

Anthocyanins in 'Cabernet Gernischet' (*Vitis vinifera* L. cv.) Aged Red Wine and Their Color in Aqueous Solution Analyzed by Partial Least Square Regression

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Abstract Anthocyanins are considered one of the main color determinants in aged red wine. The anthocyanins in aged red wine made from 'Cabernet Gernischet' (*Vitis vinifera* L. cv.) grape were investigated by high performance liquid chromatography- electronic spray ionization- mass spectrometry (HPLC-ESI-MS) and their color presented in aqueous solution were evaluated using partial least square regression (PLS). The results showed that there were 37 anthocyanins identified in this wine, including 22 pyranoanthocyanins. The analysis of PLS indicated that different anthocyanins showed distinct color values: malvidin 3-*O*-(6-*O*-acetyl)-glucoside-4-vinylguaiacol (Mv3-acet-glu-vg) presented the highest color values, while malvidin 3-*O*-glucoside (Mv3-glu) showed least. Among the free non-acylated anthocyanins, peonidin 3-*O*-glucoside (Pn3-glu) showed the highest color values; the coumarylated anthocyanins presented higher color values than their corresponding acetylated anthocyanins and parent anthocyanins; pyranoanthocyanins presented also higher color values than their original anthocyanins; the color of anthocyanins depended on their structure. This work will be helpful to reveal evolution in aged red wine.

Keywords: color of anthocyanin, 'Cabernet Gernischet' red wine, partial least square regression

Introduction

Anthocyanins provide red wine with attractive color which can approximately reflect the wine age and impact the consumption behavior of customers. Until now, several hundreds of known anthocyanins have been identified from different wines or model wines, which can be classified, according to their structures, into the following groups: the non-acylated anthocyanin monoglucosides (the common anthocyanins in red wine, Fig. 1), acylated anthocyanins, pyranoanthocyanins (Fig. 1), direct flavanol-anthocyanin condensation products, acetaldehyde-mediated or other compounds-mediated flavanol-anthocyanin condensed products, and polymeric anthocyanins (1-7). Anthocyanin composition and content gradually change during winemaking and aging, which will lead to the color shift from the initial purple-red color of young red wine to the brick-red color of aged red wine.

Pyranoanthocyanins are generated during wine fermentation and aging through the condensation of anthocyanins with other compounds such as acetaldehyde, acetone, pyruvic acid, 4-vinylphenol, 4-vinylcatechol, 4-vinylguaiacol, 4-vinylsyringol, vinyl-flavanol, α -ketoglutaric acid, glyoxylic acid, hydroxycinnamic acids, and so on (1,2,4,5,8,9). These condensed products all contain a pyran ring (Fig. 1). Visible increase in pyranoanthocyanin content can be observed in the aged red wine. In comparison with their parent anthocyanins, pyranoanthocyanins make maximum of

absorption length hypsochromic or bathochromic shift. As a result, orange or blue hue in red wine increases. In addition, the pyranoanthocyanins can withstand against the changes of pH and SO₂. It is thus suggested that they might contribute more to wine color than their parent anthocyanins during the red wine aging (5,6,10,11).

The color of red wine, influenced by anthocyanins, copigmentation, wine pH, SO₂ concentration, alcohol, metal ions, polymeric pigments, and other factors, is complex (7,12-15). However, in aged red wine, the effect of pH, SO₂ on wine color becomes less important because the pyranoanthocyanins and polymeric anthocyanins predominate. The intermolecular copigmentation can account for 30-50% of the color in young red wines (13), while it decreases in aged red wines and accounted for 8-30% of the color (7).

Several reports have referred to the color of several anthocyanins presented in aqueous solution or model wine, and compared the effect of anthocyanins' structure on their color (10,16-19). However, the colors of different anthocyanins in aged red wine which mainly consist of the pyranoanthocyanins or polymeric anthocyanins, have not been compared by means of statistical method. On the other hand, the commercial anthocyanin standards are less available and very expensive, especially pyranoanthocyanins.

