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Wei Yuan

Stephen F Austin State University, Arthur Temple College of Forestry and Agriculture, yuanw@sfasu.edu

LiJing Zhou

Stephen F. Austin State University

Guangrui Deng

Ping Wang

Stephen F Austin State University, Arthur Temple College of Forestry and Agriculture, wangp@sfasu.edu

David Creech

Stephen F. Austin State University, dcreech@sfasu.edu

See next page for additional authors

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Authors

Wei Yuan, LiJing Zhou, Guangrui Deng, Ping Wang, David Creech, and Shiyu Li

Anthocyanins, Phenolics, and Antioxidant Capacity of *Vaccinium* L. in Texas, USA

Wei Yuan¹, Lijing Zhou², Guangrui Deng¹, Ping Wang¹, David Creech^{**2} and Shiyou Li^{*1}

¹ National Center for Pharmaceutical Crops, Arthur Temple College of Forestry and Agriculture, Stephen F. Austin State University, Nacogdoches, TX 75962, USA; ² SFA Gardens, Arthur Temple College of Forestry and Agriculture, Stephen F. Austin State University, Nacogdoches, TX 75962, USA

Abstract: Berries of *Vaccinium* spp. have long been an important source of food and pharmaceutical ingredients and are considered to have high antioxidant potential. Growing blueberries in Texas, United States is a substantial industry, but there is no report on their antioxidant capacity, total phenolics, and anthocyanins. This study evaluates antioxidant capacity and contents of total phenolics and anthocyanins in both fruits and leaves of 19 genotypes including five commercial cultivars and 11 selections of rabbiteye blueberry (*V. ashei*) and southern highbush (*V. corymbosum* hybrids), and three native species (*V. darrowii*, *V. arboreum*, and *V. fuscatum*) grown in Nacogdoches, Texas. Significant variations in antioxidant capacity (as measured by FRAP) and contents of total phenolics and anthocyanins in fruit were observed among different species, cultivars or selections with less variation observed among individual plants of the same genotype. Our data from rabbiteye blueberry selections and cultivars support the hypothesis that antioxidant capacity is more highly correlated to total phenolics than anthocyanins. The analysis of five species indicates that antioxidant activity decreased during ripening but total phenolics contents tended to increase with maturity. Antioxidant capacity, total phenolics, and anthocyanin content did not change significantly during storage at 4°C or -20°C for two weeks but decreased significantly when berries were oven dried at 65°C for 48 h. Antioxidant capacity and total phenolics content in leaves of each genotype were 3 to 15 times higher than those in fruits.

Keywords: Blueberry, fruits, leaves, *Vaccinium*, antioxidant activity, total phenolics, anthocyanins.

INTRODUCTION

Edible berries are rich in antioxidants, anthocyanins (ACY) and other phenolic compounds that have demonstrated the ability to reduce risk for development or treatment of cancers [1-6], cardiovascular disorders [5, 7, 8], obesity, diabetes [9, 10], aging-diseases [11, 12], urinary tract infections [13-16], and periodontal disease [15, 17, 18]. Berries and leaves of *Vaccinium* L. (family Ericaceae) have been an important source of food and pharmaceutical ingredients and are considered to have high antioxidant potential [3, 19, 20]. There are numerous products from *Vaccinium* leaf and fruit extracts utilized as dietary supplements in the world market. Fruit or leaf extracts of *Vaccinium* spp., particularly of bilberry (*V. myrtillus* L.) [2, 6], rabbiteye blueberry (*V. ashei* Reade) [3], lowbush blueberry (*V. angustifolium* Aiton) [21, 22], cranberry (*V. macrocarpon* Aiton) [23-25], and highbush cultivated blueberry (*V. corymbosum* L.) [1, 3] were found to induce apoptosis in cancer cells and to inhibit human leukemia [2, 23-25] and breast [23, 26], colon [2, 3, 6, 23-25], lung [25], and prostate [21-23] cancer cells *in*

vitro. Fruit extracts of some *Vaccinium* species exhibited anticarcinogenic activity [4]. The leaf, fruit, and stem extracts of the lowbush blueberry and leaf extract of *V. bracteatum* Thunb. indicated an opportunity for use as a complementary anti-diabetic therapy [9, 10]. Cranberries (particularly in the form of cranberry juice) have been widely used for several decades for the prevention and treatment of urinary tract infections (UTIs) [27]. Research suggests that its mechanism of action is preventing bacterial adherence to host cell surface membranes [28]. Cranberry is a safe, well-tolerated herbal supplement that does not have significant drug interactions [28]. Several recent clinical studies have demonstrated that cranberry and blueberry fruit or fruit juice may be a valuable therapeutic choice in the treatment of UTIs [13-16, 29]. Tannins isolated from cowberry (*V. vitis-idaea* L.) exhibited antioxidant activity and antimicrobial activity, and could be used for the treatment of periodontal disease [17, 18]. Recently, it was found that proanthocyanidin from blueberry (*V. virgatum* Aiton) leaves possessed strong suppressive effects against Hepatitis C virus subgenome expression in a replicon cell system [30]. Mice treated with polyphenol-rich extract of wild blueberry exhibited a significant improvement in learning and memory [12]. Several European studies indicated an improvement in night vision in humans with a variety of bilberry supplements. However, this has not been supported by some American clinical studies [31, 32]. To date, many medicinal investigations are still in the early stages, and thus the potential pre-

*Address correspondence to these authors at the National Center for Pharmaceutical Crops, Arthur Temple College of Forestry and Agriculture, Stephen F. Austin State University, Nacogdoches, TX 75962, USA; Tel: 936-468-2071, 936-468-5600; Fax: 936-468-7058; E-mail: lis@sfasu.edu

**SFA Gardens, Arthur Temple College of Forestry and Agriculture, Stephen F. Austin State University, Nacogdoches, TX 75962, USA; E-mail: dcreech@sfasu.edu

ventive and therapeutic effects of *Vaccinium* products for most diseases are not conclusive and well-designed clinical studies are still needed.

