JOURNAL OF AGRICULTURAL AND FOOD CHEMISTRY

Effect of Anthocyanin Fractions from Selected Cultivars of Georgia-Grown Blueberries on Apoptosis and Phase II Enzymes

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In recent years, considerable attention has been paid to anthocyanins due to their abilities to inhibit oxidative stress and cell proliferation. The regulations of apoptosis and the phase II enzymes glutathione-S-transferase (GST) and quinone reductase (QR) are other potential mechanisms through which flavonoids such as anthocyanins may prevent cancer. Our study confirmed that anthocyanin fractions from high bush blueberry cultivars increased apoptosis using two different methods: DNA fragmentation and caspase-3 activity. The effect of anthocyanins on the activity of the detoxifying enzymes GST and QR was also determined. Major anthocyanins identified were delphinidin, cyanidin, peonidin, petunidin, and malvidin. In Tifblue and Powderblue cultivars, DNA fragmentation increased at anthocyanin concentrations from 50 to 150 μg/mL, but cells treated with the anthocyanin fraction of Brightblue and Brightwell showed a prominent ladder at 50−100 μg/mL when compared to cells treated with 150 μg/mL. There was a significant difference in the caspase-3 activity ($P < 0.05$) between the control cells and the cells treated with anthocyanins from all of the cultivars. The response correlated positively with dose. The QR activity was lower in all cells treated with an anthocyanin fraction from Tifblue, Powderblue, Brightblue, and Brightwell cultivars than in control cells ($P < 0.05$). The activity decreased gradually when treated with increased concentrations of anthocyanin fractions (50−150 μg/mL) in the Tifblue and Powderblue cultivars. The GST activity was lower ($P < 0.05$) in cells treated with anthocyanin fractions from all of the cultivars and at all concentrations. These results indicated that apoptosis was confirmed in HT-29 cells when treated with anthocyanins from blueberry cultivars at 50−150 μg/mL concentrations, but these same concentrations decrease QR and GST activities rather than induce them.

KEYWORDS: Anthocyanins; blueberries; caspase-3; cultivars; DNA fragmentation; DNA ladder; glutathione-S-transferase; phase-II enzymes; quinone reductase

INTRODUCTION

Anthocyanins belong to a widespread class of phenolic compounds collectively named flavonoids. They are present in colored fruits and vegetables such as blueberries, red grapes, and red cabbages (*1*). The structures of individual anthocyanins differ in the number of hydroxyl groups, the nature and number of sugars, and the position of these attachments (*2*). There is evidence that flavonoids, including anthocyanins, may inhibit carcinogenesis (*3*). Kang et al. (*4*) showed that tart cherry anthocyanins reduced the proliferation of human colon cancer cell lines (HT-29) and the formation of adenomas in mice. In recent years, considerable attention has been paid to anthocyanins due to their ability to inhibit oxidative stress, decrease cell proliferation, and induce apoptosis in malignant cells (*5*−*7*).

Apoptosis is a programmed cell death characterized by a series of distinct morphological and biochemical alterations (*8*, *9*). This process is essential for morphogenesis, tissue homeostasis, and host defense (*9*) and plays a significant role in the elimination of seriously damaged cells or tumor cells by chemopreventive agents (*10*, *11*). Accumulating evidence suggests that defects in apoptosis may lead to several pathologies, including some neurogenerative disorders, ischemic injury, and some forms of cancer (*10*). Apoptosis is characterized by chromatin condensation, cytoplasmic blebbing, and DNA fragmentation (*12*, *13*). Nuclear alterations, the pre-eminent ultrastructural changes of apoptosis, are often associated with the internucleosomal cleavage of DNA (*8*), recognized as a "DNA ladder" on conventional agarose gel electrophoresis, and are considered to be a biochemical hallmark of apoptosis (*14*). Its measurement is simple and often used to determine whether a cell is apoptotic.

Caspase activity is responsible, either directly or indirectly, for the cleavage of several intracellular proteins that are

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10.1021/jf062915o CCC: $37.00
Published on Web 03/24/2007

proteolyzed during apoptosis (*14*). The activation of caspases during this process results in the cleavage of critical cellular substrates, including poly (ADP-ribose) polymerase and lamins, thus precipitating the morphological changes in apoptosis (*15*).

