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Fruit Quality, Antioxidant Capacity, and Flavonoid Content of Organically and Conventionally Grown Blueberries

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The effect of cultivation practices on fruit quality and antioxidant capacity in highbush blueberries var. Bluecrop (*Vaccinium corymbosum* L.) was evaluated from random samples of commercial late harvest fields in New Jersey. Results from this study showed that blueberry fruit grown from organic culture yielded significantly higher sugars (fructose and glucose), malic acid, total phenolics, total anthocyanins, and antioxidant activity (ORAC) than fruit from the conventional culture. In organically cultured fruit, the average values for the ORAC, total anthocyanins, and total phenolic content were 46.14 μmol of Trolox (TE)/g of fresh weight (fwt), 131.2 mg/100 g of fwt, and 319.3 mg/100 g of fwt, respectively. In conventionally cultured fruit, the average values for the ORAC, total anthocyanin, and total phenol content were 30.8 μmol of TE/g of fwt, 82.4 mg/100 g of fwt, and 190.3 mg/100 g of fwt, respectively. The organic culture also produced fruit with higher contents of myricetin 3-arabinoside, quercetin 3-glucoside, delphinidin 3-galactoside, delphinidin 3-glucoside, delphinidin 3-arabinoside, petunidin 3-galactoside, petunidin 3-glucoside, and malvidin 3-arabinoside than conventional culture. There was a significant correlation between the ORAC values and total phenolics and total anthocyanins. These results indicate that even though there were variations in phytonutrient content among individual farms within each cultural system, significant differences between two cultivation practices were evident.

KEYWORDS: ***Vaccinium corymbosum*; antioxidant activity; anthocyanin; total phenolic; flavonoid; sugar; organic acid; cultural system**

INTRODUCTION

The demand for organic food products has increased rapidly during recent years, partially due to the notion that health benefits are linked with the consumption of organic products (*1*). Organic produce is perceived to be more nutritious, better tasting, and environmentally friendlier, compared to conventionally grown crops (*2, 3*). However, research results are inconclusive, and there is insufficient evidence to claim differences in nutritional value related to cultural methods (*4*). Reports supporting both sides can be found in the literature. Asami et al. (*5*) showed that corn grown organically had 52% more ascorbic acid and significantly more polyphenols than conventionally grown corn. Organically grown tomatoes also contain higher levels of beneficial flavonoids (quercetin and kaempferol aglycones) (*6*). Olsson et al. (*7*) also found that the ratio of ascorbate to dehydroascorbate was significantly higher in the organically cultivated strawberries. The extracts from organically grown strawberries had a higher antiproliferative activity for both HT29 cells and MCF-7 cells than those from the conventionally grown fruit. Thus, the organically grown strawberries might have a higher content of secondary metabolites than the conventional strawberries. However, others claim the opposite, and many doubt that there is any difference between the two cultural methods (*1, 8–10*). Weibel et al. (*9*) showed that internal fruit quality of organic apples was either similar or slightly better than that of conventional fruit. Additionally, they did not find significant differences in fruit total vitamin C content between the two production systems. Magkos et al. (*10*) also stated that little or no hard evidence of differences in vitamin content and no significant differences with respect to minerals could be identified. Comparable total polyphenol content and similar antigenotoxic potential were found in organically and conventionally grown apples (*11*). Human consumption of either organically or conventionally grown apples did not make any difference in antioxidant capacity of

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Table 1. Cultural Systems (Organic and Conventional) and Origin of Blueberry Fruit Samples Used in This Study

farm	cultivation method	city, county in New Jersey
001	organic	Wall, Monmouth
002	organic	New Egypt, Plumsted Branch Ocean
003	organic	Clarksburg, Monmouth
004	organic	Hammonton, Atlantic
005	organic	Hammonton, Atlantic
006	conventional	Hammonton, Atlantic
007	conventional	Hammonton, Atlantic
008	conventional	Hammonton, Atlantic
009	conventional	Hammonton, Atlantic
010	conventional	Hammonton, Atlantic

low-density lipoproteins, endogenous DNA strand breaks, Fpg protein-sensitive sites, or capacity to protect against DNA damage caused by hydrogen peroxide (*11*). There have been a number of excellent reviews contrasting organic with conventionally grown foods on nutritional quality (*1, 8–10*).

Epidemiological studies have shown that diets rich in fruits and vegetables are associated with longer life expectancy, and these beneficial effects may be due to rich antioxidants contained in these produce (*12*). Fruits and vegetables have shown a remarkably high scavenging activity toward chemically generated radicals (*13*). They are effective in inhibiting oxidation of human low-density lipoproteins and thus have potential effects in preventing various human diseases (*12–14*).

