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Evaluation of Saskatoon Berry (*Amelanchier alnifolia* Nutt.) Cultivars for their Polyphenol Content, Antioxidant Properties, and Storage Stability

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The polyphenol contents and antioxidant activities were assessed for 17 Saskatoon berry cultivars grown in Canada in fresh and stored fruits at −20 °C for 9 months. The Nelson cultivar was the richest in total polyphenol, anthocyanin, and procyanidin contents (801, 382, and 278 mg/100 g fresh weight, respectively). This cultivar was characterized also by the highest antioxidant potential measured with DPPH and ABTS radicals (2.8 and 5.0 mM/100 g FW, respectively). Cultivar-dependent changes in polyphenol content after freezer storage were observed. In the Lee 2 cultivar, significant increases in anthocyanin and flavonol contents occurred, while in the Lee 3 and Martin cultivars considerable decreases were observed. During the freezer storage, the antioxidant activity remained unchanged except for the Smokey which showed to be the most sensitive cultivar during storage. The Nelson and Lee 2 were the most stable cultivars during storage. The high polyphenol content and antioxidant activity of the Nelson cultivar and its good storage stability would make this cultivar the optimal material for fruit growers and food producers.

KEYWORDS: ***Amelanchier alnifolia*** **Nutt.; Saskatoon berries; anthocyanins; procyanidins; flavonoids; hydrocinnamates; antioxidant activity; storage stability; thioacidolysis; HPLC-MS**

INTRODUCTION

The Saskatoon berry (*Amelanchier alnifolia* Nutt., Rosaceae) is native to the southern Yukon and Northwest Territories, the Canadian prairies, and the northern plains of the United States. The fruit, usually called a berry, is actually a pome (*1*). It has been commercially grown since the mid-1960s (*2*). Today, there are more than 1200 ha of Saskatoon berries planted in Canadian prairies: Saskatchewan, Manitoba, and Alberta, accounting for an estimated 6−8 million kilograms of Saskatoon berries (personal communications). Traditionally, Saskatoon berries were consumed mostly fresh, baked in pies, or processed into jams and spreads, but recent innovations in processing and freezing have greatly increased the potential for these berries to be used in industry, for example, by cereal, snack food, and ice cream processors (*3*).

Berries and fruits, including Saskatoon berries, are excellent sources of bioactive components such as anthocyanins, flavonols, procyanidins, and phenolic acids. Recently, anthocyanins along with other phenolics have attracted much interest due to their antioxidant properties and perceived health benefits, including antimicrobial, anti-inflammatory, and anticarcinogenic activities, insulin secretion ability, and neuroprotective effects (*4*).

Interest in the role of antioxidants in human health has prompted research in the field of horticultural and food science to assess fruit antioxidants such as phenolic compounds, and to determine how their content and activity can be maintained or even improved through cultivar development, production practices, postharvest storage, and food processing. Fruits are good sources of polyphenols, and since fruits are often consumed fresh, antioxidant activity is not lost due to any adverse effects of heat and oxidation during processing. Little is known, however, about the effects of fruit storage on the retention of dietary antioxidants such as polyphenols. Moreover, information available about the phenolic content of fruits is not always complete and often is restricted to a few cultivars and to a single group of phenolic compounds. Previous work has been reported on Saskatoon anthocyanins (*2, 5*), procyanidins (*6*), hydrocinnamates, and flavonols (*7*). The data reported, however, are quite limited by the small number of cultivars analyzed and the phenolic compounds quantified.

Accordingly, the aim of the present work was to identify and quantify individual phenolic compounds (**Figure 1**) in 17 cultivars of Saskatoon berries collected in the 2005 and 2006 seasons. Furthermore, the stability of polyphenol compounds in Saskatoon fruits during 9 months of storage at −20 °C was evaluated.

MATERIALS AND METHODS

Chemicals. Citric acid, sodium acetate, sodium hydroxide, methanol (ACS grade), and potassium chloride were purchased from Fisher

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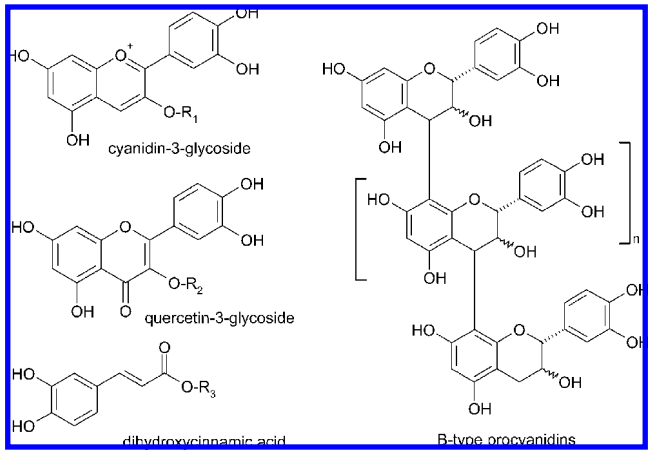

Figure 1. Polyphenol compounds from Saskatoon berries. R1 = galactose, glucose, arabinose, or xylose; R2 = galactose, glucose, arabinose, xylose, rutinose, or arabinoglucose; R3 = quinic acid.

Scientific (Ottawa, ON, Canada). Folin−Ciocalteu reagent, gallic acid, potassium persulfate, benzylmercaptan, and 2,2′-azino-*bis*(3-ethylbenzothiazoline-6-sulfonic acid) (ABTS) were purchased from Sigma (St. Louis, Mo); cyanidin-3-glucoside, cyanidin-3-galactoside, and cyanidin-3,5-diglucoside were from Indofine (Hillsborough, NJ); cyanidin-3-arabinoside was purchased from Extrasynthese (Geney Cedex, France). Chlorogenic acid, (+)-catechin, (−)-epicatechin, rutin, quercetin-3-glucoside, and quercetin-3-galactoside were purchased from ChromaDex (Santa Ana, CA).

Berry Material. Ripe Saskatoon berries from 17 varieties (Success, Lee 3, Martin, Parkhill, Forestburg, Lee 8, Lee 2, Pembina, Honeywood, Northline, Thiessen, Pasture, Nelson, Pearson, Quaker, Smokey, and Regent) were harvested in July 2005 and 2006 at the Dn'A Garden field, Elnora, Alberta, Canada. Within 2 h after harvest, whole berries were divided into two lots and stored at −20 °C prior to analysis. One lot was used for analysis of polyphenols before storage, and the other for polyphenol analysis after 9 months of storage at −20 °C. For the moisture content determination, fresh berries were ground in a food processor and dried at 110 °C until the constant weight was reached. For procyanidin content determination, fresh berry samples were ground in a food processor and freeze-dried. Chokeberries (*Aronia melanocarpa* E.) were collected at Olds College Botanical Garden. The polyphenol extract from chokeberries was prepared as described previously (*5*).

