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In Vitro Bioavailability of Phenolic Compounds from Five Cultivars of Frozen Sweet Cherries (*Prunus avium* L.)

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The bioavailability of phenolic compounds from five cultivars of frozen sweet cherries was assessed by a digestion process involving pepsin−HCl digestion (to simulate gastric digestion) and pancreatin digestion with bile salts (to simulate small intestine conditions) and dialyzed to assess serum- and colon-available fractions. After pepsin digestion, the % recovery of total phenolics, relative to the original starting material, increased, whereas the % anthocyanins did not change. Following pancreatic digestion and dialysis, the total phenolics in the IN (serum-available) fraction was about 26–30% and the OUT (colon-available) fraction was about 77–101%. The anthocyanin content in the IN fraction was 15–21%, and in the OUT fraction, it was 52–67%. Skeena, Lapins, and Sweetheart cultivars contained higher levels of total phenolics and anthocyanins, which resulted in higher concentrations of these compounds in the IN and OUT fractions. The potential bioavailability of phenolic compounds was also assessed in Bing and Lapins cultivars at three ripening stages. Immature cherries had higher % total phenolics in the IN fraction than mature or overmature cherries. However, immature cherries had the lowest concentrations of these compounds, making the actual bioavailable amounts of these compounds lower than for mature and overmature fruit. High-performance liquid chromatography analysis of Lapins cherries at three maturity stages confirmed the results obtained using spectrophotometric methods for total phenolics and anthocyanins.

KEYWORDS: Anthocyanins; cyanidin; flavonoids, total phenolics; cherry; functional food; digestion; bioavailability; maturity; ripening

INTRODUCTION

The sweet cherry (*Prunus avium* L., Rosaceae) is important commercially as a table fruit and as an ingredient for fruit cocktails and maraschino cherries. Color is the most important indicator of maturity and quality for both fresh and processed cherries. The development of red color in dark sweet cherries is an index of maturity and an important quality attribute for the consumer, through which high anthocyanins and other polyphenols contents are expressed (*1, 2*). The major polyphenols in cherries are anthocyanins and hydroxycinnamic esters (*2–4*). Anthocyanins and other polyphenols are secondary metabolites evolved by plants as a natural defense system. They are also known for their several positive effects on human health, through the prevention of coronary diseases and cancer (*5, 6*) or by acting as antioxidants toward low-density lipoproteins in liposomes (*7, 8*) and through anti-inflammatory (*9*), antimicrobial (*10*), and anticarcinogenic activities (*11, 12*). However, much of the evidence on the prevention of diseases by anthocyanins and other polyphenols is derived from epidemiological studies and/or animal experiments, which are often performed with doses much higher than those to which humans are exposed through the diet. There is also uncertainty regarding the relevance of biomarkers used as predictors of disease risk and the appropriateness of the different methods used (*6, 13*).

Prior to 1995, it was widely believed that anthocyanins and other polyphenols could not be absorbed intact after oral ingestion but were hydrolyzed to their aglycones by bacterial enzymes in the lower gastrointestinal tract. It was further suggested that the aglycones might then be partially absorbed or may undergo further biotransformation by bacteria (*14*). In 1997, Paganga and Rice-Evans (*15*) described evidence for the absorption of anthocyanins, rutin, and phloridzin and their presence in human plasma in the glycosylated form by high-

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Table 1. Total Solids Content in Frozen Cherries Used for in Vitro Digestion Experiments

type	total solids content[a] (%)
Bing	26.1 ± 0.7 a
Lapins	22.5 ± 1.4 c
Skeena	21.0 ± 0.1 d
Staccato	20.8 ± 0.2 d
Sweetheart	24.4 ± 0.2 b
Bing—immature	22.9 ± 0.4 c
Bing—mature	26.1 ± 0.7 b
Bing—overmature	29.2 ± 0.2 a
Lapins—immature	17.9 ± 0.3 c
Lapins—mature	22.5 ± 1.4 a
Lapins—overmature	21.5 ± 0.5 b

[a] Values are averages ± standard deviations ($n = 9$). Values with the same letter within vertical groups are not significantly different for $p \leq 0.05$.

performance liquid chromatography (HPLC) analysis with photodiode array detection. The polyphenols were detected in plasma from nonsupplemented humans at levels in the range of 0.5–1.6 μM. Other studies on anthocyanin absorption soon followed. Currently, there is little direct evidence of anthocyanin aglycones existing in the blood circulation or urine of humans (*16*). Most recent animal and human studies have reported that anthocyanins are absorbed intact as glycosides (*17, 18*), of which anthocyanins appear in the circulation as both intact parent glycosides and metabolized conjugates (*16, 19–22*).

Although transport and metabolic mechanisms cannot be effectively reproduced, studies can provide a simple predictive instrument to investigate the potential bioavailability of dietary compounds by assessing their stability under conditions mimicking the gastrointestinal tract. In addition, these studies allow for the screening and comparison of multiple samples, thus providing information about the influence of different food matrices on the recovery of individual compounds. Most bioavailability methods are only dependent on the diffusion rates of compounds studied and their stability.

