

1 Effect of enological treatments on phenolic and sensory characteristics of red wine during aging:
2 Micro-oxygenation, sulfur dioxide, iron with copper and gelatin fining

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25 *Abbreviations:* C, control wine; M, micro-oxygenated wine; Q, aging under “standard”
26 conditions; S, aging with high concentration of sulfur dioxide; F, aging with high concentration
27 of iron and copper; G, gelatin fining prior to aging; TT, total tannins; Sum_{mon}, sum of flavan-3-ol
28 monomers; Sum_{dim}, sum of flavan-3-ol dimers; Sum_{trim}, sum of flavan-3-ol trimers; mDP, mean
29 degree of polymerization; % P, percentage of prodelphinidins; TA, total anthocyanins; AcyAc,
30 sum of anthocyanin acetylglucosides; AcyCm, sum of anthocyanin coumaroylglucosides;
31 AcyGlc, sum of anthocyanin glucosides; CI, color intensity; H, hue; PP; polymeric pigments;
32 PCA, principal component analysis.

33 *Keywords:* wine, micro-oxygenation, sulfur dioxide, metals, gelatin fining, phenolics, Plavac
34 mali

35 Abstract:

36 This work aimed to study long-term impact of micro-oxygenation and/or different aging
37 treatments: (i) high SO₂, (ii) high Fe with Cu and (iii) gelatin fining on Plavac mali red wine
38 phenolic and in-mouthfeel sensory development in barrels and furthermore in bottles. Results
39 showed that outcomes of micro-oxygenation strongly depend on aging treatments. High SO₂
40 concentration during aging in barrels and bottles delayed typical phenolic changes and slightly
41 contributed to astringency and lower color intensity, particularly in wine that was not micro-
42 oxygenated. High metal concentrations and gelatin fining promoted intensive polymerization of
43 proanthocyanins and a lower percentage of prodelphinidins after long-term aging in barrels.
44 Also, flavan-3-ol and anthocyanins transformation rates in micro-oxygenated wines of both
45 treatments significantly differed from their controls. Gelatin fining proved to be a very effective
46 treatment for astringency reduction, particularly when combined with micro-oxygenation, but
47 fined wines after long term aging in bottles showed lower color intensity.

48

49 1. Introduction

50 Oxygen exposure of wine during winemaking and aging has a great importance on its
51 phenolic and sensory evolution (Atanasova, Fulcrand, Cheynier, & Moutounet, 2002; Cano-
52 López et al., 2008; Han, Webb, & Waterhouse, 2019). This knowledge provided the basis for
53 development of micro-oxygenation, technique that consists of intentional and controlled addition
54 of small amounts of oxygen into wine with specialized equipment allowing precise oxygen
55 dosage (Gómez-Plaza & Cano-López, 2011). Micro-oxygenation was developed as cheaper
56 alternative to oak aging, mimicking the slow uptake of oxygen that occurs in barrels. However,
57 practical application nowadays does not exclude, but complements aging in barrels (del Carmen
58 Llaudy, Canals, González-Manzano, Canals, Santos-Buelga, & Zamora, 2006), with an attempt
59 to improve red wine quality. Application benefits of this technique include stabilization of wine
60 color, improved taste and structure characteristics, improved wine aroma (Gómez-Plaza & Cano-
61 López, 2011), as well as reduced time needed to achieve some aged wine characteristics (Han et
62 al., 2019).

63 Changes in the anthocyanins concentration and/or structure, formation of ethyl-bridged-
64 pigments and pyranoanthocyanins (Atanasova et al., 2002; Cano-López et al., 2008; Gambuti,
65 Han, Peterson, & Waterhouse, 2015; Gambuti, Picariello, Moio, & Waterhouse, 2019;
66 Kontoudakis et al., 2011; Wirth et al., 2010) were found to be responsible for higher intensity
67 and/or higher color stability of micro-oxygenated wines (Cano-López et al., 2008; Cejudo-
68 Bastante, Pérez-Coello, & Hermosín-Gutiérrez, 2011; Gambuti, Rinaldi, Ugliano, & Moio, 2013;
69 González-del Pozo, Arozarena, Noriega, Navarro, & Casp, 2010; Kontoudakis et al., 2011;
70 Sánchez-Iglesias, González-Sanjosé, Pérez-Magariño, Ortega-Heras, & González-Huerta, 2009).
71 On the other hand, effects of micro-oxygenation on proanthocyanidins evolution (Atanasova et

72 al., 2002; Cano-López et al., 2008; del Carmen Llaudy et al., 2006; Kontoudakis et al., 2011) or
73 changes in wine astringency and bitterness perception are far more less studied (Cejudo-Bastante
74 et al., 2011; del Carmen Llaudy et al., 2006; Durner, Weber, Neddermeyer, Koopmann,
75 Winterhalter, & Fischer, 2010), giving rather inconsistent results. For example, some studies
76 showed that micro-oxygenation promoted polymerization of proanthocyanidins (del Carmen
77 Llaudy et al., 2006), while others indicated similar or even lower values in micro-oxygenated
78 wine than their control counterparts (Atanasova et al., 2002; Kontoudakis et al., 2011; Wirth et
79 al., 2010). However, aforementioned differences could arise from differences in phenolic
80 composition, pH, aging conditions (barrels/bottles), as well moment of sampling (Cano-López et
81 al., 2008; Kontoudakis et al., 2011). Furthermore, wines that received oxygen were perceived
82 less astringent, with more olfactory intensity, more in-mouth complexity and roundness, and
83 were the choice of consumer preference (Parpinello, Plumejeau, Maury, & Versari, 2012).
84 Earlier studies suggested that micro-oxygenation is mainly advisable for application on very
85 astringent wines or wines obtained from grapes not well ripened (del Carmen Llaudy et al.,
86 2006), as well as effective towards diminishing green tannins that were associated with
87 unripe/green flavor (Durner et al., 2010). However, great precaution must be taken as regards
88 oxygen dosage, since unfavorable changes such as overoxidation, as well as increase in dry
89 tannins (Durner et al., 2010) and astringency (Cejudo-Bastante et al., 2011) can occur. In
90 addition, only a few scientific papers have studied how previously micro-oxygenated wines
91 preformed after two or more years of aging. These studies showed that supplying oxygen
92 significantly accelerated the kinetics of degradation and transformation reactions of
93 anthocyanins, producing beneficial effects in color stability that can be kept from 20 up to 28
94 months of aging in barrels and/or bottles (González-del Pozo et al., 2010; Gambuti et al., 2019),

95 as well as positive long effects of this technique on wine astringency decrease for the wine with
96 lower pH (Gambutì et al., 2013). However, there are very limited data of how different oxido-
97 reductive conditions, like different concentrations of sulfur dioxide or metals, can impact the
98 long-term outcomes of micro-oxygenation. Namely, iron in the association with copper is an
99 essential catalyst in oxidative processes of wine, and the addition of these metals to wine was
100 found to accelerate the phenolic reactions with oxygen (Danilewicz & Wallbridge, 2010). On the
101 other hand, the presence of sulfur dioxide suppresses these reactions (Tao, Dykes, & Kilmartin,
102 2007), since sulfite reacts with hydrogen peroxide that is produced when phenols are oxidized,
103 and also largely reduces the quinones back to their original catechols (Danilewicz, Seccombe, &
104 Whelan, 2008; Danilewicz & Wallbridge, 2010). Moreover, sulfur dioxide is able to modulate
105 reactions initiated by micro-oxygenation, since micro-oxygenated wines with lower
106 concentrations of sulfur dioxide showed increase in acetaldehyde concentration, and
107 concentration of ethyl-bridged compounds and pyranoanthocyanins (Gambutì et al., 2015). Also,
108 more recent study of Gambutì et al. (2019) indicated that sulfur dioxide added before micro-
109 oxygenation slowed wine oxidation reactions during aging and affected wine color stability
110 inducing color loss. Hence since both SO₂ (Gambutì et al., 2015; Gambutì et al., 2019) and
111 metals (Morozova, Schmidt, & Schwack, 2014) showed to impact oxygen consumption rates as
112 well as some chemical and sensory characteristics of wine, they should also be taken into
113 account when oxygen management strategy is defined.

114 Another enological treatment that can be successfully used to eliminate substances of
115 colloidal nature, “soften” the wine and reduce astringency, as well as to improve wine
116 stabilization is gelatin fining. The function of gelatin fining is dominantly oriented towards
117 proanthocyanidins, their complexation with gelatin and removal through precipitation; although

118 this treatment can decrease the concentrations of low molecular flavan-3-ols (Cosme, Ricardo-
119 da-Silva, & Laureano, 2008; Cosme, Ricardo-da-Silva, & Laureano, 2009; Oberholster,
120 Carstens, & Du Toit, 2013) and slightly impact anthocyanins (Castillo-Sánchez, Mejuto, Garrido,
121 & García-Falcón, 2006). In addition, the decrease in astringency intensity by gelatin fining is
122 attributed to the decrease of proanthocyanidins concentrations as well as structural
123 characteristics, primarily polymer size (mDP) and galloylation percentage (% G) (Cosme et al.,
124 2008; Cosme, et al., 2009; Maury, Sarni-Manchado, Lefebvre, Cheynier, & Moutounet, 2001;
125 Oberholster, Carstens, & Du Toit, 2013).

126 Plavac mali is Croatian red native grapevine cultivar, originating from Dalmatia vine-
127 growing region. Among others, distinctiveness of this cultivar reflects in high concentrations of
128 tannins, particularly in the skins. Namely, concentrations of total tannins found in seeds and
129 skins can reach from 1.7-2.2 and 5.1-6.5 g/kg of fresh grapes, respectively (Ćurko, Kovačević
130 Ganić, Gracin, Đapić, Jourdes, & Teissedre, 2014), which is why application of different oxido-
131 reductive strategies and gelatin fining to wine of this cultivar represents an interesting case study
132 of application to astringent wine.

133 The aim of this research was to study long-term effect of micro-oxygenation (applied
134 before malolactic fermentation) with/or different oxido-reductive conditions (high concentration
135 of sulfur dioxide and iron with copper) and gelatin fining on development of wine phenolic and
136 sensory characteristics (astringency and bitterness intensity) during 12 months of aging in barrels
137 and furthermore after 38 months in bottles.

138 2. Materials and methods

139 2.1. Winemaking and micro-oxygenation trails

140 This study was carried out with a red wine from *Vitis vinifera* cv. Plavac mali crni grapes
141 that were harvested in their technological maturity in vintage 2010 (reducing sugars, 232.3 g/L;
142 pH, 3.45; total acidity 5.50 g/L as tartaric acid). Twenty tons of grapes were used for
143 winemaking protocol of the experimental trail that was set in “Jako vino” winery (Bol, Croatia).
144 Immediately after the harvest grapes were destemmed and crushed (Bucher vaslin, Delta E2,
145 Niederweningen, Zürich), sulfited (40 mg/L of total SO₂) and pumped into stainless steel
146 fermentation tank. After six hours, the must was inoculated with yeast (Zymaflore FX10[®],
147 Laffort, Bordeaux, France) coupled to the rehydration yeast nutrient (Dynastart[®], Laffort,
148 Bordeaux, France). At the second third of fermentation, ammonium salts and thiamine yeast
149 nutrients (Thiazote SP[®], Laffort, Bordeaux, France) were added. The fermentation/maceration
150 temperature was kept under 25 °C. After 14 days of maceration, when alcoholic fermentation
151 was finished, wine was racked and pressed (Bucher vaslin, XPF, Niederweningen, Zürich).

