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Bioactive Phenolic and Fatty Acid Composition of Canadian Wild Gooseberry and Blackcurrant

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Abstract

Wild berry varieties are widely distributed in Canada and mainly used by the indigenous population as food. Gooseberries and blackcurrant are the two tart berries with strong taste that are used to make preserves such as jams, jellies, and wine. Phenolic compounds and fatty acids are the predominant bioactive metabolites present in these berries. Recently, researchers are more interested in exploring the fruits such as berries for functional properties that benefit human health. Although wild berries are known for their health benefits, research evidence related to profiling of bioactive molecules in these wild berries such as Canadian gooseberry and blackcurrant is limited. In this study we assessed and compared the bioactive phenolic and fatty acid composition in these two berries using liquid and gas chromatography and mass spectrometry. The cyanidine glucoside, which is a polyphenolic anthocyanin present in blackcurrant is significantly higher (832 µg/g Dry wt.) compared to that in Canadian gooseberry (150 µg/g Dry wt.). Linolenic acid (20.3 %) and linoleic acid (18.2 %) were the most abundant fatty acids in blackcurrant while linoleic acid (15.0 %) and palmitic acid (11.2 %) were abundant in gooseberry. Other fatty acids such as oleic acid, linoleic, and alpha linolenic acid, which are important in cardiovascular health, were present in both blackcurrant and gooseberry.

Keywords: Berries, Bioactive, Blackcurrant, Functional foods, Gooseberry.

Introduction

Maintaining health and disease prevention are major goals driving today's consumer food choices, and there is an ever increasing demand for "healthier" or "functional foods". By definition, functional foods are "foods or part of foods" that provide medicinal or health benefits beyond basic nutritional requirements (Adefegha, 2018; Gul, Singh & Jabeen, 2016). Fruits such as berries are an abundant source of bioactive molecules providing health benefits because of their high antioxidant, vitamin, mineral, polyphenol, lipid, and fiber content. The continuing demand for "functional foods" leads to food processors in search of edible crops with high quality. For example, fruits that have a significant amount

of bioactive compounds possess antioxidant, antimicrobial, anti-inflammatory, and anti-tumorigenic activities (Ono et al., 2020) and potentially have health promoting effects when consumed on a regular basis. The American Heart Association (AHA) recommends that adults eat four to five servings of fruit per day. However, a recent study showed that the higher prices of healthy food in rural and remote communities compared to urban centers, make healthy food "beyond the reach of many families" (Wright, 2019). With the rising cost of healthy foods, it is important to identify healthy and functional foods from wild varieties that are easily accessible to rural and remote communities. In addition, it has been suggested that access to traditional food such as wild berries would help to address the disproportionately high rates of indigenous populations food insecurity and the chronic diseases (Wright, 2019).

Out of 200 species of small, fleshy, wild berries currently found in Canada, native edible wild blackcurrants (Ribes americanum) are attractive true berries with a nice fall colored shrub (Figure 1A). About 14 species of currants are found in Canada belonging to the Ribes genus in the Saxifragaceae family. These berries are red or bluish or black in colour and the plants do not have thorns or prickles (Turner, 2009). Another one is the Canadian gooseberry (Ribes oxycanthoides in Grossulariaceae Family), a wild variety (Figure 1B) that grows across the boreal region of Canada from Hudson Bay to Alaska (Carey, 1995). These plants have thorns or prickles on their stems and the berries are reddish to dark purple in colour. Many indigenous populations collect and store these berries as a food source. For example, the Ojibwa communities use these berries along with sweet corn and turn them into preserves such as jelly. Although some studies are available on Indian gooseberries (Phyllanthus emblica), documented research on Canadian gooseberries is scarce.

Figure 1.
Canadian blackcurrant (wild variety) (A) and gooseberry (B) plants with ripened berries.

