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Research Article
Phenolic Compounds and Antioxidant Activity of Wines Fermented Using Ten Blueberry Varieties

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Abstract

Objective: The aim of this study was to compare the phytochemical characteristics and *in vitro* antioxidant activities of blueberry wines of 10 widely grown blueberry cultivars and further study the effect of bioactive composition on antioxidant activity. **Methodology:** The blueberry wines of 10 blueberry cultivars from China were analyzed for their chemical composition and antioxidant activity. 'Gardenblue' wine possessed the highest content of total phenolic and tannins and the highest antioxidant activity than any other blueberry wine. 'Britewell' wine was the darkest and most purple shade of red corresponding to the highest Total Anthocyanin Value (TAC). **Results:** Variance analysis showed significant differences ($p<0.05$) in phenolic compounds (Total phenolics, anthocyanins and tannins) and antioxidant activities by DPPH, FRAP and reducing power assays among 10 blueberry wines. Correlation analysis revealed that total phenolics and tannins were distinctly responsible for the antioxidant capacity (DPPH, FRAP and reducing power). **Conclusion:** Among 10 blueberry wines, 'Gardenblue' wine can be considered as fruit wines with abundant phenolic compounds and antioxidant activities.

Key words: Blueberry wine, fermentation characteristics, antioxidants, phenolic compounds

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Competing Interest: The authors have declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Blueberry (genus *Vaccinium*, family Ericaceae) originates from North America and Europe[1]. Blueberry fruits with a dark-blue color possess a sour-sweet taste and are commonly consumed fresh or processed into products such as frozen pulp, juice, wine and jam[2]. Blueberries are not only rich in monosaccharides, amino acids, organic acids, dietary fiber, vitamins and trace elements but also contain a high contents bioactive polyphenols with potential health benefits[3-6]. So, they are a suitable raw material for wine-making[7-9].

As a health-promoting product consumed moderately, wine is an important source of natural antioxidants due to its rich phenolic compounds, including anthocyanins[10-12]. In general, the berry wine-making process is the same as making wine from grapes. Phenolic-rich compounds from berries may effectively be extracted into wines during wine processing[13]. Blueberry wines were rich in phenolic compounds including anthocyanin and thus possess high antioxidant activity[14,15]. Phenolic compounds also contributes to wines organoleptic characteristics, such as color, flavor, bitterness and astringency[16-18]. The phenolic composition and content in different wines is particularly bound up with fruit varieties. Different blueberry cultivars vary greatly in their phenolic compounds as well as antioxidant capacity[19,20].

The phenolic compounds and antioxidant capacity in berry wines were influenced by fermentation method and berry cultivars[21-23]. In this study, the chemical composition and antioxidant activity of 10 different varieties blueberry wines were evaluated and compared. The aim of this study was to compare the phytochemical characteristics and *in vitro* antioxidant activities of blueberry wines of 10 widely grown blueberry cultivars and further study the effect of bioactive composition on antioxidant activity. These results can help to identify the optimum blueberry cultivars for growth in this commercial blueberry production region and for the production of top quality fruit wine.

MATERIALS AND METHODS

Blueberry preparation and winemaking: The blueberries were handpicked in the morning and selected based on the uniformity of the size from Hefei (31°52'N, 117°17'E) blueberry plantation located in the central-eastern of China. Baldwin, Gardenblue, Britewell, Anna, O'Neal, Misty, Sharpblue, Bluecrop, Elliott, Brigitta were harvested from June 11, 2014 to August 15, 2014. At these times, berries were at their physiological maturity. All fruits samples were stored at -20°C until utilized.

