



Fruit quality, anthocyanin and total phenolic contents, and antioxidant activities of 45 blueberry cultivars grown in Suwon, Korea

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Abstract: Blueberry fruits from 45 commercial cultivars (39 northern highbush and 6 half highbush blueberry) grown in Suwon, Korea were analyzed for fruit size, soluble solids content, titratable acidity, total anthocyanin content, total phenolic content, and antioxidant activity. Fruit characteristics varied widely among the 45 blueberry cultivars. Fruit weight ranged from 0.9 to 3.6 g, soluble solids content from 8.3 to 14.3 °Brix, and titratable acidity from 0.8% to 3.6%. Antioxidant activity ranged from 0.7 to 2.1 mg of quercetin equivalents per gram of fresh berries in different blueberry cultivars. Among the 45 blueberry cultivars, high amounts of anthocyanins and polyphenols, and high antioxidant activity were observed in 'Elliott', 'Rubel', 'Rancocas', and 'Friendship'.

Key words: Northern highbush blueberry, Half highbush blueberry, Functional food, Fruit quality
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1 Introduction

The blueberry belongs to the genus *Vaccinium* and the subgenus *Cyanococcus* (Gough, 1991). In general, highbush blueberries (*V. corymbosum* L.) and rabbiteye blueberries (RB, *V. ashei* Reade) are considered commercially important blueberry types (Eck and Childers, 1966; Darnell, 2006). Highbush blueberries can be divided into three types: northern highbush blueberry (NHB), half highbush blueberry (HHB), and southern highbush blueberry (SHB).

In Korea, blueberry cultivation began in the year 2000 and has grown rapidly with increasing consumer acceptability of functional foods. The cultivation area has doubled from 546 ha in 2010 to 1042 ha in 2011

(Kim, 2011). The main cultivated blueberries in Korea are NHB and HHB cultivars. Other blueberry cultivars are planted on a small scale, especially in the southern part of Korea where they are protected from winter injury.

Blueberries are known to contain high amounts of phenolic compounds, including anthocyanins, chlorogenic acids, flavonols, and procyanidins, resulting in high antioxidant activity that provides health benefits, and conferring on blueberries the title of a functional food (Cho *et al.*, 2004; Huang *et al.*, 2012). Phenolic compounds and anthocyanins in fruit prevent or reduce the incidence of chronic diseases, such as cardiovascular disease and Alzheimer's disease (Joshapura *et al.*, 2001; Huxley and Neil, 2003; Castrejón *et al.*, 2008; Krikorian *et al.*, 2010). In North America, the use of blueberries as food and medicine has long been popular (Kalt and Dufour, 1997).

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Although many studies have investigated the fruit quality and antioxidant properties of blueberries (Wang *et al.*, 1997; Ehlenfeldt and Prior, 2001; Moyer *et al.*, 2002; Connor *et al.*, 2002a; 2002b; Kalt *et al.*, 2003), little is known about variation in fruit quality, levels of anthocyanins and phenolic compounds, and antioxidant activities in different blueberry cultivars grown in Korea. Saftner *et al.* (2008) reported that instrumental and sensory qualities of blueberries are affected by many factors, including climatic conditions, growing locations, growing seasons, and cultivars (Prior *et al.*, 1998; Connor *et al.*, 2002a; Howard *et al.*, 2003). Interestingly, variation in chemical components among cultivars was much greater than that reported between growing seasons (Howard *et al.*, 2003).

Expanding our knowledge of fruit quality, anthocyanin and polyphenol contents, and antioxidant activity in blueberries grown in Korea will benefit growers and allow them to choose the best blueberry cultivars. Therefore, this study aimed to evaluate these factors in 45 blueberry cultivars grown in Suwon, Korea.

2 Materials and methods

2.1 Materials

Fully ripe fruit samples, as judged by fully blue color at the scar, were collected in 2010 at the National Institute of Horticultural & Herbal Science (NIHHS) in Suwon, Korea. About 500 g of fruits were collected from each of 45 commercial cultivars. Three replicates were collected per cultivar (from three mature plants per cultivar). Fruits were placed in a clamshell container and transported under refrigerated conditions to the NIHHS within 1 h. Fruit samples were stored at -70°C until analyzed. Quercetin, gallic acid, 2,4,6-tri(2-pyridyl)-1,3,5-triazine (TPTZ), ferric chloride (FeCl_3), and ferric reducing antioxidant power (FRAP) were purchased from Sigma-Aldrich (St. Louis, MO, USA). All reagents were of analytical grade.

