JOURNAL OF AGRICULTURAL AND FOOD CHEMISTRY

Characterization of Cyanidin- and Quercetin-Derived Flavonoids and Other Phenolics in Mature Saskatoon Fruits (*Amelanchier alnifolia* Nutt.)

JOCELYN A. OZGA,* ASMA SAEED,† WENDY WISMER, AND DENNIS M. REINECKE

Plant Biosystems Group, Department of Agricultural, Food and Nutritional Science, 4-10 Agriculture/Forestry Centre, University of Alberta, Edmonton, Alberta, Canada T6G 2P5

In order to further characterize the anthocyanins, flavonols, and other phenolics present in mature saskatoon (*Amelanchier alnifolia* Nutt.) fruit, extracts were characterized using high-performance liquid chromatography, gas chromatography, and liquid chromatography–mass spectrometry. Cyanidin 3-*O*-galactoside, cyanidin 3-*O*-glucoside, cyanidin 3-*O*-arabinoside, and cyanidin 3-*O*-xyloside were identified as the four major anthocyanins in the mature fruit. The quercetin-derived flavonols, quercetin 3-*O*-glucoside, quercetin 3-*O*-galactoside, quercetin 3-*O*-arabinoside, quercetin 3-*O*-xyloside, quercetin 3-*O*-arabinoglucoside, quercetin 3-*O*-robinobioside, and quercetin 3-*O*-rutinoside were also identified in mature fruit extracts. In addition, two chlorogenic acid isomers (hydroxycinnamates), 3-*O*-caffeoylquinic acid and 5-*O*-caffeoylquinic acid were detected. The total content of the anthocyanin-, flavonol-, and hydroxycinnamate-type phenolics detected in mature 'Smoky' saskatoon fruit was 140, 25, and 96 mg/100 g fresh weight, respectively. These data further our knowledge of the phenolic composition of mature saskatoon fruit, and as anthocyanins, flavonols, and hydroxycinnamates exhibit antioxidant activities, the presence and levels of these classes of phenolics will aid in the understanding of the potential health-beneficial effects of saskatoon fruits in the human diet.

KEYWORDS: *Amelanchier alnifolia*; anthocyanins; caffeoylquinic acids; chlorogenic acids; cyanidin; flavonoids; flavonols; phenolics; quercetin; saskatoon fruit

INTRODUCTION

The saskatoon (*Amelanchier alnifolia* Nutt.) is an emerging North American fruit crop (*1*) that is well adapted to very cold winter temperatures and mildly alkaline soils (*2*). Its small and fleshy fruits, somewhat similar in size and color to a blueberry (*Vaccinium angustifolium* Ait.), are botanically a rosaceous pome (*3–5*). Most of the fruit production occurs in the prairie provinces of Canada, with Alberta as the current leader at approximately 1000000 kg per year (personal communication). Demand, both domestic and international, has been expanding, and production has kept pace with a 40% increase in planted acreage in the last 6 years (personal communication). Market demand is high for both fresh fruits and processed fruit products such as syrups, jams, jellies, and bakery goods. The need for quality fruit (both for processing and fresh consumption) has increased with increased consumer demand. Saskatoon fruits contain anthocyanins, which are chiefly responsible for the red, blue, and purple pigments located throughout the skin and the flesh of the fruit (*6–8*). The amount, type, and stability of the anthocyanins in saskatoon fruit are important for maintaining color in the fresh fruit and processed fruit products, an important quality parameter (*9*). Recently, anthocyanins, along with flavonols, and other phenolics have attracted much interest due to their antioxidant properties and perceived health benefits, including anticancer, anti-inflammatory, and vasoprotective effects (*10–14*). Previous studies have identified cyanidin 3-*O*-galactoside and cyanidin 3-*O*-glucoside in saskatoon fruits (**Figure 1**) by HPLC and spectrophotometry analysis (*6*), as well as the retention time and presence of the molecular ion in an HPLC-ESI/MS total ion chromatogram (*13*). Other studies have reported total anthocyanin content as determined by the pH differential method of Fuleki and Francis (*15*) and expressed as cyanidin 3-*O*-galactoside equivalents (*7*, *16*). Identification of the flavonoid, quercetin rutinoside (rutin), and the hydroxycinnamate, chlorogenic acid (5-*O*-caffeoylquinic acid), was also reported in saskatoon fruit as determined by cochromatography with a standard on HPLC and UV/vis absorption spectra (*6*). Further knowledge of the phenolic profile of saskatoon fruit would be a useful tool for food technologists and horticulturists for assessing the quality of raw and processed fruit, for establishing the nutraceutical potential of the fruit, and for beginning to understand the flavonoid biosynthesis pathways present in this fruit. The present study, therefore, aimed to

* Corresponding author. Phone: 780-492-2653. Fax: 780-492-4265. E-mail: jocelyn.ozga@ualberta.ca.

† Current address: Water and Geo-technology Section, Institute for Technical Chemistry, Forschungszentrum Karlsruhe, P.O. Box 3640, Karlsruhe, 76021 Germany.

10.1021/jf072949b CCC: $37.00
Published on Web 11/10/2007

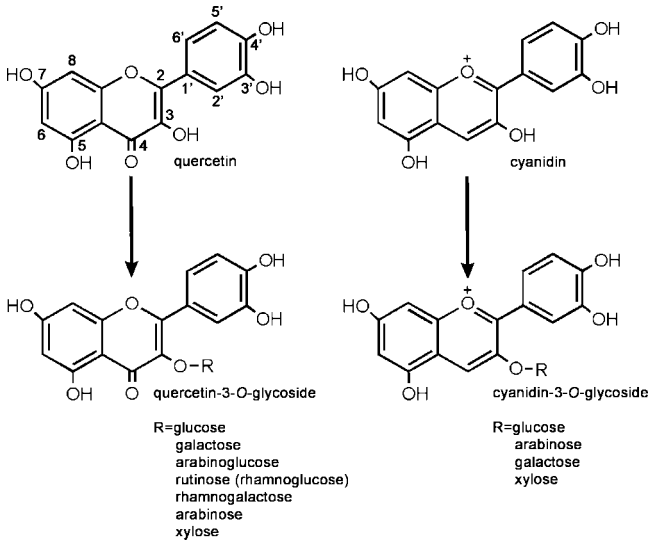

Figure 1. Cyanidin and quercetin glycosides in mature saskatoon fruit.

further characterize the phenolic profile of mature saskatoon fruit, focusing on anthocyanins and flavonols.

MATERIALS AND METHODS

Experimental Material. Saskatoon fruits (*A. alnifolia* Nutt.) from cultivars Smoky and Honeywood were collected from the University of Alberta Experimental Farm, Edmonton, Alberta, Canada, on July 22 ('Honeywood') and July 26 ('Smoky'), 2001. Fruits were harvested onto dry ice in the field, sorted for maturity on dry ice in the laboratory, and immediately stored at −80 °C.

Extraction and Purification Procedures. Mature (stage 9) (*8*) saskatoon fruits frozen in liquid nitrogen were ground in a small food processor and 4 g subsamples of the frozen ground tissue were extracted with 8 mL of an HPLC-grade solvent mixture, acetone−methanol−water−formic acid (40:40:20:0.1 v/v/v/v), and 200 mg of Celite 545 (J. T. Baker Inc., Phillipsburg, NJ). The extract was vortexed for 2 min and then filtered through Whatman No. 4 filter paper in a Buchner funnel under partial vacuum. The residue was washed with 12 mL of extraction solvent, and the extraction and wash solvent were pooled and evaporated to dryness (at <7 °C) under vacuum (0.9 kPa) using a SpeedVac concentrator (Savant, Farmingdale, NY) without heating.

The residue (615 ± 38 mg) was solubilized in 10 mL of deionized water, and a 1 mL aliquot of the solubilized extract was loaded onto a Sep-Pak C_{18} cartridge (Waters Scientific, Mississauga, Ontario, Canada) which had been preconditioned with 2 mL of 100% methanol followed by 5 mL of deionized water. The column was then washed with 5 mL of deionized water to remove sugars and organic acids. Anthocyanins and other flavonoids were eluted from the column with 10 mL of 0.1% formic acid in methanol (v/v), and this eluant was evaporated under vacuum to dryness using a SpeedVac concentrator as described above. The dried methanolic extract from the Sep-Pak C_{18} cartridge was redissolved in 100 μL (for LC-MS) or 200 μL (for C18-HPLC) of 50% aqueous methanol (v/v), and a 20 μL aliquot was chromatographed on C18-HPLC or a 5 μL aliquot was analyzed by liquid chromatography–mass spectrometry (LC-MS).

HPLC Analysis. The extracts were chromatographed on a 250 × 4.6 mm i.d. 5 μm Zorbax SB-C18 column (Agilent) fitted with a 12.5 × 4.6 mm i.d. 5 μm Zorbax SB-C18 guard column (Agilent) using a Waters (Milford, MA) 2690 separation module connected to a Shimadzu (Kyoto, Japan) SPD-M10AVP photodiode array (PDA) detector. The samples were eluted at a flow rate of 1 mL/min using a linear gradient of 5% aqueous formic acid (solvent A) and 100% methanol (solvent B) as follows: 83% A at 0 min; 77% A by 25 min; isocratic at 77% A from 25 to 45 min; 53% A by 55 min; isocratic at 53% A from 55 to 66 min; 83% A by 67 min; and isocratic at 83% A from 67 to 70 min.

