JOURNAL OF AGRICULTURAL AND FOOD CHEMISTRY

Proanthocyanidin Profile and ORAC Values of Manitoba Berries, Chokecherries, and Seabuckthorn

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Six Manitoba fruits were analyzed for their phytochemical content and antioxidant activity in order to increase their production and marketability. The major proanthocyanidins (flavanols) present in whole fruit, juice, and pulp of strawberry, Saskatoon berry, raspberry, wild blueberry, chokecherry, and seabuckthorn were measured. The extraction and purification were facilitated using flash column chromatography, while separation and identification were accomplished by using HPLC and LC−MS techniques. The total proanthocyanidin contents varied from 275.55 to 504.77 mg/100 g in the whole fruit samples. Raspberry contained the highest content, and seabuckthorn showed the lowest content of total flavanols. The highest concentration of proanthocyanidin in juice was found in Saskatoon berry (1363.34 mg/100 mL) and the lowest value in strawberry (622.60 mg/100 mL). HPLC and LC−MS results indicated that epicatechin was the most abundant flavanol followed by B2 in the berry samples, while no catechin or B1 was detected in these fruits. A series of oligomers and polymers were detected in all samples. The recovery percentage was obtained from the ratio of the unspiked samples to the area of spiked samples. Monomers, dimers, oligomers, and polymers gave recovery ranges of 83−99%. The lipophilic and hydrophilic antioxidant capacities of whole fruit, juice, and pulp extracts were measured by the oxygen radical absorbance capacity (ORAC) procedure. In whole fruits, the ORAC values varied from 135 to 479 mg/100 g TE in the MeOH fraction. The corresponding ORAC values varied from 115.30 to 733.15 mg/100 g for the acetone fraction. In juice, all berries showed the same antioxidant capacity ($P > 0.05$) (133.0−312.0 mg/100 g) in the MeOH fraction, with the exception of raspberry (603.0 mg/100 g). Overall, MeOH fractions mainly contained monomers and dimers with smaller amounts of oligomers and polymers when compared to the acetone fractions. Acetone fractions mainly contained polymers and some oligomers. Although acetone fractions contained a higher quantity of total proanthocyanidins, their antioxidant capacities were lower than MeOH fractions.

KEYWORDS: Proanthocyanidin; ORAC; Manitoba berries; chokecherries; seabuckthorn

INTRODUCTION

There is strong potential for Manitoba fruit varieties to be positioned in the marketplace. This is important for both fruit growers and fruit processing companies. Moreover, local production means a reduction in the energy costs of transport for food. These berries can be made into jam, jelly, syrup, pie filling, fruit topping, salad dressing, and alcoholic and nonalcoholic beverages. Also, they have potential for nutraceutical and functional food purposes as ingredients in dairy, baked, and snack foods. This is important for production and marketing strategies to maximize the return for berry producers in Manitoba. Berries are rich sources of essential micronutrients, particularly, vitamin C (ascorbic acid) and folic acid (*1*−*3*). They also contain proanthocyanidins (condensed tannins) that have potential health benefits such as antioxidant, anticarcinogenic, and anti-inflammatory effects (*3*−*6*). Proanthocyanidins have been reported to contribute to the phenomenon called the "French Paradox" (*7*, *8*).

Proanthocyanidins are mixtures of dimers and higher oligomers of monomeric flavan-3-ol units linked mainly through the

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10.1021/jf071163a CCC: $37.00
Published on Web 07/28/2007

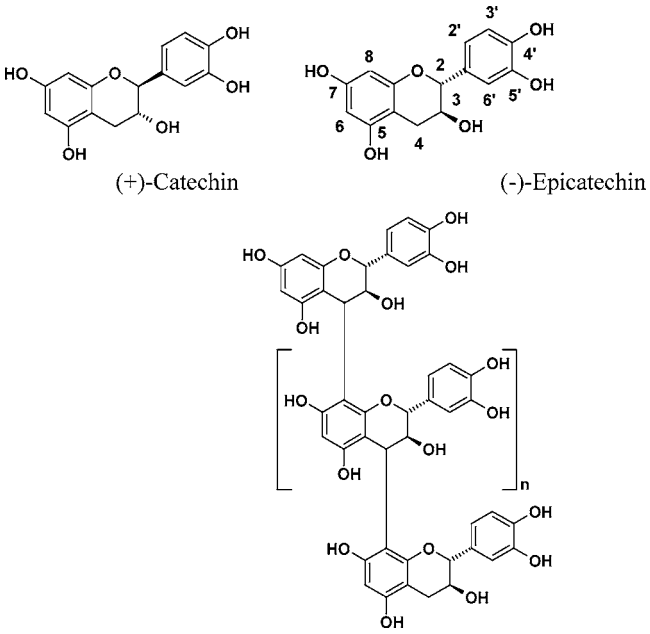

Figure 1. Structures of the flavan-3-ol monomers (+)-catechin and (–)-epicatechin and oligomeric proanthocyanidins.

