A comprehensive metabolomic-assisted investigation of bioactive phenolic and lipophilic compounds in underutilized Canadian wild berries

by

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Abstract

Prairie berries are cold hardy fruits consumed by Canadians for their perceived health benefits. Phenolic compounds, fatty acids, phytosterols and terpenes are important groups of bioactive molecules present in berries. Assessment of the bioactive compounds is essential to identify their potential as functional food. The objective of this study was to comprehensively examine the contents of phenolic compounds, fatty acids, phytosterols and terpenes content of fourteen different berries grown in prairies. The ultra-high performance liquid chromatography-high resolution mass spectrometry (UHPLC-HRMS) method was developed and used for the comprehensive and simultaneous analysis of 66 phenolic compounds in fourteen different types of Canadian wild berries. Gas chromatography-mass spectrometry (GC-MS) was used to analyze the fatty acids, phytosterols and terpenes in the aforementioned berries. *In vitro* lipid peroxidation was tested in all the selected wild berry types. Principal component analysis and analysis of variance were performed to identify the significance of the results. Wild grapes were rich in phenolic compounds such as resveratrol, while gooseberries were rich in isoquercetin and *p*-coumaric acid. Saskatoon berries were rich in chlorogenic acid. Rutin and chlorogenic acid were the most abundant phenolic compounds in chokecherry. Essential fatty acids such as linoleic and α-linolenic acids were found in wild grapes, buckthorn and Saskatoon berries. Predominant phytosterols and terpenes identified in Canadian wild berries included β-sitosterol, isofucosterol, phytol, and α-amyrin. The novel UHPLC-HRMS method for phenolic compounds and a GC-MS method for lipophilic compounds proved that the underutilized wild berries consist of unique and beneficial phenolic compounds, essential fatty acids, phytosterols and terpenes. As a source of these important bioactive compounds, these berries have the potential to function as antioxidants and antihypertensive agents. The information from this study will help in finding applications for underutilized prairie berries as potential sources of functional food in the neutraceutical and pharmaceutical industries.

Keywords: Prairie berries, bioactives, UHPLC-HRMS, GC-MS, phenolic compounds, fatty acids

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Dedication

This thesis is dedicated
To my beloved mother and father,
Priyanthi Kodikara and Upul Kodikara,
To my husband, Rockshan, for their boundless love and remarkable dedication that have shaped me into the person I am today, as well as for their affection and enduring love…

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List of Abbreviations

UHPLC-HRMS: Ultra High Performance Liquid Chromatography High Resolution Mass Spectrometry

GC-MS: Gas Chromatography Mass Spectrometry

LOD: Limit of Detection

LOQ: Limit of Quantification

SFA: Saturated Fatty Acids

MUFAs: Monounsaturated Fatty Acids

PUFAs: Polyunsaturated Fatty Acids

PCA: Principal Component Analysis

CA: Cluster Analysis

Chapter 1. Introduction

The relationship between bioactive molecules and their physiological effects within living organisms has been the subject of extensive research, particularly in promoting health and preventing diseases (Kodikara et al., 2023). Among the diverse bioactive compounds, phenolic compounds are identified as major contributors, especially in berries, where they occur as plant secondary metabolites (Rossi et al., 2022). Fatty acids in berries play a role in their nutritional and functional attributes because these compounds protect the human body from free radicals and active oxygen species, provide energy, support cell membrane structure, and have been associated with various health benefits, including anti-inflammatory properties and cardiovascular health even if they are present in minor quantities (Orsavova et al., 2015). Phytosterols are another group of bioactive compounds that have gained attention for its potential health benefits apart from the fatty acids in berries (Piironen et al., 2003; Yang et al., 2001). Terpenes are another class of plant secondary metabolites and complex compounds originating from squalene, a basic linear hydrocarbon (Kupska et al., 2016). These compounds are commonly found in various plant species and are known for their wide range of structural variations and multiple beneficial effects in biological systems. In pharmacological studies, these phytochemicals have been associated with antioxidant activity, which can help reduce the risk of chronic diseases and protect cells from oxidative damage (Szakiel et al., 2012). Berries, known for their high antioxidant capacities, have garnered significant attention due to their potential health benefits, leading to their classification as functional foods. These functional foods are crucial in promoting health, preventing diseases, and supplying essential nutrients such as carbohydrates, vitamins, proteins, and lipids (Shahidi & Ambigaipalan, 2015).

Recent scientific findings highlight the protective role of antioxidants against diseases such as diabetes, cancer, neurodegenerative, and cardiovascular diseases. Berries, in particular, emerge as functional fruits rich in essential micronutrients, notably folic acid and ascorbic acid (vitamin C) (de Souza et al., 2019). Their potential as antioxidants, anti-inflammatory agents, and anticarcinogenic sources further underline their significance in promoting human health. Phenolic compounds in berries are diverse, including phenolic acids, flavonoids, tocopherols, cinnamic acid derivatives, lignans, coumarins, tannins, and stilbenes (Shahidi & Ambigaipalan, 2015). Berries

also contain diverse fatty acids, including essential fatty acids such as α-linolenic acid and linoleic acid. Long-chain omega-3 fatty acids such as docosahexaenoic acid (DHA, 22:6n-3) and eicosapentaenoic acid (EPA, 20:5n-3) can be synthesized from α-linolenic acid, which is known to reduce the risk of heart diseases, hypertension, autoimmune disorders, and cancer (Parry et al., 2005). Phytosterols have been associated with reduced LDL (low-density lipoprotein) cholesterol levels, making them important for individuals concerned about heart health (Ogbe et al., 2015). In pharmacological studies, phytochemicals such as terpenes have been associated with antioxidant activity, which can help reduce the risk of chronic diseases and protect cells from oxidative damage (Szakiel et al., 2012).

However, analyzing these bioactive compounds in berries presents challenges due to their structural complexity, low concentrations, and vast diversity. Various methods, such as high-performance liquid chromatography (HPLC) coupled with ultraviolet-visible (UV-Vis), fluorescence, and mass spectrometry (MS), have been employed to identify and quantify phenolic compounds in berries. While UV-Vis and fluorescence spectroscopy offer advantages, mass spectrometric methods, particularly HPLC coupled with MS, provide greater selectivity and sensitivity. Recent advances in mass spectrometry, including ultra-high performance liquid chromatography mass spectrometry (UPLC-ESI-MS/MS) and high-resolution mass-spectrometry (UHPLC-HRMS), have improved accuracy, enabling the identification and quantification of a wider range of phenolic compounds (Robbins, 2003).

Despite these advancements, challenges persist, especially in the context of underutilized Canadian prairie small fruits. Existing literature predominantly focuses on well-known commercial varieties, leaving a knowledge void regarding the bioactive composition and potential health benefits of Canadian prairie-grown berries (Kodikara et al., 2023). This research aimed to address this gap by conducting targeted metabolomics and chemometric analysis of underutilized prairie fruits, providing insights into the diversity of phenolic compounds and their potential health-promoting aspects.

The primary objectives of this study involved exploring the bioactive compounds composition of underutilized Canadian prairie small fruits, understanding the diversity of these compounds, and

paving the way to explore the potential health benefits of these berries. The research also investigated the challenges of analyzing these compounds, emphasizing the development of innovative methods to overcome limitations related to structural complexity, low concentrations, and matrix effects using UHPLC-HRMS. Additionally, the investigation extended beyond phenolic compounds to explore fatty acid profiles, phytosterol content, and terpene composition in underutilized Canadian berries. The utilization of Gas Chromatography-Mass Spectrometry (GC-MS) in this comprehensive exploration aimed to offer a more thorough perspective on the nutritional value and health-promoting potential of these berries, paving the way for informed dietary choices and innovative food product development in the nutraceutical and pharmaceutical industries.

Chapter 2: Literature Review:

Canadian prairie berries: Bioactive compounds and their potential health benefits

A version of this chapter has been published in Food Reviews International Journal:

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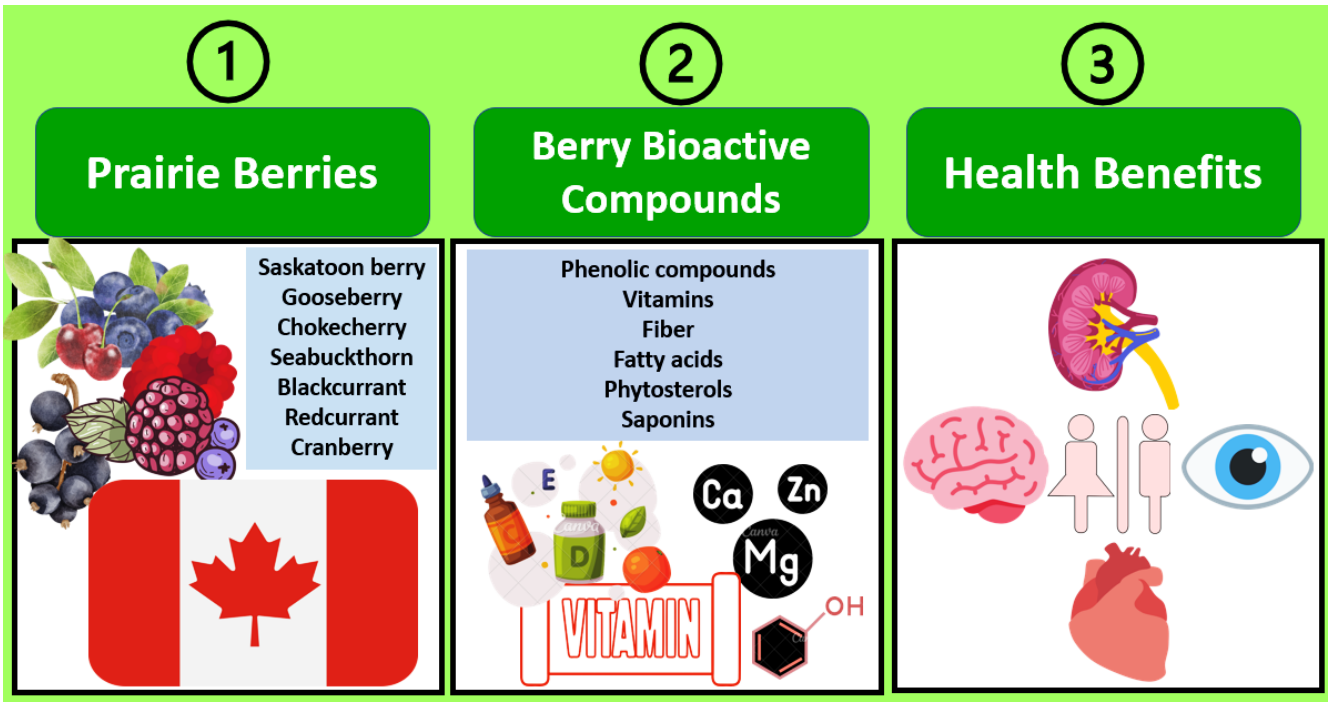
2.1 Abstract

The Canadian prairies are home to several underutilized berries, including *Vitis riparia* (wild grape), *Prunus virginiana L* (chokecherry), *Ribes hirtellum* (gooseberry), and *Amelanchier alnifolia L* (Saskatoon berry). These berries are traditionally consumed due to their perceived health benefits and are known for their ability to thrive in cold climates. One of the key reasons for their health benefits is the presence of phenolic compounds, which are one of the bioactive molecules found in berries that promote good health. Each berry species contains a diverse array of phenolic compounds such as anthocyanins, flavonols, flavan-3-ols, and proanthocyanidins, among others. These phenolic compounds contribute to the distinct flavors, colors, and aromas of the berries. Phenolic compounds are known for their high antioxidant activity, and there has been growing interest in identifying their potential health benefits. The consumption of these berries has been traditionally linked to perceived health benefits, and emerging scientific evidence supports their potential as functional foods. Studies have shown that these prairie berries may have anti-inflammatory, anti-cancer, anti-diabetic, anti-neurodegenerative, and cardiovascular health-promoting effects, among others. Additionally, their high antioxidant activity may help to reduce oxidative stress and protect against cellular damage, which could contribute to the prevention of degenerative diseases. Therefore, this review aims to provide an overview of the types of berries that are grown in the Canadian prairies, their bioactive compounds, and the related health benefits they may offer.

Keywords: Prairie berries, phenolic compounds, fatty acids, antioxidants, health benefits

Graphical Abstract



Author Contribution

Planning and supervision C.W., N.B., and T.N.; writing, and original draft preparation, and editing C.K.; review and editing, C.K., C.W., N.B., and T.N.

2.2 Introduction

Bioactive molecules are known to have physiological effects when present in a living organism. They can initiate physiological benefits such as promoting health and preventing diseases (Gnanavinthan, 2013a; Skinner & Hunter, 2013). Phenolic compounds are considered one of the major bioactive molecules found in berries, occurring as plant secondary metabolites. Due to their high antioxidant capacities, there has been an increasing interest in identifying their potential health benefits and their presence in functional foods (Khattab et al., 2015; Kylli, 2011a). Functional food can promote health, prevent disease, and provide essential nutrients to the human body, such as carbohydrates, vitamins, proteins, and lipids (Adefegha, 2018). As the name implies, functional food contains bioactive molecules consumed as a part of the normal diet (Marjanovic et al., 2021; Yan et al., 2022). Recent findings have shown that diets rich in antioxidants can protect humans against diseases such as diabetes, cancer, and neurodegenerative and cardiovascular diseases (Olas, 2018). Berries are functional fruits high in essential micronutrients, particularly folic acid and ascorbic acid (vitamin C) (Hosseinian & Beta, 2007). These soft fruits have a wide range of capacities to act as a potential antioxidant, anti-inflammatory, and anticarcinogenic source to provide health benefits to the human body.(Olas, 2018) Phenolic compounds are grouped into phenolic acids, flavonoids, tocopherols, cinnamic acid derivatives, lignans, coumarins, tannins, and stilbenes (Gnanavinthan, 2013b; Olas, 2018). Berries contain various phenolic acids: hydroxybenzoic acid and hydroxycinnamic acid derivatives. In addition, berries such as blueberries and cranberries contain high levels of proanthocyanidins. Among the berries, for instance, raspberries, blackberries, and strawberries are high in ellagitannins (Gnanavinthan, 2013b). Proanthocyanidins are present at higher levels in blueberries and cranberries, and epicatechin and catechin have been identified as the main flavonols present in berry fruit. In blueberry, lingonberry, raspberry, cloudberry, and strawberry, lignans are predominant compared to the other berries.

They are essential to consumers, mainly because of the presence of their fruit colour, nutrition, texture, size, shape, and uses (Isaak et al., 2015). Most berries are naturally grown in Canadian prairie provinces, Manitoba, Alberta, and Saskatchewan, and also in the northwestern side of the United States (Liu et al., 2018). These are consumed by people when they become sweeter, especially in the summer or fall seasons. Jams, jellies, pies, and wine are the most famous preserves

of berries with a more pungent taste. The predominant bioactive phytochemical groups present in berries are polyphenols, including anthocyanins. According to the literature, wild berries contain more antioxidants than cultivated berries (Hosseinian et al., 2007). Non-polar compounds such as triterpene hydroxycinnamates, ursolic acid, and β-sitosterol are reported to be present in berries (Ono et al., 2020). Despite fruit seeds being higher in functional lipids such as phytosterols, berries contain minor quantities of functional lipids (Fatima et al., 2012). Very few studies have examined different traditional prairie berries (Bushcraft., 2023). Thus, minimal research evidence is known about their potential health benefits as a functional food. Based on previous studies, this review extends the knowledge of prairie-grown berries, their bioactive composition, and their potential health benefits as a functional food.

2.3 Prairie berries: classification, geographical distribution and characteristics

Prairie berries are a group of small fruits that are native to North American prairies. They belong to the genus *Amelanchier* in the rose family (Rosaceae). They are particularly abundant in the Great Plains region of the United States and Canada, where they grow in open woodlands, prairies, and along stream banks. Berries are classified into several groups based on the botanical characteristics of the fruit, such as the arrangement of the flower parts, the structure of the fruit wall, and the number of seeds. Blueberries and cranberries are grouped under epigynous fruits; Saskatoon berries are identified as pomes; cherries and buffalo berries are classified as drupes; black currant, gooseberries, and elderberries are identified as true berries, and strawberries, raspberries, and blackberries are grouped as aggregate fruits. **Figure 2.1** and **Table 2.1** summarize the chemical and sensory attributes of these different types of berries. Among these soft fruits, the lowbush wild berries are native to Alberta, Canada, but they are naturally grown in all provinces because of their hardiness (Frazier, 2022). Highbush cranberry (*Viburnum opulus var. americanum*) is native to Alberta and produces cluster-type fruits. The appearance and the taste of this berry type led to identifying it as a type of cranberry even though it is not an actual type of cranberry (The Canadian Encyclopedia, 2023). However, the two plants are very different. Although both are native to North America, the highbush cranberry is a member of the *Viburnum, Caprifoliaceae,* or honeysuckle family, as opposed to the "true" or highbush blueberry, which is a member of the *Ericaceae, Vaccinium* family (Frazier, 2022).

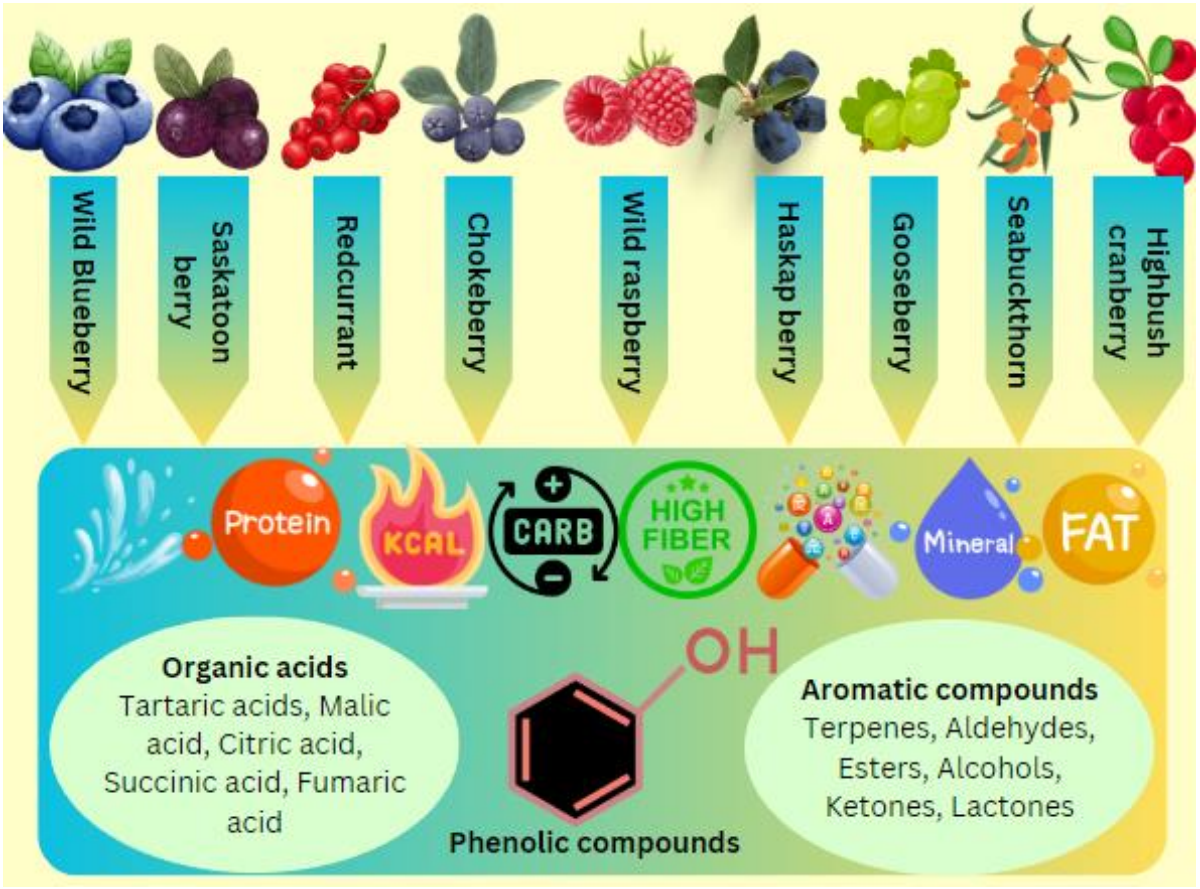


Figure 2.1 Chemical composition of berries

Table 2.1 Canadian berries and their chemical, and sensory properties and their applications in the food industry

Berry	Chemical Composition	Sensory Properties and Fruit Quality	Application in the food industry	References
Saskatoon berry (*Amelanchier alnifolia L*)	Water (%)- 80, Protein (%)- 9.7, Fat (%)- 4.2, Fiber (%)- 19.0, Calcium (%)- 0.44, Phosphorous (%)- 0.16, Potassium (%)- 1.22, Magnesium (%)- 0.20, Sulfur (%)- 0.059, Iron (ppm)- 67.03, Sodium (ppm)- 31.83, Manganese (ppm)- 67.50, Copper (ppm)- 7.23, Zinc (ppm)- 16.50, Barium (ppm)- 34.76, Molybdenum (ppm)- 0.38, Aluminium (ppm)- 74.45, Carotene (ppm)- 29.70, pH- 3.65-4.18	These are round, deep-blue, reddish-purple or black fleshy fruits. They are sweet, tangy, earthy, or acidic in taste.	Baked goods, dried berries, frozen berries, syrups and jams	(Mazza, 1982; More, 2023)
Gooseberry (*Ribes hirtellum*)	Water (%)- 87.87, Energy- 184 kJ (44 kcal), Carbohydrates- 10.18 g, Dietary fiber- 4.3 g, Fat- 0.58 g, Protein- 0.88 g, Vitamins (2%)- Thiamine (B1), Riboflavin (B2), Niacin (B3), Pantothenic acid (B5), Vitamin B6, Folate (B9), Vitamin C, Vitamin E, Minerals- Calcium (3%), Copper (4%), Iron (2%), Magnesium (3%), Manganese (%), Phosphorus (4%), Potassium (4%), Sodium (0%), Zinc (1%), pH- 2.8-3.8	Unripened berries are green in colour but ripened berries are red-purple. They are sweet-tart and sour.	Yogurt, wine, jellies and jams, wine, and fresh juice products	(Gooseberry, 2023; Gooseberry - Wikipedia, 2023)

Black chokecherry (*Prunus virginiana*)	Total Calories- 249 kcal, Vitamin A- 258.72 IU, Vitamin C- 8.47 mg, Vitamin K- 32.494 mcg, Calcium- 92.4 mg, Magnesium- 41.58 mg, Potassium- 583.66 mg, Phosphorus- 103.18 mg, Total Carbohydrates- 51.7748g, Sugars- 14.4298g, Dietary Fiber- 30.8g, Total Fat- 2.6026g, pH- 3.0-4.0	The colour of these berries ranges from dark red to deep purple. They are bitter and astringent in taste.	Wine, pancake syrup and jelly	(Choke Cherry , 2023; Nutrition Facts, 2023)
Wild grapes (*Vitis riparia*)	Water- 74.796 g, Energy- 61.740 kcal, Protein- 0.580 g, Lipid (fat)- 0.322 g, Ash- 0.524g, Carbohydrate- 15.778 g, Fibre- 0.920 g, Vitamin C- 3.680 mg, Vitamin A- 92.00 IU, Calcium- 12.880 mg, Iron- 0.267 mg, Magnesium- 4.6 mg, Phosphorus- 9.2 mg, Selenium- 0.184 mg, Potassium- 175.72 mg, pH- 3.0-3.7	These are dark purple colour fruits when ripened and have a sweet-tart flavour	Grape Vinegar	(The Forager's Guide to Wild Grapes, 2023; Recipes and Facts, 2023)
Blackcurrant/ Redcurrant (*Ribes nigrum/ Ribes rubrum*)	Water- 82g, Energy- 63kcal, Protein- 1.4g, Total lipid (fat)- 0.41g, Ash- 0.86g, Carbohydrate-15.4g, Calcium- 55mg, Iron- 1.54mg, Magnesium- 24mg, Phosphorus- 59mg, Potassium- 322mg, Sodium- 2mg, Zinc- 0.27mg, Copper- 0.086mg, Manganese- 0.256mg, Vitamin C, total ascorbic acid- 181mg, Thiamin- 0.05mg, Riboflavin- 0.05mg, Niacin- 0.3mg, Pantothenic acid- 0.398mg, Vitamin B-6- 0.066mg, Vitamin A-	The sweetest type is the blackcurrant while redcurrants are a bit sweet and bracingly tart.	Fresh frozen berries, jams, jellies and fresh juice	(St-Pierre et al., 2005; Americas Restaurant, 2023)

	12µg, Vitamin E (alpha-tocopherol)- 1 mg, Fatty acids (total saturated)- 0.034g, Fatty acids (total monounsaturated)- 0.058g, Fatty acids (total polyunsaturated)- 0.179g, pH- 2.8-3.5			
Haskap berry (*Lonicera caerulea*)	Protein- 21.2%, Carbohydrate- 0.86%, Fat- 0.01%, Ash- 0.45%, Crude fiber- 8.34%, Mineral elements (Potassium- 1777 mg/100g DW, Magnesium- 116mg/100g DW, Calcium- 2266 mg/100g DW, Sodium- 863 mg/100g DW, Iron 2909 µg/100g DW, Manganese 1301 µg/100g DW, Zinc 998 µg/100g DW, Copper 688 µg/100g DW), pH- 3.0-4.5	These berries are purple colour/dark blue. They have a tart/sweet and juicy taste.	Tea, juice, antioxidant capsules, and dried berry products	(Cheng et al., 2022)
Wild raspberry (*Rubus idaeus*)	Water- 85.8 g, Energy- 220 kJ (53 kcal), Carbohydrates- 11.94 g, Fat- 0.65 g, Protein- 1.2 g, Vitamins (Thiamine (B1)- 0.032 mg, Riboflavin (B2)- 0.038 mg, Niacin (B3)- 0.598 mg, Pantothenic acid (B5)- 0.329 mg, Vitamin B6- 0.055 mg, Folate (B9)- 21 µg, Choline- 12.3 mg, Vitamin C- 26.2 mg, Vitamin E- 0.87 mg, Vitamin K- 7.8 µg), Minerals (Calcium- 25 mg, Iron- 0.69 mg, Magnesium- 22 mg, Manganese- 0.67 mg, Phosphorus- 29 mg, Potassium- 151 mg, Zinc- 0.42 mg), pH- 3.0-3.7	These berries have a dark purple, dark pink or bright red colour. These berries have a tart and sweet taste.	Jams, wine, fruit leather, pies, muffins, smoothies, cakes and ice cream	(Vara et al., 2020; Raspberries, 2023)

Wild blueberry (*Vaccinium angustifolium*)	Water (%)- 83.3, Protein (%)- 4.9, Fat (%)- 2.4, Fiber (%)- 8.8, Calcium (%)- 0.08, Phosphorous (%)- 1.44, Potassium (%)- 0.54, Magnesium (%)- 0.041, Sulfur (%)- 0.034, Iron (ppm)- 50.15, Sodium (ppm)- 46.01, Manganese (ppm)- 35.2, Copper (ppm)- 11.4, Zinc (ppm)- 20.6, Barium (ppm)- 4.4, Aluminium (ppm)- 26.72, Carotene (ppm)- 29.30, pH- 3.3-4.5	These are round fruits with a blue-black colour. They have a flavorful, sweetness.	Supplement powder, freeze-dried raw berries, juice, fruit toppings, jelly and jam	(Sun et al., 2019; Nutrition Advance, 2023)
Chokeberry (*Aronia melanocarpa*)	Dry matter- 17-29 %, Water-insoluble material- 5-10%, Dietary fibre- 5.62 g/100 g fresh weight (FW), Organic acids- 1 – 1.5 % FW, Sugar- 16-18 %, Fat- 0.14 g/100 g FW, Protein- 0.7 g/100 g FW, Minerals and vitamins- 440 mg/100 g, Aroma components (amygdalin): 20.1 mg/100 g FW, pH- 2.5-3.5	These berries can be red, purple or orange. They have a bitter-sweet taste.	Gummies, beer, syrup, wine, jam, juice, tea, soft spreads, extracts, salsa, ice cream and tinctures	(Kulling & Rawel, 2008)
Sea Buckthorn (*Hippophae rhamnoides*)	Water- 70.6–76.9% fresh weight (FW), Solid and ash content- 8.5%–33.8% FW, Carbohydrates- 0.48%–2.87% FW, Proteins and amino acids- 0.4% to2.5% FW, Free amino acid- 0.77% to 2.19% FW (serine- 0.03%–11.12%, glutamic acid- 11.76%–16.48, and aspartic acid- 0.43%–55.68% of total amino acids), Lipids and fatty acids- 1.2%–7.8%FW (polyunsaturated fatty acids- 3.70%–24.62%, saturated- 3.70%–42.68%, and monounsaturated- 0.73%–60.37% of total fatty	These berries have a pale and silvery grey colour on the lower surface and the upper surface has a dark grey-green color. They have an intense tangy and citrusy taste.	Liquors, teas, pies, wines, jams and lotions	(Sea Buckthorn Berry 2023; Wang et al., 2022)

	acids), Total carotenoids- 0.02–0.17 g/kg, α-Tocopherol- 43-222 mg/kg, Minerals- Calcium- 0.27–3.12 (mg/kg DW), Magnesium- 0.47–2.22 (mg/kg DW), Nitrogen- 17.61–18.60 (mg/kg DW), Phosphorus- 1.50–1.71 (mg/kg DW), Potassium- 2.20–10.30 (mg/kg DW), Iron- 22–282 (mg/kg DW), Copper- 0.14–12.0 (mg/kg DW), Manganese- 8.70–16.00 (mg/kg DW), Zinc- 0.04–28.00 (mg/kg DW), and Boron- 13.61–16.30 (mg/kg DW), pH- 2.8-3.5			
Highbush cranberry (*Viburnum trilobum*)	Water- 85-90%, Calories- 40-50 cal/100g, Carbohydrates- 8-10g/ 100g, Dietary Fiber- 4-5g/ 100g, Protein- 0.5-1g/ 100g, Fat- 0.5g/ 100g, Vitamins- 15-25mg/ 100g, Minerals (Potassium, Calcium, Magnesium). Exact values for proximate composition are not available for this berry type, pH- 3.0-4.5	These are reddish-brown berries which have a tart and acidic taste.	Sauces, preserves and jams/jellies	(Moore, 2023; St-Pierre et al., 2005)
Nanny berry (*Viburnum lentago*)	Water- 70-80%, Calories- 60-70 cal/100g, Carbohydrates- 14-18g/ 100g, Fiber- 3-4g/ 100g, Protein- 1-2g/ 100g, Fat- 1g/ 100g, Vitamins (Vitamin C, Vitamin K, and Vitamin B). Exact values for proximate composition are not available for this berry type, pH- 5.0-6.5	These fruits are dark blue when ripened. They have a sweet taste.	No products are available.	(Nannyberry, 2022; Mehrabyan Nursery, 2022)

Snowberry (*Symphoricarpos albus*)	Water- 80-90%, Calories- 30-60cal/ 100g, Carbohydrates- 5-15g/ 100g, Fiber- 2-10g/ 100g, Protein- <2g/ 100g, Fat- 1g/ 100g, Vitamins and minerals (Vitamin C and potassium). Some species within the *Symphoricarpos* contain potentially toxic compounds, pH- 5.0-6.5	These are white colour fruits with a bitter taste.	No products are available. Can be used to make shampoo products.	(Krebs Creek, 2023; Native Plants PNW, 2023; Snowberry facts and health benefits, 2022)

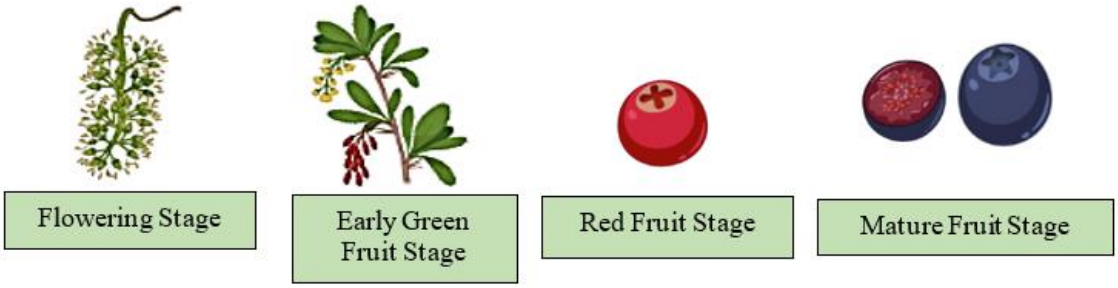
Saskatoon Berry (*Almelanchier alnifolia*) has been identified as a part of the staple foods of some indigenous communities (Hussein et al., 2020). They are very hardy plants that can be used as a natural fence, hedge, or screen. They can grow in diverse environmental conditions; for instance, they can be seen at sea level and on mountain peaks, proving their availability in western Ontario to British Columbia and the Yukon. These berries are produced commercially in North America but are mainly confined to Canada. (S. Zhao et al., 2018).

Chokecherry (*Prunus virginiana*) is one of the wild cherry types present as shrubs or small trees that grow across the southern part of Canada; its colours could vary from red to black, grow in clusters, and have a sweet taste when fully ripe. Apart from chokecherries, Canadian buffaloberry (*Sheperdia Canadensis*) and Silver buffaloberry (*S. argentia*) are two other major types of drupes present in the prairies (Frazier, 2022). Further, russet buffaloberry or soapberry (*S. canadensis*) are drupes present as reddish-orange coloured fruits, growing on deciduous shrubs from coast to coast. Although, among the soapberries, some bitter-taste fruits are used in making jelly, the indigenous communities also whip them with water to make confections in British Columbia (The Canadian Encyclopedia, 2023).

Elderberry (*Sambucus spp*) is a type of true berry, and these tree-like elderberries can be taller than 20 feet and traditionally have a sharp flavour. Some varieties are smaller and single-stemmed but often grow as a shrub-like cluster of stems (Frazier, 2022). There are two types of the most attractive true berry types, which are known as native edible wild blackcurrant (*Ribes americanum*) and wild redcurrant (*R. triste*). About 14 species of currants have been found in Canada, and they often produce aromatic shrubs with nice fall colours (Frazier, 2022). **Figure 2.2a** and **Figure 2.2b** show the common growth stages of the berries discussed above.

Wild Canadian gooseberry (*Ribes oxycanthoides*) is another berry found in the urban landscape. This berry type grows everywhere in Canada except in the Northern parts. These are good for jellies and preserves and possess a reddish to dark purple. There is also a native berry type in Canada, categorized as an aggregate berry including three types of strawberries belonging to the genus of Fragaria and the family of Rosaceae. Blackberry (*Rubus spp*) is one of them, and arctic raspberry (*Rubus articus* ssp. *acaulis*) is a creeping native aggregate berry that provides excellent ground cover. This raspberry grew in the entire breadth of Canada as a wild plant and as far north as the delta of the Mackenzie River. *Viking* and *Latham* are the two outstanding varieties identified.

Madawaska, Ottawa, Trent, Rideau, *Tweet merit* and *Madawaska* were the widely used red raspberry varieties for cultivation (Blair, 1950). Their natural habitats are woodlands, meadows, coastlines, and clearings. In addition, there are more than 12 species of blackberries growing mainly in eastern provinces and southern British Columbia (Bushcraft., 2023).



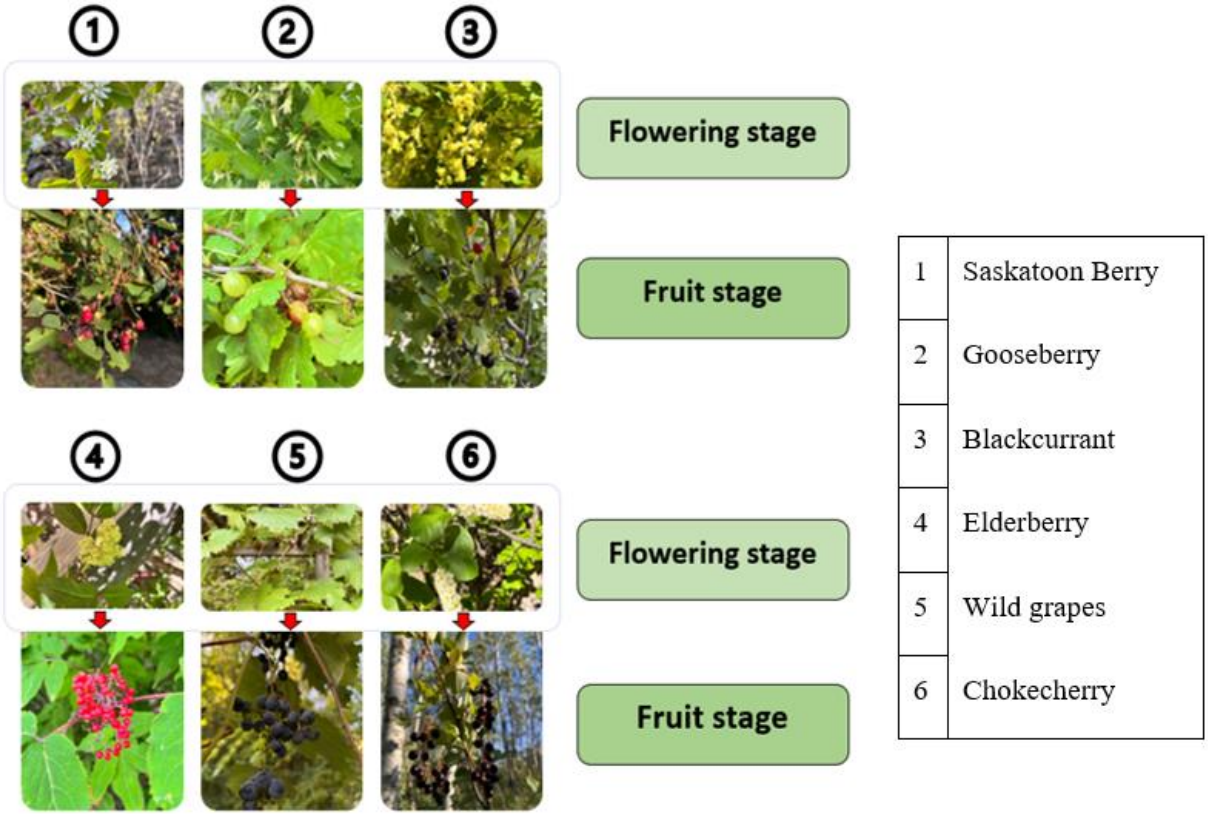


Figure 2.2a Common growth stages of the berries; the dormant stage to the mature fruit

Figure 2.2b Flowering and fruit stages of some of the prairie berries

Blackberry is found in the wild in Canada from Newfoundland westward, near the boundary of Manitoba and British Columbia. The degree to which these berries get protection during winter would determine the harvest of the berries. The places with heavy snowfalls showed the most abundant blackberries, and when the berries did not get protection from snow cover, the growth was not satisfactory. A few blackberry varieties are grown in the southwest section of Ontario and Quebec. These berries grow exceptionally well in British Columbia and some Maritime provinces' parts (Blair, 1950).

Although the gooseberry is native to Canada, it can be found in the wild almost entirely in the Arctic Circle. Gooseberry thrives in regions where the summer temperatures stay relatively cold. *Poorman*, *Clark*, and *Silvia* are the best commercial varieties. Over forty years, a Central Experimental Farm in Ottawa had tested almost all the black currant varieties. For commercial-scale planting in Central Canada, the Central Experimental Farm had identified some varieties, for instance, *Kerry*, *Magnus*, *Climax*, *Boskoop Giant*, and *Climax*, because of their larger size and high quality. Red and white currant varieties are also available; among white currants, White Dutch and White Grape are the best of the white-fruit varieties. Canadian wild berries are a popular ingredient for various products. Some common Canadian wild berry products include jams, jellies, syrups, and desserts such as pies and tarts. Blueberries, raspberries, cranberries, and wild strawberries are among the most frequently used berries in these products. These items can often be found in local markets and stores in Canada, especially in regions where wild berries grow abundantly. There is a wide range of potential to use the underutilized wild berries in different product development applications and have a significant potential due to their unique flavours, nutritional properties and natural appearance as shown in **Figure 2.3**.

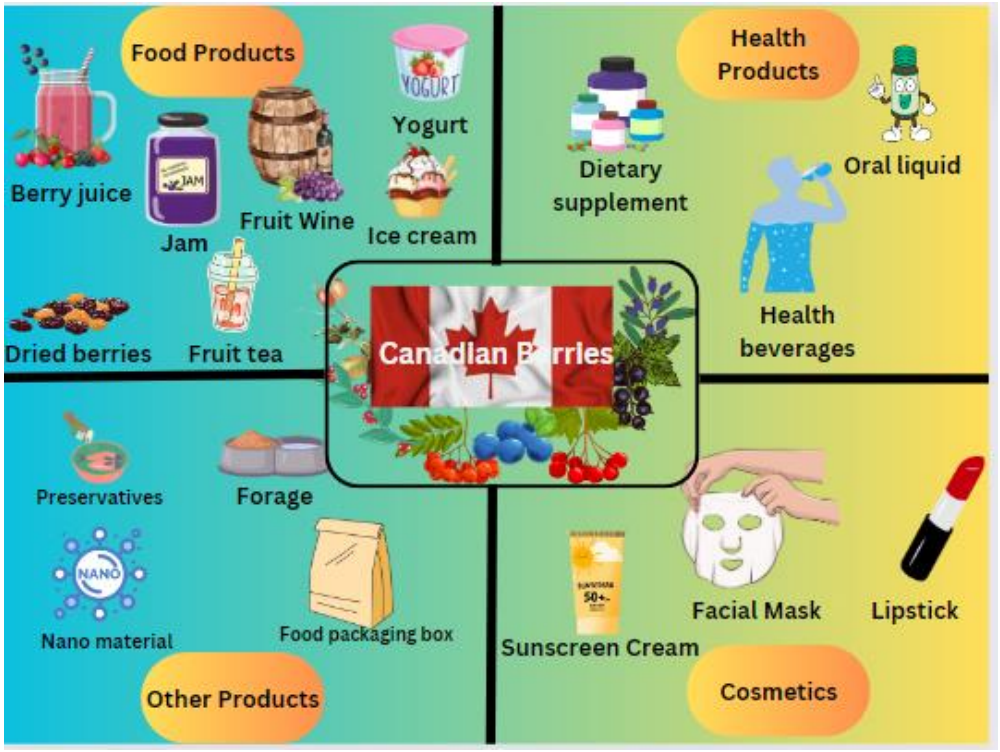


Figure 2.3 The product development application potential of Canadian wild berries

2.4 Bioactive phenolic compounds in prairie berries

Berries are a rich source of sugars and other biologically active molecules such as anthocyanins, flavonols, hydroxycinnamic acids, fibre, vitamin C, hydroxybenzoic acids, ellagitannins, stilbenes, procyanidins, and flavon-3-ols. The most abundant nutritive compounds in berries are sugar and fibre, generally between 12-15g per 100g and 2-5g per 100g, respectively (S. Zhao et al., 2018). The non-nutrient phenols are presented between 0.1 and 170 mg per 100g of dry weight. It is around 95 and 300mg per 100g in fresh weight basis as a collective value (Kylli, 2011a). Bioactive compounds in berries are shown in **Table 2.2**.

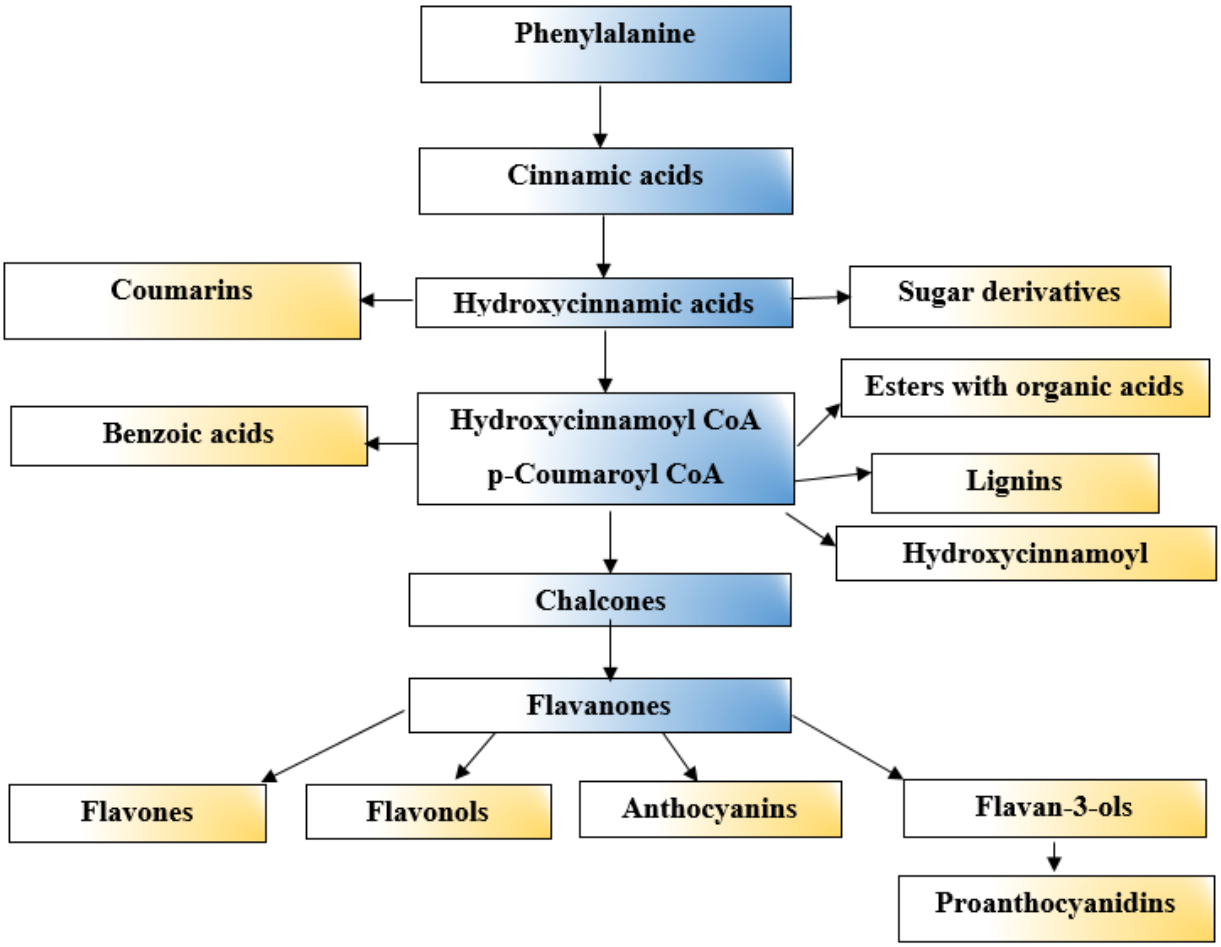
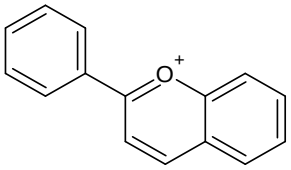
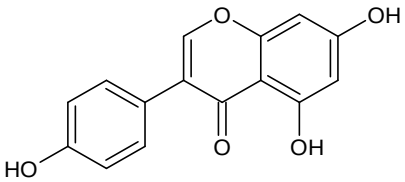
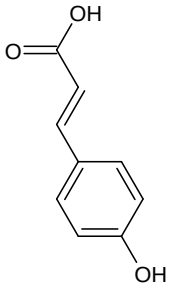


Figure 2.4 Synthesis of phenolic compounds (Kylli, 2011b)

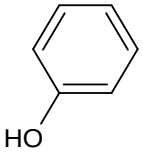
Table 2.2 Phenolic bioactive compounds in prairie berries (based on previous evidence)
Chemical structures: Source : (Bravo, 1998)

Bioactive components	Polyphenol subclasses
Anthocyanins	Flavanoids
	

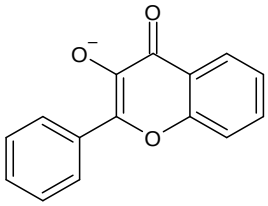
Hydroxycinnamic acids



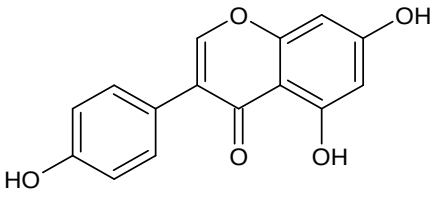
Phenolic acids



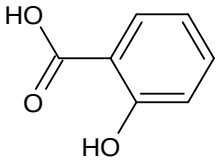
Flavonols



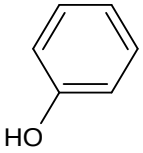
Flavonoids



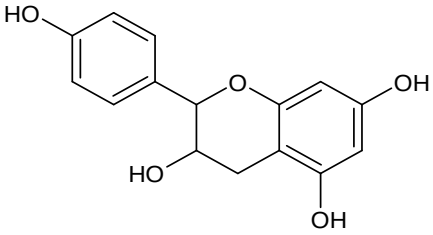
Hydroxybenzoic acids



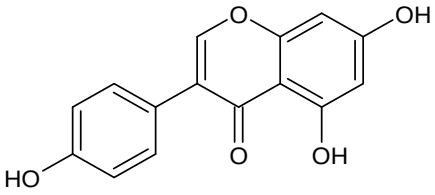
Phenolic acids



Flavon-3-ols

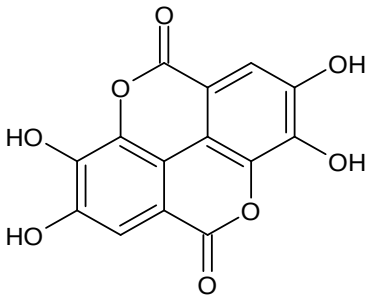


Flavonoid

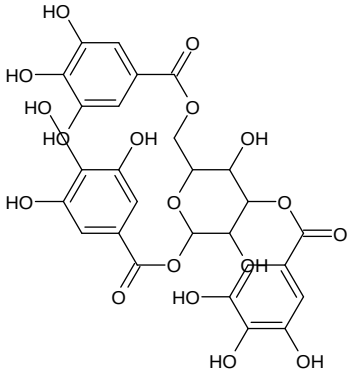


Ellagitanins, gallotannins, ellagic and coumaroyl-glycosides

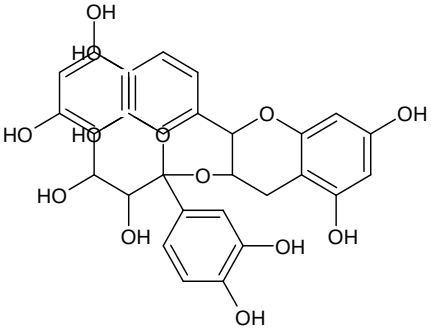
Hydrolyzable tannins



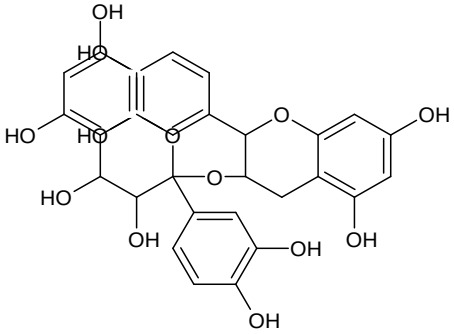
Procyanidins (flavan-3-ol polymers)



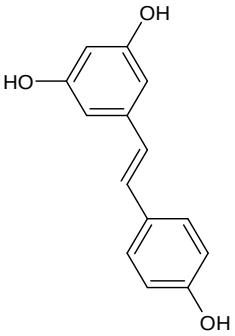
Condensed tannins

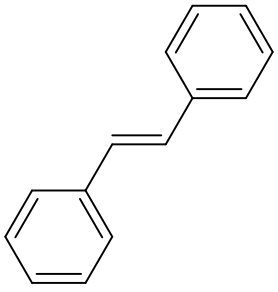


Resveratrol



Stilbenes





Phenolic compounds are derived from the amino acid phenylalanine and are considered secondary metabolites, as shown in **Figure 2.4**. Phenolics contain one or more hydroxyl groups in the aromatic ring (Kylli, 2011a). Hydroxybenzoic acids, flavonols, hydroxycinnamic acids, anthocyanins, ellagitannins, proanthocyanidins and flavon-3-ols are the main classes of phenolics present in the berry. These are formed through the shikimic acid pathway (**Figure 2.4**). First, the amino acid phenylalanine is formed. The phenylalanine enzyme is chorismate mutase-prephenate dehydratase, which generates phenylpyruvate transesterified to form phenylalanine. Then, the presence of phenylalanine ammonia-lyase catalyzes phenylalanine to form trans-cinnamic acid, one of the precursors for simple phenolic compounds, as shown in **Figure 2.4**. Finally, *p*-coumaric acid is produced by converting *trans*-cinnamic acid (Kylli, 2011a).

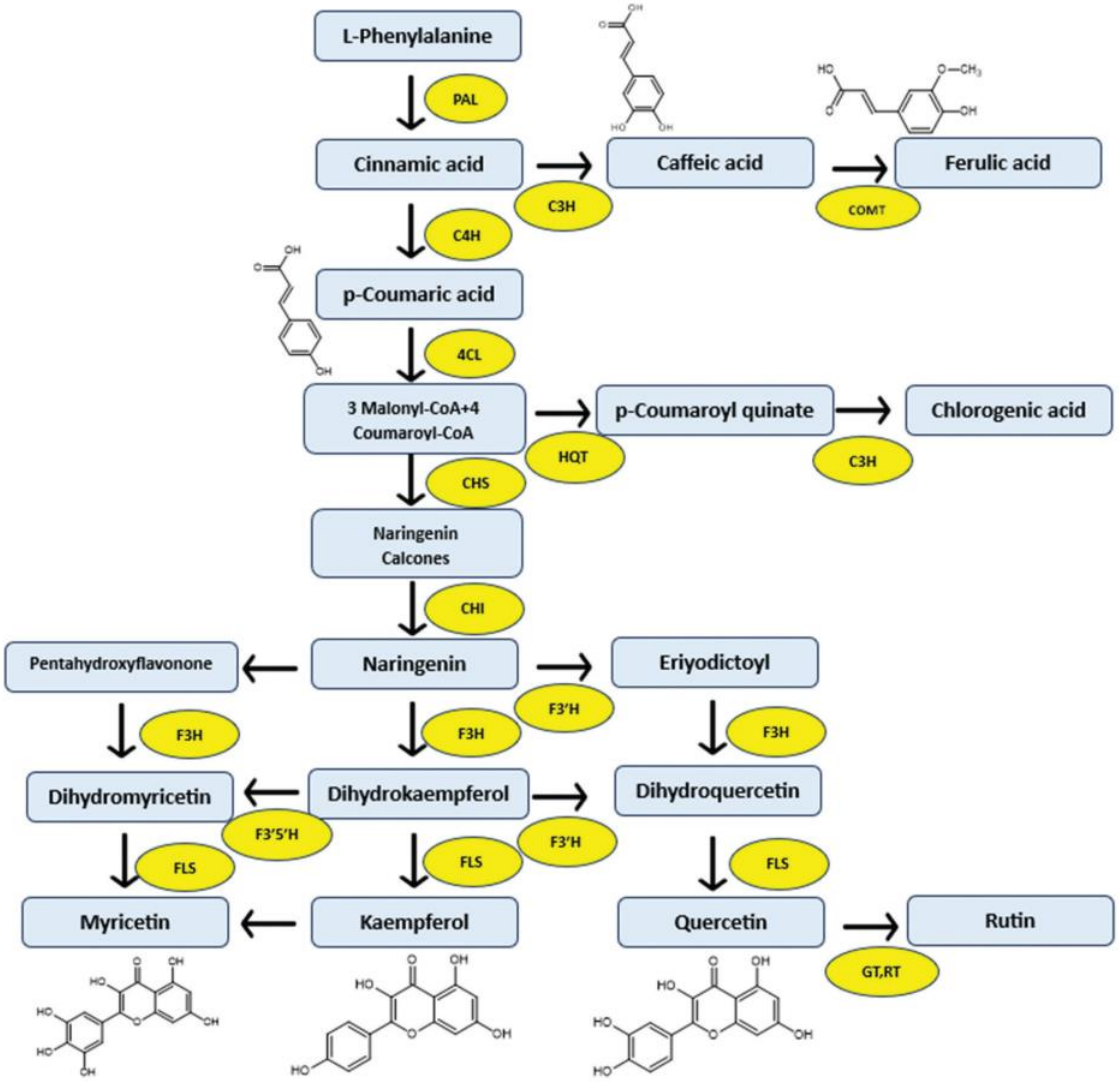


Figure 2.5 Synthesis and responsible enzymes of phenolic compounds in phenylpropanoid pathway starting from L-Phenylalanine (Bravo, 1998;Shahidi & Ambigaipalan, 2015)

The formation of sinapic acids, caffeic and ferulic acids occurs with the hydroxylated *p*-coumaric acids from hydroxycinnamic acid. Sinapic acid, caffeic acid, and ferulic acid are all formed in the phenylpropanoid pathway through a series of enzymatic reactions. Sinapic acid is synthesized from the amino acid phenylalanine via cinnamic acid and p-coumaric acid intermediates. The conversion of p-coumaric acid to sinapic acid is catalyzed by the enzyme sinapoylglucose-malate sialyltransferase (Kylli, 2011a). Caffeic acid is formed from the precursor molecule hydroxycinnamic acid, produced from phenylalanine via phenylalanine ammonia-lyase (PAL) and cinnamic acid 4-hydroxylase. Hydroxycinnamic acid is then converted to caffeic acid via the action of the enzyme caffeoyl-CoA O-methyltransferase (C4H). Ferulic acid is also synthesized from hydroxycinnamic acid, produced from phenylalanine via PAL and C4H, as shown in **Figure 2.5**. Hydroxycinnamic acid is then converted to ferulic acid via the action of the enzyme caffeic acid O-methyltransferase.(Kylli, 2011a) Cinnamic acid is then converted to 4-coumaroyl-CoA, a precursor for synthesizing various flavonoids, including anthocyanins. The 4-coumaroyl-CoA is converted to naringenin chalcone by a series of enzymatic reactions. Naringenin chalcone is then converted to dihydroflavonol by the enzyme chalcone isomerase. Dihydroflavonol is the precursor for the synthesis of various flavonoids, including anthocyanins. The synthesis of anthocyanins from dihydroflavonol involves a series of enzymatic reactions that depend on the specific type of anthocyanin being synthesized. These reactions include hydroxylation, methylation, and glycosylation, which modify the structure of the anthocyanin molecule. The final result of these reactions is the production of various anthocyanin pigments, which give berries their characteristic colours, such as blue, purple, and red. In berries, anthocyanin concentrations are generally higher in the epidermis and tissues beneath the skin than in the central part of the fruit. Anthocyanins include aglycones, anthocyanidins, and glycoside anthocyanins (Contessa et al., 2013). Anthocyanins have been reported to be the highest in blackberries and blueberries. Chokeberries, blackcurrants, saskatoon, and bilberries, also have high anthocyanin content. Bilberry alone contains around 15 anthocyanins (Kay, Neilson, & Ferruzzi, 2023). Delphinidin, peonidin, petunidin, malvidin glycosides and cyanidins' are the most abundant anthocyanins in these berries. In addition, cyanidin glucosides are the only anthocyanin group present in chokeberries,

redcurrants, lingonberries, rowanberries, red gooseberries, and blackberries. In American cranberries, cyanidins' are the predominant form of anthocyanins, while peonidin is the most predominant form of anthocyanins present in European cranberries. Procyanidins and prodelphinidins are the proanthocyanidins in strawberries and raspberries (Kylli, 2011a)

These phenolic compounds hold a C3 side chain and a phenyl ring (C6), also termed phenylpropanoids. The cleavage of two-carbon moiety from the phenylpropanoids produces the benzoic acid derivatives (Kylli, 2011a) Protocatechuic acid, syringic acid, gallic acid, dihydroxybenzoic acid, and vanillic acids are formed by the methylation and hydroxylation of hydroxybenzoic acids. These hydroxybenzoic acids are found as bound forms in most foods and often become components of hydrolyzable tannins with complex structures (Da Silva Pinto et al., 2010) Sugar derivatives and organic acids are the other forms of hydroxybenzoic acids found in foods. Figure **2.5** shows the biosynthesis of phenolic compounds through the phenylpropanoid pathway.

Berry phenolic compounds, including chlorogenic acid, a caffeic acid derivative, have generated significant interest in the recent past. In addition to berries, various other plant species produce these secondary phenolic metabolites, such as tea, roasted green beans, coffee, berries, cocoa, citrus, apples, and pears (Naveed et al., 2018). Chlorogenic acid is one of the main hydroxycinnamates, a quinic acid and caffeic acid ester. Several caffeic acid derivatives have been isolated from the plant kingdom apart from chlorogenic acids, such as glycosides, methoxylated, sulphated and esters. Ferulic acid, caffeic acid, and *p*-coumaric acid were the most common hydroxycinnamic acids found in most plants. They are present in plants in a bound form to the cell wall or as quinic acid or glucose esters. Only a small fraction is a free form in hydroxycinnamic acids. In addition, blueberries contain the highest hydroxycinnamic acids, while blueberries and cranberries contain the highest amount of flavonols, respectively. A large amount of caffeic acid derivatives are present in chokeberries, blueberries, bilberries, and Saskatoon berries. In contrast, *p*-coumaric acid is the primary hydroxycinnamic acid in redcurrants, blackcurrants, raspberries, strawberries, cranberries, lingonberries, and sea buckthorns (Eichholz et al., 2015).

Further, trans-resveratrol (3,5,4'-trihydroxystilbene) is a polyphenolic phytoalexin found in biological materials such as grapes, plums, peanuts, grape juice and red wine (Huang, Ou, et al., 2005). This compound has been of great interest for a long time due to its wide range of

physiological properties. Among the other most important bioactive compounds in berries, quercetin is a ubiquitous flavonoid belonging to the class of flavonoids. It is commonly found in onions, fruits and vegetables (Huang, Boxin, et al., 2005). Kaempferol, myricetin, isorhamnetin and quercetin are the main flavonols found in berries.

When considering the distribution of different phenolic compounds in berries, the phenolic compounds in blackberry (*Rubus spp*.) are higher compared to blueberry (*Rubus spp*), dark raspberry (*Rubus occidentalis*), and ruddy raspberry (*Rubus idaeus*) (de Souza et al., 2014). These phenolic compounds are abundant, particularly in brightly coloured berries (Boussaa et al., 2020). Individuals of various families, including Rosaceae (strawberry, raspberry, and blackberry), and Ericaceae, are among the species with the most significant concentrations of bioactive phenolic compounds (blueberry, cranberry). Besides, grape berries (class *Vitis*) and their byproducts (juice, wine) are also higher in bioactive compounds such as phenolics (Skrovankova et al., 2015). The *Vaccinium* species berries, such as blueberries, lingonberries, bilberries, *Rubus* species, strawberries, raspberries, chokeberries, and cloudberries, are abundant in hydroxybenzoic acids (Witol et al., 2018) In raspberries, cloudberries, and myrtle berries, gallic acid was the most common form of hydroxybenzoic acid. But protocatechuic acid was the most predominant compound in gooseberries, lingonberries, cranberries, blackcurrants and redcurrants (Eichholz et al., 2015). Blackcurrants, blueberries and bilberries are high in flavonols such as quercetin. Quercetin derivatives are the most common and abundant flavonols in berries, except in gooseberries, seabuckthorn, myrtle berries and blackcurrants. In blackcurrants, myricetin has been identified as the predominant flavonol group de Souza et al., 2019). In blackberries and raspberries, the most common ellagitannins are casuarictin, sanguiin H-6, potentillin, pedunculagin, and lambertianin. Ellagitannins are present at higher levels in berries and belong to the family Rosacea such as cloudberries, blackberries, strawberries and raspberries (Witol et al., 2018)

Berries contain many other bioactive compounds, including vitamin C, fibre, carotenoids, vitamin K, and folate, a type of B vitamin. Raspberry and strawberry contain significantly high levels of vitamin C, around 60 and 25mg per 100g, respectively. Even though polyphenols are non-nutrients, the importance of considering nutrient factors such as sugar, fibre and vitamins plays a significant role when focusing on the broader view of constituents within a berry or when

developing extraction methods (Caruso et al., 2015). The presence of these nutritive compound matrices can influence the analysis with extraction efficiency, compatibility with the detection systems, detection limits and chromatographic performance.

Apart from the phenolic compounds, in the last few years, there has been an increasing trend toward finding compounds that can protect the human body from reactive oxygen species and free radicals (Fatima et al., 2012). Since the lipophilic compounds in plant oils showed antioxidant activity and radical scavenging activity, research has been widely conducted to identify potential antioxidant activities in berry oils (Bederska-Łojewska et al., 2021). There is a different FA composition in berry seeds and soft parts. The seed oil has high linoleic (18:2n-6), oleic (18:1n-9), α-linolenic (18:3n-3) and palmitic (16:0) acids. However, the oil from the soft parts of the berry is mainly saturated, and they are high in palmitic and palmitoleic (16:1n-7) acids and low α-linolenic (18:3n-3) acids. Glycerophospholipids (GPL) and triacylglycerols (TAG) contain 85-95% lipophilic fraction, with the most FA in oils from seeds and berries. Defining the quality of the berries, seeds and oils is based on important bioactive compounds such as tocotrienols and tocopherols (Dulf, 2012). **Figure 2.6** shows the common forms of fatty acids present in berries. Omega-3 fatty acids are found in high levels in black currants and lingonberries (He et al., 2020). Linoleic acid is found in high levels in blackberries, raspberries, and strawberries. When considering the phytosterols, β-Sitosterol is found in high levels in blackberries, raspberries, and blueberries and stigmasterol is found in high levels in black raspberries. Saponins are another class of bioactive compounds found in various plants, including some types of berries (Zhou et al., 2022). Saponins are known for their soap-like properties, and they can have a bitter taste. Some berries that are known to contain saponins include blueberries, particularly monoglycosylated flavonoids. Blackberries are another berry that contains saponins (Güçlü-Ustündağ & Mazza, 2007). The levels of saponins in blackberries can vary depending on the variety and ripeness of the fruit. Elderberries are high in saponins, particularly a type of saponin called ursolic acid. Goji berries also contain a saponin called beta-sitosterol (Mshvildadze et al., 2004). **Figure 2.6** shows the common forms of fatty acids, phytosterols and saponins in berries.

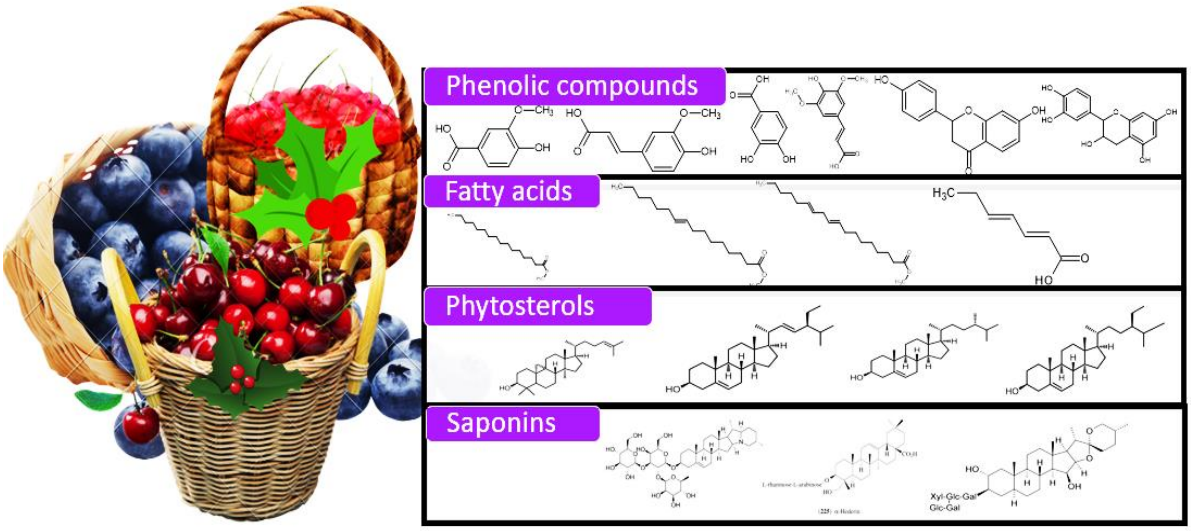


Figure 2.6 Berry bioactive compounds classes and their examples based on the literature (Cossignani et al., 2018)

Even though studies have identified berry bioactive molecules, especially phenolic compounds, only a few studies have examined the bioactive molecules present in different prairie berries. Analysis of FA in berries has been mainly restricted to sea buckthorn, wild grapes and blackcurrant (Eichholz et al., 2015). Only a few studies have been conducted to identify the phytosterols in sea buckthorn, and no studies have been conducted to identify, profile and quantify phytosterols and saponins. A knowledge gap exists, and more comprehensive studies are necessary to compare and establish whole berries as a potential functional food.

