JOURNAL OF AGRICULTURAL AND FOOD CHEMISTRY

Bioavailability of Anthocyanins from Purple Carrot Juice: Effects of Acylation and Plant Matrix

CRAIG S. CHARRON,[†] ANNE C. KURILICH,[†,‡] BEVERLY A. CLEVIDENCE,[†] PHILIPP W. SIMON,[§] DAWN J. HARRISON,[†] STEVEN J. BRITZ,[†] DAVID J. BAER,[†] AND JANET A. NOVOTNY*[,†]

Beltsville Human Nutrition Research Center, Agricultural Research Service, U.S. Department of Agriculture, Beltsville, Maryland 20705, and Vegetable Crop Research Unit, Agricultural Research Service, U.S. Department of Agriculture, Department of Horticulture, University of Wisconsin, Madison, Wisconsin 53706

Absorption of cyanidin-based anthocyanins is not fully understood with respect to dose or anthocyanin structure. In feeding studies using whole foods, nonacylated anthocyanins are more bioavailable than their acylated counterparts, but the extent to which plant matrix determines relative bioavailability of anthocyanins is unknown. Using juice of purple carrots to circumvent matrix effects, a feeding trial was conducted to determine relative bioavailability of acylated and nonacylated anthocyanins and to assess dose−response effects. Appearance of anthocyanins in plasma was measured in 10 healthy adults for 8 h following consumption of purple carrot juice. Each subject consumed 50, 150, and 250 mL of juice containing 76 μmol (65 mg), 228 μmol (194 mg), and 380 μmol (323 mg) of total anthocyanins, respectively. Acylated anthocyanins comprised 76% of total anthocyanins in the juice, yet their bioavailability was found to be significantly less than that of nonacylated anthocyanins. Peak plasma concentrations of nonacylated anthocyanins were 4-fold higher than that for acylated anthocyanins. Absorption efficiency declined across the doses administered. Because the treatments were consumed as juice, it could be discerned that the difference in bioavailability of acylated versus nonacylated anthocyanins was not primarily caused by interactions with the plant matrix.

KEYWORDS: Anthocyanin; bioavailability; absorption; carrot (*Daucus carota*)

INTRODUCTION

Anthocyanins are flavonoids found in fruits, vegetables, leaves, flowers, and grains. The presence of these polyphenols imparts bright colors of red, blue, and purple. In plants, anthocyanins offer photoprotection, scavenging of free radicals, and attraction of animals for pollination and seed dispersal (*1, 2*). Anthocyanins play a role in industry by offering a replacement for some synthetic food colorants. As dietary constituents, anthocyanins possess a variety of health benefits, including reduced risk of cardiovascular disease (*3*−*6*), decreased risk of cancer (*4*, *7*−*13*), protection against age-related neurodegenerative declines (*14*−*16*), and improved glucose regulation (*17*, *18*).

An important factor in the ability of a dietary component to provide health benefits is bioavailability. Anthocyanins appear to have low bioavailability, because recovery of anthocyanins in biological samples after volunteers have consumed anthocyanin-rich foods and extracts has been low (*19*−*25*). Anthocyanin structure is one factor that appears to affect bioavailability. Anthocyanins in nature are derivatives of six common backbone structures that are glycosylated, and the glycosylations can form linkages with aromatic acids, aliphatic acids, and methyl ester derivatives (*26*). Both glycosylation and acylation appear to affect bioavailability. A study using Caco-2 human intestinal cell monolayers showed that cyanidin 3-glucoside and peonidin 3-glucoside had higher transport efficiencies than cyanidin 3-galactoside and peonidin 3-galactoside, respectively, indicating the higher bioavailability of glucose-based anthocyanins (*27*). This lends support to the proposition that anthocyanin absorption may be mediated by the sodium-dependent glucose transporter, which is involved in the transport of the flavonoid quercetin (*28*), or the organic anion membrane carrier bilitranslocase (*29*), because the efficiency of carrier proteins in anthocyanin transport would likely be related to anthocyanin structure. In the same study, it was found that the presence of free hydroxyl groups as opposed to methoxyl groups on the aglycone was generally associated with decreased anthocyanin bioavailability (*27*). Studies have also suggested that acylation of anthocyanins can significantly affect anthocyanin absorption. Nonacylated anthocyanins from steamed red cabbage were found to be 4-fold more bioavailable than acylated anthocyanins (*30*),

* Corresponding author [telephone (301) 504-8263; fax (301) 504-9098; e-mail Janet.Novotny@ars.usda.gov].
[†] Beltsville Human Nutrition Research Center.
[‡] Present address: Quaker/Tropicana/Gatorade, Barrington, IL 60010.
[§] Vegetable Crop Research Unit.

