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Additional Information

ASSESSMENT OF VINE-SHOOTS IN A MODEL WINES AS ENOLOGICAL ADDITIVES

Cebrián-Tarancón, Cristina[1]; Sánchez-Gómez, Rosario[1]; Carot, José Miguel[2]; Zalacain, Amaya[1]; Alonso, Gonzalo L[1].; Salinas, M. Rosario[1]*

[1]Universidad de Castilla-La Mancha, E.T.S.I. Agrónomos y Montes, Cátedra de Química Agrícola, Avda. de España s/n, 02071 Albacete, Spain.

[2]Universidad Politécnica de Valencia, Departamento de Estadística Aplicada e Investigación y Calidad de Operaciones, Camino de Vera, s/n, 46022 Valencia, Spain.

*Corresponding Author, (Tel: +34 967 599210, Fax: +34 967 599238, e-mail: Rosario.Salinas@uclm.es)

ABSTRACT

For the first time, the possibility of using vine-shoots as enological additives, similar to oak chips, is proposed. Their volatile and phenolic compounds contribution to a model wine was studied. Variables such as vine-shoot vinifera (Airén and Cencibel), size (*chip* and *granule*), toasting (toasted and non-toasted), dosage (4 and 12 g/L) and maceration time (1, 7, 21, 35 and 120 days) were tested. Results showed an important transference of high-value enological compounds after 35 days of maceration with 12 g/L of toasted vine-shoots. Vanillin or guaiacol were found in concentrations always higher than their wine odor thresholds, depending on the variety and vine-shoot formats used. Moreover, *trans*-resveratrol and ellagic acid stand out for their high concentrations. This transference rate shows the enological aptitude of vine-shoots with a possibly positive contribution in real wines, both at sensory and functional level, although future studies in real wines must be carried out.

KEYWORDS: antioxidant activity, model wine, phenolic compounds, tannin, vine-shoots, volatiles.

1. INTRODUCTION

Among the enological additives, the fragments of oak wood known as chips, stand out since they contribute to the wine with aromatic characteristics similar to wines aged in oak barrels (Bautista-Ortín, Lencina, Cano-López, Pardo-Mínguez, López-Roca, & Gómez-Plaza, 2008; Cerdán, Rodríguez Mozaz, & Ancín Azpilicueta, 2002). As a result of such addition to wine, the transference of several oak compounds takes place, especially volatile and phenolic compounds (Martínez-Gil, del Alamo-Sanza, Gutiérrez-Gamboa, Moreno-Simunovic, & Nevares, 2018) which give to the wine a special highly appreciated woody aroma. In this process, the size of the oak chips, dosage, seasoning, toasting degree and time of maceration, are the main factors that determine the type and quantity of compounds released from wood to wine (Schumacher, Alañón, Castro-Vázquez, Pérez-Coello, & Díaz-Maroto, 2013).

Vine-shoots are the principal waste in viticulture, In Spain, Castilla-La Mancha region the vineyard surface is around 443.818,31 ha, which accounts for 13% of European Union (EU) and almost 6% of the world area (OIV, 2017). If it is assumed an average of 1.3 kg of vine-shoots/vine as weight for an annual average production, approximately around $2·10^7$ tons of vine-shoots are harvested per year in the world. However, the current interest for the revaluation of this wastes and the search for innovative uses to consider them as resource rather than a waste has demonstrated that vine-shoots are an important source of high value compounds with biostimulant (Sánchez-Gómez, Zalacain, Pardo, Alonso, & Salinas, 2016, 2017) and bioplaguicide effects (Sánchez-Gómez, Sánchez-Vioque, Santana-Méridas, Martín-Bejerano, Alonso, Salinas, et al., 2017). Recently, vine-shoots have been characterized and prepared to be used during winemaking in the same way that oak chips are used (Cebrián-Tarancón, Sánchez-Gómez, Gómez-Alonso,

54   Hermosín-Gutierrez, Mena-Morales, García-Romero, et al., 2018; Cebrián-Tarancón,

55   Sánchez-Gómez, Salinas, Alonso, Oliva, & Zalacain, 2018; Delgado de la Torre, 2015) .

56   Oak chips can be consider as the residues from barrel manufacturing, but their quantity

57   is insignificant in comparison with vine-shoots viticultural waste generated, but highly

58   studied due to their appreciated wine contribution. Oak chips are richer in certain furanics

59   and benzenoids volatile compounds followed by whiskey lactones and terpenes,

60   especially significant is the contribution of vanillin within the benzenoids groups, a key

61   compounds for wine organoleptic characteristics, all of them also present in toasted vine-

62   shoots (Cebrián-Tarancón, et al., 2018; Cebrián-Tarancón, Sánchez-Gómez, Salinas,

63   Alonso, Oliva, & Zalacain, 2018). It is worth mentioning that vine-shoots are a valuable

64   source of stilbenes, especially *trans*-resveratrol, which is not present in oak wood.

65   Another important difference between both woods is on the tannin fraction, being

66   ellagitannins present in oak whereas vine-shoots only have oligomeric proanthocyanidins

67   (Cebrián-Tarancón, et al., 2018).

68   However, to propose vine-shoots as a new enological additive, it is important to carry

69   out a preliminary study about the transference of compounds with enological interest from

70   vine-shoots to a model wine, which is the main objective of this work. For it, the size of

71   vine-shoot fragments, toasting effect, dosage and the maceration time within a model

72   wine were the variables tested, using vine-shoots from Airén and Cencibel *Vitis vinifera.*

73

74   **2. MATERIALS AND METHODS**

75   **2.1. Vine-shoot samples**

76   Two vine-shoot cultivars were selected, Airén (*VIVC: 157*) and Cencibel (*VIVC:*

77   *12350)*, the last one also known as Tempranillo, which represent the third and first

78   cultivars in the world respectively. Two 15 years old vineyards of O.D. Mancha (Castilla-

79 La Mancha, Spain) were selected for the experiment. Their plant density was 2000

80 vines/ha and their surface of each vineyard was 3 ha.

81   Pruning of vine-shoots was established 90 days after the last day of harvest in each

82 vineyard, in case of Cencibel was the end of August and in case of Airén by the middle

83 of September 2017. Fifty vines of each vineyard were selected randomly and 0.5 kg

84 pruned, making a total of 25 kg of vine-shoots collected for each variety and vineyard.

85 The higher accumulation of volatile and non-volatile compounds in vine-shoots takes

86 place at six months of post-pruning storage (Cebrián, Sánchez-Gómez, Salinas, Alonso,

87 & Zalacain, 2017). For this reason, vine-shoots were stored intact in the dark and at a

88 room temperature (18 ± 3 ºC) with a relative humidity ranging between 60-66%, until

89 their processing.

90   As the minimum size oak chips for winemaking is set up two millimeters (ECC, 2006),

91 two different size according to their size were tested: similar to oak chips (called *chips*)

92 with size of 2.5 cm – 3.5 cm long and a second one between 2 mm and 2 cm long (called

93 *granule*s onwards).

94   After this time, half of these vine-shoots were subjected to a toasting process in an air

95 circulation oven (Heraeus T6, Hanau Deutschland) at 180ºC and 45 min according to

96 (Cebrián-Tarancón, Sánchez-Gómez, Salinas, Alonso, Oliva, & Zalacain, 2018). Final

97 moisture was resulting 2.84%. Moreover, as grapevines are commonly treated with

98 different fungicides, a study on the evolution of these residues in toasted vine-shoots was

99 also carried out. The results confirm the disappearance of these fungicides when vine-

100 shoots are submitted to a toasting process (data not shown).

101

102

103 **2.2. Model wine and treatments**

The model wine was composed by an ethanol/water solution (12.5/87.5; v/v) and 5g/L of tartaric acid, adjusted to pH 3.5 with NaOH 1M (García-Estévez, Alcalde-Eon, Martínez-Gil, Rivas-Gonzalo, Escribano-Bailón, Nevares, et al., 2017). For each variety, Airén and Cencibel vine-shoots, were tested: both sizes (*chips* and *granule*), toasted and non-toasted, two different dosages (4 g/L and 12 g/L) and five maceration times (1, 7, 21, 35 and 120 days) starting the first day after vine-shoot samples were added in model wine.

