Food & Function

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Journal:	*Food & Function*
Manuscript ID	FO-ART-12-2021-004226.R1
Article Type:	Paper
Date Submitted by the Author:	27-Feb-2022
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Bioaccessibility, gut microbial metabolism and intestinal transport of phenolics from 100% Concord grape juice and whole grapes are similar in a simulated digestion and fecal fermentation model

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Abstract

Phenolic rich 100% grape juice has been associated with many health benefits, but its place in dietary guidance is controversial relative to whole fruit. Direct comparisons of phenolic profiles and bioavailability between these food forms are needed. Phenolic bioaccessibility and metabolism from Concord (CG) and Niagara (NG) grapes and corresponding 100% juices were investigated using an *in-vitro* digestion coupled with anaerobic gut fermentation model. Intestinal transport of resulting bioaccessible phenolics and microbial metabolites was estimated using a Caco-2 cell model. Total bioaccessible phenolics from both upper and lower digestion were similar ($P > 0.05$) between NG (400.9 ± 26.3 μmol/100g) and NGJ (349.5 ± 8.3 μmol/100g) and significantly different ($P < 0.05$) between CG (417.2 ± 24.4 μmol/100g) and CGJ (294.3 ± 45.4 μmol/100g) Total cellular transport of phenolics was similar ($P > 0.05$) between whole grapes (89.4 ± 5.3 μmol/100g for CG, and 71.8 ± 2.4 μmol/100g for NG) and 100% juices (88.0 ± 5.6 μmol/100g for CGJ, and 85.3 ± 9.4 μmol/100g for NGJ). Differences were observed between the location of phenolic metabolism, bioaccessibility and subsequent cellular transport of individual phenolics between grapes and juice matrices. Specifically, greater amounts of phenolics were transported from grape juices than whole grapes from the upper tract. However, cumulative bioaccessibility and transport from upper and lower GI digestion/fermentation together indicates that the absorbable phenolics from 100% grape juice is similar to that of whole grapes, suggesting that phenolic-mediated health benefits from consumption of whole fruit and juice may be similar.

Key Words: Grapes; Fruit; 100% Juice; Bioaccessibility; Caco-2; Microbial Metabolism

Introduction

Phenolics are broadly present in the US diet in products such as tea, cocoa, fruits, vegetables and whole grains whose consumption is often associated with prevention of chronic and degenerative disease.[1–3] Of the many diverse source of phenolics in the US diet, the 100% juices of native American grapes varietals, Concord and Niagara (*Vitis labrusca*), are rich sources of these bioactives. Combined they represent the third most widely consumed fruit juice in the United States. Both fruit and 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.[4–6] Distributed primarily in the skin and seeds of fruit, these phenolics are partially extracted through the juicing process.[1,6] Grapes, and their 100% grape juices in particular, have been well-documented with modulating oxidative and inflammatory stress and deliver impacts on vascular function in relation to both cardiovascular and neurocognitive health.[7–14] Clinical evidence specifically supports the role of 100% Concord grape juice in modulating markers of immune function, and neurocognitive and cardiovascular health.[15–20]

While evidence continues to emerge on the health benefits of 100% grape juice, the broader role of 100% fruit juice in dietary guidance remains controversial. 100% fruit juices are included in the contribution of fruit servings within the US Dietary Guidelines for Americans (DGA 2020). However, consumers and certain health professionals remain conflicted in the role of 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. Also, negative perceptions associated with commercial processing and the assumption of significant losses of fiber and vitamin C continue to impact perception of 100% juice products.[21] Despite existing compositional data demonstrating only modest differences in these factors between 100% grape juice and whole fruit,[22] 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, our group reported comparisons in phenolic species between Concord and Niagara grapes and their respective commercially produced 100% juices.[6] 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.[6] 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, between the fruit and juice forms.[6] 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.[1,4] These findings were consistent with observations for phenolics in orange fruit and 100% juice[23], and suggest that 100% grape juice and whole fruit may be quite similar in their ability to ultimately deliver bioactive phenolics.