10.1021/jf802988s
Published on Web 01/23/2009

and nonacylated anthocyanins from purple carrots were found to be 11−14-fold more bioavailable than acylated ones (*31*). Both of those studies were conducted with whole foods. Thus, the effects of anthocyanin localization in the plant matrix could not be isolated from the effects of anthocyanin structure. Plant matrix is an important factor in bioavailability of other phytonutrients. For example, β-carotene from orange fruits is substantially more bioavailable than β-carotene from green, leafy vegetables (*32*). Therefore, we conducted a study of bioavailability of acylated and nonacylated anthocyanins from purple carrot juice, an anthocyanin-rich vehicle that contains both acylated and nonacylated derivatives and which would not be vulnerable to interference by plant matrix issues. In addition, treatments were administered at three different dose levels to further elucidate anthocyanin dose−response.

MATERIALS AND METHODS

Chemicals and Materials. High-performance liquid chromatography (HPLC) grade ethyl acetate, methanol, and water were purchased from Fisher Scientific (Norcross, GA). Reagent grade formic acid and trifluoroacetic acid (TFA) were purchased from Sigma Chemical Co. (St. Louis, MO). Cyanidin 3-galactoside and malvidin 3-galactoside were purchased from Indofine Chemical Co. (Somerville, NJ). Sep-Pak Vac RC (500 mg) C18 cartridges for solid-phase extraction (SPE) were obtained from Waters Corp. (Milford, MA).

Subjects and Study Design. The study protocol was approved by the Johns Hopkins University Institutional Review Board, and written informed consent was obtained from each study subject. The 10 subjects were healthy, nonsmoking volunteers (5 males, 5 females) averaging 38 ± 15 years old and 70.0 ± 13 kg in body weight. Individuals with active disease (peripheral vascular disease, degenerative kidney disease, degenerative liver disease, cancer, acid reflux disease, or endocrine disorders) that may interfere with the study were excluded. Individuals with malabsorptive disorders or history of bariatric surgery were also excluded. Subjects' dietary history showed typical intake of three meals per day.

Three purple carrot juice treatments were administered to subjects in a crossover experimental design. All subjects received each of the treatments, and the treatment periods were separated by 4-week breaks. Subjects were randomly assigned to one of two groups, and each group had a different treatment order. Treatments consisted of 50, 150, and 250 mL of purple carrot juice and were served to fasting subjects. Blood was collected at 0, 0.5, 1, 1.5, 2, 2.5, 3, 3.5, 4, 5, 6, 7, and 8 h. Subjects were provided with an anthocyanin-free diet during the treatment day and for the prior 2 days. During the treatment day, a snack, lunch, and dinner were provided at 2, 4, and 10 h after consumption of the purple carrot juice, respectively. Caffeine consumption was prohibited on the treatment day and for 1 day prior to the treatment day. Vitamins and supplements were prohibited throughout the study.

Preparation of Purple Carrot Juice. The purple carrots were U.S. Department of Agriculture inbred B217 and were grown at the University of California Desert Research and Education Center in Holtville, CA. After the carrots had been washed, the carrot tissue was juiced in a model JE900 commercial juicer (Breville, Torrance, CA) and remaining pulp was discarded. Four batches of carrot juice were produced. Each batch was pasteurized at 82 °C, cooled in cold tap water, and frozen at −20 °C. Two days prior to use, the frozen carrot juice was thawed in a refrigerator at 4 °C.

Analysis of Anthocyanins in Purple Carrot Juice. Triplicate samples from each of the four batches were analyzed. Each sample was prepared by diluting 1 mL of purple carrot juice with 20 mL of water and then diluting a 0.5 mL aliquot of this mixture with 5 mL of methanol/10% aqueous formic acid (1:9 v/v). After this final dilution, 50 μL samples were injected onto a liquid chromatograph−mass spectrometer for anthocyanin identification and quantification.

Blood Collection and Preparation. Blood was collected into vacutainers containing EDTA and centrifuged at 2560*g* for 10 min. Plasma aliquots of 2.2 mL were combined with 1.3 mL of 0.44 M aqueous TFA in cryovials and stored at −80 °C. Anthocyanins were extracted on SPE columns as previously described (*31*). After extraction of anthocyanins, 50 μL samples were injected onto a liquid chromatograph−mass spectrometer for anthocyanin identification and quantification.

