Properties of 3-Deoxyanthocyanins from Sorghum

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There is increasing interest in natural food colorants with functional properties. Anthocyanins from black, brown (containing tannins), and red sorghums were characterized by spectrophotometric and HPLC techniques. The antioxidant activity and pH stability of the anthocyanins were also determined. Sorghum brans had 3–4 times higher anthocyanin contents than the whole grains. Black sorghum had the highest anthocyanin content (average = 10.1 mg/g in bran). The brown and red sorghum brans had anthocyanin contents of 2.8–4.3 mg/g. Only 3-deoxyanthocyanidins were detected in sorghum. These compounds are more stable to pH-induced color change than the common anthocyanidins and their glycosides. Additionally, crude sorghum anthocyanin extracts were more stable than the pure 3-deoxyanthocyanidins. The antioxidant properties of the 3-deoxyanthocyanidins were similar to those of the anthocyanins. Pigmented sorghum bran has high levels of unique 3-deoxyanthocyanidins, which are stable to change in pH and have a good potential as natural food pigments.

KEYWORDS: *Sorghum bicolor*; anthocyanins; 3-deoxyanthocyanins; pH stability; antioxidant activity; natural food color

INTRODUCTION

Anthocyanins possess both therapeutic and desirable organoleptic attributes useful in various health and food applications. Some therapeutic benefits attributed to anthocyanins include vasoprotective and anti-inflammatory properties (*1*), anticancer and chemoprotective properties (*2*), antineoplastic properties (*3*), and hypoglycemic properties (*4*). Most of the studies related to anthocyanins are based on the compounds from fruits and vegetables. However, due to a rising demand for economical sources of natural, stable pigments, there is increased interest in alternative sources of anthocyanins, especially cereals, including rice (*5*, *6*), wheat (*7*, *8*), and corn (*9*). Some pigmented sorghums are also potentially a rich source of unique anthocyanins (*10*, *11*), yet study on these sorghums is limited.

Among food plants, cyanidin is the most commonly found anthocyanidin. Other major anthocyanidins (in decreasing order of natural prevalence) include pelargonidin, peonidin, delphinidin, petunidin, and malvidin (*12*) (**Figure 1A**). These anthocyanidins exist in nature mostly as glycosides (anthocyanins). In sorghum, the most common anthocyanin types are the 3-deoxyanthocyanidins and their derivatives (*10*, *11*, *13*–*15*) (**Figure 1B**). These anthocyanins, which include luteolinidin and apigeninidin, are not commonly found in higher plants (*16*). They lack a hydroxyl group at the C-3 position and are more stable in acidic solutions than the anthocyanins found in most food plants (*17*, *18*). This suggests that sorghum is potentially a valuable source of natural food pigments. However, quantitative data and information on antioxidant and other biological properties of the sorghum anthocyanins are lacking. Such information is necessary if sorghum is to be exploited as a source of natural food color.

To effectively characterize and quantify the sorghum anthocyanins, it is important to extract them in an efficient manner in which their chemical properties are preserved. Efficiency of several solvents to extract anthocyanins and other phenols from fruits, vegetables (*19*–*21*), and cereals (*11*, *22*) has been reported. However, there is no agreement on which solvent extracts anthocyanins most efficiently. Acidified methanol and aqueous acetone are reported as most efficient by different authors. These two solvent systems were compared in this study. The objective of this study was to establish the anthocyanin composition, content, antioxidant properties, and pH color stability of various pigmented sorghums.

MATERIALS AND METHODS

Samples. Non-tannin sorghum with black pigmented pericarp, Tx430 (black); non-tannin sorghum with a bright red pigmented pericarp, Tx2911 (red) sorghum; and three tannin-containing sorghums, Sumac, SC103, and CSC3*R28 (brown), were grown in College Station, TX, between 1998 and 2002. These cultivars were chosen because they represent diverse sorghum classes with distinct differences in pericarp pigmentation. Samples were kept at −40 °C in the dark, at moisture levels of 11.5–12.5% prior to analysis. All samples were decorticated using a PRL dehuller (Nutama Machine Co., Saskatoon, Canada) to obtain bran. Bran yield was 12–15%; these bran levels were determined to give maximum phenolic concentration for these sorghum varieties (*23*). The bran fractions contained most of the pericarp, including the pigmented testa of the brown sorghums, based on visual observation of the decorticated grits. Brans and grain samples were ground through

