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Truffle aroma characterization by headspace solid-phase microextraction☆

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Abstract

In the present study, a headspace solid-phase microextraction (HS-SPME) combined to gas chromatography–mass spectrometry (GC–MS) has been used to fully characterize aroma of truffles of different species. A fiber of medium polarity (for flavors) was used to avoid discrimination towards very non-polar and polar volatile compounds. In a previous work, extraction conditions were optimized by means of an experimental design leading to the following conditions that were used in the present study: extraction temperature, 53 °C; extraction time, 13.6 min; and equilibrium time, 5 min. A comparison among different truffles species has been established in terms of qualitative and quantitative differences on volatile composition. By using the optimal extraction conditions and GC–MS it was possible to identify 89 compounds in two different truffle species such as *Tuber aestivum* and *Tuber melanosporum*. An attempt has been made in order to be able to determine the influence of different geographical origins on the aroma fraction of such fungi.

Keywords: *Tuber* spp.; Food analysis; Aroma compounds

1. Introduction

Tuber aestivum (summer truffle), *Tuber melanosporum* (black truffle of Perigord), *Tuber magnatum* (white truffle), and other truffles belonging to the genus Tuber F.H. Wigg are mycorrhizal fungi highly appreciated for their unique and characteristic aroma and for environmental and forestry applications owing to the advantages that mycorrhizae provide for host plants [1]. These truffle species can associate to numerous plants, mainly of the genus *Quercus* L., *Corylus* L., *Pinus* L., *Tilia* L., *Ostrya* Scop. y *Cistus* L. [2,3]. Their culinary and commercial value is mainly due to their organoleptic properties such as their aroma, the quality of which clearly provides the economic value of such edible fungi.

Due to the economical interest of truffles, mainly in countries such as Spain, France and Italy, it is important to develop methods that allow an objective

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evaluation of truffle aroma. Such methods can be used to identify the different truffle species to, for example, detect *T. aestivum* in products involving the mix of different truffles (to warranty the authenticity of such products) or even to determine the influence of different growing parameters in the aroma fraction of such valuable fungi. Bertault et al. [4] have suggested that the organoleptic differences seen over the geographical area of black truffles (*T. melanosporum* Vitt.) can probably be explained by environmental variation rather than by genetic factors.

Also, the study of truffle aroma has been suggested as a way of authentication of the different truffle species [5]. For example, the introduction in the European market of Asian truffles such as *Tuber indicum*, with lower aroma content and cheaper than *T. melanosporum*, but with very similar carpophore morphology and spore shape, involve the need of methods to detect Chinese truffles as adulterants of different products obtained from *T. melanosporum* [6,7].

Some research has been devoted to the identification of truffle aroma compounds and to the study of the effect of processing on the original aroma of different *Tuber* species [8–13].

The most used analytical techniques to concentrate the volatile compounds of food aroma have been obviously those based on headspace analysis [14]. For truffle aroma, techniques such as dynamic headspace coupled to gas chromatography–mass spectrometry (GC–MS) [15] and purge and trap GC–MS [16] have been used to detect black Perigord truffle and Italian white truffles aromas, respectively. Headspace solid-phase microextraction (HS-SPME) combined with GC–MS has been used to detect the volatile sulfur compounds in the aroma of white and black truffles (*T. magnatum* Pico and *T. melanosporum*, respectively). In a previous paper, we carried out the optimization, by means of an experimental design, of the extraction of volatile compounds from summer truffle aroma (*T. aestivum*) by using HS-SPME [17]. Extraction conditions such as extraction temperature, extraction time and equilibrium time have been optimized with a medium polarity fiber, to reduce discrimination toward very non-polar and polar volatile compounds.

The objective of the present investigation has been to fully characterize aroma of truffles of different species by means of HS-SPME combined with GC–MS. An objective comparison among different truffles has been established in terms of qualitative and semi-quantitative differences on volatile composition. Also, the influence of different geographical zones on the aroma fraction of the same species of truffle has been studied in an attempt to characterize, by means of the aroma, the origin of the fungi.

2. Experimental

2.1. Truffles

Truffles used in this work belong to the species *T. aestivum* and *T. melanosporum* and were collected in the Agricultural Centre of Castilla and León Community (Monasterio de la Santa Espina, Valladolid, Spain) and Navaleno (Soria, Spain). These truffles were deep frozen just after their collection and were kept at freezing temperature until extraction.

