Identification of Procyanidins and Anthocyanins in Blueberries and Cranberries (*Vaccinium Spp.*) Using High-Performance Liquid Chromatography/Mass Spectrometry

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Blueberries and cranberries were analyzed for procyanidins using normal-phase HPLC/MS. Monomers, identified as (+)-catechin and (−)-epicatechin, and a series of oligomers were detected in blueberries, and MS data confirmed that the oligomers consisted of (epi)catechin units that were exclusively singly linked (B-type). The procyanidin "fingerprints" were similar for Tifblue and Rubel but higher than that for lowbush blueberries. In whole cranberries, (−)-epicatechin was present, along with a complex series of oligomers. Both A-type (contained only one double linkage per oligomer) and B-type oligomers were present. Two commercial cranberry juices exhibited similar procyanidin profiles, except that one contained increased quantitites. There were processing effects on the procyanidin content of cranberry extract and juices when compared to those of the unprocessed fruits. Monomer, dimers, and A-type trimers were the primary procyanidins, with only trace levels of the B-type trimers and A-type tetramers and with an absence of the higher oligomers in cranberry extract and juices.

Keywords: *ORAC; phenolics; anthocyanins; flavonoids; catechins*

INTRODUCTION

Procyanidins are oligomers or polymers of polyhydroxy flavan-3-ol units that can be either singly linked at the 4 → 6 or 4 → 8 positions or doubly linked with a second interflavonoid bond formed by C−O oxidative coupling at the 2 → O^7 positions (Figures 1 and 2). Singly linked procyanidins are more common in food products, comprising the major polyphenols in chocolate, cocoa, grapes, and apples (*1*). Although found less frequently, the doubly linked procyanidins have been identified in peanuts and cinnamon (*2*−*4*).

The procyanidins are of particular interest because they are partially responsible for the organoleptic characteristics of grapes, wines, and cocoa, presumably because of interactions with salivary proteins, which might contribute to astringency (*5*). Recent reports have indicated possible health benefits, particularly to cardiovascular health, from the consumption of the procyanidins. For example, it was found in vitro that the monomeric flavan-3-ols in green tea have a regenerative and/or conservation effect on vitamin E in low-density lipoproteins, which is important in protecting against oxidation (*6*). Furthermore, procyanidins have been shown to have protective cardiovascular effects in vivo. Facino et al. (*7*) observed that supplementation of a rat diet with procyanidins made the heart less susceptible to ischemia/reperfusion damage; supplementation with procyanidins was also associated with an increase in plasma antioxidant capacity. Moreover, Yamakoshi et al. (*8*) demonstrated that a procyanidin-rich extract (fed at 0.1 or 1% of the diet) significantly reduced severe atherosclerosis in the aorta of rabbits. A polyphenolic-rich cocoa beverage suppressed ADP- or epinephrine-stimulated platelet activation and platelet microparticle formation (*9*). Wang et al. (*10*) observed trends for a dose−response increase in plasma antioxidant capacity and decreases in plasma lipid oxidation products following chocolate consumption. Recently, the oxygen radical absorbance capacity (ORAC) was found to correlate with the procyanidin content in cocoa and chocolate samples (*11*).

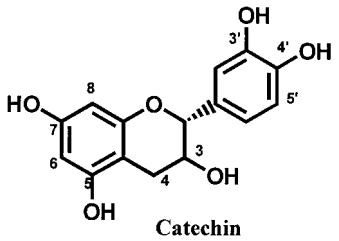

Figure 1. Structure of flavan-3-ol (catechin).

In contrast to the procyanidins, considerable work has been published on the anthocyanins in fruits and vegetables, and much of this work has been summarized by Mazza and Miniati (*12*). Quantitation of individual anthocyanins has been and continues to be limited because of the lack of availability of appropriate standards. Anthocyanins in blueberries have been characterized by Gao and Mazza (*13*, *14*). Acetylated antho-

