Potential aromatic compounds markers to differentiate between *Tuber melanosporum* and *Tuber indicum* truffles

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Running title: *T. melanosporum*, *T. indicum*, black truffle, 3-octanone, 1-octen-3-one, 3-octanol, 1-octen-3-ol

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ABSTRACT

The *Tuber indicum* (Chinese truffle) and *Tuber melanosporum* (Black truffle) species are morphologically very similar but their aromas are very different. The black truffle aroma is much more intense and complex, and it is consequently more appreciated gastronomically. This work tries to determine whether the differences between the aromatic compounds of both species are sufficiently significant so as to apply them to fraud detection. An olfactometric evaluation (GC-O) of the *T. indicum* specie was carried out for the first time. Eight important odorants were identified. In order of aromatic significance, these were: 1-octen-3-one and 1-octen-3-ol, followed by two ethyl esters (ethyl isobutyrate and ethyl 2-methylbutyrate), 3-methyl-1-butanol, isopropyl acetate, and finally the two sulfides dimethyldisulfide (DMDS) and dimethylsulfide (DMS). A comparison of this aromatic profile with that of *T. melanosporum* revealed the following differences: *T. indicum* standed out for the significant aromatic contribution of 1-octen-3-one and 1-octen-3-ol (with modified frequencies (MF%) of 82% and 69%, respectively), while in the case of *T. melanosporum* both had modified frequencies of less than 30%. Ethyl isobutyrate, ethyl 2-methylbutyrate and isopropyl acetate were also significantly higher while DMS and DMDS had low MF (30-40%) compared to *T. melanospor*um (> 70 %). The volatile profiles of both species were also studied by means of headspace solid-phase microextraction (HS-SPME-GC-MS). This showed that the family of C8 compounds (3-octanone, octanal, 1-octen-3-one, 3-octanol and 1-octen-3-ol) is present in *T. indicum* at much higher levels. The presence of 1-octen-3-ol was higher by a factor of about 100 while 1-octen-3-one was detected in *T. indicum* only (there was no chromatographic signal in *T. melanosporum*). As well as showing the greatest chromatographic differences, these two compounds were also the most powerful from the aromatic

viewpoint in the *T. indicum* olfactometry. Therefore, either of the two chromatographic methods (GC-O or HS-SPME-GC-MS), together or separately, could be used as a screening technique to distinguish between *T. indicum* and *T. melanosporum* and thus avoid possible fraud.

Keywords: *Tuber indicum*; *Tuber melanosporum*; olfactometry; 1-octen-3-one; 1-octen-3-ol; 3-octanone.

1. Introduction

There are more than a hundred different kinds of truffles around the world, most of which grow in various parts of Europe, in particular in France, Italy and Spain. In China, there are 25 species of the genus *Tuber*. One of them is the species *Tuber indicum*, found mainly in the provinces of Yunnan and Sichuan (Zhang, Zhao, Chen, Liu, Konishi, & Gao, 2007). This species, sometimes known as *Tuber sinense* or *Tuber himalayense*, is considered as one of the eight most extensively studied truffle species along with *T. melanosporum*, *T. brumale*, *T. aestivum*, *T.magnatum*, *T. borchii*, *Tirmania nivea* and *Terfezia claveryi* (Wang & Marcone, 2011).

The high prices and growing demand for truffles means that they are often the object of fraud, especially when species are morphologically similar. *T. melanosporum*, considered as the queen of truffles, is one of the most prized foods worldwide due to its organoleptic properties, especially the aroma. However, this species is very vulnerable to fraud given that other species such as *Tuber indicum* look very similar (dark gleba and black peridium) and it is difficult to tell them apart by traditional morphological observations (Douet, et al., 2004; Riousset, Riousset, Chevalier, & Bardet, 2001). Distinguishing them correctly requires microscopic observation (Janex-Favre, Parguey-Leduc, Sejalon- Delmas, Dargent, & Kulifaj, 1996) or else the use of genetic techniques (Paolocci, Rubini, Granetti, & Arcioni, 1997). Furthermore, if Chinese truffles are put in a container with *T. melanosporum* carpophores, they will go unnoticed since as well as their morphological similarity, Chinese truffles are able to absorb the aroma of black truffles. Fraud in conserves, sauces, patés and oils is quite easy because the label does not specify the species. “Black truffles” is listed in the ingredients. This suggests *T. melanosporum*, but it is quite possible that mixtures of cheaper species such as *T.*

brumale, *T. indicum* or *T. aestivum* may predominate (Mabru, Dupré, Douet, Leroy, Ravel, & Ricard, 2004), or even truffles of the *Terfezia* genera.

