Aromatic profile of black truffle grown in Argentina: characterization of commercial categories and alterations associated to maturation, harvesting date and orchard management practices

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Abstract

Black truffle (*Tuber melanosporum*) is one of the most appreciated fungi in the world mainly due to its aromatic properties. In the emerging markets such as Argentina, the aroma of locally produced truffles has not been described yet. The volatile organic compounds (VOCs) from 102 black truffles from Argentina were analyzed using solid phase microextraction gas chromatography coupled with mass spectrometer detector (SPME-GC-MS). Several factors such as commercial category, maturity stage, host tree, geographical origin, and aromatic defects detected during classification were also registered and considered. As a result, 79 VOCs were detected, among which 2-methyl-propanal, 2-butanone, 2-methyl-1-propanol, butanal-3-methyl, 3-methyl-1-butanol, 2-methyl-1-butanol were present in high percentage in fresh mature truffles, whereas immature truffles were associated with 3,5-dimethoxytoluene, 2-phenyl-2-butenal, 2,3-dimethoxytoluene. The Argentine black truffles showed significant similarities in their aromatic profile when compared with their Australian and European counterparts, but with some distinctive notes.

1. Introduction

Truffles are one of the most aromatic mushrooms known. Nowadays only a few species have commercial and culinary interest, and just some of them have been domesticated. The black truffle (*Tuber melanosporum*), also called the Périgord truffle, is cultivated in different countries, mainly in Spain (47 tons/year), France (43 tons/year) and Italy (19 tons/year) (Oliach et al., 2021) as well as in the Southern hemisphere in Australia (11 tons/year) (Čejka et al., 2020), New Zealand (data not published), Chile (1.3 tons/year) (Oliach et al., 2021), South Africa (data not published), and Argentina (0.6 tons/year, Trufas del Nuevo Mundo company data). The production in the latter countries is increasing year by year. *Tuber melanosporum* is a seasonal product, with the Southern Hemisphere production being counter-seasonal to that of the Northern Hemisphere (November to March in the latter and June-August in the former), thus practically providing fresh black truffles all-year-round.

It is also known that the aroma varies among ascocarps within the same truffle species, according to differences in the development or maturation stages (Caboni et al., 2020; Shah et al., 2020; Splivallo et al., 2012) geographical origin (Li et al., 2022; Niimi et al., 2021a), host trees (Culleré et al., 2017), and soil microbial community composition (Niimi et al., 2021b; Splivallo et al., 2011; Splivallo & Ebeler, 2015), among other external abiotic (weather conditions and soil characteristics) and biotic factors. Diverse authors suggest that the aroma profile of truffles is less likely to be affected by genetic factors than by environmental factors affecting its growing conditions (Patel et al., 2017; Splivallo et al., 2011; Vita et al., 2015). In this regard, the Chinese truffle (*Tuber indicum*) aroma analyses have shown differences among countries, within the same country, and between truffles collected from different host plants within the same orchard (Splivallo et al., 2012). Based on previous data, it is expected to detect these

aromatic differences in new producing countries such as Argentina. However, in the Southern hemisphere the aromatic profile of truffles has only been studied in Australia, the highest black truffle-producing country in the south. The Australian study showed *T. melanosporum* aromatic profiles similar to those of truffles grown in Spain and France, making these truffles suitable for Spanish and French markets during the Northern hemisphere summer. The aromatic profile was richer, earthy, maillard, vegetal with herbal aroma notes compared to European black truffle (Choo et al., 2021). Up to now, apart from Australia, there are no published data on the aromatic profile of *T. melanosporum* truffles in other producing countries outside of Europe.

Therefore, our objective was to characterize the aromatic profile of *T. melanosporum* truffles produced in Argentina and identify possible variations due to local biotic and abiotic factors in addition to previously described aromatic profiles. For this purpose, truffle developmental factors, geographic factors and factors related to the orchard management were considered (maturity stage, geographical location, host tree, commercial categorization of truffles, and aromatic defects detected during classification of truffles in commercial categories).

2. Materials and methods

2.1 Truffle orchards and sample collection

The ascocarps of *T. melanosporum* were collected in Espartillar (location 1, L1) (Saavedra, Buenos Aires, Argentina) at three different times: 6-7 June, 6-7 July and 8-9 August 2022 from the same cultivated truffle-ground; and in Azul (location 2, L2) (Azul, Buenos Aires, Argentina) in July 2022. The L1 orchard has two species of host tree planted, European oak (*Quercus robur*) and holm oak (*Quercus ilex*), whereas L2 orchard has holm oak.

Once harvested, truffles were processed and taken to the lab within 48 hours in all the samplings. Dug out truffles were immediately stored in insulated boxes with refrigeration. In the lab, the soil was removed by brushing the truffle peridium with a wet soft brush, and then rinsed with tap water and dried with absorbent paper. A qualitative selection of the best ascocarps was made by discarding those with softened texture, having coleopteran larvae inside or those damaged during the harvest by either truffle hoes or dogs. Mature truffles were classified in commercial categories: “extra”, “first” and “second” following UNECE STANDARD FFV3 (Unece, 2017). Briefly, extra truffles must have rounded shape, more or less regular and lobed, free from defects or with very slight superficial ones (appearance, shape and color). Class I might have slight defects in shape, development, coloring and bruising, whereas more notorious defects are allowed in Class II category. The minimum weight in each category is: 20 g for Extra class, 10 g for Class I, and 5 for Class II.

In our study, the maturity was determined observing the *T. melanosporum* spore morphological characteristics by microscope (Tejedor-Calvo et al., 2023a) and were compared with the description of Leonardi et al., (2021). Immature truffles were selected from discarded material observing the gleba color (Fig. 1). According to Zarivi et al., (2015) ascocarps below stage VI, also called pigmented, were considered immature. Truffle samples were processed by farmers in the same way as those with commercial quality, to avoid aroma variability due to post-harvest practices. During the commercial classification, the aroma perceived was also evaluated. In the case that a strange aroma different from “fresh black truffle” was detected, the sample/s were separated and classified as ‘aroma defects’. A truffle trained panel of 10 people (22-45 years old; 4 females and 6 males) selected an aromatic attribute to refer to each sample (Table S2).

A total of 102 truffles were analyzed, including: (i) commercial categories extra, first and second from *Q. robur* in L1; (ii) immature truffles and truffles with aromatic defects from *Q. robur* in L1; (iii) truffles harvested in localized soil amendments with a peat-based substrate, hereafter called "truffle nests" from L1 (Garcia-Barreda et al., 2020), (iv) truffles from *Q. ilex* harvested in either L1 or L2, and (v) truffles with aromatic defects detected during classification in commercial categories from L1 (Table 1).

2.2 VOCs analysis

2.2.1 VOCs extraction by SPME

The methodological approach was based on studies carried out by Culleré et al. (2012) with some modifications. A solid phase microextraction (SPME) was used to extract the aromatic compounds. For that, a fused silica fiber coated with a 50/30 mm layer of divinylbenzene/carboxen/polydimethylsiloxane from Supelco (Barcelona, Spain) was chosen. The samples (2 g of truffle) were placed in a 20 mL glass vial closed with a septum. After the vial was conditioned at 50 ºC for 10 min. The fiber was then exposed to the headspace of the vial for 20 min. Analysis were carried out by duplicate.