HPLC-DAD-MS Analysis of Anthocyanins. Anthocyanins were analyzed on an LC-MS system composed of an Agilent (Agilent Technologies, Palo Alto, CA) series 1100 LC with a 250 mm × 4.6 mm i.d., 5 μm, Zorbax SB-C18 column (Agilent), G1315A diode array detector (DAD), and G1946A mass spectrometer (MS). The LC-MS conditions and solvent system were as previously described (*31*). Selected ion monitoring was used to identify individual anthocyanins and to search for cyanidin and anthocyanin glucuronides or sulfates.

Calculations and Statistics. Malvidin 3-galactoside was used as an internal standard to account for extraction losses, which averaged 47 ± 5% (SEM). A standard curve was created using cyanidin 3-galactoside to calculate molar concentrations of individual anthocyanins expressed as cyanidin 3-galactoside equivalents. Gram concentrations of individual anthocyanins were calculated using their respective molecular weights.

The percent recovery was calculated by dividing the peak plasma anthocyanin quantity (expressed in micromoles) by the quantity (micromoles) of anthocyanins in the dose. The peak plasma quantity was determined by multiplying the plasma anthocyanin concentration by the estimated plasma volume (45 mL of plasma/kg of body mass). The data were tested for normality (using the Kolmogorov−Smirov test) and equal variance (using the Levene median test), and then a one-way repeated measures analysis of variance was used to compare plasma responses among treatments ($P < 0.05$). The Holm−Sidak method was used for pairwise multiple comparisons between treatments. The percent recoveries of acylated and nonacylated anthocyanins for each treatment were compared by *t* test ($P < 0.05$). SigmaStat software, version 3.11 (SPSS Inc., Chicago, IL), was used for these statistical analyses. The area under the plasma concentration time curve (AUC) was calculated by the trapezoidal method using Microsoft Excel 2003 v. 11.

RESULTS AND DISCUSSION

Five anthocyanins were identified in the purple carrot juice. These anthocyanins were previously detected in purple carrots in studies using mass spectrometry and nuclear magnetic resonance spectroscopy (*31*, *33*). These anthocyanins consist of a cyanidin aglycone to which a glycosidic residue is attached at the 3-position of the cyanidin: cyanidin 3-(2″-xylose-6″-glucose-galactoside), cyanidin 3-(2″-xylose-galactoside), cyanidin 3-(2″-xylose-6″-sinapoyl-glucose-galactoside), cyanidin 3-(2″-xylose-6″-feruloyl-glucose-galactoside), and cyanidin 3-(2″-xylose-6″-(4-coumaroyl)glucose-galactoside) (**Figure 1**). Cyanidin 3-(2″-xylose-6-glucose-galactoside) and cyanidin 3-(2″-xylose-galactoside) are nonacylated anthocyanins. Cyanidin 3-(2″-xylose-6″-sinapoyl-glucose-galactoside), cyanidin 3-(2″-xylose-6″-feruloyl-glucose-galactoside), and cyanidin 3-(2″-xylose-6″-(4-coumaroyl)glucose-galactoside) differ from cyanidin 3-(2″-xylose-6-glucose-galactoside) by acylation with sinapic acid, ferulic acid, and *p*-coumaric acid, respectively. All anthocyanins except cyanidin 3-(2″-xylose-6″-(4-coumaroyl)-glucose-galactoside) were also observed in plasma following consumption of purple carrot juice.

The concentrations of total anthocyanins in the purple carrot juice treatments were 76.1 μmol (64.5 mg) in 50 mL of purple carrot juice, 228.1 μmol (193.6 mg) in 150 mL of purple carrot juice, and 380.2 μmol (322.7 mg) in 250 mL of purple carrot juice (**Table 1**). This concentration of 152 μmol/100 g (129 mg/100 g) is higher than that of most vegetables, which have been analyzed whole rather than as juice and which range from 1.5 mg/100 g for red leaf lettuce and 6 mg/100 g for red beans to 113 mg/100 g for red cabbage and 116 mg/100 g for red radish (*34*). This level is similar to our previous analysis of purple carrot tap root, which contained 166 mg/100 g (*31*). By way of reference, a medium carrot weighs

