CHEMICAL COMPOSITION OF SELECT SASKATOON BERRY VARIETIES WITH AN EMPHASIS ON PHENOLICS

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ABSTRACT

In this study, three saskatoon berry varieties (Martin, Northline and Pembina) grown in Saskatchewan, Canada were analyzed for their physicochemical properties (berry size, colour, pH and % seeds), proximate composition, amino acids, major carbohydrates/polyols/galacturonic acid, major minerals, oligosaccharides, organic acids and phenolics. In addition, the phenolic subclass composition and antioxidant activities of whole fruit and pomace from commercial and laboratory scale juice production, and aqueous alcohol fractions were determined.

Fruit varieties were found to differ in colour and size but showed similar pH and °Brix values. Proximate analysis results ranged from 80.18-82.79% for moisture, 7.39-10.82% for carbohydrate, 1.13-1.79% for protein, 0.28-0.48% for lipid, 4.23-9.42% for total dietary fibre, and 0.53-0.74% for ash. Major carbohydrates and polyol identified were fructose, glucose, and sorbitol. This work represents only the second report of the detection and quantitation of sorbitol in this fruit. Oligosaccharide profiles were determined by high performance anion exchange chromatography with pulsed amperometric detection (HPAE-PAD) and capillary gas chromatography with flame ionization detection (CGC-FID) and showed the presence of a number of dextrose (DP2-5) and pectin polymers. Oligosaccharide profiles have not been reported previously. Amino acid contents ranged from 0.83-1.22 g/100 g fresh weight (FW), with arginine, aspartic acid/asparagine, glutamic acid/glutamine and leucine predominating. Major minerals quantified were calcium, magnesium, potassium and sodium, with potassium having the highest concentration that ranged from 219-248 mg/100 g FW. The major organic acids identified were malic (304.7-393.9 mg/100 g FW) and succinic (120.4-316.3 mg/100 g FW).

Phenolics from the three fruit varieties were extracted employing water, ethanol:formic acid:water, and methanol:formic acid:water (70:2:28 v:v) mixtures. The ethanol:formic acid:water (EFW) extracts from all samples were found to have the highest phenolic concentrations as determined by total phenolic content analysis. Based on total phenolic chromatographic index (TPCI) results as determined by high performance liquid chromatography with photodiode array detection (HPLC-PDA), the Northline variety had the highest TPCI at 504.2 mg/100 g FW. This variety was also shown to have the highest antioxidant activities by both the 2,2-diphenyl-1-picrylhydrazyl (DPPH) and 2,2´-azinobis-3-ethylbenzthiazoline-sulphonic acid (ABTS) radical scavenging assays, of 23.1 $1/IC_{50}$/100 mg FW and 327.5 mM TEAC/100 mg FW, respectively.

Solid phase extraction (SPE) using Amberlite® XAD16N resin and aqueous ethanol (40, 70 and 100%) was employed to produce phenolic fractions from the three fruit varieties. It was found that hydroxybenzoic acids eluted in the 40% ethanol fraction; hydroxycinnamic acids and anthocyanins eluted in the 70% ethanol fraction; and anthocyanins, flavanols and flavonols eluted in the 100% ethanol fraction. The 70% ethanol fraction had the highest TPCI and DPPH/ABTS radical scavenging abilities for all saskatoon berry varieties.

Wet and dry pomace from commercial saskatoon berry juice production had TPCI values of 404.2 mg/100 g and 250.0 mg/100 g, respectively. The ABTS values of wet and dry pomace were found to be 304.8 and 327.8 mM TEAC/100 mg, while the DPPH results were 19.4 and 16.8 $1/IC_{50}$/100 mg FW, respectively. These results show that pomace from commercial juice production was a good source of phenolics with high antioxidant capacities. Results from laboratory scale juice production of the Northline variety employing commercial conditions (i.e. time, temperature, and enzymes and dosages) showed that 29% of the phenolics remained in the pomace after juice production as determined by TPCI analysis.

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LIST OF SYMBOLS AND ABBREVIATIONS

A	Absorbance
ΔA	Absorbance value difference
ABTS	2,2′-azinobis-3-ethylbenzthiazoline-sulfonic acid
$ABTS^{\bullet+}$	2,2′-azinobis-3-ethylbenzthiazoline-sulfonic acid radical cation
ANOVA	Analysis of variance
ArO•	Aromatic hydroxyl (phenoxy) radical
ArOH	Aromatic hydroxyl
BHA	Butylated hydroxyanisole
BHT	Butylated hydroxytoluene
CGC	Capillary gas chromatography
CGC-FID	Capillary gas chromatography with flame ionization detection
cm	Centimeters
C3GE	Cyanidin-3-*O*-D-glucoside equivalents
C3GLU	Cyanidin-3-*O*-D-glucoside
°C	Degree Celsius
DP	Degree of polymerization
DPPH	2,2-diphenyl-1-picrylhydrazyl
DW	Dry weight
EFW	Ethanol-formic acid-water
F-C	Folin-Ciocalteu
F/G	Fructose/glucose ratio
FID	Flame ionization detection
FW	Fresh weight
g	Gram(s)
GAE	Gallic acid equivalents
GRAS	Generally recognized as safe
h	Hour(s)
HPAE	High performance anion exchange chromatography

HPAE-PAD	High performance anion exchange chromatography-pulsed amperometric detection
HPLC	High performance liquid chromatography
HPLC-PDA	High performance liquid chromatography-photodiode array detection
HPLC-RI	High performance liquid chromatography-refractive index detection
IC_{50}	Concentration for 50% inhibition
Kg	Kilograms
L	Litre(s)
λmax	Maximum wavelength
M	Molar
MFW	Methanol-formic acid-water
µg	Micrograms
µL	Microliters
µM	Micromolar
mAU	Milli-absorbance units
mg	Milligrams
mL	Milliliters
mm	Millimeters
mM	Millimolar
MW	Gram molecular weight
N	Normality
nm	Nanometers
NaOAc	Sodium acetate
NaOH	Sodium hydroxide
$O_2^{-}\bullet$	Superoxide anion radical
$^1O_2$	Singlet state oxygen
%	Percent
PAD	Pulsed amperometric detector
PDA	Photodiode array detector

r	Correlation coefficient
RI	Refractive index
RO•	Alkoxy radical
ROO•	Peroxyl radical
ROOH	Alkyl peroxide
ROS	Reactive oxygen species
RT	Retention time(s)
s	Seconds
s/n	Signal-to-noise ratio
TBA	Thiobarbituric acid
TBHQ	Tert-butyl hydroxyquinone
TDF	Total dietary fibre
TEAC	Trolox equivalence antioxidant capacity
TMSI	1-(trimethylsilyl)imidazole
TPC	Total phenolic content
TPCI	Total phenolic chromatographic index
UV	Ultraviolet
UV-vis	Ultraviolet-visible

1. INTRODUCTION

1.1 Summary

Saskatoon berry, *Amelanchier alnifolia* Nutt., is a pome fruit native to the Western and North central regions of North America. Saskatoon berry has shown considerable potential as a commercial product in Canada based on its consumption as a fresh fruit, and as an ingredient in foods such as jams, and as a juice/juice concentrate.

Approximately 1100 acres of saskatoon berry are currently cultivated in Saskatchewan, which accounts for 28% of Canada's orchards of this fruit, with an approximate annual economic value of two million Canadian dollars (Government of Canada, 2016; Government of Saskatchewan, 2016). A number of varieties of this fruit have been developed, which are based on fruit size, colour, yield, and ease of harvest. The most common varieties grown in Saskatchewan/Canada are Honeywood, Martin, Northline, Pembina, Regent, Smoky and Thiessen (Berkheimer and Hanson, 2001; Lavola et al., 2012).

The majority of physicochemical and chemical composition data for the aforementioned varieties are based on fruit grown in Alberta and Manitoba, with limited data on fruit grown in Saskatchewan. In addition, Saskatchewan variety data has been limited to, colour, pH, soluble solids, titratable acidity, moisture and total anthocyanin content (Zatylny et al. 2005; Hu et al. 2005).

Pomace is the residue from juice production and is composed of skin, seeds, stems, and pulp. This co-product stream can be a rich source of carbohydrates, soluble and insoluble fibre (e.g. pectin), minerals, and other nutrients such as phenolic compounds. Limited peer reviewed information is available on the pomace from saskatoon berry juice production and that available has focused on its low protein content and pH, which have been reported to make this material unsuitable as both an animal feed and as a compost to soil (White et al., 2010; Li et al., 2014). In addition, only minor amounts of saskatoon berry juice is commercially produced in Saskatchewan.

Berry fruits are considered to be rich in phenolics, and these compounds have been purported to exhibit a wide range of health benefits due to their capacity to quench free radicals,

and consequently delay oxidation stress-related diseases such as atherosclerosis, diabetes, and cancer (Turrens, 2003; Vattem et al., 2005). Also, phenolics have the capacity to minimize many of the negative impacts of lipid oxidation on the nutritional quality, flavour, colour, odour, texture, and appearance of foods.

1.2 Hypotheses and Objectives

The central hypotheses of this research were as follows: (1) that pomace from Saskatoon berry juice production is a potential source of phenolics for food and nutraceutical applications; (2) that a phenolic-rich fruit from the three saskatoon berry varieties studied could be identified; and (3) that the physicochemical and chemical composition data collected for the three saskatoon berry fruits grown in Saskatchewan would improve scientific knowledge on this fruit and geographic production region.

In addressing the hypotheses of this research project the following objectives were investigated: (1) determine the physicochemical and chemical composition of commercial Martin, Northline and Pembina varieties of saskatoon berry fruit grown in Saskatchewan for: colour, pH, % seeds, fruit size, amino acids, major carbohydrates, major minerals, oligosaccharides, organic acids, and proximate composition (i.e. ash, fibre, lipid, moisture, protein, nitrogen-free extract/total carbohydrate); (2) phenolics extraction and fractionation from Martin, Northline and Pembina varieties, and from commercial pomace from saskatoon berry juice production employing select solvent mixtures (i.e. ethanol:formic acid:water and methanol:formic acid:water; 70:2:28 v:v:v) and solid phase treatment with Amberlite® XAD16N resin; (3) determine the subclass antioxidant activities of phenolics in the aforementioned extracts and fractions employing total phenolic chromatographic index (TPCI) measurements coupled with two *in vitro* chemical assays (ABTS and DPPH); (4) conduct laboratory scale juice production on the variety with the highest phenolic content/antioxidant capacity; and (5) conduct TPCI, ABTS and DPPH analyses on the pomace from laboratory scale juice production.

2. LITERATURE REVIEW

2.1 Phenolic Compounds

Phenolics are defined as chemical compounds that have one or more hydroxyl groups directly attached to an aromatic ring (Vermerris and Nicholson, 2008). As a group, they are both structurally diverse (e.g. phenolic acids and anthocyanins) and have a wide range of molecular weights from the most simple phenolic, phenol (94.11 g/mole; Figure 2.1) to highly polymerized compounds such as proanthocyanidins (500 to >10,000 g/mole) (Balasundram et al., 2006).

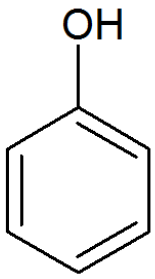


Figure 2.1 Structure of phenol, the simplest phenolic compound.

In plants, phenolics are secondary metabolites that are derived from phenylalanine, and to a lesser extent tyrosine, which are products of the shikimic acid pathway and/or acetyl coenzyme A (acetyl-CoA), which is the starting unit for the acetate pathway (Shahidi, 2000; Shahidi and Naczki, 2003). In fruit cells, phenolic compounds mainly accumulate in cell walls and vacuoles, with higher levels of these compounds being present in external (epidermal and subepidermal) over internal tissues (mesocarp and pulp) (Macheix et al, 2000).

They are most often present in their esterified (e.g. ferulic acid) or glycosylated (e.g. glucose) conjugates rather than as free phenols (Vermerris and Nicholson, 2008). In general, conjugate formation increases phenolic water solubility, improves their mobility within the plant, and provides protection against enzymatic and chemical degradation (Vattem et al., 2005).

In most plants, the key reaction in the synthesis of phenolic compounds is the conversion of phenylalanine to cinnamic acid, which is catalyzed by phenylalanine ammonia lyase (PAL), in

the shikimic acid pathway. Several abiotic and biotic factors regulate the activity of PAL. As an example, fungal infection or plant wounding triggers the transcription of messenger RNA that codes for PAL and consequently increases the biosynthesis of phenolic compounds in the plant (Özeker, 1999). Scientific studies have demonstrated that PAL in plants is encoded by multigene families (Boudet, 2007).

Phenolics are part of the defense mechanism of plants against herbivores, insects and pathogens. Upon exposure/attack, structural and chemical barriers within the plant are activated in order to prevent the pathogen's progression (Ewané, 2012). As an example, phenolics have been reported to interact with the cell membrane of *Fusarium oxysporum* in tomato root so as to inhibit the growth of this fungus (Benhamou and Lafontaine, 1995).

Phenolic biosynthesis can also be influenced by abiotic factors such as climate (e.g. solar radiation, temperature and rainfall), and agronomic practices (e.g. soil fertility, nutrient supplement, water, and pre-harvest treatment) (Kalt, 2005; Lattanzio et al., 2006; Treutter, 2010; Pombo et al., 2011). As examples, soil salinity and drought were found to enhance the production of phenolics in the rockcress plant (*Arabidopsis*) (Zhu et al., 2010); and temperature and light were shown to significantly impact the concentrations of phenolics in the seed and skin of winterberry (*Ilex verticillata*) when harvested in the winter or summer (Xu et al., 2011).

Phenolics have been reported to have a number of positive plant functions that include but are not limited to: protection against pathogens, parasites, predators, ultraviolet radiation and oxidants; cell signalling; attraction of pollinators and seed dispersing animals; and cell wall strengthening (Lattanzio, 2013; Nayak et al., 2015). Phenolics in plants exist as conjugates with aliphatic organic acids, amines, carbohydrates, lipids or other moieties, and their aromatic ring(s) contain varying levels of hydroxylation and methoxylation (Huang et al, 2010; Vladimir-Knežević et al., 2012; Giada, 2013). These variations in structure complexity contribute to the wide range of phenolic molecules in nature, where more than 8,000 different compounds have been identified (Pandey and Rizvi, 2009).

2.1.1 Phenolic Classes

Phenolics all have the basic six-carbon aromatic ring structure (i.e. C_6) with one or more hydroxyl group covalently attached (e.g. phenol). Plant phenolics have been classified into 16 major classes based on the number of carbon atoms present in their structure (Table 2.1) (Giada,

2013). According to literature, the most important classes of phenolics in fruits and vegetables include the phenolic acids and flavonoids (Vattem and Shetty, 2007).

Table 2.1 Major classes of phenolic compounds in plants. (Adapted from Giada, 2013).

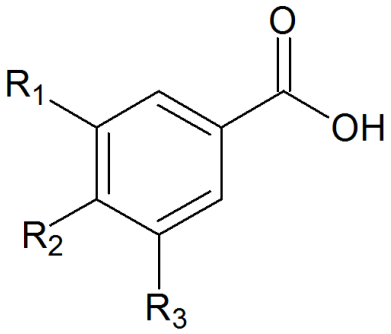
Class	Structure
Simple phenols	C_6
Benzoquinones	C_6
Phenolic acids	C_6- C_1
Acetophenones	C_6- C_2
Phenylacetic acids	C_6- C_2
Hydroxycinnamic acids	C_6- C_3
Phenylpropenes	C_6- C_3
Coumarins	C_6- C_3
Isocoumarins	C_6- C_3
Chromones	C_6- C_3
Naphthoquinones	C_6- C_4
Xanthones	C_6- C_1- C_6
Stilbenes	C_6- C_2- C_6
Anthraquinones	C_6- C_2- C_6
Flavonoids	C_6- C_3- C_6
Lignans and neolignans	$(C_6$- $C_3)_2$
Lignins	$(C_6$- $C_3)_n$

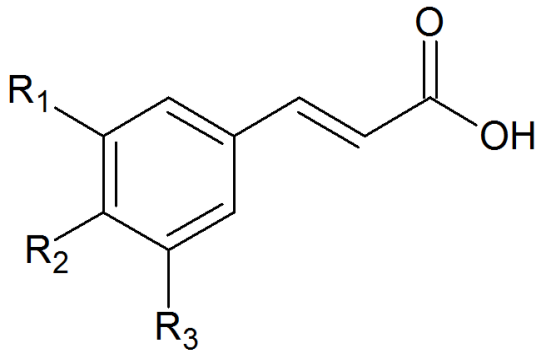
2.1.2 Phenolic Acids

Phenolic acids consist of two subclasses, hydroxybenzoic and hydroxycinnamic acid derivatives, which are structurally defined by the presence of a carboxylic acid functional group (Vermerris and Nicholson, 2008). The most common hydroxybenzoic acids in this subclass include gallic, *p*-hydroxybenzoic, protocatechuic, syringic and vanillic acids. The predominant members of this subclass present in vegetables and fruits are gallic, *p*-hydroxybenzoic and vanillic acids, which are derivatives of benzoic acid (Figure 2.2; Vermerris and Nicholson, 2008). They

are often present as phenolic-conjugates, constituents of more complex structures (e.g. tannins) and as free acids (Fleuriet and Macheix, 2003).

Hydroxycinnamic acids are derivatives of cinnamic acid (Figure 2.2). The most common plant hydroxycinnamic acids include, caffeic, ferulic, *p*-coumaric and sinapic acids (Balasundram et al., 2006). They are the major phenolic acids present in fruits, with caffeic and chlorogenic acids (5-*O*-caffeoylquinic acid) being the most abundant (Robbins, 2003; Manach et al., 2005; Figure 2.3). These compounds are most often present in nature as the hydroxyacid esters of quinic, shikimic or tartaric acids, and as phenolic-carbohydrate and protein conjugates (Fleuriet and Macheix, 2003; Figure 2.3). The presence of the site of unsaturation in the lateral chain of these compounds leads to the possible formation of two geometric isomers, Z (cis) and E (trans). Hydroxycinnamic acids found in nature are mainly present in the E configuration, however isomerization can occur during extraction, purification, and processing (Lee, 2004).





Benzoic acid derivatives	Substituents		
	R_1	R_2	R_3
Benzoic acid	H	H	H
Gallic acid	OH	OH	OH
p-Hydroxybenzoic acid	H	OH	H
Vanillic acid	CH_3O	OH	H

Cinnamic acid derivatives	Substituents		
	R_1	R_2	R_3
Caffeic acid	OH	OH	H
Cinnamic acid	H	H	H
Ferulic acid	CH_3O	OH	H
p-Coumaric acid	H	OH	H

Figure 2.2 Phenolic acids structures.

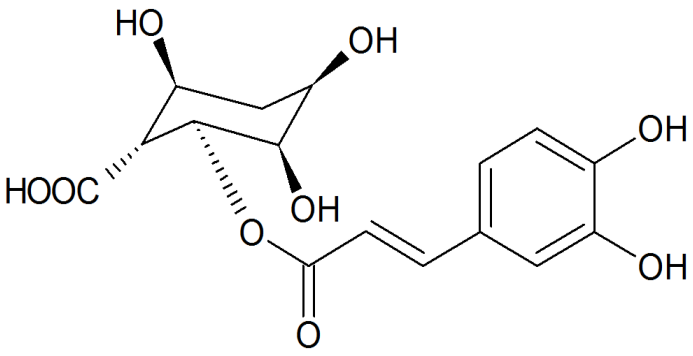


Figure 2.3 Hydroxycinnamic acid derivative: 5-*O*-caffeoylquinic acid or chlorogenic acid.

2.1.3 Flavonoids

The basic structure of a flavonoid consists of two aromatic rings (A- and B-rings) covalently linked via three carbons usually as part of a pyran ring (C-ring), resulting in a C_6–C_3–C_6 carbon configuration (C_{15}) (Figure 2.4).

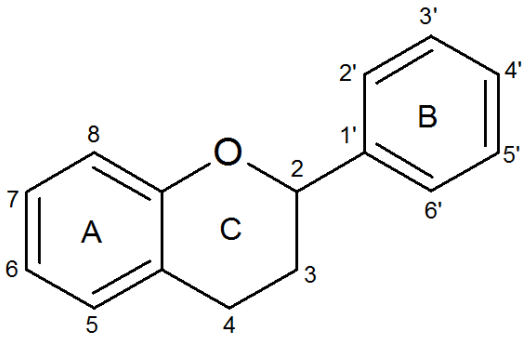


Figure 2.4 Basic flavonoid structure.

Flavonoids are divided into six major subclasses according to their degree of unsaturation and the degree of oxidation of the C-ring (i.e. pyran) into, flavonols, flavanones, flavanols, flavones, anthocyanins and isoflavones (Corradini et al., 2011; Figure 2.5). Flavonoid subclasses differ based on the number and location of hydroxyl and methoxy groups on the A and B rings (Heim et al., 2002). In addition, within these subclasses, substitution on the A and B rings include, acylation, alkylation, glycosylation, hydroxylation, oxygenation and sulfation (Heim et al., 2002; Balasundram et al., 2006, Corradini et al., 2011). As examples: flavonols (e.g. quercetin and

kaempferol), have a site of unsaturation and a hydroxyl group at the 3-position of the oxygenated C-ring; flavanones (e.g. naringenin and taxifolin) have an oxygenated C ring; flavanols (e.g. catechins) have a hydroxyl group at the 3-position of the oxygenated C ring; flavones (e.g. luteolin) have a site of unsaturation in the oxygenated C-ring; anthocyanins (e.g. cyanidin) are characterized by the presence of an oxonium ion (flavylium cation) on the C ring; and for isoflavones (e.g. genistein) the B ring is attached to the C ring at the 3-position, rather than the 2-position as is the case with all other flavonoids (Figure 2.5). Biflavonoids (e.g. theaflavins and thearubigins) are also found in nature and are polymers of two or more flavanol molecules joined covalently (Pandey and Rizvi, 2009). In addition, flavanols can polymerize to form proanthocyanidins (condensed tannins), which are oligomers and polymers of flavan-3-ol monomers.

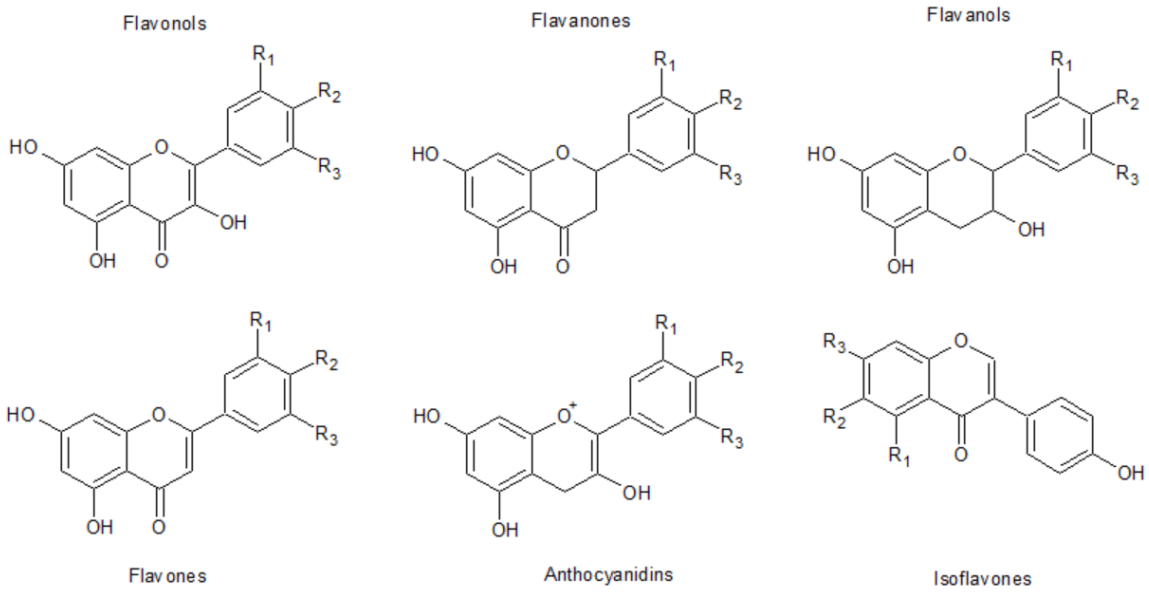


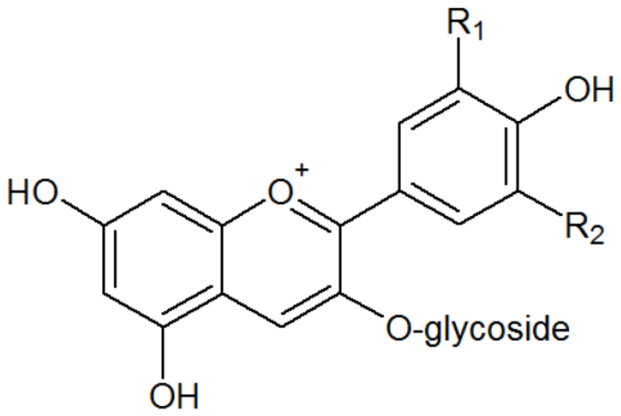
Figure 2.5 Structures of the six major subclasses of flavonoids.

Plant flavonoids are generally glycosylated, and this derivatization can occur at the C-6 and C-8 positions of the A-ring to produce *C*-glycosylflavonoids, or at the hydroxyl positions of the A-, B- and C-rings to produce *O*-glycosylflavonoids, or at the carbohydrate moiety of *C*-glycosylflavonoids to produce *O*-glycosyl-*C*-glycosylflavonoids (Lee, 2004). Carbohydrate moieties include D-arabinose, D-galactose, D-glucose, D-xylose and L-rhamnose (Heim et al.,

2002; Manach et al., 2005; Terahara, 2015). Glycosylation results in increased flavonoid water solubility and decreased free radical scavenging capacity (Corradini et al., 2011).

2.1.4 Anthocyanins

Anthocyanins are members of the flavonoid class of phenolics and are characterized by the presence of an oxonium ion on the C-ring and single/multiple glycosylation(s) at the C-3, C-5 or C-7 positions (Welch et al., 2008; Pandey and Rizvi, 2009; Figure 2.6). The basic structure is an aglycone or anthocyanidin, which is unstable because glycosylation provides increased acid hydrolysis protection for anthocyanins (Welch et al., 2008). The most common anthocyanins in fruits include, cyanidin, delphinidin, malvidin pelargonidin, peonidin and petunidin (Figure 2.6), which differ in functional group(s) and substitution patterns on the B-ring (Corradini et al., 2011).



Anthocyanin	R_1	R_2
Cyanidin	OH	H
Delphinidin	OH	OH
Malvidin	OCH_3	OCH_3
Pelargonidin	H	H
Peonidin	OCH_3	H
Petunidin	OCH_3	OH

Figure 2.6 Structures of the major naturally occurring anthocyanins.

In nature, anthocyanins are glycosides of anthocyanidins linked at the C3- and/or the C5-hydroxyl positions and in rare instances at C7. The most common carbohydrate substituent is D-glucose, however other monosaccharides including D-arabinose, D-galactose and L-rhamnose, and oligosaccharides comprised of combinations of these four carbohydrates have been identified (Brouillard, 1982; Corradini et al., 2011). In addition, they can be acylated by aliphatic and aromatic acids such as acetic, caffeic, ferulic, gallic, malic, malonic and *p*-coumaric (Vermerris and Nicholson, 2008; De Pascual-Teresa et al., 2010).

Both glycosylation and esterification with various organic acids and phenolic acids prevent anthocyanin degradation (Manach et al., 2005). Acyl groups improve anthocyanin stability as intra and/or intermolecular stacking of these groups with the pyrylium ring of the flavylium cation reduces the susceptibility of nucleophile attack by water and subsequent formation of a pseudobase or a chalcone (intramolecular copigmentation) (Williams and Grayer, 2004; Bakowska-Barczak, 2005).

Anthocyanins are water soluble pigments that are responsible for the bright red, blue and purple colours of plant parts such leaves, fruits, flowers, roots and stems (Shahidi and Naczki, 2003). The colour and stability of anthocyanin pigments are highly dependent on a number of environmental and chemical factors including pH, the presence of metal ions, and carbohydrate substitution pattern (Vermerris and Nicholson, 2008).

In addition to their colour pallet, anthocyanins have a number of other biological properties that include but are not limited to, the attraction of animal mutualists (e.g. insects) for pollination and seed dispersal, as chemical deterrents or camouflage agents against herbivores, as antibacterial agents, and as antioxidants (Kong et al., 2003; Cheynier, 2012; Lattanzio, 2013).

2.2 Phenolic Analysis

The Folin-Ciocalteu (FC) assay is both a convenient and simple analytical method that is commonly used to determine the total phenolic content (TPC) of food products (Shahidi and Naczk, 1995; Waterhouse, 2005). However, this method is not specific for phenolics, as other reducing compounds in the sample, such as amino acids (e.g. tryptophan), vitamins (e.g. ascorbic acid) and select minerals (e.g. Fe^{+2}, Mn^{+2}) contribute to the TPC results (Everette et al., 2010; Walker et al., 2010). The FC assay is based on an electron transfer reaction, where a mixture of sodium molybdate and sodium tungstate attracts an electron from the antioxidant compound (i.e.

phenolic) producing a blue colour, which can be monitored spectrophotometrically at 765 nm (Huang et al., 2005; Everette et al., 2010). Standards, usually gallic acid, are used to create a linear curve based on concentration vs. absorption and TPC values are reported as gallic acid equivalents (GAE)/weight of sample (Bakowska-Barczak et al., 2007; Everette et al., 2010).

Chromatography is the method of choice for the separation, identification, and isolation of phenolics in food/fruit samples. The most common chromatographic method for phenolic analysis is high performance liquid chromatography (HPLC) employing a C_{18} stationary phase (i.e. reversed phase chromatography) coupled with photodiode array (PDA) and/or mass spectrometry (MS) detectors. A number of HPLC-PDA methods for phenolic analysis have been published using: an end-capped C_{18} column ranging in length from 100-300 mm, 4.6 mm in diameter and with a stationary particle size of 5-10 µm and a pore size of 80-100 Å; in conjunction with, gradient elution employing an acidified aqueous phase (e.g. acetic, formic, perchloric or phosphoric acid) and an organic phase (e.g. acetonitrile or methanol) (Merken and Beecher, 2000; Robbins, 2003; Khoddami et al., 2013).

Sample preparation for phenolic analysis by HPLC can be accomplished by maceration followed by filtration, however, in the majority of cases phenolic extraction employing an acidified (e.g. formic acid) aqueous organic solvents such as acetone, diethyl ether, ethanol, ethyl acetate and methanol is used (Robbins, 2003). The selection of extraction solvent (i.e. polarity) for phenolics from a sample is based on the structures (e.g. degree of polymerization) of these compounds in the sample, as well as the possible presence of phenolic-food constituent complexes (e.g. phenolic-protein, phenolic-polysaccharide). A major advantage of the use of an extraction solvent system is improved phenolic extraction (i.e. quantity) and more complete phenolic class/subclass extraction, as the aforementioned solvents disrupt/damage the sample (i.e. fruit) cell membranes and simultaneously dissolves the phenolics (Naczk and Shahidi, 2004). The addition of weak acids to the extraction solvent results in phenolic protonation, which provides stability and improves solvent solubility (Khoddami et al., 2013). An alternate approach for phenolic isolation from a sample is through solid phase extraction employing a non-ionic polymeric absorbent hydrophobic resin (e.g. Amberlite® XAD16N). Macerated samples can be added to a resin bed or resin can first be added to the sample and mixed for a select time period to afford phenolic adsorption. A series of increasing hydrophobic mobile phases can then be passed through

the resin to remove carbohydrates, organic acids, minerals, etc., so as to produce a phenolic rich solvent fraction (Green, 2007; Bakowska-Barczak and Kolodziejczyk, 2008).

2.3 Saskatoon Berry

Amelanchier alnifolia Nutt. is a plant that is native to the Western and North central regions of North America. The fruit from this plant has a number of common names that include, saskatoon berry, serviceberry, prairie berry, shadbush berry and juneberry (Lim, 2012). The saskatoon berry plant is a deciduous large shrub/small tree, which typically grows to a height between one and eight meters (Rop et al., 2013). Saskatoon berry belongs to the family Rosaceae and is classified as a pome fruit because it develops from the gynoecial hypanthium portion of the flower (Rogiers and Knowles, 1998). Select examples of fruits belonging to the Rosaceae family include apple and pear.

The mature fruit is dark purple, has a sweet almondy flavour, and ranges in diameter from 10 to 15 mm depending upon cultivar (Zatylny et al. 2005; Ozga et al., 2007). The harvest of mature fruit occurs from July to August and the common Canadian cultivars include, Honeywood, Martin, Northline, Pembina, Regent, Smoky and Thiessen (Berkheimer and Hanson, 2001; Lavola et al., 2012).

Historically, saskatoon berry fruit was used as a food source for indigenous and European populations of the North American prairies as a fresh fruit and also as an ingredient in dried cakes, meat dishes, pemmican, soups and stews (Green and Mazza, 1986; Mazza, 2005). In addition to a food source, this fruit was/is currently used for medicinal purposes including but not limited to the treatment of, ear and eye infections, stomach ailments, and for the prevention of miscarriages (Mazza, 2005; Kraft et al., 2008).

Amelanchier alnifolia Nutt. has considerable potential as a cultivated plant in North America, Europe and Asia due to its growth adaptation to harsh winter climates and the commercial and economic potential of its fruit as a functional raw material in processed foods (e.g. jams/jellies, wine) due to its flavour and nutritional value (McGarry, 1998; Lavola et al., 2012). The application of common food/ingredient processing techniques (e.g. drying, freezing and juice production) to this fruit provides significant market potential for Canadian growers and processors (Hellström et al., 2007).

2.3.1 Chemical Composition

The chemical composition of saskatoon berry fruit varies considerably as it is dependent upon variety, maturity at harvest, and environmental growing conditions (Michalczyk and Macura, 2010). Published proximate analysis results for saskatoon berry fruit show a wide concentration range on a wet basis, as exemplified by the following research results: 0.59-0.67% ash; 17.1-22.63% carbohydrate; 0.40-0.84% lipid; 75.25-82.30% moisture; 1.05-1.94% protein; and 3.80-5.45% total dietary fibre (Mazza, 1982; Mazza and Cacace, 2003; Mazza, 2005; Hosseinian et al., 2007; Bakowska-Barczak and Kolodziejczyk, 2008; Rop et al., 2012).

2.3.2 Phenolic

The most abundant phenolic classes that have been identified in saskatoon berry fruit are the flavonoids and phenolic acids, respectively (Bakowska-Barczak and Kolodziejczyk, 2008). Within the flavonoid class, the major reported constituents are the anthocyanins, specifically cyanidin-based anthocyanins and quercetin glycosides. For phenolic acids, the major identified constituents are hydroxycinnamic acids (Mazza and Cottrell, 2008; Lavola et al., 2012).

The four major anthocyanins identified in saskatoon berry fruit, from highest to lowest concentration are, cyanidin-3-*O*-galactoside, cyanidin-3-*O*-glucoside, cyanidin-3-*O*-arabinoside, and cyanidin-3-*O*-xyloside (Bakowska-Barczak et al., 2007; Ozga et al., 2007; Bakowska-Barczak and Kolodziejczyk, 2008). Other anthocyanins identified in this fruit include cyanidin-3,5-*O*-diglucoside, petunidin-3-*O*-galactoside, and petunidin-3-*O*-glucoside, which are present in low concentrations (Kraft et al., 2008).

Within the flavonoid class, flavonols and catechin/procyanidin derivatives were also detected in saskatoon berries, and included the following, quercetin glycosides, 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 (Ozga et al., 2007; Lavola et al., 2012).

In the phenolic acid class, hydroxycinnamic acids identified in saskatoon berry fruit include, caffeic, chlorogenic (5-*O*-caffeoylquinic acid), dicaffeoylquinic, ferulic, neochlorogenic (3-*O*-caffeoylquinic acid), *p*-coumaric and sinapic acids (Mattila et al. 2006; Bakowska-Barczak and Kolodziejczyk, 2008). Although the phenolic acids analyzed in saskatoon berry fruit were mainly hydroxycinnamic acids, select hydroxybenzoic acids, including ellagic, gallic, *p*-

hydroxybenzoic, protocatechuic and vanillic acids were also detected (Mattila et al. 2006; Kraft et al., 2008; Lavola et al., 2012).

2.3.3 Health Benefits

Phenolic compounds present in fruits exhibit a wide range of purported health benefits due to their capacity in quenching free radicals (e.g. reactive oxygen species [ROS]) from biological systems and consequently preventing/delaying oxidation stress-related diseases (Vattem et al., 2005).

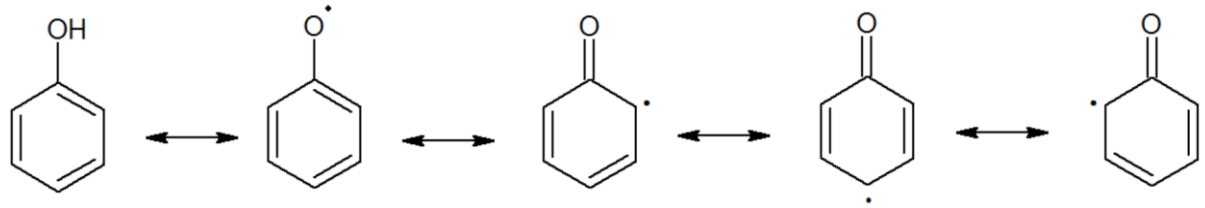
Reactive oxygen species include but are not limited to the, alkoxyl radical (RO•), hydrogen peroxide (H_2O_2), hydroxyl radical (HO•), hypochlorous acid (HOCl), peroxy radical (ROO•), singlet state oxygen ($^1O_2$) and the superoxide anion radical (O_2^-•) (Halliwell et al., 1995; Turrens, 2003). Reactive oxygen species are produced during cellular activities and are important for physiological functions such as antimicrobial activities, energy production and signal transduction for cellular communication (Noguchi and Niki, 1998). However, when ROS are in excess, they initiate autoxidation, a reaction that can oxidize unsaturated lipids, DNA, proteins/ enzymes, nucleic acids and cell membranes (Halliwell, 1991; Sidhu and Al-Zenki, 2005). Free radicals have been implicated in several pathological conditions, such as atherosclerosis, age-related macular degeneration, diabetes, cardiovascular disease, cancer development, cataracts, inflammation and other degenerative diseases (Shahidi and Naczk, 2003).

2.4 Phenolic Antioxidant Mechanisms

Phenolics (ArOH) can react with free radicals (e.g. ROO•, RO•, HO•) by donating electrons or hydrogen atoms to the radical. They are often referred to as free radical scavengers and this mechanism slows the autoxidation process, as the donation of a hydrogen atom from the phenolic occurs at a faster rate than the chain propagation reaction, with the resulting phenoxyl radical (ArO•) being stable (Bakalbassis et al., 2006):

$$RO\bullet + ArOH \rightarrow ROH + ArO\bullet$$

Phenoxyl radicals are stable and either do not participate in the propagation stage of autoxidation or do so less frequently, due to resonance stabilized delocalization of the unpaired electron over the aromatic ring as shown below.



Phenolics can transfer a single electron to an organic free radical to form a radical cation, which undergoes rapid and reversible deprotonation (Wright et al., 2001):

$RO\bullet + ArOH \rightarrow RO^{-} + ArOH^{+}\bullet$

$ArOH^{+}\bullet \rightarrow ArO\bullet + H^{+}$

$RO^{-} + H^{+} \rightarrow ROH$

Phenoxyl radicals can react with organic free radicals (e.g. R•), in termination reactions that result in non-radical products (Masuda et al., 1999):

$R\bullet + ArO\bullet \rightarrow ROAr$

$RO\bullet + ArO\bullet \rightarrow ROOAr$

Phenolics can also chelate metal ions (e.g. Fe^{+2}), which have the capacity to catalyze oxidation reactions by reacting with triplet state oxygen, and also participate in the Fenton reaction. It has been shown that phenolic acids and flavonoids complex iron and copper ions, preventing metal catalyzed reactions and thus inhibit radical formation (Heim et al., 2002; Rice-Evans et al., 1996).

Finally, phenolics have been shown to inhibit enzymes that are responsible for the generation of reactive oxygen species such as cyclooxygenases and lipoxygenases (Nijveldt et al., 2001).

2.5 Lipid Oxidation

Oxidation is an important reaction in foods that commonly occurs when lipids react with oxygen through a free radical mechanism (Kubow, 1992; Thorat et al., 2013). This reaction can occur either chemically, involving oxygen and sites of unsaturation, or enzymatically involving lipoxygenase, oxygen and sites of unsaturation (Reische et al. 1997). Lipid oxidation negatively impacts the nutritional quality, flavour, colour, texture, and appearance of foods. In addition, these reactions result in the production of reactive oxygen species and cyclic lipids that may compromise human health (Viuda-Martos et al., 2011; Thorat et al., 2013).

2.5.1 Mechanism

The direct reaction of unsaturated lipids with oxygen in its triplet state ($^3O_2$; ground state) is thermodynamically unfavourable because ground state lipids are singlet state (^1R), and chemical reactions between reactants with different states are 'not allowed' due to opposite electron spin direction forms (Frankel, 2014). Therefore, a lipid must lose a hydrogen to form a lipid radical, or an electron or a hydrogen atom transfer between an excited triplet state sensitizer (e.g. metal ion) and an unsaturated fatty acid occurs so as to produce free radicals or radical ions; or triplet state oxygen ($^3O_2$) is converted to singlet state oxygen ($^1O_2$) through energy transfer (e.g. heat, energy [UV light]) to a sensitizer (S) (Kubow, 1992; Wasowicz et al., 2004; Schaich, 2015):

$$^1S + \text{energy} \rightarrow {}^3S^*$$
$$^3S^* + {}^3O_2 \rightarrow {}^1O_2 + {}^1S$$

Peroxides are the primary products of lipid oxidation and readily decompose into various secondary oxidation products including, acids, aldehydes, alcohols, alkanes, alkenes, and esters (Xu, 2012; Frankel, 2014; Schaich, 2015). The major steps in the autoxidation reaction are (where RH is an unsaturated fatty acid; $^1O_2$ is singlet state oxygen; *I* is an initiator; ROOH is a fatty acid peroxide and • denotes a free radical):

A. Initiation:

$$RH\ (unsaturated) + {}^1O_2 \rightarrow ROOH$$

$$ROOH \rightarrow RO\bullet + HO\bullet$$

$$\text{or } RH \xrightarrow{I} IH + R\bullet$$

B. Propagation

$$R\bullet + O_2 \rightarrow ROO\bullet$$

$$ROO\bullet + RH \rightarrow R\bullet + ROOH$$

$$ROOH \rightarrow RO\bullet + HO\bullet$$

$$RO\bullet + RH \rightarrow R\bullet + ROH$$

C. Termination:

$$R\bullet + R\bullet \rightarrow RR$$

$$R\bullet + ROO\bullet \rightarrow ROOR$$

$$ROO\bullet + ROO \rightarrow ROOR + O_2$$

The first step in the autoxidation reaction between a site of unsaturation and oxygen (both in their singlet states) results in the formation of peroxides. Subsequent reactions between free radicals and unsaturated lipids occur because the methylene group allylic to a site of unsaturation and particularly between a non-conjugated unsaturated pair is reactive because the resulting allylic radical is stable, due to delocalization (Kubow, 1992). During the propagation reaction, alkyl radicals may also react with molecular oxygen to form peroxyl radicals, which in turn can further react with unsaturated lipids (Frankel, 2014). The termination step occurs when two radicals react to form a non-radical product or due to hydrogen donor or electron donor reactions (Kubow, 1992).

2.5.2 Synthetic Antioxidants

Antioxidants are compounds that at low concentrations retard the oxidation of biomolecules, such as lipids and proteins in food products, thus improving product shelf life by protection against oxidative deterioration (Karre et al., 2013). The use of antioxidants in foods reduces raw material waste and nutritional loss and increases the potential use of polyunsaturated lipids in product formulations (Thorat et al., 2013).

In order for an antioxidant to be used in a food product, it must be: nontoxic, effective at low concentrations, stable, capable of surviving processing (e.g. carry-through effect), and their

colour, flavour, and odor must be minimal (Reische et al. 1997). Also, legislation and product compatibility are factors that must be considered when determining the choice of an antioxidant in a food formulation (Thorat et al., 2013).

The most common synthetic antioxidants used to delay the oxidative deterioration of lipids in processed foods are butylated hydroxyanisole (BHA), butylated hydroxytoluene (BHT), propyl gallate (PG) and tert-buty-hydroquinone (TBHQ) (Frankel, 2014; Figure 2.7). They are most often used in concentration levels less than 0.01% (based on lipid content), and in Canada, their maximum concentration alone or in combination is 0.02% (Reische et al. 1997; Health Canada, 2015). Despite their efficacy, low cost, and high stability, the potential toxicological implications of synthetic antioxidants (e.g. carcinogenicity) and consumer demand for both natural ingredients and clean product labels, have resulted in renewed research into the identification and use of natural antioxidants derived from fruits and vegetables (Viuda-Martos et al., 2011).

Synthetic antioxidants delay autoxidation reactions by interfering in the propagation or initiation steps of lipid oxidation through the donation of hydrogen atoms to lipid alkyl, alkoxyl and peroxyl radicals (Ladikos and Lougovois, 1990; Frankel, 2014). During the propagation reaction, synthetic antioxidants are converted to a stable phenoxyl radical, which is stable due to, delocalization of the unpaired electron in the aromatic ring, stabilization of the free radical at the ortho and para positions of the aromatic ring due to electron donation by alkyl groups, and steric hindrance (Shahidi and Naczk, 2003; Frankel, 2014).

