Assessing Potential Bioavailability of Raspberry Anthocyanins Using an in Vitro Digestion System

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The bioavailability of anthocyanins from raspberry extracts was assessed using an in vitro digestion procedure that mimics the physiochemical and biochemical changes that occur in the upper gastrointestinal tract (GIT). Effectively all of the total phenol content of the raspberry extract survived gastric digestion and partitioned between the IN sample, which represents the serum available material, and the OUT sample, which represents the material that remains in the GIT and passes through to the colon. All of the anthocyanins also survived gastric digestion, but only ~5% entered the IN sample and ~70% of total anthocyanins were recovered in the IN and OUT samples. Codigestion of the raspberry extract with commonly combined foodstuffs such as bread, breakfast cereal, ice cream, and cooked minced beef gave a different pattern. The total phenol content of the IN samples was slightly reduced by codigestion with ice cream or breakfast cereal but unaffected by codigestion with bread or minced beef. In most cases, the phenol contents of the postgastric and OUT samples were reduced as compared with the expected values. However, the anthocyanin content of the IN samples was unaffected or increased by coincubation with the foodstuffs. This suggests that polyphenols transiently bind to food matrices during digestion, which protects the more labile anthocyanins from degradation, and they are free to diffuse into the IN sample. The anthocyanin composition of the bioavailability samples was monitored by liquid chromatography–mass spectrometry. All eight anthocyanins previously identified in raspberry were detected in the extract and the postgastric samples at similar yields. All eight anthocyanins could be discerned in the IN and OUT samples, but some such as cyanidin-3-O-glucoside were greatly reduced and others such as pelargonidin-3-O-glucoside were apparently increased in abundance. These differences in stability and their importance for the bioavailability of anthocyanins are discussed.

KEYWORDS: Anthocyanins; antioxidants; berry; bioavailability; health benefits; raspberry; polyphenols; stability

INTRODUCTION

Anthocyanins are the pigments responsible for the red and blue colors of plant organs such as fruits, flowers, and leaves (1). Anthocyanins are composed of six anthocyanidin aglycones linked to sugar groups at positions 3 and/or 5 (Figure 1). They can also be industrially important natural food colorings (2). The main dietary sources of anthocyanins include red fruits, certain vegetables, and red wine (3).

Dietary consumption may be as high as 200 mg/day, and berry fruits, which accumulate large quantities of anthocyanins, can provide 100–300 mg in a single portion (4–6). Like other members of the flavonoids, anthocyanins are effective antioxidants (2) but they have also been proposed to have other biological activities, which may be independent of their antioxidant capacities, that produce health benefits (7); examples range from protection of low-density lipoproteins from oxidation thus influencing cardiovascular performance (8), protection against mutagens and carcinogens (9, 10), suppression of inflammatory responses (11), and protection against age-related declines in cognitive behavior and neuronal dysfunction in the central nervous system (12).

Nevertheless, to achieve any effect in a specific tissue or organ, these bioactive compounds must be bioavailable, i.e., effectively absorbed from the gut into the circulation and delivered to the appropriate location within the body. The bioavailability of anthocyanins is open to question. Oral administration of anthocyanin-rich fruits, extracts, or pure anthocyanins has proved to have beneficial effects in preventing or suppressing diseased states in vivo (13, 14). Studies of oral administration of anthocyanins have confirmed the increased antioxidant status of the serum (13, 15–17), but this is usually accompanied by very low uptake of anthocyanins into the serum.

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(<1% of dose) and correspondingly low levels of urinary excretion as intact or conjugated forms (18–22). The apparent low bioavailability of anthocyanins seems to cast doubt on their ability to exert their proposed beneficial effects.
However, assessment of true bioavailability of any class of phytochemicals requires a synthesis of data concerning their absorption, metabolism, tissue and organ distribution, and excretion. Such studies carried out in animals or human subjects are complex, expensive, and lengthy. In this study, we assess the recovery of raspberry anthocyanins using an in vitro digestion procedure that mimics the physiochemical and biochemical conditions encountered in the gastrointestinal tract. This in vitro procedure allows the screening of multiple samples and may provide data on the relative potential bioavailability of different polyphenolic components. We also assess the effects of codigestion with common foodstuffs and monitor the stability and recovery of individual anthocyanins using liquid chromatography—mass spectrometry (LC-MS).

