CHARACTERIZATION OF BLACKCURRANT BERRIES (*Ribes nigrum*) AND THE EVALUATION OF THEIR BIOACTIVE COMPOUNDS AFTER ULTRASOUND-ASSISTED WATER EXTRACTIONS, ENZYMATIC TREATMENTS, AND FERMENTATION

BY

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THESIS

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ABSTRACT

Blackcurrants (*Ribes nigrum*) (BC) have remained relatively unknown to the US market because they were prohibited from being grown in the US from the early 20th century until the 1980s. This was due to significant losses by the lumber industry, which discovered that some native and non-native species of the *Ribes* genus could act as vectors for the fungus *Cronartium ribicola*. Currently, some US farmers have a renewed interest in this high-value crop because BC and BC products are trending worldwide. BC are known to possess higher concentrations of anthocyanins (ANC) than other similarly colored berries, such as blueberries and blackberries. BC are also a rich source of phytochemicals which are potent antioxidants, antimicrobials and have anti-inflammatory properties; all of which add to the attractiveness of BC and their use in functional foods.

The objective was to characterize the whole fruit, and its component parts, of four varieties of BC and their effect on the activities of α-amylase, α-glucosidase, DPPIV, as biochemical markers of diabetes. In addition, the antioxidant capacity was measured using 2,2- diphenyl-1-picrylhydrazyl (DPPH$^{\cdot}$), after water-based ultrasound assisted extraction, treatment with pectinase and fermentation.

BC varieties (Titania, D16-6-54, Consort, and D16-8-14) were dissected into parts (juice, seeds, skins) freeze dried, ground and kept at -20 ºC. Parts and whole berries were evaluated to determine total anthocyanins (TA), total polyphenols (TP), total condensed tannins (TT), and HPLC quantification after 2, 4, 6 h sonication extraction. For LC-ESI-MS analyses, BC samples were extracted with methanol-HCl, sonicated for 1 h, kept overnight at 4 ºC; aliquots were filtered and analyzed.

The highest concentrations of TA were found in the skins of Titania, Consort, and D16-8-14 with no statistical difference among them (19.0 ± 2.0, 19.7 ± 2.7, and 20.3 ± 3.5 mg eq C3G/g dry weight (DW), respectively) ($p > 0.05$). The largest concentrations of TP were seen in the seeds of Titania and D16-8-14 with no statistical difference among them (34.4 ± 1.3 and 34.6 ± 0.5 mg eq GA/g DW, respectively) ($p > 0.05$). Condensed tannins were found to have the largest concentrations in the skins of all BC (Titania, 391.8 ± 0.0; D16-6-54, 438.2 ± 0.1; Consort, 472.4 ± 0.0; and D16-8-14, 521.0 ± 0.5 mg eq catechin/g, DW). A total of four anthocyanins (delphinidin 3-*O*-rutinoside, delphinidin 3-*O*-glucoside, cyanidin 3-*O*-rutinoside, cyanidin 3-*O*-glucoside) were identified across all samples. All four varieties of BC showed that the dominant compounds are the rutinoside forms of both delphinidin and cyanidin. Titania skins demonstrated the greatest α -amylase inhibition at 94.8% inhibition. The seeds of Titania, D16-6-54 and Consort all had the highest % inhibition of α -glucosidase with no significant differences among them (97.9 ± 0.0, 97.9 ± 0.2, and 97.9 ± 0.0, respectively). Enzymatic treatment doses and heating times (52 °C) were evaluated for their effect on the concentration of ANC and it was determined that a dose of 400 mL/ton held for 150 min at 52 °C yielded the highest concentration of ANC. Positive correlations were noted between the total time (min) of heating (52 °C) and TT and TP ($r = 0.725$ and $r = 0.731$, respectively at $\alpha = 0.05$). Positive correlations were also noted for the fermentation temperatures (23 °C and 15 °C) for TA, TT, and TP ($r = 0.608$, $r = 0.569$, and $r = 0.546$, respectively at $\alpha = 0.05$). Juices and skins from all four cultivars had the lowest IC_{50} values (most potent) for α-amylase inhibition, while the fermented products had the lowest IC_{50} values for *α*-glucosidase inhibition. IC_{50} values for the inhibition of

DPPIV were similar for all four cultivars. All four varieties differed in concentrations of specified phenolic compounds and showed potential for biological activity.

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To my mother, Magda H. Cortez, for always believing in me.

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ABBREVIATIONS

ANC	Anthocyanins
ANC	Total anthocyanins
BC	Blackcurrant
BHT	Butylated hydroxytoluene
C3G	Cyanidin 3-glucoside
D3G	Delphinidin 3-O-glucoside
D3R	Delphinidin 3-rutinoside
DDI	Deionized water
DPPH	2,2-diphenyl-1-picrylhydrazyl
DPPIV	Dipeptidyl peptidase IV
DW	Dry weight
ESI	Electrospray ionization
GA	Gallic acid
GLP-1	Peptide-1
HCD	Higher energy C-trap dissociation
HPLC	High performance liquid chromatography
IC_{50}	Inhibition of 50%
TP	Total polyphenols
TT	Total condensed tannins
UAE	Water-based ultrasound-assisted extract
US	United States

CHAPTER 1: INTRODUCTION

1.1 Overview

Blackcurrants (*Ribes nigrum*) are small dark purple fruit that comes from medium-sized woody shrubs (**Figure 1.1**) (Corrigan, Hedderley, Langford, & Zou, 2014; Törrönen et al., 2012). These shrubs are native to colder climate areas such as northern Europe, northern Asia, and central Asia, with Poland being the primary exporter (80% - 90% of global exports) of fresh and processed blackcurrant (Michalska, Wojdyło, Łysiak, Lech, & Figiel, 2017). Production of blackcurrants (BC) depends on the genetics of each cultivar and the temperature of the growing environment. BC are well known in European markets but not in the United States (US). BC have remained relatively unknown to the US market because they were prohibited from being grown in the US from the early 20th century until the 1980s. This was due to significant losses by the lumber industry, which discovered that some native and non-native species of the *Ribes* genus could act as vectors for the fungus *Cronartium ribicola*. Since then, new cultivars have been created which do not act as vectors for the fungus, in addition to the already resistant BC cultivar 'Consort.' Currently, some US farmers have a renewed interest in this high-value crop because BC and BC products are trending worldwide. It was reported in 2017 that 3,100 new BC products appeared globally, 199 of which were from the US alone (FONA International, 2017). **Figure 1.2** highlights some commercially available BC products which can be found in the US**.** BC, which are known for their characteristic deep shades of purple, also have a characteristic bitter and astringent flavor. This is why it is quite common to find BC products with significant amounts of sugar added. BC are also known to have a high concentration of flavonoids, specifically anthocyanins, which provide the fruits with their purple color (Archaina, Leiva, Salvatori, & Schebor, 2017). BC are a

rich source of phytochemicals which are potent antioxidants, antimicrobials and have anti-inflammatory properties (Nour, Stampar, Veberic, & Jakopic, 2013). Part of the objectives were to summarize and offer an up-to-date information of the available literature regarding BC, as well as their chemical, sensorial, processing, and potential biological properties.

Our project goals were to better understand the differences among 4 cultivars of BC, fill a gap in knowledge regarding extraction methods for BC, while exploring methods using water only. Also, to understand how enzymes and the fermentation process affect concentrations of bioactive compounds. To our knowledge, this is the first report detailing the quantification of ANC in BC and BC fermented products using a strictly water-based ultrasound extraction.

The first aim of this research was to characterize 4 different cultivars of BC berries (Titania, D16-6-54, Consort, and D16-8-14) and their parts (juice, seeds, and skins) to compare ANC, condensed tannins, and polyphenol contents of the BC. Additionally, we also sought to understand if these phytochemicals are concentrated in any particular part of the berries. As consumers demand more and more healthful products that are made using green technology, there is an increased need to discover ways in which extractions of pigments and bioactive compounds can be done using water alone. The second aim of this research was to determine the time needed for water-based ultrasound-assisted extraction to obtain ANC, and other phenolic compounds, from BC. We did determine that a 2 h water-based UAE was sufficient to extract >50% of ANC from BC. Consort and D16-8-14 whole berries had the highest concentration of ANC (14.5 ± 2.8 and 14.8 ± 2.8 mg eq. C3G/g, DW) after a 2 h UAE. Consort also had the highest concentration of TP present overall. The third aim of this research was to determine the effect of a pectinase treatment on the extraction of ANC, total condensed tannins (TT), or total polyphenols (TP). The fourth, and final, aim of this research was to determine the effect of fermentation of BC mash on the

concentrations of ANC, TT, and TP. It was determined that the fermentation process did increase the ANC concentration 5 times and the pectinase treatment increased TP concentrations. All solid material remaining after the fermentation were also analyzed to determine their potential related to the inhibition of enzymes related to type-2 diabetes. All BC pomaces proved to be powerful antioxidants with lower IC_{50} values compared to the fresh whole berries. **Figure 1.3** shows a graphical summary of this research.

1.2 Figures

Figure 1.1 Blackcurrant shrubs growing in Champaign, IL

Figure 1.2. Examples of blackcurrant products

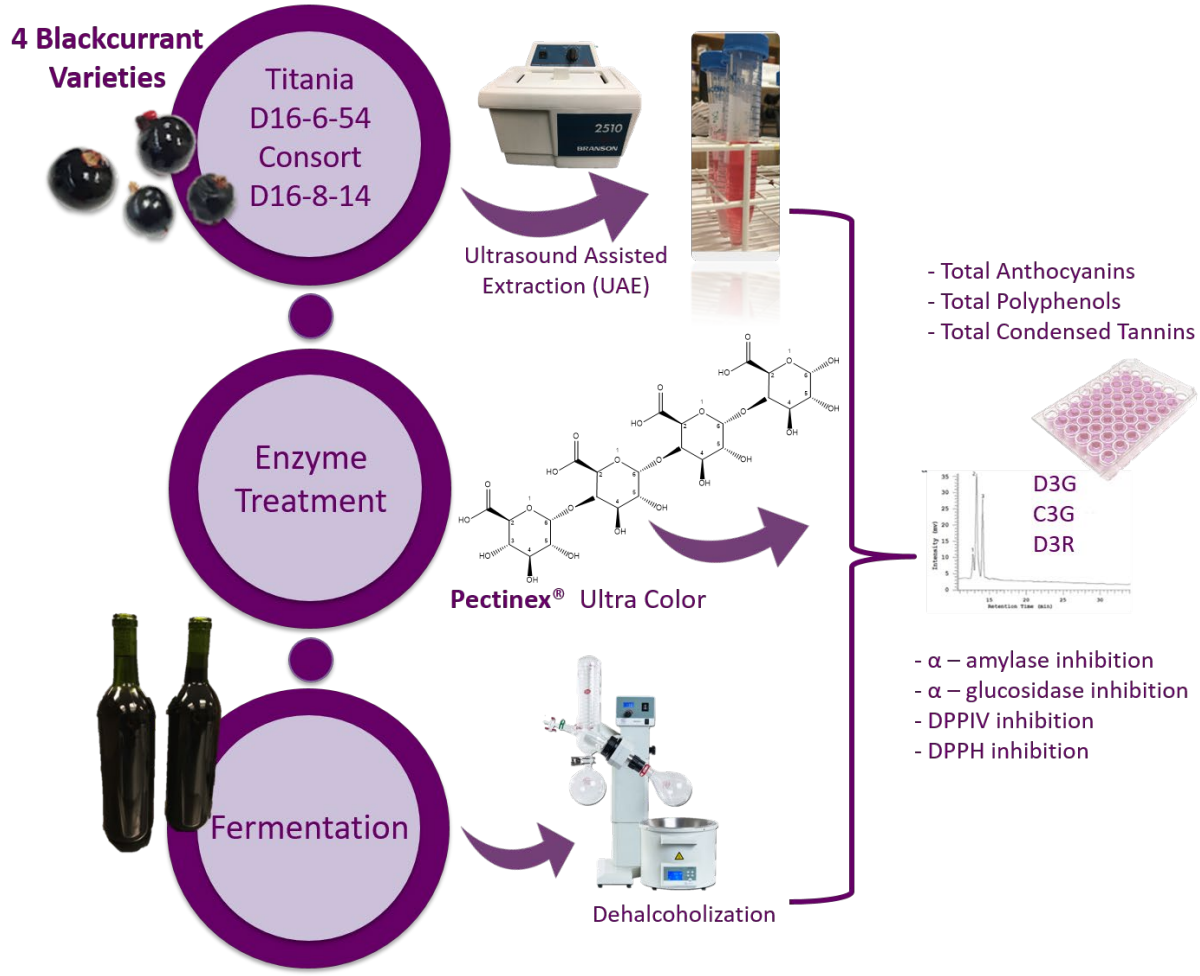

Figure 1.3. Summary of research

1.3 References

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CHAPTER 2: LITERATURE REVIEW - BLACKCURRANTS (*Ribes nigrum*): A REVIEW ON CHEMISTRY, PROCESSING AND HEALTH BENEFITS

2.1 Abstract

Blackcurrants (*Ribes nigrum*) are relatively new to the United States market; however, they are well known and popular in Europe and Asia. The use of blackcurrants has been trending worldwide, particularly in the US. We believe that demand for blackcurrants will grow as consumers become aware of the several potential health benefits these berries offer. The objectives of this review were to present an up-to-date summary of information on blackcurrants based on articles published within the last decade. Furthermore, to provide the food industry insights into possibilities for the utilization of blackcurrants. The chemistry, processing methods, and health benefits have been highlighted in addition to how the environment and variety impact the chemical constituents of blackcurrants. A search for journal publications on blackcurrants was conducted which included keywords such as chemical characterization, health benefits, processing, technologies, anthocyanins, and proanthocyanidins. This review provides the most up-to-date information available on the subject. In conclusion, blackcurrants and their products have industrial uses from which extractions can be made to produce natural pigments to be used as food additives. BC contain flavonoids, specifically anthocyanins, which provide the fruits with their purple color. BC are a rich source of phytochemicals with potent antioxidants, antimicrobials, and anti-inflammatory properties. Also, blackcurrants have the potential to improve overall human health particularly with diseases associated with inflammation and regulation of blood glucose.

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2.2 Introduction

Blackcurrants (*Ribes nigrum*) are small dark purple fruit that comes from medium-sized woody shrubs (Corrigan, Hedderley, Langford, & Zou, 2014; Törrönen et al., 2012). These shrubs are native to colder climate areas such as northern Europe, northern Asia, and central Asia, with Poland being the primary exporter (80% - 90% of global exports) of fresh and processed blackcurrant (Michalska, Wojdyło, Łysiak, Lech, & Figiel, 2017). Production of blackcurrants (BC) depends on the genetics of each cultivar and the temperature of the growing environment. BC are well known in European markets but not in the United States (US). BC have remained relatively unknown to the US market because they were prohibited from being grown in the US from the early 20th century until the 1980s. This was due to significant losses by the lumber industry, which discovered that some native and non-native species of the *Ribes* genus could act as vectors for the fungus *Cronartium ribicola*. This is the cause of the white pine blister rust, disease in pine trees, that leads to mortality of native five-needle pines, important for the US lumber industry (Tanguay, Cox, Munck, Weimer, & Villani, 2015). Since then, new cultivars have been created which do not act as vectors for the fungus, in addition to the already resistant BC cultivar 'Consort.' Currently, some US farmers have a renewed interest in this high-value crop because BC and BC products are trending worldwide. It was reported in 2017 that 3,100 new BC products appeared globally, 199 of which were from the US alone (FONA International, 2017). BC, which are known for their characteristic deep shades of purple, also have a characteristic bitter and astringent flavor. This is why it is quite common to find BC products with significant amounts of sugar added. BC are also known to have a high concentration of flavonoids, specifically anthocyanins, which provide the fruits with their purple color (Archaina, Leiva, Salvatori, & Schebor, 2017). These winter hardy berries are a rich source of phytochemicals which are potent

antioxidants, antimicrobials and have anti-inflammatory properties (Nour, Stampar, Veberic, & Jakopic, 2013). The objective of this review was to summarize and offer an up-to-date information of the available literature regarding BC, their chemical, sensorial, processing, and potential biological properties.

2.3 Chemistry and Sensory Properties of Blackcurrants

2.3.1 Chemical characterization

Blackcurrants (*Ribes nigrum*) are widely recognized for containing high levels of polyphenols, specifically anthocyanins (ANC, **Figure 2.1**) and proanthocyanidins (PAC, **Figure 2.2**), when compared with other berries (Lee et al., 2015). Both blackberries and blueberries have lower total anthocyanin concentrations compared to BC (949.4 ± 4.0, 1562.2 ± 52.4, and 1741 ± 48.8 mg/100 g, dry weight (DW), respectively) (Lee et al., 2015). Interestingly, a large degree of variability of anthocyanin concentrations was demonstrated among three BC cultivars ('Record,' 'Blackdown,' and 'Ronix') with a range from 80 to 476 mg/100 mg fresh weight (FW) (Nour et al., 2013). This variance among cultivars suggests that more research is needed to determine which cultivars contain the highest concentrations of these beneficial bioactive compounds. According to Nour et al. (2013), who performed a maceration of the berries in food grade ethanol (40%, 60%, or 96%), the compounds found in BC were delphinidin 3-*O*-glucoside (D3G), delphinidin 3-*O*-rutinoside (D3R), cyanidin 3-*O*-glucoside (C3G), cyanidin 3-*O*-rutinoside (C3R), petunidin 3- *O*-rutinoside, pelargonidin 3-*O*-rutinoside, peonidin 3- *O*-rutinoside, petunidin 3-(6-coumaroyl) glucoside, and cyanidin 3-(6-coumaroyl) glucoside (**Table 2.1**). Of the three different extractions (40%, 60%, and 96%), 60% ethanol was able to extract the highest concentrations of the four major ANC from all three cultivars, except for D3R (Nour et al., 2013). After performing ANC extraction with an 80% (v/v) aqueous methanol solution with 0.1% HCl, Lee et al. (2015) reported that the

content of ANC in blackcurrants was delpinidin-3-*O*-rutinoside (55.2%), cyanidin-3-*O*-rutinoside (23.2%), and delphinidin-3-*O*-glucoside (18.8%). This suggested that ethanol was more effective than acidified methanol for extraction of more diverse forms of ANC from BC (**Table 2.1**). **Figure 2.3** presents an HPLC profile at 520 nm for the characterization of ANC showing the presence of delphinidin 3-*O*-glucoside, delphinidin 3-*O*-rutinoside, cyanidin 3-*O*-glucoside, cyanidin 3-*O*-rutinoside (Buchert et al., 2005). According to the study by Nour et al., (2013), the concentration of total phenolics in BC ranged between 1261 to 1694 mg eq of gallic acid/L with the lesser values being from the 40% ethanol extraction and the higher values from the 96% ethanol extraction. Proanthocyanidins (PAC) present in BC, are polymers which can be divided into two categories, procyanidins (PC) and prodelphinidins (PD, **Figure 2.2**) (Laaksonen, Salminen, Mäkilä, Kallio, & Yang, 2015). PC are polymers made up of catechins (+) and epicatechins (-) and PD are also polymers made up of gallocatechins (+) and epigallocatechins (-) (**Figure 2.2**) (Laaksonen et al., 2015). ANC, are naturally hydrophilic and therefore have limited application potential in both foods and cosmetics which contain fats or oils (Cruz et al., 2018). However, there has been research done to try to improve the performance, stability, formulation properties and color of ANC from BC. One particular study sought to increase the stability of ANC from BC without a loss in bioactivity (Cruz et al., 2018). This study used the enzyme *Candida antarctica* lipase B and octanoic acid to lyophilize and esterify ANC from BC. BC extracts from skins were obtained (**Table 2.1**) and purified to only contain the four major monomeric ANC (delphinidin-3-*O*-rutinoside (43.3%), cyanidin-3-*O*-rutinoside (34.0%), cyanidin-3-*O*-glucoside (7.0%) and delphinidin-3-*O*-glucoside (15.7%) (Cruz et al., 2018). This work concluded that only the glucoside forms of cyanidin and delphinidin were acylated by the enzymes and not the rutinoside forms. A different study found that by using lauric acid, each of the four major ANC were

monoacylated successfully without an adverse effect on relative proportions (Yang, Kortesniemi, Ma, Zheng, & Yang, 2019). Each of the acylations was noted at the 6" -OH position and at the 4" -OH position of the glucosides and rutinosides, respectively (Yang et al., 2019). This process succeeded in enhancing the lipophilicity of the compounds, which makes them more compatible for use in lipid-based foods and cosmetics. While one group of researchers were able to alter the hydrophilicity of ANC from BC, results still suggest that more research is needed to address the hydrophilicity of ANC from BC so that the food and cosmetics industries may better utilize them.

2.3.2 Sensory attributes

BC are bitter and astringent; because of this, large amounts of sugar are often added to BC products to offset the bitterness and astringency. This can be problematic for companies seeking to appeal to health-conscious consumers. Pectinolytic enzymatic treatments, which increase juice yields, also increase the perception of bitterness and astringency because the enzymes increase the mean degree of polymerization of PAC (Laaksonen et al., 2015). Additionally, astringency is related to the mean degree of polymerization (mDP) of PAC, which are oligomeric and polymeric tannins with different flavan-3-ol units (Laaksonen et al., 2015). The mDP is an indicator of the average number of flavan-3-ol monomers that make up the condensed tannins (Laaksonen et al., 2015). Epicatechins, which are subunits of PC are thought to be more bitter and astringent than catechins at equal concentrations. The reason for the perception of these bitter and astringent flavors is still not fully understood (Laaksonen et al., 2015). It has historically been hypothesized, and generally accepted, that this phenomenon is due to polymeric tannins binding and precipitating salivary proteins, which in turn are perceived as a rough and drying sensation in the mucous membranes (Laaksonen et al., 2015). It is believed that proline clusters, and possibly nearby residues, are the probable sites for the PC interactions with salivary proteins (Soares et al., 2018).