A rather well-characterized defense mechanism may contribute to the chemopreventive effect of some flavonoids. This mechanism involves the induction of detoxification enzymes, including members of the glutathione S-transferase (GST) family and NAD(P)H (quinone reductase) (QR). The human body is constantly exposed to potential carcinogens in the environment. The body deals with these compounds through a system of xenobiotic-metabolizing enzymes called phase I and phase II enzymes. Phase I enzymes are members of the cytochrome P450 superfamily. They oxidize xenochemicals into electrophilic intermediates. These electrophilic intermediates are able to induce DNA damage and mutations. This accounts for the carcinogenic activity of many chemicals (*16*). Phase II detoxification enzymes are responsible for metabolizing products of the phase I metabolic reactions. They degrade reactive electrophilic intermediates through conjugation or reduction reactions, thereby protecting cells from oxidative DNA damage.

GST detoxifies carcinogens and facilitates their excretion by promoting the conjugation of electrophilic compounds with glutathione, and NAD(P)H QR, another phase II enzyme, works by catalyzing two-electron reductions on free radicals and toxic oxygen metabolites; this reduction deactivates them and protects the surrounding tissues from mutagenesis and carcinogenesis.

More than 40 compounds from dietary sources that function as phase II enzyme inducers have been identified (*17*, *18*). Many studies have shown that flavonoids such as anthocyanins can stimulate GST, a promising strategy for the prevention of colon cancer (*19*). Like GST, QR can be induced by dietary components, including the flavonoids (*20*).

For this study, we used HT-29 colon cancer cells, which are widely used in the research of colon cancer and to assess the bioactivity of flavonoids. The objectives of the present study were the following: (i) to confirm apoptosis caused by anthocyanin fractions extracted from four cultivars of blueberries (Tifblue, Powderblue, Brightblue, and Brightwell) using two methods, (a) DNA fragmentation and (b) caspase-3 activity, and (ii) to study the effect of anthocyanins on the induction of phase II enzymes GST and QR in cell culture.

MATERIALS AND METHODS

Chemicals. Pure standards of anthocyanins were purchased from Polyphenol Laboratories (AS) (Sandnes, Norway). These standards were Dp-Glc (delphinidin 3-O-β-glucopyranoside), Cy-Gal (cyanidin 3-O-β-galactopyranoside), Pt-Glc (petunidin 3-O-β-glucopyranoside), Pn-Gal (peonidin 3-O-β-galactopyranoside), and Mv-Glc (malvidin 3-O-β-glucopyranoside). Acetone, acetonitrile, methanol, O-phosphoric acid [85% purity, high-performance liquid chromatgraphy (HPLC) grade], hydrochloric acid (analytical grade), sulfuric acid, formic acid, and water (HPLC grade) were purchased from Fisher Scientific (Norcross, GA). Caspase-3 colorimetric assay kits were purchased from Chemicon International, Inc. (Temecula, CA). Apoptotic DNA ladder kits (Boehringer Mannheim, Roche) were purchased from Roche (Indianapolis, IN). GSH activity assay kits were purchased from Cayman Chemical Co. (Ann Arbor, MI). A BCA Protein assay kit was purchased from Pierce (Rockford, IL). Tween 20, FAD, NADPH, dicoumarol, potassium chloride, Tris-HCl, monobasic sodium phosphate, dibasic sodium phosphate, and EDTA-disodium salt were purchased from Sigma (St. Louis, MO). The human colorectal adenocarcinoma HT-29 cell line was purchased from ATCC (Manassas, VA).

Sample Collection. Mature blueberries were harvested from the Tifton field in 2005. The blueberry cultivars collected were Tifblue, Powderblue, Briteblue, and Britewell. The samples were frozen and stored at −40 °C until use.

Extraction and Fractionation. Anthocyanin fractions were obtained using a modified version of a procedure reported by Youdim et al. (*21*) and Oszmianski et al. (*22*). The schematic diagram of the fractionation procedure was reported by Yi et al. (*23*). Crude extracts of blueberries were obtained through homogenization of whole blueberries in acetone:methanol:water:formic acid (40:40:20:0.1, v/v/v/v). Crude extracts were applied to an activated Oasis HLB cartridge (Waters Corp., Milford, MA). They were washed with 15% methanol to remove the phenolic acids and then washed with acidified methanol (5% formic acid in methanol), which eluted the anthocyanins. The anthocyanin fraction was passed through a Sephadex LH20 column (Amersham Biosciences AB, Uppsala, Sweden). The column was then washed with 70% methanol acidified with 10% formic acid to elute the anthocyanins and flavonoids. The LH20 column was then washed with 70% acetone to elute the tannins or procyanidins. The anthocyanin and flavonoid fractions were applied to the second Oasis HLB cartridge. The cartridge was washed with 5% formic acid, followed by ethyl acetate, and then 10% formic acid in methanol. The ethyl acetate eluted the flavonoids, and the acidified methanol eluted the anthocyanins. The anthocyanin fraction was collected and concentrated in rotatory evaporator to remove the solvent at 48 °C for 2 h and left overnight at room temperature and freeze-dried using a UNITOP 600L freeze dryer (Virtis, Gardiner, NY). Extraction and fractionation were repeated five times, and the fractions were pooled together to obtain a sufficient amount for the bioassay.

