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Analytical Methods

Composition of commercial truffle flavored oils with GC–MS analysis and discrimination with an electronic nose

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ABSTRACT

Truffles are among the most expensive foods and their quality depends on their unique aroma, composed of complex mixtures of lipophilic volatile organic compounds (VOCs). There are many foods flavored with truffle, and oils are particularly common. Using DHS–GC–MS and an electronic nose (MOS), 18 samples of olive oil flavored with white and black truffles from the Italian market were subjected to a blind analysis. Qualitative and quantitative analysis with DHS–GC–MS detected the presence of 63 VOCs, 32 of which can be attributed to olive oil, also defective, and 19 to truffles, while 12 foreign compounds are of dubious origin (synthesis and/or demolition). The data obtained with the electronic nose (MOS), processed statistically, was able to discriminate the aromas coincident with the three species of truffle declared on the label (the white truffle *Tuber magnatum* and the black truffles *Tuber melanosporum* and *Tuber aestivum*), demonstrating the potential and reliability of this technique, confirming the established malpractice of the use of bismethyl(dithio)methane in black truffles flavorings.

1. Introduction

Truffles are subterranean fruiting bodies produced by some species of fungi of the genus *Tuber* (Ascomycota, Pezizales); in nature they must be eaten by animals to disperse their spores. For this reason, when ripe, intense aromas develop to attract animals (Pacioni, Bologna, & Laurenzi, 1991). The composition of these aromas is very complex, and there are different metabolic pathways involved in their production as has been clarified by studying the genome of *Tuber melanosporum* Vittad. (Martin et al., 2010).

Some species of truffles are among the most expensive foods available and owe their value precisely to the complex emanating aroma, even if today, unlike in the past, due to their high price, truffles are usually used as a flavoring rather than as food.

The scent of truffles for flavoring has been the subject of numerous investigations and the subject of patents since the beginning of the last century (Morel-Lautier, 1904, see References S Patent), when it was offered as an olive oil flavored with black truffle (*T. melanosporum*). Later, thanks to a combined gas chromatograph mass spectrometer (GC–MS) analysis of the headspace Fiecchi, Kienle, Scala, and Cabella (1967), were able to identify bis(methylthio)methane (BMDTM), the volatile compound responsible for the smell impact of white truffles (*Tuber magnatum* Pico). This molecule, a thioether also known as 2.4-ditiopenthane, was easily available as it was derived from the oil industry, used in organic syntheses and as a solvent. Because of its low-cost, highly effective olfactory, solubility and stability and persistence, low toxicity, it was used immediately as "natural flavoring", according the European regulation, for the production of "truffle" oil and various flavored food products. This compound, in fact, although correctly identified as the characteristic aroma of white truffle (*T. magnatum*), was used in abnormal amounts to strengthen the aroma of other truffle species, such as *T. melanosporum* and *Tuber aestivum* Vittad., which are characterised by completely different odours.

Also subjected to various investigations by the Technical Committee Joint FAO/WHO, it was finally validated as a food additive (IPCS INCHEM 1999 – Technical Fiche JECFA n.533).

Based on the results of improved analytical techniques for volatile organic compounds (VOCs), a series of investigations followed that have continued to characterise the flavor profile not only of white truffle, but of other species of truffle as well.

Particularly studied are the most important species in terms of commercial value: white truffle (*T. magnatum*), black truffle (*T. melanosporum*), and summer truffle (*T. aestivum*) (see Tables 1 and 1S).

From these investigations, directly or indirectly, the chemical additives were also more similar to the actual composition of the aroma of both white (*T. magnatum*) and black truffle (*T. melanosporum*); such additives are often protected by patents (see References S Patent).

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In addition to oils, truffle flavorings have found applications in a range of innovative food products such as chocolate, honey, and salt, as well as butter, pasta, salted meats, cheeses, liqueurs, and pickles.

In parallel with the formulation of widely-used synthetic flavorings, a range of truffle oils obtained in a totally natural manner, often using patented techniques, has also been developed for Asian truffles (see References S Patent). However, the composition of the VOCs of these natural products is most likely not very constant because of the variability of aroma shown by individual truffles (Mauriello, Marino, D'Auria, Cerone, & Rana, 2004).

However, these differences are not always perceived by the human sense of smell, and in fact humans are exceptionally sensitive to some volatiles, but insensitive to many others (Leffingwell & Associates, 2009; Morales, Luna, & Aparicio, 2005). A person's ability to detect odours is also influenced by several other factors (genetic variability, olfactory fatigue, and ambient conditions). For this reason, considerable interest exists in the development of alternative instrumental techniques (non-invasive and non-destructive) to allow more objective, faster, and less expensive assessments of the sensory quality of olive oil products (Sinelli, Cerretani, Di Egidio, Bendini, & Casiraghi, 2010). Metal oxide semiconductor (MOS) sensors for electronic noses are largely applied to food (Berna, 2010; Servili et al., 2008) and recently MOS sensors have been used to study aroma in virgin olive oil (VOO) (García-González & Aparicio, 2010) to monitor on-line the accumulation of volatile compounds in the head space malaxer chamber during malaxation (Esposto et al., 2009), to detect oxidative status evolution (Lerma-García, Simó-Alfonso, Bendini, & Cerretani, 2009), evaluate a variety of sensory defects in accordance with trained panellists (Lerma-García et al., 2010), and to authenticate VOOs according to varietal or geographical origin of olive fruits (Tena, Lazzez, Aparicio-Ruiz, & García-González, 2007). These MOS sensors do not provide a quali-quantitative analysis of volatile compounds of samples, but respond to the entire set of volatiles in a unique digital pattern. These patterns represent a signature of the particular set of aromatic compounds as these should determine a specific olfactory perception (Zhang, Chang, Wang, & Ye, 2008).

