Effect of the presence of different oak ellagitannins in their own disappearance under oxidative or inert atmosphere

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Abstract

The disappearance of the *C*-glycosidic ellagitannins over time can occur even in absence of oxygen and their disappearance rate seems to be affected by the presence of other ellagitannins in the media. The objective of this work was to study the influence of the presence of other ellagitannins and/or oxygen on the individual evolution of the main oak ellagitannins in simple model systems in order to understand their behaviours in more complex media, such as wine. In all the studied conditions, vescalagin disappeared faster than castalagin, highlighting its greater reactivity. Oxygen increased the individual disappearance rate, as also occurred when more than one type of ellagitannin was present, above all if the additional ellagitannin(s) contained the same conformation in C1 as vescalagin. Experimental data were fitted to a kinetic model considering both the oxygen-dependent and oxygen-independent reactions, making possible the comparison between individual compounds in different scenarios.

Keywords.

Oak ellagitannins, vescalagin, castalagin, HPLC-MS-MRM, oxygen, kinetic model, oxidation reactions.

1. Introduction

Wines are aged in oak wooden barrels in order to improve their overall quality. Interactions between the wine, the wood and the surrounding air can take place inside the barrel (Feuillat. Perrin & Keller, 1994; Ribéreau-Gayon, Glories, Maujean, & Dubourdieu, 2000). Wine can first interact with the wood of the barrel by extracting wood compounds that will integrate, from that moment onwards, into wine composition. On the other hand, wine constituents can be adsorbed onto the wood surface, also causing a change in the initial wine composition. In addition, oxygen can penetrate inside the barrel and interact with all these compounds present in the wine, thus increasing wine complexity. Vivas and Glories (1997) estimated that 63% of the oxygen penetrates via the gaps between staves and only 16% passes through the wood. Recently, Nevares, Crespo, González, & del Álamo-Sanza (2014) were able to visualise the diffusion of oxygen through oak wood, demonstrating the permeability of wood to oxygen and the influence of the moisture content of the wood on the oxygen transmission rate (OTR) through the wood. In addition, when wood enters in contact with wine, the oxygen that is trapped in the void spaces of the wood is released to the wine and it has been reported that this oxygen can be very relevant during the first moments of contact between the wood and wine (García-Estévez et al., 2017a).

Molecular oxygen is a weak oxidant and it is assumed that the oxidation reactions do not directly involve oxygen, but certain substances that can fix oxygen and then act as oxidants (Ribéreau-Gayon et al., 2000). *C*-glycosidic ellagitannins are among the compounds that can be extracted from the oak wood during barrel ageing that could take part in these oxidative reactions (Ribéreau-Gayon et al., 2000). In fact, among the phenolic compounds released from wood, ellagitannins can be considered as the main oxygen consumers (García-Estévez et al., 2017a). Their levels are conditioned by the levels of oxygen (García-Estévez et al., 2017a), which, in turn, can affect wine organoleptic properties. Astringency and colour are among the wine organoleptic properties that can be modulated by ellagitannins. These properties can be affected, either directly, due to their ability to precipitate

58 salivary proteins (Glabasnia & Hofmann, 2006) or to form coloured anthocyano-ellagitannin hybrids
59 (Chassaing et al., 2010; García-Estévez et al., 2013), or indirectly, by favouring the interaction
60 between different wine constituents responsible for these organoleptic properties (Vivas & Glories,
61 1996). For instance, ellagitannins seem to promote the acetaldehyde-mediated condensation reactions
62 between flavanols or between flavanols and anthocyanins. Consequently, the flavanol and/or pigment
63 compositions would be modified, also causing changes in wine astringency and/or colour.
64 The levels of ellagitannins in wine first depend on the levels on the wood. They can constitute up to
65 10% of the dry weight of oak heartwood (Puech, Feuillat, Mosedale, & Puech, 1996), although several
66 factors, such as the botanical origin of the wood, can determine their contents on wood (Scalbert,
67 Monties, & Favre, 1988; Masson, Moutounet & Puech, 1995). Consequently, the type of oak barrel
68 employed for ageing will influence the levels in wine (García-Estévez, Escribano-Bailón, Rivas-
69 Gonzalo & Alcalde-Eon, 2017b). The second important factor conditioning the levels of ellagitannins
70 in wine is the extractability, which refers to the ability of the compounds to be released from the wood
71 to the wine. Both wood content and extractability can be affected by cooperage. Thus, the technique
72 employed to manufacture the stave and the type and length of the drying and toasting processes
73 (Masson et al., 1995; Cadahía et al., 2001) will also condition the amounts of ellagitannins released
74 from the wood to the wine. Recent studies have reported that they can represent circa 80% of the
75 extractable phenolic compounds from French oak wood (García-Estévez et al., 2017a). However,
76 once extracted to the wine the levels do not remain stable. In the different studies that have evaluated
77 the evolution of the ellagitannin levels in different barrel-aged wines (Jourdes, Lefeuvre & Quideau,
78 2009; García-Estévez et al., 2017b) it was observed that the levels increased and reached a maximum
79 content after three months of stay in the barrel and then, they start decreasing. This evolution is the
80 consequence of the co-existence of two processes, the ellagitannin extraction from the wood and the
81 ellagitannin disappearance, with predominance of the extraction process during the first 3 months and
82 predominance of disappearance from then onwards. Due to the simultaneous occurrence of both