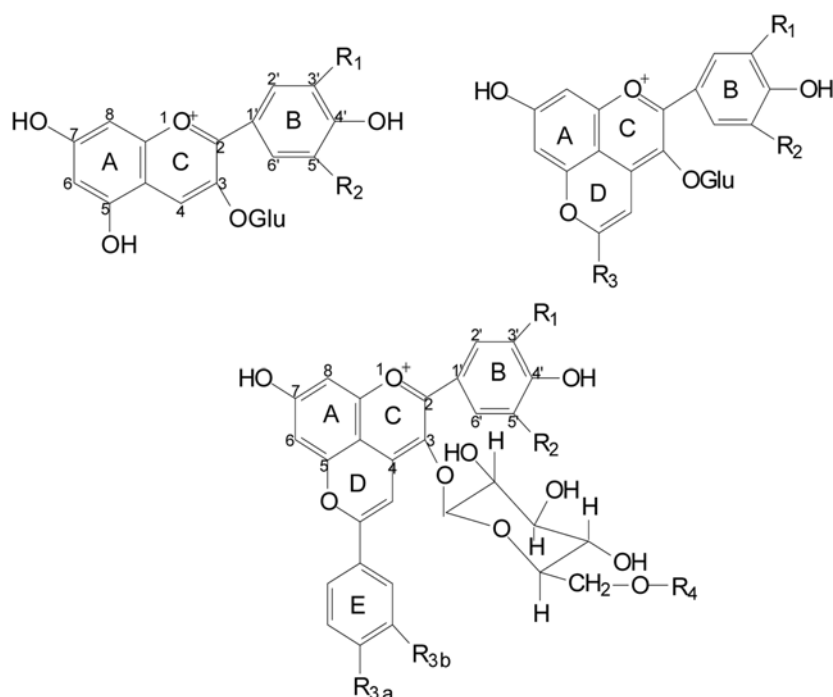
'Cabernet Gernischet' (*Vitis vinifera* L. cv.) is a Chinese characteristic wine grape variety that was imported from Europe in 1894 and naturally selected in China. Until now, little is known about the anthocyanins from the 'Cabernet Gernischet' aged red wine and their present color. For instance, whether or not substituent difference between various anthocyanins determines color nuance and which substituent makes greater contribution to the color of

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Received November 12, 2008; Revised January 5, 2009;

Accepted January 8, 2009



Anthocyanins	R ₁	R ₂	R ₃	R _{3a}	R _{3b}	R ₄
Dp3-glu	OH	OH	-	-	-	-
Cy3-glu	OH	H	-	-	-	-
Pt3-glu	OCH ₃	OH	-	-	-	-
Pn3-glu	OCH ₃	H	-	-	-	-
Mv3-glu	OCH ₃	OCH ₃	-	-	-	-
Mv3-glu-acetal	OCH ₃	OCH ₃	H	-	-	-
Pn3-glu-pa	OCH ₃	H	COOH	-	-	-
Mv3-glu-vg	OCH ₃	OCH ₃	-	OH	OCH ₃	H
Mv3-acet-glu-vc	OCH ₃	OCH ₃	-	OH	OH	Acetyl
Mv3-coum-glu-vp	OCH ₃	OCH ₃	-	OH	H	Coumaryl

Fig. 1. Structures of 5 common anthocyanins (Dp3-glu, Cy3-glu, Pt3-glu, Pn3-glu, and Mv3-glu) and some pyranoanthocyanins (Mv3-glu-acetal, Pn3-glu-pa, Mv3-glu-vg, Mv3-acet-glu-vc, and Mv3-coum-glu-vp). Dp3-glu, delphinidin 3-*O*-glucoside; Cy3-glu, cyanidin 3-*O*-glucoside; Pt3-glu, petundin 3-*O*-glucoside; Pn3-glu, peonidin 3-*O*-glucoside; Mv3-glu, malvidin 3-*O*-glucoside; Mv3-glu-acetal, malvidin 3-*O*-glucoside-acetaldehyde; Pn3-glu-pa, peonidin 3-*O*-glucoside-pyruvic acid; Mv3-glu-vg, malvidin 3-*O*-glucoside-4-vinylguaiacol; Mv3-acet-glu-vc, malvidin 3-*O*-(6-*O*-acetyl)-glucoside-4-vinylcatechol; Mv3-coum-glu-vp, malvidin 3-*O*-(6-*O*-coumaryl)-glucoside-4-vinylphenol.

anthocyanins. In the present study, anthocyanin composition was analyzed using high performance liquid chromatography-electronic spray ionization-mass spectrometry (HPLC-ESI-MS) in 'Cabernet Gernischt' aged red wine which is of good quality in China, and a statistical method, partial least square regression (PLS) analysis, was introduced to evaluate the color of various anthocyanins in aqueous solution, and further to understand the effect of different substituents of anthocyanins on appearance color of anthocyanins. This work will provide an important basis for comprehending the mechanism of red wine color changing.