It is well-known that the volatile organic compounds (VOC) profile of *T. melanosporum* and *T. indicum* is dominated by alcohols (48-57%), aldehydes (4 – 27%) and other aromatic compounds (9-30%) (Splivallo, Bossi, Maffei, & Bonfante, 2007a). According to these authors, *T. melanosporum* can be distinguished from *T. indicum* due to its higher aroma content and larger variety of sulfur containing compounds. Some volatile compounds seem to be specific to one of these species, as is the case of 1,2-dimethoxybenzene, 2-phenyl-2-buten-1-al and 5-methyl-2-phenyl-2-hexenal (Splivallo et al., 2007a). However, none of these volatile compounds are characterized by relevant aromatic properties.

Therefore, the main aim of the present work is to evaluate if it is possible to differentiate these species of black truffle (*T. indicum* and *T. melanosporum*) from an aromatic point of view and to try to find some specific aromatic compounds which can act as markers of these species, enabling their discrimination. For this purpose, gas chromatography-olfactometry (GC-O) and gas chromatography-mass spectrometry (GC-MS) strategies were used to provide a complete aromatic profile of *T. indicum* and to compare it with that corresponding to *T. melanosporum*. This comparison will reveal if some aromatic compounds may be considered as potential markers of *T. indicum*. This information could be very useful in order to reduce fraud.

2. Materials and Methods

2.1. Ascocarps of *Tuber indicum* and *Tuber melanosporum*

The ascocarps of *T. indicum* were bought at a local supplier. This truffle species was sold without covering soil but once in the laboratory we cleaned and selected the ascocarps. The truffles were rinsed with tap water and dried in a fluid laminar cabinet. Qualitative selection of the ascocarps was made by discarding truffles with softened texture, dipters and coleoptera larva or those damaged during the harvest (by shovel or dog's teeth). A group of three truffle experts selected from the different samples those showing the most typical aroma characteristics.

The ascocarps of *T. melanosporum* were collected from cultivated truffle grounds in Sarrión (Teruel, Spain) and processed as described in Cullere et al. (2010, 2012)

2.2. Reagents

Solvents: dichloromethane and methanol were purchased from Merck (Darmstadt, Germany); water was purified in a milliQ system from Millipore (Bedford, MA). Resins: Lichrolut® EN resins (non polar resins) and polypropylene cartridges (0.8 cm internal diameter, 3 mL internal volume) were supplied by Merck (Darmstadt, Germany).

Standards: the standards used for identifications were supplied by Aldrich (Steinheim, Germany), Fluka (Buchs, Switzerland), PolyScience (Niles, USA), Lancaster (Strasbourg, France), AlfaAesar (Karlsruhe, Germany). An alkane solution (C8-C28), 20 mg L^{-1} in dichloromethane, was employed to calculate the linear retention index (LRI) of each analyte.

2.3. Gas Chromatography-Olfactometry (GC-O)

2.3.1. Preparation of extracts. Five ascocarps of *T. indicum* truffles were cut into thin slices using a sharp knife and mixed in order to obtain a homogeneous sample that can be considered as representative of this truffle species.

The volatiles of the truffle were collected using a purge and trap system following the same methodology as that used previously in order to characterize the *T. melanosporum* aromatic profile (Culleré, Ferreira, Chevret, Venturini, Sánchez-Gimeno, & Blanco, 2010). A Lichrolut® EN cartridge (400 mg) (Merck) was placed on the top of a bubbler flask containing 21 g of truffle cut up into pieces of approximately one gram and a half. The truffles were purged by a stream of nitrogen at 25 ºC during 7.5 hours. The LiChrolut® EN trapping cartridge was kept at 0ºC during the purging time. Volatile truffle constituents released in the headspace were trapped in the cartridge containing the sorbent and were further eluted with 3.2 mL of dichloromethane containing 5% methanol. A concentration step was not necessary in this case.

2.3.2. GC-O analysis. All sniffing experiments were carried out in a Trace gas chromatograph from TermoQuest, equipped with a flame ionization detector (FID) and a sniffing port (ODO-1 from SGE) connected by a flow splitter to the column exit. The column was a DB-WAX from J&W (Folsom, CA, USA), 30m, 0.32mm i.d., 0.5 mm film thickness. A constant pressure of 52 kPa was maintained throughout the analysis time. The carrier was H_2. Two microlitres were injected in splitless mode for 1min splitless time. Injector and detector were both kept at 250 ºC. The temperature program was 40 ºC for 5 min, then raised by 4 ºC min^{-1} to 100 ºC followed by 6 ºC min^{-1} to 220 ºC, and finally kept at this temperature for 20 min. To prevent condensation of high boiling compounds on the sniffing port, the port was heated sequentially with a laboratory-made rheostat.

A panel of six judges carried out the sniffing of the extract. Sniffing time was approximately 30 min, and each judge carried out one session per day. The panelists were asked to score the intensity of each aromatic stimulus using a 4-point scale (0 = not detected, 1 = weak, 2= clear but not intense note, 3 = intense note). The signal obtained was modified frequency (MF(%)), a parameter which was calculated with the formula proposed in (Dravnieks, 1985): $MF(\%)=(F(\%)I(\%))^{1/2}$where F(%) is the detection frequency of an aromatic attribute expressed as a percentage of the total number of judges and I(%) is the average intensity expressed as a percentage of the maximum intensity.

