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

Eva Campo, Pedro Marco, Rosa Oria, Domingo Blanco and María E. Venturini *

[1] Plant Food Research Group, Instituto Universitario de Investigación Mixto Agroalimentario de Aragón (Universidad de Zaragoza - CITA). Miguel Servet 117, 50013 Zaragoza, Spain.

(*) To whom correspondence should be addressed

Phone: 34 976-761584

Email: ugeventu@unizar.es (M.E. Venturini)

RUNNING TITLE: Aroma changes in preserved black truffles

Abstract

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

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

1. Introduction

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

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

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

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

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

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

2. Material and Methods

2.2. Truffle collection and processing

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

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

Ten truffles were arranged in five polypropylene trays (250 mL) (Borden, S.A., Alicante, Spain) each containing two ascocarps. The upper part of the package (96 cm^2) was heat sealed with a microperforated film (two 90 × 50 μm holes) (Amcor Flexibles, Ledbury, U.K.) to achieve internal atmosphere gaseous concentrations of approximately 10% CO_2/10% O_2 at 4 ºC. These conditions decrease the truffle metabolism and the microbial growth rate and also delay the development of superficial mycelial growth, avoiding the presence of off-odours and maintaining the characteristic aroma very close to that of the freshly harvested truffles (Rivera, Blanco, Salvador, & Venturini, 2010). The rest (n=40) of the ascocarps were sliced into about 2–3 mm and mixed together in order to obtain a pooled sample. 10 g of this fresh sliced pool was immediately submitted to olfactometric analysis as described in section 2.3. The

sampling pool was then divided into four portions (around 250 g each) at random. These were processed by different preservation methods:

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

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

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

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

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

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

2.3. Analysis of odor-compounds

2.3.1. Preparation of aroma extracts by SPME

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

2.3.2. Gas chromatography-olfactometry

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

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

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

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

2.4. Sensory analysis

2.4.1. Panel training and formal measurements.

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

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

2.4.2. Data analysis

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

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

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

3. Results and Discussion

3.1. Prior considerations regarding truffle sampling

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

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

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

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

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

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

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

3.2. Sensory changes induced by the preservation method

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

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

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

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

3.3. Correlation between odour and aroma profiles

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

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

4. Conclusions

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

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References

Al-Ruqaie, I. (2005). Effect of different processes and preservation methods on the quality of truffles I. Conventional methods (drying/freezing). *Journal of Food Processing and Preservation, 30*, 335-351.

Culleré, L., Ferreira, V., Chevret, B., Venturini, M.E., Sánchez-Gimeno, A.C., & Blanco, D. (2010). Characterization of aroma active compounds in black truffles (*Tuber melanosporum*) and summer truffles (*Tuber aestivum*) by gas chromatography-olfactometry. *Food Chemistry 122,* 300-306.

Culleré, L., Ferreira, V., Venturini, M.E., Marco, P., & Blanco, D. (2012). Evaluation of gamma and electron-beam irradiation on the aromatic profile of black truffle (*Tuber melanosporum*) and summer truffle (*Tuber aestivum*). *Innovative Food Science and Emerging Technologies 13,* 151-157.

Culleré, L., Ferreira, V., Venturini, M.E., Marco, P., & Blanco, D. (2013). Chemical and sensory effects of the freezing process on the aroma profile of black truffles (*Tuber melanosporum*). *Food Chemistry 136:* 518-525.

Campo, E., Ballester, J., Langlois, J., Dacremont, C., & Valentin, D. (2010). Comparison of conventional descriptive analysis and a citation frequency-based descriptive method for odor profiling: An application to Burgundy Pinot noir wines. *Food Quality and Preference 21,* 44-55.

Benucci, G.M.N., & Bonito, G. (2016). The truffle microbiome: species and geography effects on bacteria associated with fruiting bodies of hypogeous pezizales. *Microbial Ecology 72 (1),* 4–8.

Dravnieks, A. (1985). Atlas of odor character profiles. Philadelphia: ASTM.

