

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

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12 **Running title:** *T. melanosporum*, *T. indicum*, black truffle, 3-octanone, 1-octen-3-one,
13 3-octanol, 1-octen-3-ol

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25 **ABSTRACT**

26 The *Tuber indicum* (Chinese truffle) and *Tuber melanosporum* (Black truffle) species
27 are morphologically very similar but their aromas are very different. The black truffle
28 aroma is much more intense and complex, and it is consequently more appreciated
29 gastronomically. This work tries to determine whether the differences between the
30 aromatic compounds of both species are sufficiently significant so as to apply them to
31 fraud detection. An olfactometric evaluation (GC-O) of the *T. indicum* specie was
32 carried out for the first time. Eight important odorants were identified. In order of
33 aromatic significance, these were: 1-octen-3-one and 1-octen-3-ol, followed by two
34 ethyl esters (ethyl isobutyrate and ethyl 2-methylbutyrate), 3-methyl-1-butanol,
35 isopropyl acetate, and finally the two sulfides dimethyldisulfide (DMDS) and
36 dimethylsulfide (DMS). A comparison of this aromatic profile with that of *T.*
37 *melanosporum* revealed the following differences: *T. indicum* stood out for the
38 significant aromatic contribution of 1-octen-3-one and 1-octen-3-ol (with modified
39 frequencies (MF%) of 82% and 69%, respectively), while in the case of *T.*
40 *melanosporum* both had modified frequencies of less than 30%. Ethyl isobutyrate, ethyl
41 2-methylbutyrate and isopropyl acetate were also significantly higher while DMS and
42 DMDS had low MF (30-40%) compared to *T. melanosporum* (> 70 %). The volatile
43 profiles of both species were also studied by means of headspace solid-phase
44 microextraction (HS-SPME-GC-MS). This showed that the family of C8 compounds (3-
45 octanone, octanal, 1-octen-3-one, 3-octanol and 1-octen-3-ol) is present in *T. indicum* at
46 much higher levels. The presence of 1-octen-3-ol was higher by a factor of about 100
47 while 1-octen-3-one was detected in *T. indicum* only (there was no chromatographic
48 signal in *T. melanosporum*). As well as showing the greatest chromatographic
49 differences, these two compounds were also the most powerful from the aromatic

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

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

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75 **1. Introduction**

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

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

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

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

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

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124 **2. Materials and Methods**

125 **2.1. Ascocarps of *Tuber indicum* and *Tuber melanosporum***

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

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

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136 **2.2. Reagents**

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

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

147 **2.3. Gas Chromatography-Olfactometry (GC-O)**

148 **2.3.1. Preparation of extracts.** Five ascocarps of *T. indicum* truffles were cut into thin
149 slices using a sharp knife and mixed in order to obtain a homogeneous sample that can
150 be considered as representative of this truffle species.

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

161 **2.3.2. GC-O analysis.** All sniffing experiments were carried out in a Trace gas
162 chromatograph from ThermoQuest, equipped with a flame ionization detector (FID) and
163 a sniffing port (ODO-1 from SGE) connected by a flow splitter to the column exit. The
164 column was a DB-WAX from J&W (Folsom, CA, USA), 30m, 0.32mm i.d., 0.5 mm
165 film thickness. A constant pressure of 52 kPa was maintained throughout the analysis
166 time. The carrier was H₂. Two microlitres were injected in splitless mode for 1min
167 splitless time. Injector and detector were both kept at 250 °C. The temperature program
168 was 40 °C for 5 min, then raised by 4 °C min⁻¹ to 100 °C followed by 6 °C min⁻¹ to 220
169 °C, and finally kept at this temperature for 20 min. To prevent condensation of high
170 boiling compounds on the sniffing port, the port was heated sequentially with a
171 laboratory-made rheostat.

