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Phenolic Profiles of *Vitis davidii* and *Vitis quinquangularis* Species Native to China

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ABSTRACT: The accumulation of phenolics in the skins of two *Vitis davidii* cultivars ('Ziqiu' and 'Xiangzhenzhu') and one *Vitis quinquangularis* cultivar ('Xiangshan No. 4') native to China was followed during ripening. It was found that the anthocyanin composition of all three grapes was dominated by anthocyanidin 3,5-*O*-diglucosides. The cultivar 'Xiangshan No. 4' (*V. quinquangularis*) contained a high level of 3′,4′-substituted anthocyanins and low levels of flavonols and 3′,4′-substituted flavan-3-ols, indicating that the F3′H branch pathway was the principal carbon pathway synthesizing mainly 3′,4′-substituted anthocyanins. No myricetin-type flavonols were found in this cultivar, but in both 'Ziqiu' and 'Xiangzhenzhu' (*V. davidii*) cultivars the F3′5′H branch pathway was dominant, resulting in malvidin-based diglucoside anthocyanins. Cyanidin-based and petunidin-based anthocyanins were not detected in the 'Ziqiu' cultivar. Principal component analysis revealed that *V. davidii* grapes had abundant flavonols by the early middle developmental stages and a high level of malvidin-type anthocyanins by the late developmental stage. In contrast, the *V. quinquangularis* cultivar contained other anthocyanins instead of malvidin-type anthocyanins.

KEYWORDS: *anthocyanins, flavonols, flavan-3-ols, Vitis davidii, Vitis quinquangularis*

INTRODUCTION

China has very abundant *Vitis* germplasms in diverse species, which are distributed extensively within the country. Among them, some native species, such as *Vitis davidii*, *Vitis quinquangularis*, and *Vitis amurensis*, have relatively strong disease resistance and good adaptability to local climatic conditions.[1−3] *V. davidii* grapes were originally grown in subtropical areas, such as the Yunnan-Guizhou Plateau and the Yangtze River Basin, and were typically found in valleys at <1500 m altitude. The high-yielding vines have large stems and long ear-like leaves (above 200 mm), and the late ripening berries have thick dark-red skins. The wines produced from *V. davidii* grapes are dark purple or ruby red and have a distinct aroma reflecting the taste of the variety. *V. quinquangularis*, known locally as the pentagon-leafed grape, is distributed south of the Yellow River in regions that have sufficient sunshine and are at an altitude of <1500 m. The grape berries of *V. quinquangularis* ripen with a low sugar content and high acid but a dark-colored skin. The wines have a characteristic varietal aroma and pronounced acid and tannic taste.[1] Both *V. davidii* and *V. quinquangularis* grapes can resist disease, but the species have lower cold resistances.[1−3] The vines are susceptible to downy mildew disease, but they have strong resistance to powdery mildew and botrytis. In addition, these two species possess good adaptability to high-temperature and high-humidity conditions.[1,2] Cultivation of *V. davidii* and *V. quinquangularis* grapes have been rapidly expanding, and it is expected that high-quality wines will be produced. However, the flavor compounds in these indigenous Chinese *Vitis* have yet to be fully determined.

Phenolics are critical components in relation to grape and wine quality. They have several functions in plants including protection from UV radiation, defense against invading pathogens, pigmentation, and attraction of pollinators.[4,5] Grape phenolics can be classified as flavonoids and non-flavonoids. The former has a common C6C3C6 structure, whereas the latter group has C6C1, C6C3, or C6C2C6 structures. Both classes are synthesized from phenylalanine through phenylpropanoid metabolism and flavonoid metabolism. The majority of nonflavonoids found in grapes are composed of hydroxycinnamic acids, hydroxybenzonic acids, and stilbenes, and these compounds are localized in both skin and pulp, which are considered to be the major constituents of pulp.[6] Only 20−25% of phenolic acids are present in free forms, and the majority of them exist as esters with glycosides or organic acids.[7,8] The hydroxybenzoic acids and hydroxycinnamic acids in grapes have aroused great interest due to possible health benefits, such as antibacteria and anti-inflammatory activities.[9] Flavonoids in grapes are mainly composed of three classes: flavonols, flavan-3-ols, and anthocyanins. Flavonols in grapes predominantly consist of three basic aglycons, quercetin, kaempferol, and myricetin, with the 3-*O*-glucosides being the main derivatives followed by 3-*O*-galactosides and 3-*O*-glucuronides.[10,11] Flavonols are important cofactors for color enhancement in copigmentation and also act as a natural sunscreen in the skins of grape berries.[12,13] Flavan-3-ols are the basic building block for grape tannins.[14] The monomers and oligomers of flavan-3-ols contribute bitterness to wine, and their polymers contribute to astringency.[15] The

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anthocyanins comprise 3-*O*-monoglucosides and, in some non-*vinifera* varieties, 3,5-*O*-diglucosides as well, of cyanidin, delphinidin, petunidin, peonidin, and malvidin, together with the corresponding acetyl, *p*-coumaroyl, and caffeoyl derivatives.[16,17] They impart red, purple, and black colors to grapes.[15] Anthocyanins can be degraded to give lower molecular weight molecules or transformed into more stable pigments during winemaking and aging.[18] These flavonoids are synthesized by one of two branch pathways: one is mediated by flavonoid 3′-hydroxylase (F3′H) to produce 3′-hydroxylated flavonoids with a B-ring dihydroxyl group at the C3′ and C4′ positions, such as quercetin-type flavonols and cyanidin-type anthocyanins; the other is mediated by flavonoid 3′,5′-hydroxylase (F3′5′H) to produce 3′,5′-hydroxylated flavonoids with a trihydroxylated B-ring with an additional hydroxyl group at the C5′ position, as found in myricetin-type flavonols and delphinidin-type anthocyanins.[19] Because phenolics are not only highly associated with the quality of grape and wines but also because the antioxidant properties are beneficial for human health,[4,5] knowing the composition and content of various phenolic components of wild grape species will enable their better utilization.

Several previous studies on wild grape germplasms have dealt only with the quantitative determination of total anthocyanin content.[20,21] There are also several studies focusing on different anthocyanin fingerprints of wild genotypes of *V. vinifera* from different regions.[22,23] Less attention was paid to the comprehensive analysis of phenolics profiles in indigenous grapes, especially poor in the developmental pattern of phenolics over the whole growth period. To the best of our knowledge, the evolution of individual phenolic compounds in *V. davidii* and *V. quinquangularis* grapes during maturation has not yet been elucidated.

In the present paper, the composition and content of phenolics in the berry skins of 'Ziqiu' (*V. davidii*), 'Xiangzhenzhu' (*V. davidii*), and 'Xiangshan No. 4' (*V. quinquangularis*) native to China was investigated by means of HPLC-DAD-ESI-MS/MS, with an aim of leading to an increased appreciation of Chinese grape germplasms and a better understanding of their possible contribution to the utilization of grape phenolics.

MATERIALS AND METHODS

Materials. Berries of 'Ziqiu' (*V. davidii*) and 'Xiangzhenzhu' (*V. davidii*) were collected fortnightly from 4 weeks after flowering until commercial harvest in 2009 in the experiment station of Hunan Agricultural University, midsouth China (8.12° N, 112.59° E). The vineyard of *V. davidii* grapes is located in a subtropical monsoon climate with abundant sunshine, an annual frost-free period of about 275 days, an average temperature of 17.0 °C, and an annual rainfall of 1422.4 mm. In 2009 berries of 'Xiangshan No. 4' (*V. quinquangularis*) were sampled every other week from 5 weeks after flowering until commercial harvest in the vineyard of Horticulture Research Institute of Guangxi Academy of Agricultural Sciences, south China (22.84° N, 108.33° E). The vineyard of *V. quinquangularis* grapes is located south of the Tropic of Cancer with a humid subtropical monsoon climate, plenty of sunshine, and abundant rainfall. The major climate condition of the *V. quinquangularis* grapes vineyard is characterized by hot and humid with little frost, an annual average temperature of 21.6 °C, an average relative humidity of 79%, and an average annual rainfall of 1304.2 mm. Veraison occurred at 10 week after flowering in *V. quinquangularis* and at 11 weeks after flowering in the two *V. davidii* cultivars in the same year. Commercial harvesting was done at 15 weeks after flowering for the *V. quinquangularis* cultivar and 1 week later for the two *V. davidii* cultivars. Seven samples of the *V. davidii* cultivar and six samples for the *V. quinquangularis* cultivar were obtained for analysis during fruit development in all. Despite being in different regions, similar viticultural management practices with respect to fertilization, irrigation, pruning, and disease control were used.

Approximately 300 berries in total were selected at random from at least 30 grapevines in different rows for each cultivar, excluding the edge rows and the first few vines in every row. About 80−100 berries within each variety from different positions were considered as one replicate resulting in three replications. The berries were immediately transported on ice to the laboratory. The skins were stripped off manually, rapidly frozen in liquid nitrogen, ground under liquid nitrogen to a fine powder, and then lyophilized for 24 h at −50 °C using an LGJ-10 freeze-dryer. The dried powder samples were stored at −40 °C for subsequent extraction.

Reagents and Standards. Quercetin, quercetin-3-*O*-glucoside, quercetin-3-*O*-galactoside, kaempferol-3-*O*-glucoside, gallic aid, caffeic acid, *trans*-resveratrol, (+)-catechin, (−)-epicatechin, (−)-epigallocatechin, (−)-epicatechin-3-*O*-gallate, cyanidin-3-*O*-glucoside, peonidin-3-*O*-glucoside, delphinidin-3-*O*-glucoside, malvidin-3-*O*-glucoside, and phloroglucinol standards were purchased from Sigma Chemical Co. (St. Louis, MO, USA). Methanol, formic acid, acetonitrile, and acetic acid (HPLC grade) were obtained from the Fisher Co. (Fair Lawn, NJ, USA). Ethyl acetate, ascorbate, and acetone (analytical grade) were sourced from Beijing Chemical Reagent Plant (Beijing, China). Deionized water was from Wahaha Co. (Hangzhou, China). All other chemicals were purchased from Sigma-Aldrich Co. unless otherwise noted.

