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Bioactive Polyphenols in Leaves, Stems, and Berries of Saskatoon (*Amelanchier alnifolia* Nutt.) Cultivars

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ABSTRACT: The Saskatoon berry is currently cultivated in many parts of the world for its suitability for various food products and due to its high content of nutrients and polyphenols. To determine the phytochemical profile of a Saskatoon plant, polyphenols from leaves, stems, and berries were screened from four cultivars grown in Finland using HPLC-DAD and HPLC-ESI/MS. The phenolic composition and concentrations varied among plant parts and cultivars. The main berry components were cyanidin-based anthocyanins (63% of the phenols), quercetin-derived flavonol glycosides, and hydroxycinnamic acids. The total anthocyanin content varied between 258.7 and 517.9 mg/100 fresh weight among cultivars. Protocatechuic acid was found for the first time in Saskatoon berries. The leaves consisted of quercetin- and kaempferol-derived glycosides (41% of the phenols), hydroxycinnamic acids (36%), catechins, and some neolignans. Quercetin 3-galactoside and 3-glucoside, (−)-epicatechin, and chlorogenic acid were the main phenolics in the leaves of all cultivars. The stem components were flavanone and flavonol glycosides (55% of the phenols), catechins (38%), and hydroxybenzoic acids. Concentrations of the main compound, eriodictyol 7-glucoside, varied among cultivars from 3.3 to 6.5 mg/g of stem dry weight. Very high proanthocyanidin contents were found in stems and leaves (10−14% of dry biomass), whereas berries contained a low amount of proanthocyanidins (3% of dry biomass). The findings reveal that leaves and stems of Saskatoon cultivars possess high amounts of various phenolic compounds that may offer new functional raw materials for a wide range of food and health products.

KEYWORDS: *polyphenols, Saskatoon berry, Amelanchier alnifolia, eriodictyol 7-glucoside*

INTRODUCTION

The Saskatoon (*Amelanchier alnifolia* Nutt., Rosaceae), native to the Canadian prairies and the northern plains of the United States, bears berry-like pome fruits that were picked in the wild and used as one of the main food sources and medicines by the early settlers in the North American prairies.[1] Some four decades ago, commercial orchard production began in Canada, and currently the planted acreage of Saskatoon is the second largest after the strawberry. The popularity of Saskatoon cultivation is based on its great potential as a functional raw material in a wide range of food products due to high nutrient (iron, manganese, calcium, vitamin C, and carotene) and polyphenol contents in the berries, exhibiting strong antioxidant activity.[1−5] Large-scale commercial Saskatoon cultivation has encouraged plant breeders to develop several cultivars, which perform well in diverse environments.[1] It is only very recently that Saskatoon berries were introduced for commercial cultivation in Finland, and currently over the half of the production areas are located in the eastern part of Finland (North Karelia). The most popular Canadian cultivars have been 'Smoky', 'Honeywood', 'Thiessen', 'Northline', 'Regent', and 'Martin'. Farming experience suggests that the Saskatoon plant has adapted well to Finland's climatic conditions: maximum yields have been about 5 t/ha in North Karelia (unpublished information), and there have been no major attacks by plant diseases or insects.

Saskatoon berries are reported to be good sources of many bioactive phenolic components, such as anthocyanins, flavonols, procyanidins, and phenolic acids.[4−7] As much as 382 mg/100 g based on fresh weight of anthocyanins has been reported in Saskatoon berries,[5] which are among the highest amounts in cultivated berries.[8,9] Anthocyanin- and flavonoid-rich foods have been demonstrated to have a beneficial protective function in combating certain cancers,[10−14] inflammation and cardiovascular diseases,[15−19] type II diabetes,[20,21] obesity,[22] and age-related macular degeneration.[23]

Many biologically active types of polyphenols are also found in the leaves of several food plants, and products made from leaves are an important source of polyphenols in the human diet.[24−26] Leaves of black currants have for a long time been used in a wide variety of applications. Black currant leaves contain abundant amount of polyphenols, vitamin C, and salicylic acid,[26,27] and proanthocyanidins isolated from leaves have been demonstrated to possess an anti-inflammatory function both in vitro and in vivo.[27] Correspondingly, the leaves of sea buckthorn are very rich in a variety of flavonoids compared with the berries.[28] However, although the leaves of several plant species are used in herbal medicines and may contain high concentrations of phytochemicals of potential importance for nutraceutical and other food and nonfood

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applications, only limited information is available on the phytochemical content of the leaves of major crops.

Previously, most of the phytochemical analyses published have been limited to the berries of Saskatoon, and hardly anything is known about the chemical composition of other parts of this important berry species. The aim of this work was to provide new information about the phenolic composition of different organs of the Saskatoon plant. In addition to berries, the leaves and stems of four Saskatoon cultivars grown in Finland were studied for their phenolic compounds using HPLC-DAD and HPLC-MS.

