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

Natka Ćurko, Karin Kovačević Ganić, Marina Tomašević, Leo Gracin, Michael Jourdes, Pierre-Louis Teissèdre

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

Natka Ćurko[a], Karin Kovačević Ganić[a], Marina Tomašević[a], Leo Gracin[b] Michael Jourdes[cd], Pierre-Louis Teissedre[cd]*

[a] University of Zagreb, Faculty of Food Technology and Biotechnology, Pierottijeva 6, 10000 Zagreb, Croatia

[b] University of Split, University Department of Marine Studies, Ulica Ruđera Boškovića 37, 21000 Split, Croatia

[c] University of Bordeaux, ISVV, EA 4577 Œnologie, 210 Chemin de Leysotte, 33140 Villenave d'Ornon, France

[d] INRA, ISVV, USC 1366 Œnologie, 210 Chemin de Leysotte, 33140 Villenave d'Ornon, France

* Corresponding author. Tel.: + 33 (0)5 57 57 58 50; fax + 33 (0)5 57 57 58 13

E-mail: pierre-louis.teissedre@u-bordeaux.fr (P.L Teissedre).

Other authors e-mails:

natka.curko@pbf.unizg.hr (Natka Ćurko)

karin.kovacevic.ganic@pbf.unizg.hr (Karin Kovačević Ganić)

marina.tomasevic@pbf.unizg.hr (Marina Tomašević)

lgracin@unist.hr (Leo Gracin)

michael.jourdes@u-bordeaux.fr (Michael Jourdes)

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; 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; TA, total anthocyanins; AcyAc, sum of anthocyanin acetylglucosides; AcyCm, sum of anthocyanin coumaroylglucosides; AcyGlc, sum of anthocyanin glucosides; CI, color intensity; H, hue; PP; polymeric pigments; PCA, principal component analysis.

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

Abstract:

This work aimed to study long-term impact of micro-oxygenation and/or different aging treatments: (i) high SO_2, (ii) high Fe with Cu and (iii) gelatin fining on Plavac mali red wine phenolic and in-mouthfeel sensory development in barrels and furthermore in bottles. Results showed that outcomes of micro-oxygenation strongly depend on aging treatments. High SO_2 concentration during aging in barrels and bottles delayed typical phenolic changes and slightly contributed to astringency and lower color intensity, particularly in wine that was not micro-oxygenated. High metal concentrations and gelatin fining promoted intensive polymerization of proanthocyanins and a lower percentage of prodelphinidins after long-term aging in barrels. Also, flavan-3-ol and anthocyanins transformation rates in micro-oxygenated wines of both treatments significantly differed from their controls. Gelatin fining proved to be a very effective treatment for astringency reduction, particularly when combined with micro-oxygenation, but fined wines after long term aging in bottles showed lower color intensity.

1. Introduction

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

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

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

as well as positive long effects of this technique on wine astringency decrease for the wine with lower pH (Gambuti et al., 2013). However, there are very limited data of how different oxido-reductive conditions, like different concentrations of sulfur dioxide or metals, can impact the long-term outcomes of micro-oxygenation. Namely, iron in the association with copper is an essential catalyst in oxidative processes of wine, and the addition of these metals to wine was found to accelerate the phenolic reactions with oxygen (Danilewicz & Wallbridge, 2010). On the other hand, the presence of sulfur dioxide suppresses these reactions (Tao, Dykes, & Kilmartin, 2007), since sulfite reacts with hydrogen peroxide that is produced when phenols are oxidized, and also largely reduces the quinones back to their original catechols (Danilewicz, Seccombe, & Whelan, 2008; Danilewicz & Wallbridge, 2010). Moreover, sulfur dioxide is able to modulate reactions initiated by micro-oxygenation, since micro-oxygenated wines with lower concentrations of sulfur dioxide showed increase in acetaldehyde concentration, and concentration of ethyl-bridged compounds and pyranoanthocyanins (Gambuti et al., 2015). Also, more recent study of Gambuti et al. (2019) indicated that sulfur dioxide added before micro-oxygenation slowed wine oxidation reactions during aging and affected wine color stability inducing color loss. Hence since both SO_2 (Gambuti et al., 2015; Gambuti et al., 2019) and metals (Morozova, Schmidt, & Schwack, 2014) showed to impact oxygen consumption rates as well as some chemical and sensory characteristics of wine, they should also be taken into account when oxygen management strategy is defined.

Another enological treatment that can be successfully used to eliminate substances of colloidal nature, “soften” the wine and reduce astringency, as well as to improve wine stabilization is gelatin fining. The function of gelatin finning is dominantly oriented towards 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

This study was carried out with a red wine from *Vitis vinifera* cv. Plavac mali crni grapes that were harvested in their technological maturity in vintage 2010 (reducing sugars, 232.3 g/L; pH, 3.45; total acidity 5.50 g/L as tartaric acid). Twenty tons of grapes were used for winemaking protocol of the experimental trail that was set in "Jako vino" winery (Bol, Croatia). Immediately after the harvest grapes were destemmed and crushed (Bucher vaslin, Delta E2, Niederweningen, Zürich), sulfited (40 mg/L of total SO_2) and pumped into stainless steel fermentation tank. After six hours, the must was inoculated with yeast (Zymaflore FX10®, Laffort, Bordeaux, France) coupled to the rehydration yeast nutrient (Dynastart®, Laffort, Bordeaux, France). At the second third of fermentation, ammonium salts and thiamine yeast nutrients (Thiazote SP®, Laffort, Bordeaux, France) were added. The fermentation/maceration temperature was kept under 25 °C. After 14 days of maceration, when alcoholic fermentation was finished, wine was racked and pressed (Bucher vaslin, XPF, Niederweningen, Zürich).