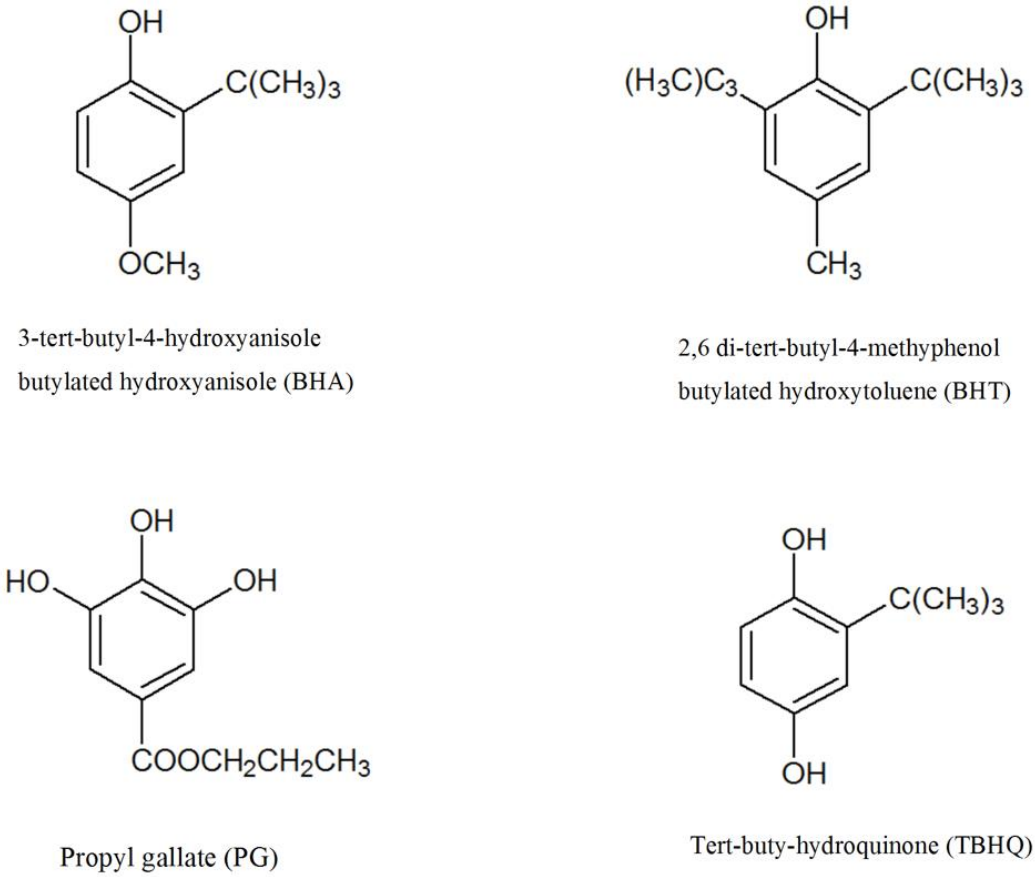


Figure 2.7 Structures of the major synthetic antioxidants used in processed food (Adapted from Frankel, 2014).

2.6 Fruit Juice Processing

A literature review found no published information on juice production from saskatoon berry. However, commercial fruit juice production from pome fruits such as apple and pear generally involves the following processing stages: (a) the fruit is washed with water to remove external contaminants; (b) the fruit is mashed by milling or crushing (e.g. hammer mill, horizontal crusher) and heated (50 to 90^{o}C for 30-60 s to destroy native enzymes (e.g. polyphenol oxidase); (c) the mash is treated with an enzyme cocktail containing amylase(s), hemicellulase(s) and pectinase(s) (i.e. carbohydrases), which aid in cell wall breakdown and increase juice yield; (d) juice extraction employing belt and/or hydraulic presses; (e) extracted juice clarification via sedimentation which may include treatment with additional carbohydrases (i.e. pectinase

treatment) followed by filtration; and (e) juice pasteurized (70 to 100°C for 6 to 40 s) for direct packaging or juice pasteurization and evaporation (multiple effect evaporator) to produce a concentrate (70-71 °Brix) (Spanos and Wrolstad, 1992; Bates et al., 2001; Fang et al., 2006; Ribeiro et al., 2010).

2.7 Co-product Stream from Fruit Juice Processing

Pomace is the material that remains following juice extraction from fruit and is considered to be a co-product of the fruit juice industry. In general, pomace represents 25–30% of the total weight of processed fruits following extraction and this co-product consists of pulp, skins, seeds, and stems (Bhushan et al., 2008; White et al., 2010). This co-product has a rich chemical composition that is comprised of, soluble and insoluble carbohydrates, proteins, minerals and other compounds including phenolics (Zheng and Shetty, 2000; Shalini and Gupta, 2010; Martín-Sánchez et al., 2014).

In the food industry, co-product streams such as pomace are often used as fertilizers and/or animal feed (Laufenberg et al., 2003; Vattem et al., 2005). However, value-added products such as pectin are extracted from citrus peel and apple pomace for use as a gelling agent in the food and beverage industries (Monspart-Senyi, 2006; Viuda-Martos et al., 2011). In addition, new applications for pomace are being explored which include but are not limited to their use as a substrate in microbial processes for the production of chemicals such as ethanol, organic acids and pigments (Zheng and Shetty, 2000, White et al., 2010).

To date, there has been only one published paper (Li et al., 2014) on phenolic compound identification and free radical scavenging capacity of dry pomace from saskatoon berry. As such, there is a lack of scientific information on phenolic composition and concentrations, antioxidant capacity and use of either the pomace directly or solvent fractions from pomace in food formulations containing polyunsaturated lipids.

3. MATERIALS AND METHODS

3.1 Samples

Martin, Pembina (2015 crop), and Northline (2015 and 2016 crops) saskatoon berry varieties (all "jam quality"), pomace from commercial saskatoon berry juice production (wet pomace [Northline variety] and dry pomace [combined Martin, Northline and Thiessen varieties], 2013 crops) were purchased from Prairie Berries Inc. (Keeler, SK). Fruit and pomace samples were stored at -30°C ± 2°C until analyzed.

Commercial enzymes used for pear juice production were provided by Novozymes (Bagsvaerd, Denmark) and included the following: Pectinex Ultra Clear, Pectinex Ultra Mash, and Amylase AG 300. All enzyme solutions were stored at 4 ± 1°C.

3.2 Chemicals

All solvents used in this research were of American Chemical Society (ACS) grade or higher. The following chemicals were obtained from Sigma-Aldrich Canada Ltd. (Oakville, ON): α-amylase solution (A-3306); amyloglucosidase solution (A-9913); arbutin; 2,2'-azinobis (3-ethylbenzothiazoline-6-sulfonic acid) (ABTS); caffeic acid; catechin; celite; cellobiose (*O*-β-D-glucopyranosyl-(1→4)-*O*-D-glucopyranose); cellotriose (*O*-β-D-glucopyranosyl-(1→4)-*O*-β-D-glucopyranosyl-(1→4)-*O*-D-glucopyranose); chlorogenic acid; *p*-coumaric; cyanidin-3-*O*-glucoside; cyanidin-3-*O*-rutinoside; digalacturonic acid (*O*-α-D-galactopyranuronosyl-(1→4)-D-galacturonic acid); 2,2'-diphenyl-1-picrylhydrazyl (DPPH); ellagic acid; epicatechin; ferulic acid; Folin-Ciocaulteu reagent (FC); D-fructose; D-galacturonic acid; gallic acid; D-glucose; 4-hydroxybenzoic acid; isomaltotriose (*O*-α-D-glucopyranosyl-(1→6)-α-D-glucopyranosyl-(1→6)-*O*-D-glucopyranose); maleic acid; D-malic acid; malonic acid; maltose (*O*-α-D-glucopyranosyl (1→4)-*O*-D-glucopyranose); narigenin; pectin from apple; phloridzin; potassium persulfate; protease solution (P-3910); quercetin; quinic acid; raffinose (*O*-α-D-galactopyranuronosyl-(1→6)-α-D-glucopyranosyl-β-D-fructofuranoside); rutin; starch from wheat; succinic acid, Sylon TP (TMSI + pyridine, 1:4); and trigalacturonic acid (*O*-α-D-galactopyranuronosyl-(1→4)-*O*-α-D-

galactopyranuronosyl-(1→4)-D-galacturonic acid).

The following chemicals were purchased from Santa Cruz Biotechnology (Dallas, TX, USA): D-galacturonic acid methyl ester, 6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid (Trolox) and vanillic acid. Maltopentaose (*O*-α-D-glucopyranosyl-(1→4)-α-D-glucopyranosyl-(1→4)-α-D-glucopyranosyl-(1→4)-α-D-glucopyranosyl-(1→4)-D-glucopyranose) and maltotriose (*O*-α-D-glucopyranosyl-(1→4)-α-D-glucopyranosyl-(1→4)-Glc(1→4)-D-glucopyranose) were purchased from Supelco Analytical (Bellefonte, PA, USA).

Chemicals obtained from BDH (Edmonton, AB) were acetic and ascorbic acids. Cyanidin-3-*O*-xyloside was obtained from Toronto Research Chemicals Inc. (Toronto, ON) and cyanidin-3-*O*-arabinoside from Extrasynthase S. A. (Genay, France). 1,2-Dithio-DL-threitol was purchased from Fluka Chemie GmbH (Buchs, Switzerland).

Chemicals obtained from Fisher Scientific (Edmonton, AB) were: acetone; acetonitrile; boric acid; formic acid; hydrochloric acid; Kjel catalyst mixture (copper sulphate and sodium persulfate); methanol; 2-(4-N-morpholino)ethanesulfonic acid; N-point indicator; oxalic acid; petroleum ether; phosphoric acid; potassium chloride; sodium acetate trihydrate; sodium carbonate; sodium hydroxide solution (50% w:w); sulfuric acid and tris(hydroxymethyl)aminomethane (MES-TRIS). Ethanol (95% (v:v) and anhydrous) was obtained from Commercial Alcohols Inc. (Brampton, ON) through the College of Agriculture and Bioresources chemical stores (Saskatoon, SK). The water used in this research was produced from a Millipore Milli-Q™ water system (Millipore Corporation, Milford, MA, USA).

3.3 Physicochemical Analysis

3.3.1 Sample Preparation

Saskatoon berry fruits were thawed at 4 ± 1°C for 24 h and the juice was obtained by manually pressing approximately 50 g of fruit between multiple layers of cheesecloth (Fischer Scientific, Edmonton, AB). This juice was used for all physicochemical analyses except fruit size.

3.3.2 Colour

Juice colour parameters were measured employing a ColorFlex EZ colorimeter (Hunterlab Labscan 6000, Hunter Associates Laboratory Inc., Reston, VA, USA). The colorimeter was standardized using a white tile (standard no. LS-13903) with colour coordinates L* = 92.73, a* =

-0.93 and b*= 0.67. Colour parameters (L*: lightness/darkness; a*: red/green; b*: yellow/blue) were determined on 20 mL of saskatoon berry juice for each variety. The observed angle was set at 10° and three separate measurements were taken following rotation of the sample cup by ~30°. Hue angle and chroma were determined by calculating $\tan^{-1}$ (b*/a*) and the square root of $(a^*)^2 + (b^*)^2$, respectively. Juice colour was measured immediately after sample preparation as outlined in section 3.3.1. All samples were analyzed in triplicate (n = 3).

3.3.3 pH

Juice pH was measured employing a SympHony SP70P pH meter (VWR International, Edmonton, AB). The pH meter was calibrated using a three point standard with pH buffers of 2.00, 4.00 and 7.00 (Fischer Scientific). All samples were analyzed in triplicate.

3.3.4 % Seed Weight

Whole fruit (10 to 30 g) was thawed overnight at 4°C and accurately weighed (±0.0001) using an analytical balance (Mettler-Toledo Inc., Mississauga, ON). The fruit was carefully ground using a mortar and pestle maintaining the seeds intact. Seeds were separated manually and transferred into pre-dried and pre-weighed (±0.0001 g) aluminum dishes (70 mm, Fischer Scientific) and dried overnight (12-16°C) at 105°C in a forced air oven (Isotemp Premium Ovens 700 Series, Fisher Scientific, Dubuque, IA, USA). Dried seeds were then allowed to cool in a desiccator for 1h and weighed. Sample % seed weight was determined as follows:

$$\% \text{ Seed weight} = \frac{(\text{weight of dried seeds + dish}) - \text{dish weight}}{\text{weight of whole fruit sample}} \times 100$$

3.3.5 Size

Both frozen and thawed fruit size was measured using an electronic digital caliper (VWR International) on 10 randomly selected fruits for each variety. Fruit diameter was measured on frozen berries (-20 ± 2°C) and the same berries after thawing at room temperature (20-21°C) for 45 min.

3.4 Proximate Analysis

3.4.1 Sample Preparation

Frozen saskatoon berry fruit was thawed at 4 ± 2°C for 24 h and then macerated using a blender (Osterizer™, Sunbeam Canada, Toronto, ON) at speed #10 for 2 min. Wet and dry representative pomace samples were individually ground (IKA A11 Basica, North Chase, NC, USA) for three separate 15 s intervals, with mixing between each grinding regimen. Samples were stored at -30 ± 2°C until analyzed.

3.4.2 Ash

Sample ash content was determined gravimetrically employing the dry ashing method as outlined in AOAC Method 940.26 (AOAC, 2000). Samples (2-3 g) were accurately weighed (±0.0001 g) and transferred into pre-weighed porcelain crucibles (50 mL; Fisher Scientific), and were initially pre-heated on a hot plate (VWR International) until the sample darkened and the resulting grey/black smoke was no longer visible. The pre-heated samples were then transferred to a muffle furnace (Thermolyne Corporation, Dubuque, IA, USA) at 550°C for 12-16 h. Samples were transferred to, and allowed to cool to room temperature in a desiccator for 1 h and were weighed. All samples were analyzed in triplicate. Sample ash content was determined as follows:

$$\% \text{ Ash} = \frac{(\text{weight of crucible + sample}) - \text{weight of dry crucible}}{\text{weight of sample before ashing}} \times 100$$

3.4.3 Moisture

Sample moisture content was determined gravimetrically by forced air oven drying according to AOAC Method 925.10 (AOAC, 2000). Moisture content was conducted on both macerated fruit samples and wet and dry pomace. Whole fruit was thawed at 4 ± 2°C for 24 h prior to analysis. Aluminum dishes (70 mm, Fischer Scientific) were pre-dried in a forced air draft oven at 105°C for 12 h and were allowed to cool in a desiccator for 1 h. Fruit samples (3-4 g) were accurately weighed (± 0.0001 g) into pre-weighed and dried aluminum dishes followed by drying at 105°C for 16-20 h. The dried samples were then allowed to cool in a desiccator for 1 h and weighed. All samples were analyzed in triplicate. Sample moisture content was determined as follows:

$$\% \text{ Moisture} = \frac{(\text{weight of dried sample + dish}) - \text{dish weight}}{\text{weight of sample before drying}} \times 100$$

3.4.4 Protein

Sample protein content was determined employing the Kjeldahl nitrogen analysis method according to AOAC Method 920.152 (AOAC 2000). Nitrogen content was converted to percent protein using the common factor of 6.25. Samples (2-3 g) were accurately weighed (±0.0001 g) on nitrogen-free weighing paper (Fischer Scientific) and the filter paper containing the sample was quantitatively transferred to a Kjeldahl digestion flask (KDF), followed by the addition of two tablets of Kjel catalyst ($CuSO_4$ + $Na_2S_2O_8$) and 20 mL of concentrated sulfuric acid. Sample digestion was carried out at setting #8 for 15 min followed by increased heating to setting #10 for 1 h. Following digestion, the KDF was placed on a cooling rack for 10-20 min, and 70 mL of water was added, followed by steam distillation with 80 mL of a 30% (w:w) sodium hydroxide solution for approximately 5 min. The distillate was collected in a 250 mL Erlenmeyer flask containing 30 mL of 4% boric acid and 4 drops of N-point indicator. The resulting solution was titrated with 0.10 N hydrochloric acid. A blank consisting of all reagents and filter paper, and a standard of glycine (0.50 ± 0.01 g) were run in conjunction with all samples. All samples were analyzed in triplicate. Sample nitrogen and protein contents were determined as follows:

$$\% \text{ N} = \frac{(\text{mL HCl sample - mL HCl blank}) \times 0.1 \times 14.007}{1000 \times \text{sample weight}} \times 100$$

$$\% \text{ Protein} = \%\text{N} \times 6.25$$

Where:

0.10 = HCl Normality

14.007 = molecular mass of nitrogen (g/mole)

6.25 = conversion factor

3.4.5 Lipid

Sample lipid content was determined employing a continuous extraction protocol (Goldfish) according to AOAC Method 960.39 (AOAC 2000) with petroleum ether as the solvent. Fruit samples were dried and ground prior to lipid analysis. The dried ground samples (3-4 g) were accurately weighed (±0.0001 g) on filter paper (12.5 cm; Whatman #1; VWR International), which was folded and placed into a cellulose extraction thimble (VWR International). Approximately 50 mL of petroleum ether was added to a pre-dried and pre-weighed fat extraction beaker (FAB) and the extraction thimble and FAB were attached to the Goldfish apparatus (Labconco, Kansas City, MO, USA). Extraction was conducted for 8 h under controlled heating so as to obtain a condensation rate of ~1 drop/sec. The majority (>90%) of the petroleum ether was recovered and the remainder of the solvent was removed in the fume hood employing a hot plate (VWR International) for ~15 min. The FAB was transferred to a forced air oven at 105^{o}C for 30 min, and was then allowed to cool to room temperature in a desiccator for 1 h followed by gravimetric analysis. Crude lipid content (dry weight basis) was determined as follows:

$$\% \text{ Lipid} = \frac{(\text{weight of FAB + lipid}) - \text{FAB weight}}{\text{weight of dried sample}} \times 100$$

3.4.6 Total Dietary Fibre

Sample total dietary fibre content was determined by enzymatic digestion according to AOAC Method 991.43 (AOAC, 2000). Pre-dried and defatted samples (1 g) were accurately weighed (±0.0001 g) and transferred into a 400 mL beaker followed by the addition of 50 mL of 0.05 M MES-TRIS buffer [19.52 g of 2-(4-N-morpholino)ethanesulfonic acid and 14.2 g of tris(hydroxymethyl)aminomethane in 1.7 L of water, followed by pH adjustment to 8.2 with the dropwise addition of 6.0 N NaOH. Fifty microliters of α-amylase enzyme solution (A-3306, Sigma-Aldrich, St. Louis, MO) was added and the beaker was covered and placed in a water bath (Haake D1, Berlin, Germany) at 95-100°C for 30 min. The resulting mixture was cooled to <60 °C followed by the addition of 100 µL of protease enzyme solution (P-3910, Sigma-Aldrich) and the covered beaker was placed in the shaking/agitating water bath at 60°C for 30 min. To this mixture was added 5.0 mL of 3.0 M acetic acid solution and 100 µL of amyloglucosidase solution (A-9913, Sigma-Aldrich) followed by incubation in the same water bath at 60°C for 30 min.

Following enzymatic hydrolysis, ~220 mL of 95% ethanol was added, and the beaker was covered with aluminum foil and kept static at room temperature for 10-12 h. The resulting precipitate was collected by vacuum filtration using a dried and pre-weighed (±0.0001 g) filtration crucible (Pyrex no. 32940, coarse ASTM 40~60 µm, 50 mL; Fisher Scientific) containing ~0.5 g of celite, followed by sequential treatment with ~8 mL portions of 78% ethanol, 95% ethanol and acetone. The filtration crucible plus residue was transferred to a forced-air oven and dried at 105°C for 10-12 h, and was allowed to cool in a desiccator for 1 h followed by gravimetric analysis. Total dietary fibre (dry weight basis) was determined as follows:

$$\% \text{ Total dietary fibre} = \frac{\text{crucible with residue weight - crucible and celite weight}}{\text{sample weight}} \times 100$$

3.4.7 Carbohydrate/Total Soluble Solids (°Brix)

Sample total soluble solids expressed as °Brix was determined by refractometry (Auto Abbe Refractometer, Model 10504, Leica Inc., Buffalo, USA) with temperature compensation, according to AOAC method 932.12 (AOAC, 2000). Total soluble solids content was conducted on juice samples, and wet and dry pomace. Ground wet and dry pomace (IKA A11) were diluted with water (1:2, w:w basis) and then mixed by blending (Sunbeam Canada). All samples were analyzed in triplicate (n = 3).

3.5 Amino Acids

The amino acid composition of each saskatoon berry variety was determined employing AOAC Official Methods 985.2 and 988.15 (AOAC, 2003). Briefly, 20 g of saskatoon berry fruit was dried overnight at 105°C (16 h) and then ground to produce a fine powder. To individual 20 x 150 mm screw cap Pyrex tubes 2 g of saskatoon berry powder was added followed by the addition of 15.00 mL of 6 N HCl for total amino acids, or 15 mL of 10 M NaOH for tryptophan. Samples were then flushed with N_2. Tubes were capped and oven heated at 110°C ± 0.5°C for 20 h. Following acid/base protein digestion, individual amino acids were quantified using high performance liquid chromatography employing the pico-tag amino acid analysis system (Waters Corporation, Milford, MA). All samples were analyzed in duplicate (n = 2).

3.6 Carbohydrates/Polyols

Saskatoon berry juice was prepared as described in section 3.3.1. The total soluble solids content of the expressed juice was determined by refractometry (Auto Abbe refractometer; Leica Inc., Buffalo, NY) followed by a 1:1 dilution with water to reach 6.0-8.0 ± 0.1 °Brix. The resulting diluted sample was syringe filtered (nylon, 0.2 µm pore size; 13 mm diameter; Chromatographic Specialties, Brockville, ON) prior to analysis. Major carbohydrate, including fructose, glucose, sucrose and sorbitol (polyol) concentrations, were determined by high performance liquid chromatography with refractive index detection (HPLC-RI) on an Agilent 1100 series HPLC system (Agilent Technologies Canada Inc., Mississauga, ON). The components of the HPLC system were the solvent degasser, quaternary pump, auto sampler, column heater, refractive index detector (RI) and system control by Chemstation LC-3D software (Revision B.04.01). Carbohydrate separation was performed on a Capcell-Pak 5 µm NH2 UG-80S5 column, 250 x 4.6 mm, with a CapCell guard cartridge (Phenomenex, Torrance, CA) employing an isocratic mobile phase of acetonitrile:water (75:25, v:v) at a flow rate of 1.0 mL/min and the sample injection volume was 5.0 µL. Samples were syringe filtered (nylon, 0.2 µm pore size, 13 mm diameter, Chromatographic Specialties, Brockville, ON, Canada) into 2 mL amber HPLC vials (Chromatographic Specialties) prior to analysis. Carbohydrate detection was by refractive index maintained at 25 ± 1°C. Calibration curves for fructose, glucose, sorbitol and sucrose were constructed at concentrations ranging from 5.00-50.00 g/L in water, and had correlation coefficients ≥0.999. All samples were analyzed in triplicate.

Due to the low sucrose concentration and validation for the presence of sorbitol in these samples, they were also analyzed employing a Dionex ICS 5000 HPLC system (Thermo Fisher Scientific, Waltham, MA, USA) equipped with a Dionex AS autosampler, and an ICS 5000 electrochemical cell with a disposable gold electrode. The potentials and durations of the gold electrode were as follows: E1 = 0.10 V, t1 = 0.00 s; E2 = −2.00 V, t2 = 0.41 s; E3 = 0.60 V, t3 = 0.43 s; E4 = −0.10 V, t4 = 0.44 s; E5 = −0.10 V, t5 = 0.50 s and data acquisition was carried out using Dionex Chromeleon 7.0 software (Revision B.04.01). Carbohydrate separation was accomplished using a Dionex CarboPac PA1 column (4 x 250 mm) in series with a CarboPac PA1 guard column (4 x 50 mm) at room temperature in conjunction with an isocratic mobile phase of 80 mM NaOH. The flow rate was 1.0 mL/min and the sample injection volume was 20.0 µL. Samples were syringe filtered into 2 mL amber HPLC vials (Chromatographic Specialties) prior

to analysis. All samples were analyzed in triplicate (n = 3).

3.7 Galacturonic Acid and Oligosaccharides

Galacturonic acid and oligosaccharide analysis were carried out using a Dionex ICS 5000 HPLC system (Thermo Fisher Scientific, Waltham, MA, USA) as described in section 3.6. Separation was accomplished using a Dionex CarboPac PA1 column (4 x 250 mm) in series with a CarboPac PA1 guard column (4 x 50 mm) at room temperature (21–23°C). A linear gradient elution program was used for oligosaccharide separation where solvent A was 160 mM sodium hydroxide (NaOH), solvent B was 160 mM NaOH/1.0 M sodium acetate (NaOAc) and solvent C was 1.0 M NaOH. The gradient conditions were 100.0% A for 1.5 min; linear gradient to 75.0% B at 75.0 min; 100.0% B at 75.1 min with a hold until 78.0 min; 100.0% C at 78.1 min with a hold until 80.0 min; 100.0% A at 80.1 min with a hold until 90.0 min (total sample run time including column equilibrium was 80 min). The flow rate was 0.7 mL/min. The injection volume was 20.0 µL. Samples were syringe filtered into 2 mL amber HPLC vials (Chromatographic Specialties) prior to analysis. Standard curves for galacturonic acid, maltose, maltotriose, and maltopentaose were prepared in water at concentrations ranging from 1.0 to 100.0 ppm with correlation coefficients >0.999. All samples were analyzed in triplicate. To confirm oligosaccharide identification, samples were also analyzed by capillary gas chromatography with flame ionization detection (CGC-FID) on an Agilent 6890 gas chromatograph equipped with an Agilent 6890 series autosampler (Agilent Technologies Canada Inc., Mississauga, ON, Canada) as previously described by Willems and Low, 2014. Briefly, 150 µL aliquots of each sample were freeze-dried (Heto Lab Equipment, Allerod, Denmark) in individual 12 x 32 mm glass vials (Chromatographic Specialties Inc.). To the resulting dried sample, 500 µL of Sylon TP was added and the vials were capped and transferred to a block heater (Denville Scientific Inc., Metuchen, NJ, USA) maintained at 70.0°C for 1 h with shaking every 10–15 min.

3.8 Major Minerals

Sample major mineral (i.e. Ca, K, Mg, and Na) content was determined by inductively coupled plasma (ICP) optical emission spectroscopy according to AOCS Ca 17-01 and AOCS-Ca 20-99. Briefly, 20 g of saskatoon berry fruit was dried overnight at 105°C (16 h) and then ground to a fine powder. The dried powder (2 g) was then digested in 10 mL of a 1:1 mixture (v:v) of

concentrated nitric and sulphuric acids on a hot plate (VWR International) for 6 hours. The mixture was taken to almost dryness followed by the addition of 2.0 mL of concentrated nitric acid and the resulting solution was quantitatively transferred to a 25 mL volumetric flask and brought to volume with water. All samples were analyzed in duplicate (n = 2).

3.9 Organic Acids

The organic acid content of saskatoon berry juice samples (section 3.3.1) was determined by high performance liquid chromatography with photodiode array detection (HPLC-PDA) on an Agilent Technologies 1100 Series HPLC system (section 2.1.6). Organic acid separation was performed on a Restek Allure organic acids column (250 x 4.6 mm, 5 µm, 60 Å; Chromatographic Specialties) employing an isocratic mobile phase of 100 mM K_2HPO_4 adjusted to pH 2.5 with 50 mM ortho-phosphoric acid maintained at a flow rate of 0.7 mL/min. Organic acid detection was achieved employing a PDA detector with monitoring at 226 nm with reference at 360 nm. The sample injection volume was 20.0 µL. Samples were syringe filtered into 2 mL amber HPLC vials prior to analysis. The reducing agent, 1,2-dithiothreitol (1.5 mg/2 mL) was added to samples and standards prior to HPLC analysis, so as to ensure that sample ascorbic acid was in the reduced state. Standard curves were prepared with the major organic acids that have been identified in fruits including, acetic, ascorbic, citric, maleic, malic, malonic, oxalic, quinic and succinic acids, which were analyzed individually and as a mixture. Sample organic acid identification and quantification were conducted by comparisons of retention times and calibration curves to standards, respectively. The concentration of organic acid standards ranged from 20-5000 mg/L in water. Standard curves had correlation coefficients ≥0.999. All samples were analyzed in triplicate.

3.10 Phenolics Extraction

3.10.1 Aqueous Extract

Frozen saskatoon berry fruit and pomace (wet and dry) were thawed at 4 ± 2°C for 24 h. Aqueous extracts were prepared for each saskatoon berry variety and also for wet and dry pomace following AOAC Method 920.149 (AOAC, 2000) with modification. Briefly, for whole fruit, 25 g (±1.00 g) was homogenized with 50 mL of water by mechanical blending the fruit at speed #10 for 3 min; for pomace, 5 g (±0.50 g) was ground (IKA A11) for three separate 15 sec intervals. The resulting macerate or ground material was quantitatively transferred to a 250 mL beaker with

water washing to a total volume of 80 mL. This solution was brought to boil at 100°C for ~ 30 min with constant stirring @600 rpm on a hotplate/stirrer (VWR International). The water lost due to evaporation was replaced at 10-15 min intervals. The resulting solution was cooled to room temperature (22 ± 2°C) for 30 min and was vacuum filtered (VWR-413, 12.5 cm), and washed with approximately 20 mL of water. The filtrate was quantitatively transferred to a 100 mL volumetric flask and brought to volume with water. Extracts were stored at -30 ± 2°C until analyzed. All samples were analyzed in triplicate (n = 3).

3.10.2 Ethanol-and Methanol-Formic Acid-Water Extracts

Ethanol-and methanol-formic acid-water extracts (EFW and MFW, 70:2:28% (v:v:v)) were prepared for each saskatoon berry variety, and for wet and dry pomace using 25 g (±1.00 g) and 5 g (±0.50 g) of pomace, respectively. The fruit/pomace sample was weighed into a 250 mL beaker and 40 mL of solvent (EFW or MFW) was added followed by homogenization as outlined above. The homogenate was covered with parafilm and stored at 4°C for 12-16 h with constant stirring at 600 rpm. The resulting solution was vacuum filtered and washed with approximately 10 mL of solvent, and the filtrate was retained. The sediment was removed from the filter paper and re-suspended in 40 mL of solvent and stirred for 10 min at room temperature at 600 rpm. The resulting mixture was then filtered and the sediment was washed with 10 mL of solvent. Filtrates were combined and quantitatively transferred to a 100 mL volumetric flask and brought to volume with the appropriate solvent. Extracts were stored at -30 ± 2°C until analyzed. All samples were analyzed in triplicate (n = 3).

3.11 Fractionation of Saskatoon Berry and Pomace EFW Extracts

Amberlite® XAD16N resin was used to fractionate saskatoon berry and wet pomace EFW extracts (section 3.10.2). The resin was initially hydrated in 50% aqueous ethanol (i.e. 50% ethanol) for 30 min with slow agitation. Sufficient hydrated resin was then transferred to glass column (50 × 2.5 cm) to produce a resin bed of approximately 33 mL. The resin bed was washed sequentially with 90 mL of water, 90 mL of 90% (v:v) aqueous ethanol (i.e. 90% ethanol) and 90 mL of water. Individual 10.0 mL aliquots of each of the previously prepared saskatoon berry sample and wet pomace EFW extracts (section 3.10.2) were evaporated (30°C) to dryness (BUCHI Labortechnik AG, Switzerland) and weighed. Dried extracts were then re-dissolved in 5.0 mL of

water for Martin, Northline and Pembina samples and 2.5 mL of 100% ethanol + 2.5 mL water for wet pomace. Each diluted sample was quantitatively transferred to the resin column followed by sequential fractionation with 90 mL each of water (fraction 1), 40% (v:v) ethanol (fraction 2), 70% (v:v) ethanol (fraction 3), and 100% ethanol (fraction 4). Individual fractions were concentrated to dryness at 30°C by rotoevaporation and freeze drying (Heto-Holten A/S, Allerod, Denmark). Freeze dried fractions were stored in lightproof containers at -30°C.

3.12 Total Phenolic Content

Total phenolic content (TPC) by the Folin-Ciocalteu method and total phenolic chromatographic index (TPCI) by HPLC-PDA were determined for all three saskatoon berry varieties, and wet and dry pomace extracts (section 3.10), and all saskatoon berry and pomace fractions (section 3.11).

3.12.1 Total Phenolic Content (TPC) by Folin-Ciocalteu Assay

The total phenolic content for all samples was determined using the Folin-Ciocalteu (FC) assay. Sample dilutions were as follows: the aqueous extract was diluted 1/10 with water; the ethanol-formic acid extract was diluted 1/20 with 70% ethanol; and the methanol-formic acid extract was diluted with 1/15 70% methanol, so as to obtain results that were well within standard curves. The following reagent addition sequence was used and must be followed to avoid precipitate formation so as to obtain accurate and reproducible results. A 250 µL aliquot of each diluted sample was placed in individual 13 x 100 mm glass test tubes (VWR International), followed by the addition of 2.5 mL of 0.2 N FC solution and 1.0 mL of a 15% (w:v) aqueous sodium carbonate solution. The resulting mixture was vortexed at setting 6 for 10-15 s. Samples were then placed in the dark for 2 h at room temperature, followed by spectroscopic analysis (Genesys 10S UV-visible; Fisher Scientific) at 765 nm. Blanks were prepared which contained all of the reagents and 250 µL of water, or 70% ethanol or 70% methanol in place of the diluted water, EFW and MFW extracts, respectively. Standard curves were prepared using 250 µL aliquots of an aqueous gallic acid solution at concentrations ranging from 5.0-60.0 ± 0.1 mg/L. Standard curves had correlation coefficients ≥0.999. All samples and standards were analyzed in triplicate (n = 3) and the results were reported as gallic acid equivalent (GAE; g/100 g of fresh fruit or 100 g of pomace).

3.12.2 Total Phenolic Chromatographic Index (TPCI)

Sample total phenolic chromatographic index (TPCI) was determined on the aqueous and EFW and MFW (3.10) extracts and phenolic fractions (3.11) employing high performance liquid chromatography with photodiode array detection (HPLC-PDA). Phenolics (i.e. chromatographic peaks @280 nm) were identified and placed into specific phenolic subclasses (e.g. hydroxybenzoic acids, hydroxycinnamic acids, flavanols) based upon their UV-visible spectra comparison to reference standards. The concentration for each phenolic subclass was determined by chromatographic area comparison to reference standards, and extract TPCI was determined by the area summation of all subclasses.

Chromatography was performed on an 1100 series HPLC system (Agilent Technologies Canada Incorporated, Mississauga, ON). Sample phenolic separation was achieved using a 250 x 4.6 mm Prodigy ODS-3 5 μm, C_{18} column (Phenomenex, Torrance, CA, USA) in series with a C_{18} guard cartridge (Phenomenex), at 25.0 ± 1.0°C.

The gradient mobile phase system used for phenolic compound separation consisted of: solvent A was 10 mM formic acid and solvent B was 70% acetonitrile: 30% solvent A (v:v). The linear gradient program was as follows: 100% A for 3 min, to 4% B at 6 min, to 10% B at 15 min, to 15% B at 30 min, to 20% B at 35 min, to 23% B at 50 min, to 25% B at 60 min, to 30% B at 66 min, to 50% B at 80 min, to 80% B at 85 min, which was held at 80% B for 5 min. The injection volume was 20 μL and the mobile phase flow rate was 0.8 mL/min. All samples were syringe filtered prior to HPLC analysis.

Phenolic compound detection was achieved employing a PDA detector with monitoring at 254, 280, 360 and 520 nm, with reference at 360, 400, 700 and 700 nm, respectively. Phenolic standards, arbutin, caffeic acid, catechin, chlorogenic acid, cyanidin-3-*O*-glucoside, cyanidin-3-*O*-rutinoside, ellagic acid, epicatechin, ferulic acid, 4-hydroxybenzoic acid, gallic acid, naringenin, p-coumaric acid, phloridzin, quercetin, rutin and vanillic acid, were prepared at 100.0 (± 0.2) ppm and were used to identify sample spectral (i.e. UV-visible profiles), retention time and quantitation parameters. For quantification, standard curves were prepared and ranged from 0.1 to 100.0 ppm and regression equations had correlation coefficients ≥0.990. The concentration of each phenolic subclass was then summed to calculate sample TPCI. All samples were analyzed in triplicate.

3.13 Total Anthocyanin Content

Sample (section 3.10.2) monomeric anthocyanin content was determined by the pH differential method (AOAC Method 2005.02; AOAC, 2000). In this assay, sample absorption was determined at two different pH values and these solutions were prepared as follows: (a) pH 1.00 buffer was prepared by dissolving 1.86 g of potassium chloride (0.025 M) in ~960 mL of water in a 1 L beaker, followed by pH adjustment to 1.00 (±0.05) using concentrated 2 N HCl. The solution was quantitatively transferred to a 1 L volumetric flask and brought to volume with water; and (b) pH 4.50 buffer was prepared by dissolving 54.43 g of sodium acetate (0.4 M) in ~960 mL of water in a 1 L beaker, followed by pH adjustment to 4.50 (±0.05) using concentrated 2 N HCl. The solution was quantitatively transferred to a 1 L volumetric flask and brought to volume with water. A 2.0 mL aliquot of each fruit/pomace extract sample was added to separate 50 mL volumetric flasks and brought to volume with the aforementioned pH 1.00 and 4.50 buffer solutions and the resulting solutions were held static at room temperature in the dark for 2 h. A sample blank was prepared with water. Sample absorbance at 512 and 700 nm was measured by UV-visible spectroscopy (Genesys 10S UV-visible; Fisher Scientific). The difference in absorbance (ΔA) between the pH 1.00 and pH 4.50 buffers was calculated as follows:

$$\Delta A = (A_{512nm}\ \text{pH 1.00} - A_{700nm}\ \text{pH 1.00}) - (A_{512nm}\ \text{pH 4.50} - A_{700nm}\ \text{pH 4.50})$$

The A_{700nm} was used to correct for background absorbance due to sample turbidity. Total anthocyanin content was expressed as mg cyanidin-3-*O*-glucoside/100 g fresh berries or 100 g of pomace and calculated as follows:

$$\text{Total anthocyanin content} = \frac{\Delta A \times MW \times 25 \times 1000}{\varepsilon \times 1}$$

Where:

MW: molecular weight of cyanidin-3-*O*-glucoside (449.2 g/mol)

25: dilution factor

1000: conversion of g to mg

ε: molar absorbance/extinction coefficient of cyanidin-3-*O*-glucoside (26900 $L.mol^{-1}.cm^{-1}$)

1: cuvette path length (cm)

3.14 Qualitative and Quantitative Anthocyanin Analysis

Sample EFW extracts (section 3.10.2) were subjected to solid phase extraction using a C_{18} Sep-Pak to isolate sample anthocyanins (Waters Corporation, Milford, MA). The solid phase cartridge was pre-conditioned by washing with 5 mL of methanol/0.01% HCl followed by 2 mL of water/0.01% HCl. One mL of sample EFW extract was added to the cartridge followed by treatment with 2 mL of water/0.01% HCl and sample anthocyanins were eluted using 2.0 mL of methanol-0.01% HCl (Green, 2007).

Samples were analyzed by high performance liquid chromatography with photodiode array detection (HPLC-PDA). Anthocyanin separation was afforded using the previously described analytical column (section 3.2.4) with a mobile phase system consisting of aqueous 4.0% (v:v) phosphoric acid at pH 1.4 (solvent A) and acetonitrile (solvent B) used under the following gradient conditions: initial, 6% B for 12 min, followed by a linear gradient to 20% B at 66 min, and then held at 20% B for 18 min. The mobile phase flow rate was 0.8 mL/min. The sample injection volume was 20 μL and all samples were syringe filtered (nylon, 0.2 μm pore size; 13 mm diameter; Chromatographic Specialties) prior to analysis. Analyte detection was achieved using a PDA detector with monitoring at 520 nm with reference at 700 nm. Anthocyanin standards used for identification were: cyanidin-3-*O*-galactoside (ideain), cyanidin-3-*O*-glucoside (kuromain), cyanidin-3-*O*-arabinoside and cyanidin-3-*O*-xyloside. Sample anthocyanin identification was afforded by RT comparison to standards and spiking experiments, and standard curves (10-300 mg/L; correlation coefficients ≥0.990) were employed to determine anthocyanin concentrations.

3.15 *In Vitro* Radical Scavenging Assays

ABTS and DPPH in vitro scavenging assays were determined for all three saskatoon berry varieties, and wet and dry pomace extracts (section 3.10), and saskatoon berry and pomace fractions (section 3.11).

3.15.1 ABTS (2.2-azino-bis-3-ethylbenzthiazoline-6-sulfonic acid

Radical cation ($ABTS^{\bullet+}$) solutions were prepared by mixing 4.00 mL of 7.0 mM ABTS and 2.00 mL 7.0 mM potassium persulfate in water. The resulting mixture was maintained at room

temperature for 12 h in the dark to afford $ABTS^{\bullet+}$ formation. The resulting $ABTS^{\bullet+}$ radical cation solution was diluted approximately 1 in 50 with 70% methanol so as to give an absorbance reading of 0.75 ± 0.05 at 734 nm. The following dilutions were used for sample analysis: (a) aqueous extracts were diluted, 1/3, 1/4, 1/5, 1/10, 1/20 with water; and (b) methanol and ethanol-formic acid extracts were diluted, 1/5, 1/10, 1/20, 1/30 with 70% methanol. The Trolox standard was prepared in 70% methanol at the following concentration range: 0.1 mg/mL (0.47 mM) to 0.5 mg/mL (2.06 mM). The assay was conducted by mixing 10 µL of the sample solution with 1.00 mL of $ABTS^{\bullet+}$ solution. The control solution consisted of 10 µL of 70% methanol in 1.00 mL $ABTS^{\bullet+}$ solution. The blank was 70% methanol. Samples and blanks were vortexed for 6 min, and their absorbance was determined at 734 nm. Percent ABTS radical scavenging activity was calculated as follow:

$$\% \text{ ABTS radical scavenging activity} = \left[1 - \frac{A_{734}\ \text{sample}}{A_{734}\ \text{control}}\right] * 100$$

A_{734} sample = sample absorbance at 734 nm

A_{734} control = control absorbance at 734 nm

The % $ABTS^{\bullet+}$ inhibition was plotted as a function of sample concentration and linear regression equations were determined. Correlation coefficients of the linear regression equations were ≥ 0.950. All samples were analyzed in triplicate (n = 3). The % $ABTS^{\bullet+}$ inhibition of 1 mM Trolox was determined from linear regression curves of the Trolox standards. The sample concentration equivalent to the inhibition activity of 1 mM Trolox was calculated. The Trolox equivalent antioxidant capacity (TEAC) was expressed as the equivalent activity of Trolox (mM)/100 mg sample as follows:

$$TEAC = 100/Y_{TE}$$

TEAC = Trolox equivalent antioxidant capacity (Trolox equivalents/100 mg sample)

100 is the conversion factor to standardize the sample to 100 mg/mL

Y_{TE} = sample concentration (mg/mL) producing an $ABTS^{\bullet+}$ inhibition equivalent to 1 mM

Trolox.

3.15.2 DPPH (2,2-diphenyl-1-picrylhdrazyl)

A 500μM DPPH solution was prepared by dissolving 9.8 ± 0.2 mg of DPPH in a 50 mL volumetric flask with 70% methanol:water (v:v) followed by sonication (20 ± 3 °C; Bransonic, Danbury, CT, USA). Fresh DPPH solution was prepared daily for sample analysis. The following dilutions were used for sample analysis to produce DPPH radical scavenging levels ranging from approximately 10 to 85%: (a) aqueous extracts were diluted, 1/2, 1/3, 1/5, 1/10, 1/20 with water; and (b) ethanol- and methanol-formic acid-water extracts were diluted, 1/5 1/10, 1/20, 1/30 with 70% methanol. Diluted sample (250 μL) aliquots were added to 13 x 100 mm glass test tubes followed by the addition of 1.00 mL of DPPH solution. A control sample was prepared by adding 250 μL of 70% methanol to 1.00 mL DPPH solution, and the blank was 70% aqueous methanol. Samples and blanks were vortexed for 10 to 15 seconds. The samples were kept static at room temperature in the dark for 15 min before their absorbance at 517 nm was determined. Percent DPPH radical scavenging activity was calculated as follows:

$$\%\ \text{DPPH radical scavenging activity} = \left[1 - \frac{A_{517}\ \text{sample}}{A_{517}\ \text{control}}\right] * 100$$

A_{517} sample = sample absorbance at 517 nm
A_{517} control = control absorbance at 517 nm

The 50% radical inhibition concentration (IC_{50}) was determined by plotting the % DPPH radical scavenging versus concentration for each sample by linear regression. The IC_{50} value was expressed as mg solids/mL of DPPH solution and the antioxidant activity was reported as 1/ IC_{50}. Regression equations had correlation coefficients ≥0.910. All samples were analyzed in triplicate (n = 3).

3.16 Laboratory Scale Saskatoon Berry Juice Production

Laboratory scale juice was prepared using the Northline variety (2016 crop) purchased from Prairie Berries Inc. (Keeler, SK) employing commercial juice production enzymes following

the method of Willems and Low (2016). In brief, the berries were thawed at 4 ± 2°C for 24 h and then blanched by hot water treatment at 100°C/3 min, in order, to inactivate native polyphenol oxidase activity. To 100.0 g of blanched fruit, 30.0 mL of water was added and the resulting mixture was blended at speed #10 at room temperature for 2 min [fruit mashing stage]. The resulting mixture was quantitatively transferred to a 500 mL beaker and 4.5 µL of Pectinex Ultra Mash enzyme (Novozymes, Bagsvaerd, Denmark; 11000 units/mL) for 90 min at room temperature. The resulting mixture was brought to 50 ± 2°C in an Aqua-Therm shaking water bath (New Brunswick Scientific Co. Inc., Edison, NJ, USA) and 4.5 µL of both Pectinex Ultra Clear (Novozymes; 7900 units/mL) and Amylase AG 300 L (Novozymes; 300 units/mL) with sample agitation at 500 rpm for 90 min [mash enzyme stage]. Samples were immediately heated for 10 min at 90°C for enzyme inactivation. The juice was separated from the pulp by vacuum filtration with VWR 417 filter paper [juice and pomace stage]. Laboratory scale juice production was conducted in triplicate.