Extraction of Phenolic Compounds from Grape Skins. The extraction of anthocyanins in grape skins was carried out according to a previously published method in the laboratory.[24] Grape skin powder (0.5 g) was suspended in 10 mL of methanol solution containing 2% formic acid and extracted with ultrasound for 10 min followed by shaking in the dark for 30 min at 25 °C. The residue was repeatedly extracted with the same solvent four times. The organic fraction was pooled, evaporated by a vacuum rotary evaporator, and finally redissolved in 10 mL of 10% ethanol solution (pH 3.7).

The extraction of nonanthocyanin phenolic compounds (flavonols, hydroxybenzoic acids, hydroxycinnamic acids, and stilbenes) was made according to the method described in the previous paper.[25] Powdered grape skin (2.00 g) was macerated with 5 mL of distilled water in advance and then mixed with 45 mL of ethyl acetate in darkness for 30 min. The extraction with ethyl acetate was repeated four more times. The pooled extracts were evaporated to dryness by a rotary evaporator at 30 °C, and the remaining residue was redissolved in 2 mL of methanol.

To determine total content of various flavan-3-ol units, acid cleavage was conducted in the presence of excess phloroglucinol to obtain the component units of their oligomers and polymers.[26] For this, 0.1 g of grape skin was mixed with 1 mL of phloroglucinol buffer (0.5 g of phloroglucinol, 258 μL of concentrated HCl, 10 mL of methanol), extracted in a 70 °C water bath for 20 min, and subsequently neutralized with 1 mL of sodium acetate (200 mM, pH 7.5). This extraction procedure was repeated twice. After that, the supernatants were combined and stored at low temperature for analysis. For free flavan-3-ol monomers, to 0.1 g of the dried grape skin was added 0.005 g of ascorbic acid to protect against oxidation, and then the mixture was mixed with 1 mL of extraction solution (acetone/water, 7:3, v/v) and centrifuged for 15 min (8000*g*) at low temperature. This was repeated, and the combined supernatants of 400 μL were dried rapidly, resuspended in 200 μL of methanol (1% HCl), and then neutralized with 200 μL of sodium acetate (200 mM, pH 7.5).

All of the samples were filtered through 0.22 μm filters prior to HPLC analysis.

Analysis of Phenolic Compounds. Analysis of phenolic compounds was carried out using an Agilent 1200 series high-performance liquid chromatograph (HPLC) equipped with a G1322A degasser, a G1312B bin pump, a G1367C HiP-ALS, a G1316B TCC, and a G1314C VWD. The mass spectrometric acquisition parameters were as follows: nebulizer pressure, 30 psi; dry gas flow, 10 mL/min;

Table 1. Anthocyanins Found in the Skins of 'Ziqiu' (*V. davidii*), 'Xiangzhenzhu' (*V. davidii*), and 'Xiangshan No. 4' (*V. quinquangularis*) during the Sampling Period[a]

retention time t_R (min)	anthocyanin	$[M]^+$(fragment-MS^2) (m/z)	ref(s)	Ziqiu	Xiang-zhenzhu	Xiangshan No. 4
2.63	delphinidin-3,5-*O*-diglucoside	627 (465, 303)	20, 27, 33	−	+	+
3.41	cyanidin-3,5-*O*-diglucoside	611 (449, 287)	20, 27, 33	−	−	+
3.63	petunidin-3,5-*O*-diglucoside	641 (479, 317)	20, 27, 33	−	+	+
3.99	delphinidin-3-*O*-monoglucoside	465 (303)	20, 27, 28, 30, 33, 34	−	−	+
5.18	peonidin-3,5-*O*-diglucoside	625 (463, 301)	20 27 33,	+	+	+
5.50	malvidin-3,5-*O*-diglucoside	655 (493, 331)	20, 27, 33	+	+	+
6.32	delphinidin-3-*O*-acetylglucoside-5-*O*-glucoside	669 (507, 465, 303)	33	−	−	+
7.02	cyanidin-3-*O*-monoglucoside	449 (287)	20, 27, 28, 30, 33, 34	−	+	+
7.55	peonidin-3-*O*-monoglucoside-pyruvid acid	531 (463, 369)	28, 42	+	+	−
7.79	cyanidin-3-*O*-acetylglucoside-5-*O*-glucoside	653 (611, 449, 287)	20	−	−	+
7.84	peonidin-3-*O*-caffeoylglucoside	625 (463, 301)	20, 27	−	+	−
8.28	petunidin-3-*O*-acetylglucoside-5-*O*-glucoside	683 (641, 479, 317)	20	−	−	+
9.25	peonidin-3-*O*-monoglucoside	463 (301)	20, 27, 28, 30, 33, 34	−	−	+
10.28	malvidin-3-*O*-monoglucoside	493 (331)	20, 27, 28, 30, 33, 34	+	+	−
11.43	delphinidin-3-*O*-*cis*-coumaroylglucoside-5-*O*-glucoside	773 (627, 611, 465, 303)	20, 27, 33	−	−	+
11.80	malvidin-3-*O*-acetylglucoside-5-*O*-glucoside	697 (655, 535, 493, 331)	20	+	+	−
13.77	delphinidin-3-*O*-*trans*-coumaroylglucoside-5-*O*-glucoside	773 (627, 611, 465, 303)	20, 27, 33	+	+	+
17.44	cyanidin-3-*O*-coumaroylglucoside-5-*O*-glucoside	757 (611, 595, 449, 287)	20, 33	−	−	+
17.55	malvidin-3-*O*-monoglucoside-acetaldehyde	517 (355)	28, 42	−	+	−
17.99	malvidin-3-*O*-caffeoylglucoside-5-*O*-glucoside	817 (655, 493, 331)	20	+	−	−
18.11	petunidin-3-*O*-coumaroylglucoside-5-*O*-glucoside	787 (641, 625, 479, 317)	20, 27, 33	−	+	+
18.95	peonidin-3-*O*-*trans*-coumaroylglucoside-5-*O*-glucoside	771 (625, 609, 463, 301)	20, 27, 33	−	−	+
19.49	malvidin-3-*O*-*cis*-coumaroylglucoside-5-*O*-glucoside	801 (655, 639, 493, 331)	20, 27, 33	+	+	−
19.63	delphinidin-3-*O*-coumaroylglucoside	611 (465, 303)	27, 28, 30, 33, 34	−	−	+
21.69	malvidin-3-*O*-*trans*-coumaroylglucoside-5-*O*-glucoside	801 (655, 639, 493, 331)	20, 27, 33	+	+	+
23.21	cyanidin-3-*O*-coumaroylglucoside	595 (449, 287)	20, 27, 28, 30, 33, 34	−	−	+
23.54	malvidin-3-*O*-caffeoylglucoside	655 (493, 331)	20, 30, 34	+	+	−
28.24	peonidin-3-*O*-coumaroylglucoside	609 (463, 301)	20, 27, 28, 30, 33, 34	−	−	+
28.77	malvidin-3-*O*-coumaroylglucoside	639 (493, 331)	20, 27, 28, 30, 33, 34	+	+	+

[a]'+' indicates that the compound was detected in at least one sampling time. '−' indicates that the compound was not detected during any sampling period.

dry gas temperature, 325 °C; scan at m/z 100−1000. The ion trap mass spectrometer was operated in positive ion mode for anthocyanin and in negative ion mode for nonanthocyanin phenolic compounds.

Anthocyanin extract (30 μL) was injected onto a Kromasil C18 column (250 × 4.6 mm, 5 μm).[24] The mobile phase was composed of (A) 6% (v/v) acetonitrile containing 2% (v/v) formic acid and (B) 54% acetonitrile containing 2% (v/v) formic acid. The following gradient elution was applied: 10% B for 1 min, from 10 to 25% B for 17 min, isocratic 25% B for 2 min, from 25 to 40% for 10 min, from 40 to 70% for 5 min, from 70 to 100% for 5 min. Other conditions were as follows: flow rate, 1.0 mL/min; detection wavelength, 525 nm; and column temperature, 50 °C.

Nonanthocyanin phenolic compounds were assessed by using (A) 1% (v/v) acetic acid in water and (B) 1% (v/v) acetic acid in acetonitrile gradient at a flow rate of 1.0 mL/min.[25] The selected column was a Zorbax SB-C18 column (50 × 3 mm, 1.8 μm), and the column temperature was maintained at 25 °C. The injection volume was 2 μL, and the detection wavelength was 280 nm. The gradient conditions were as follows: 0 min, 5% B; 10 min, 8% B; 18 min, 10% B; 40 min, 15% B; 50 min, 20% B; 53 min, 30% B; 58 min, 50% B; 62−66 min, 100% B.

Flavan-3-ol units (25 μL) were separated on a reversed phase column (Zorbax SB-C18, 250 × 4.6 mm, 5 μm) at a flow rate of 1 mL/min and monitored at 280 nm at 25 °C.[26] Mobile phase A was 0.2% (v/v) acetic acid in water, and mobile phase B was 4:1 acetonitrile/0.2% acetic acid. The elution conditions were as follows: 0 min, 10%; 20 min, 10%; 30 min, 15%; 40 min, 20%; 50 min, 33%; 55 min, 40%; 58 min, 100%; 63 min, 100%; 64 min, 10%.

Qualitative and Quantitative Analysis of Phenolic Compounds. Some compounds were identified after comparison of MS information, elution order, and retention times to those of the commercially available standards, including quercetin, quercetin-3-*O*-glucoside, quercetin-3-*O*-galactoside, kaempferol-3-*O*-glucoside, gallic aid, caffeic acid, *trans*-resveratrol, (+)-catechin, (−)-epicatechin, (−)-epigallocatechin, (−)-epicatechin-3-*O*-gallate, cyanidin-3-*O*-glucoside, peonidin-3-*O*-glucoside, delphinidin-3-*O*-glucoside, and malvidin-3-*O*-glucoside. The remaining phenolic compounds were identified mainly by comparing molecular ions, product ions, and the elution orders of these compounds with those available in the published literature.[10,11,27−29]

The quantification of phenolic compounds was obtained by the use of external standards. The relative content of each anthocyanin, flavonol, hydroxybenzoic acid, hydroxycinnamic acid, or stilbene compound was obtained as the equivalent of malvidin-3-*O*-glucoside, quercetin, gallic acid, caffeic acid, and *trans*-resveratrol, respectively, using HPLC peak areas at the detection wavelength. Quantification of the flavan-3-ols was based on (+)-catechin, (−)-epicatechin, (−)-epigallocatechin, and (−)-epicatechin-3-*O*-gallate standards.