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

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

Once the micro-oxygenation process was completed, lactic acid bacteria (Lactoenos 450 PreAc®, Laffort, Bordeaux, France) were added to both, control (C) and micro-oxygenated wine (M). After malolactic fermentation was over, SO_2 was corrected in C and M wine to leave the wines with a free SO_2 content of 25 mg/L.

2.2. Oak-barrel wine aging trails

After malolactic fermentation was over, the control (C) and micro-oxygenated (M) lot were distributed in oak barrels for aging. Conventional wine analyses of these wines were performed according to official OIV methods using standard equipment (OIV 2009). The initial parameters of C wine were: density, 0.9903 g/L; ethanol, 14.05 (% vol); dry extract, 32.9 g/L; reducing sugars, 3.39 g/L; total SO_2, 38 mg/L; free SO_2, 26 mg/L; pH, 3.75; total acidity 4.73 g/L (as tartaric acid); volatile acidity, 0.25 g/L (as acetic acid); lactic acid, 1.03 g/L; while malic acid was not detected. The initial parameters of M wine were: density, 0.9903 g/L; ethanol, 14.06 (% vol); dry extract, 32.7 g/L; reducing sugars, 3.21 g/L; total SO_2, 37 mg/L; free SO_2, 24 mg/L; pH, 3.77; total acidity 4.75 g/L (as tartaric acid); volatile acidity, 0.30 g/L (as acetic acid); lactic acid, 1.14 g/L; while malic acid was not detected.

Different enological treatments were applied to C and the same to the M wines prior/during the aging in barrels. The treatments applied were: (i) Treatment Q - aging under standard aging conditions; (ii) Treatment S - aging with high content of SO_2, (iii) Treatment F – aging with high content of iron and copper and (iv) Treatment G – gelatin fining prior to aging. Detailed description of treatments with doses employed are shown in Table 1. Oak-barrel wine 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 SO_2 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 Ćurko 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

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

2.6. Structural characterization of wine proanthocyanidins

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

2.7. HPLC analysis of anthocyanins

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

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

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

2.9. Data analysis

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

3. Results and discussion

3.1. Effect of enological treatments on proanthocyanidins concentrations and structural 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 SO_2) 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

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

Aging with 40 mg/L of free SO_2 represented as Treatment S (higher SO_2 content), did not influence the development of total tannins as much as flavan-3-ols. In general, CS and MS wines of Treatment S evolved in similar manner as earlier described for CQ and MQ of Treatment Q, with slightly higher concentrations in CS than MS wine. Furthermore, changes of concentrations of flavan-3-ols during aging in barrels were suppressed in wines containing higher levels of SO_2, which became even more pronounced with the time. For instance, results obtained after 12 months of aging in barrels showed significantly higher concentrations of flavan-3-ol monomers and dimers in CS than CQ wine, as well as in MS than MQ wine, receptively. Earlier study of Tao et al. (2007) also showed strong decrease of flavan-3-ols in wine with lower concentration of SO_2, and demonstrated moderating effect of SO_2 on the interaction of oxygen with polyphenols due to its ability to reduce oxidized polyphenols and remove peroxide (Danilewicz et al., 2008). More recently, Gambuti et al. (2015) reported that SO_2 and glutathione alter the outcome of

343 micro-oxygenation. Aforementioned results imply that free SO_2 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).

Gelatin fining (Treatment G) applied prior to aging, showed significant impact on the evolution of total tannins and flavanols during aging (Table 2). Wines of this treatment, particularly micro-oxygenated wine (MG), showed significantly lower concentrations of total tannins compared to wines of Treatment Q. Contrary to total tannins, trends in the evolution of flavan-3-ols among control and micro-oxygenated wines of Treatment G were quite different from those observed in Treatment Q. Namely, concentrations of flavan-3-ol dimers and trimers at the end of aging in barrels and bottles were significantly higher in MG than CG wine, which was not the case for MQ and CQ wines. Identical evolution among control and micro-oxygenated wines were earlier noticed for Treatment F, but in the case of Treatment G significant differences were kept even in bottles. As earlier explained, degradation of ethyl-linked compounds (del Carmen Llaudy et al., 2006) might be responsible for observed results in micro-oxygenated wines. However, specific evolution of flavan-3-ol dimers and trimers in MG wine imply that intensity of aforementioned changes in micro-oxygenated can be affected by gelatin fining. Moreover, this can be further supported by the fact that very different pattern was observed when gelatin fining was applied on control wine (CG). In addition, CG wine compared to CQ wine showed significantly lower concentrations in the sum of dimers and trimers after 12 months of aging, as well as significantly lower concentrations in the sum of monomers, dimers and timers after aging in bottles. Contrary, MG wine compared to MQ wine showed significantly higher concentrations in the sum of dimers, but still slightly lower concentrations in the sum of trimers after 12 months of aging, while after aging in bottles MG wine was characterized with significantly lower concentrations of dimers and just slightly lower concentrations of monomers than MQ wine. Hence, despite the specific evolution, particularly of MG wine, both wines of 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 SO_2
413 contributed to the significantly lower mDP, particularly in CS wine, which was expected since
414 SO_2 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 where 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 O_2 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 SO_2
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

moderating effect of SO_2 on the interaction of oxygen with wine polyphenols were previously noticed in the experiments with high SO_2 content (Gambuti et al., 2015; Gambuti et al., 2019; Tao et al., 2007). SO_2 protects polyphenolic compounds from oxidation (because it reacts with hydrogen peroxide, and thus prevents the formation of acetaldehyde) and also reduces the resulting quinones back to their original catechol (Danilewicz et al., 2008; Danilewicz & Wallbridge, 2010). Furthermore, high SO_2 concentrations induced significant changes in chromatic characteristics, particularly significantly lower color intensity and % blue , as well as higher hue and % yellow, as previously shown (Gambuti et al., 2015; Tao et al., 2007). Obtained results can partly be explained by bleaching effect of SO_2 on anthocyanins, by nucleophilic addition at the C4 position in the C ring of the anthocyanin in the flavylium cation (Danilewicz et al., 2008). Also, possibility that high SO_2 concentrations during aging supported predominance of more stable pigments like carboxypyranoanthocyanins, represented by vitisin A that caused shift towards orange coloration cannot be excluded (Wirth et al., 2010).

