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Comparison of the anthocyanins composition of five wine-making grape cultivars cultivated in the Wujiaqu area of Xinjiang, China

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ABSTRACT

Anthocyanins biosynthesis are essentially determined by grape cultivar genotype. However, specific environmental and vine management conditions could alter the accumulation of anthocyanins. This study selected five wine-making grape cultivars cultivated in the Wujiaqu area of the Xinjiang region of China to investigate the profiles of anthocyanins. Results showed that a total of 15 anthocyanins were identified. Marselan contained 15 anthocyanins, whereas only 14 anthocyanins were present in Cabernet-Sauvignon and Cabernet Franc. Syrah and Petit Verdot were found to only contain 13 anthocyanins. Cabernet-Sauvignon and Cabernet Franc appeared to show the highest and lowest levels of the total anthocyanins, respectively. Cabernet-Sauvignon possessed the highest levels of the total monomeric and acylated anthocyanins, respectively. Malvidin-3-O-glucoside and its acylated derivatives (malvidin-3-O-(6-O-acetyl)-glucoside and malvidin-3-O-(6-O-coumaryl)-glucoside) were the dominant anthocyanins in these grape cultivars. Principal component analysis revealed that the acylated anthocyanins were the major anthocyanins that differentiated these grape cultivars.

KEYWORDS

anthocyanins, grapes, Xinjiang Region, principal component analysis, HPLC-MS

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INTRODUCTION

Grapes are one of the most popular fruits in the world and have gained much interest in the field of food and nutritional sciences (Bakels, 2002). It has been confirmed that the consumption of grapes and wines could significantly lower the incidence of multiple chronic diseases, including obesity, hypertension, diabetes and cancers (Costa *et al.*, 2017; Iriti and Varoni, 2014). Such health benefits in grapes and wines mainly result from their high levels of antioxidants (Frederiksen *et al.*, 2007).

Polyphenols are the major antioxidants in grapes and wines and these secondary metabolites play a vital role in the determination of the sensory attributes of grapes (Zhao *et al.*, 2010). In addition, polyphenols, as the major antioxidants, also determine the nutritional and bioactive functions of grapes and wines (He and Giusti, 2010). Anthocyanins are a major polyphenol group and they have been confirmed to exert a significant role in the appearance of grapes and wines (Frederiksen *et al.*, 2007). During the grape development period, anthocyanins are mainly biosynthesized and accumulated in the skin of grapes through the conjugation of anthocyanidins with sugar moiety (Filippetti *et al.*, 2015). The major anthocyanins in *Vitis vinifera* L. grapes include cyanidin-3-O-glucoside, petonidin-3-O-glucoside, dephinidin-3-O-glucoside, peonidin-3-O-glucoside and malvidin-3-O-glucoside. These monomeric anthocyanins further experience acylation and/or polymerization to form anthocyanins derivatives in grapes (Hrazdina and Franzese, 1974). It has been accepted that the grape cultivar genotype essentially determines the anthocyanins composition, whereas environmental and cultivation conditions could play a role in affecting the accumulation of anthocyanins in grapes through regulating the polyphenols metabolisms (de Freitas and Mateus, 2011).

The Wujiaqu area of northern Tianshan mountain region is an important wine production area in Xinjiang, with the same latitude as California and Bordeaux. The area possesses a high day-to-night temperature difference with a long sunshine duration and a high sunshine intensity. The major soil type in the area consists of sandy and/or rocky soils. These conditions are extremely suitable for grape cultivation. In recent years, the major varieties produced in this area are Cabernet-Sauvignon and Merlot. Although some studies have reported on the performance of Cabernet Franc, Marselan and Petit Verdot, there is no related research in the Wujiaqu area. The previous studies indicated that the anthocyanin content was affected by environment conditions, soil status and viticulture and the anthocyanin biosynthesis pathway was altered by difference climate conditions (Kliewer, 1970; Mori *et al.*, 2005; Xing *et al.*, 2015) Therefore, the anthocyanin compounds and profiles of five red grapes cultivated in the Wujiaqu area might be different to grapes from other regions. The purpose of this study was to investigate the anthocyanin compounds of five wine grapes in the climactic conditions of the Wujiaqu area to help improve the quality of the grape berries using appropriate cultivation techniques, to evaluate the winemaking potential of the different grape cultivars and to enrich variety of grape cultivars.

MATERIALS AND METHODS

1. Chemicals and reagents

The external standard malvidin-3-O-glucoside was purchased from ExtraSynthesis SA (Genay, France) with a purity of 95.0 %. The HPLC grade methanol, acetonitrile, formic acid and acetic acid were purchased from Fisher Scientific (Fairlawn, NJ, USA). The analytical grade ethyl acetate was from Beijing Chemical Reagent Company (Beijing, China). Deionized water was purified using the Milli-Q purification system (Millipore, Burlington, MA, USA). The other reagents used in the study were of analytical grade unless specifically noted.