2.2 Evaluation of fruit quality

Damaged berries were discarded from harvested fruit, and the fresh weight (FW) of 20 blueberry fruits from each cultivar was measured using a digital bal-

ance. Ten fruits, randomly collected from each cultivar, were placed in cheesecloth and pressed to make juice; this was carried out three times such that three replicates for each cultivar were measured. The soluble solids content (SSC) of the juice was measured using a hand refractometer (N1, Atago, Tokyo, Japan). The titratable acidity (TA) of fruit juice was assayed by titration with 0.05 mol/L NaOH. Firmness measurements were obtained using a 5-mm flat-tipped probe and applying the probe perpendicularly at 10 mm/s to the each of 20 fruits with skin, using a rheometer (LF Plus, Lloyd Instruments Ltd., England). The blueberries were placed on their sides with the stem end to the left and the calyx end to the right. Firmness results are reported as the mean force (N) of penetration.

2.3 Extraction

Fruit extraction for measuring total anthocyanin and total phenol contents and antioxidant activity was carried out following the modified method of Barnes *et al.* (2009). Five grams of pooled blueberry samples (10 fruits) were homogenized with 5 ml of a mixture of ethanol, distilled water, and HCl (70:30:1, v/v/v). The homogenate was then centrifuged at $14000\times g$ for 20 min at 4°C (VS-550, Vision Scientific, Korea). The extraction was repeated twice with three replications. The supernatants were combined, and the final volume was increased to 20 ml with extraction solution.

2.4 Total anthocyanin content

Total anthocyanin content was determined following the method of Connor *et al.* (2002b). Extract solution was diluted (1:99, v/v) in acidified methanol to obtain an absorbance between 0.200 and 1.000 at 530 nm. The values are expressed as mg cyanidin-3-glucoside equivalents per 100 g FW using a molar extinction coefficient of 29600.

2.5 Total phenolic content

Total phenolic content was determined following the modified method of Singleton and Rossi (1965). Briefly, 1 ml of 1 mol/L Folin-Denis reagent was added to 1 ml of the extract. Five minutes after mixing, 1 ml of a saturated sodium carbonate solution and 8 ml of distilled water were added. After a 2-h incubation, the absorbance was measured at 725 nm with a spectrophotometer (U best-30, JASCO Co.,

Tokyo, Japan). The values are expressed in gallic acid equivalents using a gallic acid (0–0.1 mg/ml) standard curve.

2.6 Antioxidant activity

Antioxidant activity was measured by the FRAP method following the procedure of Doshi *et al.* (2006). A volume of 100 µl of the extract, appropriately diluted with 10% methanol, was added to 1 ml FRAP assay solution, which was prepared by mixing 25 ml of 300 mmol/L acetate buffer (pH 3.6), 2.5 ml of TPTZ solution (10 mmol/L TPTZ in 40 mmol/L HCl), and 2.5 ml of 20 mmol/L FeCl₃. Antioxidant activity was calculated as the difference in absorbance at 593 nm after 0 and 6 min measured using a spectrophotometer (Agilent-8453, Agilent, USA). The values are expressed in quercetin equivalents using a quercetin (0–0.5 mg/ml) standard curve.

2.7 Statistical analysis

Fruit constituents were evaluated in a completely randomized design with three replications. Differences were considered significant if *P*-values were under 0.05, and means were compared by Tukey's test using SPSS program version 12.0 (SPSS Inc., Chicago, IL, USA).

3 Results and discussion

3.1 Fruit characteristics of 45 blueberry cultivars

The average fruit weight was 2.0 g (range, 0.9–3.6 g) for NHB and 1.6 g (range, 0.8–2.2 g) for HHB (Table 1). Fruit weight varied considerably among cultivars. The large fruited cultivars (≥ 3 g/fruit) were 'Sunrise', 'Brigitta', 'Herbert', 'Toro', and 'Nui' from the NHB group. A prior study of 64 blueberry cultivars grown in Tokyo also showed significant variation in fruit weight among cultivars (Che *et al.*, 2009).