Samples were taken at the following processing stages for analysis: blending (i.e. fruit mashing stage); following Pectinex Ultra Clear and Amylase AG 300 L treatment (mash enzyme stage); juice; and pomace. All sampling was done in triplicate. Samples from the juice mashing stage, mash enzyme stage and pomace were centrifuged at 6000 rpm (Clinical 200 centrifuge, VWR International, Edmonton, AB, Canada) for 15 min and the supernatant was separated from the pellet and used for all analyses. Samples taken at each processing stage were analyzed for select physicochemical properties, moisture content, total phenolic content (TPC), and antioxidant activities (ABTS and DPPH) as outline in sections 3.3; 3.4.3; 3.12.1; 3.15.1; and 3.15.2, respectively. Samples were appropriately diluted with 70% ethanol v:v and analyzed for their TPCI as outlined in section 3.12.2.

3.17 Statistical Analysis

Statistical analysis of experimental data was performed using software SPSS for Windows version 22.0 (IBM SPSS Inc., Chicago, IL, USA). Data were analyzed using a one-way analysis of variance (ANOVA). The difference between means ($p<0.05$) was determined using the multiple-comparison Tukey's HSD (honestly significant difference) multiple comparison test.

4. RESULTS AND DISCUSSION

4.1 Physicochemical Analysis

Mean and standard deviation physicochemical analysis results for hand pressed juices (section 3.3.1) and whole berries (i.e. size) from Martin, Northline and Pembina saskatoon berry varieties are shown in Table 4.1.

Table 4.1 Mean and standard deviation physicochemical analytical results for juices from Martin, Northline and Pembina saskatoon berry varieties.

Physicochemical Parameter		Variety		
		Martin	Northline	Pembina
Colour	L*	8.51 ± 0.07[1c]	3.69 ± 0.13[b]	3.26 ± 0.04[a]
	a*	9.98 ± 0.27[c]	7.46 ± 0.09[b]	5.17 ± 0.23[a]
	b*	2.89 ± 0.14[b]	1.21 ± 0.24[a]	1.55 ± 1.16[a]
	Chroma (C*)	10.40 ± 0.26[b]	7.87 ± 0.59[a]	16.60 ± 2.70[c]
	Hue angle (θ)	16.17 ± 0.86[b]	10.27 ± 2.10[a]	16.53 ± 2.69[b]
pH[2]		3.95 ± 0.06[a]	4.02 ± 0.02[ab]	4.10 ± 0.05[b]
Size (mm)[3]		13.49 ± 1.45[b]	9.16 ± 0.79[a]	12.40 ± 1.13[b]
Size (mm)[4]		12.29 ± 1.16[b]	8.29 ± 0.51[a]	11.75 ± 1.17[b]

[1]Mean ± standard deviation results of triplicate sample analysis.

[2]pH measure for hand pressed juices (section 2.1.3.1).

[3]Diameter measured before thawing.

[4]Diameter measured after thawing.

[a-c]Mean values in the same row followed by a common letter were not statistically different ($p<0.05$) by Tukey's HSD multiple range test.

These physicochemical analysis results provide a number of quality control parameters for the fruit, which are important for selecting appropriate varieties for commercial production and for food processing (e.g. juice production).

The Hunterlab system measures colour employing the following tristimulus scale, L* is a measure of sample lightness (i.e. 0 for black and 100 for white), a* measures sample redness when positive and greenness when negative, and b* measures sample yellowness when positive and blueness when negative. In addition, the hue angle indicates the predominant colour of the sample, and the intensity of this colour is represented by the chroma value (Voss, 1992; Green and Low, 2013; Pathare et al., 2013).

Samples had L* values that ranged from 3.26-8.51 indicating dark coloured juices with Martin (L* = 8.51) being significantly lighter than Northline (L* = 3.69) and Pembina (L* = 3.26) varieties. These L* values were much lower than those reported in literature for whole fruits of, 21.4, 17.6 and 17.0, for Martin, Northline and Pembina, respectively (Zatylny, 2005). However, the L* results obtained were in agreement with the rankings of these three varieties with respect to sample lightness. Possible reasons for the observed differences in juice lightness within these samples, and between these samples and those reported in literature include, fresh fruit vs. juice, phenolic content, polyphenol oxidase activity, and environmental growth conditions.

Sample juice colour is mathematically described by the hue angle, where a decrease in hue angle value is concomitant with a shift from red to red-purple (Green and Mazza, 1986). Hue angles for these juices ranged from 10.27 to 16.53, with Northline (10.27) being significantly different from both Martin (16.17) and Pembina (16.53), indicating that the juice produced from the Northline variety was a dark purple. The hue angle values reported for Martin, Northline and Pembina in literature of 18.0, 15.4-17.9 and 18.2, respectively, were higher than the results found in this study (Green and Mazza, 1986; Zatylny et al., 2005). It has been reported that hue angle values of saskatoon berry (saskatoon berry variety non-specified) was dependent upon variety, and that it decreased with ripening, resulting in a colour shift from red to red-purple (Green and Mazza, 1986).

The observed differences in saskatoon berry juice colour amongst the three varieties studied, with Northline being deeper red-purple and Martin and Pembina red, could be exploited by producers and processors for the development of food products/ingredients as colour is often associated with both product recognition and quality.

The pH results for Martin, Northline and Pembina berry juice showed that all were acidic and had individual values of, 3.95, 4.02 and 4.10, respectively. These values agreed with those reported in literature for these three varieties of, 3.71, 3.78-4.07 and 3.95, for Martin, Northline

and Pembina, respectively (Green and Mazza, 1986; Mazza and Cacace, 2003; Zatylny et al., 2005).

Mean diameter fruit size of the three saskatoon berry cultivars ranged from 8.29-12.29 mm (Table 4.1), with Northline being significantly smaller than Martin and Pembina. The mean diameter for saskatoon berries reported in literature ranges from 5-15 mm (Zatylny et al. 2005; Shaw et al., 2004; Ozga et al., 2007). The results from this study are consistent (i.e. within the standard deviation) with those previously reported for Martin and Pembina varieties of 13.80 mm and 11.40 mm, however, the literature value of 12.20 mm for Northline was significantly higher than that found in this study of 8.29 mm. Possible non-varietal reasons for the smaller fruit size of Northline could be due to environmental growth conditions. It has been reported that the size of saskatoon berries is an important physical characteristic for the selection of new varieties and the commercialization of the fruit based on ease of harvest and the general relationship between fruit size and yield (McGarry et al., 2001).

4.2 Proximate Composition

Mean and standard deviation proximate analysis results for Martin, Northline and Pembina varieties of saskatoon berry on a wet basis are shown in Table 4.2. Sample proximate analysis provides both chemical composition and nutritional information on foods and ingredients by the analytical measurement of moisture, ash, lipid, protein and total dietary fibre content. In addition, the nitrogen free extract, which is often expressed as the carbohydrate content of the sample is determined by subtraction of the aforementioned analytical measurements from 100%.

Table 4.2 Mean and standard deviation proximate analysis results for Martin, Northline and Pembina saskatoon berry varieties.

Component (%wet basis)	Variety		
	Martin	Northline	Pembina
Moisture	81.62 ± 0.11[1a]	80.18 ± 0.93[a]	82.79 ± 0.98[a]
Carbohydrate[2]	10.08 ± 1.19[b]	7.39 ± 0.85[a]	10.89 ± 0.55[b]
Total dietary fibre	6.27 ± 0.21[b]	9.42 ± 0.27[c]	4.23 ± 0.33[a]
Protein	1.22 ± 0.08[a]	1.79 ± 0.13[b]	1.13 ± 0.09[a]
Ash	0.53 ± 0.03[a]	0.74 ± 0.04[c]	0.64 ± 0.01[b]
Lipid	0.28 ± 0.01[a]	0.48 ± 0.03[c]	0.32 ± 0.01[b]
Total soluble solids (°Brix)	15.72 ± 0.03[c]	12.79 ± 0.09[a]	14.59 ± 0.03[b]

[1]Mean ± standard deviation results of triplicate sample analysis.

[2]Determined by difference from the analytical results for ash, fibre, lipid, moisture and protein.

[a-c]Mean values in the same row followed by a common letter were not statistically different ($p<0.05$) by Tukey's HSD multiple range test.

Water was found to be the major constituent for all three saskatoon berry varieties and its concentration ranged from 80.18 to 82.79%. These analytical values agreed with those reported in literature of 81-84% for both Martin and Northline, and 82% for Pembina (Mazza and Cacace, 2003; Bakowska-Barczak and Kolodziejczyk, 2008; Rop et al., 2012). These values also agreed with those of other pome fruits such as apple (*Malus domestica*) and pear (*Pyrus communis* L.), with values of 81-86% and 82-85%, respectively (Carbonaro et al., 2002; Ferreira et. al, 2002; Kheiralipour et al., 2008; Egea et al., 2012). The moisture content of fruits plays an important role as a reactant and reaction medium, which impacts product texture, storage, and microbiological stability (e.g. shelf life) (Belitz et al., 2009; Sánchez et al., 2010).

Plant dietary fibre is a mixture of complex organic polymeric substances that includes cellulose, hemicellulose, lignin, pectin, and gums/mucilage that are resistant to human enzymatic digestion (Dhingra et al., 2012). Total dietary fibre (TDF) results for Pembina, Martin and Northline varieties were 4.23, 6.27 and 9.42%, respectively. In addition, each of the TDF results was found to be statistically different. There is no published TDF data for the three varieties analyzed in this study, however, the fibre content of saskatoon berries (non-specified varieties)

have been reported to range from 3.80 to 5.45% (Mazza, 1982; Mazza, 2005). The observed high TDF value for Northline of 9.43% when compared to those of Martin and Pembina may be explained by the thicker fruit cuticle (i.e. peel) and higher seed weight in this variety of 4.27% when compared to that of Martin (1.93%) and Pembina (3.01%). Also, the fact that the berry size of the Northline variety (8.29 mm) was significantly smaller than those of Martin (12.29 mm) and Pembina (11.75 mm) results in a larger surface area of skin (i.e. fibre) to berry, which would also contribute to the observed higher fibre content of this variety. Saskatoon berry is a good source of fibre when compared to orange (*Citrus sinenis*; 1.60-2.39%), grapes (*Vitis vinifera*; 0.88-1.36%), blueberry (*Vaccinium corymbosum* L.; 1.46-2.40%) and other pome fruits such as apple and pear with range values of 0.80-3.74% and 2.80-5.20%, respectively (Lintas and Cappelloni, 1992; Chang et al., 1998; Senser et al., 1999; Gorinstein et al., 2001; Mazza, 2005; Mahattanatawee et al., 2006; Feliciano et al., 2010; Abdualrahman, 2015; USDA, 2016).

Protein content results for the three varieties ranged from 1.13 to 1.79%, with Pembina containing the least protein and Northline the greatest. The protein content results obtained in this study were similar to data reported for non-specified saskatoon berry varieties that ranged from 1.05 to 1.94% (Mazza, 1982; Mazza and Cacace, 2003; Mazza, 2005; Hosseinian et al., 2007). saskatoon berries contain a higher protein content than other pome fruits, such as apple (0.06-0.26%) and pear (0.2-0.5%) (Senser et al., 1999; Barroca et al., 2006; USDA, 2016; Feliciano et al., 2010). The Kjeldahl method used in this study quantifies sample nitrogen content, which was converted to percent protein using the factor 6.25 (i.e. assumption of an average nitrogen content of proteins of 16.0%).

Ash is the inorganic residue remaining after sample moisture and organic matter have been removed by combustion. The ash contents of Martin, Pembina and Northline varieties in this study were 0.53, 0.64 and 0.74%, respectively. These values agreed with ash content results for saskatoon berry samples from literature that ranged from 0.59-0.67% (Mazza, 1982; Mazza, 2005; Hosseinian et al., 2007). When compared to other fruits, the ash content of each of these three varieties were higher than those reported for blueberry (*Vaccinium corymbosum* L; 0.19-0.24%) and strawberry (*Fragaria virginiana*; 0.35-0.40%), and two commercial pome fruits, apple (0.25-0.44%) and pear (0.29-0.43%) (Mazza, 2005; Barroca et al., 2006; Skupień, 2006; Onibon et al., 2007; Campeanu et al., 2009; Giampieri et al., 2012; Marjanović-Balaban et al. 2012). The observed significant variation in saskatoon berry variety ash content is most likely due to the fruit

variety, degree of ripening, soil mineral content, and agronomic practices (Yang and Kallio et al. 2002; Ozga et al., 2006; Zatylny et al, 2005).

Proximate analysis of sample lipid content includes, but is not limited to the measurement of, triacylglycerols, oil-soluble pigments such as chlorophyll and carotenoids, waxes, free fatty acids and phospholipids. The crude lipid content of the three saskatoon berry varieties ranged from 0.28 to 0.48%, with statistically different results for each. The maximum lipid content of 0.48% was found for the Northline variety, which was approximately 1.7x greater than that observed for Martin. The observed higher concentration of lipid in the Northline variety was in agreement with the hypothesized increased cuticle thickness of this variety, and the observed high concentration of lipids in fruit cuticles (Martin and Rose, 2014). Also, the lipid content of the Northline variety was the only result that was in agreement with the literature value range of 0.40-0.84% for saskatoon berry (Mazza, 1982; Mazza, 2005; Hosseinian et al., 2007). When compared to other pome fruits, the lipid content of these saskatoon berry samples were generally found to be higher than apple and pear with concentrations of 0.1-0.2% and 0.1-0.3%, respectively (Barroca et al., 2006; USDA, 2016).

Sample total soluble solids (TSS) are reported as °Brix, which is the grams of sucrose per one hundred grams of sample (i.e. % carbohydrate). However, in foods the TSS results are comprised of all soluble compounds (e.g. organic acids) in the sample, therefore this result is an overestimation of sample carbohydrate content. The observed differences between TSS and total carbohydrate content results (Table 4.2) for each of the saskatoon berry varieties analyzed in this study is clearly demonstrated by a comparison of these values. As an example, the TSS and total carbohydrate content values for the Martin variety were 10.92% and 15.72 °Brix, respectively, resulting in a carbohydrate difference of 1.4x. The mean TSS results for Northline, Pembina and Martin varieties were, 12.79, 14.79 and 15.72 °Brix, respectively, with each value being significantly different. The lowest observed TSS value was for Northline, which also agreed with this variety having the lowest carbohydrate content (Table 4.1), and these results may be explained by the possible lack of maturity of this fruit at harvest, although no red berries were observed in the sample, and/or environmental growth conditions. Literature TSS results for Martin, Northline and Pembina have been reported as, 15.3, 14.9-16.5 and 20.1 °Brix were in close agreement to the mean of 15.72 °Brix (based on all three varieties) obtained in this study, however, the TSS mean result of 14.79 °Brix found for the Pembina variety was much lower than that reported for this

variety in literature of 20.1 °Brix (Zatylny et al., 2005). The TSS results for these three saskatoon berry varieties were similar to those reported for apple (12.2-13.8 °Brix), blueberry (10-14 °Brix) and pear (13.2-17.6 °Brix) (Fourie et al., 1991; Kalt and McDonald, 1996). Fruit TSS values are dependent on variety, degree of maturity and environmental growth conditions (Zatylny et al., 2005).

4.3 Moisture and Total Soluble Solids Contents of Saskatoon Berry Pomace

The mean and standard deviation results for both moisture and total soluble solids contents of wet and dry saskatoon berry pomace are shown in Table 4.3.

Table 4.3 Mean and standard deviation moisture and total soluble solids contents results for wet and dry pomace.

Component (% w:w)	Wet Pomace	Dry Pomace
Moisture Content	68.00 ± 0.87[1]	7.94 ± 0.02
Total Soluble Solids (°Brix)	8.11 ± 0.56	3.26 ± 0.27

[1]Mean ± standard deviation results of triplicate sample analysis.

Wet and dry pomace were obtained from commercially produced saskatoon berry juice (wet pomace from the Northline variety and dry pomace from a blend of Martin, Northline and Thiessen varieties), and the moisture contents of these samples were similar to the ranges reported for apple pomace of 70-76% (wet) and 7-10% (dry) (Sudha et al., 2007; García et al., 2009). The total soluble solids for wet and dry pomace from saskatoon berry juice production were 8.11 and 3.26 °Brix, respectively, indicating incomplete juice extraction during their commercial processing. The TSS for wet pomace from saskatoon berry juice production was much higher than those reported for commercial juices in literature. As examples, the wet pomace TSS literature values for cranberry and grape are 1.38 and 3.6 °Brix, respectively (Park and Zhao, 2006; Deng and Zhao, 2011). No literature information was found on the TSS contents for dry fruit pomace from commercial juice production. The total soluble solids content of pomace is an important parameter, as it indicates the effectiveness of juice extraction from the fruit during processing.

4.4 Amino Acids

The amino acid profiles for each saskatoon berry variety (section 3.5) are shown in Table

4.4 (normalized to 100%).

Table 4.4 Mean results for the amino acid contents of Martin, Northline and Pembina saskatoon berry varieties.

Amino Acids	Martin	Northline	Pembina
Alanine	5.75[1,2]	4.98	5.11
Arginine	6.50	7.42	7.61
Aspartic acid + Asparagine	12.80	13.21	11.25
Cysteine	1.85	1.96	1.88
Glycine	6.21	6.20	6.26
Glutamic Acid + Glutamine	21.65	24.10	24.19
Histidine	3.13	2.37	2.92
Isoleucine	4.14	3.75	4.07
Leucine	7.41	7.18	7.40
Lysine	1.53	1.94	1.88
Methionine	0.96	0.96	1.04
Phenylalanine	4.55	4.40	4.59
Proline	5.86	5.22	5.21
Serine	5.60	5.46	5.53
Threonine	3.14	2.77	2.92
Tryptophan	0.44	0.41	0.42
Tyrosine	3.36	3.02	2.92
Valine	5.12	4.65	4.80
Total Amino Acids	100	100	100

[1]Percent.

[2]Mean results of duplicate sample analysis.

The predominant individual and combined amino acids in Martin, Northline and Pembina saskatoon berry varieties were glutamic acid + glutamine (21.7-24.2%), aspartic acid + asparagine (11.3-13.2%), leucine (7.2-7.4%) and arginine (6.5-7.6%). These results agreed with those

reported for the Smoky variety, which was the only literature value found for saskatoon berries (Mazza, 2005).

The total amino acid contents of the Northline, Pembina and Martin varieties were, 1.22 g, 0.84 g, and 0.83 g/100 g fresh weight (FW), respectively. The observed amino acid concentration for the Northline variety was similar to that reported in literature for the Smoky variety of 1.12 g/100 g FW. Specific concentrations of essential amino acids such as leucine are important as they can identify food protein sources that may be beneficial to human health. The total amino acid content of the saskatoon berry samples analyzed in this study were higher than those reported for blueberry (0.50 g/100 g FW), and other pome fruits such as apple (0.20 g/100 g FW) and pear (0.30 g/100 g FW) (Health Canada, 2016; USDA, 2016).

4.5 Major Carbohydrates/Sorbitol

The major carbohydrate and sorbitol contents of the expressed saskatoon berry juice samples (section 3.3.1) were qualitatively and quantitatively determined by high performance liquid chromatography-refractive index detection (HPLC-RI) employing retention time (RT) comparison to external standards, spiking experiments and peak area/height comparison to standard curves (Table 4.5).

Table 4.5 Mean and standard deviation results for fructose, glucose, sucrose and sorbitol concentrations in Martin, Northline and Pembina saskatoon berry varieties.

Variety	Fructose (F)	Glucose (G)	Sorbitol	Sucrose	Total	F/G
Martin	5.09 ± 0.05[1,2ab]	4.33 ± 0.12[a]	2.12 ± 0.14[a]	ND[3]	11.54 ± 0.56[a]	1.18
Northline	5.36 ± 0.13[b]	4.76 ± 0.08[b]	1.86 ± 0.15[a]	ND	11.98 ± 0.51[a]	1.13
Pembina	4.75 ± 0.05[a]	4.04 ± 0.06[a]	2.15 ± 0.09[a]	ND	10.94 ± 0.12[a]	1.18

[1]g/100 g FW.

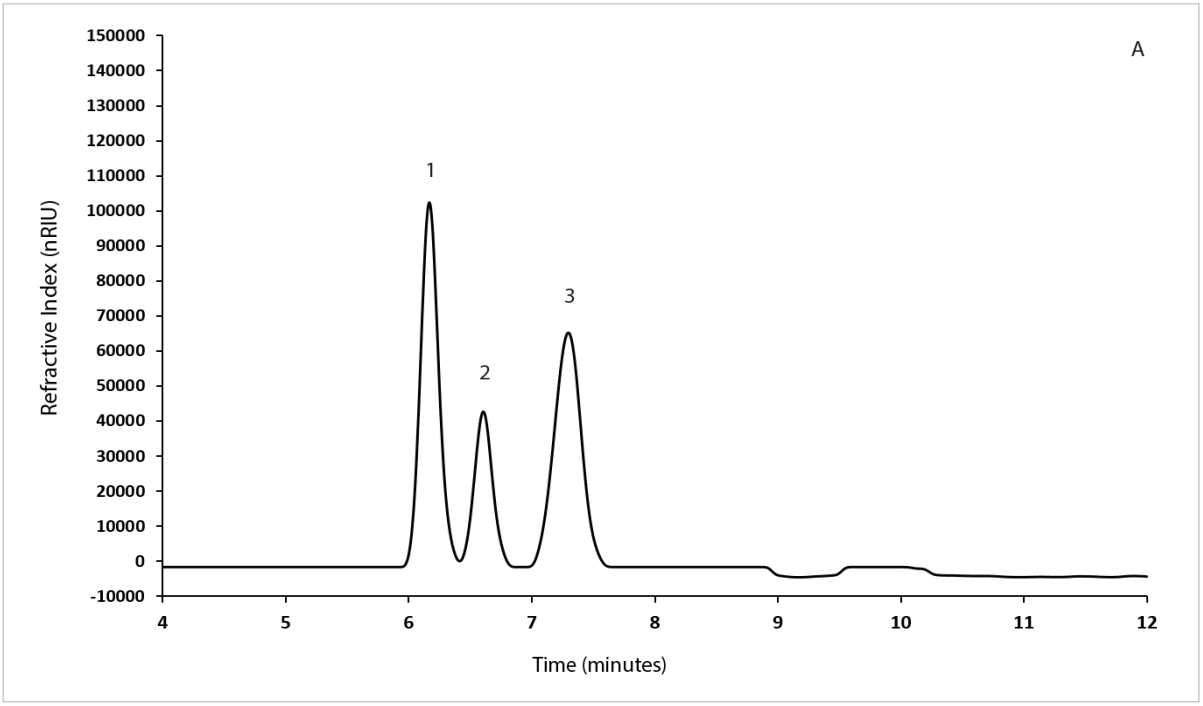
[2]Mean ± standard deviation results of triplicate sample analysis.

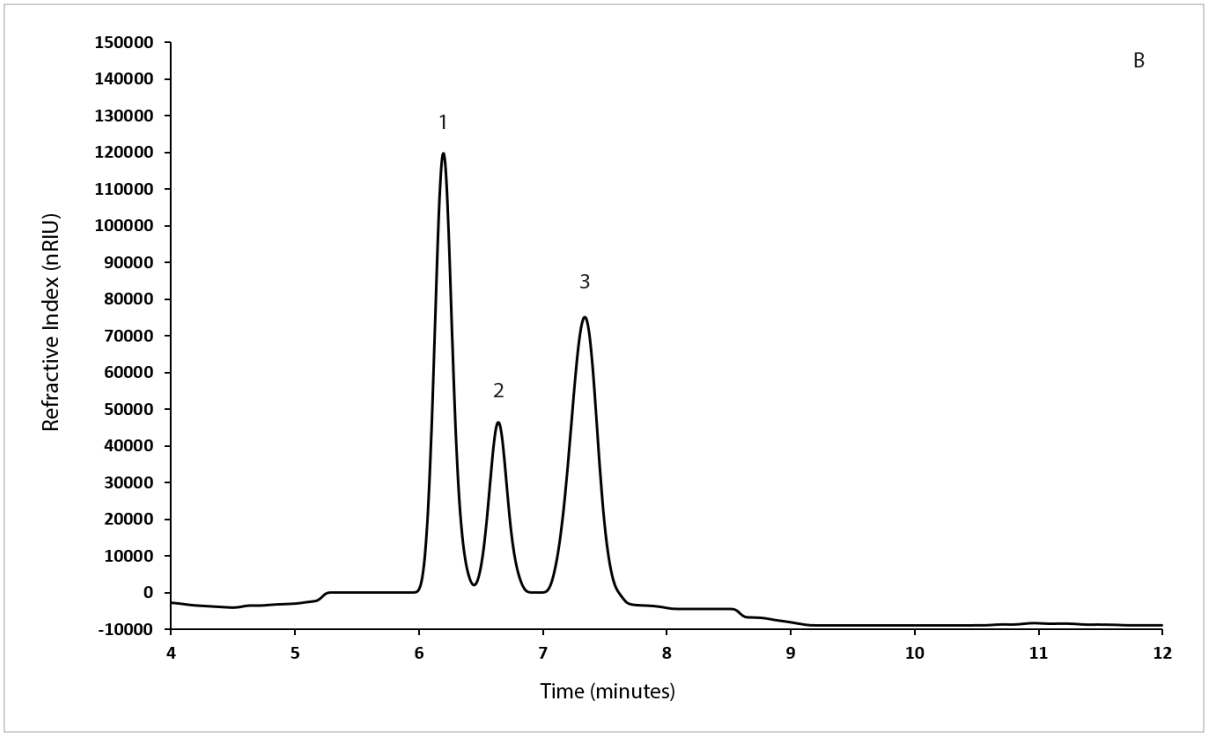
[3]ND, not detected <0.05% detection limit).

[a-c]Mean values in the same column followed by a common letter were not statistically different ($p<0.05$) by Tukey's HSD multiple range test between saskatoon berry varieties.

The major carbohydrates and polyol identified in these samples were fructose, glucose

and sorbitol (Figure 4.1).





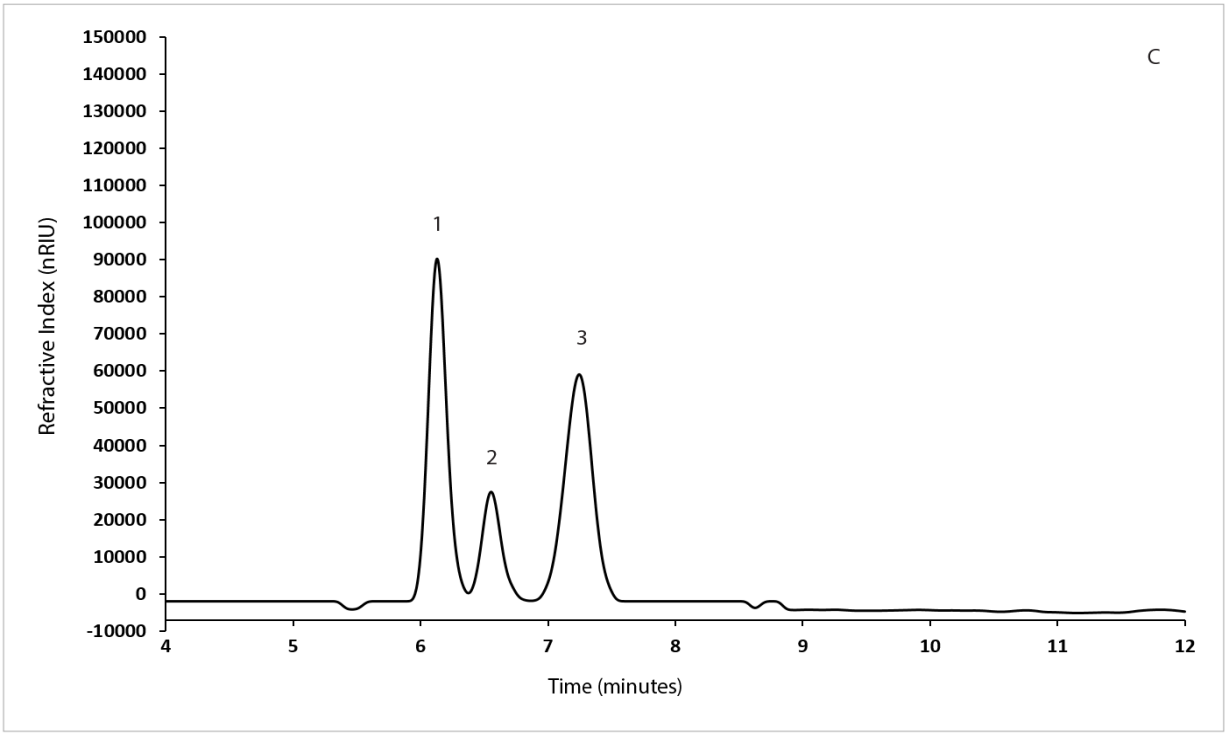


Figure 4.1 HPLC-RI chromatograms showing the major carbohydrates and polyol in saskatoon berry juices. Chromatogram identification: A. Martin; B. Northline; and C. Pembina varieties. Peak identity: 1. fructose; 2. sorbitol; and 3. glucose.

Fructose was identified as the major carbohydrate in all samples with a concentration range of 4.75 to 5.36 g/100 g FW, which accounted for ~44% of the total carbohydrate/polyol content of the expressed juice. The mean concentration of 5.07 g/100 g FW for fructose in the samples analyzed in this study was lower than those reported for apple and pear (i.e. pome fruit) of 5.90 and 6.42 g/100 g FW respectively (Health Canada, 2016; USDA, 2016).

Glucose concentration in the three samples ranged from 4.04 to 4.76 g/100 g FW, which accounted for ~40% of the total carbohydrate/polyol content of the expressed juice. The Northline variety was found to have a significantly higher concentration of glucose (4.76%) when compared to the Martin and Pembina varieties. The mean concentration of 4.38 g/100 g FW for glucose in the samples analyzed in this study was higher than those reported for apple and pear of 2.43 and 2.60% respectively (Health Canada, 2016; USDA, 2016).

Fructose to glucose (F:G) ratios are commonly reported for fruits and fruit products and are generally >1.0 (Elkins et al., 1988). The F:G ratio can be used as a means of detecting the

debasing (i.e. adulteration) of fruit juices/products with a commercial inexpensive sweetener such as invert sugar (F:G of 1:1) or high fructose corn syrup (F:G of 0.7, 1.2 and 9.0 for HFCS 42, 55 and 90, respectively) (Willems and Low, 2012; Ruiz-Matute et al., 2013). The F:G ratios for the saskatoon berry juices in this study were all >1.0 but were significantly lower than those reported for apple and pear of 3.2-4.2 and 2.1-3.2, respectively (Elkins et al., 1988; Willems and Low, 2014). The F:G ratio is also an important parameter for fruit juice flavour as fructose is much sweeter (sweetness value [sv]: 1.5) than sorbitol (sv: 1.0) and glucose (sv: 0.7), and these compounds balance juice sweetness with the organic acids (i.e. sour taste response) present in fruits.

Sucrose was not detected in the expressed juice samples based on HPLC-RI analysis (detection limit of ≤0.05%), and was supported by high performance anion exchange chromatography-pulsed amperometric detection (HPAE-PAD) analysis, which had a lower detection limit for this compound of ≤0.0005%. Interestingly, a sucrose concentration of 0.17 g/100 g FW has been reported for the Smoky variety of saskatoon berry (Mazza, 2005). This discrepancy in sucrose results could only be due to the variety and/or the maturity stage of the samples analyzed, however, maturity stage appears to be unlikely due to the °Brix values of the samples analyzed in this study. Sucrose concentrations reported for other pome fruits such as apple and pear are 0.30 and 2.30%, respectively (Health Canada, 2016; USDA, 2016).

The majority of literature data on the carbohydrate content of saskatoon berries were determined by difference (section 4.2), however, a published report (Mazza, 2005) on the major carbohydrate content of the Smoky variety was determined by HPLC. In this paper, fructose, glucose and sucrose concentrations were reported as 5.94, 5.23 and 0.17 g/100 g FW, with no mention of sorbitol. A comparison of these results with those found in this study show much higher (17-19%) values for fructose and glucose, no detection of sorbitol and detection of sucrose. Interestingly, the total carbohydrate content reported by the author of 11.36 g/100 g FW was very similar to the mean value (based on all three varieties) of 11.49 g/100 g FW found in this study. This similarity in total carbohydrate/polyol results may be explained by the presence of sorbitol in the Smoky variety that was not identified by the author as it co-eluted with fructose/glucose with the stationary phase used for this analysis (not reported but most likely a polar stationary phase with amino or diol functionality).

The presence of sorbitol in saskatoon berry has only been reported once in literature

(Rogiers and Knowles, 1997). The concentration of sorbitol in the saskatoon berry varieties studied in this research was significant and accounted for ~18% of the total carbohydrate/polyol concentration of the expressed juice. The presence and concentrations of sorbitol in the analyzed samples was confirmed by capillary gas chromatography with flame ionization detection (CGC-FID) through retention time comparison to standards, spiking experiments and standard curves. The mean sorbitol concentration of 2.04 g/100 g FW determined in the saskatoon berry varieties analyzed in this study falls within the range of 1.73 to 3.25 g/100 mL reported for commercial pear juice (Willems and Low, 2014).

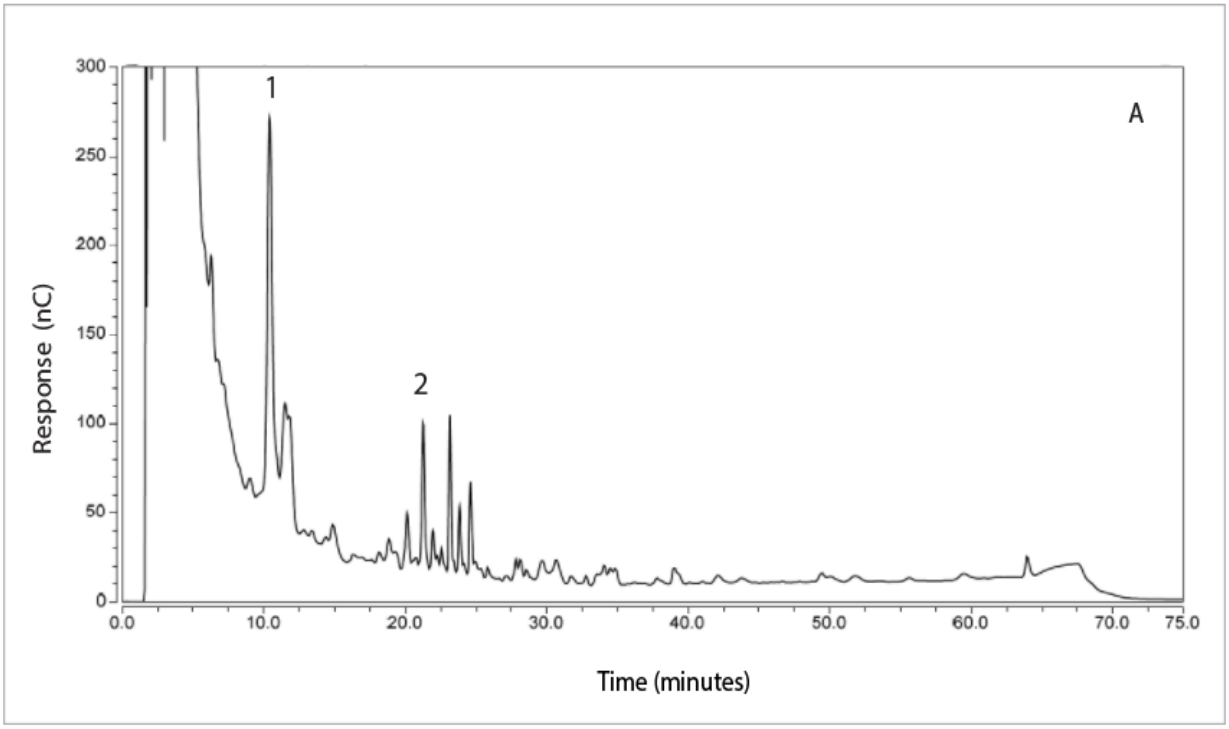
Based on their mean total carbohydrate concentration of 11.49 g/100 g FW, saskatoon berries are richer in these compounds when compared to apple (10.39 g/100 g FW) and pear (9.75 g/100 g FW), which are both used in commercial fruit juice production. As such, it is reasonable to postulate that based on carbohydrate content alone that saskatoon berry could have a possible place in the fruit juice market as a stand-alone juice or as a blending agent (e.g. apple-saskatoon berry).

The difference between total carbohydrate results for expressed juices obtained by HPLC-RI of 10.94 to 11.98 g/100 g FW (Table 4.5) to the ^{o}Brix values obtained from the macerated whole fruit of 12.79 to 15.72 (Table 4.2) can be explained by the presence of chemical compounds in the berries (e.g. pectin, organic acids) that contribute to total soluble solids results that are not detected by HPLC-RI or the experimental conditions employed, and/or are not eluted from the berries by physical expression.

4.6 Galacturonic Acid and Oligosaccharides

Representative HPAE-PAD chromatograms for the expressed juice from Martin, Northline and Pembina saskatoon berry varieties (section 3.3.1) are shown in Figure 4.2. Chromatographic profiles for all three samples were virtually identical qualitatively with only peak heights/areas being different (i.e. quantitative). Major carbohydrates eluting after glucose and fructose (i.e. retention times >10.0 minutes), were maltose (~10.5 min), galacturonic acid (~21.3 min) and a group of oligosaccharides (~22.5 to 25.0 min) with retention times that corresponded to glucose polymers (degree of polymerization [DP] of 3-5; Figure 4.3). Other possible fruit oligosaccharides including, raffinose, digalacturonic acid and trigalacturonic acid were eliminated as possibilities based on unmatched HPAE-PAD retention times (Figure 4.3). The presence of galacturonic acid

and maltose were confirmed by spiking experiments and by CGC-FID analysis. Quantitative results for these two compounds are reported in Table 4.6. The chromatographic conditions employed of an anion exchange stationary phase-basic (i.e. 100 mM NaOH) mobile phase coupled with a gold electrode maintained at redox potentials for carbohydrate oxidation (section 3.7), also supports the presence of galacturonic acid, maltose and a series of unidentified oligosaccharides in each of the juice samples. This is the first report of the presence of maltose and other oligosaccharides in saskatoon berry/juice.



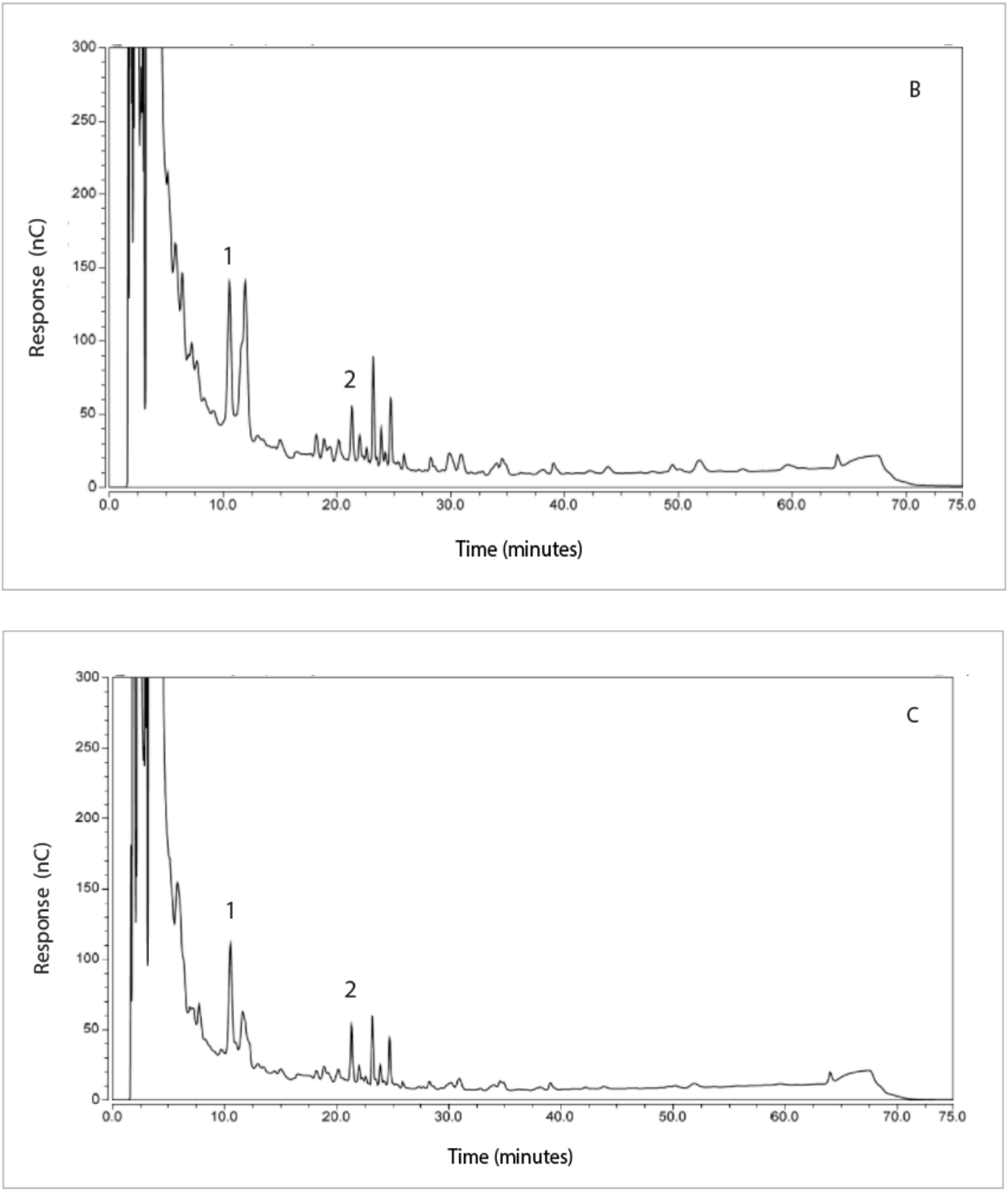


Figure 4.2 HPAE-PAD chromatograms showing the presence of galacturonic acid, maltose and other oligosaccharides in saskatoon berry juices. Chromatogram identification: A. Martin; B. Northline; and C. Pembina varieties. Peak identity: 1. maltose; 2. galacturonic acid.

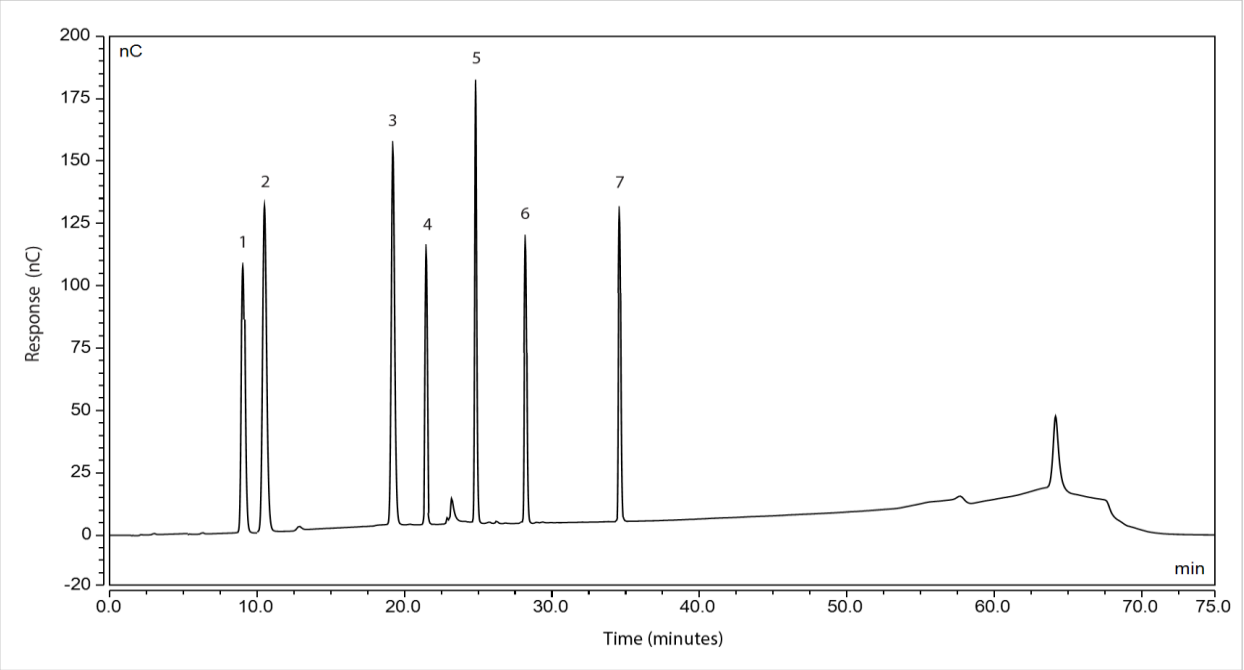


Figure 4.3 HPAE-PAD chromatogram of carbohydrate/oligosaccharide standards. Peak identity: 1. raffinose; 2. maltose; 3. galacturonic acid; 4. maltotriose; 5. maltopentaose; 6. digalacturonic acid; and 7. trigalacturonic acid (concentrations ranged from 100 to 200 mg/L).

Table 4.6 Mean and standard deviation results for galacturonic acid and maltose concentrations in Martin, Northline and Pembina saskatoon berry varieties.

	Martin	Northline	Pembina
Galacturonic Acid	10.2 ± 3.0[1,2]	7.6 ± 0.6	8.7 ± 1.0
Maltose	15.0 ± 4.9	10.1 ± 1.4	8.6 ± 1.4

[1]mg/100 g FW.

[2] Mean ± standard deviation results of triplicate sample analysis.

The concentration of galacturonic acid in the three saskatoon berry varieties ranged from 7.6 to 10.2 mg/100 g FW, with the lowest concentration found for the Northline variety. The concentration of free galacturonic acid in whole fruits is typically very low (McClendon et al., 1959), although it is a major constituent of pectin (Kashyap et al., 2001). The presence of low concentrations of this compound in these expressed juices most likely arises from the interaction

between natural pectinases and pectin, which occurred during the physical manipulation of this fruit.

The concentration of maltose in the saskatoon berry samples ranged from 8.6 to 15.0 mg/100 g FW, with the lowest observed in the Pembina variety. The presence of maltose in whole fruit can be explained by enzymatic starch hydrolysis, which also explains the possible presence of maltotriose (DP3), maltotetraose (DP4) and maltopentaose (DP5) in these samples. The presence of these compounds in expressed juices can be explained by naturally present amylases coming into contact with fruit starch.