For anthocyanin aglycon identification by C18-HPLC, the same column as described above was used with a Varian (Walnut Creek, CA) VISTA 5000 liquid chromatograph equipped with a Waters 486 UV/vis variable wavelength detector (VWD). The samples were eluted at a flow rate of 1 mL/min using a linear gradient of 5% aqueous formic acid (solvent A) and 100% methanol (solvent B) as follows: 86% A at 0 min; 77% A by 25 min; isocratic at 77% A from 25 to 45 min; 53% A by 55 min; isocratic at 53% A from 55 to 58 min; 86% A by 59 min; and isocratic at 86% A from 59 to 64 min. The anthocyanin peaks were detected at 520 nm.

Quantitation of the hydroxycinnamates (at 325 nm), flavonols (at 350 nm), and anthocyanins (at 520 nm) was accomplished by comparison of the HPLC-PDA peak areas in the samples with that in a standard curve generated by injecting increasing amounts of standard onto the C18-HPLC column. The levels of the hydroxycinnamates are given in 5-*O*-caffeoylquinic acid equivalents, flavonols in quercetin 3-*O*-galactoside equivalents, and anthocyanins in cyanidin 3-*O*-glucoside equivalents.

LC-MS. Identification of the phenolics was further confirmed by LC-MS. A 5 μL aliquot of stage 9 'Smoky' fruit processed through the Sep-Pak C_{18} cartridge step was injected onto a 50 × 2.00 mm i.d. 2.5 μm Phenomenex Luna C18 column connected to an Agilent 1100 LC-MS system fitted with an electrospray ionization (ESI) interface. The system consisted of a binary HPLC pump equipped with a variable wavelength detector set at 280 nm (from 0 to 70 min), or at 280 nm (from 0 to 35 min) and 350 nm (from 35 to 70 min), and an Agilent 1946 MSD single quadruple mass spectrometer. For gradient 1, the extracts were eluted at 250 μL/min using the same linear gradient profile of solvent A (5% formic acid in water) and solvent B (100% methanol) as described for the C18-HPLC−PDA analysis above. For gradient 2, the extracts were eluted at 250 μL/min using a linear gradient consisting of solvent A (0.5% acetic acid in water) and solvent B (1% acetic acid in 100% methanol) as follows: 95% A at 0 min; 90% A by 10 min; 5% A by 20 min; isocratic to 35 min. The variable wavelength detector was set at 280 nm for samples eluted using gradient 2. The MS data were acquired in the positive ionization mode with a scan range of 120−1200 amu with the fragmentor voltage ramped linearly from 60 V at the low mass to 200 V at the high mass. For ESI operation, nitrogen was used for nebulization and drying, the capillary voltage was 4 kV, and the spray chamber temperature was 350 °C.

Anthocyanin Sugar Conjugate Analysis. For identification and quantification of the sugars conjugated to the anthocyanidins, fruit extracts were acid hydrolyzed, and the freed sugars were identified as their alditol acetates following the modified procedures of Blakeney et al. (*17*), Kraus et al. (*18*), and Lein et al. (*19*). In order to determine the normal complement of soluble sugars in the saskatoon extract (zero time control) as well as the optimal duration for acid hydrolysis of the extract fractions, the saskatoon fruit flavonoid extract (processed through the Sep-Pak C_{18} step) was subjected to acid hydrolysis at 110 °C for 0, 15, 30, 45, 60, 90, 120, 150, and 180 min. To specifically identify the sugars conjugated to the anthocyanidins in peaks 1A, 1B, 1C, and 1D (**Figure 2**), HPLC fractions eluting at the retention times of these peaks were collected individually for acid hydrolysis and analysis. Fruit extracts or anthocyanin HPLC fraction samples (50 μL per reaction) were treated with 250 μL of 2.4 N HCl, and the hydrolysis was allowed to proceed at 110 °C (30 min optimal timing was used for HPLC fractions). The acid-hydrolyzed samples were cooled in an ice bath, and 75 μL of concentrated ammonium hydroxide was added. Subsequently, a 1 mL sodium borohydride solution (30 mg of sodium borohydride in 1 mL of anhydrous dimethyl sulfoxide) was added to the hydrolysate, and reduction was allowed to proceed for 90 min at 40 °C in a water bath. The excess sodium borohydride was decomposed by the addition of 300 μL of glacial acetic acid. This was followed by the addition of 200 μL of 1-methylimidazole and 2 mL of acetic anhydride. The mixture was mixed well, and acetylation was allowed to proceed for 10 min at room temperature. The excess acetic anhydride was decomposed by adding 5 mL of water. Alditol acetates were partitioned into 4 mL of dichloromethane by vigorous shaking. The mixture was centrifuged at 1400 rpm for 5 min, the upper water layer was removed, and the lower layer of dicholoromethane containing alditol acetates was washed twice with 5 mL of water followed by centrifugation and then evaporated to dryness under a stream of nitrogen.

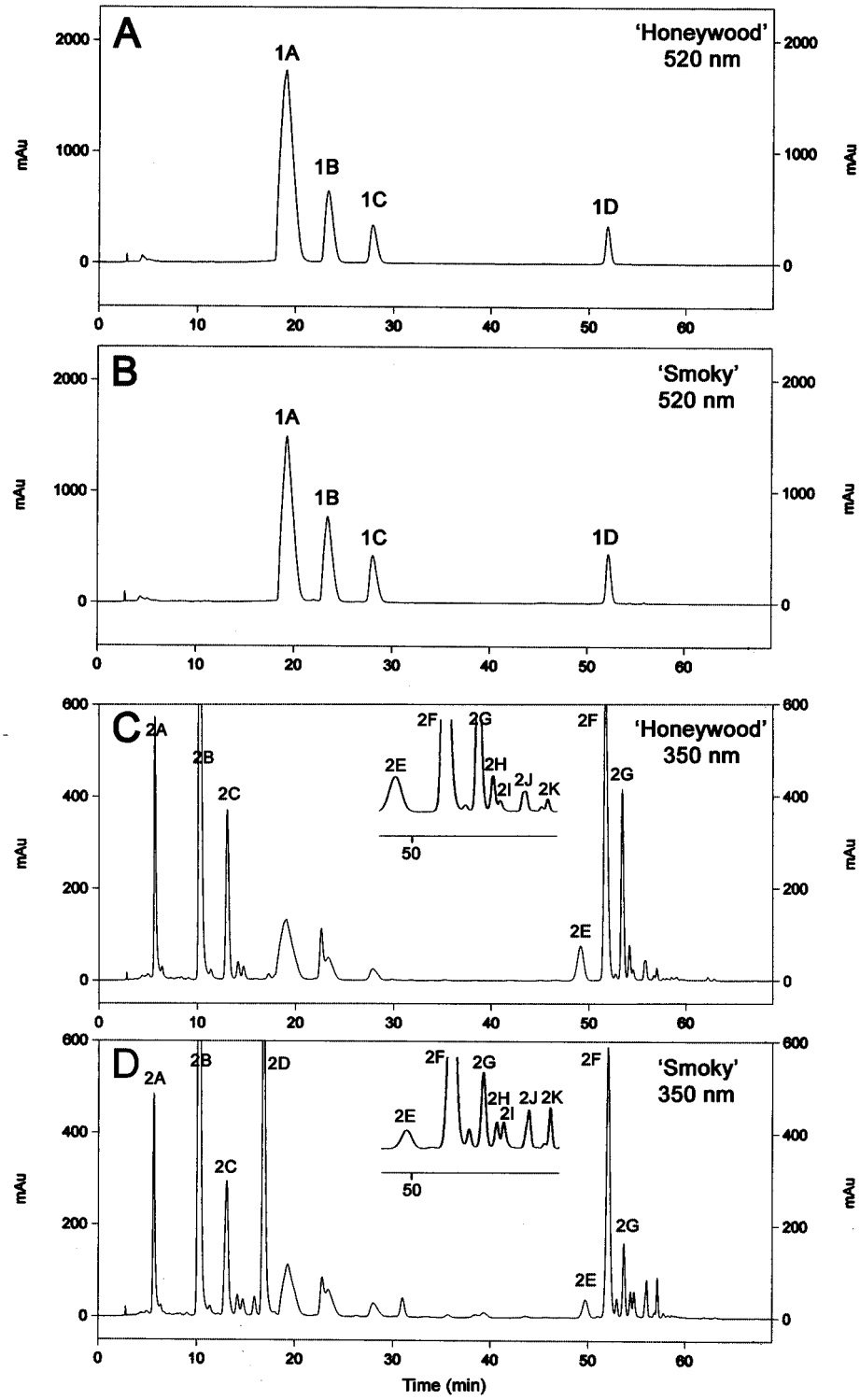

Figure 2. C18-HPLC profile of anthocyanin (520 nm), flavonol (350 nm), and other phenolic (350 nm) compounds present in mature saskatoon fruit of 'Honeywood' (**A** and **C**) and 'Smoky' (**B** and **D**). Peaks: 1A (cyanidin 3-*O*-galactoside), 1B (cyanidin 3-*O*-glucoside), 1C (cyanidin 3-*O*-arabinoside), 1D (cyanidin 3-*O*-xyloside), 2A (3-*O*-caffeoylquinic acid), 2B (5-*O*-caffeoylquinic acid), 2C (unknown), 2D (unknown hydroxycinnamate), 2E (quercetin 3-*O*-arabinoglucoside), 2F (quercetin 3-*O*-galactoside), 2G (quercetin 3-*O*-glucoside), 2H (quercetin 3-*O*-robinobioside), 2I (quercetin 3-*O*-rutinoside), 2J (quercetin 3-*O*-arabinoside), and 2K (quercetin 3-*O*-xyloside).