Considering the analysis of oils and foods flavored with truffles, there is virtually no research to date, although the subject has been brought to the attention of the competent offices and public opinion on the use of these synthetic flavorings, often in exaggerated quantities (http://www.nytimes.com/2007/05/16/dining/16truf.html?pagewanted=all).

For this reason, we considered it useful to examine, in terms of qualitative and quantitative composition, oils flavored with truffles, a product widely present in the Italian market and strongly exported in foreign markets, but also produced in other European countries and beyond, including USA (http://whatscookingamerica.net/Vegetables/TruffleOil.htm).

The purpose was to ascertain, using advanced analytical techniques (DHS–GC–MS and electronic olfactory system), the composition of some of the most representative products of the current commercial products and check the reliability of analytical systems in discriminating the quality of flavorings.

2. Experimental

2.1. Samples

We analysed 14 commercially-available oils flavored with truffles. Samples were numbered consecutively in an indiscriminate manner for blind analysis. Each sample was divided into two aliquots of 50 ml each, which were analysed with GC–MS and EOS. On the basis of labels, samples TO1, TO4, TO6, TO10, TO11, TO12 and TO13 were flavored with the essence of *T. magnatum*; TO5, TO7, TO9 with *T. melanosporum*; TO2 and TO8 with *T. aestivum*; sample TO3 marked "Tartufo" ("Truffle") did not specify any species of truffle, while sample TO14 was flavored with a sauce of *T. melanosporum*.

2.2. Headspace sampling

The headspace sampling technique used was the same described by Barcarolo and Casson (1997). Seven g of sample was weighed into a 10 ml vial. An internal standard (isooctane, 0.02 µl, Merck) was added to the sample. Vials were sealed with an aluminium–rubber septum (Supelco Inc., Bellefonte, PA, USA) and kept at 35 °C for 30 min before analysis. The sample was purged by bubbling helium: stripping was carried out for 180 s with helium at a rate of 10 ml/min. Volatile components were driven into a capillary tube that was inside a cryogenic trap (liquid nitrogen) maintained at −110 °C, and connected in a on-column mode to a capillary gas chromatograph (Carlo Erba GC 8000, 20090 Milan, Italy). The connection to the analytical column was not direct, and a "Y" press fit was inserted and connected to a vapour exit valve. During the sampling step, helium was back-flushed through the analytical column with an outlet in the afore mentioned vapour exit device. This had the aim of avoiding any contamination of the analytical column.

2.3. Analysis with coupled gas chromatography–mass spectrometry

At the end of sampling (purging) time, desorption of volatile components takes place by heating the trap to 240 °C in 5 s and then by transferring the volatiles to the capillary column in 15 s. The analytical column used was a capillary fused-silica column 50 m × 0.32 mm I.D., coated with PS 264 (Mega, 20090 Milan, Italy) with a 3 µm film thickness.

The capillary gas chromatography system was coupled directly to a MD 800 mass spectrometer (Carlo Erba, 20090 Milan, Italy). Gas chromatography conditions were as follows: oven initial temperature 40 °C, hold for 6 min, then programmed to 180 °C at a rate of 5 °C/min, followed by 5 min at 180 °C, and 7 °C/min to 200 °C with 5 min of final isotherm. The transfer line temperature was kept at 250 °C.

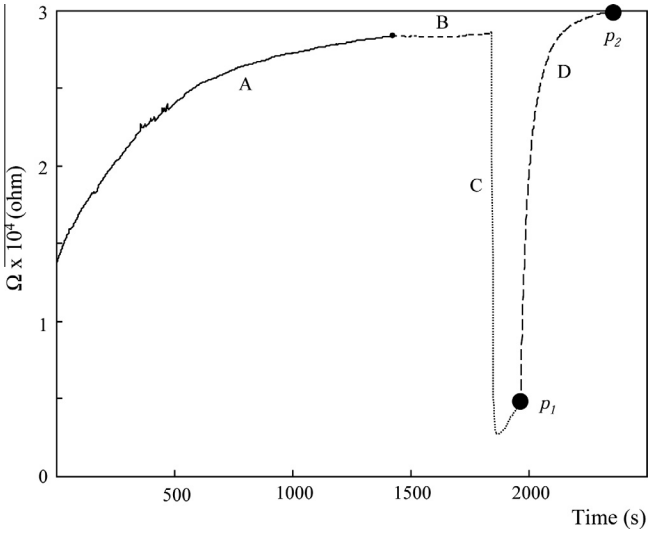

Fig. 1. Plot representing the electric resistance (Ω) of a MOS sensor during oil evaluation: (A) conditioning phase, (B) before injection phase, (C) measurement cycle and (D) recovery phase.

The mass spectrometer scanned from m/z 29 to m/z 300 at 0.5 s cycle time. The ion source was set at 180 °C and spectra were obtained by electron impact (70 eV).

The tentative identification of compounds was carried out by a comparison of the MS spectra with members of the NBS library.

2.4. Instrumentation and working conditions of olfactory system

An electronic olfactory system (EOS 507, Sacmi Imola S.C., Bologna, Italy) composed of a measuring chamber with 6 metal oxide sensors and a personal computer was used for the acquisition and analysis of the data generated by the EOS 507. The sensors used were: sensor 1 (SnO_2), sensor 2 (SnO_2 + SiO_2), sensor 3, 4, and 5 (catalysed SnO_2 with Au, Ag, and PD, respectively) and sensor 6 (WO_3). During the analysis, sensors were maintained at a temperature range of 350–450 °C. The EOS 507 was controlled by an integrated PDA equipped with proprietary software, and was connected to an automatic sampling apparatus (Model HT500H) that had a carousel of 10 sites for loading samples. Samples were kept at controlled temperature (37 °C) and placed in a chamber that removes humidity from the surrounding environment.

