Phenolic Compound Profiles in Grape Skins of Cabernet Sauvignon, Merlot, Syrah and Marselan Cultivated in the Shacheng Area (China)

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The phenolic compounds in the grape skins of Cabernet Sauvignon (CS), Merlot (ML), Syrah (SY) and Marselan (MS) from Shacheng, in China, were compared using HPLC-MS/MS. The results showed that the types and levels of phenolic compounds varied greatly with cultivars. Malvidin derivatives were the main anthocyanins. CS and ML showed a higher content of malvidin-3-*O*-(6-*O*-acetyl)-glucoside than malvidin-3-*O*-(*trans*-6-*O*- coumaryl)-glucoside, while SY and MS differed from CS and ML. ML had higher delphinidin and cyanidin derivatives, SY had higher peonidin derivatives, while malvidin and petunidin were higher in MS. The total content of flavonols, flavan-3-ols, phenolic acids and stilbenes in grape skins showed no difference among CS, ML and MS. Isorhamnetin-3-*O*-glucoside (CS, ML, MY), quercetin-3-*O*-glucoside (SY), procyanidin trimer (SY, MS), procyanidin dimer (CS, ML), syringetin-3-*O*-glucoside, *trans*-cinnamic acid and resveratrol were the most abundant non-anthocyanin phenolic compounds. Cluster analysis showed that CS and ML, and SY and MS had similar phenolic profiles.

INTRODUCTION

Phenolic compounds in red grape skins are one of the most important parameters in determining red wine character and quality, and directly influence consumers' overall acceptance (Li *et al*., 2011). They contribute to the mouthfeel, colour and stability of red wines; some of them also exhibit potent biological activities (Gómez-Alonso *et al*., 2007). Phenolic compounds, mostly originating from grape berries, are transferred into wine during the winemaking process (Salas *et al*., 2003). The main phenolic compounds in red grapes and red wine are anthocyanins and non-anthocyanins, which include flavonols, flavan-3-ols, phenolic acids (including hydroxycinnamic acids and hydroxybenzoic acids) and stilbenes (Monagas *et al*., 2005; Li *et al*., 2011; Zhang *et al*., 2015). Anthocyanins, which are directly responsible for red grape and red wine colour (Mateus *et al*., 2002; Revilla *et al*., 2009), are composed of the monoglucosides of five anthocyanidins, namely delphinidin, cyanidin, petunidin, peonidin and malvidin, along with the corresponding acetyl, *p*-coumaroyl and caffeoyl derivatives (Liang *et al*., 2008; Raúl *et al*., 2009). Flavonols, which influence red wine coloration by co-pigmentation (Boulton, 2001), consist of the glycosides of myricetin, quercetin, kaempferol, isorhamnetin, syringetin and laricitrin (Raúl *et al*., 2009). The flavan-3-ols found in the skin and seed are mainly catechin, epicatechin, gallocatechin, epigallocatechin and their corresponding polymers. Flavan-3-ols are mainly responsible for the astringency, bitterness and structure of wines (Monagas *et al*., 2005), and they also play an important role in the stabilization of the red colour in wines (Sun *et al*., 2007). Stilbenes (mainly resveratrol) exhibit significant antioxidant properties in the prevention of arteriosclerosis and coronary heart disease (Sun *et al*., 2002).

The amount of phenolic compounds in grapes is influenced by the grape variety, along with viticultural and environmental factors (which are usually described by the French term "terroir"), which include soil type, geographical location and weather conditions (Douglas *et al*., 2001; Brescia *et al*., 2002; Xing *et al*., 2015). Temperature plays a direct and important role in the formation of phenolic compounds; temperatures higher than 30°C are not conducive to anthocyanin synthesis (Spayd *et al*., 2002; Tarara *et al*., 2008). Lower night temperatures can result in greater accumulation of anthocyanins (Mori *et al*., 2005). Several studies have shown a positive association between

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sunlight exposure and flavone accumulation (Downey *et al*., 2004). Li *et al*. (2011) reported that "terroir" characteristics affect the flavonoid biosynthesis in grape berries, eventually leading to the difference in the phenol profile of wines from different regions.

Shacheng region is a traditional vine-growing region in the Hebei province of China. It is located in the Sangyang basin, which is a warm, semiarid region. The mean annual temperature is 12.5°C. Active accumulated temperature (≥ 10°C) is more than 3 500°C. Annual rainfall is 400 mm. Solar radiation is high (146.36 kcal/cm^2), and the annual frostless period is 160 days. The area under wine grapes in Shacheng region is 8 300 ha, and the main cultivars are Cabernet Sauvignon, Merlot, Syrah, Marselan, etc. However, there is little information available on the phenolic compounds of wine grapes in the Shacheng region.