Materials and Methods

Samples 'Cabernet Gernischt' (*Vitis vinifera* L. cv.) red wine was produced in 2004 vintage in the city of Yantai, Shandong Province, China. The red wine was stored in oak

barrel 15 months, then bottled and stored in a cellar. The separation of anthocyanins from wine samples was performed according to the method of Alcalde-Eon *et al.* (4) with some modification. The samples (30 mL) were added into TSK Toyopearl gel HW-40(S) column (200×15 mm i.d., Tosoh, Tokyo, Japan) and eluted with solvent A (H₂O: MeOH: CH₃COOH, 80:18:2, v/v/v) (H₂O, distilled water; MeOH, AR grade, Beijing Chemical Works, Beijing, China; CH₃COOH, HPLC grade, Fisher, Pittsburg, PA, USA) of about 100 mL, and then with solvent B (H₂O: MeOH: CH₃COOH, 18: 80:2, v/v/v) until eluate from the column was non-colored. The flow rate was 0.5 mL/min. All eluted fractions were divided into 5 parts according to the color, which was freeze-dried under vacuum conditions (Vacuum Freeze Drying Machine LGJ-10; Four-Ring Science Instrument, Beijing, China) and then was dissolved with 5 mL of aqueous 2%(v/v) formic acid. These parts were used for color evaluation and HPLC-MS analysis.

This separation was independently repeated 4 times. (The last separation was divided into 4 parts, thus a total of 19 parts for all analysis).

Analysis of CIE L a*b* values CIE L a*b* values were analyzed according to the method described by Ayala *et al.* (20,21). All the wine fractions were filtered through 0.45- μ m cellulose acetate and nitrocellulose (CAN) filter (BFC, Beijing, China) prior to analysis. CIE L a*b* measurement was carried out by means of a Shimadzu UV-2450 spectrophotometer (Kyoto, Japan), 0.2 cm path length, at 440, 530, and 600 nm, respectively. Distilled water was used as the blank. All analyses were replicated twice.

HPLC-MS analysis An Agilent 1200 series LC-MSD (Trap VL; Santa Clara, CA, USA), equipped with a UV detector and reversed phase column (Kromasil C18, 250 \times 4.6 mm 5 μ m, Eka Chemicals, Bohus, Sweden), was used. The solvents were (A) aqueous 2% formic acid (HPLC grade, Fisher), and (B) acetonitrile (HPLC grade, Fisher) containing 2% formic acid. The gradient was from 6 to 10% B for 4 min, from 10 to 25% B for 8 min, isocratic 25% B for 1 min, from 25 to 40% for 7 min, from 40 to 60% for 15 min, from 60 to 100% for 5 min, from 100 to 6% for 5 min, at a flow rate of 1.0 mL/min. Injection volumes were 30 μ L, and the detection wavelength was 525 nm. MS conditions were as follows: electron spin ionization (ESI) interface, positive ion model, 35 psi nebulizer pressure, 10 L/min dry gas flow rate, 350°C dry gas temperature, and scans at m/z 100-1,000 and 500-1,500. All analyses were replicated twice.

Statistical analysis It is difficult for multiple linear regression to correctively compare and explain the meaning of partial regression coefficients because of the multico-linearity (high correlation among the independent variables). Instead, analysis of partial least square regression (PLS) combines the properties of multiple linear regressions with principal component analysis to produce a technique that is able to accept collinear data and to reduce the number of required calibration samples. The optimum number of terms (anthocyanins, independent variables) in the PLS calibration models was determined by examining the proportion of total variance explained by each component and the physical meaning of all partial regression coefficients in order to avoid over-fitting of the model because the regression model aimed at the comparison of importance of independent variables. Models were cross-validated by the leave-one-out method. All variables were normalized prior to the analysis. Some related theory of PLS can be found elsewhere (22,23). The contents of various anthocyanins were expressed as their peak area in HPLC. If the anthocyanin was not detected in a sample, it was represented as zero in the data matrix. PLS was performed by the statistical software of SAS V8 (SAS Institute, Cary, NC, USA).

Results and Discussion

Anthocyanins in 'Cabernet Gernischt' red wine According to the retention time and the fragment characteristics of their molecular structure (4), 40

