

Antioxidant Capacity, Vitamin C, Phenolics, and Anthocyanins after Fresh Storage of Small Fruits

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Fresh strawberries (*Fragaria × ananassa* Duch.), raspberries (*Rubus idaeus* Michx.), highbush blueberries (*Vaccinium corymbosum* L.), and lowbush blueberries (*Vaccinium angustifolium* Aiton) were stored at 0, 10, 20, and 30 °C for up to 8 days to determine the effects of storage temperature on whole fruit antioxidant capacity (as measured by the oxygen radical absorbing capacity assay, Cao et al., *Clin. Chem.* **1995**, *41*, 1738–1744) and total phenolic, anthocyanin, and ascorbate content. The four fruit varied markedly in their total antioxidant capacity, and antioxidant capacity was strongly correlated with the content of total phenolics (0.83) and anthocyanins (0.90). The antioxidant capacity of the two blueberry species was about 3-fold higher than either strawberries or raspberries. However, there was an increase in the antioxidant capacity of strawberries and raspberries during storage at temperatures >0 °C, which was accompanied by increases in anthocyanins in strawberries and increases in anthocyanins and total phenolics in raspberries. Ascorbate content differed more than 5-fold among the four fruit species; on average, strawberries and raspberries had almost 4-times more ascorbate than highbush and lowbush blueberries. There were no ascorbate losses in strawberries or highbush blueberries during 8 days of storage at the various temperatures, but there were losses in the other two fruit species. Ascorbate made only a small contribution (0.4–9.4%) to the total antioxidant capacity of the fruit. The increase observed in antioxidant capacity through postharvest phenolic synthesis and metabolism suggested that commercially feasible technologies may be developed to enhance the health functionality of small fruit crops.

Keywords: *Anthocyanins; phenolics; ascorbate; strawberries; raspberries; blueberries; ORAC*

INTRODUCTION

A preponderance of epidemiological studies provide convincing evidence of the beneficial role of fruits and vegetables in the diet for the maintenance of health and prevention of disease (Ames et al., 1993). More recently, evidence is accumulating from several fields of science, including epidemiology, human medicine, and nutrition, to suggest that fruit and vegetable antioxidants play an important role in reducing the risk of degenerative diseases such as cardiovascular disease, various cancers, and neurological diseases. Ascorbate, an essential vitamin found in fruits and vegetables, has been particularly well studied in its role as an antioxidant and is suggested to serve several physiological functions including (1) preventing free-radical-induced damage to DNA (Fraga et al., 1991), (2) quenching oxidants which can lead to the development of cataracts (Mares-Perlman, 1997), (3) improving endothelial cell dysfunction (Levine et al. 1996), and (4) decreasing LDL-induced leukocyte adhesion (Lehr et al., 1995).

The significance of fruit and vegetable phenolics as dietary antioxidants has recently been suggested by several research groups. Their studies illustrate how phenolics, and especially flavonoids, (1) have substantial

antioxidant capacity (Prior et al., 1998), (2) make a significant contribution to the diet (Hertog et al., 1993), (3) may reduce the risk of cardiovascular disease (Schramm and German, 1998), and (4) have a stability that exceeds that of ascorbate (Miller and Rice-Evans, 1997).

Interest in the role of antioxidants in human health has prompted research in the fields of horticulture and food science to assess fruit and vegetable antioxidants, such as ascorbate, carotenoids, tocopherols, and phenolics, and to determine how their content and activity can be maintained or even improved through cultivar development, production practices, postharvest storage, and food processing. Fruits are good sources of both phenolics and ascorbate, and since fruits are often consumed fresh, antioxidant capacity is not lost due to any adverse effects of heat and oxidation during processing. Little is known, however, about the effects of fresh fruit storage on the retention of dietary antioxidants such as phenolics and ascorbate.

