THE ROLE OF PHENOLIC COMPOUNDS ON OLIVE OIL AROMA RELEASE

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24 **ABSTRACT**

25

26 In this study, atmospheric pressure chemical ionisation-mass spectrometry (APCI-MS) was
27 successfully applied to understand the effect of phenolic compounds on the release of olive oil
28 aroma compounds. Eight aroma compounds were monitored under *in-vivo* and *in-vitro* dynamic
29 conditions in olive oil with and without the addition of virgin olive oil (VOO) biophenols. Three
30 model olive oils (MOOs) were set up with identical volatile compounds concentrations using a
31 refined olive oil (ROO). Phenolics were extracted from VOOs and were added to two MOOs in
32 order to obtain two different concentrations of phenolic compounds (P+ = 354 mg kg^{-1}; P++ = 593
33 mg kg^{-1}). Another MOO was without VOO biophenols (P-). Phenolic compounds impacted both the
34 intensity and time of aroma release. In the *in-vivo* study, 1-penten-3-one, *trans*-2-hexenal and esters
35 had lower release in the presence of higher levels of biophenols after swallowing. In contrast,
36 linalool and 1-hexanol had a greater release. The more hydrophobic compounds had a longer
37 persistence in the breath than the hydrophilic compounds. VOO phenolics-proline-rich proteins
38 complexes could explain the binding of aroma compounds and consequently their decrease during
39 analysis and during organoleptic assessment of olive oil.

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46 **Keywords:** Virgin olive oil; APCI-MS; Phenolic compounds; saliva; Aroma release; *In-vivo*
47 study; volatile compounds.

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52 **1. Introduction**

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54 Virgin olive oil (VOO) is one of the most appreciated fat products of the Mediterranean diet, and
55 many positive nutritional properties have been associated with its consumption (Keys, 1995;
56 Psaltopoulou, Kosti, Haidopoulos, Dimopoulos, & Panagiotakos, 2011). The popularity of VOO is
57 linked both to its health properties and pleasant aroma. While its health properties are attributed to
58 phenolic compounds, which are also responsible for its bitterness and pungency, its pleasant aroma
59 is due to the presence of volatile aroma compounds (Aparicio, Morales, & Alonso, 1996; Servili *et*
60 *al*., 2009). The latter are composed large number (about 100) of different volatiles. These are
61 concentration dependent and vary with variety, growing conditions, and post-harvest processing
62 (Reiners & Grosch, 1998). The lipoxygenase pathway accounts for the main enzymatic reaction
63 producing the most of the aroma compounds of olive oil, which are mainly C_6 and C_5 aldehydes,
64 alcohols and esters (Sánchez-Ortiz, Pérez, & Sanz, 2013).

65 Olive oil phenolic compounds are mainly phenolic acids, simple phenols like tyrosol and
66 hydroxytyrosol, secoiridoid derivatives of the glycosides oleuropein and ligstrodide, lignans,
67 flavonoids, and hydroxyl-isochromans (Servili *et al*., 2009).

68 Olive oil bitterness can be classified by its biophenol content into four categories. A quantity of
69 phenolic compounds equal or lower than 220 mg kg^{-1} corresponds to non-bitter oils or almost
70 imperceptible bitterness. Slight bitterness of VOO corresponds to 220–340 mg kg^{-1} of phenolic
71 compounds. On the contrary, bitter oils have biophenols levels ranging from 340 to 410 mg kg^{-1},
72 while phenolics higher than 410 mg kg^{-1} correspond to quite bitter or very bitter oils (Beltran,
73 Ruano, Jimenez, Uceda, & Aguilera, 2007).