The identification of the odorants was carried out by a comparison of their odors, chromatographic retention index in both DB-WAX and VF-5MS columns and MS spectra with those of pure reference compounds.

2.3.3. Identification of 1-octen-3-ol as responsible for the odor zone at LRI $_{\text{DB-WAX}}$ 1463 by a multidimensional system (GC-O-GC-O-MS).

This multidimensional chromatographic system was used to verify the identity of some odorants detected previously by gas chromatography-olfactometry (GC–O). The extract obtained by the purge and trap system was concentrated to 200 µL. After this, a volume of fifty microlitres was injected in a multidimensional GC–O–GC–O–MS system from Varian (Walnut Creek, CA, USA). The system consisted of two independent gas chromatographs interconnected by a thermoregulated transfer line kept at 200 ºC and equipped with a Deans valve switching system (Valco Instruments, Houston, TX), two olfactory ports and FID and MS detectors, as described previously (Culleré et al., 2010). Chromatograph 1 was equipped with a DB-Wax column (polyethylene glycol) from J&W,30 m x 0.32 mm I.D. with 0.5-lm film thickness. The oven temperature program

was 40 ºC for 5 min, then raised by4 ºC min^{-1} to 100 ºC, followed by 6 ºC min^{-1} to 220 ºC, and finally held at this temperature for 40 min. Initially, the GC–O extract (50 µL) was monitored by olfactometry in the first chromatograph to select the fraction containing the target odorant. In further chromatographic runs, selective heart-cutting was made to isolate the odorant of interest which was transferred to the second chromatograph equipped with a Factor Four VF-5MS column from Varian (30 m x 0.32 mm; 1 µm film thickness). In this second oven, the isolated odorant was trapped in a CO_2cryotrapping unit and monitored by olfactometry with simultaneous MS detection. Two minutes after the heart-cutting, the CO_2 flow was removed at the same time that the temperature program (4 ºC min^{-1} up to 200 ºC and then 50 ºC min^{-1} up to 300 ºC) of the second oven was activated. The MS parameters were: transfer line 170 ºC; ion trap 150 ºC, and trap emission current 30 µA. The global run time was recorded in full scan mode (m/z 40–250 mass range). FID and MS data were registered and processed with Workstation 6.30 software equipped with the NIST 98 (US National Institute of Standards and Technology) MS library (NIST, Gaithersburg, MD). The programmable temperature vaporising injector (PTV) conditions, delay time and heart-cutting interval were the same as those used in a previous paper (Culleré, Escudero, Pérez-Trujillo, Cacho, & Ferreira, 2008).

The identity of the odorant was determined from the odor description, mass spectrum and linear retention indices on both columns (DB-Wax and VF-5MS). The identity was confirmed by injection of the pure reference standard, when available.

2.4. Headspace solid-phase microextraction (HS-SPME)

The methodology applied for this analysis was used in a recently published previous work (Culleré, Ferreira, Venturini, Marco, & Blanco, 2012). Approximately 2 grams of sample was placed in a 20 mL vial closed with a plastic film. Once the desired

temperature (53ºC) had been reached, the vial was allowed to condition for the equilibrium time (5 min). After this time, the fiber (a 50/30 µm layer of divinylbenzene/carboxen/polydimethylsiloxane from Supelco) was introduced into the vial and exposed to the headspace of the sample during 13.6 minutes. Thermal desorption of the compounds from the fiber coating took place in the GC injector at 200 ºC for 15 minutes.

2.5. Gas Chromatography-Mass Spectrometry (GC-MS) conditions

The analyses were performed with a CP-3800 chromatograph coupled to a Saturn 2200 ion trap mass spectrometric detection system from Varian (Sunnyvale, CA, USA). A DB-WAXETR capillary column (J&W Scientific, Folsom, CA,USA) of 60m x 0.25 mm I.D., film thickness 0.25 µm was used, preceded by a3 m x 0.25 mm uncoated (deactivated, intermediate polarity) precolumn from Supelco (Bellefonte, PA, USA). Helium was the carrier gas at a flow rate of 1 mL min^{-1}. The oven temperature was initially 40ºC during 5 minutes, then raised by 4ºC min^{-1} to 140ºC, followed by a rate of 10ºC min^{-1} to 220ºC and finally held at this temperature for 10 minutes. The MS transfer line and chamber ionization temperature was 200ºC, and the trap emission current was 80 µA. The global run time was recorded in full scan mode (45-250 m/z mass range). The injection was in splitless mode (splitless time 5 min) at a temperature of 200ºC. A desorption time of 15 minutes was used.

