

Assessment of Volatile Compound Profiles and the Deduced Sensory Significance of Virgin Olive Oils from the Progeny of Picual x Arbequina Cultivars

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1 **Abstract**

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Volatile compounds are responsible for most of the sensory qualities of virgin olive oil and they are synthesized when enzymes and substrates come together as olive fruit is crushed during the industrial process to obtain the oil. Here we have studied the variability among the major volatile compounds in virgin olive oil prepared from the progeny of a cross of Picual and Arbequina olive cultivars (*Olea europaea* L.). The volatile compounds were isolated by SPME, and analyzed by HRGC-MS and HRGC-FID. Most of the volatile compounds found in the progeny's oil are produced by the enzymes in the so-called lipoxygenase pathway, and they may be clustered into different groups according to their chain length and polyunsaturated fatty acid origin (linoleic and linolenic acids). In addition, a group of compounds derived from amino acid metabolism and two terpenes also contributed significantly to the volatile fraction, some of which had significant odor values in most of the genotypes evaluated. The volatile compound content of the progeny was very varied, widely transgressing the progenitor levels, suggesting that in breeding programs it might be more effective to consider a larger number of individuals within the same cross than using different crosses with fewer individuals. Multivariate analysis allowed genotypes with particularly interesting volatile compositions to be identified and their flavor quality deduced.

Keywords: *Olea europaea* L., virgin olive oil, volatile compounds, variability, segregation, quality

36 **1. Introduction**

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38 Olive oil is one of the oldest known plant oils and it is unique as it can be consumed as a fruit
39 juice called virgin olive oil (VOO). This product represents the primary source of lipids in the
40 Mediterranean diet, which has been linked to positive health benefits. Indeed, this diet reduces
41 the risk from a number of diseases, mainly those containing an inflammatory component such
42 as cardiovascular diseases, certain types of cancer, diabetes, metabolic syndrome, arthritis and
43 Alzheimer's disease [1-6]. Thus, increased VOO consumption, one of the main distinguishing
44 features of the Mediterranean diet, is likely to have a positive impact on the general
45 population's health and consequently, on the budgets allocated to healthcare systems.

46 However, the increase in the demand for high-quality VOO may not only be attributed to its
47 potential health benefits but also, to its excellent organoleptic properties [7]. In this sense,
48 volatile compounds are responsible for the aroma of VOO, which is characterized by a unique
49 balance of green and fruity attributes spiced with some other positive aromas that make it a
50 distinctive edible oil. The size, shape, conformation, type and position of the functional
51 groups in volatile compounds are features that strongly influence odor perception and
52 pleasantness [8, 9]. On the other hand, concentration and odor threshold of each volatile
53 compound define its sensory attributes. These parameters determine the odor activity value
54 (OAV), the ratio between the concentration of the volatile compound and its odor threshold,
55 whereby volatile compounds with an OAV below one do not contribute to VOO aroma. An
56 interesting approach to understand the relationship between volatile compounds and odor
57 attributes is the statistical sensory wheel (SSW) developed by Aparicio and Morales [10],
58 which compiles the sensory attributes evaluated by trained VOO sensory panels across
59 Europe. The resulting information allows sensory notes with a similar semantic description to
60 be clustered into a number of sectors that contain the volatile compounds generally identified
61 by a given sensory perception, and that among the most relevant to VOO aroma may include
62 green or ripe fruit odor notes.

63 Most of the volatile compounds present in VOO are synthesized when the enzymes and
64 substrates come together when the olive fruit is crushed during the industrial process to obtain
65 this product. The lipoxygenase (LOX) pathway participates in the biosynthesis of six straight-
66 chain carbons (C6) compounds in the volatile fraction of VOO [11] and from a quantitative or
67 qualitative point of view, C6 aldehydes and alcohols, as well as their corresponding esters, are
68 the most important compounds in VOO aroma [12, 13]. These compounds are synthesized

