



EFFECT OF PHENOLIC COMPOUNDS ON ANTIOXIDANT ACTIVITY IN 8 BLUEBERRY (*VACCINUM SPP.*) JUICES

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ABSTRACT

Phenolic compounds of juices from 8 blueberry cultivars, Baldwin, Gardenblue, Anna, O'Neal, Misty, Bluecrop, Elliott and Brigitta, were evaluated. Antioxidant capacity (by DPPH, FRAP, and reducing power assays) of 8 juices were also investigated. Eight blueberry juices contained phenolics levels of 597.23–1417.45 mg gallic acid equivalents/L juice, flavonoids levels of 102.80–546.25 mg rutin equivalents/L juice and anthocyanins levels of 138.60–492.62 mg cyanidin-3-glucoside equivalents/L juice, respectively. Among these juices, the juice from 'Gardenblue' cultivar possessed the highest content of phenolics, flavonoids, and anthocyanins. Variance analysis showed significant differences ($P < 0.05$) in phenolic compounds (phenolics, flavonoids, anthocyanins) and antioxidant activities among 8 blueberry juices. Correlation analyses revealed phenolics, flavonoids, and anthocyanins were distinctly responsible for antioxidant capacity. A strong correlations was found between flavonoids and antioxidant capacity of 8 cultivars (DPPH, $r = 0.95$; FRAP, $r = 0.97$; reducing power, $r = 0.89$).

1. Introduction

Blueberry (genus *Vaccinium*, family Ericaceae) originates from North America and Europe (Rimando et al., 2004). Blueberries not only contain essential nutrients but also possess abundant phenolic compounds, such as anthocyanins, flavonoids and chlorogenicacids (Prior et al., 1998; Manach et al., 2005; Giovanelli et al., 2013). The phenolic compounds can protect organisms against oxidative stress induced by free radicals (Manach et al., 2005) and exhibit a wide range of biological properties, including cardioprotective, anti-inflammatory, and anticarcinogenic properties (Nohynek et al., 2006; Tsuda, 2012).

Blueberry fruits are commonly consumed in fresh or processed food, such as juice, wine, jam and so on (Anna and Grzegorz, 2015; Nindo et al., 2005). Processed blueberry juice not only increases the berries' commercial life but also allows wider consumer access to this diet-enriching food. Blueberry juice with higher amounts of phenolic compounds and stronger antioxidant capacity has increased nutritional and health benefits for the consumer (Nindo et al., 2005; Rossi et al., 2003). However, processing method and blueberry cultivars vary greatly in their phenolic compound content as well as antioxidant capacity (Prior et al., 1998; Sapers et al., 1984; Rodrigues et al., 2011;

Wang et al., 2012a; Wang et al., 2012b; Xin et al., 2015). Previous researches mainly focused on phenolic compound content and antioxidant capacity of different varieties blueberry fruits (Giovanelli and Buratti (2009); You et al., 2011; Howard et al., 2003), only a few data concern the differences of bioactive compounds from blueberry juices.

Because of blueberry's unique nutrition, blueberry species have been successfully propagated and cultivated in China since 1989. With the increase in production, growers require additional options, and processing into blueberry juice is an effective way to maximize usage and markets. The aim of this work was to investigate the bioactive compositions of juices from 8 blueberry (*Vaccinium spp.*) cultivars and further study the effect of bioactive composition on antioxidant activity

2. Materials and methods

2.1. Preparation of blueberry juices

Blueberries of uniform size and at physiological maturity were handpicked in the morning from a commercial blueberry plantation located in Hefei (31°52'N, 117°17'E) in central-eastern China. Eight cultivars, namely Baldwin, Gardenblue, Anna, O'Neal, Misty, Bluecrop, Elliott, and Brigitta, were harvested from June 11, 2014 to August 15, 2014, respectively. All fruits were collected and stored at -20 °C until used.

Frozen blueberries were thawed and crushed into mashes, respectively. The mash was macerated with pectinase (Laffort, Sydney, Australia) at a concentration of 0.07 g/kg fruit for 2 h at room temperature, then squeezed with 200 mesh of silk cloth and centrifuged at $3100 \times g$ for 15 min to obtain blueberry juice.

2.2. Chemicals

Folin–Ciocalteu reagent and gallic acid were obtained from Sinopharm Chemical Reagent (Shanghai, China). 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). Other general chemicals with analytical grade were obtained from local suppliers.