The purpose of this study was to investigate the phenolic compounds of the main wine grapes in the Shacheng region to help improve the quality of the grape berries by appropriate cultivation techniques, and to evaluate the winemaking potential of the different grape cultivars.

MATERIALS AND METHODS

Materials

The study was carried out at Donghuayuan town, which is located in the Shacheng region of Hebei province, China. The soil type is clay and sandy. The climate, which is influenced by abundant sunshine, moderate heat, a high temperature difference between day and night and cool weather in the summer, provides a good environment for vine growing.

Four red *Vitis vinifera* grapes, of the varieties Cabernet Sauvignon (CS), Merlot (ML), Syrah (SY) and Marselan (MS), were collected in 2010. The grapevines of each cultivar were four years old, planted at a spacing of 2.5 m × 1.0 m (row, vine). The grape berries were harvested at technological ripeness, depending on the sugar and acid content. The fresh grape samples were placed in freezer bags and taken to the laboratory immediately. Grape skins were peeled from the berries and frozen in liquid nitrogen, followed by grinding and lyophilisation. The grape skin powder was stored at -40°C until used (Jin *et al*., 2009).

Chemicals and standards

Malvidin-3-*O*-glucoside standard was purchased from Extrasynthese SA (Genay, France). Quercetin, (+)-catechin, gallic acid, caffeic acid and *trans*-resveratrol standards were all purchased from Sigma Company (St. Louis, MI, USA). Ethyl acetate (analytical grade) was obtained from Xian Chemical Reagent Plant (Xian, China). HPLC-grade methanol, formic acid, acetic acid and acetonitrile were obtained from Fisher Company (Fairlawn, NJ, USA).

Extraction and analysis of anthocyanins

Grape skin powder (0.50 g) was immersed in methanol (10 mL) containing 2% formic acid. The extraction was performed for 10 min with the assistance of ultrasonic vibration, and then the mixture was shaken in the dark at a rate of 150 rpm for 30 min at 25°C . The extract was centrifuged at 8 000 *g* for 10 min and the supernatant was collected. The residues were extracted another three times. All supernatants were mixed and evaporated to dryness using a rotary evaporator. Then the dry residual was re-dissolved in 10 mL mobile phase (A:B = 9:1) (A: aqueous 2% (vol) formic acid, B: acetonitrile containing 2% (vol) formic acid). The final samples were filtered through 0.45 μm filters (cellulose acetate and nitrocellulose, CAN) prior to HPLC-MS analysis. All extractions were done in triplicate.

An Agilent 1100 series LC-MSD trap VL instrument equipped with a diode array detector and reverse phase column (Kromasil C18, 250 mm × 4.6 mm, 5 μm) was used for qualitative and quantitative analyses of anthocyanin in the extracts. Elutions included two solutions: (A) aqueous 2% (vol) formic acid and (B) acetonitrile containing 2% (vol) formic acid. The gradient was as follows: 4 min, 6% to 10% B; 8 min, 10% to 25% B; 1 min, isocratic 25% B; 7 min, 25% to 40% B; 15 min, 40% to 60% B; 5 min, 60% to 100% B; then 5 min, 100% to 6% B. The flow rate was 1.0 mL/min. Injection volumes were 30 μL and the detection wavelength was 525 nm. MS conditions were as follows: Electrospray ionisation (ESI) interface, positive ion mode, 35 psi nebuliser pressure, 10 mL/min dry gas flow rate, 350°C dry gas temperature and scans at *m*/*z* 100 to 1 000 (Jin *et al*., 2009). All analyses were performed in duplicate.