After analyses of five different cultivars, Mortti, Mikael, Marski, Ola, and Breed15, it was discovered that samples that had undergone an enzymatic treatment prior to processing showed not only a significantly higher mDP but also demonstrated higher concentrations of PAC (both PC and PD) (Laaksonen et al., 2015). BC juices that contain higher concentrations of PAC, could be viewed as undesirable due to their flavor, despite their benefits (Laaksonen et al., 2015). This does, however, offer unique opportunities for extractions since the diversity of ANC in BC is not complex. Sensory evaluations of other aspects of BC have also been conducted to explore the use of BC pomace as a source of dietary fiber. In one study, consumers were blindly tested for acceptance of a 50% wheat flour, 30% buckwheat flour, and 20% corn flour crackers versus crackers made up of the same ingredients with the addition of blackcurrant pomace (10%, 20%, and 30%) (Schmidt, Geweke, Struck, Zahn, & Rohm, 2018). The 20% pomace cracker scored a 4.17 on an acceptance scale of 1 to 7, while the reference scored a 4.37 on the same scale (Schmidt et al., 2018). However, the 30% pomace crackers produced a stiffer dough, which led to a lower hardness trait due to high water absorption; thus, the pomace restricted the ability of a strong protein network to form. The poor formation of a protein network also resulted in at least a 57% decreased volume of the 20% and 30% pomace crackers versus the 10% pomace and reference crackers (Schmidt et al., 2018). Changes to the color of the cracker were also noted as the blackcurrant pomace changed the color of the crackers from the traditional light tan color to a deep shade of red. There were only slight differences between the structures and appearances of the 20% and 30% pomace crackers versus the reference with no pomace and 10% pomace crackers (Schmidt et al., 2018). Despite this, there was virtually no difference between consumer preferences of crackers, which shows blackcurrant pomace is a viable option to replace a significant amount of wheat flour in baked goods (Schmidt et al., 2018). The ANC in BC berries

and juices provide rich colors to commercial products, some of which are recorded in **Table 2.2**. The color parameters of some commercially available BC beverages were measured in our laboratory using the CIELAB color scale. Both hue angle and chroma were calculated using the L*a*b* values and the following formulas: Chroma $C^* = \sqrt{(a*)^2 + (b*)^2}$ and Hue angle $h_{ab} = \tan^{-1}\left(\frac{b*}{a*}\right)$. Results are presented in **Table 2.2**

Chroma is the saturation or richness of a color and hue angle referrers to the color perceived based on the wavelength (Cortez, Luna-Vital, Margulis, & Gonzalez de Mejia, 2017).

2.4 Environmental and Variety Impact on Chemical Composition

While it is clear that there are several benefits to be gained by using BC in food products, both the environment and genetics play critical roles in the production, chemistry, and nutritional quality of the BC fruits. A study, which investigated the environmental effects on blackcurrants, was conducted in Denmark (55° 18′ N 10° 26′ E) from October 2014 to April 2015. It was demonstrated in this study that there was a significant decrease in the number of flowers when the experimental plots were warmed to an average temperature of 1.3 °C than the control plot (ambient temperature) (Andersen et al., 2017). The temperature of the control plots did not vary the height of the plants; however, air temperature of warmed plots led to lower height of 50 cm and 80 cm (on average by 0.4 °C and 0.7 °C, respectively). Both cultivars (Narve Viking and Zusha) grown in ambient temperatures produced more flowers per plant (451 and 491, respectively) and had higher berry yields, total berries per plant, and produced berries with greater individual weights (Andersen et al., 2017). When comparing each of these two cultivars, warmer temperatures did not physically damaged them. This ultimately led to the conclusion that the environment does not lead to a direct correlation between the crop and production, but rather, has an effect on the genes

of growth and development. This, consequently, leads to changes such as fewer flowers and less beneficial health properties, for instance, a decrease in flavanol and anthocyanin content (Andersen et al., 2017). Not only does temperature affect decreasing the aforementioned bioactive compounds, but there was a clear correlation between higher concentrations of gallic acid and the colder temperatures in the control plots (Andersen et al., 2017). Production of blackcurrant berries depends on the genetics of each cultivar and the temperature of the growing environment. High temperatures during the growing season are also associated with the inhibition of various biochemical processes during blackcurrant development, which in turn decreases the amount of ascorbic acid produced (Woznicki et al., 2017). High temperatures (12 – 24 °C) have been shown to reduce the amount of ascorbic acid and the overall sugar content by 27% in blackcurrants (Woznicki et al., 2017). Although, higher temperatures lessen not all properties of the blackcurrants; citric acid has been shown to increase (Woznicki et al., 2017). It can be concluded from these studies that growing BC plants in colder climates produce berries, which have higher concentrations of beneficial bioactive compounds such as phenolics, which add value to an already high-value fruit. Another recent study also examined the effects of growing temperature and day length from a metabolomics approach (Xu et al., 2019). The recorded data from this work confirmed earlier observations by Woznicki et al. (2017) by concluding that growing temperature significantly affected a total of 365 metabolites constituting a wide variety of chemical classes. A comparison between ambient conditions and controlled conditions (planted in pots outdoors with ambient summer conditions, 59°40’N) demonstrated that ripening BC berries had accumulated a total of 34 additional metabolites under ambient conditions, the majority of which were ANC and flavonoids (Xu et al., 2019). Additionally, a significant up-regulation of 100 metabolites (linear increase) was noted with increased cultivation temperatures, and 42 metabolites experienced a

linear decrease. It is particularly interesting to note that phenylalanine was one of the up-regulated metabolites (with increased cultivation temperatures) and it is also the main precursor for the synthesis of flavonoids. However, it is not the limiting factor for synthesis efficiency (Xu et al., 2019). While this information does provide great insight into ideal BC growing conditions for the maximization of various polyphenolic compounds, it also offers producers an opportunity to adjust growing methods as temperatures become extreme with the changing global climate.

2.5 Technological Methods for Blackcurrant Processing

2.5.1 Enzymatic treatments

Generally, the reasons for berry fruit processing are to maximize juice yields, inactivate microorganisms, inactivate enzymes, and to maintain the sensory qualities of the finished product (Mäkilä, Laaksonen, Kallio, & Yang, 2017). The use of enzymatic treatments in juice production is quite common, especially in the processing of berry juices because it can increase juice yields up to approximately 91% (Laaksonen et al., 2014) (**Table 2.3**). These treatments improve the juice yield, decrease the viscosity of the juice, and also significantly increase the extraction of bioactive compounds such as phenolics (Bender, Killermann, Rehmann, & Weidlich, 2017; Laaksonen et al., 2014). The contents of procyanidins (PC) and prodelphinidins (PD) are significantly higher with enzymatic maceration than without (Laaksonen et al., 2015). A possible explanation for this phenomenon is that bioactive compounds, which are trapped in the networks of the pectins, are liberated with the effects of the enzymes. Employment of the enzymatic process increases the nutritional value of BC juices because of the increase in what is an already high concentration of bioactive compounds. A Finnish research group demonstrated a 151 mg/100 mL increase in total PAC and a 121 mg/100 mL increase of total PD with the utilization of an enzymatic process for BC juice production (Laaksonen et al., 2015). In 2017, a different study demonstrated that the total

ANC concentration of BC juice could be increased by 584 mg/100 g before pasteurization and 524 mg/100 g after pasteurization with the use of enzymatic pectinases (Mäkilä et al., 2017). Enzymatic treatments achieve these higher extraction rates as a result of the enzymes demolishing cell wall structures, which happens by cleaving pectins and causing the degeneration of soluble pectins (Bender et al., 2017). Berries are known to have higher viscosities during processing for juice making, than other fruits. After the berries have been crushed the higher viscosity complicates the pressing process and causes a great deal of inefficiency, which is why it is necessary to use enzymatic treatments in the production of berry juices (Bender et al., 2017).

2.5.2 Processing methods

BC are relatively expensive fruits containing concentrated amounts of compounds in the skins and seeds that are beneficial to health, which are typically discarded. Included in these compounds are not only polyphenols, but also polyunsaturated fatty acids (PUFA), tocopherols, phytosterols, polycosanols, and I (Basegmez et al., 2017). One way of maximizing the many benefits of BC is to find ways in which the BC pomace can be processed and repurposed for use. Pomace is the material that remains after the BC have been processed for juice, which consists mainly of skins and seeds. At present, much of the pomace, which is acidic, is discarded as waste and has the potential to become an environmental hazard when it is disposed of in landfills (Basegmez et al., 2017). In addition, it is quite wasteful to discard such an enriched material. Basegmez et al. (2017) discovered a remedy for these issues that has many advantages. This is the use of supercritical fluid extraction with carbon dioxide (SFE-CO_2) along with response surface methodology (RSM) and central composite design (CCD) (Basegmez et al., 2017) (**Table 2.1**). This is a green technology method for the recovery of high-value fractions. This process is rapid, automatable, selective, non-flammable, involves no toxic solvents, does not allow exposure to light

or oxygen during extraction, and produces solvent-free extracts and residues (Basegmez et al., 2017). As the name implies, SFE-CO_2 involves the use of carbon dioxide, which is of low toxicity and generally recognized as safe (GRAS) by the Food and Drug Administration (Basegmez et al., 2017). Another study utilized performed ultrasound-assisted extractions (UAE) of BC pomace by using water acidified with citric acid (Archaina et al., 2017). In this work, it was documented that UAE is a viable method for the extraction of bioactive compounds from BC pomace. Furthermore, in this same study, the investigators used a maltodextrin carrier matrix to spray dry the extracts. It was concluded in this research that the obtained powder maintained high levels of total ANC and total phenolic contents (63.01 ± 1 mg eq C3G/100 g dry material and 116.87 ± 5 mg eq gallic acid/100 g dry material, respectively) (Archaina et al., 2017). Conventional drying methods, such as convective drying, freeze-drying, and microwave vacuum drying, offer less expensive alternatives to SFE- CO_2 for the recovery and use of BC pomace, however, they do have some limitations. It was discovered that the dehydration of BC pomace by freeze-drying reduced the total phenolics by 76% and with convective drying (90°C) by 90% compared to fresh pomace (Michalska et al., 2017). Interestingly, samples that were dried using convection exhibited the most significant decrease in total flavonols when dried between 50°C and 60°C (Michalska et al., 2017). The use of BC pomace (BCP) as a means of adding I to processed foods offers an attractive incentive for BC producers and processors alike. A recent characterization study on BCP sourced from two different countries (Lucozade-Ribena-Suntory, UK and GreenField Natural Ingredients, Warsaw, Poland) reported that 25-30% of BCP is soluble dietary I (SDF) (e.g., pectin and some hemicelluloses), while approximately 47% is insoluble dietary I (IDF) (e.g., cellulose or lignin) (Alba et al., 2018). Pure IDF was measured as being approximately 61%. Ratios for IDF/SDF were calculated for the BC from the UK and the BC from Poland as 1.9 and 1.6, respectively (Alba et

al., 2018). The main cell wall component noted in this research was Klason lignin, which was the major insoluble I in both BCP (Alba et al., 2018). This characterization of BCP was preceded by fractions of constituent soluble and insoluble fractions followed by extractions of pectins (acid-soluble and calcium-soluble), alkali-soluble lignin, alkali-soluble hemicelluloses, and cellulose (**Table 2.1**) (Alba et al., 2018). This study confirmed that downstream waste from BC processing could be fractioned, used as food ingredients for added benefits, and potentially increasing the ability to make health claims. Another study was able to demonstrate how BC pomace (skins and other solid material that remain after juice processing) can be used to create hair dyes that are an intense blue color by employing entirely sustainable technology (Rose et al., 2018). It should also be noted that the resulting colorant was entirely biodegradable, which is attractive for consumers (Rose et al., 2018). A similar study found an environmentally friendly way to extract ANC from BC waste to be used as colorants (Farooque, Rose, Benohoud, Blackburn, & Rayner, 2018).

2.6 Health Benefits of Bioactive Compounds from Blackcurrant

Xu et al. (2018) characterized the effects of ultrasound irradiation on the bioactivities of BC polysaccharides. During this characterization process, three different BC polysaccharide solutions were assessed for the effects from ultrasound treatments and its impact on antioxidant activity, free radical scavenging activities, inhibition of lipid peroxidation, protection from DNA damage and the inhibition of α-amylase and α-glucosidase activities. It was concluded that the higher wattage of ultrasound power produced a higher reducing sugar content along with improved thermal stability. While there was an increase in reducing sugar content, six species of monosaccharides (galacturonic acid, galactose, mannose, glucose, arabinose, and rhamnose) were found in the treated sample. The same six monosaccharides were also found in the control suggesting that the ultrasound treatments did not produce any significant structural changes.

However, the study concluded that ultrasound irradiation improves the antioxidant capacity, and the percent inhibitions of both α-amylase and α-glucosidase, likely because there was a degradation of polysaccharides present. The degraded polysaccharide U-600 W (M_w = 1.32 x 10^4 kDa) ultrasound treated sample exhibited the best results for all assays performed when compared to the polysaccharide which received a lower wattage treatment.

Ashigai et al. (2018) demonstrated the effectiveness of oral intake of BC cassis polysaccharide on reducing skin dehydration caused by ultraviolet light in mice. They also reported decreases on markers of inflammation, such as those of interleukin-6 and matrix metalloprotein transcription levels in the skin of hairless mice.

BC are high in ascorbic acid (50-280 mg / 100 g or 300 mg/100 mL of juice), this together with a high flavonoid content bolsters the antioxidant capacity of the berries and increases their potential to promote health benefits (Bladé et al., 2016; Castro-Acosta et al., 2016; Lee et al., 2015; Nour et al., 2013; Woznicki et al., 2017). By comparison, BC have a much higher concentration of ascorbic acid than both raspberries and blueberries (Bender et al., 2017). According to Nour et al. (2013), ascorbic acid concentration can be found in a range from 50 to 280 mg/100 g FW, adding to the attractiveness of BC for the food and beverage industries. Not only is ascorbic acid an antioxidant, but it also facilitates the biosynthesis of collagen, and aids in the production of some peptide hormones (Woznicki et al., 2017). The antioxidant properties of BC are attributed to the polyphenols (which include ANC and PAC) present in the flesh and skin of the berries. Polyphenols are known to be responsible for the scavenging, or trapping, of free radicals, which are responsible for oxidative stress. Results from the study by Nour et al. (2013) indicated that there was a high correlation (r = 0.85) between antioxidant activity and the total concentration of ANC (**Table 2.4**). Generally speaking, ANC are supposed to increase the antioxidant capacity;

however, BC exhibit a lesser antioxidant capacity than both blackberries and blueberries (Lee et al., 2015). This reduced antioxidant capacity in BC can likely be attributed to the presence of other polyphenols that are not ANC such as phenolic acids, PAC, tannins, and flavonoids (Lee et al., 2015). Additionally, the lower antioxidant capacity of BC could be attributed to the specific structures of the ANC present and perhaps steric hindrance by glycones attached to the B-ring (Lee et al., 2015). A different study found that the antioxidant capacity can be increased by first completing a mash enzymatic maceration of the berries (Bender et al., 2017). It has recently been reported that BC extracts were able to produce hypocholesterolemic effects in mice with diet-induced obesity (Benn et al., 2014; Kim et al., 2018) (**Table 2.4**). A separate study was conducted to evaluate the effects of BC extracts on mRNA and protein expression of genes of Caco-2 cells, which are human epithelial colorectal adenocarcinoma cells (Kim et al., 2018). This study demonstrated that BC extracts increased low-density lipoprotein receptors (LDLR) without any changes to the cell mRNA. Overall, the data suggested that BC extracts increased the transport of cholesterol via the enterocytes, which suggests that the BC extracts play a part in the hypocholesterolemic effects (Kim et al., 2018). The exact mechanism of action was not determined in this study, which means that further *in vivo* studies are needed to characterize the mechanisms. A significant decrease in mean arterial pressure and total peripheral resistance in 15 endurance-trained male cyclists that received 600 and 900 mg BC extract supplement per day during 2 h of prolonged exercise (Cook, Myers, Gault, Edwards, & Willems, 2017) (**Table 2.4**). In a separate study, the mean fat oxidation in endurance-trained females increased by 27% during 120 min of moderate-intensity cycling when ingested 600 mg/day of blackcurrant extract in comparison to placebo (Strauss, Willems, & Shepherd, 2018).

In addition to being able to affect cholesterol levels positively, BC have been reported to lower blood glucose levels and ameliorate glucose tolerance in both mice and rats, and also to decrease postprandial blood glucose concentrations in humans (Iizuka et al., 2018). A recent study reported that dietary forms of blackcurrant extracts (BCE), which are heavily concentrated with delphinidin 3-rutinoside (D3R), can significantly reduce blood glucose levels and improve glucose tolerance in type 2 diabetic mice (Iizuka et al., 2018). The mechanistic changes that produced these effects were due to an increased secretion of glucagon-like peptide-1 (GLP-1) in plasma. Also, it was due to the upregulation of intestinal prohormone convertase 1/3 (PC1/3) expression and the activation of adenosine monophosphate-activated protein kinase (AMPK) mediated translocation of the insulin-regulated glucose transporter (Glut4) in the skeletal muscle of type 2 diabetic mice (Iizuka et al., 2018).

It was reported by Nanashima et al. (2018) that treatments with BCE increased collagen, elastin and hyaluronic acid in human skin fibroblasts and ovariectomized rats. This study used normal human female skin fibroblast cells (TIG113), OVX female Sprague-Dawley rats (12 weeks old) which had their ovaries removed to simulate menopausal women, and sham surgery rats (Nanashima et al., 2018). The TIG113 cells were treated with blackcurrant extracts (BCE) with 1.0 μg/mL for microarray gene expression profiling and either 1.0 μg/mL or 10.0 μg/mL for reverse phase polymerase chain reaction (RT-qPCR) assays. The rats were fed AIN-93M diets, with and without 3% BCE (Nanashima et al., 2018). Results from this study indicated that TIG113 cells that were exposed to BCE had similar effects to TIG113 cells that have been exposed to estradiol. The results from the OVX rat study indicated that the thickness of the collagen was significantly greater in those treated with 3% BCE (1156 ± 36 μm) and in sham rats (845 ± 36 μm) (Nanashima et al., 2018). Therefore, it was made evident that BCE, particularly the four major

compounds (D3G, D3R, C3G, and C3R) in BCE, produce phytoestrogen effects, which are favorable for the skin (**Table 2.4**).

2.7 Summary and Perspectives

There is an increased interest in blackcurrants in the US because of their unique flavor and biological characteristics (**Table 2.5**). Various reports have concluded that there are four major anthocyanins present in blackcurrants (rutinoside and glucoside forms of delphinidin and cyanidin) while other anthocyanins may be present in much smaller concentrations. Further evaluations are needed to examine and document the differences in bioactive compounds in all known cultivars and varieties of blackcurrants. The characteristic bitter and astringent flavors in blackcurrants can be attributed to proanthocyanidins with the intensity of the taste being determined by the mean degree of polymerization of the compounds. While proanthocyanidins are known to promote health (up to a certain molecular size) more research is needed to understand how to overcome the challenges that astringency and bitterness present for the formulation of desirable food products without the addition of sugar. Furthermore, it is equally important that the solution to this issue does not negate the health benefits of these complex compounds. It is not only the berries that have industrial uses, but also the pomace from which extractions can be made to produce natural pigments to be used as food additives. These products are in demand by consumers and can also minimize environmental impacts. Extractions of anthocyanins and other bioactive compounds yield significant concentrations depending on the method, and there is a need to develop additional green and food safe methods for blackcurrants. Drying methods, particularly freeze-drying and convection drying, significantly reduce the concentration of phenolics in blackcurrants. This also presents a gap in knowledge which needs to be addressed to preserve the beneficial aspects of these healthful fruits. Current research has demonstrated that blackcurrants have great potential to

improve overall health particularly with diseases associated with inflammation and regulation of blood glucose. The use of blackcurrants in the cosmetics industry is also attractive due to their ability to activate estradiol pathways and decrease the appearance of wrinkles on the skin. Concentrations of anthocyanins and other bioactive compounds are dependent on the genetics and growing conditions of the berries. However, BC exhibited much higher levels of phenolic compounds when grown in cooler climates. More research is needed to fully understand the breadth of health benefits to be gained from blackcurrants and how these berries can be incorporated into foods.

2.8 Tables and figures

Table 2.1. Extraction methods, solvents, and compounds to obtain phenolics from blackcurrants

Extraction Methods and Starting Materials	Solvents	Compounds Extracted	References
Whole fresh blackcurrant berries were macerated. Phenomenex Gemini C18 (150 x 4.60 mm, 3 µm) column, protected with Phenomenex security guard column	Food grade ethanol (40%, 60%, or 96%), 1% formic acid with 5% acetonitrile in water, 100% acetonitrile	Delphinidin 3-glucoside, delphinidin 3-rutinoside, cyanidin 3-glucoside, cyanidin 3-rutinoside, petunidin 3-rutinoside, pelargonidin 3- rutinoside, peonidin 3-rutinoside, petunidin 3-(6-coumaroyl) glucoside, and cyanindin 3-(6-coumaroyl) glucoside	Nour et al., 2013
Whole freeze-dried blackcurrant berries. Sep.-Pak C18 Plus Short SPE cartridge (Waters, Milford, MA)	80% (v/v) aqueous methanol solution with 0.1% HCl & rinses of water, ethyl acetate, and acidic MeOH	Delphinidin-3-*O*-rutinoside (55.2%), cyanidin-3-*O*-rutinoside (23.2%), and delphinidin-3-*O*-glucoside (18.8%)	Lee et al., 2015
Blackcurrant Juice	Acidified MeOH, ethyl acetate	Delphinidin glycosides, cyanidin glucosides, glucosides of anthocyanins, rutinosides of anthocyanins, anthocyanin degradation products, flavonol glycosides, flavonol aglycones, myricetin glycosides, quercetin glycosides, kaempferol glycosides, isorhamnetin glycosides, glucosides of flavonols, rutinosides of flavonols, and various hydroxycinnamic acids	Mäkilä et al., 2017
Blackcurrant Pomace. SFE-CO2; Helix 1 SFE system with a 50 mL stainless cylindrical extractor vessel (id = 14 mm, length = 320 mm) filled with 15g BC pomace, Soxhlet	SFE-CO_2, hexane, acetone, ethanol:water, pressurized ethanol, pressurized water	Fatty acids (myristic, palmitic, palmitic, palmitoleic, heptadecanoic, stearic, oleic, linolelaidic, linoleic, arachidic, γ-linolenic, cis-11, 14-eicosenoic, linolenic, cis-11, 14-eicosadienoic, behenic, cis-11, 14,17-eicosatrienoic, lignoceric)	Basegmez et al., 2017

Table 2.1 (cont.)