152 Initial wine after alcoholic fermentation was distributed into two stainless steel tanks of
153 5431 L (1.5 m diameter, 3.1 m height). One tank was subjected to the micro-oxygenation
154 treatment while the other was used as control. Micro-oxygenation was applied before malolactic
155 fermentation using Viso 6 equipment (Vivelys, Villeneuve-lès-Maguelone, France). The tank
156 dimensions and the placement of a technical ceramic diffuser close to the bottom of the tank
157 ensured sufficient height necessary for dissolution of oxygen microbubbles. The amount of
158 oxygen addition was established according to initial sensory characteristics of the wine, and
159 modulated according to wine development during micro-oxygenation. Doses applied were 10
160 mL/L/month for 18 days followed with 5 mL/L/month for 12 days. The temperature in the
161 control and micro-oxygenated tank was kept at 18 °C. The dissolved oxygen were measured
162 three days after the micro-oxygenation has finished, using a luminescent dissolved oxygen

163 sensor (Hach Lange, HQ40d, LDO101, Düsseldorf, Germany), and amounted below 0.1 mg/L in
164 micro-oxygenated and control wine, respectively.

165 Once the micro-oxygenation process was completed, lactic acid bacteria (Lactoenos 450
166 PreAc[®], Laffort, Bordeaux, France) were added to both, control (C) and micro-oxygenated wine
167 (M). After malolactic fermentation was over, SO₂ was corrected in C and M wine to leave the
168 wines with a free SO₂ content of 25 mg/L.

169 2.2. Oak-barrel wine aging trails

170 After malolactic fermentation was over, the control (C) and micro-oxygenated (M) lot
171 were distributed in oak barrels for aging. Conventional wine analyses of these wines were
172 performed according to official OIV methods using standard equipment (OIV 2009). The initial
173 parameters of C wine were: density, 0.9903 g/L; ethanol, 14.05 (% vol); dry extract, 32.9 g/L;
174 reducing sugars, 3.39 g/L; total SO₂, 38 mg/L; free SO₂, 26 mg/L; pH, 3.75; total acidity 4.73
175 g/L (as tartaric acid); volatile acidity, 0.25 g/L (as acetic acid); lactic acid, 1.03 g/L; while malic
176 acid was not detected. The initial parameters of M wine were: density, 0.9903 g/L; ethanol, 14.06
177 (% vol); dry extract, 32.7 g/L; reducing sugars, 3.21 g/L; total SO₂, 37 mg/L; free SO₂, 24 mg/L;
178 pH, 3.77; total acidity 4.75 g/L (as tartaric acid); volatile acidity, 0.30 g/L (as acetic acid); lactic
179 acid, 1.14 g/L; while malic acid was not detected.

180 Different enological treatments were applied to C and the same to the M wines
181 prior/during the aging in barrels. The treatments applied were: (i) Treatment Q - aging under
182 standard aging conditions; (ii) Treatment S - aging with high content of SO₂, (iii) Treatment F –
183 aging with high content of iron and copper and (iv) Treatment G – gelatin fining prior to aging.
184 Detailed description of treatments with doses employed are shown in Table 1. Oak-barrel wine
185 aging experiment was set in triplicate for C and M wine of each treatment. Wines matured in two

186 years' old medium toasted barrels of 225 L (French oak, Nadalie, Ludon-Médoc, France) during
187 the period of 12 months. The temperature of the cellar was kept around 15 °C during aging.
188 Level of SO₂ was measured every two weeks and corrected if necessary. Also, the concentration
189 of iron and copper were determined by ICP-MS method as described by Vinković Vrček et al.
190 (2011). The metals were analyzed prior to their addition to wine, amounting to 0.989 ± 0.004
191 mg/L for the former and 0.081 ± 0.000 mg/L for the latter. On this basis, the amount which was
192 finally added to wine was defined to obtain the 10 mg/L of iron and 0.5 mg/L of copper. In the
193 last sampling point, the concentration was 9.441 ± 0.012 and 8.462 ± 0.008 mg/L of iron and
194 0.132 ± 0.001 and 0.157 ± 0.000 mg/L of copper in control and micro-oxygenated wine,
195 respectively; which is below the maximum allowed concentration according to European
196 regulations. Sampling was performed at 3 points: after 1, 6, and 12 months of aging in barrels.

197 2.3. Bottling and storage

198 After 12 months of aging in barrels, both C and M wines of Treatment Q, S, F and G
199 (Table 1) were bottled in 750 mL dark glass bottles with natural cork stoppers (24 mm diameter
200 x 45 mm length; produced by River Cork, Cortiças, Unipessoal Lda, Rio Meão, Portugal). Wine
201 aged in the same wine cellar room, where humidity and temperature conditions were controlled
202 throughout the aging period and amounted around 75% and 16 °C, respectively. Bottle aging
203 experiment was set in triplicate for C and M wine of each treatment. Analyses were conducted
204 after 38 months of aging.

205 2.4. Phenolic and chromatic characterization by spectrophotometry

206 Spectrophotometric analyses were conducted on the UviLine 9400 (Schott Instruments,
207 Mainz, Germany) single beam spectrophotometer. The concentration of total tannins (TT) was

208 determined by the acid hydrolysis method (Ribéreau-Gayon & Stonestreet, 1966). The
209 concentration of total anthocyanins was measured by the bisulfite bleaching procedure
210 (Ribéreau-Gayon & Stonestreet, 1965). Polymeric pigments (PP) were analyzed as the
211 contribution ratio of color fraction resistant to bisulfite bleaching (Glories, 1984b). Chromatic
212 characteristics were determined by optical density measured at three wavelengths: 420 nm
213 (yellow), 520 nm (red) and 620 nm (blue) in path length of 1 mm; from which color intensity
214 (CI) (Glories, 1984a), hue (H) (Sudraud, 1958) and contribution of red (% red), yellow
215 (% yellow) and blue color (% blue) (Glories, 1984a) were calculated. All spectrophotometric
216 analyses were conducted in triplicate.

217 2.5. HPLC-Fluo-MS analysis of monomeric and oligomeric flavan-3-ols

218 Analysis was performed on Finningan Surveyor Plus HPLC (Thermo-scientific,
219 Massachusetts, USA) instrument with fluorescence detector (FL Plus Detector) coupled to a
220 ChromQuest software data system as well as mass detector (LCQ Deca XP, quadrupole mass
221 spectrometer with ESI mode) coupled to a Xcalibur software data system. Separation was
222 conducted on LiChrospher RP-18 (250 mm × 4 mm, 5 µm) column (Merck, Darmstadt,
223 Germany). The mobile phase used consisted of solvent A: water/formic acid (99.5:0.5, v/v) and
224 solvent B: acetonitrile/formic acid (99.5:0.5, v/v) according to the method of Čurko et al. (2014),
225 with small modifications in gradient conditions: 3% B isocratic from 0-3 min, 3-5% B linear
226 from 3-10 min; 5% B isocratic from 10-14 min, 5-7% B linear from 14-20 min, 7-10% B linear
227 from 20-22 min, 10% B isocratic from 22-27 min, 10-12% B linear from 27-32 min, 12-14% B
228 linear from 32-34 min, 14-25% B linear from 34-45 min, 25-100% B linear from 45-46 min,
229 100% B isocratic from 46-50 min, 100-3% B linear from 50-51 min, with re-equilibration of the
230 column from 51-55 min under initial gradient conditions. Detection and identification of

231 monomeric and oligomeric flavan-3-ols [(+)-catechin (CAT), (-)-epicatechin (EC), procyanidin
232 dimers B1, B2, B3, B4 and trimers C1 and T2] was performed as previously shown (Chira,
233 Pacella, Jourdes, & Teissedre, 2011; Ćurko et al., 2014), while quantification was done using
234 external standards calibration curves. Sum of flavan-3-ol monomers (Sum_{mon}) was calculated as
235 sum of catechin and epicatechin. Sum of flavan-3-ol dimers (Sum_{dim}) was calculated as sum of
236 procyanidin dimers B1, B2, B3 and B4. Sum of flavan-3-ol trimers (Sum_{trim}) was calculated as
237 sum of procyanidin trimers C1 and T2.

238 2.6. Structural characterization of wine proanthocyanidins

239 Analysis was performed on Finningan Surveyor Plus HPLC (Thermo-scientific,
240 Massachusetts, USA) instrument with diode array detector (PDA Plus Detector) and mass
241 detector (LCQ Deca XP) coupled to a Xcalibur software data system. Structural characteristics of
242 proanthocyanidins mean degree of polymerization (mDP) and percentage of prodelphinidins
243 (% P) were analyzed by the phloroglucinolysis method based upon the acid-catalyzed cleavage
244 of flavan-3-ol interflavanoid bonds in presence of phloroglucinol as the nucleophilic reagent
245 (Drinkine, Lopes, Kennedy, Teissedre, & Saucier, 2007). The reaction cleavage products were
246 determined as earlier proposed by Chira et al. (2011).

247 2.7. HPLC analysis of anthocyanins

248 Analysis was performed on Accela HPLC (Thermo-scientific, Massachusetts, USA)
249 instrument with diode array detector (Accela PDA Detector) coupled to a Xcalibur software data
250 system. Anthocyanins were separated on Nucleosil C18 (250 mm × 4.6 mm, 5 μm) column
251 (Phenomenex, Torrance, USA) using solvent A: water/formic acid (95:5, v/v) and solvent B:
252 acetonitrile/formic acid (95:5, v/v) according to the method earlier described (Ćurko et al.,

253 2014). Detection and identification were conducted at 520 nm. Quantification was done using
254 external standard calibration curve of malvidin-3-*O*-glucoside chloride. Sum of anthocyanin
255 glucosides (AcyGlc) was calculated as sum of 3-*O*-glucosides of delphinidin, cyanidin,
256 petunidin, peonidin and malvidin. Sum of anthocyanin acetylglucosides (AcyAc) was calculated
257 as sum of 3-*O*-(6-*O*-acetyl)glucosides of peonidin and malvidin. Sum of anthocyanin
258 coumaroylglucosides (AcyCm) was calculated as sum of 3-*O*-(6-*O*-*p*-coumaroyl)glucosides of
259 peonidin and malvidin.

260 2.8. Sensory analysis

261 The sensory analysis comprised the analysis of astringency and bitterness intensity of
262 wine by the method of Quantitative Descriptive Analysis (QDA) (Chira et al., 2011; Čurko et al.,
263 2014). The overall in-mouth perception of both descriptors was evaluated using the 0-7 point
264 scale (0 = absence of perception, 1 = low, 2 = slight, 3 = moderate, 4 = strong, 5 = intensive,
265 6 = very intensive, 7 = excessive). It is important to note that overall astringency was assessed as
266 a complex sensation combining three distinct aspects: drying of the mouth, roughing of oral
267 tissues, and puckering or drawing sensations felt in the cheeks and muscles of the face. A panel
268 consisting of 14 judges (wine professionals) from the University of Bordeaux participated in
269 sensory evaluation. Training of judges was carried out during 12 sessions in 4 consecutive weeks
270 as earlier described by Čurko et al. (2014), employing aluminum sulfate and quinine sulfate as
271 the referent standards for astringency and bitterness, respectively. The ability of judges to
272 recognize and distinguish astringency mouth-feel and bitter taste in concentrations above known
273 thresholds was first tested. Afterwards, judges were trained to rank the intensity of astringency
274 and bitterness perception in different concentrations of standard solutions by ranking tests.
275 Solutions were first prepared as individual standards, while after, wine model solutions

276 containing both standards were assessed. Finally, astringency and bitterness intensity was
277 evaluated in the wine model standard solutions of aluminum sulfate (0-4 g/L), quinine sulfate (0-
278 15 mg/L) and the wine model commercial tannin solutions (0-4 g/L) using an QDA and intensity
279 scale of 0-7.

280 The samples were tasted in coded black glasses, at room temperature, with the presence
281 of referent standard solutions. The judges took 20 mL of the sample in their mouth, held it for
282 30 s, spat it out and rated its astringency and bitterness intensity using a 0-7 point scale. Special
283 precautions in the sensory analysis assessment were taken in order to avoid the “carryover”
284 effect (increase in astringency upon the repeated stimulation). Hence, the panelists were obliged
285 to eat plain crackers, rinse their mouth with water, and finally wait for 30 s between samples.
286 Sensory analysis was performed in duplicate.