The SSC of fruit of the cultivars ranged from 8.3 to 14.3 °Brix (Table 1). The highest SSC (14.3 °Brix) was observed in 'Friendship' from the HHB group. The TA of blueberry juice at harvest showed large variation among cultivars, ranging from 0.8% to 3.6% (Table 1). The TA was highest in 'Sunrise' (3.6%) and lowest in 'Bluecrop' (0.8%). Fruit firmness

ranged from 3.5 N ('Friendship' from the HHB group) to 12.4 N ('Nui' from the NHB group) among the 45 cultivars. Similar results were obtained for 64 blueberry cultivars grown in Tokyo (Che *et al.*, 2009).

3.2 Functional constituents of blueberries

The anthocyanin content of blueberries is an indicator of the nutrient quality of the fruit as a functional food, compared to other nutrients in fruit (Kalt and Dufour, 1997). Anthocyanins are found in various fruits, including blackberries, blackcurrants, and blueberries (Moyer *et al.*, 2002; Huang *et al.*, 2012). They have also been reported to contribute to antioxidant activity in fruit (Kalt and Dufour, 1997; Moyer *et al.*, 2002). The total anthocyanin content of each of the 45 blueberry cultivars in our study is presented in Table 2. A large variation was observed among cultivars for total anthocyanin content, ranging from 167.6 to 677.8 mg cyanidin-3-glucoside per 100 g FW. The highest total anthocyanin content was recorded in 'Friendship' at 677.8 mg cyanidin-3-glucoside per 100 g FW. 'Elliott' was also a good source of anthocyanin (Table 2). Dragović-Uzelac *et al.* (2010), however, reported that the cultivar 'Sierra' contained more total anthocyanin than 'Elliot' in blueberries grown in Croatia. Overall, Cho *et al.* (2004) reported total anthocyanin contents (143–822 mg cyanidin-3-glucoside per 100 g FW) similar to the values we observed (167.6–677.8 mg cyanidin-3-glucoside per 100 g FW), whereas other researchers reported lower values (93.1–235.4 mg cyanidin-3-glucoside per 100 g FW) (Prior *et al.*, 1998). The observed differences were probably due to different extraction methods, environmental growing conditions, and genotypes. Indeed, environmental growing conditions can affect the ability of blueberries to synthesize anthocyanin (Howard *et al.*, 2003). Kalt *et al.* (2001) also reported that syntheses of anthocyanins and phenolic compounds can be influenced by biotic and abiotic factors, such as irradiation, temperature, and pathogen attacks.

The total phenolic content plays an important role in antioxidant activities, as measured by FRAP, 2,2-diphenyl-1-picrylhydrazyl (DPPH), and 2,2'-azino-bis(3-ethylbenzothiazoline-6-sulphonic acid) (ABTS) methods (Connor *et al.*, 2002b), and is thought to be influenced more by genotype than by environmental

Table 1 Physicochemical properties of 45 blueberry cultivars grown in Suwon, Korea

