Application of Differential Colorimetry To Evaluate Anthocyanin–Flavonol–Flavanol Ternary Copigmentation Interactions in Model Solutions

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ABSTRACT: The combined effect of anthocyanin–flavanol–flavonol ternary interactions on the colorimetric and chemical stability of malvidin-3-glucoside has been studied. Model solutions with fixed malvidin-3-glucoside/(+)-catechin ratio (MC) and variable quercetin-3-β-D-glucoside concentration (MC+Q) and solutions with fixed malvidin-3-glucoside/quercetin-3-β-D-glucoside ratio (MQ) and variable (+)-catechin concentration (MQ+C) were tested at levels closer to those existing in wines. Color variations during storage were evaluated by differential colorimetry. Changes in the anthocyanin concentration were monitored by HPLC-DAD. CIELAB color-difference formulas were demonstrated to be of practical interest to assess the stronger and more stable interaction of quercetin-3-β-D-glucoside with MC binary mixture than (+)-catechin with MQ mixture. The results imply that MC+Q ternary solutions kept their intensity and bluish tonalities for a longer time in comparison to MQ+C solutions. The stability of malvidin-3-glucoside improves when the concentration of quercetin-3-β-D-glucoside increases in MC+Q mixtures, whereas the addition of (+)-catechin in MQ+C mixtures resulted in an opposite effect.

KEYWORDS: ternary copigmentation, malvidin-3-glucoside, (+)-catechin, quercetin-3-β-D-glucoside, differential colorimetry

INTRODUCTION

Anthocyanins, flavanols, and flavonols are grape flavonoids of special interest in enology due to their influence on important sensory properties of red wines such as color, bitterness, astringency, and stability. Whereas anthocyanins and their derivatives are the pigments directly responsible for the red color, flavanols and flavonols have been shown to contribute to astringency and bitterness. Also, by combining with anthocyanins, colorless flavonoids are involved in the long-term color stability through copigmentation interactions. In nature, anthocyanins are frequently found participating in noncovalent associations with other colorless substances (the so-called copigments) when they coexist in an aqueous medium such as red wine. This weak association is termed intermolecular copigmentation and represents one of the most complex and efficient mechanisms of anthocyanin chromophore stabilization. The basic role of copigments is to protect the colored flavylum cation against the nucleophilic attack of water, peroxide, and sulfur dioxide bleaching and pH changes, thus preventing the formation of the colorless hemiacetal and chalcone forms. Furthermore, this interaction could be an initial step leading to the formation of anthocyanin-derived pigments, which better preserve the wine color. Among red wine components, phenolics derived from seeds (mainly flavanols) and skins (mainly flavonols and flavanols) appear to be the most abundant compounds to act as anthocyanin copigments. As a consequence, the proportions of these compounds are frequently modulated by applying vinification techniques to ensure more stable color formation.

Given the importance of grape flavonoids on wine color, the effectiveness of flavanols and flavonols as copigments has been widely studied in model solutions, and the color stabilization that they induce has been extensively demonstrated. However, most studies focused on copigmentation have considered individual compounds at concentrations and ratios generally much greater than those found in red wines, which makes it difficult to extrapolate the results. Most of these studies stated that individual flavonoids (either flavanols or flavonols) cause more intense and stable color of anthocyanins than the pigments alone, when tested isolated (binary pigment–copigment mixtures). However, controversial or even opposite effects have been recently described when the same phenolic copigments were studied in conjunction. For example, Rustioni et al. reported that certain combinations of flavonoids extracted from different grape tissues demonstrated a strong copigmentation effect, whereas others surprisingly produced a decrease in the anthocyanin color solution. These findings suggest a possible “anti-copigmentation” effect between
colorless flavonoids and anthocyanins when these compounds coexist together in a complex food matrix such as red wines.

In red wine, copigmentation occurs in a “multiple” way, and more than one copigment can interact simultaneously with anthocyanins. Under these conditions, it is expected that there will be competition between the copigments and pigments in the same solution. The preference or order for these associations could result in positive or negative synergies on the color and color stability, as has been previously reported for other important properties of phenolic compounds such as antioxidant activity.18

Studies focused on the mechanism of these multiple interactions in model solutions and its effects on copigmentation are scarce,19,22 and some aspects are still not well understood such as the possible additive, synergistic, or antagonistic effects among the components present in multiple mixtures and the way to quantify the extent of the process.