The chromatographic data were analyzed by Varian Saturn GC-MS Version 5.2 software. The identity of the odorants was determined by a comparison of their chromatographic retention index and MS spectra with those of pure reference compounds. The data is expressed in area percentages.

Five ascocarps of *T. indicum* were cut into thin slices using a sharp knife. A total of three replicates of each truffle species were analyzed in order to confirm the

variability associated to each truffle. The resulting reproducibility was satisfactory, given that an SPME technique was used and, moreover, that some of the compounds were studied in very low concentrations. Thus, relative standard deviations values (RSD %) lower than 10% were obtained in all cases.

3. Results and discussion

3.1. Semiquantitative GC-O experiments

The results of the experiments carried out with *T. indicum* samples are summarized in Table 1. This table provides the chromatographic retention data of the different aromatic compounds detected in the olfactometric experiments, the odor description of these compounds given by the trained sensory panel, the chemical identity of the odorant responsible for the odor and the olfactometric scores (as modified frequency in %) given by the sensory panel. Only the odorants that reached a MF(%) higher than 30% in *T. indicum* truffles are included in the table. The extracts for the olfactometric experiments were obtained following a mild procedure trying to imitate as closely as possible the exact aroma composition of the vapors emitted by the truffles. Therefore, the MF(%) of the odorants given in the table should give a reasonable estimate of the potential importance of the compound in the aroma profile of each truffle species. As can be seen, eight odorants can be considered as relevant from an aromatic point of view. The most important of them was the 1-octen-3-one, characterized by mushroom nuances, given its high modified frequency (82%). This was followed by 1-octen-3-ol (69%), ethyl isobutyrate (65%) and 3-methyl-1-butanol and ethyl 2-methylbutyrate (both with 53%). The identification of the compound responsible for the aromatic zone described as having a clear odor of mushroom with LRI $_{\text{DB-WAX}}$ 1463 required the help of a multidimensional chromatographic system (GC-O-GC-O-MS), following the procedure described above. This aroma may be explained by the 1-octen-3-ol or else by the 1-nonen-3-one, given that both compounds are eluted in zones which are quite close in both columns (especially in DB-WAX). After capturing the zone of interest in the dual system and transferring it to the second column, the chromatographic peak shown in Figure 1 was obtained. The spectrum

297 clearly corresponds to 1-octen-3-ol. In this way it was possible to identify beyond doubt
298 an odorant which is so significant in the aromatic profile of *T. indicum*.
299 A comparison of the olfactometric profile of *T. indicum* with that of *T. melanosporum*,
300 described in a previous publication (Culleré et al., 2010) and in table 1, reveals the
301 following differences: **1)** *T. melanosporum* is characterized by a slightly more complex
302 aromatic profile, having 11 odorants with a MF $\geq$ 30% while *T. indicum* has only 8 such
303 compounds; **2)** the aromatic profile of *T. indicum* differs from that of *T. melanosporum*
304 by the high intensities found in the former of 5 odorants (1-octen-3-one, 1-octen-3-ol,
305 ethyl isobutyrate, ethyl 2-methylbutyrate and isopropyl acetate). Just as March,
306 Richards, & Ryan, (2006) suggested that some esters could be used to distinguish
307 certain species from others (i.e. ethyl 4-methylpentanoate for *T. melanosporum*), our
308 olfactometric study suggests that the above-mentioned two esters and the acetate could
309 be important aromatic discriminators between the two species. Leaving aside the
310 potential aromatic contribution of these three compounds, the important sensory role of
311 the 1-octen-3-one and the 1-octen-3-ol should be emphasised (both have a very
312 characteristic mushroom aroma). In fact, a series of C8 aliphatic compounds, such as 1-
313 octen-3-one, 3-octanol, 1-octen-3-ol, E-2-octen-1-ol, and 3-octanone have been reported
314 to be the major contributors to the characteristic flavor of diverse mushrooms (Cronin &
315 Ward, 1971; Cho, Namgung, Choi, & Kim, 2008; Fischer & Grosch, 1987; Pyysalo &
316 Suinhko, 1976; Venkateshwarlu, Chandravadana, & Tewari, 1999). The compound 1-
317 octen-3-one was reported to be the most potent key odorant in both the pileus and the
318 stipe (Venkateshwarlu, Chandravadana, & Tewari, 1999). It is interesting to know that
319 these C8 compounds are mainly formed by the oxidation of linoleic and linolenic acids
320 in the presence of enzymes, such as lipoxygenase and hydroperoxidelyase (Assaf,
321 Hadar, & Dosoretz, 1997). Furthermore, 1-octen-3-ol (along with other C8 volatiles) is

