

Free Radical-scavenging Activity and Anthocyanin Profiles of Cabernet Sauvignon and Merlot Wines from Four Wine Grape-growing Regions in China

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The present study focused on the free radical-scavenging activity and anthocyanin profiles of Cabernet Sauvignon and Merlot wines produced from four different regions in China. The anthocyanin profiles in all wine samples were analysed by HPLC-MS/MS, while the free radical-scavenging activity was estimated by the DPPH assay. The results show that the contents of phenolic subclasses and the levels of antioxidant activity in all wine samples varied greatly among cultivars and environmental factors of vine growth, and these values were the most prominent in Yuquanling regional wines. As the main components in anthocyanins, the percentages of malvidin-3-*O*-glucoside and its derivatives showed differences within grape cultivars in the different regional wines; these monomeric anthocyanins (not present simultaneously in the four regional wines studied within grape cultivars) had concentrations below 10 mg Mv/L. The significant correlation was obtained between DPPH-scavenging ability and the total phenolic, flavonoid and anthocyanin content. It can be concluded that this information could be used as a biochemical marker for the authenticity of the single-cultivar red wines that were produced from the four regions above.

INTRODUCTION

As a class of phenolic compounds originating from wine grapes, anthocyanins are responsible for grape and wine colour (Tang *et al.*, 2017). The colour of wine is one of the most important sensory properties. Therefore, it has long been recognised that the colour intensity of young red wines to some extent correlates positively with the overall wine quality. Anthocyanins accumulate in the grape skin after véraison via the phenylpropanoid biosynthetic pathway (Downey *et al.*, 2006), and those of wine are usually extracted from the skins of grapes during crushing, pressing and fermentation.

Red wines made from *Vitis vinifera* L. grapes normally contain five main monomeric anthocyanins, namely Dp, Cy, Pt, Pn and Mv (Fig. 1). Moreover, the main anthocyanins in the skins of red wine grape varieties are five primitive monoglucosides and their acetylated or coumaroylated derivatives, which are formed by combination with coumaric or caffeic acid (Monages *et al.*, 2003). Most of anthocyanins are Mv and its derivatives. In addition, the anthocyanins can also be classified according to either the number of hydroxyl groups (3'-substituted anthocyanins and 3',5'-substituted anthocyanins) or methoxyl group on the B-ring, or the type

of acylation (aliphatic or aromatic) (Fig. 1) (Gómez-Alonso *et al.*, 2007). The colour characteristics of anthocyanins

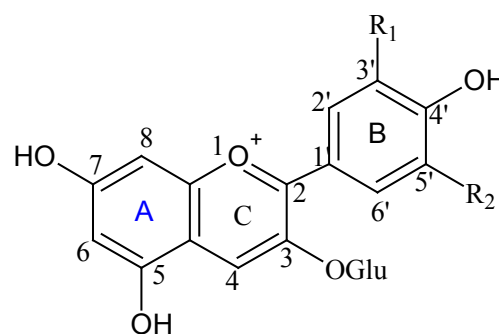


FIGURE 1

Structure of five primitive anthocyanin monoglucosides. Note: Delphinidin-3-*O*-glucoside (Dp): R₁-OH, R₂-OH; Cyanidin-3-*O*-glucoside (Cy): R₁-OH, R₂-H; Petundin-3-*O*-glucoside (Pt): R₁-OCH₃, R₂-OH; Peonidin-3-*O*-glucoside (Pn): R₁-OCH₃, R₂-H; Malvidin-3-*O*-glucoside (Mv): R₁-OCH₃, R₂-OCH₃.

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usually vary with these substituents. Besides genotype, management factors, winemaking and ageing conditions, and so on, it is known that climate and soil conditions are considered to be the main environmental factors determining wine characteristics and quality, resulting in different wine types. Like most other phenolic compounds, the composition and concentration of anthocyanins in red wine grapes and their corresponding wines vary with the climate and soil conditions of the vineyard. Significant differences in anthocyanin concentration between shaded clusters and sun-exposed clusters of Pinot Noir grape were found by Cortell and Kennedy (2006). Cheng *et al.* (2014) suggest that Cabernet Sauvignon berries from soils with less water and organic matter contain higher levels of 3',5'-substituted, *O*-methylated and acylated anthocyanins, which represent a positive characteristic conferring more stable pigmentation to the corresponding wine. In addition, it has been indicated that the total anthocyanins of the Malbec variety (*Vitis vinifera* L.) increased dramatically with higher elevations in Mendoza, Argentina (Bajda, 2007), while the skins were five times thicker at 1 500 m than at 850 m. The content of total phenolic compounds, total flavonoids and total anthocyanins increased with altitude in Cabernet Sauvignon and Merlot wines (Jin *et al.*, 2017).

Due to large phenolic compounds in wine, wine is considered to possess the ability to scavenge excess radicals (Liu *et al.*, 2018). Positive correlations between total phenolics and antioxidant capacity have been reported (Gómes-Plaza *et al.*, 2006; Orak, 2007; Jin *et al.*, 2017). Many studies indicate that the consumption of small amounts of red wine on a regular basis reduces the risk of cardiovascular disease, atherosclerosis and certain types of cancers; this benefit is attributed to the antioxidant properties of the polyphenolic compounds (Mazza *et al.*, 1999; Yilmaz & Toledo, 2004). Still, it is very important to determine which group of phenolic compounds is most influential in these properties of wine. Between *in vitro* and *in vivo* research trials, anthocyanins have demonstrated a noticeable ability to reduce cancer cell proliferation and to inhibit tumour formation (Hou, 2003; Lila, 2004). Anthocyanins (Kähkönen & Heinonen, 2003) and extracts containing anthocyanins (Shirahigue *et al.*, 2010) were also reported to show radical-scavenging activity and the ability to prevent lipid peroxidation.

Unlike most other wine-producing countries, China's wine-producing areas are very scattered, with a distance of over 2 000 kilometres from west to east (Li *et al.*, 2011). Moreover, these wine grape-growing regions of China display unique ecological conditions. All the different climate and soil characteristics give them the capability to produce various types of wines. Cabernet Sauvignon and Merlot are the world's most well-known *Vitis vinifera* red grape cultivars. Due to their premium quality and stronger adaptability to arid and barren lands than other grape varieties, both of them have spread across the old world and new world wine countries, from France and Italy to New Zealand, Australia and South Africa. Cabernet Sauvignon and Merlot are still the most popular varieties in each of China's wine grape-growing regions (Wang *et al.*, 2018). However, the differences in the anthocyanin profiles of each

of the monovarietal wines from four wine grape-growing regions in China remain unclear.

The aim of this work was therefore to (1) investigate and analyse the differences in the anthocyanin compounds from four regional Cabernet Sauvignon and Merlot wines, and to elucidate these wines' characteristics using their anthocyanin profiles, defined as the percentage content of each anthocyanin, and (2) evaluate the free radical-scavenging activity of four regional Cabernet Sauvignon and Merlot wines, and establish the relationship between four subclasses of phenolics and antioxidant activity in wine by correlation analysis. The present results will provide an essential basis for further fingerprinting research on these regional wines. In addition, these results also will be helpful in discovering some valuable information that could result in the production of high-quality wine in China.

MATERIALS AND METHODS

Chemicals and reagents

Folin-Ciocalteu's phenol reagent, gallic acid, catechin, 2,2'-diphenyl-1-picrylhydrazyl (DPPH), 6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid (Trolox), *p*-dimethylaminocinnamaldehyde (DMACA) and malvidin-3-*O*-glucoside were purchased from Sigma-Aldrich (St. Louis, MO, USA). Methanol (HPLC grade) was obtained from Fisher Co. (Fairlawn, NJ, USA). All other chemicals and solvents were analytical reagent grade and were purchased in China.

Sample collection and vinification

The present study was conducted using Cabernet Sauvignon and Merlot vines grafted onto SO₄ rootstock from four regions that are harvested from west to east: Yuquanying of Ningxia (hereafter NXYQY) is a cool and semi-arid area in Western China; Xiangning of Shanxi (hereafter SXXN) belongs to the arid area in Northern China; Shacheng of Hebei (hereafter HBSC) is a warm and semi-arid area in Eastern China; Changli of Hebei (hereafter HBCL) is a wetter area in Eastern China. The climate data and soil conditions of the four experimental regions during the growing season of wine grapes are listed in Table 1. Vines were grown for eight years, drip irrigated, Dulong trained, with a vine spacing of 3.0 × 1.2 m. Rows were east-west oriented. The vineyard was managed in accordance with the standard agronomic practices in the four regions.