Blueberries were crushed into mash and then treated at 16°C for 24 h with pectinase (0.03 g kg^{-1}, Laffort, Sydney, Austrilia) and potassium metabisulphite (60 mg kg^{-1}), then a part of these mashes were sampled for analysis (Table 1) and other mashes were adjusted to 20 °Brix with food-grade pure sucrose and inoculated *S. cerevisiae* (Zymaflore F15, Laffort, Sydney, Austrilia) of 0.25 g kg^{-1}. Alcoholic fermentation was carried out at 25°C and ended when the residual sugar content was below about 10.0 g L^{-1}, which took place about 6-8 days period and density controls were maintained during this period. Separation of the wine pomace was performed at the end of alcoholic fermentation and 60 mg kg^{-1} of potassium metabisulphite were added and then the wine samples were aged at 16°C for 6 months until analyzed. Wine making process flow path chart was presented in Fig. 1.

Chemical reagents: Folin Ciocalteu reagent, gallic acid, gallotannic acid and ascorbic acid were obtained from

Table 1: Quality parameters including alcoholicity, dry extract, volatile acid, TSS, pH and RS of 10 different varieties blueberry wines

	Oenological parameters						
	Fermentation 0 day		Six months of bottle aging				
Varieties	pH	TSS (°Brix)	pH	RS (g L^{-1})	Alcoholicity (v/v, %)	Dry extract (g L^{-1})	Volatile acid (g L^{-1})
O'Neal	3.46 ± 0.13^{gh}	10.8 ± 0.4^{ab}	3.43 ± 0.05^{ab}	6.83 ± 0.18^{abc}	12.0 ± 0.1^{c}	18.97 ± 0.1^{c}	0.79 ± 0.03^{c}
Anna	3.60 ± 0.06^{h}	14.8 ± 0.2^{f}	3.43 ± 0.07^{ab}	6.80 ± 0.17^{abc}	11.3 ± 0.2^{a}	19.00 ± 0.3^{c}	1.05 ± 0.05^{b}
Misty	3.07 ± 0.12^{ef}	10.7 ± 0.5^{ab}	3.32 ± 0.02^{a}	7.03 ± 0.22^{c}	12.1 ± 0.3^{c}	18.77 ± 0.3^{a}	1.06 ± 0.01^{b}
Sharpblue	3.36 ± 0.05^{g}	13.2 ± 0.3^{e}	3.34 ± 0.02^{ab}	6.73 ± 0.16^{abc}	11.5 ± 0.1^{ab}	19.07 ± 0.1^{d}	1.01 ± 0.04^{b}
Bluecrop	2.74 ± 0.10^{abc}	10.1 ± 0.2^{a}	2.93 ± 0.04^{ab}	6.92 ± 0.07^{bc}	11.8 ± 0.2^{bc}	18.88 ± 0.1^{b}	1.08 ± 0.04^{ab}
Elliott	2.59 ± 0.12^{a}	12.3 ± 0.3^{d}	2.84 ± 0.01^{ab}	6.79 ± 0.03^{abc}	11.5 ± 0.1^{ab}	19.01 ± 0.2^{c}	0.86 ± 0.03^{c}
Brigitta	2.60 ± 0.13^{ab}	11.4 ± 0.5^{bc}	2.87 ± 0.02^{b}	6.47 ± 0.32^{a}	12.0 ± 0.4^{c}	19.33 ± 0.7^{f}	1.16 ± 0.01^{a}
Baldwin	2.87 ± 0.13^{cd}	12.0 ± 0.6^{cd}	3.05 ± 0.01^{ab}	6.78 ± 0.27^{abc}	12.2 ± 0.2^{c}	19.02 ± 0.3^{cd}	1.00 ± 0.03^{b}
Gardenblue	2.96 ± 0.06^{de}	14.6 ± 0.3^{f}	3.28 ± 0.03^{ab}	6.62 ± 0.17^{ab}	11.5 ± 0.3^{ab}	19.18 ± 0.4^{e}	0.85 ± 0.03^{c}
Britewell	3.15 ± 0.10^{f}	$13.1\pm0.3_{e}$	3.19 ± 0.04^{ab}	6.91 ± 0.13^{bc}	11.5 ± 0.3^{ab}	18.89 ± 0.2^{b}	0.85 ± 0.03^{c}

