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Study of the Interaction of Anthocyanins with Phenolic Aldehydes in a Model Wine Solution

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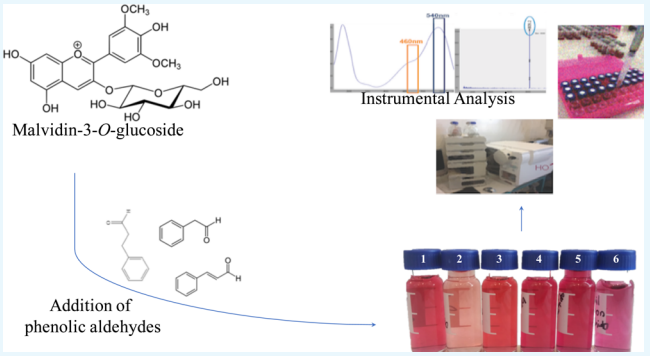
ABSTRACT: Aldehydes may be present in wines as a result of metabolic processes during wine fermentation or through oxidation and extraction from wood during wine aging in oak barrels. Apart from acetaldehyde, the most abundant aldehyde in wine, other aldehydes such as furfural and more recently vanillin have shown to contribute to the formation of more stable pigments. The copigmentation effect of phenolic molecules, including flavanols and anthocyanins themselves, has been previously evaluated in wine and model solutions, and even the effect of aldehydes related to wine aging has been documented at different pHs and molar ratios. The copigmentation phenomenon is observed by hyperchromic effects and bathochromic shifts of λ_{max}, and, in the same time, the presence of larger molecular weight pigments, potentially less susceptible to degradation, was followed up. This experimental work intended to evaluate the potential of five different aldehydes, all of which are safe for human consumption and are used in the food industry, to the formation of pyranoanthocyanin-like and polymeric pigments in the model solution.



1. INTRODUCTION

There are different metabolic pathways in which ethanal, also called acetaldehyde, can be produced during winemaking. The first pathway is from the pyruvate coming from glucose as a part of the glycolysis pathway[1] or coming from malic acid as a part of the maloalcoholic fermentation.[2] Another pathway is from ethanol through the reversible acetaldehyde−ethanol step with activity from the cytosolic and mitochondrial alcohol deshydrogenases.[3] Finally, ethanal can also be directly produced by the chemical peroxidation of ethanol that takes place during wine oxidation during the process of barrel aging or when microoxygention is applied.[4]

Despite the way in which acetaldehyde is produced, this metabolite and pyruvate may produce vitisins from their condensation with anthocyanins, process that varies in function of the fermentative yeast strains used for winemaking.[5] Vitisins A and B are one type of stable pigments formed in red wines by cycloaddition with pyruvate and acetaldehyde, respectively, during fermentation and during wine aging. Acetaldehyde can also participate in the formation of other stable pigments such as vinyl-pyranoanthocyanins (portisins) and flavanol-anthocyanins ethyl-linked adducts.[6] Important to mention is that acetaldehyde can also produce adducts from the reaction with sodium bisulfite added to wine, reducing the ability of acetaldehyde to produce stable pigments.

Besides the fact that acetaldehyde is a metabolite present in wines during fermentation and wine oxidation, there may be other aldehydes in wine coming from amino acids when the amount of molecular oxygen is excessive;[7] the aldehydes formed through this chemical process studied by these authors are methional from methionine and phenylacetaldehyde (PHAC) from phenylalanine. The presence of aldehydes from oxidation-related and oak aging-related processes has been previously reported;[8] regarding the first process, the aldehydes found in wines are methional, benzaldehyde, PHAC, hexenal, heptenal, octenal, and nonenal; with regards to the oak aging process, the aldehydes found are furfural, 5-methylfurfural, 5-hydroxymethylfurfural, and syringaldehyde (SYRN).[8] The concentration of the aldehydes in wines depends on different factors such as the age of the wine and the type of aging. PHAC for example has been found at concentrations of 11 μg/L in young red wines and up to 78.7 μg/L in Port wines and 91.2 μg/L in red wines with long aging.[9] Vanillin, in another example, has been reported in concentrations at sub-threshold without having an impact in the aroma profile of wines but, enough to react with other compounds to produce color changes and reducing their impact in wine.[10]