69 from polyunsaturated fatty acids containing a (Z,Z)-1,4-pentadiene structure, such as linoleic
70 (LA) and linolenic (LnA) acids. In the first step of this pathway, LOX produces the
71 corresponding 13-hydroperoxide derivatives that are subsequently cleaved heterolytically by
72 hydroperoxide lyase (HPL) to C6 aldehydes [11, 14, 15]. C6 aldehydes can then be reduced
73 by alcohol dehydrogenases (ADHs) to C6 alcohols [11, 16] and finally, they can be
74 transformed into the corresponding esters by alcohol acyltransferases (AATs) [11, 17].
75 Compounds with five straight-chain carbons (C5 compounds) are also relevant to the aroma
76 of olive oil [13] and they are generated through an additional branch of the LOX pathway that
77 involves the production of a 13-alkoxyl radical by LOX, as demonstrated in soybean seeds
78 [18]. This radical can undergo subsequent homolytic non-enzymatic β -scission to form a 1,3-
79 pentene allylic radical that can be chemically dimerized to generate pentene dimers (PD), or
80 that reacts with a hydroxyl radical to form C5 alcohols. The latter represents the origin of the
81 C5 carbonyl compounds present in the volatile fraction of olive oil through enzymatic
82 oxidation by ADH, as believed to occur in soybean leaves [19].
83 New cultivars with improved sensory quality might further stimulate VOO consumption and
84 although olive breeding programs have traditionally focused mainly on the improvement of
85 agronomic traits [20], more recent breeding studies have addressed selection for the sensory
86 and nutritional qualities of VOO [21, 22]. Considering the significance of the aroma to VOO
87 quality, the aim of the present work was to assess the variation in the volatile fraction of VOO
88 and to deduce the aroma properties in a segregating population of a cross of Picual x
89 Arbequina olive cultivars. This study was carried out in the framework of an olive breeding
90 program that aimed to identify new olive genotypes that give rise to oils with improved
91 sensory and nutritional attributes.

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94 **2. Materials and Methods**

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96 *2.1. Plant material*

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98 A total of 136 olive genotypes (*Olea europaea* L.) from a Picual x Arbequina cross were
99 considered in the present study. The two parental cultivars, Picual and Arbequina, were grown
100 in the same orchard as the seedling progeny. The cross was made in spring 2001 and the
101 seedlings obtained were submitted to the habitual protocol followed on the breeding program
102 [23]. Initial seedling growth was forced in a greenhouse by means of drip fertirrigation,

103 temperature control and continuous light. The plants were then established in open field in
104 September 2003 with a spacing of 1.5 x 4 m, trained to form a canopy at 160 cm height and
105 then allowed to develop freely. Drip irrigation and standard culture practices were followed to
106 ensure tree growth without limitations. All trees were grown in the same edaphoclimatic
107 conditions at the experimental orchards of IFAPA (Alameda del Obispo, Córdoba, Spain).
108 Fruit was picked by hand on three consecutive years (2008-2010) when it reached an average
109 ripening index of 2.5 (turning stage) to better compare the genotypes (according to El Riachy
110 *et al.* [24]).

111 112 2.2. Olive oil extraction

113
114 Olive oil was extracted using an Abencor analyzer (Comercial Abengoa, S.A., Seville, Spain)
115 that simulates the industrial process of VOO production on a laboratory scale [25]. Milling of
116 the olive fruit was performed using a stainless steel hammer mill operating at 3000 rpm and
117 with a 5 mm sieve. Malaxation was carried out for 30 min with the Abencor thermobeater
118 operated at 30 °C according to industrial recommendations. Centrifugation of the kneaded
119 paste was performed in a basket centrifuge at 3500 rpm for 1 min and after centrifugation, the
120 oils were decanted and paper filtered. Oils were stored under nitrogen at -20 °C until they
121 were analyzed.

