Chemical and colorimetric study of the influence of grape soluble polysaccharides on the color and stability of malvidin 3-*O*-glucoside solutions

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A R T I C L E I N F O	A B S T R A C T
	Red wine color is mainly due to anthocyanins' flavylium cation. In wine, these pigments are involved in a pH-dependent equilibrium involving other forms, both colored and non-colored. This equilibrium is affected by the presence of other phenolic compounds, the so-called copigments. Moreover, other non-phenolic compounds that can be found in wine, such as polysaccharides, can also affect wine color. In this work, molecular dynamics simulations, tristimulus colorimetry and HPLC-DAD analyses have been performed to assess the effect of soluble polysaccharides (P) on the color and stability of malvidin 3-*O*-glucoside (Mv) solutions at different pH values, both in presence and absence of other compounds that can establish copigmentation interactions with the pigment. Results showed that P shifts the color of flavylium solutions towards a more intense color with bluer hues, which can be related to a stabilization of the blue quinoidal forms of anthocyanins. This effect is similar to that observed for quercetin 3-*β*-glucopyranoside (QG), although, in the case of P, it seems to be more relevant. This behavior could be due to the presence of hydrophobic regions in P that allows the interaction with the quinoidal forms, which in the case of the polysaccharide might be related to the type I rhamnogalacturonans.

1. Introduction

Color is one of the main attributes of red wine that can be related to its quality since it affects the wine acceptance by consumers. The intense red color of young wines is due to the flavylium cation of anthocyanins. However, flavylium structure is very unstable due to its deficiency on electrons, which explains the high reactivity of anthocyanins. At slightly higher pH values, the anthocyanins participate in a complex equilibrium involving the quinonoid bases (blue), that are formed by a deprotonation of phenol groups in the flavylium structure, the hemiketal form (colorless), formed by hydration of the flavylium cation and the chalcones (yellow), which are given raised by tautomerization of the hemiketal and isomerization reactions (Brouillard & Delaporte, 1977). Although the flavylium cation is only stable at acidic pH (pH $\leq$ 2), in wine matrix it is stabilized by the interactions with other wine compounds (the so-called copigments), through the copigmentation

phenomenon (Boulton, 2001; He et al., 2012). Hence, the copigmentation phenomenon is essential to improve the chemical colorimetric stability of anthocyanins, which, in turn, is the main reason of the stability of wine color, being responsible for 30–50% of the coloration in young red wines (Trouillas et al., 2016). This phenomenon occurs due to intermolecular interactions through non-covalent hydrophobic molecular associations between the aromatic nuclei of the colored forms of anthocyanins and the copigments (usually colorless) (Mazza & Brouillard, 1990; Santos-Buelga & de Freitas, 2009), which can take place in aqueous medium like red wine. Also, intramolecular interactions (Dangles et al., 1993) and self-association, where copigmentation involved just anthocyanin molecules, can play an important role in wine color (Trouillas et al., 2016).

In wine matrix, there are different compounds that can act as copigments. Flavonoids such as flavonols and flavanols, and non-flavonoids, such as hydroxycinnamic acids (Gómez-Míguez et al., 2006), are the main copigments found in wine. In binary pigment:

Abbreviations

(Mv)	malvidin 3-*O*-glucoside
(P)	polysaccharide
(PC)	phenolic compounds
(QG)	quercetin 3-*β*-glucopyranoside
(CA)	caffeic acid
(E)	(−)-epicatechin
(C)	(+)-catechin
(GA)	gallic acid
(Mv:P)	malvidin 3-*O*-glucoside:polysaccharide binary interaction
(Mv:QG)	malvidin 3-*O*-glucoside:quercetin 3-*β*-glucopyranoside binary interaction
(Mv:CA)	malvidin 3-*O*-glucoside:caffeic acid binary interaction
(Mv:E)	malvidin 3-*O*-glucoside:epicatechin binary interaction
(Mv:C)	malvidin 3-*O*-glucoside:catechin binary interaction
(Mv:GA)	malvidin 3-*O*-glucoside:gallic acid binary interaction
(Mv:PC:P)	malvidin 3-*O*-glucoside:each phenolic compounds:polysaccharide ternary interaction
(Mv:QG:P)	malvidin 3-*O*-glucoside:quercetin 3-*β*-glucopyranoside:polysaccharide ternary interaction
(Mv:CA:P)	malvidin 3-*O*-glucoside:caffeic acid:polysaccharide ternary interaction
(Mv:E:P)	malvidin 3-*O*-glucoside:epicatechin:polysaccharide ternary interaction
(Mv:C:P)	malvidin 3-*O*-glucoside:catechin:polysaccharide ternary interaction
(Mv:GA:P)	malvidin 3-*O*-glucoside:gallic acid:polysaccharide ternary interaction

copigment complexes, it has been reported that both flavonoids and non-flavonoids copigments give rise to a more stable color of anthocyanins, although in a different extend (Baranac et al., 1997; Gonçalves et al., 2013; Gordillo et al., 2012). From a molecular point of view, the copigmentation complex made up of the pigment and the copigment shows a sandwich configuration in which the most important forces are hydrophobic interactions (π-π stacking). The flavylium cation is protected from nucleophilic water attack due to this sandwich configuration, which implies the stabilization of the colored forms of the anthocyanin and, in turn, leads to a higher intensity of color (hyperchromic effect). Moreover, in some cases, the presence of copigments also leads to a displacement in the maximum absorption wavelength (bathochromic effect), *i.e.*, to bluer solutions (Gordillo et al., 2012).

Polysaccharides are biopolymers constituted by the union of 10 or more sugar units through glycosidic bonds. These compounds are one of the main groups of macromolecules present in wine (Jones-Moore et al., 2021), since they can be found at concentrations ranging from 200 to 1.500 mg/L. Polysaccharides can be released from yeast during autolysis or be extracted from grape cell wall during winemaking. The cell wall of grapes is mainly composed by three types of polysaccharides: hemicellulose, cellulose and pectins (Jones-Moore et al., 2021; Zhang et al., 2021). The last two represent 30–40% of the polysaccharide components of the cell wall (Nunan et al., 1997), being pectins the most important group regarding wine composition. Pectin composition mainly includes homogalacturonans and rhamnogalacturonans I and II (Caffall & Mohnen, 2009; Guadalupe et al., 2015). Polysaccharides are important in wine since, among others, they can play a key role in organoleptic properties of wines, such as color or astringency. Wine anthocyanins can interact with pectic polysaccharides, which can directly affect their color by increasing the intensity (Fernandes, Oliveira, et al., 2020), or increase their physico-chemical stability, by increasing their colloidal stability (Jones-Moore et al., 2021), and by