Immediately before analysis, ∼1.5 g of truffle was cut from the frozen sample, allowed to thaw at ambient temperature for 15 min, and cut into thin slices of truffle flesh using a sharp knife. A minimum of two samples of each truffle species have been analyzed in order to confirm the variability associated to each truffle.

2.2. Headspace solid-phase microextraction

An SPME holder (Supelco, Bellefonte, PA, USA) was used to perform the experiments. A fused silica fiber coated with a 50/30 μm layer of divinylbenzene–Carboxen–polydimethylsiloxane (Supelco) was chosen to extract the volatile components from the truffles.

The fiber was conditioned following the manufacturer's instructions previous to its use.

Approximately 1 g of sample was placed in a 4 ml vial closed with a plastic film. Once the desired temperature (53 °C) had been reached in a water bath, the vial was placed inside the bath and was allowed to condition for the equilibrium time (5 min, no fiber exposition). After the equilibrium time, the fiber was introduced into the vial and exposed to the headspace of the sample during 13.6 min. These conditions were selected from a previous work where a complete optimization of the extraction conditions was carried out

[17]. The reproducibility of the whole method (extraction and analysis) has been calculated using six replicates providing a R.S.D. value of around 12%.

2.3. Aroma analysis by gas chromatography–mass spectrometry

An Agilent-6890 GC system coupled to an Agilent-5873 mass spectrometer was used to perform all the GC analyses. The capillary column used in the GC–MS was a 50 m × 0.25 mm i.d. fused silica capillary column coated with a 0.2 μm layer of Carbowax 20M. Thermal desorption of the compounds from the fiber coating took place in the GC injector at 200 °C for 15 min in splitless mode for 10 min. Other operating conditions were as follows: detector temperature, 250 °C; oven temperature program from 40 to 60 °C at 10 °C min^{-1} and then to 200 °C (15 min at constant temperature) at 3 °C min^{-1}. Helium at 15 psig was used as carrier gas with a flow rate of 1.0 ml min^{-1} (1 psi = 6894.76 Pa).

Compounds were tentatively identified by comparison of the spectra with those in a mass spectrometry library (Wiley) and with data found in the literature.

3. Results and discussion

Different truffle species such as *T. aestivum* (from two different geographical zones of Spain, Valladolid and Soria) and *T. melanosporum* (from Soria) were extracted by using the optimal HS-SPME conditions obtained in a previous work done in our laboratory [17] and analyzed by GC–MS. Figs. 1–3 show the total ion current (TIC) mass chromatograms of the three truffles studied in the present work. No peaks appeared in the blank runs, thus indicating that no compounds due to the fiber or contamination are expected. Also, by analyzing a minimum of two samples of each of the truffle species studied and comparing the chromatographic profiles and the relative areas of the compounds detected, we can establish that the differences observed among the different truffles are due to a different geographical origin and/or specie. By using the described procedure, it was possible to identify 89 compounds in the two different truffle species that are listed in Table 1 along with their relative percentages (as normalized areas). By comparing *T. melanosporum* and *T. aestivum* it can be easily seen that the first one has a stronger aroma (in terms of total amount and number