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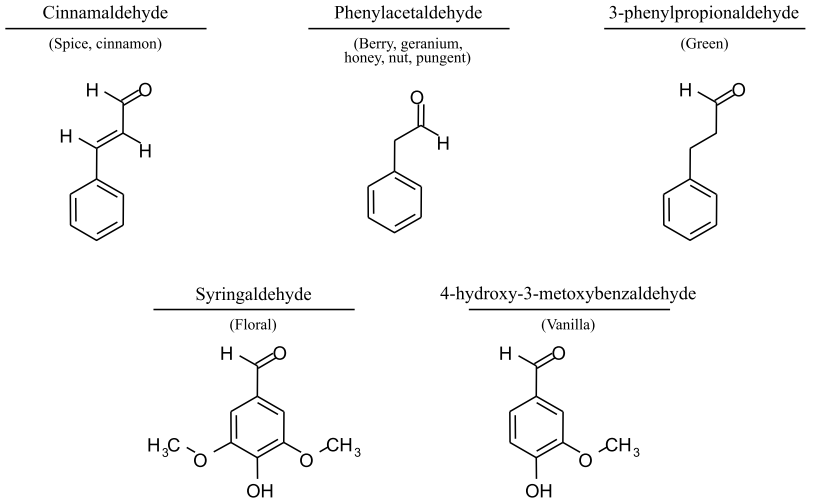

Figure 1. Phenolic aldehydes used in the experimental design. Names and aroma profiles are listed above each 2D structure. Aldehydes abbreviations used along the experiment: CINN—cinnamaldehyde; PHAC—phenylacetaldehyde; PHPR—3-phenylpropionaldehyde; SYRN—syringaldehyde; HMBZ—4-hydroxy-3-methoxybenzaldehyde.

It has been reported that furfural can replace acetaldehyde in the polymerization process in the condensation of (+)-catechin and anthocyanins observed in wines aged in oak barrels,[11] and it has also been shown that methylfurfural reacts with malvidin-3-*O*-glucoside to form furanic aldehyde adducts.[12] The aldehydes, mainly acetaldehyde, are believed to form a carbocation as a result of the protonation of the carbonyl group under acidic conditions.[13] This reaction is followed by the nucleophilic addition to flavanols or to anthocyanins units.

The presence of phenolic aldehydes could also change the color of anthocyanins by copigmentation phenomena in a similar way that other possible copigments such as hydroxycinnamic acids[14] or flavonoids including flavan-3-ols and anthocyanins through noncovalent interactions such as the intermolecular interaction and the self-association.[15]

Beyond the fact that some of the before-mentioned aldehydes have a negative contribution to aroma profile of red wines, an experimental design was conducted with different aldehydes, all of which are safe for human consumption and are used in the food industry as flavoring agents, in order to evaluate their contribution to color modification of red wine model solutions with special interest in the production of pyranoanthocyanin-like pigments and polymeric pigments. The aldehydes selected for the evaluation were cinnamaldehyde (CINN), PHAC, phenylpropionaldehyde, SYRN, and 4-hydroxy-3-methoxybenzaldehyde (HMBZ) in which the structures are shown in Figure 1. SYRN and HMBZ may also be present in wines because these aldehydes could be extracted from the oak barrels during wine aging[16] together with coniferaldehyde and sinapaldehyde. All of these five aldehydes have a phenolic moiety that, associated with anthocyanins, are expected to affect the absorption properties of the pigments and thus the color that these molecules may have.

2. RESULTS AND DISCUSSION

2.1. Color Evolution. The change in color was monitored with instrumental color measurements; the spectrophotometric technique recorded the variations in absorbance at different wavelengths (420 nm—yellow, 520 nm—red, and 620 nm—blue). During the month that the experiment spanned, the percentage at these three maximum absorbance wavelengths remained steady for the control (Figure 2A). On the other hand, variations in the color have been observed when using different aldehydes. The use of 3-phenylpropionaldehyde (PHPR) decreased the percentage of red, whereas on the other hand, blue and yellow increased over time. CINN and PHAC have the largest amount of yellow among all aldehydes and the lowest contribution from red; regarding blue fraction, CINN decreases over time, whereas PHAC increases it. SYRN and HMBZ had the greater values for red percentage after the control with a slight decrease toward the end of the evaluation; SYRN has similar values for yellow percentage than the control, whereas HMBZ has slightly higher values; regarding the blue content, both aldehydes have larger percentage of blue with a trend to increase it with SYRN and to decrease with HMBZ toward the end of the evaluation. These changes in color measurements with different absorption wavelengths may be attributed to the interaction of the phenolic aldehyde molecules and the anthocyanins in the model solutions. The shifts observed are different among the samples.

