Oxygen Radical Absorbing Capacity, Anthocyanin and Phenolic Content of Highbush Blueberries (*Vaccinium corymbosum* L.) during Ripening and Storage

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ADDITIONAL INDEX WORDS. ORAC, fruit, maturity, postharvest, pigment, antioxidant

ABSTRACT. The antioxidant properties of blueberries have been examined only in ripe fruit, although fruit of different maturities are used in processed food products. In this study, highbush blueberry cultivars Bergitta, Bluegold, and Nelson highbush blueberry fruit at different stages of ripeness were examined to characterize differences in oxygen radical absorbing capacity (ORAC) and the phenolic components responsible for ORAC. Underripe fruit at different stages of maturity were also stored at 20 °C for up to 8 days to assess changes in ORAC and phenolic content. Anthocyanin content was substantially higher in fruit of more advanced stages of ripeness. In contrast, the phenolic content and ORAC were lower in the riper fruit. Anthocyanins continued to form during storage, although rate of pigment formation declined after about 4 days. Less anthocyanin pigment was formed in the less ripe fruit. After 8 days of storage, the anthocyanin content of fruit harvested 5% to 50% or 50% to 95% blue exceeded that of ripe fruit. Up to 60% of the total phenolic content could be accounted for by anthocyanins. ORAC was positively correlated with total phenolic content (R^2 = 0.78), but not with anthocyanin content.

Reports of the human health benefits of dietary antioxidants have encouraged researchers to examine factors that influence the content of phytochemical antioxidants in fruit and vegetables (Farnham et al., 1999; Goldman et al., 1999; Grusak et al., 1999; Kochian and Garvin,1999). Foods such as apples, onions and tea, contribute significant quantities of flavonoid antioxidants to the diet (Hertog et al., 1993). Other foods such as blueberries, although not consumed in large volumes, have a high antioxidant capacity due to their high content of both flavonoid and nonflavonoid antioxidants (Cao et al., 1996; Prior et al., 1998; Wang et al., 1996). Essentially all of the studies on dietary antioxidants examine these compounds in food plants that are at their optimal stage of maturity. However many fruit products consumed in processed forms contain fruit of various stages of ripeness.

The phenolic components of blueberry fruit are not entirely known. In ripe blueberries, the hydroxycinnamate derivative, chlorogenic acid, is the major nonflavonoid phenolic (Schuster and Herrmann, 1985) while anthocyanin pigments are the major flavonoid phenolics (Machiex et al., 1990). Other monomeric flavonoids such as flavonol glycosides and catechins are present in much lower quantities (Kühnau, 1976; Machiex et al., 1990). During ripening, the concentration of cinnamate derivatives in fruit generally decreases, although the absolute content may increase due to fruit enlargement (Herrmann, 1989). However, slightly unripe lowbush blueberries have the same concentration of chlorogenic acid as fully ripe and overripe fruit (Kalt and McDonald, 1996). There is a rapid increase in the content and concentration of anthocyanins during blueberry ripening. Anthocyanin pigments are formed in the peel and are responsible for the change of blueberry fruit surface color from yellow-white to dark blue. During blueberry ripening, the anthocyanin concentration increases from 0 to ≈11 mg·g⁻¹ dry weight of fruit (Kalt and McDonald, 1996).

Anthocyanins have substantial antioxidant capacity, as measured by the oxygen radical absorbing capacity (ORAC) assay (Wang et al., 1997). Chlorogenic acid is also an effective antioxidant, with an ORAC [μmol trolox equivalents (TE)/mg] similar to flavan-3-ols and anthocyanidins (Prior and Cao, 1999). In ripe blueberry fruit, both anthocyanins and total phenolics are strongly correlated with ORAC values (Prior et al., 1998). Although anthocyanins contribute substantially to the pool of phenolic components in ripe blueberries, it is not known how phenolic compounds and their antioxidant capacity are influenced by fruit maturity.