2. Experimental site and grape cultivars

Fully ripened grapes of five cultivars – Cabernet Franc (No.327, Grafted 5BB), Cabernet-Sauvignon (No.338 Own-rooted), Marselan (C980, Grafted 1130P), Petit Verdot (Grafted 1130P) and Syrah (No.100, Grafted 1130P) – were hand harvested in a 2017 vintage at the experimental stage at the Tangting Xianlu winery in the northern foothills of the Tianshan Mountains in the Wujiaqu area (44 20’ north, 87 53’ east, altitude 440.5m). For the experiment, we used *V. vinifera L. CV*. grapevines that were root planted in 2013. Row and vine spacing were set to 3.0 m and 1.0 m, respectively, with rows oriented in the east–west direction. A modified vertical shoot positioned system was employed, with sloping trunks and with horizontal cordons positioned 0.5 m above ground. All vines were

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spur-pruned with 15–18 nodes per linear meter and were normalized to one or two bunches per shot. Nutrition and pest management was carried out according to industry standards for the specified cultivar and region, as previously described (Cheng *et al.*, 2014). The detailed harvest information of these cultivars is listed in Table 1. The harvested grape samples were placed in plastic bags with dry ice and immediately transported to our laboratory for further analysis.

3. Physicochemical indexes measurements

The berry weight of each grape cultivar was calculated by weighing 100 randomly picked berries. A PAL-2 digital Brix refractometer was used to measure the total soluble solids (°Brix) of the grape berries. The titratable acidity of each grape cultivar was analyzed by titrating the grape juice pH to 8.2 with NaOH and was expressed as gram tartaric acid equivalents per liter of juice.

4. Anthocyanins extraction

The extraction of anthocyanins from these grape cultivars followed a published method with minor modifications (He *et al.*, 2010b). In brief, the grape skins were hand-peeled and then freeze-dried using liquid nitrogen. The resultant skins were grounded into powder then the skin powder (0.1 g) was placed into a plastic centrifuge tube and then mixed with 1 mL 50 % formic acid solution. The mixture was sonicated at room temperature for 20 min and then centrifuged at 8,000 rpm for 5 min to collect the supernatant. The sediment was mixed with 1 mL of 50 % formic acid solution and sonicated at room temperature for another 20 min. The resultant mixture was then centrifuged at 8,000 rpm for 5 min and the supernatants were combined. Subsequently, the combined supernatant was evaporated to dryness under a rotary evaporator at 37 °C for 20 min to dryness. The resulting dryness was mixed with 2 mL of methanol and then stored at -80 °C for further analysis. Each grape cultivar was extracted in triplicate.

5. HPLC-DAD-MS/MS

An Agilent 1200 HPLC system coupled with a diode array detector and an MSD trap VL mass spectrometry (Agilent Technologies, Santa Clara, CA, USA) was used to analyze the composition of anthocyanins in these grape cultivars. A 250 × 4.6 mm, 5.0 µm Zorbax Eclipse SB-C18 column (Agilent Technologies, Santa Clara, CA, USA) was used to separate anthocyanins under a 1 mL/min flow rate. The mobile phase consisted of (A) 5.0 % v/v formic acid in water and (B) 5.0% v/v formic acid in acetonitrile. An elution gradient was programed as follows: 0–1 min, 3 % B isocratic; 1–12 min, 3 %–5 % B; 12–24 min, 15 %–25 % B; 24–28 min, 25 %–30 %B; and 28–32 min, 30 %B–40 %B. The column was maintained at 25°C during the elution program and the wavelength on the diode array detector was set at 525 nm. The anthocyanins extract was filtered through 0.45 µm filters and a 20 µL extract was injected into HPLC. A positive electrospray ionization was used under a 30 psi nebulizer, a 12 mL/min drying gas and at a 300°C drying temperature. A full mass scan with 100–1500 m/z was used to record the mass spectrum of anthocyanins. Anthocyanins were tentatively identified by comparing their mass spectrum with a published method (He *et al.*, 2010b). Each grape cultivar anthocyanin extract was analyzed in triplicate. The external standard, malvidin-3-O-glucoside, was used to quantify these anthocyanins and results were expressed as mg/g dry grape skin.

6. Statistical analysis

Data were expressed as the mean ± standard deviation of triplicate tests. One-way analysis of variance (ANOVA) was applied to judge the means difference under a Turkey multiple range test with a significant level of 0.05, using SPSS Statistical 19.0 (SPSS Inc., Chicago, IL, USA). Principal component analysis was performed using these detected anthocyanins as variables to unveil the similarity of these wine-making grape cultivars using MetaboAnalyst 2.0 (http://www.metaboanalyst.ca/) with an auto-scaling normalization.