The first in vitro digestion was described by Miller et al. (*23*), correlating the iron content in IN samples with serum iron bioavailability in vivo. Later, this methodology was adapted for determining the potential bioavailability of anthocyanins and other phenolics in different fruit products (*24–28*). These studies found a good correlation with the bioavailability observed. Several factors can influence the content of anthocyanins and other phenolics in cherries; they include cultivar, maturity, geographic location, and environmental factors such as light, temperature, and various stresses (*3, 4, 29*). Studies on the composition of anthocyanins in cultivars of sweet cherries have shown that they are a rich source of cyanidin 3-glucoside and cyanidin 3-rutinoside (*3*). Information about the bioavailability of these compounds is required to advance our understanding so that frozen cherries or other cherry products may be included among "functional foods" and considered as healthy components of our daily diet.

A simulated digestion procedure based on published works (*24, 27*) was used in the present study to evaluate the bioavailability of anthocyanins and other polyphenols from five cultivars of frozen sweet cherries (Bing, Lapins, Skeena, Staccato, and Sweetheart). Bing and Lapins cherries, harvested at three different stages of maturity (immature, mature, and overmature), were also studied, and untreated cherries and samples that were pepsin−HCl-digested (to simulate gastric digestion), pancreatin-digested with bile salts (to simulate small intestine conditions), and dialyzed in the IN (serum-available) fraction and the OUT (colon-available) fraction were analyzed by HPLC and spectrophotometric methods.

MATERIALS AND METHODS

Cherry Samples. Samples of five cultivars of sweet cherries (*P. avium* L.) were harvested at commercial maturity (mature) from the orchards of the Pacific Agri-Food Research Center at Summerland, British Columbia, during July and August 2006. The cultivars were Bing, Lapins, Skeena, Staccato, and Sweetheart. For the Bing and Lapins cultivars, fruit was also harvested at two other stages of ripeness (immature and overmature). Immature cherries were picked about 1 week early, whereas overmature cherries were picked about 1 week late. To achieve a more consistent sample, cherries of a uniform color were selected at each harvest date. Within 1 h of harvest, stems were removed, and the cherries were packed in plastic bags and frozen. The fruit was kept at –25 °C for about 2 months until analyzed.

Chemicals. Pepsin (product number P-7125), pancreatin (product number P-7545), bile extract porcine (product number B-8631), caffeic acid, chlorogenic acid, and gallic acid were purchased from Sigma Chemical Co. (St. Louis, MO). Cyanidin 3-*O*-glucoside chloride, cyanidin 3-*O*-rutinoside chloride and peonidin 3-rutinoside chloride were purchased from Extrasynthese (Genay, France).

Sample Preparation. Distilled water (100 g) was added to 100 g of pitted and quartered frozen cherries, and the mixture was blended for 3 × 15 s in a semimicro stainless steel blender jar using a Waring blender. Three samples of about 10 g each of the homogenate were set aside for total solids determination. The rest of the homogenate was acidified to pH 2 by adding 5 M HCl. For digestion, two samples of acidified homogenate were taken. One sample consisted of 100 g, which was placed in a 500 mL polypropylene screw-cap container, and the other control (Original) sample of 50 g was placed in a 90 mL polypropylene screw-cap container. Containers were made by Starplex Scientific Inc. (Etobicoke, ON, Canada). For each sample of cherries, three replicate homogenate samples were prepared.

In Vitro Bioavailability Procedure. The procedure used was modified from the methods reported by Gil-Izquierdo et al. (*27*) and McDougall et al. (*24*) and consisted of an initial pepsin−HCl digestion (to simulate gastric digestion) followed by a pancreatin digestion with bile salts (to simulate small intestine conditions). To the 100 g acidified homogenate sample, 31500 units of pepsin were added (one unit of pepsin will produce a ΔA_{280} of 0.001/min, at pH 2.0 and 37 °C, measured as trichloroacetic acid-soluble products using hemoglobin as the substrate). The homogenate was then incubated in a shaking water bath at 37 °C and 100 rpm for 2 h (Precision Scientific, model 25, Winchester, VA). The 50 g acidified homogenate sample (original) was also incubated under the same conditions, but no pepsin was added.

The 100 g postgastric digest homogenate was next divided into four 20 g samples, which were placed into 90 mL polypropylene screw-cap containers with 5 mL of a 4 mg/mL pancreatin and 25 mg/mL porcine bile extract solution added to each container. The pancreatin and bile extract solutions were prepared fresh daily. The leftover postgastric digest was frozen immediately and saved as the after pepsin digestion (after) sample.

One of these ~25 g postgastric mixtures was used to determine the amount of 1 M $NaHCO_3$ needed to neutralize the postgastric digest. The mixture was titrated with 1 M $NaHCO_3$ to reach a pH of 7.5, and this volume of 1 M $NaHCO_3$ was recorded. For each of the three samples, a 30 cm segment of cellulose dialysis tubing from Sigma (product number D-9777) was prepared. The dialysis tubing was rated to retain >90% of cytochrome C (MW 12400) in solution over a 10 h period. To the dialysis tubing, a total volume of 25 mL of $NaHCO_3$ solution was added. This solution consisted of the volume of 1 M $NaHCO_3$ required to reach pH 7.5, and distilled water was added to make up a volume of 25 mL. This allowed the volume of tubing to contain the same volume as the postgastric digest mixture.