Antioxidant capacity (AC) in *Vaccinium* with variables including varieties, cultivation, process, and storage has been of interest to consumers and the industry. The AC of *Vaccinium* (as determined by oxygen radical absorbance capacity (ORAC) or ferric reducing antioxidant power (FRAP)) is primarily due to total phenolics (TPH) [19, 20, 33]. Among phenolics, ACY is of particular interest because of their frequent occurrence in crops considered to have high nutritional value. ACY are water-soluble pigments responsible for red, purple, and black colors of *Vaccinium* berries. ACY is composed of an aglycone anthocyanidin and a sugar moiety mainly attached at the 3-position on the C-ring, e.g., delphinidin, cyaniding, petunidin, peonidin, pelargogidin, and malvidin [34]. Other major phenolics include gallic acid, *p*-hydroxybenzoic acid, caffeic acid, *p*-coumaric acid, ferulic acid, ellagic acid, (+)-catechin, (-)-epicatechin, myricetin, quercetin, and kaempferol [19].

It has been reported that TPH content and AC of *Vaccinium* are significantly affected by genotype and growing season [20, 33, 35-38]. TPH and ACY contents are more affected by genotype than growing season, whereas ORAC_{FL} values are more affected by growing season [35]. Wild berries usually have much higher nutritional values than those in cultivation [20, 39]. ORAC and TPH levels in leaves are significantly higher than in fruits of highbush blueberry [40]. Recently, there have been some studies of extraction and purification of some flavonoids or ACY from leaves of alpine bilberry (*V. uliginosum* L.) [41-44] and sea bilberry (*V. bracteatum* Thunb.) [45, 46].

Investigations on AC, TPH, and ACY contents in *Vaccinium* are numerous in the last decade. However, existing research is primarily restricted to certain species grown in North America, Europe, East Asia, and Australia, e.g., low-bush wild blueberry [20, 33, 39, 47-50], highbush cultivated blueberry [20, 33, 36, 39, 40, 47, 51-58], half-highbush blueberry (*V. angustifolium* × *V. corymbosum*) [54], rabbiteye blueberry [20, 33, 51], bilberry [20, 59], black huckleberry (*V. membranaceum* Douglas ex Torr.) [33, 54, 60], cascade huckleberry (*V. deliciosum* Piper) [54, 60], velvet-leaf blueberry (*V. myrtilloides* Michx.) [33, 50], oval-leaf blueberry (*V. ovalifolium* Smith) [33, 54, 60], evergreen huckleberry (*V. ovatum* Pursh) [33, 54], cranberry [8], wild cranberry (*V. oxycoccus* L.) [54], red huckleberry (*V. parvifolium* Smith) [33, 54], *V. reticulatum* Smith [61], *V. calycinum* Smith [61], alpine bilberry [41-44, 54], cowberry [17, 62], sea bilberry [10, 46], and Caucasian blueberry (*V. arctostaphylos* L.) [63].

The United States produces about 60% of the world blueberry production with 199,127 metric tons reported in 2008 [64]. There are more than 63,000 ha of blueberries in the United States, and the leading states in blueberry production are Maine, Michigan, New Jersey, North Carolina, Oregon, Washington, Georgia, and Florida [64]. Studies in the United States are primarily on wild or cultivated blueberries in Northeast or Pacific Northwest [20, 33, 39, 40, 47, 48, 50, 53, 54, 65]. There are only a few studies involving the nutraceutical properties of blueberries grown in the southern United States (e.g., Georgia, Florida, and Arkansas), e.g.,

southern highbush blueberry (*V. corymbosum* hybrids, introgressed with Darrow's blueberry (*V. darrowii* Camp.)) [19, 66] and rabbiteye [19, 33], but none from Texas. Existing reports on the relationship of fruit size and AC are somewhat contradictory [20, 33, 39, 40, 47, 67, 68] and further investigation is needed to clarify their effects. *Vaccinium* leaf extracts could be an important source of pharmaceutical products. However, most studies on AC, TPH, and ACY contents of *Vaccinium* focus on fruits rather than leaves. Long-term frozen storage of blueberries has not significantly affected the AC, TPH, and ACY [56, 65, 69]. It was further reported that the ACY content in dried blueberries is reduced in comparison to that found in fresh blueberries, while AC of the extracts did not differ from that of the fresh fruit [69]. Little is known, however, about the effect of storage process on the content of phenolic compounds of blueberries. This problem must be addressed in the development of blueberry extract products.

This study is to evaluate AC, TPH, and ACY contents in both fruits and leaves of major commercial cultivars in Texas and some important selections. Changes in AC, TPH, and ACY contents in fruits by different storage methods were also investigated. Data on both cultivars and storage method will provide groundwork for the development of *Vaccinium* pharmaceutical products (both berries and dietary supplement products) with high antioxidant activity and nutritional level for domestic and overseas markets.

MATERIALS AND METHODS

Chemicals

2,4,6-tripyridyl-s-triazine (TPTZ), gallic acid, Folin & Ciocalteu's phenol reagent were purchased from Sigma-Aldrich (St. Louis MO). Sodium acetate, FeCl₃·H₂O, ACS grade methanol and other unspecified reagents were purchased from VWR International (West Chester, PA).

Sample Preparation

Fruit and leaf samples of *Vaccinium* were collected from three plants each of 19 genotypes including five commercial cultivars, 11 selections of *V. ashei* and *V. corymbosum*, and three native species (*V. darrowii* Camp., *V. arboreum* Marsh., and *V. fuscatum* Aiton) in Nacogdoches, Texas, USA in 2009 and 2010, Fig. 1, Table 1). The five commercial cultivars included 'Brightwell', 'Climax', 'Tifblue', 'Premier', and 'Powderblue' (rabbiteye blueberries (*V. ashei*), and 11 breeding selections including rabbiteyes MS 63, T 342, and T 38 and the southern highbush (*V. corymbosum* hybrids) MS 108, MS 122, MS 179, MS 231, MS 343, MS 546, and MS 548, Darrow's blueberry (*V. darrowii*) ('Nativeblue'), and wild sparkleberry (*V. arboreum*) (leaves only) and black highbush blueberry (*V. fuscatum*, synonym: *V. arkansanum* Ashe). In addition, three commercially available samples ('Organic', 'Driscolls', and 'Naturipe') were purchased from a local grocery store in Nacogdoches, Texas, USA.

To investigate the relationship between fruit size and AC, TPH, and ACY contents, mature berries from each of the three plants of 'Tifblue' and 'Premier' were divided by berry size. Berry size was determined by counting the number of berries per 100 g of sample.