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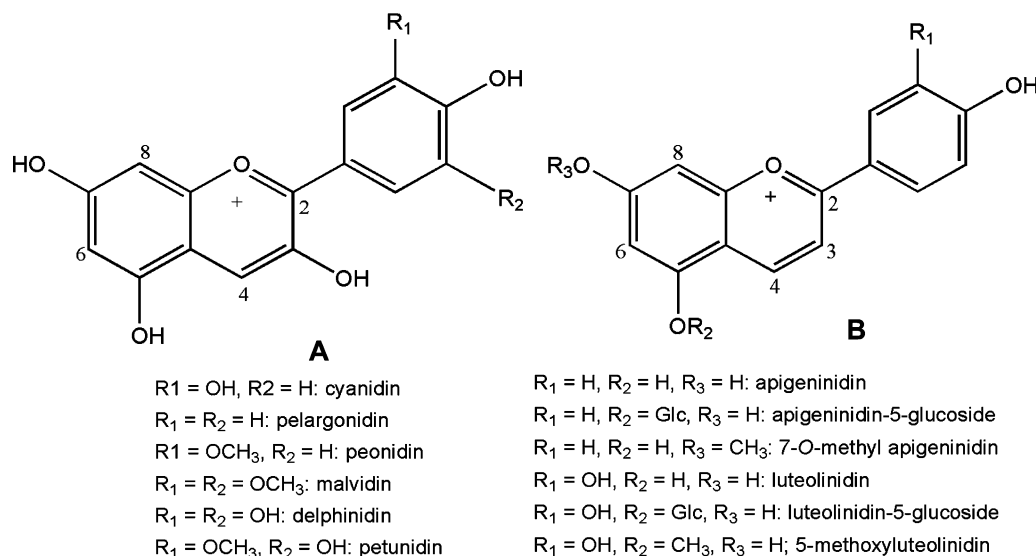
Figure 1. Common anthocyanidins (**A**) compared to the 3-deoxyanthocyanidins and their derivatives found in sorghum (**B**).

a UDY mill (1 mm mesh) before extraction and analysis. All analyses were conducted in triplicate.

Anthocyanin standards were obtained from ChromaDex Inc. (Santa Ana, CA). They included chloride salts of luteolinidin, apigeninidin, peonidin, pelargonidin, pelargonidin-3,5-diglucoside, cyanidin, cyanidin-3-glucoside, cyanidin-3,5-diglucoside, and cyanidin-3-rutinoside. Trolox was obtained from Aldrich (Milwaukee, WI). 2,2′-Azinobis(3-ethyl-benzothiazoline-6-sulfonic acid) diammonium salt (ABTS) and gallic acid were obtained from Sigma (St. Louis, MO), and 2,2-diphenyl-1-picrylhydrazyl (DPPH) was acquired from Acros Organics (Morris Plains, NJ).

Sample Extraction. Two extraction solvents were used: 1% HCl in methanol and 70% aqueous acetone. The extraction procedure involved the addition of 10 mL of solvent to 0.5 g of sample in 50 mL centrifuge tubes and shaking the samples for 2 h at low speed in an Eberbach shaker (Eberbach Corp., Ann Arbor, MI). Samples were then centrifuged at 7000g for 10 min and decanted. Residues were rinsed with two additional 10 mL volumes of solvent with shaking for 5 min, centrifuging at 7000g for 10 min, and decanting in each case. The three aliquots were combined and used for analysis.

For HPLC analysis of anthocyanins, sample extracts were initially evaporated to near dryness under vacuum at 35 °C (Brinkmann Rotovapor R110, Brinkmann Instruments, Westbury, NY) and then redissolved in 5 mL of water. The samples were then loaded in a C-18 Sep-Pak preparative column that had been prerinsed with 2 mL of 100% MeOH and 5 mL of water. The column was then rinsed with 5 mL of water, and anthocyanins were eluted with 10 mL of 1% formic acid in MeOH. The Sep-Pak cleaned samples were compared with directly injected samples for peak resolution. However, there was no significant gain in peak resolution when the Sep-Pak was used. Hence, all samples were subsequently directly injected after filtration through a 0.45 μm nylon membrane (Millipore Corp., Billerica, MA). Use of preparative Sep-Pak cartridges may be more suitable for samples with significant levels of sugars and phenolic acids, as is common with fruit extracts.

Total Phenol and Anthocyanin Contents. Samples were analyzed for phenolic content using the modified Folin−Ciocalteu method of Kaluza et al. (*24*). The pH differential method as reported by Fuleki and Francis (*25*) and Giusti and Wrolstad (*26*) was used for quantitative determination of anthocyanins with minor modifications. One of two 0.2 mL aliquots was diluted with 2.8 mL of pH 1.0 buffer (125 mL of 0.2 N KCl, and 385 mL of 0.2 N HCl) and the other with pH 4.5 buffer (400 mL of 1 N sodium acetate, 240 mL of 1 N HCl, and 360 mL of distilled water). The absorbance was measured by scanning with a Cary 300 Bio UV−vis spectrophotometer (Varian Co., Walnut Creek, CA) from 300 to 700 nm. Total anthocyanin pigments were determined from absorbance in pH 1.0 buffer. Monomeric anthocyanins could not be estimated because we observed a significant absorbance for purified monomeric sorghum 3-deoxyanthocyanidins in pH 4.5 buffer. Extinc-

tion coefficients for anthocyanin standards were determined using the formula described by Fuleki and Francis (*25*).