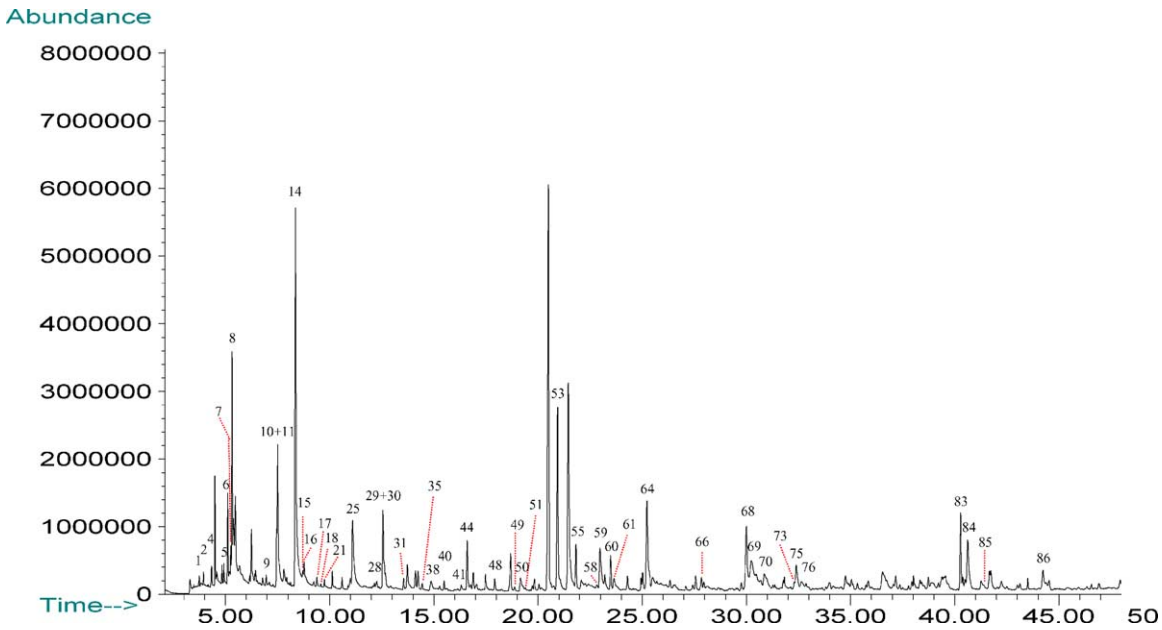

Fig. 1. Total ion current (TIC) mass chromatogram of a HS-SPME of *T. aestivum* (Navaleno, Soria). Chromatographic conditions: injector temperature, 200 °C for 15 min in splitless mode for 5 min; detector temperature, 250 °C; oven temperature program, 40 to 60 °C at 10 °C min^{-1}, to 200 °C (15 min constant) at 3 °C min^{-1}; time scale (min). Peak assignment as in Table 1.

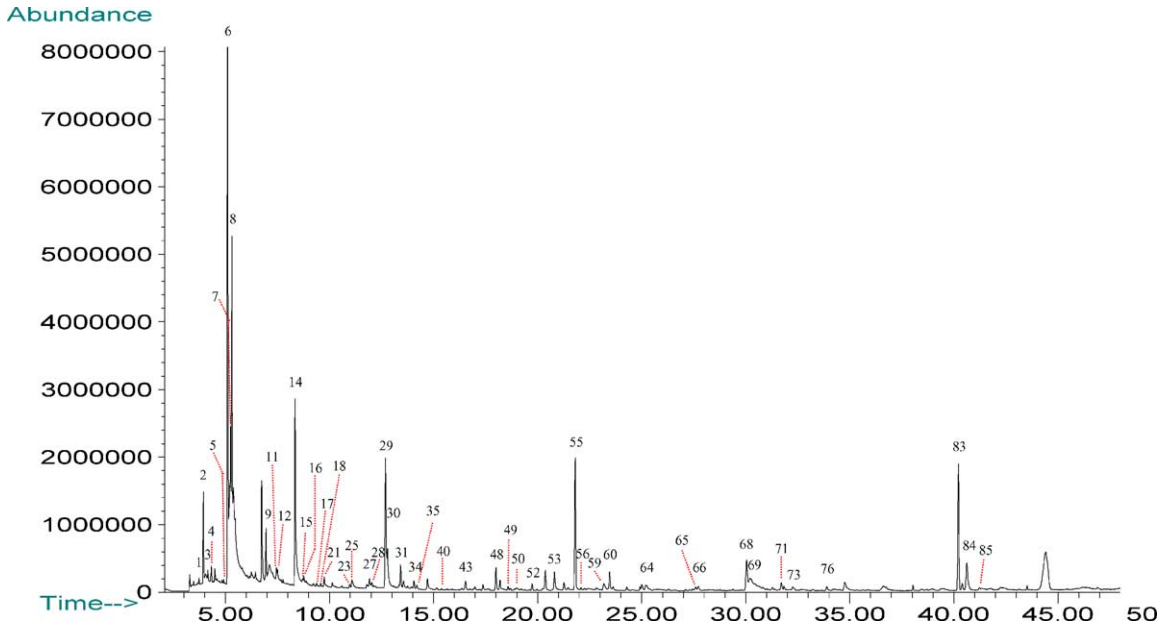

Fig. 2. Total ion current (TIC) mass chromatogram of a HS-SPME of *T. aestivum* (Monasterio de la Santa Espina, Valladolid). Chromatographic conditions as mentioned in Fig. 1. Peak assignment as in Table 1.