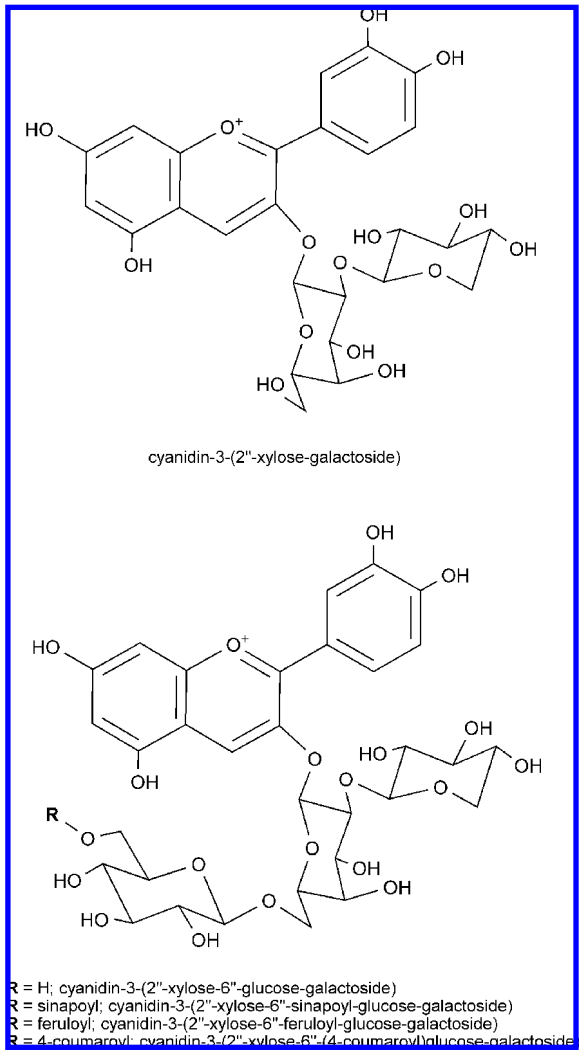

Figure 1. Chemical structures of anthocyanins detected in purple carrot juice. Structures are represented as previously reported (*33*).

Table 1. Acylated and Nonacylated Anthocyanin Content of Treatments[a]

	μmol per treatment		
treatment	acylated	nonacylated	total
50 mL	57.7 ± 3.3	18.3 ± 1.3	76.1 ± 4.5
150 mL	173.1 ± 9.8	55.0 ± 3.8	228.1 ± 13.5
250 mL	288.6 ± 16.4	91.7 ± 6.4	380.2 ± 22.5

[a] Values are expressed as means ± SEM.

61 g, on average, and the juice yield was about half the mass of the carrot. Acylated anthocyanins comprised 76% of total anthocyanins in each treatment, and nonacylated anthocyanins comprised 24%. This distribution is similar to our previous findings for whole purple carrots, in which 86% of anthocyanins were acylated (*31*).

The mean concentration of total anthocyanins in plasma following consumption of purple carrot juice is represented in **Figure 2**. Anthocyanins were detected at the first time point (0.5 h) for each treatment, with the most rapid accumulation of anthocyanins in blood occurring between 0 and 2 h, and anthocyanins were still detectable at the final time point (8 h). The maximum mean concentrations of total anthocyanins were measured at 2 h for the 50- and 250-mL treatments and at 1 h for the 150-mL treatment.

Plasma total anthocyanin response is represented by peak concentration, percent recovered at peak, and area under the plasma concentration−time curve (AUC) (**Table 2**). The peak

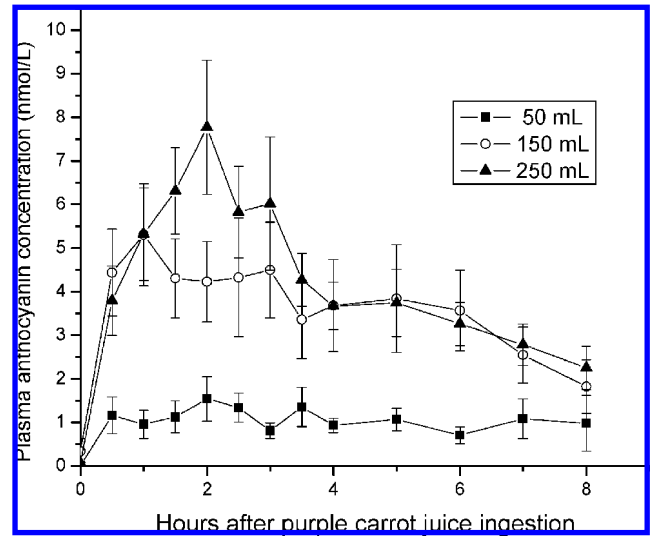

Figure 2. Mean plasma total anthocyanin concentration following consumption of 50, 150, or 250 mL of purple carrot juice. Error bars represent ± SEM ($n = 10$).