MATERIALS AND METHODS

Chemicals. The solvents, methanol, tetrahydrofuran, and butanol, were of HPLC grade and purchased from Lab Scan (Dublin, Ireland); orthophosphoric acid (VWR Int., Briane, France), formic acid (Merck, Darmstadt, Germany), and hydrochloric acid (Merck) were of analytical reagent quality. Commercial standards benzoic acid, chlorogenic acid, (+)-catechin, (−)-epicatechin, and picein were purchased from Sigma-Aldrich Chemie (Steinheim, Germany); protocatechuic acid, eriodictyol, eriodictyol 7-glucoside, and quercetin 3-galactoside were from Roth (Karlsruhe, Germany), and quercetin 3-glucoside was from Extrasynthese (Geney Cedex, France). Cyanidin 3-glucoside and cyanidin 3-galactoside were purchased from Polyphenols Laboratories AS (Sandnes, Norway).

Experimental Material. The samples were collected from four different cultivars ('Smoky', 'Thiessen', 'Honeywood', and 'Northline') of the Saskatoon plants (*A. alnifolia* Nutt., Rosaceae) grown in Finland after a growing season in August 2010. Leaf and stem samples were taken from two clonal plants grown on four agricultural farms near the city of Joensuu (62° 35′ N, 29° 46′ E), eastern Finland, and berry samples were collected from three of these farms. Thus, the number of leaf and stem samples for each cultivar was 8 (4 sites × 2 plants). Collected berry samples represented the yield of each cultivar, from which four subsamples were randomly taken for extraction and, thus, the number of analyzed samples in each cultivar was 12 (3 sites × 4 subsamples). Both the mature leaves and the stems (15 cm from the top of the stem) were dried in a drying room at 10% relative humidity and 23 °C and then milled to a powder with a Precellys24 homogenizer (Bertin Technologies, Montigny, France). Ripe berries were frozen immediately after harvesting and stored at −20 °C until analyzed. For phenolic analyses the frozen berries were homogenized with a Precellys24. For proanthocyanidin analyses, berry samples were freeze-dried and then milled to a powder.

Extraction Procedures. Anthocyanins were extracted from 200 mg of powdered leaf and stem material and 400 mg of homogenized fresh berry material with 1 mL of acidified acetone (1% formic acid in 70% acetone) by homogenizing vigorously for 20 s in a Precellys24 homogenizer and then centrifuging for 3 min (15700 rcf, 4 °C; Eppendorf 5415, Hamburg, Germany). The procedure was repeated four times; supernatants were combined and concentrated until almost dry (acetone-free) by using a rotary concentrator (Eppendorf 5301, Hamburg, Germany). Berry anthocyanidin extracts were redissolved in 20 mL and leaf and stem extracts in 2 mL of 1% formic acid before the HPLC analyses. Other phenolics were extracted similarly using 20 mg of dried leaf and stem material and 80 mg of fresh berry material with 5 × 0.6 mL of 100% methanol. The procedure was repeated five times, and combined supernatants were evaporated to dryness and redissolved in 0.6 mL of Milli-Q water/methanol (1:1) before the HPLC analyses.

HPLC/DAD-ESI-MS Determination of Individual Polyphenols. The polyphenols were analyzed from their extracts using the HP 1100 series LC-MS system (Agilent Technologies, Palo Alto, CA) equipped with a diode array detector (DAD) coupled with a single-quadrupole mass spectrometer with an electrospray ionization (ESI) interface. The reverse-phase separation was performed on a Hypersil ODS 75 mm × 4.6 mm i.d. RP C18 column in quantitative analyses and on a Hypersil ODS 100 mm × 2.1 mm i.d. RP C18 column in qualitative analyses as described in Julkunen-Tiitto and Sorsa.[29] Anthocyanins were separated by gradient elution, using 5% v/v formic acid (A) and methanol (B) as eluents. The elution system was as follows: 0−5 min, 5% B; 5−40 min, 20% B; 40−50 min, 100% B; 50−52 min, 5% B.[30] Other phenols were separated by elution using aqueous 1.5% THF and 0.25% orthophosphoric acid (A) and methanol (B), and the elution system was as follows: 0−10 min, 15% B; 10−20 min, 30% B; 30−50 min, 50% B; 50−60 min, 100% B.[29] The flow rate was 2 mL/min and the injection volume, 15 μL.

The identification and quantification of benzoic acid, chlorogenic acid, picein, (−)-epicatechin, protocatechuic acid, eriodictyol, eriodictyol 7-glucoside, quercetin 3-glucoside, quercetin 3-galactoside, cyanidin 3-glucoside, and cyanidin 3-galactoside was based on their commercial standards. Quantifications of other phenolics were based on the standards as follows: Chlorogenic acid was used for all hydroxycinnamic acids, (+)-catechin for catechin/procyanidin derivatives and neolignans, quercetin 3-galactoside for the rest of the quercetin-derived flavonols, and cyanidin 3-galactoside for the rest of the cyanidin-based compounds. The amount of all the standards used for the single-point quantification was 1 μg in injection volume of 20 μL. The tentative identification for the compounds not having commercial standards was based on the retention time, UV spectrum, molecular ions (Table 1), and literature.[6]

Proanthocyanidin Analysis. Proanthocyanidins (condensed tannins) were determined from 0.5−1.5 mg of the powdered plant material by means of a butanol−HCl test,[31] which was standardized with purified tannins from the leaves of *Betula nana*. Quantification of proanthocyanidins was based on oxidative polymerization of catechin/epicatechin units in acid−butanol. Briefly, ground samples were dissolved in methanol and mixed with a butanol/HCl (20:1) solution and 2% $FeNH_4(SO_4)_2$ reagent. The samples were incubated at 100 °C for 50 min and cooled at room temperature, and the absorbance was measured at 550 nm. The amount of berry proanthocyanidins is transformed into corresponding units of fresh weight in Table 2.