C4−C8 bond and, to a lesser extent, through a C4−C6 linkage (both are called B-type) (*2*, *9*). The flavan-3-ol units can also be linked, albeit less commonly, by an additional ether bond between C2 and O7 (A-type) (*10*). Their physicochemical and biological characteristics depend on their structure and, particularly, on their degree of polymerization (*10*). Procyanidins represent the largest class of proanthocyanidins and consist of epicatechin and catechin subunits (**Figure 1**).

Generally, proanthocyanidins are defined in terms of their degree of polymerization (DP) indicating the number of monomers that are linked (*11*). The individual oligomers are commonly referred to as dimers, trimers, tetramers, pentamers, hexamers, and so forth (*11*). To date, there is little established literature for Manitoba fruits to characterize and compare their nutritional and phytochemical values. The USDA database includes the proanthocyanidin content of some fruits; however, there are varietal and geographical differences in phytochemical composition of fruits (*1*). The distribution and the structural characteristics of proanthocyanidins in most of the foods worldwide remain unknown. In addition, there is a great variation in units (e.g., μmol, mg/L) used to report the procyanidin content of fruits in the literature, making comparison difficult.

There is a growing interest in finding alternative antioxidant food preservatives, specifically, natural products that may also have the potential to provide additional health benefits. Berry fruit procyanidins are natural antioxidants and may have potential health benefits associated with their consumption. This additional role may also provide a value-added economic benefit to fruit growers.

The major objective of this study was isolation and quantification of major proanthocyanidins in six different Manitoba fruits.

MATERIALS AND METHODS

Six Manitoba fruits (strawberry, Saskatoon berry, raspberry, wild blueberry, chokecherry, and seabuckthorn) were collected at the peak of ripeness, transported to our laboratory within 12 h of picking, chilled on route and upon receipt, and freeze-dried in a Virtis Genesis freeze dryer (SP Industries, Gardiner, NY). Berries were hand picked during the period of June to August of 2006 from orchards surrounding Winnipeg (Manitoba, Canada). Strawberries, raspberries, and chokecherries were harvested when they were completely red and fully ripened. Wild blueberry and Saskatoon berries were harvested when completely dark blue in color. Seabuckthorn berries were harvested via cuttings when they were fully ripe and bright yellow in color. The trees were pruned especially for the project, but it was part of the main harvest. Each of the berries came from one variety. The fruits were randomly picked from several trees in the orchard and then combined for further analyses. The analyses were conducted on the dry powders obtained after grinding the freeze-dried samples. The approximate composition of whole fruit samples was determined using official methods (*12*) (**Table 1**). The data were expressed on a wet-weight basis.

Table 1. Approximate Composition of Fruits (Fresh Weight Basis)[a]

fruit sample	moisture %[b]	protein %	fat %	ash %	CHO %[c]
strawberry	87.53 b	0.76 e	0.26 d	0.48 c	10.97 e
Saskatoon	75.25 e	1.05 c	0.48 c	0.59 b	22.63 b
raspberry	88.61 a	1.28 a	0.93 b	0.51 c	8.67 f
wild blueberry	85.71 c	0.31 f	0.34 dc	0.15 d	13.49 c
chokecherry	66.83 f	1.15 b	0.10 e	0.82 a	31.10 a
seabuckthorn	81.38 d	0.91 d	4.41 a	0.78 a	12.52 d
LSD	0.40	0.08	0.15	0.06	0.40

[a] $P < 0.05$ using Fisher's least significance difference (LSD). [b] Different letters in the same column are used to show significant differences among these fruits. [c] CHO = carbohydrate, by difference (100 − sum of approximate composition).

Juice Processing. Frozen berries were thawed at room temperature and weighed. Strawberries, blueberries, and raspberries were processed into juice using a Moline American classic pulp ejector juicer Model # 104 (Moline Manufacturing Co. Ltd., San Dimas, CA). The pulp fractions from these berries were collected and centrifuged (Sorvall RC5C, Sorvall Instruments, MANDEL Scientific Company Inc., Guelph, ON) at 5000 rpm (GS-3-rotor) for 20 min to further extract juice. The juice fractions were then combined. For fruits containing stones such as seabuckthorn, chokecherries, and Saskatoon, a mechanical press (F. Dick, Germany) was used to extract the juice. The centrifugation step was not required for these fruits. All juices were stored at −18 °C until analyzed. Pulp fractions were freeze-dried for 54 h using a Virtis Genesis freeze dryer (SP Industries, Gardiner, NY) and stored at −18 °C until analyzed.

Chemicals. The solvents acetone, methanol, acetonitrile, and acetic acid were HPLC grade (Fisher Scientific Co., Ottawa, ON). The standards (+)-catechin, (−)-epicatechin, and procyanidin dimers B1 (epicatechin-(4-*b*-8)-catechin) and B2 (epicatechin-(4-*b*-8)-epicatechin) were purchased from Extrasynthese (Genay Cedex, France). A composite procyanidin oligomer containing monomers through hexamers was provided by Amino Up Chemical Co. Ltd (Japan).

