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# Influence of Growing Season on Phenolic Compounds and Antioxidant Properties of Grape Berries from Vines Grown in Subtropical Climate

Changmou Xu,[†,∥] Yali Zhang,[†,∥] Lei Zhu,[†] Yu Huang,[‡] and Jiang Lu*[,†,§]

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

[‡]Guangxi Academy of Agricultural Sciences, Nanning 530007, People's Republic of China

[§]Center for Viticulture and Small Fruit Research, Florida Agricultural and Mechanical University, Tallahassee, Florida 32317, United States. [∥] These authors contributed equally to this paper.

**ABSTRACT:** The influence of growing season (winter vs summer) on the synthesis and accumulation of phenolic compounds and antioxidant properties was studied in five grape cultivars for three consecutive years. Four phenolic compound parameters (total phenols, flavonoids, flavan-3-ols, and anthocyanins) and three antioxidant property parameters [2,2-diphenyl-1-picrylhydrazyl radical scavenging, 2,2-azinobis(3-ethylbenzothiazolinesulfonic acid) radical scavenging, and ferric reducing antioxidant power] were investigated. Results showed that both phenolic compounds and antioxidant properties in the seed and skin of winter berries were significantly ($p < 0.05$) higher than those of summer berries for all of the cultivars investigated. The anthocyanin profiles of berry skins appeared to be extremely consistent in different years for the same crop, whereas they varied greatly between the two crops within the same year (winter vs summer). Winter berries contained richer glucosides of delphinidin, cyanidin, peonidin, and malvidin than summer berries. These seasonal variations of phenolic compounds and antioxidant properties on grape berries were largely contributed by climatic factors such as temperature, solar radiation, rainfall, and hydrothermic coefficient between different growing seasons.

**KEYWORDS:** Growing seasons, climatic factors, summer and winter berries, two crops, total phenols, anthocyanins, temperature, DPPH

## INTRODUCTION

Grapevine is one of the most widely grown fruit crops in the world. It is commonly grown in temperate areas where the culture practice is one-crop-a-year that allows 3−5 months of vine dormancy from late fall to early spring. In recent years, grape production has been expanded to warmer subtropical areas where the climate conditions allow the production of two crops per year. This practice, in addition to making more efficient use of the land, can increase income for grape growers. In the two-crop-a-year grape culture system, the growing season for the first, or summer, crop is usually from February to June, and that of the second, or winter, crop is from August to December.

It has been reported that, for the one-crop-a-year culture system, phenolic compositions and concentrations of grape berries varied in different growing seasons, even when the same cultivar was grown in the same vineyard for two consecutive years.[1] It is understandable that these variances should have much to do with the seasonal difference of climatic conditions. The impact of climatic factors on phenolic compositions and concentrations of grape berries has been widely studied. For example, Mori et al.[2] demonstrated that high temperature (35 °C daytime, 20 °C nighttime) during the day from veraison to harvest reduced the total anthocyanins content to less than half of that in the control (25 °C daytime, 20 °C nighttime) in 'Cabernet Sauvignon' berries. Solar radiation significantly increased the expression of flavonoid biosynthetic genes[3,4] and the accumulation of flavonoid.[5] In a shaded and exposed treatment study, the flavonol concentration in 'Pinot noir' berry skins increased linearly with the increase of fruit cluster exposure to the sunlight.[6] Water availability was also found to influence the accumulation and composition of phenolic compound in grape berries. In one phytotron study, for example, biosynthesis of proanthocyanins and anthocyanins increased when water was deficient between veraison and harvest maturity.[7] Although manipulating a single microclimate factor would help to understand its effects on phenolic biosynthesis, studies on the effects of actual macroclimatic conditions on grape berry quality would be more useful in viticulture practice.

Several studies have demonstrated the effects of yearly difference on grape phenolic compositions and concentrations, such as flavonoids[1] and anthocyanins.[8] The seasonal variation of grape phenolic compositions and concentrations within a year, to the best of our knowledge, has not been reported. The two-crop-a-year culture practice, due to the drastic contrast of climate conditions, is a great system to study the influence of climate conditions on grape berry qualities and antioxidant properties.

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## MATERIALS AND METHODS

**Chemicals and Standards.** Folin−Ciocalteu's phenol reagent, gallic acid (≥98%, UV, HPLC), procyanidin (≥95%), malvidin 3,5-diglucoside (≥98%), rutin (≥98%), 2,4,6- tripyridyl-*s*-triazine (TPTZ) (≥99%), 2,2′-azinobis(3-ethylbenzothiazoline-6-sulfonic acid) diammonium salt (ABTS) (≥98%), and 2,2-diphenyl-1-picrylhydrazyl (DPPH) (≥97%) were obtained from Sigma-Aldrich (St. Louis, MO). Sodium carbonate and vanillin were purchased from Merck (Darmstadt, Germany). 6-Hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid (Trolox) (≥99%) was obtained from Alexis (Axxora, Switzerland). HPLC-grade solvents and other analytical grade solvents were purchased from Beijing Huagong (Beijing, China).

**Research Vineyard.** Research was carried out in 2007, 2008, and 2009 in the Experimental Vineyard of Guangxi Academy of Agricultural Sciences, located in southern China (latitude 22.47 °N, longitude 108.21 °E). The climate is typically subtropical monsoon with mild winters; rainfall is around 800−1600 mm per year, mainly distributed from June to September. The period from March to May is "plum rains" season, whereas October−February is the dry season. The vines were planted in east−west oriented rows, spaced 1.5 m (between vines) × 2.0 m (between rows). Five-year-old vines were trained upward on vertical shoot positioning with short spur pruning.

**Grape Materials.** Fully ripened colored wine grapes of 'Kyoho' (Euro-American hybrid, used as wine grape in Guangxi production area) and 'NW196' (*Vitis quinquangularis* Rehd. × *Vitis vinifera* L., Euro-Asian hybrid) were collected twice a year (summer and winter) for three consecutive years. Another three white Euro-American wine grape cultivars 'Kamabikaki', 'Orion White', and 'Sirius White' were collected in the same vineyard in 2009 only. Three to four clusters per vine, and 10 vines per cultivar were randomly picked and shipped immediately to China Agricultural University, where analysis was performed.