Statistical Analysis. Statistical analysis was performed using StatView (Mac OS) software. All of the chemical analyses of berries were repeated five times ($n = 5$) and those of leaves and stems four times ($n = 4$) for all four Saskatoon cultivars. The data were subjected to analysis of variance to detect the significant differences ($p < 0.05$) between cultivars for individual compounds and compound groups (total).

RESULTS AND DISCUSSION

Small Molecular Weight Phenolics in Berries. The total anthocyanin content of the berries varied from 258.7 to 517.9 mg/100 g fresh weight (1300−2490 mg/100 g dry weight), being lowest in cv. 'Smoky' and highest in cv. 'Thiessen' (Table 2). These are high concentrations in relation to those reported in Canadian Saskatoons[2−6] or in most other berries and fruits.[9,32−35] Anthocyanins, the major class of flavonoid compounds, accounted for about 63% of the small molecular weight phenols. Five different cyanidin-based anthocyanins were detected, which is in line with earlier studies.[2−6] Cyanidin 3-galactoside and cyanidin 3-glucoside occurred in high quantities in all of the cultivars, comprising about 60 and 20%, respectively, of all the anthocyanins (Table 2). Furthermore, cyanidin 3-arabinoside and cyanidin 3-xyloside were found in minor amounts, and a cyanidin 3,5-diglucoside in very low concentrations (maximum 14 μg/g FW). Interestingly, although the number of anthocyanins is much lower than that found in other berries (5 in Saskatoon compared to 10 in crowberry or 15 in bilberry),[30,34,35] the Saskatoon cultivars grown in Finland seem to produce total concentrations of anthocyanins that are as high as those found in other northern black−blue berries. Northern berries are known to contain higher concentrations of polyphenols such as anthocyanins and flavonols than berries grown in lower latitudes.[30,36,37] This may

Table 1. Retention Times (t_R), UV−Vis Maxima, and Mass Fragment Ions of Compounds in Saskatoon Extracts As Determined by LC-MS Analyses

compound	t_R (min)	λ_{max} (nm)	molecular ion (M^+)
protocatechuic acid	2.1	216, 256, 294	155[a] (100), 177[b] (80)
picein	2.9	212, 266	321[b] (100)
protocatechuic acid deriv[c] 1	3.9	216, 258, 290	
neochlorogenic acid	5.9	218, 235, 299 sh, 324	355[a] (100), 377[b] (80)
cyanidin 3,5-diglucoside	6.1	514	611, 499[a], 287[d]
benzoic acid deriv	6.7	237, 276	
neolignan 1	9.3	230 sh, 284	
(+)-catechin	9.6	257 sh, 280	291[a] (100)
chlorogenic acid	9.7	218, 236, 300 sh, 326	355[a] (79), 377[b] (100)
cinnamic acid deriv 1	10.6	218, 244, 300, 326	
proanthocyanidin deriv	10.8	230 sh, 280	
neolignan 2	11.0	230 sh, 282	
cinnamic acid deriv 2	11.8	216, 234, 298, 328	
(−)-epicatechin	12.1	226 sh, 280	291[a] (100)
benzoic acid	13.5	230, 275	123[a] (1), 145[b] (100)
procyanidin deriv 1	13.8	220, 270, 294 sh	
catechin deriv 1	14.7	235, 280	
cinnamic acid deriv 3	15.0	218, 242, 300 sh, 328	
procyanidin deriv 2	15.6	220, 280	
cyanidin 3-galactoside	16.6	514	449[a] (50), 287[d] (100)
cinnamic acid deriv 4	17.0	218, 242, 300 sh, 330	
quercetin diarabinoglucuronide	17.1	256, 298 sh, 342	765[a] (100), 303[e] (25)
eriodictyol 7-glucoside	17.2	226, 284, 330	451[a] (100), 473[b] (97), 289[f] (98)
quercetin arabinoglucuronide	17.5	256, 352	611[a] (95), 633[b] (100), 303[e] (23)
quercetin diglycoside	18.1	256, 354	303[e]
quercetin arabinoglucoside	19.1	256, 355	597[a] (35), 619[b] (100), 303[e] (33)
cyanidin 3-glucoside	19.5	514	449[a] (100), 287[d] (4)
cinnamic acid deriv 5	19.9	216, 233, 300 sh, 328	
quercetin 3-galactoside	20.4	256, 354	465[a] (50), 487[b] (100), 303[e] (39)
quercetin glycoside	20.7	256, 356	611[a] (25), 633[b] (100), 303[e] (59)
cyanidin 3-arabinoside	20.8	514	419[a] (100), 287[d] (69)
quercetin 3-glucoside	20.9	256, 354	465[a] (67), 487[b] (100), 303[e] (60)
cinnamic acid deriv 6	21.8	218, 246, 300 sh, 328	
quercetin 3-rutinoside	22.0	258, 356	611[a] (100), 303[e] (80)
quercetin 3-arabinoside	22.6	258, 356	435[a] (2), 457[b] (5), 303[e] (100)
quercetin 3-xyloside	23.3	256, 352	441[a] (100), 463[b] (3), 303[e] (29)
isorhamnetin	23.7	256, 302 sh, 354	
kaempferol 3-glucoside	23.9	264, 350	449[a] (28), 471[b] (100), 287[g] (26)
kaempferol deriv 1	24.1	266, 325	287[g]
isorhamnetin deriv 1	24.6	252, 305 sh, 356	
eriodictyol	24.9	228, 288	289[a]
kaempferol deriv 2	26.2	266, 296, 352	
cinnamic acid deriv 7	29.1	218, 244, 302 sh, 328	
cyanidin 3-xyloside	29.5	515	419[a] (100), 287[d] (16)
isoquercetin-monocoumaroyl	29.6	256, 268 sh, 326	