Extraction and Purification. The extraction and purification of procyanidins were accomplished according to a modification of the method by Naczk and Shahidi and Lee et al. (*14*, *15*). Whole fruit (5 g), fresh juice (2 g), and pulp (3 g) of the berry samples were mixed with 20 mL of acetone, water, and acetic acid (70:29.5:0.5, v/v), sonicated for 20 min, and the solvent was removed. Sephadex-LH 20 with 60% methanol in water was added to a column (50 × 4.5 cm) and conditioned for 4 h. The whole extracted sample (reconstituted in 2−4 mL of methanol) was added to the top of the column. Fractionation of procyanidins was performed by adding 200 mL of 60% (v/v) methanol in order to remove most of the monomers, dimers, and the lower molecular weight procyanidin oligomers. The individual oligomers are commonly referred to as dimers, trimers, tetramers, pentamers, hexamers, up to decamers; however, our classification of oligomers was trimer up to hexamer based on our standards. Procyanidins with a degree of polymerization (DP) > 6 are reported together as polymers, while the monomers and dimers are reported individually. An amount of 200 mL of acetone, water, and acetic acid (70:29.5:0.5, v/v) was used to obtain polymer fractions and residues from methanol fractions. Acetone was evaporated under partial vacuum at 40 °C. Analytical HPLC was used to separate and isolate the compounds of interest.

HPLC Analysis. The HPLC method was based on a modification of methods from the literature (*13*, *15*). Analyses were conducted on a

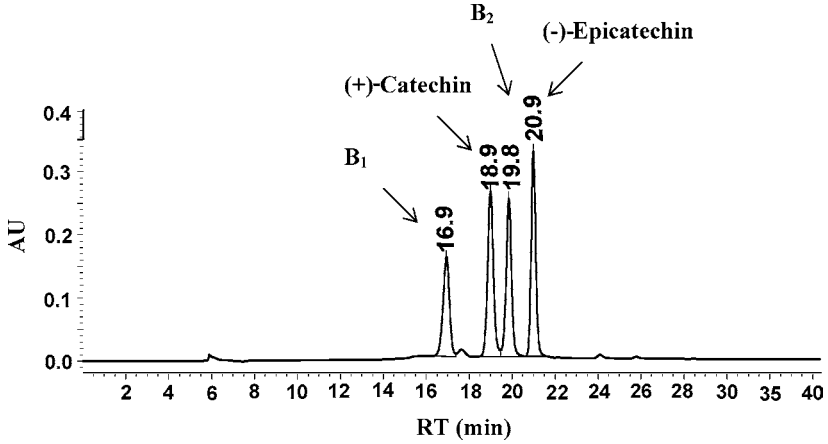

Figure 2. HPLC chromatograms of procyanidin monomer ((+)-catechin and (–)-epicatechin) and dimer (B1, B2) standards.

HPLC (Waters 2695) system equipped with a photodiode array detector (Waters 996), Empower software (Waters Corp., Milford, MA), and an autosampler (Waters 717 plus). The separation of proanthocyanidins was accomplished on a Symmetry C18 column (4.6 × 150 mm, i.d. 5μm). Mobile phases with a gradient condition were A, 0.1% acetic acid in deionized water, and B, 1% acetic acid in acetonitrile. The gradient condition was 0−5 min, 90% A; 5−25 min linear decrease to 60% A; 25−50 min linear increase to 90%. Elution of the compounds of interest was monitored at a wavelength of 320 nm for anthocyanins and at 280 nm for other phenolics. Also, spectral data (254−600 nm) were collected for all samples. The flow rate was 0.5 mL/min, and the injection volume was 10 μL for each sample. Under the current chromatographic conditions, the limit of detection (LOD) was determined according to the signal (height of peak)-to-noise (background) ratio (S/N), and the limit of quantification (LOQ) was determined by direct injection of each standard. The LOD was S/N > 5, and the LOQ was in μg/mL ranges.

Ultra Performance Liquid Chromatography (UPLC) coupled with ESI−MS/MS. LC separation was performed on an ACQUITY UPLC system consisting of a binary pump, a sample manager, and a PDA detector set at 280 nm (Waters Corp, Milford, Massachusetts). The ACQUITY UPLC BEH C18 column (1.0 × 100 mm, 1.7 μm) was used for the separation of phenolics (0.2 mL/min). The LC with a PDA detector and Acquity UPLC BEH C18 column (1.7 μm, 1 × 100 mm) was applied to separate the individual phenolics. The solvents were 0.1% formic acid (solvent A) and 100% methanol (solvent B). Prior to MS analysis, a binary mobile phase consisting of 0.1% formic acid (A) and 100% methanol (B) was used under the following gradient conditions: 0−8 min, 13−24 %B linear; 8−12 min, 24 %B isocratic; 12−22 min, 24−100 %B linear followed by 4 min of re-equilibration of the column before the next injection. The eluting stream from the UPLC was introduced into a Waters Quatro Micro API mass spectrometer (Waters Corp, Milford, MA) equipped with an ESCi multimode ionization probe (ESI APCI). We optimized the MS parameters using the procyanidin oligomer mixture. All spectra were obtained in negative-mode ESI, and the scan was set at a *m*/*z* of 100−1900. MS parameters were as follows: capillary voltage, 3 kV; cone voltage, 30 V; extractor voltage, 3.3 V; source temperature, 100 °C; desolvation temperature, 210 °C; cone gas flow, 50 L/h; desolvation gas flow, 600 L/h. Nitrogen gas was used for desolvation and the cone.

