EVALUATION OF THE IMPACT OF INITIAL RED WINE COMPOSITION ON CHANGES IN COLOR AND ANTHOCYANIN CONTENT DURING BOTTLE STORAGE

COLOR & ANTHOCYANIC COMPOSITION CHANGES IN WINES DURING BOTTLE STORAGE

José-Miguel Avizcuri[a,b], María-Pilar Sáenz-Navajas[b], José-Federico Echávarri[c], Vicente Ferreira[b] & Purificación Fernández-Zurbano[a,c*]

[a]Instituto de Ciencias de la Vid y del Vino (UR-CSIC-GR), Carretera de Burgos Km. 6, Finca La Grajera; 26007 Logroño, La Rioja, Spain.

[b]Laboratorio de análisis del aroma y enología (LAAE). Department of Analytical Chemistry, Universidad de Zaragoza, Instituto Agroalimentario de Aragón (IA2) (UNIZAR-CITA), Associate unit to Instituto de Ciencias de la Vid y el Vino (UR-CSIC-GR), c/ Pedro Cerbuna 12, 50009 Zaragoza, Spain.

[c]Department of Chemistry, Universidad de La Rioja (UR), c/ Madre de Dios 51, 26006 Logroño, La Rioja, Spain.

*Correspondence should be addressed to:

Purificación Fernández Zurbano

Phone: +34 941 299 622

Email: puri.fernandez@unirioja.es

ABSTRACT

Sixteen commercial red wines, selected to cover a different range of color and total polyphenols index (TPI), were stored at 25ºC during 6 months under controlled and different oxygen additions (0, 1.1, 3.1, 10.6 and 30.4 mg L^{-1}) during the bottling process. Changes in color and the anthocyanic composition were evaluated using transmittance spectra and UPLC-MS-UV/Vis respectively. Results reveal a general pattern in the evolution of wines. However, different patterns of evolution related to initial wine composition, especially to TPI, were observed. Wines with higher TPI had a lower evolution, whereas wines with lower TPI showed a higher evolution and greater variability in behavior. In general, oxygen seemed to accelerate all changes observed during aging although the oxygen effect was more limited than the effect of the storage time. These results are relevant for wine experts and help explain the evolution of wine at the bottling stage.

KEYWORDS

Wine; Bottling storage; Oxygen; Color; Anthocyanins

1. INTRODUCTION

During their time in the bottle wines undergo changes that greatly influence the organoleptic features that determine their quality. These changes depend on the time in the bottle, on access to oxygen and, of course, on the composition of the wine before it is bottled. Regarding the time in the bottle, the Designation of Origin regulations usually determine the minimum time wines must remain in the bottle to reach the specific sensory attributes required. However, managing the amounts of oxygen is still a challenge in the manufacturing process, although it is widely accepted that the presence of moderate amounts of oxygen in the wine can be beneficial, whereas too little or too much can make the wine either not develop the intended attributes or develop undesired oxidation notes. In the aging process in the bottle there are two key moments for wine to be oxygenated: at bottling and the transfer through the stopper. Oxygen intervenes in complex reactions- including those undergone by the wine's phenolic composition- which play an important role in some of the sensory attributes sought during aging in the bottle, such as the reduction of astringency and color stabilization. Color is one of the main attributes involved in appearance evaluations and thus in the construction of the consumers´ concept of quality. Color provides information about the type of wine, winemaking or aging processes. Color can often predispose the perception of other sensory characteristics as it allows one to anticipate the taste and/or odor properties based on the previous experience of the consumer (De Simón, Cadahia, Sanz, Poveda Pérez-Magariño, Ortega-Heras & González-Herta, 2008). This explains the importance of wine color in the acceptability of products (Morrot, Brochet & Dubourdieu, 2001).

The change of color during aging from red–purple to brick red hues is attributed to the progressive formation of new pigments as anthocyanins react with other compounds (Somers, 1971; Timberlake & Bridle, 1971; Dallas & Laureano, 1994, Atasanova, Fulcrand, Cheynier & Moutounet, 2002). The progress of these chemical reactions during winemaking and wine

69 aging depends on multiple factors, such as the concentration of anthocyanins and flavanols,
70 acetaldehyde and other yeast metabolites, as well as pH, temperature, the presence of oxygen or
71 sulfur dioxide among others (Somers & Evans 1986; Dallas et al., 1994; Romero & Bakker;
72 2000a, 2000b; Fulcrand, Dueñas, Salas & Cheynier, 2006). The mechanisms involved in the
73 formation of these pigments have been reported to be the result of direct condensation of
74 anthocyanins with other molecules such as flavanols (Vivar-Quintana, Santos-Buelga, Francia-
75 Aricha & Rivas-Gonzalo, 1999; Salas, Atanasova, Poncet-Legrand, Meudec, Mazauric &
76 Cheynier, 2004), mediated mainly by acetaldehyde- although other carbonylic compounds have
77 also been described (Fulcrand, Cheynier, Oszmiansky & Moutounet, 1997; Escribano-Bailón,
78 Álvarez-García, Rivas-Gonzalo, Heredia & Santos-Buelga, 2001; Pisarra, Mateus, Rivas-
79 Gonzalo, Santos-Buelga & De Freitas, 2003; Fulcrand et al., 2006) or by cycloaddition
80 reactions between anthocyanins and other molecules such as acetaldehyde, pyruvic acid or
81 vinylphenol among others (Bakker et al, 1997; Fulcrand, Cameira do Santos, Sarni-Manchado,
82 Cheynier & Favre-Bonvin, 1996; Fulcrand, Benabdeljalik, Rigaud, Cheynier & Moutounet,
83 1998). In wine, acetaldehyde and pyruvic acid are products of microbial metabolism. The
84 former may also be produced during aging by ethanol oxidation coupled with autoxidation of
85 ortho-diphenols (Wildenradt & Singleton, 1974), although it has been recently shown that the
86 increase in acetaldehyde during oxidation can be attributed to the cleavage of the hydroxyethyl
87 sulfonate already present in the wine, as consequence of the depletion of SO_2 caused by
88 oxidation (Carrascon, Fernández-Zurbano, Bueno & Ferreira, 2015).
89 In general, pigments derived from anthocyanins are more resistant to pH changes and
90 bleaching by bisulfite than the precursor anthocyanins (Sarni-Manchado, Fulcrand, Souquet,
91 Cheynier & Moutounet, 1996). This helps in understanding the stabilization of color that wine
92 undergoes during a correct aging process. In this stage, oxygen plays an important and essential
93 role in the formation of anthocyanin-derived compounds and therefore decisive in the stability

of the wine's color (Fulcrad et al., 1998; Perez-Magariño&Gonzalez-San Jose, 2004; Wirth, Morel-Salmi, Souquet, Dieval, Aagaard, Vidal, Fulcrand&Cheynier, 2010).

Color stability during the aging process in the bottle is discussed in several studies performed on red wines, where color intensity (CI) did not change during the time of the study (Gambuti, Rinaldi, Ugliano &Moio, 2013; Pérez-Magariño & González-San José, 2004). However, other aging experiments also carried out on red wines with the aim of evaluating the impact of exposure to different oxygen doses during bottling stage show that wines stored with higher oxygen transferrates (OTR) had higher color intensity (CI) (Wirth et al., 2010; Caillé, Samson, Wirth, Diéval, Vidal & Cheynier, 2010; Han, Ugliano, Currie, Vidal, Diéval & Waterhouse, 2014). This increase in CI for wines stored under higher OTRs may be attributed to (1) a higher increase in the formation of different pigments as a result of oxygen exposure, (2) the release of pigments bound to SO_2 as consequence of the higher consumption of sulfites in higher OTR wines (Wirth et al. 2010). Specifically, Carrascon et al. (2015) found that the increase in absorbances to 520, 420 and 620 nmis limits the consumption of oxygen when the level of free SO_2 is above 5 mg/L.

Knowledge concerning the evolution of color and anthocyanin composition that red wines undergo during their time in the bottle based on their initial composition is currently a scientific challenge and a demand made by wine experts. In order to delve deeper into the relation between the initial composition of wines and the sensory and chemical changes they may undergo during aging in the bottle, a larger project was undertaken. To this purpose 16 red wines with different sensory attributes and different TPI were chosen and stored for 6 months at varying oxygen levels, dosed when they were bottled. A paper already published by our research group contains the section corresponding to the changes in aroma and taste (Sáenz-Navajas, Avizcuri, Ferreira & Fernández-Zurbano, 2014). This paper focuses on studying the changes that wine undergoes in color and the anthocyanin composition during aging in the

bottle. The goals of this paper are to determine the existence of behavior models for wine during aging in the bottle depending on their initial composition, as well as the influence of the dissolution of controlled oxygen levels during the bottling process.

2. MATERIALS AND METHODS

2.1 Reagents

Bovine serum albumin (BSA, fraction V powder) was purchased from Sigma. Glacial acetic acid, HPLC-grade acetone, HPLC–MS-grade acetonitrile, formic acid reagent grade, absolute ethanol and sodium chloride were obtained from Scharlau (Barcelona), and potassium metabisulfite from Panreac (Madrid, Spain). Oenin-chloride was obtained from Extrasynthese. Deionized water was purified with a Milli-Q water system (Millipore, Molsheim, France) prior to use.

2.2 Commercial wines

Sixteen different Spanish commercial red wines from different wine making areas were selected to cover a suitable range of total phenolic index (TPI) and color intensity (CI). The detailed list of samples, including sample information and basic compositional data obtained following standard operating procedures, is shown in Table 1. The wines were coded so that the first two letters refer to the name of the wine, an underscore followed by a letter that indicates the Designation of Origin of the wine and two numbers for the year it was made (MG_V05).

2.3 Storage of wine samples under different initial oxygen doses

For each wine there were 7 bottles with 750 mL capacity which were placed in an anoxic glove box equipped with a vacuum chamber (Jacomex, Dagneux, France) where oxygen was under 0.002%. In this chamber, the contents of these 7 bottles were mixed in a big beaker and stirred until the oxygen level in the 5250 mL of total wine dropped to 0.00 mg L^{-1} as measured with a fluorescence oxygen meter OptiOx SG-98 from Mettler Toledo (Barcelona, Spain). This amount of wine was distributed into 5 air-tight amber bottles, 1150 mL capacity, supplied by

144 Sigma-Aldrich. Thus, for each of the 16 chosen wines, 5 bottles were prepared, filled with 1035
145 mL of the same wine, different oxygen regimens and 115 mL of Argon headspace. The bottles
146 were closed with an internal silicone septum, a crimp cap, a second silicone septum and an
147 external screw cap. Known volumes of oxygen were introduced through the internal septum
148 (with bottles upside down so that the oxygen passed through the wine) with a Hamilton gas-
149 tight syringe (Samplelock™ syringe). Bottles were kept upside down about 15 minutes to
150 ensure oxygen contact with the entire volume of wine. Oxygen was introduced into the vacuum
151 chamber of the anoxic glove box in Tedlar gas sampling bags supplied by Sigma-Aldrich. The
152 oxygen volumes introduced were equivalent to the theoretical concentrations of 0, 1.1, 3.1, 10.6
153 and 30.4 mg of oxygen per liter of wine. This range covers the normal levels introduced during
154 normal wine bottling operations and extends it to two unrealistic extreme situations (0 and 30.4
155 mg L^{-1}). All the oxygen was introduced in a single dose at the time of bottling. Lastly, the
156 resulting eighty (16 wines x 5 oxygen levels) 1150 mL bottles were double sealed under
157 vacuum into two plastic bags (with known oxygen permeability (< 9 cm^3m^{-2} 24 h) supplied by
158 Amcor (Barcelona, Spain). The eighty bottles were taken out of the anoxic glove box and
159 stored in the dark at 25 °C during 6 months in an incubator (Climas GROW 360). The overall
160 permeability of the systems was independently checked with control samples containing a
161 solution of indigo carmine following the procedure developed by Lopes et al. (2009). Results
162 suggested that the total external atmospheric oxygen that penetrated into the samples after the 6
163 months of storage was 0.9 ± 0.6 mg, which can be considered air-tight enough for the purposes
164 of the experiment. Taking into consideration this permeability data during 6 months, the
165 oxygen levels in the wines were 0.9 (level 0); 2.0 (level 1); 4.0 (level 2); 11.5 (level 3) and 31.3
166 mg L^{-1} (level 4).