[a] $[M + H]^+$ (ion abundance). [b] $[M + Na]^+$. [c] deriv = derivative. [d] Cyanidin. [e] Quercetin. [f] Eriodictyol. [g] Kaempferol.

be explained by the long days in the north during summer time, since exposure to light has a considerable effect on most flavonoids, but other climatic and environmental factors may also affect the concentrations (see, e.g., refs 36 and 38).

Several other flavonoids, such as flavonols and catechin/procyanidin derivatives (Table 2), were also detected from Saskatoon (not fully identified). Eight flavonols were quercetin-derived glycosides, of which the major component, quercetin 3-galactoside, comprised about 50% of all the flavonols. The three minor glycosides of quercetin were all found in similar quantities (about 0.1 mg/g on average), whereas three other glycosides were found in even lower amounts (Table 2). Seven of these main flavonol components have been detected also earlier in Saskatoon berries,[5,6] but in this work we tentatively identified also the monocoumaroyl-isoquercetin. The total content of quercetin glycosides in Saskatoon berries ranged from 44.0 mg/100 g FW for cv. 'Smoky' to 92.3 mg/100 g FW for cv. 'Thiessen', which is relatively high compared to the reported quercetin contents of most berries and fruits.[5,32,37−39]

The phenolic acids analyzed in Saskatoon berries were mainly hydroxycinnamic acids, but minor amounts of hydroxybenzoic acids were also detected (Table 2). Hydroxycinnamic acids comprised 18−26% of the small molecular weight phenolics, which is a relatively low proportion of phenolic acids compared to those measured in several other cultivated or naturally growing berries.[39−41] However, the concentrations in chlorogenic acids were quite similar to what has earlier been reported in the Canadian Saskatoon cultivars.[5,6] Chlorogenic acid is the most dominant of cinnamic acids in berry fruits, for which the highest amounts are reported for blueberries (0.5−2 mg/g fresh weight).[32,39,42] Together with anthocyanins, it has proven to be the most important antioxidant in berries.[5,32,42] Although the concentrations of hydroxycinnamic acids depend on botanical families and the size of the fruit, their levels in berries generally decrease during ripening [5,25]

A small amount of protocatechuic acid was detected as a new component of hydroxybenzoic acids for the first time in Saskatoon berries (Table 2). Previously, the protocatechuic acid has been found as both free and bound forms in European juneberry (*Amelanchier ovalis*) among other hydroxybenzoic acids.[41] In this work, the concentration of free protocatechuic acid (3−6 μg/g) was found markedly lower than that detected in European juneberry (10 mg/g FW) or other black−blue berries.[41,43,44] The Saskatoon berries also contained two protocatechuic acid derivatives (data not collected); on the basis of their longer and shorter HPLC retention times, they are probably bound as esters or glycosides to protocatechuic acid, respectively, as was found in ref 41.