Genotype	Cultivar	Harvest date*	FW [#] (g)	SSC ^Δ (°Brix)	TA ^Δ (%)	pH ^Δ	Firmness (N) [#]	
Northern highbush	Berkeley	Jul. 7	2.20 d-f	12.1 a-g	0.89 l-s	3.8 a-e	5.7 n-u [▲]	
	Bluecrop	Jul. 7	1.83 g-j	9.8 f-h	0.78 q-s	4.0 a-d	6.5 h-q	
	Bluegold	Jul. 7	1.96 f-i	9.8 f-h	2.0 f-i	3.6 b-e	6.9 f-n	
	Bluehaven	Jun. 29	1.96 f-i	11.0 b-h	2.0 f-i	3.4 b-e	5.2 p-v	
	Bluejay	Jun. 24	2.45 cd	11.5 a-g	2.5 cd	3.5 b-e	6.1 i-s	
	Blueray	Jun. 29	2.47 cd	10.3 c-h	2.5 cd	3.4 b-e	6.2 i-s	
	Bluetta	Jun. 22	2.02 e-g	10.1 d-h	2.0 e-g	3.3 b-e	5.8 k-t	
	Brigitta	Jul. 12	3.42 ab	11.7 a-g	3.4 ab	3.5 b-e	7.6 d-h	
	Burlington	Jul. 7	1.66 h-k	12.9 a-e	1.7 h-k	3.6 b-e	6.2 i-s	
	Collins	Jun. 24	1.83 g-j	10.9 c-h	1.8 g-j	3.5 b-e	7.7 d-h	
	Coville	Jul. 1	1.64 i-k	11.5 a-g	1.6 i-k	3.9 a-d	5.8 k-t	
	Croatan	Jun. 25	1.38 k-m	12.3 a-g	1.4 k-m	4.2 a-c	4.9 s-v	
	Darrow	Jun. 29	2.27 d-f	10.6 c-h	2.3 d-f	3.0 de	6.8 g-o	
	Dixi	Jul. 7	2.51 cd	11.8 a-g	2.5 c-d	3.8 a-e	5.1 q-v	
	Duke	Jun. 24	1.81 g-j	9.7 f-h	1.8 g-j	3.9 a-e	9.0 bc	
	Earliblue	Jun. 22	2.21 d-f	11.6 a-g	2.2 d-f	2.8 e	7.1 e-k	
	Elliott	Jul. 27	1.83 g-j	10.5 c-h	1.8 g-j	3.4 b-e	5.6 n-u	
	Elizabeth	Jul. 6	2.26 d-f	13.1 a-d	2.3 d-f	3.9 a-e	7.6 d-h	
	Evelyn	Jun. 29	1.42 kl	12.6 a-f	1.4 kl	3.5 b-e	5.7 l-t	
	Herbert	Jul. 1	3.17 b	10.5 c-h	3.2 b	3.7 b-e	4.1 vw	
	Jersey	Jul. 7	1.96 f-i	10.8 c-h	2.0 f-i	3.7 b-e	7.0 e-l	
	Lateblue	Jul. 27	2.46 cd	10.6 c-h	2.5 cd	3.2 e	6.2 i-r	
	Meader	Jun. 29	2.28 d-f	9.7 f-h	2.3 d-f	3.5 b-e	7.4 e-i	
	Nelson	Jun. 29	2.75 c	10.7 c-h	2.7 c	3.9 a-e	4.4 u-w	
	Nui	Jun. 24	3.13 b	10.3 c-h	3.1 b	3.3 b-e	12.4 a	
	Olympia	Jun. 29	1.59 jk	11.7 a-g	1.6 jk	0.6 f	6.2 i-s	
	Pacific	Jun. 29	1.05 mn	10.0 e-h	1.0 mn	4.1 a-d	3.9 vw	
	Patriot	Jun. 24	2.32 de	9.8 f-h	2.3 de	3.3 b-e	9.8 b	
	Pioneer	Jul. 6	0.93 n	12.0 a-g	0.9 n	4.3 ab	4.5 t-w	
	Puru	Jun. 24	1.53 jk	11.3 a-h	1.5 j-k	3.5 b-e	7.2 e-j	
	Rancocas	Jul. 7	1.34 k-m	12.3 a-g	1.3 k-m	4.5 a	8.0 c-g	
	Reka	Jun. 25	1.65 i-k	10.7 c-h	1.6 i-k	3.4 b-e	8.2 c-f	
	Rubel	Jun. 29	1.06 mn	13.2 a-c	1.1 mn	3.4 b-e	6.5 h-p	
	Sierra	Jun. 29	2.40 cd	11.5 a-g	2.4 cd	3.3 b-e	8.2 c-e	
	Spartan	Jun. 22	1.57 jk	12.3 a-g	1.6 jk	3.9 a-d	5.9 j-s	
	Sunrise	Jul. 6	3.58 a	12.7 a-f	3.6 a	3.5 b-e	8.9 b-d	
	Toro	Jul. 7	3.14 b	9.3 gh	3.1 b	3.9 a-e	4.9 r-v	
	Washington	Jul. 6	1.82 g-j	10.9 c-h	1.8 g-j	3.7 a-e	8.2 c-e	
	Weymouth	Jun. 25	1.31 k-m	11.4 a-h	1.3 k-m	3.3 b-e	5.5 o-u	
	Half highbush	Chippewa	Jul. 7	2.01 e-h	8.3 h	0.99 k-r	3.5 b-e	4.1 vw
		Friendship	Jun. 29	1.08 l-n	14.3 a	2.14 a	3.4 b-e	3.5 w
		Northblue	Jun. 24	2.21 d-f	9.7 f-h	1.05 i-r	3.3 b-e	7.0 e-m
		Northcountry	Jun. 24	0.86 n	14.0 ab	1.32 c-j	3.5 b-e	5.7 m-t
		Northland	Jun. 25	1.65 i-k	9.8 f-h	1.32 c-j	3.6 b-e	6.1 j-s
		Polaris	Jun. 24	1.82 g-j	11.8 a-g	1.26 c-k	3.1 de	6.9 f-n