74 The flavour notes, derived from volatile and phenolic compounds, are the main features
75 evaluated in the organoleptic assessment of VOO. This assessment identifies mainly positive
76 attributes and defects in the oil, and it is critical for the oil's quality classification according to
77 European legislation (EEC Reg. 2568/91 and further amendments UE Reg. 1348/2013) and the

78 International Olive Council (IOC, 2015). Bitter and pungency notes of VOO are very desirable,
79 even if it is not considered important in commodity classification (De Santis & Frangipane, 2015).
80 Furthermore, the aroma release from VOO could be also affected by saliva during an
81 organoleptic assessment, the ability of salivary constituents to interact with aroma compounds has
82 recently been reviewed (Ployon, Morzel, & Canon, 2017). Mucin and α-amylase are the most
83 important proteins of saliva. It has been reported that such proteins affect the volatility of some
84 aroma compounds by their capacity to trap volatiles through with hydrophobic interactions (Friel &
85 Taylor, 2001; Pagès-Hélary, Andriot, Guichard, & Canon, 2014). In the case of mucin, covalent
86 interactions with aldehydes and ketones have also been shown (Friel & Taylor, 2001). Moreover, it
87 has been also reported that human saliva has a stronger effect compared to artificial saliva probably
88 due to the presence of different proteins and the activity of certain enzymes (Buettner 2002; Pagès-
89 Hélary *et al.*, 2014).
90 Phenolic compounds are known to interact reversibly with proteins and aroma compounds. For
91 example, for wine the interactions between phenolics and salivary proteins (Baxter, Lilley, Haslam, &
92 Williamson, 1997) or phenolics and volatile compounds (Pozo-Bayon & Reineccius, 2009) are
93 reported to affect the wine aroma release (Munoz-Gonzalez et al., 2014; Esteban-Fernández, Muñoz-
94 González, Jiménez-Girón, Pérez-Jiménez, & Pozo-Bayón, 2018). So far, only a few studies have reported
95 on VOO aroma-phenolic-salivary protein interactions. A study on the interaction effect between
96 VOO biophenols and salivary mucin showed that VOO phenolic extracts had a greater interaction
97 with mucin than individual phenolic compounds, even at low concentration (about 300 mg kg^{-1})
98 (Quintero-Flórez, Sánchez-Ortiz, Gaforio Martínez, Jiménez Márquez, & Beltrán Maza, 2015). In
99 an another study by SPME, a low-medium level of VOO phenolic compounds (about 300 mg kg^{-1})
100 was shown to affect the release of olive oil aroma compounds in the presence of human saliva. The
101 results have also shown the lowest headspace release of volatile compounds belonging to the
102 chemical class of ethyl esters, acetates, alcohols and ketones (Genovese, Caporaso, Villani,
103 Paduano, & Sacchi, 2015).

104 Therefore, the presence of biophenols may play a significant role during organoleptic
105 assessment.
106 During the sensory evaluation of VOO (considered a dynamic oral process) the polyphenol-
107 aroma and polyphenol-salivary protein interactions may alter the VOO-air partitioning (volatility)
108 of the aroma compounds thereby affecting aroma release. This has been proved for other types of
109 food and drink such as wine (Villamor & Ross, 2013).
110 Breath-by-breath by atmospheric pressure chemical ionisation-mass spectrometry (APCI-MS) is
111 a very useful tool designed primarily to monitor real-time changes in the concentration of known
112 volatiles while eating (Taylor, Linforth, Harvey, & Blake, 2000) and the impact of conscious and
113 subconscious control of muscles while swallowing and subsequent breathing (Rabe, Linforth,
114 Krings, Taylor, & Berger, 2004; Gierczynski, Labouré, Sémon, & Guichard, 2007).
115 The aim of this work was therefore to study key aroma compounds from VOO and to investigate
116 how VOO phenolic compounds influence aroma release. For this purpose, three model olive oils
117 (MOOs) with identical concentrations of volatile compounds, differing only for biophenols, were
118 used. This allowed us to study aroma release from products with differences in phenolic compounds
119 content but without major differences in the VOO composition. Aroma release was first determined
120 by APCI-MS under *in-vivo*. Then, *in-vitro* dynamic measurements by APCI-MS were performed
121 without and with the addition of artificial saliva to understand, the mechanism involved in the
122 release of aroma compounds during consumption.
123
124 **2. Material and Methods**
125
126 2.1. *Samples, standards and reagents*
127

