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What is the best method for preserving the genuine black truffle (*Tuber melanosporum*) aroma? An olfactometric and sensory approach

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RUNNING TITLE: Aroma changes in preserved black truffles

26 **Abstract**

27 The aim of this work was to evaluate the effects of different preservation methods
28 (freeze-drying, hot-air drying, freezing and canning) on the aroma profile of *T.*
29 *melanosporum* truffles. Volatile organic compounds (VOCs) were extracted by solid-
30 phase microextraction (SPME) and analysed by gas-chromatography olfactometry to
31 monitor changes occurring in key-aroma compounds. Samples were also submitted to
32 descriptive sensory analysis by a panel of trained judges, with the aim of correlating
33 both sets of data. Freeze-drying – and to a lesser extent hot-air drying – were the only
34 treatments able to retain key-compounds such as dimethylsulphide (DMS) and
35 dimethyldisulphide (DMDS), evoking the aroma typically associated with fresh truffle.
36 Principal component analysis (PCA) performed on the descriptive data showed the
37 sensorial proximity between fresh and freeze-dried truffle, and also the differences
38 between them and those frozen and canned. Despite some differences in the odour
39 volatile profile of fresh and freeze-dried truffles (mainly the lack of 2,3-butanedione
40 and branched ethyl esters), freeze-drying is the most suitable technique for preserving
41 the overall original aroma of fresh truffle. Several key-odour compounds – mainly
42 unsaturated linear chain carbonyl compounds, sulphur and pyrrole derivatives – emerge
43 as biomarkers of the studied technologies.

44 *Keywords:* freeze-drying, hot-air drying, freezing, canning

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49 **1. Introduction**

50 *Tuber melanosporum*, known as the “black”, “winter” or “Périgord” truffle and
51 commonly referred to as the “black diamond of cuisine”, is one of the most highly
52 appreciated truffle species. Due to its intense and complex aroma, *T. melanosporum* is
53 considered the queen of truffles, and is one of the most highly prized foods worldwide.
54 Despite the fact that more than 300 volatiles have been described from about eleven
55 species to date (Splivallo, Ottonello, Mello, & Karlovsky, 2011) only a few actually
56 play an active role on the aroma of *T. melanosporum* (Culleré et al., 2010). Whether
57 truffles or microbiomes, the so called microbial communities inhabiting fruiting bodies,
58 are responsible for shaping the aroma of a given species is currently a source of debate
59 (see Vahdatzadeh, Deveau, & Splivallo, 2015 and Splivallo, & Cullere, 2016). These
60 works highlighted that volatiles common to several truffle species may be of mixed
61 origin while more specific ones may strictly be derived from microbes (mainly
62 bacteria).

63 Truffles exhibit their maximum sensorial properties when fresh. With a shelf-
64 life of 7-10 days, truffles quickly lose their flavour intensity and start to spoil. In recent
65 years, some common postharvest preservation technologies have been tested to extend
66 postharvest shelf-life. For example, the combination of a decontamination step with
67 modified atmosphere packaging prolonged the shelf-life of *T. melanosporum* from 14 to
68 28 days (Rivera, Venturini, Oria, & Blanco, 2011a). Gamma and electron-beam
69 ionizing radiation have also been used to significantly reduce the microorganisms
70 present in the peridium and therefore minimize the microbial growth (Rivera, Venturini,
71 Marco, Oria, & Blanco, 2011b). These irradiation treatments did not improve the shelf-
72 life of *T. melanosporum* truffles beyond one month, which is insufficient to satisfy the
73 continuous demand of black truffle throughout the year.

74 Such limitations beg for long-term preservation technologies. Canning (C) is a
75 simple, common long-term preservation method usually employed by companies
76 dedicated to the production and commercialization of truffles. However, the
77 consequences of the thermal treatment for the organoleptic properties of these ascocarps
78 are severe. Their texture becomes soft, the gleba veins disappear and the aroma changes
79 dramatically, resulting in a heat-treated product which is barely reminiscent of the
80 original (Murcia et al., 2003). Hot air drying (HAD) or dehydration of truffles is
81 another classical preservation method that reduces the water content and microbial
82 growth, slowing enzymatic and chemical activities. However, this method is not exempt
83 from aroma quality depreciation (Al-Ruqaie, 2005). Freezing (FZ) is a long-term
84 storage technology frequently applied to truffles, but it has some limitations with
85 respect to aroma quality, which is seriously affected. Research by (Culleré, Ferreira,
86 Venturini, Marco, & Blanco, 2013) revealed that after only 24 h, frozen samples were
87 richer in diacetyl, 1-octen-3-one, 1-octen-3-ol, 2-methylisoborneol and
88 dimethyltrisulphide, and poorer in isoamyl alcohol, ethyl 3-methylbutyrate and
89 methanethiol.

90 In light of the observed limitations, freeze-drying (FD) or lyophilisation could
91 be an interesting alternative to these traditional preservation methods. Although it is an
92 expensive technique when compared to traditional dehydration methods, it provides
93 higher quality products with minimal nutritional and organoleptic changes. In the case
94 of *Pleurotus eryngii*, lyophilisation better maintained the quality of tasty compounds in
95 the processed product compared to dehydration (Li et al, 2015). Palacios, Guillamon,
96 García-Lafuente, & Villares, 2012) showed that some of the volatile compounds were
97 lost after the lyophilisation of *T. melanosporum* but were almost totally recovered after
98 rehydration.