*The first harvest date for mature, fully colored, unblemished fruit; [#] Values represent the mean of 20 fruits; ^Δ Values represent the mean of three replicates using 10 fruits each; [▲] Means followed by different letters in the same column are significantly different at $P < 0.05$, with comparisons performed using Tukey's test

Table 2 Total anthocyanin and total phenolic contents and antioxidant activities of 45 blueberry cultivars grown in Suwon, Korea

Genotype	Cultivar	Harvest date*	Total anthocyanin content (mg cyanidin-3-glucoside/100 g FW) [#]	Total phenolic content (mg gallic acid/100 g FW) [#]	Antioxidant activity (mg quercetin/g FW) [#]	
Northern highbush	Berkeley	Jul. 7	250.5 d-i	195.1 m-o	0.89 l-s [▲]	
	Bluecrop	Jul. 7	227.2 f-i	205.2 k-o	0.78 q-s	
	Bluegold	Jul. 7	308.1 c-i	289.8 e-j	1.37 c-h	
	Bluehaven	Jun. 29	206.4 hi	195.0 m-o	0.89 m-s	
	Bluejay	Jun. 24	243.5 e-i	189.4 no	0.83 o-s	
	Blueray	Jun. 29	225.1 f-i	200.2 m-o	0.87 n-s	
	Bluetta	Jun. 22	267.1 d-i	206.4 k-o	1.02 j-r	
	Brigitta	Jul. 12	215.5 hi	173.8 o	0.86 n-s	
	Burlington	Jul. 7	361.8 c-g	340.0 c-e	1.56 bc	
	Collins	Jun. 24	265.5 d-i	302.4 d-g	1.41 c-g	
	Coville	Jul. 1	242.6 e-i	188.8 no	0.97 k-s	
	Croatan	Jun. 25	285.4 c-i	262.3 e-n	1.25 d-k	
	Darrow	Jun. 29	262.8 d-i	201.5 l-o	1.03 i-r	
	Dixi	Jul. 7	234.4 e-i	219.4 h-o	1.00 k-r	
	Duke	Jun. 24	250.8 d-i	217.6 h-o	0.80 q-s	
	Earliblue	Jun. 22	302.9 c-i	266.6 e-n	0.97 k-s	
	Elliott	Jul. 27	552.7 ab	434.5 b	1.72 b	
	Elizabeth	Jul. 6	222.1 g-i	229.9 g-o	0.86 n-s	
	Evelyn	Jun. 29	376.0 c-e	273.3 e-m	1.24 d-k	
	Herbert	Jul. 1	191.0 hi	170.9 o	0.66 s	
	Jersey	Jul. 7	227.4 f-i	190.4 no	0.77 rs	
	Lateblue	Jul. 27	325.2 c-h	249.1 f-o	1.14 f-o	
	Meadar	Jun. 29	246.0 e-i	216.9 i-o	0.76 rs	
	Nelson	Jun. 29	255.0 d-i	209.0 j-o	0.82 p-s	
	Nui	Jun. 24	263.5 d-i	232.0 f-o	0.97 k-s	
	Olympia	Jun. 29	298.6 c-i	283.5 e-l	1.08 h-q	
	Pacific	Jun. 29	278.5 c-i	299.9 d-h	1.11 g-p	
	Patriot	Jun. 24	208.7 hi	210.3 i-o	1.03 i-r	
	Pioneer	Jul. 6	312.9 c-h	259.8 e-n	1.20 e-l	
	Puru	Jun. 24	223.4 g-i	223.9 g-o	1.03 i-r	
	Rancocas	Jul. 7	416.3 bc	385.7 bc	1.50 b-e	
	Reka	Jun. 25	241.7 e-i	251.8 f-o	1.02 i-r	
	Rubel	Jun. 29	419.4 bc	375.0 b-d	1.53 b-d	
	Sierra	Jun. 29	286.3 c-i	260.7 e-n	1.16 f-n	
	Spartan	Jun. 22	274.4 c-i	266.3 e-n	1.01 j-r	
	Sunrise	Jul. 6	394.6 cd	313.7 c-f	1.43 b-f	
	Toro	Jul. 7	167.6 i	187.6 no	0.76 rs	
	Washington	Jul. 6	368.9 c-f	314.5 c-f	1.33 c-i	
	Weymouth	Jun. 25	226.7 f-i	291.7 e-i	1.19 f-m	
	Half highbush	Chippewa	Jul. 7	217.8 g-i	230.0 g-o	0.99 k-r
		Friendship	Jun. 29	677.8 a	523.8 a	2.14 a
		Northblue	Jun. 24	256.4 d-i	247.8 f-o	1.05 i-r
		Northcountry	Jun. 24	281.8 c-i	313.5 c-f	1.32 c-j
		Northland	Jun. 25	319.4 c-h	287.1 e-k	1.32 c-j
		Polaris	Jun. 24	280.0 c-i	263.1 e-n	1.26 c-k