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

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

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185 **2.3.3. Identification of 1-octen-3-ol as responsible for the odor zone at LRI_{DB-WAX}**
186 **1463 by a multidimensional system (GC-O-GC-O-MS).**

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

197 was 40 °C for 5 min, then raised by 4 °C min⁻¹ to 100 °C, followed by 6 °C min⁻¹ to 220
198 °C, and finally held at this temperature for 40 min. Initially, the GC–O extract (50 µL)
199 was monitored by olfactometry in the first chromatograph to select the fraction
200 containing the target odorant. In further chromatographic runs, selective heart-cutting
201 was made to isolate the odorant of interest which was transferred to the second
202 chromatograph equipped with a Factor Four VF-5MS column from Varian (30 m x 0.32
203 mm; 1 µm film thickness). In this second oven, the isolated odorant was trapped in a
204 CO₂ cryotrapping unit and monitored by olfactometry with simultaneous MS detection.
205 Two minutes after the heart-cutting, the CO₂ flow was removed at the same time that
206 the temperature program (4 °C min⁻¹ up to 200 °C and then 50 °C min⁻¹ up to 300 °C) of
207 the second oven was activated. The MS parameters were: transfer line 170 °C; ion trap
208 150 °C, and trap emission current 30 µA. The global run time was recorded in full
209 scan mode (m/z 40–250 mass range). FID and MS data were registered and processed
210 with Workstation 6.30 software equipped with the NIST 98 (US National Institute of
211 Standards and Technology) MS library (NIST, Gaithersburg, MD). The programmable
212 temperature vaporising injector (PTV) conditions, delay time and heart-cutting interval
213 were the same as those used in a previous paper (Culleré, Escudero, Pérez-Trujillo,
214 Cacho, & Ferreira, 2008).

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

218 **2.4. Headspace solid-phase microextraction (HS-SPME)**

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

222 temperature (53°C) had been reached, the vial was allowed to condition for the
223 equilibrium time (5 min). After this time, the fiber (a 50/30 µm layer of
224 divinylbenzene/carboxen/polydimethylsiloxane from Supelco) was introduced into the
225 vial and exposed to the headspace of the sample during 13.6 minutes. Thermal
226 desorption of the compounds from the fiber coating took place in the GC injector at 200
227 °C for 15 minutes.

228 **2.5. Gas Chromatography-Mass Spectrometry (GC-MS) conditions**

229 The analyses were performed with a CP-3800 chromatograph coupled to a
230 Saturn 2200 ion trap mass spectrometric detection system from Varian (Sunnyvale, CA,
231 USA). A DB-WAXETR capillary column (J&W Scientific, Folsom, CA, USA) of 60m x
232 0.25 mm I.D., film thickness 0.25 µm was used, preceded by a 3 m x 0.25 mm uncoated
233 (deactivated, intermediate polarity) precolumn from Supelco (Bellefonte, PA, USA).
234 Helium was the carrier gas at a flow rate of 1 mL min⁻¹. The oven temperature was
235 initially 40°C during 5 minutes, then raised by 4°C min⁻¹ to 140°C, followed by a rate of
236 10°C min⁻¹ to 220°C and finally held at this temperature for 10 minutes. The MS
237 transfer line and chamber ionization temperature was 200°C, and the trap emission
238 current was 80 µA. The global run time was recorded in full scan mode (45-250 m/z
239 mass range). The injection was in splitless mode (splitless time 5 min) at a temperature
240 of 200°C. A desorption time of 15 minutes was used.

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

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

247 variability associated to each truffle. The resulting reproducibility was satisfactory,
248 given that an SPME technique was used and, moreover, that some of the compounds
249 were studied in very low concentrations. Thus, relative standard deviations values (RSD
250 %) lower than 10% were obtained in all cases.

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272 3. Results and discussion