128 The refined olive oil (ROO) and virgin olive oil (VOO) from *Rotondella*, *Carpellese* and
129 *Nostrale di Felitto* cultivars were supplied, respectively, by Dorella Oleificio Candela srl
130 (Castellamare di Stabia, Napoli, Italy) and Azienda Agricola Marco Rizzo (Felitto, Salerno, Italy).
131 Ethyl butyrate (98%), *cis*-3-hexenylacetate (98%), ethyl acetate (99%), hexanal (97%), *trans*-2-
132 hexenal (95%), 1-hexanol (98%), linalool (97%), and 1-penten-3-one (95%) were food grade and
133 were supplied by Sigma–Aldrich (St.Louis, USA). The following reagents were used for the
134 analysis: hexane (99%), distilled water, supplied by VWR International (Milan, Italy). HPLC grade
135 methanol (>99.9% purity), hexane (>95%), Folin–Ciocalteu reagent, sodium carbonate anhydrous
136 (>99.5%), caffeic acid (97%) were bought from Sigma–Aldrich (St.Louis, USA). Food grade ethyl
137 alcohol (96%) was bought from Selex S.p.A. (Trezzano sul Naviglio, Milano, Italy).
138
139 2.2. *Samples preparation*
140
141 To study the effect of phenolic compounds on the release of olive oil aroma compounds, three
142 MOOs were set up with identical volatile compounds concentrations using a refined olive oil
143 (ROO). Phenolics were extracted from VOOs and were added to ROO in order to obtain MOOs
144 with two different concentrations of phenolic compounds (P++ and P+). Another MOO was built
145 with volatile compounds but without VOO biophenols (P-). The MOOs were stored at ambient
146 conditions (19 °C) avoiding light exposure and high temperatures in order to prevent oxidation and
147 were used within three months from their preparation. For each system, blank solutions without
148 volatile compounds were also tested.
149
150 2.2.1. *Preparation of the refined olive oil sample with added virgin olive oil phenolic compounds*
151
152 The phenolic extract was obtained from a blend of three VOOs obtained from *Rotondella*,
153 *Carpellese* and *Nostrale di Felitto* olive cultivars, respectively. An aliquot of the oil sample (50 g)

154 was dissolved in hexane (100 mL). A subsequent extraction was carried out using a water/methanol
155 mixture (40/60 v/v) in a separating funnel (500 mL) after having shaken it vigorously for 15 min in
156 a 500 mL bottle. This step was repeated twice using a total of 140 mL solvent. Subsequently, the
157 obtained hydro-alcoholic extract was washed with hexane to remove any oil contamination and was
158 centrifuged for 5 min at 3500 rpm (ALC International srl, PK-120, Milan, Italy). The organic phase
159 was removed from the sample, and the hydro-alcoholic phase was collected in the flask and
160 evaporated under vacuum in a rotary evaporator at 35 °C (Heidolph, VV 2000). The phenolic
161 compounds were suspended using 10 mL ethyl alcohol (food grade). A total of 1.750 kg of VOO
162 was used to extract phenolics. A total of 350 mL of biophenols extract in ethyl alcohol was obtained
163 and subsequently concentrated up to a final volume of 100 mL using a rotary evaporator at 35 °C
164 (Heidolph, VV 2000). 100 mL phenolic extract was added in a flask with 1500 g of refined olive
165 oil. The oil mixture was stirred and treated in an ultrasonic bath for 5 min. Then, ethanol was
166 evaporated in a vacuum evaporator (Heidolph VV 200) at 35 °C (Genovese *et al*., 2015).

167 The amount of total phenolic compounds added to the ROO (593±33 mg kg^{-1}) was chosen in
168 order to reproduce very bitter oil (P++). An aliquot of this sample was diluted with ROO (50:50) in
169 order to obtain MOO with a level of total phenolic compounds of 354±14 mg kg^{-1} in order to
170 reproduce a bitter oil (P+) as indicated by Beltrán *et al*. (2007).

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172 2.2.2. *Preparation of the refined olive oil sample*

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174 In the control sample (P-) phenolic extract was not added, 66 mL ethanol food grade was added
175 in a flask with 1000 g of refined olive oil. Then, the oil mixture was subjected to the same protocol,
176 previously described, for the addition of the phenolic compounds.

