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ORIGINAL ARTICLE

The growing season impacts the accumulation and composition of flavonoids in grape skins in two-crop-a-year viticulture

Lei Zhu[1,2] · Yu Huang[3] · Yali Zhang[4] · Changmou Xu[4] · Jiang Lu[2] · Ying Wang[1]

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Abstract The influence of growing season (winter vs. summer) on the flavonoid accumulation and composition was studied in the skins of three grape cultivars for two consecutive years under a two-crop-a-year viticulture practice in Southwest China. The total anthocyanin, flavonol and flavan-3-ol contents in winter berry skins were significantly higher than those in summer berry skins for 'Kyoho' and 'Muscat Hamburg'. Reversely, the content of anthocyanin in 'NW196' winter berry was lower than summer berry. However, the percentage of diglycosylated, trihydroxylated, methylated, and acylated anthocyanins, trihydroxylated and methylated flavonols, and flavan-3-ol polymers were higher in the summer berry skins than the winter berry skins among all the three grape cultivars. Winter climatic conditions were favorable to flavonoid accumulation for the non native grapes 'Kyoho' and 'Muscat Hamburg', while the summer climatic conditions were beneficial to anthocyanin accumulation for 'NW196' that has 50% genetic background from a local wild grape species *Vitis quinquangularis*. These seasonal variations of flavonoid accumulations and compositions in the grape skins were primarily contributed by different climatic factors, such as temperature, solar radiation, and rainfall.

Keywords Growing seasons · Flavonoids · Accumulation · Composition · Climatic factors

Introduction

The grape flavonoid compounds typically include anthocyanins, flavonols and flavan-3-ols. The contents and compositions of these compounds greatly contribute to grape and wine quality, and can also benefit human health. Climatic factors, such as temperature, solar radiation and rainfall, play important roles in flavonoid synthesis and accumulation during grape maturation (Downey et al. 2006). There have been several studies on the impact of a single climatic factor on concentrations and compositions of flavonoids in berry skins of *Vitis vinifera* and American grapes (Mori et al. 2007a, b; Price et al. 1995). However, neither studies have focused on the comprehensive effects of multiple climatic factors nor on grape cultivars with East Asian origin.

In recent years, with the expansion of grape production to low latitudes, the techniques used for two-crop-a-year viticulture have attracted much attention in tropical and subtropical regions. In China, two-crop-a-year viticulture has been successfully practiced in the south of the Nanling Mountains (24°00′–26°30′N and 110°–116°E). The growing season of the first (or summer) crop is from late February to June, when the climate is hot/humid with relatively short sunlight time because of more cloudy and

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✉ Jiang Lu
Jiang.lu@sjtu.edu.cn

1 College of Food Science and Technology, Heilongjiang Bayi Agricultural University, Daqing 163319, People's Republic of China

2 School of Agriculture and Biology, Shanghai Jiaotong University, Shanghai 200240, People's Republic of China

3 Guangxi Academy of Agricultural Sciences, Nanning 530007, People's Republic of China

4 College of Food Science and Nutritional Engineering, China Agricultural University, Beijing 100083, People's Republic of China

rainy days. The second (or winter) crop grows from late August to December, when the climate is cooler/drier with more sunlight hours and larger temperature difference between day and night.

These two crops in a year, grown under dramatically contrasting climatic conditions, provide materials to study the integrated influences of multiple climatic factors on grape berry qualities, particularly flavonoid characteristics. In a previous study, we demonstrated that the total phenolic contents and antioxidant properties in the skins and seeds of winter grapes were significantly higher than those in summer grapes for five grape cultivars (Xu et al. 2011). This study investigates the effects of growing season (winter vs. summer) on the accumulation and composition of flavonoid compounds in grape skins among different genotypes, including a Euro–Asian hybrid.

Materials and methods

Materials

Three grape cultivars with different genetic backgrounds were investigated during two consecutive years (2009 and 2010): 'Kyoho' (KH, *V. vinifera* × *V. labrusca* L.), 'Muscat Hamburg' (MH, *V. vinifera*) and 'NW196' (NW, *V. quinquangularis* Rehd. × *V. vinifera*). The grapes were cultivated under field conditions at the Experimental Vineyard of Guangxi Academy of Agricultural Sciences, located in southwest China (22°47′N and 108°21′E and 72 m above sea level). The type of soil is yellow clay soil. The local climate is typically subtropical monsoon with mild winters. The average annual temperature is approximately 21.6 °C, the lowest average monthly temperature is 12.8 °C in January, and the highest average monthly temperature is 28.2 °C in July and August. The average annual rainfall is 1304.2 mm, primarily concentrated from April to September.

All vines were grown on their own roots. The 5-year-old 'Kyoho' vine spacing was 2 m in east/west oriented rows, with 3.3 m between rows. The 4-year-old vines of 'Muscat Hamburg' and 'NW196' were spaced 1.5 m in east/west oriented rows with 2.5 m between rows. At harvest, mature berries were collected during summer and winter growing seasons in 2009 and 2010. The harvest dates for the three grape cultivars were largely consistent, which were based on sugars and sensory quality of the grape berries. At least 30 clusters were collected at similar positions from 10 vines for each cultivars. Two 100-berry batches were randomly selected from the top, middle, and bottom portions of the clusters. Each group of berries was considered as one replication, resulting in two replications per grape cultivar. The skins were manually separated, freeze-dried (LGJ-12, Songyuan Huaxing Corporation, Beijing, China) and ground (FW-135, Taister Corporation, Tianjin, China) into a fine powder. The final skin samples were stored in vacuum-packaged polyethylene pouches at −20 °C for subsequent analysis.

