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Changes in Fruit Antioxidant Activity among Blueberry Cultivars during Cold-Temperature Storage

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Antioxidant activity, total phenolic content, anthocyanin content, and six other fruit characters including titratable acid concentration, soluble solids, firmness, and percentage of bruised berries were determined for nine blueberry (*Vaccinium* L. sp.) cultivars at harvest and at various postharvest intervals after storage at 5 °C. Berries from MSU-58, Brigitta, and Legacy stored successfully for 7 weeks, Bluegold stored for 3−5 weeks, Bluecrop, Elliott, and Nelson stored for 3 weeks, and Jersey and Little Giant stored for fewer than 3 weeks. During the time they retained marketable quality, one cultivar (MSU-58) demonstrated a 29% increase in antioxidant activity. None of the cultivars showed a significant decrease from the harvest antioxidant activity value during storage. Antioxidant activity, total phenolic content, and anthocyanin content were strongly correlated with each other ($r = 0.87-0.99$, $P < 0.01$). All three parameters were moderately correlated with soluble solids ($r = 0.47$, $P \leq 0.05$; $r = 0.44$, $P \leq 0.05$; and $r = 0.64$, $P \leq 0.01$, respectively), and antioxidant activity and total phenolic content were both moderately correlated with pH ($r = 0.53$ and 0.49, respectively; $P \leq 0.05$). However, antioxidant activity, total phenolic content, and anthocyanin content showed no correlation with firmness, percent severely bruised berries, or weight loss. Antioxidant activity and total phenolic content at harvest both correlated with titratable acidity at harvest ($r = 0.68$, $P \leq 0.05$ and $r = 0.70$, $P \leq 0.05$, respectively) on a cultivar mean basis. Berries from Elliott were also harvested from plants at two levels of bush ripeness (30−50% and 60−80% ripe berries on plants) and separated into three fruit maturity classes on the basis of percent blue color. The level of bush ripeness had no significant effect on antioxidant activity, total phenolic content, or anthocyanin content; however, fruit maturity had a significant effect on antioxidant activity, total phenolic content, and anthocyanin content, and bush ripeness × fruit maturity interactions were significant for these three traits. Berries with 50−75% blue coloration harvested from bushes with 60−80% mature fruit showed a significant increase in antioxidant activity, total phenolic content, and anthocyanin content during the first 3 weeks in storage. Our results demonstrate that increases in antioxidant activity, total phenolic content, and anthocyanin content may occur in the blueberry during cold storage and are cultivar-dependent. The increases that occur in immature fruit, such as in Elliott, may be advantageous for producers who wish to delay marketing of the fruit.

KEYWORDS: ***Vaccinium*** **sp.; anthocyanins; phenolics; postharvest**

INTRODUCTION

The increased interest in fruit and vegetable antioxidant compounds has stimulated research into the basis of variation in antioxidant activity that exists within a crop. Factors that may impact antioxidant activity include maturity at harvest, season of maturity, genetic differences, preharvest environmental conditions, postharvest storage conditions, and processing. For blueberry (*Vaccinium* L. sp) fruit, which ranks high in antioxidant activity among fresh fruits (*1*, *2*), genetic differences have been demonstrated in several studies (*1*, *3*, *4*). Some of the other factors have been preliminarily explored. For example, late harvests of *V. ashei* Reade cultivars Tifblue and Brightwell were reported to demonstrate higher antioxidant activity than early harvests, as determined by their oxygen radical absorbance capacity (ORAC) (*1*). Additionally, purée extraction temperature, adjusted pH of squeezed blueberry juice, and introduction of oxygen into juice were demonstrated to affect the ORAC of lowbush blueberry products (*5*). Kalt et al. (*6*) reported that *V.*

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corymbosum L. cultivar Bluecrop demonstrated a 1.2-fold increase in anthocyanin content over an 8 day period when stored at 20 °C, which was accompanied by a 1.2-fold increase in ORAC. Storage for the same period at 0, 10, or 30 °C did not result in significant changes in anthocyanins, ascorbate, total phenolics, or ORAC in this cultivar. In the same study, lowbush blueberry clones did not show a significant change in ORAC during 8 days of storage at any of the four temperatures tested, despite a 27% loss in ascorbate at 20 and 30 °C. Kalt and McDonald (*7*) reported a mean 18% increase in anthocyanins in three lowbush (*V. angustifolium* Ait.) cultivars, each harvested at three different maturities, when held at 1 °C for 2 weeks.