2.5. MOS sensor array procedure

For each sample, 15 g were placed in 100 mL Pyrex vials equipped with a pierceable silicon/Teflon cap. For each sensor, signal was divided in four parts (see Fig. 1): (A) conditioning phase (25 min period employed to obtain a constant baseline), (B) before injection phase (in which samples were incubated at 37 °C for 7 min before injection), (C) measurement cycle (in which the oil headspace, sampled with an automatic syringe was then pumped over the sensor surfaces for 2 min during which the sensor signals were recorded; in this phase, sensors were exposed to filtered air at a constant flow rate of 50 sccm (standard cubic cm per min) to obtain the baseline) and (D) recovery phase (another 7 min period applied to restore the original MOS conditions). Ambient air filtered with activated silica and charcoal was used as a reference gas during the recovery phase of the measurement cycle. The previous conditions ensured that the baseline reading had indeed been recovered before the next analysis was performed.

The experimental conditions adapted from Camurati, Tagliabue, Bresciani, Sberveglieri, and Zaganelli (2006) were used, and each sample was evaluated in triplicate on different days.

2.6. Data treatment and statistical analysis

The data from the electronic nose was extracted and analysed with the statistical package "Nose Pattern Editor" (Sacmi Imola S.C.). A feature extraction algorithm called "classical after feature" was applied to the data before other statistical treatments.

The response extracted by each sensor was defined by:

$$X = p_1/p_2$$

where p_1 is the resistance of a sensor in the presence of the volatile compounds emitted from the VOO headspace, p_2 is the resistance of the sensor after measurement, and X is the response of each sensor recorded.

2.7. Detrended fluctuation analysis (DFA)–principal component analysis (PCA)

Detrended-fluctuation analysis (DFA) (Peng et al., 1994) aims to improve the evaluation of correlations in a time series by eliminating linear trends from the data obtained by "classical after feature" (Section 2.5). The data obtained this way have been statistically treated by principal component analysis (PCA) to separate different kinds of samples.

Table 1
Selected volatile components (expressed in μg/kg on commercial truffle flavored olive oil samples. These VOCs has been recorded in the three main commercial species of truffles and found in the truffle flavored oils.

Compounds	TO1	TO2	TO3	TO4	TO5	TO6	TO7	TO8	TO9	TO10	TO11	TO12	TO13	TO14
Acetaldehyde	58.17 ± 6.54	94.64 ± 5.84	48.66 ± 285	13.73 ± 1.01	17.32 ± 0.8	21.1 ± 1.7	16.22 ± 0.78	40.51 ± 2.6	30.63 ± 1.98	29.38 ± 1.29	19.63 ± 2.17	42.52 ± 6.07	20.98 ± 1.84	36.71 ± 3.69
Ethanol	2844 ± 182	4407 ± 383	4298 ± 229	1459 ± 114	713.9 ± 39.3	843.5 ± 27.4	851.8 ± 94	3008 ± 209	1564 ± 10.4	1559 ± 61	920.6 ± 49.8	2060 ± 14.7	887.3 ± 22.8	2395 ± 323
Acetone	430.4 ± 49.4	596.7 ± 18.3	1140 ± 74.2	70.77 ± 5.17	677.1 ± 34.6	756.9 ± 19.9	1674 ± 54	461 ± 22.8	784.5 ± 50.6	398.4 ± 24.5	62.75 ± 7.33	308.5 ± 14.5	619.6 ± 34.6	381.7 ± 9.1
Dimethylsulfide	8.08 ± 0.3	10.46 ± 0.96	21.91 ± 2.71	3.85 ± 0.47	26456 ± 700	5568 ± 396	**33656** ± 1530	43.39 ± 2.72	30891 ± 639	123.9 ± 18.1	32.7 ± 4.5	112 ± 10.04	3028 ± 218	n.d.
1-Propanol	144.9 ± 3.72	180.5 ± 12.2	139.6 ± 11.7	38.45 ± 2.24	n.d.	n.d.	n.d.	130.9 ± 4.31	n.d.	137.2 ± 4.88	12.32 ± 1.72	944.2 ± 58.12	4165 ± 325	1653 ± 55.2
Isobutanal	16.07 ± 2.03	18.82 ± 2.18	19.27 ± 1.47	5.37 ± 0.34	18289 ± 159	5042 ± 337	**23168** ± 1364	17.05 ± 0.67	20190 ± 1943	27.5 ± 1.48	n.d.	91.34 ± 6.53	1112 ± 83.3	180.9 ± 12.8
2-Butanone	103.2 ± 9.95	139.1 ± 7.42	96.53 ± 4.69	12.33 ± 0.31	23782 ± 348	5661 ± 357	26767 ± 338	102 ± 5.38	26147 ± 1793	1273 ± 69.2	38.89 ± 1.37	1005 ± 56.5	32943 ± 122	2512 ± 311
Hexane	46.85 ± 2.25	564.5 ± 49.4	121.4 ± 4.49	57.33 ± 4.32	n.d.	n.d.	n.d.	60.73 ± 0.07	n.d.	339.6 ± 28.3	103.1 ± 6.14	100.1 ± 9.24	281.7 ± 18.7	1142 ± 8.77
Ethylacetate	706.6 ± 93.8	834.2 ± 4.17	570.1 ± 37.1	199.2 ± 1.12	273.8 ± 16.4	85.48 ± 6.76	66.78 ± 3.7	489.4 ± 16.7	656.7 ± 81.4	318.3 ± 26.2	69.71 ± 3.94	276.4 ± 7.71	57.15 ± 3.88	87.88 ± 4.85
Isobutanol	160.1 ± 21.1	115.1 ± 7.31	36.7 ± 3.17	19.29 ± 1.13	55.2 ± 4.95	23.49 ± 3.26	1445 ± 54.6	32.06 ± 0.82	300.1 ± 24.3	n.d.	n.d.	53.46 ± 5.81	16.39 ± 0.63	1254 ± 4.6
3-Methylbutanal	124.4 ± 13.5	134.04 ± 13.1	102.9 ± 8.16	26.02 ± 0.34	n.d.	n.d.	n.d.	102.9 ± 6.47	n.d.	72.27 ± 9.7	4.8 ± 0.16	58.37 ± 3.31	32.87 ± 0.85	79.32 ± 8.95
2-Methylbutanal	194.1 ± 19.5	175.1 ± 13.4	126.4 ± 10.2	32.39 ± 1.53	n.d.	n.d.	n.d.	145.6 ± 5.51	n.d.	86.21 ± 8.08	4.43 ± 0.27	68.81 ± 5.52	9.36 ± 0.95	99.3 ± 3.11
3-Methyl-1-butanol	80.8 ± 5.54	59.11 ± 4.52	17.56 ± 1.43	20.17 ± 1.43	n.d.	1.13 ± 0.12	n.d.	101.5 ± 10.4	20.48 ± 1.21	19.26 ± 1.53	4.18 ± 0.4	9.89 ± 0.29	5.45 ± 0.62	24.39 ± 1.84
2-Methyl-1-butanol	75.02 ± 8.96	78.95 ± 3.73	20.59 ± 1.1	23.37 ± 1.14	n.d.	5.74 ± 0.51	n.d.	59.67 ± 5.27	33.3 ± 0.3	20.31 ± 3.13	5.33 ± 0.06	16.81 ± 0.32	7.71 ± 0.04	51.1 ± 2.7
Dimethyldisulfide	524.9 ± 45.6	46.49 ± 2.18	917.7 ± 77.1	133.1 ± 3.16	442.1 ± 33.1	169.2 ± 10.9	284.1 ± 13.3	1724 ± 19.7	276.2 ± 28.9	1013 ± 18.9	125.7 ± 11.1	222.4 ± 0.99	143.1 ± 11.4	12.44 ± 0.42
Bis(methylthio)methane	4390 ± 132	1027 ± 104	4343 ± 72.7	1532 ± 21.6	10399 ± 821	6614 ± 268	12594 ± 740	5260 ± 26.9	10418 ± 791	10737 ± 376	5056 ± 407	6697 ± 60	5659 ± 179	n.d.
Dimethyltrisulfide	1.35 ± 0.2	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	0.83 ± 0.05	0.27 ± 0.02	n.d.	n.d.	n.d.	n.d.	n.d.
Nonanal	3.18 ± 0.23	3.85 ± 0.13	5.46 ± 0.7	1.95 ± 0.16	n.d.	2.49 ± 0.29	n.d.	5.47 ± 0.35	2.53 ± 0.41	n.d.	2.46 ± 0.15	4.87 ± 0.35	3.54 ± 0.14	n.d.
Methyl methylthiomethyldisulfide	n.d.	2.81 ± 0.04	n.d.	n.d.	n.d.	2.38 ± 0.19	n.d.	n.d.	n.d.	2.35 ± 0.1	1.93 ± 0.11	n.d.	2.28 ± 0.23	n.d.