Significance testing among the different samples was performed by one-way ANOVA followed by Duncan's range test. Different superscripts between rows represent significant differences between samples ($p<0.05$), TSS: Total soluble solids, RS: Residual sugars

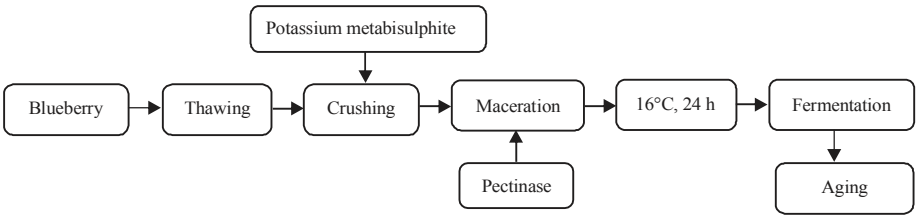

Fig. 1: Wine making process flow path chart

Sinopharm Chemical Reagent (Shanghai, China). The 2, 2-diphenyl-1-picrylhydrazyl (DPPH) and 2,4,6-tris (2-pyridyl)-1, 3,5-triazine (TPTZ) were purchased from Sigma-Aldrich (St. Louis, MO, USA). Sucrose is food-grade pure (≥99.5%) and other general chemicals with analytical grade were obtained from local suppliers.

Standard analysis of wines: The alcoholicity, dry extract, volatile acidity, Total Soluble Solids (TSS), pH and Residual Sugars (RS) were analyzed according to the methods proposed by the National Standard of the People's Republic of China[24].

Chromatic characteristics: Color was determined with ColorQuest XE (Hunter Associates Laboratory, Inc. Reston, VA, USA) (the 10° Standard Observer and Standard Illuminant D65), according to the OIV recommendations[25]. The color characteristics were L* (lightness, ranging from 0 black to 100 white), a* (positive values for the direction of redness and negative values for the direction of the complement green) and b* (positive for yellowness and negative for blueness). The values a* and b* were used to calculate the hue angle [H = arctan (b*/a*)].

Total Anthocyanin Content (TAC): The TAC was estimated using the pH differential method. Briefly, each sample was diluted with pH 1.0 and 4.5 buffers to attain the same dilution. The absorbance was measured at 510 and 700 nm in both pH 1.0 and 4.5 buffers. Then, the TAC was calculated using the following equation:

$$A = (A_{510}\text{-}A_{700})_{pH\ 1.0}\text{-}(A_{510}\text{-}A_{700})_{pH\ 4.5} \quad (1)$$

$$TAC = \frac{(A \times MW \times DF \times V_e \times 1000)}{\varepsilon \times 1 \times M} \quad (2)$$

where, MW is the molecular weight of cyanidin-3-glucoside (449 g mol^{-1}), DF is the dilution factor, V_e is the extract volume, ε is the molar extinction coefficient of cyanidin-3-glucoside (29,600) and M is the mass of the berries extracted. The results were expressed as milligram cyanidin-3-glucoside (C3G) equivalents per liter of blueberry wine (mg C3G L^{-1}).

Total Phenolic Content (TPC): The TPC was determined according to Folin Ciocalteu method[26] with some modifications. The absorbance of the sample was determined at 765 nm. The equation obtained for the calibration curve of gallic acid (20-180 mg L^{-1}) was Y = 0.0047X+0.0909 (r = 0.9996). The results were expressed as milligram Gallic Acid Equivalents (GAE) per liter of blueberry wine (mg GAE L^{-1}).

Total Tannin Content (TTC): The TTC was measured by Folin Denis method[27]. The absorbance was measured at 700 nm. The equation obtained for the calibration curve of gallotannic acid (0.2-130 mg L^{-1}) was Y = 0.0236X+0.65 (r = 0.9995). The results were expressed as milligram Gallotannic Acid (GA) equivalent per liter of blueberry wine (mg GA L^{-1}).