2.5 Chemistry of Berry Bioactive Compounds and their Potent Health Benefits

2.5.1 Hydroxycinnamic Acids and Hydroxybenzoic Acids

Hydroxycinnamic and hydroxybenzoic acids comprise a six-carbon atom (C_6) phenyl ring. They differ by the number of carbons on their side chains (Bravo, 1998). The difference between these two acids is that hydroxybenzoic acids have only a single carbon side chain denoted as C1 and hydroxycinnamic acids have a three-carbon side chain denoted as C3. Both the acid types terminate with a carboxyl group. The most common hydroxybenzoic acids are protocatechuic, syringic, gallic, and vanillic. Hydroxycinnamic acids and hydroxybenzoic acids are derived from the shikimate pathway, a metabolic pathway found in plants, fungi, and bacteria. Hydroxycinnamic acids are derived from the amino acid phenylalanine, which is first converted to cinnamic acid by

phenylalanine ammonia-lyase (PAL). Cinnamic acid is then hydroxylated by cytochrome P450 enzymes, which can add one or more hydroxyl groups at different positions, forming various hydroxycinnamic acids such as caffeic acid, ferulic acid, and sinapic acid. These hydroxycinnamic acids are commonly found in plants and are important plant cell wall components. On the other hand, hydroxybenzoic acids are derived from the amino acid tyrosine. The first step is the conversion of tyrosine to p-hydroxyphenyl pyruvic acid by the action of tyrosine aminotransferase. p-hydroxyphenyl pyruvic acid is then converted to p-hydroxybenzoic acid by a series of enzymatic reactions involving decarboxylation, reduction, and hydroxylation (Bravo, 1998). Hydroxycinnamic and hydroxybenzoic acids are important in plants, acting as antioxidants, UV-absorbing agents, and signal molecules.

2.5.2 Flavonols, Flavan-3-ols, Proanthocyanidins and Anthocyanins

The subclasses of flavonoids are anthocyanins, flavan-3-ols and flavonols. The basic structure of anthocyanins consists of two linked aromatic rings, A-ring and B-ring combined by a heterocycle consisting of three carbons and one oxygen known as C-ring, and each aromatic ring possesses at least one aromatic hydroxyl group. Based on the hydroxylation and methoxylation of the B-ring and functional groups of the C-ring, flavonols, flavan-3-ols and anthocyanins are subdivided into subclasses. The C-ring unsaturation is the most distinguishable feature of the separation. For instance, anthocyanins contain double bonds between carbon 1-2 and 3-4. There are 2-3 double bonds between carbons in flavonols, and there is no unsaturation in the C-ring of flavon-3-ols. The interflavan linkages between flavon-3-ol units form the proanthocyanidins, while procyanidins are formed by epicatechin units (Bravo, 1998). Procyanidins are a class of flavan-3-ols formed from the polymerization of epicatechin and/or catechin monomers. The procyanidins can be formed through two pathways: the B-type procyanidins and the A-type procyanidins (Bravo, 1998). The B-type procyanidins are formed through the interflavan bond formation between the C4 carbon of one epicatechin and the C8 carbon of another epicatechin unit, forming a 4-8 linkage. The reaction can continue to polymerize, forming dimers, trimers, tetramers, and larger oligomers. The B-type procyanidins are characterized by their terminal epicatechin units, which have a free 3-hydroxyl group. The A-type procyanidins are formed through the interflavan bond formation between the C4 carbon of one epicatechin and the C6 carbon of another epicatechin unit, forming a 4-6 linkage. The reaction can also continue to polymerize, forming dimers, trimers, tetramers, and larger

oligomers. The A-type procyanidins are characterized by their terminal epicatechin units, which have a closed 3-hydroxyl group due to forming a cyclic bond between the C2 and C3 carbons (Bravo, 1998). In addition, flavan-3-ols are most commonly non-glycosylated. The most common flavon-3-ols are (-)-epicatechin, epigallocatechin, gallocatechin and (+)-catechin. Flavonols and anthocyanins are naturally glycosylated. Anthocyanins have enhanced stability due to their glycosylated form.

2.5.3 Ellagic acids, Ellagitannins and Hyolyzable Tannins

Ellagic acid, ellagitannins, and hydrolyzable tannins are all formed from the oxidation of polyphenolic compounds such as flavonoids and phenolic acids. Ellagic acid is formed from the oxidative coupling of gallic acid molecules, which can occur in the presence of oxygen and metal ions such as iron and copper. The reaction forms a dimeric molecule called ellagic acid, which can further react with other polyphenolic compounds to form larger molecules called ellagitannins (Bravo, 1998). Ellagic acid and ellagitannins are commonly found in pomegranates, strawberries, and raspberries.

Hydrolyzable tannins are another class of polyphenolic compounds formed through the esterification of polyphenolic acids with sugars. The reaction forms a complex molecule that can be hydrolyzed by acid or enzymes to release the polyphenolic acid and the sugar moiety. Ellagitannins are a subclass of hydrolyzable tannins formed from the reaction between ellagic acid and sugars. The reaction forms a complex molecule with multiple ellagic acid units, which can be further hydrolyzed to release ellagic acid or converted into other derivatives such as urolithins. The formation of ellagic acid, ellagitannins, and hydrolyzable tannins depends on the availability of the starting polyphenolic compounds and the presence of enzymes and metal ions and other reactants that can facilitate the oxidative or esterification reactions (Avula et al., 2023).

2.5.4 Fatty acids

Fatty acid synthesis in berries, as in most plants, occurs through de novo fatty acid biosynthesis, which occurs in the cells' plastids. The process involves the conversion of acetyl-CoA into long-chain fatty acids (He et al., 2020). The primary source of acetyl-CoA in plants is derived from the

breakdown of glucose through glycolysis. The acetyl-CoA produced in glycolysis is transported to the plastids, serving as the starting material for fatty acid synthesis. The synthesis of fatty acids involves a series of enzymatic reactions catalyzed by different enzymes (Fărcaș et al., 2022). The first step involves the conversion of acetyl-CoA to malonyl-CoA by the enzyme acetyl-CoA carboxylase. Malonyl-CoA then undergoes a series of condensation reactions with acetyl-CoA, catalyzed by the enzyme fatty acid synthase (FAS), to form long-chain fatty acids. The specific fatty acids that the plant synthesizes depend on the type and activity of the FAS enzyme complex (Zorzi et al., 2020). Palmitic acid is generally synthesized through a series of condensation and reduction reactions involving malonyl-CoA and acetyl-CoA. Oleic acid is formed through the desaturation of palmitic acid, which is catalyzed by the enzyme stearoyl-ACP desaturase. Linoleic acid is formed through the desaturation of oleic acid by the enzyme oleate desaturase (Yan et al., 2022). The fatty acids synthesized in the plastids are then transported to other parts of the cell or other cells, where they are used for energy production, membrane synthesis, or storage compounds. In berries, fatty acids play important roles in seed development, flavour, and aroma.

2.5.5 Phytosterols

Phytosterols are synthesized through the mevalonate pathway in plants, a metabolic pathway for the biosynthesis of isoprenoids (Piironen et al., 2003). Isoprenoids are a large class of natural compounds that include terpenes, carotenoids, and phytosterols. The mevalonate pathway begins with the condensation of acetyl-CoA to form HMG-CoA (3-hydroxy-3-methylglutaryl-coenzyme A), which is then reduced to mevalonic acid. Mevalonic acid is then phosphorylated to form mevalonate-5-phosphate, which is converted to isopentenyl pyrophosphate (IPP) and dimethylallyl pyrophosphate (DMAPP) through a series of enzymatic reactions (Trautwein & Demonty, 2007). IPP and DMAPP are the basic building blocks for synthesizing all isoprenoids, including phytosterols. Phytosterols are synthesized from IPP and DMAPP through a series of enzymatic reactions that involve cyclization, hydrogenation, and other chemical modifications. The specific enzymes involved in phytosterol synthesis vary depending on the plant species and the type of phytosterol being synthesized. However, some key enzymes involved in phytosterol synthesis in berries include squalene synthase, cycloartenol synthase, and sterol C-24 methyltransferase (Trautwein & Demonty, 2007).

2.6 Metabolism of berry bioactive compounds in the human body and their potential health benefits

Berries are a rich source of various nutrients, including fibre, vitamins, and minerals, other than the phenolic compounds, all of which contribute to their potential health benefits (Rahman et al., 2021). Dietary fibre is essential in maintaining digestive health, regulating blood sugar levels, and reducing the risk of chronic diseases such as heart disease and diabetes. Fiber also helps to promote satiety and can aid in weight management. A study found that higher dietary fibre intake was associated with a lower risk of cardiovascular disease, type 2 diabetes, and certain cancers. Apart from that, berries are also a good source of vitamins C, K, and B. Vitamin C, in particular, is a powerful antioxidant that can help to protect against oxidative stress and inflammation and is important for maintaining healthy skin, immune function, and collagen production. A review showed that vitamin C intake was inversely associated with the risk of cardiovascular disease and certain cancers (Bento-Silva et al., 2020). Berries also contain various minerals, including potassium, magnesium, and calcium, which are important for maintaining healthy bones, muscles, and blood pressure. Potassium, in particular, has been shown to help regulate blood pressure and reduce the risk of stroke and heart disease. Increased potassium intake was associated with a reduced risk of stroke and cardiovascular disease. Phenolic compounds are special in berries compared to vitamins, minerals, and fibre because they offer unique health benefits not provided by other nutrients (Rahman et al., 2021). While vitamins, minerals, and fibre also play important roles in overall health, phenolic compounds offer additional health benefits.

Phenolic compounds are metabolized in the body through complex reactions that depend on the specific compound and the individual's metabolic pathways (Bravo, 1998). The first step in metabolizing phenolic compounds is often the breakdown of the glycoside bond that links the sugar moiety to the phenolic aglycone. This reaction is typically catalyzed by enzymes called glycosidases, which are present in the gut microbiome and the liver. After the glycoside bond is broken, the phenolic aglycone can be absorbed into the bloodstream and transported to the liver, which undergoes further metabolism. The liver is the primary site of metabolism for phenolic compounds, and the reactions there depend on the specific compound and the individual's metabolic pathways (Rahman et al., 2021). Common metabolic pathways for phenolic compounds in the liver include methylation, sulfation, glucuronidation, and oxidation. Methylation and

sulfation reactions are typically used to increase the water solubility of the phenolic compounds, making them easier to excrete in urine. Glucuronidation involves the attachment of a glucuronic acid molecule to the phenolic compound, which also increases water solubility. Oxidation reactions in mitochondria can form reactive oxygen species, which can have beneficial or harmful effects on the body depending on the compound and the dose. Once the phenolic compounds are metabolized in the liver, they can be excreted in urine or bile (Bento-Silva et al., 2020). Some phenolic compounds can also be metabolized by the gut microbiome, which can produce secondary metabolites such as urolithins, which have been shown to have beneficial health effects. Therefore the metabolism of phenolic compounds in the body is a complex and dynamic process that depends on the specific compound, the individual's metabolic pathways, and the presence of enzymes and microbiota in the gut and liver (Redan et al., 2016). Studies have shown that the bioavailability of phenolic compounds varies from berry to berry, which may also be influenced by the processing method. Food processing procedures, such as high-temperature treatments, have been identified as a major factor responsible for the destruction or modification of natural phytochemicals, which can affect the antioxidant properties of foods (Tyagi et al., 2022). However, this reduction can be offset by degrading high molecular weight phenolic compounds into smaller ones with higher antioxidant properties. For example, phenolic compounds' antioxidant activity helps protect cells against oxidative damage caused by free radicals, which may reduce the risk of chronic diseases such as heart disease, cancer, and neurodegenerative diseases. In recent years, the consumption of berries and fruits, in general, has increased globally. Research shows that increased consumption of these fruits and berries may be associated with a lower incidence of disorders caused by reactive oxygen species, including cardiovascular disease, cancer and inflammatory processes (Kylli, 2011b). Studies have shown that dietary polyphenols are biologically active substances with therapeutic effects on cells and/or tissues. In addition to their well-described free radical scavenging properties, the presence of hydrophobic and hydrophilic domains in polyphenols allows them to interact and diffuse with biological membranes and bind to receptors and receptors in enzymes to affect intracellular signaling (Golovinskaia & Wang, 2021). Furthermore, the specific biological activities of phenolics in berries depend on various factors, including the phenolic group, their concentration, the type of berry, and how they are consumed, such as fresh berries, juice, wine, jam, oil or medicinal products etc. Fresh berries and various products, jams, juices, wine and berry extracts, are an integral part of the human diet and

act as functional foods (Golovinskaia & Wang, 2021). They also have a pleasant taste and low-calorie content. Furthermore, fresh berries and their products have high phenolic compounds: flavonoids such as anthocyanins and non-flavonoids such as stilbenes and phenolic acids. Since berries are often eaten raw, these compounds are not inactivated by cooking. From a nutritional point of view, one hypothesis that has emerged to explain the antioxidant effects of polyphenols is that they act as mild toxins, stimulate a general xenobiotic and/or antioxidant response in target cells, and activate many defence genes (Golovinskaia & Wang, 2021). Dietary antioxidants that can react with those free radicals can significantly impact the prevention and progression of various diseases associated with oxidative stress (Zhao et al., 2020). Phenolic compounds in berries have also been shown to have anti-inflammatory effects, which may help to prevent or slow the progression of inflammatory diseases.

Chlorogenic acid is a well-known powerful antioxidant agent; this compound and its derivatives exhibited significant bioactivity against various disorders (Agunloye et al., 2019). The anti-obesity and anti-diabetic properties of chlorogenic acids have been linked to glucose metabolism. It has also been suggested that human and animal models' reduced body weight and glucose uptake are associated with chlorogenic acid-rich diets. This action may occur due to the inhibition of glucose release by inhibiting the activity of glucose-6-phosphatase in the liver and by inhibiting glucose-6-phosphatase by absorption in the small intestine via inhibiting phosphate translocase (Naveed et al., 2018). In addition, chlorogenic acid has been identified to enhance AMP-activated protein kinase phosphorylation, adiponectin and adiponectin receptor, and reduce liver glucose-6-phosphatase expression. Chlorogenic acid, one of the most abundant dietary polyphenolic compounds, has been reported to exhibit potent anti-inflammatory potential, and it was also found to prevent Con A-induced hepatitis in mice effectively. Furthermore, administering chlorogenic acid to injured mice can accelerate wound healing without causing adverse effects on the heart and kidneys (Chen et al., 2019). It is also widely recognized that chlorogenic acid has several health benefits as an antihypertensive agent. Caffeic acid exhibited several potent biological activities in vitro and in vivo, including anticancer, immunosuppressant, antioxidant, anti-inflammatory, anti-HIV, and others. Caffeic acid derivatives, especially caffeic acid phenethyl ester, were claimed to exhibit therapeutic effects, including an anti-diabetic and hepato-protective agent and an anti-cancer drug for treating human breast cancer (Agunloye et al., 2019).

Many studies have shown that oxidative stress is involved in the pathogenesis of cardiovascular diseases due to excessive production of reactive oxygen species and/or a deficiency in antioxidant defences. This implies a potential interest in antioxidant molecules to limit the development of cardiovascular disease. Resveratrol has been reported to inhibit intracellular and extracellular reactive oxygen species production by enhancing glutathione, an intracellular free radical scavenger (Kuršvietienė et al., 2016). Resveratrol has been shown to enter cells that can exhibit antioxidant properties. It appears to be capable of scavenging hydroxyl radicals produced by a Fenton reaction. It has been shown to reduce lipid peroxidation, improve lipid profile, reduce platelet aggregation, increase vasodilation, and lower blood pressure (Hosseinian & Beta, 2007).

Quercetin, a flavonoid, acts as a powerful reducing agent and other food-reducing agents, such as vitamins C, E and carotenoids, to protect body tissues from oxidative stress. Commonly known as a powerful antioxidant, it prevents organisms from various diseases associated with oxidative stress, such as cancer, cardiovascular disease, inflammation and other degenerative diseases. Through years of research, researchers claim that flavonoids are effective in reducing the leading causes of death from cancer and other degenerative diseases based on the fact that flavonoids have been found to have antioxidant and pro-oxidant effects. For instance, Quercetin has been attributed to having anti-inflammatory, antibacterial and muscle relaxant activities and antiviral activity (Ozga et al., 2008). The mechanism of its antiviral activity is related to its ability to bind to viral proteins, thereby interfering with the synthesis of viral nucleic acids; For example, methyl quercetin was found to block poliovirus replication by interfering with the single-stranded RNA replication intermediate involved in blocking cellular protein synthesis. Food-derived flavonoids, especially quercetin, modulate various immune functions (Deepika & Maurya, 2022). The ability of quercetin to modulate the metabolism of carcinogens through inhibition or induction of enzymes involved in the biotransformation of carcinogens is an important mechanism believed to play a protective role against cancer. Flavonoids, including quercetin, are so-called bifunctional inducers that induce phase I and II biotransformation enzyme activity at the gene expression level (Deepika & Maurya, 2022).

In berries, anthocyanins have been shown to prevent and treat various cardiometabolic diseases. For example, observational data for dietary anthocyanin intake is inversely associated with cardiovascular disease risk in European and US populations. The research investigated the effect of berry-derived anthocyanin supplements on serum lipid profiles in dyslipidemic patients (Jordheim et al., 2007). These authors reported that anthocyanin consumption increased high-density lipoprotein (HDL)-cholesterol concentrations and decreased low-density lipoprotein (LDL)-cholesterol concentrations. In addition, studies have shown that anthocyanin supplementation significantly reduced serum levels of LDL- cholesterol, triglycerides, apolipoprotein, and apo C-III and increased HDL- cholesterol (Sandoval-Ramírez et al., 2021). In clinical trials, consumption of chokeberry extract resulted in slight but statistically significant reductions in total cholesterol, LDL-cholesterol, and TAG levels, and increased HDL- cholesterol and decreased lipid peroxidation in plasma of patients with metabolic syndrome (Ma et al., 2018). In addition, anthocyanins have also been shown to have neuroprotective effects, which may help to protect against age-related cognitive decline and neurodegenerative diseases such as Alzheimer's disease (Bento-Silva et al., 2020). Phenolic compounds in berries have also been found to have anti-cancer effects, inhibiting the growth and spread of cancer cells and promoting apoptosis (programmed cell death). **Figure 2.7** shows the potent physiological effects of bioactive compounds. It is important to note that more research is needed to fully understand the mechanisms and health effects of the various compounds in berries and that individual results may vary based on factors such as age, sex, and health status. Its presence and concentration vary from berry to berry, which may explain different health-promoting activities (Heinonen, 2007).

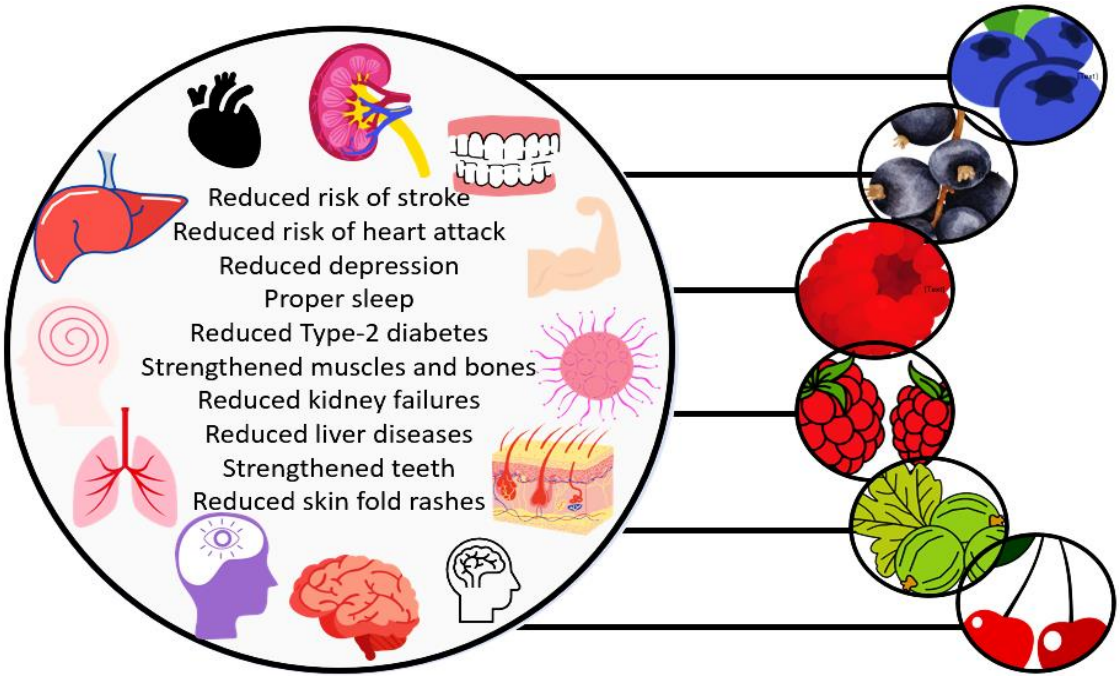


Figure 2.7 Berry bioactive molecules and their health benefits

In addition to those phenolic bioactive molecules, berries contain fatty acids, phytosterols and saponins which have been identified for their properties in pharmacology, toxicity, physiology and nutrition (S. Zhao et al., 2018). The body's metabolism of berry fatty acids, phytosterols, and saponins can vary depending on the specific compound and the individual's metabolism. Fatty acids are metabolized through beta-oxidation, which occurs in the mitochondria of cells. This process breaks down fatty acids into acetyl-CoA molecules, which can then enter the citric acid cycle (known as the Krebs cycle) to produce energy. Fatty acids from berries can be incorporated into cell membranes or used for energy production (Mshvildadze et al., 2004; Murru et al., 2022) Previous studies have found that berry seed oil is rich in linoleic acid and oleic acid, which can help lower blood cholesterol levels, whereas soft berry parts contain more saturated fatty acids such as palmitic and palmitoleic acid (Dulf, 2012;Fărcaș et al., 2022) In addition, omega-3 fatty acids have anti-inflammatory and heart-healthy properties.

Phytosterols are plant sterols, are structurally similar to cholesterol and can compete with cholesterol for absorption in the digestive tract (Zhao et al., 2020). Phytosterols are primarily

metabolized in the liver, which can be converted into bile acids and excreted in the feces. Some phytosterols may also be incorporated into cell membranes or transported in the blood (Ogbe, 2015). These berry compounds also provide human health benefits, including anti-cancer, anti-aging, anti-inflammatory, antioxidant and anti-proliferative activities (Zhao et al., 2018). A summary of the potential health benefits of berries is shown in **Figure 2.7**. Plant sterols have been shown to reduce serum concentrations of total and LDL cholesterol when sterol-rich foods that provide 1.5-2.0 g of plant sterols per day are consumed (Yang et al., 2001). In addition, the antioxidant properties of phytosterols can help lower cholesterol in the blood. Furthermore, epidemiological and experimental studies suggest that dietary phytosterols may protect against various types of cancer (Piironen et al., 2003). These results have sparked a general interest in identifying the importance of plant sterols in food. **Figure 2.6** shows the common forms of phytosterols present in berries. However, little is known about the effects of ingesting plant sterols from unfortified foods (Da Silva Pinto et al., 2010). It is commonly known that vegetable oils and grains are the best natural sources of dietary plant sterols. However, high consumption of fruits and vegetables, which rarely have sterol concentrations greater than 200–300 mg/kg fresh weight, contributes significantly to total sterol intake. The total intake of plant sterols from vegetables, fruits and berries forms many individual sterols (Piironen et al., 2003). **Table 2.3** summarizes the health benefits identified in some of the Canadian berries grown in prairies. Particularly, in Saskatoon berries, it has been found that beta-sitosterols are the predominant type of phytosterols present and are considered a plant sterol with the highest ability to produce cholesterol-lowering products and drugs with steroidal drugs (Fang, 2020).

Table 2.3 A list of berry fruits and their potential health benefits

Berry	Bioactive compounds	Health Benefits
Saskatoon berry (*Amelanchier alnifolia L*)	Polyphenolic compounds, anthocyanins, phenolic acids, flavonols, flavan-3-ols, triterpenoids, carotenoids, chlorophylls and tocopherols.	-Protective activities include antioxidant, anti-radical and anti-carcinogenic, anti-inflammatory, anti-diabetic, vasoprotective and neuroprotective activities -Preventive and therapeutic effects on many diseases such as cancer, inflammation and cardiovascular diseases, obesity, neurodegenerative diseases and muscle degeneration. (Fang, 2020)
Gooseberry (*Ribes hirtellum*)	Phenolic compounds (Chlorogenic acid, rutin, caftaric acid) and carotenoids, including vitamins, betalains and bio-compatible xanthophylls	-Anti-aging properties keep the body energetic and strengthen immunity. -Removes toxins from the body and strengthens the liver. -Prevents inflammation and is useful in diarrhea and dysentery. -Prevent acid reflux as it soothes the intestinal lining. -Helps the body's cells produce insulin to prevent diabetes (Gooseberry Health Benefits, M. U., 2022).

Red chokecherry & Black chokecherry (*Prunus virginiana &*)	Data was not found	-Free radical scavenging potential than strawberries, raspberries, blueberries, Saskatoon berries and sea buckthorn. -Rich in antioxidants, flavonoids, anthocyanins, and proanthocyanins, each of which is said to have high value in fighting allergens, viruses, and cancer-causing elements. -Contains high doses of quinic acid; prevents urinary tract infections. -Reduce lipopolysaccharide-induced pro-inflammatory cytokines (Benefits of Prunus Virginiana, 2022).
Wild grapes (*Vitis riparia*)	Stilbenes, phenolic acids, anthocyanins, flavonoids, and lipids	-Anti-inflammatory and antioxidant properties: Ex. Resveratrol; can slow or prevent cancer growth in the lymph node, liver, stomach, breast, colon, skin cancer and leukemia. -Lipid-lowering and anti-inflammatory actions that may help reduce the risk of cardiovascular disease. -Preventing the buildup of platelets and lowering blood pressure and the risk of irregular heart rhythms. (Canadian Wild Berries, 2023)

Blackcurrant (*Ribes nigrum*)	Phenolic compounds and vitamin C	-Antioxidant, anti-inflammatory, neuroprotective actions and anticancer properties. -Vascular protective effects, hypocholesterolemic effects, and enhancement of fat oxidation. -Improvement of brain function, gut microbiota modulation and postprandial blood glucose reduction (Kruszewski et al., 2021).
Red currant (*Ribes rubrum*)	Phenolic compounds, minerals (Fe, K) and vitamin C	-Source of iron, which is essential for the formation of red blood cells. -Act against colds and the formation of certain types of cancer. -Potassium in red currants is a heart-healthy mineral that plays a vital role in cardiovascular health. It also helps prevent high blood pressure and lower blood pressure. -Antioxidant carotenoid lycopene; helps reduce the risk of heart disease and cancer. -Protects the body from free radical stress that can damage DNA and other cellular structures (Currant: Health Benefits, 2023).

Haskap berry (*Lonicera caerulea*)	Phenolic compounds	-Reduced cancer, improved hyperglycemia and insulin resistance, and decreased bone loss and neurocognitive improvement. -Helps lower non-HDL plasma lipids and normalized triglyceride concentrations. -Improved lactase activity and improved insulin resistance. -Reduce nitrosamine-induced DNA damage in normal human lung epithelial cells (Amararathna et al., 2020).
Wild raspberry (*Rubus idaeus*)	Especially ellagitannins, anthocyanins and other polyphenolic compounds	-Reduce adiposity and lipid accumulation in vitro in rat models of diet-induced obesity -Preventing metabolic disturbances associated with high energy intake rather than reversing the metabolic effects of obesity. -Inhibiting the expression of adipogenesis-related genes to prevent adipocyte differentiation and lipid accumulation. -Anti-hypertensive, anti-inflammatory and anti-cancer abilities. Improve metabolic resilience in an obese environment (Bederska-Łojewska et al., 2021).

Wild blueberry (*Vaccinium angustifolium*)	Phenolic acid, anthocyanins and vitamins	-Favourably affects insulin sensitivity and early biomarkers of cardiovascular diseases, such as blood pressure, endothelial function, and oxidative stress. -Cardiovascular disease prevalence and mortality. -Improved insulin resistance as determined by the euglycemic hyperinsulinemic clamp in insulin-resistant men and women (Martineau et al., 2006).
Chokeberry (*Aronia melanocarpa*)	Phenolic compounds, anthocyanins and procyanidins	-Potent more free radical scavenging activities to cure a variety of chronic conditions, including diabetes, cancer, heart disease, metabolic disorders, and Alzheimer's, than blueberries -Reduced oxidative stress caused by a class of antipsychotic drugs. -Reduce the risk of heart disease by lowering cholesterol levels and blood pressure (Hosseinian et al., 2007).

Sea Buckthorn (*Hippophae rhamnoides*)	Unsaturated fatty acids, phenolic compounds (flavonoids), phytosterols and vitamins (A, C and E)	-The butanol fraction containing the highest amount of phenolic compounds showed the highest radical scavenging activity and the strongest β-glucosidase inhibitory effect. -Acts as an antioxidant, reduces lipid oxidation, helps relieve pain, aids collagen synthesis and epithelialization, prevents bleeding; promotes wound healing; anti-ulcer effect maintains cell membrane integrity, accelerates collagen synthesis, stimulates cell repair and nerve regeneration, supports cell proliferation, acting as a cofactor for enzymes and improve the use of vitamin A, reduce the risk of heart attack and stroke, Immunostimulant, stimulates cell repair and nerve regeneration. -Sea buckthorn seed oil has significant antiatherogenic properties. -Cardio-protectivity of the seabuckthorn oil may be due to the presence of phytosterols, unsaturated fatty acids, and vitamins that have synergistic effects on cardiovascular health when administered in combination (Hosseinian et al., 2007).

Highbush cranberry (*Viburnum trilobum*)	Phenolic acids, terpenes, flavonols, anthocyanins, proanthocyanidins	-Positive effect on arterial cholesterol profiles and reduce several cardiometabolic risk factors. -Proanthocyanidins isolated from cranberries inhibited the growth of lung cancer cells. -Exert bioactive chemoprevention by influencing cancer cell viability, proliferation, adhesion, kinetics, apoptosis, signalling pathways, oxidative status and inflammation. -The cranberry proanthocyanidin-rich fraction, and the flavonoid-rich fraction reduced cell viability or density in 41 cancer cell lines of 16 types (Da Silva Pinto et al., 2010).
Nanny berry (*Viburnum lentago*)	Data was not found	-Useful in treating respiratory diseases, and digestive and menstrual problems. -Helps those suffering from digestive disorders and menstrual problems, as well as relieve pain and anxiety. -Beneficial to treat digestive diseases (Nannyberry Facts and Health Benefits, 2022).

Snow berry (*Symphoricarpos albus*)	Saponins	-Treat a variety of ailments, but especially as an external skin wash. -For the treatment of diarrhea. -For the treatment of skin rashes. -Poultices of parts of the plant including leaves, fruits and bark are used to treat burns, wounds, cuts, cracks and injured skin (Snowberry facts, 2023).

Saponins are metabolized in the intestines by gut bacteria, which can hydrolyze the glycosidic bond and release the aglycone form of saponin. This aglycone can then be absorbed into the bloodstream and metabolized by the liver. Some saponins may have anti-inflammatory and antioxidant effects, while others may interfere with the absorption of certain nutrients (Xue et al., 2022). While saponins can have potential health benefits, it's important to note that they can also have side effects in some people, such as digestive discomfort or irritation of the intestinal lining. Yet, much work needs to be done to establish anti-diabetic, anti-inflammatory and anti-cancer activities and improve cardio-metabolic and cognitive performance following the consumption of berries (Yao et al., 2004).

Although research has been conducted on the health benefits of berry fatty acids, phytosterols, some gaps still need to be addressed (Kopylov et al., 2021). There is a need for further research to fully understand the mechanisms behind the potential health benefits of berry fatty acids, particularly concerning their anti-inflammatory properties. In addition, there is a lack of research investigating the bioavailability of berry fatty acids and their potential health benefits. Phytosterols have been extensively studied for their cholesterol-lowering effects, but there is a lack of research on their potential effects on other aspects of health, such as inflammation and oxidative stress. Finally, while saponins have been studied for their potential health benefits, including anti-inflammatory and anti-cancer effects, there is a need for further research to understand their mechanisms of action and potential therapeutic uses fully.

2.7 Commercialization of prairie berries

Canada is a significant producer and exporter of a variety of berries. Some of Canada's most commonly produced and exported berries include blueberries, cranberries, strawberries, raspberries, and blackberries. Canada is the world's largest producer and exporter of Blueberries (Vaccinium angustifolium), with exports worth an estimated $239 million reaching over 30 countries in 2018 (Skinner, Margot., & Hunter, 2013). Canada is one of the largest producers of blueberries in the world, with the majority of production coming from the provinces of British Columbia and Quebec. In 2020, Canada produced approximately 207,900 metric tons of blueberries (Agriculture and Agri-Food Canada Publications, 2023). Canada is the second-largest producer of cranberries in the world after the United States. The majority of Canadian cranberry

production comes from the provinces of Quebec and British Columbia. In 2020, Canada produced approximately 196,400 metric tons of cranberries. Strawberries are one of the major growing berry types in Canada, where the majority of production comes from Ontario and Quebec. According to Agriculture and Agri-Food Canada Publications, Canada produced around 45,800 metric tons of strawberries in 2020 (Agriculture and Agri-Food Canada Publication, 2023). The provinces of British Columbia and Ontario were the primary producers of Canadian raspberries, with a production of approximately 12,800 metric tonnes in the same year. Canada is a significant producer of blackberries, with the majority of production coming from the provinces of British Columbia and Ontario. In 2020, Canada produced approximately 5,300 metric tonnes of blackberries. Most exports go to the United States from Canada compared to the other exporting countries (Agriculture and Agri-Food Canada Publications, 2023).

In recent years, the Saskatoon berry industry has been growing, with efforts to commercialize the berry through research and development, marketing, and value-added products. According to a report by Agriculture and Agri-Food Canada, the Saskatoon berry industry in Canada is valued at around $20 million, with most of the production occurring in Saskatchewan. The industry is primarily made up of small-scale producers, with some larger operations emerging in recent years. Most Saskatoon berries are sold fresh, with the remainder used for processing into value-added products such as jam, juice, and wine. When considering Saskatoon berries, research and development have been crucial to the growth of the Saskatoon berry industry. Organizations such as the Prairie Fruit Development Centre (PFDC) and the University of Saskatchewan have researched the berry's genetics, cultivation, and processing, providing valuable information for growers and processors. The PFDC has also worked with the industry to develop new value-added products, such as Saskatoon berry powder and puree (Prairie Fruit Growers Association, 2023). Marketing efforts have also been important for the commercialization of the Saskatoon berry. The Saskatchewan Berry Growers Association (SBGA) promotes the berry and its health benefits through various channels, including social media, trade shows, and partnerships with other organizations. The SBGA also provides resources and support for growers, such as workshops and market research (Saskatchewan Fruit Growers Association, 2023). Promoting underutilized berries such as Saskatoon berry, chokecherry, and gooseberry in the prairie region of Canada and commercializing them can be challenging, but several strategies can be employed. One example

of such research is the exploration of underutilized berries to understand their nutritional value, culinary uses, and medicinal properties. This research can facilitate the development of new products and their marketing to consumers. Collaboration and cooperation with farmers can also be beneficial by educating them on the production process of underutilized berries, involving them in developing new products, and working together to create effective marketing strategies. Creating value-added products, such as jams, jellies, juices, and extracts from underutilized berries, can help generate demand for these fruits and enhance their commercial viability. Above all, conducting awareness campaigns to educate consumers on the benefits of underutilized berries can be done through social media, blogs, and other digital platforms, as well as through print and television advertising. Collaborating with food industry partners such as restaurants, bakeries, and cafes to introduce the underutilized berries in their products can help to create awareness around the berries and generate interest among consumers. Participating in local and regional events such as food fairs, farmers markets, and trade shows to showcase the underutilized berries and create awareness among the public, seeking government support in the form of grants, subsidies, and tax incentives to promote the growth and commercialization of underutilized berries are some of the other strategies to accelerate the popularity of prairie berries. By employing these strategies, underutilized berries in the prairie region of Canada can be promoted and commercialized, helping to create a new market and boost the local economy while providing many health benefits to the consumers.

2.8 Conclusion

Different types of berries are grown in the prairies, such as Saskatoon berries, gooseberries, chokecherries, blackcurrants, blueberries, cranberries, nannyberries, raspberries, and haskap berries. Phenolic compounds are an essential group of bioactive molecules in berries that promote good health. Dietary antioxidants can significantly impact the prevention and progression of various diseases associated with oxidative stress. Berries contain various compounds with antioxidant properties, including phenolic compounds, flavonoids, anthocyanins, stilbenes, unsaturated fatty acids, phytosterols, saponins, terpenes, alkaloids, and procyanidins. These berries have a wide range of capacities to act as a potential source of antioxidants, anti-inflammatories, and anti-carcinogens, providing health benefits to the human body. Studies have revealed that the consumption of berries has health benefits, such as beneficial effects on the prevalence of

cardiovascular disease and influencing cancer cell viability, proliferation, adhesion, kinetics, apoptosis, signalling pathways, oxidative status, and inflammation. Despite this, very few studies have been conducted on the bioactive profiles of prairie berries grown in Canada, including the profiles of phenolic compounds, fatty acids, phytosterols, saponins, alkaloids, terpenes, and their pharmacological and anti-nutritional properties. Therefore, conducting further metabolomics-assisted investigations to identify all the potential bioactive molecules present in these underutilized wild berries grown in prairies and conducting preclinical and clinical studies to identify their potential health benefits is essential.

2.9 References

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Chapter 3: A new UHPLC-HRMS metabolomics approach for the rapid and comprehensive analysis of phenolic compounds in blueberry, raspberry, blackberry, cranberry and cherry fruits

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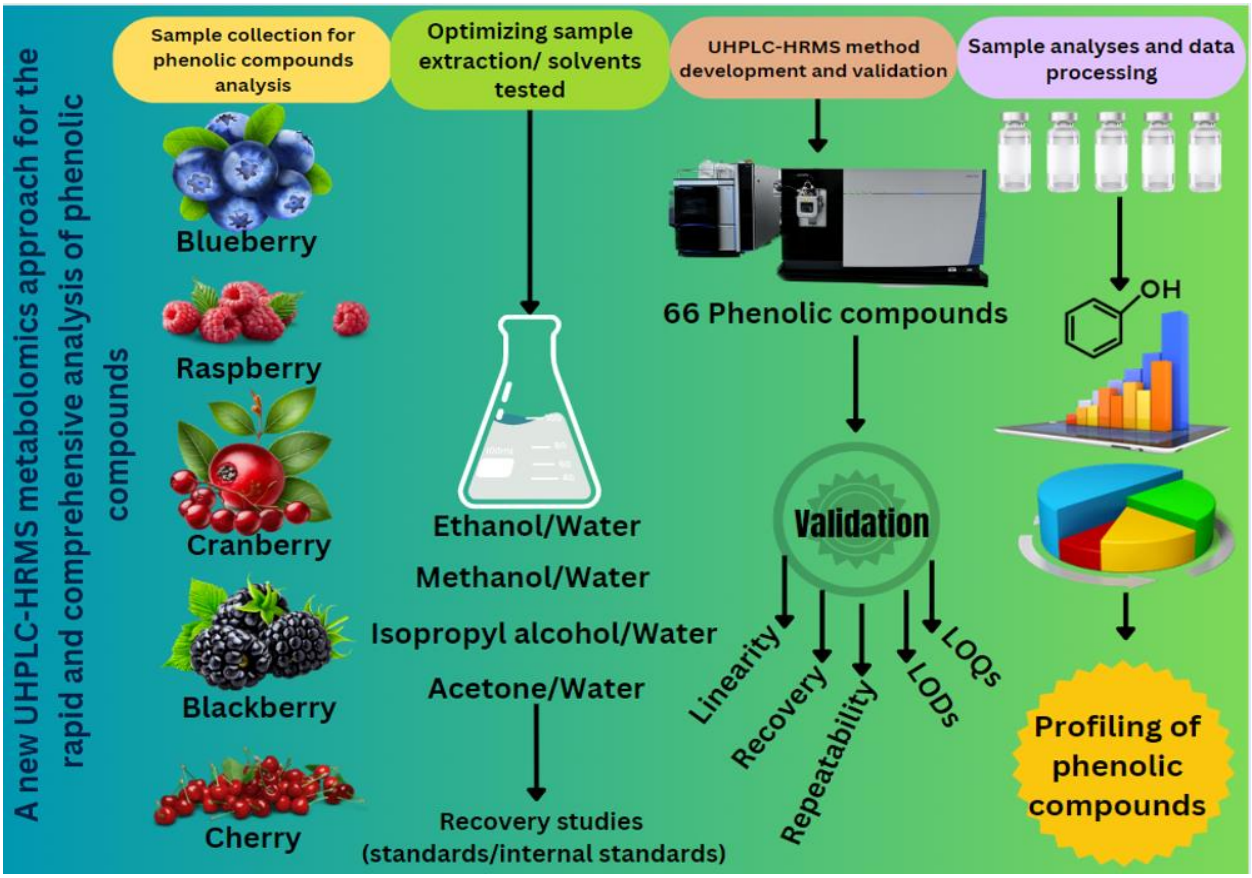
3.1 Abstract

Phenolic compounds are considered an important group of bioactive molecules that are present in abundant quantities in fruits such as berries and cherries; hence, the analysis and quantification of these compounds are of significant interest to the scientific community. The current study aimed to develop a novel analytical method using liquid chromatography and high-resolution mass spectrometry (UHPLC-HRMS) for the rapid, comprehensive and simultaneous analysis of 66 phenolic compounds optimized for the selected five types of fruits commercially available in Canada. Bioactive compounds that could potentially be metabolite markers for each berry were identified. Various phenolic compounds were identified and quantified in all five selected fruits. Notably, blackberries were rich in anthocyanins such as cyanidin-3-glucoside (368.4±6 µg/g), while blueberries were rich in peonidin-3-glucoside (1083±9 µg/g). In addition, raspberries and cherries contained significant amounts of cyanidin-3-rutinoside, at 3156±36 µg/g and 301.3±2 µg/g, respectively, while cranberries contained the highest concentrations of petunidin at 829.7±3 µg/g. The newly developed and validated UHPLC-HRMS method proved helpful in comprehensively analyzing phenolic compounds in blueberry, raspberry, cranberry, blackberry and cherry. Identifying and quantifying bioactives can lead to applications in neutraceutical and pharmaceutical industries by using phenolic-rich berry extracts in functional foods, supplements, or pharmaceutical products.

Keywords: High-resolution mass spectrometry, phenolic compounds, anthocyanins, berries, cherries, functional foods

Graphical Abstract



Author Contribution

Funds acquisition: CW and TN; Conceptualization: CK, SS and CW; Methods: SS and CK; Analysis: CK and SS; Writing the first draft: CK; Editing and reviewing: SS, CW, NB and CK; Final version review: SS, CW, CK, TN and NB; Research Supervision: SS and CW; Student academic supervision: CW and NB.

3.2 Introduction

Berries (*Vaccinium spp*) are a group of widely consumed fruits consisting of approximately 450 distinct species, including well-known varieties such as blueberries, blackberries, raspberries and cranberries (Jiang et al., 2022; Tyagi et al., 2022). Sugar and fibre are berries' most abundant nutritive compounds, ranging from 12-15 g per 100 g and 2-5 g per 100 g, respectively. Phenols are contributed for 0.1 and 170 mg per 100 g on a dry weight basis as the non-nutrient component (Kodikara et al., 2023). Phenolic compounds are naturally occurring secondary metabolites found in plants and are known to possess various biological activities such as antioxidant, anti-inflammatory and anticancer properties. Phenolic compounds are grouped into phenolic acids, flavonoids, tocopherols, cinnamic acid derivatives, lignans, coumarins, tannins and stilbenes (Huang et al., 2016; Jiang et al., 2022).

The most abundant phenolic compounds present in *Vaccinium* genus berries are procyanidins, phenolic acids, stilbene derivatives, flavonoids and anthocyanins such as malvidin, cyanidin, delphinidin, petunidin and peonidin. Piceatannol, resveratrol, and viniferin have been identified as the common stilbenes found in berries (Błaszczyk et al., 2019). Some of these phenolic compounds, especially anthocyanins, including glycosides of cyanidin, pelargonidin and delphinidin contribute to fruit colours, e.g., the bluish colours of blueberries and the reddish colour of raspberries (Hosseinian and Beta, 2007). Raspberries (*Rubus idaeus*) also contain diverse phenolic compounds, contributing to the plant's inherent self-defence mechanisms and potential health benefits to human beings (Lopez-Corona et al., 2022). In addition, cranberry fruits also consist of various bioactive compounds that significantly benefit human health. These berries also contain essential vitamins (A, C and E), minerals (potassium, sodium, selenium) and lutein and β-carotene. Cranberries contain polyphenols, such as flavonols, anthocyanins, proanthocyanidins, phenolic acids and resveratrol (Lopez-Corona et al., 2022). Similar to (+)-catechins, (-)-epicatechins are commonly abundant in most berries and contribute to their antioxidant capacity. Epigallocatechin is often found in smaller amounts in berries than in green tea, but it still contributes to the overall antioxidant properties of berries (Jiang et al., 2022; Tyagi et al., 2022). Similar to berries, cherries belonging to the *Rosaceae* family and *Prunus* genus are notably rich in phenolic compounds, with a prominent presence of anthocyanins that confer various health

benefits. Cherries contain phenolic compounds such as phenolic acids, flavonoids and stilbene. Notably, the flavonoids class of compounds, including anthocyanins, dihydroflavonols, flavanols, flavanones, flavones, flavonols and isoflavonoids, are known to be present in significant concentrations (Hu et al., 2021).

Metabolite profiling of berries is of significant interest in food and pharmaceutical research. However, analyzing berry phenolic compounds is challenging due to their structural complexity, low concentration and vast diversity (Jiang et al., 2022; Tyagi et al., 2022). Various methods have been developed for the identification and quantification of phenolic compounds in berries, using analytical tools such as high-performance liquid chromatography (HPLC) coupled with various detectors, including ultraviolet-visible (UV-Vis), fluorescence and mass spectrometry (MS). For example, UV-Vis spectroscopy is a commonly used technique for detecting berry phenolic compounds (Tyagi et al., 2022). Fluorescence spectroscopy has higher sensitivity than UV-Vis spectroscopy but has low selectivity, resulting in the inclusion of non-phenolic compounds, leading to an overestimation or exclusion of phenolic compounds, leading to an underestimation of total phenolic content (Latchoumane et al., 2022; Tyagi et al., 2022). On the other hand, mass spectrometric methods offer greater selectivity and sensitivity due to the capability to perform fragmentation of parent analytes into unique fragments, enabling the identification of each analyte with greater accuracy. HPLC coupled with MS has proven to be a powerful method for analyzing phenolic compounds, accurately identifying and quantifying multiple analytes. Suppression and enhancement of signals due to co-eluting compounds from the sample matrix is of major concern when identifying and quantifying analytes using MS (Remane et al., 2010; López-fernández et al., 2020).

Recent advances in MS helped improve analyte detection with greater accuracy, even in very complex matrices, minimizing confounding factors that can enhance or suppress signals (Remane et al., 2010). Recently developed methods to identify phenolic compounds using UPLC-ESI-MS/MS have improved the sensitivity, selectivity and accuracy of phenolic compound analysis in berries compared to traditional methods but the number of compounds detected in the existing methods are limited to 15-20 (Li et al., 2022). Despite these improvements, effective sample preparation still plays a vital role in removing interferences, concentrating target compounds and

minimizing matrix effects (Li et al., 2022). Using heated electrospray ionization (HESI) techniques improves analyte ionization and increases signal strength (Lu et al., 2008). Ultra-high performance liquid chromatography coupled with high-resolution MS (UHPLC-HRMS) is a novel technique, allowing for the identification and quantification of a wide range of phenolic compounds in complex matrices with greater confidence. In addition, this technique can be optimized for specific phenolic classes (Lucci et al., 2017). Matrix effects due to the presence of other compounds in the berry matrix, such as sugars, organic acids and pigments for instance chlorophyll, can interfere with the separation and detection of phenolic compounds, as a result of co-elution and leading to ion suppression or enhancement (Alara et al., 2021a; Constantin & Istrati, 2022a). Lack of selectivity may detect non-phenolic compounds, leading to inaccurate quantification of phenolic content in berries (Avula et al., 2023; Constantin & Istrati, 2022b). In addition, the analytical methods may require more time due to complex sample preparation steps, which could increase the risk of sample degradation and introduce errors in the analysis (Alara et al., 2021b). Currently in literature, organic solvents such as methanol, acetone and ethanol and their mixtures with water, with additives such as formic acid and acetic acid, were the most widely used solvents and solvent combinations for phenolic compound extraction (Boeing et al., 2014). Because the recovery of the phenolic compounds depends on their polarity and the solvents used for the extraction, there is a need to develop new methods for extracting and analyzing phenolic compounds in berries to overcome the aforementioned various limitations and provide accurate and reliable data.

To our knowledge, this is the first method for the simultaneous determination of 66 different phenolic compounds present in the phenylpropanoid pathway belonging to the phenolic compound classes including hydroxybenzoic acids, hydroxycinnamic acids, flavonols, flavans, stilbenes and anthocyanins in different fruit matrices such as cherry, blueberry, blackberry, cranberry and raspberry using a UHPLC-HRMS. The main objective of this study was to develop a rapid sample extraction method and an analytical method using UHPLC-HRMS to comprehensively detect and quantify phenolic compounds in raspberry, blueberry, blackberry, cranberry and cherries.

3.3 Materials and Methods

3.3.1 Analytical reagents and standards

Phenolic compound standards were purchased from Toronto Research Chemicals (Toronto, Canada), Sigma-Aldrich (ON, Canada) and Cayman Chemical (Michigan, USA) (**Table 3.1 and Table 3.S2**). Four stable-isotope labeled standards ($^{13}C_6$-resveratrol, $^{13}C_3$-caffeic acid, D_3-ferulic acid and D_2-gallic acid) were purchased from Toronto Research Chemicals (ON, Canada). LC-grade formic acid (99%), acetonitrile, methanol and dimethyl sulfoxide were purchased from Fisher Scientific (ThermoFisher, Mississauga, ON, Canada). Ultrapure water (18.2 MΩ.cm, total organic carbon < 3 ppb) was generated from the Milli-Q purification system (Millipore, Bedford, MA, USA). Stock solutions of each analyte (1000 mg/L) were prepared using acetonitrile except for apigenin, luteolin, vitexin, isovitexin, vitexin-2-rhamnoside, daidzein, genistein, daidzin and aromadendrin, where a solvent mixture of dimethyl sulfoxide (10%) in acetonitrile was used. All stocks and working solutions were stored in amber glass vials at -20 °C until analysis to minimize degradation.

3.3.2 Fruit samples

Blackberry (*Rubus fruticosus,* PLU 4239), blueberry (*Vaccinium spp*., PLU 4240), cranberry (*Vaccinium macrocarpon,* PLU 4242), raspberry (*Rubus idaeus,* PLU 4054) and cherry (*Prunus spp*., PLU 4045) samples were purchased from Loblaw supermarkets from 3 different locations in Manitoba, Canada. Each type of fruit was pooled into a composite sample and a sub-sample was collected to determine moisture content using the standard oven drying method. The pooled berries and cherries were surface washed, freeze-dried and ground using mortar and pestle in a liquid nitrogen medium. The ground samples were stored at -80 °C until further analysis. Replicate sub-samples were taken from each composite sample for phenolic compound profiling.

3.3.3 Extraction of phenolic compounds

The extraction of phenolic compounds in berries and cherries was conducted according to Mustafa et al. (2022), with modifications and optimization of the parameters such as the sample amount, extraction solvent and the solvent/sample evaporation method to obtain higher recovery values for all the analyzed phenolic compounds. The phenolic compound extraction efficiency from the berries was tested with four different organic solvent mixtures as follows: methanol/water (60/40, v/v), methanol/water (80/20, v/v), acetone/water (60/40, v/v), acetone/water (80/20, v/v),

ethanol/water (60/40, v/v), ethanol/water (80/20, v/v), isopropanol/water (60/40, v/v) and isopropanol/water (80/20, v/v). All extractions were performed in replicates for method validation (n=11) and sample analysis using the validated method was performed in triplicates (n=3). The above solvents and ratios were selected based on previous studies conducted to extract phenolic compounds from different berry matrices (Boeing et al., 2014; Alara et al., 2021b). The sample extraction was optimized to quantify the maximum number of phenolic compounds at higher recovery levels. Freeze-dried berry samples (500 mg) were weighed into clean 15 mL Falcon tubes followed by the addition of a solvent mixture (6 mL) containing isopropanol: water (80:20, v/v) to achieve a matrix-to-solvent ratio of 1:12. A mixture (100 µL) of two internal standards (resveratrol $^{13}C_6$ and ferulic acid D_3) at a concentration of 1mg/mL was added to the sample-solvent mixture before extraction. The mixture was mixed thoroughly by inversion and extracted on a rotatory shaker at 60 rpm for 30 min, followed by sonication at 30 °C for 30 min. Then the samples were centrifuged (4000 ×g) at -4 °C for 30 min, separating the supernatant and filtered through a 0.2 µm nylon syringe-filters (Phenomenex, Torrance, USA) attached to a 10 mL disposable syringe into a clean 10 mL flat-bottomed tube. An aliquot (1.5 mL) of the extract was evaporated until complete dryness under a vacuum using a sample evaporator (Rocket Evaporator, Thermo Fisher, Mississauga, ON, Canada). The dried extract was reconstituted in methanol: water (50:50, v/v, 500µL) by sonicating (5 sec) and vortexing. After reconstitution, the extract was filtered again using a 0.2 µm nylon syringe filter into a clean LC vial. A mixture of internal standards (caffeic acid $^{13}C_3$ and gallic acid D_2) was added to the reconstituted extract and analyzed using a UHPLC (Vanquish, Thermo Fisher Scientific, Mississauga, Canada) coupled with an HRMS (ID-X Tribrid Orbitrap, Thermo Fisher Scientific, Mississauga, Canada).

3.3.4 Analytical instrumentation

Data acquisition using UHPLC-HRMS was achieved within 24 h of sample extraction and the samples were maintained at 4 °C in the sample compartment during analysis. A reverse-phase biphenyl column (2.6 µm, 100 x 2.1 mm, Kinetex, Phenomenex, USA) was used to separate analytes in the extract before mass spectrometric analysis. The column was maintained at 35 °C for the duration of the analysis and a solvent gradient with water as mobile phase A and acetonitrile as mobile phase B, both containing 0.1% formic acid as a modifier at a constant flow rate of 0.25

mL/min was used to elute the analytes through the column. The solvent gradient was as follows: 5% B; 0-0.5 min, 55% B; 0.5-14 min, 100% B; 14-16 min, isocratic conditions; 16-18.5 min, 5% B; 18.5-19 and returned to the initial conditions of 5% B; 19-20. The total run time of the method was 20 min. During the analysis, an autosampler injected 10 µL of sample extract into the column.

High-resolution mass spectrometer conditions were as follows: A heated electrospray ionization (HESI–II) was used to condition the solvent spray from the column using sheath, aux and sweep gas to achieve a stable, homogenous and charged spray. The simultaneous identification of 66 compounds was based on the m/z using the accurate masses of parent ions. The confirmation of each compound was based on the presence of fragment ions for each compound. A spray voltage of 3500 V for positive ions and 2200 V for negative ions was used in positive and negative ionization modes, respectively. The ion transfer tube was maintained at 325 °C while the vaporizer was set at 350 °C. Data was acquired using full scan mode for m/z 150 to 900 at 120 k resolution, followed by the intensity and data-dependent MS2 fragmentation at 30 k resolution. Both MS and MS2 data were acquired using Orbitrap. Fragmentation of parent ions was achieved by high-efficiency collision-induced dissociation (HCD) set at 20, 30 and 40%. Depending on the analyte, one or two fragment ions were chosen for analyte confirmation, while the accurate mass of the parent ion was used for quantitation. All analytes were infused in pure solvent to obtain fragmentation patterns in both negative and positive ionization modes.

3.3.5 Method validation parameters

The optimized method was validated according to the European Medicines Agency guidelines (Medicines Agency, 1922) on bioanalytical method validation for the LC-HRMS method using the linearity, limits of detection (LODs), limits of quantification (LOQs), recovery, stability, accuracy and precision as shown in **Table 3.2**. Linearity of the calibration standards range from 0.001, 0.002, 0.005, 0.01, 0.025, 0.05, 0.1, 0.2 and 0.25 µg/mL was tested using linear regression model with a weighting factor of $1/x^2$ and coefficient of correlation (r) was calculated and expressed as r^2 for all analytes (**Table 3.2**). Analyte stability was tested by analyzing samples immediately after sample extractions and reanalyzing them after holding them for 24 hours at 4 °C. The accuracy was measured by subtracting the actual concentration from the calculated concentration and expressing it in percentage values and coefficient of variance. The intra-day and

inter-day variation was measured using repeated injections of calibration standards on the same day (intra-day) and three consecutive days (inter-day), respectively. The inter-day standards were maintained at -20 °C after analysis each day. All accuracy and precision values were expressed as averages of relative standard deviations (RSD% = (s / x) × 100, where s = standard deviation and x = compound mean concentrations). The LODs and LOQs were measured using the calibration standards for the instrument LODs and LOQs. The LOD was calculated using the formula LOD = 3.3 × σ / S; where S is the slope of the calibration curve and σ is the standard deviation of the response. The LOQ was calculated by multiplying the LOD by 3.3. Fortification experiments were carried out to measure the recoveries for different berry matrices and cherries, tested at spiking levels of 25, 50 and 100 ng/mL. This was conducted by spiking the berry and cherry ground material at the concentrations and calculating recovery values.

3.3.6 Statistical analyses

All analyses were performed in 11 replicates for method validation parameters and in 3 replicates for sample analyses. The concentrations of all analytes were expressed on dry weight (DW) basis and all the results were expressed as mean ± standard deviation (SD). The linearity of the calibration curves was confirmed by performing regression analysis. One-way analysis of variance (ANOVA) was performed for individual phenolic compounds, followed by a Tukey's test for the multiple comparison. Box plots were used to summarize a data set for each phenolic compound, including mean and SD. Principal component analysis (PCA) was conducted at the correlation of α 0.05 to reduce the data set's dimensionality and identify the most important dimensions that capture the most variance among the tested berry types based on the concentrations of all quantified phenolic compounds. The quantified 66 phenolic compounds were treated as independent variables and five principal components were calculated. The number of principal components was determined based on the scree plot. A cluster analysis (CA) was performed, followed by the PCA to identify distinct patterns within the data set and to group berries based on the similarity level of 80%. All statistical analyses were performed using the Minitab software (version 17, Minitab LLC, PA, USA) and graphical representation was generated using Origin software (version 2022b MA, USA).

3.4 Results and Discussion

3.4.1 Optimizing the sample extraction

The extraction efficiency of the phenolic compound from various fruit matrices highly depends on the solvent used and its polarity (Alothman et al., 2009). Identifying the best solvent for extracting phenolic compounds from berry matrices is one of the most critical aspects of food and pharmaceutical research and their applications. Different phenolic compounds have varying solubility properties and may be found in different parts of the berry matrix (e.g., skin, seeds, pulp). Choosing the suitable solvent ensures efficient extraction of a wide range of phenolics, maximizing the yield and representing the actual phenolic content of the fruits tested. In the current study, four different organic solvents (methanol, ethanol, acetone and isopropanol), in combination with water at different ratios, were tested with stable-isotope labelled and non-labelled standard compounds. The recoveries (**Figure 3.1**) of different solvent combinations revealed that there is a significant ($p<0.05$) effect on the phenolic compounds extraction recoveries from the type of solvent used and the ratio of solvent/water. The lowest recoveries (44-66 %) were obtained from the ethanol/water combinations for the phenolic compounds and their internal standards used in the current study. Higher and reproducible recoveries (97±1.33% to 107±1.02%) were obtained with isopropanol/water (80/20, v/v) and these recoveries were significantly higher ($p<0.05$) compared to the other solvent combinations (**Figures 3.1 and 3.S2**). Even though the recoveries obtained from acetone/water (80/20, v/v) (recovery, 77% to 95%) were similar to isopropanol/water (80/20, v/v); however, higher variation was observed (±13.4 SD) in the case of acetone/water. The standard deviations were lower in isopropanol/water (80/20, v/v). The recoveries for resveratrol $^{13}C_6$ and ferulic acid D_3 are shown in (**Figures 3.1a and 3.1b**). Organic solvents, when used as pure extraction solvents, methanol had better extraction efficiency compared to water, ethanol and acetone (Alothman et al., 2009). However, aqueous solutions of organic solvents are considered the most effective in extracting phenolic compounds compared to pure solvents. Venkatesan et al. (2019) found that isopropanol with water was most efficient in extracting phenolic compounds among other solvents tested. In our study, isopropanol/water (80/20, v/v) showed higher extraction efficiency than the other solvent combinations for the compounds tested. For instance, afzelin, herbacetin and quercetin recoveries were 98%, 87% and 95%, respectively. Even though acetone/water (80/20, v/v), combinations achieved higher extraction efficiencies for kaempferol

and caffeic acid, the variation (±9.22 vs ±0.21) between samples was high compared to isopropanol/water (**Figures 3.S2b and 3.S2f**). Ethanol and methanol solvents in combination with water showed the lowest extraction efficiencies compared to the isopropanol and acetone solvent combinations with water (p<0.05). Therefore, this study optimized the analytical method further using an isopropanol/water (80/20, v/v) solvent mixture for 66 phenolic compounds from different berry matrices. The recoveries obtained with different berry and cherry matrices ranged from 83% to 126% with the combination of isopropanol/water (80/20, v/v) (**Table 3.2 and Figure 3.S2**). The results indicated that the isopropanol/water (80/20, v/v) solvent mixture efficiently extracted phenolic compounds identification and quantification in these small fruits.

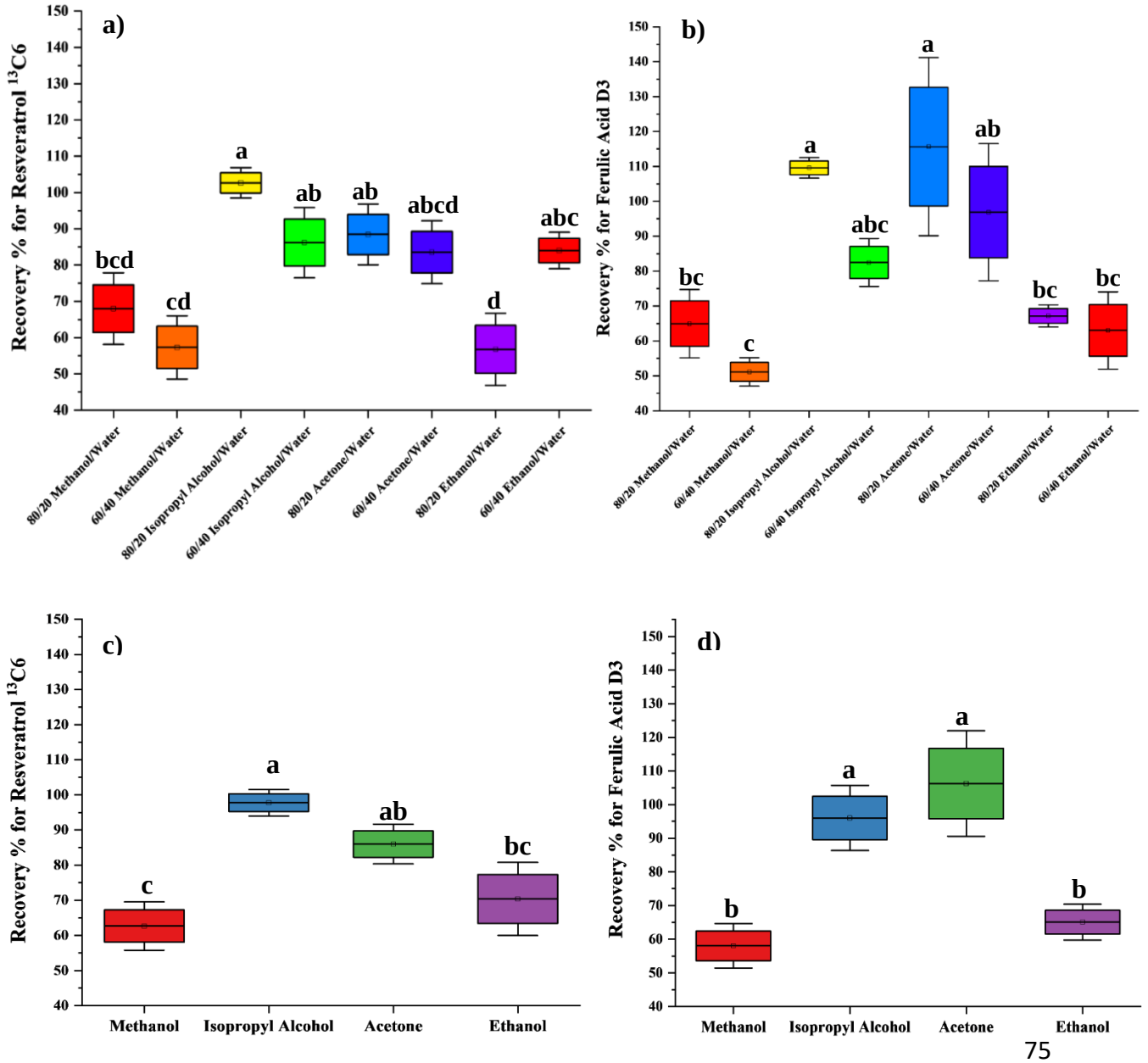


Figure 3.1: Recovery percentages of resveratrol $^{13}C_6$ and ferulic acid D_3 obtained from the different solvent-water combinations; methanol/water (60/40, v/v), methanol/water (80/20, v/v), acetone/water (60/40, v/v), acetone/water (80/20, v/v), ethanol/water (60/40, v/v), ethanol/water (80/20, v/v), isopropanol/water (60/40, v/v) and isopropanol/water (80/20, v/v). **a)** Recovery percentages obtained for resveratrol $^{13}C_6$; **b)** Recovery percentages obtained for ferulic acid D_3; **c)** Recovery percentages comparison among methanol, isopropanol, acetone and ethanol for resveratrol $^{13}C_6$; and **d)** Recovery percentages comparison among methanol, isopropanol, acetone and ethanol for ferulic acid D_3; The line within the box plots shows the mean recovery percentage. The box plots in each graph contain different letters if there is a significant difference ($p<0.05$) in the mean values of the recovery percentages based on Tukey's test

3.4.2 Optimization of the UHPLC-HRMS for phenolic compound analysis

All phenolic compound analytes were separated chromatographically using UHPLC with a bi-phenyl column enabling identification and quantification, with $^{13}C_6$-resveratrol, $^{13}C_3$-caffeic acid, D_3-ferulic acid and D_2-gallic acid as stable-isotope labelled internal standards. The analytical method was optimized to achieve high and reproducible recoveries with the ability to identify and quantify various analytes in the fruit matrices. Two chromatographic columns, namely, the C18 column (Phenomenex, Torrance, CA, USA) and the reverse-phase Kinetex biphenyl column, which has a particle size of 2.6 μm, 100 x 2.1 mm (Phenomenex, Torrance, CA, USA) were tested to obtain better separation and resolution of analyte peaks. Along with columns, different solvent mixtures (acetonitrile/water 100/0 (v/v), acetonitrile/water 50/50 (v/v), methanol/water 100/0 (v/v), methanol/water 50/50 (v/v) for the sample extract resuspension and gradient flow rates (0.25 and 0.3 mL/min) were tested. Nonetheless, the stationary phase significantly impacts the sensitivity and selectivity of phenolic compounds (Liaudanskas et al., 2017). Based on the obtained data, the biphenyl column showed the best separation of phenolic compounds compared to the C18 column with the methanol/water 50/50 (v/v) combination at a flow rate of 0.25 mL/minute. A gradient program of 5% B; 0-0.5 min, 55% B; 0.5-14 min, 100% B; 14-16 min, isocratic conditions; 16-18.5 min, 5% B; 18.5-19, isocratic condition; 19-20 was tested with two different combinations of mobile phases; 0.1% formic acid in water as the mobile phase A and 0.1% formic acid in methanol as the mobile phase B and 0.1% formic acid in water as the mobile phase A and 0.1% formic acid in acetonitrile as the mobile phase B. The optimum separation of the 66 phenolic compounds was achieved with the mobile phase combination of 0.1% formic acid in water as the mobile phase A and 0.1% formic acid in acetonitrile as the mobile phase B and the separation of compounds with symmetric peaks was achieved within 20 minutes. Phenolic compound separation at different compound concentration levels was achieved with two stationary phases and chromatograms were obtained to compare the peak intensities and peak separation and resolutions. Apart from the early eluting compounds, the chromatogram obtained from the C18 column was lower in intensity for each eluted phenolic compound than the bi-phenyl column (data not shown). The chromatograms obtained on the bi-phenyl column showed higher intensities and better separation, with symmetric peaks. Therefore, the bi-phenyl column was used in the current phenolic compounds analysis.