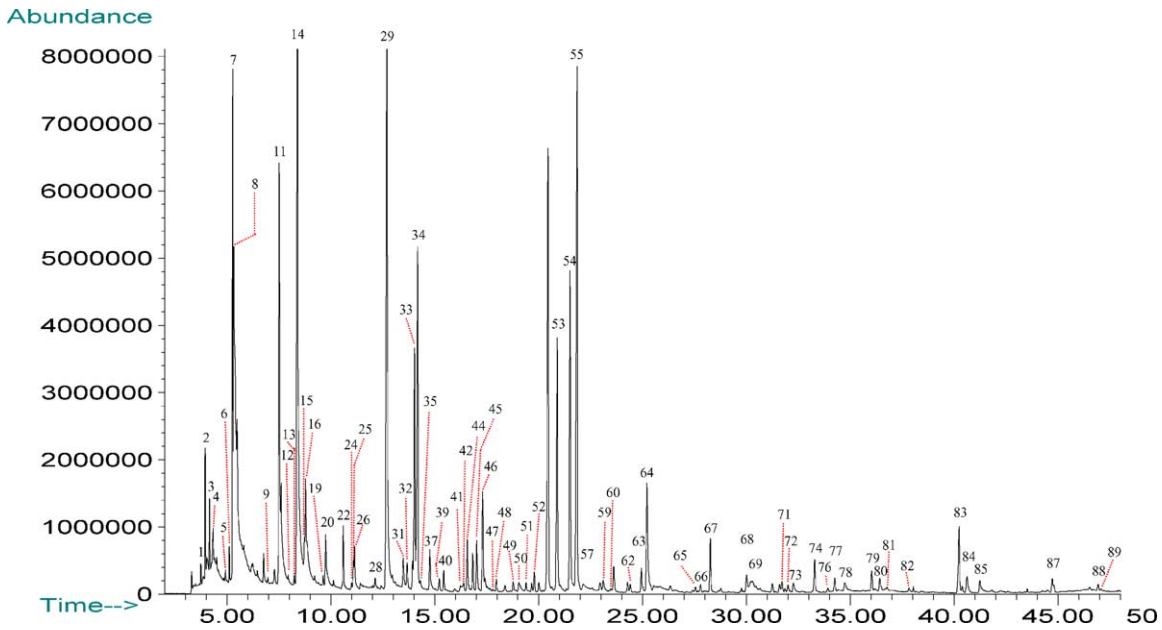

Fig. 3. Total ion current (TIC) mass chromatogram of a HS-SPME of *T. melanosporum* (Navaleno, Soria). Chromatographic conditions as mentioned in Fig. 1. Peak assignment as in Table 1.

Table 1
Identified compounds and relative percentages of *T. aestivum* (Soria) and (Valladolid) and *T. melanosporum* (Soria) aroma compounds extracted by HS-SPME using *A* (%) for flavors fiber