DPPH assay: The DPPH free radical-scavenging capacity was estimated using the following method. About 2.95 µL of 0.1 mM DPPH methanolic solution was added to 50 µL of the sample extracts. The mixture was thoroughly mixed and kept in the dark for 30 min. The absorbance of the reaction mixture was measured at 517 nm. The results were expressed in milligram vitamin C equivalent antioxidative capacity per liter of blueberry wine (mg VC L^{-1}).

Ferric Reducing Antioxidant Power (FRAP) assay: The FRAP assay was carried according to the procedure described. The FRAP reagent was prepared in acetate buffer (adjusted pH to 3.6 by acetic acid), TPTZ solution (10 mM in 40 mM HCl) and 20 mM iron (III) chloride solution in the proportion of 10:1:1 (v/v), respectively. The reagent was prepared fresh daily, each sample (90 µL) was mixed with 3.0 mL of the FRAP reagent and incubated for 10 min at 37°C. The absorbance was read at 593 nm. The results were expressed as milli mole ferrous ion per liter of blueberry wine (mmol Fe^{2+} L^{-1}).

Reducing Power (RP) assay: The RP of sample was determined referring to the method. The diluted samples (1 mL) were added into phosphate buffer (2.5 mL 0.2 M, pH 6.6) and potassium ferricyanide (2.5 mL, 1%). The mixture was incubated (50°C, 20 min), 5 mL of 10% trichloroacetic acid was added to the mixture, then centrifuged for 10 min at 3000 rpm and 2.5 mL aliquot of the supernatant was mixed with ultrapure water (2.5 mL) and 0.5 mL of 0.1% $FeCl_3$. The absorbance was measured at 700 nm after standing for 2 min, the final result was thus expressed as milligram vitamin C equivalent per liter of blueberry wine (mg VC L^{-1}).

Statistical analysis: Data were expressed as the Means±Standard Deviation (SD) of triplicate determinations. Mean differences were determined by one-way ANOVA followed by Tukey's test using Prism™ v6.0 software. The differences were considered significant when $p<0.05$ and denoted by different letters. Linear regression plots were generated and correlations were computed as Pearson's correlation coefficient (r) using Prism™ v6.0 software.

RESULTS AND DISCUSSION

Primary physicochemical parameters of wines fermented with ten blueberry varieties: Several primary physicochemical parameters of 10 blueberry wines are shown in Table 1. Ten blueberry wines had an alcohol strength of 11.3-12.2% and Residual Sugars (RS) ranged from 6.47-7.03 g L^{-1}. Because blueberry mashes were adjusted to 20 °Brix before alcoholic fermentation, so alcohol content of wines were not significant difference. The residual sugar content ranged from 4-12 g L^{-1} in commercial semi-dry wine[28]. The pH ranged from 2.84-3.43 and these results agreed with the pH range for commercial blueberry wines[8] between 2.8 and 3.7. The dry extract averaged around 19.01 g L^{-1} and the differences among 10 blueberry wines were lower than 0.56 g L^{-1}. The dry extract are considered markers for grape content with the legal limit in grape wines[28] exceed to 16 g L^{-1}, volatile acid, expressed as acetic acid, ranged from 0.79-1.16 g L^{-1}. Volatile acid are considered markers for spoilage with the legal limit in grape wines[28] set to 1.2 g L^{-1}.

Phenolic compounds and chromatic characteristics of wines: Phenolic compounds can determine the color quality of fruit wines. Table 2 presents phenolic compounds and chromatic characteristics of 10 wines via 6 months aging. The Total Phenols (TP), total anthocyanins (TAC) and Total Tannins Content (TTC) content ranged from 506.81-1205.11 mg GAE L^{-1}, 41.08-316.44 mg C3G L^{-1} and 16.08-129.51 mg GA L^{-1} in wines, respectively. The L* parameter varied from 15.92-38.43 and H° ranged from 28.33-34.92 in 10 blueberry wines (Table 2).