Interestingly, the whole phytochemical profile and the concentrations of the chemicals in Saskatoon berries resemble closely those reported in chokeberries (*Aronia melanocarpa*)

Table 2. Concentrations of Individual Phenolic Compounds (mg/g FW; Mean ± SE; $n = 12$) in Berries of Saskatoon Cultivars[a]

polyphenol	'Smoky' a	'Thiessen' b	'Honeywood' c	'Northline' d
protocatechuic acid	0.005 ± 0.001	0.006 ± 0.001	0.003 ± 0.000	0.003 ± 0.001
neochlorogenic acid	0.093 ± 0.007 bc	0.129 ± 0.007 a	0.128 ± 0.013 a	0.095 ± 0.012
chlorogenic acid	0.529 ± 0.032 bc	0.742 ± 0.062 ad	0.721 ± 0.100 ad	0.483 ± 0.038 bc
cinnamic acid deriv 2	0.324 ± 0.057 cd	0.400 ± 0.031 c	0.559 ± 0.026 ab	0.516 ± 0.059 a
cinnamic acid deriv 3	0.040 ± 0.006 bcd	0.079 ± 0.014 ac	0.124 ± 0.021 ab	0.099 ± 0.010 a
cinnamic acid deriv 4	0.049 ± 0.007 c	0.061 ± 0.009	0.094 ± 0.017 a	0.079 ± 0.014
cinnamic acid deriv 5	0.019 ± 0.003 b	0.004 ± 0.002 acd	0.027 ± 0.004 b	0.021 ± 0.004 b
cinnamic acid deriv 6	0.025 ± 0.008 c	0.007 ± 0.004 c	0.048 ± 0.011 abd	0.036 ± 0.008 c
phenolic acids total	1.084	1.428	1.704	1.332
cyanidin 3-galactoside	1.769 ± 0.300 bcd	3.314 ± 0.418 a	3.076 ± 0.163 a	3.277 ± 0.055 a
cyanidin 3-glucoside	0.400 ± 0.025 bc	1.022 ± 0.206 ad	0.804 ± 0.118 a	0.451 ± 0.032 b
cyanidin 3-arabinoside	0.269 ± 0.040 bc	0.515 ± 0.070 ad	0.447 ± 0.035 a	0.332 ± 0.005 b
cyanidin 3-xyloside	0.149 ± 0.017 bc	0.328 ± 0.056 ad	0.289 ± 0.030 a	0.194 ± 0.008 b
anthocyanins total	2.587	5.179	4.616	4.254
quercetin arabinoglucoside	0.059 ± 0.010 bcd	0.120 ± 0.013 a	0.109 ± 0.006 ad	0.149 ± 0.003 ac
quercetin 3-galactoside	0.259 ± 0.006 bcd	0.519 ± 0.028 a	0.503 ± 0.075 a	0.411 ± 0.019 a
quercetin glycoside	0.025 ± 0.009 bcd	0.123 ± 0.008 acd	0.093 ± 0.013 ab	0.067 ± 0.003 ab
quercetin 3-glucoside	0.051 ± 0.001 bcd	0.096 ± 0.007 a	0.092 ± 0.014 a	0.116 ± 0.004 a
quercetin 3-rutinoside	0.018 ± 0.002 b	0.031 ± 0.002 acd	0.021 ± 0.002 b	0.015 ± 0.000 b
quercetin 3-arabinoside	0.012 ± 0.002 bcd	0.024 ± 0.002 a	0.033 ± 0.006 a	0.026 ± 0.001 a
quercetin 3-xyloside	0.014 ± 0.002 bd	0.009 ± 0.001 a	0.012 ± 0.002	0.007 ± 0.000 a
monocoumaroyl-isoquercetin	0.002 ± 0.001	0.001 ± 0.001 c	0.004 ± 0.001 bd	0.000 ± 0.000 c
flavonols total	0.440	0.923	0.867	0.791
catechin deriv 1	0.052 ± 0.007 bc	0.208 ± 0.033 ad	0.150 ± 0.043 a	0.058 ± 0.016 b
procyanidin deriv 1	0.019 ± 0.003 b	0.062 ± 0.013 ac	0.034 ± 0.010 b	0.042 ± 0.003
procyanidin deriv 2	0.057 ± 0.007	0.099 ± 0.015	0.124 ± 0.058	0.101 ± 0.013
catechin derivatives total	0.128	0.369	0.308	0.201
proanthocyanidins	6.911 ± 0.582	7.317 ± 1.493	6.472 ± 0.674	6.487 ± 0.317

[a]Statistically significant differences among cultivars are marked with different letters ($p < 0.05$). deriv = derivative.

(see, e.g., ref 45). However, there were significant differences among cultivars in the accumulation of berry polyphenols (Tables 1−3). The cv. 'Smoky' had significantly lower concentrations of several low molecular weight phenolics compared to the other three cultivars, 'Thiessen', 'Honeywood', and 'Northline', supporting the observations made on the cultivars grown in Canada.[2−6]