The measurement of these three fixed absorption wavelengths also allowed us to follow up the changes in color intensity (CI) and hue (*N*) shown in Figure 2B,C, respectively. As it can be seen, the control has a steady CI and *N* during the experiment, whereas the addition of phenolic aldehydes produces changes in these two parameters. The aldehydes PHPR, PHAC, and SYRN increase CI over time; on the other hand, CINN and HMBZ had a decreasing trend. Nonetheless, CI values are higher than the control after 4 weeks for the trials with aldehydes as a result of the increased absorptions in yellow and blue fractions. The pronounced gain in yellow percentage by addition of CINN and PHAC (Figure 2A) has resulted in an increase of *N* three times higher than the control with a trend to have larger *N* values over time; SYRN and HMBZ had N values similar to the control with a slight increase over time (Figure 2C). Having larger *N* values is generally related to wine ageing processes where red wine pigments may undergo copigmentation phenomena with phenolic compounds found in wine, yielding molecules with lower maximum absorbance wavelength (ca. 505 nm), toward brick-red hue.[20] Shifts in the maximum absorbance wavelength of a pigment, toward higher wavelengths, are known as the bathochromic effect also named the bluing effect in wines.[21]

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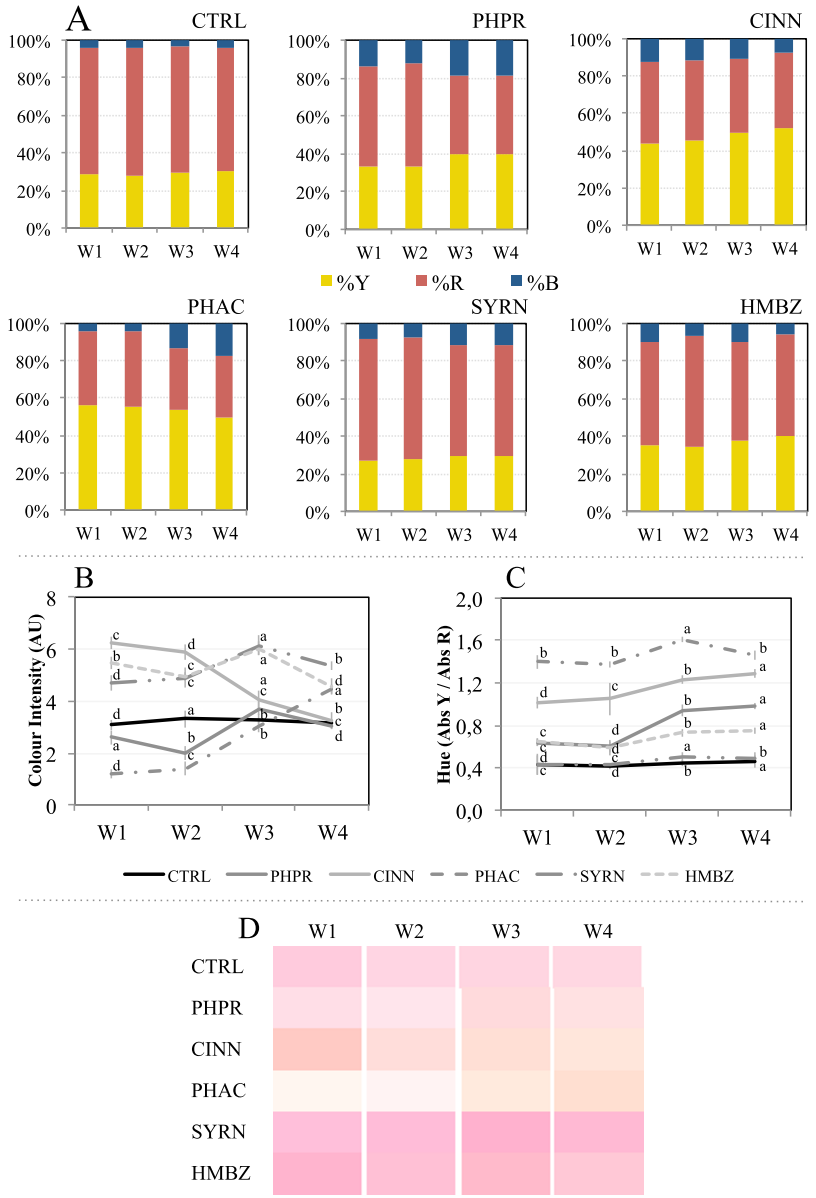