The observed oligosaccharide profiles and their concentrations in the three saskatoon berry varieties are of significant importance as the presence and/or absence of oligosaccharides can be used as fingerprints to detect juice authenticity/adulteration and processing (Low and Wudrich, 1993; Willems and Low, 2016). Saskatoon berry juice would be an ideal candidate for adulteration with commercial inexpensive sweeteners such as high fructose corn syrup (HFCS) and invert sugar, based on the cost of the fruit, its high carbohydrate content, and its fructose:glucose ratio. However, due to the low levels of naturally occurring oligosaccharides in saskatoon berry, it is possible that both HPAE-PAD and CGC-FID methods could be developed to detect these types of adulteration in juice or juice blends produced from this fruit.

4.7 Major Minerals

The major mineral analysis results for Martin, Northline and Pembina varieties of saskatoon berry as determined by inductively coupled plasma (ICP) (section 3.8) are shown in Table 4.7.

Table 4.7 Mean and standard deviation results for calcium, magnesium, potassium and sodium concentrations in Martin, Northline and Pembina saskatoon berry varieties.

Minerals	Martin	Northline	Pembina
Calcium (Ca)	34.3 ± 4.7[1,2a]	82.7 ± 6.0[c]	54.0 ± 2.4[b]
Magnesium (Mg)	19.3 ± 2.7[a]	36.7 ± 0.5[b]	18.7 ± 1.7[a]
Potassium (K)	219.0 ± 8.5[a]	248.4 ± 13.7[a]	225.3 ± 12.4[a]
Sodium (Na)	2.2 ± 0.3[a]	3.5 ± 0.6[a]	2.5 ± 0.4[a]

[1]mg/100 g FW.

[2]Mean ± standard deviation results of duplicate sample analysis.

[a-c]Mean values in the same column followed by a common letter were not statistically different ($p<0.05$) by Tukey's HSD multiple range test between saskatoon berry varieties.

The mean calcium concentrations of the three saskatoon berry varieties ranged from 34.3 to 82.7 mg/100 g FW with the highest being found for the Northline variety. These results were similar to those reported in literature for five varieties (Brnensky, Martin, Smoky, Thiessen and Tisnovsky) of this fruit, which ranged from 41.9 to 98.2 mg/100 g FW (Mazza, 2005; Jurikova et al., 2012; Rop et al., 2012), with no literature results for Northline and Pembina varieties being reported. The calcium content of the saskatoon berry varieties in this study were higher than those reported for apple, blueberry and pear whole fruits of 7, 6 and 9 mg/100 g edible portion, respectively (Health Canada, 2016; USDA, 2016).

The mean magnesium concentration of the three saskatoon berry varieties ranged from 18.7 to 36.7 mg/100 g FW with the highest found for the Northline variety. These results were similar to those reported in literature for the aforementioned five saskatoon berry varieties of 21.0 to 31.6 mg/100 g FW (Mazza, 2005; Jurikova et al., 2012; Rop et al., 2012) with no reported literature results for the Northline and Pembina varieties. The magnesium content of the saskatoon berry varieties in this study were higher than those reported for apple, blueberry and pear whole fruits of 5, 6 and 7 mg/100 g edible portion, respectively (Health Canada, 2016; USDA, 2016).

Potassium was found to be the most abundant mineral in the samples analyzed with a mean concentration range of 219.0 to 248.4 mg/100 g FW, with no significant differences observed between these three varieties. These results were within the range of those reported in literature

for the aforementioned five saskatoon berry varieties of 162.1 to 431.7 mg/100 g FW (Mazza, 2005; Jurikova et al., 2012; Rop et al., 2012). No literature results for the potassium content of Northline and Pembina saskatoon berry varieties were found. The potassium content of saskatoon berries found in this study were higher than those reported for apple, blueberry and pear of 109, 77 and 116 mg/100g edible portion, respectively (Health Canada, 2016; USDA, 2016).

The mean sodium concentration of the three saskatoon berry varieties ranged from 2.2 to 3.5 mg/100 g FW, with no significant differences observed between the three varieties. These results were in agreement with those reported in literature for the aforementioned five saskatoon berry varieties of 0.48 to 2.5 mg/100 g FW (Mazza, 2005; Jurikova et al., 2012; Rop et al., 2012). No literature results for the sodium content of Northline and Pembina saskatoon berry varieties were found. The sodium content of saskatoon berries found in this study were higher than those reported for apple, blueberry and pears of 1 mg of sodium/100 g edible portion (Health Canada, 2016; USDA, 2016).

The observed differences in mineral concentrations between the three varieties studied, and between the mean results for these three varieties and those reported in literature are most likely due to fruit variety, agronomic practices, and environmental growth conditions such as soil fertility, temperature and humidity (Hornick, 1992).

4.8 Organic Acids

The major organic acids present in the expressed saskatoon berry juice samples (section 3.3.1) were qualitatively and quantitatively determined by high performance liquid chromatography-photodiode array detection (HPLC-PDA) employing retention time (RT) comparison to external standards, spiking experiments and peak area/height comparison to standard curves (Table 4.8). Organic acid standards chosen for comparison/identification were based on their literature reported presence in saskatoon berry. A representative HPLC-PDA chromatogram for the seven organic acids used as standards in this study is shown in Figure 4.4.

Table 4.8 Mean and standard deviation results for individual and total organic acids in Martin, Northline and Pembina saskatoon berry varieties.

	Martin	Northline	Pembina
Ascorbic Acid	10.4 ± 1.2[1,2a]	10.6 ± 1.7[a]	16.0 ± 1.5[b]
Malic Acid	304.7 ± 1.0[a]	393.9 ± 2.2[a]	333.2 ± 1.7[c]
Maleic Acid	34.0 ± 1.5[a]	39.6 ± 1.2[b]	41.3 ± 1.5[b]
Oxalic Acid	28.5 ± 1.5[b]	51.5 ± 0.2[c]	8.8 ± 0.3[a]
Quinic Acid	51.4 ± 2.4[c]	42.9 ± 0.9[b]	26.2 ± 1.1[a]
Succinic Acid	120.4 ± 4.3[b]	316.3 ± 1.3[a]	200.4 ± 1.4[c]
Total	549.3 ± 9.6[a]	854.8 ± 6.1[c]	625.8 ± 6.6[b]

[1]mg/100 g FW.

[2]Mean ± standard deviation results of triplicate sample analysis.

[a-c]Mean values in the same row followed by a common letter were not statistically different ($p<0.05$) by Tukey's HSD multiple range test between saskatoon berry varieties.

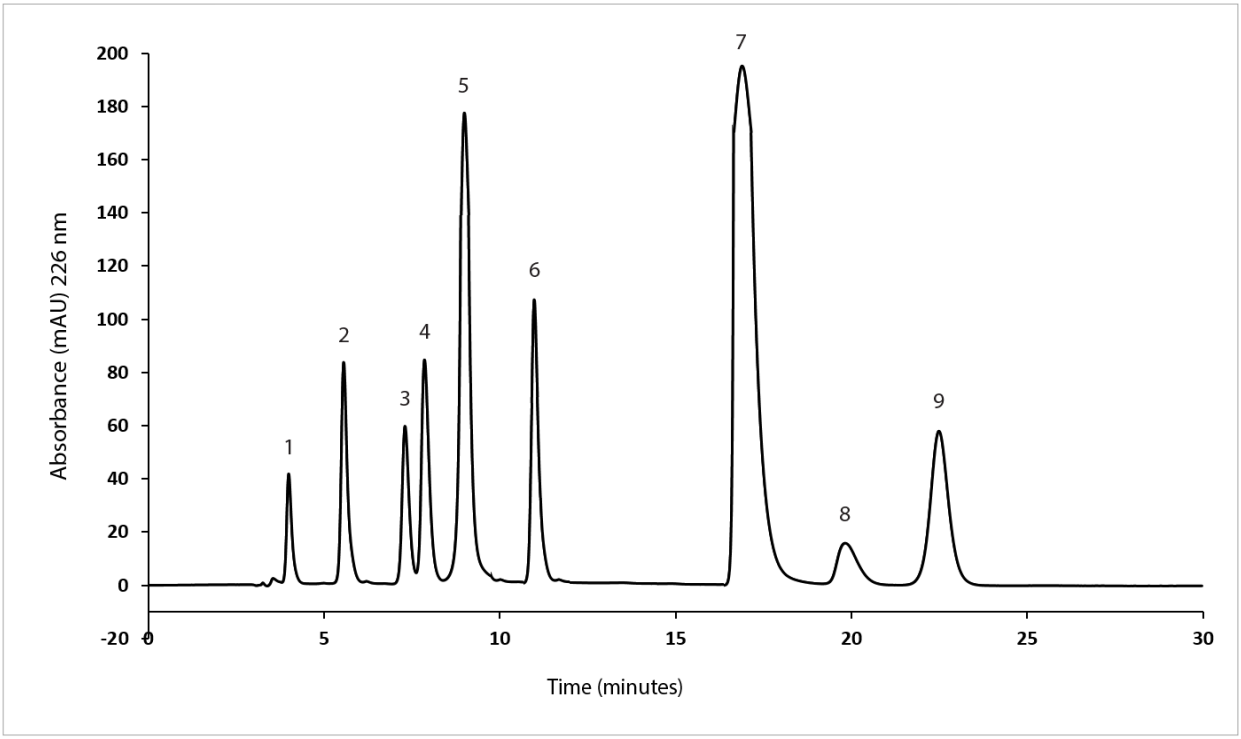
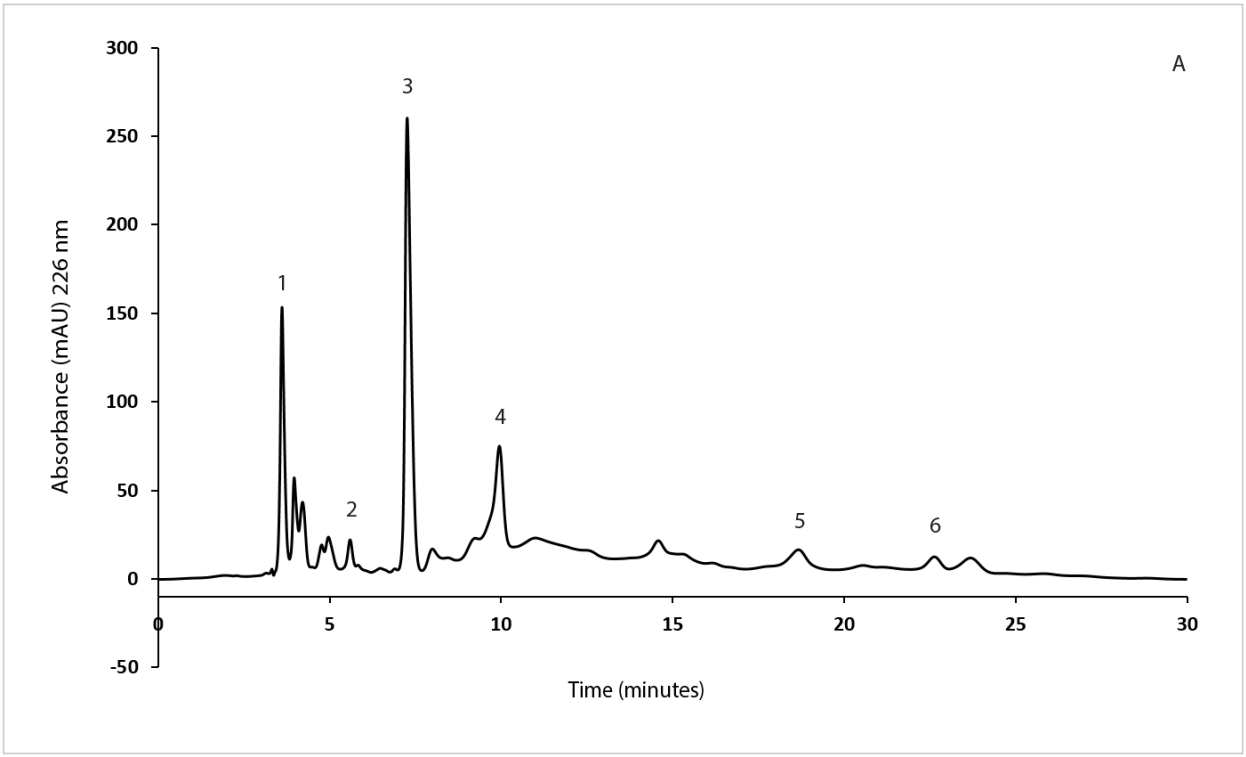


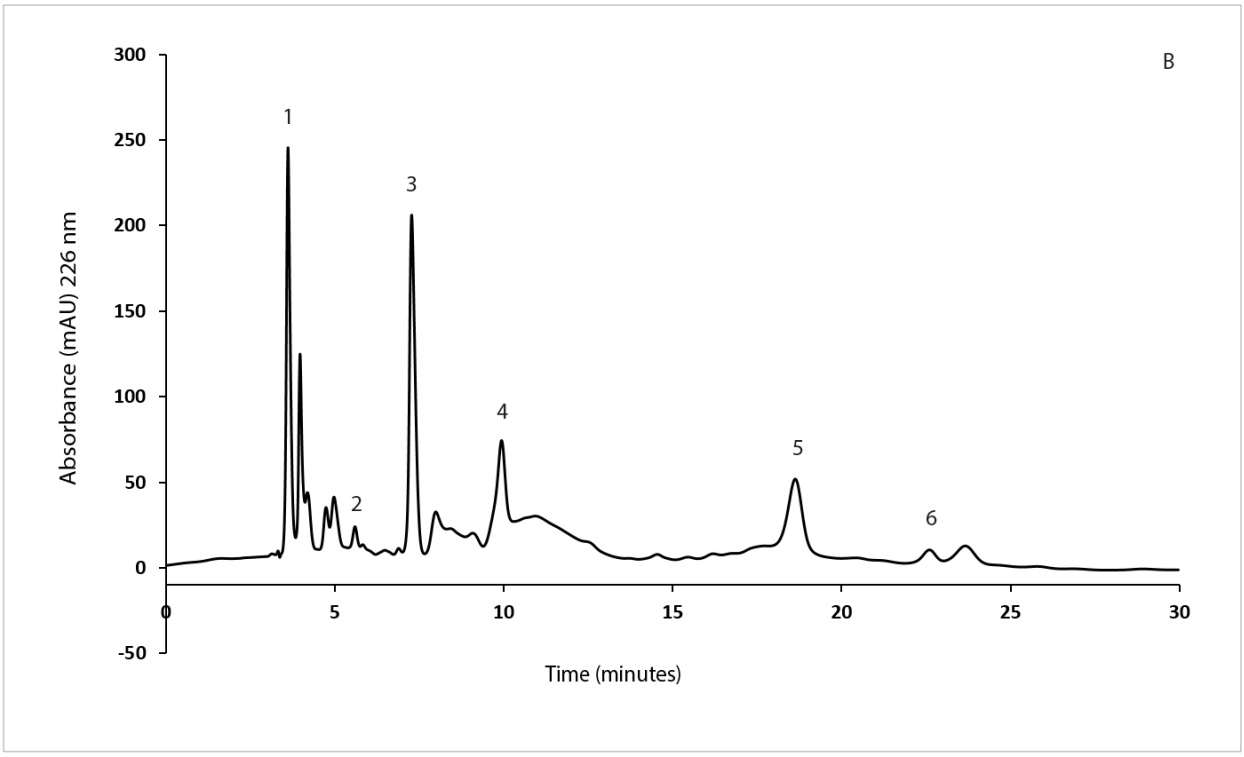
Figure 4.4 HPLC-PDA chromatogram of the organic acid standards. Peak identity: 1. oxalic acid; 2. quinic acid; 3. malic acid; 4. malonic acid; 5. ascorbic acid; 6. acetic acid; 7. citric acid; 8. succinic acid; and 9. maleic acid (concentrations ranged from 100 to 500 mg/L).

Experimental results showed that the mean total organic acid concentrations in the three expressed saskatoon berry juices ranged from 549.3 to 854.8 mg/100 g FW (Table 4.8; Figure 4.5). The Northline variety contained the highest mean organic acids content of 854.8 and Martin the lowest at 549.3 mg/100 g FW, respectively. These two values were significantly different and show that this 1.6x difference in acidity affords juices with a range of sourness that can be exploited for consumer taste preferences. The total organic acid results for these samples were within the broad literature range for saskatoon berries of 580 to 1790 mg/100 g FW (Mazza, 2005; Jurikova et al., 2012).

The major organic acids present in each of the samples studied were malic and succinic acids, with concentration ranges of 304.5 to 394.0, and 120.4 to 316.3 mg/100 g FW, respectively. The malic acid content in these samples accounted for 46-55% of the total organic acids content. Ascorbic acid was identified in each of the saskatoon berry juices with a concentration range of

10.4 to 16.0 mg/100 g FW, which was greater than those reported for apple and pear of 4.6 and 4.3 mg/100 g FW, respectively (USDA, 2016).





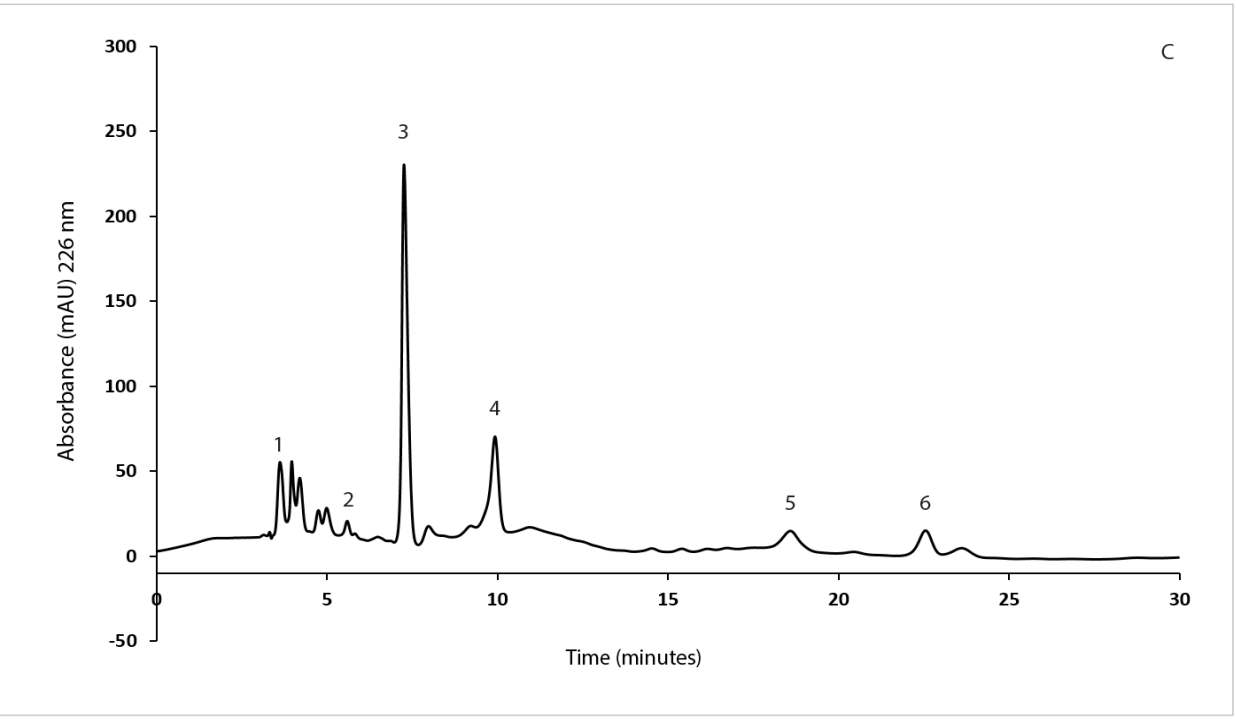


Figure 4.5 HPLC-PDA chromatograms showing the organic acid composition of saskatoon berry juices. Chromatogram identification: A. Martin; B. Northline; and C. Pembina. Peak identity: 1. oxalic acid; 2. quinic acid; 3. malic acid; 4. ascorbic acid; 5 succinic acid; and 6. maleic acid.

From literature, the major organic acid composition and concentration ranges for Northline and Smoky varieties were, malic acid (350-562 mg/100 g FW), succinic acid (100-300 mg/100 g FW), and quinic acid (60-90 mg/100 g FW) (Wolfe and Wood, 1971; Rogiers and Knowles, 1997). Minor organic acids identified in these two varieties included citric acid (10-25 mg/100 g FW), *cis*-aconitic acid (0.5-1.3 mg/100 g FW), pyruvic acid (5-25 mg/100 g FW), oxalic (2.0-2.7 mg/100 g FW) and fumaric acids (0.0005-0.01 mg/100 g FW) (Rogiers and Knowles, 1997). Results from this study were in general agreement with the aforementioned with respect to major organic acid composition, however, differences were observed in minor organic acid content and concentrations.

Literature values for the malic acid concentration range in apples and pears are 522.2 to 1993.7, and 151 to 478 mg/100 g FW, respectively, and are dependent upon fruit variety (Chinnici et al., 2005).

4.9 Total Phenolic Content of Saskatoon Berry Varieties and Pomace Extracts as Determined by the Folin-Ciocalteau Assay

Sample total phenolic content was determined employing the Folin-Ciocalteau (FC) assay, which measures the total reducing capacity of the sample. This method is based on an electron transfer reaction between sample reducing compounds (e.g. phenolics) and the phosphomolybdic-phosphotungstic acid reagent, which results in a sample solution colour change from yellow to blue (Singleton et al., 1999; Huang et al., 2005) that is measured spectrophotometrically at 765 nm. The FC reagent reacts preferentially with phenolics under the basic conditions (pH ~10) of this assay (15% w:v, Na_2CO_3) as the phenolic hydroxyl group dissociates to form the phenolate anion which reduces the FC reagent (Huang et al., 2005), and the colour change is proportional to the concentration of the phenolic(s). It has been reported that other sample constituents, such as such as vitamins (e.g. Vit C), amino acids and copper complexes can artificially inflate total phenolic content results (Wrolstad, 1993; Singleton et al., 1999; Everette et al., 2010).

Sample total phenolic content was expressed as milligrams of gallic acid (a phenolic acid that is a member of the hydroxybenzoic acid subclass) equivalents (GAE) per 100 g of fresh fruit weight (FW), or wet or dry pomace (mg GAE/100 g) (Table 4.9).

Table 4.9 Mean and standard deviation total phenolic content results for extracts of Martin, Northline and Pembina saskatoon berry varieties and wet and dry pomace.

	Total Phenolics				
Extracts	Martin[1]	Northline[1]	Pembina[1]	Wet Pomace[2]	Dry Pomace[2]
Aqueous	222.6 ± 9.9[3Ab]	251.1 ± 4.9[Ac]	119.5 ± 2.2[Aa]	410.6 ± 0.5[A]	212.1 ± 3.3[A]
EFW	313.1 ± 11.4[Ca]	526.8 ± 5.3[Cb]	296.2 ± 6.7[Ca]	587.8 ± 6.1[C]	585.6 ± 4.9[C]
MFW	249.8 ± 5.8[Ba]	457.7 ± 11.5[Bc]	252.4 ± 1.5[Ba]	506.0 ± 3.9[B]	338.6 ± 2.8[B]

[1]mg GAE/100 g FW.

[2]mg GAE/100 g pomace.

[3]Mean ± standard deviation results of triplicate sample analysis.

[A-C]Mean values in the same column followed by a common letter were not statistically different ($p<0.05$) by Tukey's HSD multiple range test between the type of extracts.

[a-c]Mean values in the same row followed by a common letter were not statistically different ($p<0.05$) by Tukey's HSD multiple range test between the varieties and pomace.

Analytical results show that the Northline variety had the highest total phenolic content (TPC) in all three solvent extracts followed by Martin and Pembina. In addition, the ethanol-formic acid-water (EFW) extract was found to contain a significantly higher (~527 GAE) TPC when compared to those observed in the aqueous (~251 GAE) and the methanol-formic acid-water extracts (MFW; ~458 GAE). This trend in higher TPC values with EFW versus MFW extracts was observed for all samples. The significantly higher TPC in the EFW extract can be explained by improved phenolics extraction via cell wall damage, the increased solubility of intermediate hydrophobic phenolics in this solvent (i.e. dielectric constant of 24.30 for ethanol versus 32.63 for methanol) and the role of formic acid in compound protonation, which results in improved aqueous-alcohol solubility (Weast, 1978). A similar trend in increased TPC and extraction solvent was observed for both wet and dry pomace samples. These results clearly show that the pomace co-product stream from juice production provide a good source of phenolic compounds that can be extracted and used as food ingredients and as health products (i.e. nutraceuticals). However, as this pomace was not produced from the berries used in this study, a direct comparison of TPC results was not possible. Although TPC results provide no information on extract

phenolic/reducing compound composition and structure, these results show the important relationship between extraction solvent composition and phenolics extraction.

According to literature, TPC results for saskatoon berries extracted with 80% methanol containing 0.1% formic acid ranged from 405 to 801 mg GAE/100 g fresh weight (Fukumoto and Mazza, 2000; Mazza and Cacace, 2003; Mazza, 2005; Bakowska-Barczak and Kolodziejczyk, 2008). Based on specific varieties, TPC literature reports for 80% methanol extracts from Martin, Northline and Pembina varieties were 724.0, 498-604, and 577.04 mg GAE/100 g fresh weight, respectively (Mazza, 2005; Bakowska-Barczak and Kolodziejczyk, 2008). These values were all higher than those found in this study, with those reported for Martin and Pembina greater by approximately 43% and 51%, respectively. It is not clear why these values are much higher than those observed in this study, however, fruit chemical compound composition and concentration differences are known to be impacted by environmental factors such as light, temperature and agronomic practices (Ozga et al., 2006; Bakowska-Barczak et al., 2007; Bakowska-Barczak and Kolodziejczyk, 2008). As presented previously, total phenolic content as measured by the FC assay includes the total reducing activities of a variety of sample constituents including but not limited to, ascorbic acid, amino acids and copper complexes. Therefore, concentration differences in these compounds between the fruit samples analyzed in this study and those in literature would also contribute to the observed TPC differences.

The wet pomace TPC results were higher for each of the three extraction solvents when compared to the three saskatoon berry varieties. The observed differences in TPC values between the fruit varieties and wet pomace were not due to moisture content as 25 g of fruit (mean moisture content of 81.5%; Table 4.2) and 5 g of wet pomace (moisture content of 68.0%; Table 4.3) was used in the FC assay, resulting in a solids ratio of approximately 3:1 (fruit:pomace). Therefore, the observed differences in TPC values were due to the fruit chemical composition and concentrations in the pomace. The observed TPC for saskatoon berry pomace was lower than those reported in literature for a selection of fruit pomaces (unreported moisture contents) that are considered to be rich in phenolic compounds including, bilberry, blackberry, cranberry and raspberry, with values of 1116.24, 804.50, 600 and 637.77 mg of GAE/100 g of pomace, respectively (Lee and Wrolstad, 2004; White et al., 2010; Vulić et al., 2011).

Dried pomace TPC results were found to be significantly lower in both the aqueous and MFW extracts when compared to wet pomace. As these samples were not produced from the same

raw fruit (e.g. Martin, Northline and Thiessen varieties for dry pomace, and Northline variety for wet pomace), it is not possible to directly compare these results. The EFW total phenolic content results for wet and dried pomace were not significantly different, however, there is a significant difference in their moisture contents of 68.0% versus 7.9% (Table 4.3). Therefore, it was expected that the EFW TPC for the dry pomace should have been higher than that for the wet pomace as 5 g of each material was used in these experiments. As this was not observed in these samples, it can be hypothesized that the reducing powers of the more hydrophobic phenolic compounds in the dry pomace sample were negatively impacted by the drying process.

4.10 Total Phenolic Chromatographic Index/Indices (TPCI) of Saskatoon Berry Varieties and Pomace Extracts

Sample phenolics classification was accomplished by high performance liquid chromatography with photodiode array detection (HPLC-PDA). For these analyses, all sample compounds exhibiting a detector response (i.e. chromatographic peak with a signal to noise ratio ≥3x) at 280 nm were assumed to be phenolics. In conjunction with sample analysis a standard containing eleven of the most common phenolics reported to be present in fruits was also analyzed under the same chromatographic conditions so as to afford specific sample compound identification based on retention time comparisons to this standard (Figure 4.6). Sample chromatographic peaks that did not match the retention times of external standards were assigned a phenolic subclass based on the comparison of their UV-visible spectral profile with those of the external standards (Figure 4.7).

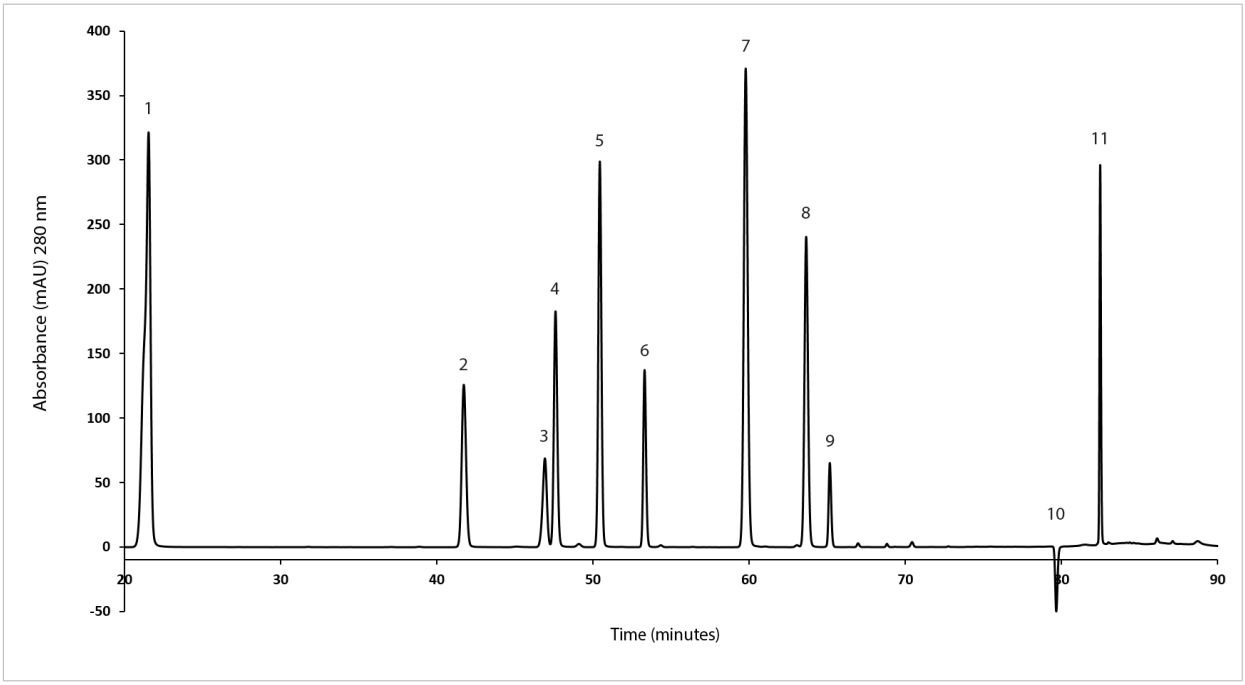


Figure 4.6 HPLC-PDA chromatogram of the eleven most common phenolics present in fruits. Peak identities: 1. gallic acid; 2. 4-hydroxybenzoic acid; 3. catechin; 4. chlorogenic acid; 5. caffeic acid; 6. epicatechin; 7. *p*-coumaric acid; 8. ferulic acid; 9. rutin; 10. quercetin; and 11. naringenin. (standard concentrations ranged from 100 to 200 ppm).

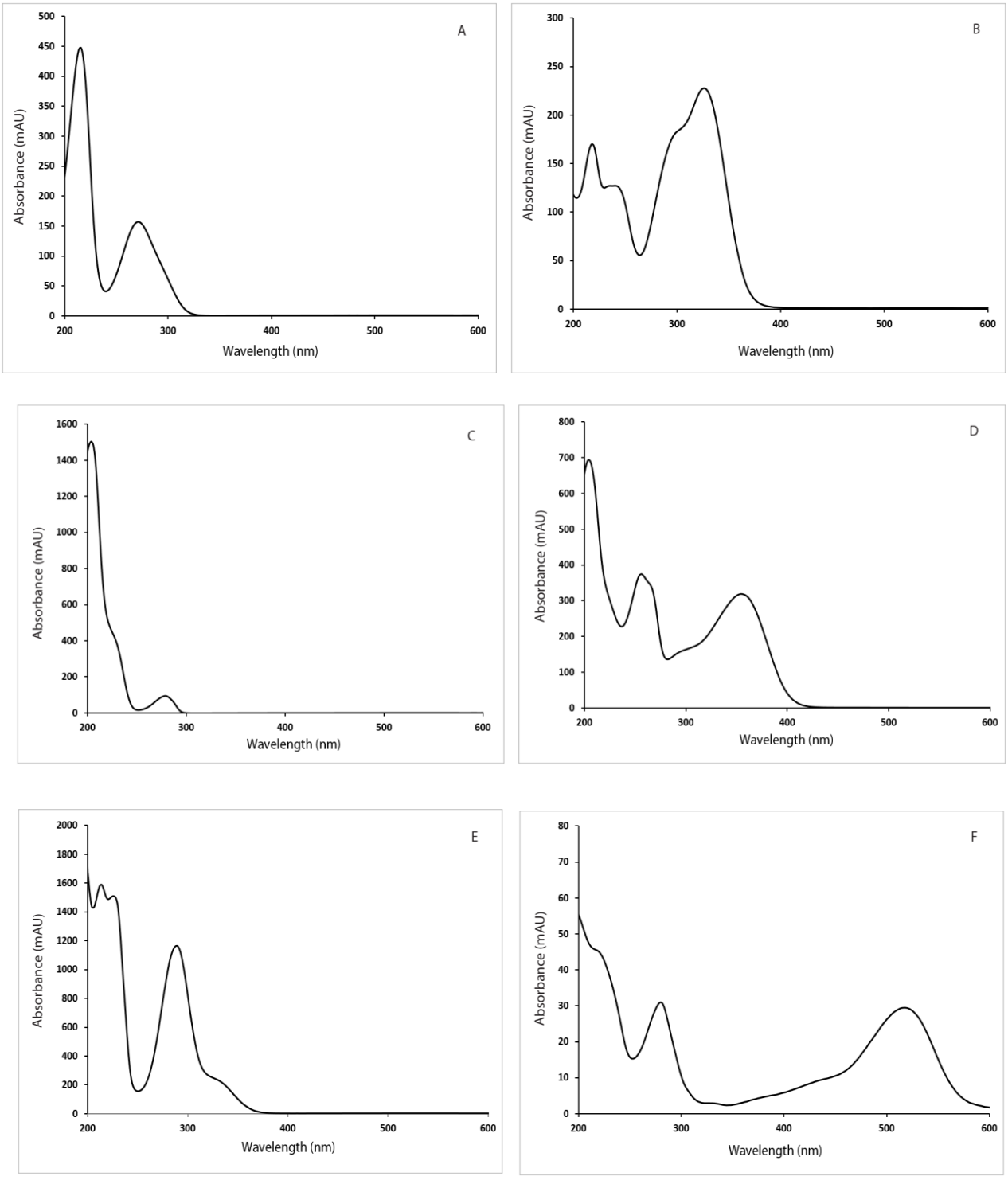


Figure 4.7 UV-visible spectra profiles of phenolic standards. A: gallic acid, a hydroxybenzoic acid; B. chlorogenic acid, a hydroxycinnamic acid; C. catechin, a flavanol; D. rutin, a flavonol; E. naringenin, a flavanone; F: cyanidin-3-*O*-rutinoside, an anthocyanin.

Representatives of the five major subclasses of fruit phenolics were also analyzed by HPLC-PDA so as to determine the total phenolic chromatographic index for each sample extract. These representative compounds, their subclasses and approximate retention times were: gallic acid/hydroxybenzoic acids/~20 min; chlorogenic acid/hydroxycinnamic acids/~37 min; cyanidin-3-*O*-rutinoside/anthocyanins/~39 min; epicatechin/flavanols/~48 min; and rutin/flavonoids/~67 min (Figures 4.8 and 4.9).

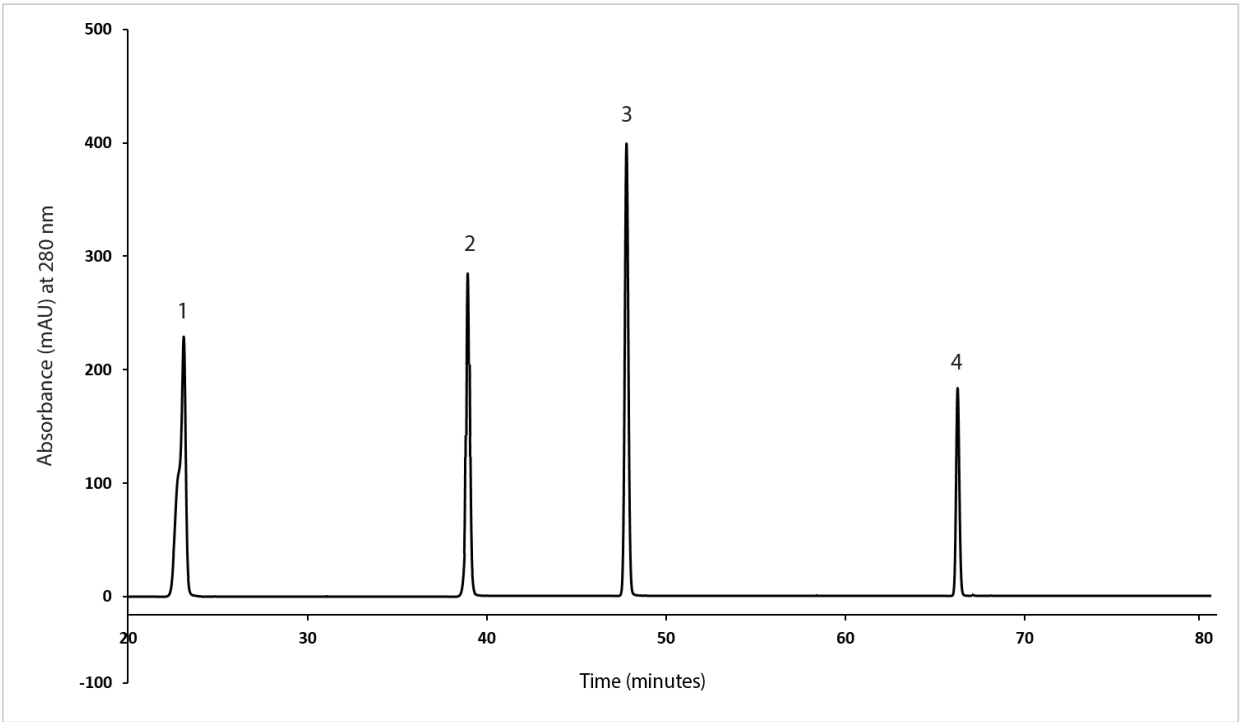


Figure 4.8 HPLC-PDA chromatogram of phenolic standards representing four major subclasses of fruit phenolics. Peak identities and subclasses: 1. gallic acid, hydroxybenzoic acids; 2. chlorogenic acid, hydroxycinnamic acids; 3. epicatechin, flavanols; and 4. rutin, flavonols. (standard concentrations ranged from 100 to 200 ppm).

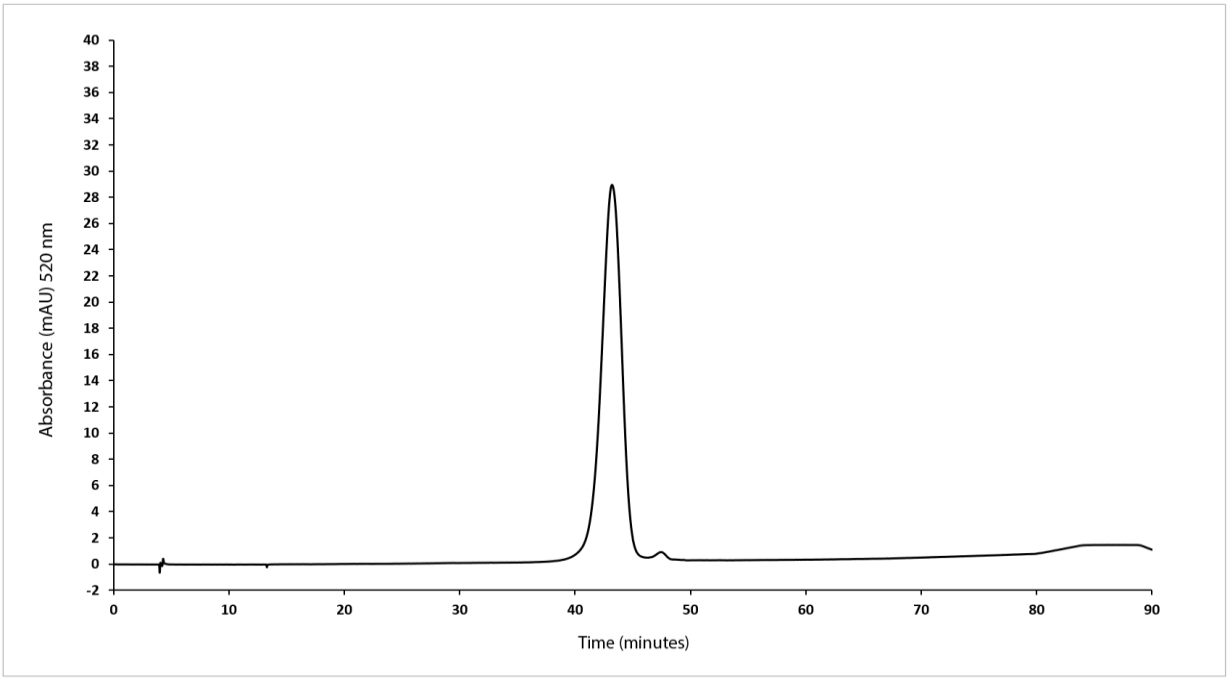
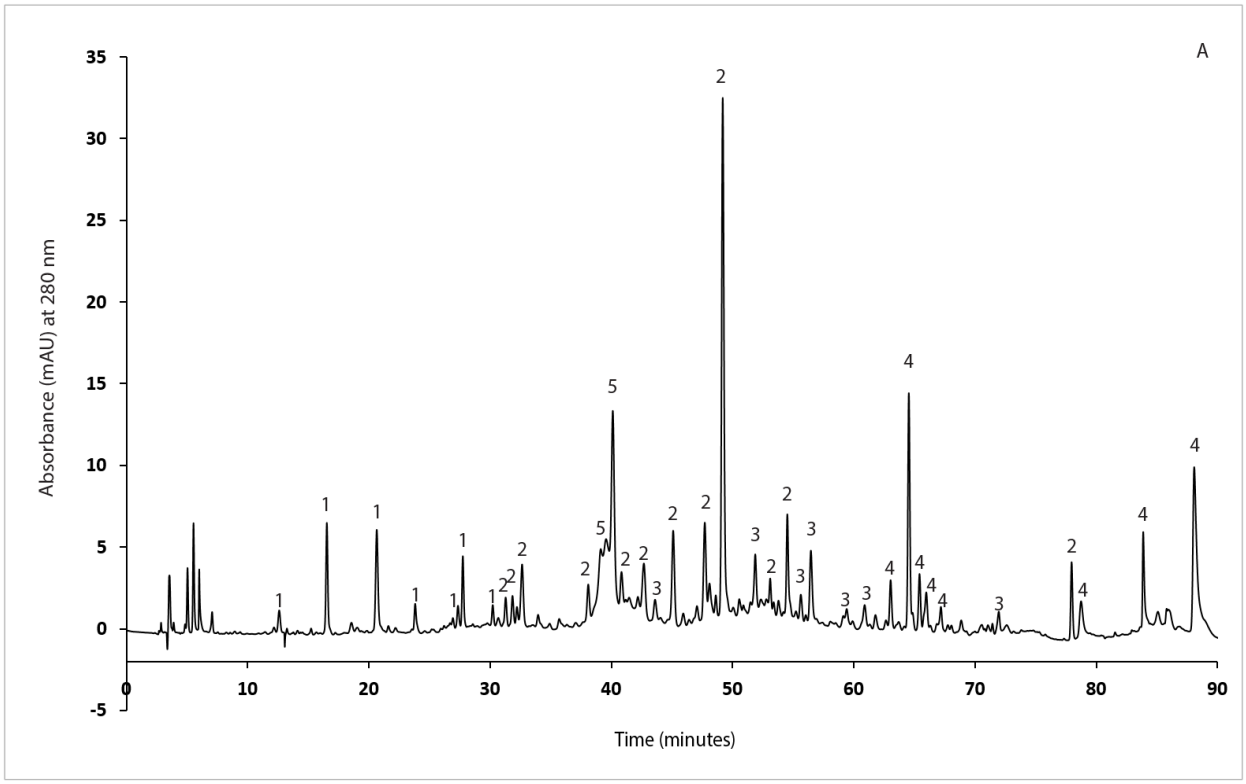
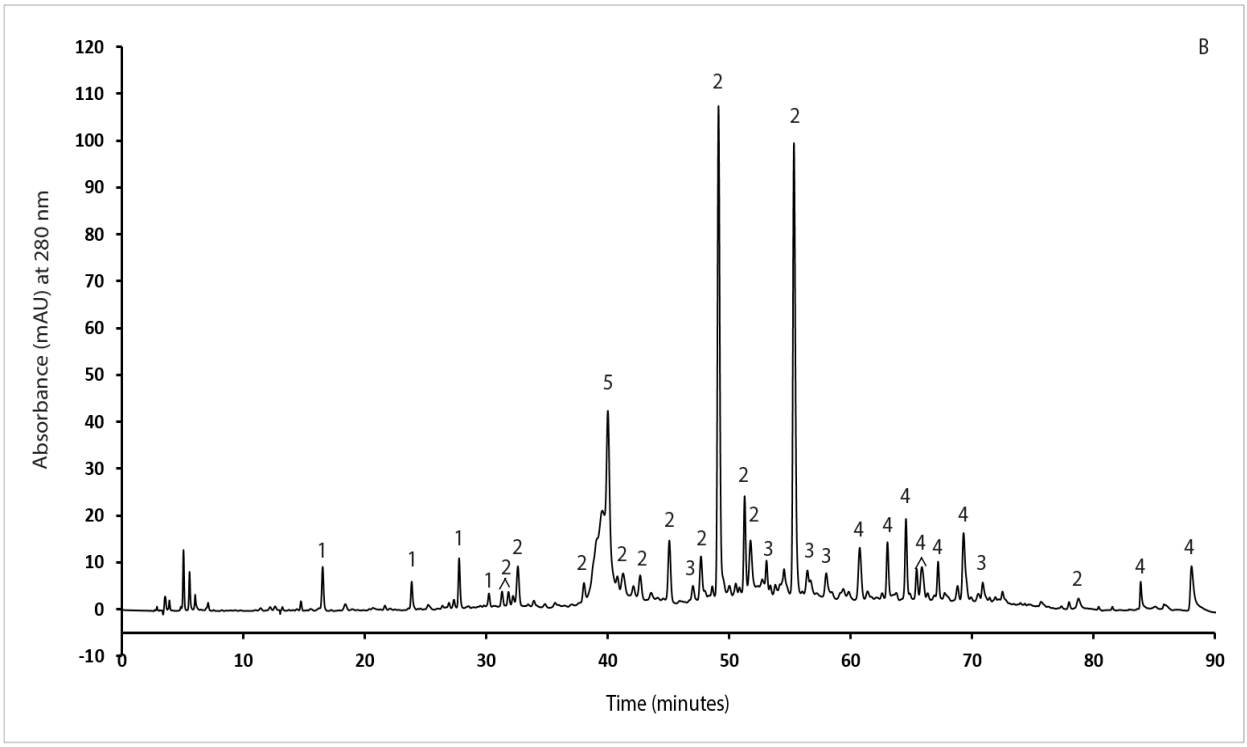


Figure 4.9 HPLC-PDA chromatogram of cyanidin-3-*O*-rutinoside (100 ppm; identified as 5) representing the anthocyanin subclass.

