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Article

Relevance of the Concentrations and Sizes of Oligomeric Red Wine Pigments to the Color Intensity of Commercial Red Wines

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ABSTRACT: Color is a major sensorial characteristic of red wines. Numerous monomeric and some small oligomeric pigments have been characterized from red wines but the contribution of larger oligomeric pigments to the color intensity has not been established by direct measurements. We measured the color intensity of 317 commercial red wines and semiquantified the malvidin glycosides and eight different adduct groups derived from the malvidin glycosides by ultra-performance liquid chromatography−tandem mass spectrometry. Two of these groups were oligomeric pigments consisting of proanthocyanidins and malvidin glycosides with either direct or methylmethine linkages. The carboxypyranomalvidins and the oligomeric pigments were found to be major contributors to the color intensity. Besides the concentrations, the sizes of the oligomeric pigments had a positive and significant connection to the color intensity. The 1-year-old wines were studied separately and, even in the youngest of wines, the adducts of the malvidin glycosides were the major contributors to the color intensity.

KEYWORDS: *anthocyanins, chromatographic fingerprints, polymeric pigments, proanthocyanidins, tannins*

INTRODUCTION

Red wines contain oligomeric or even polymeric pigments, which are thought to be important for the wine color.[1−3] These oligomers are formed via reactions between proanthocyanidins (PA), i.e., the main tannins in red wines, and anthocyanins, which are naturally occurring pigments in the grape skin. The most predominant anthocyanins in red wines are structurally derived from malvidin glycosides (Mv), with the main individual compounds being malvidin glucoside, malvidin acetylglucoside, and malvidin coumaroylglucosides (Figure 1).[4,5] In the various structural subgroups of the proanthocyanidin−malvidin glycoside adducts, the Mv unit can be the terminal unit in the oligomer (PA−Mv^+) or the PA and Mv units can be linked via a methylmethine bridge (PA−methylmethine−Mv^+; Figure 1). Red wines contain numerous individual monomeric anthocyanin adducts as well (e.g., those in Figure 1), which are formed via reactions between the anthocyanins and small wine components or yeast metabolites.[4]

Anthocyanins are in constant structural equilibrium in aqueous solutions and the mole fractions of the various forms in solution are greatly dependent on the pH.[6,7] Some of the anthocyanin structures are colored, while others are not and, therefore, an understanding of their thermodynamic properties is needed to determine their relevance to the wine color. Indeed, the thermodynamic and chromatic properties of many anthocyanins and monomeric anthocyanin adducts are nowadays well understood and the same goes for the dimeric adducts belonging to the PA−Mv^+ and PA−methylmethine−Mv^+ adduct groups.[4,8−10] However, the PA−Mv^+ and PA−methylmethine−Mv^+ adducts are virtually by definition thought to exist in red wines as mixtures of oligomers or even polymers. It has been stated that the dimers could serve as markers for many related larger compounds[3] but the problem is that the properties of the dimers, and their contents in red wines, may not necessarily represent the whole compound groups and the higher oligomers. For example, it was demonstrated with dimers and a trimer consisting of a pyranomalvidin glucoside and catechin units that the trimer had a bathochromic shift of 8 nm in the wavelength of the maximum absorbance compared to the dimers, and the molar absorptivity of the trimer increased significantly more than the absorptivity of the dimers upon a pH change from 1.0 to 3.6.[11] Typically, the molar absorptivities of anthocyanin-derived pigments increase only slightly or they drop when pH is changed from very acidic to less acidic conditions.[11] Intramolecular copigmentation by the catechin moieties was suggested to cause the observed differences in the properties of the dimers and the trimer, which gives reason to believe that the degree of oligomerization of other oligomeric pigments could have an impact on the wine color as well. Additionally, when it comes to the contribution of pigments to the color intensity, it would be beneficial to study the red wine pigments in their natural environment, i.e., in an actual wine matrix, and to measure the concentrations of many different types of pigments at once. Then, it is possible to find out how changes in the concentrations of the pigments affect the intensity of the observed color and how the contributions of various pigment groups compare to one another.