From a sensory point of view, the objective assessment of copigmentation seems to be critical for understanding the relationship between grape composition and color in young red wines.21 It is assumed that positive effects of copigmentation include greater color stability and also quantitative and qualitative color changes. Therefore, an adequate description of the color variations caused by copigmentation requires considering the changes produced in the entire visible spectral curve (380–770 nm), and not only in its visible λmax portion. The color characterization of the multiple copigmentation process in the CIELAB color space by differential colorimetry provides the opportunity of assessing this phenomenon in a global way by considering the modifications in the whole visible spectrum, and conclusions related to the later color stability could be extracted. Research into the industrial evaluation of color variations based on differential colorimetry has shown significant progress in recent years.23–25 The CIELAB color attributes lightness, chroma, and hue, and their related color-difference formulas are widely used for industrial applications that require accurate measurement of color changes related to perception and acceptability. Nonetheless, there is very little previous information on the application of differential colorimetry into the study of copigmentation.21 Because of the CIELAB colorimetric attributes of color, and to the interpretation of the copigmentation effects on its visual assessment, both in model solutions and in wines.21,15,22,27

Thus, with the aim to further the knowledge of the copigmentation interactions and their implication in the chemical and colorimetric stability of the color of red wine anthocyanins, we have studied the combined effect of anthocyanin–flavanol–flavonol ternary mixtures by differential colorimetry. The levels of flavanols and flavonols in the multiple mixtures were modified to simulate the effect of applying such enological procedures as adding white skins (flavanols) or seeds (flavonols) during the vinification of red wines. The pigment considered in this study represents the main anthocyanin of red grapes and wines (malvidin-3-glucoside). The copigment (+)-catechin was selected as it is one of the main monomeric flavanols present in grape seed, and quercetin-3,3′-d-glucoside was representative of flavonols present in grape skin.

## MATERIALS AND METHODS

### Chemicals and Standards

Malvidin-3-glucoside (≥97%) was purchased from Extrasynthese (Genay, France). The copigments (+)-catechin (≥96%) and quercetin-3,3′-d-glucoside (≥90%) were purchased from Sigma-Aldrich (Madrid, Spain). All chromatographic solvents (acetonitrile and formic acid) were of HPLC grade (Merck, Darmstadt, Germany). Purified water was obtained from a NANOpure Diamond system (Barnsted Inc., Dubuque, IA, USA). All other chemicals (methanol, tartaric acid, and sodium chloride) were of analytical grade and supplied by Panreac Química (Barcelona, Spain).

### Preparation of Ternary Copigmented Solutions

Copigmented solutions were used in a wine-like medium containing 5 g/L tartaric acid in 12% ethanol with ionic strength adjusted to 0.2 M by the addition of sodium chloride and pH adjusted to 3.6 with NaOH.

Two copigmented solutions based on binary combinations of malvidin-3-glucoside with (+)-catechin (MC) and malvidin-3-glucoside with quercetin-3,3′-d-glucoside (MQ) were prepared at the same pigment/copigment concentration (200:200 mg/L). MC and MQ solutions were filtered (0.45 μm Millipore-AP20 filters) and stored closed in darkness at 25 °C for 2 h to reach equilibrium. Then, quercetin-3,3′-d-glucoside was added in concentrations 50, 100, and 200 mg/L to three aliquots of the MC solution to obtain the following ternary mixtures: MC+Q50, MC+Q100, and MC+Q200. In the same way (+)-catechin was added in concentrations of 50, 100, and 200 mg/L to three aliquots of the MQ solution to obtain the following ternary mixtures: MQ+C50, MQ+C100, and MQ+C200.

All of the copigmented solutions, in both binary and ternary combinations, were prepared in triplicate and stored closed in darkness at 25 °C. The final anthocyanin concentration was the same in all cases (200 mg/L). Samples (n = 24) were taken periodically for the colorimetric and chemical analysis at 2, 24, and 72 h and at 1, 2, and 3 weeks.