Vine-shoot samples were introduced in infusion bags, in similar way as the oak chips are introduced in real winemaking, and keeping the bags submerged in the model solution with the help of inert glass balls. The maceration step was carried out in one litter amber glass bottles, standing, at dark and under temperature control conditions (16 ± 1ºC). The studied conditions were choose taken in account the traditional use of oak chips in model wines (Bautista-Ortín, Lencina, Cano-López, Pardo-Mínguez, López-Roca, & Gómez-Plaza, 2008; Schumacher, Alañón, Castro-Vázquez, Pérez-Coello, & Díaz-Maroto, 2013). A total of 40 different wine model solutions of each variety were prepared in duplicate.

2.3. Chemical composition analysis

2.3.1. Volatile compounds

Volatiles were determined according to (Sánchez-Gómez, Zalacain, Alonso, & Salinas, 2014). Their extraction was carried out by means of Stir Bar Sorptive Extraction (SBSE) with a polydimethylsiloxane twister bar (10 mm length, 0.5 mm film thickness). Samples were stirred at 500 rpm during 60 minutes later analysis was performed using an automated thermal desorption unit (TDU, Gerstel, Mülheim and der Ruhr, Germany) mounted on an Agilent 7890A gas chromatograph system (GC) coupled to a quadrupole Agilent 5975C electron ionization mass spectrometric detector (MS, Agilent

129 Technologies, Palo Alto, CA, USA), equipped with a fused silica capillary column (BP21

130 stationary phase, 30-m length, 0.25 mm I.D. and 0.25 μm film thickness) (SGE,

131 Ringwood, Australia). The carrier gas was helium with a constant column pressure of

132 20.75 psi.

133   The stir bars were thermally desorbed in a stream of helium carrier gas at a flow rate

134 of 75 mL/min with the TDU programmed from 40 to 295 °C (held 5 min) at a rate of 60

135 °C/min, splitless desorption mode. The analytes were focused in a programmed

136 temperature vaporizing injector (PTV) (CIS-4, Gerstel), containing a packed liner (20 mg

137 tenax TA), held at - 40 °C with cryo cooling prior to injection. After desorption and

138 focusing, the CIS-4 was programmed from -40 to 260 °C (held for 5 min) at 12 °C/min

139 to transfer the trapped volatiles onto the analytical column. The GC oven temperature was

140 programmed to 40 °C (held for 2 min), raised to 80 °C (5 °C/min, held for 2 min), raised

141 to 130 °C (10 °C/min, held for 5 min), raised to 150 °C (5 °C/min, held for 5 min), and

142 then raised to 230 °C (10 °C/min, held for 5 min). The MS was operated in scan

143 acquisition (27−300 u) with an ionization energy of 70 eV. The temperature of the MS

144 transfer line was maintained at 230 °C.

145   MS data acquisition was carried out at positive scan mode, although to avoid matrix

146 interferences, the MS quantification was performed in the SIM mode using their

147 characteristic m/z values. Compounds identification was performed using the NIST

148 library and confirmed by comparison with the mass spectra and retention time of their

149 pure standards. The standards used to identify and quantify volatile (GC-MS) were

150 purchased in Sigma-Aldrich (Steinheim, Germany), the numbers in brackets indicates the

151 m/z used for quantification: benzaldehyde (m/z = 106), citronellol (m/z = 69), citronellal

152 (m/z = 69), ethyl hexanoate (m/z = 101), ethyl octanoate (m/z = 101), 4-ethylphenol (m/z

153 = 107), eugenol (m/z = 164), farnesol (m/z = 69), furfural (m/z = 96), geranyl acetone (m/z

154 = 43), guaiacol (m/z = 109), 1-hexanol (m/z = 56), hexyl acetate (m/z = 43), 5-

155 hydroxymethylfurfural (m/z = 97), β-ionone (m/z = 177), β-ionol (m/z = 205), linalool

156 (m/z = 71), 5-methylfurfural (m/z = 110), nonanal (m/z = 57), 1-octen-3-ol (m/z = 57),

157 syringol (m/z = 154), vanillin (m/z = 151), 4-vinylguaiacol (m/z = 151) and *trans/cis*-

158 whiskey lactones (m/z = 99). As internal standard 3-methyl-1-pentanol (220 μl/25 mL

159 model wine) was used. Quantification was based on calibration curves of the respective

160 standards in the model wine at five different concentrations (2 μg/L–15 mg/L) (R^2 = 0.95-

161 0.99). All analyzes were made in triplicate.

162

163 *2.3.2. Low molecular weight phenolic compounds (LMWPC)*

164   The samples were filtered through a PVDF Durapore filter of 0.22 μm (Millipore,

165 Bedford, MA, USA) and 20 μL were injected into an Agilent 1200 HPLC chromatograph

166 (Palo Alto, California, USA) equipped with a Diode Array Detector (Agilent G1315D)

167 and atmospheric pressure chemical ionization (MM-ESI/ APCI-MS) system, coupled to

168 an Agilent ChemStation (version B.03.01) data-processing station. Separation was

169 performed on a reverse phase ACE Excel 3 C18-PFP (4.6 mm × 150 mm, 3 μm particle

170 size) and a precolumn ACE Excel HPLC Pre-colum Filter 1PK (0.5 μm particle size) at

171 30 °C. The HPLC proportion of solvents used was water/formic acid/acetonitrile

172 (97.5:1.5:1 v/v/v) as solvent A and acetonitrile/formic acid/solvent A (78.5:1.5:20 v/v/v)

173 as solvent B. The elution gradient was set up for solvent B as: 0 min, 5%; 8.40 min, 5%;

174 12.50 min, 10%; 19 min, 15%; 29 min, 16%; 30 min, 16.5%; 34.80 min, 18%; 37.20 min,

175 32%; 42 min, 62%; 52 min, 90%; 54 min, 100%; 56 min, 100%; 60 min, 5%; 65 min,

176 5%.

177   All compounds detection was carried out by means of DAD detector by comparison

178 with the corresponding UV–vis spectra and retention time of their pure standards (Sigma-

179   Aldrich, Steinheim, Germany). Although other LMWPC were looked for, the compounds

180   quantified and identified at different wavelength were: (+)-catechin, (-)-epicatechin,

181   gallic acid, protocatechuic acid at 280 nm; ellagic at 256 nm; conyferaldehyde and

182   sinapaldehyde at 324 nm; *trans*-piceid (*t*-resveratrol-3-glucoside) and *trans*-resveratrol

183   at 308 nm. Quantification was based on calibration curves of the respective pure standards

184   in the model wine at five different concentrations achieved by UV-vis signal (0.40 – 260

185   mg/L) (R^2 = 0.96-1.00). All analyzes were made in triplicate.

186

187   *2.3.3. Tannin fraction*

188   The tannin level was measured in samples according to the methylcellulose

189   precipitation method by (Sarneckis, Dambergs, Jones, Mercurio, Herderich, & Smith,

190   2006). Tannin precipitation was performed using a 0.04% methylcellulose solution (w/v,

191   in deionized water). The tannin concentration was determined in triplicate by the

192   difference in absorption at 280 nm between the sample without the addition of

193   methylcellulose and the methylcellulose precipitated. The tannin content was quantified

194   as (-)-epicatechin.

195

196   *2.3.4. Antioxidant capacity*

197   The scavenging activity against the 1, 1-diphenyl-2-picrylhydrazyl (DPPH) free

198   radical was determined according to (Braca, De Tommasi, Di Bari, Pizza, Politi, &

199   Morelli, 2001). Samples (0.5 mL) were mixed with 2.5 mL of DPPH in methanol (0.04

200   mg/mL), incubated 1 hour at room temperature and the absorbance measured at 517 nm

201   in a Perkin-Elmer Lambda 20 UV-visible Spectrophotometer. Distilled water and (+)-

202   catechin standard were used as negative and positive control of the assay, respectively.

203 The reduction of DPPH was tested in triplicate for five randomly different volumes: 10,

204 20, 40, 80 and 100 µl of the wine model solutions.

205

206 **2.4. Statistical analysis**

207 To guarantee quality of data, firstly a complete descriptive analysis was carried out.

208 Special attention was paid to detect potential outliers. For that, firstly each group was

209 analyzed of the three repetitions comparing their variability. Subsequently, numeric and

210 graphic (box-whiskers) descriptive analysis were carried out. Finally, a detailed residual

211 analysis was done in each one of the analysis of variance. Due to the experimental plan,

212 the statistical analysis was done with the average values of each one of the experimental

213 conditions. For each compound, the Tukey test was used to analyze significant differences

214 among the selected treatments ($\alpha < 0.05$). To achieve a general view and compare the

215 effect of each treatment, afactor analysis of correlations was used. For that, variables

216 selected were the more important aroma compounds related with the classical sensory

217 profile of wood aged wines.As a method of extraction, the principal component method

218 was applied with varimax rotation.