RESULTS AND DISCUSSION

Grape cultivar genotype plays an essential role in the determination of the composition of phenolic compounds (including anthocyanins) in grape berries (Mattivi *et al.*, 2006). Additionally, different environmental conditions could also alter the biosynthesis of phenolic compounds in grapes (Song *et al.*, 2015; Yang *et al.*, 2009). It has been known that anthocyanins and other phenolic compounds are the secondary metabolites synthesized in some critical genes and enzymes in grapes. Environmental alteration and management differences might activate or

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inhibit the expression of genes in grape, which could further alter the activity of enzymes that regulate the accumulation of anthocyanins (Figueiredo-González *et al.*, 2012). The wine-making section in the Wujiaqu area of the Xinjiang region, compared to other major wine-making regions in China, possesses significant climate and soil differences, which might result in the differences in the accumulation of anthocyanins in wine-making grape cultivars. Previous studies have only focused on the major wine-making cultivars in this area, including Cabernet-Sauvignon and Merlot (Wang, 2015). Both the phenolic compounds composition and volatiles profile of these two cultivars have been well documented (Wang, 2015). However, the anthocyanins composition of the other grape cultivars, such as Cabernet Franc, Marselan, Petit Verdot and Syrah, have not been well studied or analyzed. Therefore, it is necessary to understand the anthocyanins composition of these cultivars because this directly affects the quality (appearance) of these grape cultivars and their corresponding wine.

1. Climate condition

Wujiaqu country is a traditional vine-growing region in the Xinjiang province of China. It is located in the northern foothills of the Tianshan mountain area, which is a mid-temperature continental climate. The mean annual temperature is 6.5 °C and the active accumulated temperature is > 3700 °C. Annual rainfall is 190mm and the frostless period is 180 days. Figure 1 shows the climate data in the Wujiaqu country, including rainfall, sunshine, average temperature, days of maximum temperature and minimun temperature, average relative humidity and temperature difference. The data was taken from the China Meteorological Data Sharing Service System. The area have high maximum temperature and full sunshine periods were during grape growing and there was a large temperature difference range from 7 °C to 19.2 °C. The average temperature was higher than 20 °C most days.

2. Physicochemical indexes

Table 1 shows the physicochemical indexes of the five wine-making grape cultivars grown in the northern foothills of Tianshan Mountains in the Xinjiang region. Syrah had the highest berry weight, followed by Cabernet Franc and Petit Verdot. The lowest berry weight was observed in the Marselan cultivar. The total soluble solids of these grape cultivars were in a similar range of 19.5 to 21.0 °Brix. There was a significant difference in the titratable acidity of the grape cultivars (Table 1). For example, the Cabernet-Sauvignon cultivar appeared to exhibit the highest titratable acidity (10.2 g tartaric acid/L), followed by Syrah and Petit Verdot. The Marselan and Cabernet Franc cultivars had titratable acidity of 7.2 g and 7.8 g tartaric acid/L, respectively.

3. Total anthocyanins content and anthocyanins composition

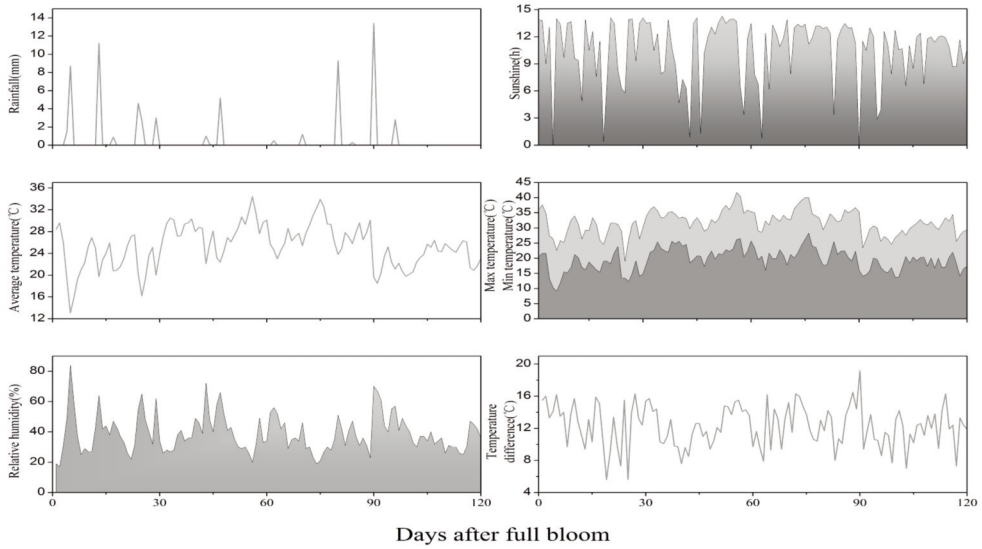


FIGURE 1. The climate parameters in Wujiaqu area during grape ripening from June to September in 2017

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TABLE 1. Sampling data, berry weight (100 berries), total soluble solids and titratable acidity of five wine-making grape cultivars cultivated in the northern foothills of the Tianshan Mountains in the Xinjiang region of China.