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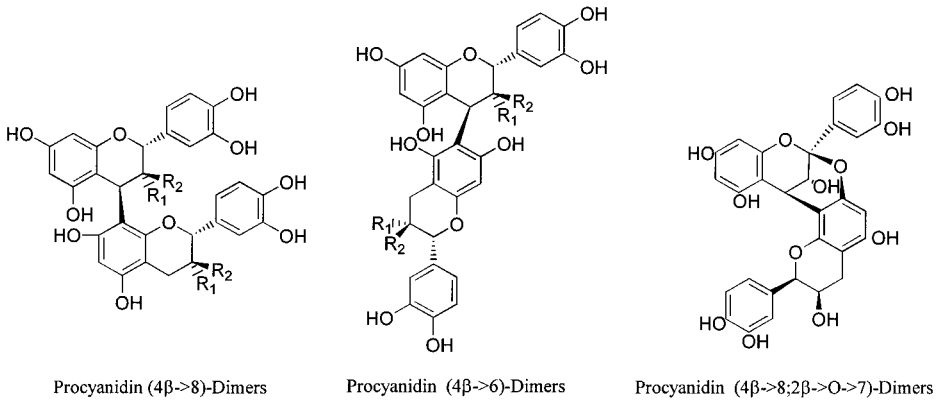

Figure 2. Representative structures of the singly linked (B-type) and doubly linked (A-type) procyanidin dimers.

cyanins are prevalent in lowbush, but generally not in cultivated, blueberries.

Fruits and vegetables have been identified as a primary source of dietary flavonoid intake; therefore, identification of anthocyanins and procyanidins in these foods could help partially explain the correlations observed between fruit and vegetable consumption and the decreased risk of cardiovascular disease. In the present study, the procyanidin content in blueberries, cranberries, and products derived from these berries were analyzed using the normal-phase high performance liquid chromatography/mass spectrometry (HPLC/MS) method reported by Hammerstone et al. (*15*). Cranberries were chosen because the observed protective effects against urinary tract infections have recently been attributed to the procyanidin content (*16*). Blueberries were selected because of their high antioxidant capacity compared to those of other fresh fruits and vegetables (*17*−*19*).

MATERIALS AND METHODS

Chemicals. *R*-Phycoerythrin (*R*-PE), ascorbic acid, gallic acid, chlorogenic acid, epicatechin, rutin hydrate, and acetonitrile (HPLC grade) were purchased from Sigma (St. Louis, MO). Kuromanin chloride was obtained from Indofine Chemical Co., Inc. (Somerville, NJ). 6-Hydroxy-2,5,7,8-tetramethyl-2-carboxylic acid (Trolox) was obtained from Aldrich (Milwaukee, WI). 2,2′-Azobis (2-amidinopropane) dihydrochloride (AAPH) was obtained from Wako Chemicals USA, Inc. (Richmond, VA). Methanol and methylene chloride (HPLC grade) were from Fisher Scientific (Boston, MA). HPLC-grade water and acetic acid were obtained from J.T. Baker Inc. (Phillipsburg, NJ).

Samples. Fresh, ripe **s**amples of lowbush (wild) blueberries (*Vaccinium angustifolium*) were obtained from regions and clones in Maine and Canada and frozen immediately after harvest. A composite sample was made from the samples from the different regions by pooling together quantities proportional to those normally produced in the different regions. The mixed composite sample represented more than 500 kg of blueberries. From this composite, approximately 2 kg was freeze-dried and ground for analysis. Highbush blueberries (Rubel variety) (*Vaccinium corymbosum*) were obtained from MBG Marketing (Grand Junction, MI). The Tifblue variety of blueberries (*Vaccinium ashei* Reade, Rabbiteye) was obtained from Dr. G. Krewer (Tifton, GA). Rubel and Tifblue blueberries were harvested at the usual state of maturity for machine harvest for the area of the country. Approximately 1 kg was shipped overnight without freezing in an insulated container to the USDA-HNRCA laboratory where they were frozen, freeze-dried, and stored at −70 °C until being analyzed. Cranberries (*Vaccinium macrocarpon* Ait.) and cranberry juice cocktail were purchased from a local supermarket. The cranberries represented berries grown in Massachusetts, but the variety was not determined. Spray-dried cranberry extract was obtained from Triarco Industries (Wayne, NY). Freeze-dried blueberries and cranberries were pulverized in a blender.