White

Pink

Pink

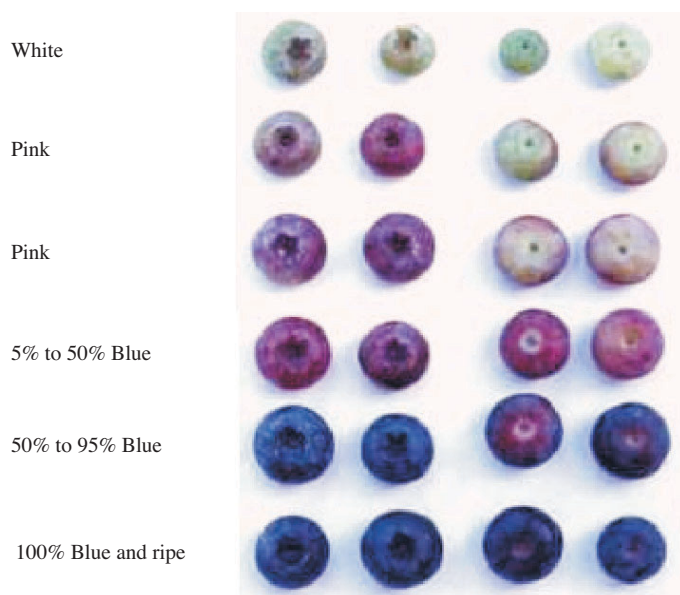5% to 50% Blue

50% to 95% Blue

100% Blue and ripe

Fig. 1. Fruit color at different stages of ripeness based on the surface color of the fruit at the stem and calyx end. Fully ripe fruit were distinguished from 100% blue fruit by a depression at the point of attachment of the stem.

The postharvest synthesis of anthocyanins has been previously documented in fruit and varies among fruit species (Kalt et al., 1993, 1999). In *Vaccinium* species, increases in anthocyanin content during storage have been reported for lowbush blueberries (*V. angustifolium* Aiton) (Kalt and McDonald, 1996), rabbiteye blueberries (*V. asheii* Reade) (Basiouny and Chen, 1988) and highbush blueberries (Kalt et al., 1999).

Because of the potential health benefits attributed to blueberry phenolics, including their anthocyanins, the factors which influence phenolic antioxidant content and profile should be investigated. Thus, objective of this study was to determine how fruit maturity and storage influences the total phenolic and anthocyanin content, as well as the antioxidant capacity, of highbush blueberries.

Materials and Methods

SAMPLES. Highbush blueberry cultivars Bergitta, Bluegold and Nelson were harvested from a commercial acreage near Kentville, Nova Scotia, at various stages of ripeness. Samples consisting of a minimum of 20 fruit were placed in the following ripeness categories based on their surface color at harvest: 1) white, 2) pink, 3) 5% to 50% blue, 4) 50% to 95% blue 5)100% blue, and 6) fully ripe (Fig. 1). Fruit that were either 100% blue, or fully ripe, were evaluated only at the time of harvest. The other four ripeness stages were evaluated at harvest, and again after 1, 2, 4, and 8 d of 20 °C storage in the dark. Humidity of the storage chamber was maintained at 0.212 kPa using solutions of glycerol-water as described by Forney and Brandl, (1992). The size of fruit (cm³/fruit) was measured in each sample by determining the volume of water displaced by 10 fruit submerged in a known volume of water in a graduated cylinder. After this measurement was taken, surface water was removed and fruit were frozen and stored at –70 °C until extraction.

SAMPLE PREPARATION. Fruit samples were crudely chopped, while frozen, in a food processor and then stored at –40 °C. Once all frozen samples were crudely chopped, a weighed amount of chopped frozen fruit was ground for 2 min in a Virtis Shear Homogenizer (The Virtis Co., Gardiner, N.Y.) in three volumes of extraction solvent containing 40 methanol : 40 acetone : 20 water : 0.1 formic acid. The extract was vacuum filtered through a Whatman #4 filter (Fisher Scientific, Nepean, Ont.), then brought to a fixed volume with extraction solvent, and stored at –40 °C for no longer than 1 month before total phenolic and anthocyanin analysis. For the measurement of antioxidant capacity, an aliquot of extract was dried under vacuum at 30 °C, and resolubilized in water.

MEASUREMENT OF ANTHOCYANINS, PHENOLICS, AND ANTIOXIDANT CAPACITY. Total anthocyanin content of the extracts was determined by the spectrophotometric method of Wrolstad (1976). Depending on their anthocyanin content, samples were either concentrated under vacuum or diluted, before measurement. Total anthocyanin content was calculated using the extinction coefficient for cyanidin 3-glucoside (29, 600). Total dissolved phenolics was determined using the Folin-Ciocalteu method, absorbance was measured at 700 nm, and results were expressed as mg gallic acid equivalents/ g dry weight. (Singleton and Rossi,1965).

Anthocyanin content was calculated as a percentage of total dissolved phenolics by assaying known quantities of pure cyanidin 3-glucoside (Extrasynthese, Genay, France) using the methods of Wrolstad (1976) and Singleton and Rossi (1965). This produced a linear relationship ($R^2 = 0.998$) of: $y = 0.9368x + 13.445$, where y was phenolic content in milligram gallic acid equivalents per liter, and x was anthocyanin content in milligram cyanidin 3-glucoside per liter.