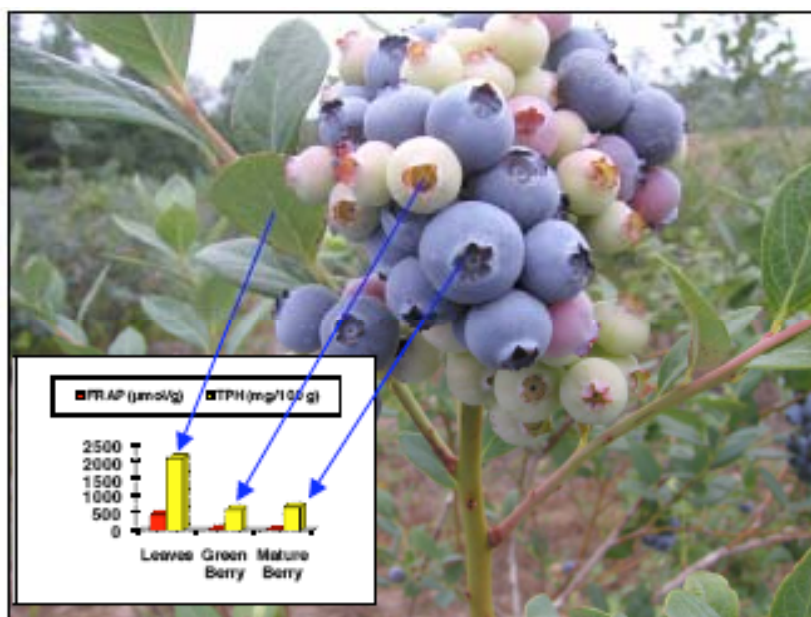


Fig. (1). Antioxidant capacity (AC) (in measurement of FRAP) and total phenolics (TPH) contents in immature and mature blueberries (photo by S.Y. Li).

Fruit samples of each collection were divided into four groups for different treatments: fresh (extracted and analyzed within 3 h), stored at 4°C for two weeks, frozen at -20°C for two weeks, and dried at 65°C for two weeks. Leaf samples were collected in the morning and freshly extracted and analyzed the same day.

Ten gram berry or leaf samples were added to 50 mL conical tubes and amended with 1:1 (w/v) ice-cold acidified methanol (0.1% HCl). The samples were then homogenized with a Pro250 homogenizer (Pro Scientific Inc, Monroe, CT, USA) for 1 min. After 30 min, the homogenate was centrifuged under 3000 rpm for 10 min. 1.5 mL supernatant was transferred to eppendorf tubes and centrifuged at 14,000 rpm for 15 min. The supernatant was diluted for further analysis, normally 100× for ferric-reducing ability of plasma (FRAP) assay and total phenolic assay and 20× for anthocyanin content assay.

Antioxidant Capacity (AC) Assay

The FRAP assay was performed as described by Benzie and Strain (1996) [70] and adapted for using 96-well plate spectrophotometer. Briefly, the antioxidants reduce ferric/TPTZ complex to a blue-colored ferrous form at 37°C in pH 3.6 sodium acetate buffer. The increased absorbance at 593 nm is proportional to the ferric reducing antioxidant power. The results were expressed as micromoles (µmol) of ferric iron reduced per gram fresh fruit.

Total Phenolics (TPH) Content

The Folin-Ciocalteu method [71] was used to determine total phenolic content as described by Singleton and Rossi in 1965. An adaptation was made to use a 96-well microplate. After 120 min incubation for color development, absorption was read at 755 nm by a µQuant spectrophotometer (Bio-Tek Instruments, Inc.). Results are expressed as mg gallic acid equivalents/100 g material.

Total Anthocyanins (ACY) Content

ACY content was determined by AOAC official pH differential method [72]. Results are expressed as mg cyanidin 3-glucoside equivalents/100g fresh fruit using a molar extinction coefficient of 29,600.

Sugar Contents

Total sugar was determined by a modified phenol-sulfuric acid method [73]. On a 96-well plate, the following solutions were added successively: 40 µL standard glucose solution, 20 µL 5% phenol, 140 µL concentrated sulfuric acid. The plate was then incubated at 85°C for 50 min. After cooling to 25°C for 30 min, the plate was read under 490 nm with BioTek µQuant Microplate Spectrophotometer (Winooski, VT). To establish a standard curve of linear correlation, a series of standard glucose solutions at 20, 50, 70, 100, 250, 375, and 500 µg/mL were prepared using 100 mL volumetric flasks. Total sugar content of the berry samples were calculated with the established standard glucose curve.

Statistical Analysis

While an ANOVA test was conducted by SAS 9.2 for Windows, the authors agreed that the differences in plant material collection dates (early-ripening berries harvested in May and late-ripening in July) and the different times of sample runs in the laboratory must be given consideration as an important unaccounted for variable and their impact on actual data, and, thus, the appropriateness of the test. These two variables (time of collection, time of lab run) certainly influence the actual values obtained for AC, ACY, FRAP, etc. That said, “the large number of genotypes, samples and analyses certainly suggests some confidence in the major trends found in this study and in the conclusions reached.”

Table 1. Size of Fruits of 19 Genotypes of *Vaccinium* in Texas

Variety/Genotype	Berry Number/100 g	
	2010	2009
<i>V. darrowii</i> (Darrow's)*	362	397
<i>V. arboreum</i> (sparkleberry)**	-	-
<i>V. fuscatum</i> (black highbush)**	654	-
<i>V. ashei</i> (rabbiteye)*	-	-
Brightwell	68	79
Climax	74	105
Tifblue	76	77
Premier	67	100
Powderblue	125	82
MS 63	56	58
T 342	74	54
T 38	72	129
<i>V. corymbosum</i> hybrids (Southern highbush)*		
MS 108	63	81
MS 122	88	82
MS 179	62	-
MS 231	75	66
MS 343	168	-
MS 546	137	-
MS 548	166	-
MS 611	122	-

*in cultivation; ** wild collected.