Extraction Methods and Starting Materials	Solvents	Compounds Extracted	References
Blackcurrant Pomace. Separation by centrifugation	HCl/KCl buffer (ph 2.0, 0.1M), 95% ethanol, isopropanol, deionized water	Acid-soluble pectins	Alba et al., 2018
Blackcurrant Pomace. Separation by centrifugation	0.25% w/v ammonium oxalate (pH 4.6) (solid to liquid ratio 1:40)	Calcium-bound pectins	Alba et al., 2018
Blackcurrant Pomace. Separation by centrifugation	6% v/v H_2O_2 (60 pH 11.5) and 3 g L^{-1} of $NaBH_4$ (solid to liquid ratio 1:20)	Alkali-soluble lignin, alkali-soluble hemicelluloses, cellulose	Alba et al., 2018
Solid-phase extraction (Amberlite XAD-7HP (120 g) column, rotary evaporator, high vacuum	Acidified water (0.01% v/v HCl), acidified ethanol (0.01% v/v HCl)	Blackcurrant skins yielded a blackcurrant extract (amorphous violet solid or purified blackcurrant extract)	Cruz et al., 2018
Blackcurrant Pomace. Solid-phase extraction (Amberlite XAD-7HP, 60g).	Water acidified with 0.01% v/v HCl	Dark violet amorphous solid	Rose et al., 2018
Blackcurrant Pomace. Fruit to solvent ratio = 1:3. Ultrasound-assisted extraction with an amplitude range between 0 and 100% (UP100H, Teltow, Germany), 0.50, 5.25, and 10 min.	Water, 50:50 water with ethanol (96%), (85:15) water with citric acid (1 M, 2 M, 1.5 M, 3.0 M), (85:15) ethanol and HCl (1.5 M)	Sonicated extract	Archaina et al., 2017
Blackcurrant Skins. Separation with Buchner funnel loaded with RP-C18 silica gel and lyophilization	Dissolved in 100 mL acidified water (2% HCl), extracted with ethyl acetate (3 x 100 mL), elution solvent (water/methanol 70:30 (v/v) and acidified (2% HCl)	Purified blackcurrant extract (amorphous violet solid) yielded a dark red solid	Cruz et al., 2018

Table 2.2. Color Parameter Measurements of Commercially Available Blackcurrant Beverages.

* Black Box Cabernet Sauvignon red wine added for a comparison between blackcurrant products and a red wine. Ribena was diluted 1:4 with water.

Beverage	Manufacturer and Origin	Ingredients	L*	a*	b*	Hue Angle	Chroma	Color Square
Mathilde Cassis	Ars-sur-Formans, France	Noir de Bourgogne, Blackdown	16	38	20	28 ± 0.1	43 ± 0.01	
Briottet Crème de Cassis	Dijon, France	Blackcurrants, sugar, alcohol	11	32	13	22 ± 0.01	34 ± 0.0	
Cassis Lambic	Vlezenbeek, Belgium	Barley, unmalted wheat, blackcurrant juice, aged hops, wild airborne yeast.	47	36	47	53 ± 0.02	60 ± 0.04	
Pomona Kir	Pamona, IL	Blackcurrants, Southern Illinois apples	60	31	53	60 ± 0.02	61 ± 0.04	
Cider Kir	Nelson, New Zealand	Carbonated cider, 84% apple juice, 10% blackcurrant (Upper Moutere) juice, 5% water, 1% cane sugar, ascorbic acid	39	47	46	44 ± 0.02	66 ± 0.01	
Wasosz Beer	Konopiska, Poland	Water, pilsner malt, caramel malt, hops, yeast, currant juice	60	24	42	60 ± 0.04	49 ± 0.0	
Black Mead	White Winter Winery, Iron River, WI	Honey, blackcurrant, natural flavors	29	40	42	47 ± 0.02	58 ± 0.01	
Ribena	Stockley Park, Uxbridge (England)	Water, sugar, blackcurrant juice from concentrate (23%), citric acid, vitamin C, preservatives (potassium sorbate, sodium bisulphite), color (anthocyanins)	69	35	17	26 ± 0.01	39 ± 0.0	
Fortuna Czarna Porzeczka Nektar	Warsaw, Poland	Water, blackcurrant juice from concentrate, sugar, natural blackcurrant flavor	27	44	38	40 ± 0.01	58 ± 0.0	
Black Box Cabernet Sauvignon*	Madera, CA	Red wine from grapes	13	35	15	23 ± 0.01	38 ± 0.01	

Table 2.3. Examples of processing methods, treatments, and changes in composition of blackcurrant juice.

Processing methods	Treatments	Changes in composition	References
Enzymatic maceration	Pectinase 714L, Biocatalysts Ltd., Cardiff, UK (dosage= 57 mg of enzyme/380 g of berry mash	Increase in mDP (increases astringency and bitterness)	Mäkilä et al., 2017
Heat and enzymatic treatments	50°C, 85°C, Pectinex® Ultra Color	Heat treatment had no effect on juice yield, enzymatic treatment reduced turbidity and viscosity	Bender et al., 2017
Enzymatic maceration	Pectinase 714L, Biocatalysts Ltd., Cardiff, UK (dosage= 57 mg of enzyme/380 g of berry mash	Increase in procyanidins and prodelphinidins (dimers and trimers)	Laaksonen et al., 2015

* All enzymatic treatments increased overall juice yields. Mean degree of polymerization (mDP)

Table 2.4. Examples of health benefits and associated compounds found in blackcurrant products

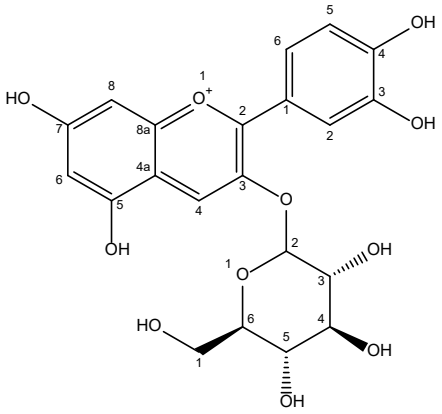
Sample description	Compounds	Properties Beneficial to Health	References
Whole berry and juice	Phenolic compounds (Cyanidin 3-glucoside, cyanidin 3-rutinoside, delphinidin 3-glucoside, delphinidin 3-rutinoside) and ascorbic acid	1. Antioxidant (reduction in oxidative stress by scavenging of free radicals) (biochemical), 2. anti-inflammatory (*in vitro*), 3. hypocholesterolemic (mice & rats), 4. increase in cellular LDL uptake, decrease postprandial blood glucose 5. phytoestrogenic (*in vitro*), ameliorate glucose tolerance (mice & rats), (humans), 6,7. increases fat oxidation (humans), 8. biosynthesis of collagen and production of some peptide hormones	1. Bender et al., 2017; 2. Benn et al., 2014; 3. Cook et al., 2017; 4. Kim et al., 2018; 5. Nanashima et al., 2018; 6. Nour et al., 2013; 7. Strauss et al., 2018; 8. Woznicki et al., 2017
Seeds	Gamma linoleic acid	Antioxidant (reduction in oxidative stress by scavenging of free radicals) (biochemical), potential attenuation of inflammatory responses	Nour et al., 2013; Sergeant, Rahbar, & Chilton, 2016
Leaves	Phenolic compounds (gallic, chlorogenic, caffeic, p-coumaric, feulic, sinapic, salicylic) and flavonoids (rutin, myricetin, quercetin)	Diaphoretic, diuretic, anti-inflammatory (biochemical)	Nour et al., 2013

Each sample description may also represent an extract from that particular source material.

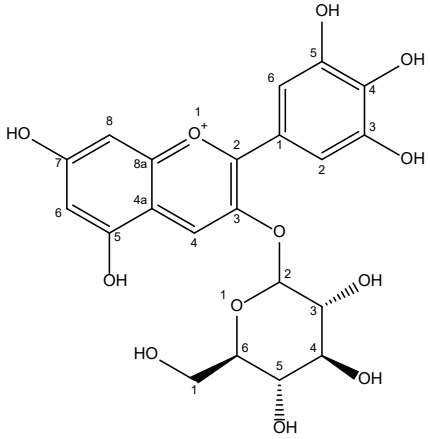
Table 2.5. Examples of blackcurrant products.

Name	Type of Product	Origin	Ingredients	Health Claims	Price in US Dollars
Monin blackcurrant syrup	Premium gourmet syrup	Clearwater, FL	Pure cane sugar, water, natural blackcurrant flavor	None	$9.95 for 750 mL glass bottle
St. Dalfour blackcurrant all natural fruit spread	Fruit spread	Chambord, France	Blackcurrants, concentrated grape juice, fruit pectin	None	$9.89 for 283.5 g
Pepsi Co 1893 blackcurrant cola	Soda/soft drink	Purchase, NY USA	Carbonated water, sugar, caramel color, natural flavor, phosphoric acid, sodium citrate, potassium sorbate, caffeine, gum Arabic, kola nut extract	None	$1.79 for 355 mL
Gabriel Boudier crème de casis	Liqueur	Dijon, France	Blackcurrants, ethanol, sugar	None	$32.99 for 375 mL
Ribena blackcurrant concentrate and ready to drink beverages	Drink	Uxbridge, England	Water, sugar, blackcurrant juice from concentrate (6%), vitamin C, citric acid, color (anthocyanins)	Daily dose of vitamin C	$1.66 for 1 L
Harney and Sons Fine Teas blackcurrant tea	Tea	New York, USA	Black tea, currants, blackcurrant flavor, contains natural flavors	None	$5.99 for 40 g
Standard Process blackcurrant seed oil supplements *Not FDA approved	Nutritional supplements	Wisconsin, USA	Blackcurrant seed oil, gamma-linolenic acid, gelatin, glycerin, water	Encourages proper eicosanoid synthesis, supports the body's normal tissue repair process, supports normal blood flow, supports healthy immune system function	$16.50 for 60 perles

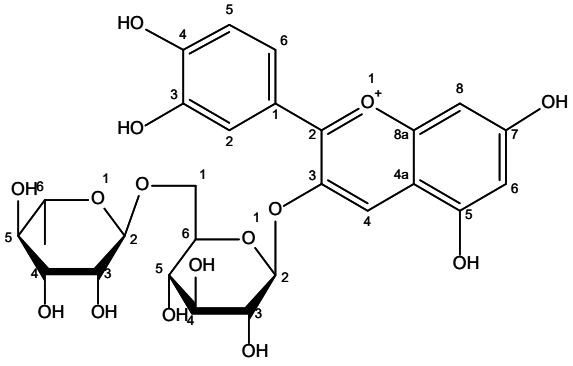
*All prices listed were obtained in 2017



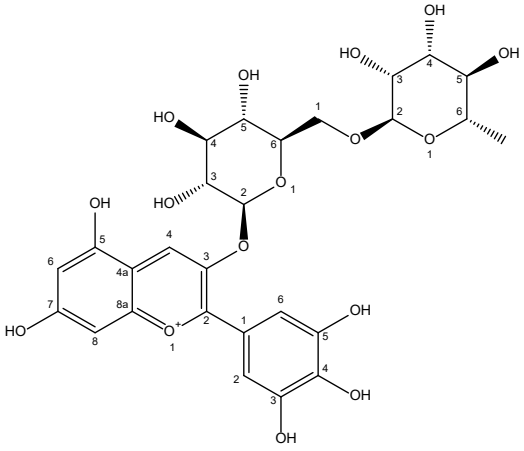
Cyanidin 3-*O*-glucoside



Delphinidin 3-*O*-glucoside



Cyanidin 3-*O*-rutinoside



Delphinidin 3- *O*-rutinoside

Figure 2.1. The four main anthocyanins found in blackcurrants.

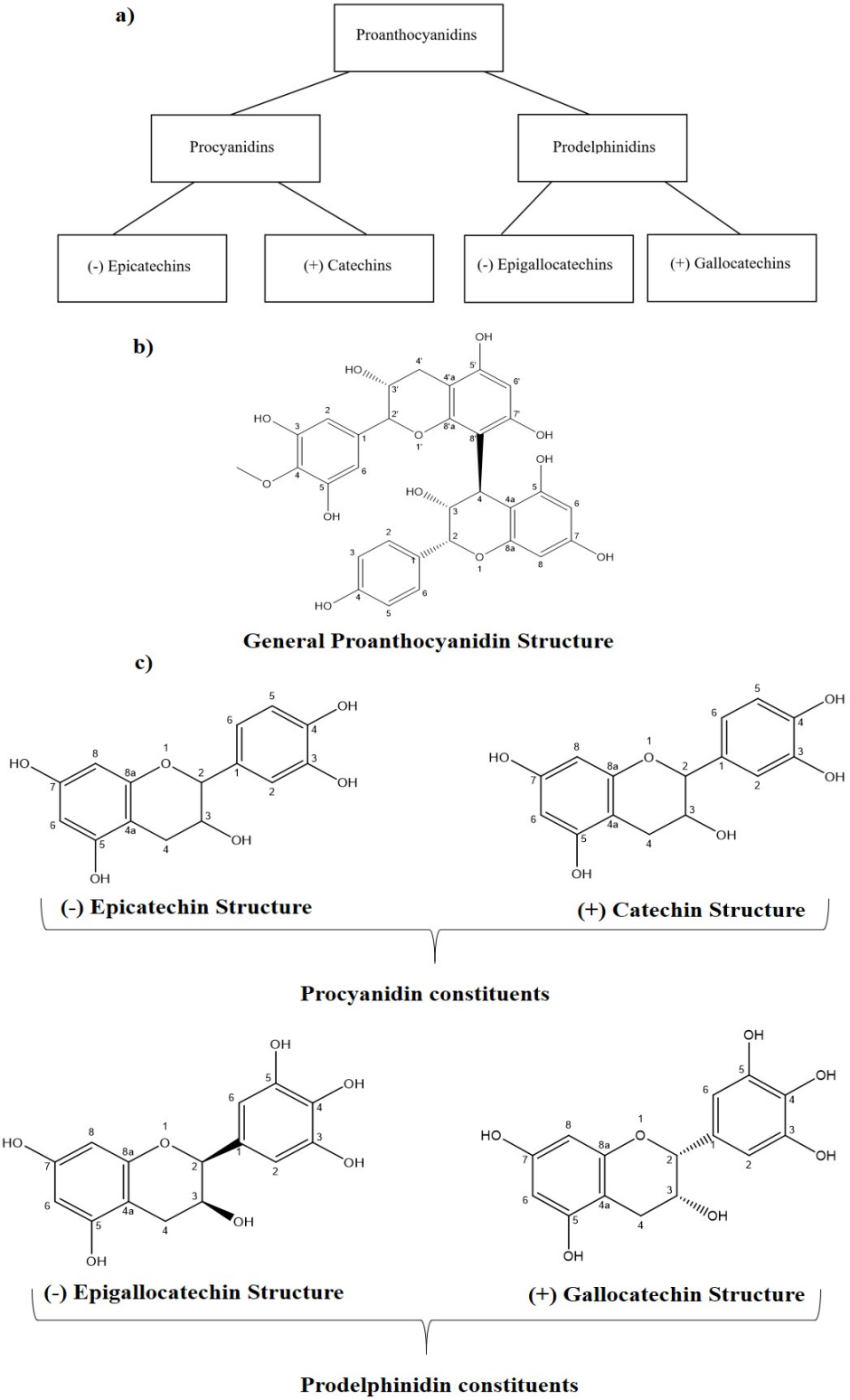
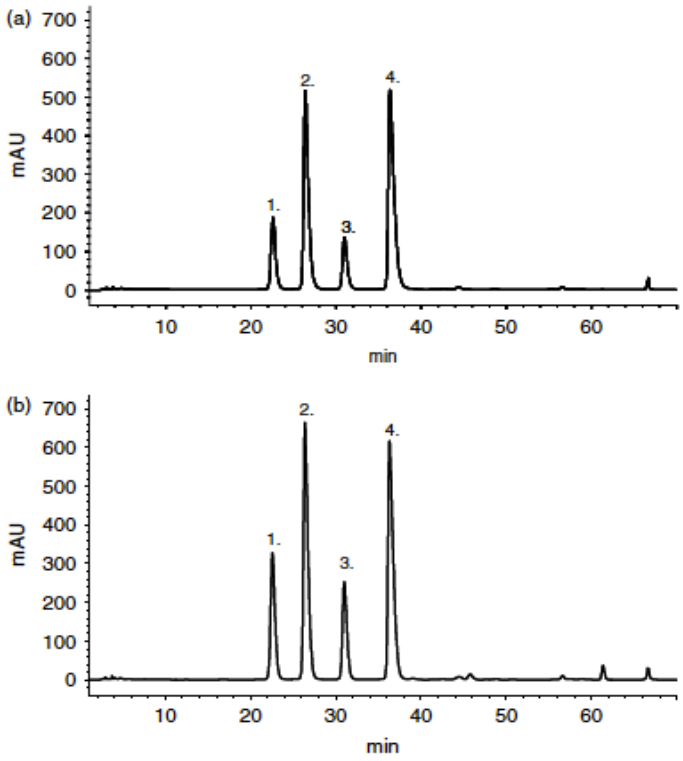


Figure 2.2. Chemical structures of proanthocyanidins and their constituents.



HPLC chromatograms (520 nm) of anthocyanins in black currant juices: (a) reference juice (no enzyme treatment); (b) enzyme-treated (Pectinex BE-3L) juice. Peak identification:
1. Delphinidin 3-O-glucoside,
2. Delphinidin 3-O-rutinoside,
3. Cyanidin 3-O-glucoside,
4. Cyanidin 3-O-rutinoside.

Figure 2.3. Reported HPLC characterization of blackcurrants before and after pectinase treatment. Buchert et al., 2005

2.9 References

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CHAPTER 3: HYPOTHESIS AND OBJECTIVES

3.1 Long-term goal

To provide information about the differences between blackcurrant cultivars and potential uses, particularly as natural pigments with the value-added benefit of having beneficial bioactive compounds.

3.2 Central hypothesis

Bioactive compounds present in blackcurrants can be extracted using a 2 h, water-based ultrasound-assisted method, and that anthocyanins and other phenolic compounds from both fresh berries and fermented berries can inhibit enzymes related to type-2 diabetes.

3.3 Overall objective

To characterize the components of four varieties of BC and their effect on the activities of *α*-amylase, *α*-glucosidase, dipeptidyl peptidase IV (DPPIV), and their 2, 2-diphenyl-1-picrylhydrazyl ($DPPH^{\cdot}$) radical scavenging capacity after water-based ultrasound-assisted extraction, treatment with pectinase, and fermentation.

3.4 Specific aims

Aim 1: To determine the effect of a water-based ultrasound-assisted extraction method on concentration of anthocyanins, and other phenolic compounds, from blackcurrants (**Figure 3.1a**).

Aim 2: To characterize four different cultivars of BC berries (Titania, D16-6-54, Consort, and D16-8-14) and their parts (juice, seeds, and skins) and compare anthocyanins, condensed tannins, and polyphenol concentration of the BC. In addition, their effect on the activities of *α*-amylase, *α*-glucosidase, dipeptidyl peptidase IV (DPPIV), and their 2, 2-diphenyl-1-

picrylhydrazyl (DPPH·) radical scavenging capacity after water-based ultrasound-assisted extraction (**Figure 3.1b**).

Aim 3: To determine the effect of a pectinase treatment and the fermentation of BC mash on concentrations of anthocyanins, total condensed tannins, or total polyphenols. In addition, their effect on the activities of α-amylase, α-glucosidase, dipeptidyl peptidase IV (DPPIV), and their 2, 2-diphenyl-1-picrylhydrazyl (DPPH·) radical scavenging capacity after water-based ultrasound-assisted extraction.