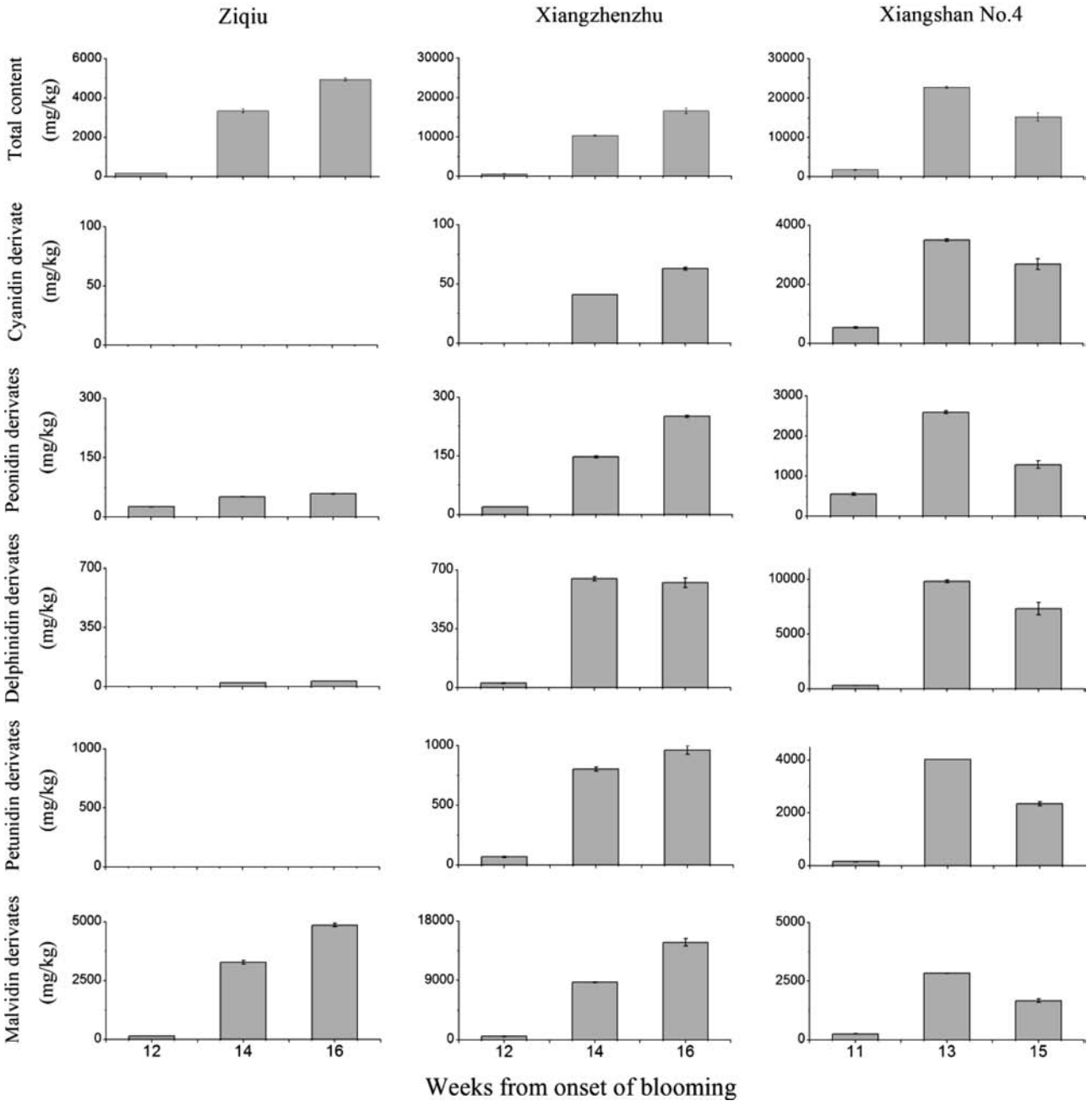

Figure 1. Accumulation of five types of anthocyanins in the skins of 'Ziqiu' (*V. davidii*), 'Xiangzhenzhu' (*V. davidii*), and 'Xiangshan No. 4' (*V. quinquangularis*) during berry maturation.

The method of calculating the mean degree of polymerization (mDP) was performed according to a previously published method in our laboratory, and proanthocyanidin was analyzed by acid cleavage.[26] The flavan-3-ol terminal subunits were released from the proanthocyanidin oligomers and polymers, and the flavan-3-ol extension subunits were released as their corresponding flavan-3-ol phloroglucinol adducts. The content of the terminal subunits was calculated by subtracting the content of the free monomers in the non-acid-cleaved sample from the total free monomers of the acid-cleaved sample. The mDP was calculated as the ratio of the total terminal plus the total extension subunits divided by terminal subunits.

Statistical Analyses. SPSS 11.5 software (Chicago, IL, USA) was used for statistical analyses. Each data point, expressed as milligram equivalent of the respective standard per kilogram of dried grape skin, was the average of three replications.

RESULTS AND DISCUSSION

Comparison of Anthocyanins in the Three Grape Cultivars. A total of 29 anthocyanins were found from the grape skins of 'Ziqiu', 'Xiangzhenzhu', and 'Xiangshan No. 4' during their development (Table 1). These included 4 anthocyanidin monoglucosides, 5 anthocyanidin diglucosides, 6 acyl anthocyanidin monoglucosides, 12 acyl anthocyanidin diglucosides, and 2 polymeric anthocyanidin monoglucosides. Of the ones in Table 1, 17 anthocyanins are 3,5-*O*-diglucosides. The majority of them were acetylated and coumarylated at the C3′-position, and a minority was caffeoylated. This is quite different from *V. vinifera* grapes, the species most extensively used in the global wine industry. The composition of anthocyanins in *V. vinifera* grapes has been characterized by

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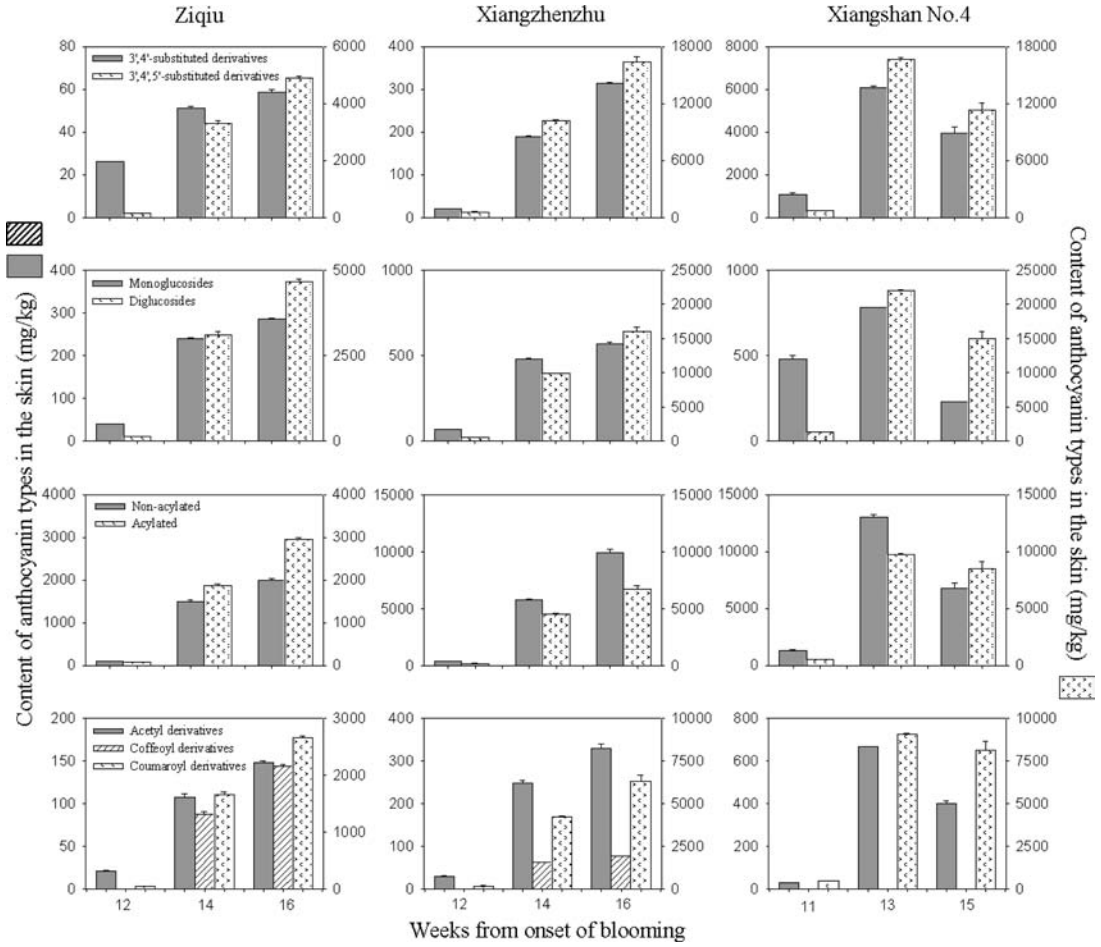

Figure 2. Accumulation of various groups of anthocyanins in the skins of 'Ziqiu' (*V. davidii*), 'Xiangzhenzhu' (*V. davidii*), and 'Xiangshan No. 4' (*V. quinquangularis*) during berry maturation. The scale of the gray columns and the patterned columns is at the left and that of the white columns with arrows is at the right.

their trace amount of diglucosides.[30,31] However, another grape species native to China, *V. amurensis*, has also been demonstrated to be rich in anthocyanidin diglucosides, without any acyl diglucosides and acyl monoglucosides being detected.[32] The *V. amurensis* vines are mostly distributed in northeastern China and show strong tolerance to temperatures as low as −40 °C. Besides, the *Vitis labrusca* grapes, native to northern America, contain plenty of anthocyanidin diglucosides and anthocyanidin monoglucosides with a high proportion of coumaroylated derivaties and a small amount of acetylated derivatives.[33] Furthermore, anthocyanidin diglucosides are greater in abundance than anthocyanidin monoglucosides in both *Vitis riparia* and *Vitis rupestris* grapes.