83 phenomena, it is difficult to study each separately during barrel ageing. The use of model systems
84 can be an adequate approach to carry out this type of studies, as it was done for studying the extraction
85 process (García-Estévez, Alcalde-Eon, Le Grottaglie, Rivas-Gonzalo, & Escribano-Bailón, 2015).
86 From that study, it was observed that two main steps compose the ellagitannin extraction and that the
87 entire process can be adjusted to a kinetic model. Regarding the disappearance process, a recent study
88 also carried out in model systems containing oak chips (García-Estévez et al., 2017a) with different
89 oxygen levels has exposed the complexity of the process. That study first confirmed the existence of
90 the interaction between oxygen and ellagitannins, which was assessed from the relationship between
91 the reductions of their levels. This interaction with oxygen is mainly due to their structure with many
92 hydroxyl groups in *ortho* position (Vivas & Glories, 1996). Another relevant finding of that study
93 (García-Estévez et al., 2017a) was that in the absence of oxygen, the decrease in the levels of
94 ellagitannins also took place. In addition, it was observed that these oxygen-independent reactions
95 were also present in oxygen containing media. A kinetic model that correctly fitted the experimental
96 data in all the scenarios (absence or presence of oxygen at different levels) was proposed to study the
97 evolution of the ellagitannin levels. According to the model, three processes occurring simultaneously
98 govern the levels: the extraction from the wood, the interaction with oxygen and the participation in
99 reactions independent of oxygen. Thus, in the absence of oxygen, the decrease in the levels of the
100 ellagitannins extracted from the oak wood were exclusively due to oxygen-independent reactions,
101 whereas in the presence of oxygen, the decrease in the levels was due to both oxygen-dependent and
102 –independent reactions, explaining the faster decreasing rates. Furthermore, it was observed that in
103 the presence of oxygen, the contribution of oxygen-independent reactions also increased, pointing to
104 a possible indirect contribution of oxygen to this type of reactions. Taking into account this
105 complexity and the coexistence of the extraction process, simpler model systems are needed to study
106 in detail the ellagitannin disappearance. In the present work, different model systems containing pure
107 ellagitannins in the absence and in the presence of oxygen were prepared and the concentrations

determined by HPLC-MS^n-MRM. Considering the differences in the behaviour reported for the main oak ellagitannins (García-Estévez et al., 2017a), model systems containing one single ellagitannin were prepared to evaluate their disappearance in the absence and presence of oxygen. Moreover, model systems containing a mixture of the main oak ellagitannins were also prepared to study how the evolutions of the different ellagitannins were modified by the coexistence of other ellagitannins in the model system. These experiments will be helpful to understand the behaviours of oak ellagitannins in more complex media, such as wine and therefore, to estimate the possible impact of the barrels (based on their ellagitannin composition) on the wine reactivity, which would directly affect important wine organoleptic properties, like colour and astringency and, consequently, wine quality.

2. Materials and Methods

2.1. Oak wood ellagitannins

Oak ellagitannins were extracted and purified from *Quercus petraea* (Matt.) Liebl. wood as previously described (García-Estévez, Escribano-Bailón, Rivas-Gonzalo, & Alcalde-Eon, 2010).

2.2. Samples

Four types of model solutions containing i) castalagin (**C**), ii) vescalagin (**V**), iii) an equimolar mixture of castalagin and vescalagin (**M**), or iv) a mixture of the four main oak ellagitannins (castalagin, vescalagin, grandinin and roburin E (**El**)) were prepared in triplicate under both inert (N_2) or oxidative (air saturated) atmosphere. Each model solution contained a total ellagitannin concentration of 40 mg/L, which is in the order of the concentrations that can be reached in wine aged in oak barrels at the moment of maximum ellagitannin concentration (García-Estévez et al., 2017b). In **El** model solutions, both castalagin and vescalagin represented each one *ca*. 40% of total ellagitannin whereas grandinin and roburin represented each one *ca.* 10% of total ellagitannin content. The model solutions were prepared by dissolving the ellagitannin(s) in ultrapure water in amber glass

133 vials hermetically closed with butyl rubber stoppers and open centre crimp seals. The inert and
134 oxidative atmospheres were maintained during all the experiment by periodically sparging either
135 Nitrogen or compressed air, respectively, into the different solutions through the septum by means of
136 a syringe (needle size: 0.8×40 mm) coupled to the gas dispensers. A second syringe needle was placed
137 through the septum to allow the gas exchange (exit of the displaced gases out from the vial). Model
138 systems were kept in darkness and at controlled temperature (23ºC) and were periodically sampled
139 (days 0, 2, 6, 9, 12 and 31) through the septum with a syringe (needle size: 0.5×16 mm) until
140 ellagitannin disappearance. (-) Gallocatechin (Sigma-Aldrich; St. Louis, MO) was added to the
141 samples as internal standard (final concentration of 0.015 mg/mL) and then, the samples were filtered
142 (0.45 μm hydrophilic PVDF Clarinert Syring Filters, Agela Technologies, Wilmington, DE, USA)
143 prior to the HPLC-MS^n-MRM analyses.

144 **2.3. HPLC-MS^n-MRM analyses**

145 Ellagitannins were analysed by HPLC-MS^n-MRM with the methodology previously developed and
146 validated in our laboratory (García-Estévez, Escribano-Bailón, Rivas-Gonzalo & Alcalde-Eon,
147 2012). Samples were injected in a Hewlett-Packard 1200 series LC (Agilent Technologies,
148 Waldbronn, Germany) using an AQUA C-18 reversed-phase, 5 mm, 150 mm × 4.6 mm column
149 (Phenomenex®, Torrance, CA, USA) thermostatted at 35ºC. An aqueous solution (2.5%) of acetic
150 acid (AnalaR, Normapur, VWR International, Fontenay-sous-Bois, France), 100% HPLC-grade
151 isopropanol (HiPerSolv® Chromanorm, VWR International, Fontenay-sous-Bois, France) and 100%
152 HPLC-grade methanol (Macron Fine Chemicals, Avantor, Gliwice, Poland) were used as eluents
153 following the gradient previously reported (García-Estévez et al., 2010). The mass spectrometer was
154 connected to the HPLC system via the DAD cell outlet. MS detection was performed in negative
155 mode with an API 3200 Qtrap equipped with an ESI source and a triple-quadrupole linear ion trap
156 mass analyser controlled by Analyst 5.1 software (Applied Biosystems, Darmstadt, Germany).
157 Previously validated mass conditions (García-Estévez et al., 2012) were employed for the qualitative