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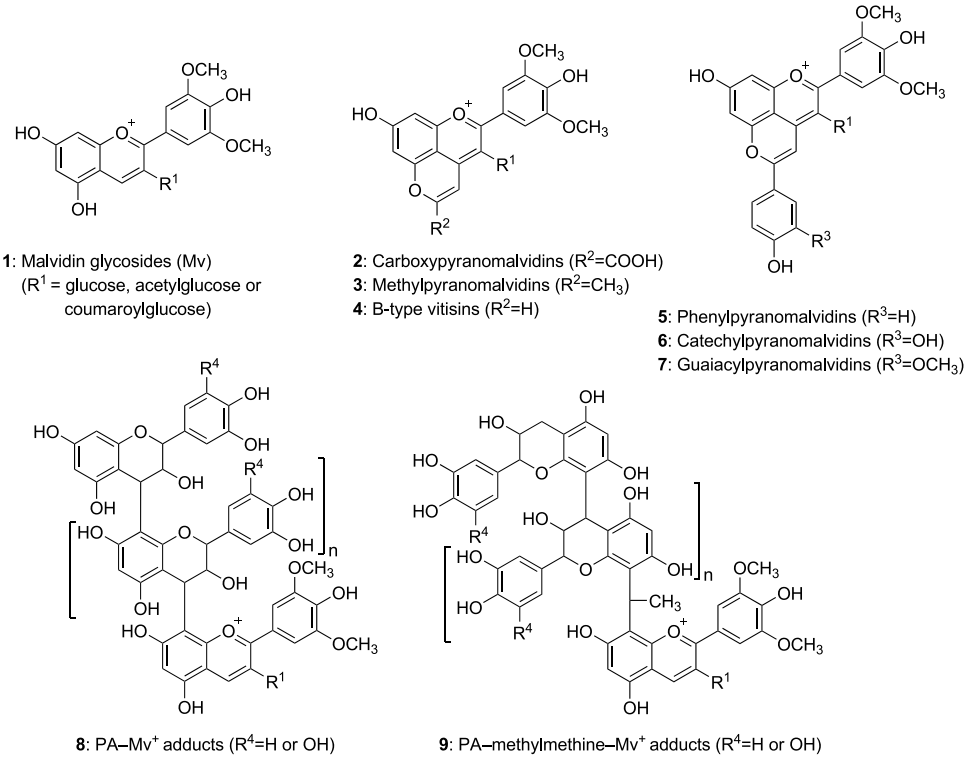

Figure 1. Malvidin-derived pigment groups, which were semiquantified from the red wines. The R^1 in all structures refer to the same substituents as in **1**.

We recently published a group-specific ultra-performance liquid chromatography−tandem mass spectrometry (UPLC−MS/MS) method that enables rapid detection and semi-quantification of malvidin glycosides and eight different malvidin-based pigment groups in red wines (Figure 1).[12] Two of these groups are oligomeric, and of both of these groups, the method is able to detect separately small oligomeric adducts (SOA), medium-sized oligomeric adducts (MOA), and large oligomeric adducts (LOA; Figures 2 and 3). Briefly, the method produces fragment or marker ions of the targeted compound groups by in-source collision-induced dissociation and the marker ions are then detected with multiple reaction monitoring (MRM). This methodology produces two-dimensional (2D) chromatographic fingerprints, which provide both qualitative and quantitative information about the targeted compound groups (Figures 2 and 3). Quantitative information about the sizes of the oligomeric adducts in a sample can be acquired by calculating the relative proportions of the SOAs, MOAs, and LOAs (SOA-%, MOA-%, and LOA-%), which reveal how much the adducts of different sizes contribute to the concentration (Figure 3). The LOA-% is the most interesting of the parameters because it directly reflects how a large proportion of the concentration of the oligomeric adducts is comprised of the largest detectable adducts. Therefore, it can be used as a metric of the degree of oligomerization or polymerization. For instance, should the LOA-% correlate with the color intensity, the degree of oligomerization or polymerization would have a positive connection to the color intensity. The unprecedented analytical accuracy regarding the oligomeric pigments makes it possible to arrive at specific conclusions about certain types of oligomeric adducts rather than just discussing polymeric pigments on a general level, as is often done in the literature.

In this paper, we measured the color intensity of 317 commercial red wines and set out to establish connections between the pigment composition and color intensity in red wines. Our goal was to discover how precisely the color intensity can be explained based on the pigment composition, how the contributions of the two oligomeric pigment groups compare against the contribution of the monomeric pigments, and whether the sizes of the oligomeric pigments have an effect on the color intensity. Finally, by focusing only on the 1-year-old wines, we tested whether the color intensity was explained by the same features in the youngest of wines as it was in the complete data set. The wine set was heterogeneous, since the wines originated from 13 countries (84 regions), and included 36 different primary grape varieties; 176 red wines were single-cultivar wines and 141 were blends and the wines were 1−8 years old at the time of their sampling (Table 1). Thus, the wine set was optimal to be used in discovering general patterns related to the color of commercial red wines.

MATERIALS AND METHODS

Red Wines. Some of the 317 wine samples were collected by the Natural Chemistry Research Group (n = 45) and some were provided by Alko Inc. (n = 272), a Finnish national alcoholic beverage retailing company. Aliquots of the red wines were sampled from freshly opened bottles and they were stored at −80 °C.

Semiquantitative Analyses. The UPLC−MS/MS system consisted of a Waters Acquity ultra-performance liquid chromatograph (UPLC; Waters Corporation, Milford, MA), which was coupled to a Xevo triple quadrupole mass spectrometer (Waters Corporation, Milford, MA). The UPLC system consisted of a binary solvent manager, a sample manager, a column oven, and a diode array detector. The column was an Acquity UPLC BEH Phenyl column (100 × 2.1 mm i.d., 1.7 μm; Waters Corporation, Wexford, Ireland). The concentrations of pigment groups **1**−**9** (Figure 1) were semiquantified using the UPLC−MS/MS method of Laitila et al.[12]