All grape berries were harvested manually at optimum technological maturity for these vineyards in September of the year. Pre-fermentation treatments and winemaking were performed as described by Li (2002). Briefly, grapes were crushed in an experimental destemmer-crusher and then transferred to stainless steel containers. Thirty litres of each wine were produced in three replicates. Sulphur dioxide (1.5 g) and pectinase (Lallzyme Ex) (0.9 g) were added to the musts and the contents were mixed by hand. After maceration of the musts for 24 h, they were inoculated with *Saccharomyces cerevisiae* EC-1118 (Lallemand, Danstar Ferment AG, Switzerland) (6.0 g) according to commercial specifications. Alcoholic fermentation was carried out at 20°C to 25°C to dryness (reducing sugar < 4 g/L), which took place over a period of six to eight days, and density controls

TABLE 1
Climate data and soil conditions for the four experimental regions during the growing season of wine grapes (April to September).

Analytical index	Cabernet Sauvignon				Merlot			
	NXYQY	SXXN	HBSC	HBCL	NXYQY	SXXN	HBSC	HBCL
Residual sugar (g/L)	2.0 ± 0.1 ^b	2.1 ± 0.0 ^b	2.9 ± 0.1 ^a	2.8 ± 0.2 ^a	1.9 ± 0.0 ^b	2.1 ± 0.1 ^b	3.1 ± 0.3 ^a	2.9 ± 0.3 ^a
Titrate acidity ^a (g/L)	7.3 ± 0.1 ^a	7.1 ± 0.3 ^{ab}	6.7 ± 0.2 ^{abc}	6.3 ± 0.5 ^c	8.0 ± 0.0 ^a	6.7 ± 0.4 ^b	8.3 ± 0.3 ^a	6.7 ± 0.1 ^b
pH	3.1 ± 0.0 ^b	3.1 ± 0.1 ^b	3.5 ± 0.2 ^a	3.6 ± 0.1 ^a	3.0 ± 0.0 ^b	3.3 ± 0.2 ^{ab}	3.5 ± 0.1 ^a	3.6 ± 0.1 ^a
Alcohol (%)	12.0 ± 0.2 ^a	12.0 ± 0.2 ^a	12.4 ± 0.2 ^a	12.2 ± 0.1 ^a	11.0 ± 0.1 ^b	10.8 ± 0.0 ^b	11.5 ± 0.2 ^a	11.0 ± 0.1 ^b
Total phenolics (mg GAE/L)	2 710.4 ± 200.5 ^a	1 129.8 ± 79.6 ^c	2 330.2 ± 120.3 ^b	1 313.0 ± 45.2 ^c	1 656.5 ± 161.7 ^a	860.2 ± 45.7 ^c	1247.7 ± 110.1 ^b	941.2 ± 66.4 ^c
TFO (mg CTE/L)	2 290.3 ± 157.2 ^a	859.9 ± 55.5 ^d	1 906.4 ± 132.9 ^b	1 189.1 ± 89.1 ^c	1375.4 ± 76.4 ^a	697.5 ± 23.1 ^c	1 031.4 ± 95.4 ^b	660.6 ± 18.5 ^c
TFA (mg CTE/L)	532.3 ± 79.8 ^a	277.7 ± 34.3 ^b	666.4 ± 84.6 ^a	342.7 ± 47.6 ^b	497.4 ± 16.4 ^a	279.8 ± 24.1 ^c	361.1 ± 10.3 ^b	272.8 ± 40.0 ^c
Total anthocyanin (mg Mv/L)	400.3 ± 16.3 ^a	286.8 ± 27.9 ^b	372.6 ± 4.2 ^a	261.5 ± 42.5 ^b	350.3 ± 54.8 ^a	259.4 ± 0.8 ^b	216.7 ± 20.8 ^{bc}	157.5 ± 17.5 ^c
Radical scavenging (µmol/L)	6 179.4 ± 110.3 ^a	4 832.2 ± 45.8 ^c	5 342.3 ± 201.5 ^b	4 670.4 ± 157.0 ^c	5 304.1 ± 144.7 ^a	3 857.0 ± 88.9 ^c	4 852.6 ± 122.4 ^b	3 890.3 ± 95.4 ^c

The results were expressed as mean values ± SD of triplicate samples; different letters within a row for the same parameters indicate significant differences among four regional wines by Tukey's test ($p < 0.05$); ^a Values expressed as grams of tartaric acid equivalents per litre (g/L).

were maintained during this period. At the end of alcoholic fermentation, the wines were separated from the pomace, after which SO₂ (1.5 g) was added. After fermentation, the wine samples were bottled and stored at 10°C to 15°C prior to analysis. All the samples were five months old for the analysis. Residual sugar, total acidity, pH and ethanol were analysed (Office International de la Vigne et du Vin [O.I.V.], 2018) (Table 2).

Determination of phenolic compounds

The total phenol (TP) content was determined by the Folin-Ciocalteu colorimetric method (Rapisarda *et al.*, 1999). The TP concentration was expressed as milligrams of gallic acid equivalents per litre basis (mg GAE/L). The total flavonoid (TFO) and total flavanol (TFA) contents were measured separately according to a colorimetric assay (Kim *et al.*, 2003) and the DMACA method (Li *et al.*, 1996; Morrourgh *et al.*, 1996), both of which were expressed as milligrams of catechin equivalents per litre basis (mg CTE/L). The total anthocyanin (TA) content was determined by the pH-differential method (Giusti & Worsltad, 2001) using two buffer systems – potassium chloride buffer, pH 1.0 (0.025 M) and sodium acetate buffer, pH 4.5 (0.4 M). The contents were calculated as malvidin-3-*O*-glucoside and expressed as milligrams of malvidin-3-*O*-glucoside (Mv) equivalents per litre basis (mg Mv/L).

Free radical-scavenging activity

The ability to scavenge DPPH free radicals was determined. Scavenging activity was based on the slightly modified method (Brand-Williams *et al.*, 1995). Briefly, 0.1 mL of sample solution (with appropriate dilution as needed) was added to 3.9 mL of a 60 µM solution of DPPH in methanol. A control sample, containing the same volume of solvent in place of extract, was used to measure the maximum DPPH absorbance. After the reaction was allowed to take place in the dark for 30 min, the absorbance at 515 nm was recorded to determine the concentration of remaining DPPH. Results were expressed as Trolox equivalent antioxidant capacity (µM TE/L).

Quantitative analyses by HPLC-MS/MS

Qualitative and quantitative analyses of anthocyanin compounds were performed at the Centre for Viticulture and Oenology, College of Food Science & Engineering, China Agricultural University. The wine samples obtained above were filtered through 0.45 µm filters (cellulose acetate and nitrocellulose, CAN) and the resulting filtrates were directly used for qualitative and quantitative analyses. For the anthocyanin compounds, an Agilent 1100 series LC-MSD trap VL, equipped with a DAD detector and reversed phase column (Kromasil C18, 250 × 4.6, 5 µm), was used. The solvents were: (A) aqueous 2% formic acid, and (B) acetonitrile containing 2% formic acid. The gradient was from 6% to 10% B for 4 min, from 10% to 25% B for 8 min, isocratic 25% B for 1 min, from 25% to 40% for 7 min, from 40% to 60% for 15 min, from 60% to 100% for 5 min, and from 100% to 6% for 5 min, with a flow rate of 1.0 mL/min. Injection volumes of 30 µL were used, and the detection wavelength was 525 nm. MS conditions were as

TABLE 2
General composition and radical-scavenging activity of Cabernet Sauvignon and Merlot wines from four different wine grape-growing regions.