177

178 2.2.3. *Preparation of aroma solutions*

179

180 Only well known significant volatile key aroma compounds of virgin olive oils were considered
181 in our study when preparing the solutions of aroma compounds (Aparicio *et al.*, 1996). They
182 included 2 aldehydes, 2 acetates, 1 ester, 1 alcohol, 1 ketone and 1 terpene (Table 1). Volatile
183 compounds were dissolved in the target olive oil and homogeneously mixed by magnetic stirring.
184 Two aroma solutions were prepared and analysed separately by APCI-MS. The aroma solution was
185 added to oil sample 1 day before the analysis in order to avoid its oxidation. The final concentration
186 for each volatile compound in oil sample is reported in Table 1.
187
188 2.2.4. *Preparation of artificial saliva*
189
190 Artificial saliva was composed of recommended ingredients (Genovese, Piombino, Gambuti, &
191 Moio, 2009): 5.2 g $NaHCO_3$, 1.37 g $K_2HPO_4 \cdot 3H_2O$, 0.90 g NaCl, 0.5 g KCl, 0.44 g $CaCl_2 \cdot 2H_2O$,
192 0.5 g NaN_3, 2.2 g mucin (type 1-S from bovine submaxillary glands; Sigma, Milan, Italy) and
193 200,000 units α-amylase (DFP-treated, Type I-A from porcine pancreas; Sigma, Milan, Italy) in 1 L
194 of distilled water (adjusted to pH 7). The saliva was freshly prepared and heated gently to 37 °C
195 prior to experimentation.
196
197 2.3. *Extraction and analysis of phenolic compounds*
198
199 The extraction and quantification of total phenolic compounds was carried out by using the
200 Folin–Ciocalteau colorimetric essay according to Sacchi, Caporaso, Paduano & Genovese (2015). It
201 was performed to confirm the quantity of added phenolics in the MOOs. The concentration of
202 phenolic compounds in the three MOO was statistically different ($p < 0.05$).
203
204 2.4. *APCI-MS analysis*
205

206 Headspace or breath was sampled into a MS Nose interface (Micromass, Manchester, UK) fitted
207 to a Quattro Ultima mass spectrometer (Milford, Waters) at flow rates of 5 and 40 mL/min
208 respectively (transfer line temperature 100 °C). The analytes present in the gas phase were ionized
209 by a 4kV corona discharge (sample cone voltage 15V) in the source (75 °C) before passing them
210 into the analyser region of the mass spectrometer. The compounds were monitored in selected ion
211 mode using dwell time 0.2 s for headspace analysis and 0.01 s for breath-by-breath analysis. The
212 ions monitored were the protonated molecular ion (MH+) with the exception of linalool and 1-
213 hexanol, which dehydrated to form the (MH+) - H2O ion (Table 1). Two aroma solutions were
214 prepared and separately analysed (Table 1). A signal was observed when only a solution containing
215 the test compounds were present; while neither signals were observed for control samples (olive oil
216 with and without biophenols with no added volatiles), nor interference was found in naturally
217 compounds present in the breath.

218

219 2.4.1. *Breath-by-breath measurements*

220

221 Panellists were instructed to consume a 3.5 mL aliquot of oil solution from a small plastic cup,
222 and exhale (via the nose) into a "T" piece mounted onto the end of the MS Nose transfer line.
223 The third port of the T piece served as an outlet for excess breath. Thirteen exhalations were
224 studied for the olive oil sample, so that the changes in breath volatile concentration (nosespace)
225 could be followed over time. The first three exhalations were made with the sample retained in the
226 mouth (named stripping) while the other breaths (named breath) occurred after the swallowing of
227 the sample.
228 The panellist was asked to regulate his breathing and strip before each analysis. Accordingly, the
229 panellist started with regular breathing. At a certain time, while breathing in, the panellist brought
230 the sample his mouth cavity using a small plastic cup (20 mL), mixed the oil sample and saliva in
231 mouth with tongue moving for 10 s without swallowing and without breathing, the panellist then