Of the 29 anthocyanins found, 5 were cyanidin (Cy), 6 peonidin (Pn), 6 delphinidin (Dp), 3 petunidin (Pt), and 9 malvidin (Mv) derivatives. Of the 29, 11 were found in 'Ziqiu', 16 in 'Xiangzhenzhu', and 21 in 'Xiangshan No. 4'. In the 'Ziqiu' skins there were derivatives of Mv, Pn, and Dp, with 4 being monoglucosides and 7 diglucosides. They included acylated and nonacylated mono- and diglucosides of Mv together with Pn-3,5-*O*-diglucoside, Pn-3-*O*-monoglucoside-pyruvic acid, and Dp-3-*O*-*trans*-coumaroylglucoside-5-*O*-glucoside. No Cy derivatives or Pt derivatives have been detected. Of the 16 anthocyanins found in the skins of 'Xiangzhenzhu', 10 anthocyanins were common with those found in 'Ziqiu', and the missing one was Mv-3-*O*-caffeoylglucoside-5-*O*-glucoside. Both of the *V. davidii* cultivars contained a wide variety of Mv derivatives and a few Cy, Dp, Pt, and Pn derivatives, which may be a characteristic of *V. davidii*. In contrast, 'Xiangshan No. 4' fruits were high in various derivatives of Cy, Dp, Pt, and Pn, but had only a few Mv derivatives. Of the 21 anthocyanins found in 'Xiangshan No. 4', only 3 Mv derivatives were found, being Mv-3,5-*O*-diglucoside, Mv-3-*O*-coumaroyl glucoside, and Mv-3-*O*-*trans*-coumaroyl glucoside-5-*O*-glucoside, respectively. No caffeoyl anthocyanins were found in 'Xiangshan No. 4' grapes.

All of the anthocyanins found in the three grape cultivars were classified into five groups according to their anthocyanidin unit. The evolution of total anthocy-anins and five anthocyanidin derivatives during berry maturation is shown in Figure 1. The total anthocyanin content in 'Ziqiu' grapes was much lower than in the other two cultivars. Apart from Cy derivatives and Pt derivatives absent in 'Ziqiu' grapes during the sampling period, other anthocyanidin derivatives and the total content of anthocyanins in the two *V. davidii* cultivars showed similar trends with a gradual increase during berry maturation. Correspondingly, the five groups of anthocyanins in 'Xiangshan No. 4' grapes all showed an increase followed by a decrease, as did the total anthocyanin content, which means that the rate of anthocyanin degradation must have been greater than the rate of biosynthesis during the late maturation stage of 'Xiangshan No. 4' (Figure 2). Furthermore, the content of Mv derivatives in either 'Ziqiu' or 'Xiangzhenzhu' was much greater than in

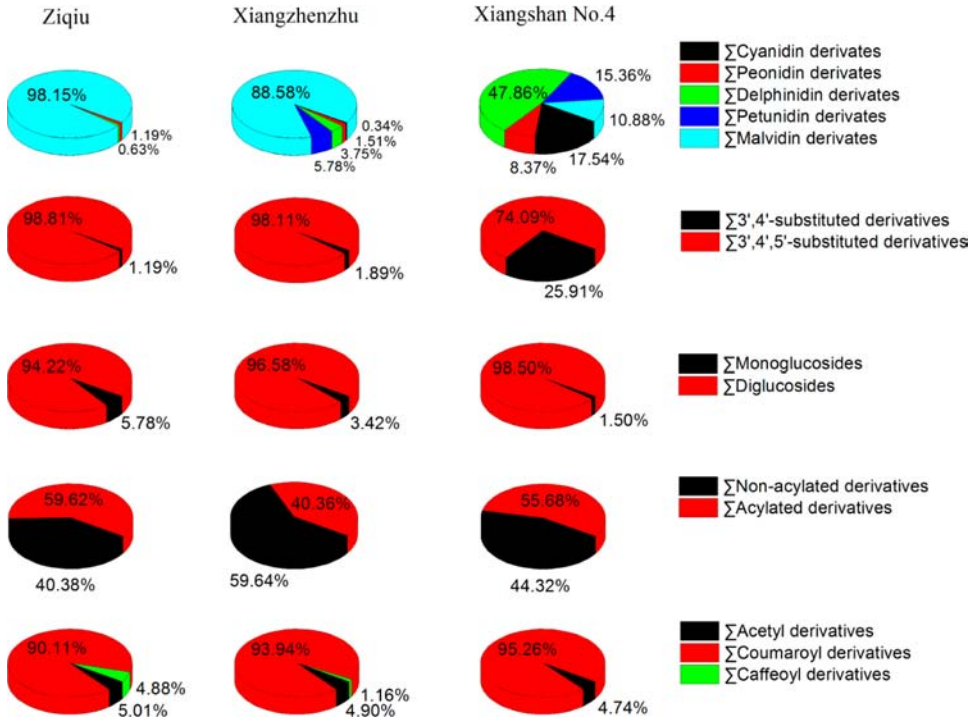

Figure 3. Percentages of various groups of anthocyanins to total anthocyanins in the skins of 'Ziqiu' (*V. davidii*), 'Xiangzhenzhu' (*V. davidii*), and 'Xiangshan No. 4' (*V. quinquangularis*) at commercial harvest date.

'Xiangshan No. 4', whereas four other groups of anthocyanidin derivatives all showed significantly lower levels when compared with those in the 'Xiangshan No. 4' grapes (Figure 3). The changes in the five groups of anthocyanidin derivatives were due to the dramatic variation in their diglucosides and coumaroyl derivatives. In contrast to these native Chinese grape cultivars, the anthocyanin content in *V. vinifera* grapes generally shows an continuing increase until commercial ripeness, due to a continuous increase of monoglucosides and acyl anthocyanins, especially Mv-3-*O*-monoglucoside, Mv-3-*O*-acetylmonoglucoside, and Mv-3-(6-*O*-*trans*-coumaroyl)-monoglucoside.[34] The maximum absorption wavelength of various Dp, Pt, and Mv derivatives that had trisubstituted B rings ranged from 518 to 520 nm, corresponding to the area of red-purple hues in the CIELab space, whereas Cy and Pn derivatives with disubstituted B rings had maxima at approximately 512 nm, corresponding to the orange area.[35] Additionally, anthocyanins with more methoxyl groups displayed a hypsochromic shift moving toward purple hues.[35] Therefore, it is suggested that the resulting wine from the two *V. davidii* cultivars with their abundant Mv derivatives would display a more purple hue.

To assist with understanding the influence of anthocyanin composition, we grouped these anthocyanins into different types according to their substitution patterns. As shown in Figure 2, the anthocyanins found have been classified into two groups according to the B-ring substitution (3′,4′ and 3′,4′,5′), whereas the monoglucosides and diglucosides relate to the level of glycosylation of anthocyanins. They were also classified into nonacylated and acylated forms according to the absence or presence of acyl moieties on the glucosides and further classified into acetyl, caffeoyl, and coumaroyl derivatives on the basis of the acylated molecule. Regardless of the grouping, the various anthocyanin groups in 'Ziqiu' and 'Xiangzhenzhu' grapes all showed a continuous increase with berry maturation, and those in 'Xiangshan No. 4' grapes exhibited a sharp increase followed by a decline (Figure 2). In the two *V. davidii* cultivars, the content of 3′,4′,5′-substituted derivatives was at least 80-fold that of 3′,4′-substituted derivatives, but the proportion of 3′,4′,5′-substituted to 3′,4′-substituted derivatives in the content was only about 3-fold in 'Xiangshan No. 4' grapes. These three cultivars were all characterized by extremely high levels of anthocyanidin diglucosides relative to monoglucosides, with similar levels of nonacylated and acylated anthocyanins, and very high levels of coumaroyl anthocyanins. Unlike *V. vinifera* grapes, acetyl derivatives represented a prominent proportion of the acyl anthocyanins in most cases.[36] Additionally, the acylation of anthocyanins causes the color shift and the variation in hue.[37] When compared to nonacetylated derivatives, the acetylated anthocyanins displayed a bathochromic effect shifting slightly toward an orange hue, whereas the coumaroyl anthocyanins showed a hypsochromic effect shifting toward a purple hue.[37] Hence, these three cultivars studied here may have a more intense pigmentation (hyperchromic effect) with a higher proportion of coumaroyl anthocyanins.

