Black truffle aroma transfer kinetics to food matrices

Eva Tejedor-Calvo[1]*, Sergi García-Barreda[1], María Ángeles Sanz[2], Ana Pilar Gracia[3], Sergio Sánchez[1], Pedro Marco[1]

[1] Department of Plant Science, Agrifood Research and Technology Centre of Aragón (CITA). Avda. Montañana 930, 50059 Zaragoza, Spain.

[2] Laboratories and Technological Assistance, Agrifood Research and Technology Centre of Aragon (CITA), Avda. Monta˜nana, 50059, Zaragoza, Spain

[3] Plant Food Research Group, Department of Food Technology, University of Zaragoza–IA2 (Zaragoza University-CITA), C/Miguel Servet 177, 50013, Zaragoza, Spain.

Keywords: *Tuber melanosporum*, aromatization, aromatic compounds, olfactometry.

Abbreviations: GC-O, chromatography-olfactometry, SPME-GC-MS, solid phase microextraction gas chromatography mass spectrometry

***Corresponding author:**

Eva Tejedor-Calvo

etejedor@cita-aragon.es

Abstract

Nowadays black truffles are so highly valued that truffled products are available in supermarkets whereas fresh truffle is mainly used in the restaurants. It is known that truffle aroma can change because heat treatments, but there is no scientific evidence about what molecules are transferred, in which concentration, and how much time is needed to aromatize products with truffle. In this study, four different fat-based food products (milk, sunflower oil, grapeseed oil and egg's yolk), were used to study black truffle (*Tuber melanosporum*) aroma transference for 14 days. Gas chromatography and olfactometry results showed different volatile organic compounds profile depending on the matrix used. After 24 hours, some key truffle aromatic compounds were detected in all the food matrices. Among them, grape seed oil was the most aromatized product probably because of its odorless properties. According to our results, dimethyl disulphide, 3-methyl-1-butanol and 1-octen-3-one odorants showed the highest aromatization power.

1. Introduction

Truffles have a worldwide interest mainly due to their aromatic properties. The number of products with truffle added has recently increased in supermarkets and restaurants. Traditionally, it is believed that truffle key aromatic compounds can be retained by fatty products, such as butter or oil. In fact, some of the truffle aroma compounds show lipophilic character (Tejedor-Calvo et al., 2021; Tejedor-calvo et al., 2023b; Wernig et al., 2018). In this regard, the greatest challenge of the food industry is to obtain truffle-flavored products with real truffles; food preservation technologies dramatically modify some of the key truffle aromatic compounds (Tejedor-calvo et al., 2023a). To counter the loss, the food industry usually adds synthetic or natural, but not extracted from truffles, food flavorings. Hence, 2,4-dithiapentane, or bis(methylthio)methane, is commonly used as truffle aroma substitute (Campo et al., 2018; Torregiani et al., 2017), but this compound is only characteristic of white truffle (*Tuber magnatum*). A mix of DMS (dimethyl sulphide) and 2-methylbutanal tries to mimic the aroma of *T. melanosporum* (Talou, Delmas, & Gaset, 2011). These chemical additives used as "truffle flavoring" in some restaurants and truffled products decrease truffles' prestige and confuse the consumer (Tejedor-Calvo, et al 2022).

More than 200 volatile organic compounds (VOCs) have been reported from truffles (Campo et al., 2017; Splivallo, Ottonello, Mello, & Karlovsky, 2011; Tejedor-Calvo et al., 2021). Nevertheless, the aroma of black truffles is only composed of about 10-20 main odorants (Culleré, Ferreira, Venturini, Marco, & Blanco, 2013). Dimethyl disulphide (DMDS), DMS, 2,3-butanodione, ethyl 2-methylbutyrate, ethyl 3-methylbutyrate, 1-octen-3-one, 2-acetyl-1-pyrroline, acetic acid, methional, *(E, Z)*-2,6-nonadienal, *(E, Z)*-2,4-nonadienal, and 3-ethylphenol are the most relevant (Campo et al., 2017). Because of these compounds, black truffle aroma can range from mild to intense, and can vary from

cheese-like, earthy, garlicky, pungent, vanilla-like, creamy, leathery, dusty, to gasoline like, among others (Campo et al., 2017; Culleré et al., 2010; Tejedor-Calvo et al., 2021). The research related with truffle aroma has been mainly focused in quality improvement, preservation techniques and, more recently, in the factors involved in aroma development (Choo et al., 2021; Niimi, Deveau, & Splivallo, 2021; Splivallo et al., 2011). Only few articles describe home-made truffled products, in which the products were made adding truffles (Beara et al., 2021; Wernig et al., 2018; Tejedor-calvo et al., 2023a). A common technique used in cuisine is aromatization, usually applied with contact and other techniques such as ultrasounds (Karoui, Wannes, & Marzouk, 2010), but products also can be aromatized without contact. No information about truffle aroma transfer without contact to other products is described in the literature. With this study, authors pretend to understand the truffle aroma transfer to food matrices using a home-made aromatization method available to scientist aside from cooking field.

Therefore, the aim of this study was to evaluate the black truffle aroma changes during storage time along with, for the first time, study the aroma transfer to different fat-based matrices (milk, sunflower oil, grapeseed oil and egg's yolk), in an attempt to identify the molecules with most aromatizing power as well as select the optimal time to aromatize a product. For this, two semi- instrumental techniques: solid phase microextraction gas chromatography mass spectrometry (SPME-GC-MS) and gas chromatography-olfactometry (GC-O) analysis approach were employed.

2. Materials and methods

2.1 Truffles and food matrices selection

Tuber melanosporum ascocarps were collected at Anento (Zaragoza province, eastern Spain). The following day, truffles were taxonomically identified by morphological features (Montecchi & Sarasini, 2000; Riousset, 2001), selected to avoid those damaged

by abiotic or biotic factors and conserved under refrigeration (Rivera et al., 2011b). Moreover, ripeness of the truffles was individually assessed following Zeppa et al. (2002).

The selected matrices were chosen regarding their fat content since many truffled products are fat-based products (Tejedor-Calvo, et al 2022). Cow milk, sunflower oil (SO) and eggs were purchased from local supermarket (EROSKI brand, Zaragoza, Spain). Grapeseed oil (GO) was purchased from Dietisa company (Barcelona, Spain). According to labelling, fat content of the selected matrices was 3.6% in milk, 9.5% in eggs, 91% in sunflower oil and 100% in grapeseed oil.

2.2 Experimental design

To characterize the transfer kinetics, an experimental approach was adopted using the matrices (milk, SO, GO and egg yolk), that were tested and compared to truffle. Measures were taken at nine different time points: day 0 (before the beginning of the experiment) and days 1, 2, 3, 4, 5, 7, 10 and 14.

Portions of mature truffles were placed in trays up to 100 g per tray along with the fat-based matrices (200 g of each matrix in a glass box per tray) (Figure S1) and kept hermetically closed at 4 °C during the experiment. The glass box was previously treated by sterilization process to avoid microorganism contamination and possible remaining aromas. In case of the egg yolk experiment, ten fresh eggs were included in the tray in order to use one egg, as sample, for each day. Also, an absorbing paper sheet was included and changed every 24 h, to retain humidity and avoid microbiological proliferation on truffles.

2.3 Volatile compounds analysis

2.3.1 Extraction by SPME

The aromatic compounds were extracted by SPME technology (Gómez, Lavega-gonzález, Tejedor-calvo, Pérez-Clavijo, & Carrasco, 2022). Briefly, a fused silica fiber coated with a 50/30 mm layer of divinylbenzene/carboxen/polydimethylsiloxane from Supelco (Barcelona, Spain) was chosen. For sampling, the tray was open, and 2 grams of truffle material and matrix (from every tray) were placed into a 20 mL glass vial hermetically closed with a septum. Six replicates of each sample were used to the following analysis: three for GC-MS and three for GC-O. In the case of egg yolk, egg white and shell were removed, so the sample for GC-O analysis was taken from yolk part. In all cases GC-O analyses were carried out immediately after sampling. After the vial was conditioned at 50 ºC for 5 min, the fiber was then exposed to the vial headspace for 30 min.

