This compound was not found in this present work. these differences may be attributed to analytical procedure or harvest site.

Analyses of carpophores of *Tuber aestivum* and *T. aestivum* f. *uncinatum* are given in Table IV (Figure 3). Dimethysulfide was found with the highest frequency of 85% in the samples. Butanone and 3-methylbutanal were also identified in 60% and 55% of specimens, respectively. VOCs found in discrete amounts in some samples were dimethylsulfide, 1-methoxy-3-methylbenzene, butanone, ethanol, and ethyl acetate. The amounts of dimethylsuphide were always lower in *T. aestivum* f. *uncinatum* than in *T. aestivum*.

The results stated are in substantial agreement with those published by Agostinone et al., and the same main VOCs in both truffle entities were found (7). On the contrary, other VOCs (such as 3- and 2-methylbutanal, 2-phenylethanol, and 3- and 2-methyl-1-butanol) found by Bellesia in *T. uncinatum* were not presently detected (16).

SPME–gas chromatography (GC)–mass spectrometry (MS) analysis of white truffle samples (*T. magnatum*) detected only the seven VOCs given in Table V (Figure 4). Dimethylsulfide was present in all the samples examined, with area percentages varying from 0.4–16.6%. With the exception of 2- and 3-methylbutanal, which was present in two samples, only sulfur compounds were found. The most abundant VOC was

Table II. VOCs Identified in *T. excavatum* and Corresponding Area Percent

	Compound	t_R (min)	Sample 1	2	3	4
2	Ethanol	1.80		38.9	2.3	
7	Dimethylsulfide	2.03		2.4	3.4	2.2
15	2-Methyl-1-propanol	2.47			4.4	
16	Acetic acid	2.56		4.5	0.4	
18	3-Methylbutanal	2.76	4.8			
21	*cis*-Methylpropenylsulfide	3.53				1.5
22	3-Methyl-1-butanol	3.69			5.1	
24	2-Methyl-1-butanol	3.87			2.8	
26	Toluene	4.43			0.3	
34	1,3-Dimethylbenzene	6.60			0.1	
37	Styrene	7.08			0.3	
39	3-Octanone	7.91	6.4	5.2	17.4	
40	Benzaldehyde	8.00	7.2			
44	1-Octen-3-ol	9.06	48.5			
45	2-Pentylfuran	9.15	0.7			
47	3-Octanol	9.24	6.2		31.3	
59	Decane	12.84		1.1	1.3	
63	Benzo[c]thiophene	13.34			0.2	

Table III. VOCs Identified in *T. borchii* and Corresponding Area Percent

	Compound	t_R (min)	Sample 1	2	3	4	5
2	Ethanol	1.80	0.7		0.8		
4	1,2-Pentadiene	1.95	63.1	5.7		6.4	
5	2-Methyl-1,3-butadiene	1.99			42.7		2.3
11	2-Butanol	2.26				0.1	
14	2-Methylfuran	2.45	0.2		2.5	0.2	
15	2-Methyl-1-propanol	2.47			0.2		
17	2-Methylbutanal	2.74		0.7	0.6	0.3	
18	3-Methylbutanal	2.76	0.1		2.8		
19	Pentanal	2.80				1.0	
20	1-Methylpropyl formate	3.06		8.6			
22	3-Methyl-1-butanol	3.69			2.3		
24	2-Methyl-1-butanol	3.87		1.8	0.6	0.5	
26	Toluene	4.43		0.3		0.1	
29	3-Methylthiophene	4.98	0.3		0.3	0.3	
34	1,3-Dimethylbenzene	6.60		0.2		0.1	
37	Styrene	7.08		0.2		0.1	
39	3-Octanone	7.91			0.6	6.6	
48	β-Ocimene	9.48	0.7		0.3	1.2	
49	Decane	9.50		1.7	0.5	0.9	
63	Benzo[c]thiophene	13.34		0.2		0.4	
68	Tetradecane	17.03		23.2		0.1	
69	Tetradecanal	17.20		17.2		1.2	