3.5 Figures

Figure 3.1a. Aim 1 and Aim 2 Experimental Design

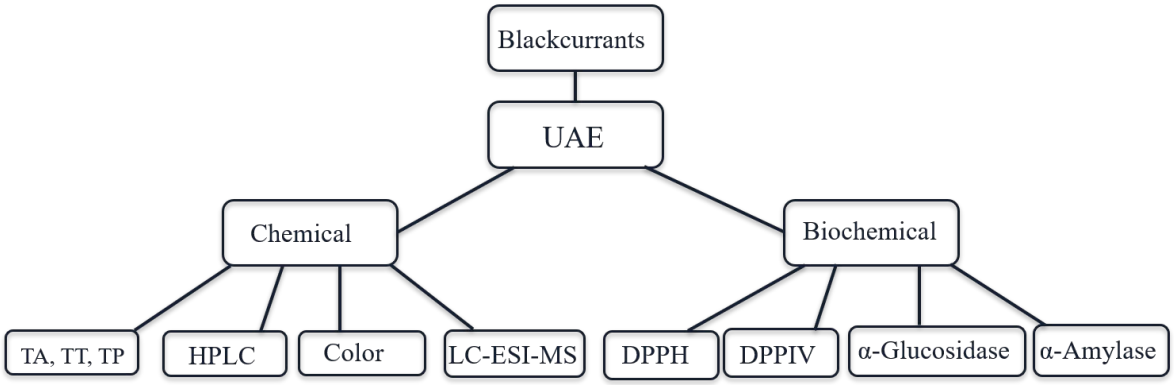
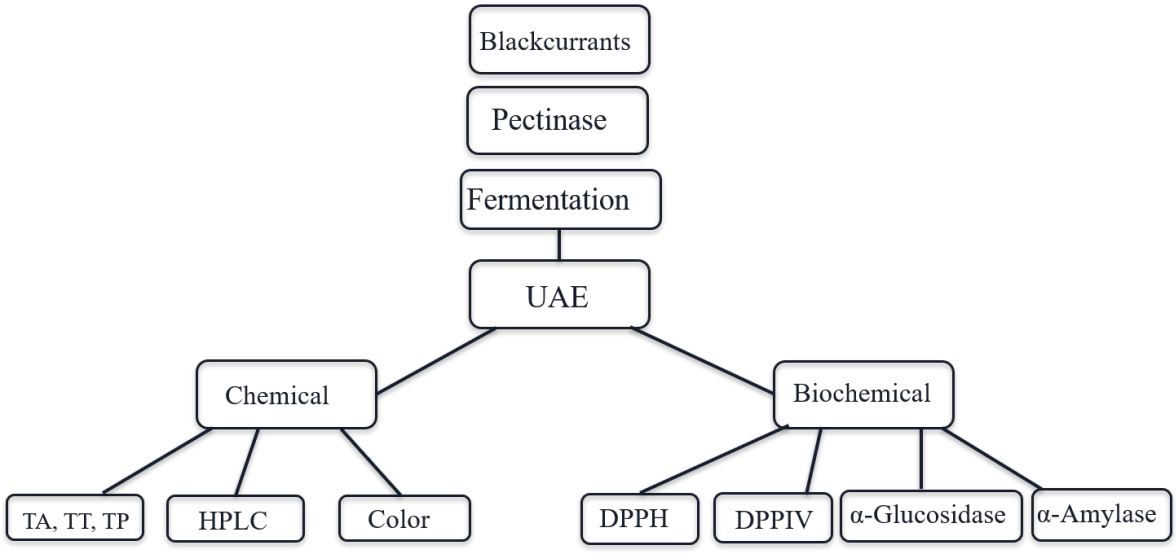


Figure 3.1b. Aim 3 Experimental Design



CHAPTER 4: CHARACTERIZATION OF BLACKCURRANT BERRIES (*Ribes nigrum*) AND THE EVALUATION OF THEIR BIOACTIVE COMPOUNDS AFTER ULTRASOUND-ASSISTED WATER EXTRACTIONS, ENZYMATIC TREATMENT, AND FERMENTATION

4.1 Abstract

The aim was to characterize the chemical composition of four varieties of blackcurrants (BC), and the whole berries in comparison to the parts, after a water-based ultrasound-assisted extraction. Their *α*-amylase, *α*-glucosidase, and dipeptidyl peptidase IV (DPPIV) inhibitory potentials were also evaluated. BC varieties (Titania, D16-6-54, Consort, and D16-8-14) were dissected into parts (juice, seeds, skins), treated with pectinase, and fermented. The highest concentration of total anthocyanins (ANC) were found in the skins of Titania, Consort, and D16-8-14 (19.0 ± 2.0, 19.7 ± 2.7, and 20.3 ± 3.5 mg eq C3G/g dry weight, DW, respectively). The largest concentration of total polyphenols (TP) was in the seeds of Titania and D16-8-14 (34.4 ± 1.3, and 34.6 ± 0.5 mg eq GA/g DW, respectively). Condensed tannins had the largest concentration in the skins of all BC. LC-ESI-MS analysis detected three anthocyanins in all four cultivars and parts (delphinidin 3-*O*-rutinoside, delphinidin 3-*O*-glucoside, and cyanidin 3-*O*-rutinoside). In general, juice, whole, and skins, had the lowest IC_{50} (mg/g of whole dry fruit or DW) of all cultivars for *α*-amylase. Dealcoholized wine-mix- 15 °C had the lowest IC_{50} for *α*-glucosidase across all cultivars, parts, and fermented products. During pectinase treatment, positive correlations were found between heating time (min) at 52 °C and total tannins (TT) and TP (r=0.725 and r=0.731, respectively, p=0.05).

This chapter, with modifications, will be submitted for publication: Cortez, R., Berhow, M., & de Mejia, E.G. (2019*). Food Research International*.

Fermentation increased ANC approximately 5 times. UAE is a viable method for the extraction of ANC from BC and offers the food industry a value-added alternative to synthetic food colorants. The present research also offers valuable information on the chemical differences among four cultivars of blackcurrants, their parts, and their potential biological activities.

4.2 Introduction

Blackcurrants (BC) (*Ribes nigrum*) are berry fruits which are known to have properties beneficial to human health. BC species are woody shrubs that thrive in temperate climates and produce dark purple berries. They are native to northern Europe, northern Asia, and widely cultivated in New Zealand (Corrigan, Hedderley, Langford, & Zou, 2014; Törrönen et al., 2012). Over the past decade, BC have grown in popularity, particularly in the United States (US), because of their unique flavor, appearance and potential health benefits (Millar, 2014; Mortimer, 2014). BC contain bioactive compounds (polysaccharides, unsaturated fatty acids, flavanols, anthocyanins and vitamins) that have potential health benefits such as reducing the incidence of non-communicable diseases such as type 2 diabetes (Shaw, Nyanhanda, McGhie, Harper, & Hurst, 2017). Anthocyanins (ANC), the compounds that give BC their color, are water-soluble glycosides and acylglycosides of anthocyanidins in the form of polyhydroxylated and polymethoxylated heterosides derived from flavylium or 2-phenylbenzopyrilium ions (de Mejia et al., 2015). The colors of anthocyanins are dependent on the acylation, substitutions in the B-ring of the aglycon; ANC are red at an acidic pH, colorless at pH ~4, and blue in neutral pH ranges (Cortez, Luna-Vital, Margulis, & Gonzalez de Mejia, 2017). Delphinidin 3-*O*-rutinoside, one of the main ANC present in BC, has been reported to improve glucose tolerance (Tani, Nishikawa, Kato, & Tsuda, 2017). This is because berries, which contain a high concentration of phenolic compounds, can inhibit starch degrading enzymes such as α-amylase and α-glucosidase making them an option for

the management of type 2 diabetes by reducing glucose absorption (Castro-Acosta et al., 2017; Fan, Johnson, Lila, Yousef, & De Mejia, 2013). Similarly, dipeptidyl peptidase IV (DPPIV) has been considered a pharmaceutical target for the treatment of type 2 diabetes due to its incretin hormone regulatory effects (Fan et al., 2013). Previous studies have established that phenolic compounds, specifically ANC, from different berry species have the ability to inhibit the proteolytic cleavage activities of the DPPIV enzyme (Fan et al., 2013).

The antioxidant capabilities of ANC from berries are often evaluated by analyzing their DPPH· (2, 2-diphenyl-1-picrylhydrazyl) radical scavenging activity. It is important to examine how BC are capable of scavenging free radicals and thus understand their potential to relieve oxidative stress as an added health benefit.

While BC commercial products, especially beverages, are quite common in Europe and other parts of the world, they are not in the US. A challenge, and probably an opportunity, to the food industry is that BC tend to be much bitterer than other berries such as blackberries, blueberries and strawberries. The mean degree of polymerization of proanthocyanidins (condensed tannins), or the average number of flavan-3-ol monomeric units present, dictates the level of perceived bitterness and astringency in BC (Laaksonen, Salminen, Mäkilä, Kallio, & Yang, 2015).

Nine individual ANC (delphinidin 3-*O*-glucoside, delphinidin 3- *O*-rutinoside, cyanidin 3- *O*-glucodside, cyanidin 3- *O*-rutinoside, petunidin 3- *O*-rutinoside, pelargonidin 3- *O*-rutinoside, peonidin 3- *O*-rutinoside, petunidin 3-(6-coumaroyl)-glucoside and cyanidin 3-(6-coumaroyl)-glucoside) have been detected in BC through the use of ethanolic extracts (Nour, Stampar, Veberic, & Jakopic, 2013). Of the reported studies of BC, organic solvents have been used to obtain the extracts. To our knowledge, the present research is the first determination of ANC from BC using a strictly water-based ultrasound-assisted extraction (UAE). The current knowledge

about BC is limited, therefore research is needed to fully understand BC and unlock their biological potential. The objective was to characterize the whole fruit, and its component parts, of four varieties of BC and their effect on the activities of α-amylase, α-glucosidase, DPPIV, as biochemical markers of diabetes. In addition, the antioxidant capacity was measured using 2,2-diphenyl-1-picrylhydrazyl (DPPH$^{\cdot}$), after water-based ultrasound assisted extraction, treatment with pectinase and fermentation.

4.3 Materials and methods

4.3.1 Chemicals

Standards for HPLC analysis were purchased from Alkemist Laboratories (Garden Grove, CA). Their purities were as follows: tulipanin chloride (delphinidin 3-*O*-rutinoside, D3R) $\geq$ 95%, myrtillin chloride (delphinidin 3-*O*-glucoside, D3G) $\geq$ 95%, keracyanin chloride (cyanidin 3-*O*-rutinoside, C3R) $\geq$ 96%, and kuromanin chloride (cyanidin 3-*O*-glucoside, C3G) $\geq$ 96% . Pectinex® Ultra Color (EC 3.2.1.67 – galacturan 1,4-alpha-galacturonidase) was purchased from Novozymes North America (Franklinton, NC). Lavlin 71B (*Saccharomyces cerevisiae*, I.N.R.A - Narbonne), Go-Ferm Protect Evolution ™ , and Fermaid K were purchased at www.amazon.com. DPPIV assay kits were purchased from Promega (Madison, WI). All other chemicals and reagents were purchased from Sigma Aldrich (Saint Louis, MO) unless otherwise stated.

4.3.2 Blackcurrant berries

Titania, D16-8-14 and D16-6-54 BC cultivars were purchased from Highland Valley Farm, (Bayfield, WI, http://www.bayfieldblues.com/), located at latitude 46.832891 and longitude -90.67736. The Consort variety was grown at the South Farms of the University of Illinois at Urbana-Champaign and kindly provided to us by the Department of Crop Sciences. The BC

growing season begins in autumn and the harvest is usually early to midsummer. According to US climate data, the average temperature in Bayfield, WI during the growing season ranged from average lows at 2.3 ℃ in October (2016), to -16.0 ℃ in January (2017), to 13.1 ℃ in July (2017). The average high temperatures ranged from 12.5 ℃ in October (2016), to -6.2 ℃ in January (2017), to 25.0 ℃ in July (2017). The average annual rainfall for the year was 851 mm. Weather conditions in Champaign, IL were warmer with average lows at 5.1 ℃ in October (2016), to -8.9 ℃ in January (2017), to 17.1 ℃ in July (2017). Average high temperatures in Champaign, IL were 18.4 ℃ in October (2016), to 0.4 ℃ in January (2017), to 28.9 ℃ in July (2017) and the average annual rainfall was 1008 mm. All BC were received, frozen, and stored at -20 ℃ until use.

4.3.3 Sample preparation

Samples were prepared by thawing 100 g of each variety of BC at 4 °C. The skins were manually separated from the berries and stored at -20 °C. The pulp was pressed through a 500 µm sieve and the juice collected and stored at -20 °C. Seeds that remained on top of the sieve were rinsed and sonicated with distilled and deionized water (DDI) for 45 min (water bath with ice) using a Branson 2510 Ultrasonic Cleaner (142 Watts, 40 kHz frequency transduction). The seeds were also stored at -20 °C. After all samples were thoroughly frozen and freeze-dried, they were then ground using a coffee grinder, mortar and pestle, and finally, passed through a 500 µm sieve (**Figures 4.1, 4.2, 4.3, and 4.4**).

4.3.4. Pectinase treatment

Preliminary enzyme treatment studies were performed with equal parts of each cultivar (Titania, D16-6-54, Consort, and D16-8-14) mixed together in each of 12 bags (**Figures 4.5 and 4.6).** The BC were then thawed at 4 ℃, then mashed by hand and preheated to 52 ℃. Pectinex®

Ultra Color was added at various doses (0, 400, 600, 800 mL/ton) to catalyze the hydrolysis of α (1→4)-linked D-galacturonic bonds. Samples of each enzyme dose were held at 52 ℃ for different periods of time 90, 120, and 150 min. The contents of each bag were poured into fine mesh nylon bags, which were then pressed. Each of the juices collected was then frozen at -20 ℃ and lyophilized. The remaining solid material was ground and 25.0 mg of each sample was dispersed into 10 mL of ultra-pure water. A water-based ultrasound-assisted extraction of anthocyanins was then conducted in an ice bath using a Branson 2510 Ultrasonic Cleaner (142 Watts, 40 kHz frequency transduction) for 2 h.

4.3.5 Fermentation process

The frozen BC berries were first divided into 2 groups by separating the Consort (labeled as C) cultivar from the Titania, D16-6-54, and D16-8-14 cultivars (mixed batch, labeled as M). The Titania, D16-6-54, and D16-8-14 were all combined together for a total weight of 23.8 kg, and the total Consort weight was 9.0 kg. Both batches were divided into smaller portions in preparation for the enzymatic pre-treatment (**Figure 4.7**). The batch M was split into 15 bags, each with a mass of 1.6 kg. The C batch was split into 6 bags, each with a mass of 1.5 kg. All of the bags of BC were held at 4 °C to allow the berries to thaw. Next, all of the BC were mashed by hand while remaining in their bags. All of the bags of mashed BC were then heated to 52 °C using a water bath. After each of the bags of mash reached 52 °C, pectinase (Pectinex® Ultra Color) was added to each of the bags (600 mL/ton). The bags were removed from the water bath after 120 min and briefly held at 4 °C to allow the mash to cool. The cooled batches of BC mash were added to Cambro containers which were lined with fine mesh Eagle Brewing bags (BAG26, 29” x 29”). Go-Ferm Protect Evolution™ (30 g/100 L) was added to 500 mL of ultra-pure water at 42 ºC to provide a combination of protective and nutritive benefits for optimal fermentation. Lavlin 71B

yeast (25 g/100 L) was added to the Go-Ferm Protect Evolution™ solution when it cooled to 37 ºC and was gently stirred. Fermaid K was added on the second day of fermentation according to the manufacturer's instructions. Total º Brix of each batch was adjusted after the addition of water, with yeast and nutrients, by adding 1.8 – 13.5 g of white sugar to the mash. No other sugar or water was added for the fermentation. Each of the batches (M and C) were fermented at both 15 °C and 23 °C. The duration for all fermentations ranged between 14 and 21 days. All batches were mixed ("punched down") twice a day during fermentation. After the fermentations stopped, juices were pressed out using the fine mesh bags and all of the pomaces were collected and also analyzed. All fermented juices were placed in glass carboys and airlocked for an additional 14 days and SO_2 (50 ppm) was added to prevent spoilage. All fermented juices were bottled and stored at 23 °C. BC enzyme treated samples (BCE), finished *as is* wine (AW), blackcurrant pomace (BCP), and dealcoholized wine (DA) were all lyophilized and kept at -20 °C until analysis (**Figure 4.7**). The remaining solid material was ground and 25.0 mg of each sample was dispersed into 10 mL of ultra-pure water. A water-based ultrasound-assisted extraction of anthocyanins was then conducted in an ice bath (0 °C) using a Branson 2510 Ultrasonic Cleaner (142 Watts, 40 kHz frequency transduction) for 2 h.

4.3.6 Water-based ultrasound-assisted extraction

UAE is an ideal method for the extraction of ANC from BC because it is a green technology that requires less time, does not involve toxic solvents, requires less energy, and most importantly, is safer for human consumption (Galván D'Alessandro, Dimitrov, Vauchel, & Nikov, 2014; He et al., 2016). This extraction method is also faster and more efficient because the ultrasonic waves produce a cavitation effect in the solvent (water), which produces accelerated particle movement, and allows the solvent to better penetrate the material (He et al., 2016) (**Figure 4.3**). All freeze-

dried samples were extracted by mixing 25.0 mg of dry material with 10 mL of ultra-pure water. This mixture was sonicated (142 Watts, 40 kHz frequency transduction) in an ice water bath for cycles of 2 h, centrifuged at 1853 *g* for 30 min, refrigerated and allowed to settle for approximately 12 h. Aliquots of the resulting clear liquid were removed from the tubes and used as the 2 h BC extracts. Additional ultra-pure water was added to each of the pellets that remained in the sample tubes to a total volume of 10 mL. They were then sonicated in an ice water bath for an additional 2 h, centrifuged for 30 min at 1853 *g* and left to settle for approximately 12 h. Aliquots of the resulting clear liquid were removed from the tubes and used as the 4 h BC extracts. Ultra-pure water was added again to each of the remaining pellets from the previous extraction that remained in the sample tubes to a total volume of 10 mL. They were then sonicated in an ice water bath for an additional 2 h and centrifuged for 30 min at 1853 *g* and left to settle for approximately 12 h. Aliquots of resulting clear liquid were removed from the tubes and were used as the 6 h BC extracts. UAE extracts were then stored at -20 °C and were analyzed within one week. The efficacy of water-based UAE of ANC was determined by extracting the same samples in 3 cycles of 2 h (6 h total), until virtually no color was present (**Figure 4.4**).

4.3.7 Colorimetric measurements

All colorimetric measurements were made using the CIELab L^* a^* b^* color system as previously reported (Haggard et al., 2018). The CIELab L^* a^* b^* color system is the most commonly used method to measure colors in foods because of the uniform distribution of colors and because it is the color space which is closest to the way in which humans perceive color (D. Wu & Sun, 2013). CIELab is also an established international standard for color measurements (D. Wu & Sun, 2013). This method has also been previously used to understand the colorimetric

properties of beverages, such as wines and others that use ANC extracts as natural pigments (Gordillo et al., 2014; Haggard et al., 2018).

4.3.8 Quantification of total anthocyanins, total condensed tannins, and total polyphenols

The objective for the evaluation of total anthocyanins (ANC), total condensed tannins (TT), and total polyphenols (TP) was solely to quantify compounds after a water-based UAE based on the methods used, not the optimization of extraction of ANC, TP, or TT. The concentration of ANC was determined in triplicate using an AOAC Official Method as described by a previous study (Lee et al., 2015). Absorbance was read at 520 and 700 nm using a Synergy 2 multi well plate reader (BioTek, Winooski, VT). ANC results were expressed as mg equivalents of cyanidin-3-glucoside (C3G) per g of freeze-dried material (DW). Concentrations of total condensed tannins (TT), were obtained using the vanillin assay (Chen, Somavat, Singh, & Gonzalez de Mejia, 2017; Schofield, Mbugua, & Pell, 2001). All BC extracts were evaluated in triplicate. The absorbance values were then read at 500 nm, with a filter from 492 – 520 nm in a Synergy 2 multi well plate reader (BioTek, Winooski, VT). Catechin was used as the standard and results were reported as mg equivalents of catechin per g of freeze-dried material (DW). An equation ($y = 0.302x – 0.03$) for a standard curve with a range of catechin concentrations from 0.1 mg/mL to 0.8 mg/mL, was used to calculate the TT of each BC extract ($r^2 = 0.99$). The concentration of TP was determined by the Folin-Ciocalteu method (Johnson, Lucius, Meyer, & Gonzalez De Mejia, 2011). Absorbance was read at 690 nm using a Synergy 2 multi well plate reader (BioTek, Winooski, VT). Results were expressed as mg equivalents of gallic acid per g of freeze-dried material (DW). Concentrations of TP were calculated using a standard curve of $y = 0.018x + 0.044$ ($r^2 = 0.99$).

4.3.9 LC-ESI-MS analysis

BC extracts for LC-ESI-MS analysis were prepared by first dissolving 123.6 mg of lyophilized BC parts and whole (powders) in 4 mL of 0.1% HCl in methanol. The samples were then sonicated for approximately 1 h and then stored at 4 °C overnight. All samples were filtered using 0.45 μm, PVFD syringe filters before analysis. All BC extracts were evaluated using a Thermo Electron LTQ Orbitrap Discovery Mass Spectrometer (linear ion trap, LTQ XL) MS, coupled to a high precision electrostatic ion trap (Orbitrap) MS with a higher energy C-trap dissociation (HCD) cell attached, with an Ion Max electrospray ionization (ESI) source; a Thermo Scientific ACCELA series HPLC system (ACCELA 1250 UHPLC pump; ACCELA1 HTC cool stack autoinjector; and a ACCELA 80 Hz PDA detector); all running under Thermo Scientific Xcalibur 2.1.0.1140 LC-MS software. For the HPLC conditions, the column was a 3 mm x 150 mm Inertsil reverse phase C-18, ODS 3, 3 μm column (Metachem, Torrance, CA). The anthocyanin analysis protocol used 10% methanol as the initial solvent water with 0.1% formic acid at a flow rate of 0.25 mL per min. After injecting 1 μL or less, the column was held at the initial conditions for 2 min, then developed with a linear gradient to 100% methanol and 0.1% formic acid over an additional 50 min. The column effluent was monitored at 520 nm with the PDA detector. Mass spectrometry was run with the ESI probe in the positive mode. The source inlet temperature was set to 300 ºC and the sheath gas rate was set at 50 arbitrary units while the auxiliary gas rate was set at 5 arbitrary units and the sweep gas rate at 2 arbitrary units. The maximal mass resolution was 30,000 and the spray voltage 3.0 kV, while the tube lens was 100 V. The MS was calibrated on a weekly basis, with a standard calibration mixture recommended by Thermo Scientific. Signal detection was optimized by running the auto tune software feature as needed. Other parameters were determined and set by the calibration and tuning process. The

software package was set to collect mass data between 100-2000 AMU and the most significant sample ions generated under these conditions were $[M]^+$.

4.3.10 HPLC analysis

All HPLC analysis of BC extracts were performed in triplicate using a Hitachi HPLC System (Hitachi High Technologies America, Inc. Schaumburg, IL) with a L-7100 pump and multi-wavelength detector according to a slightly modified protocol .[7] The analysis was conducted using an injection volume of 20 μL and a flow rate of 1 mL/min with helium bubbled mobile phases. A gradient from A. 2% formic acid in water and B. 0% acetonitrile to 40% acetonitrile in a linear fashion using a Hichrom Prevail 5 μL, 250 x 4.6 mm, C18-Select column for 35 min. ANC concentrations were determined with 7-point calibration curves from 0.005 mg/mL to 0.06 mg/mL for delphinidin 3-*O*-glucoside (D3G), delphinidin 3-*O*-rutinoside (D3R), cyanidin 3-*O*-glucoside (C3G), and cyanidin 3-*O*-rutinoside (C3R). Points were plotted as the area under the curve versus the concentration of standard for both C3G and D3G ($r^2 = 1.00$ and $r^2 = 0.99$, respectively). Peaks from BC extracts and their corresponding compounds were verified by comparisons with current literature and the evaluation of pure standard compounds. Peaks were identified at 520 nm based on retention times for standards.(De Mejia et al., 2015) HPLC analysis of BC extracts were also performed at 280 nm for comparison and verification of phenolics.