2.3. Chemical analyses

Total soluble solids (expressed as °Brix) were measured on a digital refractometer (TD-45, TOP Instrument Co., Ltd., Zhejiang, China) at $25 \pm 1^\circ\text{C}$ and results were expressed in °Brix. The titratable acidity and total sugars were also determined AOAC methods (1980).

2.4. Total phenolic content (TPC)

The TPC was determined according to Folin–Ciocalteu method (Sanchez-Patan et al., 2015). The absorbance of the sample was determined at 765 nm. The results were expressed as mg gallic acid equivalents (GAE) per one liter of juice (mg GAE/L juice).

2.5. Total flavonoid content (TFC)

The TFC were measured using a colorimetric assay adapted from Mahmood et al. (2012). The absorbance was measured at 510 nm. The results were expressed as mg rutin equivalent (RE) per one liter of juice (mg RE/L juice).

2.6. Total anthocyanin content (TAC)

The TAC was estimated using the pH differential method. Aliquots of each sample were diluted with pH 1.0 or pH 4.5 buffers to the same dilution. The absorbance was measured at 510 nm and 700 nm in both pH 1.0 and pH 4.5 buffers. The TAC was calculated by using Eq. (1).

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

Where A is the difference in absorbance between pH 1.0 and 4.5, MW is the molecular weight of cyanidin-3-glucoside (449 g/mol); 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 blueberry extracted.

The results were expressed as mg cyanidin-3-glucoside (C3G) equivalents per one liter of juice (mg C3G/L_{juice}).

2.7. Free radical scavenging capacity (DPPH)

The DPPH free radical-scavenging capacity was estimated by adding 2.95 microliters of 0.1 mM DPPH methanolic solution to 50 μ L of the sample extracts. The solution was thoroughly mixed and in dark for 30 min. The absorbance was measured at 517 nm. The results were expressed in mg VC equivalent antioxidative capacity per one liter of juice (mg VC/L_{juice}).

2.8. Ferric reducing antioxidant power (FRAP) assay

FRAP reagent was prepared fresh daily in acetate buffer (adjusted pH to 3.6 by acetic acid) by mixing TPTZ solution (10 mM in 40 mM HCl) and 20 mM iron chloride solution in the proportion of 10:1:1, respectively. Each sample (90 μ L) 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 mmol ferrous ion per one liter of juice (mmol Fe²⁺/L_{juice}).

2.9. Reducing power assay (RP)

The RP was determined by adding diluted samples (1 mL) 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). Five mL of 10 % trichloroacetic acid was added to the mixture before centrifugation for 10 min at 2400 \times g. A 2.5 mL aliquot of the supernatant was mixed with ultrapure water (2.5 mL) and 0.5 mL of 0.1% FeCl₃. The absorbance was read at 700 nm after standing for 2 min; the final result was expressed as mg VC equivalent per one liter of juice (mg VC/L_{juice}).

2.10. Statistical analysis

Data were expressed as the means \pm the standard deviation (SD) of triplicate determinations. Mean differences were determined by one-way ANOVA followed by Duncan's range test using Prism™ v6.0 software. The differences were considered significant when $P < 0.05$ and are denoted by different letters. Linear regression plots were generated and correlations between antioxidant activities and phenolic, flavonoid and anthocyanin contents were computed as Pearson's correlation coefficient (r) using Prism™ v6.0 software.

3. Results and discussions

3.1. Quality attribute of blueberry juices

The quality and acceptability of juice are related to its total soluble solids (TSS) content, acidity and ratio of total soluble solids to acidity. Six quality attributes of juices from 8 common blueberry cultivars, namely pH, titratable acidity (TA), total soluble solids (TSS), total sugars (TS), and the ratio of total soluble solids / titratable acidity (TSS/TA), were measured (Table 1).

As shown in Table 1, eight blueberry juices contained TSS levels of 10.1 °Brix (Bluecrop)–14.8 °Brix (Anna), TA levels of 2.45 (Anna)–18.58 g/L_{juice} (Elliott) and TSS/TA levels of 0.66 (Elliott)–6.04(Anna),

respectively (Table 1). TS varied from 86.33 (Bluecrop) to 137.66 g/L juice (Anna). The pH values ranged from 2.59 (Elliott) to 3.60 (Anna).