anthocyanins were detected from 'Cabernet Gernischt' red wine including 4 non-acylated anthocyanins (delphinidin 3-*O*-glucoside, Dp3-glu; petunidin 3-*O*-glucoside, Pt3-glu; peonidin 3-*O*-glucoside, Pn3-glu; malvidin 3-*O*-glucoside, Mv3-glu), 3 acylated anthocyanins (petunidin 3-*O*-(6-*O*-acetyl)-glucoside, Pt3-acet-glu; malvidin 3-*O*-(6-*O*-acetyl)-glucoside, Mv3-acet-glu; malvidin 3-*O*-(6-*O*-coumaryl)-glucoside, Mv3-coum-glu), 22 pyranoanthocyanins, 11 condensed anthocyanins with (epi)catechin (4 malvidin 3-*O*-glucoside-4-ethyl-(epi)-catechin, Mv3-glu-ethyl-(epi)c; malvidin 3-*O*-glucoside-4-vinyl-catechin, Mv3-glu-4-vinyl-c; malvidin 3-*O*-(6-*O*-acetyl)-glucoside-4-vinyl-catechin, Mv3-acet-glu-4-vinyl-c; malvidin 3-*O*-glucoside-4-vinyl-(epi)catechin, Mv3-glu-4-vinyl-(epi)c; malvidin 3-*O*-(6-*O*-acetyl)-glucoside-4-vinyl-(epi)catechin, Mv3-acet-glu-4-vinyl-(epi)c; malvidin 3-*O*-glucoside-catechin, Mv3-glu-c; malvidin 3-*O*-glucoside-propyl-catechin, Mv3-glu-propyl-c; malvidin 3-*O*-(6-*O*-acetyl)-glucoside-ethylflavanol, Mv3-acet-glu-ethylflavanol) (Table 1 and 2). Pyranoanthocyanins were mainly formed by reaction of anthocyanins with acetaldehyde, pyruvic acid, 4-vinylphenol, 4-vinylcatechol, or 4-vinylguaiacol.

As is shown in Table 1, pyranoanthocyanins detected in 'Cabernet Gernischt' red wine were main products of condensing reactions of anthocyanins with acetaldehyde, pyruvic acid, 4-vinylphenol, 4-vinylcatechol or 4-vinylguaiacol. The molecular and product ions of peaks No. 14 and 15 at m/z 595 and 433 (lose 162) were identified as Pt3-glu-vp and Pn3-glu-vc according to their different retention time. While the molecular and product ion of the peak No. 18 at m/z 637 and 433 (lose 204) should correspond to the acetylated compound of peak No. 14 or 15, and the peak No. 18 should be concluded as being Pn3-acet-glu-vc or Pt3-acet-glu-vp. The peak No. 16 was identified as Mv3-coum-glu and the peak No. 17 was Mv3-glu-vc for which molecular and product ion was at m/z 625 and 463. Similarly, the peak No. 20 was identified as 2 kinds of molecular and product ions, which were Mv3-glu-vp and Mv3-acet-glu-vc. The molecular ion of the peak No. 21 and 23 respectively lost 162 and 204 to form the same product ions at m/z 477, and these 2 compounds were identified as Mv3-glu-vg and Mv3-acet-glu-vg. While the peak No. 22 and 24 respectively lost 204 and 308 to form the same product ion at m/z 447, thus, they were identified as Mv3-acet-glu-vp and Mv3-coum-glu-vp (4).

Some condensed compounds were detected by MS scans at m/z 500-1,500. As is shown in Table 2, the molecular and product ions at m/z 809, 647, and 519 appeared 4 times with different retention times. This was tentatively identified as the condensed compound between Mv3-glu and catechin or epicatechin mediated by acetaldehyde, namely Mv3-glu-4-ethyl-(epi)catechin, and the ion fragments at m/z 647 and 519 were formed by removal of glucoside (-162) and catechin (-290). There are 2 molecular configurations (S and R) in these kinds of compounds because there is an asymmetric carbon at ethyl; Mv3-glu moiety may react with catechin or epi-catechin. In addition, Mv3-glu may link to catechin or epi-catechin through C6 or C8 of Mv3-glu, or through C6 or C8 of catechin, or through C6 or C8 of epi-catechin (8,24-26). Thus, it is speculated that this kinds of compounds contain 16 isomers.

Table 1. Anthocyanins in 'Cabernet Gernischt' red wine detected by MS scans at m/z 100-1,000¹⁾

No.	RT (min)	RT-STD	Molecular and products ions (m/z)	Anthocyanins
1	3.846	0.084	465,303	Dp3-glu
2	6.243	0.096	479,317	Pt3-glu
3	8.669	0.100	463,301	Pn3-glu
4	9.596	0.043	493,331	Mv3-glu
4 ²⁾	7.904		531,369	Pn3-glu-pa
5 ³⁾	11.840	0.041	561,399	Mv3-glu-pa
6	13.183	0.026	517,355	Mv3-glu-acetal
7 ³⁾	14.677	0.019	603,399	Mv3-acet-glu-pa
8	15.479		521,317	Pt3-acet-glu
9	16.700	0.008	559,355	Mv3-acet-glu-acetal
10	19.844	0.042	535,331	Mv3-acet-glu
11	20.180		581,419	Dp3-glu-vc
12	22.454	0.035	611,449	Pt3-glu-vc
13	25.954	0.001	533,371	Dp3-glu-pa
14	26.009	0.022	595,433	Pt3-glu-vc
15	26.511	0.021	595,433	Pn3-glu-vc
16	27.303		639,331	Mv3-coum-glu
17	27.307	0.012	625,463; 639,331	Mv3-glu-vc, Mv3-coum-glu
18	28.407		637,433	Pn3-acet-glu-vc or Pt3-acet-glu-vc
19	28.757	0.225	579,417	Pn3-glu-vc
20	29.380	0.007	609,447; 667,463	Mv3-glu-vc, Mv3-acet-glu-vc
21	30.324	0.008	639,477	Mv3-glu-vc
22	31.402	0.007	651,447	Mv3-acet-glu-vc
23	32.165	0.009	681,477	Mv3-acet-glu-vc
24	33.421	0.008	755,447	Mv3-coum-glu-vc

