Anthocyanins, Phenolics, and Antioxidant Capacity of Processed Lowbush Blueberry Products

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ABSTRACT: Temperature, pH, and oxygenation of extracted blueberries were examined to determine how processing may affect the antioxidant capacity of blueberry food products. Extraction of fruit at 60 °C resulted in higher recovery of anthocyanins and antioxidant capacity, compared to extracts obtained at 25 °C. Subsequent room temperature storage resulted in losses in anthocyanins and antioxidant capacity only in those extracts obtained at 60 °C. Antioxidant capacity was greatest in pH 1 extracts, compared to extracts at pH 4 and 7. Oxygenation was detrimental to both anthocyanins and antioxidant capacity. Antioxidant capacity of processed products was positively correlated with anthocyanin (R = 0.92) and phenolic content (R = 0.95), and negatively correlated with % polymeric color (R = −0.64). In general, products that had experienced less processing had a higher antioxidant capacity. Simple colorimetric tests for anthocyanins and phenolics proved to be useful indicators of antioxidant capacity in processed products.

Key Words: antioxidant capacity, ORAC, pH, blueberry, *Vaccinium angustifolium*

Introduction

Cumulative results of epidemiological and in vitro research suggest that the consumption of fruit and vegetables antioxidants may decrease the risk of cardiovascular disease and various forms of cancer by providing enhanced antioxidant protection in the human body (Ames and others 1993). Fruit and vegetable antioxidants, which include vitamin C, carotenoids, tocopherols, and phenolics, vary greatly in their content and profile among various fruits and vegetables. Compared to other fruits and vegetables, a high antioxidant capacity has been reported for lowbush blueberries (*Vaccinium angustifolium* Ait.) (Prior and others 1998). The antioxidant capacity is most significantly correlated with the contents of total phenolics and anthocyanins, while vitamin C makes a small contribution to the total antioxidant capacity (Prior and others 1998).

The effects of food processing on anthocyanins has been studied from the standpoint of the color quality as affected by pigment stability under various conditions. Heat, pH, oxygen, and various storage conditions are known to have marked effects on anthocyanins (Francis 1989).

The reports of high antioxidant capacity of wild blueberries (Prior and others 1998) has sparked considerable popular interest in these fruits by consumers who want to know how blueberries can contribute to the nutritional quality of their diet. To date, no food supplement products containing North American wild lowbush blueberry are commercially available. This fruit continues to be consumed primarily as a ingredient in various beverage, dairy, pastry, and cereal products.

Since high antioxidant capacity is a desirable characteristic for foods, the aim of this study was to examine the effects of various processing factors on the antioxidant capacity of blueberry juice and purée. Also, commercially processed blueberry products were analyzed for their antioxidant capacity.

Results and Discussion

Blueberry purée extraction at 25 and 60 °C

The yield of puréed blueberry fruit anthocyanins, total phe- nolics, and antioxidant capacity was markedly affected by both the extraction time and temperature (Fig. 1). After 60 min, the content of monomeric anthocyanins in solution had increased 2.6-fold in the 25 °C extracts and almost 15-fold in the 60 °C extracts. After the same period, the total phenolic content was about 2 times greater after extraction at 60 °C compared to 25 °C. Since anthocyanin flavonoids and hydroxycinnamic acids (for example, chlorogenic acid) make the major contribution to the total phenolic content of blueberry fruit, the results suggest that the extraction of hydroxycinnamic acids from the fruit flesh was less affected by temperature than was the extraction of anthocyanins from the fruit peel. This may be due to the increased permeabili-
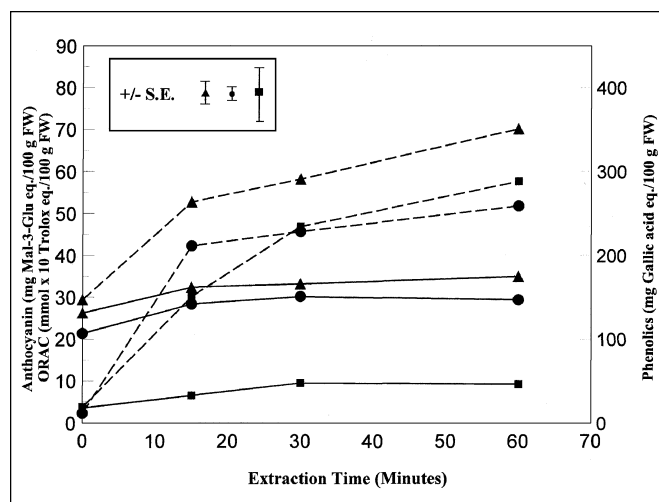