Accurate masses and m/z of all parent and fragment ions were obtained by infusing all the analytes (**Table 3.1)**. The compounds which shared the same parent masses (nicotiflorin and vicenin-2, afzelin and vitexin/isovitexin, herbacetin and quercetin, apigenin and genistein, luteolin and kaempferol) were separated based on their respective major fragment ions as well as retention time on the column (**Figure 3.S1**). (+)-Catechin, (-)-epicatechin pair and isovitexin, vitexin pair had similar parent masses and retention times. Therefore, those compounds were quantified together in the current analysis, as differentiation was impossible. Few other compounds had the same parent mass but different major fragment ions. For example, nicotiflorin and vicenin-2 with m/z 593.1511 had fragment ions m/z 285.0397 for nicotiflorin, while m/z 473.1082, m/z 503.1188 and m/z 353.0661 for vicenin-2. Similarly, kaempferol-3-glucoside and orientin have the same parent mass of m/z 447.0932, but the fragments were m/z 284.0323, m/z 285.0402, m/z 327.0808 for kaempferol-3-glucoside and m/z 327.0506, m/z 357.061 for orientin, respectively. Afzelin, isovitexin and vitexin share the same mass of m/z 431.0983, but afzelin has fragments that are different from vitexin and isovitexin (afzelin, m/z 285.0401, isovitexin and vitexin, m/z 311.0556 and m/z 341.0659). Herbacetin and quercetin have the same mass of m/z 301.0353 but have different fragment ions of m/z 272.0279 and m/z 178.9983, m/z 151.0033, respectively. Apigenin and genistein have the same parent mass of m/z 269.0455 but the fragments included m/z 225.0551, m/z 201.0551 and m/z 149.0239 for apigenin and m/z 173.4916, m/z 159.0446, m/z 133.0291, m/z 225.0551, m/z 241.0503 for genistein. Lastly, fisetin, kaempferol and luteolin have the same parent mass of m/z 285.0404 but have different fragment ion profiles. For instance, fisetin has the fragment ions of m/z 163.0034, m/z 135.0085, m/z 257.0452 and m/z 241.0503, while kaempferol has the fragment ions of m/z 151.0034 and m/z 257.0452. Even though luteolin has the same parent mass as fisetin and kaempferol it has fragments of m/z 241.0499, m/z 199.0895, m/z 175.0395 and m/z 157.0031. The degree to which the changes in intensities and profiles of the different fragment ions form in compounds that share the same parent mass depends on the compound structures and the stability of the compounds and different structures inevitably fragment differently (Li et al., 2022). Despite the effectiveness of advanced HRMS tools, certain substantial issues exist without resolution, leading to potential drawbacks in the reliable identification of the analytes. Notably, the influence of ionic enhancement/suppression effects and the presence of isomeric compounds that elute at same retention time and similar fragments. Consequently, ensuring the proficient chromatographic separation of all analytes of interest

including isomers, emerges as an utmost consideration (Lucci et al., 2017). **Figure 3.S1** shows the spectral information of the compounds with the same parent m/z but different major fragments.

The HESI ionization of the parent ions was monitored in both deprotonated $[M-H]^-$ or protonated $[M+H]^+$ forms for each compound's ability to form stable ions. Among the 66 compounds analyzed, 53 were more stable and sensitive in the negative ionization mode, while 13 compounds, including all anthocyanins, were monitored in the positive ionization mode (**Table 3.1**). The newly developed UHPLC-HRMS method has greater advantages compared to the existing methods because the number of compounds analyzed with a relatively short elution gradient compared to the existing methods. Some of the existing methods developed with LC-MS techniques are confined only to a few phenolic compound classes such as flavonoids (Vankova et al., 2019), phenolic acids and aglycones (Subbiah et al., 2021). For instance, the newly developed HPLC-MS/MS method by Mustafa et al. (2022), could analyze only 36 compounds within 32 min. Further, the developed methods using LC-ESI-QTOF-MS/MS showed a total method run time of 85 min to detect around 65 phenolic compounds (Subbiah et al., 2021). In addition, most of the studies focused on in vitro analysis of the total contents of phenolic, flavonoids and anthocyanin without in-detailed analyses of composition of different phenolic classes (De Souza et al., 2014).

3.4.3 Method validation

The method validation parameters were obtained as stated in the European Medicines Agency guidelines before the actual sample analysis. The method for 66 phenolic compounds was validated on various parameters (**Table 3.2**). The calibration curves for each analyte with 0.001-0.25 µg/mL had a linearity of R^2 =0.99. The stability of the compounds was tested under freeze-thaw cycles and stable temperature (at 4 ^oC) and in both conditions, the RSD% values varied ≤1% (**Table 3.2**). The relative standard deviation (RSD%) values for the intra-day and inter-day accuracy ranged from 0.02%-3.92% for intra-day and 0.52%-4.98% for inter-day, respectively. The spiking of low, medium and high concentration levels of all 66 analytes was analyzed with acceptable values for accuracy in repeated measurements. Lower RSD% values indicate the stability of the tested compounds and the repeatability of the method. The LODs for compounds ranged from 0.0004-0.05 µg/mL. The LOQs were at the ranges of 0.001-0.1 µg/mL. Obtaining

lower values for both LODs and LOQs shows the higher sensitivity of the instrument to detect the selected compounds. The recoveries obtained for all the berry and cherry matrices ranged from 75%-130%, confirming the efficiency of the phenolic compounds extraction method (**Table 3.2)**. In the current study, the developed UHPLC-HRMS method exhibited acceptable accuracy, reliability and specificity in quantifying the phenolic compounds in these small fruits. By employing multiple standards, we could identify and quantify a more comprehensive range of phenolic compounds present in blueberries, raspberries, cranberries, blackberries and cherries, including those that may have been overlooked in traditional analyses with only one standard as a reference. This approach substantially reduced the risk of underestimating or overestimating the phenolic content in berries, thus providing more reliable and accurate results. On the other hand, the developed method could detect and quantify phenolic compounds in both berries and cherries matrices.

Table 3.1 The 66 phenolic compounds acquisition parameters for the UHPLH-HRMS analysis

Sl. No.	Retention time (min)	Compound Name	Ionization mode	Accurate molecular mass	Precursor m/z	Fragments m/z*
1	8.10	Afzelin[3]	Negative	432.1062	431.0984	285.0401
2	10.33	Apigenin[1]	Negative	269.0456	269.0456	225.0551, 201.0551, 149.0239
3	1.82	Arbutin[3]	Negative	272.0902	271.0823	161.0452, 203.9364
4	8.07	Aromadendrin[3]	Negative	288.0639	287.0561	259.0608, 243.0659
5	4.93	Caffeic acid[1]	Negative	180.0428	179.035	135.0449
6	3.80	Caftaric acid[1]	Negative	312.0487	311.0409	179.0344, 149.0086
7	5.43	(+)-Catechin[1]	Negative	290.0785	289.0718	245.0811, 205.0499, 173.4917, 179.0344
8	4.68	Chlorogenic acid[1]	Negative	354.0956	353.0878	191.0559
9	4.50	Cyanidin[3]	Positive	287.055	285.040	246.9308, 213.0542, 259.0598
10	5.49	Cyanidin-3-arabinoside[1]	Positive	419.0973	419.0973	284.0325
11	5.08	Cyanidin-3-glucoside[1]	Positive	449.1078	449.1078	287.0547
12	4.93	Cyanidin-3-rutinoside[1]	Positive	595.1658	595.1658	287.054
13	8.78	Daidzein[1]	Negative	254.0585	253.0506	227.0698, 199.0748
14	6.17	Daidzin[1]	Negative	416.1113	415.1035	255.0647, 338.3414
15	6.73	Delphinidin[1]	Positive	303.0499	303.0499	257.044, 229.0492
16	3.00	Delphinidin-3-glucoside[1]	Positive	465.1033	465.1033	337.0562, 301.0352, 418.9732
17	6.69	Ellagic acid[2]	Negative	302.0068	300.999	257.0089, 229.014, 185.0242
18	5.48	(-)Epicatechin[1]	Negative	290.0796	289.0718	245.0815, 205.0502
19	7.13	(-)-Epicatechin gallate[1]	Negative	442.0895	441.0827	289.0712, 169.0318
20	3.56	(-)-Epigallocatechin[1]	Negative	306.0734	305.0667	179.0347, 221.0453
21	6.76	Ferulic acid[2]	Negative	194.0585	193.0506	149.0603
22	10.39	Fisetin[3]	Negative	286.0483	285.0405	163.0034, 135.0085, 257.0452, 241.0503
23	2.20	Gallic acid[1]	Negative	170.0221	169.0143	125.0241
24	10.31	Genistein[1]	Negative	270.0534	269.0456	159.0446, 133.0291, 225.0551, 241.0503
25	6.27	Glycitein[3]	Negative	284.0684	283.0606	351.0483
26	6.24	Glycitin[3]	Negative	446.1219	445.114	283.0612, 282.053

27	8.90	Herbacetin[3]	Negative	302.0432	301.0354	272.0279
28	10.92	Hesperetin[3]	Negative	302.0796	301.0718	286.0483
29	6.79	Isoquercetin[1]	Negative	464.096	463.0882	301.0346
30	10.68	Isorhamnetin[3]	Negative	316.0589	315.051	315.0513, 300.0278
31	8.04	Isovitexin[1]	Negative	431.0984	431.0984	311.0556, 341.0659
32	10.49	Kaempferol[1]	Negative	286.0483	285.0405	151.0034, 257.0452
33	7.27	Kaempferol-3-glucoside[3]	Negative	448.1011	447.0933	284.0323, 285.0402, 327.0808
34	9.23	Liquiritingenin[3]	Negative	256.0741	255.0663	135.0086
35	10.43	Luteolin[1]	Negative	286.0472	285.0405	241.0499, 199.0895, 175.0395, 157.0031
36	4.67	Malvidin[1]	Positive	331.0819	331.0819	315.0499, 316.0579, 299.0552, 287.0552
37	5.59	Malvidin-3-glucoside[1]	Positive	493.1326	493.1326	331.0802
38	4.71	Malvin[1]	Positive	655.1854	655.1854	493.1328, 331.0802
39	5.90	Myricetin[1]	Negative	318.037	317.0303	178.9982, 151.0033
40	7.69	Naringin[3]	Negative	580.1798	579.1719	459.114
41	5.50	Nicotiflorin[1]	Negative	594.159	593.1512	285.0397
42	7.29	Orientin[3]	Negative	448.1011	447.0933	327.0506, 357.061
43	8.03	Okanin[3]	Negative	288.0633	287.0561	135.0440
44	6.07	Para-coumaric acid[1]	Negative	164.0479	163.0401	119.0499
45	10.29	Pelargonidin[3]	Positive	271.0594	271.0594	193.1306
46	8.78	Peonidin[3]	Positive	301.0712	301.0712	286.0468, 284.0321
47	5.57	Peonidin-3-glucoside[1]	Positive	463.1235	463.1235	301.0702
48	7.08	Petunidin[3]	Positive	317.0655	317.0655	302.042
40	7.76	Piceatannol[3]	Negative	244.0741	243.0663	225.0557, 201.0556
50	6.84	Polydatin[3]	Negative	390.132	389.1242	227.0712
51	7.44	Procyanidin A2[3]	Negative	576.1273	575.1195	423.0718, 449.0873, 289.0712
52	5.07	Procyanidin B2[3]	Negative	578.143	577.1352	425.0878, 407.0765, 289.0712
53	6.35	Procyanidin C2[3]	Negative	866.2064	865.1985	695.1401, 577.1348, 739.166, 713.166
54	3.24	Protocatechuic acid[1]	Negative	154.0272	153.0193	109.0291
55	8.93	Quercetin[1]	Negative	302.0432	301.0354	151.0033
56	6.80	Quercetin-3-galactoside[1]	Negative	464.096	463.0882	301.0349, 300.0271

57	6.87	Resveratrol[1]	Negative	228.0792	227.0714	185.0605
58	7.72	Rhapontin[3]	Negative	420.1426	419.1348	257.0817
59	6.60	Rutin[1]	Negative	610.1528	609.1461	301.0349
60	6.93	Sinapic acid[1]	Negative	224.069	223.0612	179.071, 164.0475
61	5.21	Syringic acid[1]	Negative	198.0534	197.0456	182.0216, 153.0551, 138.0317
62	6.96	Taxifolin[3]	Negative	304.0589	303.051	285.04
63	6.09	Vanillic acid[2]	Negative	168.0428	167.035	123.0446
64	5.49	Vicenin-2[3]	Negative	594.159	593.1512	473.1082, 503.1188, 353.066
65	8.11	Vitexin[1]	Negative	432.1062	431.0984	311.0552
66	6.43	Vitexin-2-rhamnoside[1]	Negative	578.163	577.1563	413.0871

All the fragment ions identified in the study were used for compound confirmations.

[1]Chemicals purchased from Toronto Research Chemicals (Toronto, Canada); [2]Chemicals purchased from Sigma-Aldrich (ON, Canada); [3]Chemicals purchased from Cayman Chemical (Michigan, USA)

[*] Fragments are listed in the order of observed intensities

Table 3.2 Method validation parameters for the 66 phenolic compounds using ultra-high performance liquid chromatography high-resolution mass spectrometry

Sl. No.	Compound Name	Concentration range (µg/mL)	Linearity (R^2)	LOD (µg/mL)	LOQ (µg/mL)	Recovery (%)*					Repeatability (RSD %, n=3)	
						Blackberry	Blueberry	Cherry	Raspberry	Cranberry	Intra-day	Inter-day
1	Afzelin	0.001-0.25	0.9991	0.0009	0.0027	126	121	115	121	117	0.02	0.88
2	Apigenin	0.002-0.25	0.9987	0.0014	0.0042	110	113	110	118	120	0.01	0.92
3	Arbutin	0.005-0.25	0.9971	0.0031	0.0094	88	86	87	81	91	0.18	0.99
4	Aromadendrin	0.002-0.25	0.9923	0.0012	0.0036	103	110	101	112	109	0.02	0.52
5	Caffeic acid	0.005-0.25	0.9981	0.0021	0.0064	93	90	97	99	100	1.14	2.78
6	Caftaric acid	0.002-0.25	0.9997	0.0018	0.0055	98	87	99	98	97	0.76	1.69
7	(+)-Catechin	0.002-0.25	0.9977	0.0011	0.0034	120	100	112	118	121	2.13	3.21
8	Chlorogenic acid	0.002-0.25	0.9969	0.0017	0.0052	115	118	109	100	119	3.92	4.14
9	Cyanidin	0.005-0.25	0.9968	0.0029	0.0089	103	101	98	101	100	1.98	3.43
10	Cyanidin-3-arabinoside	0.005-0.25	0.9963	0.0025	0.0077	108	102	101	110	103	2.11	4.12
11	Cyanidin-3-glucoside	0.005-0.25	0.9976	0.0024	0.0073	115	107	110	118	120	1.89	3.98
12	Cyanidin-3-rutinoside	0.005-0.25	0.9978	0.0029	0.0089	127	119	120	124	110	2.14	4.67
13	Daidzein	0.002-0.25	0.9993	0.0012	0.0037	100	100	98	101	110	0.02	1.12
14	Daidzin	0.005-0.25	0.9931	0.0034	0.0104	98	87	97	96	100	0.01	0.99
15	Delphinidin	0.010-0.25	0.9952	0.0063	0.0189	86	90	87	89	100	1.14	1.98
16	Delphinidin-3-glucoside	0.005-0.25	0.9956	0.0042	0.0128	88	91	98	88	101	1.78	3.56
17	Ellagic acid	0.050-0.25	0.9953	0.0328	0.0993	89	87	90	88	87	2.34	4.11
18	(-)-Epicatechin	0.002-0.25	0.9997	0.0012	0.0029	128	110	120	121	100	2.89	3.76

19	(-)-Epicatechin gallate	0.005-0.25	0.9997	0.0025	0.0075	103	110	104	99	100	2.67	4.18
20	(-)-Epigallocatechin	0.005-0.25	0.9976	0.0041	0.127	109	114	112	100	98	2.13	3.98
21	Ferulic acid	0.005-0.25	0.9906	0.0049	0.1504	93	98	100	99	97	1.13	1.97
22	Fisetin	0.005-0.25	0.9956	0.0034	0.0091	86	90	92.4	87	86	0.05	1.15
23	Gallic acid	0.005-0.25	0.9973	0.0033	0.01	78	75	81	79	91	0.17	1.99
24	Genistein	0.005-0.25	0.9973	0.0029	0.0088	101	99	104	98	105	0.04	1.08
25	Glycitein	0.005-0.25	0.9956	0.0032	0.0057	99	100	98	104	110	0.08	1.17
26	Glycitin	0.005-0.25	0.9924	0.0022	0.0062	97	98	100	98	113	0.19	1.36
27	Herbacetin	0.005-0.25	0.9975	0.0025	0.0077	88	90	100	97	88	1.03	2.37
28	Hesperetin	0.001-0.25	0.9996	0.0006	0.0017	83	78	91	79	98	0.09	2.19
29	Isoquercetin	0.001-0.25	0.9997	0.0004	0.0011	109	113	105	100	107	0.98	1.99
30	Isorhamnetin	0.001-0.25	0.9995	0.0004	0.0012	97	98	100	97	100	1.12	2.79
31	Isovitexin	0.002-0.25	0.9991	0.0012	0.003	104	100	100	101	112	0.06	1.47
32	Kaempferol	0.002-0.25	0.9951	0.0019	0.0058	107	98	100	114	115	0.36	2.98
33	Kaempferol-3-glucoside	0.001-0.25	0.9996	0.0009	0.0026	110	100	102	107	99	0.57	1.69
34	Liquiritingenin	0.001-0.25	0.9991	0.0008	0.0025	100	100	97	101	115	0.08	1.19
35	Luteolin	0.002-0.25	0.9952	0.0019	0.0057	94	110	101	98	100	0.17	2.18
36	Malvidin	0.005-0.25	0.9964	0.0033	0.01	89	90	79	101	103	1.34	3.45
37	Malvidin-3-glucoside	0.002-0.25	0.9972	0.001	0.004	87	92	77	99	99	1.86	2.97
38	Malvin	0.010-0.25	0.9991	0.0068	0.0206	99	100	104	99	100	1.97	4.11
39	Myricetin	0.005-0.25	0.9992	0.0037	0.0111	100	89	97	112	102	0.11	2.11
40	Naringin	0.001-0.25	0.9995	0.0008	0.0025	104	98	102	98	112	0.65	1.97
41	Nicotiflorin	0.002-0.25	0.9995	0.0014	0.0042	111	100	103	107	98	0.88	2.36
42	Okanin	0.002-0.25	0.9987	0.0011	0.0034	121	101	98	77	80	0.26	1.12
43	Orientin	0.001-0.25	0.9994	0.0009	0.0027	85	78	89	92	99	0.76	2.67
44	Para-coumaric acid	0.005-0.25	0.9931	0.0023	0.007	90	92	79	80	95	0.92	3.11
45	Pelargonidin	0.005-0.25	0.9948	0.0043	0.0272	79	88	89	79	100	1.45	2.23

46	Peonidin	0.001-0.25	0.9997	0.0008	0.0023	79	85	90	91	94	2.11	3.75
47	Peonidin-3-glucoside	0.002-0.25	0.9998	0.0018	0.0055	82	91	78	85	89	2.67	3.89
48	Petunidin	0.050-0.25	0.9912	0.0325	0.0985	85	77	84	90	87	2.53	2.97
49	Piceatannol	0.005-0.25	0.9983	0.0027	0.0083	119	100	99	115	121	0.97	2.31
50	Polydatin	0.005-0.25	0.9995	0.0023	0.0059	120	101	109	100	118	0.53	1.99
51	Procyanidin A2	0.005-0.25	0.9987	0.0035	0.0105	127	120	100	107	119	0.33	2.16
52	Procyanidin B2	0.002-0.25	0.9994	0.0013	0.0041	121	119	109	101	120	0.54	1.98
53	Procyanidin C2	0.005-0.25	0.9956	0.0045	0.0135	119	121	118	104	119	0.68	1.67
54	Protocatechuic acid	0.020-0.25	0.9987	0.0188	0.0568	97	89	79	81	100	0.83	2.49
55	Quercetin	0.005-0.25	0.9907	0.0025	0.0077	108	101	99	100	99	0.97	1.74
56	Quercetin-3-galactoside	0.005-0.25	0.9912	0.0042	0.0122	110	106	101	99	111	0.42	1.39
57	Resveratrol	0.010-0.25	0.9981	0.0087	0.0294	94	89	79	78	81	0.11	0.98
58	Rhapontin	0.002-0.25	0.9964	0.0017	0.054	100	98	104	107	116	0.73	1.11
59	Rutin	0.005-0.25	0.9969	0.0021	0.0063	125	131	128	120	121	1.29	3.26
60	Sinapic acid	0.005-0.25	0.9938	0.0032	0.0999	77	80	91	98	100	2.18	3.77
61	Syringic acid	0.010-0.25	0.9937	0.0053	0.1546	78	81	94	101	98	2.76	4.57
62	Taxifolin	0.002-0.25	0.9991	0.0014	0.0032	114	107	101	113	100	0.04	1.13
63	Vanillic acid	0.01-0.25	0.9923	0.0082	0.0249	82	79	90	77	100	1.18	3.55
64	Vicenin-2	0.002-0.25	0.9952	0.0011	0.0029	119	103	110	99	113	0.03	1.00
65	Vitexin	0.002-0.25	0.9991	0.0012	0.003	120	117	102	120	122	0.09	1.21
66	Vitexin-2-rhamnoside	0.005-0.25	0.9929	0.0043	0.0121	114	101	98	102	118	0.02	0.98

LOD: Limit of Detection; LOQ: Limit of Quantification; RSD%: Relative Standard Deviation; Intra-day Repeatability: Analysed within the same day; Inter-day Repeatability: Analysed during three consecutive days; *Spiking level 0.025 µg/mL into the berry powder

3.4.4 Quantification of the phenolic compounds in blackberry, blueberry, raspberry, cranberry and cherry

3.4.4.1 Flavonols

Several flavonols were identified, including quercetin, isoquercetin, quercetin-3-galactoside, kaempferol, kaempferol-3-glucoside, myricetin, fisetin and rutin in berries (**Figure 3.2a)**. The highest total flavonol content reported in the current study was in blackberries (137.6 mg/kg), followed by cherries (127.8 mg/kg). Blueberries had a total flavonol content of 53.74 mg/kg and raspberries had a content of 19.3 mg/kg. These contents were similar to the results obtained by Liu et al. (2020), that the total flavonol content in blueberries is almost twice the flavonols found in raspberries. Cranberries contained the lowest (10.3 mg/kg) total flavonol content among all the tested berries. Among the total flavonols, rutin had the highest concentration across all the berries. It was followed by quercetin, quercetin-3-galactoside and isoquercetin in decreasing order of concentrations. Cherries contained the highest rutin content at 109±3 mg/kg (p<0.05), compared to berries. Damin et al. (2019) reported similar concentrations of rutin in the range of 43.2 mg/kg and 162.4 mg/kg in cherries, followed by blackberries with a rutin content of 69.8±0.34 mg/kg. There was no significant difference in rutin content between blueberries (6.94±0.09 mg/kg) and raspberries (6.13±0.09 mg/kg). Previously, Diaconeasa et al. (2014), reported rutin content in blueberries at 65.96±1.28 mg (rutin/chlorogenic acid eq.± sd/100 g on fresh weight, FW), approximately 10 times higher than the current study. This was due to the inclusion of chlorogenic acid equivalent under rutin (Diaconeasa et al., 2014). In the same study, the authors also reported that rutin concentrations were higher compared to previous studies. Among all the berries tested, cranberries had the lowest rutin content, reported as 3.35 mg/kg. The quercetin content was also significantly different among each berry type, as shown in **Table 3.3**. The highest was in blackberries (35.3±0.13 mg/kg) followed by raspberry (10.0±0.18 mg/kg), cherry (4.20±0.01 mg/kg), blueberry (1.55±0.02 mg/kg) and cranberry (0.30 mg/kg). The highest quercetin-3-galactoside and myricetin contents at 20.6 mg/kg and 3.5±0.10 mg/kg, respectively, were found in blueberries. Fisetin, kaempferol and kaempferol-3-glucoside were found in lower quantities than the other flavonols among all berries. The highest kaempferol content was detected in raspberries (1.78 mg/kg) (p<0.05), while the highest kaempferol-3-glucoside content was found in blackberries (1.59 mg/kg) (p<0.05). In blackberries, previous studies have found that in 100g of

ripened blackberries (dry weight basis), the content of isoquercetin was 1888.51 ± 2.76 µg, kaempferol (399.48 ± 5.03 µg), quercetin (5509.61 ± 135.05 µg) and rutin in minor quantities (Schulz et al., 2019). The content of kaempferol and kaempferol -3-glucoside content found in the current study was very similar to the content found in Shulz et al. (2019). Lopez-Corona et al. (2022) reported the presence of high contents of quercetin-3-glucoside and kaempferol-3-glucoside which were responsible for the high antioxidant activities of the raspberries. The highest fisetin content was found in raspberries, which was at 0.08 mg/kg) (p<0.05). Myricetin, quercetin and kaempferol are flavonols often found in greater quantities in blueberries and raspberries (De Souza et al., 2014; Mustafa et al., 2022).

3.4.4.2 Flavones

Flavones included afzelin, apigenin, arbutin, herbacetin, isorhamnetin, isovitexin, luteolin, orientin, taxifolin, vicenin-2, vitexin and vitexin-2-rhamnoside in the current study (**Table 3.3**). Total flavones detected in blackberries, raspberries, cherries, blueberries and cranberries were 37.1 mg/kg, 22.4 mg/kg, 10.5 mg/kg, 4.60 mg/kg and 2.0 mg/kg, respectively. Literature data was not available for the total flavone content in berries; however, afzelin (12.2±1.2 µg/g on dry weight, DW±SD) and arbutin (1519±10.3 µg/g DW±SD) on a dry weight basis were reported in the early stages of lingonberries and at the non-vegetative stage of the plant the arbutin content was reduced to 69.3±3.2 µg/g DW±SD (Vilkickyte & Raudone, 2021) while 0.024 to 0.030 mg/kg (afzelin) and 0.157 to 0.580 mg/kg (arbutin) were observed in the berries tested in the current study, significantly lower than those in lingonberries. Arbutin was reported below the limit of detection in blueberries by Durak et al. (2020). Herbacetin, on the other hand, is mostly found in herbal plants such as *Rhodiola rosea L.* (genus *Rhodiola*) (Diniz et al., 2020). Among the flavones tested in the five commercial berry types, herbacetin was found in higher quantities (**Figure 3.S3b**). The highest herbacetin content (p<0.05) was found in blackberries (34.6±0.85 mg/kg), followed by raspberry (10.3±0.05 mg/kg), cherry (7.69±0.03 mg/kg), blueberry (1.34±0.02 mg/kg) and cranberry (0.30 mg/kg). The highest orientin content (p<0.05) was also found in blackberries (1.49 mg/kg) compared to the other tested berries. The highest isorhamnetin content was found in blueberries (2.11 mg/kg), while cherries had the highest (p<0.05) vicenin-2 content (0.18 mg/kg) compared to the tested berry types.

3.4.4.3 Flavans

The total flavans found in the current study showed that the highest total content of flavans is present in cranberry (62.02 mg/kg), followed by blackberry (46.85 mg/kg). Cherry contained a total flavan content of 31.14 mg/kg, while the lowest content was found in blueberry (2.81 mg/kg). Raspberry had a total flavan content of 16.82 mg/kg. Among these flavans, (-)-epigallocatechin was present in smaller quantities compared to (+)-catechin and (-)-epicatechin in berries, as shown in **Figure 3.2e**. The highest (+)-catechin and (-)-epicatechin content were found in cranberry (p<0.05) (both (+)-catechin and (-)-epicatechin accounting for 61.9±0.80 mg/kg), but the highest (p<0.05) (-)-epigallocatechin at 0.77 mg/kg was found in blueberry among all the tested berries. The higher contents of (+)-catechin (3.3–6.1 mg/kg FW) and (-)-epicatechin (24.5–44.6 mg/kg FW) were previously reported by Nemzer et al. (2022) in cranberry. The lowest content of (+)-catechin was found in cherry (0.02 mg/kg), which was not significantly different from the amount detected in blackberry (0.03 mg/kg) (Nemzer et al., 2022). Berries, such as blueberries and cranberries, are rich sources of procyanidins and these compounds have been studied for their potential health benefits, including anti-inflammatory and anticancer effects (Hu et al., 2021). Nemzer et al. (2022) also found catechin and epicatechin in different varieties of cranberry berry samples where (-)-epicatechin constitutes the primary component of proanthocyanidins, with (+)-catechin and (-)-epigallocatechins present in minor quantities. Among the cranberry species studied, *Vaccinium vitis-idaea* displayed the highest total content, with 15.48 mg/100 g FW for the European variety and 17.68 mg/100 g FW for the Canadian variety (Nemzer et al., 2022). *Vaccinium macrocarpon* varieties showed a range of 2.80–5.05 mg/100 g FW, while the lowest concentrations were observed in *Vaccinium oxycoccus* varieties, ranging from 0.55 to 1.94 mg/100 g FW. These findings indicate that depending on the variety and the geographical locations flavans contents could be different. The amount found in the Canadian variety shows similar results as identified in the current study. As shown in **Figure 3.2f,** procyanidin A2, B2 and C2 were detected in berries; however, compared to the B2 and C2, A2 was presented in smaller amounts. Procyanidin B2 was found to be the most abundant procyanidin present in all the berries tested and with the highest content (p<0.05) in blackberries (90.5±1.15 mg/kg). The highest (p<0.05) procyanidin C2 content was found in cranberries (80.6±0.14 mg/g).

3.4.4.5 Hydroxybenzoic acids

Hydroxybenzoic acid is a benzoic acid derivative with one or more hydroxyl (-OH) groups attached to the benzene ring (Alara et al., 2021a). These can be found in berries, along with other fruits and vegetables. These are part of the larger group of polyphenolic compounds and contribute to the antioxidant capacity of these foods. Berries, being rich in polyphenols, are known to contain various hydroxybenzoic acids such as gallic acid, protocatechuic acid, vanillic acid and syringic acid (Alara et al., 2021a; Mustafa et al., 2022). In the current study, cherries had the highest total hydroxybenzoic acids content (3.14 mg/kg), followed by blackberries (2.01 mg/kg), blueberries (1.92 mg/kg), raspberries (0.68 mg/kg) and cranberries (0.21 mg/kg). Among the hydroxybenzoic acids tested, protocatechuic acid is the most abundant, followed by gallic acid, vanillic acid and syringic acid, as shown in **Figure 3.2c**. The highest content of protocatechuic acid, vanillic acid and syringic acid was found in cherries ($p<0.05$; 2.59±0.04 mg/kg, 0.48 mg/kg and 0.05 mg/kg, respectively), but the highest gallic acid content was found in blueberries ($p<0.05$; 1.30 mg/kg). The variation in the presence of hydroxybenzoic acid in blueberries follows the previous research findings described by Mustafa et al. (2022). Blueberries consist mainly of p-coumaroylquinic acid (1860–2080 mg/kg FW) as a hydroxybenzoic acid. Additionally, they contain p-hydroxybenzoic acid-O-glucoside (4–5 mg/kg), gallic acid-4-O-glucoside (2–9 mg/kg FW) and protocatechuic acid-4-O-glucoside (3–6 mg/kg) which leads to a different hydroxybenzoic acid profile present in blueberries (Bento-Silva et al., 2020).

3.4.4.6 Hydroxycinnamic acids

Hydroxycinnamic acids are a group of organic compounds in the phenolic acid family. They are derived from cinnamic acid by adding hydroxyl groups (-OH) to the cinnamic acid structure (Jiang et al., 2022; Tyagi et al., 2022). Hydroxycinnamic acids are commonly found in various plant-based foods, including fruits, vegetables, grains and beverages such as coffee and tea. Researchers have shown a growing interest in phenolic acids and their derivatives because of their nutritional and antioxidant properties in various food sources (Mustafa et al., 2022). The most common hydroxycinnamic acids found in berries and fruits were chlorogenic, p-coumaric, caffeic and ferulic acids (Mustafa et al., 2022). The current study analyzed caffeic acid, caftaric acid, chlorogenic acid, ferulic acid, p-coumaric acid and sinapic acid in the tested commercial berry types, as shown in **Figure 3.2d**. The highest total hydroxycinnamic acid was found in cherries

(211.4 mg/kg), while the lowest content was found in raspberries (7.52 mg/kg). Blueberry contains the second-highest total hydroxycinnamic content (170.1 mg/kg), which was higher than cranberries (52.48 mg/kg) and blackberries (26.93 mg/kg). Chlorogenic acid was the highly abundant hydroxycinnamic acid type found in all berries; however, the highest chlorogenic content (p<0.05) was found in cherries (199±3.87 mg/kg) compared to the berries. The abundance of chlorogenic acid in higher contents among the other hydroxycinnamic acids in berries has been previously reported (Jiang et al., 2022; Mustafa et al., 2022; Rossi et al., 2022). Blueberry (154±2.00 mg/kg) contained the highest chlorogenic acid content, followed by cranberry (51.2±0.20 mg/kg). Similar results were obtained by Mustafa et al. (2022), where it was found that the highest chlorogenic acid content was present in blueberries compared to the other tested berries. According to their study, chlorogenic acid (344.04 mg/kg) was the most dominant phenolic acid in blueberry, representing 92.2% of the total phenolic acid content, however, its content is highly variable within blueberry varieties ranging from 34.3 to 113.8 mg/100 g FW (Mustafa et al., 2022). The highest (p<0.05) caffeic acid and ferulic acid contents were found in blueberry (5.20±0.08 mg/kg and 10.4±0.35 mg/kg, respectively), whereas the highest caftaric acid was found in cranberry (0.05 mg/kg). *P*- coumaric acid content in cranberry was 2.09±0.08 mg/kg, the highest (p<0.05) among all the analyzed berry types. Raspberries had the lowest (p<0.05) hydroxycinnamic acids; however, they had the highest sinapic acid content (p<0.05) at (0.65 mg/kg) compared to other berries.

3.4.4.7 Anthocyanins

Anthocyanins play a pivotal role in providing the red, blue, blue and black pigments found in berries, standing out as the most significant polyphenols in these fruits. Among them, the most common anthocyanins present in berries are cyanidins, peonidins, malvidins, pelargonidins, petunidins and delphinidins (Mustafa et al., 2022). In the current study, 12 most abundant anthocyanins out of the total anthocyanin profile in berries were identified, including cyanidin, cyanidin-3-arabinoside, cyanidin-3-glucoside, cyanidin-3-rutinoside, delphinidin, delphinidin-3-glucoside, malvin, malvidin-3-glucoside, pelargonidin, peonidin, peonidin-3-glucoside and petunidin (**Figure 3.2b**). The most abundant anthocyanin compound in berries among the other anthocyanins was cyanidin-3-rutinoside. The current study detected the highest delphinidin content in blueberries at 45.5±0.82 mg/kg, followed by blackberries at 26.7±0.29 mg/kg. The

highest delphinidin-3-glucoside content was reported in blueberry at 94.4±0.56 mg/kg, followed by blackberry at 18.0±0.21 mg/kg. As reported in the literature, the blue colour berries, such as blueberry and blackberry, contained the highest amount of delphinidin and delphinidin-3-glucoside content ($p<0.05$) compared to the other red colour berries tested (raspberry and cranberry) and cherries (Hosseinian and Beta, 2007). Similar results were observed by Mustafa et al. (2022), where they found a distinctive anthocyanin profile present in blueberries and most abundantly delphinidin-3-galactoside followed by malvidin-3-galactoside, petunidin-3-glucoside, cyanidin-3-glucoside, pelargonidin-3-glucoside and delphinidin-3,5-diglucosides as the prominent anthocyanins present in blueberries. The most abundant anthocyanins observed in blueberries were delphinidin-3-galactoside and pelargonidin-3-glucoside (381.28 mg/kg and 180.20 mg/kg, respectively). The red colour berries, such as raspberries and cranberries and also cherries showed higher contents of cyanidin compounds compared to the blue colour berries ($p<0.05$) (**Figure 3.S4**). For instance, the highest content of cyanidin-3-rutinoside was found in cherry (316±3.66 mg/kg), followed by raspberry (310±1.63 mg/kg). The highest pelargonidin content ($p<0.05$) was found in cranberry (0.06 mg/kg) and the lowest amount was found in blueberry (0.03 mg/kg). The highest ($p<0.05$) malvidin-3-glucoside content (253±2.13 mg/kg) and peonidin-3-glucoside content (108±9.81 mg/kg) were also found in blueberries. A high amount of malvidin-3-glucoside in blueberries was reported previously (Huang et al., 2016). The highest content of cyanidin-3-glucoside was found in blackberries (368±6.99 mg/kg), followed by blueberries (165±0.39 mg/kg). The highest ($p<0.05$) petunidin content (830±3.88 mg/kg) and peonidin content (1.07mg/kg) were found in cranberries and these findings following the results found previously for the anthocyanin contents in cranberries (Brown & Shipley, 2011). The composition and quantity of anthocyanins are contingent upon factors such as varieties, fruit size, species, ripening stage, pre-harvest environmental factors, climatic conditions and storage conditions (Mustafa et al., 2022).

3.4.4.8 Stilbenes

In the current study, the highest total stilbene content found in cherries (2.53 mg/kg) was significantly higher than the other tested berry types. For instance, the second highest total stilbenes content was found in cranberries (0.11 mg/kg) followed by blackberries (0.09 mg/kg), blueberries (0.08 mg/kg) and raspberries (0.08 mg/kg) (**Table 3.3**). Stilbenes are mostly found in grapes compared to berries and studies have shown that they are located in the skin of the grapes

(Błaszczyk et al., 2019). Piceatannol, polydatin and rhapontin were identified in commercial berry types. Among them, rhapontin was the most abundant stilbene found compared to the others (**Table 3.3).** The highest ($p<0.05$) rhapontin content was found in cherries (0.07 mg/kg), followed by raspberries, cranberries, blueberries, and blackberries, which had similar concentration (~0.04 mg/kg). The piceatannol content was not significantly different among the tested berry types ($p>0.05$). The highest ($p<0.05$) polydatin content was also found in cranberry (2.44 mg/kg), whereas the lowest content (< 0.02 mg/kg) of polydatin was found in blueberry and raspberry ($p>0.05$). Resveratrol was not found in any berries tested in the current study, even though recoveries from the extraction method in spiked samples were 78-84 % (**Table 3.2**). This could be due to the higher detection limit for resveratrol than the actual amounts in berries. Sebastià et al., (2014) noted resveratrol at 0.41-0.45 µg/g, 0.02 µg/g and 0.01µg/g in blueberries, highbush blueberries and lowbush wild blueberries, respectively. The LOQ for resveratrol in the current study was 0.08 µg/g, which could have been low to quantify in the samples. The quantitative and qualitative composition of stilbenes in edible berries is influenced by various factors. These include the type of soil, cultivar, climatic conditions, genotype, developmental stage and storage conditions. As secondary metabolites, stilbenes exhibit a dynamic profile shaped by a multitude of interacting elements in the berry's environment (Sebastià et al., 2014).

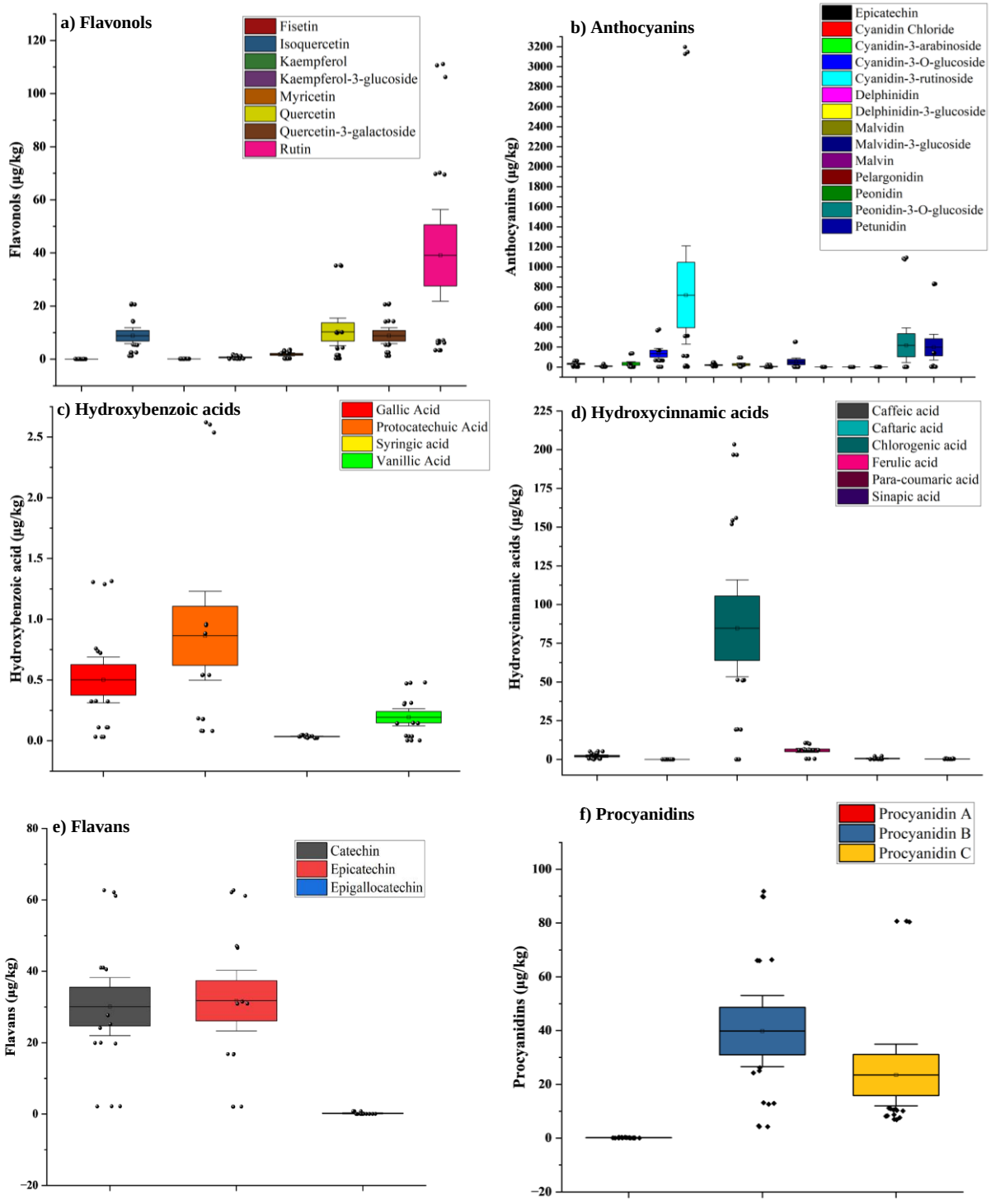


Figure 3.2 Box plots for concentrations of phenolic compounds belonging to different phenolic compound classes tested in all berries (blueberry, raspberry, blackberry, cranberry and cherry fruits); a) Flavonols; b) Anthocyanins; c) Hydroxybenzoic acids; d) Hydroxycinnamic acids; e)

Flavans; and f) Procyanidins. The horizontal line in each box plot shows the mean content of each phenolic compound of all the tested berries. The data points indicate concentrations of different phenolic compounds of each berry type and the whiskers represent standard error of the concentrations.

Table 3.3 Phenolic compounds content (µg/kg dry weight ± SD (n=3)) in berries analyzed by the developed UHPLC-HRMS method within 20 minutes

Compound	Class of the compound	Blackberry (µg/kg)	Blueberry (µg/kg)	Cranberry (µg/kg)	Raspberry (µg/kg)	Cherry (µg/kg)
Afzelin	Flavones	25.70±1.22^{b}	24.19±0.03^{b}	29.55±1.67^{a}	24.12±0.01^{b}	24.94±0.67^{b}
Apigenin	Flavones	12.13±0.01^{b}	12.13±0.00^{b}	12.81±0.37^{b}	12.31±0.16^{b}	13.61±0.43^{a}
Arbutin	Flavones	423.4±18.4^{c}	544.2±10.74^{b}	156.7±0.63^{e}	263.6±2.68^{d}	580.4±12.2^{a}
Aromadendrin	Isoflavones	19.86±3.13^{b}	14.83±0.22^{b}	17.81±1.50^{b}	18.61±0.76^{b}	44.15±2.46^{a}
Caffeic acid	Hydroxycinnamic acid	797.6±2.53^{c}	5204±80.2^{a}	319.7±27.8^{d}	586.7±15.8^{cd}	3906±84.7^{b}
Caftaric acid	Non-flavonoid phenolic	18.42±0.21^{b}	17.40±1.10^{b}	52.97±0.10^{a}	17.93±0.86^{b}	18.66±1.23^{b}
(+)-Catechin*	Flavans	40850±885^{b}	2127±27.4^{e}	61980±803^{a}	19860±1130^{d}	25640±1832^{c}
Chlorogenic acid	Hydroxycinnamic acid	19340±873^{d}	154001±2007^{b}	51180±205^{c}	55.05±0.86^{e}	198900±388^{a}
Daidzein	Isoflavones	ND	2.90±0.00^{a}	ND	ND	ND
Daidzin	Isoflavones	78.98±1.77^{c}	46.84±1.50^{d}	57.44±0.33^{d}	615.4±12.8^{a}	384.3±12.0^{b}
Ellagic acid	Ellagitannin	14830±255^{a}	94.57±2.24^{c}	70.87±1.99^{c}	8932±201^{b}	97.32±3.81^{c}
(-)-Epicatechin*	Flavans	46820±264^{b}	2042±14.5^{e}	61980±803^{a}	16770±790^{d}	31120±333^{c}
(-)-Epigallocatechin	Flavans	34.84±0.92^{cd}	773.3±9.56^{a}	45.03±0.08^{bc}	49.38±1.46^{b}	25.72±0.20^{d}
Ferulic acid	Hydroxycinnamic acid	6404±183^{b}	10420±1348^{a}	421.3±1.63^{d}	5876±69.2^{c}	6149±88.3^{bc}
Fisetin	Flavonols	61.87±0.97^{b}	39.33±0.36^{e}	57.88±1.40^{c}	89.16±2.03^{a}	49.52±1.44^{d}
Gallic acid	Hydroxybenzoic acid	738.7±18.4^{b}	1302±13.0^{a}	109.5±0.07^{d}	323.6±1.5^{c}	31.42±0.39^{e}
Genistein	Isoflavones	14.50±1.79^{a}	ND	13.23±0.15^{a}	13.26±0.52^{a}	14.27±0.45^{a}
Glycitein	Isoflavones	24.70±1.27^{d}	36.73±0.59^{a}	29.87±1.33^{c}	33.63±1.26^{b}	33.41±0.45^{b}
Glycitin	Isoflavones	138.9±5.17^{a}	69.37±1.47^{c}	64.67±3.21^{c}	87.89±4.31^{b}	73.06±1.78^{c}
Herbacetin	Flavones	34560±853^{a}	1343±258^{d}	300.8±0.76^{d}	10330±540^{b}	7688±368^{c}
Hesperetin	Flavanones	17.86±0.82^{b}	17.28±0.33^{b}	17.29±0.16^{b}	16.85±0.16^{b}	68.22±2.82^{a}
Isoquercetin	Flavonols	14250±722^{b}	20670±179^{a}	2512±2.22^{d}	1252±4.16^{e}	5418±95.1^{c}
Isorhamnetin	Flavones	328.2±8.37^{c}	2109±86.9^{a}	83.84±2.42^{e}	196.9±2.95^{d}	617.1±10.6^{b}
Isovitexin*	Flavones	25.69±1.22^{b}	24.16±0.04^{bc}	28.89±0.34^{a}	24.11±0.01^{c}	25.21±0.16^{bc}
Kaempferol	Flavonols	148.8±1.75^{b}	122.5±0.74^{d}	138.0±1.76^{c}	178.2±2.89^{a}	140.1±3.33^{c}
Kaempferol-3-glucoside	Flavonols	1585±172^{a}	283.7±3.49^{c}	1230±4.67^{b}	126.2±1.56^{c}	232.1±1.45^{c}
Liquiritingenin	Flavanones	15.11±0.54^{a}	ND	ND	14.35±0.07^{b}	ND

Luteolin	Flavones	65.58±1.02^{b}	43.12±0.36^{e}	61.66±1.08^{c}	92.63±2.67^{a}	53.24±1.45^{d}
Myricetin	Flavonols	2270±31.02^{c}	3437±106^{a}	210.7±2.15^{d}	276.2±3.35^{d}	3008±144^{b}
Naringin	Flavanones	34.27±1.32^{b}	23.65±0.38^{c}	96.90±0.40^{a}	98.44±2.18^{a}	24.99±0.38^{c}
Nicotiflorin	Flavonols	3818±74.19^{c}	106.2±2.07^{d}	48.52±0.57^{d}	8525±222^{b}	71220±2100^{a}
Okanin	Chalcones	22.26±1.28^{b}	13.40±0.43^{d}	17.68±0.95^{c}	17.71±0.64^{c}	38.74±1.37^{a}
Orientin	Flavones	1488±7.35^{a}	281.1±2.08^{c}	1234±2.34^{b}	97.14±4.21^{e}	138.1±1.27^{d}
Para coumaric acid	Hydroxycinnamic acid	164.8±2.19^{c}	123.4±2.96^{c}	367.1±2.35^{b}	348.2±5.72^{b}	2092±82.7^{a}
Piceatannol	Stilbene	14.38±0.09^{a}	14.37±0.04^{a}	13.53±0.88^{a}	13.57±1.33^{a}	14.14±0.96^{a}
Polydatin	Stilbene	34.43±0.92^{c}	25.85±0.49^{c}	62.03±1.55^{a}	25.33±2.27^{c}	2441±7.71^{a}
Procyanidin A2	Procyanidin	292.2±8.57^{a}	41.51±0.86^{d}	93.7±1.98^{c}	92.54±11.8^{c}	180.6±0.92^{b}
Procyanidin B2	Procyanidin	90490±1149^{a}	4363±199^{e}	66140±1844^{b}	25160±9803^{c}	12930±2833^{d}
Procyanidin C2	Procyanidin	10300±981^{b}	8372±279^{c}	80600±1145^{a}	7149±407^{d}	10980±1762^{b}
Protocatechuic acid	Hydroxybenzoic acid	931.1±42.6^{b}	540.1±1.21^{c}	80.9±0.44^{e}	179.7±3.38^{d}	2586±44.0^{a}
Quercetin	Flavonols	35250±938^{a}	1554±21.2^{d}	303.9±4.86^{e}	10040±108^{b}	4200±188^{c}
Quercetin-3-galactoside	Flavonols	14250±721^{b}	20690±150^{a}	2511±2.48^{d}	1248±3.24^{e}	5418±95.5^{c}
Resveratrol	Stilbene	ND	ND	ND	ND	ND
Rhapontin	Stilbene	37.81±1.21^{bc}	37.24±1.3^{c}	37.53±0.63^{bc}	40.26±1.65^{b}	74.34±0.22^{a}
Rutin	Flavonols	69810±349^{b}	6946±96.4^{c}	3352±2.58^{d}	6130±99.6^{cd}	109300±2700^{a}
Sinapic acid	Hydroxycinnamic acid	222.1±5.17^{d}	317.2±2.41^{c}	198.5±1.34^{e}	649.9±5.69^{a}	362.7±2.51^{b}
Syringic acid	Hydroxybenzoic acid	36.83±1.02^{b}	35.65±1.48^{b}	22.11±1.08^{c}	32.94±2.68^{b}	47.18±1.42^{a}
Taxifolin	Flavones	85.44±1.42^{c}	196.3±4.85^{b}	50.68±1.07^{d}	179.0±2.56^{b}	1148±15.9^{a}
Vanillic acid	Hydroxybenzoic acid	306.9±6.87^{b}	37.53±2.08^{d}	2.22±0.15^{e}	144.2±2.32^{c}	475.7±4.38^{a}
Vicenin-2	Flavones	57.30±1.12^{b}	27.18±1.15^{c}	49.21±0.42^{b}	ND	180.1±10.5^{a}
Vitexin*	Flavones	25.71±1.22^{b}	24.22±0.02^{b}	29.64±1.61^{a}	24.12±0.01^{b}	25.36±0.06^{b}
Vitexin-2-rhamnoside	Flavones	25.67±0.30^{b}	ND	26.00±1.69^{b}	26.72±1.55^{b}	49.06±0.43^{a}
Cyanidin	Anthocyanin	30950±110^{a}	1547±4.51^{e}	1823±5.07^{d}	5977±23.4^{b}	3868±6.38^{c}
Cyanidin-3-arabinoside	Anthocyanin	134800±2292^{a}	23790±121^{b}	790.6±4.55^{c}	223.7±3.59^{c}	2349±4.71^{c}
Cyanidin-3-glucoside	Anthocyanin	368400±6991^{a}	165500±3977^{b}	1744±3.14^{d}	65630±135^{c}	66060±776^{c}
Cyanidin-3-rutinoside	Anthocyanin	110400±1679^{b}	2385±14.7^{c}	14280±204^{d}	310300±1630^{a}	315600±3666^{a}
Delphinidin	Anthocyanin	26740±998^{b}	45360±821^{a}	10360±977^{c}	5204±248^{e}	7025±169^{d}

Delphinidin-3-glucoside	Anthocyanin	18020±211[b]	94440±560[a]	2467±4.33[d]	2180±56.4[d]	8624±141[c]
Malvin	Anthocyanin	ND	540.0±10.9[a]	ND	144.6±3.51[b]	ND
Malvidin-3-glucoside	Anthocyanin	90.63±1.34[b]	252500±2127[a]	84.24±2.86[b]	88.04±6.03[b]	54.54±0.58[b]
Pelargonidin	Anthocyanin	54.45±1.27[b]	29.91±0.91[d]	61.28±0.42[a]	30.45±0.83[d]	42.46±1.5[c]
Peonidin	Anthocyanin	512.2±2.67[c]	396.7±4.12[d]	1068±3.19[a]	385.9±10.51[d]	644.2±31.4[b]
Peonidin-3-glucoside	Anthocyanin	666.7±4.93[b]	108400±9812[a]	81.53±0.04[b]	1175±727[b]	2258±126[b]
Petunidin	Anthocyanin	2733±164[d]	140400±2006[b]	829700±3880[a]	1638±243[d]	13600±485[c]

ND. Not Detected

The values containing different letters have significantly different (p<0.05) mean concentrations based on Tukey's mean separation.

*(+)-Catechin, (-)-epicatechin and vitexin, isovitexin were calculated together as a summation of both isomers.

3.4.5 Principal component analysis (PCA)

The current trend in polyphenol studies extends beyond traditional quantitative and qualitative analyses, emphasizing the exploration of compositional profiles and fingerprints for authentication and classification purposes. The application of chemometric data analysis has shown a substantial increase, particularly with the use of LC-MS and LC-HRMS, providing datasets of higher quality (Lucci et al., 2017). These datasets are effectively utilized through chemometric methods such as principal component analysis and partial least square-discriminant analysis. Beyond classification, chemometrics proves to be valuable in the initial stages of analytical method development, streamlining sample treatments and chromatographic separations (Lucci et al., 2017). According to the current study of PCA of LC-HRMS data, the first three principal components showed 83% of the total data variation (**Figure 3.3a**). Accordingly, the tested five different berries were separated into three groups, with cherry and blueberry as individual groups, indicating no similarities in phenolic profiles between cherry and blueberry and the other tested berries. Raspberry, blackberry and cranberry were grouped, indicating more similarities in phenolic compound profiles among these three berries. (**Figure 3.3a**). The first principal component explained a variation of 34% in the phenolic compound profiles and grouped blueberry individually mainly due to the negative loadings among the anthocyanins such as delphinidin, delphinidin-3-glucoside, malvin, malvidin, malvidin-3-glucoside and other phenolic compounds including gallic acid, quercetin-3-galactoside (**Figure 3.3b and Table 3.S1**). Raspberry, blackberry and cranberry grouped because of the positive correlation among the phenolic compounds such as (+)-catechin, (-)-epicatechin, caftaric acid, procyanidin B2, procyanidin C2 and quercetin as well as anthocyanins such as cyanidin compounds. These findings follow the data reported in Hosseinian & Beta (2007). The second principal component described the data variation of 28% and the responsible variables contributing to group cherry individually. This was due to positive loadings between compounds such as vanillic acid, syringic acid, p-coumaric acid and stilbenes, including rhapontin and polydatin (**Table 3.S1**). These results show how the different berry types could be categorized based on their presence of different phenolic compound profiles and quantities. Identifying and quantifying the phenolic compounds with analytical techniques helps differentiate berry and cherry fruits. In addition, there is a potential to use chemometrics studies to reveal common chemical profiles among berries from the same region or cultivar. This technique could help distinguish authentic berries from potentially adulterated ones

or genetically modified cultivars from native berries. Given the diverse nature of polyphenols in the plant kingdom, the intricate composition of food matrices, and the abundance of data, especially from HRMS instruments, the future anticipates a notable increase in publications leveraging chemometric studies for polyphenol analysis.In addition, these analytical methods can help researchers and food producers set up quality control measures to ensure that the product's characteristics align with specific standards and affirm food safety.

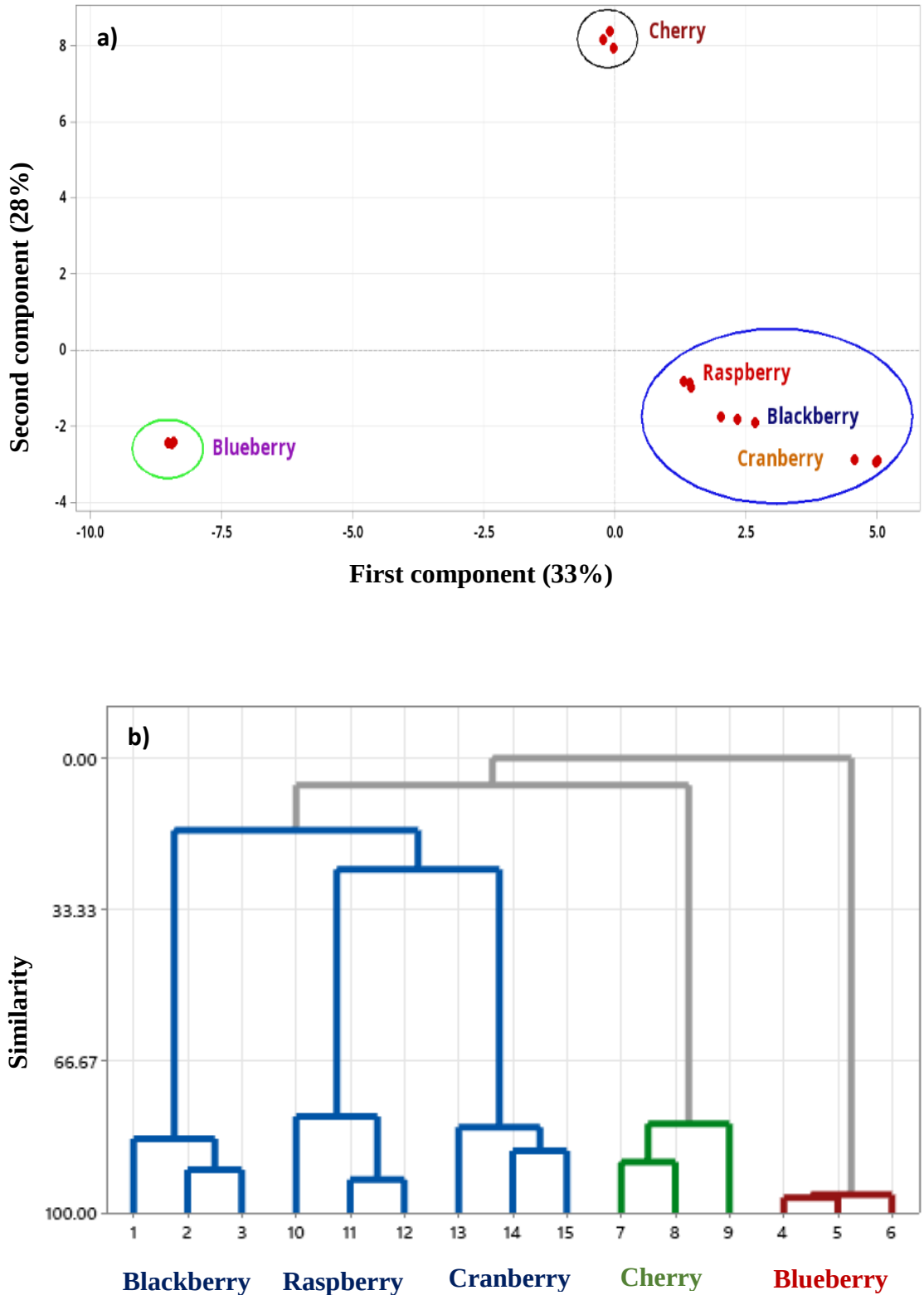


Figure 3.3 a) Principal Component Analysis (PCA) and b) Cluster Analysis (CA) of phenolic compounds in cherry, blueberry, raspberry, blackberry and cranberry in different similarity level

3.4.6 Cluster analysis (CA)

Principal Component Analysis known to be the favored method for conducting exploratory studies on food properties (Lucci et al., 2017). However, CA is used to provide supplementary information concerning the distribution of samples into groups (Lucci et al., 2017). CA was performed to generate a dendrogram (**Figure 3.3b)** to confirm the similarities in phenolic compounds observed in berries as indicated in the PCA analysis. The lines indicated in the same colour belong to one cluster. The generated clusters were marked differently based on their phenolic compound profiles and concentrations. As shown in PCA, cluster analysis generated the dendrogram grouping raspberry, blackberry and cranberry together as the first cluster, which showed 15.95% similarity compared to the other two clusters, which had only one berry type each, cherry and blueberry as clusters 2 and 3, respectively. The major phenolic compounds responsible for cluster 1 were (+)-catechin, (-)-epicatechin, herbacetin, procyanidin B2, procyanidin C2, quercetin, rutin, cyanidin-3-arabinoside, cyanidin-3-glucoside, cyanidin-3-rutinoside and petunidin. These results indicated that raspberry, cranberry and blackberry have similar phenolic compound profiles and quantities of the above-mentioned phenolic compounds compared to cherry and blueberry. Even within cluster 1, raspberry and cranberry showed more similarities (24.5%) than blackberry. The responsible phenolic compounds for these similarities between raspberry and cranberry are the presence of caffeic acid, daidzein, (-)-epigallocatechin, gallic acid, glycitien, isoquercetin, isorhamnetin, myricetin, quercetin-3-galactoside, cyanidin-3-glucoside, delphinidin, delphinidin-3-glucoside, malvidin and peonidin-3-glucoside.

On the other hand, apigenin, aromadendrin, chlorogenic acid, daidzin, hesperetin, nicotiflorin, okanin, *p*-coumaric acid, polydatin, protocatechuic acid, rhapontin, rutin, syringic acid, taxifolin, vanillic acid, vicenin-2, vitexin-2-rhamnoside and cyanidin-3-rutinoside, contributed to the cluster 2 to group cherry individually emphasizing the presence of these compounds in cherry make it unique compared to the other tested berry types. De Souza et al. (2014) observed similar findings in cherries. Similarly, cluster 3 consisted of only blueberry based on its differences in the phenolic compound profiles and their concentrations. Similar observations were made in wild blueberries from Manitoba and analyzed using similar methods as the current study (Kodikara et al., unpublished data). The content of afzelin, caftaric acid, fisetin, isovitexin, kaempferol, luteolin,

naringin, procyanidin C2, sinapic acid, vitexin, peonidin, petunidin makes blueberry different from the other tested berry types and grouped individually. The presence of these compounds in blueberries has been reported in previous studies (De Souza et al., 2014; Mustafa et al., 2022; Shi et al., 2017).

3.5 Conclusion

Several factors, such as the variety, ripening stage, environmental conditions, cultivation practices, harvesting, post-harvest handling, processing and cooking, storage conditions and the genetics of varieties, can influence the phenolic profiles and concentrations in various fruits, including berries and other plant-based materials. These factors are crucial to consider when studying or analyzing phenolic compounds. This novel UHPLC-HRMS method has been successfully developed and validated for the simultaneous detection and quantification of 66 phenolic compounds belonging to different distinct chemical groups such as flavonols, flavanones, anthocyanins, procyanidins, hydroxybenzoic acids, hydroxycinnamic acids and stilbenes in both berries and cherries with an optimized phenolic compounds extraction procedure compared to the existing methods. All parameters related to UHPLC-HRMS were optimized, enabling sensitive, quantitative analysis of the target analytes within 20 min. Compared to existing methods, the newly developed UHPLC-HRMS approach offers a remarkable advancement in the quantification of berry phenolic compounds. Most conventional methods are time-consuming and require a longer analysis period, often taking several hours to complete. On the other hand, the new method stands out by enabling the quantification of 66 phenolic compounds within a 20-minute timeframe, significantly reducing the analysis time, which could avoid the potential degradation of some of the unstable phenolic compounds. Resolving such complexities in different chemical groups while maintaining a fast analysis time was a significant challenge. The validation results demonstrate that the method is sensitive enough to comprehensively analyze phenolic compounds in large sample sets, even with limited sample volumes. Furthermore, the utilization of UHPLC-HRMS provides superior resolution and accuracy in compound identification compared to traditional techniques. This method's novelty lies in its ability to comprehensively analyze a wide range of phenolic compounds in a highly efficient and time-effective manner. This innovation accelerates research and quality control processes and enhances the precision and sensitivity of phenolic compound

quantification in berries. As a result, it serves as an invaluable tool for building a definitive polyphenol composition database specific to berries, cherries and related foods.

The current study identified phenolic compounds in blueberry, raspberry, blackberry, cranberry and cherry. One of its primary applications will be identifying the phenolic compound and quantities present in different berries, which would help identify the different phenolic compound profiles concerning their different geographical locations, growing conditions and genetic and environmental factors. This UHPLC-HRMS method holds immense significance for the berry and cherry industry as it could play a crucial role in verifying the authenticity of berry fruits. In the analyses of various berries, it was noted that the abundance of different phenolic compounds and their quantities vary depending on the type of berry. Principal component analysis and cluster analysis showed that the cherry and blueberry have unique phenolic compound profiles and quantities compared to raspberry, blackberry and cranberry. By identifying and quantifying these compounds, the nutritional potential of these berries was evaluated to improve their utilization, leading to the diversification of diets and promoting sustainable agriculture by supporting the cultivation of a wider variety of crops.

Further, in the food industry, fast and reliable analytical methods are essential for quality control and standardization of berry and cherry-based products. The changes in the phenolic compound profiles during the fruit ripening stages warrant further investigation. Future studies will be needed to comprehensively assess the relative changes in phenolic compounds and effects due to environment and varieties. Such research will contribute to a deeper understanding of the phenolic composition of fruits and their potential implications for human health and nutrition. The knowledge generated in this study will potentially lead to innovative applications of phenolic compounds, such as incorporating phenolic-rich berry extracts into functional foods, supplements, or pharmaceutical products.