Compound	RT (min)	λ_{max} (nm)	MS; MS ² (m/z)	Cabernet Sauvignon				Merlot			
				SXXN	NXYQY	HBCL	HBSC	SXXN	NXYQY	HBSC	HBCL
Delphinidin-3-O-glucoside	8.03	524	465 (M ⁺); 303	23.4 ± 0.1 ^c (7.21%)	35.2 ± 0.4 ^b (9.89%)	41.7 ± 2.2 ^a (6.83%)	5.4 ± 0.1 ^d (1.16%)	14.2 ± 0.2 ^c (4.33%)	23.0 ± 1.3 ^b (6.42%)	8.9 ± 0.4 ^b (3.52%)	35.3 ± 0.8 ^a (9.01%)
Cyanidin-3-O-glucoside	10.27	516	449 (M ⁺); 287	2.7 ± 0.2 ^c (0.83)	1.7 ± 0.1 ^d (0.48%)	7.4 ± 0.3 ^a (1.21%)	3.6 ± 0.4 ^b (0.77%)	2.3 ± 0.3 ^b (0.70%)	3.3 ± 0.1 ^a (0.92%)	1.2 ± 0.1 ^c (0.47%)	1.3 ± 0.0 ^c (0.33%)
Petunidin-3-O-glucoside	11.65	524	479 (M ⁺); 317	20.4 ± 0.9 ^b (6.29%)	30.2 ± 1.3 ^{ab} (8.49%)	27.6 ± 1.1 ^b (4.52%)	33.2 ± 2.0 ^b (7.13%)	19.3 ± 0.5 ^c (5.88%)	27.9 ± 1.4 ^b (7.78%)	12.2 ± 0.7 ^d (4.82%)	36.8 ± 2.4 ^a (9.39%)
Peonidin-3-O-glucoside	13.72	518	463 (M ⁺); 301	12.0 ± 1.1 ^{bc} (3.70%)	9.3 ± 0.1 ^c (2.61%)	36.4 ± 1.8 ^a (5.97%)	13.8 ± 0.2 ^b (2.96%)	7.6 ± 0.2 ^c (2.32%)	16.7 ± 0.1 ^a (4.66%)	5.7 ± 0.1 ^d (2.25%)	15.2 ± 0.2 ^b (3.88%)
Malvidin-3-O-glucoside	14.72	528	493 (M ⁺); 331	138.6 ± 1.9 ^d (42.73%)	157.8 ± 3.7 ^e (44.34%)	226.5 ± 0.7 ^a (37.12%)	180.3 ± 2.0 ^b (38.73%)	147.1 ± 0.3 ^a (44.83%)	126.0 ± 3.3 ^b (35.16%)	101.2 ± 1.8 ^c (40.02%)	124.4 ± 0.7 ^b (31.75%)
Delphinidin-3-O-(6-O-acetyl)-glucoside	15.28	526	507 (M ⁺); 303	9.4 ± 0.2 ^c (2.90%)	13.5 ± 0.2 ^b (3.79%)	11.0 ± 1.3 ^c (1.80%)	19.6 ± 0.5 ^a (4.21%)	9.0 ± 0.7 ^c (2.74%)	19.8 ± 1.4 ^a (5.52%)	5.3 ± 0.1 ^d (2.10%)	11.2 ± 0.8 ^b (2.86%)
Peonidin-3-O-glucoside-pyruvic acid	15.78	506	531 (M ⁺); 369	trace	nd	nd	nd	7.1 ± 0.2 ^a (2.16%)	1.3 ± 0.1 ^c (0.36%)	2.0 ± 0.0 ^b (0.79%)	nd
Malvidin-3-O-glucoside-pyruvic acid	16.04	526	561 (M ⁺); 399	1.5 ± 0.3 ^c (0.46%)	4.5 ± 0.1 ^b (1.26%)	1.3 ± 0.2 ^c (0.21%)	6.5 ± 0.3 ^a (1.40%)	5.7 ± 0.1 ^c (1.74%)	8.6 ± 0.3 ^a (2.40%)	7.7 ± 0.5 ^b (3.04%)	2.3 ± 0.2 ^d (0.59%)
Cyanidin-3-O-(6-O-acetyl)-glucoside	17.40	522	491 (M ⁺); 287	1.2 ± 0.0 ^c (0.37%)	1.3 ± 0.2 ^{bc} (0.37%)	1.6 ± 0.2 ^b (0.26%)	3.6 ± 0.1 ^a (0.77%)	trace ^c	7.1 ± 1.0 ^b (1.98%)	2.5 ± 0.1 ^b (0.99%)	1.4 ± 0.5 ^b (0.36%)
Malvidin-3-O-glucoside-acetaldehyde	17.71	508	517 (M ⁺); 355	3.6 ± 0.2 ^c (1.11%)	1.6 ± 0.1 ^d (0.45%)	4.5 ± 0.1 ^b (0.74%)	11.7 ± 0.2 ^a (2.51%)	2.1 ± 0.4 ^b (0.64%)	3.8 ± 0.2 ^a (1.06%)	2.3 ± 0.0 ^b (0.91%)	1.4 ± 0.2 ^c (0.36%)
Peonidin-3-O-(6-O-acetyl)-glucoside-pyruvic acid	17.78	528	573 (M ⁺); 369	1.4 ± 0.1 ^b (0.43%)	3.8 ± 0.2 ^a (1.07%)	1.5 ± 0.2 ^b (0.25%)	nd	3.4 ± 0.2 ^b (1.04%)	1.7 ± 0.1 ^c (0.47%)	4.6 ± 0.4 ^a (1.82%)	1.1 ± 0.2 ^c (0.28%)
Malvidin-3-O-(6-O-acetyl)-glucoside-pyruvic acid	18.04	516	603 (M ⁺); 399	trace ^c	9.9 ± 0.2 ^b (2.78%)	9.9 ± 0.4 ^b (1.62%)	13.2 ± 0.4 ^a (2.84%)	nd	trace ^c	5.0 ± 0.1 ^b (1.98%)	10.2 ± 0.3 ^a (2.60%)
Petunidin-3-O-(6-O-acetyl)-glucoside	18.35	522	521 (M ⁺); 317	7.2 ± 0.4 ^a (2.22%)	3.0 ± 0.2 ^b (0.84%)	1.8 ± 0.3 ^b (0.29%)	7.8 ± 1.3 ^a (1.68%)	2.5 ± 0.1 ^b (0.76%)	12.6 ± 1.6 ^a (3.52%)	1.1 ± 0.3 ^c (0.43%)	1.1 ± 0.1 ^c (0.28%)
Malvidin-3-O-((6-O-acetyl)-glucoside-ethyl-(epi)catechin	18.59	528	809 (M ⁺); 519, 357	1.4 ± 0.4 ^d (0.43%)	3.3 ± 0.1 ^b (0.93%)	4.6 ± 0.2 ^a (0.75%)	2.5 ± 0.2 ^c (0.54%)	2.7 ± 0.2 ^b (0.82%)	4.6 ± 0.1 ^a (1.28%)	2.1 ± 0.3 ^c (0.83%)	1.5 ± 0.1 ^d (0.38%)
Delphinidin-3-O-(cis-6-O-coumaryl)-glucoside	19.18	532	611 (M ⁺); 303	8.0 ± 0.4 ^b (2.47%)	8.7 ± 0.8 ^b (2.44%)	14.2 ± 0.6 ^a (2.33%)	5.4 ± 0.2 ^c (1.16%)	7.9 ± 0.3 ^c (2.41%)	11.5 ± 0.6 ^b (3.21%)	nd	23.9 ± 2.1 ^a (6.10%)
Malvidin-3-O-(6-O-acetyl)-glucoside-acetaldehyde	19.28	526	559 (M ⁺); 355	1.9 ± 0.3 ^c (0.59%)	4.1 ± 0.5 ^b (1.15%)	3.8 ± 0.1 ^b (0.62%)	6.6 ± 0.3 ^a (1.42%)	2.5 ± 0.3 ^b (0.76%)	2.2 ± 0.2 ^b (0.61%)	4.5 ± 0.3 ^a (1.78%)	5.0 ± 0.7 ^a (1.28%)
Peonidin-3-O-(6-O-acetyl)-glucoside	20.15	522	505 (M ⁺); 301	1.6 ± 0.2 ^b (0.49%)	1.8 ± 0.1 ^b (0.51%)	1.6 ± 0.1 ^b (0.26%)	11.4 ± 2.3 ^a (1.42%)	2.8 ± 0.2 ^c (0.76%)	3.8 ± 0.1 ^b (0.61%)	6.4 ± 0.5 ^a (1.78%)	1.3 ± 0.1 ^d (1.28%)
Malvidin-3-O-(6-O-acetyl)-glucoside	20.86	528	535 (M ⁺); 331	53.1 ± 0.6 ^f (16.37%)	56.3 ± 3.5 ^e (15.82%)	130.7 ± 3.7 ^a (21.42%)	72.9 ± 4.0 ^b (15.66%)	55.8 ± 2.8 ^a (17.01%)	32.4 ± 0.9 ^b (9.04%)	33.9 ± 0.3 ^b (13.40%)	52.1 ± 0.6 ^a (13.30%)
Peonidin-3-O-(6-O-caffeoyl)-glucoside	21.33	520	625 (M ⁺); 301	trace ^c	1.6 ± 0.3 ^b (0.45%)	3.0 ± 0.2 ^a (0.49%)	3.4 ± 0.1 ^a (0.73%)	2.0 ± 0.1 ^{cd} (0.61%)	2.5 ± 0.2 ^{bc} (0.70%)	4.8 ± 0.3 ^a (1.90%)	1.6 ± 0.1 ^d (0.41%)
Cyanidin-3-O-(6-O-coumaryl)-glucoside	21.47	520	595 (M ⁺); 287	2.1 ± 0.1 ^b (0.65%)	2.9 ± 0.2 ^a (0.81%)	1.4 ± 0.2 ^c (0.23%)	1.0 ± 0.0 ^d (0.21%)	3.1 ± 0.2 ^c (0.94%)	4.3 ± 0.1 ^a (1.20%)	3.8 ± 0.2 ^b (1.50%)	1.5 ± 0.1 ^d (0.38%)
Malvidin-3-O-(6-O-coumaryl)-glucoside-pyruvic acid	21.70	518	707 (M ⁺); 399	nd	nd	nd	6.0 ± 0.3 (1.29%)	nd	nd	3.3 ± 0.1 (1.30%)	nd
Malvidin-3-O-(6-O-caffeoyl)-glucoside	21.98	532	655 (M ⁺); 331	2.2 ± 0.1 ^c (0.68%)	3.7 ± 0.3 ^b (1.04%)	nd	5.6 ± 0.3 ^a (1.20%)	2.7 ± 0.1 ^c (0.82%)	4.5 ± 0.2 ^a (1.26%)	3.4 ± 0.2 ^b (1.34%)	2.4 ± 0.0 ^c (0.61%)