99 Some works have investigated the influence of preservation methods on the
100 physico-chemical and microbial parameters in truffles. Pennazza, Fanali, Santonico,
101 Lugo, Cucchiarini, Dachà et al. (2013) studied the volatile composition of *Tuber*
102 *magnatum Pico* under different storage conditions (wrapped in blotting paper and
103 covered by rice at 4°C and 8°C). These authors monitored the abundance of a total of 84
104 volatile compounds by means of head-space solid phase micro extraction (HS-SPME)
105 coupled to gas chromatography – mass spectrometry. Saltarelli, Ceccaroli, Cesari,
106 Barbieri, & Stocchi (2008) evaluated possible alterations during truffle preservation
107 (frozen and sterilised by autoclave) in terms of the biochemical and microbiological
108 profiles of several species, including *T. melanosporum*. However, as far as we are
109 aware there are no previous studies providing a simultaneous comparison of the
110 influence of different technologies on volatiles (in terms of both number and nature)
111 relevant for the aroma perceived by humans, which can only be addressed by means of
112 olfactometric studies in combination with sensory analysis.

113 Therefore, the aim of this study was to evaluate the impact of canning,
114 dehydration, freezing and freeze-drying preservation methods on the odour compounds
115 and aromatic profile of black truffles compared to the original fresh product, in an
116 attempt to identify which technology would be the most successful for preserving the
117 genuine truffle aroma. For this purpose, a dual olfactometric and sensory analysis
118 approach was employed.

119

120 **2. Material and Methods**

121 *2.2. Truffle collection and processing*

122 *T. melanosporum* ascocarps (n=50; approximately 25 g each) were collected in
123 cultivated truffle-grounds under holm oak trees (*Quercus ilex* subsp. *ballota*) in Sarrión

124 (Teruel, Spain), with the help of a trained dog. The truffles were harvested in January
125 and shipped to the laboratory with covering soil in insulated boxes with ice packs. The
126 samples were brushed with a wet soft brush, rinsed with tap water and forced-air dried
127 for 15 min in a laminar cabinet. A qualitative selection of the ascocarps was made by
128 discarding truffles with softened texture, coleopteran larvae or damaged during the
129 harvest. Maturity was determined for each fruiting body by microscopic observation
130 and calculating the ratio between the number of asci containing melanized spores and
131 the total number of asci. The degree of maturation of the ascocarps was defined using
132 the following categorised stages, on the basis of the percentage of asci-containing
133 mature spores: stage 0 = 0–5%, stage 1 = 6–30%, stage 2 = 31–70%, and stage 3 = 71–
134 90% (Zeppa et al., 2002). The maturation stage of the spores was defined by a
135 morphological method.. The mature spores are dark, dull brown, have an ellipsoidal
136 shape and are decorated with very sharp spines, often curved, 2-3 (5) microns in size.

137 Ten truffles were arranged in five polypropylene trays (250 mL) (Borden, S.A.,
138 Alicante, Spain) each containing two ascocarps. The upper part of the package (96 cm²)
139 was heat sealed with a microperforated film (two 90 × 50 μm holes) (Amcors Flexibles,
140 Ledbury, U.K.) to achieve internal atmosphere gaseous concentrations of approximately
141 10% CO₂/10% O₂ at 4 °C. These conditions decrease the truffle metabolism and the
142 microbial growth rate and also delay the development of superficial mycelial growth,
143 avoiding the presence of off-odours and maintaining the characteristic aroma very
144 close to that of the freshly harvested truffles (Rivera, Blanco, Salvador, & Venturini,
145 2010). The rest (n=40) of the ascocarps were sliced into about 2–3 mm and mixed
146 together in order to obtain a pooled sample. 10 g of this fresh sliced pool was
147 immediately submitted to olfactometric analysis as described in section 2.3. The

148 sampling pool was then divided into four portions (around 250 g each) at random.

149 These were processed by different preservation methods:

150 a) **Canning (CA)**: slices were placed in 50 mL glass jars (20 g per jar) and 20 mL
151 of hot (85 °C) distilled water was added. The jars were then airtight sealed and
152 autoclaved (Micromar-Mini autoclave, Marrodán, Lodosa, Spain) at 121 °C for
153 30 min.

154 b) **Hot air-drying (HAD)**: slices were laid on perforated trays in a forced air
155 convection oven (Digitronic-TFT, Selecta, Barcelona, Spain) and dried at 50 ± 1
156 °C with maximum air speed. The drying samples were weighed each hour until
157 the moisture content remained unchanged. They were then equilibrated to room
158 temperature and vacuum-packed in polyethylene bags (Oriented
159 Polyamide/Polypropylene, 15/65, 80 µm (Orved, Musile di Piave, Italy) with a
160 VM-12 vacuum sealer (Tecnotrip, Barcelona, Spain) until analysis.

161 c) **Freezing (FZ)**: slices were vacuum-packed as described above and frozen at -
162 80 °C in a MDFU3286S freezer (Sanyo Electric Co., Tokyo, Japan).

163 d) **Freeze-drying (FD)**: slices were placed in a freeze drier (HETO DW8,
164 Barcelona, Spain) and frozen at -20 °C for 15 min, and then dehydrated for 28 h
165 (primary drying at -5 °C for 2 h, 0 °C for 4 h, 5 °C for 4 h, 10 °C for 4 h, 15 °C
166 for 4 h and 20 °C for 4 h, and secondary drying at 25 °C for 4 h). The truffle
167 samples were then vacuum-packed as explained above until analysis.