In recent years, the importance of interactions/metabolism of fruit phenolics with gut microbial communities and resulting impacts on human health effects have become apparent.[24–27] 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.[28–30] 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.[24–26] 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.[25,27,31–33]

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[6], 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.

Material and Methods

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)-γ-valerolactone, γ-valerolactone, phenylacetic acid, 3-hydroxyphenylacetic acid, 3-hydroxybenzoic acid, 4-hydroxybenzoic acid, 3-hydroxyphenylproponoic 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), α-amylase (A3176), pepsin (P7125), bile (B8631), pancreatin (P7547), lipase (L3126), and bovine serum albumin (A8806) were purchased from Sigma-Aldrich (St. Louis, MO, USA).

Experimental Design

This experiment was designed to provide direct comparisons between phenolic bioaccessibility from whole grapes and 100% juice in the upper GI, lower GI and ultimately intestinal transport of bioaccessible phenolics/metabolites in both compartments (Figure 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 in the small intestine. Resulting crude digesta (uncentrifuged digesta fraction) and pellet fractions (combined at a 20:80 volume 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.

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°C prior to shipment to the Plants for Human Health Institute (Kannapolis, NC, USA). Grapes were stored at −40°C until further processing and analysis. In an effort to compare grapes more accurately with their respective juices, Concord and Niagara grapes were processed in a manner simulating commercial 100% grape juice production conditions (Supplemental Figure 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°C) and pectinase (Pectinexx Ultracolor – 80 µL/kg) treatment for 60 minutes. Juice was then extracted from the hot grape mash using a Breville Juicer (Sydney, Australia). Free run and extracted grape juices were coarse filtered through muslin. Course filtered grape juice was then subjected to another pectinase (Pectinexx BEXXL – 50 µL/L) treatment for 30 minutes at room temperature (~21°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°C for 2 min). Following pasteurization, bottles were cooled and stored at 4°C for a minimum of two weeks to allow for further clarification and tartaric acid stabilization before analysis. From 2 kg of Concord grapes, ~1.2 kg (1.2 L) of Concord juice was produced, while for 2 kg of Niagara grapes, ~1.1 kg (1.1 L) of Niagara juice was produced. The final grape juice products from each type of grape had similar color and sugar content (~16° brix) as single strength, commercially produced 100% Concord or Niagara juice, respectively.

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.[6] was used to simulate 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. ~2.5 g 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-bioaccessible) 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.

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_2, 5% H_2, 90% N_2) with O_2 and H_2 levels monitored using a CAM-12 Anaerobic Monitor (Coy Laboratory Products, Grass Lake, MI, USA). A palladium catalyst was used to scavenge residual O_2 and heat the chamber. Anaerobic conditions were as follows: O_2: 0-50 ppm, H_2: 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 (N_2-sparged) phosphate-buffered saline.[34] 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.[34–36] One day prior to fermentations, in 250mL of distilled water, a solution of peptone water (2 g), yeast extract (2 g), NaCl (0.1 g), K_2HPO_4 (40 mg), $MgSO4•7H_2O$ (10 mg), Na_2HPO_4 (40 mg), $NaHCO_2$ (2 g), $CaCl2•6H_2O$ (10 mg), Tween 80 (2 mL), Haemin (50 mg), Vitamin K1 (10 µL), and bile salts (0.5 g) were prepared. Another solution of resazurin (4 mL) and L-cysteine (0.5 g) in 250 mL of DI water was prepared. Both solutions were pH buffered to 6.8 ± 0.1, brought to a volume of 500 mL individually 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 1:1 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 prepared from each Concord and Niagara grape/juice digested sample. Briefly, 40 mL 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, 10 mL of pellet mixture equates to the insoluble portion of 40 mL of crude digesta). For each Concord and Niagara grape/juice fermentation, 4 mL of the respective pellet mixture (representing the insoluble fraction from 16 mL crude digesta) and 4 mL 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 (1 g inulin/reaction) were included. For each fermentation experiment, 0.84 mL of fecal slurry was added and brought to a final volume of 42 mL with fermentation media (1:50 fecal slurry dilution). 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, 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 2).