Mixed berries randomly picked from the top, middle, and bottom portions of each cluster were used for this analysis. Seeds and skins were separated manually and immediately freeze-dried in a freeze-dryer (LGJ-18, Ruibang Xinye Corp., Beijing, China). Dried specimens were stored in vacuum-packaged polyethylene pouches at −20 °C until analysis. Freeze-dried grape seeds were crushed and then defatted using our previously reported method.[9] The defatted grape seeds and skins were put in a mortar in liquid nitrogen and ground into a fine powder for subsequent analysis.

**Preparation of Grape Seed and Skin Extracts.** Total phenols were extracted from grape seeds and skins using our previously reported method.[9] Briefly, 0.2 g of freeze-dried and ground seeds or skins was weighed into 50 mL centrifuge tube with 8 mL of methanol/water/hydrochloric acid (70:29:1, v/v/v) in an orbital shaker at 300 rpm for 100 min at 25 °C. After the supernatant had been poured out, the precipitate was re-extracted with 8 mL of the same solvent two more times. Supernatants were combined in a 50 mL tube and centrifuged at 2800*g* for 20 min (Beckman Coulter Ltd., Palo Alto, CA). The supernatant was then filtered on a filter paper, and the filtrate was collected. All extracts were stored at 4 °C in the dark until further analysis (normally within 2 days). Extractions were performed in three replicates for each individual powder−solvent combination.

Anthocyanins of grape skins used for HPLC-MS/MS analysis were extracted as follow: 0.5 g of freeze-dried and ground skins was weighed into a 50 mL centrifuge tube with 8 mL of methanol/water/formic acid (70:29:1, v/v/v) in an orbital shaker at 300 rpm for 100 min at 25 °C. After the supernatant had been poured out, the precipitate was re-extracted with 8 mL of the same solvent two more times. Supernatants were combined in a 50 mL tube and centrifuged at 2800*g* for 20 min. The supernatant was then filtered on a filter paper, and the filtrate was collected. The combined filtrates were evaporated to dryness (≤30 °C) in a rotary evaporator (Ika-Werke RV06-ML, Germany), and the residue was redissolved in chromatographic grade methanol. All extracts were stored at 4 °C in the dark until further analysis (normally within 2 days). Extractions were performed in three replicates for each individual powder−solvent combination.

**Photometric Determination of Total Phenols, Flavonoids, Flavan-3-ols, and Anthocyanins.** The total phenol content in grape seeds or skins was determined according to the Singleton et al.[10] method on a UV−vis double-beam UNICO UV-2800 spectrometer (UNICO, New York). Gallic acid (GA) was used as standard and expressed as gallic acid equivalents (mg GAE/g DM, mg gallic acid/g of dry defatted matter) through the calibration curve of gallic acid. The linearity range of the calibration curve was 50−1000 $\mu$g/mL ($r$=0.9998). The total flavonoid content was determined using the colorimetric method described previously by Dewanto et al.[11] The results were calculated and expressed as micrograms of rutin equivalents (mg RAE/g DM) using the calibration curve of rutin. The linearity range of the calibration curve was 100−1000 $\mu$g/mL ($r$=0.9992). The total flavan-3-ol content was determined using the vanillin assay[12] with procyanidin as the standard and expressed as procyanidin equivalents (mg PAE/g DM) through the calibration curve of procyanidin. The linearity range of the calibration curve was 10−250 $\mu$g/mL ($r$ = 0.9998). The total anthocyanin content was determined following the procedure described by Lohachoompol et al.[13] using malvidin 3,5-diglucoside as the standard and expressed as malvidin 3,5-diglucoside equivalents (mg MAE/g DM) through the calibration curve of malvidin 3,5-diglucoside. The linearity range of the calibration curve was 100−1000 $\mu$g/mL ($r$ = 0.9995). All analyses were replicated twice with the mean ± SD being reported.

**HPLC-DAD-ESI-MS/MS Analysis of Anthocyanins.** Analysis of anthocyanins were carried out according to the following method by using an Agilent 1200 series LC and LC-MSD Trap VL mass spectrometer (Agilent Technologies, Palo Alto, CA) equipped with an electrospray ionization (ESI) interface. The LC system includes a G1379A online degasser, a G1311A quaternary pump, a 1313A autosampler, a G1316A thermostatic column control, and a G1315A DAD, all of which were controlled by Agilent ChemStation version 5.2 software. The HPLC separation was performed on a reversed-phase Zorbax SB-$C_{18}$ column (250 mm × 4.6 mm i.d., 5 $\mu$m particle size, Agilent Technologies) at 25 °C. The mobile phase consisted of 2% methanoic acid in water (solvent A) and 2% methanoic acid in acetonitrile (solvent B) at the following gradient: 0−1 min, 3% B; 1−12 min, 3−15% B; 12−24 min, 15−25% B; 24−28 min, 25−30% B; 28−32 min, 30−4% B. The flow rate was 1.0 mL/min. The injection volume was 20 $\mu$L with the UV detector set to an absorbance wavelength of 525 nm. The ESI parameters were as follows: nebulizer, 30 psi; dry gas ($N_2$) flow, 12 mL/min; dry gas temperature 300 °C; the ion trap mass spectrometer was operated in negative ion mode with a scanning range from $m/z$ 100 to 1500. Identification of individual anthocyanins was made with retention time and UV−vis spectral data.

**Antioxidant Properties of Grape Seed and Skin Extracts.** Free radical scavenging activity on DPPH assay was based on the method of Brandwilliams et al.[14] For the free radical scavenging activity on ABTS assay, a procedure from Re et al.[15] was used. The radical scavenging activities of the test samples were expressed as Trolox equivalent antioxidant capacity ($\mu$M TE/g DM, $\mu$M trolox/g of dry defatted matter) on their percentage inhibitions. Determination of ferric reducing antioxidant power (FRAP assay) was done according to the method of Benzie et al.[16] The antioxidant power of the test samples was also expressed as Trolox equivalent antioxidant capacity ($\mu$M TE/g DM). Trolox standard solutions in all assays were prepared at a concentration ranging from 100 to 1000 $\mu$M. The $r$ values of these calibration curves were 0.9998, 0.9994, and 0.9992, respectively.