Chemicals and standards

The standards, including malvidin-3-*O*-glucoside, quercetin and catechin, were obtained from Sigma-Aldrich (St. Louis, MO, USA). The high-performance liquid chromatography (HPLC)-grade reagents, including acetonitrile, formic acid, acetic acid and methanol, were obtained from Thermo Fisher Scientific (Fairlawn, NJ, USA). All other analytical-grade reagents and chemicals were purchased from Lanyi Chemical Co. (Beijing, China).

Determination of berry weight, total soluble solids, titratable acids and pH

The mature grape berries (30) from each replicate sample were weighed and recorded. The total soluble solids were determined using a hand-held refractometer (Wancheng Co., Beijing, China), and the pH was assayed using a pH meter (pH 211, HANNA, Italy). To determine the titratable acids, approximately 20 g of berries for each replicate sample was crushed, and the volume was diluted to 200 mL with distilled water. A total of 20 mL of filtered solution was titrated using 0.1 N NaOH to a pH endpoint of 8.1 (Margaret et al. 2007).[5]

Extraction of phenolic compounds

Total phenolic compounds were extracted from grape skins according to our previously reported method with slight modifications (Xu et al. 2010). Briefly, 0.5 g of skin powder was extracted with 20 mL of methanol/water/acetic acid (70:29:1, v/v/v) solvent in a shaker (SHZ-88A, Taicang Experiment Equipment Factory, Jiangsu, China) for 2 h. The extracts were evaporated to dryness (RE-52A, Yarong Biochemistry Instrument Factory, Shanghai, China) at 30 °C and were re-dissolved in the above extraction solvent to a unified volume of 5 mL for subsequent use for anthocyanin analysis.

The non-anthocyanin phenolic compounds were extracted according to the Jin, et al. method (Jin et al. 2009). Briefly, 2 g of the skin powder was weighed in a 100-mL conical flask with 20 mL ethyl acetate and 4 mL distilled water in a shaker for 30 min. Subsequently, the extracts and solids were separated. This process was repeated five times. The collected extracts for each sample

were evaporated to dryness at 30 °C and re-dissolved with methanol to a unified volume of 2 mL.

Analysis of phenolic compounds

The anthocyanin concentrations were analyzed using an Agilent 1100 series LC-MSD trap (Jin et al. 2009). The samples were injected (30 μL) directly after filtration through a 0.45-μm inorganic membrane on a Kromasil-C18 reversed-phase column (250 × 4 mm, 6.5 μm) at 50 °C. The solvent system consisted of an aqueous solution containing 2% formic acid (phase A) and an acetonitrile solution containing 2% formic acid (phase B), and the flow rate was 1 mL/min. The linear gradient for solvent B was: 0–1 min, 10% B; 2–17 min, 10–25% B; 18–20 min, 25% B; 21–30 min, 25–40% B; 31–35, 40–70% B; and 36–40 min, 70–100% B. The UV detector was set to an absorbance wavelength of 525 nm. For identification, electrospray ionization tandem mass spectrometry (ESI–MS/MS) was used, employing the following parameters: positive ionization mode; dry gas, N_2, 10 mL/min; drying temperature, 350 °C; nebulizer, 35 psi; and scan range, 100–1000 *m/z*. Anthocyanins were quantified using malvidin-3-O-glucoside (MGE) as a standard and are expressed as μg MGE per g dry weight of grape skins (DW).

The non-anthocyanin phenolic compounds were analyzed using a Waters ACQUITY Ultra Performance Liquid Chromatography (UPLC) System combined with tandem XEVO TQ mass spectrometry. The samples were injected (2 μL) directly after filtration through a 0.22-μm nylon membrane onto a ZORBAX SB-C18 column (3 × 50 mm, 1.8 μm) at 25 °C. The solvent system consisted of a water solution containing 1% acetic acid (phase A) and an acetonitrile solution containing 1% acetic acid (phase B), and the flow rate was 1 mL/min. The linear gradient for solvent B was:[7] 0–10 min, 5–8%; 10–18 min, 8–10%; 18–40 min, 10–15%; 40–50 min, 15–20%; 50–53 min, 20–30%; 53–58 min, 30–50%; 58–62 min, 50–100%; and 62–66 min, 100%. The UV detector was set to an absorbance wavelength of 280 nm. The MS analysis employed the following parameters: negative ionization mode; dry gas, N_2, 10 mL/min; drying temperature, 325 °C; nebulizer, 35 psi; and scan range, 100–1000 *m/z*. Flavonols and flavan-3-olswere quantified using quercetin equivalence (QE) and catechin equivalence (CE) as standards and are expressed as μg QE/g DW and μg CE/g DW, respectively.