A: Source: http://www.hort.cornell.edu

B: Source: https://d2dvlr1w1gfn2q.cloudfront.net

The health benefits of fruits are attributed to their phenolic compounds, which are secondary metabolites of plant metabolism. The bioactive phenolics in various berries include flavanoids (anthocyanins, flavonols and flavanols), condensed tannins, hydrolysed tannins, stilbenoids, phenolic acids and lignans (Fatima et al., 2012). There is ample evidence to suggest an association of berry phenolics with health benefits. For example, epigallocatechin, epicatechin gallate, gallic acid, tannic acid, epicatechin gallate, geraniin, quercetin, and rutin have been reported to have anti-mutagenic properties (Bhargava & Westfall, 1969; Kumar et al., 2018). In addition, many lipid groups in berries (unsaturated fatty acids, sterols, terpenoids and others) have also demonstrated high biological activity and could potentially be important contributors in cardio-metabolic health. Berry seed oils are found to be concentrated with essential polyunsaturated fatty acids. Other bioactive compound including sterols and phenolic compounds are known to possess cardio-protective functions in aged rats (Charnock, Crozier & Wodhouse, 1994; Jurgoński et al., 2018).

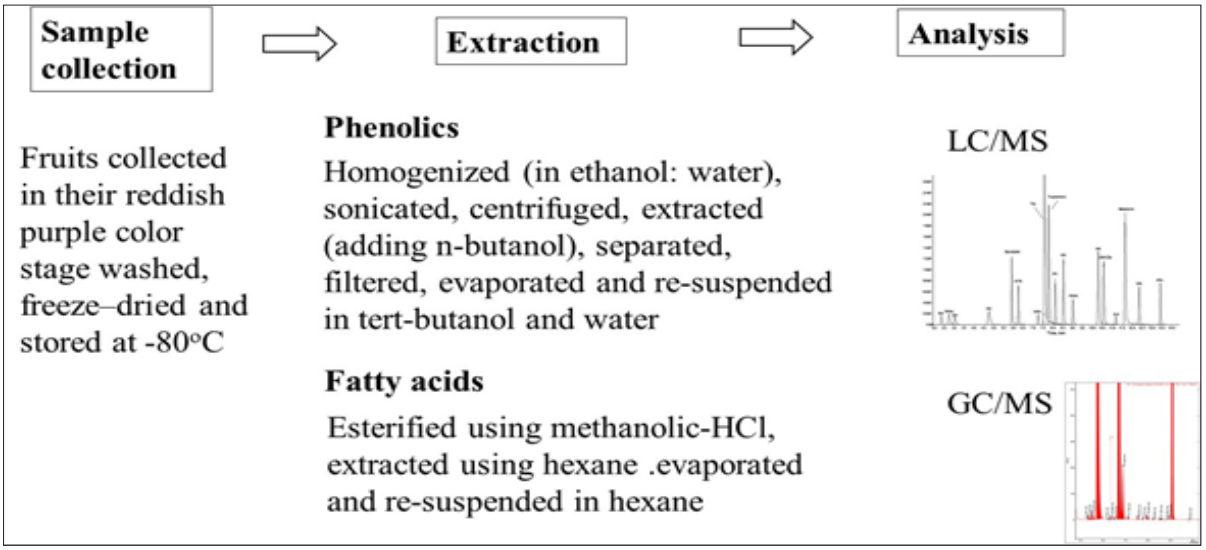
Parker et al., (2014) found that blackcurrant fruits contain two major anthocyanins, delphinidin and cyanidin. These two anthocyanins occurred as glucoside and rutinoside conjugates while other anthocyanins constituted a smaller fraction. The authors also noted the differences in anthocyanin contents between various cultivated blackcurrant varieties. Blackcurrants are also considered as a good source of lipids such as polyunsaturated fatty acids (Basegmez et al., 2017). For example, these authors confirmed lipophilic extracts of the berries were rich in polyunsaturated fatty acids (linoleic 46.89%, γ-linolenic 14.02%), while polar fractions showed a strong antioxidant capacity. Hence, further evaluation of not only cultivated varieties but also wild ones will help harness the full potential of these berry fruits that have promising health benefits. Investigating wild varieties also provide insights into unexplored germplasm for breeding purposes (Migicovsky & Myles, 2017). In this study we investigated the bioactive composition of Canadian gooseberry and blackcurrant with primary focus on phenolics and fatty acids compounds.