446 Hazelwood, L.A., Daran, J.M., Maris, A.J.A., van Pronk, J.T., & Kickinson, J.R.
447 (2008). The Ehrlich pathway for fusel alcohol production : a century of research on
448 *Saccharomyces cerevisiae* metabolism. *Applied and Environmental Microbiology*
449 *74,* 2259-2266.

450 Hofmann, T., & Schieberle, P. (1998). Flavor contribution and formation of the intense
451 roast-smelling odorants 2-propionyl-1-pyrroline and 2-propionyltetrahydropyridine
452 in Maillard-type reactions. *Journal of Agricultural and Food Chemistry, 46(7),*
453 2721-2726.

454 ISO 11035, (1994). Sensory analysis: identification and selection of descriptors for
455 establishing a sensory profile by a multidimensional approach. International
456 Organization for Standardization, Geneva, Switzerland.

457 Li, X., Feng, T., Zhou, F., Zhou, S., Liu, Y., Li, W., […], & Yang, Y. (2015). Effects of
458 drying methods on the tasty compounds of *Pleurotus eryngii*. *Food Chemistry 166,*
459 358-364.

460 Liu, R.S., Zhou, H., Li, H.M., Yuan, Z.P., Chen, T., & Tang, Y.J. (2013). Metabolism
461 of L-methionine linked to the biosynthesis of volatile organic sulfur-containing
462 compounds during the submerged ferementation of *Tuber melanosporum*. *Applied*
463 *Microbiology and Biotechnology 97*, 9981-992.

464 Martin, F., Kohler, A., Murat, C., Balestrini, R., Coutinho, P.M., Jaillon, […], &
465 Wincker, P. (2010). Périgord black truffle genome uncovers evolutionary origins
466 and mechanisms of symbiosis. *Nature 464*, 1033-1038.

467 Murcia, M.A., Martinez-Tomé, M., Vera, A., Morte, A., Gutiérrez, A., Honrubia, M., &
468 Jiménez, A.M. (2003). Effect of industrial processing on desert truffles *Terfezia*

claveryi Chatin and *Picoa juniperi* Vittadini): proximate composition and fatty acids. *Journal of the Science of Food and Agriculture, 83,* 535-541.

Palacios, I., Guillamon, E., García-Lafuente, A., & Villares, A. (2012). Effects of freeze-drying treatment on the aromatic profile of *Tuber* spp. truffles. *Journal of Food Processing and Preservation 38,* 768-773.

Penazza, G., Fanali, C., Santonico, M., Dugo L, Cucchiarini, L., Dachà, M., […], & Costa, R. (2013). Electronic nose and GC-MS analysis of volatile compounds in *Tuber magnatum Pico*: Evaluation of different storage conditions. *Food Chemistry 136,* 668-674.

Poehlmann, S., & Schieberle, P. (2013). Characterization of the aroma signature of styrian pumpkin seed oil (cucurbita pepo subsp. Pepo var. Styriaca) by molecular sensory science. *Journal of Agricultural and Food Chemistry. 61,* 2933-2942.

Rivera, C.S., Blanco, D., Salvador, M.L., & Venturini, M.E. (2010). Shelf-life extension of fresh *Tuber aestvum* and *Tuber melanosporum* truffles by modified atmosphere packaging with microperforated films. *Journal of Food Science 75,* 225-233.

Rivera, C.S., Venturini, M.E., Oria, R., & Blanco, D. (2011a). Selection of a decontamination treatment for fresh *Tuber aestivum* and *Tuber melanosporum* truffles packaged in modified atmospheres. *Food Control 22,* 625–632

Rivera, C.S., Venturini, M.E., Marco, P., Oria, R., & Blanco, D. (2011b). Effects of electron beam and gamma irradiation treatments on the microbial populations, respiratory activity and sensory characteristics of *Tuber melanosporum* truffles packaged under modified atmospheres. *Food Microbiology 28,* 1252-1260.