Postharvest storage of fruit crops is interesting in that metabolism continues to occur, although the fruit is detached from the plant (i.e., the nutrient source). The goal of postharvest storage technology is to manipulate fruit and vegetable metabolism during storage to extend product shelf life. In most cases, this involves slowing respiratory metabolism through low-temperature storage or storage in a high carbon dioxide atmosphere (Hardenburg et al., 1986). The notion of manipulating

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Table 1. Content of Phenolics, Anthocyanins, and Ascorbate and Antioxidant Capacity (ORAC_{ROO}) of Four Fruit Species at Harvest^a

fruit	phenolics μmol of gallic acid/g FW	anthocyanins μmol of Mal-3-glu/g FW	ascorbate $\mu\text{mol/g}$ FW	ORAC _{ROO} μmol of trolox eq/g FW
strawberry	5.08 (0.438)	0.155 (0.028)	1.96 (0.147)	20.6 (2.33)
raspberry	7.10 (0.188)	0.840 (0.053)	1.23 (0.066)	21.4 (2.24)
highbush blueberry	22.7 (0.804)	2.67 (0.097)	0.489 (0.031)	60.1 (2.81)
lowbush blueberry	27.7 (1.09)	4.35 (0.160)	0.358 (0.014)	64.4 (3.68)
<i>F</i> probability	<0.001	<0.001	<0.001	<0.001
S.E.	0.566	0.0359	0.0281	2.25

^a Standard error values are given in parentheses. For each fruit $n = 4$.

postharvest metabolism to enhance the health functionality of fruits and vegetables has not been explored.

The following study describes the responses of four small fruit crops to storage at various temperatures with regard to their phenolic, anthocyanin, and ascorbate content and consequent antioxidant capacity. Results suggest that storage at temperatures >0 °C may actually enhance antioxidant capacity and, therefore, the health functionality of certain small fruit crops.

MATERIALS AND METHODS

Chemicals. *R*-Phycoerythrin (*R*-PE), ascorbic acid, and gallic acid were purchased from Sigma Chemical Co. (St. Louis, MO). 6-Hydroxy-2,5,7,8-tetramethyl-2-carboxylic acid (Trolox) was purchased from Aldrich Chemicals (Milwaukee, WI). 2,2'-Azobis(2-amidinopropane) dihydrochloride (AAPH) was purchased from Wako Chemicals USA, Inc. (Richmond, VA). Glycerol was purchased from VWR, Richmond, BC. Methanol (HPLC grade), metaphosphoric acid, glacial acetic acid, and hydrochloric acid were purchased from Fisher Scientific (Nepean, ON).

Fruit Samples. Strawberry (*Fragaria* \times *ananassa* Duch., cv. Kent), raspberry (*Rubus idaeus* Michx., cv. Nova), and highbush blueberry (*Vaccinium corymbosum* L., cv. Bluecrop) fruit were each harvested from two different commercial growers near the Agriculture and Agri-Food Canada Research Centre in Kentville, NS, Canada. At least 20 clones of wild blueberries (*Vaccinium angustifolium* Aiton) were harvested from each of two sites in the blueberry production region approximately 100 km from Kentville. Fruit harvests were conducted twice, for a total of four replicate samples (two locations, two harvests) for each species. In all cases, fully colored fruit were selected for storage; over-ripe (deeply colored), damaged, and underripe fruit were removed.

Storage Treatments. For each fruit species, four samples were packaged separately in half pint or one pint polystyrene 'clam shell' containers. Approximate sample weights were as follows: strawberries 300 g, raspberries 200 g, highbush blueberries 350 g, and lowbush blueberries 200 g. Packaged samples were stored in a stainless steel chamber (0.67 m³) which was sealed with an airtight Plexiglas lid. Chambers were stored in the dark in controlled temperatures rooms set at 0, 10, 20, and 30 °C (± 0.5 °C). A constant vapor pressure deficit of 0.212 kPa was maintained in the chambers using solutions of glycerol-water as described by Forney and Brandl (1992). Samples (four samples per species) were removed after 0, 2, 4, and 8 days of storage.