168 For each preservation treatment, five sub-portions (≈ 50 g) were separately packed
169 for use in the sensory training. All processed samples were stored for fifteen days.
170 Regarding sample conditioning prior to olfactometric and sensory analysis, the
171 dehydrated and freeze-dried samples were rehydrated by adding Mili-Q water (3 mL
172 per truffle gram) and incubated for 10 min at room temperature in order to favor water

173 absorption. The frozen truffles were tempered to room temperature before opening the
174 vacuum package.

175 *2.3. Analysis of odor-compounds*

176 *2.3.1. Preparation of aroma extracts by SPME*

177 The methodological approach was based on works carried out by Culleré,
178 Ferreira, Venturini, Marco, & Blanco (2012). A fused silica fiber coated with a 50/30
179 μm layer of divinylbenzene/carboxen/polydimethylsiloxane from Supelco (Barcelona,
180 Spain) was chosen to extract the aromatic compounds. Two grams of finely sliced
181 truffle (around 2 mm thick) were placed in a 20 mL glass vial closed with a septum and
182 conditioned at 53 °C for 5 min. The fiber was then exposed to the headspace of the
183 truffle for 13.6 min. In all cases GC-O analysis was carried out immediately after
184 sampling. A total of four SPME extracts were prepared per preservation method, one
185 per GC-O judge.

186 *2.3.2. Gas chromatography-olfactometry*

187 GC-O analysis was carried out in a gas chromatograph HP 4890 (Termost,
188 Milan, Italy) with a flame ionization detector (FID) and an olfactometric port ODO-I
189 supplied by SGE (Ringwood, Australia). This instrument was equipped with a capillary
190 column DB-WAX (polyethylene glycol) supplied by J&W Scientific (Folsom, CA) of
191 30 m, 0.32 mm i.d., 0.5 μm film thickness, and a precolumn (3 m; 0.32 mm i.d.) from
192 Supelco (Bellefonte, PA). The chromatographic conditions were: nitrogen as the carrier
193 (3.5 mL min^{-1}); splitless injection (splitless time 60 s); injector and detector temperature
194 220°C. The oven temperature program was: 40 °C for 5 min, then raised at $6 \text{ }^\circ\text{C min}^{-1}$ to
195 220 °C, maintained during 15 min for cleaning purposes.

196 A panel of four judges (two women and two men, ranging from 29 to 45 years
197 of age) with long experience in olfactometry performed the sniffing analysis. They were

198 asked to provide a descriptor of each eluted odor and to rate it using a 7-point intensity
199 scale (0 = no odor; 1 = weak, low intensity; 2 = clear perception, strong intensity; 3 =
200 extremely strong; intermediate values of 0.5, 1.5, and 2.5 being allowed).

201 The data processed were a mixture of the intensity and the frequency of
202 detection of an odorant. This parameter is known as “modified frequency” (MF) and is
203 calculated with the formula proposed by Dravnieks (1985): $MF (\%) = [F (\%)*I (\%)]^{1/2}$,
204 where F (%) is the detection frequency of an aromatic odorant expressed as a
205 percentage of the total number of judges and I (%) is the average intensity expressed as
206 a percentage of the maximum intensity. This strategy provides data of semiquantitative
207 value and makes it possible to identify potentially important aroma compounds in
208 truffle (Culleré et al., 2010). The odorants were identified by comparison of their odors
209 and chromatographic retention index in a DB-WAX column with those of pure
210 reference compounds, when available. Additionally, the identity of compounds was
211 checked by comparing the sequence of LRI with that of other published databases. In
212 particular, we used the database compiled for Styrian pumpkin seed oil (Poehlmann &
213 Schieberle, 2013), as many of our target, low-odour threshold volatiles were previously
214 detected in samples of this pumpkin seed oil.

215 2.4. Sensory analysis

216 2.4.1. Panel training and formal measurements.

217 Seven truffle experts (producers, retailers and food scientists) were trained in the
218 aromatic description of fresh and preserved truffles during five 1-h sessions following
219 the ISONORM 11035. In the first session, the tasters evaluated 8 samples of fresh and
220 preserved truffles to generate the most pertinent aroma terms. This preliminary list was
221 presented to the panelists in the second session during which the attributes of the same
222 samples were assessed, this time using a 10-point scale ranging from 0 (not present) to

223 10 (very intense). Principal component analysis (PCA) was performed to visualize
224 correlations among terms (synonyms and antonyms), and the results were shown to the
225 panelists in the third session. This was divided into two parts. First, they compared their
226 individual responses from the former session with the average value given by the rest of
227 the panelists, which helped in concept alignment. Secondly, they discussed the
228 pertinence of the attributes and agreed on the terms of the final list, which included:
229 “global aroma intensity”, “truffle-like typical aroma”, “black olives”, “mushroom”,
230 “animal-leather”, “baked potato” and “nut-seeds”. In session four, different aroma
231 references were provided to illustrate the terms on the list. In case of disagreement
232 among panelists, a discussion was established until a consensus was achieved. Session
233 five was devoted to the evaluation of 5 truffles in duplicate. From these data, the
234 panel’s performance was checked regarding the ability to discriminate among products
235 and in terms of reproducibility and the homogeneity of the panel in the use of the
236 descriptors, as described by Campo, Ballester, Langlois, Dacremont, & Valentin
237 (2010). Based on these indicators, the panel was deemed successfully trained. A final
238 session was devoted to evaluating, in duplicate, the 5 truffle samples of the study. All
239 the samples were presented in closed opaque containers coded with a three-digit
240 number, in random order. Participants were not aware of the nature of the truffle
241 samples under study.

