

# What Gives a Wine Its Strong Red Color? Main Correlations Affecting Copigmentation

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**ABSTRACT:** Copigmentation and enological parameters were studied in a collection of 250 red wines. Although several copigmentation studies have been performed with model solutions, little is known about the actual consequences directly in wine of anthocyanin interactions. To date, some studies have considered relationships between copigmentation and natural wine constituents, but none correlates copigmentation measurements with the real wide concentration in wine. In this work, published hypotheses based on model solutions such as phenolic acid copigmentation ability or the influence of copigmentation factors such as flavonols are empirically evaluated in a large sample of wines for the first time. The study confirms previous results obtained from solutions, whereas other factors suggested as being relevant seem to be unrelated to the studied effect at the concentration range naturally occurring in the wines studied. For instance, the important role of flavonols and hydroxycinnamic acids has been ratified, whereas ethanol, gallic acid, and some metals show significant inverse correlations with copigmentation. Unexpectedly, magnesium content in wine correlates with color, whereas the concentration of traditional copigments, such as quercetin, does not show any correlation with copigmentation.

**KEYWORDS:** wine, copigmentation, anthocyanin, color, copigment

## ■ INTRODUCTION

Wine copigmentation intensifies its red color and produces bathochromic shifts. Copigmentation effects are observed in the  $\lambda_{\max}$  of the absorption spectrum and along the entire visible spectral curve. Copigmentation occurs as a result of anthocyanins' ability to form associations between themselves or with copigments based on weak interactions, mainly van der Waals or hydrophobic forces. Copigmentation complexes are easily formed but can be dissociated by dilution or by an increase of wine cosolvents such as ethanol or acetic acid.<sup>1</sup>

Nucleophilic attack from water reducing the formation of the hydrated colorless carbinol and increasing the number of flavylum cation colored bodies is prevented by copigmentation.<sup>2</sup> Different molecules have been proposed to act as copigments, from monomeric anthocyanin themselves to phenolic acids or flavonoids among other compounds.<sup>3</sup> Flavonols and hydroxycinnamic acids are traditionally considered to be the main wine copigments. The effectiveness and intensity of copigmentation depend on numerous factors, such as content, molar ratio, or chemical structure.<sup>4</sup> The copigments present in wine can act in competition between themselves, resulting in the process known as anti-copigmentation. This phenomenon has a significant influence when a copigment replaces another less stable copigment, inducing a global loss of color.<sup>5</sup> This process might also take place when a copigment associates with any colorless form of anthocyanin or with cyclodextrins.<sup>1</sup>

Copigmentation has been analyzed in enological conditions<sup>6</sup> and in model solutions,<sup>7</sup> but how to enhance or reduce it is still not completely understood. The structure of the compounds seems to affect the rate and degree of copigmentation, as well as the ionic strength, temperature, or pigment to copigment molar ratio.<sup>8</sup> The above scenarios are tested here by studying a large

data set of red wines with a wide range of copigmentation and compound concentrations. This is, to the best of our knowledge, the most ambitious study of this kind, as far as the above paradigms are mainly based on laboratory tests but wines have rarely been directly evaluated. Empirical correlations are tested here between the main factors thought to affect copigmentation, which allow the evaluation of the strength of some of the proposed relationships and their validity in real data (i.e., red wine vs model solutions).

## ■ MATERIALS AND METHODS

The bulk of studies concerning copigmentation come from test models based on solutions (Table 1); the best examples are Miniati et al.,<sup>8</sup> Mirabel et al.,<sup>9</sup> Lambert et al.,<sup>10</sup> Gordillo et al.,<sup>11</sup> Malaj et al.,<sup>12</sup> and Zhang et al.<sup>13</sup> Some authors have considered extracts,<sup>2,5,14</sup> and others have designed experiments by adding specific compounds to the wines.<sup>15–19</sup> Given the richness of the wine matrix, the above claims should be tested directly in the product because model solutions or extracts cannot consider every possible interaction. This study includes 250 bottled young and aged red wines from the Canary Islands (Spain) and encompasses different cultivars and vintages (from 2004 to 2012). Composition details according to variety or average values for phenolics and copigmentation can be found in Heras-Roger et al.<sup>20</sup>

**Copigmentation and Color.** The estimation of cofactors and the copigmentation contribution to color was estimated by using the Boulton procedure,<sup>21</sup> which is based upon the dissociating effect of dilution at pH 3.6. To this end, color including copigmentation was directly obtained from the absorbance spectrum of the wines, whereas color without copigmentation was estimated from a 20 rate dilution spectrum and multiplication by the dilution factor (20), as this latter