167 As this experiment was part of a bigger project, only those samples that underwent
168 significant sensory changes during wine aging were chemically analyzed. Discriminant tests

169 (triangle tests) were performed to evaluate sensory differences between samples stored under
170 oxygen levels 1 and 3. Only when the triangle test was not significant ($P > 0.05$), samples of
171 this wine stored under both oxygen levels were not submitted for chemical and sensory
172 characterization. In these cases the wine sample stored at oxygen level 2 was used for chemical
173 and sensory characterization (Sáenz-Navajas et al. 2014). According to this premise, 86 (16
174 original wines + 70 aged wines) samples out of 96 (16 original wines + 80 aged wine samples)
175 were analyzed in terms of color and anthocyanic composition and thus reported in this paper.
176 For the wines with different oxygen regimes, we added the numbers 0 to 4 to their initial
177 wine code, where 0 (MG_V05_0) is for the sample stored during 6 months in the bottle without
178 adding oxygen and 4 (MG_V05_4) is for the sample stored during 6 months in the bottle with
179 the highest addition of oxygen.

180 **2.4 Conventional oenological parameters**

181 Ethanol content, pH, reducing sugars, (total) titratable and volatile acidities were determined by
182 Infrared Spectrometry with Fourier Transformation (FTIR) with a WineScan™ FT 120
183 (FOSS), which was calibrated with wine samples analyzed in accordance with official OIV
184 practices (OIV 2005). Malic and lactic acid were determined by enzymatic methods in
185 accordance with official AOAC analysis methods (AOAC, 2002). Total polyphenol index (TPI)
186 was determined as absorbance at 280 nm (Ribéreau-Gayon, 1970). Free and combined sulfur
187 dioxide analyses were performed by the OIV method (OIV 2005). Color intensity (CI) was
188 calculated as the sum of absorbances at 420, 520 and 620 nm. Tonality (T) was calculated as
189 the relation between absorbances at 420 and 520 nm.

190 **2.5 Analysis of polymeric pigments and copigmented anthocyanins**

191 The procedure for the determination of large polymeric pigments (LPP) and small polymeric
192 pigments (SPP) was based on the procedure developed by Harbertson, Picciotto, & Adams
193 (2003). Copigmented anthocyanins were estimated according to the method developed by

194 Boulton (2001). This method is based on diluting the wine to induce the dissociation of
195 copigmented complexes

196

197

198 **2.6 Color measurements**

199 The wine transmittance spectra were measured by Perkin-Elmer Lambda 6
200 spectrophotometer (Perking-Elmer Corp., Norwalk, CT) using 0.2 cm path-length quartz
201 cuvettes. Measurements were taken every 1 nm between 380 and 780 nm. Wine samples were
202 centrifuged and filtered through 0.45 μm filter prior to analysis. From the spectra, the color
203 coordinates were calculated using the CIELAB method (C.I.E., 2004) with the CIE 1964 10°
204 standard observer and the illuminant D65 as reference, according to the OIV (O.I.V., 2005).
205 The color coordinates correspond to wine lightness (L^*_{10}) and green-red ($a^*_{10} < 0$ to $a^*_{10} > 0$)
206 and blue-yellow ($b^*_{10} < 0$ to $b^*_{10} > 0$) axes. The chroma coordinates (C^*_{ab}) and hue (h_{ab}) were
207 calculated based on the coordinates a^*_{10} and b^*_{10}, $C^*_{ab} = [(a^*_{10})^2 + (b^*_{10})^2]^{1/2}$ *and* $h_{ab} =$
208 $arctan\left(b^*_{10}/a^*_{10}\right)$ (Resolution OENO 1/2006, 2006).

209 **2.7 UPLC–UV/Vis-MS anthocyaninsanalysis**

210 The analyses of anthocyanins were performed following the previously reported method by
211 González-Hernández, Avizcuri-Inac, Dizy & Fernández-Zurbano (2014). Each sample was
212 analyzed in duplicate with a Waters Acquity Ultra Performance LC system (Milford, MA,
213 USA) by direct injection of wine samples. UPLC separation was achieved using an acquity
214 BEHC18 column (100 mm × 2.1 mm, i.d., 1.7 μm particle size, Waters) kept at 40 °C. Mobile
215 phase flow rate was 0.45 mL min^{-1} and the injection volume was 7.5 μL. Solvents were (A)
216 water/formic acid (5%) and (B) acetonitrile/formic acid (5%). The identity assignation of

compounds was carried out by comparison of their retention time (t_R), MS and MS/MS spectra (Table 2). Quantification by UV/Vis was performed with a variable wavelength detector at 520 nm. The concentration of anthocyanins has been expressed as mg L^{-1} of malvidin-3-O-glucoside (lineal range: 0.054-58.3 mg/L; area = 0.0270 [malvidin-3-O-glucoside] - 0.0037; R^2 = 0.999). Once the anthocyanin compounds were individually quantified, they were grouped by chemical similarity, verifying that they undergo similar changes (increase or decrease) during aging. These groups include: no acylated anthocyanins, acylated anthocyanins, pyranoanthocyanins and malvidin-3-glc-ethyl-(epi)catechin. Table 2 shows the individual compounds of each of these groups.

MS and MS/MS analyses were performed by coupling the Waters Acquity Ultra Performance LC chromatograph system described above to a Microtof-Q (Q-TOF) mass spectrometer from Bruker Daltonik (GMBH, Germany) with an electrospray interface. The MS/MS analyses were performed by applying 20-50 eV. Chromatographic separation was performed under the same conditions described above. Electrospray ionization was carried out in positive mode using a capillary voltage of -4.5 kV. A coaxial nebulizer N_2 gas flow with a dry gas of 9.0 L min^{-1} at 180 °C and 4.0 bar of pressure around the ESI emitter was used to assist the generation of ions. The mass spectrometer was calibrated across the mass range of 50–1200 m/z using sodium formate internal references.

2.8 Data analysis

Analyses were performed in duplicate with the results expressed as the average of the two measurements. All analyses were carried out with the SPSS program for Windows (SPSS inc, v 19, Chicago, USA). Data were studied with the ANOVA one-way linear model analysis of variance and significant differences among means ($P< 0.05$) were determined by Duncan's multiple range tests.

241 A first PCA was calculated with data from the chemical composition of the 16 wines
242 analyzed before the aging process. In order to choose the number of factors that should be
243 retained, dimensions with an eigenvalue higher than the mean eigenvalue (Kaiser condition)
244 were calculated for PCA spaces. The Hierarchical Cluster Analysis (HCA) with the Ward
245 criteria was applied to the main components of the wines in the space defined by the previously
246 calculated PCA. The clusters identified by truncating the tree diagram were identified by using
247 the test-value parameter (Morineau, lebart, &Piron, 1995). The test-value corresponds to a
248 statistical criterion akin to a standardized variable (zero mean and unit variance). Significance
249 is obtained when the absolute test-value is ≥1.96, which corresponds to an error threshold of
250 5%. A ranking of the terms according to their test-values provides a quick characterization of
251 each cluster (Morineau, 1984). The statistical software package used for these analyses was
252 SPAD software (version 5.5, CISIA-CESRESTA, Montreuil, France).

253 For the 16 wines (global storage effect) and for wines belonging to the same cluster
254 (storage effect for each cluster), differences between the average for each parameter before and
255 after storage (average of oxygen doses) were calculated. Significances of these differences
256 were evaluated by*t*-test.

257 **3. RESULTS**

258 **3.1 Color parameters, anthocyanic composition and TPI of wines before storage**

259 Tables 2 and 3 show the TPI data, color parameters, anthocyanins and anthocyanin
260 derivatives for the 16 wines before aging. These wine samples presented a range of CI going
261 from 7.3 to 19.3 absorbance units (AU) (SO_C07 and MG_V05, respectively). Their TPI
262 (Table 1) ranged from 45.4 in BE_R10 to 83.3 in MG_V05. Given the variability among
263 commercial wines (different origin, vintage, type of vinification, variety, etc…) samples
264 presented differences in hue, color coordinates and in polymeric pigment, copigmented
265 anthocyanins, anthocyanins and anthocyanin derivative concentrations.

A PCA was calculated on 16 chemical variables (pH and TPI together with anthocyanins, anthocyanin derivatives and color parameters shown in Table 2). Figure 1a shows the distribution of the samples on the first two PCs. PC1 was positively contributed by non-acylated (93%) and acylated anthocyanins (92%), ethylidene-linked malvidin-3-glucoside-(epi)catechin dimer (84%) and copigmented anthocyanins (79%), and negatively contributed by tonality (86%). This component appeared to be related to wine vintage, since youngest wines were projected on the right side of the plane. These samples were characterized by higher concentrations of non-acylated, acylated, ethylidene-linked malvidin-3-glucoside-(epi)catechin dimer and copigmented anthocyanins and lower values for the hue variable. The second PC was positively contributed by the concentration of free sulfur dioxide (49%) and negatively contributed by CI (87%), small polymeric pigments (SPP) (86%) and pyranoanthocyanins (70%). Thus, wines plotted on the top part of the plane were characterized by lower CI, higher concentrations of SPP, pyranoanthocyanins and higher concentration of free sulfur dioxide.

The correlation matrix stemming from PCA revealed that CI was highly correlated to the concentration of pyranoanthocyanins, SPP, the a^*_{10} coordinate ($P < 0.001$ in all cases) and a tendency ($P < 0.1$) with LPP concentration.

Acylated and non-acylated anthocyanins, pyranoantocyanins and ethylidene-linked malvidin-3-glucoside-(epi)catechin dimer were negatively correlated to the b^*_{10} coordinate ($P < 0.05$ in all cases). Similarly, copigmented anthocyanins and free sulfur dioxide concentration ($P < 0.05$) presented negative correlations with the b^*_{10} coordinate. Thus, wines with higher levels of free sulfur dioxide and copigmented anthocyanins presented lower yellow nuances, given that h_{ab} presented higher values. On the one hand, this suggested that the presence of sulfur dioxide capable of reducing the quinones generated during the oxidation of ortho-diphenols would avoid the reactions yielding yellow compounds (Singleton, 1987; Laurie et al., 2012; Nikolantonaki & Waterhouse, 2012). On the other hand, copigmentation, which generates a

bathocromic shift from red to blue-purple color in wine (Boulton, 2001), would be involved in the decrease of h_{ab} coordinate and thus in the yellow color of wine.

Concerning a^*_{10} coordinate, it was primarily correlated with copigmented anthocyanins, SPP, LPP, acylated anthocyanins, ethylidene-linked malvidin-3-glucoside-(epi)catechin dimer and pyranoanthocyanins, suggesting an important contribution of these compounds to the red color of these wines as a result of the relationship between the coordinate a^*_{10} and the coordinate C^*_{10}.