¹⁾RT, retention time; STD, standard deviation; Dp, delphinidin; Cy, cyanidin; Pt, petunidin; Pn, peonidin; Mv, malvidin; glu, glucoside; acet, acetyl; coum, coumaryl; caff, caffeoyl; acetal, acetaldehyde; pa, pyruvic acid; vg, 4-vinylguaiacol; vc, 4-vinylcatechol; vp, 4-vinylphenol; c, catechin.

²⁾Detected in different fraction.

³⁾Not selected into the PLS equations because of the inverse sign of regression coefficients.

Table 2. Anthocyanins detected by MS scans at m/z 500-1,500

RT (min)	Molecular and products ions (m/z)	Anthocyanins
15.0	809,647,519	Mv3-glu-ethyl-(epi)c
16.0	809,647,519	Mv3-glu-ethyl-(epi)c
17.3	809,647,519	Mv3-glu-ethyl-(epi)c
18.6	809,647,519	Mv3-glu-ethyl-(epi)c
26.1	805,643	Mv3-glu-4-vinyl-c
28.2	847,643	Mv3-acet-glu-4-vinyl-c
28.7	805,643	Mv3-glu-4-vinyl-(epi)c
29.5	847,643	Mv3-acet-glu-4-vinyl-(epi)c
4.2	781,619	Mv3-glu-c
16.7	985,823,661	Mv3-glu-propyl-c
24.0	851,561	Mv3-acet-glu-ethylflavanol
31.4 ¹⁾	621,417	Pn3-acet-glu-vc
31.8 ¹⁾	771,463,447	
	771,447	Mv3-caff-glu-vc
	771,463	Mv3-coum-glu-vc

¹⁾Detected by MS at m/z 100-1,000.

The molecular ion and ion fragment at m/z 805 and 643 were respectively identified as Mv3-glu-4-vinyl-catechin and Mv3-glu-4-vinyl-(epi)-catechin with loss of glucoside (-162), while the molecular ions and ion fragments at m/z

847 and 643 were identified as their corresponding acetylated compounds, that is Mv3-acet-glu-4-vinyl-c and Mv3-acet-glu-4-vinyl-(epi)-c (2,27).

The molecular ion and ion fragment at m/z 781 and 619

Table 3. Anthocyanins and color parameters of the 5 fractions of first separation

		Fractions ¹⁾				
		1A	1B	1C	1D	1E
Color parameters	L*	83.82	79.77	52.43	63.67	95.63
	a*	19.95	36.66	53.15	42.35	4.33
	b*	12.82	7.61	24.20	31.85	7.28
	C*ab	23.72	37.44	58.40	52.99	8.47
	H*ab	32.72	11.72	24.48	36.95	59.27
Anthocyanins (content expressed as peak area)	Dp3-glu	-	-	57.66	-	-
	Pt3-glu	-	8.59	69.25	-	-
	Pn3-glu	-	13.58	24.32	-	-
	Mv3-glu	65.24	1,466.35	582.33	78.81	8.21
	Mv3-glu-pa	82.30	-	-	-	-
	Mv3-glu-acetal	19.69	117.07	47.03	12.02	-
	Mv3-acet-glu-pa	-	36.62	-	-	-
	Mv3-acet-glu-acetal	-	8.25	-	-	-
	Pt3-acet-glu	-	-	7.75	-	-
	Mv3-acet-glu	20.08	-	294.43	18.77	-
	Dp3-glu-vc	-	-	-	7.09	-
	Pt3-glu-vc	-	-	-	30.62	-
	Pt3-glu-vc	-	-	-	15.30	-
	Pn3-glu-vc	-	-	-	24.80	-
	Mv3-coum-glu	-	-	42.05	-	-
	Mv3-glu-vc, Mv3-coum-glu	-	-	-	489.90	-
	Pn3-acet-glu-vc or Pt3-acet-glu-vc	-	-	-	3.99	-
	Pn3-glu-vc	-	-	-	4.85	-
	Mv3-glu-vc, Mv3-acet-glu-vc	-	-	108.34	317.57	-
	Mv3-glu-vc	-	-	18.92	5.65	-
	Mv3-acet-glu-vc	-	-	16.27	66.95	-
Mv3-acet-glu-vc	-	-	4.15	-	-	
Mv3-coum-glu-vc	-	-	-	9.10	-	