protecting them from discoloration by pH changes and SO_2-bleaching (Weilack et al., 2023). With regards wine astringency, it seems that polysaccharides can both interact with wine flavanols and reduce their content in wine, which can be related to a reduction in wine astringency levels (Manjón et al., 2023). Moreover, polysaccharides can affect the interaction between salivary proteins and tannins, reducing their aggregation and, therefore, reducing astringency (Carvalho et al., 2006; Brandão et al., 2017). However, the direct role of polysaccharides in copigmentation reactions, acting as sort of copigments by their own and their potential indirect effect on color, affecting the copigmentation interactions between phenolic compounds and anthocyanins are still fully unraveled. Moreover, the wine industry generates approximately 110 million tons of pomace after fermentation, which is an economic and ecological problem (Apolinar-Valiente et al., 2015). Thus, the re-valorization of these by-products from the winemaking (for instance, the recovery of polysaccharides from grape pomace) may be an important advantage for wine industry. Hence, the use polysaccharide obtained from winemaking by-products such as grape pomace could be an interesting approach for the stabilization of wine color, which could, in turn, could imply the re-valorization of winemaking by-products. To better understand copigmentation process, it is necessary an analysis of the spectral variations due to the noncovalent interactions. Tristimulus Colorimetry allows, by using the color space CIELAB, the characterization of the copigmentation effect in the whole visible spectral (380–770 nm), both in a qualitative and a quantitative way. Moreover, Molecular Dynamics (MD) simulations complement the experiments by using molecular models to provide a better knowledge on the copigmentation behavior between the pigment and the copigments. Indeed, MD simulations envisage the preferred number of molecules involved in the interactions and use several conformations to obtain an estimation of the Gibbs energies association ($\Delta G_{binding}$) (Trouillas et al., 2016).

In this work, we have studied the effect of soluble polysaccharides (P) on the color of flavylium cation form of anthocyanins by assessing the effect of P on the color and stability of malvidin 3-*O*-glucoside (Mv) solutions at different pH values. Furthermore, to evaluate if the effect of polysaccharide on Mv color can be affected by other wine compounds that can establish copigmentation interactions with the pigment, we have studied whether the presence of phenolic compounds (PC), known as wine copigments, could modify the effect of P on the color of the solutions. To achieve these objectives, this work was performed on model solutions at two different pH values: at pH 1.1, to avoid the existence of other equilibrium forms than flavylium cation and at pH 3.6, the most usual pH value found in red wines. MD simulations were performed to study the copigmentation molecular interactions and tristimulus colorimetry and HPLC-DAD to assess the color and stability of the pigment solutions, respectively. Thus, in this paper, a comprehensive study of the role of soluble polysaccharides on the color of the anthocyanin solutions is performed for the first time, by evaluating both the direct effect of these compounds on the colored forms of anthocyanins and the effect on their stabilization by copigmentation.

2. Materials and methods

2.1. Chemicals

Quercetin 3-*β*-glucopyranoside was purchased from Cymit Quimica. com (Barcelona, Spain). (−)-Epicatechin (≥90%), (+)-catechin hydrate (≥98%), pullulan standard set, 1-phenyl-3-methyl-5-pyrazolone (PMP) and trifluoroacetic acid (99%) were purchased from Sigma-Aldrich (St. Louis, MO). Caffeic acid (≥99%) and gallic acid were purchased from ACROS organics (Morris Plains, NJ) and from Merck (Darmstadt, Germany), respectively. 2-Deoxy-ᴅ-ribose ʟ-rhamnose, D-(+)-galacturonic acid, D-(+)-glucose, D-(+)-xylose, D-(+)-galactose, D-(+)-mannose, ᴅ-galacturonic acid, D-(+)-fucose, D-(−)-arabinose standards were acquired from Sigma Aldrich (St Louis, MO) and Alfa-Aesar (Karlsruhe, Germay). Methanol, acetonitrile (UHPLC-MS grade) and formic acid

(98%) were purchased from Macron Fine Chemicals (Deventer, Netherlands), from Scharlab S.L. (Barcelona, Spain) and from VWR Prolabo (Paris, France), respectively. LiNO$_3$ was purchased from Thermo Scientific (Waltham, MA). Ethanol, ammonium acetate, ammonia solution and chloroform were purchased from Scharlab S. L. (Barcelona, Spain), VWR Prolabo (Paris, France) and Labken (Madrid, Spain). Purified water was obtained from a MiliQ Gradient water purification system (Millipore, Billerica, MA). Malvidin 3-O-glucoside was isolated in the laboratory and its extraction and purification process is outlined below.

2.2. Isolation of malvidin 3-O-glucoside

The pigment malvidin 3-O-glucoside (Mv) was extracted from the skins of *Vitis vinifera* cv Tempranillo red grapes using acidic methanol (methanol/HCl 0.5N; 95:5 *v/v*) as described by García-Estévez et al. (García-Estévez et al., 2017). Mv purification was performed as described in García-Estévez et al. (García-Estévez et al., 2013): the concentrated extract was loaded onto a Sephadex LH-20 (Sigma-Aldrich, St. Louis, MO) column, previously conditioned by acidic water (HCl 3N, pH 1.1). Elution was carried out using the same aqueous HCl solution. The first fraction eluted, which corresponds to Mv, was collected and freeze-dried to furnish a reddish purple powered whose purity (>97%) was assessed by HPLC-DAD.

2.3. Obtaining and analysis of soluble grape skin cell wall material (CWM)

Pomace obtained from red grape (*V. vinifera* cv Tempranillo) were manually separated to remove any seed or stem. Then, the isolated skins were put into boiling water for 2h (30g grape skins per liter) for polysaccharide extraction, based on the procedure described by Manjón and coworkers (Manjón et al., 2023). The extract was freeze-dried and the polysaccharides characteristics, *i.e.* its molecular weight and monosaccharide composition, were determined by HPLC. The molecular weight of the polysaccharides was estimated by using high resolution size exclusion chromatography coupled to a refractive index detector (HRSEC-RID). Sample was dissolved in the same solvent employed at mobile phase (0.1 M LiNO$_3$). HRSEC was performed using an Agilent 1260 Infinity HPLC system (Agilent Technologies, Palo Alto, CA) equipped with a refractive index detector (RID), using two serial Shodex OHpak SB-803 HQ and SB-804 HQ columns (8 mm Å, 300 mm) (Showa Denko Europe GmbH, Munich, Germany) thermostatted at 60 °C. Elution was performed using a flow rate of 1 mL/min of 0.1 M LiNO$_3$, as previously described Guadalupe et al. (Guadalupe & Ayestarán, 2007). Calibration was carried out with several pullulan molecular weight standards (P1, Mw = 342 Da; P2, Mw = 1320 Da; P3, Mw = 6200 Da; P4, Mw = 10,000 Da; P5, Mw = 21,700 Da; P6, Mw = 48,800 Da; P7, Mw = 113,000 Da; P8, Mw = 200,000 Da; P9, Mw = 348,000 Da; P10, Mw = 805,000 Da). The analysis was performed in triplicate.