287 2.9. Data analysis

288 The statistical data analysis was carried out using the Analysis of Variance (ANOVA) by
289 Statistica V.7 software (Statsoft Inc., Tulsa, USA). Tukey’s HSD and Duncan’s tests were used
290 as a comparison test when samples were significantly different after ANOVA ($p < 0.05$) for
291 chemical and sensory analyses. The principal component analysis (PCA) was performed on the
292 correlation matrix using the attributes of chemical and sensory analysis in order to examine any
293 possible grouping of samples by different treatments applied during aging.

294 3. Results and discussion

295 3.1. Effect of enological treatments on proanthocyanidins concentrations and structural
296 characteristics during aging

297 The effect of different enological treatments on proanthocyanidins concentrations and
298 structural characteristics of Plavac mali wine are reported in Table 2. In agreement with the
299 literature (Chira et al., 2011; Gambuti et al., 2013; Sánchez-Iglesias et al., 2009), decrease in the
300 content of total tannins and flavan-3-ols (monomers, dimers and trimers) was observed in wines
301 of all treatments in barrels and furthermore in bottles over time. Namely, during aging
302 proanthocyanidins undergo spontaneous cleavage and formation of new interflavanic bonds,
303 polymerization with anthocyanins, as well as precipitation of large insoluble polymers formed
304 (Es-Safi, Fulcrand, Cheynier, & Moutounet, 1999; Fulcrand, Dueñas, Salas, & Cheynier, 2006).
305 The observed loss in concentrations of tannins and low molecular flavanols can be attributed to
306 their participations in subsequent reactions. However, intensity of aforementioned loss, showed
307 to be treatment-dependent (Table 2).

308 Significantly higher concentration of tannins and in the sum of flavan-3-ol monomers,
309 dimers and trimers was found in control (CQ) than in micro-oxygenated (MQ) wine of Treatment
310 Q (aging with 25 mg/L of free SO₂) but only after the first month of aging in barrels (Table 2).
311 Lower concentrations of these compounds in MQ wine can be associated with more pronounced
312 polymerization reactions among flavanols, as well as flavanols and anthocyanins mediated by
313 acetaldehyde which are known to be favored during micro-oxygenation (Atanasova et al., 2002;
314 Cano-López et al., 2008; González-del Pozo et al., 2010). Nevertheless, majority of differences
315 among two wines of Treatment Q afterwards tended to disappear, showing comparable or only
316 slightly higher amounts in CQ wines. Similar remarks were given by other authors indicating
317 that effects of micro-oxygenation on some chemical parameters, like tannins and flavan-3-ols,
318 decrease as wines ages in barrels and/or bottles (del Carmen Llaudy et al., 2006; Gambuti et al.,
319 2013; Sánchez-Iglesias et al., 2009). However, in case of flavan-3-ol dimers significant

320 differences among CQ and MQ wines were kept throughout all period of aging in barrels,
321 although this effect could not be attributed to the particular individual procyanidin dimer
322 (Supplementary Table 1), but the overall sum of dimers (Table 2). These results imply that the
323 sum of flavan-3-ol dimers was highly susceptible to the changes induced by oxygen. Indeed,
324 earlier study of Dallas, Ricardo-da-Silva, and Laureano (1996) reported that rate of loss of
325 dimeric procyanidins in wine-like solution containing malvidin-3-glucoside and acetaldehyde,
326 and hence “oxidative condition”, was greater than that of monomers that reacted more slowly.
327 However, trends found in the sum of dimers were lost after long period of ageing in the bottles.
328 In addition, the same study (Dallas et al., 1996) proposed highest degradative reaction rate in the
329 case of trimers, which is in accordance to very sharp drop of almost 40% that occurred in the
330 sum of trimers during first 6 months of aging in barrels (Table 2).

331 Aging with 40 mg/L of free SO₂ represented as Treatment S (higher SO₂ content), did not
332 influence the development of total tannins as much as flavan-3-ols. In general, CS and MS wines
333 of Treatment S evolved in similar manner as earlier described for CQ and MQ of Treatment Q,
334 with slightly higher concentrations in CS than MS wine. Furthermore, changes of concentrations
335 of flavan-3-ols during aging in barrels were suppressed in wines containing higher levels of SO₂,
336 which became even more pronounced with the time. For instance, results obtained after 12
337 months of aging in barrels showed significantly higher concentrations of flavan-3-ol monomers
338 and dimers in CS than CQ wine, as well as in MS than MQ wine, receptively. Earlier study of
339 Tao et al. (2007) also showed strong decrease of flavan-3-ols in wine with lower concentration of
340 SO₂, and demonstrated moderating effect of SO₂ on the interaction of oxygen with polyphenols
341 due to its ability to reduce oxidized polyphenols and remove peroxide (Danilewicz et al., 2008).
342 More recently, Gambuti et al. (2015) reported that SO₂ and glutathione alter the outcome of

343 micro-oxygenation. Aforementioned results imply that free SO₂ in the concentration of 40 mg/L
344 can delay decrease of flavan-3-ols during long-term aging in barrels. Nevertheless, differences
345 observed at the end of 12 months of aging in barrels were less pronounced after 38 months of
346 aging in bottles (Table 2).

347 On the contrary, the addition of iron and copper in Treatment F altered the outcomes of
348 micro-oxygenation by inducing some opposite trends among CF and MF wines (Table 2). For
349 instance, higher concentrations of all flavan-3-ol monomers and dimers were established in MF
350 after 12 months of aging in barrels. As earlier explained (del Carmen Llaudy et al., 2006), this
351 might result from the degradation of ethyl-linked flavanols and pigments. Namely, ethyl-bridged
352 compounds are rapidly formed in oxidative conditions (Cano-López et al., 2008; Gambuti et al.,
353 2013), but also rapidly broke down; and therefore as a result of their degradation, flavan-3-ols
354 and anthocyanins can be released (Es-Safi et al., 1999; Escribano-Bailón, Álvarez-García, Rivas-
355 Gonzalo, Heredia, & Santos-Buelga, 2001). Moreover, addition of iron and copper to wine
356 accelerates the reaction with oxygen (Danilewicz & Wallbridge, 2010). Thus, higher
357 concentrations of flavan-3-ols in MF compared to CF wine was no surprise, since iron and
358 copper were found to play important catalytic roles in oxidative processes of wine, promoting the
359 formation of *o*-quinones and acetaldehyde (Danilewicz et al., 2008). In general, wines of
360 Treatment F, compared to Treatment Q, showed slightly lower concentrations of tannins and
361 flavan-3-ols, indicating that addition of iron and copper can slightly accelerate decrease of these
362 compounds during aging. However, impact of addition of iron and copper was more evident
363 during aging in barrels than bottles. These results can be further supported with the more recent
364 finding showing that oxidative storage increases the most oxidative catalytic form of the metal
365 (Kontoudakis, Guo, Scollary, & Clark, 2017).

366 Gelatin fining (Treatment G) applied prior to aging, showed significant impact on the
367 evolution of total tannins and flavanols during aging (Table 2). Wines of this treatment,
368 particularly micro-oxygenated wine (MG), showed significantly lower concentrations of total
369 tannins compared to wines of Treatment Q. Contrary to total tannins, trends in the evolution of
370 flavan-3-ols among control and micro-oxygenated wines of Treatment G were quite different
371 from those observed in Treatment Q. Namely, concentrations of flavan-3-ol dimers and trimers
372 at the end of aging in barrels and bottles were significantly higher in MG than CG wine, which
373 was not the case for MQ and CQ wines. Identical evolution among control and micro-
374 oxygenated wines were earlier noticed for Treatment F, but in the case of Treatment G
375 significant differences were kept even in bottles. As earlier explained, degradation of ethyl-
376 linked compounds (del Carmen Llaudy et al., 2006) might be responsible for observed results in
377 micro-oxygenated wines. However, specific evolution of flavan-3-ol dimers and trimers in MG
378 wine imply that intensity of aforementioned changes in micro-oxygenated can be affected by
379 gelatin fining. Moreover, this can be further supported by the fact that very different pattern was
380 observed when gelatin fining was applied on control wine (CG). In addition, CG wine compared
381 to CQ wine showed significantly lower concentrations in the sum of dimers and trimers after 12
382 months of aging, as well as significantly lower concentrations in the sum of monomers, dimers
383 and trimers after aging in bottles. Contrary, MG wine compared to MQ wine showed significantly
384 higher concentrations in the sum of dimers, but still slightly lower concentrations in the sum of
385 trimers after 12 months of aging, while after aging in bottles MG wine was characterized with
386 significantly lower concentrations of dimers and just slightly lower concentrations of monomers
387 than MQ wine. Hence, despite the specific evolution, particularly of MG wine, both wines of
388 Treatment G compared to Treatment Q finished the aging with lower concentrations in the sum

389 of flavan-3-ol monomers and dimers and trimers. In addition, this was especially pronounced in
390 the sum of flavan-3-ol dimers, since sharp drop in concentration occurred in both CG and MG
391 wine. More intensify changes in fraction of dimers compared to monomers or trimers can be due
392 to the gelatin physicochemical characteristics such as molecular weight distribution, that were
393 found to have great impact to fining outcomes (Cosme et al., 2009). Among determined flavan-
394 3-ols, (-)-epicatechin and procyanidin B1 in Plavac mali wine showed to be highly susceptible to
395 long-term effect of gelatin fining, due to the significantly lower concentrations of these
396 compounds in Treatment G than Treatment Q (Supplementary Table 1). Although some authors
397 observed significant decrease in concentration of flavan-3-ol monomers, dimers and trimers by
398 gelatin fining (Cosme et al., 2008), other reported little or no effect (Oberholster et al., 2013).
399 Nevertheless, these literature data investigated short-term effects of gelatin fining (within 4
400 months after the fining), while on the other hand, our results demonstrated long-term effect of
401 gelatin fining on flavan-3-ol evolution.

402 Changes of mDP showed that proanthocyanidins underwent structural rearrangements
403 (interflavan bond cleavage and formation), as well as precipitations during aging in barrels and
404 bottles (Table 2). The first six months of aging in barrels were characterized with more intensive
405 polymerization (increase in the mDP), similar as earlier reported (Atanasova et al., 2002; Cano-
406 López et al., 2008); while major fall of mDP in all wines was observed after long-term bottle
407 aging (Chira et al., 2011). Micro-oxygenated wines of all treatments showed only slightly higher
408 values of mDP than those of their control counterparts. Literature data considering the effect of
409 micro-oxygenation after maturing/aging are rather inconsistent, some indicating that micro-
410 oxygenation induced the polymerization of proanthocyanidins (del Carmen Llaudy et al., 2006),
411 while others found similar values in control and micro-oxygenated wines (Atanasova et al.,

412 2002; Kontoudakis et al., 2011). At the end of bottle aging, higher concentrations of SO₂
413 contributed to the significantly lower mDP, particularly in CS wine, which was expected since
414 SO₂ has the ability to reduce oxidized polyphenols back to their reduced forms and to remove
415 peroxide (Danilewicz & Wallbridge, 2010). On the other hand, significantly higher values of
416 mDP were obtained in CF and MF wines after 12 months of aging, indicating that higher content
417 of iron and copper promoted polymerization reactions of proanthocyanidins. These results were
418 not surprising, since oxidation of ethanol by hydrogen peroxide showed to be dependent on
419 metals such as iron and copper to generate hydroxyl radicals by the way of the Fenton reaction
420 (Danilewicz, 2003). However, very sharp drop of mDP, resulting in significantly lower values
421 occurred during reductive aging in bottles of both wines of Treatment F. In addition, wine of
422 Treatment G, compared to Treatment Q were characterized with significantly lower mDP during
423 first six, and opposite significantly higher mDP during last six months of aging in barrels. Also,
424 it is interesting to note that both Treatment F and G showed increase in the mDP value during
425 last six months of aging in barrels, contrary to both Treatment Q and S where drop of mDP was
426 noticed in this point. In addition, this was particularly enhanced in MF and MG wines which as
427 earlier explained showed very specific evolution of flavan-3-ols at the end of aging in barrels.
428 This indicates that both Treatment F and G, particularly when combined with micro-oxygenation
429 promoted more intensive reactions among proanthocyanidins. Nevertheless, values of mDP
430 analyzed after long period of aging in bottles in Treatment G were significantly lower than in
431 Treatment Q. The obtained results were in accordance to earlier studies demonstrating that
432 polymer size as well as percentage of galloylation can be significantly reduced by gelatin
433 addition (Cosme et al., 2008; Cosme et al., 2009; Maury et al., 2001; Oberholster et al., 2013),
434 although aforementioned studies investigated short-term effects of gelatin fining.