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.[37] 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 amino acids, 1% v/v HEPES, 1% v/v streptomycin/penicillin, 0.1% v/v gentamicin. Cells were seeded at a density of $1.25x10^5$ cells per well on Transwell inserts (Corning polyester membrane, 0.4 μm pore size, 24 mm diameter) and allowed to differentiate for 21-25 days post-confluency at 37°C under CO_2/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 $\Omega\ cm^2$) values using a voltameter (Millicell ERS-2 Epithelial Volt-Ohm Meter), 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). 2 mL 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, 1 mL 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).

Polyphenol Extraction

Phenolic extraction methodologies were adapted from Moser et al.[38] and Mengist et al.[39] 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 mL) 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 2 mL of 0.1% formic acid in water for solid phase extraction (Oasis HLB 1cc-30mg 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 3 mL 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.

Polyphenol and Metabolite Analysis by UPLC-MS/MS

Dried extracts were reconstituted in 50 µL to 1000 µL of methanol, water, formic acid (50:49.9:0.1), filtered with 0.45 µm PTFE filters, and analyzed by UPLC-MS/MS. Phenolic compounds and metabolites were resolved with an Acquity UPLC BEH C18 1.7 µm (2.1 x 50 mm) 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.[6] 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-). Anthocyanins,5-(3,4-dihydroxyphenyl)-γ-valerolactone, and γ-valerolactone were detected under ESI+ 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 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 and using multi-level response curves for a structurally similar anthocyanin (e.g. curves for cyanidin-3-glucoside were used to estimate acylated cyanidin forms, delphinidin-3-glucoside for acylated delphinidin forms, etc.) semi-quantitative estimates for acylated forms for each major class of anthocyanidin were calculated.

Statistical Analyses

All data are presented as a mean ± 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

298 transported content for a compound to the total apical content from the treatment (aqueous or
299 fermenta) expressed as a percentage.
300
301 Percent Relative Bioaccessibility = (bioaccessible or absolute content ÷ total content) x 100
302
303 Percent Transport Efficiency = (cumulative basolateral content ÷ total apical content) x 100

Results and Discussion

Content of Phenolics from 100% Grape Juice are lower than that of whole grape profiles

Concord and Niagara grapes had a greater total phenolic content, summed from individual species quantified through LC-MS, (214.8 ± 22.4 mg/100g and 29.8 ± 2.3 mg/100g, respectively) than their respective 100% juices (39.8 ± 1.7 mg/100g for Concord and 29.1 ± 1.4 mg/100g for Niagara) (Figure 2A, B, Supplemental Table 2). 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 2A, B, Supplemental Table 2). Predominant anthocyanins include cyanidin, peonidin, and delphinidin derivatives, with the majority of anthocyanin content being attributed to acylated forms (Figure 2A). Total content of anthocyanins, acylated anthocyanins, and anthocyanin-glycosides were significantly greater ($P < 0.05$) in Concord grapes than in Concord juice (Figure 2A). Total flavan-3-ol content, including catechin and epicatechin, was significantly greater ($P < 0.05$) in Concord and Niagara grapes than their respective juices (Figure 2A, B). Flavonol content, primarily quercetin derivatives, did not significantly ($P > 0.05$) differ between Concord grapes and juice, but were significantly lower in Niagara grapes compared to Niagara juice (Figure 2A, B). 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 2A, B). Finally, phenolic acids, primarily hydroxycinnamic acids and their tartaric acid esters, were greater ($P < 0.05$) in Concord and Niagara juices than their respective Concord and Niagara grapes (Figure 2A, B). These results are in agreement with previously published reports,[6,40] 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.[41,42] The higher apparent phenolic acid content in juices relative to fruit is also consistent with previous observations[6] 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.[43]