**Statistical Analysis.** Experimental results were expressed as the mean ± SD of three parallel measurements. Data were subjected to ANOVA, and differences among cultivars were tested by post hoc

**Table 1. Phenolic Compounds in Seeds and Skins among Five Grape Cultivars in Different Years and Seasons**

| | | total phenols[a] (mg GAE/g DM) | | total flavonoids[a] (mg RAE/g DM) | | total flavan-3-ols[a] (mg PAE/g DM) | | | |
|---|---|---|---|---|---|---|---|---|---|
| cultivar/year | season | seeds | skins | seeds | skins | seeds | skins | skin anthocyanins[a] (mg MAE/g DM) | color (visual) |
| **Kyoho** | | | | | | | | | |
| 2007 | summer[b] | 50.11 ± 1.51 g | 22.21 ± 1.58 e | 45.52 ± 2.80 fg | 17.65 ± 1.46 hi | 42.30 ± 2.15 efg | 15.42 ± 1.05 g | 2.58 ± 0.09 i | red |
| | winter[c] | 60.68 ± 1.90 f | 43.28 ± 1.72 b | 56.80 ± 2.33 e | 38.45 ± 1.71 c | 53.26 ± 2.17 d | 30.21 ± 1.11 c | 12.14 ± 0.40 d | black |
| 2008 | summer | 51.60 ± 3.98 g | 24.36 ± 1.65 e | 46.75 ± 2.41 f | 18.50 ± 0.77 hi | 44.21 ± 2.72 ef | 18.30 ± 0.87 f | 2.64 ± 0.16 i | red |
| | winter | 69.01 ± 2.30 e | 46.58 ± 2.15 a | 61.22 ± 3.38 e | 42.50 ± 1.25 b | 58.84 ± 2.00 d | 37.51 ± 1.20 b | 13.70 ± 0.46 c | black |
| 2009 | summer | 52.92 ± 2.72 g | 40.38 ± 2.10 b | 48.56 ± 2.11 f | 34.74 ± 0.90 d | 46.01 ± 1.45 e | 26.90 ± 1.11 d | 10.44 ± 0.44 f | red |
| | winter | 75.12 ± 2.27 d | 49.75 ± 2.42 a | 70.60 ± 2.13 d | 46.92 ± 1.15 a | 65.30 ± 1.45 c | 40.55 ± 0.55 a | 18.00 ± 0.51 a | black |
| **NW196** | | | | | | | | | |
| 2007 | summer | 38.93 ± 2.20 i | 24.21 ± 1.80 e | 36.90 ± 3.25 hi | 12.50 ± 0.47 j | 36.00 ± 1.60 hi | 10.90 ± 1.11 h | 7.70 ± 0.35 h | black |
| | winter | 41.36 ± 1.68 hi | 32.50 ± 2.05 c | 39.75 ± 2.85 gh | 20.74 ± 1.25 f | 39.02 ± 1.11 gh | 15.11 ± 0.93 g | 10.76 ± 0.25 ef | black |
| 2008 | summer | 38.54 ± 1.65 i | 22.01 ± 1.91 e | 28.74 ± 2.13 j | 12.12 ± 0.97 j | 26.60 ± 2.07 k | 8.45 ± 0.45 i | 9.34 ± 0.28 g | black |
| | winter | 46.19 ± 2.90 h | 30.85 ± 1.32 cd | 42.96 ± 2.55 fg | 19.28 ± 0.55 gh | 40.12 ± 1.62 fgh | 15.02 ± 0.71 g | 14.11 ± 0.40 c | black |
| 2009 | summer | 38.18 ± 1.55 i | 27.44 ± 1.75 d | 32.10 ± 1.65 ij | 18.50 ± 0.70 hi | 29.55 ± 2.08 jk | 9.44 ± 0.29 hi | 11.46 ± 0.55 e | black |
| | winter | 42.30 ± 1.95 hi | 30.78 ± 1.66 cd | 35.76 ± 2.36 hi | 19.69 ± 0.70 gh | 33.45 ± 2.38 ij | 15.92 ± 0.80 g | 16.94 ± 0.76 b | black |
| **Orion White** | | | | | | | | | |
| 2009 | summer | 68.71 ± 1.82 e | 9.88 ± 0.61 g | 68.20 ± 1.80 d | 4.42 ± 0.30 k | 55.00 ± 2.51 d | 1.29 ± 0.07 L | ND[d] | white |
| | winter | 79.78 ± 2.09 c | 10.72 ± 0.71 g | 75.84 ± 2.66 c | 5.50 ± 0.45 k | 67.29 ± 3.32 c | 3.30 ± 0.40 k | ND | white |
| **Kamabikaki** | | | | | | | | | |
| 2009 | summer | 88.70 ± 4.27 b | 24.23 ± 2.15 e | 81.50 ± 2.57 b | 16.74 ± 0.90 i | 78.90 ± 2.15 b | 10.40 ± 1.11 h | ND | white |
| | winter | 99.18 ± 3.36 a | 30.58 ± 1.19 cd | 90.47 ± 4.35 a | 28.88 ± 1.72 e | 88.09 ± 4.54 a | 21.33 ± 1.30 e | ND | white |
| **Sirius White** | | | | | | | | | |
| 2009 | summer | 72.03 ± 2.10 de | 14.52 ± 0.92 f | 70.32 ± 2.92 d | 5.80 ± 0.35 k | 54.01 ± 2.01 d | 5.52 ± 0.50 j | ND | white |
| | winter | 89.28 ± 2.91 b | 17.08 ± 1.21 f | 86.88 ± 3.76 a | 10.71 ± 0.55 j | 77.67 ± 3.25 b | 6.99 ± 0.31 j | ND | white |

[a] Values represent means of triplicate determination ± SD. [b] Summer berries (first crop, harvested in late June). [c] Winter berries (second crop, harvested in late December). [d] ND, not determined.