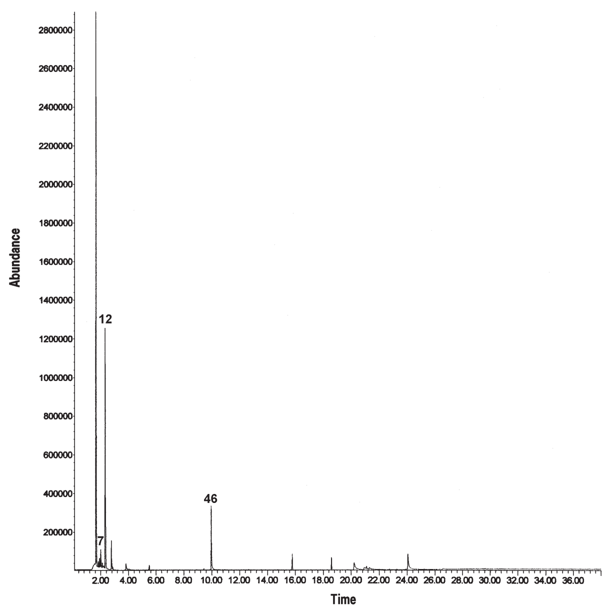

Figure 3. Chromatogram of a sample of *T. aestivum* f. *uncinatum*: (7) dimethylsulfide, (12) butanone, and (45) 1-methoxy-3-mehylbenzene.

2,4-dithiopentane, a result in agreement with those reported by Hansen, Kühne, and Bellesia (10,14). 1,2,4-Trithiolane, methyl(methylthio)methyldisulfide, and *tris*(methylthio) methane, as reported by Pelusio, were not found in this study (30).

Twenty-eight VOCs were found in *T. brumale* and *T. brumale* f. *moschatum* and are listed in Table VI (Figure 5). The most representative compounds found in *T. brumale* were dimethyl-

Table V. VOCs Identified in *T. magnatum* and Corresponding Area Percent

	Compound	t_R (min)	Sample 1	2	3	4	5
7	Dimethylsulfide	2.03	16.6	0.4	9.4	0.7	3.8
17	2-Methylbutanal	2.74		0.6	0.1		
18	3-Methylbutanal	2.76		1.7	0.1		
23	Dimethyldisulfide	3.86	0.6		0.3		
33	2,4-Dithiopentane	6.59			57.0	2.1	6.4
41	Dimethyltrisulfide	8.15			0.1		
62	Trimethyltrisulfide	13.05			1.2		

Table IV. VOCs Identified in Samples of *Tuber aestivum* and Its Form *uncinatum and Corresponding Area Percent**

	Compound	t_R (min)	1*	2*	Sample 3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20
2	Ethanol	1.80			0.4	9.3	22.6	22.9				1.9										0.1
3	Propanone	1.93			0.6					0.8												
7	Dimethylsulfide	2.03	2.9	2.2	7.9	24.8		5.2	4.0	5.5		8.0	6.9		11.3	5.5	3.7	24.1	16.1	16.2	10.4	6.3
8	1-Propanol	2.05				2.1																
9	2-Methylpropanal	2.13							1.0	1.5												
11	2-Butanol	2.26				2.6																
12	Butanone	2.35	18.9	2.3	54.5					3.3		4.0	2.2		37.7			6.9	2.5	10.7	4.8	6.2
13	Ethyl acetate	2.44			0.1		2.6													47.4	15.9	
15	2-Methyl-1-propanol	2.47			0.9	2.4	3.4															
17	2-Methylbutanal	2.74							0.9	4.3	1.2			1.1						0.5	0.9	0.1
18	3-Methylbutanal	2.76			0.3				1.9	9.4	3.1	0.9	1.7	3.8		1.1				0.4	1.5	0.7
20	1-Methylpropyl formate	3.06			0.4																	
22	3-Methyl-1-butanol	3.69				2.1	3.8			1.2		1.2										
24	2-Methyl-1-butanol	3.87			1.1	7.9	3.9	3.5														
25	1-Methylpropyl acetate	4.25			0.5																	
26	Toluene	4.43	0.4																			
28	1,3-Butandiol	4.97					1.4															
32	2,3-Butandiol	5.80					1.4					0.5										
39	3-Octanone	7.91					0.8															0.4
40	Benzaldehyde	8.00								1.0												
45	2-Pentylfuran	9.15								0.7												
46	1-Methoxy-3-methylbenzene	9.23	9.6	1.4					28.7	0.5		5.2										8.0
57	2-Phenylethanol	11.88					1.3															