Table 2. Plasma Total Anthocyanin Response[a]

treatment	peak concentration (nmol/L)	% recovered at peak	plasma AUC (nmol h/L)
50 mL	2.5 ± 0.6 a	0.0102 ± 0.0025 a	7.3 ± 1.7 a
150 mL	6.6 ± 1.3 b	0.0090 ± 0.0017 a	28.8 ± 6.6 b
250 mL	9.6 ± 1.7 c	0.0078 ± 0.0013 a	32.0 ± 5.0 b

[a] Values are expressed as means ± SEM. Means followed by different letters within a column are significantly different, $P < 0.05$. Note that **Figure 2** shows the mean of anthocyanin concentrations at each time point and this table shows the mean *peak* concentration for total anthocyanins. Because the peak values did not occur at the same time for all subjects, the values in this table do not match those in **Figure 2**.

total anthocyanin concentration increased significantly with increasing dose consumed. The peak concentration was 2.5 nmol/L when 50 mL of purple carrot juice was ingested and was 1.6-fold higher for the 150-mL dose level (i.e., 3-fold higher dose) and 2.8-fold higher for the 250-mL dose level (i.e., 5-fold higher dose). Thus, on a percentage basis, the increase in peak concentration was lower than the increase in dose level. Although the plasma AUCs at the 150- and 250-mL dose levels were significantly higher than at the 50-mL dose level, the percent recovered at peak did not vary significantly with dose level, probably due to high coefficients of variation. The similar values for AUC at the 150- and 250-mL dose levels suggest that anthocyanin absorption mechanisms began to saturate somewhere between these two levels of purple carrot juice consumption.

Previous studies of anthocyanin dose response have had mixed results with respect to saturation. A study of strawberry anthocyanins showed no change in absorption efficiency over three dose levels ranging from 15 to 60 μmol (*35*). A study of red cabbage anthocyanins showed that absorption of anthocyanins increased with increasing dose, but with decreasing absorption efficiency, over doses ranging from 138 to 414 μmol (*30*). A study of whole purple carrot anthocyanins demonstrated no increase in total anthocyanins absorbed when dose was doubled, suggesting saturation of absorption at doses over the range of 357−714 μmol (*31*). Considering these studies together, absorption efficiency was most greatly affected by dose at the higher dose ranges. The anthocyanin dose range for this study (76−380 μmol) was chosen to be below the dose range of our

Table 3. Plasma Acylated and Nonacylated Anthocyanin Response[a]

	peak concentration (nmol/L)		% recovered at peak	
treatment	acylated	nonacylated	acylated	nonacylated
50 mL	1.2 ± 0.3 a	1.6 ± 0.4 a	0.0067 ± 0.0019 aA	0.0264 ± 0.0073 aB
150 mL	3.0 ± 0.7 b	4.1 ± 0.9 b	0.0055 ± 0.0013 aA	0.0225 ± 0.0041 aB
250 mL	3.4 ± 0.7 b	6.3 ± 1.2 c	0.0037 ± 0.0007 aA	0.0211 ± 0.0035 aB

[a] Values are expressed as means ± SEM. Means followed by different lower case letters within a column are significantly different, $P < 0.05$. For % recovered at peak, means followed by different upper case letters within a row are significantly different, $P < 0.05$. Note that **Figure 2** shows the mean of anthocyanin concentrations at each time point and this table shows the mean *peak* concentrations for acylated and nonacylated anthocyanins. Because the peak values did not occur at the same time for all subjects, the values in this table do not match those in **Figure 2**. In addition, the peak concentration for acylated anthocyanins did not occur simultaneously with the peak concentration for nonacylated anthocyanins. Thus, summing the peak values for acylated and nonacylated anthocyanins from this table does not yield the peak value for total anthocyanins in **Table 2**.

previous study of anthocyanin bioavailability from whole purple carrots because absorption of anthocyanins appeared to be saturated through the dose range used for the previous study (357−714 μmol) (*31*). We also chose the highest dose level of this study (380 μmol) to be close to the lowest dose level of the previous study (357 μmol) (*31*) so that the two studies would together provide a wide dose range tested for a clearer picture of where saturation might occur. Thus, the 250-mL dose of juice provided a similar dose of anthocyanins as the 250-g dose of carrot sticks in the previous study (250 g of cooked purple carrot sticks delivered 357 μmol of anthocyanins and 250 mL of juice provided 380 μmol of anthocyanins) (*31*). Taken together, the studies suggest that absorption of cyanidin-based anthocyanins saturates between dose levels of approximately 250 and 350 μmol.