RESULTS AND DISCUSSION

Variations of AC, ACY, and TPH in Fruits

With Genotype

There were significant variations in AC (FRAP), TPH, and ACY contents in fruits among different species, cultivars, or selections (Tables 2-4). For both harvest years, the fruit of cultivated Darrow's blueberry had higher mean FRAP values (78.26 and 113.94 $\mu\text{mol/g}$ in 2009, and 2010, respectively), followed by rabbiteyes (37.38 and 76.19 $\mu\text{mol/g}$, respectively) and then the southern highbush blueberry (26.67 and 52.45 $\mu\text{mol/g}$, respectively) (Table 2). Among rabbiteye cultivars and selections, MS 63 has highest AC values in fruits in both harvest years (45.76 and 91.75

$\mu\text{mol/g}$ in 2009 and 2010, respectively), followed by T 38 (86.22 $\mu\text{mol/g}$ in 2010 and 40.01 $\mu\text{mol/g}$ in 2009) and 'Tifblue' (33.05 and 88.19 $\mu\text{mol/g}$ in 2009 and 2010, respectively). Among the five commercial cultivars, 'Tifblue' had the highest AC values while 'Premier' had the lowest AC values (36.36 and 55.30 $\mu\text{mol/g}$ in 2009 and 2010, respectively). This result was further confirmed by the comparison of data from both big and small berries (Table 5). The TPH and ACY contents in fruits generally showed a similar pattern (Tables 3-5).

In 'Tifblue' and 'Premier', less variation among FRAP, TPH, and ACY in berries was observed between individual plants of the same genotype than among genotypes (Table 5).

With Fruit Size

ACY contents are significant contributors to AC of *Vaccinium* berries and are confined primarily to the skin of the fruit in Section *Cyanococcus* (highbush, lowbush, rabbiteye) [20, 47]. In our study, ACY in the mature berries of 'Powderblue' (rabbiteye) was primarily found in skins, but was also found in flesh (Table 6). In 'Powderblue', AC (FRAP, $\mu\text{mol/g}$) and TPH concentrations (mg/100 g) in the pulp were about 5.2% and 10.5% of those in the skin and seed, respectively (Fig. 2, Table 6). Thus, smaller berries with higher specific surface area have higher AC and TPH. Smaller berries of 'Tifblue' and 'Premier' had significantly higher AC (by 16-43%) than bigger berries (Table 5). Statistical analysis by two-way ANOVA with genotypes and sizes as factors revealed significant differences ($p < 0.005$) between the berry size and the values of FRAP and TPH (no significant differences for ACY, $p > 0.05$). The regression analysis results also indicated that fruit size was significantly correlated to AC and TPH contents in these cultivars (Fig. 3).

Several studies suggest that ACY content is the major contributor to the higher antioxidant activity in smaller berries [20, 33, 40, 47]. An inverse relationship was found between fruit size and ACY content in highbush [33, 40] and cranberry [68]. However, in other studies no relationship between fruit size and ACY content in highbush [39, 67] or lowbush blueberries [39] was found. It is further reported that fruit size was not correlated to ACY in rabbiteye, lowbush, velvet-leaf blueberry, oval-leaf blueberry, evergreen huckleberry, red huckleberry, black huckleberry and *V. constablaei* [33]. Moyer *et al.* (2002) reported that TPH was more highly correlated to AC than ACY [33]. Our results from rabbiteye blueberries ('Tifblue' and 'Premier') support this hypothesis (Table 5).

Fruit size is an important factor for differences in antioxidant activity within a genotype. In addition to fruit size, skin thickness, light condition, and other ecological factors might also influence AC when comparing different genotypes. It has been reported that wild *Vaccinium* usually have much higher AC than those in cultivation [20, 39]. This hypothesis cannot be tested without comparative study of the same varieties in both wild and cultivated conditions. Evaluations of cultivated and wild huckleberries (*V. membranaceum*, *V. deliciosum*, and *V. ovalifolium*) in the Pacific Northwest [60] indicates that wild berries do not necessarily have higher antioxidant activity than cultivated berries of the same species. In this study, cultivated native *V. darrowii*

Table 2. Antioxidant Capacity (FRAP) of Berries of Some Species, Cultivars, and Selections of *Vaccinium* in Texas

Species/ Cultivars/Selections	FRAP ($\mu\text{mol/g}$) (Mean \pm SD)				
	Fresh (2010)	Fresh (2009)	Refrigerated (2009) (2 Weeks)	Frozen (2009) (2 Weeks)	Oven-Dried (2009) (2 Weeks)
<i>V. darrowii</i> (Darrow's)*	113.94 \pm 4.24	78.26 \pm 4.53	26.76 \pm 2.48	37.10 \pm 1.57	20.97 \pm 0.72
<i>V. fuscatum</i> (black highbush)**	85.13 \pm 4.74	48.01 \pm 1.60	-	-	-
<i>V. ashei</i> (rabbiteye)*	76.19 \pm 12.01	37.38 \pm 6.64	46.61 \pm 12.98	37.73 \pm 10.01	13.84 \pm 2.65
'Brightwell'	71.79 \pm 1.12	24.69 \pm 3.05	45.73 \pm 1.98	24.81 \pm 0.92	11.41 \pm 0.69
'Climax'	75.16 \pm 1.30	35.98 \pm 5.41	49.07 \pm 2.66	43.37 \pm 3.02	12.62 \pm 1.40
'Tifblue'	88.19 \pm 2.07	33.05 \pm 4.02	28.89 \pm 0.47	49.35 \pm 1.13	13.03 \pm 2.59
'Premier'	55.30 \pm 1.38	36.36 \pm 1.64	37.26 \pm 8.07	42.68 \pm 2.01	10.86 \pm 0.61
'Powderblue'	69.31 \pm 2.43	39.02 \pm 2.15	61.29 \pm 7.27	36.26 \pm 1.21	12.94 \pm 0.60
MS 63	91.75 \pm 3.35	45.76 \pm 5.27	50.06 \pm 2.19	55.67 \pm 2.03	17.44 \pm 0.25
T 342	71.83 \pm 4.41	44.13 \pm 2.53	66.38 \pm 2.04	31.48 \pm 1.45	18.13 \pm 2.16
T 38	86.22 \pm 0.73	40.01 \pm 5.16	34.22 \pm 0.98	34.20 \pm 4.20	14.32 \pm 0.40
<i>V. corymbosum</i> hybrids (southern highbush)*	52.45 \pm 8.16	26.67 \pm 9.30	41.67 \pm 6.31	29.93 \pm 7.35	11.57 \pm 2.56
MS 108	41.97 \pm 0.42	16.94 \pm 2.65	35.73 \pm 0.93	31.79 \pm 4.83	9.44 \pm 0.66
MS 122	56.12 \pm 1.76	27.59 \pm 3.32	48.29 \pm 0.88	36.17 \pm 1.83	10.86 \pm 1.05
MS 179	52.81 \pm 0.62				
MS 231	43.99 \pm 1.42	35.47 \pm 2.74	40.98 \pm 1.42	21.82 \pm 2.07	14.41 \pm 0.10
MS 343	66.08 \pm 1.77	-	-	-	-
MS 546	57.75 \pm 1.05	-	-	-	-
MS 548	55.38 \pm 2.24	-	-	-	-
MS 611	45.51 \pm 1.08	-	-	-	-
Others					
'Organic'***	-	35.63 \pm 3.11	-	-	-
'Driscolls'***	-	24.72 \pm 3.37	-	-	-
'Naturipe'***	-	35.96 \pm 1.59	-	-	-
Mean	67.24 \pm 1.85	38.06 \pm 3.13	43.72 \pm 2.61	37.06 \pm 2.19	13.87 \pm 0.93