Extraction. Alcoholic extraction of Saskatoon berry polyphenols was performed as previously described (*5*). Briefly, compounds were extracted by homogenizing for 30 s 10 g of frozen fruits in 70 mL of 80% aqueous methanol (0.1% formic acid). The mixture was sonicated for 20 min and centrifuged for 30 min (1000*g*, 4 °C). The samples were extracted once more with 70 mL of 80% aqueous methanol (0.1% formic acid) using the same procedure. For spectrophotometric analysis, the supernatants were combined, adjusted to the known volume, and filtered through 0.45 μm filter, while for high-performace liquid chromatography (HPLC) analysis the combined supernatants were evaporated at 40 °C under vacuum to remove solvents. The residue was dissolved in water (0.1% formic acid) and applied to a column of Amberlite XAD-16 nonionic polymeric absorbent (Rohm and Hass, Philadelphia, PA). After washing with water (0.1% formic acid), the polyphenol fraction was collected by elution with methanol (0.1% formic acid) and then evaporated to dryness using a rotary evaporator. The residue was dissolved with HPLC grade water (0.1% formic acid).

Determination of Total Polyphenols. The total polyphenol content of the extracts was determined using the Folin−Ciocalteu colorimetric method as described by Singleton et al. (*8*) with modifications. One mL of berry extract and 1 mL of Folin−Ciocalteu reagent were pipetted into a 100 mL volumetric flask. After 3 min, 10 mL of a 20% aqueous solution of sodium carbonate (Na_2CO_3) was added, and the flask was brought to volume with distilled water. The absorbance at 765 nm was measured after 1 h, and the results were expressed as milligrams of gallic acid equivalents per 100 g of fresh weight (FW). Data are reported as a mean value for three measurements.

Determination of Antioxidant Activity. The antioxidant activity of hydroalcoholic extracts was determined using the Trolox equivalent antioxidant capacity (TEAC) assay with ABTS and DPPH radicals. The TEAC assay with ABTS radicals was carried out according to Re and co-workers (*9*), while the TEAC assay with DPPH radicals was according to the procedure described by Yen and Chen (*10*). TEAC results are expressed as millimoles of Trolox equivalents per 100 g of FW of Saskatoon berry. Data are reported as a mean value for four measurements.

HPLC-ESI-MS/MS Determination of Individual Polyphenols. The chromatographic system consisted of a 1100 Series Agilent Technologies LC/MSD system equipped with a diode array detector (DAD) coupled to a mass spectrometer (quadrupole analyzer) equipped with an electrospray ionization (ESI) interface (Agilent Technologies, Mississagua, ON, Canada). The reversed-phase separation was performed on a 250 mm × 4.6 mm i.d. RP C18 column (Grace Vydac, Hespedia, CA). The compounds were separated with gradient elution using 4.5% aqueous formic acid (A) and 80% acetonitrile in solution A (B) as eluents. The elution system was as follows: 0−7 min 15% B, 7.0−15 min 20% B, 15−16 min 100% B, 16−24 min 0.0% B. MS parameters were as follows: capillary voltage, 4000 V; drying gas temperature, 350 °C; gas flow (N_2), 12 L/min; nebulizer pressure, 60 psi. The instrument was operated in both positive and negative ion mode scanning from *m*/*z* 100 to 1500 at a scan rate of 2.0 s/cycle.

Determination of Individual Antioxidant Potential of Saskatoon Polyphenols. One mL of Saskatoon extract (polyphenol concentration 1 mg/mL) prepared as presented above and 0−200 μL of $ABTS^{\cdot+}$ solution (3 mM concentration) were mixed to react for 3 h and then passed through a 0.5 μm filter and injected for HPLC assay. Blanks of extract with water and $ABTS^{\cdot+}$ with water were also analyzed.

Determination of Procyanidins Content. The procyanidin content in Saskatoon berries was determined by the thioacidolysis method described by Guyot et al. (*11*). Briefly, freeze-dried and grounded samples (30 mg) were dissolved in methanol and mixed with a 5% solution of benzylmercaptan in methanol. The vials were sealed and incubated at 40 °C for 30 min with agitation every 10 min and then cooled in an ice bath. Reverse-phase HPLC analyses were performed with an Agilent 1100 liquid chromatograph (Agilent Technologies, Mississagua, ON, Canada) system equipped with a diode array detector and the same column as above. The thioacidolysis products were separated with gradient elution using 2.5% aqueous acetic acid (A) and acetonitrile (B) as eluents. The elution system was as follows: 0 min 3% B, 5 min 15% B, 15 min 16% B, 45 min 50% B. Epicatechin and catechin were quantified using external standards of the authentic compounds. Catechin was used as a standard for quantification of epicatechin benzylthioethers. The average degree of polymerization (DP) was measured by calculating the molar ratio of all the flavan-3-ol units (thioether adducts + terminal units) to (+)-catechin and (−)-epicatechin corresponding to terminal units. Data are reported as a mean value for three measurements.

Statistical Analysis. Statistical analysis was performed using STATGRAPHIC software (StatPoint, Inc., Sainte-Fey, QE, Canada). Data were subjected to one-way analysis of variance for mean comparison, and intercultivar significant differences were calculated according to Tukey's HSD multiple range test. Correlations were calculated on cultivar mean basis, according to Pearson's test. Differences at $p < 0.05$ were considered to be statistically significant.

RESULTS AND DISCUSSION

The present study clarifies the role of the genotype (cultivar) and the effect of freezer storage on the content of bioactive polyphenol compounds in Saskatoon berries. The dry matter content in Saskatoon berries differed strongly between cultivars (**Table 1**). For instance, the Quaker cultivar has less than half the amount of dry matter compared to the Nelson cultivar (13.8 vs 21.4%, respectively).

Table 1. Total Polyphenol Content and Antioxidant Activity of Saskatoon Berry Cultivars before and after 9 Months of Storage at −20 °C

		total polyphenols[a]		ABTS[b]		DPPH[b]	
cultivar	dry matter [%]	before storage	after storage	before storage	after storage	before storage	after storage
Success	15.2	574.17f	553.73f	3.84c	3.85c	2.23b	2.13b
Lee 3	14.3	652.07d	640.71d	4.14b	4.04b	2.31b	2.35ab
Martin	15.7	724.01b	713.80b	4.10b	3.96bc	2.63a	2.62a
Parkhill	16.1	554.47f	535.45f	3.58cd	3.49cd	2.34ab	2.34ab
Forestburg	14.2	588.61f	563.52f	3.85c	3.63cd	2.20bc	2.39ab
Lee 8	15.8	629.94d	620.38d	3.95bc	3.83c	2.53a	2.82a
Lee 2	18.3	701.33b	684.04b	3.72c	3.64c	2.09b	2.10b
Pembina	17.7	577.04f	549.91f	3.63c	3.55c	2.11bc	2.21b
Honeywood	15.4	775.29a	744.64a	4.23b	4.33b	2.53a	2.90a
Northline	16.4	604.90e	588.29e	3.69c	3.48cd	2.24b	2.34ab
Thiessen	15.2	702.64b	675.17b	3.78c	3.5cd	2.04bc	2.34ab
Pasture	16.1	684.61c	679.59c	4.02b	3.66c	2.08bc	2.12bc
Nelson	21.4	801.37a	798.83a	4.98a	5.00a	2.76a	2.98a
Pearson	15.5	614.68de	593.21de	3.84c	3.75c	2.34ab	2.54a
Quaker	13.8	697.24b	674.50b	4.34b	4.25b	2.27b	2.27b
Smokey	16.3	623.47d	601.92d	3.36d	3.01d	1.90c	1.65c
Regent	14.7	559.66f	543.53f	4.03b	3.91bc	2.76a	2.70a

[a] Data are expressed as milligrams of gallic acid equivalents per 100 g of fresh weight. [b] Data are expressed as millimoles of Trolox equivalents per 100 g of fresh weight. Means within columns are significantly different ($p < 0.05$).