Antioxidant Activity. The antioxidant activity of fruits was measured using the oxygen radical absorbance capacity (ORAC) according to the procedures described by Huang et al. (*16*) and modified by Li et al. (*17*). An FLx800 microplate fluorescence reader (Bio-Tek Instruments, Inc., Winooski, VT) was used with fluorescence filters for an excitation wavelength of 485/20 nm and an emission wavelength of 528/20 nm. The plate reader was controlled by KC4 3.0 software (version 29). Dilution of the sample, rutin control, and the Trolox standard was done manually. The quantity of 300 μL of each of the buffer solution (blank) and diluted sample solution, rutin control, and Trolox standard was transferred to a 96 well flat-bottom polystyrene microplate (Corning Incorporated, Corning, NY) by hand according to their designated positions. A full automation of plate-to-plate liquid transfer was programmed by using a Precision 2000 microplate pipetting system (Bio-Tek Instruments, Inc., Winooski, VT). A peroxyl radical was generated by AAPH during measurement, and fluorescein was used as the substrate. All of the reaction mixtures were prepared in the measured plate in duplicate, and at least three independent assays were performed for each sample.

Final ORAC values were calculated by using a regression equation between the Trolox concentration and the net area under the fluorescence decay curve. The area under curve (AUC) was calculated as follows

$$\mathrm{AUC} = 0.5 + f_1/f_0 + ... + f_i/f_0 + ... + f_{49}/f_0 + 0.5(f_{50}/f_0)$$

where f_0 = initial fluorescence reading at 0 min and f_i = fluorescence reading at time i min.

The net AUC was obtained by subtracting the AUC of the blank from that of the sample. ORAC values were expressed as Trolox equivalents by using the standard curve. Final results were calculated and expressed as mg TE per 100 g of fruit samples.

Statistical Analysis. Samples were analyzed in triplicate, and one-way analysis of variance was performed using SAS, version 9.1. The Fisher's least significant differences (LSD) at $P < 0.05$ was used to determine significant differences among samples.

RESULTS AND DISCUSSION

Proximate Analysis. The proximate analysis composition of whole berry fruits is shown in **Table 1**. These values are close to values reported previously (*2*, *3*, *18*−*20*). Raspberry showed the highest protein value (1.28%), and wild blueberry showed the lowest value (0.31%). Seabuckthorn had the highest fat content (4.41%), and chokecherry had the lowest content (0.1%). There was no difference ($P > 0.05$) between the ash content of chokecherry (0.82%) and seabuckthorn (0.78%). Seabuckthorn is a shrub used in nutraceutical products in Europe and Asia, whereas it is virtually unknown in North America (*20*). It has potential in the cosmetic industry and as a health food due to its unique chemical and nutritional composition (*21*). Chokecherry showed the highest carbohydrate content (31.10%), while raspberry had the lowest content (8.67%).

HPLC and UPLC−MS. For HPLC, peak identification of each procyanidin was based on the comparison of the relative retention time (RT), the percentage peak area, and the UV spectral profile of proanthocyanidin standards (**Figure 2**). Monomers, dimers, and oligomers up to the hexamer were quantified based on our standard. Procyanidins with a degree of polymerization (DP) > 6 are reported together as polymers. Each dimer (B1 and B2) was detected earlier than the corresponding monomer standard (catechin and epicatechin) in