### HPLC-DAD Analysis

The content of malvidin-3-glucoside in the model solutions and its evolution over the storage period were evaluated by HPLC-DAD using a Hewlett-Packard 1200 series liquid chromatograph (Palo Alto, CA, USA), equipped with a quaternary pump, column heater, UV–vis diode array detector, automatic injector, and ChemStation software. A Zorbax SB C18 column (4.6 mm × 250 mm, 4.6 μm particle size) was thermostated at 38 °C. The solvents used were (A) acetonitrile/formic acid/water (3:10:87) and (B) acetonitrile/formic acid/water (50:10:40). The elution profile was as follows: 0–5 min, 94% A; 5–7.5 min, 70% A; 7.5–12.5 min, 60% A; 12.5–16 min, 55% A; 16–19 min, 50% A; 19–20 min, 94% A. The flow rate was 1 mL/min, and the injection volume was 50 μL. UV–vis spectra were recorded from 200 to 800 nm with a bandwidth of 2.0 nm. The quantification was performed at 252 nm by HPLC-DAD using calibration curves of malvidin-3-glucoside standard. All analyses were carried out in duplicate, and results were expressed as milligrams per liter.

### Analysis of Copigmentation by Colorimetric Measurements

The absorption spectra (380–770 nm) of all solutions were recorded at constant intervals (Δt = 2 nm) with a Hewlett-Packard UV–vis HP8453 spectrophotometer, using 5 mm path length glass cells and distilled water as a reference. The CIELAB parameters were calculated from the absorption spectra by using the original software CromLab28 following the recommendations of the Commission International de l’Eclairage: CIE 1964 10° Standard Observer and the Standard Illuminant D65, corresponding to natural daylight.29 CIELAB parameters were calculated: L* (the correlate of lightness; ranging from 0, black, to 100, white) and b* (positive for yellowish colors and negative for bluish ones). From these coordinates, other color parameters are defined: the hue angle (h) (the correlate of chromaticity or tone, and the chroma C′* hues are the correlate of saturation or intensity of color). Moreover, L*, C′* and h* can be distinguished as quantitative or qualitative parameters as they indicate quantitative (L* and C′* hues) or qualitative (h*) attributes of color.

The color changes of solutions induced by copigmentation and their color stability over the storage period were evaluated by differential colorimetry according to the methodology described in Gordillo et al.15 which is based on the application of various different
color-difference formulas. The color difference between pairs of samples was computed as the Euclidean distance between two points on the CIELAB space.

**RESULTS AND DISCUSSION**

### Effect of Multiple Copigmentation on Color Characteristics by Differential Colorimetry

Table 1 shows the influence of increasing concentrations of quercetin-3-β-D-glucoside and (+)-catechin on the colorimetric parameters ($L^*$, $a^*$, $b^*$, $C^*_ab$, and $h_{ab}$) and total color of MC and MQ solutions, respectively, after 2 h of storage. Results showed that the two copigments studied have quite different abilities to interact with malvidin-3-glucoside in multiple copigmentation conditions. In particular, the addition of quercetin-3-β-D-glucoside caused an immediate increase in the total color of the MC solutions, which reflects the preferential binding between quercetin-3-β-D-glucoside and the flavylum ions and the shift of the equilibrium toward the colored forms. The magnitude of this effect, expressed as the relative increase of the Total color, was significantly ($p < 0.05$) larger at higher concentrations of quercetin-3-β-D-glucoside. Specifically, the total color was increased by 5, 12, and 20% at 0.5, 1, and 2 mg/L of quercetin-3-β-D-glucoside. Thus, quercetin-3-β-D-glucoside was demonstrated to have a strong affinity for malvidin-3-glucoside in the presence of (+)-catechin. Also, the addition of quercetin-3-β-D-glucoside to MC solutions produced a progressive increase in lightness and hue values ($L^*$ and $h_{ab}$), followed by an increase of chroma ($C^*_ab$), which means a gradual and significant intensification of the color.

In contrast, the addition of (+)-catechin did not greatly influence the colorimetric characteristics of MQ solutions at any tested level, with the exception of the $b^*$ and hue values, which were significantly ($p < 0.05$) decreased at 200 mg/L. These slight variations observed in the qualitative attributes of color ($b^*$ and hue values) denote a bluish effect. In any case, no statistically significant differences were found in the total color, chroma, and lightness (quantitative attributes) between MC and MQ+C solutions, indicating similar color intensity in both binary and ternary mixtures. Thus, in comparison to quercetin-3-β-D-glucoside, (+)-catechin was demonstrated to have a much lower affinity for malvidin-3-glucoside in multiple conditions.