Total Polyphenol Content. The results of the Folin−Ciocalteu assay are shown in **Table 1**. Nelson and Honeywood cultivars had the highest gallic acid equivalents (801 and 775 mg/100 g FW, respectively), followed by Martin, Thiessen, Lee 2, and Quaker cultivars (724, 703, 701, and 697 mg/100 g FW, respectively). The lowest polyphenol contents were measured in Forestburg, Pembina, Success, Regent, and Parkhill cultivars (589, 577, 574, 560, and 554 mg/100 g FW, respectively).

In contrast, wild strawberry contained a lower amount of polyphenols, 438 mg/100 g FW (*5*). Also red and black raspberries, belonging to Rosaceae, contained a lower amount of total polyphenols, 455 and 412 mg/100 g FW, respectively (*5*). A previous report on the phenolic content in Saskatoon berries showed a lower concentration of total polyphenols in fruits, 405 mg of chlorogenic acid/100 g FW (*12*). The differences in analytical methods and extraction procedures may have contributed to the differences in phenolic concentrations between the two studies.

This is the first systematic study on polyphenol variation in Saskatoon berries. The results of HPLC-ESI-MS/MS analysis are presented in **Tables 2−4** as a mean value from 2005 and 2006 harvest seasons, since data obtained in 2 years were not significantly different.

The identification of polyphenol compounds in Saskatoon berries was based on comparison of their retention times and mass spectrometric data with those of pure standard and chokeberry (*Aronia melanocarpa* E.) polyphenol extracts. Chokeberry and Saskatoon berry belong to the Rosaceae family, and their polyphenol composition is almost identical. The chokeberry extract was chosen, since its polyphenol composition is well-known (*13, 14*).

Anthocyanin Composition and Concentration in Saskatoon Berries. The HPLC analysis showed that Saskatoon berry contained four main anthocyanin compounds (**Figure 2**). By comparing their mass spectra and retention times with standards and chokeberry extract, they were identified as cyanidin-3-galactoside, cyanidin-3-glucoside, cyanidin-3-arabinoside, and cyanidin-3-xyloside. Additionally, the fifth minor anthocyanin was identified as cyanidin-3,5-diglucoside. This is the first report about the presence of cyanidin-3,5-diglucoside in Saskatoon berries. The Saskatoon anthocyanin identification presented herein is in agreement with the data reported previously (*2, 7*).

A similar anthocyanin pattern for all Saskatoon berry cultivars was obtained by HPLC. Cyanidin-3-galactoside consistently accounted for the largest percentage of the total peak area (from 65% for Forestburg to 78% for Lee 8), followed by cyanidin-3-glucoside (from 9% for Lee 8 to 23% for Pembina), cyanidin-3-arabinoside (from 8% for Northline to 13% for Pearson), cyanidin-3-xyloside (from 0.4% for Lee 8 to 11% for Forestburg), and cyanidin-3,5-diglucoside (from 0.1% for Pasture to 0.3% for Forestburg).

The anthocyanin profile was identical in all cultivars, but the proportions of each compound were variable. High anthocyanin content was associated primarily with a high cyanidin-3-galactoside content, especially for Northline and Lee 8 cultivars. Nelson had the highest concentration of all the main anthocyanins. Forestburg had the high amounts of cyanidin arabinoside and xyloside.

The high concentration of anthocyanins in the Nelson cultivar was reported before by Zatylny et al. (*15*) who determined the anthocyanin composition and concentration in 16 Saskatoon cultivars grown in the province of Saskatchewan (Canada) in the 1999 and 2000 seasons. They reported that Pearson, Quaker, Smokey, and Success contained the lowest concentrations of anthocyanins. The authors also reported that Northline had the lowest cyanidin-3-glucoside content of the cultivars, which is in agreement with the present data.

Previously, Mazza (*2*) analyzed anthocyanin composition in Saskatoon berries by HPLC and identified cyanidin-3-galactoside, cyanidin-3-glucoside, and cyanidin-3-xyloside in Honeywood and Smokey cultivars. The proportion of anthocyanins reported was similar to that currently found, although the amounts were twice as low as presented herein.

Fruit of the Nelson variety in this study had the highest anthocyanin content, 382.1 mg/100 g FW, followed by Martin and Lee 3, 342.5 and 319.6 mg/100 g FW, respectively (**Table 2**). The Pearson and Smokey berries had the lowest anthocyanin content, 190.0 and 189.7 mg/100 g FW, respectively. The anthocyanin concentration in Saskatoon berry cvs Northline and Smokey reported by Mazza (*2*) was lower than that in the present study (124.2 and 86.2 mg/100 g FW, respectively). Recently, Ozga et al. (*7*) analyzed the anthocyanin content in Saskatoon berry cv Smokey and reported 136.8 mg/100 g FW of anthocyanins. Also, Hu et al. (*16*) reported low concentrations of anthocyanins in Saskatoon berry cvs Smokey and Thiessen, as 41 and 60 mg/100 g, respectively. The discrepancy in anthocyanin contents between the four studies may be due to differences in methodology, growing location and climate, fruit maturity levels, and storage conditions prior to analysis.

Hydroxycinnamic Acids Composition and Concentration in Saskatoon Berries. Hydroxycinnamic acid derivatives were the second largest group of polyphenols after anthocyanins that contributed to the final concentration of polyphenols in Saskatoon berries. The HPLC-ESI-MS/MS data showed the presence of chlorogenic (5-*O*-caffeoylquinic acid), neochlorogenic (3-*O*-caffeoylquinic acid), and dicaffeoylquinic acids in Saskatoon extracts (**Figure 2**). Chlorogenic acid was identified by comparing the retention time and MS data with those of a pure standard. The neochlorogenic acid was identified by retention time comparison with the chokeberry phenolics chromatogram and MS data. The present identification is in agreement with previously described data (*2, 7*). The dicaffeoylquinic acid was identified by MS data. The presence of dicaffeoylqunic acid in Saskatoon fruit was not reported before. Interestingly, two additional