Qualitative and Quantitative HPLC Analysis of Anthocyanins. A Waters system (Milford, MA) was used for HPLC analysis. The system included a model 600 pump, with a 600E control system, a 996 PDA detector, and a 717 autosampler. Data collection/manipulation was via Waters Millenium software. The gradient profile was modified from that of Wang et al. (*27*). Separation was on a 250 × 4.6 mm i.d. reversed phase Spherisorb ODS-2 5 μm column (Waters). Flow rate was 0.5 mL/min; injection volume, 20 μL; column temperature, 35 °C; and detection, 210−600 nm. The mobile phase was (A) 10% formic acid in water and (B) acetonitrile/water/formic acid (5:4:1). The gradient was as follows: 0−3 min, 12% B isocratic; 3−10 min, 12−30% B; 10−15 min, 30% B isocratic; 15−20 min, 30−40% B; 20−30 min, 40% B isocratic; 30−40 min, 40−100% B; 40−60 min, 100% B isocratic; and 60−63 min, 100−12% B; 63−75 min, 12% B isocratic. Identification of anthocyanins was by matching peak retention times and spectral characteristics with those of standards. HPLC quantification was by use of a standard curve obtained by injecting different concentrations of luteolinidin standard. Peak areas were obtained at 480 nm.

Antioxidant Analysis. The DPPH and ABTS methods were used on the basis of the findings of Awika et al. (*28*). The DPPH• radical was dissolved in methanol and kept at −20 °C in the dark prior to use. Sample extracts (150 μL) were reacted with 2850 μL of the DPPH solution for 8 h with shaking. Trolox was used as a standard. The ABTS assay was performed in both aqueous and organic solvent systems. For ABTS•+ generation from ABTS salt, 3 mM of $K_2S_2O_8$ was reacted with 8 mM ABTS salt in distilled, deionized water for 16 h at room temperature in the dark. The ABTS•+ solution was then diluted with a pH 7.4 phosphate buffer solution containing 150 mM NaCl (PBS) or absolute ethanol to obtain an initial absorbance of 1.5 at 730 nm. Fresh ABTS•+ solution was prepared for each analysis. Samples and standards (100 μL) were reacted with the ABTS•+ solution (2900 μL) for 30 min in the PBS (ABTS−PBS) or for 8 h in ethanol (ABTS−EtOH) as detailed by Awika et al. (*28*).

pH Stability of Sorghum Anthocyanins. The pH values of the crude sorghum anthocyanin extracts and the standards were adjusted in aqueous sodium acetate buffer to values between 1.0 and 7.0, at concentrations of 15−20 mg/L. Under the test conditions all of the anthocyanin standards obeyed Beer's law at the concentrations used (5−40 mg/L). Samples were equilibrated for 30 min under laboratory light conditions. Absorption spectra were recorded from 300 to 700 nm after 30 min of equilibration at 24 °C (*18*) under laboratory light conditions. Statistical analyses were by SAS (SAS Institute, Cary, NC).

Table 1. Anthocyanin Levels[a] in Pigmented Sorghum Grains and Brans

| | grain | | | | bran | | |
| | spectrophotometry[b] | | spectrophotometry | | HPLC[c] | | |
	MeOH[d]	Me₂CO[e]	MeOH	Me₂CO	luteolinidin	apigeninidin	total[f]
Black 1999	2.7	1.1	10.4	4.8	1.8	0.4	6.1
Black 2001	2.3	1.4	8.9	4.0	1.3	1.4	5.4
Black 2002	2.8	1.1	11.0	5.2	1.5	1.3	6.0
Red	0.7	0.1	3.6	1.3	tr[g]	0.3	1.6
SC103 (brown)	1.0	nd[h]	2.8	nd	0.3	0.4	1.6
Sumac (brown)	1.3	0.3	4.3	2.3	0.7	0.6	2.8
CSC3*R28 (brown)	1.0	0.4	2.9	1.6	0.4	0.6	2.0
CV%[i]	2.5	4.7	2.5	4.7	1.4	1.4	1.4