Statistical analysis

All results are expressed as the means ± standard deviations (S.D.) and were subjected to *T* test using SPSS 19.0 (IBM Corporation, New York, USA.) at the 95% confidence level.

Results

Variation of basic fruit qualities between the summer- and winter-grown mature grapes

There were significant differences in basic fruit qualities between the summer and winter mature berries of all of the grape cultivars in 2009 and 2010 (Table 1). Summer crops had larger berries than winter crops. The soluble solids and titratable acidity of summer berries were significantly lower than those of winter berries. In summer growing season, the soluble solids of grape berries could only reach 16–18°Brix because of less sunlight and small diurnal temperature range.

Table 1 The basic fruit qualities of mature grape berries in different years and seasons

Cultivars	Yeas	Berry weight[a] (g)		Soluble solids[a] (°Brix)		Titrable acidity[a] (g/L)	
		Summer[b]	Winter[c]	Summer[b]	Winter[c]	Summer[b]	Winter[c]
Khoyo	2009	7.96 ± 1.43 b	5.77 ± 1.11 a	16.27 ± 0.12 a	18.07 ± 0.31 b	3.73 ± 0.13 a	6.91 ± 0.32 b
	2010	11.66 ± 1.74 b	7.03 ± 1.52 a	17.27 ± 0.12 a	19.07 ± 0.25 b	3.38 ± 0. 19 a	6.63 ± 0.64 b
Muscat	2009	3.95 ± 0.83	3.45 ± 0.61	16.17 ± 0.21 a	19.93 ± 0.81 b	3.72 ± 0.03 a	7.69 ± 1.35 b
Hamburg	2010	3.99 ± 0.76	3.28 ± 0.09	16.47 ± 0.50 a	18.30 ± 0.26 b	3.64 ± 0.21 a	4.82 ± 0.72 b
NW196	2009	1.09 ± 0.32	0.70 ± 0.18	18.33 ± 0.12 a	21.83 ± 0.41 b	2.41 ± 0.3 a	10.45 ± 0.44 b
	2010	1.07 ± 0.13	0.96 ± 0.14	17.40 ± 0.40 a	21.83 ± 0.41 b	2.39 ± 0.06 a	6.53 ± 0.22 b

[a] Different letters are significantly different between summer and winter grapes for the same grape cultivar in each year at 0.05 level. No letter represented no significant differences between summer and winter grapes

[b] Summer berries (first crop, harvested in late June)

[c] Winter berries (second crop, harvested in late December)

[b] Summer berries (first crop, harvested in late June)

[c] Winter berries (second crop, harvested in late December)

Variations of anthocyanins between summer- and winter-grown grape skins

Anthocyanins initially accumulate in grapes during veraison and gradually increased as the grape berry getting mature. The total anthocyanin contents (TAC) and the percentages of each anthocyanin type in the skins of 'Kyoho', 'Muscat Hamburg' and 'NW196' during both years are shown in Table 2.

There were significant differences in the variations of TACs between summer and winter berries. For 'Kyoho', more anthocyanin compounds were identified in winter grape skins than in summer grape skins, and the content of each compound in winter berry skin was higher than that in summer berry skin (Supporting information). Thus, the skin of winter berry had significantly higher TAC than the summer berry skin.

For 'Muscat Hamburg', the compound number identified in winter berry skins were richer than those in summer berry skins. Although the contents of some minor anthocyanin compounds in summer berry skin were higher than in the winter berry skin (Supporting information), the TAC in winter grapes was generally higher than that in summer grapes.

Different patterns of TAC between the summer and winter berries were observed in 'NW196' of which the TAC in summer berry skin was obviously higher than that in winter berry skin during both years.

Although the winter grape skin possessed more detectable anthocyanin compound types, the contents of major anthocyanins, such as malvidin-3,5-diglucosides, malvidin-3-(6-coumaroyl)-glucoside-5-glucoside and malvidin-3-(6-coumaroyl)-glucoside, were significantly less in the winter berry skins than those in summer berry skins (Supporting information).

The anthocyanins in the skins of 'Kyoho', 'Muscat Hamburg' and 'NW196' primarily consisted of glucosides and acylated glucosides of delphinidin (dp), cyanidin (cy), petunidin (pt), peonidin (pn) and malvidin (mv). However, pelargonidin (pg)-3-glucoside was identified in 'Muscat Hamburg' winter grape skins at veraison and maturity in 2010. In a previous study, we detected pg-3,5-diglucosides in *V. rotundifolia* 'Noble' and 'Alachua', and pg-3-glucoside in *V. labrusca* 'Niagara Rosada' and *V. aestivalis* 'Black Spanish' (Zhu et al. 2012). Furthermore, the pg-monoglucoside or pg-diglucosides were also detected in muscadine grapes (Sandhu et al. 2010) and a few cultivars of *V. vinifera* (He et al. 2010), American grapes/Euro–American hybrids (Tian et al. 2005; Wang et al. 2003) and East Asian grapes/Euro–Asian hybrids (Zhao et al. 2010) in other studies. In the present study, we reported the first detection of pg-3-glucoside in 'Muscat Hamburg' (*V.vinifera*) skins.