158 and quantitative analyses of the oak ellagitannins. MRM analyses allowed the detection of the
159 targeted transitions for each ellagitannins (933/631 for castalagin, 933/301 for vescalagin and
160 1065/249 for grandinin and roburin E) and for the internal standard (305/125). The signals of those
161 transitions were used for quantification following the previously developed methodology (García-
162 Estévez et al., 2012).
163 In order to make possible the comparison between the disappearance process of a same ellagitannin
164 in the different model systems (isolated, equimolar mixture with a second ellagitannin or mixed with
165 the other three ellagitannins), the proportion of the initial content remaining at each sampling point
166 was calculated.

167 **2.4. Statistical analyses**

168 The IBM-SPSS Statistics 23 for Windows software package (Armonk, NY, USA) was used to
169 perform the statistical analysis. The percentages of a same ellagitannin in the absence and in the
170 presence of another(s) ellagitannins at each sampling point were analysed by means of one-way
171 analysis of variance (ANOVA) and Tukey's honestly significant difference test to assess the
172 significance of the differences observed among samples ($p < 0.05$).

173 **2.5. Data modelling**

174 Microsoft Excel 2013 (Redmond, WA, USA) and SOLVER function were used for regression
175 analysis. Experimental data were fitted to a model comprising two different processes following a
176 first-order kinetic each one, as described by the following equation (eq. 1):

177 $$C_{ellag} = C_o - C_i*(1-e^{-Ki*t})-C_d*(1-e^{-Kd*t})$$

178 In this equation, C_{ellag} is the ellagitannin concentration at each time, C_o is the theoretical initial
179 ellagitannins concentration (mg/L), C_i and C_d are the theoretical ellagitannins concentrations that
180 disappear due to the reaction not directly (oxygen-independent) and directly (oxygen-dependent)
181 related to oxygen, respectively; k_i and k_d (day^{-1}) are the kinetic constants of each one of these two
182 process and t is the time of storage (day).

183 Fitting was done by nonlinear regression, minimizing the squared errors by using an iteration protocol
184 based on the robust and reliable generalized reduced gradient (GRG) method. The goodness of fit of
185 the models was assessed using R^2_{adj}. The behaviours of the different ellagitannins in the different
186 scenarios tested in the present study were then evaluated from the comparison of the relevance of
187 each of the two considered processes and from comparison of the constants of each process for each
188 ellagitannin.
189
190 **3. Results and discussion**
191 Figure 1 shows the evolution of the percentage of the initial ellagitannin content over time for
192 castalagin (a and c) and vescalagin (b and d) in inert atmosphere ("N_2": a and b) and in the presence
193 of oxygen ("O_2": c and d). Furthermore, the influence of the presence of additional ellagitannin(s) on
194 the individual evolution in the different scenarios was also studied (blue line: a single ellagitannin;
195 orange line: equimolar mixture of castalagin and vescalagin; grey line: a mixture of the four main oak
196 ellagitannins).
197 A disappearance of the ellagitannins over time was observed in all the model systems, even in those
198 without oxygen, which pointed out to the existence of reactions not directly related to oxygen that
199 can cause ellagitannin disappearance. However, as it was expected, the rates were lower in the model
200 systems that did not contain oxygen than in those containing oxygen. In fact, whereas in the absence
201 of oxygen the complete ellagitannin disappearance took about 30 days, in the presence of oxygen it
202 took less than 12 days. In the latter ones, the disappearance can be related, as it will be explained
203 bellow, to two different types of reactions: oxygen-independent and oxygen-dependent reactions.
204 *3.1. Inert model systems*
205 The differences on the behaviour of castalagin and vescalagin in what respects the oxygen-
206 independent reactions could be assessed from their evolutions in inert model systems (Fig. 1a and
207 1b). In these inert model systems containing only one compound (blue line), vescalagin was the

208 ellagitannin whose disappearance was faster. For instance, at day 2, whereas 86% of the initial content
209 still remained in the model system containing castalagin, almost 30% of the initial content was lost
210 in the case of vescalagin. Similarly, at the end of the study, only 2% of the initial content of vescalagin
211 remained in its model system, but that containing castalagin showed more than 6% of the initial
212 content. These results are in accordance with the behaviours reported in model solutions containing
213 oak chips (García-Estévez et al., 2017a) and with the greater reactivity previously reported for
214 vescalagin than for castalagin (Viriot, Scalbert, Hervé du Penhoat, & Moutounet, 1994; Vivas,
215 Laguerre, Pianet de Boissel, Vivas de Gaulejac, & Nonier, 2004; Quideau et al., 2005; Jourdes et al.,
216 2009). According to Vivas and co-workers (2004), this greater reactivity of vescalagin could be
217 explained from the differences observed in the electronic distribution over the nonahydroxytriphenic
218 area of vescalagin and castalagin. The faster disappearance of vescalagin in relation to castalagin was
219 also reported in 40 and 70% ethanol-water (*v/v*) model systems containing a single purified
220 ellagitannin (Puech, Mertz, Michon, Le Guernevé, Doco, & Hervé du Penhoat, 1999). However, some
221 of the reactions taking part in those model systems (such as the formation of derivatives of castalagin
222 and vescalagin after reaction with ethanol) cannot occur in the model systems of the present study
223 considered in this section since in the present study the ellagitannins were dissolved in ultrapure water
224 and were kept in inert atmosphere. On the contrary, hydrolysis reactions of castalagin and vescalagin
225 leading to castalin and vescalin respectively could occur and bearing in mind their different electronic
226 distributions (Vivas et al., 2004), a greater trend of vescalgin to hydrolyse to vescalin than castalagin
227 to castalin seems also reasonable, which would partly explain the faster disappearance of vescalagin
228 than that of castalagin. Despite the theoretical lower reactivity of the solution employed in the present
229 study, the evolution observed for vescalagin was very similar to those reported by Puech and co-
230 workers (1999) and was even faster for castalagin in the present study. This means that ellagitannins
231 in solution are quite reactive, even in the absence of ethanol or oxygen.