435 In addition, micro-oxygenation itself did not have major effect on the % P during aging in
436 barrels and bottles. However, addition of metals forced the evolution of % P within specific
437 ways. For instance, MF wine showed significantly lower values after 1 month of aging, while
438 results obtained for both CF and MF wine were generally lower compared to wines of all other
439 treatments after 12 months of aging (particularly CQ and MQ wine), as well as slightly lower
440 after aging in bottles. Interesting analogy can be made with the results of Ferreira, Carrascon,
441 Bueno, Ugliano, and Fernandez-Zurbano (2015) who demonstrated higher decrease of % P in the
442 fastest O₂ consuming wines, since epigallocatechin groups are the most reductive amongst all
443 phenolic functional groups. Also, our results (Table 2) showed significantly lower concentration
444 of % P in MG wine after 1 month and both CG and MG wines of Treatments G after 12 months
445 of aging in barrels. Similarly, Cosme et al. (2009) reported significantly lower % P within the
446 polymeric proanthocyanidin fraction with mDP value 4.9. However, differences among
447 treatments were lost after 38 months of aging in bottles.

448 3.2. Effect of enological treatments on anthocyanins concentrations and chromatic characteristics 449 during aging

450 The decrease of total and free anthocyanins (glucosides and acyl-derivatives) during
451 aging in barrels and furthermore bottles (Table 3) is in agreement with earlier reported results
452 (Alcalde-Eon, Escribano-Bailón, Santos-Buelga, & Rivas-Gonzalo, 2006; Gambuti et al., 2013;
453 Sánchez-Iglesias et al., 2009). These changes primarily arise from direct and indirect
454 condensation reactions of anthocyanins with flavanols and due to the formation of anthocyanin-
455 derived pigments, pyranoanthocyanins (Alcalde-Eon et al., 2006). Indeed, loss of anthocyanins
456 throughout an aging period was followed by formation of polymeric pigments and changes in
457 chromatic characteristics, particularly increase in hue and yellow color (%) and decrease in the

458 contribution of red color (%), as presented in Table 4. Namely, modifications of pigment profile
459 during aging are responsible for changes in color from purple-red in young wine toward more
460 red-orange hues in aged ones. Condensation reactions of anthocyanins and flavanols, primarily
461 ethyl-bridged ones, cause bathochromic shifts (bluish red hues); while formation of
462 pyranoanthocyanins primarily causes a hypsochromic shift (orange hues) (García-Puente Rivas,
463 Alcalde-Eon, Santos-Buelga, Rivas-Gonzalo, & Escribano-Bailón, 2006).

464 In Treatment Q, significant differences among CQ and MQ in concentrations of
465 anthocyanins after 12 months of aging were established only in the sum of anthocyanin
466 glucosides, but afterwards diminished (Table 3). Although, the loss of free anthocyanins is
467 expected to be higher in micro-oxygenated wine due to formation of ethyl-bridged compounds
468 and pyranoanthocyanins (Cano-López et al., 2008; Kontoudakis et al., 2011), some differences
469 between micro-oxygenated wines and their control wines can be lost with time (Sánchez-Iglesias
470 et al., 2009). Nevertheless, micro-oxygenation induced some favorable long-term effects on red
471 wine chromatic characteristics (González-del Pozo et al., 2010), that is significantly higher color
472 intensity and % blue with lower % yellow after 12 months of aging in barrels, as well as
473 significantly higher % of polymeric pigments and % blue with lower % yellow in bottles
474 (Table 4).

475 CS and MS wine of Treatment S evolved more or less in parallel, with only slightly lower
476 concentrations of anthocyanins and improved chromatic characteristics in MS wine after 12
477 months in barrels, that were lost in bottles (Tables 3 and 4). Higher concentration of SO₂
478 significantly delayed the loss of anthocyanins during aging. For instance, wines of Treatment S
479 showed significantly higher concentrations of anthocyanins compared to Treatment Q, in both
480 barrels and bottles (Table 3 and Supplementary Table 2). The identical trends as well as

481 moderating effect of SO₂ on the interaction of oxygen with wine polyphenols were previously
482 noticed in the experiments with high SO₂ content (Gambutì et al., 2015; Gambutì et al., 2019;
483 Tao et al., 2007). SO₂ protects polyphenolic compounds from oxidation (because it reacts with
484 hydrogen peroxide, and thus prevents the formation of acetaldehyde) and also reduces the
485 resulting quinones back to their original catechol (Danilewicz et al., 2008; Danilewicz &
486 Wallbridge, 2010). Furthermore, high SO₂ concentrations induced significant changes in
487 chromatic characteristics, particularly significantly lower color intensity and % blue , as well as
488 higher hue and % yellow, as previously shown (Gambutì et al., 2015; Tao et al., 2007). Obtained
489 results can partly be explained by bleaching effect of SO₂ on anthocyanins, by nucleophilic
490 addition at the C4 position in the C ring of the anthocyanin in the flavylum cation (Danilewicz
491 et al., 2008). Also, possibility that high SO₂ concentrations during aging supported predominance
492 of more stable pigments like carboxypyrananthocyanins, represented by vitisin A that caused
493 shift towards orange coloration cannot be excluded (Wirth et al., 2010).

494 On the other hand, higher concentration of metals induced specific evolution of
495 anthocyanins, particularly in MF wine, that showed significantly higher concentrations of
496 anthocyanins than CF wine (Table 3 and Supplementary Table 2). Cleavage of unstable ethyl-
497 bridged anthocyanin-flavanol compounds (Alcalde-Eon et al., 2006; Escribano-Bailón et al.,
498 2001) was proposed to be responsible for higher levels of free anthocyanins in some micro-
499 oxygenated wines (del Carmen Llaudy et al., 2006). Namely, MF wine is expected to induce
500 more ethyl-bridged polymerization reactions, not just due to the micro-oxygenation (Atanasova
501 et al., 2002; Cano-López et al., 2008), but also due to the catalytic role of metals in wine
502 oxidative processes (Danilewicz, 2003). Also, significantly lower % red was found in CF than
503 MF wine in the last point in barrel and bottles. Nevertheless, both wines of Treatment F finished

504 the aging in bottles with significantly higher concentrations of free anthocyanins (AcyGlc) and
505 % blue than wines of Treatment Q. More interestingly, constant increase in % blue throughout
506 the aging in barrels as well as aging in bottles was noticed for both wines of Treatment F.

507 The similar trend earlier explained among CF and MF wine, was noticed among CG and
508 MG wine but in the latter case it was far less pronounced. MG wine showed significantly higher
509 concentrations of anthocyanin-3-*O*-glucosides and anthocyanin-3-*O*-(6-*p*-coumaroyl)glucosides
510 than CF wine or both wines of Treatment Q after 12 months of aging in barrels (Table 3 and
511 Supplementary Table 2). Obtained results imply that formation and furthermore cleavage of
512 ethyl-bridged compounds was more intense in MG than MQ wine, as well as that long-term
513 outcomes of gelatin fining depend on wine phenolic composition. However, after 38 months of
514 aging in the bottles, differences among Treatment Q and G diminished. In addition, gelatin fined
515 wines of Treatment G showed lower color intensity throughout all aging period, while other
516 chromatic characteristics were similar to those of untreated wines. Earlier studies also found a
517 decrease in color intensity with gelatin fining (Cosme et al., 2009; Castillo-Sánchez et al., 2006).

518 3.3. Effect of enological treatments on astringency and bitterness perception during aging

519 Decrease of astringency intensity (Table 5) was detected during aging in barrels and
520 furthermore in bottles. Gradual diminution of astringency during aging, as proposed in literature,
521 is due to decrease of proanthocyanidins concentrations, as well as their structural rearrangements
522 (decrease of polymer size and galloylation percentage) (Chira et al., 2011; Gambuti et al., 2013;
523 Sánchez-Iglesias et al., 2009); and formation of different tannin-like polymeric pigments (Vidal,
524 Francis, Noble, Kwiatkowski, Cheynier, & Waters, 2004). Overall changes of polyphenolic
525 compounds observed during aging of Plavac mali wine are in a good accordance with presented
526 studies. Furthermore, decrease of bitterness intensity was noticed only during first 6 months of

527 aging in barrels (Table 5), but furthermore, no consistent trends were observed except in CG
528 wine of Treatment G. This wine showed sharp drop of bitterness intensity during last six months
529 in barrels, that continued even in bottles. However, the same trend was not noticed for MG wine,
530 probably since the more intensive drop of bitterness intensity occurred even earlier, during first
531 six months of aging in the barrels, while afterwards no significant changes in the bitterness
532 intensity were observed.

533 Micro-oxygenation contributed to significantly lower astringency intensity in treatments
534 Q, S and F after 12 months of aging in barrels, as well as treatment G after 1 and 6 months, but
535 all these differences were lost during aging in bottles (Table). Bibliography data are rather
536 inconsistent about the effect of micro-oxygenation on in-mouth tannic characteristics of wine.
537 Some authors reported diminishing of “green” tannins that were associated with unripe/green
538 flavor (Durner et al., 2010) and drastically lower astringency after long period of aging (del
539 Carmen Llaudy et al., 2006). In some cases, although differences among wines were not noticed
540 at first, they were observed after 42 months of aging in bottles (Gambutí et al., 2013). On the
541 other hand, Sánchez-Iglesias et al. (2009) established that positive sensory effects of micro-
542 oxygenation were lost after six months of aging in barrels; while others even reported an increase
543 of astringency with micro-oxygenation (Cejudo-Bastante et al., 2011). However, some of these
544 negative results were supposed to arise from application of higher oxygen doses and possible
545 overoxidation. Nevertheless, the development of mouth-feel characteristics of wine during
546 storage, among others also showed to be affected by wine phenolic composition and conditions
547 during aging (Cano-López et al., 2008; Gambuti et al., 2013), which particularly reflect in our
548 results. In addition, literature data are also inconsistent about the effect of micro-oxygenation on
549 the bitterness intensity perception. While some previous studies reported lower bitterness

550 intensity in micro-oxygenated wines (Cejudo-Bastante et al., 2011), others show little or no
551 impact (del Carmen Llaudy et al., 2006). Furthermore, both wines of Treatment S contributed to
552 slightly higher astringency intensity than CQ wine, as well as significantly higher astringency
553 intensity than MQ wine after 38 months of aging in bottles. Moreover, significantly higher
554 bitterness intensity was found in CS wine compared to wines of all treatments. These results are
555 consistent with earlier studies (Gambutí et al., 2019; Tao et al., 2007) proposing that
556 concentrations of SO₂ can affect the polyphenol chemistry and wine astringency. Also, slightly
557 lower astringency intensity was found in wines of Treatment F than Treatment Q after 12 months
558 of aging, but these effects were lost in bottles. Wines subjected to gelatin fining showed in
559 general much lower astringency intensity, which was not surprising, knowing the impact of this
560 treatment to proanthocyanidins concentration and composition. Lower astringency in gelatin-
561 treated wines due to the lower tannin levels was earlier established (Maury et al., 2001). Also,
562 these wines achieved generally higher ratings for organoleptic quality, due to the significantly
563 lower “fine emery” and “adhesive”, meaning that gelatin non-treated wines had rougher surface
564 smoothness compared to treated ones (Oberholster et al., 2013). Moreover, gelatin fining was
565 particularly effective in MG wine.