Dialysis tubing containing $NaHCO_3$ was added to each of the postgastric digest mixtures, and the screw-cap containers were closed. The containers were then incubated at 37 °C and 100 rpm shaking for 2 h. The 50 g acidified homogenate sample (original) was also incubated under the same conditions without any addition of enzymes. Following

Table 2. Total Phenolics and Anthocyanins in Frozen Cherry Cultivars Following in Vitro Digestion

	Bing	Lapins	Skeena	Staccato	Sweetheart
		total phenolics[a]			
original (mg gallic acid equiv/kg)	1804 ± 110 c	2003 ± 201 b	2853 ± 132 a	1580 ± 99 d	1824 ± 84 c
after (% original)	127.0 ± 4.0 ab	124.8 ± 8.0 ab	121.0 ± 5.3 b	128.3 ± 7.5 a	122.7 ± 3.5 ab
IN (% original)	26.9 ± 1.6 b	28.9 ± 1.3 ab	26.9 ± 2.7 b	29.2 ± 2.4 a	28.4 ± 1.7 ab
OUT (% original)	100.8 ± 7.9 a	95.0 ± 6.8 b	76.8 ± 4.4 d	88.8 ± 6.8 c	91.4 ± 2.9 bc
		total anthocyanins[a]			
original (mg cyanidin 3-rutinoside equiv/kg)	858 ± 43 d	1589 ± 262 b	3199 ± 166 a	1278 ± 38 c	1500 ± 67 b
after (% original)	99.8 ± 6.4 b	106.8 ± 9.5 a	98.7 ± 4.1 b	98.5 ± 5.1 b	99.8 ± 1.8 b
IN (% original)	14.9 ± 1.6 c	21.4 ± 1.8 a	19.7 ± 2.5 ab	19.3 ± 1.8 b	20.1 ± 1.3 ab
OUT (% original)	59.6 ± 6.5 b	66.9 ± 7.6 a	52.4 ± 3.4 c	53.8 ± 3.6 c	61.7 ± 2.8 b

[a] Values are averages ± standard deviations ($n = 9$). Values with the same letter within a row are not significantly different for $p \leq 0.05$.

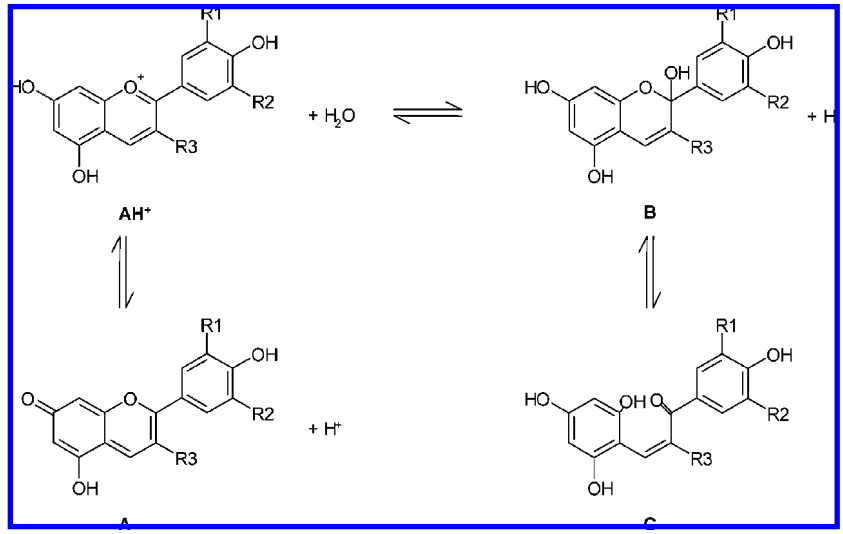

Figure 1. Structural transformations of anthocyanins.

incubation, all containers were cooled to room temperature. The weights and pH of the material inside the dialysis tubing (IN) and outside the dialysis tubing (OUT) were measured and recorded. According to this method, at the end of incubation, the solution that entered the dialysis tubing (IN) represented the material that entered the serum, and the solution outside the dialysis tubing (OUT) represented the material that remained in the gastrointestinal tract and could pass to the colon (*24*). Samples from the various fractions as well as the starting material (original) were frozen and stored at –25 °C until further analysis.

Total Solids Content. The solids content of homogenates was measured by drying samples (~10 g) in aluminum pans in a vacuum oven at 70 °C and 125–175 torr for 22–24 h until the weights stabilized. The solids content was calculated as weight of dry sample/weight of wet sample × 100. The solids content was then corrected to find the solids content for frozen cherries (solids content of homogenate/2). The solids content of the homogenate was divided by 2 to get the solids content of the frozen cherries, since the homogenate was a mixture of cherry and water (1:1, w/w).