Fig. 1—Effect of extraction temperature on yield of monomeric anthocyanins (mg malvidin 3-glucoside 100 g FW) ■, total phenolics (mg gallic acid/ 100 g FW) ▲, and total antioxidant capacity (ORAC) (mmoles x 10 Trolox eq/ 100 g FW) ● of blueberry purée. Solid line = extraction at 25 °C; dashed line = extraction at 60 °C. Standard error: monomeric anthocyanins = 4.03; total phenolics = 9.11; total antioxidant capacity = 0.326. Time 0 indicates values immediately after puréeing of fruit.

ty of membranes in the macerated peel tissue at higher temperatures, which would facilitate anthocyanin release during extraction (Spanos and others 1990). Also the decreased solubility of oxygen at higher temperature may reduce oxidative degradation of anthocyanins during extraction. (Spanos and others 1990). The antioxidant capacity (for example, the oxygen radical absorbing capacity, ORAC) of the 60 °C extract was about 1.8 times greater than the 25 °C extract after 60 min of extraction.

Although extraction of blueberry fruit at 60 °C yielded solutions with a higher level of anthocyanins, total phenolics, and a higher antioxidant capacity, these extracts experienced losses in all of these components during 20 °C storage (Table 1). In the 60 °C extract, anthocyanin content declined by more than half during storage, and the total phenolic content decreased by 30%. Accompanying the losses in anthocyanins and total phenolics was a 30% loss in total antioxidant capacity. The detrimental effects of high temperature on anthocyanins have been recognized; a half life of 1 h for strawberry pigments at 100 °C is reported, and a logarithmic relationship between temperature and pigment destruction has been described (Francis 1989). In contrast to the 60 °C extracts, samples that were extracted at 25 °C were positively affected by storage. Extraction of components from the solid material of the purée continued to occur in the 25 °C extract during storage, so that anthocyanin content had increased 1.7-fold, and total phenolics 1.5-fold after 2 wk. As a result, the antioxidant capacity of the extract increased 1.5-fold. Clearly, the combination of time and temperature must be optimized in the development of blueberry food processes when the aim is to maximize the antioxidant capacity of the final product. High temperature/short time treatments have been studied and are recommended to protect anthocyanins from the action of glycosidases, which generate unstable anthocyanidins (aglycones) during pectolytic treatment (Wightman and Wrolstad 1996).

Blueberry juice at pH 1, 4, and 7

The adjusted pH of blueberry juice affected the content of monomeric anthocyanins, % polymeric color, total phenolic content and antioxidant capacity (Table 2). There were no significant effects of 1 and 2 wk storage on the pH-adjusted samples (data not shown), and therefore only data obtained at 0 wk is included.

Most striking was the effect of pH on the monomeric anthocyanin content which was about 10-fold higher at pH 1 than at pH 4 and 7. The high level of anthocyanin at pH 1 is consistent with the presence of the flavylium cation which is most intensely colored, compared to the quinonoidal pseudobase, and chalcone forms, which are pale or colorless. These pH-dependent ionic forms of anthocyanins have been well characterized, and it is known that some time may be required to achieve equilibrium among structural forms at a given pH (Mazza and Miniati 1993). It is possible that in the pH 4 and 7 samples, equilibrium had not been achieved during colorimetric analysis in the pH 1 and 4.5 buffers, which may have contributed to the apparent low level of anthocyanins at pH 4 and 7. For samples at pH 7, irreversible changes (for example, degradation following chalcone formation) may have also contributed to the apparent losses (Francis 1989). This possibility is substantiated by the lower antioxidant capacity of the pH 7.0 samples. Irreversible loss of anthocyanin is suggested by the lower total phenolic content in both the pH 4 and 7 samples. Since color intensity of anthocyanins is greatest at low pH (Francis 1989), and ORAC is also greatest at low pH (Table 2), processes which are designed to maximize color intensity, including acidification, may also ensure maximum antioxidant capacity in finished products.

The high % polymeric color in juice of pH > 1 indicated greater self-association of anthocyanin molecules at high pH (Table 2). In this experiment it is unclear whether anthocyanin self-association affects antioxidant capacity, since pH 4 and 7 had different ORAC levels but the same % polymeric color. However, results of the blueberry food product survey which are presented later, indicate that antioxidant capacity and % polymeric color were negatively correlated.