566 Overall wine chemical and sensory data obtained through aging in barrels and bottles
567 were processed by principal component analysis (PCA) in Fig. 1. The first two principal
568 components accounted for 85.90% of the total variance. Variable plots including chemical and
569 sensory parameters were mainly distributed according to the first principal component (PC 1)
570 that can be interpreted as “aging” component (Fig. 1A). This component showed very high
571 negative correlation with total tannins, flavan-3-ol monomers, dimers and trimers, total and free
572 anthocyanins, % red and astringency intensity, as well as very high positive correlation with

573 % polymeric pigments, hue and % yellow. The second principal component (PC 2) showed a
574 high positive correlation with mDP, color intensity and % blue, and although less important
575 (12.16% of the variance) than the first one, could be referred as the “oxygenation or treatment”
576 component. The effect of storage time was clearly reflected in PCA analysis. Namely, effect of
577 aging can be seen by the distribution of samples around the PC 1 (Fig. 1B). Wine samples after 1
578 and 38 months of aging were placed on the left and right side of first factorial plane,
579 respectively; while wine samples obtained after 6 and 12 months of aging were placed between
580 the two groups mentioned (Fig. 1B). These results indicate an important decrease in
581 concentrations of flavanols and anthocyanins occurred during aging, which was accompanied
582 with the formation of polymeric pigments, increase of hue and % yellow and decrease of % red
583 and astringency. Generally, phenolic and sensory differences among control wines and their
584 micro-oxygenated counterparts were particularly pronounced after 1 and 12 months of aging in
585 barrels, and partly lost during aging in bottles. Effects of Treatment S stated to be visible after 6
586 months of aging, and furthermore were even pronounced over time. Displacement of CS and MC
587 samples was primarily due to the higher concentrations of flavanols, anthocyanins, hue and
588 % yellow, higher astringency, as well as lower mDP, color intensity and % red and % blue. On
589 the other hand, grouping of MF, MQ and MG wines due to the higher mDP, % blue and color
590 intensity was noticed after 12 months of aging. Aging in bottles reduced the differences among
591 wines of Treatments Q, F and G. Nevertheless, in the end of aging, overall chemical and sensory
592 characteristics of wines of Treatment F were closer to the ones of wine MQ, while those of
593 Treatment G to the CQ wine.

594

595 4. Conclusions

596 Changes in phenolic composition and sensory characteristic of Plavac mali wine were
597 affected both by micro-oxygenation and different aging conditions (SO₂, metals and gelatin
598 fining). High concentration of SO₂ (40 mg/L of free SO₂) compared to standard concentration
599 (25 mg/L of free SO₂) slowed down decrease of flavan-3-ols and particularly anthocyanins.
600 Wines of this treatment finished the aging with lower color intensity and higher astringency,
601 particularly control wine. The addition of metals (10 mg/L of Fe with 0.5 mg/L of Cu)
602 accelerated decrease of flavan-3-ols and anthocyanins during aging in barrels, but only in control
603 wine of this treatment, while micro-oxygenated wine showed higher concentration of these
604 compounds. Also, higher concentration of metals promoted continuous polymerization among
605 proanthocyanidins, but only in barrels, as well as lower % P after 12 months of aging. Moreover,
606 this treatment was characterized with continuous increase in % blue in barrels and bottles, while
607 it had little or no impact to wine astringency and bitterness. Gelatin fining primarily affected
608 wine proanthocyanidins, promoted constant increase of mDP in barrels, but very sharp drop in
609 bottles. Nevertheless, it produced wines with significantly lower astringency through all aging
610 period, particularly the micro-oxygenated one; as well as lower color intensity after long-term
611 aging in bottles.

612 Acknowledgements

613 The authors wish to thank Jako vino (Brač, Croatia) for experimental setup and for providing the
614 wine samples used for the analysis.

615 Conflict of interests

616 The authors declare no conflict of interest.

617 References

- 618 Alcalde-Eon, C., Escribano-Bailón, M. T., Santos-Buelga, C., & Rivas-Gonzalo, J. C. (2006). Changes in
619 the detailed pigment composition of red wine during maturity and ageing: A comprehensive
620 study. *Analytica Chimica Acta*, *563*, 238-254. <https://doi.org/10.1016/j.aca.2005.11.028>.
- 621 Atanasova, V., Fulcrand, H., Cheynier, V., & Moutounet, M. (2002). Effect of oxygenation on
622 polyphenol changes occurring in the course of wine-making. *Analytica Chimica Acta*, *458*, 15-27.
623 [https://doi.org/10.1016/S0003-2670\(01\)01617-8](https://doi.org/10.1016/S0003-2670(01)01617-8).
- 624 Cano-López, M., Pardo-Mínguez, F., Schmauch, G., Saucier, C., Teissedre, P.-L., López-Roca, J. M., &
625 Gómez-Plaza, E. (2008). Effect of micro-oxygenation on color and anthocyanin-related
626 compounds of wines with different phenolic contents. *Journal of Agricultural and Food*
627 *Chemistry*, *56*, 5932-5941. <https://doi.org/10.1021/jf8006147>.
- 628 Castillo-Sánchez, J., Mejuto, J., Garrido, J., & García-Falcón, S. (2006). Influence of wine-making
629 protocol and fining agents on the evolution of the anthocyanin content, colour and general
630 organoleptic quality of Vinhão wines. *Food Chemistry*, *97*, 130-136.
631 <https://doi.org/10.1016/j.foodchem.2005.03.030>.
- 632 Cejudo-Bastante, M., Pérez-Coello, M., & Hermosín-Gutiérrez, I. (2011). Effect of wine micro-
633 oxygenation treatment and storage period on colour-related phenolics, volatile composition and
634 sensory characteristics. *LWT-Food Science and Technology*, *44*, 866-874.
635 <https://doi.org/10.1016/j.lwt.2010.10.015>.
- 636 Chira, K., Pacella, N., Jourdes, M., & Teissedre, P. L. (2011). Chemical and sensory evaluation of
637 Bordeaux wines (Cabernet-Sauvignon and Merlot) and correlation with wine age. *Food*
638 *Chemistry*, *126*, 1971-1977. <https://doi.org/10.1016/j.foodchem.2010.12.056>.
- 639 Cosme, F., Ricardo-da-Silva, J. M., & Laureano, O. (2008). Interactions between protein fining agents
640 and proanthocyanidins in white wine. *Food Chemistry*, *106*, 536-544.
641 <https://doi.org/10.1016/j.foodchem.2007.06.038>.
- 642 Cosme, F., Ricardo-da-Silva, J. M., & Laureano, O. (2009). Effect of various proteins on different
643 molecular weight proanthocyanidin fractions of red wine during wine fining. *American Journal of*
644 *Enology and Viticulture*, *60*, 74-81.
- 645 Ćurko, N., Kovačević Ganić, K., Gracin, L., Đapić, M., Jourdes, M., & Teissedre, P.-L. (2014).
646 Characterization of seed and skin polyphenolic extracts of two red grape cultivars grown in
647 Croatia and their sensory perception in a wine model medium. *Food Chemistry*, *145*, 15-22.
648 <https://doi.org/10.1016/j.foodchem.2013.07.131>.
- 649 Dallas, C., Ricardo-da-Silva, J. M., & Laureano, O. (1996). Interactions of oligomeric procyanidins in
650 model wine solutions containing malvidin-3-glucoside and acetaldehyde. *Journal of the Science*
651 *of Food and Agriculture*, *70*, 493-500. [https://doi.org/10.1002/\(SICI\)1097-0010\(199604\)70:4<493::AID-JSFA528>3.0.CO;2-7](https://doi.org/10.1002/(SICI)1097-0010(199604)70:4<493::AID-JSFA528>3.0.CO;2-7).
- 653 Danilewicz, J. C. (2003). Review of reaction mechanisms of oxygen and proposed intermediate reduction
654 products in wine: Central role of iron and copper. *American Journal of Enology and Viticulture*,
655 *54*, 73-85.
- 656 Danilewicz, J. C., Secombe, J. T., & Whelan, J. (2008). Mechanism of interaction of polyphenols,
657 oxygen, and sulfur dioxide in model wine and wine. *American Journal of Enology and*
658 *Viticulture*, *59*, 128-136.
- 659 Danilewicz, J. C., & Wallbridge, P. J. (2010). Further studies on the mechanism of interaction of
660 polyphenols, oxygen, and sulfite in wine. *American Journal of Enology and Viticulture*, *61*, 166-
661 175.
- 662 del Carmen Llaudy, M., Canals, R., González-Manzano, S., Canals, J. M., Santos-Buelga, C., & Zamora,
663 F. (2006). Influence of micro-oxygenation treatment before oak aging on phenolic compounds
664 composition, astringency, and color of red wine. *Journal of Agricultural and Food Chemistry*, *54*,
665 4246-4252. <https://doi.org/10.1021/jf052842t>.

666 Drinkine, J., Lopes, P., Kennedy, J. A., Teissedre, P.-L., & Saucier, C. (2007). Ethylidene-bridged flavan-
667 3-ols in red wine and correlation with wine age. *Journal of Agricultural and Food Chemistry*, *55*,
668 6292-6299. <https://doi.org/10.1021/jf070038w>.

669 Durner, D., Weber, F., Neddermeyer, J., Koopmann, K., Winterhalter, P., & Fischer, U. (2010). Sensory
670 and color changes induced by microoxygenation treatments of Pinot noir before and after
671 malolactic fermentation. *American Journal of Enology and Viticulture*, *61*, 474-485.
672 <https://doi.org/10.5344/ajev.2010.09122>

673 Es-Safi, N.-E., Fulcrand, H., Cheynier, V., & Moutounet, M. (1999). Studies on the acetaldehyde-induced
674 condensation of (-)-epicatechin and malvidin 3-O-glucoside in a model solution system. *Journal*
675 *of Agricultural and Food Chemistry*, *47*, 2096-2102. <https://doi.org/10.1021/jf9806309>.

676 Escribano-Bailón, T., Álvarez-García, M., Rivas-Gonzalo, J. C., Heredia, F. J., & Santos-Buelga, C.
677 (2001). Color and stability of pigments derived from the acetaldehyde-mediated condensation
678 between malvidin 3-O-glucoside and (+)-catechin. *Journal of Agricultural and Food Chemistry*,
679 *49*, 1213-1217. <https://doi.org/10.1021/jf001081l>.

680 Ferreira, V., Carrascon, V., Bueno, M., Ugliano, M., & Fernandez-Zurbano, P. (2015). Oxygen
681 consumption by red wines. Part I: Consumption rates, relationship with chemical composition,
682 and role of SO₂. *Journal of Agricultural and Food Chemistry*, *63*, 10928-10937.
683 <https://doi.org/10.1021/acs.jafc.5b02988>.

684 Fulcrand, H., Dueñas, M., Salas, E., & Cheynier, V. (2006). Phenolic reactions during winemaking and
685 aging. *American Journal of Enology and Viticulture*, *57*, 289-297.

686 Gambuti, A., Han, G., Peterson, A. L., & Waterhouse, A. L. (2015). Sulfur dioxide and glutathione alter
687 the outcome of microoxygenation. *American Journal of Enology and Viticulture*, *66*, 411-423.
688 <https://doi.org/10.5344/ajev.2015.15005>.

689 Gambuti, A., Picariello, L., Moio, L., & Waterhouse, A. L. (2019). Cabernet Sauvignon aging stability
690 altered by microoxygenation. *American Journal of Enology and Viticulture*, *70*, 323-331.
691 <https://doi.org/10.5344/ajev.2019.18061>.