4.3.11 α-Amylase inhibition

Evaluation of α-amylase inhibition was conducted according to the following slightly modified method.[24] Briefly, a negative extract control to account for any potential interference (500 μL BC extract + 1% starch solution + 96 mM 3,5-dinitrosalicylic acid solution, no enzyme added) was prepared. A positive extract control (no BC extract, 1% starch solution, α-amylase, 96

mM 3,5-dinitrosalicylic acid and phosphate buffer). A 500 μL BC extract or 1 mM acarbose (for comparison), a commonly used drug for management of type 2 diabetes, were added to each test tube followed by 500 μL of α-amylase solution (0.02 M sodium phosphate buffer with 0.006 M sodium chloride and 13 U/mL of α-amylase) and incubated at 25 °C for 3 min (Johnson et al., 2013) A 1% soluble starch solution (500 μL) in 0.02 sodium phosphate buffer, pH 6.9 with sodium chloride was then added to each tube and incubated for an additional 3 min at 25 °C. The reaction was then stopped with 1.0 mL of dinitrosalicylic acid color reagent and then placed in a 100 °C water bath for 10 min. Each of the mixtures in the tubes was diluted 1:10 ratio with ultra-pure water. The absorbance was then measured at 540 nm using a Synergy 2 multi well plate reader (BioTek, Winooski, VT). An additional blank consisting only of ultra-pure water was also measured to aid in the calculation of results. The calculations for α-amylase results were adjusted taking into consideration any interference that may have been present and to provide a more accurate result. The calculation was as follows:

$$\% \text{ Inhibition} = \left[\text{Abs}_{\text{Positive Extract Control}} - \frac{(\text{Abs}_{\text{BC Extracts}} - \text{Abs}_{\text{Negative Extract Control}})}{\text{Abs}_{\text{Positive Extract Control}}}\right] \times 100$$

4.3.12 α- Glucosidase inhibition

BC extracts were analyzed for percent inhibition of α-glucosidase according to a slightly modified procedure.[24] Briefly, 50 μL of BC extract, a blank (0.1 M phosphate buffer, pH 6.9), or 1 mM acarbose (for comparison) was added to each well of a 96 well plate, followed by 100 μL of α-glucosidase solution (1.0 U/mL in 0.1 M phosphate buffer, pH 6.9), then incubated at 25 °C for 10 min. Next, 50 μL of 5 mM p-nitrophenyl- α-D-glucopyranoside in 0.1 M phosphate buffer (pH 6.9) was added to each well and incubated for 5 min at 25 °C. The absorbance was read at 405 nm using a Synergy 2 multi well plate reader (BioTek, Winooski, VT). The calculation for percent inhibition was as follows:

$$\% \text{ inhibition} = \left(\frac{\text{Absorbance}_{\text{BC Extract}} - \text{Absorbance}_{\text{BC Extract No Enzyme}}}{\text{Absorbance}_{\text{BC Extract}}}\right) \times 100$$

4.3.13 Dipeptidyl peptidase IV (DPPIV) inhibition

Dipeptidyl peptidase IV (DPPIV) is a proteolytic enzyme that when inhibited, it reduces glucose levels.[25] The DPPIV-GloTM protease assay (G8351, Promega, Madison, WI) was used to measure DPPIV activity inhibition following the manufacturer's protocol. Briefly, 50 μL of DPPIV-GloTM reagent was added to each well of a white walled 96-well plate containing 50 μL of blank (DPPIV-GloTM reagent + vehicle control for enzyme treatment agent or inhibitor), positive control (DPPIV-GloTM reagent + vehicle control + purified DPPIV enzyme), or BC extracts (DPPIV-GloTM reagent + BC extracts + purified DPPIV enzyme). Luminescence was recorded 30 min after the addition of the DPPIV-Glo^{TM} reagent with a Synergy 2 multi well plate reader (BioTek, Winooski, VT).

4.3.14 Radical scavenging activity determination by 2,2- diphenyl-1-picrylhydrazyl (DPPH·)

All BC extracts (0.31 mg/mL, 0.63 mg/mL, 1.25 mg/mL, and 2.50 mg/mL) were analyzed using a Synergy 2 multi well plate reader (BioTek, Winooski, VT) to determine their radical scavenging activity using a modified DPPH· assay (Kedare & Singh, 2011; Mishra, Ojha, & Chaudhury, 2012). A 152.16 µM DPPH· solution made with 80% MeOH was used (Fukumoto & Mazza, 2000). A gallic acid (GA) standard curve with five concentrations ranging from 50 to 600 µM was used to quantify BC extracts antioxidant activity. GA was chosen as the standard antioxidant because it was determined that it is approximately six times more efficient as an antioxidant than sesamol and has greater antiradical properties than both ascorbic acid and butylated hydroxytoluene (BHT) (Mishra et al., 2012). The standard curve's regression equation ($r^2 = 0.99$) at 515 nm was as follows: $y = 0.0007x + 0.0038$**;** where: y is the absorbance read at 515

nm and x is the concentration of GA (μM) in 100% MeOH. This equation was used to calculate the μM eq GA/mg DW of the extracts. Briefly, 20 μL of GA (GA + DPPH·) for the standard curve, BC extracts (BC extracts + DPPH·), positive control (80% MeOH + DPPH·) and negative control (BC extracts + ultra-pure water) were added to each well of a 96-well plate as previously described (Fukumoto & Mazza, 2000). Next, 200 μL of 152.16 μM DPPH· solution were added to the 96-well plate, while taking measures to protect the reaction from vaporization and light (parafilm and aluminum foil). The 96-well plate was then incubated at room temperature for 30 min. Absorbance was then read at 515 nm (Mishra et al., 2012).

4.3.15 Statistical analysis

Data are expressed as the mean ± standard deviation of at least three independent measurements. A one-way ANOVA analysis was conducted to compare data between groups, UAE times, pectinase treatments, and fermentation products using JMP version 13.0. The Tukey-Kramer test was conducted to determine statistical differences among means, which were considered significant at $p < 0.05$. Enzymatic inhibition essays were analyzed using Graph Pad Prism 8. Graph Pad was used for the interpolation of the IC_{50} values and also for the Pearson correlations.

4.4 Results and discussion

4.4.1 Berry sizes

Consort and D16-6-54 were the smallest of the four cultivars with an average diameter of 0.97 ± 0.1 cm and 1.04 ± 0.1 cm, respectively. The diameters of the other cultivars were D16-8-14 (1.23 ± 0.2 cm) and Titania (1.37 ± 0.1 cm). Consort and D16-6-54 had the highest degree of

soluble solids, (20.6 ± 0.6 and 19.0 ± 0.3 °Bx, respectively). The other Brix° measurements were Titania (15.0 ± 0.2 °Bx), and D16-8-14 (18.3 ± 0.5 °Bx) (**Figure 4.8**).

A study has reported diameters of fruits from fifteen blueberry cultivars ranging from 12.8 mm to 18.7 mm (Johnson et al., 2011). Whole berries from all 4 cultivars, which were mashed, showed that their average pH was 2.9, with an average °Brix of 18.2 ºBx, which is consistent with the findings of others (Kikas et al., 2017; Kozák, Békássy-Molnár, & Vatai, 2009). A different study, which compared organic versus conventional BC, reported that their Titania berries had a °Brix range from 14.3 to19.3 with the lower values coming from organic fruits (Kikas et al., 2017). It has also been noted that BC cultivated at higher latitudes (66° 34' N) contain higher amounts of volatiles (Marsol-Vall, Kortesniemi, Karhu, Kallio, & Yang, 2018).

4.4.2 Component yields

The yields of each of the individual BC components (juice, seeds and skins) varied depending on the cultivar. Titania had the cleanest separation of skin from the berry and as a result had the largest percent mass of skins (31.3%) in comparison with the average of the four cultivars (19.6%). D16-8-14 presented the highest percent mass of juice (48.5%) in comparison with the average of the four cultivars (31.3%), and the Consort cultivar had skins that are thinner and weaker. A different study, which analyzed six different cultivars of BC, reported an average juice yield of 58.1% (Landbo & Meyer, 2004). Enzymatic treatments, such as the use of pectinases, are effective means to increase juice yields and reduce turbidity. It has also been indicated that the use of pectinases, cloned from *Aspergillus niger* and *Aspergillus aculeatus*, can produce BC juice yields as high as 78.9% (Landbo & Meyer, 2004).

4.4.3 Colorimetric measurements

BC extracts, with an average pH = 2.9, appeared to be a reddish color to the naked eye. The hue angles increased in value as sonication time of the remaining solids from the previous extraction increased, indicating a significant shift and overall loss in color to an almost clear appearance (**Table 4.1**). After three consecutive extractions of the remaining solid material, most of the color compounds were extracted. The same comparison of chroma, which indicates the intensity or richness of color, showed a sharp decrease as sonication times increased (**Table 4.1**). An increase in the L^* value was observed across all samples, except for the seeds. The overall loss of color indicates a successful extraction of color compounds. There was a 215% difference in hue angles between the color averages of whole berries after 2 h and 4 h extractions and a 640% difference between 2 h and 6 h. The difference in chroma between 2 h and 4 h was 80% and a comparison of color parameters after the 2 h, 4 h, and 6 h water-based UAE extractions. The color fading with extraction time indicated an efficient extraction of colored pigments. Titania, D16-6-54, Consort and D16-8-14 whole berries had ΔE values of 22.9, 19.6, 19.4 and 22.3, respectively which were the color differences between the 2 h UAE and 6 h UAE. Color differences (ΔE) between the 2 h UAE and 4 h UAE for whole berries were calculated as being 20.6 (Titania), 14.0 (D16-6-54), 15.4 (Consort) and 16.0 (D16-8-14). Interestingly, recent research has demonstrated that ANC rich extractions from BC skin waste can be used as alternative to synthetic hair dyes, which speaks to the power of BC pigments (Rose et al., 2018).

4.4.4 Total anthocyanins, condensed tannins, and polyphenols concentrations

BC extracts were evaluated to determine differences in ANC between 2, 4, and 6 h of UAE extraction. ANC represent the majority of bioactive compounds (approximately 55%) found in BC and are water soluble, making a water-based UAE a reasonable option for extraction (Farooque,

Rose, Benohoud, Blackburn, & Rayner, 2018). It was determined that > 50% of ANC can be extracted from BC when using a 2 h UAE extraction process (**Table 4.2**). After 2 h UAE, Consort and D16-8-14 whole berries had the highest concentration of ANC (14.5 ± 2.8 and 14.8 ± 2.8 mg eq. C3G/g, DW). It was also discovered that there was no statistical difference in ANC among Titania, Consort, and D16-8-14 skins (19.0 ± 2.0, 19.7 ± 2.7, and 20.3 ± 3.5 mg eq. C3G/g, DW, respectively) ($p > 0.05$) after a 2 h UAE extraction. The Consort whole and juice had the highest ANC concentration after 4 h UAE (whole 7.5 ± 0.6 and juice 7.1 ± 0.0 mg eq. C3G/g, DW) ($p < 0.05$). Results from 6 h UAE showed no statistical differences among samples, except for D16-8-14 whole and seeds, both of which had lesser ANC concentrations. A previous study first extracted ANC from BC using sulfured water (SO_2 concentrations in the range of 28 to 1372 ppm) and then quantified ANC (Cacace & Mazza, 2002). The average result of 15.3 mg eq C3G/100 g (dry basis) in this study, did not differ significantly from the juice in our study that used 2 h water-based UAE extraction. Another research examined two different BC cultivars (Öjebyn and Titan) and reported a ANC range of 756 ± 12 mg eq C3G/100 g to 1064 ± 8 mg eq C3G/100 g after BC extraction using 70% acetone as solvent (Nour et al., 2013). These results are similar to the findings in the present research, which was done without the use of organic solvents. Pectinase treatments, regardless of dose used, increased the concentration of ANC in comparison to the juice for cultivars Titania and D-16-8-14 **(Figure 4.9)**. Pearson correlations revealed that there was no correlation between ANC and pectinase treatment. After comparing the selected Pectinex® dosage and heating time with the AW fermentation products it was concluded that fermentation increased ANC by 5 times.

TT concentrations after 2 h UAE extraction were evaluated (**Table 4.3**). Consort, D16-6-54, and D16-8-14 whole berries had the highest concentrations of TT and were not statistically

different ($p > 0.05$). There was no statistical difference in TT among the seeds of all cultivars ($p > 0.05$). A comparison of the average of the whole fruit for all cultivars, with enzymatic treatment and fermentation products, TT did not increase which is consistent with Laaksonen et al. (2015) (**Figure 4.9**). However, Pearson correlations showed that the heating of the BC mash for the pectinase treatment did increase TT concentrations ($r^2 = 0.535$), while the fermentation process reduced TT by half. BC are rich in condensed polymeric tannins (proanthocyanidins), which are comprised of less than 10 subunits if they have not undergone enzymatic treatment (Laaksonen et al., 2015). Any extraction methods for the quantification of proanthocyanidins should not involve the use of acetone because ANC may react with the acetone to produce proanthocyanidins, which would give a false result (X. Wu, Gu, Prior, & McKay, 2004a). Proanthocyanidins are what impart the astringency in the characteristic flavor and taste of BC. This perception of astringency occurs because of the interaction of proanthocyanidins (2 to 8 units interacting with proteins) (Fennema O. R., 1993). While there is not much known about the exact mechanism that produces these sensations, there is speculation that the perception of astringency and bitterness is due to the binding of proanthocyanidins to salivary proteins which precipitate (Laaksonen et al., 2015). A recent study concluded that this phenomenon is produced by the interaction of proanthocyanidins with proline clusters and nearby residues (Soares et al., 2018). These compounds are generally categorized as monomers, dimer, trimers, tetramers, oligomers and polymers, depending on their degree of polymerization (Chen et al., 2017). It is thought that compounds with a degree of polymerization larger than 4 have very low to no bioavailability because of their large structures (Chen et al., 2017). While these compounds may not be absorbed, they eventually reach the colon where they are transformed into metabolites by the microbiota present, which may explain their positive health effects (Chen et al., 2017).

TP from BC extracts were evaluated after 2 h UAE extraction (**Table 4.3**). The Consort cultivar had the highest concentration of TP, except for their seeds (whole 22.0 ± 0.5, juice 23.1 ± 1.0, and skins 24.0 ± 0.6 µg eq. GA/mg DW). Consort was by far the dominant cultivar in TP concentration for the whole fruit and the juice. No statistical difference was noted between juice samples from Titania, D16-6-54 and D16-8-14 ($p > 0.05$). However, it has been reported that BC leaves have 37% more TP than berries (Teleszko & Wojdyło, 2015). An average of 1480.5 mg eq GA/L for a 60% ethanolic (1 L) extraction, performed by the maceration of 600 g of BC berries over a three-week period, has been reported in a separate investigation (Nour et al., 2013). In comparison, the water-based UAE method was only able to extract ~1.5% of TP (Nour et al., 2013). A much different result was achieved by Lee et al. (2015) when extracting BC using 160 mL of 80% (*v*/*v*) aqueous methanol with 0.1% HCl to remove compounds from 8 g of freeze-dried BC. They prepared an ANC fraction using Sep-Pak C18 Plus Short SPE cartridge and then quantified TP. Their results (2382.4 ± 60.8 mg eq GA/100 g, DW), are roughly equivalent to the concentration of TP quantified in our study for Consort juice after water-based UAE. It is possible to bypass the use of organic solvents and still achieve high results by the water-based UAE method (Lee et al., 2015). A comparison of the average of the whole fruit for all cultivars with pectinase treated BC mash increased the TP concentrations (**Figure 4.9**). These results were confirmed with Pearson correlations ($r^2 = 0.521$). The fermentation procedure increased the TP.

4.4.5 LC-ESI-MS analysis

The LC-ESI-MS analysis of BC confirmed that four major ANC (delphinidin 3-*O*-glucoside, delphinidin 3-*O*-rutinoside, cyanidin 3-*O*-glucoside and cyanidin 3-*O*-rutinoside) are present in BC extracts, as previously reported by other investigators (Farooque et al., 2018; Schofield et al., 2001) (**Figure 4.10**). A study from 2003 reported that BC contain delphinidin 3-

O-rutinoside (MS- 611 (303+162+146) (m/z)) and delphinidin 3-*O*-glucoside (MS 462 (303+162) (m/z)) with 979 and 538 mg/kg, by fresh weight, respectively, which made up the majority of ANC present (Määttä, Kamal-Eldin, & Riitta Törrönen, 2003). This study reported cyanidin 3-*O*-rutinoside (MS- 595 (287+162+146, m/z) and cyanidin 3-*O*-glucoside (MS- 449 (287+162,m/z) as being 1163 and 331 mg/kg, by fresh weight, respectively (Määttä et al., 2003). It was also noted that no other ANC were recorded, all flavonol glycosides were reported as 102 mg/kg, by fresh weight, and all hydroxycinnamic acid derivatives as 84 mg/kg, by fresh weight (Määttä et al., 2003). Our research revealed that Titania was the only cultivar in which peonidin 3-*O*-rutinoside was detected; a finding consistent with previous studies (X. Wu, Gu, Prior, & McKay, 2004b). A number of other phenolic peaks with absorbances at wavelengths 280 nm and 340 nm were seen in the extracts, but at relatively low concentrations. Proanthocyanins were also present in these extracts (Fan et al., 2013; Kähkönen et al., 2001; Mikulic-Petkovsek, Slatnar, Stampar, & Veberic, 2012; Wu et al., 2004b).

One previous BC characterization study was able to identify a total of 14 compounds, again, with the glucoside and rutinoside forms of delphinidin and cyanidin being the four major compounds present (X. Wu et al., 2004b). Other phenolic compounds were reported present in BC, regardless of their relative abundance, in black, green, red and white currant juices (Kikas et al., 2017; Mishra et al., 2012; Teleszko & Wojdyło, 2015). While these results suggest that BC might not have the most diverse ANC species, this would be advantageous to anyone seeking to extract and isolate pure compounds.

4.4.6 HPLC analyses

HPLC analyses determined that D16-8-14 had the highest concentrations of D3G, D3R, and C3G in skins of all 4 cultivars (65.2 ± 0.4, 247.6 ± 2.8, and 180.9 ± 3.6 mg/g, DW,

respectively) (**Table 4.4**). Analysis showed that the Pectinex dose of 400 mL/ton held for 150 min at 52 °C provided some of the highest overall concentration of D3R (153.5 ± 1.3 mg/g, DW). Results revealed that there was no statistical difference between 400 mL/ton held for 150 min and 600 mL/ton held for 120 min for D3G concentration values. The same is true for C3R concentrations between 400 mL/ton held for 150 min and 800 mL/ton held for 150 min. The as is wine- Consort- 23 °C had the highest concentrations of D3G, D3R, and C3R of all of the fermented products and byproducts. A previously reported HPLC analysis confirmed the existence of four major compounds (delphinidin 3-glucoside, delphinidin 3-rutinoside, cyanidin 3-glucoside and cyanidin 3-rutinoside) in six different BC cultivars (Ben Alder, Ben Nevis, Ben Lomond, Ben Tirran, Titania and Ukraine) (X. Wu et al., 2004b). These results differ from the present work in that C3G did not resolve, which could possibly be explained by the tendency for ionization of compounds by ultrasound. However, MeOH/H_2O/AcAc were used for the extraction of phenolics from BC by Wu et al. (2004), which could account for any differences in the concentrations and the presence of ANC (Cacace & Mazza, 2002).

4.5 Diabetes Related Enzymes

4.5.1 IC_{50} α-Amylase

Table 4.5 presents the half maximal inhibitory concentration of α-amylase (IC_{50}) for BC extracts and BC fermentation products. Juices and skins from all cultivars had the lowest IC_{50}values, (). These results suggest that fresh frozen BC are more potent inhibitors of α-amylase activity than fermented products. A study, which evaluated the ability of blackberry/blueberry wine to inhibit α-amylase activity, demonstrated that neither ANC nor proanthocyanidins significantly contributed to the inhibition of the enzyme (Fan et al., 2013).

4.5.2 IC_{50} α-Glucosidase

BC fermented products demonstrated to be more potent inhibitors of α-glucosidase activity than that of α-amylase. As "is wine" and dealcoholized wine had the lowest IC_{50} regardless of fermentation temperature (**Table 4.5**). A previous publication found that α-glucosidase inhibition was greater when the concentration of TP was higher which is consistent with our findings. A positive correlation was seen between TP and α-glucosidase (r^2= 0.426). A similar study reported that ANC and proanthocyanidins can inhibit α-glucosidase activity (Fan et al., 2013).

4.5.3 IC_{50} Dipeptidyl peptidase IV inhibition

Although some statistical differences were detected, DPP-IV values were very consistent among all samples (**Table 4.5**). The inhibition of DPPIV limits proteolytic cleavage and therefore increases the concentration of endogenous incretins, like peptide-1 (GLP-1), in a similar fashion as (Vinayagam, Jayachandran, & Xu, 2016). After food is ingested, GLP-1 is secreted by intestinal L-cells as a thirty-amino-acid hormone (GlaxoSmithKline, 2006). This hormone gets cleaved by DPPIV and becomes inactive, but it is the active form that helps to regulate postprandial blood glucose levels by stimulating the secretion of insulin (GlaxoSmithKline, 2006). Therefore, food interventions that inhibit DPPIV activity are desirable.

4.6 Antioxidant Activity

4.6.1 IC_{50} DPPH· assay and radical scavenging

The IC_{50} of DPPH· free radical scavenging capacity of BC extracts and fermented products is presented in **Table 4.5**. There was no statistical difference among D16-6-54 seeds (0.01 ± 0.02 mg/g, DW), BC Pomace- Consort- 15 °C (0.01 ± 0.01 mg/g, DW), and BC Pomace- Mix- 15 °C (0.01 ± 0.02 mg/g, DW). BC pomace (0.31 mg/ml) gave a DPPH inhibition of 83% in comparison

with gallic acid (0.10 mg/ml) that gave 100% inhibition; therefore, pomace is a promising inhibitor of DPPH· activity. It has been confirmed that polysaccharides present in BC are good for scavenging free radicals, such as DPPH·, *in vitro* (Xu et al., 2016). A positive correlation was noted between TP and DPPH· (r^2= 0.279). IC_{50} results from the study on BC polysaccharides were fairly consistent with the results in the present research (Xu et al., 2016). A different study evaluated the difference in radical scavenging activities between D3R, C3R, and their acylated products and it was concluded that their acylated products was significantly higher ($p < 0.05$) (Yang, Kortesniemi, Ma, Zheng, & Yang, 2019).