Total Phenolics and Anthocyanin Assays. Samples of the original, after, IN, and OUT fractions from in vitro digestion were thawed as required, and aliquots were transferred to 2 mL centrifuge tubes. For original and after samples, there were three replicates from the experiments that were analyzed in tripicate. For IN and OUT samples, there were nine replicates from the experiments (three replicates for each of the three homogenates produced). All 2 mL tubes of sample were centrifuged at 14000 rpm for 10 min using a MiniSpin plus centrifuge (Eppendorf, Hamburg, Germany), and the supernatant was used for total phenolic and anthocyanin determination.

The total phenolic content was measured using a modified version of the Folin−Ciocalteu procedure described by Singleton and Rossi (*30*). Briefly, 50 μL of sample or standard and 150 μL of distilled water were added to a test tube. To the test tube, 1 mL of a 1/30 solution of Folin−Ciocalteu's phenol reagent from Sigma was added followed by 0.8 mL of Na_2CO_3 solution (75 g/L). The solution was mixed and allowed to sit for 60–120 min before reading at A_{765} nm. Gallic acid was used as the standard in concentrations from 0 to 250 mg/L in distilled water. The results were converted to express total phenolics as mg of gallic acid equiv/kg of frozen fruit. The concentrations for the after, IN, and OUT fractions were also converted to % recovery relative to the original fraction as reported by McDougall et al. (*24, 25*). The recovery for the original fraction would be 100%.

The total anthocyanin concentration was estimated using a modified version of the Glories' method (*31*). Briefly, the method consisted of adding 0.25 mL of sample or standard, 0.25 mL of 0.1% HCl in 95% ethanol, and 4.5 mL of 2% HCl to a test tube. The solution was allowed to sit for at least 15 min before reading at A_{280}, A_{320}, A_{360}, and A_{520} nm. The anthocyanin content was quantified at 520 nm using standards of 0–250 mg/L in 70% methanol. The results were converted to express anthocyanin content as mg of cyanidin 3-glucoside or cyanidin 3-rutinoside equiv/kg of frozen fruit. The concentrations for the after, IN, and OUT fractions were also converted to % recovery relative to the original fraction.

HPLC Analysis. Selected samples of the original, after, IN, and OUT fractions from in vitro digestion of Lapins cherries were thawed as required. Aliquots of the original and after fractions were transferred to 2 mL centrifuge tubes. Aliquots (3.5 g) of IN and OUT fractions were combined with 0.5 mL of 10.5% trifluoroacetic acid and allowed to sit for 30 min before transferring samples to 2 mL centrifuge tubes. IN and OUT fractions were found to be unstable unless acidified prior to HPLC analysis. All 2 mL tubes were centrifuged at 14500 rpm for 5 min using a MiniSpin plus centrifuge just prior to HPLC analysis. The supernatants were transferred to HPLC vials for analysis.

HPLC analysis was performed using an Agilent 1100 liquid chromatography system (Agilent Technologies Inc., Palo Alto, CA)