a potential signal molecule produced by both truffle mycelium and fruiting bodies (Menotta, Giocchini, Amirucci, Buffalini, Sisti, & Stocchi, 2004; Splivallo, Novero, Bertea, Bossi, & Bonfante, 2007b), as well as by most other fungi. At high concentrations it shortens the primary root and exerts generally toxic effects on plants, inducing the loss of chlorophyll, probably through oxidative stress (Splivallo et al., 2007b). At lower concentrations, this compound has been reported to induce plant defense genes (Kishimoto, Matsui, Ozawa, & Takabayashi, 2007), potentially modulating the fitness of the host plant. **3)** Therefore, both odorants (1-octen-3-one and 1-octen-3-ol) could be considered as markers of *T. indicum* and thus avoid confusion between the two species and prevent possible fraud. **4) A**nother noteworthy difference is that the sulphides, dimethylsulfide and dimethyldisulfide, have been detected in *T. indicum* with much lower modified frequencies (<40% MF) then those found in *T. melanosporum* (>70% MF), as would be expected in the light of the work of Splivallo et al., (2007a). **5)** Finally, it is important to mention the exclusive presence of three phenols in the olfactometric profile of *T. melanosporum*: 3-ethyl-5-methylphenol, 3-ethylphenol and 3-proypylphenol. These could also be considered as aroma markers for this species of black truffle.

To conclude, it is clear that the olfactometric differences are sufficiently marked for the purposes of distinguishing between the two species using the chromatographic method described.

3.2. Volatile composition analysis by Head Space Solid-Phase Micro Extraction (HS-SPME)

A more detailed study of the volatile composition of *T. indicum* was carried out in addition to the olfactometric analysis. Table 2 shows the data obtained (expressed in area percentages) for the compounds which appeared in average quantities above or

347 equal to 0.7 %. A total of 17 compounds met this criterion. The most abundant by a
348 substantial margin was 1-octen-3-ol with an area percentage of 37%, followed by 3-
349 methyl-1-butanol (13.9 %) and dimethylsulfide (8.3 %). These three odorants were also
350 considered significant in the olfactometric study, together with 1-octen-3-one (which
351 appears here at the bottom of the table with only 0.7% abundance). It is also worth
352 pointing out that 6 of the 17 compounds listed in Table 2 contain 8 carbon atoms (3-
353 octanone, Z-5-octen-1-ol, 3-octanol, octanal, 1-octen-3-ol and 1-octen-3-one). This
354 family of compounds is characterised by a high degree of aromatic potential, with low
355 threshold values. As can be seen in Table 3, the most powerful odorants in this series
356 are 1-octen-3-one with a threshold value estimated in water of only 0.005 μg L^{-1},
357 followed by octanal with 0.7 μg L^{-1} and 1-octen-3-ol (1 μg L^{-1}). Although these values
358 were not calculated for the truffle, they provide an indication of the aromatic importance
359 of these compounds.
360 The abundance of this family of compounds may also be a marker to differentiate *T.*
361 *indicum* from *T. melanosporum*, as Figure 2 suggests. The chromatogram in Figure 2
362 refers to samples of *T. melanosporum* analysed in a previous study (Culleré, Ferreira,
363 Venturini, Marco, & Blanco, 2012). The figure shows HS-SPME-GC-MS
364 chromatograms of the profiles of both species. It is clear that they can be distinguished
365 one from the other by the high content of C8 compounds in *T. indicum*. The greatest
366 differences correspond to 1-octen-3-one (with a peak in *T. indicum* only) and 1-octen-3-
367 ol, whose chromatographic signal is about a hundred times greater in the Chinese
368 species.
369 These results demonstrate the importance of this group of compounds and are consistent
370 with a previous study (Splivallo et al., 2007b). These authors concluded that the
371 contribution of E-2-octenal, 1-octen-3-ol, 3-octanol, 3-octanone and some other

372 compounds, not including 1-octen-3-one, accounted for 71 ± 17% in *T. indicum*, whilst
373 in the case of *T. melanosporum* the contribution was only 52 ± 15%. On the other hand,
374 other authors (Bellesia, et al., 2002) have found 3-methyl-1-butanol as the major
375 component present in *T. indicum* instead of 1-octen-3-ol.

376 **4. Conclusions**

377 This study demonstrates the important role played by the family of C8 compounds in
378 the aroma of the *T. indicum* species. The abundance of the majority of these compounds
379 was already known (Splivallo et al., 2007b). However, for the first time a comparison
380 has been made between the aromatic profiles of *T. indicum* and *T. melanosporum*
381 including both the most relevant aromatic compounds and the composition in volatiles.
382 The olfactometric study (GC-O) shows the aromatic importance in the aromatic profile
383 of *T. indicum* of 1-octen-3-one and 1-octen-3-ol in particular, both having a
384 characteristic mushroom aroma. It is worth noting that while 1-octen-3-ol is the most
385 abundant compound (37.1%), 1-octen-3-one only constitutes 0.7% and yet from the
386 olfactometric point of view it is top of the list of key odorants. This shows the
387 importance of carrying out a GC-O study if a list of compounds according to their
388 aromatic relevance is required.