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

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

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

intensity in micro-oxygenated wines (Cejudo-Bastante et al., 2011), others show little or no impact (del Carmen Llaudy et al., 2006). Furthermore, both wines of Treatment S contributed to slightly higher astringency intensity than CQ wine, as well as significantly higher astringency intensity than MQ wine after 38 months of aging in bottles. Moreover, significantly higher bitterness intensity was found in CS wine compared to wines of all treatments. These results are consistent with earlier studies (Gambuti et al., 2019; Tao et al., 2007) proposing that concentrations of SO_2 can affect the polyphenol chemistry and wine astringency. Also, slightly lower astringency intensity was found in wines of Treatment F than Treatment Q after 12 months of aging, but these effects were lost in bottles. Wines subjected to gelatin fining showed in general much lower astringency intensity, which was not surprising, knowing the impact of this treatment to proanthocyanidins concentration and composition. Lower astringency in gelatin-treated wines due to the lower tannin levels was earlier established (Maury et al., 2001). Also, these wines achieved generally higher ratings for organoleptic quality, due to the significantly lower "fine emery" and "adhesive", meaning that gelatin non-treated wines had rougher surface smoothness compared to treated ones (Oberholster et al., 2013). Moreover, gelatin fining was particularly effective in MG wine.

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

4. Conclusions

Changes in phenolic composition and sensory characteristic of Plavac mali wine were affected both by micro-oxygenation and different aging conditions (SO_2, metals and gelatin fining). High concentration of SO_2 (40 mg/L of free SO_2) compared to standard concentration (25 mg/L of free SO_2) slowed down decrease of flavan-3-ols and particularly anthocyanins. Wines of this treatment finished the aging with lower color intensity and higher astringency, particularly control wine. The addition of metals (10 mg/L of Fe with 0.5 mg/L of Cu) accelerated decrease of flavan-3-ols and anthocyanins during aging in barrels, but only in control wine of this treatment, while micro-oxygenated wine showed higher concertation of these compounds. Also, higher concentration of metals promoted continuous polymerization among proanthocyanidins, but only in barrels, as well as lower % P after 12 months of aging. Moreover, this treatment was characterized with continuous increase in % blue in barrels and bottles, while it had little or no impact to wine astringency and bitterness. Gelatin finning primary affected wine proanthocyanidins, promoted constant increase of mDP in barrels, but very sharp drop in bottles. Nevertheless, it produced wines with significantly lower astringency through all aging period, particularly the micro-oxygenated one; as well as lower color intensity after long-term aging in bottles.

Acknowledgements

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

Conflict of interests

The authors declare no conflict of interest.

References

Alcalde-Eon, C., Escribano-Bailón, M. T., Santos-Buelga, C., & Rivas-Gonzalo, J. C. (2006). Changes in the detailed pigment composition of red wine during maturity and ageing: A comprehensive study. *Analytica Chimica Acta, 563*, 238-254. https://doi.org/10.1016/j.aca.2005.11.028.

Atanasova, V., Fulcrand, H., Cheynier, V., & Moutounet, M. (2002). Effect of oxygenation on polyphenol changes occurring in the course of wine-making. *Analytica Chimica Acta, 458*, 15-27. https://doi.org/10.1016/S0003-2670(01)01617-8.

Cano-López, M., Pardo-Mínguez, F., Schmauch, G., Saucier, C., Teissedre, P.-L., López-Roca, J. M., & Gómez-Plaza, E. (2008). Effect of micro-oxygenation on color and anthocyanin-related compounds of wines with different phenolic contents. *Journal of Agricultural and Food Chemistry, 56*, 5932-5941. https://doi.org/10.1021/jf8006147.

Castillo-Sánchez, J., Mejuto, J., Garrido, J., & García-Falcón, S. (2006). Influence of wine-making protocol and fining agents on the evolution of the anthocyanin content, colour and general organoleptic quality of Vinhão wines. *Food Chemistry, 97*, 130-136. https://doi.org/10.1016/j.foodchem.2005.03.030.

Cejudo-Bastante, M., Pérez-Coello, M., & Hermosín-Gutiérrez, I. (2011). Effect of wine micro-oxygenation treatment and storage period on colour-related phenolics, volatile composition and sensory characteristics. *LWT-Food Science and Technology, 44*, 866-874. https://doi.org/10.1016/j.lwt.2010.10.015.

Chira, K., Pacella, N., Jourdes, M., & Teissedre, P. L. (2011). Chemical and sensory evaluation of Bordeaux wines (Cabernet-Sauvignon and Merlot) and correlation with wine age. *Food Chemistry, 126*, 1971-1977. https://doi.org/10.1016/j.foodchem.2010.12.056.

Cosme, F., Ricardo-da-Silva, J. M., & Laureano, O. (2008). Interactions between protein fining agents and proanthocyanidins in white wine. *Food Chemistry, 106*, 536-544. https://doi.org/10.1016/j.foodchem.2007.06.038.