122 123 2.3. Analysis of volatile compounds

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125 Olive oil samples were conditioned to room temperature and then placed in a vial heater at 40
126 °C. After a 10 min equilibration, volatile compounds from the headspace were adsorbed onto
127 SPME fiber DVB/Carboxen/PDMS 50/30 µm (Supelco Co., Bellefonte, PA). The sampling
128 time was 50 min at 40 °C and desorption of volatile compounds trapped in the SPME fiber
129 was performed directly into the GC injector. Volatile compounds were identified out on a
130 7820A/GC-5975/MSD system (Agilent Technologies), equipped with a DB-Wax capillary
131 column (60 m × 0.25 mm i.d., film thickness, 0.25 µm: J&W Scientific, Folsom, CA) and
132 under the following conditions: the injection port was operated in splitless mode at 250 °C;
133 He was used as the carrier gas and the flow rate was 1 mL/min; column was held for 6 min at
134 40 °C and then ramped up at 2 °C min⁻¹ to 168 °C; the mass detector was operated in the
135 electronic impact mode at 70 eV, the source temperature was set at 230 °C and the mass

136 spectra were scanned at 2.86 scans/s in the m/z 40-550 amu range (see a sample in Figure S1
137 and Table S1 in Supporting Information). The compounds were matched to the Wiley/NBS
138 and NIST libraries and against the GC retention time of available standards. VOO volatile
139 compounds were analyzed three times on a HP-6890 gas chromatography apparatus (Agilent
140 Technologies), which was equipped with a similar column and operated under the following
141 operating conditions in order to obtain quite similar retention times for volatile compounds
142 such as those obtained with the 7820A/GC-5975/MSD system: N₂ as the carrier gas at a
143 constant pressure of 17 psi; injector and detector at 250 °C; column held for 6 min at 40 °C
144 and then programmed at 2 °C min⁻¹ to 168 °C. Individual calibration curves for each
145 compound were used for quantification by adding known amounts of the different compounds
146 to redeodorized high-oleic sunflower oil.

147 The volatile compounds were clustered into different groups and subgroups according to the
148 polyunsaturated fatty acid and the origin of the LOX pathway branch, and as the terpene and
149 branched-chain (BC) volatile compounds from amino acid metabolism (Table 1).

150 151 *2.4. Statistical analysis*

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153 The data were evaluated using STATISTICA (Statsoft Inc., Tulsa, OK, USA). Correlations
154 among volatile compounds or groups of volatile compounds were analyzed using Pearson's
155 correlation coefficients. Principal component (PCA) and cluster analysis were used to
156 evaluate the associations among the volatile compounds from the progeny.

157 158 159 **3. Results and Discussion**

160
161 Taking into account the proven relationship between volatile compounds and the
162 sensorial quality of VOO [7], the volatile fraction of the oils produced from the segregating
163 progeny of a Picual x Arbequina cross was assessed over three consecutive years. These data
164 have now been deposited at the Oleagen web page (<https://chirimoyo.ac.uma.es/oleagen>). In
165 terms of the content of volatile compounds, the progeny displayed a high degree of variability
166 between individuals, widely transgressing the progenitor levels (Figure 1 and Table 1). A
167 wide variability in such progeny has been reported previously for fruit traits [26] and for other
168 oil components [27]. However, as far as we know there have been no previous reports on the

169 segregation of the volatile compound content in VOO. As mentioned in the Introduction, most
170 of the volatile compounds found in the oils of the progeny are produced by the enzymatic
171 systems including in the so-called LOX pathway [11], and they may be clustered into
172 different groups according to chain length and the polyunsaturated fatty acid (C6/LnA,
173 C6/LA, C5/LnA, C5/LA), and the origin of the esters (LOX esters). Previous findings suggest
174 that the synthesis of these compounds depends on the availability of substrates to be
175 catabolized through the LOX pathway during the process of obtaining the oil. Moreover,
176 LOX activity is an important limiting factor for the synthesis of these volatile compounds,
177 although such limitations do not seem to occur in the same direction among the different olive
178 cultivars [28, 29]. The differences observed in the limitation of the HPL reaction among the
179 olive cultivars seem to be more closely related to the variation in the amount of
180 hydroperoxides synthesized during the oil extraction process of each cultivar than to the HPL
181 activity during olive processing [30]. In addition, a group of compounds that featured a
182 branched-chain (BC) chemical structure derived from amino acid metabolism and two
183 terpenes also contributed significantly to the volatile fraction in the oils isolated from the
184 progeny.