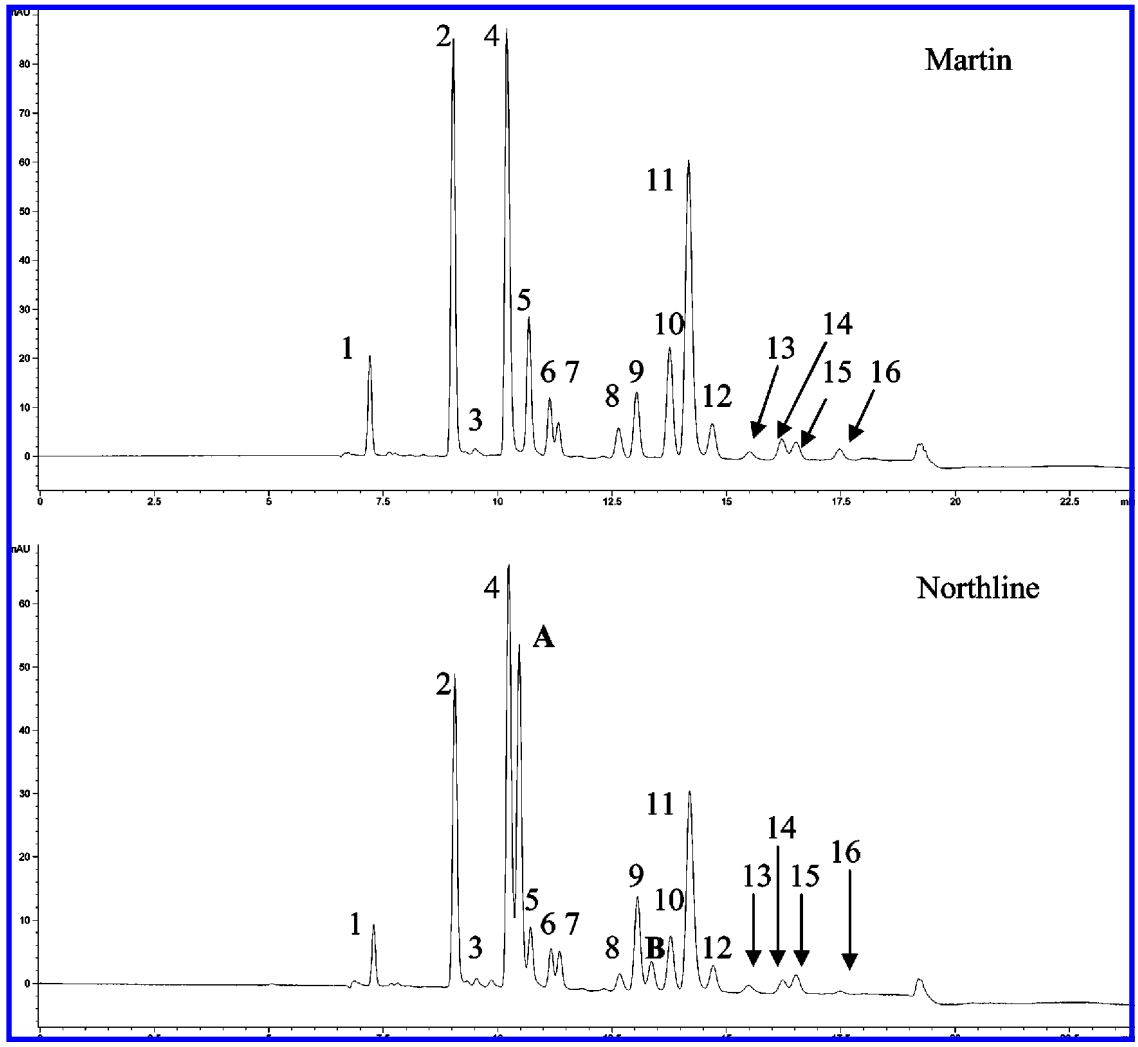

Figure 2. HPLC profile of Saskatoon berry (cv Martin and Northline) polyphenols (360 nm): (1) neochlorogenic acid, (2) chlorogenic acid, (3) cyanidin-3,5-diglucoside, (4) cyanidin-3-galactoside, (5) cyanidin-3-glucoside, (6) cyanidin-3-arabinoside, (7) unknown hydroxycinnamic acid derivative, (8) cyanidin-3-xyloside, (9) quercetin-3-vicianoside, (10) quercetin-3-robinobioside, (11) quercetin-3-galactoside, (12) quercetin-3-glucoside, (13) quercetin-3-pentoside, (14) quercetin-3-pentoside, (15) dicaffeoylquinic acid, (16) unknown flavonol, (A) unknown hydroxycinnamic acid derivative, and (B) unknown hydroxycinnamic acid derivative.

hydroxycinnamates were found in 11 Saskatoon cultivars. The identification of these acids was difficult because of coelution with cyanidin-3-galactoside and quercetin-3-vicianoside. The presence of an unknown hydroxycinnamate was reported before (*7*) in the Smokey cultivar. The concentration of hydroxycinnamic acid derivatives varied significantly among Saskatoon cultivars (**Table 3**). The Nelson cultivar contained the highest amount of chlorogenic acid (130 mg/100 g FW), while Pembina and Lee 2 were richest in neochlorogenic acid (62 and 55 mg/100 g FW, respectively). Success contained the lowest amount of chlorogenic acid (42 mg/100 g FW), whereas Northline had the lowest amount of neochlorogenic acid (5 mg/100 g FW). At the same time, Northline contained the highest concentration of unknown hydroxycinnamic acid (56 mg/100 g FW). Concentrations of neochlorogenic and unknown acids were similar to those presented previously (*7*), but the concentration of chlorogenic acid published by the authors was twice as low as that presented herein.

Flavonol Composition and Concentration in Saskatoon Berries. Six flavonols were identified in Saskatoon polyphenol extracts and quantified. All were quercetin derivatives, with different sugar substitutions. Peaks 11 and 12 (**Figure 2**) coeluted with the standards of quercetin-3-galactoside and quercetin-3-glucoside, respectively. The identification of quercetin-3-vicianoside and quercetin-3-robinobioside was done on the basis of MS/MS information and data published by Slimestad et al. (*14*). Peaks 13 and 14 had the same molecular weight and fragment mass (*m*/*z* 435, 303), indicating quercetin bonded with a pentose. Previously, quercetin-3-arabinoside and quercetin-3-xyloside were identified in Saskatoon berries (*7*).

The cultivars showing the largest content of flavonols were Nelson, Pasture, and Lee 3, whereas Parkhill was the cultivar showing the smallest flavonoid content. All the cultivars analyzed showed the same flavonol pattern. Quercetin-3-galactoside was the main flavonol in Saskatoon fruits. The concentration of quercetin-3-galactoside ranged from 13.5 mg/100 g FW for the Regent cultivar to 38.5 mg/100 g FW for the Pasture cultivar. Ozga et al. (*7*) reported lower amounts of flavonols in Smokey and Honeywood cultivars than those presented in this study; however, the HPLC pattern of flavonols was similar.

Procyanidins Content in Saskatoon Berries. The procyanidin concentration in Saskatoon berries was analyzed by the thiolysis method. Thiolytic degradation of Saskatoon procyanidins released catechin and epicatechin as the only free flavan-3-ols and epicatechin benzylthioether as the only flavan-3-ol adduct, suggesting that these procyanidins essentially consist

Table 2. Quantification of Individual Anthocyanins and Total Anthocyanin Content[a] in Saskatoon Berry Cultivars before and after Storage at −20°C