The total phenolic chromatographic index (TPCI) is defined as the sum of all extractable phenolics as analyzed by HPLC-PDA, and is calculated by determining the area sum for all identified phenolic subclasses found in the sample (Escarpa and Gonzalez, 2001). Initially, the phenolic subclass of each peak was identified by UV-visible comparison to standards (Figure 4.7), and then subclass concentration was determined by area summation and comparison with the phenolic standard of that subclass through linear regression (i.e. concentration vs. peak area). Representative HPLC-PDA chromatograms for each sample extract with subclass identification of peaks are shown in Figure 4.10. This method was also used to determine phenolic sublcass concentration and TPCI values for each of the three solvent extracts (i.e. water, EFW and MFW) for each of the three saskatoon berry varieties and wet and dry pomace, and these results are reported in Tables 4.10-4.12. Sample anthocyanins and flavonoids were quantified at wavelengths that were close to their maximum absorbances of 520 and 360 nm, respectively. All other phenolic subclasses were quantified at 280 nm.


A
Absorbance (mAU) at 280 nm
Time (minutes)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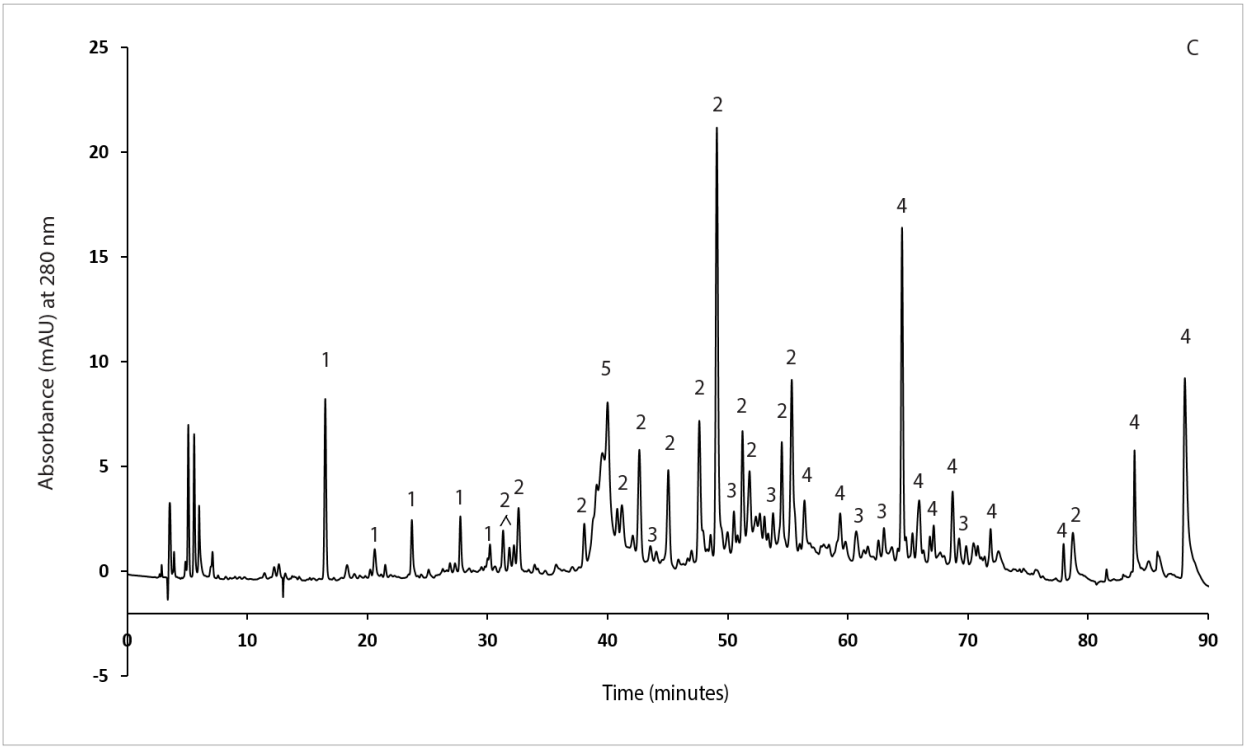
Time (minutes)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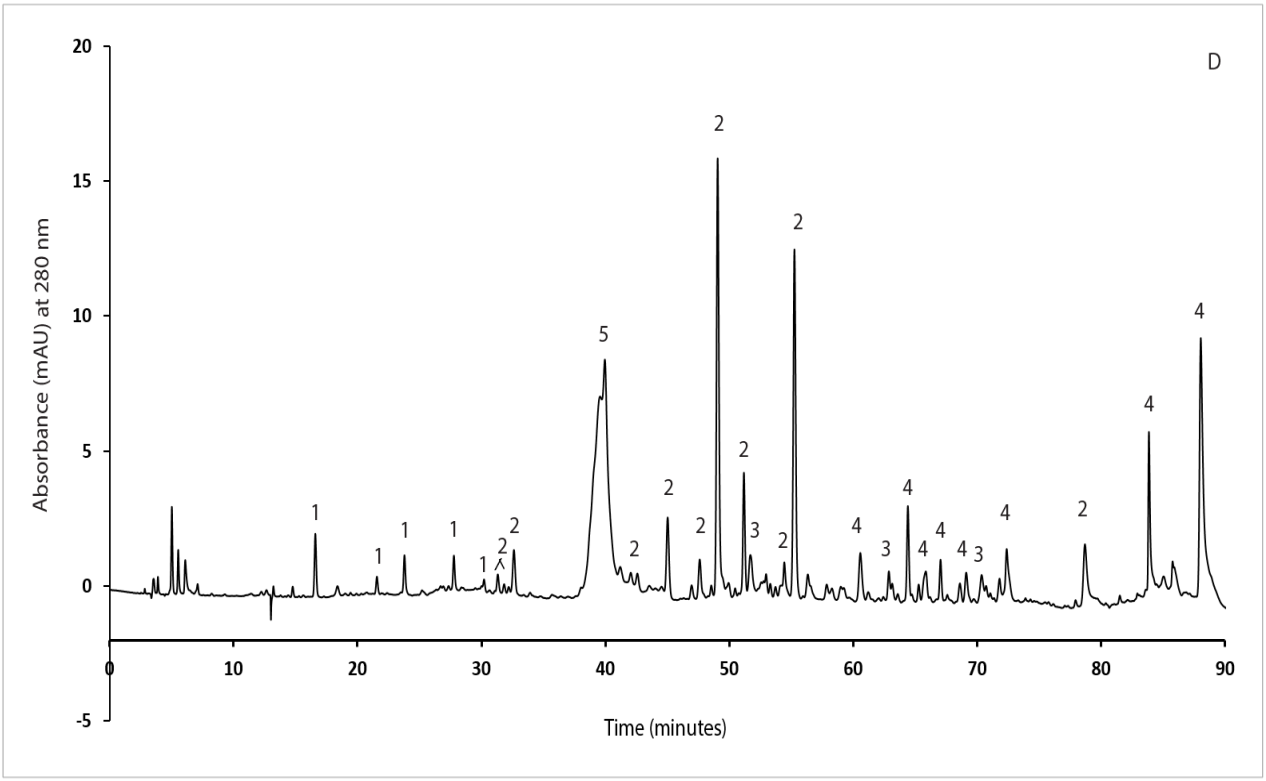
Absorbance (mAU) at 280 nm
Time (minutes)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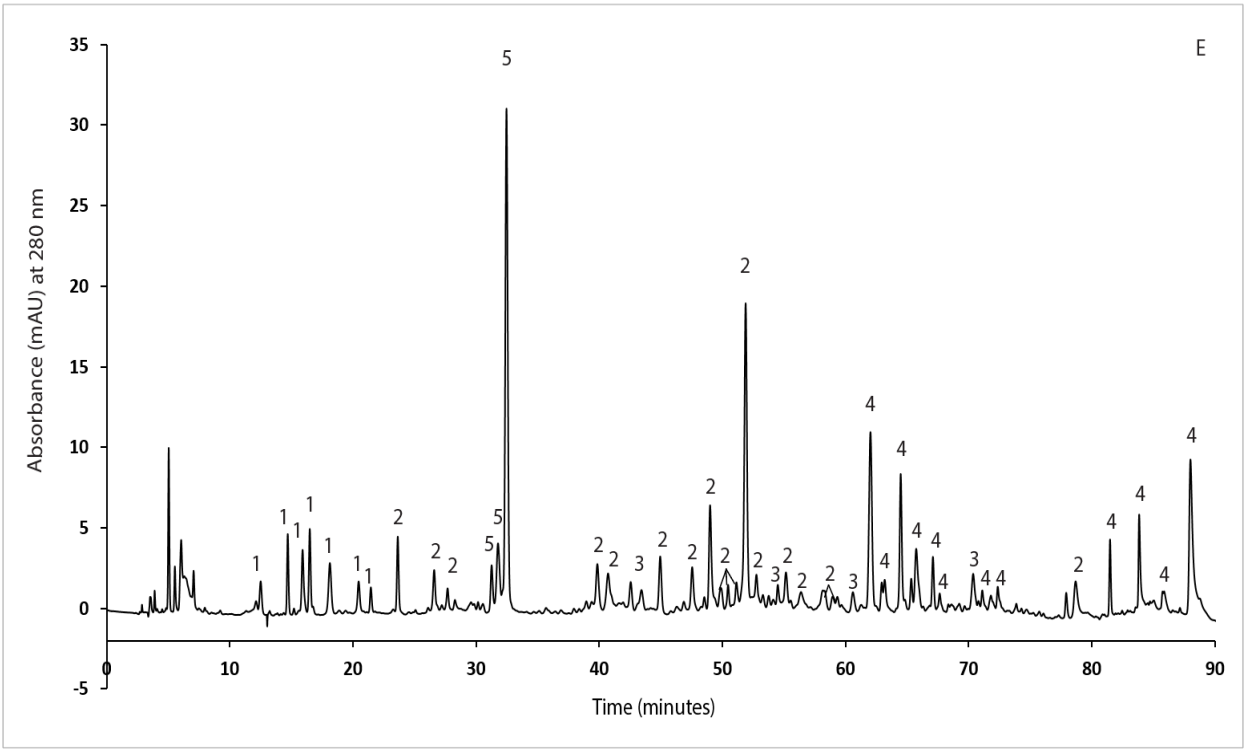
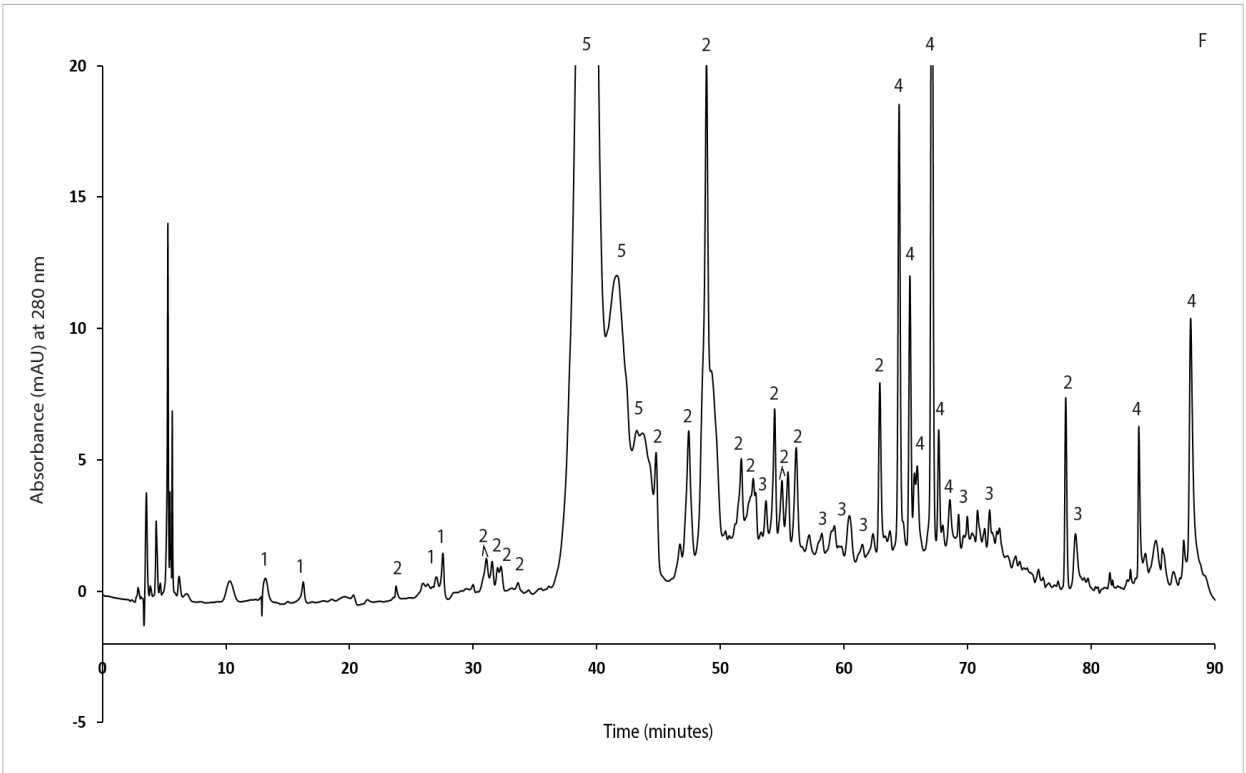
Time (minutes)


E
Absorbance (mAU) at 280 nm
Time (minutes)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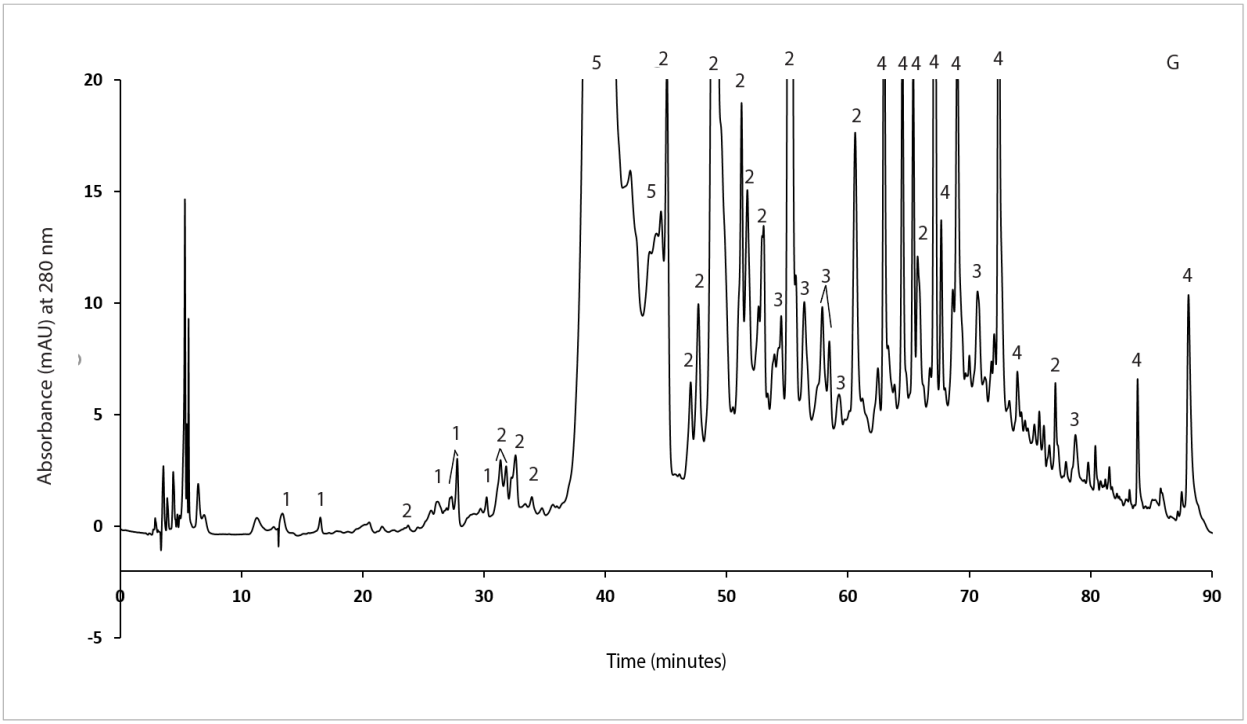
Time (minutes)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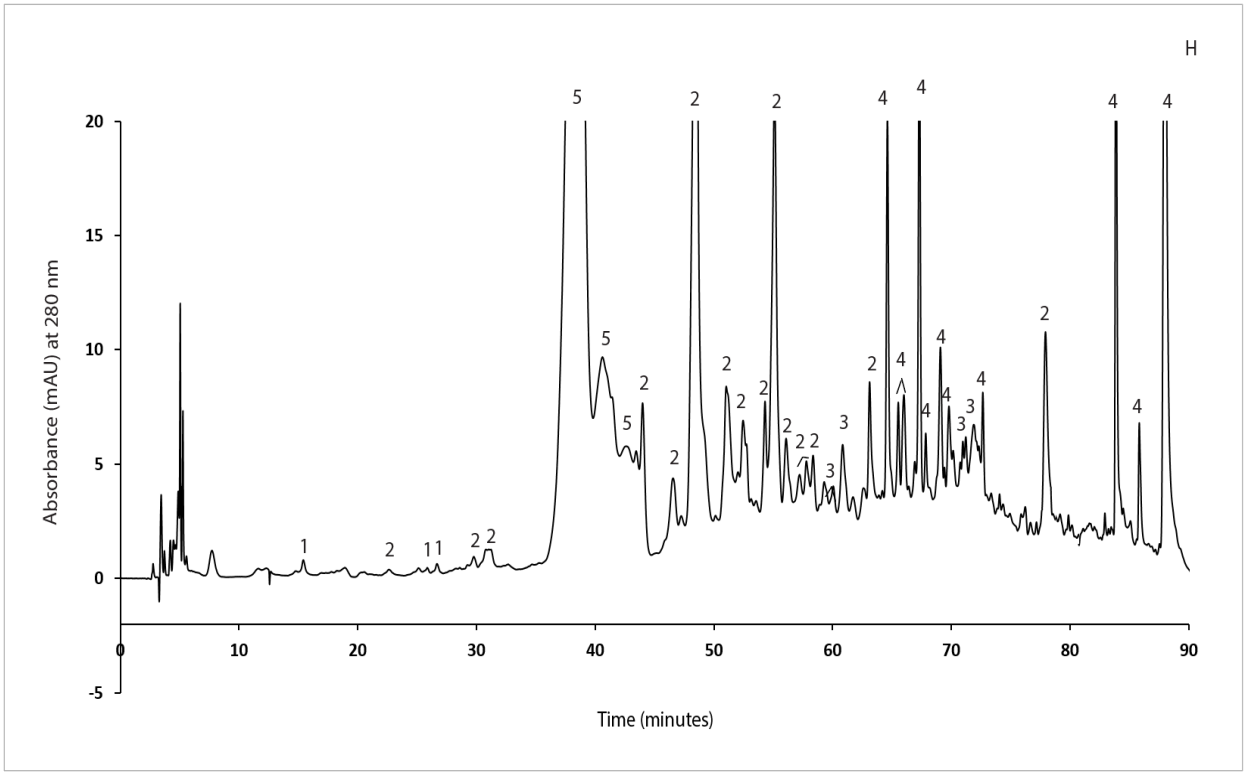
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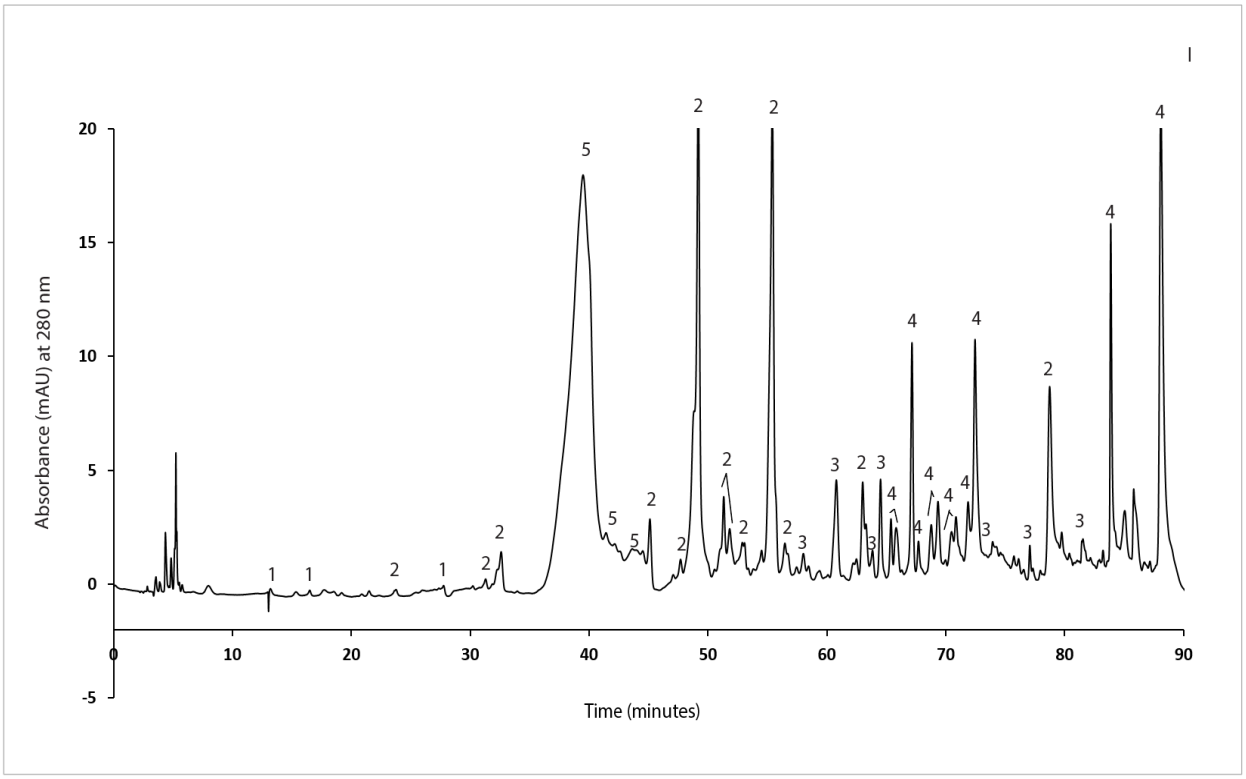
Time (minutes)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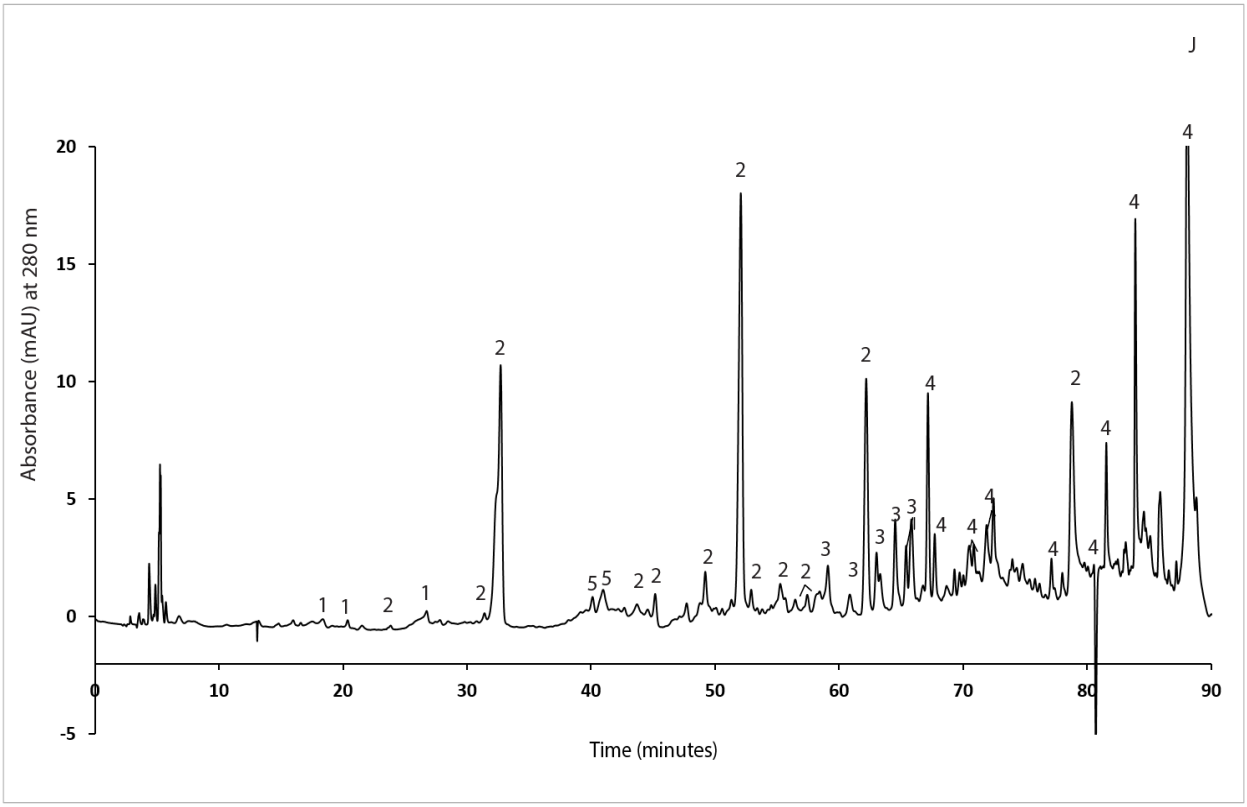
Time (minutes)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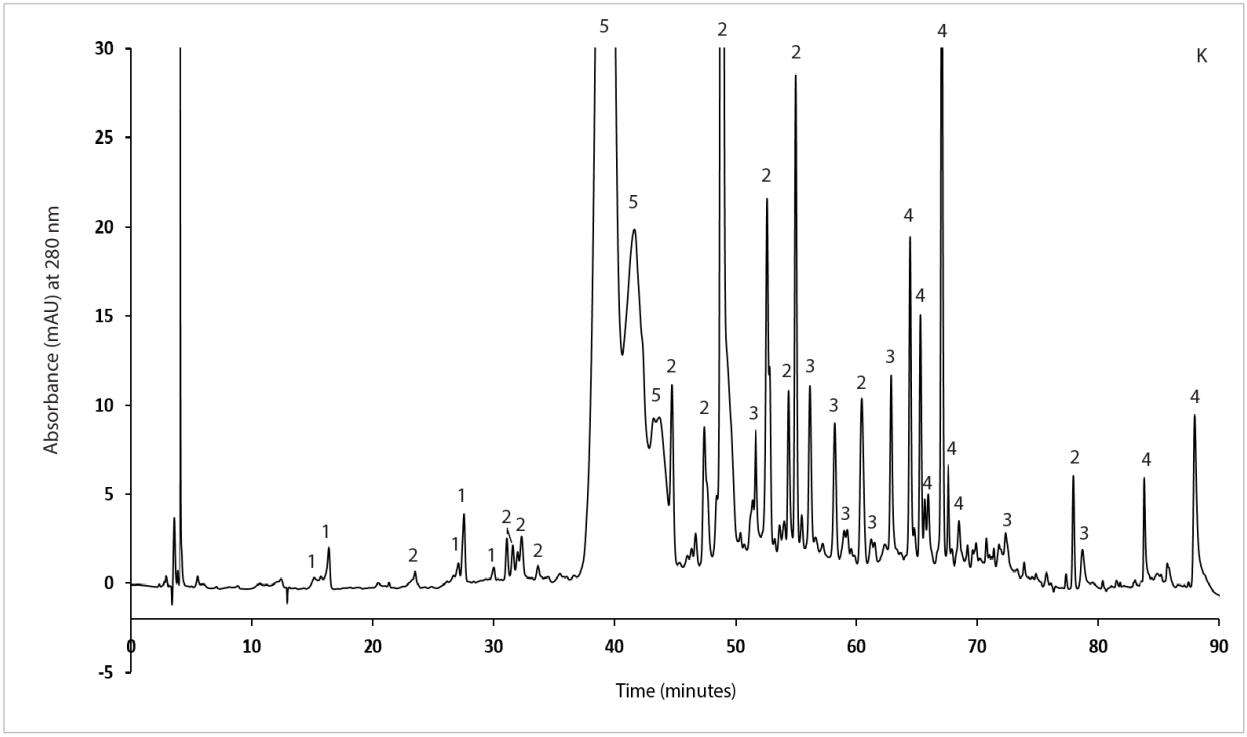
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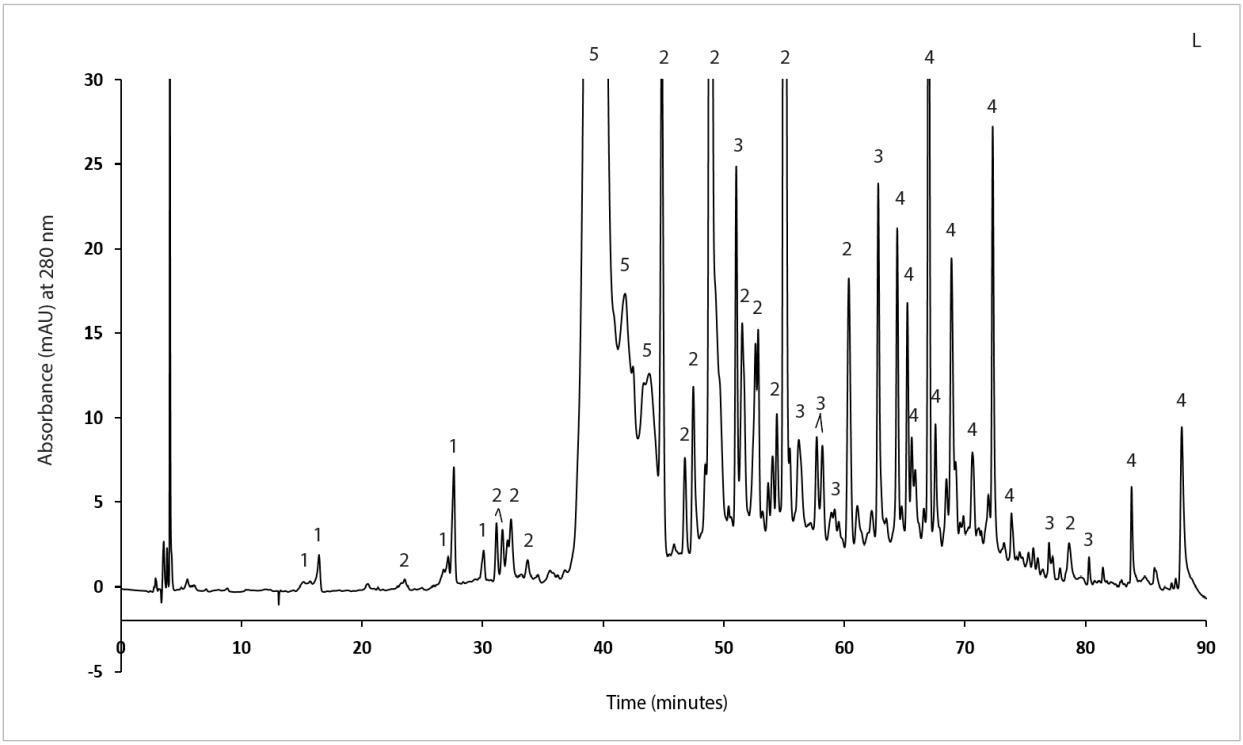
Time (minutes)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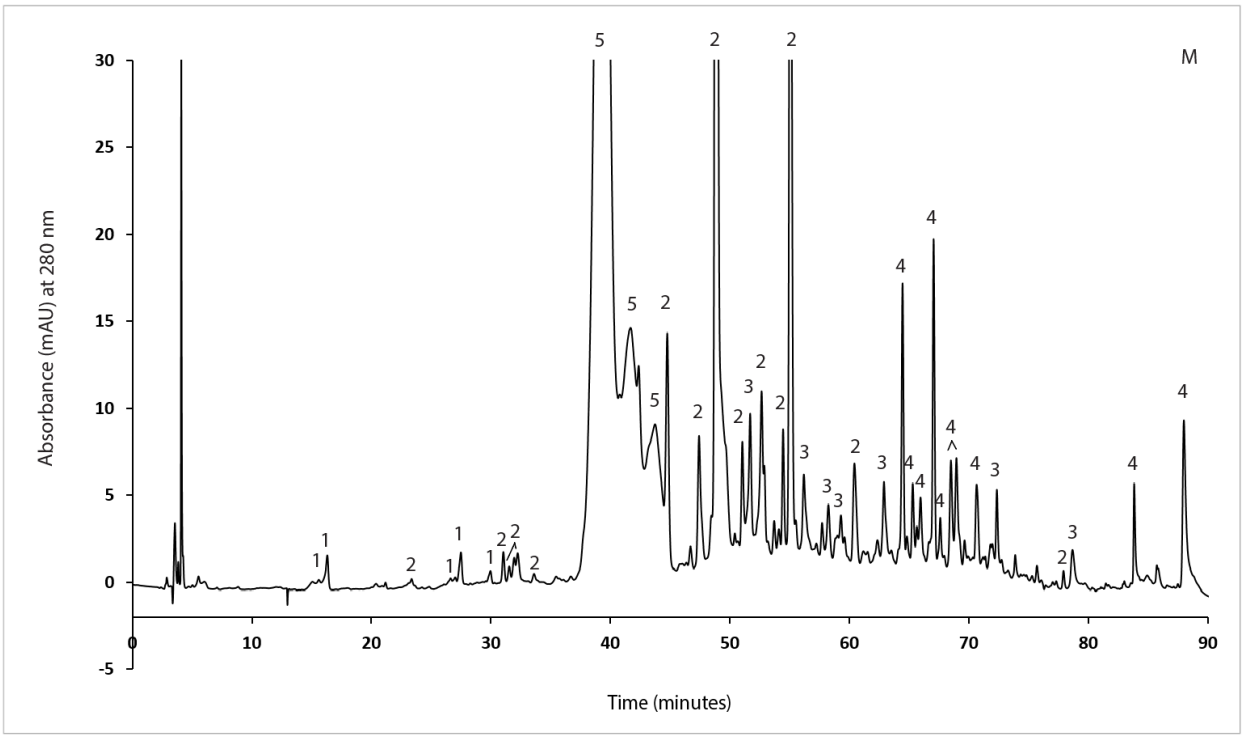
Time (minutes)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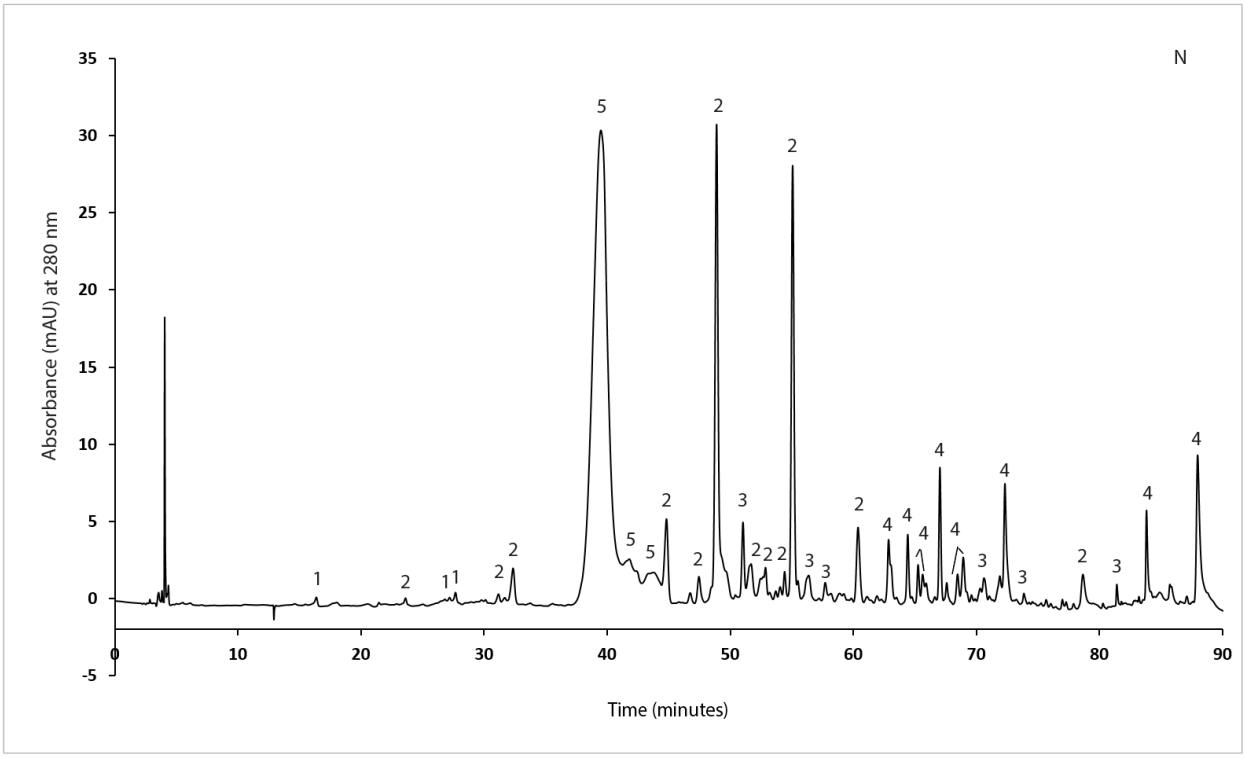
Time (minutes)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Time (minutes)

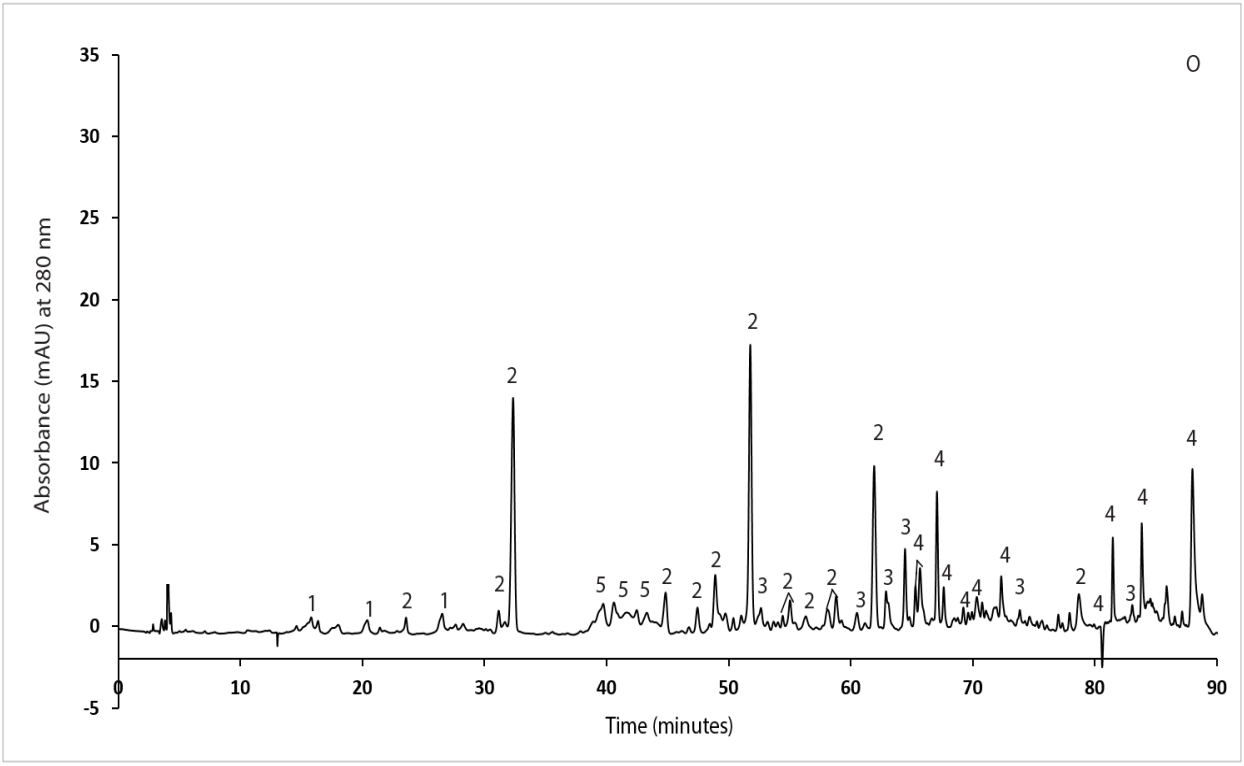


Figure 4.10 HPLC-PDA chromatograms showing the identification of peak phenolic subclasses in saskatoon berry and pomace samples based on their UV-visible spectrums. Chromatogram identification: A-E (aqueous extracts), A. Martin, B. Northline, C. Pembina, D. wet pomace, and E. dry pomace; F-J (EFW extracts), F. Martin, G. Northline, H. Pembina, I. wet pomace, and J. dry pomace; K-O (MFW extracts), K: Martin, L. Northline, M. Pembina, N. wet pomace, and O: dry pomace. Peak phenolic subclass assignments: 1. hydroxybenzoic acids; 2. hydroxycinnamic acids; 3. flavanols; 4. flavonols; 5. anthocyanins.

Table 4.10 Mean and standard deviation TPCI results for the five major phenolic subclasses for aqueous extracts of Martin, Northline and Pembina saskatoon berry varieties and wet and dry pomace.

	Martin[2]	Northline[2]	Pembina[2]	Wet Pomace[3]	Dry Pomace[3]
Hydroxy-benzoic acids	6.5 ± 0.4[4]	9.0 ± 0.1	8.1 ± 0.1	7.3 ± 0.3	8.3 ± 0.4
Hydroxy-cinnamic acids	13.0 ± 0.6	27.8 ± 1.2	11.6 ± 0.5	38.6 ± 2.8	17.3 ± 0.6
Flavanols	8.3 ± 1.0	7.3 ± 0.3	8.0 ± 0.4	22.3 ± 1.3	14.8 ± 0.9
Flavonols	20.0 ± 1.4	52.0 ± 2.5	16.2 ± 0.3	16.6 ± 2.0	8.2 ± 0.1
Anthocyanins	6.4 ± 0.7	22.3 ± 0.1	5.7 ± 0.1	42.8 ± 0.4	2.8 ± 0.1
TPCI[1]	54.1 ± 0.1	118.4 ± 10.4	49.8 ± 0.2	127.7 ± 12.2	51.4 ± 1.1

[1]Total Phenolic Chromatographic Index = sum of all identified and quantified phenolic peaks.

[2]mg/100 g FW.

[3]mg/100 g of pomace.

[4]Mean ± standard deviation results of triplicate sample analysis.

Table 4.11 Mean and standard deviation TPCI results for the five major phenolic subclasses for EFW extracts of Martin, Northline and Pembina saskatoon berry varieties and wet and dry pomace.

	Martin[2]	Northline[2]	Pembina[2]	Wet Pomace[3]	Dry Pomace[3]
Hydroxy-benzoic acids	5.0 ± 0.6[4]	4.5 ± 0.1	4.1 ± 0.1	5.1 ± 0.7	2.6 ± 0.2
Hydroxy-cinnamic acids	56.7 ± 1.4	105.7 ± 9.1	38.1 ± 0.5	76.1 ± 1.9	66.3 ± 2.1
Flavanols	25.5 ± 0.2	18.7 ± 0.1	17.2 ± 0.1	52.6 ± 3.2	58.1 ± 2.0
Flavonols	84.1 ± 1.3	102.3 ± 10.2	36.4 ± 0.3	115.9 ± 1.1	108.3 ± 1.6
Anthocyanins	122.7 ± 9.1	273.1 ± 12.1	118.9 ± 0.2	154.6 ± 12.2	14.8 ± 0.6
TPCI[1]	294.1 ± 9.9	504.2 ± 13.8	215.5 ± 10.6	404.2 ± 15.7	250.0 ± 12.5

[1]Total Phenolic Chromatographic Index = sum of all identified and quantified phenolic peaks.

[2]mg/100 g FW.

[3]mg/100 g of pomace.

[4]Mean ± standard deviation results of triplicate sample analysis.

Table 4.12 Mean and standard deviation TPCI results for the five major phenolic subclasses for MFW extracts of Martin, Northline and Pembina saskatoon berry varieties and wet and dry pomace.