Extraction and analysis of non-anthocyanins

Grape skin powder (2.00 g) was macerated with distilled water (5 mL) and ethyl acetate (45 mL), and shaken in the dark for 30 min. The supernatant was collected and the residue was extracted four times. All of the supernatants were mixed together and evaporated to dryness using a rotary evaporator, and then re-dissolved in methanol (2 mL). All extractions were done in triplicate. The determination and quantification of non-anthocyanins were carried out using HPLC-MS/MS, as described by Jin *et al*. (2009). Each sample was first filtered through a 0.22 μm organic membrane and then injected into an Agilent 1200 series HPLC-MSD trap VL instrument, equipped with a variable wavelength detector and a reverse phase column (Zorbax SB-C18 column 3 mm × 50 mm, 1.8 μm). Samples were eluted at a gradient using two mobile phases: (A) aqueous 1% acetic acid and (B) acetonitrile containing 1% acetic acid. The gradient was 5 min, 5% to 8% B; 2 min, 8% to 12% B; 5 min, 12% to 18% B; 5 min, 18% to 22% B; 2 min, 22% to 35% B; 2 min, 35% to 100% B; 4 min, 100% B, then 2 min, 100% to 5% B, at a flow rate of 1.0 mL/min. The detection wavelength was 280 nm and the injection volume was 2 μL. The column temperature was 25°C. The MS conditions were as follows: ESI, negative ion mode; nebuliser, 35 psi; dry gas flow, 10 mL/min; dry gas temperature, 325°C; scan, 100 *m*/*z* to 1 000 *m*/*z*. All analyses were done in duplicate.

Quantification of phenolic compounds

Anthocyanins, flavonol, flavan-3-ols, hydroxybenzoic acid, hydroxycinnamic acids and stilbenes were calculated by using an external standard calibration curve for each compound, and were expressed as mg of malvidin-3-*O*-glucoside (ME), quercetin, (QE), (+)-catechin (CE), gallic acid (GAE), caffeic acid (CAE) and *trans*-resveratrol (RE) equivalents per kg of dry grape skin.

Statistical analysis

SPSS 16.0 for Windows was used for the variance and cluster analysis. Data were expressed as mean ± standard deviation of three independent experiments with two replicates.

RESULTS AND DISCUSSION

The anthocyanin composition of grape skin

As shown in Fig. 1, the total content of anthocyanin varied from 10 847.02 mg ME/kg (ML) to 20 790.75 mg ME/kg (MS). There was a significant difference in the anthocyanin contents in the grape skins of the four varieties, with a decreasing order: MS > SY > CS > ML. It has been reported that there are no significant differences in the anthocyanin contents of Cabernet Sauvignon and Merlot (Jensen *et al*., 2008), which is inconsistent with our results. In the grape cultivars studied, malvidin and its derivatives (46.33% to 71.76% of total anthocyanins) are the main anthocyanins present in the skin. Compared with other cultivars, ML had higher delphinidin, cyanidin and their derivatives; SY had higher peonidin and its derivatives; and MS had higher malvidin and petunidin and their derivatives.

A total of 21 different anthocyanins were identified from four grape cultivars using HPLC-ESI-MS/MS (Table 1), including five glucosides, five acetyl glucosides, seven coumaroyl glucosides, two caffeoyl glucosides and two polymeric anthocyanins, all of which could be classified into the monoglucosides and derivatives of five anthocyanidins: delphinidin, cyanidin, petunidin, peonidin and malvidin. As in previous studies (Revilla *et al*., 2001; Kallithraka *et al*., 2005; Raúl *et al*., 2009), malvidin-3-*O*-glucoside was the most abundant anthocyanin in CS, ML, SY and MS, accounting for 36.51%, 28.64%, 26.52% and 38.95% of the total anthocyanin content respectively. In addition to this compound, delphinidin-3-*O*-glucoside (CS, ML, MS) and peonidin-3-*O*-glucoside (SY) were the second most abundant non-acylated anthocyanins. It is worth mentioning that malvidin-3-*O*- glucoside-pyruvic acid was only detected in SY, and malvidin-3-(6-*O*-caffeoyl)-glucoside was not detected in ML and MS. Mazza *et al*. (1999) reported that malvidin-3-*O*- (6-*O*-acetyl)-glucoside, along with malvidin-3-*O*-(6-*O*-coumaryl)-glucoside, was one of the most important derivatives for the characterisation of varieties. In our study, CS and ML showed a high content of malvidin-3-*O*-(6-*O*-acetyl)-glucoside, followed by malvidin-3-*O*-(*trans*-6-*O*-coumaryl)-glucoside, as in a previous study (Revilla *et al*., 2001). However, SY and MS had higher contents of malvidin-3-*O*-(*trans*-6-*O*-coumaryl)-glucoside (SY: 4324.94 mg ME/kg, 23.73% of total anthocyanins; MS: 3596.14 mg ME/kg, 17.30% of total anthocyanins) than malvidin-3-*O*-(6-*O*-acetyl)-glucoside (SY: 2058.55 mg ME/kg, 11.30% of total anthocyanins; MS: 3127.17 mg ME/kg, 15.04% of total anthocyanins).