¹⁾-, not detected 1A-1E; the 5 fractions of first separation in sequence (alphabetic order).

was identified as the direct condensed product between Mv3-glu and catechin (27). The compound of molecular ion and ion fragment at m/z 771, 463, and 447 was detected using MS, which was probably a mixture of Mv3-coum-glu-vc (771,463) and Mv3-caff-glu-vc (771,447). The compound of molecular ion and ion fragment at m/z 851 and 561 was tentatively identified as the Mv3-acet-glu-ethyl-flavanol (27), while the compound displaying the signal at m/z 985, 823, and 661 may be related to the pigment of Mv3-glu-propyl-cat for which the molecular ion was at m/z 823 (28). In addition, the compound of molecular ion and ion fragment at m/z 621 and 417 were identified as Pn3-acet-glu-vc (4). The mass spectrum information about these compounds is presented in Table 2.

Color of various fractions As can be seen from Table 3, the L* (lightness) value gradually decreased from fraction 1A to 1C, and then increased from fraction 1C to 1E. Conversely, the values of a* (presenting red) and C*ab (chroma) gradually increased and then decreased. The change of the b* (presenting yellow) value first decreased, then increased, then decreased again from fraction 1A to 1E, while H*ab (hue) value first decreased, then increased.

The change of these color parameters was in accordance with that of anthocyanins in these fractions. In fraction 1A, the anthocyanins mainly included Mv3-glu, Mv3-glu-pa, Mv3-glu-acetal, and Mv3-acet-glu, while the fraction 1B had Pt3-glu, Pn3-glu, Mv3-glu, Mv3-glu-acetal, Mv3-acet-glu-pa, and Mv3-acet-glu-acetal. Both types and contents (peak area expressed) of anthocyanins in fraction 1B were shown different from fraction 1A. As a result, the L*, b*, and H*ab values decreased and a* and C*ab values increased. Fraction 1C included the anthocyanins that were mainly Dp3-glu, Pt3-glu, Pn3-glu, Mv3-glu, Mv3-glu-acetal, Pt3-acet-glu, Mv3-acet-glu, Mv3-coum-glu, Mv3-glu-vc (Mv3-acet-glu-vc, coelution), Mv3-glu-vc, Mv3-acet-glu-vc, and Mv3-acet-glu-vc, and its L* value was lowest and the a*, b*, C*ab values were highest. As for fraction 1D, the anthocyanins monoglucoside decreased and the pyranoanthocyanins increased. The b* and H*ab values, as expected, increased and the L*, a*, and C*ab values decreased because pyranoanthocyanins made the wine a more yellow color than the anthocyanins monoglucoside. In fraction 1E, no anthocyanins was detected except the Mv3-glu, and the L* and H*ab values increased, while the a*, b*, and C*ab values decreased.

Table 4. The contribution of 23 anthocyanins to color parameters analyzed by partial least square regression¹⁾

Anthocyanins	Peak No.	L*	a*	b*	C*ab	H*ab
Mv3-acet-glu-vg	23	-0.5281	0.5408	0.3556	0.6386	-0.0570
Pn3-acet-glu-vc or Pt3-acet-glu-vp	18	-0.4292	0.4396	0.2890	0.5190	-0.0464
Pt3-acet-glu	8	-0.3510	0.3594	0.2363	0.4244	-0.0379
Dp3-glu-vp	11	-0.3238	0.3316	0.2180	0.3916	-0.0350
Dp3-glu-pa	13	-0.2273	0.2328	0.1531	0.2749	-0.0246
Pt3-glu-vp	14	-0.1674	0.1714	0.1127	0.2024	-0.0181
Pn3-glu-vp	19	-0.1496	0.1532	0.1007	0.1809	-0.0162
Pn3-glu-pa	4 ²⁾	-0.1232	0.1262	0.0830	0.1490	-0.0133
Mv3-glu-vg	21	-0.1203	0.1232	0.0810	0.1454	-0.0130
Mv3-coum-glu-vp	24	-0.1083	0.1109	0.0729	0.1309	-0.0117
Pn3-glu-vc	15	-0.0999	0.1024	0.0673	0.1209	-0.0108
Pt3-glu-vc	12	-0.0933	0.0955	0.0628	0.1128	-0.0101
Pn3-glu	3	-0.0838	0.0859	0.0564	0.1014	-0.0091
Dp3-glu	1	-0.0610	0.0625	0.0411	0.0738	-0.0066
Mv3-coum-glu	16	-0.0389	0.0398	0.0262	0.0470	-0.0042
Pt3-glu	2	-0.0386	0.0395	0.0260	0.0467	-0.0042
Mv3-acet-glu-vp	22	-0.0335	0.0343	0.0226	0.0405	-0.0036
Mv3-acet-glu	10	-0.0132	0.0136	0.0089	0.0160	-0.0014
Mv3-glu-vp, Mv3-acet-glu-vc	20	-0.0068	0.0070	0.0046	0.0082	-0.0007
Mv3-glu-vc, Mv3-coum-glu	17	-0.0053	0.0055	0.0036	0.0064	-0.0006
Mv3-glu-acetal	6	-0.0043	0.0044	0.0029	0.0053	-0.0005
Mv3-acet-glu-acetal	9	-0.0012	0.0013	0.0008	0.0015	-0.0001
Mv3-glu	4	-0.0005	0.0005	0.0005	0.0006	-0.0001