Blueberries have been known to have a high content of antioxidants (*15, 16*), thus making them effective in inhibiting oxidation of human low-density lipoproteins and preventing or alleviating various human diseases caused by oxidative stress (*14*). Differences in content of various antioxidants and other flavonoids affecting health could result from differences in organic and conventional farming. However, no research has been done to compare fruit quality, antioxidant capacity, and content of flavonoids in organically and conventionally grown blueberries. The objectives of this investigation were to evaluate the effects of cultural systems, organic or conventional, on sugar, organic acid, anthocyanin, and phenolic contents and antioxidant activity as well as flavonoid content in blueberries.

MATERIALS AND METHODS

Chemicals. 2′,2′-Azobis(2-amidinopropane) dihydrochloride (AAPH) was obtained from Wako Chemicals USA Inc. (Richmond VA). 6-Hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid (Trolox) and fluorescein disodium were obtained from Aldrich Chemical Co. (Milwaukee, WI). Acetonitrile, methanol, acetone, and water were of HPLC grade and were purchased from Baxter (Muskegon, MI). Chlorogenic acid, resveratrol, and myricetin 3-arabinoside were obtained from Sigma Chemical Co. (St. Louis, MO). Quercetin 3-galactoside, quercetin 3-glucoside, cyanidin 3-galactoside, malvidin 3-galactoside, and malvidin 3-glucoside were obtained from Indofine Chemical Co., Inc. (Somerville, NJ). Delphinidin 3-glucoside and petunidin 3-glucoside were obtained from Polyphenols Laboratories AS (Sandners, Norway). Other authentic standards were obtained from Sigma Chemical Co. and Fisher Scientific (Suwanee, GA).

Fruit Sample Handling. Highbush blueberries cv. Bluecrop were used in this study. Organically cultured samples were harvested and collected from five certified organic farms in New Jersey including farms 001−005 (**Table 1**). Samples of conventionally cultured blueberries were harvested from five conventionally operated orchards including farms 006−010 (**Table 1**). All of these farms are in the surrounding area with comparable environmental factors. Only commercially mature fruits were harvested on the basis of the development of fully blue color in early August. Fruits were picked from the full sunny side of the tree and selected for uniform size and color. The sizes of harvested fruit were comparable from various orchards regardless of cultural systems. Undamaged, composite, random berries were selected and collected from each farm. Samples were collected in triplicate from each site and kept separate for analysis and used as replicates to strengthen the statistical analysis. Approximately 800 g−1 kg (fresh berries) of total fruit was harvested from each farm, and the fruits were transported in a cooler to Beltsville, MD, on the same day. Berries were cut into small slices, mixed, divided into 5 g aliquots, frozen in liquid nitrogen, and then stored at −80 °C until they were used for assays.

Production Systems of Conventional and Organic Blueberry Farming. Blueberries are grown on sandy mineral soils having pH between 4 and 5. Although each farm is different in many small, specific applications of fertilizers and pesticides, there is a general similarity among the organic producers and a general similarity among the conventional producers and little similarity between both groups in agricultural methods. Both groups use different USDA registered materials for fertility and pest management. The growers with conventional fields generally utilized urea and ammonium sulfate as nitrogen fertilizers at standard rates of approximately 30 kg of synthetic nitrogen per acre. Direct uptake of these water-soluble chemicals occurs in the root zone of the crop (*17–19*). In contrast, growers with organic fields utilize a "feed-the-soil" system that incorporates cover crops, peat, compost, fish meal, humus, and manures rich in naturally produced nitrogen. Fertilizer recommendations are based on foliar analysis, and the foliar nitrogen levels in organic blueberry culture are generally maintained between 1.6 and 2.2%. These natural nutrients promote the growth of beneficial soil microorganisms and do not harm the symbiotic endo-mycorrhizal fungi associated with the highbush Bluecrop blueberry root system. These decomposers process biomass materials and indirectly relay nitrogen, phosphorus, potassium, and other available plant nutrients through the crop rhizosphere (*17–19*).

Weeds are a major problem in all commercial blueberry fields. Conventional growers utilize an arsenal of pre-emergent herbicides such as Devrinol, Princep, Sinbar, Surflan, Solicam, and Kerb as well as postemergent herbicides such as Poast, Fusilade, Glyphosate, and Gramoxone to directly control weed problems (*17–19*). In contrast, organic blueberry growers do not use these synthethic herbicides but rely on a weed management system that includes horticultural practices as cover crops, smother crops, mulching, and mechanical cultivation. Organic herbicides such as natural crop oils, vinegar, and soaps were used as well (*17–19*).

Insect pests and disease pathogens are controlled by conventional growers with numerous synthethic insecticides and fungicides registered and approved by the U.S. EPA. Organic growers may not use these materials to remain certified. Instead, they depend upon preventative measures and organically derived/Organic Material Review Institute (OMRI)-approved compounds such as minerals, botanicals, and bacteriologicals (*17–19*).