The non-anthocyanin composition of grape skin

The non-anthocyanin composition of the CS, ML, SY and MS grape skins obtained from Donghuayuan is summarised in Table 2. A total of 25 non-anthocyanin phenolic compounds were identified and quantified in these samples, including 14 flavonols, four flavan-3-ols, four hydroxybenzoic acids, two hydroxycinnamic acids and one stilbene.

The flavonol composition

Flavonols exist mainly as the four main aglycones: myricetin, quercetin, kaempferol and isorhamnetin (Monagas *et al*., 2005). As shown in Table 2, 14 flavonol compounds in the grape skins varied significantly among the grape cultivars studied. Myricetin-3-*O*-glucuronide was detected only in ML, but no myricetin-3-*O*-galactoside was detected in ML.

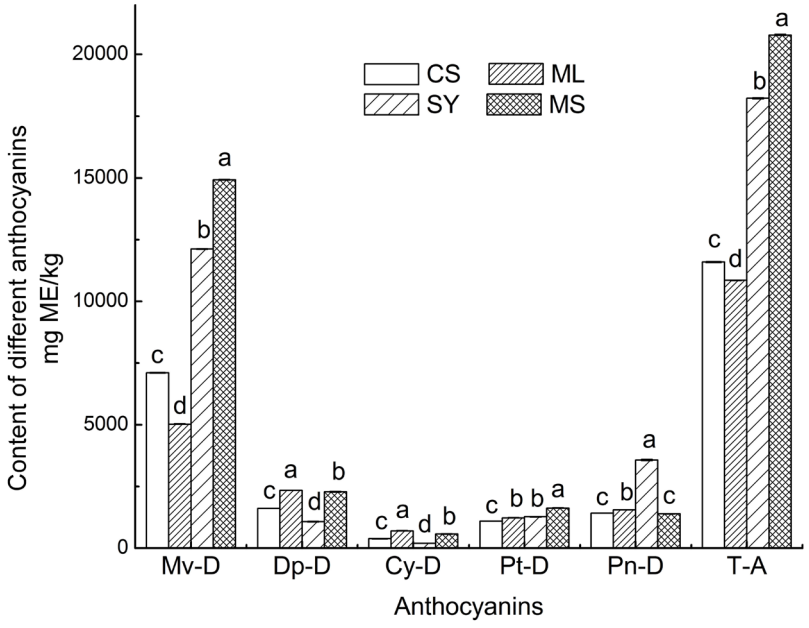

FIGURE 1

The content of different anthocyanin derivatives of four grape cultivars. ME: malvidin-3-O-glucoside; Mv-D: malvidin and its derivatives; Dp-D: delphinidin and its derivatives; Cy-D: cyanidin and its derivatives; Pt-D: petunidin and its derivatives; Pn-D: peonidin and its derivatives; T-A: total anthocyanins. Different small letters indicate a significant difference at $P < 0.05$.

TABLE 1
The content of anthocyanin compounds in the berry skins of four grape cultivars.