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Table 1. Copigmentation Results from Previous Research and Comparison with Results from the Present Study

reference	matrix considered	conclusion	effect observed?
Thakur and Arya, 1989 <sup>37</sup>	grape juices	sugar and its degradation products increase color loss and decrease anthocyanin stability	yes
Miniati et al., 1992 <sup>8</sup>	model solutions	copigmentation is affected by compound, concentration, ethanol, and temperature; the most stable solutions contain quercetin and gallic acid, and the least stable, catechin	factors, yes quercetin, no gallic, no
Baranac et al., 1996 <sup>38</sup>	model solutions	rutin copigment role depends on molar ratio and temperature	yes
Mirabel et al., 1999 <sup>9</sup>	model solutions	copigmentation occurs with epicatechin and leads to new pigments	yes
Gonnet, 1999 <sup>39</sup>	model solutions	color effects of the copigmentation of cyanin by rutin increase with copigment/pigment ratio	yes
Darias-Martin et al., 2001 <sup>15</sup>	addition to wine	caffeic acid contributes to young wine color, and catechin leads to a slight decrease in color	yes
Darias-Martin et al., 2002 <sup>16</sup>	addition to wine	caffeic acid addition during initial winemaking stages produces color enhancement	yes
Eiro and Heinonen, 2002 <sup>33</sup>	model solutions	greatest copigmentation takes place in malvidin 3-glucoside solutions with ferulic acids	
Hermosín and González, 2003 <sup>1</sup>	reconstituted wine	each 2% increase in ethanol content leads to a perceivable color change	yes
Schwarz et al., 2005 <sup>17</sup>	addition to wine	rutin is the best copigmentation agent, whereas coumaroylated anthocyanins might prevent it	yes
Gómez-Míguez et al., 2006 <sup>3</sup>	model solutions	flavanols are the least effective copigments, whereas flavonols are the best, ahead of caffeic and coumaric acid	yes
Gris et al., 2007 <sup>14</sup>	grape extracts	caffeic acid addition increases the stability of anthocyanins	yes
Kunsági-Máté et al., 2008 <sup>36</sup>	model solutions	the impact of ferrous and ferric ions is negligible in the presence of caffeic acid	no
Sun et al., 2010 <sup>40</sup>	raspberry juice	copigmentation is more favorable at low temperatures, pH 4.0, cyanidin 3-glucoside, and with ferulic acid	
Kopjar and Pilizota, 2011 <sup>30</sup>	addition to juice	catechin copigmentation is lower than gallic acid	no
Lambert et al., 2011 <sup>10</sup>	model solutions	quercetin produces the strongest copigment, whereas quercetin-3-glucoside reduces its effect; copigmentation with flavanols and chlorogenic and caffeic acid is also assessed	flavanols, yes caffeic, yes quercetin, no
Rustioni et al., 2012 <sup>4</sup>	grape extracts	anti-copigmentation is possible with quercetin, tannins, and caffeic acid; flavonols are the best copigmentation cofactors	yes
Czibulya et al., 2012 <sup>18</sup>	addition to wines	enhanced color intensity is observed in the presence of potassium ions	yes
Gordillo et al., 2012 <sup>11</sup>	model solutions	copigmentation effects between catechins/caffeic acid and malvidin 3-glucoside depend on pH and molar ratio	yes
Malaj et al., 2013 <sup>12</sup>	model solutions	syngic acid is a slightly more efficient copigment than coumaric acid	yes
Ghasemifar and Saeidian, 2014 <sup>19</sup>	addition to juice	copigmentation effects increase with catechin content	yes
Zhang et al., 2015 <sup>43</sup>	model solutions	syngic acid has a stronger copigmentation effect than protocatechuic and gallic acids	yes

operation dissociates any copigmentation complex. Copigmentation effects can be directly quantified by using the absorbance at 520 nm, either as total copigmentation (Copigm) in units of absorbance (UA) or as a percentage of color as a result of copigmentation ( $X_{COP}$ ):

$$\text{Copigm (UA)} = A_{\text{wine}}^{520} - A_{\text{dilution 1:20}}^{520} \quad (1)$$

$$X_{COP} (\%) = \left( \frac{A_{\text{wine}}^{520} - A_{\text{dilution 1:20}}^{520}}{A_{\text{wine}}^{520}} \right) \times 100 \quad (2)$$

Color parameters were obtained by analyzing the absorption spectra between 370 and 700 nm, whereas color intensity (ICM) was quantified by addition of  $A_{420}$ ,  $A_{520}$ , and  $A_{620}$ . CIELab differences between copigmented and non-copigmented wine color were obtained by applying the method of García-Marino et al.<sup>22</sup> This procedure allows the quantification of the above effects over the entire visible range. The color variation induced by copigmentation ( $\Delta E_{ab}$ ) in CIELab units (CU) and the changes in color intensity in absorbance units (AU) were assessed as follows:

$$\Delta E_{ab} (\text{CU}) = \left[ (L^*_{COP} - L^*_{NCOP})^2 + (a^*_{COP} - a^*_{NCOP})^2 + (b^*_{COP} - b^*_{NCOP})^2 \right]^{0.5} \quad (3)$$

$$\begin{aligned} \Delta \text{ICM (UA)} &= \text{ICM}_{COP} - \text{ICM}_{NCOP} \\ &= (A_{420} + A_{520} + A_{620})_{COP} - (A_{420} + A_{520} + A_{620})_{NCOP} \end{aligned} \quad (4)$$