2.2.2 GC-MS analysis

The VOCs profile of the different samples was analyzed using a gas chromatograph Perkin Elmer Clarus 600 Series coupled with a Perkin Elmer Clarus 600 mass spectrometer detector (Chatsworth, California, United States). This SPME-GC-MS instrument was equipped with a capillary column HP-5MS of 30 m, 0.32 mm i.d., 0.25 μm film thickness and a flow of 1 mL/min with helium as a carrier gas. The samples were injected in splitless mode. The oven temperature was 45 ºC held for 2 min, 45–200 ºC at a rate of 4 ºC/min, 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.

2.2.3 Data analysis

Peak identification of the VOCs was achieved by comparison of the mass spectral with mass spectral data from the NIST MS Search Program 2.0 library, and by comparison of previously reported Retention Indexes (RI) with those calculated using an n-alkane series (C6–C20) under the same analysis conditions. The n-alkane series and standards for MS identification (all standards of purity higher than 95%). A semi-quantification was done by integrating the area of one ion characteristic of each compound and normalization by calculating the relative percentage using OpenChrom ® (V. 1.5.0) program. This allowed comparison of each eluted compound between samples.

2.3 Sensory analysis

A panel of ten trained tasters evaluated the aroma of the samples from the different host trees (*Q. robur* and *Q. ilex*) from L1 samples. Tasters were previously trained for two sessions of 45 min. The analyses were conducted according to the ISO 11035:1994. A preliminary sensory analysis test was carried out to detect differences among samples from different host trees. The samples were presented in equal quantities (0.20 g of *T. melanosporum*) laminated with sunflower oil, in opaque and odorless glass vials, thus preventing panelists from recognizing the mushrooms by any other sense than smell. The sunflower oil was selected to avoid that color or shape interfere in the sample selection for sensory analysis. Two truffle samples from each host tree were selected. Samples were randomly coded with three digits and left to reach room temperature (20 °C) for one hour before being presented to the panelists. The trained panel also selected the sensory attributes to those samples externally included in the “mature” category but

158 classified as ‘with defects’. All procedures were performed in compliance with relevant
159 laws and institutional guidelines and that the appropriate institutional committee have
160 approved them. The consent has been given by the sensory panel used within
161 manuscript.

162

163 *2.4 Statistical analysis*

164 The sensory data was analyzed with one-way ANOVA followed by Tukey’s multiple
165 comparison test. Differences were evaluated at 95% confidence level ($P \leq 0.05$). The
166 VOCs were analyzed with Principal Component Analysis (PCA), performed and
167 visualized in RStudio February 1, 1335 (RStudio Team, 2019) using R version 3.6.1 and
168 the factoextra package (Kassambara & Mundt, 2017).

169 **3. Results and discussion**

170 *3.1 Aroma profiling of commercial fresh black truffle from Argentina*

171 In the 102 analyzed black truffles (Table 1), a total of 79 VOCs were identified (Table
172 2, Fig. S1). Among them, 2-methyl-propanal (C6), 2-butanone (C7), 2-methyl-1-
173 propanol (C8), butanal-3-methyl (C9), 3-methyl-1-butanol (C12), 2-methyl-1-butanol
174 (C13), 2-ethyl-4-penatenal (C15), hexenal (C16) and 4-methyl-3-pentenal (C18) were
175 detected in high relative area percentage (Table S1). Apart from them, other truffle key
176 aromatic compound, such as dimethyl sulfide, were found.

177 The data obtained were compared to the published aroma profiles of *T. melanosporum*
178 truffles grown in Europe and Australia (Table S1). Overall, the VOCs profile of
179 Australian truffles was dominated by 2-butanone (3.3%), 2-methyl-1-butanol (22.8%),
180 3-methyl-1-butanol (15.8%), 2-methyl-1-propanol (2.8), benzene,1-methoxy-3-methyl
181 (7.4%) and dimethyl-sulfide (8.8%) (Choo et al., 2021). The Argentine samples

contained similar values of 2-butanone (1.9%), 2-methyl-butanol (22%), 3-methyl-1-butanol (11.45%), 2-methyl-1-propanol (1.5%) and dimethyl-sulfide (7.7%). However, hexanal percentage in Argentina truffles (9.7%) was higher than in Australian (<0.11%) or Spanish (2.4%) truffles (Choo et al., 2021; Tejedor-Calvo et al., 2021). The aroma of hexanal is characterized by grass green, and fatty aroma (Table 2) (Tejedor-Calvo et al., 2021). Therefore, truffles from Argentina have green and fatty notes compared with the European and Australian truffles.

Mauriello et al. (2004) listed 17 main identified VOCs that represent black truffles grown in Italy, 5 of which were identified in Australian truffles (Choo et al., 2021) and 11 in our samples. March et al. (2006) reported 23 main VOCs that represents the aroma profile of French truffles, 5 of which were detected in the current study (2-methyl-1-butanol, acetaldehyde, carbon dioxide, 2-butanone, and dimethyl sulfide). Comparing with the 68 VOCs detected in Spanish truffles, 29 of them were found in Australia (Choo et al., 2021) and 28 in the Argentinian samples. Comparing with the 44 VOCs in Australian truffles, 18 were found in our samples. If we compare the percentages, truffles from Argentina matched 43 and 42% with the Australian and Spanish VOCs. These results suggested that even though black truffles from Argentina, Australia and Europe are the same species and very similar in terms of VOCs, some VOCs differences were still detected (Table S1). These differences could be related to variations in the soil, the climatic regime during truffle growth and maturation, the host tree species, or the composition of the soil microbiota (Culleré et al., 2017; Splivallo & Ebeler, 2015). However, for a precise comparison, it would be necessary to use the same harvesting method, VOCs extraction, GC method and data treatment, as well as considering that harvesting times are quite different in the northern and southern hemisphere.

206 The main VOCs proportion over the three commercial categories (Fig. 2) were
207 heterogenous within the commercial qualities. Some compound levels such as 2-
208 butanone decreased whereas 2-ehtyl-pentenal increased during the season. Truffles key
209 VOCs profile belonging to the second category showed differences in the 3-methyl-1-
210 butanol proportion compared with extra and first categories. The difference in VOCs
211 proportions might be because the extra and first categories go through a strict physical
212 selection criterion during the postharvest procedure, according to FFV Unece standard
213 norm (Unece, 2017), and therefore second category truffles can be more heterogeneous.
214 The variability in the relative area percentage (%) of volatiles among samples could also
215 be due to maturity variations or anomalies as affected by temperature during harvest
216 (Caboni et al., 2020). Besides these minor differences, truffle samples from the same
217 category showed similar aromatic profiles, indicating that the commercial classification
218 carried out in Argentina is objective.