4.7 Conclusions

In summary, to our knowledge, this is the first report detailing the quantification of ANC in BC and BC fermented products using a strictly water-based ultrasound extraction. BC fruits have potential to offer many solutions for current issues facing the food industry such as consumer demand for more healthful colored processed foods. ANC and other bioactive compounds from BC can successfully be extracted by waster-based UAE which could be used as safe and natural pigments with the potential health value-added benefit. As demand for BC and BC products increases, we believe the present research provides industry with insight into how BC products and byproducts might better be utilized to produce higher quality food products that contain health promoting bioactive compounds.

4.8 Tables and figures

Table 4.1. Measurement of color parameters of four cultivars of blackcurrants after 2, 4, 6 h water-based UAE.

	2 h UAE Extraction						4 h UAE Extraction						6 h UAE Extraction					
	L*	a*	b*	Hue Angle	Chroma	Color Square	L*	a*	b*	Hue Angle	Chroma	Color Square	L*	a*	b*	Hue Angle	Chroma	Color Square
Titania																		
Whole	87	17	2	8 ± 0.0 ^{b}	18 ± 0.0 ^{d}		94	1	1	45 ± 0.3 ^{a}	2 ± 0.0 ^{d}		98	1	2	67 ± 0.1 ^{d}	2 ± 0.0 ^{d}	
Juice	86	20	2	6 ± 0.0 ^{d}	20 ± 0.1 ^{d}		93	7	1	6 ± 0.0 ^{d}	7 ± 0.1 ^{c}		100	0	0	47 ± 1.0 ^{d}	0.3 ± 0.0 ^{c}	
Seeds	89	9	3	19 ± 0.1 ^{d}	10 ± 0.7 ^{a}		89	1	6	82 ± 0.1 ^{b}	6 ± 0.0 ^{b}		94	1	6	84 ± 0.0 ^{b}	6 ± 0.0 ^{c}	
Skins	70	45	12	15 ± 0.7 ^{a}	47± 0.0 ^{a}		86	9	3	18 ± 0.0 ^{c}	10 ± 0.3 ^{b}		95	1	3	70 ± 0.0 ^{a}	3 ± 0.0 ^{d}	
D16-6-54																		
Whole	83	20	4	10 ± 0.9 ^{a}	21 ± 0.1 ^{c}		84	7	5	35 ± 0.1 ^{b}	8 ± 0.0 ^{a}		89	2	7	77 ± 0.0 ^{a}	7 ± 0.0 ^{b}	
Juice	75	36	8	13 ± 0.1 ^{a}	36 ± 0.1 ^{b}		83	5	5	41 ± 0.0 ^{b}	7 ± 0.0 ^{b}		98	0	1	85 ± 0.0 ^{a}	1.2 ± 0.0 ^{a}	
Seeds	88	8	5	32 ± 0.1 ^{b}	9 ± 0.0 ^{b}		78	3	13	78 ± 0.0 ^{d}	13 ± 0.1 ^{a}		75	3	15	81 ± 0.0 ^{c}	16 ± 0.0 ^{a}	
Skins	77	34	6	11 ± 0.0 ^{c}	34 ± 0.9 ^{d}		74	4	2	34 ± 0.1 ^{a}	4 ± 0.1 ^{d}		95	2	4	61 ± 0.2 ^{b}	4 ± 0.0 ^{c}	
Consort																		
Whole	78	29	6	11 ± 0.0 ^{a}	29 ± 0.1 ^{b}		90	6	2	24 ± 0.1 ^{c}	6 ± 0.0 ^{b}		85	2	9	76 ± 0.0 ^{b}	10 ± 0.0 ^{a}	
Juice	73	41	8	11 ± 0.1 ^{b}	42 ± 0.0 ^{a}		91	7	1	11 ± 0.0 ^{c}	7 ± 0.1 ^{a}		99	0	0	64 ± 1.7 ^{b}	0.3 ± 0.0 ^{b}	
Seeds	91	6	2	23 ± 0.0 ^{c}	9 ± 0.0 ^{b}		90	1	5	81 ± 0.1 ^{c}	13 ± 0.1 ^{a}		93	1	6	79 ± 0.1 ^{d}	16 ± 0.0 ^{a}	
Skins	70	44	11	14 ± 0.3 ^{b}	45 ± 0.0 ^{b}		91	6	2	16 ± 0.1 ^{d}	6 ± 0.1 ^{c}		93	4	4	46 ± 0.1 ^{d}	5 ± 0.0 ^{a}	
D16-8-14																		
Whole	78	33	6	11 ± 0.0 ^{a}	34 ± 0.0 ^{a}		89	5	2	21 ± 0.0 ^{d}	5 ± 0.1 ^{c}		96	1	2	73 ± 0.1 ^{c}	2 ± 0.0 ^{c}	
Juice	82	27	4	8 ± 0.0 ^{c}	27 ± 0.3 ^{c}		92	1	1	52 ± 0.1 ^{a}	1 ± 0.0 ^{d}		100	0	0	58 ± 0.8 ^{c}	0.1 ± 0.0 ^{d}	
Seeds	90	4	4	43 ± 0.3 ^{a}	5 ± 0.1 ^{d}		87	1	5	84 ± 0.0 ^{a}	5 ± 0.1 ^{d}		91	1	8	85 ± 0.0 ^{a}	8 ± 0.0 ^{b}	
Skins	72	43	10	13 ± 0.1 ^{b}	44 ± 0.0 ^{c}		83	9	4	23 ± 0.1 ^{b}	10 ± 0.0 ^{a}		93	3	4	52 ± 0.22 ^{c}	5 ± 0.0 ^{b}	

All values are mean ± standard deviation of the mean. Different letters in each column indicate significant differences among groups for each variety ($p < 0.05$).

Table 4.2. Total anthocyanin (ANC) concentration in blackcurrant cultivars after 2, 4, 6 h UAE.

	2 h ANC Average (mg eq C3G/g DW)			
Cultivar	**Whole**	**Juice**	**Seeds**	**Skins**
Titania	5.3 ± 1.9 ^{c,C}	7.4 ± 0.6 ^{c,B}	3.8 ± 1.5 ^{c,C}	19.0 ± 2.0 ^{a,A}
D16-6-54	9.5 ± 1.1 ^{b,B}	17.7 ± 1.3 ^{a,A}	6.1 ± 0.2 ^{a,C}	16.1 ± 2.7 ^{b,A}
Consort	14.5 ± 2.8 ^{a,B}	15.5 ± 3.2 ^{a,B}	4.7 ± 0.4 ^{b,C}	19.7 ± 2.7 ^{a,A}
D16-8-14	14.8 ± 2.2 ^{a,B}	11.0 ± 3.5 ^{b,C}	3.5 ± 0.6 ^{c,D}	20.3 ± 3.5 ^{a,A}
	4 h ANC Average (mg eq C3G /g DW)			
Cultivar	**Whole**	**Juice**	**Seeds**	**Skins**
Titania	1.6 ± 0.0 ^{d,C}	3.3 ± 1.0 ^{c,B}	0.6 ± 0.4 ^{c,D}	5.9 ± 1.3 ^{b,A}
D16-6-54	4.8 ± 0.8 ^{b,B}	5.4 ± 0.9 ^{b,B}	2.4 ± 0.0 ^{a,C}	7.0 ± 2.3 ^{b,A}
Consort	7.5 ± 0.6 ^{a,AB}	7.1 ± 0.0 ^{a,B}	1.9 ± 0.2 ^{b,C}	7.8 ± 0.6 ^{a,A}
D16-8-14	4.2 ± 0.2 ^{c,B}	1.4 ± 2.1 ^{d,C}	2.0 ± 0.2 ^{b,C}	8.1 ± 1.5 ^{a,A}
	6 h ANC Average (mg eq C3G /g DW)			
Cultivar	**Whole**	**Juice**	**Seeds**	**Skins**
Titania	2.7 ± 0.0 ^{a,A}	0.9 ± 0.6 ^{a,C}	1.3 ± 0.4 ^{a,B}	0.2 ± 0.2 ^{b,D}
D16-6-54	3.1 ± 0.8 ^{a,A}	2.0 ± 0.2 ^{a,B}	1.4 ± 0.0 ^{a,C}	0.9 ± 0.4 ^{a,D}
Consort	3.0 ± 0.7 ^{a,A}	1.8 ± 0.2 ^{a,B}	1.2 ± 0.4 ^{a,C}	0.7 ± 0.4 ^{a,D}
D16-8-14	2.0 ± 2.0 ^{b,A}	1.4 ± 0.2 ^{a,AB}	0.4 ± 0.2 ^{b,B}	0.6 ± 0.9 ^{a,B}

All values are mean ± standard deviation of the mean. Different uncapitalized letters in each column indicate significant differences among parts of different cultivars ($p < 0.05$). Different capitalized letters in each row indicate significant differences among parts of the same cultivar ($p < 0.05$). All results were quantified by dry weight (concentration of BC extracts = 2.5 mg/mL). Total anthocyanin (ANC) concentrations are expressed as µg equivalents of cyanidin-3-glucoside (C3G) per gram of dry weight material (DW). Absorbance was read at 520 nm and 700 nm.

Table 4.3. Concentration of condensed tannins (TT) and polyphenols (TP) in blackcurrant cultivars after 2 h UAE.

Total Condensed Tannins Average (mg eq Catechin/g DW)				
Cultivar	Whole	Juice	Seeds	Skins
Titania	173.3 ± 0.0 ^{b,C}	240.6 ± 0.0 ^{c,B}	153.4 ± 0.0 ^{a,D}	391.8 ± 0.0 ^{d,A}
D16-6-54	348.8 ± 0.1 ^{ab,C}	367.6 ± 0.1 ^{b,B}	283.7 ± 0.0 ^{a,D}	438.2 ± 0.1 ^{c,A}
Consort	461.4 ± 0.0 ^{a,A}	438.2 ± 0.0 ^{a,B}	238.4 ± 0.0 ^{a,C}	472.4 ± 0.0 ^{b,A}
D16-8-14	306.8 ± 0.0 ^{ab,B}	192.1 ± 0.0 ^{d,D}	232.9 ± 0.0 ^{a,C}	521.0 ± 0.5 ^{a,A}
Total Polyphenols Average (mg eq GA/g DW)				
Cultivar	Whole	Juice	Seeds	Skins
Titania	12.4 ± 0.5 ^{c,D}	15.3 ± 3.5 ^{b,C}	34.4 ± 1.3 ^{a,A}	19.3 ± 0.6 ^{c,B}
D16-6-54	13.0 ± 0.8 ^{c,C}	13.4 ± 0.6 ^{b,C}	23.0 ± 0.6 ^{b,A}	17.9 ± 0.6 ^{c,B}
Consort	22.0 ± 0.5 ^{a,A}	23.1 ± 1.0 $^{a.A}$	18.6 ± 0.9 ^{b,B}	24.0 ± 3.6 ^{a,A}
D16-8-14	15.7 ± 0.9 ^{b,BC}	13.4 ± 1.7 ^{b,C}	34.6 ± 0.5 ^{a,A}	21.3 ± 0.6 ^{b,B}

All values are mean ± standard deviation of the mean. Different uncapitalized letters in each column indicate significant differences among cultivars ($p < 0.05$). Different capitalized letters in each column indicate significant differences among parts of the same cultivar ($p < 0.05$). All results were quantified by dry weight (concentration of BC extracts = 2.5 mg/mL). Total polyphenols and total condensed tannins concentrations are expressed as μg equivalents of gallic acid (GA) per gram of freeze-dried material and mg equivalents of catechin per gram of dry weight material (DW), respectively. Absorbance was read at 500 nm, with a filter from 492-520 nm for total condensed tannins, and at 690 nm for total polyphenols.

Table 4.4. Concentration of delphinidin 3-*O*-glucoside (D3G), delphinidin 3-*O*-rutinoside (D3R), and cyanidin 3-*O*-rutinoside (C3R), in blackcurrant extracts and blackcurrant fermentation products from HPLC after a 2 h water-based UAE.

Materials	Cultivars/parts		D3G (mg/g, DW)	D3R (mg/g, DW)	C3R (mg/g, DW)
BC Cultivars	Titania	Whole	13.4 ± 4.8 ^{nop}	130.0 ± 1.6 ^{jklm}	48.8 ± 16.0 ^{no}
		Juice	19.9 ± 4.6 ^{lm}	125.4 ± 1.0 ^{jklm}	61.2 ± 7.7 ^{klmn}
		Seeds	30.4 ± 0.5 ^{efghi}	165.8 ± 2.4 ^{bcd}	87.8 ± 1.8 ^{ef}
		Skins	12.4 ± 0.3 ^{nop}	84.6 ± 1.7 ^{mno}	58.7 ± 1.0 ^{lmn}
	D16-6-54	Whole	13.7 ± 0.1 ^{no}	95.8 ± 1.7 ^{lmn}	65.2 ± 0.6 ^{jklm}
		Juice	5.8 ± 0.4 ^{qrst}	30.5 ± 1.2 ^{rstu}	19.8 ± 0.4 r^{st}
		Seeds	21.6 ± 0.1 ^{klm}	148.4 ± 0.4 ^{defg}	**104.0 ± 1.2 ^{cd}**
		Skins	25.4 ± 0.1 ^{ijkl}	115.3 ± 1.8 ^{ijkl}	70.4 ± 1.2 ^{ijkl}
	Consort	Whole	36.3 ± 0.3 ^{cd}	174.9 ± 1.9 ^{bc}	**140.8 ± 2.2 ^{cd}**
		Juice	4.8 ± 0.4 ^{rst}	22.5 ± 0.7 ^{tu}	13.5 ± 0.3 st
		Seeds	33.8 ± 0.4 ^{cdef}	182.5 ± 0.7 ^{b}	**116.0 ± 2.5 ^{c}**
		Skins	10.0 ± 0.4 ^{opqr}	62.8 ± 0.9 ^{opq}	53.0 ± 1.5 ^{mno}
	D16-8-14	Whole	17.9 ± 0.1 ^{mn}	107.7 ± 0.3 ^{klm}	87.0 ± 0.7 ^{ef}
		Juice	8.1 ± 0.4 ^{pqrs}	41.1 ± 3.0 ^{qrst}	34.1 ± 1.9 ^{pq}
		Seeds	21.6 ± 0.1 ^{hijk}	164.3 ± 0.7 ^{bcde}	**133.6 ± 1.2 ^{b}**
		Skins	**65.2 ± 0.4 ^{a}**	**247.6 ± 2.8 ^{a}**	**180.9 ± 3.6 ^{a}**
Enzyme Treatments at 52 ºC (mL/ton)/min	No heat/No enzyme		23.2 ± 0.8 ^{jklm}	121.3 ± 0.5 ^{hijk}	73.7 ± 0.3 ^{ghijk}
	0 (mL/t)/90 (min)		30.2 ± 0.8 e^{fghi}	133.1 ± 0.8 ^{fghij}	81.2 ± 1.1 ^{efghi}
	0 (mL/t) /120 (min)		**32.2 ± 1.9 ^{cdefg}**	142.6 ± 2.4 ^{defgh}	84.0 ± 1.4 ^{efgh}
	0 (mL/t) /150 (min)		26.3 ± 2.4 ^{hijk}	129.6 ± 0.7 ^{fghijk}	77.8 ± 0.7 ^{fghij}
	400 (mL/t) /90 (min)		**32.4 ± 1.9 ^{cdefg}**	139.1 ± 0.9 ^{efghi}	82.2 ± 0.5 ^{efghi}
	400 (mL/t) /120 (min)		**32.6 ± 0.6 ^{cdefg}**	131.5 ± 0.9 ^{fghijk}	78.0 ± 0.8 ^{fghij}
	400 (mL/t) /150 (min)		**37.4 ± 1.7 ^{c}**	153.5 ± 1.3 ^{cdef}	91.8 ± 1.8 ^{de}
	600 (mL/t) /90 (min)		27.2 ± 1.9 ^{ghij}	125.1 ± 8.4 ^{ghijk}	73.0 ± 5.3 ^{hijk}
	600 (mL/t) /120 (min)		**34.1 ± 0.7 ^{cde}**	141.0 ± 2.2 ^{defgh}	78.6 ± 1.4 ^{fghi}
	600 (mL/t) /150 (min)		25.9 ± 1.1 ^{hijk}	120.4 ± 1.5 ^{hijkl}	70.4 ± 0.9 ^{ijkl}
	800 (mL/t) /90 (min)		28.0 ± 2.0 ^{fghij}	124.8 ± 1.2 ^{ghijk}	73.4 ± 0.9 ^{hijk}
	800 (mL/t) /120 (min)		29.1 ± 1.3 ^{efghi}	128.0 ± 1.3 ^{ghijk}	75.5 ± 1.5 ^{fghij}
	800 (mL/t) /150 (min)		31.4 ± 1.5 ^{defgh}	144.4 ± 1.6 ^{defgh}	86.2 ± 1.2 ^{efg}
Fermentation Products	As is Wine- Consort- 23 °C		**43.4 ± 0.6 ^{b}**	132.0 ± 2.2 ^{fghi}	**140.2 ± 1.5 ^{b}**
	As is Wine- Consort- 15 °C		1.1 ± 0.3 ^{t}	18.1 ± 1.4 ^{tu}	8.3 ± 0.5 ^{t}
	As is Wine- Mix- 23 °C		4.9 ± 1.2 ^{rst}	48.0 ± 0.5 ^{qrs}	26.9 ± 0.3 ^{qr}
	As is Wine- Mix- 15 °C		4.2 ± 0.6 st	49.6 ± 0.8 ^{pqr}	27.9 ± 1.1 ^{qr}
Dealcoholized Fermentation Products	Dealcoholized Wine- Consort- 23 °C		2.1 ± 0.2 ^{t}	24.6 ± 0.9 ^{rstu}	9.9 ± 0.7 ^{t}
	Dealcoholized Wine- Consort- 15 °C		1.4 ± 0.6 ^{t}	24.2 ± 0.4 ^{stu}	12.5 ± 0.1 st
	Dealcoholized Wine- Mix- 23 °C		11.1 ± 3.9 ^{opq}	84.3 ± 0.7 ^{mno}	51.2 ± 0.7 ^{no}
	Dealcoholized Wine- Mix- 15 °C		11.0 ± 1.1 ^{opq}	73.5 ± 0.9 ^{nop}	44.9 ± 1.2 ^{op}
Fermentation Byproducts	BC Pomace- Consort- 23 °C		8.3 ± 0.5 ^{opqrs}	36.7 ± 1.5 ^{rst}	18.0 ± 0.6 ^{rst}
	BC Pomace- Consort- 15 °C		6.2 ± 1.6 ^{qrst}	25.1 ± 1.2 ^{rstu}	9.7 ± 0.5 ^{t}
	BC Pomace- Mix- 23 °C		4.9 ± 0.5 ^{rst}	42.0 ± 1.2 ^{qrst}	24.8 ± 0.6 ^{qrs}
	BC Pomace- Mix- 15 °C		0.7 ± 0.2 ^{t}	10.7 ± 0.2 ^{u}	8.2 ± 0.1 ^{t}

All values are mean ± standard deviation of the mean. All results were quantified by dry weight (concentration of BC extracts = 2.5 mg/mL). Different uncapitalized letters in each column indicate significant differences ($p < 0.05$).