The alditol acetate samples were redissolved in 1 mL of dichloromethane, and approximately 0.5 μL was injected onto a 30 m $\times$ 0.25 mm i.d. DB-17 capillary column (J & W Scientific, Folson, CA) connected to a Varian 3400 gas chromatograph equipped with a flame ionization detector for identification and quantitation of sugars. Helium was used as the carrier gas at a rate of 1.5 mL/min. The injector temperature was programmed from 60 to 270 °C at 150 °C/min and then maintained for 20 min. The oven temperature was increased from 50 to 190 °C at 30 °C/min, maintained for 3 min, then increased to 270 °C at 5 °C/min, and then maintained for 5 min. The detector temperature was set at 270 °C. The amount of sugar released over time by acid hydrolysis was determined using *myo*-inositol as an external standard.

Table 1. Retention Times (t_R) and UV/Vis Maxima of Standards and Saskatoon Fruit Hydroxycinnamate-, Anthocyanin-, and Flavonol-Type Phenolics Separated on C18-HPLC

peak[a]	compound	sample[b]	t_R (min)	λ_{max} (nm)
2A	3-*O*-caffeoylquinic acid	'Smoky' extract	5.6	247, 300 sh, 324
		'Honeywood' extract	5.7	247, 300 sh, 324
2B	5-*O*-caffeoylquinic acid	standard	10.2	246, 300 sh, 325
		'Smoky' extract	10.2	249, 300 sh, 325
		'Honeywood' extract	10.2	248, 300 sh, 325
2C	unknown	'Smoky' extract	13.1	246, 288, 333
		'Honeywood' extract	13.1	246, 286, 337
2D	unknown hydroxycinnamate	'Smoky' extract	16.8	247, 300 sh, 327
		'Honeywood' extract	nd	nd
1A	cyanidin 3-*O*-galactoside	standard	19.2	245, 279, 516
		'Smoky' extract	19.3	245, 279, 516
		'Honeywood' extract	19.1	245, 279, 516
1B	cyanidin 3-*O*-glucoside	standard	23.0	245, 279, 516
		'Smoky' extract	23.5	245, 279, 516
		'Honeywood' extract	23.4	245, 279, 516
1C	cyanidin 3-*O*-arabinoside	'Smoky' extract	28.0	245, 279, 517
		'Honeywood' extract	27.9	245, 279, 517
2E	quercetin 3-*O*-arabinoglucoside	standard	49.2	246, 354
		'Smoky' extract	49.7	245, 354
		'Honeywood' extract	49.2	245, 354
2F	quercetin 3-*O*-galactoside	standard	51.8	245, 354
		'Smoky' extract	52.0[c]	246, 280 sh, 352, 519
		'Honeywood' extract	51.7[c]	245, 280 sh, 355, 519
1D	cyanidin 3-*O*-xyloside	'Smoky' extract	52.2[c]	246, 280 sh, 355, 519
		'Honeywood' extract	51.9[c]	245, 280 sh, 356, 519
2G	quercetin 3-*O*-glucoside	standard	53.7	245, 354
		'Smoky' extract	53.7	245, 355
		'Honeywood' extract	53.4	246, 355
2H	quercetin 3-*O*-robinobioside	'Smoky' extract	54.4	245, 353
		'Honeywood' extract	54.2	245, 354
2I	quercetin 3-*O*-rutinoside	standard	54.3	256, 355
		'Smoky' extract	54.7	246, 354
		'Honeywood' extract	54.5	245, 354
2J	quercetin 3-*O*-arabinoside	'Smoky' extract	56.0	246, 354
		'Honeywood' extract	55.9	246, 355
2K	quercetin 3-*O*-xyloside	'Smoky' extract	57.1	247, 350
		'Honeywood' extract	57.0	246, 353
	cyanidin[d]	standard	57.2	246, 275, 528
	quercetin[d]	standard	63.8	255, 370

[a] Peak number as designated in **Figure 2**. [b] Extracts of stage 9 'Smoky' and 'Honeywood' fruit or standards. [c] Cochromatographed in the C18-HPLC−PDA system and gave overlapping spectra. [d] Not detected in stage 9 fruit extracts; nd = not detected.

The following commercial standards of anthocyanins, other flavonoids, and phenolics were used for comparison of retentions times, UV/vis absorption spectra, and MS fragment ions for HPLC and LC-MS analyses: cyanidin chloride, delphinidin chloride, pelargonidin chloride, peonidin chloride, malvidin chloride, quercetin, myricetin, cyanidin 3-*O*-galactoside, cyanidin 3-*O*-glucoside, quercetin 3-*O*-galactoside, quercetin 3-*O*-glucoside, quercetin 3-*O*-arabinoglucoside, and quercetin 3-*O*-rutinoside (Extrasynthese SA, Genay Cedex, France); chlorogenic acid (5-*O*-caffeoylquinic acid) (Sigma-Aldrich, St. Louis, MO). The sugar standards, glucose, mannose, *myo*-inositol, and galactose were purchased from Sigma-Aldrich; arabinose, xylose, and rhamnose were purchased from Fisher Scientific (Pittsburgh, PA); and the sugar derivatization chemicals were purchased from Sigma-Aldrich.

RESULTS AND DISCUSSION

The anthocyanin profile for any given plant species and tissue is distinctive (*20*). Separation of anthocyanins in food and plant extracts by HPLC has been monitored by several authors at the wavelength of 520 nm, which has been reported to be close to the maximum absorbance wavelength (λ_{max}) for many anthocyanins (*21*). The HPLC profile of the flavonoid fraction of the saskatoon fruit cvs. Smoky and Honeywood showed four major peaks when monitored at 520 nm (**Figure 2A**,**B**). Peaks 1A and 1B and the anthocyanin standards cyanidin 3-*O*-galactoside and cyanidin 3-*O*-glucoside, respectively, cochromatographed on C18-HPLC and exhibited identical spectroscopic properties (**Table 1**).

When HPLC fractions eluting at the retention time of peaks 1A, 1B, 1C, and 1D (**Figure 2**) were acid hydrolyzed and rechromatographed on C18-HPLC under the same solvent and gradient conditions, only one significant peak (greater than 91% of total peak area) was detected at the wavelength of 520 nm in each fraction, which chromatographed at the same retention time as the aglycon cyanidin standard (peak 2 at 58.9−59.2 min) (**Figure 3A**). An acid-hydrolyzed extract of stage 9 'Smoky' fruit processed through the Sep-Pak C_{18} cartridge step was also chromatographed on the HPLC and monitored at 520 nm. Again, the only significant peak detected (91% of total peak area) was at the same retention time as the aglycon cyanidin standard (**Figure 3A**). Several very small peaks were detected between the retention times of 50 and 70 min, where anthocyanin aglycons elute in this HPLC system, specifically, peak 1 at 53.0 min, peak 3 at 60.7 min, and peak 4 at 67.5 min in the acid-hydrolyzed extract and peak 4 at 67.6 min in fraction 1D (**Figure 3A**). However, these small peaks did not coelute at the retention times of anthocyanin aglycons, delphinidin (54.2 min), pelargonidin (61.8 min), peonidin (62.7 min), or malvidin (64.4 min). When the acid-hydrolyzed extract of stage 9 'Smoky' fruit was chromatographed on the C18-HPLC-PDA