Cosme, F., Ricardo-da-Silva, J. M., & Laureano, O. (2009). Effect of various proteins on different molecular weight proanthocyanidin fractions of red wine during wine fining. *American Journal of Enology and Viticulture, 60*, 74-81.

Ćurko, N., Kovačević Ganić, K., Gracin, L., Đapić, M., Jourdes, M., & Teissedre, P.-L. (2014). Characterization of seed and skin polyphenolic extracts of two red grape cultivars grown in Croatia and their sensory perception in a wine model medium. *Food Chemistry, 145*, 15-22. https://doi.org/10.1016/j.foodchem.2013.07.131.

Dallas, C., Ricardo-da-Silva, J. M., & Laureano, O. (1996). Interactions of oligomeric procyanidins in model wine solutions containing malvidin-3-glucoside and acetaldehyde. *Journal of the Science 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.

Danilewicz, J. C. (2003). Review of reaction mechanisms of oxygen and proposed intermediate reduction products in wine: Central role of iron and copper. *American Journal of Enology and Viticulture, 54*, 73-85.

Danilewicz, J. C., Seccombe, J. T., & Whelan, J. (2008). Mechanism of interaction of polyphenols, oxygen, and sulfur dioxide in model wine and wine. *American Journal of Enology and Viticulture, 59*, 128-136.

Danilewicz, J. C., & Wallbridge, P. J. (2010). Further studies on the mechanism of interaction of polyphenols, oxygen, and sulfite in wine. *American Journal of Enology and Viticulture, 61*, 166-175.

del Carmen Llaudy, M., Canals, R., González-Manzano, S., Canals, J. M., Santos-Buelga, C., & Zamora, F. (2006). Influence of micro-oxygenation treatment before oak aging on phenolic compounds composition, astringency, and color of red wine. *Journal of Agricultural and Food Chemistry, 54*, 4246-4252. https://doi.org/10.1021/jf052842t.

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

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

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

Escribano-Bailón, T., Álvarez-García, M., Rivas-Gonzalo, J. C., Heredia, F. J., & Santos-Buelga, C. (2001). Color and stability of pigments derived from the acetaldehyde-mediated condensation between malvidin 3-*O*-glucoside and (+)-catechin. *Journal of Agricultural and Food Chemistry, 49*, 1213-1217. https://doi.org/10.1021/jf0010811.

Ferreira, V., Carrascon, V., Bueno, M., Ugliano, M., & Fernandez-Zurbano, P. (2015). Oxygen consumption by red wines. Part I: Consumption rates, relationship with chemical composition, and role of SO_2. *Journal of Agricultural and Food Chemistry, 63*, 10928-10937. https://doi.org/10.1021/acs.jafc.5b02988.

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

Tao, J., Dykes, S. I., & Kilmartin, P. A. (2007). Effect of SO_2 concentration on polyphenol development during red wine micro-oxygenation. *Journal of Agricultural and Food Chemistry, 55*, 6104-6109. https://doi.org/10.1021/jf070625h.

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

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

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

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.

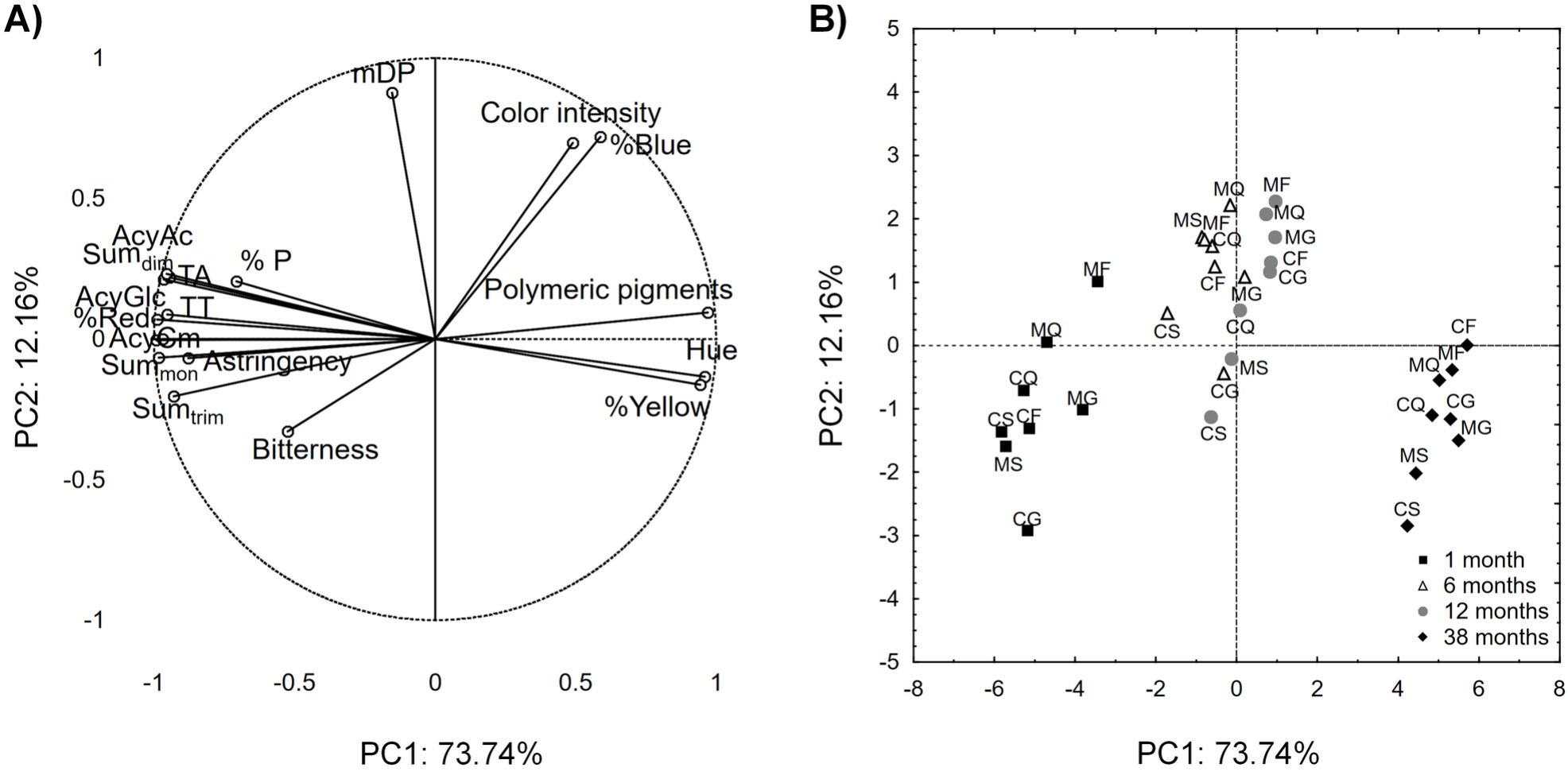
A)
mDP
Color intensity
%Blue
AcyAc
Sum dim
% P
TA
AcyGlc
TT
%Red
AcyCm
Sum mon
Astringency
Sum trim
Bitterness
Polymeric pigments
Hue
%Yellow
PC2: 12.16%
PC1: 73.74%
B)
1 month
6 months
12 months
38 months
PC2: 12.16%
PC1: 73.74%