Anthocyanins	$[M^+]$ (Frag. MS^2 *m*/*z*)	Content (mg ME/kg)			
		CS	ML	SY	MS
Delphinidin-3-*O*-glucoside	465 (303)	1307.52 ± 13.32	2028.26 ± 8.64	745.14 ± 8.93	1355.59 ± 6.92
Cyanidin-3-*O*-glucoside	449 (287)	239.48 ± 5.69	541.06 ± 5.15	146.53 ± 3.27	144.01 ± 4.88
Petunidin-3-*O*-glucoside	479 (317)	792.60 ± 6.12	830.02 ± 6.32	970.85 ± 7.92	1198.82 ± 5.63
Peonidin-3-*O*-glucoside	463 (301)	824.27 ± 5.23	239.54 ± 2.11	1105.45 ± 8.68	691.62 ± 4.32
Malvidin-3-*O*-glucoside	493 (331)	4231.81 ± 6.24	3107.13 ± 8.93	4833.27 ± 10.67	8098.94 ± 7.89
Delphinidin-3-*O*-(6-*O*-acetyl)-glucoside	507 (303)	297.21 ± 7.32	232.81 ± 4.58	157.23 ± 3.43	354.80 ± 5.44
Peonidin-3-*O*-glucoside-pyruvic acid	531 (301)	35.90 ± 3.45	42.05 ± 2.46	53.51 ± 5.32	60.46 ± 3.87
Malvidin-3-*O*-glucoside-pyruvic acid	603 (399)	nd	nd	26.39 ± 2.29	nd
Cyanidin-3-*O*-(6-*O*-acetyl)-glucoside	491 (287)	79.54 ± 4.61	118.89 ± 3.38	46.34 ± 3.59	68.67 ± 5.44
Petunidin-3-*O*-(6-*O*-acetyl)-glucoside	521 (317)	270.28 ± 5.38	247.98 ± 4.32	265.46 ± 3.15	394.64 ± 1.59
Delphinidin-3-*O*-(6-*O*-coumaryl)-glucoside	611 (303)	tr	81.93 ± 2.78	163.37 ± 2.98	571.25 ± 4.44
Peonidin-3-*O*-(6-*O*-acetyl)-glucoside	505 (301)	329.68 ± 6.24	576.65 ± 3.84	835.08 ± 4.69	48.31 ± 3.61
Malvidin-3-*O*-(6-*O*-acetyl)-glucoside	535 (331)	2023.29 ± 8.98	1079.31 ± 7.43	2058.55 ± 6.33	3127.17 ± 7.65
Peonidin-3-*O*-(6-*O*-caffeoyl)-glucoside	625 (301)	27.31 ± 4.21	151.85 ± 2.74	233.00 ± 2.15	163.46 ± 3.33
Cyanidin-3-*O*-(6-*O*-coumaryl)-glucoside	595 (287)	61.88 ± 5.33	37.46 ± 3.24	tr	361.98 ± 5.68
Malvidin-3-(6-*O*-caffeoyl)-glucoside	655 (331)	79.45 ± 4.27	nd	622.91 ± 5.68	nd
Petunidin-3-*O*-(6-*O*-coumaryl)-glucoside	625 (317)	25.89 ± 3.16	150.44 ± 2.11	35.98 ± 3.38	31.79 ± 2.92
Peonidin-3-*O*-(*cis*-6-*O*-coumaryl)- glucoside	609 (301)	25.89 ± 2.58	41.53 ± 1.58	56.52 ± 2.73	44.18 ± 3.47
Malvidin-3-*O*-(*cis*-6-*O*-coumaryl)-glucoside	639 (331)	52.64 ± 4.32	55.38 ± 3.23	280.37 ± 3.11	97.55 ± 4.32
Peonidin-3-*O*-(*trans*-6-O-coumaryl)-glucoside	609 (301)	169.90 ± 5.84	501.63 ± 4.32	1260.80 ± 6.32	381.36 ± 7.88
Malvidin-3-*O*-(*trans*-6-*O*-coumaryl)-glucoside	639 (331)	715.19 ± 6.11	783.10 ± 4.88	4324.94 ± 5.66	3596.14 ± 6.52

Values are means of duplicate determination ± S.D. nd: not detected. tr: trace. ME: malvidin-3-*O*-glucoside

Kaempferol-3-*O*-galactoside was not detected in MS. Among the flavonols, isorhamnetin-3-*O*-glucoside was the most abundant in CS, ML and MS, but SY showed a higher level of quercetin-3-*O*-glucoside, consistent with Jin *et al*. (2009). Total flavonol contents ranged from 2 285.5 mg QE/kg (ML) to 2 871.00 mg QE/kg (SY), higher than previously described (Mattivi *et al*. 2006; Jin *et al*., 2009). The total flavonol content was higher than in other non-anthocyanins, accounting for from 82.18% (SY) to 86.14% (ML) of total non-anthocyanins content. Moreover, the total flavonol content of SY was significantly higher than that of ML ($P < 0.05$) (Fig. 2a).

The flavan-3-ol composition

Four flavan-3-ols detected in the grape skins of CS, ML, SY and MS are shown in Table 2. They are gallocatechin, procyanidin dimer, (+)-catechin and procyanidin trimer. No gallocatechin was found in SY and MS. Procyanidin trimer was the most abundant in SY and MS, while CS and ML had higher levels of procyanidin dimer and (+)-catechin respectively.

Total flavan-3-ol contents ranged from 156.79 mg CE/kg (MS) to 200.59 mg CE/kg (SY), accounting for 5.56% to 6.57% of total non-anthocyanin contents, a little higher than that recorded in the study of Jin *et al*. (2009), which was done at the foot of Qi-lian Mountain in northwest China. It appears that a warm climate is beneficial to producing a high content of flavan-3-ols (Rodriguez *et al*., 2006; Fernandez *et al*., 2007). Nevertheless, no significant ($P < 0.05$; $P < 0.01$) differences were found among the grape cultivars studied (Fig. 2b).

