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Volatile compounds from six species of truffle – head-space analysis and vapor analysis at high mass resolution

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Abstract

Head-space analysis at high mass resolution has been carried out of volatile compounds emanating from each of six species of truffle so as to identify those compounds that reveal to dogs, pigs, and flies (genus *Suillia*) the truffle's subterranean habitat. The six species of truffle examined were *T. aestivum*, *T. brumale*, *T. melanosporum*, *T. miesentericum*, *T. rufum*, and *T. simonea*. The truffle species were obtained from Ayme Truffe of Grignan, 26230 France. Of the 36 volatile compounds identified, 15 of these compounds were observed from all 6 species. The 7 alcohols identified formed a homologous series over the molecular weight range MW = 46–88, while a second homologous series over the molecular weight range MW = 74–144 was formed by the 16 esters identified. It is proposed that variation of the incidence of esters can provide a method for differentiating between the six truffle species examined. Dimethyl sulfide was identified from all species except *T. brumale*.

Keywords: Truffle volatile; Accurate mass; Head-space analysis; Mass spectrometry; Gas chromatography

1. Introduction

Truffles (Tuber spp.) are rounded, ugly and potato-shaped mushrooms with a subterranean habitat. European truffle species which are appreciated highly because of their unique and characteristic aroma are used widely in French and Italian cuisines where they are considered as prized delicacies, particularly the black Périgord truffle (*T. melanosporum*) and the summer truffle (*T. aestivum*). The taste of a truffle can be likened to a blend of garlic together with a pungent mushroom flavor. Truffles are served usually uncooked; they are shaved into foods such as pasta, pizza, omelette, and salads. The culinary value of truffles is due to their organoleptic properties which are of such high quality that these edible fungi have assumed an appreciable economic value.

Truffles are the fruiting bodies of mychorrhizal fungi associated principally with the roots of oak trees in forests and oak plantations. Trained pigs and dogs through their ability to detect and recognize the volatile aromatic chemicals produced by the truffles determine the underground locations of truffles. The locations of truffles can be detected also by observing those locations where the flies (genus *Suillia*) hover; they lay eggs on the ground above truffles that, in turn, provide food for the larvae [1]. While pigs have the keener nose for truffles, they tend to eat the truffles they detect, they tire quickly and are difficult to transport; thus, dogs are preferred because they can detect truffles from 30 to 50 m and have little appetite for mushrooms. No race of dog looks instinctively for truffles; while several types of dog can be trained to look for truffles, hunting dogs are not used because of their primary interest in finding game [1].

In 1981, Claus et al. [2] demonstrated the presence, at 40–60 ng/g in black truffles, of a steroidal pheromone having a musk odor. It was shown later in field studies [1] that buried samples of the pheromone solution at a concentration 10 times that in fresh truffles remained undetected. In the light of this somewhat inconsequential debut to the identification of truffle aromas, it should be borne in mind that Bertault et al. [3] have suggested that environmental variation rather than genetic factors may explain the organoleptic differences in black truffles (*T. melanosporum*) observed over a geographical area. Thus,

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the country of truffle origin should be borne in mind in the comparison of analyses of volatile organic compounds emanating from truffles. Such analysis has been carried out using a variety of methods.

Gas chromatography (GC) has been combined with dynamic head-space concentration and coupled with each of mass spectrometry (MS) and olfactometry; in this latter case, analysis was carried out on freshly harvested truffles and the odorous effluent from a gas chromatographic column was assessed by experts according to an olfactory reference. More than 50 volatile components were identified in black truffles [4] for which less than 9 are reported by human experts to be key-flavor compounds. Dimethyl sulfide appeared to be the key-odor compound for truffle localization by truffle hunting animals [1] but two other sulfurous, and three C_8 compounds were reported to be attractive for truffle flies [5]. Head-space analysis was applied to both whole fresh truffles and to stored truffle aroma [6–8] and to canned truffles [9].

Dynamic head-space coupled with GC/MS [10,11] and purge-and-trap GC/MS [12,13] have been used for the identification of volatile compounds from black Périgord truffle from France and Italian white truffle. The detection of volatile sulfur compounds emanating from Spanish white (*T. magnatum* Pico) and black (*T. melanosporum*) truffles has been carried out using head-space solid-phase microextraction (HS-SPME) combined with GC/MS analysis [14–16]. Recently, the HS-SPME technique combined with ion trap mass spectrometry has been applied to the analysis of volatile organic compounds from six species of Italian truffles, both black and white [17]. Some 36 volatile organic compounds, consisting of alkanes, alcohols, esters, aldehydes, ketones, terpenes, etc., of widely ranging polarity and molecular weight were identified for *T. magnatum*, 29 for *T. borchii*, 34 for *T. driophyllum*, 46 for *T. brumale*, 40 for *T. miesentericum*, and 66 for *T. aestivum*. However, the individual compounds were not reported; rather the average mass spectrum for an entire ion chromatogram acquired over a period in excess of 50 min represented a fingerprint for a given species. The six resulting fingerprints were subjected to stepwise factorial discrimination analysis leading to the successful identification of truffle species.