Blueberry juice storage in air

The introduction of oxygen when blueberry juice samples were shaken in air had a marked effect on monomeric anthocyanins, total phenolics, and antioxidant capacity (Fig. 2). In vials that were completely filled and had little oxygen-containing head space, there was no loss in anthocyanins after 6 h, while in the half-full vessels, 76% of the monomeric anthocyanins were lost during the same period. Similarly, total phenolic content and antioxidant capacity did not decrease in sealed vials but decreased 30% and 46%, respectively in those with air, over the same period. Oxygen has been reported to be an important factor in destabilizing anthocyanins in processed products (Francis 1989), and also clearly had a negative effect on the antioxidant capacity of blueberry juice (Fig. 2). In addition to direct oxidation, anthocyanins with an o-diphenolic B ring (for example, cyanidin and delphinidin) may be degraded by o-quinones formed by the oxidation of chlorogenic acid by polyphenol oxidase (Kader and others 1997). Anthocyanins without o-diphenolic B ring can form adducts with the o-quinones. Possible copigmentation effects of these adducts may explain the apparent rise in anthocyanin content after 2 and 4 h of exposure to oxygen (Fig. 2). The oxidation of anthocyanins may be most significant in fruit juices or beverages, because the aqueous matrix can dissolve large amounts of oxygen during processing, as compared to products with low water content (for example, dried fruit). Methods to eliminate dissolved oxygen such as nitrogen sparging or vacuum deaeration may be beneficial for retention of color and antioxidant capacity in processed blueberry products.

Survey of blueberry food products

Commercial lowbush blueberry food ingredients and finished products were surveyed for their anthocyanin and phenolic content and total antioxidant capacity. These foods have undergone a variety of processes and are available in many different forms. Since these products have widely varying moisture levels, differences in ORAC are discussed on the basis of product dry weight (DW) (Table 3). The highest antioxidant capacity was
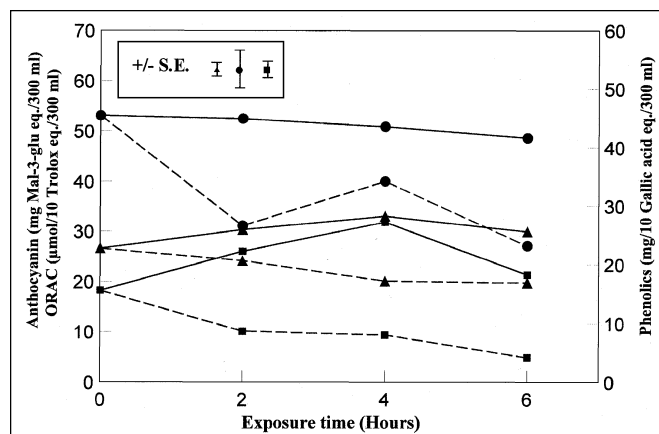
Fig. 2—Effect of oxygenation on content of monomeric anthocyanins (mg malvidin 3-glucoside/300 ml) ■, total phenolics (mg gallic acid/300 ml) ▲, and total antioxidant capacity (ORAC) (μmoles Trolox eq/300 ml) ● of blueberry juice during mixing. Solid line = 100% full; dashedline = 50% full. Standard Error: monomeric anthocyanins = 0.984; total phenolics = 6.58; ORAC = 2.69

Table 1—Effect of 60 min extraction at 25° or 60° C, followed by 2 wk storage at 20° C, on the content of monomeric anthocyanins, total phenolic content and antioxidant capacity (ORAC) of blueberry purée

| Component | Units | Extraction at 25 °C | | Extraction at 60 °C | | F prob | S.E. |
		60 min	+ 2 wk	60 min	+ 2 wk		
Anthocyanins	mg M 3-g*/100g FW	12.8	22.2	79.6	31.3	<.001	4.03
Phenolics	mg gallic acid eq/100g FW	175	258	351	213	<.001	9.11
ORAC	mmol Trolox eq/ 100 g FW	2.94	4.54	5.18	3.65	<.001	0.133

*malvidin 3-glucoside

Table 2—Effect of pH on the content of monomeric and polymeric anthocyanins, total phenolic content and antioxidant capacity of blueberry juice

pH	Anthocyanins (μg[a]/ml)	Polymeric Color (%)	Phenolics (μg GA[b]/ml)	ORAC (μmol Trolox eq/ml)
1	118	19.6	1064	20.4
4	15.7	71.2	756	20.4
7	8.50	70.7	816	14.9
S.E.	7.66	2.44	56.5	2.20
F prob	<.001	<.001	<.001	.004

[a] malvidin 3-glucoside
[b] gallic acid equivalents

recorded for fresh blueberry fruit. Commercial samples of individually quick-frozen fruit (IQF 1 & 2) were somewhat lower in their ORAC. Interestingly, frozen fruit which contained lower levels (60% to 80%) of blue, fully ripe fruit were comparable to fresh fruit in their ORAC. These results illustrate the potential range in ORAC values that may be present among similar fresh and frozen whole blueberry fruit samples and supports earlier results on variation in ORAC based on genotype and source (Prior and others 1998). The results also indicate that underripe blueberry fruit which have a relatively low anthocyanin content may still have a high level of ORAC, suggesting that other phenolic components present in underripe fruit contribute substantially to ORAC.