The different abilities of quercetin-3-β-D-glucoside and (+)-catechin to modify the copigmentation equilibrium in multiple conditions can be explained in terms of their specific binding strengths and concentrations resulting in variable effects in color. With respect to the MQ solutions, our results suggest that the strong interaction initially established between quercetin-3-β-D-glucoside and malvidin-3-glucoside prevents the subsequent binding with (+)-catechin. As expected, quercetin-3-β-D-glucoside offers larger extended π-conjugation that makes the molecule more planar and facilitates the π-π interactions to form stable copigmentation complexes, so the availability of the flavylum ion to interact with (+)-catechin could be strongly limited.
limited. Similar results were obtained by Rustioni et al., who concluded that some flavonols are capable of... bluish e... on September 1, 2015 | http://pubs.acs.org 
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MQ Solutions, Calculated as Mean Values at 2 h and 3 Weeks of Storage (25 °C)

<table>
<thead>
<tr>
<th></th>
<th>MC solutions$^a$</th>
<th>MQ solutions$^b$</th>
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<tbody>
<tr>
<td></td>
<td>MC+Q50/MC</td>
<td>MQ+CS0/MQ</td>
</tr>
<tr>
<td></td>
<td>MC+Q100/MC</td>
<td>MQ+C100/MQ</td>
</tr>
<tr>
<td></td>
<td>MC+Q200/MC</td>
<td>MQ+C200/MQ</td>
</tr>
<tr>
<td>2 h of Storage</td>
<td></td>
<td></td>
</tr>
<tr>
<td>$\Delta E^*_{ab}   $</td>
<td>2.9</td>
<td>0.6</td>
</tr>
<tr>
<td>$\Delta L^*        $</td>
<td>−1.7</td>
<td>1.2</td>
</tr>
<tr>
<td>$\Delta C^*_{ab}   $</td>
<td>+1.8</td>
<td>−0.8</td>
</tr>
<tr>
<td>$\Delta h_{ab}     $</td>
<td>−2.4°</td>
<td>1.0°</td>
</tr>
<tr>
<td>3 Weeks of Storage</td>
<td></td>
<td></td>
</tr>
<tr>
<td>$\Delta E^*_{ab}   $</td>
<td>10.0</td>
<td>3.8</td>
</tr>
<tr>
<td>$\Delta L^*        $</td>
<td>−8.7</td>
<td>6.0</td>
</tr>
<tr>
<td>$\Delta C^*_{ab}   $</td>
<td>+4.4</td>
<td>+1.9</td>
</tr>
<tr>
<td>$\Delta h_{ab}     $</td>
<td>−3.8°</td>
<td>−10.0°</td>
</tr>
</tbody>
</table>

$^a$MC, Mv 3-gluc/(+) cat (200:200 mg/L); MC+Q50, MC+Q100, MC+Q200, MQ solution +50,+100,+200 mg/L of querc 3-gluc. $^b$MQ, Mv 3-gluc/ querc 3-gluc (200:200 mg/L); MQ+CS0, MQ+C100, MQ+C200, MQ solution +50, +100, +200 mg/L of (+)-cat.

Effect of Multiple Copigmentation on Color Stability by Differential Colorimetry. The evolution of the total color of solutions during the 3 weeks of storage in darkness at 25 °C is shown in Figure 2, where the mean value and standard deviation of samples are included. It can be observed that the addition of quercetin-3-β-D-glucoside is more effective in maintaining the total color of the MQ solutions than the addition of (+)-catechin to the MQ solutions. As shown in Figure 2A, the total color hardly decreased in MC binary solutions after 2 weeks of storage (around 28%), whereas in the ternary solutions containing 50, 100, and 200 mg/L of quercetin-3-β-D-glucoside the total color losses were 13.6, 10.4, and 4%, respectively. Contrary to that, higher decreases of total color were observed in MQ+Q ternary solutions as the (+)-catechin ratio increased in the mixtures (Figure 2B).

The assessment of changes in the CIELAB parameters during storage was also decisive to establish the copigment having the greatest impact on the stability of the solutions (MC+Q and MQ+Q). Independently of the concentration tested, the global variations of lightness values were always below 3.5% increases (positive values), resulting in more saturated solutions. Thus, it was possible to evaluate which color attribute was the most influenced (Figure 1). Taking into account only solutions with the largest perceptible color changes (MC+Q100 and MC+Q200), it can be observed that quercetin-3-β-D-glucoside induced similar color effects independently of the concentration tested. Results showed that the lightness was the most overall influential parameter (%Δ$^2$L ∼ 50%), whereas the weights of the chroma and hue modifications were in general comparatively lower, but similar among them (%Δ$^2$C and %Δ$^2$H ∼ 25%).
(+)-Catechin showed a lower protective effect on the quantitative attributes of color, especially for chroma variations (Figure 4A, B). During storage, the chroma values notably decreased about 13% with concentrations of (+)-catechin up to 100 mg/L. Thus, surprisingly, the binary solution MQ showed greater color stability over time than in the presence of increasing concentrations of (+)-catechin. These results highlight that adding copigments does not always lead to a stabilizing effect, but depends on the type of copigments as well as on its concentration.