cultivar	storage time[b]	Cy-3,5-digl	Cy-3-gal	Cy-3-gl	Cy-3-ara	Cy-3-xyl[c]	total
Success	0	0.43c	141.09e	30.53d	16.47e	15.15d	203.67f
	9	0.98a	151.73e	37.33c	26.20c	14.78d	231.02fe
Lee 3	0	0.78ab	204.08c	59.92a	33.00bc	21.85c	319.63bc
	9	0.62b	187.58d	55.42a	35.32b	15.92d	294.86d
Martin	0	0.70b	238.36b	52.84ab	34.01bc	16.56d	342.47b
	9	0.50bc	205.63c	49.52b	37.51b	19.71c	312.87c
Parkhill	0	0.46bc	135.70e	33.29d	18.24de	12.17de	199.86f
	9	0.49bc	121.11f	28.72de	22.16d	12.96de	185.44f
Forestburg	0	0.86a	148.28e	53.53ab	33.93bc	29.17ab	265.77de
	9	0.76ab	139.49e	47.12b	32.66bc	15.92d	235.95ef
Lee 8	0	0.53bc	216.94c	24.19de	24.22cd	10.40e	276.28de
	9	0.58b	207.10c	26.73de	28.27c	13.62de	276.30de
Lee 2	0	0.36c	122.95f	40.04c	19.41d	12.02de	194.78fg
	9	0.64b	131.28ef	50.42b	34.61b	12.96 de	229.91e
Pembina	0	0.47bc	115.47f	46.23b	21.99d	15.30d	199.46f
	9	0.78b	103.31 g	40.40c	23.92cd	15.90d	184.31f
Honeywood	0	0.73b	147.86e	56.66a	31.31c	16.90d	253.46e
	9	0.83ab	143.39e	62.50a	45.19a	22.73bc	274.64de
Northline	0	0.59b	225.74bc	25.94de	25.88c	15.10d	293.25d
	9	0.33c	209.72c	28.31de	20.87d	11.00e	270.23de
Thiessen	0	0.53bc	180.72d	47.82b	26.70c	19.63c	275.40de
	9	0.40c	171.16d	41.02c	24.72cd	17.48d	254.78d
Pasture	0	0.27c	168.87d	55.54a	27.26c	21.92c	273.86de
	9	0.83a	172.45d	53.01ab	36.84b	21.10c	284.23d
Nelson	0	0.72b	247.85a	60.03a	41.47ab	32.02a	382.09a
	9	0.93a	251.57a	63.93a	46.67a	25.03b	388.13a
Pearson	0	0.51bc	114.94f	36.48a	24.21cd	13.87d	190.01fg
	9	0.76b	108.12fg	32.31ab	23.82cd	14.84d	179.85 g
Quaker	0	0.57b	140.56e	33.32d	18.99de	11.57de	205.01f
	9	0.59b	143.65e	34.34d	36.27b	15.37d	230.22ef
Smokey	0	0.51bc	111.39fg	40.41c	23.83cd	13.57d	189.71fg
	9	0.62b	97.06 g	32.71d	17.49e	10.00e	157.88 h
Regent	0	0.62b	193.06d	51.95ab	26.99c	18.14cd	290.76d
	9	0.98a	181.73d	47.33b	26.20c	14.78d	271.02de

[a] All data are expressed as milligrams per 100 g of fresh weight. [b] Months. [c] Content estimated as cyanidin-3-arabinoside equivalent. Means within columns are significantly different ($p < 0.05$).

Table 3. Quantification of Individual Hydroxycinnamic Acids and Total Hydroxycinnamic Content[a] in Saskatoon Berry Cultivars before and after Storage at −20°C

cultivar	storage time	neochlorogenic acid	chlorogenic acid	unknown	dicaffeoylquinic acid	total
Success	0	12.13e	42.19f	27.64bc	0.29fg	82.25d
	9	10.88e	44.75f	19.65c	0.32fg	75.60de
Lee 3	0	18.08d	103.63b	T	0.79e	122.50b
	9	8.32ef	70.54d	T	1.65bc	80.51d
Martin	0	12.82e	100.66b	ND	0.46f	113.94bc
	9	5.75fg	92.43bc	ND	0.41f	98.59c
Parkhill	0	11.12e	117.41ab	ND	0.55ef	129.08b
	9	6.31f	78.81cd	ND	0.53ef	85.65d
Forestburg	0	17.28d	103.82b	21.89c	0.66ef	143.65a
	9	12.13e	84.21c	12.86d	0.49f	109.69c
Lee 8	0	5.81fg	71.50d	54.69a	0.82e	132.82b
	9	4.97f	56.39ef	35.16b	0.79e	97.31c
Lee 2	0	54.99a	67.68de	ND	1.76bc	124.43b
	9	53.15a	68.16de	ND	1.92b	123.23b
Pembina	0	61.52a	75.14d	ND	1.82bc	138.48d
	9	37.25b	47.24f	ND	2.08b	86.57b
Honeywood	0	7.42f	86.81c	6.25e	1.21d	101.69c
	9	6.32f	76.59d	3.12f	1.57c	87.60d
Northline	0	4.90 g	67.70e	55.54a	1.19d	129.33b
	9	3.35 g	54.42ef	25.65bc	0.79e	84.21d
Thiessen	0	14.08de	83.83c	T	0.20 g	98.11c
	9	9.12ef	55.97ef	T	0.09 h	65.18e
Pasture	0	9.34ef	79.69c	ND	1.20d	90.23cd
	9	5.48fg	64.84e	ND	0.91de	71.23de
Nelson	0	37.51b	129.70a	ND	1.32d	168.53a
	9	31.62c	107.32b	ND	3.47a	142.41b
Pearson	0	12.51e	108.54b	25.72bc	0.87e	147.64ab
	9	7.04f	83.64c	13.01d	0.72e	104.41c
Quaker	0	19.96d	88.70c	T	1.43c	110.09c
	9	11.14e	70.46d	0.00	1.57c	83.17d
Smokey	0	11.46e	96.55bc	21.86c	1.22d	131.09b
	9	1.46 h	42.34f	11.20d	1.32d	56.32f
Regent	0	15.43d	72.00d	12.81d	0.95de	101.19c
	9	10.88e	44.75f	9.30de	0.95de	65.88e

[a] All data are expressed as milligrams of chlorogenic acid per 100 g of fresh weight. Means within columns are significantly different ($p < 0.05$).

of (−)-epicatechin units with (+)-catechin present only in a low proportion as a terminal unit. The Saskatoon berry procyanidin oligomers were characterized previously (*6*) revealing that Saskatoon berry procyanidins consisted mainly of epicatechin units linked by β-type bonds, which agrees with our results.

The concentration of procyanidins in Saskatoon berries varied from 128 mg/100 g FW for Northline to 295 mg/100 g FW for Thiessen (**Figure 3**). The procyanidins in the Success cultivar had highest degree of polymerization, followed by Regent, Pearson, and Lee 2. The lowest degree of polymerization was found in Pembina.

Antioxidant Activity of Saskatoon Berries. The antioxidant potential of the Saskatoon berries was determined on the basis of the scavenging activity of the stable free radicals DPPH and ABTS (**Table 1**). The Nelson cultivar which contained the highest level of polyphenols was characterized by the highest antioxidant activity, 5.0 and 2.8 mM/100 g FW for ABTS and DPPH, respectively. Smokey showed the lowest antioxidant potential among the cultivars tested, 3.4 and 1.9 mM/100 g FW for ABTS and DPPH, respectively. The wild Saskatoon berry showed similar antioxidant activity (4.0 mM/100 g FW with ABTS) as compared to cultivated Saskatoon fruits (*5*). The high antioxidant potential of Saskatoon berries has been reported previously (*12*), and the high antioxidant properties of the Saskatoon berry correlated with its high content of anthocyanins and chlorogenic acid. Recently, the antioxidant potential of Saskatoon berry cvs Thiessen and Smokey with DPPH and ABTS radicals was analyzed (*16*). Thiessen had a significantly higher antioxidant activity than Smokey, which is in agreement with our results. The antioxidant activity of Saskatoon berries was higher than the antioxidant potential of wild red raspberries, black raspberries, and wild strawberries (3.2, 3.3, and 2.0 mM/100 g FW with ABTS, respectively) but lower than the antioxidant activity of honeysuckle fruits (9.6 mM/100 g FW with ABTS) (*5*).