Fruit purée was relatively high in its antioxidant capacity, falling within the range of the whole fresh and frozen fruit samples. There was a difference between the ORAC of blueberry fruit in pies prepared from fresh fruit versus canned pie filling, with the ORAC of the pie filling fruit having only about one third of the ORAC of fresh baked fruit. Dried blueberry fruit varied in its ORAC level, and appeared to be lower in fruit that had been dried more thoroughly. The ORAC value of juice concentrate (± 65°Brix) was only about 65% that of the fresh and frozen fruit, which is ± 12 °Brix (Kalt and McDonald 1996) suggesting that substantial damage and/or losses of antioxidants occurred during processing. The pomace by-product from juice extraction had about 16% of the ORAC value of the fresh and frozen fruit.

Among the commercial blueberry products tested, anthocyanins and total phenolics were both strongly and positively correlated with ORAC (Table 4). The % polymeric color was negatively correlated (−0.64) with ORAC. Together, the results suggest that, in spite of processing losses, anthocyanins make a significant contribution to the antioxidant capacity of blueberry food products.

Table 3—Antioxidant capacity (ORAC) of commercial lowbush blueberry products

| Category | Product | Dry Weight % | ORAC | |
			mmol Trolox eq/100 g FW	mmol Trolox eq/100 g DW
Fresh	Fresh	17.7	6.34	52.9
Frozen	IQF 1	15.5	4.60	31.2
	IQF 2	12.2	4.80	39.3
	60% Blue, IQF	16.9	9.33	52.9
	80% Blue, IQF	12.6	8.53	50.7
	Flour coated fruit	26.7	5.99	22.3
Purée	Purée	12.8	5.37	42.0
Baked	Fresh Pie	28.5	5.38	19.1
	Pie Filling, rebaked	35.8	2.20	6.13
	Muffin	46.9	2.83	6.04
Canned	Pie Filling	36.6	2.37	6.49
	Fruit	25.4	4.76	18.7
	Light syrup	21.4	3.03	14.2
	Jam	27.3	2.88	10.6
Dried	Intermediate moisture	66.8	17.1	25.5
	Low moisture	83.2	12.6	15.1
	Cereal Fruit	92.2	2.74	2.97
	Sugar infused fruit	71.8	8.11	11.3
	Blended powder	90.0	6.40	7.44
Sorbet	Sorbet	23.7	2.24	9.54
Juice	Concentrate	49.5	5.54	29.4
	Juice pomace	46.2	3.40	7.41

Table 4—Spearman correlation of commercial blueberry food anthocyanins, phenolics, and % polymeric color and antioxidant capacity (ORAC) (dry weight basis)

Variate 1	Variate 2	R²
Monomeric Anthocyanin	ORAC	0.92**
Phenolics	ORAC	0.95**
% Polymeric Color	ORAC	-0.64**

** $P < 0.01$

In fruits such as blueberries, where phenolic components and, more specifically, anthocyanins, make a significant contribution to total antioxidant capacity, simple colorimetric tests such as the Folin-Ciocalteu test (Singleton and Rossi 1965) for phenolics or the Wrolstad anthocyanin test (Wrolstad 1976) can be useful in estimating losses in antioxidant capacity. These tests can be used in process development aimed at preserving antioxidant capacity.

Materials and Methods

Blueberry purée extraction at 25 ° and 60 °C

Fresh lowbush blueberry fruit (from at least 20 clones) was extracted in (1:1, w/v) water. Glass vials containing aliquots of purée were held in a water bath at either 25 or 60 °C. One vial was removed after 0, 15, 30, and 60 min. A second vial was removed after 60 min and was stored at 20 °C for 2 wk in the dark. All vials were frozen immediately after treatment and stored at −70 °C until the aqueous fractions were analyzed as described below. This experiment was repeated 3 times.

Blueberry Juice at pH 1, 4 ,and 7

Juice was obtained from fresh blueberries using a table top juicer. Aliquots (25 ml) of squeezed blueberry juice were adjusted to either pH 1, 4 or 7 using HCl or NaOH, and then brought to 50 ml with water. Triplicate samples at each pH were stored in darkness at 20 °C for 0, 1, and 2 wk, in individu-

al glass vials with no headspace. After removal samples were stored at −70 °C.