242 2.4.2. Data analysis

243 *Discriminant attributes:* A one-way analysis of variance (ANOVA) in which the
244 preservation method (n=5) was the factor and the judges (n=7; average of two
245 replicates) were considered as repetitions was performed on the descriptive analysis
246 data. All analyses were performed with SPSS 15.0 (SPSS Inc., Chicago, IL, USA). For

247 attributes exhibiting a “preservation treatment” effect, a Duncan test ($P = 0.05$) was run
248 in order to establish differences between means.

249 *Product characterization:* Standardized Principal Component Analysis (PCA) was
250 performed on the mean ratings of the odour compounds identified by GC-O and each
251 type of truffle (correlation matrix). In order to incorporate sensory information in the
252 PCA plot, the average attribute’s score for each sample was considered as a
253 supplementary variable in the PCA dataset. Analyses were carried out with SPAD
254 software (version 5.5, CISIA-CERESTIA, Montreuil, France).

255

256 **3. Results and Discussion**

257 *3.1. Prior considerations regarding truffle sampling*

258 Sampling for GC-O and sensory analysis was made by mixing slices from
259 different ascocarps ($n=40$ for truffles submitted to preservation) in a composite pool.
260 Several works have shown that volatile organic compounds (VOCs) can vary between
261 and within the same truffle species and harvesting origin. Splivallo et al., (2012)
262 showed that in an orchard of *T. uncinatum*, truffles producing different concentrations
263 of C8-VOCs clustered around distinct host trees as a consequence of fungal genotype.
264 More recent works by Splivallo et al., (2015a) and Splivallo & Ebeler (2015b) show
265 that bacteria associated with truffle-ascocarps contribute to truffle aroma, and in
266 particular sulphur-containing (see line 41) volatiles such as thiophene derivatives. The
267 presence of a diverse bacterial community inside *Tuber spp.* on gleba tissue was also
268 recently shown by Benucci & Bonito (2016). Hence these findings suggest that the
269 laboratory sampling technique may have an influence on the odour profile derived from
270 our study. Independently of variations among ascocarps, truffles exhibit their maximum
271 sensorial properties as a fresh product. It is expected, therefore, that preservation

272 methods will be responsible for major changes in the aroma of truffles allocated to
273 conservation. Mixing different ascocarps in a composite pool reflects, nevertheless, the
274 reality of what often happens in the truffle industry. Since a mix of ascocarps of
275 different geographical origins is likely to occur among batches, our sampling approach
276 aims to provide a real picture of the major differences observed between fresh and
277 preserved commercial truffles.

278 *3.1. Differences in the odour profile of fresh and preserved truffles*

279 The major goal at this stage was to screen the odour composition of fresh black
280 truffle and its homologues preserved under four conditions (canning, dehydration,
281 freezing and freeze-drying) to examine the influence of the preservation process on the
282 chemical odour profile of the samples. Table 1 presents the chemical identity, odour
283 description, chromatographic retention data and abundances of the detected odorants
284 with MF (%) >25. Only three compounds in the table were common to the five
285 samples; 1-octen-3-one, methional and E,E-2,4-nonadienal.

286 ***Fresh (Control)***. This sample presented the most complex profile in terms of odorants
287 (17 in total) and chemical families. Two sulphur compounds exhibiting black olive
288 aromas, dimethylsulfide (DMS) and dimethyldisulfide (DMDS), received very high
289 scores. In particular, DMDS reached almost a 100% MF, which means that it was
290 perceived by all the judges at the maximum intensity. DMS and DMDS have been
291 reported in most truffle species and are thought to derive from the catabolism of L-
292 methionine through the Ehrlich pathway (Splivallo et al., 2011; Liu et al., 2013). A
293 similar pattern was observed for ethyl 2-methylbutyrate. It is important to point out that
294 some compounds were only detected in the fresh sample: acetic acid, β -phenylethanol,
295 δ -decalactone, and an unknown compound (LRI= 1555) with a potent aroma of
296 Roquefort-type cheese (MF=67%). The odour diversity of these four compounds

297 contributes to the complexity of fresh truffle aroma. Culleré et al., (2010) identified β -
298 phenylethanol as one of the key-odorants of *T. melanosporum*. In baker's yeast it is
299 derived from the catabolism of phenylalanine amino acid through the Ehrlich pathway
300 (Hazelwood, Daran, Maris, Pronk, & Kickinson, 2008). Despite the fact that candidate
301 genes potentially involved in this biosynthetic route were proposed for *T.*
302 *melanosporum* in 2010 (Martin et al., 2010), the precise Ehrlich pathway has not yet
303 been characterized in truffles.

304 **Freeze-drying (FD).** This sample presented similar odour intensities of DMS and
305 DMDS (above 80% MF) to that of the control sample, which indicates that the freeze-
306 drying treatment was successful for preserving both sulphur compounds. Palacios et al.,
307 (2011) reported that the freeze-drying process was able to retain DMS, but not DMDS,
308 on lyophilized samples. Freeze-dried truffle was the only sample with 3-ethylphenol. In
309 contrast, there are some differences with respect to the fresh product. For example, no
310 judge detected the volatiles with creamy and fruity notes (2,3-butanedione and
311 branched ethyl esters, respectively). Other volatiles were very intense in the freeze-
312 dried truffle, mainly aldehydes: 2-methylbutanal, hexanal, Z-4-heptanal and especially
313 methional. In a work studying the effects of freeze-drying on the aromatic profile of
314 *Tuber* spp. truffles (*T. melanosporum*, *T. indicum* and *T. aestivum*), these authors
315 concluded that the *T. melanosporum* aroma profile was little affected by the freeze-
316 drying process, which is somewhat inconsistent with the data observed here. This may
317 be due to the fact that the authors analysed truffle aroma in terms of quantitative mass
318 spectral data, and not through olfactory-based screening techniques, which makes it
319 difficult to detect molecules of low odour thresholds present at trace levels. 2-acetyl-
320 pyrroline, a powerful compound well-known for its implications in roasted aroma,
321 deserves special attention (Hoffmann & Schieberle, 1998). This molecule exhibits a

322 characteristic popcorn-like odour and an extremely low odour threshold of 0.02 ng/L in
323 air (Schieberle, 1995), which gives an idea of its aroma potential, as well as the benefits
324 of using an olfactometric approach on this work.