Extending the shelf life of small fruit is usually achieved through low-temperature or controlled atmosphere storage, but for the blueberry, little has been published regarding cultivar differences in antioxidant activity during low-temperature storage. Whether antioxidant activity correlates with changes in fruit quality or whether differences in fruit maturity at the time of harvest have an impact on the antioxidant activity of blueberries stored at low temperature is unknown. In this paper, we describe cultivar differences in antioxidant activity, total phenolic content, and anthocyanin content that occur during storage and correlate these variables with titratable acid concentrations and changes in fruit firmness, bruising, and soluble solids concentration.

MATERIALS AND METHODS

Chemicals. 2,4,6-Tripyridyl-s-triazine (TPTZ) and chlorogenic acid were purchased from Sigma Chemical (St. Louis, MO). Trolox (6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid) was purchased from Aldrich Chemical (Milwaukee, WI). Ferrous ammonium sulfate was purchased from Fisher Scientific, (Pittsburgh, PA), sodium acetate was purchased from J. T. Baker (Phillipsburg, NJ), and ferric chloride was purchased from EM Science (Gibbstown, NJ).

Fruit. Mid- and late-season cultivars of commercial importance, nationally or in the Great Lakes region, were chosen. With the exception of Little Giant, which is grown primarily for processing, they are grown for fresh consumption as well as for processing. Several cultivars were of particular interest because they maintained high quality during prolonged cold storage in preliminary studies and may have the potential for extended marketing. Approximately 5 kg of ripe, sound berries were harvested in July and August 2000 from mature plants of cultivars Bluecrop, Jersey, Elliott, and the selection MSU-58 grown at Benton Harbor, MI (latitude 42°4′N) and from cultivars Bluegold, Brigitta, Nelson, Legacy, and Little Giant grown at Grand Junction, MI (latitude 42°24′N). Fruit was harvested when 30−40% of the fruit on the bush was ripe. For Elliott only, additional fruit was harvested when 60−80% of the fruit on the bush was ripe, and berries harvested at both stages were separated into three groups based on visual estimation of surface color: (i) at least 50% but less than 75% blue color, (ii) at least 75% but less than 100% blue coloration, and (iii) 100% blue. Only fully mature (100% blue) fruit was used in comparisons among cultivars. Berries were held for the duration of storage in a flow-through system using 0.18 L plastic containers at 5 °C and 90% relative humidity with an airflow rate of 50 mL per minute, under ambient O_2 and CO_2. Four 150−200 g samples of berries per cultivar were evaluated for fruit quality attributes after 0, 3, 5, 6, and 7 weeks of storage, and berry weight was determined on a random 25 berry sample. Not all parameters were evaluated at all time points; titratable acid was evaluated in all cultivars at harvest only, and for most cultivars, pH was evaluated at harvest and at 3 weeks postharvest only. In some instances, retention of sufficient fruit for other evaluations precluded pH determination at 3 weeks postharvest. After assessment, 30−50 g of sound fruit from each sample, if available, was frozen at −80 °C. Frozen berries were shipped on dry ice to Minnesota without thawing and held at −80 °C until extraction, for determination of antioxidant activity, total phenolic content, and anthocyanin content. A fruit sample was considered of marketable quality if the majority of the fruit appeared "sound", without visual signs of external damage and softening. Further testing was abandoned when there was not enough sound fruit to make quality determinations.

Quality Attributes. Soluble solids, pH, and titratable acidity were measured in each of four replicates, using juice extracted from a 25 berry sample blended at high speed in a tissue homogenizer. Soluble solids were determined using a hand-held refractometer. Results are reported in percent soluble solids (mass/mass) on a fresh weight basis. Titratable acidity was determined from 10 mL of juice diluted to 100 mL with distilled water, titrated with 0.1 N NaOH to pH 8.2, and expressed as the percent citric acid (mass/mass) on a fresh weight basis. Firmness was assessed using a portable firmness-measuring instrument (*8*). An internal condition assessment was made by cutting 25 fruits in half using a stainless steel knife and rating the relative degree of bruising as 0, 0−25, 25−50, or greater than 50%.