The concentration of anthocyanins and polyphenols was found to correlate with the antioxidant activity of the berries (*17*−*19*). In the present study, a good correlation between antioxidant activity and total anthocyanin and polyphenol contents was found ($r_{ABTS} = 0.93$, $r_{DPPH} = 0.83$ for total anthocyanins and $r_{ABTS} = 0.73$, $r_{DPPH} = 0.69$ for total polyphenols). According to Rice-Evans et al. (*20*), the antioxidant potential of polyphenols is influenced by their chemical structures. The hydroxylation in the B ring and glycosylation have a strong influence on the antioxidant potential of phenolic compounds. In order to check the individual antioxidant capacity of the main polyphenols found in Saskatoon berries, the HPLC analysis was run before and after the ABTS radical addition to Saskatoon polyphenol extracts. The results obtained showed that Saskatoon berry anthocyanins demonstrated the highest antioxidant potential among polyphenols. Among anthocyanins, pentose-containing molecules showed higher scavenging activity (95−96% reduction of peak area after 3 h reaction with 200 μL of ABTS) than hexose-containing molecules (92−93% reduction of peak area), which correlates with the results reported previously (*20*). The

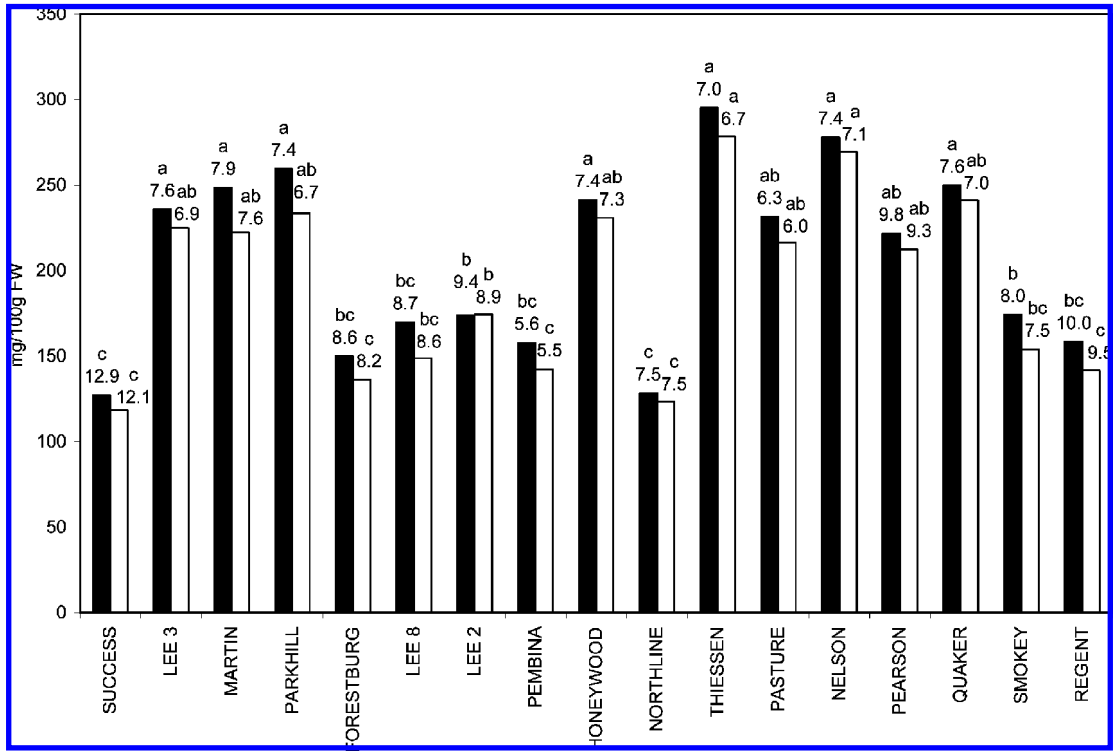

Figure 3. Procyanidin content of the 17 Saskatoon berry cultivars before and after 9 months of storage at −20 °C. Numbers over the bars represent average degree of polymerization. Bars with different letters are significantly different ($p < 0.05$).

lower antioxidant potential of chlorogenic acid (22% reduction of peak area) also agrees with the data presented therein. Interestingly, quercetin-3-galactoside showed low free radical scavenging activity (23% reduction of peak area). The antioxidant activity of quercetin-3-galactoside was expected to be close to the antioxidant activity showed by cyanidin-3-galactoside. The internal interactions between polyphenols and other extract components could reduce the antioxidant activity of quercetin-3-galactoside. Further investigation of interactions between polyphenols and other berry components and their influence on the biological activity of berries may be helpful in explanation of this phenomenon.

Storage Stability of Saskatoon Berry Polyphenols. Berries, including Saskatoon berries, are very perishable and are characterized by a very short shelf life. Fresh Saskatoon berries are available only for a few weeks in the year; therefore, their shelf life can be extended by freezing.

The obtained results with 17 Saskatoon berry cultivars studied show that the effect of freezing on anthocyanins varies among cultivars. In some Saskatoon cultivars such as Success, Lee 2, Honeywood, Pasture, Nelson, and Quaker, an increase in anthocyanin content was observed, but only in cv Lee 2 the increase was significant. In the other cultivars, a decrease in anthocyanin concentration was detected; in Lee 3, Martin, and Smokey, the changes were statistically significant (**Table 2**). The HPLC analysis of individual anthocyanins in stored fruits showed that the instability of cyanidin-3-galactoside was mainly responsible for the decrease in total anthocyanin concentration in some cultivars. On the other hand, the increase of anthocyanin content in Saskatoon berries after storage can be related to the increase in the amount of cyanidin-3-arabinoside (59% in Success, 38% in Quaker, and 35% in Pasture). Previously, a 2.5-fold increase in anthocyanins in raspberries after 8 days of storage at 20 °C was reported (*21*). At the same time, smaller changes in anthocyanin content were reported after storage at lower temperature (10 and 0 °C). A 23% increase in anthocyanins of raspberry cv Heritage and a 10% decrease in anthocyanins of cv Rubi after 9 months of storage at −20 °C were reported previously (*22*). Recently, anthocyanin contents of frozen blueberries were found stable over 3 months of storage at −20 °C (*23*).

The increase in anthocyanin content in some Saskatoon cultivars was not correlated with total phenolic content. The total polyphenol concentrations in Saskatoon fruits were not affected by storage at −20 °C. A slight decrease, less than 5%, in total phenolics was observed in all cultivars tested (**Table 1**). No significant decrease in total polyphenols in strawberry Dover and Campineiro cultivars stored at 6, 16, and 25 °C for 2−3 days was reported before (*24*). In another study with Spanish raspberries (*25*), it was demonstrated that the freezing process had little effect on total phenolic content during 12 months of frozen storage.

The hydroxycinnamic acid derivatives were less stable than flavonoids during the 9 months of freezer storage (**Table 3**). No significant changes of hydrocinnamate content after freezer storage were noticed in Lee 2, Pasture, Success, and Martin. The highest (65%) losses of chlorogenic acid were observed in Smokey. In Success, Martin, Northline, and Lee 2, no significant changes in chlorogenic acid were observed. The highest decrease in neochlorogenic acid content occurred in Smokey, lower in Pembina and Lee 3, while in Lee 2, Lee 8, and Nelson minimal changes in neochlorogenic acid were observed. The content of the unknown hydroxycinnamic acid derivative decreased in all Saskatoon cultivars after 9 months of storage at −20 °C.