Extraction of Polyphenols from Blueberries and Cranberries. For extraction, 15 g of the freeze-dried material was added to 135 mL of an extraction mixture containing acetone, water, and acetic acid (70:29.5:0.5 v/v). The mixture of solvent and freeze-dried material was vortexed, sonicated for 5 min in a water bath at 50 °C, and kept at 50 °C for 30 min, after which the mixture was centrifuged at 3300 rpm for 15 min. The resulting supernatant was concentrated using a rotary evaporator at 40 °C under partial vacuum; the concentrated material (approximately 12 mL) was diluted to 25 mL with 20% methanol/water (v/v). Twenty-milliliter columns (Supelco, Bellefonte, PA) containing 5 g of Sephadex LH-20 (Sigma Chemical Co., St. Louis, MO) were hydrated for more than 2 h in 25 mL of water and then packed by elution with water. Five milliliters of the extracted material was loaded onto the column. The columns were then eluted with 25 mL of 20% methanol/water (v/v) to remove sugars and phenolic acids, followed by 40 mL of 60% methanol/water (v/v) to elute the flavonols and anthocyanins and finally by 90 mL of 100% methanol for elution of the procyanidins. The three methanol fractions were collected separately and sampled (1 mL) for determination of total phenolics, anthocyanins, and ORAC (*17*). Total recoveries were calculated based on the volume of extract applied to the column and the total volume in each of the fractions eluted from the Sephadex LH-20 column. Separate fractions from three Sephadex columns were pooled and concentrated by rotary evaporation, and the concentrated material from the 100% methanol fraction was diluted to a final volume of 15 mL with 100% methanol. The other fractions were freeze-dried. For the cranberry juice cocktails, 80 mL was loaded directly onto the hydrated Sephadex LH-20 column and eluted using the series of methanol/water washes described above.

Recovery Studies of Phenolic Standards. A set of known standards was prepared by dissolving chlorogenic acid, 700 μg/mL, epicatechin, 940 μg/mL, rutin, 700 μg/mL, and kuromanin chloride, 360 μg/mL, in 10 mL of 25% methanol/water. Five milliliters of this mixture was loaded onto a LH-20 Sephadex column and eluted as described earlier. The three fractions collected were concentrated by rotary evaporation and reconstituted to a total volume of 5 mL with 20% methanol. Samples were analyzed on an HP1100 series HPLC instrument (Hewlett-Packard, Palo Alto, CA) by injecting 2 μL onto a 5-μm Hypersil ODS column (200 × 2.1 mm) and eluting at 26 °C with a linear gradient from 92% solvent A (3% v/v formic acid in water) and 8% solvent B (100% acetonitrile) to 30% solvent B in 20 min at a flow rate of 0.4 mL/min. UV data were collected by using a diode array detector set at 280, 320, 360, and 520 nm for detection and quantification of epicatechin, chlorogenic acid, rutin, and kuromanin chloride, respectively. Recoveries of each of the standard components were calculated by the areas in each of the three fractions collected from the Sephadex column.

HPLC/MS Analyses of Polyphenols. Chromatographic analyses were performed on an HP1100 series HPLC instrument equipped with an autosampler/injector, quaternary HPLC pump, column heater, diode array detector, fluorescence detector, and HP ChemStation for data collection and manipulation. Normal phase separations of the procyanidin oligomers were performed on a 5 μ Luna silica column (250 × 4.6 mm) (Phenomenex, Torrance, CA). UV detection was recorded at 280 nm. For fluorescence detection, the excitation and emission wavelengths were 276 and 316 nm, respectively. The ternary mobile phase was as described by Hammerstone et al.,[15] consisting of (A) dichloromethane, (B) methanol, and (C) acetic acid and water (1:1 v/v). For HPLC/MS analyses, the HPLC apparatus was interfaced to an HP series 1100 mass-selective detector (model G1946A) equipped with an atmospheric pressure ionization electrospray chamber. Ten millimolar ammonium acetate in methanol was used as an ionization reagent at a flow rate of 0.1 mL/min and added via a tee in the eluant stream of the HPLC apparatus just prior to the mass spectrometer by an auxiliary HP 1100 series HPLC pump. Conditions for analysis in the negative ion mode included a capillary voltage of 3.5 kV, a fragmentor voltage of 85 V, a nebulizing pressure of 25 psig, and a drying gas temperature of 350 °C. Data were collected on an HP ChemStation using scan mode over a mass range of m/z 220−2200 at 2.12 s per cycle.

HPLC/MS/MS of Anthocyanins. Anthocyanins were separated on a Zorbax C18 column (150 × 4.6 mm) (Agilent Technologies, Wilmington, DE). Anthocyanins (20 μL injected) were eluted with a gradient of 5% formate (mobile phase A) and 100% methanol (mobile phase B). The gradient was from 5 to 35% mobile phase B at a flow rate of 1.0 mL/min over a 40 min period. The mass spectrometer was a Bruker model Esquire-LC multiple ion trap mass spectrometer. Mass spectral data were collected with the Topaz software, which also controlled the instrument and was integrated with the HP ChemStation software, which collected the diode array signal. Conditions for mass spectral analysis in the positive ion mode included a capillary voltage of 4000 V, a nebulizing pressure of 30.0 psi, a drying gas flow of 9.0 mL/min, and a temperature of 300 °C.