Analysis of Sugars and Organic Acids. Three 5 g samples of blueberries from each farm were extracted twice with 15 mL of imidazole buffer (20 mM, pH 7.0) using a Polytron homogenizer (Brinkmann Instruments, Westbury, NY). The extraction, purification, and derivatization procedures for nonstructural sugars and organic acids have been described previously (*20*). A Hewlett-Packard 5890 gas chromatograph (Hewlett-Packard, Palo Alto, CA) equipped with a flame ionization detector and a fused silica capillary (dimethylsilicone fluid, 12.5 m × 0.2 mm) (Hewlett-Packard) was used for the separation of sugars and organic acids. Sugars and organic acids were quantified by comparing peak area with those of standards.

Total Anthocyanins and Total Phenolic Content. Three 5 g samples of blueberries from each farm were extracted with 20 mL of 80% acetone containing 0.2% formic acid using a Polytron (Brinkmann Instruments, Inc.). The homogenized samples from the acetone extracts were then centrifuged at 14000*g* for 20 min at 4 °C. The supernatants were transferred to vials, stored at −80 °C, and later used for the determination of total anthocyanins, soluble phenolics, and oxygen radical absorbance capacity assay (ORAC) analysis.

Total anthocyanin contents in blueberry extract were determined by using the pH differential method (*21*). Absorbance was measured in a Shimadzu spectrophotometer (Shimadzu UV-160) (Shimadzu Scientific Instruments, Inc., Colmbia, MD) at 510 and 700 nm in buffers at pH

1.0 and 4.5, using $A = [(A_{510} - A_{700})_{pH1.0} - (A_{510} - A_{700})_{pH4.5}]$ with a molar extinction coefficient for cyanidin 3-glucoside (29600) for blueberries. Results were expressed as milligrams of cyanidin 3-glucoside equivalent per 100 g of fresh weight (fwt).

Total soluble phenolics in the fruit extract were determined with Folin−Ciocalteu reagent according to the method of Slinkard and Singleton (*22*) using gallic acid as a standard. Results were expressed as milligrams of gallic acid equivalent (GAE), in the blueberry extract, per 100 g of fwt.

High-Performance Liquid Chromatography (HPLC) Analysis of Berry Anthocyanins and Phenolic Compounds. HPLC was used to separate and determine individual anthocyanins and phenolic compounds in berry tissue samples. The supernatants (18 mL) from the above extracts were concentrated to dryness using a Buchler Evapomix (Fort Lee, NJ) in a water bath at 35 °C and were dissolved in 4 mL of acidified water (3% formic acid) and then passed through a C_{18} Sep-Pak cartridge (Waters), which was previously activated with methanol followed by water and then 3% aqueous formic acid. Anthocyanins and other phenolics were adsorbed onto a column, whereas sugars, acids, and other water-soluble compounds were eluted with 10 mL of 3% formic acid. Anthocyanins and other phenolics were then recovered with 2.0 mL of acidified methanol containing 3% formic acid. The methanol extract was passed though a 0.45 μm membrane filter (Millipore, MSI, Westboro, MA), and 20 μL was analyzed by HPLC. The samples were determined using a Waters (Waters Corp., Milford, MA) HPLC system coupled with a photodiode array detector (Waters 990 series) and equipped with two pumps (600E system controller). Samples were injected at ambient temperature (20 °C) into a reverse phase Nova-Pak C_{18} column (150 × 3.9 mm, particle size = 4 μm) with a guard column (Nova-Pak C_{18}, 20 × 3.9 mm, particle size = 4 μm) (Waters Corp.). The mobile phase consisted of 5% aqueous formic acid (A) and HPLC grade acetonitrile (B). The flow rate was 1 mL/min, with a gradient profile consisting of A with the following proportions (v/v) of B: 0−1 min, 4%; 1−10 min, 4−6% B; 10−15 min, 6% B; 15−35 min, 6−18% B; 35−40 min, 18−20% B; 40−42 min, 20−45% B; 42−45 min, 45−100% B; 45−50 min, 100% B. The phenolic compounds in fruit extracts were identified by their UV spectra, recorded with a diode array detector and by chromatographic comparison with authentic markers (*23–29*). Retention times and spectra were compared to those of the pure standards, and the results were confirmed by co-injection with authentic standards. Individual phenolic acids, flavonols, and anthocyanins were quantified by comparison with an external standard of chlorogenic acid, resveratrol, myricetin, quercetin, cyanidin 3-galactoside, delphinidin 3-glucoside, petunidin 3-glucoside, malvidin 3-galactoside, and malvidin 3-glucoside. Each standard was dissolved in methanol at a concentration of 1 mg/mL, and five dilute solutions from these stock solutions were used to prepare calibration curves of each standard. Recoveries were measured by extracting the recovered amounts of pure substances added to frozen blueberries before the experiment. Three replicates from each sample were used for HPLC analyses. Scanning between 250 and 550 nm was performed, and data were collected by the Waters 990 3-D chromatography data system.