Vapor and head-space analyses of the volatile compounds emitted by six species of French truffles have been carried out in the present study. The Tuber species of truffle examined were *T. aestivum*, *T. brumale*, *T. melanosporum*, *T. miesentericum* (aka American truffles), *T. rufum*, and *T. simonea*; the truffle samples were obtained from Ayme Truffe of Grignan, 26230 France. The dogs used by Ayme Truffe appear to detect each of the six truffle species with equal facility. One sample of each of six truffles was obtained from Ayme Truffe in August 2004; the truffle species were identified by M. Ayme. Each sample was sealed hermetically in a plastic envelope at source and was stored subsequently in a freezer at about −7 °C. The samples were transported in an ice-cooled container. The samples were examined in January 2005.

It should be noted that the results presented here are somewhat preliminary. With the proven analytical technique, the investigation should be extended to an exploration of the impact of the different stages of truffle maturity, truffle condition, state of hydration, storage, etc., on the volatility profiles of the truffles examined here. In addition, the investigation should be extended to an exploration of the differences among the volatile organic compounds from each species of truffle over a geographical area.

2. Experimental methods

Head-space analysis was carried out for each of six species of truffle using pressure balanced head-space sampling (Turbomatrix, Perkin-Elmer, Beaconsfield, UK) and a GC/TOF-MS instrument (ThermoElectron, Hemel Hempstead, UK). The samples were equilibrated for 10 min at 80 °C. The injection was carried out over 6 s at 14 psi and an inlet temperature of 180 °C. A helium carrier gas was used at a constant flow of 1.5 mL/min with a DB-FFAP column 30 m × 0.32 mm i.d. × 0.5 μm film thickness. The oven temperature profile was 40 °C for the first 3 min, rising to 130 °C at the rate of 10 °C/min and then to 250 °C at 30 °C/min and held for 1 min. In addition, the vapor surrounding each species sealed hermetically in a blister pack was sampled using a gas-tight syringe. Analysis was performed on a GC/TOF-MS instrument (Micromass, Manchester, UK) with high mass resolution. Separation of vapor components was achieved using a helium carrier gas at a constant flow of 1 mL/min on a ZB-5 column 30 m × 0.25 mm i.d. × 0.25 μm film thickness. The inlet (Optic 3, ATLAS GL, Veldoven, The Netherlands) was maintained at 200 °C. The oven temperature profile was 40 °C for the first 3 min, rising to 120 °C at the rate of 10 °C/min and then to 250 °C at 30 °C/min and held for 1 min. Mass spectra were acquired in the presence of a perfluorokerosene calibrant, with the mass scale locked on 218.9856 Th.

3. Discussion of results

The observation of numerous volatile compounds from gas chromatographic/mass spectrometric examination of each of the six species of truffle in turn is seen clearly from the total ion chromatogram (TIC) obtained for each sample. Six TICs are shown as insets in Fig. 1a–f, one for each of *T. aestivum*, *T. brumale*, *T. melanosporum*, *T. miesentericum*, *T. rufum*, and *T. simonea*, respectively. While the duration of each gas chromatographic run was varied in the range 17–25 min, the TICs shown in insets in Fig. 1 depict the range of elution time from 1 to 7.5 min. The total ion signal intensity of each TIC was calculated as the sum of the ion signal intensity at the peak maxima of the identified peaks. Each TIC is set as an inset to the summed mass spectrum obtained at high mass resolution for the corresponding TIC acquired over a period of 7.5 min. This procedure is similar to that reported in reference [17]. The summed mass spectra could be subjected to stepwise factorial discrimination analysis [17] but this procedure was not carried out.

From the sum of mass spectra observed throughout a single peak in the TIC was subtracted the background in the immediate vicinity of the peak; the resulting mass spectrum was compared with NIST webBook mass spectra (http://webBook.nist.gov/chemistry) and/or with reference mass spectra. In Fig. 2 is demonstrated the process for identification of a volatile com-