185 When VOO was obtained from non-sound fruits, such as infested olives or olives
186 collected from the ground, or if VOO was inadequately processed or stored, the profile of
187 volatile compounds in VOO may include compounds that are responsible for off-flavors,
188 predominantly carboxylic acids or aliphatic C8-C11 carbonyls and alcohols [31, 32]. The
189 presence of such compounds is commonly associated with sensory defects in the oils and they
190 are mainly produced by chemical oxidation or through the activity of exogenous enzymes,
191 usually due to microbial activity present in non-sound fruits. In this study, only hand-picked,
192 sound fruit was used in the turning stage, and mild operation conditions were employed to
193 avoid the synthesis of such compounds that cause defects in the oils.

194 With very few exceptions, most of the volatile compounds in the oils of the progeny
195 were six straight-chain carbon compounds derived from linolenic acid (C6/LnA), the content
196 of which was 2-160 times higher on average than that of the rest of the groups of volatile
197 compounds in the oils (Fig. 1). The C6/LnA compounds varied from 0.50 to 51.99 $\mu\text{g/g}$ oil, a
198 variability that greatly exceeded that found for oils produced from other olive cultivars. The
199 accumulation of C6 compounds studied in 39 monovarietal VOOs obtained from trees
200 cultivated in the same orchard under the same pedoclimatic conditions was in the range of
201 2.52-18.11 $\mu\text{g/g}$ oil [33]. Among the C6/LnA compounds, the aldehyde group (C6/LnA

202 aldehydes) was the most abundant, (*E*)-hex-2-enal being the main contributor (88% of total
203 C6/LnA compounds on average: Figure 2). The mean content of this compound in the oils
204 was 14.59 µg/g oil, ranging from 0.35-43.38 µg/g oil. These large amounts of (*E*)-hex-2-enal
205 and the relatively low odor threshold (Table 1) mean that this C6/LnA compound is likely to
206 be one of the main contributors to the aroma of the olive oils produced from the progeny.
207 Indeed, this seems to be a common feature of the oils obtained from different olive cultivars
208 [33].

209 Only the C5/LnA group displayed comparable contents to those of the C6/LnA group
210 of compounds when the pentene dimers were included among them. Pentene dimers are
211 thought to be synthesized during the oil extraction process through the same branch of the
212 LOX pathway as the C5 compounds [34]. A strong relationship between the pentene dimer
213 content and that of the rest of C5/LnA compounds has been observed in this study, which will
214 be discussed later. Pentene dimers represented on average 86% of the C5/LnA content in the
215 progeny oils (Fig. 2), although the sensory contribution of pentene dimers to the VOO aroma
216 seems to be quite low or negligible given the estimated odor thresholds for these compounds.
217 While no odor thresholds have been reported for pentene dimers, estimates can be made from
218 the average published values for the structurally related C6-C10 dienes [35], which on
219 average represent 13,500 ng/g oil. Although displaying comparatively lower contents than
220 pentene dimers, the rest of C5/LnA compounds seem to have a notable involvement in the
221 VOO aroma according to their OAVs. Among them, pent-1-en-3-one and the pent-2-en-1-ols
222 are especially noteworthy, in close agreement with earlier findings [36], and all the genotypes
223 displayed pent-1-en-3-one contents above its odor threshold (0.73 ng/g oil: Table 1). The
224 aroma of this compound is described as green pungent and it is considered to provide an
225 unpleasant sensation [37]. However, the aroma of pent-2-en-1-ol is described as green fruity,
226 the typical basic perception of virgin olive oils, reminiscent of healthy, fresh olive fruits
227 harvested at the right ripening stage. Most of the genotypes of the progeny have (*Z*)-pent-2-
228 en-1-ol contents below its threshold concentration, suggesting this component is generally of
229 little relevance in terms of contributing to VOO aroma. Conversely, 63% of the oils from the
230 genotypes had a (*E*)-pent-2-en-1-ol content above its estimated odor threshold.