Figure 2. Color evolution in the trials in terms of: (A) percentage of yellow, red, and blue measured with UV−vis; (B) CI; (C) hue (*N*); (D) visual representation of the CIE *L*a*b** coordinates used to show the changes in color during the span of the evaluation. Mean values (n = 3). Different letters denote a significant difference for each solution with 95% confidence level (LSD test).

Table 1. Mean Values of ΔA_{520} and CIE *L*a*b Differences ΔE^*_{ab}, ΔL^*, ΔC^*_{ab}, and Δh_{ab} after 4 Weeks[a,b]**

	PHPR	CINN	PHAC	SYRN	HMBZ
ΔA_{520}	−0.8 ± 0.002^{e}	−0.8 ± 0.001^{d}	−0.6 ± 0.001^{c}	+1.1 ± 0.002^{a}	+0.3 ± 0.001^{b}
ΔE^*_{ab}	12.0 ± 0.01^{d}	13.9 ± 0.01^{b}	16.7 ± 0.02^{a}	13.0 ± 0.01^{c}	5.7 ± 0.18^{e}
ΔL^*	+0.6 ± 0.01^{b}	+3.0 ± 0.03^{a}	−0.5 ± 0.01^{c}	−9.1 ± 0.02^{e}	−4.9 ± 0.03^{d}
ΔC^*_{ab}	−8.4 ± 0.02^{e}	−7.0 ± 0.02^{c}	−8.4 ± 0.01^{d}	+8.0 ± 0.01^{a}	+2.8 ± 0.01^{b}
Δh_{ab}	2.3 ± 0.08^{d}	11.7 ± 0.01^{b}	14.4 ± 0.02^{a}	5.0 ± 0.03^{c}	1.4 ± 0.06^{d}

[a]Average and SD (n = 3). Different letters denote a significant difference with 95% confidence level (LSD test). [b]PHPR—3-phenylpropionaldehyde; CINN—cinnamaldehyde; PHAC—phenylacetaldehyde; SYRN—syringaldehyde; HMBZ—4-hydroxy-3-methoxybenzaldehyde.

The values from the CIE *L*a*b** coordinates allowed us to build up a color palette chart (Figure 2D) that shows the evolution of color over the span of the experiment. The lightness (L^*) is larger for PHAC followed by PHPR after one week, and it has similar values for PHPR, CINN, and PHAC at the end of the evaluation; higher lightness values may be related to lower color stability because lightness decrease in wine samples over time indicates higher color stabilization;[22] these authors correlate the decrease in L^* with an addition of oenological tannins, increasing the amount of pigments in the wine. The reduction of stability observed in the experimental trials, and therefore an increase in L^* values, might be explained by the fact