TABLE 2 (CONTINUED)

Compound	RT (min)	λ_{max} (nm)	MS; MS ² (m/z)	Cabernet Sauvignon				Merlot			
				NXYQY	SXXN	HBCL	HBSC	NXYQY	SXXN	HBSC	HBCL
Malvidin-3-O-(6-O-coumaroyl)-glucoside-acetaldehyde	22.12	530	663 (M ⁺); 355	nd	1.6 ± 0.1 (0.49%)	nd	5.6 ± 0.3 (1.20%)	1.8 ± 0.2 ^c (0.55%)	2.4 ± 0.1 ^b (0.67%)	3.4 ± 0.3 ^a (1.34%)	nd
Petunidin-3-O-(6-O-coumaroyl)-glucoside	22.80	530	625 (M ⁺); 317	2.2 ± 0.1 ^b (0.62%)	2.0 ± 0.3 ^b (0.62%)	1.6 ± 0.2 ^b (0.26%)	6.5 ± 0.3 ^a (1.40%)	2.5 ± 0.2 ^b (0.76%)	4.1 ± 0.4 ^a (1.14%)	3.5 ± 0.2 ^a (1.38%)	1.3 ± 0.1 ^c (0.33%)
Peonidin-3-O-(cis-6-O-coumaroyl)-glucoside	23.64	524	609 (M ⁺); 301	2.4 ± 0.3 ^b (0.67%)	2.3 ± 0.2 ^b (0.71%)	4.6 ± 0.1 ^a (0.75%)	4.6 ± 0.3 ^a (0.99%)	2.3 ± 0.1 ^a (0.70%)	3.2 ± 0.2 ^c (0.89%)	5.9 ± 0.3 ^a (2.33%)	4.0 ± 0.2 ^b (1.02%)
Malvidin-3-O-(cis-6-O-coumaroyl)-glucoside	23.98	536	639 (M ⁺); 331	1.9 ± 0.1 ^c (0.53%)	3.3 ± 0.2 ^b (1.02%)	9.7 ± 1.2 ^a (1.59%)	3.5 ± 0.3 ^b (0.75%)	1.5 ± 0.1 ^{ab} (0.46%)	2.0 ± 0.2 ^a (0.56%)	1.9 ± 0.1 ^{ab} (0.75%)	1.3 ± 0.4 ^b (0.33%)
Peonidin-3-O-(trans-6-O-coumaroyl)-glucoside	24.86	522	609 (M ⁺); 301	2.9 ± 0.2 ^c (0.81%)	3.4 ± 0.3 ^c (1.05%)	17.7 ± 2.0 ^a (2.90%)	4.8 ± 0.3 ^b (1.03%)	1.5 ± 0.1 ^a (0.46%)	6.9 ± 0.3 ^a (1.93%)	3.5 ± 0.1 ^b (1.38%)	2.1 ± 0.1 ^c (0.54%)
Peonidin-3-O-glucoside-4-vinylcatechol	25.00	504	595 (M ⁺); 433	nd	nd	nd	1.7 ± 0.2 (0.37%)	nd	nd	nd	nd
Malvidin-3-O-(trans-6-O-coumaroyl)-glucoside	25.72	530	639 (M ⁺); 331	10.3 ± 2.0 ^c (2.89%)	12.2 ± 0.3 ^c (3.76%)	24.4 ± 1.4 ^a (4.00%)	16.7 ± 0.3 ^b (3.59)	10.5 ± 0.2 ^c (3.20%)	14.9 ± 0.2 ^b (4.16%)	8.6 ± 0.1 ^c (3.40%)	39.3 ± 2.3 ^a (10.03%)
Malvidin-3-O-(6-O-ferulyl)-glucoside	26.42	532	669 (M ⁺); 331	1.3 ± 0.1 (0.37%)	trace	nd	nd	nd	trace	nd	nd
Peonidin-3-O-glucoside-4-vinylphenol	27.46	508	579 (M ⁺); 417	2.0 ± 0.2 ^a (0.56%)	nd	1.4 ± 0.1 ^b (0.23%)	1.7 ± 0.1 ^{ab} (0.37%)	nd	nd	nd	1.1 ± 0.1 (0.28%)
Malvidin-3-O-glucoside-4-vinylphenol	28.43	504	609 (M ⁺); 447	1.9 ± 0.2 ^b (0.53%)	1.4 ± 0.1 ^c (0.43%)	3.6 ± 0.2 ^a (0.59%)	1.5 ± 0.1 ^{bc} (0.32%)	1.4 ± 0.1 ^a (0.43%)	1.0 ± 0.1 ^a (0.28%)	1.2 ± 0.2 ^a (0.47%)	1.3 ± 0.2 ^a (0.33%)
Peonidin-3-O-(6-O-acetyl)-glucoside-4-vinylphenol	29.13	504	621 (M ⁺); 417	2.0 ± 0.1 ^a (0.56%)	1.5 ± 0.2 ^b (0.46%)	1.6 ± 0.1 ^{ab} (0.26%)	1.6 ± 0.2 ^{ab} (0.34%)	1.6 ± 0.1 ^b (0.49%)	2.1 ± 0.1 ^a (0.59%)	1.7 ± 0.1 ^{ab} (0.67%)	1.7 ± 0.3 ^{ab} (0.43%)
Malvidin-3-O-glucoside-4-vinylguaiacol	29.86	508	639 (M ⁺); 477	nd	trace	nd	1.3 ± 0.2 (0.28%)	nd	1.4 ± 0.2 (0.39%)	nd	nd
Malvidin-3-O-(6-O-acetyl)-glucoside-4-vinylphenol	30.92	508	651 (M ⁺); 447	1.9 ± 0.1 ^b (0.53%)	1.9 ± 0.2 ^b (0.59%)	3.7 ± 0.3 ^a (0.61%)	nd	1.4 ± 0.1 ^b (0.43%)	1.5 ± 0.0 ^b (0.42%)	1.3 ± 0.1 ^b (0.51%)	3.1 ± 0.1 ^a (0.79%)
Malvidin-3-O-(6-O-acetyl)-glucoside-4-vinylguaiacol	31.80	502	681 (M ⁺); 477	1.4 ± 0.1 ^b (0.39%)	1.5 ± 0.2 ^b (0.46%)	9.9 ± 0.3 ^a (1.62%)	1.2 ± 0.2 ^b (0.26%)	2.3 ± 0.1 ^a (0.70%)	trace ^b	nd	2.3 ± 0.2 ^a (0.59%)
Malvidin-3-O-(6-O-coumaroyl)-glucoside-4-vinylphenol	32.18	502	755 (M ⁺); 447	1.5 ± 0.1 ^a (0.42%)	1.6 ± 0.1 ^a (0.49%)	1.5 ± 0.2 ^a (0.25%)	1.3 ± 0.2 ^a (0.28%)	1.5 ± 0.1 ^b (0.46%)	1.3 ± 0.3 ^b (0.36%)	nd	3.3 ± 0.1 ^a (0.84%)
Sum-3-monoglucoside				234.2 ± 2.6 ^b (65.81%)	197.1 ± 3.6 ^c (60.76%)	339.6 ± 2.0 ^a (55.65%)	236.3 ± 6.1 ^b (50.75%)	190.5 ± 0.6 ^b (58.06%)	196.9 ± 4.4 ^b (54.94%)	129.2 ± 0.5 ^c (51.08%)	213.0 ± 2.3 ^a (54.36%)
Sum-3-gl-acetyl				78.9 ± 0.7 ^c (28.74%)	72.7 ± 2.8 ^a (25.31%)	170.7 ± 1.5 ^a (29.76%)	120.8 ± 2.1 ^b (30.17%)	75.0 ± 1.2 ^b (25.60%)	68.0 ± 2.4 ^c (24.49%)	63.1 ± 0.3 ^d (27.04%)	80.8 ± 2.0 ^a (23.48%)
Sum-3-gl-coumaroyl				29.9 ± 0.5 ^d (9.19%)	36.5 ± 0.3 ^c (11.26%)	75.1 ± 0.6 ^a (12.31%)	55.4 ± 0.8 ^b (11.90%)	32.6 ± 2.7 ^c (9.94%)	50.6 ± 1.5 ^b (14.12%)	33.9 ± 2.4 ^c (13.38%)	76.7 ± 1.3 ^a (15.97%)
Sum-3-gl-caffeoyl				5.3 ± 0.5 ^b (1.49%)	2.2 ± 0.4 ^c (0.68%)	3.0 ± 0.7 ^c (0.49%)	9.0 ± 0.6 ^a (1.93%)	4.7 ± 1.0 ^b (1.43%)	7.0 ± 0.3 ^b (1.96%)	8.2 ± 0.7 ^a (3.24%)	4.0 ± 0.4 ^c (1.02%)