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Chapter 4: Targeted Metabolomic Investigation of 66 Bioactive Phenolic Compounds in 14 different Small Fruits from Canadian Prairies using UHPLC-HRMS

A version of this chapter is in-progress to submit to Journal of Food Composition and Analysis:

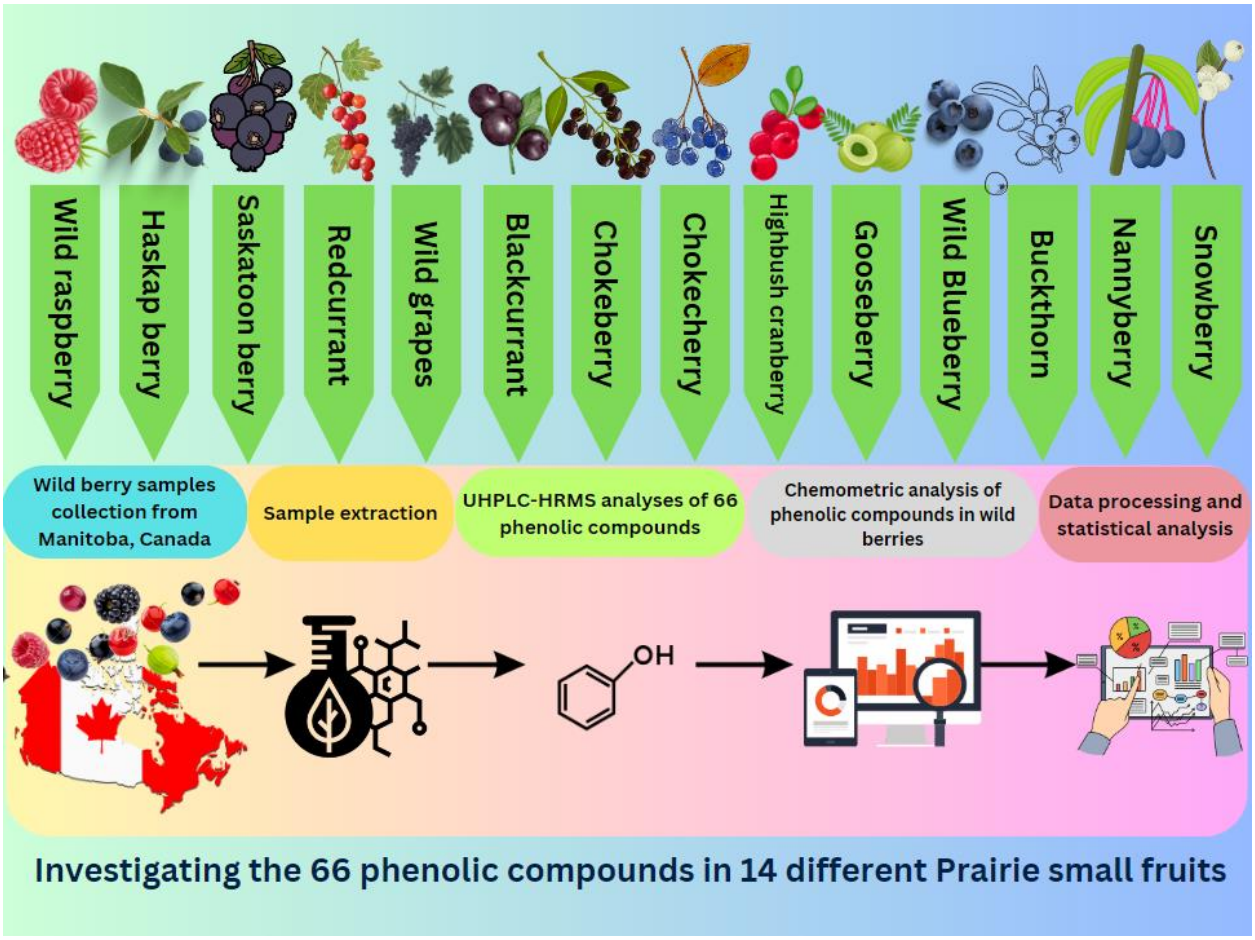
Kodikara C, Netticadan T, Bandara N, Sura. S., Wijekoon C. (2024). Targeted Metabolomics Investigation of Sixty Six Bioactive Phenolic Compounds in Fourteen Different Prairie Small Fruits using UHPLC-HRMS

4.1 Abstract

Traditional small fruits such as berries, widely distributed in the Canadian prairies have gained interest to researchers and consumers, due to their potential health benefits. The current study explores the presence and diversity of phenolic compounds in fourteen different Canadian prairie small fruits. A targeted metabolomics approach was performed utilizing ultra-high-performance liquid chromatography and high-resolution mass spectrometry to identify and quantify a total of 66 phenolic compounds and their diversity in small fruits. Following the phenolic compound identification and quantification in each fruit, a chemometric analysis was performed to identify the trends and variations of these fruits based on their phenolic compound contents. While most small fruits has all major phenolic compounds, their concentrations greatly varied between them. Saskatoon berries and gooseberries exhibited distinct phenolic compound profiles compared to the other fruits. The highest (p<0.05) total phenolic content from the analyzed compounds was identified in Saskatoon berries (2100 mg/kg), followed by chokeberries (1350 mg/kg) and wild blueberries (1190 mg/kg). Anthocyanins were identified as the most abundant phenolic compounds belonging to flavonoids. The analysis also revealed an abundance of different phenolic compounds, including flavonoids, phenolic acids, and other polyphenols. The K-means cluster analysis of phenolic compound profiles in various fruit clusters revealed distinct phenolic compound profile patterns. Blackcurrants and haskap berries clustered together due to the elevated levels of isoflavones, flavonols, flavans, and hydroxycinnamic acids, contributing to a unique phenolic compound profile. A comprehensive investigation of phenolic compound profiles allowed for a deeper understanding of the potential bioactive which further could lead to possible applications in functional foods, nutraceuticals, and pharmaceuticals.

Keywords: Bioactive phenolic compounds, UHPLC-HRMS, Canadian small fruits, chemometric analysis, metabolomics, berries

Graphical Abstract



Author contribution

Funds acquisition: CW and TN; Conceptualization: CK, SS and CW; Methods: SS and CK; Analysis: CK and SS; Writing the first draft: CK; Editing and reviewing: SS, CW, NB and CK; Final version review: SS, CW, CK, TN and NB; Research Supervision: SS and CW; Student academic supervision: CW and NB.

4.2 Introduction

Bioactive molecules, particularly phenolic compounds, are recognized for their pivotal role in promoting health and preventing or delaying chronic diseases such as cancer, diabetes, heart problems in human beings and animals (Zorzi et al., 2020). Small fruits such as berries, known for their rich content of bioactive compounds, have become centre of food research, primarily due to their potential health benefits. Functional foods, defined by their ability to promote health and prevent diseases, are rich in essential nutrients for maintaining overall well-being (Adefegha, 2018). Small fruits adundant in essential micronutrients such as folic acid and vitamin C, in addition to various phenolic compounds, are ideal candidates for functional foods with diverse functions including antioxidant, anti-inflammatory, and anticarcinogenic agents (Hosseinian & Beta, 2007; Kodikara et al., 2023; Olas, 2018a). Some of these small fruits, exhibiting diverse classifications, geographical distributions, and unique characteristics, are endemic to Canadian prairie provinces and the northwestern United States, and serve as an important dietary supplement for local populations, especially during summer and fall seasons (Hosseinian & Beta, 2007; Kodikara et al., 2023; Zhao et al., 2020). The distribution of these berries varies across Canada, influenced by factors that select for these small fruits such as cold-hardiness, temperature, and water availability.

Targeted metabolomic analysis of phenolic compounds is a specialized approach and focuses on the quantitative measurement of a predefined suite of phenolic compounds within a biological sample (Roberts et al., 2012). Unlike untargeted metabolomics, which aims to identify and semi-quantify a broad range of metabolites, targeted metabolomic analysis specifically refines in on a selected group of compounds relevant to a particular biological pathway or physiological process. This method employs advanced analytical techniques such as mass spectrometry or nuclear magnetic resonance spectroscopy to accurately identify and quantify the concentrations of specific metabolites, providing detailed insights into the metabolic profile of a sample. Targeted metabolomics is particularly valuable in studies where a precise understanding of specific metabolic pathways or biomarkers is crucial, offering a more focused and comprehensive analysis of metabolite dynamics (Roberts et al., 2012).

Recent research has emphasized the significance of phenolic compounds present in fruits, demonstrating their capacity to initiate physiological benefits such as antioxidant activity, anti-inflammatory effects, and anticarcinogenic properties (Marjanovic et al., 2021; Olas, 2018b). Various studies demonstrated the association between the consumption of fruits rich in phenolic compounds and various health benefits in humans (Hosseinian & Beta, 2007; Kodikara et al., 2023; Zhao et al., 2020). Phenolic acids, cinnamic acid derivatives, lignans, flavonoids, tocopherols, coumarins, stilbenes, and tannins were the major contributors to the bioactive profile of small fruits such as berries (Del Rio et al., 2010; Rahman et al., 2022). The interest in these compounds was further increased by their high antioxidant capacities, prompting investigations into their potential health benefits upon incorporation into other functional foods (Skinner & Hunter, 2013). Moreover, small fruit consumption has increased in recent years, correlating with a lower incidence of cardiovascular diseases, cancers, and inflammatory processes that are related to the formation of reactive oxygen species (Burton-Freeman et al., 2016). Dietary polyphenols, including phenolic compounds in small fruits such as berries, exhibit therapeutic effects on cells and tissues, affecting intracellular signaling and modulating immune functions (Bravo, 1998). Specific phenolic compounds such as chlorogenic acid, resveratrol, quercetin, and anthocyanins demonstrated diverse health benefits, including anti-inflammatory, anti-obesity, anti-diabetic, and cardioprotective properties (Galiniak et al., 2019; Naveed et al., 2018; Ozga et al., 2007). Epigallocatechin, epicatechin gallate, gallic acid, quercetin, geraniin, tannic acid, and rutin have been reported to have anti-mutagenic properties (Bhargava and Westfall, 1969; Kumar et al 2018). Tannins such as ellagic acid have exhibited anti-bacterial, anti-fungal, and anti-viral activities (Ratnoff and Crum, 1964). Further studies also revealed that fruit phenolic compounds affect four risk factors of cardiovascular diseases: platelet aggregation, elevated blood pressure, vascular dysfunction, and hyperlipidemia (Chong et al. 2010). Therefore these phenolic compounds, found in fruits, have been linked to the prevention and/or delay of the onset of various chronic diseases, making berries a valuable component of a health-promoting diet (Vahapoglu et al., 2022).

Despite the growing interest in the health benefits of small fruits, there remains a research gap, particularly concerning underutilized Canadian prairie small fruits/berries in their phenolic compositions, relative concentrations, and functional properties. Existing literature predominantly focuses on well-known commercial varieties, leaving a knowledge void for the bioactive

composition and potential health benefits of Canadian prairie-grown berries and small fruits (Kodikara et al., 2023; The Canadian Encyclopedia, 2022). This study aimed to address this knowledge gap by providing a comprehensive analysis of phenolic compounds in small fruits commonly grown in Canadian prairies. This was achieved by conducting targeted metabolomics using high-resolution mass spectrometric (HRMS) data acquisition and multivariate data analysis of phenolic compound profiles in small fruits and understanding their occurrence, diversity, and extent.

4.3 Materials and Methods

4.3.1 Reagents and Materials

Analytical standards of 37 of total 66 phenolic compounds including apigenin, caffeic acid, caftaric acid, (+) catechin, chlorogenic acid, cyanidin-3-galactoside, daidzein, daidzin, delphinidin, delphinidin-3-glucoside, (-) epicatechin, (-) epicatechingallate, (-) epigallocatechin, (-) epigallocatechin gallate, gallic acid, genistein, isoquercetin, isovitexin, kaempferol, luteloin, malvidin, malvin, malvidin-3-glucoside, myricetin, nicotiflorin, p-coumaric acid, pelargonidin, peonidin-3-o-glucoside, protocatechuic acid, quercetin, quercetin-3-galactoside, resveratrol, rutin, syringic acid, sinapic acid, vitexin, and vitexin-2-O-rhamnoside were commercially available (Toronto Research Chemicals, Toronto, Canada). Three standards namely, Vanillic acid, ferulic acid, and ellagic acid were purchased from Sigma-Aldrich (Reidster, Steinhem). Other 26 phenolic compounds standards (**Table 4.S1**) were obtained from Cayman Chemical (Michigan, USA). Individual pure stock solutions (1000 mg/L) of each compound were prepared in acetonitrile except apigenin, daidzein, daidzin, genistein, luteolin, vitexin, isovitexin, vitexin-2-O-rhamnoside, , and aromadendrin, where a mixture of dimethyl sulfoxide: acetonitrile (10:90, v/v) was used. All stock solutions were prepared fresh in amber glass vials and stored at -20°C. A working standard mixture was prepared from the individual standard stock solutions at the concentration of 100 µg/mL using a mixture of methanol: water (50:50, v/v) for preparing calibration standards. An 8-point calibration curve was established using calibration standards prepared using working standard mixture at 1 ng/mL, 2 ng/mL, 5 ng/mL, 10 ng/ mL, 25 ng/mL, 50 ng/mL, 100 ng/mL and 250 ng/mL.

4.3.2 Small fruit samples

Fourteen small fruit samples were collected from various locations within a radius of 100 km of Winnipeg, Manitoba, Canada as described in Kodikara et al., (submitted to Food and Function). Small fruits samples included chokecherries (*Prunus virginiana*), chokeberries (*Aronia melanocarpa*), Saskatoon berries (*Amelanchier alnifolia*), redcurrants (*Ribes rubrum*), wild grapes (*Vitis riparia*), snowberries (*Symphoricarpos albus*), gooseberries (*Ribes hirtellum*), highbush cranberries (*Viburnum trilobum*), blackcurrants (*Ribes nigrum*), wild raspberries (*Rubus idaeus*), haskap berries (*Lonicera caerulea*), wild blueberries (*Vaccinium angustifolium*), buckthorn (*Hippophae rhamnoides*) and nannyberries (*Viburnum lentago*) (**Figure 4.S1**). Each fruit type collected from different locations was combined into a composite sample, and a sub-sample was used to determine the moisture content using the standard oven drying method (Reeb & Milota, 1999). The combined berries were sorted to remove debris, followed by freeze-drying (~150 h) to remove the moisture. Samples (2g) were ground using a mortar and pestle in the presence of liquid nitrogen to preserve sample phenolic compound composition. All fruit samples included their seeds. The ground samples were stored in an air-tight falcon tube at -80 °C until further analysis.

4.3.3 Extraction of phenolic compounds

Ground fruit samples were extracted for phenolic compounds using the methodology described in Kodikara et al. (2024). Briefly, a sub-sample (500mg) was placed in clean 15-mL Falcon tubes, and a solvent mixture (6mL) of isopropanol: water (80:20, v/v) was added. An aliquot (100µL) of a mixture of two internal standards (resveratrol $^{13}C_6$ and ferulic acid D_3; 1mg/mL each) was added to the sample before extraction. The sample-solvent mixture was thoroughly mixed, and extracted on a rotary shaker at 60 rpm (30 min), followed by sonication at 30 °C (30 min). Subsequently, the sample-solvent mixture was centrifuged (4000 ×g, 30 min) at -4 °C and the supernatant was filtered through a 0.2-µm nylon syringe-filters (Phenomenex, Torrance, USA) attached to a disposable syringe (10 mL) into a clean flat-bottomed tube (10 mL). An aliquot of the extract (1.5 mL) was completely evaporated under a gentle vacuum using a sample evaporator (Rocket Evaporator, Thermo Fisher, Mississauga, Canada). The dried extract was resuspended in 500 µL methanol:water (50:50, v/v) by sonicating and vortexing (5 s, each). After resuspension, the extract

was filtered again using a nylon syringe filter (0.2 µm) into a clean amber glass LC vial for analysis. Sample analysis was completed within 24 hours of the extraction process.

4.3.4 Sample analysis and data acquisition

Prior to analysis, a mixture of two internal standards (caffeic acid $^{13}C_3$ and gallic acid D_2) was added to the reconstituted extract, and sample analysis and data acquisition were performed as described in Kodikara et al. (2024), using a UHPLC (Vanquish, Thermo Fisher Scientific, Mississauga, Canada) coupled with an HRMS (ID-X Tribrid Orbitrap, Thermo Fisher Scientific, Mississauga, Canada). Briefly, a reverse-phase biphenyl column (2.6 µm, 100 x 2.1 mm, Kinetex, Phenomenex, USA) at a column temperature of 35 °C was used for analyte separation in the presence of water (mobile phase A) and acetonitrile (mobile phase B), both containing 0.1% formic acid and at a constant flow rate of 0.25 mL/min. Data acquisition, peak detection, and quantification of all analytes were achieved using HRMS using conditions described in Kodikara et al. (2024). Briefly, the spray voltage for positive and negative electrospray ionization were set at 3500 V and 2200 V, respectively, with the ion transfer tube and vaporizer temperatures at 325 °C and 350 °C, respectively. Data was acquired for all analytes using full scan mode at 120k resolution, followed by intensity and data-dependent MS2 fragmentation at 30k resolution using orbitrap. All 66 compounds were identified and quantified based on the parameters including column retention time, m/z using accurate masses of parent ions, and presence of fragment ions using TraceFinder software (v4.1, Thermo Fisher Scientific, Mississauga, Canada) (**Table 4.S1**).

4.3.5 Statistical analysis

Sample analysis for 66 phenolic compounds in all small fruits was carried out in triplicates and the data were expressed as the mean ± standard deviation (SD). One-way analysis of variance (ANOVA) was performed for each phenolic compound with a subsequent mean separation using Tukey's test. Data was summarized using column graphs and box plots, presenting mean values along with standard deviations. The chemometric analysis of 66 phenolic compounds across the 14 different Candian small fruits, was performed using principal component analysis (PCA) to reduce the dimensionality of the whole data set. PCA analysis was conducted with the contents of quantified phenolic compound classes as independent variables, resulting in the computation of

five principal components. Following the PCA, K-means cluster analysis was performed collectively for all identified phenolic compound classes after dividing 66 individual phenolic compounds into the phenolic classes based on their structural similarity. The determination of the number of principal components was determined by hierarchical cluster analysis. All statistical analyses were performed using Origin software (v2022b MA, USA).

4.4 Results and Discussion

4.4.1 Targeted metabolomics characterization of phenolic compounds

The moisture content of all the small fruits was determined to be in the range of 70% to 90% (**Tables 4.1a and 4.1b**). The phenolic compounds composition of 14 different Canadian small fruits normalized to dry weight (DW) was presented in **Tables 4.1a and 4.1b**. The total phenolic content varied among different fruit types with the highest concentration in Saskatoon berry (2102.45 mg/kg), followed by chokeberry (1354.26 mg/kg), and blueberry (1190.85 mg/kg). Saskatoon berry has been previously reported for its abundant phenolic content. For example, Dudonné et al. (2015), reported a total phenolic content of 148±2.91mg/100g FW (fresh weight), equivalent to 7400 mg/kg DW, assuming an 80% moisture content, in Canadian Saskatoon berry analyzed using Folin-Ciocalteu method. The differences in the identified values could be due to the extraction solvents and analytical methods used. Folin-Ciocalteu method analyses for the total phenolic compound are non-specific and could include other nonphenolic reducing agents and could lead to overestimation (Perez et al., 2023; Munteanu and Apetrei, 2021) whereas HRMS methods as one used in this study help in the characterization of phenolic compounds composition and avoid overestimation, however, not all phenolic compounds were accounted rather those with potential health benefits. Similarly, only small differences were noted in phenolic compound content among different fruit types analyzed in this study. For example, highbush cranberry (140.29 mg/kg) and nannyberry (252.19 mg/kg) had lower phenolic content on a fresh weight basis compared to those found by Dudonné et al. (2015) in berries (762±4.21mg/100g FW). In addition, other factors including variations in genetic profiles, physiological conditions, environmental conditions, agronomic conditions, and ripening stage could play an important role (Juríková et al., 2013; Liu et al., 2020). Wild grapes (388.36 mg/kg) and chokecherry (577.27 mg/kg) demonstrated moderate phenolic content among the tested berry types in the current study. The total phenolic content of each fruit type ranged from 140.3 mg/kg to 2102 mg/kg and was significantly different from each other except buckthorn and wild grapes (p<0.05) and was in the following decreasing order: Saskatoon berry > chokeberry > wild blueberry > Gooseberry > Haskap berry > Blackcurrant > redcurrant > chokecherry > buckthorn = wild grapes > gooseberry > snowberry > nannyberry > wild raspberry> highbush cranberry (**Figure 4.1**). These results contribute valuable insights into the phenolic diversity of Canadian small fruits and their potential implications for

human health. The variations observed in the phenolic content among different berries highlight the diversity of their chemical profiles and emphasize the need for a comprehensive approach when interpreting and comparing phenolic data. A matrix bubble plot (**Figure 4.2**) was created to represent data on berries and their phenolic compounds, offering a comprehensive overview of multiple variables in a single graph. The absence of bubbles for specific compounds indicates their relatively low concentrations within the berries. Among the different phenolic compound classes, anthocyanins were the major type of phenolic compound class present in all the tested small fruits.

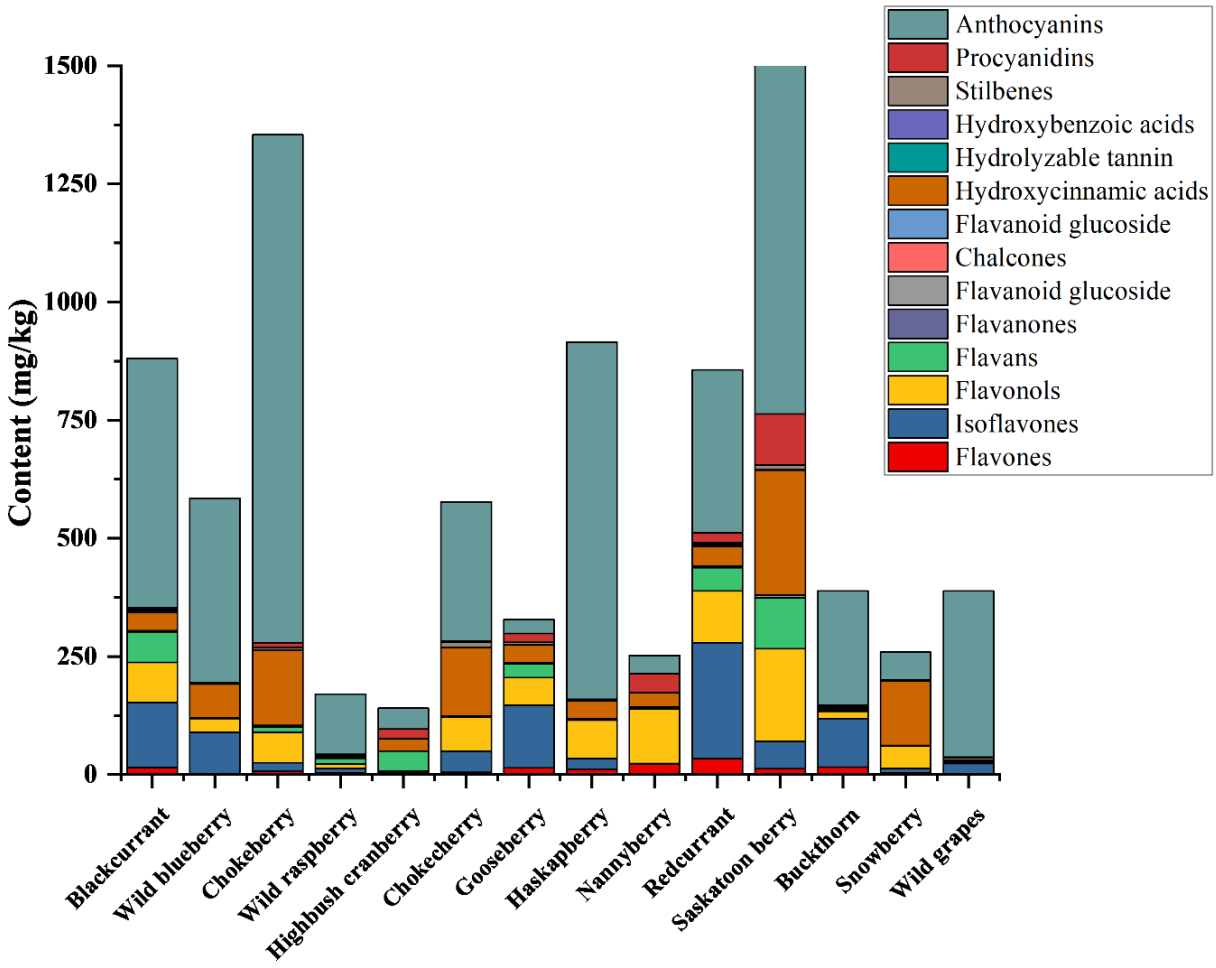


Figure 4.1. Distribution of different phenolic compound classes in Canadian small fruits. Each bar represents the combination of different phenolic classes contribute to the total phenolic content in each analyzed small fruit

Table 4.1a. Phenolic compounds content (mg/kg dry weight ± standard deviation (n=3)) in 14 different types of Canadian small fruits analyzed by the UHPLC-HRMS method

Phenolic compound	Blackcurrant	Wild blueberry	Chokeberry	Wild raspberry	Highbush cranberry	Chokecherry	Gooseberry
	<---------- Mean ± standard deviation, mg/kg dry weight ---------->						
Afzelin	0.778±0.008^{b}	0.227±0.007^{c}	0.102±0.005^{e}	0.016±0^{f}	NF	0.099±0.002^{e}	0.154±0.003^{d}
Apigenin	0.032±0^{b}	NF	NF	NF	NF	NF	0.023±0.002^{c}
Arbutin	0.032±0.002^{h}	NF	0.993±0.01^{f}	1.642±0.003^{e}	0.844±0.007^{fg}	2.332±0.018^{d}	9.055±0.321^{b}
Luteolin	0.14±0.006^{e}	0.019±0.001^{h}	0.018±0.001^{h}	0.025±0.002^{h}	0.018±0.001^{h}	0.019±0.002h	0.178±0.005^{c}
Isorhamnetin	NF	0.06±0.003^{b}	0.003±0.001^{fg}	0.003±0^{fg}	0.026±0.003^{cde}	NF	0.383±0.014^{a}
Vitexin	1.178±0.018^{a}	0.254±0.002^{d}	0.148±0.006^{ef}	0.032±0.003^{kl}	0.003±0^{l}	0.103±0.004^{g}	0.34±0.014^{c}
Vitexin-2-O-rhamnoside	1.513±0.12^{d}	0.925±0.029^{e}	1.479±0.028^{d}	0.262±0.01^{gh}	0.304±0.004^{fg}	0.272±0.002^{g}	0.382±0.009^{fg}
Isovitexin	1.187±0.003^{b}	0.251±0.003^{d}	0.138±0.005^{f}	0.032±0.003^{k}	0.003±0^{l}	0.099±0.007^{gh}	0.341±0.014^{c}
Herbacetin	1.493±0.013^{b}	0.006±0.001^{e}	0.005±0.001^{e}	0.005±0.001^{e}	0.006±0.002^{e}	0.005±0^{e}	0.016±0.004^{de}
Taxifolin	4.975±0.057^{a}	0.134±0.005^{gh}	0.629±0.01^{f}	0.194±0.006^{g}	0.03±0.001^{i}	0.046±0.005^{i}	1.076±0.027^{e}
Orientin	2.705±0.038^{c}	0.178±0.005^{f}	0.308±0.005^{ef}	0.296±0.005^{f}	0.005±0^{f}	0.898±0.04d^{e}	1.101±0.017^{d}
Vicenin-2	0.655±0.018^{e}	0.054±0.002^{gh}	3.54±0.055^{b}	0.087±0.003^{gh}	0.005±0^{h}	0.18±0.008^{fg}	0.746±0.008^{e}
Total flavones	**14.69**	**2.11**	**7.36**	**2.60**	**1.24**	**4.05**	**13.80**
Aromadendrin	0.374±0.005^{b}	0.01±0.001^{d}	0.006±0.001^{d}	0.033±0.001^{d}	0.005±0^{d}	0.004±0^{d}	0.217±0.005^{c}
Daidzein	NF	NF	NF	NF	NF	NF	0.01±0.001^{c}
Daidzin	107.6±0.671^{b}	47.14±60.933^{cd}	14.94±0.737^{cd}	6.27±0.142^{d}	1.235±0.02^{d}	44.652±1.958^{cd}	129.7±0.528^{b}
Genistein	0.033±0.001^{c}	NF	NF	NF	NF	NF	0.041±0.002^{b}
Glycitein	29.39±0.311^{b}	0.08±0.003^{e}	0.693±0.014^{de}	3.689±0.023^{c}	0.028±0.004^{e}	0.407±0.033^{de}	0.378±0.003^{de}
Glycitin	NF	40.42±0.967^{b}	1.753±0.042^{de}	NF	NF	NF	2.834±0.012^{d}
Total isoflavones	**137.42**	**87.65**	**17.39**	**9.99**	**1.27**	**45.06**	**133.13**
Quercetin	1.493±0.012^{b}	0.006±0.001^{ef}	0.006±0^{ef}	0.006±0.001^{ef}	NF	0.005±0^{ef}	0.016±0.004^{de}
Quercetin-3-galactoside	10.08±0.182^{c}	4.514±0.047^{f}	6.433±0.298^{d}	0.541±0.007^{ij}	0.768±0.007^{i}	5.633±0.152^{e}	2.998±0.063^{g}
Isoquercetin	10.08±0.182^{c}	4.647±0.191^{f}	6.433±0.298^{e}	0.541±0.007^{ij}	0.772±0.022^{ij}	5.554±0.133^{e}	3.052±0.051^{g}
Kaempferol	0.142±0.005^{cd}	NF	0.021±0.001^{h}	0.025±0.001^{gh}	0.019±0.001^{h}	0.022±0.002^{h}	0.178±0.006^{b}
Kaempferol-3-glucoside	5.382±0.197^{c}	0.651±0.012^{ij}	1.272±0.044^{fgh}	0.547±0.015^{j}	0.623±0.01^{ij}	0.929±0.009^{hi}	1.521±0.059^{f}

Myricetin	16.41±0.287^{a}	0.673±0.011^{ef}	0.551±0.008^{fg}	0.627±0.017^{ef}	NF	0.912±0.085^{e}	1.904±0.024^{d}
Rutin	41.69±0.113^{f}	18.43±0.762^{g}	50.60±1.293^{e}	7.328±0.259^{h}	2.155±0.006^{i}	59.55±0.264^{d}	48.032±1.608^{e}
Fisetin	0.098±0.002^{bc}	0.003±0.001^{i}	0.002±0.001^{i}	0.011±0.001h^{i}	0.003±0^{i}	0.005±0.001^{hi}	0.104±0.005^{b}
Total flavonols	**85.39**	**28.92**	**65.32**	**9.63**	**4.34**	**72.61**	**57.81**
(+)-Catechin	8.481±0.218^{e}	0.774±0.003^{ghi}	5.321±0.218^{f}	5.832±0.157^{f}	23.27±0.576^{b}	0.199±0.007^{i}	10.41±0.666^{d}
(-)-Epicatechin	46.82±0.264^{b}	0.775±0.003^{fg}	5.321±0.218^{e}	5.832±0.157^{e}	19.49±0.397^{c}	0.199±0.007^{g}	18.16±0.845^{d}
(-)-Epicatechin Gallate	7.429±0.173^{b}	0.022±0.001^{e}	0.051±0.004^{e}	0.219±0.004^{cd}	0.018±0.001^{e}	0.058±0.003^{e}	0.114±0.006^{de}
(-)-Epigallocatechin	1.579±0.021^{a}	0.095±0.004^{gh}	0.066±0^{h}	0.168±0.003^{f}	0.085±0.001^{h}	0.112±0.006^{gh}	0.951±0.057^{c}
Total flavans	**64.31**	**1.67**	**10.76**	**12.05**	**42.87**	**0.57**	**29.64**
Naringin	0.272±0.006^{hi}	0.25±0.007^{i}	0.152±0.001^{jk}	1.459±0.015^{b}	0.058±0.001^{l}	2.217±0.051^{a}	0.681±0.006^{e}
Liquiritingenin	0.023±0.001^{c}	NF	0.004±0^{d}	NF	NF	NF	NF
Hesperetin	1.308±0.027^{b}	NF	0.005±0^{c}	NF	NF	NF	NF
Total flavanones	**1.602**	**0.250**	**0.161**	**1.459**	**0.058**	**2.217**	**0.681**
Nicotiflorin	0.646±0.023^{e}	0.052±0.003^{g}	3.433±0.152^{b}	0.084±0.003^{g}	0.006±0.002^{g}	0.179±0.006^{fg}	1.098±0.033^{cd}
Total flavonoid glucoside	**0.646**	**0.052**	**3.433**	**0.084**	**0.006**	**0.179**	**1.098**
Okanin	0.268±0.007^{b}	0.006±0.001^{f}	NF	0.025±0.002^{ef}	NF	0.001±0^{f}	0.153±0.01^{d}
Total chalcones	**0.268**	**0.006**	**0.000**	**0.025**	**0.000**	**0.001**	**0.153**
Caffeic Acid	0.053±0.003^{c}	0.886±0.011^{d}	0.262±0.007^{d}	0.349±0.009^{d}	0.087±0.003^{b}	0.668±0.01^{d}	0.78±0.007^{a}
Caftaric Acid	1.892±0.043^{c}	0.583±0.011^{d}	0.97±0.033^{d}	0.488±0.007^{d}	5.084±0.125^{b}	0.742±0.011^{d}	26.42±1.006^{a}
Chlorogenic Acid	36.06±0.11^{b}	69.47±1.401^{b}	158.1±206.5^{ab}	0.041±0.001^{b}	20.98±0.37^{b}	142.6±4.327^{ab}	10.45±0.209^{b}
Ferulic Acid	0.032±0.002^{bc}	0.11±0.004^{a}	0.007±0.003^{e}	0.003±0^{e}	0.006±0.002^{e}	0.006±0.001^{e}	0.006±0.002^{e}
Para-coumaric Acid	0.167±0.003^{cd}	0.019±0.001^{f}	0.029±0.003^{ef}	0.021±0.003^{f}	NF	0.189±0.01^{c}	0.105±0.014^{de}
Sinapic Acid	NF	NF	0.002±0^{d}	0.006±0.001^{c}	NF	0.017±0.002^{a}	0.018±0.001^{a}
Total hydroxycinnamic acids	**38.20**	**71.07**	**159.35**	**0.91**	**26.16**	**144.14**	**37.77**
Ellagic Acid	1.526±0.012^{b}	NF	NF	NF	NF	NF	NF
Total hydrolyzable tannin	**1.526**	**0.000**	**0.000**	**0.000**	**0.000**	**0.000**	**0.000**
Gallic Acid	1.542±0.023^{b}	0.041±0.001^{f}	0.006±0.001^{f}	0.036±0.003^{f}	0.005±0^{f}	0.031±0.002^{f}	0.188±0.005^{de}
Protocatechuic Acid	0.211±0.009^{d}	0.064±0.003^{gh}	0.08±0.001^{fg}	0.018±0.002^{i}	0.107±0.004^{ef}	0.12±0.006e	0.099±0.005^{ef}
Syringic Acid	0.4±0.008^{a}	0.01±0^{efg}	0.001±0^{h}	0.006±0.001^{fgh}	0.003±0^{gh}	0.046±0.002^{d}	NF
Vanillic Acid	0.038±0.001^{d}	0.006±0.001^{h}	0.024±0.003^{e}	0.002±0^{hi}	NF	0.089±0.003^{a}	0.007±0.001^{gh}

Total hydroxybenzoic acids	**2.191**	**0.122**	**0.110**	**0.062**	**0.115**	**0.285**	**0.295**
Piceatannol	0.093±0.002^{c}	0.077±0.003^{e}	0.068±0.003^{g}	0.069±0.002^{fg}	NF	0.073±0.002^{ef}	NF
Polydatin	1.333±0.04^{ef}	0.621±0.005^{ghi}	4.555±0.06^{c}	3.444±0.049^{d}	0.094±0.003^{i}	11.18±0.599^{a}	0.82±0.023^{fgh}
Resveratrol	NF	NF	0.077±0.001^{e}	0.047±0.002^{f}	0.104±0.002^{d}	0.029±0^{g}	0.101±0.011^{d}
Rhapontin	1.182±0.021^{b}	0.037±0.001^{f}	0.074±0^{ef}	0.346±0.004^{d}	0.16±0.006^{def}	0.183±0.006^{def}	4.28±0.264^{a}
Total stilbenes	**2.608**	**0.735**	**4.775**	**3.906**	**0.358**	**11.471**	**5.201**
Procyanidin A	0.687±0.027^{c}	0.14±0.002^{j}	0.391±0.007^{f}	0.221±0.003^{hi}	0.034±0.002^{k}	1.092±0.036^{a}	0.521±0.022^{e}
Procyanidin B	1.325±0.041^{cd}	0.917±0.024^{def}	1.089±0.016^{de}	0.185±0.003^{g}	0.952±0.01^{def}	0.534±0.696^{efg}	0.495±0.017^{fg}
Procyanidin C	1.374±0.023^{gh}	0.31±0.011^{i}	9.097±0.253^{f}	1.534±0.024^{g}	18.69±0.382^{c}	0.41±0.022^{hi}	17.38±0.523^{d}
Total procyanidins	**3.385**	**1.368**	**10.577**	**1.941**	**19.673**	**2.036**	**18.39**
Cyanidin Chloride	7.178±0.158^{e}	2.914±0.098^{hi}	17.34±0.841^{c}	1.698±0.039^{j}	4.067±0.04^{g}	3.567±0.02^{gh}	0.536±0.017^{k}
Cyanidin-3-arabinoside	9.136±0.397^{d}	64.52±0.573^{c}	470.4±4.006^{a}	0.892±0.032^{e}	1.145±0.034^{e}	1.849±0.026^{e}	0.599±0.01^{e}
Cyanidin-3-O-glucoside	308.6±1.293^{bc}	106.5±4.813^{cd}	286.7±70.07^{bcd}	46.4±1.198^{cd}	3.916±0.059^{d}	77.98±1.294^{cd}	5.238±0.333^{d}
Cyanidin-3-rutinoside	178.2±1.09^{a}	2.208±0.091^{i}	3.994±0.013^{i}	54.22±1.212^{e}	2.566±0.016^{i}	146.7±1.34^{b}	3.088±0.048^{i}
Delphinidin	6.42±0.152^{de}	23.16±0.342^{c}	222.1±2.028^{a}	3.784±0.02^{hij}	5.3±0.108^{ghi}	21.68±0.919^{c}	3.45±0.078^{ij}
Delphinidin-3-glucoside	10.39±0.209^{h}	82.36±2.159^{a}	62.33±1.325^{c}	3.327±0.032^{j}	9.755±0.507^{h}	29.54±0.926^{e}	5.241±0.292^{j}
Malvidin	2.303±0.073^{hi}	9.362±0.583^{a}	5.353±0.205^{c}	9.679±0.238^{a}	3.312±0.044^{ef}	8.648±0.491^{b}	2.685±0.053^{fgh}
Malvidin-3-glucoside	0.417±0.02^{c}	391.3±206.1^{a}	1.226±0.033^{c}	1.665±0.049^{c}	1.246±0.031^{c}	1.107±0.035^{c}	0.488±0.024^{c}
Malvin	0.144±0.002^{d}	0.979±0.038^{cd}	0.139±0.001^{d}	0.31±0.004^{d}	0.348±0.007^{d}	0.376±0.003^{d}	2.339±0.036^{c}
Pelargonidin	0.609±0.005^{h}	1.88±0.192^{cd}	0.663±0.017^{h}	0.921±0.026^{g}	3.14±0.021^{a}	1.294±0.044^{f}	0.934±0.04^{g}
Peonidin	1.128±0.009^{fgh}	1.433±0.026^{ef}	0.878±0.018^{ghi}	1.172±0.018^{fg}	1.658±0.037^{de}	0.503±0.026^{i}	0.726±0.012^{hi}
Peonidin-3-O-glucoside	1.905±0.033^{e}	306.6±8.66^{a}	0.56±0.014^{e}	2.614±0.021^{e}	0.605±0.009^{e}	0.423±0.025^{e}	1.036±0.014^{e}
Petunidin	1.277±0.02^{ghi}	3.878±0.096^{c}	3.329±0.048^{cd}	0.9±0.017^{hi}	7.135±0.187^{b}	1.014±0.092^{hi}	3.46±0.074^{cd}
Total anthocyanins	**527.70**	**996.9**	**1075.02**	**127.58**	**44.19**	**294.65**	**29.82**
Total phenolic content	**880.2**	**1191**	**1354**	**170.3**	**140.3**	**577.3**	**327.9**
Moisture content (%)	**75.72±3**	**84.10±2**	**82.95±3**	**85.78±3**	**82.35±3**	**76.36±2**	**85.92±4**

NF: Not Found

[a-i]The values containing different letters have significantly different (p<0.05) mean concentrations based on Tukey's mean separation across the rows for different small fruits included in Tables 1a and 1b

Table 4.1b. Phenolic compounds content (mg/kg dry weight ± standard deviation (n=3) in 14 different types of Canadian small fruits analyzed by the UHPLC-HRMS method

Phenolic compound	Haskapberry	Nannyberry	Redcurrant	Saskatoon berry	Buckthorn	Snowberry	Wild grapes
	<---------- Mean ± standard deviation, mg/kg dry weight ---------->						
Afzelin	0.16±0.009^{d}	0.042±0.002^{f}	3.086±0.06^{a}	0.026±0.002^{f}	0.007±0.001^{f}	0.026±0.001^{f}	0.032±0.002^{f}
Apigenin	0.023±0.002^{c}	0.266±0.003^{a}	0.023±0.002^{c}	0.013±0.001^{d}	NF	NF	NF
Arbutin	9.055±0.321^{b}	16.34±0.13^{d}	0.035±0.001^{h}	0.035±0.001^{h}	8.416±0.094^{c}	0.496±0.015^{g}	NF
Luteolin	0.044±0^{g}	0.521±0.003^{a}	0.131±0.007^{e}	0.087±0.005^{f}	0.153±0.005^{d}	0.192±0.001^{b}	0.039±0.002^{g}
Isorhamnetin	0.029±0.002^{cd}	0.014±0^{ef}	0.068±0.005^{b}	0.069±0.002^{b}	0.031±0.001^{c}	0.026±0.002^{cde}	0.016±0.001^{de}
Vitexin	0.172±0.002^{e}	0.073±0.003^{hi}	3.934±0.027^{a}	0.131±0.007^{fg}	0.103±0.004^{gh}	0.068±0.003^{ij}	0.042±0.004^{jk}
Vitexin-2-O-rhamnoside	0.507±0.017^{f}	2.089±0.007^{c}	5.211±0.204^{a}	2.619±0.129^{b}	0.053±0.001^{hi}	0.015±0.001^{i}	0.259±0.011^{gh}
Isovitexin	0.172±0.002^{e}	0.073±0.003^{hi}	3.944±0.026^{a}	0.131±0.007^{f}	0.101±0.003^{g}	0.067±0.002^{ij}	0.041±0.003^{jk}
Herbacetin	0.008±0^{e}	0.029±0.004^{cd}	2.095±0.015^{a}	0.01±0.001^{e}	0.005±0.001^{e}	0.033±0.001^{c}	0.005±0^{e}
Taxifolin	0.069±0.006^{hi}	0.593±0.004^{f}	2.566±0.033^{b}	1.386±0.049^{d}	1.961±0.024^{c}	0.022±0.001^{i}	0.03±0.001^{i}
Orientin	1.101±0.017^{d}	1.305±0.019^{d}	11.19±0.739^{a}	1.49±0.072^{d}	3.608±0.017^{b}	2.492±0.021^{c}	0.049±0.005^{f}
Vicenin-2	0.111±0.007^{gh}	0.271±0.007^{f}	1.22±0.045^{d}	5.696±0.134^{a}	1.419±0.054^{c}	NF	0.067±0.002^{gh}
Total flavones	**11.45**	**21.62**	**33.51**	**11.69**	**15.86**	**3.44**	**0.58**
Aromadendrin	0.056±0.005^{d}	0.057±0.003^{d}	0.269±0.014^{bc}	NF	2.635±0.158^{a}	NF	0.004±0^{d}
Daidzein	NF	NF	0.041±0.002^{b}	0.048±0.007^{a}	NF	NF	NF
Daidzin	16.73±1.071^{cd}	0.265±0.005^{d}	203.2±3.243^{a}	57.34±2.206^{c}	44.16±0.986^{cd}	9.411±0.162^{cd}	19.033±0.82^{cd}
Genistein	NF	0.267±0.008^{a}	0.024±0.004^{d}	0.014±0.002^{e}	NF	0.006±0.002^{f}	NF
Glycitein	0.116±0.008^{e}	0.151±0.004^{e}	41.57±0.869^{a}	1.061±0.037^{d}	0.439±0.023^{e}	0.492±0.007^{e}	0.488±0.006^{e}
Glycitin	5.484±0.23^{c}	1.014±0.026^{de}	NF	NF	55.88±3.132^{a}	NF	2.937±0.089^{cd}
Total isoflavones	**22.38**	**1.75**	**245.06**	**58.47**	**103.12**	**9.91**	**22.46**
Quercetin	0.009±0^{ef}	0.028±0.002^{cd}	2.092±0.011^{a}	0.01±0.001^{ef}	0.006±0.001^{ef}	0.034±0.001^{c}	NF
Quercetin-3-galactoside	2.707±0.068^{g}	0.006±0.001^{j}	12.42±0.422^{b}	20.35±0.336^{a}	0.257±0.002^{ij}	1.801±0.052^{h}	0.512±0.026^{ij}

Isoquercetin	2.734±0.046^{g}	1.841±0.171^{h}	12.11±0.246^{b}	20.12±0.433^{a}	0.26±0.008^{j}	1.862±0.007^{h}	0.887±0.008^{i}
Kaempferol	0.042±0.005^{f}	0.516±0.01^{a}	0.132±0.006^{d}	0.086±0.005^{e}	0.153±0.005^{c}	0.191±0.004^{b}	0.039±0.002^{fg}
Kaempferol-3-glucoside	1.111±0.028^{gh}	1.316±0.018^{fg}	16.61±0.188^{a}	3.067±0.042^{e}	6.494±0.179^{b}	4.833±0.268^{d}	0.092±0.008^{k}
Myricetin	0.451±0.014^{fg}	2.172±0.014^{d}	8.912±0.314^{b}	2.63±0.09^{c}	0.731±0.019^{ef}	NF	0.217±0.004^{gh}
Rutin	74.23±1.789^{c}	108.9±0.688^{b}	57.59±1.706^{d}	149.9±17.54^{a}	7.343±0.179^{h}	38.53±1.146^{f}	0.212±0.005^{i}
Fisetin	0.017±0.002^{gh}	0.462±0.011^{a}	0.07±0.003^{d}	0.055±0.004^{e}	0.029±0.002^{f}	0.088±0.003^{c}	0.026±0.001^{fg}
Total flavonols	**81.30**	**115.30**	**109.94**	**196.23**	**15.27**	**47.34**	**1.99**
(+)-Catechin	0.746±0.046^{ghi}	1.424±0.022^{g}	20.15±0.028^{c}	53.14±0.914^{a}	0.33±0.026^{hi}	NF	1.373±0.047^{gh}
(-)-Epicatechin	0.737±0.037^{fg}	1.449±0.041^{f}	20.17±0.06^{c}	53.162±0.885^{a}	0.329±0.026^{g}	NF	1.406±0.015^{f}
(-)-Epicatechin Gallate	0.017±0.001^{e}	0.032±0.004^{e}	8.55±0.013^{a}	0.041±0.003^{e}	0.057±0.002^{e}	0.028±0.001^{e}	0.345±0.015^{c}
(-)-Epigallocatechin	0.148±0.002^{fg}	0.302±0.009^{e}	NF	1.388±0.017^{b}	0.182±0.007^{f}	0.756±0.019^{d}	0.143±0.009^{fg}
Total flavans	**1.65**	**3.21**	**48.87**	**107.73**	**0.90**	**0.78**	**3.27**
Naringin	1.102±0.026^{b}	0.992±0.019^{c}	0.304±0.006^{d}	0.163±0.002^{g}	0.383±0.016^{d}	0.109±0.001^{g}	0.346±0.009^{f}
Liquiritingenin	NF	NF	NF	0.051±0.005a	0.043±0.002b	0.001±0d	NF
Hesperetin	0.007±0.001^{c}	0.03±0.001^{c}	2.038±0.036^{a}	NF	NF	0.022±0.002^{c}	NF
Total flavanones	**1.109**	**1.023**	**2.343**	**0.214**	**0.427**	**0.133**	**0.346**
Nicotiflorin	0.113±0.003^{fg}	0.273±0.004^{f}	1.207±0.023^{c}	5.816±0.173^{a}	0.956±0.024^{d}	0.004±0^{g}	0.017±0^{g}
Total flavonoid glucoside	**0.113**	**0.273**	**1.207**	**5.816**	**0.956**	**0.004**	**0.017**
Okanin	0.046±0.005^{e}	0.05±0.002^{e}	0.187±0.008^{c}	0.017±0^{f}	2.328±0.027^{a}	0.002±0^{f}	0.002±0^{f}
Total chalcones	**0.046**	**0.050**	**0.187**	**0.017**	**2.328**	**0.002**	**0.002**
Caffeic Acid	0.512±0.005^{d}	0.299±0.019^{c}	2.297±0.022^{d}	3.089±0.018^{d}	0.725±0.063^{d}	35.16±0.311^{b}	0.541±0.016^{d}
Caftaric Acid	0.5±0.018^{d}	1.895±0.016^{c}	0.943±0.047^{d}	0.534±0.035^{d}	0.727±0.039^{d}	5.175±0.071^{b}	0.458±0.04^{d}
Chlorogenic Acid	36.97±1.319^{b}	26.85±0.81^{b}	38.14±1.509^{b}	255.8±3.308^{a}	0.142±0.005^{b}	96.45±0.455^{ab}	NF
Ferulic Acid	0.009±0.004^{e}	0.006±0.002^{e}	0.023±0.001^{d}	0.036±0.004^{b}	0.026±0.001^{cd}	0.005±0.001^{e}	0.01±0.002^{e}
Para-coumaric Acid	0.013±0.003f	0.172±0.002^{cd}	0.572±0.004^{b}	4.349±0.098^{a}	0.617±0.009^{b}	0.132±0.002^{cd}	0.01±0.001^{f}
Sinapic Acid	NF	NF	NF	NF	0.014±0.001^{b}	NF	NF
Total hydroxycinnamic acids	**38.00**	**29.22**	**41.97**	**263.84**	**2.25**	**136.92**	**1.02**
Ellagic Acid	0.007±0de	0.03±0.001^{c}	2.102±0.017^{a}	NF	NF	0.021±0.001^{cd}	0.005±0de
Total hydrolysable tannin	**0.007**	**0.030**	**2.102**	**0.000**	**0.000**	**0.021**	**0.005**
Gallic Acid	0.005±0.001^{f}	0.017±0.001^{f}	1.883±0.029^{a}	0.219±0.01^{d}	1.108±0.05^{c}	0.023±0.002^{f}	0.148±0.004^{e}

Protocatechuic Acid	0.024±0.002^{i}	0.186±0.004^{d}	0.297±0.008^{c}	1.007±0.02^{a}	0.761±0.027^{b}	0.034±0.002^{hi}	0.078±0.002^{fg}
Syringic Acid	0.039±0.002^{d}	0.013±0.001^{ef}	0.163±0.006^{b}	0.085±0.004^{c}	0.01±0.001^{efg}	0.018±0.001^{e}	0.018±0.001^{e}
Vanillic Acid	0.011±0.001^{g}	NF	0.056±0.001^{c}	0.061±0.002^{b}	0.018±0.001^{f}	NF	0.007±0.001^{gh}
Total hydroxybenzoic acids	**0.079**	**0.216**	**2.399**	**1.372**	**1.896**	**0.075**	**0.251**
Piceatannol	0.071±0.002^{fg}	0.15±0.002^{a}	NF	0.072±0^{fg}	0.084±0.001^{d}	NF	0.123±0.002^{b}
Polydatin	0.409±0.01^{hi}	0.476±0.02^{hi}	1.499±0.036^{e}	8.648±0.11^{b}	0.346±0.005^{hi}	1.144±0.048^{efg}	4.905±0.379^{c}
Resveratrol	0.005±0.001^{h}	0.242±0.002^{b}	0.038±0.001^{fg}	0.307±0.011^{a}	0.097±0.004^{d}	NF	0.183±0.006^{c}
Rhapontin	0.257±0.005^{ef}	0.331±0.009^{de}	1.147±0.031^{b}	1.055±0.039^{b}	0.173±0.003^{h}	0.34±0.011^{d}	0.57±0.01^{c}
Total stilbenes	**0.742**	**1.199**	**2.683**	**10.082**	**0.700**	**1.484**	**5.780**
Procyanidin A	0.259±0.003^{h}	0.057±0.001^{k}	0.742±0.011^{b}	0.716±0.025^{bc}	0.58±0.006^{d}	0.311±0.003^{g}	0.183±0.013^{ij}
Procyanidin B	0.524±0.016^{efg}	2.115±0.026^{b}	4.972±0.067^{a}	1.743±0.092^{bc}	0.058±0.001^{g}	0.022±0.001^{g}	0.191±0.007^{g}
Procyanidin C	1.393±0.028^{gh}	37.71±0.532^{b}	16.03±0.239^{e}	105.023±0.936^{a}	0.057±0.004^{i}	NF	0.755±0.033^{ghi}
Total procyanidins	**2.177**	**39.885**	**21.739**	**107.482**	**0.695**	**0.333**	**1.129**
Cyanidin Chloride	19.05±0.221^{b}	1.401±0.032^{jk}	5.596±0.139^{f}	13.21±0.32^{d}	34.93±0.852^{a}	1.625±0.044^{j}	2.045±0.05^{ij}
Cyanidin-3-arabinoside	1.425±0.017^{e}	0.366±0.011^{e}	3.779±0.069^{e}	141.3±1.787^{b}	2.617±0.054^{e}	2.032±0.06^{e}	0.552±0.01^{e}
Cyanidin-3-O-glucoside	577.1±3.96^{ab}	4.211±0.1^{d}	188.6±3.227^{cd}	612.6±13.972^{a}	37.65±1.314^{cd}	13.28±0.7^{cd}	73.67±1.107^{cd}
Cyanidin-3-rutinoside	22.48±0.75^{f}	2.47±0.018^{i}	107.4±1.846^{c}	1.286±0.042^{i}	74.23±1.714^{d}	12.19±0.487^{h}	18.25±1.166^{g}
Delphinidin	13.48±0.41^{d}	9.002±0.473^{f}	10.59±0.34^{ef}	45.99±1.36^{b}	12.71±0.589^{de}	5.847±0.244^{gh}	2.54±0.046^{j}
Delphinidin-3-glucoside	20.93±0.402^{f}	5.915±0.051^{ij}	16.95±0.405^{g}	78.51±1.323^{b}	20.16±0.543^{f}	8.571±0.263^{hi}	53.07±1.468^{d}
Malvidin	2.455±0.036^{ghi}	3.815±0.047^{de}	2.337±0.029^{ghi}	4.339±0.21^{d}	1.83±0.037^{i}	3.022±0.076^{fg}	2.678±0.063^{fgh}
Malvidin-3-glucoside	0.458±0.053^{c}	0.275±0.01^{c}	1.227±0.024^{c}	0.205±0.008^{c}	0.354±0.006^{c}	0.269±0.008^{c}	124.9±2.444^{b}
Malvin	1.553±0.031^{cd}	0.309±0.006^{d}	0.244±0.012^{d}	0.366±0.011^{d}	5.334±0.184^{b}	0.176±0.006^{d}	29.74±1.833^{a}
Pelargonidin	2.162±0.014^{b}	3.063±0.055^{a}	0.508±0.024^{h}	1.592±0.054^{e}	2.021±0.065^{bc}	1.7±0.037^{de}	1.857±0.056^{cd}
Peonidin	2.085±0.012^{c}	3.751±0.033^{b}	1.21±0.037^{fg}	2.015±0.034^{cd}	26.73±0.51^{a}	1.739±0.035^{cde}	1.983±0.068^{cd}
Peonidin-3-O-glucoside	90.28±1.597^{b}	3.142±0.041^{e}	3.456±0.037^{de}	1.741±0.048^{e}	10.49±0.503^{d}	6.728±0.254^{de}	37.339±0.749^{c}
Petunidin	2.296±0.028^{ef}	0.638±0.032^{i}	2.227±0.046^{ef}	1.372±0.02^{gh}	12.64±0.78^{a}	1.692±0.088^{fg}	2.837±0.06^{de}
Total anthocyanins	**755.75**	**38.36**	**344.13**	**904.48**	**241.69**	**58.87**	**351.51**
Total phenolic content	**914.9**	**252.2**	**856.3**	**2102**	**388.4**	**259.3**	**388.4**
Moisture (%)	**83.45±3**	**79.34±2**	**77.34±2**	**78.34±2**	**70.67±1**	**79.23±2**	**77.83±2**

NF: Not Found

[a-i]The values containing different letters have significantly different (p<0.05) mean concentrations based on Tukey's mean separation across the rows for different small fruits included in Tables 1a and 1b.

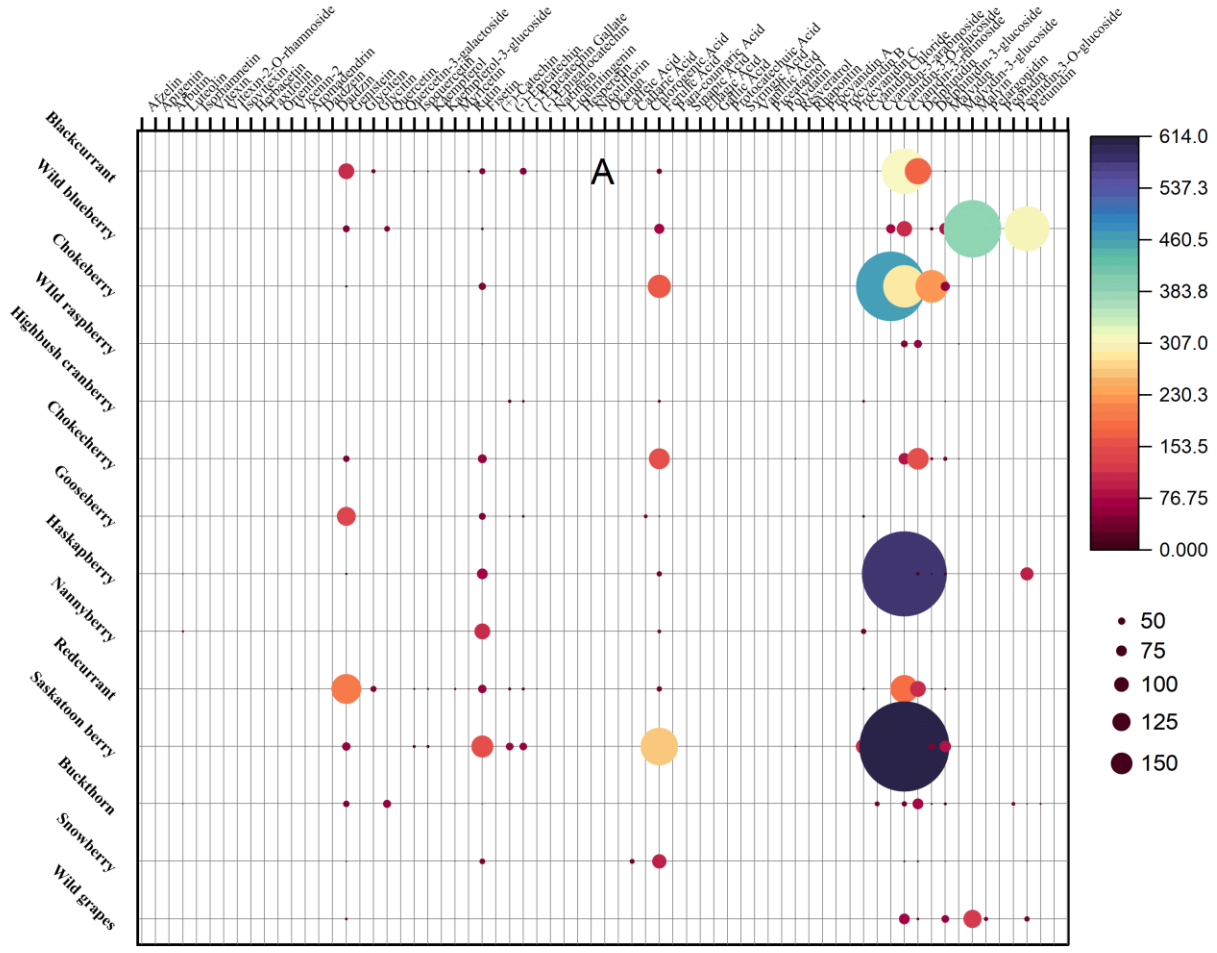


Figure 4.2 Matrix bubble shows the most abundant phenolic compounds among 66 phenolic compounds among 14 different types of Canadian small fruits and the compounds present in relatively low berries. The X-axis represents various phenolic compounds and the Y-axis represents the different types of Canadian small fruits. The size of each bubble represents the concentration or quantity of the respective phenolic compound in a particular small fruit. Larger bubbles indicate higher concentrations. Different bubble colors represent different phenolic compounds. Larger bubbles indicate higher levels of the presence of different phenolic compounds providing a visual cue for an extra layer of information.

4.4.2 Quantification of phenolic compounds in Canadian wild berries

4.4.2.1 Flavonoids

Flavonoids, a diverse group of polyphenolic compounds, consist of several classes that contribute to the wide array of plant pigments and antioxidants (Yao et al., 2004). Common classes within the flavonoid group include flavanols, flavones, flavanols, flavanones, isoflavones, and anthocyanins (Del Rio et al., 2010). Each class possesses unique chemical structures and biological properties, collectively play significant roles in the health benefits associated with various berries and other fruits and vegetables (Yao et al., 2004). Anthocyanins, belonging to a subclass of flavonoids, are known for their antioxidant properties, contributing more than 50% to the overall flavonoid content in berries (Brown & Shipley, 2011; Castañeda-Ovando et al., 2009). In the current study, 47 phenolic compounds were identified belong to the flavonoids class. Among these, anthocyanins contributed significantly to the variations in total flavonoid contents and also to the total phenolic compound contents of tested small fruits.

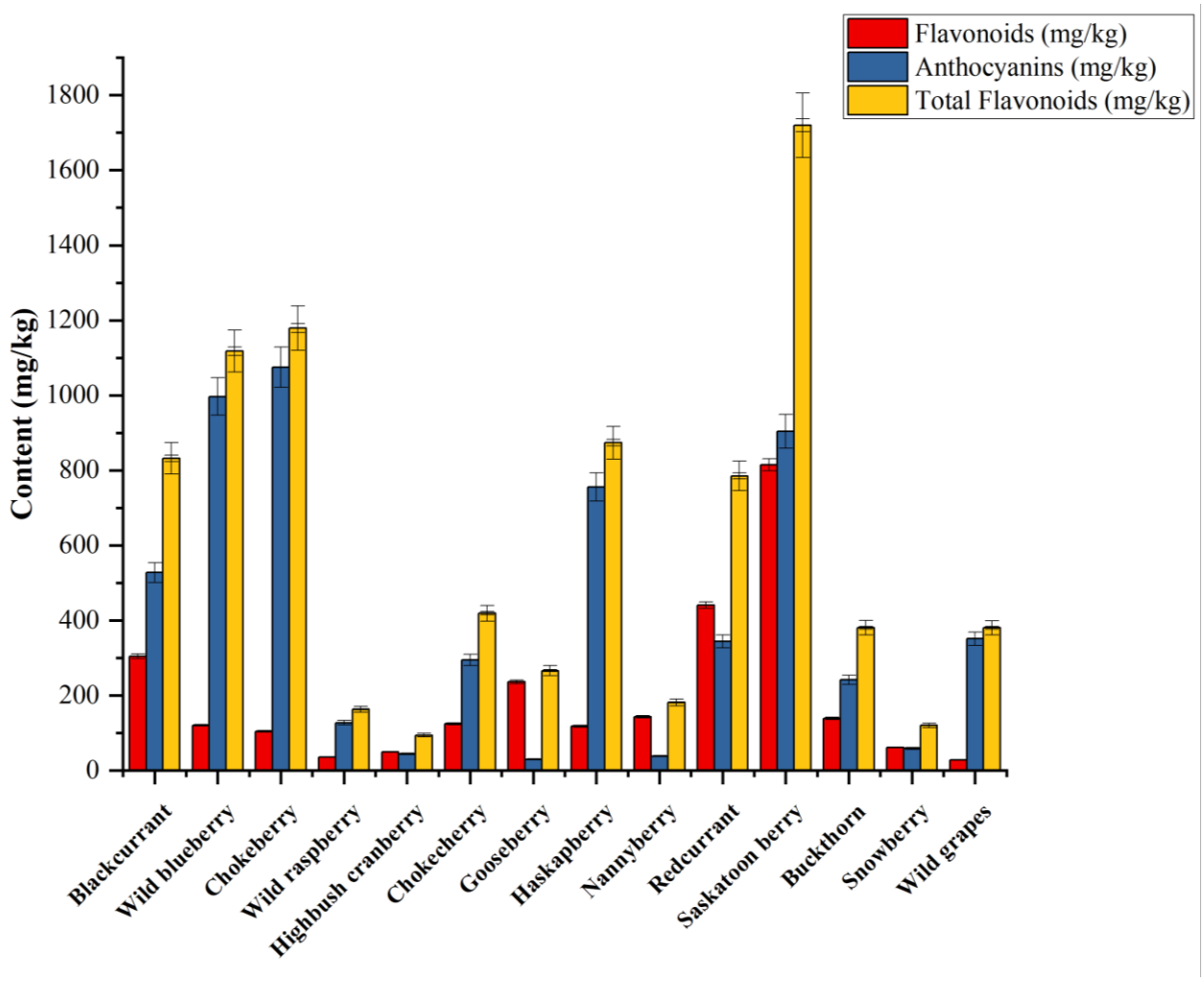


Figure 4.3 Bar graph showing the variation in the distribution of flavonoid compounds (flavonoid compounds without anthocyanins, and total flavonoid content along with the anthocyanins contents) among 14 different types of Canadian small fruits.

As shown in **Figure 3**, results indicate a significant relationship between the total flavonoid content in various berries and the presence of anthocyanins. The contributions of the anthocyanins for the total flavonoid contents were 94.8% (blueberries), 92.5% (wild grapes), 91.2% (chokeberries), 86.5% (haskap berries), 78.1% (raspberries), 70.3% (chokecherries), 63.51% (buckthorns), 63.4% (blackcurrants), 52.6% (Saskatoon berries), 48.9% (snowberries), 47.0% (highbush cranberries), 43.8% (redcurrants), 21.1% (nannyberries) and 11.20% (gooseberries) highlighting the significant contribution of anthocyanins to the total flavonoid content. Saskatoon berry had the highest total flavonoid content among the listed berries (1719.66 mg/kg), primarily due to a significant anthocyanin contribution (904.48 mg/kg, 52.6% of the total flavonoid content). Among the tested fruits, Saskatoon berries showed a balance between anthocyanins and other flavonoids (52.6% of anthocyanin contribution to the total flavonoid content). Chokeberry had the second-highest total flavonoid content of 1179 mg/kg, showcasing a considerable contribution from its total anthocyanins (1075 mg/kg, 91.2% of anthocyanins in total flavonoid content). The high anthocyanin levels contribute significantly to the overall flavonoid profile compared to the other flavonoid classes, especially in the berries such as blueberries, haskap berries and chokeberries, which were in the range of 80-90%. A similar trend was found in wild blueberries, haskap berries, blackcurrant, chokecherry, wild grapes, and wild raspberries where a significant contribution was from anthocyanins to the total flavonoid content of the berries. An opposite trend was observed in redcurrants, gooseberries, snowberries and nannyberries where other flavonoids' contribution was above 50% to the total flavonoid content and they were higher than the anthocyanins. For example, redcurrants had a total flavonoid content without anthocyanins of 441.1 mg/kg, and the total anthocyanin content was found as 344.1 mg/kg. Previous studies also found that anthocyanins were typically the primary and abundant phenolic compounds found in berries (Amararathna et al., 2020; Jin et al., 2015; Lorenzo Rodriguez et al., 2022). Genetic factors governing redcurrants might prioritize the production of other flavonoid subclasses or have regulatory mechanisms that limit the accumulation of anthocyanins (Ma et al., 2018). Environmental factors also play a crucial role in the formation of flavonoid composition (Witol et al., 2018). For instance, berries exposed

to intense sunlight may produce higher levels of anthocyanins as a protective response to UV radiation. The environmental conditions specific to the growth and cultivation of each berry type contribute to the observed variations in their flavonoid content. Furthermore, the physiological stage of the berries during harvesting can impact their flavonoid composition. Anthocyanin levels may increase during ripening, affecting the overall flavonoid profile (Castañeda-Ovando et al., 2009; De Pascual-Teresa & Sanchez-Ballesta, 2008). Understanding these factors is crucial not only but also for optimizing the cultivation and utilization of these small fruits with desirable flavonoid profiles for nutritional and health purposes.

Another subclass of flavonoids are flavones and in the current study, afzelin, apigenin, arbutin, luteolin, isorhamnetin, vitexin, isovotexin, vitexin-2-rhamnoside, herbacetin, taxifolin, orientin and vicenin-2 were identified (**Table 1a and b**). Redcurrants were high (p<0.05) in total flavone content at 33.51 mg/kg (**Figure 4a**) compared to the other fruits. This berry showcases significant concentrations of several flavones, including vicenin-2 (5.696 mg/kg) and vitexin-2-rhamnoside (5.211 mg/kg). Compared to the total phenolic contents, the contribution from the total flavone content is less than 10% in all the studied berries. For instance, even though redcurrants had the highest total flavone content, the contribution to the total phenolic content was only 8.57%. On the contrary, highbush cranberry appears to have the lowest total flavone content at 1.24 mg/kg. While this berry contains lower concentrations of individual flavones compared to other varieties, it's important to note that the nutritional value of berries extends beyond flavones, and they may still contribute to a balanced diet. Examining specific flavones, the compound arbutin demonstrates noteworthy variations across different berries. Nannyberry, chokecherry, and buckthorn display particularly high concentrations of arbutin, reaching 16.34 mg/kg, 9.055 mg/kg, and 8.416 mg/kg, respectively. In terms of individual flavones contributing to the total flavone profile, taxifolin shows considerable variability. Blackcurrant had the highest taxifolin content at 4.975 mg/kg, whereas snowberry, while still notable, exhibits a lower content at 0.022 mg/kg. potentially influencing their overall health benefits. Arbutin is recognized for its potential health benefits, including antioxidant properties and skin-lightening effects, suggesting that the consumption of these berries may offer these specific advantages (Durak et al., 2020).