Sample Preparation. After removal from the storage chamber and separation from damaged or decayed fruit, an approximately 25 g subsample of whole fruit was immediately homogenized in 10 volumes of a cold solution of 3% (w/v) metaphosphoric acid, 8% glacial acetic acid (v/v), in water (pH 1.5) for 1 min, using a Waring Blender (Waring Products, New Hartford, CT). Because of their large size, strawberry fruit were first cut in half and one-half was immediately ground as described above. A uniform suspension of homogenate was stored at -70 °C for ascorbate analysis. Fruit extracts for phenolic analysis were obtained by grinding fruit (1:2 w/v) for 2 min in hot methanol, using a Vitis Homogenizer (The Virtis

Co., Gardiner, NY). Fruit extracts for anthocyanin analysis were obtained by grinding fruit (1:10) in methanol acidified with 0.01% HCl (v/v) for 2 min, followed by storage at room temperature in the dark for at least 12 h. Phenolic and anthocyanin extracts were stored at -40 °C prior to analysis. The methanolic extract obtained for phenolic analysis was also used for measurement of antioxidant capacity by drying an aliquot of extract under vacuum at 30 °C and resolubilizing in water. Depending on the fruit, aqueous extracts were further diluted between 100- and 400-fold prior to the antioxidant capacity assay.

Measurement of Anthocyanins, Phenolics, Ascorbate, and Antioxidant Capacity. Total anthocyanin content of the appropriately diluted extracts was determined by the spectrophotometric method of Wrolstad (1976), and data were calculated using the extinction coefficient for malvidin 3-glucoside (28 000). Total phenolic content was measured by the Folin-Ciocalteu method at 700 nm, using gallic acid as a standard (Singleton and Rossi, 1965).

Ascorbate homogenates were centrifuged at 12 000 rpm at 4 °C for 5 min. Ascorbate was analyzed by paired-ion RP-HPLC, with electrochemical detection (Martin and Frei, 1997). An aliquot of the acidic supernatant was chromatographed on an LC8 column (150 mm \times 4.6 mm i.d., 3 μm particle size; Supelco, Bellefonte, PA) using deionized water containing 1% methanol in 40 mM sodium acetate and 1.5 mM dodecyltri-ammonium phosphate as the mobile phase (Q12 ion pair cocktails; Regis, Morton Grove, IL). Ascorbate was detected at an applied potential of +0.6 V by an LC 4B amperometric electrochemical detector (Bioanalytical Systems, West Lafayette, IN).

Antioxidant capacity was measured as oxygen radical absorbing capacity (ORAC) according to the method of Cao et al. (1995) using a COBAS-FARA II centrifugal analyzer (Roche Diagnostic, Nutley, NJ). The ORAC assay (400 μL) contained 16.7 nM *R*-phycoerythrin (*R*-PE; Sigma, St. Louis MO), 4 mM AAPH, and 20 μL of sample. After initiation of the assay with AAPH, a peroxy radical generator) fluorescence of *R*-PE was read on duplicate samples, blanks, and standards of Trolox, a water-soluble vitamin E analogue. Readings were taken at 2 min intervals over a 70 min period. The area under the curve was calculated and expressed as micromole equivalents of Trolox per gram of fresh weight.

Statistical Analysis. The analysis of variance procedure of Genstat 5 (Genstat 5 Committee, 1993) was used to analyze results. Unless otherwise indicated, only differences of $P < 0.05$ are discussed. Bivariate regression analysis was used to correlate anthocyanin, phenolic, ascorbate, and ORAC results.

RESULTS

Phenolics, Anthocyanins, Ascorbate, and Antioxidant Capacity. Strawberry, raspberry, highbush blueberry, and lowbush blueberry were different ($P \leq 0.001$) from each other in their contents of phenolics, anthocyanins, and ascorbate and their antioxidant capacity (Table 1). The total phenolic content of highbush and lowbush blueberries was about 4-fold higher than in strawberries and raspberries. The anthocyanin content of the four species varied more than 25-fold with

Table 2. Statistical Significance of Changes in Content of Phenolics, Anthocyanins, and Ascorbate and Antioxidant Capacity against the Peroxyl Radical (ORAC_{ROO}) among Four Fruit Species, during Storage^a

fruit	factor	F probability			
		phenolics	anthocyanin	ascorbate	ORAC _{ROO} ^c
strawberry	days ^b	NS	<0.001	NS	0.032
	temperature ^c	NS	<0.001	NS	<0.001
raspberry	days	<0.001	<0.001	<0.001	0.003
	temperature	<0.001	<0.001	0.013	0.002
highbush blueberry	days	NS	0.014	NS	NS
	temperature	NS	<0.001	NS	NS
lowbush blueberry	days	NS	NS	0.022	NS
	temperature	NS	NS	0.004	NS

^a For each combination of days and temperature, $n = 4$. ^b Stored for 0, 1, 2, 4, and 8 days. ^c Stored at 0, 10, 20, or 30 °C.