To better understand the variations in the anthocyanin composition, the identified anthocyanin compounds were grouped based on the number and type of substituents in the molecular structure. According to the numbers of B-ring substituents of anthocyanidins, the grape anthocyanins primarily consisted of 3′,4′-substituents (cy- and pn-derivatives) and 3′,4′,5′-substituents (dp-, pt- and mv-derivatives). In 'Muscat Hamburg', 3′,4′-substituents were predominant. In contrast, in 'Kyoho' and 'NW196', 3′,4′,5′-substituents were the predominant anthocyanin type. The winter berries had higher 3′,4′- substituent proportions than summer berries for all the cultivars in both years. The reverse trend was observed for the proportions of 3′,4′,5′-substituents.

The methylated (pt-, pn- and mv-derivatives) anthocyanins were predominant compared with the non-methylated (pg-, dp- and cy-derivatives) in all the cultivars investigated. The proportions of methylated compounds in winter berries significantly decreased compared with those in summer berries for all samples studied.

For the glucoside, only monoglucosidic anthocyanins were detected in *V. vinifera* 'Muscat Hamburg', both monoglucosidic and diglucosidic anthocyanins were detected in interspecific hybrids 'Kyoho' and 'NW196'. Our study found that the diglucosidic percentages in 'Kyoho' and 'NW196' increased in summer berries and decreased in winter berries. The proportions of monoglucosidic and diglucosidic anthocyanins differed obviously between the summer and winter berries. The percentages of monoglucosidic anthocyanins in winter berry skins were significantly higher than those in summer berry skins, and the opposite trend was observed for diglucosidic anthocyanins, except for the 'Kyoho' samples at veraison in 2009, in which no marked difference was observed.

The 6-OH on the glucoside of anthocyanins may be acylated in grapes. In the present study, acylated anthocyanins included acetyl, coumaryl and caffeoyl derivatives. More importantly, the total percentages of these compounds in the TAC in winter berries were significantly lower than in summer berries for all of the cultivars during both years, although no obvious variations were observed in mature 'Kyoho' samples in 2009. This change was consistent with the variations observed for coumaryl derivatives, the major acylated anthocyanins detected in most samples analyzed, between summer and winter berries. However, summer berries of 'Muscat Hamburg' was an exception, in which acetyle derivatives were main in their less acylated anthocyanins.

Variation of flavonols between summer- and winter-grown grape skins

For 'Kyoho' and 'Muscat Hamburg', the flavonol content (TFO) in skins of winter berries were generally higher compared with summer berries. 'NW196' had unique

Table 2 The contents and compositions of anthocyanins among three grape cultivars in different years and seasons

	2009[ab]		2010[ab]	
	Summer	Winter	Summer	Winter
Kyoho				
Total anthocyanins (μg MGE/g DW)[c]	2210.20 ± 185.42 a	7185.63 ± 459.62 b	359.36 ± 2.00 a	11,081.50 ± 16.11 b
3′4′-substituted %[f]	15.45 ± 0.09 a	22.86 ± 0.14 b	22.18 ± 0.61	23.56 ± 0.09
3′4′5′-substituted %	84.55 ± 0.09 b	77.14 ± 0.14 a	77.82 ± 0.61	76.44 ± 0.09
Non-methoxylated %[g]	4.99 ± 0.31 a	12.99 ± 0.00 b	nd	17.43 ± 0.03
Methoxylated %	95.01 ± 0.31 b	87.01 ± 0.00 a	100.00 ± 0.00 b	82.57 ± 0.03 a
Monoglucoside %[h]	37.16 ± 0.30 a	73.98 ± 0.80 b	26.17 ± 1.50 a	69.38 ± 0.03 b
Diglucosides %	62.84 ± 0.30 b	26.02 ± 0.80 a	73.83 ± 1.50 b	30.62 ± 0.03 a
Non-acylated %[i]	72.62 ± 0.02	72.53 ± 1.13	58.06 ± 0.77 a	72.36 ± 0.06 b
Acetylated %	0.65 ± 0.32	0.89 ± 0.70	2.39 ± 0.03 b	1.30 ± 0.01 a
Cinnamylated %	26.73 ± 0.30	22.23 ± 1.85	33.98 ± 1.24 b	26.10 ± 0.06 a
Caffeicylated %	Nd	4.34 ± 0.03	5.57 ± 0.44 b	0.25 ± 0.02 a
Muscat Hamburg				
Total anthocyanins (μg MGE/g DW)	543.02 ± 19.17 a	6462.62 ± 231.75b	1692.42 ± 44.10 a	6138.02 ± 28.94 b
4′-substituted %	Nd	Nd	Nd	0.49 ± 0.03
3′4′-substituted %	62.30 ± 1.57 a	81.01 ± 0.33 b	72.09 ± 0.40 a	88.74 ± 0.33 b
3′4′5′-substituted %	37.70 ± 1.57 b	18.99 ± 0.33 a	27.91 ± 0.40 b	10.77 ± 0.29 a
Non-methoxylated %	9.94 ± 0.51 a	14.88 ± 1.33 b	5.95 ± 0.16 a	17.88 ± 0.18 b
Methoxylated %	90.06 ± 0.51 b	85.12 ± 1.33 a	94.05 ± 0.16 b	82.12 ± 0.18 a
Non-acylated %	95.48 ± 0.08 a	97.57 ± 0.09 b	92.61 ± 0.45 a	96.34 ± 0.25 a
Acetylated %	Nd	1.10 ± 0.20	1.69 ± 0.03	2.29 ± 0.11
Cinnamylated %	4.52 ± 0.08 b	1.07 ± 0.13 a	4.79 ± 0.40 b	1.16 ± 0.12 a
Caffeicylated %	nd	0.25 ± 0.17	0.90 ± 0.03	0.21 ± 0.02
NW196				
Total anthocyanins (μg MGE/g DW)	6262.87 ± 69.95 b	5583.84 ± 115.76 a	9017.53 ± 17.13 b	6102.31 ± 31.12 a
3′4′-substituted %	7.43 ± 0.02 a	12.17 ± 0.03 b	7.38 ± 0.00 a	12.76 ± 0.26 b
3′4′5′-substituted %	92.57 ± 0.02 b	87.83 ± 0.03 a	92.62 ± 0.00 b	87.24 ± 0.26 a
Non-methoxylated %	3.86 ± 0.10 a	13.47 ± 0.08 b	2.22 ± 0.09 a	14.37 ± 0.29 b
Methoxylated %	96.14 ± 0.10 b	86.53 ± 0.08 a	97.78 ± 0.09 b	85.63 ± 0.29 a
Monoglucoside %	13.58 ± 0.08 a	34.03 ± 0.09 b	9.86 ± 0.02 a	43.16 ± 0.29 b
Diglucosides%	86.42 ± 0.08 b	65.97 ± 0.09 a	90.14 ± 0.02 b	56.84 ± 0.29 a
Non-acylated %	54.15 ± 0.68 a	77.96 ± 0.05 b	61.63 ± 0.03 a	74.44 ± 0.29 b
Acetylated %	0.30 ± 0.02	0.73 ± 0.12	1.10 ± 0.03 a	3.11 ± 0.12 b
Cinnamylated %	36.35 ± 0.09 b	17.20 ± 0.08 a	30.29 ± 0.11 b	16.14 ± 0.01 a
Caffeicylated %	9.20 ± 0.75 b	4.10 ± 0.01 a	6.98 ± 0.12	6.31 ± 0.18