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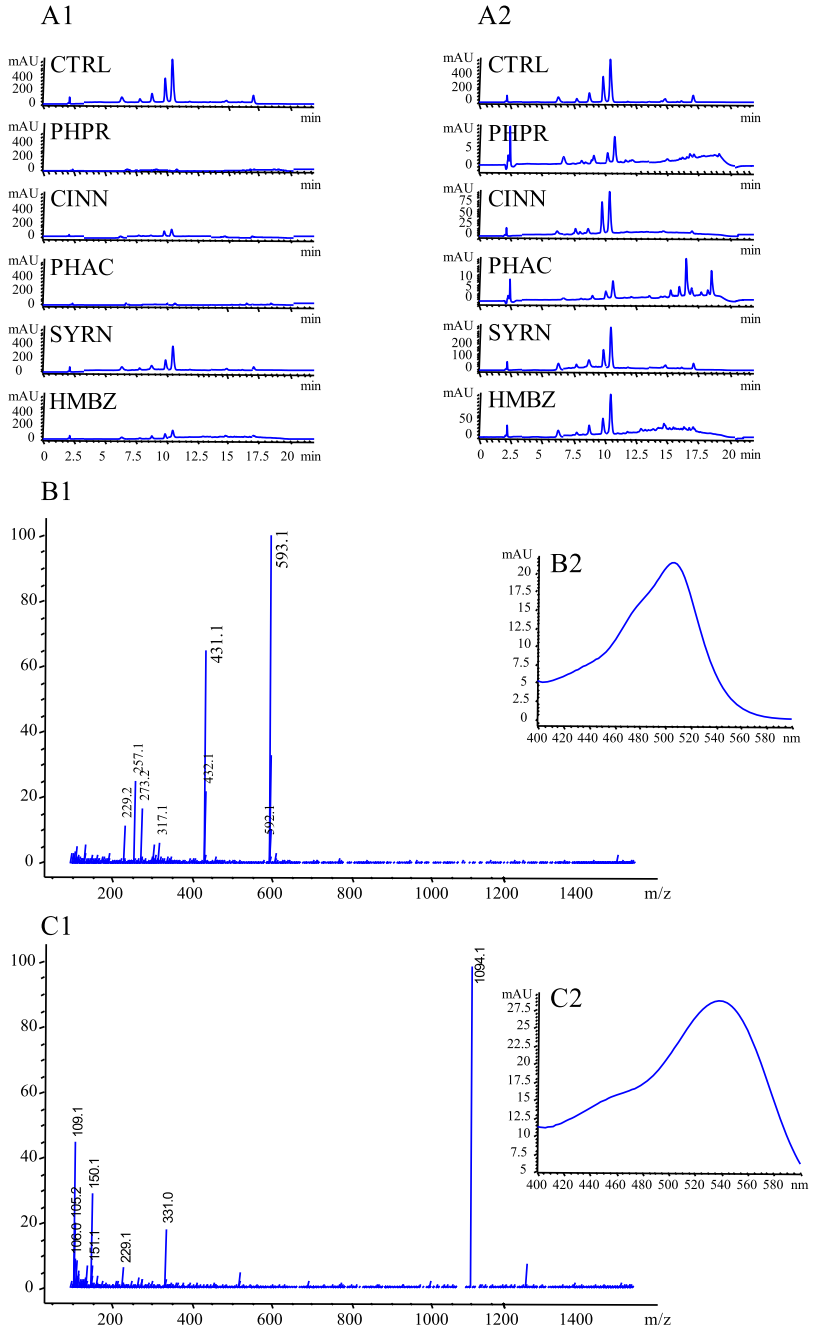

Figure 3. DAD signal at 525 nm for HPLC chromatograms at same scale (A1) and at full scale (A2); the DAD signals correspond to the measurement after 4 weeks. The mass spectrum relative abundance (B1) and DAD signal at 525 nm (B2) for peak identified as potential malvidin-3-*O*-glucoside-phenylacetaldehyde with t_R of 16.622 min; mass spectrum relative abundance (C1) and DAD signal at 525 nm (C2) for peak identified as potential anthocyanin dimer condensed with HMBZ with t_R of 13.326 min.

that aldehydes in excess promote the precipitation of pigments in wine model solutions.

The CIE $L^*a^*b^*$ values for the control trial were considered as initial reference conditions for determining differences among samples after 4 weeks. Absolute color difference (ΔE^*_{ab}), variations in lightness (ΔL^*), chroma (ΔC^*_{ab}), and hue (Δh^*_{ab}) are shown in Table 1. Positive values for ΔA_{520} are the effect of having fortified CI as a consequence of the addition of copigments and an increase in absorbance at 520 nm known as the hyperchromic effect or fortified CI;[23] this hyperchromic effect may be the result of shifting the anthocyanin molecule equilibrium toward the flavylium-colored form.[24] The smaller the ΔE^*_{ab}, the less the color variation observed between samples; in this way, HMBZ had the least color variation from the control, whereas PHAC had the greatest. The change in color in all samples having aldehydes was perceived by the human eye, and this can be explained analytically because the value for ΔE^*_{ab} was greater than 3 CIE $L^*a^*b^*$ units, which was experimentally determined with red wines and a panel of observers with normal color vision.[25] In terms of lightness (ΔL^*), PHPR and CINN had positive variation because of an increase in L^*, whereas the others had negative values for lower L^* values. The greater variation in hue (Δh^*_{ab}) was observed in CINN and PHAC. Another study observed a bathochromic effect at low pH values