Automated ORAC Assay. The automated ORAC assay was carried out on a COBAS FARA II spectrofluorometric centrifugal analyzer (Roche Diagnostic System Inc., Branchburg, NJ) with a 565-nm emission filter. The procedure was based on a previous report of Cao and co-workers (*20*), as modified for the COBAS FARA II instrument (*21*). Briefly, in the final assay mixture (0.4 mL total volume), *R*-phycoerythrin (*R*-PE) (16.7 nM) was used as a target of free radical (or oxidant) attack, with 2,2′-azobis (2-amidinopropane) dihydrochloride (AAPH) (4 mM) as a peroxyl radical generator. Trolox (1.0 μmol/L final concentration), a water-soluble analogue of vitamin E, was used as a control standard. The analyzer was programmed to record the fluorescence of *R*-PE every 2 min after addition of AAPH. All fluorescence measurements are expressed relative to the initial reading. Final results were calculated using the differences of the areas under the *R*-PE decay curves between the blank and a sample, and expressed as micromoles of Trolox equivalents (TE) per gram of fresh weight.

Total Anthocyanins and Phenolics. Total anthocyanins were estimated by a pH differential method (*22*) that was adapted for measurement on the COBAS FARA II instrument. Absorbance was measured at 510 and 700 nm in buffers at pH 1.0 and 4.5, using $A = (A_{510} - A_{700})_{pH1.0} - (A_{510} - A_{700})_{pH4.5}$ with a molar extinction coefficient of 29 600. Results were expressed as milligrams of cyanidin-3-glucoside equivalent per gram of fresh or dry weight. Total soluble phenols in the fractions were determined with Folin-Ciocalteu reagent by the method of Slinkard and Singleton (*23*), which was adapted to run on the COBAS FARA II analyzer.

RESULTS AND DISCUSSION

Because cranberries and blueberries contain large amounts of sugars, phenolic acids, flavonols, and anthocyanins, a solid-phase extraction procedure using Sephadex LH-20 was developed to crudely remove these compounds and obtain a procyanidin-enriched extract. Method development was done using a representative compound from each phenolic group, and recovery experiments revealed high yields for each class of compounds (Table 1). Although it was originally predicted that many of the phenolic acids would elute in the 20% MeOH fraction, chlorogenic acid actually eluted primarily in the 60% MeOH fraction. Chlorogenic acid was chosen as a representative phenolic acid because it is found in large amounts in blueberries; however, it is not a typical phenolic acid because it contains an ester bond, and this unusual chemical structure might account for the difference in elution properties. Nonetheless, the solid-phase extraction procedure was successful in providing a procyanidin-enriched fraction and in removing the majority of the other phenolic components.

Table 1. Recovery of Standards [(−)-Epicatechin, Chlorogenic Acid, Rutin, and Kuromanin Chloride], Total Phenolics, Total Anthocyanins, and Total ORAC in Blueberry and Cranberry Samples from Sephadex LH-20 Column

compound	recovery (%)			
	20% MeOH fraction	60% MeOH fraction	100% MeOH fraction	total recovery
standards				
(−)-epicatechin	0.0	4.0	98.4	102.4
chlorogenic acid	6.4	92.8	0.8	100.0
rutin	0.0	72.0	29.0	101.0
kuromanin chloride	0.0	84.0	8.2	92.2
total phenolics				
lowbush extract	11.4	54.5	36.7	103
lowbush blueberry[a]	7.6 ± 3.0	47.7 ± 5.9	40.4 ± 9.2	96 ± 10
highbush (rubel)[a]	4.5 ± 0.9	49.0 ± 4.5	31.9 ± 7.7	85 ± 8
mean ± SEM	7.8 ± 2.0	50.4 ± 2.1	36.3 ± 2.5	95 ± 5
cranberry extract	3.0	17.2	69.5	90
cranberry[b]	25.3 ± 0.1	26.4 ± 0.1	48.4 ± 0.1	113 ± 0
mean	14.2	21.8	59.0	102
anthocyanins				
lowbush extract	5.1	114.0	18.0	137
lowbush blueberry[a]	1.5 ± 1.5	94.0 ± 1.9	17.9 ± 4.7	113 ± 7
highbush (rubel)[a]	2.2 ± 0.5	92.1 ± 6.5	7.8 ± 2.8	101 ± 15
mean ± SEM	2.9 ± 1.1	100.0 ± 7.1	14.6 ± 3.4	117 ± 11
cranberry extract	2.6	82.5	35.4	121
cranberry[b]	24.5 ± 0.1	67.6 ± 0.0	7.9 ± 0.0	116 ± 0
mean	13.6	75.1	21.7	118.5
total ORAC				
lowbush extract	7.7	57.8	25.0	91
lowbush blueberry[a]	4.8 ± 0.9	41.5 ± 7.4	33.3 ± 4.8	80 ± 3
highbush (rubel)[a]	3.9 ± 0.7	36.6 ± 13.3	36.9 ± 9.5	77 ± 15
mean ± SEM	5.5 ± 0.8	45.3 ± 1.9	31.7 ± 1.4	83 ± 3
cranberry extract	5.0	30.1	72.3	107
cranberry[b]	13.4 ± 0.0	32.6 ± 0.0	54.0 ± 0.0	98 ± 0
mean	9.2	31.4	63.2	102.5