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

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,[6] the relative (%) bioaccessibility of phenolics was greater, in most cases, from juice compared to their respective grapes (Figure 3). For example, primarily seed-derived flavonoids (flavan-3-ols) had a significantly ($P < 0.05$) higher percent bioaccessibility in Concord (29%) and Niagara (50%) juices than either Concord (0.1%) or Niagara (0.1%) grapes (Figure 3). Similarly, relative bioaccessibility of total anthocyanins was significantly ($P < 0.05$) greater in Concord juice (56%) than Concord grapes (5%) (Figure 3). This 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 (37.5 ± 1.1 mg/100g for Concord, 19.9 ± 0.4 mg/100g for Niagara) was significantly ($P < 0.05$) greater than that of grapes (10.1 ± 2.0 mg/100g for Concord, 0.3 ± 0.2 mg/100g for Niagara), exhibiting broader differences in bioaccessible content than was previously observed between grapes and juices for both Concord and Niagara varietals (Figure 2C, D).[6] 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, 5.09-5.66 mg/100g for Niagara; summed from individual compounds).[6] 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. in our previous experiment,[6] 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 more representative comparison of processing effects. 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 – 10.80 mg/100g for 100% Niagara juice from previous assessments in 2017/2018.[6] Despite these differences, these results demonstrate that absolute bioaccessible contents of flavan-3-ols, phenolic acids, anthocyanins, and flavanols (Figure 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 upper gut and, juice likely provides a matrix with an increased proportion of bioaccessible phenolics available in the small intestine.

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 conjugated metabolites generated from incubation of aqueous fraction (AQ) derived from Concord and Niagara juice/grapes digesta are shown in Figure 4. The majority of native phenolic compounds transported include flavonols (quercitin-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 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 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).

Despite differences in relative bioaccessibilities (Figure 3) the overall content of bioaccessible flavonols remains comparable between grapes (2.4 ± 1.1 μmol/L digesta for Concord, 2.9 ± 1.9 μmol/L Niagara) and juice (5.1 ± 1.0 μmol/L for Concord, 3.6 ± 0.6 μmol/L for Niagara). While total bioaccessibility anthocyanins levels are significantly different ($P < 0.05$)

between Concord grapes (188.8 ± 32.3 μmol/L) and juice (338.3 ± 10.2 μmol/L), both are within the same order of magnitude and quite high. Overall, 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.[43] 100% grape juice has low fiber content[22] 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.[32,44] 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,[45,46] though none were observed above the limit of detection of 3.4nM. Delphinidin-3-glucuronide (Supplemental Table 4) was observed but only in low levels. This low level of Phase II conjugation produced by Caco-2 in a three compartment model is consistent with some previous reports[47] 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.[45,46]

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 4). Phenylacetic acid and phenylpropionic acids were found in AQ of digested grapes and juice (Supplemental Table 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 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 4), this is most likely a function of the amount present in the AQ treatment (Supplemental Table 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 are 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. 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.

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* anerobic fermentation. Fermenta pH (Supplemental Figure 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.[48] Concord and Niagara juice and grapes digesta samples with fecal inoculum saw similar drops in pH (5.2 – 5.5). A small pH drop was also observed in fecal-free Concord and Niagara controls (~6.5 pH), though this is primarily attributed to CO_2 absorption and formation of carbonic acid, and potentially chemical degradation releasing acids or decarboxylation.

Panels A and C of Figure 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.[32,44] 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 degrade 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.[24] 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 µmol/L for Niagara, 1.62 µmol/L for Concord) had a greater aglycone flavonol content at 12 hours than juices (3.27 µmol/L Niagara, 1.35 µmol/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 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 6 presents a simplified scheme to summarize the pathways responsible for generation of phenolic microbial metabolites. Panels B and D of Figure 4 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, higher concentrations of 3-hydroxybenzoic acid, 5-(3,4-dihydroxyphenyl)-γ-valerolactone (3,4diHP-γ-valerolactone), γ-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[32,44], while 3,4diHP-γ-valerolactone[49,50] and γ-valerolactone[50] 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 5).

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 5). Differences in 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, releases additional phenolics from seeds/skins allowing for comparable levels of native phenolic species and subsequent microbial metabolites between grapes and grape juices. The notable exception to this are flavan-3-ols/procyanidins and their microbial metabolites. Procyanidins and their dimers/monomers are known to be extensively metabolized by microbiota.[44] 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 more accurately estimate available metabolite pools in humans.[49,50] In particular, γ-valerolactone is known to be a very late (> 24 hours) appearing microbial metabolite,[50] and the presence of flavan-3-ol monomers/dimers and 3,4diHP-γ-valerolactone, an intermediate metabolite in the formation of γ-valerolactone,[50] 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.