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Table 2. Anthocyanins in Trials after Four Weeks with Retention Times (t_R) and Spectral Features in LC−DAD−ESI/MS Analysis[a]

anthocyanin	t_R	λ_{max} (nm)	$[M]^+$ (m/z)	MS (m/z)
CTRL				
delphinidin-3-*O*-glucoside	7.43	524	465	303
cyanidin-3-*O*-glucoside	8.67	518	449	287
petunidin-3-*O*-glucoside	9.50	526	479	317
peonidin-3-*O*-glucoside	10.48	518	463	301
malvidin-3-*O*-glucoside	10.97	528	493	331
delphinidin-3-*O*-(6-acetyl)-glucoside	12.45	530	507	303
cyanidin-3-*O*-(6-acetyl)-glucoside	13.57	528	491	284
petunidin-3-*O*-(6-acetyl)-glucoside	14.07	530	521	317
peonidin-3-*O*-(6-acetyl)-glucoside	15.09	522	505	301
malvidin-3-*O*-(6-acetyl)-glucoside	15.29	530	535	331
delphinidin-3-*O*-(6-*p*-coumaroyl)-glucoside	15.51	532	611	303
cyanidin-3-*O*-(6-*p*-coumaroyl)-glucoside	16.46	524	595	287
petunidin-3-*O*-(6-caffeoyl)-glucoside	16.76	534	625	317
malvidin-3-*O*-(6-*p*-coumaroyl)-glucoside	17.67	532	639	331
PHAC				
unidentified	13.62	506	613	453
unidentified	14.85	515	701	347
unidentified	15.99	502	563	401
peak 1[b]	16.62	508	593	431
PHPR				
unidentified	18.48	537	915	603
SYRN				
unidentified	16.03	538	655	
HMBZ				
peak 2[c]	13.33	542	1094	331
unidentified	14.40	540	1189	657/521
unidentified	17.43	542	899	563/317

[a]Notes: CTRL groups monomeric anthocyanins and acyl derivatives found in all trials. [b]Concentration accounts for 0.28 mg/L. [c]Concentration accounts for 0.63 mg/L.

(1−3) for vanillic, syringic, and conyferil aldehydes and also a hyperchromic effect at higher pH values (from 3 to 5); the molar ratio of pigment/copigment also showed to have an influence on the hyperchromic effect being the molar ratio 1:100 the one having greater effect.[26]

From the overall results of the color evolution assessment, those obtained with SYRN and HMBZ are interesting because N is steadier with respect to the control and have, at the same hand, larger CI, positive chroma values and hyperchromic effect under this concrete pH value and aldehyde concentration. These two aldehydes, unlike the rest, have multiple functional groups (see Figure 1).

2.2. Anthocyanins Analysis by High-Performance Liquid Chromatography (HPLC). The number of anthocyanins, including the nonacylated, the acetyl-, *p*-coumaroyl-, and caffeoyl-derivatives, decreased in all cases when aldehydes were present in the model media after 4 weeks (Figure 3A1) in the same time that some precipitates began to appear in the bottom of the vials; SYRN experienced less pigment reduction, whereas on the contrary, PHPR yielded the lowest concentration of pigments after the evaluation and it had coloring matter deposited in the vials. A full scale chromatogram from the samples shown in Figure 3A1 is given in Figure 3A2 to zoom in on the images and stress out the peaks present in the trials even though their concentration is low. The DAD signal and mass spectra (MS) from the HPLC−DAD−ESI/MS analysis allowed the identification of the peaks observed on Figure 3A2 at full scale. The following anthocyanins were identified in all six samples: delphinidin-3-*O*-glucoside, cyanidin-3-*O*-glucoside, petunidin-3-*O*-glucoside, peonidin-3-*O*-glucoside, malvidin-3-*O*-glucoside, delphinidin-3-*O*-(6-*p*-coumaroyl)-glucoside, cyanidin-3-*O*-(6-*p*-coumaroyl)-glucoside, petunidin-3-*O*-(6-caffeoyl)-glucoside, and malvidin-3-*O*-(6-*p*-coumaroyl)-glucoside. Besides these peaks, other peaks with unidentified nature are also reported in samples PHAC, PHPR, SYRN, and HMBZ (Table 2). In the case of PHAC, the unidentified peaks had maximum absorbance wavelengths between 502 and 515 nm, lower than those of monomeric anthocyanins but similar to the range of absorption seen for the vitisins.[27,28] One of these peaks, labeled peak 1 in Table 2 had a molecular ion $[M]^+$ at (m/z) 593 and fragment ion with at (m/z) 431 (Figure 3B1) which may potentially correspond to the intramolecular interaction of a malvidin-3-*O*-glucoside moiety and the PHAC molecule; this peak has a λ_{max} of 508 nm (Figure 3B2) and t_R of 16.62 min.