Blueberry juice storage in air

Glass vials (40 ml) were filled either half or completely full with fresh blueberry juice, so that juice was exposed to different amounts of air. Vials were sealed and shaken on a tilt shaker for 0, 2, 4, 6, and 8 h at room temperature in darkness After removal of triplicate samples from the shaker at the prescribed times, samples were stored at −70 °C until analysis.

Blueberry foods survey

A variety of processed blueberry food products were obtained from various commercial sources. Samples included fresh, frozen, pureed, baked, canned, dried, juiced products and blueberry sorbet. Blueberries were removed before extraction from the products where they were used as an ingredient (for example, baked products). Since product formulations were not available, no correction could be made for ingredients (for example, sugar or starch) associated with the blueberry fruit used for extraction. For analysis of phenolic, anthocyanin, and total antioxidant capacity, fruit products were homogenized in three volumes (dry weight basis) of solvent (40:40:20:0.1, acetone, methanol, water, formic acid) for 2 min using a Vitis Shear Homogenizer (The Virtis Co., Gardiner, N.Y., U.S.A.) Extracts were vacuum filtered, then brought to 50 ml with extraction solvent and stored at −70 °C until further analysis.

Chemicals

R-phycoerythrin and gallic acid were purchased from Sigma Chemical Company (St. Louis, Mo., U.S.A.). 6-Hydroxy-2,5,7,8-tetramethyl-2-carboxylic acid (Trolox) was purchased from Aldrich Chemicals (Milwaukee, Wis., U.S.A.). 2,2'-Azobis(2-amidinopropane) dihydrochloride (AAPH) was purchased from Wako Chemicals USA, Inc. (Richmond, Va., U.S.A.). Folin-Ciocalteu reagent was obtained from BDH Scientific (Toronto, Ontario, Can.) and all other chemicals were obtained from Fisher Scientific (Toronto, Ontario, Can.).

Measurement of anthocyanins, phenolics, and antioxidant capacity assays

Total monomeric anthocyanin content was determined on appropriately diluted extracts by the pH differential spectrophotometric method of Wrolstad (1976). Diluted pigment samples were further diluted 10-fold with either a 3:1 mixture of 0.2M HCl and 0.2M KCl (pH 1) or 0.4M Na-acetate buffer (pH 4.5). Measurements were carried out on a microplate reader (Molecular Devices, Menlo Park Calif., U.S.A,). Anthocyanin content was quantified using the extinction coefficient for malvidin 3-glucoside (28,000). The percent contribution by polymerized anthocyanins was determined according to Wrolstad (1976) using absorbance values at the specified wavelengths, determined with and without added potassium metabisulfite.

Total phenolic content was measured on a microplate reader equipped with a 700 nm filter using the Folin-Ciocalteu method, and gallic acid as a standard (Singleton and Rossi 1965). At 700 nm, the absorption for the phenolic-based chromagen is > 90% of its absorption at 760 nm (Singleton and Rossi 1965).

To measure antioxidant capacity, an aliquot of the acetone/MeOH/water/formic acid (40:40:20:0.1) extracts of blueberry food samples were dried at 30 °C under vacuum using a centrifugal evaporator (Savant Instruments, Hicksville, N.Y., U.S.A.), and redissolved in same volume of water. Aqueous samples (for example, juice) were used directly, without solvent removal. Total antioxidant capacity was measured as oxygen radical absorbing capacity (ORAC) according to the method of Cao and others (1995) using a COBAS-FARA II centrifugal analyzer (Roche Diagnostic, Nutley, N.J., U.S.A.). The ORAC assay (400 μL) contained 16.7 nM R-phycoerythrin, 4 mM AAPH, and 20 μL of diluted sample. Samples were diluted between 200 and 2000-fold for the ORAC assay. After initiation of the assay with AAPH, a peroxyl radical generator, fluorescence of R-phycoerythrin was read on appropriately diluted duplicate samples, blanks and Trolox standards. Trolox is a water-soluble vitamin E analog. Readings were taken at 2 min intervals over a 70 min period. The area under the curve was calculated and expressed as equivalents of Trolox/ unit of sample tested as described by Cao and others. (1995).

Statistical analysis

The analysis of variance procedure of Genstat 5 (Genstat Committee 1993) was used to analyse results. The Spearman Rank Correlation Coefficients of Genstat 5 was used to determine the relationships among variates. The Spearman procedure measures the association between the ranking of two variables. Unless otherwise indicated only differences of $P < 0.05$ are discussed.

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