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**Table 2. Anthocyanins Identified in Grape Skins of 'Kyoho' and 'NW196' by LC-UV-MS/MS[a]**

| peak | RT (min) | anthocyanin | K-07-S[b] | K-07-W | K-08-S | K-08-W | N-07-S | N-07-W | N-08-S | N-08-W |
|---|---|---|---|---|---|---|---|---|---|---|
| 1 | 4.6 | delphinidin 3-*O*-glucoside | ND | 0.46 | ND | 0.55 | ND | 0.63 | ND | 0.76 |
| 2 | 5.8 | peonidin 3,5-*O*-diglucoside | 0.12 | 0.34 | 0.18 | 0.31 | ND | 1.02 | ND | 1.44 |
| 3 | 6.2 | malvidin 3,5-*O*-diglucoside | 0.28 | 0.66 | 0.04 | 0.69 | 2.76 | 2.46 | 2.91 | 3.98 |
| 4 | 7.5 | cyanidin 3-*O*-glucoside | 0.06 | 0.42 | 0.03 | 0.46 | ND | 0.65 | ND | 0.76 |
| 5 | 10.2 | peondin 3-*O*-glucoside | 0.09 | 0.55 | 0.05 | 1.10 | ND | 1.01 | ND | 0.94 |
| 6 | 11.5 | malvidin 3-*O*-glucoside | 0.34 | 1.64 | 0.07 | 1.91 | 0.09 | 1.81 | 0.22 | 1.99 |
| 7 | 12.8 | malvidin 3-*O*-(6-*O*-acetyl)-glucoside (*cis*) | 0.08 | 0.12 | 0.05 | 0.14 | 0.03 | ND | 0.05 | ND |
| 8 | 19.5 | malvidin 3-*O*-(6-*O*-caffeoyl)-5-*O*-glucoside | 0.22 | 0.21 | 0.02 | 0.08 | 0.44 | ND | 0.41 | ND |
| 9 | 21.3 | delphinidin 3-*O*-(6-*O*-coumaryl)-glucoside | 0.09 | 0.52 | 0.04 | 0.54 | 0.04 | 0.13 | 0.05 | 0.21 |
| 10 | 22.6 | peondin 3-*O*-(6-*O*-acetyl)-glucoside | 0.13 | 0.19 | 0.02 | 0.38 | ND | ND | ND | ND |
| 11 | 23.5 | malvidin 3-*O*-(6-*O*-coumaryl)-5-*O*-glucoside | 0.70 | 1.24 | 0.21 | 0.89 | 1.49 | 0.02 | 0.64 | 0.22 |
| 12 | 25.2 | petunidin 3-*O*-(6-*O*-coumaryl)-glucoside | ND | 0.19 | ND | 0.19 | ND | 0.05 | ND | 0.06 |
| 13 | 25.6 | peonidin 3-*O*-(6-*O*-coumaryl)-glucoside (*cis*) | 0.13 | ND | 0.05 | ND | 0.02 | ND | 0.12 | nD |
| 14 | 26.4 | malvidin 3-*O*-(6-*O*-coumaryl)-glucoside (*cis*) | 0.27 | 0.64 | 0.05 | 0.51 | 0.11 | 0.11 | 0.18 | 0.16 |
| 15 | 29.8 | peonidin 3-*O*-(6-*O*-coumaryl)-glucoside (*trans*) | 0.17 | 0.49 | 0.13 | 0.60 | ND | 0.12 | ND | 0.16 |
| 16 | 30.2 | malvidin 3-*O*-(6-*O*-coumaryl)-glucoside (*trans*) | 0.86 | 1.85 | 0.32 | 1.66 | 0.36 | 0.32 | 0.68 | 0.38 |
| | | total | 2.91 | 9.52 | 1.26 | 10.01 | 5.34 | 8.33 | 5.26 | 11.06 |

[a] mg MAE/g DM; values represent means of triplicate determination. ND, not determined. [b] 'Kyoho' (K), 'NW196' (N), summer berries (S), winter berries (W), 2007 (07), 2008 (08).

comparison test (Student−Newman−Keuls) at $p < 0.05$ with Microsoft Excel 2003 and SPSS 16.0 for Windows.

## RESULTS AND DISCUSSION

**Variation of Phenolic Compounds between the Summer- and Winter-Grown Grape Berries.** *Total Phenols, Flavonoids, and Flavan-3-ols.* The total phenolic compounds in grape seeds of winter berries were significantly ($p < 0.05$) higher than those of summer berries in all of the cultivars studied (Table 1). The most significant increase of total phenolic compounds was found in the seeds of tetraploid 'Kyoho', a red Euro-American hybrid, which increased 32% on average from the summer berries to the winter berries, followed by Euro-American hybrids 'Orion White', 'Kamabikaki', and 'Sirius White', and the least increase was found in Euro-Asian hybrid 'NW196' (average of 12% increase). The lesser phenolic variation between the winter and summer crops in 'NW196' was probably because a parent of 'NW 196' is local wild species, which makes 'NW196' more adapted to the local climates. The scale of difference between the summer and winter crops varied in different years. Similar distributions were also found in total flavanoids and total flavan-3-ols between the summer and winter berries in all of the cultivars studied.

The phenolic contents in grape skins of winter berries were also significantly ($p < 0.05$) higher than that of summer berries among all of the cultivars studied (Table 1). Similar to the seeds, skins of 'Kyoho' had the most increase of phenol contents (2-fold) from the summer berries, whereas the increase of Euro-Asian hybrid 'NW196' was intermediate of the white Euro-American hybrids 'Orion White', 'Kamabikaki', and 'Sirius White'.

*Anthocyanins.* The anthocyanin content from the winter berry skins was significantly ($p < 0.05$) higher than that of the summer berry (Table 1). For example, the total anthocyanin content in 'Kyoho' skin in 2008 winter berries was 13.70 mg MAE/g DM, which was about 5-fold higher than the value of corresponding summer berries (2.64 mg MAE/g DM). For the same crop (summer or winter), the anthocyanin content also varied in different years. For instance, the 'Kyoho' summer berries in 2009 possessed about 4-fold higher anthocyanins than those in 2007 or 2008.

