

## ABSTRACT

MOHAMEDSHAH, ZULFIQAR YUSUF. Comparative Assessment of Phenolic Bioaccessibility and Bioavailability from 100% Juice and Whole Fruit – A Preclinical Approach. (Under the direction of Dr. Mario Ferruzzi).

The consumption of fruit is critical to meeting nutritional needs and in the prevention of chronic disease. As a nutrient dense food, fruit is a key source of micronutrients and dietary phytochemicals. However, less than a quarter of Americans consume the recommended daily fruit servings, driven by cost, convenience and other factors. 100% fruit juices are a cost effective and available option that accounts for ~35% of daily fruit consumption. Despite this, current dietary guidance favors consumption of whole fruit over 100% fruit juices for factors including loss of fiber and perceived increased sugar intake from juice products. However, there may be benefits to 100% fruit juice consumption as its' liquid matrix, and the extensive mechanical and enzymatic action of the commercial juicing process, may provide an equivalent or better method for delivery of bioactive compounds, specifically phenolic species. By comparing phenolic-rich whole grapes and 100% grape juice, the objective of this research was to draw direct comparisons between fruit forms and investigate the impact of food matrix and processing (mastication of whole fruit vs. juice) has on bioavailability of phenolics and their secondary metabolites produced throughout human digestion.

To understand if 100% grape juice can provide a matrix with highly bioaccessible phenolics relative to whole fruit, differences in phenolic content and bioaccessibility from commonly consumed table, Concord and Niagara grapes and their 100% juices were compared. Phenolic content in whole grapes and 100% juices were determined by LC-MS prior to *in-vitro* digestion to determine phenolic bioaccessibility. Concord and Niagara grape seeds had the highest concentration of phenolics in the forms of flavan-3-ols and larger procyanidins. Purple Concord

grape skins were rich in anthocyanins and flavanols, while grape pulp had low quantities of phenolic species. While phenolic content of whole grapes was significantly ( $p < 0.01$ ) greater than their 100% grape juices, following simulated digestion, absolute bioaccessible content of phenolics were found to be similar between grapes and 100% juice. Differences in bioaccessible content were driven by high relative bioaccessibility of anthocyanins in Concord juice compared to grapes as well as for flavan-3-ols and phenolic acids from grape juices to whole grapes. A greater fraction of skin and seed phenolics were extracted through juicing and made bioaccessible making 100% grape juice and whole fruit similar in overall phenolic delivery to consumers.

In a second study, phenolic bioaccessibility and metabolism from Concord and Niagara grapes and corresponding 100% juices was assessed in both the upper and lower GI tract using an *in-vitro* digestion coupled with anaerobic gut fermentation model. Intestinal transport of resulting bioaccessible phenolics and metabolites was estimated using a Caco-2 cell model. Total bioaccessible phenolics from both upper and lower tract digestion was similar between whole grapes and 100% juices. Total cellular transport of phenolics was also similar between whole grapes and 100% juices. Some differences were observed between the location of phenolic metabolism, bioaccessibility and subsequent cellular transport of phenolics between grapes and juice. Specifically, greater amounts of flavonoids were transported from grape juices than whole grapes from the upper tract, which aligns with reported mechanisms for acute responses to grape juice consumption. Yet, cumulative bioaccessibility and transport from upper and lower GI digestion/fermentation together indicates that the absorbable phenolics from 100% grape juice is reflective of that of whole grapes, suggesting that phenolic-mediated health benefits from consumption of whole fruit and juice may be similar.

These findings provide a mechanistic and compartmentalized framework that compares delivery characteristics of bioactive phenolics species between 100% grape juice and respective whole grapes. Furthermore, these results help to better understand the role 100% fruit juices play in a health promoting diet with respect to benefits from consuming fruits.

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Comparative Assessment of Phenolic Bioaccessibility and Bioavailability from 100% Juice and Whole Fruit – A Preclinical Approach

by  
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## DEDICATION

*To faith, family, and friends.*

## **BIOGRAPHY**

Zulfiqar Yusuf Mohamedshah was born on June 24<sup>th</sup>, 1996, in Montgomery, Maryland. He completed his Bachelor of Science in Chemistry at the University of Virginia in May 2018. Beginning in the summer of 2018, Zulfiqar worked as a Laboratory Technician under Dr. Mario Ferruzzi at the Plants for Human Health Institute in Kannapolis, North Carolina. Becoming intrigued by his work on the impacts of food processing on phenolic bioavailability, Zulfiqar transitioned to graduate studies under Dr. Ferruzzi. Upon completion of his master's degree, Zulfiqar will pursue a doctorate in Chemistry at the University of California, San Diego.

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## CHAPTER 1. Review of Relevant Literature

### 1.1 Introduction

The benefits of fruit consumption, including their contribution to meeting nutrient needs and in prevention of chronic disease, have been well established. As a key source of select micronutrients (vitamins and minerals), as well as dietary phytochemicals (carotenoids and phenolics), fruit products offer consumers nutrient dense options that, beyond traditional nutrition, have been linked to numerous beneficial health effects. This includes prevention of chronic and degenerative diseases such as cardiovascular disease, coronary heart disease, hypertension, stroke, asthma, obesity, type II diabetes, chronic obstructive pulmonary disease, cognitive impairment, osteoporosis, eye disease, and arthritis (Zhan et al. 2017; Gan et al. 2015; L. Wu, Sun, and He 2016; Hu et al. 2014; Seyedrezazadeh et al. 2014; Schwingshackl et al. 2015; Y. Wu et al. 2015; Kaluza et al. 2017; Jiang et al. 2017; Tucker et al. 1999; M. Li et al. 2019; K. K. Ho, Ferruzzi, and Wightman 2019).

Recognizing the importance of fruit as part of a healthy diet, the Dietary Guidelines for Americans, 2020-2025 continues to recommend 1-2 cups per day of fruit consumption varying with age, gender, and physical activity level (USDA & HHS 2020). Current recommendations further state that this can be achieved by consuming whole fruit, 100% fruit juice, or other fruit forms (i.e., dried fruit, canned fruit). However, driven partially by concerns with dietary sugar, potential for excessive fluid calorie intake as well as real and perceived changes in fiber and/or other nutrient content through juice processing, efforts to separate whole fruit and 100% juice in dietary guidance have intensified.

Current dietary guidance does in fact place a preference towards whole fruit relative to 100% juice stating:

*“The fruit food group includes whole fruits and 100% fruit juice... At least half of the recommended amount of fruit should come from whole fruit, rather than 100% juice... Although 100% fruit juice without added sugars can be part of a healthy dietary pattern, it is lower in dietary fiber than whole fruit. Dietary fiber is a dietary component of public health concern. With the recognition that fruit should mostly be consumed in whole forms, the amount of fruit juice in the USDA Food Patterns ranges from 4 fluid ounces at the lower calorie levels and no more than 10 fluid ounces at the highest calorie levels.” (Dietary Guidelines for Americans, 2020-2025)*

Meeting daily fruit servings, and by extension leveraging the health benefits of fruit, remains a challenge for a majority of Americans. Less than 24% of the American population meet current guidelines for fruit consumption with average intake of <0.5 cups per day (USDA & HHS 2020). Considering the high cost, seasonal variability in quality, and availability as well as the perishable nature of fresh fruit, 100% juice remains a cost effective and available option across all socioeconomic strata. Reported estimates point to the fact that 100% juice contributes up to 35% of daily fruit intake and contributions of juice to overall fruit intake increases for lower income individuals (Drewnowski and Rehm 2015). As such, efforts to modify current guidance based on negative perceptions of 100% juice, may prove counterproductive and negatively impact different segments of consumer and their ability to meet recommendations for fruit intake.

As defined by the US Code of Federal Regulations, 100% juices are:

*“directly expressed from a fruit or vegetable (i.e., not concentrated and reconstituted) shall be considered to be 100% juice and shall be declared as ‘100% juice’” (21CFR101.30)*

If fruit juice has been reconstituted from a concentrate, 100% fruit juice is defined by its soluble solids content (i.e. °Brix) specific to individual fruit according to the US FDA (21CFR101.30). In this context the main factor legally defining 100% juice is that it is (a) derived from a plant product and (b) it is ultimately linked directly to the natural sugar content of the whole fruit and the ability to extract the sugar through processing.

Negative perception of 100% fruit juice appears to be driven by several factors including the high natural sugar content of 100% juice, the perceived negative effects of processing, and weak associations with weight gain. Furthermore, the concern that overconsumption of liquid (juice) versus solid (whole fruit) calories continues. Combined, these perceptions have likely contributed somewhat to the significant (>20%) decline in 100% fruit juice consumption in recent years (Byrd-Bredbenner et al. 2017; Rehm et al. 2016).

However, perceptions and reality may occasionally conflict. For example, sugar content between common whole fruit and 100% juice products are more similar than perceived on an equivalent serving basis (Table 1.1). The similarity in whole fruit and 100% fruit juice across most nutrients (including sugar) is evident both in 100g comparisons and in ½ cup servings. Also, a meta-analysis of studies found that 100% juice consumption was not associated with weight gain in children 7–18 years and, in fact, 100% fruit juice consumption was associated with higher overall diet quality (Auerbach et al. 2017; O’Neil et al. 2012; Agarwal, Fulgoni III, and Welland 2019). Similar levels of macronutrients, vitamins, and minerals are compared across three fruits

and their 100% juices (Table 1.1). A key exception is for vitamin C and fiber content. While vitamin C loss is mostly attributed to the thermal processing during pasteurization of juice, fiber loss relates greatly to the physical aspects of juicing including the separation of seed and skin components from juice derived primarily from flesh. This is indeed a consequence of processing and is widely used as a factor to support consumption of whole fruit versus 100% juice even with clear similarities in other macro and micronutrient profiles.

Placing these differences in context is critical. While vitamin C content is reduced through juice processing, this is consistent with vitamin C thermal and oxidative sensitivity, and variation exists even in fresh fruit content. However, actual changes in fiber content needs to be placed in context of actual differences in fiber from ½ cup servings amount to 0.4, 0.5, and 1.8g in total for grape, apple and orange juice versus fruit (Table 1.1). While significant, these losses are rather modest considering the role of fruit in overall fiber intake. Overall fruits and vegetables (excluding 100% juice) contribute only 12 and 16% of the total dietary fiber intake in the United States, with a majority of dietary fiber intake coming from whole grains. (Hoy and Goldman 2014). Furthermore, it has been reported no significant differences in dietary fiber intake between children (2-18 years) who are consumers of 100% fruit juice and non-consumers (Nicklas, O’Neil, and Fulgoni 2015). However, vitamin C, magnesium, potassium and overall diet quality increased with increased fruit juice consumption (Nicklas, O’Neil, and Fulgoni 2015). Such data suggest that even considering these losses through processing, nutritional density of whole fruit and 100% juice may not be as different as commonly perceived, and that nutritional impact of delivering fruit serving from 100% fruit juice can be meaningful.

**Table 1.1.** Comparison of macronutrients, minerals, and vitamins in apple, orange, and grapes and their 100% fruit juices. (Adapted from Ho, Ferruzzi, and Wightman 2019)

Database value	Unit	Apple	Apple juice	Apple (1/2 cup slices)	Apple juice (1/2 cup)	Orange	Orange juice	Orange (1/2 cup sections without membranes)	Orange juice (1/2 cup)	Grapes	Grape juice	Grapes (16 grapes) <sup>b</sup>	Grape juice (1/2 cup)
Database Number <sup>a</sup>		09004	09016	09004	09016	09203	09207	09203	09207	09132	09135	09132	09135
Weight	g	100	100	55	124	100	100	92.5	124.5	100	100	78.4	126.5
<b>Macronutrients</b>													
Water	g	86.67	88.24	47.67	109.42	87.14	87.72	80.6	109.21	80.54	84.51	63.14	106.91
Energy	kcal	48	46	26	57	46	47	43	59	69	60	54	76
Protein	g	0.27	0.1	0.15	0.12	0.7	0.68	0.65	0.85	0.72	0.37	0.56	0.47
Total lipid (fat)	g	0.13	0.13	0.07	0.16	0.21	0.15	0.19	0.19	0.16	0.13	0.13	0.16
Carbohydrate, by difference	g	12.76	11.3	7.02	14.01	11.54	11.01	10.67	13.71	18.1	14.77	14.19	18.68
Fiber, total dietary	g	1.3	0.2	0.7	0.2	2.4	0.3	2.2	0.4	0.9	0.2	0.7	0.3
Sugars, total	g	10.1	9.62	5.55	11.93	9.14	8.76	8.45	10.91	15.48	14.2	12.14	17.96
<b>Minerals</b>													
Calcium, Ca	mg	5	8	3	10	43	10	40	12	10	11	8	14
Iron, Fe	mg	0.07	0.12	0.04	0.15	0.09	0.1	0.08	0.12	0.36	0.25	0.28	0.32
Magnesium, Mg	mg	4	5	2	6	10	10	9	12	7	10	5	13
Phosphorus, P	mg	11	7	6	9	12	17	11	21	20	14	16	18
Potassium, K	mg	90	101	50	125	169	184	156	229	191	104	150	132
Sodium, Na	mg	0	4	0	5	0	4	0	5	2	5	2	6
Zinc, Zn	mg	0.05	0.02	0.03	0.02	0.08	0.04	0.07	0.05	0.07	0.07	0.05	0.09
<b>Vitamins</b>													
Vitamin C, total ascorbic acid	mg	4	0.9	2.2	1.1	45	30.1	41.6	37.5	3.2	0.1	2.5	0.1
Thiamin	mg	0.019	0.021	0.01	0.026	0.1	0.039	0.092	0.049	0.069	0.017	0.054	0.022
Riboflavin	mg	0.028	0.017	0.015	0.021	0.04	0.021	0.037	0.026	0.07	0.015	0.055	0.019
Niacin	mg	0.091	0.073	0.05	0.091	0.4	0.201	0.37	0.25	0.188	0.133	0.147	0.168
Vitamin B-6	mg	0.037	0.018	0.02	0.022	0.051	0.031	0.047	0.039	0.086	0.032	0.067	0.04
Folate, DFE	µg	0	0	0	0	17	24	16	30	2	0	2	0
Vitamin B-12	µg	0	0	0	0	0	0	0	0	0	0	0	0
Vitamin A, RAE	µg	2	0	1	0	11	9	10	11	3	0	2	0
Vitamin A, IU	IU	38	1	21	1	225	175	208	218	66	8	52	10
Vitamin E (alpha-tocopherol)	mg	0.05	0.01	0.03	0.01	0.18	0.2	0.17	0.25	0.19	0	0.15	0
Vitamin D (D2 + D3)	µg	0	0	0	0	0	0	0	0	0	0	0	0
Vitamin D	IU	0	0	0	0	0	0	0	0	0	0	0	0
Vitamin K (phylloquinone)	µg	0.6	0	0.3	0	0	0.1	0	0.1	14.6	0.4	11.4	0.5

<sup>a</sup>Macronutrient, mineral, and vitamin data were obtained from the USDA National Nutrient Database for Standard Reference (<https://ndb.nal.usda.gov/ndb/>)

<sup>b</sup>Based on MyPlate (<https://www.choosemyplate.gov/fruit>) which specifies that 16 grapes = ½ cup equivalent of fruit. USDA National Nutrient Database for Standard Reference (<https://ndb.nal.usda.gov/ndb/>) indicates that ½ cup of grapes is ~15.41 grapes.

“NA” indicates that data was not available

In addition to traditional nutrients, fruits and 100% fruit juice are also a key dietary source of bioactive phytochemicals including carotenoids and phenolics (Manach et al. 2004; Saini, Nile, and Park 2015). Another common perception is that whole fruits remain a better source of these bioactives compared to their 100% juices. In a manner similar to the comparison for key nutrients, a comparison of phenolic content in whole fruit (orange, apple and grape) to 100% fruit juice was made by Ho et al. (2019) (Table 1.2). Using values derived from the USDA flavonoid database (Bhagwat, Haytowitz, and Holden 2014) and Phenol Explorer (Phenol Explorer, 2015), the content of major phenolic species in whole fruit and corresponding 100% fruit juices were reported to differ somewhat with levels in juice being similar but subtly lower than those in whole fruit. However, it is important to consider edible portions and the localization of phenolics in the fruit for these comparisons to be more meaningful. Apples, commonly consumed as whole fruit and juice, contain anthocyanins, predominantly in the skin, as well as flavan-3-ols, and flavonols in skin, flesh, and core (Harnly et al. 2006). Berries, particularly dark fruits such as purple grapes and berries, are key sources of anthocyanins, flavanols, and flavan-3-ols including polymeric procyanidins (Howard and Hager 2007; Ivanova, Stefova, and Chinnici 2010). Though present throughout the fruit, these polyphenols are generally concentrated in the skins and seeds of grapes and berries (Xu et al. 2011). If one considers the fact that seeds are typically not consumed as part of the whole fruit; then a substantial portion of the polyphenols would not be in fact consumed, particularly flavan-3-ols and large procyanidin polymers.

Citrus fruits are well known as a source of flavanones including as naringenin and hesperetin (Manach et al. 2004; Manach et al. 2003). Similar to berries, the concentration of phenolics is greatest in the peels of citrus fruits and is therefore reduced in consumption fresh fruit as the peels are generally discarded (Manach et al. 2004; Gil-Izquierdo, Gil, and Ferreres 2002).

With concentrations of bioactives, such as phenolics, greatest in skins and seeds of many fruits, it is critical to consider how juicing may serve to transfer these compounds from the whole fruit to the juice thereby making phenolics available to consumers that otherwise would not be consumed.

**Table 1.2.** Comparison of phytonutrients in apple, orange, and grape and their 100% fruit juice. (Adapted from Ho, Ferruzzi, and Wightman 2019)

Database value	Unit	Apple <sup>a</sup>	Apple juice <sup>b</sup>	Apple (1/2 cup, slices) <sup>a</sup>	Apple juice (1/2 cup) <sup>b</sup>	Oranges, raw, navels (Citrus sinensis) <sup>a</sup>	Juice, orange, chilled, includes from concentrate <sup>a</sup>	Orange (1/2 cup sections without membranes) <sup>a</sup>	Orange juice (1/2 cup) <sup>a</sup>	Grapes, red, raw <sup>a,c</sup>	Grapes (16 grapes) <sup>a,c</sup>	Grape juice <sup>a</sup>	Grape juice (1/2 cup) <sup>a</sup>
USDA Database Number/Phenol-Explorer Entry		09504, 09503, 09501, 09502, 09003, and 09500	(Apple [Cider], pure juice)	09504, 09503, 09501, 09502, and 09500	(Apple [Cider], pure juice)	09202	09209	09202	09209	97074	97074	09135, 99436	09135, 99436
Weight	g	100	100	100	124	100	100	92.5	124.5	100	78.4 <sup>e</sup>	100	126.5
<b>Anthocyanidins</b>													
Cyanidin	mg	0-4.9	NA	0-2.695	NA	0	0	0	0	0.08-1.16	0.06-0.91	0.04-0.89	0.05-1.13
Delphinidin	mg	NA	NA	NA	NA	0	NA	0	NA	2.27	1.78	0.1-1.92	0.13-2.43
Malvidin	mg	NA	NA	NA	NA	0	NA	0	NA	0.10-39	0.07-30.58	0.08-11.17	0.10-0.13
Pelargonidin	mg	NA	NA	NA	NA	0	NA	0	NA	0.02	0.02	0.02	0-0.03
Peonidin	mg	NA	NA	NA	NA	0	NA	0	NA	0.014-3.62	0.01-2.84	0.17-1.06	0.22-1.34
Petunidin	mg	NA	NA	NA	NA	0	NA	0	NA	1.97	1.54	0.1-1.02	0.127-1.29
<b>Flavan-3-ols</b>													
Epicatechin	mg	1.8-19.16	9.03	0.99-10.54	11.20	0	NA	0	NA	0.01-0.96	0.01-0.75	0-0.56	0-0.71
Catechin	mg	0-3.4	4.61	0-1.87	5.7164	0	NA	0	NA	0.013-0.82	0.01-0.64	0.17-0.82	0.22-1.04
Gallocatechin	mg	NA	0	NA	0	0	NA	0	NA	NA	NA	0	0
Procyanidins <sup>d</sup>	mg	14.56-93.96	9.02-20.47	8.01-51.68	11.91-27.02	0	0	0	0	46.69	36.60	46.69	59.06
<b>Flavonols</b>													
Kaempferol	mg	NA	NA	NA	NA	0.13	NA	0.12025	NA	0.003	0.003	0.01	0.01
Myricetin	mg	NA	NA	NA	NA	0.15	NA	0.138	NA	0.01	0.008	0.7	0.89
Quercetin	mg	0.52-19.76	1.04	0.29-10.87	1.2896	0.45	0.4	0.42	0.498	0.021-1.04	0.016-0.815	0.09-0.72	0.11-0.91
<b>Flavanones</b>													
Hesperetin	mg	NA	NA	NA	NA	21.87	16.38	20.23	20.39	NA	NA	NA	NA
Naringenin	mg	NA	NA	NA	NA	7.1	2.56	6.56	3.187	NA	NA	NA	NA
Naringin	mg	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
<b>Stilbenes</b>													
Resveratrol	mg	NA	NA	NA	NA	NA	NA	NA	NA	0.001	0.00078	NA	NA

<sup>a</sup>Phytochemical values were derived from the USDA Database for the Flavonoid Content of Selected Foods

<sup>b</sup>Phytochemical values were derived from Phenol-Explorer (<http://phenol-explorer.eu>)

<sup>c</sup>Values obtained from O'connor et al. (2013)

<sup>d</sup>Values obtained from the USDA Database for Proanthocyanidin Content of Selected Foods

<sup>e</sup>Based on MyPlate (<https://www.choosemyplate.gov/fruit>) which specifies that 16 grapes = 1/2 cup equivalent of fruit. USDA National Nutrient Database for Standard Reference (<https://ndb.nal.usda.gov/ndb/>) indicates that 1/2 cup of grapes is ~15.41 grapes.

“NA” Indicates that data was not available from the references used.

There is reason to believe that differences in ultimate bioavailability of micronutrients and phytochemicals would exist between whole fruit and 100% juice. For example, the fact that many phenolics are concentrated in the non-edible seeds (and potential skin) would serve to minimize the potential absorbability of these component. Beyond physical location, the whole fruit matrix itself may serve to limit the bioavailability of phenolics potentially due to entrapment of micronutrients and bioactive species in fiber or cellular structures that could serve to limit their digestive release and availability for absorption or interaction with the microbiota (Palafox-Carlos, Ayala-Zavala, and González-Aguilar 2011). Interestingly, a single *in vitro* study reported that both carotenoid and flavonoid bioaccessibility was greater from orange juice processed by different methods, (fresh, flash-pasteurized, and pasteurized) compared to coarse cut orange fruit (Aschoff et al. 2015). From this preclinical result it can be hypothesized that differences in bioavailability and potentially metabolism by both host and gut microbial communities would exist between whole fruit and 100% juice. This is likely a result of interactions within the juice matrix and the type and extent of juice processing whereby phenolics are more effectively extracted from the fruit matrix into the juice and made bioaccessible for either uptake by the human intestine or metabolism by the microbiota.

Ultimately, phenolic bioavailability from whole fruit would require efficient human digestion to release phenolics in the GI tract making them available for absorption or interaction with the microbiota. In juice, the processing enables this release and by virtue of this, enhances the bioaccessibility and potential delivery of phenolics and their bioactive metabolites. Further, interactions between phenolics and macronutrients including non-starch polysaccharides/fiber can serve to limit bioaccessibility of phenolics (Palafox-Carlos, Ayala-Zavala, and González-Aguilar 2011). As juice processing may break these interactions during extraction and further reduce fiber

levels, it is plausible to believe that these products may differ in ultimate bioavailability in humans and could help explain, in part, the differences between health associations observed with fruit juices and whole fruit.

Further, within this paradigm, potential differences may also exist in metabolism of phenolics by both host systems and microbial communities. Microbial metabolism is understood to be key in the delivery of fruit derived phenolic metabolites that are actually found in circulation and in urine and are believed to be associated with health benefits of fruit. These are in fact small molecular weight microbial catabolites of native flavonoids and phenolics from fruit/juice (Neilson and Ferruzzi 2011; Redan et al. 2016; Kay 2010; Aura 2008). Considering both the enhanced bioaccessibility generating higher concentration and different ratios of phenolics in the gut lumen, differences in macronutrient content, fermentable fiber in particular, it is plausible to consider that circulating and urinary metabolite profiles in response to whole fruit or juice consumption might differ. In fact, similar 2 h urinary phenolic metabolite profiles in humans consuming either whole blueberries or juice were reported (Langer, Kennel, and Lodge 2018). While promising, this study did not likely capture the broad profiles of microbial metabolites which peak at 18-36 h after consumption. Broader assessment of urinary patterns including quantitative 24-48 h profiling and changes over long-term consumption are needed to better establish any equivalency of whole fruit and 100% juice.

Differences in the metabolic processing of fruit phenolics could serve to help us better understand if fruit juice and whole fruit impart benefits through similar or different mechanisms. It can also be used as a metric by which fruit juice products (including 100% juice and other improved but not 100% fruit juice products) can be comparatively assessed to ensure alignment with benefits of whole fruit. It can further be used to develop into “metabotypes” as has been done

for ellagitannin rich pomegranate and urolithin metabolism to predict responsiveness to treatment for blood lipids linked to cardiovascular risk (González-Sarriás et al. 2017). Expanding this concept to better refine response from fruit and juice would likely prove useful in designing future functional studies but also in setting recommendations for consumption.

In an effort to provide mechanistic links between fruit phenolics and their health benefits of fruit consumption, significant efforts have been placed on the study of phenolic digestion, absorption, metabolism, and tissue distribution from fruit using both preclinical and clinical models. While the literature currently lacks studies specifically designed to assess bioavailability and metabolism of fruit phenolics directly comparing whole fruit and juice, it is critical to consider that such studies would in fact offer the possibility of drawing direct comparisons of the “delivery” characteristics between forms. With this in mind, the focus of this thesis research was to profile the phenolic content and assess the phenolic bioavailability through both the upper (small intestinal) and lower (large intestinal/colonic) digestive tracts for commonly consumed berries and their 100% juices.

While nutrient and phytochemical bioavailability is best measured through *in-vivo* animal and clinical settings, *in-vitro* models have proven useful surrogates to estimate the factors affecting phytochemical release, absorption, and metabolism through digestion (Hur et al. 2011; Bohn et al. 2018; Artursson, Palm, and Luthman 2001; Hubatsch, Ragnarsson, and Artursson 2007). As a particular advantage, the use of compartmentalized *in-vitro* models allows for a mechanistic and segmented understanding of bioactive digestion and absorption, which is difficult to obtain using *in-vivo* models. This work will provide a preclinical framework to compare the delivery of phenolics by consumption of grapes versus 100% grape juices designed to support future *in-vivo* and clinical studies in an effort to better understand the role 100% fruit juice products play in a

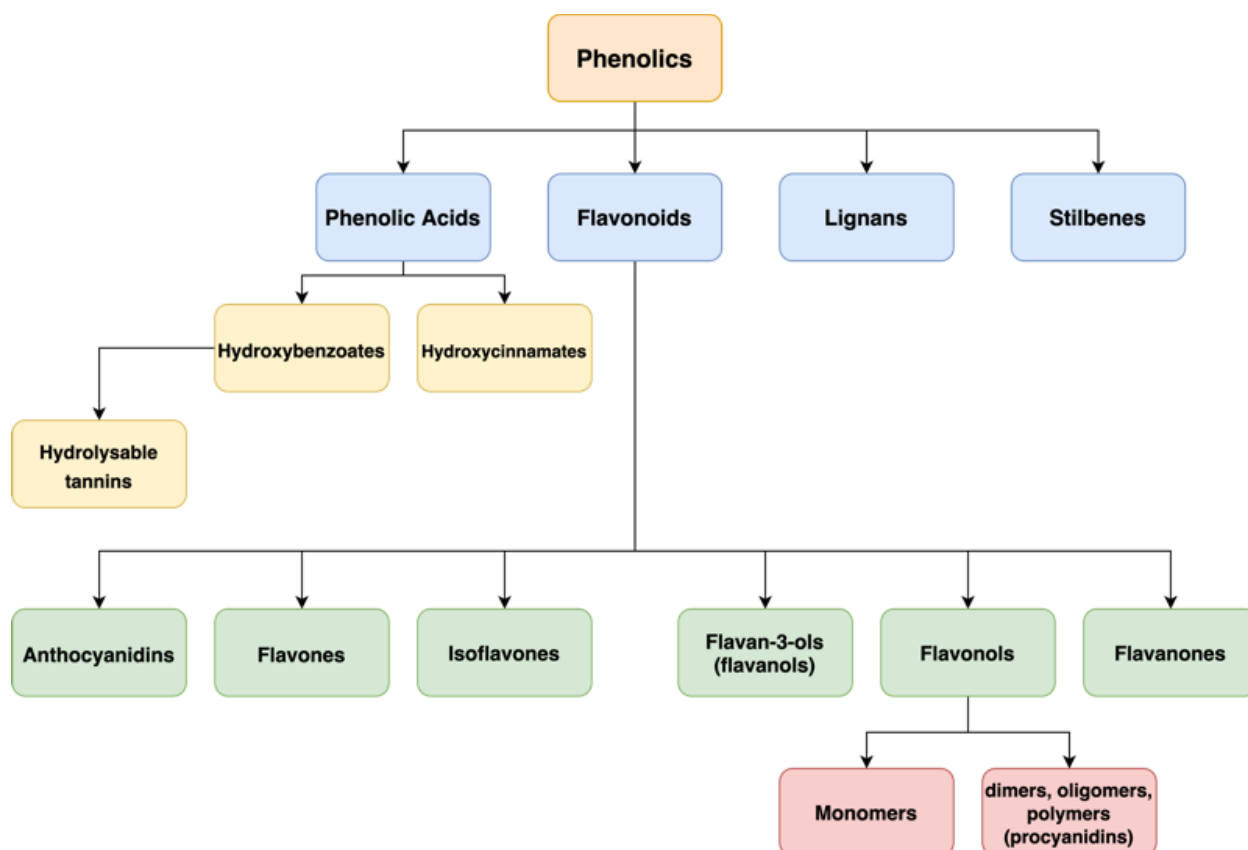
healthy diet. In support of this effort, the focus of this literature review is to provide a background into grape-derived phenolics, the commercial production of 100% grape juice, and insights to phenolic bioavailability and the *in-vitro* models (small intestinal, large intestinal, and cellular flux) used to determine phenolic bioavailability.

## 1.2. Introduction to Phenolics

Phenolic species represent one of the largest class of plant secondary metabolites consumed by humans, with daily intake reaching as high as 1 g per day (Kuhnau 1976; Scalbert, Johnson, and Saltmarsh 2005; Zamora-Ros et al. 2016). Common dietary sources of phenolics include coffee, tea, chocolate, fruits (particularly berries), grains, and vegetables, though the type and concentration of individual phenolic species varies greatly (Tsao 2010; Manach et al. 2004; El Gharras 2009; Bravo 1998). Biosynthesis of polyphenols occurs in higher plants through complex biochemical routes based on the shikimate, phenylpropanoid, and flavonoid pathways (A. Crozier, Jaganath, and Clifford 2006; Bravo 1998). Over 8,000 individual phenolic species have been identified and play diverse roles in plants, including pigmentation, UV protection, disease resistance, nitrogen fixation, and growth (Bravo 1998; A. Crozier, Jaganath, and Clifford 2006).

Phenolics are defined by having at least one aromatic ring with one more hydroxyl (alcohol) groups substitutions. Furthermore, phenolic species, particularly flavonoids, are typically found conjugated to various organic acids and simple/complex carbohydrates. Phenolic compounds can range from simple, single-ringed aromatic compounds to large complex polymers such as proanthocyanidins. Due to the exceedingly large number of phenolic compounds, they have been classified and grouped into a variety of categories and subcategories based on source, biological function, and chemical structure (Figure 1.1). For the purposes of this review, phenolics

are broadly classified in one of two groups: flavonoids or nonflavonoids. Major flavonoid subcategories include anthocyanidins, flavones, isoflavones, flavan-3-ols (flavanols), flavonols, and flavanones. Nonflavonoids are comprised of major categories including phenolic acids (hydroxybenzoates and hydroxycinnamates), stilbenes, and lignans.



**Figure 1.1.** Breakdown of major phenolic categories and subcategories.

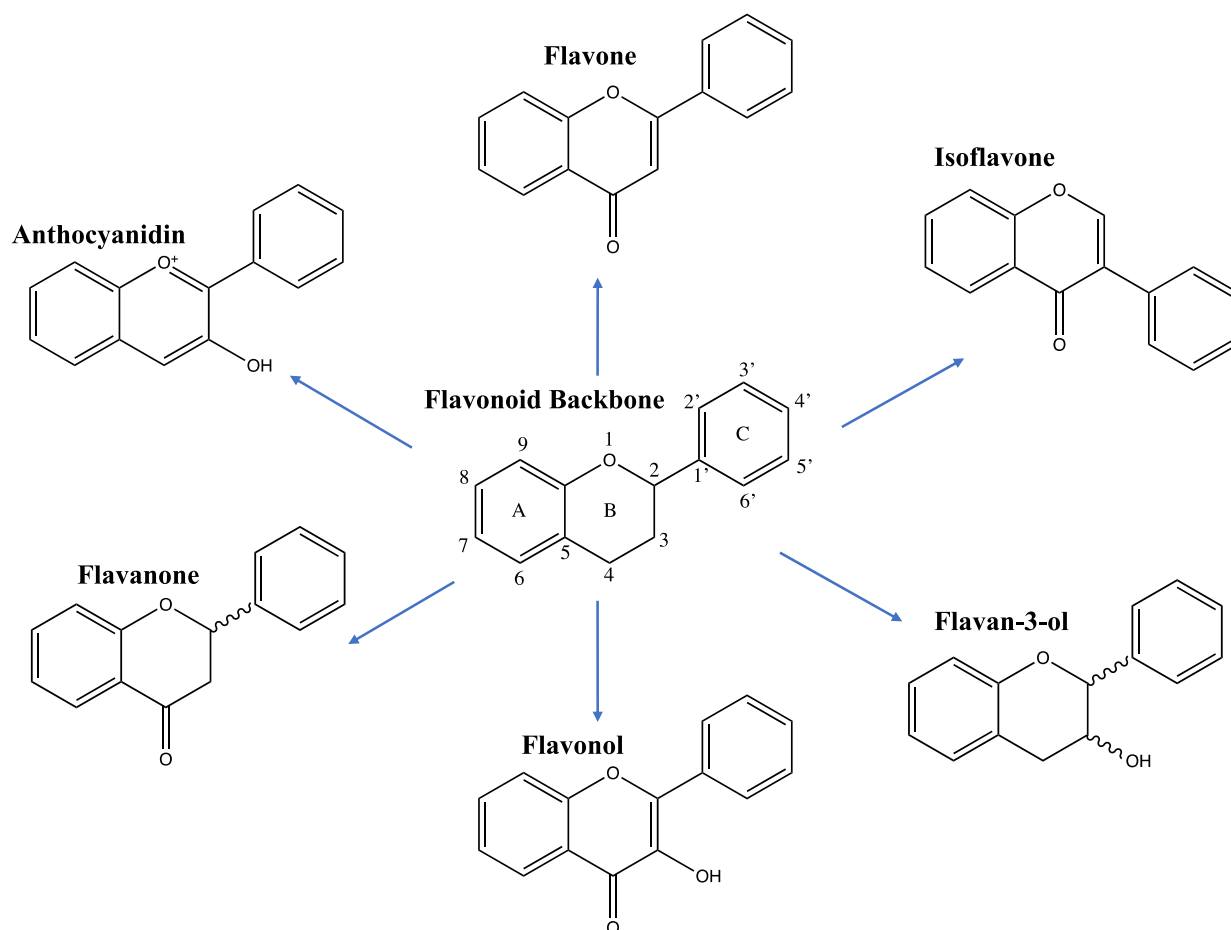
Due to their abundance in the human diet and antioxidant/free-radical scavenging capacities for some phenolics, there has been increasing interest in understanding the role phenolic species may play in human health. Dietary phenolics have been studied for their potential ability to modulate oxidative stress through a variety of mechanisms including free radical scavenging, inhibition of oxidative enzymes, influencing the cell cycle, induction of endogenous antioxidant mechanisms, and impacts to signal transduction (Han, Shen, and Lou 2007). Through the

modulation of oxidative stress, phenolics may play roles in cardiovascular protection, neuroprotection, antiinflammation, anti-cancer, immune function, diabetes, and gastrointestinal health (Quiñones, Miguel, and Aleixandre 2013; Vita 2005; Silva and Pogačnik 2017; Stoner and Mukhtar 1995; Kampa et al. 2007; Duthie, Duthie, and Kyle 2000; González-Gallego et al. 2010; Ding, Jiang, and Fang 2018; Xiao and Hogger 2015; Bahadoran, Mirmiran, and Azizi 2013). However, the specific mechanisms through which phenolics may actually affect human health is complex and remains unclear. Some incongruence between translating results observed *in vitro* with *in vivo* animal and clinical studies exists (Han, Shen, and Lou 2007; Perez-Vizcaino and Fraga 2018; Fraga et al. 2019). Nonetheless, research into the bioactivity of phenolics remains pertinent as clinical evidence supports numerous positive health benefits from phenolic-rich diets/foods, including to cardiovascular health, anti-inflammatory status, immune function, cognitive function, and gastrointestinal health among others (Rowe et al. 2011; Lamport, Lawton, et al. 2016; Park, Kim, and Kang 2004; Copetti et al. 2018; Lockyer et al. 2017; Loo et al. 2020).

### **1.3. Flavonoids**

Flavonoids represent the most commonly consumed phenolic species, comprising of roughly two-thirds of dietary phenolics (Laura et al. 2019; Manach et al. 2004; Bravo 1998). They are ubiquitous across the plant kingdom particularly found in leaves, flowering structures, barks, stems, and the skins and seeds of fruits, playing varied roles as secondary metabolites within the plants (A. Crozier, Jaganath, and Clifford 2006; El Gharras 2009; Claudine Manach et al. 2004). The basic structure of flavonoids is composed of two aromatic rings (A and C rings) bridged by three carbons (B ring) (Figure 1.2). Variations in the hydroxylation patterns and substituents among the B and C rings help to further classify flavonoids into smaller subclasses of which the major six are: anthocyanidins, flavones, isoflavones, flavan-3-ols, flavanols, and flavanones

(Figure 1.2). While for most flavonoid subclasses the C ring is connected to the B ring at C2, for some classes this varies such as for isoflavones where the C ring is connected at C3 (Figure 1.2). Differences in modification (ex. hydroxylation, methylation, acetylation, glycosylation, etc.) and stereochemistry at various carbons on the subclass backbones accounts for the wide variety of individual species of flavonoids found across the plant kingdom (Figure 1.2).



**Figure 1.2.** Flavonoid and flavonoid subclass backbone structures.

### 1.3.1. Anthocyanidins

Anthocyanidins, more commonly their glycosylated derivatives, anthocyanins, are responsible for the deep red, purple, blue, and pink hues observed across much of the plant

kingdom (A. Crozier, Jaganath, and Clifford 2006). The color of anthocyanins is highly pH dependent and is further influenced by various modifications to structure including hydroxylation, methylation, glycosylation, and esterification patterns (Tsao 2010; Manach et al. 2004). Principle anthocyanidins include cyanidin, malvidin, peonidin, petunidin, delphinidin, and pelargonidin which are further glycosylated, esterified, and acylated to produce a myriad of anthocyanin derivatives. Cyanidin-based forms remain the most common in nature (Manach et al. 2004). While anthocyanins are found in a variety of food sources such as cereal, maize, and vegetables (ex. carrots, cabbage, beans, onions), they are most concentrated in fruits and fruit derived products (Manach et al. 2004; Tsao 2010). Anthocyanins can be found in high concentrations in darkly colored berries (ex. blueberries, strawberries, grapes etc.), pomegranates, cherries, wine, and fruit juices with levels reaching as high as 2-4 g/kg fw in blackcurrants or blackberries (Manach et al. 2004). Anthocyanins are widely studied due to their implication with numerous potential health benefits including alleviating oxidative stress, prevention of cardiovascular disease, anticarcinogenic properties, prevention of weight gain and obesity, and diabetes among many others (He and Giusti 2010; Ramirez-Tortosa et al. 2001; Renaud and de Lorgeril 1992; Wallace 2011; Jing et al. 2008; Y. Zhang et al. 2008; Tsuda 2008; Ghosh and Konishi 2007).

### 1.3.2. Flavones

Flavones are a subclass of flavonoids found predominantly as glycosylated forms of luteolin and apigenin. The most significant sources of these compounds being reported in the diet are celery, parsley, and some herbs (A. Crozier, Jaganath, and Clifford 2006; Claudine Manach et al. 2004). Notably, hydrophobic, polymethoxylated flavones such as nobiletin and tangeretin are found in large amounts in citrus peels (up to 6.5 g/L in oil of mandarin) (Shahidi and Naczki 1995;

S.-C. Ho and Kuo 2014). Citrus-derived flavones have become of increasing interest due their potential for general anti-inflammatory/oxidant capacity and role in neuroprotection (S.-C. Ho and Kuo 2014; X.-M. Chen, Tait, and Kitts 2017; D. Zhang, Hou, and Peng 2018; Fatima et al. 2017; Fatima and Siddique 2019).

### 1.3.3. Isoflavones

Isoflavones represent a fairly unique subclass of flavonoids primarily entering the human diet from soybeans and soya-derived products. The three main forms are genistein, daidzein, and glycitein on which various glycosylations and acetylations may be present at up to 3.8 g/kg fw in soya beans (A. Crozier, Jaganath, and Clifford 2006; Claudine Manach et al. 2004). Though not a steroidal hormone, isoflavones exhibit potent estrogenic activity (Vitale et al. 2013). This is due to structural similarity of isoflavones and human 17- $\beta$ -estradiol as isoflavones are hydroxylated on C8 and C4' conferring the ability to bind both  $\alpha$  and  $\beta$  estrogen receptors on a variety of target organs. This capacity to mimic estrogen may exert health benefits including chemoprevention of prostate and breast cancers (Vitale et al. 2013).

### 1.3.4. Flavan-3-ols

Flavan-3-ols represent a complex subclass of flavonoids ranging from simple monomers such as (+)-catechin and (-)-epicatechin to larger oligomers and polymers dubbed condensed tannins or proanthocyanidins (Claudine Manach et al. 2004; A. Crozier, Jaganath, and Clifford 2006). Unlike most other flavonoid subclasses, flavan-3-ols have two chiral centers at the C2 and C3 positions on the B ring, giving the possibility of four stereoisomers for a single monomer unit upon hydroxylation of the B ring at these locations. The most common naturally occurring isomers

are (+)-catechin (2*R*, 3*S*) and (-)-epicatechin (2*S*, 3*R*), with the (-)-catechin (2*R*, 3*R*) and (+)-epicatechin (2*S*, 3*S*) forms being extremely rare in nature (A. Crozier, Jaganath, and Clifford 2006). Monomer flavan-3-ols are commonly further hydroxylated to form gallocatechins and can also undergo esterification with gallic acid (ex. epigallocatechin gallate) and are not glycosylated in foods (Manach et al. 2004). Proanthocyanidins can range from dimers to polymers of up to 50 units (A. Crozier, Jaganath, and Clifford 2006). Proanthocyanidins composed of only (epi)catechin monomer units are named procyanidins, which are the most abundant type of polymeric flavan-3-ols found in plants (A. Crozier, Jaganath, and Clifford 2006). Catechin and epicatechin are found in high concentrations in numerous fruits like apricots, cherries, apples, and peaches (up to 250 mg/kg, 220 mg/kg, 120 mg/kg, 140 mg/kg respectively) while gallocatechins and gallocatechin gallates are found in grapes, legumes, and importantly green tea in very high concentrations (up to 800 mg/kg in tea) (Manach et al. 2004; Bravo 1998; A. Crozier, Jaganath, and Clifford 2006). Procyanidins are found in high concentrations in wine, grape seed, and chocolate (A. Crozier, Jaganath, and Clifford 2006). Flavan-3-ol monomers, oligomers, and polymers are of heavy interest for their biological activity as they and their gut microbial metabolites play roles in mitigating diabetes, gastrointestinal disorders, cardiovascular disease, and immune modulation among many other functions (Márquez Campos, Jakobs, and Simon 2020; Raman et al. 2019; Burton-Freeman et al. 2019; Nawrot-Hadzik et al. 2021; Hooper et al. 2012; Monagas et al. 2010).

### 1.3.5. Flavonols

Flavonols are perhaps the most ubiquitous and most common class of flavonoids in foods, with the main species being quercetin, kaempferol, and myricetin (A. Crozier, Jaganath, and Clifford 2006; Claudine Manach et al. 2004). Flavonols are typically found glycosylated with

various sugar moieties (ex. glucose, rhamnose, galactose, etc.), being conjugated most frequently at the C3 position of the B-ring, though other locations for glycosylation have been reported (A. Crozier, Jaganath, and Clifford 2006). Flavonol rich food sources include red wine, tea, onions, leafy green vegetables, broccoli, blueberries, and leeks (Manach et al. 2004). Due to their significant antioxidant potential, flavanols, particularly quercetin, have been extensively studied for their antidiabetic, anti-inflammatory, anticarcinogenic, antarthritic, cardioprotective, and neuroprotective benefits (Boots, Haenen, and Bast 2008; Salehi et al. 2020; Rather and Bhagat 2020; Guillermo Gormaz, Quintremil, and Rodrigo 2015; G.-J. Shi et al. 2019; Tahir et al. 2021; Xie, Huang, and Su 2016).