Internal standard isooctane at 0.02 μl = 13.8 μg.
Samples: TO1: *T. magnatum*; TO2: *T. aestivum*; TO3: truffle; TO4: *T. magnatum*; TO5: *T. melanosporum*; TO6: *T. magnatum*; TO7: *T. melanosporum*; TO8: *T. aestivum*; TO9: *T. melanosporum*; TO10: *T. magnatum*; TO11: *T. magnatum*; TO12: *T. magnatum*; TO13: *T. magnatum*; TO14: *T. melanosporum* from pate.
"n.d.", not detected; **bold**, abnormal values.

Table 2
Other volatile components (expressed in μg/kg) on commercial truffle flavored olive oil samples.

Rt	Compounds	TO1	TO2	TO3	TO4	TO5	TO6	TO7	TO8	TO9	TO10	TO11	TO12	TO13	TO14
3.9	Methanol	431.3 ± 42.8	517.5 ± 5.37	681.1 ± 18.38	179.1 ± 10.3	118.7 ± 8.14	252.4 ± 24.1	56.14 ± 2.59	569.1 ± 0.35	367 ± 30.3	307.8 ± 27.4	184.4 ± 8.28	671.3 ± 42.1	181.2 ± 11.9	356.5 ± 4.8
4.54	Methanethiol	208.3 ± 2.71	20.73 ± 1.91	750.5 ± 18.5	28.22 ± 0.43	2073 ± 54.9	1087 ± 68.4	1211 ± 58.6	684.2 ± 58.1	494.4 ± 11.2	1185 ± 55.4	942.4 ± 63.8	546.8 ± 68.8	840 ± 5.87	0.26 ± 0.02
4.66	Methylformate	21.06 ± 2.71	33.08 ± 3.83	52.28 ± 3.41	17.25 ± 1.3	35.75 ± 2.21	12.17 ± 0.83	3.87 ± 0.21	36.12 ± 1.89	27.67 ± 1.1	31.3 ± 4.61	9.14 ± 0.21	60.7 ± 3.42	8.33 ± 0.52	23.53 ± 0.52
6.31	2-Propenal (acrolein)	n.d.	59.1 ± 2.3	32.29 ± 0.46	9.35 ± 0.67	28.98 ± 2.04	28.08 ± 1.43	10.18 ± 1.01	n.d.	4.51 ± 0.32	n.d.	14.96 ± 1.05	18.4 ± 1.21	28.94 ± 1.71	19.6 ± 1.55
6.57	Propanal	252.1 ± 11.3	703.3 ± 63.1	691.5 ± 96.5	280.7 ± 20.15	n.d.	322.1 ± 36.1	n.d.	291.1 ± 5.16	n.d.	416.2 ± 29.4	41.23 ± 3.01	486.5 ± 20.7	194.9 ± 13.4	446 ± 57.7
6.79	Pentane	116.1 ± 12.3	2147 ± 166	593.5 ± 49.6	591.8 ± 34.1	184.9 ± 0.28	418.8 ± 28.4	377.6 ± 7.21	4365 ± 65	1041 ± 67.5	5168 ± 346	118.2 ± 5.51	228.6 ± 23.7	161.2 ± 6.1	796 ± 84.8
7.54	Ethylformate	56.65 ± 5.4	70.91 ± 5.37	90.58 ± 3.82	37.1 ± 2.5	8.77 ± 0.11	10.33 ± 0.71	2.61 ± 0.27	95.82 ± 4.7	49.12 ± 2.97	50.9 ± 4.02	4.53 ± 0.72	60.48 ± 1.82	4.99 ± 0.3	48.54 ± 6.66
8.11	Methylacetate	263.5 ± 9.97	487.9 ± 17.8	255.8 ± 11.9	74.07 ± 6.2	n.d.	n.d.	n.d.	208.3 ± 1.7	n.d.	161.8 ± 12.3	16.46 ± 1.73	178.8 ± 4.17	n.d.	85.1 ± 2.65
8.61	Carbon disulfide	n.d.	n.d.	n.d.	0.66 ± 0.08	46.97 ± 3.24	8.25 ± 0.71	21.45 ± 1.11	4.47 ± 0.3	4.04 ± 0.14	n.d.	n.d.	1.67 ± 0.1	4.87 ± 0.49	n.d.
8.71	Dimethylsulfone	5.75 ± 0.42	4.27 ± 0.01	9.3 ± 0.48	1.41 ± 0.04	n.d.	5.31 ± 0.52	n.d.	1.99 ± 0.14	n.d.	4.63 ± 0.07	1.93 ± 0.11	3.46 ± 0.21	3.67 ± 0.15	15.75 ± 1.48
9.98	2-Butenal (crotonal)	21.83 ± 1.51	396.1 ± 4.38	45.77 ± 2.08	5.33 ± 0.7	285.7 ± 12.5	n.d.	118.1 ± 8.41	7.95 ± 0.08	n.d.	112.5 ± 7.57	n.d.	19.1 ± 0.09	18.97 ± 1.16	16.4 ± 0.27