2.3.2 GC-MS analysis

Two SPME extracts were prepared per sample. The VOCs profile of different truffles species was analyzed by static GC-MS using a gas chromatograph Agilent 6890N (Termoquest, Milan, Italy) coupled with a mass spectrometer detector. This instrument was equipped with a capillary column HP-5MS (Agilent Technologies, California, USA) of 30 m, 0.32 mm i.d., 0255 μm film thickness and a flow of 1 mL/min with helium as a carrier gas. The oven temperature was 45 ºC held for 2 min, then raised at 4 ºC/min to 200 ºC, and finally to 225 ºC at 10 ºC/min, and held for 5 min. The MS used the electron impact mode with an ionization potential of 70 eV and an ion source temperature of 200 ºC. The interface temperature was 220 ºC. The MS scanning was recorded in full scan mode (35–250 m/z). A TurboMass software was used for controlling the GC-MS system. Peak identification of the VOCs was achieved by comparison of the mass spectra with the NIST MS Search Program 2.0 library mass spectral data, and by comparison of

134 previously reported Retention Index (RI) with those calculated using an n-alkane series
135 (C6–C20) under the same analysis conditions. The n-alkanes series and standards for MS
136 identification (all standards of purity higher than 95%) were purchased from Sigma-
137 Aldrich (Madrid, Spain). The semiquantification was done by integrating the area of one
138 ion characteristic of each compound and normalization by calculating the relative
139 percentage. This allowed the comparison of each eluted compound between samples.
140 2.3.3 GC-O analysis
141 A total of three SPME extracts were prepared per sample, one per GC-O judge. The
142 judges who performed the sniffing analysis (three women from 26 to 31 years of age)
143 have long experience in olfactometry. Previously, standard compounds from truffles,
144 marked as [a] in Table 1, were used for the judges training.
145 The GC-O analysis was carried out in a gas chromatograph HP 4890 (Termoquest, Milan,
146 Italy) with a flame ionization detector (FID) and an olfactometric port ODO-I supplied
147 by SGE (Ringwood, Australia). This instrument was equipped with a capillary column
148 DB-WAX (polyethylene glycol) supplied by J&W Scientific (Folsom, CA) of 30 m, 0.32
149 mm i.d., 0.5 μm film thickness, and a precolumn (3 m; 0.32 mm i.d.) from Supelco
150 (Bellefonte, PA). The chromatographic conditions were nitrogen as gas carrier (3.5
151 mL/min); splitless injection (splitless time: 60 s); injector and detector (temperature: 220
152 ºC). The oven temperature program was: 40 ºC for 5 min, then raised at 6 ºC/min to 220
153 ºC, maintained during 15 min for cleaning purposes.
154 The data obtained was a mixture of the intensity and the frequency of the odorants
155 detected/identified (Campo et al., 2017). This parameter is known as "modified
156 frequency" (MF) and is calculated by the following formula $MF\ (\%) = [F\ (\%)*I\ (\%)]^{1/2}$,
157 where F (%) is the detection frequency of an aromatic odorant expressed as the percentage
158 of the total number of judges, and I (%) is the average intensity expressed as the

159 percentage of the maximum intensity. The odorants were identified by comparison of
160 their odors and chromatographic retention index in a DB-WAX column with those of pure
161 reference compounds, when available. Additionally, the identity of compounds was
162 checked by comparing the sequence of LRI with that of other published databases. The
163 n-alkanes series and standards for MS identification (all standards of purity higher than
164 95%) were purchased from Sigma-Aldrich (Madrid, Spain).

165 *2.4. Statistical analysis*

166 The MF values for the odorants in the samples were analyzed with principal component
167 analysis (PCA) performed using R version 3.6.1 (RStudio Team, 2019) and the factoextra
168 package (Kassambara & Mundt, 2017). One PCA was built to analyze all the samples
169 together and then other PCAs for each of the matrices to gain insight in their specific time
170 patterns. PERMANOVA and PERMDISP analysis were also used to analyze all the
171 samples together and assess the differences of position and dispersion in the PCA among
172 the various matrices, using the R package vegan (Oksanen et al., 2020).

173

174 **3. Results and discussion**

175 *3.1 Aromatic profile of truffle and matrices by SPME-GC-MS*

176 A total of eighty-eight compounds were identified with SPME-GC-MS throughout the
177 study (Table 1 and Figure 1). All of them were found alternately in truffles and the food
178 matrices, except hexane that was exclusive from the matrices. At day 0, before the
179 aromatization process, the food matrices did not contain as many VOCs numbers as
180 truffle: 65 compounds in truffles, 35 in each oils, 20 in milk and 10 in egg yolk. In truffle
181 samples, three alcohols (2-methyl-1-pronanol, methyl-1-butanol and 2-methyl-1-butanol)
182 and two aldehydes (3-methyl-butanal and 2-methyl-butanal) were reported in truffles (day
183 0) as highest relative percentage. Those and other aromatic compounds (dimethyl

sulphide, 2-butanone) are known as key truffle compounds (Campo et al., 2017; Culleré et al., 2013; Tejedor-Calvo et al., 2021). Methylene chloride was common in all the food matrices, but some acids were only found in grapeseed oil (acetic acid) and sunflower oil (hexanoic acid and butyric acid) whereas others (tetradecanoic, pentadecanoic, hexadecenoic and octadecanoic acids) had higher levels in milk and yolk samples in comparison with the two oils studied.

Truffle aroma is in constantly change since many factors are involved, *e.g.* respiratory rate, preservation technique, storage time, microbiological composition (Choo et al., 2021; Niimi et al., 2021; Savini et al., 2020; Vahdatzadeh, Deveau, & Splivallo, 2019), so is difficult the stablish the maximum days of use. In this study, some changes were observed in truffle aroma during storage time (Table 2): an increasing of several compounds such as 2-methyl-1-propanol, hexanal, ehtly-2-methylbutanoate, anisole, 3-methyl-acid butanoic, and 2-methyl-butanoic acid; and a decreasing of 3-mehtly-1-butanol and 2-mehtyl-1-butanol. However, butanal-3-methyl and butanal-3-methyl maintained their similar values during storage time. These results indicate clear compounds levels changes that could potentially have a large impact on the overall perceived aroma character of the truffles. In agreement with that, Niimi et al. (2021) indicated that some molecules did not change in their amounts as a function of storage time. They explained that could be done because the compounds were not metabolised by any of the bacterial species detected or due to a continuous production over time. Freshness volatile markers were identified in *T. aestivum* samples (days 3, 6, and 9), including DMS, 2-butanone, ethyl acetate and 2,3-pentadione. By contrast, spoilage markers comprised for instance 2-phenylacetaldehyde, 2 and 3-methyl-1-butanol along with butanoic acid and ethyl butanoate (Vahdatzadeh et al., 2019). Our results obtained

agreed with some of them (DMS, 2-butanone), but others such as 2- and 3-methyl-1-butanol did not probably because those results were obtained from summer truffle.

After the aromatization process, many key truffle VOCs were retained into the food matrices (Figure 1). Some of their values increased (*e.g.* 2-methyl-1-butanol) by days while others decreased (*e.g*. dimethyl sulphide, 2-butanone). Certain molecules, as 3-methyl-1-butanol, showed different behaviours during aromatization process depending on the food matrix. Indeed after 24 hours, key truffle compounds showed higher levels in milk, followed by both oils and yolk. According to several reports, oil, protein and polysaccharides as well as their combinations in emulsions can retain different type of molecules (E. Guichard, 2002; Elisabeth Guichard, 2006; Mao, Roos, Biliaderis, & Miao, 2017). In this study, milk and yolk are a more complex mix of proteins, lipids and carbohydrates than the two oils. Regarding our results, same VOCs pattern was observed in both vegetable oils probably since they have similar composition (fatty acids profile and sterols composition). Wernig et al. (2018) prepared home-made truffle-flavored oils (50,100 and 200 mg/mL of *T. magnatum*) and it was observed higher levels of some aldehydes (3-methylbutanal, 2-methylbutanal, 2-pentenal), ketones (2-butanone and 3-pentanone), and sulfur compounds (DMS and 2,4-dithiapentane), similar compounds as in our results.

Some of the identified key black truffle VOCs (2-methyl-propanal, hexanal, ethyl 2-methylbutanoate, ethyl 3-methylbutanoate, heptanal) revealed low levels or absence in the matrices for the first days. The reason might be due to molecules mass, volatile capacity, hydrophobicity, or lipophilic characteristics, among other reasons (E. Guichard, 2002; Elisabeth Guichard, 2006; Mao et al., 2017). So, depending on the target molecules, aromatization process could improve by modifying some parameters (*i.e.* temperature, forced air, close system) in order to trap other molecules.