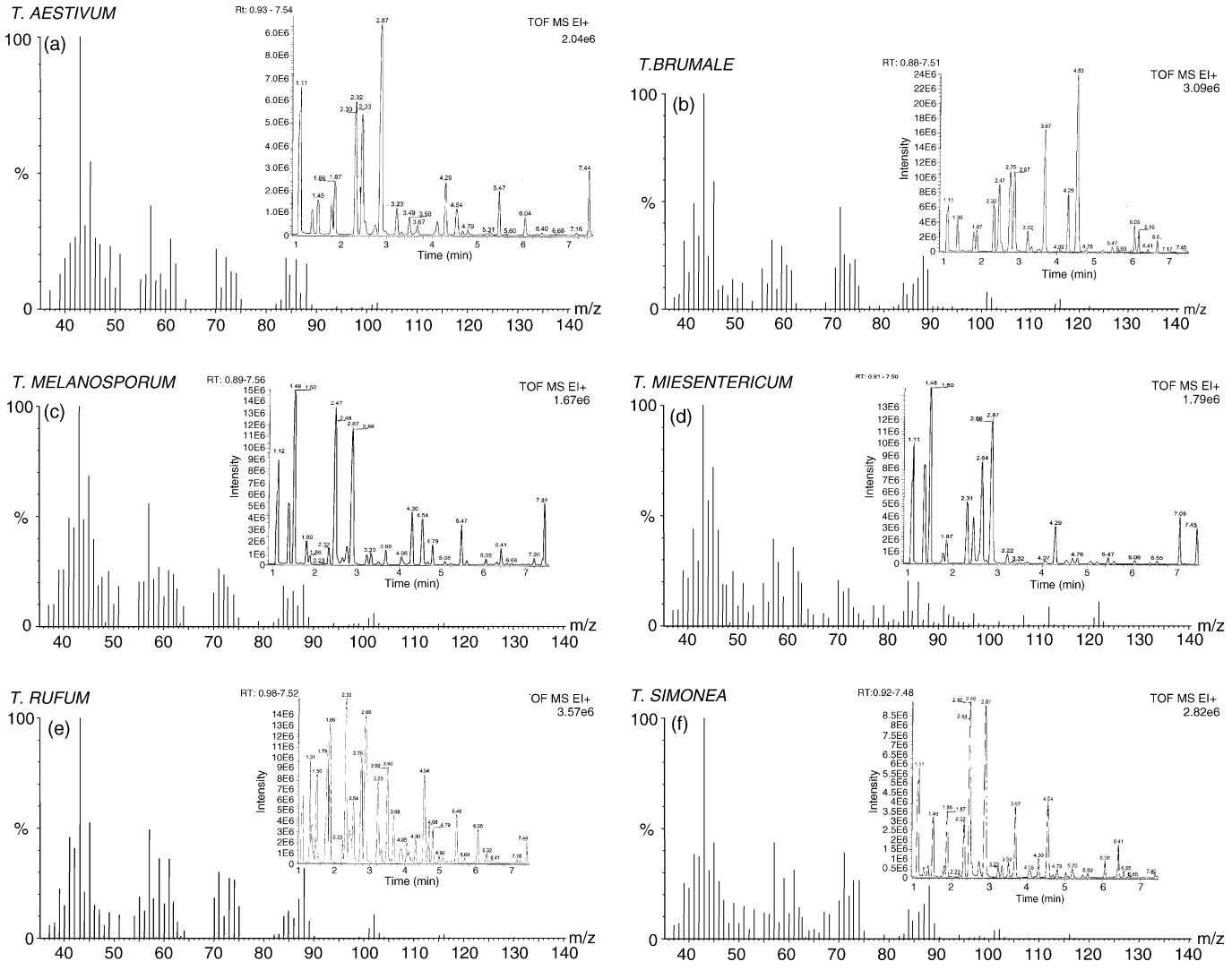

Fig. 1. The summed mass spectrum obtained at high mass resolution for the corresponding total ion chromatogram acquired over a period of 1–7.5 min; the corresponding total ion chromatogram is shown in inset. Summed mass spectra and total ion chromatograms of volatile compounds for six samples of truffle: (a) aestivum (4.5×10^7), (b) brumale (11×10^7), (c) melanosporum (8.2×10^7), (d) miesentericum (8.1×10^7), (e) rufum (15×10^7), and (f) simonea (4.9×10^7). Each total ion chromatogram corresponds to the elution period 1–7.5 min. The total ion signal intensity of those peaks that contributed ≥1% of the sum of peak maxima is given in square parentheses for each truffle species.

pound. The raw data are shown in Fig. 2a together with a library entry in Fig. 2b; the difference between the raw data and the library entry is shown in Fig. 2c and the structure of pentanoic acid, 4-methyl, ethyl ester is shown in Fig. 2d. The SI (a direct matching factor, matching an unknown to the library mass spectrum, i.e., are the peaks in the unknown compound present in the library mass spectrum?) and RSI (a reverse search matching factor, matching the library mass spectrum with that of the unknown compound, i.e., are the peaks in the library mass spectrum present in that of the unknown?) values for the identification of pentanoic acid, 4-methyl, ethyl ester are 887 and 902, respectively. Generally, the SI values ranged from 600 to 960 except for carbon dioxide **1** for which SI = 559 and 2H-1-benzopyran-2-one, 7-[(3,7-dimethyl-2,6-octadienyl)oxy]- **36** for which SI = 436. The respective RSI values were 922 and 855. Generally, the RSI values ranged from 819 to 999. A perfect fit would have a score of 1000. Major peaks of the observed mass spectra were obtained at high mass resolution (7000 FWHM at *m*/*z* 614) for verification of compound identity. An example is shown in Fig. 3 wherein the base peak of the upper mass spectrum was determined as *m*/*z* 88.0551. The lower mass spectrum is the NIST mass spectrum for pentanoic acid, 4-methyl, ethyl ester. The raw data for the experimental mass spectrum agree well with the NIST mass spectrum for pentanoic acid, 4-methyl, ethyl ester, $C_8H_{16}O_2$, (the lower mass spectrum in Fig. 3). If the base peak is due to the loss of C_4H_8 from pentanoic acid, 4-methyl, ethyl ester, the calculated mass/charge ratio for the product ion $C_4H_8O_2^+$ is 88.0525 leading to a mass defect of +2.6 mDa. Thus, this compound was identified as pentanoic acid, 4-methyl, ethyl ester.