Small Molecular Weight Phenolics in Leaves. The phenolics in leaves consisted of flavonols (about 41% of the small molecular weight phenols), catechins (flavan-3-ols; about 18%), hydroxycinnamic acids (about 36%), and neolignans (Table 3). The flavonoid content comprised about 3% of the leaf dry mass. Cultivars differed also in the accumulation of leaf phenolics (Table 3): cv. "Smoky" had the lowest and cv. 'Honeywood' the highest total content of small molecular weight phenolics. Nine individual flavonols were identified in leaves, and all of the main components were quercetin glycosides, as was also found in berries. The total amount of quercetins in Saskatoon leaves was very high, from 24 mg/g DW in cv. 'Northline' to 32 mg/g DW in cv. 'Honeywood' (Table 3). Furthermore, kaempferol 3-glucoside and two kaempferol derivatives were found as minor components (total of kaempferols being below 1 mg/g leaf DW). In addition, high concentrations of a flavan 3-ol (−)-epicatechin were detected in Saskatoon leaves, about 0.6% of dry mass in cv. 'Smoky' and 1% of dry mass in cv. 'Northline' (Table 3). In general, quercetin and kaempferol are fairly widespread in plants and are usually detected at concentrations of 15−30 mg/kg FW in the edible parts of most vegetables and fruits, whereas the highest (−)-epicatechin contents have been reported for apples, >100 mg/kg FW.[25,46] In tea-leaf products, catechins are present in 0.9−3.7% by leaf weight ((−)-epicatechin content varying from 0.01 to 0.09 mg/g FW),[46] and the most efficiently extractable quercetin and kaempferol glycosides are reported to be present in maximum total contents about 2.4 and 1.7 mg/g, respectively.[24] Furthermore, the leaves of black currants, blueberries, and bilberries have been reported to contain higher amounts of flavonols (quercetins, kaempferols, and myricetins) and hydroxycinnamic acids compared to berries. The total flavonol contents in leaves varies generally between 1 and 4 mg/g DW and between 0.2 and 0.5 mg/g DW in berries, respectively.[26,47] In Saskatoon leaves, hydroxycinnamic acid concentrations were twice to those detected in *Vaccinium* species,[47] and the main compound, chlorogenic acid, accounted for as much as 2% of dry leaf mass (Table 3). The polyphenolic concentrations of leaves seems to be much higher than those in berries (Tables 1 and 2) and, thereby, higher antioxidant capacities for leaves are expected than in berries,[18,26,33] implicating that leaves of berry plants could be more widely exploited as a functional food ingredients than used today.

Small Molecular Weight Phenolics in Stems. The entire phenolic composition of stems differed markedly from that of

Table 3. Concentrations of Individual Phenolic Compounds (mg/g DW; Mean ± SE; n = 8) in Leaves of Saskatoon Cultivars[a]

polyphenol	'Smoky' a	'Thiessen' b	'Honeywood' c	'Northline' d
protocatechuic acid	0.094 ± 0.004 b	0.059 ± 0.006 acd	0.100 ± 0.006 b	0.088 ± 0.008 b
neochlorogenic acid	0.915 ± 0.043 bc	1.494 ± 0.082 acd	1.175 ± 0.080 b	1.106 ± 0.035 b
chlorogenic acid	17.64 ± 0.531 c	18.51 ± 0.956	20.16 ± 1.231 a	17.55 ± 0.784
cinnamic acid deriv 1	0.605 ± 0.027	0.576 ± 0.077	0.637 ± 0.025	0.589 ± 0.059
cinnamic acid deriv 3	0.741 ± 0.085 bcd	1.605 ± 0.032 a	1.365 ± 0.075 a	1.552 ± 0.223 a
cinnamic acid deriv 6	2.737 ± 0.141 b	2.087 ± 0.169 ac	3.145 ± 0.217 b	2.490 ± 0.039
cinnamic acid deriv 7	0.047 ± 0.013 bc	0.101 ± 0.036 acd	0.164 ± 0.024 ab	0.115 ± 0.008 b
phenolic acids total	22.78	24.43	26.75	23.49
quercetin 3-galactoside	9.762 ± 0.598 bc	12.19 ± 0.162 ad	11.85 ± 1.009 ad	8.779 ± 0.799 bc
quercetin 3-glucoside	8.260 ± 0.299 bcd	6.165 ± 0.201 ad	6.591 ± 0.523 ad	4.718 ± 0.480 abc
quercetin 3-arabinoside	1.896 ± 0.120 bcd	0.630 ± 0.036 acd	1.341 ± 0.099 ab	1.086 ± 0.074 ab
quercetin 3-xyloside	0.231 ± 0.019 bcd	0.170 ± 0.015 ad	0.146 ± 0.020 a	0.094 ± 0.011 ab
quercetin diarabinoglucuronide	0.506 ± 0.036 bcd	0.015 ± 0.011 acd	0.231 ± 0.019 ab	0.251 ± 0.003 ab
quercetin arabinoglucuronide	0.669 ± 0.062 bcd	0.037 ± 0.004 a	0.051 ± 0.007 a	0.052 ± 0.013 a
quercetin arabinoglucoside	3.882 ± 0.254 cd	3.531 ± 0.286 cd	7.242 ± 0.849 ab	6.199 ± 0.439 ab
quercetin glycoside	0.000 ± 0.000 bcd	4.743 ± 0.378 a	2.936 ± 0.203 a	2.787 ± 0.042 a
quercetin diglycoside	0.408 ± 0.038 bcd	0.035 ± 0.005 acd	0.062 ± 0.005 ab	0.043 ± 0.011 ab
kaempferol 3-glucoside	0.458 ± 0.027 bd	0.302 ± 0.043 a	0.376 ± 0.022	0.293 ± 0.018 a
kaempferol deriv 1	0.298 ± 0.016 bc	0.225 ± 0.016 acd	0.400 ± 0.032 ab	0.329 ± 0.029 b
kaempferol deriv 2	0.124 ± 0.007 b	0.027 ± 0.004 acd	0.137 ± 0.013 b	0.108 ± 0.021 b
monocoumaroyl-isoquercetin	0.129 ± 0.011 bc	0.124 ± 0.032 bc	0.248 ± 0.018 ab	0.233 ± 0.021 ab
flavonols total	26.62	28.19	31.61	24.97
catechin deriv 1	2.168 ± 0.153 bcd	2.865 ± 0.162 cd	4.229 ± 0.474 ab	4.231 ± 0.153 ab
(−)-epicatechin	5.800 ± 0.336 bcd	9.198 ± 0.760 a	8.181 ± 0.404 a	10.06 ± 0.719 a
catechins total	7.968	12.06	12.41	14.29
neolignan 1	1.682 ± 0.143 b	0.755 ± 0.099 acd	1.828 ± 0.189 b	1.726 ± 0.159 b
neolignan 2	0.916 ± 0.056 c	0.870 ± 0.128 c	1.233 ± 0.073 ab	1.145 ± 0.057
lignans total	2.598	1.625	3.061	2.871
picein	0.106 ± 0.006 bcd	0.053 ± 0.007 acd	0.178 ± 0.006 ab	0.153 ± 0.013 ab
proanthocyanidins	124.7 ± 4.656	134.4 ± 6.902	124.8 ± 8.978	142.8 ± 7.311