Isoflavones are another subgroup of flavonoids, which are naturally occurring polyphenolic compounds found in plants. In the current study, aromadendrin, daidzein, daidzin, genistein, glycitein, and glycitin were identified as the isoflavones present in the Canadian small fruits (**Table 1a and b**). Redcurrants had the highest (p<0.05) total isoflavone content at 245.06 mg/kg, primarily influenced by the presence of significant amounts of daidzin (203.2 mg/kg) and glycitein (41.57 mg/kg). The contribution of total isoflavones content to the total phenolic content in redcurrants is only 28.6% while in gooseberries it was 40.6%. Blackcurrant follows closely to redcurrants with a total isoflavone content of 137.42 mg/kg, prominently featuring daidzin (107.6 mg/kg). The presence of a high amount of daidzin currants has previously also been reported by (Witol et al., 2018). Compared to the other small fruit types currants including both black and red are significantly rich in isoflavones (**Figure 4b**). Conversely, some berries exhibit lower concentrations of specific isoflavones. For example, wild grapes and chokecherries had the lowest concentration of aromadendrin at 0.004 mg/kg, and daidzein, genistein and glycitin were absent in most of the tested small fruits emphasizing that berries are not rich sources of these compounds specifically. These isoflavones are rich in other plant-based sources such as soy and legumes (Singh et al., 2023). While these berries may not be as rich in isoflavones as other small fruits, it's essential to consider their overall flavonoid profile and diverse nutritional contributions.

Flavonols are another subclass of flavonoids that contribute to diverse biological activities and health benefits. In the current study flavanols identified included quercetin, quercetin-3-galactoside, isoquercetin, kaempferol, kaempferol-3-glucoside, myricetin, rutin and fisetin were identified as flavanols (**Table 1a and b**). Rutin was found as the most abundant flavanol compound present in all the studied Canadian small fruits. Among all fruits tested, Saskatoon berries exhibited the highest (p<0.05) total flavanol content at 631.23 mg/kg (**Figure S2c**). This berry showed substantial levels of rutin (584.9 mg/kg), followed by quercetin-3-galactoside (20.35 mg/kg) and isoquercetin (20.12 mg/kg). The significant presence of these flavanols highlights Saskatoon berry as a potential nutritional powerhouse, offering a diverse array of health-promoting compounds. Redcurrants and chokecherries also demonstrated significant flavanol content. Chokecherry, in particular, exhibits high levels of rutin (59.55 mg/kg) while redcurrant had a rutin content of 57.59 mg/kg and kaempferol-3-glucoside content of 16.61 mg/kg. The presence of kaempferol and

kaempferol-3-glucoside is notable in redcurrants and blackcurrants, emphasizing the diversity in flavanol composition among different berries.

Flavans, flavanones, and flavonoid glucosides are other distinct subclasses within the broader category of flavonoids, which are polyphenolic compounds found abundantly in various plant sources (citation). These compounds are widely distributed in fruits, vegetables, and herbs, contributing not only to the vibrant pigmentation of these plants but also to their defense mechanisms (citation). Flavans are recognized for their antioxidant, anti-inflammatory, and potentially anticancer properties (Hollman & Arts, 2000). In terms of flavans, (+)-catechin, (-)-epicatechin, (-)-epicatechin gallate and (-)-epigallocatechin were the studied flavans found in Canadian small fruits in the current study (**Table 1a and b**). Saskatoon berry had the highest total flavan content at 107.7 mg/kg, highlighting its richness in this flavonoid class. Following Saskatoon berries, blackcurrants (64.31 mg/kg) and redcurrants (48.87 mg/kg) also displayed high levels of flavans, contributing to their overall flavonoid diversity. (+)-catechin and (-)-epicatechin were the most abundant flavans contributing to the total flavan content of almost all the small fruit types (**Figure S2b**). Naringin, liquiritigenin, and hesperetin were studied as flavanones, and nicotiflorin was studied as the flavonoid glucoside in the Canadian small fruits. Both total flavanones and flavonoid contents were in the range of 0.1-5.8 mg/kg and did not significantly contribute to the total flavonoid profile of the studied small fruits.

Procyanidins are another class of flavonoids known for their antioxidant properties. Procyanidins A, B, and C were the tested procyanidins in Canadian small fruits in the current study (**Table 4.1a and b**). Total procyanidins are present in varying concentrations across the tested berries. Saskatoon berries, buckthorn, and chokecherries exhibit relatively high levels of total procyanidins, with Saskatoon berries having the highest ($p<0.05$) content (10.5 mg/kg) (**Figure 4.4g**). These findings align with existing literature, highlighting the variability of procyanidin content among different berry species (Hellström et al., 2007). Among the procyanidins, procyanidin C was significantly higher in Saskatoon berries (107.5 mg/kg) compared to the other tested small fruit types where all of them had procyanidin contents lower than 20 mg/kg. Procyanidin B, another subclass of procyanidins, shows diverse concentrations in the tested berries. Redcurrants and nannyberries displayed relatively higher levels of Procyanidin B, with

redcurrant being the richest source. Chokecherries, wild blueberries, and snowberries showed lower concentrations compared to the aforementioned small fruits.

Anthocyanins, primarily pigments, were the major type of flavonoid compound present in berries and contributed significantly to the flavonoid profile of berries (Ma et al., 2018). These pigments are responsible for the vibrant red (the abundance of cyaniding compounds) purple, and blue colors observed in many different berries (Hosseinian & Beta, 2007). In berries, anthocyanins contribute not only to the visual colour of the fruit but also to the potential health benefits. The specific types and concentrations of anthocyanins vary among different berry species. Common anthocyanins found in berries include cyanidin, delphinidin, malvidin, pelargonidin, peonidin, and petunidin (Lorenzo Rodriguez et al., 2022; Rossi et al., 2022). In the current study, 13 different anthocyanins were quantified in the small fruits including, cyanidin (cyanidin-3-arabinoside, cyanidin-3-glucoside, and cyanidin-3-rutinoside), delphinidin, delphinidin-3-glucoside, malvidin, malvidin-3-glucoside, malvin, pelargonidin, peonidin, peonidin-3-glucoside and petunidin (**Table 4.1a and 4.1b**). In the comprehensive exploration of anthocyanin profiles across various berries, chokeberries showed the highest (p<0.05) total anthocyanin content (1075 mg/kg) followed by wild blueberries (996.9 mg/kg), saskatoon berries (904.5 mg/kg), haskap berries (755.8 mg/kg) and blackcurrants (527.7 mg/kg) (**Figure 4.5h**). Chokeberry distinguishes itself with high contents of cyanidin-3-arabinoside (470.4 mg/kg) and cyanidin-3-glucoside (286.7 mg/kg). Blueberries were previously also reported for their high anthocyanin content at the concentration ranges from 65 to 222 mg/ 100g FW (Rossi et al., 2022). An interesting trend emerged with malvidin-3-glucoside, a key anthocyanin contributing to deep purple hues. For instance, blueberries and wild grapes were considerably higher in malvidin-3-glucoside contents (391.3 mg/kg and 124 mg/kg, respectively) compared to the other tested small fruits. The content of malvidin-3-glucoside was less than 2 mg/kg in other small fruits analyzed in the current study. A similar trend was observed in peonidin-glucoside in blue color berries where the highest (p<0.05) was found in blueberries (306.6 mg/kg) followed by haskap berries (90.28 mg/mg) and wild grapes (37.3 mg/kg). Conversely, cyanidin-3-glucosides, cyanidin-3-arabinosides and delphinidin compounds were higher (p<0.05) in red color berries such as saskatoon berries (612.6 mg/kg, 141.3 mg/kg and 124.5 mg/kg, respectively) compared to the other tested small fruit (**Table 4.1a and 4.b**). Cyanidin-3-rutinoside was found in high quantities (p<0.05) in black color berries such as blackcurrants (178.2

mg/kg), chokecherries (146.7 mg/kg) and buckthorns (74.2 mg/kg) compared to the other tested blue, purple and red color berries which emphasized that different anthocyanins profiles contributed to different colors of Canadian small fruits and also their possibilities of usage in different health implications (**Figure 4.S3**). The comparative analysis of different flavonoid classes in small fruits highlights the diversity and complexity of their flavonoid profiles. Each berry type exhibits unique combinations and concentrations of flavonoids within each class, offering consumers a variety of bioactive compounds with potential health benefits (Rossi et al., 2022). The factors including genetic composition, environmental, and physiological conditions contributes to the distinctive flavonoid compositions observed in these small fruits, emphasizing the importance of incorporating a diverse range of fruits into one's diet to access the full spectrum of these health-promoting compounds.

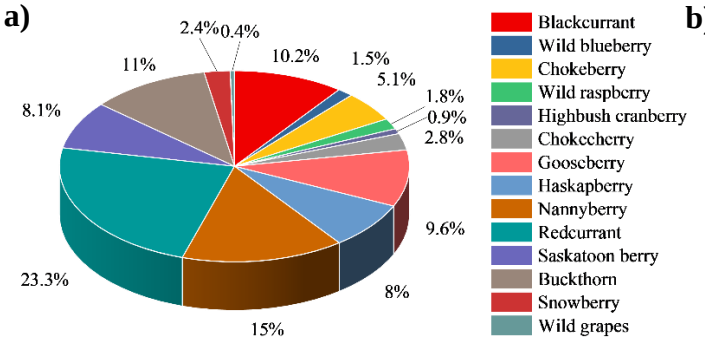
4.4.2.2 Hydroxycinnamic acids

Hydroxycinnamic acids quantified in small fruits included caffeic acid, caftaric acid, chlorogenic acid, ferulic acid, p-coumaric acid, and sinapic (**Table 4.1a and 4.1b**). The hydroxycinnamic acids profile among the small fruits showed variations indicating distinctive chemical compositional characteristic of each small fruit type (**Figure 4.4d**). The total hydroxycinnamic acids greatest in Saskatoon berries (263.8 mg/kg, $p<0.05$), followed by chokeberries (159.4 mg/kg) and chokecherries (144.1 mg/kg) while the lowest was found in wild raspberries (0.91 mg/kg) and wild grapes (1.02 mg/kg). Chlorogenic Acid, a major contributor to the antioxidant activity, is particularly abundant in all the tested Canadian small fruits with greatest in Saskatoon berries (255.837 mg/kg), followed by chokeberries (158.1 mg/kg) and chokecherries (142.6 mg/kg) and lowest in wild grapes (96.447 mg/kg) and Redcurrant (38.14 mg/kg) . When considering the other hydroxycinnamic acids present in small fruits, the highest caffeic acid content (35.16 mg/kg) was found in snowberries while the highest ($p<0.05$) caftaric acid content (26.42 mg/kg) was found in gooseberries. The highest *p*-coumaric acid content was found in Saskatoon berries (4.349 mg/kg) while the presence of *p*-coumaric acid content was not that significant in all the other tested Canadian small fruits. Among the hydroxycinnamic acids, ferulic acid was the lowest one across all berries. acid, chlorogenic acid, p-coumaric acid, ferulic acid, and sinapic acid were the most commonly detected and reported hydroxycinnamic acids in fruits (Ruiz et al., 2015). All these hydroxycinnamic acids were detected at various concentrations in all fruits as reported above.

4.4.2.3 Hydroxybenzoic acids

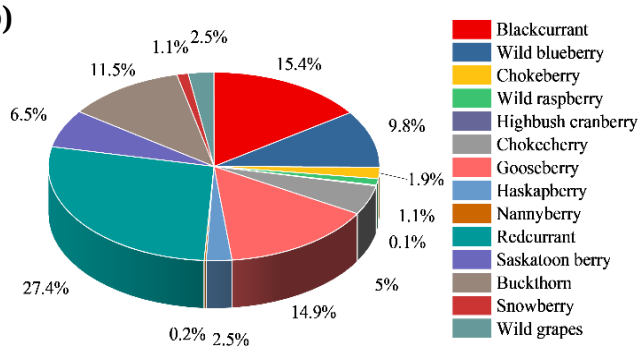
In the current study, gallic acid, protocatechuic acid, syringic acid and vanillic acids were identified as hydroxybenzoic acids present in Canadian small fruits (**Table 4.1a and 4.1b**). The presence of hydroxybenzoic acids was not abundant in all the studies of small fruit types. The highest was found in redcurrants (2.394 mg/kg), followed by blackcurrants (2.191 mg/kg) while the lowest was found in wild raspberries (0.062 mg/kg). Among the tested hydroxybenzoic compounds, only gallic acid and protocatechuic acids were found in most of the tested berries. The highest gallic acid content was found in redcurrants (1.883 mg/kg) followed by blackcurrants (1.542 mg/kg).

a)



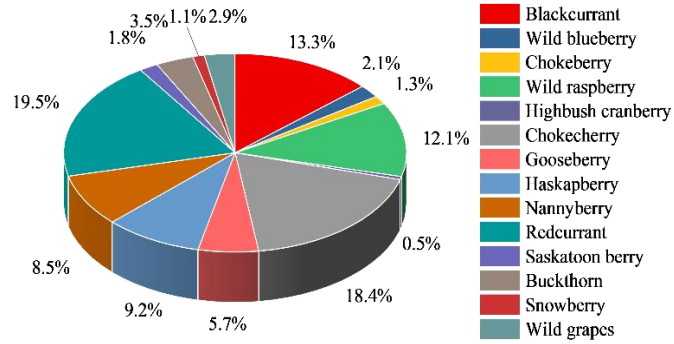
Flavones

b)



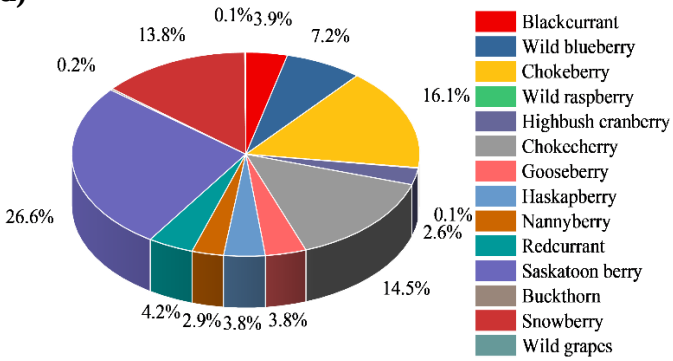
Isoflavones

c)



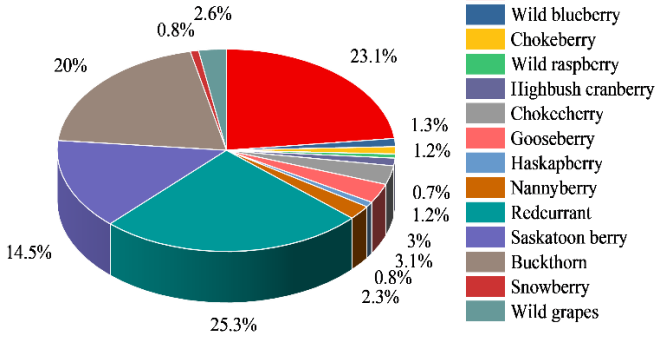
Flavanones

d)



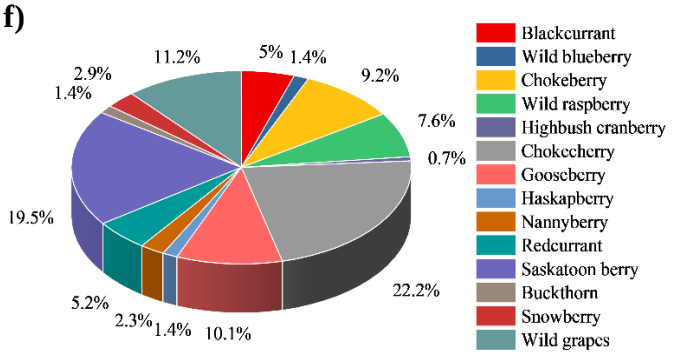
Hydroxycinnamic acids

e)



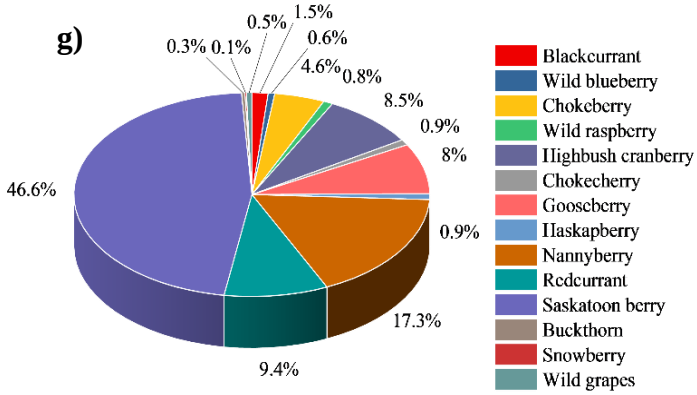
Hydroxybenzoic acids

f)



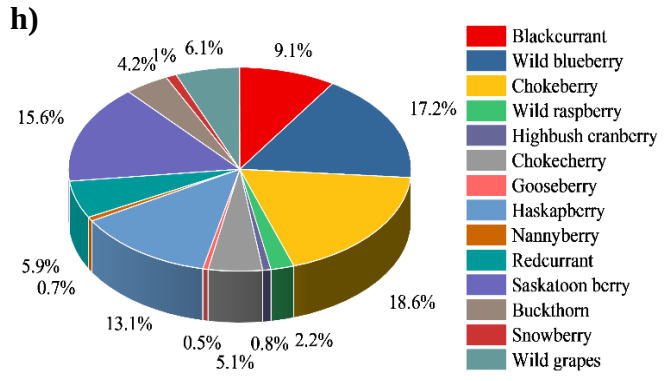
Stilbenes

g)



Procyanidins

h)



Anthocyanins

Figure 4.4 Pie charts showing the distribution of different phenolic compound classes among 14 different types of Canadian small fruits. a) flavones, b) isoflavones, c) flavanones, d) hydroxycinnamic acids, e) hydroxybenzoic acids, g) procyanidins, and h) anthocyanins distribution among Canadian small fruits.

4.4.2.4 Stilbenes

Stilbenes include resveratrol, piceatannol, polydatin, and rhapontin and are known for their antioxidant properties and have gained attention for their potential health benefits (Błaszczyk et al., 2019). Chokecherries were found to contain the greatest concentrations of total stilbenes among tested fruit types at 11.47 mg/kg) followed by saskatoon berries (10.08 mg/kg) and wild grapes (5.78 mg/kg) (**Table 4.1a and 4.1b**). Among various stilbenes, polydatin was the most abundant stilbene found in the studied small fruits, however, resveratrol was found as another abundant stilbene compound in wild grapes. Piceatannol was present in several berries, with significant contents in blackcurrants, haskap berries, and wild grapes ranging from 0.03 to 0.2 mg/kg. Piceatannol has been associated with various health benefits, including anti-inflammatory and anti-cancer properties (Błaszczyk et al., 2019). Resveratrol, although not detected in some berries such as wild blueberries, blackcurrants and snowberries, is found in others, including chokecherries.

4.4.3 Principal component analysis and hierarchical cluster analysis of Canadian small fruits based on their phenolic contents

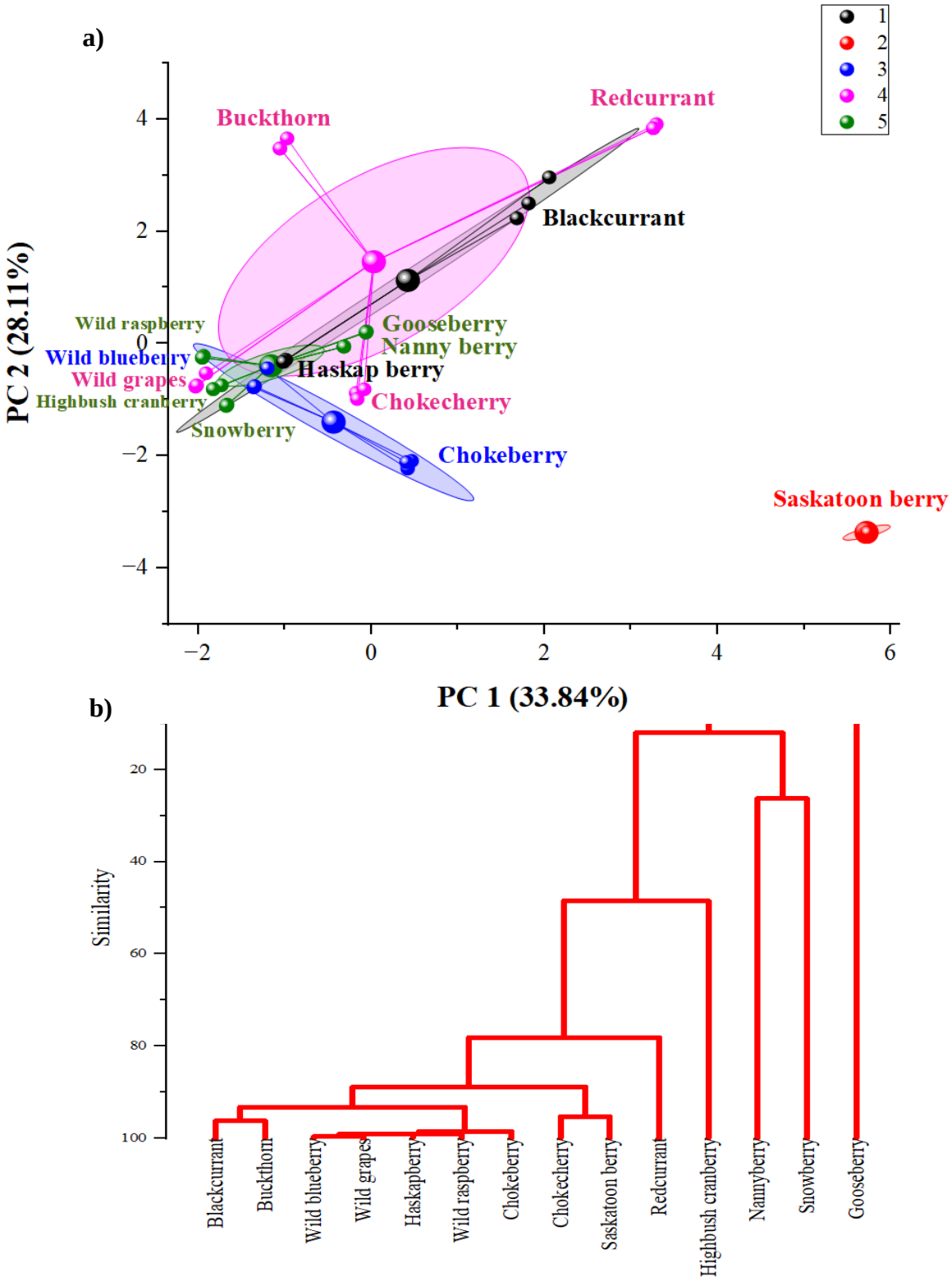


Figure 4.5 Chemometric analysis of phenolic compounds present in Canadian small fruits. a) K-means cluster analysis (1-5 cluster numbers) and b) dendrogram showing the formation of hierarchical clusters based on similarities of phenolic compounds profiles and quantities of Canadian small fruits.

Principal Component Analysis (PCA) is a statistical technique used to simplify the complexity of high-dimensional data while retaining trends and patterns (Boeing et al., 2014). In this analysis of Canadian small fruit phenolic data, the correlation matrix provides insights into the relationships among different phenolic compounds. The eigenvalues and eigenvectors offered information on the variability and directions of principal components. Additionally, the scores of the berries on the first two principal components provide a graphical representation of their overall phenolic composition (**Figure 4.S4**). When considering the correlation matrix, the positive values between compounds such as flavones and isoflavones suggest a positive correlation, indicating that these compounds tend to co-occur. Conversely, negative values between flavones and hydroxycinnamic acids indicate an inverse relationship. The eigenvalues represent the proportion of variance explained by each principal component. In this study, the first two principal components contribute significantly to the variance (61.95%), indicating that they effectively capture the major patterns in the data. The high percentage of cumulative variance suggests that the first few principal components can adequately represent the overall phenolic profile of the berries. Examining the coefficients of the extracted eigenvectors for PC1 and PC2, it can be identified the compounds that contribute most to each principal component. For instance, PC1 is influenced by compounds such as flavones, isoflavones, and flavonoid glucoside, while PC2 is characterized by hydroxycinnamic acids and hydrolyzable tannin. The scores of the small fruits on the first two principal components provide a basis for their comparison. For example, blackcurrants have positive scores on both PC1 and PC2, indicating a higher concentration of the compounds associated with these components. In contrast, wild blueberries have negative scores on both, suggesting a different phenolic profile. In the context of berries, the interpretation of the PCA results should consider the biological significance of the phenolic compounds. For instance, blackcurrant might be distinguished by its higher levels of flavones and isoflavones, potentially contributing to its unique phenolic profile compared to other berries. Conversely, wild blueberries might be characterized by lower levels of these compounds but higher concentrations of hydroxycinnamic acids and hydrolyzable tannins.

Saskatoon berries stand out on PC2 with the highest positive score, indicating higher contents of hydroxycinnamic acids and hydrolyzable tannin compared to other berries. This suggests that the Saskatoon berry has a unique phenolic profile dominated by these specific compounds. When considering wild raspberries, chokeberries, and highbush cranberries, they have relatively moderate scores on both PC1 and PC2, suggesting a balanced composition of the contributing phenolic compounds. Chokeberry, in particular, has a positive score on PC2, indicating a slightly higher concentration of hydroxycinnamic acids and hydrolyzable tannin.

The above results are further supported by the k-means cluster analysis. The K-means cluster analysis reveals distinctive patterns in the phenolic compound profiles of different clusters of berries (**Figure 4.5a**). Each cluster is characterized by specific levels of various compounds, providing insights into the chemical diversity among berries. Cluster 1, which consists of blackcurrants and haskap berries (shown in black color), stands out for its elevated levels of isoflavones, flavonols, flavans, and hydroxycinnamic acids. These compounds contribute to the unique phenolic profile of berries in this cluster. While other compounds have moderate to low concentrations, the abundance of isoflavones and flavonols is particularly noteworthy within this cluster. Cluster 2, which consists only of Saskatoon berries (shown in red) is marked by high levels of flavonols, hydroxycinnamic acids, and anthocyanins. This cluster exhibits low concentrations of flavones, isoflavones, and flavans. The relatively low within-cluster sum of squares, along with the low average and maximum distances, suggests a tight grouping of observations within cluster 2, emphasizing its internal homogeneity as triplicates. Cluster 3, which consists of wild blueberries and chokeberries (shown in blue color) displays significant levels of isoflavones, flavonols, and hydroxycinnamic acids. The moderate levels of flavans and anthocyanins contribute to the overall composition of this cluster. Cluster 4 consists of buckthorns, redcurrants, wild grapes, and chokecherries (shown in pink color), and exhibits high levels of isoflavones, flavones, flavans, hydroxycinnamic acids, and anthocyanins. With a higher within-cluster sum of squares, this cluster appears to have greater variability compared to others. The compounds contributing to this variability are key in distinguishing the berries within Cluster 4. Lastly, cluster 5 consists of wild raspberries, gooseberries, highbush cranberries, nannyberries, and snowberries (shown in green color), is the biggest cluster, and showcases moderate levels of flavones, flavonols, flavans, and

hydroxycinnamic acids. Notably, isoflavones and anthocyanins are present in lower concentrations. This cluster represents a unique phenolic profile, distinct from the other clusters. The distances between the final cluster centers provide additional insights. Cluster 2 which consists only of saskatoon berries is notably isolated from the other clusters, suggesting a clear separation in its phenolic composition. The ANOVA results indicate significant variations in flavonols, flavans, hydroxycinnamic acids, and anthocyanins among the clusters, emphasizing the importance of these compounds in differentiating the berries.

The hierarchical cluster analysis developed a dendrogram as shown in Figure 5b for berries based on their phenolic compound profiles revealing interesting patterns and similarities among different clusters. The dendrogram illustrates the stages of merging and the distances between clusters, offering insights into the chemical similarities among the berries. In the early stages of clustering, small groups of berries with similar phenolic profiles gradually merge. Significant clusters include the combination of wild blueberries and wild grapes, as well as the merging of haskap berries and wild raspberries, emphasizing the phenolic similarities between these berries. As the clustering process progresses, more significant mergers occur, bringing together clusters with shared phenolic characteristics. Chokeberry and Saskatoon berry has a similarity value of 1.46 indicating a notable similarity between these two types of berries. Blackcurrant and Buckthorn have the same similarity value of 3.7, suggesting they are equally dissimilar from the other berries studied. Redcurrant and highbush cranberry are more similar than other variables, as indicated by the lower similarity value of 21.8. Gooseberry has a high dissimilarity value of 100 suggesting significant difference from other berries. This dissimilarity could be attributed to the absence of a high content of anthocyanins and the presence of a high content of isoflavones compared to the tested other types.

4.5 Conclusion

In this study, a UHPLC-HRMS approach was used to conduct a comprehensive analysis of 66 phenolic compounds in underutilized Canadian small fruits to identify, quantify, and characterize the phenolic compound profiles of 14 small fruits. Examining phenolic compound concentrations across different berries further exposed different trends in the phenolic compound profiles present in Canadian small fruits. For instance, redcurrant exhibited high levels of various compounds,

including flavones, isoflavones, and anthocyanins, contributing to its overall high phenolic content. Saskatoon berry was identified as a small fruit with a distinctive profile rich in flavonols and total phenolic compounds. Further employing both K-means and hierarchical cluster analyses, clustering patterns based on phenolic compound profiles were also achieved. The K-means cluster analysis identified distinct groupings of berries characterized by specific concentrations of phenolic compounds. The hierarchical cluster analysis revealed a dynamic merging process, emphasizing the gradual merging of clusters based on phenolic similarities. The dendrogram provided a visual representation of hierarchical relationships, indicating stages of merging and influential variables at each step. This method highlighted chemical affinities between these underutilized small fruits, concluding in the formation of a single cluster, suggesting an overarching similarity among all berries. Therefore, this comprehensive study not only highlights the chemical diversity among Canadian small fruits but also demonstrates the utility of clustering analyses in revealing patterns and relationships within the dataset. The identified clusters and influential compounds contribute valuable information for researchers, nutritionists, and industries interested in leveraging the potential health benefits and unique flavor profiles of these berries. The results pave the way for further exploration and utilization of these small fruits in various applications.

4.6 References

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Chapter 5: Lipophilic bioactive compounds and lipid peroxidation of Canadian wild berries

A version of this has been submitted to Journal of Food and Function:

Kodikara C, Netticadan T, Joseph S., Bandara N, Wijekoon C. (2024). Lipophilic bioactive compounds, lipid peroxidation and anti-hypertensive activity of Canadian wild berries- Submitted-in peer review (Journal of Food and Function- Manuscript ID: FO-ART02-2024-000665)

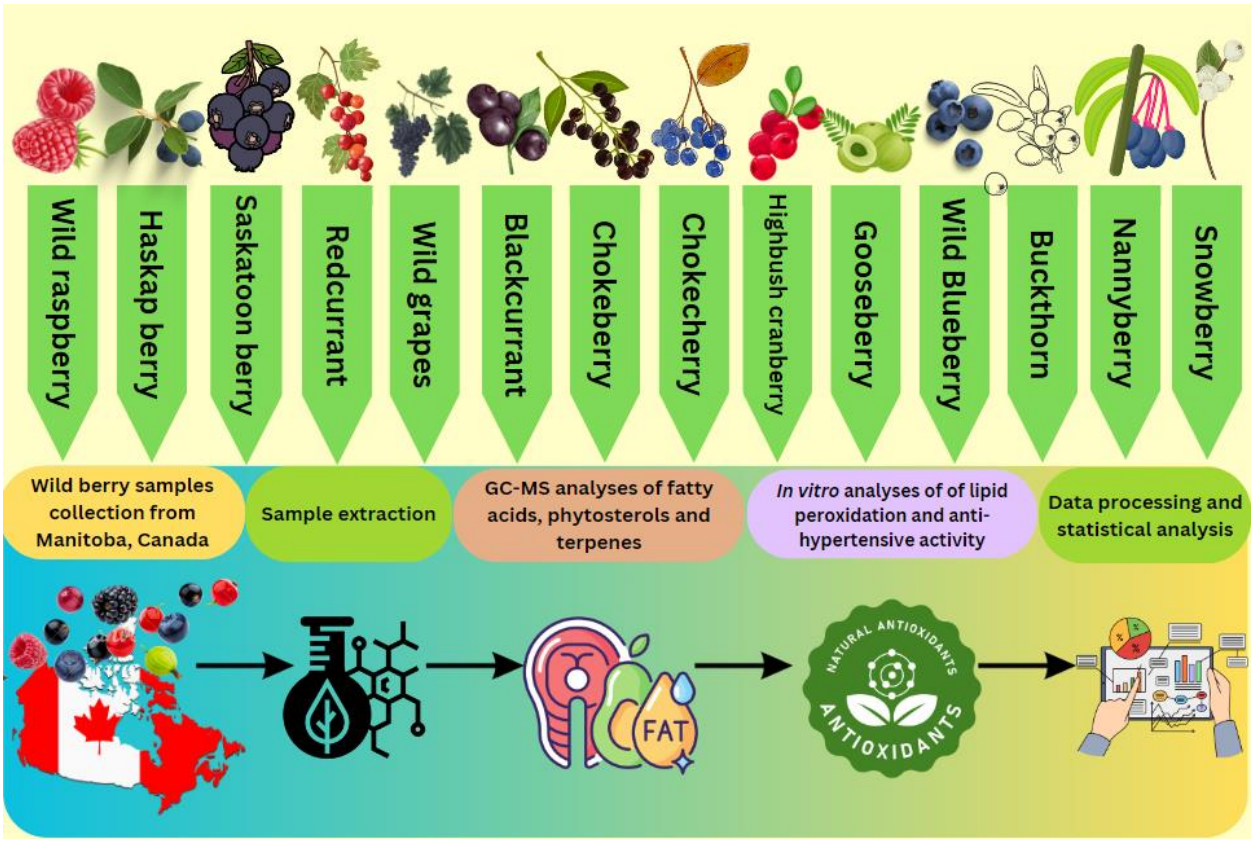
5.1 Abstract

Traditional berries are small fruits consumed by Canadians, including the indigenous population, and are widely distributed in the Canadian prairie provinces. The current study investigates the lipophilic bioactive compounds such as fatty acids, phytosterols, and terpenes, and their bioactivities, such as lipid peroxidation as well as the anti-hypertensive activities of fourteen underutilized Canadian wild berries. These berries include Saskatoon berries (*Amelanchier alnifolia*), gooseberries (*Ribes hirtellum*), wild grapes (*Vitis riparia*), blackcurrants (*Ribes nigrum*), redcurrants (*Ribes rubrum*), haskap berries (*Lonicera caerulea*), wild raspberries (*Rubus idaeus*), wild blueberries (*Vaccinium angustifolium*), chokeberries (*Aronia melanocarpa*), buckthorn (*Rhamnus cathartica*), highbush cranberries (*Viburnum trilobum*), chokecherries (*Prunus virginiana*), nannyberries (*Viburnum lentago*) and snowberries (*Symphoricarpos albus*). The fatty acids, phytosterols, and terpenes were identified using Gas Chromatography-Mass Spectrometry (GC-MS). Lipid peroxidation was determined using *in vitro* methods. Notably, wild grapes exhibited the highest (p<0.05) total fat content (7659±312 µg/g DW), followed by haskap berries (4650±184 µg/g DW). Polyunsaturated fatty acids (PUFAs) were highest (p<0.05) in wild grapes (74%). Predominant phytosterols and terpenes identified in Canadian wild berries included β-sitosterol, isofucosterol, phytol, and α-amyrin. Saskatoon berries and gooseberries showed a distinct phytosterol and terpene profile compared to the other wild berries. Snowberries demonstrated the highest (p<0.05) lipid peroxidation. This research provides valuable insights into the lipophilic bioactive compounds and their potential activities *in vitro* of the Canadian wild

berries, offering a foundation for further exploration and potential applications in functional foods or nutraceuticals.

Keywords: Canadian wild berries, fatty acids, phytosterols, terpenes, GC-MS, lipid peroxidation

Graphical Abstract



Author contribution

Funds acquisition: CW and TN; Conceptualization: CK, and CW; Methods: CK, CW and SJ, Analysis: CK; Writing the first draft: CK; Editing and reviewing: CW, NB, TN, SJ and CK; Final version review: CW, TN, NB, SJ and CK; Research and Academic Supervision: CW and NB.

5.2 Introduction

Canada is endowed with a rich diversity of underutilized berries, which are small fruits found mostly in prairies in North America. Belonging to the rose family (*Rosaceae*), wild berries are a valuable yet largely unexplored natural resource (Bere, 2007a; Fang, 2021; Kodikara et al., 2023). Wild berries are non-cultivated plants that have not been bred for the protection of the plants or for selective breeding. The phytochemical profile of these wild berries is modulated by the biotic (microbial pathogens, neighboring plants, canopy composition etc.) and abiotic (moisture, soil fertility, snow cover in winter, temperature, light etc.) environmental stresses. Therefore, there is potential for many phytochemicals involved with plant defensive mechanisms to accumulate in these wild berries compared to cultivated berries (Bajramova & Spégel, 2022a). In this era of increasing interest in functional foods and nutraceuticals, the unique combination of bioactive compounds found in these berries presents an opportunity for the development of novel, health-promoting products (Kay et al., 2022; Kodikara et al., 2023).

Both hydrophilic and lipophilic phytochemicals are present in significant quantities within berries (Kay et al., 2022). Consequently, the collective impact of combining numerous bioactive compounds in these berries, either complementing or synergizing with each other, or the additive effects of all the bioactive compounds present in diets rich in berries are considered to be responsible for the underlying health benefits (Szakiel et al., 2012). Phenolic compounds are the major bioactive phytochemical group of berries (Zorzi et al., 2020a). Fatty acids in berries play a crucial role in their nutritional and functional attributes because these compounds protect the human body from free radicals and active oxygen species, provide energy, support cell membrane structure, and have been associated with various health benefits, including anti-inflammatory properties and cardiovascular health (Orsavova et al., 2015). Berries contain diverse fatty acids, including essential fatty acids such as α-linolenic acid and linoleic acid. Long-chain omega-3 fatty acids such as docosahexaenoic acid (DHA, 22:6n-3) and eicosapentaenoic acid (EPA, 20:5n-3) can be synthesized from α-linolenic acid, which is known to reduce the risk of heart diseases, hypertension, autoimmune disorders, and cancer (Parry et al., 2005). Essential fatty acids cannot be synthesized in the human body and should be provided through the diet (Vaitkevičienė et al., 2019). The composition of the fatty acids in these wild berries can vary among species and

environmental conditions (Basu et al., 2010). Fatty acid composition in the soft parts of the berry is different than that in the seed. For instance, oleic (18:1n-9), palmitic (16:0) acid, linoleic (18:2n-6), and α-linolenic (18:3n-3) are high in seed oil but the soft parts of the berry consist mainly of saturated fatty acids such as palmitoleic (16:1n-7) acid and are low in α-linolenic acid (Zorzi et al., 2020b). In addition, researchers have found that the α- linolenic acid content present in berries is similar to the levels found in purslane and other α-linolenic-rich plants, and the content is considerably higher than in other commonly eaten fruits such as banana, orange, and apple (Bere, 2007a).

Phytosterols are another group of bioactive compounds that have gained attention for its potential health benefits apart from the fatty acids in berries (Piironen et al., 2003a; Yang et al., 2001). Phytosterols are plant-derived compounds structurally similar to cholesterol, and are known for their ability to lower cholesterol absorption in the human body, making them valuable for cardiovascular health (Bajramova & Spégel, 2022a). Phytosterols have been associated with reduced LDL (low-density lipoprotein) cholesterol levels, making them important for individuals concerned about heart health (Ogbe et al., 2015). In berries, the presence of phytosterols contributes to their nutritional value. Phytosterols such as stigmasterol, campesterol, sitosterol, and avenasterols, which belong to the class of 4-desmethylsterols are the most common type of sterols found in fruits and vegetables (Piironen et al., 2003b). These compounds may vary in concentration depending on the berry species and growing conditions (Lagarda et al., 2006). For instance, β-sitosterol was found in Saskatoon berries, raspberries, blueberries, and blackberries, while black raspberries had high levels of stigmasterol. Among the phytosterols, β-sitosterols are the type of plant sterols that can potentially be used to develop steroidal drugs and cholesterol-lowering products (Cossignani et al., 2018).

Terpenes are another class of plant secondary metabolites and complex compounds originating from squalene, a basic linear hydrocarbon (Kupska et al., 2016). These compounds are commonly found in various plant species and are known for their wide range of structural variations and multiple beneficial effects in biological systems. In pharmacological studies, these phytochemicals have been associated with antioxidant activity, which can help reduce the risk of chronic diseases and protect cells from oxidative damage (Szakiel et al., 2012). As well, antimicrobial, anti-

inflammatory, anti-ulcer, hepatoprotective, immunomodulatory, hypolipidemic and cholesterol-reducing, anti-atherosclerotic, wound-healing, anticoagulant, and anticancer properties have been reported for these compounds (Szakiel et al., 2012).

Berries may be susceptible to lipid peroxidation, a process where the fats undergo cell damage due to oxidative stress. This susceptibility is pivotal in comprehending the potential of berries to reduce oxidative damage within the human body. However, the emphasis lies in understanding how Canadian wild berries, as a holistic entity, might undergo this oxidative process (Freese et al., 2002). If the fats within the berries degrade through lipid peroxidation before consumption, it could impact their availability and efficacy as antioxidants within the body, potentially limiting their capacity to counteract oxidative stress. Therefore, the susceptibility of these wild berries to lipid peroxidation holds significance in assessing their potential contribution to mitigating oxidative damage in the human body (Kodikara et al., 2023).

While a significant amount of research has focused on the lipophilic compounds in commonly consumed berries, comprehensive data are scarce such as fatty acid profiles, phytosterol content, terpenes composition and lipid peroxidation susceptibility of various underutilized wild berries (Kodikara et al., 2023). Existing research is limited to a few common berry types, such as blueberries, raspberries, and seabuckthorn, leaving numerous other species unexplored (Bajramova & Spégel, 2022b; Celik & Ercisli, 2009). When considering the berry types, seabuckthorn is the most widely studied berry type among the buckthorn species for its fatty acid and phytosterol content (Dudau et al., 2021; Yang & Kallio, 2001a). Common buckthorn, known for its thorny nature and purgative properties, plant derives its common name from its historical use as a laxative. In North America, it exhibits a robust and invasive presence (Woodland Trust, 2024). Understanding the differences among these berry species is important for maximizing their potential utility because they are not widely cultivated or commercialized. Closing this research gap by investigating the fatty acid composition, phytosterols, and terpenes contents of underutilized Canadian berries can offer a more thorough perspective on their nutritional value and health-promoting potential vital to unlocking their full potential as functional foods or for the development of natural products with health benefits. It can also provide a foundation for making informed dietary choices and creating innovative food products that harness the benefits of both

the phenolic compounds and fatty acids in these berries. Therefore, the current research aimed at conducting a comprehensive exploration of 14 different types of underutilized berries found in Canada, focusing on their fatty acid profile, phytosterol content and terpene composition using Gas Chromatography-Mass Spectrometry (GC-MS) as well as assessing their susceptibility to lipid peroxidation.

5.3 Materials and methods

5.3.1 Reagents and materials

Fatty acid methyl ester mix (Supelco 37 FAME Mix), fatty acid internal standard, margaric acid (C:17) and GC derivatization reagent N, O-Bis(trimethylsilyl)trifluoroacetamide with trimethylchlorosilane (silylating mixture V, BSTFA + TMCS) were purchased from Millipore Sigma (St. Louis, MO, USA). Phytosterols standards (campesterol, stigmasterol, sitosterol, brassicasterol, fucosterol, delta-5-avenasterol and delta-7-avenasterol and the internal standard (5α-cholestane-3β-ol), Thiobarbituric Acid Reactive Substances (TBARS) kit was purchased from Cayman Chemical (Michigan, USA). Solvents, including hexane and methanolic hydrochloric acid, were all HPLC and LC grade and were purchased from Fisher Scientific (Waltham, MA, USA). Ultrapure water (18.2 MΩ.cm, total organic carbon < 3 ppb) was generated from the Milli-Q purification system (Millipore, Bedford, MA, USA).

5.3.2 Berry samples

Wild berries were collected from various locations at their ripened stage, from parks and landscapes in Manitoba, Canada. A list of wild berries collected is shown in **Table 5.1**. Each berry type was pooled separately into a composite sample, and moisture content was determined by the standard oven-drying method using a sub-sample. The pooled berries were cleaned, and freeze-dried for one week until they were of constant weight, and ground using a mortar and pestle with liquid nitrogen. The powdered samples were stored at -80 °C until further analysis. Triplicate sub-samples were taken from composite samples to analyze fatty acids, phytosterols, and terpenoids.

Table 5.1 A list of berry fruits collected from Manitoba, Canada, for the analyses of fatty acids phytosterols and terpenoids

Berry	Identification	Location
Saskatoon berry (*Amelanchier alnifolia L*)		49.80553° N, 97.18542° W 49.79877° N, 97.12236° W
Gooseberry (*Ribes hirtellum*)		49.88262° N, 97.12863° W
Wild grapes (*Vitis riparia*)		49.88432° N, 97.13019° W

Blackcurrant (*Ribes nigrum*)		49.88122° N, 97.12610° W
Red currant (*Ribes rubrum*)		49.83036° N, 97.11420° W
Haskap berry (*Lonicera caerulea*)		49.41302° N, 96.28796° W
Wild raspberry (*Rubus idaeus*)		49.41301° N, 96.28647° W

Wild blueberry (*Vaccinium angustifolium*)		49.41302° N, 96.28796° W
Chokeberry (*Aronia melanocarpa*)		49.78797° N, 97.20374° W
Common Buckthorn (*Rhamnus cathartica*)		49.80646° N, 97.17751° W
Highbush cranberry (*Viburnum trilobum*)		49.80656° N, 97.17818° W

Black chokecherry (*Prunus virginiana*)		49.80497° N, 97.17778° W
Nanny berry (*Viburnum lentago*)		49.80647° N, 97.17789° W
Snowberry (*Symphoricarpos albus*)		49.80660° N, 97.17836° W

5.3.3 Total fatty acids extraction

The analysis of fatty acids was performed using a one-step extraction and methylation protocol, as described by Wijekoon et al. (2021), with additional modifications. Powdered berry samples (200 mg) were weighed into 12 mL Pyrex tubes, and 40 µL of the internal standard (C17:0, margaric acid) was added at a concentration of 1mg/mL. Then, 2 mL of 3N methanolic-HCl was added and tubes were vortexed for 30 seconds before incubation in a dry bath (Boekel Scientific, PA, USA)

for 45 min at 80◦C. After that, the samples were removed and cooled at room temperature. Then, 4 mL of hexane was added, vortexed for 30 seconds, and left under dark conditions for 15 min to extract FAMEs. The hexane layer (supernatant) was transferred into a new disposable glass tube and evaporated to complete dryness at 45 °C for one hour using a Vacufuge Plus sample concentrator (Eppendorf AG, Hamburg, Germany). Then, the extract was reconstituted in 1 mL hexane, vortexed for 30 seconds, filtered through a 0.2 µm nylon syringe filter, and transferred into GC vials for the FAMEs analysis with GC-MS. The amount of each fatty acid was calculated in µg/g using the following equation;

$$Amount\ (x) = \frac{A_x\ \div\ A_{is} * C}{m}$$

Where A_x is the peak area of the fatty acid, A_{is} is the peak area of the internal standard (C:17, margaric acid), C is the concentration (µg/mL) of the internal standard, and m is the mass (g) of the wild berry sample.

5.3.4 Phytosterols and terpenes extraction

The extraction of phytosterols and other triterpenes was done according to the method described by Winkler-Moser (2011). First, 300 mg of the berry powder was weighed and 1N methanolic KOH solution was prepared with a ratio of 1: 10 of KOH in methanol. Subsequently, 6 mL of the methanolic KOH solution was mixed with 94 mL of HPLC-grade MeOH. Then 3 mL of this mixture was mixed with 80 µL of internal standard (5α-cholestane-3β-ol, 2 mg/mL) and added to the powdered sample, followed by vortexing for 1 minute. The samples underwent a heat treatment in a dry bath at 90 °C for 1 hour for complete saponification and then were allowed to cool at room temperature for 30 min. Further steps included the addition of 3ml petroleum ether and 1.5 mL of milli-Q water, vortexing, and extraction of the supernatant into glass vials. Then, the evaporation was carried out for 30 min at 45 °C using a Vacufuge Plus sample concentrator (Eppendorf AG, Hamburg, Germany). After the evaporation, 1 ml hexane (HPLC grade) was added into the glass vial, mixed, and transferred (1 ml) into GC vials. After a subsequent drying step, 50µL of the GC derivatizing reagent BSTFA + TMCS (silylation reagent) and 150µL of LC-grade hexane were added to the vials, which were then sealed and incubated in a dry bath for 1 hour at 65°C. The final extracts were transferred into inserts and subjected to GC-MS analysis, providing a comprehensive

insight into the phytosterols present in the berry samples. The amount (µg/g) of each phytosterol and terpene was calculated in µg/g using the following equation;

$$Amount\ (x) = \frac{A_x\ \div\ A_{is} * C}{m}$$

Where A_x is the peak area of the fatty acid, A_{is} is the peak area of the internal standard (5α-cholestane-3β-ol), C is the concentration (µg/mL) of the internal standard, and m is the mass (g) of the wild berry sample.

5.3.5 Gas chromatography-mass spectrometric (GC-MS) analysis

FAMEs were analyzed using Bruker 436-GC equipped with EVOQ-TQ-MS (Bruker Daltonics, Germany) (**Supplementary Table 5.1** (**Table 5.S1** and **Table 5.S2)**) following the method described in Wijekoon et al. (2021). The compounds were separated using Rt-2560 capillary column (100 m × 250 µm × 20) (Restek, Bellefonte, PA, USA) (**Supplementary Figure 5.1** (**Figure 5.S1**)). The carrier gas was high-purity Helium, with a 0.8 mL/min flow rate. The oven program was as follows: The initial temperature of 100 °C for 4 min was ramped up to 250 °C at 3 °C/min. It was held for 8 min at a temperature of 250 °C. The total run time for the fatty acid methyl esters was 62 min, whereas for the phytosterols and terpens, the total run time was 80 min per sample. The ion source and transfer line temperatures were 230 °C and 180 °C, respectively. The mass spectra were acquired in the 50-500 amu range in the full scan mode using Bruker Daltonics MS Workstation (Version 8.2.1) software. The identification and quantification of the FAMEs depended on the retention times of the FAMEs standard mixture (Supelco 37 Component FAME Mix, Millipore Sigma, Saint Louis, MO, USA), and the NIST (National Institute of Standards and Technology) Library. The quantification of the fatty acids was based on the internal standard method.

5.3.6 Extraction of berries for lipid peroxidation

The methodology for extracting berries followed the approach described in Kodikara et al. (2023). Briefly, freeze-dried berry samples (500mg) were placed in clean 15-mL falcon tubes, and a solvent mixture (6mL) of isopropanol: water (80:20, v/v). The mixture was facilitated with thorough mixing, extraction on a rotary shaker at 60 rpm for 30 min, followed by sonication at 30

°C for 30 min. Subsequently, the samples were centrifuged (4000 ×g) at -4 °C for 30 min, separating the supernatant, which was then filtered through a 0.2 µm nylon syringe-filters (Phenomenex, Torrance, USA) attached to a 10-mL disposable syringe into a clean 10 mL flat-bottomed tube.

5.3.7 Analyses of lipid peroxidation in berries

The analysis of lipid peroxidation was conducted in berries using the protocol described in the TBARS Assay Kit (Cayman Chemical, 2023). Briefly, the Malondialdehyde (MDA) standard was used to generate the calibration curve at 0, 0.625, 1.25, 2.5, 5, 10, 25 and 50 µM concentrations. Then 100 µL from each standard, berry extracts (obtained by method 2.6), sodium dodecyl sulfate (SDS) solution, and 4mL of color reagent were added to 5mL vials and mixed. Then, the tubes were boiled using a water bath (Thermo Scientific, Mississauga, ON, Canada) at 100 °C for one hour. After one hour, the tubes were removed immediately and placed in an ice bath to stop further reactions. After 10 min, vials were centrifugated (1600g) at 4 °C. Then the vials were kept for 30 min at room temperature to stabilize the samples before loading onto a clear plate, and the absorbance was read at 530-540 nm using a microplate reader (FLUOstar Omega, Germany). After plotting the MDA colorimetric standard curve, the absorbance values were calculated using the following equation; MDA (µM) = (Corrected absorbance - y intercept)/ Slope.

5.3.8 Statistical analysis

Analyses of wild berries were performed in triplicate for fatty acids, phytosterols, terpenes, lipid peroxidation, and anti-hypertensive activity. All the results were expressed as average ± standard deviation (SD). One-way ANOVA was performed for individual fatty acids, phytosterols, terpenes, lipid peroxidation, and anti-hypertensive activity, followed by a Tukey's test for the mean comparison. Column graphs and box plots, including mean values and standard deviations, were used to summarize the data. Principal component analysis (PCA) was conducted to reduce the data set's dimensionality and identify the most important dimensions that capture the greatest variance among the tested berry types based on the concentrations of all quantified fatty acids, phytosterols, and terpenes. The quantified lipophilic compounds were treated as independent variables and five principal components were calculated. Following the PCA, K-means cluster analysis was

performed collectively for all the identified lipophilic compounds and separately for fatty acids and combined phytosterols and terpenes. The number of principal components was determined based on the hierarchical cluster analysis. All statistical analyses were performed using the Minitab software (version 17, Minitab LLC, PA, USA), and graphical representation was generated using Origin software (version 2022b MA, USA).

5.4 Results and discussion

5.4.1 Fatty acid profiles and quantities of wild berries

The current comprehensive investigation of the fatty acid composition in of a diverse array of wild berries grown in Canada is shown in **Table 5.2**. The fatty acid composition of wild berries found in in Manitoba is a blend of saturated (SFAs) and unsaturated fatty acids, categorized based on the number of unsaturated bonds as monounsaturated (MUFAs) or polyunsaturated fatty acids (PUFAs), including omega-3 (n-3) and omega-6 (n-6) fatty acids. Wild berries exhibit increased levels of MUFA and PUFA, elevated concentrations of n-6 and n-3 fatty acids, an acceptable ratio of SFA to PUFA, a diminished ratio of n-6 to n-3 fatty acids, and a high amount of n-3 fatty acids (Bere, 2007b). The total fatty acid content found in the tested wild berry types was different ($p<0.05$) among different berries. The order of the total fat content was as follows: wild grapes> haskap berry> wild raspberry> gooseberry> Saskatoon berry> blackcurrant> wild blueberry> snowberry> redcurrant> nannyberry> chokecherry> chokeberry> buckthorn> highbush cranberry (**Figure 5.S3**). The highest total fat content ($p<0.05$) was found in wild grapes (7659±312 µg/g DW) followed by haskap berry (4650±184 µg/g DW) and wild raspberry (3321±133 µg/g DW). While the overall fat content adhered to the abovementioned order, variations in the total content of SFAs, MUFAs, and PUFAs among berries were observed. For example, although wild grapes exhibited the highest total fat content, haskap berries demonstrated higher levels of total SFAs than wild berries. Conversely, despite haskap berries having the second-highest total fat content, their total MUFAs were lower than those in wild grapes and wild raspberries (**Table 5.2**). When considering the fatty acid profiles present in each wild berry type, α-linoleic acid (PUFA) is the most abundant fatty acid, followed by linolenic acid (PUFA), palmitic (MUFA) and oleic acid (MUFA) among all the other fatty acids (**Figure 5.1**). Previous studies on seabuckthorn found that in oils extracted from berry pulp/peel, the primary fatty acids, listed in descending order, are oleic

(20-40%), palmitic (17-27%), palmitoleic (10-22%), linoleic (10%-20%), and α-linolenic (4-9%) (Yang & Kallio, 2001a, 2001b). The chemical compositions of seed and pulp/peel oils vary widely based on subspecies, fruit harvesting time, and climatic conditions. Seed oil is rich in unsaturated fatty acids, particularly linoleic (30-40%) and α-linolenic (20-35%), while pulp/peel oil is saturated, with palmitoleic (16-54%) and palmitic acids (17-47%) as the predominant fatty acids (Dulf, 2012). In addition, a study conducted by Bajramova & Spégel (2022c) found that goji berries have a rich content of oleic acid (cis18:1) MUFAs and PUFAs, while α-linolenic acid was notably high in strawberries, followed by goji berries. Linoleic acid stood out in goji berries and white mulberries. Conversely, white mulberries showed higher trans-fatty acid elaidic acid (Bajramova & Spégel, 2022c; Parry et al., 2005). Cranberries displayed elevated levels of very long-chain fatty acids (number of carbons <22). The unsaturated-to-saturated fatty acid ratio (UFA/SFA) was higher in strawberries and goji berries than in other fruits. Linoleic acid, abundant in superfruits such as berries, was linked to reduced cardiovascular disease risk but raised concerns about inflammation (Stote et al., 2023). Studies suggested replacing saturated fats with MUFAs and PUFAs to lower the risk of type 2 diabetes and cardiovascular diseases (Parry et al., 2005). When considering the grapes, the grape seeds' oil content was identified in the 7 to 160 g/Kg DW range, with linoleic acid, oleic acid, and palmitic acid as major constituents, comprising over 92–97% of total fatty acids (Sabra et al., 2021). However, the distribution of fatty acids in each berry type varies depending on its plant source, assessing their impact on human health is crucial, considering the distinct influences on health and risks of serious diseases (Sabra et al., 2021).

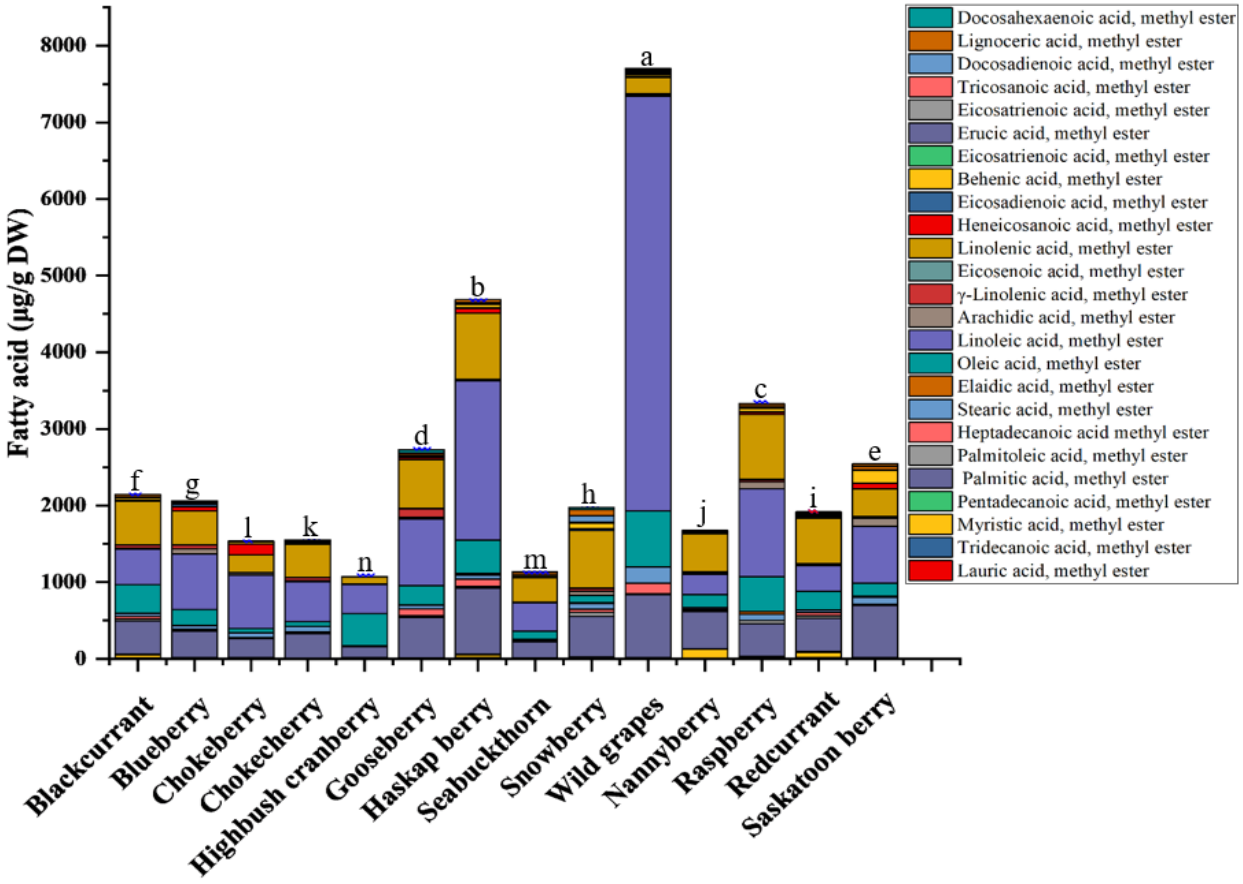


Figure 5.1. A stacked column of the total fatty acid distribution in Canadian wild berries based on the data from GC-MS analysis of fatty acid methyl esters. The letters above the columns indicate significant differences (p<0.05) within a column (total fat content of each berry type) obtained from a one-way analysis of variance (ANOVA), and mean separation was performed using Tukey's test.

5.4.1.1 Saturated fatty acids (SFAs)

Short and medium-chain saturated fatty acids have fewer than 12 carbon atoms (He et al., 2020). In the examined berries, the identified and quantified saturated fatty acids (SFAs) include lauric acid (C12:0), tridecanoic acid (C13:0), myristic acid (C14:0), pentadecanoic acid (C15:0), palmitic acid (C16:0), heptadecanoic acid (C17:0), stearic acid (C18:0), arachidic acid (C20:0), heneicosanoic acid (C21:0), behenic acid (C22:0), tricosanoic acid (C23:0), and lignoceric acid (C24:0). SFAs constitute 16-47% of total fatty acid methyl esters (FAMEs) on dry weight basis,

with haskap berries having the highest SFA content (1252 µg/g DW), followed by wild grapes (1236 µg/g DW) and Saskatoon berries (1199 µg/g DW). Among these, Saskatoon berries had the highest proportion (47%) of SFAs in total fat content, primarily represented by palmitic acid (C16:0, 676.6± µg/g DW), followed by behenic acid (C22:0, 165.9 µg/g DW) and arachidic acid (C20:0, 104.8± µg/g DW), consistent with existing literature. The consideration of the proportional values are important because the total fat content in berries ranging from Palmitic acid (C16:0) was the most abundant SFA in haskap (863.9±18.5 µg/g DW) and wild grapes (817.6±19.4 µg/g DW), similar to Saskatoon berries (**Table 5.2**). Snowberry and nannyberry also had high proportions of SFAs in total fat content (44% and 43%, respectively). Wild grapes had the second-highest total SFAs (16% DW out of the total fat content). Redcurrants, blackcurrants, gooseberry, haskap berry, buckthorn, wild raspberry, and wild blueberry had total SFAs ranging from 21%-36% out of the total fat content on dry weight basis. Although the proportions of SFAs did not differ significantly, the total SFA content varied significantly among the berry types (**Table 5.2**). The order of total content of SFAs present in berries was as follows: haskap berry> wild grapes> Saskatoon berry> snowberry> gooseberry> wild raspberry> nannyberry> redcurrant> blackcurrant> wild blueberry> chokeberry> chokecherry> buckthorn> highbush cranberry.

5.4.1.2 Monounsaturated fatty acids (MUFAs)

In the current study, oleic acid (C18:1), elaidic acid (C18:1 Trans), and palmitoleic acid (C16:1) were identified as the predominant types of MUFAs in the berry samples (**Table 5.2**). Except for chokecherry and chokeberry, most analyzed samples exhibited a variations in the proportion of MUFAs in their FAME composition, ranging from 4% to 39%. Notably, highbush cranberry displayed the highest percentage (39%) of MUFAs from the total fat content compared to other wild berries, such as Blackcurrant (19%), wild raspberry (16%), redcurrant (15%), wild blueberry and nannyberry (11%), wild grapes, buckthorn, haskap berry, gooseberry, snowberry (9%), and Saskatoon berry (8%). Despite highbush cranberries having the highest proportion, wild grapes showed the highest ($p<0.05$) MUFA content among all tested wild berries (758.9 µg/g, DW, 10% of the total fat content DW), followed by wild raspberries (547.1 µg/g DW, 16% of the total fat content DW) and haskap berries (482.9 ± µg/g DW, 10% of the total fat content DW). These obtained data align with the existing literature based on the wild grapes' MUFA contents (Ju et al., 2021). Data is lacking in most of the wild berries to compare the obtained data but considering the

most common berries the values are consistent with the literature (Parry et al., 2005; Yang & Kallio, 2001a). Conversely, chokeberries exhibited the lowest MUFA content (65.05 µg/g DW, 4% of the total fat content DW). In most wild berry samples, oleic acid (C18:1, n-9) emerged as the predominant MUFA, with wild grapes having the highest (p<0.05) content (725.2 µg/g DW), followed by wild raspberries (454.4 µg/g DW) and haskap berries (436.2 µg/g DW). Erucic acid (C22:1n-9) was detected in trace amounts exclusively in haskap berries (2.25±0.16 µg/g DW), nannyberries (1.41±0.21 µg/g DW), and wild raspberries (1.23 µg/g DW). The order of total content of MUFAs present in berries was as follows: wild grapes> wild raspberry> haskap berry> highbush cranberry> blackcurrant> redcuarrant> gooseberry> wild blueberry> Saskatoon berry> nannyberry> snowberry> buckthorn> chokecherry> chokeberry (**Figure 5.S5**).

5.4.1.3 Polyunsaturated fatty acids (PUFAs)

Polyunsaturated fatty acids (PUFAs) are a type of fatty acid that contains more than one double bond in its hydrocarbon chain. Linoleic acid (C18:2n-6), α-linolenic acid (C18:3n-3), eicosadienoic acid (C20:2), and γ-linolenic acid (C18:3n-6) were the major type of PUFAs found in the tested wild berry types (**Table 5.2**). PUFAs, particularly omega-6 and omega-3 essential fatty acids, play vital roles in various biological processes (Dulf et al., 2012; Piironen et al., 2003c). The highest proportion (74% out of the total fat content) of PUFAs was found in wild grapes, followed by chokecherry (64%) and haskap berry (63%). Similar to the proportions of the total fat contents, in the actual PUFAs contents, the same order was observed when comparing only the PUFA content found in the tested wild berry types. For instance, the highest (p<0.05) PUFA content was found in wild grapes (758.9 µg/g DW), followed by wild raspberries (547.1 µg/g DW) and haskap berries (482.9 µg/g DW). The major PUFA found in all tested berries was α-linoleic acid (C18:2n-6), an omega-3 fatty acid. The highest (p<0.05) content was found in wild grapes (5447±138 µg/g DW), followed by haskap berries (2034±37.42 µg/g DW), wild raspberries (1155±60.7 µg/g DW) and gooseberries (871.8±6.97 µg/g DW). The α-linoleic acid (C18:2n-6) content found in wild blueberries (729.3±6.23 µg/g DW) was not different (p>0.05) from Saskatoon berries (740.7±32.4 µg/g DW) and chokeberries (692.7±4.16 µg/g DW). A-linolenic acid (C18:3n-3), an omega-3 fatty acid, was the second most abundant PUFA in the tested berry types. The highest (p<0.05) content of α-linolenic acid (C18:3n-3) was found in haskap berries (868.9±5.4 µg/g DW), followed by snowberries (747.2±11.86 µg/g DW) and gooseberries

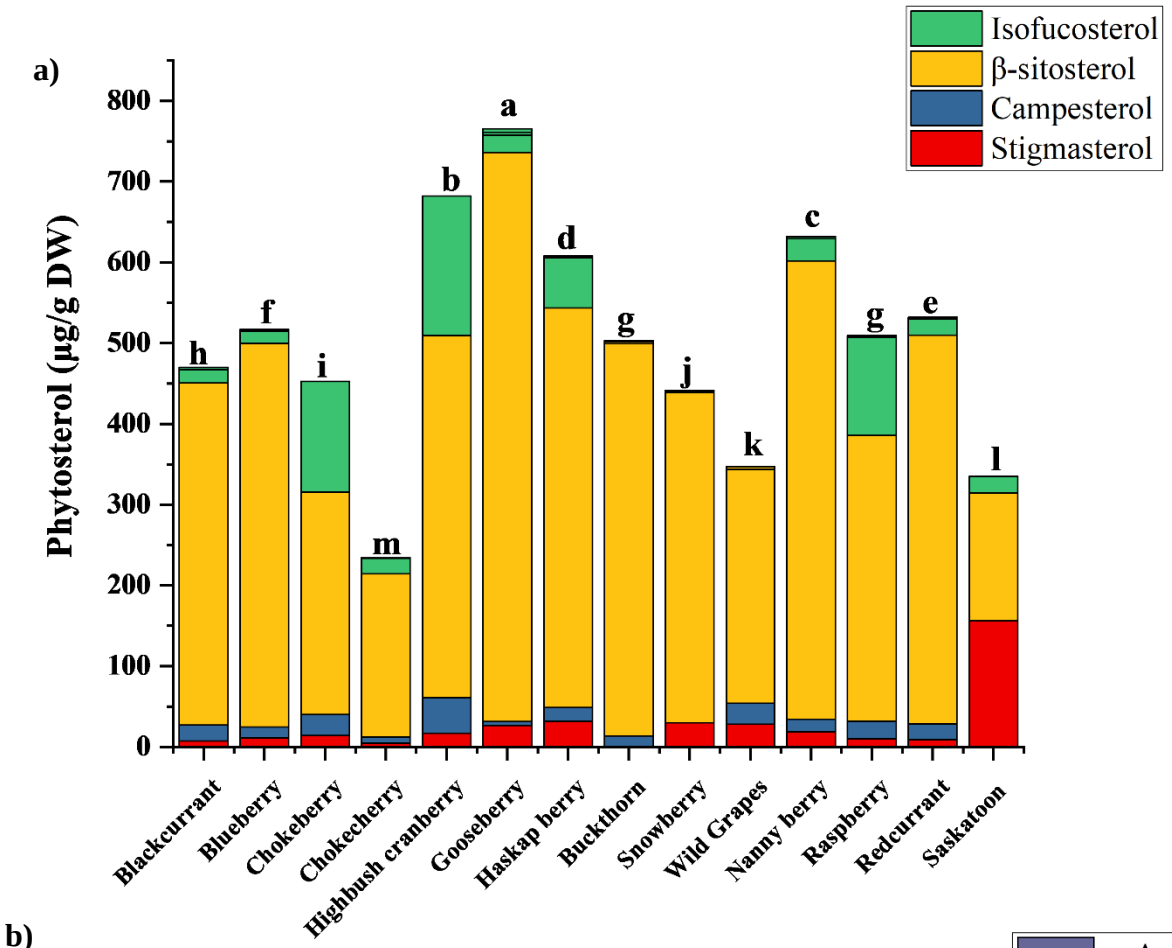
(645.1±7.09 µg/g DW). The linolenic acid (C18:3n-3) content found in wild blueberries (435.6±8.27 µg/g DW) was not different (p>0.05) from the content present in nannyberries (455.4±27.65 µg/g DW) and chokecherries (429.3±3.28 µg/g DW). The order of total content of PUFAs present in berries was as follows: wild grapes> wild raspberry> haskap berry> gooseberry> wild blueberry> Saskatoon berry> blackcurrant> chokecherry> redcuarrant> chokeberry> snowberry> nannyberry> buckthorn> highbush cranberry. Previous studies on berry seeds, a major byproduct in fruit juice manufacturing, have been studied for their content of α-linolenic acid and natural antioxidants (Parry et al., 2005). Cold-pressed raspberry seed oil contained 35% α-linolenic acid and exhibited significant antioxidant activities. Similarly, cranberry seed oil, a rich source of essential fatty acids, contained 35-44% linoleic acid (18:2n-6) and 23-35% α-linolenic acid (Parry et al., 2005). The omega-6/omega-3 fatty acid ratio is considered a pivotal factor for balanced eicosanoid synthesis, with its nutritional importance frequently discussed concerning dietary patterns. In the wild berry samples analyzed, all the berries had an acceptable omega-6/omega-3 fatty acid range of 1:1 to 3:1, which was in agreement with the recommended ratios as mentioned by Dulf et al. (2012), except for wild grapes, which had 26:1 due to high values of omega-6 fatty acids compared to the omega-3 fatty acids. Studies indicate that the typical Western diet maintains an omega-6 to omega-3 fatty acid ratio of approximately 15–17:1, but for optimal health, the recommended essential fatty acid balance (omega –6/ omega –3) should fall between 2.5 and 5.0 (Dulf et al., 2012).