233 Although truffle is an aromatic product containing more than 200 VOCs, only some of
234 them have odoriferous properties. To detect them is needed a complementary technique,
235 as olfactometry. SPME-GC-MS and -GC-O are complementary techniques very useful to
236 identify the aroma profile. The first is an instrumental technique that reports objectively
237 the VOCs compounds or aromatic profile, whereas the second, a semi-instrumental
238 technique, determined those volatile compounds that humans can detect or hedonic
239 profile.
240
241 *3.2 Hedonic profile of truffles and changes during storage time by SPME-GC-O*
242 In order to determine the matrix with more trapping power, a deeply investigation and
243 analysis through aromatic compounds have been carried out using SPME-GC-O. Thirty-
244 six odor compounds were detected and identified in the GC-O study; those with MF
245 values lower than 20 were directly discarded from the analysis (Table 2, Table S1). Fresh
246 truffle was composed by 28 odor compounds (day 0). Among them, DMS (truffle), 3-
247 methyl-butanal (rancid), ethyl 2-methylbutanoate (strawberry) and 3-methyl-1-butanol
248 (malty) ranged above 80% MF. Other key compounds, such as 2,3-butanodione (buttery),
249 1-hexen-3-one (metallic), 1-octen-3-one (mushroom-like), 2-acetyl-1-pyrroline (toasted
250 almond) and methional (baked potato) (Campo et al., 2018; Campo et al., 2017; Culleré
251 et al., 2013) were detected with high MF values, but lower than 80%.
252 Some of these aromatic compounds maintained their initial high MF values during the
253 storage time (*e.g.* DMS, 3-methyl-butanal and 3-methyl-1-butanol) whereas others, with
254 lower initial MF value, increased their values above 90 % (*e.g.* methyl 2-methylbutanoate,
255 1-octen-3-one, acetic acid and non-identified compound 8 - sweaty). Three compounds
256 with pungent/almond-like (pentanal), yeast-like (non-identified 3) and citric (non-
257 identified 4) attributes disappeared the third storage day. These aromatic changes during

storage time might be due to various reasons. One of them is associated with senescence, due to a development of mycelial growth on the surface, followed by a superficial degradation and therefore a firmness loss (Benucci & Bonito, 2016; Culleré, Ferreira, Venturini, Marco, & Blanco, 2012). Ketones and methional are the odorant markers of the freezing process in black truffles (Culleré et al., 2010), but also a reduction of 2,3-butanedione and ethyl esters compounds and encouraged a powerful geranium odor (Z-1,5-octadien-3-one) (Campo et al., 2017).

Our results indicate that freezing process was not applied since Z-1,5-octadien-3-one was not detected, however low temperatures (4ºC) might have affected truffle aroma to a lesser extent.

3.3 Hedonic profile of food matrices and changes during storage time by SPME-GC-O

In comparison with truffle, the selected matrices contained less compounds compared with GC-MS, only 1 to 5 odor compounds (day 0) (Table S1). Before the aromatization process, milk was characterized by truffle (DMS), metallic (1-hexen-3-one), green (1-butanol), sweaty (non-identified compound 8) and caramel-like (furaneol) odors, but all of them with values less than 51% MF. SO matrix was characterized by yeast-like (non-identified compound 3), metallic (1-hexen-3one), baked potato (methional) and cheese (3-methylbutanoic acid) aromatic attributes, but their MF levels were lower than in the truffle sample. However, in GO sample only mushroom-like odor (1-octen-3-one) was detected (20% MF), indicating that was the odorless matrix. Egg yolk revealed malty (3-methyl-1-butanol) and toasted almond (2-acetyl tetrahydropyridine) attributes with low MF levels compared to the truffle sample.

The milk matrix retained from the first day truffle (DMDS), buttery (2,3-pentanodione), malty (3-methyl-1-butanol) and mushroom-like (1-octen-3-one) attributes. All these

283 molecules/odorants maintained high MF values throughout the experiment. Other odors
284 such as rancid (3-methyl-butanal), apple-like (methyl 2-methylbutanoate), strawberry
285 (ethyl-2-methybutanoate), fish (Z-4-heptenal), roasty (2-acetyl-1-pyroline) and toasted
286 almond (3-3-isobutyl-2-methoxypyrazine) were detectable from the third to the tenth day
287 in milk samples. In total, milk was able to trap from 8 to 14 odor compounds, depending
288 on the time exposure to truffle. Similarly, GO retained from 5 to 12 compounds. Among
289 them, truffle (non-identified compound 2 and DMDS), rancid (3-mehtyl-1-butanal) and
290 malty (3-methyl-1-butanol) aromas were trapped from the first day of exposure. From the
291 third day, eight compounds were trapped in GO matrix. Conversely, SO and yolk showed
292 low trapping power detecting only 9 and 6 compounds, respectively, throughout the
293 experiment. Truffle (non-identified compound 2 and DMDS), butter (2,3-pentanodione),
294 strawberry (ethyl 2-methylbutanoate) and malty (3-methyl-1-butanol) were detected
295 before the third day in SO, whereas only yeast-like (non-identified compound 3) and
296 vinegar (acetic acid) were noticed in yolk. In general, truffle, malty, buttery and
297 mushroom like aromatic attributes corresponding to DMDS, 3-methyl-1-butanol, 2,3-
298 pentanodione and 1-octen3-one, respectively, showed the highest aromatization power in
299 the food matrices tested.
300 During storage time, the matrices acted as a trap material and several volatiles were
301 transferred to them. A selection of the four most representative key aromatic compounds
302 is shown in Figure 2. A continuous increasing of MF values was observed in the non-
303 identified compound with truffle aroma (Figure 2a) until the fourteen days, except for
304 milk sample showing no holding capacity. Dimethyl sulfide (truffle) enhanced the highest
305 MF different day depending on the matrix: day 1 – SO, day 4 – GO and milk, day 5 - yolk
306 (Figure 2b). On the contrary, 3-methyl-1-butanol (malty) MF levels were similar within

307 the first three days in all matrices (Figure 2c). Only grapeseed oil and milk matrices
308 displayed 1-octen-3-ol (mushroom) in the first four days.
309 During the aromatization process, volatile compounds can be trapped differently
310 depending on the matrix composition. The aroma interactions with non-volatile
311 macromolecules such as sugars, proteins and lipids have been thoroughly reviewed
312 (Karoui et al., 2010; van Ruth, Frasnelli, & Carbonell, 2008). Many studies have been
313 done on the protein–flavour interactions, showing hydrophobic binding of most volatile
314 compounds tested (ketones, alcohols, aldehydes, terpenes) with proteins such as bovine
315 serum albumin and β-lactoglobulin, for instance (Elisabeth Guichard, 2006). That means
316 that milk could be a potential trap material due to its composition, which agrees with our
317 results since milk trapped more molecules than the remaining matrices tested. The aroma
318 trapping in the rest of the matrices, as they are mainly fat-based (oils and yolk), might be
319 due to an interaction with other fat-based or hydrophilic compound since the protein
320 content is low or null. According to that, Druaux et al., (1998) studied the transfer rate of
321 volatiles at the liquid-water interface, reporting that it mainly depended on the
322 hydrophobicity of the aromatic compounds.
323 It was expected to obtain similar results by both methodologies: GC-O and -MS. Some
324 of the key truffle compounds (DMS, 3-mehtyl-1-butanol, 3-methyl-butanal) reported high
325 levels in both techniques, indicating high levels as well as strong odor. However, others
326 such as 1-octen-3-ol or ethyl 2-methylbutanoate despite their strong aroma (MF %)
327 showed low ratio with GC-MS technique. This might be due to these molecules are easily
328 detectable by human nose because its aromatic potential, even at low doses. Therefore,
329 olfactometry is a powerful and necessary technique when aromatic compounds are the
330 target compounds.

A PCA was used to explore the possible correlations of the truffle aroma detected by CG-O and the truffled matrices odor throughout storage (Figure 3). The PCA analysis explained 50.5 % of the data variability with the two first PCA components. The first component allowed to clearly separate the aroma profile of truffles from all the matrices, with the former being characterized by a higher contribution of many compounds to the olfactometric profile (e. g. DMS, 3-methyl-butanal, 2-methylbutanoate, 2-acetyl-pyroline, 3-isobutyl-2-methoxypyrazine), thus indicating a much more complex aroma (Figure 3). This shows that the aromatic compounds were far from being completely transferred to any of the matrices. It may be interesting to test other techniques such as heat or ultrasound to increase the odor transference.