Table 4.5. IC_{50} of Blackcurrant extracts and fermentation products on *α*-amylase, *α*-glucosidase, DPPIV, and DPPH·

Materials	Cultivar/parts		*α*-amylase IC_{50} (mg DW/g whole fruit)	*α*-glucosidase IC_{50} (mg DW/g whole fruit)	DPPIV IC_{50} (mg DW/g whole fruit)	DPPH· IC_{50} (mg DW/g whole fruit)
BC Cultivars	Titania	Whole	2.26 ± 0.01 ^{g}	0.75 ± 0.00 ^{bcd}	1.21 ± 0.38 ^{p}	0.10 ± 0.01 ^{j}
		Juice	**0.64 ± 0.02** ^{lm}	0.75 ± 0.00 ^{bcd}	1.33 ± 0.26 ^{fghi}	0.05 ± 0.08 ^{lm}
		Seeds	2.39 ± 0.02 ^{h}	0.78 ± 0.00 ^{abc}	1.12 ± 0.52 ^{q}	0.50 ± 0.01 ^{a}
		Skins	**0.59 ± 0.04** ^{lmn}	0.77 ± 0.00 ^{bcd}	1.30 ± 0.59 ^{jkl}	0.17 ± 0.01 ^{gh}
	D16-6-54	Whole	0.76 ± 0.01 ^{l}	0.78 ± 0.00 ^{abc}	1.30 ± 0.69 ^{jkl}	0.08 ± 0.02 ^{jk}
		Juice	**0.54 ± 0.02** ^{mn}	0.77 ± 0.00 ^{bcd}	1.35 ± 0.64 ^{efg}	0.14 ± 0.02 ^{i}
		Seeds	ND	0.83 ± 0.00 ^{a}	1.44 ± 0.30 ^{a}	**0.01 ± 0.02** ^{n}
		Skins	**0.66 ± 0.08** ^{lm}	0.80 ± 0.00 ^{ab}	1.31 ± 0.95 ^{ij}	0.16 ± 0.02 ^{gh}
	Consort	Whole	**0.54 ± 0.01** ^{mn}	0.73 ± 0.00 ^{cd}	1.32 ± 0.69 ^{hi}	0.24 ± 0.02 ^{de}
		Juice	**0.44 ± 0.03** ^{n}	0.73 ± 0.00 ^{cd}	1.35 ± 0.69 ^{de}	0.17 ± 0.03 ^{gh}
		Seeds	62.32 ± 0.03 ^{b}	0.77 ± 0.00 ^{abcd}	1.31 ± 0.63 ^{ijk}	0.38 ± 0.01 ^{bc}
		Skins	**0.61 ± 0.03** ^{lmn}	0.75 ± 0.00 ^{bcd}	1.33 ± 0.41 ^{ghi}	0.25 ± 0.01 ^{d}
	D16-8-14	Whole	0.70 ± 0.04 ^{lm}	0.75 ± 0.00 ^{bcd}	1.27 ± 0.98 ^{m}	0.05 ± 0.02 ^{l}
		Juice	**0.55 ± 0.06** ^{mn}	0.75 ± 0.00 ^{bcd}	1.30 ± 0.37 ^{jkl}	0.07 ± 0.02 ^{k}
		Seeds	23.47 ± 0.02 ^{d}	0.78 ± 0.00 ^{abc}	**1.23 ± 0.45** ^{op}	0.40 ± 0.01 ^{b}
		Skins	**0.68 ± 0.00** ^{lm}	0.75 ± 0.00 ^{bcd}	**1.27 ± 1.08** ^{mn}	0.17 ± 0.01 ^{g}
Fermentation Products	As is Wine- Consort- 23 °C		1.67± 0.02 ^{i}	**0.65 ± 0.00** ^{ef}	1.30 ± 0.28 ^{jkl}	0.23 ± 0.00 ^{e}
	As is Wine- Consort- 15 °C		1.03 ± 0.02 ^{k}	**0.63 ± 0.00** ^{f}	1.37 ± 0.15 ^{bcd}	0.21 ± 0.00 ^{f}
	As is Wine- Mix- 23 °C		1.48 ± 0.02 ^{j}	**0.65 ± 0.00** ^{ef}	1.35 ± 0.50 ^{def}	0.15 ± 0.01 ^{hi}
	As is Wine- Mix- 15 °C		1.52 ± 0.04 ^{ij}	**0.71 ± 0.00** ^{de}	1.34 ± 0.12 ^{efgh}	0.14 ± 0.01 ^{i}
Dealcoholized Fermentation Products	Dealcoholized Wine- Consort- 23 °C		1.19 ± 0.06 ^{k}	**0.60 ± 0.00** ^{f}	1.28 ± 0.23 ^{lm}	0.39 ± 0.02 ^{b}
	Dealcoholized Wine- Consort- 15 °C		1.01 ± 0.00 ^{k}	**0.64 ± 0.00** ^{f}	1.32 ± 0.28 ^{hij}	0.37 ± 0.01 ^{c}
	Dealcoholized Wine- Mix- 23 °C		1.03 ± 0.02 ^{k}	**0.63 ± 0.01** ^{f}	1.36 ± 0.07 ^{c}	0.26 ± 0.01 ^{d}
	Dealcoholized Wine- Mix- 15 °C		1.07 ± 0.01 ^{k}	**0.53 ± 0.00** ^{g}	1.29 ± 0.07 ^{klm}	0.20 ± 0.01 ^{f}
Fermentation Byproducts	BC Pomace- Consort- 23 °C		1.44 ± 0.01 ^{j}	0.73 ± 0.00 ^{cd}	**1.25 ± 2.06** ^{no}	0.08 ± 0.02 ^{jk}
	BC Pomace- Consort- 15 °C		4.14 ± 0.01 ^{f}	0.76 ± 0.00 ^{bcd}	1.33 ± 1.41 ^{fghi}	**0.01 ± 0.01** ^{n}
	BC Pomace- Mix- 23 °C		12.26 ± 0.01 ^{e}	0.75 ± 0.01 ^{bcd}	1.38 ± 1.36 ^{bc}	0.03 ± 0.00 ^{m}
	BC Pomace- Mix- 15 °C		24.27 ± 0.01 ^{c}	0.77 ± 0.00 ^{bcd}	1.39 ± 1.36 ^{b}	**0.01 ± 0.02** ^{n}

All values are mean ± standard deviation of the mean. Different uncapitalized letters in each column indicate significant differences among blackcurrant extracts and blackcurrant fermentation products ($p < 0.05$). ND = not determined.

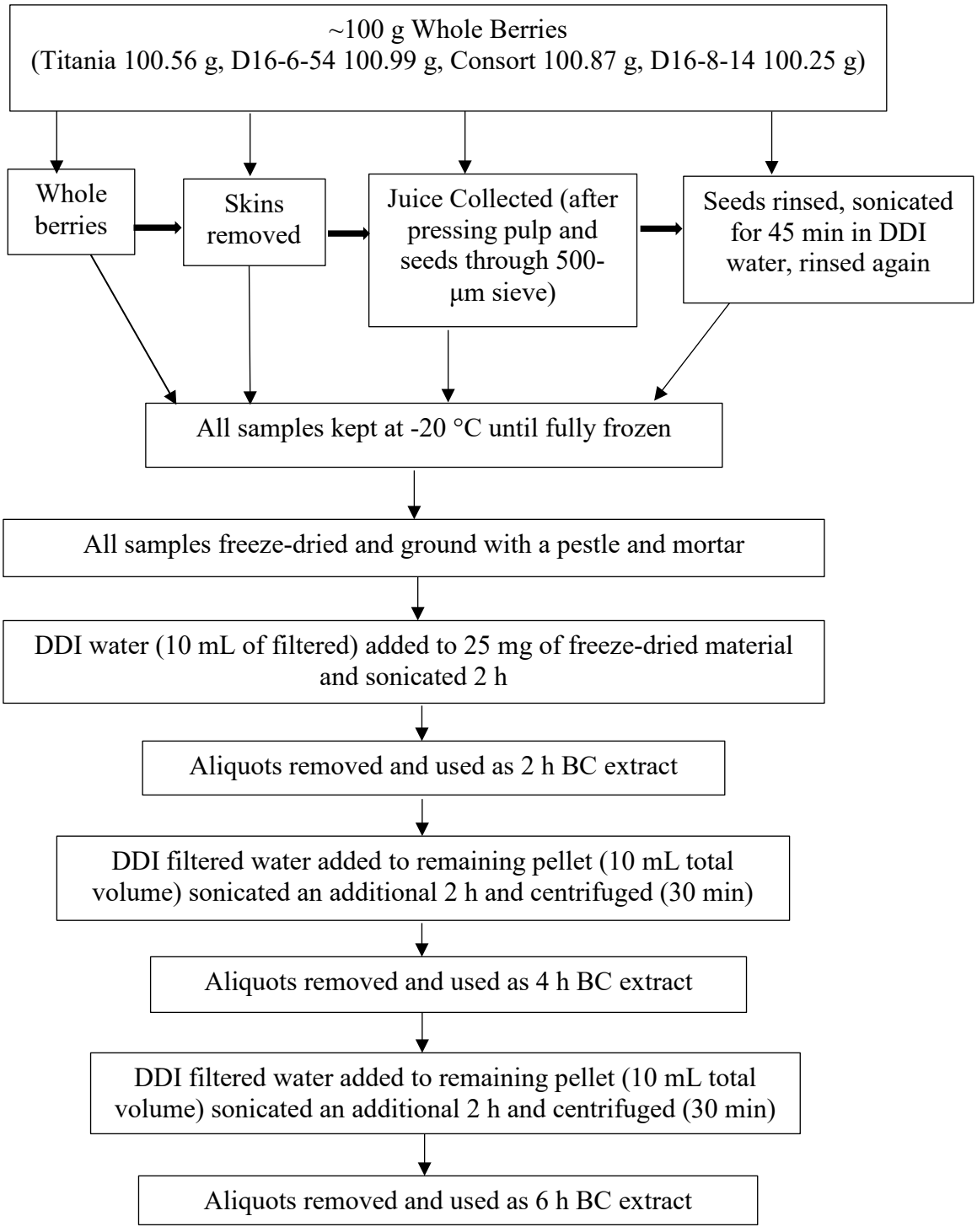


Figure 4.1. Flow diagram of sample preparation

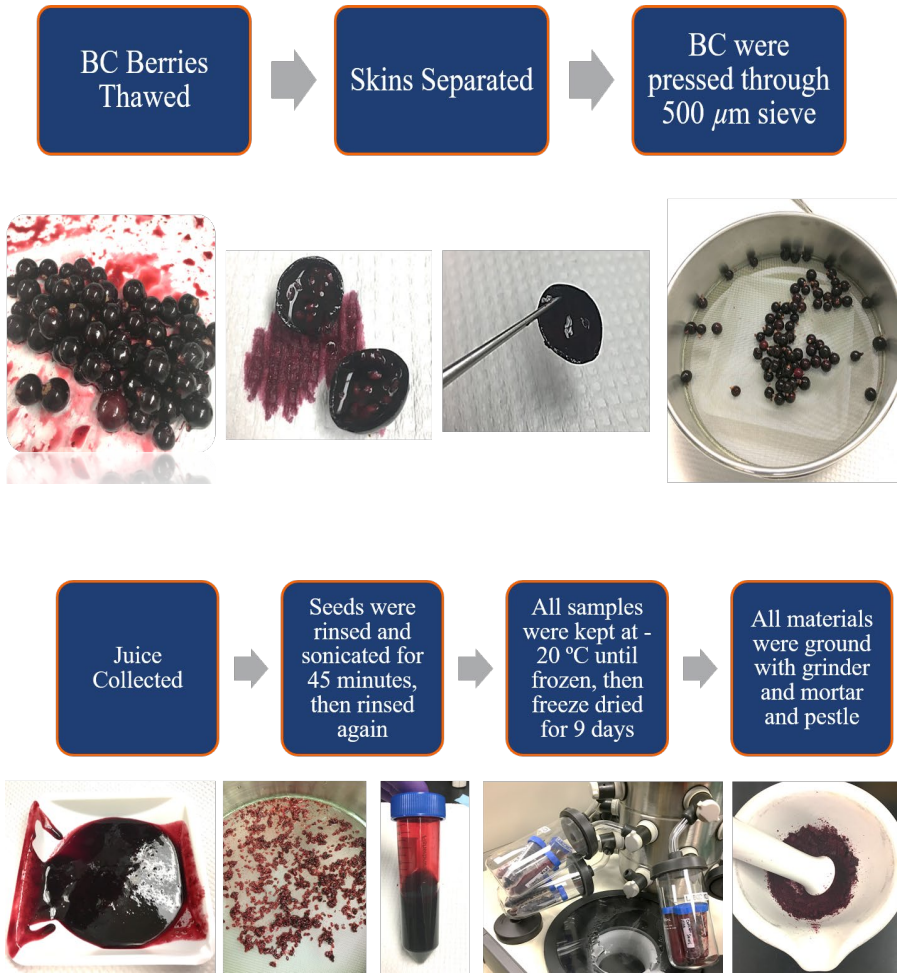


Figure 4.2. Aim 1 and 2 sample preparation

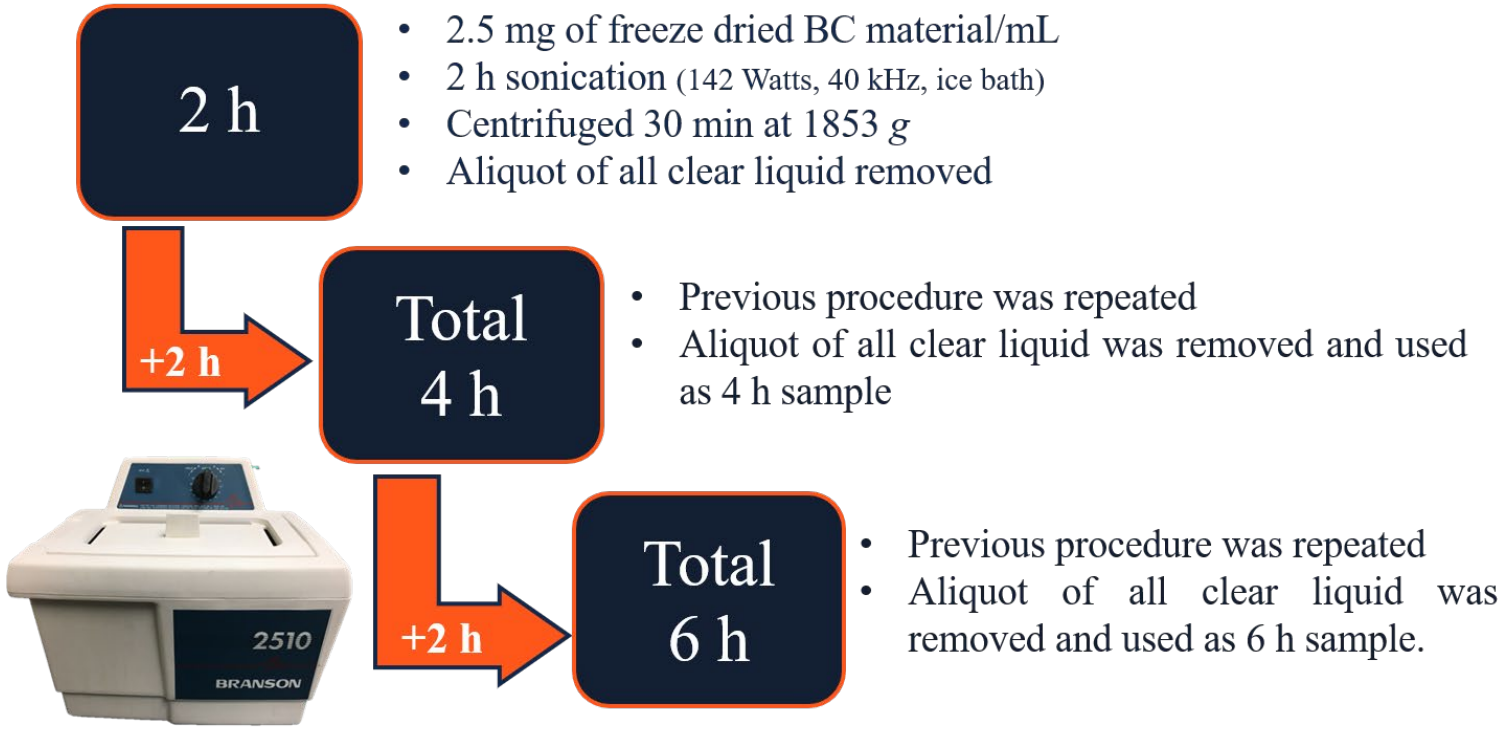


Figure 4.3. Water-based ultrasound-assisted extractions for characterization.

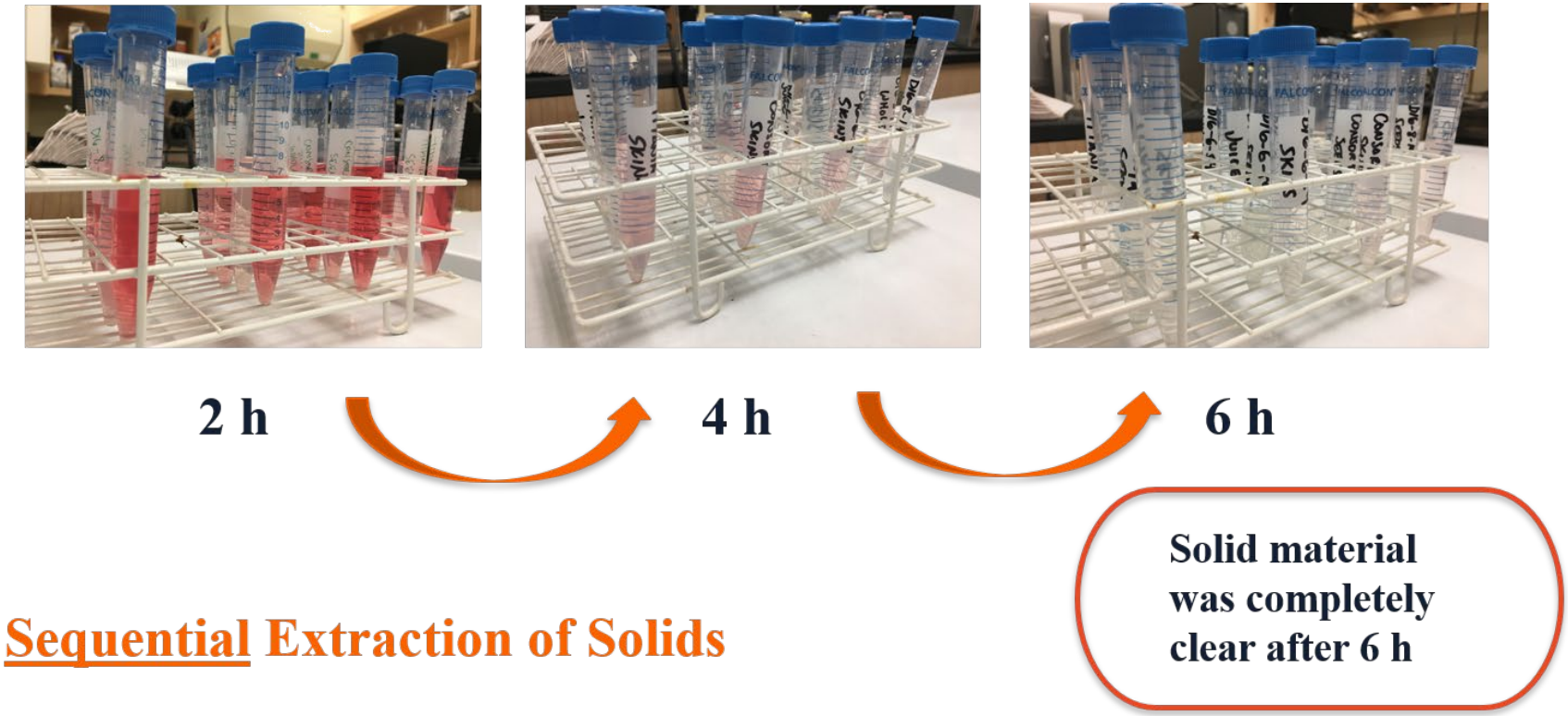


Figure 4.4. Blackcurrant extracts after 2, 4, and 6 h water-based ultrasound-assisted extractions.

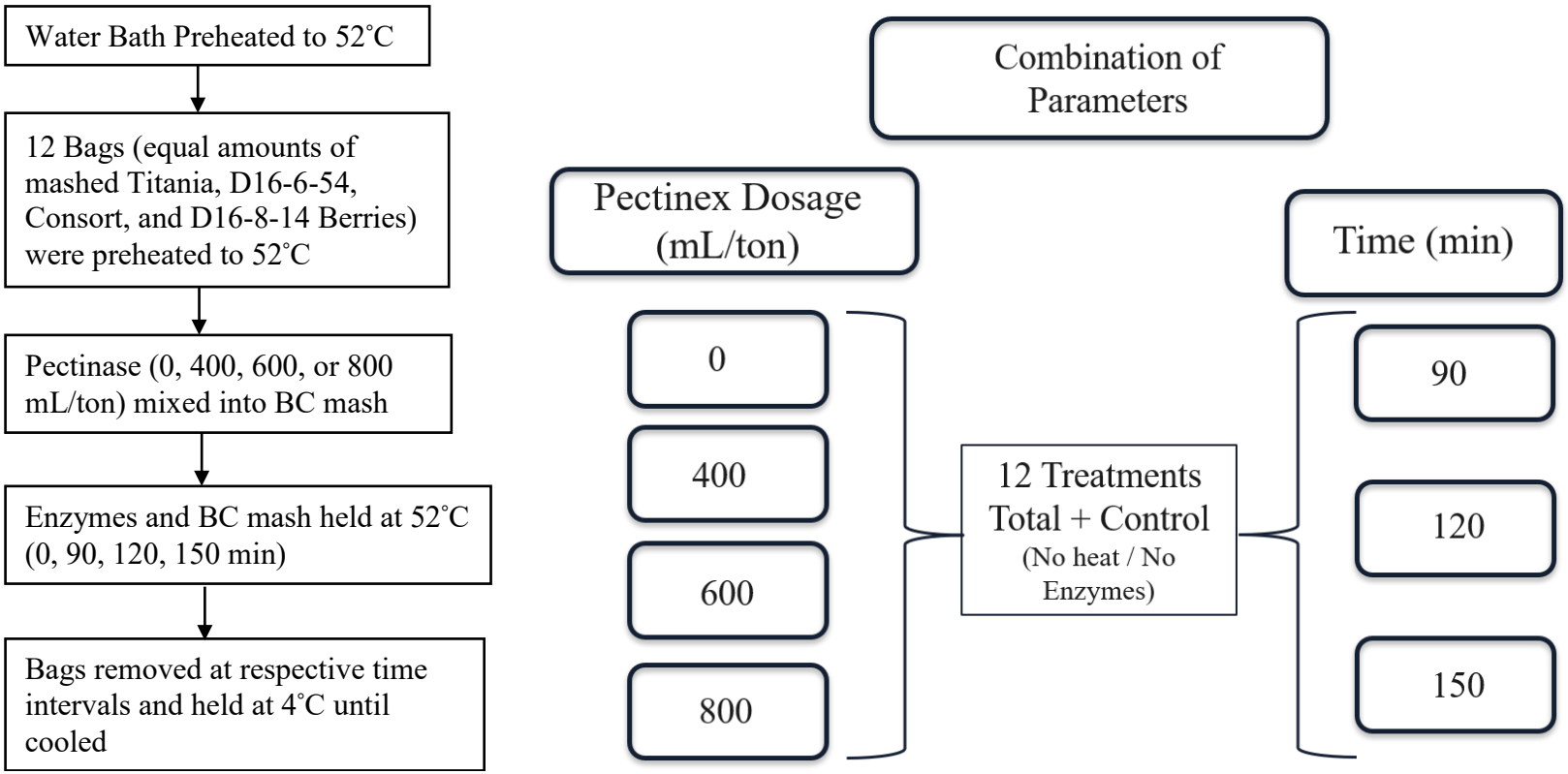
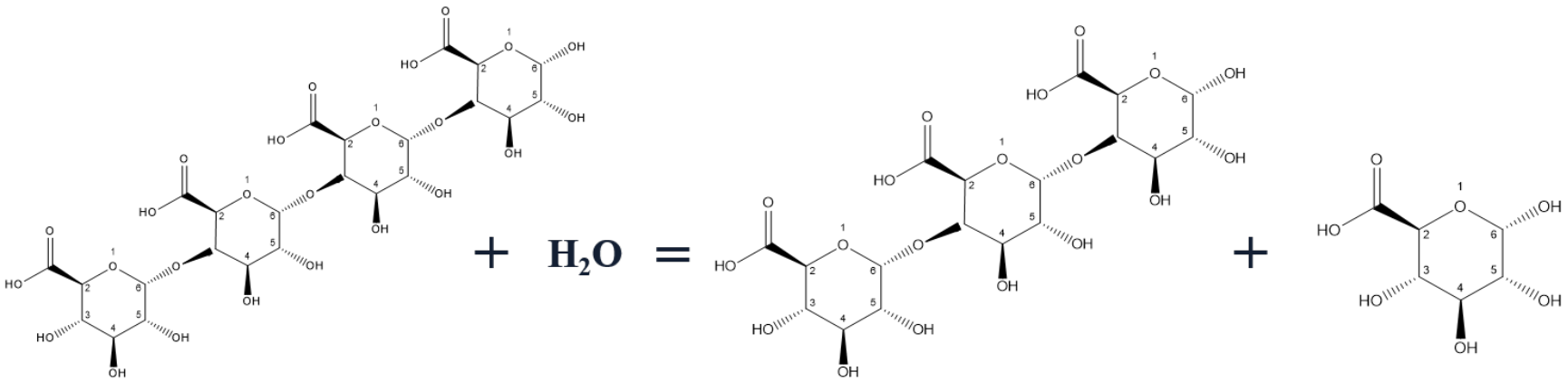


Figure 4.5. Flow diagram of pectinase treatment process.