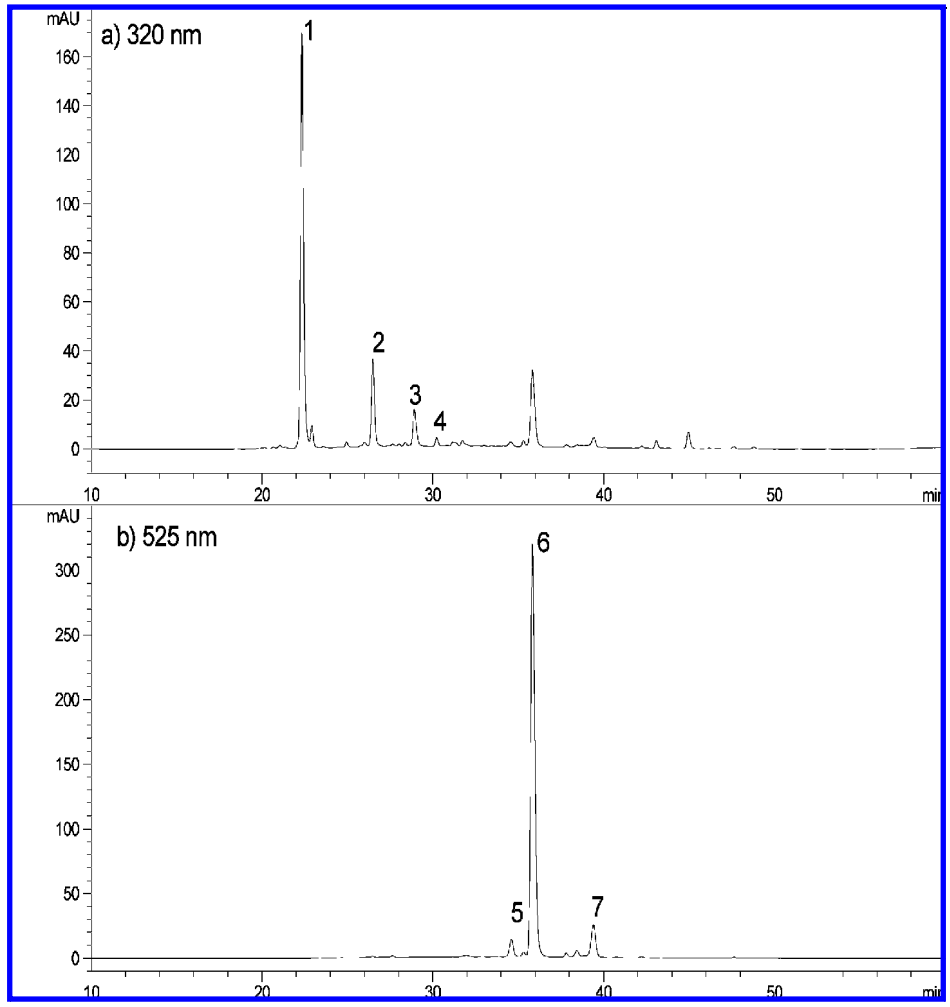

Figure 2. HPLC chromatograms at (**a**) 320 and (**b**) 525 nm for original mature Lapins extract. Peak 1, neochlorogenic acid; peak 2, *p*-coumaroylquinic acid; 3, chlorogenic acid; 4, caffeic acid; 5, cyanidin 3-glucoside; 6, cyanidin 3-rutinoside; and 7, peonidin 3-rutinoside.

Table 3. Total Phenolics and Anthocyanins in Frozen Bing and Lapins Cherries at Three Ripening Stages Following in Vitro Digestion

	Bing			Lapins		
	immature	mature	overmature	immature	mature	overmature
			total phenolics[a]			
original (mg gallic acid equiv/kg)	1366 ± 36 c	1804 ± 110 b	2138 ± 43 a	1249 ± 87 c	2003 ± 201 b	2134 ± 72 a
after (% original)	114.2 ± 4.5 b	127.0 ± 4.0 a	122.7 ± 6.5 a	131.9 ± 7.0 a	124.8 ± 8.0 b	124.1 ± 6.3 b
IN (% original)	27.7 ± 2.2 a	26.9 ± 1.6 a	22.7 ± 1.5 b	35.7 ± 2.9 a	28.9 ± 1.3 b	28.1 ± 2.8 b
OUT (% original)	101.6 ± 5.3 a	100.8 ± 7.9 a	97.3 ± 3.6 a	99.9 ± 6.1 a	95.0 ± 6.8 ab	91.9 ± 3.4 b
			total anthocyanins[a]			
original (mg cyanidin 3-rutinoside equiv/kg)	290 ± 27 b	858 ± 43 a	884 ± 30 a	434 ± 39 b	1589 ± 262 a	1546 ± 104 a
after (% original)	92.7 ± 6.6 b	99.8 ± 6.4 a	92.5 ± 5.8 b	112.1 ± 10.6 a	106.8 ± 9.5 ab	101.5 ± 3.0 b
IN (% original)	12.7 ± 2.2 b	14.9 ± 1.6 a	11.7 ± 1.4 b	23.1 ± 1.9 a	21.4 ± 1.8 a	19.4 ± 2.1 b
OUT (% original)	73.7 ± 6.6 a	59.6 ± 6.5 b	63.6 ± 4.9 b	65.3 ± 3.3 ab	66.9 ± 7.6 a	60.5 ± 3.6 b

[a] Values are averages ± standard deviations ($n = 9$). Values with the same letter within a row for either Bing or Lapin are not significant.

equipped with a photodiode array detector. Samples of 5 μL were injected onto a reversed-phase Zorbax SB-C18, 5 μm, 250 mm × 3.0 mm column from Agilent preceded by a Zorbax SB-C18, 5 μm, 12.5 mm × 4.6 mm guard column from Agilent. A gradient solvent system using solvent A (50 mM phosphoric acid) and solvent B (methanol) at a flow rate of 0.4 mL/min was used. The proportion of solvent B used was as follows: 0−5 min, 5%; 5−51 min, 55%; 51−61 min, 100%; 61−68 min, 100%; 68−73 min, 5%; and 73−83 min, 5%. The HPLC run was monitored at 200–700 nm. Chromatograms at 320 and 525 nm were analyzed to determine colorless phenolic compounds and anthocyanins, respectively. Chlorogenic acid at 0–300 mg/L in 70% methanol was used as a standard for colorless phenolics at 320 nm, and cyanidin 3-glucoside and cyanidin 3-rutinoside at 0–300 mg/L in 70% methanol were used as standards for anthocyanins at 525 nm. Peaks were quantified based on areas.

Statistical Analysis. Data were analyzed using the SAS (SAS Institute Inc., Cary, NC, version 9) analysis of variance procedure. Duncan's multiple range tests were used to compare means. Differences with $p \leq 0.05$ were considered significant.