692 Gambuti, A., Rinaldi, A., Ugliano, M., & Moio, L. (2013). Evolution of phenolic compounds and
693 astringency during aging of red wine: Effect of oxygen exposure before and after bottling.
694 *Journal of Agricultural and Food Chemistry*, *61*, 1618-1627. <https://doi.org/10.1021/jf302822b>.

695 García-Puente Rivas, E., Alcalde-Eon, C., Santos-Buelga, C., Rivas-Gonzalo, J. C., & Escribano-Bailón,
696 M. T. (2006). Behaviour and characterisation of the colour during red wine making and
697 maturation. *Analytica Chimica Acta*, *563*, 215-222. <https://doi.org/10.1016/j.aca.2005.11.044>.

698 Glories, Y. (1984a). La couleur des vins rouges. 1ere partie : Les équilibres des anthocyanes et des tanins.
699 *OENO One*, *18*. <https://doi.org/10.20870/oeno-one.1984.18.3.1751>.

700 Glories, Y. (1984b). La couleur des vins rouges. 2e partie : Mesure, origine et interprétation. *OENO One*,
701 *18*. <https://doi.org/10.20870/oeno-one.1984.18.4.1744>.

702 Gómez-Plaza, E., & Cano-López, M. (2011). A review on micro-oxygenation of red wines: Claims,
703 benefits and the underlying chemistry. *Food Chemistry*, *125*, 1131-1140.
704 <https://doi.org/10.1016/j.foodchem.2010.10.034>.

705 González-del Pozo, A., Arozarena, Í., Noriega, M.-J., Navarro, M., & Casp, A. (2010). Short- and long-
706 term effects of micro-oxygenation treatments on the colour and phenolic composition of a
707 Cabernet Sauvignon wine aged in barrels and/or bottles. *European Food Research and*
708 *Technology*, *231*, 589-601. <https://doi.org/10.1007/s00217-010-1311-x>.

709 Han, G., Webb, M. R., & Waterhouse, A. L. (2019). Acetaldehyde reactions during wine bottle storage.
710 *Food Chemistry*, *290*, 208-215. <https://doi.org/10.1016/j.foodchem.2019.03.137>.

711 Kontoudakis, N., González, E., Gil, M., Esteruelas, M., Fort, F., Canals, J. M., & Zamora, F. (2011).
712 Influence of wine pH on changes in color and polyphenol composition induced by micro-
713 oxygenation. *Journal of Agricultural and Food Chemistry*, *59*, 1974-1984.
714 <https://doi.org/10.1021/jf103038g>.

715 Kontoudakis, N., Guo, A., Scollary, G. R., & Clark, A. C. (2017). The impact of aging wine in high and
716 low oxygen conditions on the fractionation of Cu and Fe in Chardonnay wine. *Food Chemistry*,
717 229, 319-328. <https://doi.org/10.1016/j.foodchem.2017.02.065>.

718 Maury, C., Sarni-Manchado, P., Lefebvre, S., Cheynier, V., & Moutounet, M. (2001). Influence of fining
719 with different molecular weight gelatins on proanthocyanidin composition and perception of
720 wines. *American Journal of Enology and Viticulture*, 52, 140-145.

721 Morozova, K., Schmidt, O., & Schwack, W. (2014). Impact of headspace oxygen and copper and iron
722 addition on oxygen consumption rate, sulphur dioxide loss, colour and sensory properties of
723 Riesling wine. *European Food Research and Technology*, 238, 653-663.
724 <https://doi.org/10.1007/s00217-013-2142-3>.

725 Oberholster, A., Carstens, L. M., & Du Toit, W. J. (2013). Investigation of the effect of gelatine, egg
726 albumin and cross-flow microfiltration on the phenolic composition of Pinotage wine. *Food*
727 *Chemistry*, 138, 1275-1281. <https://doi.org/10.1016/j.foodchem.2012.09.128>.

728 OIV. 2009. Compendium of International Methods of Analyses of Wines and Musts. Organisation
729 Internationale de la Vigne et du Vin, Paris.

730 Parpinello, G. P., Plumejeau, F., Maury, C., & Versari, A. (2012). Effect of micro-oxygenation on
731 sensory characteristics and consumer preference of Cabernet Sauvignon wine. *Journal of the*
732 *Science of Food and Agriculture*, 92, 1238-1244. <https://doi.org/10.1002/jsfa.4688>.

733 Ribéreau-Gayon, P., & Stonestreet, E. (1965). Le dosage des anthocyanes dans les vins rouges. *Bulletin*
734 *de la Société Chimique de France*, 9, 2649-2652.

735 Ribéreau-Gayon, P., & Stonestreet, E. (1966). Dosage des tanins du vin rouge et détermination de leur
736 structure. *Chimie Analytique*, 48, 188-196.

737 Sánchez-Iglesias, M., González-Sanjosé, M. L., Pérez-Magariño, S., Ortega-Heras, M., & González-
738 Huerta, C. (2009). Effect of micro-oxygenation and wood type on the phenolic composition and
739 color of an aged red wine. *Journal of Agricultural and Food Chemistry*, 57, 11498-11509.
740 <https://doi.org/10.1021/jf902173d>.

741 Sudraud, P. (1958). Interpretation des courbes d'absorption des vins rouges. *Annals of Technology*
742 *Agriculture*, 7, 203-208.

743 Tao, J., Dykes, S. I., & Kilmartin, P. A. (2007). Effect of SO₂ concentration on polyphenol development
744 during red wine micro-oxygenation. *Journal of Agricultural and Food Chemistry*, 55, 6104-6109.
745 <https://doi.org/10.1021/jf070625h>.

746 Vidal, S., Francis, L., Noble, A., Kwiatkowski, M., Cheynier, V., & Waters, E. (2004). Taste and mouth-
747 feel properties of different types of tannin-like polyphenolic compounds and anthocyanins in
748 wine. *Analytica Chimica Acta*, 513, 57-65. <https://doi.org/10.1016/j.aca.2003.10.017>.

749 Vinković Vrček, I., Bojić, M., Žuntar I., Mendaša G. & Medić-Šarić, M. (2011) Phenol content,
750 antioxidant activity and metal composition of Croatian wines deriving from organically and
751 conventionally grown grapes. *Food Chem.* 124, 354-361.
752 <https://doi.org/10.1016/j.foodchem.2010.05.118>.

753 Wirth, J., Morel-Salmi, C., Souquet, J. M., Dieval, J. B., Aagaard, O., Vidal, S., Fulcrand, H., &
754 Cheynier, V. (2010). The impact of oxygen exposure before and after bottling on the
755 polyphenolic composition of red wines. *Food Chemistry*, 123, 107-116.
756 <https://doi.org/10.1016/j.foodchem.2010.04.008>.

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758 Figure 1. Contribution of chemical and sensory variables (A) and distribution of control and
759 micro-oxygenated Plavac mali wines (B) during aging in barrels (1, 6 and 12 months) and bottles
760 (38 months) in two dimensional coordinate system defined by the two principal components
761 (PC 1 and PC 2).

762 Table 1. Wine aging experimental design (barrel aging followed by aging in bottles).

763 Table 2. Effect of different enological treatments on proanthocyanidins concentrations and
764 structural characteristics in Plavac mali wine during aging in barrels followed by aging in bottles.

765 Table 3. Effect of different enological treatments on anthocyanins concentrations in Plavac mali
766 wine during aging in barrels followed by aging in bottles.

767 Table 4. Effect of different enological treatments on chromatic characteristics in Plavac mali
768 wine during aging in barrels followed by aging in bottles.

769 Table 5. Effect of different enological treatments on astringency and bitterness intensity in
770 Plavac mali wine during aging in barrels followed by aging in bottles.

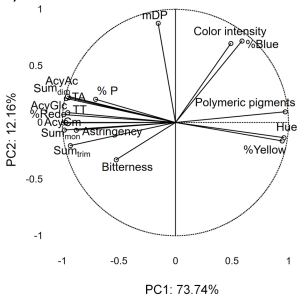
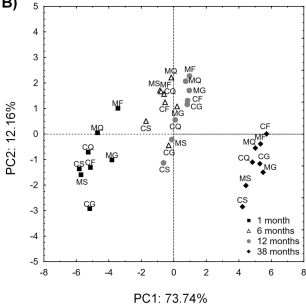
A)**B)**

Table 1. Wine aging experimental design (barrel aging followed by aging in bottles)

Treatment			
Micro-oxygenation	Aging	Description of the aging treatment*	Wine code**
Control wine (C)	Q	aging under standard conditions: 25 mg/L of free SO ₂	CQ
Micro-oxygenated wine (M)			MQ
Control wine (C)	S	aging with high content of sulfur dioxide: 40 mg/L of free SO ₂	CS
Micro-oxygenated wine (M)			MS
Control wine (C)	F	aging with high content of iron and copper: addition of 10 mg/L Fe and 0.5 mg/L Cu prior to aging (Fe was added as FeSO ₄ x 7H ₂ O, Cu was added as CuSO ₄ x 5H ₂ O); 25 mg/L of free SO ₂	CF
Micro-oxygenated wine (M)			MF
Control wine (C)	G	gelatin fining prior to aging [100 mL/hL of Gecoll supra® (Laffort, Bordeaux, France)]; 25 mg/L of free SO ₂	CG
Micro-oxygenated wine (M)			MG

*Concentrations of free SO₂ were measured/corrected every two weeks during aging in barrels and prior to bottling.

**Aging in barrels (12 months), followed by aging in bottles (38 months).

Table 2. Effect of different enological treatments on proanthocyanidins concentrations and structural characteristics in Plavac mali wine during aging in barrels followed by aging in bottles