MATERIALS AND METHODS

Plant Material and Extraction. Raspberries (10 kg of Rubus idaeus L. variety Glen Ample) were purchased from local farmers. Batches (250 g) were frozen at −20°C and used within a week. The defrosted material was homogenized in an equal volume of acetonitrile in a Waring blender (full power; 3 x 15 s). After filtration through sheets of muslin, the extracts were dried by rotary evaporation. The dried material was homogenized in an equal volume of acetonitrile in a Waring blender (full power; 15 s). After centrifugation (5000 g, 20 min, 4 °C), the supernatant was removed and dried to a syrup in a rotary evaporator. The syrup was stored frozen at −20°C.

Bioavailability Procedure. The procedure was adapted from the method outlined by Gil-Izquierdo et al. (23), which itself was adapted from the work of Miller et al. (24). This work showed significant correlation between in vitro and in vivo measurements of iron bioavailability, but no similar correlation has yet been obtained for other components such as polyphenols. The method consists of two sequential steps: an initial pepsin/HCl digestion for 2 h at 37 °C to simulate gastric conditions followed by a digestion with bile salts/pancreatin for 2 h at 37 °C to simulate small intestine conditions. The raspberry syrup was diluted in distilled water to a working concentrate that was eight times more concentrated than the concentration of soluble phenols found in the raspberry extract.

Codigestion Experiments. Different procedures were used for dry or wet foodstuffs. White bread was crumbled in a Waring Blender, 5 g was added to 17.5 mL of distilled water, and then 2.5 mL of the working raspberry concentrate was added. Breakfast cereal (Shredded Wheat, Nestle UK Ltd.) was crumbled in a Waring Blender, and 5 g was used as above. Minced beef steak was cooked using a microwave oven and 5 g wet weight made up to 17.5 mL with distilled water before the raspberry concentrate was added. The ice cream (TESCO value, TESCO Ltd., Cheshunt, United Kingdom) contained 4 g of protein, 26 g of sugars, and 7 g of fat per 100 g fresh weight. After thawing, 10 g was made up to 17.5 mL with distilled water before the raspberry concentrate was added. The slurries were put through the normal bioavailability procedure. Foodstuff alone control digestions were carried out to monitor changes in soluble phenols. These solutions were compared with raspberry + foodstuff and raspberry alone controls to obtain a % value for the difference between the expected phenol content (= raspberry alone + foodstuff alone) values and the observed raspberry + foodstuff phenol content. The data for total recovery of phenols are presented in this manner because the digestion of the foodstuffs produced 10-fold increases in phenol content making graphical comparisons of % recovery figures difficult. No anthocyanins were detected in the foodstuff digestions; therefore, any anthocyanins were assumed to have come from the raspberry extracts.

Anthocyanin and Phenol Assays. The total anthocyanin concentration was estimated by a pH differential absorbance method (5). The absorbance value was related to anthocyanin content using the molar extinction coefficient calculated in-house for pure cyanidin-3-O-glucoside = 29000 M−1 cm−1 (purchased from Extrasyntese, Genay, France). The method of total anthocyanin determination is based on the recovery of the red flavylium form at pH 1.0, but it is known that chalcone to flavylmion reversal is slow. Therefore, the anthocyanin recovery values for the IN and OUT samples, where at pH 7–8 the chalcone form would be predominant, may be underestimated. However, allowing for complete reversal of chalcone to flavylmion (leaving samples at pH 1.0 for 12 h at room temperature; 25), did not alter the apparent anthocyanin recovery.