No.	Retention time (min)	Compound	*A* (%) *T. aestivum* (Soria)	*A* (%) *T. aestivum* (Valladolid)	*A* (%) *T. melanosporum* (Soria)
1	3.74	Acetaldehyde	0.2798	0.0278	0.0699
2	3.94	Dimethylsulfide	0.2668	0.4560	0.5245
3	4.16	Propanal	–	0.0390	0.2444
4	4.33	2-Propanone	0.5749	0.1067	0.0948
5	4.93	Ethyl acetate	0.4703	0.0158	0.0361
6	5.11	2-Butanone	2.1852	38.1709	1.3517
7	5.27	2-Methyl-butanal	0.6362	19.3657	19.1255
8	5.33	3-Methyl-butanal	7.5634	32.0520	38.3154
9	6.95	2-Butanol	0.2342	0.3094	0.0277
10	7.46	Methylbenzene	–	0.0479	–
11	7.51	2-Butenal	7.9041	0.0606	3.4546
12	7.94	Ethyl-3-methylbutanoate	0.2113	–	0.0901
13	8.23	Dimethyldisulfide	0.0579	–	0.0437
14	8.39	Hexanal	17.6341	1.8416	5.9278
15	8.73	2-Methyl-1-propanol	0.5876	0.0536	0.3050
16	8.79	2-Methyl-2-butenal	1.3518	0.0768	1.2245
17	9.38	Ethylbenzene	0.3328	0.0257	–
18	9.58	1,3-Dimethylbenzene	0.1077	0.0217	–
19	9.63	Butyl-2-methylbutanoate	–	–	0.0388
20	9.75	3-Penten-2-one	–	–	0.4421
21	9.74	1,4-Dimethylbenzene	0.2385	0.0783	–
22	10.60	2-Methylpropyl 2-methylbutanoate	–	–	0.2907
23	10.97	1,2-Dimethylbenzene	–	0.0288	–
24	11.00	2-Methylpropyl-3-methylbutanoate	–	–	0.0304
25	11.08	Heptanal	5.3558	0.1251	0.1573
26	11.14	3-Methylbutyl-2-methylpropanoate	–	–	0.2708
27	11.92	3-Methyl-3-penten-2-one	–	0.1061	–
28	12.13	2-Pentylfuran	0.3545	0.0542	0.0665
29	12.70	2-Methyl-1-butanol	3.7360	1.1602	4.9360
30	12.79	3-Methyl-1-butanol	–	0.4016	–
31	13.48	3-Octanone	0.3818	0.1711	0.2006
32	13.66	5-Methyl-2-heptanone	–	–	0.2139
33	14.02	6-Dodecanol	–	–	1.4844
34	14.17	2-Methylbutyl-2-methylbutanoate	–	–	2.4996
35	14.30	1,2,4-Trimethylbenzene	0.2017	0.0459	0.0692
36	14.71	2-Octanone	–	0.1239	–
37	14.76	Pentyl-3-methylbutanoate	–	–	0.2861
38	14.87	Octanal	0.8590	–	–
39	15.22	2,3-Dihydro-4-methylfuran	–	–	0.0759
40	15.43	Octa-1,5-dien-3-ol	0.3222	0.0113	0.1188
41	16.23	2,3-Octanedione	0.0551	–	0.0590
42	16.39	3-Hydroxy-2-butanone	–	–	0.0597
43	16.54	1,3,4-Trimethyl-2-pyrazoline	–	0.0988	–
44	16.57	2-Heptenal	2.1574	–	0.3549
45	17.01	3-Octen-2-one	–	–	0.3743
46	17.30	Methoxybenzene	–	–	0.8269
47	17.80	2-Ethyl-1,4-dimethylbenzene	–	–	0.0056
48	17.96	1-Hexanol	0.5090	0.2079	0.0724
49	18.78	Dimethyltrisulfide	0.1771	0.0201	0.0731
50	19.05	Nonanal	1.1640	0.0486	0.1189
51	19.39	3-Octanol	0.1114	–	0.0462

Table 1 (*Continued*)

No.	Retention time (min)	Compound	*A* (%) *T. aestivum* (Soria)	*A* (%) *T. aestivum* (Valladolid)	*A* (%) *T. melanosporum* (Soria)
52	19.79	3-Ethyl-4,5-dihydro-1H-pyrazole	–	0.0549	0.1318
53	20.89	2-Octenal	8.2730	0.2091	1.8688
54	21.51	1-Methoxy-3-methylbenzene	–	–	2.2948
55	21.85	1-Octen-3-ol	1.8082	1.1694	4.0489
56	22.09	1-Heptanol	–	0.0141	–
57	22.14	1-Methoxy-3-methylbenzene	–	–	0.1047
58	22.83	2-Furancarboxaldehyde	0.1812	–	–
59	23.00	Acetic acid	3.1710	0.1227	–
60	23.47	2-Ethyl-1-hexanol	1.3032	0.1941	0.0188
61	23.64	Decanal	1.1520	–	–
62	24.41	4-Mercapto-4-methyl-2-pentanone	–	–	0.0593
63	24.94	2-(1-Methylethyl)phenol	–	–	0.1992
64	25.21	Benzaldehyde	6.9354	0.1358	1.4436
65	27.54	Sulfinylbismethane	–	0.0469	0.0379
66	27.85	2-Undecanone	0.6202	0.0557	0.0491
67	28.26	4-Hydroxycroman	–	–	0.3824
68	30.01	2(3H)-Dihydrofuranone	4.4916	0.4278	0.2518
69	30.30	Phenylacetaldehyde	1.8540	0.1754	0.2710
70	30.86	2-Propenoic acid	1.9972	–	–
71	31.71	2-Methylhexanoic acid	–	0.0889	0.0786
72	32.01	3-Methyl-1H-pirazol	–	–	0.0689
73	32.27	2,4-Nonadienal	0.2358	0.0602	0.1088
74	33.28	1,2-Dimethoxybenzene	–	–	2.6553
75	32.40	Dodecanal	1.7074	–	–
76	33.92	Naphtalene	0.7081	0.0796	0.0442
77	34.25	1,3-Dimethoxybenzene	–	–	0.1513
78	34.73	2,4-Decadienal	–	–	0.1360
79	36.04	2,5-Dimethoxytoluene	–	–	0.1708
80	36.42	3,4-Dimethoxytoluene	–	–	0.1156
81	36.76	1-Methoxy-4-(1-propenyl)-benzene	–	–	0.0385
82	37.83	2-Methoxy-4-ethyl-6-methylphenol	–	–	0.0293
83	40.23	2,6-Bis(1,1-dimethylethyl)-4-methylphenol	3.7377	1.3151	0.5952
84	40.62	Phenylethanol	3.8257	0.4601	0.1887
85	41.24	α-Ethylidene-phenylacetaldehyde	0.6037	0.0372	0.1835
86	44.24	Phenol	1.3732	–	–
87	44.72	1,2-Dimethoxy-4-(2-propenyl)benzene	–	–	0.1645
88	46.94	1-Methyl-4-(phenylmethyl)benzene	–	–	0.0738
89	47.07	*p*-Cresol	–	–	0.0270