Cultivar	Sampling date	Berry weight (g/100 berries)	Total soluble solids (°Brix)	Titratable acidity (tartaric acid g /L)
Cabernet Franc	09/04/17	148.20 ± 4.38	20.10 ± 0.05 c	7.80 ± 0.06 c
Cabernet-Sauvignon	8/16/2017	114.50 ± 2.55	20.30± 0.06 b	10.20± 0.75 a
Marselan	8/16/2017	74.00 ± 1.98	20.40 ± 0.07 b	7.20 ± 0.42 c
Petit Verdot	09/07/17	131.40± 4.95	21.00 ± 0.06 a	9.20 ± 0.20 b
Syrah	09/06/17	288.80 ± 6.65	19.50 ± 0.15 d	9.70 ± 0.15 ab

Data are mean ± standard deviation of triplicate tests. Different letters in each row represent significant difference at a significant level of 0.05.

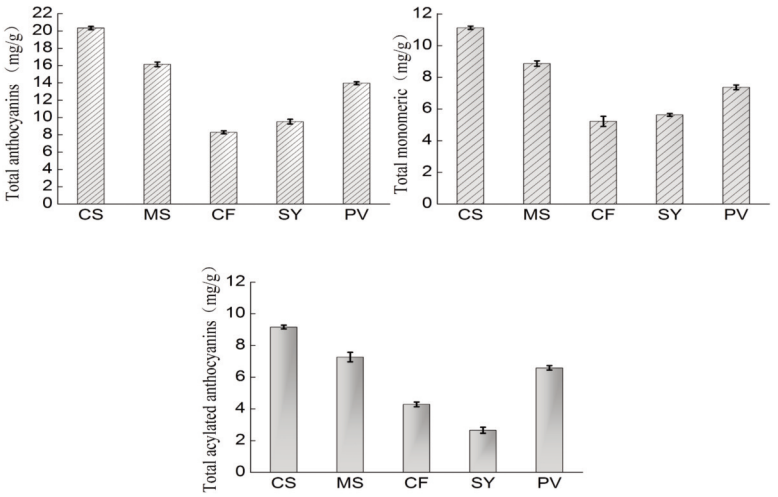


FIGURE 2. Anthocyanin content of total anthocyanins, total monomeric anthocyanins and total acylated anthocyanins in berry dry skin of five red grapes in the Wujiaqu area of Xinjiang region.
CS, Cabernet-Sauvignon, MS, Marselan, CF, Cabernet franc, SY, Syrah, PV, Petit Verdot.

Figure 2 shows the total anthocyanins content in these grape cultivars, which ranged from 8.29 to 20.34 mg/g, with the increasing order CF<SY<PV<MS<CS. It has been reported elsewhere that there are significant differences in the anthocyanins content of Cabernet-Sauvignon, Syrah and Marselan, with a decreasing order of MS>SY>CS, which is inconsistent with our results (Shi *et al.*, 2016). In addition, the previous studies in the Beijing region of China indicated that the anthocyanins content of Syrah was higher than in Cabernet Franc and Marselan (Xing *et al.*, 2018). Comparing these grape cultivars with those cultivated in other major wine-making regions of China, there was a difference in the total anthocyanins content of Cabernet-Sauvignon depending on location (Jiang *et al.*, 2014) and the total anthocyanins content of Cabernet-Sauvignon was lower than in our study.

Yang *et al.* reported that the total anthocyanins content in Marselan and Petit Verdot cultivars grown in the Changli region of Heibe province were also lower than in our results (Yang *et al.*, 2017). Previous studies have found that increased fruit sunlight will generally improve grape anthocyanins composition. It has been reported that sunshine intensity and duration might be the critical factors that affect the biosynthesis of anthocyanins in grapes during the grape development period (Zhao *et al.*, 2015). Such variations were also mainly seen from the climate and soil differences among different wine-making regions, as suggested by Liang *et al.* (2014). The results indicated that the five grapes in the Wujiaqu area have higher

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anthocyanins content duet to their unique climate.

A total of 15 anthocyanins were identified in the grape cultivars in this study (Figure 3): the Marselan grape cultivar contained these 15 anthocyanins ; Cabernet-Sauvignon and Cabernet Franc cultivars contained 14 anthocyanins but did not contain malvindin-3-O-(6-O-coumaryl)-glucoside ; Petit Verdot and Syrah were only found to contain 13 anthocyanins and contained neither malvidin-3-O-(6-O-coumaryl)-glucoside nor malvindin-3-O-(6-O-acetyl) – glucoside.

According to their structural nature, anthocyanins can be classified into monomeric anthocyanins, acylated anthocyanins and polymerized anthocyanins (Hrazdina and Franzese, 1974). We further compared the individual anthocyanins content of the grape cultivars under their sub-groups.