[a] Milligrams per gram of luteolinidin equivalents (dry basis), determined from absorbance in pH 1, 0.2 N KCl buffer. [b] Estimated from absorbance in pH 1 buffer. [c] Peak areas were determined at 480 nm; samples were extracted in acidified methanol. [d] Acidified methanol (1% HCl) extract. [e] Seventy percernt aqueous acetone extract. [f] Includes unidentified anthocyanin peaks. [g] Trace. [h] Not detected at significant levels. Samples were extracted for 2 h at room temperature. [i] Coefficient of variation (percent relative standard deviation of each sample within column). Values represent means of triplicate analysis.
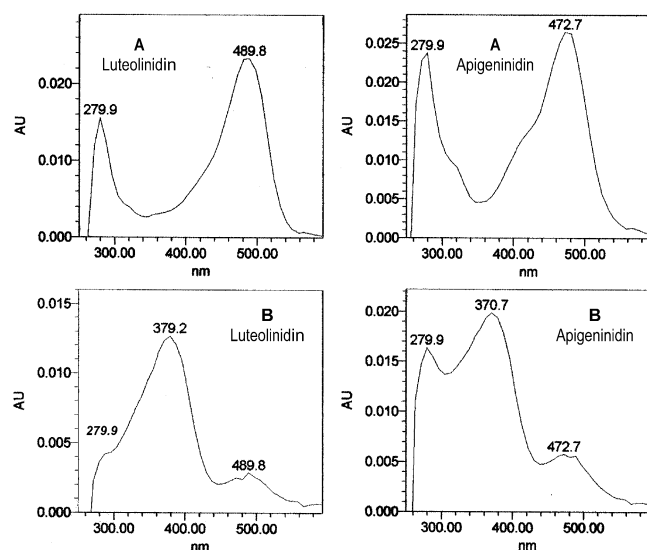

Figure 2. Spectral characteristics of 3-deoxyanthocyanidins isolated from black sorghum by HPLC: (**A**) from acidified methanol (1% HCl) extracts; (**B**) from 70% aqueous acetone extracts. Note the significant modification of the spectra from aqueous acetone extracts, which suggests formation of new compounds (*21*).

RESULTS AND DISCUSSION

Sorghum Anthocyanin Extraction and Quantification. The total anthocyanin content of acidified methanol extracts were on average 60% higher than those of aqueous acetone extracts (**Table 1**). Several authors reported that aqueous acetone was better than various alcoholic solvents for fruit procyanidins, anthocyanins, and other phenols (*19, 20*). However, Lu and Foo (*21*) observed significant anthocyanin interaction with aqueous acetone to form pyranoanthocyanidins, which significantly lowered quantities of detectable anthocyanins. The extent of this reaction depended on the duration of anthocyanin−acetone interaction and temperature. Such anthocyanin−solvent reactions do not occur in acidified methanol (*21*). The spectral characteristics of the HPLC-isolated sorghum 3-deoxyanthocyanidins in this study also indicated significant solvent modification by acetone, but not acidified methanol (**Figure 2**), probably due to formation of pyranoapigeninidin and pyranoluteolinidin. We observed similar peak modification of the commercial 3-deoxy-anthocyanidin standards when they were dissolved in aqueous acetone and allowed to equilibrate for 2 h (data not shown). This implies that if acetone is to be used as a solvent for sorghum 3-deoxyanthocyanins, extraction time and temperature

have to be kept to a minimum to minimize solvent−anthocyanin reactions. However, acidified methanol is a better solvent for sorghum 3-deoxyanthocyanins because it does not seem to react with these compounds once extracted. Consequently, only quantitative data for acidified methanol extracts are discussed.

The sorghum anthocyanin contents estimated by HPLC generally correlated with those estimated by spectrophotometry but were on average 43% lower (**Table 1**). Although specrophotometry is less precise than HPLC for anthocyanin determination, the HPLC method most likely underestimated anthocyanin content because the fixed wavelength used to obtain the luteolinidin standard curve and quantify peak areas (480 nm) may have resulted in reduced relative molar response of the anthocyanins represented by some of the isolated peaks as previously observed (*27*). Wang et al. (*27*) found that the HPLC method generally underestimated the anthocyanin content of blueberries when compared with MALDI-TOF MS analysis.

Black sorghum had more than twice the levels of anthocyanins in the red and brown sorghums (**Table 1**). The sorghum brans had on average 3−4 times the levels of anthocyanins in grains. Black sorghum brans had the highest anthocyanin content (average = 10.1 mg/g) compared to the brown (average = 3.6 mg/g) and the bright red (3.6 mg/g) sorghum brans (spectrophotometry data). The black sorghum has the highest potential as a source of natural food pigments. The sorghum grains and brans had higher anthocyanin content than other specialty cereals (*5, 7−9*) and most fruits and vegetables (*16, 29−32*). Sorghum has cost advantages over the fruits and vegetables in terms of production, yield, storage stability, and ease of processing. Additionally, it may be possible to manipulate environment and genetics to maximize anthocyanin accumulation in sorghum (*33−36*).