231 On average, terpenes were the third major group of volatile compounds in the oils of
232 the progeny of Picual x Arbequina cross, although their levels were very variable (8-19,653
233 ng/g oil) compared to those of C5/LnA compounds. In general, limonene seems not to be an
234 important contributor to VOO aroma since only four of the genotypes had an OAV above 1

235 for this terpene (Table 1). However, around 65% of the progeny seemed to have significant
236 levels of ocimene, suggesting that this compound might make an important contribution to the
237 aroma of the oils (OAV>1) should the odor threshold of ocimene be similar to that of the
238 structurally cycled isomer limonene (250 ng/g oil). As far as we know, no thresholds for
239 ocimene in oil have been reported. Nevertheless, the fact that non-cyclic terpenes generally
240 display lower odor thresholds than their cyclic counterparts suggests that the contribution of
241 ocimene to VOO aroma could be even more prominent.

242 As mentioned previously, esters convey the fruity odor notes to the oils that are much
243 appreciated by consumers, especially LOX esters. The LOX ester content in the progeny was
244 871 ng/g oil on average and in a range of 8-7438 ng/g oil. Only a few genotypes had a hexyl
245 acetate and (*E*)-hex-2-en-1-yl acetate contents consistent with them contributing to VOO
246 aroma (OAV > 1: Table 1), although (*Z*)-hex-3-en-1-yl acetate did seem to be an important
247 contributor to VOO aroma in around 67% of the progeny.

248 The levels of the BC compounds in the different genotypes of the progeny are also
249 noteworthy. Although these compounds are found at low concentrations in the oils (averaging
250 overall around 100 ng/g oil and in the range of 15-901 ng/g oil), they could still have a
251 profound impact on the aroma of the oils. The 2-methyl-butan-1-ol content seems to be
252 related to the fusty defects of VOO aroma [38], yet this BC compound was generally found
253 below its odor threshold. Conversely, the 2 and 3-methyl-butanal content suggests that these
254 BC aldehydes did contribute to the VOO aroma, since in all the genotypes of the progeny the
255 OAV for both these BC aldehydes was >1. Indeed, both BC aldehydes were located in the
256 ripe fruit sector of the SSW [10].

257 When the relationships among the different of groups of volatile compounds in the oils
258 from the Picual x Arbequina cross progeny were studied, the main classes of volatile
259 compounds (C6, C5 and terpenes) were significantly correlated with the total content of
260 volatile compounds in the oils (Table 2). Of particular note was the strong and very
261 significant correlation between the C6/LnA aldehydes ($r = 0.91$) and the total content of
262 volatile compounds in the oils, as well as the moderate correlation of the C5/LnA carbonyls (r
263 = 0.49) and the pentene dimers ($r = 0.70$) with this parameter. By contrast, BC compounds
264 and esters display a weak negative correlation (BC aldehydes) or almost no correlation with
265 the total volatile compound content of the oils. This is noteworthy given that these
266 compounds may have a strong impact on the aroma of VOO, especially the LOX esters and
267 BC aldehydes. Moreover, these groups of compounds were not strongly correlated with any

268 other group of compounds. While this is reasonable for BC compounds given that they do not
269 share any synthetic metabolic pathway, it was unexpected for the LOX esters. These esters
270 were weakly correlated with C6/LA alcohol ($r = 0.33$) but not with their main C6/LnA
271 precursor alcohols ($r = 0.02$), which suggests that the activity of the alcohol acyltransferase
272 (AAT) is limited to a large extent among the individual progeny. In fact, we previously found
273 AAT activity to be limited in both progenitors of this progeny, albeit more so in Picual than in
274 Arbequina fruit [39]. It was also concluded that the origin of the low volatile esters arising
275 from the LOX pathway in oils of Arbequina and Picual cultivars was largely due to a
276 limitation on alcohol synthesis during VOO production than to dampened AAT activity. In
277 this sense, there was a weak correlation between the content of the C6/LnA alcohols and their
278 metabolic precursors, the C6/LnA aldehydes ($r = 0.29$: Table 2), as also observed for the
279 saturated fraction of C6/LA alcohol and C6/LA aldehyde ($r = 0.42$). These data are in
280 accordance with the strong limitation on ADH activity found in the Picual and Arbequina
281 progenitors during oil extraction [39].