325 **Hot air drying (HAD).** This was characterized by the absence of high volatility
326 compounds, except for E,E-2,4-nonadienal. Similarly to freeze-drying, dehydration was
327 successful in preserving low volatile sulphur compounds – although intensity scores
328 were much lower for this sample (61 and 32 % MF, respectively) – and enhancing 2-
329 acetyl-1-pyrroline. Dehydration, together with freeze-drying, presented important levels
330 of the branched chain aldehyde 2-methylbutanal.

331 **Freezing (FZ).** Some compounds are probably induced or degraded as a consequence
332 of a freezing step, irrespective of whether the method is freeze-drying or conventional
333 freezing. Both samples lacked 2,3-butanedione and ethyl esters. Truffles produce
334 numerous C8 volatiles with a characteristic fungal odor which are important
335 contributors to aroma variability (Splivallo et al., 2012). 1-octen-3-one was clearly
336 perceived in both the fresh and preserved samples, reaching maximum olfactometric
337 scores in the frozen sample. This is in agreement with results reported by Cullere et al.,
338 (2010) which pointed to this ketone as the odorant marker of the freezing process in
339 black truffles. Methional was perceived with maximum scores in both the freeze-dried
340 and the frozen samples, tripling the intensity found in fresh black truffle. The freezing
341 process was characterized by high levels of Z-1,5-octadien-3-one (MF=83%), a potent
342 odour compound that smells like geranium. Interestingly, this compound was not
343 detected on the fresh and freeze-dried product. In canned and hot air dried samples it
344 reached scores of 33% and 41% (MF), respectively.

345 **Canning (C).** Three major considerations characterized the odour profile of this sample.
346 a) canned truffle was the only one lacking 2-acetyl-1-pyrroline; b) dimethyl-trisulfide –

347 evoking an unpleasant, gas-like odor – was only detected in this sample, suggesting it
348 could be a molecular marker of the canning process, and *c*) canning was the only
349 storage method that preserved ethyl esters (ethyl 2- and 3- methyl butyrate) in the
350 truffle.

351 Several compounds identified by olfactometry are reported for the first time in
352 *T. melanosporum*: Z-4-heptenal, 2-acetylpyrroline, Z-1,5-octadien-3-one, isopropyl-
353 and isobutylmethoxypyrazine, E,Z-2,6-, E,Z-2,4-, and E,E-2,4- nonadienals and δ -
354 decalactone. Carbonyl compounds emerge as important odour molecules greatly
355 affected by the preservation technology. Whereas Z-4-heptenal and Z-1,5-octadien-3-
356 one were not detected in the fresh truffle, they appear as important biomarkers of the
357 freezing process. In contrast, the three nonadienal isomers are essential constituents of
358 fresh *T. melanosporum*, being affected in different ways by the preservation
359 methodology applied.

360 3.2. Sensory changes induced by the preservation method

361 The single term that did not vary significantly among the samples was the global
362 aroma intensity (Figure 1). However, major differences in the nature of the aroma
363 evoked by the five samples were observed. The fresh product exhibited the most intense
364 typical truffle aroma, followed by the freeze-dried one. It is worth noting that in the
365 case of the freeze-dried sample the intensity of this attribute decreased by around 40 %
366 with respect to the fresh product. Frozen truffles presented a dramatic loss of the typical
367 truffle aroma. This is in agreement with Culleré and co-workers (2013), who pointed
368 out that after 24 h of freezing a significant loss of the characteristic truffle aroma was
369 observed.

370 A similar pattern was observed for “black olives” which was similarly perceived
371 in the freeze-dried and dehydrated truffles, although with a significant loss with respect

372 to the fresh truffle. In contrast, neither the truffle nor the black olives aromas were
373 evoked by the frozen and canned samples. Animal-leather notes were also perceived in
374 the fresh and lyophilized truffles, although most intensely in the latter. Major changes
375 in the sensory profile were observed for the frozen truffles, as they evoked an
376 extremely intense baked potato attribute and, to a lesser extent, mushrooms. The
377 combination of both attributes yielded a clearly distinguishable aromatic profile, far
378 from the genuine fresh truffle aroma. The nut-seed odour was very intense in the
379 canned sample, followed by the dehydrated one.

380 These results suggest that, from an overall sensory viewpoint, the freeze-drying
381 treatment was the most successful for preserving the overall aroma quality and
382 complexity of fresh truffle, although with a lower intensity. According to panel
383 descriptions, frozen and canned truffles smell like a totally different product dominated
384 by the baked potato and nut-seed odours, respectively.