*in cultivation; ** wild collected; *** purchased from a local grocery store; - no data collection.

('Nativeblue') had higher levels in FRAP, TPH, and ACY contents in both fruits and leaves than the native species *V. fuscatum* in the wild (Tables 2-4).

With Fruit Maturity

To date there has been limited research on the development of phenolic compounds and antioxidant activity during fruit maturation and ripening [74]. Castrejon *et al.* reported that antioxidant activity and TPH content tended to decrease

during ripening [74]. However, in our analysis of the six species/cultivars grown in Texas, antioxidant activity of fruits decreased during ripening but TPH contents tended to increase with maturity. On average, mature berries have only 83% of the FRAP of green berries but have 114% of the TPH content found in green berries (Fig. 1, Tables 2-4, 7). This suggests that other non-phenolics are responsible for the higher antioxidant activity in green berries.

Table 3. Total Phenolics (TPH) of Berries of Some Species, Cultivars, and Selections of *Vaccinium* in Texas

Species/ Cultivars/Selections	TPH (mg/100g) (Mean ± SD)				
	Fresh (2010)	Fresh (2009)	Refrigerated (2009) (2 Weeks)	Frozen (2009) (2 Weeks)	Oven-Dried (2009) (2 Weeks)
<i>V. darrowii</i> (Darrow's)*	598.42 ± 28.35	659.61 ± 47.08	746.60 ± 33.47	407.0 ± 18.95	202.55 ± 8.74
<i>V. fuscatum</i> (black highbush)**	593.42 ± 26.67	649.57 ± 88.53	-	-	-
<i>V. ashei</i> (rabbiteye) *	425.34 ± 44.11	599.76 ± 114.39	550.65 ± 122.55	510.61 ± 147.61	232.79 ± 86.95
'Brightwell'	351.13 ± 37.50	529.57 ± 60.88	511.00 ± 66.47	291.48 ± 41.98	236.49 ± 18.17
'Climax'	391.84 ± 21.55	709.38 ± 60.69	567.38 ± 21.90	567.25 ± 16.11	266.58 ± 20.92
'Tifblue'	494.24 ± 51.04	671.79 ± 45.70	363.80 ± 13.48	602.74 ± 15.29	71.86 ± 25.41
'Premier'	417.74 ± 10.12	700.06 ± 22.04	479.42 ± 23.93	540.32 ± 24.84	148.55 ± 5.69
'Powderblue'	410.32 ± 28.64	733.36 ± 79.21	649.95 ± 31.68	474.76 ± 5.17	229.08 ± 8.28
MS 63	453.40 ± 19.58	456.16 ± 41.98	582.99 ± 11.95	778.63 ± 6.02	345.97 ± 35.89
T 342	424.42 ± 10.71	515.88 ± 45.43	768.91 ± 24.13	430.07 ± 23.22	305.59 ± 8.93
T 38	459.59 ± 28.03	481.90 ± 52.69	481.71 ± 37.65	399.63 ± 6.30	258.17 ± 18.79
<i>V. corymbosum</i> hybrids (southern highbush) *	332.33 ± 54.16	378.96 ± 52.11	517.68 ± 86.72	452.88 ± 34.06	198.56 ± 23.79
MS 108	282.16 ± 12.23	434.21 ± 44.42	452.11 ± 4.95	416.37 ± 16.80	178.95 ± 8.65
MS 122	298.89 ± 5.66	330.69 ± 23.85	616.01 ± 24.97	483.79 ± 6.10	225.02 ± 12.73
MS 179	310.39 ± 28.04				
MS 231	266.19 ± 21.44	371.97 ± 15.47	484.93 ± 22.06	458.49 ± 13.30	191.72 ± 18.88
MS 343	396.70 ± 29.55	-	-	-	-
MS 546	419.74 ± 22.22	-	-	-	-
MS 548	336.68 ± 19.89	-	-	-	-
MS 611	347.87 ± 23.51	-	-	-	-
Others					
'Organic'***	-	591.81 ± 79.73	-	-	-
'Driscolls'***	-	371.03 ± 9.00	-	-	-
'Naturipe'***	-	533.68 ± 9.67	-	-	-
Mean	391.75 ± 23.42	549.29 ± 45.40	510.29 ± 23.08	487.55 ± 16.17	221.71 ± 15.92

*in cultivation; ** wild collected; *** purchased from a local grocery store; - no data collection.

With Storage

On average, FRAP, ACY, and TPH values had no significant changes in storage at 4°C or freezing for two weeks. These results are consistent with other studies [56, 65, 69]. However, fruit dried at 65°C significantly reduced FRAP by 56.6-73.2%, TPH by 47.6-69.3%, and ACY by 38.1-67.4% in comparison to fresh berries (Tables 2-4).

Variations of AC, ACY, and TPH in Leaves

AC (FRAP) and TPH contents in leaves were significantly (3 to 15 times) higher than in fruit of the same geno-

types which is consistent with previous studies [40]. However, the variation among of FRAP and TPH contents in leaves between the two harvest years were significantly greater than the variation attributed to genotypes: (Table 8). MS 343 and 'Tifblue' had the highest AC and TPH contents among the test genotypes.

Total Soluble Sugars

In general, there were no differences in soluble sugar among most genotypes, although 'Darrow's blueberry tended to have lower sugars (Table 9).