HPLC Analysis. Anthocyanin fractions were hydrolyzed by dissolving them in a 50% methanol solution containing 2 N HCl (50 mL of methanol + 33 mL of water + 17 mL of 37% HCl). The samples were placed in a water bath at 80 °C and shaken at 200 rpm for 2 h to allow for acid hydrolysis of anthocyanins. HPLC was performed with a Hewlett−Packard (Avondale, PA) model 1100 liquid chromatograph with quaternary pumps and a diode array UV−visible detector. The mobile phase was solvent A, O-phosphoric acid/methanol/water (5:10:85, v/v/v), and solvent B, acetonitrile. The flow rate was 0.5 mL/min. The gradient for the separation was a linear gradient of 100 to 50% for solvent A and 0−50% for solvent B over 25 min, followed by 5 min postrun with HPLC-grade water. Anthocyanins were detected at 520 nm and identified with authentic standards as previously described (*24*).

Cell Culture. The human colorectal adenocarcinoma HT-29 cancer cells were cultured in an ATCC McCoy's medium with 10% fetal bovine serum. Cells were incubated at 37 °C with 5% CO_2 (Harris model #HWO 701T-ABA, Norwalk, CT). The medium was changed 2−3 times per week.

Induction of Apoptosis. Exponentially growing cells were harvested by centrifugation and resuspended in a fresh medium to achieve a culture density of 2×10^5 cells/mL. Apoptosis was induced with different concentrations of anthocyanins (50, 100, and 100 μg/mL) for 6 h. These cells were used for the DNA fragmentation assay, a caspase-3 colorimetric assay, protein assay, and GST and QR activity assays.

Electrophoretic Analysis of DNA Fragmentation. The untreated cells (control) and anthocyanin-treated cells (2×10^6) were harvested, washed in phosphate-buffered saline (PBS), and then lysed using lysis buffer. The samples were incubated at 15−25 °C for 10 min. The lysed sample was poured into a filter tube containing glass fiber fleece. Apoptotic DNA bound quickly to glass fiber fleece in the presence of a chaotropic salt, guanidine hydrochloride (guanidine HCl). After cellular impurities were washed off the fleece, the DNA was released from the fleece using a low salt buffer. The DNA quantification was done at 260 nm using UV−visible spectrophotometer (Shimadzu UV-1601, Norcross, GA). The DNA samples were electrophoresed at 100 V for 1 h in 1.5% (w/v) agarose gels (Sigma) complemented with ethidium bromide (1 μg/mL, Sigma). Separated DNA fragments (DNA ladders) were visualized using UV transilluminator (254 nm, UltraLum Electronic UV Transilluminator, Claremont, CA).

Caspase-3 Colorimetry Assay. The untreated cells (control) and anthocyanin-treated cells were harvested (2×10^6), washed in PBS, and centrifuged at 1500 rpm for 10 min. The pellet was resuspended in lysis buffer and incubated at room temperature for 10 min. After incubation, the samples were centrifuged for 5 min in a microcentrifuge (10000*g*). The cytosol collected was used for protein analysis and for

Table 1. Individual Anthocyanins in Frozen Blueberries[a]

	frozen blueberries (mg/100 g of fruit weight)				
	dp-gly	cy-gly	pn-gly	pt-gly	mv-gly
Tifblue	8.5 ± 0.7	23.5 ± 2.8	31.5 ± 1.4	6.5 ± 0.7	39.0 ± 2.8
Powderblue	9.0 ± 0.0	18.0 ± 0.7	28.0 ± 2.1	5.5 ± 2.1	38.0 ± 5.7
Brightblue	8.5 ± 0.6	17.5 ± 0.7	35.0 ± 2.0	3.8 ± 2.1	43.1.0 ± 5.7
Brightwell	15.5 ± 2.1	20.6 ± 3.5	50.8 ± 1.4	5.0 ± 1.4	17.5 ± 0.7