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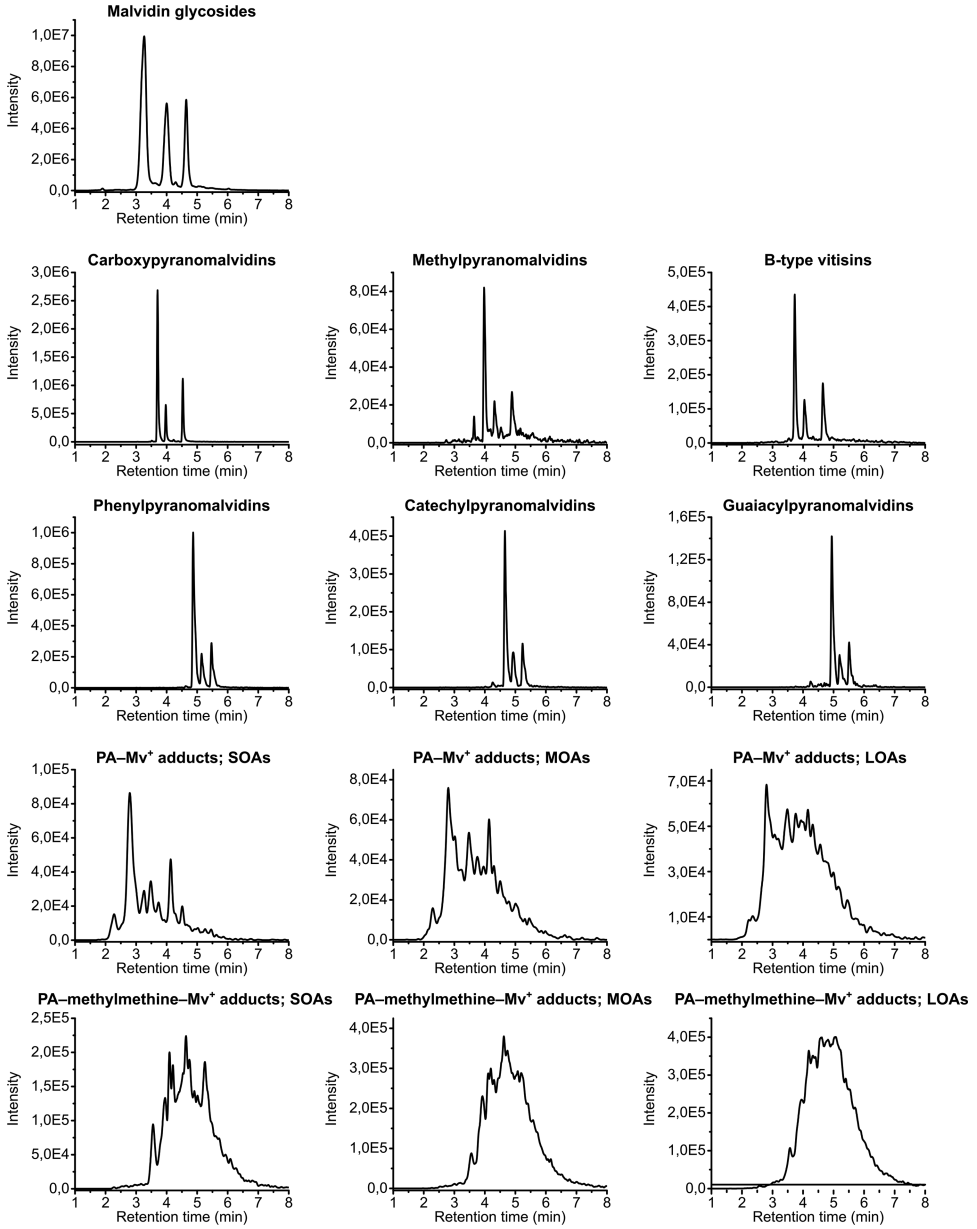

Figure 2. Examples of all measured 2D chromatographic fingerprints from a single red wine. Similar 2D fingerprints were measured from each wine. The shape of the 2D fingerprints provides qualitative information about the pigment composition, whereas the areas provide quantitative information. The areas were further transformed into relative concentrations with calibration curves and they were used to model the color intensity. Abbreviations: PA, proanthocyanidin; Mv, malvidin glycoside; SOA, small oligomeric adduct; MOA, medium-sized oligomeric adduct; LOA, large oligomeric adduct.

The compound groups were detected by the quantitative transitions of the group-specific MRM methods. The chromatogram areas were transformed into relative concentrations with calibration curves, which were prepared from a single reference wine, a JP Chenet Merlot 2015. In other words, the concentrations were reported as percentages of the concentrations in the reference wine. This was done to take into account the nonlinear response in some of the compound groups. Refer to Laitila et al.[12] for details. A diluted external standard wine, an Alamos Tempranillo 2015, was analyzed after every 10 injections to monitor and account for the natural fluctuation in the performance of the MS/MS system. The responses of malvidin glycosides, carboxypyranomalvidins, phenylpyranomalvidins, PA−Mv^+ adducts, and PA−methylmethine−Mv^+ adducts were monitored in the external standard and their responses were used to calculate a correction coefficient to correct the raw responses of pigment groups **1**−**9** in the actual samples. Carboxypyranomalvidins, B-type vitisins, and methylpyranomalvidins were corrected with the correction coefficient calculated from the responses of the carboxypyranomalvidins and all three pinotin groups (**5**−**7**) were corrected based on the responses of the phenylpyranomalvidins. The responses in the external standard wine at the time of the analysis of the calibration curves were used as reference points, to which the areas of the pigment groups in the external standards during the quantitative analyses were compared to obtain the correction coefficient. The concentrations of the oligomeric pigments were calculated from the summed total chromatogram areas of the SOAs, MOAs, and LOAs. The LOA-% of the oligomeric pigments were calculated as ratios between the areas of the LOAs and the total summed chromatogram areas (Figure 3). The wines were analyzed as such after filtration by a 0.2 μm PTFE filter. Other instrumentational details, operating parameters, and methodological details are described in Laitila et al.[12]