RESULTS AND DISCUSSION

In Vitro Bioavailability of Phenolic Compounds in Five Cultivars of Mature Cherries. The total solids content of

Table 4. Selected Phenolics at 320 nm Using HPLC in Frozen Lapins Cherries at Three Ripening Stages Following in Vitro Digestion

	immature	mature	overmature
	peak 1, neochlorogenic acid[a]		
original (mg chlorogenic acid equiv/kg)	343 ± 41 b	384 ± 42 a	342 ± 32 b
after (% original)	113.2 ± 16.2 a	102.6 ± 1.2 b	101.0 ± 2.3 b
IN (% original)	31.5 ± 3.0 a	26.2 ± 2.8 b	27.1 ± 2.9 b
OUT (% original)	48.4 ± 1.7 a	48.4 ± 4.5 a	45.1 ± 4.5 a
	peak 2, *p*-coumaroylquinic acid[a]		
original (mg chlorogenic acid equiv/kg)	85 ± 6 a	86 ± 8 a	80 ± 4 a
after (% original)	107.7 ± 8.2 a	101.3 ± 3.4 b	100.2 ± 4.1 b
IN (% original)	32.4 ± 3.3 a	26.7 ± 4.7 b	29.8 ± 2.9 ab
OUT (% original)	50.1 ± 1.6 a	53.3 ± 5.8 a	50.6 ± 3.9 a
	peak 3, chlorogenic acid[a]		
original (mg chlorogenic acid equiv/kg)	43 ± 5 a	41 ± 4 a	37 ± 3 b
after (% original)	109.6 ± 11.1 a	103.6 ± 5.3 ab	96.9 ± 6.9 b
IN (% original)	34.9 ± 3.7 a	33.8 ± 3.5 a	36.9 ± 4.4 a
OUT (% original)	49.9 ± 2.9 b	58.2 ± 6.1 a	56.1 ± 5.9 a
	peak 4, caffeic acid[a]		
original (mg chlorogenic acid equiv/kg)	6.0 ± 0.7 b	7.9 ± 0.6 a	8.2 ± 1.3 a
after (% original)	112.6 ± 11.4 a	102.0 ± 7.7 b	102.8 ± 9.8 b
IN (% original)	421.5 ± 34.3 a	346.7 ± 68.3 b	347.0 ± 54.2 b
OUT (% original)	494.0 ± 44.2 a	468.6 ± 103.5 a	436.2 ± 46.9 a

[a] Values are averages ± standard deviations ($n = 9$). Values with the same letter within a row are not significantly different for $p \leq 0.05$. Peaks are as in **Figure 2**.

Table 5. Selected Anthocyanins at 525 nm Using HPLC in Frozen Lapins Cherries at Three Ripening Stages Following in Vitro Digestion

	immature	mature	overmature
	peak 5, cyanidin 3-glucoside[a]		
original (mg cyanidin 3-rutinoside equiv/kg)	13 ± 2 c	58 ± 8 a	41 ± 5 b
after (% original)	112.2 ± 14.0 a	99.2 ± 4.4 b	99.8 ± 4.8 b
IN (% original)	16.2 ± 1.5 a	12.7 ± 2.3 b	12.7 ± 2.7 b
OUT (% original)	25.0 ± 1.8 a	25.6 ± 5.0 a	23.0 ± 6.8 a
	peak 6, cyanidin 3-rutinoside[a]		
original (mg cyanidin 3-rutinoside equiv/kg)	373 ± 43 c	1407 ± 161 a	1123 ± 95 b
after (% original)	111.7 ± 14.0 a	101.8 ± 2.6 b	98.8 ± 3.5 b
IN (% original)	17.8 ± 2.1 a	14.6 ± 2.4 b	15.3 ± 3.0 b
OUT (% original)	34.7 ± 1.4 a	36.1 ± 6.2 a	33.4 ± 7.8 a
	peak 7, peonidin 3-rutinoside[a]		
original (mg cyanidin 3-rutinoside equiv/kg)	15 ± 2 b	125 ± 13 a	126 ± 8 a
after (% original)	97.2 ± 8.7 a	99.6 ± 2.3 a	99.0 ± 2.2 a
IN (% original)	16.3 ± 1.7 a	13.8 ± 2.2 b	14.7 ± 2.3 ab
OUT (% original)	34.2 ± 3.0 a	37.9 ± 5.8 a	35.8 ± 5.8 a

[a] Values are averages ± standard deviations ($n = 9$). Values with the same letter within a row are not significantly different for $p \leq 0.05$. Peaks are as in **Figure 2**.

mature Bing, Lapins, Skeena, Staccato, and Sweetheart and immature, mature, and overmature Bing and Lapins cherries are reported in **Table 1**. Because the major component of solids in cherries is sugar, the total solids content gave a good indicator of the soluble solids of the fruit. The total solids content ranged from 21 to 26%. Bing cherries had the highest level while Staccato and Skeena had the lowest. Cherries were all harvested within days of commercial maturity and so had similar total solids. For Bing and Lapins cherries, there was more variation in the solids content, which possibly is a reflection of greater variability in maturity among cherries harvested from these cultivars.