*The first harvest date for mature, fully colored, unblemished fruit; [#]Values represent the mean of three replicates using 10 fruits each;[▲] Means followed by different letters in the same column are significantly different at $P < 0.05$, with comparisons performed using Tukey's test

conditions (Connor *et al.*, 2002a). Generally, antioxidant activity has been shown to correlate with total phenolic content (Ehlenfeldt and Prior, 2001; Connor *et al.*, 2002b). In our study, total phenolic content ranged from 170.9 to 434.5 mg gallic acid per 100 g FW in the NHB group and from 230.0 to 523.8 mg gallic acid per 100 g FW in the HHB group. The differences in total phenolic contents between some cultivars were statistically significant, similar to previous reports (Ballington *et al.*, 1984; Dragović-Uzelac *et al.*, 2010). ‘Friendship’ contained the highest amount of total phenolic compounds at 523.8 mg gallic acid per 100 g FW. Among the NHB cultivars, ‘Elliott’, ‘Rancocas’, and ‘Rubel’ contained high amounts of total phenolic compounds. Ehlenfeldt and Prior (2001) reported similar results.

The antioxidant activity of fruit is an indication of the fruit’s functional value (Rice-Evans *et al.*, 1996). Antioxidant activity measured by FRAP assay depends on the redox potential of the phenolic compounds found in the sample and the structural properties of these compounds (Pulido *et al.*, 2000). In this study, the antioxidant activity of 45 blueberry cultivars showed large variation, ranging from 0.7 to 2.1 mg quercetin/g FW (Table 2). Scalzo *et al.* (2005) and Prior *et al.* (1998) also reported that antioxidant activity differed among cultivars.

The antioxidant activity of ‘Friendship’ was the highest among the 45 blueberry cultivars examined. Our results were similar to those of previous studies, which showed that small berries contained proportionally more anthocyanins and phenolic compounds than large berries, being concentrated in the epidermal tissue (Connor *et al.*, 2002a; Howard *et al.*, 2003). Among the NHB cultivars, ‘Elliot’, ‘Burlington’, ‘Rubel’, and ‘Rancocas’ had the highest antioxidant activity. ‘Herbert’ had the lowest antioxidant activity among all 45 blueberry cultivars. Similar results were obtained in different cultivation areas (Ehlenfeldt and Prior, 2001; Connor *et al.*, 2002a; Howard *et al.*, 2003).

Based on the above results, fruit quality profiles were varied among 45 blueberry cultivars. We conclude that blueberry cultivars grown in Korea are good sources of natural antioxidants and contain large amounts of polyphenolic compounds and anthocyanins.

Compliance with ethics guidelines

Jin Gook KIM, Hong Lim KIM, Su Jin KIM, and Kyo-Sun PARK declare that they have no conflict of interest.

This article does not contain any studies with human or animal subjects performed by any of the authors.

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