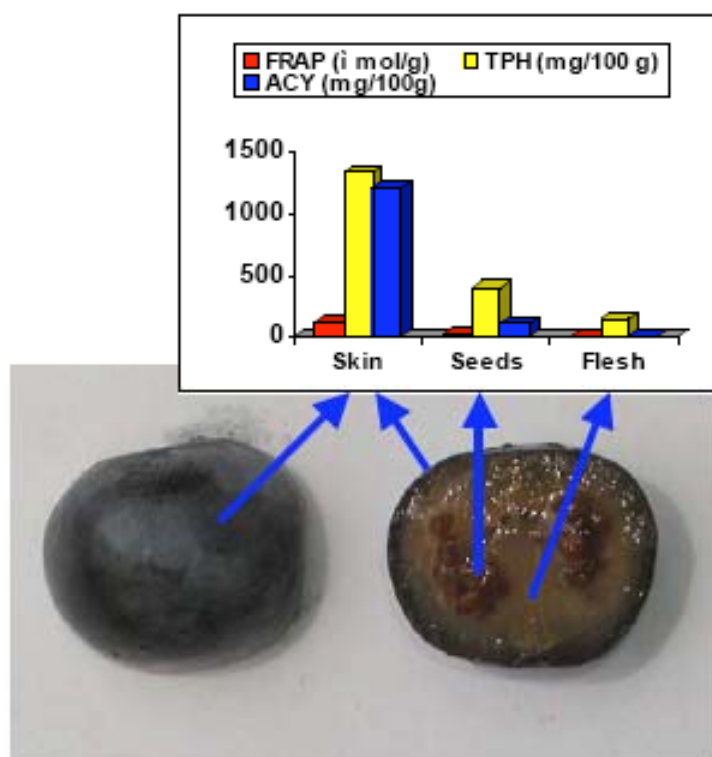
Table 4. Total Anthocyanins (ACY) of Berries of Some Species, Cultivars, and Selections of *Vaccinium* in Texas

Species/ Cultivars/Selections	ACY (mg/100g) (Mean ± SD)				
	Fresh (2010)	Fresh (2009)	Refrigerated (2009) (2 Weeks)	Frozen (2009) (2 Weeks)	Oven-Dried (2009) (2 Weeks)
<i>V. darrowii</i> (Darrow's)*	305.82 ± 25.43	516.86 ± 23.37	164.64 ± 8.50	424.00 ± 19.60	168.34 ± 21.72
<i>V. fuscatum</i> (black highbush)**	206.19 ± 45.24	-	-	-	-
<i>V. ashei</i> (rabbiteye) *	277.88 ± 54.46	392.92 ± 57.19	364.93 ± 95.82	326.97 ± 95.99	153.86 ± 40.53
'Brightwell'	213.63 ± 3.11	300.79 ± 30.81	372.33 ± 4.00	181.97 ± 6.60	141.36 ± 8.93
'Climax'	304.00 ± 2.22	419.31 ± 44.35	391.93 ± 10.56	327.56 ± 5.39	121.35 ± 6.34
'Tifblue'	277.68 ± 7.62	361.45 ± 5.62	245.42 ± 40.91	476.85 ± 9.24	101.32 ± 9.33
'Premier'	225.49 ± 3.69	463.48 ± 54.22	270.80 ± 18.11	315.63 ± 9.06	131.57 ± 9.64
'Powderblue'	370.28 ± 19.89	363.63 ± 59.86	388.82 ± 1.29	279.05 ± 0.23	152.83 ± 7.54
MS 63	281.43 ± 6.00	385.74 ± 42.85	413.57 ± 59.38	452.56 ± 75.91	203.49 ± 18.68
T 342	227.08 ± 5.69	473.66 ± 82.23	544.20 ± 95.48	301.91 ± 15.95	157.61 ± 25.69
T 38	323.42 ± 19.52	375.32 ± 41.23	292.33 ± 64.58	280.26 ± 18.51	221.32 ± 19.98
<i>V. corymbosum</i> hybrids (southern highbush) *	188.71 ± 42.64	264.88 ± 57.99	359.67 ± 55.24	276.85 ± 109.56	163.96 ± 77.05
MS 108	130.87 ± 3.69	210.27 ± 38.66	295.98 ± 15.43	296.75 ± 10.39	247.31 ± 12.56
MS 122	142.20 ± 1.18	258.64 ± 59.28	394.52 ± 1.31	375.10 ± 7.76	149.26 ± 14.69
MS 179	182.98 ± 6.34	-	-	-	-
MS 231	180.83 ± 5.88	325.74 ± 19.43	388.52 ± 83.15	158.70 ± 5.82	95.32 ± 8.65
MS 343	263.81 ± 4.86	-	-	-	-
MS 546	211.40 ± 3.62	-	-	-	-
MS 548	218.38 ± 6.72	-	-	-	-
MS 611	179.19 ± 5.71	-	-	-	-
Others					
'Organic'***	-	607.58 ± 105.92	-	-	-
'Driscolls'***	-	375.88 ± 4.64	-	-	-
'Naturipe'***	-	537.15 ± 10.71	-	-	-
Mean	237.56±7.72	398.37 ± 41.55	343.11 ± 33.56	322.53 ± 15.35	157.59 ± 13.65

*in cultivation; ** wild collected; *** purchased from a local grocery store; - no data collection.

Table 5. Antioxidant Activity, TPH, and ACY Contents of Different Size of Berries of ‘Tifblue’ and ‘Premier’

Plant Sample	Number/100g		FRAP ($\mu\text{mol/g}$) (mean \pm SD)		TPH (mg/100g) (mean \pm SD)		ACY (mg/100g) (mean \pm SD)	
	Big	Small	Big	Small	Big	Small	Big	Small
‘Tifblue’	90	180	76.45 \pm 1.06	101.98 \pm 7.46	505.26 \pm 14.45	623.20 \pm 29.74	242.27 \pm 10.43	267.15 \pm 5.70
#1	94	192	77.68 \pm 3.76	109.09 \pm 4.60	519.24 \pm 20.39	656.97 \pm 33.78	253.59 \pm 30.47	263.86 \pm 26.00
#2	120	189	75.89 \pm 4.26	102.62 \pm 3.99	490.39 \pm 15.33	611.74 \pm 20.73	233.05 \pm 27.80	263.86 \pm 11.93
#3	90	159	75.79 \pm 1.58	94.22 \pm 3.14	506.14 \pm 14.12	600.89 \pm 17.48	240.16 \pm 33.40	273.73 \pm 4.79
‘Premier’	83	171	56.54 \pm 5.51	71.65 \pm 2.38	397.63 \pm 37.89	513.91 \pm 14.08	234.10 \pm 8.23	249.64 \pm 9.71
#1	82	165	62.37 \pm 1.67	72.60 \pm 4.94	425.96 \pm 8.28	516.55 \pm 15.64	241.34 \pm 8.89	242.53 \pm 1.18
#2	90	185	55.84 \pm 4.50	73.41 \pm 2.11	412.34 \pm 6.23	526.48 \pm 15.94	225.15 \pm 23.74	245.69 \pm 8.97
#3	78	162	51.42 \pm 2.11	68.95 \pm 1.47	354.59 \pm 26.15	498.70 \pm 23.24	235.81 \pm 5.34	260.70 \pm 19.40