Extractions. Separate extractions were performed on each of the four samples per cultivar per storage time. Extractions were performed under reduced light conditions. For each extraction, approximately 10 g of frozen berries was weighed and counted and allowed to thaw partially at −20 °C. Extracts for all assays were prepared using acidified methanol (0.1% HCl). This solvent maximizes extraction of anthocyanins (*9*), and studies in our lab demonstrated that acidified methanol was superior to methanol/formic acid/water (*10*) for the recovery of anthocyanins (data not shown). Preliminary studies (data not shown) also demonstrated no significant difference in total phenolic content between extracts prepared in acidified methanol and those prepared in 80% ethanol (*11*). Following 1:1 (w/v) addition of ice-cold acidified methanol, the fruit was homogenized with a Polytron (Kinematica, Luzern, Switzerland) homogenizer for 2 min. The homogenizer probe was rinsed with an additional identical volume of acidified methanol, and the rinsate was added to the homogenate. The homogenate was filtered by gravity through 11 μm filter paper (P5, Fisher Scientific), and the residue was mixed with a third volume of acidified methanol. This was refiltered, and the filtrates were combined and standardized to a final volume of 30 mL with acidified methanol. An 8 mL aliquot was stored at −80 °C until the assays for antioxidant activity, total phenolic content, and anthocyanin content were performed. Each extract was tested in duplicate for each of the assays.

Antioxidant Activity Assay. Antioxidant activity was determined using a modification (*12*) of the ferric-reducing antioxidant power (FRAP) assay (*13*), originally referred to as the ferric-reducing ability of plasma. Briefly, the antioxidant capacity of dilute berry extract is determined by its ability to reduce ferric iron to ferrous iron in a solution of TPTZ prepared in sodium acetate at pH 3.6. The reduction of iron in the TPTZ−ferric chloride solution (FRAP reagent) results in the formation of a blue-colored product (ferrous tripyridyltriazine complex), the absorbance of which is read spectrophotometrically at 593 nm 4 min after the addition of appropriately diluted berry extract or antioxidant standard to the FRAP reagent. Two antioxidant standard curves, using Trolox (25−500 μM) and ferrous ammonium sulfate (50−1000 μM) were run with each assay. The standard curves were linear in the ranges tested. Trolox demonstrated activity approximately twice that of ferrous ammonium sulfate, similar to that previously reported (*13*). The 1% (v/v) aqueous dilution of the blueberry extracts, when used in the final reaction at a 1:9 dilution with the FRAP reagent, did not produce significant color interference at 593 nm. Results are expressed using the Trolox standard only, as μmol Trolox equivalents (TE) g^{-1} fresh fruit.

Total Phenolic Content. The Folin Ciocalteu-based method as applied by Coseteng and Lee (*11*) was used, with an incubation time of 90 min for color development. Results are expressed as mg chlorogenic acid equivalents/100 g fresh fruit, rather than using gallic acid as a standard. Chlorogenic acid is the predominant phenolic acid in blueberry (*10*), which possesses very little gallic acid. Under the conditions used, chlorogenic acid equivalents were approximately 1.8× gallic acid equivalents.

Anthocyanin Content. Berry extracts were diluted (1:99) in acidified methanol to obtain an absorbance between 0.200 and 1.000 at 530 nm. Because the extracts were freshly prepared from frozen fruit and did not undergo extensive browning, a pH differential method was

Table 1. Mean Values[a] and Tukey's HSD for Mean Separation for Antioxidant Activity, Total Phenolic Content, Anthocyanin Content, and Quality Attributes in Fully Ripe Blueberry Fruit Harvested and Stored at 5 °C for the Periods Specified