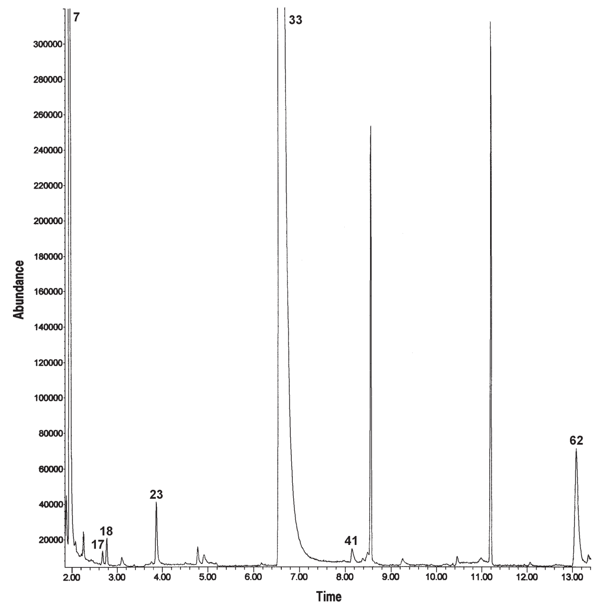

Figure 4. Chromatogram of a sample of *T. magnatum*: (7) dimethylsulfide, (17) 2-methylbutanal, (18) 3-methylbutanal, (23) dimethyldisulfide, (33) 2,4-dithiopentane, (41) dimethyltrisulfide,and (62) trimethyltrisulfide.

sulfide, butanone, 2-methylbutanal, 1-methylpropyl formate, 2-methylpropanal, and 1,4-dimethoxybenzene. Dimethylsulfide was detected in lower amounts in *T. brumale* f. *moschatum* than in *T. brumale*. Other main VOCs found were 2-methylpropanal, butanone, 2-methylbutanal, and 1,4-dimethoxybenzene. These results fit those reported by Bellesia (14). Furthermore, in this study, six compounds never before found (1-methoxy-3-methylbenzene, decane, anisole, 3-ethyl-5-methylphenol, 1,4-dimethoxybenzene, and 1,2,4-trimethoxybenzene) were detected; whereas, 1-propanol, 3-octanol, 3-methyl-1-butanol, 5-hexen-2-ol, 3-nonanol, benzylic alcohol, methylphenols, and 3-methylbutylamine were absent.

Although only two samples of *T. melanosporum* were analyzed, the results of the analyses performed were uniform. As illustrated in Table VII, small amounts of dimethysulfide were found. The main VOCs detected were 2-methylbutanal, 2-methylpropanol, and 2-methyl-1-butanol. The following six esters, never detected before (2,5,8,12,14,17), were also found: ethyl 2-methylbutanoate, 2-methylpropyl 2-methylbutanoate, 2-methylbutyl 2-methylbutanoate, 2-methylpropyl 2-methylpropanoate, and 3-methylbutyl 2-methylpropanoate. It is well known that the organic esters are strictly related to flavor (33). Therefore, the mentioned compounds could play an important role in defining the aroma of the truffle species.

The results of the SPME–GC–MS analyses of *T. oligospermum* are collectively presented in Table VIII. Only propanone was detected in two samples. The other VOCs were present with the former mixed with additional organic substances.

Finally, Table IX summarizes the results obtained from the analyses of *T. panniferum* (Figure 5). As in *T. magnatum*, the main constituent of *T. panniferum* aroma is 2,4-dithiopentane.

Table VI. VOCs Identified in Samples of *Tuber brumale* and Its Form *moschatum* and Corresponding Area Percent

		t_R	Sample						
	Compound	(min)	1*	2*	3	4	5	6	7
1	Acetaldehyde	1.72						0.1	
2	Ethanol	1.80		0.2		0.2	0.7	0.3	0.7
7	Dimethylsulfide	2.03	15.3	9.0	0.6	1.2	3.0	3.4	6.7
9	2-Methylpropanal	2.13	4.2	0.8	3.0	1.9	2.4	11.6	2.4
10	Methylethyl formate	2.17	4.3	4.3	1.1	5.7	10.7	0.8	1.1
12	Butanone	2.35	2.5	1.5	7.3	0.8	1.3	11.8	2.7
13	Ethyl acetate	2.44	0.3	1.1	0.3	0.1	0.2	0.2	
15	2-Methyl-1-propanol	2.47	0.8			0.8	3.3	1.9	1.3
17	2-Methylbutanal	2.74	12.8	18.6	5.2	7.2	3.0	18.6	8.6
18	3-Methylbutanal	2.76	0.7	0.4	0.4	0.5	0.3	2.2	0.4
20	1-Methylpropyl formate	3.06	21.7	8.8	2.1	4.3	16.5	4.0	8.7
24	2-Methyl-1-butanol	3.87	1.0			1.1	1.1	2.5	1.6
25	1-Methylpropyl acetate	4.25						0.1	
26	Toluene	4.43						0.1	
34	1,3-Dimethylbenzene	6.60						0.1	
37	Styrene	7.08						0.2	
38	Anisole	7.10	0.1	0.3	0.3	0.2		0.8	0.3
39	3-Octanone	7.91						0.1	
44	1-Octen-3-ol	9.06						0.1	
46	1-Methoxy-3-Methylbenzene	9.23	2.7	0.2		0.4			2.6
49	Decane	9.50	0.1					0.8	
50	1-Methoxy-4-methylbenzene	9.98						0.1	
51	3-Ethyl-5-methylphenol	10.16	0.2			0.2			0.3
52	Limonene	10.18						0.04	
54	1-Propynylbenzene	10.54						0.1	
60	1,4-Dimethoxybenzene	12.90	8.8	6.2		31.1	5.1	7.4	17.5
64	Benzo[b]thiophene	13.55						0.5	
66	1,2,4-Trimethoxybenzene	16.59	1.3	5.6	4.5	9.0	0.5	3.5	0.3