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 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. Despite levels of flavonoids being detected in 12 hr fermenta samples (Figure 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.[44] 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 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. These data suggest that for 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.

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 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 digesta matrix, but perhaps less so in the juice digesta matrix. Interactions between phenolics and cell wall polysaccharide interactions have been documented and are typically stabilized by non-covalent and ionic interactions.[51] These interactions, while enhancing apparent bioaccessibility may serve to limit the ability of phenolics to interact at the intestinal brush boarder affecting absorption of nutrients and phenolics themselves.[52] This has also been observed with oat cereals with high levels of beta-glucan.[53] Following anaerobic gut fermentation, transport rates did not differ between whole grape and juice digesta, suggesting a reduction of those factors by fermentation and 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 5), aligned with cellular transport of metabolites from 12-hour fermenta (Figure 7). Considering the low bioavailability of many native flavonoid species, long-term health implications associated with grape polyphenol consumption, have been more recently associated with microbial phenolic metabolites.[25,27,31–33] 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 12 hr fermenta samples. As indicated earlier, there were key differences in the microbial metabolite production from fermentation of flavan-3-ols and their larger polymers between juices and grapes (Figure 5). If later hour (24 or 48 hr) fermenta samples were used as treatments for absorption and transport studies more pronounced differences between grape versus juice microbial metabolite absorption and transport may be observed, perhaps suggesting differential health implications for chronic consumption of grapes versus 100% grape juice.

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 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 μmols/100g of starting grape/juice material. Therefore, despite differences between the fruit and juice matrices, their starting phenolic content (Figure 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. Overall, these results suggest that the bioavailability and metabolism of phenolics between whole grape and 100% grape juice consumption are 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.

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.[20,54,55] 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.[20,40,54–56] 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. 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.[26,27,31] Benefits on cardiovascular health, type-2 diabetes, some cancers, neurological health, inflammation, and pulmonary health have been associated with circulating phenolic microbial metabolites.[57–59] 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.

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.

Conflicts of Interest

MGF has received research funding from Welch's Foods in the past 3 years.

Acknowledgements

MGF was supported by the United States Department of Agriculture National Institute of Food and Agriculture, Hatch Project NC 02618. The authors would also like to acknowledge Lauren Essenmacher and Lisard Iglesias for aid with anaerobic fermentations and Jolynne D. Wightman of Welch's Food Inc. for supplying Concord and Niagara grape samples.

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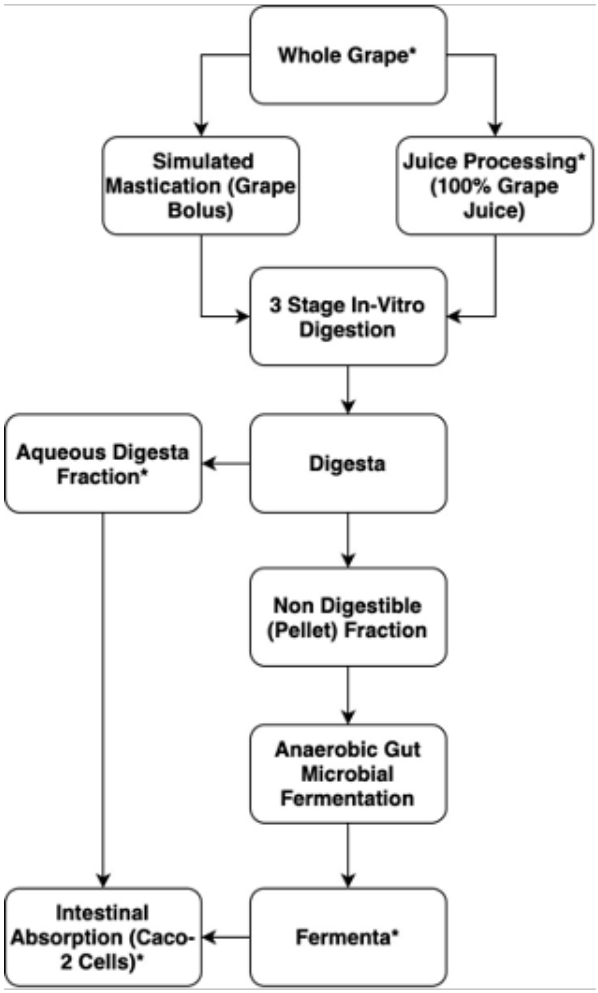
841 **Figures**