Hierarchical cluster analysis was calculated on the PCA dimensions to classify wines according to their initial composition. Figure 1b shows the tree diagram and the three clusters obtained. For each cluster, the closest wine to the center of gravity was identified as the most typical specimen of the group and so their color characteristics. These samples were MC_R09 (cluster 1), CD_C10 (cluster 2) and CT_B07 (cluster 3).

Table 4 shows the global values of the parameters for the whole set of wines (average among the 16 studied wines) and the average values for each cluster.

Cluster 1 comprised five wines (MC_R09, AR_A08, BE_R10, RN_R09 and SC_R10): all of them with TPI lower than the average, presenting the highest concentrations of free sulfur dioxide and copigmented anthocyanins, the lowest concentration of SPP and LPP, and the lowest values for CI and b^*_{10}.

Cluster 2 was comprised of 5 samples: CH_R10, BO_B10, RM_R10; GC_B10, CD_C10, all of them belonging to the youngest vintage: 2010. This cluster presented higher TPI than the average. These wines contained the highest concentrations of ethylidene-linked malvidin-3-glucoside-(epi)catechin dimer, pyranoanthocyanins, acylated and non-acylated anthocyanins, LPP, SPP and the highest values for the a^*_{10} coordinate and CI, as well as the lowest tonality value.

315 Cluster 3 consisted of six wines (CZ_D08, SO_C07, RB_R06, CT_C07, AY_C05 and
316 MG_V05) belonging to the oldest vintages. This cluster presented TPI values higher than the
317 average and showed the highest values of tonality and b^*_{10} coordinate and the lowest
318 concentrations of ethylidene-linked malvidin-3-glucoside-(epi)catechin dimer, acylated, non-
319 acylated and copigmented anthocyanins.
320
321
322
323 **3.2 Evolution of color parameters and anthocyanic composition during bottle aging**
324 **3.2.1 Global Evolution**
325 The effect of aging was assessed by comparing color parameters of the 16 wines before
326 and after bottle aging during 6 months at 25 °C in the presence of five different oxygen levels.
327 Considering the global evolution of the sample set (Table 5), a decrease in the
328 concentration of both free (-14.8 mg L^{-1}) and combined (-20.1 mg L^{-1}) sulfur dioxide and an
329 increase of their yellow nuances, measured as tonality (+0.13) and b^*_{10} coordinate (+10.27)
330 during aging, were observed. The variation of hue and b^*_{10} coordinate values during aging was
331 positively correlated with wine vintage ($P < 0.05$); thus, the older the wine, the smaller the
332 increase in these parameters.
333 On the other hand, neither CI nor pH seemed to globally vary during aging. Regarding the
334 anthocyanic composition, the concentration of LPP increased during aging. In contrast, an
335 important decrease in the concentration of anthocyanins (non-acylated and acylated) was
336 observed, while pyranoanthocyanins and the ethylidene-linked malvidin-3-glucoside-
337 (epi)catechin dimer underwent a slight decrease. Copigmented anthocyanins and SPP remained
338 constant after bottle storage.

339 Although a general evolution pattern was observed for the overall sample set, some
340 departures from this trend were observed depending on the initial composition of wines. The
341 evolution of each of the 3 clusters described above is further commented upon.

342 **3.2.2 Evolution of cluster 1**

343 The evolution of the five wines included in cluster 1 (MC_R09, AR_A08, RN_R09,
344 BE_R10, SC_R10) can be observed in Figure 2a. The evolution (increase or decrease) of their
345 color parameters and the anthocyanic composition are shown in Table 5.

346 Wine samples in this cluster were characterized by high concentrations of free sulfur
347 dioxide and copigmented anthocyanins as well as low concentrations of SPP and TPI values,
348 which were lower than the average (Table 4). These wines underwent substantial changes
349 during aging, and the injection of different oxygen concentrations at the bottling stage also
350 induced changes in color and the pigment composition. It is important to note that in all cases
351 the effect of aging time was more important than the effect of the oxygen levels studied, as
352 Figure 2a shows. The two dimensional PCA (Figure 2a) shows a displacement of all wines after
353 6 months of aging to the left of their corresponding original wine (MC_R09, AR_A08,
354 RN_R09,BE_R10, SC_R10) with a decrease in the first PCA dimension. This shift was mainly
355 due to the increase in hue (+0.2 on average) and b^*_{10} coordinate (+12.0 on average) during the
356 bottle aging period, as well as to the decrease in the concentrations of both non-acylated (-61.7
357 mg L^{-1} on average) and acylated anthocyanins (-13.4 mg L^{-1} on average).

358 The effect of aging on PC2 was not as clear as for PC1, since in this case three out of the
359 five wines from this cluster underwent a negative displacement (lower scores of PC2). One
360 sample presented a positive displacement (AR_A08), while no shift on PC2 was observed for
361 sample MC_R09. According to Table 5, these changes could mainly be explained in terms of
362 increase in wine color intensity for most wines (except for SC_R10).

363 The presence of different oxygen levels (levels 0-4) resulted in a displacement of the
364 samples to the left side of the plot (lower scores for PC1). This suggested that higher
365 concentrations of oxygen applied at bottling favored the formation of yellow nuances and
366 caused a greater decrease of acylated and non-acylated anthocyanins. Furthermore, as for the
367 global effect observed for the overall sample set, the presence of increased oxygen
368 concentrations did not generate similar variations of color or anthocyanic composition during
369 the aging storage according to PC2 displacements. Thus, a positive displacement (to positive
370 PC2 values) of samples aged with the highest oxygen doses (MC_R09_4, SC_R10_4,
371 RN_R09_4, BE_R10_4) was observed in four wines with respect to samples aged in the
372 absence of oxygen (MC_R09_0, SC_R10_0, RN_R09_0, BE_R10_0), while the opposite effect
373 was found for samples AR_A08_4 and AR_A08_0.

374 **3.2.3 Evolution of cluster 2**

375 The evolution of the five wines included in cluster 2 (CH_R10, BO_B10, RM_R10,
376 GC_B10, CD_C10) is shown in Figure 2b. The evolution (increase or decrease) of their color-
377 parameter and anthocyanin composition is shown in Table 5.

378 Wines belonging to cluster 2 were characterized by high concentrations of ethylidene-
379 linked malvidin-3-glucoside-(epi)catechin dimer, pyranoanthocyanins and LPP, and high
380 values of a^*_{10} coordinate, CI and low hue value. All samples presented TPI higher than the
381 average. These wines underwent substantial changes during aging. As shown in Figure 2b, after
382 6 months of aging, all wines were plotted on the left part of the PCA plot with respect to their
383 original wine (before storage: RM_R10, GC_B10, CD_C10, CH_R10, BO_B10). These
384 changes during aging were associated to the increase in hue value (+0.1) and b^*_{10} coordinate
385 (+10.5), while also associated to the decrease in the concentrations of non-acylated (-64.6 mg
386 L^{-1} on average) andacylated (-14.7 mg L^{-1} on average) anthocyanins as well as of ethylidene-

linked malvidin-3-glucoside-(epi)catechin dimer (-0.3 mg L^{-1} on average) (Table 5). Changes observed along PC2 were quite limited (Figure 2b).

The presence of different oxygen doses caused similar effects to the storage in terms of anthocyanic composition and color evolution but the magnitude of these changes was more discrete. This could be observed in Figure 2b, where wines stored with higher oxygen levels (level 4) were plotted on the left section of the plot with respect to the lowest oxygen levels (level 0). These displacements were in any case lower than those observed during wine aging (original wines vs level 0). However, in PC2 only a slight displacement was observed along PC2 in the case of samples bottled with the highest oxygen levels (level 4). A common increase of the hue value was observed for the 5 wines belonging to cluster 2. However, differences among these 5 wines in the evolution of other parameters were also observed. A negative displacement to lower PC2 scores, involving a decrease of red nuances (coordinate C^*_{10}) and a decrease in CI, was observed for samples CH_R10 and BO_B10. This fact occurred in wines with lower TPIs and not aged in barrel. Conversely, GC_B10 and RM_R10 wines, with higher TPIs and aged in oak barrels (4 and 8 months, respectively), showed an increase of a^*_{10} coordinate with higher doses of oxygen. In view of the data in Table 5, the variation in the concentration of pyranoanthocyanins was the main variable responsible for the shifts undergone by samples belonging to cluster 2.

3.2.4 Evolution of cluster 3

The evolution of the six wines belonging to cluster 3 (CZ_D08, SO_C07, RB_R06, CT_B07, AY_C05, MG_V05) is shown in Figure 2c. The evolution (increase or decrease) of color parameters and anthocyanic composition for wines of this cluster is shown in Table 5.

Wines of this cluster, which were the oldest of the sample set studied, presented higher TPI than the average and were also characterized by high values of hue and b^*_{10} coordinate as well as low concentrations of ethylidene-linked malvidin-3-glucoside-(epi)catechin dimer, acylated,

412 non-acylated and copigmented anthocyanins. Important changes were observed for these
413 samples during aging. A negative displacement (to the left section of the plot) along PC1 was
414 observed for all wines (Figure 2c). This shift was attributed to an increase of hue and a decrease
415 in the concentration of the acylated anthocyanins, pyranoanthocyanins and ethylidene-linked
416 malvidin-3-glucoside-(epi)catechin dimer.

417 In the second component, a negative displacement during aging was observed for all wines
418 and it was higher in wines with lower TPI (RB_R06 and CT_B07). In view of the results in
419 Table 5, the displacement seemed to be mainly due to a decrease of non-acylated anthocyanin
420 concentrations.

421 Similarly, the effect of different oxygen concentrations at bottling could only be observed
422 for wines with lower TPI (RB_R06 and CT_B07) and attributed to an increase in hue values
423 and a decrease of anthocyanins with increasing oxygen doses. The effect of oxygen on the rest
424 of wines of this cluster was quite limited as samples were projected close together on the plot
425 (Figure 2c).

426 The effect of oxygen on the MG_V05 wine was especially remarkable. Lower oxygen
427 levels (levels 0-3) caused a small decrease in CI (unlike the other wines of the cluster) and an
428 increase in yellowish hues (Supplementary material Table 1). However, the highest oxygen
429 level (level 4) caused a high decrease of CI. This fact could be explained by the precipitation of
430 the coloring matter observed in this wine, which was exclusively observed for the highest
431 oxygen concentration (level 4). This could explain that this wine presented a different trend
432 compared to the other wines of this cluster.

433 A strategy for exploring the effect of oxygen on compositional data and color parameters
434 could be a comparison of the Euclidean distances between the highest and the lowest oxygen
435 levels applied at bottling (level 4 vs level 0) (Supplementary material Table 2). As reported
436 above, for the three wine clusters or groups, dose 4 presented the lowest value for PC1 when

437 compared with dose 0, even if this shift was different amongst them. Thus, wines belonging to
438 clusters 1 and 2 showed similar average displacement for this PC, whereas their standard
439 deviation (sd) was different. Wines with lower TPI (cluster 1) showed higher sd (1.12) than
440 wines of cluster 2 (0.51), these samples being the youngest among the sample set. This
441 indicated that in the presence of oxygen, wines with lower TPI (average = 51.6) showed higher
442 variability in their evolution than wines with higher TPI (average = 65.1).

443 The wines of cluster 3 (aged wines with similar TPI (average = 63.8) to cluster 2 showed a
444 smaller shift in PC1 (-0.51) than wines belonging to the other two clusters and a similar
445 evolution regarding the sd (0.65) calculated with the Euclidean distance between level 4 and
446 level 0.