designation	storage interval (weeks)	AA[b]	TPH[c]	ACY[d]	wt[e] (g)	% with <25% bruising[f]	% with >50% bruising[g]	firmness (g mm^{-1})	SS[h] (%)	pH	TA[i] (%)
Bluecrop	0	11.1	402	123	58.0	73	9	136	11.1	3.36	1.16
	3	10.3	388	119	52.6	57	21	148	9.2	3.42	
Bluegold	0	13.1	492	181	47.2	80	7	159	12.0	3.26	2.10
	3	16.3	574	198	50.8	58	20	180	10.4	3.10	
Brigitta	0	9.0	335	132	67.0	84	3	173	12.6	3.22	1.40
	3	9.3	358	131	70.7	74	12	227	10.6		
	5	10.1	380	143	67.2	68	20	237	10.7		
	7	8.5	347	126	58.0	58	23	241	10.0		
MSU-58	0	14.2	494	202	48.4	92	2	197	12.7	3.42	1.36
	3	18.3	605	220	47.7	87	6	209	11.3	2.98	
	5	16.6	566	212	46.8	62	20	261	11.2	2.67	
	6	14.7	524	199	57.2	47	28	230	10.8		
	7	10.8	406	131	40.7	47	26	274	10.5		
Elliott[j]	0	14.3	515	191	43.4	79	12	175	11.3	3.16	2.46
Jersey	0	7.8	336	121	40.6	62	9	159	11.2	3.70	0.92
	3	8.5	355	110	36.8	44	43	152	8.8		
Legacy	0	12.2	470	143	45.8	84	4	173	11.6	3.63	1.52
	3	14.7	529	183	44.8	68	13	232	11.2	3.60	
	5	14.1	512	168	49.1	60	32	207	11.1	3.16	
	6	13.2	505	159	46.7	52	24	218	11.0		
	7	14.5	529	170	44.8	55	28	217	11.9		
Little Giant	0	17.6	595	280	16.6	45	14	150	14.5	3.14	2.01
	3	16.3	542	238	16.8	7	86	141	12.4		
Nelson	0	9.0	376	93	63.0	54	18	140	11.5	3.13	1.52
	3	11.1	426	112	63.4	37	30	163	10.5	3.13	
Tukey's HSD		3.7	118	52	8.6	32	24	38	2.4	0.41	1.14

[a] Mean values for all determinations based on $n = 4$, except Jersey and Little Giant at 0 weeks storage, where mean AA, TPH, and ACY values were based on $n = 3$. [b] Antioxidant activity, expressed as μmol TE g^{-1} fresh fruit. [c] Total phenolic content, expressed as mg chlorogenic acid equivalents/100 g fresh fruit. [d] Anthocyanin content, expressed as mg cyanidin 3-glucoside equivalents/100 g fresh fruit. [e] Weight of a 25 berry sample. [f] Percentage of a 25 berry sample with less than 25% internal bruising. [g] Percentage of a 25 berry sample with greater than 50% internal bruising. [h] Soluble solids, expressed on fresh weight basis. [i] Titratable acid, expressed on fresh weight basis. [j] Insufficient sample remained for AA, TPH, and ACY determinations after 3 weeks of storage; only data from harvest are presented.

considered unnecessary. Results are expressed as mg cyanidin 3-glucoside equivalents/100 g fresh fruit using a molar extinction coefficient of 29 600.

Statistical Analyses. Analyses of variance (ANOVA) were performed with cultivar−storage interval combinations, bush ripeness, and fruit maturity as fixed effects and replications (extracts) as random effects. Comparisons of treatment means were made using Tukey's Studentized range test (HSD) based on harmonic mean sample size. For all analyses, $P = 0.05$. Statistical analyses were performed using SPSS for Windows, version 8.0 (Chicago, IL). Correlations were calculated on a cultivar−storage interval (treatment combination) mean basis or a cultivar mean basis as indicated below.

RESULTS

Of the nine cultivars, Jersey and Little Giant maintained market quality for fewer than 3 weeks, Bluecrop, Elliott, and Nelson stored successfully for 3 weeks, Bluegold stored successfully for between 3 and 5 weeks, and Brigitta, MSU-58, and Legacy stored successfully for 7 weeks (data not presented).