[a] Values are means of duplicate determination ± S.D (n = 2). nd means not detected. The content of each anthocyanin compound are shown in the supplementary material

[b] Different letters in each year are significantly different between summer and winter berries for the same grape cultivar at 0.05 level. No letter represented no significant differences between summer and winter grapes

[c] Summer berries (first crop, harvested in late June)

[d] Winter berries (second crop, harvested in late December)

[e] Anthocyanin contents are expressed as μg malvidin-3-O-glucoside equivalence (MGE) per g of dry weight of grape skins (DW)

[f] Anthocyanin compounds in grape skin are divided into 4′-substituents (pelargonidin-derivatives), 3′,4′-substituents (cyanidin- and peonidin-derivatives) and 3′,4′,5′-substituents (delphinidin-, petunidin- and malvidin-derivatives) according to the number of B-ring substituents

[g] Or anthocyanin compounds in grape skin are divided into non-methylated (pelargonidin-, delphinidin- and cyanidin-derivatives) and methylated (petunidin-, peonidin- and malvidin-derivatives) anthocyanins depending on whether the hydroxyls in B-ring are methylated

[h] Or anthocyanin compounds in grape skin are divided into monoglucosidic and diglucosidic anthocyanins according to the number of glucose groups

[i] Or anthocyanin compounds in grape skin are divided into non-acylated, acetylated, coumarylated and caffeoylated derivatives depending on the acylated groups

pattern of TFO accumulation where no significant differences were observed between the summer and winter berries in both years (Table 3).

Six major flavonol aglycones, quercetin (Q), kaempferol (K), myricetin (M), isorhamnetin (I), syringetin (S) and laricitrin (L), were identified in 'Kyoho', 'Muscat Hamburg' and 'NW196' skins. The flavonols in grapes could also be divided into 4′-substituents (K-derivatives), 3′,4′-substituents (Q- and I-derivatives) and 3′,4′,5′-substituents (M-, L- and S-derivatives) according to the B-ring substituent numbers (Downey et al. 2004). The 3′,4′-substituents, primarily Q-derivatives, were the most abundant flavonols in the skins in all the grape cultivars. The variations in the flavonol compositions between summer and winter berries were similar for all of the grape cultivars during both years. During grape maturation, the proportions of 3′,4′-substituents were higher in winter berries than in summer berries, and the opposite trend was observed for the proportions of 3′,4′,5′-substituent, although statistically differences were not observed in some samples (Table 3).

In the skins of the grape cultivars examined in the present study, the methylated flavonols, including S-, I- and L-derivatives, were the minor types. The proportions of methylated flavonols were significantly higher in summer berry skins than the winter berry skins for the three cultivars.