447 Besides the displacement along PC1, the addition of different oxygen levels to the sixteen
448 wines studied generated shifts on PC2, the most important being for the highest level applied
449 (level 4). This change along PC2 was different among the three groups of wines studied
450 (Supplementary material Table 2); however, its interpretation was deemed difficult. This was
451 mainly because, unlike PC1, PC2 presented different (positive or negative, depending on the
452 cluster) correlations with variables (such as pH or a^*_{10}), even if the CI variable was positive in
453 the three cases.

454 **4. DISCUSSION**

455 The major aim of this work was to evaluate the changes in color parameters and the
456 anthocyanin composition of a relatively large number of red wines during bottle storage (6
457 months at 25 °C) in air-tight containers under five different oxygen doses mimicking real and
458 extreme bottling situations. Results showed that there was a general pattern of evolution of
459 color parameters and anthocyanin composition of red wines during bottle aging. This general
460 pattern was mainly characterized by a decrease in the concentration of free and combined sulfur
461 dioxide and an increase in yellow nuances during aging measured by the increase in hue and

462 b^*_{10} coordinate, both parameters related to wine aging (Negueruela, Echavarri, & Pérez, 1995;
463 García-Puente, Alcalde-Eon, Santos-Buelga, Rivas-Gonzalo & Escribano-Bailón, 2006; Perez-
464 Magariño & González-San José, 2006). These aging changes have been widely described in
465 literature (Wirth et al., 2010; Wirth, Caille, Souquet, Samson, Dieval, Vidal, Fulcrand &
466 Cheynier, 2012; Negueruela et al., 1995; García-Puente et al., 2006; Perez-Magariño et al.,
467 2006). Thus, when there are low concentrations of sulfur dioxide in wines, the formation of
468 quinones and hydrogen peroxide is favored as a consequence of the oxidation of ortho-
469 diphenols (Danilewicz, 2012). Those quinones that are not reduced due to insufficient content
470 in SO_2 can participate in polymerization reactions, which lead to an increase in the yellow color
471 of wines (Singleton, 1987).
472 Likewise, it has also been observed that the variation of hue during aging presented a
473 positive correlation with vintage ($P < 0.01$), indicating that young wines presented a pigment
474 composition that seem to be less stable during aging. These results agree with the fact that the
475 coloring matter of aged wines is more stable to changes produced by time, temperature or
476 available oxygen during aging (McRae et al., 2012). Furthermore, the general pattern of
477 evolution involved an increase in the concentration of LPP and a decrease in free anthocyanins
478 (acylated and non-acylated), pyranoanthocyanins and ethylidene-linked malvidin-3-glucoside-
479 (epi)catechin dimer pigments. The decrease in free anthocyanins during aging has been widely
480 described in literature (Atanasova, Fulcrand, Cheynier & Moutounet, 2002; Monagas et al.,
481 2005; García-Falcón, Pérez-Lamela, Martínez-Carballo & Simal-Gándara, 2007; Giovanelli &
482 Brenna, 2007; Wirth et al., 2010, 2012; Chira, Pacella, Jourdes & Teissedre, 2011; Gambuti et
483 al., 2013; Gómez-Gallego, Gómez García-Carpintero, Sánchez-Palomo, González-Viñas &
484 Hermosín-Gutiérrez, 2013). As expected, this decrease was more pronounced for non-acylated
485 and acylated anthocyanins than for pyranoanthocyanins and ethylidene-linked malvidin-3-
486 glucoside-(epi)catechin dimer. This fact is very interesting in order to preserve wines with a

487 less evolved color for longer, since pyranoanthocyanins and ethylidene-linked malvidin-3-
488 glucoside-(epi)catechin dimer appear to be opposed to the increase in yellow nuances (Sáenz-
489 Navajas, Echavarri, Ferreira & Fernández-Zurbano, 2011). The contribution of these
490 compounds to the color of aged wines has been widely demonstrated (Escribano-Bailón et al.,
491 2002; Wirth et al., 2010; Sáenz-Navajas et al., 2011). These works agree in finding a decrease
492 in non-acylated and acylated anthocyanins, while there is a disagreement in the reported
493 evolution of compounds such as pyranoanthocyanins and ethylidene-linked malvidin-3-
494 glucoside-(epi)catechin dimer. There are some research papers that show an increase in the
495 concentration of these compounds, especially during the early stages of bottle aging (Wirth et
496 al., 2012; García-Falcón et al., 2007; Atanasova et al., 2002), while others (Monagas et al.,
497 2005) show a slight decrease. As reported in the bibliography, pyranoanthocyanins are more
498 stable than their anthocyanic precursors, but depending on factors such as wine composition or
499 aging period, among others, an increase or slight decrease in the concentration of these
500 compounds may occur. In this experiment, results showed that once the concentration of
501 pyranoanthocyanins and ethylidene-linked malvidin-3-glucoside-(epi)catechin dimer decreased,
502 an increase in the concentration of SPP polymeric pigments and especially of LPP were
503 observed. The decrease observed for the former compounds could be due to reactions between
504 pyranoanthocyanins and vinyl-flavanol adducts as proposed by Mateus, Silva, Rivas-Gonzalo,
505 Santos-Buelga & De Freitas (2003), leading to pigments with higher molecular weight.
506 Even if a general pattern of evolution was observed, different trends have been found
507 depending on the initial composition of wine samples such as color or polyphenolic
508 composition (TPI). Thus, the youngest wines (cluster 2) underwent more important changes in
509 the color parameters studied. These wines showed a slight decrease in TPI, a reduction in all
510 the families of monomeric anthocyanins, while no variation in copigmented anthocyanins or
511 SPP were found. Furthermore, the wines of this cluster presented a different evolution of CI

depending on their TPI. Thus, a decrease in CI was observed in wines with lower TPI; whereas for wines with higher TPI, this parameter increased with aging in wines with higher TPI. Wirth et al., (2012) and Caille et al., (2010) related this increase in CI to the decline of sulfur dioxide; however, the decrease of this antioxidant was similar in all wines, suggesting that the release and contribution of these compounds should be similar. Thus, this effect could be attributed to the condensation of polyphenolic compounds such as flavanols and proanthocyanidins with anthocyanins, which would lead to an increase in the concentration of red pigments. The reduction of anthocyanins minimizes the irreversible transformation of the anthocyanins through the chalcone series into colorless phenolic acids, leading to an irreversible decrease in wine color (Ribéreau-Gayon, 1970). These results are in accordance with the fact that wines with higher TPIs are the most resistant to oxidation and the most suitable for aging (Jaffré, Valentin, Dacremont & Peyron, 2009).

Contrary to young wines, samples of older vintages (cluster 3) experienced an unexpected increase in copigmented anthocyanins during aging. This fact is difficult to explain since these compounds have been reported to contribute mostly to the color of young wines (Boulton, 2001). Lastly, the wines in cluster 1, which belonged to intermediate vintages and presented low TPI, were characterized by a decrease in red nuances when CI increased. This decrease in red color was attributed to the decrease in copigmented anthocyanins, non-acylated and acylated anthocyanins, while the increase in CI could be linked to an increase in the absorbance at 420 nm and also in the b^*_{10} coordinate. The observed increase in the yellow color of these intermediate vintage wines was (1.5 units) higher than that experienced by young wines (cluster 2), possibly due to their lower TPI (Table 4). Similarly, Gambuti et al. (2013) observed a slight decrease of color in wines with less TPI.

The second main result of this dataset was that the role of the initial oxygen level at bottling was minimal compared to the storage time. Oxygen appears to slightly accentuate the

processes observed during aging. It should be considered that the wine samples used in this experiment were red wines with relatively high TPIs and able to consume all the oxygen in just a few days. Nevertheless, all wines showed the most important compositional changes between the highest and the lowest oxygen levels applied at bottling (level 4 and level 0). There were certain compounds that experienced remarkable changes when the level of oxygen was increased. Moreover, in the presence of oxygen, the evolution of wines with lower TPI showed higher variability (in terms of the chemical variables analyzed) than wines with higher TPI, which showed a more homogeneous evolution among them.

Wine MG_V05 was the only wine that experienced a precipitation of coloring matter with the highest dose of oxygen, together with a decline in CI and, most importantly, a reduction of its yellow color measured by the hue. The formation of yellow, large and insoluble polymeric pigments (Habertson et al., 2003; Sun, Barradas, Leandro, Santos & Spranger, 2008) described to occur during wine aging, could explain the apparition of this precipitation, the reduction in CI and the yellow color of this wine sample.

5. CONCLUSIONS

In conclusion, the wines stored in this study showed a general pattern in the evolution of their characteristics and color composition. This pattern of evolution depended on both the initial composition of the wine and its TPIs. Thus, wines with higher TPI from older vintages had the most stable coloring matter and experienced a small evolution. Wines with lower TPI showed a more important evolution and greater variability in the behavior, even if they were not young wines.

More important changes in wine composition and related to oxygen doses were expected; however, these changes were less marked than those related to aging time. This could be related to the fact that the total addition of oxygen (in the initial headspace) was carried out at once at bottling, while the injection of oxygen along the storage period (through closure) is

562 expected to induce a different evolution of wine samples. Similarly, the content and the
563 consumption rate of sulfur dioxide seemed to be responsible for the reactions of polyphenolic
564 compounds; hence, these issues are currently being considered in our laboratory.

565 The results presented in this paper are relevant to wine experts since they help to
566 understand the evolution of color properties of wine during bottling. This study may help to
567 develop strategies to manage this stage in winemaking with objective criteria.

568

569 **6. ACKNOWLEDGEMENTS**

570 This work was funded by the Spanish Ministry of Economy and Competitiveness with the
571 (Proyects AGL2010-22355 and AGL2014-59840) J.M.A. acknowledges the Government of
572 Navarra for his predoctoral (FPI) fellowship and M.P.S.N. the Spanish Ministry of Economy
573 and Competitiveness (M.E.C.) for her postdoctoral fellowship (Formación Posdoctoral 2013,
574 former Juan de la Cierva).

575

576 **7. REFERENCES**

577 AOAC, Method 993.05: L-Malic acid/total malic acid ratio in apple juice. In Official Methods
578 of Analysis, 2002.

579 Atanasova, V., Fulcrand, H., Cheynier, V., & Moutounet, M. (2002). Effect of oxygenation on
580 polyphenol changes occurring in the course of wine-making. Analitycal Chimica Acta. 428, 15-
581 27.

582 Boulton, R. (2001). The copigmentation of anthocyanins and its role in the color of red wine: a
583 critical review. American Journal of Enology and Viticulture. 52, 67-87.

584 Caillé, S., Samson, A., Wirth, J., Diéval, J. B., Vidal, S., & Cheynier, V. (2010). Sensory
585 characteristics changes of red Grenache wines submitted to different oxygen exposures pre and
586 post bottling. Analytical Chimica Acta. 660, 35-42.