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847 **Figure 1. Experimental Design.** Overall flow of studies conducted, including coupled 3 stage *in-vitro* digestion
848 and anaerobic gut microbial fermentation. The presence of * indicates fractions and studies where phenolic and
849 metabolic species were quantified by LC-MS. Masticated whole grapes (grape bolus) was compared with 100%
850 grape juice for both Concord and Niagara grape varietals throughout all performed experiments. For the
851 anaerobic fermentation experiments, an 80:20 pellet fraction to digesta fraction treatment was used.

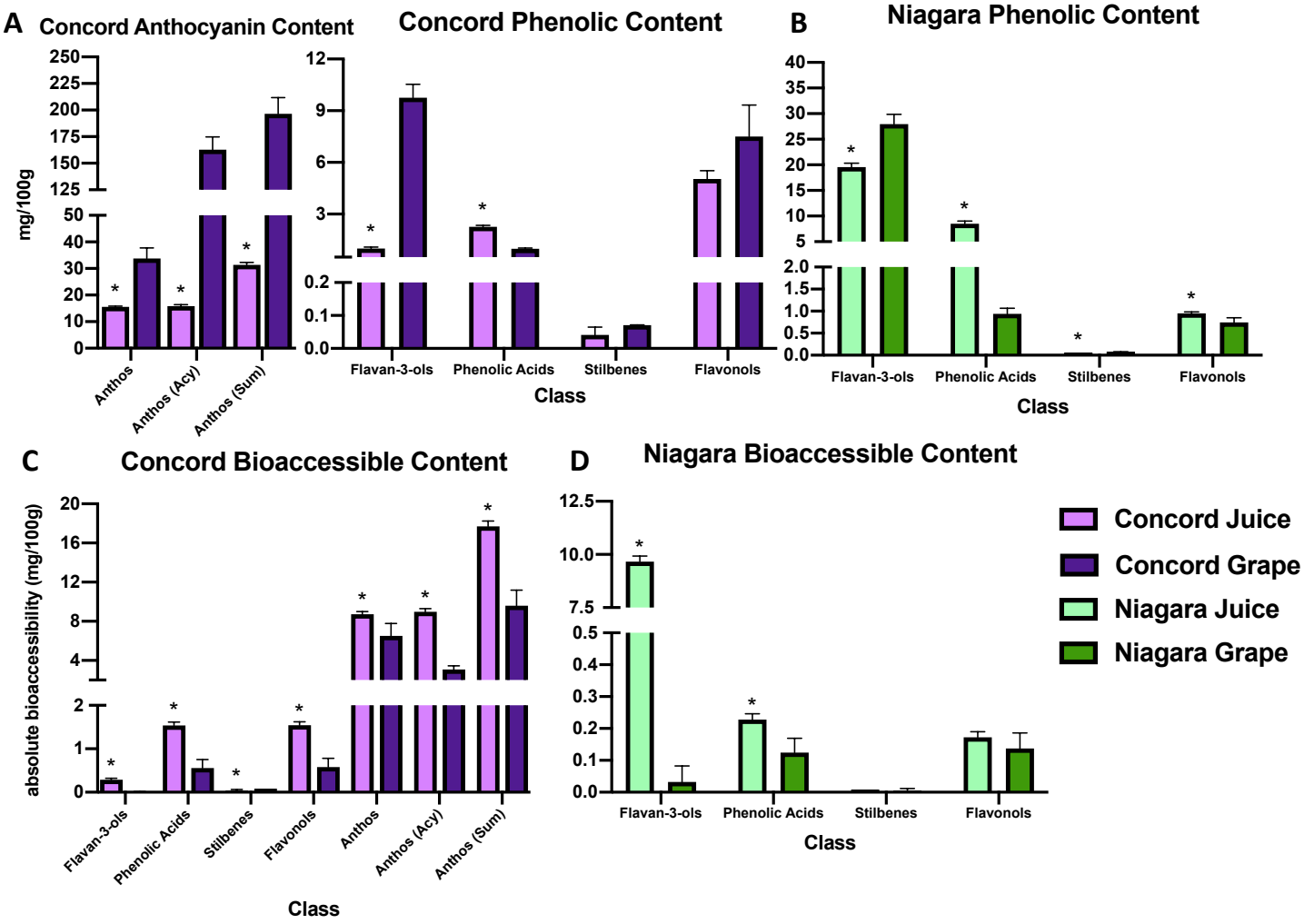

Figure 2. Comparison of total and bioaccessible content of phenolics between whole juicing grapes and 100% grape juice. Panels A and B depict starting contents of