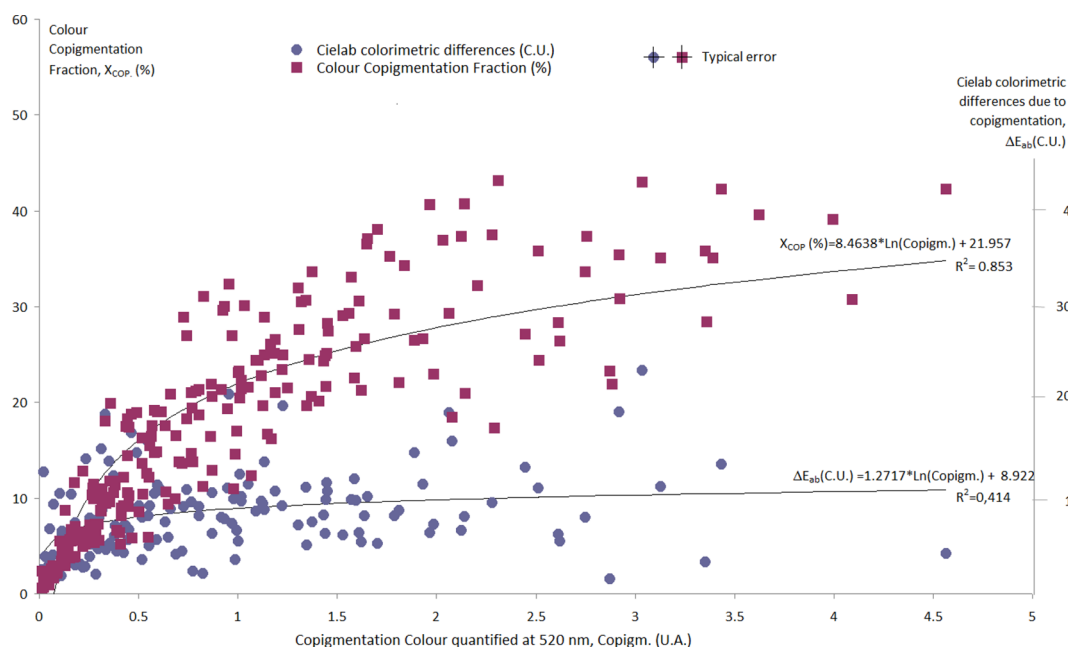
Spectrophotometric measurements were obtained by means of a 225 PerkinElmer spectrophotometer and quartz cuvettes of 1 or 0.1 cm path length depending on the saturation of the signal. Copigmentation was estimated by using the 1:20 wine dilution (taking in account the dilution factor) and measurements of the wine absorbance applying eqs 1–4.

Pyrananthocyanins and polymeric pigments are another source of red wine color. Polymeric pigments can be estimated by applying the Boulton procedure<sup>21</sup> using the absorbance measured at 520 nm after the addition of 160  $\mu\text{L}$  of 5%  $\text{SO}_2$  solution to 2 mL of wine sample and applying the following equations:

$$\text{polymeric pigment (UA)} = A_{\text{wine}}^{520} - A_{\text{SO}_2}^{520} \quad (5)$$

$$X_{\text{polymeric pigment}} (\%) = \left( \frac{A_{\text{wine}}^{520} - A_{\text{SO}_2}^{520}}{A_{\text{wine}}^{520}} \right) \times 100 \quad (6)$$

**Phenolic Quantification.** Wine was injected (15  $\mu\text{L}$ ) in a Nova-Pak C-18 reversed phase column (3.9  $\times$  150 mm, 4  $\mu\text{m}$ ) from a Waters 2690 separation system with a photodiode array detector.



**Figure 1.** Relationships between magnitudes commonly used for wine copigmentation quantification.

Ibern-Gómez et al.<sup>23</sup> methodology was followed; therefore, chromatograms were extracted at 280 nm (hydroxybenzoic acids), 320 nm (hydroxycinnamic acids), 360 nm (flavonols), and 520 nm (anthocyanins). Compounds available were directly compared with external standards (Sigma-Aldrich, USA) and the remaining identified by relative retention times and characteristic spectral data in the 200–700 nm range.<sup>24,25</sup> All standards showed linear calibration curves within the concentration range considered (0.5–15 mg resveratrol/L, 0.5–100 mg quercetin/L and caffeic acid/L, 0.5–200 mg gallic acid/L and mg catechin/L, 0.5–400 mg oenin/L).