Oxygen Radical Absorbance Capacity (ORAC) Assay. The ORAC assay was carried out using a high-throughput instrument platform consisting of a robotic eight-channel liquid handling system and a microplate fluorescence reader (*30*). Final ORAC values were calculated using the regression equation between Trolox concentration and the net area under the curve (AUC) and were expressed as micromoles of Trolox equivalents (TE) per gram of fwt.

Statistical Analysis. To compare cultural systems the data were analyzed as one-factor general linear models using Proc Mixed (SAS Institute) with cultural system type as the factor. As no farm had both cultural systems, the farms were analyzed separately by system as one-factor general linear models with farm as the factor. The assumptions of the linear model were checked. For the cultural system analysis, the chlorogenic acid results were log transformed and the transformed values used in the analysis; its means are reported in the original units. For the organic farms analysis, the malic acid, resveratrol, and petunidin 3-arabinoside results each had a farm at which all of the values were equal; that is, the variance was zero. Therefore, those farms were not included in the analysis. When necessary, the variance grouping technique was used on the variables to correct variance heterogeneity for the means comparisons. Means comparisons were done with Sidak adjusted p values so that the experiment-wise error rate was 0.05.

Correlation coefficients between antioxidant activity (ORAC), total phenolics, and total anthocyanins were calculated using Excel (Microsoft Corp., Redmond, WA) and are reported as R^2 values.

RESULTS AND DISCUSSION

Results from this investigation of blueberries grown from different cultural systems (organic or conventional) are shown in **Tables 2–5**. Fructose and glucose were found to be the major sugars in blueberries (**Table 2**). Sucrose constituted a minor amount (data not shown). The proportions of fructose, glucose, and sucrose are important in the perception of fruit quality as fructose is 1.8 times sweeter than sucrose (*32*), whereas the sweetness of glucose is only 60% of that of sucrose (*33, 34*). A comparison between mean fruit sugar contents showed that blueberry fruit grown from organic culture yielded higher mean values of sugars (fructose and glucose) than fruit from the conventional culture (**Table 2**). In organic culture, blueberries from farms 001 and 005 contained higher amounts of fructose and glucose than blueberries from other farms (**Table 2**). Citric acid was the major organic acid in blueberries. Organically cultured fruit contained higher amounts of malic acid. However, cultivation methods showed no effect on citric acid content (**Table 2**).

The effects of organic and conventional cultures on anthocyanins, total phenolics, and antioxidant activity (expressed as ORAC values) in blueberry fruit are shown in **Table 2**. Substantial variations were detected in these substances among various farms. In organic culture, blueberries from farms 001 and 003 had higher ORAC values, total phenolics, and anthocyanins than fruit from other farms. In conventional culture, blueberries from farm 010 had the highest ORAC values compared to other farms (**Table 2**). However, high total phenolic content was found in blueberries from farms 009 and 010, and high total anthocyanin content was found in fruit from farms 007, 009, and 010 compared to fruit from other conventional farms. Farm location affected total phenolics, total anthocyanins, and ORAC values (**Table 2**). This may reflect differences in cultural practices among locations, including differences in water stress, mineral nutrient availability, and ultraviolet radiation (*35*). The mean ORAC values in blueberries from organic and conventional cultures were 46.14 and 30.76 μmol/g of fwt, respectively. The mean values for total phenolic content in blueberries were 319.3 and 190.3 mg/100 g of fwt and for total anthocyanins were 131.0 and 82.36 mg/100 g of fwt in blueberries from organic and conventional cultures, respectively. There was a linear relationship between ORAC values and total phenolic or anthocyanins. In this study, the correlation coefficient for ORAC (x-axis) versus total phenolics (y-axis) was 0.8158 ($y = 3.7477x - 35.772$), and that for ORAC (x-axis) versus total anthocyanins (y-axis) was 0.7487 ($y = 8.0382x - 59.841$). These results suggest that the antioxidant capacity of blueberry fruit could have been derived from the contribution of phenolic and anthocyanin compounds. Positive correlations between ORAC and total phenolic or anthocyanin content have also been reported previously in various berries (*36, 37*).