Isolation of Sorghum Anthocyanins. Apigeninidin and luteolinidin represented on average 36−50% of the total black and brown sorghum anthocyanins identified by HPLC (**Table 1**; **Figure 3**). A major unidentified peak in these sorghums (representing 25% of total anthocyanin pigments) was spectrally similar to luteolinidin and is most probably a methyl ether or glycoside of luteolinidin (*10, 15*). Six other relatively minor peaks were present in the black and brown sorghum anthocyanin profiles (**Figure 3**). All of these peaks had spectral characteristics that were very similar to apigeninidin and luteolinidin, implying they are structural derivatives of the 3-deoxyanthocyanidins. The red sorghum had no detectable luteolonidin, whereas apigeninidin represented 19% of its total anthocyanins (**Table 1**; **Figure 3**). Additionally, a major peak that was

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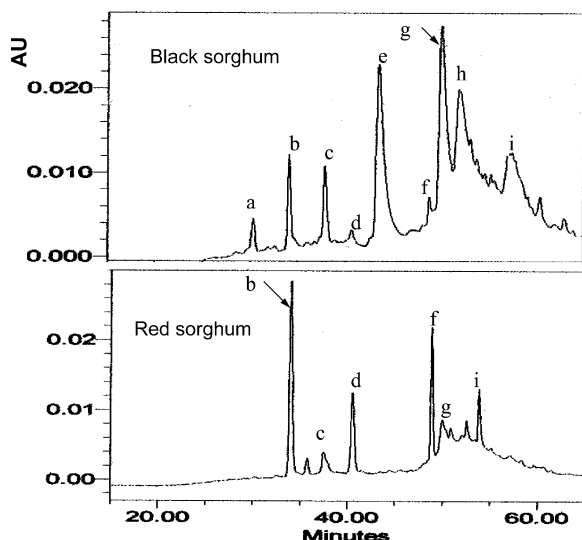
Figure 3. Typical HPLC profiles of black and red sorghum 3-deoxyanthocyanins detected at 480 nm. Peaks e and g are luteolinidin and apigeninidin, respectively; peaks a, d, f, h, and i were tentatively identified as derivatives of luteolinidin; peaks b and c were tentatively identified as derivatives of apigeninidin. Peaks were identified on the basis of matching retention times and spectral characteristics with commercial standards.

spectrally very similar to apigeninidin represented 40% of total anthocyanins in the red sorghum. On the basis of its elution time and spectral characteristics, the peak was probably a glycoside of apigeninidin (*10*, *13*). The rest of the peaks were spectrally similar to luteolinidin and were most likely its derivatives. Nip and Burns (*10*, *13*) identified 5-*O*-glucosides of both luteolinidin and apigeninidin in sorghum. The caffeic acid acyl ester of apigeninidin (*14*), 5-methoxyluteolinidin (*15*), and 7-methoxyapigeninidin (*37*) have also been identified in sorghum grain. Thus, unlike other cereals and most food plants, sorghum grain does not seem to have 3-hydroxylated anthocyanins but is a uniquely rich food source of the 3-deoxyanthocyanins. With the high levels of these compounds measured in especially the black sorghum, the commercial potential of sorghum as a source of 3-deoxyanthocyanins is promising.

Properties of the Sorghum 3-Deoxyanthocyanidins Compared to Anthocyanidins. The 3-deoxyanthocyanidins produce a yellow (apigeninidin) and orange (luteolinidin) color in acidic solvents and are distinctly different from the anthocyanins and their aglycons, which are mostly reddish to purple in acidic media. The 3-deoxyanthocyanidins showed absorption maxima lower than 480 nm, whereas the anthocyanins and their aglycons have absoption maxima above 500 nm in pH 1 buffer (**Table 2**). The 3-deoxyanthocyanidins are thus potentially useful as replacements for artificial yellow and orange pigments.

The molar absorptivity of the 3-deoxyanthocyanidins (in pH 1.0 KCl buffer) were generally higher than those of the anthocyanidins except for cyanidin-3,5-diglucoside (**Table 2**). This indicates the 3-deoxyanthocyanidins require a lower concentration than the anthocyanins to give a desired absorbance.

pH Stability of the 3-Deoxyanthocyanins. Monomeric anthocyanins show virtually no absorbance at pH 4−5 (*25*, *26*); this principle is used to estimate monomeric anthocyanin content in fruits. However, luteolinidin and apigeninidin, the major 3-deoxyanthocyanidins in sorghum, showed significant absorbance at pH 4−5) (**Table 3**; **Figure 4**). This indicates that the pH differential method of Fuleki and Francis (*25*) is not appropriate for estimating monomeric anthocyanins in sorghum.