Antioxidant capacity was measured as ORAC following the automated method of Cao et al. (1993) and Cao et al. (1995) with a COBAS-FARA II centrifugal analyzer (Roche Diagnostic, Nutley, N.J.). The assay solution (400 μL) contained 16.7 nmol·L⁻¹ of ß-phycoerythrin (PE) (Sigma, St. Louis, Mo.), 4 mmol·L⁻¹ 2,2' azobis (2-amidinopropane) dihydrochloride (AAPH) (Wako Chemicals, Richmond, Va.) and 20 μL of sample. The reaction was initiated by adding AAPH, which is a peroxyl generator. Trolox (6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid) (Sigma, St. Louis, Mo.), a water-soluble analogue of vitamin E, was used as a standard for antioxidant activity at a concentration of 1.0 mmol·L⁻¹. Reagents were dissolved in 75 mM phosphate buffer, pH 7.0. Fluorescence of ß-PE was read on duplicate samples every 2 min after addition of AAPH. The total run time for the ORAC assay was 70 min. Area under the curve was calculated and expressed as μmol TE/g dry weight. Samples were extracted and analyzed in a prescribed randomized fashion to eliminate any effects of frozen storage on analytes.

STATISTICAL ANALYSIS. Anthocyanins (expressed as $\log_{10}$ cyanidin 3-glucoside equivalents), phenolics (milligram gallic acid equivalents), logit % (anthocyanin:phenolic), and ORAC were analyzed using a mixed linear model. This model included the fixed effects of cultivar, maturity, a quadratic polynomial for storage time and the interactions between these terms. The model also included the random effects of harvest and the harvest by cultivar interaction. This model was fit using restricted maximum likelihood (REML) as implemented in the MIXED procedure in SAS (SAS Institute, Cary, N.C.). Transformations of anthocyanins and anthocyanin/phenolic were included to ensure that the assumptions of the mixed linear model were not violated. Nonsignificant quantitative terms were removed from the model and the simplest hierarchal polynomial was used to describe changes over time. The mean content for each of the responses at harvest were estimated as well as the change in the response over time. All comparisons were made on the transformed scale (where appropriate) and

back transformed for presentation purposes. Unless otherwise indicated, only results with $P < 0.05$ are discussed.

Results

FRUIT COMPOSITION AT HARVEST. Size of the highbush blueberry fruit differed among the various stages of ripeness. Fruit of the two most ripe stages (100% blue and fully ripe) were more than 2.5 times larger (cubic centimeters per fruit) than the white fruit (Table 1). Among the three cultivars, the percent dry weight was between 14% and 21% higher in the fully ripe fruit, compared to the white fruit (Table 1). Since differences in dry weight were relatively small compared to differences in fresh weight, the content of anthocyanins, phenolics and antioxidant capacity are expressed on a dry weight basis. Although the cultivars did not differ in fruit size (cm³), they did differ marginally in their percent dry weight ($P = 0.021$), with 'Bluegold' > 'Nelson' > 'Bergitta'.

Anthocyanin content differed among the ripeness stages ($P < 0.0001$), and ranged from near 0 in white fruit, to approximately 8 mg cyanidin 3-glucoside equivalents/g dry weight in fruit that

was 100% blue or fully ripe at harvest (Table 2). There was an interaction between ripeness and cultivar ($P < 0.0001$). However, the anthocyanin content was only marginally different among the three cultivars ($P = 0.049$). When anthocyanin content was expressed as a proportion of the total phenolics, a significant effect of ripeness and combinations of ripeness by cultivar were observed, although the cultivars did not differ from each other ($P = 0.15$). 'Bluegold' had a higher anthocyanin content (cyanidin 3-glucoside equivalents/g dry weight) than 'Nelson' ($P = 0.033$) and 'Bergitta' ($P = 0.030$) although 'Nelson' and 'Bergitta' were not different from each other.

Total phenolic content decreased as the fruit became ripe ($P = 0.0003$), and ranged from about 11 to 28 mg gallic acid equivalents/g dry weight among fruit of the various cultivar × ripeness combinations. The cultivars differed in their total phenolic content ($P = 0.013$) and there was a significant interaction between cultivar and ripeness ($P = 0.040$). 'Bergitta' had a lower phenolic content than the other two cultivars ($P < 0.01$). Phenolic content of 'Bluegold' and 'Nelson' did not differ from each other ($P = 0.246$).

The anthocyanin content of the fruit ranged between 1% and 60%

Table 1. Size and dry weight content of highbush blueberry fruit harvested at different stages of ripeness.