385 *3.3. Correlation between odour and aroma profiles*

386 Results from the PCA are shown in Figure 2 (correlation circle) and Figure 3
387 (sample projection). The first two PCs accounted for 66% of the total variance. The first
388 component discriminates among high-volatility sulphur compounds/alcohols/branched
389 ethyl esters and aldehydes/ketones. The second component contrasts the above-
390 mentioned families (with the single exception of ethyl esters) with DMTS and 1-octen-
391 3-ol. The correlation circle shows the projection of the sensory attributes as
392 supplementary variables to better interpret the relationships among the odour molecules
393 and aroma notes. The black olives (evoked by DMS and DMDS) and animal-leather
394 notes were highly correlated to the perception of the typical truffle-like aroma. δ -
395 decalactone and acetic acid appear as clear contributors to the genuine truffle aroma. In
396 contrast, the baked potato and mushroom-like odours are far from the general image of

397 fresh truffle (Figure 3). These are directly related to the presence of methional and 1-
398 octen-3-one, respectively, which explains the proximity of both samples in the fourth
399 quarter of the PC plot. Differences observed with respect to the dehydrated truffle could
400 be explained in terms of the simultaneous high levels of Z-1,5-octadien-3-one and E,E-
401 2,4-nonadienal.

402

403 **4. Conclusions**

404 The preservation technology has a huge impact on both the chemical and
405 sensory profiles of the *T. melanosporum* truffle. Freeze-drying emerges as the most
406 suitable method for truffle preservation as it is able to retain key-odour compounds such
407 as DMS or 3-ethylphenol. Ketones, aldehydes and sulphur compounds play a major role
408 in shaping the aroma of truffles submitted to preservation methods. Other molecules are
409 reported for the first time in this work as potential markers of some of the studied
410 preservation methods (2-acetylpyrroline for freeze-drying and hot air drying, and Z-1,5-
411 octadien-3-one for freezing). These results should be of interest for the truffle industry,
412 which would benefit from being able to evaluate the degree of deviation of the aroma of
413 preserved truffles with respect to that of the original, genuine, fresh product.

414

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419

420 **Ethics statement:** Use of human subjects for this study was reviewed by the University
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422

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1 **Table 1.** Gas chromatography-olfactometry analysis of fresh, freeze-dried, hot air-dried, frozen and canned *T. melanosporum* truffles: chemical
 2 identity, CAS number, odor descriptor, linear retention index (LRI), retention time and modified frequency percentage.
 3

Compound number	Identity	CAS number	Odor descriptor	LRI DB-WAX	Retention time (min)	Modified frequency (%) ^a				
						Fresh	Freeze-dried	Hot air-dried	Frozen	Canned
1	Dimethylsulphide-(DMS) ^b	75-18-3	black olives, truffle	<1000	3.53	84	80	61	-	-
2	2-Methylbutanal ^b	96-17-3	fusel	<1000	4.15	-	51	76	-	-
3	Dimetildisulphide-(DMDS) ^b	624-92-0	black olives, truffle	915	5.59	97	82	32	-	-
4	2,3-butanedione ^b	431-03-8	butter,-cream	989	8.15	66	-	76	-	67
5	Ethyl-2-methylbutyrate ^b	7452-79-1	strawberry	1066	10.45	93	-	-	-	67
6	Ethyl-3-methylbutyrate ^b	108-64-5	strawberry, pineapple	1074	11.01	51	-	-	-	50
7	Hexanal ^b	66-25-1	bush, leaf	1097	11.46	-	67	71	83	-
8	Z-4-heptenal ^b	6728-31-0	fish	1256	17.30	-	48	35	83	-
9	1-Octen-3-ona ^b	4312-99-6	mushroom	1319	19.33	50	58	94	97	33
10	2-Acetyl-1-pyrroline ^b	99583-29-6	popcorn, toasted bread	1360	20.49	17	83	95	52	-
11	Z-1,5-octadien-3-one ^b	65767-22-8	geranium	1394	21.53	-	-	41	83	33
12	Dimethyltrisulfide-(DMTS) ^b	3658-80-8	gas,-garbage	1415	22.29	-	-	-	-	100
13	3-Isopropyl-2-methoxypyrazine ^b	25773-40-4	bell-pepper	1458	23.45	-	52	-	67	-
14	Acetic acid ^b	64-19-7	vinegar	1470	24.06	42	-	-	-	-
15	Methional ^b	3268-49-3	baked potato	1482	24.27	33	100	87	100	83
16	1-Octen-3-ol ^b	3391-86-4	mushroom	1525	25.39	-	-	20	-	50
17	ni		Roquefort cheese	1555	26.28	67	-	-	-	-
18	3-Isobutyl-2-methoxypyrazine ^b	24683-00-9	bell pepper	1570	26.53	52	-	-	17	-
19	2-Acetyl tetrahydropyridine ^c	27300-27-2	toasted-almond	1574	26.58	-	-	-	50	-
20	E,Z-2,6-nonadienal ^b	557-48-2	cucumber	1624	28.19	51	-	-	34	-
21	ni		truffle	1628	28.25	-	33	-	-	-
22	E,Z-2,4-nonadienal ^b	5910-87-2	rancid, broth	1721	30.47	50	67	-	68	54
23	ni		roses	1725	30.53	-	50	-	-	-
24	Ethyl phenylacetate ^b	101-97-3	honey	1768	31.57	-	-	-	52	-
25	E,E-2,4-nonadienal ^b	5910-87-2	rancid	1797	32.39	23	39	60	50	54
26	β -phenylethanol ^b	60-12-8	floral	1933	37.03	53	-	-	-	-
27	3-Ethylphenol ^b	620-17-7	animal, leather	>2000	42.15	54	17	-	-	-
28	δ -decalactone ^b	705-86-2	dried peach	>2000	43.03	54	-	-	-	-

4 ^aAverage data from four olfactometry judges (n=4).