Table 2. Molar Absorptivity and Antioxidant Activity of Anthocyanin Standards

standard	$\lambda_{max}{}^a$ $(\epsilon)^b$	antioxidant activity[c] ABTS−PBS	ABTS−EtOH	DPPH
luteolinidin	482 (31700 ± 1100)	4.8	4.1	1.5
apigeninidin	468 (30400 ± 500)	4.2	2.9	0.5
peonidin	516 (27200 ± 1000)	3.6	3.3	1.0
pelargonidin	506 (28100 ± 200)	3.3	2.8	1.4
Pg-3,5-diglucoside	498 (28800 ± 1100)	2.9	3.4	0.9
cyanidin	516 (24800 ± 60)	5.4	4.5	1.6
Cy-3-glucoside	512 (28600 ± 800)	4.6	4.0	2.7
Cy-3,5-diglucoside	510 (33900 ± 1700)	6.3	4.4	2.1
Cy-3-rutinoside	514 (26100 ± 1700)	4.2	3.4	2.0
CV%[d]		1.5	3.6	4.5

[a] Maximum absorption wavelength in 0.2 M KCl, pH 1.0, buffer. [b] Extinction coefficient in the pH 1.0 buffer ± standard deviation, $n = 3$; all standards showed >98% peak purity by HPLC. [c] Moles of Trolox/mole. [d] Coefficient of variation (percent relative standard deviation of each sample within column). Analyses were conducted in triplicates.

More importantly, it shows that the 3-deoxyanthocyanidins are more stable at higher pH than monomeric anthocyanins.

Apigeninidin and luteolinidin standards showed <50% reduction in absorbance in pH 4 relative to pH 1 buffer (**Table 3**). Gous (*11*) reported an even higher pH stability (30% loss of absorbance in pH 4 relative to pH 1) for apigeninidin and luteolinidin purified from a black sorghum variety. The 3-hydroxylated anthocyanidins and their glycosides, on the other hand, showed >99% loss of absorbance in pH 4 relative to pH 1 buffer (**Table 3**). The exception was cyanidin, which showed an 88% loss of absorbance at pH 4. At pH 7, the 3-deoxyanthocyanidins still showed substantially higher absorbance retentions than the anthocyanins (**Table 3**). Both apigeninidin and luteolinidin retained 41% of their original absorbance at pH 7, whereas the anthocyanidins retained on average 3−17% of their original absorbance at the neutral pH. Thus, without acylation, such anthocyanins are not useful colorants at pH >3.5. However, because most anthocyanins of commercial interest exist naturally in acylated forms, the stability of the acylated anthocyanins relative to sorghum 3-deoxyanthocyanins is discussed below.

Higher pH stability was observed for crude sorghum 3-deoxyanthocyanins than for the deoxyanthocyanidin standards (**Table 3**). The crude sorghum 3-deoxyanthocyanin extracts retained 77% of their absorbance at pH 4 relative to pH 1. Gous (*11*) reported a similar high stability for a black sorghum anthocyanin extract (84% color retention at pH 4 relative to pH 2). The high stability of the crude sorghum anthocyanins relative to the purified aglycons may be due to copigmentation and complexation with other extracted phenolic constituents. Even though the 3-deoxyanthocyanins exist in nature substantially as aglycons as indicated by our data (**Table 1**) and previously reported data (*16*), glycosides (*10*, *13*) and acylated forms (*14*) have been reported in sorghum. Acylated anthocyanins, mostly with phenolic acids, are far more stable than the nonacylated forms (*38*−*40*) and are the major natural forms of anthocyanins that are currently used commercially as food pigments (e.g., red cabbage and purple carrot anthocyanins). Despite the increased stability, however, a substantial decrease in the absorbance of the acylated anthocyanins is still observed as the pH increases. Giusti and Wrolstad (*41*) reported a decrease in the absorbance of potato (pelargonidin-3-rutinoside-5-glucoside acylated with a cinnamic acid) and radish (pelargonidin-sophoroside-5-glucoside acylated with a cinnamic or malonic acid) anthocyanins

Table 3. pH Stability of Black Sorghum 3-Deoxyanthocyanin Extract and 3-Deoxyanthocyanin Standards Compared to Anthocyanidins (Sodium Acetate Buffer)

	crude extract[a]		luteolinidin		apigeninidin		cyanidin		peonidin		pelargonidin	
pH	Abs[b]	%[c]	Abs	%	Abs	%	Abs	%	Abs	%	Abs	%
1	0.93	100	0.60	100	0.68	100	0.43	100	0.74	100	0.44	100
2	0.86	92	0.58	97	0.65	96	0.20	47	0.32	73	0.19	42
3	0.79	85	0.48	80	0.42	62	0.07	16	0.01	01	0.02	05
4	0.72	77	0.31	52	0.35	51	0.05	12	0.01	01	0.01	01
5	0.58	62	0.21	36	0.28	41	0.06	13	0.02	03	0.00	00
7	0.55	60	0.24	41	0.28	41	0.07	17	0.04	05	0.01	3
CV%[d]	4.2		2.5		2.5		2.5		2.5		2.5	