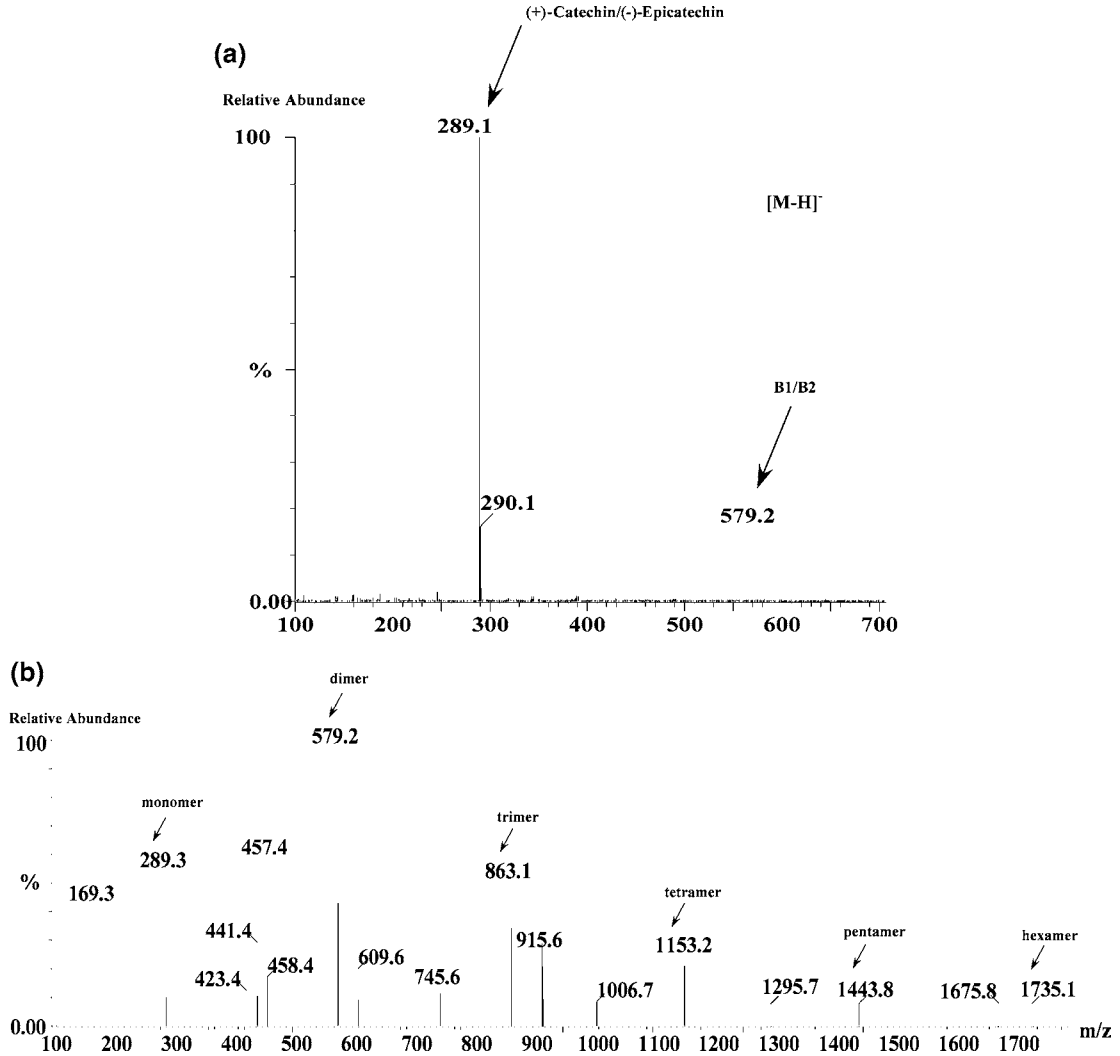

Figure 3. (**a**) Spectra of (+)-catechin/(–)-epicatechin and B1/B2 standards in the negative ESI mode. (**b**) Spectra of the hexameric standard in the negative ESI mode.

HPLC, indicating a higher polarity of the dimer than that of the monomer (**Figure 2**). A hexamer standard was used to measure the presence of oligomers, and all major peaks after the hexamer were counted as polymers. The RT and UV profiles of the polymers were useful tools for identifying the peaks corresponding to each polymer. The polymers exhibited UV absorption at 280 and 320 nm and were distinguishable from the UV absorption of monomers and dimers (320 nm). Additional data from the literature (*2*, *10*, *14*, *22*, *23*) assisted in the measurement of polymers and individual phenolics. The identity of each phenolic compound in samples and standards was confirmed by the used of an ACQUITY UPLC−MS/MS equipped with an electrospray ionization source. **Figure 3a** shows a MS spectrum of a mixture of monomeric and dimeric proanthocyanidin standards in negative ESI mode. The spectra of the hexameric standards (**Figure 3b**) were used to measure the presence of oligomers in the fruit samples. When the spectra patterns of the proanthocyanidin present in the berries were compared with the spectra of the standards, they showed the same fragmentation patterns and, thus, supported the identification of each compound.

Proanthocyanidin Contents. Monomers, dimers, oligomers, and polymers of the flavanols of berries are listed in **Table 2**. Different flavanols were present in the MeOH and acetone fractions. We concluded that monomers and dimers were mainly eluted in the MeOH fraction, and most of the oligomers and polymers were mainly eluted in the acetone fraction. The total proanthocyanidin contents varied from 258.60 to 504.77 mg/100 g in the whole fruit samples (**Table 2**). Raspberry contained the highest content, and wild blueberry showed the lowest content of total flavanols. There was no difference ($P > 0.05$) between the procyanidin content of chokecherry (285.91 mg/100 g) and seabuckthorn (275.55 mg/100 g). The content of procyanidin in blueberry, chokecherry, and strawberry was 331.9, 363.7, and 145 mg/100 g of fresh fruit, respectively, indicating validation of the method of this study and a high recovery of all procyanidins, as reported by other workers (*11*, *24*). The procyanidin content of total methanol and acetone fractions of juice and pulp for each berry is shown in **Tables 2** and **3**, respectively. The highest concentration of proanthocyanidin in juice was found in Saskatoon berry (1363.34 mg/100 mL) and the lowest value in strawberry (622.60 mg/100 mL). The corresponding procyanidin values in pulp were 1230.50 and 831.60 mg/100 g for seabuckthorn and Saskatoon, respectively. The procyanidin levels indicated remnants of juice inside of the pulp during juicing (*11*). Consumption of berries, in general, can provide a good source of dietary phenolics and likely multihealth benefits. Also, seabuckthorn is reported to have a high content of vitamin C (4.2−13.2 g/l) (*20*). This study demonstrated that Saskatoon berry has high levels of phenolics. Saskatoon berries represent an expanding crop in Manitoba with long-term industry potential. Historically, Saskatoon berries have