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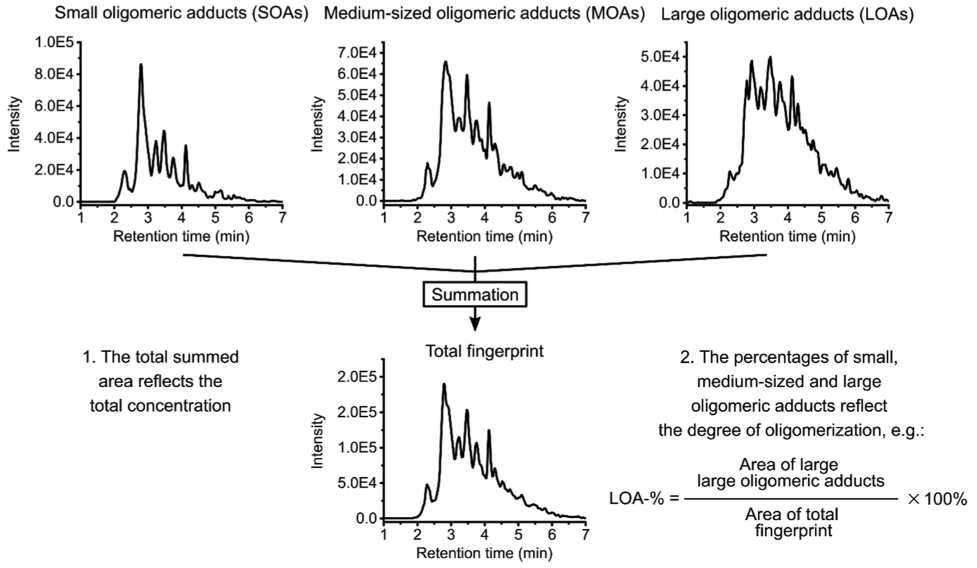

Figure 3. Examples of 2D chromatographic fingerprints of the PA−Mv^+ adducts. The UPLC−MS/MS method produces separate 2D fingerprints of small oligomeric adducts (SOAs), medium-sized oligomeric adducts (MOAs), and large oligomeric adducts (LOAs), which can be summed to form total fingerprints. The areas of the total fingerprints of the oligomeric pigments were transformed into relative concentrations with calibration curves and they were used to model the color intensity. The proportions of the small, medium-sized, and large oligomeric adducts of the total summed areas (SOA-%, MOA-%, and LOA-%) provided relative information about the average sizes of the oligomeric adducts.

Table 1. Summary of the Commercial Red Wines Utilized in the Present Study (n = 317)[f]

countries	regions	primary grape varieties[c]	age in years[e]
France (90)	Douro (40)	Pinot Noir (52)	1 (78)
Portugal (46)	Languedoc-Roussillon (30)	Shiraz (48)	2 (71)
Australia (40)	Beaune (24)	Merlot (39)	3 (44)
Italy (32)	Pfalz (19)	Cabernet Sauvignon (31)	4 (28)
Germany (20)	South Eastern Australia (19)	Touriga Ciol (29)	5 (11)
Spain (19)	Listrac-Medoc (18)	Blaufrankisch (13)	≥6 (8)
USA (15)	Barossa Valley (15)	Tempranillo (11)	not known (77)
others[a] (55)	others[b] (152)	others[d] (94)	

[a]Six other countries and five wines from unknown countries. [b]77 Other regions and nine wines from unknown regions. [c]Secondary grape varieties were used in 141 wines. [d]29 Other grape varieties and 27 wines with unknown primary grape variety. [e]At the time of sampling. [f]The numbers in parentheses represent the numbers of wines.

Color Measurements. The absorbance of each red wine was measured as such at 415, 520, and 620 nm with a 96-well plate reader (Multiskan Ascent, Thermo Fisher, Waltham). The absorbances were measured in duplicate and 125 μL of wine was pipetted to each well. The intensity of the color was defined as the sum of the three individual absorbances.[13,14] Typically, 420 nm is used as one of the detection wavelengths but, because of instrumentational limitations, 415 nm was used in this study.

Statistical Analyses. All statistical analyses were performed with R (version 3.5.3) in Rstudio integrated development environment (version 1.2.1335).[15,16] Partial least-squares regression (PLSR) models were utilized to study the connections between the pigment groups (predictors) and the intensity of the color (response). The predictors and the response were log-transformed prior to model fitting to meet the assumption of the linear correlations and the variables were autoscaled by subtracting the means from the variables and dividing them by their standard deviations. The variables were also log-transformed for the correlation analysis. The PLSR analyses were performed with the "*plsdepot*" package in R.[17] The optimal number of latent variables was chosen based on the predicted residual sums of squares (PRESS) and the residual sums of squares (RSS) as well as the coefficient of determination (R^2) and the cross-validated R^2 (Q^2). The normal QQ plot of the y-residuals and the scatter plot of the y-residuals and the predicted values were visually inspected to ensure that the residuals were symmetrically distributed and homoscedastic. Separate PLSR models were made for the whole data set and for the 1-year-old wines to test whether the color was determined by the same features in the whole set as well as in the youngest of commercial wines.