Cultivar	Parameter	White	Pink	5% to 50% Blue	50% to 95% Blue	100% Blue	Ripe
				Ripeness stage			
Bergitta	Fruit size[z,y] (cm³)	0.992	0.94	1.50	1.69	2.31	2.45
	SE	0.0942	0.0893	0.143	0.161	0.219	0.232
Bluegold	Fruit size[z,y] (cm³)	0.799	1.06	1.23	1.41	1.88	2.41
	SE	0.0759	0.101	0.117	0.134	0.179	0.229
Nelson	Fruit size[z,y] (cm³)	0.813	1.16	1.39	1.57	2.21	2.23
	SE	0.0772	0.110	0.132	0.149	0.21	0.212
Bergitta	Dry wt[x] (%)	10.1	11.0	11.9	10.6	13.6	12.9
	SE	0.0485	0.528	0.571	0.509	0.652	0.619
Bluegold	Dry wt[x] (%)	12.3	13.7	12.2	11.9	14.4	15.2
	SE	0.590	0.658	0.586	0.571	0.691	0.730
Nelson	Dry wt[x] (%)	12.2	11.7	12.1	12.0	13.2	14.3
	SE	0.586	0.562	0.581	0.576	0.634	0.686

[z]Back transformed from $\log_{10}$.
[y]F probability: ripeness $P < 0.0001$; cultivar $P = 0.158$.
[x]F probability: ripeness $P < .0001$; cultivar $P = 0.021$.

Table 2. Anthocyanin, total phenolic content, and oxygen radical absorbing capacity (ORAC) of highbush blueberry fruit harvested at different stages of ripeness.

Cultivar	Parameter	White	Pink	5% to 50% Blue	50% to 95% Blue	100% Blue	Ripe
				Ripeness stage			
Bergitta	Anthocyanin[z]	0.016 ± 0.004	0.122 ± 0.028	1.59 ± 0.360	7.81 ± 1.79	7.41 ± 2.26	8.19 ± 2.49
Bluegold	Anthocyanin	0.045 ± 0.011	0.460 ± 0.107	2.72 ± 0.630	7.37 ± 1.69	8.38 ± 2.55	9.23 ± 2.81
Nelson	Anthocyanin	0.030 ± 0.011	0.075 ± 0.017	1.63 ± 0.370	5.51 ± 1.27	8.67 ± 2.64	8.45 ± 2.57
Bergitta	Phenolics[y]	15.7 ± 2.10	18.7 ± 2.09	12.5 ± 2.10	13.5 ± 1.98	10.9 ± 2.04	11.7 ± 2.44
Bluegold	Phenolics	23.9 ± 1.22	20.0 ± 2.03	26.8 ± 2.73	28.0 ± 2.20	18.5 ± 2.34	19.6 ± 2.29
Nelson	Phenolics	24.5 ± 2.12	22.5 ± 2.21	20.2 ± 2.12	23.1 ± 2.16	18.7 ± 2.39	14.1 ± 2.21
Bergitta	Anthocyanin[x]	2.85 ± 0.620	2.80 ± 0.610	12.5 ± 2.40	43.2 ± 5.50	60.5 ± 5.90	47.1 ± 7.60
Bluegold	Anthocyanin	1.33 ± 0.290	4.02 ± 0.870	10.5 ± 2.10	22.3 ± 3.90	39.7 ± 6.20	40.4 ± 6.30
Nelson	Anthocyanin	1.37 ± 0.300	1.51 ± 0.330	8.48 ± 1.71	23.1 ± 3.80	39.3 ± 6.20	50.8 ± 6.37
Bergitta	ORAC[w]	305 ± 50.0	279 ± 38.0	318 ± 49.0	293 ± 27.0	228 ± 71.0	206 ± 41.0
Bluegold	ORAC	502 ± 61.0	419 ± 40.0	439 ± 50.0	475 ± 40.0	385 ± 51.0	423 ± 82.0
Nelson	ORAC	524 ± 49.0	431 ± 49.0	309 ± 49.0	373 ± 41.0	347 ± 68.0	333 ± 77.0