[a] Mean ± SEM of two separate fractionations. [b] Mean ± SEM of four separate fractionations.

Data on the recovery of total phenolics are presented in Table 1. Major differences existed between cranberry and blueberry samples in the proportion of the total phenolics isolated in the 100% methanol fraction; cranberries had a higher percentage compared to blueberries (48 vs 36%). Conversely, in the 60% methanol fraction, the proportion of total phenolics in the blueberry was increased relative to that in the cranberry (50 vs 26%). Chlorogenic acid was a major contributor to the total phenolics in this fraction in blueberries. Total antioxidant capacity (ORAC) parallels the total phenolic measurement. Flavonoids, including anthocyanins, have been shown to have a relatively high antioxidant

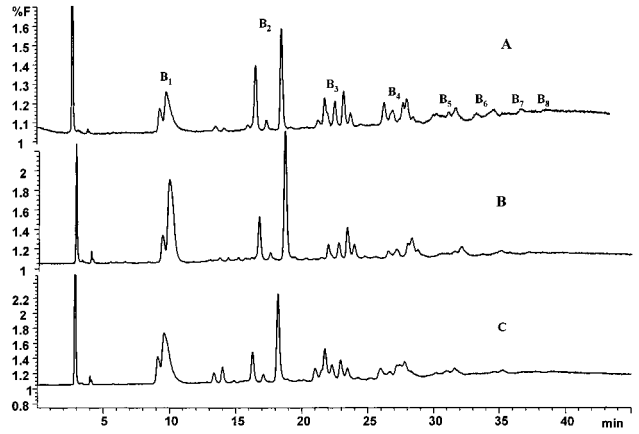

Figure 3. Normal-phase HPLC fluorescence traces of the procyanidin content in the 100% MeOH wash of (A) lowbush, (B) Rubel, and (C) Tifblue blueberries. B_1-B_8 correspond to the procyanidin monomers through octamers, respectively. The letter B denotes that the oligomers were formed by single linkages (B-type).

capacity. (*24*, *25*) The HPLC data confirm other data indicating that there are more total procyanidins and less total anthocyanins in cranberry than in blueberry.

Total recoveries of phenolics, anthocyanins, and ORAC were more variable than the standards, as might be expected, and it appears that some ORAC activity is lost during the process of fractionation, particularly in blueberries, as recovery was only 83%. More than 100% of the anthocyanins appeared to be recovered, but this is probably due to the low sensitivity of the spectrophotometric anthocyanin assay and errors associated with measuring the low concentrations in the 20 and 100% methanol fractions. On the basis of the ORAC activity in the 100% methanol fraction, it appears that up to 32 and 54% of the antioxidant capacity in blueberries and cranberries, respectively, can be accounted for by procyanidins. This represents a very significant portion of the total antioxidant capacity. What is not know is whether these relatively large procyanidin molecules are absorbed and can contribute to in vivo antioxidant status. Available data indicate that some components in procyanidin preparations and/or metabolites are absorbed, which can increase in vivo antioxidant capacity (*7*, *10*).