219 *3.2 Differences associated to immaturity*

220 The VOCs profile of immature truffles was compared with that of mature commercial
221 truffles in order to evaluate the differences and target compounds (Fig. 3). Immature
222 samples showed high amounts of 3-methyl-1-butanol (C12) (18-50%), followed by 2-
223 butanone (C7) (up to 20%), and 4-methyl-pentenal (C18) (up to 18%) (Fig. S1). Other
224 compounds such as 2-octanone (C37), 3-octanol (C38), 1-butamine, 2-methyl-N-(2-
225 methylbutylidene) (C42), Benzene, 1,2-dimethoxy- (C53) and (E)-2-nonenal (C54)
226 were detected in higher concentration than in mature commercial categories. These
227 compounds were completely different from the mature truffles profile, therefore might
228 be related with immature conditions (Culleré et al., 2010; Strojnik et al., 2020). Some
229 mature truffle samples showed similar fingerprint than immature ones, such as S1-2,
230 N1-2, R-5, F3-3, D1, L2-5 (Fig. S1), indicating that despite their external appearance

231 the aroma profile can be classified as immature. Truffles from nests did not show VOCs
232 profiles different from truffles from the bulk soil.

233 Shah et al. (2020) studied the aroma changes in summer truffles (*T. aestivum*), showing
234 an increasing of the C8-VOCs (1-octen-3-ol) from 7.27 to 16.64%, as well as an
235 increase in alcoholic compounds and decrease in aldehyde compounds upon maturity.
236 Some of the C8-VOCs were characterized as mushroom-like aroma (Culleré et al.,
237 2013). Agreeing to that, in our samples some aldehydes as 2-ethyl-4-pentenal and
238 hexenal showed lower intensities in mature truffles, whereas alcoholic compounds like
239 3-octanol and 3-methyl-penol showed higher (Fig. S1). These compounds might
240 contribute to the Argentine truffle aroma with mushroom-like notes.

241 Molinier et al. (2015) described that 2-butanone was linked to truffle maturation.
242 However, no such relationship has been detected in either our study or *T. aestivum*
243 samples (Strojnik et al., 2020). Some authors described that the increase of 3-methyl-1-
244 butanol could be due to the catabolism of leucine via Ehrlich pathway during the amylic
245 fermentation of the polysaccharides by yeast and microbes that contribute to the
246 degradation of truffle (Caboni et al., 2020; Splivallo et al., 2007; Vahdatzadeh et al.,
247 2019). In our fresh samples, as well as in Australian samples, these compounds were
248 found in high levels (22.13 and 15.82 % respectively). In accordance with that, the
249 Australian VOCs study described that 3-methyl-1-butanol increased to 17.39 and 18.23
250 after 7 and 14 storage days. Also, Vahdatzadeh et al. (2019) described this compound as
251 marker for truffle degradation. Nevertheless, the Argentine truffle samples were
252 analyzed only 24 hours after harvesting, therefore if there is any increasing of 3-methyl-
253 1-butanol might not be because of the spoilage.

254 A principal components analysis (PCA) for each harvesting time was used to explore
255 the possible correlations of VOCs with mature and immature truffles (Fig. 3). The PCAs

analysis explained 44.2, 37.1 and 39% of the data variability with the two first components for June (Fig. 3A), July (Fig. 3B) and August (Fig. 3C) respectively. In the first harvesting time (June), the aroma profile of immature truffles was mainly separated of mature truffles by the first PCA. The compounds that showed the more positive loadings with the first PCA component (associated with immature truffles) were 2-penthyl-2-butenal (C69) and (E)-4-nonenal (C47), whereas those showing the more negative loadings were 2-butanone (C7) and butanal-3-methyl (C9) (Fig. 3A). Most compounds with high relative abundance were positively associated with mature truffles. Among immature samples, two groups could be clearly separated, with IN1-1 and IN5-1 in the right end and the remaining samples in the middle of the biplot. This suggests a different stage of maturity, with the former group apparently being farther from maturity.

In the second harvesting time (July), the PCA also allowed to clearly separate immature from mature truffles, although in this case only two immature truffles were analyzed. The compounds that showed the more positive association with immature truffles were methylpropylformate (C11), 2-methyl-2-pentenal (C19), 2-hexenal (C20) and 1,2-dimethoxybenzene (C53), none of which appeared in June associated with immature truffles. The July PCA also clearly separated one truffle classified as commercial (ES2-2). Most mature truffles clustered in the lower right end of the PCA, positively associating with compounds that in June also positively associated with mature truffles (Fig. 3A). However, sample ES2-2 positively associated to compounds that in June positively associated to immature truffles, suggesting that the aromatic profile of this truffle could be classified as unripe despite its gleba color.

In the third harvesting month (August), as in the others PCAs, butanal-3-methyl (C9) showed the more positive association with the larger group of commercial mature

281 truffles, in this case characterized by positive loadings for the first PCA component. On
282 the other hand, 2,5-dimethoxyethylbenzene (C73) and 2,4-nonadienal (C62) showed the
283 more negative loadings for PCA1. The second PCA more positive loadings
284 corresponded to 1,2,4-trimethoxybenzene (C75) and 3,4,5-trimethoxy-toluene (C77),
285 whereas 4-methyl-3-pentenal (C18), methyl butyl acetate (C25) and limonene (C41) to
286 the negative loadings. In this PCA, the immature samples were placed indistinctly, with
287 IN4-3, IN3-3 and IN6-3 showing a positive association with PCA2. One mature sample
288 (F4-3) was near these immature samples, suggesting an immature aroma. Regarding the
289 key compounds (red arrows), they were more widely spread in the PCA in August
290 samples than in previous months. This indicates that there were fewer differences
291 between truffles classified as immature and mature towards the end of the harvesting
292 season.

293 *3.3 Differences associated to host tree species*

294 Truffles harvested under *Q. ilex* (QI) and *Q. robur* (QR) in the same location and
295 harvesting time, presented 79 compounds (Table 2) in common. A PCA was used to
296 explore whether the differences among these samples could be linked to the host tree
297 species. This PCA explained 43.3% of the data variability with the two first components
298 (Fig. 4A). The first PCA component was negatively associated with compounds that
299 had previously been associated to immature truffles (Fig. 3), such as 2,5-
300 dimethoxyethylbenzene (C73), 3,5-dimethoxytoluene (C68), 2,4-nonadienal (C62),
301 dodecane (C60) or tetradecane (C76). This suggests that truffles such as QI-3, QI-6 and
302 QR-2 have not completely developed the characteristic aroma of mature truffles yet.
303 Most of the remaining truffles clustered in the right end of the biplot (Fig. 4A),
304 positively associated to VOCs that had previously been associated with mature truffles
305 (Fig. 3). Within this group, the QI truffles (QI-1, QI-2, QI-5, QI-7) tended to show

306 higher PCA1 and PCA2 scores, although a few QR truffles also did. Thus, mature QI
307 truffles showed a slightly higher association to compounds such as 2-ethyl-4-pentenal
308 (C15), hexanal (C16), or 2-octene (C17), whereas most mature QR truffles showed
309 slightly higher association to compounds such as 2butanone (C7), 2-methyl-1-propanol
310 (C8) or 3-methyl-butanal (C9).
311 Culleré et al. (2017) reported that truffles from Portuguese oak (*Quercus faginea*) had a
312 more intense animal aroma than those of holm oak truffles, mainly due to 3-methyl-1-
313 butanol and 3-ethyl-5-methylphenol compounds. Only the former 3-methyl-1-butanol
314 (C12) was detected in our samples, slightly more associated to QR than to QI truffles
315 (Fig. 4A). Furthermore, the same authors observed that the levels of the other two
316 phenols identified (p-cresol and 3-ethylphenol) were slightly higher in samples taken
317 from Portuguese oak than in the rest. However, those compounds were not detected in
318 the analyzed truffles from Argentina. Additionally, during the sensory analysis it was
319 check whether QI-QR differences were detected by humans. From 36 answers of the
320 trained panel, only 20% of the results were correct (data not shown), indicating that
321 there were no statistical differences noticeable by humans between QR and QI truffles.