**Enological Parameters.** Conventional parameters of enological importance, such as alcoholic degree, organic acids, and mineral profile, were obtained following standard procedures.<sup>26</sup> Free acetaldehyde, ammonia, glucose+fructose, and all organic acids except tartaric were quantified using specific enzymatic techniques (TDI, Spain) in a LISA200 automatic analyzer (Hycl Diagnostics, France). Calibration curves for these compounds were linear, and almost all wines were directly analyzed. Only those whose concentration was over the calibration range were previously diluted (calibration range: 0.05–1.25 g acetic acid/L, 0.1–2 g gluconic acid/L, 0.1–5 g L-malic/L and g L-lactic/L, 0.1–6 g glucose+fructose/L, 10–250 mg/L for nitrogen ammonia, citric acid, and acetaldehyde). All of these techniques are based on sample absorption at 340 nm before and after specific enzymatic reactions take place.

Tartaric acid was quantified at 480 nm in the same instrument by using a colorimetric sequential technique based on its reaction with vanadium salts in acid media (TDI, Spain). Minerals were quantified by atomic absorption spectrometry using air/acetylene except for magnesium, which needed an acetylene/nitrous oxide flame. The wine was diluted to fit the calibration range for Na, K, and Mg, whereas Fe, Cu, Co, and Mn were directly quantified with a Varian Spectraa spectrometer. No digestion procedures were applied, but for Fe, Cu, and Mn ethanol was previously removed by heating ( $T \sim 60^\circ\text{C}$ ,  $t \sim 24$  h) as recommended by OIV standards.<sup>26</sup>

**Statistical Analysis.** All parameters were measured in triplicate and systematically repeated when the standard deviation of the three measurements exceeded 5% of the average value quantified. Correlations were evaluated using the Pearson coefficient ( $r$ ), and one-way analysis of variance (ANOVA) was applied. The level of significance was established at 0.01 or 0.05, not considering any relationship above these limits relevant. Due to the high number of samples evaluated ( $n = 250$ ) some correlation factors might not seem

meaningful at first sight, but the relationships detailed in this study are significant at the level specified in each table.

## RESULTS

Some wine features are considered to be potentially involved in copigmentation, and therefore their relationships with Copigm,  $X_{\text{COP}}$ , and  $\Delta E_{\text{ab}}$  were investigated. Figure 1 shows these three magnitudes according to the copigmentation measured in the wines. Parameters were well related but not completely proportional, mainly due to the dissimilar effects produced by copigmentation at different points of the spectral curve.<sup>27</sup> In fact,  $\Delta E_{\text{ab}}$  registers color differences as a result of copigmentation in the whole spectral curve, whereas Copigm and  $X_{\text{COP}}$  quantify the hyperchromic effect exclusively on the basis of 520 nm absorbances.

**Polyphenolic Content and Pigment/Copigment Molar Ratio.** As observed in model solutions, copigmentation phenomena are related to anthocyanin and flavonoid content (Table 1). Table 2 summarizes the correlations between copigmentation and phenol compounds obtained in this study. Oenin displays the highest correlation with copigmentation among the anthocyanins, whereas rutin is the most relevant copigment among the flavonols, in agreement with model solution studies. Caffeic and coumaric acids significantly correlate with copigmentation, confirming previous results from grape extracts.<sup>14</sup> Caffeic acid and *p*-coumaric acid can also participate in the formation of pyranoanthocyanins.<sup>28</sup> Vaadia<sup>29</sup> obtained a better anthocyanin–copigmentation correlation by subtracting the malvidin-3-glucoside content from the total anthocyanin quantification. Nevertheless, according to the results in the present study, the correlation between copigmentation and total monomeric anthocyanins is better when malvidin-3-glucoside is included ( $r \sim 0.69 > r \sim 0.59$ ).

Caffaric and tartaric acid show inverse correlations, as far as we know, this situation is described here for the first time. A similar behavior is observed for gallic and protocatechuic acids, which based on model solutions could have been considered suitable copigments.<sup>30</sup> Interestingly, Zhang et al.<sup>13</sup> revealed that

Table 2. Phenolic Compounds Range Concentration and Relationships with Copigmentation/Color<sup>a</sup>