10.87	2-Butenone	3.77 ± 0.52	8.49 ± 0.21	7.84 ± 0.49	1.52 ± 0.01	2.11 ± 0.17	3.38 ± 0.35	2.28 ± 0.24	7.87 ± 0.75	n.d.	5.97 ± 0.74	7.35 ± 0.76	9.25 ± 0.2	8.27 ± 0.57	3.11 ± 0.25
11.13	Butanal	7.12 ± 1.02	24.98 ± 0.47	47.82 ± 2.78	5.17 ± 0.49	591.2 ± 17.11	79.6 ± 3.73	593.1 ± 17.9	62.91 ± 4.66	487.3 ± 33.2	19.53 ± 1.28	10.09 ± 1.23	35.79 ± 0.76	61.23 ± 3.33	9.33 ± 0.77
12.76	2-Thiabutane	909.1 ± 64.4	555.6 ± 41.2	3590 ± 240	379.4 ± 3.11	4628 ± 187	2212 ± 87	4356 ± 575	2283 ± 123	1974 ± 70.4	3711 ± 265	1879 ± 34	3308 ± 66.3	1990 ± 261	57.12 ± 2.22
14.91	Butylformate	15.39 ± 1.03	3.54 ± 0.36	1.6 ± 0.06	3.42 ± 0.33	n.d.	n.d.	n.d.	5.43 ± 0.7	n.d.	6.51 ± 0.9	0.69 ± 0.08	2.44 ± 0.18	2.58 ± 0.24	1.03 ± 0.16
15.94	1-Penten-3-ol	77.17 ± 7.06	50.86 ± 2.48	56.03 ± 0.37	63.32 ± 4.72	4.28 ± 0.45	13.51 ± 1.17	6.02 ± 0.71	195.3 ± 6.86	84.67 ± 8.41	69.59 ± 2.57	5.24 ± 0.15	130.7 ± 4.1	11.23 ± 0.56	146.5 ± 11.6
16.74	Cyclopentanol	291.7 ± 30.9	364.7 ± 8.84	282 ± 12.2	91.15 ± 0.97	100.4 ± 6.05	120.3 ± 13.3	146.5 ± 7.41	643.4 ± 20.8	331.1 ± 3.18	239 ± 22.8	50.32 ± 2.23	268.9 ± 4.52	113.4 ± 7.03	381.5 ± 14.7
16.93	Heptane	86.5 ± 7.72	338.4 ± 14.1	70.29 ± 3.53	39.28 ± 2.76	40.1 ± 3.35	135.1 ± 15	81.16 ± 3.22	102.1 ± 5.74	84.68 ± 7.25	209.4 ± 18.3	41.98 ± 5.28	45.92 ± 3.85	45.74 ± 1.8	43.45 ± 3.44
17.22	2-Oxa-4-thiapentane	16.01 ± 2.21	n.d.	n.d.	94.03 ± 2.54	29.59 ± 1.41	19.76 ± 1.6	152.1 ± 6.07	3.38 ± 0.44	2.16 ± 0.23	n.d.	32.67 ± 3.52	n.d.	14.58 ± 1.8	n.d.
17.45	Ethylpropionate	8.52 ± 1.11	3.85 ± 0.48	1.57 ± 0.17	1.98 ± 0.16	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	2.19 ± 0.08	n.d.	n.d.
17.54	Methylmethacrylate	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	73.34 ± 5.99	n.d.	n.d.	n.d.	n.d.
17.63	Propylacetate	12.63 ± 1.46	n.d.	n.d.	n.d.	50.25 ± 2.92	7.23 ± 0.68	16.61 ± 0.57	n.d.	18.31 ± 1.67	n.d.	n.d.	n.d.	4.41 ± 0.55	n.d.
18.44	Diethylacetal	n.d.	3.72 ± 0.32	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
20.53	2-Pentenol	14.12 ± 1.13	10.75 ± 1.06	n.d.	12.41 ± 0.6	n.d.	7.69 ± 0.8	n.d.	61.83 ± 0.76	n.d.	10.72 ± 0.8	4.3 ± 0.47	19.71 ± 2	6.29 ± 0.29	13.46 ± 1.5
21.17	3-Methylthio-1-propanol	2.62 ± 0.13	n.d.	n.d.	n.d.	13.14 ± 0.53	5.34 ± 0.57	33.42 ± 1.06	n.d.	1.05 ± 0.14	n.d.	8.62 ± 0.75	n.d.	3.19 ± 0.36	n.d.
21.53	2-Hexanone	1.89 ± 0.25	1.63 ± 0.17	3.45 ± 0.13	0.32 ± 0.03	2.75 ± 0.06	0.93 ± 0.09	n.d.	2.01 ± 0.18	1.08 ± 0.08	n.d.	0.94 ± 0.13	0.87 ± 0.07	0.57 ± 0.06	3.36 ± 0.26
21.93	3-Hexenal	8.7 ± 1.04	3.89 ± 0.18	n.d.	1.36 ± 0.11	n.d.	n.d.	3.42 ± 0.14	19.15 ± 1.44	3.02 ± 0.33	8.23 ± 0.54	n.d.	1.99 ± 0.11	0.68 ± 0.05	2.49 ± 0.31