The phenolic acid and stilbene composition

Six phenolic acids (four hydroxybenzoic acids, two hydroxycinnamic acids) and one stilbene were identified and quantified in the four wine grape cultivars (Table 2). The hexose ester of protocatechuic acid was not detected in CS, and no dimer (epi)gallocatechin-(epi)catechin was detected in MS. Syringetin-3-*O*-glucoside was the most abundant hydroxybenzoic acid. Total hydroxybenzoic acid contents ranged from 88.44 mg GAE/kg (CS) to 116.36 mg GAE/kg (SY), and this was about 3.10% to 3.51% of the total non-anthocyanins. As observed for the flavan-3-ol concentration, the level of total hydroxybenzoic acids showed no significant ($P < 0.05$; $P < 0.01$) differences among the grape cultivars studied (Fig. 2c).

trans-Cinnamic acid was the most abundant hydroxycinnamic acid among the grape cultivars studied. Total hydroxycinnamic acid contents ranged from 7.31 mg CAE/kg (CS) to 16.38 mg CAE/kg (SY), which was about 0.26% to 0.49% of the total non-anthocyanins. SY had a higher content of hydroxycinnamic acids than CS, while the others had no significant differences (Fig. 2d).

Stilbenes are phytoalexins that are directly related to environmental stress. Resveratrol was identified and quantified in the CS, SY and MS. The content of resveratrol

TABLE 2
The content of non-anthocyanin compounds in the berry skins of four grape cultivars (mg/kg).

Non-anthocyanin phenolic compounds	$(M-H)^-$ (Frag. MS^2 *m/z*)	Content			
		CS	ML	SY	MS
Flavonols:					
Quercetin-3-*O*-hexoside	463 (301)	61.57 ± 11.38	39.88 ± 0.15	43.53 ± 0.38	50.71 ± 1.50
Myricetin-3-O-glucuronide	477 (301)	nd	228.54 ± 5.61	nd	nd
Myricetin-3-*O*-galactoside	479 (317)	206.52 ± 6.56	nd	167.34 ± 6.04	221.29 ± 0.03
Isorhamnetin-3-*O*-galactoside	477 (315)	64.83 ± 8.75	118.25 ± 4.16	47.36 ± 2.73	30.46 ± 4.50
Myricetin-3-*O*-glucoside	479 (317, 179, 151)	233.86 ± 5.96	141.72 ± 4.03	200.97 ± 17.42	248.2 ± 44.31
Dihydroquercetin-3′-*O*-rhamnoside	449 (285, 303)	72.51 ± 3.87	122.87 ± 22.75	62.08 ± 2.95	71.42 ± 18.12
Quercetin-3-*O*-galactoside	463 (301)	70.76 ± 6.43	94.17 ± 12.32	120.1 ± 3.56	71.75 ± 0.01
Quercetin-3-*O*-glucuronide	477 (301)	128.41 ± 18.40	215.81 ± 15.40	213.67 ± 4.43	146.87 ± 1.00
Quercetin-3-*O*-glucoside	463 (301)	245.65 ± 34.76	418.38 ± 23.62	772.01 ± 18.70	238.38 ± 6.85
Laricitrin-3-*O*-glucoside	493 (331)	55.27 ± 3.55	62.2 ± 24.56	72.42 ± 5.02	50.07 ± 0.38
Kaempferol-3-*O*-galactoside	447 (285)	56.74 ± 6.05	74.39 ± 14.17	55.6 ± 3.58	nd
Kaempferol-3-*O*-glucoside	447 (285)	423.22 ± 74.88	281.95 ± 59.50	360.42 ± 12.56	371.54 ± 0.08
Isorhamnetin-3-*O*-glucoside	477 (315)	584.27 ± 77.89	427.61 ± 33.19	698.67 ± 77.72	622.26 ± 81.97
Quercetin-3-*O*-rutinoside	609 (301)	254.90 ± 24.98	59.8 ± 0.04	56.88 ± 0.05	300.20 ± 2.03
Flavan-3-ols:					
Gallocatechin	305 (179, 217, 137, 125)	29.99 ± 6.60	29.49 ± 2.06	nd	nd
Procyanidin dimer	577 (425, 289)	64.38 ± 7.42	44.1 ± 4.48	42.66 ± 4.96	19.08 ± 4.21
(+)-Catechin	289 (245)	41.3 ± 12.86	50.43 ± 5.56	63.8 ± 0.80	35.56 ± 0.01
Procyanidin trimer	865 (695, 577, 287)	48.85 ± 3.75	50.23 ± 2.35	94.12 ± 52.86	102.15 ± 6.79
Hydroxybenzoic acids					
Dimer (epi)gallocatechin-(epi)catechin	593 (425, 289, 407)	1.29 ± 0.59	0.55 ± 0.02	0.14 ± 0.01	nd
Hexose ester of protocatechuic acid	315 (153)	nd	10.69 ± 1.53	10.65 ± 5.09	tr
Hexose ester of vanillic acid	329 (191, 167)	25.55 ± 7.03	22.93 ± 0.80	29.05 ± 2.07	10.32 ± 3.54
Syringetin-3-*O*-glucoside	507 (345)	61.60 ± 10.90	57.02 ± 26.86	76.52 ± 45.13	87.61 ± 11.86
Hydroxycinnamic acids:					
Hexose ester of ferulic acid	355 (193)	3.81 ± 0.83	2.98 ± 0.08	3.83 ± 0.07	4.22 ± 0.07
trans-Cinnamic acid	147	3.51 ± 0.34	6.13 ± 2.97	12.55 ± 4.84	9.72 ± 0.54
Stilbenes:					
Resveratrol	227 (185, 159)	110.77 ± 20.94	93.67 ± 16.47	121.63 ± 9.90	75.73 ± 9.89