of volatile compounds found), therefore, being culinary and economically the most appreciated.

In the literature, there is no complete agreement about the compounds responsible for the truffle aroma impact. For example, 2-methylpropanol, 3-methylbutanol and dimethylsulfide have been described as key components of truffle aroma [18]. Also, Talou et al. [19] described dimethylsulfide and 2-methylbutanol as responsible for sulfurous and pungent notes and determine both to have a great importance on the final aroma impression. A research dealing with the truffle aroma imitation suggested, in order to obtain a truffle aroma close to the original, high levels of dimethylsulfide, 2-methylbutanal, acetaldehyde and 2-butanone, low levels of 2-methylpropanal and zero levels of acetone, 2-methyl-1-propanol, 2-methyl-1-butanol and ethanol; these last four compounds seemed to play a role in providing a higher powerfulness in the truffle aroma. Pelusio et al. [13] associates the presence of sulfur compounds to the unique aroma of white truffle.

Among the compounds detected and tentatively identified in the present work in *T. melanosporum*, the major are: 3-methylbutanal and 2-methylbutanal accounting for more than 50% of the total aroma (57%). Other compounds such as hexanal, 2-methyl-1-butanol, 2-butanol, 1-octen-3-ol, 2-butenal, 1,2-dimethoxybenzene, 2-methylbutyl-2-methylbutanoate, 1-methoxy-3-methylbenzene, 2-octenal, 6-dodecanol, benzaldehyde, 2-butanone and 2-methyl-2-butenal are also found but at lower concentrations. It is important to emphasize the lack of 2,3-butanedione, compound previously found in some black truffles of Italian origin but not described in the French ones. The fact that this compound cannot be found in the black truffles of Spanish origin could be associated to a higher resemblance of both, Spanish and French species of black truffles. Nevertheless, this possibility has to be deeply explored by an exhaustive characterization of both species by using similar extraction procedures. Another explanation for not finding 2,3-butanedione could be associated to the preservation of the truffles under freezing conditions before analysis [20].

Other compounds detected in *T. melanosporum* (Soria) and previously identified are the following: 2-methylpropyl-3-methylbutanoate, 5-methyl-2-heptanone, 2-methylbutyl-2-methylbutanoate, pentyl-3-methylbutanoate, 2,3-dihydro-4-methylfuran, 3-octen-2-one, methoxybenzene, 1-methoxy-3-methylbenzene, 1,2-dimethoxybenzene, 1,3-dimethoxybenzene, 2,5-dimethoxytoluene, 3,4-dimethoxytoluene, and *p*-cresol. In the black truffle, some other compounds have been identified for the first time such as butyl-2-methylbutanoate, 3-penten-2-one, 3-methylbutyl 2-methylpropanoate, 6-dodecanol, 3-hydroxy-2-butanone, 2-ethyl-1,4-dimethylbenzene, 4-mercapto-4-methyl-2-pentanone, 2-(1-methylethyl)phenol, 4-hydroxycroman, 3-methyl-1H-pyrazole, 2,4-decadienal, 1-methoxy-4-(1-propenyl)-benzene, 2-methoxy-4-ethyl-6-methylphenol, 1,2-dimethoxy-4-(2-propenyl)-benzene and 1-methyl-4-(phenylmethyl)benzene.