219

220 **3. RESULTS AND DISCUSSION**

221 *3.1. Vine-shoot conditions selection*

222 It is well known that the compounds extracted when wine is in contact with oak wood

223 are of great importance for wine quality, since the aroma is markedly modified (Cerdán,

224 Rodríguez Mozaz, & Ancín Azpilicueta, 2002; García-Estévez, et al., 2017). Oak wood

225 is always submitted to a toasting process and volatile compounds such as vanillin,

226 guaiacol or whiskey lactones are significantly generated. These volatiles have also been

227 identified in toasted and non-toasted vine-shoots (Cebrián, Sánchez-Gómez, Salinas,

228   Alonso, & Zalacain, 2017; Cebrián-Tarancón, Sánchez-Gómez, Salinas, Alonso, Oliva,

229   & Zalacain, 2018; Delgado De La Torre, Priego-Capote, & Luque De Castro, 2015;

230   Sánchez-Gómez, Zalacain, Alonso, & Salinas, 2014, 2016a), contributing with a vanilla

231   aroma, smoked and toasted notes or a characteristic coconut aroma respectively (Schranz,

232   Lorber, Klos, Kerschbaumer, & Buettner, 2017). But there are other aroma which are not

233   in common in both woods, as for example linalool, which has been only quantified in

234   vine-shoots but not in oak chips (Cebrián-Tarancón, Sánchez-Gómez, Salinas, Alonso,

235   Oliva, & Zalacain, 2018). It is a well-known vine compound, which can be accumulate

236   in grapes and to be extracted along winemaking, contributing to the varietal aroma of

237   wine and its typicality.

238   In order to determine the best transference conditions of such compounds (vanillin,

239   guaiacol, *cis* and *trans* whiskey lactones and linalool) from vine-shoots to the model wine,

240   a factor analysis was performed (Table 1) considering their size, dosage, toasting and

241   maceration time in contact with the model wine.

242   The factorial analysis for both vine-shoot varieties (Airén and Cencibel) provided in

243   both cases, two factors with a similar structure (Table 1). The first factor gathers the

244   information of the variables *trans*-whiskey lactone, *cis*-whiskey lactone and linalool,

245   while the second was generated by guaiacol and vanillin. Figures 1 and 2 shows the scores

246   of Airén and Cencibel experiments carried out in the factors obtained, allowing a global

247   view and where it is possible to compare the effects of the sizes, dosages, toasting and

248   maceration time within the model wines. It is noteworthy that for the two varieties, the

249   two underlying factors obtained were the same; however, the response to the treatments

250   (dosages, toasting and maceration time) in each variety was different as it can be observed

251   in Figures 1 and 2. All variables had a positive weight, which means that the positioning

252   of the model wines on the right side of the first factor implies simultaneously greater

values of *trans*-whiskey lactone, *cis*-whiskey lactone and linalool. In the second factor, the positioning in the upper part implies greater values of guaiacol and vanillin. In this way, the treatments with a higher extractability capacity were the ones positioned in the upper right of the two graphs: in case of Airén variety (Figure 1), the treatment with *chips* was in the right part of factor 1, which means that it generally presents greater values of *trans*-whiskey lactone, *cis*-whiskey lactone and linalool. In the axis, it is observed that model wines placed in the upper and right part and therefore with greater values of vanillin and guaiacol, correspond to different combinations of dosages, toasting and maceration time, suggesting that interactions of higher order are produced between the tested experimental conditions. So, according to these results, the best treatments for this variety would be add 12 g/L of toasted *chips* and macerate during 21 and 35 days (model wines named as C-T-12-21 and C-T-12-35) (Figure 1). In case of Cencibel variety (Figure 2), a very different behaviour was observed between *chips* and *granule* formats, but with an opposite response to that of Airén. Here, the model wines in contact with *granules* have a greater value of *trans*-whiskey lactone, *cis*-whiskey lactone and linalool, due to the position of the scores in the factor 1. In factor 2, again, it was observed that the best treatments to achieve greater values of vanillin and guaiacol, correspond to the different combinations of treatments. The selection of the best conditions for the vine-shoots was carried out by analysing the positioning of the model wines on the factorial plans. In addition, for Cencibel variety, in contrast by Airén variety, it has been observed that the two factors present certain correlation. Looking at loading values (Table 1), it is concluded that the best treatments were the five located in the upper right, all of them with a dosage of 12 g/L and *granule* size. It is due that they were located on the main direction marked by almost all the variables and that was located on the quadrant bisector. Although the most efficiency treatments for Cencibel variety were 35 and 120 days of

278   maceration time (Figure 2), the last one was not significantly different, so 35 days was

279   select for Cencibel vine-shoots (model wine G-T-12-35). In addition, such results suggest

280   that after 35 days of maceration, the kinetics of extraction of the studied compounds

281   remains constant. Moreover, the maceration treatment for 21 days (model wine G-T-12-

282   21) also will be studied in detail, since it was the second most important treatment and

283   for to compare with the Airén vine-shoots.

284

285   *3.2. Detailed composition of the selected model wine solutions*

334   Table 2 shows a detailed analysis of the transferred compounds from the two varieties

335   of vine-shoots, when 12 g/L of toasted pieces (*chips* and *granules*) were macerated during

336   21 and 35 days in the model wine. In case of pH (Table 2), it was observed lower values

337   when higher maceration times were used, independently of the variety and vine-shoot

338   formats, in relation to the control model wine. In case of Airén model wines macerated

339   21 days, an increment of 0.7 units were observed and about 0.48 units in case of 35

340   maceration days. In case of Cencibel model wines macerated 21 days, an increment of

341   0.73 units were observed, and about 0.55 units in case of 35 maceration days. Such pH

342   increment should be taking into account when vine-shoots will be added to real wines.

343   Compounds were grouped according to their chemical structure as volatiles and low

344   molecular weight phenolic compounds.

345   The total volatile composition of the resulting model wines was clearly different for

346   both varieties according to the formats and maceration time used. When Airén *chips* were

347   used, in the case of the lowest maceration time (21 days), it was observed a significant

348   higher level of compounds than in the rest of treatments. By contrast, the transfer of

349   compounds from Cencibel *chips* to model wine was higher when they were macerated for

350 a longer period of time (35 days) and such contribution showed a similar content than in

351 case of the *granule* format.

352 Furanics were the most abundant volatile compounds in wines, reaching value ranges

353 of 2891.68 – 5516.08 µg/L for Airén and 2825.02 – 3625.62 µg/L for Cencibel ones.

354 Such compounds result from the hemicellulose degradation during the toasting process

355 (Chatonnet, Boidron, & Pons, 1989) and can contribute to wine aroma with caramel,

356 toasted and honey like notes. The high presence of these compounds was expected by

357 their high affinity to ethanol/water (model wine) (Alañón, Alarcón, Marchante, Díaz-

358 Maroto, & Pérez-Coello, 2017). However, its content was lower than their corresponding

359 wine odor thresholds (Table 2). It is accepted that compounds whose Odor Activity Value

360 (ratio between the concentration of a compounds and its odor threshold, OAV) is ≥ 1

361 contribute individually to the matrix aroma (Guth, 1997), so such furanics do not

362 participate in the perception of the aroma of these wine model solutions (Table 2).

363 Volatile phenols were the second most abundant group, where the most important

364 compounds, from the point of view of its contribution to the wine aroma, were vanillin

365 and guaiacol. Both compounds come up from lignin degradation while wood toasting

366 takes place (Cebrián-Tarancón, Sánchez-Gómez, Salinas, Alonso, Oliva, & Zalacain,

367 2018). In case of vanillin, similar trend was found for both varieties, the highest

368 concentrations were observed when *granule* format was used. The most significant

369 increase with maceration time was observed in Cencibel wines from *granule* format,

370 where vanillin concentration reached 227.40 µg/L and 261.25 µg/L after 21 and 35 days,

371 respectively. The OAV of vanillin in all wine model solutions was > 1 (Table 2).