322 *3.4 Differences associated to geographical location*

323 We performed a PCA analysis to explore whether there were aromatic differences
324 between truffles harvested under holm oak among the two different geographical
325 locations. The PCA explained 50.5% of the data variability with the first two
326 components (Fig. 4B). The first PCA component was positively associated with VOCs
327 that had previously been linked to immature truffles (Figs. 3, 4). Truffles from *Q. ilex* in
328 the Espartillar orchard (L1) were again separated in two groups, as shown above (Fig.
329 4A). Truffles from the Azul orchard (L2) clustered together between these two groups,
330 showing a progression along the PCA1 axis. The only exception was truffle L2-5, which

correlated with other compounds also related previously with immaturity, such as 1,4-dimethoxy-3-methylbenzene (C65), including some aldehydes (E)-2-nonenal (C54), (E)-4-decenal (C58), and (E)-2-decenal (C67). Following the PCA some compounds (arrows pointing at the top left of the PCA) were more correlated with L2 samples, whereas other ones (arrows pointing at the bottom left) were more correlated with L1 (Fig. 4B). Although there is not an exclusive compound for each location, the relative area percentage of these groups of VOCs might indicate where the sample was taken.

Several authors have studied the differences of nutritional compounds or aromatic volatiles in truffles in relation to geographical regions (Al-Laith, 2010; Garcia-Barreda et al., 2019; Niimi et al., 2021b; Šiškovič et al., 2021; Vita et al., 2015; Wu et al., 2021). In our case, with only two locations (distant 200 km apart and with similar climatic conditions within the Pampean grasslands), and likely without genetic variability between both locations (the black truffle has been introduced in both cases), differences related to maturity stage appear much more clearly that differences between locations, despite the fact that edaphoclimatic factors or soil microbial populations can influence truffle development (Li et al., 2022; Niimi et al., 2021a). It has been suggested that content of C8-VOCs (1-octen-1-ol, 1-octen-3-one, 2-octenal) could be controlled at the genetic level and not depend on the maturation process (Šiškovič et al., 2021; Splivallo et al., 2012).

Furthermore, VOCs differences within *T. aestivum* truffles in Slovenia were described by Šiškovič et al. (2021). Their results showed strong differences between one location with sub-Mediterranean climate, where the peak truffle production is during late spring to early summer, and other locations with a later production peak (autumn to winter). The content of 2-methylbutan-1-ol (15–25%) and 1-methoxy-3-methylbenzene (≤1%) allowed to distinguish *T. aestivum* geographical origin. As far as we know, *T.*

356 *melanosporum* truffles studies involving different origins have been focused on other
357 fields such as genetics (García-Cunchillos et al., 2014), climate (Garcia-Barreda et al.,
358 2019) and bioactive compounds (Tejedor-Calvo et al., 2020). However, aroma or
359 metabolomics might be able to detect geographical origin differences.

360 *3.5 Aroma defects*

361 We expected to identify the compound corresponding to each "aromatic attribute"
362 through the VOCs analysis. For each defective sample, a unique profile, different from
363 the commercial category samples, was observed (Fig. S2). The first defective sample
364 (D1), with an aroma described as 'medicine', contained high amounts of propanal-2-
365 methyl (malt, green), 3-methyl-1-butanol (whiskey), (E)-2-nonenal (fatty, green,
366 cucumber), linalyl acetate (sweet, fruity) and 2-undecanone (creamy, cheese,
367 pineapple). It might be possible that the odor perceived was a combination of some of
368 these compounds, instead of only one. High levels of 3-methyl-1-butanal and 3-methyl-
369 1-butanol have been used as marker of truffle over-ripeness (Culleré et al., 2012; March
370 et al., 2006; Splivallo & Ebeler, 2015; Tejedor-Calvo et al., 2023b; Tejedor-Calvo et al.,
371 2023c; Torregiani et al., 2017).

372 The second sample (D2), described as with 'amonia' smell, contained high percentage
373 of 2-butanone. The smell emitted by this molecule has been described as ether, sweet or
374 solvent, but not ammonium. So, maybe other compounds or the synergic effect of them
375 are given the ammonium aroma detected by the trained panel. The third sample (D3)
376 was related with bacterial aroma, and the VOCs analysis showed similar content as D1
377 except for benzene, 1,2-dimethoxy- (musty), benzene, 1,4-dimethoxy-3-methyl- (green),
378 and (E)-2-decenal (fatty, earthy). This VOCs profile might indicate that truffle bacteria
379 content was higher despite having a normal external appearance. The aroma of sample
380 D4 was classified as fishy. Apparently, none of the compounds in Table S2 is usually

associated to fish odor. Some authors related z-4-heptenal to the fish odor descriptor (Campo et al., 2017; Tejedor-Calvo et al., 2021). However, this compound was not detected in Argentine truffles, so the fish odor description might be due to other minor compound or to some combination of compounds.

The odor from samples D5 and D6 were both described as solvent. They presented similar VOCs content compared with commercial truffles, except for 4-methyl-3-pentenal and 2-octanone compounds. Since none of them has aromatic properties, the solvent description might be due to compounds with similar percentage in both samples, *i.e*. 2-butanone.

The aroma of D7 sample was described as rotten, and the VOCs profile presented high percentages of C8-VOCs such as 2-octanone, 3-octanol (nut, mushroom) or octanal (green, citrus), but also acetaldehyde (pungent, ether) and 2,4-nonadienal (green). It has been described that acetic acid, ethanol, 2-methyl-1-butanol,2-methyl-1-propanol, acetaldehyde, 3-methylbutanal, 2-methylbutanal, propanal, hexanal or 2-methyl-2-butenal formed by truffle fermentation are possible markers of product spoilage (Costa et al., 2015). Among them, only 2-methyl-2-butenal was found in high percentage in the D7 sample, compared to other samples. Thus, the aroma profile of this sample might be due to truffle fermentation.

Harki et al. (2006) stated that the formation and increase of acetaldehyde through amino acid degradation does contribute to the fragrance of truffle. However, Choo et al. (2021) indicated that acetaldehyde high levels in truffles might result in a fruity and floral aroma. The D7 sample showed high level of acetaldehyde, indicating that an amino acid degradation might have happened.