232 In the model systems containing a mixture of ellagitannins (**M** and **El** model systems, orange and
233 grey line, respectively), the levels of castalagin and vescalagin underwent a greater initial decrease
234 than when they were alone in the solutions. At day 2, castalagin and vescalagin decreased about 20%
235 more in the model systems containing mixtures. The decrease seemed to be greater as the number of
236 additional ellagitannins increased, although in some cases differences between these model systems
237 (**M** *vs* **El** model systems) were not significant. In the model systems containing an equimolar mixture
238 of castalagin and vescalagin (**M** model systems; Fig. 1a and 1b, orange line), it could be observed
239 that the presence of vescalagin had a greater impact on the levels of castalagin than the presence of
240 castalagin on the evolution of vescalagin, highlighting again the greater reactivity of vescalagin. Thus,
241 whereas the presence of castalagin only caused an additional loss of 4% of vescalagin from day 6
242 onwards, the presence of vescalagin caused an additional decrease of 16% of castalagin.
243 In the model systems containing the four ellagitannins (**El** model systems, Fig. 1a and 1b, grey line),
244 the additional decrease caused by the additional presence of roburin E and grandinin was more
245 noticeable in the evolution of vescalagin than in the case of castalagin. In the case of the evolution of
246 castalagin, the differences between **M** and **El** model systems were not significant. In the case of
247 vescalagin, differences were significant. This pointed to a higher reactivity of grandinin and roburin
248 E in relation to castalagin.
249 With regard to grandinin and roburin E, their behaviours (Fig. 2) were more similar to that showed
250 by vescalagin than by castalagin, which might be related to the fact that both grandinin and roburin
251 E have the same *C*-1 conformation than vescalagin, which has been pointed out as the main reason
252 for the higher reactivity of vescalagin (Vivas et al., 2004; Quideau et al., 2005). Taking into account
253 the results obtained in these model systems with inert atmosphere, it can be concluded that the
254 disappearance of ellagitannins can take place even in absence of oxygen and ethanol. Furthermore, it
255 has been demonstrated that the rate of this disappearance can be increased by the presence of other
256 ellagitannins, above all if they have a β-orientation in C1.

257 *3.2. Oxidative model systems*

258 As previously indicated, in the air-saturated model systems the decreases observed for castalagin and
259 vescalagin were faster than in the model systems prepared under inert atmosphere. This was in
260 accordance with the results obtained in model systems containing oak chips (García-Estévez et al.,
261 2017a) and it is related to the ability of ellagitannins to interact with oxygen as a consequence of their
262 structures, with many hydroxyl groups in *ortho* position (Vivas & Glories, 1996). *Ortho*-diphenols
263 can react with oxygen, yielding H_2O_2 and *ortho*-quinones at the end of the cascade (Cheynier,
264 Atanasova, Fulcrand, Mazauric, & Moutounet, 2002). These *ortho*-quinones can, in turn, act as
265 powerful oxidants and electrophilic species, being readily reduced through oxidation of lower redox
266 potential molecules (Cheynier et al, 2002). Thus, in the case of ellagitannins under oxidative
267 atmosphere, their disappearance can also be due to the reactions with oxygen, through the formation
268 of transient *ortho*-quinones. These *ortho*-quinones can take part in the reactions with other molecules
269 of ellagitannins, contributing to their disappearance, or they can even react with the hydrolysis
270 products, since they still have in their structure hydroxyls in *ortho* position. In fact, a higher
271 oxidisability has been reported for these hydrolysis products (vescalin and castalin) in relation to their
272 parents (vescalagin and castalagin) (Vivas et al., 2004). Furthermore, it has been recently reported
273 that the presence of oxygen seems to favour the hydrolysis reactions of the ellagitannins, as higher
274 levels of the hydrolysis products were observed in the model systems containing higher oxygen levels
275 (García-Estévez et al., 2017a). The combination of the hydrolysis and oxidation reactions could,
276 therefore, increase the disappearance rate of the ellagitannins, through the increase of the number of
277 possible chain reactions. Moreover, the oxidation of the phenols is much easier from the phenolate
278 anion than from the unionised phenol, being then, faster at higher pH values (Cheynier et al, 2002).
279 Considering the predicted pK_a (4.07 ± 0.40) reported for vescalagin (Donno, Mellano, Prgomet,
280 Cerutti, & Beccario, 2017) and the pH of the model systems (pH=5.5) of the present study, the