232 inhaled air through the mouth (semi-closed) 3 times in a rapid succession (stripping) and pushed air
233 in through his nose, simulating a VOO sensory assessment. Then the panellist swallowed the entire
234 sample at once and paused for 2 s, and subsequently, exhaled 10 times but every 3 breaths
235 swallowed his saliva. The swallowing of saliva during MOO assay was necessary as olive oil
236 stimulates saliva production.-Such exhalations represent the aroma persistence of olive oil sample.
237 The panellist washed the mouth with water and ate bread to clean the mouth from the bitter
238 biophenols. The analysis of each sample lasted about 1.8 min. All analyses were performed in
239 triplicate and were repeated on three different days (n=9).
240 In Figure 1A an example of a breath-by-breath release profile collected from the panellist after
241 VOO consumption is shown. Acetone (m/z 59) is generated in the liver, and considerable amounts
242 of it are transferred in exhaled breath. Therefore, it is useful as a marker for exhalation events
243 (Linforth, Martin, Carey, Davidson, & Taylor, 2002). Other ions are protonated molecular ions
244 (MH+) of 1-penten-3-one (m/z 85), *trans*-2-hexenal (m/z 99), ethyl butyrate (m/z 117) and *cis*-3-
245 hexenyl acetate (m/z 143). The length of exhalation and the intensity of released flavour during
246 exhalation correspond to quantity of volatiles reaching the olfactory region. The intensity of each
247 exhalation was reported as a peak area (in Figure 1A each peak has a different colour). After the
248 initial stripping period, a MOO sample previously brought into the mouth was swallowed at
249 approximately 0.3 min, afterwards volatile delivery was measured for other 1 min with ten breaths.
250 An example of normalized time-release curve for ion 143 (*cis*-3-hexyl acetate) obtained from two
251 MOOs, with and without phenolics addition, is shown in Figures 1B.
252 The concentration used for each volatile compound for breath-by-breath analysis is reported in
253 Table 1. The chromatograms generated in the MassLynx software (v4.1) (Micromass, Manchester,
254 U.K.) were integrated so that peak areas and corresponding times could be extracted. Then the
255 extracted data was processed using CDC-2000 (Cut, Delete and Calibration for APCI-MS analysis)
256 and Microsoft Excel (Microsoft Corporation, USA) software without the application of smoothing

257 algorithm. Changes in headspace concentrations in the *in-vivo* experimental system were expressed
258 as changes on a relative percentage scale where the first breath was considered as 100 %.

259

260 2.4.2. *Dynamic headspace measurements*

261

262 Aliquots of oil (25 g) were placed in 100-mL flasks (Schott bottle; Fischer Scientific,
263 Loughborough, UK), each fitted with a lid with three ports. Headspace was sampled via the central
264 port into the MS Nose at a flow rate of 5 mL/min while nitrogen was bubbled through the sample
265 (65 mL/min) via a tube into another inlet port of the lid. Measurements were made for a total of 13
266 min on model solutions containing aroma compounds at different concentrations (Table 1). Samples
267 were allowed to equilibrate at room temperature (21 °C) for 3 h before measurement. To simulate
268 the oral process, 5 mL of artificial saliva was added to each oil sample. The ratio of saliva to olive
269 oil was 1/5, as previously defined (Genovese *et al.*, 2015) and was chosen in order to approximate,
270 as much as possible, the real oral conditions according to literature (Roberts & Acree, 1995). For
271 the first two min of the sampled headspace, oil and saliva were stirred at 300 rpm. Data was
272 processed using MassLynx (v4.1) (Micromass Ltd., Manchester, U.K.), CDC-2000 (Cut, Delete and
273 Calibration 2000 for APCI-MS analysis), and Microsoft Excel (Microsoft Corporation, USA). The
274 ion current intensity was not subjected to smoothing algorithm. Changes in headspace
275 concentrations in the dynamic experimental system were expressed as changes on a relative
276 percentage scale, where the highest height was considered as 100 %. All the analyses were
277 performed in quadruplicate.