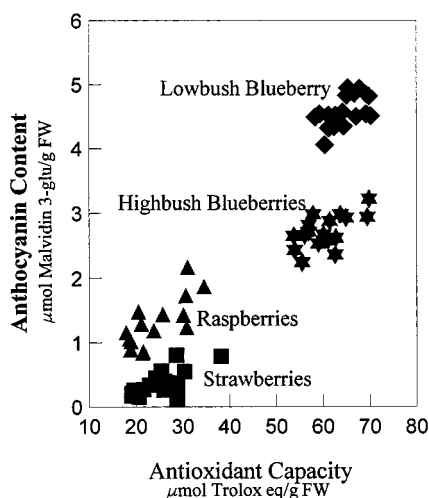


Figure 1. Relationship between anthocyanins and antioxidant capacity in four fruits after storage at different temperatures and for different periods. Each data point is the mean of four samples per temperature and storage period combination.

lowbush blueberry > highbush blueberry > raspberry > strawberry. Ascorbate varied more than 5-fold with strawberry > raspberry > highbush blueberry > lowbush blueberry. The total antioxidant capacity of the two blueberry species was about 3-fold higher than strawberries or raspberries, which were similar to each other (Table 1).

Using results from all species, storage temperatures, and storage periods, anthocyanin content was strongly correlated with the total phenolic content of the fruit, ($R = 0.91$). Phenolics and anthocyanins were both strongly correlated to antioxidant capacity. In bivariate regression of anthocyanin and antioxidant capacity for the four species, for all storage temperatures and days in storage, $R = 0.90$ (Figure 1). The correlation for total phenolics and antioxidant capacity was 0.83. Because the anthocyanin content changed more than the phenolic content among the fruits during storage (Table 2), only the anthocyanin content of fruit in relation to antioxidant capacity (Figure 1) and storage time and temperature (Figures 2–5) is shown.

Ascorbate content and antioxidant capacity were negatively correlated ($R = -0.80$) since ascorbate levels were low in the fruit where antioxidant capacity was high (Table 1). On the basis of an ORAC value for ascorbate of 5.6 μmol of Trolox equiv per 1 mg of ascorbate (Prior et al., 1998), the antioxidant capacity due to ascorbate was 9.4% for strawberries, 6.1% for raspberries, 0.8% for highbush blueberries, and 0.6% for lowbush blueberries.

Storage Temperature Effects. Strawberry anthocyanin content and antioxidant capacity were affected by storage time and temperature (Table 2). Strawberry anthocyanin content increased an average of 4.3-fold after 8 days, and the magnitude of the increase was related to temperature (Figure 2). When strawberries were stored at 0 °C for 8 days, anthocyanins increased 1.7-fold, while for the same period at 30 °C, the apparent increase was 6.8-fold. The antioxidant capacity of strawberry fruit during the 8 day period increased by an average of 1.5-fold, with the greatest increase occurring at 10 and 20 °C.