The phenol content was measured using a modified Folin–Ciocalteau method (5). Phenol contents were estimated from a standard curve of gallic acid. All results have been corrected for the presence of phenols in the pancreatic/bile salts mixture.

Samples from the bioavailability procedure were acidified to 0.5% (v/v) trifluoroacetic acid (TFA) by slow addition of 10% TFA and mixed well. After centrifugation, insoluble material was retained for further analysis. The soluble material was passed through C18 solid-phase extraction (SPE) cartridges (1000 mg capacity, Phenomenex Ltd.), which had been preequilibrated in ultrapure water (UPW) containing 0.25% (v/v) TFA. After a wash with 2 volumes of TFA/UPW, the bound material was eluted by the addition of 0.25% (v/v) TFA in 25% (v/v) acetonitrile. This concentration afforded complete separation of total phenolics from the bile salts present in the IN and OUT samples. The recovery of cyanidin-3-O-glucoside from the SPE procedure was around 90%. The phenol and anthocyanin contents of the bound fractions and the “insoluble” material, which was soluble in methanol, were assayed. The fractions were then dried in a speed-vac (Thermo-Finnegar Ltd.) equivalent phenol concentrations to facilitate comparison after LC-MS analysis.

LC-MS. Samples (containing 40 μg gallic acid equivalents by Folin assay) were analyzed on a LCQ-DECA system, comprised of a Surveyor autosampler, pump, and photodiode array detector (PDAD) and a ThermoFinnigan mass spectrometer iontrap. The PDAD scanned three discrete channels at 200, 365, and 520 nm. Samples were eluted over a gradient of 5 (0.5% formic acid) to 25% acetonitrile (0.5% formic acid) on a C18 column (Synergi Hydro C18 with polar end capping, 4.6 mm x 150 mm, Phenomenex Ltd.) over 60 min at a rate of 400 μL/min. The LCQ-Deca LC-MS was fitted with an ESI (electrospray ionization) interface and analyzed the samples in positive ion mode. There were two scan events; full scan analysis followed by data-dependent MS/MS of most intense ions. The data-dependent MS/MS used collision energies (source voltage) of 45% in wideband activation mode. The MS detector was tuned against prominent anthocyanin peaks in the raspberry extract.
RESULTS

The recovery of phenols from the bioavailability procedure was generally higher than the recovery of anthocyanins (Tables 1 and 2). On average, allowing for losses and errors, the recovery of phenols in the IN and OUT samples approached 100% of the amount in the extract. The recovery of anthocyanins was lower, and only 70% could be recovered in the IN and OUT samples. Although there was some experiment to experiment variation, the pattern of recovery of anthocyanins (Table 2) was generally lower than phenols in the IN sample (denoting serum bioavailability) suggesting that raspberry anthocyanins were particularly poorly recovered as compared with bulk polyphenols in this procedure.

Codigestion with common foodstuffs changed the bioavailability of phenols (Figure 2) and anthocyanins (Figure 3). Codigestion with ice cream and Shredded Wheat slightly reduced the serum available phenol content (IN sample) whereas bread and minced beef caused no effect and an increase, respectively. Codigestion with all foodstuffs reduced the total phenol content of the postgastric samples, and the total phenol content of the OUT samples was reduced in all but the ice cream experiment (Figure 2). The anthocyanin content of the IN samples was either unaffected or increased by codigestion with the foodstuffs (Figure 3). If the phenols and anthocyanins bind to the foodstuffs during gastric and pancreatic digestion, this would protect the anthocyanins but still allow these particularly labile compounds to diffuse into the IN sample.