[a] Each value was expressed as a mean ± SD, $n = 3$.

caspase-3 activity. The assay mixture was prepared in a 96 well plate using cytosol from treated cells and untreated cells and a caspase-3 substrate. The plate was incubated at 37 °C for 1 h, and the samples were read at 415 nm using Bio-Rad model 680 Microplate Reader (Hercules, CA). Increases in caspase-3 activity were determined by comparing the OD reading from the induced apoptotic sample with the OD reading of the uninduced control.

Cell Preparation for Enzyme Activity Assays. Following trypsinization and harvesting, the cell suspension was centrifuged at 750*g* for 5 min (Beckman T-J6; Palo Alto, CA). The supernatant was discarded. The cells were resuspended in 2 mL of PBS and centrifuged again at 750*g* for 5 min. The cells were then disrupted using a sonicator (Branson Sonifier 450, St. Louis, MO) for 30 s at 20% power. The homogenate was combined with an equal amount of homogenizing buffer and centrifuged (Beckman J2HS, JS-7.5 swinging bucket rotor) at 10000*g* for 20 min at 4 °C. The supernatant was transferred to a polycarbonyl centrifuge tube, and the weight-matched tubes were ultracentrifuged (Beckman Optima LE-80K Ultracentrifuge) at 100000*g* for 1 h and 10 min at 4 °C. The supernatant (cytosol) was divided into three tubes and frozen at −80 °C until analysis.

Protein Assay. The amount of protein in the cells was measured using a BCA protein assay kit at 590 nm with a Bio-Rad model 680 Microplate Reader. Briefly, this method combined the reduction of Cu^{++} to Cu^{+} using a protein in alkaline media with a highly sensitive and selective colorimetric detection of Cu^{+} using bicinchoninic acid. Enzyme activities for GST and QR were expressed per mg protein. All samples were run in duplicate.

QR Assay. The QR activity was also measured in the cytosol using methods described by Kore et al. (*25*) with 12 mmol/L 2,6-dichloroindophenol as the substrate (DPIP). The QR activity was measured in triplicate with a spectrophotometer (Beckman DU 650, Beckman Instruments Inc., Fullerton, CA).

GST Assay. The GST activity was measured using 10 mM 1-chloro-2, 4-dinitrobenzene (CDNB) as a substrate. Twenty microliters of cytosolic sample, 150 μL of potassium-phosphate buffer (0.1 mol/L), and the 20 μL reduced form of glutathione (GSH; MW = 307.3) were added to each microplate well. To initiate the assay, 10 μL of CDNB was added to each well. The rate of change in absorbance for each sample was read at 340 nm using a Bio-Rad model 680 Microplate Reader. This assay indirectly measured the enzyme activity by measuring the conjugation of CDNB with glutathione by GST. All samples were run in triplicate, and average values were reported.

Statistical Analysis. Statistical analysis was done with the SAS software package (*26*). One-way analysis of variance (ANOVA) was performed to determine differences in enzyme activity. When *F* values for the ANOVA were significant, differences in means were determined using Duncan's multiple range tests as a procedure for mean separation ($P < 0.05$).