5 ^bIdentification based on coincidence of gas chromatographic retention with those of the pure compounds available in the laboratory.

6 ^cTentative identification based on comparison with LRI databases published in the literature.

7 ni: not identified; -: not detected

Table 2. Significance of the factor “preservation method” in the sensory aroma attributes of fresh, freeze-dried, hot air-dried, frozen and canned *T. melanosporum* truffles according to one-way analysis of variance (judges as repetitions; n=7). Different letters indicate the existence of a significant difference between samples. (Duncan test, 5% confidence level).

Aroma attributes	P	Fresh	Freeze-dried	Hot air dried	Frozen	Canned
Global aroma intensity	0.1926					
Truffle-like typical aroma	0.0008	d	c	b	a	a
Black olives	0.0035	c	b	b	a	a
Mushroom	0.0431	c	bc	ab	a	a
Animal-leather	0.0062	c	b	a	a	a
Baked potato	0.0009	a	a	a	b	a
Nut-seeds	0.0076	a	ab	bc	a	c

Figure 1. Sensory aroma attributes of fresh, freeze-dried, hot air-dried, frozen and canned *T. melanosporum* truffles. Data corresponds to the average of seven judges (mean values of 2 replicates per judge). Notations *, ** and *** indicate the existence of a significant difference ($p < 0.05$, 0.01 and 0.001, respectively) between preservation methods according to one-way analysis of variance (ANOVA).

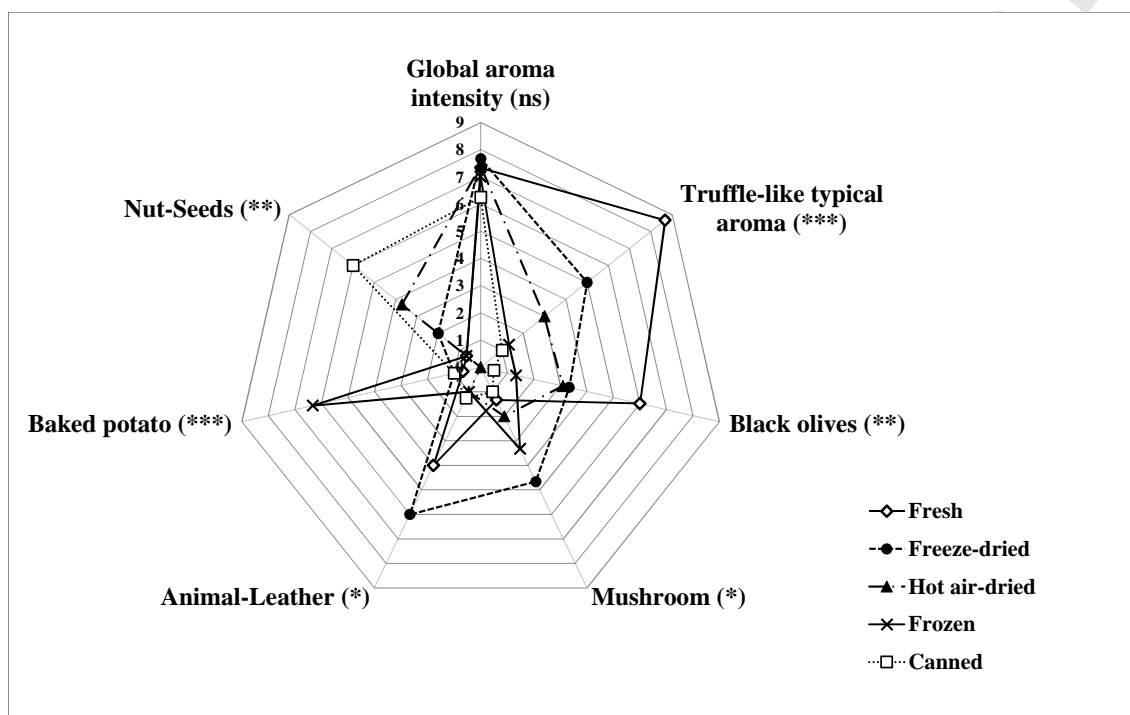


Figure 2. Circle of correlation for gas chromatography-olfatometry descriptors on principal components 1 and 2 of fresh, freeze-dried, hot air-dried, frozen and canned *T. melanosporum* truffles. Sensory attributes (in grey) are projected as illustrative variables.