587 Carrascón, V., Fernández-Zurbano, P., Bueno, M., & Ferreira, V., (2015). Oxygen consumption
588 by red wines. Part II: Differential effects on color and chemical composition caused by oxygen
589 taken in different sulfur dioxide-related oxidation contexts. Journal Agriculture and Food
590 Chemistry. 63, 10938-10947.
591 Chira, K., Pacella, N., Jourdes, M., & Teissedre, P.L. (2011). Chemical and sensory evaluation
592 of Bordeaux wines (Cabernet-Sauvignon and Merlot) and correlation with wine age. Food
593 Chemistry. 126, 1971-1977.
594 C.I.E. Colorimetry, 2nd ed. Central Bureau of the Commission Internationale de L´Eclairage,
595 Viena, Publication C.I.E. 2004, No. 15, 2.
596 Dallas, C., & Laureano, O. (1994). Effects of pH, sulfur dioxide, alcohol content, temperature
597 and storage time on colour composition of a young Portuguese red table wine. Journal of the
598 Science of Food and Agriculture, 65, 477- 485.
599 Danilewicz, J.C. (2012). Review of oxidative processes in wine and value of reduction
600 potentials in enology. American Journal of Enology and Viticulture. 63, 1-10.
601 De Simón, B. F., Cadahia, E., Sanz, M., Poveda, P., Perez-Magariño, S., Ortega-Heras, M., &
602 Gonzalez-Herta, C. (2008). Volatile compounds and sensorial characterization of wines from
603 four Spanish denominations of origin aged in Spanish Rebollo (Quercus pirenaica Willd.) oak
604 wood barrels. Journal Agriculture and Food Chemistry. 56, 9046-9055.
605 Escribano-Bailón, T., Álvarez-García, M., Rivas-Gonzalo, J.G., Heredia, F.J. & Santos-Buelga,
606 C. (2001). Color and stability of pigments derived from the acetaldehyde-mediated
607 condensation between malvidin 3-O-glucoside and (+)-catechin. Journal Agriculture and Food
608 Chemistry. 49, 1213-1217.
609 Fulcrand, H., Cameira do Santos, P.-J., Sarni-Manchado, P., Cheynier; V. & Favre-Bonvin, J.
610 (1996). Structure of new anthocyanin-derived wine pigments. Journal of the Chemical Society -
611 Perkin Transactions 1, 7, 735-739.

612 Fulcrand, H., Cheynier, V., Oszmianski, J., & Moutounet, M., (1997). An oxidized tartaric acid
613 residue as a new bridge potentially competing with acetaldehyde in flavan-3-ol condensation
614 Phytochemistry, 46, 223-227.
615 Fulcrand, H., Benabdeljalil, C., Rigaud, j., & Cheynier, V., (1998). A new class of wine
616 pigments generated by reaction between pyruvic acid and grape anthocyanins. Phytochemistry,
617 47, 1401-1407.
618 Fulcrand, H., Dueñas, M., Salas, E., & Cheynier, V., (2006). Phenolic reactions during
619 winemaking and aging. *American Journal of Enology and Viticulture,* 57, 289-297.
620 Gambuti, A., Rinaldi, A., Ugliano, M., & Moio, L. (2013). Evolution of phenolic compounds
621 and astringency during aging of red wine: effect of oxygen exposure before and after bottling.
622 Journal Agriculture and Food Chemistry. 61, 1618-1627.
623 García Falcón, M.S., Pérez Lamela, C., Martínez Carballo, E., & Simal Gándara, J. (2007).
624 Determination of phenolic compounds in wines: Influence of bottle storage of young red wines
625 on their evolution. Food Chemistry. 105, 248-259.
626 García Puente, E., Alcalde Eon, C., Santos Buelga, C., Rivas Gonzalo, J. C., & Escribano
627 Bailón, M. T. (2006). Behaviour and characterisation of the colour during red wine making and
628 maturation. Analitycal Chimica Acta, 563, 215-222.
629 Giovanelli, G., & Brenna, O. V. (2007). Oxidative stability of red wine stored in packages with
630 different oxygen permeability. European Food Research and Technology. 226, 169-179.
631 Gómez Gallego, M. A., Gómez García Carpintero, E., Sánchez Palomo, E., González Viñas,
632 M.A. Hermosín Gutiérrez, I. (2013). Evolution of the phenolic content, chromatic
633 characteristics and sensory properties during bottle storage of red single-cultivar wines from
634 Castilla La Mancha region. Food Research International. 51, 554-563.
635 González-Hernández, M., Avizcuri-Inac, J. M., Dizy, M., & Fernández-Zurbano, M. P. (2014).
636 Ultra performance liquid chromatography coupled to ultraviolet-Vis and mass spectrometry

637 detector for screening of organic acids and polyphenols in red wine. High-Performance Liquid
638 Chromatography (HPLC): Principles, Practices and Procedures. Nova Science Publisher. New
639 York-USA. 267-297.
640 Habertson, J. F., Picciotto, E. A., & Adams, D. O. (2003). Measurement of polymeric pigments
641 in grape berry extracts and wines using a protein precipitation assay combined with bisulfite
642 bleaching. American Journal of Enology and Viticulture. 54, 301-306.
643 Han, G., Ugliano, M., Currie, B. Vidal, S., Dieval J-B. & Waterhouse, A.L., (2014). Influence
644 of clouse, phenolic levels and microoxygenation on Cabernet Sauvignon wine composition
645 after 5 years' bottle storage. Journal Science Food Agriculture, 95, 36-43.
646 Jaffré, J., Valentin, D., Dacremont, C., & Peyron, D. (2009). Burgundy red wines:
647 representation of potential for aging. Food Quality and Preference. 20, 505-513.
648 Laurie, V.F., Zúñiga, M.C., Carrasco-Sánchez, V., Santos, L. S., Cañete, A., Olea-Azar, C.,
649 Ugliano, M., Agosin, E. (2012). Reactivity of 3-sulfanyl-1-hexanol and catechol-containing
650 phenolics in vitro. Food Chemistry. 131, 1510-1516.
651 Lopes, P., Silva, M.A., Pons, A., Tominaga, T., Lavigne, V., Saucier, C., Darriet, P., Cabral,
652 M., Teissedre, P. L., & Dubourdiu, D. (2009). Impact of oxygen dissolved at bottling and
653 transmitted through closures on the composition and sensory properties of a Sauvignon Blanc
654 wine during bottle storage. Journal Agriculture and Food Chemistry. 57, 10261–10270.
655 Mateus, N., Silva, A.M.S., Rivas-Gonzalo, J.C., Santos-Buelga, C., De Freitas, V. (2003). A
656 new class of blue anthocyanin-derived pigments isolated from red wines. Journal Agriculture
657 and Food Chemistry. 51, 1919-1923.
658 Mazza, G., Fukumoto, L., Delaquis, P., Girard, B. & Ewert B. (1999. Anthocyanins,
659 Phenolics, and Color of Cabernet Franc, Merlot, and Pinot Noir Wines from British Columbia.
660 Journal Agriculture and Food Chemistry. 47, 4009–4017.

661 McRae, J.M., Dambergs, R.G., Kassara, S., Parker, M., Jeffery, D. W., Herderich, M.J., &
662 Smith, P.A. (2012). Phenolic compositions of 50 and 30 year sequences of Australian red
663 wines: the impact of wine age. Journal Agriculture and Food Chemistry. 60, 10093-10102.
664 Monagas, M., Gómez-Cordovés, C., & Bartolomé, B. (2005). Evolution of polyphenols in red
665 wines from Vitis vinífera L. during aging in the bottle – I. Anthocyanins and
666 pyranoanthocyanins. European Food Research and Technology. 220, 607-614.
667 Morineau, A. (1984). Note sur la caractérisation statistique d'une classe par les valeurs-test. In
668 Bulletin Technique Centre Statistique Informatique Appliquées, 2, 20-27.
669 Morineau, A., Lebart, L., Piron, M. (1995). Statistique exploratoire multidimensionnelle. Paris:
670 Dunod.
671 Morrot, G., Brochet, F., & Dubourdieu, D. The color of odors. Brain and Language, 2001, 79,
672 309-320.
673 Negueruela, I., Echavarri, J. F., & Pérez, M. M. (1995). A study of correlation within
674 enological colorimetric indexes and CIE colorimetric parameters in red wine. American Journal
675 of Enology and Viticulture. 46, 353-356.
676 Nikolantonaki, M., Waterhouse, A.L. (2012). A method to quantify quinone reaction rates with
677 wine relevant nucleophiles: A key to the understanding of oxidative loss of varietal thiols.
678 Journal Agriculture and Food Chemistry. 60, 8484-8491.
679 O.I.V. Recueil des methodes internationales d´analyse des vins et des mouts. Vienne, 2005.
680 Perez-Magariño, S., & González-San José, M. L. (2004). Evolution of flavanols, anthocyanins,
681 and their derivatives during the aging of red wines elaborated from grapes harvested at different
682 stages of ripening. J. Agric. Food Chem., 2004, 52, 1181-1189.
683 Perez-Magariño, S., & González-San José, M. L. (2006). Polyhenols and colour variability of
684 red wines made from grapes harvested at different ripeness grade. Food Chemistry. 96, 197-
685 208.

686 Pissarra, J., Mateus, N., Rivas-Gonzalo, J., Santos Buelga, C., & De Freitas, V. (2003).
687 Reaction between malvidin 3-glucoside and (+)-catechin in model solutions containing
688 different aldehydes. Journal Food Science. 476-481.
689 Puértolas, E., Saldaña, G., Condón, S., Álvarez, I., & Raso, J. (2010). Evolution of
690 polyphenolic compounds in red wine from Cabernet Sauvignon grapes processed by pulsed
691 electric fields during aging in bottle. Food Chemistry. 119, 1063-1070.
692 Resolution OENO 1/2006. Determination of the chromatic characteristics of wine according to
693 CIELab. 2006, Paris, France.
694 Ribéreau-Gayon, P. (1970). Le dosage des composes phénoliques totaux dans les vins rouges.
695 Analytical Chimica Acta. 52, 627-631.
696 Romero C. & Bakker, J. (2000a). Effect of storage temperature and pyruvate on kinetics of
697 anthocyanin degradation, visitin A derivate formation and colour characteristics of model
698 solution. Journal Agriculture and Food Chemistry. 48, 2135-2141.
699 Romero C. & Bakker, J. (2000b). Effect of acetaldehyde and several acids on the formation of
700 visitin A in model wine anthocyanin and colour evolution. Journal of Food Science and
701 Technology 34, 129-140.
702 Sáenz-Navajas, M. P.; Avizcuri, J. M.; Ferreira, V.; Fernández-Zurbano, M. P. (2014). Sensory
703 changes during bottle storage of Spanish red wines under different initial oxygen doses. Food
704 Research International. 66, 235-246.
705 Sáenz-Navajas, M.P., Echavarri, J.F., Ferreira, V., & Fernández-Zurbano, M.P. (2011).
706 Pigment composition and color parameters of commercial Spanish red wines samples: Linkage
707 to quality perception. European Food Research and Technology. 232, 877-887.
708 Salas, E., Atanasova, V., Poncet-Legrand, C., Meudec, E., Mazauric, J.P., & Cheynier, V.
709 (2004). Demonstration of the occurrence of flavanol–anthocyanin adducts in wine and in model
710 solutions. Analytical Chimica Acta. 513, 325-332.