The compounds identified for each species of truffle are listed in Table 1. The total number of compounds detected was 36. In a given TIC, summed mass spectra were examined for only those peaks that contributed ≥0.1% of the sum of peak maxima. How-

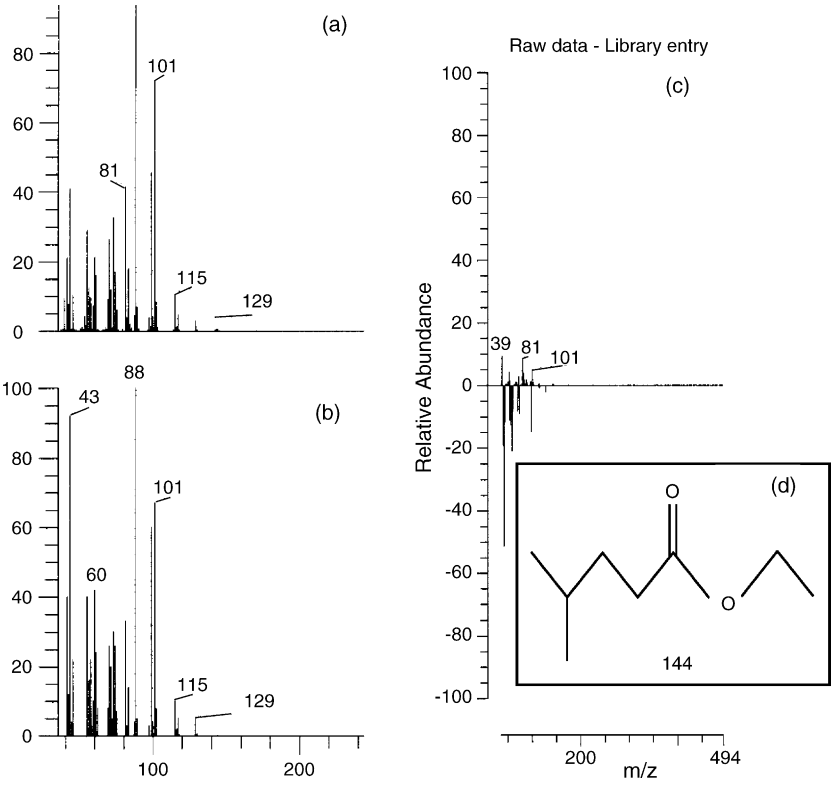

Fig. 2. Identification of pentanoic acid, 4-methyl, ethyl ester: (a) experimentally observed average of 10 mass spectra obtained over the retention time 7.18–7.21 min and background subtracted; (b) library mass spectrum of pentanoic acid, 4-methyl, ethyl ester; (c) raw data—library entry; and (d) structure of pentanoic acid, 4-methyl, ethyl ester.

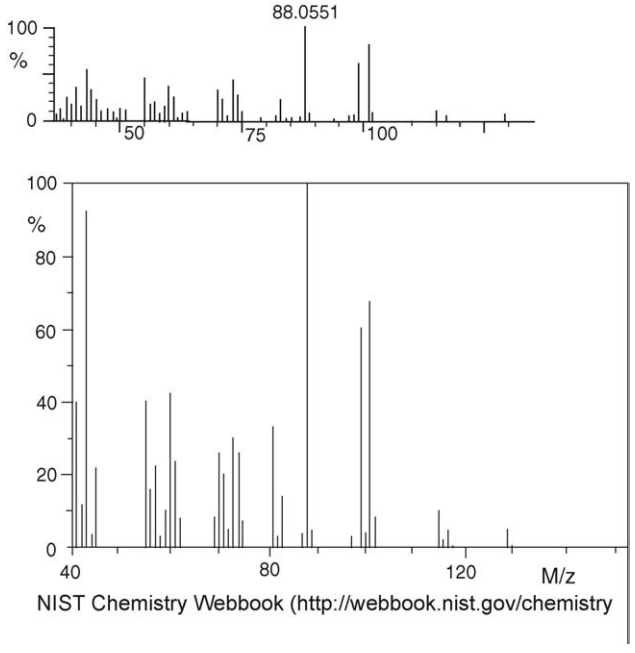

Fig. 3. High mass resolution identification of pentanoic acid, 4-methyl, ethyl ester: upper, experimentally observed high mass resolution average of 10 mass spectra obtained over the retention time 7.18–7.21 min, background subtracted, base peak observed at *m/z* 88.0551; lower, NIST webBook mass spectrum for pentanoic acid, 4-methyl, ethyl ester.

ever, when the temporal variation of ion signal intensity of a low intensity (ion count $<10^5$) peak matched closely that of an identified peak of higher intensity and the signal-to-noise ratio of the low intensity peak was ≥ 3, the two peaks were assumed to be due to the single identified compound. In such cases, the identified compound was listed in Table 1 as being present in trace amounts. All six species examined exhibited 19–25 compounds each with *T. aestivum* exhibiting the lowest number (19) and *T. rufum* exhibiting the highest number (25). On the basis of total ion count, *T. aestivum* showed the lowest ion signal intensity while the ion count for *T. rufum*, which showed the highest ion count, was some 3.3 times that of *T. aestivum*.