At commercial harvest ripeness, there was a great difference in the proportion of 3′,4′,5′-substituted anthocyanins to the sum between *V. davidii* species and *V. quinquangularis* species, but only a minor difference between the two *V. davidii* cultivars (Figure 3). The 3′,4′,5′-substituted anthocyanins, of the Dp, Pt, and Mv derivatives, accounted for >98% of the total anthocyanin content of 'Ziqiu' and 'Xiangzhenzhu' and for approximately 74% in 'Xiangshan No. 4'. It should be noted here that the 3′,4′,5′-substituted anthocyanins in the 'Ziqiu' cultivar were principally Mv derivatives and a trace amount of Dp-3-*O*-*trans*-coumaroyl glucoside-5-*O*-glucoside. The percentage of Mv derivatives reached 88% in the 'Xiangzhenzhu' cultivar, but only 11% in the 'Xiangshan No. 4' grapes. The 3′,4′-substituted and 3′,4′,5′-substituted anthocyanins were

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Table 2. Individual Nonanthocyanin Phenolic Compound from the Grape Skins of 'Ziqiu' (*V. davidii*), 'Xiangzhenzhu' (*V. davidii*), and 'Xiangshan No. 4' (*V. quinquangularis*) during the Sampling Period[a]

retention time t_R (min)	nonanthocyanin phenolic compd	$[M]^+$(fragment-MS^2)(m/z)	ref(s)	Ziqiu	Xiangzhenzhu	Xiangshan No. 4
	flavonols					
7.57	dihydrokaempferol-3-*O*-glucoside	449 (287)	42	+	+	+
17.97	dihydrokaempferol-3-*O*-rhamnoside	433 (269, 179, 151)	42	+	+	−
24.83	kaempferol-3-*O*-galactoside	447 (285)	10, 11, 33	+	+	−
25.73	kaempferol-3-*O*-glucoside	447 (285, 255, 327)	10, 11, 33	−	+	−
29.02	kaempferol-3-*O*-rutinoside	593 (285)	42	+	+	−
36.26	kaempferol-3-*O*-rhamnoside	431 (285)	42	+	+	−
17.25	kaempferol-*O*-hexoside	447 (285)	42	−	−	+
12.70	dihydroquercetin-*O*-hexoside	465 (339, 151, 447)	42	+	+	+
20.29	quercetin-3-*O*-galactoside	463 (301)	10 11 33,	+	+	−
20.94	quercetin-3-*O*-glucuronide	477 (301)	11, 33	+	+	+
22.17	quercetin-3-*O*-glucoside	463 (301)	10, 11, 33	+	+	+
22.73	quercetin-3-*O*-rutinoside	609 (301)	11, 33	+	+	−
23.04	quercetin-*O*-xyloside	433 (301)	42	+	+	−
31.61	dihydroquercetin-*O*-xyloside	435 (285, 303, 151)	42	+	−	−
28.34	quercetin-3-*O*-rhamnoside	447 (301)	11, 33	+	+	−
39.17	isorhamnetin-3-*O*-rhamnoside	461 (315)	11, 33	+	+	−
24.50	laricitrin-3-*O*-glucoside	493 (331)	10, 11, 33	+	+	−
33.31	syringetin-3-*O*-glucoside	507 (345)	10, 11, 33	+	+	−
19.13	myricetin-3-*O*-glucoside	479 (317, 179)	10, 11, 33	+	−	−
13.39	myricetin-3-*O*-galactoside	479 (317)	10, 11, 33	−	+	−
	flavan-3-ols					
18.50	catechin	289 (245)	26	+	+	+
32.91	epicatechin	289 (245)	26	+	+	+
14.24	epigallocatechin	305 (179, 137)	26	+	+	+
46.90	epicatechin-3-*O*-gallate	441 (289, 169)	26	+	+	+
	hydroxybenzoic acid					
2.06	hexose exter of protocatechuic acid	315 (153)	42	+	+	+
4.37	hexose ester of vanillic acid	329 (191, 167)	42	+	+	+
	hydroxycinnamic acid					
1.30	caftaric acid	311 (179, 149)	33, 42	+	+	−
3.41	ferulic acid	193	42	+	+	+
3.65	coumaric acid	163 (119)	33, 42	+	+	+
2.77	caffeic acid	179 (135)	33, 42	+	−	+
5.12	glucoside exter of coumaric acid	325 (163, 145, 119)	42	+	+	+
5.57	hexose exter of ferulic acid	355 (193, 217, 175)	33, 42	+	+	+
10.65	cinnamic acid	147 (129)	42	+	+	+
4.60	ethyl caffeate	207 (179, 135)	42	−	+	−
	stilbenes					
50.20	pallidol	453 (359, 265)	42	−	−	+

[a]'+' indicates that the compound was detected in at least one sampling time. '−' indicates that the compound was not detected during any sampling period.

generated through the F3′H-mediated and F3′5′H-mediated branch pathways, respectively. Such differences in the ratio of 3′,4′-substituted to 3′,4′,5′-substituted derivatives reflect the differences in the activities of F3′H and F3′5′H to some extent. Although the total anthocyanin content from these three cultivars varied, in all three the diglucoside forms were far more abundant than monoglucosides, with coumaroyl derivatives totaling >90%. Diglucosides also existed in high proportion in *V. amurensis* grapes, but no acylated derivatives have been reported.[32] These three cultivars studied here all contained about 40−60% of acyl anthocyanins, which is slightly higher than the percentage of acylated anthocyanins (<40%) in *V. vinifera* grapes.[36] García-Beneytez et al. collected 12 red grape cultivars (*V. vinifera*) at harvest grown in Spain and found that the contents of acyl anthocyanins in the skin were lower than those of nonacylated derivatives with the percentage of >60%.[30] De Rosso et al. detected the anthocyanins in grape skin from 21 hybrid red varieties produced by crossing *V. vinifera*, *V. riparia*, *V. labrusca*, *V. lincecumii*, and *V. rupestris* species and found that the percentage of acyl anthocyanins was <30%.[38] Besides, a minor amount of caffeoyl derivatives was detected in the two *V. davidii* cultivars.

Comparison of Flavonols in the Three Grape Cultivars. A total of 20 flavonols was found from the three

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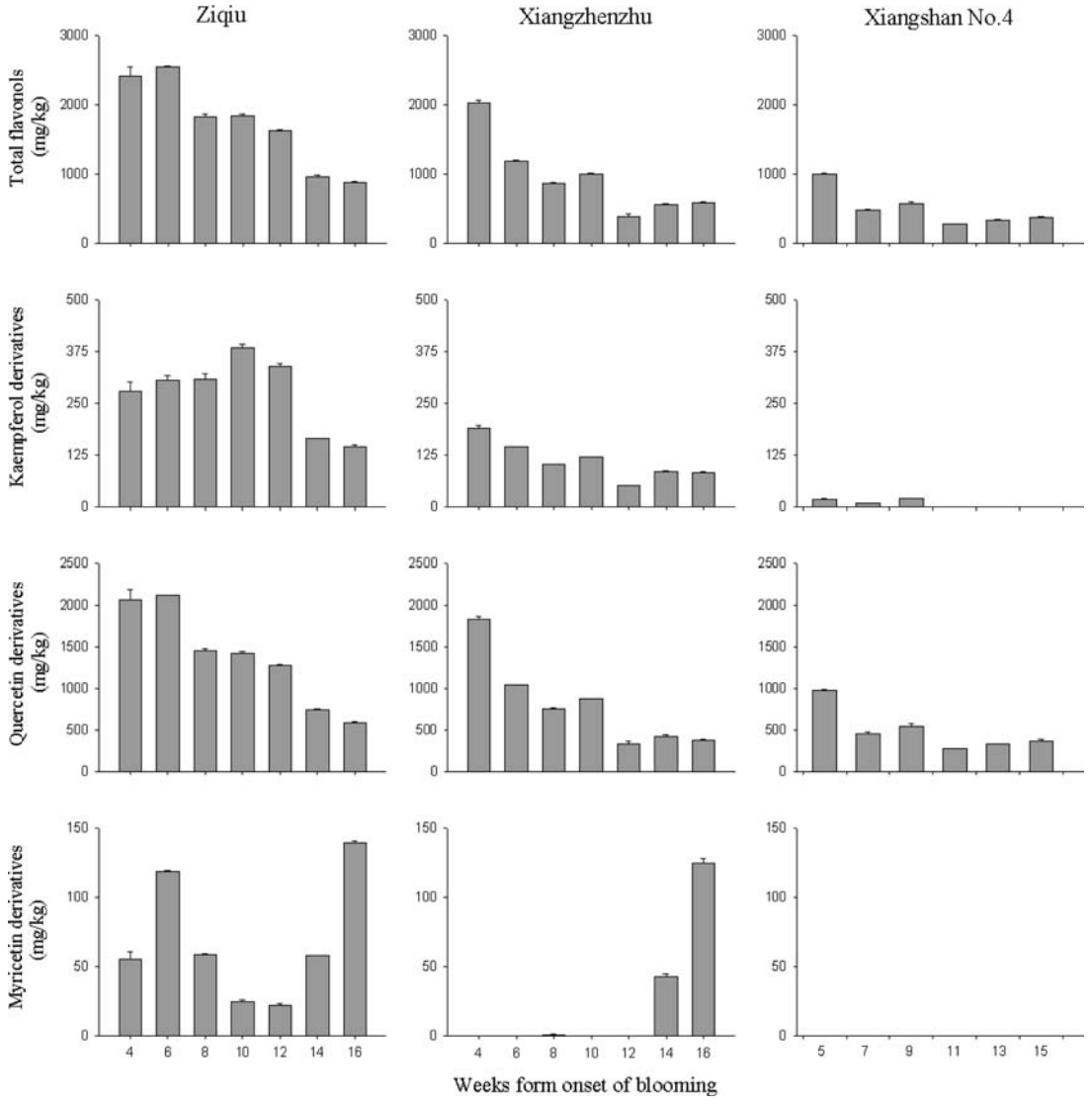

Figure 4. Accumulation of total flavonols, kaempferol derivatives, quercetin derivatives, and myricetin derivatives in the skins of 'Ziqiu' (*V. davidii*), 'Xiangzhenzhu' (*V. davidii*), and 'Xiangshan No. 4' (*V. quinquangularis*) during berry development.

cultivars during berry development (Table 2). On the basis of their biosynthetic pathway and molecular structure, these flavonols were divided into three groups: kaempferol (Kf) derivatives, quercetin (Qu) derivatives, and myricetin (My) derivatives. 'Ziqiu' grapes contained 17 flavonols, including 5 Kf derivatives, 9 Qu derivatives, and 3 My derivatives, whereas 'Xiangzhenzhu' had 17 flavonols including 6 Kf derivatives, 8 Qu derivatives, and 3 My derivatives. However, only 2 Kf derivatives and 3 Qu derivatives were found in 'Xiangshan No. 4' grapes, and no My derivatives were detected at any stage during maturation. My-type flavonols, together with Dp-type, Pt-type, and Mv-type anthocyanins, were the products of the F3′5′H-mediated branch pathway, belonging to the 3′,4′,5′-substituted metabolites. The findings were quite different from that in *Vitis* species previously studied, which could be an interesting topic worthy of further study.