Regarding trials PHPR, SYRN, and HMBZ, the unidentified peaks found had higher λ_{max}, toward bluish tonality. The weight of the ions $[M]^+$ of these unidentified peaks resembles that of polymeric pigments as well as the shift of wavelengths as a result of a bathochromic effect.[20] A second peak, peak 2 (Table 2), with $[M]^+$ at (m/z) 1094 and λ_{max} at 542 nm found in the trial HMBZ is similar to that reported for the formation of an oligomeric unit from the reaction of malvidin-3-*O*-glucoside and (+)-catechin in the presence of vainillic aldehyde;[10] such pigment formed had $[M]^+$ at (m/z) 917 and λ_{max} 549 (purple color); the vainillic aldehyde was found to form an aryl linkage. In the current case study, the reaction may have taken place

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between two anthocyanin units because the $[M]^+$ got for peak 2 was at (m/z) 1094 and would potentially correspond to two anthocyanins moieties linked by one moiety of HMBZ; a second fragment ion at (m/z) 331 suggests that one of these moieties correspond to a molecule of malvidin-3-*O*-glucoside. The mass spectrum relative abundance and the DAD signal at 525 nm for this peak are shown in Figure 3C1,C2, respectively. Nonetheless, the molecular ions give information regarding the nature of the components; the techniques used do not allow the identification of molecular structures produced.

3. CONCLUSIONS

The color evolution after the addition of phenolic aldehydes in model wine solution has happened through different mechanisms, and it is visible to human eye. The addition of aldehydes contributed to diminish the concentration of pigments in solution to a different extent and to precipitate coloring matter; what is not, yet clear is the nature of the interaction of the aldehydes and the anthocyanins to produce such effect. On the other hand, some aldehydes have apparently contributed to the formation of pyranoanthocyanin-type pigments with lower λ_{max} and larger hue values, whereas others have contributed to having a bathochromic effect after forming pigments with larger λ_{max} and potential oligomeric/polymeric conformation. In this way, besides polymerization, the copigmentation phenomena may have happened intermolecular wise. The further study of the effect of phenolic aldehydes on the yeast metabolism and, therefore, on the color stabilization during wine fermentation would provide further information on this type of stable pigment occurrence.

4. MATERIALS AND METHODS

4.1. Model Wine Solution. The model media were prepared by using a hydro-alcoholic solution (14% v/v ethanol) with pH 3.5 adjusted by the addition of tartaric acid using a magnetic stirrer. Natural grape color powder—enocyanin—(EV-12 Red Shade, E-163) from the company Secna (Valencia, Spain) was added to the model solution to have a concentration of 100 mg/L anthocyanins.

Five different aldehydes were used in the experimental design, CINN 98+% A14689 (Alfa Aesar, Germany), PHAC 95% A14263 (Alfa Aesar, Germany), PHPR P0217 (TCI, Germany), SYRN S29672 (Merck, Germany), and 4-hydroxy-3-metoxybenzaldehyde 99% (Aldrich, Germany). The model solution was then divided into six different treatments in the function of the aldehydes used: (1) control (CTRL), (2) CINN, (3) PHAC, (4) PHPR, (5) SYRN, and (6) 4-hydroxy-3-metoxybenzaldehyde (HMBZ). Each model solution is having aldehydes in a molar ratio of 1:10 with respect to the pigment molar concentration; the molar concentration for enocyanin considers the molecular weight of the malvidin-3-*O*-glucoside as a reference. The solutions were kept in a 100 mL brown glass flask at constant 20 °C in the dark with a headspace of 10 mL. Each week, for 1 month, 2 mL of each sample was taken from each flask and filtered with 0.45 μm methylcellulose membrane to perform anthocyanins evolution with HPLC−DAD/MS−ESI and color determinations with UV−vis spectrophotometry. No measurements for intake of oxygen were determined during the experimentation.