2.4. Monosaccharide characterization by HPLC-DAD-MS

The monosaccharide composition of the obtained polysaccharides was determined by HPLC-DAD-MS (Agilent Technologies, Palo Alto, CA), after acid hydrolysis of the extracted polysaccharides and subsequent derivatization with 1-phenyl-3-methyl-5-pyrazonone (PMP) following an adaptation of the method reported by Ruiz-Garcia et al. (Ruiz-Garcia et al., 2014). Briefly, polysaccharides were hydrolyzed in TFA 2M at 100 °C for 3h and the hydrolysate was submitted to a derivatization with 0.5M 1-phenyl-3-methyl-5-pyrazolone (PMP) in 1M NH$_4$OH for 1h at 70 °C, using deoxyglucose as internal standard. The excess of PMP was removed by a liquid-liquid extraction with chloroform and the monosaccharide derivatives were analyzed by HPLC-DAD-MS-MRM. The monosaccharides were characterized according to their mass spectrum, fragmentation pattern and retention

times, and were compared with the available sugar standards. Quantification of the monosaccharide derivatives was performed using the chromatograms were registered at 250 nm and the calibration curves constructed for L-rhamnose, D-(+)-galacturonic acid, D-(+)-glucose, D-(+)-xylose, D-(+)-galactose, D-(+)-mannose, D-galacturonic acid, D-(+)-fucose, D-(−)-arabinose standards under the same analysis conditions. All analyzes were carried out in triplicate. The results were expressed as the mean ± standard deviation of the triplicate corresponding to each sample analyzed (see Supplementary material for further details).

2.5. Model solutions

All the model solutions were prepared both at pH 1.1 and at pH 3.6 (wine pH). The pigment concentration was the same in all cases (50 μM). Binary mixtures containing Mv and the isolated soluble polysaccharides (P) were prepared at molar ratios 1:1 (for both pH studied) and 1:0.5 (pH 3.6). Moreover, five solutions based on binary combinations of Mv with different phenolic compounds (PC), namely quercetin 3-β-glucopyranoside (Mv:QG), 3,4-dihydroxycinnamic acid (Mv:CA), (−)-epicatechin (Mv:E), (+)-catechin hydrate (Mv:C) and gallic acid (Mv:GA) were prepared at four different pigment/copigment concentrations, corresponding to 1:1, 1:2, 1:3 and 1:4 M ratio. A reference solution of anthocyanin at each pH was also prepared. Ternary mixtures containing Mv, each PC and P were also prepared at 1:1:0.5 M ratio (pH 3.6). All solutions were prepared in triplicate and stored in the dark at 25 °C for 2h to reach equilibrium. Furthermore, solutions at pH 1.1 were stored in the dark 25 °C for 5 weeks to monitor color and pigment stability.

2.6. Colorimetric measurements

The absorption spectra of the solutions were recorded on a Hewlett-Packard UV–Vis HP3853 spectrophotometer (Agilent Technologies, Waldbronn, Germany) at constant intervals (Δλ = 1 nm) using 10 mm path length quartz cells and acidic water (pH 1.1 and 3.6) as a reference. CIELAB color parameters (L^*, a^*, b^*, C^*_{ab}, and h_{ab}) were obtained from the visible spectra (380–770 nm), employing as references the CIE 1964 standard observer (10° visual field) and the Standard Illuminant D65. The CIELAB parameters were calculated using the software Cromalab™ (Heredia et al., 2004).

The effect of copigmentation interactions on color was evaluated by differential colorimetry (Gordillo et al., 2012, 2015), comparing the color of the pigment solution in absence and in presence of the different compounds assayed as copigments. Color difference (ΔE^*_{ab}) between samples was calculated using the CIELAB color difference formula $\Delta E^*_{ab} = [(\Delta L^*)^2 + (\Delta a^*)^2 + (\Delta b^*)^2]^{1/2}$ where ΔL^*, Δa^*, and Δb^* are the differences between the CIELAB parameters. The total color was also calculated as the color difference between the L^*, a^* and b^* values of each sample respect of those of the water as an achromatic reference (L^* = 100, a^* = 0 and b^* = 0), following the equation:

$$\text{Total color} = [(L^*-100)^2 + (a^*-0)^2 + (b^*-0)^2]^{1/2}$$

In addition, relative contribution of each color attribute (ΔL^*, ΔC^*_{ab} and Δh_{ab}) was calculated and used to evaluate the trend of the individual attribute (Gordillo et al., 2012, 2015):

$$\%\Delta L = [(\Delta L^*) / (\Delta E^*_{ab})] \times 100$$

$$\%\Delta C = [(\Delta C^*_{ab}) / (\Delta E^*_{ab})] \times 100$$

$$\%\Delta H = [(\Delta H) / (\Delta E^*_{ab})] \times 100$$

Where the ΔH value is deduced from: $\Delta H = [\Delta E^*_{ab} - ((\Delta L)^2 + (\Delta C)^2)]^{1/2}$

2.7. HPLC-DAD analysis

The chemical stability of Mv was monitored by means of HPLC-DAD,

using a Hewlett-Packard 1100 series liquid chromatograph (Agilent Technologies, Waldbronn, Germany), following a previously developed methodology (Alcalde-Eon et al., 2006). Briefly, an AQUA C18 reversed-phase, 5 μm, 150 mm × 4.6 mm column (Phenomenex®, Torrance, CA, USA) thermostatted at 35 °C was used. The flow rate was set a 0.5 mL min^{-1} and the solvents employed were an aqueous solution (0.1%) of trifluoroacetic acid (A) and 100% acetonitrile (B), establishing the following gradient: from 10 to 15% B for 10 min, isocratic at 15% B for 5 min, 15–18% B for 5 min and 18–35% B for 10 min, followed by column washing and re-equilibration to initial conditions. Detection was carried out at 520 nm as preferred wavelength. Spectra were recorded from 220 to 600 nm. Identification of anthocyanins and derived pigments was carried out from the chromatographic retention times.