The second PCA component clearly separated truffles of early days (days 0-4) from those of late days (days 5-14). Early days were associated to relatively higher MF values of ethyl 2-methylbutanoate (strawberry), 1-hexanol (green, flowery), methional (baked potato), 3-2-acetyl tetrahydropyridine (toasted almond), 2-methylbutanoic acid (cheese) and *(E, E)*- 2,4-nonadienal (rancid), whereas late days were associated to 3-methyl-butanal (rancid), methyl 2-methylbutanoate (apple-like), 3-methylbuyl acetate (banana-like), 3-methyl-1-butanol (malty), 2-acetyl-1-pyroline (toasted almond), 3-isobutyl-2-methoxypyrazine (bell pepper), acetic acid (vinegar), 1-octen-3-ol (mushroom) and non-identified compound 9 (rancid). The same pattern was also observed for the matrices, although much less markedly. Some of the rancid, toasted almond and mushroom attributes were associated with compounds found in truffle spoilage (Rivera et al., 2011a). PERMANOVA analysis was performed on this data, showing a significant effect of matrix ($F = 11.9$, $P < 0.001$, $R^2 = 0.52$) and exposure time ($F = 4.7$, $P = 0.002$, $R^2 = 0.05$) on the olfactometric profile. These results confirm that the matrix is the factor which shows a higher correlation with the variability in the samples, and points that the matrices

356 show either significant differences in the position of their PCA centroids or significant
357 differences in the dispersion of their corresponding samples (or both of them). The
358 confidence ellipses for the centroids of each matrix confirm the first scenario, with truffle
359 being clearly separated from all the matrices, whose centroid clustered relatively close to
360 each other (Figure S2). To assess the second scenario, a PERMDISP analysis was carried
361 out, showing a significant effect of the matrix ($F = 16.1$, $P < 0.001$), which indicates that
362 there were significant differences among matrices regarding the dispersion of their
363 corresponding samples. The dispersion of the truffle samples was significantly higher
364 than those of the GO, milk and SO samples, and these showed significantly higher
365 dispersion than the yolk samples (Figure S2). This dispersion is linked to the changes
366 throughout storage, thus confirming the lower ability of yolk to trap the truffle volatile
367 compounds. Normally, yolk is able to trap aromatic compounds and in cuisine truffle
368 aroma is noticeable when yolk is warm up. For that, further experiments considering
369 temperature as well as consumers should carry out.
370
371 *3.4 Time trends in the odor composition of matrices*
372 In order to evaluate the potential of the matrices to trap truffle aromatic compounds, an
373 individualized analysis was carried out for each matrix. Thus, a PCA was applied to each
374 matrix to gain insight into the process of aromatic compounds transfer from truffles
375 detected by GC-O (Figure 4). With these results, authors pretend to select the best matrix
376 to trap the truffle aroma.
377 The PCA analysis of the different matrices explained from 56.9 to 67.5% of the data
378 variability with the two first components. In all cases, the first PCA component associated
379 with the temporal trend of the transfer process (Figure 4, Table S1). In the milk, day 0
380 was characterized by relatively higher MF values of 1-hexen-3-one; days 1-2 by DMS,

ethyl-3-methybutanoate and furaneol; days 3-4 by 2,3-pentanodione and acetic acid; days 5-7 by 3-methyl-1-butanol and 1-octen-3-one; day 10 by ethyl 2-methylbutanoate, Z-4-heptenal, and 3-isobutyl-2-methylpirazyne; and day 14 by 3-methylbutyl acetate and 1-octen-3-ol (Figure 4).

The SO showed similar patterns. Day 0 was characterized by 3-methyl-butanoic acid, whereas days 1-2 by 1-hexen-3-one. Ethyl-3-methyl butanoate and non-identified compound 3 characterized days 3-4. Day 5 was related with DMS, DMDS, 3-methylbutyl acetate and 1-octen-3-one (Figure 4). In the yolk, samples from days 0 to 4 were associated with higher MF values of methional and 3-isobutyl-2-methoxypyrazine, whereas days 7 and 10 were associated with non-identified compound 2 (truffle odor) and Z-4-heptenal (Figure 4). Finally, the pattern shown by the GO samples was similar. Odor intensity was low until day 5, with non-identified compound 1, DMDS and 2,3-pentanodione as the characteristic compounds. Then, days 7, 10 and 14 were clearly different and associated with relatively higher MF values of 2-methyl-butanoic acid, 1-hexanol and methyl 2-methylbutanoate (Figure 4). This matrix showed a high trap potential from the seventh day, which could be increased adding other techniques: ultrasounds, aromatization with contact or supercritical fluid extraction (Tejedor-Calvo et al., 2021), for instance. Also, this matrix is the more odorless among those studied, therefore the less likely to interfere with the truffle aroma.

Generally, until the fourth day similar aromatic compounds were detected, however since day 5 a profile change was detected mainly due to more trapping compounds. Indeed, except for grape seed oil days from 1-4 were placed in different position on PCA than 5 onwards. Therefore, four days is recommended as maximum of aromatization process for milk and yolk because change their profile from fifth day ahead, whereas one more day can be applied to oils (five days of aromatization process).

406

407 **4. Conclusions**

408 Black truffle (*Tuber melanosporum*) aromatic compounds were able to be transferred
409 passively through air into milk, sunflower oil, grapeseed oil and egg's yolk. Among the
410 identified VOCs, only 36 of them had odorous properties. After 24 hours of aromatization
411 process, DMDS, 2,3-pentanodione, 3-methyl-1-butanol and 1-octen-3-one were capture
412 by all the food matrices. Despite some of the key truffle aromatic compounds were highly
413 detected at the end of the study (day 14), no more than 4 days is recommended to made
414 home-made products due to compounds with negative odor attributes are transferred.
415 Further studies are needed to develop different products with genuine truffle aroma and
416 avoid the truffle market frauds existing.

417

418 **Acknowledgments**

419 This research was supported by Diputación Provincial de Zaragoza (DPZ) in Spain
420 (project "Mejora de la Eficiencia de la Producción y la Innovación Agroalimentaria de la
421 Trufa en la Provincia de Zaragoza") and Fondo Europeo Agrícola de Desarrollo Rural
422 2014-2022 (FEADER) in Spain (project "Organización, valorización y promoción de la
423 Trufa Negra de Aragón'').

424

425 **CRediT author statement**

426 **Eva Tejedor-Calvo:** Conceptualization, Data curation, Investigation, Methodology,
427 Software, Writing - original draft. **Sergi García-Barreda:** Methodology, Software,
428 Writing - review & editing. **María Ángeles Sanz:** Formal analysis, Methodology. **Ana**
429 **Pilar Gracia:** Formal analysis, Methodology. **Sergio Sánchez:** Project administration,

Writing - review & editing. **Pedro Marco:** Conceptualization, Supervision, Project administration, Writing - review & editing.