Variation of flavan-3-ols between summer- and winter-grown grape skins

For 'Kyoho' and 'Muscat Hamburg' skins, the total flavan-3-ol contents (TFA) in winter berry skin was richer than that in summer berry skin. For 'NW196' skins, no variation of TFA was observed between summer and winter berries in both years. The identified flavan-3-ol monomers were primarily consisted of catechin and epicatechin, and the polymers included dimmers and trimers among the three grape cultivars investigated. Flavan-3-ols in 'Kyoho' skins were evenly split between monomers and polymers, while polymers were the main flavan-3-ol type in 'Muscat Hamburg' skins and monomers, accounting for almost 100%, were predominant in 'NW196' skins. In general, the monomer proportions increased in the winter berries in comparison to the summer berries for 'Kyoho' and 'Muscat Hamburg'. The opposite was true for the polymer proportions, although statistically significant differences were not observed in some samples (Table 3).

Discussions and conclusions

The climatic conditions during the summer and winter growing seasons of 2009 and 2010 were shown in Appendix 3 and 4 in supplementary material. For climatic features of premium wine regions in China, the grapes grown under winter climate in Nanning were more conducive to high-quality wine production which primarily relies on the rich content of phenolic compounds in grape berries. Consistent with this observation, the same trend was noted in the previous study (Xu et al. 2011). In the present study, the anthocyanin concentrations in the samples for HPLC–MS/MS were increased so that more types of anthocyanin compounds were detected. As expected, new and interesting variations in the skin flavonoid contents and compositions were observed for grapes with different genetic backgrounds between summer and winter growing seasons.

'Muscat Hamburg' (*V. Vinifera*) cultivar and 'Kyoho' (*V. vinifera* × *V. labrusca*) are introduced grape cultivars in Nanning. The 'NW196', a new wine grape hybrid, was generated using a *V. quinquangularis* grape as the female parent and a *V. vinifera* wine grape cultivar as the male parent at the Guangxi Academy of Agricultural Sciences. The flavonoid contents in summer versus winter berry skin displayed different features during ripening between exotic and local genetic backgrounds. The different variation potentially reflect *V. quinquangularis*, a wild grape species of Southwest China, which accumulates more phenolic compounds compared with exotic grape species (*V. vinifera* and *V. labrusca*) during the summer growing season, when the climate of its natural habitat is humid and hot. However, variations in flavonoid compositions in the skins of three grapes with different genetic backgrounds showed the same characteristics between summer and winter growing seasons. Maybe because the responses of the downstream modifications in the flavonoid biosynthetic pathway, such as hydroxylation, glucosylation, methylation and acetylation, to the variations in climatic conditions are similar.

For the variation of anthocyanin contents in summer and winter berry skins of 'NW196', we got a different result from the previous study. In the present study, 32 anthocyanin compounds were identified in the concentrated anthocyanin extract, but only 12 anthocyanin compounds were found in the previous study (Xu et al. 2011). Some anthocyanin compounds with low levels in 'NW196' were not detected in the summer berry skins (Xu et al. 2011), such as dp-3-glc, pn-3,5-diglc, pn-3-glc, pt-3-cmglc and pn-3-trans-cmglc (Appendix 1 in supplementary material). More importantly, the concentrations of Mv-3,5-diglc and mv-3-cmglc-5-glc, the main anthocyanin compounds in 'NW196', in summer berries were significant higher than those in winter berries. This results were consistent in the samples of 2007 (Xu et al. 2011), 2009 and 2010 (Appendix 1 in supporting information). The concentrations of the two compounds between summer and winter berries existed a big differences in 2009 and 2010. As a result,

Table 3 The contents and compositions of flavonols and flavan-3-ols among three grape cultivars in different years and seasons