[z]Milligram cyanidin 3-glucoside equivalents/gram dry weight, back transformed from $\log_{10}$; F probability: ripeness $P < 0.0001$; cultivar $P = 0.049$; cultivar × ripeness $P < 0.0001$.
[y]Milligram gallic acid equivalents/gram dry weight; F probability: ripeness $P = 0.0003$; cultivar $P = 0.013$; cultivar × ripeness $P = 0.040$.
[x]Percent of total phenolics, back transformed from logit; F probability: ripeness $P < 0.0001$; cultivar $P = 0.060$; cultivar × ripeness $P = 0.0002$.
[w]Oxygen radical absorbing capacity, micromole trolox equivalents/gram dry weight; F probability: ripeness $P = 0.001$; cultivar: $P = 0.011$; cultivar × ripeness: $P = 0.172$.
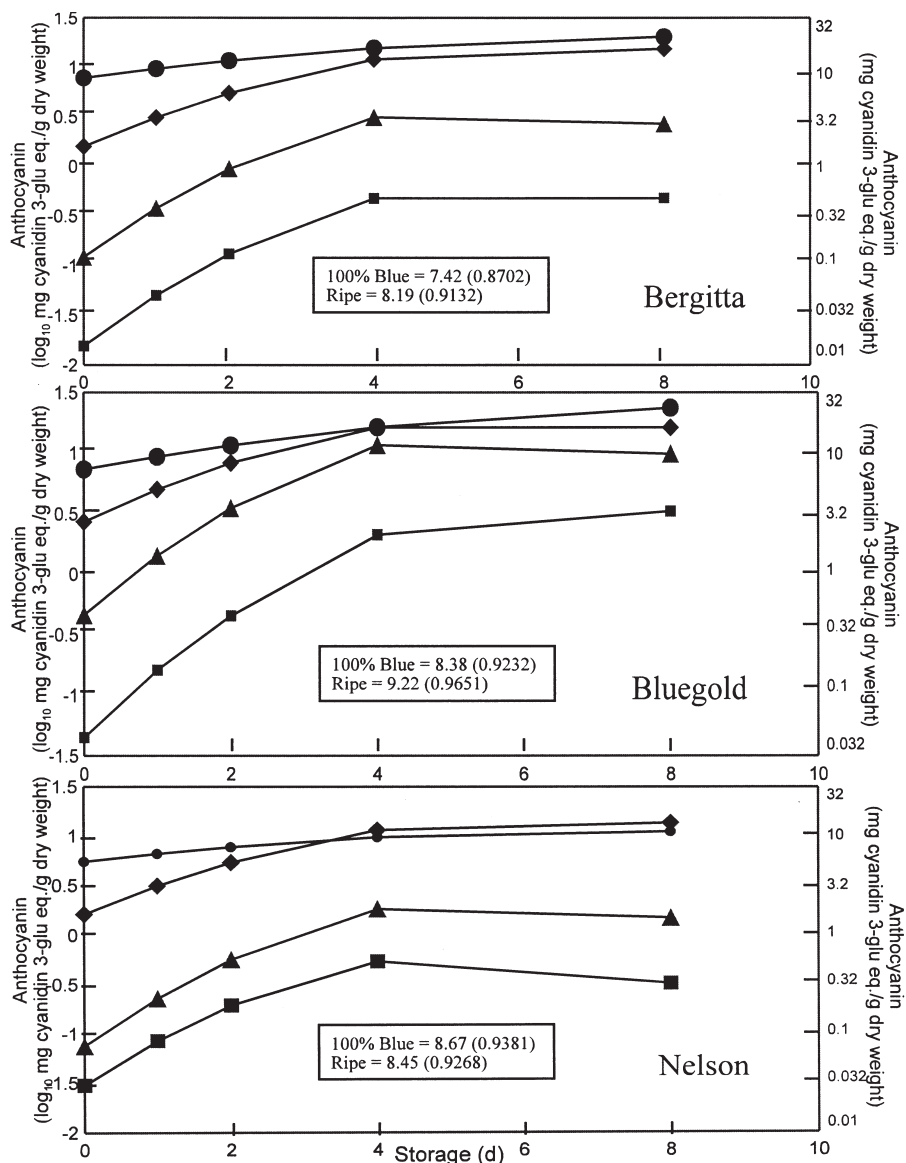
Fig. 2. Changes in anthocyanin content (milligram cyanidin 3-glucoside equivalents/g dry weight) of highbush blueberry cultivars during storage at 20 °C. White (■); Pink (▲); 5% to 50% Blue (◆); 50% to 95% Blue (●).

increase in the anthocyanin content ($\log_{10}$ cyanidin 3-glucoside equivalents/g dry weight) in fruit of the four underripe stages during storage at 20 °C. The increase in anthocyanin content could be fitted to a linear model, which depended on both ripeness and cultivar ($P < 0.0001$) or a quadratic model, which depended only on ripeness ($P < 0.0001$) (Fig. 2). Anthocyanin accumulation during storage appeared linear in the fruit that were 5% to 50% and 50% to 95% blue at harvest. During the 8 d storage period, the anthocyanin content of the 5% to 50% blue-harvested 'Nelson' increased by more than 5-fold, while the increase was only about 2.8- and 1.3-fold in 'Bluegold' and 'Bergitta', respectively (Fig. 2). 'Bluegold', 'Bergitta' and 'Nelson' fruit that were 50% to 95% blue at harvest had increased their anthocyanin content by 2.6-, 2.5-, and 1.3-fold, respectively, after 8 d of storage (Table 2, Fig. 2). The accumulation of anthocyanin in white and pink fruit during storage appeared to conform to a quadratic function. The pink and white fruit never attained a pigment level as high as that of the 100% blue or ripe fruit at harvest.

Changes in the content of dissolved phenolics in fruit stored for 8 d best fit a linear model ($P < 0.0001$), although they depended on the particular cultivar and maturity combination ($P = 0.0192$). In general, phenolic content increased during storage, however among the cultivar and ripeness combinations, different patterns of change were observed.