Conclict of interest

None

435 **References**

436 Beara, I., Majkić, T., & Torović, L. (2021). Bioguided design of new black truffle
437 (Tuber aestivum Vittad.) product enriched with herbs and spices. *LWT*, *138*,
438 110637. https://doi.org/10.1016/j.lwt.2020.110637
439 Benucci, G. M. N., & Bonito, G. M. (2016). The Truffle Microbiome: Species and
440 Geography Effects on Bacteria Associated with Fruiting Bodies of Hypogeous
441 Pezizales. *Microbial Ecology*, *72*(1), 4–8. https://doi.org/10.1007/s00248-016-
442 0755-3
443 Campo, E., Guillén, S., Marco, P., Antolín, A., Sánchez, C., Oria, R., & Blanco, D.
444 (2018). Aroma composition of commercial truffle flavoured oils: does it really
445 smell like truffle? *Acta Horticulturae*, (1194), 1133–1140.
446 https://doi.org/10.17660/ActaHortic.2018.1194.162
447 Campo, E., Marco, P., Oria, R., Blanco, D., & Venturini, M. E. (2017). What is the best
448 method for preserving the genuine black truffle (Tuber melanosporum) aroma? An
449 olfactometric and sensory approach. *LWT - Food Science and Technology*, *80*, 84–
450 91. https://doi.org/10.1016/j.lwt.2017.02.009
451 Choo, K. S. O., Bollen, M., Ravensdale, J. T., Dykes, G. A., & Coorey, R. (2021).
452 Effect of chitosan and gum Arabic with natamycin on the aroma profile and
453 bacterial community of Australian grown black Périgord truffles (Tuber
454 melansoporum) during storage. *Food Microbiology*, *97*, 103743.
455 https://doi.org/10.1016/j.fm.2021.103743
456 Culleré, L., Ferreira, V., Chevret, B., Venturini, M. E., Sánchez-Gimeno, A. C., &
457 Blanco, D. (2010). Characterisation of aroma active compounds in black truffles
458 (Tuber melanosporum) and summer truffles (Tuber aestivum) by gas
459 chromatography–olfactometry. *Food Chemistry*, *122*(1), 300–306.

https://doi.org/10.1016/J.FOODCHEM.2010.02.024

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*(JANUARY), 151–157. https://doi.org/10.1016/j.ifset.2011.09.003

Culleré, L., Ferreira, V., Venturini, M. E., Marco, P., & Blanco, D. (2013). Potential aromatic compounds as markers to differentiate between Tuber melanosporum and Tuber indicum truffles. *Food Chemistry*, *141*(1), 105–110. https://doi.org/10.1016/J.FOODCHEM.2013.03.027

Druaux, C., Le Thanh, M., Seuvre, A.-M., & Voilley, A. (1998). Application of headspace analysis to the study of aroma compounds-lipids interactions. *Journal of the American Oil Chemists' Society*, *75*(2), 127–130. https://doi.org/10.1007/s11746-998-0022-y

Gómez, I., Lavega-gonzález, R., Tejedor-calvo, E., Pérez-Clavijo, M., & Carrasco, J. (2022). Odor Profile of Four Cultivated and Freeze-Dried Edible Mushrooms by Using Sensory Panel , Electronic Nose.

Guichard, E. (2002). Interactions between flavor compounds and food ingredients and their influence on flavor perception. *Food Reviews International*, *18*(1), 49–70. https://doi.org/10.1081/FRI-120003417

Guichard, E. (2006). Flavour retention and release from protein solutions. *Biotechnology Advances*, *24*(2), 226–229. https://doi.org/10.1016/J.BIOTECHADV.2005.11.003

Karoui, I. J., Wannes, W. A., & Marzouk, B. (2010). Refined corn oil aromatization by Citrus aurantium peel essential oil. *Industrial Crops and Products*, *32*(3), 202–207.

https://doi.org/10.1016/j.indcrop.2010.04.020

Mao, L., Roos, Y. H., Biliaderis, C. G., & Miao, S. (2017). Food emulsions as delivery systems for flavor compounds: A review. *Critical Reviews in Food Science and Nutrition*, *57*(15), 3173–3187. https://doi.org/10.1080/10408398.2015.1098586

Niimi, J., Deveau, A., & Splivallo, R. (2021). Aroma and bacterial communities dramatically change with storage of fresh white truffle Tuber magnatum. *LWT*, *151*, 112125. https://doi.org/10.1016/J.LWT.2021.112125

Oksanen, J., Blanchet, F.G., Friendly, M., Kindt, R., Legendre, P., McGlinn, D., Minchin, P.R., O'Hara, R.B., Simpson, G.L., Solymos, P. and Stevens, M.H.HOksanen, J., Blanchet, F.G., Friendly, M., Kindt, R., Legendre, P., McGlinn, D., Minchin, P.R., O'Ha, M. H. . (2020). vegan: Community Ecology Package. R package version 2.5-6. 2019. Retrieved from https://cran.r-project.org/package=vegan

Rivera, C. S., Venturini, M. E., Marco, P., Oria, R., & Blanco, D. (2011a). 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*(7), 1252–1260. https://doi.org/10.1016/j.fm.2011.05.002

Rivera, Venturini, M. E., Oria, R., & Blanco, D. (2011b). Selection of a decontamination treatment for fresh Tuber aestivum and Tuber melanosporum truffles packaged in modified atmospheres. *Food Control*, *22*(3–4), 626–632. https://doi.org/10.1016/J.FOODCONT.2010.10.015

Savini, S., Longo, E., Servili, A., Murolo, S., Mozzon, M., Romanazzi, G., & Boselli, E. (2020). Hypobaric Packaging Prolongs the Shelf Life of Refrigerated Black Truffles (Tuber melanosporum). *Molecules*, *25*(17), 3837.

https://doi.org/10.3390/molecules25173837

Splivallo, R., Ottonello, S., Mello, A., & Karlovsky, P. (2011). Truffle volatiles: From chemical ecology to aroma biosynthesis. *New Phytologist*, *189*(3), 688–699. https://doi.org/10.1111/j.1469-8137.2010.03523.x

Talou, T., Delmas, M., & Gaset, A. (2011). Direct Capture of Volatiles Emitted From Entire Black Perigord Truffle. *Journal of Essential Oil Research*, *1:6*, 281–286. https://doi.org/10.1080/10412905.1989.9697799

Tejedor-Calvo, E., García-Barreda, S., Sánchez, S., Morales, D., Soler-Rivas, C., Ruiz-Rodriguez, A., … Marco, P. (2021). Supercritical CO2 extraction method of aromatic compounds from truffles. *LWT*, *150*, 111954. https://doi.org/10.1016/j.lwt.2021.111954

Tejedor-Calvo, E., García-Barreda, S., Felices-Mayordomo, M., Blanco, D., Sánchez, S., Marco, P. (2022). Truffle flavored commercial products veracity and sensory analysis from truffle and non-truffle consumers. *Food control*, manuscript accepted - in production

Tejedor-Calvo, E., Morales, D., Sanz, M. Á., Sánchez, S., Marco, P., & García-Barreda, S. (2023a). Aromatic changes in home-made truffle products after heat treatments. *Food Research International*, 164, 112403.

Tejedor-Calvo, E., Marco, P., Spègel, P., & Soler-Rivas, C. (2023b). Extraction and trapping of truffle flavoring compounds into food matrices using supercritical CO2. Food Research International, 112422.

Torregiani, E., Lorier, S., Sagratini, G., Maggi, F., Vittori, S., & Caprioli, G. (2017). Comparative Analysis of the Volatile Profile of 20 Commercial Samples of Truffles, Truffle Sauces, and Truffle-Flavored Oils by Using HS-SPME-GC-MS. *Food Analytical Methods*, *10*(6), 1857–1869. https://doi.org/10.1007/s12161-016-

0749-2

Vahdatzadeh, M., Deveau, A., & Splivallo, R. (2019). Are bacteria responsible for aroma deterioration upon storage of the black truffle Tuber aestivum: A microbiome and volatilome study. *Food Microbiology*, *84*, 103251. https://doi.org/10.1016/J.FM.2019.103251

van Ruth, S. M., Frasnelli, J., & Carbonell, L. (2008). Volatile flavour retention in food technology and during consumption: Juice and custard examples. *Food Chemistry*, *106*(4), 1385–1392. https://doi.org/10.1016/J.FOODCHEM.2007.08.093

Wernig, F., Buegger, F., Pritsch, K., & Splivallo, R. (2018a). Composition and authentication of commercial and home-made white truffle-flavored oils. *Food Control*. https://doi.org/10.1016/j.foodcont.2017.11.045

Wernig, F., Buegger, F., Pritsch, K., & Splivallo, R. (2018b). Composition and authentication of commercial and home-made white truffle-flavored oils. *Food Control*, *87*, 9–16. https://doi.org/10.1016/J.FOODCONT.2017.11.045

Zeppa, S., Guidi, C., Zambonelli, A., Potenza, L., Vallorani, L., Pierleoni, R., … Stocchi, V. (2002). Identification of putative genes involved in the development of Tuber borchii fruit body by mRNA differential display in agarose gel. *Current Genetics*, *42*(3), 161–168. https://doi.org/10.1007/s00294-002-0343-6

554 **TABLES**

555 **Table 1.** List of VOCs identified by SMPE-GC-MS in truffles samples and food matrices.