282 Significant correlations were also found between the pentene dimers content and that
283 of C5/LnA carbonyls and alcohols ($r = 0.71$ and 0.61 , respectively). This was similar to that
284 of the latter groups of compounds when compared with each other ($r = 0.76$), suggesting a
285 strong metabolic relationship between these groups of compounds. Indeed, these data support
286 the hypothesis that pentene dimer synthesis during oil extraction process occurs in the same
287 way as C5 alcohols are synthesized, involving the formation of an alkoxy radical from a
288 polyunsaturated fatty acid following the activity of a LOX protein [34]. The fact that the
289 pentene dimer content was not correlated with the C5/LA carbonyl and alcohol content ($r = -$
290 0.04 and -0.15 , respectively) suggests that pentene dimers were only produced from LnA and
291 not from LA.

292 Most of this variability among the VOO volatile profiles seems to correspond
293 exclusively to the genotype, since the genotypes could not be grouped in terms of harvest year
294 when analyzed by a PCA using either all the volatile compounds as the variables or only those
295 that are most important from a sensorial point of view (Figure S2 in Supporting Information).
296 It should be noted that the progeny and progenitors were all grown in the same orchard, under
297 the same edaphoclimatic conditions, and that the oils were extracted in exactly the same way,
298 with no *a priori* criterion to select the genotypes tested on each of the three sampling years.
299 Indeed, similar results were found when analyzing the phenolic profiles of these progeny oils

300 [27], although it has been possible to detect the differentiation of distinct groups when a
301 reduced number of genotypes from an olive cross were compared over consecutive years [40].

302 A PCA was performed to explain the correlations among the different volatile
303 compounds assessed in the oils of the progeny of the Picual x Arbequina cross (Figure 3). The
304 first two PCs carried a moderate amount of important information, with the first factor
305 explaining 25.73 % of the variance whereas the second factor explained 11.47 %. We
306 previously found quite similar values when assessing the content of the main phenolic
307 compounds in the oils of this progeny (24.94% and 16.46% for factor 1 and 2, respectively)
308 [27]. Most of the C6 and C5 compounds could be grouped separately from the other
309 compounds (Figure 3A) and especially, the C6 aldehydes and the C5 compounds derived
310 from LnA that cover a region between the second and third quadrants whose variances are
311 basically explained by factor 2. The only exception is the position of (*Z*)-pent-2-en-1-ol (5C-
312 5), clearly distanced from its isomer (*E*)-pent-2-en-1-ol (5C-6), which suggests a different
313 origin: (*E*)-pent-2-en-1-ol would be synthesized through the homolytic branch of the LOX
314 pathway, whereas the origin of its (*Z*)-isomer could be more closely related to chemical
315 oxidation. However, this is not consistent with the location in the plot of their theoretical
316 metabolic products, (*Z*) and (*E*)-pent-2-enal (5C-2 and 5C-3, respectively). The C6 aldehyde
317 (hexanal, 6C-8) and the C5 compounds derived from LA (5C-14, 5C-15 and 5C-16) were also
318 clearly separated from the main group of compounds derived from the LOX pathway (C6
319 aldehydes and the C5 compounds derived from LnA). Again and although at least partially
320 synthesized through the LOX pathway, the origin of these compounds could be more closely
321 related to pure chemical oxidation processes. Indeed, there may be two different modes of
322 hexanal formation during VOO extraction, through enzymatic and non-enzymatic pathways
323 [41]. The latter may be boosted by the use of high temperatures and longer times during the
324 malaxation of the olive paste.