5.4.2 Phytosterols and terpenes

In this investigation, phytosterol content in wild berries was identified and quantified (**Figure 5.S2**). The sample preparation method facilitated the determination of phytosterols, encompassing the terpenic compounds. Additionally, the values for other terpenic compounds incorporate diterpene, triterpenes, and sesquiterpene, which were identified through mass spectral data as terpenes. Phytosterols, resembling cholesterol, are abundant in berries and play a role in hindering the absorption of intestinal cholesterol, including the reabsorption of endogenous biliary cholesterol (Lagarda et al., 2006; Trautwein & Demonty, 2007). Nine common compounds, including, β-sitosterol, campesterol, stigmasterol, isofucosterol, α-amyrin, β-amyrin, uvaol, phytol, and farnesol were identified in studied wild berry samples (**Table 5.3**). Notably, β-sitosterol and isofucosterol were identified as the most abundant phytosterols identified in the

berries compared to campesterol and stigmasterol. Among the terpenes, phytol and α-amyrin were identified in higher quantities compared to other terpenic compounds. The highest (p<0.05) total phytosterol and terpenic content was found in nannyberries (1024±98.4 µg/g DW) followed by buckthorn (1016±88.6 µg/g DW) where the total content was not different from each other. Compared to the other wild berries tested haskap berries (968.9±40.6 µg/g DW) had the third highest (p<0.05) content of phytosterols and terpenes content. The total phytosterols and terpenes content in both wild raspberries (616.4±25.4 µg/g DW) and chokeberries (612.2±35.9 µg/g DW) were not significantly different from each other (**Table 5.3**). Even though wild grapes contained the highest total fatty acid content (**Figure 5.1**), the amount of phytosterols and terpenes contents are not considerably higher when compared with the other tested wild berries. However, specific phytosterol compositions were observed in certain samples. For instance, isofucosterols were exclusively present in all the wild berries except snowberries, wild grapes and buckthorn. Moreover, stigmasterol was not present in buckthorn and campesterol was not found in snowberries and Saskatoon berries. But stigmasterol was found in high quantities in Saskatoon berries compared to the other tested wild berries. In literature, the concentrations of plant sterols in fresh berries varied, ranging from 60 mg/kg FW in red currant to 279 mg/kg, Fresh Weight (FW) in lingonberry, with cultivated berries such as blackcurrant, redcurrant, and strawberry showing significantly lower levels compared to wild berries such as lingonberry and blueberry (Piironen et al., 2003c). Previous studies on buckthorn found that both berry seeds and pulp/peel exhibited high oil content, containing significant amounts of phytosterols (up to 23 g/kg in seed oil, up to 29 g/kg in pulp/peel oil) (Dulf, 2012). Among the cultivated berries, raspberries displayed a higher sterol content than the others. Similar to vegetables and fruits, there was substantial variability in sterol contents when expressed on a dry weight basis, ranging from 372 to 2160 mg/kg. Notably, blackcurrant and redcurrant, and to a lesser extent, strawberry, exhibited lower sterol levels than other berries (**Figure 5.S4**). Surprisingly, in the current study, the observed differences in sterol content among the wild berries could not be attributed to factors such as seed size, seed proportion, or oil content. For instance, lingonberry and blueberry, despite having a significantly lower proportion of seeds (14 and 29 versus 101 g/kg) and lower oil contents (41 and 85 g/kg fresh berries) compared to raspberry (223 g/kg), were comparable to raspberry as sources of sterols. In literature, the predominant sterol in berries was identified as β-sitosterol, as shown in the current study constituting 61–93% of the total sterols, with the lowest proportion found in lingonberry

(Bajramova & Spégel, 2022a). Lingonberry and strawberry displayed considerable proportions of other sterols, exceeding 20% of the total sterols. Historically, interest in berries as plant sterol sources has been limited. In earlier studies, strawberry was found to contain 120 mg sterols kg^{-1} FW (Piironen et al., 2003c). Additionally, Bulgarian berry seed oils exhibited varying sterol contents, with chokeberry seed oil containing 1.2 g/kg, blackcurrant seed oil containing 1.4 g/kg, and rosehip seed oil containing only 0.4 g/kg. In rosehip seed, the total oil content was the lowest, constituting only one-third of that in the other two berries. More recently, buckthorn berries were found to contain sterols 340–520 mg/kg, with corresponding values in seeds and fresh pulp/peel at 1200–1800 mg/kg and 240–400 mg/kg, respectively (Piironen et al., 2003c). Additionally, previous studies on raspberry and blueberry berry seeds showcased notable levels of β-sitosterol level when the seeds were cold-pressed for oil (Parry et al., 2005). Previous research suggests that β-sitosterol possesses antioxidant properties, as well as antigenotoxic and immuno-stimulant potential (Piironen et al., 2003c; Yang et al., 2001). Additionally, studies demonstrated the anti-proliferative and pro-apoptotic effects of β-sitosterol in human leukemic cells (U937), highlighting its potential therapeutic benefits (Dulf et al., 2012). When considering phytosterols and terpenes compounds separately, the results vary in terms of total content. Nannyberries and buckthorn have higher total phytosterols and terpenes content, but when considering only the phytosterol content, gooseberries exhibit the highest ($p<0.05$), followed by highbush cranberry and nannyberries (**Figure 5.2a**). The distribution of terpenes content also differs ($p<0.05$) from the total phytosterols content, with Saskatoon berries having the highest, followed by buckthorn and nannyberries (**Figure 5.2b**). Among the terpenes, phytol was found in all the tested wild berry types, but the other terpenoid compounds were not found in all the other wild berries tested. For instance, β-amyrin was not found in snowberries while, α-amyrin was not found in chokecherries, gooseberries, wild grapes, snowberries and blackcurrants. Farnesol was not found in chokeberries, chokecherries, haskap berries and wild grapes. Uvaol was not found in chokecherries, gooseberries, haskap berries and blackcurrants. In recent years, the crucial role of terpenes and terpenoids in promoting human health has been identified (Masyita et al., 2022). These bioactive compounds' diverse therapeutic potential has been explored in numerous studies, both in vitro and in vivo, showcasing their efficacy as antimicrobials, anticancer agents, anti-inflammatories, antiallergics, antioxidants, anti-aggregators, neuroprotective agents, anticoagulants, sedatives, and analgesics (Masyita et al., 2022).


a)
Isofucosterol
β-sitosterol
Campesterol
Stigmasterol
Phytosterol (µg/g DW)
0
100
200
300
400
500
600
700
800
h
f
i
m
b
a
d
g
j
k
c
g
e
l
Blackcurrant
Blueberry
Chokeberry
Chokecherry
Highbush cranberry
Gooseberry
Haskap berry
Buckthorn
Snowberry
Wild Grapes
Nanny berry
Raspberry
Redcurrant
Saskatoon

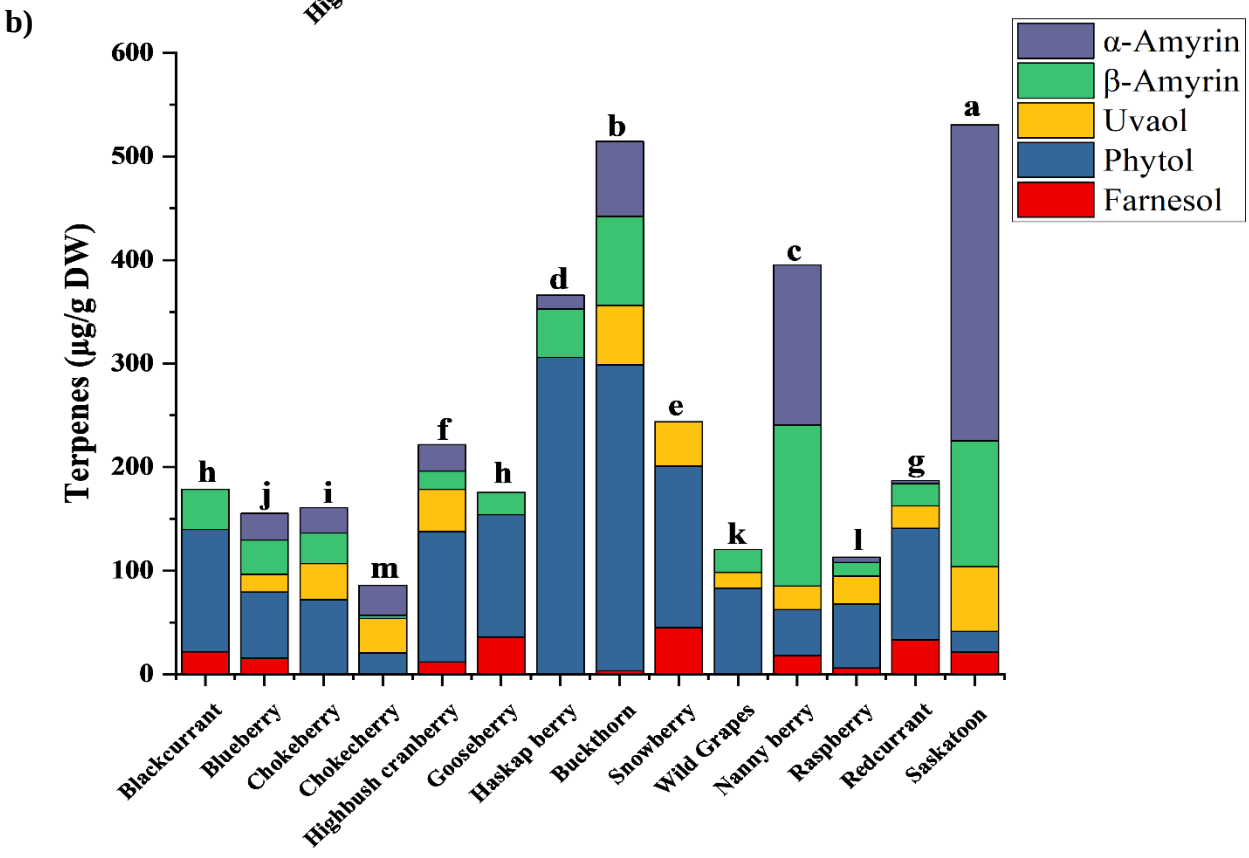

b)
α-Amyrin
β-Amyrin
Uvaol
Phytol
Farnesol
Terpenes (µg/g DW)
0
100
200
300
400
500
600
h
j
i
m
f
h
d
b
e
k
c
l
g
a
Blackcurrant
Blueberry
Chokeberry
Chokecherry
Highbush cranberry
Gooseberry
Haskap berry
Buckthorn
Snowberry
Wild Grapes
Nanny berry
Raspberry
Redcurrant
Saskatoon

Figure 5.2 A stacked column of the total phytosterols and terpenes distribution in Canadian wild berries based on the data from GC-MS analysis of phytosterols and terpenes compounds. **a)** Stacked column of the total phytosterols content present in these wild berries; **b)** Stacked column of the total terpenes content present in Canadian wild berries. The letters above the columns indicate significant differences ($p<0.05$) within a column obtained from a one-way analysis of variance (ANOVA), and mean separation was performed using Tukey's test.

Table 5.2 Fatty acid content (µg/g dry weight ± RSD (n=3)) in wild blueberry, chokecherry, highbush cranberry, haskap berry, buckthorn, snowberry, wild grapes, nannyberry, wild raspberry, redcurrant, blackcurrant and Saskatoon berries analyzed by GC-MS

Fatty acid	Wild blueberry (µg/g)	Chokeberry (µg/g)	Chokecherry (µg/g)	Highbush cranberry (µg/g)	Gooseberry (µg/g)	Haskap berry (µg/g)	Buckthorn (µg/g)
C12:0	3.356±0.16^{f}	7.808±0.16^{d}	1.656±0.03^{gh}	7.614±0.19^{d}	1.441±0.09^{gh}	14.72±0.34^{b}	1.132±0.06^{h}
C13:0	6.819±0.21^{b}	ND	ND	ND	ND	2.547±0.47^{d}	ND
C14:0	5.978±0.44^{gh}	6.402±0.17^{gh}	3.53±0.09^{h}	11.63±0.11^{e}	7.725±0.22^{fg}	39.36±0.25^{c}	4.649±0.16^{gh}
C15:0	2.531±0.33^{ef}	2.635±0.06^{e}	1.051±0.05^{g}	0.928±0.03^{g}	4.561±0.21^{c}	3.582±0.28^{d}	5.64±0.23^{b}
C16:0	344.6±5.1^{h}	246.7±1.2^{j}	324.6±4.28^{i}	137.3±4.58^{l}	528.8±8.37^{d}	863.9±8.35^{a}	211.2±0.38^{k}
C16:1	17.28±0.28^{e}	2.095±0.08^{gh}	3.48±0.17^{g}	1.36±0.11^{h}	16.16±0.8^{e}	21.33±0.2^{d}	2.856±0.03^{gh}
C17:0	5.696±0.13^{ef}	14.36±0.2^{de}	14.8±0.18^{de}	ND	93.34±0.73^{b}	100.5±2.6^{b}	2.54±0.02^{ef}
C18:0	46.31±1.09^{i}	58.72±1.21^{f}	70.34±0.33^{e}	11.59±0.37^{m}	48.53±1.22^{h}	51.56±0.5^{g}	21.69±0.26^{l}
C18:1trans	2.54±0.31^{ghi}	4.138±0.04^{fg}	ND	2.027±0.17^{hi}	4.465±0.44^{f}	17.96±0.92^{b}	1.469±0.02^{ij}
C18:1	207.6±5.42^{h}	57.58±2.45^{n}	65.60±1.28^{m}	416.4±6.1^{d}	248.3±2.73^{f}	436.2±4.93^{c}	106.2±3.1^{k}
C18:2n-6	729.3±6.23^{ef}	692.7±4.16^{f}	519.5±2.33^{g}	379.5±3.4^{i}	871.8±6.97^{d}	2034±37.42^{b}	372.8±7.3^{i}
C20:0	63.57±2.11^{c}	29.44±0.23^{e}	16.66±0.23^{fg}	4.12±0.2^{k}	18.31±0.54^{f}	14.94±0.35^{gh}	8.477±0.45^{j}
C18:3n-6	50.22±1.13^{b}	ND	39.62±0.06^{c}	ND	112.3±0.87^{a}	ND	0.724±0.03^{f}
C20:1	5.575±0.39^{d}	1.239±0.15^{fg}	1.797±0.1^{f}	4.478±0.25^{de}	5.833±0.43^{d}	5.225±0.44^{d}	1.44±0.03^{fg}
C18:3n-3	435.6±8.27^{ef}	233.1±4.72^{i}	429.3±3.28^{f}	87.41±2.55^{j}	645.1±7.09^{c}	868.9±5.04^{a}	313.6±5.56^{h}
C21:0	61.70±1.19^{c}	142.5±0.24^{a}	23.57±0.27^{f}	4.302±0.42^{h}	20.73±0.57^{f}	57.57±1.61^{d}	3.682±0.32^{hi}
C20:2	31.62±0.42^{a}	1.209±0.13^{f}	3.292±0.23^{e}	ND	6.312±0.12^{cd}	7.16±0.62^{c}	4.321±0.5^{de}
C22:0	14.52±0.19^{e}	22.36±0.36^{d}	15.42±0.35^{e}	3.421±0.46^{f}	14.12±0.49^{e}	43.21±0.33^{c}	25.14±0.88^{d}
C20:3n-6	ND	ND	ND	ND	ND	ND	ND
C22:1n-9	ND	ND	ND	ND	ND	2.252±0.16^{a}	ND

C20:3n-3	1.207±0.18^{ef}	0.673±0.58^{fg}	2.742±0.13^{c}	1.672±0.38^{de}	2.442±0.1^{cd}	4.124±0.41^{b}	0.888±0.02^{efg}
C23:0	11.53±0.3^{c}	3.316±0.67^{f}	ND	ND	8.886±0.68^{d}	21.97±1.39^{a}	5.387±0.16^{e}
C22:2n-6	ND	ND	ND	ND	ND	ND	ND
C24:0	11.74±0.2^{fg}	7.113±0.54^{gh}	15.71±0.27^{ef}	1.667±0.15^{h}	23.84±0.39^{d}	38.57±2.18^{c}	37.46±6.58^{c}
C22:6n-3	ND	ND	ND	ND	ND	ND	ND
SFAs	578.5^{j} (28%)	541.3^{k} (35%)	487.4^{l} (31%)	182.6^{n} (17%)	770.3^{e} (28%)	1252^{a} (27%)	327.0^{m} (29%)
MUFAs	232.9^{h} (11%)	65.05^{n} (4%)	70.88^{m} (5%)	424.3^{d} (39%)	274.8^{g} (10%)	482.9^{c} (10%)	111.9^{l} (10%)
PUFAs	1247^{e} (61%)	927.7^{j} (60%)	994.4^{h} (64%)	468.6^{n} (44%)	1681^{d} (62%)	2914^{b} (63%)	692.3^{m} (61%)
n-3 PUFAs	435.6^{h} (21%)	233.1^{l} (15%)	429.3^{i} (28%)	87.41^{n} (8%)	688.2^{d} (25%)	868.9^{a} (19%)	313.6^{k} (28%)
n-6 PUFAs	779.5^{e} (38%)	692.6^{g} (45%)	559.1^{h} (36%)	379.5^{j} (35%)	984.1^{d} (36%)	2034^{b} (44%)	373.5^{k} (33%)
n-6/n-3	1.790	2.971	1.302	4.341	1.430	2.341	1.191
n-6:n-3	2:1	3:1	1:1	4:1	1:1	2:1	1:1
Total fat content	**2059±101^{g}**	**1534±123^{l}**	**1553±112^{k}**	**1075±85^{n}**	**2679±210^{d}**	**4650±184^{b}**	**1131±90.4^{m}**

Fatty acid	**Snowberry (µg/g)**	**Wild grapes (µg/g)**	**Nannyberry (µg/g)**	**Wild raspberry (µg/g)**	**Redcurrant (µg/g)**	**Blackcurrant (µg/g)**	**Saskatoon berry (µg/g)**
C12:0	9.475±0.23^{c}	2.21±0.07^{g}	5.98±0.59^{e}	9.251±0.45^{c}	16.33±0.48^{a}	9.94±0.74^{c}	6.042±0.16^{e}
C13:0	ND	8.264±0.42^{a}	2.125±0.28^{d}	2.062±0.09^{d}	3.182±0.09^{c}	3.299±0.09^{c}	ND
C14:0	16.11±0.59^{d}	5.002±0.36^{gh}	115.6±3.69^{a}	15.96±0.66^{d}	64.89±1.26^{b}	38.33±2.52^{c}	10.46±0.69^{ef}
C15:0	ND	1.616±0.18^{fg}	3.684±0.61^{cd}	2.41±0.06^{ef}	8.028±0.37^{a}	6.364±0.19^{b}	3.99±0.69^{cd}
C16:0	532.7±15.59^{d}	817.6±9.44^{b}	485.1±4.33^{e}	429.9±5.69^{g}	437.1±13.86^{f}	435.9±10.51^{f}	676.6±31.32^{c}
C16:1	52.03±1.92^{a}	16.29±0.61^{e}	16.17±0.62^{e}	42.86±1.61^{b}	30.72±0.44^{c}	21.15±0.62^{d}	9.932±0.75^{f}
C17:0	25.17±17.32^{d}	138.1±2.77^{a}	11.17±0.35^{ef}	3.49±0.71^{ef}	44.33±0.95^{c}	41.47±2.26^{c}	11.74±1.37^{def}
C18:0	78.13±6.23^{c}	204.4±1.28^{a}	23.25±0.67^{l}	75.85±9.6^{d}	28.38±0.8^{k}	33.72±0.36^{j}	88.81±9.09^{b}
C18:1trans	10.69±1.05^{d}	3.882±0.33^{fg}	1.362±0.3^{ij}	39.34±0.42^{a}	6.163±0.58^{e}	3.207±0.16^{fgh}	12.47±1.21^{c}
C18:1	93.91±5.69^{l}	725.2±11.63^{a}	165.2±1.9^{j}	454.4±11.34^{b}	240.4±10.96^{g}	374.3±10.45^{e}	176.4±1.87^{i}

C18:2n-6	ND	5447±138^{a}	267.8±3.65^{j}	1155±60.7^{c}	335.2±12.03^{i}	464.5±21.6^{h}	740.7±32.44^{e}
C20:0	55.99±3.64^{d}	15.52±0.64^{gh}	15.21±0.9^{gh}	87.29±7.48^{b}	11.60±0.54^{i}	14.63±0.32^{h}	104.8±11.29^{a}
C18:3n-6	29.99±0.46^{d}	ND	19.67±0.07^{e}	31.87±7.12^{d}	14.81±0.79^{e}	34.39±2.21^{cd}	17.37±0.99^{e}
C20:1	10.52±1.23^{b}	13.58±0.53^{a}	3.586±0.41^{e}	9.301±0.34^{bc}	ND	1.844±0.01^{f}	8.816±1.01^{c}
C18:3n-3	747.2±11.86^{b}	212.4±3.73^{i}	455.4±27.65^{e}	846.9±15.54^{a}	593.1±19.68^{d}	574.23±22.5^{d}	363.6±21.4^{g}
C21:0	ND	2.671±0.62^{hi}	13.37±4.18^{g}	27.56±1.21^{e}	16.66±0.91^{g}	14.16±0.57^{g}	72.94±7.52^{b}
C20:2	21.45±1.12^{b}	4.615±0.78^{de}	4.347±1.84^{de}	5.062±0.22^{de}	ND	ND	3.238±0.19^{e}
C22:0	74.10±6.34^{b}	22.73±1.09^{d}	14.85±3.32^{e}	46.83±5.98^{c}	17.64±1.12^{e}	25.49±0.3^{d}	165.9±13.49^{a}
C20:3n-6	ND	ND	2.096±1.66^{a}	ND	ND	ND	ND
C22:1n-9	ND	ND	1.419±0.21^{b}	1.231±0.18^{c}	ND	ND	ND
C20:3n-3	14.76±0.43^{a}	0.699±0.61^{fg}	1.027±0.03^{ef}	1.377±0.38^{ef}	ND	1.167±0.03^{ef}	1.389±0.06^{ef}
C23:0	ND	5.762±0.71^{e}	2.454±0.65^{f}	6.35±0.81^{e}	18.78±0.11^{b}	11.59±0.31^{c}	10.21±0.54^{cd}
C22:2n-6	ND	ND	ND	ND	ND	ND	ND
C24:0	80.90±5.48^{a}	11.64±0.68^{fg}	4.8±1.9^{h}	25.85±0.69^{d}	21.26±0.64^{de}	33.56±8.31^{c}	47.91±1.62^{b}
C22:6n-3	27.38±0.68^{a}	ND	ND	ND	ND	ND	ND
SFAs	872.6^{d} (44%)	1236^{b} (16%)	697.6^{g} (43%)	732.8^{f} (22%)	688.2^{h} (36%)	668.5^{i} (31%)	1199^{c} (47%)
MUFAs	167.2^{k} (9%)	758.9^{a} (10%)	187.7^{j} (11%)	547.1^{b} (16%)	277.3^{f} (15%)	400.5^{e} (19%)	207.7^{i} (8%)
PUFAs	927.1^{k} (47%)	5664^{a} (74%)	750.3^{l} (46%)	2041^{c} (61%)	943.2^{i} (49%)	1074^{g} (50%)	1126^{f} (44%)
n-3 PUFAs	774.6^{c} (39%)	212.4^{m} (3%)	455.4^{g} (28%)	846.9^{b} (26%)	593.1^{e} (31%)	574.2^{f} (27%)	363.6^{j} (14%)
n-6 PUFAs	29.99^{n} (2%)	5447^{a} (71%)	287.5^{m} (18%)	1187^{c} (36%)	350.1^{l} (18%)	498.8^{i} (33%)	758.1^{f} (30%)
n-6/n-3	0.039	25.649	0.631	1.402	0.590	0.869	2.085
n-6:n:3	0:1	26:1	1:1	1:1	1:1	1:1	2:1
Total fat content	**1881±110^{h}**	**7659±312^{a}**	**1636±114^{j}**	**3321±133^{c}**	**1909±193^{i}**	**2143±142^{f}**	**2534±172^{e}**

The superscript letters indicate significant differences ($p<0.05$) within a row obtained from a one-way analysis of variance (ANOVA), and mean separation was performed using Tukey's test; Not detected indicated as ND; SFA, Saturated Fatty Acids; MUFAs, Monou

saturated Fatty Acids; PUFAs, Polyunsaturated Fatty Acids; n-3 PUFAs, Omega-3 Polyunsaturated Fatty Acids; n-6 PUFAs, Omega-6 Polyunsaturated Fatty Acids. The proportion of different fatty acids to the total fat content is given in parentheses.

Table 5.3 Phytosterols and terpenes content (µg/g dry weight ± RSD (n=3) in wild blueberry, chokecherry, highbush cranberry, haskap berry, buckthorn, snowberry, wild grapes, nannyberry, wild raspberry, redcurrant, blackcurrant and Saskatoon berries analyzed by GC-MS

Compounds	Stigmasterol	Campesterol	β-sitosterol	Isofucosterol	β-Amyrin	α-Amyrin	Phytol	Farnesol	Uvaol	Total
Wild blueberry	11.75±0.96^{fg}	13.67±0.45^{g}	474.1±11.7^{f}	14.98±0.64^{h}	34.55±1.25^{f}	25.25±0.47^{d}	63.66±0.79^{i}	15.16±0.52^{f}	16.98±0.9^{g}	**670.2±27.4^{h}**
Chokeberry	13.13±1.11^{f}	24.98±1.16^{b}	275.6±14.5^{l}	135.7±2.39^{b}	29.73±0.93^{g}	24.92±0.42^{d}	72.08±0.53^{h}	ND	35.85±1.02^{d}	**612.2±35.9^{j}**
Chokecherry	5.36±0.44^{i}	7.11±0.36^{h}	201.8±8.63^{m}	19.88±0.37^{f}	27.11±0.24^{g}	ND	21.31±1.06^{l}	ND	ND	**282.6±12.5^{l}**
Highbush cranberry	17.42±1.37^{e}	43.63±5.44^{a}	448.3±10.7^{g}	171.5±11.3^{a}	17.37±0.39^{i}	25.48±0.46^{d}	127.4±11.8^{d}	12.25±0.47^{g}	41.76±2.07^{c}	**905.1±44.9^{d}**
Gooseberry	27.55±0.87^{d}	4.936±0.83^{i}	707.7±12.8^{a}	21.12±0.15^{f}	20.85±0.6^{h}	ND	119.0±10.3^{e}	37.26±5.32^{b}	ND	**938.5±29.4^{c}**
Haskap berry	32.99±1.1^{b}	16.78±0.22^{e}	492.7±12.0^{c}	64.58±2.42^{d}	44.79±1.93^{d}	13.87±0.54^{e}	303.1±21.3^{a}	ND	ND	**968.9±40.6^{b}**
Buckthorn	ND	14.10±0.11^{fg}	487.5±15.8^{d}	ND	86.74±3.09^{c}	73.02±0.39^{c}	295.2±10.5^{b}	3.373±0.34^{i}	56.37±0.97^{b}	**1016±88.6^{a}**
Snowberry	30.61±0.54^{c}	ND	409.6±21.8^{i}	ND	ND	ND	156.2±3.66^{c}	45.04±3.88^{a}	41.64±2.35^{c}	**683.1±32.6^{g}**
Wild grapes	29.19±0.75^{cd}	25.72±1.35^{b}	291.2±11.4^{k}	ND	22.88±0.98^{h}	ND	83.87±8.73^{g}	ND	15.88±0.64^{g}	**468.7±24.3^{k}**
Nannyberry	19.48±0.44^{e}	15.40±0.2^{ef}	566.8±31.9^{b}	27.67±0.45^{e}	156.5±11.2^{a}	153.3±11.3^{b}	45.11±4.48^{k}	18.01±0.75^{e}	22.66±0.45^{f}	**1024±98.4^{a}**
Wild raspberry	11.03±0.76^{fg}	21.24±0.71^{c}	353.7±10.3^{j}	121.4±11.3^{c}	12.86±0.65^{j}	4.68±0.58^{f}	60.36±4.36^{j}	5.49±0.47^{h}	25.59±0.99^{e}	**616.4±25.4^{j}**
Redcurrant	9.821±0.55^{gh}	18.94±0.69^{d}	482.3±16.5^{e}	19.26±1.02^{fg}	21.82±0.34^{h}	3.475±0.19^{f}	107.1±9.61^{f}	33.08±1.99^{c}	22.08±0.51^{f}	**717.9±46.6^{f}**
Blackcurrant	7.708±0.22^{h}	19.87±0.19^{cd}	424.4±19.8^{h}	15.98±0.68^{gh}	39.45±0.46^{e}	ND	116.6±8.24^{e}	21.52±1.14^{d}	ND	**645.7±25.3^{i}**
Saskatoon berry	156.3±8.54^{a}	ND	158.4±9.87^{n}	19.92±0.78^{f}	121.1±8.67^{b}	304.1±21.7^{a}	20.69±4.58^{l}	20.96±1.38^{d}	63.15±0.78^{a}	**864.6±39.4^{e}**

The superscript letters indicate significant differences (p<0.05) within a column obtained from a one-way analysis of variance (ANOVA), and mean separation was performed using Tukey's test; Not detected indicated as ND.

5.4.3 Lipid peroxidation of Canadian Wild Berries

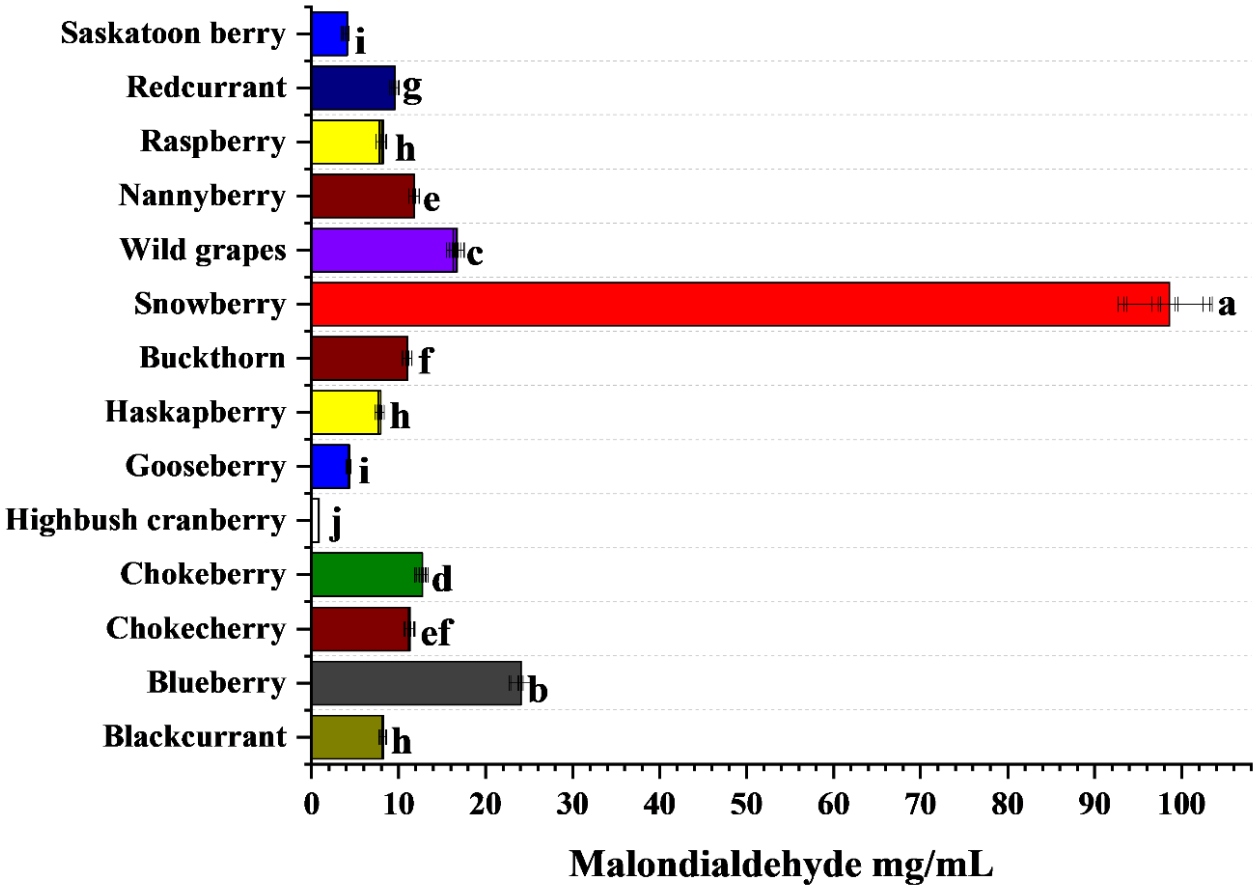


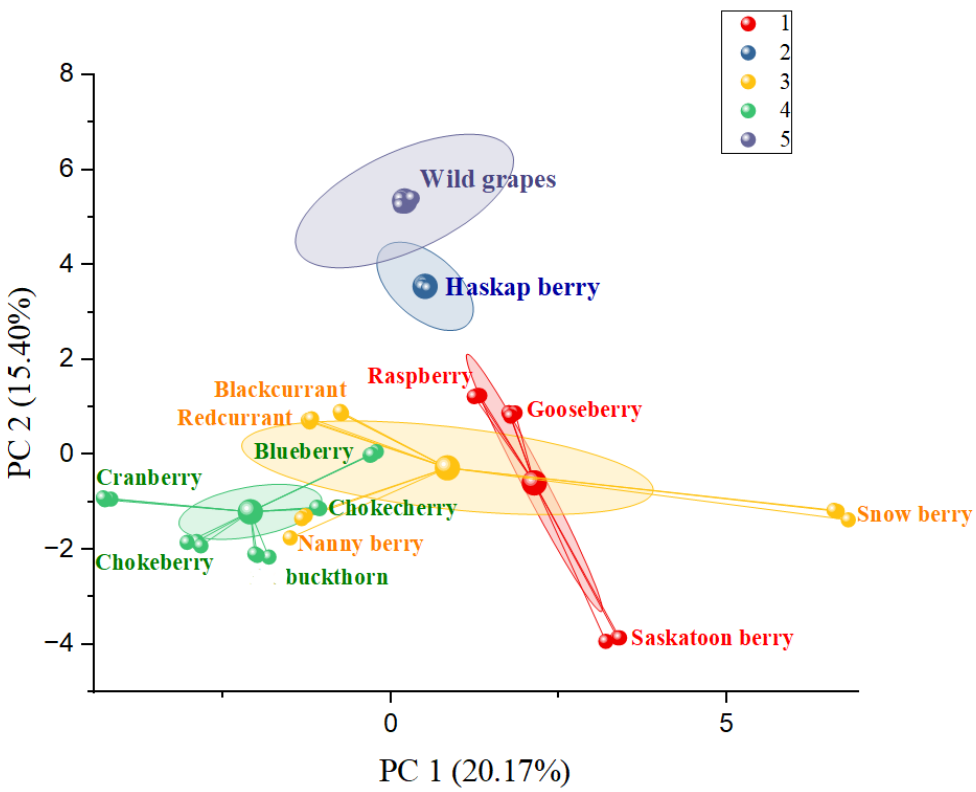
Figure 5.3 Lipid peroxidation of Canadian wild berries. The letters above the columns indicate significant differences (p<0.05) within a column obtained from a one-way analysis of variance (ANOVA), and mean separation was performed using Tukey's test.

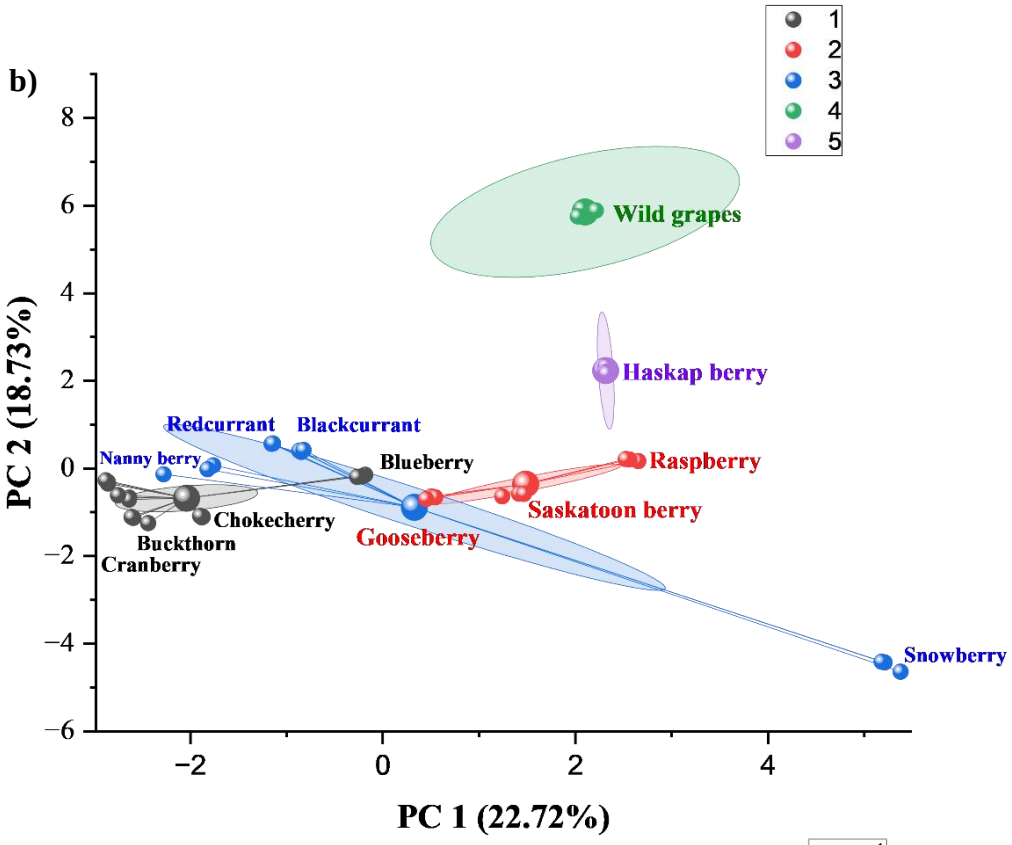
Lipid peroxidation of Canadian wild berries were evaluated (**Figure 5.3a**). Snowberry exhibited the highest (p<0.05) lipid peroxidation value (98.15±1.88 MDA mg/mL), indicating a potentially higher susceptibility to oxidative damage. Wild blueberries, chokecherries, chokeberries, wild grapes, and nannyberries displayed moderate to high lipid peroxidation values, suggesting a potential range of antioxidant capacities among these berries. Blackcurrants, gooseberries, haskap berries, raspberries, redcurrants, and Saskatoon berries show moderate lipid peroxidation values, indicating a potential protective effect against oxidative damage. Highbush cranberries stand out with the lowest (p<0.05) lipid peroxidation value (0.821±0.04 MDA mg/mL), suggesting a strong potential for antioxidant protection against lipid peroxidation. When comparing these results, it's

essential to consider the inherent variability in natural products, which can be influenced by factors such as growing conditions, ripeness, and genetic diversity. The observed variations in lipid peroxidation levels among different berries highlight the diverse antioxidant capacities of these wild berries, supporting the perception that different berries may confer varying degrees of protection against oxidative stress. Literature on antioxidant properties of berries generally aligns with the observed results, with high-antioxidant berries such as blueberries and highbush cranberries exhibiting lower lipid peroxidation values, while moderate to high-antioxidant berries such as chokeberries and wild grapes fall within the expected range (Mylnikov et al., 2005; Shewfelt & Del Rosario, 2000). The results suggest that these berries may serve as potential sources of natural antioxidants, with implications for health and nutrition. Further research could delve into the specific bioactive compounds responsible for the observed antioxidant effects in each berry type.

5.4.4 Principal component analysis (PCA) and cluster analysis (CA) of wild berries based on their fatty acids, phytosterols and terpenes contents

a)




b)
1
2
3
4
5
Wild grapes
Haskap berry
Redcurrant
Blackcurrant
Nanny berry
Blueberry
Raspberry
Saskatoon berry
Chokecherry
Gooseberry
Buckthorn
Cranberry
Snowberry
PC 2 (18.73%)
PC 1 (22.72%)

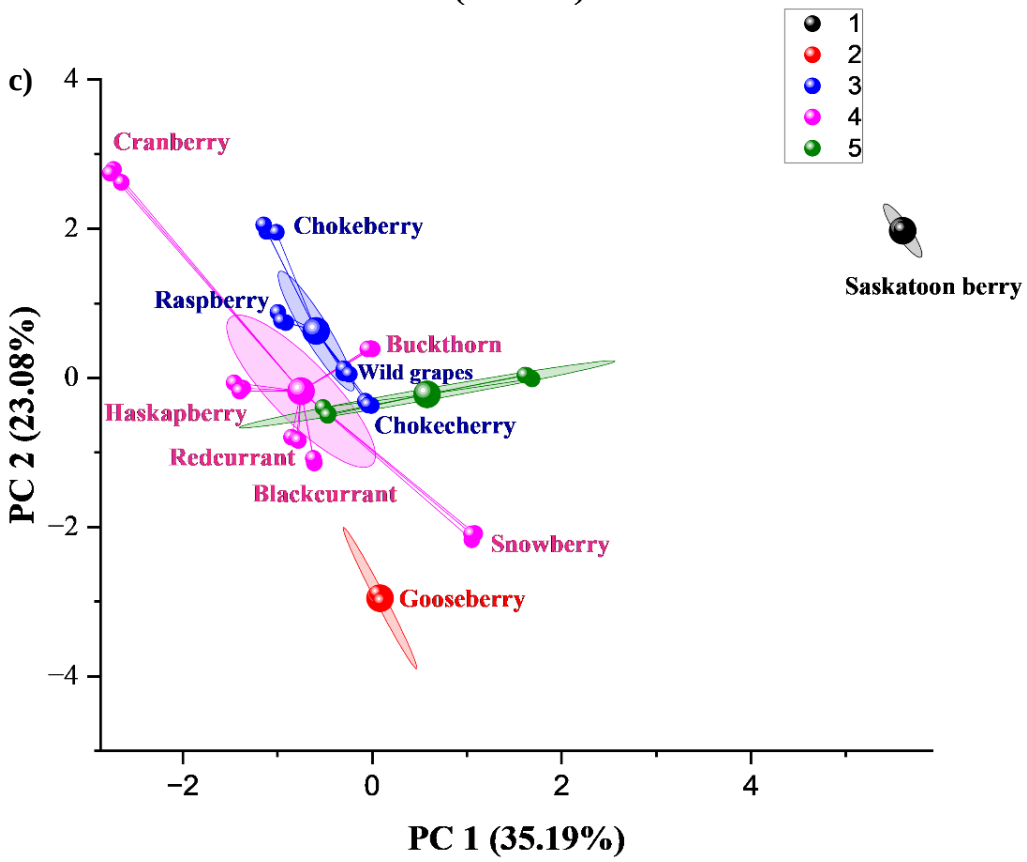

c)
1
2
3
4
5
Cranberry
Chokeberry
Saskatoon berry
Raspberry
Buckthorn
Wild grapes
Haskapberry
Chokecherry
Redcurrant
Blackcurrant
Snowberry
Gooseberry
PC 2 (23.08%)
PC 1 (35.19%)

Figure 5.4 Cluster analyses of wild berries based on their **a)** total fatty acids, phytosterols, and terpenes contents, **b)** total fatty acids, and **c)** phytosterol and terpenes contents

The results of the principal component analysis (PCA) (**Figure 5.S6**) provide valuable insights into the variance and contribution of each principal component (PC) in explaining the variability within the dataset. The eigenvalues represent the variance captured by each PC, with higher eigenvalues indicating greater significance. In this analysis, the first PC (PC1) has the highest eigenvalue of 7.06, explaining 20.17% of the total variance. The cumulative percentage of the first three PCs describes 49.87% of the total data variation. This suggests that the first few PCs can effectively represent a substantial portion of the dataset's variability. Examining the coefficients of PC1, it becomes evident that certain fatty acids and phytochemicals contribute significantly to the composition of PC1. Notably, C16:0, C16:1, C18:1, C20:1 fatty acids, phytols, and farnesols have higher positive coefficients compared to the other PCs, indicating their positive association with PC1. Conversely, C22:0, C22:1n-9, and α-amyrin have negative coefficients, suggesting a negative association with PC1. This implies that berries such as Saskatoon berries, nannyberries, buckthorn, and chokeberries with higher levels of the positively weighted compounds and lower levels of the negatively weighted compounds contribute more to the variability captured by PC1.

Moving to PC2, which has an eigenvalue of 5.39 and explains an additional 15.40% of the total variance, the coefficients reveal a different set of influential compounds. C13:0, C14:0, C16:0, C18:0, and C20:0 fatty acids and stigmasterol and β-sitosterol have positive coefficients, indicating their positive association with PC2. On the other hand, C15:0, C18:1trans, C20:3n-6, and Uvaol have negative coefficients, suggesting a negative association. Therefore, berries such as highbush cranberries, blackcurrants, and redcurrants with higher levels of the positively weighted compounds and lower levels of the negatively weighted compounds contribute more to the variability captured by PC2.

Figure 5.4 represents the results of a K-means cluster analysis conducted on 14 different berries, characterized by their total fatty acids, phytosterols, and terpenes content. **Figure 5.4a** clusters represent the grouping of the wild berries based on their total fatty acids, phytosterols, and terpenes contents. The 5 clusters exhibit distinct profiles in terms of these chemical constituents. The

content of individual fatty acids, phytosterol, and terpene within each cluster offers insights into the chemical diversity among the berries. Examining cluster1, which includes three different types of berries tested, including raspberries, gooseberries, and Saskatoon berries (shown in red color, **Figure 5.4a**), it is characterized by relatively high levels of C16:0, C18:1, C18:2n-6, and C20:1 fatty acids. Additionally, phytol, stigmasterol, and campesterol are prominent in this cluster. The relatively high content of C16:0 and C18:1 suggests a prevalence of palmitic and oleic acids, respectively. These fatty acids and the identified phytosterols contribute to the distinctive chemical fingerprint of berries in cluster 1. In Cluster 2, which contained only haskap berries (shown in blue color, **Figure 5.4a**), higher concentrations of C14:0, C16:0, C18:1, and C18:2n-6 fatty acids are observed. Notably, this cluster is characterized by substantial levels of total fatty acids and phytosterols and terpenes such as stigmasterol and β-amyrin.

The elevated levels of these compounds contribute to the overall chemical profile of berries within cluster 2. When considering cluster 3 (shown in yellow color, **Figure 5.4a**), which consisted of four different types of berries, including blackcurrants, redcurrants, nannyberries, and snowberries, had elevated levels of C14:0, C16:0, C18:2n-6, and C20:3n-6. Interestingly, this cluster exhibits a higher concentration of C18:3n-3 compared to the other clusters. Various fatty acids, phytosterols and terpenes, such as campesterol and β-amyrin, contribute to the unique chemical composition of berries in cluster 3. Cluster 4 (shown in yellow color, **Figure 5.4a**), which is the largest cluster consisting of highbush cranberries, blueberries, chokeberries, chokecherries, and buckthorn, stands out with relatively lower levels of total fatty acids, particularly C18:2n-6 and C18:3n-6. This cluster is characterized by a distinct phytosterol and terpene profile, including higher concentrations of farnesol and β-sitosterol. The lower fatty acid content and specific terpene composition contribute to the chemical distinction of berries in cluster 4. Lastly, cluster 5 (shown in purple color, **Figure 5.4a**), consisting of only wild grapes, exhibits a unique profile with high C16:0, C16:1, C18:1, and C20:1 fatty acids. The presence of the highest amount of C18:2n-6 and the absence of C18:3n-6 fatty acids are responsible for the distinguished separation of this cluster only with wild grapes.

Moreover, a significant presence of terpenes, such as α-amyrin and stigmasterol, further contributes to the distinct chemical composition of the wild grapes in cluster 5. The pairwise

distance matrix quantitatively measured dissimilarity between clusters based on their chemical profiles. The lower values within each cluster suggested similarities in the composition of the analyzed compounds. In addition, these results highlight the statistical significance of differences in fatty acid content among the clusters. For instance, the F-values for C16:0, C18:2n-6, C18:3n-3, C18:1, and several other fatty acids are markedly high (all with $p<0.0001$), indicating substantial variations among clusters. Conversely, some fatty acids, such as C20:2 and C20:3n-6, exhibit lower F-values with higher *p*-values, suggesting less significant differences among clusters. This information aids in understanding the relationships and differences between the berry clusters, providing a valuable scientific basis for further investigations into these berries' nutritional and bioactive compounds.

After the cluster analysis was performed on 14 distinct berry types, considering their fatty acids, phytosterols, and terpenes content, subsequent analyses were performed for fatty acids, phytosterols, and terpenes combined. Interestingly, the overall cluster analysis exhibited similarities with the fatty acids analysis (**Figure 5.4b**) but deviated significantly from the analysis focused solely on phytosterols and terpenes (**Figure 5.4c**). This divergence in cluster patterns indicates a significant difference in the composition-based clustering of Canadian berries, emphasizing the impact of considering different biochemical components on the resulting classifications. The clusters in **Figure 5.4b** for individual fatty acids reveal patterns and statistical significance in the distribution of fatty acid content across different clusters. As previously identified in **Figure 5.4a**, the distinct clusters indicate the unique fatty acid compositions in different types of Canadian wild berries. Similarly, the analysis of phytosterols and terpenes revealed diverse cluster patterns denoted by distinct phytosterols and terpenes compositions. The ANOVA results complemented the cluster analysis, emphasizing the statistical significance of the observed differences in phytosterol and terpene content among clusters. For instance, in the phytosterol clusters, Cluster 4 (shown in dark pink color, **Figure 5.4c**) consisted of 6 different types of wild berries, including highbush cranberries, buckthorn, haskap berries, redcurrants, blackcurrants and snowberries, demonstrated higher concentrations of phytol, β-sitosterol, and α-amyrin compared to other clusters, providing valuable information on the specific phytosterols and terpenes that contribute to the cluster formations. Cluster 3 (shown in blue color, **Figure 5.4c**), which contained four different berry types such as chokeberries, chokecherries, wild grapes, and

raspberries, consisted of a diverse composition of phytosterols and terpenes and a significant variation in the content of each component such as β-sitosterol and phytol. On the other hand, cluster 2 (shown in red color, **Figure 5.4c**) consisted only of gooseberries demonstrating a unique composition with elevated levels of β-sitosterol, farnesol, and phytol. Previous studies on gooseberries identified them as the fruit containing the most diverse terpenes (Kupska et al., 2016). Further, cluster 1 (shown in black color, **Figure 5.4c**), which consists only of Saskatoon berries, showed a significant grouping pattern and unique composition based on their phytosterols and terpenes contents compared to all the other wild berries tested in this study. A high amount of α-amyrin content (304.1±1.47 µg/g DW) made Saskatoon berries significantly different from the other tested berries. The α-amyrin and β-amyrin compounds were known to have pharmacological potentials such as anxiolytic, anti-inflammatory, hepatoprotective, and antitumor effects (Viet et al., 2021). These findings collectively emphasize the heterogeneity in the phytosterols' and terpenes' composition across different berry clusters, providing valuable insights into the biochemical diversity within the studied berry types. Further investigations could explore the biological implications of these compositional variations regarding nutritional or functional attributes. This information aids in understanding the relationships and differences between the berry clusters, providing a valuable scientific basis for further investigations into these berries' nutritional and bioactive properties.

5.5 Conclusion

The current study comprehensively explores lipophilic bioactive compounds such as fatty acid, phytosterols, and terpenes content in fourteen underutilized Canadian wild berries using GC-MS. Wild grapes emerged with the highest total fat content, followed by haskap berries and wild raspberries. Saturated fat contents varied among berries, with highbush cranberries leading MUFAs. Wild grapes, chokecherries, and haskap berries exhibited the highest percentages of PUFAs. While omega-3/omega-6 fatty acid ratios generally met standards, wild grapes deviated due to elevated omega-6 fatty acid levels. Key phytosterols and terpenes identified included β-sitosterol, isofucosterol, phytol, and α-amyrin. Snowberries displayed high lipid peroxidation suggesting potential negative health impacts. The relationships between bioactive compounds and health benefits emphasize the complexity of these interactions, calling for further molecular investigations. Applying k-means cluster analysis to fatty acid, phytosterol, and terpene datasets

enhances our understanding of chemical diversity among wild berries. These identified clusters provide a basis for future investigations into these Canadian berries' biological significance, nutritional value, and potential applications. Researchers and nutritionists can leverage this information for informed decision-making in scientific and practical contexts, contributing to advancements in berry-related research and applications in the pharmaceutical and nutraceutical industries.

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Zorzi, M., Gai, F., Medana, C., Aigotti, R., Morello, S., & Peiretti, P. G. (2020a). Bioactive compounds and antioxidant capacity of small berries. *Foods*, 9(5). https://doi.org/10.3390/foods9050623

Zorzi, M., Gai, F., Medana, C., Aigotti, R., Morello, S., & Peiretti, P. G. (2020b). Small Berries. *Foods*, 9, 623, 1–13. https://doi.org/10.3390/foods9050623

Chapter 6: Conclusions and future research directions

6.1 Conclusion

The comprehensive analysis of phenolic compounds, fatty acids, phytosterols, and terpenes in underutilized Canadian small fruits, mainly berries, has provided valuable insights into their chemical diversity and potential health benefits. The newly developed UHPLC-HRMS method for phenolic compound analysis offers a significant advancement in efficiency and precision, allowing for identifying and quantifying 66 phenolic compounds within a short timeframe. This method holds immense significance for the berry and cherry industry, enabling the verification of authenticity and paving the way for establishing a definitive polyphenol composition database specific to these fruits.

The study revealed distinct phenolic compound profiles and quantities in blueberry, raspberry, blackberry, cranberry, and cherry, indicating variations based on geographical locations, growing conditions, and genetic and environmental factors. Principal component and cluster analysis highlighted unique profiles for cherry and blueberry compared to raspberry, blackberry, and cranberry. This information is crucial for understanding the nutritional potential of these berries, promoting sustainable agriculture, and diversifying diets.

Moreover, exploring lipophilic bioactive compounds such as fatty acids, phytosterols, and terpenes in fourteen underutilized Canadian wild berries using GC-MS unveiled diverse chemical compositions. Identifying key compounds such as β-sitosterol, isofucosterol, phytol, and α-amyrin contributes to our understanding of the nutritional value of these berries. The study emphasized the complexity of interactions between bioactive compounds and health benefits, suggesting the need for further molecular investigations. The K-means cluster analysis applied to fatty acid, phytosterol, and terpene datasets revealed distinct groupings among wild berries, providing a foundation for future research into their biological significance and potential applications. The clusters identified could serve as a basis for tailored nutritional strategies, considering factors such as lipid peroxidation.

Recommendations for future research include continued metabolomics-assisted investigations to identify all potential bioactive molecules, both phenolic and lipophilic, in underutilized wild berries grown in prairies. Preclinical and clinical studies should be conducted to explore the health benefits of these bioactive compounds. Additionally, investigating changes in phenolic compound profiles during fruit ripening stages and assessing the effects of environmental factors and varieties will contribute to a deeper understanding of berry composition.

Future directions should also explore innovative applications of phenolic compounds, such as incorporating phenolic-rich berry extracts into functional foods, supplements, or pharmaceutical products.

Therefore, this comprehensive study has laid the groundwork for advancing research, quality control, and standardization in the berry and cherry industry. The identified chemical diversity and clusters among underutilized Canadian small fruits provide a platform for further exploration and utilization in various applications, contributing to advancements in both scientific and practical contexts.

6.2 Recommendations for future studies

The current study opens up exciting possibilities for future applications in various fields, ranging from agriculture and food industries to pharmaceuticals and nutraceuticals. Here are some potential future applications based on the findings:

- Pharmaceutical and Nutraceutical Industries:

✓ Nutraceuticals/Functional Food Development Development: The identified phenolic compounds, fatty acids, phytosterols, and terpenes in underutilized Canadian small fruits may be a source for developing new pharmaceutical compounds. The compounds with potential health benefits, such as antioxidant and anti-inflammatory properties, could be investigated further for their therapeutic applications.

✓ Functional Foods and Supplements: The study suggests incorporating phenolic-rich berry extracts into functional foods or dietary supplements could provide a convenient and

concentrated form of bioactive compounds. This could lead to developing products with enhanced nutritional profiles and potential health benefits.

- Agriculture and Sustainable Farming:

✓ Crop Diversification: Understanding the chemical diversity among underutilized Canadian small fruits can encourage farmers to diversify their berry crops. This diversification promotes sustainable agriculture and contributes to food security by expanding the range of available crops.

✓ Cultivation Practices: The knowledge gained from the study can inform cultivation practices, helping optimize growing conditions to enhance the production of specific bioactive compounds. This could lead to increased yields of berries with desired nutritional properties.

- Quality Control in the Food Industry:

✓ Verification of Authenticity: The UHPLC-HRMS method developed in this study for phenolic compound analysis holds great potential for quality control in the food industry. It could be employed to verify the authenticity of berry and cherry-based products, ensuring consumers receive products with the claimed polyphenol composition.

✓ Standardization of Products: The ability to rapidly and accurately quantify 66 phenolic compounds provides a robust tool for standardizing berry-based products. This ensures consistency in the bioactive content of products, meeting regulatory requirements and consumer expectations.

- Personalized Nutrition:

✓ Tailored Dietary Recommendations: The identified clusters of bioactive compounds in berries, especially those related to fatty acids, phytosterols, and terpenes, could be used to tailor dietary recommendations based on individual health needs.

- Further Research and Innovation:

✓ Exploration of Novel Applications: The study provides a foundation for researchers to explore novel applications of bioactive compounds. This could include developing

innovative food products, novel drug formulations, or integrating berry extracts into unconventional applications, such as cosmetics or functional textiles.

- ✓ Molecular Investigations: Future research could delve deeper into the molecular interactions between bioactive compounds and their health implications. Understanding these interactions at the molecular level could uncover new therapeutic targets and contribute to advancements in the fields of medicine and nutrition.

Therefore, the future applications of the current study are diverse and extend across multiple industries. From improving human health through pharmaceutical advancements to enhancing agricultural practices and food quality control, the findings can drive innovation and contribute to the development of a range of products with tangible benefits for both consumers and industries.