492 Saltarelli, R., Ceccaroli, P., Cesari, P., Barbieri, E., & Stocchi, V. (2008). Effect of
493 storage on biochemical and microbiological parameters of edible truffle species.
494 *Food Chemistry*, *109*, 8-16.

495 Schieberle, P. (1995). Quantitation of important roast-smelling odorants in popcorn by
496 stable isotope dilution assays and model studies on flavor formation during
497 popping. *Journal of Agricultural and Food Chemistry*. *43*, 2442−2448.

498 Splivallo, R., Ottonello, S., Mello, A., & Karlovsky, P. (2011). Truffle volatiles: from
499 chemical ecology to aroma biosynthesis. *New Phytologist 189*, 688-699.

500 Splivallo, R., Valdez, N., Kirchhoff, N., Ona, M.C., Schmidt, J.P., Feussner, I., &
501 Karlovsky, P. (2012). Intraspecific genotypic variability determines concentrations
502 of key truffle volatiles. *New Phytologist, 194(3),* 823-835.

503 Splivallo, R., Deveau, A., Valdez, N., Kirchhoff, N., Frey-Klett, P., & Karlovsky, P.
504 (2015a). Bacteria associated with truffle-fruiting bodies contribute to truffle aroma.
505 *Environmental Microbiol*ogy *17(8),* 2647-2660.

506 Splivallo, R., & Ebeler, S.E. (2015b). Sulfur volatiles of microbial origin are key
507 contributors to human-sensed truffle aroma. *Applied Microbiology and*
508 *Biotechnology 99(6),* 2583-2592.

509 Splivallo, R., & Cullere, L. (2016). The smell of truffles: from aroma biosynthesis to
510 product quality. In True Truffle (*Tuber spp.*) in the World. Soil biology 47.
511 Zambonelli, A., Iotti, M., Murat, C. (Eds.). Springer International Publishing. doi:
512 10.1007/978-3-319-31436-5.

513 Vahdatzadeh, M., Deveau, A., & Splivallo, R. (2015). The role of microbiome of
514 truffles in aroma formation: a meta-analysis approach. *Applied and Environmental*
515 *Microbiology 81(20),* 6946-6952.

516 Zeppa, S., Guidi, C., Zambonelli, A., Potenza, L., Vallorani, L., Pierleoni, R., Sacconi,
517 C., & Stocchi, V. (2002). Identification of putative genes involved in the
518 development of *Tuber borchii* fruit body by mRNA differential display in agarose
519 gel. *Current Genetics 42(3)*, 161-168.

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

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-methoxipyrazine[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-methoxipyrazine[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	-	-	-	-

[a]Average data from four olfactometry judges (n=4).

[b]Identification based on coincidence of gas chromatographic retention with those of the pure compounds available in the laboratory.

[c]Tentative identification based on comparison with LRI databases published in the literature.

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).