Raspberries were most affected by storage; changes ($P < 0.05$) due to storage time and temperature were observed in all variates measured (Table 2). Raspberry phenolics and anthocyanins increased by about 1.5- and 2.5-fold, respectively, after 8 days of storage at 20 °C (Figure 3). Changes in these components were less after 10 and 30 °C storage and least at 0 °C (Figure 3). The increase in raspberry phenolic and anthocyanin content at 20 °C was accompanied by an almost 2-fold increase in antioxidant capacity. During the same period at 20 °C, ascorbate content decreased by 22%. At 30 °C, 46% of the ascorbate was lost by the end of the storage period. After the 8 day storage period, particularly at higher temperatures, raspberry tissues were beginning to break down and some whole fruits had to be discarded. In highbush blueberries, only anthocyanins were significantly affected by storage time ($P = 0.014$) and temperature ($P < 0.001$) (Table 2). The greatest increase in anthocyanin content occurred at 20 °C, where after 8 days the level was 1.2-fold higher than immediately after harvest (Figure 4). Under the same storage conditions, ORAC increased by 1.2-fold, although there was no change in the highbush blueberry ORAC after storage at any other temperature (Figure 4). Lowbush blueberries were the only fruit where anthocyanin content did not increase during storage nor were there changes in total phenolics or antioxidant capacity (Table 2). Ascorbate declined in lowbush blueberries after 8 days of storage at 20 and 30 °C (Table 2; Figure 5).

DISCUSSION

Phenolics, Anthocyanins, Ascorbate, and Antioxidant Capacity. No previous study has directly compared strawberries, raspberries, highbush blueberries, and lowbush blueberries, with respect to phenolics, anthocyanins, ascorbate, and antioxidant capacity during storage. However, phenolics, anthocyanins, and antioxidant capacity were recently examined in highbush and lowbush blueberries (Prior et al., 1998). Anthocyanins, phenolics, and antioxidant capacity were more than 30% lower in the Nova Scotia lowbush

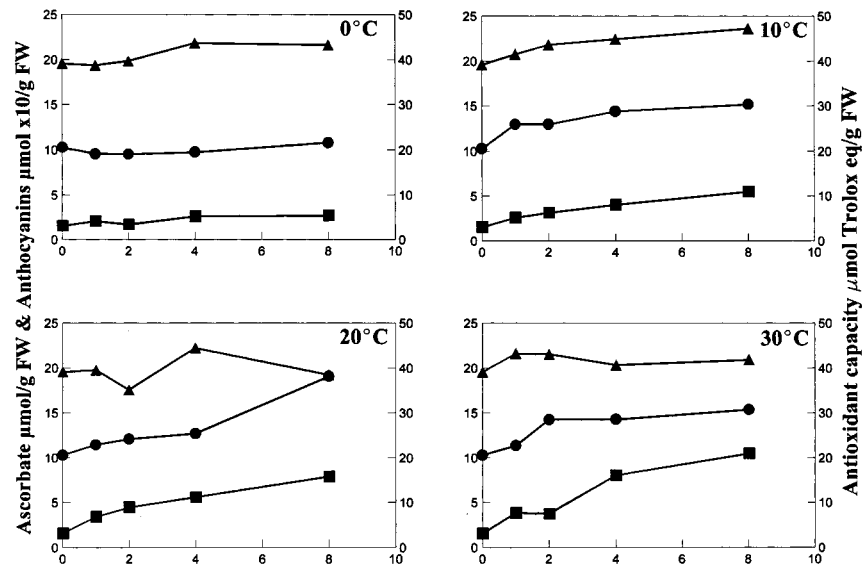


Figure 2. Changes in strawberry anthocyanins (■), ascorbate (▲), and ORAC (●) during storage at 0, 10, 20, and 30 °C. Four samples were removed after 1, 2, 4, and 8 days of storage. Standard error (SE) for anthocyanins = 0.028, ascorbate = 0.147, and ORAC = 2.23.