	Martin[2]	Northline[2]	Pembina[2]	Wet Pomace[3]	Dry Pomace[3]
Hydroxy-benzoic acids	3.9 ± 0.1[4]	4.4 ± 0.2	4.3 ± 0.3	8.0 ± 1.8	3.5 ± 0.6
Hydroxy-cinnamic acids	30.5 ± 0.3	68.9 ± 4.1	38.2 ± 5.7	90.1 ± 3.9	34.9 ± 0.6
Flavanols	24.9 ± 0.1	23.4 ± 0.5	10.2 ± 0.8	24.3 ± 0.2	24.5 ± 0.1
Flavonols	48.5 ± 1.1	106.8 ± 9.5	56.8 ± 1.6	61.3 ± 1.4	88.9 ± 2.4
Anthocyanins	95.2 ± 2.1	215.3 ± 15.1	104.5 ± 13.1	176.4 ± 10.2	14.7 ± 1.4
TPCI[1]	203.0 ± 10.7	418.7 ± 11.8	214.1 ± 10.7	361.1 ± 12.2	165.1 ± 11.5

[1]Total Phenolic Chromatographic Index = sum of all identified and quantified phenolic peaks.

[2]mg/100 g FW.

[3]mg/100 g of pomace.

[4]Mean ± standard deviation results of triplicate sample analysis.

The TPCI results for the three saskatoon berry variety extracts clearly show that as the extraction solvent composition changes from aqueous only, to those containing both formic acid and appreciable levels of ethanol and methanol, the TPCI values increase dramatically. This observed increase in TPCI was in agreement with the TPC results previously reported and supports the hypothesis of increased solubility of phenolics as a function of the alcohol (i.e. ethanol and methanol) used, and the role of formic acid in phenolic protonation, which results in improved aqueous-alcohol solubility. The highest saskatoon berry variety TPCI results were found for Northline of 424.18 and 418.70 mg/100 g FW for the EFW and MFW extracts, respectively. These results agreed with those determined for the TPC results previously reported where the Northline variety was significantly higher than those observed for Martin and Pembina. The higher phenolic content of the Northline fruit can be explained by a combination of varietal differences (i.e. genetic) and the surface (i.e. skin) to volume differences between the berries; as berry skin is phenolic rich. Of interest was the similarity in EFW and MFW TPCI results for the Pembina variety of 215.53 and 213.97 mg/100 g FW, respectively, and for Northline, as presented above.

Based on the large differences in TPCI results between the aqueous and EFW/MFW extracts it can be hypothesized that all three saskatoon berry sample analyzed contained appreciable concentrations of extractable intermediate hydrophobic phenolics (i.e. flavonols, flavanols, anthocyanins) and that phenolic protonation improved total phenolics extraction.

Literature TPCI results obtained by solvent extraction (methanol:formic acid:water, 80:0.1:19.9, v:v:v) for Martin (524.03 mg/100 g FW), Northline (467.11 mg/100 g FW) and Pembina (381.68 mg/100 g FW) extracts were all higher than the EFW and MFW results for these varieties in this study (Bakowska-Barczak and Kolodziejczyk, 2008). These differences are best explained by environmental growth conditions, which can result in significant differences in TPCI results for the same variety from year to year.

The lowest TPCI values for all five samples analyzed in this study were in their aqueous extracts (Table 4.10). Chromatographic results show much lower extraction of flavanols, flavonols and anthocyanins phenolic subclasses (Figure 4.10- A-E) under these extraction conditions when compared to those obtained by EFW and MFW solvent systems (Figure 4.10- F-O). These results clearly show that the addition of formic acid-ethanol/methanol resulted in improved extraction of more hydrophobic subclasses of phenolics (i.e. flavonols, flavanols, anthocyanins) in these fruit extracts. The role of solvent composition in phenolic subclass extraction is supported by the aqueous TPCI results obtained for the hydroxybenzoic acid subclass as the combined mean result of 7.85 mg/100 g FW (Table 4.10) for the three saskatoon berry varieties was higher than those determined for the EFW and MFW extracts of 4.53 and 4.21 mg/100 g FW (Tables 4.11 and 4.12), respectively. It has been reported in literature that hydroxybenzoic acid and other members of this phenolic subclass have higher solubility in water than in ethanol or methanol (Yalkowsky and He, 2003; Galanakis, et al., 2011).

The major phenolic subclasses identified in the EFW and MFW extracts analyzed in this study, based on their percent composition of the TPCI value were, anthocyanins (41-54%), flavonols (16-28%), and hydroxycinnamic acids (14-24%). These results were in agreement with those reported in literature for saskatoon berries using HPLC-ESI-MS/MS of, anthocyanins (59-64%), flavonols (8-12%), and hydroxycinnamic acids (17-39%) (Bakowska-Barczak and Kolodziejczyk, 2008). As an example, HPLC/LC-MS determined phenolic subclass composition for the Northline variety was, anthocyanins (62-65%), flavonols (10-12%) and hydroxycinnamic acids (21-28%) (Lavola et al., 2012).

The TPCI results from this study identified anthocyanins as the major phenolic subclass in all samples. The majority of sample anthocyanins were found in the EFW and MFW extracts, and amongst the three saskatoon berry varieties, their concentration in Northline was consistently higher than those found in Martin and Pembina.

The TPCI analytical results for the dried pomace extract sample showed much lower anthocyanin concentrations of approximately 14.7 mg/100 g for the EFW and MFW (Tables 4.11 and 4.12). These results show that the commercial heating step required to reduce the moisture content of pomace to approximately 8% (Table 4.3) resulted in phenolic destruction, particularly the anthocyanins.

When compared to the fruits, the wet pomace extract from commercial juice production was second only to the Northline variety in TPCI value and anthocyanin content. Although direct comparison of these results with the Northline variety (raw fruit) used in this study was not possible as the actual fruit samples came from different growing seasons (2013 versus 2015), these result show that this co-product stream from commercial juice production would be a good source of phenolics in general, and in anthocyanins in particular, as a natural antioxidant in food formulations, as a possible colouring agent/ingredient for foods, natural health products and pharmaceuticals, and/or as a nutraceutical. No literature information on the TPCI values of pomace from saskatoon berry juice production was available for comparison to the results obtained in this study.

4.11 Total Anthocyanin Content of Saskatoon Berry Varieties and Pomace as Determined by the pH Differential Method

Based on their major contribution to TPCI results, the anthocyanin content of aqueous, EFW and MFW extracts were determined by the pH differential method. This method is based on sample absorbance measurements at pH 1.00 and 4.50 at both 512 and 700 nm, and is commonly used to determine the total monomeric anthocyanin content of fruits/fruit extracts (Wrolstad, 1993; Lee et al., 2008; Castañeda-Ovando et al., 2009). Absorbance at these wavelengths is based on the structural properties of monomeric anthocyanins, which are highly coloured at pH 1.00 due to the presence of the oxonium ion (flavylium cation) form of these molecules, while at pH 4.50 they are essentially colourless in their hemiketal form (Figure 4.11).

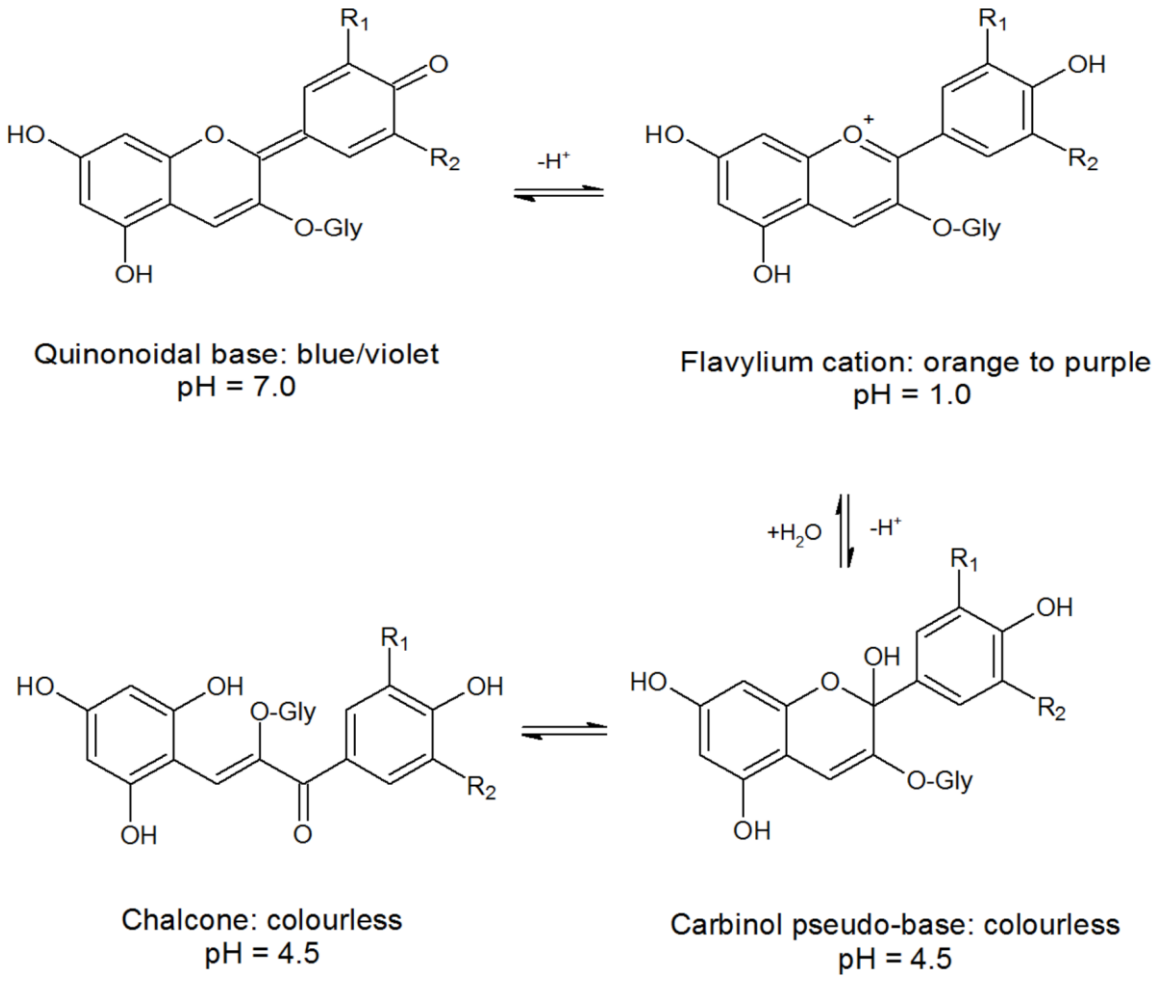


Figure 4.11 Predominant structural forms of anthocyanins at select pH values (adapted from Castañeda-Ovando et al., 2009).

In the visible spectrum, anthocyanins show maximum absorbance at 512 nm and the measurement at 700 nm is used to account for sample turbidity (Wrolstad, 1993; Lee et al., 2005). The difference in absorbance of the diluted extracts between these two pH values is divided by a standard anthocyanin molar absorbance coefficient, typically cyanidin-3-*O*-D-glucoside (C3GLU; the most common anthocyanin identified in nature) so as to determine sample total anthocyanin content.

The total anthocyanin content of saskatoon berry aqueous, EFW and MFW extracts expressed as milligrams of cyanidin-3-*O*-glucoside equivalents (C3GE) are shown in Table 4.13.

Table 4.13 Mean and standard deviation total anthocyanin content results for Martin, Northline and Pembina saskatoon berry and wet pomace extracts.

	Total Anthocyanins			
Extracts	Martin[1]	Northline[1]	Pembina[1]	Wet Pomace[2]
Aqueous	24.2 ± 0.1[3Ab]	49.4 ± 0.1[Ac]	16.7 ± 0.1[Aa]	107.6 ± 0.7[A]
EFW	118.5 ± 0.3[Cb]	180.3 ± 0.3[Bc]	98.7 ± 0.1[Ca]	179.4 ± 1.2[C]
MFW	81.2 ± 0.2[Bb]	178.8 ± 3.0[Bc]	72.4 ± 0.2[Ba]	146.6 ± 0.7[B]

[1]mg C3GE/100 g FW.

[2]mg C3GE/100 g wet pomace.

[3]Mean ± standard deviation results of triplicate sample analysis.

[A-C]Mean values in the same column followed by a common letter were not statistically different ($p<0.05$) by Tukey's HSD multiple range test between the type of extracts.

[a-c]Mean values in the same row followed by a common letter were not statistically different ($p<0.05$) by Tukey's HSD multiple range test between the varieties and pomace.

The EFW extract of the Northline variety showed the greatest anthocyanin content of all samples analyzed with a value of 180.3 mg C3GE/100 g FW, followed by Martin and Pembina varieties with values of 118.5 and 98.7 mg C3GE/100 g FW, respectively (Table 4.13). According to literature, the anthocyanin content of saskatoon berry samples using the pH differential method ranged from 25 to 388.13 mg C3GE/100 g FW, with a specific range for Northline of 138 to 204 mg C3GE/100 g FW (Mazza and Miniati, 1993; Rogiers and Knowles,1997; Kwok et al., 2004; Mazza, 2006). Anthocyanin content values for the Martin and Pembina varieties by the pH differential method have not been reported in literature, however, total anthocyanin contents as determined by HPLC analysis of 184.31 (Martin) and 54.4 - 342.5 mg C3GE/100 g (Pembina) have been reported (Zatylny et al., 2005; Bakowska-Barczak and Kolodziejczyk, 2008).

The EFW and MFW extracts for all three saskatoon berry varieties showed significantly higher total anthocyanin content when compared to the aqueous extract. In addition, the EFW total anthocyanin results were found to be statistically higher for both the Martin and Pembina varieties when compared to MFW. Anthocyanins contain both an aromatic-hydrocarbon backbone, which is hydrophobic, and covalently bound hydroxyl groups and carbohydrates that are hydrophilic

(Castañeda-Ovando et al., 2009). As such, improved anthocyanin extraction would be expected in EFW and MFW as they contained the more nonpolar organic solvents (i.e. ethanol and methanol), and an acid for anthocyanin protonation and water. Also, as previously discussed, improved phenolics (e.g. anthocyanin) extraction due to fruit cell wall damage would be expected with the more hydrophobic solvent ethanol when compared to water. Results from this study are supported by those in literature as both Konecsni (2011) and Li et al. (2014), reported higher anthocyanin extraction from saskatoon berry samples with EFW and MFW versus water. The results from this study clearly show the importance of extraction solvent choice on anthocyanin solubility from fruits. As both ethanol and formic acid are approved for food use, the EFW extract could be used directly or further stabilized (e.g. encapsulation, acylation) as a food colourant, a natural antioxidant, or as a nutraceutical.

The total anthocyanin content of the wet pomace EFW extract as determined by the pH differential method of 179.4 mg C3GE/100 g, was not significantly different from that observed for the EFW of the Northline raw fruit of 180.3 mg C3GE/100 g FW. As discussed previously, although this pomace was not produced from this raw fruit, and the total soluble solids for the raw fruit was greater than that used for the pomace (i.e. 25 g/80.18% moisture vs. 5 g/68.00% moisture), these results show that this co-product stream would be a valuable source of anthocyanins. As the food industry is under pressure from consumers to reduce and/or eliminate the use of artificial colourants and antioxidants in foods, anthocyanins from pomace could aid in the current food colourant and antioxidant trend from artificial to natural (Sigurdson et al., 2017). No published information on the anthocyanin content of pomace from commercial saskatoon berry juice production was identified, however, total anthocyanin results for pomace from commercial fruit juices including cranberry, raspberry and strawberry of 116.90, 65.21 and 19.48-28.29 mg/100 g FW, respectively, have been reported, which are all lower than those determined in this study for saskatoon berry (White et al., 2010; Vulić et al., 2011; Šaponjac et al., 2015). However, the total anthocyanin results for the three saskatoon berry varieties reported in this study were lower than that reported for blueberry pomace of 194.5 mg/100 g FW (Lee and Wrolstad, 2004). It should be noted that the moisture contents of the aforementioned fruit pomaces were not reported, which limits the direct comparison of these literature results to those obtained for the saskatoon berry varieties analyzed in this study.

4.12 Total Qualitative and Quantitative Anthocyanin Analysis by HPLC-PDA

Mean and standard deviation chromatographic results for total and individual anthocyanins from the EFW extracts of saskatoon berry varieties and wet and dry pomace are shown in Table 4.14. Chromatographic analysis showed that each saskatoon berry EFW extract sample contained cyanidin-3-*O*-galactoside (retention time [RT]~ 40.7 min), cyanidin-3-*O*-glucoside (RT: ~ 43.5 min), cyanidin-3-*O*-arabinoside (RT: ~ 45.8 min) and cyanidin-3-*O*-xyloside (RT: ~ 53.2 min) (Figure 4.12). Qualitative analysis was afforded by HPLC-PDA retention time comparison to standards and spiking experiments and quantitative analysis by peak area/height comparison to standard curves (section 3.14).

Table 4.14 Mean and standard deviation anthocyanin content for Martin, Northline and Pembina saskatoon berry varieties as determined by HPLC-PDA.

	Martin[1]	Northline[1]	Pembina[1]	Wet pomace[2]	Dry pomace[2]
Cyanidin-3-*O*-galactoside	71.8 ± 1.0[3a]	205.1 ± 0.3[b]	70.7 ± 1.7[a]	113.5 ± 0.4	4.6 ± 0.1
Cyanidin-3-*O*-glucoside	24.1 ± 2.9[b]	30.6 ± 1.3[c]	16.8 ± 0.8[a]	20.9 ± 0.9	4.8 ± 0.1
Cyanidin-3-*O*-arabinoside	15.2 ± 0.9[a]	24.9 ± 0.7[b]	13.8 ± 0.2[a]	12.5 ± 0.3	3.2 ± 0.2
Cyanidin-3-*O*-xyloside	10.0 ± 0.4[a]	15.8 ± 0.7[b]	8.6 ± 1.0[a]	8.5 ± 0.3	2.5 ± 0.4
Total	121.0 ± 1.2[b]	276.4 ± 2.3[c]	109.9 ± 1.6[a]	115.3 ± 0.3	15.0 ± 0.4

[1]mg/100 g FW.

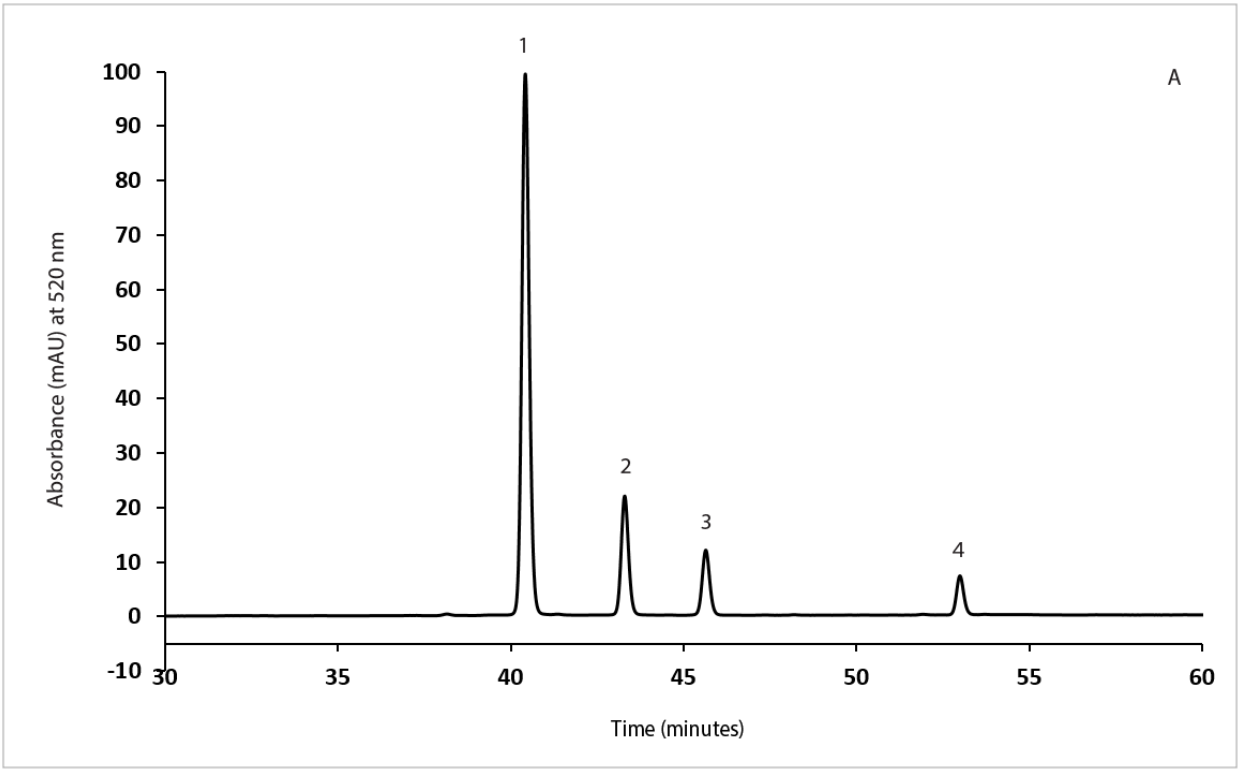
[2]mg/100 g pomace.

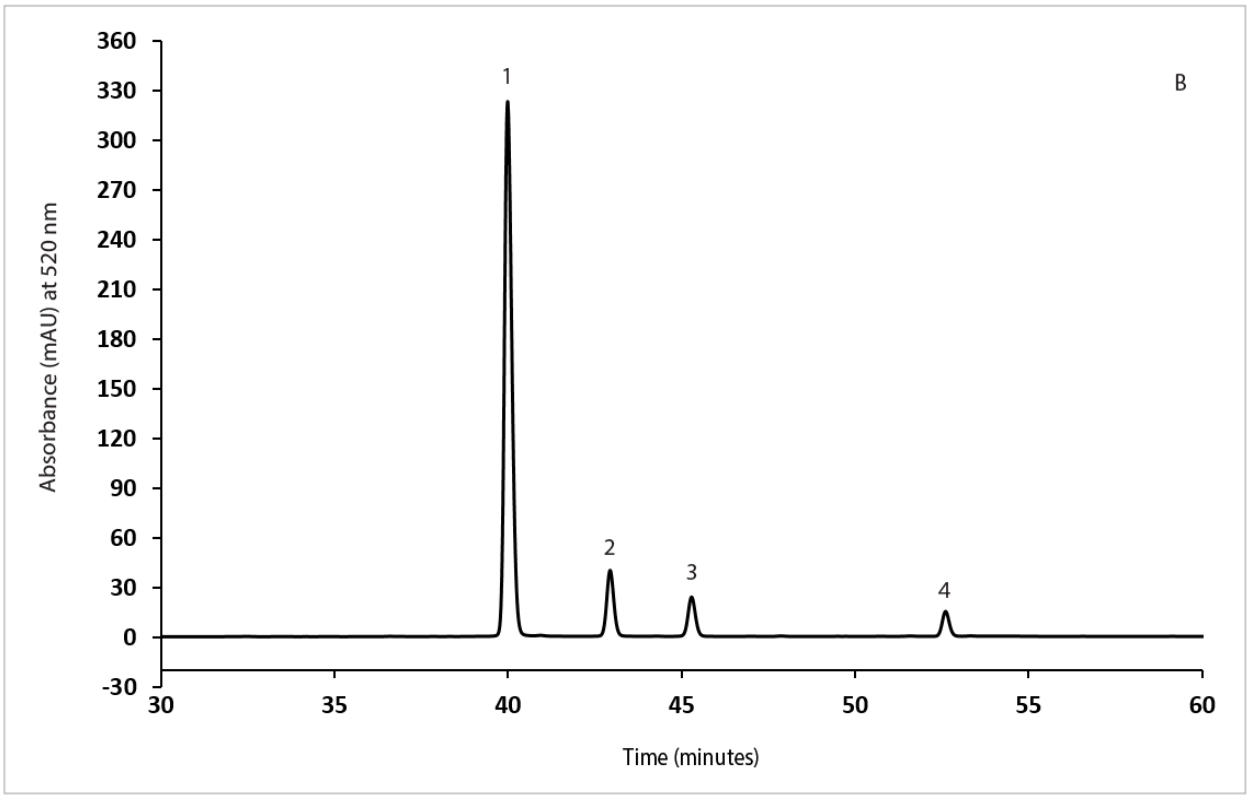
[3]Mean ± standard deviation results of triplicate sample analysis.

[a-c]Mean values in the same row followed by a common letter were not statistically different. ($p<0.05$) by Tukey's HSD multiple range test between saskatoon berry varieties.

The Northline variety had the highest total anthocyanin content as determined by HPLC-PDA analysis 276.4 mg/100 g FW, followed by the Martin and Pembina varieties at 121.0 and 109.9 mg/100 g FW, respectively. The percent anthocyanin composition in the Northline variety

was found to be, 74.2% cyanidin-3-*O*-galactoside, 11.1% cyanidin-3-*O*-glucoside, 9.0% cyanidin-3-*O*-arabinoside and 5.7% cyanidin-3-*O*-xyloside. A fifth anthocyanin, cyanidin-3,5-*O*-diglucoside has been reported in saskatoon berry at much lower concentrations (0.33-0.78 mg/100g FW), however, this compound was not identified in the samples analyzed in this study. The HPLC-PDA profile of the dry pomace (Figure 4.12 E) showed additional peaks, which could have resulted from structural changes (e.g. deglycosylation). These structural changes would not include the cyanidin breakdown products 2,4,6-trihydroxybenzaldehye (PGA) and 3,4-dihydroxybenzoic acid (PCA) based on detector wavelength (i.e. 520 nm) (Kay et al., 2009).

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Absorbance (mAU) at 520 nm
Time (minutes)
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2
3
4
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300
270
240
210
180
150
120
90
60
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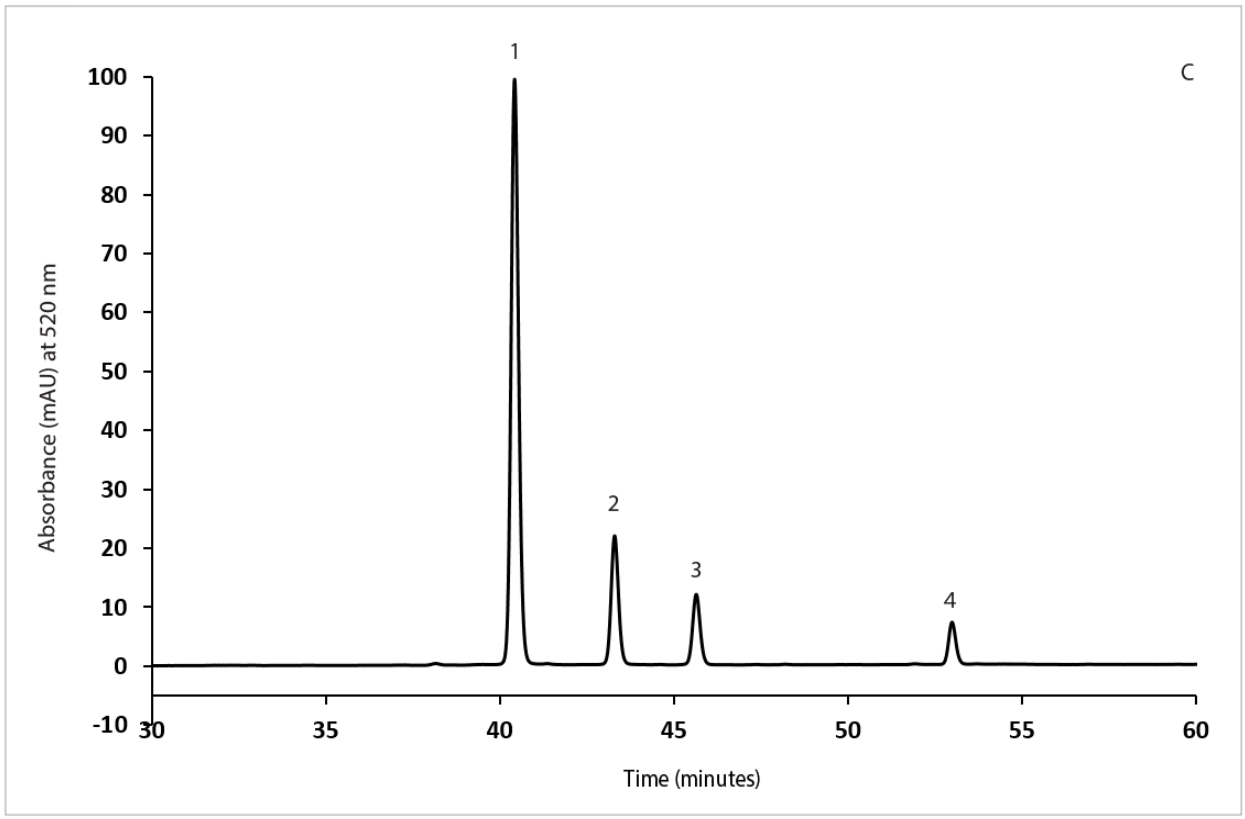
35
40
45
50
55
60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Absorbance (mAU) at 520 nm
Time (minutes)
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2
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4
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80
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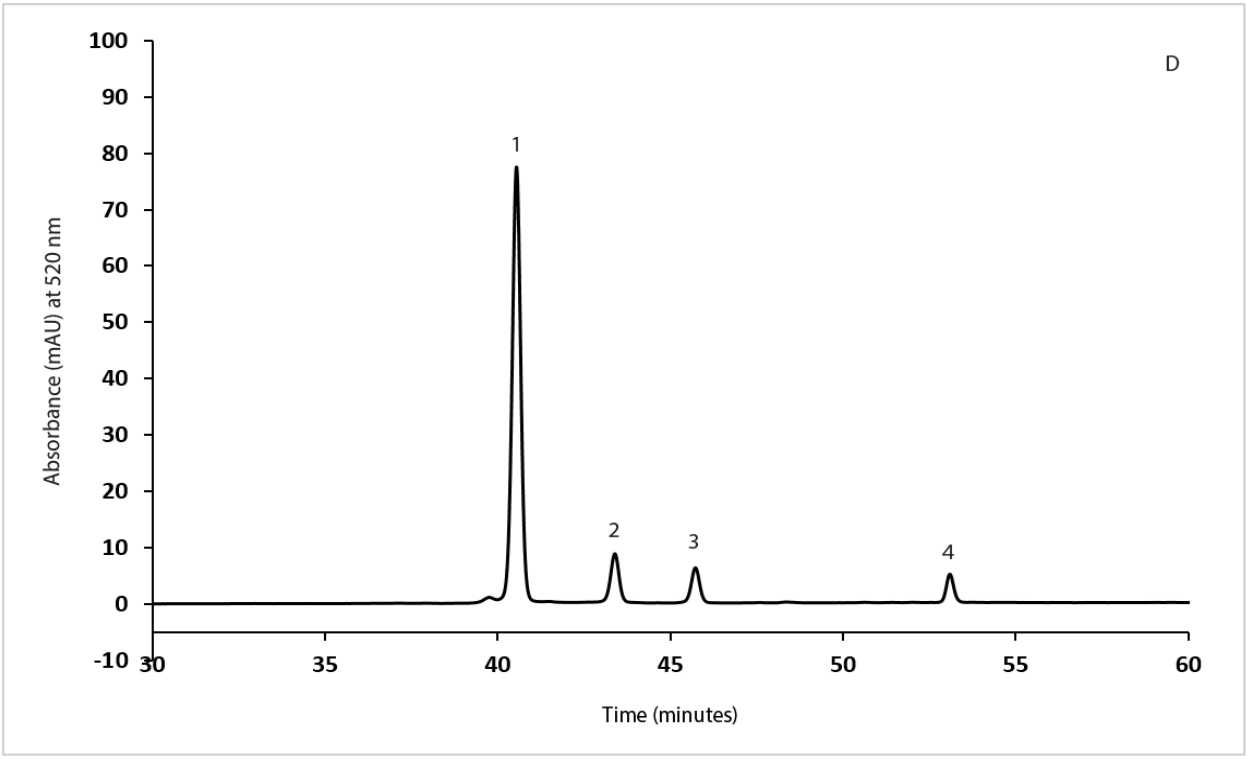

D
Absorbance (mAU) at 520 nm
100
90
80
70
60
50
40
30
20
10
0
-10
1
2
3
4
30
35
40
45
50
55
60
Time (minutes)

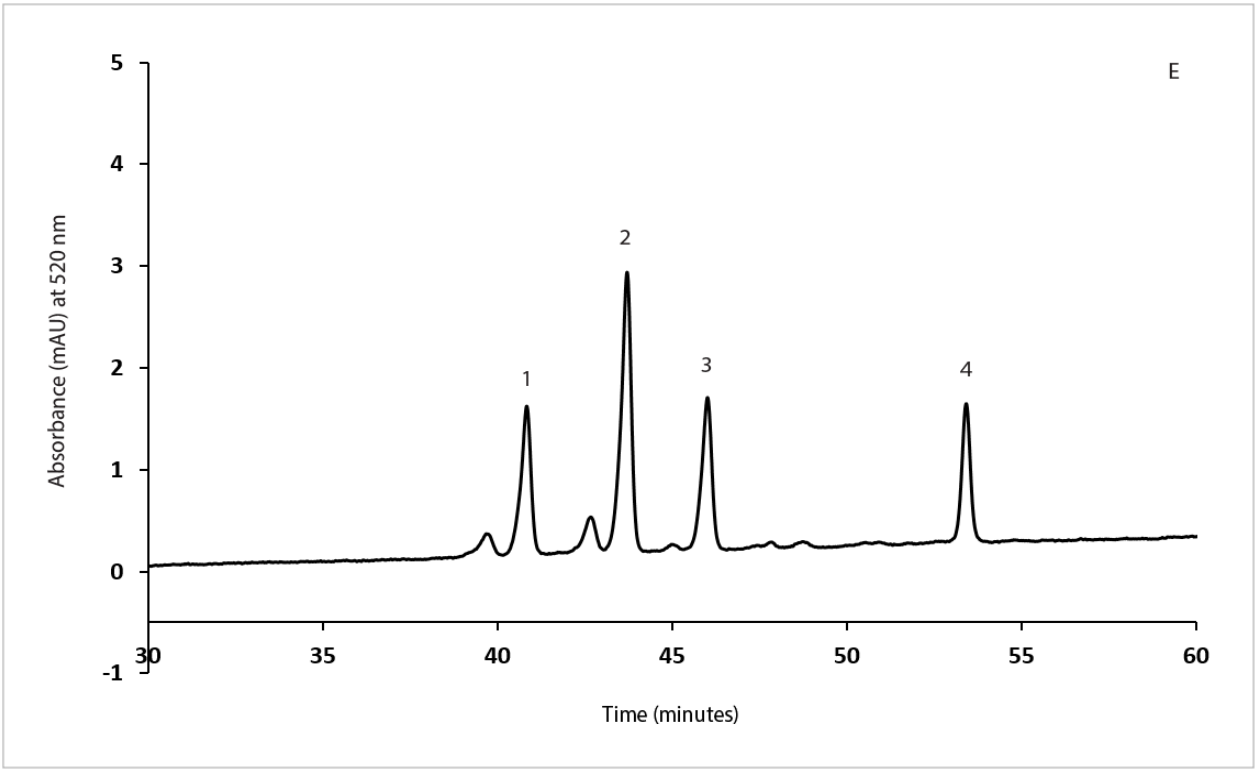


Figure 4.12 HPLC-PDA chromatograms showing the anthocyanin composition of saskatoon berry and pomace samples. Chromatogram identification (EFW extracts): A. Martin; B. Northline; C. Pembina; D. wet pomace; and E. dry pomace. Peak identity: 1. cyanidin-3-*O*-galactoside; 2. cyanidin-3-*O*-glucoside; 3. cyanidin-3-*O*-arabinoside; and 4. cyanidin-3-*O*-xyloside.

Literature results for total anthocyanin contents of saskatoon berry vary greatly, as supported with the following data: (a) 55.4 to 312.9 mg/100 g FW for Martin; (b) 69.9 to 425.4 mg/100 g FW for Northline; and (c) 159.4 to 184.3 mg/100 g FW for Pembina (Zatylny et al., 2005; Bakowska-Barczak and Kolodziejczyk, 2008; Lavola et al., 2012; Jin et al., 2015). However, all literature supports the finding in this research study that cyanidin-3-*O*-galactoside was the major anthocyanin in the three saskatoon berry varieties studied. Literature concentration ranges for this anthocyanin in these varieties are, 11.7-49.5 for Martin, 48.3-327.7 for Northline, and 98.2-103.3 mg/100 g FW (56.07%) for Pembina (Zatylny et al., 2005; Bakowska-Barczak and Kolodziejczyk, 2008; Lavola et al., 2012; Jin et al., 2015). The cyanidin-3-*O*-galactoside value for Northline was within the literature range for this variety, however, the values of 71.8 for Martin

and 70.7 mg/100 g FW for Pembina, were higher and lower, respectively, when compared to these literature range values. These observed differences in cyanidin-3-*O*-galactoside concentrations may be explained by one or more of the following, extraction solvent (i.e. EFW) employed environmental growth conditions, geographical origin, fruit maturity, and storage conditions.

The total anthocyanin content of the EFW wet pomace extract from commercial saskatoon berry juice production was 155.3 mg/100 g FW, and contained all four of the major anthocyanins, with a cyanidin-3-*O*-galactoside concentration of 73.1 mg/100 g FW. These results clearly show that pomace from juice production would be a possible source of anthocyanins for use as natural colourants and antioxidants in foods and/or as nutraceuticals. The total anthocyanin content of dry pomace from commercial saskatoon berry juice production was much lower at 15.0 mg/100 g FW. The lower concentration of anthocyanins in the dried material clearly shows the role of temperature on their stability (Larrauri et al., 1997; Patras et al., 2010). The identification and quantitation of anthocyanins in saskatoon berry pomace by HPLC-PDA have not been previously reported.

4.13 Antioxidant Activities of Saskatoon Berry and Pomace Extracts

4.13.1 ABTS (2,2-azino-bis-3-ethylbenzthiazoline-6-sulfonic acid) Radical Scavenging Assay

The ABTS radical scavenging or Trolox equivalent antioxidant capacity (TEAC) results for aqueous, EFW and MFW extracts of the three saskatoon berry and pomace samples analyzed in this study and expressed as mM Trolox activity equivalent (TEAC)/100 mg sample are reported in Table 4.15.

Table 4.15 Mean and standard deviation ABTS radical scavenging activity data for extracts of Martin, Northline and Pembina saskatoon berry varieties and wet and dry pomace.

	ABTS Radical Scavenging Activity				
Extracts	Martin[1]	Northline[1]	Pembina[1]	Wet Pomace[2]	Dry Pomace[2]
Aqueous	146.4 ± 2.2[3Ab]	185.1 ± 3.3[Ac]	84.4 ± 2.2[Aa]	238.0 ± 8.9[A]	66.2 ± 6.4[A]
EFW	177.1 ± 4.4[Bb]	327.5 ± 5.2[Cc]	159.8 ± 5.4[Ba]	304.8 ± 7.1[B]	327.8 ± 5.2[C]
MFW	155.8 ± 5.7[Aa]	264.1 ± 3.8[Bc]	151.4 ± 4.6[Ba]	263.0 ± 6.1[A]	186.1 ± 8.1[B]

[1]Reported as mM Trolox activity equivalent (TEAC)/100 mg FW.

[2]Reported as mM Trolox activity equivalent (TEAC)/100 mg pomace.

[3]Mean ± standard deviation results of triplicate sample analysis.

[A-C]Mean values in the same column followed by a common letter were not statistically different ($p<0.05$) by Tukey's HSD multiple range test between the type of extracts.

[a-c]Mean values in the same row followed by a common letter were not statistically different ($p<0.05$) by Tukey's HSD multiple range test between the varieties and pomace.

The EFW extract of the Northline variety showed the greatest ABTS radical scavenging activity of all samples analyzed with a TEAC value of 327.5, which was significantly higher than those found for the Martin and Pembina varieties with values of 155.8 and 151.4 mM Trolox equivalent (TEAC)/100 mg FW, respectively (Table 4.15). From literature, the TEAC values for saskatoon berry fruit vary as indicated by, (a) results from non-specified varieties, which ranged from 84.4-322.8 (Kwok et al., 2004; Hu et al., 2005; Konecsni, 2011); and (b) those for Martin, Northline and Pembina, of 41.0, 36.9 and 36.3, respectively (Bakowska-Barczak and Kolodziejczyk, 2008). This range in TEAC values may be explained by differences in analytical methods employed (i.e. temperature and reaction time), phenolic extraction protocols used (e.g. solvent) and sample phenolic content.

The TEAC values for the alcohol extracts of the fruits were significantly higher than those observed for the aqueous extracts. These results were consistent with higher TPC, TPCI and DPPH values for these same samples, and are consistent with the hypothesis of improved total and intermediate polarity phenolics (e.g. flavonoids) extraction employing ethanol and methanol aqueous acid solvent mixtures versus water alone. As an example, the solubility of the hydrophobic

flavonoid rutin was shown to decrease from ~38% in ethanol to ~0.5% (w:w) in water (Krewson and Naghski, 1952; Zi et al., 2007). Therefore, greater phenolics extraction through cell wall damage coupled with improved hydrophobic subclass solubility in the alcohol extracts can explain the higher TEAC results found for the EFW and MFW extracts when compared to the water extract.

The TEAC values found for the saskatoon berry fruit samples analyzed in this study were higher than those reported in literature for blackberry, red raspberry, and strawberry of 192, 145 and 115, respectively, but were lower than that of black raspberry at 438 mM Trolox equivalent/100 mg FW (Ozgen et al., 2006).

The TEAC values found for the solvent extracts of wet pomace were consistent with those found for the fruits (i.e. EFW>MFW>aqueous) and ranged from 238.0 (water) to 304.8 (EFW). Although not directly comparable to the fresh fruit samples, it can be concluded from these results that the pomace resulting from juice production would be a valuable source of phenolics and provides evidence of the structural integrity of these fruit phenolics under frozen storage (~3 years in this case) as their ABTS radical scavenging activity was still present.

4.13.2 DPPH (2,2-diphenyl-1-picrylhydrazyl) Radical Scavenging Assay

The DPPH free radical scavenging activity results for aqueous, EFW and MFW extracts of the three saskatoon berry varieties and pomace expressed as $1/IC_{50}$ (1/100 mg of sample weight) are shown in Table 4.16.

Table 4.16 Mean and standard deviation DPPH radical scavenging activity data for extracts of Martin, Northline and Pembina saskatoon berry varieties and wet and dry pomace.

	DPPH Radical Scavenging Activity				
Extracts	Martin[1]	Northline[1]	Pembina[1]	Wet Pomace[2]	Dry Pomace[2]
Aqueous	8.3 ± 1.9[3Aa]	15.0 ± 1.5[Ac]	7.5 ± 1.6[Aa]	17.5 ± 1.2[A]	10.9 ± 1.6[A]
EFW	14.7 ± 1.7[Bb]	23.1 ± 1.3[Bc]	12.1 ± 1.2[Ba]	19.4 ± 1.8[C]	16.8 ± 1.0[C]
MFW	14.9 ± 1.5[Bb]	24.2 ± 1.7[Bc]	13.0 ± 1.9[Ba]	18.5 ± 1.3[B]	14.3 ± 1.7[B]

[1]Reported as $1/IC_{50}$ (1/100 mg FW sample for 50% DPPH radical inhibition).

[2]Reported as $1/IC_{50}$ (1/100 mg pomace for 50% DPPH radical inhibition).

[3]Mean ± standard deviation results of triplicate sample analysis.

[A-C]Mean values in the same column followed by a common letter were not statistically different ($p<0.05$) by Tukey's HSD multiple range test between the type of extracts.

[a-c]Mean values in the same row followed by a common letter were not statistically different ($p<0.05$) by Tukey's HSD multiple range test between the varieties and pomace.

For the three fruits, the MFW extract of the Northline variety showed the greatest DPPH radical scavenging activity with a $1/IC_{50}$ value of 24.2, followed by Martin and Pembina varieties with values of 14.0 and 13.0, respectively (Table 4.16). According to literature, the DPPH radical scavenging results for saskatoon berries (non-specified varieties) ranged from 2.7 to 15.8 ($1/IC_{50}$), which were lower than the results reported in this study (Hu et al., 2005, Li et al., 2009). These differences were most likely due to the assay procedure followed by these authors, where a lower reaction time was used, which often leads to incomplete quenching of the DPPH radical (Sánchez-Moreno, 2002).

The EFW and MFW extracts showed significantly higher DPPH values when compared to the aqueous extract. These differences in the DPPH assay results for these extracts would be due to their phenolic composition, which is dependent upon the solvent extraction efficiency of intermediate hydrophobic phenolics (e.g. flavonoids), which are supported by their TPCI results (Tables 4.11 and 4.12). Also, alcohol (EFW and MFW) afford more effective breakdown of cell membranes to release phenolics. The flavonoid class has been reported to exhibit greater radical

scavenging capacity when compared to other phenolic classes such as hydroxycinnamic acids (Rice-Evans et al., 1996; Heim et al., 2002; Villaño et al., 2007). Therefore, the more nonpolar solvents such as ethanol and methanol are able to better solubilize these compounds when compared to water, and consequently extracts/fractions containing these phenolics would yield higher DPPH free radical scavenging activity values.

The observed antioxidant activities of the three saskatoon berry variety samples were lower than those reported in literature for blueberry and cranberry fruit of 60 and 80 expressed as 1/100 mg FW, respectively, however, they were greater than that reported for raspberry of 7.46 ($1/IC_{50}$) (Schlesier et al., 2002; Novaković et al., 2011). Limitations exist when comparing literature DPPH results as the methods used are unstandardized, which leads to the use of different reaction times, temperatures, reference standards and expressed units (Sánchez-Moreno, 2002).

Wet and dry pomace from commercial saskatoon berry $ju_{ic}e$ production showed higher DPPH radical scavenging activities in their EFW extracts of 19.4 and 16.8 ($1/IC_{50}$), respectively. Wet pomace showed a higher radical scavenging ability than dry pomace, which may have been due to the fruit used in juice production, and/or structural changes (e.g. decomposition) in phenolics due to drying. As previously discussed, direct comparison of these two pomace samples were not possible. There is a lack of scientific information on the free radical scavenging ability of saskatoon berry pomace, with the only scientific report based on the oxygen radical absorbance capacity (ORAC assay) test for aqueous, 70% methanol and 70% ethanol extracts with values of 3.04, 11.94, 10.31 mM Trolox activity equivalent/100 mg dry pomace (Li et al., 2014).