711 Sánchez-Iglesias, M., González-San José, M. L., Pérez-Magariño, S., Ortega-Heras, M., &
712 Gonzalez-Huerta, C. (2009). Effect of micro-oxygenation and wood type on the phenolic
713 composition and color of an aged red wine. Journal Agriculture and Food Chemistry. 57,
714 11498-11509.
715 Sarni-Manchado, P., Fulcrand, E., Souquet, J. M, Cheynier, V., & Moutounet, M. (1996).
716 Stability and color of unreported wine anthocyanin-derived pigments. Journal of food Science,
717 61, 938-941.
718 Singleton, V. L. Oxygen with phenols and related reactions in musts, wines, and model
719 systems: Observations and practical implications. Am J. Enol. Vitic., 1987, 38, 69-77.
720 Sun, B., Barradas, T., Leandro, C., Santos, C., & Spranger, I. (2008). Formation of nex stable
721 pigments from condensation reaction between malvidin 3-monoglucoside and (-)-epicatechin
722 mediated by acetaldehyde: Effect of tartaric acid concentration. Food Chemistry. 110, 334-351.
723 Somers, T. C., (1971). The polymeric nature of wine pigments. *Phytochemistry,* 10, 2175-2186.
724 Timberlake, C. F. & Bridle, P. (1976). Interactions between anthocyanins, phenolic
725 compounds, and acetaldehyde and their significance in red wine. *American Journal of Enology*
726 *and Viticulture,* 27, 97-105.
727 Somers, T. C. & Evans, M. E. (1986). Evolution of red wines I. Ambient influences on colour
728 composition during early maturation. *Vitis,* 25, 31-39.
729 Villamor, R., Haberson, J., & Ross, C. (2009). Influence of tannin concentration, storage
730 temperature, and time on chemical and sensory properties of Cabernet Sauvignon and Merlot
731 wine. American Journal of Enology and Viticulture. 60, 442-449.
732 Vivar-Quintana, A.M., Santos-Buelga, C., Francia-Aricha, E., & Rivas-Gonzalo J.C. (1999).
733 Formation of anthocyanin-derived pigments in experimental red wines. Food Science and
734 Technology International. 5, 347-352.

735 Wirth, J., Morel-Salmi, C., Souquet, J. M., Dieval, J. B., Aagaard, O., Vidal, S., Fulcrand, H.,
736 & Cheynier, V. (2010). The impact of oxygen exposure before and after bottling on the
737 polyphenolic composition of red wines. Food Chemistry. 123, 107-116.
738 Wildenradt, H.L. & Singleton, V.L (1974). The production of aldehydes as a result of oxidation
739 of phenolic compounds and its relation to wine aging. American Journal of Enology and
740 Viticulture, 25, 119-126.
741 Wirth, J., Caille, S., Souquet, J. M., Samson, A., Dieval, J. B., Vidal, S., Fulcrand, H., &
742 Cheynier, V. (2012). Impact of post-bottling oxygen exposure on the sensory characteristics
743 and phenolic composition of Grenache rose wines. Food Chemistry. 132, 1861-1871.
744
745
746 **FIGURE CAPTIONS**
747 **Figure 1. a)** Projection of the 16 wines on the first two principal component of the PCA and **b)**
748 Tree diagram and the three clusters derived from the Hierarchical cluster analysis calculated on
749 three dimensions of the PCA performed with the 16 wine samples.
750 **Figure 2**. **a)** Projection of the 5 wines of cluster 1 on the first two principal component of the
751 PCA, **b)** projection of the 5 wines of cluster 2 on the first two principal component of the PCA
752 and **c)** projection of the 6 wines of cluster 3 on the first two principal component of the PCA

753

Table 1. The sixteen studied commercial wines and their original oenological parameters.

wine code	origin	vintage year	grape variety	Months in barrels	TPI[a]	pH	TA[b]	VA[c]	RS[d]	MA[e]	LA[f]	Alcohol (% v/v)
MG_V05	DO Dominio de Valdepusa	2005	Cabernet Sauvignon	12	83.4 ± 0.7	3.65 ± 0.01	4.91 ± 0.02	0.56 ± 0.01	4.35 ± 0.09	0.29 ± 0.10	0.77 ± 0.02	15.2 ± 0.02
AY_C05	DO Cariñena	2005	Merlot, Tempranillo,Cabernet Sauvignon	10	74.3 ± 0.3	3.52 ± 0.00	5.86 ± 0.01	0.69 ± 0.01	3.39 ± 0.18	0.33 ± 0.10	1.00 ± 0.03	14.3 ± 0.07
RB_R06	DOCa Rioja	2006	Tempranillo, Garnacha	18	49.4 ± 0.3	3.49 ± 0.01	5.37 ± 0.01	0.57 ± 0.01	2.23 ± 0.07	0.23 ± 0.14	1.45 ± 0.01	14.3 ± 0.00
CT_B07	DO Borja	2007	Garnacha	15	59.1 ± 0.3	3.47 ± 0.00	5.66 ± 0.01	0.51 ± 0.00	4.34 ± 0.18	0.30 ± 0.02	0.75 ± 0.03	13.9 ± 0.07
SO_C07	DO Cariñena	2007	Garnacha, Tempranillo, Cabernet Sauvignon	18	54.9 ± 1.4	3.53 ± 0.00	5.66 ± 0.01	0.75 ± 0.00	3.81 ± 0.03	0.18 ± 0.04	1.21 ± 0.01	13.8 ± 0.05
AR_A08	DO Arlanza	2008	Tempranillo	12	53.0 ± 0.2	3.73 ± 0.00	5.57 ± 0.01	0.63 ± 0.01	1.98 ± 0.10	0.24 ± 0.06	2.79 ± 0.03	13.6 ± 0.03
CZ_D08	DO Duero	2008	Tempranillo	18	62.0 ± 0.1	3.65 ± 0.01	5.33 ± 0.01	0.57 ± 0.01	1.71 ± 0.18	0.35 ± 0.07	2.47 ± 0.01	13.4 ± 0.05
MC_R09	DOCa Rioja	2009	Tempranillo,Graciano, Mazuelo	12	52.3 ± 0.3	3.64 ± 0.01	4.92 ± 0.02	0.52 ± 0.01	2.09 ± 0.14	0.21 ± 0.02	2.11 ± 0.05	13.7 ± 0.03
RN_R09	DOCa Rioja	2009	Tempranillo, Garnacha	18	49.7 ± 0.4	3.65 ± 0.01	5.35 ± 0.01	0.66 ± 0.01	1.67 ± 0.15	0.18 ± 0.10	2.14 ± 0.01	13.6 ± 0.03
BO_B10	DO Borja	2010	Garnacha, Syrah, Tempranillo	0	61.0 ± 0.9	3.66 ± 0.01	5.04 ± 0.01	0.47 ± 0.00	2.68 ± 0.20	0.17 ± 0.06	1.07 ± 0.01	14.8 ± 0.07
CH_R10	DOCa Rioja	2010	Tempranillo, Viura	0	60.3 ± 0.4	3.88 ± 0.00	4.45 ± 0.01	0.62 ± 0.01	1.77 ± 0.14	0.20 ± 0.03	3.30 ± 0.02	14.1 ± 0.03
CD_C10	DO Cariñena	2010	Garnacha, Tempranillo, Cabernet Sauvignon	0	66.4 ± 0.4	3.63 ± 0.00	5.30 ± 0.01	0.53 ± 0.01	2.57 ± 0.16	0.24 ± 0.17	0.90 ± 0.01	13.5 ± 0.07
SC_R10	DOCa Rioja	2010	Tempranillo, Garnacha	0	57.8 ± 0.3	3.72 ± 0.02	4.84 ± 0.01	0.48 ± 0.01	2.32 ± 0.08	0.18 ± 0.04	2.52 ± 0.01	13.4 ± 0.03
GC_B10	DO Borja	2010	Garnacha	4	71.4 ± 0.3	3.43 ± 0.01	6.14 ± 0.01	0.42 ± 0.01	3.61 ± 0.11	0.25 ± 0.02	0.68 ± 0.02	14.7 ± 0.04
RM_R10	DOCa Rioja	2010	Graciano	8	66.4 ± 1.7	3.57 ± 0.00	5.80 ± 0.01	0.41 ± 0.01	2.31 ± 0.21	0.19 ± 0.06	1.45 ± 0.02	14.8 ± 0.04
BE_R10	DOCa Rioja	2010	Tempranillo, Garnacha	0	45.4 ± 1.0	3.61 ± 0.00	5.09 ± 0.01	0.25 ± 0.01	1.52 ± 0.15	0.18 ± 0.02	1.86 ± 0.02	13.9 ± 0.02

Data expressed as the mean ± SD (n = 2).
[a]Total Polyphenol Index
[b]Total titratable acidity expressed in g L^{-1} of tartatic acid
[c]Volatile acidity expressed in g L^{-1} of acetic acid
[d]Reducing sugars expressed in g L^{-1}
[e]Malic acid expressed in g L^{-1}
[f]Lactic acid expressed in g L^{-1}

Table 2. Identification, pigment groups, MS and MS^2 spectrum data (M^+: positive charged molecular ion), retention time (t_R), chemical identity, maximum (max.), average and minimum (min.) concentrations (expressed in mg L^{-1} of malvidin-3-O-glucoside) for anthocyanins analyzed by UPLC–UV/Vis-MS.

peak	pigment groups	M^+ (m/z)	MS^2	t_R (min)	compound	max.	mean	min.
1	pyranoanthocyanin	489	327	1.2	B-type vitisin of Dp-3-glc	0.22	0.13	0.05
2	non acylated anthocyanins + A-F adduct	465/781	303/619	2.0	Dp-3-glc + Mv-3-glc-(epi)catechin	17.00	8.72	0.47
3	non acylated anthocyanins	449	287	2.4	Cy-3-glc	2.12	1.11	0.30
4	non acylated anthocyanins	479	317	2.7	Pt-3-glc	17.80	8.25	0.33
5	non acylated anthocyanins	463	301	3.2	Pn-3-glc	6.52	3.02	0.10
6	non acylated anthocyanins	493	331	3.4	Mv-3-glc	72.43	36.35	2.48
7	acylated anthocyanins	507	303	3.6	Dp-3-acylglc	0.96	0.29	0.05
8	pyranoanthocyanin	561	399	3.7	Vitisin A	4.21	2.76	1.98
9	pyranoanthocyanin	517	355	4.0	Vitisin B	0.18	0.08	0.05
10	pyranoanthocyanin + acylated anthocyanins	559/491	355/287	4.2	B-type vitisin of Mv-3-acylglc + Cy-3-acylglc	0.82	0.25	0.05
11	acylated anthocyanins	521	317	4.6	Pt-3-acylglc	2.14	0.52	0.08
12	ethylidene-linked malvidin-3-glucoside-(epi)catechin dimer	809	357	4.9	Ethylidene-linked malvidin-3-glucoside-(epi)catechin dimer	0.71	0.34	0.12
13	acylated anthocyanins	611	303	5.3	Dp-3-*p*-coumglc	3.48	1.82	0.08
14	acylated anthocyanins	535	331	5.8	Mv-3-acylglc	10.63	3.31	0.07
15	pyranoanthocyanin	707	399	5.9	A-type vitisin of Mv-3-*p*-coumglc	0.86	0.42	0.23
16	acylated anthocyanins	625	317	6.7	Pt-3-*p*-coumglc	2.76	1.16	0.05
17	acylated anthocyanins	639	331	7.3	Mv-3-*p*-coumglc *cis*	1.97	0.52	0.18
18	acylated anthocyanins	609	301	8.0	Pn-3-*p*-coumglc	2.05	0.97	0.05
19	acylated anthocyanins + pyranoanthocyanin	639/771	331/463	8.2	Mv-3-*p*-coumglc *trans* + Mv-3-p-coumglc 4-vinylcatechol adduct	7.62	4.04	0.53
20	pyranoanthocyanin	651	447	9.4	Mv-3-acylglc 4-vinylphenol adduct	2.28	0.50	0.14
21	pyranoanthocyanin	755	447	10.7	Mv-3-*p*-coumglc 4-vinylphenol adduct	0.07	0.05	0.05

Dp: delphinidin; Cy: cyanidin; Pt: petunidin; Pn: peonidin; Mv: malvidin; glc: glucose; acylglc: *6''*-acetylglucoside; *p*-coumglc: *6''-p*-coumaroylglucoside

9 **Table 3.** Original color parameters and anthocyanins concentration of wines studied.