372 However, while in Airén model wines from *granule* format the OAV values were 2.73

373 and 2.84 for 21 and 35 maceration days respectively, in Cencibel model wines with

374 similar format, the OAV values were 3.79 and 4.35, respectively. These results suggest

13

375   that *granule* toasted vine-shoots from both varieties, could increase wine vanillin content

376   with an important vanilla aromatic note. Such possibility also was presented when *chip*

377   format was used, but in a less extension. Moreover, vanillin concentration in all model

378   wines studied were comparable to the levels reported for others works when oak barrels

379   and fragments were tested (Navarro, Kontoudakis, Gómez-Alonso, García-Romero,

380   Canals, Hermosín-Gutíerrez, et al., 2018). According with previous works, the size of

381   wood fragment may have a relevant effect on the formation of vanillin during the toasting

382   of the pieces of vine-shoots (Campbell, Sykes, Sefton, & Pollnitz, 2005).

383   Guaiacol showed different behavior regardless of the vine-shoots variety, but not

384   significant differences were observed between the concentrations achieved by the best

385   treatments. For model wines in contact with Cencibel vine-shoots, the higher

386   concentration of guaiacol was found when *granule* size was used, 15.21 μg/L and 16.30

387   μg/L for 21 and 35 days of maceration, respectively. In contrast, *chip* size contributed

388   with a greater quantity of guaiacol to the wine, 17.98 μg/L and 18.25 μg/L, in case of

389   Airén variety (Table 2) for 21 and 35 days of maceration. Very low concentrations of this

390   compound when oak *chips* were used in other previous works (Bautista-Ortín, Lencina,

391   Cano-López, Pardo-Mínguez, López-Roca, & Gómez-Plaza, 2008). Moreover, in the

392   same way that vanillin, the guaiacol OAV values were ≥ 1, so vine-shoots used in wines

393   in the conditions of this study, could be contribute with its "smoked" or "toasted" notes

394   to the wine aroma (Schranz, Lorber, Klos, Kerschbaumer, & Buettner, 2017), as oak

395   barrels and chips (Chira & Teissedre, 2013). With similar aroma descriptors, syringol and

396   4-vinylguaiacol also were detected in model wine solutions, but their OAV values were

397   < 1. However, synergies effects may exist between volatile compounds, especially in

398   those with similar aromatic nuances (Ferreira & Cacho, 2009) . Eugenol, a volatile phenol

399   with a characteristic clove aroma (Ferreira, López, & Cacho, 2000), had a different

behave according to the vine-shoot varieties and formats, but in all cases the concentration was lower than its odor threshold. The highest and lowest content were in model wines from Cencibel and Airén *granule* sizes, respectively. Very low concentration of 4-ethylphenol was detected in the different model wines, ranging between of 2.43 - 4.01 µg/L, fall far below its odor threshold, so its leather and horse note should not be detected (Bautista-Ortín, Lencina, Cano-López, Pardo-Mínguez, López-Roca, & Gómez-Plaza, 2008).

Terpenoids, a group of compounds that are associated with the grape variety, with roses, citrusy or lilac notes , also showed the highest extraction when the *granule* format was used. No significant differences were found between both times of maceration in Airén model wines for total terpenoids, but in case of Cencibel, the highest concentration,127.46 µg/L according to Table 2, was reached after 35 days of maceration (Table 2). It is important to highlight the presence of β-ionol, which was not described previously in toasted vine-shoots (Cebrián-Tarancón, Sánchez-Gómez, Salinas, Alonso, Oliva, & Zalacain, 2018), which showed up five times content when *granule* format was used. This suggest that a minor size of particle favors its extraction. Compounds such as citronellal, linalool, farnesol, among others terpenoids, were also detected in all model wines, being linalool the most abundant when the *granule* format of Cencibel was used. At individual level, the OAV values were < 1 for all of them, although the information about the odor threshold for β-ionol was not found in the literature (Table 2). However, the synergist effect that may exist between them, could enhance the wine varietal aroma if vine-shoots were used as enological additives. On the other hand, if the β-ionone precursor, β-ionol, is considered as a powerful odorant, these results could suggest an important contribution of its positive floral aroma to the wines, since the contents found were much higher than the odor threshold of the β-ionone. C_6 compounds, also known for

425 their herbaceous aroma contribution to wine aroma, are the products of the enzymatic

426 breakdown of unsaturated fatty acids (Aubert & Chalot, 2018). In this case, 1-hexanol

427 was the only one representing this chemical family and showed a different behavior

428 depending of the vine-shoot varieties used. Model wine in contact with Airén vine-shoots

429 showed the highest concentration after 21 days of maceration, independently of the size.

430 However, wines in contact with Cencibel vine-shoots showed the highest concentration

431 when the *chip* format was used, independently of the maceration time.

432 The influence of vine-shoots in the total esters and "others" group compounds content

433 (Table 2) was not relevant.

434 At sensory level, the lactones (*cis* and *trans*- whiskey lactone isomers) found in the

435 model wines were not relevant, since their content were much lower than their

436 corresponding odor thresholds (Table 2). Nevertheless, it is worth discussing them,

437 because until recently, they were considered exclusive as oak compounds, but according

438 to (Sánchez-Gómez, Zalacain, Alonso, & Salinas, 2016b) , they were also present in

439 toasted vine-shoots. Such compounds may contribute with a woody coconut aroma,

440 which is positively correlated with the *cis*-isomer (Chatonnet, Boidron, & Pons, 1990) or

441 an herbaceous and also woody aroma (Bautista-Ortín, Lencina, Cano-López, Pardo-

442 Mínguez, López-Roca, & Gómez-Plaza, 2008), which is attributed to the *trans*-isomer.

443 In our work, both isomers were quantified when the *chip* format was used, being

444 necessary a long time of maceration in case of Cencibel vine-shoots. Higher

445 concentrations were found in the case of Cencibel cultivar, 8.89 µg/L, when the *granule*

446 format was tested.

447 Summarising, in an attempt to extrapolate the actual results to real wines and

448 considering the volatiles with OAV >1, we suggest that: 1) If wines with vanilla notes are

449 desired, then vine-shoots with a *granule* format must be used independently of the variety,

macerating them during 35 days; 2) If wines with toasted notes (guaiacol) are desired, Airén vine-shoots must be used in *chips* format, macerating during 21 days; 3) If wines with both aromas (vanilla and toasted) are desired, Cencibel vine-shoot must be used in *granule* formats, macerating during 35 days.

The low molecular weight phenolic compounds identified in model wines from the selected treatments were also shown in Table 2, grouped according to their chemical phenolic families such as flavanols, phenolic acids, stilbenes and aldehydes. All quantified compounds were also found in non-toasted vine-shoots (Cebrián, Sánchez-Gómez, Salinas, Alonso, & Zalacain, 2017) although their content decreased with toasting, except for *trans*-resveratrol compound which increased (Cebrián-Tarancón, Sánchez-Gómez, Salinas, Alonso, Oliva, & Zalacain, 2018). (+)-Catechin and (-)-epicatechin were the flavanols detected and only quantified when *granule* format was used for both varieties, reaching concentrations of 18.24 mg/L and 7.34 mg/L in Airén and Cencibel, respectively. These contents were in the same range than in real wines which have not been in contact with any wood (Gordillo, Baca-Bocanegra, Rodriguez-Pulído, González-Miret, García Estévez, Quijada-Morín, et al., 2016; Sánchez-Gómez, Zalacain, Pardo, Alonso, & Salinas, 2017). Ellagic acid was the most abundant phenolic compound transferred from vine-shoots to model wines, whereas gallic and protocatechuic acids were also transferred but in very small quantities. Such acids were previously detected in vine-shoots, toasted and non-toasted, being ellagic acid the most significant (Cebrián-Tarancón, Sánchez-Gómez, Salinas, Alonso, Oliva, & Zalacain, 2018). The mean content of ellagic acid in all model wines was approximately 33 mg/L and not relevant differences were observed among format, varieties and maceration time. The content of ellagic acid in real wines was lower than in the studied model wines. However, it has recently been shown that the addition of ellagic acid in pre-fermentative

475 stage of winemaking, at concentrations of 300 mg/L, markedly improves the color of red

476 wines, acting as an important cofactor of anthocyanins, but also stabilizing the phenolic

477 profile (Zhang, He, Zhang, Reeves, Liu, Zhao, et al., 2018). This result suggests the

478 interest of using vine-shoots as enological additives, both before and after the alcoholic

479 fermentation of wines.