273 3.1. Semiquantitative GC-O experiments

274 The results of the experiments carried out with *T. indicum* samples are
275 summarized in Table 1. This table provides the chromatographic retention data of the
276 different aromatic compounds detected in the olfactometric experiments, the odor
277 description of these compounds given by the trained sensory panel, the chemical
278 identity of the odorant responsible for the odor and the olfactometric scores (as
279 modified frequency in %) given by the sensory panel. Only the odorants that reached a
280 MF(%) higher than 30% in *T. indicum* truffles are included in the table. The extracts for
281 the olfactometric experiments were obtained following a mild procedure trying to
282 imitate as closely as possible the exact aroma composition of the vapors emitted by the
283 truffles. Therefore, the MF(%) of the odorants given in the table should give a
284 reasonable estimate of the potential importance of the compound in the aroma profile of
285 each truffle species. As can be seen, eight odorants can be considered as relevant from
286 an aromatic point of view. The most important of them was the 1-octen-3-one,
287 characterized by mushroom nuances, given its high modified frequency (82%). This
288 was followed by 1-octen-3-ol (69%), ethyl isobutyrate (65%) and 3-methyl-1-butanol
289 and ethyl 2-methylbutyrate (both with 53%). The identification of the compound
290 responsible for the aromatic zone described as having a clear odor of mushroom with
291 LRI_{DB-WAX} 1463 required the help of a multidimensional chromatographic system (GC-
292 O-GC-O-MS), following the procedure described above. This aroma may be explained
293 by the 1-octen-3-ol or else by the 1-nonen-3-one, given that both compounds are eluted
294 in zones which are quite close in both columns (especially in DB-WAX). After
295 capturing the zone of interest in the dual system and transferring it to the second
296 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

322 a potential signal molecule produced by both truffle mycelium and fruiting bodies
323 (Menotta, Giocchini, Amirucci, Buffalini, Sisti, & Stocchi, 2004; Splivallo, Novero,
324 Berteza, Bossi, & Bonfante, 2007b), as well as by most other fungi. At high
325 concentrations it shortens the primary root and exerts generally toxic effects on plants,
326 inducing the loss of chlorophyll, probably through oxidative stress (Splivallo et al.,
327 2007b). At lower concentrations, this compound has been reported to induce plant
328 defense genes (Kishimoto, Matsui, Ozawa, & Takabayashi, 2007), potentially
329 modulating the fitness of the host plant. **3)** Therefore, both odorants (1-octen-3-one and
330 1-octen-3-ol) could be considered as markers of *T. indicum* and thus avoid confusion
331 between the two species and prevent possible fraud. **4)** Another noteworthy difference is
332 that the sulphides, dimethylsulfide and dimethyldisulfide, have been detected in *T.*
333 *indicum* with much lower modified frequencies (<40% MF) than those found in *T.*
334 *melanosporum* (>70% MF), as would be expected in the light of the work of Splivallo et
335 al., (2007a). **5)** Finally, it is important to mention the exclusive presence of three
336 phenols in the olfactometric profile of *T. melanosporum*: 3-ethyl-5-methylphenol, 3-
337 ethylphenol and 3-propylphenol. These could also be considered as aroma markers for
338 this species of black truffle.

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

342 **3.2. Volatile composition analysis by Head Space Solid-Phase Micro** 343 **Extraction (HS-SPME)**

344 A more detailed study of the volatile composition of *T. indicum* was carried out
345 in addition to the olfactometric analysis. Table 2 shows the data obtained (expressed in
346 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 $\mu\text{g L}^{-1}$,
357 followed by octanal with 0.7 $\mu\text{g L}^{-1}$ and 1-octen-3-ol (1 $\mu\text{g L}^{-1}$). 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 $71 \pm 17\%$ in *T. indicum*, whilst
373 in the case of *T. melanosporum* the contribution was only $52 \pm 15\%$. 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