2.8. Molecular modeling and molecular dynamics (MD) simulations

The computational protocol of modelling and MD simulations were performed as in our previous study (Torres-Rochera et al., 2023), but in this study, in addition to the malvidin 3-*O*-glucoside flavylium form, the quinoidal base (Mv-quinoid) structures were also considered. The three possible tautomers of the Mv quinoidal base (keto grupo at C7, C5 and C4′) are hereinafter called Mv-C7, Mv-C5 and Mv-C4' for simplicity. Therefore, each system was composed by 4 Mv, 1 quinoidal base and 11 molecules of PC, based on the theoretical flavylium:quinoinal proportions that could be found at that pH (Cruz et al., 2022) and to reproduce the experimental conditions. Three MD simulations for each PC were carried out to include a different quinoidal base tautomeric form in each system. The Amber 12 simulation package (Case et al., 2012) was used to carry out the optimizations and MD simulations. The details of the MD procedure are provided in the Supplementary material. The prevalent pigment:copigment (1:1) aggregate of the systems with caffeic acid, epicatechin and quercetin 3-*β*-glucopyranoside was used as starting geometry of a further MD simulation to clarify the binding mode between both molecules. The binding energy of each complex was determined using the Molecular Mechanics/Poisson Boltzmann Surface Area (MM/PBSA) approach (Humphrey et al., 1996). A total of 100 structures of each MD simulation were used for the analysis. The results are present as relative enthalpic binding energies ($\Delta\Delta H_{binding}$) with respect to the most stable complex.

2.9. Statistical analysis

The statistical significance of the differences between the obtained results were evaluated by one-way analysis of variance (ANOVA) and posthoc Turkey test, by using the software packing for Windows IBM SPSS 26 (SPSS, Inc. Chicago, IL). Differences were considered statistically significant at $p < 0.05$.

3. Results and discussion

3.1. Pectic polysaccharide characterization

Once the polysaccharides (P) were extracted from red grape pomace, their molecular weight (Mw) was calculated by using size exclusion chromatography (HRSEC-RID) and their carbohydrate composition was determined by HPLC-DAD-MS (for further information, see Supplementary material). The results showed that the Mw of P was 21.3 KDa. Regarding the carbohydrate composition (see Table S1 in the Supplementary material), the most abundant monosaccharides of P were arabinose, galactose and mannose (these three monosaccharides represent *ca.* 65% of the total monosaccharide content). Therefore, the families of polysaccharides found in the highest proportion in P were MP (mannoproteins) and pectic PRAGs (polysaccharides rich in arabinose and galactose). Furthermore, the high level of galactose in P suggests the presence of galactan-type side chains. Indeed, in our study, the galactose/arabinose ratio was close to one (>0.99), which, according to the

literature, indicates the presence of arabinogalactan side chains (Slavov et al., 2017). Galacturonic acid only represents 5% of total monosaccharides, indicating low presence of homogalacturonan chains. Moreover, the galacturonic acid/rhamnose ratio determined was very low (1.64), which suggests an important presence of rhamnogalacturonan I in our polysaccharide (Fernandes, Oliveira, et al., 2020; Slavov et al., 2017). Also, according to Apolinar-Valiente and coworkers, the ratio of (arabinose + galactose)/rhamnose can be used to estimate the relative importance of the neutral side-chains to the rhamnogalacturonan backbone. The ratio obtained in the case of P is higher (14.10), than those reported previously (6.52–8.10) (Apolinar-Valiente et al., 2015), which may indicate that rhamnogalacturonan molecules that composed the polysaccharide P has a high proportion of neutral side-chains in its structure.

3.2. Effect of the presence of P or PC on the color of flavylium cation solutions (pH 1.1)

Color analysis was performed using the CIELAB space at pH 1.1 in order to study the effect of the copigments on the color of solutions of the flavylium cation without the interferences of the presence of other anthocyanins forms, since, at that pH, the existence of other molecular forms apart from the flavylium cation of Mv is avoided. First, the effect of P was assessed by studying the color parameters (L^*, a^*, b^*, C^*_{ab}, h_{ab}) of the binary mixture Mv:P compared to Mv control solution after 2h of storage. Results (Table 1) showed a significant decrease for all color parameters of the solutions due to the presence of P. The presence of P leads to a decrease in L^* value, *i.e.* to darker solutions. However, the most affected parameter is the hue value (h_{ab}), *ca.* 7 CIELAB units, indicating a displacement of the color of the solution toward bluer hues. Thus, the presence of P shifts the color of flavylium solution towards a darker and, mostly, towards a bluer color. Also, there is a decrease of chroma value, that is, the solution has a less pure color, which could be related to the existence of different colored forms that show different colors. This can indicate that the polysaccharide could be favoring the presence of quinoidal forms of the anthocyanin (blue forms) in the solution, along with the flavylium cation (red). This variation of color could be related to the Mv:P interaction, which may involve the hydrophobic domains of the polysaccharides, such as rhamnogalacturonan-I domains that could participate in the formation of complexes with the pigment (Fernandes, Oliveira, et al., 2020).

The color of Mv:PC solutions prepared at different molar ratios (Mv: PC, Mv:PC$_2$, Mv:PC$_3$ and Mv:PC$_4$) was also determined using the CIELAB space. As expected, the PC that most changed the flavylium cation color was QG (data not shown), which is in agreement with the results obtained in a previous study (Torres-Rochera et al., 2023). To go deeper on the study of these color changes, the color differences between Mv control and Mv:P or Mv:PC solutions were calculated, as well as the relative contribution of each color attribute in those color differences (Table 2). This also allows to determine which color variations could be detected by human eye, since values higher than 3 are appreciable by the human eye (Martínez et al., 2001). A higher value of 3 for ΔE^*_{ab} can be observed for Mv:P and Mv:QG at all pigment:PC ratios assayed. It is worth noting that, in the case of QG (a PC recognized as a good copigment), to obtain a difference of color of similar magnitude to that

Table 1
Color parameters (L^*, a^*, b^*, C^*_{ab}, h_{ab}) obtained at pH 1.1 for Mv control and Mv:P (1:1) solutions after 2h of storage.

	L^*	a^*	b^*	C^*_{ab}	h_{ab}
Mv	66.9 ± 0.3 [a]	61.5 ± 0.1 [a]	14.2 ± 0.3 [a]	63.1 ± 0.2 [a]	13 ± 0.3 [a]
Mv:P	66.2 ± 0.1 [b]	59.9 ± 0.3 [b]	6.2 ± 0.1 [b]	60.3 ± 0.3 [b]	5.9 ± 0.1 [b]

Mv: malvidin 3-*O*-glucoside. P: polysaccharide. Different letters within each column indicate statistical differences (p < 0.05, n = 3).

Table 2

Color differences (ΔE^*_{ab}) at pH 1.1 between malvidin (Mv) control solution and the solutions added with phenolic compounds, at four different molar ratios (1:1, 1:2, 1:3 and 1:4), or polysaccharides and relative contribution of each color attribute (%ΔL, %ΔC, %ΔH).