The percent (logit % anthocyanins) of the total phenolics present as anthocyanins in the under-ripe fruit changed during storage (Fig. 3). Changes could be fitted to a linear model ($P < 0.0001$) depending on the cultivar ($P < 0.0001$) and cultivar and maturity combination ($P = 0.0233$). A linear increase in the percentage of phenolics present as anthocyanins was most apparent in the white and the 50% to 95% blue fruit. A quadratic model could also be applied to the changes in the percent anthocyanins during storage ($P < 0.0001$), which depended only on the maturity of the fruit ($P = 0.0154$). A curvilinear increase in percent of phenolics as anthocyanins was most apparent in the pink and the 50% to 95% blue fruit.

There was a linear increase in the ORAC during fruit storage ($P = 0.0001$), which was independent of cultivar and ripeness. The ORAC value for all cultivar and maturity combinations increased by an estimated 10.6 μmol TE/g dry weight per day, and after 8 d of storage ORAC had increased between 20% and 30% among the three cultivars (Fig. 4).

Over all cultivars, ripeness stages, and storage treatments, there was a strong, positive relationship between ORAC and total dissolved phenolics ($R^2 = 0.78$). However there was no relationship between ORAC and anthocyanin content ($R^2 < 10^{-4}$). Anthocyanins and phenolics also were not correlated ($R^2 = 0.058$).

of the total dissolved phenolics in the fruit (Table 2). There was a significant interaction between cultivar and maturity ($P = 0.0002$), which was apparent at several ripeness stages. The percentage of phenolics present as anthocyanins was higher in 'Bergitta' fruit than the other two cultivars, in 10 of the 12 cultivar × ripeness combinations ($P \le 0.016$). 'Bluegold' and 'Nelson' only differed at the pink fruit color stage ($P < 0.0001$). All the cultivars had a similar percentage of phenolics present as anthocyanins at the ripe stage (i.e. commercial maturity).

ORAC was different ($P = 0.001$) among the six ripeness stages at the time of harvest. Fruit of more advanced ripeness were generally lower in their ORAC values than less ripe fruit (Table 2). The cultivars also differed in their ORAC ($P = 0.011$). The ORAC of 'Bergitta' was significantly lower than either 'Bluegold' ($P = 0.0048$) or 'Nelson' ($P = 0.0125$), while 'Bluegold' and 'Nelson' did not differ from each other ($P = 0.242$). There was no interaction between cultivar and ripeness for ORAC.

CHANGES IN FRUIT DURING STORAGE. There was a substantial

Discussion

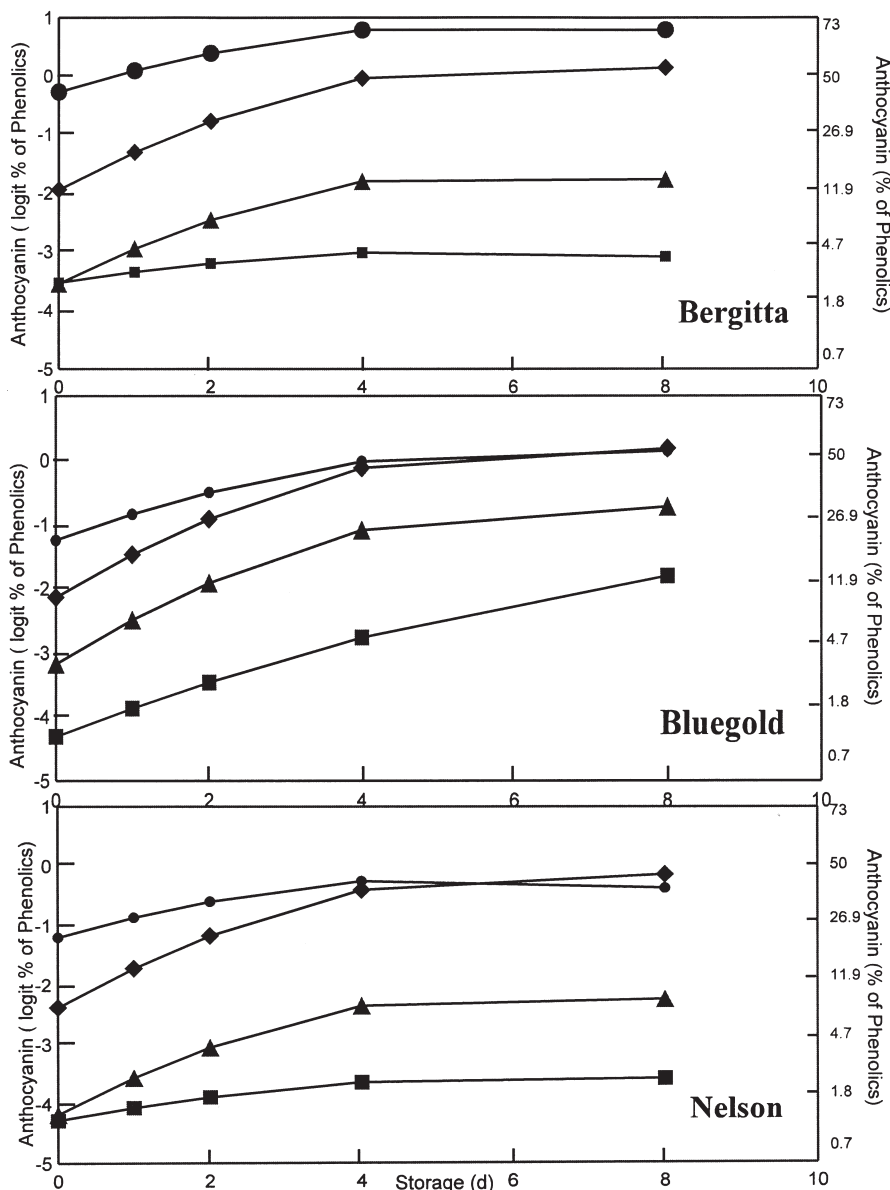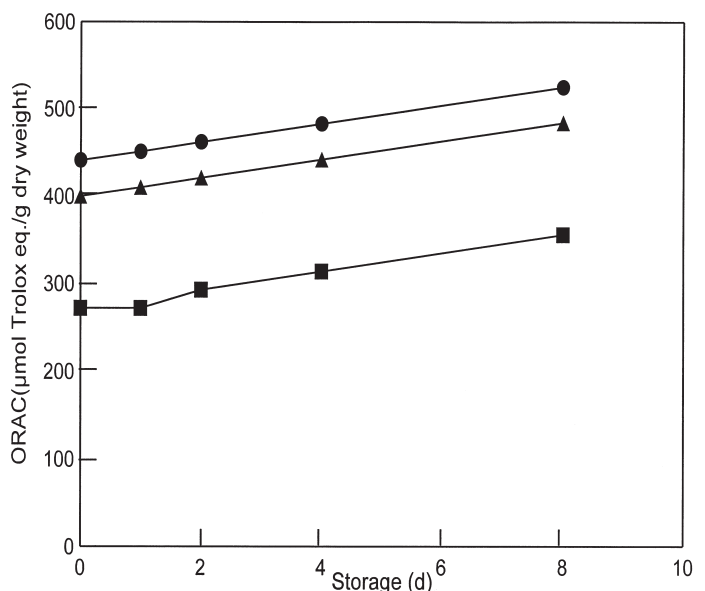Compared to many fruit and vegetables, blueberries have a