281 existence of ellagitannins in phenolate forms and consequently, the formation of transient *ortho*-
282 quinones might be possible.
283 At day 2, the solutions containing one single ellagitannin under oxidative conditions (Fig. 1c and 1d,
284 blue line) showed more important decreases than under inert atmosphere. To be precise, at day 2,
285 castalagin and vescalagin levels decreased 32% and 42% more, respectively, under oxidant
286 atmosphere compared to the decrease observed under inert atmosphere. From these results, it seems
287 that vescalagin is initially more affected than castalagin by the presence of oxygen in the solution,
288 thus pointing out to a higher trend of vescalagin to be oxidized. This higher oxidisability of vescalagin
289 in solution was in accordance with the results reported by Vivas and co-workers (2004), which also
290 demonstrated higher thermodegradability and higher polarity for vescalagin than for castalagin. The
291 differences on the orientation of the OH group of *C*-1 of the glucose between vescalagin (exo-
292 positioned; β-oriented) and castalagin (endo-positioned; α-oriented) (Jourdes et al., 2009) might
293 explain the different reactivity towards oxygen.
294 Under oxidative atmosphere, the presence of additional ellagitannins also increased the rate of
295 disappearance (**M** and **El** model systems, Fig. 1c and 1d, orange and grey line, respectively).
296 However, in this case, the effect was less noticeable than under inert atmosphere, probably due to the
297 participation of all the ellagitannins in the interactions with oxygen. In the model systems containing
298 only one additional ellagitannin (**M** model systems), castalagin, once again, was more affected than
299 vescalagin by the presence of the other ellagitannin (vescalagin or castalagin, respectively) in the
300 solution. In the model systems containing a mixture of the four ellagitannins (**El** model systems),
301 vescalagin seemed to be more affected than castalagin by the additional presence of grandinin and
302 roburin E, similarly to what it was observed in the solutions under inert atmosphere. Thus, it seems
303 that the ellagitannins showing the same conformation as vescalagin in C-1 of glucose (i.e. grandinin
304 and roburin E, which show a β-oriented substituent in C-1) have a higher ability to affect the stability
305 of the other ellagitannins present in the solution.

306 Regarding the evolutions of grandinin and roburin E levels under oxidative atmosphere (Fig. 2) in **El**
307 model systems, it could be observed that oxygen also caused a faster and greater decrease than that
308 observed in inert atmosphere. Furthermore, as occurred under inert atmosphere, their evolutions were
309 more similar to that of vescalagin than to that of castalagin. Thus, at day 2, only about 15% of the
310 initial content of grandinin and roburin E remained in the solution, which is almost the same observed
311 for vescalagin (Fig. 1). On the contrary, in the same model system at the same day the content of
312 castalagin almost reached 35% of the initial content.

313 *3.3. Kinetic model*

314 In a previous study carried out in model systems containing oak chips (García-Estévez et al., 2017a)
315 it was possible to fit the evolution of the ellagitannin to a kinetic model that comprised three terms,
316 corresponding respectively to the extraction process, to the disappearance due to the oxygen-
317 independent reactions and to the disappearance due to the oxygen-dependent reactions. Taking into
318 account that the model systems employed in the present study were simpler and that the extraction
319 process was not taking place, a kinetic model that only comprised the two terms related to
320 disappearance reactions was built from the experimental data to assess the disappearance rates of the
321 different ellagitannins studied in the different scenarios considered in the present work. Thus, the
322 model described by the equation 1 was used to fit the experimental data. Table 1 shows the values of
323 the theoretical maximum concentration, constants and rates of both, the oxygen-dependent and
324 oxygen-independent processes for each ellagitannin in each model system. From the observation of
325 these values in the model systems containing a single ellagitannin (**C** or **V**) in inert atmosphere, it can
326 be concluded that oxygen independent reactions were much faster for vescalagin than for castalagin
327 (mean k_i= 0.174 day^{-1} and 0.089 day^{-1}, respectively) which is in accordance, as previously
328 commented, with the higher reactivity described for vescalagin. In air-saturated atmosphere,
329 disappearance reactions involving oxygen were also present and this type of reactions was always
330 faster than oxygen-independent ones for each ellagitannin. Vescalagin showed again higher rates than

castalagin for both types of reactions. Differences between castalagin and vescalagin could also be observed in what respects the amount of ellagitannin that is involved in each process. In the case of castalagin, most of the initial content (*circa* 70%) would disappear as a consequence of the oxygen-dependent reactions whereas in the case of vescalagin these reactions would only be responsible for 41% of the decreases, being the oxygen-independent reactions the predominant ones. However, it is important to remark that, as indicated above, oxygen caused initially a greater decrease in the levels of vescalagin than in those of castalagin. Thus, according to the reactivity of vescalagin and castalagin, oxygen could initially oxidise vescalagin faster than castalagin originating earlier the oxidised products, which, in turn, would take part in the oxygen-independent reactions earlier. Consequently, the oxygen-independent reactions would be more favoured in the model systems containing vescalagin than in those containing castalagin. Furthermore, this fact points to a possible indirect effect of oxygen in the oxygen-independent reactions, by the formation of oxidised ellagitannins that would also participate in the disappearance of the native ellagitannins. This indirect role of oxygen in the oxygen-independent reactions would also explain why in the presence of oxygen, the rates of the oxygen-independent reactions were increased in relation to the rates observed in inert atmosphere (Table 1).

Respecting the model systems containing an equimolar mixture of both ellagitannins (**M**), it could be first observed that in general, the presence of an additional ellagitannin increased the rates of disappearance (Table 1). In the model systems with inert atmosphere, the rate of disappearance of castalagin in the presence of vescalagin increased 1.6-fold in relation to the rate observed for castalagin when it was alone, whereas that of vescalagin in the presence of castalagin only increased 1.2-fold. This fact highlights again the greater reactivity of vescalagin (or the products resulting from its own disappearance) in relation to castalagin. Under oxidative atmosphere, the rates of the oxidative reactions (k_d) increased in relation to those obtained for the model systems with one single ellagitannin. In the case of vescalagin, the percentage of the initial content whose decrease can be