	ΔE^*_{ab}	%ΔL	%ΔC	%ΔH
Mv:P	8.2 ± 0.1 [a,b]	0.8 ± 0.2 [b]	12.3 ± 2.1 [a,b]	86.9 ± 2.3 [a]
Mv:QG	3.4 ± 0.2 [d]	2.4 ± 2.1 [b]	4.5 ± 2.1 [a,b]	93.1 ± 1.6 [a]
Mv:QG₂	6.1 ± 0.2 [c]	2.6 ± 0.6 [b]	3.2 ± 1.1 [a,b]	94.3 ± 0.9 [a]
Mv:QG₃	7.6 ± 0.2 [b]	5.5 ± 3.0 [a,b]	1.5 ± 1.3 [b]	93.0 ± 3.6 [a]
Mv:QG₄	9.5 ± 0.2 [a]	3.0 ± 0.2 [b]	1.9 ± 1.2 [a,b]	95.0 ± 1.3 [a]
Mv:CA	0.8 ± 0.6 [e]	35.6 ± 35.8 [a,b]	24.5 ± 32.3 [a,b]	39.9 ± 46 [b,c,d]
Mv:CA₂	1.3 ± 0.6 [e]	25.5 ± 26.1 [a,b]	53.4 ± 42.5 [a,b]	21.1 ± 16.5 [b,c,d]
Mv:CA₃	1.6 ± 0.4 [e]	21.8 ± 17.0 [a,b]	22.8 ± 17.8 [a,b]	55.4 ± 22.3 [a,b,c]
Mv:CA₄	2.1 ± 0.2 [d,e]	10.1 ± 3.5 [a,b]	27.2 ± 6.9 [a,b]	62.7 ± 7.3 [a,b]
Mv:E	1.4 ± 0.4 [e]	22.4 ± 1.7 [a,b]	68.6 ± 6.4 [a,b]	8.9 ± 4.7 [d]
Mv:E₂	1.1 ± 0.4 [e]	36.5 ± 32.6 [a,b]	58.7 ± 29.5 [a,b]	4.8 ± 3.1 [d]
Mv:E₃	1.0 ± 0.3 [e]	41.4 ± 35.9 [a,b]	35.2 ± 25.4 [a,b]	23.4 ± 12.3 [b,c,d]
Mv:E₄	1.1 ± 0.3 [e]	12.4 ± 9.2 [a,b]	28.2 ± 22.7 [a,b]	59.4 ± 22.7 [a,b,c]
Mv:C	1.3 ± 0.6 [e]	30.7 ± 26.1 [a,b]	65.1 ± 20.1 [a,b]	4.1 ± 6.1 [d]
Mv:C₂	0.7 ± 0.2 [e]	77.3 ± 23.2 [a]	19.9 ± 22.4 [a,b]	2.8 ± 1.4 [d]
Mv:C₃	1.3 ± 0.3 [e]	20.1 ± 10.7 [a,b]	70.1 ± 9.1 [a,b]	9.8 ± 2.8 [d]
Mv:C₄	1.5 ± 1.3 [e]	55.1 ± 45.5 [a,b]	38.1 ± 47.6 [a,b]	6.8 ± 2.8 [d]
Mv:GA	1.1 ± 0.4 [e]	51.6 ± 46.5 [a,b]	42.8 ± 41.7	5.6 ± 5.2 [d]
Mv:GA₂	1.7 ± 0.4 [e]	34.2 ± 5.7 [a,b]	61.8 ± 7.6	4.0 ± 1.8 [d]
Mv:GA₃	1.4 ± 0.1 [e]	17.6 ± 16.1 [a,b]	77.1 ± 18.5 [a]	5.3 ± 2.5 [d]
Mv:GA₄	1.1 ± 0.5 [e]	24.3 ± 25.8 [a,b]	56.6 ± 41.4	19.2 ± 17.3 [c,d]

Mv: malvidin 3-*O*-glucoside. QG: quercetin 3-*β*-glucopyranoside. CA: caffeic acid, E: epicatechin, C: catechin. GA: gallic acid. Different letters in each column indicate significant differences ($p < 0.05$, n = 3).

obtained for Mv:P at a molar ratio 1:1 (8.2 CIELAB units), a molar ratio of Mv:QG higher than 1:3 (7.3 CIELAB units) is needed. This highlights the important effect on color of the presence of P in the Mv solutions. It was also observed that, for Mv:P and Mv:QG solutions, the absolute color differences were mainly qualitative (%ΔH), whereas for the rest of phenolic compounds in solution with Mv, in general, the main relative contribution was quantitative (%ΔL and %ΔC). This indicates that the main change on the color of solutions due to the presence of P or QG leads to bluer solutions, which might be related to a stabilization of blue forms of anthocyanins, mainly in the case of P, where the change in the chroma attribute is also relevant.

3.3. Effect of the presence of P and/or PC on the color of mv solutions at wine pH (pH 3.6)

The influence of the presence of P on the color of Mv solutions at wine pH (pH 3.6) has been assessed both in absence and presence of the different PC assayed as copigments, in order to assess the potential role of these compounds for the different anthocyanin forms that are present at wine pH. Table 3 shows the results of this colorimetric study. The addition of P causes important changes in color, which are similar to those caused by QG. It seems that the interaction between P and Mv and that between QG and Mv lead to similar changes in color, since the solutions added with P or QG showed lower L^* values, higher C^*_{ab} values and more negative values for h_{ab} attribute, that its, darker and more vivid bluish hues, although there were significant differences between both of them regarding the achieved values. Taking into account that flavonols as QG or rutin are considered to be one of the best copigments in wine (Gómez-Míguez et al., 2006; Kunsági-Máté et al., 2006; Teixeira et al., 2013), these results point out the important role that P could have in wine color.

Going further, in general, ternary interactions showed significant differences in all the colorimetric parameters for all PC studied, with lower L^* values, higher C^*_{ab} values and more negative values for h_{ab} attribute, which, once again, can be related to darker and more vivid bluish hues. As an exception, in the case of QG, certain additive effect could be observed, since the decrease in hue values is higher in the ternary solution than in the binary ones (although differences are not

Table 3

Color parameters (L^*, a^*, b^*, C^*_{ab}, h_{ab}) obtained at pH 3.6 for Mv control and for binary (Mv:P and Mv:PC) and ternary (Mv:PC:P) solutions.