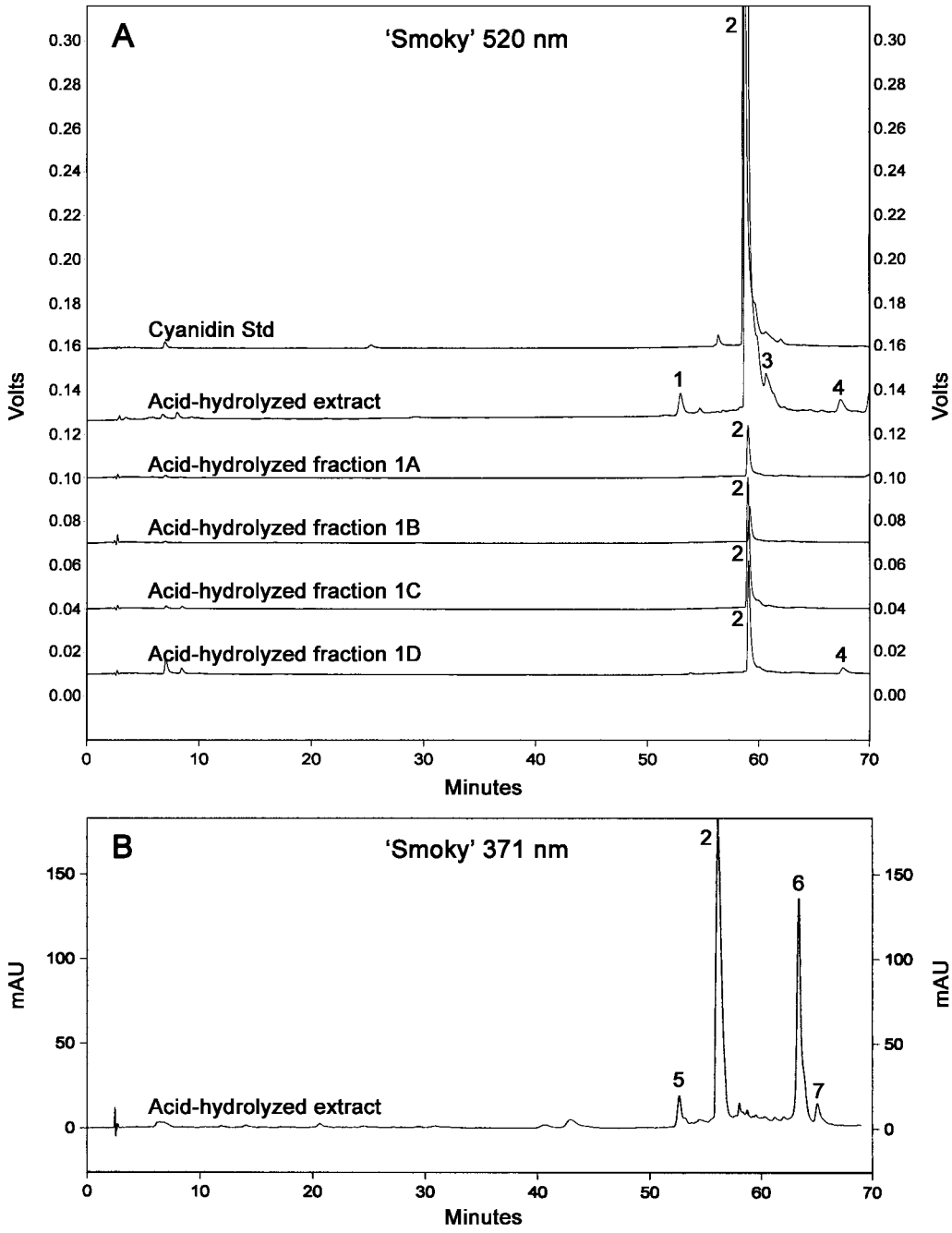

Figure 3. Anthocyanin aglycon identification by C18-HPLC after acid hydrolysis of mature saskatoon fruit extracts. (**A**) Acid-hydrolyzed samples of C18-HPLC fractions eluting at the retention time of peak 1A, peak 1B, peak 1C, and peak 1D in **Figure 2** or an extract of stage 9 'Smoky' fruit (acid hydrolyzed after processing through the Sep-Pak C_{18} cartridge step) monitored by C18-HPLC at 520 nm (retention time of peak 2, 59.0–59.2 min). (**B**) Hydrolysate of the extract of stage 9 'Smoky' fruit monitored by C18-HPLC at 371 nm.

system, in addition to the cyanidin peak at 56.2 min (peak 2; λ absorption maxima 247, 274, 528 nm) (**Figure 3B**), a peak corresponding to quercetin at 63.4 min (peak 6; λ absorption maxima 256, 371 nm) (**Figure 3B**) was detected, confirming the presence of quercetin glycosides as discussed below. Two very minor peaks at the retention times of 52.7 min (peak 5) (**Figure 3B**) and 65.0 min (peak 7) (**Figure 3B**) were also detected. Peak 5 had absorption maxima of 246 and 367 nm, characteristic of a flavonol; however, it did not cochromatograph with the flavonol myricetin (57.1 min), and it is likely not kaempferol, as the latter is less polar than myricetin and should elute after myricetin in this C18-HPLC system (**Figure 3B**). Peak 7 had a complex absorption spectrum (279, 373 sh, 453 sh, 540 nm) and therefore may be a mixture of anthocyanidin and flavonol-type compounds (**Figure 3B**).

For identification of the sugar moieties conjugated to the anthocyanin aglycon, saskatoon fruit flavonoid extracts, processed through the Sep-Pak C_{18} cartridge step, were initially subjected to acid hydrolysis at 110 °C for 0−180 min, and identification and quantification of sugars were carried out by GC analysis (**Figure 4**). The zero time control demonstrated that relatively small amounts of xylose, arabinose, glucose, and galactose were present as soluble sugar constituents in the saskatoon fruit Sep-Pak C_{18} extract in the relative proportion

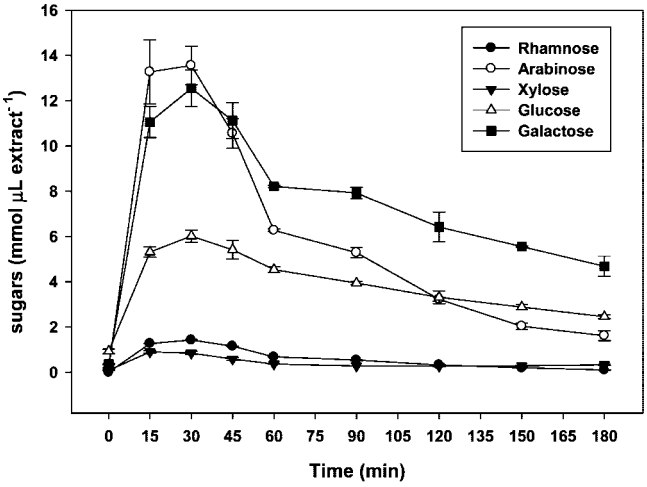

Figure 4. Determination of optimal acid hydrolysis duration for identification of anthocyanidin sugar conjugates as their alditol acetates by GC analysis.

of 1:2:11:4, respectively. After 30 min of acid hydrolysis, arabinose, glucose, and galactose levels in the extract increased substantially, while xylose and rhamnose levels remained relatively low (**Figure 4**). Acid hydrolysis for periods longer than 30 min resulted in degradation of hydrolyzed sugars in the extract. These data demonstrate that these sugars can be released from compounds in this fruit extract by acid hydrolysis, with arabinose, glucose, and galactose making up the majority of the conjugated sugars. Additionally, these data show that 30 min is the optimal timing for acid hydrolysis of the conjugated sugars. Using a 30 min duration for acid hydrolysis, the major soluble sugars in the acid-hydrolyzed HPLC fractions were identified as galactose in fraction 1A, glucose in fraction 1B, and arabinose in fraction 1C (**Figure 5A**). These data are consistent with the identities of peak 1A as cyanidin 3-*O*-galactoside, peak 1B as cyanidin 3-*O*-glucoside, and peak 1C as cyanidin 3-*O*-arabinoside. A wide collection window was employed for isolating the 520 nm peak 1D, which was subsequently shown to overlap with the 350 nm peaks 2E, 2F, and 2G (**Figure 2**). The acid-hydrolyzed HPLC fraction 1D contained the soluble sugars, galactose, glucose, arabinose, and xylose (**Figure 5B**). As described below, the relative levels of galactose, glucose, and arabinose are accounted for as sugars conjugated to quercetin in this fraction (peaks 2E, 2F, and 2G) (**Figure 2**), suggesting that the pentose sugar xylose is the sugar conjugated to cyanidin in peak 1D. Furthermore, the relative retention time of peak 1D compared to those of cyanidin 3-*O*-galactoside (peak 1A), cyanidin 3-*O*-glucoside (peak 1B), and cyanidin 3-*O*-arabinoside (peak 1C) is consistent with the identification of peak 1D as cyanidin 3-*O*-xyloside, as reported using C18-HPLC chromatography in apple (*Malus domestica* Borkh.), a large-fruited pome species (*22*), and chokeberry (*Aronia melanocarpa* Elliott), a small-fruited species like saskatoon (*23*), both members of the Rosaceae family, as is saskatoon.