556 Values are given in relative percentage.

Number	RT (min)	Identity	CAS number	RI $_{exp}$	RI $_{lit}$	Mass (*m/z*)		
1	1.33	Ethanol	64-17-5	<500	427	45	46	43
2	1.37	2-propanone	67-64-1	<500	500	43	58	42
3	1.47	Dimethyl sulphide	75-18-3	521	521	62	61	47
4	1.50	Methylene chloride	75-09-2	532	531	49	86	84
5	1.56	1-propanol	71-23-8	555	548	31	29	42
6	1.57	Propanal-2-methyl	78-84-2	558	560	43	72	41
7	1.62	2,3-butanedione	431-03-8	577	587	43	87	86
8	1.64	Isopropyl formate	625-55-8	582	-	45	73	42
9	1.67	Butanal	123-72-8	595	598	44	72	57
10	1.68	3-methyl-2-butanone	563-80-4	597	-	43	86	41
11	1.69	Acetic acid	64-19-7	600	602	43	60	45
12	1.71	2-butanone	78-93-3	603	602	43	72	57
13	1.72	Hexane	110-54-3	604	-	57	86	56
14	1.77	Ethyl acetate	141-78-6	609	607	43	80	70
15	1.87	2-methyl-1-propanol	78-83-1	622	626	43	42	41
16	1.90	Acetaldehyde	75-07-0	624	-	44	43	42
17	2.08	Butanal-3-methyl	590-86-3	645	646	44	71	58
18	2.10	Butanol	71-36-3	648	656	56	55	43
19	2.12	Butanal-2-methyl	96-17-3	653	653	57	86	58
20	2.22	2-propanone-1-hydroxy	116-09-6	662	-	43	74	42
21	2.23	1-penten-3ol	616-25-1	663	680	57	58	55
22	2.31	Metylpropylformate	589-40-2	672	-	45	73	59
23	2.37	2-pentanone	107-87-9	679	687	43	86	71
24	2.45	2,3-pentadione	600-14-6	689	696	43	100	57
25	2.47	Pentanal	110-62-3	691	704	44	58	57
26	2.48	2-pentanol	6032-29-7	692	700	45	73	55
27	2.61	2-butanone,3-hidroxy	513-86-0	704	707	45	88	55
28	2.96	3-methyl-1-butanol	123-51-3	723	737	55	70	57
29	3.04	2-methyl-1-butanol	137-32-6	728	743	57	70	56
30	3.10	Dimethyl disulphide	624-92-0	731	733	94	79	61
31	3.18	2-methyl-Pentanal	123-15-9	735	-	43	58	41
32	3.43	Propanoic-ac-2methyl-esther	97-62-1	775	760	43	29	71
33	3.74	Isobutylacetate	110-19-0	783	770	43	56	41
34	3.99	Propanoic-ac-2methyl	79-31-2	735	753	43	88	73
35	4.10	1,3-butanediol	107-88-0	787	785	43	28	57
36	4.34	Octane	111-65-9	800	800	43	85	71
37	4.38	Hexanal	66-25-1	802	801	44	57	56
38	4.44	Ethylbutanoate	105-54-4	803	803	71	43	29
39	5.39	Furfural	98-01-1	830	830	96	95	67
40	5.64	2-methylthio-ethanol	5271-38-5	873	-	61	92	47
41	6.09	Ethyl 2-methylbutanoate	7452-79-1	849	853	102	85	74
42	6.21	Ethyl l-3-methylbutanoate	108-64-5	853	851	88	85	60
43	6.39	4-pentenal	2100-17-6	858	-	55	29	41
44	6.75	Hexanol	111-27-3	868	867	56	69	55
45	7.16	2-methyl-butyl-acetate	624-41-9	880	880	43	70	55
46	7.57	2-heptanone	110-43-0	890	889	43	71	59
47	7.94	Heptanal	111-71-7	902	894	70	57	55
48	8.11	Methional	3268-49-3	906	908	48	104	47

49	8.48	Anisol	100-66-3	916	918	108	93	78
50	8.55	Isobutyl isobutyrate	97-85-8	917	914	71	89	57
51	8.80	Dimethyl-sulfone	67-71-0	924	924	79	15	94
52	10.09	Isobutyl butyrate	539-90-2	957	961	71	56	43
53	10.10	Benzaldheyde	100-52-7	957	961	77	106	105
54	10.62	1-heptanol	111-70-6	971	967	70	69	56
55	10.97	1-octen-3-ol	3391-86-4	980	978	57	72	55
56	11.24	3-octanone	106-68-3	987	988	43	57	29
57	11.49	2-octen-4-ona	4643-27-0	993	-	69	41	84
58	11.56	3-octanol	589-98-0	995	994	59	101	83
59	11.76	Hexanoic acid, ethyl esther	123-66-0	1000	998	88	29	27
60	11.80	Butyric acid	2445-67-2	1002	1002	57	85	103
61	11.82	Octanal	124-13-0	1002	1003	43	84	56
62	12.02	3-methyl-acid butanoic	589-59-3	1003	1004	85	57	41
63	12.38	3-methylanisol	100-84-5	1018	1028	122	107	92
64	13.28	Benzeneacetaldehyde	122-78-1	1043	1047	91	120	92
65	13.80	E-2-octenal	2548-87-0	1058	1059	41	83	70
66	14.29	1-octanol	111-87-5	1071	1067	56	84	70
67	14.34	Linalool oxide cis	60047-17-8	1073	1074	59	94	93
68	14.50	3-methyl-phenol	108-39-4	1088	1083	118	107	79
69	14.89	Linalool oxide trans	34995-77-2	1088	1090	59	94	55
70	15.03	2-nonanone	821-55-6	1091	1090	43	58	41
71	15.33	Isoamyl-2methylbutyrate	27625-35-0	1101	1103	70	103	85
72	15.42	Nonanal	124-19-6	1103	1106	57	98	70
73	15.45	2-methyl-butanoic acid	2445-78-5	1104	1105	70	57	85
74	15.72	Benzeneethanol	60-12-8	1113	1113	91	122	65
75	16.88	Benzene, 1,2-dimethoxy-	91-16-7	1147	-	138	95	77
76	17.51	Benzene, 1,3-dimethoxy-	151-10-0	1167	-	138	109	95
77	18.52	Ethyl caprylate	106-32-1	1198	1196	88	101	57
78	18.99	2,4-nonadienal	5910-87-2	1213	1214	81	41	67
79	19.79	3,2dimethoxytoluene	4463-33-6	1240	-	57	41	29
80	20.11	2,5-dimethoxytoluene	24599-58-4	1250	-	137	152	109
81	21.41	2-undecanone	112-12-9	1293	1296	68	43	59
82	22.01	Benzene,1,2,3-trimethoxy	634-36-6	1314	1315	168	153	110
83	28.57	Dodecanoic acid	143-07-7	1560	1557	73	60	41
84	33.26	Tetradecanoic acid	554-63-8	1754	1763	73	60	55
85	35.46	Pentadecanoic acid	1002-84-2	1859	1857	73	43	60
86	37.56	Hexadecanoic acid	57-10-3	1960	1960	43	73	60
87	41.46	Octadecanoic acid	57-11-4	2150	2159	43	73	129

RT= retention time

RI $_{exp}$ = Retention Index experimental.

RI $_{lit}$ = Retention Index Literature database NIST

560 **Table 2**. List of odor compounds obtained by GC-O analysis: retention time (RT),

561 chemical identity, CAS number, odor descriptor and linear retention index (LRI).