According to literature, a positive relationship exists between total phenolic content (TPC) and ABTS and DPPH free radical scavenging assay values (Sánchez-Moreno, 2002; Dudonné et al., 2009). The results obtained for the three saskatoon berry varieties in this study support this conclusion; for example, the EFW extract of the Northline variety had statistically higher values for TPC (526.8 mg GAE/100 g FW), ABTS (327.5 mM of Trolox equivalents/100 mg of FW) and DPPH (23.1 $1/IC_{50}$ [1/100 mg FW]) when compared to the Martin and Pembina varieties.

4.14 Analysis of Saskatoon Berry and Pomace Amberlite® XAD16N Resin-Ethanol Fractions

4.14.1 Total $Phenol_{ic}$ Content by the Folin-Ciocalteau Method

Total phenolic content (TPC) results for the fractions produced from resin-percent ethanol treatment of the three saskatoon berry EFW extracts (section 3.11), showed the following: (1) the

Northline variety had the highest combined (i.e. all three fraction values) of 579.8 mg GAE/100 g FW; and (2) the 70% aqueous ethanol fraction TPC values were significantly higher than the 40% aqueous ethanol and 100% ethanol fractions for all three varieties (Table 4.17).

Table 4.17 Mean and standard deviation total phenolic content results for EFW fractions of Martin, Northline and Pembina saskatoon berry varieties and wet pomace.

	Total Phenolic Content			
Fractions	Martin[1]	Northline[1]	Pembina[1]	Wet Pomace[2]
40% ethanol	9.6 ± 1.1[3a]	10.3 ± 0.5[a]	7.9 ± 1.3[a]	15.1 ± 1.5[a]
70% ethanol	242.6 ± 15.6[c]	418.8 ± 12.7[c]	233.6 ± 14.2[c]	326.7 ± 14.7[c]
100% ethanol	117.5 ± 8.9[b]	150.7 ± 9.0[b]	123.6 ± 10.4[b]	158.7 ± 7.1[b]

[1]mg GAE/100 g FW.

[2]mg GAE/100 g pomace.

[3]Mean ± standard deviation results of triplicate sample analysis.

[a-d]Mean values in the same column followed by a common letter were not statistically different ($p<0.05$) by Tukey's HSD multiple range test between the varieties and pomace.

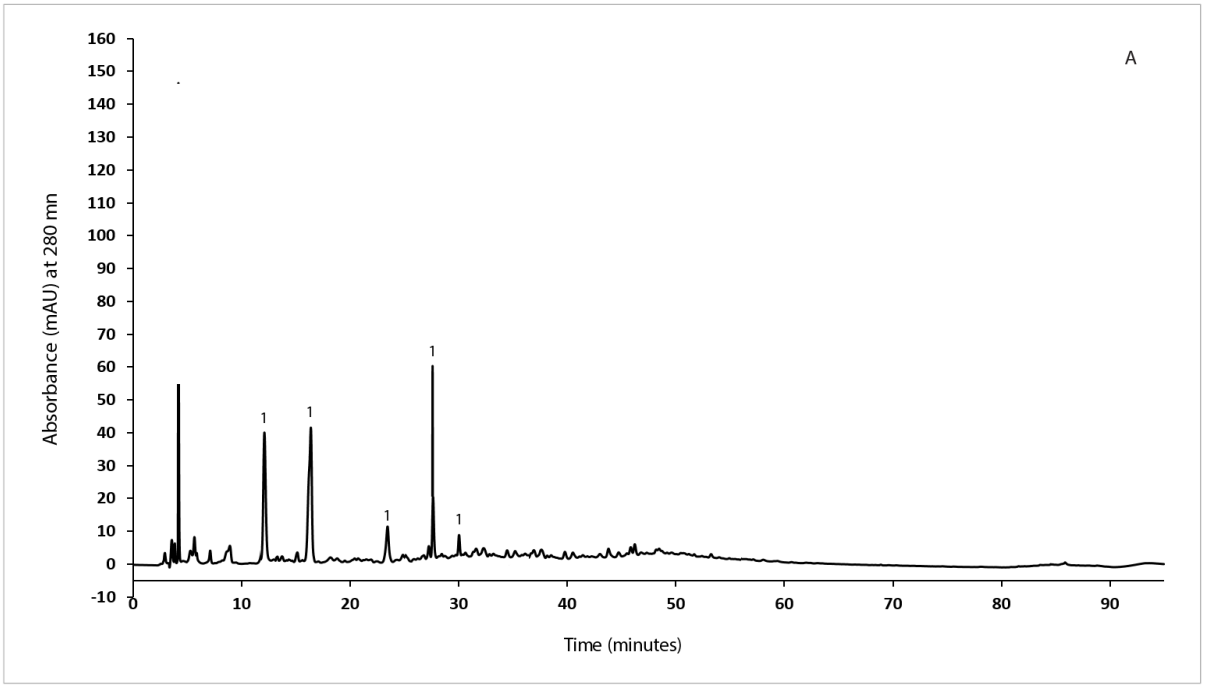
The total phenolic content (TPC) results for the wet pomace EFW extracts (section 4.9) showed the same trend with respect to fractionation solvent and provided further evidence that this material would be a good source of phenolics (i.e. combined fraction values of 540.56) as a food ingredient and/or health supplement.

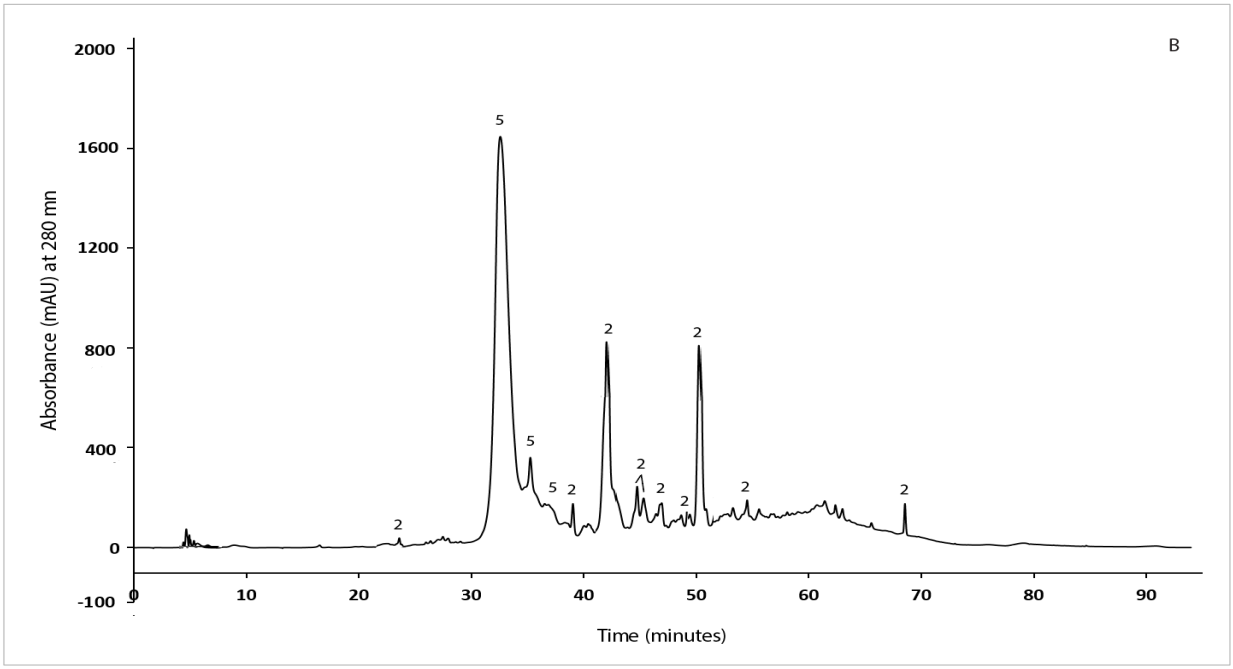
Although TPC results provided no specific information on phenolic structure, the fact that the three fractions had significantly different TPC values clearly shows that solvent hydrophobicity resulted in the extraction of different phenolics subclasses.

4.14.2 Total Phenolic Chromatographic Index/Indices (TPCI)

The major phenolic subclasses in each fraction were identified by TPCI (section 3.12.2) and representative chromatograms for the Northline variety are shown in Figure 4.13. Individual chromatographic peaks were identified/classified based on their retention times and UV-visible spectral profiles as compared to standards. A representative chromatogram showing the HPLC-

PDA analysis of the water fraction for the Northline variety is shown in Figure 4.14. Chromatograms for each fraction of Martin, Pembina and wet pomace can be found in Appendix A.l. The use of ND (non-detected) for phenolic subclasses is used when the detector response for a chromatographic peak had a peak height less than 6x the signal-to-noise ratio (S/N), which is commonly used by analytical chemists.





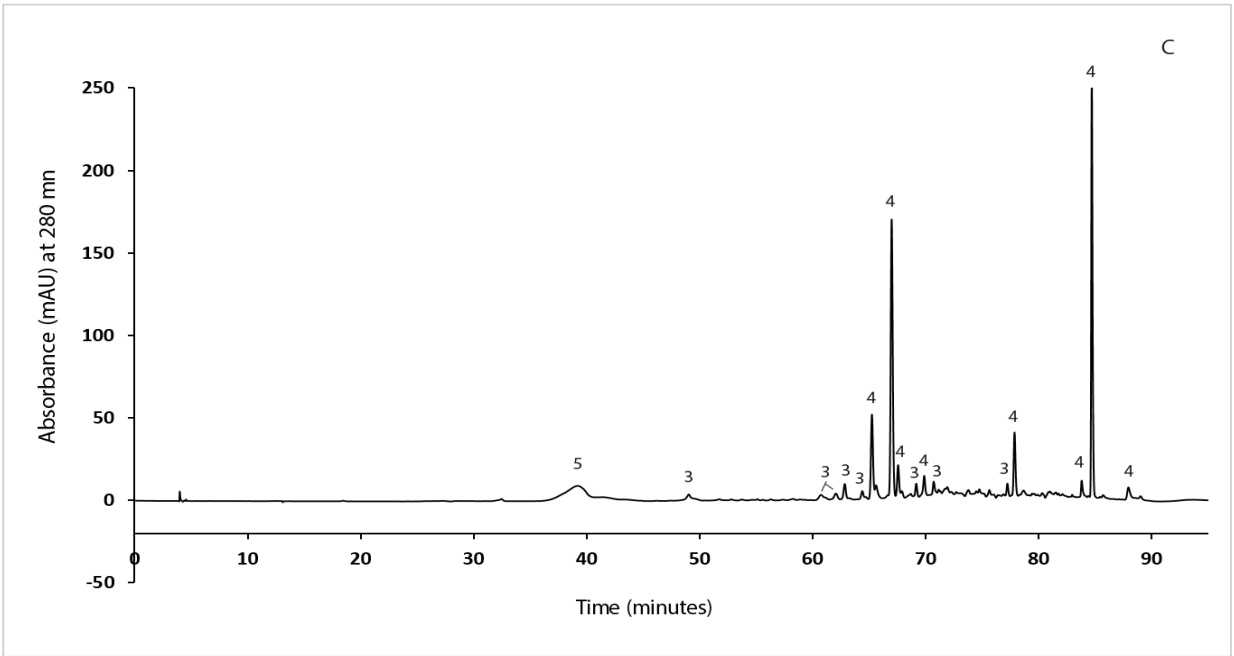


Figure 4.13 HPLC-PDA chromatograms showing the identification of peak phenolic subclasses produced from resin-ethanol fractionation of the Northline variety based on their UV-visible spectrums. Chromatogram identification: A. 40% ethanol; B. 70% ethanol; and C. 100% ethanol. Peak subclass assignments: 1. hydroxybenzoic acids; 2. hydroxycinnamic acids; 3. flavanols; 4. flavonols; 5. anthocyanins.

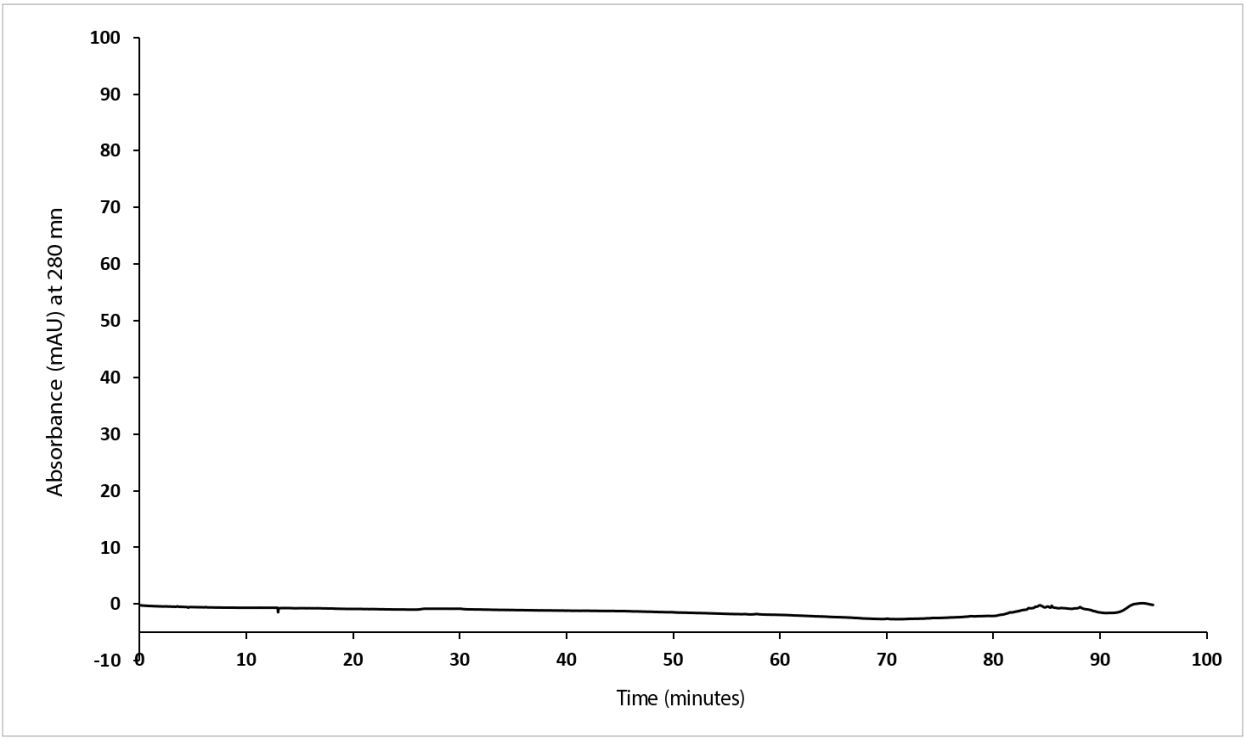


Figure 4.14 HPLC-PDA chromatograms for the water fraction of the Northline variety.

Based on TPCI results, fractions produced from resin-ethanol treatment for each of the three saskatoon berry and pomace EFW extracts (section 3.11), showed the following: (1) the water extract showed minimal extraction of compounds (e.g. phenolics) with an absorbance at 280 nm; (2) sample hydroxybenzoic acids eluted in the 40% ethanol fraction: (3) hydroxycinnamic acids and anthocyanins eluted in the 70% ethanol fraction; and (4) anthocyanins, flavanols and flavonols eluted in the 100% ethanol fraction (Tables 4.18-4.20). Analytical results clearly demonstrate that phenolic subclass fractionation is possible for fruit extracts using Amberlite® XAD16N resin coupled with varying concentrations of aqueous ethanol.

Table 4.18 Mean and standard deviation TPCI results for the five major phenolic subclasses for the 40% ethanol fractions of Martin, Northline and Pembina saskatoon berry varieties and wet and dry pomace.

	Martin[2]	Northline[2]	Pembina[2]	Wet Pomace[3]
Hydroxybenzoic acids	7.2 ± 0.5[4]	9.2 ± 1.9	6.1 ± 1.1	10.3 ± 1.3
Hydroxycinnamic acids	ND[5]	ND	ND	ND
Flavanols	ND	ND	ND	ND
Flavonols	ND	ND	ND	ND
Anthocyanins	ND	ND	ND	ND
TPCI[1]	7.2 ± 0.5[4]	9.2 ± 1.9	6.1 ± 1.1	10.3 ± 1.3

[1]Total Phenolic Chromatographic Index = sum of all identified and quantified phenolic peaks.

[2]mg/100 g FW.

[3]mg/100 g of pomace.

[4]Mean ± standard deviation results of triplicate sample analysis.

[5]ND represents no observable peak (< 6 x S/N).

Table 4.19 Mean and standard deviation TPCI results for the five major phenolic subclassses for the 70% ethanol fractions of Martin, Northline and Pembina saskatoon berry varieties and wet and dry pomace.

	Martin[2]	Northline[2]	Pembina[2]	Wet Pomace[3]
Hydroxybenzoic acids	ND[5]	ND	ND	ND
Hydroxycinnamic acids	64.2 ± 7.6	125.4 ± 7.9	48.1 ± 4.5	85.1 ± 0.9
Flavanols	ND	ND	ND	ND
Flavonols	ND	ND	ND	ND
Anthocyanins	168.7 ± 25.1	292.7 ± 15.1	180.9 ± 11.2	225.6 ± 1.2
TPCI[1]	232.9 ± 21.6	418.1 ± 13.8	229.0 ± 10.6	310.7 ± 15.7

[1]Total Phenolic Chromatographic Index = sum of all identified and quantified phenolic peaks.

[2]mg/100 g FW.

[3]mg/100 g of pomace.

[4]Mean ± standard deviation results of triplicate sample analysis.

[5]ND represents no observable peak (< 6 x S/N).

Table 4.20 Mean and standard deviation TPCI results for the five major phenolic subclassses for the 100% ethanol fractions of Martin, Northline and Pembina saskatoon berry varieties and wet and dry pomace.

	Martin[2]	Northline[2]	Pembina[2]	Wet Pomace[3]
Hydroxybenzoic acids	ND[5]	ND	ND	ND
Hydroxycinnamic acids	ND	ND	ND	ND
Flavanols	45.8 ± 5.2	32.0 ± 3.5	30.2 ± 2.8	64.3 ± 2.2
Flavonols	100.4 ± 3.6	120.5 ± 4.5	92.8 ± 2.6	115.3 ± 4.4
Anthocyanins	5.4 ± 0.8	6.9 ± 0.7	4.5 ± 0.5	6.4 ± 0.2
TPCI[1]	151.6 ± 8.3	159.4 ± 6.8	127.5 ± 5.7	186.0 ± 6.2

[1]Total Phenolic Chromatographic Index = sum of all identified and quantified phenolic peaks.

[2]mg/100 g FW.

[3]mg/100 g of pomace.

[4]Mean ± standard deviation results of triplicate sample analysis.

[5]ND represents no observable peak (< 6 x S/N).

4.15 Antioxidant Activity of Saskatoon Berry and Pomace Resin-Ethanol Fractions

4.15.1 ABTS (2,2-azino-bis-3-ethylbenzthiazoline-6-sulfonic acid) and DPPH (2,2-diphenyl-1-picrylhydrazyl) Radical Scavenging Assay

The ABTS and DPPH free radical scavenging activity results of Amberlite® XAD16N fractions expressed as mM Trolox activity equivalent (TEAC)/100 mg FW and $1/IC_{50}$ 1/100 mg FW, respectively, are shown in Tables 4.21 and 4.22.

Table 4.21 Mean and standard deviation ABTS radical scavenging activity data for EFW fractions of Martin, Northline and Pembina saskatoon berry varieties and wet pomace.

	ABTS Radical Scavenging Activity			
Fractions	Martin[1]	Northline[1]	Pembina[1]	Wet Pomace[2]
40% ethanol	71.5 ± 4.1^{1Ab}	112.3 ± 9.5^{Ad}	52.2 ± 1.3^{Aa}	101.2 ± 1.5^{Ac}
70% ethanol	235.4 ± 10.6^{Cb}	336.3 ± 12.7^{Cd}	216.3 ± 14.2^{Ca}	317.2 ± 14.7^{Cc}
100% ethanol	121.9 ± 8.9^{Bb}	162.1 ± 2.0^{Bd}	101.6 ± 10.4^{Ba}	159.2 ± 7.1^{Bc}

[1]Reported as mM Trolox equivalents (TEAC)/100 mg FW.

[2]Reported as mM Trolox equivalents (TEAC)/100 mg pomace.

[3]Mean ± standard deviation results of triplicate sample analysis.

[A-C]Mean values in the same column followed by a common letter were not statistically different ($p<0.05$) by Tukey's HSD multiple range test between the type of extracts.

[a-c]Mean values in the same row followed by a common letter were not statistically different ($p<0.05$) by Tukey's HSD multiple range test between the varieties and pomace.

Table 4.22 Mean and standard deviation DPPH radical scavenging activity data for EFW fractions of Martin, Northline and Pembina saskatoon berry varieties and wet pomace.

	DPPH Radical Scavenging Activity			
Fractions	Martin[1]	Northline[1]	Pembina[1]	Wet Pomace[2]
40% ethanol	0.7 ± 1.1^{3Aa}	1.3 ± 0.5^{Ab}	0.6 ± 1.3^{Aa}	1.1 ± 1.5^{Ab}
70% ethanol	19.4 ± 2.6^{Ca}	28.8 ± 12.7^{Cd}	21.6 ± 14.2^{Cc}	26.7 ± 14.7^{Cb}
100% ethanol	15.9 ± 0.9^{Bb}	17.7 ± 2.0^{Bc}	12.6 ± 10.4^{Ba}	18.9 ± 7.1^{Bd}

[1]Reported as $1/IC_{50}$ (1/100 mg FW sample for 50% DPPH radical inhibition).

[2]Reported as $1/IC_{50}$ (1/100 mg pomace for 50% DPPH radical inhibition).

[3]Mean ± standard deviation results of triplicate sample analysis.

[A-C]Mean values in the same column followed by a common letter were not statistically different ($p<0.05$) by Tukey's HSD multiple range test between the type of extracts.

[a-c]Mean values in the same row followed by a common letter were not statistically different ($p<0.05$) by Tukey's HSD multiple range test between the varieties and pomace.

The results obtained for Amberlite® XAD16N fractions for both antioxidant activity assays showed similar trends. The highest free radical scavenging activity was observed in the 70% EFW fraction for all saskatoon berry varieties and wet pomace (Tables 4.21 and 4.22). These results can be explained by this fraction having both the highest phenolics and anthocyanin contents/concentrations as shown by TPCI results, coupled with the fact that anthocyanins are reported to have the highest free radical scavenging ability of fruit phenolics (Lavola et al., 2012).

The anthocyanin, flavonol and flavone phenolic subclasses have high radical scavenging abilities because of the unsaturation in their C-rings, which provide increased electron delocalization and stabilization of the phenoxy radical when compared to the hydroxybenzoic and hydroxycinnamic acids phenolic subclasses (Rice-Evans et al., 1996).

The combined total ABTS and DPPH antioxidant activities for all three Amberlite® XAD16N fractions (i.e. 40% + 70% + 100% ethanol), were higher than those found for the original EFW extracts for all three varieties. As an example, the EFW extract of the Northline variety had antioxidant values of 327.5 (TEAC)/100 mg FW and 23.1 $1/IC_{50}$ 1/100 mg FW, for the ABTS and DPPH assays, respectively (Tables 4.15 and 4.16). Whereas, the ABTS and DPPH antioxidant assays results for the combined ethanol fractions of the Northline variety were 610.7 (TEAC)/100

mg FW and 47.8 $1/IC_{50}$ 1/100 mg FW, respectively (Tables 4.21 and 4.22). The large differences in these results can be explained by the increased concentration of phenolics in the ethanol fractions when compared to the EFW extract, which would also contain sample carbohydrates, organic acids, minerals, etc. The majority of these non-phenolic compounds were removed in the water fraction during sample Amberlite® XAD16N treatment, resulting in more purified phenolic fractions (i.e. 40, 70 and 100% ethanol). Similar results have been reported on antioxidant assay differences between fruit extracts and their fractions in literature (Re et al., 1999; Green, 2007; Konecsni, 2011).

4.16 Laboratory Scale Saskatoon Berry Juice Analysis

Research results obtained in this thesis from wet and dry pomace samples from commercial saskatoon berry juice production indicated that this co-product stream would be a good source of phenolics for industrial food/nutraceutical applications. However, the original fruit that was used in juice production was not available for analysis. In order to conclusively prove this hypothesis, fresh (i.e. 2016 crop) raw fruit from the Northline variety, which was shown to have the highest phenolics content by TPC and TPCI analyses, and ABTS/DPPH free radical scavenging ability of the three varieties studied. Therefore, Northline fruit (2016 crop) was subjected to laboratory scale juice production employing commercial processing steps, time/temperature conditions, and enzyme preparations and dosages.

4.16.1 Physicochemical Analysis

Mean and standard deviation physicochemical analysis results for laboratory scale juice produced from the Northline saskatoon berry variety (2016 crop) are shown in Table 4.23.

Table 4.23 Mean and standard deviation physicochemical analytical results for laboratory scale juice produced from the Northline saskatoon berry variety.

Physicochemical Parameter		Juice
Colour	L*	3.81 ± 0.04[1]
	a*	9.88 ± 0.18
	b*	3.07 ± 0.38
	Chroma (C*)	10.35 ± 0.06
	Hue angle (θ)	19.20 ± 2.54
pH[2]		12.92 ± 2.34
Total soluble solids (°Brix)		3.88 ± 0.06
Yield (%)		74.51 ± 4.23

[1]Mean ± standard deviation results of triplicate sample analysis.

Laboratory scale juice had a mean L* value of 3.81, which was similar to that observed for the Northline hand pressed juice sample (2015 crop; Table 4.1) of 3.69. The hue angle value was 19.20, which is indicative of a red coloured juice, and this value was much higher than that observed for the Northline hand pressed juice (Table 4.1) of 10.27. These differences in colour values between the two Northline variety samples could be due to the juice processing conditions employed (i.e. hand pressing vs. enzymatic treatments), sample phenolic content, sample polyphenol oxidase activity, and fruit environmental growth conditions.

The total soluble solids of the laboratory scale juice was 12.92 °Brix (note: without correction for the 30.0 mL of water added during the fruit mashing stage) which was consistent with the value obtained for the hand pressed juice (Table 4.2) of 12.79 °Brix. As previously reported, the °Brix value for this laboratory scale produced juice was within the range reported for commercial apple juice of 12.2-13.8 (Fourie et al., 1991). Similarities between pH values for the laboratory scale juice of 3.88 and that determined for the hand pressed juice (Table 4.1) of 4.02 were also found.

Juice yield from laboratory scale processing employing enzymes, enzyme dosages and temperature and time conditions that were consistent with those used in commercial apple and pear

juice production (Willems and Low, 2016) was ~75% (w:w), excluding the 30.0 mL of water added during the fruit mashing stage. This value was within the range of yields generally obtained from industrial apple juice production of 60 to 85% (w:w), which is dependent on fruit variety and maturity, extraction equipment employed, pressing aids, time/temperature relationships used for enzyme treatment, and concentrations of enzymes used (Root and Barrett, 2005). This juice extraction volume result provides further evidence that saskatoon berry has great potential for commercial juice production.

The mean and standard deviation pH, moisture and total soluble solids contents of the pomace from laboratory scale juice production were 3.68 ± 0.05; 70.35 ± 0.54% (w:w); and 3.99 ± 1.84 °Brix, respectively. The moisture content value was similar to that found for the commercial wet pomace sample (Table 4.3) of 68.0%, however, the total soluble solids value was much lower than the 8.11 °Brix determined for the commercial sample (Table 4.3). Possible reasons for this large difference in °Brix values between pomace samples could be explained by the total soluble solids contents of the starting fruit, more efficient juice release from the fruit through enzymatic treatments, and/or more efficient soluble solids removal during the vacuum filtration step in laboratory scale juice processing versus that used in industry (e.g. belt press).

4.16.2 Total Phenolic Content and Free Radical Scavenging Activity

Mean and standard deviation total phenolic content (TPC), and ABTS and DPPH free radical scavenging activity values for samples at each stage of laboratory scale juice production and pomace are reported in Table 4.24.

Table 4.24 Mean and standard deviation for total phenolic content (TPC), and ABTS and DPPH radical scavenging activity results for laboratory scale juice processing stage and pomace of the Northline saskatoon berry variety.

Processing stage	TPC[1]	ABTS[2]	DPPH[3]
Mash	551.5 ± 13.5[4c]	305.7 ± 3.3[c]	32.3 ± 1.4[c]
After enzyme treatment	579.7 ± 12.0[d]	362.6 ± 5.7[e]	38.2 ± 2.1[d]
Juice	398.2 ± 15.4[b]	246.9 ± 6.1[b]	24.4 ± 0.3[b]
Pomace	178.4 ± 11.2[a]	119.1 ± 2.6[a]	13.5 ± 1.2[a]

[1]mg GAE/100 g FW or juice.

[2]mM TEAC/100 mg FW sample or juice.

[3]$1/IC_{50}$ (1/100 mg FW sample or juice for 50% DPPH inhibition).

[4]Mean ± standard deviation results of triplicate sample analysis.

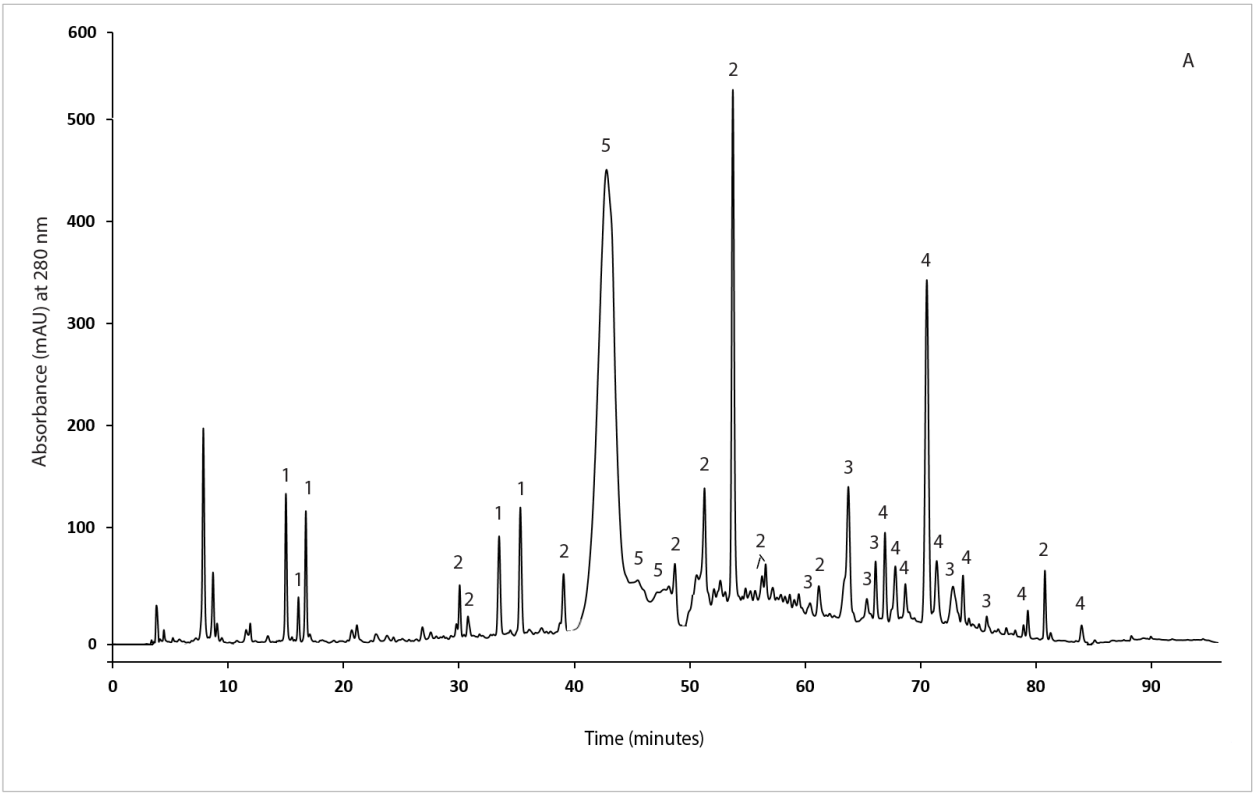
[a-e]Mean values in the same column followed by a common letter were not statistically different ($p<0.05$) by Tukey's HSD multiple range test between the processing stages.

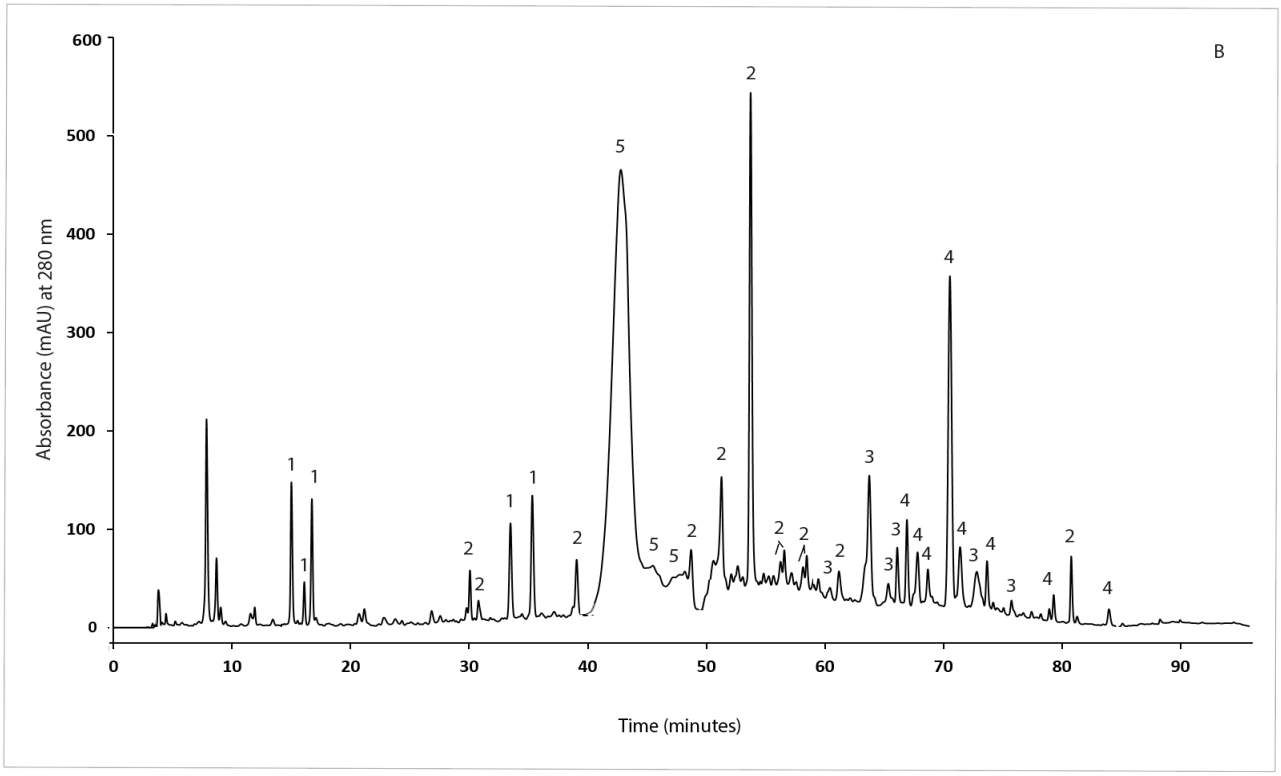
Generally, analytical results for TPC, ABTS, and DPPH were found to decrease as the fresh fruit was converted to juice (i.e. from the mash to juice stages of processing). As an example, the mean TPC value of the fruit (i.e. mash stage) decreased from 551.5 mg GAE/100 g FW to 398.2 mg GAE/100 g juice weight, which may be explained by losses in phenolics and changes in phenolic structure due to temperature/time processing (i.e. heating stages). The observed increases in TPC, ABTS, and DPPH values following the enzyme treatment stage may be explained by improved exposure of sample phenolics to the colourimetric reagents used in these assays (e.g. Folin and Ciocalteu's phenol reagent) via the removal of polysaccharides (i.e. pectin and hemicelluloses) that can form non-covalent bonds with sample phenolics, the depolymerization of phenolics (e.g. proanthocyanidins), the breakdown of cellular constituents through temperature and enzyme treatments that release bound phenolics (e.g. protein-phenolic complexes), and/or improved phenolic extraction from fruit cells due to the time/temperature conditions employed (Dewanto et al., 2002; Suzme et al., 2014).

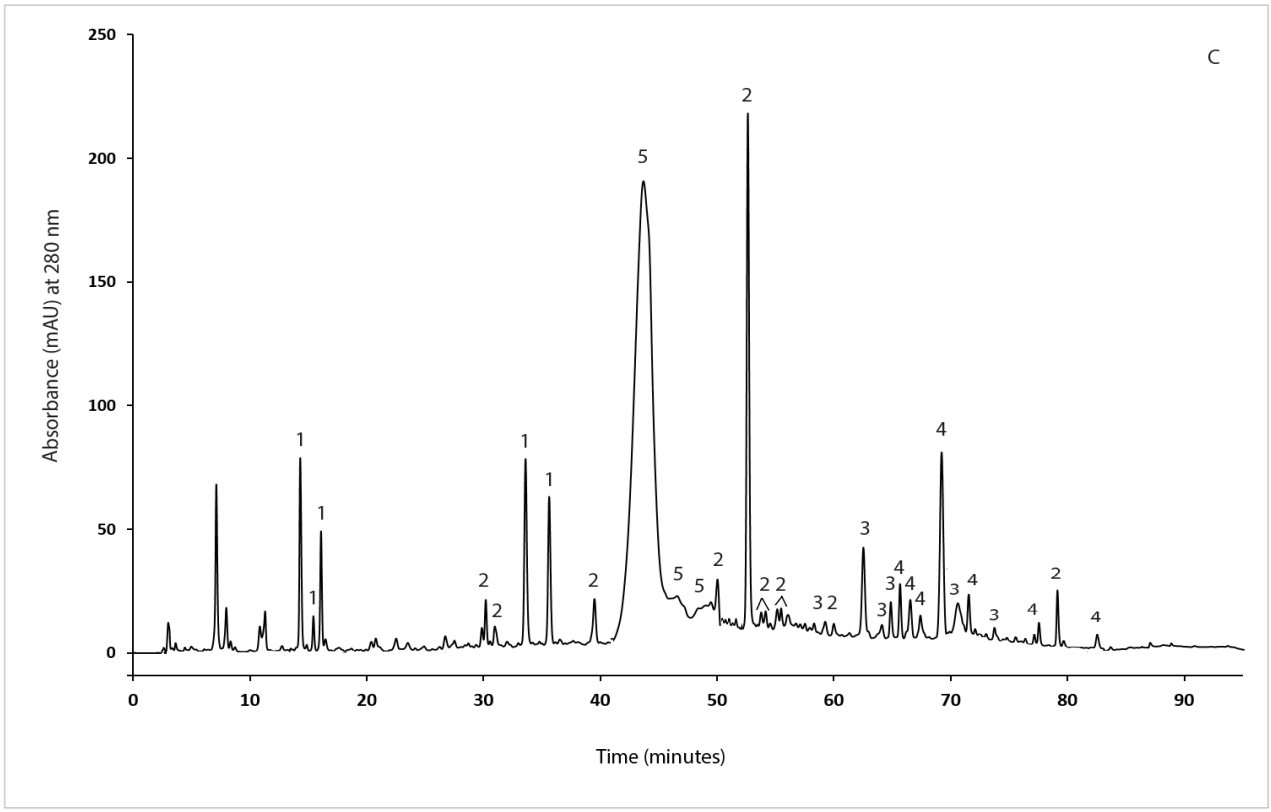
The pomace produced during laboratory scale juice production was found to have a mean TPC value of 178.4 mg GAE/100 g of juice. With a correction for moisture content differences between the mash of 81.5% (Table 4.2) vs. the pomace of 70.4%, approximately 29% of the original TPC value was present in the pomace. In addition, the pomace showed free radical

scavenging ability by both the ABTS and DPPH assays, with a concomitant decrease in activity when compared to the fresh fruit. These results support those reported previously (section 4.13) that the pomace from commercial saskatoon berry juice production is a valuable source of phenolics with free radical scavenging abilities. Published studies on the TPC value of the pomace from apple and cranberry following juice production were ~17-58% of the original value (Guyot et al. 2003; White et al., 2011).

Representative HPLC-PDA chromatograms (i.e. TPCI) for each processing stage of laboratory scale juice production with phenolic subclass assignments are shown in Figure 4.15.




B
Absorbance (mAU) at 280 nm
Time (minutes)


C
Absorbance (mAU) at 280 nm
Time (minutes)

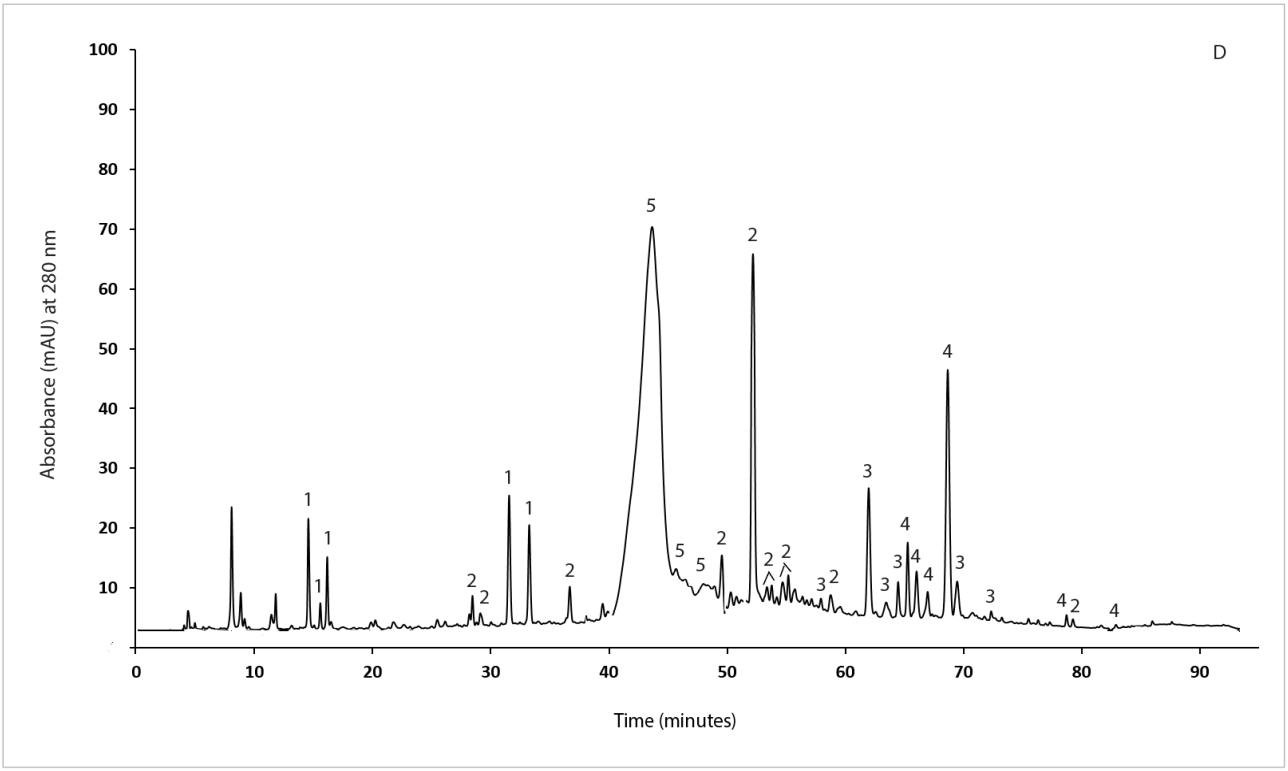


Figure 4.15 HPLC-PDA chromatograms showing the identification of peak phenolic subclasses for samples from each stage of laboratory scale juice production and pomace from the Northline variety of saskatoon berry based on their UV-visible spectrums. Chromatogram identification: A. mash; B. after enzyme treatment; C. juice; and D. pomace. Peak subclass assignments: 1. hydroxybenzoic acids; 2. hydroxycinnamic acids; 3. flavanols; 4. flavonols; 5. anthocyanins.

Five major fruit phenolic subclasses were identified in the samples from each stage of laboratory scale juice production and the pomace (Table 4.25). A decrease in both phenolic subclass TPC and TPCI values was found between the raw fruit and the juice produced employing commercial enzyme preparations and conditions. These results were consistent with those reported in Table 4.24 with the same trend in an increase in both TPC and TPCI for each subclass between the mash and the mash + enzyme stages, where the aforementioned explanations also hold.

As reported previously for the three saskatoon berry varieties studied in this research project (Tables 4.10-4.12), the major phenolic subclass in both the juice and pomace was anthocyanins, which accounted for ~60 and ~40% of the total, respectively. These compounds are

negatively impacted by high temperature/pH/time conditions as shown by a decrease (with a correction for moisture content differences) in their TPCI values of ~29% from the mash to juice stages of laboratory scale processing.

Table 4.25 Mean and standard deviation TPC and TPCI results for the five major phenolic subclasses for laboratory scale juice production stage and pomace of the Northline saskatoon berry variety.

	Mash[2]	After enzyme treatment[2]	Juice[3]	Pomace[4]
Hydroxybenzoic acids	5.1 ± 0.1[5]	5.4 ± 0.3	4.3 ± 0.3	1.5 ± 1.8
Hydroxycinnamic acids	115.6 ± 9.1	125.9 ± 7.1	85.2 ± 5.7	38.1 ± 3.9
Flavanols	16.7 ± 0.1	20.4 ± 0.5	4.2 ± 0.8	15.3 ± 0.2
Flavonols	107.4 ± 8.2	109.8 ± 9.5	58.8 ± 1.6	49.3 ± 1.4
Anthocyanins	280.1 ± 12.1	300.3 ± 15.1	230.5 ± 13.1	70.4 ± 10.2
TPCI[1]	524.9 ± 10.8	561.8 ± 13.8	383.0 ± 15.7	174.6 ± 12.2

[1]Total Phenolic Chromatographic Index = sum of all identified and quantified phenolic peaks.