Full-scan LC-MS analysis of the saskatoon flavonoid extract of 'Smoky' fruit (**Table 2**), when compared to standards, confirmed the identity of peak 1A as cyanidin 3-*O*-galactoside and peak 1B as cyanidin 3-*O*-glucoside. Both peaks 1A and 1B eluted at the retention times of their respective cyanidin glycoside, and they both showed a molecular ion mass of 449 atomic mass units (amu) with a fragment mass of 287 amu confirming the aglycon cyanidin (287 amu) with an attached hexose sugar (287 + 162 = 449 amu) (**Table 2**). Peaks 1C and 1D were identified as cyanidin pentoses, exhibiting a molecular ion mass of 419 amu with a fragment mass of 287 amu confirming the aglycon cyanidin (287 amu) with an attached pentose sugar (287 + 132 = 419 amu) (**Table 2**). Again, the relative retention times of peaks 1C and 1D on the LC-MS were consistent with the identification of peak 1C as cyanidin 3-*O*-arabinoside and peak 1D as cyanidin 3-*O*-xyloside. The aglycon of cyanidin was not detected in the C18-HPLC−PDA traces of 'Smoky' or 'Honeywood' mature fruit extracts (**Figure 2**). However, a small amount of cyanidin was detected in the LC-MS scan of the 'Smoky' fruit extract (**Table 2**). The presence of the cyanidin aglycon in this extract is most likely a result of hydrolysis of the cyanidin glycosides during sample preparation as suggested by Adhikari et al. (*13*).

With these LC-MS data, along with data on the C18-HPLC retention times, maximum absorption spectra, identification of the anthocyanin aglycon and the acid-hydrolyzed soluble sugars derived from these anthocyanin fractions, and confirmation with known standards and/or with data presented in the literature, we identify peak 1A as cyanidin 3-*O*-galactoside, peak 1B as cyanidin 3-*O*-glucoside, peak 1C as cyanidin 3-*O*-arabinoside, and peak 1D as cyanidin 3-*O*-xyloside.

For cyanidin 3-*O*-galactoside and cyanidin 3-*O*-glucoside, these data are consistent with those reported by Mazza (*6*) and Adhikari et al. (*13*) in mature saskatoon fruit extracts. However, the C18-HPLC fraction containing peak 1C identified in this study as cyanidin 3-*O*-arabinoside was previously reported by Mazza (*6*) as cyanidin 3-*O*-xyloside in mature saskatoon fruit extracts. Since the previous study (*6*) did not report the time of acid hydrolysis, it is possible that the sample was hydrolyzed for a period long enough for significant arabinose degradation (**Figure 4**). In addition, Mazza (*6*) also detected a fourth late-eluting compound on C18-HPLC-UV (280 nm) that had spectroscopic properties similar to a monoglycosylated cyanidin compound, identified in this study as cyanidin 3-*O*-xyloside, but no further identification was attempted in the earlier study.

The amount of anthocyanins extracted from mature saskatoon fruit was similar in both cultivars tested (**Table 3**), with a total content of 140 mg/100 g fwt in 'Smoky' and 131 mg/100 g fwt in 'Honeywood'. These values for total anthocyanin content are within the range (70−178 mg/100 g fwt) of those reported for mature saskatoon fruit as determined by the pH differential method of Fuleki and Francis (*15*) and expressed as cyanidin 3-*O*-galactoside equivalents (*7*, *16*). In comparison to other selected fruits in the Roseaceae family, the levels (in mg/100 g fwt) of anthocyanins in mature saskatoon fruit were significantly greater than that in apple (5.3), peach (*Prunus persica* [L.] Batsch.) (4.8), and nectarine (*P. persica* [L.] Batsch.) (6.8), similar to that in black plum (*Prunus salicina*) (125), and less than that in chokeberry (1480) (*24*).

Quercetin glycosides were also identified in mature saskatoon flavonoid extracts. Peaks 2E, 2F, 2G, and 2I (**Table 1** and **Figure 2**) cochromatographed with the standards of quercetin 3-*O*-arabinoglucoside, quercetin 3-*O*-galactoside, quercetin 3-*O*-glucoside, and quercetin 3-*O*-rutinoside, respectively. The identity of these quercetin glycosides was confirmed by LC-MS with standards, which yielded an aglycon molecular ion mass of 303 amu and molecular and fragment ions characteristic of each quercetin glycoside (**Table 4**). Peak 2E eluted at the retention time of quercetin 3-*O*-arabinoglucoside and exhibited a molecular ion mass of 597 amu (aglycon + glucose + arabinose), the sodium adduct molecular ion mass at 619 amu (addition of sodium and loss of one proton, M^+ plus 22), and fragment masses at 303 amu confirming the aglycon quercetin

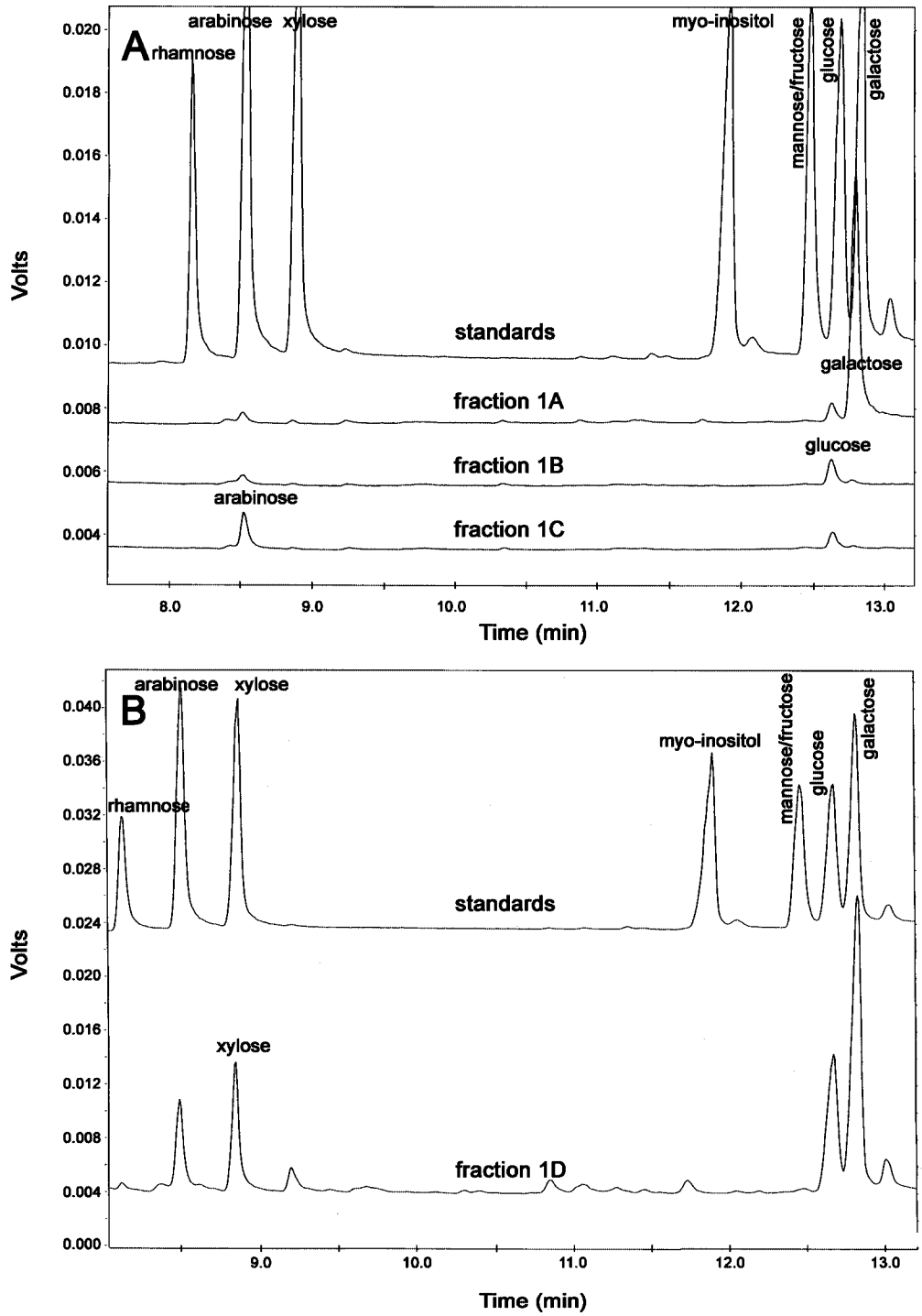

Figure 5. Identification of sugars conjugated to the anthocyanidin moiety. C18-HPLC fractions of (**A**) peaks 1A, 1B, and 1C and (**B**) peak 1D (**Figure 2**) identified as their alditol acetates by GC analysis.

and at 465 amu confirming the aglycon with an attached glucose sugar. Peak 2F eluted at the retention time of quercetin 3-*O*-galactoside and exhibited a molecular ion mass of 465 amu (aglycon + galactose), the sodium adduct molecular ion mass of 487 amu, and a fragment mass at 303 amu confirming the aglycon quercetin. Peak 2G eluted at the retention time of quercetin 3-*O*-glucoside and exhibited a molecular ion mass of 465 amu (aglycon + glucose), the sodium adduct molecular ion mass of 487 amu, and a fragment mass at 303 amu confirming the aglycon quercetin. Peak 2I eluted at the retention time of quercetin 3-*O*-rutinoside and exhibited a molecular ion mass of 611 amu (aglycon + glucose + rhamnose), the sodium adduct molecular ion mass of 633 amu, and fragment masses at 303 amu confirming the aglycon quercetin and at 465 amu confirming the aglycon with an attached glucose sugar. Peak 2H exhibited the same molecular ion mass (611 amu, aglycon + hexose + rhamnose) and characteristic fragment ions and spectroscopic properties (**Table 1**) of quercetin 3-*O*-rutinoside but eluted slightly before that of quercetin 3-*O*-rutinoside (**Tables 1** and **4**). As quercetin 3-*O*-rhamnogalactoside (also called quercetin 3-*O*-robinobioside) eluted prior to that of quercetin 3-*O*-rutinoside (quercetin 3-*O*-rhamnoglucoside) using C18-HPLC chromatography (*25*), we have assigned the identity of peak 2H to quercetin 3-*O*-robinobioside. Peaks 2J and 2K exhibited a molecular ion mass of 435 amu (aglycon + pentose), the sodium adduct molecular ion mass of 457 amu, and a