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 $FeSO_4 \times 7H_2O$, Cu was added as $CuSO_4 \times 5H_2O$); 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 SO_2 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*	***CQ***	5.95 ± 0.08 a	5.00 ± 0.06 a	4.80 ± 0.12 ab	3.89 ± 0.09 a
	MQ	5.40 ± 0.13 bc	5.01 ± 0.08 a	4.73 ± 0.07 ab	3.69 ± 0.06 abc
	CS	6.02 ± 0.17 a	5.06 ± 0.14 a	4.95 ± 0.08 a	3.93 ± 0.05 a
	MS	5.69 ± 0.19 ab	5.01 ± 0.05 a	4.82 ± 0.08 ab	3.88 ± 0.10 ab
	CF	5.38 ± 0.02 bc	4.99 ± 0.18 ab	4.67 ± 0.08 b	3.84 ± 0.04 ab
	MF	5.28 ± 0.07 c	4.96 ± 0.14 ab	4.35 ± 0.05 c	3.69 ± 0.02 abc
	CG	5.21 ± 0.15 cd	4.67 ± 0.07 bc	4.30 ± 0.07 c	3.59 ± 0.08 bc
	MG	4.86 ± 0.13 d	4.46 ± 0.14 c	4.24 ± 0.09 c	3.47 ± 0.24 c
Sum_{mon}**	***CQ***	85.59 ± 0.67 ab	67.66 ± 0.96 bc	63.33 ± 1.07 b	53.61 ± 0.31 a
	MQ	77.58 ± 0.92 d	65.75 ± 0.35 cd	61.77 ± 0.48 b	52.18 ± 0.71 ab
	CS	86.99 ± 0.19 a	70.51 ± 0.57 a	67.43 ± 0.16 a	53.84 ± 3.55 a
	MS	83.28 ± 0.49 c	68.98 ± 1.13 ab	66.12 ± 0.60 a	53.45 ± 2.01 a
	CF	84.52 ± 1.22 bc	66.35 ± 0.27 c	59.33 ± 0.22 c	47.90 ± 0.85 bc
	MF	83.13 ± 0.18 c	66.31 ± 0.60 c	62.29 ± 1.42 b	48.96 ± 0.58 bc
	CG	83.92 ± 0.22 bc	63.72 ± 1.84 d	62.39 ± 1.16 b	46.01 ± 0.65 c
	MG	82.73 ± 0.31 c	63.67 ± 0.20 d	62.80 ± 0.45 b	48.88 ± 1.14 bc
Sum_{dim}**	***CQ***	78.83 ± 0.91 ab	65.37 ± 0.99 ab	57.97 ± 0.82 c	26.61 ± 0.30 b
	MQ	71.86 ± 0.58 f	61.16 ± 1.06 cd	55.49 ± 0.32 d	25.57 ± 0.38 b
	CS	80.49 ± 0.48 a	68.10 ± 0.68 a	64.11 ± 1.08 a	27.85 ± 0.33 a
	MS	76.86 ± 0.31 c	64.26 ± 1.05 bc	60.71 ± 0.49 b	26.05 ± 0.38 b
	CF	76.49 ± 0.97 cd	61.67 ± 0.13 cd	54.08 ± 1.22 d	23.29 ± 0.21 cd
	MF	74.89 ± 0.32 de	63.26 ± 0.74 bcd	58.35 ± 0.95 bc	24.40 ± 0.22 c
	CG	77.26 ± 0.37 bc	60.57 ± 2.26 d	55.01 ± 1.08 d	21.23 ± 0.72 e
	MG	73.80 ± 0.76 e	60.54 ± 1.51 d	57.81 ± 0.97 c	22.83 ± 0.35 d
Sum_{trim}**	***CQ***	32.13 ± 0.35 ab	18.31 ± 0.78 abc	17.16 ± 0.24 ab	7.75 ± 0.19 ab
	MQ	29.26 ± 0.93 c	17.82 ± 0.26 bcd	16.98 ± 0.19 ab	7.49 ± 0.12 b
	CS	33.27 ± 1.09 a	19.88 ± 0.12 a	18.34 ± 0.89 a	8.04 ± 0.21 a
	MS	32.69 ± 0.31 a	19.57 ± 0.18 ab	17.28 ± 0.36 ab	7.66 ± 0.18 ab
	CF	30.91 ± 0.58 bc	18.18 ± 0.87 abcd	16.03 ± 0.69 b	7.50 ± 0.11 b
	MF	30.80 ± 0.35 bc	17.99 ± 0.55 abcd	16.11 ± 0.51 b	7.59 ± 0.04 b
	CG	30.19 ± 0.35 c	17.05 ± 1.61 cd	14.70 ± 0.08 c	6.65 ± 0.16 c
	MG	29.23 ± 0.51 c	16.26 ± 0.32 d	16.07 ± 0.40 b	7.36 ± 0.12 b