RESULTS AND DISCUSSION

Initial Analysis. The major anthocyanins in four cultivars (Tifblue, Powderblue, Brightblue, and Brightwell) are shown in **Table 1**. The major anthocyanin found in all of the cultivers (Tifblue, Powderblue, Brightblue, and Brightwell) was malvidin-glycoside (mv-gly) followed by peonidin glycoside (pn-gly) > cyanidin glycoside (cy-gly) > delphinidin glycoside (dp-gly) > petunidin glycoside (pt-gly). The different anthocyanins are expressed based on the specific weight of the anthocyanins, including dp-gly, cy-gly, pt-gly, pn-gly, and mv-gly, because most of the anthocyanins in blueberry are monoglycosides (i.e., galactosides, glucosides, or arabinosides) (*23*). Mv-gly was found to be the predominant anthocyanin in all of the cultivars, but Brightwell had pn-gly as the predominant anthocyanin. The content of mv-gly was 39.0, 38.0, 43.1, and 17.5 mg/100 g of frozen blueberry in Tifblue, Powderblue, Brightblue, and Brightwell, respectively. Pn-gly was the second largest anthocyanin. The total content of pn-gly was 31.5, 28.0, 35.0, and 50.8 mg/100 g of frozen blueberry in Tifblue, Powderblue, Brightblue, and Brightwell, respectively. There are not many reports available for anthocyanin content of the above cultivars; however, malvidin has been reported as the predominant anthocyanin in many blueberry varieties (*27*, *28*). Major anthocyanins in the anthocyanin fractions of four cultivars (Tifblue, Powderblue, Brightblue, and Brightwell) are shown in **Table 2**. Mv-gly was the predominant anthocyanin in all of the cultivars, but Brightwell had peonidin-glycoside as the predominant anthocyanin. The content of mv-gly was 40.5, 44.4, 44.4, and 20.0 mg/100 mg of anthocyanin fraction in Tifblue, Powderblue, Brightblue, and Brightwell, respectively. Peonidin-glycoside was the second largest anthocyanin. The total content of pn-gly was 23.5, 25.2, 23.4, and 35.8 mg/100 mg of anthocyanin fraction in Tifblue, Powderblue, Brightblue, and Brightwell, respectively. There was considerable loss in dp-gly content during extraction. There were certain unidentified peaks not considered for calculation. Delphinidin-glycoside and cyanidin-glycoside were lower in concentration than previously reported (*24*, *29*). These variations may be due to the storage conditions or handling of raw material or due to environmental factors such as light, temperature, agronomic practices, and various stresses.

Table 2. Individual Anthocyanins in the Anthocyanin Fraction of Different Cultivars of Frozen Blueberries[a]

	anthocyanin fraction[b]					
	dp-gly	cy-gly	pn-gly	pt-gly	mv-gly	total
Tifblue	1.8 ± 0.3	15.6 ± 2.1	23.5 ± 0.7	6.1 ± 0.1	40.5 ± 1.3	87.4±0.1
Powderblue	2.6 ± 0.8	9.7 ± 1.2	25.2 ± 0.6	4.7 ± 0.6	44.4 ± 1.9	86.0±0.1
Brightblue	4.6 ± 0.4	10.4 ± 1.7	23.4 ± 0.6	2.0 ± 0.1	44.4 ± 0.9	87.7 ± 0.4
Brightwell	8.0 ± 0.1	11.6 ± 0.9	35.8 ± 2.6	4.7 ± 0.4	20.0 ± 1.0	79.2 ± 0.4

[a] Each value was expressed as a mean ± SD, $n = 3$. [b] Expressed as mg/100 mg of anthocyanins.

DNA Fragmentation. The induction of apoptosis in tumor cells has been shown to be a common anticancer mechanism of many cancer therapies; therefore, finding potential therapeutic antitumor compounds with potent and selective apoptotic effects would be valuable (*30*). DNA fragmentation is a primary physiological characteristic of apoptosis and a relatively late event in apoptosis. Following agarose gel electrophoresis of HT-29 cells treated with anthocyanins from different cultivars, a typical ladder pattern of internucleosomal fragmentation was observed. **Figure 1** shows the DNA fragmentation in cells undergoing apoptosis. The characteristic cleavage of DNA into oligonucleosome fragments can be seen as DNA laddering. In Tifblue and Powderblue cultivars, DNA fragmentation increased at anthocyanin concentrations of 50−150 μg/mL, but cells treated with the anthocyanin fraction of Brightblue and Brightwell showed a prominent ladder at 50−100 μg/mL when compared to cells treated with 150 μg/mL. Necrosis may be the predominant process at the highest anthocyanin concentrations, with fewer cells undergoing apoptosis. Peonidin was the predominant anthocyanin in Brightwell; the remaining cultivars had malvidin as the predominant anthocyanin. Some, but not all, researchers have reported that malvidin stimulates apoptosis