**Fig. (2).** Antioxidant capacity (AC) (in measurement of FRAP), total phenolics (TPH), and anthocyanins (ACY) contents in different parts of mature blueberries (photo by S.Y. Li).**Table 6.** Antioxidant Activity, TPH, and ACY Contents of Different Parts of Berries of ‘Powderblue’

Part	FRAP ($\mu\text{mol/g}$) (Mean \pm SD)	TPH (mg/100g) (Mean \pm SD)	ACY (mg/100g) (Mean \pm SD)
Seeds	20.23 \pm 0.80	398.83 \pm 53.79	113.76 \pm 8.80
Skin	118.14 \pm 3.41	1340.57 \pm 83.25	1210.33 \pm 146.49
Flesh	6.22 \pm 0.33	140.24 \pm 7.17	-*
Whole Berry	47.99 \pm 3.17	504.91 \pm 40.87	435.72 \pm 22.75

*not detectable; - no data collection.

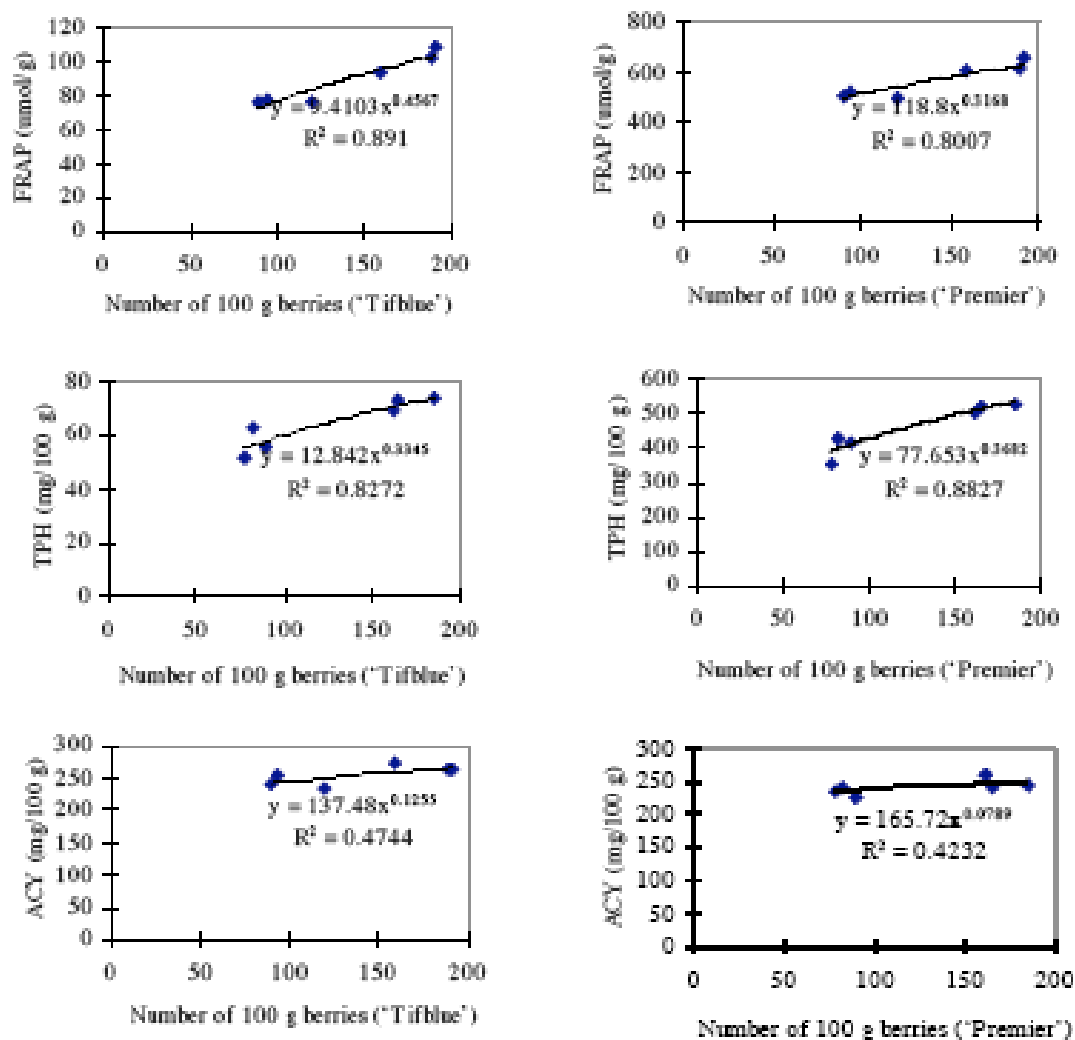


Fig. (3). 1a. The correlations between fruit size and antioxidant capacity (AC) (in measurement of FRAP) (1a: 'Tifblue'; 1b: 'Premier'), total phenolics (TPH) (2a: 'Tifblue'; 2b: 'Premier'), and total anthocyanins (ACY) (3a: 'Tifblue'; 3b: 'Premier'), respectively.

Table 7. Green Berries of Different Species, Cultivars, and Selections of *Vaccinium*

Species/ Cultivars/Selections	FRAP (µmol/g) (Mean ± SD)	TPH (mg/100g) (Mean ± SD)
<i>V. darrowii</i> (Darrow's)*	45.74 ± 1.97	552.94 ± 45.01
<i>V. fuscatum</i> (black highbush)**	62.81 ± 3.16	852.43 ± 106.64
<i>V. ashei</i> (rabbiteye) *		
'Climax'	57.27 ± 1.50	565.79 ± 26.93
'Tifblue'	52.61 ± 2.17	519.77 ± 25.86
'Premier'	42.62 ± 1.20	509.88 ± 19.44
'Powderblue'	64.76 ± 2.66	618.40 ± 40.37
Mean	54.30 ± 2.11	603.20 ± 44.04

*in cultivation; ** wild collected.