The results are expressed as mean values ± SD of triplicate samples; different letters within a row for the same compound indicate significant differences among four regional wines by Tukey's test ($p < 0.05$); "nd", not detected.

follows: electrospray ionisation (ESI) interface, positive ion model, 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. The anthocyanin compounds were identified by their order of elution and retention time with respect to malvidin-3-*O*-glucoside, and the weight of molecular ion and the fragment ion compared with standards and references (He *et al.*, 2010).

Quantification analyses of anthocyanin

All the monomeric anthocyanins were quantified using malvidin-3-*O*-glucoside as standard. Gradients with eight concentrations of mixture standard (malvidin-3-*O*-glucoside) were set with three replications, while one group of standard curve of the concentration was made based on the average area of the malvidin-3-*O*-glucoside compound, and all the monomeric anthocyanins were qualified by the external standard method.

Statistical analysis

Data were expressed as the mean \pm SD. Statistical analysis of the data was performed using SPSS 17.0 for Windows, with three replications of the same sample. Significant differences between wines from the four different regions were determined by Tukey's test ($p < 0.05$).

RESULTS AND DISCUSSION

Total content of phenolic subclasses in wine samples

The content of total phenols (TP), flavonoids (TFO), flavanols (TFA) and anthocyanins (TA) was determined for all Cabernet Sauvignon and Merlot wines from the above four regions, as listed in Table 2. The phenol concentrations vary widely in the wine samples tested.

The Cabernet Sauvignon and Merlot wines with the highest amount of TP were both from the NXYQY region, whereas both monovarietal wine samples tested from the SXXN region displayed the lowest values. For the Cabernet Sauvignon wines, the TP content in the NXYQY region was about 2.0 to 2.4 times those in the HBCL and SXXN regions, being approximately 1.2 times that in the HBSC region. For the Merlot wines, the content of TP in the NXYQY region was 1.7 to 1.9 times those in the HBCL and SXXN regions. For both monovarietal wines, the TP content in the HBCL region was not significantly ($p < 0.05$) higher than that in the SXXN region.

The TFO contents of Cabernet Sauvignon and Merlot wines in the NXYQY region were the highest among the four regions, followed by the HBSC region, and the HBCL and SXXN regions. Unlike the Cabernet Sauvignon wines, the Merlot wines from the HBCL and SXXN regions contained a non-significantly ($p < 0.05$) lower content of TFO. The content of TFA varied from 277.7 to 666.4 mg CTE/L for the Cabernet Sauvignon wines, and from 272.8 to 497.4 mg GAE/L for the Merlot wines. The TFA content of the Cabernet Sauvignon wines in the NXYQY and HBSC regions was almost double that in the SXXN region, and its content in the NXYQY regional Merlot wines was more than 70% that in the HBCL and SXXN regions. In addition, for the two monovarietal wines, there was a non-significant ($p < 0.05$) difference in TFA content between the HBCL and

SXXN regions.

Anthocyanins are an important quality parameter for red grapes because of their significance in determining the colour of the resulting wines. In the present study, the content of TA varied from 261.5 to 400.3 mg Mv/L, with the average value being 330.3 mg Mv/L for the Cabernet Sauvignon wines, and from 157.5 to 350.3 mg Mv/L, with an average value of 246.0 mg Mv/L for the Merlot wines. The TA contents of the two monovarietal wines in the NXYQY region were the highest, and the contents in the HBCL region were the lowest. Furthermore, for the Cabernet Sauvignon wines there were non-significant ($p < 0.05$) differences in TA content between the NXYQY and HBSC regions, as well as between the HBCL and SXXN regions. A high concentration of anthocyanins in wine is essential for good colour, resulting in high-quality wine.

Monomeric anthocyanins are the most labile phenolic compounds in wine, typically decreasing at a rate of about 50% per year (Munoz-Espada *et al.*, 2004). Anthocyanin extraction and stability are affected by winery production practices. Monomeric anthocyanins usually decline during maceration and fermentation, but the process may continue throughout the life of a wine. The characteristics of wine, such as SO₂, pH and acetaldehyde, can influence these processes and anthocyanin interactions with other phenolic compounds. The stability of anthocyanins can be enhanced through so-called co-pigmentation. Co-pigmentation therefore plays a crucial role in wine ageing and maturation. Acylated anthocyanins containing two or more aromatic acyl groups may affect the colour through intramolecular co-pigmentation (Vivar-Quintana *et al.*, 2002).

In order to identify the influence of ecological condition on the phenolic contents of wine samples from the four wine grape-growing regions, all the raw materials (grape berries) and wines were kept under the same conditions, including the same cultivation management and vintage, and the same winemaking techniques and ageing conditions. The results further confirm a variation in phenolic content among the wine samples tested. As is well known, the amounts of phenolic materials vary considerably in different wine grape-growing regions, depending on the grape variety and environmental factors affecting vine growth (Villaño *et al.*, 2006; Shi *et al.*, 2016; Martínez-Gil *et al.*, 2018). The range of data obtained is in agreement with the available literature (Li *et al.*, 2009, 2011).

HPLC analysis of anthocyanin compounds in wine samples

A total of 37 anthocyanins were identified in both the Cabernet Sauvignon and Merlot wines, including five primitive anthocyanins and 32 derivatives (Table 3). The anthocyanin profile of grapes and wine, determined by the relative proportions of the different anthocyanins, are characteristic for each grape variety and corresponding wine. Moreover, the concentrations of different compounds varied significantly within grape cultivars according to environmental conditions (Williams & Graver, 2004; Villaño *et al.*, 2006).

The details of these anthocyanins, listed in Table 3, exhibited discrepancies in the monomeric anthocyanin

TABLE 3
Content (mg Mv/L) and percentage of each anthocyanin in Cabernet Sauvignon and Merlot wines from four different wine grape-growing regions.