Conclusion

This study has shown that SPME represents a useful, noninvasive method to determine volatile organic compounds in aromas. SPME analysis involves no chemical manipulation of the samples, which may explain the absence of previously detected truffle VOCs. The results presented here may support the hypothesis that they are artifacts.

The most complete homogeneous study on the volatile organic compounds in the flavor of truffles has been compiled and is useful because it can give information on the actual composition of the flavor of truffles. These data could be useful in the formulation of artificial aroma of truffles.

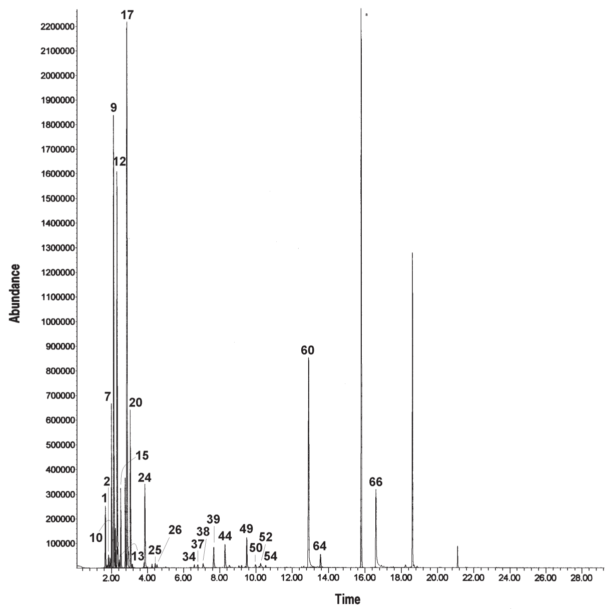

Figure 5. Chromatogram of a sample of *T. brumale*: (1) acetaldehyde, (2) ethanol, (7) dimethylsulfide, (9) 2-methylpropanal, (10) methylethyl formate, (12) butanone, (13) ethyl acetate, (15) 2-methyl-1-propanol, (17) 2-methylbutanal, (18) 3-methylbutanal, (20) 1-methylpropyl formate, (24) 2-methyl-1-butanol, (25) 1-methylpropyl acetate, (26) toluene, (34) 1,3-dimethylbenzene, (37) styrene, (38) anisole, (39) 3-octanone, (44) 1-octen-3-ol, (49) decane, (50) 1-methoxy-4-methylbenzene, (52) limonene, (54) 1-propynylbenzene, (60) 1,4-dimethoxybenzene, (64) benzo[b]thiophene, and (66) 1,2,4-trimethoxybenzene.

Table VII. VOCs Identified in T. melanosporum and Corresponding Area Percent

	Compound	t_R (min)	Sample 1	Sample 2
1	Acetaldehyde	1.72	2.0	1.1
2	Ethanol	1.80	3.4	1.6
7	Dimethyllsulfide	2.03	1.4	2.1
9	2-Methylpropanal	2.13	3.2	4.7
12	Butanone	2.35	1.3	1.3
15	2-Methylpropanol	2.47	12.7	9.8
17	2-Methylbutanal	2.74	9.6	18.4
18	3-Methylbutanal	2.76	1.3	1.1
20	1-Methylpropyl formate	3.06	0.3	0.8
22	3-Methyl-1-butanol	3.69	0.9	
24	2-Methyl-1-butanol	3.87	24.9	25.5
27	Hexanal	4.77	0.7	0.7
31	Ethyl 2-methylbutanoate	5.76	0.2	0.3
36	2-Methylpropyl 2-methylpropanoate	7.01		0.3
38	Anisole	7.10		0.1
42	2-Methylpropyl 2-methylbutanoate	8.67	0.9	0.7
43	2-Methylbutyl 2-methylpropanoate	8.91	0.2	0.5
53	2-Methylbutyl 2-methylbutanoate	10.43	0.4	1.4