These blueberry wines showed higher total polyphenol content compared to white wines from grapes with the content[29] of 191-306 mg GAE L^{-1}. The TAC of these blueberry wines (41.08-316.44 mg C3G L^{-1}) were very higher than previous values observed for blueberry wines of 10.71-37.29 mg L^{-1} [8]. Cultivars play a more important role in influencing total phenolics and total anthocyanins in berry wines[23,30]. Cheng *et al.*[31] stated the variability in total phenols, total anthocyanins and total tannins content in various grape species.

The presence of specific anthocyanins in wines produced from berries may provide more color to the finished wine product. The L* value is an indicator of darkening and Hue angle (H°) can be considered as an indicator of browning[32]. The 'Anna' cultivar was characterized by a significantly high lightness (38.43) and the highest H° (34.92) but by the lowest TAC. The 'Britewell' wine was the darkest (15.92) and had the lower H° (29.90) corresponding to the highest TAC value.

Table 2: The TPC, TAC and TTC, L* and H° of bottle-aged (6 months) wines from 10 blueberry cultivars

	Phenolic compounds			Chromatic characteristics	
Cultivars	TPC (mg GAE L^{-1})	TAC (mg C3G L^{-1})	TTC (mg GA L^{-1})	L*	H°
O'Neal	538.72±2.13^{f}	75.98±2.23^{g}	25.29±0.41^{h}	25.01±0.03^{d}	33.68±0.03^{c}
Anna	674.89±4.73^{d}	41.08±1.16^{i}	80.47±0.28^{c}	38.43±0.01^{a}	34.92±0.03^{a}
Misty	784.47±6.07^{c}	139.44±3.50^{e}	77.87±0.63^{d}	19.02±0.02^{j}	33.07±0.03^{d}
Sharpblue	888.72±7.06^{b}	174.09±1.41^{d}	66.02±0.38^{e}	28.64±0.03^{c}	28.33±0.07^{i}
Bluecrop	548.30±3.17^{f}	110.21±1.19^{f}	16.08±0.61^{i}	30.44±0.02^{b}	30.22±0.04^{g}
Elliott	579.15±1.97^{e}	141.94±2.02^{e}	33.01±0.43^{g}	20.37±0.04^{g}	34.40±0.05^{b}
Brigitta	506.81±2.12^{g}	49.26±1.78^{h}	16.08±0.50^{i}	22.09±0.02^{f}	30.02±0.03^{h}
Baldwin	886.60±6.07^{b}	199.13±2.75^{c}	55.87±0.63^{f}	24.48±0.02^{e}	30.48±0.06^{f}
Gardenblue	1205.11±8.06^{a}	210.41±1.73^{b}	129.51±1.00^{a}	19.33±0.03^{h}	31.07±0.08^{e}
Britewell	884.47±6.93^{b}	316.44±1.83^{a}	88.03±0.57^{b}	15.92±0.02^{j}	29.90±0.03^{h}

Significance testing among the different samples was performed by one-way ANOVA followed by Duncan's range test. Different superscripts between rows represent significant differences between samples ($p<0.05$), TPC: Total phenolic content, TAC: Anthocyanin content, TTC: Tannin content, L*: Lightness and H°: Hue angle

Antioxidant capacity of wines: The antioxidant activities estimated by three different assays varied considerably in different final bottled wines, depending on the blueberry variety (Table 3). The DPPH, FRAP and RP were 12.33-41.23 mg VC L^{-1}, 5.29-20.97 mmol Fe^{2+} L^{-1} and 1.69-4.39 mg VC L^{-1}, respectively (Table 3). There were obvious differences in DPPH among 10 cultivars ($p<0.05$). 'Gardenblue' displayed the highest FRAP in these cultivars, followed by 'Misty' and 'Anna', respectively. Similar to the FRAP value, the highest DPPH and the highest RP were found in 'Gardenblue' cultivar, followed by the 'Anna' cultivars. The FRAP of 10 blueberry wines was similar to the blackberry wine with the FRAP ranged[33] from 7.8-15.8 mmol L^{-1}. Many studies showed the antioxidant activities of berry wines were primarily influenced by berry cultivars[8,13,14,23,29].