Whole lowbush blueberries were analyzed for their procyanidin content and compared to two cultivated varieties, Rubel and Tifblue. These two varieties were chosen because previous studies revealed that the Rubel variety had a high ORAC value in contrast to the Tifblue variety, which generally exhibited lower ORAC (*17*). Using the normal-phase HPLC/MS method reported by Hammerstone et al. (*15*) with fluorescence detection as modified by Lazarus et al. (*1*), the procyanidin-enriched fractions were analyzed. The fluorescence response curves are shown in Figure 3. As can be seen, a series of oligomers was detected, and the mass spectral data confirmed that the oligomers consisted of (epi)catechin units that were exclusively singly linked [B_1 (monomer) through B_8 (octamer)]. The fluorescence trace shows two peaks corresponding to monomers that were identified as (+)-catechin and (−)-epicatechin (B_1) by comparison of retention times with those of authentic standards. Interestingly, the peak areas using the fluorescence response were similar for the Tifblue and Rubel varieties, suggesting that they contain similar procyanidin

Table 2. Total Procyanidin Content of Blueberries, Cranberries, and Cranberry Juice

source	concentration[a,b]
blueberries	
lowbush (wild)[a]	3
Rubel[a]	6
Tifblue[a]	8
cranberries	
whole cranberries[a]	17
cranberry extract[a]	5
cranberry juice 1[b]	223
cranberry juice 2[b]	216

[a] In units of μg/g of dry weight. [b] In units of μg/100 mL.

compositions. Although at this point our primary objective was not in the quantitation of the procyanidins, we did use a cocoa standard to estimate quantities of procyanidins present (*11*). On a dry matter basis, the total procyanidin concentration was higher in cranberries than in blueberries (Table 2). On a dry matter basis, ORAC was highest in the lowbush composite sample and lowest in the Rabbiteye blueberry samples. Thus, there appeared to be an inverse relationship between antioxidant capacity and procyanidin content within the three blueberry varieties. Additional work is needed to explain this apparent inverse relationship. It might represent a divergence in the relative activities of the synthetic pathways for procyanidins relative to other polyphenolics.

Previously, Cao et al. fed a water extract of blueberries to rats to study the effects on oxygen toxicity (*26*). This extract was made by homogenizing in a blender 20 g of frozen blueberries with 200 mL of water. The resulting homogenate was centrifuged at 3000 rpm for 20 min. The supernatant was frozen and then crushed and freeze-dried. This extract had levels of procyanidins equivalent to those in the other blueberry samples, although total phenolics and anthocyanins were less on a dry matter basis than those in the other blueberry samples. Although preliminary, data suggest that it is possible that both anthocyanins and procyanidins present in the extract might have been active in protecting the integrity of the capillaries in rats exposed to 100% oxygen for 48 h. In other studies, the procyanidin fraction of lowbush blueberry has been shown to exhibit anticarcinogenic activity as evaluated by in vitro screening tests (*27*).

Whole cranberries were also analyzed to confirm the presence, type, and size of procyanidins in cranberries as reported by Howell et al. (*16*). The fluorescence response curve of the HPLC chromatogram of whole cranberries is shown in Figure 3A and reveals a complex series of oligomers. The pattern indicates the presence of predominantly one monomer, which was assigned as (−)-epicatechin (B_1) by confirmation with an authentic standard. Mass spectral data indicated that both A-type (containing at least one double linkage) and B-type (exclusively singly linked) oligomers were present. In the cases where A-type oligomers were identified, only one double linkage per oligomer was observed, which is in contrast to other species, which have been reported to contain oligomers with more than one double linkage (*1*, *28*). For comparison, a commercial cranberry extract and two brands of cranberry juice were analyzed to determine their procyanidin contents, and the results are shown in Figure 4B and C and Table 2. Only one cranberry juice chromatogram is shown because the two juices analyzed contained an identical composition

Table 3. Antioxidant Capacity (ORAC), Total Phenolics, and Anthocyanins in Samples of Blueberries, Cranberries, and Cranberry Juice

item	ORAC (μmol TE/g (mL)) wet wt.[a]	ORAC (μmol TE/g (mL)) DM	total phenolics (mg/g (mL)) wet wt.[a]	total phenolics (mg/g (mL)) DM	total anthocyanins (mg/g (mL)) wet wt.[a]	total anthocyanins (mg/g (mL)) DM	dry matter (%)
			blueberry				
lowbush composite	44.5	264	6.3	37.6	1.7	10.0	16.9
lowbush extract	–	189	–	19.0	–	2.6	–
highbush-Rubel	31.1	220	4.5	32.0	2.2	15.5	14.2
rabbiteye-Tifblue	35.8	175	8.0	39.1	2.8	13.7	20.4
			cranberry				
whole	37.4	275	17.2	104.5	3.6	26.2	13.6
extract	–	106	–	17.4	–	1.6	–
cranberry juice 1	5.0	–	0.61	–	0.012	–	–
cranberry juice 2	6.7	–	0.36	–	0.015	–	–

[a] Data from freeze-dried samples was converted to a fresh weight basis based upon dry matter content of samples.