Materials and Methods

Sample collection

Ribes americanum and Ribes oxyacanthoides berry fruits were collected in their reddish and purple colour stage, respectively from various locations including parks and landscapes in Winnipeg, in the province of Manitoba, Canada. Fruits were washed, freeze–dried and stored at -80^{o}C within 24 hrs until further analysis. A composite sample was prepared by grinding the freeze-dried sample and the ground material was used for extraction of phenolic and fatty acid compounds (Fig. 2).

Figure 2.
Schematic diagram of the process of berry bioactive molecules analysis.



Sample extraction

Phenolic compounds

Metabolites from freeze-dried and homogenized samples were extracted using the methodology adopted from Hosseinian, Li & Hydamaka et al. (2007), Olas et al. (2018) and Rozalska, Sadowska & Zuchowski (2018). Briefly, sample material (2 g) was homogenized using a handheld homogenizer in a bath of 10 mL ethanol:water (80:20 v/v) in 50-mL centrifuge tubes. Then the extract was subjected to liquid-liquid extraction by addition of n-butanol and shaking the mixture vigorously. The mixture was dried by evaporating n-butanol and re-suspended in a solvent mixture of ethanol:water (80% v/v) and sonicated (1 h). Then the mixture was centrifuged (14000 rpm for 25 min) to separate the solvent and fruit material (pellet). The supernatant, containing phenolic compounds, was separated, filtered, and evaporated to complete dryness, using a rotatory evaporator. The dried residue was re-suspended in 2 mL ethanol: water (80:20 v/v). This constituted the polar fraction of phenolic compounds. The pellet from the earlier step of centrifugation, consisting of mainly non-polar metabolites was re-suspended in ethanol, and dried using a rotatory evaporator. The residue was re-suspended in a mixture of tert-butanol and water (non-polar fraction). Both polar and non-polar fractions together were fortified with labelled internal standards before analysis.

Fatty acids

Samples were extracted for fatty acids using a one-step extraction-methylation protocol adapted from (Carvalho, Teixeira & Brodelius, 2011, Vrinten et al., 2005) with some modifications. Briefly, fatty acids in freeze-dried and ground fruit material (200 mg) were esterified using methanolic-HCl (2 mL of 3 molar/L) by incubating at 85°C for 90 min. Methylated fatty acids esters were extracted in two steps using hexane (2 mL in each step)

under dark conditions. The hexane layer (top layer) was quantitatively transferred to a new disposable glass tube and evaporated to dryness using sample concentrator (Vacufuge Plus, Eppendorf AG, Hamburg, Germany). The dried extract was re-suspended in hexane (1 mL) and vortexed before transferring into a GC vial for analysis.

Quantification of phenolic compounds

Sample extracts were analyzed using high resolution mass spectrometer (Orbitrap ID-X Tribrid Mass Spectrometer, Thermo Fisher Scientific, Mississauga, Canada) in positive mode, coupled with ultra-high performance liquid chromatography (UHPLC-HRMS) (Vanquish, Thermo Fisher Scientific, Mississauga, Canada). Separation of analytes was achieved using a reverse-phase biphenyl column (particle size 2.6 µm, 100 x 2.1 mm, Kinetex, Phenomenex, USA) held at 35^{o}C. Gradient elution was achieved with 100% water (mobile phase A) and 100% acetonitrile (mobile phase B) both containing 0.1 % formic acid at a flow rate of 0.25 mL min-1 with a runtime of 20 min. The Extract was subsequently passed through heated electrospray ionization. An orbitrap acquisition method with full scan for m/z 150 to 800 at 120000 resolution followed intensity and data dependent MS2 fragmentation at 30000 resolution was used. A stepped higher-energy C-trap dissociation collision energies at 15, 25, and 35 % were used to achieve fragmentation. Method detection and quantification limits ranged from 0.1 to 0.25 ng mL-1 and 0.25 to 0.5 ng mL-1, respectively. Five points (0.1 to 200 ng mL-1 calibration curves were prepared using a mixture of pure analytical standards for quantification of analytes in the extracts. Identification and quantification of analytes was performed using TraceFinder (Software version 4.1, Thermo Fisher Scientific).