Table 2.[a]

fruit sample	monomer[b] mg/100 g	dimer mg/100 g	oligomer mg/100 g	polymer mg/100 g	total mg/100 g
(a) Proanthocyanidin Content (Fresh Weight Basis) of the Whole Fruit–Methanol Fraction					
strawberry	22.39 b	20.62 f	76.51 b	86.33 a	205.86 b
Saskatoon	23.55 b	108.70 a	35.03 d	19.40 d	186.68 c
raspberry	43.29 a	68.14 b	92.44 a	36.61 c	240.48 a
wild blueberry	4.87 e	31.64 c	35.02 d	48.87 b	120.41 e
chokecherry*	12.47 d	23.78 e	22.31 e	54.66 b	113.21 e
seabuckthorn	19.67 c	27.13 d	52.15 c	36.56 c	135.51 d
LSD	1.41	2.27	1.29	7.36	7.80
(b) Proanthocyanidin Content (Fresh Weight Basis) of the Whole Fruit–Acetone Fraction					
strawberry	8.76 c	18.67 b	78.04 c	135.39 a	240.87 b
Saskatoon	6.55 d	20.94 a	93.72 b	61.48 e	182.69 c
raspberry	13.99 a	13.19 d	130.55 a	106.56 b	264.29 a
wild blueberry	5.17 e	2.17 f	50.80 e	80.05 d	138.19 e
chokecherry*	5.37 e	8.48 e	67.67 d	91.18 c	172.69 d
seabuckthorn	11.54 b	15.21 c	31.79 f	81.49 d	140.03 e
LSD	0.70	1.11	3.10	2.72	5.90
(c) Proanthocyanidin Content (Fresh Weight Basis) of the Whole Fruit–Total					
strawberry	31.16 b	39.29 d	154.56 b	221.71 a	446.72 b
Saskatoon	30.10 b	129.65 a	128.75 c	80.88 e	369.37 c
raspberry	57.28 a	81.33 b	222.99 a	143.16 b	504.77 a
wild blueberry	10.05 d	33.81 e	85.81 e	128.93 c	258.60 e
chokecherry*	17.84 c	32.25 e	89.97 d	145.84 b	285.91 d
seabuckthorn	31.21 b	42.34 c	83.94 e	118.06 d	275.55 d
LSD	1.67	2.96	2.72	8.18	10.40

[a] $P < 0.05$ using Fisher's least significance difference (LSD). [b] Different letters in the same column are used to show significant differences among these fruits.

Table 3.[a]

fruit sample	monomer[b] mg/100 mL	dimer mg/100 mL	oligomer mg/100 mL	polymer mg/100 mL	total mg/100 mL
(a) Proanthocyanidin Content (Fresh Weight Basis) of the Juice–Methanol Fraction					
strawberry	101.46 c	4.29 d	8.83 e	24.53 e	139.12 d
Saskatoon	4.42 e	11.52 d	28.24 d	62.75 d	106.93 e
raspberry	109.14 b	205.59 a	153.49 a	186.98 a	655.21 a
wild blueberry	148.65 a	160.86 b	89.85 b	75.06 c	474.42 b
chokecherry*	ND[c]	ND	ND	ND	ND
seabuckthorn	49.03 d	58.48 c	52.04 c	146.74 b	306.28 c
LSD	6.03	2.27	9.29	10.8	23.9
(b) Proanthocyanidin Content (Fresh Weight Basis) of the Juice–Acetone Fraction					
strawberry	3.22 d	77.19 b	210.68 b	192.39 d	483.48 e
Saskatoon	116.55 a	111.81 a	280.61 a	747.44 a	1256.41 a
raspberry	7.30 bc	15.29 d	18.06 e	74.70 e	115.35 d
wild blueberry	14.04 b	6.67 d	185.58 c	391.58 c	597.87 c
chokecherry*	ND	ND	ND	ND	ND
seabuckthorn	9.85 bc	32.49 c	61.16 d	558.16 b	661.66 b
LSD	9.39	11.32	17.43	16.32	24.12
(c) Proanthocyanidin Content (Fresh Weight Basis) of the Juice–Total					
strawberry	104.68 c	81.48 e	219.51 c	216.93 e	622.60 e
Saskatoon	120.97 b	123.33 c	308.86 a	810.19 a	1363.34 a
raspberry	116.44 b	220.88 a	171.55 d	261.68 d	770.55 d
wild blueberry	162.69 a	167.53 b	275.43 b	466.64 c	1072.29 b
chokecherry*	ND	ND	ND	ND	ND
seabuckthorn	58.88 d	90.97 d	113.20 e	704.90 b	967.94 c
LSD	3.23	3.54	5.91	7.31	17.43

[a] $P < 0.05$ using Fisher's least significance difference (LSD). [b] Different letters in the same column are used to show significant differences among these fruits. [c] ND = not detected.