After 9 months of storage at −20 °C, an increase in flavonol content was observed in Nelson, Lee 2, Honeywood, and Regent cultivars (**Table 4**). In Forestburg, Martin, Northline, and Lee 3, a significant decline in flavonol content occurred. In the other cultivars, no significant changes in flavonol concentration were observed. The quercetin glycosides showed differences in their stability with quercetin-3-galactoside being the most stable (except for Forestburg, Martin, and Lee 3 cultivars) and

Table 4. Quantification of Individual Flavonols and Total Flavonol Content[a] in Saskatoon Berry Cultivars before and after Storage at −20°C

cultivar	storage time	Q-3-vic[b]	Q-3-rob[b]	Q-3-gal	Q-3-gluc	Q-3-pent[c]	total
Success	0	7.03cd	7.82cd	29.26c	5.33c	5.84b	55.28b
	9	6.85cd	7.88cd	26.78cd	5.55c	5.90b	52.96bc
Lee 3	0	8.30c	13.78a	38.02a	5.47c	5.49bc	71.06a
	9	6.04d	11.27b	28.17c	4.02d	4.27cd	53.77bc
Martin	0	9.01b	10.90b	35.92ab	7.47a	4.32cd	67.62a
	9	7.26cd	9.30c	31.27c	4.32d	3.82de	55.97b
Parkhill	0	3.67f	2.01 h	14.22f	4.30d	6.02b	30.22ef
	9	3.85f	2.41gh	13.96f	3.93d	4.63cd	28.78f
Forestburg	0	5.24de	4.61f	27.71cd	5.32c	8.61a	51.49bc
	9	3.58f	2.87 g	18.78e	3.12e	4.43cd	32.78e
Lee 8	0	9.99ab	6.53de	26.05cd	3.68de	4.22cd	50.47bc
	9	9.03ab	6.47de	24.71d	3.50de	3.60de	47.31c
Lee 2	0	6.45d	5.91e	21.92de	4.16d	4.11d	42.55d
	9	8.07c	7.31d	24.97d	4.98cd	5.08c	50.41bc
Pembina	0	6.36d	6.23e	22.17de	4.35d	4.63cd	43.74d
	9	6.51d	6.22e	20.28e	4.19d	4.29d	41.49d
Honeywood	0	4.67e	7.50d	28.90c	3.64de	5.71bc	50.42bc
	9	4.83e	7.69d	30.56c	4.00d	6.33b	53.41bc
Northline	0	9.38b	5.93e	23.78d	3.71de	3.73e	46.53c
	9	8.63bc	5.30ef	21.39de	3.09d	3.12e	41.53d
Thiessen	0	9.28b	9.93bc	31.22c	5.07cd	3.92d	59.42b
	9	8.02c	8.91c	27.56cd	4.12d	3.58de	52.19bc
Pasture	0	7.92c	9.64c	38.51a	8.16a	6.90b	71.13a
	9	8.09c	9.97bc	38.99a	8.10a	6.09b	71.24a
Nelson	0	10.88a	9.86c	34.42ab	7.06b	8.51a	70.73a
	9	11.57a	10.09bc	36.29a	7.33b	8.44a	73.72a
Pearson	0	3.39f	3.08 g	19.21e	3.44de	5.32c	34.44ef
	9	3.00fg	2.79 g	17.15ef	2.82e	4.79c	30.55f
Quaker	0	6.76cd	12.12ab	29.03c	4.21d	3.97d	56.09b
	9	6.14d	11.13b	25.91cd	3.63de	3.91d	50.72bc
Smokey	0	3.79f	3.30fg	20.70e	3.55de	5.72bc	37.06de
	9	3.27f	3.00 g	18.50ef	2.77e	5.39c	32.93e
Regent	0	3.29f	3.76f	13.45f	5.13cd	6.17b	31.80e
	9	6.85cd	7.88cd	19.78e	5.55c	5.90b	45.96d

[a] All data are expressed as milligrams per 100 g of fresh weight. [b] Content estimated as quercetin-3-rutinoside equivalent. [c] Content estimated as quercetin-3-glucoside equivalent. Means within columns are significantly different ($p < 0.05$).

quercetin pentosides being the most sensitive during frozen storage. Previously, the good stability of quercetin-3-galactoside during apple storage was reported (*26*). Another group observed significant decreases in quercetin content (40%) in bilberries and lingonberries but not in black currants or red raspberries during 9 months of storage at −20 °C (*27*).

Saskatoon berry procyanidin contents were not greatly affected by storage (**Figure 3**). The highest decrease occurred in the Smokey cultivar (12%). Storage at −20 °C for 9 months had no effect on the degree of polymerization of procyanidins.

One possible explanation for the unexpected increases in polyphenol content in stored Saskatoon berries could be that phenolic compounds in frozen Saskatoon berries become more easily extractable during storage. This might be due to degradation of cell structures during storage. On the other hand, the losses of phenolic compounds in some Saskatoon berry cultivars can be related to increases of polyphenol oxidase (PPO) enzyme activity during storage in these fruits. In a previous study with four raspberry cultivars (*25*), the cultivar-dependent changes in PPO enzyme activity during freezer storage were presented. The investigation of PPO enzyme activity in Saskatoon berry cultivars and its changes during freezer storage should be helpful in explanation of the differences in polyphenol storage stability in Saskatoon berry cultivars.

Freezer storage showed no effect on the antioxidant potential of Saskatoon berries (**Table 1**). A significant decrease of antioxidant potential was observed only in the Smokey cultivar, in which a decline of procyanidin compounds occurred. The changes in polyphenol concentration after freezer storage did not influence the antioxidant activity of stored Saskatoon berries. Probably the storage changes of antioxidant compounds other than polyphenols (vitamins, carotenoids), not studied in this work, balanced the antioxidant activity of stored Saskatoon berries. The data published previously (*27*) showed decreases in antioxidant activity of strawberries after storage at 6, 16, and 25 °C for 2−6 days. For apples stored both at refrigerator temperature and under controlled atmosphere conditions, no changes in antioxidant activity were detected (*28*). Also, the antioxidant activity of raspberries was not affected during 1 year storage at − 20 °C (*22*).

In conclusion, Saskatoon berries are a rich source of polyphenols, mostly anthocyanins and flavonols. The polyphenol concentrations varied considerably within Saskatoon berry cultivars, with Nelson being the richest one. The high polyphenol content correlated with the high antioxidant potential of Saskatoon berries. Nelson, followed by Honeywood, Lee 3, and Martin cultivars demonstrated the highest antioxidant potential. The storage stability of polyphenols in Saskatoon berries was cultivar-dependent. Smokey showed to be the most sensitive Saskatoon berry cultivar during cold storage, while Nelson and Lee 2 were more stable. The high polyphenol content and antioxidant activity of the Nelson cultivar and its good storage stability would make this cultivar the optimal material for fruit growers and food producers. The excellent stability of bioactive compounds of Saskatoon berries during freezer storage allows prolonging the storage time of these berries. Moreover, the longer time of berry storage would enable the processing of Saskatoon berries during the winter.