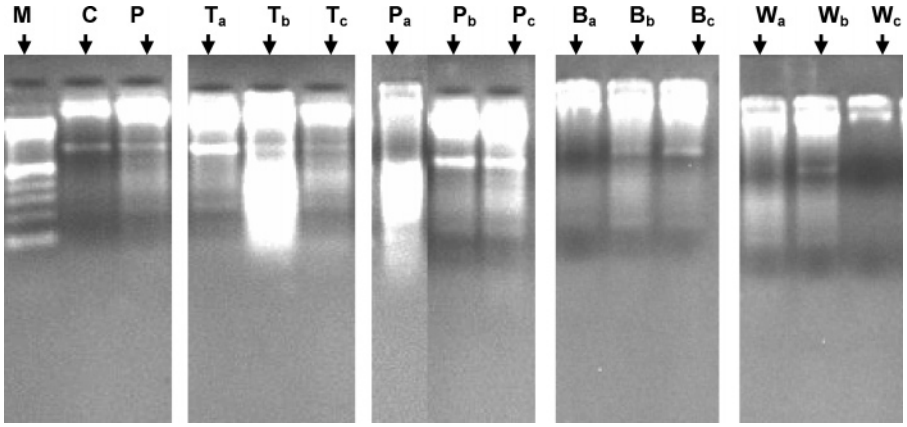

Figure 1. Apoptotic DNA fragmentation in HT-29 cells after 6 h of treatment of anthocyanin fractions from four cultivars of blueberries: Tifblue, Powderblue, Brightblue, and Brightwell. Concentrations of anthocyanins used were 50, 100, and 150 μg/mL of medium. Abbreviations: M, DNA marker; C, control; P, positive control; T_a, T_b, and T_c, DNA from cells treated with Tifblue anthocyanin concentrations of 50, 100, and 150 μg/mL of medium, respectively; P_a, P_b, and P_c, DNA from cells treated with Powderblue anthocyanin concentrations of 50, 100, and 150 μg/mL of medium, respectively; B_a, B_b, and B_c, DNA from cells treated with Brightblue anthocyanin concentrations of 50, 100, and 150 μg/mL of medium, respectively; and W_a, W_b, and W_c, DNA from cells treated with Brightwell anthocyanin concentrations of 50, 100, and 150 μg/mL of medium, respectively.

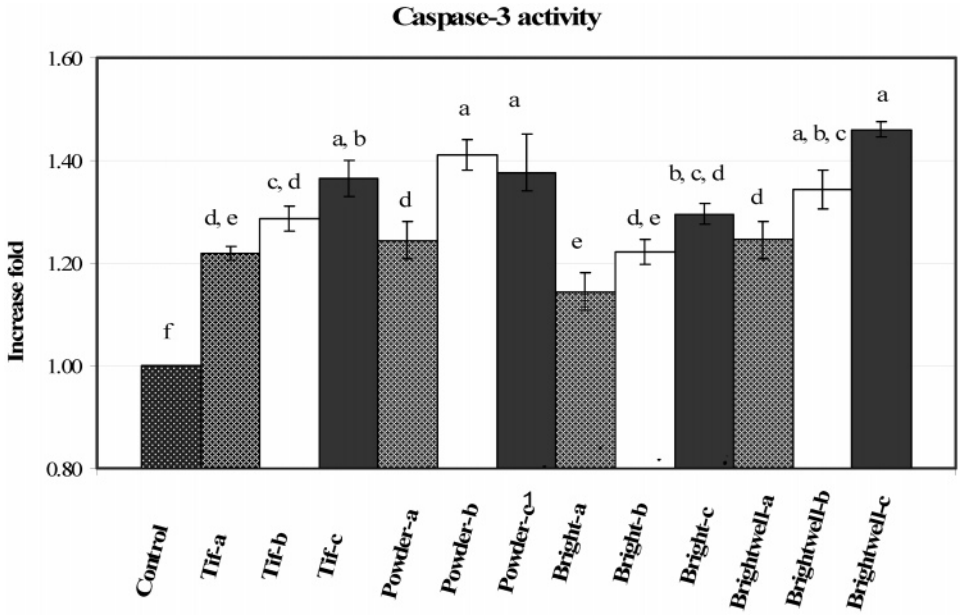

Figure 2. Caspase-3 activity in HT-29 cells after 6 h of treatment of anthocyanin fractions from four cultivars of blueberries: Tifblue, Powderblue, Brightblue, and Brightwell. Concentrations of anthocyanins used were 50, 100, and 150 μg/mL of medium. The *Y*-axis represents the fold increase in the caspase-3 activity as compared to the control. Abbreviations: Tif-a, -b, and -c, cells treated with Tifblue anthocyanin concentrations of 50, 100, and 150 μg/mL of medium, respectively; Powder-a, -b, and -c, cells treated with Powderblue anthocyanin concentrations of 50, 100, and 150 μg/mL of medium, respectively; Bright-a, -b, and -c, cells treated with Brightblue anthocyanin concentrations of 50, 100, and 150 μg/mL of medium, respectively; and Brightwell-a, -b, and -c, cells treated with Brightwell anthocyanin concentrations of 50, 100, and 150 μg/mL of medium, respectively.

in HL-60 cells (*31*), although some have reported that malvidin is unable to induce apoptosis due to the absence of a dihydroxyphenyl structure on the B ring of malvidin (*32*). In this study, we worked with the mixture of anthocyanins; thus, our results are not specific to malvidin.