Correlation between the phenolic compounds and antioxidant capacity of wines: Figure 2 shows that a good correlation between TPC and antioxidant capacity of the wines was noted (DPPH, r = 0.714, RP, r = 0.828), TTC showed very good correlations with DPPH values (r = 0.742) and with RP values (r = 0.849) and the weaker correlation was obtained

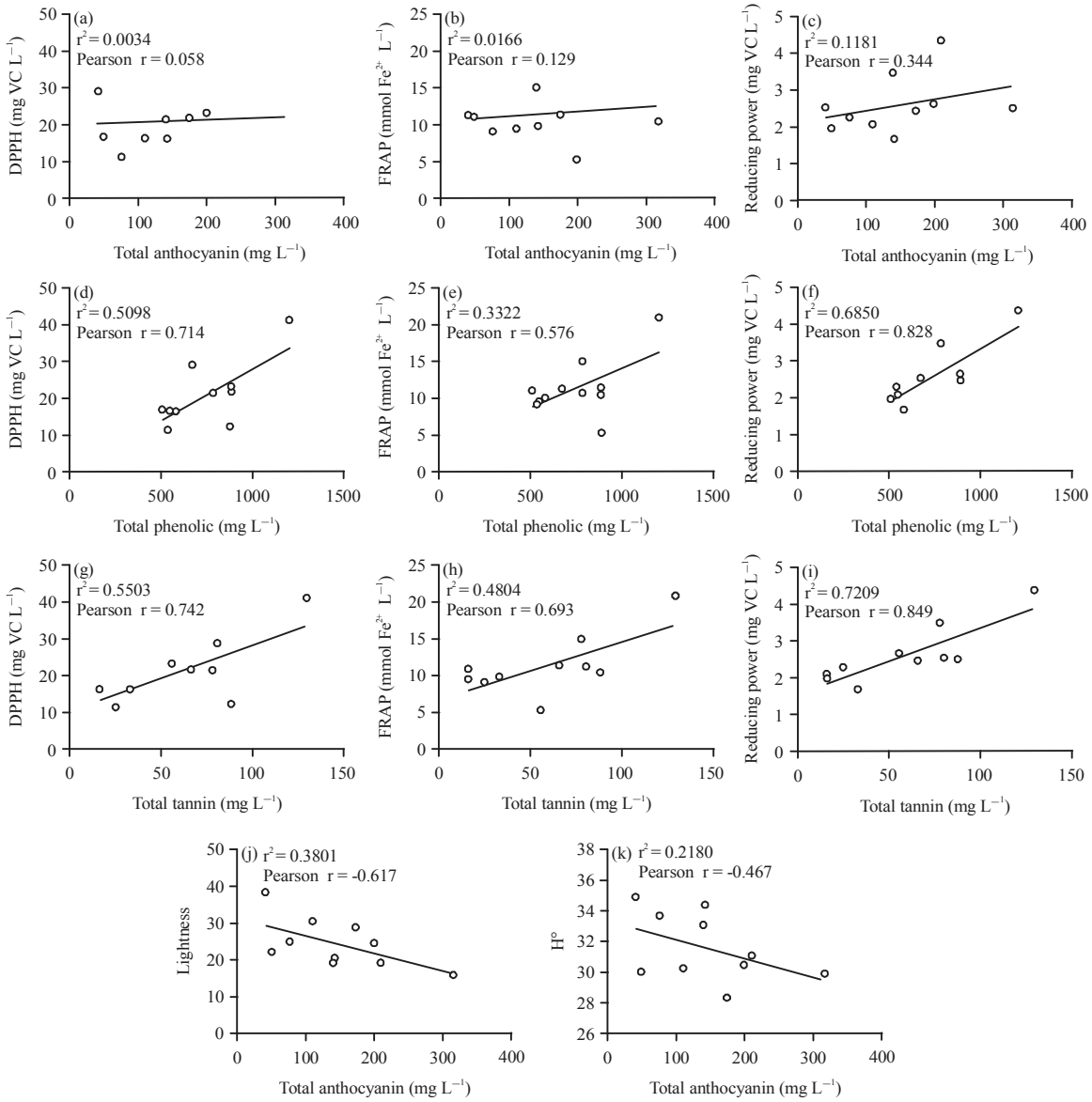

Fig. 2(a-k): Pearson's correlation coefficients ($p<0.05$) between free radical scavenging capacity (DPPH), Ferric Reducing Antioxidant Power (FRAP), Reducing Power (RP) and Total Anthocyanin Content (TAC), phenolic content (TPC), tannin content (TTC), lightness (L*) and hue angle (H°) of bottle-aged (6 months) wines from ten blueberry cultivars

Table 3: Free radical scavenging capacity (DPPH), ferric reducing antioxidant power (FRAP) and reducing power (RP) from 10 blueberry wines

Cultivars	DPPH (mg VC L^{-1})	FRAP (mmol Fe^{2+} L^{-1})	RP (mg VC L^{-1})
O'Neal	11.30±0.50^{e}	9.07±0.41^{f}	2.27±0.11^{d}
Anna	28.98±1.60^{b}	11.29±0.52^{c}	2.55±0.02^{c}
Misty	21.44±0.90^{c}	15.03±0.51^{b}	3.50±0.02^{b}
Sharpblue	21.67±0.10^{c}	11.43±0.55^{c}	2.47±0.02^{c}
Bluecrop	16.34±0.80^{d}	9.51±0.43^{ef}	2.08±0.02^{de}
Elliott	16.27±0.10^{d}	9.89±0.37^{def}	1.69±0.01^{f}
Brigitta	16.83±0.09^{d}	10.98±0.13^{cd}	1.98±0.03^{e}
Baldwin	23.22±0.45^{c}	5.29±0.22^{g}	2.65±0.12^{c}
Gardenblue	41.23±1.08^{a}	20.97±0.11^{a}	4.39±0.12^{a}
Britewell	12.33±0.39^{e}	10.49±0.28^{cde}	2.52±0.03^{c}

Significance testing among the different samples was performed by one-way ANOVA followed by Duncan's range test. Different superscripts between rows represent significant differences between samples ($p<0.05$)

between TAC and the three measures of antioxidant capacity (DPPH, r = 0.058, FRAP, r = 0.129, RP, r = 0.344).