Mean values for antioxidant activity, total phenolic content, anthocyanin content, and quality attributes for each cultivar and storage interval are shown in **Table 1**. When data were analyzed in a two-way ANOVA for effects of cultivar and storage interval, variation among cultivars was significant for antioxidant activity, total phenolic content, and anthocyanin content across all storage intervals, but there were cultivar × storage interval interactions for all three traits with rank and scale changes among cultivars (analyses not presented). Brigitta, MSU-58, and Legacy demonstrated the lowest percentage of berries with severe bruising at harvest and 3 weeks postharvest (**Table 1**). These three cultivars and Elliott were the firmest cultivars at harvest and throughout the postharvest period, consistently maintaining firmness measurements above 200 g/mm. Only Brigitta demonstrated a significant loss in berry weight during storage, at 7 weeks postharvest. The berry weight of MSU-58 at 6 weeks postharvest was greater than at harvest, but at 7 weeks, it was less than at harvest, possibly due to sampling variation.

Of the five cultivars that ranked highest for antioxidant activity at harvest, four—Bluegold, Legacy, Little Giant, and MSU-58—also ranked highest 3 weeks postharvest (**Table 1**) and were distinguishable from the three lowest-ranking cultivars at that time. Two of the four cultivars—Legacy and MSU-58—maintained high antioxidant activity levels after 5 and 7 weeks of storage. The lowest-ranking cultivars at harvest remained so through the third and fifth weeks of storage.

Cultivar rankings for total phenolic content were very similar to those for antioxidant activity at all postharvest intervals, but anthocyanin content rankings did not resemble the antioxidant activity rankings as closely (**Table 1**). However, for all three traits, the same four highest-ranking cultivars were separable from the remaining four cultivars by Tukey's HSD at 3 weeks postharvest.

Antioxidant activity was 24, 21, and 23% higher in the first postharvest interval (up to 3 weeks postharvest) than at harvest for Bluegold, Legacy, and Nelson, respectively, but only the 29% increase demonstrated by MSU-58 during this interval was significant. In the second postharvest interval (3−5 weeks postharvest), cultivars did not change in antioxidant activity. Of the three cultivars that stored successfully for 7 weeks, only MSU-58 showed a decrease from the maximum antioxidant activity attained during storage. However, antioxidant activity

Table 2. Pearson's Correlation Coefficients[a] for Antioxidant Activity, Total Phenolic Content, Anthocyanin Content, and Quality Attributes at Time of Harvest, Based on Genotype Storage Time Combinations for Nine Blueberry Cultivars Stored for up to 7 Weeks at 5 °C

	AA[b]	TPH[c]	ACY[d]	% wt loss	severe bruising[e]	firmness	SS[f]	pH
AA	1.00	0.99**	0.91**	–0.44	–0.13	0.13	0.47*	–0.53*
n =	25	25	25	16	25	25	25	17
TPH		1.00	0.87**	–0.46	–0.10	0.11	0.44*	–0.49*
n =		25	25	16	25	25	25	17
ACY			1.00	–0.45	–0.13	0.07	0.64**	–0.41
n =			25	16	25	25	25	17

[a] *,** Significant at $P \leq 0.05$ and 0.01, respectively. [b] Antioxidant activity. [c] Total phenolic content. [d] Anthocyanin content. [e] On the basis of percentage of berries with greater than 50% internal bruising. [f] Soluble solids.

at 7 weeks postharvest was not lower than at harvest for this cultivar. MSU-58 and Legacy both ranked relatively high for antioxidant activity at harvest, demonstrated substantial increases in antioxidant activity during storage, and stored successfully for 7 weeks, whereas Brigitta, which also stored successfully for seven weeks, was relatively low in antioxidant activity at harvest (**Table 1**) and showed no change in antioxidant activity during storage.

Individual cultivars showed trends for total phenolic content similar to those for antioxidant activity (**Table 1**), with Bluegold, Legacy, Nelson, and MSU-58 demonstrating total phenolic content that was 13−23% higher at 3 weeks postharvest than at harvest, but these increases were not significant. The changes in anthocyanin content values over the postharvest period were also similar in trend to those for antioxidant activity for individual cultivars; however, none of the changes in anthocyanin content in the first postharvest interval were significant.