Peaks are as follows: (1) cyanidin-3-O-glucoside, (2) peonidin-3-O-glucoside, (3) delphidin-3-O-glucoside, (4) petunidin-3-O-glucoside, (5) cyanidin-3-O-(6-O-acetyl)-glucoside, (6) malvidin-3-O-glucoside, (7) peonidin-3-O-(6-O-acetyl)-glucoside, (8) delphinidin-3-O-(6-O-acetyl)-glucoside, (9) petunidin-3-O-(6-O-acetyl)-glucoside, (10) malvidin-3-O-(6-O-acetyl)-glucoside, (11) cyanidin-3-O-(6-O-coumaryl)-glucoside, (12) peonidin-3-O-(6-O-coumaryl)-glucoside, (13) delphidin-3-O-(6-O-coumaryl)-glucoside, (14)

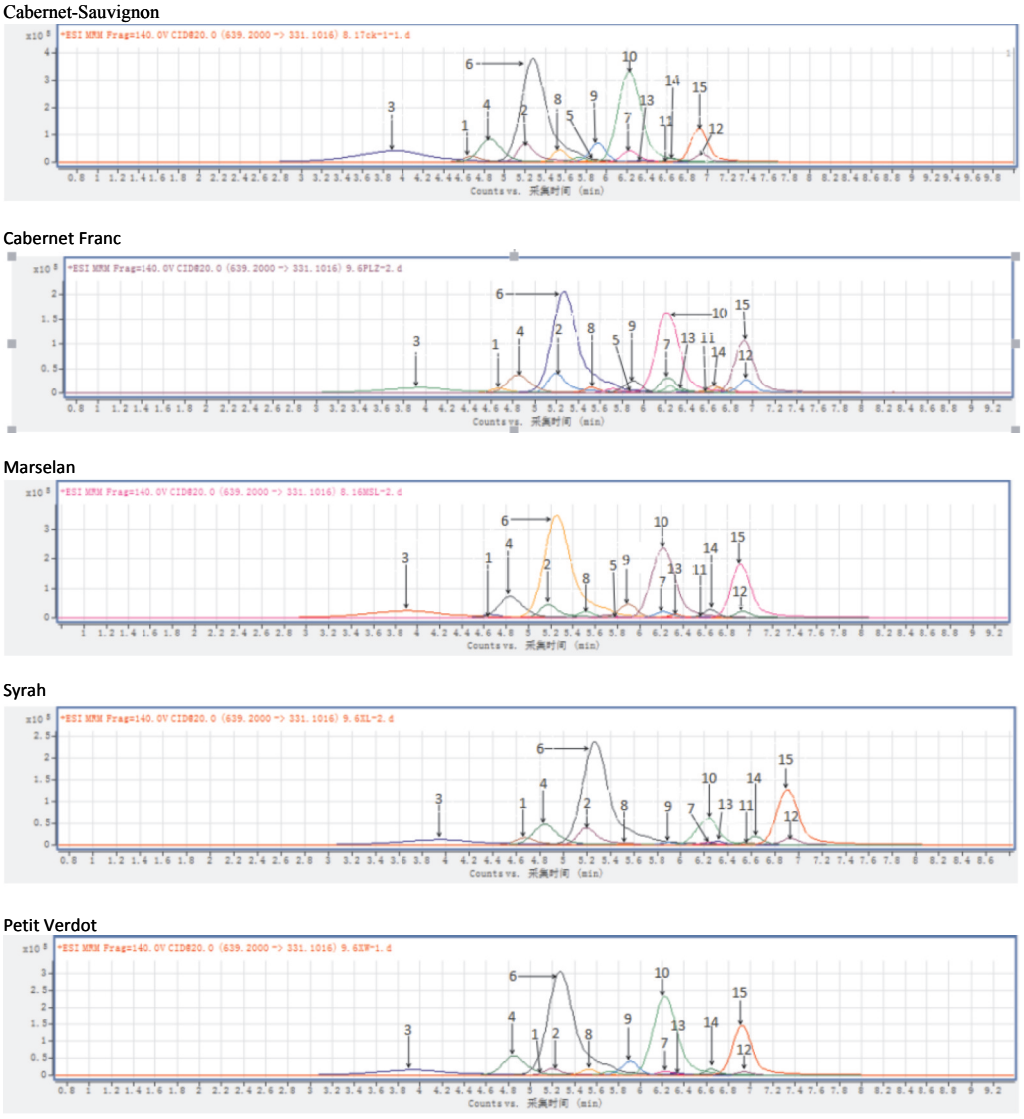


FIGURE 3. High-performance liquid chromatography of anthocyanins in five wine-making grape cultivars cultivated in the Wujiaqu area in the Xinjiang region of China.

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petunidin-3-O-(6-O-coumaryl)-glucoside and (15) malvidin-3-O-(6-O-coumaryl)-glucoside.

4. Monomeric anthocyanins

Regarding the total monomeric anthocyanins content in Figure 2, the Cabernet-Sauvignon and Syrah cultivars exhibited the highest level of the total monomeric anthocyanins, whereas the lowest total monomeric anthocyanins content was found in the Petit Verdot cultivar. The proportion of individual anthocyanins that were analyzed by HPLC are shown in Table 2.

It has been confirmed that *Vitis vinifera* L. grapes contain five monomeric anthocyanins (Alcalde-Eon *et al.*, 2006). In the present study, all these grape cultivars were found to contain five basic monomeric anthocyanins, including dephinidin-3-O-glucoside, cyanidin-3-O-glucoside, petunidin-3-O-glucoside, peonidin-3-O-glucoside and malvidin-3-O-glucoside (Table 2).