The eight most common identified compounds exhibited by all species examined and based on the sum of relative intensities were, in order of decreasing total ion intensity, ethanol **15**, carbon dioxide **1**, 2-butanone **10**, ethyl acetate **8**, acetaldehyde **3**, butanoic acid, methyl ester **18**, acetic acid, methyl ester **6**, and 2-butanol **21**.

Listed in Table 2 are those compounds that are unique to one of the six species of truffle. A compound is defined as being unique to a given species of truffle when there was either no detectable signal from the other species of truffle or the signal detected had a signal/noise ratio < 3. According to Pierre Ayme from whom the truffle samples were obtained, his dogs do not differentiate between species of truffle in that they detect all six of the species examined here. However, because the study of truffle aroma has been suggested as a means of authentication of the various truffle species [11], a compound unique to such

Table 1
Identified compounds listed in order of increasing retention time for six truffle species (*T. aestivum*, *T. brumale*, *T. melanosporum*, *T. miesentericum*, *T. rufum*, and *T. simonea*); the percentage relative intensity is given for each GC peak

No.	RT	Compound	Cas #	*T. aestivum*	*T. brumale*	*T. melanosporum*	*T. miesentericum*	*T. rufum*	*T. simonea*
1	1.12	Carbon dioxide	124-38-9	14.1	5.76	11.1	11.7	4.13	11.2
2	1.24	1,3-Pentadiene	504-60-9	–[a]	–	–	–	–	1.16
3	1.36	Acetaldehyde	75-07-0	2.35	3.63	6.18	9.65	6.26	1.35
4	1.49	Dimethyl sulfide	75-18-3	3.63	–	17.5	17.0	5.42	6.17
5	1.80	Acetone (2-propanone)	67-64-1	3.20	2.30	2.33	0.93	6.65	0.96
6	1.86	Acetic acid, methyl ester	79-20-9	5.12	2.75	0.82	2.44	8.58	6.94
7	2.23	Acetic acid, propyl ester	109-60-4	–	–	–	–	1.29	–
8	2.32	Acetic acid, ethyl ester	141-78-6	12.8	5.76	1.75	6.05	10.1	5.78
9	2.43	Acetic acid, 1-methylethyl ester	108-21-4	–	–	–	–	2.06	–
10	2.47	2-Butanone	78-93-3	11.5	8.24	15.6	4.65	1.55	17.9
11	2.54	Propanoic acid, methyl ester	544-12-1	–	–	–	–	3.87	–
12	2.64	Butanal, 3-methyl-	590-86-3	–	–	–	9.88	–	–
13	2.72	Propanoic acid, 2-methyl, methyl ester	547-63-7	–	–	–	–	–	1.73
14	2.75	Isopropyl alcohol	67-63-0	–	9.84	1.75	–	6.65	–
15	2.87	Ethanol	64-17-5	20.3	9.84	13.5	14.1	9.03	17.5
16	3.23	Propanoic acid, ethyl ester	105-37-3	2.77	2.48	1.05	2.21	5.03	0.96
17	3.50	4-Hydroxy-3-methyl-2-butanone	9006-26-2	1.71	0.35	0.41	tr[b]	5.94	1.73
18	3.68	Butanoic acid, methyl ester	623-42-7	0.96	14.6	1.52	0.12	3.03	7.13
19	3.86	Acetic acid, butyl ester	123-86-4	–	–	–	–	0.90	–
20	4.05	Butanoic acid, 2-methyl-, methyl ester	868-57-5	–	–	0.82	0.35	1.29	0.77
21	4.30	2-Butanol	78-92-2	5.34	4.52	5.25	3.72	1.61	2.12
22	4.53	Butanoic acid, ethyl ester	105-54-4	–	21.3	–	–	5.42	7.90
23	4.54	1-Propanol	71-23-8	2.56	-	4.66	0.35	-	-
24	4.68	Propanoic acid, propyl ester	106-36-5	0.43	0.22	0.35	0.58	2.20	0.39
25	4.79	Butanoic acid, 2-methyl-, ethyl ester	7452-79-1	0.53	0.27	1.98	0.64	2.26	0.87
26	5.46	Ethenamine, *N*-methylene-	38239-27-9	–	–	–	–	3.03	-
27	5.47	1-Propanol, 2-methyl-	78-83-1	4.48	0.62	4.08	0.58	–	0.29
28	6.04	Acetic acid, 2-methylbutyl ester	624-41-9	1.71	–	–	–	–	-
29	6.06	Butanoic acid, propyl ester	105-66-8	–	3.46	0.58	0.23	2.19	1.93
30	6.16	Butanoic acid, 1-methylpropyl ester	819-97-6	–	2.75	tr	–	tr	tr
31	6.41	1-Butanol	71-36-3	0.32	0.35	1.75	0.17	0.13	3.47
32	7.06	2-Pentene, 3-ethyl-2-methyl-	19780-67-7	–	–	0.12	4.65	–	–
33	7.20	Pentanoic acid, 4-methyl-, ethyl ester	25415-67-2	–	–	0.70	–	–	–
34	7.44	1-Butanol, 2-methyl-	137-32-6	6.19	0.27	6.18	3.49	1.42	tr
35	11.08	Benzene, 1-methoxy-3-methyl-	100-84-5	–	0.71	–	6.51	–	–
36	16.30	2H-1-benzopyran-2-one, 7-[(3,7-dimethyl-2,6-octadienyl)oxy]-	495-02-3	–	–	–	–	–	1.73