4.2. Color Determination. The color of the trials has been determined with the use of a UV−visible spectrophotometer 8453 from Agilent Technologies (Palo Alto, CA, USA) with a photodiode array detector and the use of a 1 mm path length cuvette. The absorption at three different wavelengths (420, 520, and 620 nm) was used to compare CI and hue (N) in all trials after sample collection. The results are also expressed as the percentage of yellow (Y %), red (R %), and blue (B %) fractions present in each sample, obtained from the different absorption signals.[17] The percentage obtained is the relative ratio of each absorbance to the sum of all them.

The color differences were monitored with the ΔE_{ab} as the Euclidian distance between two points in the CIE Laboratory coordinates. These coordinates were also used to evaluate quantitative differences in L^* (lightness), C^*_{ab} (chroma) and qualitative differences of h_{ab} (hue).[18] All CIE $L^*a^*b^*$ values were also determined with the same spectrophotometer from Agilent Technologies. The formulae used for the calculations are

$$\Delta E_{ab} = [(\Delta L^*)^2 + (\Delta a^*)^2 + (\Delta b^*)^2]^{1/2}$$

$$\Delta h_{ab} = [(\Delta E_{ab})^2 - (\Delta L)^2 + (\Delta C_{ab})^2]^{1/2}$$

$$\% \Delta L = [(\Delta L)^2/(\Delta E_{ab})^2] \times 100$$

$$\% \Delta C = [(\Delta C)^2/(\Delta E_{ab})^2] \times 100$$

$$\% \Delta h = [(\Delta h_{ab})^2/(\Delta E_{ab})^2] \times 100$$

4.3. HPLC Analysis. HPLC with a diode array detector and electrospray ionization coupled to MS (DAD−ESI/MS) has been used to identify and characterize known anthocyanins in control trial as well as the follow up the evolution of anthocyanin profile in all other trials; an Agilent Technologies 1100 (Palo Alto, CA, USA) chromatograph with a column RP Kinetex C18 (100 × 4.6 mm; 2.6 μm) (Phenomenex, Torrance, CA, USA) was used for this purpose. Two solvents were used: solvent A (water/formic acid 95:5 v/v) and solvent B (methanol/formic acid 95:5 v/v) with the following gradient of solvent B (0.8 mL/min): from 20 to 50% from time 0 to 27 min; 50% from time 27 to 28 min; and finally from 50 to 20% from time 28 to 29 min until reaching a steady state. The malvidin-3-*O*-glucoside has been used as an external standard at a wavelength of 525 nm for the quantification of all pigments,[19] whereas the identification was carried out with MS positive scanning from 100 to 1500 m/z from time 0 to 23 min. The conditions for the electrospray ionization (ESI) used are as follow: drying gas (N_2) flow rate 10 mL/min; temperature 350 °C; nebulizer pressure 380 Pa (55 psi); and capillary voltage 4000 V. Detection limit was set to 0.1 mg/L.[6]

4.4. Statistical Analysis. Means and standard deviations were calculated, and differences examined using ANOVA and the least significant difference (LSD) test. All calculations were made using PC Statgraphics v.XI software (Graphics Software Systems, Rockville, MD, USA). Significance was set at $P < 0.05$.

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C.E. performed the analysis, collected test data, interpreted the results, and drafted the manuscript. A.M. designed the study and

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interpreted the results. F.Z. designed the study and interpreted the results. I.L. collected data and interpreted results. J.M.d.F. collected data and helped with interpretation of results. J.A.S.-L. helped with the interpretation of the results.

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Notes

The authors declare no competing financial interest.

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■ ABBREVIATIONS

CTRL, control; CINN, cinnamaldehyde; PHAC, phenylacetaldehyde; PHPR, 3-phenylpropionaldehyde; SYRN, syringaldehyde; HMBZ, 4-hydroxy-3-methoxybenzaldehyde

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