[2]mg/100 g FW.

[3]mg/100 g of juice.

[4]mg/100 g of pomace.

[5]Mean ± standard deviation results of triplicate sample analysis.

5. GENERAL CONCLUSIONS

The central hypotheses of this research were to improve the scientific knowledge on the physicochemical and chemical composition of saskatoon berry fruit grown in Saskatchewan, identify a saskatoon berry variety rich in phenolics, and to determine if pomace from commercial juice production is a potential source of phenolics for food and nutraceutical applications.

The first segment of this research focused on the physicochemical properties Martin, Northline, and Pembina varieties of saskatoon berry grown in Saskatchewan. The fresh pressed juice tristimulus colour values and ranges were, L* (3.26-8.51), a* (5.17-9.98), b* (1.21-2.89), and hue angle (10.27-16.53). The diameter of the three varieties ranged from 8.29-12.29 mm, pH from 3.95-4.10 and °Brix values from 12.79-15.72.2.

The proximate composition of Martin, Northline and Pembina varieties of saskatoon berry grown in Saskatchewan was also determined. Proximate analysis results for the three varieties ranged from 80.18-82.79% for moisture, 7.39-10.82% for carbohydrate, 1.13-1.79% for protein, 0.28-0.48% for lipid, 4.23-9.42% for total dietary fibre, and 0.53-0.74% for ash. Major carbohydrates and sorbitol concentrations were determined by HPLC-RI and ranged from 4.75-5.36% for fructose, 4.04-4.76% for glucose, and 1.86-2.15% sorbitol. Fruit oligosaccharide profiles as determined by HPAE-PAD showed the presence of both dextrose (DP2-5) and pectin polymers. Amino acid concentrations ranged from 0.83-1.22 g/100 g fresh weight (FW). Major minerals were quantified by ICP and their concentration ranges were, calcium/34.3-82.7 ppm, magnesium/18.7-36.7 ppm, potassium/219.0-248.4 ppm and sodium/2.2-3.5 ppm. Organic acids were determined by HPLC-PDA with ascorbic, malic, maleic, oxalic, quinic, succinic and identified, and the major acids being malic (304.7-393.9 mg/100 g FW) and succinic (120.4-316.3 mg/100 g FW). This data represents a significant addition of scientific information on the chemical composition of three saskatoon berry varieties grown in Saskatchewan, Canada.

The second segment of this research focused on phenolic profiling and free radical scavenging abilities of extracts from Martin, Northline and Pembina fruit varieties and wet and dry pomace from commercial juice production. Phenolics were extracted using water, ethanol:formic

acid:water, and methanol:formic acid:water (70:2:28 v:v) mixtures. The ethanol:formic acid:water (EFW) phenolic extracts for each variety were shown to have the greatest TPC and TPCI values, and those for the Northline variety were the highest at 526.8 mg GAE/100 g FW, and 504.2 mg/100 g FW, respectively. The TPC and TPCI results for the Martin and Pembina varieties were, 313.1 and 296.2 mg GAE/100 g FW, and 294.1 and 215.5 mg/100 g FW, respectively. The ABTS and DPPH free radical scavenging abilities of the EFW extracts were determined for all three varieties with the greatest observed for Northline of 327.5 mM (TEAC)/100 mg FW and 23.1 $1/IC_{50}$/100 mg FW, respectively. The ABTS and DPPH radical scavenging results for Martin and Pembina were, 177.1 and 159.8 mM (TEAC)/100 mg FW, and 14.7 and 12.1 $1/IC_{50}$/100 mg FW, respectively. The Northline variety was identified as phenolic-rich by both TPC and TPCI results. These results can be explained by the greater surface area (i.e. skin to fruit) of the Northline variety when compared to Martin and Pembina, and the fact that the berry skin contains a high concentration of phenolics.

Based on their phenolic composition as determined by TPCI, the major phenolic subclass identified in the three saskatoon berry varieties was anthocyanins at 41-54% of the total phenolics content. Analysis of EFW extracts for their anthocyanin content and concentration by HPLC-PDA showed the presence of cyanidin-3-*O*-galactoside, cyanidin-3-*O*-glucoside, cyanidin-3-*O*-arabinoside, and cyanidin-3-*O*-xyloside; with cyanidin-3-*O*-galactoside being the major anthocyanin with a concentration range of 70.7-205.1 mg/100 g FW.

The EFW extracts for each variety were fractionated employing Amberlite® XAD-16 resin and aqueous ethanol mobile phases ranging from 40-100%. Analysis of individual fractions by HPLC-PDA for their TPCI showed that hydroxybenzoic acids eluted in 40% ethanol fraction; hydroxycinnamic acids and anthocyanins eluted in the 70% ethanol fraction; and anthocyanins, flavanols and flavonols eluted in the 100% ethanol fraction. The ABTS and DPPH free radical scavenging abilities of each fraction were determined with the greatest observed for the 70% ethanol fraction for all three varieties. Northline had the highest free radical scavenging ability for both ABTS and DPPH assays of, 336.3 mM (TEAC)/100 mg FW and 28.8 $1/IC_{50}$/100 mg FW, respectively.

Wet and dry pomace from commercial saskatoon berry juice production had TPCI values of 404.2 mg/100 g FW and 250.0 mg/100 g FW, respectively. The ABTS values for wet and dry pomace were found to be 304.8 and 327.8 mM (TEAC)/100 mg FW, while the DPPH values were

19.4 and 16.8 $1/IC_{50}$/100 mg FW, respectively. These results showed that the wet pomace from juice production could be a good source of phenolics for food and nutraceutical formulations.

The last segment of this research studied the physicochemical analysis, phenolic profile and free radical scavenging ability of laboratory scale juice produced using commercial conditions (i.e. temperature, time, enzymes and dosages) from the Northline variety. The major findings from this research showed that: (a) approximately 29% of the phenolic compounds in the original fruit were in the pomace as determined by TPCI values of 524.9 and 174.6 mg/100 g FW value for mashed fruit and pomace (with a correction for moisture content differences), respectively; (b) the ABTS and DPPH values for pomace had approximately 38 and 41% of the initial free radical scavenging abilities of the original fruit.

6. FUTURE STUDIES

This research determined the phenolic content from saskatoon berry varieties and pomace from commercial and laboratory scale juice production, including the concentration of each five phenolic subclasses (hydroxybenzoic acids, hydroxycinnamic acids, flavanols, flavonols and anthocyanins) in extracts and isolated phenolic fractions. However, the structure identities of specific phenolic compounds in both extracts and phenolic fractions remain unknown. Future research aimed at the structure identification and quantification of specific phenolic compounds in both saskatoon berry varieties and pomace could be obtained by HPLC-PDA coupled with mass spectrometry (MS) and/or nuclear magnetic resonance spectroscopy (NMR). This work could provide information on the roles that fruit variety plays on phenolic profile/concentrations and the role of specific phenolic compounds on the antioxidant capacity of the extracts/phenolic fractions.

The individual isolated phenolic fractions could be used to investigate the potential of delaying the oxidation of polyunsaturated oils. This could be accomplished by, for example rancimat method, peroxidase value determination or other modern techniques such as electro-paramagnetic spectroscopy (EPR) and differential scanning calorimetry (DSC). The structure elucidation of phenolic fractions could explain the relationship between the phenolic subclasses or specific phenolics and their antioxidant mechanism of action in delaying oxidation in polyunsaturated oil. One challenge facing this approach is the solubility of phenolic fractions in polyunsaturated oils. This problem could be overcome by using either emulsifiers, to help interface solubility of oil-phenolics, or deglycosylation of phenolic compounds, which would improve hydrophobicity and consequently the solubility of phenolics in oil. Isolated phenolic fractions could be used to replace synthetic antioxidants, which have been reported to have toxicological implications (e.g. carcinogenicity). The use of natural antioxidant would help to meet consumers' demand for natural ingredients and clean product labels.

In addition, isolated phenolic fractions could be used to investigate *in vitro* and *in vivo* antitumor activities. These studies could help understand the molecular mechanism of action of specific phenolic compounds against cancer cell lines. One possible approach to improve targeted

delivery of phenolic compounds in animal models could be the encapsulation of phenolic fractions to create nanoparticles.

The application of saskatoon berry pomace in ready to eat products containing high concentration of polyunsaturated lipids could be investigated by either applying it directly (formulation of food products) or applying phenolics isolated from pomace. This could improve product appearance (i.e colour) and enhance shelf life (i.e delay oxidation). The extraction of value-added ingredients from pomace could also increase economic feasibility for the food industry due to reduction of waste and aggregation value to a cheap co-product stream.

In this study, for the first time, the oligosaccharide profile of saskatoon berry was investigated. The presence and/or absence of oligosaccharides can be used as fingerprints to detect juice authenticity/adulteration and processing. In addition, further information on the effect of juice processing on the phenolic profile and concentration could be obtained by HPLC-PDA-MS. Specific fingerprint compounds (i.e oligosaccharides and phenolics) could be identified and this information be used for authenticity of juice products. It may be possible that this work could be expanded to other fruit juices.

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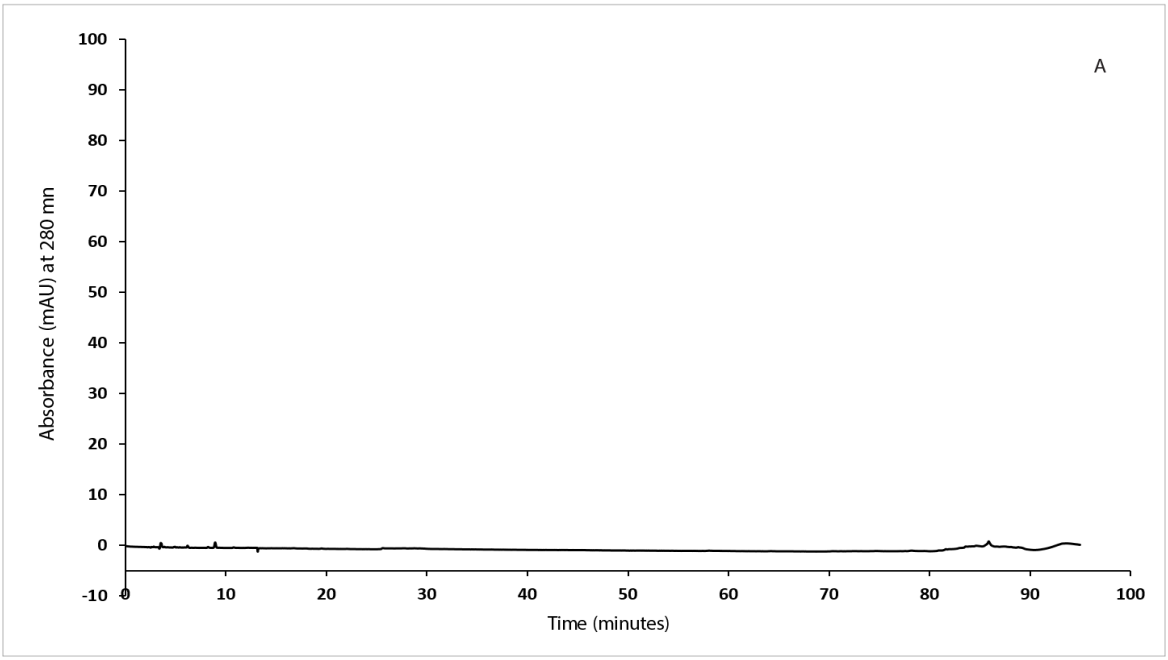
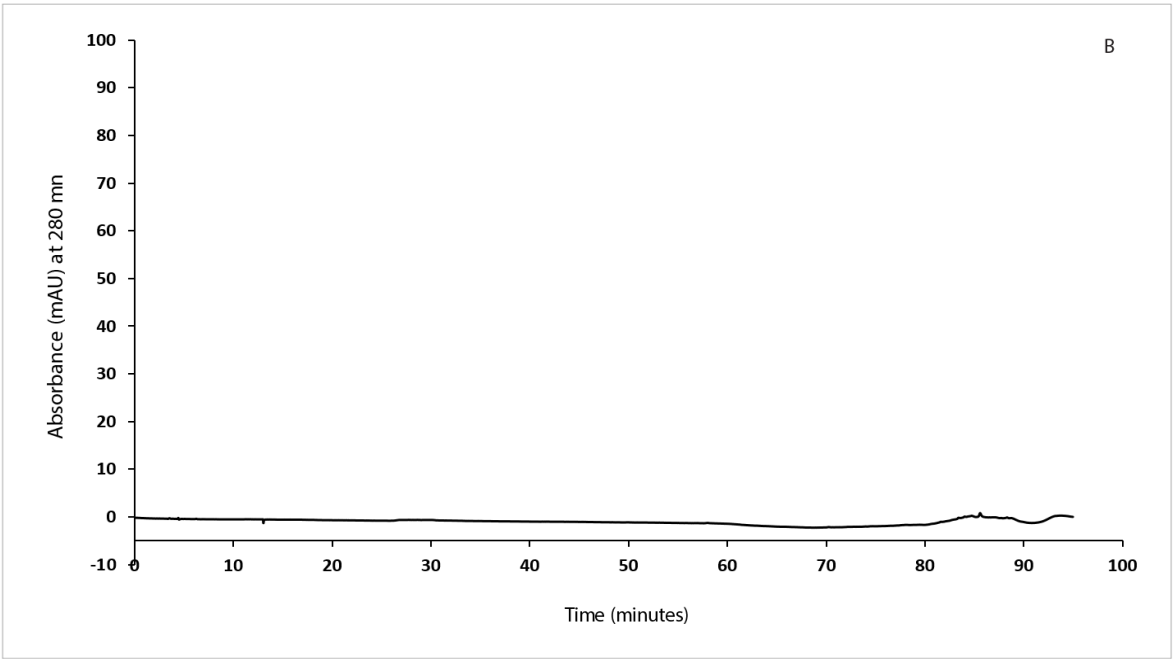
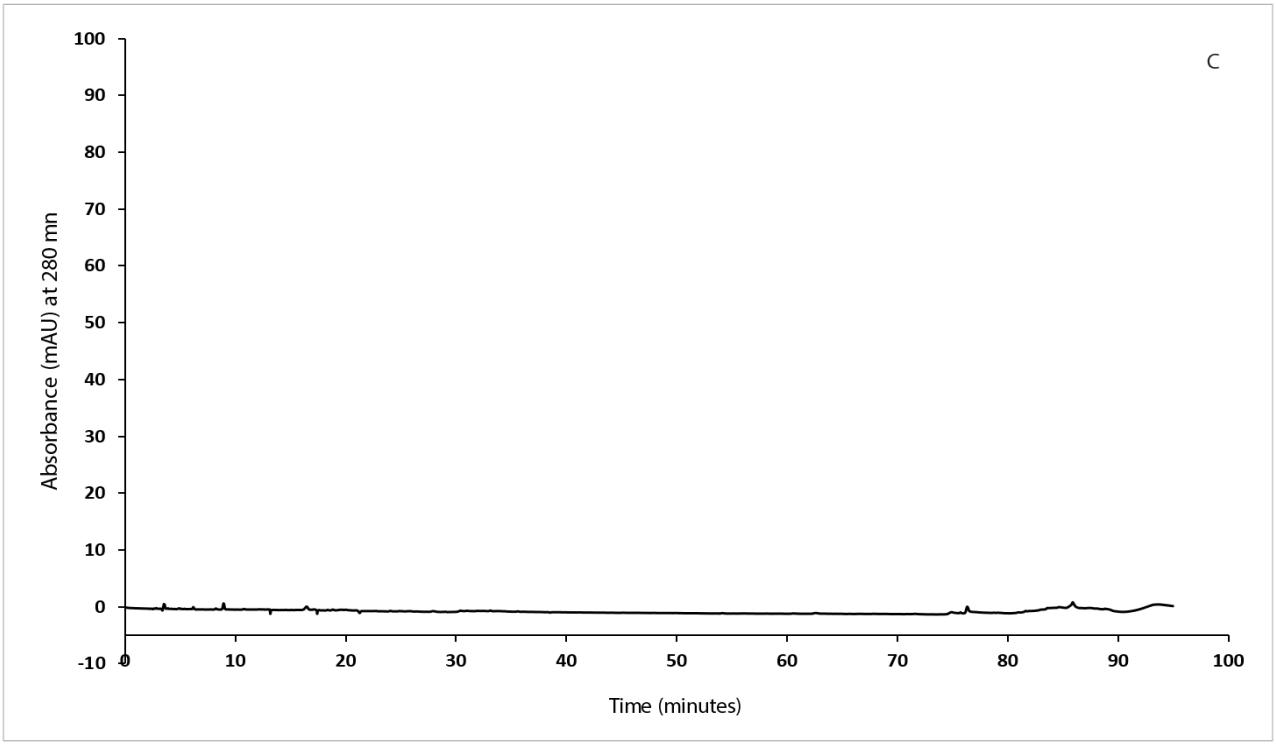
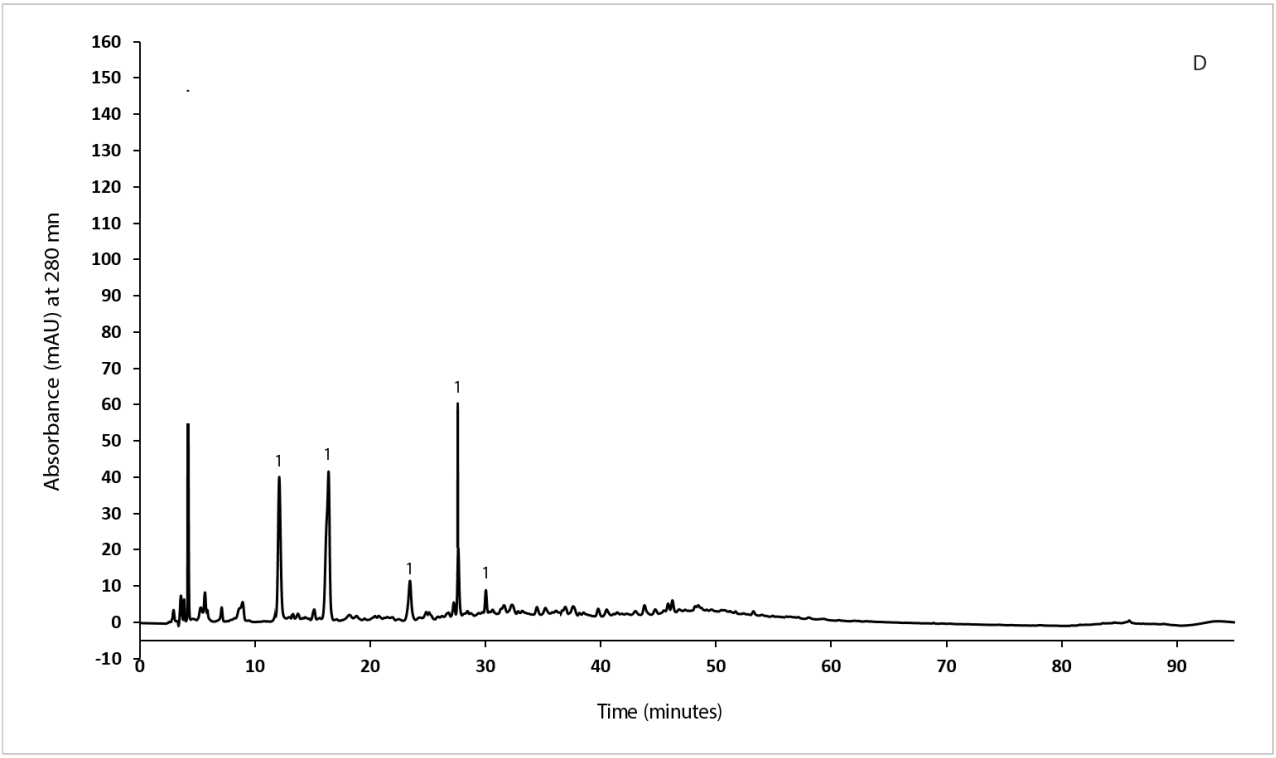
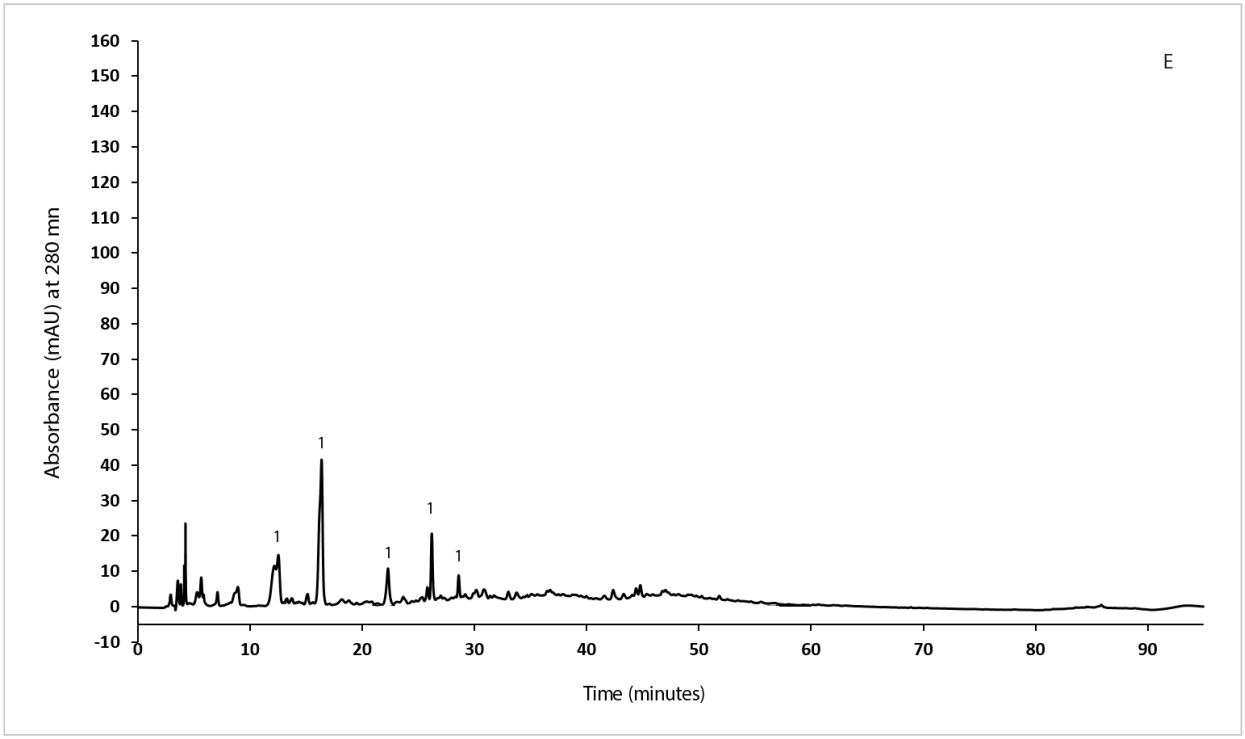
8. APPENDIX

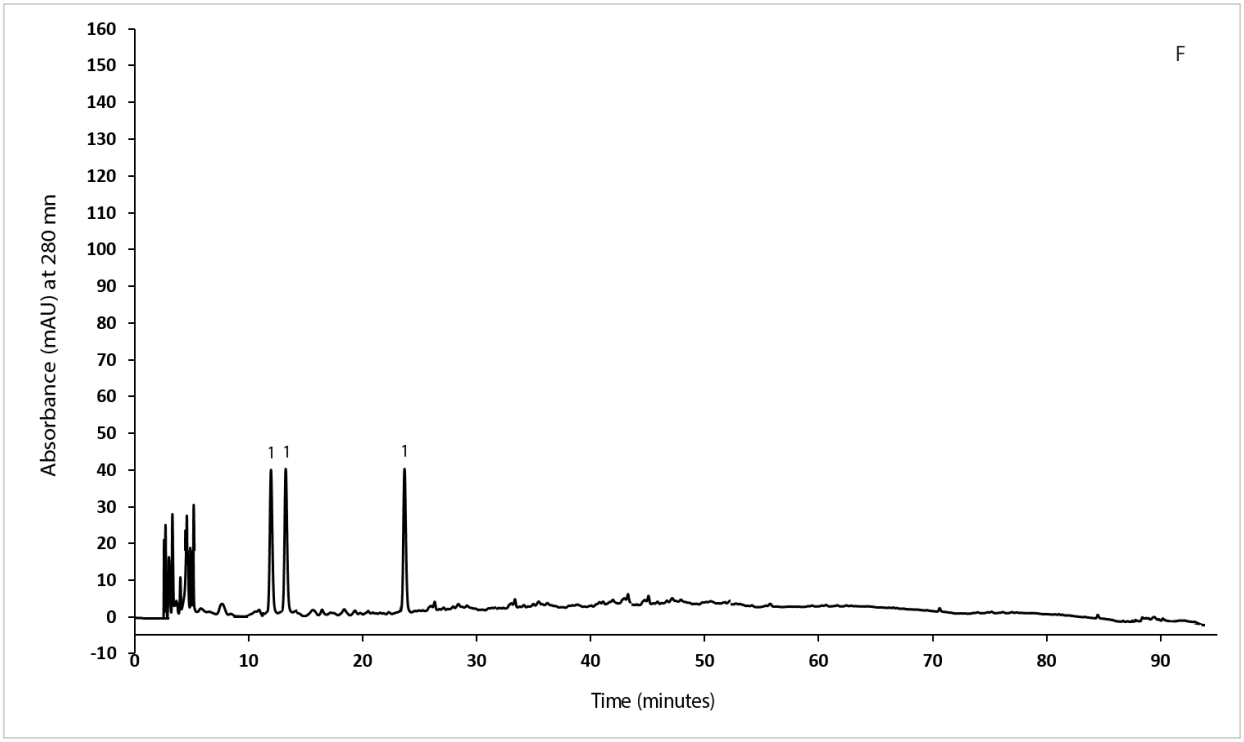

A
Absorbance (mAU) at 280 mn
Time (minutes)
100
90
80
70
60
50
40
30
20
10
0
-10
0
10
20
30
40
50
60
70
80
90
100

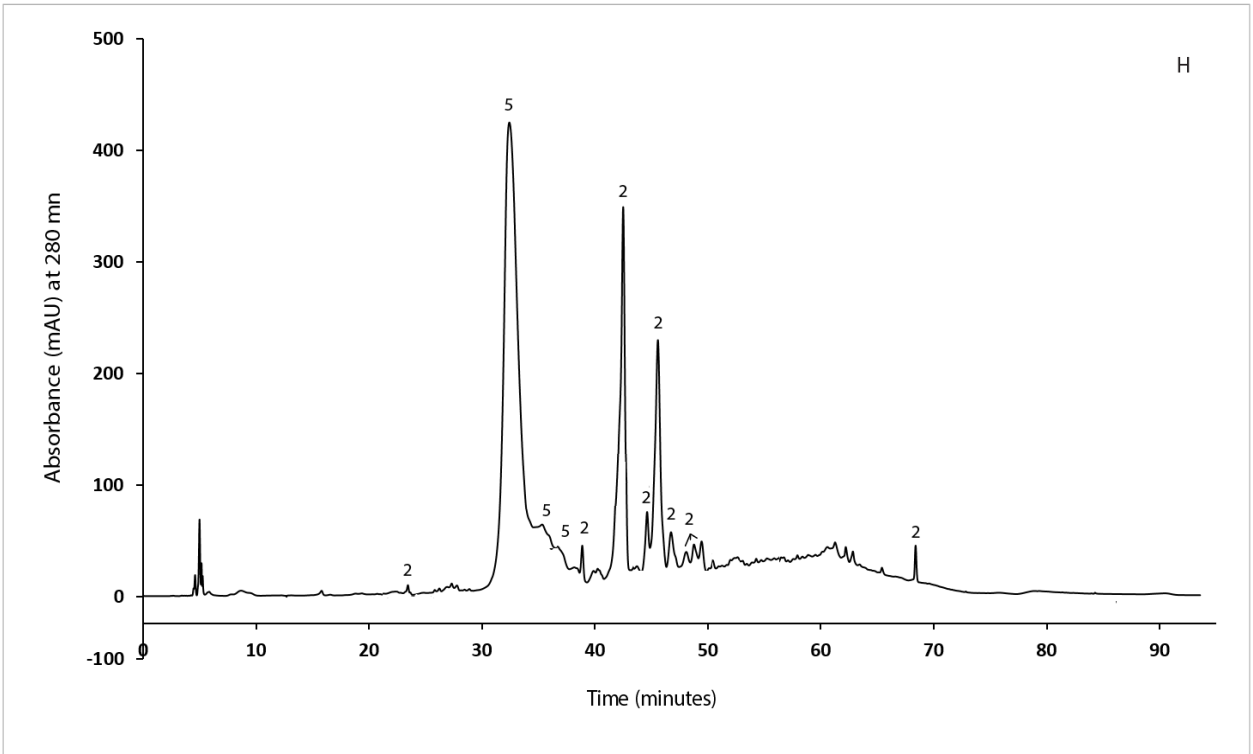

B
Absorbance (mAU) at 280 mn
Time (minutes)
100
90
80
70
60
50
40
30
20
10
0
-10
0
10
20
30
40
50
60
70
80
90
100

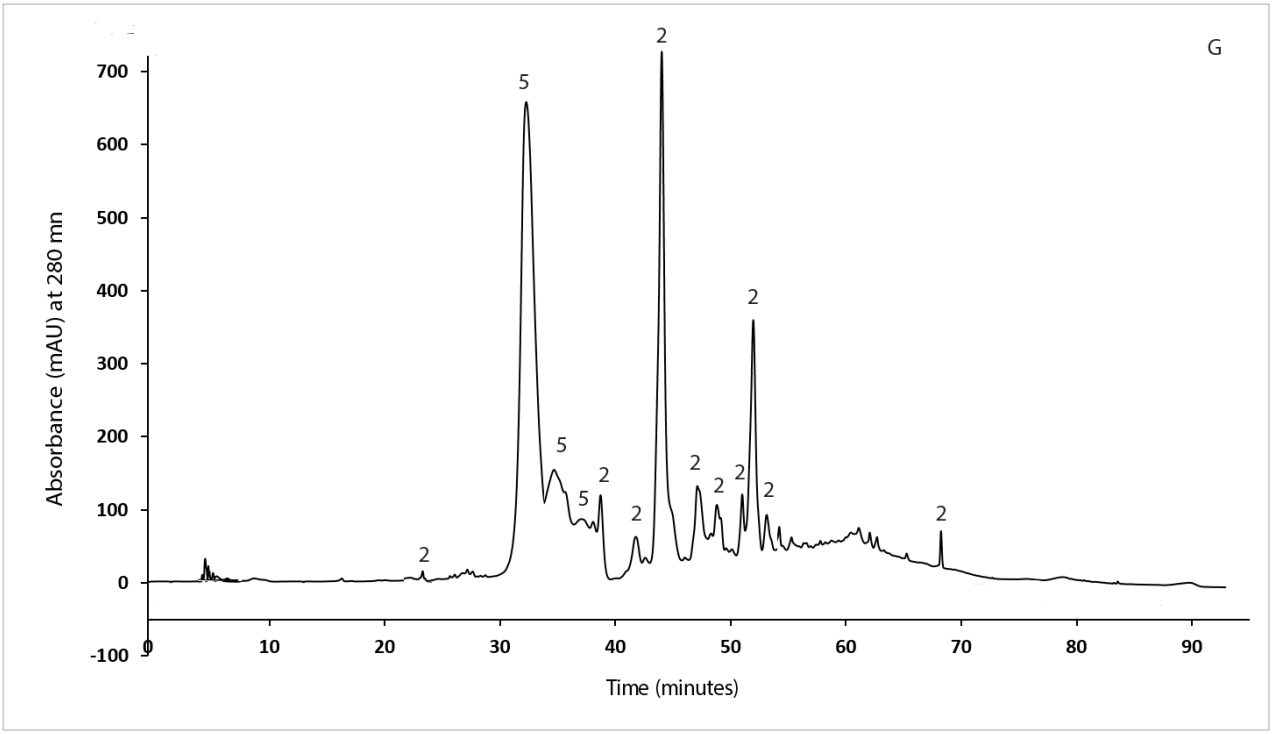

C
Absorbance (mAU) at 280 mn
Time (minutes)

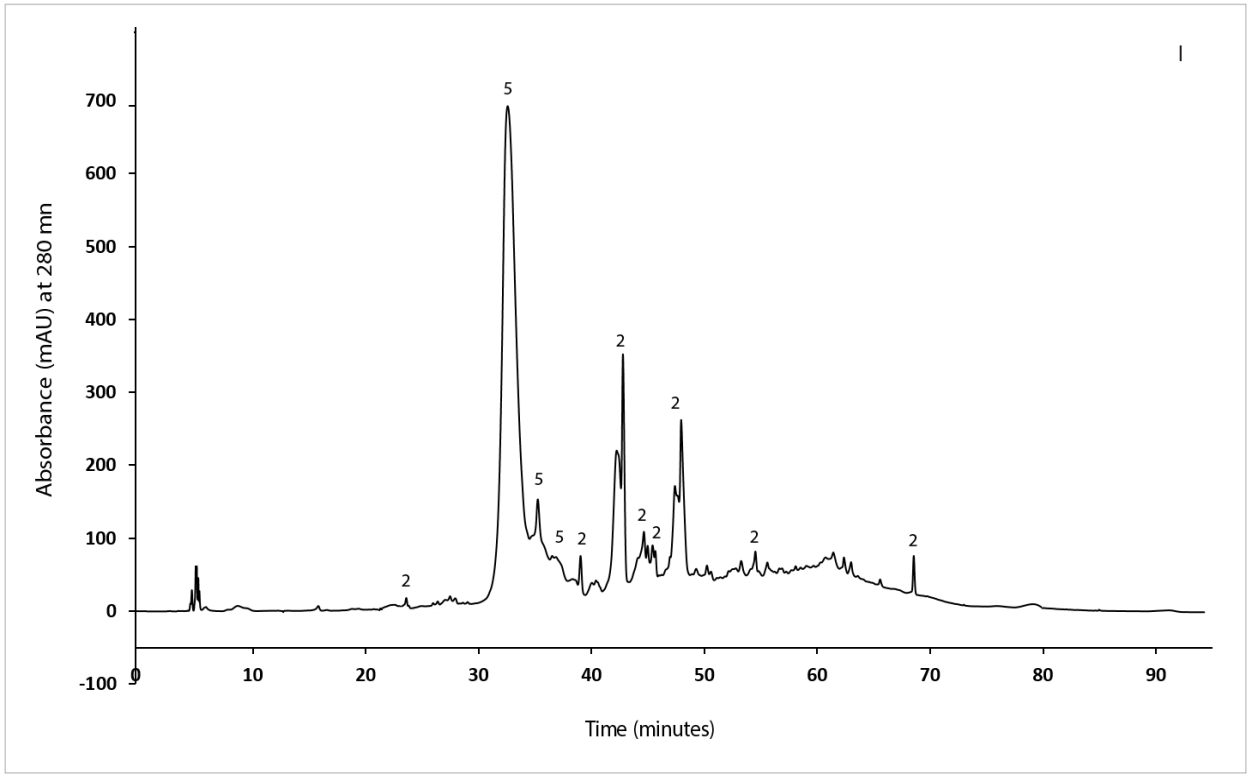

D
Absorbance (mAU) at 280 mn
Time (minutes)
1
1
1
1
1

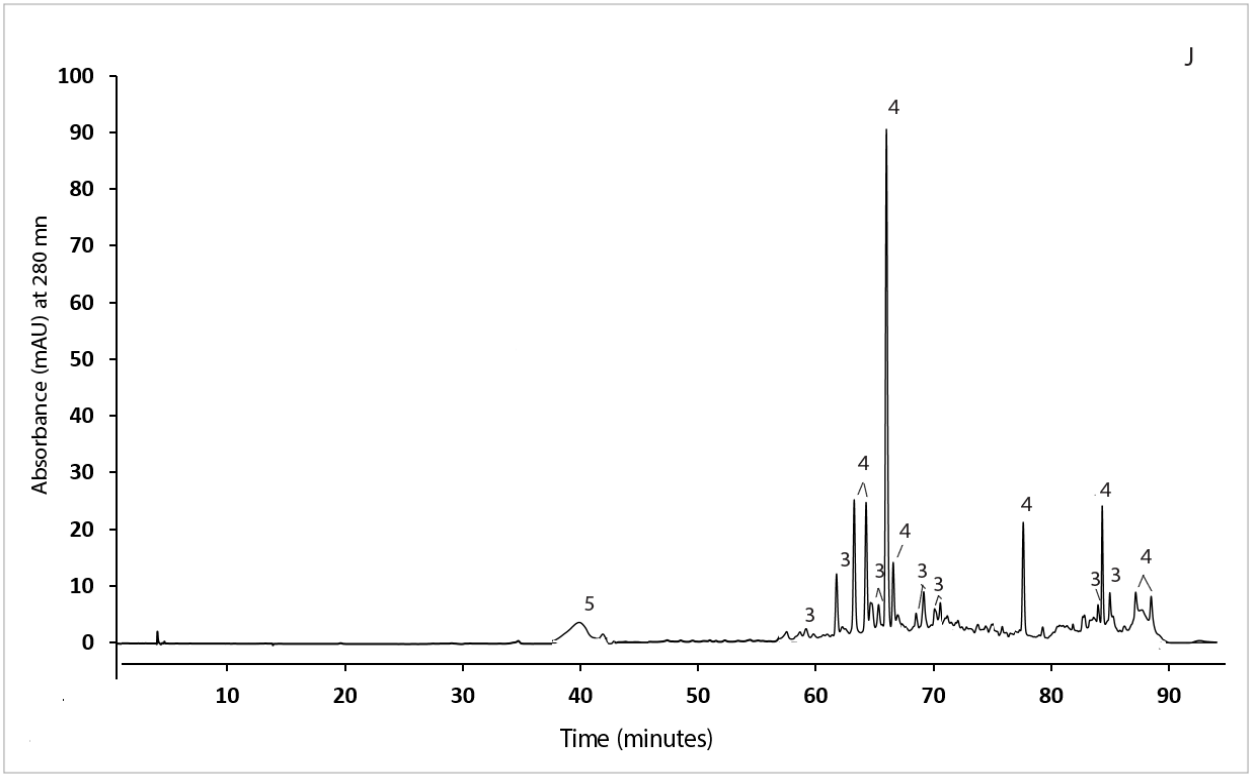

E
Absorbance (mAU) at 280 mn
Time (minutes)
1
1
1
1
1

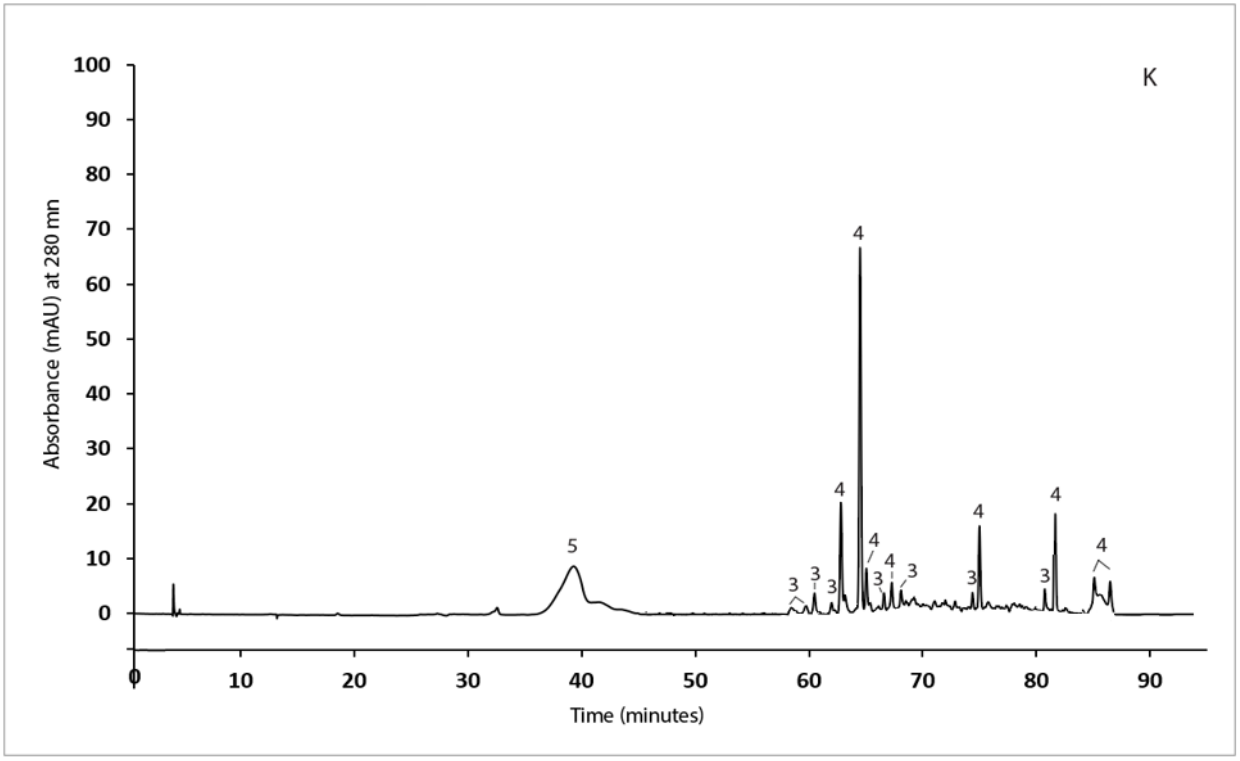

F
Absorbance (mAU) at 280 mn
Time (minutes)
1
1
1


H
Absorbance (mAU) at 280 mn
Time (minutes)
500
400
300
200
100
0
-100
0
10
20
30
40
50
60
70
80
90
5
2
2
2
5
5
2
2
2
2
2
2


G
Absorbance (mAU) at 280 mn
Time (minutes)
700
600
500
400
300
200
100
0
-100
0
10
20
30
40
50
60
70
80
90
5
2
2
5
5
2
2
2
2
2
2
2
2


I
Absorbance (mAU) at 280 mn
Time (minutes)


J
Absorbance (mAU) at 280 mn
Time (minutes)


K
Absorbance (mAU) at 280 nm
Time (minutes)
100
90
80
70
60
50
40
30
20
10
0
0
10
20
30
40
50
60
70
80
90
5
4
3

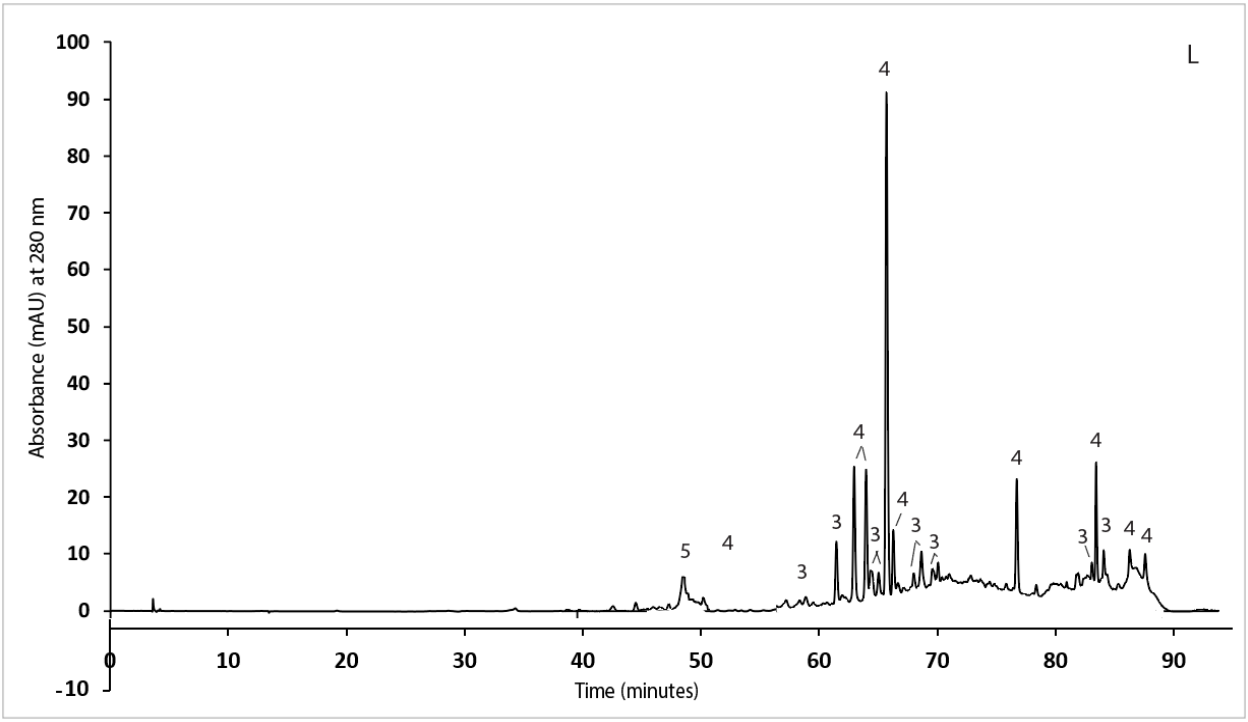


Figure 8.1 HPLC-PDA chromatograms showing the identification of peak phenolic subclasses produced from resin-ethanol fractionation of Martin and Pembina saskatoon berry varieties and wet pomace samples based on their UV-visible spectrums. Chromatogram identification: A-C (water fraction), A. Martin, B. Pembina, and C. wet pomace; D-F (40% ethanol), D. Martin, E. Pembina, and F. Wet pomace; G-I (70% ethanol), G. Martin, H. Pembina, and I. Wet pomace; J-L (100% ethanol), J: Martin, K. Pembina, and L. Wet pomace. Peak subclass assignments: 1. hydroxybenzoic acids; 2. hydroxycinnamic acids; 3. flavanols; 4. flavonols; 5. anthocyanins.