22.06	Octane	580.5 ± 28.6	2097 ± 166	656.4 ± 14.9	401.4 ± 3.25	111 ± 6.5	329.5 ± 22.9	116.6 ± 6.33	881.1 ± 8.7	348.7 ± 15.5	1110 ± 19	104.6 ± 6.9	572 ± 8.27	284.4 ± 11.5	429.8 ± 42.4
22.35	4-Octene	5.45 ± 0.32	32.12 ± 1.56	5.29 ± 0.29	5.21 ± 0.18	2.86 ± 0.18	2.23 ± 0.19	1.62 ± 0.12	13.79 ± 0.47	2.29 ± 0.04	10.2 ± 1.3	1.14 ± 0.16	2.76 ± 0.2	1.29 ± 0.21	3.12 ± 0.07
22.61	Butylacetate	5.25 ± 0.75	n.d.	5.4 ± 0.23	0.71 ± 0.1	4.14 ± 0.19	n.d.	n.d.	n.d.	1.38 ± 0.13	n.d.	n.d.	n.d.	n.d.	n.d.
23.01	2-Thiahexane	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	1.22 ± 0.22	1.68 ± 0.04	n.d.	n.d.	n.d.	n.d.
23.31	Hexylmethylether	42.44 ± 3.33	51.74 ± 3.81	30.93 ± 1.81	1.35 ± 0.19	n.d.	n.d.	n.d.	n.d.	8.4 ± 0.65	11.04 ± 0.83	1.45 ± 0.13	4.87 ± 0.22	0.66 ± 0.06	2.4 ± 0.05
23.53	3-Methylhexenylether	22.43 ± 2.65	19.03 ± 0.98	20.81 ± 0.68	1.08 ± 0.14	n.d.	n.d.	n.d.	n.d.	4.38 ± 0.27	12.34 ± 1.38	0.8 ± 0.05	1.14 ± 0.06	0.63 ± 0.06	n.d.
24.14	4-Methyl-4-hydroxy-2-pentanone	n.d.	n.d.	27.04 ± 2.5	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
24.68	2-Hexenal	285.7 ± 12.4	298.8 ± 29.8	87.43 ± 4.64	546.3 ± 28.1	38.86 ± 2.2	90.6 ± 10.6	3.38 ± 0.19	1737 ± 8.27	386.7 ± 21.8	492.2 ± 19.6	72.21 ± 4.33	628.3 ± 79.7	131.8 ± 5.09	507.6 ± 19.5
24.85	3-Hexenol	80.93 ± 6.74	42.38 ± 2.88	17.94 ± 1.28	187.3 ± 21.1	n.d.	n.d.	n.d.	296.7 ± 25.2	28.51 ± 1.93	34.18 ± 3.7	11.97 ± 0.87	24.25 ± 2.26	6.78 ± 0.42	16.1 ± 2.14
25.23	4-Thia-1,6-heptadiene	n.d.	n.d.	n.d.	n.d.	57.84 ± 1.24	318.7 ± 20.8	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	912 ± 89	n.d.
25.27	2-Hexenol	66.05 ± 6.46	64.36 ± 4.64	n.d.	57.72 ± 5.64	n.d.	n.d.	n.d.	n.d.	20.35 ± 1.38	41.89 ± 2.08	12.17 ± 1.02	62.15 ± 2.22	n.d.	63.61 ± 4.38
26.01	1,4-Dithiane	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	0.4 ± 0.04	0.67 ± 0.04	n.d.	n.d.	0.37 ± 0.03	n.d.
26.29	1,2-Bis(methylmercapto)ethane	2.03 ± 0.16	n.d.	n.d.	n.d.	21.1 ± 0.5	11.16 ± 0.3	19.88 ± 1.35	n.d.	n.d.	n.d.	8.31 ± 0.56	3.53 ± 0.31	6.14 ± 0.61	n.d.
26.37	2-Heptanone	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	5.71 ± 0.85	0.48 ± 0.04	n.d.	n.d.	n.d.	n.d.	n.d.
28.46	1,2,3-Trithiolane	8.84 ± 0.8	4.4 ± 0.2	4.2 ± 0.26	5.33 ± 0.1	n.d.	n.d.	n.d.	27.22 ± 0.46	n.d.	9.58 ± 0.52	n.d.	20.19 ± 0.99	2.18 ± 0.36	n.d.
29.34	2-Heptenal	7.04 ± 0.97	7.75 ± 1.15	0.99 ± 0.02	2.28 ± 0.2	n.d.	1.26 ± 0.1	n.d.	7.58 ± 0.7	n.d.	5.75 ± 0.37	1.39 ± 0.03	2.64 ± 0.33	1.43 ± 0.22	1.68 ± 0.21
32.84	Limonene	9.1 ± 0.6	5.25 ± 0.8	4.18 ± 0.4	1.38 ± 0.04	n.d.	4.59 ± 0.25	n.d.	7.51 ± 0.82	3.47 ± 0.24	n.d.	n.d.	n.d.	7.34 ± 0.6	15.17 ± 0.32