RESULTS AND DISCUSSION

The UPLC−MS/MS method produces semiquantitative data and the relative concentrations can be compared between samples within the compound groups. In general, in electrospray ionization mass spectrometry different analytes are ionized with different efficiencies inside the ion source and the ionized analytes are converted from eluent to gas-phase ions with different efficiencies as well.[18,19] Furthermore, the analytes are fragmented twice with the utilized UPLC−MS/MS method: first inside the ion source to produce the marker ions and then in the collision chamber during the MRM. The fragmentation in both situations is more efficient with some analytes and less efficient with some. All of this adds up, meaning that the comparison of the responses of the 2D fingerprints or the semiquantified concentrations is both uninformative and meaningless between the compound groups. However, the concentrations can still be compared between samples within the compound groups and the variation in the concentrations can be linked to the variation in the color intensity with suitable statistical methods. Simple linear correlation coefficients provide some information about the associations between the pigment groups and the color intensity (Figure S1) but statistical partial least-squares regression (PLSR) models provide a far more powerful statistical framework for the analysis of multivariate and

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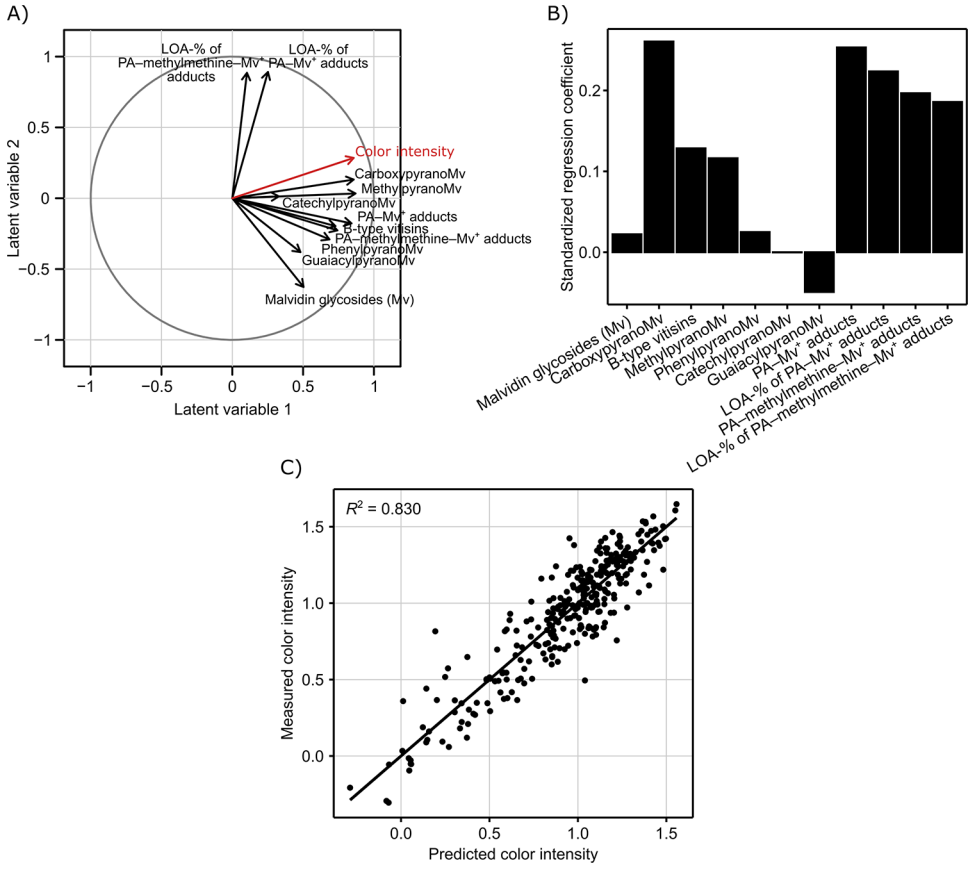

Figure 4. Summary of the partial least-squares regression (PLSR) model explaining the color intensity in the whole wine set (n = 317). Panel A shows the correlation biplot of the original variables and the first two latent variables, panel B shows the standardized regression coefficients of the PLSR model with three latent variables, and panel C shows the scatter plot of the predicted color intensities of the three-component model and the measured color intensities. Refer to Table S1 for numerical values of the correlation coefficients and the regression coefficients.

collinear chemical data. All available data can be incorporated into PLSR models simultaneously to reveal how well the data explains the color intensity and which pigment groups are the most important in modeling the color intensity.