278

279 2.5. *Statistical analysis of data*

280

281 Significant biophenols differences among the different MOOs were determined by one-way
282 ANOVA statistical analysis. Fisher's test was used to discriminate among the means of the

283 variables. Data elaboration was carried out using Minitab statistical software (version 17.2.1,
284 Minitab Inc).
285
286 **3. Results and discussion**
287
288 Breath-by-breath APCI-MS measurements for eight volatile compounds from three MOOs with
289 different phenolic compounds concentrations are reported in Figure 2. The time-release curves were
290 expressed as polynomial functions to better show the trend. They were extracted from the
291 normalized breath-by-breath curves (Supplementary material).
292 The highest release of aroma compounds in exhaled air occurred directly after swallowing the
293 olive oil sample (0.3 min), followed by a decrease over subsequent exhalations. Swallowing induces
294 the opening of the velum (Buettner, Beer, Hannig, & Settles, 2001) and thus, it is at this time that
295 the major part of the aroma is released from the oral cavity to the pharynx (Buettner, Otto, Beer,
296 Mestres, Schieberle, & Hummel, 2008).
297 The highest level of olive oil biophenols (P++) showed a lower initial release rate of 1-penten-3-
298 one and *trans*-2-hexenal compared to the other two samples (P+ and P-). After 0.8 min the release
299 was similar for all the samples (Figure 2).
300 Concerning esters (ethyl butyrate and *cis*-hexenyl acetate), the P++ sample exhibited a lower
301 release than the P- and P+. On the contrary, to the previous compounds, the persistence of these
302 aroma compounds in the breath at 0.8 min showed an increase in the presence of biophenols (Figure
303 2). Similar behaviour is also shown by ethyl acetate, although the differences are less evident.
304 Linalool and 1-hexanol showed a salting out effect in the P++ sample at 0.3 min. While for 1-
305 hexanol at 0.8 min the release decreased until becoming similar to the other samples (P+ and P-),
306 linalool (P++) had enhanced persistence in the breath for the whole duration of the analysis (Figure
307 2). Finally, for hexanal no important differences were reported.

308 These results are in agreement with our previous work on olive oil aroma except for 1-hexanol
309 and linalool (Genovese *et al.*, 2015). The authors reported that the VOO phenolic compounds
310 reduced the headspace concentration of different volatile compounds, among them there were ethyl
311 butyrate and *cis*-hexenyl acetate, 1-penten-3-one and *trans*-2-hexenal. However, the above
312 mentioned work was not an *in-vivo* real-time study but it simulated the retronasal conditions using
313 SPME technique with the addition of human saliva and a time sampling of aroma release of 4 min.
314 No other data has been published so far about the interaction between olive oil phenolics and
315 volatile compounds. On the contrary, in other studies on wine, sensory approaches were employed
316 to explore the changes in wine aroma perception due to the action of polyphenols. In general, it has
317 been stated that the intensities of fruity and floral aromas seem to decrease when the level of
318 polyphenols increases (Goldner, Lira, van Baren, & Bandoni, 2011). Moreover, the addition of
319 grape seed extracts (about 80% consists of proanthocyanidins and polymers of catechin) to wine not
320 only changes astringency, but also enhances the woody/earthy aroma and reduces the fruity aroma
321 (Cliff, Stanich, Edwards, & Saucier, 2012). Aronson & Ebeler (2004) found that the presence of
322 both gallic acid and naringin decreased the perceived aroma intensity of 2-methylpyrazine, and
323 naringin had a greater negative effect on ethyl benzoate. Lorrain, Tempere, Iturmendi, Moine, de
324 Revel, & Teissedre (2013) found that catechin significantly altered the sensory perception of most
325 esters (ethyl isobutyrate, ethyl butyrate and ethyl octanoate). Jung & Ebeler (2003) showed a
326 significant reduction in the headspace of hexanal and ethyl hexanoate by catechin. Esteban-
327 Fernández et al. (2018) found that wine phenolic acids favoured the intra-oral release of β-
328 phenylethanol, linalool and β-ionone, while wine flavonoids induced lower intra-oral release of
329 certain esters. Wine polyphenols have been reported to interact with aroma compounds in solution,
330 mainly by non-covalent binding, and to directly affect wine aroma release. This interaction can also
331 occur through hydrogen binding, hydrophobic or hydrophilic interactions, as recently reviewed by
332 Villamor & Ross (2013).