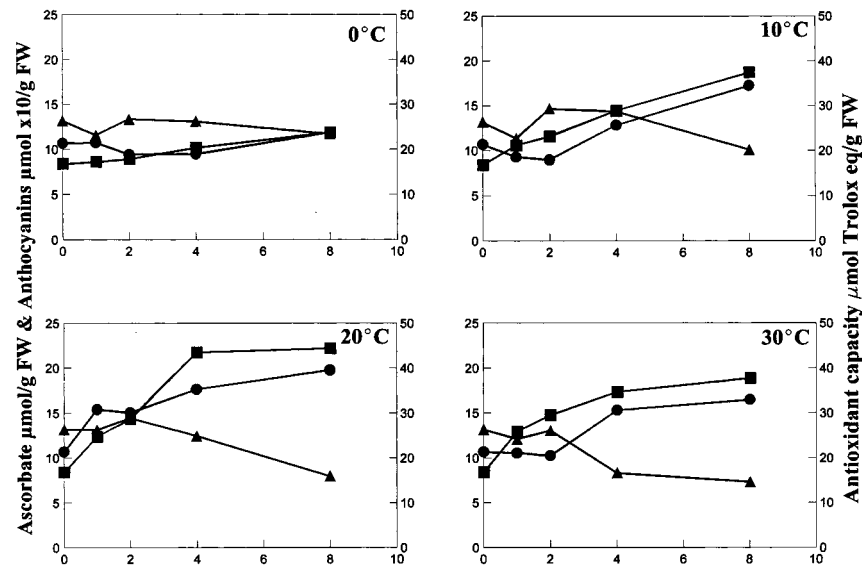


Figure 3. Changes in raspberry anthocyanins (■), ascorbate (▲), and ORAC (●) during storage at 0, 10, 20, and 30 °C. Four samples were removed after 1, 2, 4, and 8 days of storage. SE for anthocyanins = 0.053, ascorbate = 0.066, and ORAC = 2.24.

blueberries samples surveyed by Prior et al. (1998) compared to the present study. In the study by Prior et al. (1998) lowbush blueberry fruit from Maine had a 25–30% lower ORAC value compared to those from Nova Scotia and Prince Edward Island. Compared to the present study, the highbush blueberry cultivar Bluecrop in the study by Prior et al. (1998) was 70% lower in its antioxidant capacity, 50% lower in anthocyanins, and 40% lower in total phenolics. Environmental factors which influence the formation of anthocyanins (e.g., light, temperature, agronomic practices, various stresses) may have contributed to the differences between the two studies. Single genotypes of lowbush blueberries were reported to differ in their anthocyanin content by 30% between two seasons (Kalt and McDonald, 1996).

The antioxidant capacity of strawberries determined using the ORAC assay and reported by Wang et al. (1996) is about 25% lower than the value determined for strawberries in the present study. This may be due, in part, to the use of different strawberry genotypes in

the two studies. Despite the quantitative differences between the studies of Wang et al. (1996) and Prior et al. (1998) with respect to blueberries and strawberries, the same pattern emerged, i.e., that phenolic and anthocyanin content were significantly correlated with antioxidant capacity. The correlation between anthocyanins and antioxidant capacity was higher in the present study ($R = 0.90$) compared to that of Prior et al. (1998) ($R = 0.77$) and is probably due to the greater range in both sets of values that occurred among the four fruit species. The four species split into two distinct groups—those with high phenolics, anthocyanins, and antioxidant capacity and low ascorbate (i.e. highbush and lowbush blueberry) and those with lower phenolics, anthocyanins, and antioxidant capacity and high ascorbate (i.e., strawberry and raspberry) (Table 1).

Ascorbic acid in strawberries and raspberries has been previously reported as 60 and 25 mg/100 g FW, respectively (Mapson, 1970). This is 1.7-fold higher than the value determined in the present study for strawber-