[a] Acidified methanol anthocyanin extract from black sorghum. [b] Absorbance at λ_{max}. [c] Percent absorbance retention normalized to absorbance in pH 1.0. [d] Coefficient of variation (percent relative standard deviation of each sample within column). Analyses were conducted in triplicates.
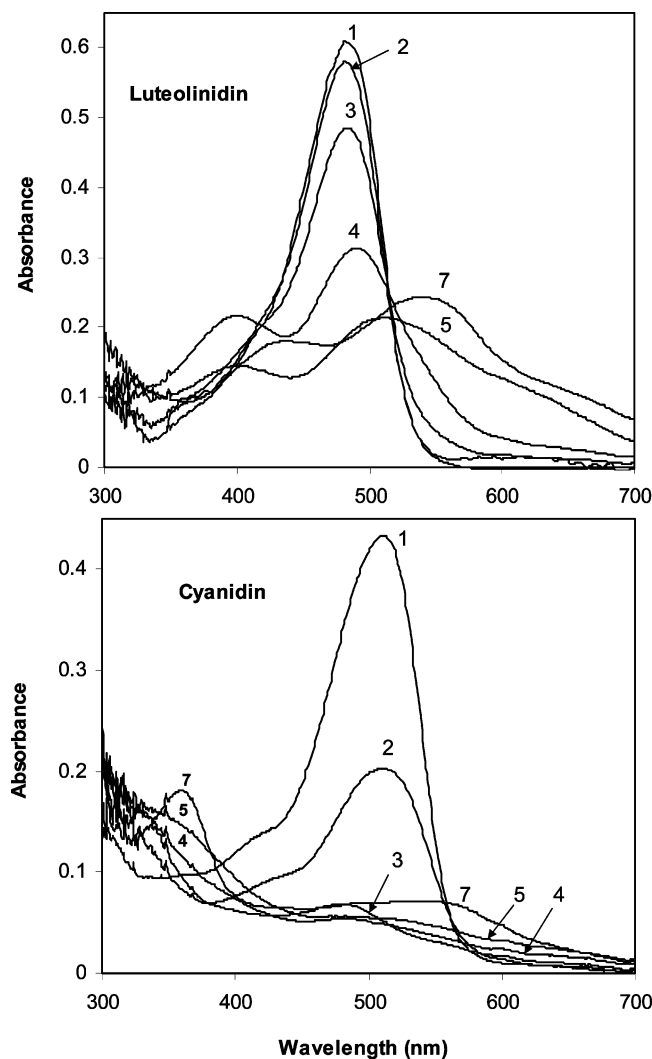
Figure 4. UV–vis spectra of luteolinidin and cyanidin at different pH values, illustrating the relative stability of the 3-deoxyanthocyanidins to change in pH. Solutions were equilibrated for 30 min at 24 °C under laboratory light conditions. Numbers represent pH values for each spectrum.

by 85% (pH 4; 0.1 M citrate buffer) and 50% (pH 5−6; 0.1 M phosphate buffer), respectively, relative to pH 1 buffer. The sorghum 3-deoxyanthocyanin extracts are apparently more pH stable than even the acylated forms of anthocyanins from other sources. Gous (*11*) also reported that a crude black sorghum anthocyanin extract was relatively stable to temperature and light, with no change in absorbance when subjected to 70 °C for up to 36 h, and only a 9% reduction in absorbance when subjected to 1000 μ-einstein (equivalent to half sunlight

intensity) at 24 °C for 48 h. The high stability, coupled with the high levels of the anthocyanins measured in sorghum relative to other commodities, further establishes a need to explore the use of sorghum anthocyanins as food colorants.

Various authors have reported on the color stability of 3-deoxyanthocyanidins relative to anthocyanidins (*17, 18, 42*). However, these reports were based on synthetic forms of these compounds with various substitution patterns, most of which have not been identified in nature. Because the current drive toward use of anthocyanins is largely motivated by the fact that they are "natural", the past findings that the synthetic forms of 3-deoxyanthocyanidins are more stable did not produce the desired impact. Additionally, levels of the 3-deoxyanthocyanidins in any food plants had not been reported. We hope that our results will stimulate a strong interest in these unique anthocyanidins as food colorants and for other biological applications.