[a] Signal/noise ratio < 3.
[b] Signal/noise ratio > 3, but ion count < 10^5.

a valued species as *T. melanosporum* may provide a method for distinguishing this species from, for example, *T. indicum* which has a similar carpophore morphology and spore shape [18,19] but with a lower aroma content such that it commands a lower price than does *T. melanosporum*.

It is significant that the alcohols and esters identified here each form a homologous series as shown in Figs. 4 and 5, respectively. In each of these figures, the identifying number of an alcohol or ester from Table 1 is given immediately above the structure of the compound; below the structure is given the compound name followed by its molecular weight in parentheses. The alcohols observed were ethanol **15**, the 1- and 2-propanols **23** and **14**, respectively, the three butanol isomers **27**, **31**, and **21** together with one C_5-alcohol identified as 1-butanol, 2-methyl **34**. It is reasonable to assume that the sequentially synthesised homologous series of seven alcohols can be oxidised to the corresponding aldehydes, ketones, and acids. The aldehydes observed here were acetaldehyde **3** and butanal, 3-methyl **12**, while the ketones observed were acetone **5** and 2-butanone **10**. The acids formed will then react with the initial alcohols to produce a homologous series of esters of some 16 esters such as that shown in Fig. 5.

With respect to the distribution of the alcohols observed among the six species of truffle, ethanol **15**, the three butanol isomers **27**, **31**, and **21,** and 1-butanol, 2-methyl **34** were common to all species; the sole exception being the lack of 1-propanol, 2-methyl **27** in *T. rufum*. However, 1-propanol **23** was observed only from *T. aestivum*, *T. brumale*, *T. melanosporum*, and *T.*

Table 2
Identified compounds that are unique[a] for a given species of truffle

Truffle	Compound	No.[b]
T. aestivum	Acetic acid, 2-methylbutyl ester	28
T. brumale	Butanoic acid, 1-methylpropyl ester	30
T. melanosporum	Pentanoic acid, 4-methyl-, ethyl ester	33
T. miesentericum	Butanal, 3-methyl-	12
T. rufum	Acetic acid, propyl ester	7
	Acetic acid, 1-methylethyl ester	9
	Propanoic acid, methyl ester	11
	Acetic acid, butyl ester	19
	Ethenamine, *N*-methylene-	26
T. simonea	1,3-Pentadiene	2
	Propanoic acid, 2-methyl, methyl ester	13
	2H-1-Benzopyran-2-one, 7-[(3,7-dimethyl-2,6-octadienyl)oxy]-	36

[a] A compound is defined as being unique to a given species of truffle when there was either no detectable signal from the other species of truffle or the signal detected had a signal/noise ratio < 3.
[b] The number given to each compound as shown in Table 1.

miesentericum, while 2-propanol **14** was observed only from *T. brumale*, *T. melanosporum*, and *T. rufum*; neither 1-propanol **23** nor 2-propanol **14** was observed from *T. simonea*. With respect to the distribution of aldehydes and ketones observed among the six species of truffle, butanal, 3-methyl **12** was observed only from *T. miesentericum*, while acetaldehyde **3** and the ketones acetone **5** and 2-butanone **10** were observed in all cases.

While the variation in the distribution of esters among the truffle species examined affords the opportunity for differentiating between species, as shown in Table 2, 7 of the 16 esters identified were observed from all 6 species.