mDP	***CQ***	4.19 ± 0.10 bc	5.15 ± 0.10 ab	4.54 ± 0.01 d	4.23 ± 0.06 ab
	MQ	4.66 ± 0.12 a	5.18 ± 0.16 ab	4.79 ± 0.18 c	4.34 ± 0.08 a
	CS	4.18 ± 0.06 bc	5.14 ± 0.17 ab	4.46 ± 0.06 d	3.94 ± 0.06 cd
	MS	4.47 ± 0.13 ab	5.41 ± 0.12 a	4.51 ± 0.01 d	4.25 ± 0.01 ab
	CF	4.26 ± 0.08 abc	5.13 ± 0.15 ab	5.14 ± 0.14 ab	4.13 ± 0.05 b
	MF	4.45 ± 0.29 ab	5.29 ± 0.05 a	5.36 ± 0.03 a	4.14 ± 0.05 b
	CG	3.87 ± 0.13 c	4.58 ± 0.22 c	4.98 ± 0.02 bc	3.90 ± 0.08 d
	MG	4.46 ± 0.14 ab	4.85 ± 0.11 bc	5.17 ± 0.03 ab	4.09 ± 0.05 bc
%P	***CQ***	28.27 ± 1.27 a	25.95 ± 0.36 a	27.95 ± 0.54 a	22.07 ± 0.39 abc
	MQ	28.75 ± 1.24 a	26.21 ± 1.28 a	26.90 ± 1.01 ab	22.50 ± 0.35 ab
	CS	27.87 ± 0.18 a	25.92 ± 0.70 a	24.98 ± 0.24 cd	22.10 ± 0.42 abc
	MS	28.46 ± 0.72 a	26.67 ± 0.09 a	25.41 ± 0.29 bc	22.81 ± 0.25 a
	CF	28.04 ± 0.15 a	25.75 ± 1.15 ab	23.54 ± 0.68 d	21.72 ± 0.07 c
	MF	21.26 ± 0.95 b	27.35 ± 0.41 a	23.78 ± 0.22 d	21.79 ± 0.22 bc
	CG	26.47 ± 0.15 a	23.24 ± 1.56 b	25.60 ± 0.58 bc	21.75 ± 0.14 bc
	MG	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	***CQ***	321.6 ± 7.1 b	242.9 ± 8.3 b	208.8 ± 15.4 c	63.8 ± 1.2 bc
	MQ	292.4 ± 3.6 cd	236.5 ± 6.3 b	217.3 ± 16.7 bc	65.4 ± 0.9 bc
	CS	360.6 ± 10.9 a	293.1 ± 1.8 a	277.3 ± 9.7 a	72.9 ± 1.7 a
	MS	313.0 ± 3.0 bc	284.3 ± 1.1 a	274.7 ± 11.5 a	74.2 ± 1.4 a
	CF	288.5 ± 1.1 d	227.5 ± 7.1 a	208.0 ± 7.9 c	67.1 ± 1.1 b
	MF	281.1 ± 15.0 d	247.2 ± 7.3 b	239.3 ± 3.8 b	71.5 ± 1.1 a
	CG	294.3 ± 9.8 cd	239.3 ± 5.6 b	207.7 ± 2.1 c	61.7 ± 0.8 c
	MG	286.7 ± 7.9 d	236.9 ± 11.8 b	219.9 ± 8.6 bc	64.8 ± 1.8 bc
AcyGlc	***CQ***	161.40 ± 0.87 c	97.69 ± 1.53 cde	83.72 ± 0.75 e	9.81 ± 0.40 d
	MQ	159.48 ± 0.38 cd	93.24 ± 0.52 de	87.68 ± 1.27 d	9.78 ± 0.06 d
	CS	174.64 ± 1.86 a	121.04 ± 1.18 a	113.44 ± 1.31 a	16.27 ± 1.46 a
	MS	173.82 ± 1.53 a	115.05 ± 2.85 b	110.81 ± 0.59 b	18.06 ± 1.15 a
	CF	149.57 ± 0.30 e	92.45 ± 2.76 e	79.39 ± 0.74 f	12.85 ± 0.41 bc
	MF	149.32 ± 0.84 e	103.06 ± 0.55 c	91.32 ± 1.10 c	13.97 ± 0.66 b
	CG	166.50 ± 1.36 b	98.80 ± 3.26 cd	85.61 ± 0.71 de	9.69 ± 0.95 d
	MG	156.67 ± 1.18 d	94.63 ± 2.73 de	90.95 ± 0.34 c	11.69 ± 0.11 cd
AcyAc	***CQ***	13.64 ± 0.02 c	10.38 ± 0.10 de	9.32 ± 0.11 cd	1.22 ± 0.08 a
	MQ	14.27 ± 0.06 b	10.59 ± 0.03 cd	9.57 ± 0.10 bc	1.09 ± 0.14 a
	CS	14.74 ± 0.16 a	12.21 ± 0.10 a	11.30 ± 0.04 a	1.31 ± 0.11 a
	MS	14.93 ± 0.16 a	12.06 ± 0.10 a	10.90 ± 0.29 a	1.32 ± 0.11 a