Table 2. Characteristic Retention Times and Mass Fragment Ions of Hydroxycinnamate- and Anthocyanin-Type Phenolics in Mature 'Smoky' Saskatoon Fruit Extracts As Determined by LC-MS Analysis[a]

compound	sample	t_R(min)	aglycon (A)[b]	molecular ion (M^+)[b]	other fragment ions
3-*O*-caffeoylquinic acid	extract[c]	2.8		355 ([M]$^+$, 4),[d] 377 ([M − 1H + Na]$^+$, 79), 378 (12)	163 (100), 164 (12), 217 (15), 261 (19), 337 (29), 365 (39)
5-*O*-caffeoylquinic acid (chlorogenic acid)	extract	5.9		355 ([M]$^+$, 28), 356 (8), 377 ([M − 1H + Na]$^+$, 57), 378 (6)	163 (100), 164 (15), 337 (6), 449 (18), 731 ([2 M − 1H + Na]$^+$, 23), 732 (13)
cyanidin 3-*O*-galactoside	standard	7.4	287 ([A]$^+$, 13), 288 (1)	449 ([M]$^+$; A + gal,[e] 100), 450 (32), 451 (8), 452 (1)	489 (2), 359 (2), 305 (2), 217 (4)
	extract	6.6	287 ([A]$^+$, 9), 288 (2)	449 ([M]$^+$; A + gal, 100), 450 (36), 451 (8), 452 (1)	425 (2), 305 (1), 217 (2), 163 (3)
cyanidin 3-*O*-glucoside	standard	9.9	287 ([A]$^+$, 11), 288 (3)	449 ([M]$^+$; A + glu,[f] 100), 450 (28), 451 (6)	359 (3), 261 (2), 257 (2), 217 (5)
	extract	9.7	287 ([A]$^+$, 13), 288 (1)	449 ([M]$^+$; A + glu, 100), 450 (30), 451 (5)	489 (3), 365 (10), 337 (10), 217 (5), 149 (4)
cyanidin 3-*O*-arabinoside	extract	11.5	287 ([A]$^+$, 14), 288 (3)	419 ([M]$^+$; A + arab,[g] 100), 420 (24), 421 (5)	454 (4), 450 (3), 449 (15), 305 (5)
cyanidin 3-*O*-xyloside	extract	25.8	287 ([A]$^+$, 21), 288 (3)	419 ([M]$^+$; A + xyl,[h] 100), 420 (26), 421 (4)	449 (3), 149 (3), 147 (5), 117 (3)
cyanidin	standard	55.5		287 ([M]$^+$, 100), 288 (14)	360 (10), 359 (43), 305 (11), 271 (9), 261 (10), 217 (28), 173 (16)
	extract	55.6		287 ([M]$^+$, 100), 288 (14)	573 ([2 M − 1H]$^+$, 30), 551 (20), 359 (26), 304 (11), 303 (19), 149 (8)

[a] Gradient 1 used for LC-MS. [b] Ions and their associated [ion] + 1, [ion] + 2, or [ion] + 3 masses (due to naturally occurring isotopes) are grouped together. [c] Mature stage 9 'Smoky' saskatoon fruit extract processed through the Sep-Pak C_{18} cartridge step. [d] Ion abundance. [e] gal = galactose. [f] glu = glucose. [g] arab = arabinose. [h] xyl = xylose.

Table 3. Content of Hydroxycinnamate-, Anthocyanin-, and Flavonol-Type Phenolics in Mature Fruit of 'Smoky' and 'Honeywood'

compound	cultivar	mg/100 g fwt
Hydroxycinnamates[a]		
3-*O*-caffeoylquinic acid	'Smoky'	10.7 ± 1.8[b]
	'Honeywood'	10.0 ± 1.3
5-*O*-caffeoylquinic acid	'Smoky'	58.4 ± 7.6
	'Honeywood'	43.5 ± 4.2
unknown hydroxycinnamate	'Smoky'	27.0 ± 4.1
	'Honeywood'	nd
Anthocyanins[c]		
cyanidin 3-*O*-galactoside	'Smoky'	80.2 ± 6.0
	'Honeywood'	90.7 ± 4.1
cyanidin 3-*O*-glucoside	'Smoky'	32.9 ± 1.9
	'Honeywood'	22.3 ± 0.9
cyanidin 3-*O*-arabinoside	'Smoky'	15.3 ± 0.2
	'Honeywood'	10.5 ± 0.4
cyanidin 3-*O*-xyloside	'Smoky'	11.1 ± 0.1
	'Honeywood'	7.5 ± 0.0
Flavonols[d]		
quercetin 3-*O*-arabinoglucoside	'Smoky'	1.80 ± 0.06
	'Honeywood'	2.92 ± 0.04
quercetin 3-*O*-galactoside	'Smoky'	14.51 ± 1.26
	'Honeywood'	13.90 ± 0.74
quercetin 3-*O*-glucoside	'Smoky'	3.02 ± 0.19
	'Honeywood'	6.12 ± 0.31
quercetin 3-*O*-robinobioside	'Smoky'	1.21 ± 0.05
	'Honeywood'	1.27 ± 0.05
quercetin 3-*O*-rutinoside	'Smoky'	1.31 ± 0.01
	'Honeywood'	0.69 ± 0.03
quercetin 3-*O*-arabinoside	'Smoky'	1.46 ± 0.02
	'Honeywood'	0.79 ± 0.03
quercetin 3-*O*-xyloside	'Smoky'	1.47 ± 0.11
	'Honeywood'	0.66 ± 0.02

[a] Content estimated as 5-*O*-caffeoylquinic acid equivalents. [b] Mean value ± SE, $n = 2$. [c] Content estimated as cyanidin 3-*O*-glucoside equivalents. [d] Content estimated as quercetin 3-*O*-galactoside equivalents; nd = not detected.

fragment mass at 303 amu confirming the aglycon quercetin (**Table 4**). As quercetin 3-*O*-arabinoside eluted prior to quercetin 3-*O*-xyloside using a similar C18-HPLC chromatography system (*26*), we have identified peak 2J as quercetin 3-*O*-arabinoside and peak 2K as quercetin 3-*O*-xyloside.

The presence of a number of quercetin glycosides has been reported in the fruits of other members of the Roseaceae family. In chokeberry fruit, quercetin 3-*O*-arabinoglucoside, quercetin 3-*O*-robinobioside, quercetin 3-*O*-rutinoside, quercetin 3-*O*-galactoside, and quercetin 3-*O*-glucoside were identified using NMR, C18-HPLC, and LC-MS (*25*). In apple, quercetin 3-*O*-galactoside, quercetin 3-*O*-glucoside, quercetin 3-*O*-arabinoside, quercetin 3-*O*-xyloside, and quercetin 3-*O*-rutinoside were identified in the fruit using C18-HPLC and LC-MS (*27*). In black plum peel, quercetin 3-*O*-glucoside, quercetin 3-*O*-xyloside (tentative), quercetin 3-*O*-rutinoside, and a quercetin pentoxyl hexoside were identified using similar methods (*28*). In peaches and nectarines, quercetin 3-*O*-galactoside was tentatively identified as the only flavonol present in the flesh, and quercetin 3-*O*-galactoside, quercetin 3-*O*-glucoside, and quercetin 3-*O*-rutinoside were found in the peel (*28*).