Hydrolysis of *O*-glycosyl bond

Figure 4.6. Pectinase (EC 3.2.1.67 – Galacturan 1,4-alpha-galacturonidase) mechanism of action. Pectinase catalyzes the hydrolysis of *O*-glycosyl bonds between 1,4-alpha linkages.

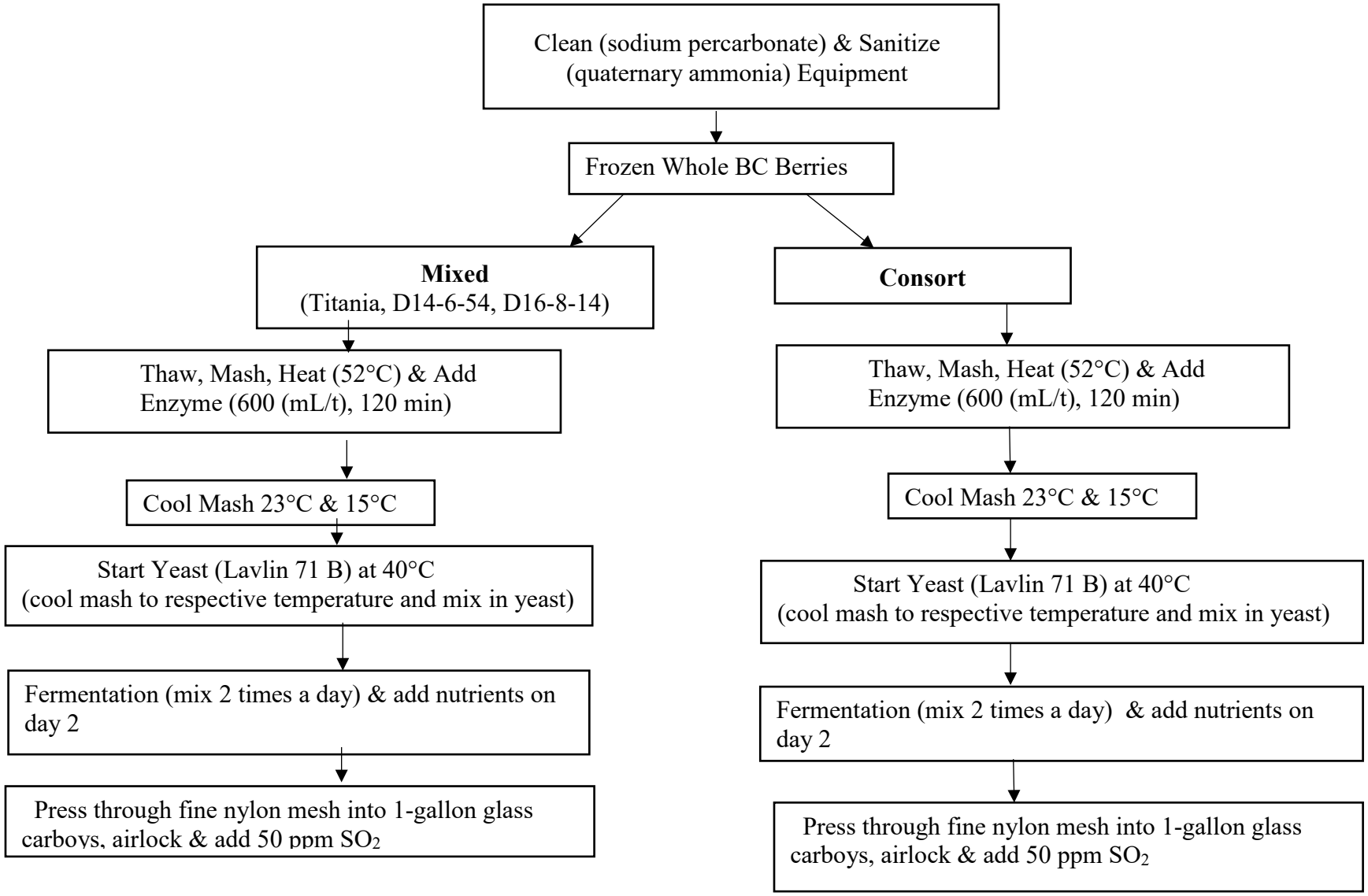
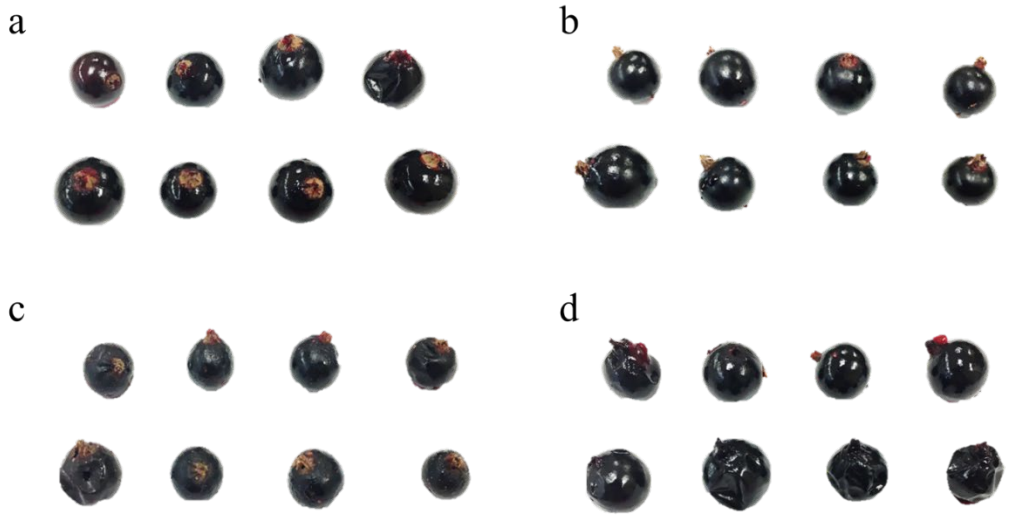


Figure 4.7. Flow diagram of fermentation.



Variety	Average Berry Size (Diameter, cm)	Brix°
Titania	1.37 ± 0.1 [a]	15.0 ± 0.2 [c]
D16-6-54	1.04 ± 0.1 [b]	19.0 ± 0.3 [ab]
Consort	0.97 ± 0.1 [b]	20.6 ± 0.6 [a]
D16-8-14	1.23 ± 0.2 [a]	18.3 ± 0.5 [b]

Figure 4.8. Representative pictures and size of the four different blackcurrant cultivars.
Different letters in each column indicate significant differences among cultivars ($p < 0.05$). Representative pictures of fresh berries of the different cultivars (a) Titania (b) D16-6-54 (c) Consort (d) D16-8-14.

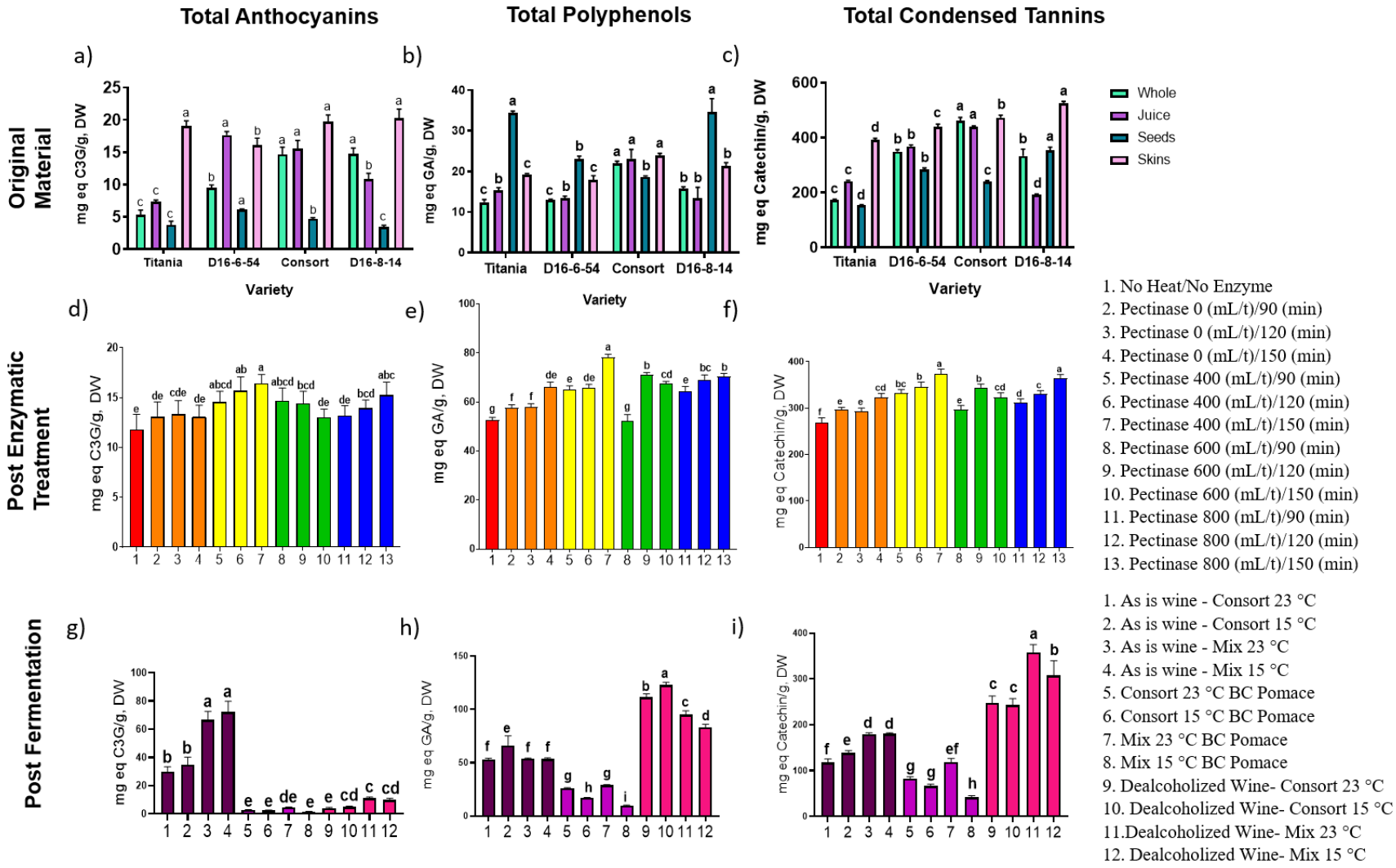


Figure 4.9. Comparison of concentrations of total anthocyanins, total polyphenols, and total condensed tannins from four different cultivars of blackcurrants and their parts (whole berries, juice, seeds, and skins), blackcurrant mash after enzymatic treatment, and fermented blackcurrant products and byproducts after a 2 h water-based ultrasound-assisted extraction. Different uncapitalized letters above each bar indicate significant differences ($p < 0.05$).

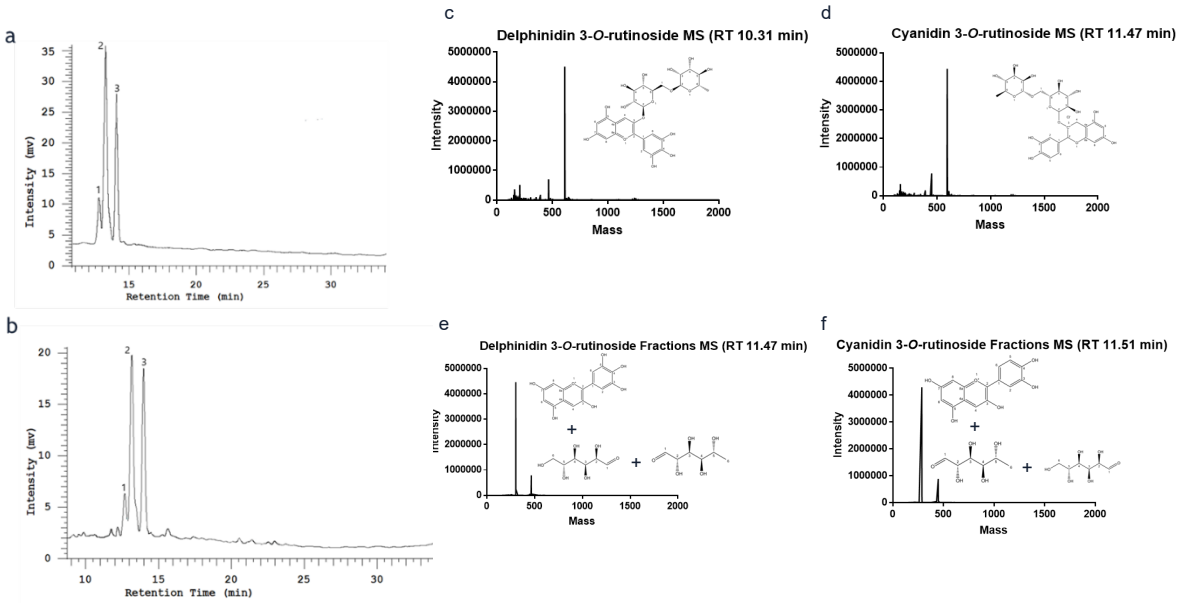


Figure 4.10. Representative HPLC and LC-ESI-MS profiles of consort and Titania whole berries. HPLC, MS, MS-MS profiles of Consort and Titania whole berries. HPLC representative chromatograms: (a) Consort whole berry at 520 nm (peak 1 = delphinidin 3-*O*-glucoside, peak 2 = delphinidin 3-*O*-rutinoside, and peak 3 = cyanidin 3-*O*-rutinoside), (b) Consort whole berry at 280 nm (peak 1 = delphinidin 3-*O*-glucosids, peak 2 = delphinidin 3-*O*-rutinoside, and peak 3 = cyanidin 3-*O*-rutinoside). Delphinidin 3-O-glucoside and Cyanidin 3-O-glucoside MS representative spectra: (c) D3R identified in Titania whole berry MS RT 10.31 and (d) C3R identified in Titania whole berry MS RT 11.47. Titania whole berry MS fractions: (e) D3R fractions (delphinidin + glucose + rhamnose) and (f) C3R fractions (cyanidin + glucose + rhamnose).

Anthocyanin	**1st Ion (M/Z)**	**T_r (min)**	**Anthocyanin**	**2nd Ion (M/Z)**	**T_r (min)**
Delphinidin 3-*O*-rutinoside	611.5 $[M]^+$	10.31	Delphinidin 3-*O*-glucoside	465.4 $[M]^+$	10.35
Cyanidin 3-*O*-rutinoside	595.5 $[M]^+$	11.47	Cyanidin 3-*O*-glucoside	449.4 $[M]^+$	11.51

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CHAPTER 5: CONCLUSIONS

- A water-based 2 h UAE was able to extract >50% of ANC from BC. Reported concentrations of ANC in the present research are comparable to concentrations reported by others that have used organic solvents for their extractions. This is evidence that there is no need for the use of organic solvents when performing extractions of BC for the purposes of human consumption.
- A comparison among Titania, D16-6-54, Consort, and D16-8-14 BC cultivars and their parts revealed that D16-8-14 and Consort whole berries had the largest concentration of ANC. Consort and D16-8-14 whole berries had the greatest concentrations of TT. Consort had the highest concentrations of TP, except in their seeds.
- Heating during pectinase treatments did increase the concentrations of both TT and TP, with a contribution of heating of the BC mash and the pectinase to function.
- LC-ESI-MS analysis confirmed the presence of four major ANC (D3G, D3R, C3G, and C3R) in all BC cultivars analyzed. Titania was the only cultivar in which peonidin 3-*O*-rutinoside was detected.
- HPLC analysis revealed that D16-8-14 skins have the highest concentrations of D3G, D3R, and C3R.
- Juices and skins from all four cultivars had the lowest IC_{50} values (most potent) for α-amylase inhibition, while the fermented products had the lowest IC_{50} values for *α*-glucosidase inhibition. IC_{50} values for the inhibition of DPPIV were similar for all four cultivars.
- Fermentation byproducts (BC pomace) proved to be a potent antioxidant when compared to 600 μM gallic acid (100% inhibition), it was still able to achieve a $DPPH^{\cdot}$ free radical scavenging capacity of 83% (at 2 mg/ml). These results provide strong evidence that BC byproducts have great potential for reutilization by the food and beverage industries.

CHAPTER 6: INTEGRATION AND FUTURE WORK

Blackcurrants are emerging as a trendy new flavor and ingredient with many potential health benefits. Consumers are increasingly demanding healthful foods that are not just calories, but also provide maximum nutritional benefit. There is also the additional demand that any additives in processed foods have dual functionality in that they meet whatever physical function required and also serve as a means of adding bioactive benefit. The goal of this research has been to understand how BC might be utilized to provide some solutions to meet these demands. We believe that it is possible to meet these goals, particularly in the case of natural pigments. Our hypothesis that bioactive compounds present in BC can be extracted using a 2 h, water-based UAE, and that ANC and other phenolic compounds from both fresh berries and fermented berries can inhibit markers of inflammation and type-2 diabetes was supported by our results. Extracting ANC from BC, using green technologies like water-based UAE, is a viable alternative for synthetic food colorants that also offers the value-added benefit of being a potent antioxidant and inhibitor of enzymes related to type-2 diabetes.

One of the challenges that BC present is that they are extremely bitter and astringent because they are heavily concentrated with condensed tannins. It is often the case that manufacturers of BC products add large amounts of sugar to offset the bitterness of the fruits, thereby reducing their health benefits. For this reason, we believe BC extracts are attractive for food product manufacturers wishing to use natural pigments coupled with the fact that BC do not have a great diversity of ANC species. There are only 4 major ANC found in BC, which are delphinidin 3-*O*-glucoside, delphinidin 3-*O*-rutinoside, cyanidin 3-*O*-glucoside, and cyanidin 3-*O*-rutinoside. It could also be argued that there are actually only 3 major ANC in BC because

cyanidin 3-*O*-glucoside is present in very low amounts compared to the other 3 compounds. Given that there are only 4 major ANC in BC, we believe that makes BC the ideal candidate for ANC extraction because the isolation methods would require minimum complexity.

The objective of this research was to characterize the components of four varieties of BC and their effect on the activities of α-amylase, α-glucosidase, DPPIV, and their DPPH· radical scavenging capacity after water-based UAE, treatment with pectinase, and fermentation. To achieve this goal, this research was divided into three aims in which BC would be characterized and evaluated. The first aim was to determine if a strictly water-based ultrasound-assisted extraction method is sufficient to extract ANC, and other phenolic compounds, from BC. As a result of this aim, it was discovered that a 2 h, water-based ultrasound-assisted extraction was capable of extracting > 50% of ANC from BC, which is comparable to ANC concentrations reported using organic solvents. Our second aim was to characterize four different cultivars of BC berries (Titania, D16-6-54, Consort, and D16-8-14) and their parts (juice, seeds, and skins) to compare ANC, condensed tannins, and polyphenol contents of the BC. The outcomes of this aim were that Consort and D16-6-54 had the smallest berry sizes with an average diameter of 0.97 ± 0.1 cm and 1.04 ± 0.1 cm, respectively. The average pH of the four cultivars was 2.9 and it was Consort and D16-8-14 whole berries that had the highest concentrations of ANC (14.5 ± 2.8 and 14.8 ± 2.8 mg eq. C3G/g, DW). Consort and D16-8-14 whole berries that had the highest concentrations of total condensed tannins, while Consort had the greatest amount of total polyphenols for all parts except seeds. It was confirmed that all four BC cultivars contain the four major ANC (D3G, D3R, and C3R) and that Titania was the only cultivar in which peonidin 3-*O*-rutinoside was detected. All other phenolic compounds detected in the four BC cultivars were seen at very low concentrations. According to our HPLC analysis, D16-8-14 skins had the highest

amounts of delphinidin 3-*O*-glucoside, delphinidin 3-*O*-rutinoside, and delphinidin 3-*O*-glucoside. The third aim was to determine what, if any, effect a pectinase treatment and the fermentation of BC mash would have on the concentrations of anthocyanins, total condensed tannins, and total polyphenols. When comparing Titania and D16-8-14 juices, it is evident that there was an increase in ANC regardless of the pectinase dose used. However, a Pearson correlation showed that there was no correlation between ANC and the pectinase treatment. The fermentation of the BC mash produced an increase of ANC by five times. There was no increase in condensed tannins because of the pectinase treatment, but a Pearson correlation revealed that there was a positive correlation ($r^2 = 0.535$) with the heating that is needed for the enzyme treatment and the fermentation process reduced condensed tannins by half. When comparing the whole fruit from all cultivars, it is evident that the pectinase treatment did increase the polyphenol content, which was confirmed by a Pearson correlation analysis ($r^2 = 0.521$). Fermentation of BC mash also increased the polyphenolic content.

Future directions of this research should be to first evaluate how these bioactive compounds from BC are metabolized and determine their bioavailability. Next, work should be done to find effective encapsulation methods which would maximize the functionality of BC extracts as food additives and also increase the bioavailability of bioactive compounds. Following encapsulation, research is needed to better understand the effects of these bioactive compounds *in vitro* and *in vivo*, starting with cell culture and moving to animal models and eventually to human studies. There is a great deal of potential for blackcurrants and as their popularity increases in the US, we believe much more research is needed to maximize the health benefits to be gained from them.