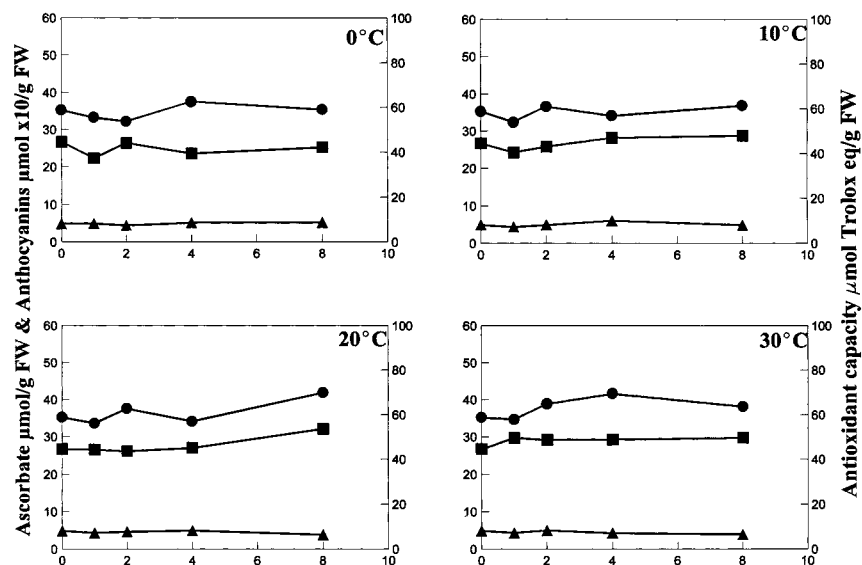


Figure 4. Changes in highbush blueberry anthocyanins (■), ascorbate (▲), and ORAC (●) during storage at 0, 10, 20, and 30 °C. Four samples were removed after 1, 2, 4, and 8 days of storage. SE for anthocyanins = 0.097, ascorbate = 0.031, and ORAC = 2.81.

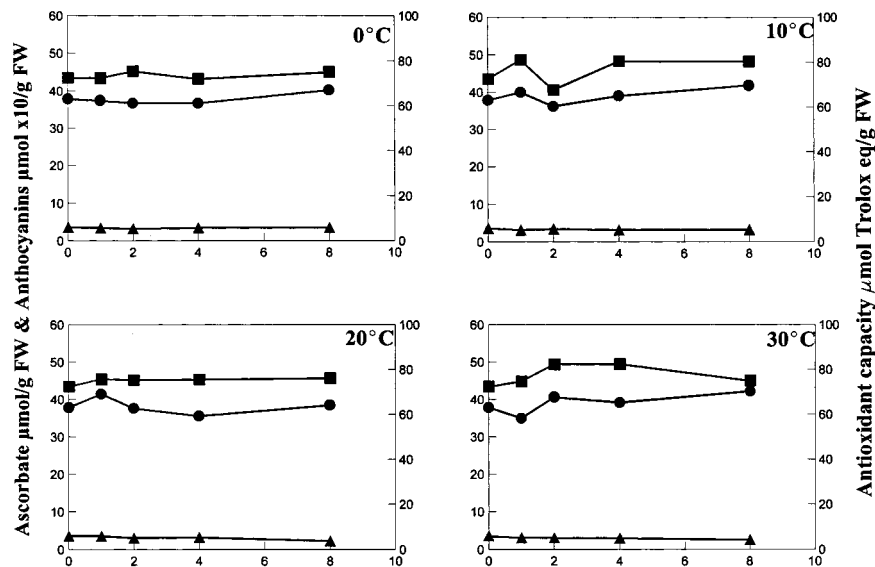


Figure 5. Changes in lowbush blueberry anthocyanins (■), ascorbate (▲), and ORAC (●) during storage at 0, 10, 20, and 30 °C. Four samples were removed after 1, 2, 4, and 8 days of storage. SE for anthocyanins = 0.160, ascorbate = 0.0137, and ORAC = 3.68.

ries, while the value for raspberries is very similar (23 mg/100 g FW). Ascorbate values reported for blueberries by Prior et al. (1998) were very similar to those of the present study. Ascorbate content in the highbush cultivar Bluecrop was virtually identical in the two studies. The mean ascorbate content of lowbush blueberries surveyed by Prior et al. (1998) was 5.5 mg/100 g FW and among our samples was 6.3 mg/100 g FW. These values were somewhat lower than the range of 7–20 mg of ascorbate/100 g FW reported for both blueberry species (Kalt and Dufour, 1997) but within the range of 1.3–16.4 mg/100 g reported by Prior et al. (1998).