325 C6 alcohols from both LA and LnA could be also grouped separately from the main
326 group of compounds derived from the LOX pathway, among which their metabolic precursors
327 could be found (Figure 3A). This distancing in the plot might be related to the aforementioned
328 weak correlation found between these groups of compounds (Table 2), and to the inactivation
329 of ADH during oil extraction [39]. The grouping of most of the esters in the fourth quadrant is
330 evident, next to but separated from the main group of compounds synthesized through the
331 LOX pathway. This might again be indicative of a disconnection with the mainstream LOX
332 pathway. In this sense, there was no correlation between the LOX esters and their main

333 precursors, the C6/LnA alcohols (Table 2), which might reflect the limitation of alcohol
334 synthesis during VOO production rather than a true dampening of AAT activity. As expected,
335 the BC compounds and terpenes grouped separately from each other and from the main group
336 of compounds derived from the LOX pathway, reflecting their different metabolic origins.

337 PCA bi-plots of the progeny oils showed strong associations between the C6 and C5
338 compounds (Figure 3A) and a number of progeny genotypes present in the third quadrant
339 (Figure 3B). Meanwhile, olive genotypes with a high content of esters and BC compounds
340 derived from amino acid metabolism were situated in the fourth quadrant. Thus, it is possible
341 to select genotypes from the progeny displaying a high level of a particular compound.
342 However, while genotypes characterized with a high content of desirable compounds like
343 C6/LnA aldehydes (6C-1 to 6C-4) could be identified in the plot, most of them were also rich
344 in non-desirable compounds like pent-1-en-3-one (5C-1), considered to provide unpleasant
345 sensations [13], or pent-1-en-3-ol (5C-4), which lies in the undesirable sector of the olive oil
346 SSW [10].

347 A PCA was performed considering the major groups of volatile compounds in the
348 progeny oils as variables in order to distinguish the genotypes that are especially rich in some
349 of these (Figure 4). Genotypes giving rise to oils with high C6/LnA aldehyde contents are
350 situated along the bisector of the first quadrant, while those producing oils with high C5/LnA
351 content, which include some non-desirable compounds from a sensorial point of view, are
352 located along the first factor axis. This distribution does not allow adequate selection of the
353 genotypes whose oils have high C6/LnA aldehydes content as well as high concentrations of
354 LOX esters or BC aldehydes., These latter groups are closely related (Figure 4A), such that
355 genotypes producing oils rich in LOX esters commonly have high BC aldehyde contents,
356 which might synergistically provide green-fruity odor notes as they are located in the green
357 and ripe fruit sectors of the SSW [10]. Thus, it is possible to select genotypes from the
358 progeny whose oils have a potential dominant green aroma, such as the genotypes UCI-55,
359 UCI-125, UCI-40 and UCI-20, or with a potent ripe fruit aroma, such as UCI-74, UCI-13,
360 UCI-135 and UCI-26.

361 As mentioned initially, the contribution of each volatile compound to the VOO aroma
362 depends on its concentration and odor threshold. Only a few volatile compounds are present
363 at levels indicating that they may contribute to the oil aroma ($OAV > 1$) of all the progeny
364 genotypes (Table 1). However, other volatile compounds contribute to just a given number of
365 the oil genotypes. PCA was performed considering only those volatile compounds that might

366 contribute to the aroma of the oil in more than 5% of the genotypes as a variable (Figure 5).
367 Most of these compounds were considered desirable for the aroma of VOO, except for hexan-
368 1-ol (6C-9), pent-1-en-3-one (5C-1), and pent-1-en-3-ol (5C-4), which provide unpleasant
369 sensations according to literature [10, 13, 38]. Factors 1 and 2 explain a good amount of the
370 data variation (41%), and a vector distribution clearly distinguishes between desirable and
371 undesirable areas in the genotype distribution plot when they are included as supplementary
372 variables (Figure 5). The vector for desirable aroma characteristics runs almost along the
373 bisector of the first quadrant, whereas the vector for undesirable aroma runs along the bisector
374 of the fourth quadrant (Figure 5A). The progenitors are located on both sides of the first factor
375 axis, although Arbequina is located in the sense of the desirable vector such that, in theory,
376 Arbequina oil aroma would be more desirable than Picual oil aroma (Figure 5B). This
377 appreciation might be related to the two-fold increase in C6/LnA compounds and the more
378 than 50% reduction of C5/LnA carbonyls in Arbequina oils compared to Picual oils. Genitors
379 differences may also be observed in the results of the cluster analysis (Figure 6). When using
380 as variables the volatile compounds that contribute to the aroma ($OAV > 1$) of the oils, the
381 genotypes from the progeny are distributed into two main groups. In each of these groups,
382 both genitors occupy a quite central position, respectively. The distribution of vectors in
383 Figure 5 allows genotypes such as UCI-41, UCI-36, UCI-39, UCI-68, UCI-133, or UCI-63 to
384 be identified, which presumably give rise to oils with remarkable sensory properties. As
385 displayed in Figure 6, most of these genotypes are included in the Picual group of the cluster
386 analysis. This information could be of interest for breeding programs aimed at producing new
387 cultivars with improved oil quality [21, 22].