Number	RT (min)	Identity	CAS number	Odor descriptor	LRI BD-WAX
1	2.83	ni- 1	-	Green	-
2	3.12	ni- 2	-	Truffle	-
3	3.53	Dimethyl sulphide-(DMS)[a]	78-18-3	Truffle	<900
4	5.59	Dimethyl disulphide[a]	624-92-0	Black olives, truffle	915
5	6.04	3-methyl-butanal[a]	96-17-3	Rancid	967
6	6.36	Pentanal[b]	110-62-3	Pungent, almond-like	972
7	8.16	2,3-pentanodione[a]	431-03-8	Buttery	990
8	9.26	Methyl 2-methylbutanoate[b]	868-57-5	Apple-like	1003
9	10.09	ni- 3	-	Yeast-like	-
10	10.38	Ethyl 2-methylbutanoate[a]	7452-79-1	Strawberry	1052
11	11.12	Ethyl 3-methylbutanoate[a]	108-64-5	Strawberry, pineapple	1066
12	11.46	1-hexen-3-one[b]	1629-60-3	Metallic	1085
13	12.51	3-methylbutyl acetate[b]	123-92-2	Banana-like	1117
14	13.48	1-butanol[b]	71-36-3	Green	1150
15	16.1	3-methyl-1-butanol[b]	123-51-3	Malty	1213
16	17.23	Z-4-heptenal [a]	6728-31-0	Fish	1255
17	19.04	ni- 4	-	Critric	-
18	19.29	1-octen-3-one[a]	4312-99-6	Mushroom-like, metalic	1315
19	20.04	1-hexanol[b]	111-27-3	Green, flowery	1334
20	20.45	2-acetyl-1-pyroline[a]	99583-29-6	Toasted almond	1356
21	21.54	Z-1,5-octadien-3-one[b]	928-96-1	Geranium	1394
22	22.33	2-propanoyl-1-pyroline[b]	133447-37-7	Roasty	1415
23	23.08	ni- 5	-	Dairy	-
24	23.32	3-Isobutyl-2-methoxypyrazine[a]	24683-00-9	Bell pepper	1439
25	23.40	ni- 6	-	Truffle	-
26	24.04	Acetic acid[a]	64-19-7	Vinegar	1463
27	24.27	Methional[a]	3268-49-3	Baked potato	1482
28	25.25	1-octen-3-ol[a]	3391-86-4	Mushroom	1516
29	26.42	2-Acetyl tetrahydropyridine[b]	27300-27-2	Toasted almond	1563
30	28.19	ni- 7	-	Earthy	-
31	29.38	ni- 8	-	Sweaty	-
32	30.37	2-methylbutanoic acid[b]	116-53-0	Cheese	1709
33	31.02	ni- 9	-	Rancid	-
34	31.57	3-methylbutanoic acid[b]	503-74-2	Cheese	1784
35	34.59	*(E, E)*- 2,4-nonadienal[a]	5910-87-2	Rancid	1895
36	42.58	furaneol[a]	3658-77-3	Caramel-like	2077

562 ni= not identified

563 [a] Identification based on coincidence of gas chromatographic retention with those of the

564 pure compounds available in the laboratory.

565 [b] Tentative identification based on comparison with LRI databases published in the

566 literature

567 **Figures**

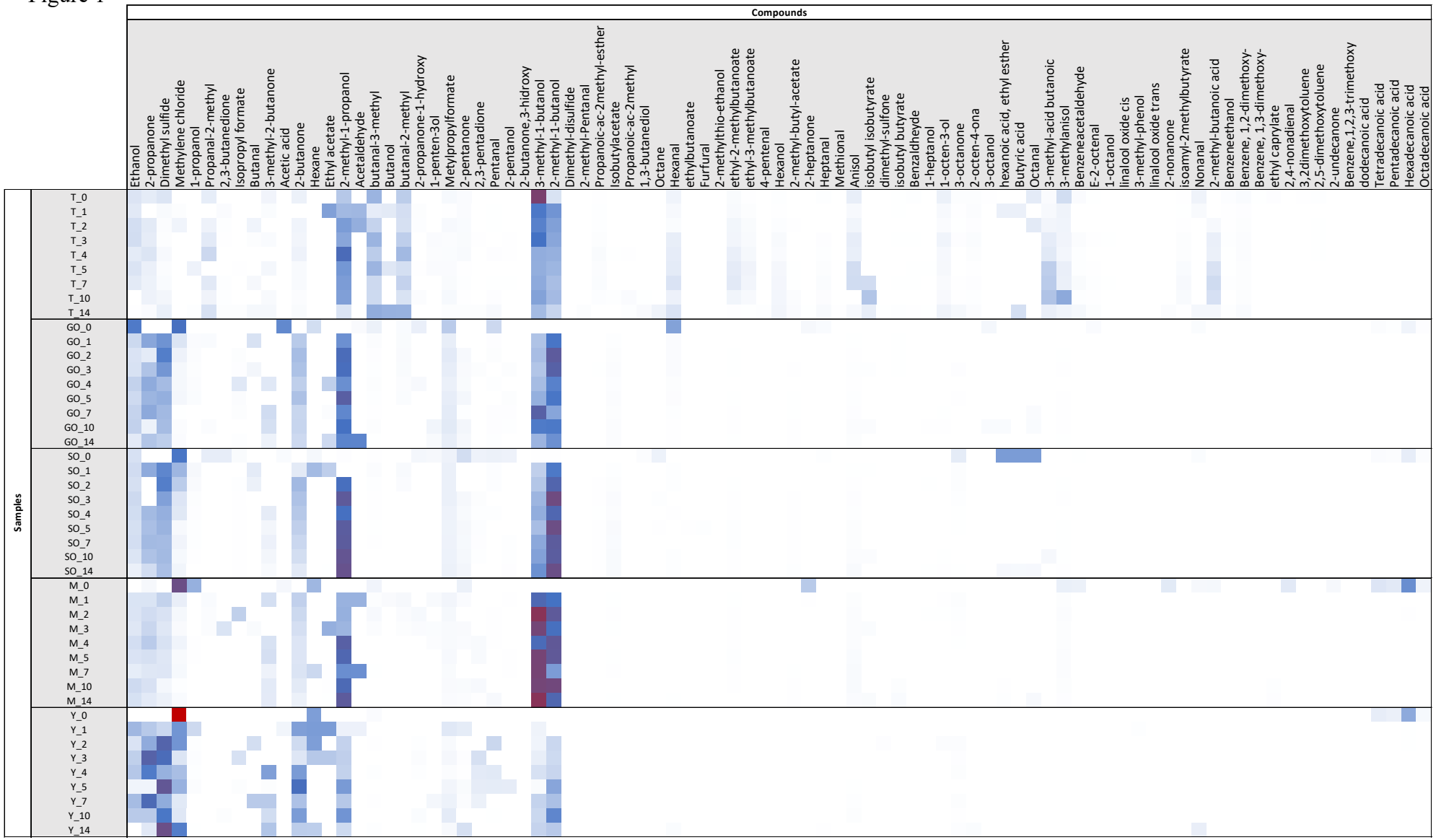
568 **Figure 1.** Heat map of VOCs detected by GC-O in truffle (T) and food matrices: milk
569 (M), sunflower oil (SO), grapeseed oil (GO) and egg yolk (Y) during 14 days. Colors
570 ranged from white (0%), blue (from 10%), red (up to 40%).

571 **Figure 2.** Evolution of four odorous compounds detected by SMPE-GC-O in truffle and
572 food matrices during storage period (14 days). Compounds correspond to number 2(A),
573 3(B), 15(C) and 18 (D) listed in Table 2. The rest of compound values are shown in Table
574 S1 in supplementary material

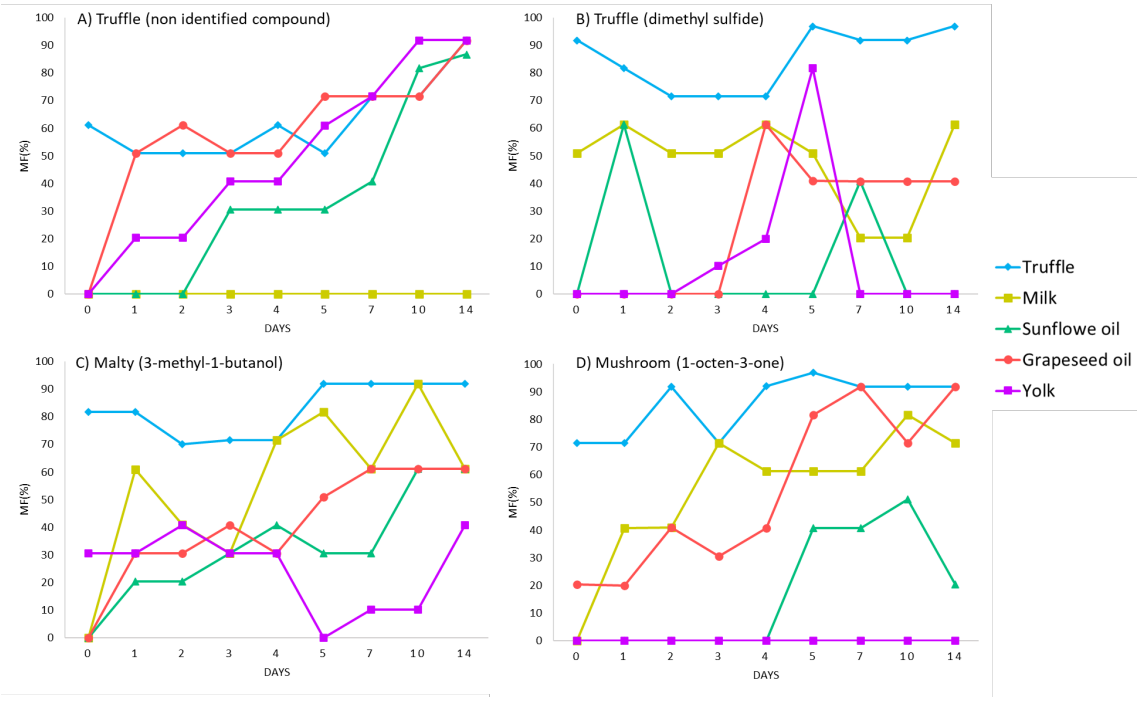
575 **Figure 3.** PCA plot corresponding to odorous attributes detected by CG-O. Odor
576 descriptors were those listed in Table 2. Arrow color indicates the contribution of a
577 compound to the PCA components (contrib) and sample color indicates the quality of
578 representation for the sample (cos2).