Storage Temperature Effects. Increases in anthocyanin content during storage have been previously reported for strawberries (Kalt et al., 1993), lowbush blueberries (Kalt and McDonald, 1996), rabbiteye blueberries (Basiouny and Chen, 1988), and raspberries (Mazza and Miniati, 1993). In *cv.* Blomidon strawberries, anthocyanin formation during storage was greater

at 20 °C than at 10 or 30 °C and white-harvested fruit formed much more anthocyanin during storage than did red-harvested fruit (Kalt et al., 1993). Different proportions of the two major strawberry anthocyanins were formed during storage, compared to when pigments were formed on the plant (Kalt et al., 1993). Since anthocyanins each have unique color characteristics, this may have contributed to apparent differences in anthocyanin content and to the darkening observed in the strawberry and raspberry fruit.

The fact that during storage both strawberries and raspberries accumulated substantial amounts of anthocyanins (Table 2; Figures 2 and 3), although only raspberries had a significant increase in phenolics (Table 2), may be interesting from the standpoint of postharvest phenolic metabolism of fruit. Raspberry titratable acidity decreases during storage (Mazza and Miniati, 1993), and organic acids, through interconversion with carbohydrates, may provide carbon skeletons

for the synthesis of phenolics, including both anthocyanin and non-anthocyanin phenolics (e.g., hydroxycinnamic acids, non-anthocyanin flavonoids). Although the increase in anthocyanins during storage was much less in raspberries (mean = 2.1-fold) compared to strawberries (mean = 4.3-fold), the increase in raspberry antioxidant capacity was slightly greater (Figures 2 and 3). The synthesis of both anthocyanin and non-anthocyanin antioxidants may have contributed to the increase in ORAC in raspberry fruit. In strawberries, where there was no significant change in phenolics, anthocyanins would have been formed from a pool of phenolic precursors with a smaller overall effect on antioxidant capacity.

There is a marked difference in the stability of ascorbate in green leafy products versus fruit. For example, in spinach, more than 90% of the ascorbate is lost within 3 days after harvest when stored at ambient temperature (Diplock et al., 1998). This is in contrast to the present study where losses in ascorbate during storage, when they occurred, were minimal. There were significant losses only in raspberries which dropped by 44% and lowbush blueberries which dropped by 27% after 8 days at 30 and 20 °C, respectively. The stability of ascorbate in fruits and fruit products is attributed to their high acid content (Mapson, 1970) and the protective effect of fruit phenolic antioxidants (Miller and Rice-Evans, 1997). The relative stability of ascorbate among the fruits in this study may also be related to intracellular compartmentation of ascorbate and phenolics. Phenolic flavonoids are localized in the cell vacuole, which is a very low pH environment and occupies the majority of the cellular volume. In fruits of high ascorbate content, most of the vitamin C will be localized in the vacuole. However, all plant cells have ascorbate in the cytosol and other cellular compartments (e.g., chloroplasts) which is not protected by phenolics and the low pH environment of the vacuole. In lowbush blueberries, which had a relatively low ascorbate content, the significant loss may be due to losses of extravacuolar vitamin C. In the case of raspberries, which had a high ascorbate content, its loss was probably due to cell disruption, since the fruits used for analysis were at the end of their storage life.

Prior et al. (1998) report the contribution by ascorbate to the antioxidant capacity of highbush blueberries as 2.3% and for lowbush blueberries as 1.5%; this is compared to values in the present study of 0.8% and 0.6% for these fruits, respectively. The difference is due to lower ORAC values determined by Prior et al. (1998). The fact that losses in ascorbate did not affect the antioxidant capacity of lowbush blueberries or raspberries is consistent with the observation that ascorbate does not contribute greatly to the antioxidant capacity of fruits.

In conclusion, this study compares and confirms the work of Wang et al. (1996) and Prior et al. (1998) in that blueberries have a high antioxidant capacity compared to strawberries and other fruit such as raspberries. The small contribution of ascorbate to the total antioxidant capacity of fruit is also confirmed in the present results. New information is presented on the effect of storage practices on fruit chemistry and antioxidant capacity which suggests that storage at ambient or above ambient temperatures will positively affect phenolic metabolism to enhance the antioxidant capacity, and therefore the health functionality of some

fruit crops. Although storage practices that were identified to be beneficial may not be commercially practical, other treatments which similarly stimulate phenolic production (e.g., UV irradiation, ozonation) may be explored for commercial storage with the aim of improving the health quality of fruit.

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