Fig. 3. Changes in anthocyanin content (percent of total phenolics) of highbush blueberry cultivars during storage at 20 °C. White (■); Pink (▲); 5% to 50% Blue (◆); 50% to 95% Blue (●).

in percent dry weight (≈17%), compared to the large increase in fruit size (≈250%), between the least ripe and most ripe stages of blueberry fruit, reflects the substantially higher water content of the riper fruit (100% blue and fully ripe) (Table 1). Although there was no difference in fruit size among the three highbush blueberry cultivars in this study, a wide range in the size of ripe fruit was reported in a survey of 80 northern and southern highbush cultivars (Kalt et al., 2001).

Both anthocyanin and phenolic concentration changed during fruit ripening. Anthocyanin content increased substantially while phenolics decreased (Table 2). Therefore, during ripening of highbush blueberry there is a shift in the pool of total phenolics toward anthocyanin synthesis, and an overall decline in the content of other phenolic components. Although specific phenolic types, other than anthocyanins, were not measured in this study, other studies report a decline in catechin monomers (Stőhr and Herrmann, 1975) and oligomers (Lister et al., 1994) during fruit ripening. Hydroxycinnamate esters, such as chlorogenic acid, which make a substantial contribution to the total phenolic content of blueberries (Kalt and McDonald, 1996), have been reported to decline during ripening of various fruit, including blueberries (Herrmann, 1989). However, the chlorogenic acid content was not lower in overripe compared to underripe lowbush blueberries (Kalt and McDonald, 1996). A decline in phenolic content during ripening does not occur in all fruit crops. Wang and Lin (2000) reported a substantially higher level of total phenolics in red ripe, compared to pink underripe, fruit of red raspberry.

Differences in the ORAC between cultivars and ripeness stages reflected differences

high ORAC (Cao et al., 1996; Prior et al., 1998; Wang et al., 1996) due to their high level of phenolic components, including anthocyanins (Kalt et al., 1999; Prior et al., 1998). Blueberry phenolic components were thought to be responsible for beneficial health effects in animals during brain aging (Youdim et al., 2000) and ischemic stroke (Sweeney et al., 2002). Blueberry phenolics have been attributed to having beneficial bioactivities against cancer (Bomser et al., 1996) and in maintenance of urinary tract health (Howell et al., 1998). Studies to date have reported a positive relationship between ORAC and both phenolics and anthocyanins (Kalt et al., 1999; Prior et al., 1998). However, the current study demonstrates that anthocyanins are not associated with the antioxidant activity of fruit at all ripeness stages.