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LITERATURE CITED

(1) Olson, A. R.; Steeves, T. A. Structural changes in the developing fruit wall of *Amelanchier alnifolia*. *Can. J. Bot.* **1982**, *69*, 1880–1887.

(2) Mazza, G. Anthocyanins and other phenolic compounds of Saskatoon berries (*Amelanchier alnifolia* Nutt.). *J. Food Sci.* **1986**, *51*, 1260–1264.

(3) Mazza, G. Compositional and functional properties of Saskatoon berry and blueberry. *Int. J. Fruit Sci.* **2006**, *5*, 101–120.

(4) Han, X.; Shen, T.; Lou, H. Dietary Polyphenols and Their Biological Significance. *Int. J. Mol. Sci.* **2007**, *8*, 950–988.

(5) Bakowska-Barczak, A. M.; Marianchuk, M.; Kolodziejczyk, P. Survey of bioactive components in western Canadian berries. *Can. J. Physiol. Pharmacol.* **2007**, *85*, 1139–1152.

(6) Hellström, J.; Sinkkonen, J.; Karonen, M.; Mattila, P. Isolation and Structure Elucidation of Procyanidin Oligomers from Saskatoon Berries (*Amelanchier alnifolia*). *J. Agric. Food Chem.* **2007**, *55*, 157–164.

(7) Ozga, J. A.; Saeed, A.; Wismer, W.; Reinecke, D. M. Characterization of Cyanidin- and Quercetin-Derived Flavonoids and Other Phenolics in Mature Saskatoon Fruits (*Amelanchier alnifolia* Nutt.). *J. Agric. Food Chem.* **2007**, *55*, 10414–10424.

(8) Singleton, V. L.; Orthofer, R.; Lamuela-Raventos, R., M. Analysis of total polyphenols and other oxidation substrates and antioxidants

by means of Folin-Ciocalteu reagent. *Methods Enzymol.* **1999**, *299*, 152–178.

(9) Re, R.; Pellegrini, N.; Proteggente, A.; Pannala, A.; Yang, M.; Rice-Evans, C. Antioxidant activity applying an improved ABTS radical cation decolorization assay. *Free Radical Biol. Med.* **1999**, *26*, 1231–1237.

(10) Yen, G.; Chen, H. Antioxidant Activity of Various Tea Extracts in Relation to Their Antimutagenicity. *J. Agric. Food Chem.* **1995**, *43*, 27–32.

(11) Guyot, S.; Marnet, N.; Sanoner, P.; Drilleau, J. F. Direct thiolysis on crude apple materials for high-performance liquid chromatography characterization and quantification of polyphenols in cider apple tissues and juices. *Methods Enzymol.* **2001**, *335*, 57–70.

(12) Fukumoto, L. R.; Mazza, G. Assessing Antioxidant and Prooxidant Activities of Phenolic Compounds. *J. Agric. Food Chem.* **2000**, *48*, 3597–3604.

(13) Oszmianski, J.; Sapis, J. C. Anthocyanins in fruits of *Aronia melanocarpa* (chokeberry). *J. Food Sci.* **1988**, *53*, 1241–1242.

(14) Slimestad, R.; Torskangerpoll, K.; Nateland, H. S.; Johannessen, T.; Giske, N. H. Flavonoids from black chokeberries *Aronia melanocarpa*. *J. Food Compos. Anal.* **2005**, *18*, 61–68.

(15) Zatylny, A. M.; Ziehl, W. D.; St-Piere, R. G. Physicochemical properties of fruit of 16 Saskatoon (*Amelanchier alnifolia* Nutt.) cultivars. *Can. J.Plant Sci.* **2005**, *85*, 933–938.

(16) Hu, C.; Kwok, B. H. L.; Kitts, D. D. Saskatoon berries (*Amelanchier alnifolia* Nutt.) scavenge free radicals and inhibit intracellular oxidation. *Food Res. Int.* **2005**, *38*, 1079–1085.

(17) Wu, X.; Gu, L.; Prior, R. L.; McKay, S. Characterization of anthocyanins and proanthocyanins in some cultivars of *Ribes*, *Aronia*, and *Sambucus* and their antioxidant capacity. *J. Agric. Food Chem.* **2004**, *52*, 7846–7856.

(18) Kähkönen, M. P.; Hopia, A. I.; Kujala, T. S.; Heinonen, M. Antioxidant Activity of Plant Extracts Containing Phenolic Compounds. *J. Agric. Food Chem.* **1999**, *47*, 3954–3962.

(19) Pantelidis, G. E.; Vasilakakis, M.; Manganaris, G. A.; Diamantidis, G. Antioxidant capacity, phenol, anthocyanin and ascorbic acid contents in raspberries, blackberries, red currant, goosberries and Cornelian cherries. *Food Chem.* **2007**, *102*, 777–783.

(20) Rice-Evans, C. A.; Miller, N. J.; Paganga, G. Structure-antioxidant activity relationships of flavonoids and phenolic acids. *Free Radical Biol. Med.* **1996**, *20*, 933–965.

(21) Kalt, W.; Forney, C. F.; Martin, A.; Prior, R. L. Antioxidant Capacity, Vitamin C, Phenolics, and Anthocyanins after Fresh Storage of Small Fruits. *J. Agric. Food Chem.* **1999**, *47*, 4638–4644.

(22) Ancos, B.; Ibanez, E.; Reglero, G.; Cano, M. P. Frozen Storage Effects on Anthocyanins and Volatile Compounds of Raspberry Fruit. *J. Agric. Food Chem.* **2000**, *48*, 873–879.

(23) Lohachoompol, V.; Srzednicki, G.; Craske, J. The changes of total anthocyanins in blueberries and their antioxidant effect after drying and freezing. *J. Biomed. Biotechnol.* **2004**, *5*, 248–252.

(24) Cordenunsi, B. R.; Genovese, M. I.; Nascimento, J. R. O.; Hassimoto, N. M. A.; Santos, R. J.; Lajolo, F. M. Effects of temperature on the chemical composition and antioxidant activity of three strawberry cultivars. *Food Chem.* **2005**, *91*, 113–121.

(25) Ancos, B.; Gonzales, E. M.; Cano, M. P. Ellagic Acid, Vitamin C, and Total Phenolic Contents and Radical Scavenging Capacity Affected by Freezing and Frozen Storage in Raspberry Fruit. *J. Agric. Food Chem.* **2000**, *48*, 4565–4570.

(26) van der Sluis, A. A.; Dekker, M.; van Boekel, M. A. J. S. Activity and Concentration of Polyphenolic Antioxidants in Apple Juice. 2. Stability during Storage. *J. Agric. Food Chem.* **2005**, *53*, 1073–1080.

(27) Hakkinen, S. H.; Karenlampi, S. O.; Mykkanen, H. M.; Torronen, A. R. Influence of Domestic Processing and Storage on Flavonol Contents in Berries. *J. Agric. Food Chem.* **2000**, *48*, 2960–2965.

(28) van der Sluis, A. A.; Dekker, M.; de Jager, A.; Jongen, W. M. F. Activity and Concentration of Polyphenolic Antioxidants in Apple: 3. Effect of Cultivar, Harvest Year, And Storage Conditions. *J. Agric. Food Chem.* **2001**, *49*, 3606–3613.

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