7.0 Supplementary material

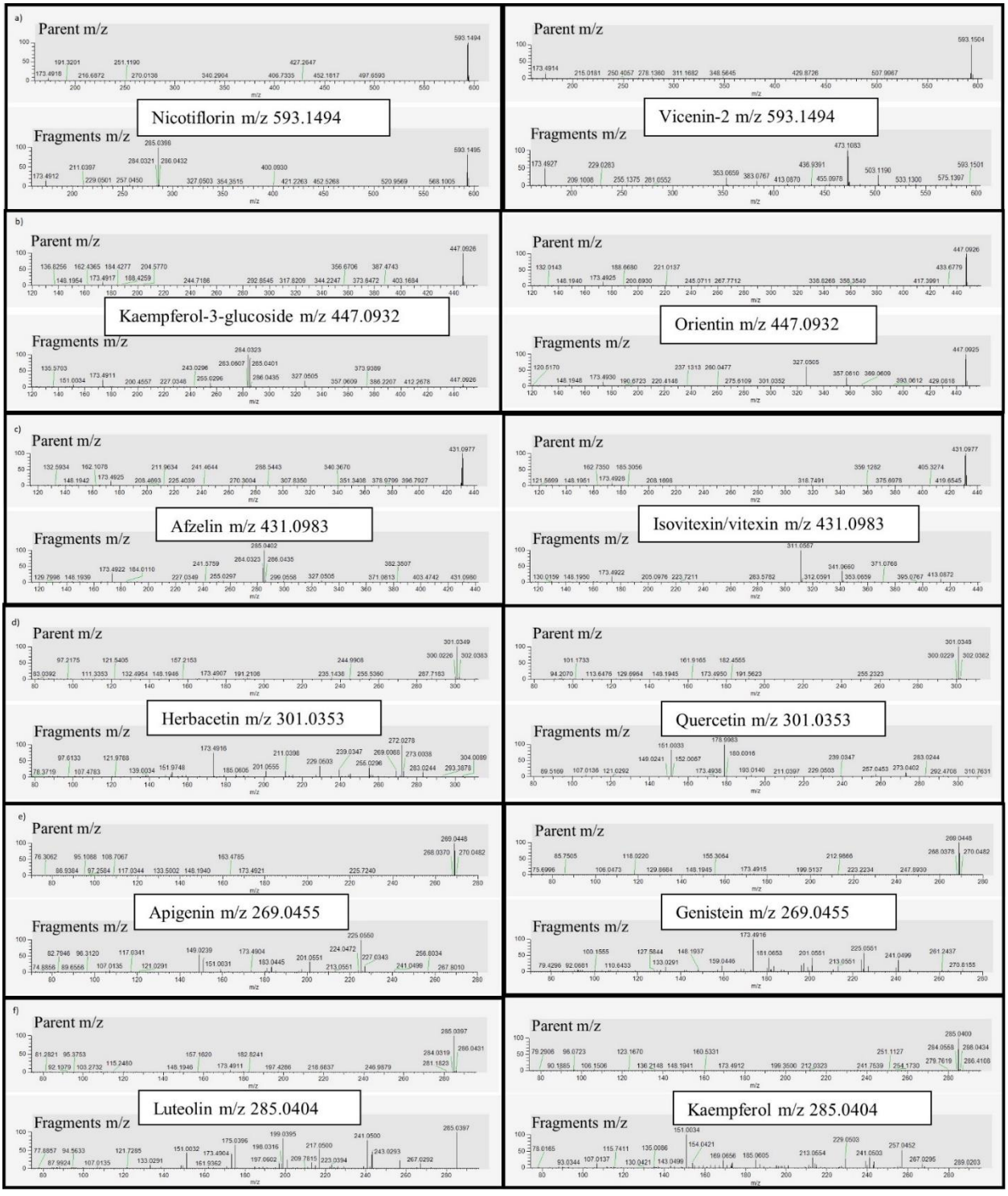
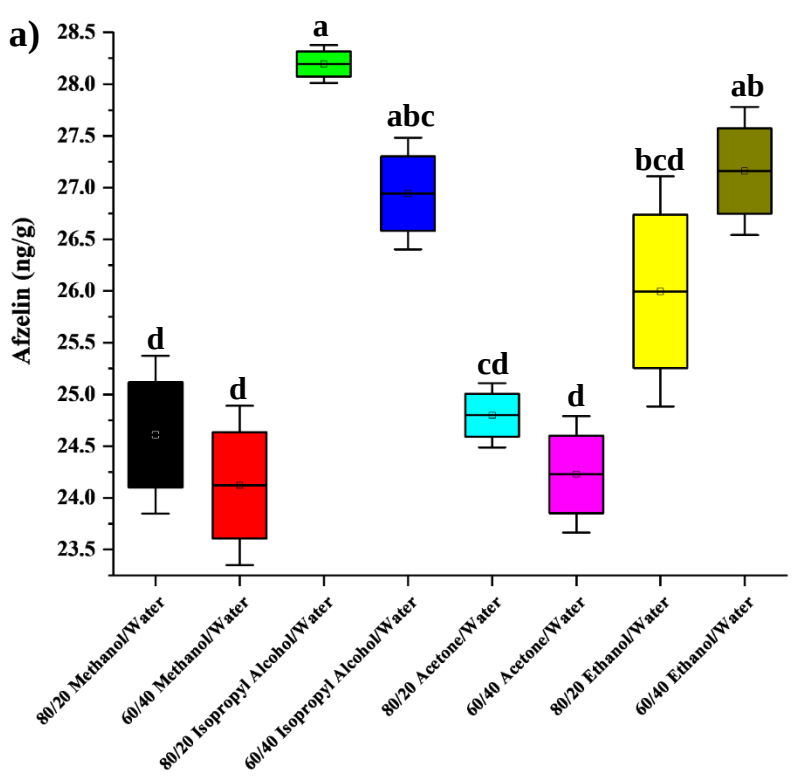
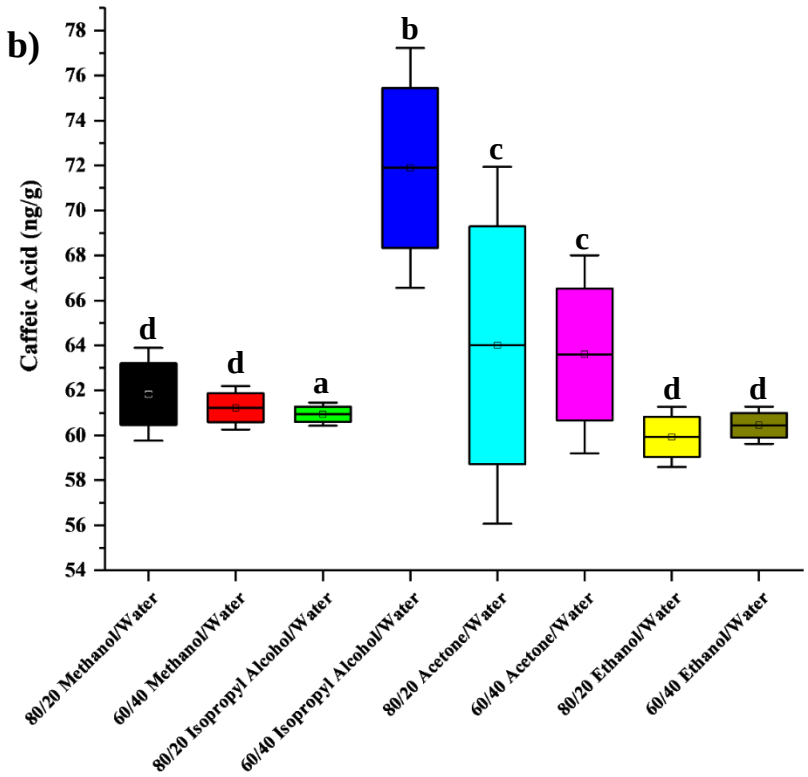
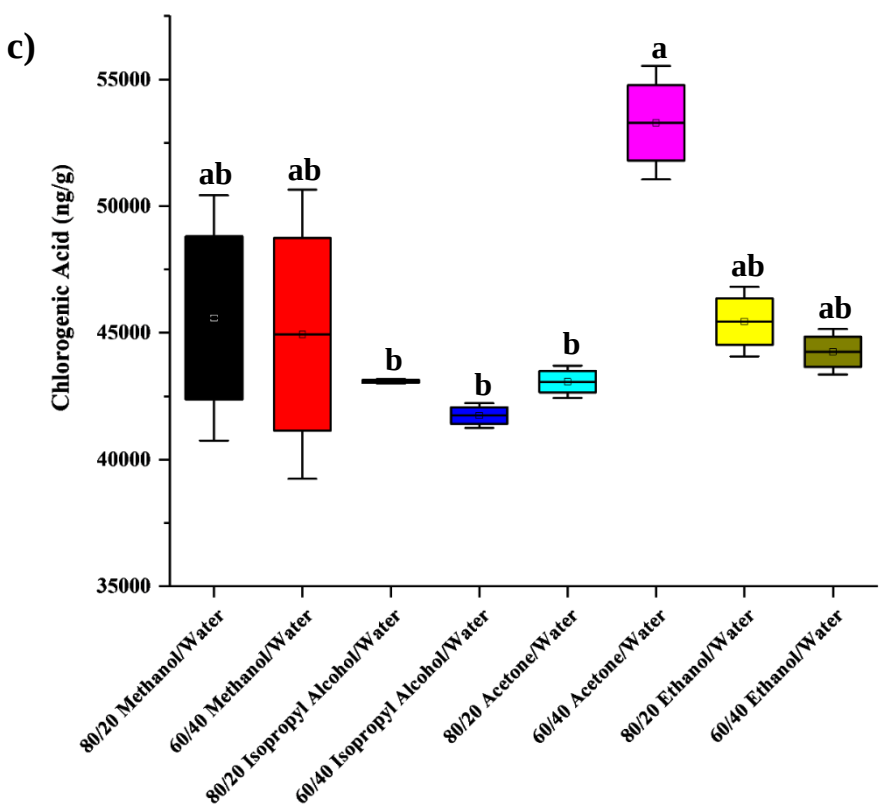


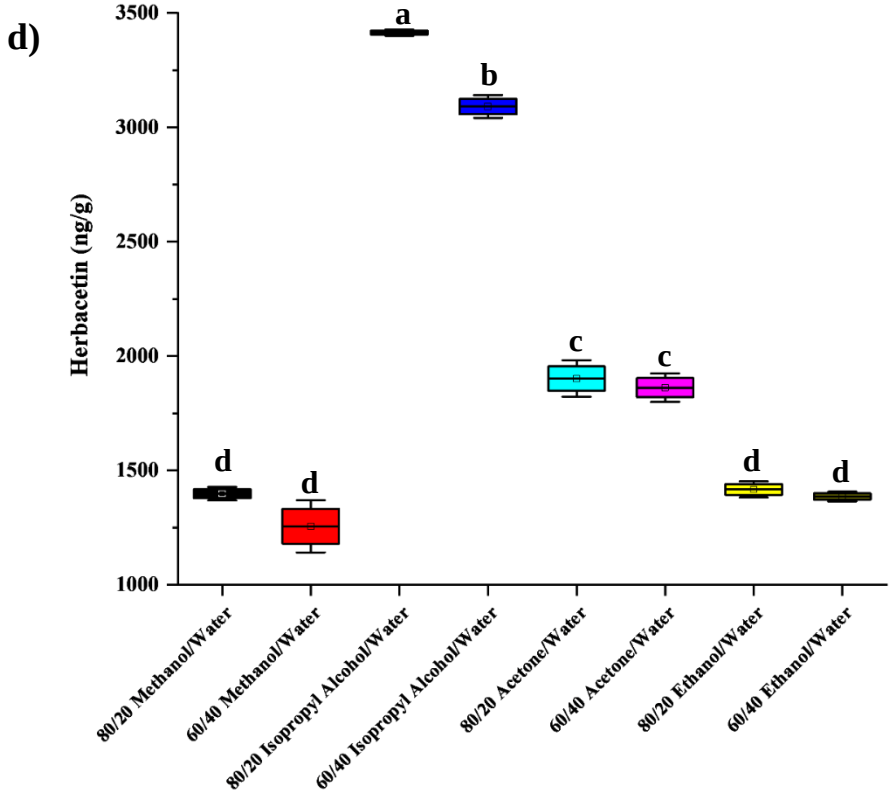
Figure 3.S1 MS^2 mass spectra of phenolic compounds showing the same parent m/z with different fragment ions used as confirmatory fragments to differentiate each phenolic compound; a) Parent

and fragments m/z for nicotiflorin and vicenin-2; b) Parent and fragments m/z for kaempferol-3-glucoside and orientin; c) Parent and fragments m/z for afzelin and isovitexin/vitexin; d) Parent and fragments m/z for herbacetin and quercetin; e) Parent and fragments m/z for apigenin and genistein; and f) Parent and fragments m/z for luteolin and kaempferol.


a)
Afzelin (ng/g)
28.5
28.0
27.5
27.0
26.5
26.0
25.5
25.0
24.5
24.0
23.5
d
d
a
abc
cd
d
bcd
ab
80/20 Methanol/Water
60/40 Methanol/Water
80/20 Isopropyl Alcohol/Water
60/40 Isopropyl Alcohol/Water
80/20 Acetone/Water
60/40 Acetone/Water
80/20 Ethanol/Water
60/40 Ethanol/Water


b)
Caffeic Acid (ng/g)
78
76
74
72
70
68
66
64
62
60
58
56
54
d
d
a
b
c
c
d
d
80/20 Methanol/Water
60/40 Methanol/Water
80/20 Isopropyl Alcohol/Water
60/40 Isopropyl Alcohol/Water
80/20 Acetone/Water
60/40 Acetone/Water
80/20 Ethanol/Water
60/40 Ethanol/Water


c)
Chlorogenic Acid (ng/g)
55000
50000
45000
40000
35000
ab
ab
b
b
b
a
ab
ab
80/20 Methanol/Water
60/40 Methanol/Water
80/20 Isopropyl Alcohol/Water
60/40 Isopropyl Alcohol/Water
80/20 Acetone/Water
60/40 Acetone/Water
80/20 Ethanol/Water
60/40 Ethanol/Water


d)
Herbacetin (ng/g)
3500
3000
2500
2000
1500
1000
d
d
a
b
c
c
d
d
80/20 Methanol/Water
60/40 Methanol/Water
80/20 Isopropyl Alcohol/Water
60/40 Isopropyl Alcohol/Water
80/20 Acetone/Water
60/40 Acetone/Water
80/20 Ethanol/Water
60/40 Ethanol/Water

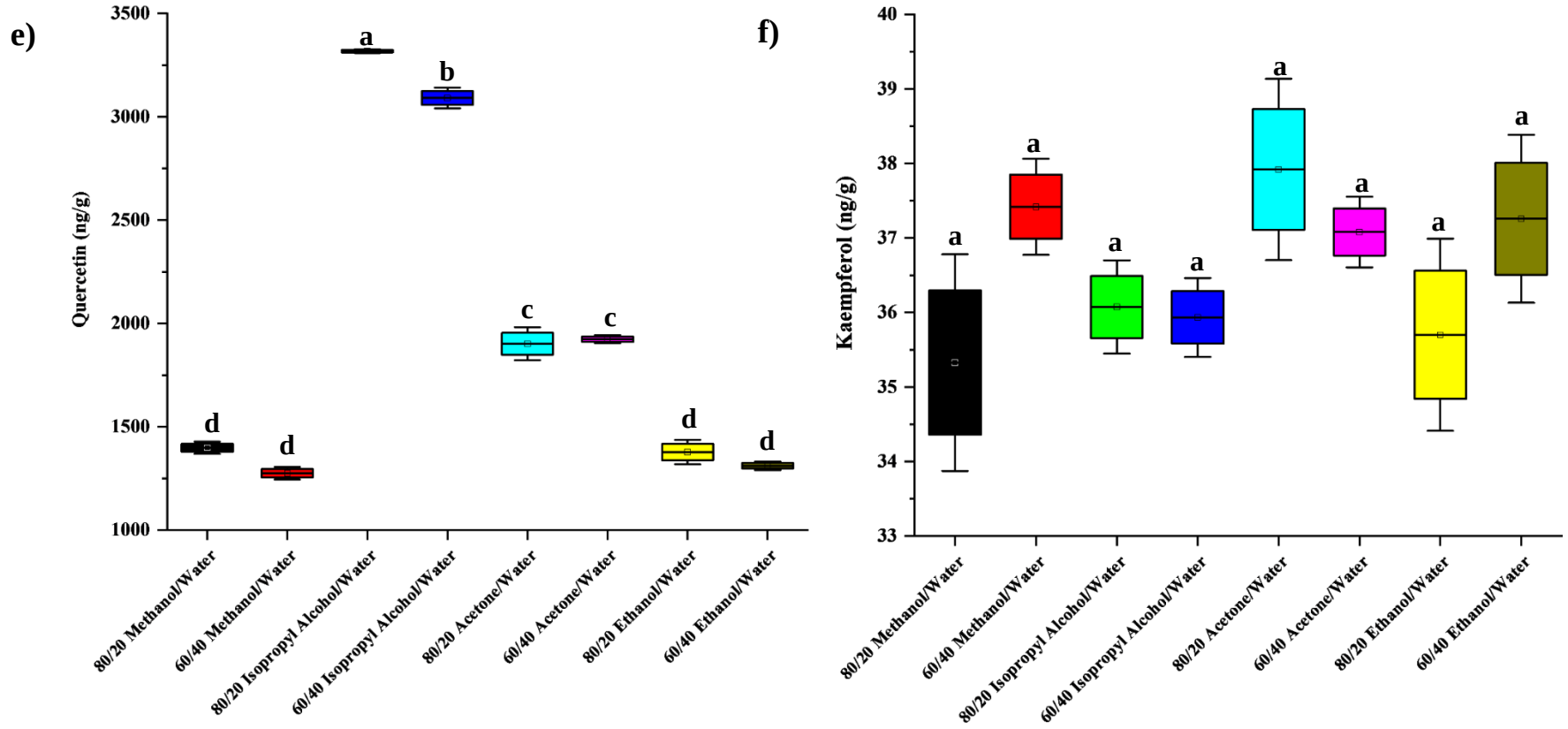


Figure 3.S2 Extracted amounts of afzelin, caffeic acid, chlorogenic acid, herbacetin, kaempferol and quercetin obtained from the different solvent-water combinations; methanol/water (60/40, V/V), methanol/water (80/20, V/V), acetone/water (60/40, V/V), acetone/water (80/20, V/V), ethanol/water (60/40, V/V), ethanol/water (80/20, V/V), isopropanol/water (60/40, V/V) and isopropanol/water (80/20, V/V) in pooled berry matrix. a) Recovered afzelin concentrations; b) Recovered caffeic acid concentrations; c) Recovered chlorogenic acid concentrations; d) Recovered herbacetin concentrations; e) Recovered kaempferol concentrations; and f)

Recovered quercetin concentrations. The line within the box plots shows the mean recovery percentage. The box plots in each graph contain different letters if there is a difference ($p<0.05$) in the mean values of the recovery percentages based on Tukey's test.

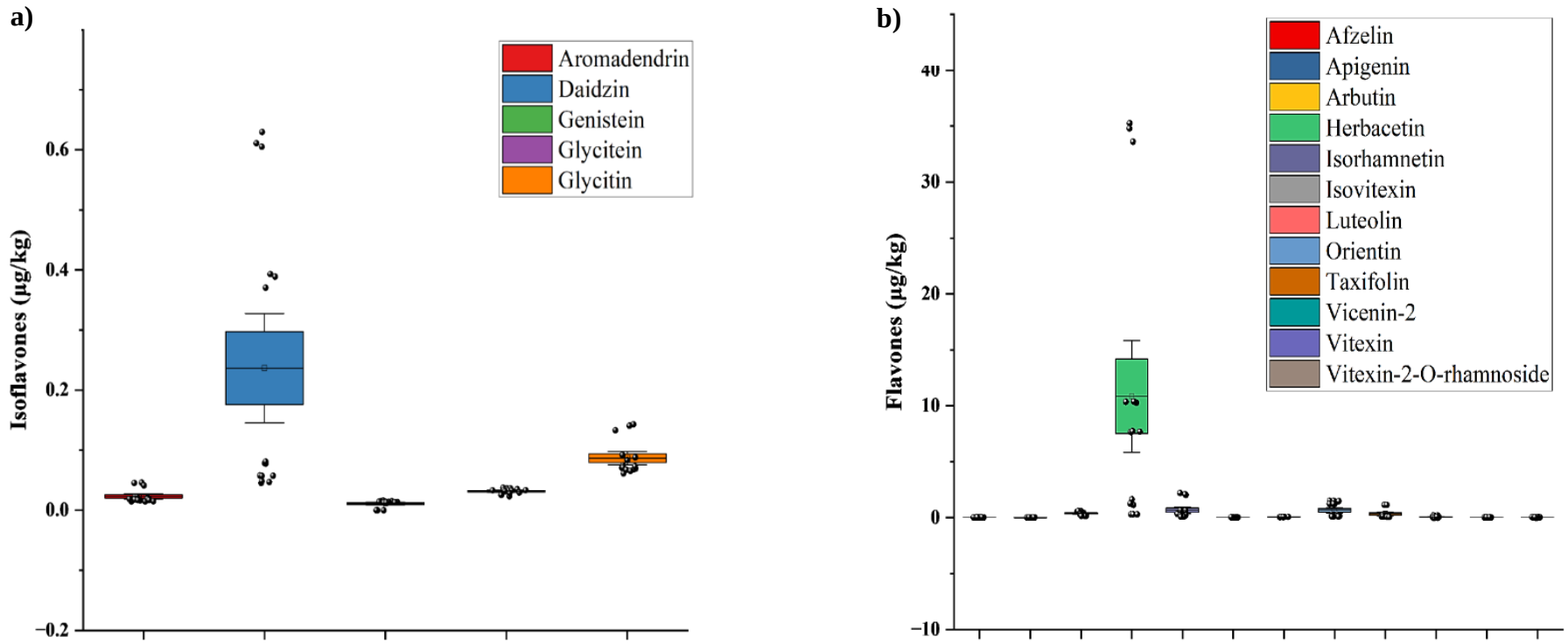


Figure 3.S3 Box plots graphs showing the total contents of different classes of phenolic compounds in berry samples. ; a) Isoflavones; b) Flavones. The horizontal line in each box plot shows the mean content of each phenolic compound in berries.

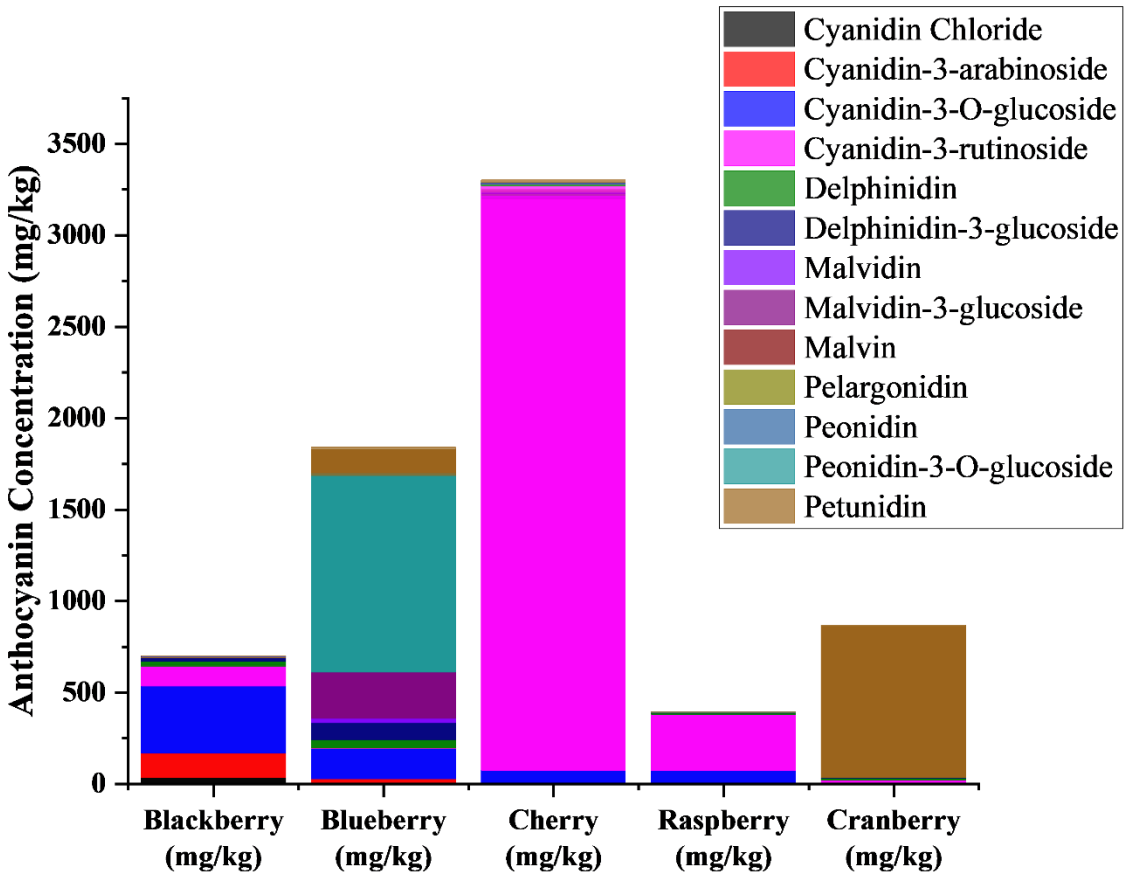
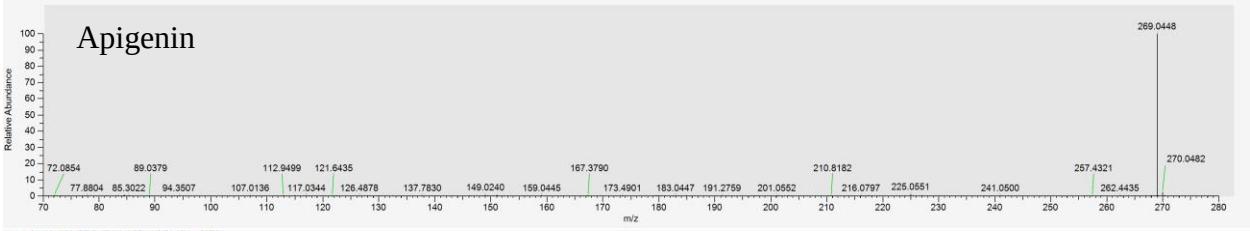
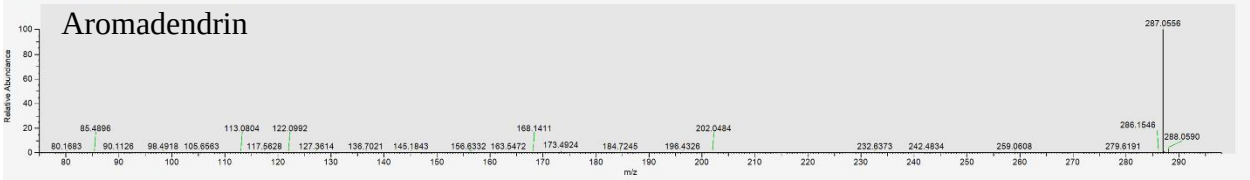


Figure 3.S4 The graph shows the differences in the distribution of anthocyanin profiles of raspberry, blueberry, cherry, blackberry and cranberry

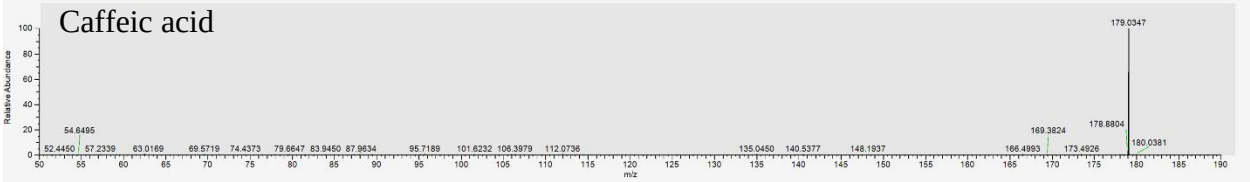
Apigenin #80 RT: 0.61 AV: 1 SB: 1 3.54 NL: 2.85E6
T: FTMS - p ESI Full ms2 269.0449@cid25.00 [70.0000-280.0000]



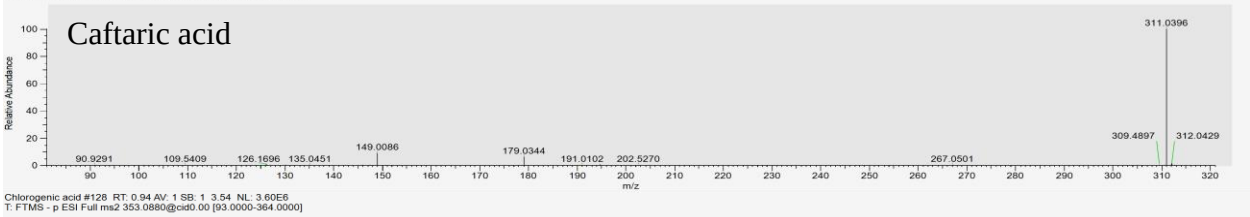
Aromadendrin #21 RT: 0.17 AV: 1 SB: 1 3.54 NL: 1.28E6
T: FTMS - p ESI Full ms2 287.0559@cid0.00 [75.0000-298.0000]



Caffeic acid #117 RT: 0.87 AV: 1 SB: 1 3.54 NL: 3.93E6
T: FTMS - p ESI Full ms2 179.0350@cid0.00 [50.0000-190.0000]



Caftaric Acid #1198 RT: 7.52 AV: 1 SB: 1 3.54 NL: 3.05E6
T: FTMS - p ESI Full ms2 311.0000@cid15.00 [81.0000-321.0000]



Chlorogenic acid #128 RT: 0.94 AV: 1 SB: 1 3.54 NL: 3.60E6
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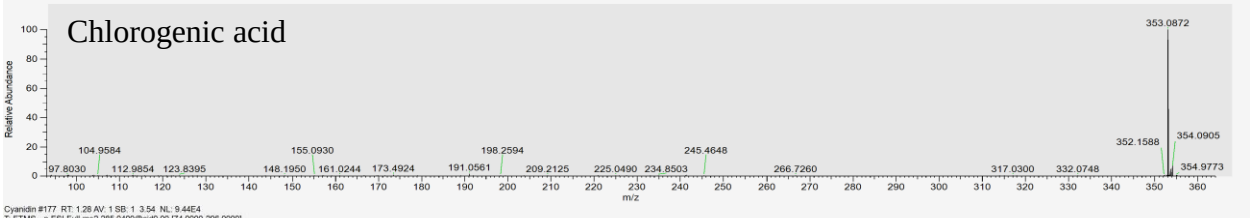

Cyanidin #177 RT: 1.28 AV: 1 SB: 1 3.54 NL: 9.44E4
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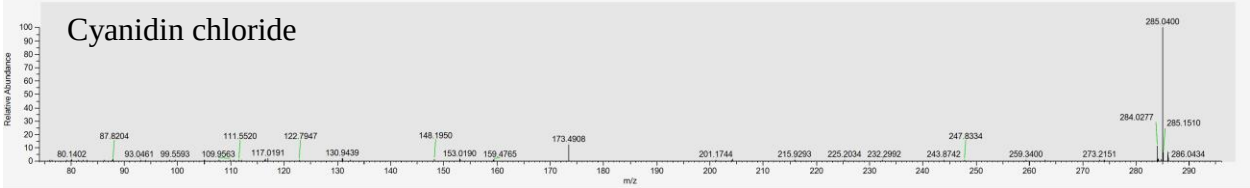
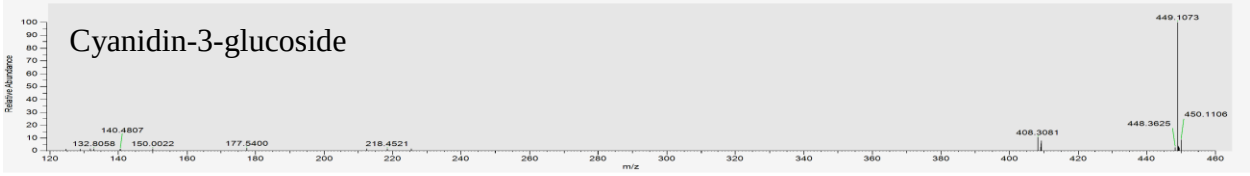


Cyanidin-3-glucoside #270 RT: 1.19 AV: 1 SB: 1 3.54 NL: 0
T: FTMS + p ESI Full ms2 449.1077@cid0.00 [119.0000-460.0000]



Cyanidin-3-o-arabinoside #58 RT: 0.24 AV: 1 SB: 1 3.54 NL: 8.85E4
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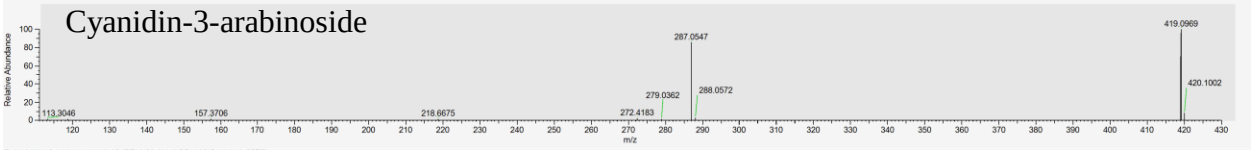

Delphinidin-3-o-glucoside #112 RT: 0.82 AV: 1 SB: 1 3.54 NL: 1.57E5
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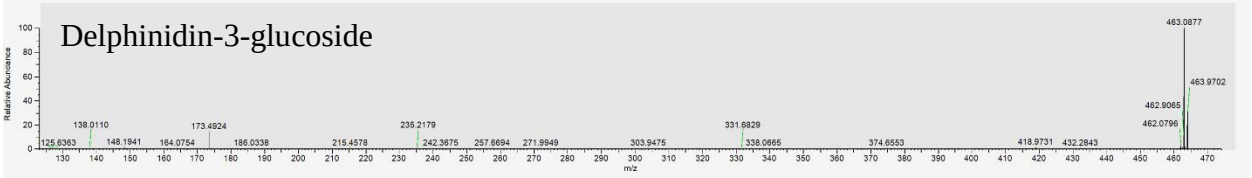

Ellagic acid #112 RT: 0.76 AV: 1 SB: 1 3.54 NL: 1.58E7
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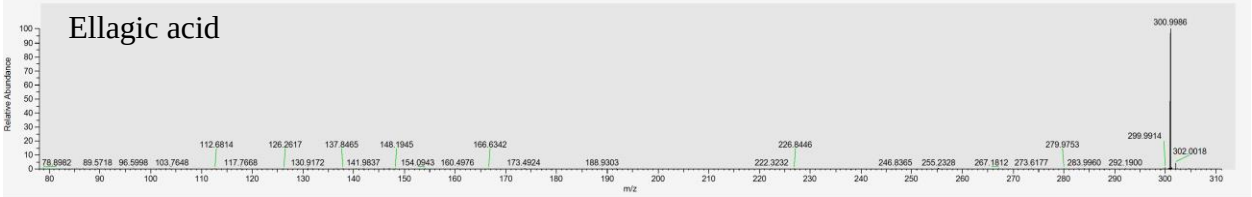

Epigallocatechin #95 RT: 0.71 AV: 1 SB: 1 3.54 NL: 2.05E5
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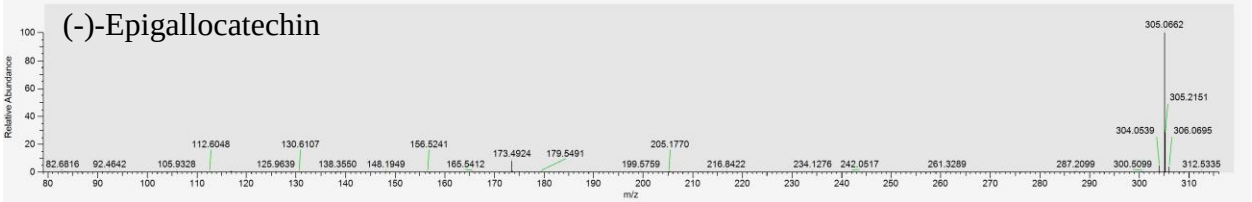

Fisetin #199 RT: 1.45 AV: 1 SB: 1 3.54 NL: 4.38E5
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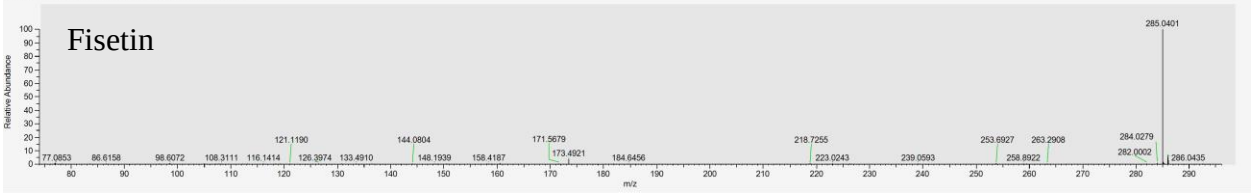

Gallic acid #127 RT: 0.93 AV: 1 SB: 1 3.54 NL: 2.65E5
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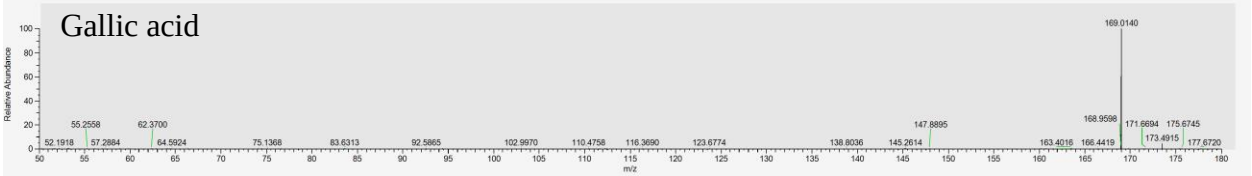

Genestein #407 RT: 2.95 AV: 1 SB: 1 3.54 NL: 8.17E5
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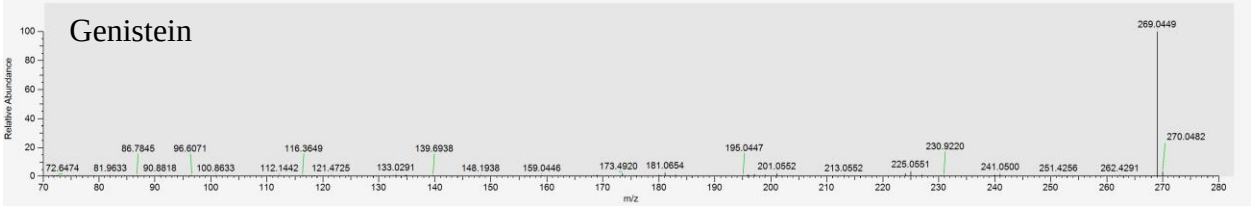

Herbacetin #95 RT: 0.68 AV: 1 SB: 1 3.54 NL: 2.63E5
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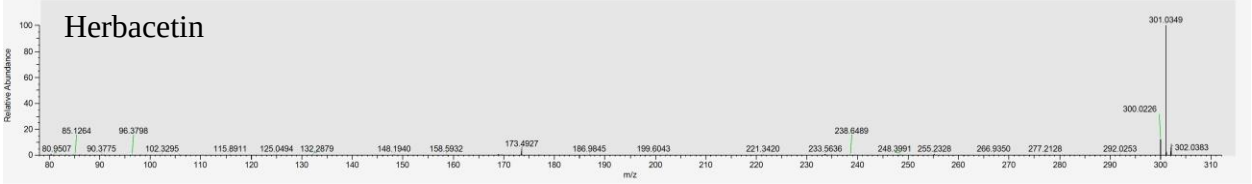

Isoquercetin #200 RT: 1.44 AV: 1 SB: 1 3.54 NL: 1.06E5
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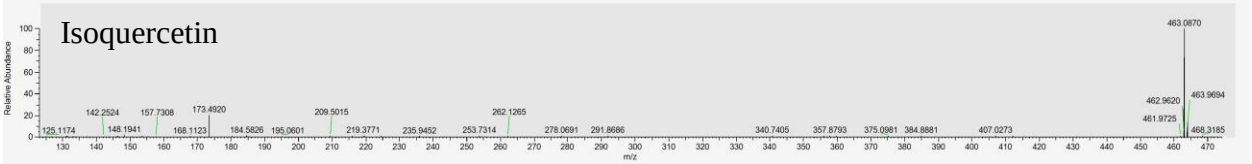

Isorhamnetin #75 RT: 0.55 AV: 1 SB: 1 3.54 NL: 3.71E5
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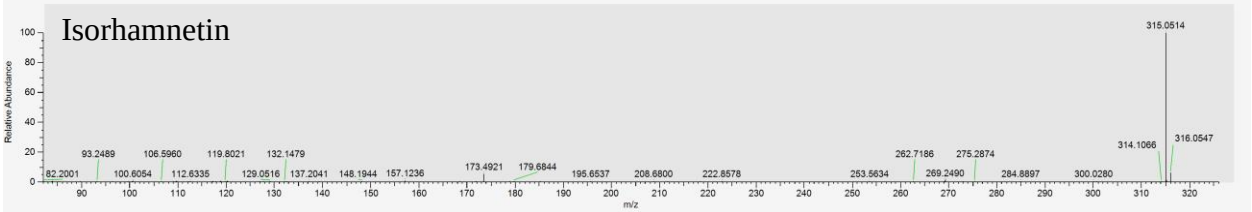

Isovitexin_20220804115239 #110 RT: 0.78 AV: 1 SB: 1 3.54 NL: 2.63E5
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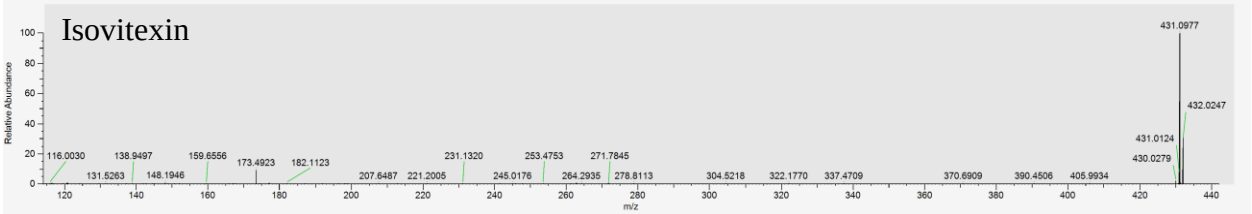

Kaempferol #165 RT: 1.27 AV: 1 SB: 1 3.54 NL: 2.67E6
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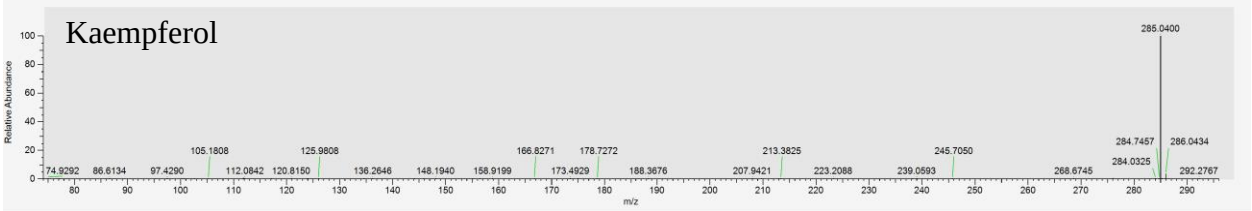

Kaempferol-3-glucoside #94 RT: 0.65 AV: 1 SB: 1 3.54 NL: 3.38E5
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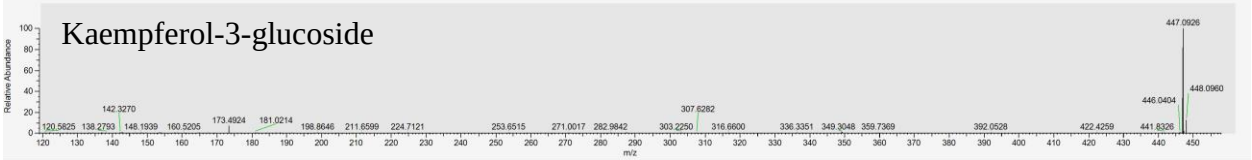

Liquiritigenin #70 RT: 0.53 AV: 1 SB: 1 3.54 NL: 1.02E6
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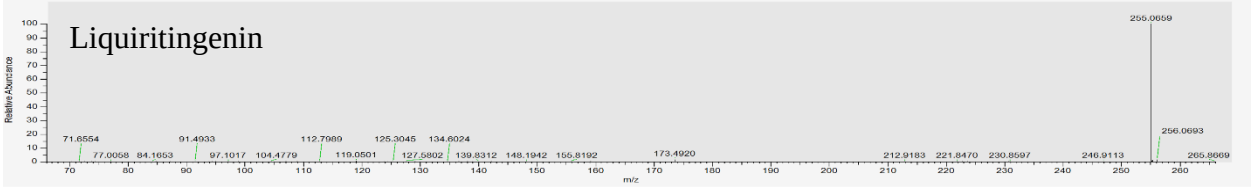

Luteolin #814 RT: 5.86 AV: 1 SB: 1 3.54 NL: 6.25E5
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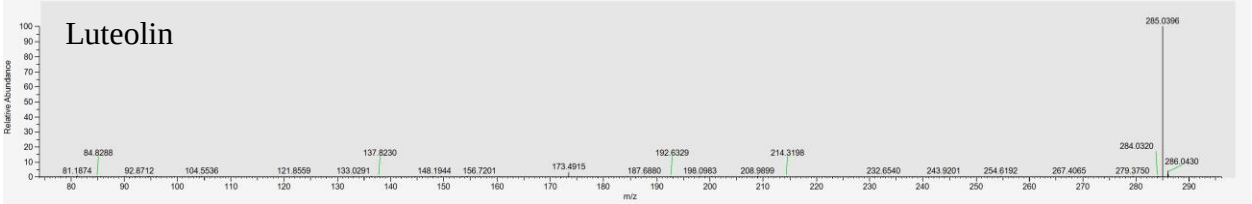
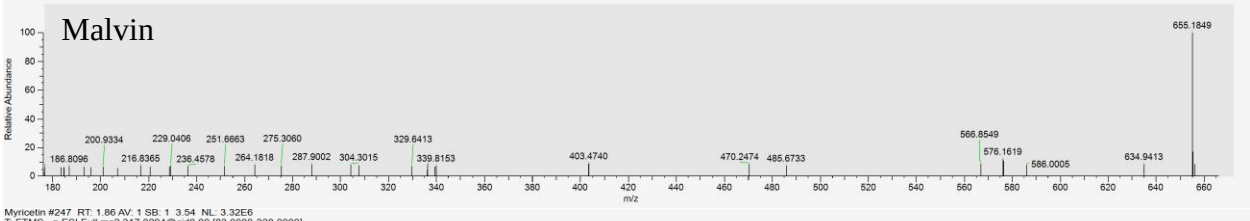


Malvin #437 RT: 3.22 AV: 1 SB: 1 3.54 NL: 1.01E4
T: FTMS + p ESI Full ms2 655.1854@cid5.00 [176.0000-666.0000]



Myricetin #247 RT: 1.86 AV: 1 SB: 1 3.54 NL: 3.32E6
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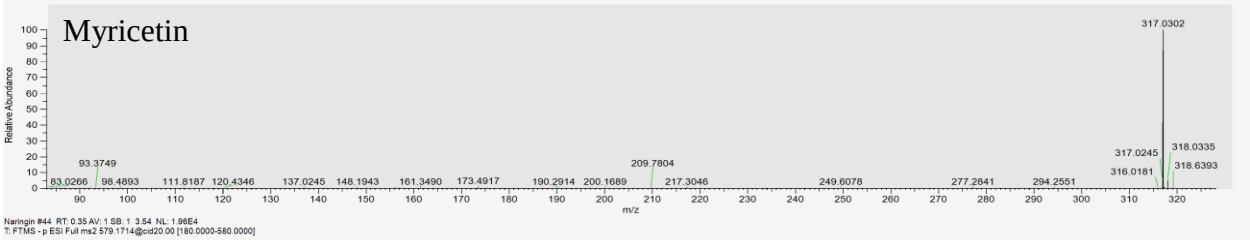
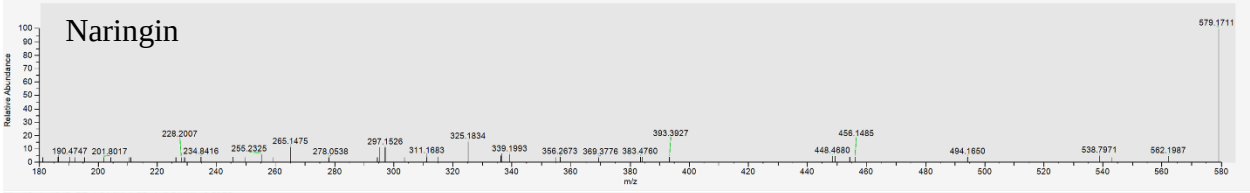


Naringin #44 RT: 0.35 AV: 1 SB: 1 3.54 NL: 1.98E4
T: FTMS - p ESI Full ms2 579.1714@cid20.00 [180.0000-580.0000]



Nicotiflorin #247 RT: 1.84 AV: 1 SB: 1 3.54 NL: 6.53E5
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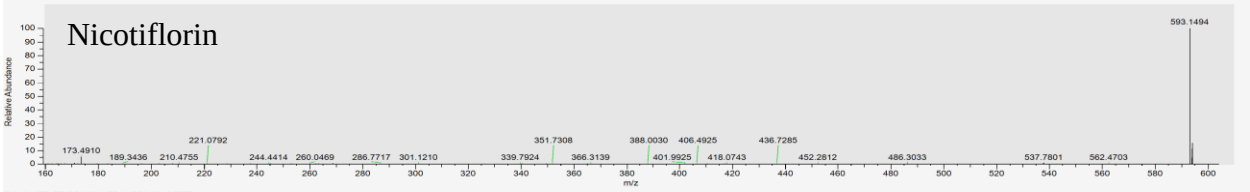

Orientin #73 RT: 0.54 AV: 1 SB: 1 3.54 NL: 1.88E5
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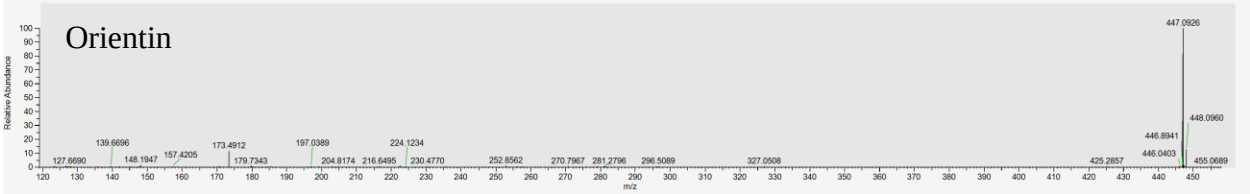
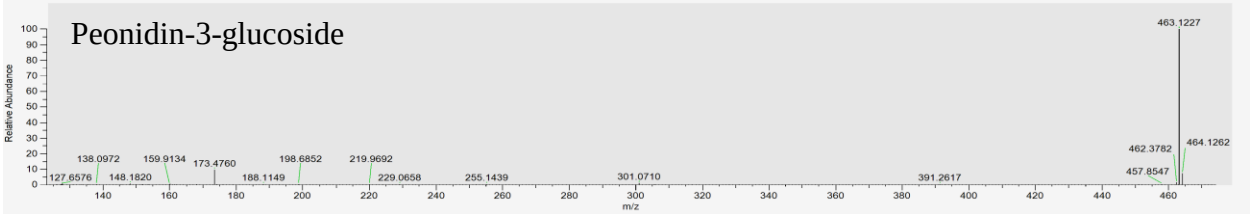
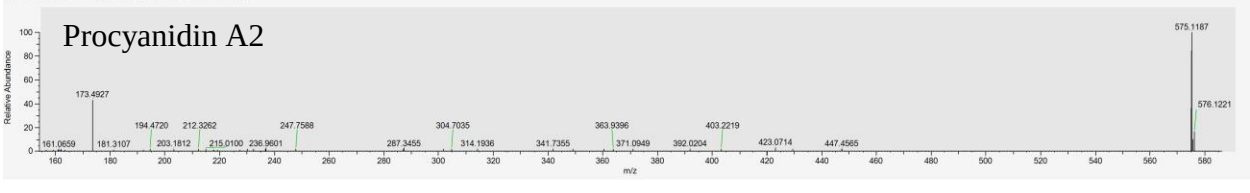


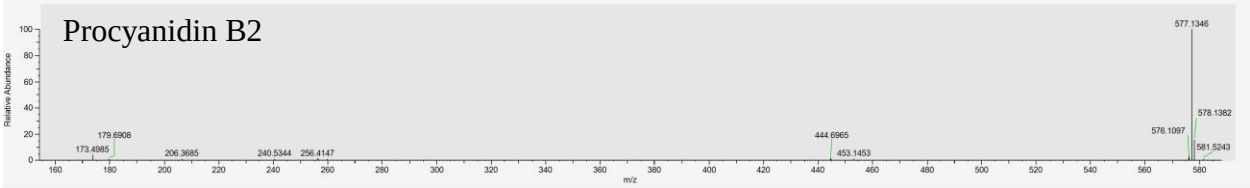
Peonidin-3-o-glucoside #79 RT: 0.56 AV: 1 SB: 1 3.54 NL: 2.63E5
T: FTMS + p ESI Full ms2 463.1227@cid0.00 [123.0000-474.0000]



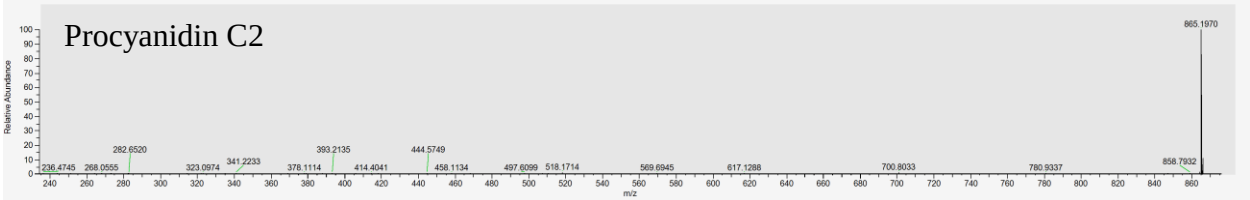
Procyanidin A2 #76 RT: 0.58 AV: 1 SB: 1 3.54 NL: 5.87E4
T: FTMS - p ESI Full ms2 575.1190@cid0.00 [154.0000-586.0000]



Procyanidin B3 #152 RT: 0.67 AV: 1 SB: 1 3.54 NL: 1.74E5
T: FTMS - p ESI Full ms2 577.1350@cid0.00 [154.0000-588.0000]



Procyanidin C1 #74 RT: 0.56 AV: 1 SB: 1 3.54 NL: 1.81E5
T: FTMS - p ESI Full ms2 865.1973@cid0.00 [234.0000-876.0000]



Protocatechuic acid #137 RT: 1.17 AV: 1 SB: 1 3.54 NL: 3.06E5
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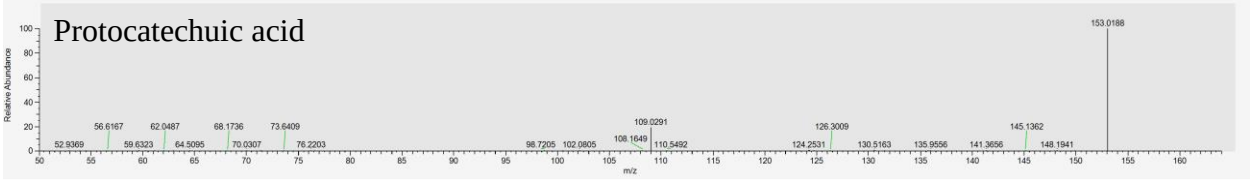
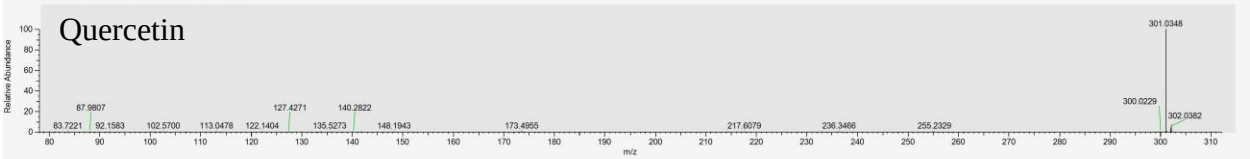


Quercetin #186 RT: 0.97 AV: 1 SB: 1 3.54 NL: 2.16E6
T: FTMS - p ESI Full ms2 301.0349@cid0.00 [78.0000-312.0000]



Quercetin-3-galactoside #75 RT: 0.55 AV: 1 SB: 1 3.54 NL: 2.34E5
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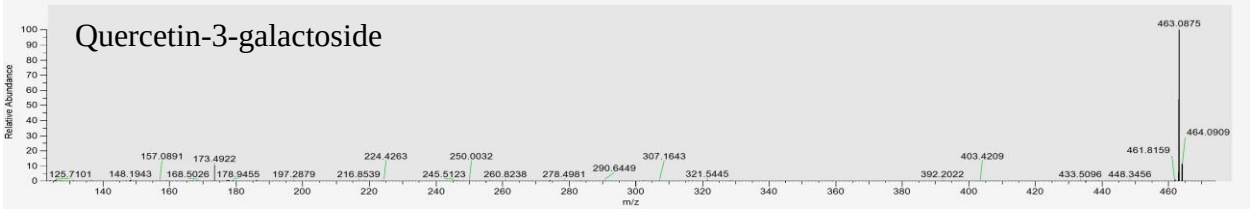
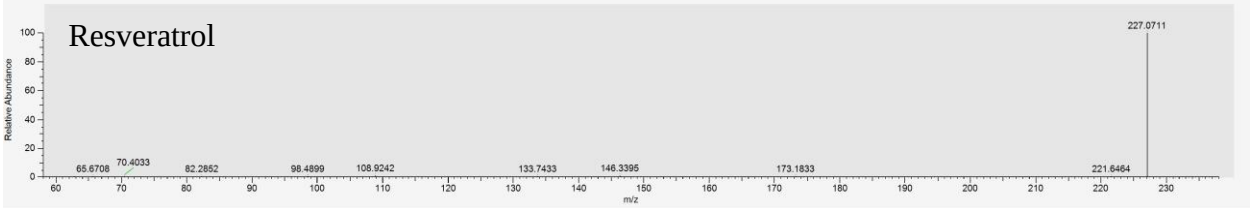
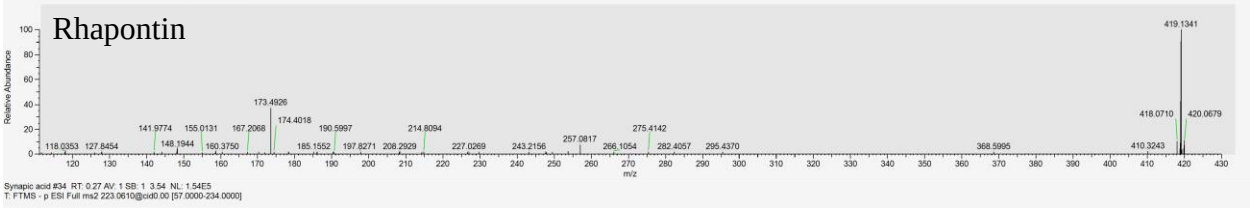


Resveratrol_20220803112723 #234 RT: 0.94 AV: 1 SB: 1 3.54 NL: 3.28E5
T: FTMS - p ESI Full ms2 227.0110@cid0.00 [58.0000-238.0000]



Rhapontin redo #79 RT: 0.56 AV: 1 SB: 1 3.54 NL: 5.00E4
T: FTMS - p ESI Full ms2 419.1345@cid0.00 [111.0000-430.0000]



Synapic acid #34 RT: 0.27 AV: 1 SB: 1 3.54 NL: 1.54E5
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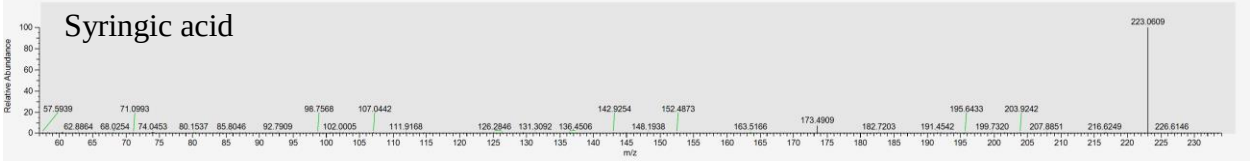


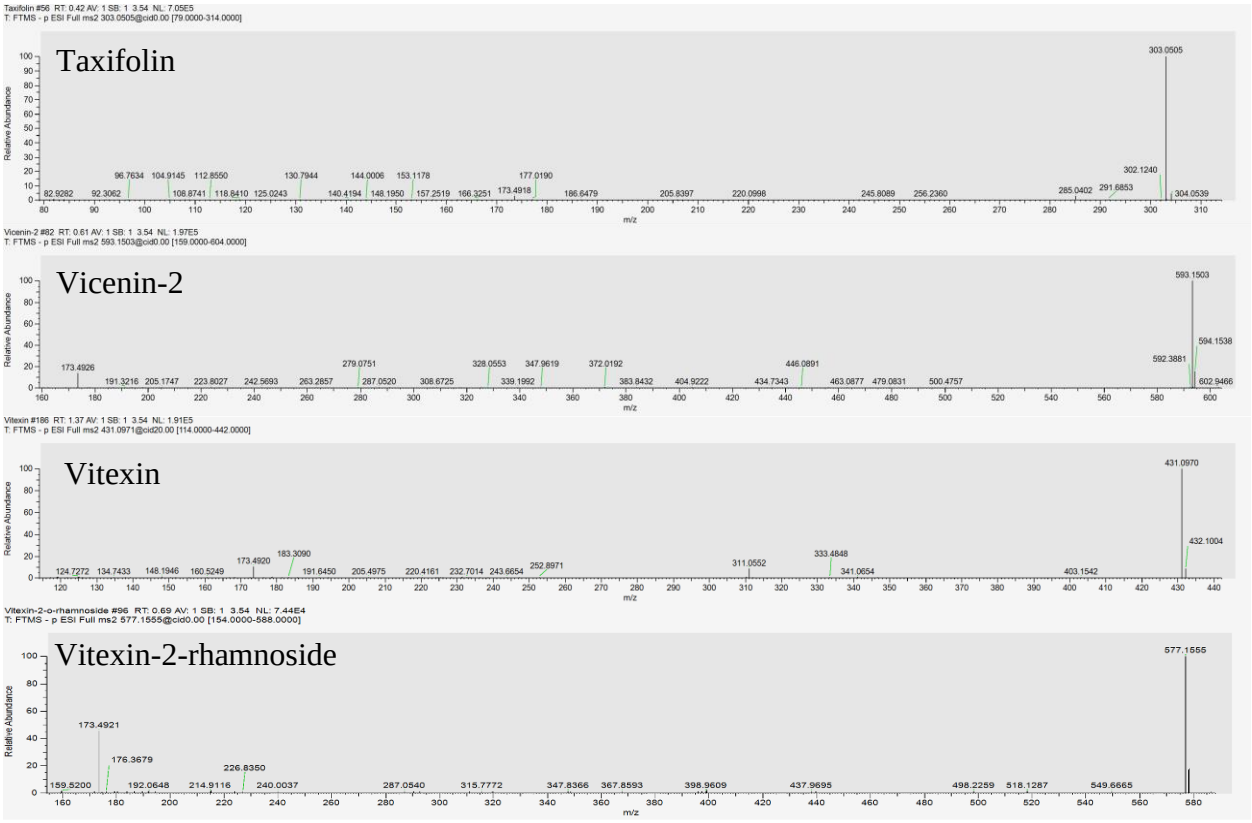


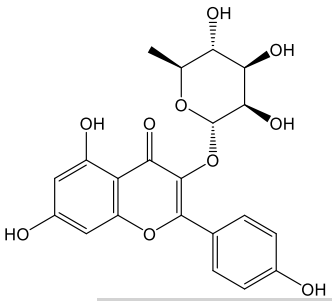
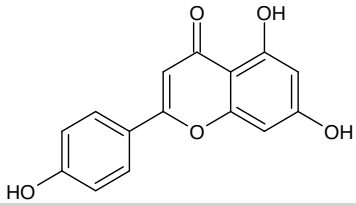
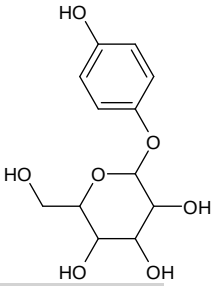
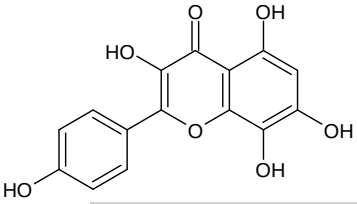
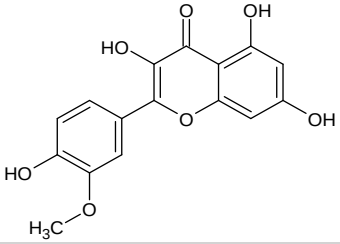
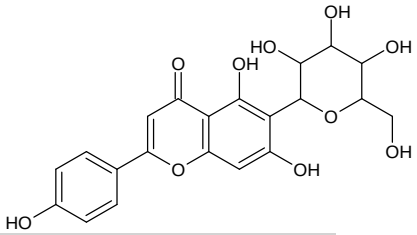
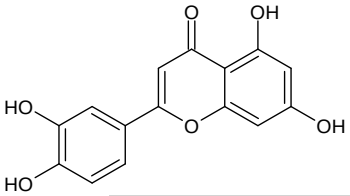
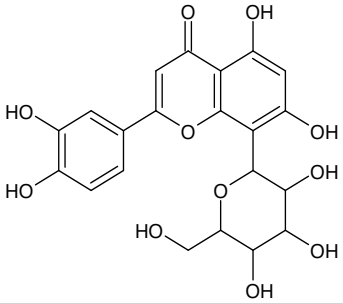
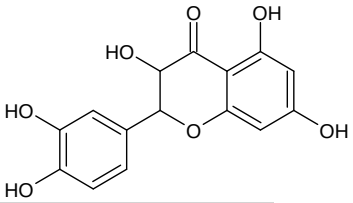
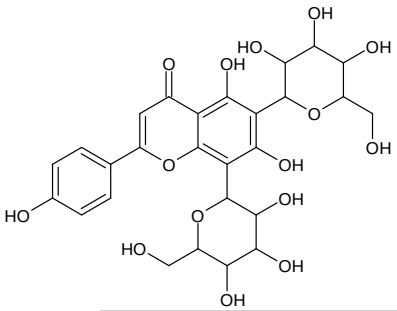
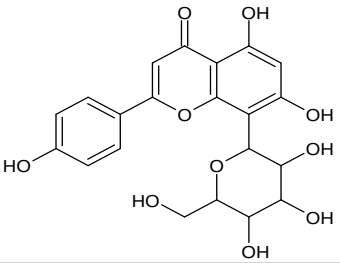
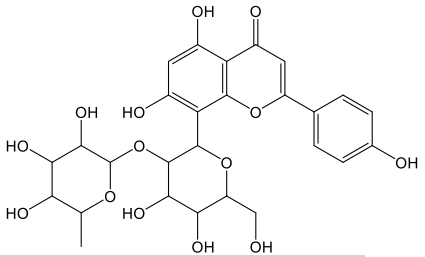
Figure 3.S5 The MS spectra of the phenolic compounds analyzed with UHPLC-HRMS

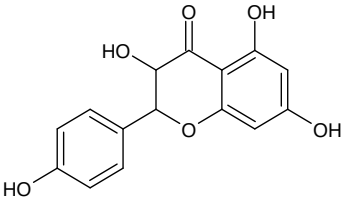
Table 3.S1 The responsible eigenvectors of the Principal Component Analysis

Variable	PC1	PC2	PC3	PC4	PC5
Afzelin	0.130	-0.065	-0.121	-0.158	-0.073
Apigenin	0.053	0.187	-0.105	-0.061	-0.036
Arbutin	-0.148	0.133	0.098	-0.077	-0.054
Aromadendrin	0.025	0.228	0.000	-0.039	0.046
Caffeic acid	-0.189	0.091	-0.031	-0.069	-0.012
Caftaric acid	0.118	-0.078	-0.181	-0.117	0.034
(+)-Catechin	0.187	-0.040	-0.043	-0.131	0.031
Chlorogenic acid	-0.125	0.153	-0.078	-0.121	-0.006
Daidzein	-0.199	-0.070	-0.038	-0.044	-0.009
Daidzin	0.036	0.107	0.003	0.278	-0.013
Ellagic acid	0.072	-0.066	0.235	0.076	-0.016
(-)-Epicatechin	0.183	-0.019	-0.017	-0.159	0.013
(-)-Epigallocatechin	-0.198	-0.074	-0.041	-0.039	-0.007
Ferulic acid	-0.188	0.021	0.119	0.044	-0.035
Fisetin	0.119	-0.040	0.049	0.252	-0.016
Gallic acid	-0.166	-0.119	0.091	-0.044	-0.023
Genistein	0.194	0.078	0.056	0.027	0.029
Glycitein	-0.148	0.049	-0.147	0.124	0.020
Glycitin	0.056	-0.042	0.256	-0.020	0.005
Herbacetin	0.064	-0.014	0.258	-0.031	-0.028
Hesperetin	-0.002	0.230	-0.020	-0.049	-0.025
Isoquercetin	-0.162	-0.063	0.108	-0.140	-0.029
Isorhamnetin	-0.208	-0.018	-0.017	-0.056	-0.019
Isovitexin	0.138	-0.051	-0.119	-0.174	-0.050
Kaempferol	0.108	-0.001	0.074	0.259	0.087
Kaempferol-3-glucoside	0.116	-0.103	0.091	-0.198	0.001
Liquiritingenin	0.072	-0.064	0.204	0.157	-0.014
Luteolin	0.119	-0.040	0.049	0.252	0.005
Myricetin	-0.157	0.092	0.089	-0.139	-0.031
Naringin	0.132	-0.096	-0.121	0.159	0.029
Nicotiflorin	0.004	0.233	-0.010	-0.014	-0.011
Okanin	0.047	0.221	0.035	-0.054	-0.037
Orientin	0.115	-0.115	0.077	-0.197	-0.004
Para-coumaric acid	0.017	0.229	-0.042	-0.027	0.002
Piceatannol	-0.064	0.021	0.088	-0.078	0.971
Polydatin	-0.001	0.230	-0.025	-0.047	-0.011
Procyanidin A2	0.095	0.060	0.214	-0.107	-0.014
Procyanidin B2	0.151	-0.099	0.110	-0.125	-0.011
Procyanidin C2	0.116	-0.076	-0.180	-0.126	0.033
Protocatechuic acid	-0.021	0.219	0.056	-0.085	-0.013
Quercetin	0.061	-0.039	0.255	-0.031	-0.033
Quercetin-3-galactoside	-0.162	-0.063	0.108	-0.140	-0.029
Rhapontin	0.002	0.232	-0.020	-0.022	-0.005
Rutin	0.025	0.192	0.123	-0.105	-0.032
Sinapic acid	-0.020	0.042	0.001	0.311	0.018
Syringic acid	-0.079	0.187	0.122	0.003	-0.021
Taxifolin	-0.025	0.231	-0.021	-0.019	-0.007
Vanillic acid	0.030	0.197	0.139	-0.026	-0.028
Vicenin-2	0.018	0.210	-0.003	-0.136	0.019

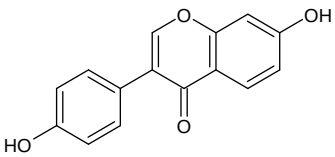
Vitexin	0.131	-0.049	-0.127	-0.164	-0.076
Vitexin-2-rhamnoside	0.132	0.183	0.010	0.009	0.019
Cyanidin	0.064	-0.041	0.248	-0.070	-0.029
Cyanidin-3-arabinoside	0.021	-0.063	0.238	-0.122	-0.032
Cyanidin-3-glucoside	-0.033	-0.054	0.248	-0.094	-0.042
Cyanidin-3-rutinoside	0.003	0.233	-0.015	-0.019	-0.018
Delphinidin	-0.163	-0.103	0.068	-0.126	-0.026
Delphinidin-3-glucoside	-0.199	-0.066	0.000	-0.070	-0.016
Malvidin	-0.198	-0.072	-0.038	-0.047	-0.009
Malvidin-3-glucoside	-0.199	-0.070	-0.038	-0.044	-0.009
Malvin	-0.196	-0.079	-0.038	0.041	-0.008
Pelargonidin	0.156	-0.032	-0.006	-0.212	0.027
Peonidin	0.130	-0.007	-0.161	-0.165	0.042
Peonidin-3-glucoside	-0.199	-0.069	-0.038	-0.044	-0.010
Petunidin	0.083	-0.094	-0.196	-0.127	0.034

Table 3.S2 Chemical structures of the phenolic compounds used in the UHPLC-HRMS analysis