¹⁾These values were partial regression coefficients (unit: per peak area) which can compare the importance of anthocyanins (independent variables) to color parameters (dependent variables).

²⁾Detected in different fraction.

Similar results were also observed in other fractions (2A-E, 3A-E, 4A-D, data not shown)

Color of various anthocyanins in aqueous solution

Because of the multico-linearity, 23 anthocyanins in Table 1 (excluding the peaks No. 5 and 7) were selected into the PLS equations according to the sign of regression coefficients. The determination coefficients of equations between these anthocyanins and color parameters (L*, a*, b*, C*ab, and H*ab) were respectively 0.8273, 0.6937, 0.7745, 0.7833, and 0.0121. Apart from the last coefficient (0.0121), the 4 other determination coefficients were significant at level of 0.05. All these determination coefficients indicated that the anthocyanins detected can explain the 82.73, 69.37, 77.45, 78.33, and 1.21 percentage of color parameters respectively, while others undetected or unidentified anthocyanins, or intermolecular copigmentation, or other factors can explain the rest of the color parameters. Twenty-three anthocyanins could explain 69.37% of the color a* value, which is in accordance with the report of Versari *et al.* (7) that anthocyanins accounted for 70-92% of the color in aged red wines.

As can be seen in Table 4, all anthocyanins were negatively correlated with the L* and H*ab values, while positively correlated with the a*, b*, and C* values. It is suggested that as the contents of these anthocyanins increased, the color becomes darker (L* value), both the red and yellow color of anthocyanin solution (a* and b* value) and the chroma (C*ab) of it also increased. In

addition, pyranoanthocyanins presented higher yellow color than their corresponding parent anthocyanins. Thus, aqueous solution characterized by the more yellow color.

Effects of substituents in B-ring on color of anthocyanins

According to the partial regression coefficients of these anthocyanins to color parameters (Table 4), Pn3-glu made the largest partial regression coefficient to color parameters per unit of peak area (absolute values for L* and H*ab parameters) among Pn3-glu (-OCH₃, -OH, -H), Dp3-glu (-OH, -OH, -OH), Pt3-glu (-OCH₃, -OH, -OH), and Mv3-glu (-OCH₃, -OH, -OCH₃) (their structures see Fig. 1), followed by Dp3-glu and Pt3-glu, while Mv3-glu made the least. It is suggested that the hydroxyl group made greater contribution in decreasing the L* and H*ab values (absolute values) and increasing a*, b*, and C*ab values than methoxyl group because the effect of hydroxyl group (auxochrome) on the B-ring was greater than the methoxyl group (auxochrome). This result is in accordance with the reports of Heredia *et al.* (16), who observed that the absorbance decreases when hydroxyl groups are substituted by methoxyl groups.

When comparing the effect of 5'-substituents of Pn3-glu (3'-OCH₃, 4'-OH, 5'-H), Pt3-glu (3'-OCH₃, 4'-OH, 5'-OH), and Mv3-glu (3'-OCH₃, 4'-OH, 5'-OCH₃) for color, it can be found that the substituents of 5'-OH and 5'-OCH₃ on B-ring of anthocyanin increased less color of a*, b*, and C*ab values and decreased less color of the L* and H*ab values than that of 5'-H did. That is, the substituents of 5'

OH and 5'-OCH₃ decreased the color compared with that of 5'-H, and 5'-OCH₃ decreased more than 5'-OH. This suggests that the positions and types of substituents are also related to the color of anthocyanins. In addition, the proportion of different forms of anthocyanins in a solution also affect their color, and the colorless forms of Mv3-glu may comprise a higher proportion, so this pigment presents the least color values (16,29).