The HPLC analysis of blueberry fruit extracts revealed that both organically and conventionally cultured berries contained chlorogenic acid, resveratrol, and the following flavonoids: myricetin 3-arabinoside, quercetin 3-galactoside, quercetin 3-glucoside, delphinidin 3-galactoside, delphinidin 3-glucoside, cyanidin 3-galactoside, delphinidin 3-arabinoside, petunidin

Table 2. Analysis of Variance, Means and Mean Comparisons of Sugars (Fructose and Glucose), Organic Acids (Citric Acid and Malic Acid), Antioxidant Activity (ORAC Value), Total Phenolics, and Total Anthocyanins for Cultivation Methods, for Farms Using Organic or Conventional Cultural System [a]

		fructose (mg/g of fwt)	glucose (mg/g of fwt)	citric (mg/g of fwt)	malic (mg/g of fwt)	ORAC (μmol/g of fwt)	total anthocyanins (mg/100 g of fwt)	total phenolics (mg/100 g of fwt)
mean								
organic cultivation		97.06a[b]	45.53a	3.47a	0.043a	46.14a	131.02a	319.3a
conventional cultivation		79.26b	29.72b	3.14a	0.029b	30.76b	82.36b	190.3b
F value		15.16	65.07	0.02	8.25	10.06	15.09	17.25
p value		0.0005	<0.0001	0.8799	0.0073	0.0034	0.0013	0.0002
				Organic Farms				
mean	001	77.55 ± 1.5d[c]	42.24 ± 1.4c	3.16 ± 0.06b	0.018 ± 0.0c	51.14 ± 2.0ab	182.48 ± 8.2a	341.83 ± 12.4b
	002	78.00 ± 1.8d	35.80 ± 2.0d	3.17 ± 0.05b	0.075 ± 0.01a	34.20 ± 1.2c	39.43 ± 5.1d	203.65 ± 13.8c
	003	110.01 ± 2.3b	51.47 ± 1.0a	4.23 ± 0.08a	0.035 ± 0.01b	55.14 ± 2.3a	167.84 ± 7.3b	431.11 ± 11.4a
	004	96.97 ± 1.3c	45.01 ± 1.7bc	4.25 ± 0.06a	0.045 ± 0.01b	49.32 ± 1.8ab	136.32 ± 7.6c	326.42 ± 13.2b
	005	112.75 ± 2.5a	53.14 ± 2.9a	2.52 ± 0.04c	0.040 ± 0.00b	40.88 ± 3.6bc	129.04 ± 6.3c	306.69 ± 8.5b
F value		1449	80.47	917	83.26	6.19	1271	54.98
p value		<0.0001	<0.0001	<0.0001	<0.0001	0.0091	<0.0001	<0.0001
				Conventional Farms				
mean	006	74.3 ± 1.8c[c]	26.3 ± 1.3c	3.31 ± 0.04b	0.025 ± 0.01bc	30.8 ± 1.4a	64.8 ± 3.1c	166.2 ± 6.7c
	007	80.4 ± 1.9b	32.9 ± 2.4ab	2.91 ± 0.08c	0.035 ± 0.01ab	29.8 ± 0.9a	90.3 ± 4.1a	128.7 ± 7.8d
	008	65.9 ± 1.6d	22.7 ± 1.9c	4.00 ± 0.03a	0.045 ± 0.01a	27.1 ± 0.7a	76.4 ± 3.2b	130.8 ± 5.6d
	009	97.0 ± 2.5a	35.4 ± 1.3a	2.58 ± 0.11d	0.015 ± 0.00c	32.4 ± 2.3a	88.9 ± 3.0a	238.2 ± 6.5b
	010	78.7 ± 2.8bc	31.3 ± 1.5b	2.90 ± 0.10c	0.025 ± 0.01bc	33.7 ± 2.4a	91.4 ± 4.2a	287.6 ± 8.9a
F value		664.0	167.7	650.3	16.40	12.55	1624.9	906.8
p value		<0.0001	<0.0001	<0.0001	<0.0001	0.0014	<0.0001	<0.0001

[a] Data expressed as mean ± SD. [b] Means within the same column of cultivation method parameter followed by different letters were significantly different at the *p* value. [c] Means within the same column of different farms (organic farms, conventional farms) followed by different letters were significantly different at the *p* value.

Table 3. Effect of Cultural System (Organic and Conventional) on Chlorogenic Acid, Resveratrol, Myricetin 3-Arabinoside, Quercetin 3-Galactoside, and Quercetin 3-Glucoside Content (Micrograms per Gram of Fresh Weight) and Analysis of Variance, Means, and Mean Comparisons of Different Cultural Systems for Different Phenolic Acids in Blueberries[a]