Quantification of fatty acids

The fatty acid composition of the sample extracts was determined using gas chromatography (Bruker 436-GC (Bruker Daltonics, Germany) coupled with flame ionization detector as methyl esters of fatty acids (FAMEs). Fatty acid methyl esters were separated on Rt-2560 capillary column (100 m x 250 µm x 0.20 µm) (Restek, USA). Helium was used as the carrier gas with a flow rate of 0.8 mL min-1. The oven was programmed with an initial temperature of 100°C for 4 min ramping up to 250°C at a rate of 3°C min-1 and held for 8 min. Identification of FAMEs was carried out by comparing the retention times with those of the analytical calibration standard mix (GLC Reference standard:463, Nu-Chek-Prep Inc, MN, USA) and results were calculated as a percentage of the calibration standard.

Statistical analysis

Data was analyzed using PROC GLM in SAS.

Results and Discussion

Analysis of phenolic composition

Concentrations of various phenolic bioactive compounds in blackcurrant and gooseberry were determined semi-quantitatively using UHPLC-HRMS (Table 1). Black currents are known to have a high concentration of flavonoids, specifically anthocyanins which provide the fruits with their purple color (Archaina et al., 2018). These winter hardy berries are considered as a rich source of phytochemicals that are potent antioxidants, antimicrobials, and have anti-inflammatory

properties (Nour et al., 2013). In our study, we detected important bioactive polyphenolic compounds in both of these Canadian wild berry types. Although most of the phenolic compounds were detected in small quantities in these two berries, the cyanidine glucoside content was abundant in both, with significantly higher amounts in blackcurrant than gooseberry (Table 1). It has been reported that cyanidine glucosides possess significant antioxidant, antidiabetic, anti-inflammatory, and cytoprotective effects against various oxidative stress-induced disorders enhancing health benefits in humans (Rahman et al., 2021).

Table 1.

Concentrations of phenolic and anthocyanin compounds of selected blackcurrant and gooseberry fruit extracts (composite samples of each) quantified using high resolution mass spectrometry coupled with liquid chromatography.

Bioactive compound	**Black current** (µg/g Dry wt.)	**Gooseberry** (µg/g Dry wt.)
Caffeic Acid	< LOQ*	< LOQ
Ferulic Acid	< LOD#	1
Chlorogenic Acid	< LOD	< LOD
Sinapic acid	< LOD	1
Benzoic acid derivatives:		
Gallic acid	1	< LOD
Flavone:		
Vitexin	< LOD	< LOD
Isoflavone:		
Genistein	< LOD	< LOD
Flavonols:		
Kaempferol	< LOD	< LOD
Quercetin	3	1
Rutin	15	20
Anthocyanins:		
Cyanidine glucoside	832	150
Luteolin	< LOD	< LOD
Myricetin	< LOD	< LOD
Pelargonidin chloride	< LOD	< LOD
Petunidin 3-O-glucoside chloride	< LOD	ND$

* LOQ: Limit of quantification = 2.5 ng/mL in extract.

LOD: Limit of detection = 1 ng/mL in extract.

$ ND: Not detected.