Sixteen individual anthocyanins were indentified in 'Kyoho' and 'NW196' berry skins including the glucosides and acylated (acetic or coumaric acids) glucoside forms of delphinidin, peonidin, malvidin, cyanidin, and petunidin (Table 2). Glucosides of malvidin and its acylated (coumaric acids) forms (peaks 3, 6, 11, 14, and 16) were the most prevalent anthocyanins in all berries (Figure 1). Delphinidin 3-*O*-glucoside (peak 1) and petunidin 3-*O*-(6-*O*-coumaryl)-glucoside (peak 12) were the signature anthocyanins for winter berries, whereas peonidin 3-*O*-(6-*O*-coumaryl)-glucoside (*cis*) (peak 13) was the signature anthocyanin for summer berries. The anthocyanin profiles of berry skins appeared to be extremely consistent in different years for the same crop, whereas they varied greatly between the two crops (summer vs winter) within the same year. For instance, glucosides or diglucosides of delphinidin, peonidin, malvidin, and cyanidin (peaks 1−6) were the main anthocyanins for the winter berries of 'NW196', whereas malvidin 3,5-*O*-diglucoside (peak 3), malvidin 3-*O*-(6-*O*-coumaryl)-5-*O*-glucoside (peak 11), and malvidin 3-*O*-(6-*O*-coumaryl)-glucoside (*trans*) (peak 16) were the main anthocyanins for the summer berries. Malvidin 3-*O*-(6-*O*-coumaryl)-glucoside (*trans*) (peak 16) was the most significant anthocyanin for 'Kyoho', and malvidin 3,5-*O*-diglucoside (peak 3) was the most significant anthocyanin for 'NW196'.

**Variation of Antioxidant Properties between the Summer- and Winter-Grown Grape Berries.** The antioxidant properties found by different assays in seeds of winter berries were significantly ($p < 0.05$) higher than those of summer berries for all of the cultivars investigated (Table 3). Similar to the phenolic contents, 'Kyoho' seeds showed the most increase of antioxidant properties in the winter berry in comparison to the summer berry (average of about 60%), whereas the smallest increase appeared in the Euro-Asian hybrid 'NW196' (average of about 10%). Increases of antioxidant properties in the Euro-American

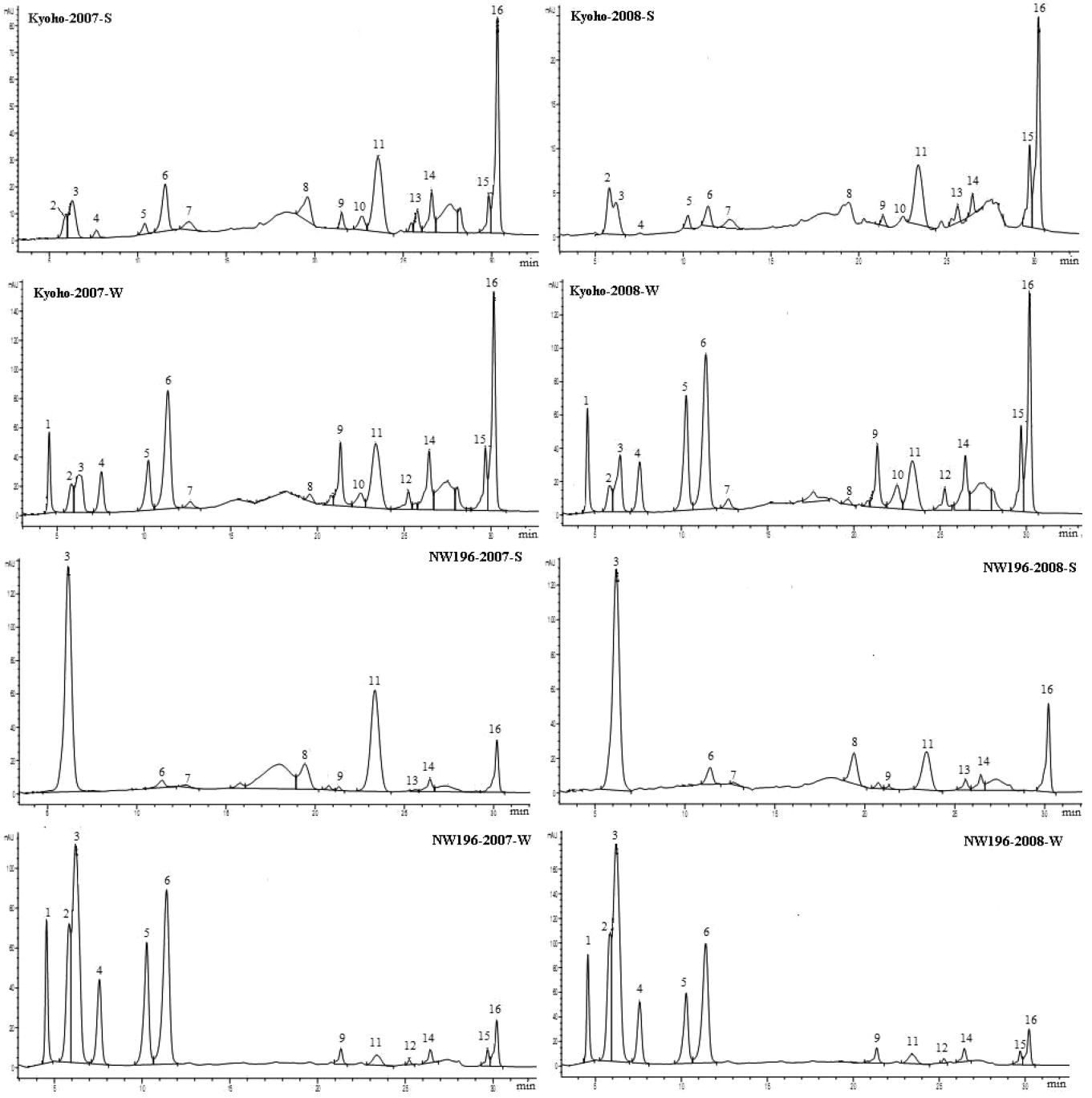


**Figure 1.** Chromatogram at 525 nm of anthocyanins in 'Kyoho' and 'NW196' grape skin extracts. Peak numbers and their corresponding anthocyanins are listed in Table 2.

hybrids 'Orion White', 'Kamabikaki', and 'Sirius White' fell between. Because the variation of antioxidant properties was closely correlated to the variation of total phenolic compounds in seeds,[17] the winter berries with richer phenolic compounds are also expected to have higher antioxidant properties.