Table 2 summarizes the total phenolics and anthocyanins in the after, IN, and OUT fractions following simulated digestion for five cherry cultivars expressed as % recovery relative to the original fraction. There was a moderate increase in the % total phenolics for all cultivars and a slight increase in % anthocyanins for Lapins following pepsin digestion (after). It is possible that pepsin digestion released phenolic compounds from the cherry fruit matrix, but further investigation would be required to confirm this. For anthocyanins, the increase could be caused by the acid conditions of the pepsin digest (pH 2), favoring the formation of some colored anthocyanin derivatives. An equilibrium exists between two colored anthocyanin species, the flavylium cation (AH^+) and quinoidal base (A), and two colorless ones, the carbinol pseudobase (B) and chalcone (C) (**Figure 1**), which depends on pH. At low pH, anthocyanins exist primarily in the red form of the flavylium cation (*32, 33*). Similar increases in anthocyanins following pepsin digestion have been seen for pomegranate juice (*28*) and raspberry extracts (*25*).

The % recovery of total phenolics in IN fractions was comparable (27–29%) for all cultivars. The % total phenolics in the OUT fractions was more variable (77–101%). Skeena

cherries had a lower recovery of phenolics in the OUT fraction than the other cultivars.

The recovery of total phenolics in the combined IN and OUT fractions therefore approached that found in the pepsin digest fraction (after) for only the Bing, Lapins, Staccato, and Sweetheart cultivars. However, when considering the actual concentrations in the original fraction (**Table 2**), the Skeena cultivar (2853 mg gallic acid equiv/kg) would be the best source of phenolics after simulated digestion followed by Lapins.

The % recovery of anthocyanins in IN fractions was comparable (19–21%) for all cultivars, except for Bing (~15%). Possibly the higher total solids content of Bing cherries (**Table 1**) influenced the diffusion rate of anthocyanins. Gil-Izquierdo et al. (*27*) found that high sugar content in samples such as jams can affect diffusion of phenolics during in vitro digestion. The % of anthocyanins in the OUT fractions was more variable (52–67%) than for the IN fractions among all cultivars. When considering the actual anthocyanin content in the original fraction (**Table 2**), Skeena (3199 mg cyanidin 3-rutinoside equiv/kg) and Lapins cultivars would also be the best source of anthocyanins.

The anthocyanin content for Skeena was about the same as its total phenolic content, but for other cultivars, the anthocyanin content was lower than the total phenolic content. Because different standards are used for the two methods, comparing the amount of anthocyanins relative to total phenolics is imprecise. However, the results suggest that anthocyanins may make up a greater proportion of phenolics in Skeena than in other cultivars.

For anthocyanins, the % recovery in the combined IN and OUT fractions was lower (18–27%) for all cultivars than in the after fraction (**Table 2**). This loss could be explained by the transformation of the red flavylium form to the colorless chalcone form at pH 7.5 or by the influence of copigments that produce a bathochromic wavelength shift and an increase in the absorbance of the visible band (*32*). The formation of insoluble complexes with particulates may also be involved (*33*).

The concentrations of anthocyanins are reported as cyanidin 3-rutinoside equivalents (**Table 2**). In the literature, results for cherry anthocyanin analyses are often given as cyanidin 3-glucoside equivalents. However, because the major anthocyanin in cherries is cyanidin 3-rutinoside (**Figure 2**), this anthocyanin was used as the standard in this study. On the basis of the standard curves made for both cyanidin 3-glucoside and cyanidin 3-rutinoside, cyanidin 3-rutinoside values can be converted to cyanidin 3-glucoside values by multiplying by a factor of 0.756.

Working with frozen strawberries, Gil-Izquierdo et al. (*27*) reported that pepsin digestion did not affect anthocyanins, whereas a significant decrease occurred after pancreatic digestion with the anthocyanin recovery in IN samples being about 12.4%. Perez-Vicente et al. (*28*) observed little or no loss of anthocyanins and phenolics following pepsin digestion of fresh pomegranate juice as compared with the initial value. They recovered 1.5% of anthocyanins and 29% of total phenolics in the IN samples. McDougall et al. (*24, 25*) used in vitro digestion for raspberry syrup and red wine. They found a recovery of phenolics in IN samples of about 10 and 7%, respectively, for raspberry syrup and wine and a recovery of anthocyanins of about 5 and 4%, respectively.

The potential bioavailability of anthocyanins and other phenolics was higher for frozen cherries than in these other food products. However, comparing in vitro studies is difficult since differences may arise from the starting material as well as the in vitro digestion procedure used. Gil-Izquierdo et al. (*27*) studied two methods of in vitro digestion. The methods basically differed in the amount of contact between the food material and the dialysis tubing. The method with less contact resulted in higher pH (around 8–9) in the IN fraction as compared to a pH of around 7.4 for the method with more contact. This consequently resulted in lower recoveries of phenolics in the IN fraction. In this present study, the pH values for IN and OUT fractions usually ranged between 7.0 and 7.5 (data not shown), which could have resulted in higher recoveries of phenolics in the IN fraction.