Antioxidant Activity of the Anthocyanins. The 3-deoxyanthocyanidins had antioxidant activities that were within the range observed for the hydroxyanthocyanins as determined by different methods (**Table 2**). All anthocyanins tested had antioxidant activity higher than that of Trolox on a molar basis, in both aqueous and organic solvent systems. The activities were lower in organic solvents compared to activity in aqueous solvent. Activities were especially low in the DPPH assay. This is partly due to color interference (*43*) and partly due to difference in reactivity of phenols against different free radicals. The correlations in antioxidant activity of the anthocyanin standards among the different methods were low ($R^2 = 0.24$ for DPPH versus ABTS−PBS and $R^2 = 0.37$ for DPPH versus ABTS−EtOH). This illustrates how the reaction environment and free radical affect the antioxidant properties of individual anthocyanins (*44*). Thus, individual anthocyanins are not likely to give broad protection against diverse oxidative stress environments in vivo as demonstrated by Lotito et al. (*45*) and Roig et al. (*46*).

The crude sorghum extracts, on the other hand, showed very strong correlations in antioxidant activity by the different methods used (ABTS versus DPPH, $R^2 = 0.96$) (**Table 4**), as previously demonstrated (*28*). This shows a consistent antioxidant profile for the natural mix of phenols present in the crude sorghum extracts and may be indicative of a broader biological activity of the crude phenol/anthocyanin mixtures compared with individual phenolics (*45, 46*). Brown sorghum extracts had exceptionally high antioxidant activity due to the presence of tannins in these sorghums, which are not present in the black and red sorghums (*28*). Total phenol content correlated most strongly with antioxidant activity ($R^2 = 0.98$ for phenols versus DPPH and $R^2 = 0.99$ for phenols versus ABTS) among the

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Table 4. Antioxidant Activity and Phenol Levels among Sorghums and Their Brans

sample	ORAC[a,b] grain	ORAC[a,b] bran	DPPH[a] grain	DPPH[a] bran	ABTS[a] grain	ABTS[a] bran	phenol[c] grain	phenol[c] bran
Tx430 (black)[d]	240	1120	47	201	65	280	5.6	26.1
Tx2911 (Red)	140	710	28	71	53	230	4.8	19.5
SC103 (brown)	nd[e]	nd	97	400	103	430	11.7	48.7
CSC3*R28 (brown)	454	2400	118	495	108	512	12.9	56.6
Sumac (brown)	868	3120	202	716	226	768	22.5	88.5
CV%[f]	6.8	6.8	5.3	5.3	3.5	3.5	4.28	4.28

[a] Micromoles of Trolox equivalents/g. [b] Awika et al. (*28*). [c] Milligrams of gallic acid equivalents/g. [d] Average of three seasons. [e] Not determined. [f] Coefficient of variation (percent relative standard deviation of each sample within column). Analyses were conducted in triplicates.

different sorghums. Among the black sorghums and their brans, anthocyanin content was also strongly correlated with antioxidant activity ($R^2 = 0.94$). Anthocyanins are the only major phenols we have observed in the black sorghum variety under the extraction procedures used in this study. In the brown and red sorghum varieties, we have observed the presence of proanthocyanidins and flavanones (e.g., naringenin), respectively, that contribute significantly to their antioxidant activity (*47*).

Various therapeutic properties reported for anthocyanins are largely attributed to their antioxidant properties. However, antioxidant activity alone is not a good predictor of potential biological benefits of the phenolic compounds because they can also modulate various biological activities depending on their molecular structure. Recently, Hou et al. (*48*) established a structure–biological activity relationship for anthocyanidins. These authors reported that substitution on the B-ring of the anthocyanidins affected their ability to suppress carcinogenesis. Such findings suggest that the 3-deoxyanthocyanidins may possess unique biological properties owing to their unique structure. To our knowledge, no biological studies have been reported for the 3-deoxyanthocyanidins. Such information is critical if the 3-deoxyanthocyanins are to be promoted as nutraceuticals.

In summary, pigmented sorghums are a rich source of unique 3-deoxyanthocyanidins that give yellow to orange color in acidic media. The compounds are greatly modified by aqueous acetone as a solvent but are stable when extracted in acidified methanol. The sorghum 3-deoxyanthocyanin pigments were more stable to pH-induced color loss than the anthocyanins commonly found in fruits and vegetables. These sorghum 3-deoxyanthocyanins are a potentially valuable source of natural food color. Additional studies are necessary to establish the color stability of the anthocyanins in actual food systems. We are currently carrying out detailed analyses to identify all of the anthocyanins in these sorghum varieties and establish their properties in various model food systems.

ABBREVIATIONS USED

ABTS, 2,2′-azinobis(3-ethylbenzothiazoline-6-sulfonic acid); DPPH, 2,2-diphenyl-1-picrylhydrazyl; ORAC, oxygen radical absorbance capacity; PBS, phosphate-buffered saline (pH 7.4); TE, Trolox equivalents.

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