	CF	13.60 ± 0.07 c	10.26 ± 0.11 e	9.04 ± 0.15 d	1.22 ± 0.01 a
	MF	13.16 ± 0.15 d	11.21 ± 0.08 b	9.84 ± 0.23 b	1.05 ± 0.18 a
	CG	14.72 ± 0.13 a	10.76 ± 0.07 c	9.56 ± 0.05 bc	1.20 ± 0.08 a
	MG	13.39 ± 0.09 cd	10.48 ± 0.09 de	9.79 ± 0.21 bc	1.12 ± 0.04 a
AcyCm	***CQ***	26.72 ± 0.24 c	13.73 ± 0.33 bc	10.85 ±0.20 c	0.57 ± 0.09 c
	MQ	26.79 ± 0.05 c	14.07 ± 0.09 bc	11.37 ± 0.14 bc	0.82 ± 0.11 abc
	CS	28.68 ± 0.43 a	16.59 ± 0.34 a	14.87 ± 0.44 a	1.02 ± 0.23 ab
	MS	28.66 ± 0.30 a	16.31 ± 0.69 a	14.63 ± 0.07 a	1.16 ± 0.29 a
	CF	25.67 ± 0.29 de	13.08 ± 0.31 c	10.14 ± 0.31 d	0.58 ± 0.11 c
	MF	25.39 ± 0.17 e	14.73 ± 0.36 b	11.91 ± 0.29 b	0.74 ± 0.10 abc
	CG	27.52 ± 0.20 b	13.89 ± 0.37 bc	10.91 ± 0.07 c	0.50 ± 0.07 c
	MG	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 (%)	***CQ***	55.83 ± 0.60 abc	69.69 ± 1.25 abc	74.54 ± 0.53 b	81.44 ± 1.04 d
	MQ	56.17 ± 0.99 ab	71.70 ± 1.41 ab	75.81 ± 0.72 ab	85.28 ± 1.10 abc
	CS	53.65 ± 0.61 d	67.07 ± 0.50 c	74.75 ± 1.42 ab	83.64 ± 1.06 bdc
	MS	54.69 ± 0.58 abcd	68.50 ± 0.43 bc	75.58 ± 0.55 ab	83.00 ± 0.67 cd
	CF	54.17 ± 0.61 cd	69.57 ± 1.97 abc	75.18 ± 1.65 ab	85.85 ± 0.85 ab
	MF	56.40 ± 0.66 a	70.10 ± 1.02 abc	78.42 ± 0.64 a	86.52 ± 0.82 a
	CG	54.52 ± 0.20 bcd	71.32 ±1.45 ab	75.29 ± 1.85 ab	82.95 ± 0.32 cd
	MG	54.79 ± 0.30 abcd	73.08 ± 1.49 a	75.61 ± 0.99 ab	83.52 ± 1.60 bcd
CI	***CQ***	7.89 ± 0.05 bc	8.85 ± 0.11 ab	8.85 ± 0.15 bc	8.89 ± 0.10 ab
	MQ	8.04 ± 0.10 b	9.04 ± 0.02 a	9.47 ± 0.10 a	8.77 ± 0.10 bc
	CS	7.44 ± 0.01 bcd	8.23 ± 0.14 de	7.68 ± 0.07 e	8.00 ± 0.11 e
	MS	7.28 ± 0.07 cd	8.25 ± 0.22 cde	8.12 ± 0.12 d	7.85 ± 0.09 e
	CF	7.90 ± 0.10 bc	8.58 ± 0.07 bc	9.23 ± 0.12 ab	9.14 ± 0.14 a
	MF	9.54 ± 0.33 a	8.43 ± 0.13 cd	9.41 ± 0.20 a	8.87 ± 0.12 abc
	CG	7.13 ± 0.07 d	8.03 ± 0.02 e	8.85 ± 0.26 bc	8.55 ± 0.07 cd
	MG	7.80 ± 0.23 bcd	8.21 ± 0.12 de	8.68 ± 0.09 c	8.41 ± 0.16 d
Hue	***CQ***	0.688 ± 0.003 ab	0.823 ± 0.006 ab	0.858 ± 0.005 bc	1.014 ± 0.018 abc
	MQ	0.674 ± 0.008 b	0.817 ± 0.008 b	0.834 ± 0.007 c	0.984 ± 0.019 c
	CS	0.708 ± 0.002 a	0.848 ± 0.017 a	0.912 ± 0.024 a	1.026 ± 0.007 ab
	MS	0.694 ± 0.013 ab	0.847 ± 0.007 a	0.880 ± 0.009 b	1.029 ± 0.009 a
	CF	0.684 ± 0.001 b	0.819 ± 0.006 b	0.873 ± 0.015 b	1.020 ± 0.015 abc
	MF	0.698 ± 0.005 ab	0.818 ± 0.009 b	0.837 ± 0.002 c	0.997 ± 0.002 abc
	CG	0.691 ± 0.006 ab	0.822 ± 0.010 ab	0.835 ± 0.003 c	0.998 ± 0.014 abc
	MG	0.687 ± 0.016 ab	0.805 ± 0.009 b	0.841 ± 0.004 c	0.990 ± 0.010 bc