Antioxidant properties were also found to be higher in skins of winter berries than summer berries for all cultivars studied (Table 3). Similar to the seeds, the most significant increase of antioxidant properties from the summer to winter berries was found in the skins of 'Kyoho', followed by 'NW196', whereas the least increases, as expected, were among the white skin cultivars 'Orion White', 'Kamabikaki', and 'Sirius White'.

**Influence of Climatic Factors on Phenolic Compounds and Antioxidant Properties of Grape Berries.** It is well-known that synthesis and accumulation of grape phenolic compounds are greatly affected by climate conditions such as temperature, solar radiation, rainfall, and hydrothermic coefficient.[1,8] Among them, temperature is considered to be one of the main factors influencing the synthesis and accumulation of phenolic compounds in grapes. The parameters of temperature include the active accumulated temperature, average temperature, temperature during the day and night, and temperature difference between day and night. An active accumulated temperature of 2800 °C was suggested to be the minimum for grape production.[18] In our study,

**Table 3. Antioxidant Activities of Grape Seed and Skin Extracts Measured by DPPH, ABTS, and FRAP Methods[a]**

| | | seeds | | | skins | | |
|---|---|---|---|---|---|---|---|
| cultivar/year | season | DPPH | ABTS | FRAP | DPPH | ABTS | FRAP |
| **Kyoho** | | | | | | | |
| 2007 | summer[b] | 333.14 ± 11.15 h | 420.24 ± 10.48 fg | 350.11 ± 12.53 gh | 158.33 ± 8.11 gh | 140.35 ± 7.00 h | 125.41 ± 6.00 g |
| | winter[c] | 578.26 ± 17.07 bc | 610.99 ± 17.69 c | 600.90 ± 22.06 c | 327.21 ± 9.53 b | 280.51 ± 7.85 b | 315.47 ± 11.18 b |
| 2008 | summer | 352.52 ± 16.05 gh | 409.62 ± 14.51 fgh | 349.76 ± 17.53 gh | 161.69 ± 7.50 fgh | 149.51 ± 6.47 h | 169.33 ± 6.58 f |
| | winter | 552.97 ± 28.22 c | 632.56 ± 21.51 bc | 572.18 ± 14.52 c | 351.78 ± 12.07 a | 313.94 ± 9.00 a | 354.47 ± 13.03 a |
| 2009 | summer | 373.21 ± 16.64 fg | 443.11 ± 16.69 f | 376.58 ± 8.82 fg | 278.07 ± 6.10 c | 258.58 ± 7.48 c | 304.75 ± 6.04 b |
| | winter | 594.24 ± 19.01 b | 654.12 ± 21.51 b | 601.00 ± 21.03 c | 349.19 ± 10.51 a | 319.47 ± 10.03 a | 370.18 ± 13.59 a |
| **NW196** | | | | | | | |
| 2007 | summer | 375.84 ± 12.02 fg | 414.28 ± 12.10 fgh | 412.33 ± 16.77 e | 173.33 ± 10.03 fg | 177.51 ± 7.59 fg | 168.04 ± 7.55 f |
| | winter | 391.70 ± 7.12 f | 420.92 ± 15.05 fg | 416.61 ± 14.60 e | 194.02 ± 11.79 e | 212.56 ± 10.00 e | 222.90 ± 9.15 c |
| 2008 | summer | 328.26 ± 14.13 h | 366.47 ± 17.50 i | 333.90 ± 20.55 h | 147.49 ± 6.17 hi | 152.86 ± 5.58 h | 134.61 ± 8.05 g |
| | winter | 396.36 ± 12.16 f | 422.98 ± 11.50 fg | 406.76 ± 6.01 ef | 221.17 ± 10.59 d | 209.86 ± 7.91 e | 222.90 ± 11.50 c |
| 2009 | summer | 345.12 ± 14.04 gh | 384.12 ± 11.25 hi | 356.12 ± 7.50 gh | 205.66 ± 8.57 d | 184.06 ± 8.08 f | 184.33 ± 8.06 e |
| | winter | 381.25 ± 17.10 fg | 401.14 ± 12.02 gh | 398.10 ± 11.80 ef | 217.30 ± 6.50 d | 229.50 ± 7.05 d | 239.19 ± 6.47 c |
| **Orion White** | | | | | | | |
| 2009 | summer | 495.24 ± 16.50 d | 525.45 ± 20.50 e | 514.76 ± 10.23 d | 92.26 ± 3.00 j | 78.12 ± 4.00 k | 51.47 ± 2.56 j |
| | winter | 514.83 ± 16.05 d | 574.00 ± 11.53 d | 585.90 ± 18.60 c | 98.56 ± 5.01 j | 82.77 ± 3.03 k | 53.61 ± 3.02 j |
| **Kamabikaki** | | | | | | | |
| 2009 | summer | 604.18 ± 18.07 b | 662.41 ± 13.03 b | 650.19 ± 13.01 b | 175.20 ± 6.63 f | 167.01 ± 6.51 g | 180.04 ± 4.59 ef |
| | winter | 662.22 ± 20.03 a | 721.96 ± 22.01 a | 747.04 ± 20.96 a | 217.26 ± 5.52 d | 190.65 ± 10.57 f | 199.76 ± 4.13 d |
| **Sirius White** | | | | | | | |
| 2009 | summer | 512.98 ± 16.17 d | 573.50 ± 17.96 d | 606.48 ± 19.64 c | 109.12 ± 4.05 j | 106.24 ± 6.02 j | 87.04 ± 4.04 i |
| | winter | 578.26 ± 15.01 bc | 646.95 ± 11.50 b | 642.04 ± 22.01 b | 133.30 ± 8.50 i | 122.50 ± 6.52 i | 102.47 ± 3.01 h |

[a] Values represent means of triplicate determination ± SD. Results are expressed as μM Trolox equivalents (μM TE/g DM). [b] Summer berries (first crop, harvested in late June). [c] Winter berries (second crop, harvested in late December).