The major volatile compounds of *T. aestivum* (Soria) are the following: hexanal, 2-octenal, 2-butenal, 3-methylbutanal, benzaldehyde and heptanal, accounting for a 53% of the total area of the chromatogram (Fig. 2 and Table 1). On the other hand, the major compounds of *T. aestivum* (Valladolid) are: 2-butanone, 3-methylbutanal and 2-methylbutanal, which account for almost 90% of the total aroma. The high percentage of hexanal and heptanal found in the summer truffle from Soria could be due to lipidic oxidation occurring during preservation of truffles at low temperatures [18]. The high concentration of 2-butanone in summer truffle from Valladolid could be associated to an aged truffle, because both, 2-butanone and methoxybenzene have been previously described as responsible for the characteristic aroma of aged truffles [15].

Among the identified compounds found in the summer truffles of different geographical origin, there are some compounds that can be considered associated to the origin (factors such as growing conditions, ecology, etc.). For example, in the summer truffle from Valladolid, some compounds such as propanal, 3-methyl-3-penten-2-one, 2-octanone, 1,3,4-trimethyl-2-pirazoline, 3-ethyl-4,5-dihydro-1H-pirazol, 1-heptanol, sulfinylbismethane and 2-methylhexanoic acid could be detected while in the summer truffle of Soria other compounds such as ethyl-3-methylbutanoate, dimethyldisulfide, octanal, 2,3-octanedione, 2-heptenal, 3-octanol, 2-furancarboxaldehyde, decanal, 2-propenoic acid, dodecanal and phenol could be exclusively found.

Among the compounds that have been only detected in *T. aestivum* of Soria, and have not been cited in different truffle species, the most important are: 2-furancarboxaldehyde, 2-propenoic acid and decanal. The 2-propenoic acid have been associated to the use of pesticides, therefore, only the two other compounds could be used to discriminate among the different origins. For the summer truffle of Valladolid, the presence of 3-methyl-3-penten-2-ona could be considered as discriminator because it has not been detected previously in any other truffle species. One isomer of this compound, the 4-methyl-3-penten-2-one has been previously described in coffee [21] but no agreement can be found about its origin because some authors described its presence in coffee as a contaminant [22].

Of the above-mentioned compounds, the most characteristics of *Tuber* spp. are the sulfur compounds, being the most important, the dimethylsulfide. This compound can be found, along with dimethyltrisulfide, in the three truffles analyzed but at very low concentrations. Table 2 shows the distribution of the sulfur compounds in the three different truffles studied in the present work. The mentioned compounds, as well as the dimethyldisulfide (not found in summer truffle of Valladolid), are very volatiles and their low

Table 2
Identified sulfur compounds and relative percentages of *T. aestivum* (Soria) and (Valladolid) and *T. melanosporum* (Soria) aroma compounds extracted by HS-SPME using *A* (%) for flavors fiber

Peak no.	Retention time (min)	Compound	*A* (%) *T. aestivum* (Soria)	*A* (%) *T. aestivum* (Valladolid)	*A* (%) *T. melanosporum* (Soria)
2	3.94	Dimethylsulfide	0.2668	0.4560	0.5245
13	8.23	Dimethyldisulfide	0.0579	–	0.0437
49	18.78	Dimethyltrisulfide	0.1771	0.0201	0.0731
62	24.41	4-Mercapto-4-methyl-2-pentanone	–	–	0.0593
65	27.54	Sulfinylbismethane	–	0.0469	0.0379

concentration in the sample can be the explanation for a loss due to evaporation [13,18]. In *T. melanosporum* and *T. aestivum* (Valladolid), a sulfur compound not previously described in truffles have been found, sulfinylbismethane, this compound could be formed from dimethylsulfide. Also, another sulfur compound not previously detected in the black truffle has been found: 4-mercapto-4-methyl-2-pentanone.

As a conclusion, *T. melanosporum* has a higher content of esters and benzene derivatives than summer truffles. Among these, *T. aestivum* (Soria) has more volatiles than the one collected in Valladolid; this could be easily correlated with the intensity of the aroma. Also, it can be concluded that the significant differences found in the summer truffles aroma could be due to the different geographical areas of origin. This species (*T. aestivum* Vitt.) has a wide geographical distribution and a diverse ecology and moreover a high level of genetic variability [4] that can clearly influence the final aroma composition of the truffles.

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