	L^*	a^*	b^*	C^*_{ab}	h_{ab}
Mv	95.3 ± 0.1 [a]	8.7 ± 0.04 [a]	−0.6 ± 0.02 [a]	8.7 ± 0.04 [a]	−3.7 ± 0.1 [a]
Mv:P	93.6 ± 0.2 [b]	10.0 ± 0.3 [b]	−2.7 ± 0.2 [b]	10.4 ± 0.2 [b]	−15.4 ± 1.4 [b]
Mv:QG	94.1 ± 0.1 [c]	10.4 ± 0.1 [c]	−2.20 ± 0.01 [c]	10.6 ± 0.1 [b]	−12.10 ± 0.01 [c]
Mv:QG:P	93.40 ± 0.01 [b]	10.7 ± 0.1 [c]	−3.3 ± 0.1 [d]	11.2 ± 0.1 [c]	−17.4 ± 0.8 [b]
Mv:CA	95.0 ± 0.1 [a]	9.0 ± 0.1 [a]	−0.8 ± 0.0 [a]	9.0 ± 0.1 [c]	−5.1 ± 0.1 [a]
Mv:CA:P	94.1 ± 0.1 [c]	9.7 ± 0.1 [b]	−2.0 ± 0.1 [c]	9.9 ± 0.1 [d]	−11.5 ± 0.4 [c]
Mv:E	95.2 ± 0.1 [a]	8.80 ± 0.03 [a]	−0.7 ± 0.1 [a]	8.80 ± 0.01 [a]	−4.6 ± 0.4 [a]
Mv:E:P	94.20 ± 0.01 [c]	9.2 ± 0.1 [c]	−1.9 ± 0.1 [c]	9.4 ± 0.1 [c]	−11.5 ± 0.7 [c]
Mv:C	95.3 ± 0.1 [a]	8.8 ± 0.01 [a]	−0.8 ± 0.01 [a]	8.80 ± 0.01 [a]	−4.9 ± 0.1 [a]
Mv:C:P	94.7 ± 0.3 [c]	9.4 ± 0.1 [c]	−1.8 ± 0.1 [c]	9.50 ± 0.01 [c]	−10.7 ± 0.9 [c]
Mv:GA	95.2 ± 0.2 [a]	8.8 ± 0.1 [a]	−0.7 ± 0.03 [a]	8.8 ± 0.1 [a]	−4.6 ± 0.2 [a]
Mv:GA:P	94.20 ± 0.01 [c]	9.5 ± 0.1 [c]	−1.9 ± 0.0 [c]	9.6 ± 0.1 [c]	−11.3 ± 0.2 [c]

Mv: malvidin 3-*O*-glucoside. P: polysaccharide. PC: phenolic compounds. QG: quercetin 3-*β*-glucopyranoside. CA: caffeic acid. E: epicatechin. C: catechin (C). GA: gallic acid. Different letters within each column indicate significant differences ($p < 0.05$, n = 3) between Mv, Mv:P and each Mv:PC and Mv:PC:P interactions.

significant when compared to Mv:P solution). Also, in this case, the chroma values of ternary solutions are the highest. This might point out, as it was observed a pH 1.1, that both QG and P could be stabilizing the blue forms of anthocyanins. Indeed, the changes in hue and chroma values observed at this pH could point to the stabilization of the blue quinoidal forms, *i.e.* the structures formed when the hydroxyl groups located in position 5, 7, or 4′ of the flavylium cation lost the proton, as it will be discussed later on MD studies. Therefore, at wine pH, the presence of P significantly affects the color of Mv solutions, both in absence and in presence of copigments. This could be explained by P-Mv interactions due to the presence of hydrophobic domains in the polysaccharides related to the less neutral sugar sidechains of the rhamnogalacturonan-I domains, which could participate in complexation with Mv (Fernandes, Oliveira, et al., 2020).

The calculated color difference values (Fig. 1) are in agreement with these results, since the highest ΔE^*_{ab} were reached for the ternary solution Mv:QG:P, followed by the binary solution Mv:P. Actually, those solutions showed color differences with regards to Mv solutions that could be detected by the human eye ($\Delta E^*_{ab} > 3$). Also, as explained before, the addition of both P and QG seems to exert an additive effect, since the color differences detected in that ternary solutions are significant higher than those detected in the corresponding binary solutions (Mv:P and Mv:QG). On the contrary, although the color differences detected in the ternary solutions containing CA, E, C or GA are higher that the corresponding binary solutions, they were lower than that calculated for Mv:P binary solution.

3.4. Color stability of mv solutions

In order to go further on the effect of the presence of P on the stability and color of flavylium form of Mv, the color and chemical composition of the solutions were studied after 5 weeks of storage. The color parameters (L^*, a^*, b^*, C^*_{ab}, h_{ab}) obtained for Mv and Mv:P solutions after the storage time for 5 weeks at pH 1.1 are shown in Table 4. It can be
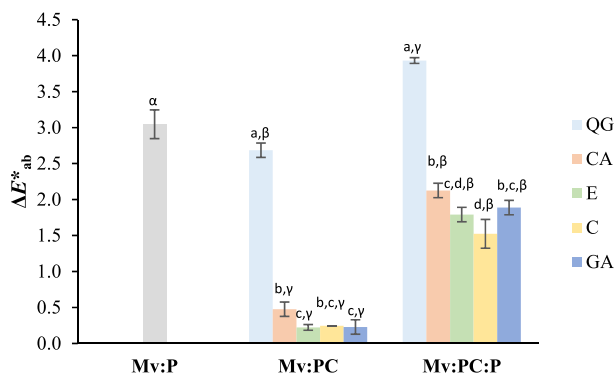
Fig. 1. Influence of polysaccharide (P) and/or phenolic compounds (PC) on ΔE^*_{ab} value (calculated respecting to malvidin 3-O-glucoside (Mv) solution) of binary and ternary solutions at pH 3.6. Different Latin letters (a,b,c,d) within each group (binary or ternary interactions) indicate significant differences ($p < 0.05$, n = 3). Different Greek letters (α,β,γ) indicate significant differences ($p < 0.05$, n = 3) between Mv:P and each Mv:PC and Mv:PC:P interactions.

Table 4
Color parameters (L^*, a^*, b^*, C^*_{ab}, h_{ab}) obtained for Mv control and for Mv:P solutions over the storage time (5 weeks, 25 °C) at pH 1.1.