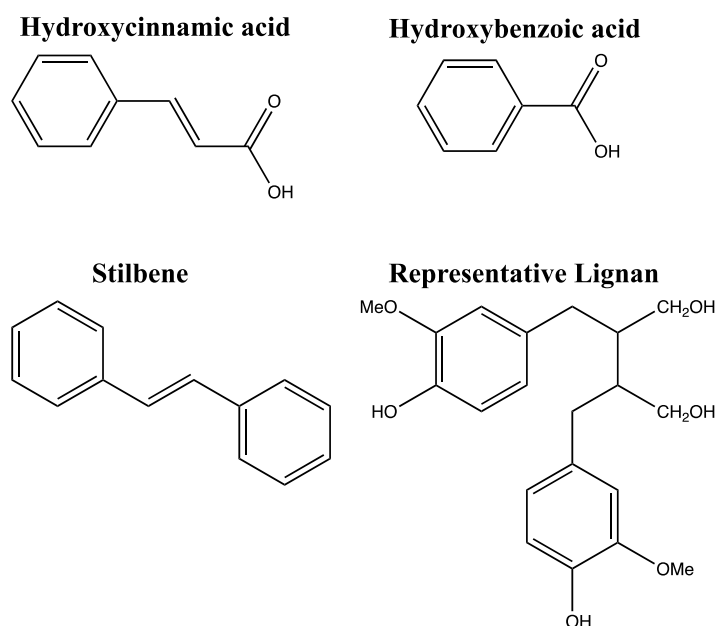
#### 1.3.6. Flavanones

As flavanones lack a double bond in the B ring, unlike other flavonoids, the C2 carbon exhibits chirality, with a majority of flavanones being in the  $\alpha$ -configuration. Flavanones are found in high concentrations only in citrus fruits, with typical aglycone forms being naringenin, hesperetin, and eriodictyol (Manach et al. 2004). Aglycone forms are rare as they are highly reactive and commonly undergo a variety of modifications including glycosylation, methylation, and hydroxylation (A. Crozier, Jaganath, and Clifford 2006). While a single glass of orange juice may contain up to 140 mg of flavanones, citrus fruits may contain up to 5x more because of high concentrations present in the normally discarded albedo and membrane segments (Manach et al. 2004; Aschoff et al. 2015). Much like other flavonoids, flavanones have been associated with numerous positive health benefits including potential as chemopreventative and therapeutic agents, prevention of cardiovascular disease, and particularly with improving cognitive and

neurological health (Cassidy et al. 2016; Mir and Tiku 2015; Lamport, Pal, et al. 2016; R. J. Kean et al. 2015; Khan et al. 2020).

#### 1.4. Nonflavonoids

The remaining major phenolic classes can be grouped into together as nonflavonoids, though these phenolic classes have no unifying backbone structure. Major nonflavonoid classes include phenolic acids as hydroxycinnamates or hydroxybenzoates, stilbenes, and lignans. Phenolic acids, having at least one hydroxyl group on the benzyl ring, are of particular importance as they account for roughly one-third of dietary phenolics (Manach et al. 2004; Laura et al. 2019). Furthermore, hydroxybenzoic acids can form large polyphenolic structures known as hydrolysable tannins (Manach et al. 2004; A. Crozier, Jaganath, and Clifford 2006; Bravo 1998). While phenolic acids and stilbenes tend to be relatively simple in structure, hydrolysable tannins and lignans may be large, complex, and diverse in structure (Figure 1.3).



**Figure 1.3.** Backbone structures or representative structures of nonflavonoid phenolic classes.

#### 1.4.1. Hydroxycinnamates

The wide variety of hydroxycinnamates are derived from cinnamic acid ( $C_6-C_3$  structure) through the phenylpropanoid pathway in plants (A. Crozier, Jaganath, and Clifford 2006). Hydroxycinnamates are commonly found in free forms such as p-coumaric, caffeic, or ferulic acids as well as glycosylated or esters of tartaric, quinic, or shikimic acids (A. Crozier, Jaganath, and Clifford 2006; Manach et al. 2004; Tsao 2010). Caffeic acid is the most abundant hydroxycinnamate found in fruits, accounting for roughly 75 to nearly 100% of total hydroxycinnamate content (Manach et al. 2004). Particularly rich fruit sources of hydroxycinnamates include plums, cherries, apples, kiwi, blackberries, and grapes at levels of up to 2 g/kg fw (Manach et al. 2004). Ferulic acid, both in soluble, free and bound forms, is found in high concentrations in the outer parts, pericarp, and aleurone layers of cereal grains, up to 2 g/kg dw in wheat (Manach et al. 2004). An ester of caffeic and quinic acid, chlorogenic acid, is perhaps the most commonly consumed phenolic species, as upwards of 350 mg can be consumed from a single cup of coffee (Manach et al. 2004; Fujioka and Shibamoto 2008). Tartaric esters of coumaric and caffeic acids (coutaric and caftaric acids, respectively) are found in high concentrations in grapes and grape derived products (Singleton, Zaya, and Trousdale 1986). Hydroxycinnamic acids, particularly chlorogenic acid, have shown potent bioactivity in cell and animal models and have been implicated with the health benefits associated with hydroxycinnamate rich foods and diets (Tajik et al. 2017; Crozier et al. 2012; Santana-Gálvez, Cisneros-Zevallos, and Jacobo-Velázquez 2017; Coman and Vodnar 2020; Sova and Saso 2020)

#### 1.4.2. Hydroxybenzoates

Hydroxybenzoates are typically found in very low quantities in plants, especially in their free forms (A. Crozier, Jaganath, and Clifford 2006; Manach et al. 2004). By far the most predominant hydroxybenzoic acid is gallic acid, with protocatechuic, vanillic, and syringic acids also being fairly common (Manach et al. 2004; Tomás-Barberán and Clifford 2000; Herrmann and Nagel 1989). Hydroxybenzoates can be found in fairly high quantities in some colored fruits (blackberries, raspberries, black currants, and strawberries), onions, tree nuts, and radishes, while tea is a significant source of gallic acid with leaves containing up to 4.5g/kg fw (Manach et al. 2004; Tomás-Barberán and Clifford 2000; Herrmann and Nagel 1989).

Hydroxybenzoates also exist in food sources in bound forms, namely as hydrolysable tannins (Bravo 1998; A. Crozier, Jaganath, and Clifford 2006; Manach et al. 2004). Hydrolysable tannins consist of numerous hydroxybenzoic acids esterified to a polyol moiety, typically a glucose (A. Crozier, Jaganath, and Clifford 2006; Bravo 1998). These esterified sugar molecules can further oxidatively condense with gallic acid to form larger polymeric polyphenolic structures (Bravo 1998). Hydrolysable tannins can be grouped as gallotannins (ex. tannic acid) being derived from gallic acid or ellagitannins being derived from ellagic acid units (Bravo 1998; Clifford 2000). As the name suggests, hydrolysable tannins are easily hydrolyzed by enzymes, acid or with mild alkali or acidic treatment, releasing the sugar moiety and gallic acid (gallotannins) or ellagic acid (ellagitannins) (Bravo 1998; A. Crozier, Jaganath, and Clifford 2006). Hydroxybenzoic acids are of great interest as they are also known to be gut microbial metabolites of other phenolics, including flavonoids, and therefore are suspected to be the mediators of the bioactivities observed from phenolic consumption (Murota, Nakamura, and Uehara 2018; Sankaranarayanan et al. 2020; Monagas et al. 2009; Serra et al. 2012; Selma, Espin, and Tomas-Barberan 2009). Furthermore,

uroolithin A, a gut microbial metabolite of ellagic acid and ellagitannins, has been heavily studied for its potent anticancer activity through mitophagy *in-vitro* and in animal models, though translation to clinical studies remains provisional (González-Sarriás et al. 2010; Ryu et al. 2016; Landete 2011).

#### 1.4.3. Stilbenes

Stilbenes share some structural similarity with flavonoids, having a 1,2-diphenylethylene (C<sub>6</sub>-C<sub>2</sub>-C<sub>6</sub>) backbone (Chong, Poutaraud, and Hugueney 2009; A. Crozier, Jaganath, and Clifford 2006). Stilbenes are known to be involved in constitutive and inducible defense mechanisms within plants particularly in response to bacterial, viral, or fungal pathogen attack (Chong, Poutaraud, and Hugueney 2009). While stilbenes can be found in many plants, the only relevant dietary source is grapes and grape-derived products, with grapes having up to 2mg/kg, primarily in the form of *trans*-resveratrol and its 3-*O*-glucoside, piceid (A. Crozier, Jaganath, and Clifford 2006; Pineiro, Palma, and Barroso 2006). Much like flavonoids, resveratrol has potent bioactivity in prevention and reducing the progression of a variety of chronic illnesses including cardiovascular disease and cancer proven through a variety of cell, animal, and clinical studies, though resveratrol suffers from extremely poor bioavailability (Baur and Sinclair 2006; Baur et al. 2006; Smoliga, Baur, and Hausenblas 2011; Roupe et al. 2006; Yu, Fu, and Wang 2012).

#### 1.4.4. Lignans

Lignans are relatively small, water soluble phenolic compounds, while lignins are very large, complex polymers that are insoluble (Heitner, Dimmel, and Schmidt 2016). They are derived from oxidative coupling of lignols, from the plant phenylpropanoid pathway (Boerjan, Ralph, and

Baucher 2003; Heitner, Dimmel, and Schmidt 2016). As lignols have nine carbons, their dimerization products, lignans, typically have a C<sub>18</sub> core giving rise a wide variety of lignan classes (Boerjan, Ralph, and Baucher 2003). Flaxseeds are the greatest dietary source of lignan with up to 3.7g/kg dw, though other seeds (sunflower, sesame), cereals (wheat, barley, oats), cruciferous vegetables, soy products, and some fruits (strawberries, apricots) also have high lignan concentrations (Manach et al. 2004; Landete 2012). Many colonic microbial communities are able to, through a variety of deglycosylation, demethylation, dehydrogenation, and dehydroxylation reactions, convert lignans to enterolactones and enterodiols, which are classified as phytoestrogens due to their estrogenic and anti-estrogenic bioactivity (Landete 2012; Adlercreutz 2007; Knight and Eden 1996; L.-Q. Wang 2002).

### **1.5. Grape and 100% Grape Juice Intake and Phenolics**

Grapes are a widely consumed phenolic-rich berry of the woody vines of deciduous flowering plant genus of *Vitis* (Kanellis and Roubelakis-Angelakis 1993; Reisch, Owens, and Cousins 2012). *Vitis* species are naturally found across the temperate regions of the northern hemisphere, consisting of about 60 different species. The most common domesticated species is *V. vinifera* from which numerous European cultivars are used for wine production, such as Cabernet Sauvignon, Graciano, Verdicchio, and Chenin blanc, or consumed as table grapes, such as Sultana/Thompson Seedless, Red Globe, Cardinal, and Black Corinth (Reisch, Owens, and Cousins 2012). In North America, the native *V. labrusca* species is widely commercialized and consumed, with cultivars such as Concord and Niagara being commonly used for 100% juices, jellies, and wine and cultivars such as Emperor and Flame Seedless are consumed as table grapes (Kanellis and Roubelakis-Angelakis 1993; Reisch, Owens, and Cousins 2012).

### 1.5.1. American Grape and 100% Grape Juice Consumption

Worldwide, grapes are one of the most widely cultivated crops, with roughly 75 million tons being produced every year (FAO & OVI 2016). About 36 million tons are used for wine product, 4 million tons for juice production, 27 million tons for table grapes, and 6 million for dried grape products (FAO & OVI 2016). Further, American domestic production of table grapes has increased by 7% over the past 5 years to 1.02 million metric tons, while consumption has increased by over 15% over the past 5 years to 1.37 million metric tons (USDA Foreign Agriculture Service 2020). The consumption of grapes, by virtue of their high phenolic content, is known to boost immunity, support cardiovascular and cognitive function, and ameliorate the impacts of obesity in clinical and animal models (Kaeberlein and Rabinovitch 2006; Dohadwala and Vita 2009; J. Lee, Torosyan, and Silverman 2017; Percival 2009; Barona, Blesso, et al. 2012; Barona, Aristizabal, et al. 2012; Leifert and Abeywardena 2008).

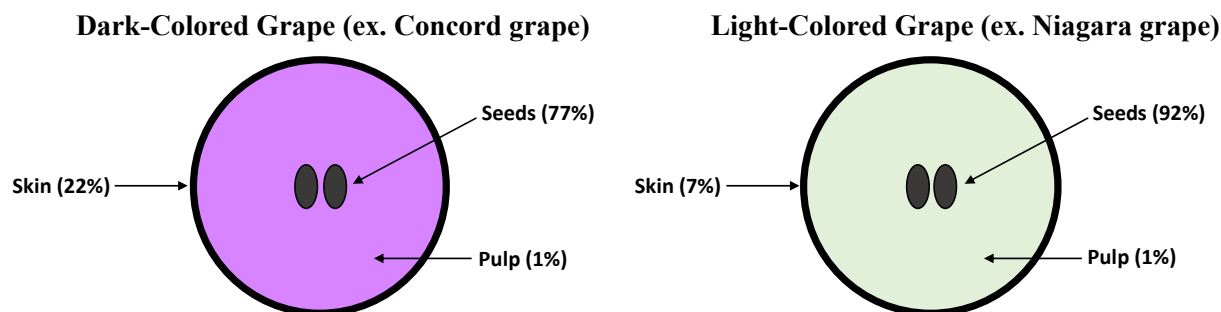
Grape juice is the third most widely consumed fruit juice in America, with 113.4 million gallons of single strength 100% grape juice being consumed in the 2019/2020 market year (USDA Economic Research Service 2020). In the last 15 years, grape juice consumption has fallen by roughly 25% (USDA Economic Research Service 2020), likely due to negative perceptions surrounding processing, high sugar content of 100% juices, and weak associations with weight gain (Byrd-Bredbenner et al. 2017; Rehm et al. 2016). However, macronutrient, micronutrient, and phytochemical composition of grapes and 100% grape juice remain comparable (Tables 1.1 and 1.2), and consumption of 100% fruit juice is associated with better overall diet quality (Auerbach et al. 2017; Agarwal, Fulgoni III, and Welland 2019; Nicklas, O'Neil, and Fulgoni 2015). Furthermore, 100% grape juice, particularly Concord grape juice, consumption has been associated with benefits to immunity, neurocognitive function, hypertension, endothelial function,

and overall cardiovascular function in animal models and clinical trials (Shanmuganayagam et al. 2007; Stein et al. 1999; Giacoppo et al. 2015; Rowe et al. 2011; Lamport, Lawton, et al. 2016; Siasos et al. 2013; Krikorian et al. 2012; Park, Kim, and Kang 2004; Haskell-Ramsay et al. 2017).

### 1.5.2. Phenolic Distribution in Grapes

Grape phenolics are localized primarily within the skin, seeds, and pulp of whole grapes in differing amounts (Figure 1.4). In dark-colored grape varieties critical to wine and juice industries (ex. Cabernet Sauvignon, Corvina, Concord, etc) the dominant polyphenolic class in the skins are anthocyanins, responsible for the deep red and purple shades observed (Liang et al. 2008). Anthocyanin species include delphinidin, cyanidin, malvidin, petunidin, and peonidin, typically found as mono-glycosides sometimes with acetyl, coumaryl, or caffeoyl substitutions (Liang et al. 2008; Revilla et al. 1999). Grape skins also contain flavonols (primarily quercetin derivatives), some phenolic acids (coumaric, caffeoyl, and coumaric acids), flavan-3-ols (free monomers and some procyanidins), and very low amounts of resveratrol (Luo et al. 2016; Katalinić et al. 2010; Pinasseau et al. 2017). It is important to note that light-colored (green or colorless) grape varieties have no anthocyanin content, significantly reducing the phenolic content within the skins compared to dark-colored grapes (Ivanova, Stefova, and Chinnici 2010; Luo et al. 2016). Phenolics are found in the highest concentration within seeds (77-92% of total grape phenolics), with flavan-3-ol monomers, oligomers, and polymers being the primary forms (Ivanova, Stefova, and Chinnici 2010; Mandić et al. 2008; J. Shi et al. 2003). Grape seeds also contain low levels of phenolic acids, resveratrol, and flavonols (Ivanova, Stefova, and Chinnici 2010; Nawaz et al. 2006; J. Shi et al. 2003). It is important to note that commonly consumed seedless table grape varieties such as Thompson seedless and Black Corinth have no seeds and therefore lack a significant portion of their

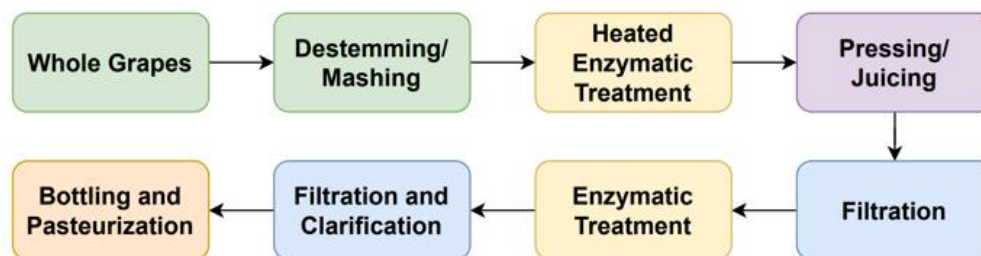
potentially bioavailable and bioactive phenolics. The pulp or flesh of whole grapes has by far the lowest concentration of phenolics ( $\leq 1\%$  of total phenolics).



**Figure 1.4.** Distribution of phenolics in grape fractions (skin, seeds, pulp) as a percentage in dark-colored and light-colored grapes. (Using data adapted from Ivanova, Stefova, and Chinnici 2010)

### 1.5.3. Impacts of 100% Grape Juicing

Figure 1.5 depicts a generalized process flow diagram for commercial juice processing for 100% grape juice. The first step in juice production is typically to select mature, ripe, high-quality fruit that can be processed typically within a reasonable time window and allowing for expression of color, flavor and nutrient content characteristic of a ripe whole fruit (Clemens et al. 2015). Conversely, many fruits intended for fresh consumption are picked mature, but not fully ripened, to allow for full ripening during storage and transportation (Prasanna, Prabha, and Tharanathan 2007). Early harvest of fruits may cause for variation in the quality and content of nutrients if consumed when not fully ripened compared to 100% fruit juice. Furthermore, the negative impacts of long-term controlled storage on labile fruit nutrients are well established (Lee and Kader 2000; Galani et al. 2017). The nature of commercial juice manufacturing is one of high efficiency of extraction, standardization, and stabilization that would limit variability and produce stable products for consumers.



**Figure 1.5.** Generalized flow diagram for commercial juice processing.

The processing of whole fruit to 100% juice is ultimately an extraction and preservation process that targets the whole fruit, including both edible and non-edible portions (seeds and skins). As concentration and distribution of nutrients and phytochemicals within fruits varies, it is important to consider how juicing and contact with all the fruit portions (i.e seed, skin and flesh) impacts the final product and nutritional quality including content of bioactives such as phenolics. In dark-colored grapes, ~77% of phenolics are localized in the seeds with an additional ~22% in the skin and ~1% in the flesh, while in light-colored grapes ~92% of phenolics are found within the seeds with 7% in the skin and 1% in the flesh (Ivanova, Stefova, and Chinnici 2010). If it is assumed that seeds are not consumed and poor efficiency of extraction from skin, then as low as 1-23% of grape phenolics may be considered available to the consumer. During commercial juicing of grapes there is extensive crushing under either cold or heated conditions to facilitate increased extraction yield (Nagy, Chen, and Shaw 1993; Fuleki and Ricardo-da-Silva 2003). This is also assisted by application of commercial enzymes systems, primarily pectinases, designed to further break the fruit matrix down and enhance recovery of juice (Demir et al. 2001). These physical and biochemical processes foster not only increased contact between phytochemical-rich seed, skin portions, but also can serve to chemically release bound or trapped phenolic species which are subsequently transferred to the juice (Manach et al. 2004; Gil-Izquierdo, Gil, and Ferreres 2002; Bai et al. 2013; Palafox-Carlos, Ayala-Zavala, and González-Aguilar 2011). This

allows for extraction of components from non-edible portions (seeds and skins) that may not be otherwise leveraged by consumers from whole fruit or even home or fresh juicing (Bai et al. 2013).

Following the extraction of grape juice, several additional processing steps are utilized to provide a stable and quality product. This includes clarification and filtration which are typically physical processes but can also be assisted through further application of additional enzyme treatments that hydrolyze cellulose and other complex carbohydrates (Kashyap et al. 2001; Demir et al. 2001). The juice may then be concentrated, usually through evaporation, to facilitate long-term storage prior to final pasteurization targeting potential pathogenic microorganisms, spoilage organisms, and enzymes to ensure a safe and stable product and maintain nutritional quality of 100% fruit juice (Petrotos and Lazarides 2001; Nagy, Chen, and Shaw 1993). While this process is traditionally a thermal process in the ranges of ~90–95°C for 30s in a hot-fill-hold configuration, other forms of thermal treatment including canning (sterilization) or aseptic processing have been widely utilized in juice processing (Bates, Morris, and Crandall 2001; Rupasinghe and Yu 2012).

When considering the impact of processing on fruit nutritional quality, though similar, differences in micronutrient and phenolic composition of 100% fruit juice and whole fruit do exist (Tables 1.1 and 1.2). These are a direct result of both variability in fruit quality as well as processing-induced changes including the well-known susceptibility of phenolics to oxidative reactions (N. Li et al. 2013; Chung et al. 2016; Bordenave, Hamaker, and Ferruzzi 2014; West and Mauer 2013). Such reactions are known to proceed through extraction, clarification, thermal treatment, and ultimately storage of juice (Margean et al. 2020; Chang et al. 2017; Capanoglu et al. 2013; Fuleki and Ricardo-da-Silva 2003; Spanos and Wrolstad 1992). For 100% grape juice, losses are variable and dependent on phenolic species, but the added benefit of enhanced extraction

leads to final levels of phenolics that are not far from those found in edible portions of whole fruits (Table 1.2).

Fresh fruits are relatively expensive and inherently perishable. Application of food processing, including juicing, allows for the ability to deliver fruit and fruit nutrients to consumers year-round and in a product (100% fruit juice) that addresses drivers of liking and consumption such as flavor, convenience, and portability. Estimates by the USDA suggest that up to 9.5 billion pounds (~25%) of fresh fruit remain uneaten at the consumer level in the United States (Buzby, Farah-Wells, and Hyman 2014). While data on fresh grape losses in the United States is difficult to obtain, especially considering the overall fruit losses, it can be assumed that post-harvest consumer level grape losses are similar to that observed in other countries of up to at least 23% (Aujla et al. 2011; Ladaniya, Wanjari, and Mahalle 2005; Blanckenberg, Opara, and Fawole 2021; Porat et al. 2018). Considering this potential loss of nutritionally dense grapes and the growing interest in food sustainability and security, impacts of juice processing of this perishable fruit in maintaining the high nutritional quality of the fruit requires further exploration.

## **1.6. Bioavailability of Phenolics**

In understanding the biological impact of dietary phenolics, it is important to not only consider the content and type of phenolics, but also the bioavailability and metabolism of those phenolics (Bravo 1998; Manach et al. 2004). Numerous factors dictate the bioavailability of individual phenolic species ranging from the food source to intestinal absorption and metabolism, ultimately determining the final biological impact of phenolics from a given dietary source (Manach et al. 2004; D'Archivio et al. 2010; Scalbert and Williamson 2000). In order to understand the potential differences in phenolic delivery between consumption of whole grapes versus 100%

grape juice, it is imperative to describe the process from digestion through absorption, metabolism, and how these stages impact the circulating forms of phenolics believed to impart health benefits.

### 1.6.1. Bioavailability, Bioactivity, and Bioaccessibility

*Bioavailability*, in nutrition, typically refers to the portion of a nutrient or bioactive component that is then available for further physiological action (Fernández-García, Carvajal-Lérida, and Pérez-Gálvez 2009). The Food and Drug Administration, from a pharmacological view point, defines bioavailability as the rate and extent to which a moiety is absorbed and becomes available at the site of action (FDA 2021). Bioavailability includes consideration of absorption, metabolism, circulation, tissue distribution and bioactivity of any given bioactive species (Fernández-García, Carvajal-Lérida, and Pérez-Gálvez 2009; Neilson and Ferruzzi 2011). However due to the difficulty in measuring bioactivity of food components on specific organs, bioavailability is also used to refer to the fraction of an oral dose of a parent compound or related bioactive metabolite that enters systemic circulation (Fernández-García, Carvajal-Lérida, and Pérez-Gálvez 2009). In the context of phenolics, bioavailability is dependent on both the food source and intrinsic host factors. Host factors address both intestinal factors, including enzyme activity and type/abundance of various colonic microbial communities, and systematic factors, such as age, gender, genetics, and overall health condition (D'Archivio et al. 2010). The impacts of food matrix, including interactions of phenolics with other food components, and food processing, such cooking, storage, and homogenization, are of growing interest when determining the bioavailability of phenolics (D'Archivio et al. 2010; Neilson and Ferruzzi 2011; Palafox-Carlos, Ayala-Zavala, and González-Aguilar 2011; Aschoff et al. 2015; Roura et al. 2008; Bordenave, Hamaker, and Ferruzzi 2014; Ferruzzi 2010). Food source factors of phenolic

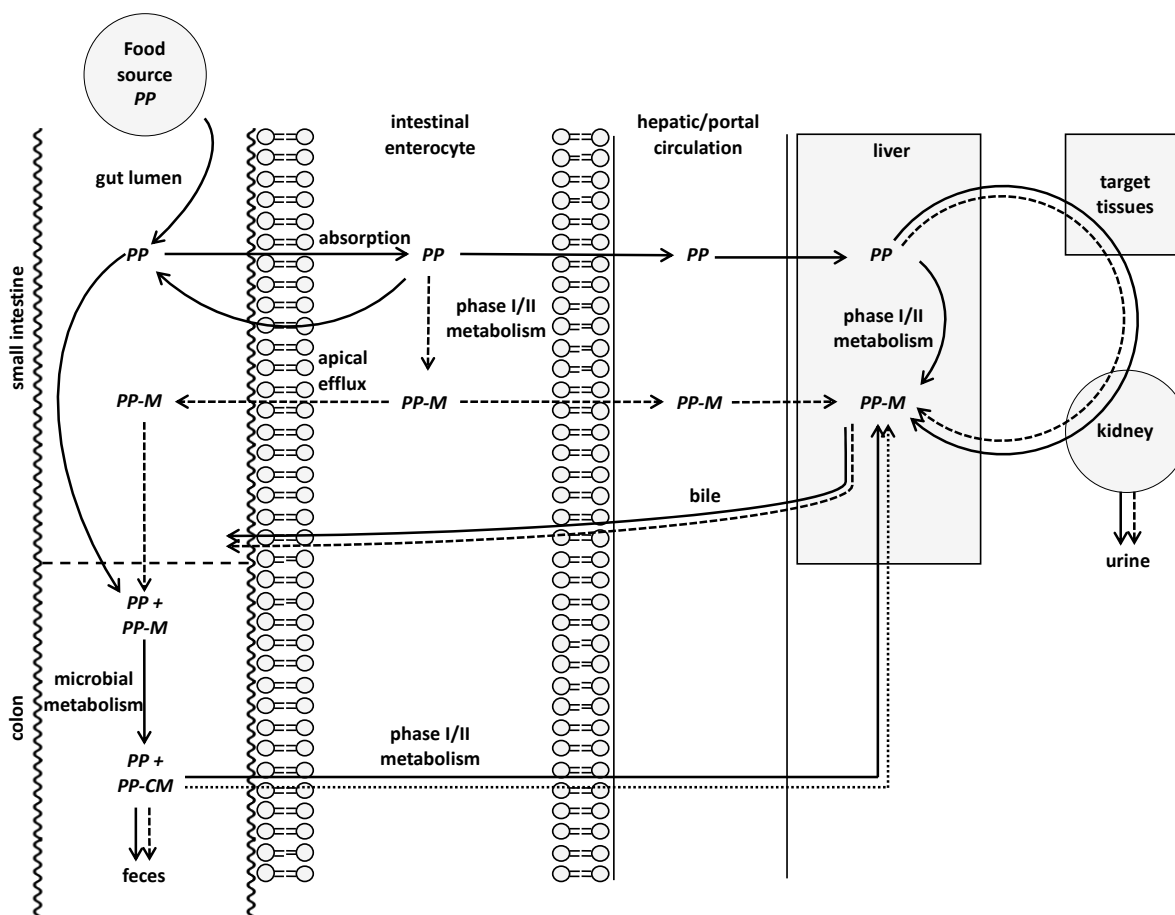
bioavailability are perhaps of greater importance, as they are more easily modified, and the impacts of modification potentially have greater implications for the broader population. Additional factors including chemical structure, pH, plant genetics, and cultivation strategies can significantly impact the absorption, delivery, and eventually biological function of phenolic species (Bravo 1998; Manach et al. 2004; D'Archivio et al. 2010; Mengist et al. 2020; Cervantes et al. 2020).

*Bioactivity* includes the transport and targeting to specific tissue, interactions with other molecules, metabolism or transformations, possible generation of biomarkers, and the ultimate physiological response of a given bioactive compound (Fernández-García, Carvajal-Lérida, and Pérez-Gálvez 2009). Bioactivity studies, including *in-vivo*, *ex-vivo*, and *in-vitro* models, are leveraged to elucidate health benefits derived from consumption of specific foods and food components, such as phenolics. *In-vitro* models can be particularly useful to understand general activity (ex. anti-cancer, anti-inflammation, modulation of enzymes, gene expression, etc.) and underlying mechanisms before translation to relevant *in-vivo* and clinical models.

*Bioaccessibility* can be defined as the fraction of a food component made available for intestinal absorption following gastrointestinal release from the food matrix (Neilson and Ferruzzi 2011; Fernández-García, Carvajal-Lérida, and Pérez-Gálvez 2009; Benito and Miller 1998). This measure encompasses the digestive processes leading to the release of compound(s) within the intestines, as well as intestinal luminal stability and absorption. Bioaccessibility is often estimated using *in-vitro* models for a wide variety of food compounds, and is used as a surrogate to both estimate bioavailability and in an effort to understand the implications of the food matrix and processing on bioavailability (Benito and Miller 1998; Shim et al. 2009; Pico et al. 2019; Neilson et al. 2009; E. G. Kean, Hamaker, and Ferruzzi 2008; Menezes et al. 2018).

### 1.6.2. Digestion and Intestinal Absorption

A general schematic of the processes involved in the metabolism and bioavailability of phenolics can be seen in Figure 1.6. Digestion begins with oral mastication of the food, which initiates the breakdown of the food matrix through the reduction of particle size by mechanical and enzymatic action, primarily due to  $\alpha$ -amylase activity (Hoebler 2000; Joubert et al. 2017). Following oral mastication, the resulting food bolus moves to the stomach, starting the gastric phase of digestion. The gastric phase is marked by a general acidification ultimately to a pH between 2 and 4, mechanical action (gastric motility), and the introduction of gastric secretions, composed of proteases, mucus, acid, and intrinsic factor (Bornhorst and Paul Singh 2014; Fruton 2002; Beveridge and Baird 2000). The combination of these factors serves to initiate release phenolics through the breakdown of the food matrix within the stomach. It is important to note that during gastric phase, phenolics, both flavonoids and nonflavonoids, are generally stable by virtue of the reduced pH that minimizes potential for oxidative processes (Rios et al. 2002; Petri et al. 2003; He, Magnuson, and Giusti 2005; X. Wu, Pittman, and Prior 2006; Bermúdez-Soto, Tomás-Barberán, and García-Conesa 2007). Following progression of gastric digestion, resulting chyme is transferred to the small intestine, beginning the small intestinal phase of digestion. Acidic gastric chyme is initially neutralized by the presence of sodium bicarbonate, and the pH of the small intestinal environment typically adjusted between 5.5 to 7.5. Pancreatic enzymes (including proteases, lipases, and amylases), bile, and mechanical action serve to fully break down the food matrix, releasing soluble nutrients, including phenolics, in the gut lumen for absorption by intestinal enterocytes (Guerra et al. 2012).



**Figure 1.6.** General schematic of the processes that affect the metabolism and bioavailability of phenolics. *PP* – phenolic species, *PP-M* – host derived phenolic metabolite, *PP-MC* – colonic microbiota derived phenolic metabolite.

The intestinal absorption of phenolics is multifactorial, affected by degree and type of glycosylation or acylation, conjugation with other phenolics (primarily acids), solubility, basic chemical structure, degree of polymerization, and molecular weight (Bravo 1998). Absorption occurs through both facilitated and passive processes in the brush border of intestinal epithelial cells (Neilson and Ferruzzi 2011; Scalbert and Williamson 2000). As nearly all flavonoids, with the notable exception of flavan-3-ols, are glycosylated to some degree from their plant sources, they are unable to passively diffuse across the phospholipid bilayer unless deglycosylated (Manach et al. 2004; Williamson, Kay, and Crozier 2018; Scalbert and Williamson 2000). Lactase phlorizin hydrolase (LPH) on the brush-boarder of enterocytes, exhibits broad substrate specificities for flavonoid glycosides, releasing the aglycone, which may then passively diffuse into the enterocytes

(Williamson, Kay, and Crozier 2018; Day et al. 2000). Glycosylated flavonoids may also be actively transported by sodium-dependent glucose transporter (SGLT1) into the enterocyte where deglycosylation may occur by cytosolic- $\beta$ -glucosidases (Williamson, Kay, and Crozier 2018; Gee et al. 2000; Manach et al. 2004). Flavan-3-ols and some hydroxycinnamic acids are thought to be transported by the monocarboxylic acid (MCT) transporter on the brush-boarder of enterocytes (Neilson and Ferruzzi 2011; Konishi and Kobayashi 2004; Konishi and Shimizu 2003; Konishi, Kobayashi, and Shimizu 2003a). Free hydroxycinnamates are rapidly absorbed, though they are rarely found in their free forms (Manach et al. 2004; Scalbert and Williamson 2000; Clifford 1999). Typically hydroxycinnamates are found esterified to sugars, acids, or plant cell wall structures, and there are no esterases in human tissues able to liberate hydroxycinnamates, thus preventing intestinal absorption (Plumb et al. 1999; Rechner et al. 2001; Manach et al. 2004). Gut microbial communities have numerous esterases, allowing for the release and subsequent absorption of free hydroxycinnamates (Andreasen et al. 2001; Couteau et al. 2001; Lafay et al. 2006). For hydroxybenzoates, particularly gallic acid, the exact absorption mechanism remains relatively unknown, though there is some evidence indicating paracellular transportation (Konishi, Kobayashi, and Shimizu 2003b; Konishi, Hitomi, and Yoshioka 2004; Mao et al. 2016). Large molecular weight phenolic polymers, particularly procyanidins, are not absorbed by the small intestinal epithelium (Manach et al. 2004; Scalbert and Williamson 2000). There is some evidence for absorption of procyanidin dimers and trimers in cell and animal models, though efficiencies are quite poor (Deprez et al. 2001; Baba et al. 2002).

In general, intestinal absorption efficiency of phenolics is low, with a significant portion of phenolics remaining in the lumen of the upper small intestine (Neilson and Ferruzzi 2011; Manach et al. 2005; Wen and Walle 2006; Walle 2004; Hollman 2004). This is also due, in part,

the ability of phenolics to be actively transported through phase III xenobiotic transport systems which would efflux intracellular phenolics back to the intestinal lumen (Neilson and Ferruzzi 2011; Williamson, Kay, and Crozier 2018). For example, while 35-80% of flavan-3-ols are absorbed by intestinal enterocytes, 11-52% may be effluxed back to the gut lumen (Yong Feng 2006; Vaidyanathan and Walle 2001; Neilson and Ferruzzi 2011). Efflux transport is facilitated by a suite of ATP-binding cassette (ABC) transporters found on the apical membrane of intestinal enterocytes (Alvarez et al. 2010). These ABC transporters, including P-glycoprotein (ABCB1), multidrug resistant protein (MRP) 1 and 2, and ABG2, play an important role in preventing potentially harmful xenobiotics, namely drugs and toxins, from entering the host (Alvarez et al. 2010; Williamson, Kay, and Crozier 2018; Takano, Yumoto, and Murakami 2006). These ABC transporters have broad specificity for a variety of substrates thereby facilitating the efflux of phenolics and related host phenolic metabolites back into the gut lumen, preventing high levels of absorption. Furthermore, ABC transporters are found within liver cells having the ability to pump phenolics and related metabolites into bile ducts back into the gastrointestinal tract, further reducing the overall bioavailability of phenolics (Alvarez et al. 2010).

### 1.6.3. The Role of Intestinal Microbiota in Modulating Phenolic Metabolism and Bioavailability

The human gastrointestinal tract, particularly the colon, is home to a densely populated microbial community, collectively containing perhaps over 100 times more genes than the human genome (Shapira 2016; Gill et al. 2006). While the gut is dominated by four bacterial phyla (*Bacteroides*, *Firmicutes*, *Actinobacteria*, and *Proteobacteria*) there is massive variation in microbial populations between individuals based on age, sex, overall health, genetics, diet and nutritional status, and environment (Arumugam et al. 2011; Yatsunencko et al. 2012; Goodrich et

al. 2014; David et al. 2014; Kau et al. 2011). The gut microbiota play fundamental roles in human health such as directly inhibiting pathogenic species, promoting enteric and immune protection, and metabolism of nutrients (Sousa et al. 2008; Clarke et al. 2014; Faderl et al. 2015). It has been reported that as little as 10% of ingested flavonoids and next to little no oligomeric or polymeric polyphenols are absorbed within in the small intestine (Murota, Nakamura, and Uehara 2018; Tao et al. 2019). It is believed that these unabsorbed phenolics reach the lower gut where they serve as substrates for microbial communities. The gut microbiota has numerous enzymes that have the ability to catalyze a myriad of reactions including hydrolysis, de/hydroxylation, oxidation, reduction, ring cleavage, isomerization, demethylation, and decarboxylation (Hervert-Hernandez and Goñi 2011). Microbial enzymes are able to act upon phenolics to produce a wide variety of lower molecular weight catabolites that can then be absorbed (Murota, Nakamura, and Uehara 2018). It is believed that these microbial metabolites of phenolics may also contribute to the health benefits associated with consumption of phenolics (Murota, Nakamura, and Uehara 2018; Aura 2008; Marhuenda-Muñoz et al. 2019).

If still conjugated (glycosylated, glucuronidated, sulfonated, etc.), flavonoids undergo a deconjugation reaction as a first step (Braune and Blaut 2016). Free flavonoids typically are subjected to a ring cleavage along the B ring by microbial action, liberating two smaller phenolic metabolites (Braune and Blaut 2016; Hervert-Hernandez and Goñi 2011). These metabolites include cinnamic acids, phenyl propionic acids, phenyl acetic acids, benzoic acids, and benzaldehydes among others (Cladis et al. 2020; Hervert-Hernandez and Goñi 2011). These phenolic metabolites can be interconverted to one another through a variety of hydrogenation, oxidation, and reduction reactions facilitated by microbes. Esterified hydroxycinnamates such as chlorogenic, caftaric, and coutaric acids may be hydrolyzed, releasing free hydroxycinnamic acids

that may be further microbially metabolized, with the final microbial phenolic metabolites being available for absorption (Couteau et al. 2001; Zhao and Moghadasian 2010). Polymeric procyanidins are known to be readily metabolized by the gut microbiome, eventually releasing dimeric and monomeric flavan-3-ols (Ou et al. 2014; Ou and Gu 2014; L. Zhang et al. 2016). Flavan-3-ols, similar to other flavonoids, undergo ring cleavage, producing a variety of phenolic acids, preferentially 3-phenylpropionic acids (Ou and Gu 2014; Deprez et al. 2001). Unique to flavan-3-ols, following ring cleavage, phenylvalerolactones, such as 5-(3',4'-dihydroxyphenyl)- $\gamma$ -valerolactone, can be produced which can undergo further dehydroxylations and  $\alpha$  and  $\beta$ -oxidations producing valeric acids, 3-phenylpropionic acids, phenylacetic acids, and benzoic acids (Sánchez-Patán et al. 2012; Ou and Gu 2014).

#### 1.6.4. Metabolism, Circulation, and Excretion

Following absorption, phenolics and their microbial metabolites may be further subjected to xenobiotic host metabolism within enterocytes and the liver (Walle 2004; Hollman 2004). While phase I metabolism (facilitated by CYPs) has been reported, flavonoids are primarily subjected to phase II metabolic conjugation processes (Walle 2004; Chen et al. 2014). Common conjugation reactions to flavonoids include glucuronidation, sulfonation, *O*-methylation, or glycation facilitated by UDP-glucuronosyltransferases, sulfotransferases, and methyltransferases (Chen et al. 2014). Individual phenolic species undergo preferential conjugation dependent on chemical structure (Walle 2004). For example, the flavan-3-ol, epicatechin gallate, only undergoes sulfate conjugation in human and rat models (Vaidyanathan and Walle 2002). While phase II metabolism of flavonoids and other phenolics is quite ubiquitous, there is increasing evidence that a portion of

anthocyanins are left conjugated with their glycosides and evade phase II metabolic processes (Walle 2004; J. Wang et al. 2013).

Phenolic compounds and their phase II conjugates circulate through the body bound to plasma proteins, primarily albumins (Manach et al. 2004). These species tend to be hydrophilic in nature requiring transporters, including organic anion transporters, organic anion-transporting peptides, and various ABC transporters, to facilitate trans-epithelial transport to wide variety target tissues and organs (Williamson, Kay, and Crozier 2018; Manach et al. 2004). Phenolics and their metabolites have been found in the brain, endothelial cells, lungs, kidney, spleen, bone, skin, heart, and bladder where they may have various activities (Claudine Manach et al. 2004; Janle et al. 2010; T.-Y. Chen et al. 2017; de Boer et al. 2005; Suganuma et al. 1998; Spencer, Abd El Mohsen, and Rice-Evans 2004; Williamson, Kay, and Crozier 2018). Phenolics are typically effluxed rapidly back into circulation from tissues by a variety of ABC transporters (Williamson, Kay, and Crozier 2018).

Excretion of phenolics and their metabolites can be through the urinary tract or the biliary route. Urinary excretion of flavonoids, phenolic acids, and their phase II conjugates has been well documented and remains a key marker for animal and clinical studies (Medina-Remón et al. 2011; Rios et al. 2003; Gonthier et al. 2003; Q. Sun et al. 2015; Mennen et al. 2008; Choudhury et al. 1999; Brevik et al. 2004). Some individual phenolic species and more extensively conjugated metabolites may be preferentially excreted through bile, which may result in enterohepatic recycling (Claudine Manach et al. 2004; Kohri et al. 2001; Liu et al. 2003; Jan et al. 2010; Zeng et al. 2016; Matsukawa, Matsumoto, and Hara 2009).

## 1.7. Models of Assessing Bioavailability

Phenolic bioavailability can be measured in a variety of ways. Human studies are regarded as the gold standard when determining bioavailability typically following a paradigm of either acute or chronic consumption of a phenolic-rich food or diet by volunteers (Williamson, Kay, and Crozier 2018). Metrics from plasma, urine, and occasionally ileal effluent samples are commonly analyzed to glean insights into phenolic bioavailability (Manach et al. 2005; Williamson, Kay, and Crozier 2018). However, human clinical trials are expensive, time consuming, and require ethical consideration. Further, in the context of understanding the impacts of juice processing and food matrix of grape versus 100% juice, human studies would remain limited in delineating the differences in absorption and metabolism from different sections of the gastrointestinal tract (small intestine vs. large intestine).

A wide variety of animal models, including mice, rats, pigs, and rabbits, have been used as relevant proxies for human phenolic absorption, metabolism, and circulation (Kim et al. 2000; Loke et al. 2010; Baur et al. 2006; Dorenkott et al. 2014; L. Chen et al. 1997; Y. Wang et al. 2017; Frederiksen et al. 2007; Gu et al. 2019). Animal models have distinct advantages over clinical trials due to the ability to manipulate nutritional and pathophysiological status, control over diet, genetic uniformity, and type of sample collection (ex. tissue). Therefore animal studies are invaluable in understanding phenolic pharmacokinetics and tissue distribution in the context of various physiological conditions (Janle et al. 2010; T.-Y. Chen et al. 2017; J. Wang et al. 2013; Cladis et al. 2020; Suganuma et al. 1998; de Boer et al. 2005; Loke et al. 2010; Khurana et al. 2013). However, differences between the physiology of humans and the variety of animal models typically employed can make correlations contentious at best (Shanks, Greek, and Greek 2009; McGonigle and Ruggeri 2014). Furthermore, similar to human trials, animal models suffer from

high cost, throughput limitations, ethical concerns, and, particular to our objectives, difficulties in delineating differences between upper and lower gastrointestinal phenolic absorption and metabolism in the context of grape juicing.

### 1.7.1. Static *In-vitro* Digestion

*In-vitro* digestion models are commonly used to mimic the gastrointestinal processes that allow for the study of the release of relevant food components due to structural changes and digestibility of a food source. These models have evolved to contain a series of digestive steps including oral, stomach, and small intestinal phases, coupling relevant mechanical and enzymatic actions to emulate digestion (Hur et al. 2011). One of the seminal studies by Miller et al (1981), describes the utilization of a digestion model with a gastric and intestinal phase to estimate the availability of dialyzable iron from a variety of foods (Miller et al. 1981). It was found that availability of iron from *in-vitro* digestion significantly correlated (0.93-0.99) with *in-vivo* data (Miller et al. 1981). From these promising results, studies utilizing *in-vitro* digestion models have exponentially increased to estimate the bioaccessibility of a variety food components, including starch, protein, lipids, and various micronutrients and phytochemicals, from various food sources with high degrees of correlation to *in-vivo* and clinical studies (Bohn et al. 2018; Carbonell-Capella et al. 2014; Marie et al. 2012; Tyssandier et al. 2003; Sayd, Chambon, and Santé-Lhoutellier 2016; Rozan et al. 1997; Monro, Mishra, and Venn 2010; Ferrer-Mairal et al. 2012).