phenol	range (mg/L)	$r_{(\text{Copigm})}$	$r_{(\text{Xcop})}$	$r_{(\Delta E_{ab})}$	$r_{(\Delta ICM)}$	$r_{(ICM)}$
<b>anthocyanins, mg oenin equiv (OE)/L</b>	18.19–889.07	0.770**	0.660**	0.260**	0.511**	0.506**
delphinidin-3-glucoside	nd–51.2	0.672**	0.584**	0.224**	0.436**	0.472**
cyanidin-3-glucoside	<1.0–66.1	0.119	0.065	0.111	0.167	0.194**
petunidin-3-glucoside	<1.0–52.1	0.716**	0.646**	0.242**	0.473**	0.442**
peonidin-3-glucoside	<1.0–69.4	0.550**	0.453**	0.168*	0.456**	0.431**
malvidin-3-glucoside (oenin)	1.5–371.2	0.752**	0.694**	0.266**	0.467**	0.379**
cyanidin-(6-acetyl)-3-glucoside	<1.0–14.2	0.523**	0.243**	0.009	0.282**	0.538**
petunidin-(6-acetyl)-3-glucoside	<0.9–27.0	0.334**	0.162*	0.098	0.339**	0.469**
peonidin-(6-acetyl)-3-glucoside	<1.0–30.4	0.385**	0.174**	0.094	0.371**	0.502**
malvidin-(6-acetyl)-3-glucoside	<0.9–86.9	0.536**	0.417**	0.334**	0.560**	0.523**
peonidin-(6-coumaroyl)-3-glucoside	<0.8–35.3	0.445**	0.257**	0.034	0.189*	0.348**
malvidin-(6-coumaroyl)-3-glucoside	<0.8–111.7	0.319**	0.252**	0.021	0.241**	0.337**
<i>nonsubstituted anthocyanins</i> (mg OE/L)	7.3–487.4	0.685**	0.469**	0.306**	0.500**	0.606**
<i>coumaroyl anthocyanins</i> (mg OE/L)	1.5–235.6	0.450**	0.312**	0.240**	0.274**	0.419**
<i>acetylated anthocyanins</i> (mg OE/L)	8.0–158.5	0.562**	0.371**	0.265**	0.545**	0.492**
<b>flavonols (mg quercetin equiv (QE)/L)</b>	6.8–127.5	0.419**	0.404**	0.261**	0.386**	0.409**
myricetin-3-glucuronide	nd–0.7	−0.111	−0.025	−0.190*	−0.120	0.182**
myricetin-3-glucoside	nd–18.6	−0.045	−0.189**	0.011	−0.138	−0.267**
laricitrin-3-glucoside	nd–4.5	0.318**	0.285**	0.284**	0.356**	0.188**
kaempferol-3-glucoside	nd–3.1	0.098	0.047	0.140	0.263**	0.167*
myricetin	<0.6–19.4	0.648**	0.570**	0.346**	0.586**	0.560**
quercetin-3-glucuronide	0.8–28.4	0.558**	0.537**	0.366**	0.454**	0.356**
quercetin-3-glucoside	0.7–20.5	0.295**	0.284**	0.165	0.220*	0.308**
rutin	nd–15.8	0.701**	0.674**	0.252**	0.468**	0.342**
isorhamnetin-3-glucoside	<0.4–10.8	0.442**	0.311**	0.194*	0.460**	0.547**
isorhamnetin	<0.6–13.9	0.353**	0.222**	0.049	0.311**	0.491**
syringetin-3-glucoside	nd–3.6	0.181**	0.077	0.172*	0.289**	0.243**
quercetin	nd–13.7	0.035	−0.044	−0.007	0.012	0.178**
<i>quercetin derivatives</i> (mg QE/L)	2.2–73.0	0.386**	0.371**	0.242**	0.356**	0.394**
<i>myricetin derivatives</i> (mg QE/L)	2.6–37.6	0.236**	0.303**	0.205*	0.220*	0.126
<i>isorhamnetin derivatives</i> (mg QE/L)	0.5–26.7	0.413**	0.278**	0.137	0.410**	0.538**
<i>nonsubstituted flavonols</i> (mg QE/L)	5.8–44.8	0.543**	0.421**	0.262**	0.515**	0.584**
<i>glucoside derivatives</i> (mg QE/L)	6.1–95.8	0.362**	0.336**	0.243**	0.308**	0.308**
<b>hydroxycinnamic acids (mg caffeic/L)</b>	10.6–240.9	0.039	0.190**	0.169*	0.095	−0.079
caftaric	1.5–92.3	−0.366**	−0.283**	−0.189*	−0.392**	−0.394**
caffeic	<0.90–61.4	0.133*	0.195**	0.162	0.075	0.020
cutaric	2.5–44.5	0.156*	0.248**	0.240**	0.274**	0.087
coumaric	<0.90–67.8	0.275**	0.262**	0.284**	0.431**	0.273**
2-S-glutathionylcaftaric	nd–1.0	0.262**	0.196**	0.054	0.343**	0.266**
<b>hydroxybenzoic acids (mg gallic/L)</b>	9.0–140.2	0.138*	0.134*	0.014	0.187*	0.154*
gallic	3.6–125.8	−0.425**	−0.290**	−0.198*	−0.305**	−0.310**
protocatechuic	<1.0–53.7	−0.195**	−0.161*	−0.021	−0.184*	0.021
syringic	2.1–20.4	0.307**	0.261**	0.134	0.250**	0.091
<b>flavan-3-ols (mg catechin/L)</b>	21.17–276.04	0.206**	0.196**	0.078	0.198*	0.325**
catechin	6.6–199.7	0.330**	0.162*	0.075	0.318**	0.421**
epicatechin	13.3–125.2	−0.175**	−0.063	−0.159	−0.082	0.140*
<b>others</b>						
resveratrol	<0.7–13.3	0.476**	0.385**	0.131	0.448**	0.387**
tyrosol	2.9–34.6	−0.326**	−0.184**	−0.101	−0.216*	−0.082
<b>pigment/copigment molar ratio</b>						
anthocyanin/hydroxycinnamics	0.1–2.6	0.775**	0.659**	0.254**	0.515**	0.513**
anthocyanin/flavonols	0.1–3.2	0.770**	0.657**	0.263**	0.516**	0.515**
anthocyanin/hydroxybenzoic	0.1–2.5	0.735**	0.616**	0.272**	0.473**	0.453**
anthocyanin/flavanols	0.1–4.2	0.657**	0.605**	0.280**	0.413**	0.341**