It was previously reported that malvidin-3-O-glucoside was the major monomeric anthocyanin in *Vitis vinifera L grapes* (Wulf and Nagel, 1978). Similarly, among these individual monomeric anthocyanins, malvidin-3-O-glucoside appeared to be the dominant monomeric anthocyanin in CF, CS, MS, PV and SY, accounting for 41.14%, 35.67 %, 40.36 %, 41.67 % and 50.13 %, respectively. As in the previous reports (Shi *et al.*, 2016), delphidin-3-O-glucoside (CS, MS) and peonidin-3-O-glucoside (SY) were the second most abundant non-acylated anthocyanins. In our studies, the delphidin-3-O-glucoside in four grape (CF, CS, MS, PV) was the most abundant non-anthocyanins. The red grapes of CS and MS are consistent with the previous studies, whereas in the present studies of Syrah is inconsistent with the previous results. The cultivars had low levels of cyanidin-3-O-glucoside, petunidin-3-O-glucoside and peonidin-3-O-glucoside. It should be noted that the Marselan cultivar contained cyanidin-3-O-glucoside. Additionally, Li *et al.* reported that the level of delphinidin-3-O-glucoside was higher than peonidin-3-O-glucoside in both Syrah and Marselan cultivated in the Gansu wine-making region of China (Li *et al.*, 2014). However, the Syrah and Marselan cultivars in the present study exhibited peonidin-3-O-glucoside at higher levels than delphinidin-3-O-glucoside. We speculated that climate variations, especially sunshine intensity and duration difference, resulted in the difference between these monomeric anthocyanins accumulations (Song *et al.*, 2015).

5. Acylated anthocyanins

Acylation is a reaction through which monomeric anthocyanins could be further stabilized in grape berries under different physiological conditions (Dixon and Steele, 1999). Basically, aromatic and/or aliphatic constituents are conjugated to the C6' position of the glucosyl groups in monomeric anthocyanins under the activity of anthocyanin acyltransferases and such conversions could further diversify anthocyanins in grape berries (Dixon and Steele, 1999; G. Mazza, . 1995). More importantly, acylation of anthocyanins in grapes could further stabilize and enhance the appearance of grape berries (Anderson, 1970). In the present study, the total acylated anthocyanins content in these grape cultivars ranged from 2.66 to 9.16 mg/g (Figure 1). Among these grape cultivars, Cabernet-Sauvignon appeared to contain the highest content of the total acylated anthocyanins, whereas the lowest total acylated anthocyanins content was found in the Syrah cultivar.

Among the individual acylated anthocyanins in these cultivars, malvidin-3-O-(6-O-acetyl)-glucoside and malvidin-3-O-(6-O-coumaryl)-glucoside were the two predominant acylated anthocyanins. Mazza *et al.* reported that malvidin-3-O-(6-O-acetyl)-glucoside and malvidin-3-O-(6-O-coumaryl)-glucoside were the most important derivatives for the characterization of red grape (Mazza, 1995). The Cabernet Franc and Syrah cultivars contained the highest and lowest level of malvidin-3-O-(6-O-acetyl)-glucoside, respectively. However, the highest content of malvidin-3-O-(6-O-coumaryl)-glucoside was found in the Syrah cultivar, at around two times higher than that in the Cabernet-Sauvignon cultivar. Additionally, these cultivars also contained dephinidin-3-O-(6-O-acetyl)-glucoside and dephinidin-3-O-(6-O-coumaryl)-glucoside, although at a low level (Table 2). The Cabernet-Sauvignon cultivar exhibited the highest level of dephinidin-3-O-(6-O-acetyl)-glucoside but the lowest level of dephinidin-3-O-(6-O-coumaryl)-glucoside compared to the other cultivars. The highest dephinidin-3-O-(6-O-coumaryl)-glucoside content was found in the Syrah cultivar, but cyanidin-3-O-(6-O-acetyl)-glucoside and cyanidin-3-O-(6-O-coumaryl)-glucoside were at

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TABLE 2. Relative amounts (%) and standard deviation (SD) for the anthocyanins in grape skin extract from five grape cultivars.