Internal standard isooctane at 0.02 μl = 13.8 μg.
Samples: TO1: *T. magnatum*; TO2: *T. aestivum*; TO3: truffle; TO4: *T. magnatum*; TO5: *T. melanosporum*; TO6: *T. magnatum*; TO7: *T. melanosporum*; TO8: *T. aestivum*; TO9: *T. melanosporum*; TO10: *T. magnatum*; TO11: *T. magnatum*; TO12: *T. magnatum*; TO13: *T. magnatum*; TO14: *T. melanosporum* from pate.
"n.d.", not detected; **bold**, abnormal values.

3. Results and discussion

3.1. GC–MS analysis of oils flavored with truffles

The purge and trap sampling technique is well suited to the analysis of liquid samples, and since it is performed at room temperature enables the acquisition of an aroma profile similar to natural olfactory perception. In our case, the volatile fraction was stripped from the samples and transferred directly to a cryogenic trap. In the cold trap, porous polymers are not present: this configuration allows resolution of typical problems of enrichment techniques as selective, incomplete or irreversible adsorption and possible artifact formation.

The volatile fraction was a complex mixture of 63 compounds, the majority of which correlated with typical flavors of virgin olive oil and aromas of truffles (Tables 1 and 2). Some obtained substances ($n = 32$) could be correlated to the typical flavor of virgin olive oil: in particular, C6 compounds (i.e. 2-hexenal) derive from an enzymatic reaction starting with the formation of 13-hydroperoxides from linoleic and linolenic acids. It can be noted that some compounds (7), even in considerable concentration, result from fermentation/oxidation processes, such as n-octane, octene, butanal, nonanal, hexanone, and hexenol, which are usually products assigned to secondary autoxidation (Procida, Giomo, Cichelli, & Conte, 2005). The presence of ethyl acetate, relevant in some samples (TO1), is the result of olive oil of poor quality, characterised by a winey–vinegary defect.

On the basis of numerous publications on the natural aromas of truffles (see Table 1S), the following considerations can be made.

Ten revealed compounds (Table 1) have been previously recorded as VOCs produced by *T. magnatum* (dimethylsulfide, isobutanal, 2-butanone, 3-methylbutanal, 2-methyl butanal, 2-methyl-1-butanol, dimethyldisulfide, bis(methylthio)methane, dimethyltrisulfide and methyl methylthiomethyldisulfide).

Seven of these compounds are VOCs in common with black truffles (*T. aestivum* and *T. melanosporum*): dimethylsulfide, isobutanal (as 2-methylpropanal), 2-butanone, 3-methylbutanal, 2-methyl butanal, 2-methyl-1-butanol, dimethyltrisulfide (Table 1). In addition to these, the VOCs found in black truffles were: acetaldehyde, ethanol, 1-propanol, hexane, ethylacetate, isobutanol, 3-methyl-1-butanol, and nonanal (Table 1).

Some VOCs were specific for different truffles: dimethyldisulphide, bis(methylthio)methane, methyl methylthiomethyldisulfide (Table 1) for *T. magnatum* and ethylacetate for *T. melanosporum*. In this last case ethylacetate can be also associated to olive oil.

In particular, bis(methylthio)methane, a compound exclusive to the white truffle, was present in most cases in oils flavored with *T. melanosporum*, in amounts much higher than those found in oils flavored with white truffle. In addition, in the oils flavored with white truffle, the amount of isobutanal, a common VOC, varied from 5.37 (TO4) to 5042 (TO6) μg/kg, whereas in *T. melanosporum* it ranged from 180.9 (TO14) to 26168 (TO7) μg/kg.

The same anomaly was found with BMDTM and involves oils flavored with *T. aestivum*. In this case, the situation was as follows: flavored oils with *T. magnatum*, 1532 (TO4) to 10737 (TO10) μg/kg; *T. aestivum* 1027 (TO2) to 5260 μg/kg (TO8); *T. melanosporum* 10399 (TO5) to 12594 μg/kg (TO7).

In sample TO14, flavored with an autoclaved pate of *T. melanosporum*, the quantities of VOCS are much lower and conform to the typical aromatic spectrum of this species, with the absence of dimethylsulfide, which probably evaporated during sterilization.