Table VIII. VOCs Identified in *T. oligospermum* and Corresponding Area Percent

	Compound	t_R (min)	Sample 1	Sample 2	Sample 3
1	Acetaldehyde	1.72			0.6
3	Propanone	1.93		0.9	2.6
17	2-Methylbutanal	2.74	0.6		
18	3-Methylbutanal	2.76	2.0		

Table IX. VOCs Identified in *T. panniferum* and Corresponding Area Percent

	Compound	t_R (min)	Sample 1	Sample 2	Sample 3
9	2-Methylpropanal	2.13	1.0	1.7	1.8
12	Butanone	2.35		16.7	3.8
17	2-Methylbutanal	2.74		1.6	1.4
18	3-Methylbutanal	2.76		1.3	1.4
23	Dimethyldisulfide	3.86		0.3	0.5
33	2,4-Dithiopentane	6.59	0.5	18.7	22.3
39	3-Octanone	7.91		3.4	
40	Benzaldehyde	8.00		1.7	1.5
72	6(*Z*),9(*E*)-Heptadecadiene	21.18		0.2	
73	1-Heptadecene	21.29		0.3	
74	1-Nonadecene	21.47			0.3

References

1. A. Fiecchi, M. Galli Kienle, A. Scala, and P. Gabella. Bis-methylthiomethane, an odorous substance from white truffle. *Tetrahedron Lett.* **18:** 1681–82 (1967).
2. K.H. Ney and W.G. Frietag. Truffle aroma. *Gordian* **80:** 214–16 (1980).
3. R. Claus, H.O. Hoppe, and H. Karg. The secret of truffles: a steroidal pheromone? *Experentia* **37:** 1178–79 (1981).
4. F. Balestreri, D. Martini, and M. Vietti. Determinazione mediante HPLC dei componenti dell'aroma del *Tuber melanosporum*. In *Atti XII Congresso Nazionale di Merceologia*. Societa Italiana di Merceologia, Torino, Italy, 1986.
5. T. Talou, M. Delmas, and A. Gaset. Principal constituents of black truffles (*Tuber melanosporum*) aroma. *J. Agr. Food Chem.* **35:** 774–77 (1987).
6. C. Bellina Agostinone, M. D'Antonio, and G. Pacioni. Odour composition of the summer truffle, *Tuber aestivum. Trans. Br. Mycol. Soc.* **884:** 568–69 (1987).
7. M. Angeletti, A. Landucci, M. Contini, and M. Bertuccioli. Caratterizzazione dell'aroma del tartufo mediante l'analisi gas cromatografia dello spazio di testa. In *Atti II Congresso Internazionale sul Tartufo*. Comune di Spoleto, Spoleto, Italy, 1988, pp. 505–509.
8. A. Fiecchi. Odour composition of truffles. In *Atti II Congresso Internazionale sul Tartufo*. Comune di Spoleto, Spoleto, Italy, 1988, pp. 497–500.
9. F. Balestrieri, D. Martini, and M. Vietti. Sulla variazione dell'aroma del *Tuber magnatum* durante il processo di maturazione. *Industrie Alimentari* **27:** 423 (1988).
10. H.P. Hanssen and B. Kühne. Aroma compounds in canned truffles. In *Atti II Congresso Internazionale sul Tartufo*. Comune di