388 In summary, this study shows that through a single cross of olive cultivars, it is
389 possible to obtain a high degree of variability for the main components responsible for the
390 aroma quality of VOO, which widely transgresses the variability in the progenitors. This
391 finding suggests that in breeding programs, it might be more effective to consider a larger
392 number of individuals within the same cross than using different crosses with fewer
393 individuals, in close agreement with earlier studies [42, 27]. The weak correlations found
394 between most of the volatile components that might influence aroma suggest the possibility of
395 obtaining new cultivars with a wide range of sensory profiles. The use of multivariate analysis
396 allows particularly interesting genotypes to be identified in terms of the volatile compound
397 composition and deduced organoleptic quality. Thus, the evaluation of the volatile profile at

398 the initial stage of selection can serve to identify potential new olive cultivars in breeding
399 programs that produce oils with improved sensory qualities.

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Figure captions

Figure 1. Content (ng/g oil) of the main groups of volatile compounds in the oils from the Picual x Arbequina progeny. The parental oils are indicated in the chart with arrows.

Figure 2. Range and distribution of the different classes of volatile compounds (ng/g oil) within the main groups in the oils from the Picual x Arbequina progeny. The squares in the interior of the boxes are the median values. The height of a box is equal to the interquartile distance, indicating the distribution for 50% of the data. The outliers (open dots) and extreme data (open triangles) are indicated outside the whiskers (the lines extending from the top and bottom of the box).

Figure 3. Bi-plot of the main volatile compounds in the oils from the Picual x Arbequina progeny. Factors 1 and 2 explain 37.20% of the data variation. A: vector distribution of the volatile compounds grouped according to their metabolic origin. B: distribution of the genotypes from the progeny.

Figure 4. Bi-plot of the main groups of volatile compounds in the oils from the Picual x Arbequina progeny. Factors 1 and 2 explain 38.32% of the data variation. A: vector distribution of the groups of volatile compounds (solid circles). B: distribution of the genotypes from the progeny, including the progenitors.

Figure 5. Bi-plot of selected volatile compounds that contribute to the aroma (OAV > 1) of the oils from the Picual x Arbequina progeny. Factors 1 and 2 explain 41% of the data variation. A: vector distribution of the volatile compounds (solid circles) and qualitative descriptors calculated from the corresponding compounds (open circles). B: distribution of the genotypes from the progeny, including the progenitors.

Figure 6. Cluster analysis of the genotypes of the Picual x Arbequina progeny using as variables the volatile compounds that contribute to the aroma (OAV > 1) of the oils. The position of the genitors (Arbequina and Picual) is marked as well as the genotypes presumably producing oils with remarkable sensory properties (*); from top to bottom: UCI-68, UCI-133, UCI-36, UCI-41, UCI-63, and UCI-39.

Supporting Information captions

Table S1. Identification of virgin olive oil volatile compounds by means of SPME-GC-MS analysis.

Figure S1. GC-MS analysis of the volatile fraction of virgin olive oil from cultivar Picual. Peak numbers are compounds listed in Table S1.

Figure S2. Principal component analysis distribution of the genotypes from the Picual x Arbequina progeny taking all the volatile compounds as variables (A) and those most important from a sensorial point of view ($OAV > 1$: B). The symbols for the genotypes have different colors according to the crop year. Prediction ellipses are displayed for each crop year (coefficient = 0.95).