Several established models for lipophilic carotenoids have been successfully adapted for the measurement of phenolics from a various food sources including grape juice, green tea, blueberries, grains, and banana flour (Garrett, Failla, and Sarama 1999; Ferruzzi, Failla, and Schwartz 2001; Green et al. 2007; M. Li et al. 2016; Moser et al. 2016; Mengist et al. 2020; Pico

et al. 2019). One of the major modifications made to the model from Garret et al (1999) includes the addition of an oral phase at the beginning of digestion (Garrett, Failla, and Sarama 1999; Moser et al. 2016). Simulated mastication better emulates chewing and results in a far more complex breakdown of physical structures leading to more accurate representation of actual release of bioaccessible phenolics compared to highly homogenized test foods. Oral phase consists of the inclusion of  $\alpha$ -amylase, mucin, and various salts and incubation for 10 minutes with gentle oscillation at 37°C. This stage is intended to not only mimic oral processing, but also the travel of the masticated food bolus down the esophagus, essentially allowing for increased duration of enzymatic activity (Thakkar et al. 2007). Following oral phase, gastric digestion is initiated by the reduction of the pH to 2.5 through the addition of HCl. A porcine pepsin solution is also added to the food bolus, providing one of the key proteases found in gastric digestion (Fruton 2002). Simulated gastric phase proceeds for 60 minutes under gentle shaking at 37°C (Thakkar et al. 2007; Moser et al. 2016). Through the combinatorial effects of physical and enzymatic action, gastric stage proves to be a fundamental step in the breakdown of the food matrix thereby facilitating the release of phenolic species (Bornhorst and Paul Singh 2014; Fruton 2002; Beveridge and Baird 2000). Small intestinal phase of the digestion model is initiated with the addition of NaHCO<sub>3</sub>, increasing the pH to 5.0. Small intestinal phase is marked by the addition of a pancreatin-lipase and bile salt solutions, providing the characteristic mixture of enzymes and emulsifying agents of small intestinal digestion (Thakkar et al. 2007; Moser et al. 2016; Green et al. 2007). pH is adjusted once again with NaHCO<sub>3</sub> to 6.5, and the simulated intestinal digestion is continued by incubation for 2 hours under gentle oscillation at 37°C, providing for more complete food matrix breakdown and release of phenolic bioactives. The aqueous fraction of the completed simulated digestion is

isolated by centrifugation and analyzed for phenolic species, thus determining the bioaccessible content.

### 1.7.2. *Ex-vivo* Anaerobic Fermentation Model

In order to understand the roles gut microbiota play, particularly in the large intestine, in human health and nutrition, a number of *in-vitro* and *ex-vivo* methodologies have been leveraged from simple batch culture to more complex multistage continuous flow models (Cinquin et al. 2006; Macfarlane and Macfarlane 2007; Payne et al. 2012; Van den Abbeele et al. 2010). Batch fermentations involve the growth of single or mixed bacterial culture (fecal material) in a specific medium, typically in a closed vessel under anaerobic conditions (Macfarlane and Macfarlane 2007; Payne et al. 2012). Batch fermentations have been utilized for a variety of dietary components including complex starches, proteins, and bioactive phenolics (Khalil et al. 2014; C. Wang et al. 2017; Pompei et al. 2008; Lesmes et al. 2008; Gumienna, Lasik, and Czarnecki 2011). The fermentation systems tend to be relatively inexpensive and practical, allowing for the processing of large numbers of samples (Macfarlane and Macfarlane 2007; Payne et al. 2012). Furthermore, batch models are particularly useful in understanding the complex relationships between the microbial metabolism of food nutrients and the production of associated microbial metabolites (Payne et al. 2012). However, due the closed-system nature of batch fermentations it is difficult to achieve steady-state conditions, making the measurement of some experimental factors difficult such as evaluating the microbial community (Macfarlane and Macfarlane 2007; Payne et al. 2012).

The batch culture anaerobic fermentation model that will be utilized in the present study was adapted from previous studies interested in the gut microbial catabolism of phenolic species from amaranth, açai, and red and white grapes (Rodríguez-Costa et al. 2018; Alqurashi et al. 2017;

Vollmer et al. 2017). Particular consideration was given to ensuring efficient and appropriate microbial fermentation of *in-vitro* digested samples (Alqurashi et al. 2017). Media is composed of a variety of salts, emulsifiers (bile salts and tween 80), peptone water, yeast extract, haemin, vitamin k1, and L-cysteine, pH buffered, and sparged overnight with nitrogen. Fecal inoculum (Fecal Microbiota Preparation for Research, FMP-R) will be sourced from two healthy donors provided by OpenBiome. The catabolism of food phenolics and the production of associated microbial phenolic metabolites will be tracked during a 48-hour anaerobic microbial fermentation of digested material modeling the large intestinal phase of digestion.

### 1.7.3. Caco-2 Intestinal Cellular Absorption and Transport

Caco-2 cells are an immortalized cell line originally obtained from human colorectal adenocarcinoma cells (Sambuy et al. 2005; Hidalgo, Raub, and Borchardt 1989). This cell line has been extensively used to model the intestinal barrier as, when cultured under specific conditions, Caco-2 cells spontaneously differentiate into a polarized heterogeneous monolayer that structurally and morphologically resembles mature intestinal enterocytes (Sambuy et al. 2005; Hidalgo, Raub, and Borchardt 1989). Confluent Caco-2 cells express microvilli, tight junctions, enzymes, and transporters characteristic to enterocytes (Hidalgo, Raub, and Borchardt 1989; Sambuy et al. 2005). Caco-2 cells have been leveraged to track the absorption and/or the apical to basolateral transport of a variety of potential pharmaceutical drugs, nutrients, and various other bioactive species (Artursson, Palm, and Luthman 2001; H. Sun et al. 2008; Glahn 2009; Amagloh et al. 2017; Moser et al. 2016; Ferruzzi, Failla, and Schwartz 2001). Utilization of Caco-2 cell monolayer is known to be highly predictive of uptake and transport across intestinal epithelium,

particularly useful in differentiating transport efficiencies and pathways between individual compounds (Artursson, Palm, and Luthman 2001; H. Sun et al. 2008; Turco et al. 2011).

Caco-2 cell monolayers, particularly the TC7 subclone, have been widely used to study the absorption and transport of phenolics and their impact (Johnston et al. 2005; Moser et al. 2016; Manzano and Williamson 2010; Vidal et al. 2005; Soler et al. 2010; Yang et al. 2014; Konishi, Kobayashi, and Shimizu 2003b). The TC7 subclone is a late passage (81-84) of the parental Caco-2 cell line that is known to be most representative of enterocytes functions, particularly regarding intercellular junctions (Sambuy et al. 2005; Turco et al. 2011). Apical to basolateral transport of phenolics is commonly measured with Caco-2 cells cultured on semipermeable, microporous membrane inserts, such as Corning Transwells (Moser et al. 2016; Redan, Chegeni, and Ferruzzi 2017; Turco et al. 2011). Numerous factors are known to influence the uptake and transport of phenolics by Caco-2 cell monolayers including chemical structure, culture practices and media, subclone type, matrix effects, and competition between individual species among others (Gómez-Juaristi et al. 2020; Sambuy et al. 2005; Knipp et al. 1997).

## **1.8. Research Objectives**

Grapes are rich sources of bioactive phenolic compounds that have been linked with many of the positive health outcomes associated with fruit consumption. Phenolics species in grapes are concentrated in seeds (primarily flavan-3-ol monomers, oligomers, and polymers) and skins (anthocyanins and flavonols), fruit fractions that are poorly digested or rarely consumed at all. Conversely, the commercial production of 100% grape juice utilizes an extraction process that involves the whole fruit, including extensive contact with fruit fractions (seeds and skin), allowing for liberation of phenolics to an absorptive-friendly aqueous matrix. Negative stigmas surrounding

consumption of 100% fruit juices, including 100% grape juice, has reduced American juice consumption in recent years, though 100% grape juice may provide for an equivalent or better matrix for the delivery of these bioactive species than whole fruit. However, there is a significant gap in the literature comparing the content and delivery potential for phenolic species between whole grapes and 100% grape juice. With this in mind, the overall intent of this research is to provide a direct comparison between the phytochemicals of whole grapes and 100% grape juice, and the impact matrix and processing (mastication of whole fruit vs. juice) has on bioaccessibility of phenolics and the secondary metabolites produced through microbial metabolism. Therefore, the following specific objectives are proposed:

*Objective I: Comparison of phenolic content and bioaccessibility between Concord/Niagara grapes and their respective 100% juices*

Hypothesis: Concord and Niagara juices will have a greater portion of accessible phenolics compared to masticated Concord, Niagara, and select commercial table grapes, though variations will exist between individual classes and species of phenolics

General Approach: Phenolic species (anthocyanins, phenolic acids, stilbenes, flavonols, and flavan-3-ols) will be quantified from separated (skins, seeds, and pulp) and whole Concord and Niagara grapes, their 100% grape juices, and table grapes by LC-MS/MS. Separated grapes were analyzed to better understand localization and concentration of phenolics within each fraction. Whole grapes (table and juicing grapes) will undergo a simulated mastication and, along with

Concord and Niagara 100% grapes juices, will be subjected to an *in-vitro* digestion model encompassing an oral, gastric, and small intestinal phase to determine phenolic bioaccessibility.

*Objective II: Exploring differences in gut microbial metabolism and small and large intestinal transport of grape phenolics and metabolites from whole grapes and 100% grape juice*

Hypothesis: 100% grape juices and whole grapes differ in phenolic profiles, bioaccessibility, and in the presence of digestible fiber (fruit pectin). With a greater portion and diversity (flavan-3-ols and anthocyanins, primarily) of bioaccessible phenolics in 100% grape juices compared to masticated whole grapes, and the presence of increased fermentable fiber in whole fruit digest, the extent of metabolism by gut microbial communities (anaerobic large intestinal fermentation) will differ between juice and whole fruit. Furthermore, cellular transport of phenolics and metabolites from small intestinal and large intestinal (anaerobic microbial fermentation) treatments will differ between grapes and juice.

General Approach: In order to make direct comparison between juicing grapes and 100% grape juice, commercial juice processing will be adapted and scaled for bench-top grape juicing to produce 100% Concord and Niagara juice. Concord and Niagara whole grapes will be masticated, and, along with their 100% grape juices, will be digested using an *in-vitro* digestion model. Aqueous digested fractions from grapes and juices will be used as treatments for Caco-2 cell monolayers to model phenolic and metabolite small intestinal transport. Combined crude and non-digestible (pellet) digestion fractions of grapes and juice will be used as treatments for *ex-vivo* microbial fermentation under anaerobic conditions using fecal inoculate to model large intestinal

colonic microbial fermentation. Microbial phenolic metabolites will be tracked and quantified during the duration of the fermentation. Lastly, fermentation fractions from grapes and juice will be used as treatments for Caco-2 cell monolayers to model phenolic and microbial metabolite large intestinal transport.

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## **Chapter 2. Comparative Assessment of Phenolic Bioaccessibility from 100% Grape Juice and Whole Grapes**

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## 2.1. Introduction

Grape juice is one of the most commonly consumed fruit juices in the United States ranking third behind apple and orange juice (USDA 2019). Native American grape varieties including Concord and Niagara are sources for the production of 100% purple and white grape juice, respectively. While providing unique flavor attributes, these native grapes and their juices are also rich sources of bioactive phenolic compounds, including flavonols, phenolic acids, stilbenes, flavan-3-ols, and anthocyanins. Grape phenolics have documented bioactivities in preclinical models including the ability to modify oxidative and inflammatory stress, modulation of glycemic properties from foods, and positive impacts on vascular function for both cardiovascular and neurocognitive endpoint (O'Byrne et al. 2002; Hogan et al. 2010; Kovaleski et al. 2019; Liu et al. 2018; Rowe et al. 2011; Décordé et al. 2009; Natella et al. 2002; Suwannaphet et al. 2010). Building on these preclinical findings, a number of clinical trials have explored the bioavailability and metabolism of individual phenolics from 100% Concord grape juice (Bitsch et al. 2004; Stalmach et al. 2012; 2011) and further reported on the beneficial effects of 100% Concord grape juice consumption on both cardiovascular and cognitive endpoints (Park, Kim, and Kang 2004; Krikorian et al. 2012; Dohadwala et al. 2010; Siasos et al. 2013; Lamport et al. 2016; Park et al. 2009; Haskell-Ramsay et al. 2017).

While promising, contradictory messages on the role of 100% grape juice in a healthy diet remain. Currently, 100% juice is included in the US Dietary Guidelines for Americans (DGA 2015) within the fruit serving category (USDA & HHS 2015). However, broad recommendations for inclusion of 100% grape juice in the diet are often met with resistance, due to the relatively high sugar content (36g per 8oz serving) and the continued belief that significant nutritional losses are experienced through juice processing thereby creating a significant difference in quality of

whole fruit versus juice. This is clearly stated in the DGA 2015 which recommends fruit servings from whole fruit more so than 100% juice due, in part, to the fiber content of whole fruit. Interestingly, direct comparisons of nutritional profiles of 100% juice and whole fruit products including table grapes remain limited. Comparisons derived from the USDA National Nutrient Database for Standard Reference, highlight the fact that grapes and grape juice contain comparable levels of major macronutrients (15.5g/100g sugar for grapes; 14.2g/100g sugar for grape juice), minerals, and vitamins, with the exception of fiber (0.9g/100g in grapes; 0.2g/100g in grape juice) and vitamin C (3.2mg/100g in grapes; 0.1mg/100g in non-fortified grape juice) (Ho, Ferruzzi, and Wightman 2019; USDA 2018).

Relative differences in composition for grape polyphenols between whole fruit and 100% juice are less clear relying primarily on qualitative comparisons. Qualitative profiles for anthocyanins, phenolic acids, and I have been reported to be similar for a broad collection of grapes, grape juices and extracts (Y. Xu et al. 2011). Variation in products exist and is likely driven by variability in fruit quality and the juicing process itself (Ho, Ferruzzi, and Wightman 2019). Makris et al (Makris, Kallithraka, and Kefalas 2006) also reported flavonol content to be greater in multiple grape cultivars compared to corresponding grape juices, though data are limited. Similarly, resveratrol content in both dark and bronze skinned muscadine grapes was reported to be greater than their corresponding juices (Ector et al. 1996). Such results help to advance the perception that fruit is a better source of phenolics than comparative juice products.

While these efforts have provided context on compositional similarities and differences, the extent to which whole fruit and 100% juice may provide bioavailable micronutrients and phenolics to consumers remains unclear. In the case of 100% grape juice, the extraction process from whole fruit involves extensive contact with skin and seed fractions, (Manach et al. 2004) rich

in phenolics but either not consumed or potentially poorly digested. Contact with these fractions is essential in the extraction of phenolics to the final juice product and provides characteristic color and flavor associated with 100% Concord and Niagara grape juices. The physical and chemical/biochemical nature of the juicing process may serve to liberate phenolics concentrated in seed and skin fractions and by extent improve the bioavailability of these compounds from fractions that are generally not consumed or contain entrapped phenolics. Aschoff et al. (Aschoff et al. 2015) reported the bioaccessibility of flavonoids from 100% orange juice relative to orange fruit homogenate and whole fruit and orange flavonoids were found to be significantly more bioaccessible from juice than whole fruit. This was attributed, in part, to the juicing process and its ability to disrupt physical entrapment and compartmentalization of phenolics to a greater extent than chewing of whole fruit. The extent to which such effects translate to common consumer grape products including 100% grape juice and common table grapes is not known. Such insights are needed to aid in refinement of guidance and provide consumers comparative assessments not only of the impacts of processing but also of performance of common consumer options that can guide selection of products that best fit their preferences and lifestyle.

With this in mind, the goal of the current study was to develop insights into the distribution of grape phenolics in American Concord and Niagara juice grapes, retention of phenolics through juice processing and to compare phenolic bioaccessibility between 100% Concord and Niagara juice, their corresponding whole fruits and to a selection of commercial table grapes using a three-stage *in vitro* digestion model.

## 2.2. Materials and Methods

### 2.2.1. Chemicals, Standards, and Solutions

Acids, salts, and extraction solvents were purchased from Fisher Scientific (Hampton, NH, USA). Chromatography solvents, including water, formic acid, and acetonitrile (ACS certified and LC-MS grade), were purchased from Fischer Scientific. Phenolic standards (p-coumaric acid, chlorogenic acid, ferulic acid, gallic acid, caffeic acid, catechin, epicatechin, epigallocatechin, resveratrol, kaempferol, quercetin, cyanidin-3-*O*-glucoside, delphinidin-3-*O*-glucoside, malvidin-3-*O*-glucoside, myricetin, and quercetin-3-glucoside) were purchased from Sigma-Aldrich (St. Louis, MO, USA). In-vitro digestion enzymes, including, mucin (M2378),  $\alpha$ -amylase (A3176), pepsin (P7125), bile (B8631), pancreatin (P7547), and lipase (L3126), were purchased from Sigma-Aldrich (St. Louis, MO, USA).

### 2.2.2. Grape Fruit, Fractions and 100% Juice Material

Whole Concord and Niagara grapes, grape fractions (skins, seeds, flesh) and 100% juice samples were provided by Welch Foods Inc (Table 2.1). Whole Concord and Niagara grapes were collected from the 2018 harvest year from Grandview, WA, USA geographic locations. Concord and Niagara grapes were handpicked on October 9, 2018 from their respective farms. For separated grapes, each berry was cut in half with a knife or scalpel. Tweezers were used to remove seeds, and the pulp was carefully separated from the skins. All samples were frozen at  $-23^{\circ}\text{C}$  and later shipped to the Plants for Human Health Institute (Kannapolis, NC) on dry ice where they were stored at  $-80^{\circ}\text{C}$  until used for analysis. 100% juices were produced from 2018 harvest year through commercial juicing and were stored at  $4^{\circ}\text{C}$  until used for analysis. 100% Concord juices and Niagara juice concentrates were produced using a commercial line that included maceration

of the grapes and exposure to mild heat and enzymatic treatment with pectinases to enhance juice extraction. Grape mass was pressed and resulting juice filtered prior to subsequent evaporation and pasteurization to generate finished experimental products (Clemens et al. 2015). Niagara grape juice samples were provided as a concentrate with or without added sulfur dioxide (potassium metabisulfite) and reconstituted to 16° Brix prior to analysis. It is important to note that juices were from commercial scale production utilizing grapes from farms across a broad region. As Concord and Niagara grapes are not typically eaten whole, select table grape varieties (Table 2.1) were obtained at a local market (Kannapolis, NC) to derive comparison between phenolic profiles and bioaccessibility of grape forms as commonly consumed.

**Table 2.1.** Grape fruit, fractions and 100% Juice Samples<sup>1</sup>

<b>Cultivar (<i>Species</i>)</b>	<b>Farm/Region</b>	<b>Product Form</b>
<b><i>Fruit</i></b>		
Concord ( <i>Vitus labrusca</i> )	Vineyard 1 Farm (Grandview, WA, USA)	Whole Grape
Concord ( <i>Vitus labrusca</i> )	Vineyard 2 Farm (Grandview, WA, USA)	Whole Grape
Niagara ( <i>Vitus labrusca</i> )	Vineyard 3 Farm (Grandview, WA, USA)	Whole Grape
Niagara ( <i>Vitus labrusca</i> )	Vineyard 4 Farm (Grandview, WA, USA)	Whole Grape
Niagara ( <i>Vitus labrusca</i> )	Vineyard 4 Farm (Grandview, WA, USA)	Skins, Seeds, Pulp
Concord ( <i>Vitus labrusca</i> )	Vineyard 1 Farm (Grandview, WA, USA)	Skins, Seeds, Pulp
<b><i>100% Juice</i></b>		
Concord	Grandview, WA, USA	16.5° Brix (Not from Concentrate)
Niagara	Grandview, WA, USA	16.0° Brix (Reconstituted)
Niagara (SO <sub>2</sub> added)	Grandview, WA, USA	16.0° Brix (Reconstituted)
<b><i>Table Grapes</i></b>		
Scarlet Royal ( <i>Vitus vinifera</i> )	Anthony's Vineyard (Bakersfield, CA, USA)	Red Seedless Whole Grape
Red Globe ( <i>Vitus vinifera</i> )	Silver Canyon (Mecca, CA, USA)	Red Seeded Whole Grape
Summer Royal ( <i>Vitus vinifera</i> )	Anthony's Vineyard (Bakersfield, CA, USA)	Black Seedless Whole Grape
Thompson ( <i>Vitus vinifera</i> )	Anthony's Vineyard (Bakersfield, CA, USA)	Green Seedless Whole Grape

### 2.2.3. Three stage *in-vitro* digestion

Phenolic bioaccessibility from aliquots of whole grapes or 100% grape juice was determined using a three-stage *in vitro* digestion model described previously by Kean et al. with modification to include a simulated mastication in the oral phase (Kean et al. 2011). Briefly, to

simulate mastication, whole grapes (~20g) were passed through a manual tenderizer (Weston Heavy Duty Meat Tenderizer, Southern Pines, NC, USA) a total of three times. The resulting crushed and sliced grape mass was lightly tapped with a food hammer 10 times to generate a crude bolus. This method resulted in a grape bolus with physical attributes (macroscopic size, extent of tissue damage and disruption of seed and skin tissues) resembling whole table grapes chewed by a human volunteer (Supplementary Figure 2.1). Following oral processing, ~1.5g aliquots of grape bolus or 100% juice were transferred to a 50mL tube along with 6mL of oral phase solution containing  $\alpha$ -amylase (10units/mg). For each sample, volume was adjusted to 30mL with saline solution (0.9% NaCl), capped and blanketed under nitrogen, vortexed, and incubated for 10 minutes at 120rpm and 37 °C. Following oral digestion, 2mL of pepsin solution (10mg/mL in 0.1M HCl) was added and the pH of each sample was adjusted to  $2.5 \pm 0.1$  with 1M HCl. Sample volume was then adjusted to 40mL with saline solution (0.9% NaCl), and samples were capped under a nitrogen blank, vortexed, and incubated for another 1 hour at 120rpm and 37 °C to simulate gastric digestion. Following gastric digestion, the small intestinal phase was initiated by adjustment of the sample pH to  $5.0 \pm 0.1$  with 0.1N NaHCO<sub>3</sub>. 2mL of pancreatin-lipase solution (20mg/mL pancreatin and 10mg/mL lipase in 100mM NaHCO<sub>3</sub>) and 3mL of bile (30mg/mL bile extract in 100mM NaHCO<sub>3</sub>) was then added to each sample. For each sample, pH was adjusted to  $6.5 \pm 0.1$  using 0.1N NaHCO<sub>3</sub>, capped under nitrogen, vortexed, and incubated for 2 hours at 120rpm and 37 °C. Following completion of the small intestinal phase of digestion, samples were centrifuged (10,000x g, 4 °C) for 1 hour to isolate the aqueous bioaccessible fraction. Aqueous fractions were collected and filtered using 0.20  $\mu$ m PTFE filter to remove small aggregates and aliquoted in cryovials, nitrogen blanked and stored at -80 °C until further extraction.

#### 2.2.4. Phenolic extraction

Whole grapes, skins, and pulp were thawed and homogenized (VWR 250 Homogenizer, 10032-766, Radnor, PA, USA) for 30 seconds at 10,000 rpm, and then aliquoted prior to phenolic extraction and subsequent analysis. Seeds were ground (Cuisinart Spice and Nut Grinder SG-10, Stamford, CT, USA) for 1 minute, and then aliquoted for phenolic extraction and subsequent analysis. Phenolic compounds were extracted utilizing a modified solid phase extraction method previously reported by Song et al. (Song et al. 2013) Briefly, ground/homogenized grape samples (~250mg) were combined with 5 mL of formic acid, water, and methanol (2:18:80). Samples were vortexed for 1 minute, sonicated for 20 minutes, vortexed for 1 minute, and finally centrifuged (4,000g) for 4 minutes. The supernatant was collected and 5 mL of 2% formic acid in methanol was added to the tubes and the remaining solid was re-extracted twice as described above. Supernatant fractions were pooled and dried under nitrogen. Dried extracts were resolubilized in 0.1% formic acid in water in preparation for solid phase extraction (Oasis HLB extraction cartridges). The cartridges were activated using sequential passes of 1% formic acid in methanol and 1% formic acid in water. Samples were loaded on the SPE cartridges, rinsed with 0.1% formic acid prior to phenolic elution with 0.1% formic acid in methanol. Phenolics were extracted from aqueous fractions by solid phase extraction (Oasis HLB extraction cartridges) as described above. Eluates were dried under nitrogen and stored at  $-80^{\circ}\text{C}$  until further analysis.

#### 2.2.5. Phenolic Analysis by LC-MS/MS

Dried extracts were resolubilized in formic acid, water, and methanol (0.1:49.9:50), filtered using a 0.45  $\mu\text{m}$  PTFE, and were analyzed by LC-MS/MS using a Waters Acquity I Class UPLC equipped with a XEVO TQD for mass spectrometer (Waters, Milford, MA, USA). Phenolics were

resolved with an Acquity UPLC BEH C18 (2.1 x 50mm) column at a flow rate of 0.5 mL/min using a gradient elution profile based on a binary phase of 0.1% formic acid in water (solvent A) and 0.1% in acetonitrile (solvent B). Separation was achieved at 40 °C using the following gradient: initially 100% A, 0-0.5min 100-94% A, 0.5-2 min 94-1% A, 2-3 min 91-87% A, 3-4.5 min 87-65%A, 4.5-5.5 min 65-100% A, 5.5-6 min 100% A. Phenolics including phenolic acids, flavan-3-ols, I and stilbenoids were detected under negative mode electrospray ionization (ESI-) with the following conditions: desolvation temperature 600 °C, desolvation gas flow 650L/hr, capillary voltage 3 kV, cone voltage 32V, and collision energy of 20V. Anthocyanins, were resolved under slightly modified conditions. Specifically, solvent A was adjusted to 2% formic acid in water and individual anthocyanins were detected in ESI+ mode with the following conditions: desolvation temperature 600 °C, desolvation gas flow 1000 L/hr, capillary voltage 3 kV, cone voltage 30V, and collision energy of 20V.

Multiple Reaction Monitoring (MRM) responses were used to quantify 35 individual phenolic compounds. Phenolic acids, stilbenoids, and flavonoids were quantified using multi-leveled response curves constructed with authentic standards for each phenolic compound with some exceptions including coumaric acid, caftaric acid, dihydrocoumaric acid, kaempferol-3-glucoside, myricetin-3-glucoside, resveratrol-3-glucoside, kaempferol-3-rutinoside, quercetin-3-rutinoside, galocatechin which were quantified using structurally similar compounds. Malvidin and delphinidin glycosides concentrations were quantified using malvidin-3-*O*-glucoside and delphinidin-3-*O*-glucoside response curves respectively. Remaining anthocyanin concentrations were determined using a calibration curve for cyanidin-3-*O*-glucoside.

### 2.2.6. Procyanidin Determination

Procyanidin content of whole grapes and grape components was determined as described by Ryan et al (Ryan et al. 2016). Briefly, ~500mg of freeze-dried grape samples were extracted with 2mL of acetic acid, water, and acetone (2:28:70) under sonication/vortexing for 15 minutes. Slurry was then centrifuged (4,000g) for 5 minutes, the supernatant collected, and the extraction repeated (3x). The combined supernatant fractions were pooled, dried under nitrogen, freeze-dried and stored at  $-80^{\circ}\text{C}$  until analysis. The 4-dimethylaminocinnamaldehyde (DMAC) colorimetric assay was performed to determine total procyanidin content. Procyanidins degree of polymerization (DP) was determined using a Hilic UPLC-MS/MS method as described by Racine et al (Racine et al. 2019).

### 2.2.7. Statistical Analysis

All data presented as a mean  $\pm$  SD were replicated with a minimum in triplicate. JMP (SAS Institute Cary, NC, USA) software was used for statistical analysis of the data. Tukey-Kramer analysis was utilized to determine significant differences among phenolics between grapes and 100% grape juices. Differences of  $p < 0.05$  were considered to be significant. For the purposes of this study, individual phenolic compounds (Supplemental Tables) were summed by compound class (phenolic acids, anthocyanins, flavan-3-ols, flavonols, and stilbenoids) and used for comparisons for clarity. Comparisons were made between the content and absolute bioaccessible fraction of phenolics in Concord grapes and Concord juice and between Niagara grapes and Niagara juice. Further comparisons were made between Concord juice and dark-colored table grapes and between Niagara juice and Thompson seedless grapes. These comparisons were made to give a better understanding of the differences in content and accessible fractions of phenolics in

grape juices and table grapes that are readily available in the market. Content and bioaccessible fractions of phenolics were expressed in mg/100g and mg/cup serving of grape sample (grape fraction, whole grape, or 100% juice), where 1 cup was defined as 151g for whole grapes (USDA 2019) and 240 mL for grape juice per the US FDA Reference Amount Commonly Consumed, to provide perspective on bioaccessibility form grape products as typically consumed. Relative bioaccessibility was calculated as the ratio of bioaccessible content for a phenolic species to the total content for that phenolic species in the fruit or juice sample expressed as a percentage.

Percent Relative Bioaccessibility = (bioaccessible or absolute content ÷ total content) x 100

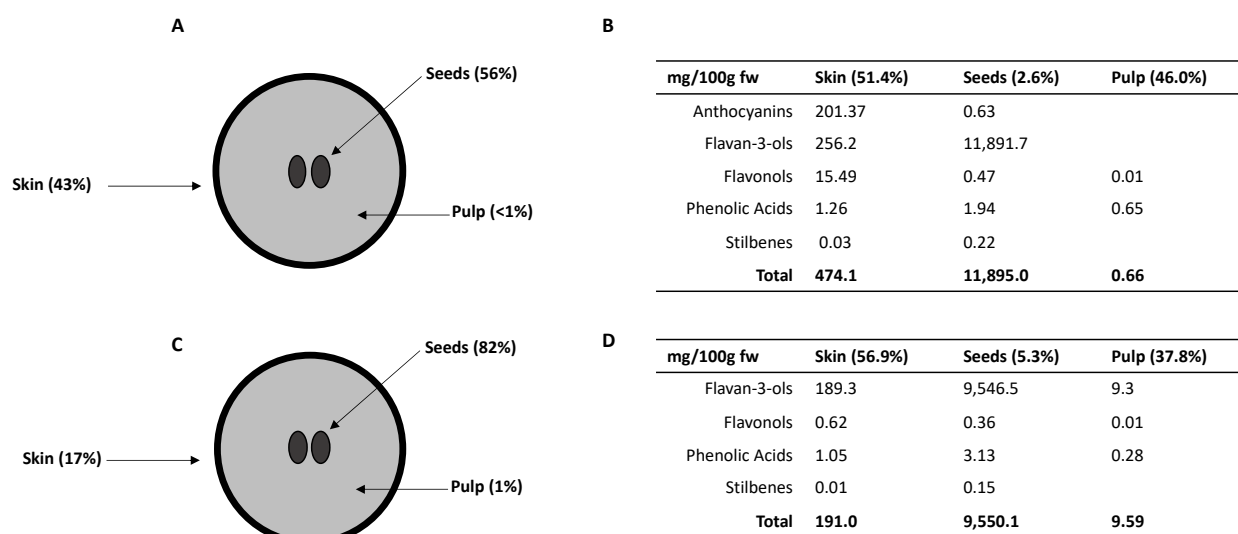
## **2.3. Results and Discussion**

### **2.3.1 Profile of phenolics in grape fractions**

In order to better understand the distribution/localization of phenolics in juice grapes, samples of both Concord and Niagara grapes were separated into seed, skin and flesh fractions and analyzed for 35 phenolic species. For ease of reporting, phenolic contents are grouped by class including phenolic acids, anthocyanins, flavan-3-ols, flavonols, and stilbenes. Content of individual phenolic species within each grape fraction (seed, skin or flesh) can be found in Supplemental Table 2.1.

Consistent with previous reports, phenolic compounds were found to accumulate in skins (Munoz-Espada et al. 2004; Liang et al. 2008; Sandhu and Gu 2010) (primarily anthocyanins and flavan-3-ols) in Concord grapes and seeds (primarily flavan-3-ols) for both Concord and Niagara grapes (Bozan, Tosun, and Özcan 2008; Rockenbach et al. 2011; Sandhu and Gu 2010; Wu, Wang, and Simon 2005). Concord grape seeds accounted for 56% of total phenolic content with flavan-3-ols being most prevalent. Concord skins accounted for 43% of total phenolics in the form of

flavan-3-ols, anthocyanins, and I (Figure 2.1). Concord grape skins contained 256.2 mg/100g flavan-3-ols, 201.37 mg/100g anthocyanins, and 15.49 mg/100g of flavonols, while the seeds contained 11.9 g/100g flavan-3-ol monomers, oligomers, and polymers (Figure 2.1). Similarly, total phenolics were concentrated in the Niagara seeds (82%) with flavan-3-ols (9.5 g/100g) being the primary form present in these grapes (Figure 2.1). The general proportion of phenolics in skin, seed, and flesh for Concord grapes was observed to be 43:56:1, while for Niagara grapes the proportion of skin, seed, and flesh was 17:82:1, with seeds accounting for a majority of all phenolics.



**Figure 2.1.** Percent of total phenolics distributed by fraction (skin, seed and pulp) within Vineyard 1 Concord grape (A and B) and Vineyard 4 Niagara grape (C and D). Inset Tables B and D present phenolic content (mg/100g fw of grape fraction). The detailed composition can be seen in Supplemental Table 2.1. Percent (%) mass contribution to whole grape is designated next to each fraction in inset Tables B and D. Flavan-3-ols include total monomeric, oligomeric, and polymeric forms quantified by DMAC analysis (Supplemental Table 2.1).

Procyanidins in particular (flavan-3-ols oligomers and polymers) are present in significant quantities in grapes, particularly in the seed fraction. In Concord grape skins and seeds DMAC analysis found 256.2 mg/100g and 11.9 g/100g total procyanidins, respectively, while total procyanidins (Hilic LC analysis) resulted in 55.39 mg/100g and 37.7 g/100g, respectively (Supplemental Table 2.1). Niagara skins, seeds, and pulp had a total procyanidin (DMAC) of 189.3

mg/100g, 9.5 g/100g, and 9.3 mg/100g, respectively, while total procyanidins (Hilic LC analysis) content was 229.55 mg/100g, 22.3 g/100g, and 1.18 mg/100g, respectively (Supplemental Table 2.1). Considering the total procyanidins by Hilic (Supplemental Table 2.1), the general proportion of phenolics in fruit compartments for Concord grapes was observed to be >99% in seeds with <1% in flesh and skins. For Niagara grapes the proportion of skin, seed, and flesh was 1:99:0.01. This effect was driven by the very high quantities of procyanidins present in the seed fractions. It is important to note that differences in total procyanidin content were consistently noted between methods. The DMAC method is a non-specific colorimetric method commonly used for other matrices such as cocoa. The Hilic LC analysis was limited by availability of standard between DP 1-10. Though total procyanidin content by Hilic in skin were in the range of those previously reported (10 – 72.1 mg/100g), (Lago-Vanzela et al. 2011; Busse-Valverde et al. 2010) this could have led to an underestimation of total procyanidins, particularly in Concord skins, by not fully capturing the diversity of compounds greater than DP 10. However, it is clear that seeds from both Niagara and Concord grapes are the fraction with the highest concentration of flavan-3-ol polymers. This is an important distinction as with consumption of whole grapes, seeds are not often consumed, and therefore a significant portion of the phenolics, primarily flavan-3-ol monomers, oligomers and polymers, would be lost at the point of consumption.

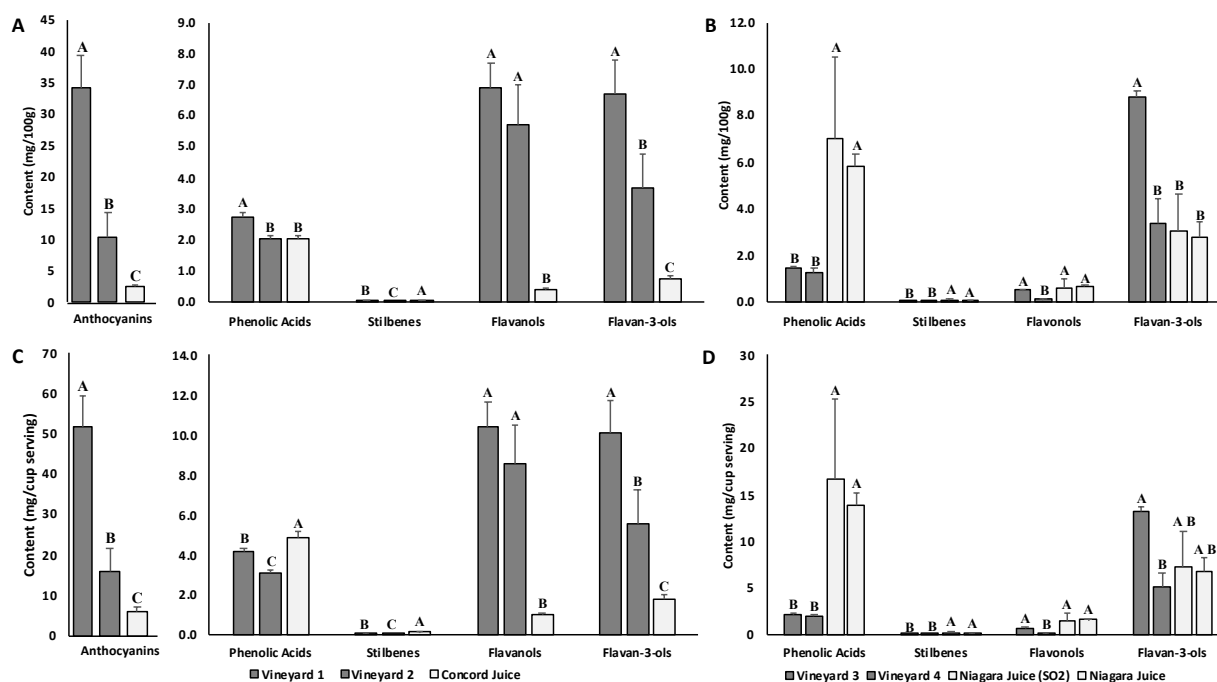
### 2.3.2. Profile of phenolics in whole grapes and 100% juice

Vineyard 1 and Vineyard 2 Concord grapes had a greater total phenolic content (50.74mg/100g and 21.91mg/100g, respectively) than 100% Concord juice (5.81 mg/100g). Phenolic species including anthocyanins, phenolic acids, flavonols, stilbenes and flavan-3-ols were all detected in both Concord grapes and 100% Concord grape juice from the same region.

Content of individual phenolic species can be found in Supplemental Table 2.2. Cyanidin-3-*O*-glucoside (0.42 – 11.66 mg/100g) and delphinidin-3-*O*-glucoside (0.97 – 11.51 mg/100g) were the most predominant anthocyanins observed in Concord grapes (Vineyard 1 and Vineyard 2) and 100% juices consistent with previous reports (Stalmach et al. 2011; Moser et al. 2016; H. Wang, Race, and Shrikhande 2003; Oh et al. 2008; Yang, Martinson, and Liu 2009). Major phenolic acids in Concord grapes and juices included hydroxycinnamic acids and their tartaric acid esters (caftaric and coutaric acid) in agreement with previous reports (Stalmach et al. 2011; Singleton, Zaya, and Trousdale 1986). Predominant flavan-3-ols included (+)-catechin and (-)-epicatechin (0.30 – 5.06 mg/100g), while quercetin derivatives composed the majority of flavonols (0.09 – 4.82 mg/100g) also in ranges consistent with previous reports (Wu, Wang, and Simon 2005; Zhu, Zhang, and Lu 2012). Very low quantities of resveratrol and resveratrol glycosides were observed in Concord juice and grapes (Stalmach et al. 2011; Y. Wang et al. 2002).

While there was significant variation between the two harvest locations (Vineyard 1 and Vineyard 2), Concord grapes were found to have significantly higher contents of flavonols ( $p < 0.0001$ ), flavan-3-ols ( $p < 0.0001$  for juice and Vineyard 1,  $p < 0.0004$  for juice and Vineyard 2), and anthocyanins ( $p < 0.0001$ ) compared to 100% Concord grape juice (Figure 2.2). However, Concord juice had a significantly higher amount of stilbenes (0.08mg/100g compared to 0.01-0.05 mg/100g;  $p < 0.0001$  between juice and Vineyard 2,  $p = 0.0411$ ) than whole grapes (Figure 2.2). Concord grape juice had comparable levels of phenolic acids to Vineyard 2 Concord grapes ( $p = 0.9822$ ), though Vineyard 1 Concord grapes were significantly greater ( $p < 0.0002$ ). On a cup/serving basis, the phenolic acids content of Concord juice was significantly greater than Vineyard 2 Concord grapes ( $p < 0.0001$ ) and comparable to Vineyard 1 Concord grapes ( $p = 0.0726$ ) (Figure 2.2). Similar trends were observed for other phenolic classes between Concord

juice and grapes on a mg/cup basis. Vineyard 1 and Vineyard 2 Concord grapes had a higher total procyanidin content through both DMAC and Hilic (376.0 – 605.5 mg/100g, 472.09 – 738.53 mg/100g, respectively) than Concord juice (119.9 mg/100g, 131.74 mg/100) (Supplemental Table 2.2).



**Figure 2.2. Comparison of phenolics between whole juicing grapes and 100% grape juice.** Panel A and C present Concord grapes (Vineyard 1 and Vineyard 2) and 100% Concord juice, while B and D present Niagara grapes (Vineyard 3 and Vineyard 4) and 100% Niagara juice. Data are expressed as mg/per 100g or mg/cup serving (151g for grapes and 240mL for juice) from a sum of individual phenolics as determined by LC-MS. Presence of different letter above error bar indicates significant difference ( $p < 0.05$ ) within individual phenolic class (anthocyanins, phenolic acids, stilbenes, flavonols, and flavan-3-ols) levels among different grapes and juices.

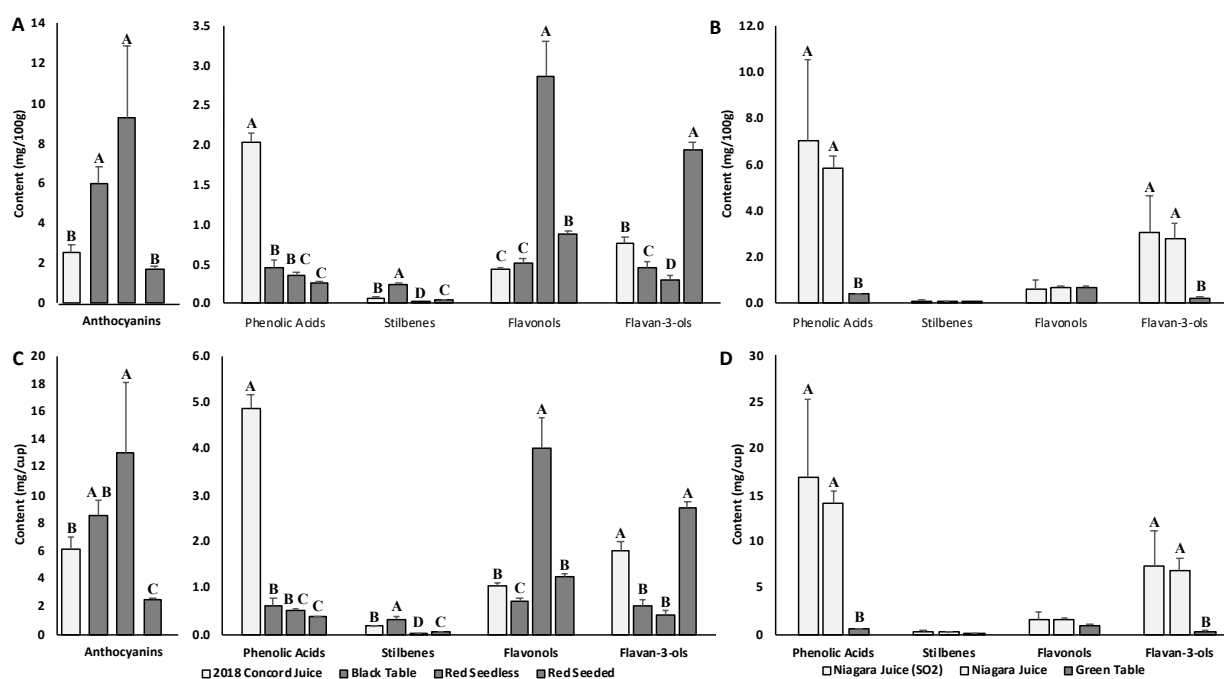
Vineyard 3 and Vineyard 4 Niagara grapes had comparable total phenolic content (10.76mg/100g and 4.85mg/100g, respectively) to both SO<sub>2</sub> Niagara and regular Niagara juice (10.80mg/100g and 9.44mg/100g, respectively). For Niagara grapes and juice, phenolic acids, stilbenes, flavonols, and flavan-3-ols were main phenolic species identified consistent with previous reports (Moser et al. 2016; Lee and Jaworski 1987). The phenolic acid profile was similar to that of Concord grapes, being composed on hydroxycinnamic acids and their tartaric acid esters (Supplemental Table 2.3). Quercetin-3-*O*-glucosides (0.12 – 0.63 mg/100g) were the predominant

flavonols present in Niagara grapes and juices. Flavan-3-ol monomers were primarily composed of (+)-catechin (0.80 – 2.25 mg/100g) and (-)-epicatechin (1.45 – 6.50 mg/100g). Quantities of phenolic acids were significantly greater in juices compared to Niagara grapes ( $p < 0.003$ ) (Figure 2.2). Flavonol and flavan-3-ols content was comparable between Niagara grapes and juices ( $p = 0.7499$  to  $0.9812$ ), though Niagara grapes (Vineyard 3) reported a significantly greater flavan-3-ols content than either juice ( $p = 0.0097$ ). However, on a per serving basis, differences in flavan-3-ol content are greater for Vineyard 3 Niagara grapes and juices are no longer significant ( $p = 0.0778$ ,  $0.0816$ ) (Figure 2.2). Similar to Concord grapes and juice, Niagara grapes had a greater total procyanidin content (269.1 – 772.5 mg/100g DMAC; 544.90 – 776.23 mg/100g Hilic) than either Niagara juice (13.7 – 18.4 mg/100g DMAC; 44.50 – 59.09 mg/100g Hilic) (Supplemental Table 2.3).