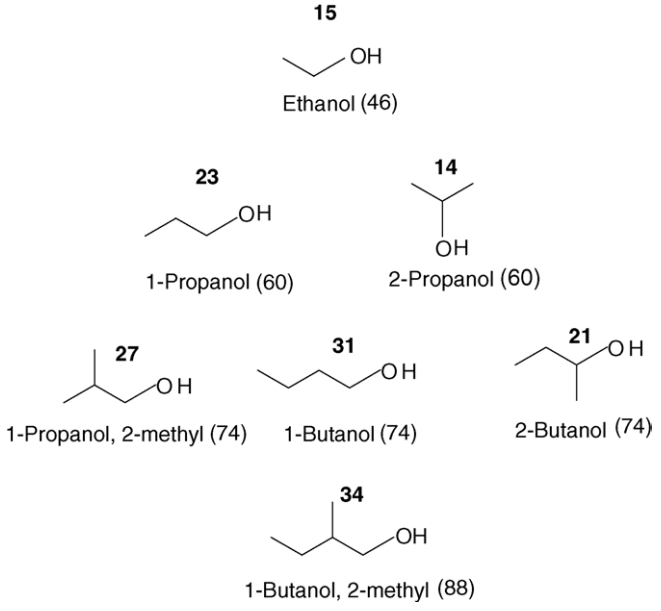

Fig. 4. Homologous series of alcohols identified from the truffle samples. The identifying number, from Table 1, for each alcohol is given immediately above the structure of the compound; below the structure is given the compound name followed by its molecular weight in parentheses.

In the HS-SPME study carried out by Diaz et al. [15] of *T. melanosporum* (from Soria, Spain) and of *T. aestivum* (from Valladolid and Soria, Spain), a total of 89 compounds that eluted over a retention time range of 3.74–47.07 min were identified. For *T. aestivum* from Soria, 23 of the total 47 compounds eluted in the first 11.13 min of observation and accounted for 51.5% of the total volatile sample; that is, approximately half of the total sample (and half the number of compounds) eluted in the first 11.13 min of observation. The corresponding observations for *T. aestivum* from Valladolid were 26/41 compounds that accounted for 97.0% of the total volatile sample, whereas for *T. melanosporum* from Soria, 29/72 compounds accounted for 81.9% of the total volatile sample. These results show clearly a significant influence of geographical location on the composition of truffle volatiles. Nevertheless, let us compare some of the results of Diaz et al. [15] for truffles from Spain with the results reported here for truffles from France.

The following eight compounds were identified both in the study by Diaz et al. [15] and in this work: acetaldehyde, **3**; dimethyl sulfide, **4**; 2-propanone, **5**; acetic acid, ethyl ester, **8**; 2-butanone, **10**; butanal, 3-methyl, **12**; 2-butanol, **21**; and 1-propanol, 2-methyl, **27** were identified. Yet these eight compounds constituted a significant, if not major, fraction of total truffle volatiles: for each species of truffle, the fractions were *T. aestivum* (Soria, Spain) 12.1%; *T. aestivum* (Valladolid, Spain) 71.7%; *T. melanosporum* (Soria) 40.7%; *T. aestivum* (Fr.) 43.3%; *T. brumale* (Fr.) 25.1%; *T. melanosporum* (Fr.) 53.4%; *T. miesentericum* (Fr.) 52.5%; *T. rufum* (Fr.) 31.6%; and *T. simonea* (Fr.) 34.6%. Trace amounts of butanoic acid, 3-methyl-, ethyl ester were found only in the two species from Soria in Spain; however, butanoic acid, 3-methyl-, ethyl ester was identified in all six truffles species from France.

Four of the above eight compounds, acetaldehyde, **3**; dimethyl sulfide, **4**; 2-propanone, **5**; 2-butanone, **10**; and 1-propanol, 2-methyl, **27**, together with ethanol, **15**, have been detected previously in black truffles (*T. melanosporum*) from France [1]. In a field study [1], only those buried solutions containing dimethyl sulphide, **4**, were located by dogs and pig with good reproducibility. In this work, dimethyl sulphide, **4**, was detected in five from five of the six truffle species where the percentage relative intensity for dimethyl sulphide, **4**, varied from 3.63 to 17.5%; dimethyl sulphide was not detected from *T. brumale*.

It is of interest to compare the quantities of 2-butanone, **10**, butanal, 2-methyl, and butanal, 3-methyl, **12**, found in truffles from Spain [15] with those found in this work from truffles from France. The first major difference is that butanal, 2-methyl was not detected in any of the six species of truffle from France. For *T. aestivum* (Soria, Spain), the percentage relative intensities of the three selected compounds were 2.2, 0.6, and 7.6%, respectively, while those for *T. aestivum* (Valladolid, Spain) were 38.2, 10.4, and 32.1%, respectively, and those for *T. melanosporum* (Soria) were 1.4, 19.1, and 38.3%, respectively. The range in percentage relative intensity is appreciable such that the sum of percentage relative intensities ranged from 10.4 to 80.7% for the two *T. aestivum* truffles from Spain. For the truffles from France, 2-butanone, **10**, was detected from each species and the range in