480 In the stilbenes group, *trans*-resveratrol was found in all model wines, while its

481 glucoside, *trans*-piceid, was only detected when *granule* formats and 35 days of

482 maceration were used. Both stilbenes were present in high concentration in vine-shoots,

483 non-toasted and toasted, but the toasting process increase them significantly, especially

484 *trans*-resveratrol (Cebrián-Tarancón, Sánchez-Gómez, Salinas, Alonso, Oliva, &

485 Zalacain, 2018). The concentration of *trans*-resveratrol in the model wines reached levels

486 of 4.05 mg/L and 2.64 mg/L for Airén and Cencibel varieties respectively, when the

487 *granule* format was used during 35 days of maceration (Table 2). The presence of *trans*-

488 resveratrol in white wines according to the bibliography may range between 0.80 to 4.61

489 mg/L (Fabjanowicz, Płotka-Wasylka, & Namieśnik, 2018) depending if there is any

490 contact with skins, seeds and grape juice during winemaking. This stilbene is considered

491 the major functional compound in red wine (Sumby, Grbin, & Jiranek, 2010). Due to the

492 fact that there was higher *trans*-resveratrol content in model wines in contact with vine-

493 shoots than in real wines, vine-shoots could be used as an enological additive to increase

494 *trans*-resveratrol and therefore, the healthy benefits of wines, being Airén *granule* size in

495 contact during 35 days, the one that may have the highest contribution in real wines. This

496 possibility does not present when oak chips are used, since this stilbene does not form

497 part of this wood.

498 Phenolic aldehydes such as coniferaldehyde and sinapaldehyde were only quantified

499 in model wines from Airén, when *chips* were macerated during 21 days, and in model

wines from Cencibel when the *granule* format was macerated during 35 days, the highest

concentrations being in this last case (Table 2).

In the pool of phenolic compounds, the tannin content was also determined. Model

wines with Cencibel, independently of the used format, had always higher tannin content

than when Airén was used. In case of Cencibel, *chips* contributed with 26.96 mg/L of (-

)-epicatechin while with granule the content was 22.53 mg/L of (-)-epicatechin. In case

of Airén chips, the tannin content within the model wines was 11.92 mg/L of (-)-

epicatechin versus the 18.55 mg/L of (-)-epicatechin for granule format. Then depending

on the variety, the contribution of tannins to the model wine solutions in terms of formats

was different.

The tannin fraction of vine-shoots is constituted only by proanthocyanins, of which

procyanidins are the majority but it is noteworthy the high amount of prodelphinidins

content in relation with other grape derivates. The mean value of the tannin fraction in

white wines is lower than in red wines, in the latest between 0.5 and 3 g/L (Sarneckis,

Dambergs, Jones, Mercurio, Herderich, & Smith, 2006). Therefore, if vine-shoots would

be used as enological additives, their contribution to the tannin sensorial properties of

wines, it does not seem to be so relevant.

3.3. Antioxidant capacity

Previously the antioxidant capacity of wines, seeds, grape pomaces or skins has been

evaluated . Such capacity is attributed to certain wine components, since they can prevent

reactions of oxidative degradation, and therefore they have a positive effect on health

(Bartolomé, Nuñez, Monagas, & Gómez-Cordovés, 2004).

The antioxidant capacity of toasted vine-shoots as a pool has not been analyzed

before, although their individual chemical characterization (Cebrián-Tarancón, Sánchez-

525 Gómez, Salinas, Alonso, Oliva, & Zalacain, 2018; Sánchez-Gómez, Zalacain, Alonso, &

526 Salinas, 2016a) suggest their antioxidant potential. In Figure 3 it is shown the percentage

527 of DPPH radical reduction of model wines solutions macerated with toasted Airén and

528 Cencibel vine-shoots as *chip* and *granule* formats during 35 days, 100 µl of these were

529 used. Independently of the variety used, the highest reduction was observed for the

530 *granule* size, reaching a 50% and 37% of DPPH radical inhibition for Airén and Cencibel

531 respectively. These results suggest that the vine-shoot formats had a direct influence in

532 the antioxidant capacity, which increase when a low particle size was used and also, they

533 were in agreement with the higher content of phenolic compounds in these model wine

534 solutions, especially stilbenes and flavanols.

535 **4. CONCLUSIONS**

536 The transference of chemical compounds with an enological interest from vine-shoots

537 to a model wine was studied for first time, in order to propose them as new enological

538 additives for wines. Such contribution depends on the vine-shoot variety and format used.

539 In all cases significant quantities of vanillin and guaiacol, above their odor thresholds,

540 were transferred from vine-shoots. *trans*-Resveratrol, the highest functional compound in

541 wines, was found in all samples in larger quantities than those found in most real wines.

542 Moreover, the ellagic acid content found make possible its participation on wine

543 copigmentation and color stabilization phenomena. Taking into account all compounds

544 and vine-shoot formats and varieties, the ones with better overall contribution was

545 Cencibel granule format macerated at 35 days.

546 Such preliminary results suggest that vine-shoots can contribute positively to modify

547 the aroma, color and healthy effect of wines, by adding to the wine their vine own

548 compounds. Future works should be addressed in real wines.

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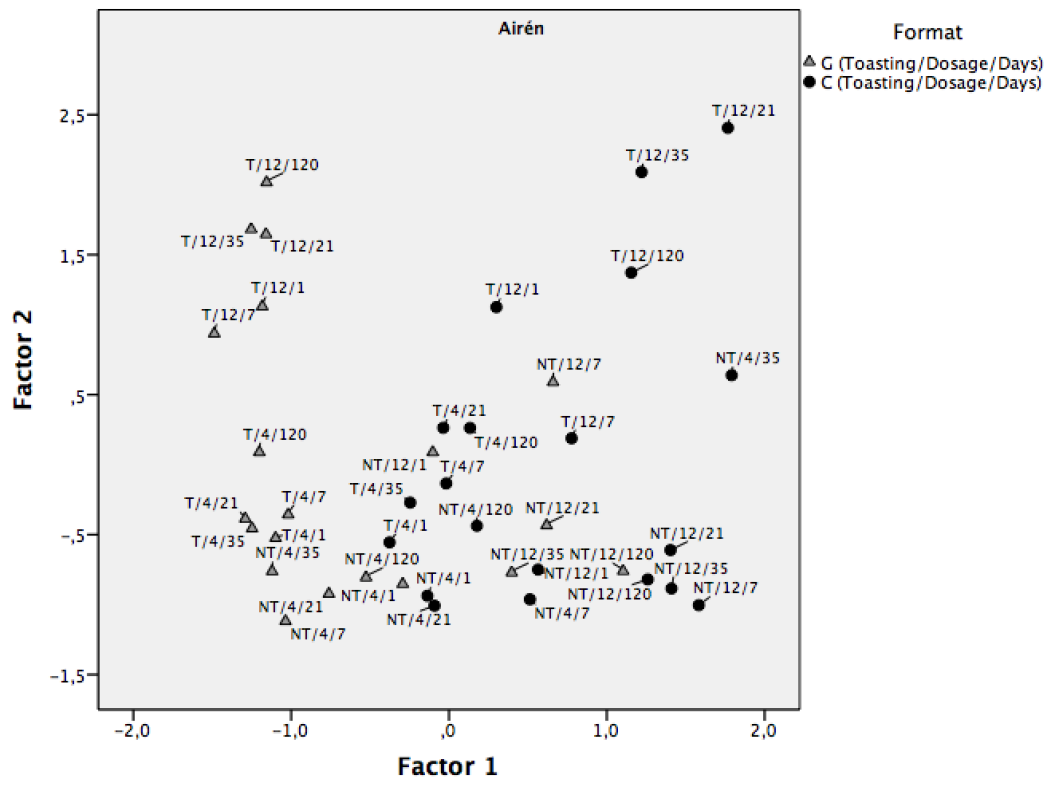701
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703 **ACKNOWLEDGEMENTS**

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708 **FIGURE CAPTIONS**

709 **Figure 1.** Scores of the forty model wines in contact with Airén vine-shoot fragments

710 obtained after the application of the Factor Analysis. Format (C: *chips*, G: *granules*);