In Vitro Bioavailability of Phenolic Compounds in Lapins and Bing Cherries at Three Ripening Stages Determined by UV–Vis Spectrophotometer. Because Bing and Lapins cherries are widely grown around the world, further studies to establish the in vitro bioavailability of anthocyanins and other phenolics in fruit at three ripening stages were carried out with these cultivars. As expected, the actual amounts of total phenolics and anthocyanins as well as total solids were higher for both cultivars in mature and overmature than immature cherries (**Tables 1** and **3**).

Generally, for both Bing and Lapins cherries, immature cherries had a higher % recovery of total phenolics in IN and OUT fractions than mature and overmature cherries. Therefore, a higher recovery of phenolics was noted in the combined IN and OUT fractions for immature Lapins than for mature and overmature cherries. Possibly the lower total solids content, mainly sugar, of immature cherries influenced diffusion rates during dialysis or changes in fruit composition with increasing maturity may have increased digestion breakdown and consequently lowered bioavailability of components. Further investigation is needed.

The % recovery of anthocyanins in IN and OUT fractions for both Bing and Lapins was lower in overmature than in immature and mature cherries. This trend would suggest that changes in the fruit matrix with maturity may be decreasing the bioavailability of anthocyanins. Further studies would be required to determine what changes are actually occurring.

In both Bing and Lapins fruit, the actual amounts of total phenolics and anthocyanins were comparable in mature and overmature (**Table 3**) as compared to the immature cherries. Because the levels in after, IN, and OUT fractions were also similar for mature and overmature cherries, amounts for total phenolics and anthocyanins were also comparable in mature and overmature cherries at any digestion stage. For Bing cherries, the amount of total phenolics was about 1.5 times and of anthocyanins about 3.0 times higher in mature and overmature than in immature cherries. For Lapins, the amount of total phenolics was about 1.6 times and of anthocyanins about 3.6 times higher in mature and overmature than in immature cherries. These results appear to be in agreement with the total phenolic and anthocyanin evolution over different ripening stages found in the Marvin-Niram sweet cherry cultivar by Serrano et al. (*34*).

Although mature cherries have better sensory quality than overmature cherries, their phenolic contents were quite comparable. Overmature cherries are usually considered to be culls for the fresh fruit market. The results for overmature cherries suggest that potentially overmature fruit could be used as a source of anthocyanins for food coloring or nutraceutical products.

In Vitro Bioavailability of Phenolic Compounds in Lapins Cherries at Three Ripening Stages Determined by HPLC. Lapins cherry extracts at three maturity stages were analyzed by HPLC to determine if the results were similar to those obtained by spectrophotometric methods. **Figure 2** shows typical HPLC chromatograms at 320 and 525 nm obtained for an extract

of original mature Lapins cherries. Only the major peaks were analyzed to simplify quantitation. At 320 nm, four peaks were identified. Neochlorogenic acid (peak 1), *p*-coumaroylquinic acid (peak 2), chlorogenic acid (peak 3), and caffeic acid (peak 4) were identified from the retention times and spectral properties of samples and standards. At 525 nm, three anthocyanin peaks were identified as reported by Gao and Mazza (*3*) and standards that were coanalyzed. These peaks were cyanidin 3-glucoside (peak 5), cyanidin 3-rutinoside (peak 6), and peonidin 3-rutinoside (peak 7).

The results for colorless phenolic and anthocyanin peaks measured by HPLC for Lapins cherries at three maturities are presented in **Tables 4** and **5**, respectively. Although the values obtained by spectrophotometric methods and HPLC were different, some similar trends were seen. For the main colorless phenolics (neochlorogenic and *p*-coumaroylquinic acids) and anthocyanins, the % recovery in IN fractions was usually higher for immature than mature and overmature fruit (**Tables 4** and **5**). In addition, the actual amounts of anthocyanins were similar in mature and overmature as compared to immature cherries (**Table 5**).

For all compounds examined except caffeic acid, the % recovery for the combined IN and OUT fractions totalled less than 100%. However, for caffeic acid (peak 4), the % recovery for the combined IN and OUT fractions totalled over 780%. Possibly the breakdown of neochlorogenic acid, chlorogenic acid, or other phenolics led to the increase in what was identified as caffeic acid. Further study would be needed to determine what changes occur.

Changes occurring for individual phenolic compounds were seen with HPLC analysis during in vitro digestion of frozen cherries. Differences were seen in how individual compounds changed at different stages of simulated digestion. The analysis was quite time-consuming though. In contrast, spectrophotometric methods like the Folin−Ciocalteu method for total phenolics gave results based on how all compounds present react with the reagent used. Singleton and Rossi (*30*) found that different phenolics react very differently with Folin−Ciocalteu reagent. Spectrophotometric methods therefore can only give a limited indication of changes occurring. The results in this study found that spectrophotometric methods can provide a quick, simple way of assessing changes for major compounds, which was confirmed by HPLC.

The in vitro model used in this study was useful to predict the potential bioavailability of phenolic compounds and anthocyanins in sweet cherries. The in vitro digestion procedure applied in this work, however, requires validation with bioavailability.

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