phenolics by class in Concord grapes, 100% Concord juice, Niagara grapes, and 100% Niagara juice. Panels C and D present bioaccessible phenolic 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 is presented as a mean ± 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 flavonols) levels between juice and their respective grapes.

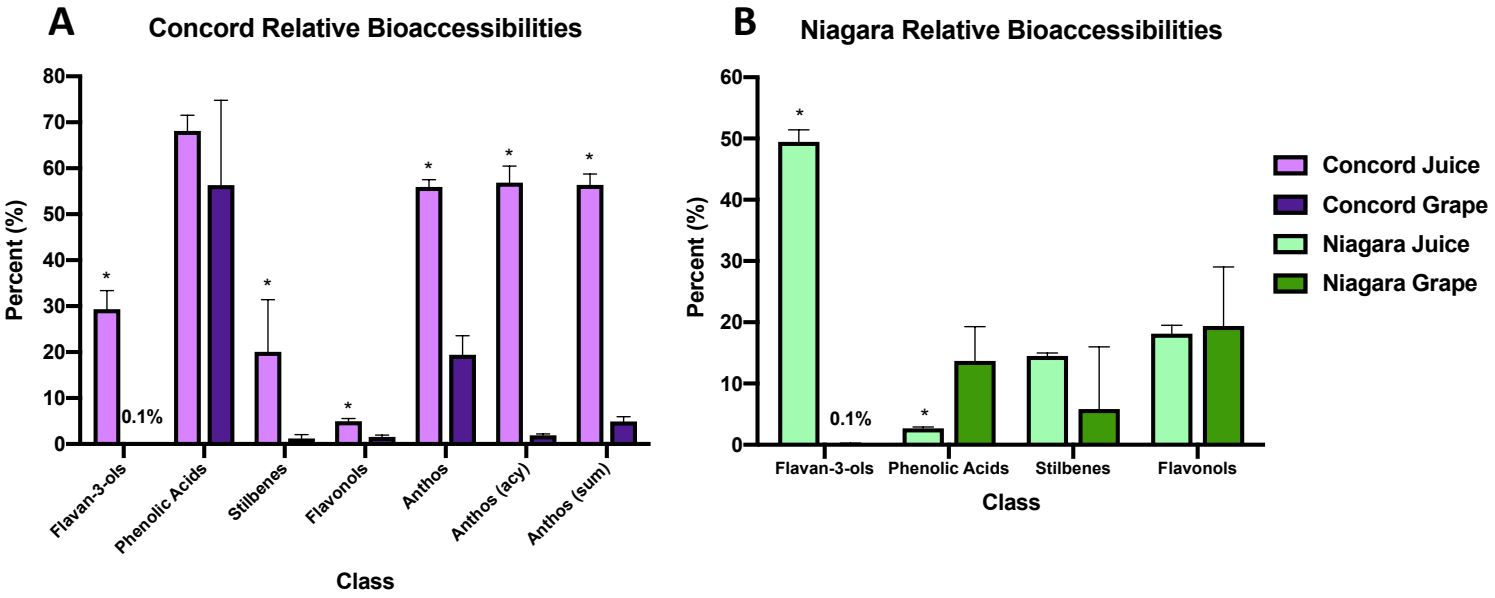

Figure 3. Relative bioaccessibilities of phenolics between whole juicing grapes and 100% grape juice. Relative bioaccessibility was calculated as the ratio of bioaccessible content (following a 3-stage *in-vitro* digestion) for a compound class to the total content for that class in the starting material 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 is presented as a mean ± 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.