Table 1. Recovery of Phenols from Raspberry Bioavailability Procedure

<table>
<thead>
<tr>
<th>Phenols</th>
<th>experiment</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>5</th>
<th>6</th>
<th>7</th>
<th>8</th>
<th>9</th>
<th>10</th>
<th>11</th>
<th>12</th>
<th>13</th>
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<th>SE</th>
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<td>100</td>
<td>100</td>
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<td>100</td>
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<td>100</td>
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</tr>
<tr>
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<td>88.3</td>
<td>84.1</td>
<td>83.0</td>
<td>106</td>
<td>97.2</td>
<td>99.6</td>
<td>88.3</td>
<td>92.3</td>
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<td>93.4</td>
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<tr>
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<td>10.7</td>
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<td>2.1</td>
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$^a$ Thirteen experiments were carried out in total, and each value within each experiment is calculated as the average of four replicate analyses. $^b$ The average of the % recovery figures for the 13 experiments is presented ± the standard error.

Table 2. Recovery of Anthocyanins in Raspberry Bioavailability Procedure

<table>
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<tr>
<th>Anthocyanins</th>
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<th>4</th>
<th>5</th>
<th>6</th>
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<th>12</th>
<th>13</th>
<th>AV$^a$</th>
<th>SE</th>
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<td>100</td>
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<td>110.8</td>
<td>94.9</td>
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<td>107.5</td>
<td>100.4</td>
<td>107.5</td>
<td>110.8</td>
<td>90.7</td>
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<td>109.2</td>
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<td>3.5</td>
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<tr>
<td>IN</td>
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<td>12.3</td>
<td>12.6</td>
<td>6.9</td>
<td>3.8</td>
<td>3.6</td>
<td>5.3</td>
<td>3.99</td>
<td>12.6</td>
<td>8.1</td>
<td>4.4</td>
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<td>2.9</td>
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<td>0.8</td>
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<tr>
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<td>86.9</td>
<td>70.3</td>
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<td>35.4</td>
<td>39.9</td>
<td>61.9</td>
<td>4.5</td>
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$^a$ Thirteen experiments were carried out in total, and each value within each experiment is calculated as the average of four replicate analyses. $^b$ The average of the % recovery figures for the 13 experiments is presented ± the standard error.

Figure 2. Recovery of phenols from codigestion experiments. Figures illustrate the percentage difference between the observed phenol content of the raspberry + foodstuff and the expected value = raspberry alone + foodstuff alone experiments. Each value is the average of four experiments, and error bars show standard errors; SW = shredded wheat.

Figure 3. Recovery of anthocyanins from codigestion experiments. The % recovery values for each IN sample are given on the diagram for ease of comparison. Each figure is the average of four replicate experiments, and the error bars represent standard errors; SW = shredded wheat.
3-O-glucoside. This peak may be due to kaempferol glucoside, which is present in raspberries (3) and gives this characteristic MS/MS profile (27). Also, the scan at $m/z = 433$ yielded two peaks at room temperature = 42.43 and 49.34, the first of which was due to mass ion of 433.0, which gave a MS$_2$ product of 271.2, consistent with the aglycone pelargonidin and loss of mass 162 = glucose, but the second peak gave MS$_2$ products of 317.0 and 271.2 suggesting that it was not due to pelargonidin-3-O-glucoside.

The peaks in at 520 nm can be assigned as follows. Peak 1 is composed of cyanidin-3-O-sophoroside, peak 2 is composed of cyanidin-3-O-(2G)-glucosylrutinoside, peak 3 is composed of cyanidin-3-O-glucoside and pelargonidin-3-O-sophoroside, peak 4 is composed of cyanidin-3-O-rutinoside and pelargonidin-3-O-(2G)-glucosylrutinoside, peak 5 is composed of pelargonidin-3-O-glucoside, and peak 6 is composed of pelargonidin-3-O-rutinoside. Using the peak areas calculated from the MS data, the order of abundance is cyanidin-3-O-sophoroside ≥ cyanidin-3-O-(2G)-glucosylrutinoside ≥ cyanidin-3-O-glucoside ≥ cyanidin-3-O-rutinoside = pelargonidin-3-O-sophoroside ≥ pelargonidin-3-O-(2G)-glucosylrutinoside = pelargonidin-3-O-glucoside ≥ pelargonidin-3-O-rutinoside with a 20-fold difference in apparent abundance between the most and the least abundant.