The TPC and antioxidant capacity had a good correlation in wines made with fruits[8,9,34]. Present results are similar to previous reports that the anthocyanins had lower impact in antioxidant capacity in comparison with the tannins in tropical highland blackberries (*Rubus adenotricus*)[14,23,35]. The rabbiteye cultivar 'Gardenblue' possessed the highest content of phenolic compounds (phenolics and tannins), corresponding to the highest value of antioxidant activity (DPPH, FRAP and RP). Figure 2 shows the correlation analyses found there was a negative correlation between the lightness and TAC (r = - 0.617) and the Hue angle was also negatively correlated with TAC for10 blueberry wines (r = - 0.467), corroborating the results in berry wines of other authors[8,36,37].

CONCLUSION

The present study demonstrated that wines from 10 blueberry cultivars widely grown in China possessed significantly different phenolic compounds and antioxidant activities. All 10 wines belong to semi-dry wine and their fermentation characteristics are considered acceptable in wine. Britewell wine was the darkest and most purple shade of red corresponding to the highest TAC value. Correlation analysis revealed that total phenolics and tannins were distinctly responsible for the antioxidant capacity (DPPH, FRAP and Reducing power). Among 10 blueberry wines, 'Gardenblue' wine possessed both the highest content of total phenolic and tannins and the highest antioxidant activity, indicating that this wine could provide an important dietary source of phytochemicals comprising a host of antioxidant polyphenols in this commercial blueberry production region.

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