356 attributed to oxygen-dependent reactions increased in the presence of castalagin. On the contrary, the
357 presence of vescalagin in the model system containing castalagin increased the relevance of the
358 oxygen-independent reactions and decreased that of the oxygen-dependent ones. This fact
359 corroborates again the different behaviour of castalagin and vescalagin
360 In the model systems containing a mixture of the four ellagitannins (**El**) under inert atmosphere, the
361 disappearance rates increased again for both ellagitannins in relation to those observed in **M** model
362 systems (Table 1). It has to be noted that the other two additional ellagitannins in **El** model systems,
363 grandinin and roburin E, have the same conformation of the C-1 of the glucose than vescalagin, which
364 seems to condition their behaviours (García-Estévez et al., 2017a). Oxygen-independent reactions
365 were faster for these two ellagitannins (grandinin and roburin E, Table 1) than for the other two
366 (castalagin and vescalagin), which, in turn, could have promote a faster establishment of this type of
367 reactions in the latter ones. In the presence of oxygen, the rates of the oxygen independent reactions
368 increased in relation to those observed in **M** model systems for castalagin and vescalagin, which can
369 probably be due to the great participation of the additional ellagitannins in **El** model systems in
370 oxygen-dependent reactions. This higher participation of grandinin and roburin E in oxygen-
371 dependent reactions was previously observed in model systems containing oak chips instead of the
372 isolated compounds (García-Estévez et al., 2017a). Thus, the oxidised products of grandinin and
373 roburin E might boost the oxygen-independent reactions of castalagin and vescalagin. On the
374 contrary, in the presence of these two additional ellagitannins, the rates of disappearance of castalagin
375 and vescalagin due to the oxygen-dependent reactions were reduced, probably due to the greater
376 consumption of oxygen by grandinin and roburin E.
377 Figure 3 shows, for castalagin and vescalagin in each model solution under oxidative atmosphere, the
378 percentage of the loss that is due to oxygen-dependent (orange) and oxygen–independent (blue)
379 processes at each moment of the evolution studied in the present work calculated from the kinetic
380 model. It can be seen that oxygen-dependent reactions are more important at the initial steps, but, as

time goes by, oxygen-independent reactions get more and more relevance, and at the end of the study, all the ellagitannin disappearance can be attributed to them. This supports the mechanism proposed for the disappearance of the ellagitannins in the presence of oxygen: during the first moments, oxygen can be the main starter of the disappearance of the ellagitannins through the formation of *ortho*-quinones. Simultaneously, oxygen might promote hydrolysis reactions of the ellagitannins, releasing hydrolysis products which are more oxidisable than their parents. As a result, the solution starts containing compounds that can initiate by themselves chain oxidation reactions, thus amplifying the initial effect of oxygen. At the end of the study, most of the ellagitannins are involved in reactions independent of oxygen. In general, oxygen-dependent reactions were more important in the evolution of castalagin than in that of vescalagin and were still present at the end of the study. On the contrary, in the case of vescalagin, oxygen-independent reactions governed its disappearance from day 9 to the end of the study. Vescalagin is initially more sensitive to oxygen, but once the reaction products are formed, the relevance of oxygen on its disappearance is reduced. This fact points to a lower reactivity of the compounds resulting from the oxidation of castalagin in relation to those of vescalagin, which is in accordance with the results observed in **M** model systems: vescalagin was less affected by the presence of castalagin than castalagin by the presence of vescalagin.

The results of the present study carried out in model systems highlight the relevance of the ellagitannin composition and extractability from the oak barrels employed during wine ageing on oxidative reactions taking place in wine. Thus, a greater involvement of ellagitannins in these reactions would be expected as the proportions of vescalagin, grandinin or roburin E increase. As a result, wine polyphenols might be more protected against oxidation and some reactions leading to new derivative polyphenols might be favoured (Vivas & Glories, 1996), which could affect the organoleptic properties of wines. Further research has to be done to assess the evolution of different types of polyphenols in the presence of different mixtures of ellagitannins.

4. Conclusions

The results of the present study have demonstrated that the main oak *C*-glycosidic ellagitannins are quite reactive in solution, even in aqueous medium and in inert atmosphere, disappearing in less than 30 days at the concentrations usually determined in wine. Under oxidative conditions the disappearing rate was increased, pointing out to a participation of oxygen in the transformation of the native ellagitannins into *ortho*-quinones, which, in turn, might start new reaction chains and promote the transformation of other ellagitannins. In the present study, it has been possible to adjust the experimental data to a kinetic model that comprises two terms, one corresponding to the reactions that occur in the absence of oxygen and the other corresponding to the reactions directly related to oxygen. The comparison of the parameters supplied by the kinetic model for each scenario has allowed the comparison between the behaviours of the main oak ellagitannin in the different conditions. In the model systems containing a single ellagitannin, the higher reactivity of vescalagin in relation to castalagin has been demonstrated. In the model systems containing more than one type of ellagitannin, a higher disappearance of the individual ellagitannins was observed, demonstrating the participation of the additional ellagitannins in the disappearance reactions of the others. Furthermore, a greater impact of the ellagitannins possessing the conformation at C1 similar to vescalagin on the ellagitannin disappearance was also observed, highlighting again the relevance of the conformation of the substituent at C1 of the glucose moiety in what respects reactivity.

Conflicts of interest

There are no conflicts to declare

Founding source

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Figure captions

Figure 1. Evolution of the percentages over the initial content for castalagin (a and c) and vescalagin (b and d) under inert (a and b) and oxidative atmosphere (c and d) in the different model systems (blue line: model systems containing a single ellagitannin; orange line: model systems containing an equimolar mixture of castalagin and vescalagin (**M**); grey line: model systems containing a mixture of castalagin, vescalagin, grandinin and roburin E (**El**)).

Figure 2. Evolution of the percentages over the initial content for grandinin (G) and roburin E (R) under inert (N_2) and oxidative atmosphere (O_2) in **El** model systems.