Because the anthocyanin level in the 250-mL dose of juice provided in this study was not different from the anthocyanin level in the 250-g dose of whole purple carrots used in the previous study (*31*), comparison of the anthocyanin response after ingestion of these treatments provides information about the overall role of the plant matrix in affecting anthocyanin absorption. Percent recovery of anthocyanins in the juice at peak plasma concentration was approximately double that for the carrot sticks, but the AUCs were similar [32.0 ± 5.0 nmol h/L for juice vs 26.6 ± 3.5 nmol h/L for whole carrots (*31*), mean ± SEM, difference not statistically significant]. These results suggest that the total anthocyanins absorbed were similar for carrot juice and whole carrots providing equivalent amounts of anthocyanins, but anthocyanin absorption from juice was more rapid. The time required for digestive processes to liberate anthocyanins from the plant matrix may account for the slower rate of anthocyanin absorption from whole carrots.

The peak concentrations of nonacylated anthocyanins increased significantly with increasing dose size. In contrast, for acylated anthocyanins, the peak concentrations at the 150- and 250-mL dose levels did not differ from one another, but were higher than the peak concentration corresponding to the 50-mL dose level (**Table 3**). (Note that **Figure 2** shows the mean of anthocyanin concentrations at each time point and **Table 3** shows the mean *peak* concentrations for acylated and nonacylated anthocyanins. Because the peak values did not occur at the same time for all subjects, the values in **Table 3** do not match those in **Figure 2**. In addition, the peak concentration for acylated anthocyanins did not occur simultaneously with the peak concentration for nonacylated anthocyanins. Thus, summing the peak values for acylated and nonacylated anthocyanins from **Table 3** does not yield the peak value for total anthocyanins in **Table 2**.) The percentages recovered at peak for nonacylated anthocyanins were 2.9-, 3.1-, and 4.7-fold higher than those of acylated anthocyanins for the 50-, 150-, and 250-mL doses, respectively. This difference in recovery is similar to that found in a study of red cabbage anthocyanins (*30*). Acylated anthocyanins from carrot sticks were also found to be significantly less bioavailable than nonacylated anthocyanins (*31*). The previous studies comparing absorption of acylated and nonacylated anthocyanins used whole foods, in which the anthocyanins would have been compartmentalized in intact plant cell vacuoles. Thus, it could not be determined if the reduction in bioavailability was strictly related to anthocyanin structure or if the more important factor was the association of acylated versus nonacylated anthocyanins with the plant matrix. In this study, we processed the carrots through juicing, thus removing the anthocyanins from the plant matrix and insoluble fiber. Therefore, differences in bioavailability of different anthocyanin forms can be attributed primarily to chemical structure.

In conclusion, nonacylated anthocyanins are more bioavailable than acylated anthocyanins from juiced carrots, a finding consistent with our previous observation from whole carrots and red cabbage. Because this study was performed using juiced carrots, thus having the plant matrix removed, the findings demonstrate that the lower relative bioavailability of acylated anthocyanins compared to nonacylated counterparts is not primarily related to differential hindrance of acylated anthocyanins within the plant matrix. In addition, absorption efficiency decreased over increasing anthocyanin dose. These findings related to dose and acylation should be considered in the development of dietary guidance related to anthocyanin intake. Adjustments should be included in dietary guidance so that delivery of desired levels of anthocyanins can be achieved.

LITERATURE CITED

(1) Neill, S. O.; Gould, K. S. Anthocyanins in leaves: light attenuators or antioxidants. *Funct. Plant Biol.* **2003**, *30*, 865–873.

(2) Gould, K. S. Nature's Swiss army knife: the diverse protective roles of anthocyanins in leaves. *J. Biomed. Biotechnol.* **2004**, *5*, 314–320.

(3) Bell, D. R.; Gochenaur, K. Direct vasoactive and vasoprotective properties of anthocyanin-rich extracts. *J. Appl. Physiol.* **2006**, *100*, 1164–1170.

(4) Dai, J.; Patel, J. D.; Mumper, R. J. Characterization of blackberry extract and its antiproliferative and anti-inflammatory properties. *J. Med. Food* **2007**, *10*, 258–265.

(5) Kaplan, M.; Hayek, T.; Raz, A.; Coleman, R.; Dornfeld, L.; Vaya, J.; Aviram, M. Pomegranate juice supplementation to atherosclerotic mice reduces macrophage lipid peroxidation, cellular cholesterol accumulation and development of atherosclerosis. *J. Nutr.* **2001**, *131*, 2082–2089.