579 **Figure 4.** PCA plot corresponding to odorous attributes detected by CG-O in different
580 matrices. Odor descriptors were those listed in Table 2. Arrow color indicates the
581 contribution of a compound to the PCA components (contrib) and sample color indicates
582 the quality of representation for the sample (cos2).

583 Figure 1


Compounds
Samples
Ethanol
2-propanone
Dimethyl sulfide
Methylene chloride
1-propanol
Propanal-2-methyl
2,3-butanedione
Isopropyl formate
Butanal
3-methyl-2-butanone
Acetic acid
2-butanone
Hexane
Ethyl acetate
2-methyl-1-propanol
Acetaldehyde
butanal-3-methyl
Butanol
butanal-2-methyl
2-propanone-1-hydroxy
1-penten-3ol
Metylpropylformate
2-pentanone
2,3-pentadione
Pentanal
2-pentanol
2-butanone,3-hidroxy
3-methyl-1-butanol
2-methyl-1-butanol
Dimethyl-disulfide
2-methyl-Pentanal
Propanoic-ac-2methyl-esther
Isobutylacetate
Propanoic-ac-2methyl
1,3-butanediol
Octane
Hexanal
ethylbutanoate
Furfural
2-methylthio-ethanol
ethyl-2-methylbutanoate
ethyl-3-methylbutanoate
4-pentenal
Hexanol
2-methyl-butyl-acetate
2-heptanone
Heptanal
Methional
Anisol
isobutyl isobutyrate
dimethyl-sulfone
isobutyl butyrate
Benzaldheyde
1-heptanol
1-octen-3-ol
3-octanone
2-octen-4-ona
3-octanol
hexanoic acid, ethyl esther
Butyric acid
Octanal
3-methyl-acid butanoic
3-methylanisol
Benzeneacetaldehyde
E-2-octenal
1-octanol
linalool oxide cis
3-methyl-phenol
linalool oxide trans
2-nonanone
isoamyl-2methylbutyrate
Nonanal
2-methyl-butanoic acid
Benzeneethanol
Benzene, 1,2-dimethoxy-
Benzene, 1,3-dimethoxy-
ethyl caprylate
2,4-nonadienal
3,2dimethoxytoluene
2,5-dimethoxytoluene
2-undecanone
Benzene,1,2,3-trimethoxy
dodecanoic acid
Tetradecanoic acid
Pentadecanoic acid
Hexadecanoic acid
Octadecanoic acid
T_0
T_1
T_2
T_3
T_4
T_5
T_7
T_10
T_14
GO_0
GO_1
GO_2
GO_3
GO_4
GO_5
GO_7
GO_10
GO_14
SO_0
SO_1
SO_2
SO_3
SO_4
SO_5
SO_7
SO_10
SO_14
M_0
M_1
M_2
M_3
M_4
M_5
M_7
M_10
M_14
Y_0
Y_1
Y_2
Y_3
Y_4
Y_5
Y_7
Y_10
Y_14

584 Figure 2

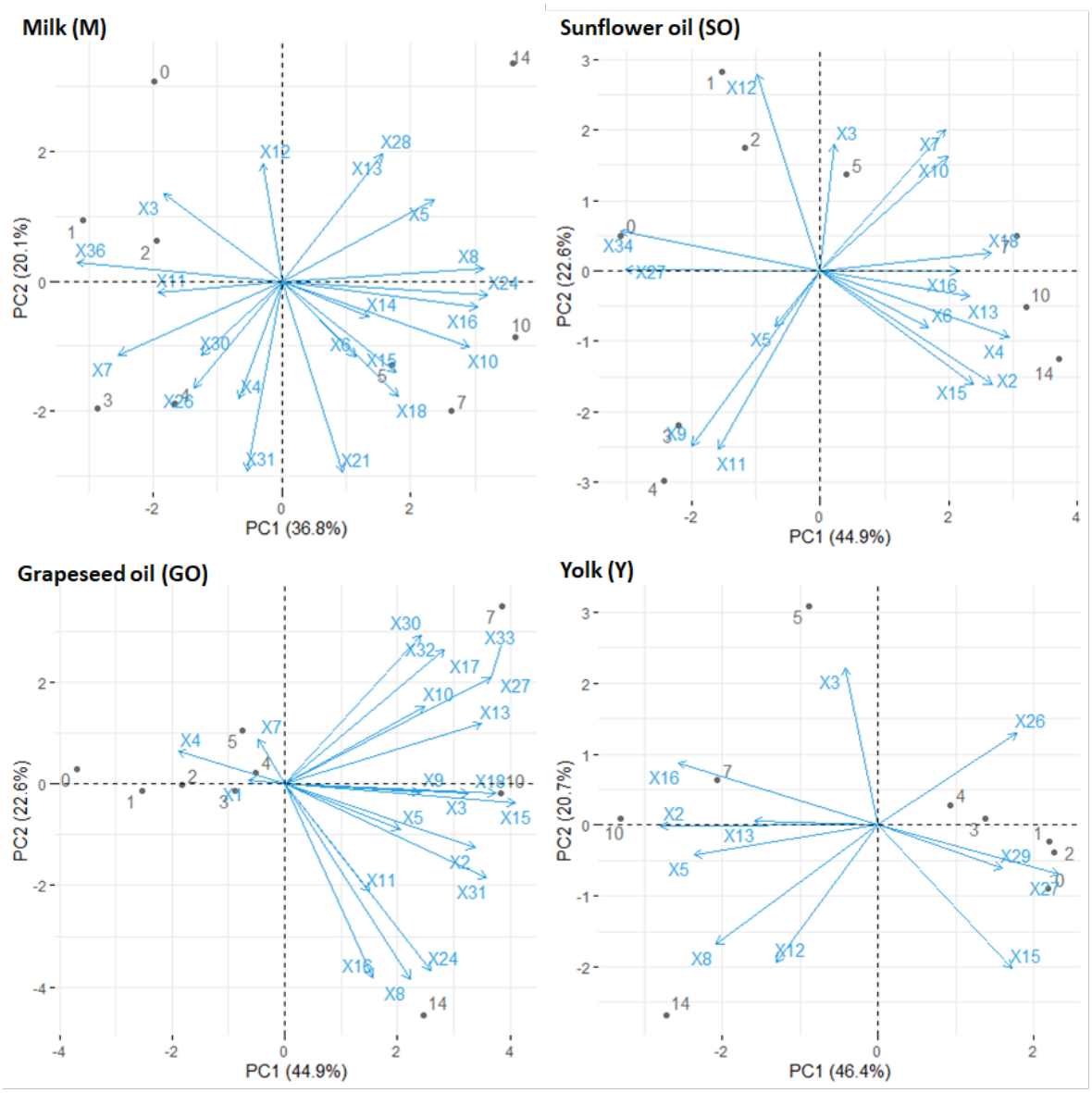


585

586 Figure 3

587

Figure 4


Milk (M)
PC2 (20.1%)
PC1 (36.8%)
Sunflower oil (SO)
PC2 (22.6%)
PC1 (44.9%)
Grapeseed oil (GO)
PC2 (22.6%)
PC1 (44.9%)
Yolk (Y)
PC2 (20.7%)
PC1 (46.4%)