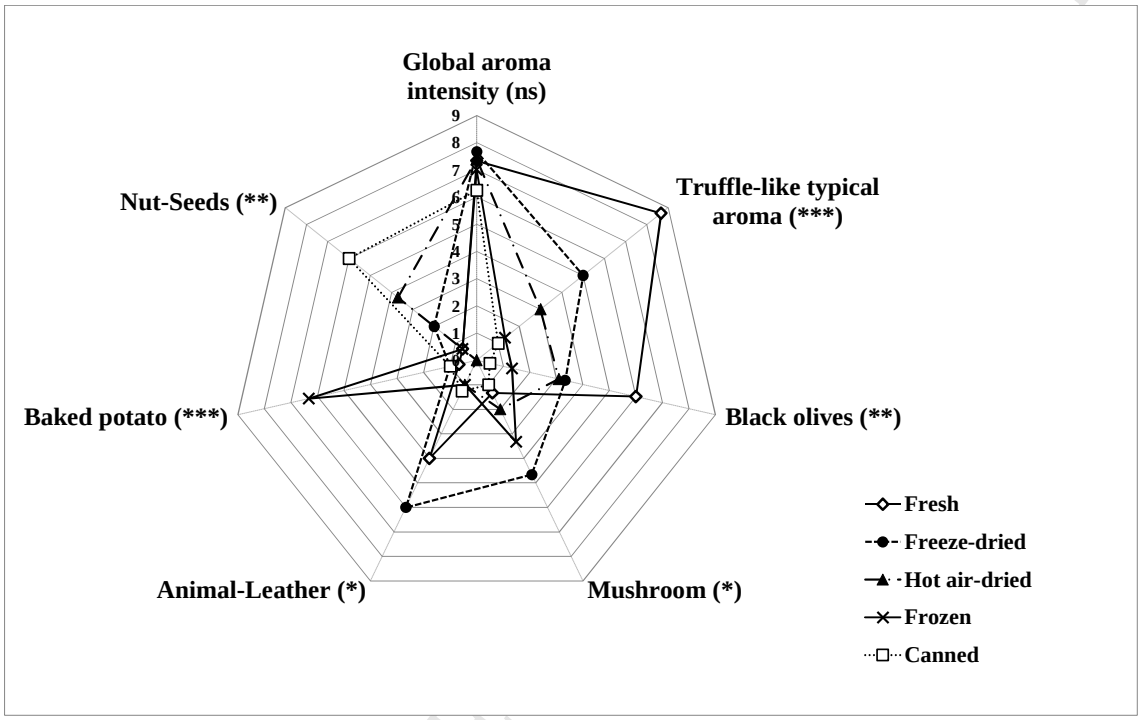


Figure 2. Circle of correlation for gas chromatography-olfatometry descriptors on principal components 1 and 2 of fresh, freeze-dried, hot air-dried, frozen and canned *T. melanosporum* truffles. Sensory attributes (in grey) are projected as illustrative variables.