333 In order to verify if there is a binding effect between phenolic compounds and aroma
334 compounds, which could explain the reduction of the VOO compounds, the three MOOs were also
335 analysed *in-vitro* dynamic conditions without and with the addition of artificial saliva.
336 Figure 3 shows the dynamic headspace curves of volatile compounds in three MOOs without the
337 addition of artificial saliva. Among them linalool, 1-hexanol and hexanal showed a difference in the
338 headspace release demonstrating a binding effect due to the presence and the level of phenolics in
339 olive oil. *cis*-3-Hexenyl acetate showed a very low binding effect but only at the highest
340 concentration of biophenols. Figure 4 shows the dynamic headspace release curves of volatile
341 compounds from MOOs at different concentration of phenolic compounds with the addition of
342 artificial saliva. In this emulsion system, VOO phenolic compounds in the presence of α-amylase
343 and mucin caused modifications in the dynamic headspace release only for linalool, 1-hexanol and
344 hexanal similar to our previous *in-vitro* test (Figure 3). These *in-vitro* tests could indicate that VOO
345 phenolic compounds could interact with some volatile compounds through non-covalent bonds and
346 that could be released slowly over time. Moreover, the possible interaction between VOO phenolics
347 and mucin (Quintero *et al.*, 2015) did not involve any significant change in aroma release, probably
348 it is a very weak interaction.
349 For wine polyphenols the reactivity is due its numerous hydroxyl functional groups and its
350 aromatic rings (Jung, de Ropp, & Ebeler, 2000) but it is also suggested that steric hindrance may
351 reduce the magnitude of the interaction (Lorrain *et al.*, 2013). The latter might explain the minor
352 interaction which occurs between the *trans*-2-hexenal and polyphenols if compared to hexanal,
353 which is explained by its steric hindrance due to the double bond.
354 However, these *in-vitro* tests did not explain the results obtained from the *in-vivo* test.
355 Particularly, the volatile compounds, which demonstrated in the *in-vivo* test a lower release in the
356 presence of VOO biphenols, but did not interact with the polyphenols *in-vitro*. In contrast, volatile
357 compounds that demonstrated a higher release interacted with VOO phenolic compounds.

358 This inconsistency could be explained by specific interactions of VOO phenolic with proline-rich
359 proteins (PRPs) that were not included in the artificial saliva in our *in-vitro* study. The PRPs
360 comprise up to 70% of the proteins in human parotid saliva (Baxter *et al.*, 1997). For instance, PRPs
361 have demonstrated a high affinity for tannins, resulted by their extended conformation (Canon *et*
362 *al.*, 2013). Smaller polyphenols (propyl gallate and epicatechin) can bind with one phenolic ring
363 stacked against each proline residue, whereas larger polyphenols occupy two or three consecutive
364 prolines (Baxter *et al.*, 1997). As supposed for red wines (Munoz-Gonzalez *et al.*, 2014; Esteban-
365 Fernández et al., 2018), the formation of VOO phenolic compounds-PRPs complexes could retain
366 volatile compounds in the hydrophobic cavities and therefore, decrease aroma release into the
367 headspace. The influence of VOO phenolics-PRPs complexes on aroma release also depends on the
368 physiochemical properties of the aroma compounds. On the contrary, the aroma compounds,
369 hydrophobically retained by VOO phenolic compounds, could be released when phenolics interact
370 with the PRPs during the olive oil assessment generating a salting out effect, *i.e.* linalool and 1-
371 hexanol. Linalool also had the greatest persistence in the breath for the whole duration of the
372 analysis while esters showed a persistence but lower than the highest release obtained at 0.3 min
373 (Figure 2). Generally, the more hydrophobic and less volatile compounds have been reported to
374 persist longer in the breath than hydrophilic (Repoux *et al.*, 2012).