The total amount of quercetin glycosides extracted from mature saskatoon fruit was similar in both cultivars tested (**Table 3**), with 24.8 mg/100 g fwt detected in 'Smoky' and 26.4 mg/100 g fwt in 'Honeywood'. Relative to other fruit in the Roseaceae family, the total flavonol level in mature saskatoon fruit is approximately five times greater than that reported for apple (5.7 mg/100 g fwt) (*27*), over five times greater than that in ripe yellow-fleshed peach and nectarine cultivars (average in peel, 5.8−7.3 mg/100 g fwt; in flesh, 1.2−1.7 mg/100 g fwt)

Table 4. Characteristic Retention Times and Mass Fragment Ions of Flavonols in Mature Saskatoon Fruit Extracts As Determined by LC-MS Analysis[a]

compound	sample	t_R (min)	aglycon (A)[b]	molecular ion (M)[b]	other fragment ions
quercetin 3-*O*-arabinoglucoside	standard	39.5	303 ($[A]^+$, 38),[c] 304 (7), 465 ($[A + glu]^+$, 14)	597 ($[M]^+$; A + glu[d] + arab,[e] 40), 598 (13), 619 ($[M - 1H + Na]^+$, 100), 620 (29), 621 (9)	435 (10), 318 (7)
	extract[f]	40.3	303 ($[A]^+$, 60), 465 ($[A + glu]^+$, 22)	597 ($[M]^+$; A + glu + arab, 49), 619 ($[M - 1H + Na]^+$, 100), 620 (30), 621 (18)	318 (18), 287 (36), 149 (24)
quercetin 3-*O*-galactoside	standard	43.3	303 ($[A]^+$, 88), 304 (13)	465 ($[M]^+$; A + gal,[g] 35), 487 ($[M - 1H + Na]^+$, 100), 488 (17)	951 (13), 287 (18), 261 (9), 217 (9)
	extract	43.6	303 ($[A]^+$, 100), 304 (15)	465 ($[M]^+$; A + gal, 37), 466 (11), 487 ($[M - 1H + Na]^+$, 64), 488 (21), 489 (11)	951 (9), 629 (6), 287 (10)
quercetin 3-*O*-glucoside	standard	47.4	303 ($[A]^+$, 100), 304 (23)	465 ($[M]^+$; A + glu, 43), 487 ($[M - 1H + Na]^+$, 73), 488 (19)	951 (21), 287 (17), 261 (12), 217 (11)
	extract	47.6	303 ($[A]^+$, 100)	465 ($[M]^+$; A + glu, 43), 487 ($[M - 1H + Na]^+$, 52)	393 (20), 379 (26), 287 (74), 149 (25), 121 (27)
quercetin 3-*O*-robinobioside	extract	48.3	303 ($[A]^+$, 100), 304 (22), 465 ($[A + gal]^+$, 27), 466 (4)	611 ($[M]^+$; A + gal + rham,[h] 17), 633 ($[M - 1H + Na]^+$, 41), 634 (8)	487 ($[A + gal - 1H + Na]^+$, 25), 488 (7)
quercetin 3-*O*-rutinoside (rutin)	standard	51.0	303 ($[A]^+$, 58), 304 (10), 465 ($[A + glu]^+$, 36), 466 (11)	611 ($[M]^+$; A + glu + rham, 52), 612 (16), 633 ($[M - 1H + Na]^+$, 100), 634 (32), 635 (13)	287 (12), 217 (11)
	extract	51.8	303 ($[A]^+$, 77), 304 (3), 465 ($[A + glu]^+$, 31), 466 (9)	611 ($[M]^+$; A + glu + rham, 46), 612 (26), 633 ($[M - 1H + Na]^+$, 100), 634 (52), 635 (10)	981 (80), 359 (31), 287 (67)
quercetin 3-*O*-arabinoside	extract	53.3	303 ($[A]^+$, 100), 304 (17)	435 ($[M]^+$; A + arab, 16), 457 ($[M - 1H + Na]^+$, 31), 458 (9)	396 (16), 395 (45), 287 (13), 211 (16)
quercetin 3-*O*-xyloside	extract	56.4	303 ($[A]^+$, 100), 304 (14)	435 ($[M]^+$; A + xyl,[i] 17), 457 ($[M - 1H + Na]^+$, 21), 458 (7)	359 (13), 287 (12), 237 (21), 215 (16)
quercetin	standard	60.7		303 ($[M]^+$, 100), 304 (22), 325 ($[M - 1H + Na]^+$, 34), 326 (6)	360 (10), 359 (38), 287 (21), 217 (6)
	extract	nd			

[a] Gradient 1 used for LC-MS. [b] Ions and their associated [ion] + 1 or [ion] + 2 masses (due to naturally occurring isotopes) are grouped together. [c] Ion abundance. [d] glu = glucose. [e] arab = arabinose. [f] Mature stage 9 'Smoky' saskatoon fruit extract processed through the Sep-Pak C_{18} cartridge step. [g] gal = galactose. [h] rham = rhamnose. [i] xyl = xylose; nd = not detected.

(*28*), similar to levels in ripe plum peel (24.1 mg/100 g fwt) (*28*), and lower than that estimated in chokeberry (71 mg/100 g fwt) (*25*).

Peak 2B cochromatographed with and had a maximum absorption spectrum similar to the standard 5-*O*-caffeoylquinic acid (**Table 1**). Peak 2B also had molecular and fragment ions characteristic of 5-*O*-caffeoylquinic acid (a molecular ion mass of 355, the sodium adduct molecular ion mass of 377, and the base peak of 163 amu) (**Table 2**). These data confirm the presence of 5-*O*-caffeoylquinic acid in mature saskatoon extracts as previously described by Mazza (*6*). In addition, peak 2A had a maximum absorption spectrum similar to 5-*O*-caffeoylquinic acid, but it eluted substantially earlier than 5-*O*-caffeoylquinic acid in our C18-HPLC system (**Table 1**). The LC-MS molecular and fragment ions confirm that peak 2A is a caffeoylquinic acid isomer (a molecular ion mass of 355, the sodium adduct molecular ion mass of 377, and the base peak of 163 amu) (**Table 2**), and its earlier elution time compared to 5-*O*-caffeoylquinic acid in reverse-phase C18 chromatography (*28*, *29*) is consistent with identification of peak 2A as 3-*O*-caffeoylquinic acid. Peak 2D, which was only detected in 'Smoky' (**Figure 2C**,**D**), also had a maximum absorption spectrum similar to 5-*O*-caffeoylquinic acid (**Table 1**) but eluted after 5-*O*-caffeoylquinic acid. As the retention time of peak 2D was close to that of the large cyanidin 3-*O*-galactoside peak (**Figure 2A**), peak 2D was individually collected as it eluted from the C18-HPLC system, and the collected fraction was injected onto the LC-MS using a modified gradient program (gradient 2). One major peak was detected at 18.2 min with the characteristic fragment ions of a caffeic acid containing hydroxycinnamate [ion (abundance): 163 (100), 164 (*10*), 165 (*6*), 377 (*11*)] along with the other fragment ions 291 (*29*), 292 (*5*), 145 (*7*), and 139 (*11*). Interestingly, an unidentified hydroxycinnamate with a maximum absorption spectrum similar to 5-*O*-caffeoylquinic acid and a similar relative retention time (with respect to 3-*O*-caffeoylquinic acid and 5-*O*-caffeoylquinic acid) as peak 2D was reported in plum (*28*). The total amount of these caffeic acid-derived hydroxycinnamates in mature saskatoon fruit was 96 mg/100 g fwt in 'Smoky' and 53 mg/100 g fwt in 'Honeywood' (**Table 3**). Mattila et al. (*30*) found that caffeic acid was the major phenolic acid (43.2 mg/100 g fwt) in saskatoon fruit extracts as determined by HPLC after alkaline and acid hydrolyses. Although the saskatoon cultivar used was not stated by Mattila et al. (*30*), the amount of hydrolyzed caffeic acid reported corresponds well with the amount of caffeic acid-derived hydroxycinnamates found in 'Honeywood' in the current study. Other minor phenolic acids detected after alkaline and acid hydrolyses (in mg/100 g fwt) were protocatechuic acid (8.4), *p*-coumaric acid (2.9), ferulic acid (2.3), *p*-hydroxybenzoic acid (0.9), sinapic acid (0.8), and vanillic acid (0.7) (*30*).

In other selected Roseaceae fruit species, the main hydroxycinnamates detected (average values in mg/100 g fwt) were 5-*O*-caffeoylquinic acid (11.8) in apple (total hydroxycinnamates, 14.2) (*27*), 3-*O*-caffeoylquinic acid and 5-*O*-caffeoylquinic acid in chokeberry (approximately 50 mg/100 g fwt each as estimated from dry weight values using 18% dry fruit mass) (*31*), 3-*O*-caffeoylquinic acid (peel, 24.2; flesh, 11.1) in ripe black plum cv. Black Beaut (total hydroxycinnamates in peel, 30.1; flesh, 12.2) (*28*), 3-*O*-caffeoylquinic acid (peel, 5.3; flesh, 4.4), and 5-*O*-caffeoylquinic acid (peel, 22.5; flesh, 7.7) in yellow-fleshed nectarine (total hydroxycinnamates in peel, 28.4; flesh, 12.1) (*28*), and 3-*O*-caffeoylquinic acid (peel, 3.7; flesh, 4.0) and 5-*O*-caffeoylquinic acid (peel, 18.5; flesh, 7.4) in yellow-fleshed peach (total hydroxycinnamates in peel, 23.1; flesh, 11.4) (*28*).

Therefore, mature saskatoon fruits contain higher levels of hydroxycinnamates than fruits of apple, black plum, yellow-fleshed nectarine, and peach and the same or lower levels than chokeberry. Indeed, caffeic acid was found to be the major phenolic acid present after alkaline and acid hydrolyses of whole fruit extracts of apple, dark plum, peach, nectarine, chokeberry, and saskatoon (*30*).