The postgastric sample also contained six main peaks at 520 nm (Figure 4), and all eight anthocyanins were detected by “mining” at the expected masses. The anthocyanins elute in the same order, and peaks can be assigned as in Figure 1. The IN

Figure 4. Separation of anthocyanins in fractions from bioavailability procedure. The panels show the absorbance of the eluent at 520 nm of the extract, the postgastric sample, the IN sample, and the OUT sample, respectively. Six peaks can be discerned as discussed in the text.
and OUT samples also contained six peaks at 520 nm (Figure 4), and all eight anthocyanins could be detected by “mining”.

As illustrated by the increased absorbance at 520 nm, the gastric treatment enhanced the levels of all of the anthocyanins over the extract. This may be due to the stability of the flavylum cation form of anthocyanins (25) and a slight reduction in other phenolics in the sample making the anthocyanins a larger portion of the total. The IN and OUT samples contained reduced amounts of all of the anthocyanins as compared with the original extract and the postgastric samples, as illustrated by the lower $A_{520}$ values for these samples (Figure 4). However, the altered levels of the six main anthocyanin peaks suggest differences in the recovery of the individual anthocyanins. As the samples were loaded at an equal concentration of phenols (i.e., 40 µg phenols/injection), the % recovery of individual anthocyanins (calculated from the MS peak areas) in the IN and the OUT samples can be compared to the content of the same anthocyanin in the postgastric sample (see Table 3). If there were no differences in the relative stability of the individual anthocyanins, one would expect the % recovery figures to be similar. Peaks 3, 4, and 6 of the IN sample were relatively depleted as compared to the postgastric sample whereas peak 5 was apparently increased (Figure 4). The reductions in peaks 3, 4, and 6 can be explained by lower recoveries of cyanidin-3-O-glucoside (57.8% of the total amount of that anthocyanin in the postgastric sample), cyanidin-3-O-rutinoside (at 54.6%), and pelargonidin-3-O-rutinoside (61.7%). The high recovery of pelargonidin-3-O-glucoside (95.3%) in the IN sample explained the apparent increase in peak 5 in the IN sample relative to the postgastric sample.

The OUT sample also had lower levels of all of the anthocyanins, but peaks 1, 3, 4, and 6 were depleted as compared to the postgastric sample (Figure 4). The reduction in peak 1 can be explained by a lower recovery of cyanidin-3-O-sophoroside (60.0% of the amount in the postgastric sample), peak 3 by lower recoveries of cyanidin-3-O-glucoside (30.5%)
and pelargonidin-3-O-sophoroside (47.6%), peak 4 by a lower recovery of cyanidin-3-O-rutinoside (44.3%), and peak 6 by a low recovery of pelargonidin-3-O-rutinoside (63.3%). The smaller size of peak 5 in the OUT sample as compared to the IN sample may be due to the lower recovery of pelargonidin-3-O-glucoside (66.3%).

Some of the losses may be accounted for by coprecipitation of anthocyanins in the IN and OUT samples with insoluble components of the pancreatic bile salts mixture that occurred during reacidification prior to SPE. These are not recovered after acidification in the SPE cleanup step. These precipitates released anthocyanins upon treatment with acidified methanol, but this amounted to only 1−2% of the total anthocyanin content of the samples. The major polyphenolic components of the IN and OUT precipitates were ellagitannin derivatives, which presumably bound to proteins in the pancreatic enzyme mixture (results not shown).