711 toasted (T: toasted, NT: non-toasted); dosages (4: 4g/L, 12: 12g/L); time of sampling (1,

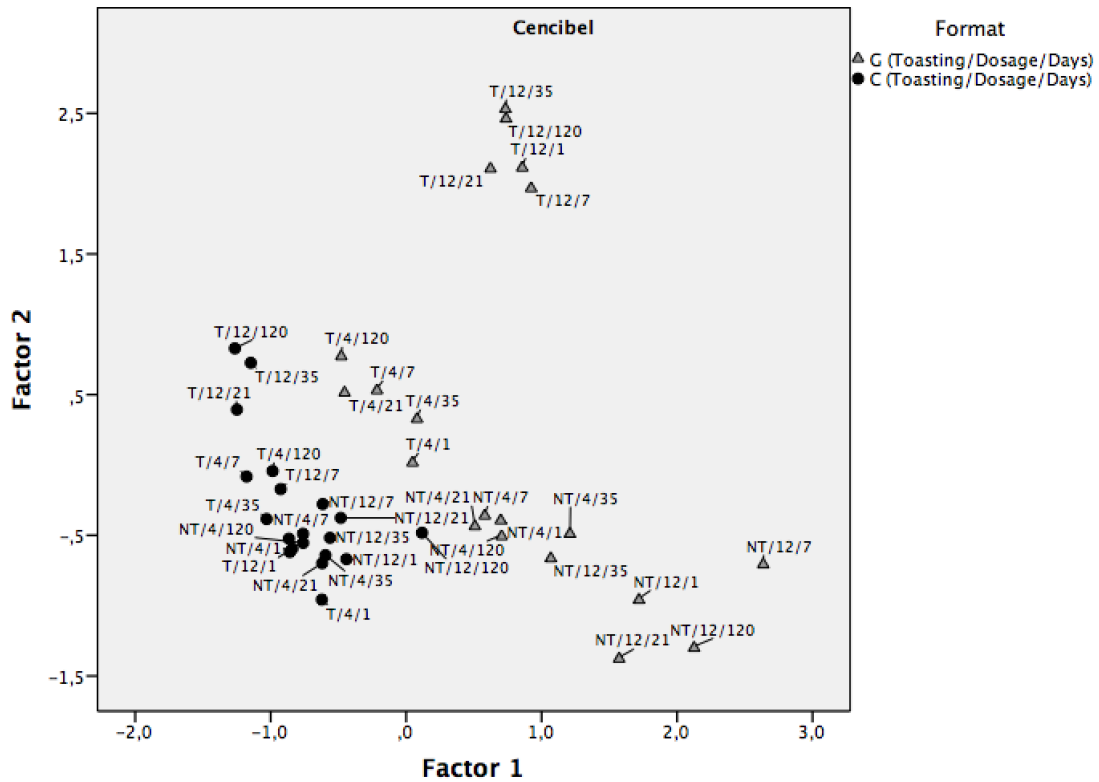712 7, 21, 35 and 120 days).

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718 **Figure 2**. Scores of the forty model wines in contact with Cencibel vine-shoot fragments

719 obtained after the application of the Factor Analysis. Format (C: *chip*s, G: *granules*);

720 toasted (T: toasted, NT: non-toasted); dosages (4: 4g/L, 12: 12g/L); time of sampling (1,

721 7, 21, 35 and 120 days).

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725 **Figure 3.** Percentages of DPPH radical inhibition (antioxidant capacity of model wines

726 in contact with 12 g/L of toasted Airén and Cencibel vine-shoots (*chip* and *granule*

727 formats) during 35 days (n=3). 100 μl of model wines.
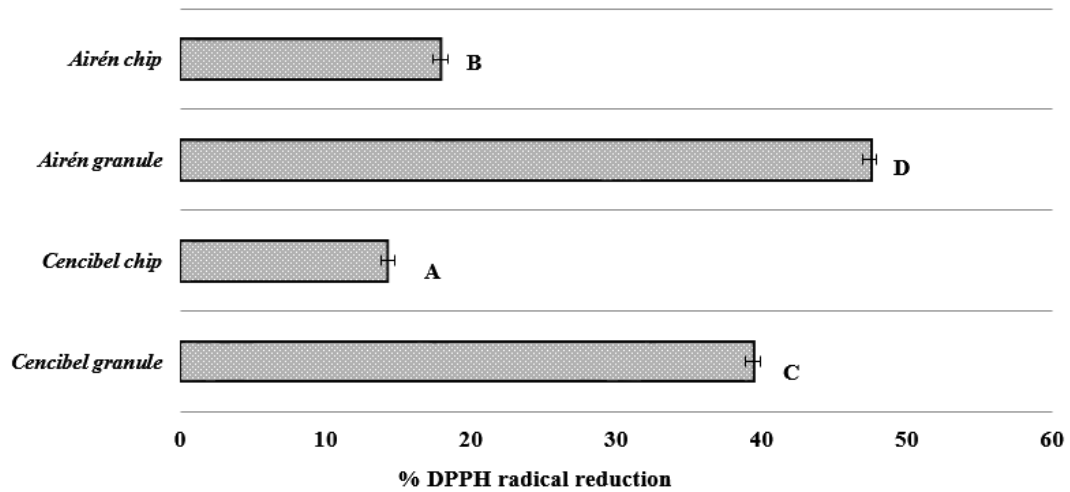
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749 **TABLES**

750 **Table 1**. Factor analysis of the model wine solutions in all of the tested conditions.

	Airén (n=40)		**Cencibel** (n=40)	
	Factor 1	*Factor 2*	*Factor 1*	*Factor 2*
% Of explained variance	51.9 %	31.9 %	81.9 %	11.4 %
trans-Whiskey lactone	0.914	0.000	0.767	0.616
cis-Whiskey lactone	0.896	-0.131	0.708	0.646
Linalool	0.891	0.097	0.950	0.218
Guaiacol	0.262	0.893	0.545	0.771
Vainillin	-0.300	0.879	0.220	0.944

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Table 2. Volatile and phenolic content (µg/L and mg/L, respectively) of model wines in contact with 12 g/L of toasted Airén and Cencibel vine-shoots (*chips* and *granules* formats) during 35 and 21 maceration days.