FRUIT COMPOSITION AT HARVEST. The relatively small increase

Fig. 4. Changes in the oxygen radical absorbing capacity of 'Bergitta' (■), 'Bluegold' (▲), and 'Nelson' (●) during storage. Changes in ORAC antioxidant capacity during storage were not different among the white, pink, 5% to 50% blue, and 50% to 95% blue ripeness stages.

in total phenolic content, but not the content of anthocyanins. Similar to the phenolic content, ORAC was lower in the fruit of more advanced ripeness, while anthocyanins were substantially higher in the riper fruit. Among the cultivars, 'Bergitta' fruit was lowest in both total phenolic ($P = 0.013$) and ORAC ($P = 0.011$), while its anthocyanin content was similar to 'Nelson'.

The results illustrate the large differences in fruit composition due to ripeness, and to a lesser degree, due to cultivar. This description of the effect of fruit maturity on the relative content of phenolics and anthocyanins, and resulting ORAC, may aid in the development of harvest and grading protocols to optimize these parameters.

Fruit composition during storage. The amount of anthocyanins produced during storage depended on the maturity of the fruit before storage with less anthocyanin synthesis occurring in less mature fruit (Table 3, Fig. 2). This suggests there may be a threshold level of ripeness, after which anthocyanin synthesis can continue either on or off the plant. If so, the results suggest the threshold ripeness would be between the pink and the 5% to 50% blue stages. This is based on the fact that the pink fruit never attained as high a pigment level as the 100% blue and fully ripe fruit at harvest, and its anthocyanin synthesis slowed markedly after ≈4 d of storage. In contrast, the 5% to 50% blue fruit accumulated anthocyanins during storage in excess of the level found in ripe fruit at harvest. Although the accumulation in 5% to 50% blue fruit also slowed after 4 d, the decline in the rate was not as great as in pink fruit (Fig. 2).

The general linear increase in phenolic content during storage of underripe fruit ($P = 0.0192$) suggests in some combinations of cultivar and ripeness, phenolic components were synthesized from nonphenolic constituents after the fruit was removed from the plant. However, the magnitude of the increase in phenolics after 8 d of storage at 20 °C was small in comparison to the large increase in anthocyanins. Therefore, under these conditions the increase in anthocyanin content after harvest was due mainly to the conversion of nonanthocyanin phenolic precursors into anthocyanins. This was also reflected in a generally similar pattern of change in the absolute content of anthocyanins, and the anthocyanin content expressed as a percentage of the total phenolics present (Figs. 2 and 3). Thus, while phenolics generally decrease in fruit that are ripened on the plant, phenolics can also increase during postharvest storage.

The postharvest accumulation of pigments in commercially ripe berry fruit has been recently reported. Although there was no change in the total phenolic content of ripe 'Bluecrop' highbush blueberries during 20 °C storage, the total phenolic content and anthocyanin content of ripe 'Nova' raspberries did increase under the same conditions (Kalt et al., 1999). Thus, an increase in total phenolic content in raspberries with ripening has been observed both on the plant (Wang and Lin, 2000) and after harvest (Kalt et al., 1999). Carbon skeletons for phenolic synthesis after harvest may be derived from carbohydrates or organic acids, both of which are abundant and whose content is rapidly changing during fruit ripening.

In the present study, the increase in ORAC during fruit storage ($P = 0.0001$) reflects the increase in total phenolic content, mostly anthocyanins, that occurred in the fruit while stored. In spite of the cultivar- and ripeness-related changes in phenolics and anthocyanins during storage, the increase in ORAC during storage was not dependent on the cultivar or the ripeness stage of the fruit. Since ORAC measures all water soluble ORAC-active compounds in the fruit extract (e.g., ascorbate, sugars) the cultivar

and ripeness related differences in phenolic components may be obscured by the contributions of other components to the ORAC measurement. Ascorbate in highbush blueberries contribute less than 1% of the total ORAC (Prior et al., 1998) while sugars in ripe fruit can contribute less than 10% (Kalt, unpublished).

In summary, since anthocyanins are the predominant phenolic in ripe blueberry fruit (Table 2, Fig. 3) (Kähkönen et al., 2001; Machiex et al., 1990), it is not surprising that anthocyanins are strongly positively correlated to ORAC in ripe blueberry fruit (Prior et al., 1998). Processed blueberry fruit products (e.g., juice) may contain lower grades of fruit, including fruit that are not fully ripe. This study indicates that underripe fruit whose color development is not complete have ORAC as high or higher than ripe fruit (Table 2). This study also demonstrates the potential to improve color and increase ORAC in blueberry fruit after fruit harvest and during storage.

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