<sup>a</sup>Correlations are expressed as Pearson coefficient: \*\*, correlation is significant at the 0.01 level; \*, correlation is significant at the 0.05 level.

the copigmentation ability of these compounds is much lower than that of syringic acid, which is a highly significant copigment according to the present study. According to the results here (+)-catechin might act as a weak copigment, in

agreement with Ghasemifar and Saeidian<sup>19</sup> and Darias-Martín et al.<sup>15</sup> Pigment/copigment molar ratio relationships with copigmentation were highly significant, being maximal for hydroxycinnamic and flavonol ratios. In this sense phenolic

Table 3. Compound Concentrations and Relationships with Copigmentation/Color<sup>a</sup>

compound	range	$r_{(\text{Copigm})}$	$r_{(\text{Xcopig})}$	$r_{(\Delta Eab)}$	$r_{(\Delta ICM)}$	$r_{(ICM)}$
ethanol (% vol)	11.00–19.54	-0.255**	-0.241**	-0.225**	0.187**	0.017
glucose + fructose (g/L)	<0.30–59	-0.220**	-0.157*	-0.163	-0.060	0.045
nitrogen ammonia (mg/L)	<10–207	-0.179**	-0.114	-0.163	-0.211*	0.056
acetaldehyde (mg/L)	<10–246	0.013	-0.024	-0.044	-0.051	-0.067
polymeric pigment (UA)	0.26–4.14	0.130	-0.251**	-0.276**	0.033	0.671**
X <sub>polymeric pigment</sub> (%)	6.1–79.5	-0.675**	-0.762**	-0.382**	-0.458**	-0.264**
<b>organic acids</b>						
acetic acid (g/L)	0.15–1.53	-0.278**	-0.184**	-0.252**	-0.331**	-0.005
tartaric acid (g/L)	1.5–4.9	-0.177**	-0.161*	-0.184*	-0.345**	-0.137*
L-malic acid (g/L)	<0.20–4.21	-0.150*	-0.094	-0.166	-0.130	0.123
L-lactic acid (g/L)	0.07–5.53	-0.093	0.057	-0.051	-0.070	0.137*
citric acid (mg/L)	<10–620	-0.063	-0.082	-0.181*	-0.194*	0.000
gluconic acid (g/L)	<0.10–2.29	-0.106	-0.100	-0.207*	-0.103	-0.029
<b>minerals (mg/L)</b>						
potassium	531–3730	-0.025	0.113	-0.150	-0.107	0.225**
magnesium	65–265	-0.126	0.079	-0.300	-0.007	0.273**
sodium	0.30–352	-0.081	-0.068	-0.012	0.019	0.105
iron	0.30–7.33	-0.190**	-0.121	-0.229*	-0.215*	0.040
copper	<0.10–6.72	-0.147*	-0.139	0.007	-0.066	-0.078
manganese	<0.10–5.07	-0.006	0.028	-0.094	-0.096	0.087
<b>wine absorbances (UA)</b>						
A <sub>280</sub>	14.61–103.07	0.280**	-0.054	-0.193*	0.067	0.541**
A <sub>320</sub>	11.84–45.2	0.164*	0.000	0.171	0.242**	0.509**
A <sub>365</sub>	2.39–14.82	0.333**	0.008	-0.088	0.126	0.663**
A <sub>520</sub>	0.72–13.78	0.573**	0.265**	0.196*	0.630**	0.957**

<sup>a</sup>Correlations are expressed as Pearson coefficient: \*\*, correlation is significant at the 0.01 level; \*, correlation is significant at the 0.05 level.

families' ability to perform a role as copigments according to their molar ratio in wine might be ranked as follows: hydroxycinnamic acids > flavonols > hydroxybenzoic acids > flavanols.