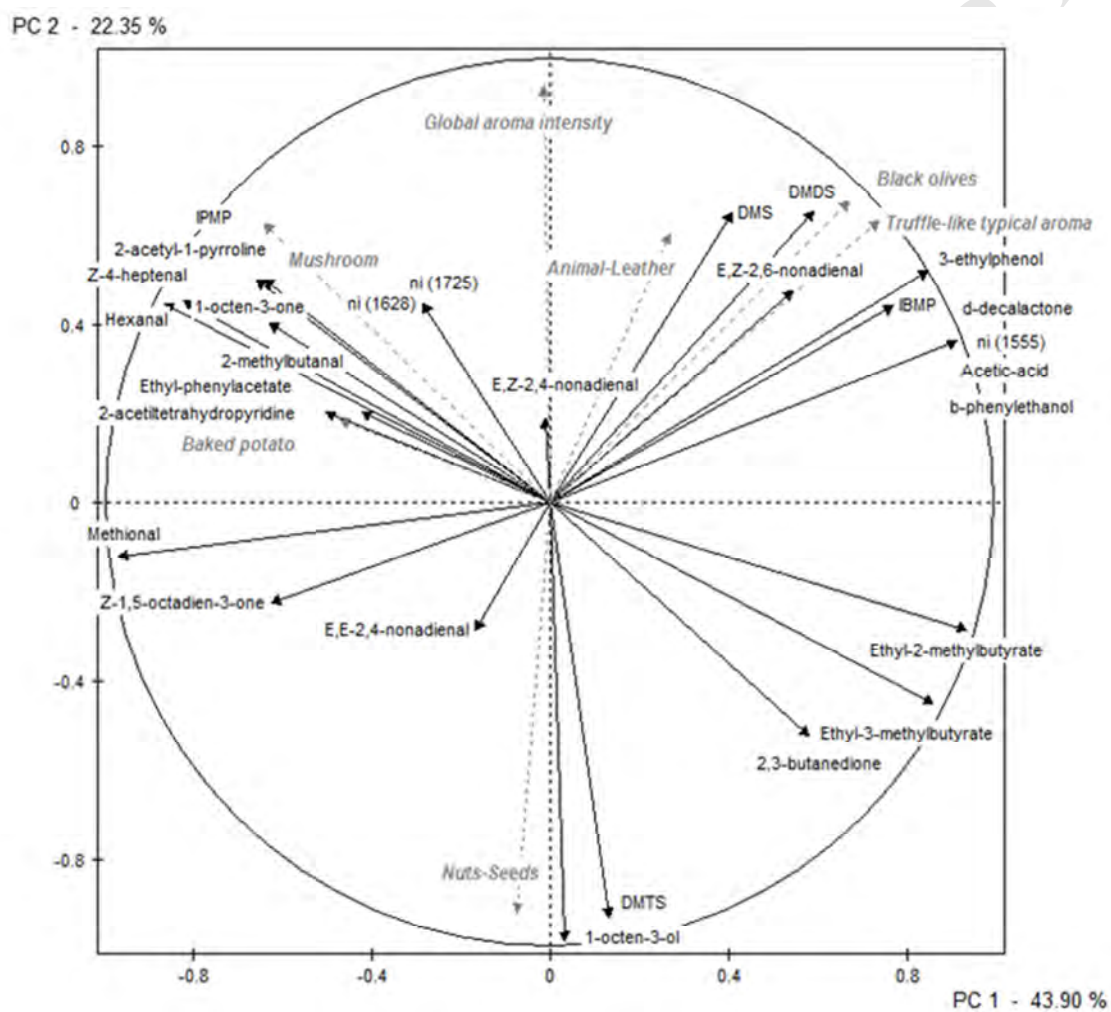
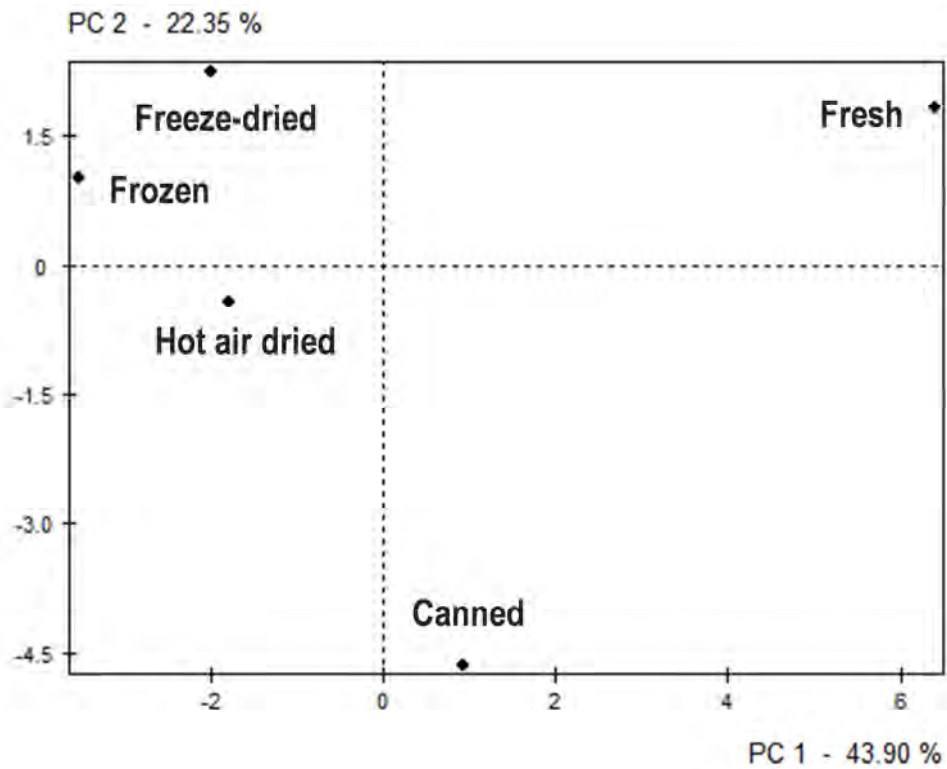


Figure 3. Projection of fresh, freeze-dried, hot air-dried, frozen and canned *T. melanosporum* truffles in the Principal Component Analysis (PCA) plot (dimensions 1 and 2) yielded by olfactometric data.



HIGHLIGHTS

- Freeze-drying arises as the most suitable preservation method
- 2-acetylpyrrolyne is a marker of the drying process
- Any freezing step involves the presence of off-odour methional
- Frozen and canned products are far from the genuine black truffle aroma

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