	2009[ab]		2010[ab]	
	Summer	Winter	Summer	Winter
Kyoho				
Total flavonols (μg QE/g DW)[e]	271.43 ± 3.86 a	366.75 ± 13.69 b	234.65 ± 9.70 a	461.62 ± 12.68 b
4′-substituted %[f]	20.13 ± 0.92	19.10 ± 0.40	17.16 ± 1.33	16.19 ± 0.38
3′4′-substituted %	55.68 ± 3.55	62.48 ± 0.02	52.25 ± 0.91 a	65.01 ± 0.45 b
3′4′5′-substituted %	24.19 ± 2.63	18.42 ± 0.42	30.59 ± 2.24 b	18.80 ± 0.08 a
Non-methoxylated %[g]	87.06 ± 2.37	89.93 ± 0.46	85.64 ± 0.03 a	90.92 ± 0.10 b
Methoxylated %	12.94 ± 2.37	10.07 ± 0.46	14.36 ± 0.03 b	9.08 ± 0.10 a
Total flavan-3-ols (μg CE/g DW)[h]	50.19 ± 0.43	68.51 ± 7.29	57.19 ± 5.68 a	124.79 ± 10.01 b
Monomers %[i]	49.86 ± 4.72	59.21 ± 1.19	46.90 ± 0.05 a	56.92 ± 2.85 b
Polymers %	50.14 ± 4.72	40.79 ± 1.19	53.10 ± 0.05 b	43.08 ± 2.85 a
Muscat Hamburg				
Total flavonols (μg QE/g DW)	291.10 ± 4.18 a	408.85 ± 14.27 b	438.77 ± 9.40 a	898.90 ± 49.69 b
4′-substituted %	21.02 ± 0.37 a	26.38 ± 1.04 b	35.51 ± 1.54	39.92 ± 0.69
3′4′-substituted %	56.96 ± 0.30	57.01 ± 1.47	47.44 ± 2.23	50.42 ± 1.19
3′4′5′-substituted %	22.02 ± 0.07 b	16.61 ± 0.43 a	17.05 ± 0.69 b	9.66 ± 0.49 a
Non-methoxylated %	77.06 ± 0.10 a	81.60 ± 0.39 b	81.71 ± 0.50 a	85.77 ± 0.02 b
Methoxylated %	22.94 ± 0.10 b	18.40 ± 0.39 a	18.29 ± 0.50 b	14.23 ± 0.02 a
Total flavan-3-ols (μg CE/g DW)	121.47 ± 3.97 a	426.16 ± 31.85 b	224.85 ± 27.06 a	428.44 ± 23.89 b
Monomers %	32.11 ± 1.12 a	41.62 ± 0.96 b	18.00 ± 0.34 a	39.34 ± 1.12 b
Polymers %	67.89 ± 1.12 b	58.38 ± 0.96 a	82.00 ± 0.34 b	60.66 ± 1.12 a
NW196				
Total flavonols (μg QE/g DW)	564.29 ± 13.11	617.73 ± 33.48	411.58 ± 6.62	571.00 ± 80.65
4′ substituted %	7.89 ± 0.26	6.12 ± 1.27	8.19 ± 1.33	10.81 ± 2.60
3′4′-substituted %	82.34 ± 0.64 a	87.59 ± 1.64 b	81.63 ± 0.59 a	86.14 ± 2.33 b
3′4′ 5′-substituted %	9.76 ± 0.37 b	6.28 ± 0.37 a	10.17 ± 0.74 b	3.05 ± 0.27 a
Non-methoxylated %	86.71 ± 0.20 a	90.95 ± 0.60 b	86.29 ± 0.85 a	92.40 ± 0.30 b
Methoxylated %	13.29 ± 0.20 b	9.05 ± 0.60 a	13.71 ± 0.85 b	7.60 ± 0.30 a
Total flavan-3-ols (μg CE/g DW)	97.16 ± 12.67	108.91 ± 2.55	75.96 ± 9.39	90.41 ± 12.96
Monomers %	100.00 ± 0.00	100.00 ± 0.00	100.00 ± 0.00 b	100.00 ± 0.00
Polymers %	Nd	Nd	Nd	Nd

[a] Values are means of duplicate determination ± S.D (n = 2). nd means not detected. The contents of each flavonol and flavan-3-ol compound are shown in the supporting information

[b] Different letters in each year are significantly different between summer and winter berries for the same grape cultivar at 0.05 level. No letter represented no significant differences between summer and winter grapes

[c] Summer berries (first crop, harvested in late June)

[d] Winter berries (second crop, harvested in late December)

[e] Flavonol contents are expressed as μg quercetin equivalence (QE) per g of dry weight of grape skins (DW)

[f] Flavonol compounds in grape skin are divided into 4′-substituents (kaempferol-derivatives), 3′,4′-substituents (quercetin- and isorhamnetin-derivatives) and 3′,4′,5′-substituents (myricetin-, laricitrin- and syringetin-derivatives) according to the B-ring substituent numbers

[g] Or flavonol compounds in grape skin are divided into non-methoxylated (kaempferol-, quercetin- and myricetin-derivatives) and methylated (syringetin-, isorhamnetin- and laricitrin-derivatives) flavonols depending on whether the hydroxyls in B-ring are methylated

[h] Flavan-3-ol contents are expressed as μg catechin equivalence (CE) per dry weight of grape skins (DW)

[i] Flavan-3-ol compounds detected in grape skin are divided into monomers (catechin and epicatechin) and polymers (dimers and trimers)

summer berries had significant higher total anthocyanin contents than winter berries in the present study, which accurately reflected the effects of growing season on anthocyanin accumulation in 'NW196' berry skins.

The correlations of the flavonoid contents and compositions in skins of the three grape cultivars and accumulated data of climatic factors in Nanning were analyzed (Appendix 5 and 6 in supplementary material). The results

indicated the differences of temperature, sunshine and rainfall between summer and winter growing seasons play an important role on flavonoid accumulation in grape skins. According to the correlation coefficients and significant level, accumulation of the anthocyanins was most sensitive to the climate changes between summer and winter. While the climate changes had less impacts on the contents of flavonols and flavan-3-ols in NW196 and the compositions of flavonols and flavan-3-ols in the three grape cultivars. The positive or negative correlations showed the change rule of the flavonoid contents in 'NW196' skins was different compared with 'Kyoho' and 'Muscat Hamburg'. In climatic conditions of Nanning, the less accumulated average monthly temperature and rainfall and the longer sunshine duration, the more flavonoid contents 'Kyoho' and 'Muscat Hamburg' skins had, while anthocyanin contents in 'NW196' was just the opposite. For the flavonoid compositions, the less accumulated average monthly temperature and rainfall and the longer sunshine duration, the more the proportions of 3′4′-substituted, non-methoxylated, monoglucoside and non-acylated anthocyanins, 3′4′-substituted flavonols and flavan-3-ol monomers the skins of the three grape cultivars possessed.