### DISCUSSION

The instability of anthocyanins in similar procedures that mimic the physiochemical and biochemical changes that occur in the upper gastrointestinal tract has already been noted (28). In pomegranates, only 2−3% of the total anthocyanins were recovered in the IN sample and 15% in the OUT sample and a similar pattern was obtained with strawberry samples (27). As eating raspberries in isolation or consuming raspberry juices makes up only a small proportion of the diet, the effect of codigestion with various foodstuffs was examined. Codigestion reduced the recovery of phenols and anthocyanins in the postgastric and OUT samples, presumably by absorption to foods. However, the recovery of phenols and anthocyanins in the IN, or serum available, sample was largely unaffected.

Anthocyanins have been recovered in very small amounts (<1% dose) in the serum after oral ingestion (18−22). The in vitro digestion procedure has not been validated against data for the in vivo bioavailability of polyphenol classes, but it is encouraging that this model also predicts low serum values for anthocyanin bioavailability and that the majority of polyphenols remain in the gut.

The low recovery of anthocyanins may have two main causes. At the pH of the small intestine (pH 7.5−8.0), anthocyanins (which exist in equilibrium of four molecular species; the colored basic flavylum cation and three secondary structures—the quinoidal bases, the carbinol pseudobase, and the chalcone pseudobase forms) are predominantly in the colorless chalcone pseudobase molecular form. The formation of the chalcone and its subsequent fission between the B and C rings (Figure 1) is also favored by elevated temperatures and results in the destruction of the anthocyanin chromophore (25). For example, the main anthocyanins in blackcurrant were increasingly unstable at pH > 4.5 (29). In addition, anthocyanins can form insoluble complexes with particulates (30) and anthocyanins bound to components of the pancreatic bile salts mixture in the IN and the OUT samples. However, these insoluble complexes contained only a small proportion of the apparent “lost” anthocyanin content of these samples. Anthocyanins were effectively stable under gastric conditions, and direct absorption from the stomach into the blood (31) may explain the rapid but transient increase in serum anthocyanins noted in animal and human studies.

Mazza et al. (15) correlated serum anthocyanin concentration after blueberry ingestion with antioxidant capacity. However, the concentration of anthocyanins was low (~nanomolar) and seemed insufficient to raise serum antioxidant capacity. Oral administration of cyanidin-3-O-glucoside to rats increased serum cyanidin-3-O-glucoside levels, but the levels of serum protocatechuic acid were eight times higher (32). Protocatechuic acid has been identified among degradation products of tart cherry anthocyanins [cyanidin-3-O-(2′G)-glucosylrutinoside > cyanidin-3-O-rutinoside > cyanidin-3-O-glucoside] maintained at pH 7.0 (33) and as a degradation product formed by B/C ring cleavage of cyanidin-3-O-glucoside (34). Further studies to identify the degradation products of anthocyanins under the neutral to alkaline conditions prevalent in the small intestine and serum are required. The majority of anthocyanins and other phenols pass intact into the colon (OUT) fraction and may be degraded into phenolic compounds (including protocatechuic acid) by gut microflora and absorbed (35−37) to contribute to the serum antioxidant capacity.

Active transport mechanisms that may occur in the small intestine (38) cannot be mimicked in the in vitro study, and partition of anthocyanins into the dialysis tubing is solely dependent on diffusion rates and their stability. Nevertheless, all eight raspberry anthocyanins were recovered in the IN sample, which denotes serum bioavailability. The instability of anthocyanins in conditions that mimic the small intestine can be rationalized, but it is more difficult to understand the apparent differences in stability of individual anthocyanins noted in this study. Some ideas about the stability of anthocyanins have been inferred from a large number of studies that focused on individual stresses such as heating, exposure to light, etc. (3, 4, 25). In general, acylated anthocyanins are more stable than nonacylated forms; 3,5-diglycosides are more stable than 3-monoglycosides; increased methoxylation of aglycone hydroxyls enhances stability, and conversely, anthocyanins with more hydroxylated aglycones are less stable. However, no such patterns can be discerned for stability based on aglycone structure or the type of glycosyl groups in this study. In fact, pelargonidin derivatives were not consistently more stable than

### Table 3. Recovery of Anthocyanins in IN and OUT Samples

<table>
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<tr>
<th>anthocyanin (pk)</th>
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<th>% recovery a IN sample</th>
<th>% recovery a OUT sample</th>
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<td>Pg-Rut (6)</td>
<td>579</td>
<td>57.4</td>
<td>69.5</td>
</tr>
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</table>

The % recovery compares the MS peak areas of the individual anthocyanins in the IN and OUT sample to those in the postgastric sample. a This refers to the peak numbers in Figure 4.