Table 1. Titratable acidity (TA), pH, total soluble solids (TSS), total sugars (TS), and the ratio of total soluble solids / titratable acidity (TSS/TA) in the juices pressed from eight blueberry cultivars of *Vaccinium* species

Cultivars	Quality attributes				
	pH	TA (g/L juice)	TSS (°Brix)	TS (g/L juice)	TSS/TA
Anna	3.60±0.06 ^h	2.45±0.12 ^a	14.8±0.2 ^e	137.66±0.43 ^g	6.04
O'Neal	3.46±0.13 ^{gh}	3.61±0.18 ^b	10.8±0.4 ^{ab}	107.14±0.18 ^c	2.99
Misty	3.07±0.12 ^{ef}	6.72±0.31 ^{cd}	10.7±0.5 ^{ab}	102.04±0.45 ^b	1.50
Bluecrop	2.74±0.10 ^{abc}	12.22±0.56 ^e	10.1±0.2 ^a	86.33±0.74 ^a	0.83
Elliott	2.59±0.12 ^a	18.58±0.21 ^g	12.3±0.3 ^d	117.56±0.29 ^e	0.66
Brigitta	2.60±0.13 ^{ab}	14.21±0.71 ^f	11.4±0.5 ^{bc}	107.14±0.35 ^c	0.80
Baldwin	2.87±0.13 ^{cd}	7.03±0.33 ^d	12.0±0.6 ^{cd}	117.10±0.33 ^d	1.71
Gardenblue	2.96±0.06 ^{de}	6.57±0.32 ^{cd}	14.6±0.3 ^e	136.36±0.87 ^f	2.22

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 ($P < 0.05$).

Table 2. The phenolic content (TPC), flavonoid content (TFC), and anthocyanin content (TAC), the antioxidant capacity (DPPH, FRAP, and RP), in the juices pressed from eight blueberry cultivars of *Vaccinium* species.

Cultivars	Phenolics			Antioxidants		
	TPC (mg/L juice)	TFC (mg/L juice)	TAC (mg/L juice)	DPPH (mg/L juice)	FRAP (mmol/L juice)	RP (mg/L juice)
Anna	1180.21±6.88 ^f	199.75±1.23 ^f	138.60±0.12 ^a	49.72±0.31 ^e	20.41±0.70 ^e	6.37±0.08 ^f
O'Neal	647.87±4.12 ^b	131.72±0.27 ^d	269.27±2.08 ^f	34.74±1.62 ^c	12.13±0.16 ^a	3.74±0.05 ^d
Misty	852.55±6.89 ^d	267.28±0.97 ^g	197.88±1.02 ^e	44.24±0.90 ^d	20.21±0.75 ^e	5.49±0.06 ^e
Bluecrop	645.11±3.43 ^b	102.80±0.72 ^a	166.15±1.16 ^b	26.68±0.57 ^b	11.61±0.48 ^a	3.19±0.01 ^c
Elliott	738.72±5.74 ^c	113.16±0.89 ^b	193.90±3.79 ^d	24.04±0.12 ^a	12.06±0.15 ^a	2.72±0.04 ^a
Brigitta	597.23±5.02 ^a	138.23±1.02 ^e	180.77±2.33 ^c	26.48±1.17 ^b	11.72±0.57 ^a	2.92±0.04 ^b
Baldwin	975.96±6.82 ^e	115.28±0.67 ^c	330.64±0.83 ^g	33.22±0.45 ^c	15.48±0.56 ^b	2.73±0.03 ^a
Gardenblue	1417.45±10.11 ^g	546.25±2.37 ^h	492.62±3.33 ⁱ	76.59±1.49 ^f	33.97±0.23 ^d	8.02±0.10 ^g

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 ($P < 0.05$).

Sweet taste positively correlated with TSS and TS, sour correlated with titratable acidity (TA), and negatively correlated with pH and TSS/TA ratio (Bett-Garber et al., 2015). 'Anna' was less acidic (TA: 2.45 g/L juice) and sweeter taste (TSS:14.8%;TS:137.66g/L_{juice}),

consequently, 'Anna' cultivar had more advantageous TSS/TA ratio (6.04). While 'Elliott' was more acidic with a TA value of 18.58 g/L juice and had lower TSS/TA ratio (0.66). Sapers et al. (1984) also noted that 'Elliott' had the higher TA and lower TSS/TA of 11 highbush cultivars.