Anthocyanin	Cultivar				
	Cabernet Franc	Cabernet-Sauvignon	Marselan	Petit Verdot	Syrah
Monomeric anthocyanin					
Delphinidin-3-*O*-glucoside	6.80 ± 0.13 ^{d}	11.86 ± 0.02 ^{a}	8.18 ± 0.12 ^{c}	6.78 ± 0.12 ^{d}	8.51 ± 0.18 ^{b}
Cyanidin-3-*O*-glucoside	0.93 ± 0.02 ^{c}	1.02 ± 0.02 ^{b}	0.64 ± 0.01 ^{d}	0.26 ± 0.07 e	1.83 ± 0.02 ^{a}
Petunidin-3-*O*-glucoside	3.11 ± 0.10 ^{d}	3.91 ± 0.02 ^{b}	3.96 ± 0.04 ^{b}	3.61 ± 0.11 ^{c}	4.43 ± 0.05 ^{a}
Peonidin-3-*O*-glucoside	2.91 ± 0.02 ^{b}	2.45 ± 0.02 ^{c}	1.81 ± 0.00 ^{d}	3.61 ± 0.11 ^{c}	3.01 ± 0.04 ^{a}
Malvidin-3-*O*-glucoside	41.14 ± 0.18 ^{b}	35.67 ± 0.16 ^{c}	40.36 ± 0.27 ^{b}	41.67 ± 0.34 ^{b}	50.13 ± 0.80 ^{a}
Acylated anthocyanin					
Delphinidin-3-*O*-(6-*O*-acetyl)-glucoside	1.64 ± 0.08 ^{b}	2.77 ± 0.01 ^{a}	1.63 ± 0.15 ^{b}	1.67 ± 0.01 ^{b}	0.30 ± 0.07 ^{c}
Cyanidin-3-*O*-(6-*O*-acetyl)-glucoside	0.29 ± 0.01 ^{b}	0.31 ± 0.01 ^{a}	0.17 ± 0.04 ^{c}	0.08 ± 0.02 ^{d}	0.10 ± 0.01 ^{d}
Petunidin-3-*O*-(6-*O*-acetyl)-glucoside	0.98 ± 0.07 ^{c}	2.10 ± 0.04 ^{a}	1.52 ± 0.11 ^{b}	1.53 ± 0.04 ^{b}	N^{D}
Peonidin-3-*O*-(6-*O*-acetyl)-glucoside	1.08 ± 0.08 ^{b}	1.30 ± 0.01 ^{a}	0.40 ± 0.02 ^{c}	N^{D}	N^{D}
Malvidin-3-*O*-(6-*O*-acetyl)-glucoside	29.59 ± 0.08 ^{a}	29.33 ± 0.08 ^{b}	24.10 ± 0.05 ^{d}	28.38 ± 0.08 ^{c}	8.97 ± 0.06 e
Delphinidin-3-*O*-(6-*O*-coumaryl)-glucoside	1.00 ± 0.02 ^{b}	0.47 ± 0.01 e	0.77 ± 0.02 ^{c}	0.67 ± 0.02 ^{d}	1.11 ± 0.04 ^{a}
Cyanidin-3-*O*-(6-*O*-coumaryl)-glucoside	0.25 ± 0.05 ^{b}	0.13 ± 0.02 ^{c}	0.12 ± 0.02 ^{c}	0.06 ± 0.01 ^{d}	0.32 ± 0.01 ^{a}
Petunidin-3-*O*-(6-*O*-coumaryl)-glucoside	N^{D}	N^{D}	0.42 ± 0.04 ^{a}	0.26 ± 0.18 ^{a}	0.23 ± 0.09 ^{a}
Peonidin-3-*O*-(6-*O*-coumaryl)-glucoside	1.06 ± 0.03 ^{a}	0.53 ± 0.03 ^{b}	0.32 ± 0.01 ^{c}	N^{D}	0.17 ± 0.04 ^{d}
Malvidin-3-*O*-(6-*O*-coumaryl)-glucoside	9.22 ± 0.45 ^{d}	8.13 ± 0.02 e	15.58 ± 0.02 ^{b}	14.65 ± 0.05 ^{c}	20.90 ± 0.48 ^{a}

Data are mean ± standard deviation of triplicate tests. “ND, not detected”. a-d significant differences at a significant level of 0.05.

a much lower level. It should be noted that the Cabernet-Sauvignon cultivar exhibited the highest level of petunidin-3-O-(6-O-acetyl)-glucoside and peonidin-3-O-(6-O-acetyl)-glucoside. It has been reported that acetyl malvidin glycosides exhibited a higher level in *Vitis vinifera* grape cultivars, whereas acetyl cyanidin glycosides were present in these cultivars at a low level (Mazza *et al.*, 1999). Our results were consistent with these reports.

He *et al.* (2010a) reported that acetyl anthocyanins could be used as critical indicators to differentiate grape cultivars. In the present study, these five cultivars possessed different compositions of acetyl anthocyanins. It should be noted that the Petit Verdot cultivar did not contain peonidin-3-O-(6-O-acetyl)-glucoside . This result was not in accordance with a previously published study where peonidin-3-O-(6-O-acetyl)-glucoside was found in Petit Verdot cultivated in the Beijing wine-making region (Yang, Chen, He and Wang, 2017). This indicated that climate differences might result in such variations in the acetyl anthocyanins composition in Petit Verdot. In terms of coumaryl anthocyanins, a significant variation among these cultivars was found in coumaryl cyanidin glycosides and coumaryl malvidin glycosides. It has been reported that Cabernet-Sauvignon cultivated in the Changli region of China contained petunidin-3-O-(6-O-coumaryl)-glucoside, whereas this anthocyanin was not present in Cabernet Franc in the Changli region (Zhang, 2011) or in the present study in the Xinjiang region. Meanwhile, cyanidin-3-O-(6-O-coumaryl)-glucoside and dephidin-3-O-(6-O-coumaryl)-glucoside were all found in Cabernet Franc, which was not consistent with a published study (Xing *et al.*, 2018). The Marselan and Petit Verdot cultivars were found to contain cyanidin-3-O-(6-O-coumaryl)-glucoside, which was not in agreement with the study reported by Yang *et al.* (2017).