Region	Altitude (m) (above sea level)	Climatic characteristics	Climate data			Soil condition			
			Annual accumulated temperature (°C)	∑ Rainfall (mm) (July-September)	Average temperature (°C) (April-September)	Sunshine hours (h) (April-September)	Soil type	Organic matter (g/kg)	pH
NXYQY	1036	Cool and semi-arid climate and a large variation in diurnal temperature	3 298-3 351	85.4	20.6	1 544.3	Gravelly soil	2.45	8.52
SXXN	1100	Cold and arid climate and a large variation in diurnal temperature	2 998	252.0	17.6	1 130.4	Clay loamy soil	9.36	8.17
HBSC	550	Warm, semi-arid climate and a large variation in diurnal temperature	3 940	289.1	19.2	2 534.9	Sandy soil	10.37	7.95
HBCL	214	Cool-warm, semi-humid climate	3 532	407.5	20.5	1 710.8	Clay and sandy soil	8.03	6.51

composition and concentration of the four regional Cabernet Sauvignon and Merlot wines. In the *Vitis vinifera* L. red cultivars there are only Dp, Cy, Pt, Pn and Mv, along with the corresponding acetyl, *p*-coumaryl, caffeoyl, and ferulyl derivatives. Cyanidin is the precursor pigment of the other anthocyanidins, and it can be transformed into peonidin by the action of a 3'-*O*-methyltransferase, or into delphinidin by the action of a 3'-hydroxylase. A 3'-5'-*O*-methyltransferase transforms delphinidin into petunidin, and petunidin into malvidin (Vivar-Quintana *et al.*, 2002). For the five primitive anthocyanins, the four different regional wines had the same composition within grape cultivars; these discrepancies of composition were only found in 14 anthocyanin derivatives, whose concentrations were basically below 10 mg Mv/L (except for Dp-3-*O*-(*cis*-6-*O*-coumaryl)-glucoside and Mv-3-*O*-(6-*O*-acetyl)-glucoside-pyruvic acid). Furthermore, the total content of monomeric anthocyanins was predominant, ranging from 197.1 to 339.6 mg Mv/L and averaging 251.8 mg Mv/L for the Cabernet Sauvignon wines, and from 129.2 to 213.0 mg Mv/L, averaging 182.4 mg Mv/L, for the Merlot wines. Next were the total content of acetylated anthocyanins, which ranged from 72.7 to 170.7 mg Mv/L and averaged 110.8 mg Mv/L for the Cabernet Sauvignon wines, and from 63.1 to 80.8 mg Mv/L, averaging 71.7 mg Mv/L, for the Merlot wines. The third class was the total content of coumarylated anthocyanins, which ranged from 29.9 to 75.1 mg Mv/L and averaged 49.2 mg Mv/L for the Cabernet Sauvignon wines, and from 32.6 to 76.7 mg Mv/L, averaging 48.5 mg Mv/L, for the Merlot wines. Blaga and Aleksandra (2010) detected 13 anthocyanins in Cabernet Sauvignon wines, with the malvidin derivatives that dominated, followed by peonidin-type anthocyanins. In the present study, we also found that malvidin derivatives dominated in all the wine samples, contributing beyond 57.65% (including Mv) to the total anthocyanins in the wines. Heredia *et al.* (1998) showed that the number of methoxyl and hydroxyl groups can affect the intensity and type of colour of anthocyanins: the more methoxyl groups there are, the less the redness; the more hydroxyl groups there are, the more bluish the shade. The contribution of each anthocyanin to the final anthocyanin profile was calculated based on the monoglucoside forms and expressed as a percentage (in parenthesis) in Table 3.

The distribution of the most common anthocyanins in the investigated Cabernet Sauvignon and Merlot wines also depends on the climatic factors of the region and on soil types: Dp was determined from 1.16 to 9.89% (mean 6.27%) in the Cabernet Sauvignon wines and from 3.52 to 9.01% (mean 5.82%) in the Merlot wines; Cy varied from 0.48 to 1.21% (mean 0.82%) in the Cabernet Sauvignon wines and from 0.33 to 0.92% (mean 0.61%) in the Merlot wines; Pt was determined from 4.52 to 8.49% (mean 6.61%) in the Cabernet Sauvignon wines and from 4.82 to 9.39% (mean 6.97%) for the Merlot wines; Pn varied from 2.6 to 5.97% (mean 3.81%) in the Cabernet Sauvignon wines and from 2.25 to 4.65% (mean 3.28%) in the Merlot wines. The most prominent anthocyanin was Mv, which accounted for 40.73% (from 37.12 to 44.34%) and 37.94% (from 31.75 to 44.82%) of the total content in the Cabernet Sauvignon and Merlot wines respectively. This is in agreement with the findings of previous research (Bouzas-Cid *et al.*, 2016; González-Neve

et al., 2016; Jin *et al.*, 2017).

The findings show that Mv was the most abundant anthocyanin in all the investigated wines. By contrast, Cy was the least abundant anthocyanin pigment, as has been demonstrated for a number of other wines (Munoz-Espada *et al.*, 2004; Kallithraka *et al.*, 2007). A similar profile has been reported for Syrah (Gómez-Míguez *et al.*, 2007), but the different patterns were also observed in Cabernet Franc and Pinot Noir wines from British Columbia (Mazza *et al.*, 1999). Another point worth mentioning is that the order of abundance based on average value of distribution if each anthocyanin was as follows: Mv > Mv-3-O-(6-O-acetyl)-glucoside > Pt > Dp > Mv-3-O-(trans-6-O-coumaryl)-glucoside or Pn.

With regard to the acylation of the glycosyl group, the distribution of non-acylated anthocyanins, with a mean of 58.24% (from 50.75 to 65.81%) for the Cabernet Sauvignon wines and 54.61% (from 51.08 to 58.06%) for the Merlot wines made it the most abundant fraction in all wine samples. The mean amount of acetylated anthocyanins was 25.50% (from 25.31 to 30.17%) for the Cabernet Sauvignon wines and 25.15% (from 23.48 to 27.04%) for the Merlot wines, followed by coumarylated anthocyanins at 11.17% (from 9.19 to 12.31%) for the Cabernet Sauvignon wines and 14.25% (from 9.94 to 19.57%) for the Merlot wines. According to the research results, it is obvious that the content of anthocyanin constituents of the monovarietal wines from four different regions differed, which may be related to the thickness of the grape skin and the climate in which the grapes grow (Crippen & Morrison, 1986; Villaño *et al.*, 2006). Environmental factors therefore could affect the biosynthesis and accumulation of each anthocyanin in the skin (Liang *et al.*, 2014). In our study, four regional Cabernet Sauvignon and Merlot wines were made from grapes harvested at different altitudes, with a higher altitude generally corresponding to more intense sunlight, lower temperature, greater temperature difference between day and night, and more extreme environmental conditions (Mateus *et al.*, 2001), all of which are important factors affecting the production of anthocyanins.

Correlation between radical-scavenging activity and phenolic subclasses in wine samples

The antioxidant activity of the wine samples was estimated by the ability of the sample to scavenge the stable DPPH free radicals (Roussis *et al.*, 2005). In the DPPH scavenging assay, antioxidants reacting with DPPH produce yellow α , α -diphenyl- β -picrylhydrazine. The degree of discoloration indicates the radical-scavenging activity of the antioxidant (Prasad *et al.*, 2010).

The free radical-scavenging activity of all the wine samples was determined by the DPPH methods, as shown in Table 2. All wines showed a higher DPPH radical-scavenging activity. For DPPH, the values of wines varied from 4 670.4 to 6 179.4 μ M TE/L for the Cabernet Sauvignon wines, and 3 857.0 to 5 304.1 μ M TE/L for the Merlot wines. The range of the data obtained is in agreement with a previous study (Jin *et al.*, 2017). The values of DPPH free radicals decreased in the order XNYQY > HBSC > SXXN > HBCL in the Cabernet Sauvignon wines and XNYQY > HBSC >

HBCL > SXXN in the Merlot wines, but the antioxidant activities of the two monovarietal wines in the SXXN and HBCL regions were non-significant ($p < 0.05$). The results of this investigation show that the higher the concentration of antioxidant, the lower the amount of remaining DPPH free radicals and the higher the free radical-scavenging activity. The percentage of DPPH radical-scavenging activity against the content of total anthocyanins, phenolics, flavonoids and flavanols of the wine samples is plotted in Fig. 2.

The different linear correlations between groups of phenolic compounds and DPPH free radical-scavenging ability of the tested wines were verified. The significant correlation was obtained between DPPH free radical-scavenging ability and TP content (Fig. 2 (A), $r^2 = 0.8360$, $p < 0.01$), TFO (Fig. 2 (B), $r^2 = 0.8457$, $p < 0.01$) and TA (Fig. 2 (D), $r^2 = 0.7254$, $p < 0.01$). There also was a linear correlation, between DPPH scavenging ability and TFA (Fig. 2 (C), $r^2 = 0.5937$, $p < 0.01$).

It is important to determine which group of phenolic compounds is more significant in the antioxidant activities of wines. Our results suggest that the amounts of TP, TFO and TA are very important for the antioxidant potency of the tested wines. However, TFA showed a weaker correlation, and these results are partially in agreement with previous reports in the literature (Minussi *et al.*, 2003; Fernández-Pachón *et al.*, 2006; Cimino *et al.*, 2007). Furthermore, the difference may be dependent on the grape varieties of the tested wine samples and the complexity of the antioxidant reaction.