farm	cultivation method	chlorogenic acid[b]	resveratrol[c]	myricetin 3-arabinoside[d]	quercetin 3-galactoside[d]	quercetin 3-glucoside[d]
001	organic	112.6 ± 1.1ab[e]	2.9 ± 0.0a	24.3 ± 0.2a	101.8 ± 7.9a	71.1 ± 7.1a
002	organic	87.1 ± 1.8bc	2.3 ± 0.2b	4.0 ± 0.3b	46.3 ± 1.0b	25.1 ± 1.1c
003	organic	167.3 ± 7.7a	1.8 ± 0.3b	15.3 ± 4.9a	35.8 ± 3.6b	37.4 ± 5.9bc
004	organic	93.5 ± 5.0bc	3.3 ± 0.4a	17.7 ± 1.3a	79.9 ± 2.0a	48.3 ± 1.6b
005	organic	44.1 ± 1.4c	3.2 ± 0.4a	21.4 ± 0.9a	77.8 ± 9.8a	61.1 ± 7.7ab
F value		1786	21.39	3319	276	40.96
p value		<0.0001	0.0021	<0.0001	<0.0001	0.0002
006	conventional	68.4 ± 4.0a[e]	2.4 ± 0.1b	9.7 ± 0.5b	55.4 ± 2.4b	39.2 ± 5.0ab
007	conventional	24.8 ± 1.2b	2.1 ± 0.1c	12.0 ± 2.0a	48.1 ± 9.8c	32.9 ± 2.9b
008	conventional	29.0 ± 0.1b	2.5 ± 0.4b	5.4 ± 0.0c	44.4 ± 1.6c	33.4 ± 0.2b
009	conventional	53.0 ± 1.4ab	1.9 ± 0.1c	9.5 ± 1.7b	60.0 ± 1.3a	44.6 ± 1.8a
010	conventional	41.6 ± 3.3ab	3.5 ± 0.1a	10.2 ± 0.3b	62.7 ± 0.6a	46.7 ± 2.0a
F alue		108.27	937.3	564.5	108.8	225.4
p alue		<0.0001	<0.0001	<0.0001	<0.0001	<0.0001
		Analysis of Variance, Means,and Mean Comparisons for Different Cultural Systems				
mean	organic	100.90a[f]	2.69a	16.54a	68.33a	48.62a
	conventional	43.36b	2.48a	9.36b	54.12a	39.36b
F alue		2.13	0.20	14.49	1.34	7.20
p alue		0.0012	0.6596	0.0015	0.2555	0.0116

[a] Data expressed as mean ± SD. [b] Data expressed as micrograms of chlorogenic acid equivalents per gram of fresh weight. [c] Data expressed as micrograms of resveratrol equivalents per gram of fresh weight. [d] Data expressed as micrograms of quercetin 3-glucoside equivalents per gram of fresh weight. [e] Means within the same column of different farms (organic farms, conventional farms) followed by different letters were significantly different at the *p* values. [f] Means within the same column followed by different letters were significantly different at the *p* values.

3-galactoside, petunidin 3-glucoside, petunidin 3-arabinoside, malvidin 3-galactoside, malvidin 3-galactoside, and malvidin 3-arabinoside (**Tables 3–5**). Chlorogenic acid content in blueberries ranged from 24.8 to 167.3 μg/g of fwt (**Table 3**). In organic culture, fruit from farms 001 and 003 had higher amounts of chlorogenic acid than fruits from other farms. Fruit from farm 006 contained the highest amount of chlorogenic acid among the conventional farms, whereas fruit from farm 007 contained the least. The mean values of chlorogenic acid content in blueberries were 100.9 and 43.36 μg/g of fwt for organic and conventional cultures, respectively (**Table 3**). Resveratrol levels in blueberries were low compared to other flavonoids. The cultural systems (organic and conventional) showed no significant effect on resveratrol content, but it was significantly affected by the farm location (**Table 3**).

The contents of myricetin 3-arabinoside, quercetin 3-galactoside, and quercetin 3-glucoside varied substantially and were significantly different among the five organic farms and six conventional farms (**Table 3**). However, the mean values of myricetin 3-arabinoside and querecetin 3-glucoside contents in fruit from organic farms were significantly higher than those from conventional farms (**Table 3**). In general, the content of quercetin-based flavonols was higher than that of myricetin flavonols in blueberries. Flavonols are effective antioxidants