Analysis of fatty acid composition

Canadian wild berries are a good source of health promoting lipids especially the unsaturated fatty acids (Szakiel et al., 2012). For example, the seed oil of Canadian-grown sea buckthorn berry cultivars contains high levels of linoleic acid and α-linolenic acid in a close to 1:1 ratio (Fatima et al, 2012). The mono- and poly-unsaturated fatty acids in Canadian blackcurrant and gooseberry fruits include palmitoleic acid, oleic acid, vaccenic acid, linoleic acid, linolenic acid, eicosatrienoic acid, and eicosapentaenoic acid (Table 2). Interestingly, blackcurrants have only few types of saturated fatty acids compared to gooseberries. For example, arachidonic acid, docosanoic acid, and lignoceric acid were not detected in blackcurrant (Table 2). According to USA Food and Drug Administration report (2018), replacing saturated fat with similar amounts of unsaturated fats may reduce the risk of heart diseases. Therefore, wild blackcurrant will be a good source of unsaturated fatty acids.

Table 2.

Fatty acid composition of selected blackcurrant and gooseberry extracts (composite samples of each) using gas chromatography coupled with flame ionization detector quantified as fatty acid methyl esters. Values represent mean of 3 samples + standard deviation).

Fatty acid		Blackcurrant	Gooseberry
Composition	Name	Mean ± sd	Mean ± sd
C16:0	palmitic acid	15.6 ± 1.7	11.2 ± 1.1
C16:1	palmitoleic acid	2.9 ± 0.2	ND*
C18:0	stearic acid	1.4 ± 0.1	0.8 ± 0.1
C18:1n9	oleic acid	12.5 ± 1.9	8.6 ± 0.9
C18:1n7c	vaccenic acid	3.6 ± 0.4	0.8 ± 0.0
C18:2n6	linoleic acid	18.2 ± 2.1	15.0 ± 1.6
C18:3n3	linolenic acid	20.3 ± 1.1	1.1 ± 0.1
C20:0	arachidonic acid	ND	1.0
C20:5	eicosapentaenoic acid	1.5 ± 0.1	2.8 ± 0.8
C20:3n3	eicosatrienoic acid	1.0 ± 0.0	2.1 ± 0.4
C22:0	Docosanoic acid	ND	0.9 ± 0.5
C24:0	lignoceric acid	ND	2.7 ± 0.7

ND - (not detected)

It would be useful to assess and compare both wild and commercially produced cultivars of blackcurrants and gooseberries for their bioactive composition. Most importantly, a comparative study between wild and cultivated varieties will help explore the untapped potential of wild berries in terms of health benefits to humans. Recent studies have shown a link between the consumption of fruits rich in polyphenolic compounds and

lipids, with reduced incidence of chronic and degenerative diseases, such as cardiovascular disease, cancer, and neurological disease (Ono et al., 2020; Cortez & Gonzalez, 2019). These studies may potentially support the use of Canadian wild berries as a good source of antioxidant bioactive compounds that promote predicted health benefits. Various potential health benefits of these berries were summarized in Figure 3 (Mandl, 2019; Link, 2020). Furthermore, characterization of these berries for antioxidant activity, bio-accessibility, and bioavailability studies will improve the value of these two types of wild berries to harness their full potential as functional foods.

Figure 3.

Potential benefits of Canadian Blackcurrants (A) and Gooseberries (B) (Mandl, 2019; Link, 2020.

A

- Fight cancer and premature aging
- Stave off inflammations and neurological disease
- Rich in antioxidants to fight harmful free radicals
- Strengthen the immune system
- Maintain integrity of mucus membranes and skin
- Promote optimal eye health
- Enhance metabolism and enrich blood cells

B

- High content of antioxidants
- Help the body fight cancer
- Good for type II diabetes, aging, and heart diseases
- Play a key role in boosting healthy aging
- Improved memory
- Better health of the urinary tract

Conclusions

Wild blackcurrant and gooseberries grown in Winnipeg, Manitoba, Canada could be very good sources of bioactive phenolics and fatty acids with potential health benefits. Comprehensive and broader characterization of bioactive molecules of these berries would benefit in understanding the relative bioactive composition changes and the influence of environmental conditions/geographic areas on bioactives. In addition, a comparison of the wild varieties with the cultivated ones would help harness the benefits by providing valuable information in the breeding process.

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