Table 8. Antioxidant Activity and TPH of Leaves of Some Species, Cultivars, and Selections of *Vaccinium* in Texas

Species/ Cultivars/Selections	FRAP (Mmol/g) (Mean \pm SD)		TPH (mg/100g) (Mean \pm SD)	
	2010	2009	2010	2009
<i>V. darrowii</i> (Darrow's)*	694.24 \pm 60.06	582.59 \pm 10.80	6092.34 \pm 848.02	1952.28 \pm 39.04
<i>V. arboreum</i> (sparkleberry) **	549.90 \pm 60.65	401.53 \pm 7.03	4927.25 \pm 240.41	3674.25 \pm 456.45
<i>V. fuscatum</i> (black highbush)**	353.77 \pm 38.89	353.32 \pm 35.50	3759.40 \pm 133.64	2756.79 \pm 534.52
<i>V. ashei</i> (rabbiteye) *				
'Brightwell'	936.43 \pm 27.61	567.95 \pm 30.42	7135.22 \pm 105.14	1939.58 \pm 47.20
'Climax'	971.27 \pm 134.40	512.14 \pm 17.57	7828.47 \pm 640.75	1747.52 \pm 38.60
'Tifblue'	1008.34 \pm 40.17	521.22 \pm 77.45	7143.04 \pm 130.34	1922.11 \pm 102.74
'Premier'	910.28 \pm 32.19	449.23 \pm 35.89	6460.05 \pm 344.19	2044.27 \pm 53.39
'Powderblue'	659.43 \pm 52.51	463.36 \pm 11.18	6456.77 \pm 376.33	2007.51 \pm 17.53
MS 63	725.05 \pm 19.97	774.96 \pm 14.24	5949.12 \pm 162.52	1998.11 \pm 58.81
T 38	924.44 \pm 30.20	555.98 \pm 16.99	7946.80 \pm 410.56	1936.62 \pm 44.47
<i>V. corymbosum</i> hybrids (southern highbush) *				
MS 108	706.01 \pm 34.66	583.01 \pm 45.75	4955.25 \pm 240.65	1679.26 \pm 117.32
MS 122	710.88 \pm 29.72	557.53 \pm 51.08	4218.20 \pm 287.88	2214.91 \pm 76.67
MS 179	300.03 \pm 7.25		2826.53 \pm 161.46	
MS 231	866.83 \pm 20.85	233.86 \pm 17.25	6603.79 \pm 450.79	1922.60 \pm 52.96
MS 343	1183.22 \pm 59.29	634.06 \pm 22.24	9941.88 \pm 513.20	1980.64 \pm 63.70
MS 546	604.51 \pm 25.49	-	6194.00 \pm 682.68	-
MS 548	448.10 \pm 72.22	-	4084.06 \pm 160.48	-
MS 611	803.58 \pm 49.03	-	6613.18 \pm 199.90	-
Mean	783.18 \pm 43.75	530.97 \pm 30.37	6279.08 \pm 354.97	2283.82 \pm 147.64

*in cultivation; ** wild collected; - no data collection.

Table 9. Soluble Sugar Contents in Mature Fruits of Different Species, Cultivars, and Selections of *Vaccinium*

Species/ Cultivars/Selections	Soluble Sugars (%, w/w, Mean \pm SD)	
	2010	2009
<i>V. darrowii</i> (Darrow's)*	10.28 \pm 0.83	12.86 \pm 1.15
<i>V. ashei</i> (rabbiteye) *		
'Brightwell'	13.96 \pm 0.65	12.00 \pm 1.64

Table 9. Contd....

Species/ Cultivars/Selections	Soluble Sugars (%, w/w, Mean \pm SD)	
	2010	2009
'Climax'	12.65 \pm 0.86	12.70 \pm 0.29
MS 108	14.00 \pm 0.58	14.68 \pm 1.56
'Tifblue'	12.90 \pm 1.41	12.55 \pm 1.12
'Premier'	12.56 \pm 1.23	13.24 \pm 1.24
'Powderblue'	9.36 \pm 1.09	12.48 \pm 1.48
MS 63	14.49 \pm 1.19	15.97 \pm 1.28
T 38	13.73 \pm 0.88	12.99 \pm 1.82
<i>V. corymbosum</i> hybrids (southern highbush) *		
MS 122	12.91 \pm 0.75	14.70 \pm 0.31
MS 179	11.10 \pm 1.71	
MS 231	13.21 \pm 0.49	13.99 \pm 1.14
MS 343	13.51 \pm 0.92	13.35 \pm 1.06
MS 546	15.36 \pm 1.23	-
MS 548	15.14 \pm 2.10	-
MS 611	14.42 \pm 0.82	-

* in cultivation; - no data collection.

CONCLUSIONS

There were significant variations in AC (as measured by FRAP), TPH, and ACY contents in fruits among different genotypes, but less variation was detected among individual plants of the same genotype. Our results from analysis of rabbiteye blueberries support the hypothesis [33] that AC was more highly correlated to TPH than to ACY. Our analysis of six species/cultivars indicated that AC of fruits decreases during ripening but TPH contents tend to increase with maturity. Our findings are consistent with previous reports [56, 65, 69] that AC, TPH, and ACY contents of berries do not change significantly during storage at 4°C or after freezing for two weeks, but decreases when dried at 65°C for 48 h or longer. AC and TPH contents in leaves of each test genotype were 3 to 15 times higher than in fruit of the same genotypes. MS 63 had the highest antioxidant activity and sugar levels in fruits, while MS 343 and 'Tifblue' were found to have the highest AC and TPH contents among the genotypes tested. These data could provide valuable information for the development of antioxidant pharmaceutical products.

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ABBREVIATIONS

AC	=	Antioxidant capacity
ACY	=	Anthocyanins
FRAP	=	Ferric reducing antioxidant power
ORAC	=	Oxygen radical absorbance capacity
TPH	=	Total phenolics
TPTZ	=	2,4,6-Tripyridyl-s-triazine

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