The Pn3-glu-vc and Pt3-glu-vc were formed by reaction of Pn3-glu and Pt3-glu with 4-vinylcatechol (vc), and the order of their color values was the same as their parent anthocyanins. That is, Pn3-glu-vc presents higher color values than Pt3-glu-vc. However, among the Dp3-glu-vp, Pt3-glu-vp, and Pn3-glu-vp, which were formed by reaction of Dp3-glu, Pt3-glu, or Pn3-glu with 4-vinylphenol (vp), the order of their color values (Dp3-glu-vp, Pt3-glu-vp, and Pn3-glu-vp) was inconsistent with that of their parent anthocyanins (Pn3-glu, Dp3-glu, and Pt3-glu). The order of color values of Dp3-glu-pa and Pn3-glu-pa was similar to that of the corresponding 4-vinylphenols pyranoanthocyanins.

All of these above mentioned indicated that the color of common anthocyanins (Pn3-glu, Dp3-glu, Pt3-glu, and Mv3-glu) depended, to a large extent, on the number, position and type of substituents on the B-ring. In addition, the pyrano-ring (D-ring) and the 4-vinylphenols of pyranoanthocyanins (Fig. 1) would also affect their color values.

Effect of acylation on color of anthocyanins From Table 4, it can be observed that among the Mv3-glu, Mv3-acet-glu, and Mv3-coum-glu, Mv3-coum-glu presented the largest color values (absolute values for L* and H*ab parameters), followed by the Mv3-acet-glu, then Mv3-glu. The color values of Pt3-acet-glu was larger than that of Pt3-glu, and Mv3-acet-glu-vg larger than Mv3-glu-vg, which indicated that the acyls would increase the color of anthocyanins, and the coumaryl contributed more than acetyl. This was probably because the aroma ring of the coumaryl stacked with the chromophore of anthocyanins to give stronger intramolecular copigmentation (6,15,30-33).

Effect of pyrano-ring on color of pyranoanthocyanins As can be seen from Table 4, among Mv3-glu, Mv3-glu-acetal, and Mv3-glu-vg (Fig. 1), Mv3-glu-vg presented the highest color values (absolute values for L* and H*ab parameters), then Mv3-glu-acetal and Mv3-glu. Apart from Mv3-acet-glu-acetal, Mv3-acet-glu-vg, and Mv3-acet-glu-vp presented larger color values than did their corresponding parent anthocyanins, while the color values of Mv3-coum-glu-vp were higher than that of Mv3-coum-glu. The pyranoanthocyanins of Dp3-glu, Pn3-glu, and Pt3-glu also presented higher color values (absolute values for L* and H*ab parameters) than themselves. These thus suggested that the pyranoanthocyanins decreased more the L* and H*ab values and increased more the a*, b*, and C*ab values (10).

The color values of Mv3-glu-vg, Dp3-glu-vp, and Pn3-glu-vp were higher than that of Mv3-glu-acetal, Dp3-glu-pa, and Pn3-glu-pa (absolute values for L* and H*ab parameters), but the Pn3-glu-vc was lower than Pn3-glu-pa. This suggests that the E-ring would further affect the color values of 4-vinylphenols pyranoanthocyanins. In

addition, Mv3-acet-glu-vg, Pn3-glu-vp, and Pt3-glu-vp presented the higher color values than their corresponding anthocyanins Mv3-acet-glu-vp, Pn3-glu-vc, and Pt3-glu-vc. Therefore, it may be speculated that the effect of methoxyl group and hydroxyl group on the E-ring (4-vinylguaiacol) to color > the effect of hydroxyl group on the E-ring (4-vinylphenol) to color > the effect of 2 hydroxyl groups on the E-ring (4-vinylcatechol) to color. This speculation was different from the contribution of substituent on the B-ring to color, and needs more research to further investigate.

Analysis of PLS showed that the hydroxyl group on the B-ring had greater effect on color values than the methoxyl group and the substituents (-OH, OCH₃) at C5' would decrease the color of anthocyanins; the coumaryl group addition on anthocyanins would increase more the color of anthocyanins than acetyl addition; pyranoanthocyanins presented the more color than their corresponding original anthocyanins.

The results obtained in this work can help to understand the color changing of anthocyanins and wine by different color values that was obtained by PLS. More research needs to be done for further validating and investigating the color change mechanism of anthocyanins and wine.

Acknowledgments

This research was supported by "948" Research Programs of China Ministry of Agriculture (Grant No. 2006-G26 to C.-Q.D).

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