	L^*	a^*	b^*	C^*_{ab}	h_{ab}
2h Mv	66.9 ± 0.3 e,f	61.5 ± 0.1 [a]	14.2 ± 0.3 [a]	63.1 ± 0.2 [a]	13.0 ± 0.3 [a]
2h Mv:P	66.2 ± 0.1 f	59.9 ± 0.3 [b,c,d]	6.2 ± 0.1 [c]	60.3 ± 0.3 [c]	5.9 ± 0.1 [c]
1W Mv	67.6 ± 0.1 d,e	60.9 ± 0.1 [a,b]	13.2 ± 0.1 [b]	62.3 ± 0.1 [a,b]	12.2 ± 0.1 [b]
1W Mv:P	67.3 ± 0.3 d,e	57.9 ± 0.3 [f]	3.4 ± 0.2 d	58.0 ± 0.3 d	3.4 ± 0.2 d
2W Mv	67.9 ± 0.1 c,d	60.3 ± 0.1 [b]	13.1 ± 0.2 [b]	61.7 ± 0.1 [b]	12.3 ± 0.1 [b]
2W Mv:P	68.1 ± 0.8 c,d	58.1 ± 0.8 [e,f]	3.0 ± 0.1 d	58.2 ± 0.8 d	3.0 ± 0.2 d
3W Mv	68.2 ± 0.2 c,d	60.5 ± 0.3 [a,b]	13.3 ± 0.2 [b]	62.0 ± 0.3 [a,b]	12.4 ± 0.2 [b]
3W Mv:P	69.8 ± 0.4 b	59.1 ± 0.3 [c,d,e]	2.1 ± 0.2 e	59.2 ± 0.3 [c,d]	2.0 ± 0.2 e
5W Mv	68.6 ± 0.1 c	60.2 ± 0.6 [b,c]	13.0 ± 0.4 [b]	61.5 ± 0.7 [b]	12.2 ± 0.3 [b]
5W Mv:P	71.3 ± 0.1 a	58.9 ± 0.2 [d,e,f]	1.8 ± 0.3 e	58.9 ± 0.2 d	1.7 ± 0.2 e

Mv: malvidin 3-O-glucoside. P: polysaccharide. Different letters within each column indicate significant differences ($p < 0.05$, n = 3).

observed that, in all cases, there was a decrease in the values of all color parameters over time. The changes in the color parametes, *i.e.*, the increase in L^* and the decrease in a^* values, along with a very little modification in b^* values in the case of Mv solutions, point to less intense colors with a less important red component, which could be interpreted as a decrease of Mv (flavylium) levels. This could be related to a partial precipitation of Mv due to its interaction with P, as it has been previously reported (Weilack et al., 2023). Moreover, the significant differences between Mv and Mv:P solutions are maintained from the beginning to the end of the experiment in almost all color parameters, but mainly in the case of chroma and hue values. Indeed, as for hue, the changes observed on color due to the presence of P increase over time. After 5 weeks, Mv:P solutions showed higher L^* values, lower C^*_{ab} values and much lower h_{ab} values than Mv solutions, *i.e.,* those solutions showed bluer but slightly less intense colors. This could point out that the stabilization of the blue quinoidal forms of Mv proposed before is stable over time, but that P cannot stabilize the flavylium form or, even, lead to a partial precipitation of this form, which is the main one at that pH. The chemical study of the solutions during storage also points in that

direction. Table 5 shows the evolution of Mv in the binary solutions. Results showed that the addition of P leads to a significant decrease in Mv content both compared to Mv control and to the other binary solutions involving the PC, which, in turn, seems to slightly stabilize the Mv. Thus, it seems that, despite the slight loss of pigments due to precipitation, P seems to stabilize blue forms of anthocyanins but not the red one.

3.5. MD simulations

In silico approaches were also employed to evaluate the copigmentation behavior between the different PC assayed against the pigment Mv. The presence of minor amounts of Mv quinoidal forms was considered in order to assess possible stabilization effect of quinoidal forms throughout the anthocyanins:copigments interaction. A cluster analysis of each MD simulation was performed to collect, in groups, all geometries of each copigmentation complex. The root-mean square deviation of the heavy atoms of the Mv, since they are the prevalent and common pigment of all systems, was used as clustering metric. The geometries with a frequency higher than 5% along the MD simulation were considered for further analysis. The number and nature of all complexes obtained from MD simulations were also studied (Table S2 in Supplementary material, in which copigmentation aggregates composed by quinoidal base forms are highlighted in parenthesis). When the 1:1 aggregates were analyzed, it can be observed that the number of Mv: copigment are much higher than those copigmentation complexes with quinoidal bases, which is expected due to the different amount of flavylium/quinoidal (4/1) molecules employed in each system. However, it seems that QG was the PC for which, proportionally, more quinoidal: PC aggregates were detected, which could explain the bluish color of the mixtures including Mv and QG. Moreover, no 1:1 aggregates with quinoidal forms were observed in the simulations with C and GA.

Further MD simulations were performed to assess the binding modes of 1:1 copigmentation complexes, involving the CA, E and QG in the interaction with the flavylium or quinoidal base forms of pigment. Fig. 2 illustrates the representative structures of the prevalent binding pose throughout each simulation, whilst Table 6 shows the enthalpic binding energies of each complex. It was verified that, in all studied complexes, the van der Waals (VdW) forces were the interactions that contribute the most to the binding and subsequent copigmentation effect (Table 6), which is in agreement with literature (Trouillas et al., 2016). According to the binding energies of the copigmentation complexes with CA, it was verified that it has rather similar affinities for the flavylium and quinoidal base (Mv-C5 and Mv-C7) forms of pigment ($\Delta\Delta H_{binding}$ of 0.34 ± 0.25 kcal/mol and -0.41 ± 0.24 kcal/mol in relation to the Mv, respectively). A higher affinity was observed for the Mv-C7:QG *vs.* Mv: QG complexes ($\Delta\Delta H_{binding}$ of -0.65 ± 0.23 kcal/mol) whereas the contrary was observed in the case of Mv:E *vs.* Mv-C5:E complexes ($\Delta\Delta H_{binding}$ of 0.68 ± 0.28 kcal/mol). This minor affinity arises from the VdW contacts (Table 6). The equivalent binding affinities are reasonable

Table 5
Mv content in the binary solutions during the storage time (5 weeks, 25 °C) at pH 1.1 expressed as percentage (%) calculated with respect to the Mv control solution.