The total flavonol content and the content of the various flavonol subgroups in 'Xiangshan No. 4' were far lower than that in the 'Ziqiu' and 'Xiangzhenzhu' cultivars (Figure 4). For all cultivars, the Qu derivatives were the major flavonol subgroup. In the two *V. davidii* cultivars, Qu-3-*O*-rhamnoside represented nearly 50% of the total content of flavonols, followed by syringetin-3-*O*-glucoside, Qu-3-*O*-glucuronide, and dihydrokaempferol-3-*O*-glucoside. In 'Xiangshan No. 4', Qu-3-*O*-glucuronide accounted for >70%. Thus, whereas the total flavonol content decreased during the maturation process, the total flavonol content of the three cultivars was always dominated by the Qu derivatives. Conversely, despite the content of My derivatives in both 'Ziqiu' and 'Xiangzhenzhu' grapes being relatively low, they reached a maximum at harvest. Furthermore, almost no My derivatives were detected in the 'Xiangzhenzhu' berries before the 14 week stage. With respect to the Kf derivatives, both dihydrokaempferol-3-*O*-glucoside and Kf-3-*O*-hexoside in 'Xiangshan No. 4' grapes were found only at the early developmental stage and disappeared postveraison. Flavonols from *V. vinifera* and *V. labrusca* grapes have displayed a qualitatively similar pattern to the three grape cultivars studied here and comprised a series of 3-*O*-glucosides, 3-*O*-galactosides, and 3-*O*-glucuronides.[10,33] In contrast, My-type flavonols accounted for approximately 50% in Bordô grapes (*V. labrusca*), and Qu-type flavonols were predominant in *V. vinifera* grapes.[10,33] Furthermore, Kf-type flavonols in both

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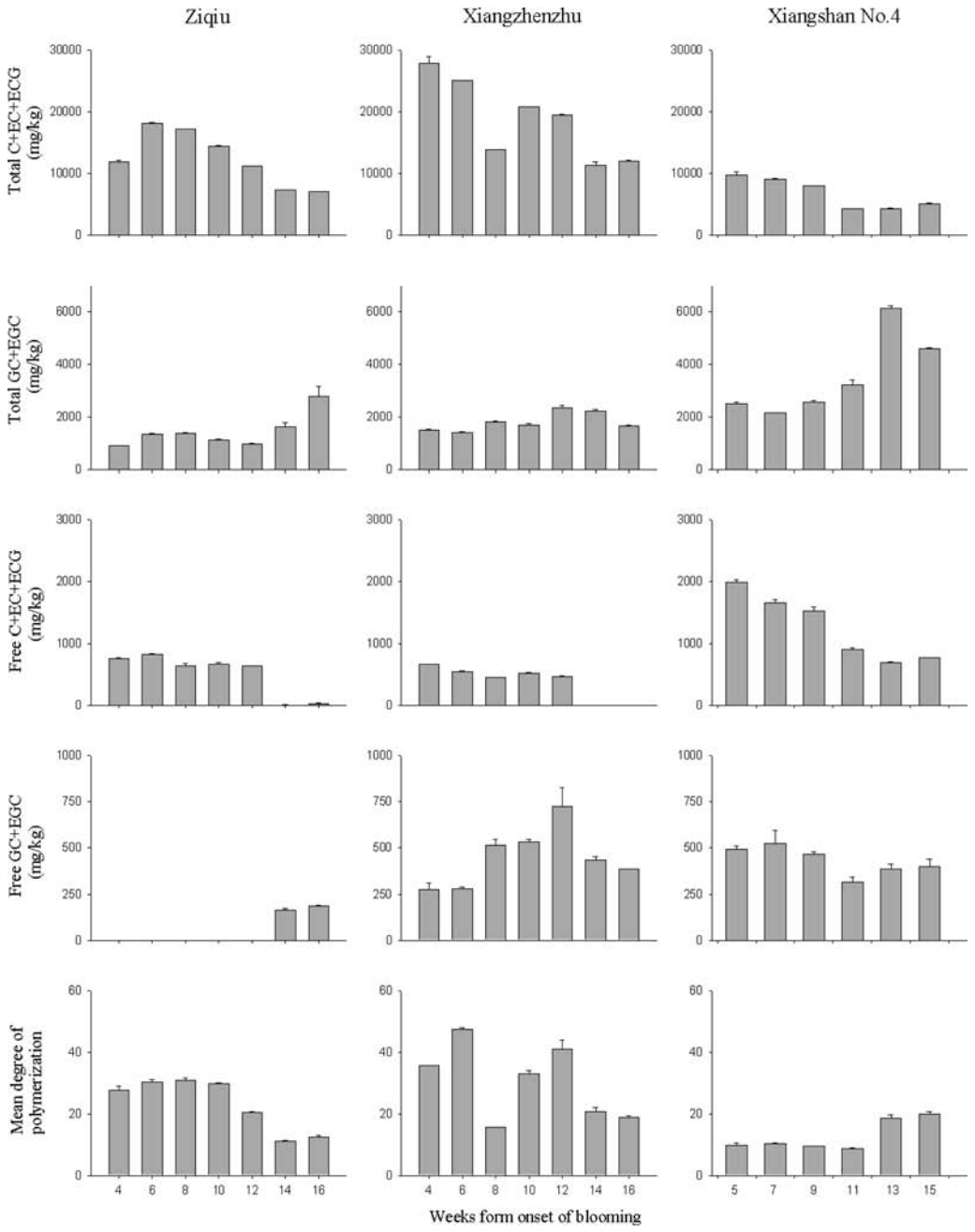

Figure 5. Evolution of total contents of 3′,4′-substituted (C+EC+ECG) and 3′,4′,5′-substituted flavan-3-ols (GC+EGC), as well as the contents of free 3′,4′-substituted and free 3′,4′,5′-substituted flavan-3-ols, and the variation of mean degree of polymerization in the skins of 'Ziqiu' (*V. davidii*), 'Xiangzhenzhu' (*V. davidii*), and 'Xiangshan No. 4' (*V. quinquangularis*) during berry development.

V. labrusca and *V. vinifera* grapes were present in minor proportions.[10,33]

Comparison of Flavan-3-ols and Mean Degree of Polymerization in the Three Grape Cultivars. Proanthocyanidins from the grape skins were acid-hydrolyzed in the presence of excess phloroglucinol, and five subunits were generated, in the three cultivars, including (+)-catechin (C), (+)-gallocatechin (GC), (−)-epicatechin (EC), (−)-epigallocatechin (EGC), and (−)-epicatechin-3-*O*-gallate (ECG) (Table 2), which was in agreement with the earlier studies on *V. vinifera* grapes.[39] The five flavan-3-ol units were also found to exist as free monomers in the grape berries. Of these flavan-3-ol compounds, GC and EGC were synthesized from the F3′5′H-mediated branch pathway and so were grouped into the 3′,4′,5′-substituted flavan-3-ols, whereas C, EC, and ECG are from the F3′H-mediated branch and were grouped into 3′,4′-substituted flavan-3-ols.

Compared with the 'Ziqiu' and 'Xiangzhenzhu' cultivars, the 'Xiangshan No. 4' cultivar had a much lower content of total 3′,4′-substituted flavan-3-ols (C, EC, and ECG) and a significantly higher content of total 3′,4′,5′-substituted flavan-3-ols (GC and EGC). As for each cultivar, the content of 3′,4′-substituted flavan-3-ols was always higher than that of 3′,4′,5′-substituted flavan-3-ols (Figure 5). There were lower levels of 3′,4′-substituted flavan-3-ols in mature berries than in the immature young berries, whereas 3′,4′,5′-substituted flavan-3-ols displayed the opposite trend. In the case of the free monomers, 3′,4′-substituted flavan-3-ols in the two *V. davidii*

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Table 3. Content of Hydroxybenzoic Acid, Hydroxycinnamic Acid, and Stilbenes in the Skins of 'Ziqiu' (*V. davidii*), 'Xiangzhenzhu' (*V. davidii*), and 'Xiangshan No. 4' (*V. quinquangularis*) at Harvest[a]

phenolic compd (mg/kg)	Ziqiu	Xiangzhenzhu	Xiangshan No. 4
hydroxybenzoic acid			
hexose ester of protocatechuic acid	27.10 ± 0.90a	76.05 ± 3.85b	tr
hydroxycinnamic acid			
caftaric acid	116.02 ± 6.05a	703.73 ± 12.06b	nd
ferulic acid	95.00 ± 3.23	tr	tr
coumaric acid	327.72 ± 19.04a	223.91 ± 4.76a	tr
glucoside ester of coumaric acid	102.81 ± 1.17b	107.88 ± 1.93c	tr
hexose ester of ferulic acid	28.23 ± 0.32a	31.53 ± 0.72a	tr
cinnamic acid	nd	37.13 ± 1.76	tr
ethyl caffeate	nd	25.58 ± 0.57	nd
total hydroxycinnamic acid	669.79 ± 14.73b	1129.75 ± 21.80c	tr
stilbene			
pallidol	nd	nd	2.61 ± 0.54

[a]tr means trace, and nd means not detected. The same letters in the same row indicate that these data have no significant difference at the 0.05 level.

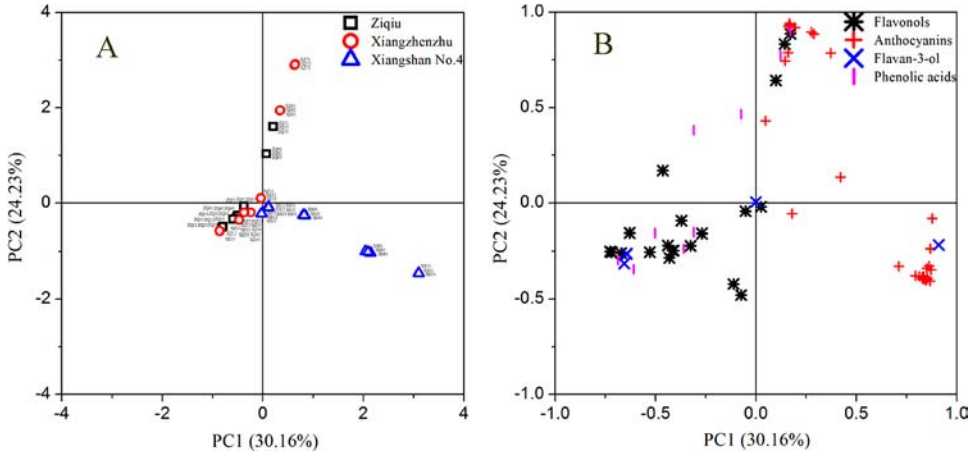

Figure 6. Principal component analysis of phenolics from 'Ziqiu', 'Xiangzhenzhu', and 'Xiangshan No. 4' during berry development: (A) scores scatter plot; (B) loading plot. The letter groups ZQ, XZ, and XS in panel A stand for the cultivars 'Ziqiu', 'Xiangzhenzhu', and 'Xiangshan No. 4', respectively. The first number means the sampling numbers according to the sampling time, and the second indicates the replicate of the samples.

cultivars almost disappeared at the commercially ripe stage, whereas no free 3′,4′,5′-substituted flavan-3-ols (GC and EGC) were found in the 'Ziqiu' berries earlier than 12 weeks.