Figure 3. Proportion of the losses of castalagin (a) and vescalagin (b) that can be attributed to oxygen-dependent (orange) and oxygen-independent (blue) reactions at the different moments considered in the present study in the different model systems (**C** or **V**: castalagin or vescalagin alone; **M**: equimolar mixture of castalagin and vescalagin: **El**: mixture of castalagin, vescalagin, grandinin and roburin E).

518 **Table 1**. Values of theoretical maximum concentrations and of the constants of each process of the
519 kinetic model described by de equation 1 for each model solutions studied.

520

	Type of medium*	C_o (mg/L)	C_i(mg/L)	k_i (day^{-1})	C_d(mg/L)	k_d(day^{-1})
Castalagin						
Ellagitannin isolated (**C**)	N_2	39.1±0.8	39.0±0.9	0.089±0.003	-	-
	O_2	38.4±0.8	12±1	0.18±0.01	26.7±0.7	0.37±0.04
C+V mixture (**M**)	N_2	19.1±0.3	19.0±0.5	0.14±0.01	-	-
	O_2	19.2±0.6	13.6±0.3	0.389±0.007	5.6±0.9	0.78±0.06
Ellagitannin mixture (**El**)	N_2	17.1±0.2	17.2±0.2	0.156±0.004	-	-
	O_2	17.7±0.3	9.7±0.3	0.42±0.02	7.94±0.05	0.70±0.08
Vescalagin						
Ellagitannin isolated (**V**)	N_2	38.6±0.4	38.7±0.5	0.174±0.003	-	-
	O_2	38±2	22±1	0.36±0.02	16.6±0.6	2.4±0.2
C+V mixture (**M**)	N_2	18.0±0.5	18.2±0.3	0.211±0.003	-	-
	O_2	19±1	7.8±0.7	0.35±0.03	11.0±0.5	3.87±0.07
Ellagitannin mixture (**El**)	N_2	13.6±0.2	13.6±0.4	0.271±0.007	-	-
	O_2	14.0±0.2	8.7±0.9	0.73±0.02	5.4±0.7	3.41±0.03
Grandinin						
Ellagitannin mixture (**El**)	N_2	5.2±0.2	5.1±0.2	0.29±0.04	-	-
	O_2	5.2±0.3	2.53±0.06	0.515±0.002	3.7±0.4	1.5±0.1
Roburin E						
Ellagitannin mixture (**El**)	N_2	4.9±0.3	5.0±0.5	0.315±0.009	-	-
	O_2	5.0±0.3	2.0±0.2	0.51±0.01	3.0±0.4	1.78±0.02

521 *N_2: inert atmosphere; O_2: air-saturated atmosphere.

522

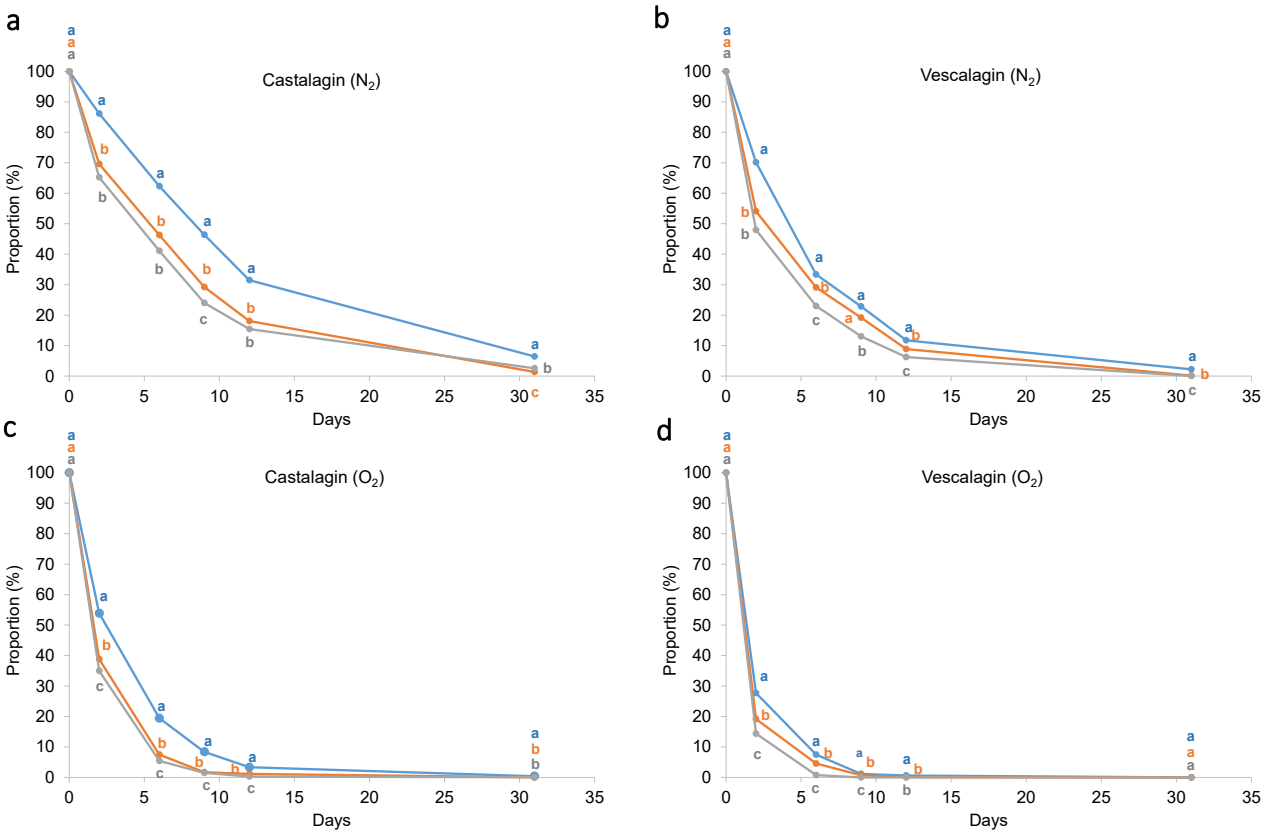

a
Castalagin (N_2)
Proportion (%)
Days
b
Vescalagin (N_2)
Proportion (%)
Days
c
Castalagin (O_2)
Proportion (%)
Days
d
Vescalagin (O_2)
Proportion (%)
Days