Table 4. Effect of Cultural System (Organic and Conventional) on Delphinidin 3-Galactoside, Delphinidin 3-Glucoside, Cyanidin 3-Galactoside, and Delphinidin 3-Arabinoside (Micrograms per Gram of Fresh Weight) and Analysis of Variance, Means, and Mean Comparisons of Different Cultural Systems for Different Phenolic Acids in Blueberries[a]

farm	cultivation method	delphinidin 3-galactoside[b]	delphinidin 3-glucoside[b]	cyanidin 3-galactoside[b]	delphinidin 3-arabinoside[b]
001	organic	311.6 ± 8.5a[c]	153.2 ± 3.7a	52.7 ± 9.2a	154.0 ± 9.8a
002	organic	14.3 ± 0.1c	11.2 ± 5.6c	9.3 ± 4.2c	23.6 ± 1.5c
003	organic	104.7 ± 8.1b	83.0 ± 8.4ab	35.3 ± 9.1ab	140.3 ± 9.8a
004	organic	122.0 ± 4.8b	46.7 ± 5.7bc	24.8 ± 0.5bc	79.8 ± 6.3b
005	organic	105.4 ± 6.2b	54.8 ± 1.6bc	23.9 ± 5.4bc	70.0 ± 1.7b
F value		2063	91.86	12.08	272
p value		<0.0001	<0.0001	0.0256	0.0002
006	conventional	38.2 ± 1.3a[c]	11.4 ± 4.6b	12.0 ± 2.2c	25.2 ± 1.5b
007	conventional	47.9 ± 4.0a	32.9 ± 0.6a	15.2 ± 1.7bc	43.8 ± 2.8a
008	conventional	37.1 ± 2.3a	25.7 ± 3.8a	17.0 ± 2.6ab	38.6 ± 7.3ab
009	conventional	49.3 ± 6.0a	29.0 ± 3.2a	19.2 ± 2.2b	47.0 ± 0.5a
010	conventional	36.2 ± 0.8a	24.2 ± 9.4a	7.8 ± 3.1d	30.4 ± 3.0ab
F value		23.53	633.4	605.2	87.05
p value		0.0050	<0.0001	<0.0001	0.0002
		Analysis of Variance and Mean Comparisons for Different Cultural Systems			
mean	organic	171.59a[d]	69.77a	29.22a	93.53a
	conventional	41.74b	24.64b	14.24b	37.00b
F value		13.61	12.66	0.46	15.41
p value		0.0022	0.0030	0.5037	0.0013

[a] Data expressed as mean ± SD. [b] Data expressed as micrograms of cyanidin 3-glucoside equivalents per gram of fresh weight. [c] Means within the same column of different farms (organic farms, conventional farms) followed by different letters were significantly different at the *p* values. [d] Means within the same column followed by different letters were significantly different at the *p* values.

Table 5. Effect of Cultural System (Organic and Conventional) on Petunidin 3-Galactoside, Petunidin 3-Glucoside, Petunidin 3-Arabinoside, Malvidin 3-Galactoside, Malvidin 3-Galactoside, and Cyanidin 3-Galactoside (Micrograms per Gram of Fresh Weight) in Blueberries[a]

farm	cultivation method	petunidin 3-galactoside[b]	petunidin 3-glucoside[b]	petunidin 3-arabinoside[b]	malvidin 3-galactoside[b]	malvidin 3-glucoside[b]	malvidin 3-arabinoside[b]
001	organic	292.3 ± 8.5a[c]	242.6 ± 1.0a	185.3 ± 7.3a	436.3 ± 8.5a	310.7 ± 4.8b	237.1 ± 5.0b
002	organic	42.5 ± 4.0c	37.7 ± 2.3e	49.5 ± 0.1b	181.4 ± 4.8c	106.6 ± 1.1d	116.2 ± 0.4c
003	organic	308.3 ± 9.5a	125.7 ± 2.7c	22.5 ± 2.9b	143.8 ± 5.3d	686.1 ± 7.4a	330.0 ± 7.9a
004	organic	131.4 ± 8.9b	83.8 ± 6.4d	113.7 ± 5.8a	332.3 ± 7.6b	152.7 ± 4.7d	210.2 ± 3.2b
005	organic	149.9 ± 6.5b	145.8 ± 4.8b	107.5 ± 3.6a	421.4 ± 6.5a	260.5 ± 8.2c	242.1 ± 4.2b
F value		66.60	427.00	78.47	765.00	1399	650.00
p value		<0.0001	<0.0001	0.0005	<0.0001	<0.0001	<0.0001
006	conventional	64.6 ± 8.5c[c]	73.2 ± 5.4a	48.1 ± 1.7a	255.3 ± 8.4c	142.0 ± 5.3d	155.5 ± 3.1c
007	conventional	87.6 ± 3.7a	89.3 ± 5.5a	76.1 ± 6.4a	350.7 ± 5.1a	226.6 ± 9.4a	229.2 ± 8.9a
008	conventional	64.0 ± 3.1c	64.5 ± 3.1a	59.7 ± 8.5a	207.3 ± 6.3d	165.0 ± 5.3c	166.2 ± 3.3b
009	conventional	86.8 ± 3.0a	85.3 ± 8.2a	76.3 ± 4.2a	302.1 ± 3.9b	181.3 ± 4.6b	226.0 ± 3.6a
010	conventional	73.3 ± 5.1b	83.2 ± 3.5a	73.3 ± 7.3a	331.5 ± 8.0ab	209.7 ± 7.5a	221.2 ± 0.1a
F value		28.09	74.60	9.19	1987.1	156.2	3730.0
p value		<0.0001	<0.0001	0.0070	<0.0001	<0.0001	<0.0001
		Analysis of Variance and Mean Comparisons for Different Cultural Systems					
mean	organic	184.86a[d]	127.13a	95.70a	303.03a	303.35a	227.12a
	conventional	75.26b	79.00b	66.70a	289.38a	184.92b	199.62b
F value		15.41	8.60	4.08	1.37	4.56	4.82
p value		0.0013	0.0097	0.0611	0.2500	0.0500	0.0357

