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
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 freeze-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, Cucchiari, 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 4°C and 8°C). 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 cm²) 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% CO₂/10% O₂ at 4 °C. 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 ºC 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 (HETE DW8, Barcelona, Spain) and frozen at -20 ºC 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
contributes to the complexity of fresh truffle aroma. Culleré et al., (2010) identified β-phenylethanol as one of the key-odorants of *T. melanosporum*. In baker’s yeast it is derived from the catabolism of phenylalanine amino acid through the Ehrlich pathway (Hazelwood, Daran, Maris, Pronk, & Kickinson, 2008). Despite the fact that candidate genes potentially involved in this biosynthetic route were proposed for *T. melanosporum* in 2010 (Martin et al., 2010), the precise Ehrlich pathway has not yet been characterized in truffles.

**Freeze-drying (FD).** This sample presented similar odour intensities of DMS and DMDS (above 80% MF) to that of the control sample, which indicates that the freeze-drying treatment was successful for preserving both sulphur compounds. Palacios et al., (2011) reported that the freeze-drying process was able to retain DMS, but not DMDS, on lyophilized samples. Freeze-dried truffle was the only sample with 3-ethylphenol. In contrast, there are some differences with respect to the fresh product. For example, no judge detected the volatiles with creamy and fruity notes (2,3-butanedione and branched ethyl esters, respectively). Other volatiles were very intense in the freeze-dried truffle, mainly aldehydes: 2-methylbutanal, hexanal, Z-4-heptanal and especially methional. In a work studying the effects of freeze-drying on the aromatic profile of *Tuber* spp. truffles (*T. melanosporum*, *T. indicum* and *T. aestivum*), these authors concluded that the *T. melanosporum* aroma profile was little affected by the freeze-drying process, which is somewhat inconsistent with the data observed here. This may be due to the fact that the authors analysed truffle aroma in terms of quantitative mass spectral data, and not through olfactory-based screening techniques, which makes it difficult to detect molecules of low odour thresholds present at trace levels. 2-acetylpyrroline, a powerful compound well-known for its implications in roasted aroma, deserves special attention (Hoffmann & Schieberle, 1998). This molecule exhibits a
characteristic popcorn-like odour and an extremely low odour threshold of 0.02 ng/L in air (Schieberle, 1995), which gives an idea of its aroma potential, as well as the benefits of using an olfactometric approach on this work.

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

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

**Canning (C).** Three major considerations characterized the odour profile of this sample. 

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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Ethics statement: Use of human subjects for this study was reviewed by the University of Zaragoza Institutional Review Board according to the Belmont Report guidelines.
References


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


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.

<table>
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<th>Compound number</th>
<th>Identity</th>
<th>CAS number</th>
<th>Odor descriptor</th>
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<th>Retention time (min)</th>
<th>Modified frequency (%)</th>
<th>Fresh</th>
<th>Freeze-dried</th>
<th>Hot air-dried</th>
<th>Frozen</th>
<th>Canned</th>
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<td>Dimethylsulphide-(DMS)</td>
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*Average data from four olfactometry judges (n=4).*

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

*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).

<table>
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<tr>
<th>Aroma attributes</th>
<th>P</th>
<th>Fresh</th>
<th>Freeze-dried</th>
<th>Hot air dried</th>
<th>Frozen</th>
<th>Canned</th>
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<tbody>
<tr>
<td>Global aroma intensity</td>
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<td>d</td>
<td>c</td>
<td>b</td>
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<td>bc</td>
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</table>
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