Color Intensity in the Whole Wine Set. First, the concentrations of compound groups **1**−**9** and the LOA-% of groups **8** and **9** were introduced into the PLSR model as predictors utilizing the whole wine set (n = 317). Three latent variables were chosen for the model as they provided a good balance between model complexity and the explanatory power of the model (Figure S2). The third latent variable was included because its addition still markedly reduced the residual sums of squares. The PLSR model consisting of three latent variables explained 64.4−93.8% of the original predictors (Table S1). The first latent variable explained 73.4% of the variation in the color intensity, the second latent variable explained 8.1%, and the third explained 1.5%, adding up to a total of 83.0%. The Q^2 of the three-component model was 0.819. The y-residuals were homoscedastic and they were symmetrically distributed.

Based on the regression coefficients of the three-component model, the concentrations of the carboxypyranomalvidins, PA−Mv^+ adducts, and PA−methylmethine−Mv^+ adducts, and the LOA-% of the PA−Mv^+ and PA−methylmethine−Mv^+ adducts were the most important variables in explaining the color intensity in the whole wine set (Figure 4). The malvidin glycosides and all three pinotin-type malvidin derivatives **5**−**7** had practically no important role in explaining the color intensity, whereas the B-type vitisins and methylpyranomalvidins had moderate correlation to the color intensity. The dimeric PA−Mv^+-type adducts consisting of catechin and malvidin glucoside units have been shown to be similar in many aspects to their precursor, the malvidin glucoside. The dimer has similar pH-dependent kinetic and thermodynamic properties as malvidin glucoside (i.e., they are mainly in colorless hemiacetal forms in the typical pH of red wines) and they are equally susceptible to bleaching by SO_2 (a chemical commonly used in winemaking).[8,20] The catechin moiety in the dimer only causes a bathochromic shift (17 nm) in the absorption maximum of the red-colored flavylium cation form compared to malvidin glucoside.[8,20] This has led to the conclusion that the transformation of malvidin glycosides into PA−Mv^+ adducts would not be as impactful on the wine color as transformations of malvidin glycosides into other types of monomeric and oligomeric pigments.[3] The significance of an observed correlation between the dimeric PA−Mv^+-type catechin−anthocyanin adducts and color intensity was even dismissed in a previous study because of the similar physicochemical properties of the directly linked dimers and anthocyanins.[21] Our method, however, detects not only the dimeric adducts but rather a much bigger portion of the PA−Mv^+ adducts consisting of numerous individual compounds with varying degrees of oligomerization.[12] Our results showed that the concentration of the PA−Mv^+ adducts had a significant connection to the color intensity (Figure 4). The dimeric PA−methylmethine−Mv^+-type adducts consisting of

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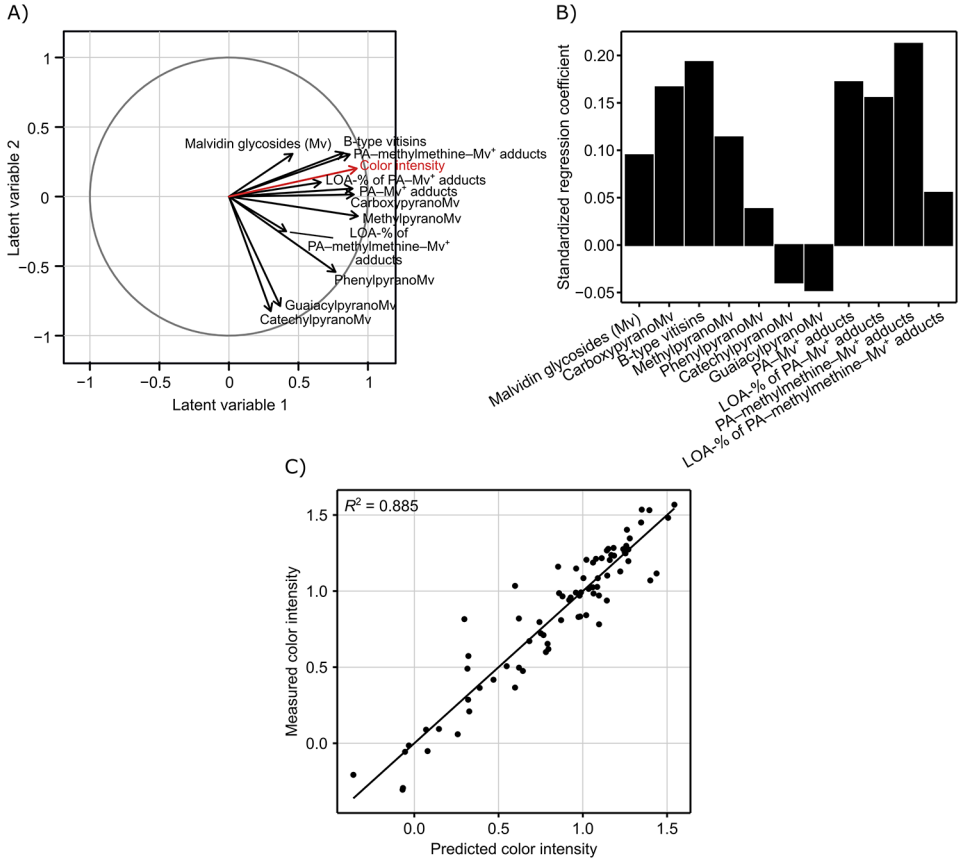