389 One of the most common frauds in this area is that many truffle-based products contain
390 fresh *T. indicum* with an artificial *T. melanosporum* aroma. These products thus contain
391 aromatic compounds characteristic of *T. indicum* truffles that are not relevant in *T.*
392 *melanosporum* ones.

393 It is therefore recommended that the GC-O method should be used and that particular
394 attention should be paid to the intensity of the C8 compounds, together with the sulfides
395 (DMS and DMDS), or that the HS-SPME-GC-MS analytical method should be used to

examine the profile of the volatiles and thus distinguish between these macroscopically similar species, *T. indicum* and *T. melanosporum*.

Acknowledgements

The authors thank the National Institute for Agricultural and Food Research and Technology (project RTA2010-00070-C02-02) for financial support.

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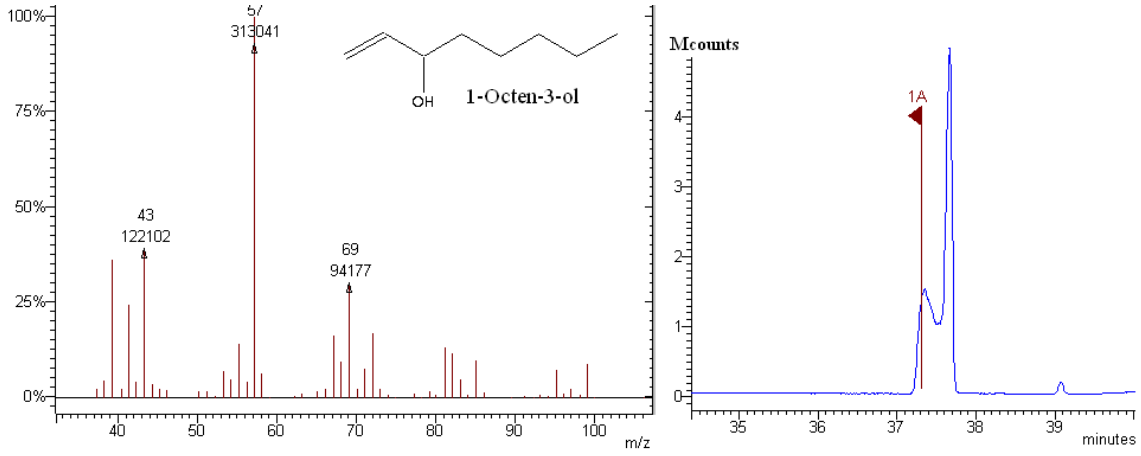
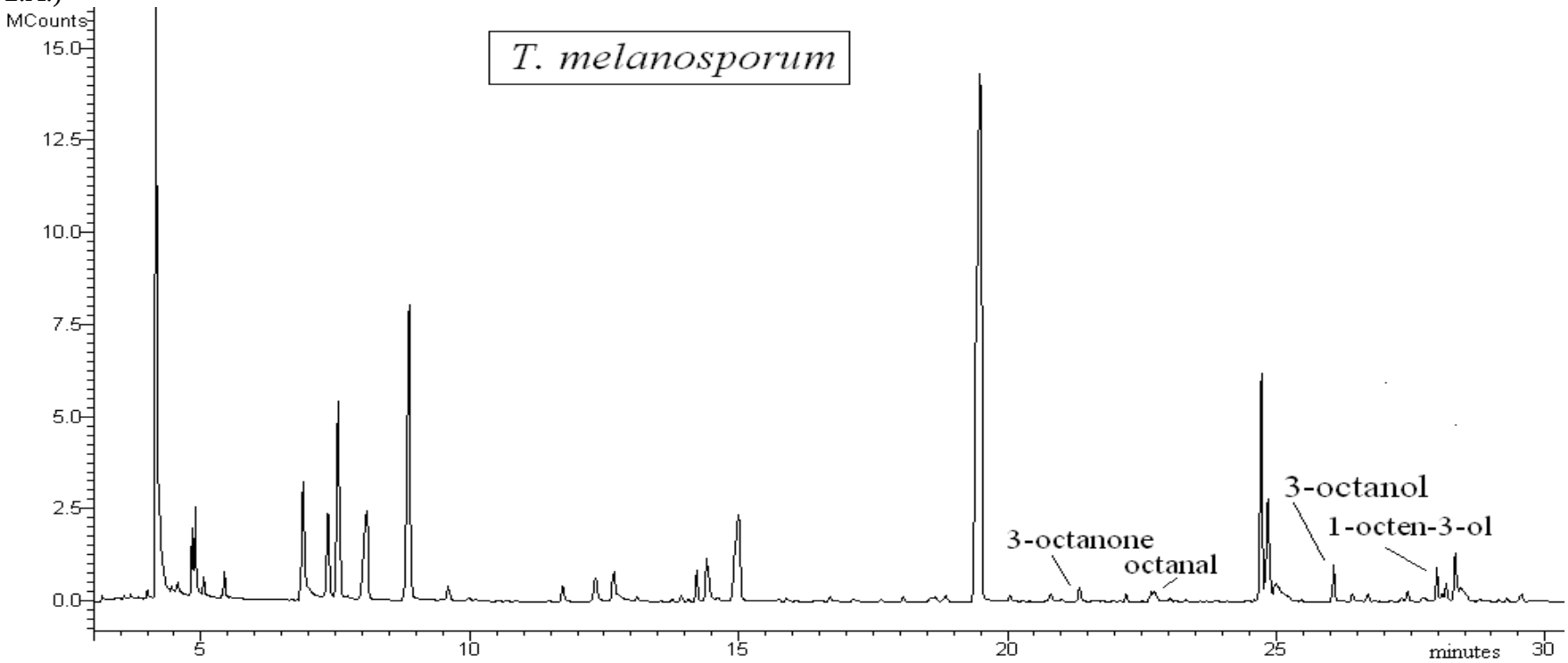
Figure captions