Volatile Compounds	Odor threshold (µg/L or mg/L)	AIRÉN Chips 21 days, 12 g/L	AIRÉN Chips 35 days, 12 g/L	AIRÉN Granules 21 days, 12 g/L	AIRÉN Granules 35 days, 12 g/L	CENCIBEL Chips 21 days, 12 g/L	CENCIBEL Chips 35 days, 12 g/L	CENCIBEL Granules 21 days, 12 g/L	CENCIBEL Granules 35 days, 12 g/L
C6 compounds (µg/L)									
1-Hexanol	8000[c]	28.17 ± 0.01 b, ε (< 0.02)	15.79 ± 0.39 a, αβ (< 0.02)	26.12 ± 4.72 b, δε (< 0.02)	21.92 ± 1.05 ab, γδ (< 0.02)	19.65 ± 1.25 B,βγ (< 0.02)	19.27 ± 1.09 B, βγ (< 0.02)	13.46 ± 1.64 A, α (< 0.02)	14.37 ± 1.80 A, αβ (< 0.02)
Total		28.17 ± 0.01 b, ε	15.79 ± 0.39 a, αβ	26.12 ± 4.72 b, δε	21.92 ± 1.05 ab, γδ	19.65 ± 1.25 B,βγ	19.27 ± 1.09 B, βγ	13.46 ± 1.64 A, α	14.37 ± 1.80 A, αβ
Esters (µg/L)									
Hexyl acetate	1500[f]	0.19 ± 0.01 b, β (< 0.02)	0.10 ± 0.00 a, α (< 0.02)	0.10 ± 0.02 a, α (< 0.02)	0.07 ± 0.01 a, α (< 0.02)	0.08 ± 0.01 A, α (< 0.02)	0.09 ± 0.02 A, α (< 0.02)	0.10 ± 0.01 A, α (< 0.02)	0.10 ± 0.01 A, α (< 0.02)
Ethyl hexanoate	14[c]	0.65 ± 0.01 b, αβγ (0.05)	0.61 ± 0.04 ab, αβ (0.04)	0.50 ± 0.06 a, α (0.04)	0.83 ± 0.05 c, βγ (0.06)	0.64 ± 0.11 B,αβγ (0.05)	0.92 ± 0.07 D, δ (0.07)	0.41 ± 0.03 A, α (0.03)	0.65 ± 0.09 C, αβγ (0.05)
Ethyl octanoate	5[c]	0.10 ± 0.01 a, αβ (0.02)	0.08 ± 0.00 a, αβ (0.02)	0.12 ± 0.05 a, αβ (0.02)	0.14 ± 0.01 a, αβ (0.03)	0.07 ± 0.01 A, αβ (< 0.02)	0.08 ± 0.00 A, αβ 0.02	0.04 ± 0.00 A, α (< 0.02)	0.27 ± 0.18 A, β (0.05)
Total		0.94 ± 0.03 b, βγδ	0.79 ± 0.04 a, αβγ	0.71 ± 0.09 a, αβ	1.04 ± 0.04 b, γδ	0.79 ± 0.12 B, αβ	1.09 ± 0.07 D, δ	0.55 ± 0.04 A, α	1.02 ± 0.18 C, γδ
Furanics (mg/L)									
Furfural	14[c]	2.63 ± 0.00 c, γ (0.26)	2.20 ± 0.013 b, β (0.16)	1.72 ± 0.42 ab, α (0.12)	1.31 ± 0.045 a, α (0.09)	1.30 ± 0.083 A, α (0.09)	1.52 ± 0.02 A, α (0.11)	1.55 ± 0.19 A, α (0.11)	1.39 ± 0.05 A, α (0.10)
5-Methylfurfural	20[b]	0.38 ± 0.00 c, ε (0.02)	0.26 ± 0.006 b, δ (< 0.02)	0.15 ± 0.04 a, αβ (< 0.02)	0.116 ± 8.99 a, α (< 0.02)	0.16 ± 0.008 A, αβγ (< 0.02)	0.18 ±0.003 AB, βγ (< 0.02)	0.18 ± 0.009 AB, βγ (< 0.02)	0.19 ± 20.91 B, βγ (< 0.02)
5- Hydroxymethylfurfural	100[b]	2.51 ± 0.00 b, β (0.04)	1.80 ± 0.14 a, α (0.02)	1.68 ± 0.30 a, α (0.02)	1.47 ± 0.09 a, α (< 0.02)	1.37 ± 0.13 A, α (< 0.02)	1.90 ± 0.19 A, α (0.02)	1.89 ± 0.25 A, α (0.02)	1.73 ± 0.25 A, α (0.02)
Total		5.52 ± 0.00 c, γ	4.25 ± 0.14 b, β	3.54 ± 0.76 ab,αβ	2.89 ± 0.14 a, α	2.83 ± 0.20 A, α	3.60 ± 0.19 B, αβ	3.62 ± 0.34 B, αβ	3.32 ± 0.20 AB, α
Lactones (µg/L)									
cis- whiskey lactone	54[h]	0.78 ± 0.01 a, β (< 0.02)	0.81 ± 0.00 b, β (< 0.02)	n.d.	n.d.	n.q.	0.27 ± 0.08 A, α (< 0.02)	3.33 ± 0.22 B, γ (0.06)	3.71 ± 0.24 B, γ (0.07)
trans- whiskey lactone	370[h]	0.69 ± 0.01 a, β (< 0.02)	1.04 ± 0.01 b, γ (< 0.02)	n.d.	n.d.	n.q.	0.39 ± 0.12 A, α (< 0.02)	4.65 ± 0.14 B, δ (< 0.02)	5.17 ± 0.08 C, ε (< 0.02)
Total		1.47 ± 0.02 β	1.84 ± 0.01 β	n.d.	n.d	n.q.	0.66 ± 0.16 A, α	7.98 ± 0.31 B, γ	8.89 ± 0.29 C, δ
Terpenoids (µg/L)									
Citronellal	*references not found*	0.62 ± 0.01 b, α	0.42 ± 0.03 a, α	0.41 ± 0.13 a, α	0.36 ± 0.02 a, α	0.48 ± 0.08 A, α	0.47 ± 0.01 A, α	0.36 ± 0.03 A, α	0.53 ± 0.23 A, α
Citronellol	100[b]	n.d.	1.89 ± 0.12 a, γ (0.02)	n.d.	n.d.	n.d.	1.20 ± 0.05 A, α (< 0.02)	n.d. (< 0.02)	1.39 ± 0.11 B, β (< 0.02)
Farnesol	1000[g]	0.69 ± 0.01 a, αβ (< 0.02)	2.25 ± 0.01 b, δ (< 0.02)	0.70 ± 0.30 a, αβ (< 0.02)	0.60 ± 0.03 a, αβ (< 0.02)	0.29 ± 0.02 A, α (< 0.02)	1.01 ± 0.09 B, β (< 0.02)	0.38 ± 0.03 A, α (< 0.02)	1.66 ± 0.40 C, γ (< 0.02)
Geranyl Acetone	60[a]	0.17 ± 0.01 a, γ (< 0.02)	0.10 ± 0.01 a, αβ (< 0.02)	0.10 ± 0.06 a, αβ (< 0.02)	0.11 ± 0.02 a, αβγ (< 0.02)	0.10 ± 0.01 B, αβ (< 0.02)	0.06 ± 0.02 A, α (< 0.02)	0.08 ± 0.01 AB, α (< 0.02)	0.15 ± 0.01 C, βγ (< 0.02)
β-ionol	*references not found*	61.34 ± 0.01 a, α	24.42 ± 0.07 a, α	138.63 ± 44.36 b, β	136.30 ± 5.11 b, β	38.95 ± 2.97 B, α	24.56 ± 2.00 A, α	110.68 ± 6.76 C, β	121.20 ± 6.69 C, β
β-ionone	0.09[e]	0.02 ± 0.01 a, α (0.20)	0.02 ± 0.01 a, α (0.20)	0.01 ± 0.00 a, α (0.15)	0.01 ± 0.00 a, α (0.15)	0.01 ± 0.00 A, α (0.15)	0.01 ± 0.00 A, α (0.15)	0.01 ± 0.00 A, α (0.15)	0.04 ± 0.00 B, β (0.43)