Table grapes including red seedless (Scarlet Royal variety), red seeded (Red Globe variety), and black seedless (Summer Royal variety) qualitatively were similar in profile to Concord grapes being rich in anthocyanins, phenolic acids, flavonols, and flavan-3-ols (Figure 2.3). Red seedless grapes had greater total phenolic content (11.42mg/100g) compared to black and red seeded grapes (7.61mg/100g and 4.60mg/100g, respectively) (Supplemental Table 2.4). This is driven by a significantly greater ( $p < 0.001$ ) content of flavonols (2.85mg/100g) in red seedless grapes compared to black (0.50mg/100g) and red seeded grapes (0.88mg/100g). Furthermore, the content of anthocyanins in red seedless (9.24mg/100g) was significantly greater ( $p < 0.0005$ ) than red seeded (1.73mg/100g) and greater than ( $p = 0.1209$ ) black seedless grapes (6.00mg/100g). It is important to note that red seeded grapes had a significantly greater ( $p < 0.001$ ) flavan-3-ol content (1.94mg/100g) than either seedless grape. Furthermore, red seeded grapes had a greater total procyanidin content (146.5 mg/100g DMAC; 241.39 mg/100g Hilic) than the

seedless table grapes (32.8 – 61.6 mg/100g DMAC; 25.68 – 41.07 mg/100g Hilic). These results further highlight seeds as primary sources of flavan-3-ol monomers and polymers in grapes, and their fate must be considered when assessing phenolics available from consumption.

By comparison, Concord grape juice had significantly greater quantities of phenolic acids than the red/black table grapes ( $p < 0.0001$ ) whereas quantities of anthocyanins, stilbenes, flavan-3-ols, and flavonols were more similar between table grapes and Concord grape juice (Figure 2.3). Green Thompson Seedless grapes contained phenolic acids, flavonols, stilbenes and flavan-3-ols. However, Niagara juice (both with and without SO<sub>2</sub> treatment) maintained a significantly greater content of phenolic acids and flavan-3-ols compared to green table grapes ( $p < 0.004$ ) (Figure 2.3).



**Figure 2.3. Comparison of phenolics between whole table grapes and 100% grape juice.** Panel A and C present colored table grapes (black, red seeded, and red seedless) and 100% Concord juice, while B and D present green table grapes and 100% Niagara juice. Data are expressed as mg/per 100g or mg/cup serving (151g for grapes and 240mL for juice) from a sum of individual anthocyanins/phenolics as determined by LC-MS. Presence of different letter above error bar indicates significant difference ( $p < 0.05$ ) within individual phenolic class (anthocyanins, phenolic acids, stilbenes, flavonols, and flavan-3-ols) levels among different grapes and juices.

Overall, these results suggest that with the possible exception of phenolic acids, initial phenolic content in whole juice grapes, Concord or Niagara, is greater than the content in their respective whole juices. This is most likely due to the nature of juicing which can only express a certain percentage of the total phenolics from the whole fruit to the juice itself. Juicing requires mechanical maceration, heat and enzymatic treatments prior to physical extraction. The juicing process also involving a thermal pasteurization and a clarification stabilization step prior to bottling finished grape juice (Turfan et al. 2011). Considering that phenolic extraction by commercial juicing is incomplete and that phenolics may be labile to both heat and physical sedimentation during clarification of the juice, (Turfan et al. 2011) it is not surprising that for most phenolic classes juice remains lower in content than corresponding whole fruit (Sólyom et al. 2014; Volf et al. 2014; Clifford 2000). Higher content of phenolic acids in juice relative to fruit are likely a result of the commercial juicing process where enzymatic treatments, including pectinases, may hydrolyze complex polysaccharide structures that could liberate certain phenolics, including phenolic acids (Kashyap et al. 2001; C. Xu et al. 2014). However, it is important to note that phenolic rich seeds are not always consumed when eaten as fresh fruit. In general, the profile and quantity of phenolics remain comparable, particularly between 100% Niagara juice and Niagara grapes, and between 100% juice and tables grapes.

### 2.3.3. Phenolic bioaccessibility from whole grapes and 100% juices

In the context of this study, bioaccessibility is defined as the fraction of phenolic compounds present in the native fruit or 100% juice that is released during normal digestion and recovered in the aqueous fraction of the small intestinal digesta thereby being considered available for intestinal absorption or further metabolism by gut microbiota (Aschoff et al. 2015;

Tagliazucchi et al. 2010). Considering the relative measure of bioaccessibility (%) provides context into the efficiency of digestive release, bioaccessible content is a derived value that represents the amount of phenolics that are bioaccessible from a specified quantity (100g) or serving (1 cup) of either fruit or juice. This value provides an estimate of the delivery characteristics of each matrix and can be used as a comparative measure between foods or to assess the impacts of processing, such as juicing.

Generally, relative bioaccessibility of phenolics was found to be greater from 100% Concord and Niagara juices compared to their respective whole grapes and table grapes (Table 2.2). Specifically, relative bioaccessibility of anthocyanins from Concord juice ranged from 86-135%, while whole Concord grapes ranged from 14-39% (Table 2.2). Flavanols had greater relative bioaccessibilities (36-43%) from 100% Concord juices than from whole Concord grapes (2-8%). While anthocyanins and flavonols remain major phenolic components for Concord grape juice, other significant differences between whole grapes and juices were observed with high bioaccessibilities for flavan-3-ols (48%-101%) from Concord and Niagara juices relative to whole grapes with intact/masticated seeds which ranged from 0-3% (Table 2.2). Phenolic acids also showed a similar trend, in that 100% juices had a higher relative bioaccessibility (39-85%) compared to whole grapes (9-67%).

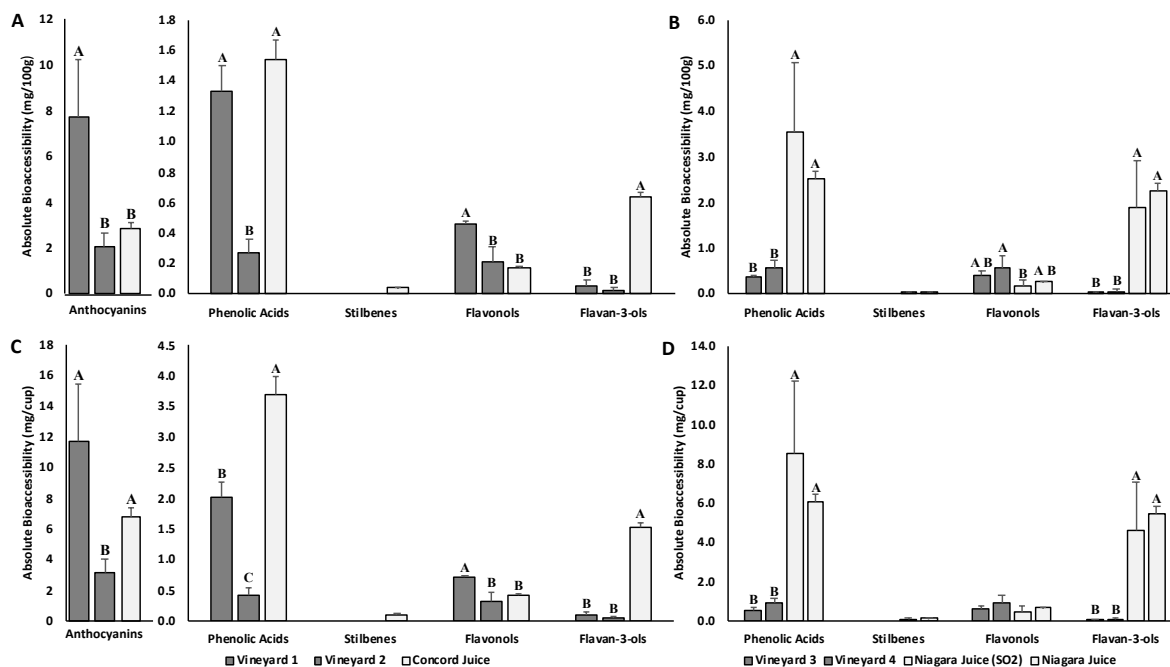
**Table 2.2.** Relative bioaccessibility (%) range of phenolics from Concord, Niagara, and table grapes and 100% Concord and Niagara grape juices.<sup>2</sup>

Grape	Farm	Anthocyanins	Phenolic Acids	Stilbenes	Flavonols	Flavan-3-ols
Concord Grape	Vineyard 1	16-32%	44-52%		6-8%	0-1%
Concord Grape	Vineyard 2	14-39%	9-17%		2-5%	0-2%
Concord Juice	Grandview, WA, USA	86-135%	70-85%	44-63%	36-43%	75-95%
Niagara Grape	Vineyard 3		21-32%		64-104%	0-1%
Niagara Grape	Vineyard 4		31-67%		394-585%	0-3%
Niagara Juice	Grandview, WA, USA		39-48%	38-52%	37-43%	63-101%
Niagara Juice SO <sub>2</sub>	Grandview, WA, USA		42-66%	25-53%	22-34%	48-76%
Red Seedless	Anthony's Vineyard	34-59%	7-26%		23-44%	4-11%
Red Seeded	Silver Canyon	20-44%	19-26%	0-19%	15-30%	2-4%
Black Seedless	Anthony's Vineyard	51-85%	39-142%	5-14%	50-153%	8-33%
Green Seedless	Anthony's Vineyard		44-62%		20-116%	2-35%

<sup>2</sup>Data are expressed as mean  $\pm$  standard deviation from  $n=3$  digestion experiments.

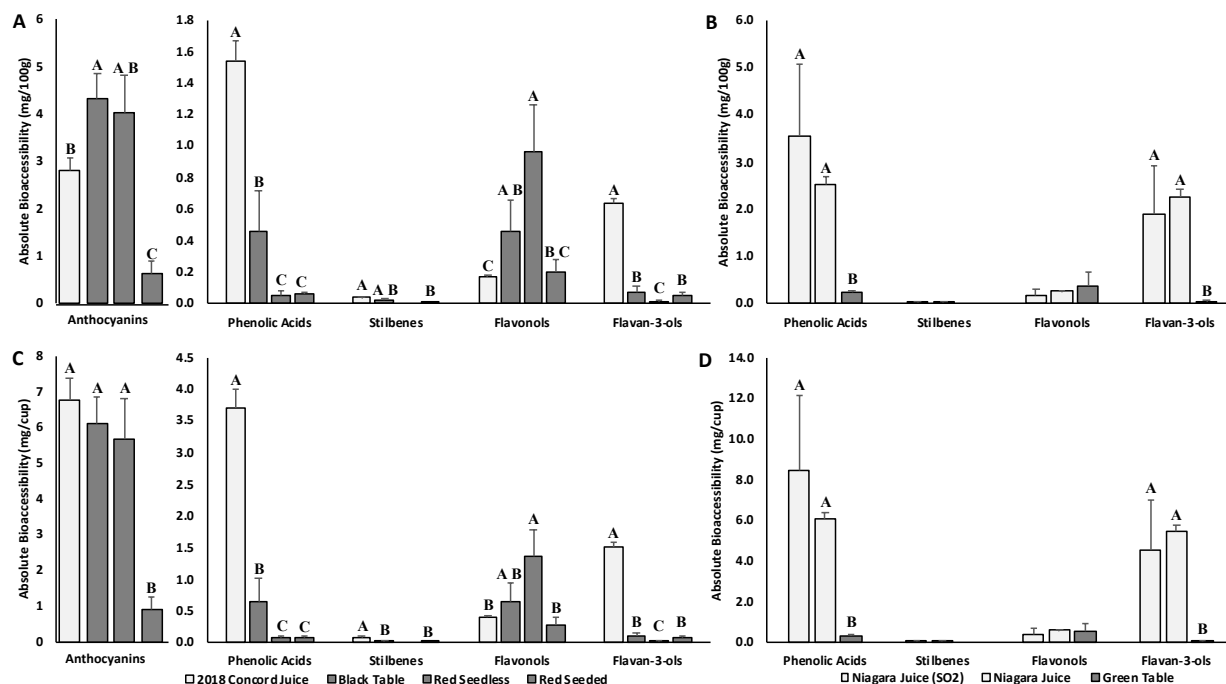
The juicing process allows for significant contact with phenolic-rich components (skins and particularly seeds) likely increasing extraction of phenolics from these components and facilitating their transfer to bioaccessible aqueous phase through digestion. Seeds are not often consumed, so phenolics (flavan-3-ols) present in seeds are not typically made accessible from whole grapes. Similarly, skins, though consumed, may not extract phenolics efficiently through mastication and normal digestion. The juicing process, aided by enzymatic and heat processing, can result in higher anthocyanin and flavonol relative bioaccessibility from Concord juice. Therefore, despite some losses through processing, the high relative proportion that is made available for absorption suggest that juice may be an equivalent if not better source of bioaccessible phenolics.

In fact, overall bioaccessible content of total phenolics was found to be similar for whole Concord grapes compared to juice (2.58 – 9.58mg/100g compared to 5.2mg/100g, respectively). Differences were observed with specific phenolic classes (Figure 2.4) and for individual phenolics (Supplemental Table 2.5). For example, bioaccessible content of flavan-3-ols was found to be significantly ( $p < 0.0001$ ) greater in Concord grape juice (0.64 mg/100g) compared to Concord grapes (0.03 – 0.05 mg/100g), contrasting the initial content of flavan-3-ols found in Concord grapes and juice (Figure 2.2). Also, bioaccessible content of phenolic acids in Concord grape juice (1.54 mg/100g) was significantly greater ( $p < 0.0001$ ) than Vineyard 2 Concord grapes (0.27 mg/100g), and comparable ( $p = 0.4860$ ) to Vineyard 1 Concord grapes (1.33 mg/100g) (Figure 2.4). Bioaccessible stilbenes were only detected following digestion of Concord grape juice. Anthocyanins were the main bioaccessible phenolic class from Concord juice (2.81 mg/100g) which was comparable ( $p = 0.8209$ ) to Vineyard 2 Concord grapes (2.07 mg/100g), though significantly lower ( $p = 0.0157$ ) than Vineyard 1 Concord grapes (7.74 mg/100g). Finally, flavonol bioaccessible content followed a similar trend with Concord juice content being comparable ( $p = 0.7772$ ) to Vineyard 2 Concord grapes, while being significantly lower ( $p = 0.01$ ) than Vineyard 1 Concord grapes (Figure 2.4).



**Figure 2.4. Comparison of absolute bioaccessible content between whole juicing grapes and 100% grape juice.** Panel A and C present Concord grapes (Vineyard 1 and Vineyard 2) and 100% Concord juice, while B and D present Niagara grapes (Vineyard 3 and Vineyard 4) and 100% Niagara juice. Data are expressed as mg/per 100g or mg/cup serving (151g for grapes and 240mL for juice) from a sum of individual bioaccessible anthocyanins/phenolics as determined by LC-MS. Presence of different letter above error bar indicates significant difference ( $p < 0.05$ ) within individual phenolic class (anthocyanins, phenolic acids, stilbenes, flavonols, and flavan-3-ols) levels among different grapes and juices.

Similar effects were observed from Niagara grape juices and whole grapes. Both SO<sub>2</sub> treated and untreated Niagara Juice had greater total bioaccessible phenolic content (5.09 – 5.66mg/100g) compared to whole Niagara grapes (0.80 – 1.18mg/100g) (Supplemental Table 2.6). Individual class differences were evident for phenolic acids (2.53 – 3.54 mg/100g) and flavan-3-ols (1.91 – 2.27 mg/100g) which were significantly greater ( $p < 0.01$ ) from Niagara juice than from grapes (Vineyard 4 and Vineyard 3) or green table (Thompson Seedless) grapes (Figure 2.4 and 5) (Supplemental Table 2.7). Flavonol bioaccessible content was comparable between Niagara juices and grapes (Vineyard 4 and Vineyard 3) (Figure 2.5). Neither Niagara (Vineyard 3 or Vineyard 4) or green table grapes had detectible quantities of bioaccessible stilbenes, while low levels (0.03 – 0.05 mg/100g) found to be bioaccessible from Niagara juices (with and without SO<sub>2</sub>) at levels similar to Concord grape/juice.



**Figure 2.5. Comparison of absolute bioaccessible phenolic content between whole table grapes and 100% grape juice.** Panel A and C present colored table grapes (black, red seeded, and red seedless) and 100% Concord juice, while B and D present green table grapes and 100% Niagara juice. Data are expressed as mg/per 100g or mg/cup serving (151g for grapes and 240mL for juice) from a sum of individual bioaccessible phenolics as determined by LC-MS. Presence of different letter above error bar indicates significant difference ( $p < 0.05$ ) within individual phenolic class (phenolic acids, stilbenes, flavonols, and flavan-3-ols) levels among different grapes and juices.

Comparing absolute bioaccessible phenolic content between red table grapes and Concord juice allows for a direct assessment of phenolic availability from two commonly consumed forms (juice versus whole grapes). Phenolic acid, flavan-3-ol, and stilbene absolute bioaccessibility were all significantly greater ( $p < 0.0043$ ) in Concord juice compared to red seeded (Red Globe), red seedless (Scarlet Royal), and black seedless (Summer Royal) table grapes (Figure 2.5). On a per serving cup basis, anthocyanin bioaccessible content of Concord juice was comparable ( $p = 0.3791$  for juice and red seedless,  $p = 0.9151$  for juice and black seedless) to that of black and red seedless table grapes and significantly greater ( $p < 0.0001$ ) than red seeded table grapes (Figure 2.5). Furthermore, the bioaccessible fraction of flavanols in Concord grape juice was comparable to table grapes, with the exception of red seedless grapes, which were significantly greater in bioaccessible flavanol content (Figure 2.5).

These data suggest that while the initial content of phenolics is highest in whole Concord and Niagara grapes, 100% grape juices and whole grapes are similar in total bioaccessible phenolics and that juice may in fact be a better vehicle for delivery of bioaccessible phenolics than whole fruit. A key factor in this observation is likely related to the liquid versus solid matrix and the benefits gained in juice extraction for certain classes of phenolics that are highly associated with skin and seed fractions that require effective mastication. Phenolics in liquid matrices have been reported to be readily available for absorption in the intestinal tract (Aschoff et al. 2015; Tagliazucchi et al. 2010). Conversely, phenolics in whole fruits, must first be extracted through normal oral processing and digestion. Mechanical action is required to break cell walls and cell membranes to release physically bound phenolics. Fiber is also present at a higher levels in whole grapes (Ho, Ferruzzi, and Wightman 2019). It is believed that solid food matrix coupled with phenolics physically entrapped or bound with fiber could potentially slow gastric transport and limit bioaccessibility (Palafox-Carlos, Ayala-Zavala, and González-Aguilar 2011). The juicing process involves extensive maceration of whole grapes, completely disrupting macro-structures such as seeds and skins, which are high in phenolic compounds. While the juicing process may in fact lead to a fraction of losses for select phenolic compounds, the remaining phenolics are present in a highly bioaccessible form. Furthermore, while whole grapes maintain higher levels of phenolics, it is likely that normal oral processing (mastication) of skins and seeds may not provide efficient enough extraction to fully release the phenolics in the matrix.

In addition to comparing whole Concord and Niagara grapes and corresponding 100% juice, it was important to consider an ecologically valid comparisons that represent direct consumer choices. Consumers have options of consuming grapes as whole fruit (mostly as table grapes) or 100% juice from juice grapes. Comparison of 100% Concord and Niagara juices to

four commercially available table grapes; Thompson seedless, Scarlet Royal seedless, Summer Royal seedless, and Red Globe seeded grapes followed similar trends observed with whole Concord and Niagara grapes. While some table grapes had relatively high initial phenolic content, the low relative bioaccessibility compared to the 100% juices made the effective delivery of these matrices similar. Of peculiar note, Thompson seedless and Summer Royal seedless grapes had relatively high relative bioaccessibility of flavan-3-ols, up to 35% (Table 2.2). This is most likely due to the flavan-3-ols being present in the flesh or skins of the grapes, as these are seedless grapes. Though the relative bioaccessibility is moderate, the actual content is quite low (Figure 2.5) and in fact Niagara grape juice was a better source of bioaccessible phenolics than Thompson seedless.

Though total procyanidin content of grape samples was estimated utilizing DMAC and Hilic techniques, the bioaccessibility of these more complex was not considered in our assessment of whole grapes and juice. The rationale for this was that it is well established that larger MW procyanidins ( $DP > 3$ ) are not absorbed in the upper GI (Ou and Gu 2014; Margalef et al. 2015). Rather, these larger MW polymers are metabolized by gut microbial communities and lead to the generation of numerous small compounds phenolic metabolites well known to be absorbed in the lower GI (Ou and Gu 2014; Margalef et al. 2015). As our model did not consider the lower GI in this phase of studies, we chose to exclude these compounds from the analysis of bioaccessibility. Future studies comparing whole grapes and juice are planned to determine the potential differences between whole fruit and juice in terms of generation and accessibility of microbial procyanidin metabolites.

Finally, it is important to note a limitation in this study with regards to comparisons between Concord and Niagara whole grapes and juice. The materials used in this study may not

reflect the broader variation across Concord and Niagara grapes as only a few locations were selected for sampling for logistical purposes. Furthermore, as we compared this to commercially processed juices, juice results are reflective of consumer products and reflect the homogeneity of these products. In the case of juice and whole grape comparisons, all efforts were made to compare products from the same geographic region. For example, Niagara juices were from commercial production sourced from grapes from multiple vineyards in Grandview, WA, USA, while they were compared to Niagara grapes from two specific vineyards in the same region. Similarly, Concord juice was from commercial production sourced from many vineyards in the Grandview, WA area, while it was compared to Concord grapes from two specific vineyards in the same region. It is well known that regional and geographical differences can compound effects on qualitative and quantitative profiles of phenolics in grapes (Jaitz et al. 2010; Jiang and Zhang 2012). Regional and geographic differences from the vineyards from which the grapes are sourced can account for the variation seen between the Concord and Niagara whole grapes and likely contribute to that from their 100% juices year to year. Though, these variations may affect the initial content of phenolics in the grapes and juices, the relative bioaccessibility results remain valid as this is a measure of the proportion of phenolics made available for intestinal absorption.

## **2.4. Conclusions**

The present results indicate that although 100% grape juice may have a lower initial phenolic content than corresponding whole fruit, juice may be able to deliver a greater percentage of phenolics for intestinal absorption compared to whole grapes. With this in mind, consumers should consider 100% grape juice as an alternative to whole grapes with regards to availability of physiologically active phenolic compounds. Future preclinical or clinical studies investigating the

bioavailability and metabolism of phenolics from 100% grapes juices compared to whole grapes are warranted to further determine if differences exist in either host or microbial metabolism as a function of grape product form.

## 2.5. References

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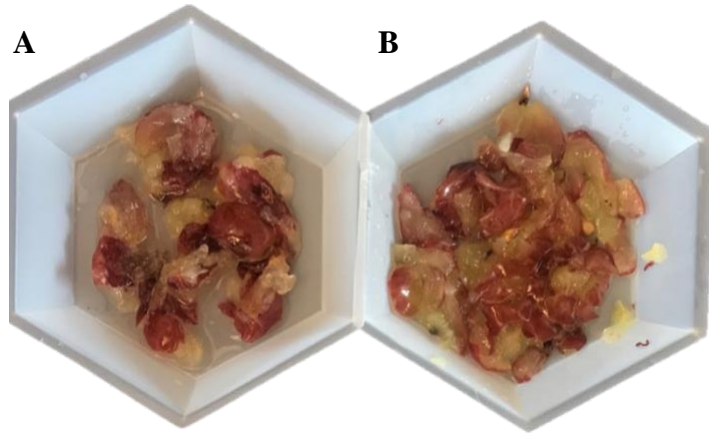
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## 2.6. Supplemental Figures/Tables



**Supplemental Figure 2.1.** Meat grinder used for simulated mastication of grapes (Weston Heavy Duty Meat Cube Tenderizer, Southern Pines, NC, USA). Images illustrate outcomes of whole grapes processed by human mastication (A) and that derived from simulated mastication (B) of red seeded table grapes.

**Supplemental Table 2.1.** Content (mg/100g of grape fraction fw) of major phenolics in Concord and Niagara grape fractions (skin, seed, or pulp).<sup>1,2</sup>

Compound Class	Compound	2018 Concord Grape (Vineyard 1)			2018 Niagara Grape (Vineyard 4)			
		Skins	Seeds	Pulp	Skin	Seeds	Pulp	
Anthocyanins	cyanidin	3-arabinoside	0.03 ± 0.01					
		3-glucoside	5.53 ± 1.71	0.03 ± 0.04				
		3-(6-p-coumaroyl-glucoside)	0.90 ± 0.26					
	peonidin	3-arabinoside	0.01 ± 0.01					
		3-glucoside	0.11 ± 0.04					
		3-(6-p-coumaroyl-glucoside)	0.07 ± 0.02					
	delphinidin	3-arabinoside	0.80 ± 0.35					
		3-glucoside	177.34 ± 77.03	0.50 ± 0.27				
		3-(6-acetyl glucoside)	7.36 ± 4.38					
	petunidin	3-arabinoside	0.02 ± 0.01					
		3-glucoside	4.73 ± 1.79	0.01 ± 0.01				
		3-(6-p-coumaroyl-glucoside)	0.40 ± 0.14					
malvidin	3-arabinoside	0.19 ± 0.04						
	3-glucoside	2.47 ± 0.80	0.08 ± 0.03					
	3-(6-p-coumaroyl-glucoside)	1.42 ± 0.44						
	<b>Total Anthocyanins</b>	<b>201.37 ± 87.03</b>	<b>0.63 ± 0.35</b>					
Phenolic Acids	coumaric acid	0.57 ± 0.22	1.46 ± 0.33	0.18 ± 0.01	0.62 ± 0.34	2.19 ± 0.29	0.18 ± 0.01	
	caftaric acid	0.34 ± 0.09	0.10 ± 0.02	0.03 ± 0.01	0.21 ± 0.07	0.09 ± 0.01	0.06 ± 0.01	
	p-coumaric acid	0.25 ± 0.05	0.27 ± 0.01	0.18 ± 0.01	0.17 ± 0.01	0.32 ± 0.01		
	gallic acid	0.04 ± 0.01	0.07 ± 0.01	0.05 ± 0.04	0.02 ± 0.01	0.48 ± 0.05	0.03 ± 0.01	
	caffeic acid	0.03 ± 0.01	0.03 ± 0.01	0.02 ± 0.01	0.02 ± 0.01	0.03 ± 0.01	0.02 ± 0.01	
	ferulic acid	0.04 ± 0.01	0.03 ± 0.01		0.03 ± 0.01	0.03 ± 0.01		
	dihydrocoumaric acid			0.20 ± 0.01				
		<b>Total Phenolic Acids</b>	<b>1.05 ± 0.43</b>	<b>1.94 ± 0.38</b>	<b>0.65 ± 0.06</b>	<b>1.05 ± 0.43</b>	<b>3.13 ± 0.37</b>	<b>0.28 ± 0.02</b>
Stilbenoids	resveratrol	0.02 ± 0.01	0.03 ± 0.03					
	resveratrol-3-glucoside	0.01 ± 0.01	0.19 ± 0.03		0.01 ± 0.01	0.15 ± 0.01		
	<b>Total Stilbenoids</b>	<b>0.03 ± 0.02</b>	<b>0.22 ± 0.06</b>		<b>0.01 ± 0.01</b>	<b>0.15 ± 0.01</b>		
Flavonols	quercetin	0.02 ± 0.01	0.01 ± 0.01		0.01 ± 0.01	0.01 ± 0.01		
	myricetin	0.17 ± 0.08	0.02 ± 0.01			0.02 ± 0.01		
	kaempferol-3-glucoside	0.01 ± 0.01			0.02 ± 0.01			
	quercetin-3-glucoside	2.17 ± 0.27	0.41 ± 0.03	0.01 ± 0.01	0.59 ± 0.24	0.25 ± 0.02	0.01 ± 0.01	
	myricetin-3-glucoside	0.34 ± 0.15	0.01 ± 0.01			0.01 ± 0.01		
	kaempferol-3-rutinoside	0.65 ± 0.16						
	quercetin-3-rutinoside	12.15 ± 3.14	0.02 ± 0.01			0.06 ± 0.06		
	<b>Total I</b>	<b>15.49 ± 3.80</b>	<b>0.47 ± 0.05</b>	<b>0.01 ± 0.01</b>	<b>0.62 ± 0.24</b>	<b>0.36 ± 0.09</b>	<b>0.01 ± 0.01</b>	
Flavan-3-ols	catechin	0.12 ± 0.09	42.40 ± 5.62	0.23 ± 0.34	0.07 ± 0.05	138.35 ± 15.39	0.35 ± 0.40	
	epicatechin	0.07 ± 0.05	179.74 ± 20.89	0.71 ± 1.20		141.32 ± 11.21	0.54 ± 0.63	
	gallocatechin	0.02 ± 0.01	0.02 ± 0.01		0.01 ± 0.01	5.82 ± 0.41		
	epigallocatechin							
		<b>Total Flavan-3-ols</b>	<b>0.21 ± 0.15</b>	<b>222.17 ± 26.53</b>	<b>0.94 ± 1.54</b>	<b>0.08 ± 0.06</b>	<b>285.48 ± 27.00</b>	<b>0.89 ± 1.03</b>
<b>Total Phenolics</b>		<b>218.36 ± 91.37</b>	<b>225.43 ± 27.37</b>	<b>1.60 ± 1.61</b>	<b>1.76 ± 0.74</b>	<b>289.25 ± 27.53</b>	<b>1.18 ± 1.06</b>	
<b>DMAC (mg/100g)</b>	<b>Total Procyanidins</b>	<b>256.2 ± 5.2</b>	<b>11,891.7 ± 594.7</b>	<b>N/A</b>	<b>189.3 ± 28.8</b>	<b>9,546.5 ± 601.4</b>	<b>9.3 ± 0.0</b>	
Hilic (mg/100g)	Procyanidins	Dimer	4.36 ± 0.30	836.71 ± 449.85	N/A	2.07 ± 0.66	1823.37 ± 36.85	0.09 ± 0.01
		Trimer	4.33 ± 0.48	3690.94 ± 246.63	N/A	6.45 ± 1.80	3092.29 ± 116.95	0.04 ± 0.01
		Tetramer	3.91 ± 1.39	1542.04 ± 315.68	N/A	7.65 ± 2.34	1047.76 ± 6.66	0.02 ± 0.01
		Pentamer	3.61 ± 1.05	1987.24 ± 548.65	N/A	11.09 ± 1.91	1128.00 ± 9.56	0.01 ± 0.01
		Hexamer	5.92 ± 1.41	3146.09 ± 878.62	N/A	30.49 ± 4.55	2114.77 ± 25.53	0.28 ± 0.11
		Heptamer	14.21 ± 2.78	8941.17 ± 4751.20	N/A	50.38 ± 7.63	4499.64 ± 19.96	0.25 ± 0.04
		Octamer	10.43 ± 1.36	12275.08 ± 4721.86	N/A	85.31 ± 18.53	6625.83 ± 1002.37	0.10 ± 0.10
		Nonamer	6.93 ± 4.30	4425.43 ± 1228.82	N/A	30.14 ± 9.79	2818.76 ± 120.97	0.10 ± 0.05
		Decamer	1.70 ± 0.02	860.39 ± 170.02	N/A	6.98 ± 2.83	341.45 ± 103.49	0.29 ± 0.17
			<b>Total Procyanidins</b>	<b>55.39 ± 13.09</b>	<b>37,705.08 ± 13,311.33</b>	<b>N/A</b>	<b>229.55 ± 50.05</b>	<b>22,280.12 ± 1,436.97</b>

<sup>1</sup>Data represents Concord and Niagara grapes cultivated in 2018 from farms in the Grandview (WA, USA) region<sup>2</sup>Data are expressed as mean ± standard deviation from n=3 independent assessments

**Supplemental Table 2.2.** Phenolic content (mg/100g fw) in Concord grapes and juice from the 2018 harvest.<sup>1,2</sup>

Compound Class	Compound	Concord Juice <sup>1</sup>	Concord Grape (Vineyard 1)	Concord Grape (Vineyard 2)	
Anthocyanins	cyanidin	3-arabinoside		0.05 ± 0.01	0.01 ± 0.01
		3-glucoside	0.42 ± 0.07	11.66 ± 1.51	2.84 ± 1.09
		3-(6-p-coumaroly-glucoside)	0.07 ± 0.01	1.56 ± 0.23	1.24 ± 0.35
	peonidin	3-arabinoside		0.02 ± 0.01	
		3-glucoside	0.01 ± 0.01	0.27 ± 0.03	0.04 ± 0.02
		3-(6-p-coumaroly-glucoside)	0.01 ± 0.01	0.11 ± 0.03	0.10 ± 0.07
	delphinidin	3-arabinoside	0.35 ± 0.05	0.72 ± 0.12	0.21 ± 0.14
		3-glucoside	0.97 ± 0.17	11.51 ± 1.90	2.18 ± 0.99
		3-(6-acetyl glucoside)		0.44 ± 0.05	0.28 ± 0.08
	petunidin	3-arabinoside		0.02 ± 0.01	0.01 ± 0.01
		3-glucoside	0.17 ± 0.05	5.95 ± 0.90	2.04 ± 0.96
		3-(6-p-coumaroly-glucoside)	0.02 ± 0.01	0.50 ± 0.09	0.58 ± 0.13
malvidin	3-arabinoside		0.06 ± 0.04	0.07 ± 0.01	
	3-glucoside	0.33 ± 0.03	1.19 ± 0.15	0.61 ± 0.14	
	3-(6-p-coumaroly-glucoside)	0.18 ± 0.02	0.29 ± 0.05	0.28 ± 0.09	
	<b>Total Anthocyanins</b>	<b>2.52 ± 0.40</b>	<b>34.34 ± 5.12</b>	<b>10.48 ± 4.06</b>	
Phenolic Acids	3,4-dihydroxycinnamic acid	0.02 ± 0.01			
	coutaric acid	0.17 ± 0.01	1.28 ± 0.11	1.03 ± 0.07	
	caftaric acid	0.92 ± 0.08	1.06 ± 0.08	0.46 ± 0.08	
	p-coumaric acid	0.43 ± 0.01	0.26 ± 0.01	0.30 ± 0.03	
	gallic acid	0.16 ± 0.01	0.10 ± 0.01	0.20 ± 0.04	
	caffeic acid	0.30 ± 0.02	0.03 ± 0.01	0.03 ± 0.01	
	ferulic acid	0.02 ± 0.01	0.03 ± 0.01	0.03 ± 0.01	
	dihydrocoumaric acid				
	<b>Total Phenolic Acids</b>	<b>2.03 ± 0.13</b>	<b>2.75 ± 0.21</b>	<b>2.04 ± 0.22</b>	
Stilbenoids	resveratrol	0.03 ± 0.01	0.02 ± 0.01		
	resveratrol-3-glucoside	0.05 ± 0.01	0.03 ± 0.01	0.01 ± 0.01	
	<b>Total Stilbenoids</b>	<b>0.08 ± 0.01</b>	<b>0.05 ± 0.01</b>	<b>0.01 ± 0.01</b>	
Flavonols	quercetin		0.01 ± 0.01	0.01 ± 0.001	
	myricetin	0.01 ± 0.01	0.08 ± 0.01	0.03 ± 0.01	
	kaempferol-3-glucoside		0.01 ± 0.01	0.01 ± 0.01	
	quercetin-3-glucoside	0.28 ± 0.02	1.53 ± 0.16	1.24 ± 0.76	
	myricetin-3-glucoside	0.03 ± 0.01	0.12 ± 0.02	0.06 ± 0.04	
	kaempferol-3-rutinoside		0.33 ± 0.03	0.20 ± 0.05	
	quercetin-3-rutinoside	0.09 ± 0.01	4.82 ± 0.57	4.16 ± 0.50	
	<b>Total I</b>	<b>0.43 ± 0.04</b>	<b>6.90 ± 0.79</b>	<b>5.71 ± 1.36</b>	
Flavan-3-ols	catechin	0.30 ± 0.01	1.63 ± 0.35	1.51 ± 0.37	
	epicatechin	0.40 ± 0.09	5.06 ± 0.78	2.15 ± 0.37	
	gallocatechin	0.02 ± 0.01			
	epigallocatechin	0.03 ± 0.01			
	<b>Total Flavan-3-ols</b>	<b>0.75 ± 0.11</b>	<b>6.70 ± 1.13</b>	<b>3.67 ± 1.12</b>	
<b>Total Phenolics</b>		<b>5.81 ± 0.69</b>	<b>50.74 ± 7.6</b>	<b>21.91 ± 6.77</b>	
<b>DMAC (mg/100g)</b>	<b>Total Procyanidins</b>	<b>119.9 ± 3.7</b>	<b>376.0 ± 13.07</b>	<b>605.5 ± 71.2</b>	
Hilic (mg/100g) Procyanidins	Dimer	21.07 ± 5.83	40.11 ± 4.49	50.96 ± 5.54	
	Trimer	29.67 ± 5.21	50.66 ± 1.60	73.10 ± 2.07	
	Tetramer	11.00 ± 0.60	23.12 ± 2.45	31.11 ± 2.85	
	Pentamer	6.00 ± 0.25	31.78 ± 1.04	38.12 ± 3.88	
	Hexamer	15.50 ± 3.03	63.82 ± 4.36	84.38 ± 9.57	
	Heptamer	11.93 ± 1.51	44.49 ± 15.77	100.83 ± 20.64	
	Octamer	25.67 ± 5.43	153.20 ± 51.76	261.40 ± 84.52	
	Nonamer	8.97 ± 0.46	56.58 ± 18.40	88.76 ± 13.26	
	Decamer	1.93 ± 1.93	8.33 ± 3.03	9.87 ± 4.20	
		<b>Total Procyanidins</b>	<b>131.74 ± 24.25</b>	<b>472.09 ± 102.9</b>	<b>738.53 ± 146.53</b>

<sup>1</sup>Data represents Concord juice from grapes cultivated in 2018 from farms in the Grandview (WA, USA) region<sup>2</sup>Data are expressed as mean ± standard deviation from n=3 independent assessments.

**Supplemental Table 2.3.** Phenolic content (mg/100g fw) in Niagara grapes and juice from the 2018 harvest.<sup>1,2</sup>

Compound Class	Compound	Niagara Juice SO <sub>2</sub> <sup>1</sup>	Niagara Juice <sup>1</sup>	Niagara Grape (Vineyard 3)	Niagara Grape (Vineyard 4)
<b>Phenolic Acids</b>	3,4-dihydroxycinnamic acid	0.18 ± 0.09	0.09 ± 0.01		
	coutaric acid	0.07 ± 0.04	0.27 ± 0.02	0.55 ± 0.07	0.51 ± 0.06
	caftaric acid	0.67 ± 0.35	2.11 ± 0.24	0.36 ± 0.03	0.30 ± 0.05
	p-coumaric acid	1.58 ± 0.81	0.67 ± 0.05	0.17 ± 0.01	0.19 ± 0.01
	gallic acid	0.67 ± 0.34	0.84 ± 0.07	0.34 ± 0.14	0.25 ± 0.08
	caffeic acid	3.67 ± 1.84	1.76 ± 0.14	0.02 ± 0.01	0.02 ± 0.01
	ferulic acid	0.14 ± 0.07	0.09 ± 0.01	0.02 ± 0.01	0.02 ± 0.01
	dihydrocoumaric acid				
	<b>Total Phenolic Acids</b>	<b>6.99 ± 3.54</b>	<b>5.83 ± 0.54</b>	<b>1.46 ± 0.25</b>	<b>1.30 ± 0.20</b>
<b>Stilbenoids</b>	resveratrol	0.05 ± 0.01	0.05 ± 0.01		
	resveratrol-3-glucoside	0.06 ± 0.03	0.06 ± 0.01	0.01 ± 0.01	0.01 ± 0.01
	<b>Total Stilbenoids</b>	<b>0.11 ± 0.04</b>	<b>0.11 ± 0.01</b>	<b>0.01 ± 0.01</b>	<b>0.01 ± 0.01</b>
<b>Flavonols</b>	quercetin	0.19 ± 0.10	0.03 ± 0.01	0.01 ± 0.01	0.01 ± 0.01
	myricetin			0.01 ± 0.01	0.01 ± 0.01
	kaempferol-3-glucoside	0.02 ± 0.01	0.01 ± 0.01	0.03 ± 0.01	
	quercetin-3-glucoside	0.43 ± 0.23	0.63 ± 0.04	0.47 ± 0.05	0.12 ± 0.01
	myricetin-3-glucoside				
	kaempferol-3-rutinoside				
	quercetin-3-rutinoside				
	<b>Total I</b>	<b>0.65 ± 0.34</b>	<b>0.68 ± 0.05</b>	<b>0.52 ± 0.05</b>	<b>0.14 ± 0.01</b>
<b>Flavan-3-ols</b>	catechin	0.80 ± 0.40	0.77 ± 0.15	2.25 ± 0.59	1.94 ± 0.58
	epicatechin	2.22 ± 1.14	2.03 ± 0.45	6.50 ± 0.67	1.45 ± 0.45
	galocatechin	0.01 ± 0.01	0.01 ± 0.01	0.02 ± 0.01	0.01 ± 0.01
	epigallocatechin	0.02 ± 0.01	0.02 ± 0.01	0.01 ± 0.01	
	<b>Total Flavan-3-ols</b>	<b>3.05 ± 1.56</b>	<b>2.82 ± 0.61</b>	<b>8.77 ± 1.25</b>	<b>3.40 ± 1.04</b>
<b>Total Phenolics</b>		<b>10.80 ± 5.48</b>	<b>9.44 ± 1.21</b>	<b>10.76 ± 1.56</b>	<b>4.85 ± 1.26</b>
<b>DMAC (mg/100g)</b>	<b>Total Procyanidins</b>	<b>18.4 ± 0.4</b>	<b>13.7 ± 0.5</b>	<b>772.5 ± 67.8</b>	<b>269.1 ± 10.7</b>
<b>Hilic (mg/100g) Procyanidins</b>	Dimer	5.54 ± 1.63	6.41 ± 0.99	59.02 ± 10.97	24.98 ± 8.39
	Trimer	27.81 ± 1.63	20.24 ± 2.76	102.55 ± 19.50	59.77 ± 8.52
	Tetramer	2.30 ± 0.52	2.96 ± 0.35	36.49 ± 5.46	32.46 ± 6.67
	Pentamer	4.53 ± 0.85	2.71 ± 0.34	47.77 ± 7.80	35.43 ± 10.13
	Hexamer	2.50 ± 0.52	1.79 ± 0.36	99.11 ± 21.14	64.64 ± 16.49
	Heptamer	1.39 ± 0.29	1.87 ± 0.28	104.00 ± 36.97	128.30 ± 79.36
	Octamer	12.68 ± 2.74	7.02 ± 1.84	205.20 ± 80.61	127.06 ± 55.00
	Nonamer	2.06 ± 0.89	1.24 ± 0.44	107.27 ± 27.41	65.19 ± 21.39
	Decamer	0.28 ± 0.11	0.26 ± 0.15	14.82 ± 6.71	7.07 ± 1.14
<b>Hilic (mg/100g)</b>	<b>Total Procyanidins</b>	<b>59.09 ± 9.18</b>	<b>44.50 ± 7.51</b>	<b>776.23 ± 216.57</b>	<b>544.90 ± 207.09</b>

<sup>1</sup>Data represents Niagara juice from grapes cultivated in 2018 in the Grandview (MA, USA) region<sup>2</sup>Data are expressed as mean ± standard deviation from n=3 independent assessments.