Figure 1. 1-Octen-3-ol spectrum (isolated from *T. indicum* truffles). Expanded MS chromatogram corresponding to the fraction isolated in the first column of the dual GC-O-GC-O-MS system.

Figure 2. Comparison of the chromatographic profiles obtained by HS-SPME-GC-MS of *T. melanosporum* (2.a.) and *T. indicum* (2.b.). The chromatogram in Figure 2.a. refers to samples of *T. melanosporum* analysed in a previous study (Culleré et al. (2012)).


100%
75%
50%
25%
0%
57
313041
43
122102
69
94177
OH
1-Octen-3-ol
40
50
60
70
80
90
100
m/z
Mcounts
4
3
2
1
0
1A
35
36
37
38
39
minutes

2.A.)


MCounts
15.0
12.5
10.0
7.5
5.0
2.5
0.0
T. melanosporum
3-octanone
octanal
3-octanol
1-octen-3-ol
5
10
15
20
25
30
minutes

2.B)

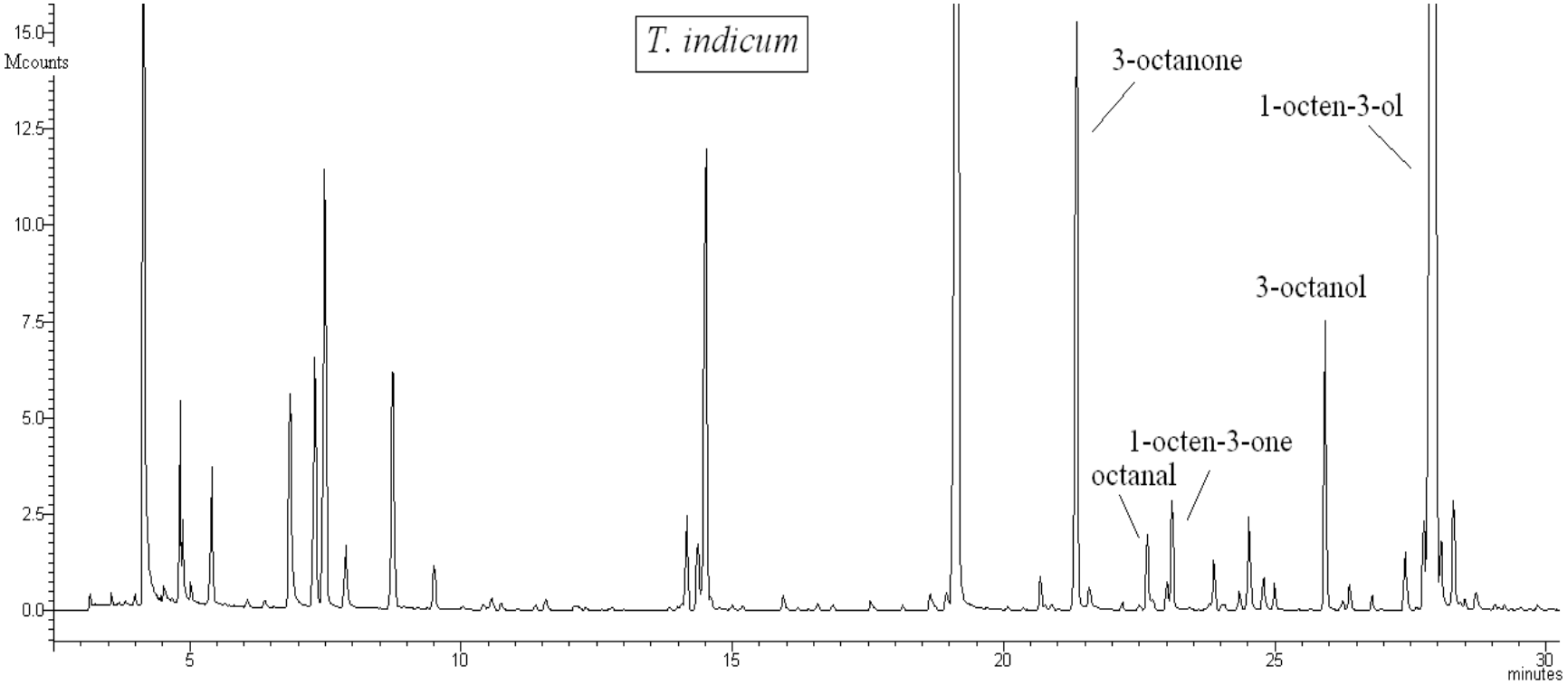

T. indicum
3-octanone
1-octen-3-ol
3-octanol
1-octen-3-one
octanal
Mcounts
15.0
12.5
10.0
7.5
5.0
2.5
0.0
5
10
15
20
25
30
minutes

Table 1. Gas chromatographic retention data, olfactory description, chemical identity and modified frequency percentage, MF(%), for each compound in *T. indicum* and *T. melanosporum* truffles.

				MF %	
LRI DBWax	**LRI** VF-5MS	**Odor descriptor**	**Identity**	***T. indicum***	***T. melanosporum*** [d]
< 900	505	Truffle, sulphur	Dimethylsulphide [a]	30	**73**
946	914	Truffle, sulphur	Dimethyldisulphide [a]	38	**76**
953	< 800	Fruit	Ethyl isobutyrate [a]	65	10
976	600	Butter, cream	2,3-Butanedione [a]	0	83
983	< 800	Fruit	Isopropyl acetate [b]	41	12
1044	801	Green apple	Ethyl butyrate [a]	0	76
1053	846	Strawberry	Ethyl 2-methylbutyrate [a]	53	12
1074	853	Fruit, anise	Ethyl 3-methylbutyrate [a]	0	35
1098	776	Metallic	1-Hexen-3-one [b]	0	35
1217	719	Cheese	3-Methyl-1-butanol [a]	53	62
1305	941	Mushroom	1-Octen-3-one [a]	**82**	16
1463	1025	Mushroom	1-Octen-3-ol [a+]	**69**	21
1521	1125	Leather	3-Ethyl-5-methylphenol [c]	0	44