% Red	***CQ***	52.4 ± 0.1 abc	48.1 ± 0.2 abc	47.1 ± 0.3 ab	43.8 ± 0.2 abc
	MQ	52.8 ± 0.4 ab	48.0 ± 0.2 abc	47.4 ± 0.3 ab	43.9 ± 0.0 abc
	CS	51.8 ± 0.1 bc	47.6 ± 0.5 bc	46.2 ± 0.6 d	43.5 ± 0.1 bc
	MS	52.6 ± 0.5 ab	47.4 ± 0.3 c	46.9 ± 0.2 bcd	43.5 ± 0.2 c
	CF	52.8 ± 0.1 ab	48.2 ± 0.2 abc	46.6 ± 0.3 cd	42.8 ± 0.2 d
	MF	51.4 ± 0.3 c	48.1 ± 0.2 abc	47.5 ± 0.1 ab	43.6 ± 0.1 abc
	CG	53.0 ± 0.1 a	48.3 ± 0.2 ab	47.8 ± 0.1 a	44.0 ± 0.3 a
	MG	52.4 ± 0.7 ab	48.4 ± 0.3 a	47.5 ± 0.1 ab	44.0 ± 0.3 ab
% Yellow	***CQ***	36.0 ± 0.1 abc	39.6 ± 0.2 bc	40.4 ± 0.0 cd	44.2 ± 0.2 ab
	MQ	35.6 ± 0.1 c	39.2 ± 0.2 c	39.5 ± 0.1 e	43.5 ± 0.2 c
	CS	36.7 ± 0.1 a	40.3 ± 0.4 a	42.1 ± 0.6 a	44.7 ± 0.2 a
	MS	36.5 ± 0.5 a	40.2 ± 0.2 ab	41.2 ± 0.3 b	44.7 ± 0.3 a
	CF	36.1 ± 0.1 abc	39.4 ± 0.2 bc	40.6 ± 0.4 bc	44.0 ± 0.2 bc
	MF	35.8 ± 0.1 bc	39.3 ± 0.3 c	39.7 ± 0.1 de	43.4 ± 0.1 c
	CG	36.6 ± 0.2 a	39.7 ± 0.3 abc	39.9 ± 0.1 cde	43.9 ± 0.3 bc
	MG	36.0 ± 0.4 abc	39.0 ± 0.3 c	39.9 ± 0.1 cde	43.6 ± 0.2 bc
% Blue	***CQ***	11.6 ± 0.1 b	12.3 ± 0.1 ab	12.5 ± 0.2 bc	12.1 ± 0.0 de
	MQ	11.6 ± 0.3 b	12.8 ± 0.2 a	13.1 ± 0.2 a	12.6 ± 0.1 bc
	CS	11.5 ± 0.0 b	12.1 ± 0.2 b	11.7 ± 0.1 e	11.8 ± 0.1 e
	MS	11.0 ± 0.3 bc	12.4 ± 0.3 ab	11.9 ± 0.1 de	11.8 ± 0.1 e
	CF	11.2 ± 0.2 bc	12.4 ± 0.0 ab	12.8 ± 0.2 ab	13.2 ± 0.3 a
	MF	12.8 ± 0.2 a	12.6 ± 0.1 a	12.9 ± 0.2 ab	13.0 ± 0.2 ab
	CG	10.4 ± 0.1 c	12.0 ± 0.2 b	12.2 ± 0.1 c	12.0 ± 0.1 de
	MG	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	***CQ***	5.8 ± 0.8 a	4.8 ± 0.5 ab	4.7 ± 0.8 a	2.9 ± 0.5 ab
	MQ	5.3 ± 0.8 ab	4.2 ± 0.7 b	3.6 ± 0.7 cd	2.8 ± 0.6 b
	CS	5.8 ± 1.0 a	5.3 ± 1.0 a	4.8 ± 1.1 a	3.5 ± 0.4 a
	MS	5.4 ± 0.9 ab	4.0 ± 1.0 b	3.8 ± 0.9 bc	3.5 ± 0.5 a
	CF	5.9 ± 0.7 a	4.7 ± 1.1 ab	4.5 ± 1.3 ab	2.9 ± 0.6 ab
	MF	5.7 ± 0.5 a	4.3 ± 1.1 b	3.2 ± 0.6 d	2.9 ± 0.7 ab
	CG	5.0 ± 0.6 b	4.1 ± 0.9 b	3.3 ± 1.0 cd	2.3 ± 0.7 bc
	MG	4.2 ± 0.7 c	3.1 ± 1.2 c	2.9 ± 0.5 d	2.0 ± 0.5 c
Bitterness intensity	***CQ***	3.3 ± 0.9 a	2.8 ± 0.8 b	3.6 ± 0.9 ab	3.0 ± 0.8 b
	MQ	3.2 ± 0.9 a	2.7 ± 1.1 b	3.0 ± 1.0 ab	3.0 ± 0.8 b
	CS	3.8 ± 1.1 a	3.8 ± 1.2 a	3.7 ± 1.2 ab	3.9 ± 0.7 a
	MS	3.8 ± 0.9 a	2.8 ± 1.3 b	3.1 ± 1.3 ab	3.0 ± 0.6 b
	CF	3.7 ± 1.2 a	3.1 ± 1.2 ab	3.9 ± 1.2 a	2.6 ± 0.8 b
	MF	3.6 ± 1.2 a	3.1 ± 0.7 ab	3.4 ± 1.2 ab	2.9 ± 0.7 b
	CG	3.6 ± 0.7 a	3.5 ± 0.9 ab	2.9 ± 1.0 ab	2.4 ± 0.7 b
	MG	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.