Values are means of duplicate determination ± S.D. nd: not detected. tr: trace.

ranged from 75.73 mg RE/kg (MS) to 121.63 mg RE/kg (SY) (Table 2). SY had a higher level of resveratrol than the three other varieties (Fig. 2e).

From the description above, the differences in anthocyanin contents among CS, ML, SY and MS were significant. However, there were no differences among CS, ML and MS for the flavonol, flavan-3-ol, phenolic acid and stilbene contents. This suggests that anthocyanins could be more useful for distinguishing grape varieties (Raúl *et al*., 2009). The phenolic composition of wines depends upon the grape variety and other factors that affect the berry development, such as soil, geographical location and weather conditions (Monagas *et al*., 2005). In the present study, under the same ecological conditions and cultivation management, the four grape cultivars displayed different phenol profiles, indicating that the biosynthesis of phenolic compounds depends largely on the genotype of the grape cultivar (Boss *et al*., 1996; Tian *et al*., 2008).

Cluster analysis

To better understand the phenolic characteristics of the four grape cultivars, cluster analysis using Ward's method was carried out on these identified phenolic compounds. As shown in Fig. 3, the four grape cultivars can be divided into two groups: CS and ML, and SY and MS. CS and ML were clustered within a short distance, indicating that CS and ML have similar phenolic compound profiles. A similar result for phenolic compounds was also observed between SY and MS. CS and ML showed great differences from SY and MS. This suggests that the profiles of the phenolic compounds in CS and ML were significantly different from those of SY and MS.