**Table 4. Climatic Factors during the Growing Seasons of the Two-Crop-a-Year Culture System in Nanning [a]**

| | summer berry (harvested in late June) | | | | | winter berry (harvested in late December) | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| year | active-T[b] (°C) | accum-T[c] (°C) | sunshine-D[e] (h) | rainfall[e] (mm) | $K$[f] | active-T (°C) | accum-T (°C) | sunshine-D (h) | rainfall (mm) | $K$ |
| 2007 | 3405.4 | 74.8 | 565.2 | 100.3 | 2.02 | 3530.6 | 55.5 | 789.9 | 23.3 | 0.51 |
| 2008 | 3377.6 | 75.1 | 448.0 | 300.6 | 1.68 | 3504.5 | 54.4 | 746.8 | 23.9 | 0.66 |
| 2009 | 3458.5 | 78.3 | 488.2 | 154.4 | 2.02 | 3630.5 | 61.3 | 798.4 | 6.2 | 0.12 |
| av | 3413.8 | 76.1 | 500.5 | 185.1 | 1.91 | 3555.2 | 57.1 | 778.4 | 17.8 | 0.43 |
| av-30[g] | 3242.4 | 76.3 | 478.4 | 207.1 | 2.41 | 3486.1 | 57.3 | 785.6 | 24.5 | 0.58 |

[a] Data from Nanning Meteorological Administration and http://www.weather.com.cn. [b] Active accumulated temperature during grape growing seasons. Calculated as $T = \sum$ti (ti ≥ 10 °C); ti was average daily temperature from February to June for summer crop and from August to December for winter crop. [c] Accumulated average monthly temperature. Calculated by adding the average temperature of the 3 months before harvesting, from April to June for the summer crop and from October to December for the winter crop. [e] Sunshine duration during grape growing seasons. Calculated by adding sunshine hours from February to June for the summer crop and from August to December for the winter crop. [e] Average monthly rainfall. Value of June for the summer crop and December for the winter crop. [f] Hydrothermic coefficient. Calculated by $K = \sum P/\sum t \times 10$, $\sum t$ was active accumulated temperature of the two months before harvesting and $\sum P$ was total rainfall in the corresponding period, from May to June for the summer crop and from November to December for the winter crop. [g] Average of 30 years (from 1971 to 2000).

the active accumulated temperatures for both growing seasons were well above the threshold, with an average of 3555.2 °C for the winter crop and 3413.8 °C for the summer crop (Table 4). This means that the temperature is sufficient to guarantee grape phenolic synthesis and accumulation. However, although the active accumulated temperature is an important climatic factor, it may not be as critical to fruit physiology as the length of time the berries are subjected to specific temperatures.[19] An accumulated average temperature for the 3 months prior to harvesting of <66 °C was reported as an important indicator for the selection of superior wine-producing areas in China.[20] The wine quality, of course, relies mostly on the contents of berry phenolic compounds. In this two-crop-a-year production area, the average 3 month accumulated temperature for the summer crop was 76.1 °C, about 10 °C above the maximal average temperature, whereas the figure was 57.1 °C (9 °C below the suggested 66 °C) for the winter crop (Table 4). The day and night temperatures as well as the difference between them were found to play an important role in grape anthocyanin synthesis and accumulation. In one phytotron study, high temperature (35 °C day, 20 °C

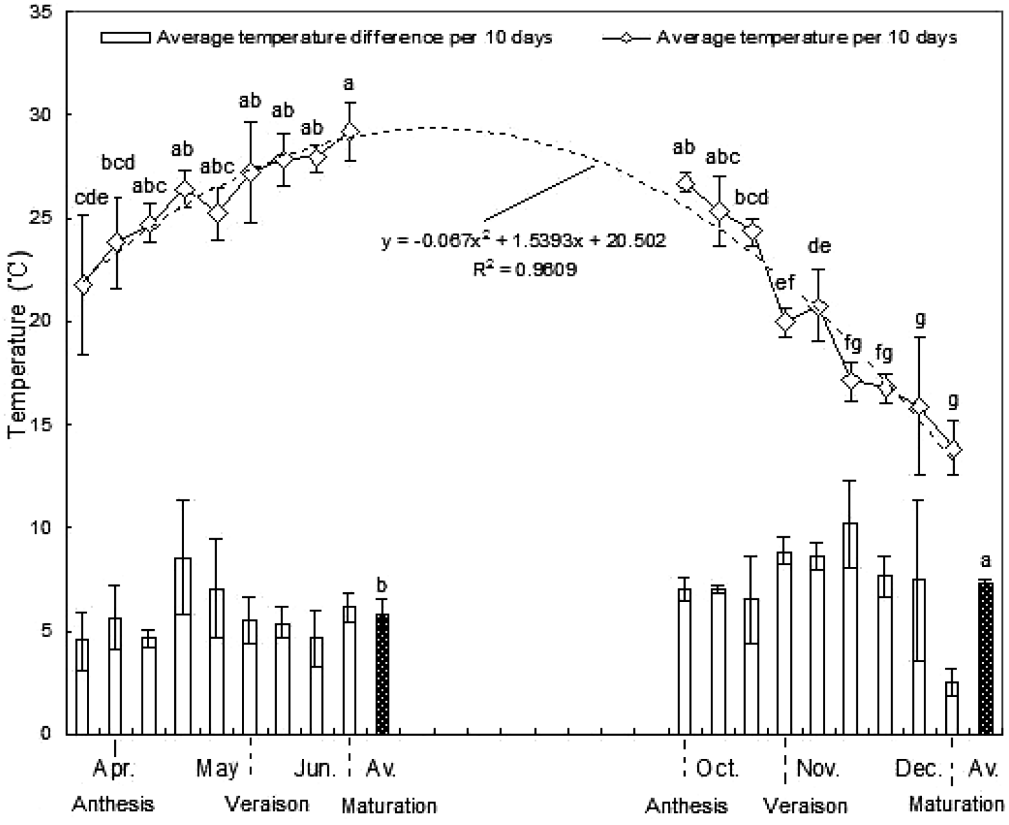


**Figure 2.** Average temperature and temperature difference (2007, 2008, and 2009) determined per 10 day periods during grape-growing seasons in Nanning. Av., average per season.

night) from veraison to harvest reduced the total anthocyanin content to less than half of that in the control (25 °C day, 20 °C night) in 'Cabernet Sauvignon' berries.[2] A similar result was also found in 'Aki Queen' berries.[21] In our study, a significantly higher temperature (averaging 31 °C day, 26 °C night) was recorded during summer crop ripening than during winter crop ripening (averaging 22 °C day, 13 °C night). In addition, the average temperature difference between day and night in the winter crop season was larger than that for the summer crop growing season (7.3 vs 5.8 °C). It was even greater during the ripening period (8.9 vs 5.4 °C) (Figure 2). All of these temperature factors should result in the synthesis and accumulation of more metabolites including the secondary metabolites of phenolic compounds in winter berries than in summer berries.