The mDP of proanthocyanidins in the grape skins varied with *Vitis* species (Figure 5). The mDP in the skins of 'Ziqiu' and 'Xiangzhenzhu' ranged from 11 to 47 during berry development, whereas the mDP in the 'Xiangshan No. 4' berry skins remained relatively constant at around 10 during the early stages of the growth and reached the maximum of about 20 at commercial harvest. The C, EC, ECG, and mDP characteristics of *V. davidii* were very similar to those found in Shiraz grapes (*V. vinifera*),[40] where C, EC, and ECG were always found in the skins and the mDP ranged from 20 to 40 during ripening. Also, the same five flavan-3-ols were found in *V. labrusca* grape skins, epicatechin being the major compound.[34,35] The mDP in Bordô grapes (*V. labrusca*) was about 12, close to the early growth of the 'Xiangshan No. 4' cultivar.

Comparison of Hydroxybenzoic Acids, Hydroxycinnamic Acids, and Stilbenes in the Three Grape Cultivars. Only two hydroxybenzoic acids, being the hexose ester of protocatechuic acid and the hexose ester of vanillic acid, were found in the skins of the three cultivars during the whole experimental period (Table 2). The hexose ester of vanillic acid was observed in both *V. davidii* cultivars at the early growth stage and disappeared from the onset of veraison. Together with the disappearance of vanillic acid ester, the protocatechuic acid ester was detectable from veraison and increased slowly to maturity. At harvest, the content of protocatechuic acid ester in 'Xiangzhenzhu' was two times higher than that in 'Ziqiu' grapes (Table 3). During the total test period, a trace amount of protocatechuic acid ester was present in 'Xiangshan No. 4' grapes.

A total of eight hydroxycinnamic acids were found in the three cultivars (Table 2). For each cultivar, the main compounds were caftaric acid, coumaric acid, the glucoside ester of coumaric acid, and ferulic acid, consistent with the results in Bordô grape skins (*V. labrusca*), in which the predominant hydroxycinnamic acids were caftaric acid and coumaric acid.[33] Similarly, caftaric acid was the main derivative found in Garnacha Tintorera grape skins (*V. vinifera*).[41] The total content of hydroxycinnamic acids in 'Xiangzhenzhu' grapes was approximately 2-fold higher than in 'Ziqiu' grapes, whereas a trace amount of hydroxycinnamic acids was observed

in 'Xiangshan No. 4' grapes (Table 3). The total content of hydroxycinnamic acids in Bordô grape skins was 483 μmol/kg, which was much lower than in *V. davidii* cultivars.[33] Some studies stated that the grape flesh was the major source of the hydroxycinnamic acids in *vinifera* cultivars, but the skin of Garnacha Tintorera grapes (*V. vinifera*) contained total hydroxycinnamic acids in higher amounts than in the flesh, and the percentage in skin was 3 times higher than in flesh.[41] No stilbenes were found in either 'Ziqiu' and 'Xiangzhenzhu', but pallidol, a resveratrol dimer, was detected in 'Xiangshan No. 4' during the whole growth period (Table 2). In contrast, resveratrol and its 3-glucoside (piceid) were found in the skin of Bordô grapes.[33]

Principal Component Analysis (PCA). PCA was used to analyze the data for the 64 phenolic compounds from the three cultivars with three replicates, taking into account the different sampling dates for each cultivar to provide an overview of the differences in phenolic profiles of these three cultivars. The first five PCs accounted for 80% of total variance, PC1, PC2, and PC3 explaining a relatively high percentage (30.2% for PC1, 24.2% for PC2, and 12.4% for PC3) of total variance. The scatter plot scores of the first two principal components (PC1 and PC2) with eigenvalue >1 are given in Figure 6A, showing the difference between the three cultivars. The corresponding loading plot is given in Figure 6B, demonstrating the relative importance of the variables.

As shown in Figure 6A, the two *V. davidii* cultivars ('Ziqiu' and 'Xiangzhenzhu') could be clearly differentiated from the *V. quinquangularis* cultivar ('Xiangshan No. 4'), but it was not possible to clearly separate the three from the other. The difference would therefore likely be species-based rather than cultivar-based. It was possible to separate 'Ziqiu' and 'Xiangzhenzhu' cultivar samples during different stages of their development. Negative scores in PC1 and PC2 corresponded to the samples at 2−12 weeks after flowering for 'Ziqiu' and at 2−10 weeks of postflowering for 'Xiangzhenzhu', whereas positive scores in PC1 and PC2 corresponded to the samples harvested at 13−16 weeks after flowering for 'Ziqiu' and at 11−16 weeks after flowering for 'Xiangzhenzhu', respectively. Moreover, both 'Ziqiu' and 'Xiangzhenzhu' (*V. davidii*) samples were distributed in the first and third quadrants and the scores in PC1 and PC2 proceeded from high negative value to high positive value along with berry development. This result indicates that the two *V. davidii* cultivars have the highest phenolic contents at maturity.

The samples of 'Xiangshan No. 4' were scattered mainly in the fourth quadrant, except for the early-picked sample (2 weeks after flowering) that was within the area of very low negative PC1 scores and very low PC2 scores. Along with berry development, the PC2 scores of these samples increased to the highest value at 13 weeks after flowering. In the plot of PCA, the position of the sample harvested at 15 weeks after flowering was close to the sample picked at 2 weeks after flowering, which was associated with a sharp decline in anthocyanins that occurred during the 2 weeks before harvest.

The corresponding loading plot showed that the majority of flavonols (excluding syringetin-3-*O*-glucoside, laricitrin-3-*O*-glucoside, and isorhamnetin-3-*O*-rhamnoside) and 3′,4′-substituted flavan-3-ols (that is, C, EC, and ECG) were concentrated in the third quadrant, which means that flavonols and 3′,4′-substituted flavan-3-ols are representative phenolics for the young grapes of 'Ziqiu' and 'Xiangzhenzhu', as well as 2 week grapes of 'Xiangshan No. 4' (Figure 6B). All of the malvidin-type anthocyanins, together with peonidin-3-*O*-monoglucoside-pyruvic acid, peonidin-3-*O*-caffeoylglucoside, and cyanidin-3-*O*-monoglucoside, were situated in the first quadrant of the loading plot, indicating that these components are characteristic phenolics of the two *V. davidii* cultivars at maturity. Apart from these, other anthocyanins were found all in the fourth quadrant. The two parts of Figure 6 showed that four types of anthocyanins other than malvidin-type anthocyanins were characteristic of the phenolic profile of 'Xiangshan No. 4' cultivars. This result corresponded highly with the relatively low content of malvidin derivatives and the high content of the derivatives of cyanidin, peonidin, delphinidin, and petunidin in this *V. quinquangularis* cultivar.

Conclusion. Large differences were found in both content and composition of the grape skin phenolics of *V. davidii* species and *V. quinquangularis* species, whereas a few differences exist between the two *V. davidii* cultivars. The three cultivars were all characterized by a high content of anthocyanidin diglucosides and a very high percentage of coumaroyl anthocyanins. The flavonoids of *V. quinquangularis* have a high percentage of 3′,4′-substituted anthocyanins generated from the F3′H branch pathway and a relatively high percentage of 3′,4′,5′-substituted flavan-3-ols synthesized through the F3′5′H branch pathway. Through PCA, the *V. davidii* grapes were determined to be characterized by abundant flavonols at the early middle developmental stages as well as a high percentage of malvidin-type anthocyanins at the late stages of development. The flavonol composition of the two *V. davidii* cultivars was more diverse compared with the *V. quinquangularis* cultivar with only a few types of flavonols being found at low levels and no myricetin-type being found in the 'Xiangshan No. 4' grapes. These results will not only provide some new insights and stimulate interest in the biosynthesis and regulation of flavonoids in the grape cultivars native to China but also help us to make use of these biological resources and to exploit their production potential in grape breeding and winemaking.

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Notes

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REFERENCES

(1) Zou, Y. Research advance in germplasm resource and utilization of *Vitis quinquangularis*. *Guangxi Agric. Sci.* **2008**, *39*, 664−667 (in Chinese with English abstract).

(2) He, P. C.; Niu, L. X. Study of cold hardiness in the wild *Vitis* native to China. *Acta Hortic. Sinica* **1989**, *11*, 81−88 (in Chinese with English abstract).