(6) Rechner, A. R.; Kroner, C. Anthocyanins and colonic metabolites of dietary polyphenols inhibit platelet function. *Thromb. Res.* **2005**, *116*, 327–334.

(7) Kang, S. Y.; Seeram, N. P.; Nair, M. G.; Bourquin, L. D. Tart cherry anthocyanins inhibit tumor development in Apc(Min) mice and reduce proliferation of human colon cancer cells. *Cancer Lett.* **2003**, *194*, 13–19.

(8) Katsube, N.; Iwashita, K.; Tsushida, T.; Yamaki, K.; Kobori, M. Induction of apoptosis in cancer cells by bilberry (*Vaccinium myrtillus*) and the anthocyanins. *J. Agric. Food Chem.* **2003**, *51*, 68–75.

(9) Lala, G.; Malik, M.; Zhao, C.; He, J.; Kwon, Y.; Giusti, M. M.; Magnuson, B. A. Anthocyanin-rich extracts inhibit multiple biomarkers of colon cancer in rats. *Nutr. Cancer* **2006**, *54*, 84–93.

(10) Ding, M.; Feng, R.; Wang, S. Y.; Bowman, L.; Lu, Y.; Qian, Y.; Castranova, V.; Jiang, B. H.; Shi, X. Cyanidin-3-glucoside, a natural product derived from blackberry, exhibits chemopreventive and chemotherapeutic activity. *J. Biol. Chem*. **2006**, *281*, 17359–17368.

(11) Yi, W.; Fischer, J.; Krewer, G.; Akoh, C. C. Phenolic compounds from blueberries can inhibit colon cancer cell proliferation and induce apoptosis. *J. Agric. Food Chem*. **2005**, *53*, 7320–7329.

(12) Hecht, S. S.; Huang, C.; Stoner, G. D.; Li, J.; Kenney, P. M.; Sturla, S. J.; Carmella, S. G. Identification of cyanidin glycosides as constituents of freeze-dried black raspberries which inhibit anti-benzo[a]pyrene-7,8-diol-9,10-epoxide induced NFkappaB and AP-1 activity. *Carcinogenesis* **2006**, *27*, 1617–1626.

(13) Netzel, M.; Netzel, G.; Kammerer, D. R.; Schieber, A.; Carle, R.; Simons, L.; Bitsch, R.; Izabela, K. Cancer cell antiproliferation activity and metabolism of black carrot anthocyanins. *Innovative Food Sci. Emerg. Technol*. **2007**, *8*, 365–372.

(14) Joseph, J. A.; Denisova, N. A.; Arendash, G.; Gordon, M.; Diamond, D.; Shukitt-Hale, B.; Morgan, D. Blueberry supplementation enhances signaling and prevents behavioral deficits in an Alzheimer disease model. *Nutr. Neurosci*. **2003**, *6*, 153–162.

(15) Joseph, J. A.; Shukitt-Hale, B.; Casadesus, G. Reversing the deleterious effects of aging on neuronal communication and behavior: beneficial properties of fruit polyphenolic compounds. *Am. J. Clin. Nutr*. **2005**, *81*, 313S–316S.

(16) Joseph, J. A.; Shukitt-Hale, B.; Denisova, N. A.; Bielinski, D.; Martin, A.; McEwen, J. J.; Bickford, P. C. Reversals of age-related declines in neuronal signal transduction, cognitive, and motor behavioral deficits with blueberry, spinach, or strawberry dietary supplementation. *J. Neurosci*. **1999**, *19*, 8114–8121.

(17) Jayaprakasam, B.; Olson, L. K.; Schutzki, R. E.; Tai, M. H.; Nair, M. G. Amelioration of obesity and glucose intolerance in high-fat-fed C57BL/6 mice by anthocyanins and ursolic acid in Cornelian cherry (*Cornus mas*). *J. Agric. Food Chem*. **2006**, *54*, 243–248.

(18) Jayaprakasam, B.; Vareed, S. K.; Olson, L. K.; Nair, M. G. Insulin secretion by bioactive anthocyanins and anthocyanidins present in fruits. *J. Agric. Food Chem*. **2005**, *53*, 28–31.

(19) Manach, C.; Williamson, G.; Morand, C.; Scalbert, A.; Remesy, C. Bioavailability and bioefficacy of polyphenols in humans. I. Review of 97 bioavailability studies. *Am. J. Clin. Nutr*. **2005**, *81*, 230S–242S.