	2h	5 weeks
% Mv:P	92.7 ± 9.5 [a]	81.8 ± 8.1 [b]
% Mv:QG	102.3 ± 1.7 [a]	111.5 ± 7.5 [a]
% Mv:CA	101.2 ± 2.9 [a]	114.9 ± 4.4 [a]
% Mv:E	101.9 ± 2.1 [a]	108.7 ± 5.5 [a]
% Mv:C	102.0 ± 2.2 [a]	108.4 ± 16.2 [a]
% MV:GA	101.2 ± 3.0 [a]	113.5 ± 4.2 [a]

Mv: malvidin 3-O-glucoside. P: polysaccharide. PC: phenolic compounds. QG: quercetin 3-β-glucopyranoside. CA: caffeic acid. E: epicatechin. C: catechin (C). GA: gallic acid. Each letter within each column indicates statistical differences (p < 0.05, n = 3).
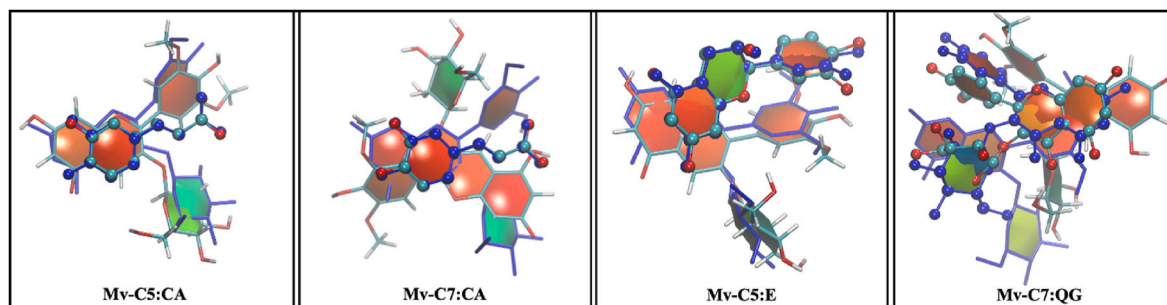
Fig. 2. Illustration of the representative geometry of the most frequent 1:1 copigmentation poses. Pigments and copigments are depicted in sticks and balls-and-sticks, respectively. As reference complexes, the Mv:copigment molecules are colored at dark blue, whilst the complexes with quinoidal bases are colored by atom type. The superimposition was carried out using the common copigment molecules. Aromatic and non-aromatic rings of all compounds are colored in orangish and green, respectively. Enthalpic binding energies of the interaction with quinoidal bases are in relation to the Mv values. (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)

Table 6
Binding energy values (kcal/mol) for some [pigment]$_1$:[copigment]$_1$ complexes.

pigment:copigment	Van der Waals	Electrostatic	Polar Solvation	Non-Polar Solvation	$\Delta H_{binding}$	$\Delta\Delta H_{binding}$
Mv:CA	-11.09 ± 0.23	-0.74 ± 0.13	2.33 ± 0.11	4.04 ± 0.16	-5.47 ± 0.19	0.00 ± 0.26
Mv-C5:CA	-10.52 ± 0.20	-0.84 ± 0.11	2.29 ± 0.10	3.95 ± 0.15	-5.13 ± 0.16	0.34 ± 0.25
Mv-C7:CA	-11.42 ± 0.15	-1.01 ± 0.10	2.33 ± 0.09	4.23 ± 0.10	-5.88 ± 0.15	-0.41 ± 0.24
Mv:E	-17.69 ± 0.19	-1.29 ± 0.12	3.20 ± 0.10	5.86 ± 0.12	-9.92 ± 0.18	0.00 ± 0.26
Mv-C5:E	-16.78 ± 0.22	-0.71 ± 0.09	2.55 ± 0.08	5.69 ± 0.12	-9.24 ± 0.21	0.68 ± 0.28
Mv:QG	-22.54 ± 0.19	-2.03 ± 0.11	4.66 ± 0.10	7.87 ± 0.10	-12.04 ± 0.18	0.00 ± 0.26
Mv-C7:QG	-23.48 ± 0.16	-1.91 ± 0.10	4.36 ± 0.07	8.34 ± 0.07	-12.69 ± 0.14	-0.65 ± 0.23

Mv: malvidin 3-*O*-glucoside. Mv-C5 and Mv-C7: malvidin 3-*O*-glucoside quinoidal bases. P: polysaccharide. PC: phenolic compounds. QG: quercetin 3-*β*-glucopyranoside. CA: caffeic acid. E: epicatechin. C: catechin (C). GA: gallic acid.

due to the structural resemblance of the Mv and respective quinoidal bases, which made similar number and nature of interactions with the solvent or copigment molecules (upon the water release). Indeed, this is highlighted by the comparison of the prevalent pigment:copigment geometries showed in Fig. 2. The Mv-C5:CA and Mv-C5:E are analogous and, in the case of Mv-C7:QG, the main intermolecular interactions are rather similar (π-π stacking and CH-π contacts). In addition, the magnitude of the effect from the different charge character is rather small because the positive charge of the Mv is delocalized along its aromatics rings as well as all copigments are non-charged polar molecules (alike the solvent). These results point out that the interaction between QG and the quinoidal forms of anthocyanins is slightly favored compared to the other PC assayed, which could be related to the structure of QG that favors the hydrophobic interactions with the anthocyanin.

4. Conclusions

In this work, we have studied the effect of soluble polysaccharides (P) obtained from red grape pomace on the color of malvidin 3-*O*-glucoside (Mv) solutions. The results show that the presence of P shifts the color of flavylium solutions towards a more intense color with bluer hues. This may indicate that P could favor the existence of quinoidal forms of the anthocyanin (blue forms) in the solution. This bluish effect is also observed for quercetin 3-*β*-glucopyranoside (QG), a phenolic compound recognized as a good copigment. However, in that case, higher levels of the phenolic compound are needed to obtain a difference of color of similar magnitude to that obtained for P. This highlight the potential relevant role that P could play in wine color. The obtained results also show that the presence of phenolic compounds slightly modifies the effect of P on the color of Mv solutions, although in the case of QG, a certain additive effect is observed.

As for the stabilization of Mv due to P, it seems that the presence of P leads to the stabilization of the blue quinoidal forms of Mv, but it cannot stabilize the flavylium form. This might be related to the interaction of P with the blue forms of the anthocyanin, in a similar way that observed for other copigments, such as QG. Indeed, MD simulation studies performed showed that the structure of QG favors the hydrophobic interactions with the quinoidal forms of the anthocyanin. Altogether, these results could indicate that the presence of hydrophobic regions of the polysaccharide (related to the the presence of type I rhamnogalacturonans) could be responsible for that behavior.

CRediT authorship contribution statement

Bárbara Torres-Rochera: Conceptualization, Methodology, Investigation, Data curation, Formal analysis, Visualization, Writing – original draft. **Natércia F. Brás:** Conceptualization, Methodology, Investigation, Formal analysis, Visualization, Writing – review & editing. **Ignacio García-Estévez:** Conceptualization, Methodology, Formal analysis, Validation, Supervision, Writing – review & editing, Project administration, Funding acquisition. **M. Teresa Escribano-Bailón:** Conceptualization, Methodology, Validation, Supervision, Writing – review & editing, Project administration, Funding acquisition.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

Data will be made available on request.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.lwt.2023.115420.

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