Changes in berry weight (up to 18%) that occurred during the postharvest period may have influenced the values for antioxidant activity, total phenolic content, and anthocyanin content, when expressed on a fresh weight basis. For instance, it is possible that a portion of the increase in these variables during storage was due to water loss. Therefore, antioxidant activity, total phenolic content, and anthocyanin content at each storage interval were also calculated based on average fresh berry weight at harvest. These recalculated values (data not presented) did not alter the results of the analyses above. In fact, the mean values for each cultivar−storage interval combination were not altered more than 0.2 μmol TE g^{-1} fresh fruit for antioxidant activity, 8 mg/100 g fresh fruit for total phenolic content, and 3 mg/100 g fresh fruit for anthocyanin content, from their original values.

Table 2 shows the correlations between antioxidant activity, total phenolic content, anthocyanin content, percent weight loss during storage, firmness, severe bruising, soluble solids, pH, and titratable acidity, based on cultivar−storage interval combination means. Correlations between antioxidant activity, total phenolic content, and anthocyanin content were high ($r = 0.87$−0.99; $P \leq 0.01$). Antioxidant activity, total phenolic content, and anthocyanin content correlated weakly with soluble solids but showed no correlation with firmness, severe bruising, or weight loss. Antioxidant activity and total phenolic content both correlated negatively with pH. Firmness, weight loss, severe bruising, and soluble solids were uncorrelated (data not presented). On a cultivar mean basis, antioxidant activity, total phenolic content, and anthocyanin content values at harvest and 3 weeks postharvest did not correlate with the soluble solids: titratable acid ratio or any of the other parameters at harvest, with the exception of antioxidant activity and total phenolic content, which both correlated with titratable acidity at harvest ($r = 0.68$ and 0.70, respectively, $P \leq 0.05$ for both; data not presented).

Table 3. Mean Values[a] and Tukey's HSD for Mean Separation for Antioxidant Activity, Total Phenolic Content, and Anthocyanin Content in Elliott Blueberry Fruit, Determined at Time of Harvest

bush ripenness[b]	fruit maturity[c]	AA[d]	TPH[e]	ACY[f]
30–40% fruit ripe	50–75% blue ($n = 3$)	6.6	300	9
	>75 to <100% blue ($n = 3$)	12.1	462	143
	100% blue	14.3	515	191
60–80% fruit ripe	50–75% blue	5.6	285	3
	>75 to <100% blue	8.8	362	79
	100% blue	16.8	596	239
Tukey's HSD ($\alpha = 0.05$)		3.0	91	53

[a] Mean values based on $n = 4$ determinations. [b] Berries harvested when 60–80% of fruit on plants was ripe. [c] Antioxidant activity, expressed as μmol TE g^{-1} fresh fruit. [d] Total phenolic content, expressed as mg chlorogenic acid equivalents/100 g fresh fruit. [e] Anthocyanin content, expressed as mg cyanidin 3-glucoside equivalents/100 g fresh fruit.

In Elliott blueberries at harvest, bush ripeness (30−40% vs 60−80% mature berries on the plant) did not affect antioxidant activity, total phenolic content, and anthocyanin content, but fruit maturity effects and bush ripeness × fruit maturity interactions were significant for all traits ($P \leq 0.001$, analysis not presented). Analysis of individual bush ripeness−fruit maturity combinations revealed that berries with greater than 75% but less than 100% blue color had lower antioxidant activity, total phenolic content, and anthocyanin content values when harvested from a bush with 60−80% mature berries than from a bush with 30−40% mature berries (**Table 3**). Fruit with other degrees of coloration (50−75 or 100%) did not differ in the levels of these traits when harvested from bushes of different maturities. When Elliott berries that were 50−75% blue from bushes with 60−80% ripe fruit were stored, increases in antioxidant activity (79%), total phenolic content (40%), and anthocyanin content (1600%) occurred during the first postharvest interval (**Table 4**).

DISCUSSION

Cultivar differences for antioxidant activity exist in ripe blueberry fruit at harvest and during cold storage, with one cultivar in our study showing an increase in antioxidant activity during storage. The increase observed is not due simply to a higher concentration in fruit that has undergone water loss during storage, as the same results were obtained when calculations were based on berry weight at harvest. Kalt et al. (*6*) reported that Bluecrop berries showed no increase in ORAC during an 8 day postharvest period when stored at temperatures from 0 to 40 °C. Our results agree, as berries from this cultivar showed no increase in antioxidant activity in over their useful storage life (3 weeks) when stored at 5 °C.