4 Conclusions

We thoroughly characterized the aroma of Argentine black truffles. The key VOCs that could be used to determine the freshness of Argentine black truffle have been identified: 2-methyl-propanal (malt, green), 2-butanone (ether), 2-methyl-1-propanol (solvent, bitter), butanal-3-methyl (malt), 3-methyl-1-butanol (whiskey), 2-methyl-1-butanol (wine, onion), 2-ethyl-4-penatenal (odorless), hexenal (grass), 4-methyl-3-pentenal (odorless). Truffles from Argentina have richer malty, vegetal and whiskey aroma notes compared to Australian and European black truffles. We identified the aroma differences that characterize immature truffles and showed that geographical origin and host tree species might slightly modify the VOCs profile. This study provides critical information for truffle growing and postharvest management of truffles in Argentina, being a baseline for further studies of truffle aroma and postharvest treatment in Argentina and the region. The VOCs profile of Argentinian truffles is similar to that of Spanish and Australian truffles, making them suitable for the global market during the Northern Hemisphere summer.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Credit authorship contribution statement

E.T-C.: Conceptualization, Research, Methodology, Data curation, Writing - original draft; S.G-B.: Software, Visualization, Writing - review & editing; S.S.: Funding acquisition; P.M.: Funding acquisition; D.P.: Methodology and data analysis for figure construction; J-S. D. Investigation, Methodology; E. N.: Conceptualization, Management and funding acquisition, Writing - review & editing.

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Tables

Table 1. Number of samples analyzed, associated host tree and qualitative categories of truffles during the harvest period.

		Location 1			Location 2
Truffle tree	Categories	June	July	August	July
Quercus robur (QR)	Extra (E)	6	6	7	-
	First (F)	7	7	6	-
	Second (S)	10	6	7	-
	Immature (IN)	6	2	6	-
	Truffle nest (N)	2	-	-	-
	With defects (D)	-	7	-	-
Quercus ilex (QI)	-	-	9	-	8

Table 2. List of volatile organic compounds (VOC) identified by SMPE-GC-MS in the truffle samples.

Nº	**Name**	**Odor***	**CAS nº**	**RT**	**RI $_{exp}$**	**RI $_{lit}$**	**Mass (*m/z*)**		
1	Carbon dioxide	-	124-38-9	1.524	588	-	44	40	44
2	Acetaldehyde	pungent, ether	75-07-0	1.599	593	-	44	43	42
3	Ethanol	sweet	64-17-5	1.694	600	459	45	43	47
4	2-propanone	caramellic, burnt	67-64-1	1.749	604	-	43	58	42
5	Dimethyl sulfide	cabbage, sulfur, gasoline	75-18-3	1.844	612	532	62	47	46
6	Propanal-2-methyl	malt, green	78-84-2	1.964	621	534	43	41	72
7	2-butanone	ether	78-93-3	2.149	634	600	43	72	47
8	2-methyl-1-propanol	solvent, bitter	78-83-1	2.334	648	618	43	39	47
9	butanal-3-methyl	malt	590-86-3	2.569	666	659	41	44	39
10	butanal-2-methyl	cocoa, almond	96-17-3	2.679	674	671	57	86	41
11	Methylpropylformate	-	589-40-2	2.874	689	-	45	41	57
12	3-methyl-1-butanol	whiskey	123-51-3	3.549	729	735	55	42	70
13	2-methyl-1-butanol	wine, onion	137-32-6	3.694	737	738	57	56	41
14	2-methyl-2-butenal	green, fruit	1115-11-3	3.739	739	-	84	55	41
15	2-ethyl-4-pentenal	-	5204-80-8	3.955	751	1034	41	55	84
16	Hexanal	grass, fat	66-25-1	4.84	800	800	44	56	41
17	2-octene	-	111-67-1	5.04	807	-	41	57	55
18	4-methyl-3-pentenal	-	5362-50-5	5.2	812	-	41	55	69
19	2-methyl-2-pentenal	green, fruity	623-36-9	5.645	828	-	41	98	39
20	2-hexenal	fat, rancid	505-57-7	6.2	847	847	41	55	39
21	2-nonanone	soap, green	821-55-6	6.365	853	1091	43	58	57
22	5-methyl-hexanal	-	1860-39-5	6.626	862	-	43	55	96
23	1-hexanol	flower, green	111-27-3	6.711	865	870	56	43	42
24	4-methyl-hexanal	-	41065-97-8	6.946	874	-	70	57	39
25	Methylbutyl acetate	fruit	624-41-9	7.061	878	877	43	70	55
26	Heptenal	fat, citrus, rancid	111-71-7	7.686	900	899	43	41	70
27	Anisole	anise seed	100-66-3	8.201	914	-	108	65	78
28	4-metyhl-1-hexanol	sweat	818-49-5	9.222	944	-	70	69	57
29	(Z)-2-heptenal	green, apple, fruity	57266-86-1	9.517	952	951	41	55	39
30	Benzaldehyde	almond, burnt sugar	100-52-7	9.597	954	960	77	106	105
31	5-methyl-2-heptanone	-	18217-12-4	9.867	962	-	43	58	71
32	1-heptanol	green	111-70-6	10.087	969	970	41	56	70
33	2-ethyl-2-hexenal	-	645-62-5	10.177	971	-	41	55	97
34	1-octen-3-one	mushroom	4312-99-6	10.332	976	976	55	70	43
35	1-octen-3-ol	mushroom	3391-86-4	10.457	979	978	55	41	72
36	3-octanone	mushroom	106-68-3	10.657	985	984	55	43	71
37	2-octanone	-	111-13-7	10.817	989	989	43	58	57
38	3-octanol	nut, mushrrom	589-98-0	11.022	995	993	59	55	83
39	Octanal	green, citrus	124-13-0	11.247	1002	1001	43	57	44