This indicates that environmental conditions might cause a regulation in the biosynthesis of these anthocyanins in grapes (de Freitas and Mateus, 2011).

6. Principal Component Analysis

In order to better understand the similarity of the composition of the anthocyanins in these grape cultivars, principal component analysis was carried out using these detected anthocyanins as variables (Figure 3). The first and second principal component (PC1 and PC2) represented 52.7% and 28.4% of the total variance, respectively. It was found that the Cabernet-Sauvignon and Cabernet Franc cultivars were positioned on the negative scale of the PC1 and PC2, whereas the Syrah cultivar was on the positive scale of the PC1 but the negative scale of the PC2. However, the Marselan and Petit Verdot cultivars were placed at around the zero

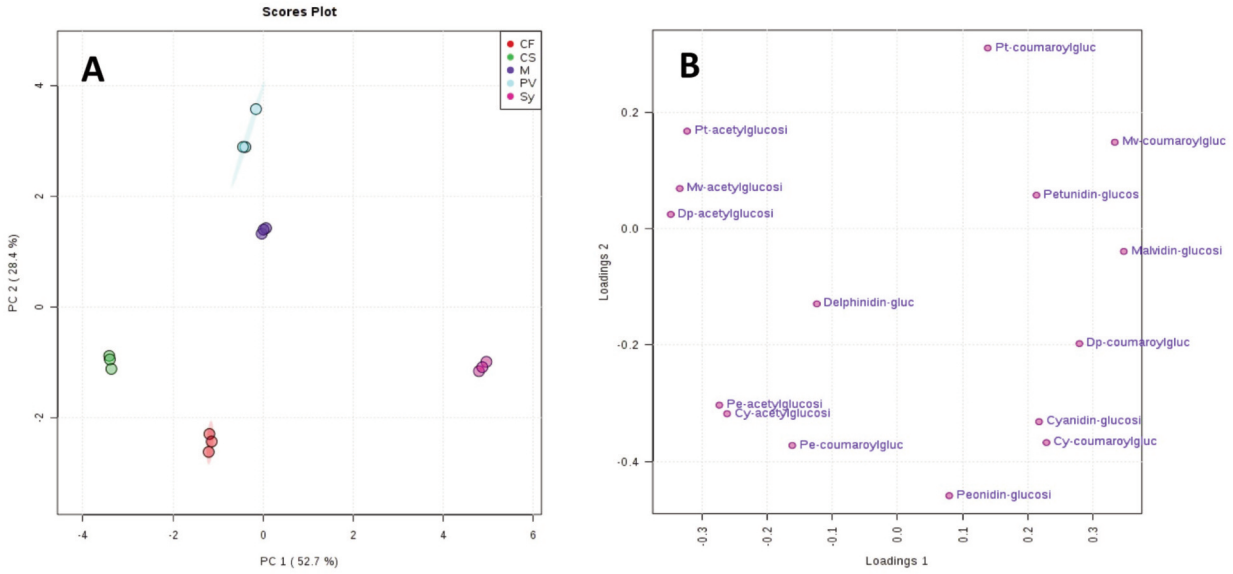


FIGURE 4. Principal component analysis of in five wine-making grape cultivars cultivated in the northern foothills of the Tianshan Mountains in the Xinjiang region of China: Score plot (A) and Loading plot (B).

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point on the PC1, and on the positive scale of the PC2 (Figure 4A). Such separations resulted mainly from the difference of the acylated anthocyanins content in these cultivars. For example, the Cabernet-Sauvignon, Cabernet Franc and Marselan mainly fitted to the area at which acetylated anthocyanins were positioned (Figure 4B).

CONCLUSIONS

In conclusion, in the northern foothills of the Tianshan Mountains in the Xinjiang region, five wine-making grape cultivars Cabernet-Sauvignon, Cabernet Franc, Marselan, Petit Verdot and Syrah were selected to investigate their composition of anthocyanins. A total of 15 anthocyanins were identified in this study. Cabernet-Sauvignon and Marselan exhibited high levels of the total anthocyanins. Malvidin-3-*O*-glucoside and its acylated derivatives appeared to be the dominant anthocyanins in these grape cultivars. Cabernet-Sauvignon and Syrah exhibited a high level of acetylated and coumarylated anthocyanins, respectively. Syrah also possessed a high level of the total monomeric anthocyanins compared to the other cultivars. Monomeric and acylated malvidin glucoside was found to be the highest in Petit Verdot, whereas Cabernet-Sauvignon showed the highest level of dephinidin monomeric and acylated glucosides. Principal component analysis revealed that the composition of anthocyanins could distinguish these grape cultivars.

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