(3) Shi, X. H.; Yang, G. S.; Xiong, X. Y.; Liu, K. Y.; Zhong, X. H.; Wang, X. R.; Ni, J. J.; Guo, G. Y. Research and utilization status quo of

germplasm resources of *Vitis davidii* Foëx in Hunan. *Hunan Agric. Sci.* **2010**, *19*, 1−4 (in Chinese with English abstract).
(4) Guilford, J. M.; Pezzuto, J. M. Wine and health: a review. *Am. J. Enol. Vitic.* **2011**, *62*, 471−486.
(5) Rice-Evans, C. A.; Miller, N. J.; Paganga, G. Structure-antioxidant activity relationships of flavonoids and phenolic acids. *Free Radical Biol. Med.* **1996**, *20*, 933−956.
(6) Mané, C.; Souquet, J. M.; Ollé, D.; Verriés, C.; Véran, F.; Mazerolles, G.; Cheynier, V.; Fulcrand, H. Optimization of simultaneous flavanol, phenolic acid, and anthocyanin extraction from grapes using an experimental design: application to the characterization of champagne grape varieties. *J. Agric. Food Chem.* **2007**, *55*, 7224−7233.
(7) Clifford, M. N. Chlorogenic acids and other cinnamates − nature, occurrence and dietary burden. *J. Sci. Food Agric.* **1999**, *79*, 362−372.
(8) Mattila, P.; Hellström, J. Phenolic acids in potatoes, vegetables, and some of their products. *J. Food Compos. Anal.* **2007**, *20*, 152−160.
(9) Duthie, G. G.; Duthie, S. J.; Kyle, J. A. M. Plant polyphenols in cancer and heart disease: implications as nutritional antioxidants. *Nutr. Res. Rev.* **2000**, *13*, 79−106.
(10) Castillo-Muñoz, N.; Gómez-Alonso, S.; García-Romero, E.; Hermosín-Gutiérrez, I. Flavonol profiles of *Vitis vinifera* red grapes and their single-cultivar wines. *J. Agric. Food Chem.* **2007**, *55*, 992−1002.
(11) Castillo-Muñoz, N.; Gómez-Alonso, S.; García-Romero, E.; Gómez, M. V.; Velders, A. H.; Hermosín-Gutiérrez, I. Flavonol 3-*O*-glycosides series of *Vitis vinifera* Cv. Petit Verdot red wine grapes. *J. Agric. Food Chem.* **2009**, *57*, 209−219.
(12) Gómez-Míguez, M.; González-Manzano, S.; Teresa Escribano-Bailón, M.; Heredia, F. J.; Santos-Buelga, C. Influence of different phenolic copigments on the color of malvidin 3-glucoside. *J. Agric. Food Chem.* **2006**, *54*, 5422−5429.
(13) Bravo, L. Polyphenols: chemistry, dietary sources, metabolism, and nutritional significance. *Nutr. Rev.* **1998**, *56*, 317−333.
(14) Dixon, R. A.; Xie, D. Y.; Sharma, S. B. Proanthocyanidins − a final frontier in flavonoid research? *New Phytol.* **2005**, *165*, 9−28.
(15) Gawel, R. Red wine astringency: a review. *Aust. J. Grape Wine Res.* **1998**, *4*, 73−95.
(16) Bakker, J.; Timberlake, C. F. Isolation, identification and characterization of new color-stable anthocyanins occurring in some red wines. *J. Agric. Food Chem.* **1997**, *45*, 35−43.
(17) García-Puente Rivas, E.; Alcalde-Eon, C.; Santos-Buelga, C.; Rivas-Gonzalo, J. C.; Escribano-Bailón, M. T. Behaviour and characterisation of the colour during red wine making and maturation. *Anal. Chim. Acta* **2006**, *563*, 215−222.
(18) González-Manzano, S.; Dueñas, M.; Rivas-Gonzalo, J. C.; Escribano-Bailón, M. T.; Santos-Buelga, C. Studies on the copigmentation between anthocyanins and flavan-3-ols and their influence in the colour expression of red wine. *Food Chem.* **2009**, *114*, 649−656.
(19) Mattivi, F.; Guzzon, R.; Vrhovsek, U.; Stefanini, M.; Velasco, R. Metabolite profiling of grape: flavonols and anthocyanins. *J. Agric. Food Chem.* **2006**, *54*, 7692−7702.
(20) Liang, Z. C.; Wu, B. H.; Fan, P. G.; Yang, C. X.; Duan, W.; Zheng, X. B.; Liu, C. Y.; Li, S. H. Anthocyanin composition and content in grape berry skin in *Vitis* germplasm. *Food Chem.* **2008**, *111*, 837−844.
(21) Pastrana-Bonilla, E.; Akoh, C. C.; Sellappan, S.; Krewer, G. Phenolic content and antioxidant capacity of muscadine grapes. *J. Agric. Food Chem.* **2003**, *51*, 5497−5503.
(22) Revilla, E.; Carrasco, D.; Benito, A.; Arroyo-García, R. Anthocyanin composition of several wild grape accessions. *Am. J. Enol. Vitic.* **2010**, *61*, 536−543.
(23) Revilla, E.; Carrasco, D.; Carrasco, V.; Benito, A.; Arroyo-García, R. On the absence of acylated anthocyanins in some wild grapevine accessions. *Vitis* **2012**, *51*, 161−165.
(24) He, J.-J.; Liu, Y.-X.; Pan, Q.-H.; Cui, X.-Y.; Duan, C.-Q. Different anthocyanin profiles of the skin and the pulp of Yan73 (Muscat Hamburg × Alicante Bouschet) grape berries. *Molecules* **2010**, *15*, 1141−1153.
(25) Jin, Z.-M.; Bi, H.-Q.; Liang, N.-N.; Duan, C.-Q. An extraction method for obtaining the maximum non-anthocyanin phenolics from grape berry skins. *Anal. Lett.* **2010**, *43*, 776−785.
(26) Liang, N. N.; He, F.; Pan, Q. H.; Wang, J.; Reeves, M. J.; Duan, C. Q. Optimization of sample preparation and phloroglucinol analysis of Marselan grape skin proanthocyanidins using HPLC-DAD-ESI-MS/MS. *Afr. J. Enol. Vitic.* **2012**, *33*, 122−131.
(27) Wu, X.; Prior, R. L. Systematic identification and characterization of anthocyanins by HPLC- ESI-MS/MS in common foods in the United States: fruits and berries. *J. Agric. Food Chem.* **2005**, *53*, 2589−2599.
(28) De Villiers, A.; Vanhoenacker, G.; Majek, P.; Sandra, P. Determinaton of anthocyanins in wine by direct injection liquid chromatography-diode array detection-mass spectrometry and classification of wines using discriminant analysis. *J. Chromatogr., A* **2004**, *1054*, 195−204.
(29) Pati, S.; Liberatore, M. T.; Gambacorta, G.; Antonacci, D.; La Notte, E. Rapid screening for anthocyanins and anthocyanin dimers in crude grape extracts by high performance liquid chromatography coupled with diode array detection and tandem mass spectrometry. *J. Chromatogr., A* **2009**, *1216*, 3864−3868.
(30) García-Beneytez, E.; Cabello, F.; Revilla, E. Analysis of grape and wine anthocyanins by HPLC-MS. *J. Agric. Food Chem.* **2003**, *51*, 5622−5629.
(31) Gómez-Plaza, E.; Gil-Muñoz, R.; Hernández-Jiménez, A.; López-Roca, J. M.; Ortega-Regules, A.; Martínez-Cutillas, A. Studies on the anthocyanin profile of *Vitis vinifera* intraspecific hybrids (Monastrell × Cabernet Sauvignon). *Eur. Food Res. Technol.* **2008**, *227*, 479−484.
(32) Zhao, Q.; Duan, C.-Q.; Wang, J. Anthocyanins profile of grape berries of *Vitis amurensis*, its hybrids and their wines. *Int. J. Mol. Sci.* **2010**, *11*, 2212−2218.
(33) Lago-Vanzela, E. S.; Da-Silva, R.; Gomes, E.; García-Romero, E.; Hermosín-Gutiérrez, I. Phenolic composition of the edible parts (flesh and skin) of Bordô grape (*Vitis labrusca*) using HPLC-DAD-ESI-MS/MS. *J. Agric. Food Chem.* **2011**, *59*, 13136−13146.
(34) Liang, Z.; Yang, C.; Yang, J.; Wu, B.; Wang, L.; Cheng, J.; Li, S. Inheritance of anthocyanins in berries of *Vitis vinifera* grapes. *Euphytica.* **2009**, *167*, 113−125.
(35) Heredia, F. J.; Francia-Aricha, E. M.; Rivas-Gonzalo, J. C.; Vicarioa, I. M.; Santos-Buelga, C. Chromatic characterization of anthocyanins from red grapes−I. pH effect. *Food Chem.* **1998**, *63*, 491−498.
(36) González-Neves, G.; Franco, J.; Barreiro, L.; Gil, G.; Moutounet, M.; Carbonneau, A. Varietal differentiation of Tannat, Cabernet-Sauvignon and Merlot grapes and wines according to their anthocyanic composition. *Eur. Food Res. Technol.* **2007**, *225*, 111−117.
(37) Torskangerpoll, K.; Nøbæk, R.; Nodland, E.; Østedal, D. O.; Andersen, Ø. M. Anthocyanin content of *Tulipa* species and cultivars and its impact on tepal colours. *Biochem. Syst. Ecol.* **2005**, *33*, 499−510.
(38) De Rosso, M.; Tonidandel, L.; Larcher, R.; Nicolini, G.; Ruqqeri, V.; Dalla Vedova, A.; De Marchi, F.; Gardiman, M.; Flamini, R. Study of anthocyanic profiles of twenty-one hybrid grape varieties by liquid chromatography and precursor-ion mass spectrometry. *Anal. Chim. Acta* **2012**, *732*, 120−129.
(39) Mattivi, F.; Vrhovsek, U.; Masuero, D.; Trainotti, D. Differences in the amount and structure of extractable skin and seed tannins amongst red grape varieties. *Aust. J. Grape Wine Res.* **2009**, *15*, 27−35.
(40) Downey, M. O.; Harvey, J. S.; Robinson, S. P. Analysis of tannins in seeds and skins of Shiraz grapes throughout berry development. *Aust. J. Grape Wine Res.* **2003**, *9*, 15−27.
(41) Castillo-Muñoz, N.; Fernández-González, M.; Gómez-Alonso, S.; García-Romero, E.; Hermosín-Gutiérrez, I. Red-color related phenolic composition of Garnacha Tintorera (*Vitis vinifera* L.) grapes and red wines. *J. Agric. Food Chem.* **2009**, *57*, 7883−7891.
(42) Monagas, M.; Bartolomé, B.; Gómez-Cordovés, C. Updated knowledge about the presence of phenolic compounds in wine. *Crit. Rev. Food Sci.* **2005**, *45*, 85−118.