40	Benzene, 1-methoxy-3-methyl	-	100-84-5	11.813	1018	-	122	91	77
41	Limonene	lemon, orange	138-86-3	12.203	1028	1026	68	93	79
42	1-butamine,2-methyl-N-(2-methylbutylidene)	-	54518-97-7	12.523	1037	-	98	43	41
43	Benzenacetaldehyde	hawthorne, honey, sweet	122-78-1	12.613	1039	1038	91	92	65
44	(E)-2-octenal	green, nut, fat	2548-87-0	13.198	1055	1059	41	55	70
45	1-nonanol	floral, rose, fresh	143-08-8	13.663	1068	1163	43	55	69
46	3-methyl-phenol	fecal, plastic	108-39-4	13.938	1075	1075	108	107	79
47	(E)-4-nonenal	-	2277-16-9	14.659	1100	-	41	55	84
48	Undecane	alkane	1120-21-4	14.854	1100	-	57	43	71
49	Nonanal	cucumber, lemon, green, citrus	124-19-6	14.924	1102	1102	57	41	55
50	2-methylbutyl-2-methylbutanoate	strawberry, pineaple	2445-78-5	15.091	1107	1103	57	70	85
51	Benzeneethanol	honey, spice, rose, lilac	60-12-8	15.199	1110	1109	91	92	122
52	3-ehtylphenol methyl ether	-	10568-38-4	15.254	1111	-	121	136	91
53	Benzene, 1,2-dimethoxy-	musty	91-16-7	16.414	1143	1150	198	95	77
54	(E)-2-nonenal	fatty, green, cucumber	18829-56-6	16.915	1157	1158	41	43	55
55	Benzene, 1,3-dimethoxy-	medicinal, chemical, root	151-10-0	17.025	1160	-	138	78	109
56	2,3-dimethoxytoluene	-	4463-33-6	17.52	1175	-	152	137	109
57	Naphthalene	pungent, dry, resinous	91-20-3	17.78	1181	1180	128	102	129
58	(E)-4-decenal	citrus, green, orange, cardamom	21662-09-9	18.17	1192	1205	41	55	84
59	Phenol,3-methyl-6-propyl-	-	31143-55-2	18.355	1197		121	122	150
60	Dodecane	alkane	112-40-3	18.46	1191	-	57	43	71
61	Decanal	sweet, waxy, orange, wheat	112-31-2	18.59	1204	1205	41	57	29
62	2,4-nonadienal	fat, wax, green	5910-87-2	18.84	1211	1216	81	41	39
63	3,4-dimetoxytoluene	-	494-99-5	19.696	1236	-	152	137	109
64	2,5-dimethoxytoluene	-	24599-58-4	19.891	1242	-	137	152	77
65	Benzene, 1,4-dimethoxy-3-methyl-	green	150-78-7	20.071	1247	-	137	152	77
66	Linalyl acetate	sweet, fruit	115-95-7	20.371	1256	1255	93	42	41
67	(E)-2-decenal	fatty, earthy	3913-81-3	20.516	1260	1259	41	43	55
68	3,5-dimethoxytoluene	-	4179-19-5	20.75	1267	1263	152	123	91
69	2-phenyl-2-butenal	oney, cocoa	4411-89-6	20.906	1271	1279	117	115	146
70	2-undecanone	creamy, cheese, pinaple	112-12-9	21.622	1292	1291	43	58	71
71	tridecane	alkane	629-50-5	21.907	1300	-	57	43	71
72	2,4-Decadienal (E,E)	fried, wax, fat	25152-84-5	22.387	1315	1314	81	41	55
73	2,5-dimethoxyethylbenzene	-	1199-08-2	22.502	1319	-	151	166	91
74	y-nonalactone	coconut, buttery	104-61-0	23.812	1359	1361	85	41	43
75	1,2,4-trimethoxybenzene	-	135-77-3	24.152	1369	-	153	168	125
76	Tetradecane	alkane	629-59-4	25.153	1400	-	57	43	71
77	3,4,5-trimethoxy-toluene	-	6443-69-2	25.2	1401	1400	182	167	139
78	2-Allyl-1,4,dimethoxybenzene	-	19754-22-4	27.9	1489	-	178	135	163
79	Phenol,2,5-bis(1,1-dimethylethyl)-	-	5875-45-6	28.589	1511	1514	191	57	41

*Odor was selected by Flavornet and Thegoodscentscompany website

RT= retention time

RI_{exp} = Retention Index experimental.

RI_{lit} = Retention Index Literature database NIST

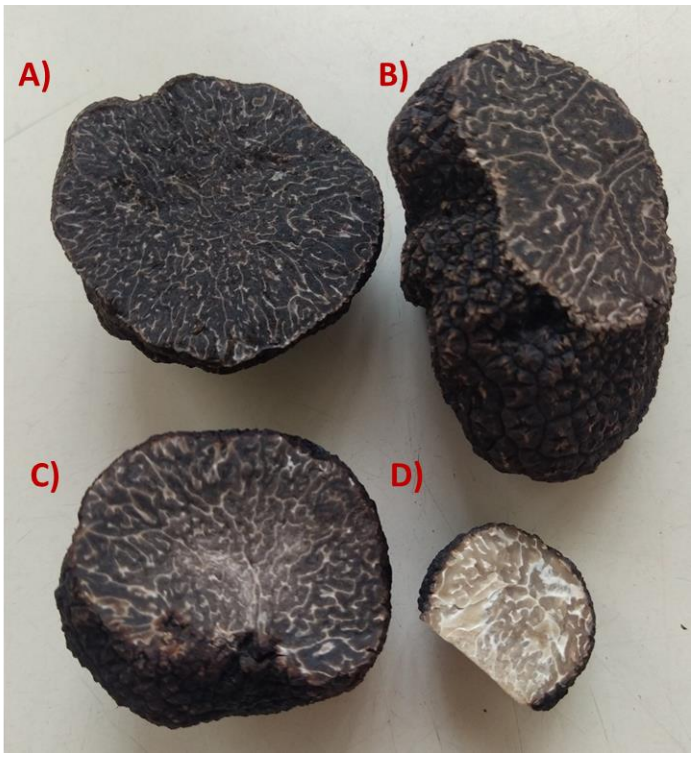
Figures

Figure 1. Commercial category samples A) Extra, B) First, C) Second, D) Immature.

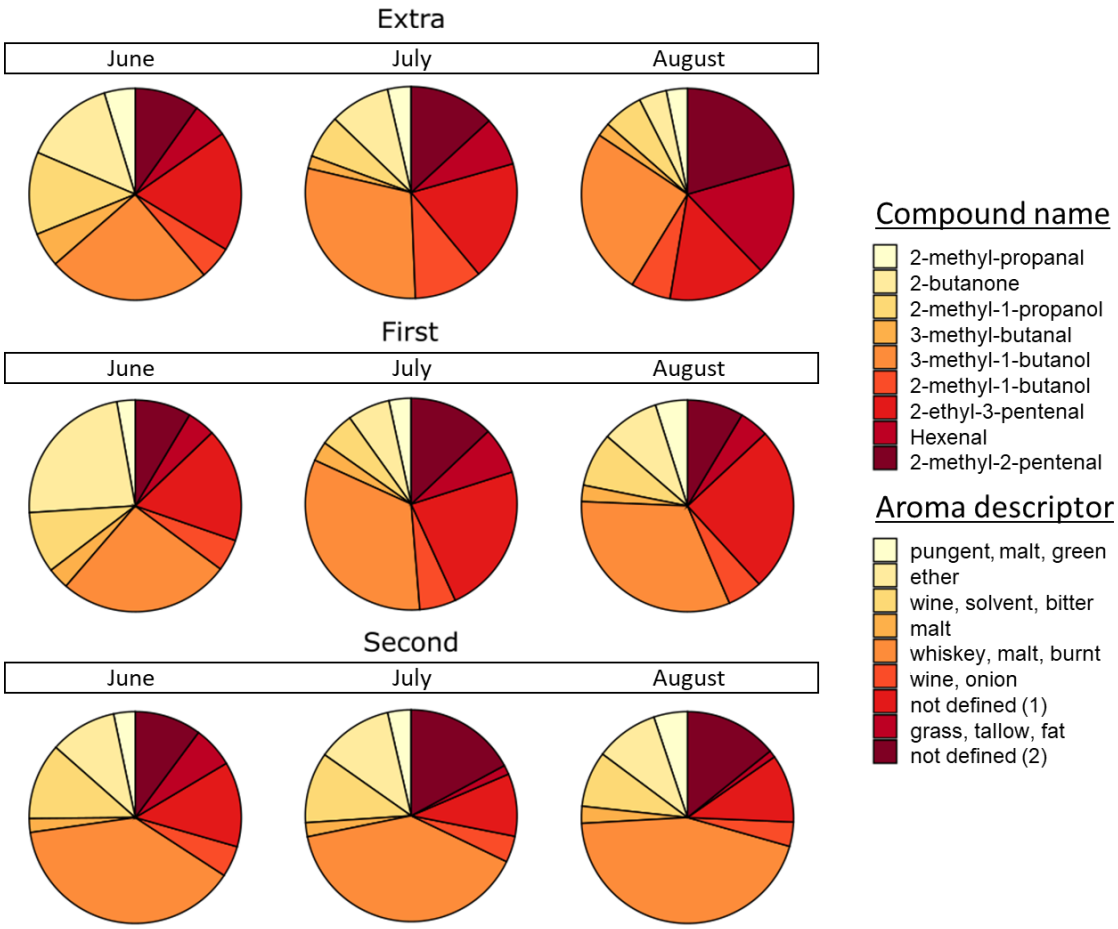
Figure 2. Proportion of key volatile compounds according to relative area percentage, in Argentine truffles based on commercial categories (extra, first, and second) and harvest months (June, July, August).