		Aging in barrels			Aging in bottles
		1 month	6 months	12 months	38 months
TT*	<i>CQ</i>	5.95 ± 0.08 a	5.00 ± 0.06 a	4.80 ± 0.12 ab	3.89 ± 0.09 a
	<i>MQ</i>	5.40 ± 0.13 bc	5.01 ± 0.08 a	4.73 ± 0.07 ab	3.69 ± 0.06 abc
	<i>CS</i>	6.02 ± 0.17 a	5.06 ± 0.14 a	4.95 ± 0.08 a	3.93 ± 0.05 a
	<i>MS</i>	5.69 ± 0.19 ab	5.01 ± 0.05 a	4.82 ± 0.08 ab	3.88 ± 0.10 ab
	<i>CF</i>	5.38 ± 0.02 bc	4.99 ± 0.18 ab	4.67 ± 0.08 b	3.84 ± 0.04 ab
	<i>MF</i>	5.28 ± 0.07 c	4.96 ± 0.14 ab	4.35 ± 0.05 c	3.69 ± 0.02 abc
	<i>CG</i>	5.21 ± 0.15 cd	4.67 ± 0.07 bc	4.30 ± 0.07 c	3.59 ± 0.08 bc
	<i>MG</i>	4.86 ± 0.13 d	4.46 ± 0.14 c	4.24 ± 0.09 c	3.47 ± 0.24 c
Sum_{mon}**	<i>CQ</i>	85.59 ± 0.67 ab	67.66 ± 0.96 bc	63.33 ± 1.07 b	53.61 ± 0.31 a
	<i>MQ</i>	77.58 ± 0.92 d	65.75 ± 0.35 cd	61.77 ± 0.48 b	52.18 ± 0.71 ab
	<i>CS</i>	86.99 ± 0.19 a	70.51 ± 0.57 a	67.43 ± 0.16 a	53.84 ± 3.55 a
	<i>MS</i>	83.28 ± 0.49 c	68.98 ± 1.13 ab	66.12 ± 0.60 a	53.45 ± 2.01 a
	<i>CF</i>	84.52 ± 1.22 bc	66.35 ± 0.27 c	59.33 ± 0.22 c	47.90 ± 0.85 bc
	<i>MF</i>	83.13 ± 0.18 c	66.31 ± 0.60 c	62.29 ± 1.42 b	48.96 ± 0.58 bc
	<i>CG</i>	83.92 ± 0.22 bc	63.72 ± 1.84 d	62.39 ± 1.16 b	46.01 ± 0.65 c
	<i>MG</i>	82.73 ± 0.31 c	63.67 ± 0.20 d	62.80 ± 0.45 b	48.88 ± 1.14 bc
Sum_{dim}**	<i>CQ</i>	78.83 ± 0.91 ab	65.37 ± 0.99 ab	57.97 ± 0.82 c	26.61 ± 0.30 b
	<i>MQ</i>	71.86 ± 0.58 f	61.16 ± 1.06 cd	55.49 ± 0.32 d	25.57 ± 0.38 b
	<i>CS</i>	80.49 ± 0.48 a	68.10 ± 0.68 a	64.11 ± 1.08 a	27.85 ± 0.33 a
	<i>MS</i>	76.86 ± 0.31 c	64.26 ± 1.05 bc	60.71 ± 0.49 b	26.05 ± 0.38 b
	<i>CF</i>	76.49 ± 0.97 cd	61.67 ± 0.13 cd	54.08 ± 1.22 d	23.29 ± 0.21 cd
	<i>MF</i>	74.89 ± 0.32 de	63.26 ± 0.74 bcd	58.35 ± 0.95 bc	24.40 ± 0.22 c
	<i>CG</i>	77.26 ± 0.37 bc	60.57 ± 2.26 d	55.01 ± 1.08 d	21.23 ± 0.72 e
	<i>MG</i>	73.80 ± 0.76 e	60.54 ± 1.51 d	57.81 ± 0.97 c	22.83 ± 0.35 d
Sum_{trim}**	<i>CQ</i>	32.13 ± 0.35 ab	18.31 ± 0.78 abc	17.16 ± 0.24 ab	7.75 ± 0.19 ab
	<i>MQ</i>	29.26 ± 0.93 c	17.82 ± 0.26 bcd	16.98 ± 0.19 ab	7.49 ± 0.12 b
	<i>CS</i>	33.27 ± 1.09 a	19.88 ± 0.12 a	18.34 ± 0.89 a	8.04 ± 0.21 a
	<i>MS</i>	32.69 ± 0.31 a	19.57 ± 0.18 ab	17.28 ± 0.36 ab	7.66 ± 0.18 ab
	<i>CF</i>	30.91 ± 0.58 bc	18.18 ± 0.87 abcd	16.03 ± 0.69 b	7.50 ± 0.11 b
	<i>MF</i>	30.80 ± 0.35 bc	17.99 ± 0.55 abcd	16.11 ± 0.51 b	7.59 ± 0.04 b
	<i>CG</i>	30.19 ± 0.35 c	17.05 ± 1.61 cd	14.70 ± 0.08 c	6.65 ± 0.16 c
	<i>MG</i>	29.23 ± 0.51 c	16.26 ± 0.32 d	16.07 ± 0.40 b	7.36 ± 0.12 b
mDP	<i>CQ</i>	4.19 ± 0.10 bc	5.15 ± 0.10 ab	4.54 ± 0.01 d	4.23 ± 0.06 ab
	<i>MQ</i>	4.66 ± 0.12 a	5.18 ± 0.16 ab	4.79 ± 0.18 c	4.34 ± 0.08 a
	<i>CS</i>	4.18 ± 0.06 bc	5.14 ± 0.17 ab	4.46 ± 0.06 d	3.94 ± 0.06 cd
	<i>MS</i>	4.47 ± 0.13 ab	5.41 ± 0.12 a	4.51 ± 0.01 d	4.25 ± 0.01 ab
	<i>CF</i>	4.26 ± 0.08 abc	5.13 ± 0.15 ab	5.14 ± 0.14 ab	4.13 ± 0.05 b
	<i>MF</i>	4.45 ± 0.29 ab	5.29 ± 0.05 a	5.36 ± 0.03 a	4.14 ± 0.05 b
	<i>CG</i>	3.87 ± 0.13 c	4.58 ± 0.22 c	4.98 ± 0.02 bc	3.90 ± 0.08 d
	<i>MG</i>	4.46 ± 0.14 ab	4.85 ± 0.11 bc	5.17 ± 0.03 ab	4.09 ± 0.05 bc
%P	<i>CQ</i>	28.27 ± 1.27 a	25.95 ± 0.36 a	27.95 ± 0.54 a	22.07 ± 0.39 abc
	<i>MQ</i>	28.75 ± 1.24 a	26.21 ± 1.28 a	26.90 ± 1.01 ab	22.50 ± 0.35 ab
	<i>CS</i>	27.87 ± 0.18 a	25.92 ± 0.70 a	24.98 ± 0.24 cd	22.10 ± 0.42 abc
	<i>MS</i>	28.46 ± 0.72 a	26.67 ± 0.09 a	25.41 ± 0.29 bc	22.81 ± 0.25 a
	<i>CF</i>	28.04 ± 0.15 a	25.75 ± 1.15 ab	23.54 ± 0.68 d	21.72 ± 0.07 c
	<i>MF</i>	21.26 ± 0.95 b	27.35 ± 0.41 a	23.78 ± 0.22 d	21.79 ± 0.22 bc
	<i>CG</i>	26.47 ± 0.15 a	23.24 ± 1.56 b	25.60 ± 0.58 bc	21.75 ± 0.14 bc
	<i>MG</i>	22.09 ± 1.21 b	25.71 ± 0.55 ab	25.39 ± 0.17 bc	22.74 ± 0.04 a

Data are presented as average value of three replications ± standard deviation (n=3). *Values expressed in g/L.

**Values expressed in mg/L. ANOVA to compare data; different letters indicate significant differences between wines of all treatments at the same time (Tukey's test, p<0.05). Abbreviations: TT, total tannins; Sum_{mon}, sum of flavan-3-ol monomers; Sum_{dim}, sum of flavan-3-ol dimers; Sum_{trim}, sum of flavan-3-ol trimers; mDP, mean degree of polymerization; %P, percentage of prodelphinidins; C, control wine; M, micro-oxygenated wine; Q, aging under "standard" conditions; S, aging with high concentration of sulfur dioxide; F, aging with high concentration of iron and copper; G, gelatin fining prior to aging.

Table 3. Effect of different enological treatments on anthocyanins concentrations in Plavac mali wine during aging in barrels followed by aging in bottles

		Aging in barrels			Aging in bottles
		1 month	6 months	12 months	38 months
TA	<i>CQ</i>	321.6 ± 7.1 b	242.9 ± 8.3 b	208.8 ± 15.4 c	63.8 ± 1.2 bc
	<i>MQ</i>	292.4 ± 3.6 cd	236.5 ± 6.3 b	217.3 ± 16.7 bc	65.4 ± 0.9 bc
	<i>CS</i>	360.6 ± 10.9 a	293.1 ± 1.8 a	277.3 ± 9.7 a	72.9 ± 1.7 a
	<i>MS</i>	313.0 ± 3.0 bc	284.3 ± 1.1 a	274.7 ± 11.5 a	74.2 ± 1.4 a
	<i>CF</i>	288.5 ± 1.1 d	227.5 ± 7.1 a	208.0 ± 7.9 c	67.1 ± 1.1 b
	<i>MF</i>	281.1 ± 15.0 d	247.2 ± 7.3 b	239.3 ± 3.8 b	71.5 ± 1.1 a
	<i>CG</i>	294.3 ± 9.8 cd	239.3 ± 5.6 b	207.7 ± 2.1 c	61.7 ± 0.8 c
	<i>MG</i>	286.7 ± 7.9 d	236.9 ± 11.8 b	219.9 ± 8.6 bc	64.8 ± 1.8 bc
AcyGlc	<i>CQ</i>	161.40 ± 0.87 c	97.69 ± 1.53 cde	83.72 ± 0.75 e	9.81 ± 0.40 d
	<i>MQ</i>	159.48 ± 0.38 cd	93.24 ± 0.52 de	87.68 ± 1.27 d	9.78 ± 0.06 d
	<i>CS</i>	174.64 ± 1.86 a	121.04 ± 1.18 a	113.44 ± 1.31 a	16.27 ± 1.46 a
	<i>MS</i>	173.82 ± 1.53 a	115.05 ± 2.85 b	110.81 ± 0.59 b	18.06 ± 1.15 a
	<i>CF</i>	149.57 ± 0.30 e	92.45 ± 2.76 e	79.39 ± 0.74 f	12.85 ± 0.41 bc
	<i>MF</i>	149.32 ± 0.84 e	103.06 ± 0.55 c	91.32 ± 1.10 c	13.97 ± 0.66 b
	<i>CG</i>	166.50 ± 1.36 b	98.80 ± 3.26 cd	85.61 ± 0.71 de	9.69 ± 0.95 d
	<i>MG</i>	156.67 ± 1.18 d	94.63 ± 2.73 de	90.95 ± 0.34 c	11.69 ± 0.11 cd
AcyAc	<i>CQ</i>	13.64 ± 0.02 c	10.38 ± 0.10 de	9.32 ± 0.11 cd	1.22 ± 0.08 a
	<i>MQ</i>	14.27 ± 0.06 b	10.59 ± 0.03 cd	9.57 ± 0.10 bc	1.09 ± 0.14 a
	<i>CS</i>	14.74 ± 0.16 a	12.21 ± 0.10 a	11.30 ± 0.04 a	1.31 ± 0.11 a
	<i>MS</i>	14.93 ± 0.16 a	12.06 ± 0.10 a	10.90 ± 0.29 a	1.32 ± 0.11 a
	<i>CF</i>	13.60 ± 0.07 c	10.26 ± 0.11 e	9.04 ± 0.15 d	1.22 ± 0.01 a
	<i>MF</i>	13.16 ± 0.15 d	11.21 ± 0.08 b	9.84 ± 0.23 b	1.05 ± 0.18 a
	<i>CG</i>	14.72 ± 0.13 a	10.76 ± 0.07 c	9.56 ± 0.05 bc	1.20 ± 0.08 a
	<i>MG</i>	13.39 ± 0.09 cd	10.48 ± 0.09 de	9.79 ± 0.21 bc	1.12 ± 0.04 a
AcyCm	<i>CQ</i>	26.72 ± 0.24 c	13.73 ± 0.33 bc	10.85 ± 0.20 c	0.57 ± 0.09 c
	<i>MQ</i>	26.79 ± 0.05 c	14.07 ± 0.09 bc	11.37 ± 0.14 bc	0.82 ± 0.11 abc
	<i>CS</i>	28.68 ± 0.43 a	16.59 ± 0.34 a	14.87 ± 0.44 a	1.02 ± 0.23 ab
	<i>MS</i>	28.66 ± 0.30 a	16.31 ± 0.69 a	14.63 ± 0.07 a	1.16 ± 0.29 a
	<i>CF</i>	25.67 ± 0.29 de	13.08 ± 0.31 c	10.14 ± 0.31 d	0.58 ± 0.11 c
	<i>MF</i>	25.39 ± 0.17 e	14.73 ± 0.36 b	11.91 ± 0.29 b	0.74 ± 0.10 abc
	<i>CG</i>	27.52 ± 0.20 b	13.89 ± 0.37 bc	10.91 ± 0.07 c	0.50 ± 0.07 c
	<i>MG</i>	26.26 ± 0.12 cd	13.80 ± 0.29 bc	11.85 ± 0.05 b	0.62 ± 0.03 bc

Data are presented in mg/L as average value of three replications ± standard deviation (n=3). ANOVA to compare data; different letters indicate significant differences between wines of all treatments at the same time (Tukey's test, p<0.05). Abbreviations: TA, total anthocyanins; AcyGlc, sum of anthocyanin glucosides; AcyAc, sum of anthocyanin acetylglucosides; AcyCm, sum of anthocyanin coumaroylglucosides; C, control wine; M, micro-oxygenated wine; Q, aging under "standard" conditions; S, aging with high concentration of sulfur dioxide; F, aging with high concentration of iron and copper; G, gelatin fining prior to aging.