Caspase-3 Activity. Caspase-3 exists as an inactive pro-caspase-3 in the cytoplasm and is proteolytically activated by multiple cleavages of pro-caspase-3 to generate the cleaved fragments in cells undergoing apoptosis. **Figure 2** shows the significant increase ($P < 0.05$) in caspase-3 activity in treated cells as compared to control. There was a significant effect ($P < 0.05$) of anthocyanins on caspase-3 activity with a response that was dose-dependent. The highest activity was observed in cells treated with the 150 μg/mL anthocyanin fraction from the Tifblue, Powderblue, and Brightblue cultivars. Cells treated with the Brightblue cultivar anthocyanin had less increase in caspase-3 activity at the 150 μg/mL concentration. The lowest response was observed with Brightblue anthocyanin. Similar results were reported in HT-29 cells when treated with natural and synthetic flavonoids (*33*).

Detoxifying Enzymes. The induction of the detoxification enzymes QR and GST is a well-characterized defense mechanism against carcinogens. In principle, the elevation of these enzymes can reduce carcinogenesis due to an enhanced removal of reactive electrophiles.

QR. QR is one of several enzymes that inactivate electrophilic carcinogens, providing a mechanism for the inhibition of carcinogenesis. **Figure 3** shows the QR activity in cells treated with anthocyanin fractions from different cultivars and control. The QR activity decreased when treated with 50−150 μg/mL

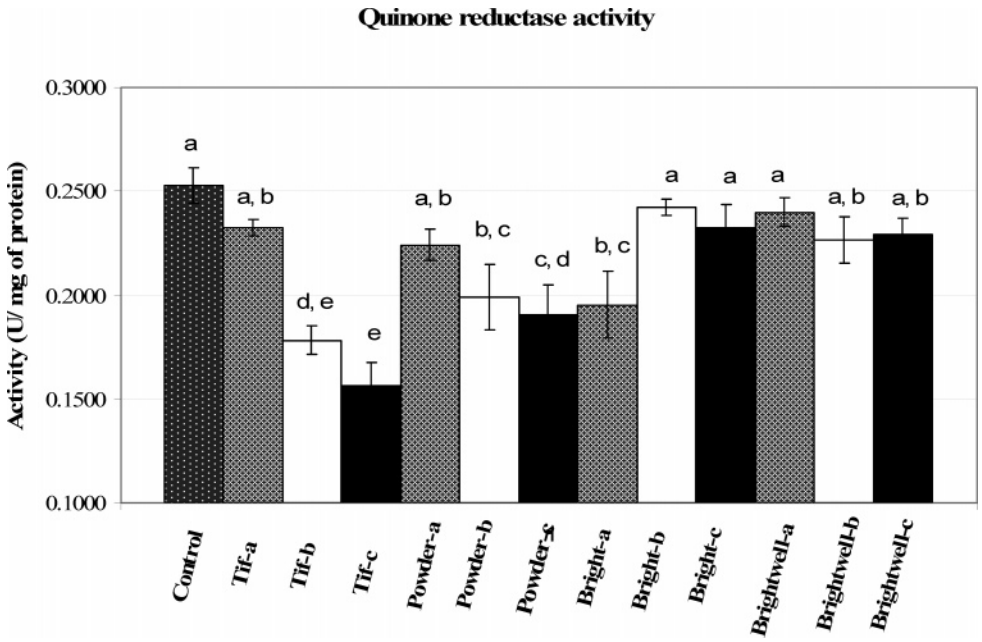

Figure 3. QR activity in HT-29 cells after 6 h of treatment with anthocyanin fractions from four cultivars of blueberries: Tifblue, Powderblue, Brightblue, and Brightwell. Concentrations of anthocyanins used were 50, 100, and 150 μg/mL of medium. The *Y*-axis represents QR activity (U/mg of protein). For abbreviations, see **Figure 2**.