Peak 2C (**Figure 2C**,**D**) was also individually collected as it eluted from the C18-HPLC system, and the collected fraction was injected onto the LC-MS using a modified gradient program (gradient 2). A number of peaks were detected in the LC-MS chromatogram, with the largest peak eluting at 18.16 min with the characteristic fragment ions of a proanthocyanin polymer (*32*) with the following $[M - 288]^+$ procyanidin sequence ions, ion (abundance) 291 (100), 579 (34), 867 (*23*), and 1155 (*11*), along with the fragment ions 139 (59), 163 (*18*), 165 (*17*), 292 (*12*), 577 (*27*), and 868 (*12*). As the maximum absorbance spectrum of peak 2C (**Table 1**) suggests that more than proanthocyanins (maximum absorbance at 280 nm) are eluting at this retention time, this peak appears to be a complex of more than one type of compound.

In conclusion, the present study confirms the presence of cyanidin 3-*O*-galactoside and cyanidin 3-*O*-glucoside as two major anthocyanins in mature saskatoon fruit. This study further identified the third most abundant anthocyanin in mature saskatoon fruit as cyanidin 3-*O*-arabinoside and the fourth as cyanidin 3-*O*-xyloside. Furthermore, we have confirmed the presence of quercetin 3-*O*-rutinoside and identified quercetin 3-*O*-robinobioside, quercetin 3-*O*-arabinoglucoside, quercetin 3-*O*-galactoside, quercetin 3-*O*-glucoside, quercetin 3-*O*-arabinoside, and quercetin 3-*O*-xyloside in mature saskatoon fruits. Two chlorogenic acid isomers, 3-*O*-caffeoylquinic acid and 5-*O*-caffeoylquinic acid, were also identified. The two cultivars tested contained similar types and amounts of anthocyanins and flavonols; however, the types of hydroxycinnamates differed, with one additional hydroxycinnamate derivative found in 'Smoky'. These data further our knowledge of the phenolic composition of mature saskatoon fruit. As anthocyanins, flavonols, and hydroxycinnamates exhibit antioxidant activities, the relatively high levels of these compounds present in mature saskatoon fruits establish this fruit as a very good source of these potentially health-beneficial compounds.

ACKNOWLEDGMENT

The authors thank Gary Sedgwick, Kelvin Lein, Alena Jin, and Don Morgan for technical assistance.

LITERATURE CITED

(1) St. Pierre, R. G. The development of native fruit species as horticultural crops in Saskatchewan. *HortScience* **1992**, *27*, 866.

(2) Finn, C. Temperate berry crops. In *Perspectives on New Crops and New Uses*; Janick, J., Ed.; ASHS Press: Alexandria, VA, 1999; pp 324–334.

(3) St. Pierre, R. G.; Steeves, T. A. Observations on shoot morphology, anthesis, flower number, and seed production in the saskatoon, *Amelanchier alnifolia* (Rosaceae). *Can. Field-Naturalist* **1990**, *104*, 379–386.

(4) McGarry, R.; Ozga, J. A.; Reinecke, D. M. Patterns of saskatoon (*Amelanchier alnifolia* Nutt.) fruit and seed growth. *J. Am. Soc. Hort. Sci.* **1998**, *123*, 26–29.

(5) McGarry, R.; Ozga, J. A.; Reinecke, D. M. Differences in fruit development among large- and small-fruited cultivars of saskatoon (*Amelanchier alnifolia*). *J. Am. Soc. Hort. Sci.* **2001**, *126*, 381–385.

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

(7) Rogiers, S. Y.; Knowles, N. R. Physical and chemical changes during growth, maturation, and ripening of saskatoon (*Amelanchier alnifolia*) fruit. *Can. J. Bot.* **1997**, *75*, 1215–1225.

(8) McGarry, R.; Ozga, J. A.; Reinecke, D. M. The effects of ethephon on saskatoon (*Amelanchier alnifolia* Nutt.) fruit ripening. *J. Am. Soc. Hort. Sci.* **2005**, *130*, 12–17.

(9) Kwok, B. H. L.; Hu, C.; Durance, T.; Kitts, D. D. Dehydration techniques affect phytochemical contents and free radical scavenging activities of saskatoon berries (*Amelanchier alnifolia* Nutt.). *J. Food Sci.* **2004**, *69*, 122–126.

(10) Clifford, M. N. Chlorogenic acids and other cinnamates—nature, occurrence, dietary burden, absorption and metabolism. *J. Sci. Food Agric.* **2000**, *80*, 1033–1043.

(11) Wang, J.; Mazza, G. Inhibitory effects of anthocyanins and other phenolic compounds on nitric oxide production in LPS/IFN-γ-activated RAW 264.7 macrophages. *J. Agric. Food Chem.* **2002**, *50*, 850–857.

(12) Lila, M. A. Anthocyanins and human health: an in vitro investigative approach. *J. Biomed. Biotechnol.* **2004**, *5*, 306–313.

(13) Adhikari, D.; Francis, J.; Schutzki, R.; Chandra, A.; Nair, M. Quantification and characterisation of cyclo-oxygenase and lipid peroxidation inhibitory anthocyanins in fruits of *Amelanchier*. *Phytochem. Anal.* **2005**, *16*, 175–180.

(14) Manach, C.; Williamson, G.; Morand, C.; Scalbert, A.; Remesy, C. Bioavailability and bioefficacy of polyphenols in humans. I. Review of 97 bioavailability studies. *Am. J. Clin. Nutr.* **2005**, *81*, 230S–242S.

(15) Fuleki, T.; Francis, F. J. Quantitative methods for anthocyanins. I. Extraction and determination of total anthocyanins in cranberries. *J. Food Sci.* **1968**, *33*, 72–77.

(16) Green, R. C.; Mazza, G. Relationships between anthocyanins, total phenolics, carbohydrates, acidity and colour of saskatoon berries. *Can. Inst. Food Sci. Technol. J.* **1986**, *19*, 107–113.

(17) Blakeney, A. B.; Harris, P. J.; Henry, R. J.; Stone, B. A. A simple and rapid preparation of alditol acetates for monosaccharides analysis. *Carbohydr. Res.* **1983**, *113*, 291–299.

(18) Kraus, R. J.; Shinnick, F. L.; Marlett, J. A. Simultaneous determination of neutral and amino sugars in biological materials. *J. Chromatogr.* **1990**, *513*, 71–81.

(19) Lein, K. A.; Sauer, W. C.; Fenton, M. Mucin output in ileal digesta of pigs fed a protein-free diet. *Z. Ernaehrungswiss.* **1997**, *36*, 182–190.

(20) Mazza, G.; Miniati, E. *Anthocyanins in Fruits, Vegetables and Grains*; CRC Press: Boca Raton, FL, 1993.

(21) Wang, J.; Kalt, W.; Sporns, P. Comparison between HPLC and MALDI-TOF MS analysis of anthocyanins in highbush blueberries. *J. Agric. Food Chem.* **2000**, *48*, 3330–3335.

(22) Mazza, G.; Velioglu, Y. S. Anthocyanins and other phenolic compounds in fruits of red-flesh apples. *Food Chem.* **1992**, *43*, 113–117.

(23) Chandra, A.; Rana, J.; Li, Y. Separation, identification, quantification, and method validation of anthocyanins in botanical supplement raw materials by HPLC and HPLC-MS. *J. Agric. Food Chem.* **2001**, *49*, 3515–3521.

(24) Wu, X.; Beecher, G. R.; Holden, J. M.; Haytowitz, D. B.; Gebhardt, S. E.; Prior, R. L. Concentrations of anthocyanins in common foods in the United States and estimation of normal consumption. *J. Agric. Food Chem.* **2006**, *54*, 4069–4075.

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

(26) Amaral, J. S.; Seabra, R. M.; Andrade, P. B.; Valentão, P.; Pereira, J. A.; Ferreres, F. Phenolic profile in the quality control of walnut (*Juglans regia* L.) leaves. *Food Chem.* **2004**, *88*, 373–379.

(27) Vrhovsek, U.; Rigo, A.; Tonon, D.; Mattivi, F. Quantitation of polyphenols in different apple varieties. *J. Agric. Food Chem.* **2004**, *52*, 6532–6538.

(28) Tomás-Barberán, F. A.; Gil, M. I.; Cremin, P.; Waterhouse, A. L.; Hess-Pierce, B.; Kader, A. A. HPLC-DAD-ESIMS analysis of phenolic compounds in nectarines, peaches, and plums. *J. Agric. Food Chem.* **2001**, *49*, 4748–4760.

(29) Gao, L.; Mazza, G. Characterization, quantitation, and distribution of anthocyanins and colorless phenolics in sweet cherries. *J. Agric. Food Chem.* **1995**, *43*, 343–346.

(30) Mattila, P.; Hellström, J.; Törrönen, R. Phenolic acids in berries, fruits, and beverages. *J. Agric. Food Chem.* **2006**, *54*, 7193–7199.

(31) Oszmiański, J.; Wojdylo, A. *Aronia melanocarpa* phenolics and their antioxidant activity. *Eur. Food Res. Technol.* **2005**, *221*, 809–813.

(32) 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.

Received for review October 5, 2007. Revised manuscript received October 12, 2007. Accepted October 12, 2007. The authors thank the Alberta Funding Consortium for funding this work.

JF072949B