2045	1096	Cotton candy	Furaneol [b]	0	33
2190	1198	Leather, animal	3-Ethylphenol [a]	0	30
2251	1292	Leather, animal	3-Propylphenol [a]	0	31

LRI: Linear retention index. [a] Identification based on coincidence of gas chromatographic retention in two different columns (DB-Wax and VF-5ms) and mass spectrometric data with those of the pure compounds available in the laboratory. [a+] Identification based on the use of a multidimensional system (GC-O-GC-O-MS).
[b] As for footnote [a], but these compounds did not produce any clear signal in the mass spectrometer because of their low concentration. [c] As for footnote [a], but in these cases pure compounds were not available in the laboratory.

[d] MF(%) data from *T.melanosporum* are published by Culleré et al. (2010)

Table 2. Volatile compounds present in *T.indicum* (HS-SPME-GC-MS analysis).

Compound	**Average area (%) (sd[**])**
1-Octen-3-ol *	37.1 (2.01)
Isoamyl alcohol *	13.9 (1.25)
Dimethylsulfide *	8.3 (1.04)
3-Octanone	4.6 (0.7)
2-Methylbutane	3.3 (0.4)
3-Methylbutanal	3.2 (0.1)
Z-5-Octen-1-ol	2.8 (0.1)
Octylcyclopropane	2.2 (0.2)
3-Octanol	2.1 (0.3)
2-Methyllbutanal	2.0 (0.1)
sec-Butylformate	1.8 (0.2)
2-Butanone	1.6 (0.06)
Benzeneacetaldehyde	1.1 (0.1)
m-Anisole	0.8 (0.10)
2,5-Dimethyl-3,4-hexanediol	0.7 (0.02)
Octanal	0.7 (0.06)
1-Octen-3-one *	0.7 (0.06)

* Odorants detected also as important from an olfactometric point of view, (sd[**]) standard deviation values.

Table 3. Odor threshold values in water and aroma descriptor of C8 volatiles.

C8 volatiles	Odor threshold in water (μg L^{-1})*	Aroma descriptor
3-octanone	28	mushroom-like
octanal	0.7	fatty/soapy
1-octen-3-one	0.005	mushroom-like
3-octanol	(n.f.)	mushroom-like/buttery
Z-5-octen-1-ol	(n.f.)	mushroom-like
1-octen-3-ol	1	mushroom-like

*Data from www.leffingwell.com, n.f.: data not found in the literature