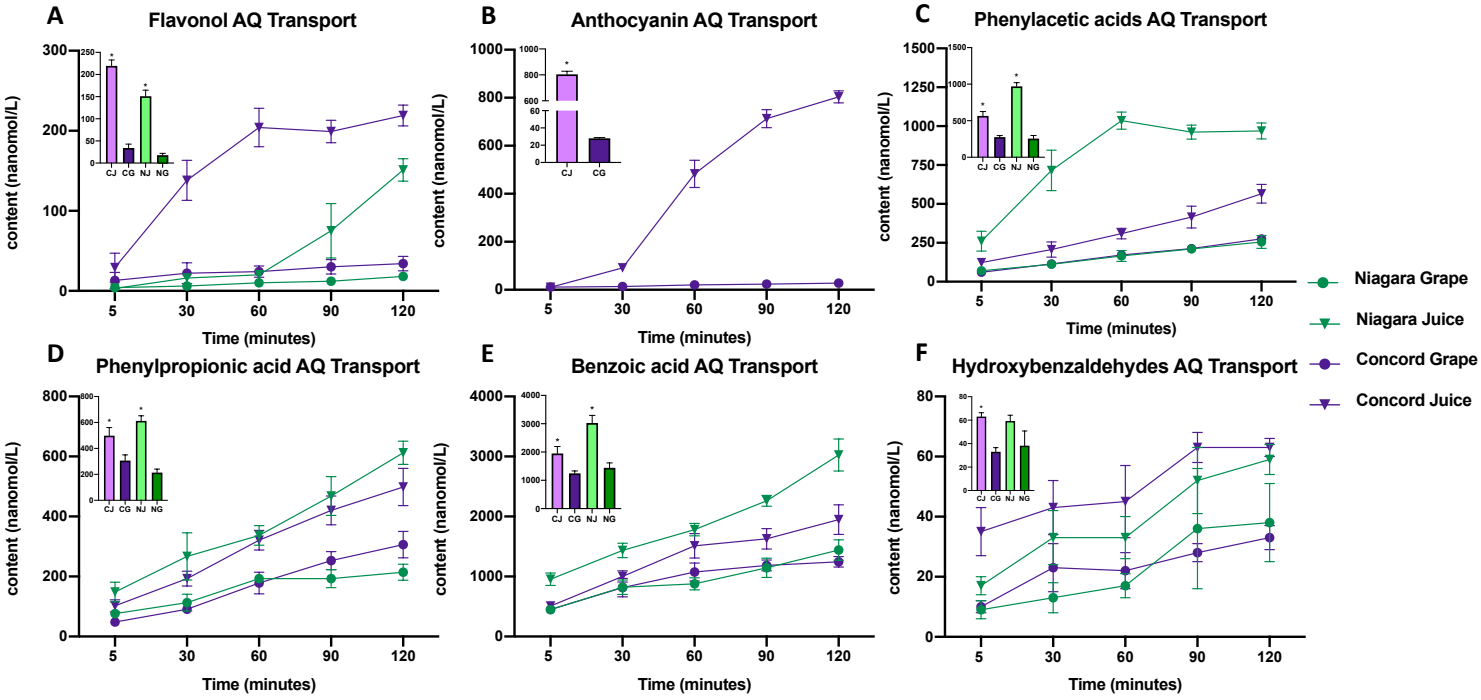

Figure 4. Cumulative phenolic apical to basolateral transport of aqueous digesta fractions of Concord and Niagara grapes and 100% grape juice. Individual panels present cumulative phenolic transport of aqueous digesta fractions over 2 hours across differentiated Caco-2 cell monolayers. Data are expressed 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 is presented as a mean ± 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.