Table 4. Effect of different enological treatments on chromatic characteristics in Plavac mali wine during aging in barrels followed by aging in bottles

		Aging in barrels			Aging in bottles
		1 month	6 months	12 months	38 months
PP (%)	<i>CQ</i>	55.83 ± 0.60 abc	69.69 ± 1.25 abc	74.54 ± 0.53 b	81.44 ± 1.04 d
	<i>MQ</i>	56.17 ± 0.99 ab	71.70 ± 1.41 ab	75.81 ± 0.72 ab	85.28 ± 1.10 abc
	<i>CS</i>	53.65 ± 0.61 d	67.07 ± 0.50 c	74.75 ± 1.42 ab	83.64 ± 1.06 bcd
	<i>MS</i>	54.69 ± 0.58 abcd	68.50 ± 0.43 bc	75.58 ± 0.55 ab	83.00 ± 0.67 cd
	<i>CF</i>	54.17 ± 0.61 cd	69.57 ± 1.97 abc	75.18 ± 1.65 ab	85.85 ± 0.85 ab
	<i>MF</i>	56.40 ± 0.66 a	70.10 ± 1.02 abc	78.42 ± 0.64 a	86.52 ± 0.82 a
	<i>CG</i>	54.52 ± 0.20 bcd	71.32 ± 1.45 ab	75.29 ± 1.85 ab	82.95 ± 0.32 cd
	<i>MG</i>	54.79 ± 0.30 abcd	73.08 ± 1.49 a	75.61 ± 0.99 ab	83.52 ± 1.60 bcd
CI	<i>CQ</i>	7.89 ± 0.05 bc	8.85 ± 0.11 ab	8.85 ± 0.15 bc	8.89 ± 0.10 ab
	<i>MQ</i>	8.04 ± 0.10 b	9.04 ± 0.02 a	9.47 ± 0.10 a	8.77 ± 0.10 bc
	<i>CS</i>	7.44 ± 0.01 bcd	8.23 ± 0.14 de	7.68 ± 0.07 e	8.00 ± 0.11 e
	<i>MS</i>	7.28 ± 0.07 cd	8.25 ± 0.22 cde	8.12 ± 0.12 d	7.85 ± 0.09 e
	<i>CF</i>	7.90 ± 0.10 bc	8.58 ± 0.07 bc	9.23 ± 0.12 ab	9.14 ± 0.14 a
	<i>MF</i>	9.54 ± 0.33 a	8.43 ± 0.13 cd	9.41 ± 0.20 a	8.87 ± 0.12 abc
	<i>CG</i>	7.13 ± 0.07 d	8.03 ± 0.02 e	8.85 ± 0.26 bc	8.55 ± 0.07 cd
	<i>MG</i>	7.80 ± 0.23 bcd	8.21 ± 0.12 de	8.68 ± 0.09 c	8.41 ± 0.16 d
Hue	<i>CQ</i>	0.688 ± 0.003 ab	0.823 ± 0.006 ab	0.858 ± 0.005 bc	1.014 ± 0.018 abc
	<i>MQ</i>	0.674 ± 0.008 b	0.817 ± 0.008 b	0.834 ± 0.007 c	0.984 ± 0.019 c
	<i>CS</i>	0.708 ± 0.002 a	0.848 ± 0.017 a	0.912 ± 0.024 a	1.026 ± 0.007 ab
	<i>MS</i>	0.694 ± 0.013 ab	0.847 ± 0.007 a	0.880 ± 0.009 b	1.029 ± 0.009 a
	<i>CF</i>	0.684 ± 0.001 b	0.819 ± 0.006 b	0.873 ± 0.015 b	1.020 ± 0.015 abc
	<i>MF</i>	0.698 ± 0.005 ab	0.818 ± 0.009 b	0.837 ± 0.002 c	0.997 ± 0.002 abc
	<i>CG</i>	0.691 ± 0.006 ab	0.822 ± 0.010 ab	0.835 ± 0.003 c	0.998 ± 0.014 abc
	<i>MG</i>	0.687 ± 0.016 ab	0.805 ± 0.009 b	0.841 ± 0.004 c	0.990 ± 0.010 bc
% Red	<i>CQ</i>	52.4 ± 0.1 abc	48.1 ± 0.2 abc	47.1 ± 0.3 ab	43.8 ± 0.2 abc
	<i>MQ</i>	52.8 ± 0.4 ab	48.0 ± 0.2 abc	47.4 ± 0.3 ab	43.9 ± 0.0 abc
	<i>CS</i>	51.8 ± 0.1 bc	47.6 ± 0.5 bc	46.2 ± 0.6 d	43.5 ± 0.1 bc
	<i>MS</i>	52.6 ± 0.5 ab	47.4 ± 0.3 c	46.9 ± 0.2 bcd	43.5 ± 0.2 c
	<i>CF</i>	52.8 ± 0.1 ab	48.2 ± 0.2 abc	46.6 ± 0.3 cd	42.8 ± 0.2 d
	<i>MF</i>	51.4 ± 0.3 c	48.1 ± 0.2 abc	47.5 ± 0.1 ab	43.6 ± 0.1 abc
	<i>CG</i>	53.0 ± 0.1 a	48.3 ± 0.2 ab	47.8 ± 0.1 a	44.0 ± 0.3 a
	<i>MG</i>	52.4 ± 0.7 ab	48.4 ± 0.3 a	47.5 ± 0.1 ab	44.0 ± 0.3 ab
% Yellow	<i>CQ</i>	36.0 ± 0.1 abc	39.6 ± 0.2 bc	40.4 ± 0.0 cd	44.2 ± 0.2 ab
	<i>MQ</i>	35.6 ± 0.1 c	39.2 ± 0.2 c	39.5 ± 0.1 e	43.5 ± 0.2 c
	<i>CS</i>	36.7 ± 0.1 a	40.3 ± 0.4 a	42.1 ± 0.6 a	44.7 ± 0.2 a
	<i>MS</i>	36.5 ± 0.5 a	40.2 ± 0.2 ab	41.2 ± 0.3 b	44.7 ± 0.3 a
	<i>CF</i>	36.1 ± 0.1 abc	39.4 ± 0.2 bc	40.6 ± 0.4 bc	44.0 ± 0.2 bc
	<i>MF</i>	35.8 ± 0.1 bc	39.3 ± 0.3 c	39.7 ± 0.1 de	43.4 ± 0.1 c
	<i>CG</i>	36.6 ± 0.2 a	39.7 ± 0.3 abc	39.9 ± 0.1 cde	43.9 ± 0.3 bc
	<i>MG</i>	36.0 ± 0.4 abc	39.0 ± 0.3 c	39.9 ± 0.1 cde	43.6 ± 0.2 bc
% Blue	<i>CQ</i>	11.6 ± 0.1 b	12.3 ± 0.1 ab	12.5 ± 0.2 bc	12.1 ± 0.0 de
	<i>MQ</i>	11.6 ± 0.3 b	12.8 ± 0.2 a	13.1 ± 0.2 a	12.6 ± 0.1 bc
	<i>CS</i>	11.5 ± 0.0 b	12.1 ± 0.2 b	11.7 ± 0.1 e	11.8 ± 0.1 e
	<i>MS</i>	11.0 ± 0.3 bc	12.4 ± 0.3 ab	11.9 ± 0.1 de	11.8 ± 0.1 e
	<i>CF</i>	11.2 ± 0.2 bc	12.4 ± 0.0 ab	12.8 ± 0.2 ab	13.2 ± 0.3 a
	<i>MF</i>	12.8 ± 0.2 a	12.6 ± 0.1 a	12.9 ± 0.2 ab	13.0 ± 0.2 ab
	<i>CG</i>	10.4 ± 0.1 c	12.0 ± 0.2 b	12.2 ± 0.1 c	12.0 ± 0.1 de
	<i>MG</i>	11.5 ± 0.3 b	12.6 ± 0.3 a	12.6 ± 0.0 bc	12.4 ± 0.1 cd

Data are presented as average value of three replications ± standard deviation (n=3). ANOVA to compare data; different letters indicate significant differences between wines of all treatments at the same time (Tukey's test, p<0.05). Abbreviations: PP; polymeric pigments; CI, color intensity; C, control wine; M, micro-oxygenated wine; Q, aging under "standard" conditions; S, aging with high concentration of sulfur dioxide; F, aging with high concentration of iron and copper; G, gelatin fining prior to aging.

Table 5. Effect of different enological treatments on astringency and bitterness intensity in Plavac mali wine during aging in barrels followed by aging in bottles

		Aging in barrels			Aging in bottles
		1 month	6 months	12 months	38 months
Astringency intensity	<i>CQ</i>	5.8 ± 0.8 a	4.8 ± 0.5 ab	4.7 ± 0.8 a	2.9 ± 0.5 ab
	<i>MQ</i>	5.3 ± 0.8 ab	4.2 ± 0.7 b	3.6 ± 0.7 cd	2.8 ± 0.6 b
	<i>CS</i>	5.8 ± 1.0 a	5.3 ± 1.0 a	4.8 ± 1.1 a	3.5 ± 0.4 a
	<i>MS</i>	5.4 ± 0.9 ab	4.0 ± 1.0 b	3.8 ± 0.9 bc	3.5 ± 0.5 a
	<i>CF</i>	5.9 ± 0.7 a	4.7 ± 1.1 ab	4.5 ± 1.3 ab	2.9 ± 0.6 ab
	<i>MF</i>	5.7 ± 0.5 a	4.3 ± 1.1 b	3.2 ± 0.6 d	2.9 ± 0.7 ab
	<i>CG</i>	5.0 ± 0.6 b	4.1 ± 0.9 b	3.3 ± 1.0 cd	2.3 ± 0.7 bc
	<i>MG</i>	4.2 ± 0.7 c	3.1 ± 1.2 c	2.9 ± 0.5 d	2.0 ± 0.5 c
Bitterness intensity	<i>CQ</i>	3.3 ± 0.9 a	2.8 ± 0.8 b	3.6 ± 0.9 ab	3.0 ± 0.8 b
	<i>MQ</i>	3.2 ± 0.9 a	2.7 ± 1.1 b	3.0 ± 1.0 ab	3.0 ± 0.8 b
	<i>CS</i>	3.8 ± 1.1 a	3.8 ± 1.2 a	3.7 ± 1.2 ab	3.9 ± 0.7 a
	<i>MS</i>	3.8 ± 0.9 a	2.8 ± 1.3 b	3.1 ± 1.3 ab	3.0 ± 0.6 b
	<i>CF</i>	3.7 ± 1.2 a	3.1 ± 1.2 ab	3.9 ± 1.2 a	2.6 ± 0.8 b
	<i>MF</i>	3.6 ± 1.2 a	3.1 ± 0.7 ab	3.4 ± 1.2 ab	2.9 ± 0.7 b
	<i>CG</i>	3.6 ± 0.7 a	3.5 ± 0.9 ab	2.9 ± 1.0 ab	2.4 ± 0.7 b
	<i>MG</i>	3.4 ± 0.8 a	2.7 ± 0.7 b	2.7 ± 0.5 b	2.8 ± 0.7 b

Data presented as average value of two repetitions ± standard deviation assessed by trained panel (n=2). ANOVA to compare data; different letters indicate significant differences between wines of all treatments at the same time (Duncan's test, p<0.05). Abbreviations: C, control wine; M, micro-oxygenated wine; Q, aging under "standard" conditions; S, aging with high concentration of sulfur dioxide; F, aging with high concentration of iron and copper; G, gelatin fining prior to aging.