Table 4. Percent Contribution of Individual Anthocyanins to Total Anthocyanins and Mass Spectral Data in Rubel and Lowbush Blueberries and Cranberries

anthocyanin	RT	blueberry lowbush %	blueberry Tifblue %	cranberry %	(m/z) total/aglycone
delphinidin-3-galactoside	14.1	7.7	12.1	–	465/303
delphinidin-3-glucoside	15.1	7.8	4.9	–	465/303
cyanidin-3-galactoside	16.5	5.0	9.9	25.2	449/287
delphinidin-3-arabinoside	17.0	5.2	6.1	–	435/303
cyanidin-3-glucoside	17.7	5.6	4.7	1.1	449/287
petunidin-3-galactoside	18.4	5.1	8.0	0.9	479/317
petunidin-3-glucoside[a]	19.5	10.7	9.2	–	479/317
cyanidin-3-arabinoside[a]	19.5	–	–	23.7	419/287
peonidin-3-galactoside	20.7	1.9	4.0	332	463/301
petunidin-3-arabinoside	21.3	3.2	3.6	–	449/317
malvidin-3-galactoside[b]	22.0	14.4	20.3	–	493/331
peonidin-3-glucoside[b]	22.0	++	++	–	463/301
malvidin-3-glucoside	23.0	14.1	9.3	–	493/331
peonidin-3-arabinoside	23.5	1.1	1.4	15.8	433/301
malvidin-3-arabinoside	24.7	5.8	6.4	–	463/331
delphinidin-6-acetyl-3-glucoside	26.4	1.4	–	–	507/303
cyanidin-6-acetyl-3-glucoside	28.9	1.6	–	–	491/287
malvidin-6-acetyl-3-galactoside	29.7	1.6	–	–	535/331
petunidin-6-acetyl-3-glucoside	30.0	1.5	–	–	521/317
peonidin-6-acetyl-3-glucoside	32.4	1.1	–	–	505/301
malvidin-6-acetyl-3-glucoside	32.9	4.6	–	–	535/331

[a] Petunidin-3-glucoside (479/317) coelutes with cyanidin-3-arabinoside (419/287) (19.5 min). Petunidin-3-glucoside predominates in lowbush and Tifblue blueberry based on the mass spectral response. In cranberry, a small amount of a compound with a mass of 577 that forms fragments of 425 and 287 colelutes with cyanidin-3-arabinoside. [b] Malvidin-3-galactoside (493/331) coelutes with peonidin-3-glucoside (463/301) (22 min). Malvidin-3-galactoside predominates in lowbush and Tifblue blueberries.

except that juice 2 had a slightly higher procyanidin concentration per 100 mL as determined by fluorescence signal. Importantly, profound processing effects on the procyanidin content of the extract and juices were observed. However, because we were not able to follow changes during the juice-making process, we cannot determine whether enzymatic or nonenzymatic oxidation or perhaps thermal degradation during the pasteurization process might have been responsible for the changes. Mass spectral data showed that the monomer, dimers, and A-type trimers were the primary procyanidins, with only trace levels of the B-type trimers and A-type tetramers and a notable absence of the higher oligomers. Furthermore, the procyanidins present in the cranberry extract were at a lower level on a dry weight basis than in the whole cranberries, as can be seen by the fluorescence response shown in Figure 4A and B and Table 2. The cranberry sample in this study had higher total phenolics and total anthocyanins than blueberries (Table 3).

The profile of anthocyanins in blueberries and cranberries is presented in Figure 5, and the percentage contribution to the total anthocyanins is presented in Table 4. Identification of individual anthocyanins becomes quite easy with retention times and UV/vis and mass spectral data. Masses for the aglycones are: delphinidin, 303; cyanidin, 287; petunidin, 317; peonidin, 301; and malvidin, 331. The order of elution of the glycosides on the C_{18} column is galactoside before glucoside, which is before the arabinoside. The red color of cranberry fruit is due to the presence of the four major anthocyanin pigments cyanidin-3-galactoside, peonidin-3-galactoside, cyanidin-3-arabinoside, and peonidin-3-arabinoside and the two minor anthocyanins cyanidin-3-glucoside and peonidin-3-glucoside (*29*) (Table 4). However, in blueberries, the total anthocyanin fraction is composed of more than 16 individual components (Table 4, Figure 5). The number of different anthocyanins might vary, with some acylated anthocyanins being reported in lowbush blueberry. Whether there is a relationship between the amount of cyanidins and procyanidin is not clear, but in cranberries, about 55% of the anthocyanins are cyanidins, whereas in blueberries, only 30−35% of the anthocyanins are in the form of cyanidins.