**Supplemental Table 2.4.** Phenolic content (mg/100g fw) in four commercial table grape varieties.<sup>1,2</sup>

Compound Class	Compound	Thompson Seedless	Black Seedless	Red Seedless	Red Seeded	
Anthocyanins	cyanidin	3-arabinoside				
		3-glucoside		0.06 ± 0.03	2.70 ± 1.44	0.40 ± 0.03
		3-(6-p-coumaroyl-glucoside)		0.01 ± 0.01	0.03 ± 0.01	0.01 ± 0.01
	peonidin	3-arabinoside				
		3-glucoside		0.25 ± 0.06	0.42 ± 0.10	0.37 ± 0.02
		3-(6-p-coumaroyl-glucoside)		0.06 ± 0.03	0.06 ± 0.02	0.09 ± 0.01
	delphinidin	3-arabinoside			0.26 ± 0.01	
		3-glucoside			0.35 ± 0.11	0.13 ± 0.01
		3-(6-acetyl glucoside)				
	petunidin	3-arabinoside				
		3-glucoside		0.94 ± 0.15	0.88 ± 0.91	0.02 ± 0.01
		3-(6-p-coumaroyl-glucoside)		0.05 ± 0.01		
malvidin	3-arabinoside		0.08 ± 0.01			
	3-glucoside		3.73 ± 0.42	1.87 ± 0.50	0.21 ± 0.02	
	3-(6-p-coumaroyl-glucoside)		0.83 ± 0.11	1.37 ± 0.35	0.24 ± 0.02	
	<b>Total Anthocyanins</b>		<b>6.00 ± 0.82</b>	<b>7.97 ± 3.44</b>	<b>1.49 ± 0.12</b>	
Phenolic Acids	3,4-dihydroxycinnamic acid					
	coumaric acid	0.19 ± 0.01	0.20 ± 0.01	0.25 ± 0.02	0.20 ± 0.01	
	caftaric acid	0.01 ± 0.02	0.04 ± 0.03	0.07 ± 0.01	0.03 ± 0.01	
	p-coumaric acid	0.17 ± 0.01	0.14 ± 0.10			
	gallic acid	0.02 ± 0.01	0.03 ± 0.01	0.01 ± 0.01	0.01 ± 0.01	
	caffeic acid	0.02 ± 0.01	0.01 ± 0.01			
	ferulic acid		0.02 ± 0.01	0.03 ± 0.01	0.02 ± 0.01	
	dihydrocoumaric acid					
	<b>Total Phenolic Acids</b>	<b>0.41 ± 0.05</b>	<b>0.44 ± 0.16</b>	<b>0.36 ± 0.05</b>	<b>0.26 ± 0.04</b>	
Stilbenoids	resveratrol	0.02 ± 0.01	0.19 ± 0.02	0.01 ± 0.01	0.03 ± 0.01	
	resveratrol-3-glucoside		0.04 ± 0.01			
	<b>Total Stilbenoids</b>	<b>0.02 ± 0.01</b>	<b>0.23 ± 0.03</b>	<b>0.01 ± 0.01</b>	<b>0.03 ± 0.01</b>	
Flavonols	quercetin	0.01 ± 0.01	0.01 ± 0.01	0.02 ± 0.01	0.01 ± 0.01	
	myricetin		0.02 ± 0.01	0.03 ± 0.01		
	kaempferol-3-glucoside	0.02 ± 0.01		0.11 ± 0.02		
	quercetin-3-glucoside	0.66 ± 0.09	0.37 ± 0.05	2.57 ± 0.41	0.78 ± 0.03	
	myricetin-3-glucoside		0.04 ± 0.01	0.05 ± 0.01	0.04 ± 0.01	
	kaempferol-3-rutinoside			0.03 ± 0.01	0.02 ± 0.01	
	quercetin-3-rutinoside		0.05 ± 0.01	0.05 ± 0.01	0.02 ± 0.01	
	<b>Total I</b>	<b>0.68 ± 0.10</b>	<b>0.50 ± 0.09</b>	<b>2.85 ± 0.47</b>	<b>0.88 ± 0.07</b>	
Flavan-3-ols	catechin	0.12 ± 0.03	0.21 ± 0.04	0.20 ± 0.04	1.26 ± 0.08	
	epicatechin	0.07 ± 0.01	0.13 ± 0.06	0.03 ± 0.01	0.64 ± 0.03	
	galocatechin	0.05 ± 0.01	0.11 ± 0.01			
	epigallocatechin			0.06 ± 0.02	0.03 ± 0.01	
	<b>Total Flavan-3-ols</b>	<b>0.24 ± 0.04</b>	<b>0.44 ± 0.11</b>	<b>0.23 ± 0.07</b>	<b>1.94 ± 0.12</b>	
<b>Total Phenolics</b>		<b>1.35 ± 0.20</b>	<b>7.61 ± 1.21</b>	<b>11.42 ± 4.04</b>	<b>4.60 ± 0.36</b>	
<b>DMAC (mg/100g)</b>	<b>Procyanidins</b>	<b>32.8 ± 4.2</b>	<b>61.6 ± 6.3</b>	<b>60.3 ± 4.2</b>	<b>146.5 ± 21.1</b>	
Hilic (mg/100g)	Procyanidins	Dimer	4.74 ± 0.23	2.71 ± 0.45	2.69 ± 0.59	14.01 ± 5.38
		Trimer	3.63 ± 0.46	3.42 ± 1.16	3.61 ± 1.52	27.56 ± 6.98
		Tetramer	1.70 ± 0.44	1.29 ± 0.35	3.04 ± 1.55	11.23 ± 2.22
		Pentamer	1.50 ± 0.40	1.79 ± 1.07	1.65 ± 0.25	13.81 ± 4.39
		Hexamer	4.01 ± 1.10	2.76 ± 0.61	4.23 ± 1.28	27.44 ± 9.65
		Heptamer	4.57 ± 2.72	5.78 ± 2.85	13.06 ± 10.13	51.41 ± 28.59
		Octamer	5.41 ± 0.81	4.20 ± 2.42	9.35 ± 5.15	58.02 ± 15.19
		Nonamer	1.14 ± 0.53	3.29 ± 1.42	2.19 ± 0.84	34.20 ± 16.70
		Decamer	0.11 ± 0.09	0.44 ± 0.35	1.25 ± 0.86	3.71 ± 1.72
<b>Hilic (mg/100g)</b>	<b>Total Procyanidins</b>	<b>26.70 ± 6.78</b>	<b>25.68 ± 10.68</b>	<b>41.07 ± 22.17</b>	<b>241.39 ± 90.82</b>	

<sup>1</sup>Data represents Table grapes cultivated in 2018 obtained from local markets in Kannapolis, NC.

<sup>2</sup>Data are expressed as mean ± standard deviation from n=3 independent assessments.

**Supplemental Table 2.5.** Bioaccessibility of individual phenolic compounds from Concord grapes and juices from 2018 harvest. <sup>1,2,3</sup>

Compound Class	Compound	Concord Juice	Concord Grape (Vineyard 1)	Concord Grape (Vineyard 2)
cyanidin	3-arabinoside		0.02 ± 0.01 (28-47%)	
	3-glucoside	0.28 ± 0.03 (56-75%)	5.00 ± 0.85 (37-52%)	0.99 ± 0.25 (27-59%)
	3-(6-p-coumaroyl-glucoside)	0.01 ± 0.01 (9-12%)	0.16 ± 0.05 (7-14%)	0.11 ± 0.03 (6-17%)
peonidin	3-arabinoside		0.01 ± 0.01 (25-76%)	
	3-glucoside		0.14 ± 0.02 (41-65%)	0.03 ± 0.03 (26-113%)
	3-(6-p-coumaroyl-glucoside)		0.02 ± 0.01 (10-33%)	0.01 ± 0.01 (7-31%)
Anthocyanins	3-arabinoside	0.24 ± 0.05 (48-86%)	0.02 ± 0.01 (2-4%)	0.02 ± 0.01 (7-11%)
	3-glucoside	1.61 ± 0.15 (125-208%)	0.19 ± 0.26 (0-4%)	0.04 ± 0.01 (1-3%)
	3-(6-acetyl glucoside)		0.07 ± 0.05 (8-31%)	0.04 ± 0.01 (11-25%)
petunidin	3-arabinoside			
	3-glucoside	0.16 ± 0.03 (81-103%)	0.75 ± 0.80 (4-29%)	0.17 ± 0.08 (4-13%)
	3-(6-p-coumaroyl-glucoside)		0.03 ± 0.02 (3-12%)	0.02 ± 0.01 (3-5%)
malvidin	3-arabinoside		0.05 ± 0.04 (88-90%)	0.08 ± 0.05 (41-211%)
	3-glucoside	0.44 ± 0.05 (110-153%)	1.21 ± 0.48 (56-139%)	0.52 ± 0.35 (24-190%)
	3-(6-p-coumaroyl-glucoside)	0.06 ± 0.01 (23-43%)	0.06 ± 0.03 (11-35%)	0.04 ± 0.01 (11-35%)
	<b>Total Anthocyanins</b>	<b>2.81 ± 0.32 (86-135%)</b>	<b>7.74 ± 2.07 (16-33%)</b>	<b>2.07 ± 0.59 (14-39%)</b>
Phenolic Acids	3,4-dihydroxycinnamic acid	0.02 ± 0.01 (58-108%)		
	coumaric acid	0.01 ± 0.01 (4-6%)	0.10 ± 0.01 (7-9%)	0.12 ± 0.03 (9-10%)
	caftaric acid	0.01 ± 0.01 (1-2%)	0.01 ± 0.01 (1%)	0.01 ± 0.01 (2-3%)
	p-coumaric acid	1.13 ± 0.14 (235-293%)	0.56 ± 0.41 (264-364%)	0.07 ± 0.06 (40-70%)
	gallic acid		0.01 ± 0.01 (9-11%)	0.01 ± 0.01 (4-6%)
	caffeic acid	0.35 ± 0.01 (115-121%)	0.34 ± 0.05 (1069-1648%)	0.04 ± 0.01 (145-209%)
	ferulic acid	0.02 ± 0.01 (116-118%)	0.03 ± 0.01 (104-132%)	0.02 ± 0.01 (92-129%)
	dihydrocoumaric acid			
	<b>Total Phenolic Acids</b>	<b>1.54 ± 0.17 (70-85%)</b>	<b>1.33 ± 0.17 (44-52%)</b>	<b>0.27 ± 0.09 (9-17%)</b>
Stilbenoids	resveratrol	0.01 ± 0.01 (22-69%)		
	resveratrol-3-glucoside	0.03 ± 0.01 (56-60%)		
	<b>Total Stilbenoids</b>	<b>0.04 ± 0.01 (44-63%)</b>		
Flavonols	quercetin		0.01 ± 0.01 (47-56%)	0.01 ± 0.01 (45-74%)
	myricetin		0.01 ± 0.01 (11-12%)	
	kaempferol-3-glucoside			0.01 ± 0.01 (43-58%)
	quercetin-3-glucoside	0.14 ± 0.01 (19-23%)	0.33 ± 0.05 (19-28%)	0.19 ± 0.08 (21-49%)
	myricetin-3-glucoside	0.03 ± 0.01 (81-92%)	0.05 ± 0.01 (30-65%)	
	kaempferol-3-rutinoside		0.03 ± 0.01 (5-10%)	
	quercetin-3-rutinoside		0.03 ± 0.03 (0-1%)	
	<b>Total I</b>	<b>0.17 ± 0.01 (36-43%)</b>	<b>0.46 ± 0.11 (6-8%)</b>	<b>0.21 ± 0.09 (2-5%)</b>
Flavan-3-ols	catechin	0.24 ± 0.03 (70-89%)	0.01 ± 0.01 (1-2%)	0.02 ± 0.02 (1-2%)
	epicatechin	0.36 ± 0.04 (79-103%)	0.05 ± 0.04 (1-2%)	0.01 ± 0.01 (1-3%)
	gallocatechin	0.02 ± 0.01 (71-115%)		
	epigallocatechin	0.02 ± 0.01 (44-70%)		
	<b>Total Flavan-3-ols</b>	<b>0.64 ± 0.08 (75-95%)</b>	<b>0.05 ± 0.05 (0-1%)</b>	<b>0.03 ± 0.03 (0-2%)</b>
<b>Total Phenolics</b>		<b>5.20 ± 0.59</b>	<b>9.58 ± 2.40</b>	<b>2.58 ± 0.80</b>

<sup>1</sup>Data are expressed as mean ± standard deviation from n=3 digestion experiments.

<sup>2</sup>Absolute bioaccessibilities are expressed as mg/100g fw

<sup>3</sup>Values in parenthesis indicate the range of relative bioaccessibility (%)

**Supplemental Table 2.6.** Bioaccessibility of individual phenolic compounds from Niagara grapes and juices from 2018 harvest. <sup>1,2,3</sup>

Compound Class	Compound	Niagara Juice SO <sub>2</sub>	Niagara Juice	Niagara Grape (Vineyard 3)	Niagara Grape (Vineyard 4)
Phenolic Acids	3,4-dihydroxycinnamic acid	0.12 ± 0.05 (53-77%)	0.09 ± 0.01 (86-105%)		
	coutaric acid		0.02 ± 0.01 (7-9%)	0.10 ± 0.01 (50-70%)	0.23 ± 0.20 (22-31%)
	caftaric acid		0.03 ± 0.01 (1-2%)	0.01 ± 0.01 (9-14%)	0.04 ± 0.01 (3-6%)
	p-coumaric acid	1.07 ± 0.57 (48-85%)	0.62 ± 0.02 (88-100%)	0.16 ± 0.06 (127-131%)	0.15 ± 0.13 (53-55%)
	gallic acid			0.05 ± 0.01 (4-8%)	0.02 ± 0.01 (3-4%)
	caffeic acid	2.25 ± 0.89 (55-82%)	1.68 ± 0.11 (87-108%)	0.05 ± 0.01 (330-485%)	0.09 ± 0.01 (145-192%)
	ferulic acid	0.10 ± 0.05 (57-84%)	0.09 ± 0.01 (91-104%)	0.03 ± 0.01 (197-265%)	0.05 ± 0.01 (97-121%)
	dihydrocoumaric acid				
<b>Total Phenolic Acids</b>		<b>3.54 ± 1.56 (42-66%)</b>	<b>2.53 ± 0.16 (39-48%)</b>	<b>0.36 ± 0.07 (21-32%)</b>	<b>0.57 ± 0.35 (31-67%)</b>
Stilbenoids	resveratrol	0.01 ± 0.01 (21-45%)	0.01 ± 0.01 (12-42%)		
	resveratrol-3-glucoside	0.02 ± 0.01 (21-59%)	0.04 ± 0.01 (58-71%)		
	<b>Total Stilbenoids</b>	<b>0.03 ± 0.01 (25-53%)</b>	<b>0.05 ± 0.01 (38-52%)</b>		
Flavonols	quercetin	0.01 ± 0.01 (3-8%)		0.01 ± 0.01 (95-135%)	0.01 ± 0.01 (55-61%)
	myricetin				
	kaempferol-3-glucoside	0.01 ± 0.01 (31-64%)	0.01 ± 0.01 (100-121%)	0.01 ± 0.01 (86-299%)	0.05 ± 0.04 (302-925%)
	quercetin-3-glucoside	0.16 ± 0.10 (7-16%)	0.24 ± 0.02 (37-43%)	0.33 ± 0.09 (71-175%)	0.51 ± 0.24 (95-224%)
	myricetin-3-glucoside			0.01 ± 0.01 (138-157%)	
	kaempferol-3-rutinoside				
	quercetin-3-rutinoside				
	<b>Total I</b>	<b>0.18 ± 0.11 (22-34%)</b>	<b>0.25 ± 0.03 (37-43%)</b>	<b>0.40 ± 0.11 (64-104%)</b>	<b>0.57 ± 0.27 (254-585%)</b>
Flavan-3-ols	catechin	0.53 ± 0.24 (58-85%)	0.89 ± 0.09 (87-139%)	0.01 ± 0.01 (0-1%)	0.01 ± 0.01 (0-2%)
	epicatechin	1.34 ± 0.77 (45-72%)	1.35 ± 0.08 (54-84%)	0.03 ± 0.01 (0-1%)	0.02 ± 0.04 (0-1%)
	gallocatechin	0.01 ± 0.01 (62-107%)			
	epigallocatechin	0.03 ± 0.01 (102-159%)	0.03 ± 0.01 (117-167%)		
	<b>Total Flavan-3-ols</b>	<b>1.91 ± 1.03 (48-76%)</b>	<b>2.27 ± 0.18 (63-101%)</b>	<b>0.04 ± 0.02 (0-1%)</b>	<b>0.04 ± 0.06 (0-2%)</b>
<b>Total Phenolics</b>	<b>5.66 ± 2.71</b>	<b>5.10 ± 0.38</b>	<b>0.80 ± 0.20</b>	<b>1.18 ± 0.68</b>	

<sup>1</sup>Data are expressed as mean ± standard deviation from n=3 digestion experiments.

<sup>2</sup>Absolute bioaccessibilities are expressed as mg/100g fw

<sup>3</sup>Values in parenthesis indicate the range of relative bioaccessibility (%)

**Supplemental Table 2.7.** Bioaccessibility of individual phenolic compounds from table grapes. <sup>1,2,3</sup>

Compound Class	Compound	Green Table	Black Table	Red Seedless	Red Seeded	
Anthocyanins	cyanidin	3-arabinoside				
		3-glucoside		0.02 ± 0.01 (22-49%)	1.38 ± 0.41 (38-71%)	0.02 ± 0.01 (3-9%)
		3-(6-p-coumaroly-glucoside)				
	peonidin	3-arabinoside				
		3-glucoside		0.10 ± 0.03 (31-52%)	0.30 ± 0.03 (49-89%)	0.23 ± 0.12 (28-88%)
		3-(6-p-coumaroly-glucoside)		0.01 ± 0.01 (14-17%)		0.01 ± 0.01 (4-16%)
	delphinidin	3-arabinoside				
		3-glucoside				
		3-(6-acetyl glucoside)				
	petunidin	3-arabinoside				
		3-glucoside		0.25 ± 0.17 (7-34%)	0.30 ± 0.21 (25-61%)	
		3-(6-p-coumaroly-glucoside)				
	malvidin	3-arabinoside		0.06 ± 0.01 (72-82%)		
		3-glucoside		3.67 ± 0.45 (98-128%)	1.88 ± 0.20 (79-124%)	0.33 ± 0.14 (87-215%)
		3-(6-p-coumaroly-glucoside)		0.19 ± 0.09 (12-36%)	0.16 ± 0.01 (8-15%)	0.03 ± 0.02 (3-18%)
<b>Total Anthocyanins</b>			<b>4.31 ± 0.76 (63-81%)</b>	<b>4.03 ± 0.87 (56-67%)</b>	<b>0.63 ± 0.31 (2-49%)</b>	
Phenolic Acids	3,4-dihydroxycinnamic acid					
	coumaric acid	0.10 ± 0.01 (51-53%)	0.10 ± 0.01 (47-53%)	0.02 ± 0.02 (1-18%)		
	caftaric acid	0.01 ± 0.01 (39-42%)	0.01 ± 0.01 (14-20%)			
	p-coumaric acid	0.14 ± 0.03 (69-96%)	0.23 ± 0.08 (91-147%)			
	gallic acid	0.01 ± 0.01 (45-60%)	0.01 ± 0.01 (37-47%)			
	caffeic acid	0.01 ± 0.01 (56-96%)	0.21 ± 0.07 (911-1448%)			
	ferulic acid		0.01 ± 0.01 (64-76%)	0.03 ± 0.01 (81-156%)	0.06 ± 0.01 (231-329%)	
	dihydrocoumaric acid					
	<b>Total Phenolic Acids</b>		<b>0.27 ± 0.04 (44-62%)</b>	<b>0.57 ± 0.17 (39-142%)</b>	<b>0.05 ± 0.03 (7-26%)</b>	<b>0.06 ± 0.01 (231-329%)</b>
	Stilbenoids	resveratrol				
resveratrol-3-glucoside			0.02 ± 0.01 (6-15%)			
<b>Total Stilbenoids</b>			<b>0.02 ± 0.01 (6-15%)</b>			
Flavonols	quercetin					
	myricetin		0.01 ± 0.01 (54-66%)	0.01 ± 0.01 (11-27%)		
	kaempferol-3-glucoside	0.01 ± 0.01 (22-172%)		0.09 ± 0.04 (42-114%)	0.01 ± 0.01 (4-138%)	
	quercetin-3-glucoside	0.36 ± 0.29 (25-118%)	0.38 ± 0.19 (62-136%)	0.76 ± 0.22 (21-37%)	0.14 ± 0.07 (13-27%)	
	myricetin-3-glucoside		0.06 ± 0.02 (89-166%)	0.01 ± 0.01 (12-37%)		
	kaempferol-3-rutinoside			0.02 ± 0.01 (34-115%)		
	quercetin-3-rutinoside			0.07 ± 0.03 (92-231%)	0.05 ± 0.03 (112-463%)	
<b>Total I</b>		<b>0.38 ± 0.30 (20-116%)</b>	<b>0.46 ± 0.21 (50-153%)</b>	<b>0.97 ± 0.31 (23-44%)</b>	<b>0.20 ± 0.11 (15-30%)</b>	
Flavan-3-ols	catechin					
	epicatechin	0.01 ± 0.01 (4-54%)			0.02 ± 0.01 (1-2%)	
	gallocatechin	0.01 ± 0.01 (18-20%)	0.04 ± 0.01 (10-67%)	0.01 ± 0.01 (26-30%)	0.03 ± 0.03 (1-11%)	
	epigallocatechin					
	<b>Total Flavan-3-ols</b>		<b>0.02 ± 0.01 (4-35%)</b>	<b>0.05 ± 0.02 (8-33%)</b>	<b>0.01 ± 0.01 (26-30%)</b>	<b>0.05 ± 0.04 (2-4%)</b>
<b>Total Phenolics</b>		<b>0.67 ± 0.35</b>	<b>5.41 ± 1.17</b>	<b>5.06 ± 1.22</b>	<b>0.94 ± 0.47</b>	

<sup>1</sup>Data are expressed as mean ± standard deviation from n=3 digestion experiments.

<sup>2</sup>Absolute bioaccessibilities are expressed as mg/100g fw

<sup>3</sup>Values in parenthesis indicate the range of relative bioaccessibility (%)

**CHAPTER 3: Bioaccessibility, gut microbial metabolism and intestinal transport of phenolics from 100% grape juice and whole grapes are similar in a simulated digestion and fecal fermentation model**

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### 3.1. Introduction

Phenolics represent one of the most commonly consumed classes of plant phytochemicals in the US diet (Manach et al. 2004). Diets rich in phenolics have been linked to the prevention of chronic and degenerative diseases and are often associated with diets rich in fruits, vegetables, whole grains, tea and cocoa products (Manach et al. 2004; Scalbert, Johnson, and Saltmarsh 2005; Chiva-Blanch and Visioli 2012). Of the many diverse sources of phenolics in the US diet, the 100% juices of native American grape varieties, Concord and Niagara (*Vitis labrusca*), are important sources of these bioactives, as combined they represent the third most widely consumed fruit juice in the United States. These grapes and their 100% juices contain a diverse array of phenolic compounds, including simple phenolic acids, stilbenes, and various flavonoids such as flavonols, flavan-3-ols, anthocyanins, and proanthocyanidin oligomers and polymers (Xu et al. 2011; Cantos, Espin, and Tomás-Barberán 2002; Mohamedshah et al. 2020). Distributed primarily in the skin and seeds of fruit, these phenolics are partially extracted through the juicing process, making them available to consumers in both liquid and solid fruit forms (Manach et al. 2004; Mohamedshah et al. 2020). Grapes, and their 100% grape juices in particular, have been well-documented as healthy beverages with the ability to modulate oxidative and inflammatory stress and deliver impacts on vascular function in relation to both cardiovascular and neurocognitive health (Barona et al. 2012; Rasines-Perea and Teissedre 2017; Leifert and Abeywardena 2008; Magrone et al. 2020; Hogan et al. 2010; Natella et al. 2002; Singh et al. 2016; Giacoppo et al. 2015). Clinical evidence specifically supports the role of 100% Concord grape juice in modulating markers of immune function, and neurocognitive and cardiovascular health (Rowe et al. 2011; Lampion et al. 2016; Siasos et al. 2013; Krikorian et al. 2012; Park, Kim, and Kang 2004; Haskell-Ramsay et al. 2017).

Evidence continues to emerge on the health benefits of 100% grape juice, but the broader role of 100% fruit juice in dietary guidance remains controversial. While 100% fruit juices continue to be included in the contribution of fruit servings within the US Dietary Guidelines for Americans (DGA 2020), consumers and certain health professionals remain conflicted in recommending 100% juice due, in part, to a perception that 100% juice products are high in sugar and lower in other nutrients relative to their whole fruit. As such, the potential benefits of 100% fruit juice are confounded by the negative perceptions associated with commercial processing and, the assumption of significant nutrient losses including fiber and vitamin C (Clemens et al. 2015). Despite existing compositional data demonstrating only modest differences in these factors between 100% grape juice and whole fruit (Ho, Ferruzzi, and Wightman 2019), the debate on differences between these product forms continues to drive consumer confusion and, serves to limit the potential public health benefits of broader 100% grape juice consumption.

Direct comparisons between grape fruit and 100% juice, in terms of broader nutritional and phenolic profiles associated with physiological benefits, remain limited. Recently, we reported comparisons in phenolic species between Concord and Niagara grapes and their respective commercially produced 100% juices (Mohamedshah et al. 2020). In general, grapes were found to have higher contents of phenolics including flavan-3-ols, flavonols, and anthocyanins relative to their respective 100% juices, with the notable exception of phenolic acids. Differences were attributed to conditions of juice extraction and, in the case of higher levels of phenolic acids, the use of pectic enzymes and heat that served to liberate these smaller molecular weight phenolics (Mohamedshah et al. 2020). While differences were evident in product content between juice and fruit, upon oral processing (mastication for whole grapes) and *in-vitro* digestion, bioaccessible content was comparable, if not greater, from 100% grape juice than from corresponding fruit

(Mohamedshah et al. 2020). This result was driven by the higher relative bioaccessibility of phenolics from juice compared to whole fruit forms that contains fractions resistant to digestion such as seeds and skins (Manach et al. 2004; Xu et al. 2011). These findings were consistent with observations for phenolics in orange fruit and 100% juice (Aschoff et al. 2015), and suggest that 100% grape juice and whole fruit may be quite similar in their ability to ultimately deliver bioactive phenolics.

However, in the context of broader clinical relevance, differences or similarities observed in the small intestinal bioaccessibility of phenolics only reflect a portion of digestion, making it most relevant to understanding the acute bioavailability of native phenolic species. In recent years, the importance of interactions/metabolism of fruit phenolics with gut microbial communities and resulting impacts to human health effects has become apparent (Parkar, Trower, and Stevenson 2013; Selma, Espin, and Tomas-Barberan 2009; Murota, Nakamura, and Uehara 2018; Kim et al. 1998). Many fruit phenolic species exhibit poor oral bioavailability in the upper gastro-intestinal tract. Food and digestive phase interactions are known to influence the rate of intestinal absorption and, in grapes versus juice, may serve to modify absorption kinetics and potentially host metabolism (Gonzales et al. 2015; Hollander and Ruble Jr 1978; Shimizu 1999). However, the poor bioavailability of many native grape phenolics is balanced by the high circulating and urinary profiles of small molecular weight phenolic metabolites generated by intestinal microbial communities primarily in the lower gastro-intestinal tract (Parkar, Trower, and Stevenson 2013; Selma, Espin, and Tomas-Barberan 2009; Murota, Nakamura, and Uehara 2018). These metabolite “signatures” of fruit and 100% juice consumption are increasingly being investigated as they represent the highest fraction of systemically available metabolite forms, and beyond being an indicator of consumption, may in fact be the main mediators of longer-term health benefits

associated with consumption of fruit products including 100% juice (Selma, Espin, and Tomas-Barberan 2009; Kim et al. 1998; Larrosa et al. 2009; Ward et al. 2004; Lee et al. 2017).

To date, differences in microbial metabolism of phenolics and ultimate bioavailability of microbial metabolites between whole fruit and 100% juice remain relatively unknown. With documented differences in upper intestinal bioaccessibility between whole fruit and 100% grape juice (Mohamedshah et al. 2020), it remains unclear the extent to which these differences impact actual intestinal uptake and/or the generation of microbial metabolite profiles. It is plausible to consider that differences in both type and quantity of phenolics present in the lower GI, as well as the presence of a highly fermentable substrate (i.e., pectin from whole grapes) could alter the response by microbial populations, and result in differences in microbial metabolite profiles between juice and fruit and potentially alter downstream bioactivity. With this in mind, the goal of this study was to directly assess if observed differences in phenolic bioaccessibility between Concord and Niagara fruit and their 100% juices alters both phenolic microbial metabolism and subsequent intestinal transport of native and microbial metabolites using an *in-vitro* digestion model that included an anaerobic fecal fermentation compartment.

## **3.2. Material and Methods**

### **3.2.1. Chemicals, Standards, and Solutions**

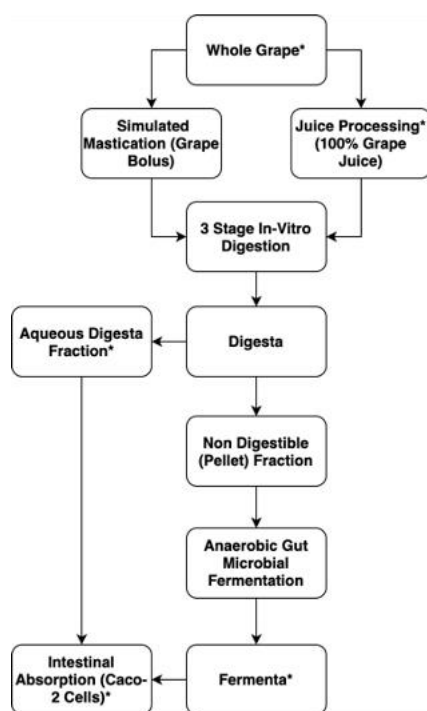
All salts, acids, and other chemicals were purchased from Fischer Scientific (Hampton, NH, USA). Chromatography solvents used (water, acetonitrile, methanol, and formic acid) were ACS certified and LC-MS grade, purchased from ThermoFischer Scientific. Authentic phenolic and metabolite standards including *p*-coumaric acid, gallic acid, caffeic acid, ferulic acid, chlorogenic acid, caftaric acid, coutaric acid, cyanidin-3-*O*-glucoside, peonidin-3-*O*-glucoside,

petunidin-3-*O*-glucoside, delphinidin-3-*O*-glucoside, malvidin-3-*O*-glucoside, (+)-catechin, (-)-epicatechin, epigallocatechin, epicatechin gallate, epigallocatechin, procyanidin B2, resveratrol, quercetin, quercetin-3-*O*-glucoside, quercetin-3-*O*-rutinoside, 5-(3,4-dihydroxyphenyl)- $\gamma$ -valerolactone,  $\gamma$ -valerolactone, phenylacetic acid, 3-hydroxyphenylacetic acid, 3-hydroxybenzoic acid, 4-hydroxybenzoic acid, 3-hydroxyphenylpropionic acid, 4-hydroxyphenylacetic acid, 3,4,-dihydroxybenzoic acid, 4-hydroxyphenylpropionic acid, hippuric acid, 3(3,4-dihydroxyphenyl)propionic acid, and 4-hydroxybenzaldehyde were purchased from Fischer Scientific or Sigma-Aldrich (St. Louis, MO, USA). Pectinases (Pectinexx BEXXL and Pectinexx Ultracolor) used for juicing were provided as a gift by Novozymes (Bagsværd, Denmark). Enzymes used for *in-vitro* digestion and cellular transport, including, mucin (M2378),  $\alpha$ -amylase (A3176), pepsin (P7125), bile (B8631), pancreatin (P7547), lipase (L3126), and bovine serum albumin (A8806) were purchased from Sigma-Aldrich (St. Louis, MO, USA).

### 3.2.2. Experimental Design

This experiment was designed to draw direct comparisons between whole grapes and 100% juice for phenolic bioaccessibility in the upper GI, lower GI and ultimately intestinal transport of bioaccessible phenolics/metabolites in both intestinal compartments (Figure 3.1). Concord and Niagara grapes and corresponding 100% juices made from the same lot of fruit were subjected to a three stage *in-vitro* digestion model including oral (with simulated mastication for fruit), gastric, and small intestinal phases. A portion of the resulting crude digesta fraction was centrifuged, resulting in an aqueous (AQ) bioaccessible fraction and pellet (insoluble) fraction. The AQ fraction was analyzed for bioaccessible phenolic species and screened for Caco-2 transport to compare grapes and juice regarding phenolic transport in the small intestine. Resulting crude digesta

(uncentrifuged digesta fraction) and pellet fractions (combined at a 20:80 ratio) were used as a substrate for an *in-vitro* anaerobic gut fermentation over 48 hours using human fecal inoculate to determine phenolic microbial metabolism and bioaccessibility in the lower GI tract. Finally, bioaccessible fractions from 12-hour fermenta were screened for Caco-2 transport to gain an understanding of potential differences between juice and fruit matrices in phenolic and metabolite lower intestinal flux.



**Figure 3.1. Experimental Design.** Overall flow of studies conducted, including coupled 3 stage *in-vitro* digestion and anaerobic gut microbial fermentation. The presence of \* indicates fractions and studies where phenolic and metabolic species were quantified by LC-MS. Masticated whole grapes (grape bolus) was compared with 100% grape juice for both Concord and Niagara grape varieties throughout all performed experiments. For the anaerobic fermentation experiments, an 80:20 pellet fraction to digesta fraction treatment was used.

### 3.2.3. Grape Fruit and Juicing Process

Freshly harvested Concord (*V. labrusca*) and Niagara (*V. labrusca*) grapes were generously provided by Welch Foods Inc. (Concord, MA, USA). Grapes were handpicked in September 2020 and stored at  $-23^{\circ}\text{C}$  prior to shipment to the Plants for Human Health Institute (Kannapolis, NC,

USA). Grapes were stored at  $-40^{\circ}\text{C}$  until further processing and analysis. In an effort to more accurately compare grapes with their respective juices, Concord and Niagara grapes were processed in a manner simulating commercial 100% grape juice production conditions (Supplemental Figure 3.1). Roughly 2kg of whole grapes were destemmed and crushed manually using a stainless-steel hand masher. Grape mash was then subjected to heat ( $55^{\circ}\text{C}$ ) and pectinase (Pectinexx Ultracolor –  $80\ \mu\text{L}/\text{kg}$ ) treatment for 60 minutes. Juice was then extracted from the hot grape mash using a Breville Juice Fountain Plus (Sydney, Australia). Free run and extracted grape juices were coarse filtered through muslin. Coarse filtered grape juice was then subjected to another pectinase (Pectinexx BEXXL –  $50\ \mu\text{L}/\text{L}$ ) treatment for 30 minutes at room temperature ( $\sim 21^{\circ}\text{C}$ ) to facilitate clarification and stabilization. Following enzymatic treatment, grape juice was filtered again using paper filters (Cytiva Whatman Grade 589/3 Quantitative Filter Paper Circles) and food-grade diatomaceous earth. Freshly filtered juice was then filled into glass bottles and pasteurized ( $85^{\circ}\text{C}$  for 2 min). Following pasteurization, bottles were cooled and stored at  $4^{\circ}\text{C}$  for a minimum of two weeks to allow for further clarification and tartaric acid stabilization before analysis. The final grape juice products from each type of grape had similar color and sugar content ( $\sim 16^{\circ}$  brix) as single strength, commercially produced 100% Concord or Niagara juice, respectively. Juicing of Niagara grapes yielded 0.62 grams of juice per gram of fresh grape, while juicing of Concord grapes yielded 0.57 grams of juice per gram of fresh grape.

It is important to note that for subsequent experiments equivalent masses of grape products (i.e. 2.5 grams of juice and whole grapes) were used, particularly for *in-vitro* digestion. Other sampling methods were considered including sampling based off of juicing yield (i.e. 0.62 grams of juice per gram of grape for Niagara, 0.57 grams of juice per gram of grape for Concord) or sampling based off of equivalent molar amounts of phenolics between juice and grapes.

Ultimately, using equivalent masses of grape products was chosen as this matches our previous study (Mohamedshah et al. 2020), and allows for easier comparisons of phenolic content, bioaccessibility, and overall transport per 100 grams between grape products that can also be easily extended to a per serving level.

#### 3.2.4. Simulated Upper GI digestion: Three Stage Oral, Gastric, and Small Intestinal Digestion.

A three stage *in vitro* digestion model as described by Mohamedshah et al (Mohamedshah et al. 2020) was used to simulated digestive breakdown and measure upper GI bioaccessibility of phenolics from whole grapes and 100% juices. Oral processing (mastication) of grapes was accomplished by three passes through a meat tenderizer (Weston Heavy Duty Meat Tenderizer, Southern Pines, NC, USA) and processing with a food hammer (10 strikes) to produce a crude grape bolus. ~2.5g of grape oral bolus or 100% juice was then introduced to the three-stage *in vitro* digestion. Following completion of the small intestinal phase, aliquots of crude digesta for each sample were centrifuged (10,000x g, 4°C) for 1 hour to isolate the aqueous (bioaccessible) fractions and pellet (non-digestible) fractions. Aqueous fractions were filtered using 0.20 µm PTFE filters to remove aggregates and crude, aqueous, and pellet fractions were aliquoted, nitrogen blanketed, and stored at -80°C for further anerobic fermentation and analysis.

#### 3.2.5. Simulated Lower GI digestion: Anaerobic Ex-vivo Fermentation Model

*Anaerobic Chamber Conditions.* To simulate the anaerobic conditions of the large intestine, a controlled atmosphere chamber (855-ACB, Plas-Labs, Lansing, MI, USA) was utilized. Anaerobic conditions were maintained using mixed gas atmosphere (5% CO<sub>2</sub>, 5% H<sub>2</sub>, 90% N<sub>2</sub>) with O<sub>2</sub> and H<sub>2</sub> levels monitored using a CAM-12 Anaerobic Monitor (Coy Laboratory Products, Grass Lake,

MI, USA). A palladium catalyst was used to scavenge residual O<sub>2</sub> and heat the chamber. Anaerobic conditions were as follows: O<sub>2</sub>: 0-50ppm, H<sub>2</sub>: 2.5-5%, Humidity: 45-60%, Temperature: ~37°C. All equipment used within the chamber were sanitized with 70% EtOH. Solutions and solvents used were either sterile-filtered or autoclaved to maintain sterile conditions within the chamber.

*Fecal Slurry, Medium Preparation, and Sample Preparation.* Aliquots of fecal material (Fecal Microbiota Preparation for Research, FMP-R) were sourced from two healthy donors provided by OpenBiome (Cambridge, MA, USA). Donors are screened through a 200-point clinical assessment followed by further testing of stool for infectious agents including viral, parasitic, and bacterial pathogens. Fecal slurry was prepared by thawing 1mL fecal aliquots from two different donors within the anaerobic chamber. Thawed fecal matter was pooled and diluted (1:10) with sterile, anaerobic phosphate-buffered saline (Vollmer et al. 2017). The resulting fecal slurry was used as inoculum for *in-vitro* gut fermentation experiments. Fermentation medium to allow for growth and proliferation of a wide variety bacteria was adapted from previously described methods (Rodríguez-Costa et al. 2018; Vollmer et al. 2017; Alqurashi et al. 2017). One day prior to fermentations, in 250mL of distilled water, a solution of peptone water (2g), yeast extract (2g), NaCl (0.1g), K<sub>2</sub>HPO<sub>4</sub> (40mg), MgSO<sub>4</sub>•7H<sub>2</sub>O (10mg), Na<sub>2</sub>HPO<sub>4</sub> (40mg), NaHCO<sub>2</sub> (2g), CaCl<sub>2</sub>•6H<sub>2</sub>O (10mg), Tween 80 (2mL), Haemin (50mg), Vitamin K1 (10µL), and bile salts (0.5g) were prepared. Another solution of resazurin (4mL) and L-cysteine (0.5g) in 250mL of DI water was prepared. Both solutions were pH buffered to 6.8 ± 0.1, brought to a volume of 500mL with DI water, and sterile filtered. The resazurin solution was boiled until colorless, and both solutions were sparged overnight with nitrogen gas. On the day of fermentation, both solutions were mixed within the chamber to generate the final fermentation medium used.

Fecal fermentations were carried out using an 80:20 (insoluble pellet: crude intestinal digesta) mixture was prepared from each Concord and Niagara grape/juice digested sample. Briefly, 40mL of crude intestinal digesta was centrifuged (as described earlier) and the pellet (insoluble portion) was isolated. The pellet was reconstituted to 10mL with sterile PBS (thus, 10mL of pellet mixture equates to the insoluble portion of 40mL of crude digesta). For each Concord and Niagara grape/juice fermentation, 4mL of the respective pellet mixture (representing the insoluble fraction from 16 mL crude digesta) and 4mL of crude digesta were combined to maintain the desired 80:20 ratio of insoluble pellet: crude digesta. Along with samples from Concord and Niagara grape/juice digestions, background controls (fecal inoculum with no grape/juice), fecal-free negative controls (grape/juice treatments, with no fecal inoculum), and positive fermentation controls (1g inulin/reaction) were included. For each fermentation experiment, 0.84mL of fecal slurry ( i.e. a 1:50 fecal slurry dilution) was added and brought to a final volume of 42mL with fermentation media. Fermentations were performed over a period of 48 hours, with fermenta aliquots collected at 0, 6, 12, 24, and 48 hours. Fermenta aliquots were immediately centrifuged (10,000x g, 4°C) for 75 minutes, to mimic centrifugation step during *in-vitro* digestion, supernatant was filtered with 0.45 µm PTFE filters, capped under nitrogen, and stored at -80°C for future analysis. Samples were also weighed at these time points to account for changes in concentration due to evaporative loss. pH of each sample was monitored every 4.5 hours using a Metrohm 855 Robotic Titrosampler (Herisau, Switzerland) (Supplemental Figure 3.2).

### 3.2.6. Cell Culture and Treatments

Intestinal transport of bioaccessible (soluble) phenolics/metabolites isolated from intestinal digesta and fermenta of grapes and 100% juice were studied using the Caco-2 (TC7) cell model (passages 81-84) as described by Redan et al (Redan et al. 2017) with modification for media containing bioaccessible fermenta fractions. Caco-2 cells were maintained in DMEM media with 10% v/v fetal bovine serum, 1% v/v nonessential vitamins, 1% v/v HEPES, 1% v/v streptomycin/penicillin, 0.1% v/v gentamicin. Cells were seeded at a density of  $1.25 \times 10^5$  cells per well on Transwell inserts (Corning polyester membrane, 0.4  $\mu\text{m}$  pore size, 24 mm diameter) and allowed to differentiate for 21-25 days post-confluency at 37°C under CO<sub>2</sub>/air (5:95) atmosphere at constant humidity.

Prior to treatment, Caco-2 cell monolayers were cultured with fresh growth media for 24 hours. Integrity of the cell monolayers was confirmed by determining transepithelial electrical resistance (180-220  $\Omega \text{ cm}^2$ ) values using a voltameter, prior to treatment and assaying of phenolic transport. Monolayers were rinsed with 0.1% fatty-acid free albumin in PBS, followed by two rinses of PBS (pH = 5.5). 2mL of PBS (pH = 5.5) was added to the basolateral chamber. 1.5 mL of Concord/Niagara grape or juice aqueous digesta (1:3 dilution with sterile PBS, pH = 5.5) or 12-hour fermenta sample (1:2 dilution with sterile PBS, pH = 5.5) was transferred to the apical chamber. Preliminary experiments suggested that acute treatment of cells with sterile filtered Concord/Niagara juice or grape aqueous fraction (1:3 dilution with sterile PBS, pH = 5.5) or bioaccessible fermenta fractions (1:2 dilution with sterile PBS, pH = 5.5) did not significantly decrease cell viability (>95%) by MTT assay (Biotium, Hayward, CA, USA) (data not shown). To monitor transepithelial transport, 1mL of basolateral media (PBS, pH = 5.5) was collected and replaced with fresh PBS (pH = 5.5) at 5, 30, 60, 90, and 120 min. One technical replicate was

performed for four biological replicates for each aqueous digesta and 12-hour fermenta for both grapes and juices. Following the 2-hour uptake period, cells were rinsed as described above, and then collected in chilled PBS (pH = 5.5). Protein levels were determined using the bicinchoninic acid method (Pierce BCA Protein Assay Kit, Thermo Fischer Scientific, Waltham, MA, USA).

### 3.2.7. Phenolic Extraction

Phenolic extraction methodologies were adapted from Moser et al (Moser et al. 2016) and Mengist et al (Mengist et al. 2020) with minor adjustments. Briefly, Concord and Niagara grapes were thawed and homogenized (VWR 250 Homogenizer, 10032-766, Radnor, PA, USA) for 30 seconds at 10,000 rpm. Phenolics were extracted from an aliquot (~0.25) using 5mL of methanol, water, and formic acid (80:18:2) by sonication (20 minutes) and vortexing (2 minutes) followed by centrifugation (4,000 x g, 5 min). The extraction was repeated twice more with 5 mL of formic acid in methanol (2:98) for the residual solids. Extracts were combined, dried under nitrogen gas and resolubilized in 0.1% formic acid in water for solid phase extraction (Oasis HLB 1cc (30mg) extraction cartridges). Cartridges were activated with sequential passes of acidified methanol (1.0% formic acid) followed by acidified water (1.0% formic acid). Following activation of the cartridges, samples were loaded, rinsed with acidified water (0.1% formic acid), and phenolic compounds were eluted with 3mL of 0.1% formic acid in methanol. Eluates were dried under nitrogen and stored at -80°C until analysis. Extraction of 100% juice, aqueous digesta fractions, fermenta samples, and cell culture media was completed by SPE as described above.

### 3.2.8. Phenolic and Metabolite Analysis by UPLC-MS/MS

Dried extracts were reconstituted in methanol, water, formic acid (50:49.9:0.1), filtered with 0.45  $\mu\text{m}$  PTFE filters, and analyzed by UPLC-MS/MS. Phenolic compounds and metabolites were resolved with an Acquity UPLC BEH C18 1.7 $\mu\text{m}$  (2.1 x 50mm) column using a Waters Acquity I Class UPLC equipped with a XEVO TQD mass spectrometer (Waters, Milford, MA, USA) as previously described by Mohamedshah et al. (Mohamedshah et al. 2020). Separations were achieved at a flow rate of 0.5 mL/min using a gradient elution based on a binary phase of acidified water (0.1% formic acid, solvent A) and acidified acetonitrile (0.1% formic acid, solvent B). Separations were achieved at 40°C with the following gradient: 0-0.5 min 100-94% A, 0.5-2.0 min 94-91% A, 2-3 min 91-87% A, 3-4/5 min 87-65% A, 4.5-5.5 min 65-100% A, 5.5-6 min 100% A. Phenolic acids, flavan-3-ols, flavonols, stilbenes, and small molecule polar metabolites were detected under negative mode electrospray ionization (ESI<sup>-</sup>). Anthocyanins, 5-(3,4-dihydroxyphenyl)- $\gamma$ -valerolactone, and  $\gamma$ -valerolactone were detected under ESI<sup>+</sup> mode, with solvent A being adjusted to 2.0% formic acid in water for elution.