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

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513 **Figure captions**

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

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

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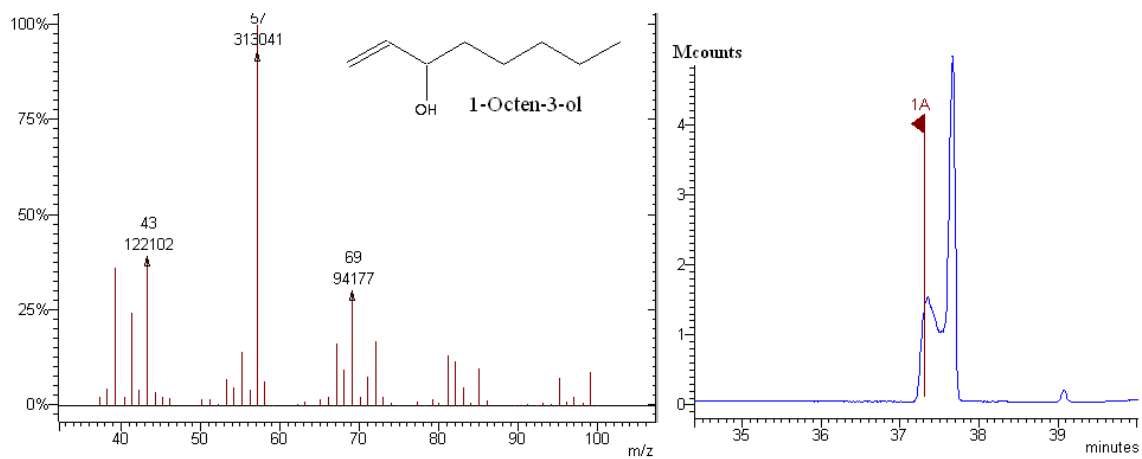
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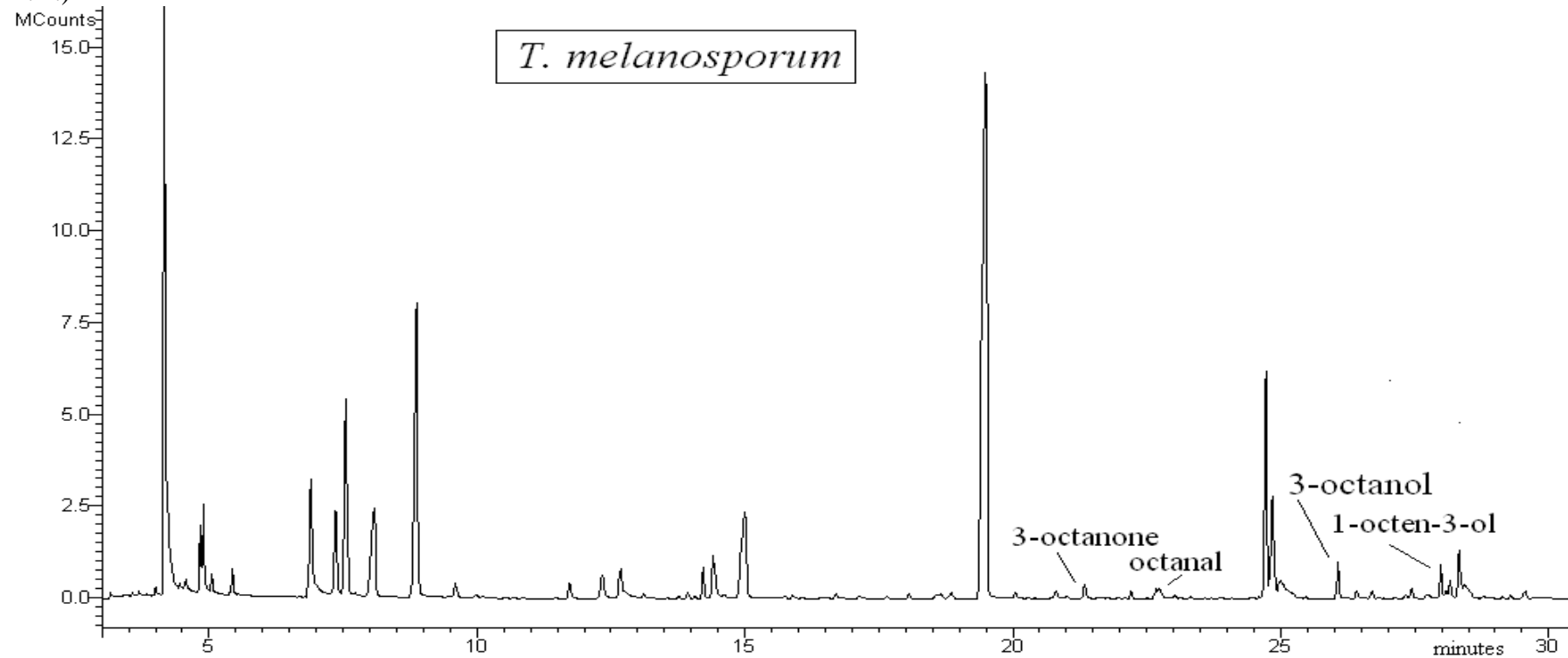
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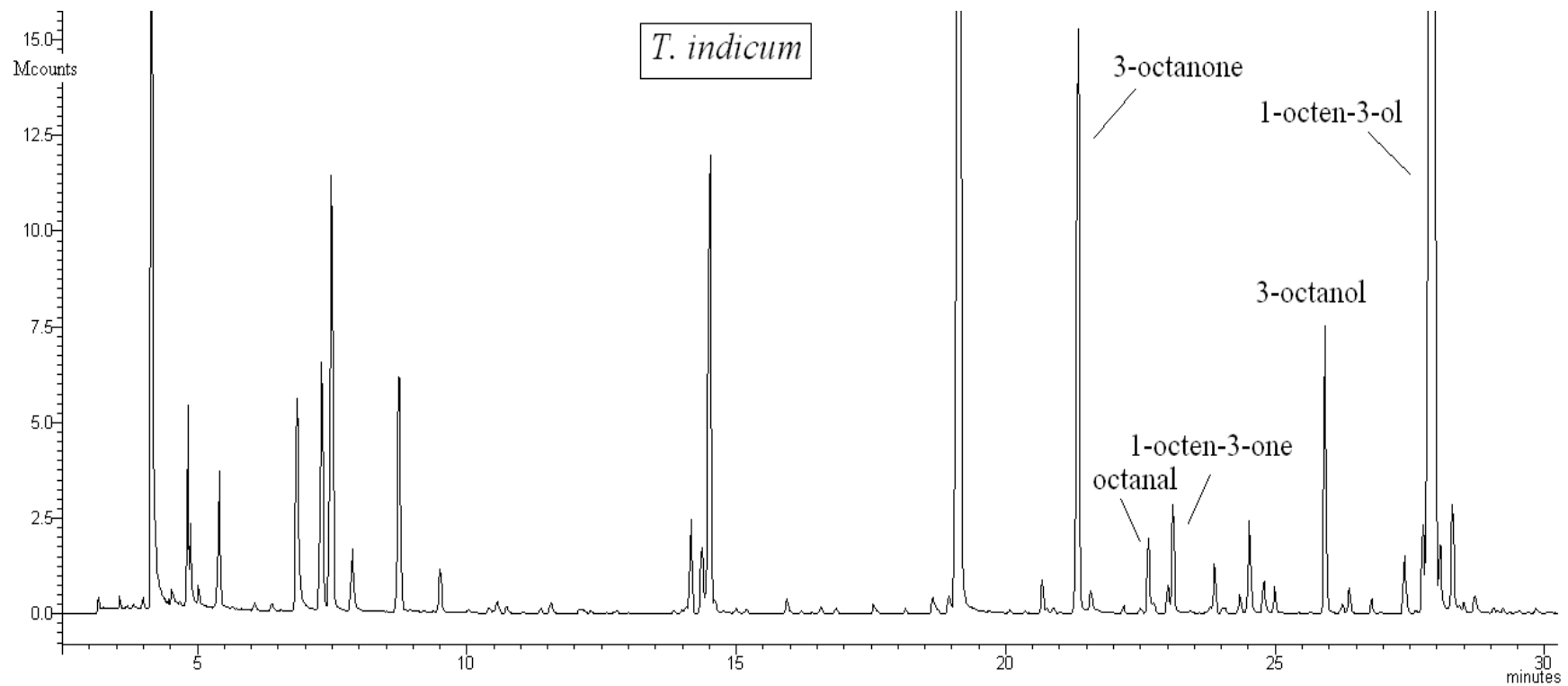
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Table 1. Gas chromatographic retention data, olfactory description, chemical identity and modified frequency percentage, MF(%), for each compound in *T. indicum* and *T. melanosporum* truffles.

LRI DBW _{ax}	LRI VF-5MS	Odor descriptor	Identity	MF %	
				<i>T.</i> <i>indicum</i>	<i>T.</i> <i>melanosporum</i> ^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-Methylbutanal	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 ($\mu\text{g L}^{-1}$)*	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