[a]Statistically significant differences among cultivars are marked with different letters ($p < 0.05$).

leaves and berries. The quercetin-derived flavonols were found in low quantities, whereas eriodictyol derivatives (flavanones) comprised about 55% and catechins (flavan-3-ols) about 38% of the phenols (Table 4). (+)-Catechin, (−)-epicatechin, and eriodictyol 7-glucoside were the main individual compounds in stems, of which eriodictyol 7-glucoside was found in especially high quantities (6.5 mg/g DW) in the cv. 'Northline' (Table 4). Cv. 'Northline' had the highest total flavonoid content in stems, whereas cv. 'Honeywood' had the lowest concentrations in several other small molecular weight phenolics. In crop plants, flavanones are present in high concentrations only in citrus fruit,[24] but they also occur in certain aromatic plants, such as the *Mentha* genus, in which eriodictyol 7-glucoside has been detected at concentrations (0.03−0.42% leaf DW)[48] similar to those now found in the Saskatoon bark (0.3−0.6% DW). Eriodictyol is a compound that has recently been found to possess very high antioxidant activity and an ability to induce a wide range of oxidative stress protectants in retinal pigment epithelial (RPE) cells.[49] Consequently, plant extracts high in eriodictyol content appear to be one of the promising new natural polyphenolic agents that induce long-term protection against oxidative stress.

All of the phenolic acids detected in stems were hydroxybenzoic acid derivatives, and they comprised about 0.1% of stem dry mass (Table 4). Hydroxybenzoic acid contents in edible plants are generally reported to be very low, about 0.02−0.3 mg/g FW, with large intra- and interspecific differences.[25,41,43] However, some herbs and spices may contain relatively high concentrations of hydroxybenzoic acids.[25,43,48] In cinnamon bark, for example, protocatechuic acid has been reported[43] to be a dominant compound (concentration = 23−27 mg/kg). In Saskatoon, the protocatechuic acid concentrations in stems and leaves varied from 48 to 100 mg/kg DW (Tables 3 and 4).

High Molecular Weight Phenolics. All of the plant parts of Saskatoon contained high amounts of proanthocyanidins (condensed tannins) as determined by the acid−butanol test. In berries, the proanthocyanidins comprised about 3% of dry biomass (32−37 mg/g DW depending on cultivar) and represented 46−69% of the total berry polyphenols (Table 2). In leaves and stems the amount of proanthocyanidins was higher (Tables 2 and 3), about 10−14% of dry biomass, which represented 64−70% of the total polyphenols in leaves and 89% of the total polyphenols in stems. The proanthocyanidin accumulations in each plant part of Saskatoon were rather similar (Tables 1−3). It has been previously demonstrated[5] that several proanthocyanidin oligomers with different degrees of polymerization occur in Saskatoon berries and that the total