Single ion responses (SIR) and Multiple Reaction Monitoring (MRM) were used to identify and quantify over 50 individual phenolic and metabolite species (Supplemental Table 3.1). Flavonoids, phenolic acids, stilbenes, and small polar metabolites were quantified using multi-leveled response curves constructed with authentic standards for each compound or a structurally similar compound. SIRs for the various anthocyanins (cyanidin, delphinidin, peonidin, petunidin, and malvidin) were used to tentatively identify acylated forms of these anthocyanins for which standards were not available. Acylated forms were summed together for a specific anthocyanin to provide semi-quantitative data for each class of anthocyanin.

### 3.2.9. Statistical Analyses

All data are presented as a mean  $\pm$  SD from quadruple replicates. For the purpose of this study, individual “native” phenolic species (phenolic acids, anthocyanins, flavan-3-ols, flavanols, and stilbenes) and metabolite species (phenylacetic acids, phenylpropionic acids, hydroxybenzaldehydes, and benzoic acids) were summed by compound class. Data for individual phenolic species/metabolites can be found in Supplemental Tables 2-6. GraphPad Prism 9 (San Diego, CA, USA) software was used for statistical analysis and visualization of the data. Unpaired t-test analysis was utilized to determine significant differences ( $P < 0.05$ ) between grapes and 100% grape juices. Comparisons were made between the content, absolute bioaccessible fraction, cumulative aqueous cell transport, anaerobic gut fermenta fraction, and cumulative fermenta cell transport of phenolic and metabolites species between Concord grapes and Concord juice and between Niagara grapes and Niagara juice. These comparisons were made to directly compare the phenolics from 100% grape juices with their respective juicing grapes throughout the *in-vitro* digestion and anaerobic fermentation models and subsequent cell transport studies. Relative bioaccessibility was calculated as the ratio of bioaccessible content for a compound to the total content for that compound in the starting material sample (grape or juice) expressed as a percentage. Cell transport efficiency was calculated as the ratio of cumulative basolateral transported content for a compound to the total apical content from the treatment (aqueous or fermenta) expressed as a percentage.

Percent Relative Bioaccessibility = (bioaccessible or absolute content  $\div$  total content) x 100

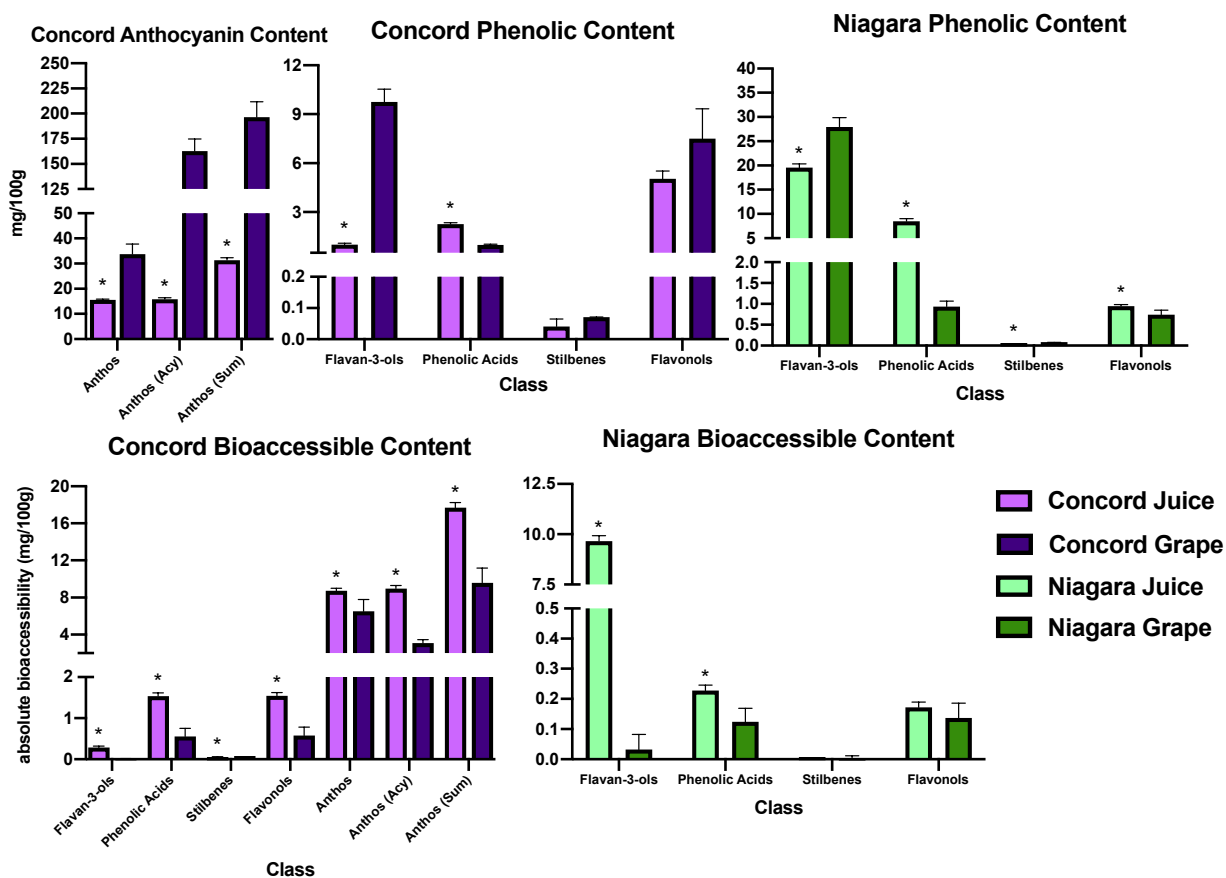
Percent Transport Efficiency = (cumulative basolateral content  $\div$  total apical content) x 100

### 3.3. Results and Discussion

#### 3.3.1. Relative and Absolute Bioaccessibility of Phenolics from 100% Grape Juice are reflective of whole grape profiles

Concord and Niagara grapes had a greater total phenolic content, summed from individual species quantified through LC-MS, (214.84 mg/100g and 29.76 mg/100g, respectively) than their respective 100% juices (39.78 mg/100g for Concord and 29.100 mg/100g for Niagara). Phenolic species quantified include flavan-3-ols, stilbenes, flavonols, and phenolic acids in both Concord and Niagara grapes/juices with anthocyanins only being detected at quantifiable levels in Concord grapes/juice (Figure 3.2, Supplemental Table 3.2). Predominant anthocyanins include cyanidin, peonidin, and delphinidin derivatives, with the majority of anthocyanin content being attributed to acylated forms (162.72 mg/100g in Concord grapes, 15.82 mg/100g in Concord juice; Figure 3.2). Total content of anthocyanins, acylated anthocyanins, and anthocyanin-glycosides were significantly greater ( $P < 0.05$ ) in Concord grapes than in Concord juice (Figure 3.2). Total flavan-3-ol content, including catechin and epicatechin, was significantly greater ( $P < 0.05$ ) in Concord (9.75 mg/100g) and Niagara (27.96mg/100g) grapes than their respective juices (1.00 mg/100g for Concord, 19.56 mg/100g for Niagara). Similarly, flavonol content, primarily quercetin derivatives, did not significantly differ between Concord (7.5 mg/100g) grapes and juice (5.04 mg/100g), but were significantly lower in Niagara grapes (0.75 mg/100g) compared to Niagara juice (0.95 mg/100g) (Figure 3.2). Stilbenes, including resveratrol and resveratrol-3-*O*-glucoside, were found in low quantities (0.4 – 0.7 mg/100g) in Concord and Niagara grapes/juices (Figure 3.2). Finally, phenolic acids, primarily hydroxycinnamic acids and their tartaric acid esters, were greater in Concord (2.28 mg/100g) and Niagara (8.54 mg/100g) juices than their respective Concord (1.03 mg/100g) and Niagara (0.98 mg/100g) grapes (Figure 3.2). These results are in agreement with

previously published reports, (Mohamedshah et al. 2020; Stalmach et al. 2011) with most phenolic classes (anthocyanins, flavan-3-ols, and flavanols) having a greater content in grapes than their respective 100% juices. This is likely a result of incomplete extraction of phenolics from the grape skins and seeds and some potential losses through mechanical and thermal treatment/processing of juice (Turfan et al. 2011; Volf et al. 2014). The higher apparent phenolic acid content in juices relative to fruit is also consistent with previous observations (Mohamedshah et al. 2020) and is attributed in large part to the enzymatic and thermal release of phenolic acids from their association with complex polysaccharides by virtue of pectinase treatments (Palafox-Carlos, Ayala-Zavala, and González-Aguilar 2011).

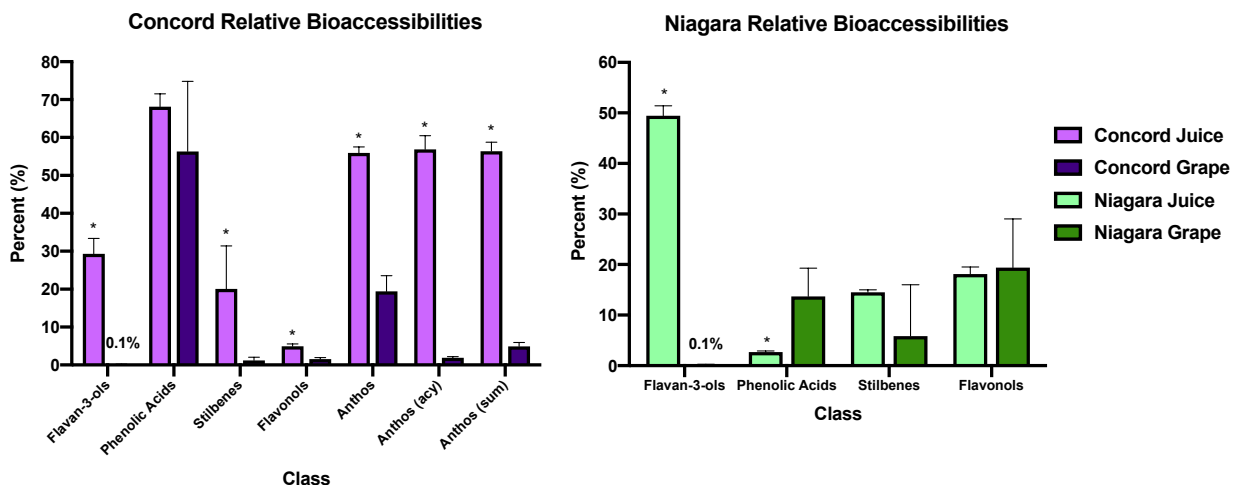


**Figure 3.2. Comparison of total and bioaccessible content of phenolics between whole juicing grapes and 100% grape juice.** Top panels depict starting contents of phenolics by class in Concord grapes, 100% Concord juice, Niagara grapes, and 100% Niagara juice. Bottom panels present bioaccessible phenolic content by class for Concord grapes, 100% Concord juice, Niagara grapes, and 100% Niagara juice. Data are expressed as mg/100g for total content or bioaccessible content for a sum of individual species by compound class as determined by LC-MS/MS. All data are presented as a mean  $\pm$  SD ( $n = 4$ , biological replicates). Presence of an \* indicates significant difference ( $P < 0.05$ ) by un-paired t-test analysis within individual phenolic class (anthocyanins, flavan-3-ols, phenolic acids, stilbenes, and I) levels between juice and their respective grapes.

Concord and Niagara grapes and their 100% juices were subjected to a three-stage *in-vitro* digestion model to estimate the upper (small) intestinal bioaccessibilities of phenolic species. Consistent with our previous report, (Mohamedshah et al. 2020) the relative (%) bioaccessibility of phenolics was greater, in most cases, from juice compared to their respective grapes (Figure 3.3). For example, primarily seed-derived flavonoids (flavan-3-ols) had a significantly higher percent bioaccessibility in Concord (29%) and Niagara (50%) juices than either Concord (0.1%) or Niagara (0.1%) grapes (Figure 3.3). Similarly, relative bioaccessibility of total anthocyanins

was significantly greater in Concord juice (56%) than Concord grapes (5%) (Figure 3.3). This further supports the notion that juice processing is able to extract phenolic species from hard to digest grape fractions such as seeds (flavan-3-ols) and skins (anthocyanins) and provides an aqueous food matrix that increases the overall availability of phenolics in the small intestine. The total bioaccessible phenolic content, summed from individual compounds by LC-MS, of juices (37.52 mg/100g for Concord, 19.87 mg/100g for Niagara) was significantly ( $P < 0.05$ ) greater than that of grapes (10.07 mg/100g for Concord, 0.30 mg/100g for Niagara), exhibiting broader differences in bioaccessible content than was previously observed between grapes and juices for both Concord and Niagara varieties (Mohamedshah et al. 2020). While the bioaccessible content for whole fruits are comparable, here higher total bioaccessible phenolic contents from juice were observed compared to a previous report (5.2 mg/100g for Concord, 5.09-5.66mg/100g for Niagara; summed from individual compounds) (Mohamedshah et al. 2020). One possible reason for this observation is that more phenolic species were assessed in the present study, including acylated anthocyanin forms and procyanidin B2, which likely account for some of the increase in overall bioaccessible phenolic content driven from seeds and skins. Furthermore, the differences observed may be a result of the matched source of grapes and 100% juice itself. Previously, (Mohamedshah et al. 2020) the 100% grape juice was sourced from a commercial juice processing facility in which the grapes are sourced from a number of vineyards and therefore the final juice may not have reflected the grape sampling. The present study utilized grapes from a single vineyard and juice made from an aliquot of those grapes. While still having natural variation, this matching of raw material and processed product provides a better direct comparison. Differences between observed levels in the present study and the previous study were also expected due to typical seasonal variation affecting phenolic content in grapes and, by extension, their 100% juices. By example, a

total phenolic content of 29.09 mg/100g for 100% Niagara juice was observed compared to 9.44 – 10.80mg/100g for 100% Niagara juice from previous assessments in 2017/2018 (Mohamedshah et al. 2020). Despite these differences, these results demonstrate that absolute bioaccessible contents of flavan-3-ols, phenolic acids, anthocyanins, and flavanols (Figure 3.2) in juices are at least comparable, if not significantly greater, than from whole grapes. These data reinforce the notion that processing of grapes to juice does not likely impact overall available levels of bioactive phenolics in the gut and, juice likely provides a matrix with an increased proportion of bioaccessible phenolics available in the small intestine.

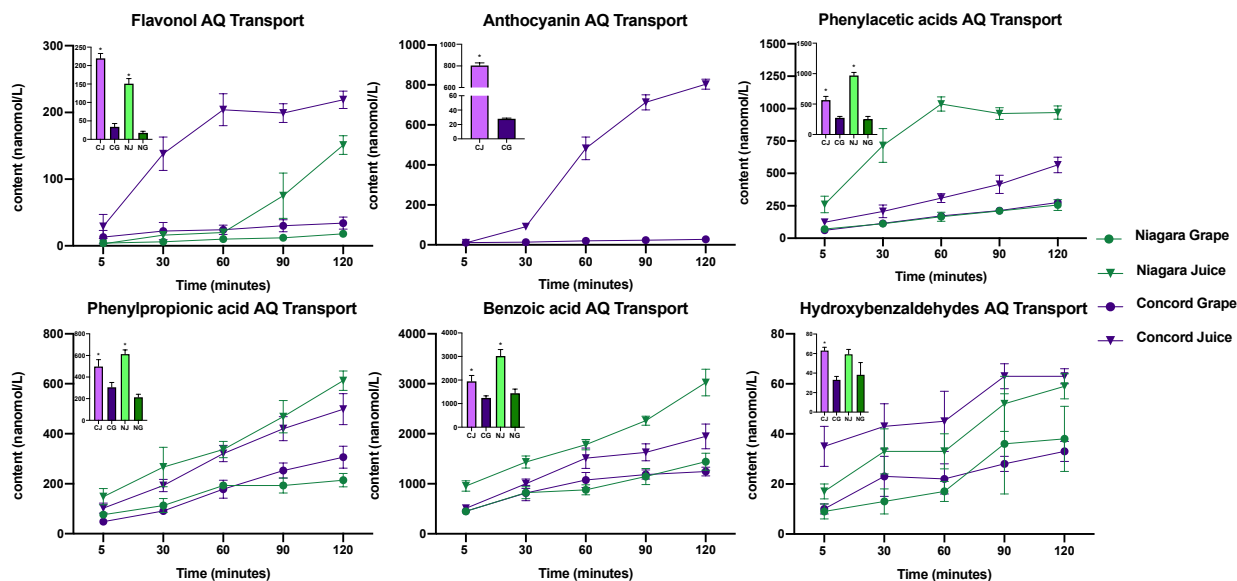


**Figure 3.3. Relative bioaccessibilities of phenolics between whole juicing grapes and 100% grape juice.** Relative bioaccessibility was calculated as the ratio of bioaccessible content (following a 3-stage *in-vitro* digestion) for a compound class to the total content for that class in the starting material sample (grape or juice) expressed as a percentage. Flavan-3-ol relative bioaccessibility was very low in both Concord and Niagara grapes at 0.1%. All data are presented as a mean  $\pm$  SD ( $n = 4$ , biological replicates). Presence of an \* indicates significant difference ( $P < 0.05$ ) by un-paired t-test analysis within individual phenolic classes (anthocyanins, flavan-3-ols, phenolic acids, stilbenes, and flavanols) between juice and their respective grapes.

### 3.3.2. Transport of Aqueous Phenolic Species differs between whole grapes and juices

Following digestion, the extent to which intestinal transport of bioaccessible phenolics might differ between grapes and 100% juice was assessed using a three compartment Caco-2 human intestinal cell model. Cumulative apical to basolateral transport from cell monolayers of

native compounds and select phase II metabolites generated from incubation of aqueous fraction (AQ) derived from Concord and Niagara juice/grapes digesta are shown in Figure 3.4. The majority of native phenolic compounds transported include flavonols (quercetin-3-glucoside) and anthocyanins (including cyanidin-3-*O*-glucoside and delphinidin-3-*O*-glucoside). Individual phenolic acids were also observed transported across cell monolayers including phenylacetic acids (primarily phenylacetic acid), phenylpropionic acids (including 3-hydroxyphenyl propionic acid and 3-(4-dihydroxy)phenylpropionic acid), and benzoic acids (mono, di, and tri-hydroxybenzoic acids). Hydroxybenzaldehyde flux was also observed. After 2 hours, phenolic transport from Concord grape AQ was significantly ( $P < 0.05$ ) lower compared to phenolics from Concord juice AQ. Specifically, 6.4x less flavonols, 2.9x less anthocyanins, 3.2x less phenylacetic acids, 1.6x less phenylpropionic acids, 1.5x less benzoic acids, and 1.9x less benzaldehydes. Similarly, following 2 hours, species from Niagara grape AQ demonstrated significantly ( $P < 0.05$ ) reduced transport compared to those from Niagara juice AQ particularly for flavonols (8.4x lower), phenylacetic acids (4.2x lower), phenylpropionic acids (2.9x lower), benzoic acids (2.1x lower), and, while not statistically significant, benzaldehydes (1.6x lower).



**Figure 3.4. Cumulative phenolic apical to basolateral transport of aqueous digesta fractions of Concord and Niagara grapes and 100% grape juice.** Individual panels present cumulative phenolic transport of aqueous digesta fractions over 2 hours across differentiated Caco-2 cell monolayers. Data are expressed as nanomols of compound transported in 1L of basolateral media. Phenolic compounds and metabolites were summed together based on compound class (flavonols, anthocyanins, phenylacetic acids, phenylpropionic acids, benzoic acids, and hydroxybenzaldehydes). Inlaid graphs in each panel show cumulative 2-hour transport across cells for Concord juice (CJ), Concord grape (CG), Niagara juice (NJ), and Niagara grape (NG). All data are presented as a mean  $\pm$  SD ( $n = 4$ , biological replicates). Presence of an \* indicates significant difference ( $P < 0.05$ ) by unpaired t-test analysis within cumulative 2hr transport for individual phenolic class levels between juice and their respective grapes.

Despite differences in relative bioaccessibilities (Figure 3.3) the overall content of bioaccessible flavonols remains comparable between grapes (2.4  $\mu\text{mol/L}$  for Concord, 2.9  $\mu\text{mol/L}$  Niagara) and juice (5.1  $\mu\text{mol/L}$  for Concord, 3.6  $\mu\text{mol/L}$  for Niagara). Similarly, total bioaccessible anthocyanin content between Concord grapes (188.8  $\mu\text{mol/L}$ ) and juice (338.3  $\mu\text{mol/L}$ ) remain relatively comparable. This indicates that initial treatment amounts of AQ flavonoids of grapes and juice for Caco-2 cell monolayers were relatively similar. However, the apparent efficiency of transport across Caco-2 monolayers was generally higher from digested juices than from digested whole grapes. Flavonol transport efficiency from grape digesta (2% Niagara, 4% Concord) was nearly 10-fold lower than from juice digesta (12% Niagara, 13% Concord), with similar trends for anthocyanins from Concord grapes (0.1%) and Concord juice

(1%). These observed differences must relate to the matrix of the food and the corresponding digest itself. Fibers and polysaccharides potentially entrap and physically reduce the availability of flavonoid and phenolic species, and these interactions may have survived digestion in some soluble or dispersed form (Palafox-Carlos, Ayala-Zavala, and González-Aguilar 2011) 100% grape juice has low fiber content (Ho, Ferruzzi, and Wightman 2019) due to extensive mechanical and enzymatic (pectinase treatments) processing and as such has less potential for such interactions.

In general, flavonoid species (particularly flavan-3-ols) are well known to be poorly absorbed in the small intestine, and the current results are in agreement with this notion (Wang et al. 2013; Ward et al. 2004). Phase II metabolites (including methylated, glucuronidated, and sulfonated conjugates) of flavan-3-ols, catechin and epicatechin, were anticipated to be transported across cell monolayers consistent with previous reports, (Chen et al. 2017; 2015) though none were observed above the LOD of 3.4nM. Delphinidin-3-glucuronide (Supplemental Table 3.4) was observed but only in low levels. This low level of Phase II conjugation by Caco-2 in a three compartment model is consistent with some previous reports (Redan, Chegeni, and Ferruzzi 2017) and may be due to the form in which phenolics were delivered as foods compared to previous studies using concentrated extracts high in flavan-3-ols monomers and polymers (Chen et al. 2017; 2015).

Though typically characterized as microbial metabolites of flavonoids, quantities of small molecular weight phenylacetic acids, phenylpropionic acids, benzoic acids, and benzaldehydes were found transported through Caco-2 cell monolayers (Figure 3.4). Phenylacetic acid and phenylpropionic acids were found in AQ of digested grapes and juice (Supplemental Table 3.3). As these compounds were not detected in starting material, nor from blank saline digestions (data not shown), observance of these metabolites likely results from chemical or potential aerobic

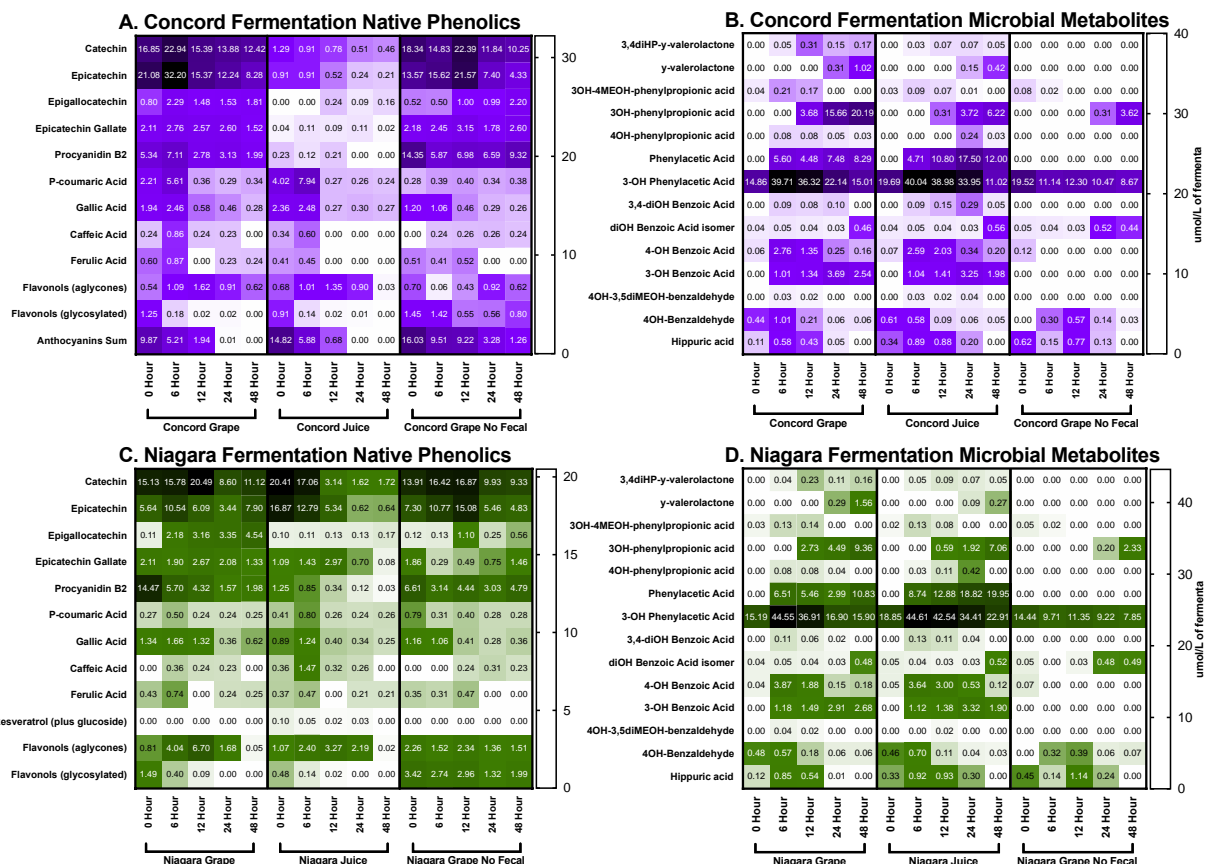
microbial degradation of native phenolic species during the *in-vitro* digestion. While it is clear that total cumulative cellular transport of phenylpropionic acids is greater in juices than grapes (Figure 3.4), this is most likely a function of the amount present in the AQ treatment (Supplemental Table 3.3), as the cellular transport efficiencies were similar between Concord grapes and juice (32% and 29%, respectively) and between Niagara grapes and juice (17% for both). Therefore, matrix effects of grape product is likely not a direct factor in the transport of phenylpropionic acids from AQ as it may have been with flavonoid transport. This may be due to the fact that chemical degradation leading to their formation would proceed from free forms that are already bioaccessible. This is supported by the higher phenylpropionic acid content in AQ material of juices compared to grapes (Supplemental Table 3.3), suggesting the food matrix impacts chemical stability of flavonoids. Benzoic acids were present in AQ fractions (primarily gallic acid and vanillic acid) through which simple chemical or enzymatic dehydroxylation mechanisms may produce the mono or dihydroxybenzoic acids transported by cell monolayers. Hydroxybenzaldehydes, not detected in AQ material, might be produced through uncharacterized mechanisms within Caco-2 from benzoic acid precursors. While this has not been previously reported, it is supported by the fact that very low quantities (33-62  $\mu\text{mol/L}$ ) were observed to be transported basolaterally (Figure 3.4). Regardless, significantly ( $P < 0.05$ ) greater amounts of phenylacetic acids, phenylpropionic acids, benzoic acids, and benzaldehydes were transported across cell monolayers from juice than grape AQ treatments, indicating, that overall, juice appears to be a more efficient matrix that promotes higher bioaccessibility and intestinal transport of native phenolics, and potentially their digestive products relative to whole grape.

### 3.3.3. In-vitro Anaerobic Gut Fermentation of Concord and Niagara Digests produce some differences in metabolite profiles

To explore potential differences between grape fruit and 100% grape juice in metabolism and ultimate availability of microbial metabolites, Concord and Niagara grape/juice samples processed by upper GI digestive conditions were further subjected to a 48-hour *in-vitro* anaerobic fermentation. Fermenter pH (Supplemental Figure 3.2) was monitored for the Concord and Niagara samples, as well as the controls, as an indication of the progression of active fermentation. pH for all fermented samples was found to decrease over the course of the 48-hour fermentation, depending on sample type. Inulin, a highly fermentable fructo-oligosaccharide used as a substrate control, had a final pH of 4.8, indicating high microbial activity as inulin fermentation is well known to generate short chain fatty acids (Macfarlane and Macfarlane 2003). Concord and Niagara juice and grapes digesta samples with fecal inoculum saw similar drops in pH (5.2 – 5.5). A pH drop was also observed in fecal-free Concord and Niagara controls (~6.5 pH), though this is primarily attributed to CO<sub>2</sub> absorption and formation of carbonic acid, and potentially chemical degradation releasing acids or decarboxylation.

Panels A and C of Figure 3.5 show concentration heat maps of select native phenolic species/classes present in the Concord and Niagara digested material through the course of the 48-hour anaerobic fermentation. Flavan-3-ol concentration, including catechin, epicatechin, epigallocatechin, epicatechin gallate, and procyanidin B2, initially increased from grapes fermentation compared to juices. Interestingly, the content of these compounds increased in the first 6 hours of fermentation for grapes and remained elevated throughout the fermentation. By comparison, juice flavan-3-ol profiles quickly decreased within the first 12 hours to low levels by the end of the fermentation. Flavan-3-ols polymers present primarily in seeds are well known to

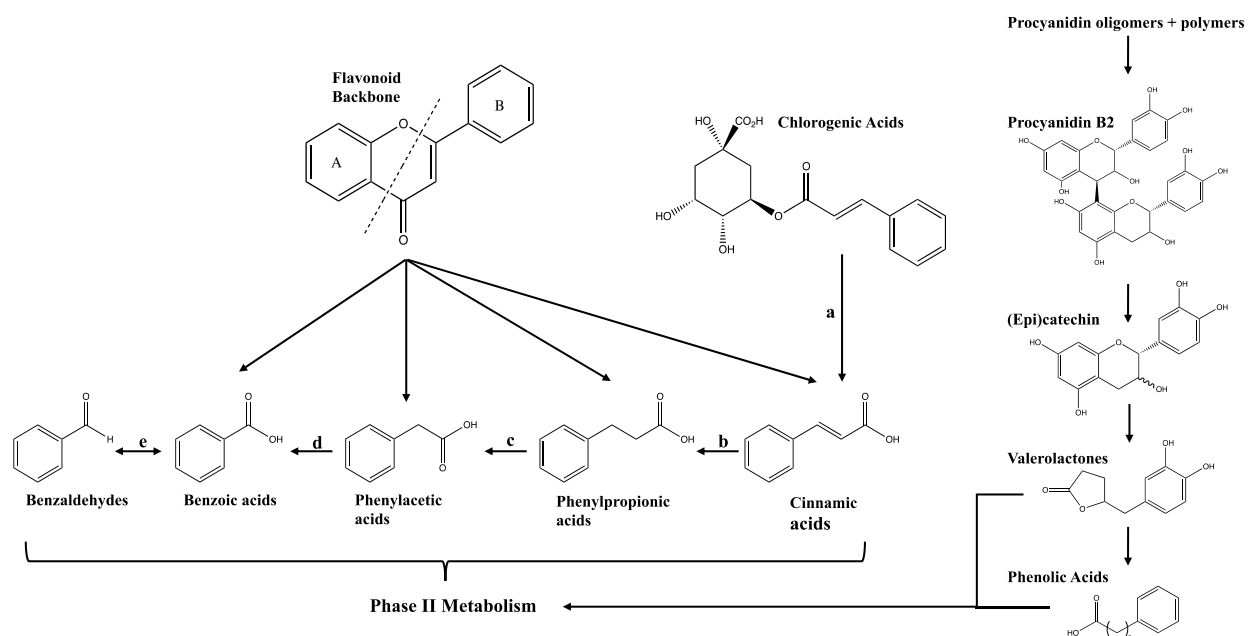
be metabolized by gut microbiota to yield both monomers and smaller molecular weight phenolic metabolites (Wang et al. 2013; Ward et al. 2004). This is consistent with the observed content of smaller procyanidins (procyanidin B2) and flavan-3-ol monomers increasing from whole grapes fermentation, suggestive of oligomer and polymer breakdown, prior to decrease. Both grapes and juice exhibit similar trends with flavonol species, as glycosylated forms quickly degrading over the first 6 hours of fermentation, with a corresponding increase of aglycone species peaking at 12 hours consistent with reported deglycosylation by fecal bacteria (Parkar, Trower, and Stevenson 2013). The levels of both glycosylated and aglycone flavonols remains relatively stable in fecal-free grape controls, suggesting the observed phenomena is primarily due to microbial metabolism and not chemical degradation for these flavonoids. It is important to note that grapes (6.70  $\mu\text{mol/L}$  for Niagara, 1.62  $\mu\text{mol/L}$  for Concord) had a greater aglycone flavonol content at 12 hours than juices (3.27  $\mu\text{mol/L}$  Niagara, 1.35  $\mu\text{mol/L}$  for Concord). Some phenolic species of the Concord and Niagara fecal-free controls did modestly decrease over the fermentation period, though not as much as samples with fecal inoculum. The lack of a significant presence of microbial metabolites (Figure 3.5, and the known susceptibility of phenolic species to heat (the chamber was 37°C) and elevated pH (> 6.4), particularly for anthocyanins, suggest that chemical degradation of phenolic species in the fecal-free controls was occurring.



**Figure 3.5. Native grape phenolics and microbial metabolites from Concord and Niagara grapes and juice over a 48-hour anaerobic fermentation.** Panels A and C show the content of native phenolics for Concord and Niagara samples over a 48-hour anaerobic microbial fermentation. Panels B and D show the content of major phenolic microbial metabolites observed from Concord and Niagara samples over the duration of the anaerobic microbial fermentation. Relevant Concord and Niagara no fecal controls are also shown. All data are presented as a mean ( $n = 4$ , biological replicates) expressed in  $\mu\text{mol/L}$  of fermenta.

Figure 3.6 presents a simplified scheme to summarize the pathways responsible for generation of phenolic microbial metabolites. Panels B and D of Figure 3.5 show the production of phenolic microbial metabolites from Concord and Niagara digested samples over the 48hr fermentation. In general, the content of microbial metabolites and trends of production and degradation of microbial metabolites remains generally similar between grapes and juice with some interesting exceptions. For example, 3-hydroxyphenylacetic acid has a high initial content (14.86 – 18.85  $\mu\text{mol/L}$ ) that increases sharply over the first 6 hours before gradually decreasing over the next 42 hours. This may correspond to the increase in phenylacetic acid observed, as it

gradually increases beginning at 6 hours. Phenylacetic acid may be produced through a dehydroxylation of 3-hydroxyphenylacetic acid, or as a breakdown product of flavonoid species (Figure 3.6). Similar trends and content are also observed between juices and grapes for the hydroxybenzoic acids. However, higher concentrations of 3-hydroxybenzoic acid, 5-(3,4-dihydroxyphenyl)- $\gamma$ -valerolactone (3,4diHP- $\gamma$ -valerolactone),  $\gamma$ -valerolactone, and 3-hydroxyphenylpropionic acid were observed in both Concord and Niagara grapes than juices after 48 hours of fermentation. These acids are known to be major microbial metabolites of flavan-3-ols (Wang et al. 2013; Ward et al. 2004), while 3,4diHP- $\gamma$ -valerolactone (Appeldoorn et al. 2009; Sánchez-Patán et al. 2012) and  $\gamma$ -valerolactone (Sánchez-Patán et al. 2012) are unique metabolites formed from the degradation of flavan-3-ols and their polymeric procyanidin forms. The differences between grapes and juice for these metabolites may correspond to the differences in available flavan-3-ol content between the forms, particularly during the late stages of the fermentation. Finally, little to no phenolic species or metabolites were observed during the fermentation of the fecal-free and Grape/Juice-free controls, indicating minimal production of phenolics or metabolites by the microbes alone (Supplemental Table 3.5).



**Figure 3.6. Simplified schematic of phenolic microbial metabolite production from grape phenolics.** Native chlorogenic acids are hydrolyzed (a) to liberate cinnamic acids. The heterocyclic ring of native flavonoids (flavan-3-ols, anthocyanins, flavonols, stilbenes, etc.) is cleaved, producing two smaller phenolic acids from the A and B rings. Further metabolism of phenolic acids includes hydrogenation (b) of cinnamic acids to phenylpropionic acids,  $\alpha$ -oxidation (c) of phenylpropionic acids to phenylacetic acids,  $\beta$ -oxidation (d) of phenylacetic acids to benzoic acids, and finally reversible reduction/oxidations (e) to benzaldehydes. Procyanidin polymers, oligomers, dimers, and monomers may also be microbially catabolized to valerolactones and finally various phenolic acids. These microbial metabolites and native grape phenolics can then be subjected to various phase II metabolic mechanisms upon absorption. Different substitutions (primarily methylations and hydroxylations) on the benzene rings produce the wide variety of metabolites and native grape phenolics observed.

While differences in phenolic content and bioaccessibility were observed between grapes and 100% juices in the small intestinal phase, the profile of native phenolics and phenolic metabolites remained relatively similar between whole grapes and their corresponding juice digesta following simulated digestion and anaerobic microbial fermentation (Figure 3.5). Much of the lowered phenolic bioaccessible content (AQ) for the whole grapes compared to grape juice is attributed to incomplete extraction of phenolic-rich grape fractions (seeds and skins), and the presence of potentially entrapping fibrous structures. As a majority of the phenolic content is not available in the small intestine from whole grapes, the “insoluble” digested fraction, once subjected to fermentation within the lower intestine, appears to further release phenolics from seeds/skins allowing for comparable levels of native phenolic species and subsequent microbial metabolites

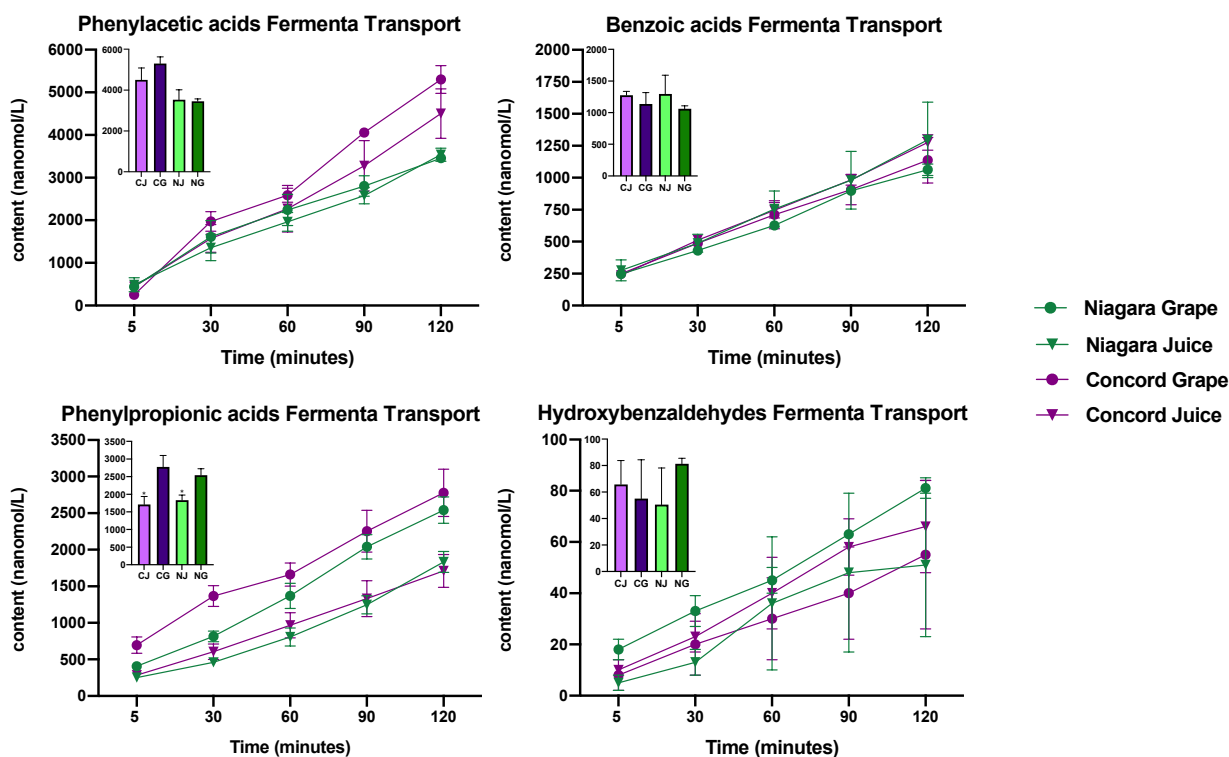
between grapes and grape juices, with the notable exception of flavan-3-ols/procyanidins and their microbial metabolites. Procyanidins and their dimers/monomers are known to be slowly metabolized by microbiota (Wang et al. 2013). The relatively high content of flavan-3-ols in the grape digesta samples during the late stages of fermentation, coupled with the production of flavan-3-ol metabolites during later fermentation hours suggests that longer fermentation times, past 48 hours, may be warranted to better estimate available metabolite pools in humans (Appeldoorn et al. 2009; Sánchez-Patán et al. 2012). In particular,  $\gamma$ -valerolactone is known to be a very late appearing microbial metabolite, (Sánchez-Patán et al. 2012) and the presence of flavan-3-ol monomers/dimers and 3,4diHP- $\gamma$ -valerolactone, an intermediate metabolite in the formation of  $\gamma$ -valerolactone, (Sánchez-Patán et al. 2012) during later hours of fermentation suggests that longer fermentation times may be warranted to truly deduce the broader differences between 100% grape juice and whole grape consumption in the large intestine.

While we believe the *in-vitro* fermentation model utilized in this study is a relevant representation of general large intestinal microbial metabolism, it is important to note some limitations. The microbial inoculum used was from fecal matter, which is more representative of the distal colonic microbial environment, rather than broader lower gut microbial communities. Furthermore, while pH was monitored during our experimentation, it was not maintained at ~6.8. Changes in pH are known to select for specific microbial strains while hampering the growth of other strains, thereby impacting the microbial community structure and activity (Duncan et al. 2009). These differences in the microbial communities in our model may have served to influence the metabolism of phenolics compared to true an *in-vivo* situation. However, as the pathways and metabolites identified are common across known gut microbial species (Parkar, Trower, and Stevenson 2013; Selma, Espin, and Tomas-Barberan 2009; Murota, Nakamura, and Uehara 2018)

results from the present study do appear to be a relevant comparison between fruit and juice matrices that merits further exploration using relevant *in-vivo* models.

#### 3.3.4. Transport of 12hr Fermenta Grape Phenolic Metabolites is similar between whole grapes and juice

In order to understand what differences may exist in intestinal absorption of phenolics released and/or metabolized by the lower GI fermentation, centrifuged fermenta containing bioaccessible phenolics/metabolites were applied to the apical surface of Caco-2 monolayers. Figure 3.7 displays cumulative apical to basolateral transport from Caco-2 cell monolayers of phenolic metabolites from 12-hour fermenta Concord and Niagara grape/juice grouped by compound class. Major transported metabolites include phenylacetic acids (phenylacetic acid, 3-hydroxy/methoxy phenylacetic acids), benzoic acids (gallic acid, 3,4-hydroxybenzoic acids), phenylpropionic acids (primarily 3-hydroxyphenylpropionic acid), and hydroxybenzaldehydes. It is also important to note that low quantities of 3,4-diHP- $\gamma$ -valerolactone were transported across monolayers (Supplemental Table 3.6). Despite levels of flavonoids being detected in 12hr fermenta samples (Figure 3.5), no flavonoids were observed to be transported across Caco-2 monolayers, likely due to low levels (below LOD) and poor overall transport efficiency by enterocytes as previously reported (Wang et al. 2013). After 2 hours, relatively similar ( $P > 0.05$ ) levels of phenylacetic acids, benzoic acids, and hydroxybenzaldehydes were transported across monolayers between Concord and Niagara grape and juice fermenta (Figure 3.7). Significantly ( $P < 0.05$ ) greater levels of phenylpropionic acids were transported from grape fermenta than juice fermenta, with Concord grape being 1.6x greater than juice and Niagara grape being 1.3x greater than juice.



**Figure 3.7. Cumulative phenolic apical to basolateral transport of 12-hour fermenta fraction of Concord and Niagara grapes and 100% grape juice.** Individual panels present cumulative phenolic transport of fermenta fractions over 2 hours across differentiated Caco-2 cell monolayers. Data are expressed as a nanomol of compound transported in 1L of basolateral media. Phenolic metabolites were summed together based on compound class (phenylacetic acids, phenylpropionic acids, benzoic acids, and hydroxybenzoic acids). Inlaid graphs in each panel show cumulative 2-hour transport across cells for Concord juice (CJ), Concord grape (CG), Niagara juice (NJ), and Niagara grape (NG). All data are presented as a mean  $\pm$  SD ( $n = 4$ , biological replicates). Presence of an \* indicates significant difference ( $P < 0.05$ ) by un-paired t-test analysis within cumulative 2hr transport for individual phenolic class levels between juice and their respective grapes.