The optimal temperature range for anthocyanin accumulation was reported between 18 (night) and 24 °C (day) by Yamane et al.[21] In a separate study, Pirie[22] drew a similar conclusion as he found that the best temperature range is between 17 (night) and 26 °C (day). In our study, the temperature range during the summer growing season (averaging 31 °C during the day and 26 °C at night) appeared well above the optimal temperature range for anthocyanin synthesis and accumulation, whereas the winter growing season (averaging 22 °C during the day and 13 °C at night) was close to the optimal temperature range. A higher anthocyanin content was therefore expected in the winter grape berries than in the summer ones.

Solar radiation also influences grape phenol synthesis and accumulation. For example, cluster shading resulted in a substantial decrease in accumulation of flavonoids in 'Pinot noir' berry.[5] With the increase of cluster exposure to the sunlight, the flavonol concentration in 'Pinot noir' berry skins increased linearly.[6] Although exposure of berry clusters to sunlight benefits the phenolic compound accumulation, the sunlight intensity should not be too strong. This is because berry temperature, as it is largely regulated by the flux density of absorbed solar radiation and convective heat loss in the field, increases linearly with incident radiation.[23] Therefore, stronger solar radiation results in higher berry temperature, which could cause a decrease of phenolic compounds such as anthocyanins in the berry.[2,21] In our study, the solar radiation and berry temperature in the summer growing season were much higher than those in the winter season, which should be considered as another factor leading to lower anthocyanin contents in the winter berries.

The sunshine duration for the winter growing season was about 1.5-fold longer than for the summer season (Table 4). This should increase photosynthesis and result in richer phenolic compound accumulation in the winter berries. In addition, the period from veraison to harvest for winter berries was also longer than that for summer berries (Figure 2). With longer sunshine duration, cooler day and night temperatures, and greater temperature difference between day and night, winter berries are expected to have richer anthocyanins than the summer ones.

Water availability was another climatic factor influencing the synthesis and accumulation of phenolic compounds in grape berries. Vine water deficits could lead to changes of berry compositions such as increases in anthocyanin and other phenolic compounds.[24,25] Water deficits also increased the expression of many genes responsible for the biosynthesis of anthocyanins and accelerated anthocyanin accumulation, particularly trihydroxylated anthocyanins.[26] Less than 100 mm of

rainfall between veraison and maturation was an important indicator for the selection of superior wine-producing areas.[20] In this two-crop-a-year production area, the average rainfall between veraison and maturation for the summer crop was 185.1 mm, which was much higher than 100 mm, whereas it was 17.8 mm for the winter crop (Table 4). It appeared that the drier winter weather would contribute to richer phenolic compounds in the winter berries.

A hydrothermic coefficient ($K$), calculated by active accumulated temperature and total rainfall in a corresponding period, was used to predict wine quality in a producing area.[27] In general, $K < 1.5$ is suitable to produce superior wines and $K = 1.5-2.0$ is suitable to produce high or moderate quality wines, whereas $K = 2.0-2.5$ is suitable to produce ordinary wines. In our study, the hydrothermic coefficient for the 2 months before harvesting was 1.91 for the summer crop and 0.43 for the winter crop (Table 4), indicating much more favorable winter climate conditions for wine production than the summer climates in Nanning area.

Different antioxidant activities analyzed with different methods have different reaction characteristics and mechanisms. Therefore, to accurately evaluate the antioxidant properties of grape extracts, three antioxidant assays (DPPH, ABTS, and FRAP) were used. Significant correlations between the antioxidant properties and phenolic compounds were reported in our previous study.[17] This means that while climatic factors influenced grape phenolic synthesis and accumulation, they also altered antioxidant properties of grape berries in a similar fashion (Table 3). Besides phenolic compounds and antioxidant properties, climatic conditions also affected basic berry qualities. For example, the soluble solids in 'Kyoho' summer berries were 14.2−15.6%, whereas they reached 18.8−20.2% for the winter berries. This should be attributed to longer sunshine duration, cooler day and night temperatures, and greater temperature difference between day and night of the winter crop than of the summer crop during the growing season. The tartaric acid contents were 0.52−0.61% for the 'Kyoho' summer berries, whereas they were 0.74−0.82% for winter berries. This result is consistent with previous reports that titrate acidity fell at higher rates at high temperature than at low temperature.[28,29] It is likely that the greater decline of titrate acidity in summer berries was attributed to the increase of malic acid degradation because of increased respiration. Overall, our study indicated that the winter climate in Nanning, a subtropical region, is much more suitable for producing high-quality grapes than the summer conditions.

Phenolic compound synthesis and accumulation in grapes is a complex process that is determined by multiple factors. For example, Tarara et al.[30] reported that anthocyanin accumulation and the anthocyanin profile in 'Merlot' berries were determined by a synergistic combination of solar radiation and berry temperature. Therefore, when the influences of climatic conditions on grape berry quality are analyzed, multiple factors must be coordinately considered at the same time. Because global warming is affecting many viticulture areas in the world,[31−33] a further study is necessary on the relationships between climatic conditions and grape phenol biosynthesis.

## AUTHOR INFORMATION

### Corresponding Author

*Phone: +86 10 62737465; fax: +86 10 62737465; e-mail j.lu.cau@gmail.com.

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