Table 4.[a]

fruit sample	monomer[b] mg/100 mL	dimer mg/100 mL	oligomer mg/100 mL	polymer mg/100 mL	total mg/100 mL
(a) Proanthocyanidin Content (Fresh Weight Basis) of the Pulp–Methanol Fraction					
strawberry	31.27 cb	428.78 d	101.20 b	102.63 bc	277.98 d
Saskatoon	25.23 c	339.46 d	114.49 a	79.20 c	252.87 d
raspberry	217.44 a	317.58 a	70.10 c	72.24 c	676.91 a
wild blueberry	58.40 cb	100.28 b	95.94 b	141.52 a	398.73 b
chokecherry	ND[c]	ND	ND	ND	ND
seabuckthorn	72.64 b	77.92 c	73.19	113.61 ba	252.87 d
LSD	0.44	0.20	0.12	0.34	0.52
(b) Proanthocyanidin Content (Fresh Weight Basis) of the Pulp–Acetone Fraction					
strawberry	12.42 b	53.12 cb	166.78 bc	350.26 b	582.43 c
Saskatoon	58.40 a	102.87 b	190.94 bac	226.52 d	578.73 c
raspberry	37.69 ba	36.14 c	112.94 c	118.59 e	304.76 d
wild blueberry	55.52 ba	163.50 a	271.16 a	324.45 c	691.77 b
chokecherry*	ND	ND	ND	ND	ND
seabuckthorn	46.16 ba	27.85 c	231.72 ba	537.94 a	893.54 a
LSD	0.45	0.59	0.96	0.24	0.50
(c) Proanthocyanidin Content (Fresh Weight Basis) of the Pulp–Total					
strawberry	43.68 c	95.99 c	267.98 bc	452.98 b	860.41 d
Saskatoon	83.63 cb	136.81 c	305.44 ba	305.72 c	831.60 d
raspberry	255.14 a	353.73 a	183.04 c	190.83 d	981.67 c
wild blueberry	113.92 b	266.37 b	367.10 a	465.96 b	1090.50 b
chokecherry*	ND	ND	ND	ND	ND
seabuckthorn	118.80 b	105.78 c	304.91 ba	651.55	1230.50 a
LSD	0.66	0.63	0.99	0.24	0.83

[a] $P < 0.05$ using Fisher's least significance difference (LSD). [b] Different letters in the same column are used to show significant differences among these fruits. [c] ND = not detected.

been a widely used native prairie fruit crop. There are now more than 3000 acres of Saskatoon berries planted in Saskatchewan, Manitoba, and Alberta, accounting for an estimated six million pounds of Saskatoon berries (*25*).

HPLC and UPLC−MS results indicated that epicatechin was the most abundant flavanol followed by B2 in the berry samples, and no catechin or B1 was detected in these fruits. Thus, the values for monomers and dimers reported in this paper (**Tables 1−3**), corresponding to epicatechin and B2, are similar to observations previously reported (*10*, *23*). A series of oligomers and polymers were detected in all samples. MS data confirmed that the oligomers consisted of (epi)catechin units that were exclusively singly linked (B-type). A recent study on a subset of 23 chocolates from different manufacturers found that (−)-epicatechin had a strong relationship with (+)-catechin and the other polyphenols but not with (−)-catechin (*23*). The procyanidin B2 dimer has epicatechin−epicatechin subunits, and thus, epicatechin concentrations can be used for predicting the concentrations of procyanidin B2 but not B1. Since (−)-catechin is the predominant form of flavanol, (−)-catechin may be effected by manufacturing process conditions forming (−)-epicatechin through epimerization (*23*). To measure the recovery percentage of major procyanidins, each sample was spiked with a known amount of catechin standard solution (1 mg/mL). The recovery percentage was obtained from the ratio of the unspiked samples to the area of spiked samples. Monomers, dimers, oligomers, and polymers gave recovery ranges of 83−99%. This indicates that procyanidins are stable under the acidic conditions that were used for both extraction and analysis by HPLC. It also validates the extraction method for analysis of major procyanidins in fruits. The total flavanol contents were lower in whole fruit and juice than in the pulps (**Table 4**). A series of oligomers and polymers were detected in all samples, and MS data confirmed that the oligomers consisted of (epi)catechin units that were exclusively singly linked (B-type). When product ion spectra patterns of phenolics present in berries were compared with the product ion spectra

Table 5.[b]

fruit sample	MeOH fraction[b] equiv of Trolox (mg/100 g)	acetone fraction equiv of Trolox (mg/100 g)
(a) ORAC for Whole Fruit (Fresh Weight Basis)		
strawberry	190.75 c	147.83 cd
Saskatoon	449.36 a	733.15 a
raspberry	167.59 c	131.16 cd
wild blueberry	331.15 b	257.35 b
chokecherry	479.46 a	213.68 cb
seabuckthorn	135.71 c	115.30 d
LSD	65.05	92.25
(b) ORAC for Fruit Juice (Fresh Weight Basis)		
strawberry	269.0 b	50.0 b
Saskatoon	133.0 b	160.0 a
raspberry	603.0 a	179.0 a
wild blueberry	312.0 b	138.0 a
seabuckthorn	217.0 b	162.0 a
LSD	182.48	40.56
(c) ORAC for Fruit Pulp (Fresh Weight Basis)		
strawberry pulp	267.24 b	118.26 cb
Saskatoon pulp	414.38 a	215.81 a
blueberry pulp	253.87 b	104.02 c
seabuckthorn pulp	77.47 c	153.45 b
LSD	33.86	37.74