Figure 3. PCA plot corresponding to VOCs attributes detected by SPME-GC-MS listed in Table 2 for the three harvest times in mature and immature truffles (colored in yellow) from *Quercus robur*: A) June, B) July, C) August. Arrows indicate the contribution of a compound to the PCA components (contrib.) and sample indicates the quality of representation for the sample (cos2). Arrows marked in red correspond to the compounds with highest relative abundance and samples marked in yellow the immature truffles. Names correspond to extra (E), first (F), second (S), immature (IN), truffle nest (N). The number after the letters correspond to the replicates, and -1, -2, or -3 correspond to June, July, or August respectively.

Figure 4. PCA plot corresponding to VOCs attributes detected by SPME-GC-MS listed in Table 2 for A) different host tree: *Quercus robur* (QR-green) and *Quercus ilex* (QI-yellow) and B) for truffles from *Quercus ilex* of two locations: Espartillar (L1-orange), Azul (L2-green). Arrow color indicates the contribution of a compound to the PCA components (contrib). In A) QR samples correspond to extra, first and second truffles from July in L1, and in B) L1 samples correspond to QI samples (see Table 1).

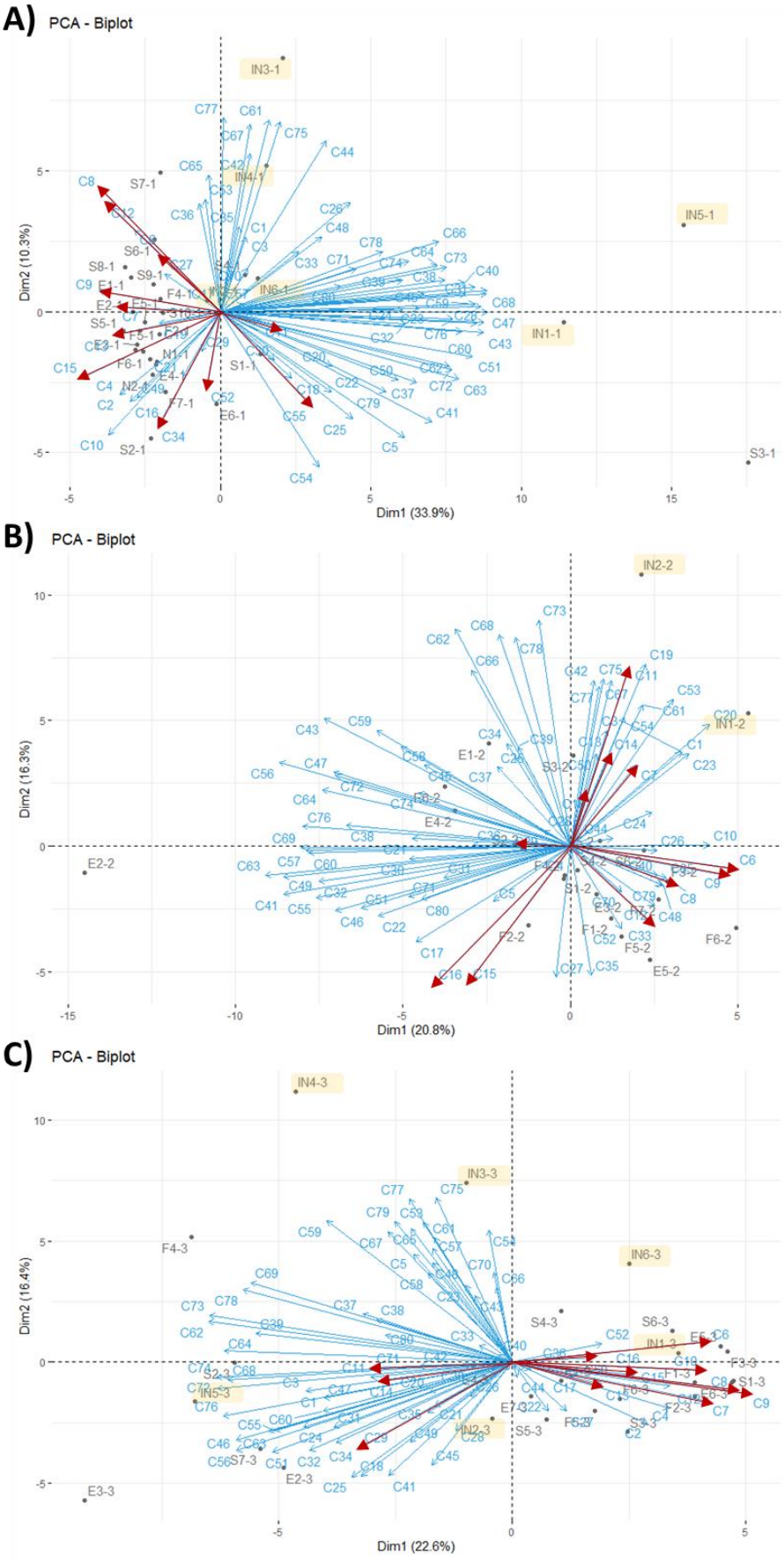
627 

628 **Figure 1**



629

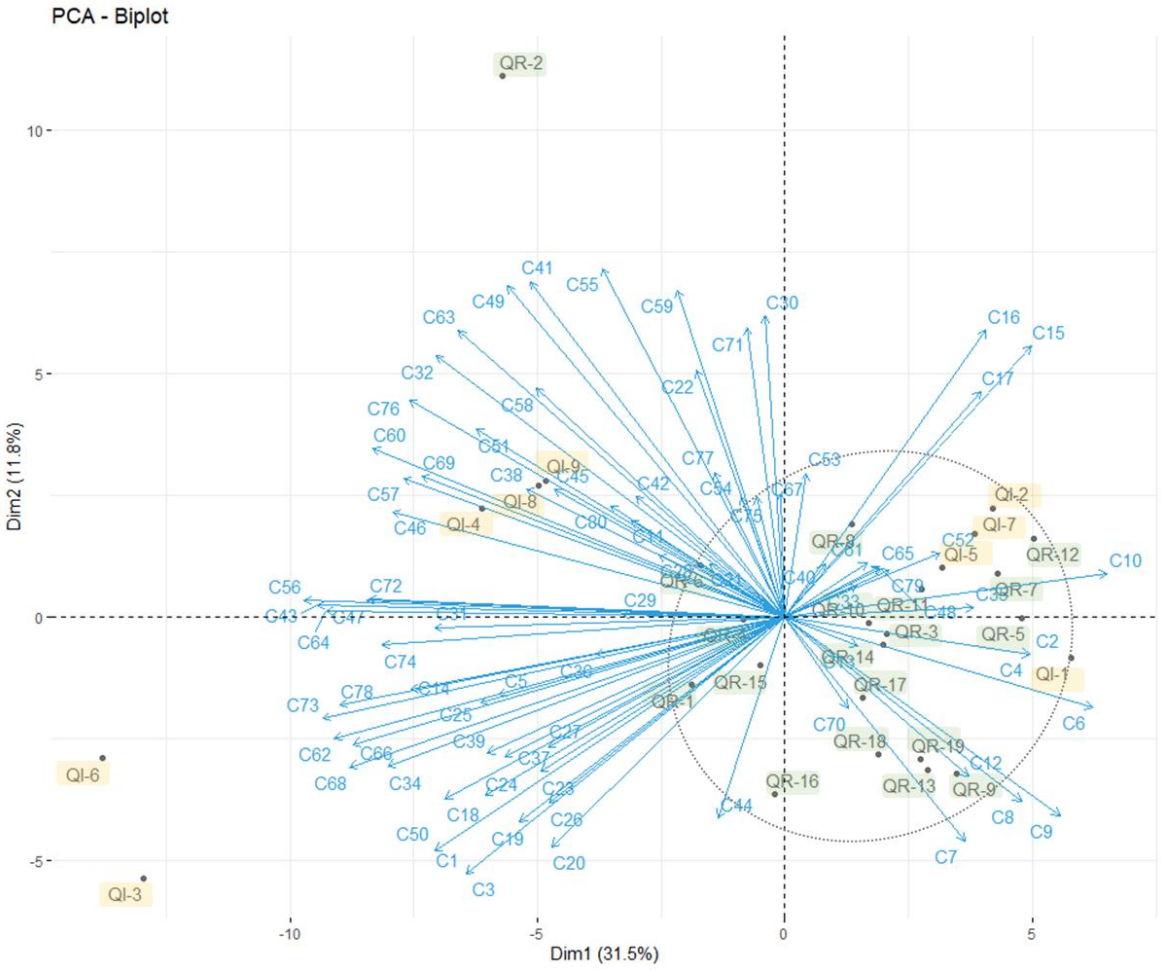
630 **Figure 2**



631

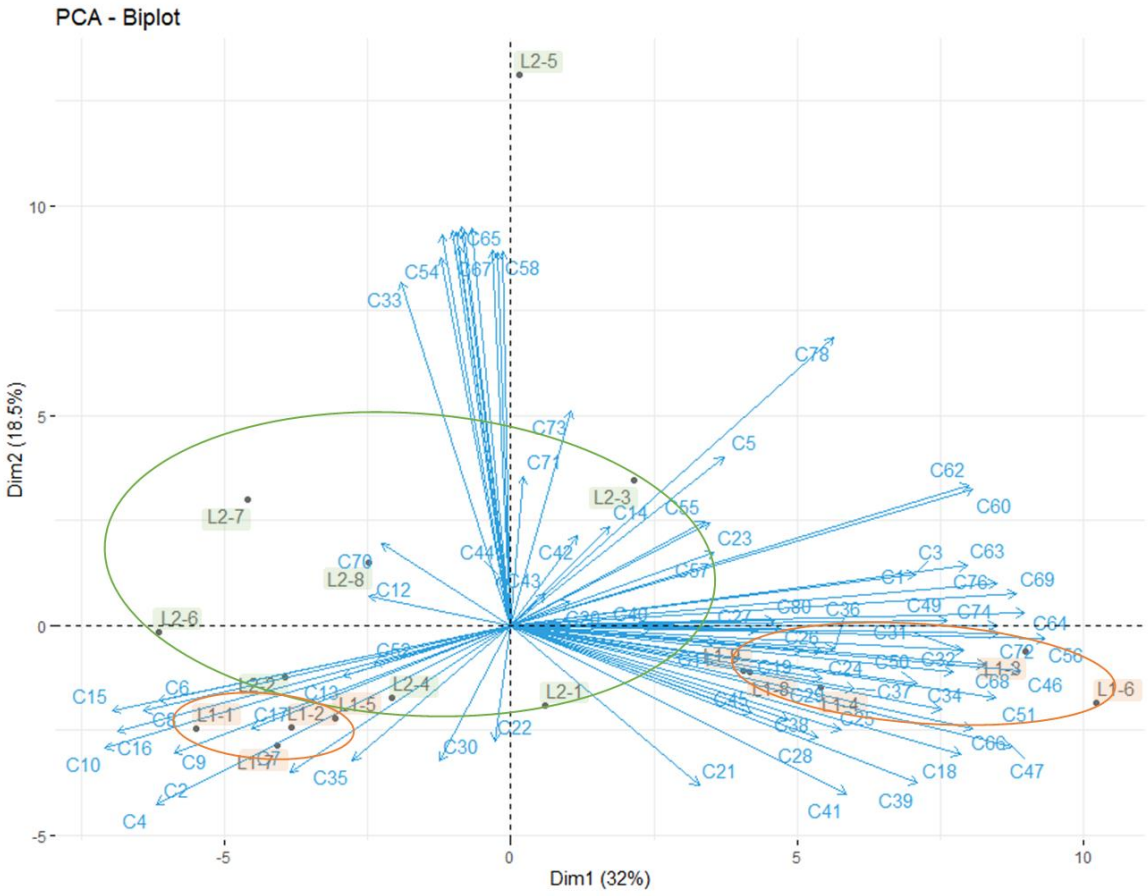
632 **Figure 3**

633 A)


PCA - Biplot
Dim2 (11.8%)
Dim1 (31.5%)
10
5
0
-5
-10
-5
0
5
QR-2
QI-6
QI-3
QI-4
QI-8
QI-9
QI-2
QI-7
QI-5
QR-12
QR-7
QR-11
QR-3
QR-5
QI-1
QR-14
QR-15
QR-17
QR-1
QR-18
QR-19
QR-16
QR-13
QR-9
C41
C55
C49
C59
C30
C63
C71
C16
C15
C17
C22
C32
C58
C76
C60
C51
C69
C38
C45
C42
C77
C54
C67
C53
C57
C46
C80
C52
C65
C10
C56
C72
C29
C43
C47
C64
C74
C5
C30
C73
C78
C14
C25
C62
C66
C39
C37
C68
C34
C24
C23
C18
C26
C50
C19
C1
C20
C3
C70
C12
C44
C8
C9
C7
C6
C4
C2
C48
C79
C33
C40

634

B)


PCA - Biplot
Dim2 (18.5%)
Dim1 (32%)
L2-5
L2-7
L2-6
L2-8
L2-3
L2-4
L2-1
L1-1
L1-2
L1-5
L1-7
L1-6
C65
C54
C67
C58
C33
C78
C73
C71
C5
C62
C60
C14
C55
C23
C3
C63
C1
C76
C69
C44
C42
C43
C67
C70
C12
C80
C36
C49
C74
C64
C40
C27
C31
C26
C72
C56
C50
C32
C24
C19
C68
C46
C15
C6
C13
C37
C34
C51
C16
C10
C9
C35
C30
C22
C21
C28
C18
C47
C66
C2
C4
C41
C39

Figure