Spoleto, Spoleto, Italy, 1988, pp. 501–504.
11. I. Flament, C. Chevallier, and C. Dobonneville. Analysis of volatile flavor constituents of Perigord black truffle (*Tuber melanosporum* Vitt.). *Riv. Ital. EPPOS* **9:** 280–99 (1990).
12. G. Pacioni, C. Bellina Agostinone, and M. D'Antonio. Odour composition of *Tuber melanosporum* complex. *Mycol. Res.* **94:** 201–204 (1990).
13. T. Talou, M. Delmas, and A. Gaset. Advances in the detection of black truffles. *Riv. Ital. EPPOS* **9:** 300–16 (1990).
14. F. Bellesia, A. Pinetti, A. Bianchi, and B. Tirillini. I composti solforati dell'aroma del tartufo: loro evoluzione durante la conservazione. *Atti Soc. Nat. Mat. Modena* **127:** 177–87 (1996).
15. F. Bellesia, A. Pinetti, A. Bianchi, and B. Tirillini. The volatile organic compounds of white truffle (*Tuber magnatum* Pico) from middle Italy. *Flavour Fragr. J.* **11:** 239–43 (1996).
16. F. Bellesia, A. Pinetti, A. Bianchi, and B. Tirillini. The volatile organic compounds of *Tuber uncinatum* from middle Italy. *J. Essent. Oil Res.* **10:** 483–88 (1998).
17. F. Bellesia, A. Pinetti, A. Bianchi, and B. Tirillini. The volatile organic compound of black truffle (*Tuber melanosporum* Vitt.) from middle Italy. *Flavour Fragr. J.* **13:** 56–58 (1998).
18. B. Tirillini, G. Verdelli, F. Paolocci, P. Ciccioli, and M. Frattoni. The volatile organic compounds from mycelium of *Tuber borchii* Vitt. *Phytochemistry* **55:** 983–85 (2000).
19. F. Bellesia, A. Pinetti, B. Tirillini, and A. Bianchi. Temperature-dependent evolution of volatile organic compounds in *Tuber borchii* from Italy. *Flavour Fragr. J.* **16:** 1–6 (2001).
20. Z. Zhang and J. Pawliszyn. Headspace solid phase microextraction. *Anal. Chem.* **65:** 1843–52 (1993).
21. J. Pawliszyn. *Solid-Phase Microextraction: Theory and Practice*. VCH, New York, NY, 1997.
22. A. Boyd-Boland, M. Chai, Y. Luo, Z. Zang, M. Yang, J. Pawliszyn, and T. Gorecki. Solvent free sample preparation techniques based on fiber and polymer technologies. *Environ. Sci. Technol.* **28:** 569A–74A (1994).
23. X. Yang and T. Peppard. Solid-phase microextraction for flavor analysis. *J. Agr. Food Chem.* **42:** 1925–30 (1994).
24. H. Chin, R. Bernhard, and M. Rosemberg. Solid phase microextraction for cheese volatile compounds analysis. *J. Food Sci.* **61:** 1118–29 (1996).
25. A. Matich, R. Rowan, and N. Banks. Solid-phase microextraction for quantitative headspace sampling of apple volatiles. *Anal. Chem.* **68:** 4114–18 (1996).
26. J. Clark and J. Bunch. Qualitative and quantitative analysis of flavor additives on tobacco products using SPME-GC-MS. *J. Agr. Food Chem.* **45:** 844–49 (1997).
27. S. Elmore, M. Erbahdir, and D. Mottram. Comparison of dynamic headspace concentration on tenax with SPME for analysis of aroma volatiles. *J. Agr. Food Chem.* **45:** 2638–41 (1997).
28. J. Song, B. Gardner, J. Holland, and R. Beaudry. Rapid analysis of volatile flavor compounds in apple fruit using solid-phase microextraction and GC-time-of-flight mass spectrometry. *J. Agr. Food. Chem.* **45:** 1801–1807 (1997).
29. A. Steffen and J. Pawliszyn. Analysis of flavor volatiles using headspace solid phase microextraction. *J. Agr. Food Chem.* **44:** 2187–91 (1997).
30. F. Pelusio, T. Nilsson, L. Montanarella, R. Tilio, B. Larsen, S. Facchetti, and J.Ø. Madsen. Heaspace solid-phase microextraction analysis of volatil organic sulfur compounds in black and white truffle aromas. *J. Agr. Food Chem.* **43:** 2138–43 (1995).
31. G. Cerone, E. Alba, M. Galante, and S. Frisullo. Specie fungine del genere *Tuber* in Basilicata. *Micologia Italiana* **1:** 9–16 (1994).
32. G. Cerone, R. Marino, and G.L. Rana. Studi sui funghi ipogei e la tartuficoltura della Basilicata. *Rivista di Micologia* **43(4):** 333–46 (2000).
33. C. Sell. Ingredients for the modern perfumery industry. In *The Chemistry of Rragrances.* D. Pybus and C. Sell, Eds. Royal Society of Chemistry, Cambridge, United Kingdom, 1999, pp. 51–124.

Manuscript accepted May 5, 2004.