3.2. Phenolic compounds and antioxidant capacity

Phenolic compounds (phenolics, flavonoids, anthocyanins) and antioxidant capacity were shown in Table 2. Eight blueberry juices contained total phenolic content (TPC) of 597.23–1417.45 mg GAE /L juice, total flavonoid content (TFC) of 102.80–546.25 mg RE/L juice and total anthocyanin content (TAC) of 138.60–492.62 mg C3G/L juice, respectively (Table 2). TPC in 8 blueberry juices varies widely. TFC and TAC had significant differences ($P < 0.05$) among 8 cultivars. ‘Gardenblue’ possessed all the highest content of TPC, TFC and TAC in these cultivars. The free radical scavenging capacity (DPPH), ferric reducing antioxidant power (FRAP) and reducing power (RP) were also investigated. DPPH, FRAP and RP were 24.04–76.59 mg VC/L juice, 11.61–33.97 mmol Fe²⁺/L juice and 2.72–8.02 mg VC/L juice, respectively (Table 2). There were obvious differences in DPPH among 8 cultivars ($P < 0.05$). ‘Gardenblue’ displayed the highest scavenging effect (76.59 mg VC/L juice) in these cultivars, followed by ‘Anna’ and ‘Misty’, respectively. Similar to the DPPH value, the highest FRAP (33.97 mmol Fe²⁺/L) and the highest RP (8.02 mg/L) were found in ‘Gardenblue’ cultivar, followed by the ‘Anna’ and ‘Misty’ cultivars. Jessica *et al.* (2013) research showed that anthocyanin contents of blueberry fruits were primarily influenced by cultivar. Connor *et al.* (2002) and Rodrigues *et al.* (2011) reported total phenolic and anthocyanin contents presented significant differences among blueberry cultivars. Lee *et al.* (2004) and Gunduz *et al.*, (2015) stated the variability in total phenolic content, total anthocyanin content and antioxidant activity in various *Vaccinium* species. Wang *et al.* (2012b) found considerable variation was in flavonoid content and antioxidant activity among 42

blueberry (*Vaccinium spp.*) cultivars. Cultivars play a more important role in influencing total phenolics, total anthocyanins, total flavonols and oxygen radical-absorbing capacity in blueberries (Howard *et al.*, 2003). Koca and Karadeniz (2009) also reported that FRAP values varied from 7.41 to 57.92 $\mu\text{mol/g}$ for six lowbush and four highbush blueberry fruits. It has been argued that the antioxidant activity of a product cannot be reasonably validated by a single method due to the complex nature of phytochemicals and their interactions, so it was important to use multiple assay systems measuring different indices (Pérez-Jiménez *et al.*, 2008).

3.3. Correlations between phenolic compounds and antioxidant activity in blueberries

Blueberry phenolics were reported as constituents, which are responsible for their high radical scavenging capacity (Giovanelli *et al.*, 2009; Wang *et al.*, 2008). In order to evaluate the antioxidant potential of 8 blueberry juices in terms of their polyphenol contents, linear regression plots were generated and the Pearson correlation coefficients were calculated (Figure 1). A very good correlation was noted between the RP values and the TPC ($r = 0.84$). Nevertheless, DPPH assay and FRAP assay showed striking correlations with TPC (DPPH, $r = 0.90$; FRAP, $r = 0.92$). Ramful *et al.* (2011) reported that TPC of the pulp extracts also correlated strongly with the antioxidant activities using the FRAP assays. TFC showed strong correlations with DPPH values ($r = 0.95$), with FRAP values ($r = 0.97$) and with RP values ($r = 0.89$). Considerable variation was found in flavonoid content, antioxidant activity, and their contribution to total antioxidant activity among 42 blueberry cultivars reported by Wang *et al.* (2012b).

A good correlation was obtained between FRAP assay values and TAC ($r = 0.71$). However, a moderate correlation was noted

between TAC and antioxidant capacity of the cultivars (DPPH, $r = 0.69$; RP, $r = 0.46$).