Furthermore, the assessment of the color differences \( \Delta E^*_{ab} \) occurring at 24 and 72 h and at 1, 2, and 3 weeks, in relation to samples taken at 2 h, provides an objective evaluation of the chromatic stability for each solution over time (Figure 5). The lower values of \( \Delta E^*_{ab} \) during storage in MC+Q ternary solutions than in MC binary solutions indicate lower color variation and, thus, higher color stability (Figure 5A). Considering the whole period of storage, the lowest color differences corresponded to MC+Q200 and MC+Q100 solutions compared to MC+Q50 and MC (\( \Delta E^*_{ab} = 9.8, 10.5, 12.1, \) and 16.4, respectively). In contrast, growing \( \Delta E^*_{ab} \) values in MQ+C ternary solutions relative to those in MQ binary solution indicate higher color variation and, thus, lower color stability (Figure 5B). Nevertheless, this negative effect seemed to greatly depend on the (+)-catechin concentration in the mixtures. It can be observed that the color modification experienced by MQ+C50 solution was not easily detectable (\( \Delta E^*_{ab} < 3 \)) until the end of the storage period. This means that (+)-catechin does not cause important color destabilization in multiple conditions at low concentrations (50 mg/L in our conditions). However, at (+)-catechin concentrations up to 100 mg/L, \( \Delta E^*_{ab} \) values were detectable from the first weeks of storage.

As a consequence of the different color stabilities over time, MC+Q and MQ+C ternary solutions could be differentiated at the end of storage period in terms of luminosity, intensity, and chromaticity from their respective MC and MQ binary solutions (Table 1). Specifically, MC+Q solutions showed
significantly lower values of $L^*$ and hue but higher values of total color and $C^*_{ab}$ than MC solution. In terms of detectable color variations (Table 2), the highest color difference value was obtained between MC and MC+Q200 solutions ($\Delta E^*_{ab} = 20.6$). The negative values of lightness and hue differences found among them ($\Delta L^* = -14.2, \Delta h_{ab} = -10.0^\circ$), but positive chroma difference ($\Delta C^*_{ab} = +13.3$), means that MC+Q200 solutions had darker and more intense color with more bluish hue than MC solutions.

On the other hand, MQ+C solutions showed significantly higher values of $L^*$ but lower total color and $C^*_{ab}$ than MQ solutions (Table 1). In this case, the trend of the lightness and chroma differences among pairs of solutions was the opposite. Positive values for $\Delta L^*$ and negative values for $\Delta C^*_{ab}$ indicate that the final color of MQ+C solutions was lighter and less saturated than that of MQ solution. Again, the color differences were most appreciable with the highest concentration of (+)-catechin (MC+Q100 and MC+Q200 solutions).
Thus, with the whole set of data taken into account, it was confirmed that the addition of increasing amounts of quercetin-3-β-D-glucoside to MC binary mixtures led to MC+Q ternary solutions with more stable color that kept their intensity and vivid bluish tonalities for a longer time, which represent color characteristics highly appreciated in young red wines. In contrast, the addition of increasing amounts of (+)-catechin to MQ binary mixtures led to MQ+C ternary solutions with less stable color that maintain their vivid red intensity for only a short time, so their value as potential additives in the wine industry could be rather questionable because the minimization of color fluctuation is essential to maintain the global quality of wines.