These data indicate that phenylpropionic acids, predominantly derived from metabolism of flavan-3-ols, are transported across monolayers in greater quantities from grape fermenta (2.9  $\mu\text{mol/L}$  Concord, 2.6  $\mu\text{mol/L}$  Niagara) than juice (1.8  $\mu\text{mol/L}$  Concord, 1.9  $\mu\text{mol/L}$  Niagara). Transport efficiency (917% Concord Juice, 149% Concord Grape, 585% Niagara Juice, and 183% Niagara Grape) of phenylpropionic acids from 12hr fermenta were over 100% indicating that Caco-2 cell metabolism and/or chemical degradation may also be contributing to observed phenylpropionic acids in the basolateral compartment. This makes it difficult to assess the impacts of starting contents of phenylpropionic acids from 12hr fermenta, though grape fermenta (3.85

$\mu\text{mol/L}$  Concord,  $2.87 \mu\text{mol/L}$  Niagara) had a greater content than juice fermenta ( $0.39 \mu\text{mol/L}$  Concord,  $0.66 \mu\text{mol/L}$  Niagara). Overall, these data suggest that for this one class of phenolic microbial metabolites, phenylpropionic acids, derived from flavan-3-ol microbial metabolism, whole grapes perhaps by virtue of their higher proportion of undigested and available substrate, exhibited higher formation and transport of phenylpropionic acids compared to juice.

Beyond phenylpropionic acids, the Caco-2 transport of microbial metabolites between grape and juice 12-hour fermenta treatments was quite similar. This was in sharp contrast to Caco-2 transport observed from the grape and 100% juice AQ treatments (Figure 3.4). Differences observed in the transport from upper intestinal AQ treatments were attributed, in part, to the potential for entrapping “accessible” phenolics by soluble fibers that would be present in the grape food matrix, but not in the liquid juice matrix. Following anaerobic gut fermentation, transport rates did not differ between whole grape and juice digesta. It is plausible to suspect that microbial fermentation of the residual undigested material from grapes led to a further release of phenolics from entrapping fibers and cell wall structures, particularly in the case of seed derived polymeric procyanidins. This would result in enhanced bioaccessibility for microbial metabolism and ultimately intestinal transport. Profiles of bioaccessible phenolic species and subsequent microbial metabolites, normalized between juice and fruit digesta (Figure 3.5), aligned with cellular transport of metabolites from 12-hour fermenta (Figure 3.7). Considering the low bioavailability of many native flavonoid species, long-term health implications associated with grape phenolic consumption, have been more recently associated with microbial phenolic metabolites (Selma, Espin, and Tomas-Barberan 2009; Kim et al. 1998; Larrosa et al. 2009; Ward et al. 2004; Lee et al. 2017). As overall bioaccessibility and intestinal transport of phenolic metabolites remains comparable between grapes and juice, effects of grape and/or juice consumption driven by

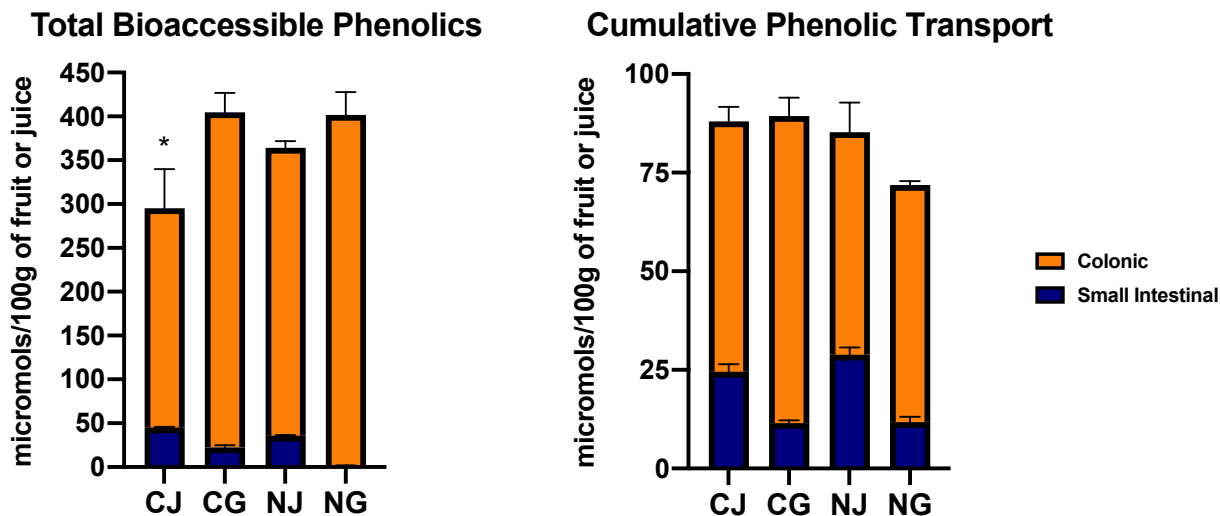
phenolic metabolites, may therefore be similar. In future studies, the chronic effects of grape fruit versus 100% grape juice consumption, due to microbial phenolic metabolites, requires further exploration, particularly with relevant *in-vivo* models and clinical trials.

It is important to note that absorption and transport studies across cell monolayers were performed only using 12hr fermenta samples. As indicated earlier, there were key differences in the microbial fermentation of flavan-3-ols and their larger polymers between juices and grapes (Figure 3.5). In particular, the levels of flavan-3-ol monomers remained relatively stable throughout fermentation for the grapes due to breakdown of larger polymers, while these compounds quickly degraded in the earlier hours of fermentation for juice treatments (Figure 3.5). This directly corresponded with the increase of flavan-3-ol specific microbial metabolites particularly at later fermentation hours, where clear differences were observed between grapes and juices (Figure 3.5). If later hour (24 or 48hr) fermenta samples were used as treatments for absorption and transport studies more pronounced differences between grape versus juice microbial metabolite absorption and transport would be observed, perhaps suggesting differential health implications for chronic consumption of grapes versus 100% grape juice.

### 3.3.5. Cumulative bioaccessibility and transport is similar between whole fruit and 100% juice

To illustrate differences in cumulative phenolic release, metabolism and subsequent intestinal flux in both upper and lower GI, total bioaccessible phenolic content and cumulative cellular transport from grapes and juice are displayed in Figure 3.8. Though variations exist, total bioaccessible phenolic content combined from both upper and lower GI digestion remains generally similar between Concord and Niagara grapes and their 100% juices. Perhaps even more telling, the cumulative phenolic flux across upper and lower GI intestinal epithelia, modeled using

Caco-2, is strikingly similar ranging between 71.8 – 89.4  $\mu\text{mols}/100\text{g}$  of starting grape/juice material. Therefore, despite differences between the fruit and juice matrices, their starting phenolic content (Figure 3.2), whole grapes and 100% grape juice deliver quite similar bioavailable phenolics and metabolites. While whole grapes have a far greater phenolic content, the combinatorial effects of phenolic extraction through juice processing to create a matrix with high bioaccessibility to the intestine and microbial communities results in relative parity between whole grapes and 100% grape juice upon simulation of both the upper and lower tracts of digestion. It is important to note that much larger amounts, in moles, of phenolic species are released and made bioavailable from lower GI digestion (including phenolic microbial metabolites) than upper GI digestion for both 100% grape juice and whole grape consumption. Overall, these results suggest that the bioavailability and metabolism of phenolics between whole grape and 100% grape juice consumption are remarkably similar. Considering health benefits potentially mediated by phenolic metabolites, it is logical to assume similar benefits can be achieved from consumption of both grape fruit and 100% juice.



**Figure 3.8. Total bioaccessible and cumulative transport of phenolics from small intestinal and colonic digestion compartments.** Bioaccessible phenolic data is expressed as 176henylprop of phenolic content (summed from individual compounds as determined by LC-MS) per 100g of starting grape material. Phenolic compounds and metabolites profiled and quantified during anaerobic fermentation are also deemed as bioaccessible, as these compounds exist in the aqueous fraction of fermenta samples, thus making these compounds available for further biological activity (such as cellular transport). The 12-hour fermenta sample data was used to determine the colonic fraction of bioaccessible phenolics, as 12-hour fermenta samples were used for cellular transport studies. Phenolic cellular transport data is expressed as micromoles of phenolic content (summed from individual compounds as determined by LC-MS) per 100g of starting grape material. All data are presented as a mean  $\pm$  SD ( $n = 4$ , biological replicates). Presence of an \* indicates significant difference ( $P < 0.05$ ) by un-paired t-test analysis within total phenolics from small intestine and colonic digestions between juice and their respective grapes.

Though the similarities in phenolic release through digestion and epithelial cellular uptake between grapes and juice are apparent, it important to note the subtle differences present, particularly in the upper GI (small intestinal) tract. Both 100% grape juices had greater total phenolic bioaccessibilities from the upper tract than their respective whole grapes. Perhaps even more important, cellular transport from upper GI digesta exhibited greater cumulative phenolic totals from the juices than grapes, including flavonoid species. The presence of circulating native and phenolic host metabolites have been known to attenuate a variety of biological effects. Numerous studies have observed health benefits including changes in cognitive performance, vascular reactivity, and serum antioxidant status following acute 100% grape juice consumption (Haskell-Ramsay et al. 2017; Copetti et al. 2018; Hashemi et al. 2010). These findings may be

driven in part by the highly bioaccessible nature of the compounds in 100% juice. The aqueous nature of the juice matrix, coupled with the efficiency of juice processing on extraction of phenolics, provide a highly bioaccessible source of native and host metabolites that can be linked to mechanism of acute effects observed from grape juice consumption (Haskell-Ramsay et al. 2017; Stalmach et al. 2011; Copetti et al. 2018; Hashemi et al. 2010; Stalmach et al. 2012). Though studies on the acute benefits of whole grape consumption are limited, the increased cellular transport levels and efficiency of flavonoids from the small intestine of 100% grape juice supports the notion that juice may be a logical matrix for the delivery of acute effects compared to whole grape consumption, which provides enhanced production of select colonic metabolites. This hypothesis would require further exploration through direct comparative assessments between 100% grape juice and grapes in studies assessing functional outcomes.

While we acknowledge that there may be some limitations with the anaerobic fermentation model used, we believe it was effective for this study, particularly for direct comparison of lower intestinal digestion/metabolism of phenolics from two distinct grape sources and physical forms. The release of accessible phenolics and microbial metabolites during *in-vitro* fermentation, emulating digestion within the lower GI tract, between grapes and juice is quite comparable. As we have detailed earlier, the activity of microbiota allowed for equivalent if not greater, particularly at later hours, phenolic species and microbial metabolites release from grapes compared to 100% grape juice. Furthermore, the cumulative transport of microbial metabolites from the lower tract remained relatively similar between juice and grapes, though grapes did exhibit greater phenylpropionic acid flux. These microbial metabolites have numerous recorded biological activities, and have been associated with the chronic health benefits associated with consumption of phenolic-rich foods, such as grapes (Murota, Nakamura, and Uehara 2018; Kim

et al. 1998; Larrosa et al. 2009). Benefits on cardiovascular health, type-2 diabetes, some cancers, neurological health, inflammation, and pulmonary health have been associated with circulating phenolic microbial metabolites (Russell and Duthie 2011; Jankowski et al. 2003; Marhuenda-Muñoz et al. 2019). The similarities observed between the release of phenolic microbial metabolites and subsequent cellular transport between grapes and 100% grape juice suggests that the benefits of chronic consumption of grapes and 100% grape juice, mediated by phenolic microbial metabolites, may be similar. Further animal and clinical studies are needed to compare phenolics from grapes and grape juice in the lower tract, particularly as this study does not delve into changing complex gut-microbial relationships (i.e., gut epithelial mucus layer) or changes in gut microbial populations best modeled in vivo. It is paramount to study such relationships for a better understanding gut/digestive health and are perhaps the mechanisms through which phenolics mediate health outcomes.

### **3.4. Conclusions**

The present studies suggest that although differences in phenolic bioaccessibility and cellular transport exist between 100% grape juice and whole grapes during various stages of digestion, particularly the small intestinal phase, the overall delivery of phenolics and associated metabolites during digestion remained similar between product forms. These results further support the notion that 100% Concord and Niagara grape juice may continue to be reasonable fruit forms for consumers with regards to delivery of bioactive phenolic compounds.

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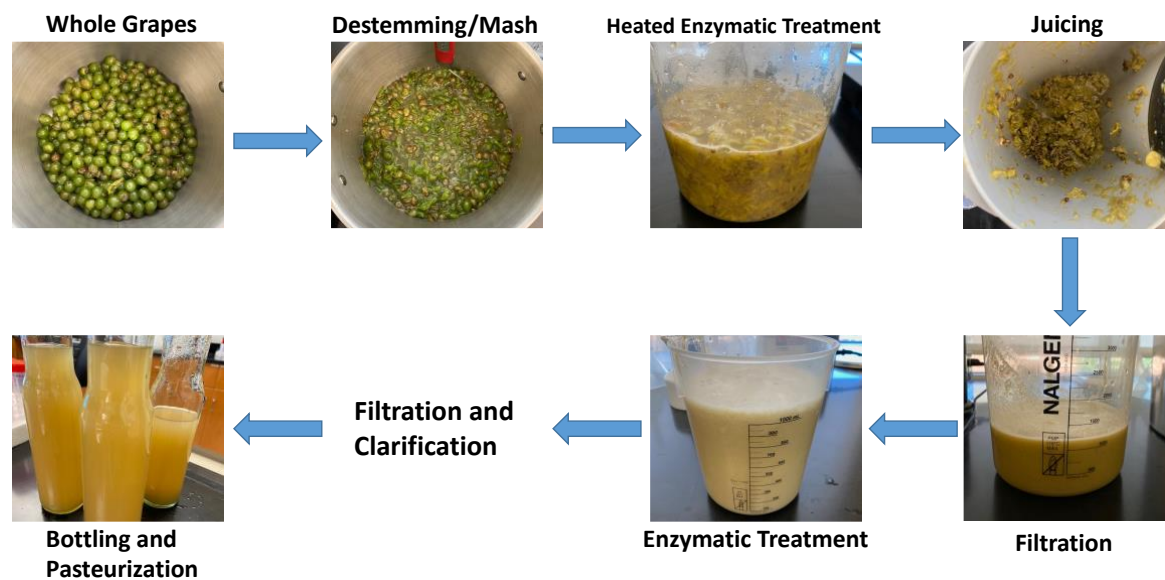
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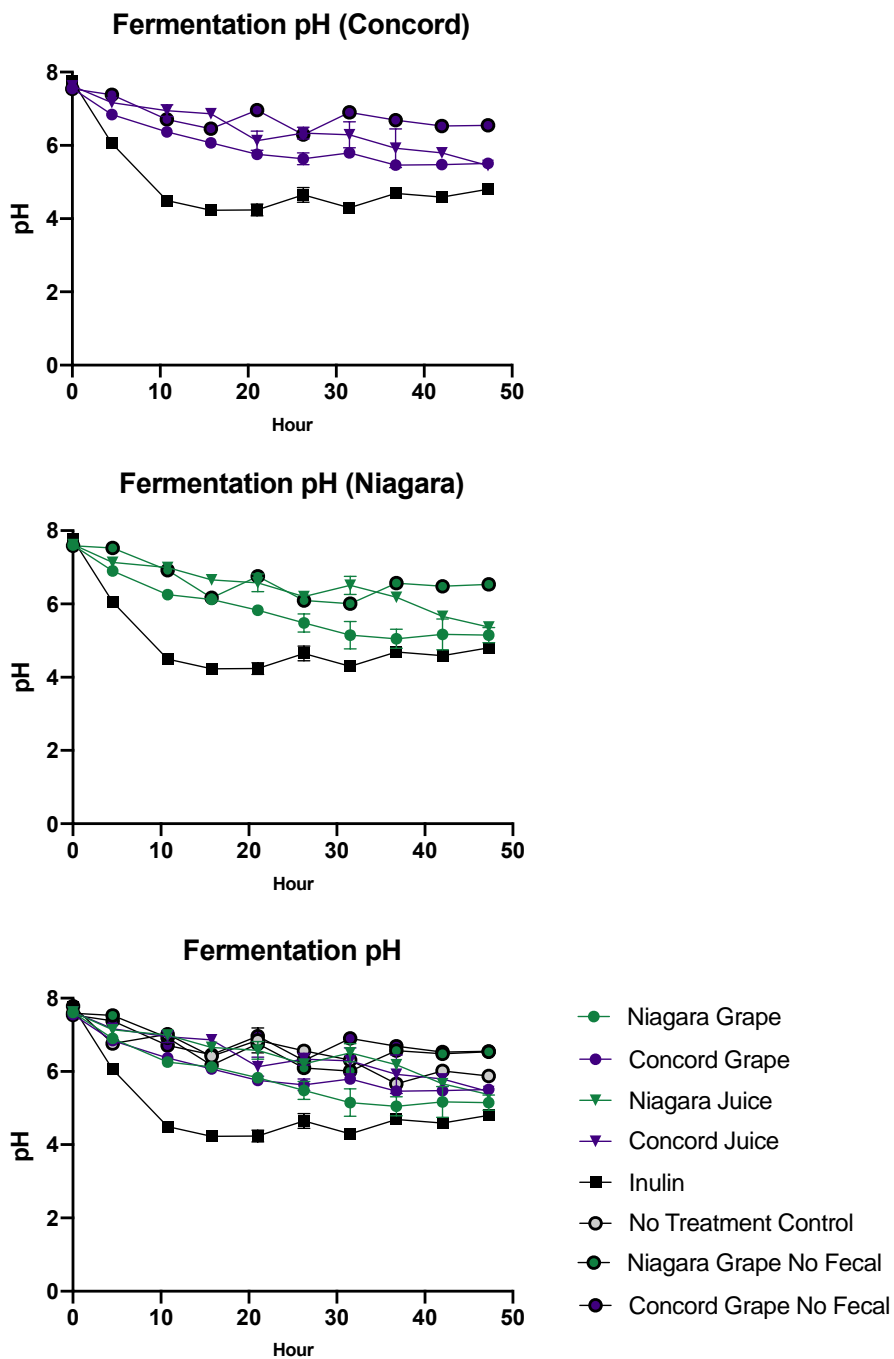
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### 3.6. Supplemental Tables/Figures



**Supplemental Figure 3.1.** Bench-top emulation of commercial 100% grape juice processing.



**Supplemental Figure 3.2.** pH of anaerobic microbial fermentation of Concord and Niagara grapes, 100% grape juices, and relevant controls over 48 hours.

**Supplemental Table 3.1.** SIR and MRM transitions for identification and quantification of phenolic compounds and metabolites.

Compound	MW (g/mol)	Detection Mode	[M-H] <sup>-</sup> (m/z)	[M+H] <sup>+</sup> (m/z)	Fragment Ion (m/z)
(+)-catechin	290	(-) ESI	289	n/a	109
(-)-epicatechin	290	(-) ESI	289	n/a	109
(+)-gallocatechin	306	(-) ESI	305	n/a	125
(-)-epigallocatechin	306	(-) ESI	305	n/a	125
(-)-epicatechin gallate	442	(-) ESI	441	n/a	169
procyanidin B2	579	(-) ESI	578	n/a	289
vanillic acid	168	(-) ESI	167	n/a	152
coutaric acid	296	(-) ESI	295	n/a	163
caftaric acid	312	(-) ESI	311	n/a	149
p-coumaric acid	164	(-) ESI	163	n/a	119
gallic acid	170	(-) ESI	169	n/a	125
caffeic acid	180	(-) ESI	179	n/a	135
ferulic acid	194	(-) ESI	193	n/a	134
phenylacetic acid	136	(-) ESI	135	n/a	91
3-hydroxyphenylacetic acid	152	(-) ESI	151	n/a	107
4-hydroxybenzoic acid	138	(-) ESI	137	n/a	93
3-hydroxybenzoic acid	138	(-) ESI	137	n/a	93
3,4-dihydroxybenzoic acid	154	(-) ESI	153	n/a	109
3-(3-hydroxyphenyl)propionic acid	166	(-) ESI	165	n/a	147
3-(3OH-4methoxyphenyl)propionic acid	196	(-) ESI	195	n/a	136
3-(4-hydroxyphenyl)propionic acid	166	(-) ESI	165	n/a	121
4OH-3,5-dimethoxybenzaldehyde	182	(-) ESI	181	n/a	166
4-hydroxybenzaldehyde	122	(-) ESI	121	n/a	92
hippuric acid	179	(-) ESI	178	n/a	77
quercetin	302	(-) ESI	301	n/a	151
myricetin	318	(-) ESI	317	n/a	151
kaempferol-3-glucoside	448	(-) ESI	447	n/a	284
quercetin-3-glucoside	464	(-) ESI	463	n/a	300
myricetin-3-glucose	480	(-) ESI	479	n/a	317
kaempferol-3-rutinoside	595	(-) ESI	594	n/a	285
quercetin-3-rutinoside	610	(-) ESI	609	n/a	300
resveratrol	228	(-) ESI	227	n/a	185
resveratrol-3-glucoside	390	(-) ESI	389	n/a	227
cyanidin-3-arabinoside	419	(+) ESI	n/a	419	287
peonidin-3-arabinoside	433	(+) ESI	n/a	433	301
delphinidin-3-arabinoside	435	(+) ESI	n/a	435	303
petunidin-3-arabinoside	449	(+) ESI	n/a	449	317
cyanidin-3-glucoside	449	(+) ESI	n/a	449	287
peonidin-3-glucoside	463	(+) ESI	n/a	464	301
delphinidin-3-glucoside	465	(+) ESI	n/a	465	303
petunidin-3-glucoside	479	(+) ESI	n/a	479	317
malvidin-3-glucoside	493	(+) ESI	n/a	493	287
cyanidin-3-galactoside	449	(+) ESI	n/a	449	287
peonidin-3-galactoside	463	(+) ESI	n/a	464	301
malvidin-3-galactoside	493	(+) ESI	n/a	493	287
3-(3,4diHP)- $\gamma$ -valerolactone	208	(+) ESI	n/a	209	148
$\gamma$ -valerolactone	100	(+) ESI	n/a	101	55
Cyanidin (acylated forms)	n/a	(+) ESI	n/a	287	n/a

\*acylated forms of anthocyanins were determined through single ion responses (SIR) of the parent anthocyanin and were identified as acylated forms due to shifts in retention

**Supplemental Table 3.2.** Raw material content (mg/100g) of phenolic species in Concord and Niagara grape and 100% juice samples.

Compound (mg/100g)	Concord Juice	Concord Grape	Niagara Juice	Niagara Grape
catechin	0.27 ± 0.02	3.91 ± 0.27	6.25 ± 0.17	16.63 ± 1.18
epicatechin	0.45 ± 0.05	4.38 ± 0.31	10.99 ± 0.44	9.62 ± 0.33
gallo catechin	n/d	n/d	0.16 ± 0.01	n/d
epigallocatechin	n/d	0.14 ± 0.02	0.16 ± 0.02	0.20 ± 0.04
epicatechin gallate	0.02 ± 0.01	0.75 ± 0.11	0.96 ± 0.05	0.68 ± 0.18
procyanidin B2	0.26 ± 0.02	0.58 ± 0.09	1.04 ± 0.11	0.83 ± 0.18
<b>Total Flavan-3-ols</b>	<b>1.00 ± 0.10</b>	<b>9.75 ± 0.82</b>	<b>19.56 ± 0.82</b>	<b>27.96 ± 1.91</b>
vanillic acid	0.01 ± 0.01	n/d	0.02 ± 0.01	n/d
coutaric acid	0.32 ± 0.02	0.10 ± 0.01	1.09 ± 0.09	0.14 ± 0.02
caftaric acid	1.70 ± 0.06	0.67 ± 0.04	5.68 ± 0.29	0.64 ± 0.11
p-coumaric acid	0.07 ± 0.01	0.04 ± 0.01	0.09 ± 0.01	0.03 ± 0.01
gallic acid	0.09 ± 0.01	0.06 ± 0.01	1.40 ± 0.16	0.06 ± 0.01
caffeic acid	0.04 ± 0.01	0.04 ± 0.01	0.22 ± 0.01	0.04 ± 0.01
ferulic acid	0.03 ± 0.01	0.08 ± 0.01	0.02 ± 0.01	0.03 ± 0.01
<b>Total Phenolic acids</b>	<b>2.28 ± 0.09</b>	<b>1.03 ± 0.06</b>	<b>8.54 ± 0.55</b>	<b>0.98 ± 0.14</b>
resveratrol	0.01 ± 0.01	0.03 ± 0.01	0.01 ± 0.01	0.03 ± 0.01
resveratrol-3-glucoside	0.03 ± 0.01	0.04 ± 0.01	0.03 ± 0.01	0.04 ± 0.01
<b>Total Stilbenes</b>	<b>0.04 ± 0.01</b>	<b>0.07 ± 0.01</b>	<b>0.05 ± 0.01</b>	<b>0.07 ± 0.01</b>
quercetin	0.01 ± 0.01	0.01 ± 0.01	0.02 ± 0.01	0.01 ± 0.01
myricetin	n/d	0.02 ± 0.01	n/d	n/d
kaempferol-3-glucoside	n/d	n/d	0.07 ± 0.01	0.02 ± 0.01
quercetin-3-glucoside	0.66 ± 0.05	0.88 ± 0.02	0.85 ± 0.03	0.63 ± 0.10
myricetin-3-glucoside	0.29 ± 0.06	0.09 ± 0.01	n/d	n/d
kaempferol-3-rutinoside	2.79 ± 0.26	1.93 ± 0.59	n/d	n/d
quercetin-3-rutinoside	1.28 ± 0.14	4.56 ± 1.21	0.01 ± 0.01	0.09 ± 0.03
<b>Total Flavonols</b>	<b>5.04 ± 0.51</b>	<b>7.50 ± 1.84</b>	<b>0.95 ± 0.04</b>	<b>0.75 ± 0.13</b>
cyanidin-3-arabinoside	0.03 ± 0.01	0.12 ± 0.01	n/d	n/d
peonidin-3-arabinoside	0.10 ± 0.01	0.42 ± 0.06	n/d	n/d
delphinidin-3-arabinoside	0.03 ± 0.01	0.13 ± 0.01	n/d	n/d
petunidin-3-arabinoside	n/d	0.04 ± 0.01	n/d	n/d
cyanidin-3-glucoside	4.86 ± 0.08	16.21 ± 1.73	n/d	n/d
peonidin-3-glucoside	1.07 ± 0.05	3.80 ± 0.48	n/d	n/d
delphinidin-3-glucoside	0.16 ± 0.01	2.80 ± 0.32	n/d	n/d
petunidin-3-glucoside	0.52 ± 0.02	4.05 ± 0.34	n/d	n/d
malvidin-3-glucoside	0.03 ± 0.01	0.10 ± 0.01	n/d	n/d
cyanidin-3-galactoside	0.21 ± 0.02	0.15 ± 0.04	n/d	n/d
peonidin-3-galactoside	6.40 ± 0.09	4.07 ± 0.78	n/d	n/d
malvidin-3-galactoside	2.21 ± 0.05	1.88 ± 0.37	n/d	n/d
cyandin (acylated)	1.85 ± 0.11	13.82 ± 1.03	n/d	n/d
peonidin (acylated)	6.80 ± 0.23	24.28 ± 2.64	n/d	n/d
delphinidin (acylated)	2.82 ± 0.13	99.72 ± 8.21	n/d	n/d
petunidin (acylated)	0.41 ± 0.02	4.47 ± 0.28	n/d	n/d
malvidin (acylated)	3.93 ± 0.18	20.43 ± 3.36	n/d	n/d
<b>Total Anthocyanins</b>	<b>15.61 ± 0.33</b>	<b>33.77 ± 4.16</b>	<b>n/d</b>	<b>n/d</b>
<b>Total Anthocyanins (Acy)</b>	<b>15.82 ± 0.66</b>	<b>162.72 ± 15.52</b>	<b>n/d</b>	<b>n/d</b>
<b>Total Anthocyanins (Total)</b>	<b>31.42 ± 0.99</b>	<b>196.49 ± 19.68</b>	<b>n/d</b>	<b>n/d</b>

\*n/d indicates that the species was not detected

\*Data are expressed as mean ± standard deviation from n=4 independent assessments.

**Supplemental Table 3.3.** Absolute bioaccessible content (mg/100g) of phenolic species in Concord and Niagara grape and 100% juice samples.

Compound (mg/100g)	Concord Juice	Concord Grape	Niagara Juice	Niagara Grape
catechin	0.16 ± 0.01	0.01 ± 0.01	5.28 ± 0.20	0.03 ± 0.04
epicatechin	0.11 ± 0.01	n/d	4.11 ± 0.14	0.01 ± 0.01
gallocatechin	n/d	n/d	0.03 ± 0.01	n/d
epigallocatechin	n/d	n/d	0.04 ± 0.01	n/d
epicatechin gallate	n/d	n/d	0.03 ± 0.01	n/d
procyanidin B2	0.02 ± 0.01	n/d	0.18 ± 0.02	n/d
<b>Total Flavan-3-ols</b>	<b>0.29 ± 0.03</b>	<b>0.01 ± 0.01</b>	<b>9.67 ± 0.37</b>	<b>0.03 ± 0.05</b>
vanillic acid	0.02 ± 0.01	n/d	0.01 ± 0.01	n/d
caftaric acid	n/d	0.03 ± 0.01	0.01 ± 0.01	0.01 ± 0.01
p-coumaric acid	1.37 ± 0.07	0.12 ± 0.11	0.07 ± 0.01	n/d
gallic acid	0.03 ± 0.01	0.13 ± 0.02	0.03 ± 0.01	0.10 ± 0.03
caffeic acid	0.09 ± 0.01	0.19 ± 0.10	0.08 ± 0.01	0.01 ± 0.01
ferulic acid	0.02 ± 0.01	0.10 ± 0.02	0.01 ± 0.01	n/d
<b>Total Phenolic Acids</b>	<b>1.54 ± 0.09</b>	<b>0.56 ± 0.26</b>	<b>0.23 ± 0.03</b>	<b>0.12 ± 0.05</b>
resveratrol-3-glucoside	0.01 ± 0.01	n/d	0.01 ± 0.01	n/d
<b>Total Stilbenes</b>	<b>0.01 ± 0.01</b>	<b>n/d</b>	<b>0.01 ± 0.01</b>	<b>n/d</b>
kaempferol-3-glucoside	n/d	n/d	0.01 ± 0.01	0.04 ± 0.02
quercetin-3-glucoside	0.14 ± 0.01	0.10 ± 0.02	0.15 ± 0.01	0.10 ± 0.03
myricetin-3-glucoside	0.06 ± 0.01	0.02 ± 0.01	n/d	n/d
kaempferol-3-rutinoside	0.05 ± 0.03	n/d	n/d	n/d
<b>Total Flavonols</b>	<b>0.25 ± 0.06</b>	<b>0.11 ± 0.03</b>	<b>0.17 ± 0.02</b>	<b>0.14 ± 0.05</b>
cyanidin-3-arabinoside	0.01 ± 0.01	n/d	n/d	n/d
peonidin-3-arabinoside	0.04 ± 0.01	n/d	n/d	n/d
delphinidin-3-arabinoside	0.01 ± 0.01	0.03 ± 0.01	n/d	n/d
cyanidin-3-glucoside	2.74 ± 0.12	0.02 ± 0.01	n/d	n/d
peonidin-3-glucoside	0.67 ± 0.03	0.03 ± 0.01	n/d	n/d
delphinidin-3-glucoside	0.04 ± 0.01	n/d	n/d	n/d
petunidin-3-glucoside	0.19 ± 0.01	n/d	n/d	n/d
malvidin-3-glucoside	0.01 ± 0.01	n/d	n/d	n/d
cyanidin-3-galactoside	0.15 ± 0.01	0.01 ± 0.01	n/d	n/d
peonidin-3-galactoside	3.65 ± 0.26	4.08 ± 0.86	n/d	n/d
malvidin-3-galactoside	1.23 ± 0.06	2.32 ± 0.41	n/d	n/d
cyandin (acylated)	1.16 ± 0.03	0.03 ± 0.02	n/d	n/d
peonidin (acylated)	4.45 ± 0.19	1.36 ± 0.16	n/d	n/d
delphinidin (acylated)	0.42 ± 0.19	0.16 ± 0.06	n/d	n/d
petunidin (acylated)	0.15 ± 0.01	0.01 ± 0.01	n/d	n/d
malvidin (acylated)	2.80 ± 0.11	1.53 ± 0.20	n/d	n/d
<b>Total Anthocyanins</b>	<b>8.73 ± 0.50</b>	<b>6.50 ± 1.30</b>	<b>n/d</b>	<b>n/d</b>
<b>Total Anthocyanins (Acy)</b>	<b>8.98 ± 0.39</b>	<b>3.09 ± 0.43</b>	<b>n/d</b>	<b>n/d</b>
<b>Total Anthocyanins (Total)</b>	<b>17.71 ± 0.88</b>	<b>9.59 ± 1.73</b>	<b>n/d</b>	<b>n/d</b>
3-hydroxyphenylpropionic acid	0.06 ± 0.01	0.04 ± 0.01	0.13 ± 0.02	0.05 ± 0.01
4-hydroxyphenylpropionic acid	0.03 ± 0.01	0.01 ± 0.01	0.05 ± 0.02	0.02 ± 0.01
phenylacetic acid	0.02 ± 0.01	0.01 ± 0.01	0.02 ± 0.01	0.01 ± 0.01

\*n/d indicates that the species was not detected

\*Data are expressed as mean ± standard deviation from n=4 independent assessments.

**Supplemental Table 3.4.** Aqueous Digesta treatment cellular transport (nanomol/L of basolateral media) of phenolic species in Concord and Niagara grape and 100% juice samples.

Compound (picomol/mL)	Concord Juice					Concord Grape					Niagara Juice					Niagara Grape				
	5 minutes	15 minutes	30 minutes	60 minutes	120 minutes	5 minutes	15 minutes	30 minutes	60 minutes	120 minutes	5 minutes	15 minutes	30 minutes	60 minutes	120 minutes	5 minutes	15 minutes	30 minutes	60 minutes	120 minutes
phenylacetic acid	7 ± 8	30 ± 35	72 ± 20	99 ± 53	194 ± 44	16 ± 6	14 ± 9	26 ± 8	28 ± 11	43 ± 7	6 ± 8	145 ± 14	220 ± 12	264 ± 11	226 ± 7	3 ± 5	7 ± 9	15 ± 7	20 ± 16	34 ± 15
3-methoxyphenylacetic acid	97 ± 9	165 ± 31	225 ± 32	304 ± 28	359 ± 40	34 ± 16	92 ± 4	138 ± 4	176 ± 9	221 ± 18	243 ± 63	558 ± 118	805 ± 56	687 ± 43	731 ± 46	56 ± 8	97 ± 14	141 ± 25	180 ± 11	210 ± 25
3-hydroxyphenylacetic acid	19 ± 6	12 ± 4	12 ± 2	13 ± 6	12 ± 4	11 ± 4	9 ± 1	8 ± 1	9 ± 3	12 ± 5	12 ± 6	13 ± 3	11 ± 2	11 ± 1	11 ± 2	11 ± 7	8 ± 2	10 ± 4	10 ± 4	11 ± 4
<b>Total phenylacetic acids</b>	<b>123 ± 16</b>	<b>207 ± 49</b>	<b>309 ± 34</b>	<b>416 ± 71</b>	<b>565 ± 60</b>	<b>61 ± 17</b>	<b>114 ± 11</b>	<b>172 ± 7</b>	<b>213 ± 7</b>	<b>276 ± 22</b>	<b>261 ± 64</b>	<b>716 ± 131</b>	<b>1036 ± 56</b>	<b>962 ± 45</b>	<b>969 ± 52</b>	<b>70 ± 13</b>	<b>112 ± 22</b>	<b>165 ± 35</b>	<b>210 ± 29</b>	<b>256 ± 42</b>
4-methoxybenzoic acid	2 ± 11	4 ± 1	4 ± 2	6 ± 3	11 ± 4	5 ± 3	11 ± 3	9 ± 2	16 ± 6	15 ± 5	90 ± 59	116 ± 61	311 ± 69	459 ± 48	677 ± 38	3 ± 1	6 ± 3	9 ± 5	16 ± 11	17 ± 7
4-hydroxybenzoic acid	142 ± 37	399 ± 61	442 ± 77	458 ± 74	549 ± 81	145 ± 22	223 ± 48	253 ± 40	320 ± 49	345 ± 40	131 ± 44	201 ± 55	258 ± 33	330 ± 68	433 ± 130	99 ± 25	139 ± 62	189 ± 26	237 ± 41	287 ± 38
3-hydroxybenzoic acid	17 ± 10	36 ± 5	55 ± 8	68 ± 14	110 ± 16	17 ± 9	39 ± 10	59 ± 23	81 ± 27	87 ± 27	21 ± 11	48 ± 19	67 ± 22	115 ± 45	133 ± 58	28 ± 12	44 ± 12	69 ± 38	44 ± 25	26 ± 25
dihydroxybenzoic acid	245 ± 48	411 ± 81	804 ± 180	840 ± 167	972 ± 208	187 ± 35	396 ± 109	551 ± 121	515 ± 89	493 ± 72	597 ± 88	890 ± 82	917 ± 23	1069 ± 75	1435 ± 132	222 ± 85	486 ± 165	413 ± 116	553 ± 118	748 ± 144
gallic acid	101 ± 4	153 ± 11	207 ± 6	257 ± 12	306 ± 14	98 ± 5	145 ± 6	201 ± 5	252 ± 4	301 ± 4	117 ± 31	180 ± 41	227 ± 38	287 ± 38	346 ± 42	96 ± 11	146 ± 6	198 ± 15	250 ± 14	298 ± 14
<b>Total benzoic acids</b>	<b>507 ± 44</b>	<b>1002 ± 93</b>	<b>1512 ± 205</b>	<b>1628 ± 171</b>	<b>1947 ± 247</b>	<b>453 ± 28</b>	<b>815 ± 155</b>	<b>1075 ± 149</b>	<b>1186 ± 92</b>	<b>1244 ± 87</b>	<b>956 ± 103</b>	<b>1436 ± 120</b>	<b>1781 ± 104</b>	<b>2262 ± 92</b>	<b>3025 ± 268</b>	<b>447 ± 72</b>	<b>822 ± 121</b>	<b>878 ± 101</b>	<b>1144 ± 161</b>	<b>1442 ± 172</b>
4-hydroxyphenylpropionic acid	25 ± 11	48 ± 12	83 ± 8	112 ± 24	138 ± 16	15 ± 4	31 ± 8	53 ± 11	67 ± 15	82 ± 10	26 ± 6	55 ± 13	75 ± 8	92 ± 7	112 ± 4	16 ± 7	25 ± 8	42 ± 7	54 ± 9	65 ± 10
3-hydroxyphenylpropionic acid	77 ± 11	146 ± 14	238 ± 27	308 ± 43	360 ± 65	32 ± 6	60 ± 13	125 ± 26	186 ± 19	224 ± 35	122 ± 31	211 ± 69	262 ± 26	375 ± 67	500 ± 38	60 ± 7	88 ± 21	151 ± 13	138 ± 25	149 ± 19
<b>Total Phenylpropionic acids</b>	<b>102 ± 20</b>	<b>193 ± 25</b>	<b>321 ± 33</b>	<b>420 ± 48</b>	<b>498 ± 62</b>	<b>48 ± 10</b>	<b>91 ± 10</b>	<b>178 ± 36</b>	<b>253 ± 30</b>	<b>306 ± 44</b>	<b>148 ± 33</b>	<b>267 ± 79</b>	<b>337 ± 33</b>	<b>468 ± 65</b>	<b>612 ± 39</b>	<b>76 ± 14</b>	<b>113 ± 28</b>	<b>193 ± 14</b>	<b>193 ± 30</b>	<b>214 ± 27</b>
4-hydroxybenzaldehyde	34 ± 8	41 ± 9	43 ± 12	60 ± 5	59 ± 4	9 ± 3	19 ± 7	19 ± 5	22 ± 5	26 ± 4	12 ± 2	28 ± 9	26 ± 9	39 ± 11	41 ± 5	7 ± 1	10 ± 6	13 ± 4	29 ± 18	31 ± 12
3-hydroxy-4-methoxybenzaldehyde	1 ± 1	2 ± 1	2 ± 1	3 ± 1	4 ± 2	1 ± 1	4 ± 1	3 ± 2	6 ± 3	7 ± 2	5 ± 2	6 ± 1	7 ± 3	13 ± 6	19 ± 5	3 ± 2	3 ± 1	4 ± 3	7 ± 3	7 ± 2
<b>Total benzaldehydes</b>	<b>35 ± 8</b>	<b>43 ± 9</b>	<b>45 ± 12</b>	<b>63 ± 5</b>	<b>63 ± 3</b>	<b>10 ± 2</b>	<b>23 ± 8</b>	<b>22 ± 6</b>	<b>28 ± 3</b>	<b>33 ± 4</b>	<b>17 ± 3</b>	<b>33 ± 9</b>	<b>33 ± 7</b>	<b>52 ± 11</b>	<b>59 ± 5</b>	<b>9 ± 3</b>	<b>13 ± 5</b>	<b>17 ± 4</b>	<b>36 ± 20</b>	<b>38 ± 13</b>
quercetin-3-glucoside	29 ± 18	138 ± 25	204 ± 24	199 ± 14	219 ± 13	13 ± 10	22 ± 13	24 ± 7	30 ± 9	34 ± 9	3 ± 1	16 ± 4	20 ± 3	75 ± 34	151 ± 14	4 ± 2	6 ± 2	10 ± 3	12 ± 2	18 ± 4
cyanidin-3-glucoside	2 ± 1	4 ± 1	24 ± 4	115 ± 6	127 ± 7	n/d	n/d	n/d	n/d	n/d	n/d	n/d	n/d	n/d	n/d	n/d	n/d	n/d	n/d	n/d
peonidin-3-Glucoside	n/d	4 ± 2	6 ± 2	4 ± 1	4 ± 1	n/d	n/d	n/d	n/d	n/d	n/d	n/d	n/d	n/d	n/d	n/d	n/d	n/d	n/d	n/d
delphinidin-3-glucoside	n/d	28 ± 11	83 ± 13	113 ± 3	208 ± 12	4 ± 1	6 ± 1	9 ± 1	11 ± 1	13 ± 1	n/d	n/d	n/d	n/d	n/d	n/d	n/d	n/d	n/d	n/d
delphinidin-3-glucuronide	2 ± 1	4 ± 3	3 ± 2	6 ± 3	6 ± 2	6 ± 1	8 ± 1	11 ± 1	13 ± 1	15 ± 1	n/d	n/d	n/d	n/d	n/d	n/d	n/d	n/d	n/d	n/d
petunidin-3-Glucoside	2 ± 1	18 ± 5	53 ± 7	63 ± 6	61 ± 6	n/d	n/d	n/d	n/d	n/d	n/d	n/d	n/d	n/d	n/d	n/d	n/d	n/d	n/d	n/d
malvidin-3-glucoside	4 ± 1	34 ± 6	315 ± 42	413 ± 34	398 ± 29	n/d	n/d	n/d	n/d	n/d	n/d	n/d	n/d	n/d	n/d	n/d	n/d	n/d	n/d	n/d
<b>Total anthocyanins</b>	<b>10 ± 1</b>	<b>91 ± 17</b>	<b>483 ± 57</b>	<b>713 ± 38</b>	<b>804 ± 25</b>	<b>11 ± 1</b>	<b>14 ± 1</b>	<b>20 ± 2</b>	<b>24 ± 1</b>	<b>28 ± 1</b>	<b>n/d</b>	<b>n/d</b>	<b>n/d</b>	<b>n/d</b>	<b>n/d</b>	<b>n/d</b>	<b>n/d</b>	<b>n/d</b>	<b>n/d</b>	<b>n/d</b>

\*n/d indicates that the species was not detected

\*Data are expressed as mean ± standard deviation from n=4 independent assessments.

**Supplemental Table 3.5.** Content (umol/L of fermenta) of phenolic species over a 48-hour anaerobic microbial fermentation in Concord grape and 100% juices and relevant fecal-free control.