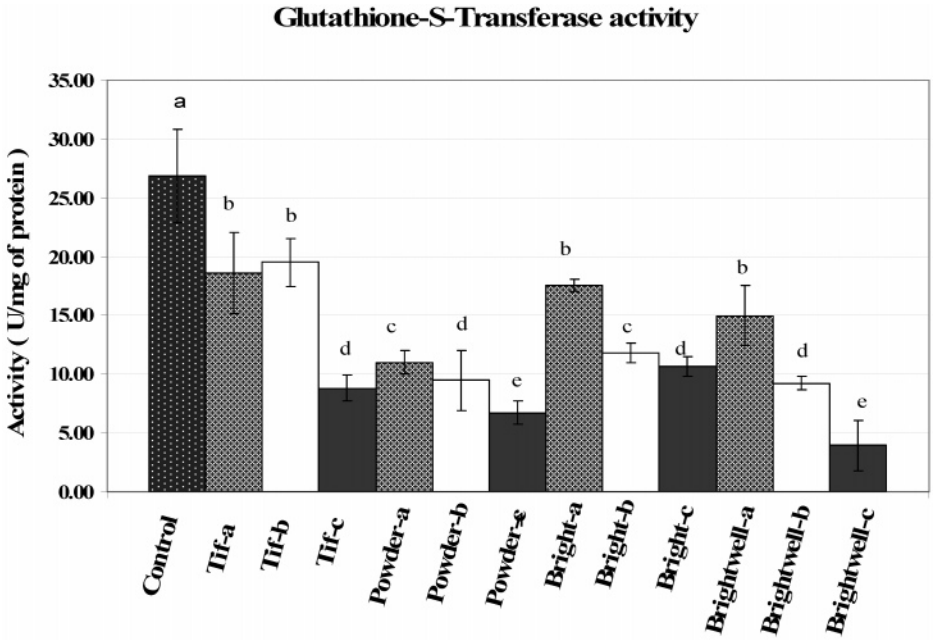

Figure 4. Glutathione-S-transferase (GST) in HT-29 cells after 6 h of treatment with anthocyanin fractions from four cultivars of blueberries: Tifblue, Powderblue, Brightblue, and Brightwell. Concentrations of anthocyanins used were 50, 100, and 150 μg/mL of medium. The *Y*-axis represents GST activity (U/mg of protein). For abbreviations, see **Figure 2**.

anthocyanin fractions from Tifblue and Powderblue cultivars. The decrease showed a dose-dependent relationship. Similarly, Bomser et al. (*34*) reported that anthocyanins fractions from lowbush blueberries were not inducers of QR activity while ethyl acetate extracts did induce QR activity. Several researchers have suggested that enzyme activity is dependent on flavonoid structure (*35*). The presence of a double bond in the heterocyclic ring (C) linking position 2 and 3 is required for QR induction capability (*35*), e.g., kaempferol and quercetin. The absence of double bond in C ring in anthocyanins may have contributed to these results. Our results may also be concentration-dependent, and these results do not rule out the possibility of different results at lower concentrations.

GST Activity. GST catalyzes the reaction with the GSH, thereby neutralizing electrophilic sites on carcinogens and rendering the products more water soluble. **Figure 4** shows the GST activity in cells treated with anthocyanin fractions from different cultivars and the control. There was a statistically significant reduction in the activity of GST in treated cells when compared with control ($P < 0.05$). A dose−response relationship was observed in all of the cultivars. Similar results with high concentrations of other flavonoid compounds were reported by earlier researchers (*36*, *37*). The effect of flavonoids on enzyme activity is correlated with the structure of the flavonoid and specific tissue (*38*). The attachment of the B ring to the C-2 position and a double bond between C-2 and C-3 may be

necessary to enhance GST activity. As with QR, these results may be concentration-dependent such that lower concentrations may have different effects.

Our results show that anthocyanin compounds may not induce detoxification enzymes in colon carcinoma cells over the same concentration ranges that increase apoptosis. Anthocyanins from four cultivars Tifblue, Powderblue, Brightblue, and Brightwell were able to induce apoptosis in a dose−response manner.

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Received for review October 11, 2006. Revised manuscript received February 16, 2007. Accepted February 20, 2007. This research study was funded by the State of Georgia's Traditional Industries Program for Food Processing Research Grant. We thank the Georgia Blueberry Growers Association for their support.

JF062915O