None of the cultivars demonstrated a decrease from its antioxidant activity value at harvest during storage. Thus, the

Table 4. Mean Values[a] and Tukey's HSD for Mean Separation for Antioxidant Activity, Total Phenolic Content, and Anthocyanin Content in Elliott Blueberry Fruit Having Greater than 50% but Less than 75% Surface Blue Coloration,[b] Stored at 5 °C for Intervals Indicated

storage interval (weeks)	AA[c]	TPH[d]	ACY[e]
0	5.6	285	3
3	10.0	398	54
5	9.6	436	54
Tukey's HSD ($\alpha = 0.05$)	1.4	54	19

[a] Mean values based on $n = 4$, except where indicated otherwise. [b] Percentage of fruit ripe on bush when berries were harvested. [c] Percentage surface blue coloration of harvested fruit. [d] Antioxidant activity, expressed as μmol TE g^{-1} fresh fruit. [e] Total phenolic content, expressed as mg chlorogenic acid equivalents/100 g fresh fruit. [f] Anthocyanin content, expressed as mg cyanidin 3-glucoside equivalents/100 g fresh fruit.

antioxidant health benefits from these berries can be retained well after harvest, until consumers eat them. Berries from one cultivar, Elliott, showed an increase in antioxidant activity, total phenolic content, and anthocyanin content during the initial postharvest period in cold storage when harvested before they had turned fully blue, even though this cultivar showed no change during storage when harvested after full color development. The increases that may occur during cold storage are advantageous to producers who may wish to delay or extend marketing.

Antioxidant activity correlated strongly with total phenolic content and anthocyanin content within and across storage periods for the nine cultivars examined. Other studies (*1*, *4*) have reported the high correlations among these parameters in blueberries, an association based on concentrations of phenolic acids, anthocyanins, and other flavonoid compounds. These three parameters also correlated weakly and positively with soluble solids, which may reflect the dependence of polyphenolic synthesis or preservation on the available energy pool (*14*, *15*). Antioxidant activity, total phenolic content, and anthocyanin content during storage showed no correlation with important fruit quality parameters such as fruit firmness, weight loss, or bruising.

Three of the four cultivars in which firmness values >200 g/mm were observed at 3 weeks postharvest stored successfully for 7 weeks, and all three of these cultivars maintained values >200 g/mm throughout the study. Fruit firmness is directly related to storage life in the blueberry but is variable from season to season and among cultivars (*16*). Although high harvest and early postharvest firmness values appeared relatively useful in predicting longer storage life in this group of cultivars, observations over several years might improve the predictive value.

The quality parameters that dictated useful storage life in these cultivars were principally firmness and bruising. Flavor and texture are also important in determining marketability following storage, but this study did not address these attributes. Also, although we did not formally assess decay in the samples, less than 2−3% of any sample showed visible evidence of decay during storage. A similar observation was made by Beaudry et al. (*17*), wherein fruit stored at 2 °C for 27 days had little rot, and only when temperatures were raised to 20 °C for 3 days were substantial levels of decay observed. Regardless, resistance to decay is considered an important factor in determining storage life, and an increasing incidence of decay has been associated with a high soluble solids:titratable acid in Wolcott blueberries at different stages of ripeness (*18*).

In conclusion, ripe fruit from most of the blueberry cultivars tested demonstrated stability of antioxidant activity, total phenolic content, and anthocyanin content during cold storage, but one cultivar demonstrated an increase during the first 3 weeks of storage. Because year-to-year effects may have a significant impact on the relative performance of some blueberry genotypes for antioxidant activity, phenolic content, and anthocyanin content (Connor, 2001), data from additional years would be useful to confirm these observations. The stage in the season at which fruits, and particularly fully blue (mature) berries, from Elliott were harvested did not affect their antioxidant activity, total phenolic content, or anthocyanin content. Elliott blueberries that were harvested when only 50−75% blue demonstrated an increase in antioxidant activity, total phenolic content, and anthocyanin content in the first 3 weeks of cold storage. Although levels of these traits did not reach those of fully mature fruit, if berries are harvested when not yet fully mature to facilitate delayed marketing, the antioxidant activity will still be appreciable when the fruit is marketed.

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