(20) El Mohsen, M. A.; Marks, J.; Kuhnle, G.; Rice-Evans, C.; Moore, K.; Gibson, G.; Debnam, E.; Srai, S. K. The differential tissue distribution of the citrus flavanone naringenin following gastric instillation. *Free Radical Res*. **2004**, *38*, 1329–1340.

(21) Wu, X.; Cao, G.; Prior, R. L. Absorption and metabolism of anthocyanins in elderly women after consumption of elderberry or blueberry. *J. Nutr*. **2002**, *132*, 1865–1871.

(22) Bitsch, I.; Janssen, M.; Netzel, M.; Strass, G.; Frank, T. Bioavailability of anthocyanidin-3-glycosides following consumption of elderberry extract and blackcurrant juice. *Int. J. Clin. Pharmacol. Ther*. **2004**, *42*, 293–300.

(23) Mulleder, U.; Murkovic, M.; Pfannhauser, W. Urinary excretion of cyanidin glycosides. *J. Biochem. Biophys. Methods* **2002**, *53*, 61–6.

(24) Netzel, M.; Strass, G.; Janssen, M.; Bitsch, I.; Bitsch, R. Bioactive anthocyanins detected in human urine after ingestion of black-currant juice. *J. Environ. Pathol. Toxicol. Oncol*. **2001**, *20*, 89–95.

(25) Bub, A.; Watzl, B.; Heeb, D.; Rechkemmer, G.; Briviba, K. Malvidin-3-glucoside bioavailability in humans after ingestion of red wine, dealcoholized red wine and red grape juice. *Eur. J. Nutr*. **2001**, *40*, 113–120.

(26) Clifford, M. N. Anthocyanins—nature, occurrence and dietary burden. *J. Sci. Food Agric*. **2000**, *80*, 1063–1072.

(27) Yi, W.; Akoh, C. C.; Fischer, J.; Krewer, G. Absorption of anthocyanins from blueberry extracts by Caco-2 human intestinal cell monolayers. *J. Agric. Food Chem*. **2006**, *54*, 5651–5658.

(28) Wolffram, S.; Block, M.; Ader, P. Quercetin-3-glucoside is transported by the glucose carrier SGLT1 across the brush border membrane of rat small intestine. *J. Nutr*. **2002**, *132*, 630–635.

(29) Passamonti, S.; Vrhovsek, U.; Mattivi, F. The interaction of anthocyanins with bilitranslocase. *Biochem. Biophys. Res. Commun*. **2002**, *296*, 631–636.

(30) Charron, C. S.; Clevidence, B. A.; Britz, S. J.; Novotny, J. A. Effect of dose size on bioavailability of acylated and nonacylated anthocyanins from red cabbage (*Brassica oleracea* L. Var. *capitata*). *J. Agric. Food Chem*. **2007**, *55*, 5354–5362.

(31) Kurilich, A. C.; Clevidence, B. A.; Britz, S. J.; Simon, P. W.; Novotny, J. A. Plasma and urine responses are lower for acylated vs nonacylated anthocyanins from raw and cooked purple carrots. *J. Agric. Food Chem*. **2005**, *53*, 6537–6542.

(32) de Pee, S.; West, C. E.; Permaesih, D.; Martuti, S.; Muhilal; Hautvast, J. G. Orange fruit is more effective than are dark-green, leafy vegetables in increasing serum concentrations of retinol and β-carotene in schoolchildren in Indonesia. *Am. J. Clin. Nutr*. **1998**, *68*, 1058–1067.

(33) Glässgen, W. E.; Wray, V.; Strack, D.; Metzger, J. W.; Seitz, H. U. Anthocyanins from cell suspension cultures of *Daucus carota*. *Phytochemistry* **1992**, *31*, 1593–1601.

(34) Wu, X.; Beecher, G. R.; Holden, J. M.; Haytowitz, D. B.; Gebhardt, S. E.; Prior, R. L. Concentrations of anthocyanins in common foods in the United States and estimation of normal consumption. *J. Agric. Food Chem*. **2006**, *54*, 4069–4075.

(35) Carkeet, C.; Clevidence, B. A.; Novotny, J. A. Anthocyanin excretion by humans increases linearly with increasing strawberry dose. *J. Nutr*. **2008**, *138*, 897–902.

Received for review September 24, 2008. Revised manuscript received December 19, 2008. Accepted December 19, 2008. This work was supported by a USDA CSREES IFAFS Grant 2000-4258.

JF802988S