Figure 5. Summary of the partial least-squares regression (PLSR) model explaining the color intensity in the 1-year-old wines (n = 78). Panel A shows the correlation biplot of the original variables and the first two latent variables, panel B shows the standardized regression coefficients of the PLSR model with two latent variables, and panel C shows the scatter plot of the predicted color intensities of the two-component model and the measured color intensities. Refer to Table S2 for numerical values of the correlation coefficients and the regression coefficients.

catechin and malvidin glucoside units, on the other hand, already have features that suggest that these types of pigments should be relevant to the wine color. Larger percentages of the dimers are in colored forms in wine pH compared to malvidin glucoside and the dimers are more protected against bleaching by SO_2.[9] As a downside, the dimers are relatively unstable at wine pH because of acid-catalyzed cleavage of the methylmethine linkages.[22] It is not currently known how well large PA−methylmethine−Mv^+ oligomers resist depolymerization but, nonetheless, the concentration of the PA−methylmethine−Mv^+ adducts had a significant connection to the color intensity as well (Figure 4).

The carboxypyranomalvidins were the most important monomeric compound group in the PLSR model explaining the color intensity in the whole wine set (Figure 4). The monomeric adducts derived from malvidin glycosides, which we semiquantified in this study, have rather similar chromatic features in the pH range of red wines as they display either a yellow (**3**) or an orange−red color (**2**, **4**−**7**) and they are mainly in colored form.[4,23] However, the concentrations of the carboxypyranomalvidins have been found to be higher in commercial wines than the concentrations of many other types of monomeric malvidin derivatives.[24−26] The UPLC−MS/MS method yields proportional information about the concentrations,[12] meaning we cannot verify if the concentrations of the carboxypyranomalvidins were indeed higher in our wine set as well compared to other types of monomeric pigments. However, as the chemical properties of the monomeric adducts of the malvidin glycosides are relatively similar in the typical red wine pH, the presumably higher concentrations might be the reason why the carboxypyranomalvidins stood out as the most important monomeric compound group (Figure 4).

The importance of the PA−Mv^+ and PA−methylmethine−Mv^+ adducts could be partially related to their concentrations in wines as well. The summed concentrations of only a few dimers belonging to pigment groups **8** and **9** have been estimated to be comparable to the concentrations of many monomeric adducts of malvidin glycosides.[26] However, these smallest possible oligomers only comprise a small portion of the whole adduct composition[12] and the true total concentrations of the two oligomeric compound groups are likely to be much higher than the concentrations of the dimers alone. These observations backed up our previous reasoning: while it is important to study and know the thermodynamic and chromatic properties of the red wine pigments, their contribution to the color intensity cannot be deduced only from the properties measured in isolated conditions.

While the majority of the variation in the color intensity was explained by the first latent variable, which mainly described the concentrations (Figure 4A and Table S1), the correlation biplot of the PLSR model clearly showed how the LOA-% of the PA−Mv^+ and PA−methylmethine−Mv^+ adducts explained a unique and significant portion of the variation in the color intensity (Figures 4A and S2). Previously, with a more limited wine set, we noted that there was a strong negative correlation between the SOA-% and LOA-% of the oligomeric adducts[12]

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and now these correlations were confirmed with a much bigger wine set (n = 317). The correlation coefficients between the SOA-% and the LOA-% were −0.95 and −0.98 for the PA−Mv^+ and PA−methylmethine−Mv^+ adducts, respectively. Similarly, the correlation coefficients between the MOA-% and LOA-% were −0.54 and −0.76. These results supported our earlier argument about the LOA-% being suitable to be used as a metric of the degree of oligomerization because wines with high proportions of LOAs were associated with lower proportions of SOAs and MOAs. Alternatively, if a large portion of the concentration was produced by the LOAs, then, subsequently, a smaller portion was produced by the SOAs and MOAs. Now, as the LOA-% of the oligomeric pigments had a positive connection to the color intensity, the chemical interpretation of the results was that an increase in the average degree of oligomerization increased the color intensity as well. In the PA−methylmethine−Mv^+ and PA−Mv^+ adducts, the PA moieties themselves do not absorb visible light, meaning that they cannot directly increase the color intensity as the degree of oligomerization increases. However, they might affect the properties of the chromophores through intramolecular copigmentation or by protecting the chromophores from the nucleophilic attack of water (or SO_2), thereby reducing the formation of the colorless hemiacetals. The latter mechanism might be especially important for the PA−Mv^+ adducts because of the restraints of the direct, less flexible linkage between the Mv and PA moieties, which likely causes the similarities in the thermodynamic and chromatic properties of PA−Mv^+-type dimers and malvidin glycosides.[8,20] Previously, the degree of oligomerization has been shown to have an effect on the chromatic properties of oligomeric pigments consisting of pyranomalvidin glucoside and catechin units.[11]

Color Intensity in the 1-Year-Old Commercial Wines. The 1-year-old wines (n = 78) were studied separately to find out whether the color intensity was explained by the same features in the youngest of commercial wines as in the whole wine set. The concentrations of pigment groups **1**−**9** and the LOA-% of groups **8** and **9** were introduced into the PLSR model as predictors and then two latent variables were chosen for the model as they provided a good balance between model complexity and explanatory power of the model (Figure S3). The inclusion of additional latent variables would have started to level and decrease the Q^2, implying of overfitting. The PLSR model consisting of two latent variables explained 23.2−88.1% of the original predictors (Table S2). The first latent variable explained 84.5% of the variation in the color intensity and the second latent variable explained 4.1%, adding up to a total of 88.5%. The Q^2 of the two-component model was 0.862. The y-residuals were homoscedastic and they were symmetrically distributed.