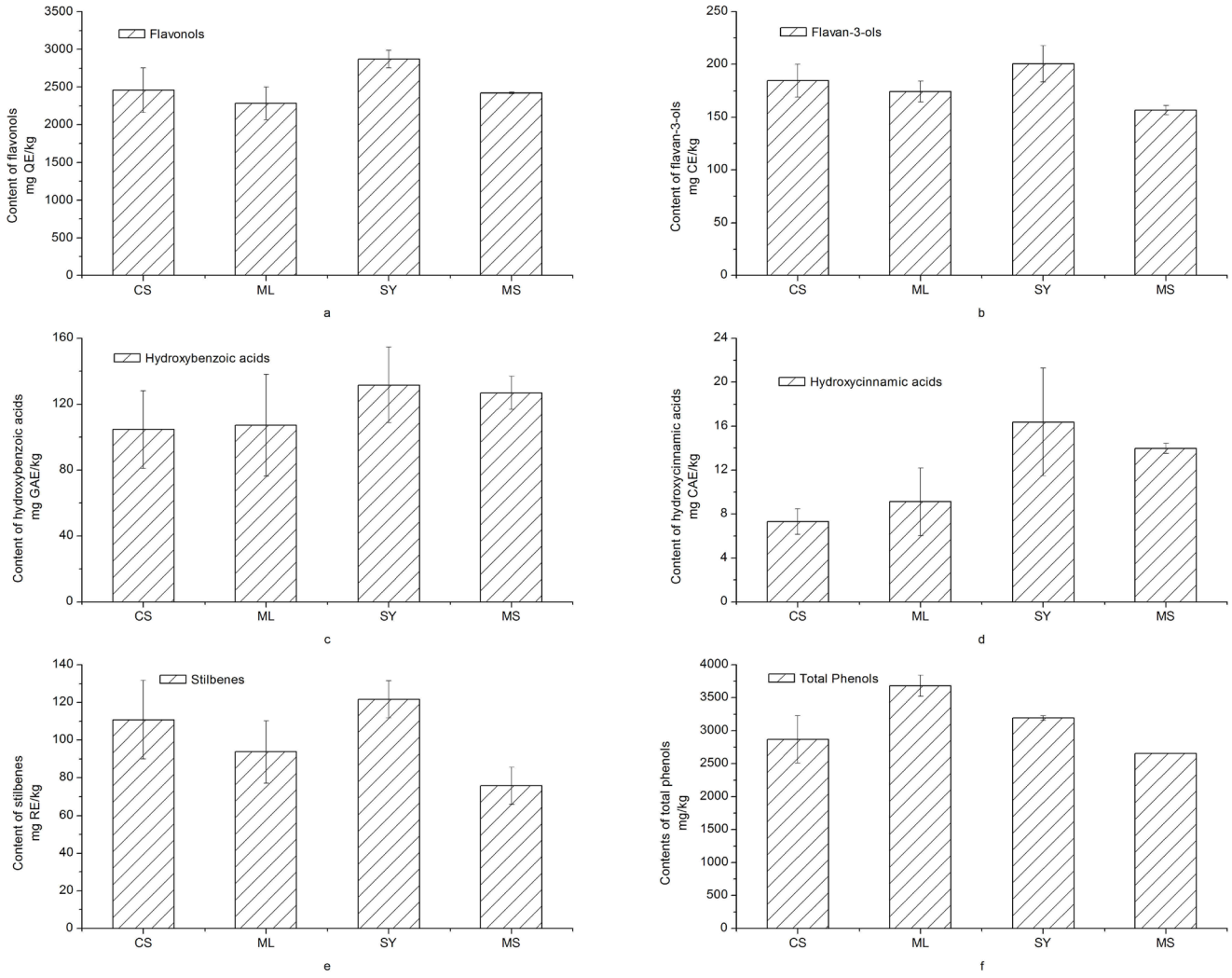

FIGURE 2
The total content of flavonols (a), flavan-3-ols (b), hydroxybenzoic acids (c), hydroxycinnamic acids (d), stilbenes (e) and total phenols (f) in the berry skins of the four grape cultivars. QE: quercetin; CE: catechin; GAE: gallic acid; CAE: caffeic acid; RE: resveratrol.

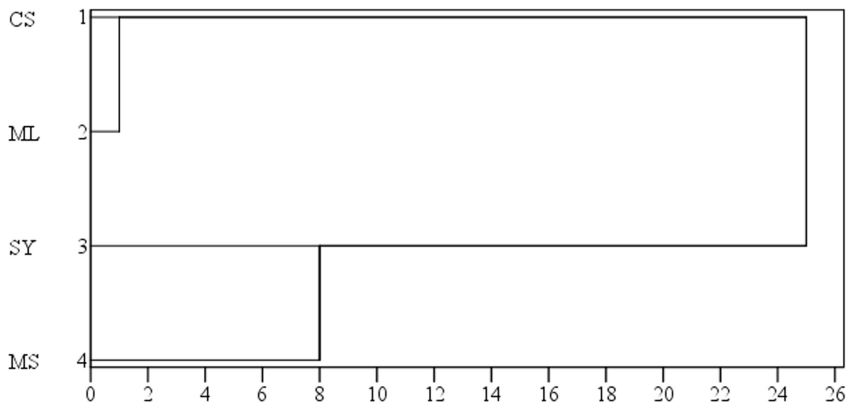

FIGURE 3
Cluster analysis of the four grape cultivars in Donghuayuan

CONCLUSIONS

In summary, 21 different anthocyanins and 27 non-anthocyanin phenolic compounds were detected in CS, ML, SY and MS grape skins from grapevines at Donghuayuan town. The composition and content of anthocyanins showed significant differences between CS and ML, and SY and MS. It was shown that malvidin and its derivatives are the main anthocyanins present in the skin. SY and MS had a higher level of total anthocyanins and malvidin and its derivatives than CS and ML. The content of non-anthocyanins showed little difference among CS, ML and MS. Cluster analysis showed that CS and ML, and SY and MS had similar phenolic compound profiles.

The findings of this study are useful for optimising winemaking processes to produce a particular geographical indication of wines' origins, depending largely on a detailed analysis of their phenolic content. This approach is especially relevant to Shacheng (China), which has a warm, semiarid climate, yet is a traditional winemaking area of which the wine typicality and "terroir" characters need to be explored.

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