[a] $P < 0.05$ using Fisher's least significance difference (LSD). [b] Different letters in the same column are used to show significant differences among these fruits.

of standards, they showed the same fragmentation patterns and supported the identification of each compound (*2*, *10*, *26*).

Antioxidant Capacity. The antioxidant capacities of whole fruit, juice, and pulp measured by the oxygen radical absorbance capacity (ORAC) procedure are shown in **Table 5**. In whole fruits, the total antioxidant capacity varied from 135 to 479 mg/100 g (Trolox equivalents) in the MeOH fraction, and no difference ($P > 0.05$) was observed between the antioxidant capacity of chokecherry (479) and Saskatoon berry (449 mg/100 g). The corresponding results of whole fruits for the acetone fraction showed that the ORAC values varied from 115 to 733 mg/100 g. There was no difference ($P > 0.05$) between the antioxidant capacity of strawberry (147), raspberry (131), and seabuckthorn (115 mg/100 g) for the acetone fraction. In juice, all berries showed the same antioxidant capacity ($P > 0.05$) (133−312 mg/100 g) in the MeOH fraction (**Table 5**), with the exception of raspberry (603.0 mg/100 g). The same observation was found in the acetone fraction, with the exception of strawberry juice (50 mg/100 g). Total procyanidins in acetone fractions were higher than MeOH fractions (**Tables 2**−**4**).

Although acetone fractions contained a higher quantity of total proanthocyanidins, their antioxidant capacities were lower than MeOH fractions. This could be due to the bulky structure and steric hindrance of polymers and oligomers when compared to those of monomers and dimers. This study demonstrated that the antioxidant capacity of polyphenols is dictated by the basic structural orientation of the compound, not just by the number of OH groups. The ring orientation will determine the ease by which a hydrogen atom from a hydroxyl group can be donated to a free radical and the ability of the compound to support an unpaired electron (*27*). Thus, the position of the OH groups in relation to one another is more important for the antioxidant capacity of phenolics (*27*, *28*) than the number of OH groups. Hydroxyl groups in close proximity, such as the OH groups in the ortho position of the B ring, appear to greatly enhance the antioxidant capacity of the phenolics (*29*, *30*). Glycosylation also decreases the antioxidant capacity of phenolics by reducing free OH and metal chelation sites (*31*). However, it is important to note that the effect of glycosylation on antioxidant capacity will depend upon the solubility of the antioxidant (e.g., water-soluble or lipid-soluble) (*27*, *28*, *30*, *32*).

Conclusion. The proanthocyanidin composition and ORAC values of whole fruit, juice, and pulp of six Manitoba fruits were measured. The fruits included strawberry, Saskatoon berry, raspberry, wild blueberry, chokecherry, and seabuckthorn. The total proanthocyanidin contents in the whole fruit samples varied from 275.55 to 504.77 mg/100 g, the values falling within the range of results previously reported. Raspberry contained the highest content, and seabuckthorn showed the lowest content of total flavanols. Epicatechin was the major flavanol followed by B2 in all samples. In all samples, MeOH fractions mainly contained monomers and dimers, with smaller amounts of oligomers and polymers when compared to those of the acetone fractions. Although acetone fractions contained a higher quantity of total proanthocyanidins, their antioxidant capacities were lower than MeOH fractions. This study demonstrates that the antioxidant capacity of a polyphenol is dictated by the basic structural orientation of the compound, not just by the number of OHs.

This study suggests that growing berries, particularly Saskatoon berry and seabuckthorn, can play a key role for fruit growers and food and nutraceutical manufacturers. It is also important as a major step in enhancing the producer-to-consumer marketing chain, giving berry producers a new avenue for large-volume distribution nationally and internationally. To achieve this goal, researchers, government, and processors should collaborate to develop a strong and vibrant fruit industry.

ACKNOWLEDGMENT

We thank the Manitoba Agri-Food Research and Development Initiative (ARDI) and Manitoba Fruit Growers Association for facilitating the provision of samples used for this study. We greatly appreciate the technical assistance provided by Denis Aminot-Gilchrist, Wan Yun Ser, and Max Abou.

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Received for review April 20, 2007. Revised manuscript received June 15, 2007. Accepted June 22, 2007. We are grateful for the financial support provided by the Manitoba Agri-Food Research and Development Initiative (ARDI), the Canada Foundation for Innovation (CFI New Opportunities Fund), and the Natural Sciences and Engineering Research Council of Canada (NSERC Discovery Grant and Canada Research Chairs Program).

JF071163A