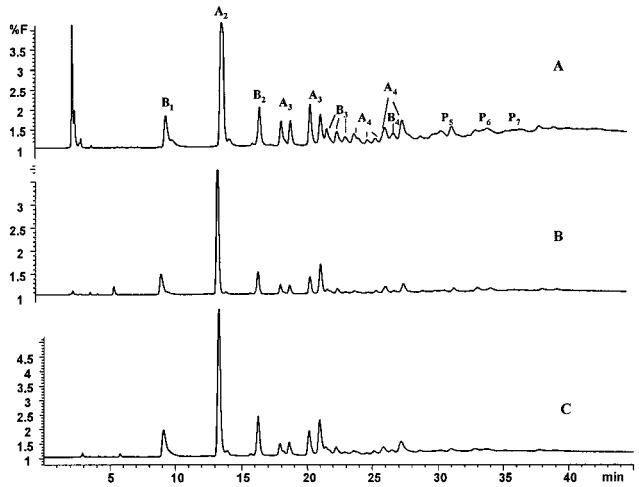

Figure 4. Normal-phase HPLC fluorescence traces of the procyanidin content in the 100% MeOH wash of (A) whole cranberries, (B) cranberry extract, and (C) cranberry juice. B_1−B_4 correspond to the procyanidin monomers through singly linked tetramers, respectively. A_2−A_4 correspond to the doubly linked (A-type) procyanidin dimers through tetramers, respectively. P_5−P_7 correspond to a mixture of doubly and singly linked procyanidin pentamers through heptamers, respectively.

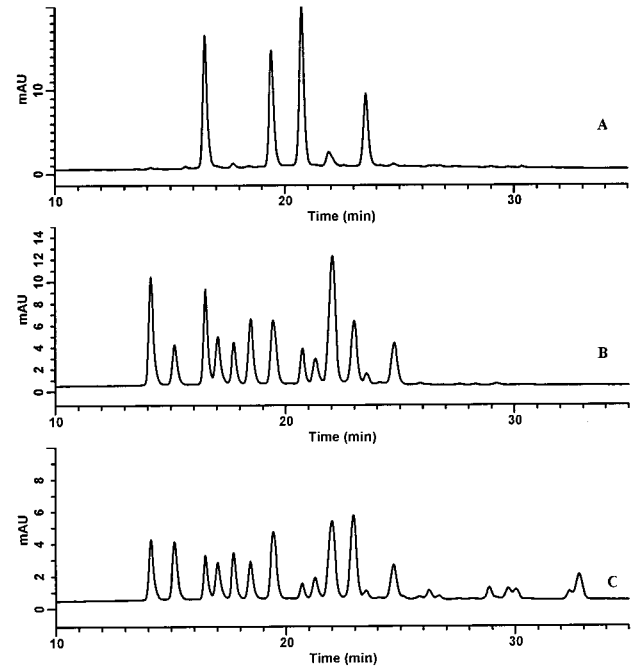

Figure 5. Anthocyanin profile using reverse-phase HPLC (520 nm) of (A) cranberries (*Vaccinium macrocarpon*), (B) highbush blueberries (Rubel; *Vaccinium corymbosum*), and lowbush blueberries (*Vaccinium angustifolium*).

CONCLUSION

This work has established the profile of procyanidins and anthocyanins in cranberries and blueberries. In cranberries, mass spectral data indicated that both A-type (containing at least one double linkage) and B-type (exclusively singly linked) oligomers are present, whereas in blueberries, only the B-type linkage was observed. Mass spectral data from two cranberry juice samples showed that the monomer, dimers, and A-type trimers were the primary procyanidins, with only trace levels of the B-type trimers and A-type tetramers and a notable absence of the higher oligomers. Processing of cranberries has a profound effect on procyanidin content. The procyanidin fraction accounts for up to 32 and 54% of the total ORAC measured in blueberries and cranberries, respectively.

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