Figure 1

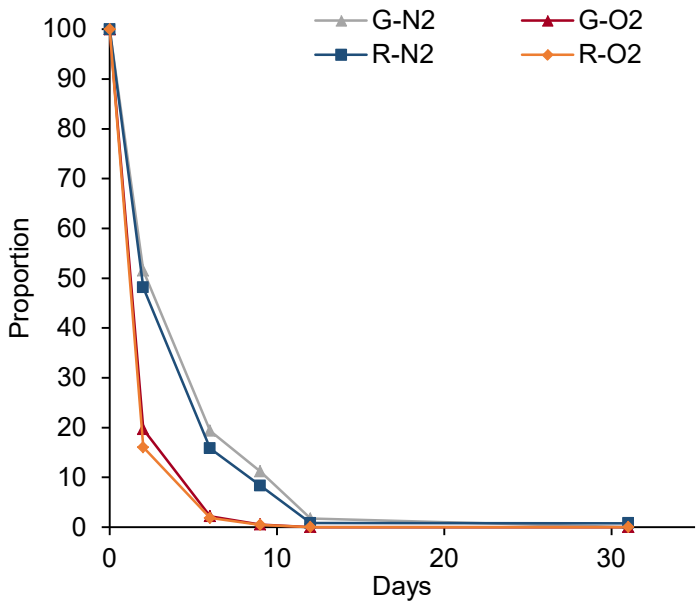
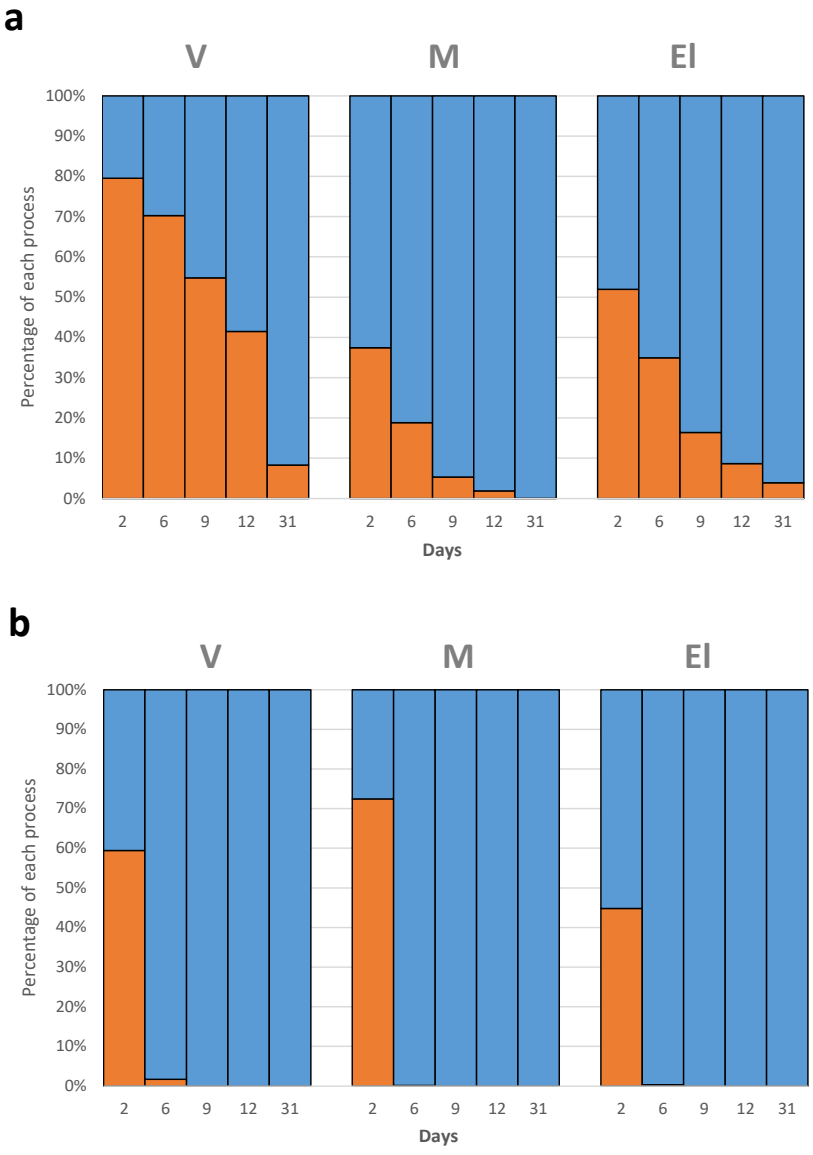

G-N2
G-O2
R-N2
R-O2
100
90
80
70
60
50
40
30
20
10
0
Proportion
0
10
20
30
Days

Figure 2


a
V
M
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100%
90%
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70%
60%
50%
40%
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20%
10%
0%
Percentage of each process
2
6
9
12
31
Days
b
V
M
El
100%
90%
80%
70%
60%
50%
40%
30%
20%
10%
0%
Percentage of each process
2
6
9
12
31
Days

Figure 3