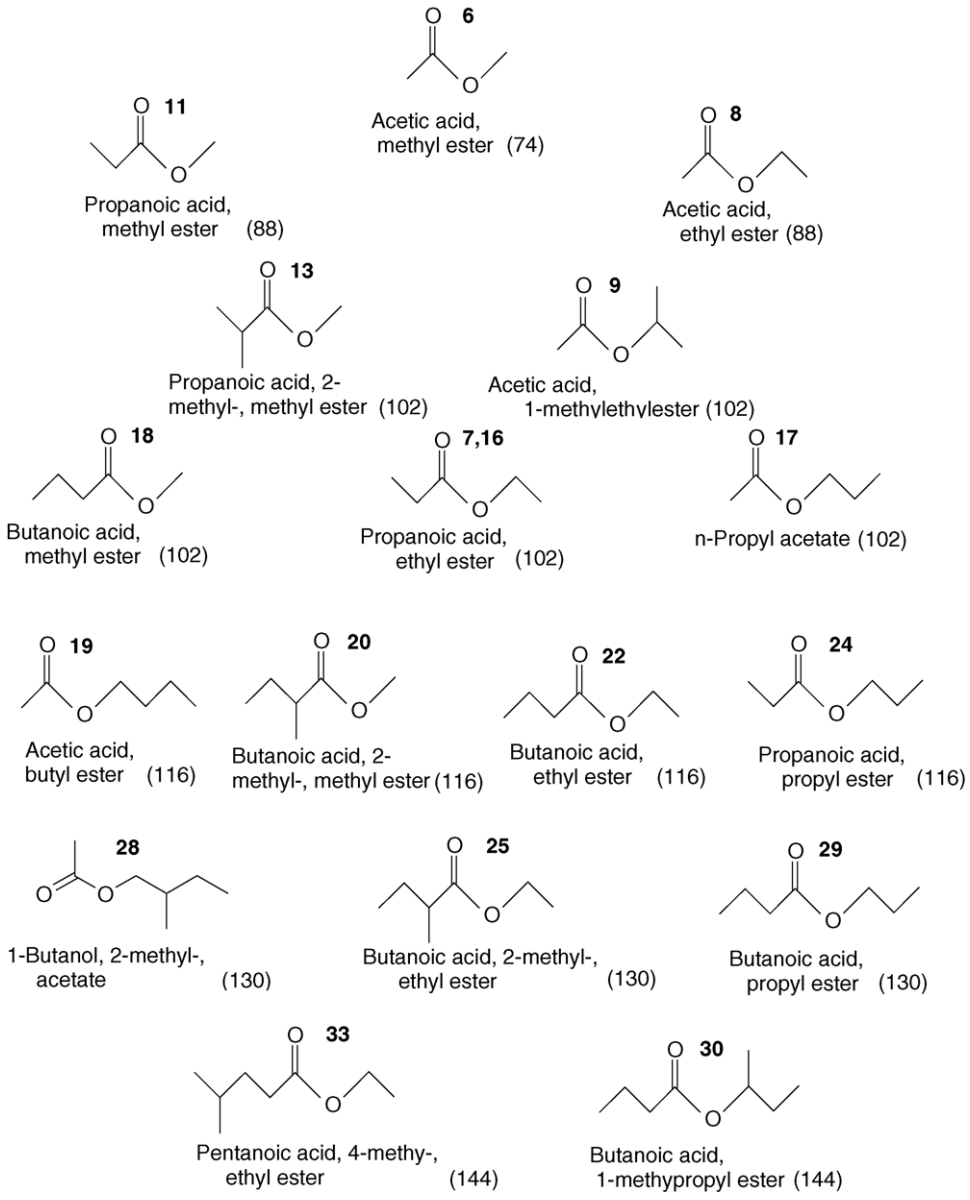

Fig. 5. Homologous series of esters identified from the truffle samples. The identifying number, from Table 1, for each ester is given immediately above the structure of the compound; below the structure is given the compound name followed by its molecular weight in parentheses.

percentage relative intensity was 1.6–17.9%; butanal, 2-methyl was not detected (as stated above); and butanal, 3-methyl, **12**, was detected only from *T. miesentericum* to the extent of 9.9%.

The presence of 2,3-butadione has been reported [12] previously in some black truffles of Italian origin but not in black truffles of French origin. 2,3-butadione was not found either in the study of truffles of Spanish origin carried out by Diaz et al. [15] or in this work on the study of truffles of French origin.

It is remarkable that the volatile fractions of the six species of truffle from France are characterized by an appreciable number of esters (17/36 compounds), more so than has been reported previously, and that six esters (acetic acid, methyl ester **6**; acetic acid, ethyl ester **8**; propanoic acid, ethyl ester **16**; butanoic acid, methyl ester **18**; propanoic acid, propyl ester **24**; and butanoic acid, 2-methyl-, ethyl ester **25**) are common to all six species examined. In addition, several alcohols (isopropyl alcohol **14**; ethanol **15**; 2-butanol **21**; 1-propanol **23**; and 1-butanol **31**) were identified.

4. Conclusions

The methods of head-space analysis and vapor analysis at high mass resolution have permitted the identification of a total of 36 compounds in the volatile fractions from 6 species of truffle from France. The form of each total ion chromatograph differentiates the six species of truffle examined without reference to any identified compound. Further differentiation of truffle species on the basis of presence/absence of identified compounds and their percentage relative intensities can be made once the compounds have been identified by their mass spectra at high mass resolution. The truffle species examined are characterized by a homologous series of esters and, to a lesser degree, by a homol-

ogous series of alcohols. Identification of a number of alcohols was not unexpected but the detection and identification of such a numerous and homologous series of esters has not been reported previously.

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