Table 4. Concentrations of Individual Phenolic Compounds (mg/g DW; Mean ± SE; n = 8) in Stems of Saskatoon Cultivars[a]

polyphenol	'Smoky' a	'Thiessen' b	'Honeywood' c	'Northline' d
protocatechuic acid	0.059 ± 0.002 b	0.048 ± 0.004 ac	0.052 ± 0.001 b	0.060 ± 0.002
protocatechuic acid deriv 1	0.032 ± 0.001 c	0.030 ± 0.002	0.038 ± 0.002 ab	0.037 ± 0.002
benzoic acid	0.839 ± 0.051 cd	0.749 ± 0.062 cd	0.423 ± 0.023 ab	0.542 ± 0.065 ab
benzoic acid deriv	0.500 ± 0.020 cd	0.516 ± 0.131 cd	0.377 ± 0.019 ab	0.386 ± 0.021 ab
phenolic acids total	1.430	1.343	0.890	1.025
eriodictyol 7-glucoside	3.926 ± 0.314 d	3.295 ± 0.507 d	3.813 ± 0.336 d	6.463 ± 0.418 abc
eriodictyol	0.331 ± 0.043 bcd	0.123 ± 0.014 a	0.128 ± 0.021 a	0.102 ± 0.011 a
quercetin 3-galactoside	0.115 ± 0.017 bcd	1.171 ± 0.260 a	0.901 ± 0.116 a	1.028 ± 0.049 a
quercetin 3-glucoside	0.111 ± 0.012	0.163 ± 0.034	0.143 ± 0.018	0.178 ± 0.010
quercetin 3-arabinoside	0.069 ± 0.007 cd	0.087 ± 0.014 d	0.112 ± 0.013 a	0.122 ± 0.003 ab
quercetin 3-xyloside	0.070 ± 0.006 bcd	0.042 ± 0.004 a	0.040 ± 0.009 a	0.043 ± 0.002 a
quercetin arabinoglucuronide	0.115 ± 0.017 bcd	0.000 ± 0.000 a	0.000 ± 0.000 a	0.000 ± 0.000 a
quercetin glycoside	0.153 ± 0.015 d	0.176 ± 0.031 d	0.204 ± 0.022 d	0.288 ± 0.009 abc
quercetin arabinoglucoside	0.245 ± 0.225	0.145 ± 0.055	0.222 ± 0.053	0.299 ± 0.013
isorhamnetin	0.068 ± 0.016	0.132 ± 0.082	0.061 ± 0.031	0.069 ± 0.007
isorhamnetin deriv 1	0.008 ± 0.003	0.024 ± 0.016	0.018 ± 0.011	0.021 ± 0.001
flavanones/flavonols total	5.211	5.358	5.642	8.613
proanthocyanidin deriv	0.629 ± 0.038 cd	0.747 ± 0.068 cd	0.426 ± 0.115 ab	0.368 ± 0.024 ab
(+)-catechin	2.421 ± 0.109 cd	3.163 ± 0.183 acd	1.782 ± 0.350 ab	1.893 ± 0.043 b
catechin deriv 1	0.622 ± 0.059 d	0.432 ± 0.071 d	0.439 ± 0.100 d	1.252 ± 0.119 abc
(−)-epicatechin	1.272 ± 0.054 bc	0.731 ± 0.052 ad	0.747 ± 0.091 ad	1.423 ± 0.061 bc
catechin derivatives total	4.944	5.073	3.394	4.936
neolignan 1	0.150 ± 0.080 bcd	0.342 ± 0.110 a	0.312 ± 0.105 a	0.317 ± 0.004 a
picein	0.062 ± 0.017 c	0.027 ± 0.002	0.020 ± 0.001 a	0.042 ± 0.005
proantocyanidins	98.54 ± 4.524	107.4 ± 4.054	96.31 ± 2.751	100.7 ± 4.838

[a]Statistically significant differences among cultivars are marked with different letters (p < 0.05).

proanthocyanidin content varies between 1.3 and 3.0 mg/g FW. Berry proanthocyanidins are found to be of the procyanidin type and consist of (−)-epicatechin units linked by β-type bonds.[5,7] The polymeric proanthocyanidin profile in Saskatoon berries is quite similar to that of chokeberries (*Aronia melanocarpa*) or other species from the Rosaceae family, in which the amounts of 40−100 mg/g DW are among the highest reported in berries.[9,45,50] However, because of even higher proanthocyanidin contents in leaves than in berries, the leaves of berry species are suggested to be the best potential sources for bioactive procyanidins and condensed tannins.[47,51]

In conclusion, several berry polyphenols, such as proanthocyanidins, anthocyanins, quercetins, and chlorogenic and benzoic acids were also found in the leaves and stems of the Saskatoon plants, whereas eriodictyols and catechins were stem-specific flavonoids and (−)-epicatechin was a leaf-specific flavonoid. Many of these polyphenols are suggested to carry beneficial health effects accompanied by reduction of the risk of inflammation, diabetes, cardiovascular diseases, and cancer.[10−21] In comparison with other plants rich in polyphenols, the composition of different phenolics, for example, flavonols and anthocyanins, in the whole Saskatoon plant is narrower, but the compounds occur in Saskatoon berries, leaves, and stems in relatively high concentrations. Saskatoon leaves appear to be suitable material for quercetin, (−)-epicatechin, or chlorogenic acid, stems for eriodictyol or catechin, and berries for cyanidin-type anthocyanin preparations. Therefore, the whole Saskatoon plant may be used as a potential source of valuable ingredients in juices, herbal teas, dietary supplements, and other products, which could open new opportunities for exploiting this crop in novel applications.

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