Temperature is one of the main factors influencing flavonoid content and composition in grape berries. Previous studies showed that moderate temperatures (Spayd et al. 2002) and dampening diurnal temperature fluctuations (Cohen et al. 2008) enhanced anthocyanin accumulation, but high temperatures reduced anthocyanin accumulation (Mori et al. 2005; Mori et al. 2007a, b; Yamane et al. 2006). However, temperature showed little to no effect on flavonol accumulation (Cohen et al. 2012a, b; Mori et al. 2005; Spayd et al. 2002). For the accumulation of flavan-3-ol polymers, a positive correlation with heat summation was observed during berry development (Cohen et al. 2008; Cohen et al. 2012a, b). On the other hand, the temperature could also alter the composition of flavonoid compounds. High temperatures (30 or 35 °C) may increase acylated anthocyanins, primarily coumaroyl derivatives (Spayd et al. 2002; Tarara et al. 2008), and decrease 3′,4′,5′-substituents and methylated derivatives (Mori et al. 2005; Mori et al. 2007a, b). Compressed diurnal temperature ranges reduced 3′,4′,5′- substituent anthocyanins and flavonols and acylated anthocyanins (Cohen et al. 2012a, b). In this two-crop-a-year production area, markedly higher temperatures were recorded during the summer growing season than in the winter growing season (averaging max 31.28 vs. 23.53 °C day, min 23.73 vs. 15.76 °C night). In addition, the diurnal temperature differences for winter crops were larger than those for summer crops during ripening (9.27 vs. 6.95 °C). In the two-crop-a-year viticulture system with multiple climatic factors, it is clear that day and night temperatures played dominant roles in both anthocyanin accumulation and the hydroxylated and methylated compositions of anthocyanins and flavonols. It is likely that day and night temperatures also influence flavan-3-ol monomer and polymer compositions. Moreover, diurnal temperature differences primarily affected the compositions of hydroxylated anthocyanins and flavonols and acylated anthocyanins.

Solar radiation exerts an important impact on flavonoid synthesis and accumulation in grape skins. Flavonol accumulation is sensitive to sunlight compared with anthocyanins and flavan-3-ols (Downey et al. 2004; Haselgrove et al. 2000; Price et al. 1995; Spayd et al. 2002). Sunlight exposure increases flavonol accumulation (Fujita et al. 2006, 2007; Matus et al. 2009; Ristic et al. 2007). When the exposure intensity exceeded a certain limit, the anthocyanin content decreased (Bergqvist et al. 2001; Chorti et al. 2010). Compared with the anthocyanin content, the anthocyanin composition was more affected by sunlight. Anthocyanins showed a proportional shift from 3′,4′-substituents to 3′,4′,5′-substituents (Downey et al. 2004; Kennedy et al. 2002; Spayd et al. 2002; Tarara et al. 2008) and from nonacylated glucosides to coumaroyl glucosides with shading (Haselgrove et al. 2000; Tarara et al. 2008). Previous studies indicated that shading decreases skin tannins (Fujita et al. 2007; Ristic et al. 2007). In the present study, the summer crop exhibited evidence of higher solar radiation intensity than the winter crop, while the sunlight time in the winter growing season was 61% longer than that in the summer growing season during both years. Thus, the sunlight time is likely a major factor influencing flavonol and tannin accumulation and hydroxylated and acylated anthocyanin compositions.

Water availability also influences the synthesis and accumulation of skin flavonoid compounds. Under water deficit, anthocyanin accumulation was enhanced (Romero et al. 2013). The anthocyanin composition was enriched in 3′,4′,5′-substituent and methoxylated derivatives by water deficit (Castellarin et al. 2007). Water deficit also increased flavonols and flavan-3-ols (Deluc et al. 2009; Esteban et al. 2001; Roby et al. 2004; Sofoa et al. 2012). In this two-crop-a-year cultivation system, the average rainfall from veraison to harvest for the summer crops was 11.4-fold higher than that for the winter crops. Even the winter crops were irrigated every week (45–75 m^3/ha) from October to November, there were much more water obtained by summer crops than by winter crops. And the winter crops were not irrigated in December. The less water availability should promote the accumulation of flavonoids, including anthocyanins, flavonols and 3′,4′,5′-substituents, in winter berry skins. Wine quality primarily relies on the quality of the grape berries, including the richness and composition of flavonoids, sugar and acid concentrations. Moderate

climate and suitable cultivation measures can produce grape berries with good quality. In general, wines made from grapes produced under cooler climatic conditions in the classic wine growing regions possess high quality. However, climatic conditions have complicated and compositive effects on grape berry qualities in the two-crop-a-year cultivation system. The increase of flavonoid contents might improve wine qualities, while the influence of alterations in flavonoid composition on wine qualities remains unknown. Moreover, the regulatory mechanisms underlying the responses to climatic conditions during flavonoid biosynthesis remain elusive. In recent years, many wine regions have been affected by global warming worldwide. Thus, the influences of multiple factors on berry quality must be coordinately considered. It is necessary to further study these subjects, which will have guiding significance for viticulture under climatic warming conditions of wine regions.

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