CONCLUSIONS

According to the results, it can be concluded that the amounts of phenolic materials and radical-scavenging activity varied considerably in the four regional Cabernet Sauvignon and Merlot wines, depending on the grape variety and the environmental factors affecting vine growth. The content of phenolic subclasses and radical-scavenging activities from the XNYQY regional wines were significantly higher than those of the other three regions tested, followed by the HBSC region. Meanwhile, a significant correlation was observed between radical-scavenging activity and phenolic subclasses (the TP, TFO and TA) for all the wine samples. The amount of phenolic subclasses is important for understanding the antioxidant potency of red wines. The mechanism by which phenolic subclasses are absorbed and metabolised in the body is currently unclear. On the other hand, their composition and concentration of anthocyanins detected in these regional Cabernet Sauvignon and Merlot wines showed discrepancies. The main components of anthocyanins, the percentage of malvidin-3-O-glucoside and its derivatives to the total content showed differences within grape cultivars from the different regional wines. Those monomeric anthocyanins that did not occur simultaneously in the four regional single-cultivar wines had concentrations below 10 mg Mv/L, except for Dp-3-O-(cis-6-O-coumaryl)-glucoside and Mv-3-O-(6-O-acetyl)-glucoside-pyruvic acid. It can be concluded that this information could be used as a biochemical marker for the authenticity of single-cultivar red wines that are produced in the above four regions (or terroirs).

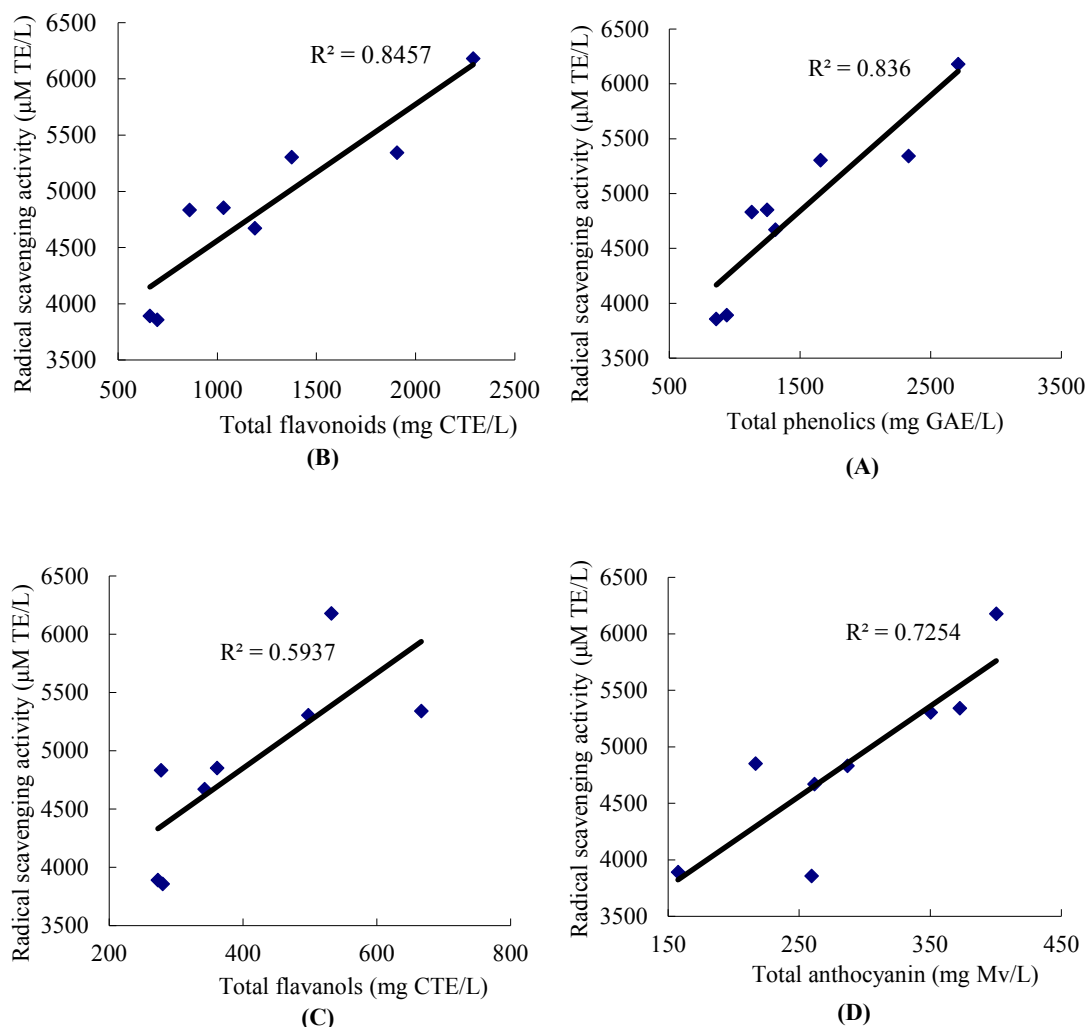


FIGURE 2

Relationship between radical-scavenging activity (RSA) and the content of phenolic subclasses in selected two monovarietal wine samples. (A) Relationship between RSA and total phenolics; (B) Relationship between RSA and total flavonoids; (C) Relationship between RSA and total flavanols; (D) Relationship between RSA and total anthocyanins.

LITERATURE CITED

- Bajda, E., 2007. Lake County seeks to elevation high altitude wines [Online]: <http://www.theelevationofwine.org> [Accessed 20th December, 2009].
- Blaga, R. & Aleksandra, R., 2010. Free radical scavenging activity and anthocyanin profile of Cabernet Sauvignon wines from the Balkan region. *Molecules* 15, 4213-4226.
- Bouzas-Cid, Y., Portu, J., Pérez-Álvarez, E.P., Gonzalo-Diago, A. & Garde-Cerdán, T., 2016. Effect of vegetal ground cover crops on wine anthocyanin content. *Sci. Hortic. Amsterdam* 57, 74-83.
- Brand-Williams, W., Cuvelier, M.E. & Berset, C., 1995. Use of a free-radical method to evaluate antioxidant activity. *LWT-Food Sci. Technol.* 28, 25-30.
- Cheng, G., He, Y.N., Yue, T.X., Wang, J. & Zhang, Z.W., 2014. Effects of climatic conditions and soil properties on Cabernet Sauvignon berry growth and anthocyanin profiles. *Molecules* 19, 13683-13703.
- Cimino, F., Sulfaro, V., Trombetta, D., Saija, A. & Tomaino, A., 2007. Radical-scavenging capacity of several Italian red wines. *Food Chem.* 103, 75-81.
- Cortell, J.M. & Kennedy, J.A., 2006. Effect of shading on accumulation of flavonoid compounds in (*Vitis vinifera* L.) Pinot Noir fruit and extraction in a model system. *J. Agric. Food Chem.* 54, 8510-8520.
- Crippen, D.D. & Morrison, J.C., 1986. The effects of sun exposure on the compositional development of Cabernet Sauvignon berries. *Am. J. Enol. Vitic.* 37, 235-247.
- Downey, M.O., Dokoozlian, N.K. & Krstic, M.P., 2006. Cultural practice and environmental impacts on the flavonoid composition of grapes and wine: A review of recent research. *Am. J. Enol. Vitic.* 57, 257-268.
- Fernández-Pachón, M.S., Villaño, D., Troncoso, A.M. & Garcia-Parrilla, M.C., 2006. Determination of the phenolic composition of sherry and table white wines by liquid chromatography and their relation with antioxidant activity. *Anal. Chim. Acta* 563, 101-108.
- Gómez-Míguez, M., González-Miret, M.L. & Heredia, F.J., 2007. Evolution of colour and anthocyanin composition of Syrah wines elaborated with pre-fermentative cold maceration. *J. Food Eng.* 79, 271-278.
- Giusti, M.M. & Wrolstad, R.E., 2001. Characterization and measurement of anthocyanins by UV-visible spectroscopy. In: *Current protocols in food analytical chemistry*. Wiley, New York. pp. 577 – 590.
- Gómes-Plaza, E., Minano, A. & Lopez-Roca, J.M., 2006. Comparison of chromatic properties, stability and antioxidant capacity of anthocyanin-