Similarly to the whole wine set, the concentrations of the monomeric carboxypyranomalvidins and the oligomeric PA−Mv^+ and PA−methylmethine−Mv^+ adducts and the LOA-% of the PA−Mv^+ adducts had a major role in explaining the color intensity (Figure 5A,B). Additionally, the B-type vitisins were more impactful on the color intensity in the young commercial wines than they were in the whole wine set. On the contrary, the LOA-% of the PA−methylmethine−Mv^+ adducts did not have a significant connection to the color intensity in young wines and, again, neither did the pinotin-type malvidin derivatives **5**−**7**. Overall, the color intensity was explained slightly better in the 1-year-old wines than it was in the whole wine set (Figures 4C and 5C). The B-type vitisins have been shown to have a similar evolutionary aging trend as the anthocyanins in red wines. Namely, the concentrations of B-type vitisins diminish as red wines age.[21,27] This evolutionary trend could be one reason why the B-type vitisins had a bigger impact on the color intensity in the 1-year-old wines compared to the whole wine set. Their concentration might be high enough in the young wines to have an impact on the color but, as the wines age, the contribution of B-type vitisins decreases as well, along with their concentrations. The lesser importance of the LOA-% of the PA−methylmethine−Mv^+ adducts might imply that there is an evolutionary trend in the composition of the PA−methylmethine−Mv^+ adducts as well, which becomes more relevant to the color intensity as wines age. The LOA-% of the PA−Mv^+ adducts was still a significant predictor, which meant that already in the young commercial wines, the degree of oligomerization of the PA−Mv^+ affected the color intensity. Interestingly, in the 1-year-old wines, the information that the LOA-% provided about the color intensity was not as exclusive and unique as it was in the whole wine set and the LOA-% was more correlated with the other predictors (Figure 5A). Even though the malvidin glycosides were more important to the color intensity in the 1-year-old wines than they were in the whole wine set, other pigment groups derived from the malvidin glycosides were still more impactful on the color (Figure 5A,B). Anthocyanins are often described to be the main contributors to the color in young red wines.[22,28,29] On the contrary, our results suggested that in the youngest commercial wines in the present wine set, the anthocyanin derivatives, mainly carboxypyranomalvidins, B-type vitisins, and the oligomeric pigments, were the primary contributors to the color intensity.

Our findings confirmed for the first time that the PA−Mv^+ and PA−methylmethine−Mv^+ adducts, first hypothesized to be present in red wines nearly 50 years ago, are on a compound group level truly important for the color intensity of red wines. Besides their concentrations in wines, their sizes, i.e., degrees of oligomerization, were shown to have a positive and important connection to the color intensity. The sizes of the oligomeric pigments explained a unique and distinctive part of the variation in the color intensity. The most important monomeric pigment group for the color intensity was the carboxypyranomalvidins and, overall, 83% of the variation in the color intensity in all 317 commercial red wines was explained by the main pigment composition. The color intensity was largely explained by the same pigment groups in the 1-year-old wines as in the whole wine set but, additionally, the B-type vitisins were major contributors to the color intensity in the youngest of wines. Moreover, the LOA-% of the PA−methylmethine−Mv^+ adducts did not have a significant connection to the color intensity in the 1-year-old wines. This implied that there could be some sort of an evolutionary trend in the composition of the PA−methylmethine−Mv^+ adducts, which becomes relevant to the wine color in older wines. The malvidin glycosides themselves, and the anthocyanins in general, might be less important for the wine color than they are generally thought to be. Even in the youngest of commercial wines, their contribution to the color intensity was minor compared to the other pigment groups. We were able to explain the vast majority of the variation in the color intensity, but the models still left some room for improvement. Red wines contain more pigment types than were analyzed in this study and their accurate analyses in the

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future could further improve our understanding of the color of red wines.

■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge at https://pubs.acs.org/doi/10.1021/acs.jafc.9b07941.

Correlation coefficients, regression coefficients, and R^2 values of the PLSR model explaining the color intensity in the whole wine set (Table S1) and in the 1-year-old wines (Table S2); Pearson's correlation coefficients of the log-transformed variables (Figure S1); and cross-validation results of the two PLSR models (Figures S2 and S3) (PDF)

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■ ABBREVIATIONS

LOA, large oligomeric adduct; LOA-%, proportion of large oligomeric adducts; MOA, medium-sized oligomeric adduct; MOA-%, proportion of medium-sized oligomeric adducts; MS, mass spectrometry; MS/MS, tandem mass spectrometry; Mv, malvidin glycoside; PA, proanthocyanidin; PLSR, partial least-squares regression; SOA, small oligomeric adduct; SOA-%, proportion of small oligomeric adducts; UPLC, ultra-performance liquid chromatography

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