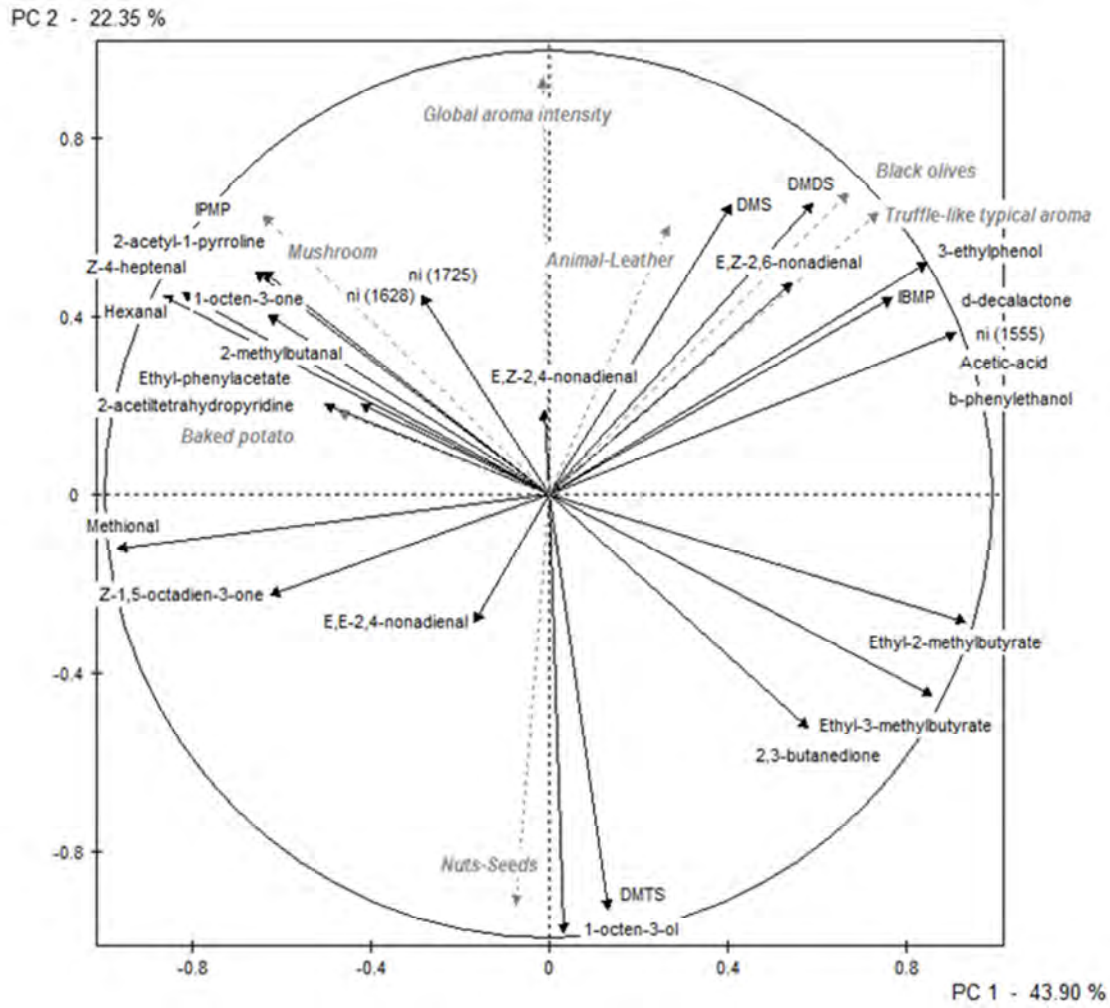
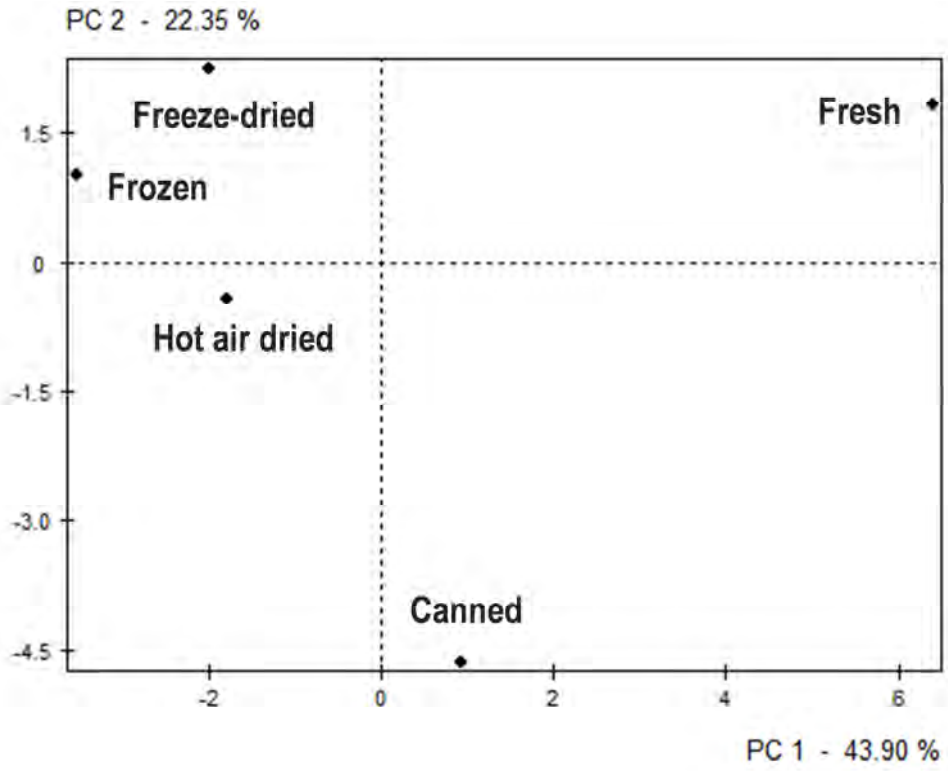


Figure 3. Projection of fresh, freeze-dried, hot air-dried, frozen and canned *T. melanosporum* truffles in the Principal Component Analysis (PCA) plot (dimensions1 and 2) yielded by olfactometric data.



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