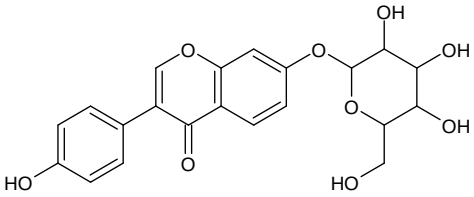
Flavonoids		
Flavones		
		
Afzelin	Apigenin	Arbutin
		
Herbacetin	Isorhamnetin	Isovitexin
		
Luteolin	Orientin	Taxifolin
		
Vicenin-2	Vitexin	Vitexin-2-rhamnoside
Isoflavones		



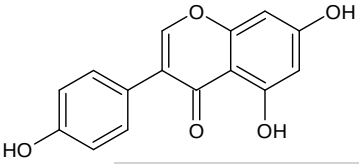
Aromadendrin



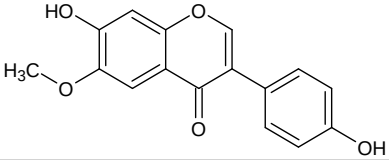
Daidzein



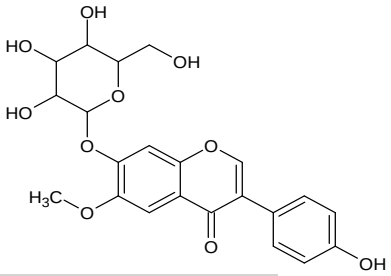
Daidzin



Genistein

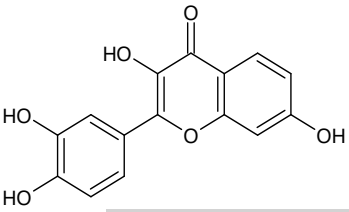


Glycitein

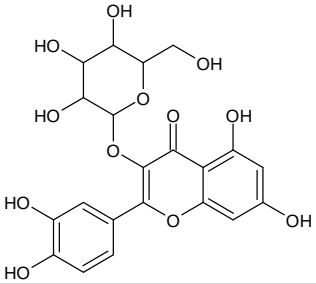


Glycitin

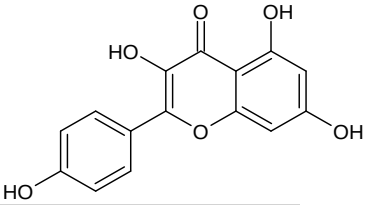
Flavonols



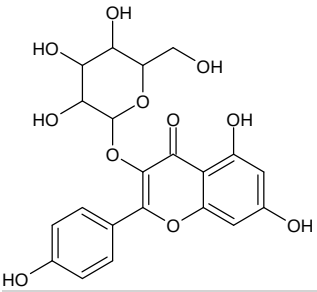
Fisetin



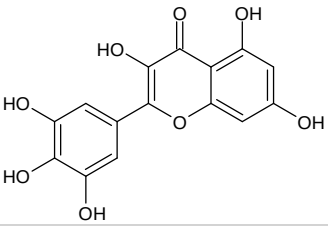
Isoquercetin



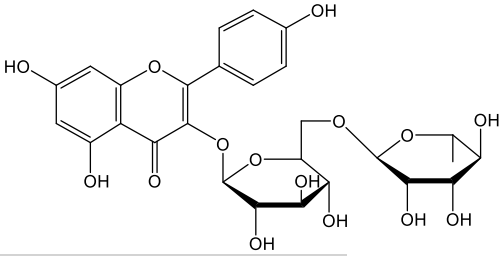
Kaempferol



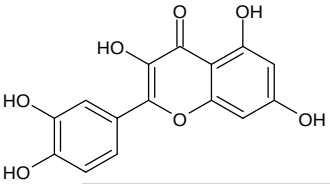
Kaempferol-3-glucoside



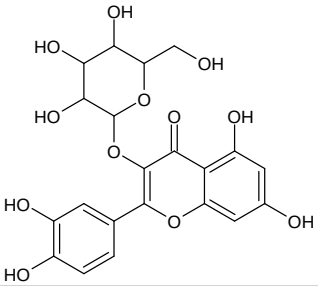
Myricetin



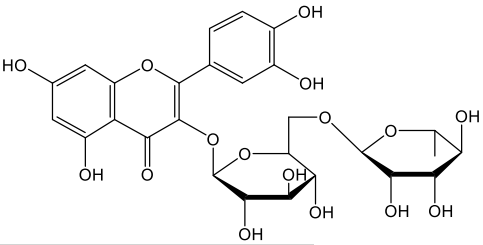
Nicotiflorin



Quercetin

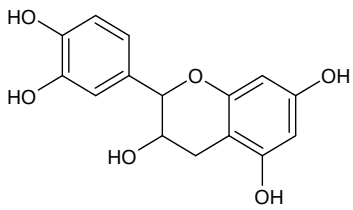


Quercetin-3-galactoside

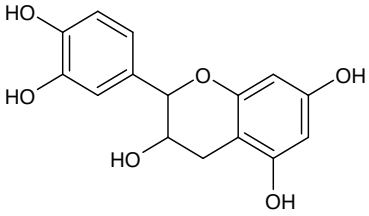


Rutin

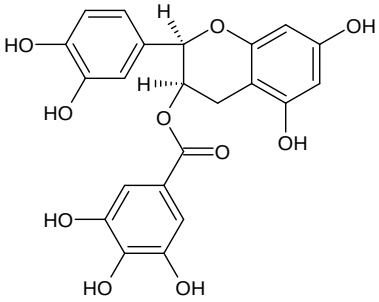
Flavans



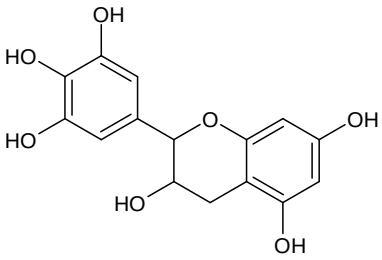
(+)-Catechin



(-)-Epicatechin

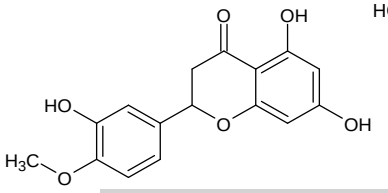


(-)-Epicatechingallate

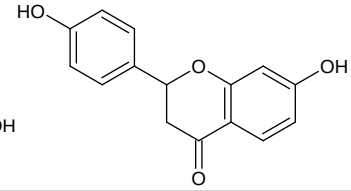


(-)-Epigallocatechin

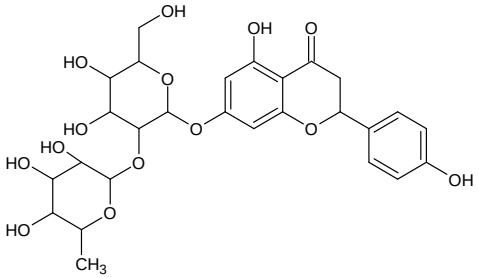
Flavanones



Hesperetin

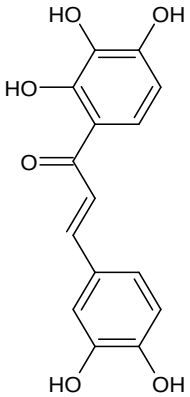


Liquiritingenin



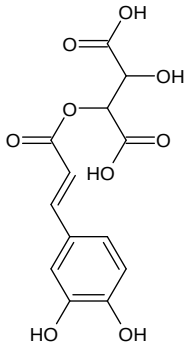
Naringin

Chalcones



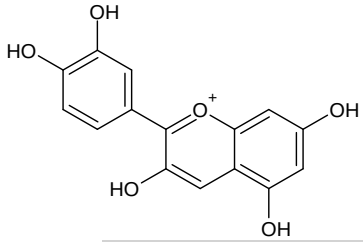
Okanin

Non-flavonoid phenolic compounds

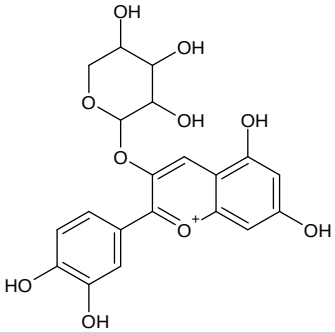


Caftaric Acid

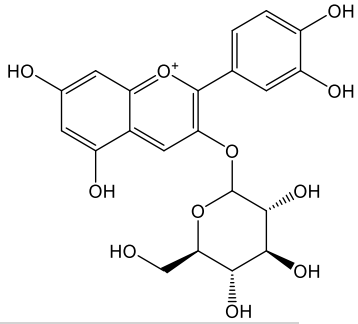
Anthocyanins



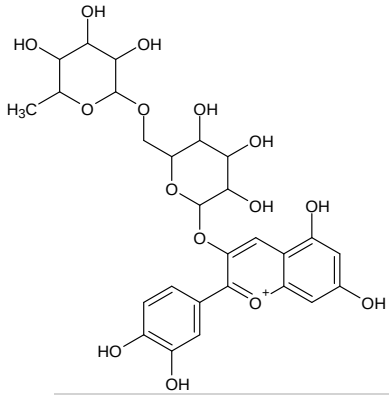
Cyanidin



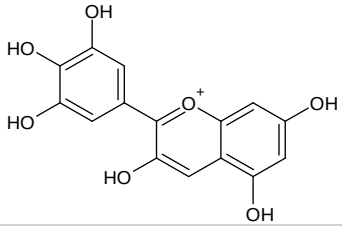
Cyanadin-3-arabinoside



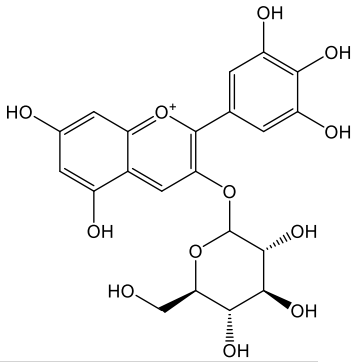
Cyanidin-3-glucoside



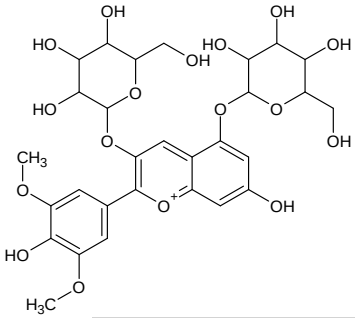
Cyanidin-3-rutinoside



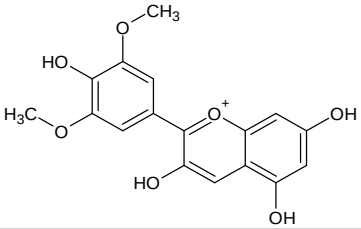
Delphinidin



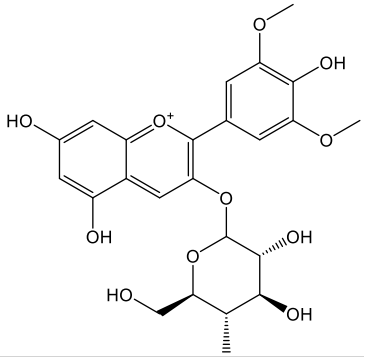
Delphinidin-3-glucoside



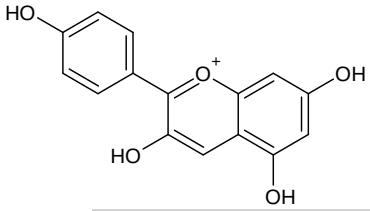
Malvin



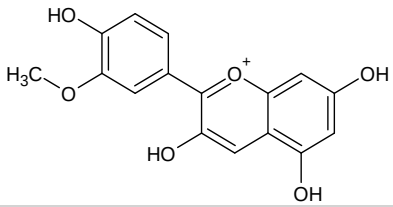
Malvidin



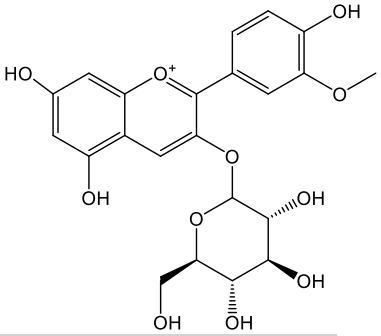
Oenin/ Malvidin-3-glucoside



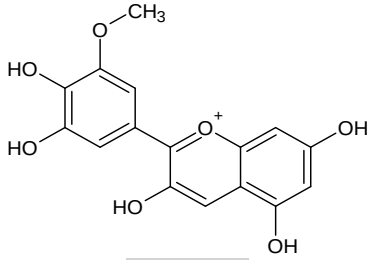
Pelargonidin



Peonidin

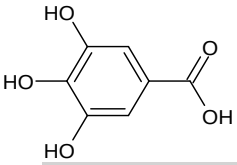


Peonidin-3-glucoside

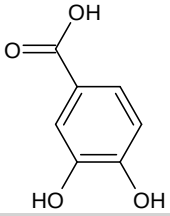


Petunidin

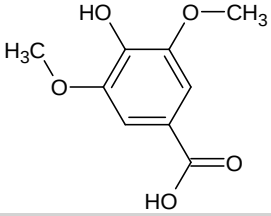
Hydroxybenzoic acids



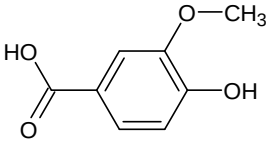
Gallic acid



Protocatechuic acid

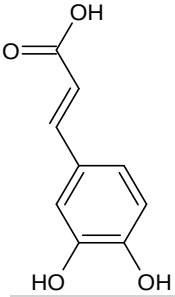


Syringic acid

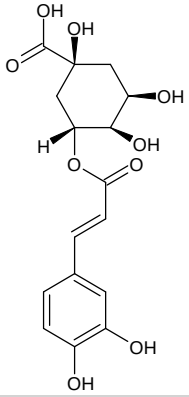


Vanillic acid

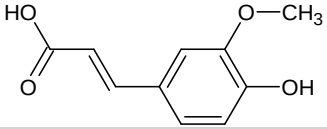
Hydroxycinnamic acids



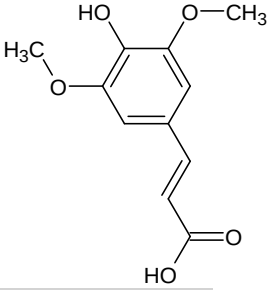
Caffeic acid



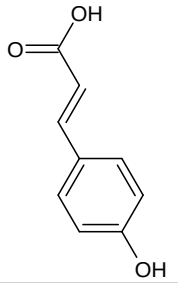
Chlorogenic acid



Ferulic acid

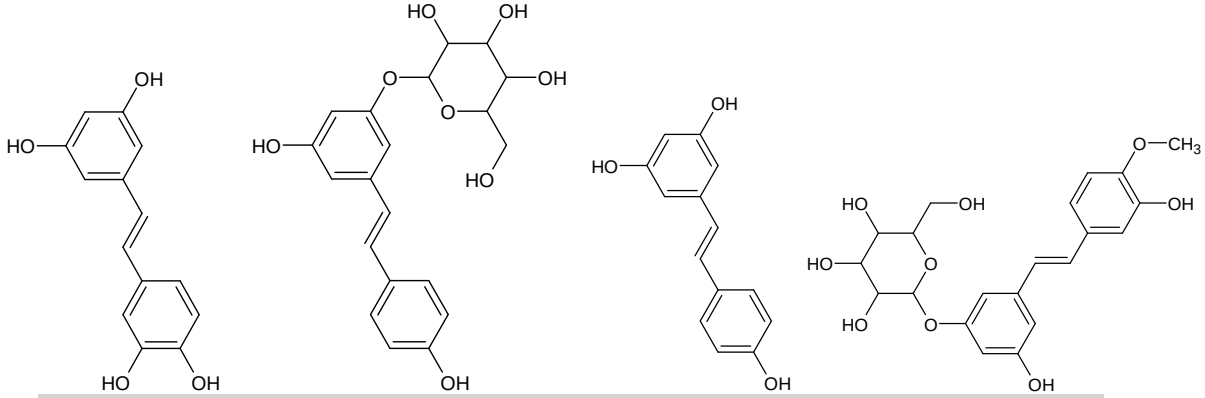


Sinapic acid



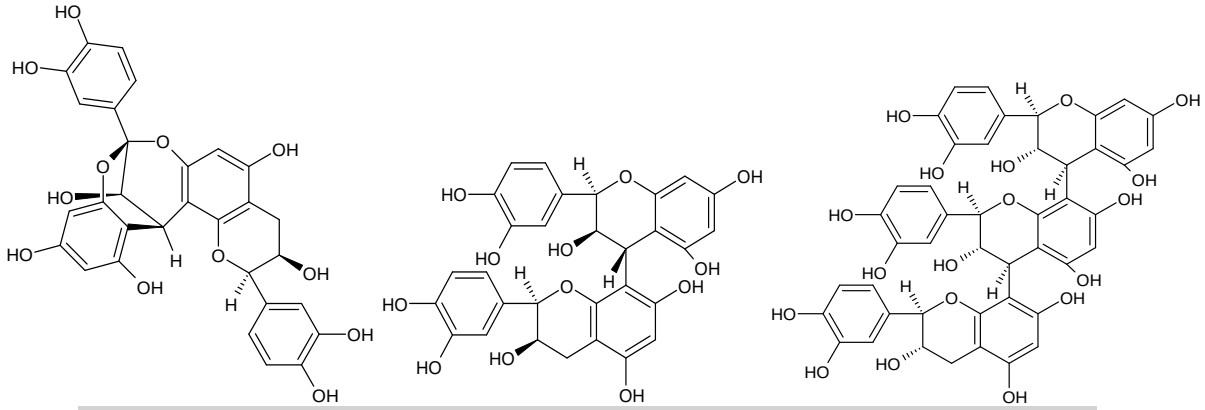
p-coumaric acid

Stilbenes



Piceatannol Polydatin Resveratrol Rhapontin

Procyanidins



Procyanidin A2 Procyanidin B2 Procyanidin C2

Figure 4.S1 Canadian small fruits collected from Manitoba for the phenolic compounds analaysis

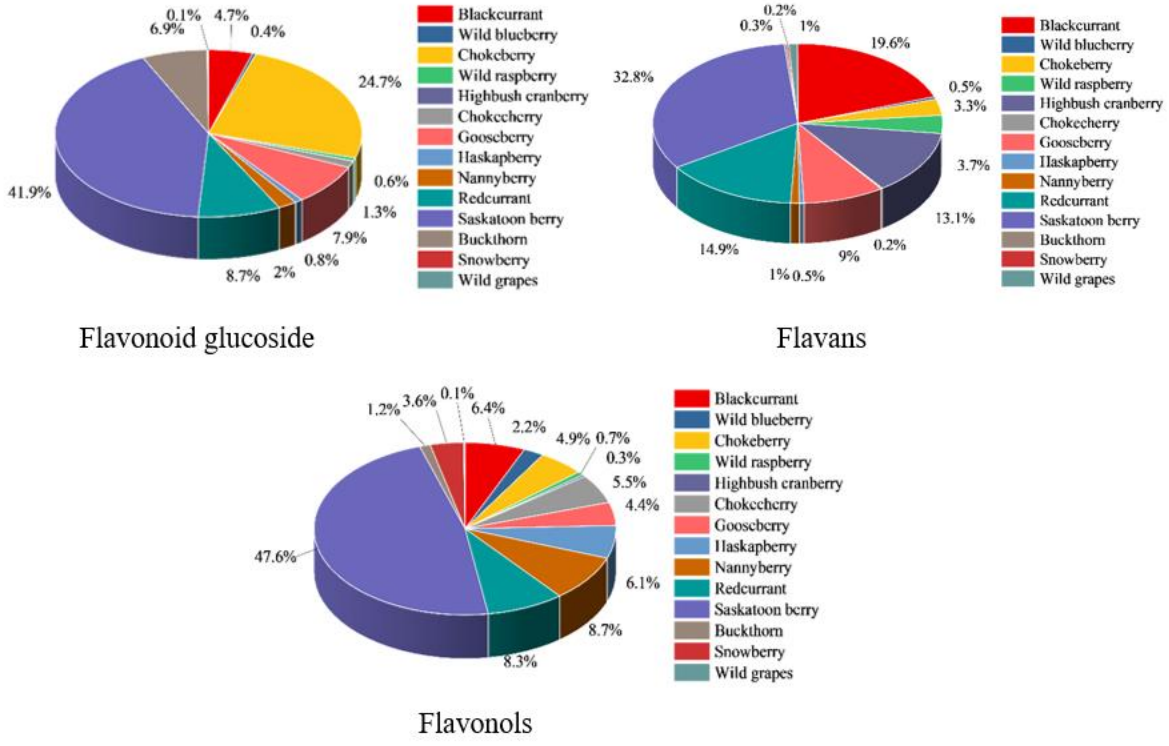


Figure 4.S2 The distribution of different phenolic compound classes in different Canadian small fruits; a) Flavonoid glucoside, b) flavans, and c) flavonols

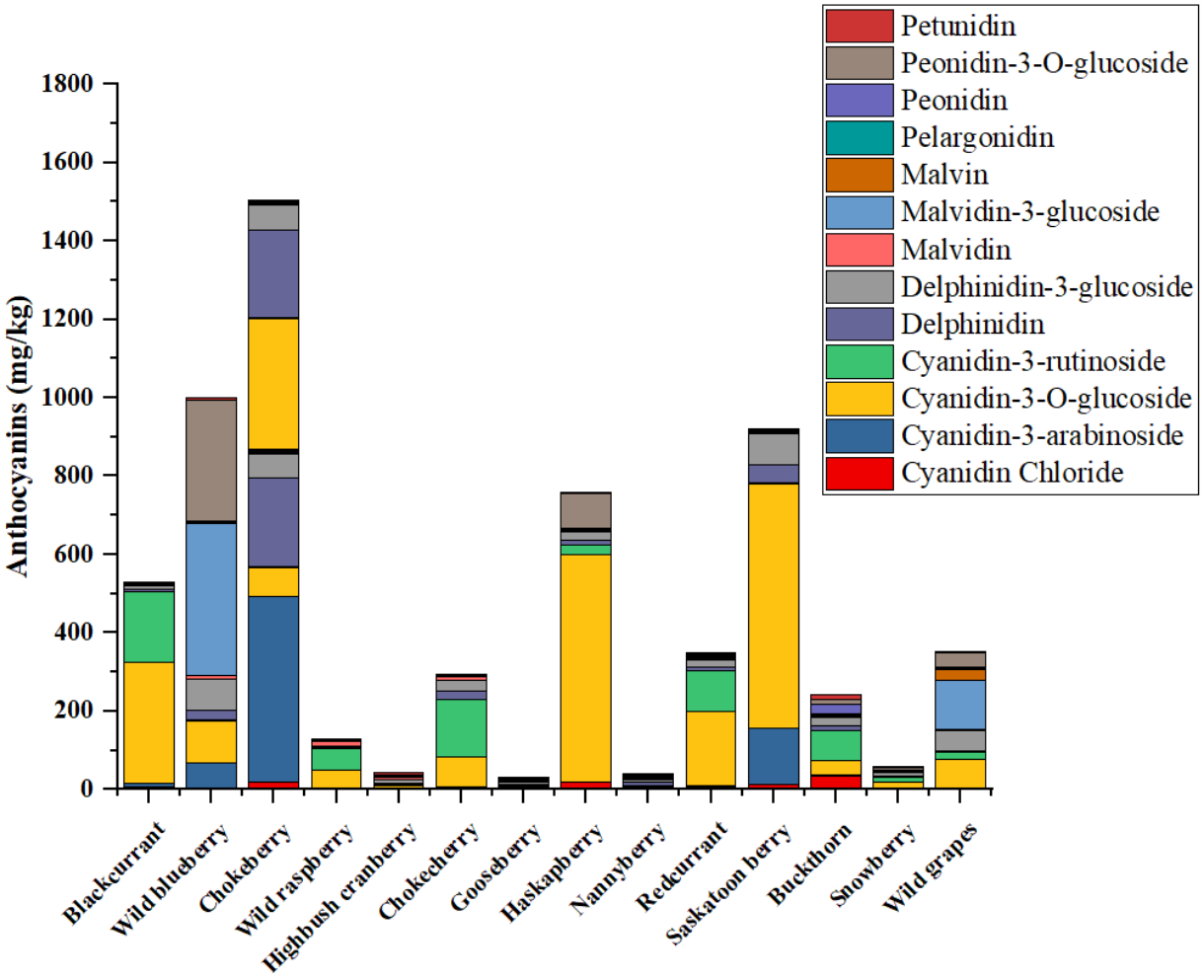


Figure 4.S3 The anthocyanin profile of Canadian small fruits

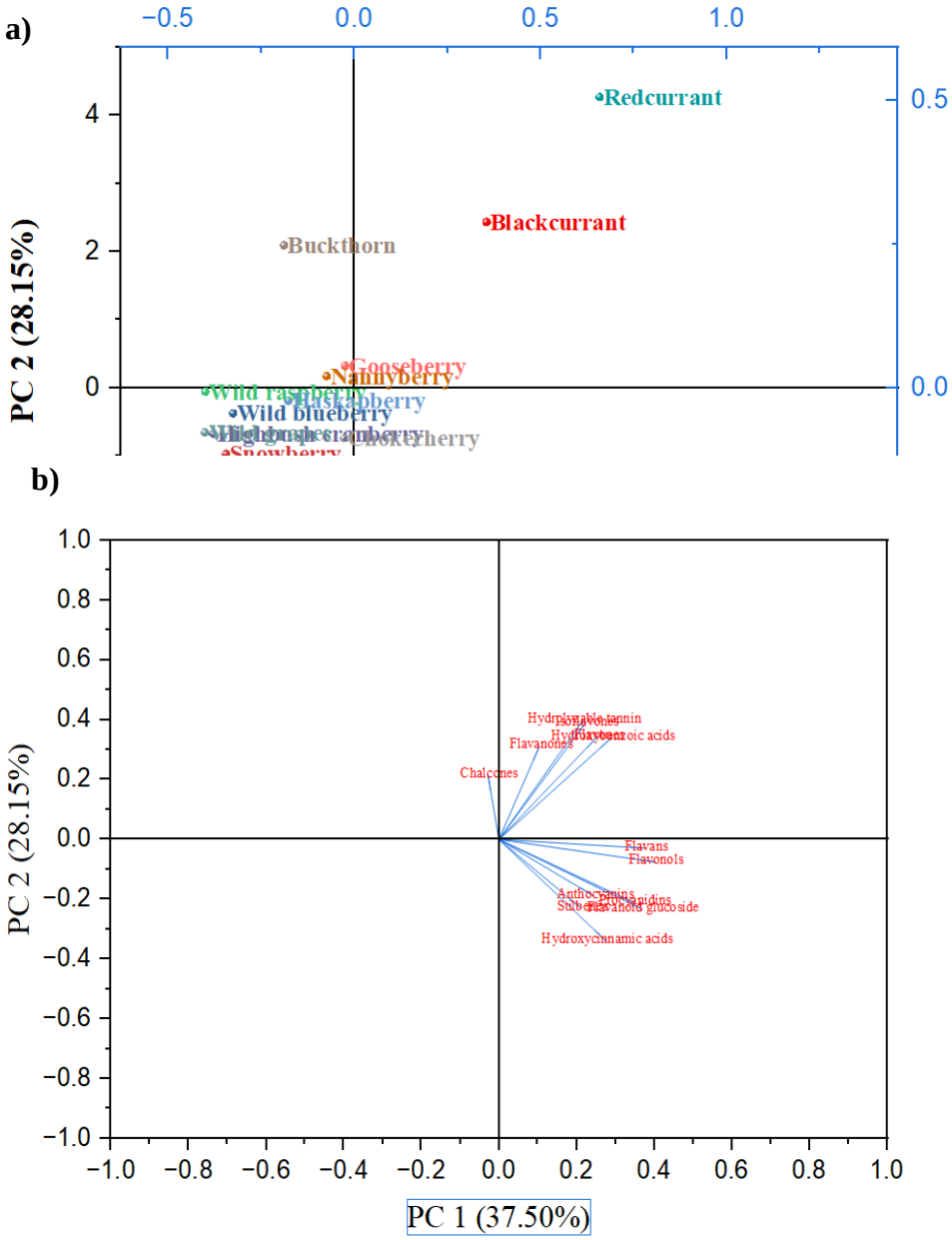


Figure 4.S4 Principal component analysis of small fruits based on their phenolic compounds contents; a) bi-plot and b) loading plot

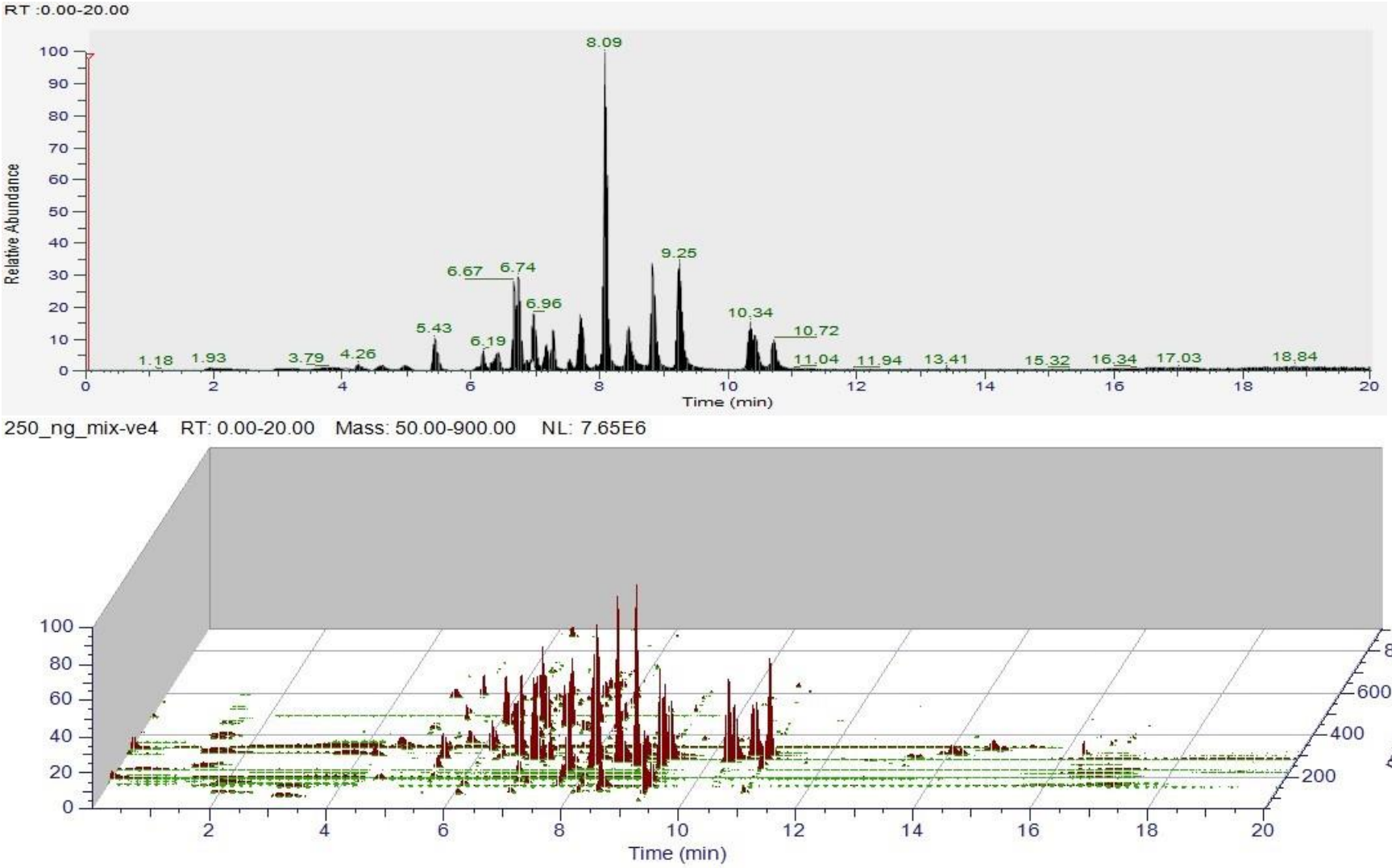


Figure 4.S5 The UHPLC-HRMS chromatogram (chromatogram and map view) of the analyzed 66 phenolic compounds in Canadian wild berries

Table 4.S1. Phenolic compounds' parent and fragment masses used in the current study

Sl. No.	Retention time (min)	Compound Name	Ionization mode	Accurate molecular mass	Precursor m/z	Fragments m/z*
1	8.10	Afzelin[3]	Negative	432.1062	431.0984	285.0401
2	10.33	Apigenin[1]	Negative	269.0456	269.0456	225.0551, 201.0551, 149.0239
3	1.82	Arbutin[3]	Negative	272.0902	271.0823	161.0452, 203.9364
4	8.07	Aromadendrin[3]	Negative	288.0639	287.0561	259.0608, 243.0659
5	4.93	Caffeic Acid[1]	Negative	180.0428	179.035	135.0449
6	3.80	Caftaric Acid[1]	Negative	312.0487	311.0409	179.0344, 149.0086
7	5.43	(+)Catechin[1]	Negative	290.0785	289.0718	245.0811, 205.0499, 173.4917, 179.0344
8	4.68	Chlorogenic Acid[1]	Negative	354.0956	353.0878	191.0559
9	4.50	Cyanidin Chloride[3]	Positive	287.055	285.040	246.9308, 213.0542, 259.0598
10	5.49	Cyanidin-3-arabinoside[1]	Positive	419.0973	419.0973	284.0325
11	5.08	Cyanidin-3-O-glucoside[1]	Positive	449.1078	449.1078	287.0547
12	4.93	Cyanidin-3-rutinoside[1]	Positive	595.1658	595.1658	287.054
13	8.78	Daidzein[1]	Negative	254.0585	253.0506	227.0698, 199.0748
14	6.17	Daidzin[1]	Negative	416.1113	415.1035	255.0647, 338.3414
15	6.73	Delphinidin[1]	Positive	303.0499	303.0499	257.044, 229.0492
16	3.00	Delphinidin-3-glucoside[1]	Positive	465.1033	465.1033	337.0562, 301.0352, 418.9732
17	6.69	Ellagic Acid[2]	Negative	302.0068	300.999	257.0089, 229.014, 185.0242
18	5.48	(-)Epicatechin[1]	Negative	290.0796	289.0718	245.0815, 205.0502
19	7.13	(-)Epicatechin Gallate[1]	Negative	442.0895	441.0827	289.0712, 169.0318
20	3.56	(-)Epigallocatechin[1]	Negative	306.0734	305.0667	179.0347, 221.0453
21	6.76	Ferulic Acid[2]	Negative	194.0585	193.0506	149.0603
22	10.39	Fisetin[3]	Negative	286.0483	285.0405	163.0034, 135.0085, 257.0452, 241.0503
23	2.20	Gallic Acid[1]	Negative	170.0221	169.0143	125.0241

24	10.31	Genistein[1]	Negative	270.0534	269.0456	159.0446, 133.0291, 225.0551, 241.0503
25	6.27	Glycitein[3]	Negative	284.0684	283.0606	351.0483
26	6.24	Glycitin[3]	Negative	446.1219	445.114	283.0612, 282.053
27	8.90	Herbacetin[3]	Negative	302.0432	301.0354	272.0279
28	10.92	Hesperetin[3]	Negative	302.0796	301.0718	286.0483
29	6.79	Isoquercetin[1]	Negative	464.096	463.0882	301.0346
30	10.68	Isorhamnetin[3]	Negative	316.0589	315.051	315.0513, 300.0278
31	8.04	Isovitexin[1]	Negative	431.0984	431.0984	311.0556, 341.0659
32	10.49	Kaempferol[1]	Negative	286.0483	285.0405	151.0034, 257.0452
33	7.27	Kaempferol-3-glucoside[3]	Negative	448.1011	447.0933	284.0323, 285.0402, 327.0808
34	9.23	Liquiritingenin[3]	Negative	256.0741	255.0663	135.0086
35	10.43	Luteolin[1]	Negative	286.0472	285.0405	241.0499, 199.0895, 175.0395, 157.0031
36	4.67	Malvidin[1]	Positive	331.0819	331.0819	315.0499, 316.0579, 299.0552, 287.0552
37	5.59	Malvidin-3-glucoside[1]	Positive	493.1326	493.1326	331.0802
38	4.71	Malvin[1]	Positive	655.1854	655.1854	493.1328, 331.0802
39	5.90	Myricetin[1]	Negative	318.037	317.0303	178.9982, 151.0033
40	7.69	Naringin[3]	Negative	580.1798	579.1719	459.114
41	5.50	Nicotiflorin[1]	Negative	594.159	593.1512	285.0397
42	7.29	Orientin[3]	Negative	448.1011	447.0933	327.0506, 357.061
43	8.03	Okanin[3]	Negative	288.0633	287.0561	135.0440
44	6.07	Para-coumaric Acid[1]	Negative	164.0479	163.0401	119.0499
45	10.29	Pelargonidin[3]	Positive	271.0594	271.0594	193.1306
46	8.78	Peonidin[3]	Positive	301.0712	301.0712	286.0468, 284.0321
47	5.57	Peonidin-3-O-glucoside[1]	Positive	463.1235	463.1235	301.0702
48	7.08	Petunidin[3]	Positive	317.0655	317.0655	302.042
40	7.76	Piceatannol[3]	Negative	244.0741	243.0663	225.0557, 201.0556
50	6.84	Polydatin[3]	Negative	390.132	389.1242	227.0712

51	7.44	Procyanidin A2[3]	Negative	576.1273	575.1195	423.0718, 449.0873, 289.0712
52	5.07	Procyanidin B2[3]	Negative	578.143	577.1352	425.0878, 407.0765, 289.0712
53	6.35	Procyanidin C2[3]	Negative	866.2064	865.1985	695.1401, 577.1348, 739.166, 713.166
54	3.24	Protocatechuic Acid[1]	Negative	154.0272	153.0193	109.0291
55	8.93	Quercetin[1]	Negative	302.0432	301.0354	151.0033
56	6.80	Quercetin-3-galactoside[1]	Negative	464.096	463.0882	301.0349, 300.0271
57	6.87	Resveratrol[1]	Negative	228.0792	227.0714	185.0605
58	7.72	Rhapontin[3]	Negative	420.1426	419.1348	257.0817
59	6.60	Rutin[1]	Negative	610.1528	609.1461	301.0349
60	6.93	Sinapic Acid[1]	Negative	224.069	223.0612	179.071, 164.0475
61	5.21	Syringic Acid[1]	Negative	198.0534	197.0456	182.0216, 153.0551, 138.0317
62	6.96	Taxifolin[3]	Negative	304.0589	303.051	285.04
63	6.09	Vanillic Acid[2]	Negative	168.0428	167.035	123.0446
64	5.49	Vicenin-2[3]	Negative	594.159	593.1512	473.1082, 503.1188, 353.066
65	8.11	Vitexin[1]	Negative	432.1062	431.0984	311.0552
66	6.43	Vitexin-2-O-rhamnoside[1]	Negative	578.163	577.1563	413.0871

All the fragment ions identified in the study were used for compound confirmations.

[1]Chemicals purchased from Toronto Research Chemicals (Toronto, Canada); [2]Chemicals purchased from Sigma-Aldrich (ON, Canada); [3]Chemicals purchased from Cayman Chemical (Michigan, USA)

[*] Fragments are listed in the order of observed intensities

Source: (Kodikara et al. 2023)

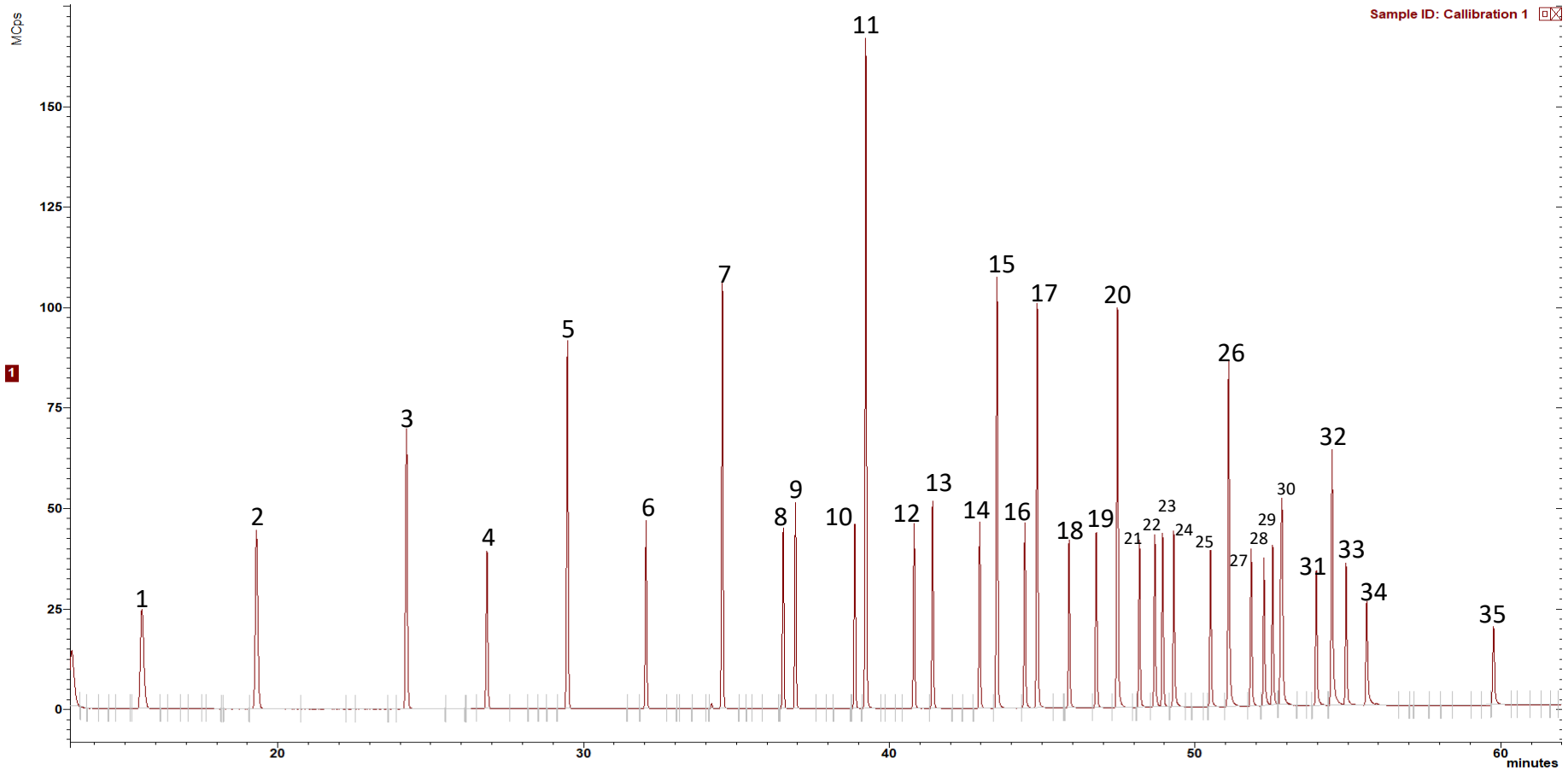


Figure 5.S1 The GC-MS chromatogram of tested fatty acid methyl esters, 1. Methyl hexanoate; 2. Methyl octanoate; 3. Methyl decanoate; 4. Methyl undecanoate; 5. Methyl laurate; 6. Methyl tri decanoate; 7. Methyl myristate; 8. Methyl myristate; 9. Methyl pentadecanoate; 10. Methyl cis-10-pentadecenoate; 11. Methyl palmitate; 12. Methyl palmitoleate; 13. Methyl heptadecanoate; 14. cis-10-Heptadecanoic acid methyl ester; 15. Methyl stearate; 16. trans-9-Elaidic acid methyl ester; 17. cis-9-Oleic acid methyl ester; 18. Methyl linolelaidate; 19. Methyl linoleate; 20. Methyl arachidate; 21. Methyl γ-linolenate; 22. Methyl cis-11-eicosenoate; 23. Methyl

linolenate; 24. Methyl heneicosanoate; 25. cis-11,14-Eicosadienoic acid methyl ester; 26. Methyl behenate; 27. cis-8,11,14-Eicosatrienoic acid methyl ester; 28. Methyl erucate; 29. cis-11,14,17-Eicosatrienoic acid methyl ester; 30. Methyl tricosanoate; 31. cis-13,16-Docosadienoic acid methyl ester; 32. Methyl lignocerate; 33. cis-5,8,11,14,17-Eicosapentaenoic acid methyl ester; 34. Methyl nervonate; 35. cis-4,7,10,13,16,19-Docosahexaenoic acid methyl ester

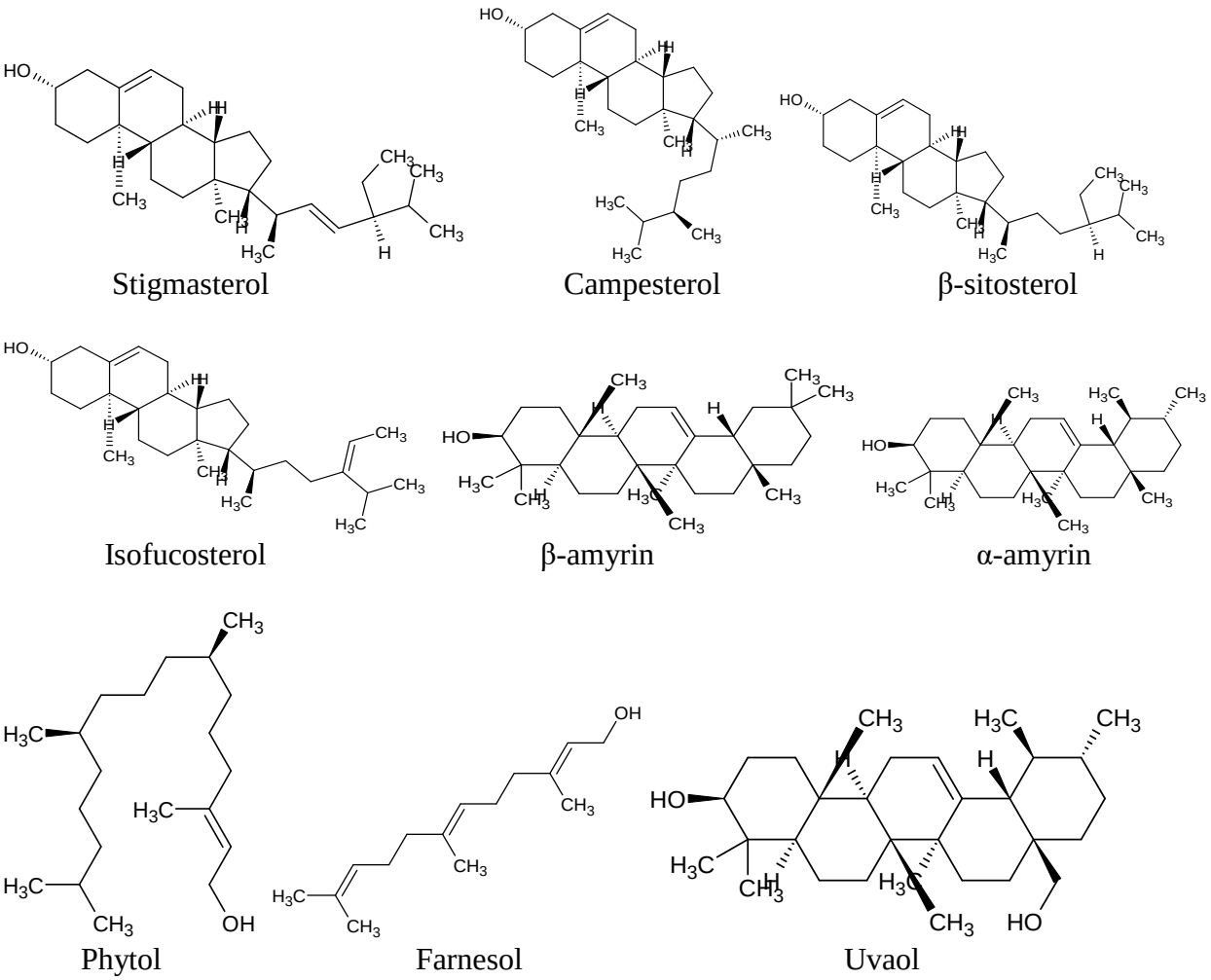


Figure 5.S2 The chemical structures of the phytosterols and terpenes identified by GC-MS in Canadian wild berries

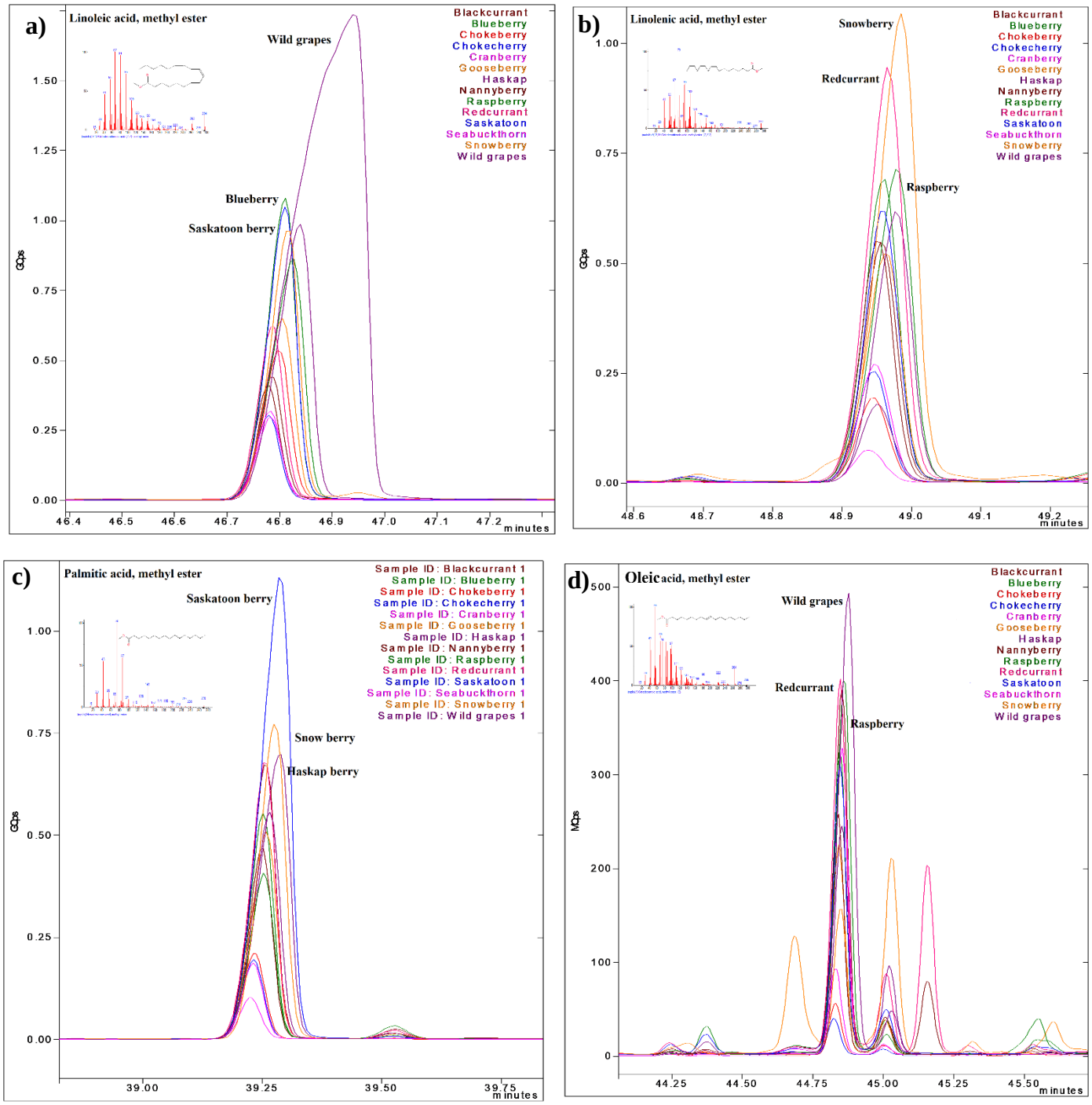


Figure 5.S3 The GC-MS chromatograms showing the distribution of **a)** Linoleic acid, b) Linolenic acid, c) Palmitic acid and d) Oleic acid in Canadian wild berries

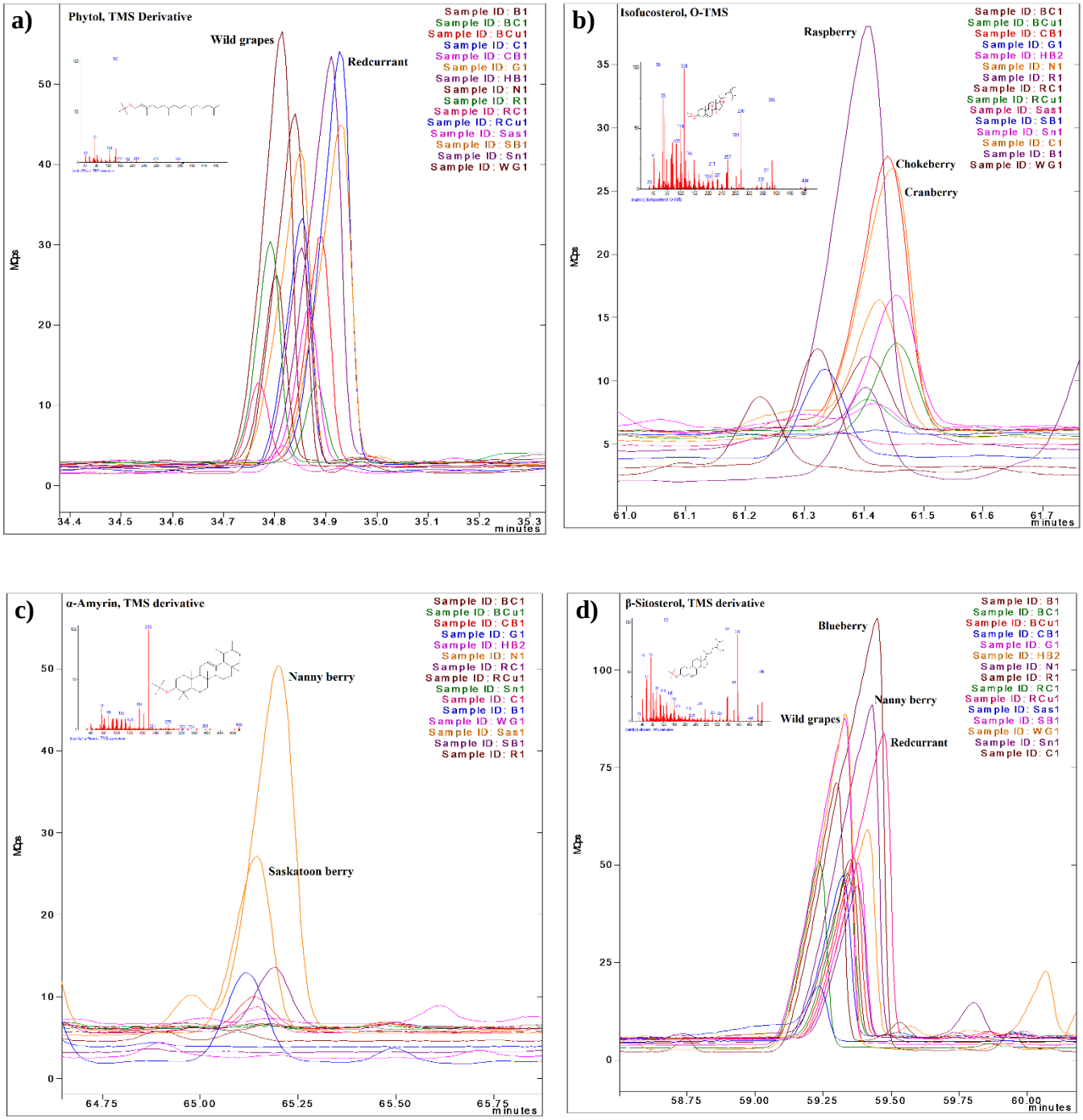


Figure 5.S4 The distribution of **a)** Phytol, **b)** Isofucosterol, **c)** α-amyrin and **d)** β-sitosterol in Canadian wild berries

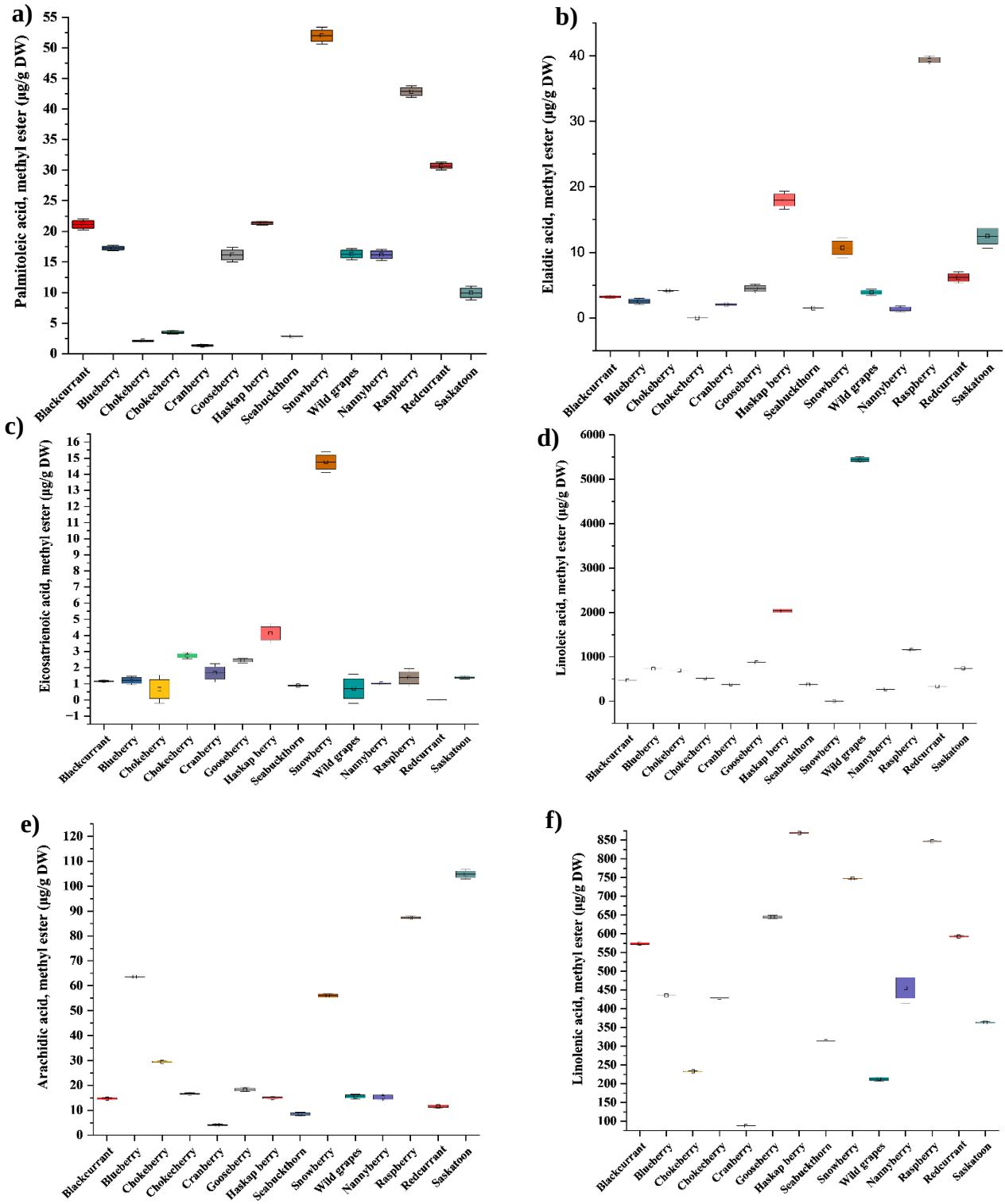


Figure 5.S5 The box plots show the distribution of a few individual fatty acids among the Canadian wild berries, **a)** Palmitoleic acid; **b)** Elaidic acid; **c)** Eicosatrienoic acid; **d)** Linoleic

acid; **e)** Arachidic acid; and **f)** Linolenic acid. The box plot line shows the mean fatty acid content of each wild berry type.

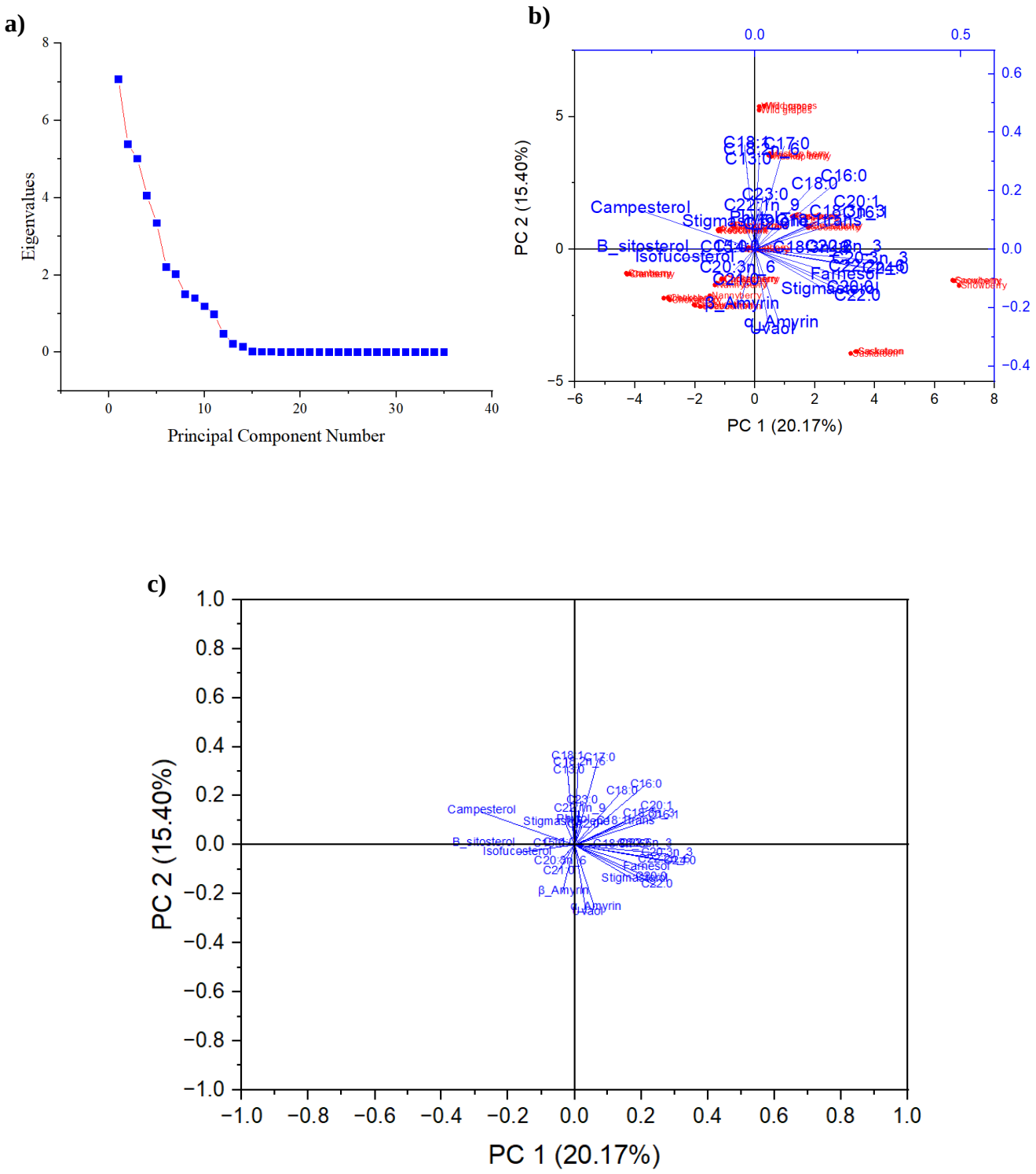
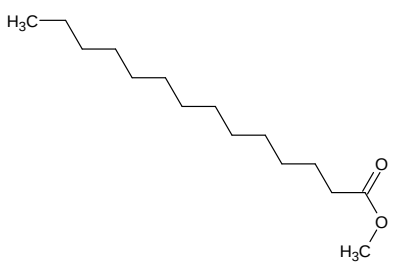
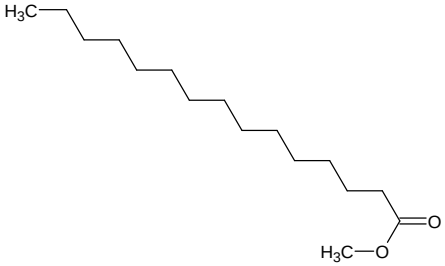
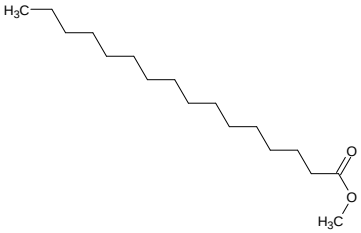
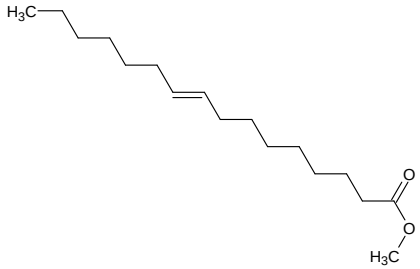
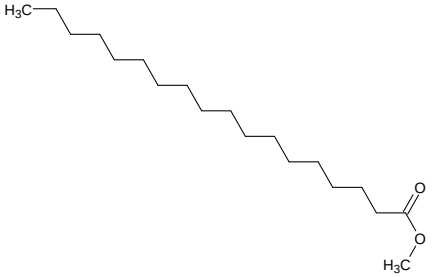
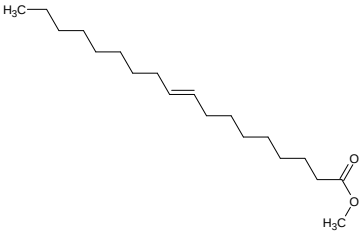
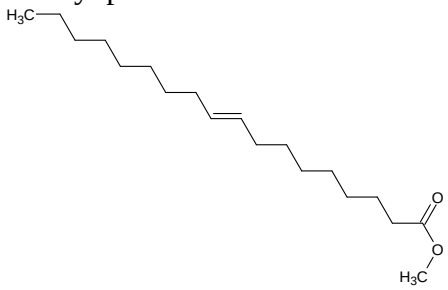
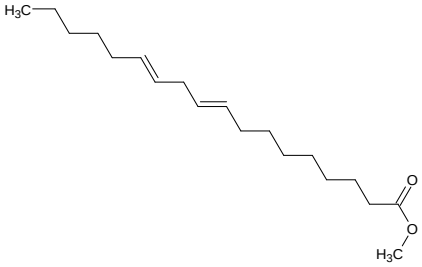
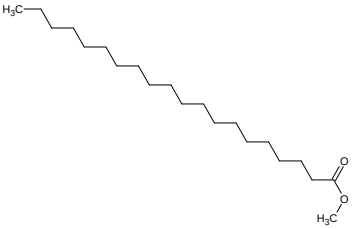
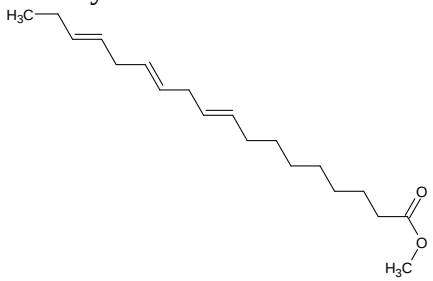
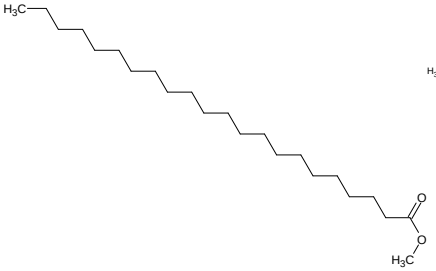
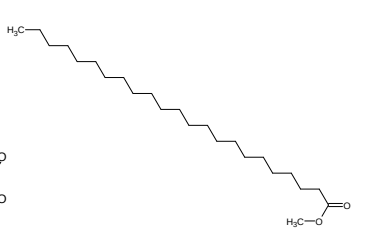
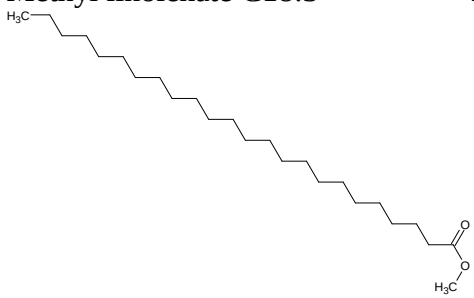
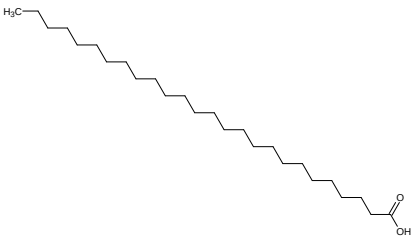
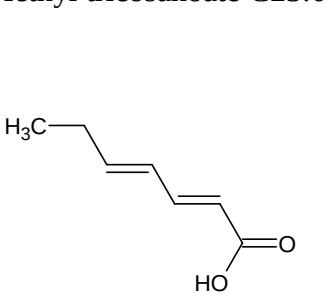

a)
Eigenvalues
Principal Component Number
b)
PC 2 (15.40%)
PC 1 (20.17%)
Campesterol
B_sitosterol
Isofucosterol
C16:0
C18:0
C20:1
C22:0
Farnesol
Stigmasterol
β_Amyrin
α_Amyrin
Uvaol
c)
PC 2 (15.40%)
PC 1 (20.17%)
Campesterol
B_sitosterol
Isofucosterol
C16:0
C18:0
C20:1
C22:0
Farnesol
Stigmasterol
β_Amyrin
α_Amyrin
Uvaol

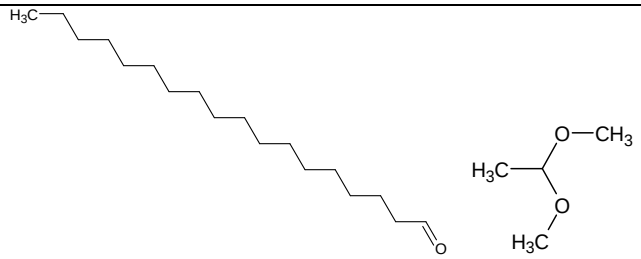
Figure 5.S6 Principal Component Analysis (PCA) of wild berries based on their fatty acids, phytosterols and terpenes content. **a)** Scree plot; **b)** bi-plot with the berries and respective compounds and **c)** bi-plot of the compound's distribution

Table 5.S1 List of the common names of the fatty acids found in Canadian wild berries

Omega name	Common name
C12:0	Lauric acid, methyl ester
C13:0	Tridecanoic acid, methyl ester
C14:0	Myristic acid, methyl ester
C15:0	Pentadecanoic acid, methyl ester
C16:0	Palmitic acid, methyl ester
C16:1	Palmitoleic acid, methyl ester
C17:0	Heptadecanoic acid methyl ester
C18:0	Stearic acid, methyl ester
C18:1trans	Elaidic acid, methyl ester
C18:1	Oleic acid, methyl ester
C18:2n-6	Linoleic acid, methyl ester
C20:0	Arachidic acid, methyl ester
C18:3n-6	γ-Linolenic acid, methyl ester
C20:1	Eicosenoic acid, methyl ester
C18:3n-3	Linolenic acid, methyl ester
C21:0	Heneicosanoic acid, methyl ester
C20:2	Eicosadienoic acid, methyl ester
C22:0	Behenic acid, methyl ester
C20:3n-6	Eicosatrienoic acid, methyl ester
C22:1n-9	Erucic acid, methyl ester
C20:3n-3	Eicosatrienoic acid, methyl ester
C23:0	Tricosanoic acid, methyl ester
C22:2n-6	Docosadienoic acid, methyl ester
C24:0	Lignoceric acid, methyl ester
C22:6n-3	Docosahexaenoic acid, methyl ester

Table 5.S2 List of Fatty Acids analyzed with GC-MS

Fatty Acid Methyl Ester/Volatile Compound Structure		
		
Methyl myristate C14:0	Methyl pentadecanoate C15:0	Methyl palmitate C16:0
		
Methyl palmitoleate C16:1	Methyl stearate C18:0	Methyl elaidate C18:1 trans
		
Methyl oleate C18:1 cis	Methyl linoleate C18:2	Methyl arachidate C20:0
		
Methyl linolenate C18:3	Methyl behenate C22:0	Methyl tricosanoate C23:0
		
Methyl lignocerate C24:0	Hexacosanoic acid C26:0	Hepta 2,4 dienoic acid



Stearaldehyde, dimethyl acetal

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