Compound (nanomol/mL)	Concord Juice					Concord Grape					Concord Grape Fecal Free Control				
	0 Hour	6 Hour	12 Hour	24 Hour	48 Hour	0 Hour	6 Hour	12 hour	24 Hour	48 Hour	0 Hour	6 Hour	12 hour	24 Hour	48 Hour
catechin	1.29 ± 0.33	0.91 ± 0.26	0.78 ± 0.23	0.51 ± 0.07	0.46 ± 0.05	16.85 ± 4.23	22.94 ± 6.61	15.39 ± 2.33	13.88 ± 1.77	12.42 ± 1.14	18.34 ± 6.51	14.83 ± 0.59	22.39 ± 0.91	11.84 ± 1.95	10.25 ± 1.1
epicatechin	0.91 ± 0.40	0.91 ± 0.26	0.52 ± 0.13	0.24 ± 0.05	0.21 ± 0.07	21.08 ± 6.63	32.20 ± 3.05	15.37 ± 2.47	12.24 ± 1.39	8.28 ± 1.79	13.57 ± 6.75	15.62 ± 2.81	21.57 ± 2.15	7.40 ± 1.80	4.33 ± 0.39
epigallocatechin	n/d	n/d	0.24 ± 0.06	0.09 ± 0.01	0.16 ± 0.04	0.80 ± 0.20	2.29 ± 0.38	1.48 ± 0.18	1.53 ± 0.45	1.81 ± 0.23	0.51 ± 0.19	0.50 ± 0.04	1.00 ± 0.31	0.99 ± 0.27	2.20 ± 0.24
epicatechin gallate	0.04 ± 0.04	0.11 ± 0.14	0.09 ± 0.04	0.11 ± 0.07	0.02 ± 0.01	2.11 ± 0.98	2.76 ± 0.48	2.57 ± 0.57	2.60 ± 0.10	1.52 ± 0.77	2.18 ± 0.18	2.45 ± 1.36	3.15 ± 0.13	1.78 ± 0.08	2.60 ± 0.31
procyanidin B2	0.23 ± 0.09	0.12 ± 0.10	0.21 ± 0.16	n/d	n/d	5.34 ± 0.68	7.11 ± 2.30	2.78 ± 0.69	3.13 ± 0.22	1.99 ± 0.31	14.35 ± 0.77	5.87 ± 0.46	6.98 ± 0.34	6.59 ± 0.31	9.32 ± 1.09
p-coumaric acid	4.02 ± 0.17	7.94 ± 0.41	0.27 ± 0.01	0.26 ± 0.01	0.24 ± 0.01	2.21 ± 0.25	5.61 ± 0.62	0.36 ± 0.01	0.29 ± 0.02	0.34 ± 0.02	0.28 ± 0.01	0.39 ± 0.04	0.40 ± 0.04	0.34 ± 0.02	0.38 ± 0.02
gallic acid	2.36 ± 0.15	2.48 ± 0.22	0.27 ± 0.02	0.30 ± 0.04	0.27 ± 0.01	1.94 ± 0.03	2.46 ± 0.06	0.58 ± 0.04	0.46 ± 0.04	0.28 ± 0.02	1.20 ± 0.04	1.06 ± 0.04	0.46 ± 0.21	0.29 ± 0.01	0.26 ± 0.01
caffeic acid	0.34 ± 0.03	0.60 ± 0.06	n/d	n/d	n/d	0.24 ± 0.02	0.86 ± 0.09	0.24 ± 0.01	0.23 ± 0.01	n/d	n/d	0.24 ± 0.01	0.26 ± 0.01	0.26 ± 0.01	0.24 ± 0.01
ferulic acid	0.41 ± 0.06	0.45 ± 0.06	n/d	n/d	n/d	0.60 ± 0.11	0.87 ± 0.11	n/d	0.23 ± 0.01	0.24 ± 0.01	0.51 ± 0.08	0.41 ± 0.01	0.52 ± 0.07	n/d	n/d
resveratrol	n/d	n/d	n/d	n/d	n/d	n/d	n/d	n/d	n/d	n/d	n/d	n/d	n/d	n/d	n/d
resveratrol-3-glucoside	0.04 ± 0.01	0.02 ± 0.01	0.04 ± 0.02	0.01 ± 0.01	n/d	n/d	n/d	n/d	n/d	n/d	n/d	n/d	n/d	n/d	n/d
quercetin	0.67 ± 0.10	0.95 ± 0.09	1.30 ± 0.26	0.85 ± 0.15	0.02 ± 0.01	0.53 ± 0.17	1.05 ± 0.11	1.57 ± 0.21	0.83 ± 0.16	0.62 ± 0.03	0.70 ± 0.03	0.06 ± 0.03	0.43 ± 0.04	0.92 ± 0.03	0.62 ± 0.01
myricetin	0.01 ± 0.01	0.05 ± 0.03	0.05 ± 0.03	0.05 ± 0.03	0.01 ± 0.01	0.01 ± 0.01	0.03 ± 0.01	0.05 ± 0.02	0.08 ± 0.01	n/d	n/d	n/d	n/d	n/d	n/d
quercetin-3-glucoside	0.58 ± 0.07	0.12 ± 0.02	0.01 ± 0.01	n/d	n/d	0.35 ± 0.09	0.07 ± 0.02	0.01 ± 0.01	n/d	n/d	0.63 ± 0.21	0.59 ± 0.18	0.55 ± 0.04	0.56 ± 0.07	0.80 ± 0.03
myricetin-3-glucoside	0.25 ± 0.01	0.02 ± 0.01	0.01 ± 0.01	0.01 ± 0.01	n/d	0.03 ± 0.01	0.02 ± 0.01	0.01 ± 0.01	0.01 ± 0.01	0.02 ± 0.01	0.03 ± 0.01	0.05 ± 0.01	n/d	n/d	n/d
quercetin-3-rutinoside	0.09 ± 0.06	n/d	n/d	n/d	n/d	0.88 ± 0.40	0.10 ± 0.07	n/d	n/d	n/d	0.79 ± 0.46	0.78 ± 0.20	n/d	n/d	n/d
peonidin-3-arabinoside	0.06 ± 0.02	n/d	n/d	n/d	n/d	0.02 ± 0.01	n/d	n/d	n/d	n/d	0.03 ± 0.01	n/d	n/d	n/d	n/d
delphinidin-3-arabinoside	0.01 ± 0.01	n/d	n/d	n/d	n/d	0.08 ± 0.01	0.07 ± 0.01	0.07 ± 0.01	n/d	n/d	0.05 ± 0.01	n/d	0.01 ± 0.01	n/d	n/d
petunidin-3-arabinoside	0.03 ± 0.01	0.01 ± 0.01	0.01 ± 0.01	n/d	n/d	0.02 ± 0.01	0.01 ± 0.01	0.01 ± 0.01	n/d	n/d	0.03 ± 0.01	0.01 ± 0.01	0.01 ± 0.01	n/d	n/d
malvidin-3-arabinoside	n/d	0.03 ± 0.02	0.04 ± 0.01	n/d	n/d	0.01 ± 0.01	0.02 ± 0.01	0.04 ± 0.01	n/d	n/d	n/d	0.03 ± 0.01	0.04 ± 0.01	n/d	n/d
cyanidin-3-glucoside	1.77 ± 0.03	1.19 ± 0.21	0.05 ± 0.01	n/d	n/d	0.33 ± 0.13	0.39 ± 0.10	0.10 ± 0.01	0.01 ± 0.01	n/d	0.34 ± 0.03	0.58 ± 0.01	0.35 ± 0.07	0.21 ± 0.02	0.19 ± 0.02
peonidin-3-glucoside	0.23 ± 0.01	0.12 ± 0.02	n/d	n/d	n/d	0.09 ± 0.06	0.02 ± 0.01	n/d	n/d	n/d	0.08 ± 0.01	0.04 ± 0.01	0.08 ± 0.06	0.10 ± 0.11	0.03 ± 0.01
delphinidin-3-glucoside	0.05 ± 0.03	0.31 ± 0.19	0.11 ± 0.06	n/d	n/d	1.80 ± 0.14	1.47 ± 0.75	0.71 ± 0.29	n/d	n/d	1.79 ± 0.33	1.15 ± 0.14	0.73 ± 0.15	0.24 ± 0.26	0.05 ± 0.01
petunidin-3-glucoside	0.18 ± 0.01	0.19 ± 0.02	0.02 ± 0.01	n/d	n/d	0.26 ± 0.07	0.36 ± 0.13	0.18 ± 0.11	n/d	n/d	0.17 ± 0.03	0.19 ± 0.05	0.31 ± 0.08	0.16 ± 0.06	0.12 ± 0.01
cyanidin-3-galactoside	0.31 ± 0.02	0.47 ± 0.06	0.04 ± 0.01	n/d	n/d	0.03 ± 0.01	0.01 ± 0.01	n/d	n/d	n/d	0.03 ± 0.01	0.03 ± 0.01	0.09 ± 0.01	0.09 ± 0.01	0.08 ± 0.01
peonidin-3-galactoside	n/d	0.01 ± 0.01	n/d	n/d	n/d	n/d	1.08 ± 0.31	0.25 ± 0.10	n/d	n/d	n/d	0.18 ± 0.10	0.13 ± 0.04	0.09 ± 0.06	n/d
delphinidin-3-galactoside	n/d	n/d	n/d	n/d	n/d	n/d	n/d	n/d	n/d	n/d	n/d	0.32 ± 0.03	0.93 ± 0.13	0.63 ± 0.69	0.13 ± 0.01
cyanidin (acylated)	0.95 ± 0.52	0.27 ± 0.13	n/d	n/d	n/d	0.61 ± 0.25	0.03 ± 0.01	0.05 ± 0.02	n/d	n/d	1.00 ± 0.72	0.45 ± 0.12	0.26 ± 0.07	0.13 ± 0.02	0.12 ± 0.01
peonidin (acylated)	5.77 ± 0.25	1.29 ± 0.63	0.16 ± 0.04	n/d	n/d	1.59 ± 0.46	0.28 ± 0.03	0.14 ± 0.07	n/d	n/d	3.43 ± 1.33	1.69 ± 0.24	1.56 ± 0.56	0.24 ± 0.26	0.06 ± 0.01
delphinidin (acylated)	1.19 ± 0.78	0.36 ± 0.23	0.12 ± 0.12	n/d	n/d	3.22 ± 0.86	0.77 ± 0.23	0.19 ± 0.03	n/d	n/d	5.19 ± 2.86	2.90 ± 0.54	2.76 ± 0.66	0.98 ± 1.05	0.21 ± 0.02
petunidin (acylated)	0.43 ± 0.15	0.19 ± 0.02	0.14 ± 0.01	n/d	n/d	0.09 ± 0.05	0.18 ± 0.02	0.13 ± 0.01	n/d	n/d	0.42 ± 0.08	0.15 ± 0.01	0.22 ± 0.02	0.17 ± 0.03	0.13 ± 0.01
malvidin (acylated)	3.85 ± 0.07	1.46 ± 0.08	n/d	n/d	n/d	1.73 ± 0.13	0.51 ± 0.23	0.07 ± 0.03	n/d	n/d	3.46 ± 1.26	1.79 ± 0.37	1.74 ± 0.52	0.25 ± 0.20	0.11 ± 0.01
phenylacetic acid	n/d	4.71 ± 1.46	10.80 ± 2.70	17.50 ± 6.85	12.00 ± 2.85	n/d	5.60 ± 1.18	4.48 ± 0.78	7.48 ± 1.25	8.29 ± 2.49	n/d	n/d	n/d	n/d	n/d
3-hydroxyphenyl acetic acid	19.69 ± 2.01	40.04 ± 2.61	38.98 ± 6.45	33.95 ± 5.04	11.02 ± 2.84	14.86 ± 3.20	39.71 ± 4.06	36.32 ± 1.36	22.14 ± 4.36	15.01 ± 1.58	19.52 ± 6.79	11.14 ± 1.06	12.30 ± 0.94	10.47 ± 0.60	8.67 ± 0.75
4-hydroxybenzoic acid	0.07 ± 0.04	2.59 ± 0.14	2.03 ± 0.16	0.34 ± 0.07	0.20 ± 0.05	0.06 ± 0.01	2.76 ± 0.35	1.35 ± 0.11	0.25 ± 0.02	0.16 ± 0.03	0.12 ± 0.03	n/d	n/d	n/d	n/d
3-hydroxybenzoic acid	n/d	1.04 ± 0.08	1.41 ± 0.15	3.25 ± 0.18	1.98 ± 0.05	n/d	1.01 ± 0.07	1.34 ± 0.15	3.69 ± 0.24	2.54 ± 0.1	n/d	n/d	n/d	n/d	n/d
3,4-dihydroxybenzoic acid	n/d	0.09 ± 0.02	0.15 ± 0.05	0.29 ± 0.02	0.05 ± 0.03	n/d	0.09 ± 0.01	0.08 ± 0.03	0.10 ± 0.01	n/d	n/d	n/d	n/d	n/d	n/d
dihydroxybenzoic acid isomer	0.04 ± 0.01	0.05 ± 0.01	0.04 ± 0.01	0.03 ± 0.01	0.56 ± 0.04	0.04 ± 0.01	0.05 ± 0.01	0.04 ± 0.01	0.03 ± 0.01	0.46 ± 0.04	0.05 ± 0.01	0.04 ± 0.01	0.03 ± 0.01	0.52 ± 0.06	0.44 ± 0.05
3,4dHP- γ-valerolactone	n/d	0.03 ± 0.01	0.07 ± 0.01	0.07 ± 0.01	0.05 ± 0.01	n/d	0.05 ± 0.01	0.31 ± 0.11	0.15 ± 0.03	0.17 ± 0.02	n/d	n/d	n/d	n/d	n/d
3-hydroxyphenylpropionic acid	n/d	n/d	0.31 ± 0.69	3.72 ± 1.94	6.22 ± 2.89	n/d	n/d	3.68 ± 2.27	15.66 ± 1.32	20.19 ± 2.27	n/d	n/d	n/d	0.31 ± 0.15	3.62 ± 2.94
3OH-4MEOHphenylpropionic acid	0.03 ± 0.01	0.09 ± 0.01	0.07 ± 0.02	0.01 ± 0.01	n/d	0.04 ± 0.01	0.21 ± 0.05	0.17 ± 0.04	n/d	n/d	0.08 ± 0.06	0.02 ± 0.01	n/d	n/d	n/d
4OH-3,5diMEOHbenzaldehyde	n/d	0.03 ± 0.01	0.02 ± 0.01	0.04 ± 0.02	n/d	n/d	0.03 ± 0.01	0.02 ± 0.01	n/d	n/d	n/d	n/d	n/d	n/d	n/d
4-hydroxyphenylpropionic acid	n/d	n/d	n/d	0.24 ± 0.04	0.03 ± 0.01	n/d	0.08 ± 0.03	0.08 ± 0.02	0.05 ± 0.01	0.03 ± 0.01	n/d	n/d	n/d	n/d	n/d
hippuric acid	0.34 ± 0.07	0.89 ± 0.17	0.88 ± 0.25	0.20 ± 0.04	n/d	0.11 ± 0.04	0.58 ± 0.20	0.43 ± 0.07	0.05 ± 0.06	n/d	0.62 ± 0.16	0.15 ± 0.05	0.77 ± 0.18	0.13 ± 0.03	n/d
4-hydroxybenzaldehyde	0.61 ± 0.07	0.58 ± 0.03	0.09 ± 0.03	0.06 ± 0.01	0.05 ± 0.02	0.44 ± 0.05	1.01 ± 0.26	0.21 ± 0.06	0.06 ± 0.01	0.06 ± 0.02	n/d	0.30 ± 0.04	0.57 ± 0.16	0.14 ± 0.03	0.03 ± 0.01
γ-valerolactone	n/d	n/d	n/d	0.15 ± 0.05	0.42 ± 0.03	n/d	n/d	n/d	0.31 ± 0.13	1.02 ± 0.20	n/d	n/d	n/d	n/d	n/d

\*n/d indicates that the species was not detected

\*Data are expressed as mean ± standard deviation from n=4 independent assessments.

**Supplemental Table 3.6.** Content (umol/L of fermenta) of phenolic species over a 48-hour anaerobic microbial fermentation in Niagara grape and 100% juices and relevant fecal-free control and no treatment controls.

Compound (nanomol/mL)	Niagara Juice					Niagara Grape					Niagara Grape Fecal Free Control					Treatment Free Control				
	0 Hour	6 Hour	12 hour	24 Hour	48 Hour	0 Hour	6 Hour	12 hour	24 Hour	48 Hour	0 Hour	6 Hour	12 hour	24 Hour	48 Hour	0 Hour	6 Hour	12 hour	24 Hour	48 Hour
catechin	20.41 ± 2.75	17.06 ± 2.32	3.14 ± 0.62	1.62 ± 0.24	1.72 ± 9.66	15.13 ± 2.73	15.78 ± 4.33	20.49 ± 1.45	8.60 ± 1.60	11.12 ± 2.35	13.91 ± 4.46	16.42 ± 5.66	16.87 ± 2.39	9.93 ± 0.77	9.33 ± 3.07	n/d	n/d	n/d	n/d	n/d
epicatechin	16.87 ± 1.00	12.79 ± 1.75	5.34 ± 0.53	0.62 ± 0.08	0.64 ± 0.19	5.64 ± 0.64	10.54 ± 1.98	6.09 ± 1.11	3.44 ± 0.66	7.90 ± 0.63	7.29 ± 1.24	10.77 ± 1.61	15.08 ± 1.16	5.46 ± 0.25	4.83 ± 1.11	n/d	n/d	n/d	n/d	n/d
epigallocatechin	0.10 ± 0.05	0.11 ± 0.03	0.13 ± 0.07	0.13 ± 0.07	0.17 ± 0.09	0.11 ± 0.03	2.18 ± 0.28	3.16 ± 0.15	3.35 ± 0.45	4.54 ± 0.40	0.12 ± 0.04	0.13 ± 0.06	1.10 ± 0.75	0.25 ± 0.07	0.56 ± 0.04	n/d	n/d	n/d	n/d	n/d
epicatechin gallate	1.09 ± 0.44	1.43 ± 0.14	2.97 ± 0.38	0.70 ± 0.19	0.08 ± 0.02	2.11 ± 0.33	1.90 ± 0.23	2.67 ± 0.34	2.08 ± 0.23	1.33 ± 0.18	1.86 ± 0.83	0.29 ± 0.11	0.49 ± 0.05	0.75 ± 0.31	1.46 ± 0.26	n/d	n/d	n/d	n/d	n/d
procyanidin B2	1.25 ± 0.11	0.85 ± 0.21	0.34 ± 0.10	0.12 ± 0.04	0.03 ± 0.01	14.47 ± 1.53	5.70 ± 0.08	4.32 ± 0.36	1.57 ± 0.11	1.98 ± 0.21	6.61 ± 1.79	3.14 ± 0.18	4.44 ± 0.36	3.03 ± 0.50	4.79 ± 2.10	n/d	n/d	n/d	n/d	n/d
p-coumaric acid	0.41 ± 0.06	0.80 ± 0.12	0.26 ± 0.01	0.24 ± 0.01	0.26 ± 0.01	0.27 ± 0.01	0.50 ± 0.06	0.24 ± 0.01	0.24 ± 0.01	0.25 ± 0.01	0.79 ± 0.08	0.31 ± 0.02	0.40 ± 0.02	0.28 ± 0.01	0.28 ± 0.01	n/d	n/d	n/d	n/d	n/d
gallic acid	0.89 ± 0.09	1.24 ± 0.06	0.40 ± 0.02	0.34 ± 0.02	0.25 ± 0.01	1.34 ± 0.26	1.66 ± 0.29	1.32 ± 0.26	0.36 ± 0.01	0.62 ± 0.09	1.16 ± 0.01	1.06 ± 0.02	0.41 ± 0.01	0.28 ± 0.04	0.36 ± 0.03	0.17 ± 0.01	n/d	n/d	n/d	n/d
caffeic acid	0.36 ± 0.06	1.47 ± 0.07	0.32 ± 0.03	0.26 ± 0.04	n/d	n/d	0.36 ± 0.03	0.24 ± 0.01	0.23 ± 0.01	n/d	n/d	n/d	0.24 ± 0.01	0.30 ± 0.06	0.23 ± 0.01	n/d	n/d	n/d	n/d	n/d
ferulic acid	0.37 ± 0.04	0.47 ± 0.06	n/d	0.21 ± 0.01	0.21 ± 0.01	0.43 ± 0.02	0.74 ± 0.15	n/d	0.24 ± 0.01	0.25 ± 0.02	0.35 ± 0.01	0.31 ± 0.01	0.47 ± 0.06	n/d	n/d	0.05 ± 0.01	0.04 ± 0.01	n/d	n/d	n/d
resveratrol	0.04 ± 0.02	0.02 ± 0.02	n/d	n/d	n/d	n/d	n/d	n/d	n/d	n/d	n/d	n/d	n/d	n/d	n/d	n/d	n/d	n/d	n/d	n/d
resveratrol-3-glucoside	0.05 ± 0.01	0.03 ± 0.01	0.02 ± 0.01	0.03 ± 0.02	n/d	0.81 ± 0.21	4.04 ± 0.90	6.70 ± 0.87	1.68 ± 0.22	0.05 ± 0.02	n/d	n/d	n/d	n/d	n/d	n/d	n/d	n/d	n/d	n/d
quercetin	1.07 ± 0.20	2.40 ± 0.35	3.27 ± 0.31	2.19 ± 0.23	0.02 ± 0.01	0.81 ± 0.21	4.04 ± 0.90	6.70 ± 0.87	1.68 ± 0.22	0.05 ± 0.02	0.67 ± 0.06	0.28 ± 0.04	1.02 ± 0.02	0.99 ± 0.01	1.00 ± 0.03	n/d	n/d	n/d	n/d	n/d
myricetin	n/d	n/d	n/d	n/d	n/d	n/d	n/d	n/d	n/d	n/d	n/d	n/d	n/d	n/d	n/d	n/d	n/d	n/d	n/d	n/d
quercetin-3-glucoside	0.48 ± 0.05	0.14 ± 0.04	0.02 ± 0.01	n/d	n/d	1.49 ± 0.32	0.40 ± 0.05	0.09 ± 0.03	n/d	n/d	1.82 ± 0.04	1.50 ± 0.22	1.64 ± 0.04	0.96 ± 0.17	1.48 ± 0.13	n/d	n/d	n/d	n/d	n/d
myricetin-3-glucoside	n/d	n/d	n/d	n/d	n/d	n/d	n/d	n/d	n/d	n/d	n/d	n/d	n/d	n/d	n/d	n/d	n/d	n/d	n/d	n/d
quercetin-3-rutinoside	n/d	n/d	n/d	n/d	n/d	n/d	n/d	n/d	n/d	n/d	n/d	n/d	n/d	n/d	n/d	n/d	n/d	n/d	n/d	n/d
peonidin-3-arabinoside	n/d	n/d	n/d	n/d	n/d	n/d	n/d	n/d	n/d	n/d	n/d	n/d	n/d	n/d	n/d	n/d	n/d	n/d	n/d	n/d
delphinidin-3-arabinoside	n/d	n/d	n/d	n/d	n/d	n/d	n/d	n/d	n/d	n/d	n/d	n/d	n/d	n/d	n/d	n/d	n/d	n/d	n/d	n/d
petunidin-3-arabinoside	n/d	n/d	n/d	n/d	n/d	n/d	n/d	n/d	n/d	n/d	n/d	n/d	n/d	n/d	n/d	n/d	n/d	n/d	n/d	n/d
malvidin-3-arabinoside	n/d	n/d	n/d	n/d	n/d	n/d	n/d	n/d	n/d	n/d	n/d	n/d	n/d	n/d	n/d	n/d	n/d	n/d	n/d	n/d
cyanidin-3-glucoside	n/d	n/d	n/d	n/d	n/d	n/d	n/d	n/d	n/d	n/d	n/d	n/d	n/d	n/d	n/d	n/d	n/d	n/d	n/d	n/d
peonidin-3-glucoside	n/d	n/d	n/d	n/d	n/d	n/d	n/d	n/d	n/d	n/d	n/d	n/d	n/d	n/d	n/d	n/d	n/d	n/d	n/d	n/d
delphinidin-3-glucoside	n/d	n/d	n/d	n/d	n/d	n/d	n/d	n/d	n/d	n/d	n/d	n/d	n/d	n/d	n/d	n/d	n/d	n/d	n/d	n/d
petunidin-3-glucoside	n/d	n/d	n/d	n/d	n/d	n/d	n/d	n/d	n/d	n/d	n/d	n/d	n/d	n/d	n/d	n/d	n/d	n/d	n/d	n/d
cyanidin-3-galactoside	n/d	n/d	n/d	n/d	n/d	n/d	n/d	n/d	n/d	n/d	n/d	n/d	n/d	n/d	n/d	n/d	n/d	n/d	n/d	n/d
peonidin-3-galactoside	n/d	n/d	n/d	n/d	n/d	n/d	n/d	n/d	n/d	n/d	n/d	n/d	n/d	n/d	n/d	n/d	n/d	n/d	n/d	n/d
delphinidin-3-galactoside	n/d	n/d	n/d	n/d	n/d	n/d	n/d	n/d	n/d	n/d	n/d	n/d	n/d	n/d	n/d	n/d	n/d	n/d	n/d	n/d
cyanidin (acylated)	n/d	n/d	n/d	n/d	n/d	n/d	n/d	n/d	n/d	n/d	n/d	n/d	n/d	n/d	n/d	n/d	n/d	n/d	n/d	n/d
peonidin (acylated)	n/d	n/d	n/d	n/d	n/d	n/d	n/d	n/d	n/d	n/d	n/d	n/d	n/d	n/d	n/d	n/d	n/d	n/d	n/d	n/d
delphinidin (acylated)	n/d	n/d	n/d	n/d	n/d	n/d	n/d	n/d	n/d	n/d	n/d	n/d	n/d	n/d	n/d	n/d	n/d	n/d	n/d	n/d
petunidin (acylated)	n/d	n/d	n/d	n/d	n/d	n/d	n/d	n/d	n/d	n/d	n/d	n/d	n/d	n/d	n/d	n/d	n/d	n/d	n/d	n/d
malvidin (acylated)	n/d	n/d	n/d	n/d	n/d	n/d	n/d	n/d	n/d	n/d	n/d	n/d	n/d	n/d	n/d	n/d	n/d	n/d	n/d	n/d
phenylacetic acid	n/d	8.74 ± 1.35	12.88 ± 1.19	18.82 ± 4.54	19.95 ± 7.21	n/d	6.51 ± 1.31	5.46 ± 1.46	2.99 ± 0.45	10.83 ± 2.52	n/d	n/d	n/d	n/d	n/d	n/d	0.22 ± 0.04	n/d	n/d	n/d
3-hydroxyphenyl acetic acid	18.85 ± 3.45	44.61 ± 3.96	42.54 ± 1.77	34.41 ± 2.77	22.91 ± 3.56	15.19 ± 3.73	44.55 ± 3.22	36.91 ± 3.26	16.90 ± 4.02	15.90 ± 4.08	14.44 ± 1.84	9.71 ± 0.72	11.35 ± 0.17	9.22 ± 0.37	7.85 ± 0.99	n/d	4.61 ± 0.53	4.87 ± 0.52	8.43 ± 0.59	13.64 ± 5.79
4-hydroxybenzoic acid	0.05 ± 0.03	3.64 ± 0.67	3.00 ± 0.30	0.53 ± 0.07	0.12 ± 0.02	0.04 ± 0.02	3.87 ± 0.23	1.88 ± 0.30	0.15 ± 0.02	0.18 ± 0.02	0.07 ± 0.01	n/d	n/d	n/d	n/d	n/d	n/d	n/d	n/d	n/d
3-hydroxybenzoic acid	n/d	1.12 ± 0.22	1.38 ± 0.09	3.32 ± 0.16	1.90 ± 0.42	n/d	1.18 ± 0.11	1.49 ± 0.29	2.91 ± 0.22	2.68 ± 0.38	n/d	n/d	n/d	n/d	n/d	n/d	n/d	n/d	n/d	n/d
3,4-dihydroxybenzoic acid	n/d	0.13 ± 0.03	0.11 ± 0.01	0.04 ± 0.01	n/d	n/d	0.11 ± 0.02	0.06 ± 0.01	0.02 ± 0.01	n/d	n/d	n/d	n/d	n/d	n/d	n/d	n/d	n/d	n/d	n/d
dihydroxybenzoic acid isomer	0.05 ± 0.01	0.04 ± 0.01	0.03 ± 0.02	0.03 ± 0.01	0.52 ± 0.02	0.04 ± 0.01	0.05 ± 0.01	0.04 ± 0.01	0.03 ± 0.01	0.48 ± 0.04	0.05 ± 0.01	n/d	0.03 ± 0.01	0.48 ± 0.08	0.49 ± 0.12	0.01 ± 0.01	0.01 ± 0.01	n/d	n/d	n/d
3,4dihp- γ-valerolactone	n/d	0.05 ± 0.01	0.09 ± 0.01	0.07 ± 0.01	0.05 ± 0.01	n/d	0.04 ± 0.01	0.23 ± 0.02	0.11 ± 0.04	0.16 ± 0.06	n/d	n/d	n/d	n/d	n/d	n/d	n/d	n/d	n/d	n/d
3-hydroxyphenylpropionic acid	n/d	n/d	0.59 ± 0.43	1.92 ± 1.03	7.06 ± 1.09	n/d	n/d	2.73 ± 1.55	4.49 ± 0.93	9.36 ± 1.31	n/d	n/d	n/d	0.20 ± 0.37	2.33 ± 0.87	n/d	n/d	n/d	n/d	n/d
3OH-4MEOHphenylpropionic acid	0.02 ± 0.01	0.13 ± 0.03	0.08 ± 0.01	n/d	n/d	0.03 ± 0.01	0.13 ± 0.04	0.14 ± 0.02	n/d	n/d	0.05 ± 0.01	0.02 ± 0.02	n/d	n/d	n/d	n/d	n/d	n/d	n/d	n/d
4OH-3,5diMEOHbenzaldehyde	n/d	n/d	0.02 ± 0.01	n/d	n/d	n/d	0.04 ± 0.01	0.02 ± 0.01	n/d	n/d	n/d	n/d	n/d	n/d	n/d	n/d	n/d	n/d	n/d	n/d
4-hydroxyphenylpropionic acid	n/d	0.03 ± 0.01	0.11 ± 0.02	0.42 ± 0.06	n/d	n/d	0.08 ± 0.02	0.08 ± 0.01	0.04 ± 0.01	n/d	n/d	n/d	n/d	n/d	n/d	n/d	n/d	n/d	n/d	n/d
hippuric acid	0.33 ± 0.17	0.92 ± 0.21	0.93 ± 0.16	0.30 ± 0.21	n/d	0.12 ± 0.13	0.85 ± 0.09	0.54 ± 0.13	0.01 ± 0.02	n/d	0.45 ± 0.032	0.14 ± 0.13	1.13 ± 0.13	0.24 ± 0.17	n/d	n/d	n/d	n/d	n/d	n/d
4-hydroxybenzaldehyde	0.46 ± 0.07	0.70 ± 0.12	0.11 ± 0.02	0.04 ± 0.01	0.03 ± 0.01	0.48 ± 0.05	0.57 ± 0.09	0.18 ± 0.03	0.06 ± 0.01	0.06 ± 0.02	n/d	0.32 ± 0.01	0.39 ± 0.10	0.06 ± 0.02	0.07 ± 0.02	n/d	n/d	n/d	n/d	n/d
γ-valerolactone	n/d	n/d	n/d	0.09 ± 0.02	0.27 ± 0.07	n/d	n/d	n/d	0.29 ± 0.07	1.56 ± 0.10	n/d	n/d	n/d	n/d	n/d	n/d	n/d	n/d	n/d	n/d

\*n/d indicates that the species was not detected

\*Data are expressed as mean ± standard deviation from n=4 independent assessments.

**Supplemental Table 3.7.** 12-hour fermenta treatment cellular transport (nanomol/L of basolateral media) of phenolic species in Concord and Niagara grape and 100% juice samples.

Compound (picomol/mL)	Concord Juice					Concord Grape					Niagara Juice					Niagara Grape				
	5 minutes	15 minutes	30 minutes	60 minutes	120 minutes	5 minutes	15 minutes	30 minutes	60 minutes	120 minutes	5 minutes	15 minutes	30 minutes	60 minutes	120 minutes	5 minutes	15 minutes	30 minutes	60 minutes	120 minutes
phenylacetic acid	n/d	835 ± 281	1189 ± 444	1664 ± 433	1952 ± 213	n/d	480 ± 73	1031 ± 260	1768 ± 239	2412 ± 298	n/d	543 ± 126	778 ± 150	936 ± 212	1276 ± 146	n/d	541 ± 92	778 ± 167	1099 ± 52	1304 ± 76
3-methoxyphenylacetic acid	175 ± 14	269 ± 15	360 ± 21	465 ± 15	563 ± 19	188 ± 14	306 ± 33	409 ± 29	520 ± 33	626 ± 45	162 ± 4	245 ± 5	396 ± 82	509 ± 94	612 ± 110	170 ± 3	282 ± 12	400 ± 10	506 ± 16	603 ± 20
3-hydroxyphenylacetic acid	265 ± 35	469 ± 66	724 ± 222	1146 ± 272	1985 ± 461	159 ± 92	1188 ± 250	1148 ± 219	1767 ± 215	2263 ± 298	323 ± 112	568 ± 153	788 ± 262	1133 ± 395	1647 ± 333	272 ± 63	790 ± 318	1061 ± 249	1198 ± 225	1551 ± 165
<b>Total phenylacetic acids</b>	<b>440 ± 41</b>	<b>1572 ± 325</b>	<b>2273 ± 544</b>	<b>3275 ± 591</b>	<b>4500 ± 584</b>	<b>251 ± 99</b>	<b>1974 ± 231</b>	<b>2588 ± 164</b>	<b>4055 ± 110</b>	<b>5302 ± 325</b>	<b>486 ± 115</b>	<b>1355 ± 105</b>	<b>1963 ± 193</b>	<b>2578 ± 613</b>	<b>3535 ± 476</b>	<b>442 ± 60</b>	<b>1613 ± 379</b>	<b>2240 ± 369</b>	<b>2804 ± 244</b>	<b>3458 ± 109</b>
4-methoxybenzoic acid	9 ± 9	18 ± 11	39 ± 26	43 ± 24	54 ± 33	7 ± 1	15 ± 4	31 ± 4	42 ± 5	61 ± 6	3 ± 1	15 ± 6	32 ± 9	45 ± 18	61 ± 10	5 ± 1	10 ± 1	28 ± 2	31 ± 3	32 ± 4
4-hydroxybenzoic acid	121 ± 10	273 ± 15	411 ± 33	549 ± 23	745 ± 54	111 ± 14	226 ± 56	336 ± 112	409 ± 92	499 ± 116	140 ± 69	250 ± 68	385 ± 122	491 ± 186	696 ± 253	102 ± 8	177 ± 37	277 ± 29	421 ± 28	520 ± 34
3-hydroxybenzoic acid	20 ± 3	81 ± 14	106 ± 23	146 ± 24	184 ± 29	23 ± 14	79 ± 26	128 ± 24	181 ± 16	242 ± 46	38 ± 17	82 ± 4	146 ± 24	193 ± 42	243 ± 50	25 ± 8	69 ± 7	88 ± 10	129 ± 15	155 ± 18
gallic acid	89 ± 9	141 ± 1	191 ± 6	245 ± 5	293 ± 3	108 ± 12	167 ± 12	216 ± 18	276 ± 24	336 ± 46	93 ± 6	141 ± 10	192 ± 13	251 ± 26	295 ± 18	116 ± 3	174 ± 6	233 ± 16	318 ± 12	355 ± 6
<b>Total benzoic acids</b>	<b>238 ± 8</b>	<b>513 ± 14</b>	<b>746 ± 60</b>	<b>983 ± 41</b>	<b>1276 ± 61</b>	<b>249 ± 13</b>	<b>487 ± 36</b>	<b>711 ± 110</b>	<b>907 ± 119</b>	<b>1138 ± 179</b>	<b>275 ± 82</b>	<b>488 ± 70</b>	<b>755 ± 140</b>	<b>980 ± 226</b>	<b>1296 ± 296</b>	<b>248 ± 2</b>	<b>430 ± 27</b>	<b>627 ± 32</b>	<b>898 ± 31</b>	<b>1062 ± 46</b>
3-hydroxyphenylpropionic acid	204 ± 38	457 ± 106	744 ± 172	1016 ± 229	1317 ± 207	573 ± 89	1148 ± 104	1318 ± 123	1829 ± 209	2264 ± 252	198 ± 45	335 ± 42	608 ± 90	986 ± 98	1513 ± 100	329 ± 41	681 ± 90	1151 ± 173	1685 ± 173	2110 ± 174
4-hydroxyphenylpropionic acid	79 ± 5	150 ± 4	223 ± 10	314 ± 22	394 ± 26	122 ± 25	219 ± 48	341 ± 49	423 ± 78	515 ± 71	54 ± 18	124 ± 29	199 ± 40	262 ± 51	321 ± 52	79 ± 27	135 ± 36	219 ± 5	356 ± 11	431 ± 24
<b>Total phenylpropionic acids</b>	<b>284 ± 41</b>	<b>606 ± 106</b>	<b>967 ± 173</b>	<b>1330 ± 247</b>	<b>1711 ± 226</b>	<b>695 ± 111</b>	<b>1366 ± 144</b>	<b>1660 ± 159</b>	<b>2252 ± 286</b>	<b>2778 ± 323</b>	<b>251 ± 54</b>	<b>459 ± 61</b>	<b>807 ± 124</b>	<b>1248 ± 123</b>	<b>1834 ± 143</b>	<b>408 ± 66</b>	<b>816 ± 74</b>	<b>1370 ± 173</b>	<b>2041 ± 168</b>	<b>2541 ± 181</b>
4-hydroxybenzaldehyde	6 ± 2	17 ± 5	24 ± 6	37 ± 10	40 ± 8	6 ± 7	12 ± 6	16 ± 8	21 ± 8	29 ± 18	3 ± 1	5 ± 2	20 ± 21	27 ± 25	28 ± 25	17 ± 4	29 ± 6	36 ± 3	47 ± 1	63 ± 3
3-hydroxy-4-methoxybenzaldehyde	4 ± 2	8 ± 6	16 ± 12	21 ± 9	26 ± 17	122 ± 25	219 ± 48	341 ± 49	423 ± 78	515 ± 71	2 ± 2	8 ± 3	16 ± 6	21 ± 6	23 ± 4	1 ± 1	4 ± 1	9 ± 2	16 ± 1	18 ± 1
<b>Total benzaldehydes</b>	<b>10 ± 1</b>	<b>23 ± 6</b>	<b>40 ± 14</b>	<b>58 ± 11</b>	<b>66 ± 18</b>	<b>8 ± 6</b>	<b>20 ± 12</b>	<b>30 ± 16</b>	<b>40 ± 18</b>	<b>55 ± 29</b>	<b>5 ± 3</b>	<b>13 ± 5</b>	<b>36 ± 26</b>	<b>48 ± 31</b>	<b>51 ± 28</b>	<b>18 ± 4</b>	<b>33 ± 6</b>	<b>45 ± 5</b>	<b>63 ± 1</b>	<b>81 ± 4</b>
3,4-diHP-γ-valerolactone	6 ± 4	8 ± 6	9 ± 5	11 ± 2	13 ± 3	5 ± 2	8 ± 3	11 ± 8	13 ± 8	17 ± 14	2 ± 2	5 ± 3	6 ± 4	6 ± 4	9 ± 5	3 ± 3	5 ± 2	8 ± 2	10 ± 3	11 ± 4

\*n/d indicates that the species was not detected

\*Data are expressed as mean ± standard deviation from n=4 independent assessments

## CHAPTER 4: Overall Conclusions and Future Directions

The benefits of fruit and vegetable consumption to the prevention of chronic disease has been well established. Despite serving as key sources of select micronutrients (vitamins and minerals) and dietary phytochemicals (carotenoids and polyphenols), intake of fruits by the average American remains significantly below recommended amounts. Current dietary guidance states that fruit servings can be achieved by the consumption of whole fruits, 100% fruit juice, or other fruits forms. Guidance does suggest a preference for consumption of whole fruits due to differences in fiber and other nutrients (i.e., vitamin C), perceived increased sugar intake, and negative connotations to food processing (commercial juice processing). However, 100% fruit juices remain an affordable and shelf-stable fruit form that may contribute up to 35% of daily fruit intake, particularly among lower income populations. Furthermore, as the nature of commercial juice processing is one of extraction and product standardization, there is evidence to suggest that 100% fruit juice products may be equivalent or better than whole fruits in the delivery of dietary phytochemicals, specifically phenolic species. Given that the associated health benefits of fruit consumption have been linked to phenolic density and their absorption and metabolism, it is critical to understand the impact processing (commercial juice processing) and food matrix (whole fruit vs. 100% juice) has on the bioavailability of bioactive polyphenols. This necessitated a systematic, compartmentalized approach leveraging an *in-vitro* digestion model, anaerobic fermentation, and Caco-2 cell models to mechanistically understand the differences in phenolic bioavailability during digestion between grapes and juice in a wholistic and localized manner.

Grapes, in both whole, table forms and as 100% juices, are widely consumed in America due to their distinctive sweet, tart taste and color. Furthermore, the consumption of grapes and grape juice has been associated with a variety of positive health outcomes, perhaps mediated by

their high phenolic content. Our findings in Chapter 2 emphasized the importance commercial juice processing may have on extraction of phenolics, ultimately leading to greater portions of bioaccessible phenolics. In particular, the typically discarded seed fractions of grapes were found to contain the greatest content of phenolics, followed by the poorly digested skin fraction, specifically for colored Concord grapes. The concentration of phenolics into fruit fractions that are rarely consumed or poorly digested indicates that consumption of whole grapes may result in a significant loss of accessible phenolics. As expected, the content of phenolics in Concord, Niagara, and some table grapes was significantly higher than their respective Concord and Niagara 100% grape juices. However, following *in-vitro* digestion, the bioaccessible content of phenolics was far more comparable, if not significantly greater, in 100% grape juices compared to the whole grapes. This was driven by relatively high percent bioaccessibilities of skin- and seed-derived phenolics of 100% grape juices compared to the whole grapes. These results indicate that a greater fraction of skin (anthocyanins and flavonols) and seed (flavan-3-ols) phenolics were extracted through juicing and made bioaccessible, making 100% grape juice and whole fruit more similar in phenolics delivery. Perhaps of greatest importance to the consumer, as Concord and Niagara grapes are normally unavailable in the market for consumption, the bioaccessible phenolic portions of table grapes were significantly lower than either 100% Concord or Niagara juice, indicating that health-conscious consumers may want to consider 100% grape juice as a healthy alternative to table grapes.

As Chapter 2 focused on the small intestinal bioaccessibility (upper tract) of phenolics, we became increasingly interested in the absorption and transport of phenolics across intestinal epithelia and the large intestinal (lower tract) accessibility and metabolism of phenolics between whole grapes and 100% grapes. Phenolics, particularly larger procyanidins, are known to be poorly

absorbed in the small intestine and are therefore common substrates for anaerobic gut microbial catabolism in the lower tract. These microbial metabolites may be subsequently absorbed and have been posited as the mediators of phenolic-associated health benefits. Leveraging our three-stage *in-vitro* digestion model, a gut microbial anaerobic fermentation model, and Caco-2 cell models, in Chapter 3, we compared the small intestinal phenolic bioaccessibility and the production of microbial phenolic metabolites and intestinal transport of both native and host/microbial metabolites in both upper and lower GI compartments. Supporting our findings in Chapter 2, while initial phenolic content was lower, the bioaccessible portion of phenolics was comparable or significantly greater in Concord and Niagara juices than their respective whole grapes driven by increased proportions of accessible seed and skin derived phenolics in the juices. The notion of increased bioaccessibility from the juice matrix is irrespective of yearly cultivation and growing differences, as 2018 grape (Chapter 2) results were very similar to that of 2020 (Chapter 3).

Our results from Chapter 3 indicate that the cumulative phenolic release, metabolism, and subsequent epithelial transport in both the upper and lower GI is quite similar. This suggests that the overall delivery of phenolics between grapes and 100% grape juices is quite comparable, though some differences exist in location, bioaccessibility, and metabolism. For example, greater phenolic cellular flux, particularly flavonoids, was observed in the upper tract from 100% grape juices than whole grapes, despite similar amounts of small intestinal bioaccessible phenolics. Phenolics were more efficiently transported from the juice matrix than the whole fruit matrix, suggesting that while phenolics are “accessible” in the fruit matrix, soluble fibers and polysaccharides present may be entrapping polyphenols that are substantially reduced in the juice matrix. This may provide evidence for a mechanism of the acute phenolic-mediated benefits associated with 100% grape juice consumption that have yet to be observed with the consumption

of grapes. As expected, due to the largely undigested seeds and their high content of flavan-3-ols and procyanidins, during anaerobic fermentation, both Concord and Niagara grapes had higher contents of released flavan-3-ols and their specific microbial metabolites than the grape juices. If fermentation was allowed to continue for longer than 48 hours, further catabolic breakdown of flavan-3-ols and polymers may have resulted in increased amounts of unique microbial metabolites, particularly in the valerolactone family. Nonetheless, the increased quantities of microbial metabolites from the grape fermenta led to an increased phenolic cellular flux of these metabolites, particularly flavan-3-ol derived metabolites, than juice fermenta samples. However, while there are subtle differences, the overall results suggest that the bioavailability and metabolism of phenolic species between whole grapes and 100% grape juice consumption are likely very similar. When considering the health benefits potentially mediated by phenolics and their metabolites, similar benefits may be achieved from consumption of both whole grapes and 100% grape juice.

The collective findings from this thesis work help to elucidate the similarities and differences in the mechanisms of phenolic bioavailability between whole grape and 100% grape juice, taking a compartmentalized, *in-vitro* digestive approach. Commercial juice processing proves to be effective in extracting phenolics from the grapes, particularly those fractions that are phenolic-rich. This translates to very comparable phenolic bioavailability throughout digestion with the consumption of whole grapes. Insights developed from this work help to emphasize the benefits food processing may have, perhaps aiding in destigmatizing the negative connotations associated with processing. Further, the results highlighting the differences in phenolic release from different food matrices (juice vs. whole fruit), particularly the impact of lacking potentially entrapping molecules, can be translated to other commodities, ranging from other fruit and

vegetable juices to novel beverage formulations. Perhaps of greatest impact, insights from this work help further support the potential benefits from 100% grape juice consumption, and potentially to other 100% fruit juices, with respect to benefits of whole fruit consumption, maybe helping maintain dietary guidance to include 100% fruit juice consumption in an effort to increase overall intake of daily fruit serving. This work is intended to provide a framework, using *in-vitro* approaches, to compare the processing and food matrix impacts between grapes and 100% grape juice on phenolic bioavailability. *In-vivo* and subsequent clinical studies are required to truly understand the differences between 100% grape juice and grapes, especially due to the health impacts mediated by phenolics from fruit consumption. Studies seeking to better understand and characterize the mechanisms of the lower GI are required; namely the impacts of various food types and processing on diversity and abundance of gut microbial populations, characterization of catabolic microbial mechanisms particularly for unique metabolites, and understanding the impact of other lower GI factors (i.e., mucus layer) on phenolic bioavailability. More detailed understanding into the genes dictating phenolic transport and metabolism within enterocytes particularly if differences in expression exist between consumption of grapes versus 100% grape juice. Other commonly consumed fruits and 100% fruit juices, particularly those with phenolics localized to specific fruit segments, must also be analyzed using similar methodologies to determine whether the impacts of juice processing observed here with grapes and grape juice can be translated to other fruits in an effort to improve dietary guidance for Americans.