Wine code	SO_2 F[a]	SO_2 C[a]	CI[b]	Tonality	color coordinates					CA[b]	SPP[b]	LPP[b]	Non-acyl[a]	Acyl[a]	Pyrano[a]	EMv-F[a]
					a^*_{10}	b^*_{10}	L^*_{10}	C^*_{ab}	h_{ab}							
MG_V05	8.0 ± 1.1	25.6 ± 0.0	19.3 ± 0.02	0.79 ± 0.03	46.5 ± 0.0	26.6 ± 0.0	34.4 ± 0.0	53.6± 0.0	29.8± 0.0	1.12 ± 0.04	1.17 ± 0.02	0.28 ± 0.01	4.03 ± 0.04	4.28 ± 0.10	5.74 ± 0.08	nd
AY_C05	6.4 ± 1.1	58.4 ± 0.0	10.8 ± 0.01	0.94 ± 0.04	41.1 ± 0.0	27.6 ± 0.1	55.3 ± 0.1	49.5± 0.1	33.9± 0.1	0.00 ± 0.01	0.60 ± 0.02	0.35 ± 0.01	5.50 ± 0.06	1.46 ± 0.02	3.31 ± 0.04	0.22 ± 0.01
RB_R06	8.0 ± 0.0	67.2 ± 1.1	8.6 ± 0.05	0.92 ± 0.03	33.2 ± 0.1	18.1 ± 0.1	60.2 ± 0.3	37.8± 0.2	28.6± 0.3	2.11 ± 0.04	0.22 ± 0.00	0.40 ± 0.05	18.08 ± 0.07	4.18 ± 0.06	3.42 ± 0.03	0.14 ± 0.01
CT_B07	15.6 ± 1.1	64.0 ± 0.0	9.2 ± 0.00	0.77 ± 0.00	42.2 ± 0.0	13.7 ± 0.0	45.7 ± 0.1	44.4± 0.1	17.9± 0.1	0.00 ± 0.06	0.61 ± 0.06	0.40 ± 0.05	29.15 ± 0.17	7.45 ± 0.05	3.53 ± 0.03	0.12 ± 0.00
SO_C07	8.8 ± 1.1	48.0 ± 0.0	7.2 ± 0.07	0.85 ± 0.10	35.1 ± 0.1	13.8 ± 0.1	64.6 ± 0.1	37.7± 0.1	21.5± 0.1	0.16 ± 0.16	0.23 ± 0.05	0.31 ± 0.01	44.89 ± 0.27	10.25 ± 0.06	5.57 ± 0.04	0.23 ± 0.00
AR_A08	23.6 ± 1.9	32.0 ± 1.1	8.8 ± 0.04	0.79 ± 0.02	37.4 ± 0.1	10.7 ± 0.0	38.9 ± 0.2	38.9± 0.1	16.0± 0.2	2.04 ± 0.05	0.54 ± 0.00	0.26 ± 0.04	58.71 ± 0.63	11.70 ± 0.06	4.58 ± 0.05	0.18 ± 0.00
CZ_D08	11.2 ± 1.7	28.8 ± 0.0	9.6 ± 0.01	0.76 ± 0.01	41.1 ± 0.0	10.5 ± 0.1	54.3 ± 0.1	42.5± 0.1	14.3± 0.1	2.77 ± 0.10	0.23 ± 0.01	0.32 ± 0.01	48.53 ± 0.21	8.84 ± 0.04	3.87 ± 0.04	0.25 ± 0.00
MC_R09	15.2 ± 0.6	35.2 ± 0.6	8.8 ± 0.00	0.80 ± 0.05	38.4 ± 0.0	10.0 ± 0.0	57.5 ± 0.0	39.6± 0.0	14.6± 0.0	2.96 ± 0.10	0.23 ± 0.01	0.32 ± 0.01	74.04 ± 0.24	15.09 ± 0.08	3.07 ± 0.06	0.29 ± 0.01
RN_R09	29.1 ± 0.0	76.8 ± 0.0	7.8 ± 0.00	0.82 ± 0.02	34.0 ± 0.0	11.3 ± 0.0	62.0 ± 0.0	35.8± 0.0	18.4± 0.0	1.98 ± 0.02	0.23 ± 0.01	0.35 ± 0.00	64.21 ± 0.44	13.92 ± 0.07	3.26 ± 0.05	0.21 ± 0.01
BO_B10	8.0 ± 0.0	19.2 ± 0.0	12.5 ± 0.00	0.66 ± 0.03	51.5 ± 0.1	9.6 ± 0.01	45.7 ± 0.1	52.4± 0.1	10.6± 0.1	1.57 ± 0.04	0.66 ± 0.03	0.32 ± 0.01	85.48 ± 0.40	21.82 ± 0.12	5.70 ± 0.03	0.71 ± 0.00
CH_R10	7.2 ± 1.1	16.0 ± 0.0	13.3 ± 0.05	0.77 ± 0.01	43.8 ± 0.0	10.5 ± 0.01	43.3 ± 0.1	45.0± 0.1	13.5± 0.1	2.81 ± 0.05	0.43 ± 0.03	0.36 ± 0.05	88.28 ± 0.15	18.90 ± 0.12	7.35 ± 0.07	0.46 ± 0.01
CD_C10	19.6 ± 0.0	64.4 ± 1.1	14.8 ± 0.00	0.66 ± 0.00	53.3 ± 0.1	13.0 ± 0.0	40.2 ± 0.1	54.9± 0.1	13.7± 0.1	2.35 ± 0.03	0.77 ± 0.01	0.49 ± 0.02	94.16 ± 0.69	26.13 ± 0.22	4.26 ± 0.04	0.62 ± 0.00
SC_R10	23.2 ± 0.6	33.6 ± 0.6	11.0 ± 0.04	0.69 ± 0.02	45.4 ± 0.0	6.7 ± 0.0	49.0 ± 0.1	46.0± 0.1	8.8± 0.1	3.29 ± 0.01	0.25 ± 0.02	0.30 ± 0.03	113.26 ± 1.49	22.27 ± 0.09	6.32 ± 0.11	0.44 ± 0.01
GC_B10	17.6 ± 0.6	51.2 ± 0.0	14.0 ± 0.01	0.64 ± 0.01	53.2 ± 0.2	10.3 ± 0.1	41.8 ± 0.2	54.2± 0.1	11.0± 0.2	1.56 ± 0.07	0.50 ± 0.02	0.52 ± 0.05	61.47 ± 0.38	14.28 ± 0.09	4.72 ± 0.04	0.41 ± 0.01
RM_R10	17.1 ± 0.0	89.2 ± 0.0	18.7 ± 0.05	0.71 ± 0.01	47.1 ± 0.1	14.0 ± 0.1	31.5 ± 0.1	49.1± 0.1	16.6± 0.1	2.84 ± 0.17	0.50 ± 0.02	0.50 ± 0.05	63.55 ± 0.53	9.14 ± 0.06	6.15 ± 0.10	0.45 ± 0.00
BE_R10	18.4 ± 1.1	33.6 ± 0.0	8.0 ± 0.00	0.71 ± 0.00	40.3 ± 0.0	5.1 ± 0.0	59.4 ± 0.0	40.6± 0.0	7.3± 0.10	2.11 ± 0.22	0.24 ± 0.02	0.24 ± 0.03	66.01 ± 0.12	14.56 ± 0.07	2.66 ± 0.07	0.33 ± 0.01

Data expressed as the mean ± SD (n = 2).

[a]: expressed in mg L^{-1}

[b]: expressed in absorbance units

nd: no detected

SO_2 F: Free sulfur dioxide

SO_2C: Combined sulfur dioxide

CI: color intensity
Hue: absorbance at 420 nm / absorbance at 520 nm
CA: copigmented anthocyanins
SPP: small weighted polymeric pigments
LPP: large weighted polymeric pigments
Non-acyl: non acylated anthocyanins
Acyl: acylated anthocyanins
Pyrano: pyranoanthocyanins
EMv-F: ethylidene-linked malvidin-3-glu-(epi)catechin dimer

Table 4. Overall average composition and composition of the cluster obtained from HCA to start. Different letters show significant differences (P <0.05)

Cluster	TPIs	pH	SO_2 F	SO_2 C[a]	CI[b]	Tonality	a^*_{10}	b^*_{10}	C^*_{ab}	h_{ab}	CA[b]	SPP[b]	LPP[b]	Non-acyl[a]	Acyl[a]	Pyrano[a]	EMv-F
Global	**60.43 ± 10.13**	3.61 ± 0.11	**14.8 ± 6.9**	31.6 ± 21.7	**11.4 ± 4.7**	**0.77 ± 1.3**	**42.7 ± 8.9**	**13.2 ± 7.2**	**40.6±6.5**	**17.3±7.7**	**1.66 ± 1.10**	**0.47 ± 0.25**	**0.36 ± 0.08**	**57.45 ± 30.84**	**12.74 ± 6.84**	**4.14 ± 1.36**	**0.32 ± 0.18**
1	51.64 ± 4.55b	3.67 ± 0.05	**21.9 ± 5.3a**	42.2 ± 19.3	8.8 ± 1.3b	0.76 ± 0.05ab	39.1 ± 3.9b	8.8 ± 2.5b	40.2±3.3b	13.0±4.3b	**2.48 ± 0.57a**	***0.27 ± 0.06b***	0.29 ± 0.05b	75.24 ± 20.59a	15.51 ± 3.74a	3.10 ± 0.26b	0.29 ± 0.10a
2	65.12 ± 8.40a	3.63 ± 0.15	13.9 ± 5.8b	48.0 ± 20.9	**14.7 ± 2.3a**	***0.69 ± 0.04b***	**49.8 ± 14.5a**	11.5 ± 1.8ab	**51.1±3.7**a	13.1±2.2ab	2.22 ± 0.63a	0.57 ± 0.13a	**0.44 ± 0.09a**	78.59 ± 14.14a	18.05 ± 6.03a	**5.63 ± 1.14a**	**0.53 ± 0.11a**
3	63.84 ± 10.89a	3.55 ± 0.08	9.7 ± 3.3b	48.7 ± 17.9	10.8 ± 4.1ab	**0.84 ± 0.07a**	39.9 ± 4.6b	**18.4 ± 6.9a**	44.2±5.8ab	**24.3±6.9**a	***0.51 ± 0.39b***	0.56 ± 0.33a	0.34 ± 0.05ab	***25.03 ± 18.28b***	***6.01 ± 3.20b***	3.77 ± 0.99b	***0.16 ± 0.09b***

underlined indicates parameters that characterize the cluster positively
Italics indicates parameters that characterize the cluster negatively
[a]: expressed in mg L^{-1}
[b]: expressed in absorbance units
nd: no detected
SO_2 F: Free sulfur dioxide
SO_2C: Combined sulfur dioxide
CI: color intensity
Hue: absorbance at 420 nm / absorbance at 520 nm
CA: copigmented anthocyanins
SPP: small weighted polymeric pigments
LPP: large weighted polymeric pigments
Non-acyl: non acylated anthocyanins
Acyl: acylated anthocyanins
Pyrano: pyranoanthocyanins
EMv-F: ethylidene-linked malvidin-3-glu-(epi)catechin dimer