375 Another important aspect to consider in an *in-vivo* test, which could affect the aroma release, is
376 the different secretion of saliva in the presence of biophenols. In fact, changes in the saliva flow and
377 composition could affect the partitioning of all aroma compounds. It is currently unknown if VOO
378 phenolic compounds are able to change the secretion of saliva both in term of composition and
379 flow. However, this type of taste stimuli is known to strongly affect salivary gland functionality and
380 therefore, could induce modifications of saliva composition (Dawes 1984). For wine, the perceived
381 intensity and duration of bitterness and astringency were affected by saliva flow rate, salivary
382 volume, salivary pH and protein composition (Fischer, Boulton, & Noble, 1994).

383

384 **4. Conclusion**

385

386 Phenolic compounds were proved to play an important role in the intensity and timing of the
387 release of certain aroma compounds during the consumption of virgin olive oil. High levels of VOO
388 phenolic compounds resulted in a smaller total release of 1-penten-3-one, *trans*-2-hexenal and
389 esters at the swallowing of olive oil sample. Probably, the complex formed between phenolics and
390 PRPs entrap aroma compounds and consequently reduce their volatility during the organoleptic
391 assessment of olive oil. Phenolic compounds were shown to interact with certain volatile
392 compounds (mainly linalool, 1-hexanol and hexanal) through proposed to be due to reversible non-
393 covalent bonds. VOO phenolic compounds could release these aroma compounds when interact
394 with PRPs generating a salting out effect and a longer persistence.

395 Although the effect of biophenols on VOO aroma release has not received as much scientific
396 attention as other foods and drinks, it is clear that it is necessary to consider it since phenolic
397 compounds may influence the release of VOO aroma compounds during its consumption, thereby
398 influencing the flavour perception and consumer acceptance. However, further sensorial studies are
399 needed to confirm our findings and better understand whether and to what extent VOO biophenols
400 affect sensory perception and consumer acceptance.

401

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404 or not-for-profit sectors.

405

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517 Figure captions:

518

519 **Figure 1**. Example of a breath-by-breath APCI-MS release profile collected from a panellist after
520 sample consumption following the instructions given in material section. m/z 59, 85, 99, 117 and
521 143 represent molecular ions of acetone, 1-penten-3-one, *trans*-2-hexenal, ethyl butyrate and *cis*-3-
522 hexenyl acetate, respectively (A). Example of normalized time-release curve for ion 143 (*cis*-3-
523 hexyl acetate) obtained from two model olive oil with and without phenolics addition (B).

524

525 **Figure 2**. Time-release curves of volatile compounds from model olive oil without phenolics (P-
526 ▬▬), with low (P+ ▪▪▪▪▪) and high concentration of phenolics (P++ ▬ ▬), expressed as
527 polynomial function. Dashed lines at 0.3 and 0.8 min indicate the swallowing of model olive oil and
528 saliva, respectively. The volatile compounds were ordered by their octanol–water partition
529 coefficients.

530

531 **Figure 3**. Dynamic headspace release of volatile compounds in P++ (♦), P+ (■) and P- (▲) model
532 olive oils. The volatile compounds were ordered by their octanol–air partition coefficients.

533

534 **Figure 4**. Dynamic headspace release of volatile compounds in P++ (♦), P+ (■) and P- (▲) model
535 olive oils with artificial saliva addition. The volatile compounds were ordered by their octanol–
536 water partition coefficients.

537

538 *Supplementary material*

539

540 Time-release curves of volatile compounds from model olive oil without phenolics (P- ▬▬▬),

541 with low (P+ ▪▪▪▪▪) and high concentration of phenolics (P++ ▬). Dashed lines at 0.3 and 0.8 min

542 indicate the swallowing of model olive oil and saliva, respectively.

543

544

545 Chemical compounds studied in this article:

546 Hexanal (PubChem CID: 6184); *trans*-2-hexenal (PubChem CID: 5281168); 1-hexanol
547 (PubChem CID: 8103); linalool (PubChem CID: 6549); ethyl butyrate (PubChem CID: 7762); ethyl
548 acetate (PubChem CID: 8857); *cis*-3-hexenyl acetate (PubChem CID: 5363388); 1-penten-3-one
549 (PubChem CID: 15394).

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