**Effect of Multiple Copigmentation Interactions on Chemical Stability.** Figure 6 shows the changes in the concentration of malvidin-3-glucoside (mg/L ± SD) in solutions over time. Although the presence of the copigments does not impede the loss of the anthocyanin content, the protective effects of quercetin-3-β-D-glucoside and (+)-catechin in multiple conditions were quite different. As observed in Figure 6A, the content of malvidin-3-glucoside in MC and MC+Q solutions is maintained more or less constant during the first 2 weeks of storage. After this, the amount of malvidin-3-glucoside declines dramatically, the rate of degradation being less intense in the presence of quercetin-3-β-O-glucoside. The global content of malvidin-3-glucoside decreased over the whole storage period by 42% in MC binary solutions. In comparison, the anthocyanin degradation was notably reduced to 24, 19.5, and 19% with the addition of 50, 100, and 200 mg/L of quercetin-3-β-D-glucoside, respectively. These observations confirm that quercetin-3-β-O-glucoside behaved as a protective copigment in multiple conditions by preventing excessive pigment loss. In general terms, the higher the concentration of quercetin-3-β-D-glucoside in MC solution, the greater protective effects against anthocyanin degradation, which was in accordance with the amplitude of the effects observed in color stability. Nevertheless, the positive effect of quercetin-3-β-O-glucoside cannot be raised to an unlimited extent because no further improvement in the anthocyanin stability was produced when its concentration exceeded a certain level (in our conditions, up to 100 mg/L). This observation was in agreement with previous studies.²,³ The analysis of variance (ANOVA, Tukey test) revealed significant differences (p < 0.05) for the final levels of malvidin-3-glucoside between MC and MC+Q solutions. MC solution showed the lowest content in malvidin-3-glucoside (126.5 ± 0.2 mg/L). As expected, no statistically significant differences were found for the anthocyanin content between MC+Q100 and MC+Q200 solutions (176.7 ± 2.8 and

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**Figure 5.** Influence of increasing concentrations of quercetin-3-β-O-glucoside and (+)-catechin on the color variation (ΔE*a) of MC and MQ binary ternary solutions, respectively, over the storage period at 25 °C: (A) MC solutions; (B) MQ solutions.

**Figure 6.** Influence of increasing concentrations of quercetin-3-β-O-glucoside and (+)-catechin on the evolution of the malvidin-3-glucoside content of MC and MQ binary ternary solutions, respectively, over the storage period at 25 °C: (A) MC solutions; (B) MQ solutions.
177.5 ± 0.3 mg/L, respectively) because the losses of malvidin-3-glucoside were very similar in both ternary solutions. Thus, the application of quercetin-3-β-D-glucoside as wine copigments appears to be promising because it tends to significantly improve the stability of the anthocyanins in multiple conditions over time.

On the other hand, the anthocyanin stability of MQ and MQ+C solutions during storage is presented in Figure 6B. It can be observed that the malvidin-3-glucoside degradation occurred more rapidly in the presence of (+)-catechin (MQ+C solutions), which is in accordance with the color evolution. In particular, more remarkable losses occurred from the earliest moment of storage at concentrations of (+)-catechin up to 100 mg/L (approximately by 24%). After 3 weeks of storage, significantly (p < 0.05) higher contents of malvidin-3-glucoside were found in MQ and MQ+C50 solutions (204.8 ± 5.9 and 187.3 ± 0.3 mg/L, respectively), whereas this value fell to 166.1 ± 1.2 and 166.9 ± 2.7 mg/L in MQ+C100 and MQ+C200 solutions. This fact seems to indicate that, at high concentrations, (+)-catechin can interfere with the existing malvidin-3-glucoside/ quercetin-3-β-D-glucoside equilibrium, diminishing their chemical stability. This antagonistic effect, clearly potentiated with time in our conditions, was also observed by Rustioni et al., who demonstrated that certain combinations of copigments (especially those from seeds, as could be (+)-catechin) can negatively affect the global copigmentation equilibrium by breaking tannin–anthocyanin interactions.

In summary, this work has shown that quercetin-3-β-D-glucoside and (+)-catechin exhibit quite different abilities to interact with malvidin-3-glucoside in ternary mixtures depending on their specific binding strength and concentration, which have important consequences on the color and chemical stability during storage. Differential colorimetry has demonstrated the fundamental role of quercetin-3-β-D-glucoside as a potential wine additive due to its immediate copigmentation effect in multiple systems and higher potential for stabilization over time. Our findings also showed the greater difficulty of (+)-catechin binding to malvidin-3-glucoside in the presence of quercetin-3-β-D-glucoside and its lower potential to lead to chemical and colorimetric stabilization. It should be borne in mind, therefore, that certain flavonoid combinations do not always result in positive effects on color by multiple copigmentation but also could cause negative effects. In this sense, further studies need to be done to better understand the mechanisms involved in the anthocyanin–flavanol–flavonol multiple interactions under different conditions (pH, temperature, sulfites, etc.) as well as to strategically select appropriate flavonoid combinations that promote synergistic interactions and avoid antagonisms.

**REFERENCES**