DHS–GC–MS analysis also revealed (Table 2) the presence of volatile compounds "unknown," or not classifiable as those typical in virgin olive oil and truffles, most likely derived from synthetic processes and/or demolition. In high concentrations the following were found: methylformate (more than 500 ppb in T01, T03, T05, T06, T07, T08, and T10); pentane (more than 500 ppb in T02, T03, T04, T08, T09, T10, and T14); 2-thiabutane; 4,thia-1,6-heptadiene. The following were present at low concentrations: carbon disulfide cyclopentanol: 2-oxa-4-thiapentane; methylmetacrylate; diethylacetal; 3-methyllthio-1-propanol; 4-methyl-4hydroxy-2-pentanone; 1,2,3-trithiolane; 2-heptenal.

3.2. MOS analysis of oils flavored with truffles

The DFA–PCA built from all samples is shown in Fig. 2, while the projection of the cases and the projection of the loadings can be seen in Fig. 3. The first component of PCA explains the 89% of variance and the second explains the remaining 10%. The PCA shows good separation of the three truffle flavored oils: indeed, the oil samples obtained from *T. melanosporum* fall between the first and fourth quadrant, while samples obtained from *T. magnatum* fell between the second and the third quadrant, and these are separate from samples of *T. aestivum* which are all in the third quadrant (Fig. 2). Fig. 3 allows an understanding of the weight of each of the 6 sensors in the classification of oils obtained from different truffles. Sensor number 5 was the most sensitive to VOCs charac-

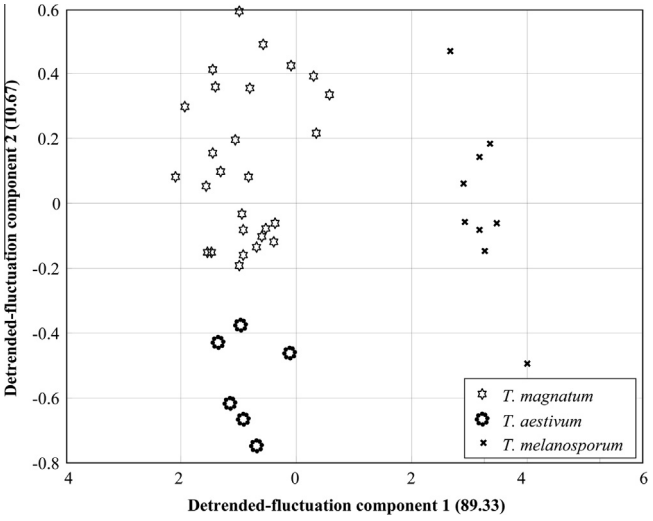

Fig. 2. Score plot on the detrended-fluctuation analysis (DFA) constructed to classify different truffle flavored oils.

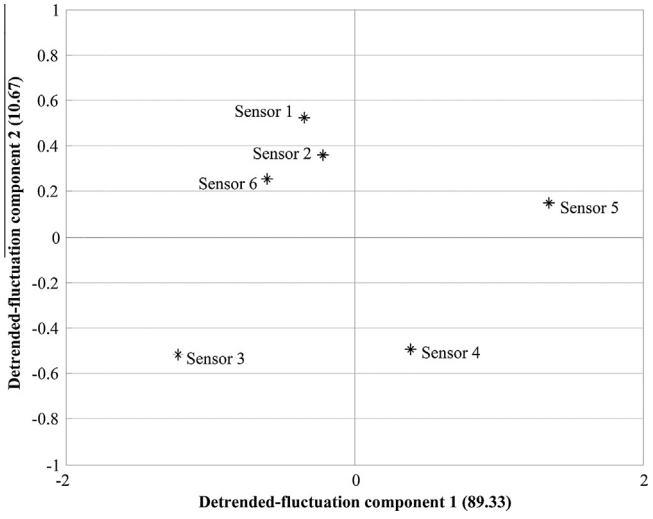

Fig. 3. Loading (sensor) plot on the detrended-fluctuation analysis (DFA) constructed to classify different truffle flavored oils.

teristic of the flavor of *T. melanosporum*, while sensor numbers 1, 2, and 6 show a higher specificity towards the volatile compounds which are most abundant in oils produced from *T. magnatum*. Sensor number 3 was the most in determining for the separation of oil flavored with *T. aestivum* compared to the other oils.

In light of the above information, it is clear that sensor number 5 should be the most sensitive to ethylacetate, which is particularly efficient in separating *T. melanosporum* from the other 2 types of flavored oils (*T. magnatum* and *T. aestivum*). In the same way, sensors 1, 2, and 6 are most sensitive to the four volatile compounds mainly characteristic of *T. magnatum*, i.e. isobutanal, dimethyldisulfide, bis(methylthio)methane, methyl methylthiomethyldisulfide.

4. Conclusions

All the oils analysed seem to have been produced with the use of complex flavorings made with the components more or less typical of the three species of truffle (*T. magnatum*, *T. melanosporum*, and *T. aestivum*) indicated on the label. However, the olive oils used were often of poor quality and defective.

In some cases, although certain substances were present in very large quantities, not occurring in nature in the specific truffle aroma, on the whole the aroma is identifiable by the instruments used. The inappropriate and excessive use of DMDTM, a compound exclusive of the natural flavoring of white truffles, in the formulation of flavoring for the black truffles should be considered an unfair practice.

In spite of this partial false formulation, the present investigation demonstrates the ability of GC–MS and the electronic nose to distinguish truffle-flavored oils produced using different raw products. Indeed, both analytical techniques show good performance in classification of samples. We can therefore assume that the MOS sensors have a qualitative sensitivity, and not quantitative, and as a consequence could be "deceived" by the use of synthetic flavors characterised by specific aromas. On the other hand, the results of DHS–GC–MS allow evaluation of the complexity of flavor determined by the use of real truffles rather than synthetic aromas.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.foodchem.2013.09.016.

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