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Linalool	25[c]	1.67 ± 0.01 c, δ (0.07)	0.70 ± 0.05 b, γ (0.03)	0.23 ± 0.11 a, αβ (< 0.02)	0.15 ± 0.02 a, α (< 0.02)	0.33 ± 0.04 A, αβ (< 0.02)	0.43 ± 0.09 A, β (0.02)	2.22 ± 0.02 B, ε (0.09)	2.48 ± 0.15 C, ζ (0.10)
	Total	64.50 ± 0.06 a, α	29.80 ± 0.11 a, α	140.09 ± 44.96 b, β	137.54 ± 5.15 b, β	40.16 ± 3.10 A, α	27.73 ± 2.07 A, α	113.73 ± 6.71 B, β	127.46 ± 6.52 C, β
Volatile phenols (µg/L)									
4-Ethylphenol	440[e]	4.01 ± 0.01 a, α (< 0.02)	2.46 ± 0.82 a, α (< 0.02)	2.97 ± 0.01 a, α (< 0.02)	2.43 ± 0.48 a, α (< 0.02)	2.98 ± 0.45 AB, α (< 0.02)	2.60 ± 0.17 A, α (< 0.02)	3.10 ± 0.53 AB, α (< 0.02)	3.89 ± 0.12 B, α (< 0.02)
Eugenol	6[c]	0.80 ± 0.01 b, β (0.13)	0.61 ± 0.03 ab, αβ (0.10)	0.58 ± 0.17 a, αβ (0.10)	0.47 ± 0.00 a, α (0.08)	0.63 ± 0.13 A, αβ (0.10)	0.85 ± 0.07 AB, βγ (0.14)	1.13 ± 0.02 B, γ (0.19)	2.03 ± 0.19 C, δ (0.34)
Guaiacol	9.5[c]	**17.98 ± 0.01 b, β (1.89)**	**18.25 ± 0.73 b, β (1.92)**	**11.51 ± 2.00 a, α (1.21)**	**11.40 ± 0.53 a, α (1.20)**	**9.23 ± 0.78 A, α (1.00)**	**11.48 ± 2.07 A, α (1.21)**	**15.21 ± 0.67 B, β (1.60)**	**16.30 ± 1.50 B, β (1.72)**
Syringol	570[e]	14.75 ± 0.01 c, δε (0.03)	9.02 ± 1.27 a, αβ (0.02)	15.47 ± 0.00 c, ε (0.03)	11.33 ± 0.81 b, βγ (0.02)	8.01 ± 1.64 A, α (< 0.02)	9.08 ± 0.88 A, αβ (0.02)	12.51 ± 1.17 C, γδ (0.02)	12.27 ± 0.48 B, γδ (0.02)
Vanillin	60[f]	**118.77 ± 0.01 a, β (1.98)**	**104.25 ± 0.71 a, αβ (1.74)**	**164.07 ± 0.00 b, γ (2.73)**	**170.62 ± 17.47 b, γ (2.84)**	**86.54 ± 1.69 A, α (1.44)**	**95.79 ± 8.41 A, αβ (1.60)**	**227.40 ± 8.07 B, δ (3.79)**	**261.25 ±15.99 C, ε (4.35)**
4-Vinylguaiacol	40[c]	1.09 ± 0.01 a, αβ (0.03)	0.56 ± 0.05 a, α (< 0.02)	1.31 ± 1.56 a, αβ (0.03)	0.97 ± 0.11 a, αβ (0.02)	0.87 ± 0.13 A, αβ (0.02)	0.67 ± 0.30 A, α (0.02)	2.16 ± 0.26 C, β (0.05)	1.57 ± 0.14 B, αβ (0.04)
	Total	157.40 ± 0.06 a, β	135.14 ± 0.45 a, αβ	195.91 ± 5.02 b, γ	197.77 ± 16.41 b, γ	108.26 ± 1.34 ,α	120.48 ± 10.07 A, α	261.50 ± 8.32 B, λ	297.32 ± 16.88 C,δ
Others (µg/L)									
Benzaldehyde	2000[d]	8.86 ± 0.01 b, γ (< 0.02)	4.93 ± 0.10 a, α (< 0.02)	7.10 ± 2.39 ab, βγ (< 0.02)	5.48 ± 0.38 a, αβ (< 0.02)	4.98 ± 0.14 A, α (< 0.02)	5.29 ± 0.06 A, αβ (< 0.02)	6.46 ± 0.26 B, αβ (< 0.02)	6.15 ± 0.17 B, αβ (< 0.02)
Nonanal	15[f]	1.70 ± 0.01 b, β (0.11)	0.80 ±0.02 a, α (0.05)	1.11 ± 0.33 a, α (0.07)	0.79 ± 0.05 a, α (0.05)	0.94 ± 0.14 A, α (0.06)	0.84 ± 0.19 A, α (0.06)	0.96 ± 0.24 0.06 A, α (0.06)	1.18 ± 0.04 A, α (0.08)
1-Octen-3-ol	3[c]	1.43 ± 0.01 c, γ (0.48)	1.10 ± 0. 13 b, βγ (0.37)	0.41 ± 0.13 a, α (0.14)	0.85 ± 0.06 b, β (0.28)	0.71 ± 0.04 A, αβ (0.24)	0.99 ± 0.07 A, β (0.33)	0.94 ± 0.01 A, β (0.31)	0.94 ± 0.32 A, β (0.31)
	Total	11.98 ± 0.03 b, β	6.83 ± 0.21 a, α	8.62 ± 2.85 ab, α	7.12 ± 0.45 a, α	6.63 ± 0.17 A, α	7.13 ± 0.13 A, α	8.36 ± 0.41 B, α	8.26 ± 0.11 B, α
Total volatile compounds (µg/L)		5876.63±0.28b, δ	4713.64±178.57a, γ	4234.96±907.27a,βγ	3511.36±19.27a, αβ	3222.50±205.9A, α	4028.73±199.36B,αβγ	4262.31±334.0B,αβγ	4060.44±177.33B,αβγ
Phenolic compounds									
Flavanols (mg/L)									
(+) -Catechin		n.d.	n.d.	n.d.	4.09 ± 0.12 a, β	n.d.	n.d.	n.d.	1.12 ± 0.01 A, α
(-) -Epicatechin		n.d.	n.d.	n.d.	14.14 ± 0.95 a, β	n.d.	n.d.	n.d.	6.23 ± 0.21 A, α
	Total	n.d.	n.d.	n.d.	18.24 ± 0.83 a, β	n.d.	n.d.	n.d.	7.34 ± 0.21 A, α
Phenolic acids (mg/L)									
Ellagic acid		33.18 ± 0.01 b, α	33.98 ± 0.07 d, γ	33.03 ± 0.00 a, α	33.53 ± 0.02 c, β	33.22 ± 0.03 A, αβ	33.54 ± 0.01 A, β	33.33 ± 0.21A, αβ	33.53 ± 0.04 A, β
Gallic acid		0.62 ± 0.03 c, γ	0.23 ± 0.01 a, β	n.d.	0.55 ± 0.01 b, γ	n.d.	n.q	n.d.	0.68 ± 0.02 B, δ
Protocatechuic acid		0.25 ± 0.00 b, γ	0.21 ± 0.00 a, β	n.d.	0.25 ± 0.02 b γ	n.d.	0.16 ± 0.00 A, α	n.d.	0.34 ± 0.01 B, δ
	Total	34.05 ± 0.03 b, βγ	34.42 ± 0.08 c, δ	33.00 ± 0.00 a, α	34.33 ± 0.03 c, γδ	33.22 ± 0.03 A, α	33.69 ± 0.03 B, β	33.33 ± 0.21 AB,α	34.54 ± 0.06 C, δ
Stilbenes (mg/L)									
trans-Piceid		n.d.	n.d.	n.d.	0.68 ± 0.00 a, α	n.d.	n.d.	n.d.	0.71 ± 0.00 A, β
trans-Resveratrol		1.16 ± 0.03 c, γ	0.74 ± 0.00 b, β	0.64 ± 0.00 a, β	4.05 ± 0.02 d, ε	0.48 ± 0.01 A, α	0.63 ± 0.00 A, β	0.48 ± 0.00 A, α	2.64 ± 0.09 B, δ
	Total	1.16 ± 0.03 c, γ	0.74 ± 0.00 b, β	0.64 ± 0.00 a, β	4.73 ± 0.02 d, ε	0.48 ± 0.01 A, α	0.63 ± 0.00 A, β	0.48 ± 0.00 A, α	3.35 ± 0.09 B, δ
Aldehydes (mg/L)									
Coniferaldehyde		0.63 ± 0.05 b, α	n.q	n.q	n.q.	n.q	n.q	n.q.	0.73 ± 0.00 A, β
Sinapaldehyde		0.20 ± 0.03 a, α	n.q	n.q	n.q	n.q	n.q	n.q	0.40 ± 0.00 A, β
	Total	0.83 ± 0.08 b, β	n.q.	n.q.	n.q.	n.q.	n.q.	n.q.	1.13 ± 0.04 A, λ
Total phenolic compounds		36.03 ± 0.14 b, γ	35.15 ± 0.08 ab, βγ	33.64 ± 0.00a, α	57.30 ± 0.84 c, ε	33.71 ± 0.04A, α	34.32 ± 0.03B, αβ	33.81 ± 0.21A, α	46.36 ± 0.02C, δ

For each compound, different small letters indicate significant differences among all treatments carried out with Airén variety, capital letters indicate significant differences among all treatments carried out with Cencibel variety. Different Greek letters indicate significant differences among all treatments, independently of the variety. Tuckey test (α < 0.05) was used for statistical analysis. The mean values (n= 3) are shown with their standard deviation. n.d: not detected. n.q. not quantified. Mean pH of model wines: Airén *chips* 21 days (4.19), Airén *chips* 35 days (3.96), Airén *granule* 21 days (4.22), Airén *granule* 35 days (4.05), Cencibel *chips* 21 days (4.04), Cencibel *chips* 35 days (3.80), Cencibel *granule* 21 days (4.42), Cencibel *granule* 35 days (4.28).

a) (Guth, 1997)

b) (Etiévant, 1991)
c) (Ferreira, López, & Cacho, 2000)
d) (Khairallah, Reynolds, & Bowen, 2016)
e) (López, Aznar, Cacho, & Ferreira, 2002)
f) (Culleré, Escudero, Cacho, & Ferreira, 2004)
g) (Franco, Peinado, Medina, & Moreno, 2004)
h) (Boidron, Chatonnet, & Pons, 1988)

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