Table 5. Differences between color parameters and anthocyanin composition before and after aging. Data in bold show significant differences ($P < 0.05$)

cluster	TPIs	pH	SO_2 F[a]	SO_2 C[a]	CI[b]	Tonality	a^*_{10}	b^*_{10}	C^*_{ab}	h_{ab}	CA[b]	SPP[b]	LPP[b]	Non-acyl[a]	Acyl[a]	Pyrano[a]	EMv-F
Global	-1.2 ± 16.2	-0.03 ± 0.16	**-14.8 ± 10.2**	**-20.1 ± 18.7**	0.37 ± 4.57	**0.13 ± 0.09**	-2.8 ± 10.6	**10.3 ± 8.5**	5,9±9,2	13.0±8.1	-0.08 ± 1.36	0.05 ± 0.28	**0.18 ± 0.13**	**-46.5 ± 31.7**	**-10.5 ± 6.9**	**-1.03 ± 0.83**	**-0.16 ± 0.11**
1	-1.7 ± 6.7	-0.04 ± 0.06	**-21.9 ± 9.2**	**-36.4 ± 17.3**	**0.77 ± 0.40**	**0.15 ± 0.09**	**-3.1 ± 2.4**	**11.9 ± 4.2**	**1,39±2,2**	**17.0±4.6**	**-1.52 ± 0.92**	**0.14 ± 0.09**	**0.17 ± 0.09**	**-61.7 ± 21.6**	**-13.4 ± 2.1**	-0.32 ± 0.86	**-0.14 ± 0.12**
2	**-2.6 ± 2.4**	-0.04 ± 0.23	**-13.9 ± 8.4**	**-29.8 ± 12.1**	-0.30 ± 3.31	**0.13 ± 0.08**	0.7 ± 12.4	**10.5 ± 2.5**	**4.0.±1,5**	10.4±7.1	0.22 ± 0.84	0.00 ± 0.12	**0.18 ± 0.16**	**-64.6 ± 12.9**	**-14.7 ± 2.6**	**-1.41 ± 1.61**	**-0.32 ± 0.10**
3	0.5 ± 11.3	-0.03 ± 0.11	**-9.7 ± 4.3**	**-37.0 ± 16.9**	-0.27 ± 4.55	**0.09 ± 0.10**	-0.8 ± 6.8	**8.6 ± 6.5**	3.3±5,1	**10.3**±3.4	**1.29 ± 0.61**	0.01 ± 0.35	**0.19 ± 0.14**	**-18.9 ± 11.5**	**-4.4 ± 3.3**	**-1.24 ± 0.85**	-0.05 ± 0.20

[a]: expressed in mg L^{-1}
[b]: expressed in absorbance units
SO_2 F: Free sulfur dioxide
SO_2 C: Combined sulfur dioxide
CI: color intensity
T: absorbance at 420 nm / absorbance at 520 nm
CA: copigmented anthocyanins
SPP: small weighted polymeric pigments
LPP: large weighted polymeric pigments
Non-acyl: non acylated anthocyanins
Acyl: acylated anthocyanins
Pyrano: pyranoanthocyanins
EMv-F: ethylidene-linked malvidin-3-glu-(epi)catechin dimer

25

Figure(s)
Click here to download Figure(s): Figures.docx

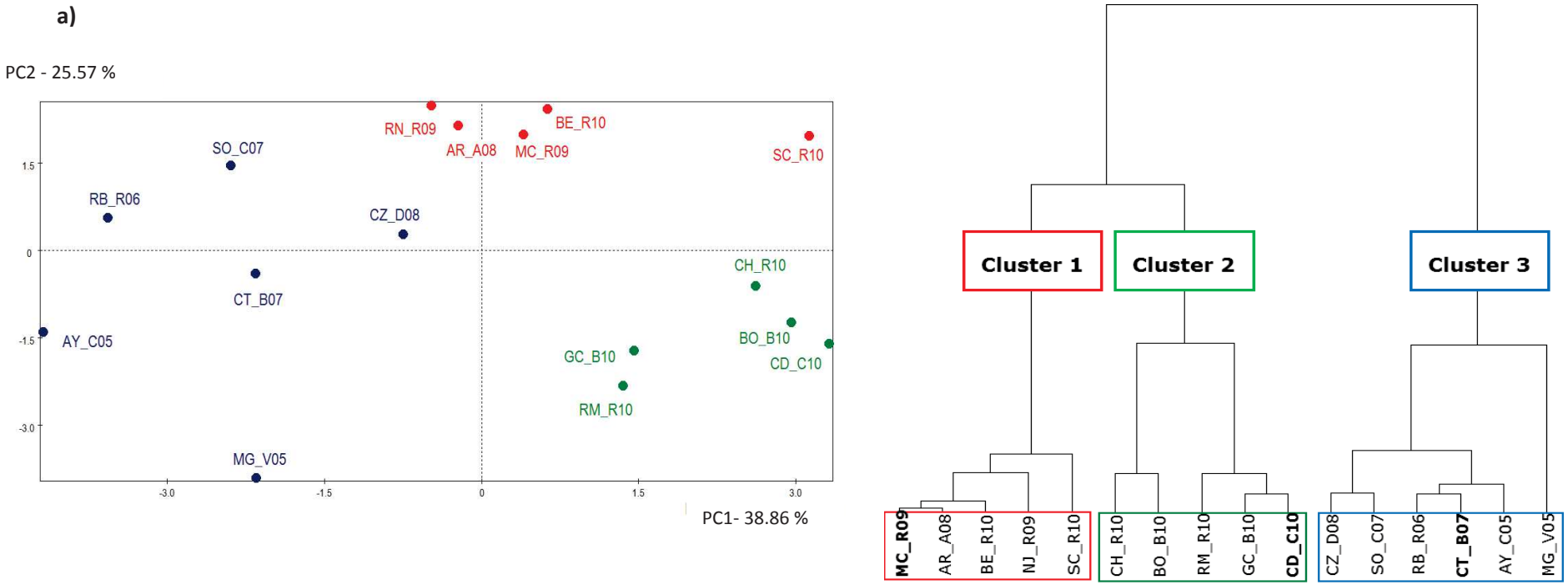

Figure 1

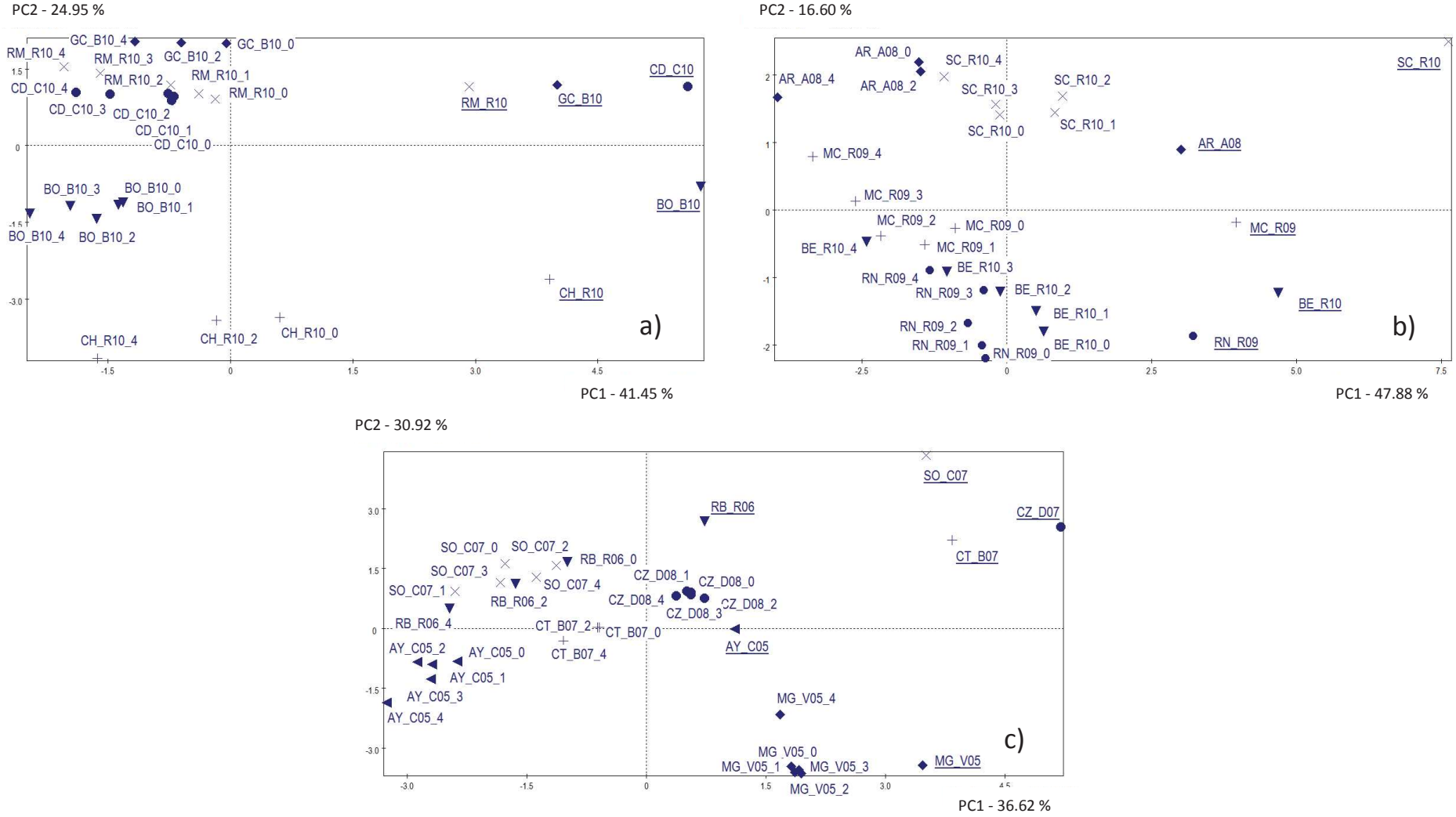
PC2 - 24.95 %
GC_B10_4
GC_B10_2
GC_B10_0
RM_R10_4
RM_R10_3
RM_R10_2
RM_R10_1
RM_R10_0
CD_C10_4
CD_C10_3
CD_C10_2
CD_C10_1
CD_C10_0
RM_R10
GC_B10
CD_C10
BO_B10_3
BO_B10_0
BO_B10_1
BO_B10_4
BO_B10_2
BO_B10
CH_R10
CH_R10_4
CH_R10_2
CH_R10_0
a)
PC1 - 41.45 %
PC2 - 16.60 %
AR_A08_0
SC_R10_4
SC_R10
AR_A08_4
AR_A08_2
SC_R10_3
SC_R10_2
SC_R10_1
SC_R10_0
AR_A08
MC_R09_4
MC_R09_3
MC_R09_2
MC_R09_0
MC_R09
BE_R10_4
MC_R09_1
RN_R09_4
BE_R10_3
RN_R09_3
BE_R10_2
BE_R10
BE_R10_1
RN_R09_2
RN_R09_1
BE_R10_0
RN_R09
RN_R09_0
b)
PC1 - 47.88 %
PC2 - 30.92 %
SO_C07
RB_R06
CZ_D07
SO_C07_0
SO_C07_2
RB_R06_0
CT_B07
SO_C07_3
CZ_D08_1
CZ_D08_0
SO_C07_4
SO_C07_1
RB_R06_2
CZ_D08_4
CZ_D08_2
CZ_D08_3
RB_R06_4
CT_B07_2
CT_B07_0
AY_C05
AY_C05_2
AY_C05_0
CT_B07_4
AY_C05_1
AY_C05_3
MG_V05_4
AY_C05_4
MG_V05_0
MG_V05_1
MG_V05_3
MG_V05
MG_V05_2
c)
PC1 - 36.62 %

Figure 2