- based aqueous extracts from grape pomace obtained from different vinification methods. *Food Chem.* 97, 87-94.
- Gómez-Alonso, S., Fernández-González, M., Mena, A., García Romero, E. & Martínez, J., 2007. Anthocyanin profile of Spanish *Vitis vinifera* L. red grape varieties in danger of extinction. *Aust. J. Grape Wine Res.* 13, 150-156.
- González-Neve, G., Favre, G., Piccardo, D. & Gil, G., 2016. Anthocyanin profile of young red wines of Tannat, Syrah and Merlot made using maceration enzymes and cold soak. *Int. J. Food Sci. Technol.* 51, 260-267.
- He, J.J., Liu, Y.X., Pan, Q.H., Cui, X.Y. & Duan, C.Q., 2010. Different anthocyanin profiles of the skin and the pulp of Yan73 (Muscat Hamburg × Alicante Bouschet) grape berries. *Molecules* 15, 1141-1153.
- Heredia, F.J., Francia-Aricha, E., Rivas-Gonzalo, J.C., Vicario, I.M. & Santos-Buelga, C., 1998. Chromatic characterization of anthocyanins from red grapes. I. pH effect. *Food Chem.* 63, 491-498.
- Hou, D.X., 2003. Potential mechanisms of cancer chemoprevention by anthocyanins. *Curr. Mol. Med.* 3, 149-159.
- Jin, X.D., Wu, X. & Liu, X., 2017. Phenolic characteristics and antioxidant activity of Merlot and Cabernet Sauvignon wines increase with vineyard altitude in a high-altitude region. *S. Afr. J. Enol. Vitic.* 38, 132-143.
- Kähkönen, M.P. & Heinonen, M., 2003. Antioxidant activity of anthocyanins and their aglycons. *J. Agric. Food Chem.* 51, 628-633.
- Kallithraka, S., Mohdaly, A.A., Makris, D.P. & Kefalas, P., 2007. Determination of major anthocyanin pigment in Hellenic native grape varieties (*Vitis vinifera* sp.): Association with antiradical activity. *J. Food Compos. Anal.* 18, 375-386.
- Kim, D.O., Chun, O.K., Kim, Y.J., Moon, H.Y. & Lee, C.Y., 2003. Quantification of polyphenolics and their antioxidant capacity in fresh plums. *J. Agric. Food Chem.* 51, 6509-6515.
- Li, H., 2002. Viticulture and practices. In: College of Enology (ed.). Research progress of vine and wine. Shaanxi Agricultural Press, Xi'an. pp. 57 – 72.
- Li, H., Wang, X.Y., Li, Y., Li, P.H. & Wang H., 2009. Polyphenolic compounds and antioxidant properties of selected China wines. *Food Chem.* 112, 454-460.
- Li, Y.G., Tanner, G. & Larkin, P., 1996. The DMACA-HCl protocol and the threshold proanthocyanidin content for bloat safety in forage legumes. *J. Sci. Food Agr.* 70, 89-101.
- Li, Z., Pan, Q.H., Jin, Z.M., Mu, L. & Duan, C.Q., 2011. Comparison on phenolic compounds in *Vitis vinifera* cv. Cabernet Sauvignon wines from five wine-growing regions in China. *Food Chem.* 125, 77-83.
- Liang, N.N., Zhu, B.Q., Han, S., Wang, J.H., Pan, Q.H., Reeves, M.J. & Duan, C.Q., 2014. Regional characteristics of anthocyanin and flavonol compounds from grapes of four *Vitis vinifera* varieties in five wine regions of China. *Food Res. Int.* 64, 264-274.
- Lila, M.A., 2004. Anthocyanins and human health: An in vitro investigative approach. *J Biomed. Biotechnol.* 5, 306-313.
- Liu, Y., Yan, J., Li, Q., Wang, J. & Shi, Y., 2018. Effect of training systems on accumulation of flavan-3-ols in Cabernet Sauvignon grape seeds at the North Foot of Mt. Tianshan. *S. Afr. J. Enol. Vitic.* 39, 35-46.
- Martínez-Gil, A.M., Gutiérrez-Gamboa, G., Garde-Cerdán, T., Pérez-Álvarez, E.P. & Moreno-Simunovic, Y., 2018. Characterization of phenolic composition in Carignan noir grapes (*Vitis vinifera* L.) from six wine-growing sites in Maule Valley, Chile. *J. Sci. Food Agric.* 98, 274-282.
- Mateus, N., Marques, S., Goncalves, A.C., Machado, J.M. & De Freitas, V., 2001. Proanthocyanidin composition of red *Vitis vinifera* varieties from the Douro valley during ripening: Influence of cultivation elevation. *Am. J. Enol. Vitic.* 52, 115-121.
- Mazza, G., Fukumoto, L., Delaquis, P., Girard, B. & Ewert, B., 1999. Anthocyanins, phenolics, and color of Cabernet Franc, Merlot, and Pinot Noir wines from British Columbia. *J. Agr. Food Chem.* 47, 4009-4017.
- Minussi, R.C., Rossi, M., Bologna, L., Cordi, L., Rotilio, D., Pastore, G.M. & Duran, N., 2003. Phenolic compounds and total antioxidant potential of commercial wines. *Food Chem.* 82, 409-416.
- Monages, M., Nunez, V., Bartolome, B. & Gomez-Cordoves, C., 2003. Anthocyanin-derived pigments in Graciano, Tempranillo, and Cabernet Sauvignon wines produced in Spain. *Am. J. Enol. Vitic.* 54, 163-169.
- Morrrough, M.I., Madigan, D. & Smyth, M.R., 1996. Semipreparative chromatographic procedure for the isolation of dimeric and trimeric proanthocyanidins from barley. *J. Agric. Food Chem.* 44, 1731-1735.
- Munoz-Espada, A.C., Wood, K.V., Bordelon, B. & Watkins, B.A., 2004. Anthocyanin quantification and radical scavenging capacity of Concord, Norton and Marechal Foch grapes and wines. *J. Agric. Food Chem.* 52, 6779-6786.
- Orak, H.H., 2007. Total antioxidant activities, phenolics, anthocyanins, polyphenoloxidase activities of selected red grape cultivars and their correlations. *Sci. Hortic.* 111, 235-241.
- Office International de la Vigne et du Vin (O.I.V.), 2018. Recueil des Méthodes Internationales D'analyse des vins et des moûts (Vol. 2). Paris. O.I.V.
- Prasad, K.N., Yang, B., Shi, J., Yu, C.Y., Zhao, M.M., Xue, S. & Jiang, Y.M., 2010. Enhanced antioxidant and antityrosinase activities of longan fruit pericarp by ultra-high-pressure-assisted extraction. *J. Pharmaceut. Biomed.* 51, 471-477.
- Rapisarda, P., Tomaino, A., Cascio, L.R., Bonina, F., Pasquale, D.A. & Saija, A., 1999. Antioxidant effectiveness as influenced by phenolic content of fresh orange juices. *J. Agric. Food Chem.* 47, 4718-4723.
- Roussis, I.G., Lambropoulos, I. & Soutli, K., 2005. Scavenging capacities of some wines and wine phenolic extracts. *Food Technol. Biotech.* 43, 351-358.
- Shi, P.B., Yue, T.X., Ai, L.L., Cheng, Y.F., Meng, J.F., Li, M.H. & Zhang, Z.W., 2016. Phenolic compound profiles in grape skins of Cabernet Sauvignon, Merlot, Syrah and Marselan cultivated in the Shacheng area (China). *S. Afr. J. Enol. Vitic.* 37, 132-138.
- Shirahigue, L.D., Plata-Oviedo, M., Alencar, D.S.M., Darce, M., Souza, T.M.F., Oldoni, T.L.C. & Contreras-Castillo, C.J., 2010. Wine industry residue as antioxidant in cooked chicken meat. *Int. J. Food Sci. Tech.* 45, 863-870.
- Tang, K., Liu, T., Han, Y., Xu, Y. & Li, J.M., 2017. The importance of monomeric anthocyanins in the definition of wine colour properties. *S. Afr. J. Enol. Vitic.* 38, 1-10.
- Villaño, D., Fernandez-Pachon, M.S., Troncoso, A.M. & Garcia-Parrilla, M.C., 2006. Influence of enological practices on the antioxidant activity of wines. *Food Chem.* 95, 394-404.
- Vivar-Quintana, A.M., Santos-Buelga, C. & Rivas-Gonzalo, J.C., 2002. Anthocyanin-derived pigments and colour of red wines. *Anal. Chim. Acta* 458, 147-155.
- Wang, X.Q., Xie, X.L., Chen, N., Wang, H. & Li, H., 2018. Study on current status and climatic characteristics of wine regions in China. *Vitis* 57, 9-16.
- Williams, C.A. & Graver, R.J., 2004. Anthocyanins and other flavonoids. *Nat. Prod. Rep.* 21, 539-573.
- Yilmaz, Y. & Toledo, R.T., 2004. Health aspects of functional grape seed constituents. *Trends Food Sci. Tech.* 15, 422-433.