**Mineral Influences.** Model solution studies suggest mineral concentrations might increase color intensity,<sup>31</sup> whereas ionic salts enhance copigmentation and sodium or magnesium enhance self-association processes.<sup>8</sup> Potassium, the most concentrated mineral in wine, has been reported to produce an increase in wine color when its content reaches 500 mg/L.<sup>18</sup> Because the samples here contained a wide range of concentrations, it was possible to test these claims (Table 3). Indeed, a significant correlation with wine color intensity was obtained, but no correlation was obtained with copigmentation.

According to Starr and Francis,<sup>32</sup> magnesium might also be involved in color equilibrium. The correlations found here for this element are similar to those for potassium, with its content being significantly related with wine color but not presenting any relationship with copigmentation.

As the samples were produced on islands and near the coast, they contained appreciable amounts of sodium, but no relationships between this mineral and copigmentation or color intensity were found. On the other hand, an inverse relationship between copigmentation and iron or copper is obtained, even though their concentrations are minor in comparison with other minerals.

**Codilution Effect: Ethanol and Acetic Acid.** Ethanol and acetic acid are described in model solutions as potential factors influencing copigmentation because their presence presumably dilutes structures formed by this phenomenon.<sup>1</sup> In this study the average wine concentration was  $13.75 \pm 1.20\%$  alcohol and  $0.59 \pm 0.24$  g acetic acid/L, and an inverse relationship at the

0.01 level was observed between both parameters and copigmentation (Table 3).

**Other Compounds.** Copigmentation is inversely related to glucose+fructose and nitrogen ammonia contents. Minor molecules such as citric or gluconic acid show no relationships, whereas more concentrated substances reveal inverse correlations significant at the 0.05 level (Table 3). The importance of polymeric pigments in wine color is revealed to be inversely correlated to the amount of copigmentation and its role in color (Table 3).

## DISCUSSION

The results here in wine generally agree with previous research performed under specific and controlled conditions. Naturally occurring compounds and copigmentation in the wines allow the validation of the assumptions directly in the matrix considering a wide copigmentation range (from 0.6 to 43.1% of color or from 1.5 to 23.3 CU).

As expected, anthocyanins showed the best correlation with copigmentation followed by flavonols. Darias-Martín et al.<sup>16</sup> prove how caffeic acid addition during initial winemaking stages produces color enhancement in the final product, whereas Schwarz et al.<sup>17</sup> examine flavonols and hydroxycinnamic acids preferment addition effects in wine. These authors say that rutin is the best copigmentation enhancement agent. This fact is supported by the present results in commercial samples. They also suggest that coumaroylated anthocyanins might prevent the action of the added copigments, which is in agreement with the results here because this type of anthocyanin is related to copigmentation to a lesser extent.

**Anthocyanins.** Cyanidin-3-glucoside shows no relationship with copigmentation at the concentration range obtained for the wines from our study, which agrees with Eiro and

Heinonen<sup>33</sup> tests under ideal conditions. Goto and Kondo<sup>34</sup> model solution studies consider that copigmentation processes with acetylated or aromatic substituted anthocyanins are more stable than those with exclusively glycosylated compounds. However, according to the present results nonsubstituted anthocyanins correlate better with wine copigmentation. The present study suggests that almost every anthocyanin participates in wine copigmentation processes, even though nonsubstituted and monoglycosylated anthocyanins, which are more concentrated in young wines, correlate the best (Table 2). This finding might be due to self-association processes normally prevented by the *p*-coumaryl group and, therefore, mostly produced in nonsubstituted anthocyanins.<sup>10</sup> In this sense the net result of intermolecular (self-association) and intramolecular copigmentation is a net color increase in addition to that exclusively due to the pigment or copigment concentration.

**Flavonols.** This group showed a high capacity for copigmentation in model solutions due to their planar  $\pi$ -electron-rich moiety.<sup>8</sup> The present results support their important role in wine. Quercetin is less stable than myricetin in experimental solutions.<sup>4</sup> According to the results here; the latter is highly related with copigmentation, whereas no correlation with the former was found, at least in the concentration range considered in this study. As both contents are similar, their dissimilar ability for copigmentation in wine might be related to stability and structural differences. Lambert et al.<sup>10</sup> solution tests describe quercetin as a stronger copigment than the same compound glycosylated at position 3. The present study suggests otherwise, as the correlation between quercetin glucuronide and glucoside with copigmentation is highly significant. These results agree with those of Rustioni et al.,<sup>5</sup> who obtained a better correlation for quercetin-3-*O*-glucoside and copigmentation in grape extracts. This fact confirms divergences between quercetin behavior in model solutions and in wine or grapes. Isorhamnetin, its glycosylated form, and larcitrin derivatives also show significant correlations with copigmentation.