[a] Data expressed as mean ± SD. [b] Data expressed as micrograms of cyanidin 3-glucoside equivalents per gram of fresh weight. [c] Means within the same column of different farms (organic farms, conventional farms) followed by different letters were significantly different at the *p* values. [d] Means within the same column followed by different letters were significantly different at the *p* values.

(*38*). They are potent quenchers of $ROO^{\bullet}$, $O_2^{\bullet -}$, and $^1O_2$ (*39*). Quercetin and other polyphenols have been shown to play a protective role in carcinogenesis by reducing the bioavailability of carcinogens (*40*). Quercetin with 3′,4′-dihydroxy substitution in the B-ring and conjugation between the A- and B-rings has a high antioxidant potential (*41*). Flavones in general have higher antioxidant activities compared to anthocyanin with the same hydroxylation patterns measured with the ORAC assay (*42*).

Blueberry fruit contained four major anthocyanins: delphinidin, cyanidin, petunidin, and malvidin. The content of malvidin-based anthocyanins in blueberries was much higher than those of petunidin-, delphinidin-, or cyanidin-based anthocyanins (**Tables 4** and **5**). Comparison of the two cultural systems showed that the chemical components of the main anthocyanin pigments were the same in both cultural systems, but there were significant quantitative differences. The mean values of the anthocyanins delphinidin 3-galactoside, delphinidin 3-glucoside, cyanidin 3-galactoside, delphinidin 3-arabinoside, petunidin 3-galactoside, petunidin 3-glucoside, petunidin 3-arabinoside, malvidin 3-galactoside, malvidin 3-glucoside, and malvidin 3-arabinoside for organic and conventional farms were 171.6 and 41.7, 69.8 and 24.6, 29.2 and 14.2, 93.5 and 37.0, 184.9 and 75.3, 127.1 and 79.0, 95.7 and 66.7, 303.0 and 289.4, 303.4 and 184.9, and 227.1 and 199.6 μg/g of fwt, respectively. Thus, fruit anthocyanins in blueberries (including delphinidin 3-galactoside, delphinidin 3-glucoside, delphinidin 3-arabinoside, petunidin 3-galactoside, petunidin 3-glucoside, and malvidin 3-arabinoside) were

significantly greater in organic than in conventional farms (**Tables 4** and **5**). Anthocyanins are glycosides that release aglycon forms (anthocyanidins) by hydrolysis (*43*). The free radical scavenging properties of anthocyanin are attributed to the phenolic hydroxyl group attached to ring structures (*43, 44*). Different flavonoids have different antioxidant capacities. Anthocyanins have been reported to help reduce damage caused by free radical activities, such as low-density lipoprotein oxidation, platelet aggregation, and endothelium-dependent vasodilatation of arteries (*13, 41*). The results of our study on sugar content, individual anthocyanins, total anthocyanins, total phenolics, and ORAC values in blueberries from conventional farms were comparable with those reported in earlier investigations (*45–47*).

In other small fruits, such as marionberries and strawberries, Asami et al. (*5*) also showed that higher levels of total phenolics were consistently found in organically grown cultivations as compared to those produced by conventional agricultural practices. However, Häkkinen and Törrönen (*48*) found that levels of flavonols and phenolic acids were similar in the cultivars of 'Polka' and 'Honeoye' cultivated by conventional or organic techniques. Only in one cultivar, 'Jonsok', did the organically cultivated berries have a 12% higher concentration of total phenolics compared to those cultivated conventionally. This difference was due to higher contents of ellagic acid and kaempferol in strawberries cultivated by organic culture than in those cultivated by conventional technique (*48*). The high content of kaempferol could be a response to pathogenic attack in organically grown strawberry because kaempferol can act as an antimicrobial compound in plants (*49*).

Collectively, our data presented here suggest that different cultural systems significantly affect blueberry fruit quality. Even though there were variations in phytonutrient content among individual farms within each cultural system, significant differences were evident between the two cultivation practices. Blueberries produced from organic culture contained significantly higher amounts of phytonutrients than those produced from conventional culture.

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