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**Note:** The table includes molecular weights (m/z) and percentage recovery of various anthocyanins in IN and OUT samples, along with standard deviations (SD) for experiments 1, 2, and 3. The data reflect the comparison of anthocyanin recoveries in the IN and OUT samples, with a focus on the postgastric sample as the reference point. The table highlights the variability in recovery percentages across different experiments, indicative of the instability of anthocyanins under simulated in vitro digestion conditions.
their cyanidin derivatives and there was no consistent effect of glycosyl groups.

Previous studies on raspberry anthocyanins have focused on their stability to particular processing techniques. For example, during heating in raspberry jam making, the most stable anthocyanins were cyanidin-3-O-(2”)-glucosylrutinoside > cyanidin-3-O-sophoroside ≥ cyanidin-3-O-rutinoside ≥ cyanidin-3-O-glucoside (39). Long-term storage of red raspberry juice concentrate at 20 °C caused a 80% loss in total anthocyanin content, which was accompanied by a drastic reduction in cyanidin-3-O-(2”)-glucosylrutinoside content, a reduction in cyanidin-3-O-sophoroside content, but an increase in cyanidin-3-O-glucoside content, apparently through breakdown of the other cyanidin glycosides under long-term acidic conditions (40).

No previous work reports on the effects of the combined stresses of elevated pH, exposure to heating, and oxygen found in the in vitro bioavailability procedure. The only report on the effect of the in vitro digestion procedure on individual anthocyanins in pomegranate gave qualitative results, but it suggested that the trihydroxylated delphinidin derivatives were the least stable (28).

There is one anomaly that deserves particular comment. Pelargonidin-3-O-glucoside was recovered in relatively higher amounts in the IN samples as compared to the other anthocyanins whereas cyanidin-3-O-glucoside was notably unstable in both the IN and the OUT samples. It is possible that the relative increase in abundance of pelargonidin-3-O-glucoside in the IN sample was caused by the breakdown of pelargonidin-3-O-rutinoside, pelargonidin-3-O-(2”)-glucosylrutinoside, and/or pelargonidin-3-O-sophoroside. However, it is difficult to rationalize why similar processes did not cause increases in cyanidin-3-O-glucoside. Cyanidin-3-O-glucoside was found to be liable to polymerization reactions during processing, fermentation, and storage of raspberry juices (40). Also, cyanidin-3-O-glucoside has a higher antioxidant capacity than other anthocyanins (19) and may be preferentially oxidized. The possibility that certain components may be sacrificially protected in complex mixtures of polyphenols requires further attention.

To summarize, anthocyanins are unstable under in vitro conditions that mimic those of the upper gastrointestinal tract. The exposure to differences in pH, oxygen, and heating combines to greatly reduce anthocyanin availability to the serum fraction. Codigestion with common foodstuffs may help protect the labile anthocyanins and certainly does not markedly decrease the levels of serum bioavailable polyphenols. All eight anthocyanins present in raspberry juice can be detected in the IN and OUT samples, but certain anthocyanins are more stable possibly due to sacrificial protection from oxidation. The low recovery of anthocyanins in the IN and OUT samples was comparable to bioavailability data obtained from human and animal feeding experiments. The in vitro digestion procedure provides a simple, rapid, and upgradeable screening method to assess the potential stability of phytochemicals from fresh, extracted, and processed foods.

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LITERATURE CITED


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