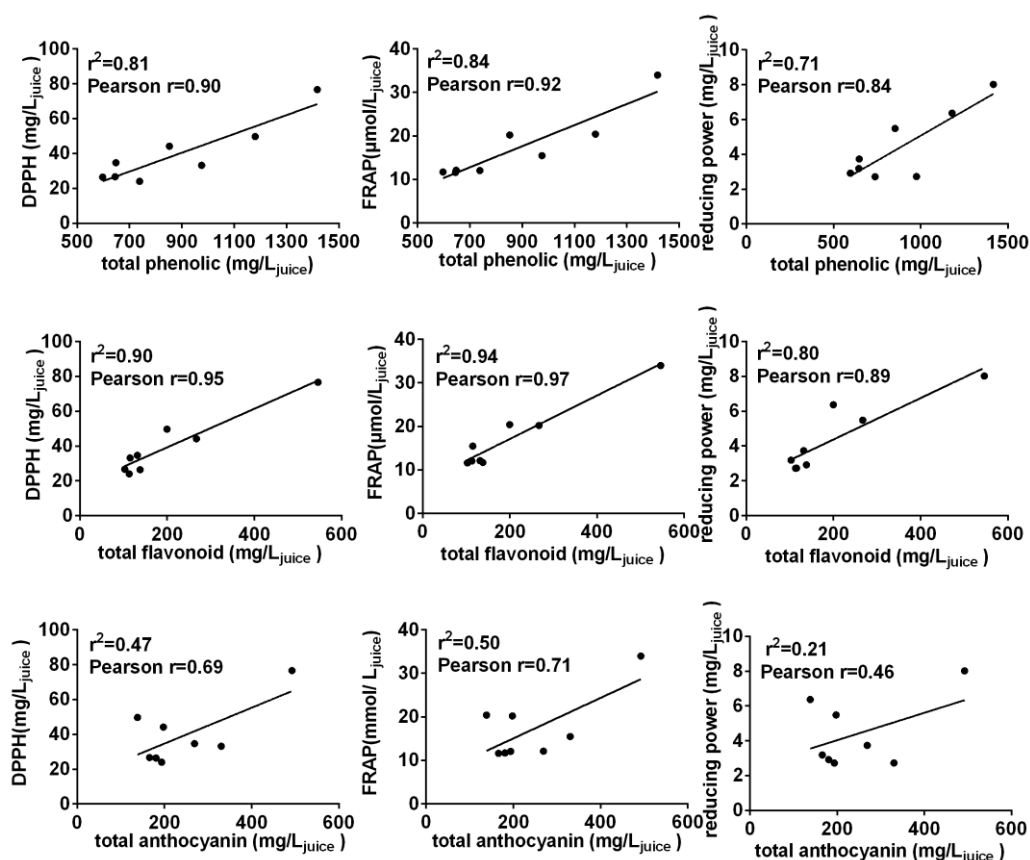


Figure 1. Linear regression plots and Pearson 's correlation coefficients of DPPH, FRAP, and reducing power values with respect to phenolics, flavonoids, and anthocyanins, of the juices pressed from eight blueberry cultivars of *Vaccinium* species

In our work, total phenolic and flavonoid had higher antioxidant potential than total anthocyanin (Figure 1). Vanessa *et al.* (2014) found the total flavonoid content was highly and positively correlated to the total monomeric anthocyanin content in berry fruits. Castrejon *et al.* (2008) reported anthocyanins in mature blueberries have lower antioxidant potential than other phenolic compounds. Giovanelli and Buratti (2009) pointed out the antioxidant activity was more related to the total phenolic rather than to the anthocyanin. The same type of linear correlation between antioxidant

activities and phenolic contents had been found in fruit juices (Gardner *et al.*, 2000).

4. Conclusions

The present study demonstrated that the juices from 8 blueberry cultivars possessed significantly different phenolic contents and antioxidant activities. Correlation analyses revealed that the phenolics and flavonoids were distinctly responsible for the antioxidant capacity, and total flavonoids showed strong correlations with DPPH, FRAP and reducing power assays (DPPH, $r = 0.95$; FRAP, $r = 0.97$; reducing power $r = 0.89$). Among 8

blueberry juices, the cultivar 'Gardenblue' possessed the highest content of phenolic compounds (phenolics, flavonoids, and anthocyanins), corresponding to the highest value of antioxidant activity (DPPH, FRAP and Reducing power).

5. References

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