**Phenolic Acids.** Hydroxycinnamic acids are considered suitable copigments on the basis of their performance in model solutions. Nevertheless, their relationships with copigmentation were less important than those for flavonols, supporting conclusions found in model solutions.<sup>4</sup> Syringic and coumaric acid comparisons in model solutions<sup>12</sup> have results similar to those obtained here, as syringic is a slightly more efficient copigment than coumaric acid. Eiro and Heinonen<sup>33</sup> considered ferulic acid copigmentation, but these compounds were not detected in any wine in the present study. Negative correlations might indicate that some hydroxybenzoic acids act as anti-copigments, with their concentrations being inversely related to copigmentation. This means that despite their valuable behavior in model solutions, their net effect in competition with stronger copigments would induce global color losses.

**Flavan-3-ols.** Mirabel et al.<sup>9</sup> suggest epicatechin may act as a bad cofactor. In fact, its content is inversely related to copigmentation, highlighting its possible role as anti-copigment.

**Pigment/Copigment Molar Ratio.** These ratios are considered critical for copigmentation processes. They normally vary in the range from 0.05 to 2.<sup>6</sup> Molar ratio correlations are higher than relationships between any individual compound and copigmentation, supporting the results of Gordillo et al.<sup>11</sup> in model solutions.

**Minerals.** Magnesium content is much lower than potassium content, but its correlation with the global color intensity was markedly higher. Therefore, it is reasonable to consider that the potassium effect in wine color observed by Czibulya et al.<sup>18</sup> could also be produced by magnesium. The present study supports the importance of metallo complexes in wine color, even though metal complexation is not possible for the most concentrated anthocyanin (oenin) and these metals are generally present at very low concentrations to prevent precipitation issues. Some authors say that iron and copper might act as color stabilization agents in anthocyanin solutions,<sup>3</sup> whereas other researchers state they might be obstacles for copigmentation because anthocyanins bind to these ions.<sup>35</sup> The results here support the findings of Kunsági-Máté et al.<sup>36</sup> on how ferric ions decrease copigmentation depending on the pigment/copigment profile, and, according to the results here, their hypothesis could be validly applied to copper as well.

**Codilution.** The present results suggest that a greater alcoholic degree or volatile acidity would imply lower copigmentation in wine, as both components probably act as dissociating cosolvents according to hypotheses based on model solutions. Acetic acid concentration is relatively minor in wine, and its correlation might be somehow influenced by aging, as aged wines (with low copigmentation) normally present higher acetic acid concentrations than young wines. This relationship was also observed when wines with <1 year of aging were exclusively considered ( $n = 140$ ), obtaining an even higher correlation factor ( $r = -0.346$ ).

**Other Compounds.** Sugars are traditionally supposed to decrease anthocyanin stability,<sup>37</sup> but small concentrations show protective effects on color.<sup>30</sup> Their relationship is particularly relevant for sweet wines and specific winemaking procedures during which concentrated must is added. Indeed, concentration appears to be critical in the study of the influence of compounds in copigmentation. Highly concentrated compounds such as tartaric or L-malic acid may be ionically bound to anthocyanins<sup>8</sup> and prevent copigmentation effects in wine, producing an effect similar to that of codilution. The contribution of polymeric pigments to wine color increases during aging, whereas copigmentation's role decreases. As stated by Rentsch et al.,<sup>28</sup> the color enhancement due to copigmentation is critically important in young wines but the contribution of polymeric pigments is crucial in aged red wines.

Copigmentation phenomena are highly affected by wine composition, which depends on initial factors (such as cultivar or climate) and final product properties (such as aging or alcoholic degree). It has been clearly shown here that some copigmentation studies carried out in model systems are not strictly applicable to wines. On the other hand, some copigmentation behaviors described in ideal solutions are reproduced in the samples here.

Phenol concentrations and molar ratios are crucial in the copigmentation process, in particular flavonols (mostly rutin and myricetin) and hydroxycinnamic acids (caffeic, coumaric, and coumaric acids). Other compounds generally considered as ideal copigments, such as quercetin, do not show any convincing relationship with this phenomenon at the concentration range observed in the wines from this study. On the other hand, the significant role of some substances believed to be important copigmentation agents according to model solutions, such as syringic acid or catechin, has been confirmed.

Anti-copigmentation and self-association processes related to compounds such as gallic acid seem to be present in wine. Unusual mineral–color relationships have been confirmed (K, Mg, Fe, Cu), and some wine composition influences in copigmentation are described (ethanol, sugar, ammonia, and some major organic acids).

These results reveal interesting relationships not only for the wine industry but also for the food science community. Indeed, they might be useful for any anthocyanin-rich product, as several factors have been investigated in such a complex matrix as wine.

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### Notes

The authors declare no competing financial interest.

## ABBREVIATIONS USED

CU, CIELab units; AU, units of absorbance;  $A_{XXX}$ , absorbance at XXX nm;  $r$ , Pearson correlation coefficient; IPT, total phenol index; ICM, global color intensity;  $X_{XX}$ , color fraction due to XX

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