

# Anthocyanins from black sorghum and their antioxidant properties

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## Abstract

A black, high anthocyanin sorghum variety (Tx430) grown in several environments was analyzed for anthocyanins by spectrophotometric and HPLC methods. The samples were also analyzed for antioxidant activity using the 2,2'-azinobis (3-ethylbenzothiaziline-6-sulfonic acid) method. Two extracting solvents, 1% HCl in methanol and 70% aqueous acetone, were compared. Sorghum brans had three to four times higher anthocyanin contents than the whole grains. The brans were a good source of anthocyanin (4.0–9.8 mg luteolinidin equivalents/g) compared to pigmented fruits and vegetables (0.2–10 mg/g), fresh weight basis. Acidified methanol extracted the anthocyanins better than aqueous acetone. Luteolinidin and apigeninidin accounted for about 50% of the anthocyanins in the black sorghums. The sorghum grains and their brans had high antioxidant activity (52–400  $\mu\text{mol TE/g}$ ) compared to other cereals (<0.1–34 mg TE/g). Black sorghum should be useful in food and other applications, because it is a valuable source of anthocyanins with good antioxidant activity.

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**Keywords:** Sorghum; Anthocyanins; Antioxidant activity; HPLC; ABTS

## 1. Introduction

Anthocyanins are becoming increasingly important not only as food colorants, but also as antioxidants. Anthocyanins are reported to have some therapeutic benefits including vasoprotective and anti-inflammatory properties (Lietti, Cristoni, & Picci, 1976), anti-cancer and chemoprotective properties (Karaivanova, Drenska, & Ovcharov, 1990), as well as anti-neoplastic properties (Kamei et al., 1995). Anthocyanins are therefore, considered to contribute significantly to the beneficial effects of consuming fruits and vegetables (Wang, Cao, & Prior, 1997). There is a rising demand for natural sources of food colorants with nutraceutical benefits (Boyd, 2000) and alternative sources of natural anthocyanins are becoming increasingly important. Speciality sorghums contain significant levels of anthocyanins and other phenols concentrated in their brans

(Awika, 2000; Gous, 1989). Black sorghum was reported to have significantly more anthocyanin pigments than other sorghums (Awika, 2000). Therefore, this sorghum has a good potential for commercial exploitation.

Anthocyanins have been extensively studied in fruits and vegetables. Limited data exists on the types and levels of anthocyanins in cereals, probably because they have never been regarded as a commercially significant source. Nip and Burns (1969, 1971) were able to isolate and identify apigeninidin, apigeninidin-5-glucoside, luteolinidin and luteolinidin-5-glucoside in red and white sorghum varieties by paper chromatography. Gous (1989) also reported luteolinidin and apigeninidin as the major anthocyanidins from a black sorghum variety. Cyanidin and pelargonidin were also reported in corn (Francis, 1989), and sorghum (Yasumutsa, Nakayama, & Chichester, 1965). However, quantitative data on sorghum anthocyanins and their antioxidant properties are hard to come by.

The most common anthocyanins in sorghum are the 3-deoxyanthocyanidins (Gous, 1989; Sweeny & Iacobucci, 1981), which comprise luteolinidin and apigeninidin. These anthocyanins have a small distribution in nature (Clifford, 2000), and are distinct from the more

*Abbreviations:* ABTS, 2,2'-azinobis (3-ethylbenzothiaziline-6-sulfonic acid); HPLC, high performance liquid chromatography; TE, trolox equivalents.

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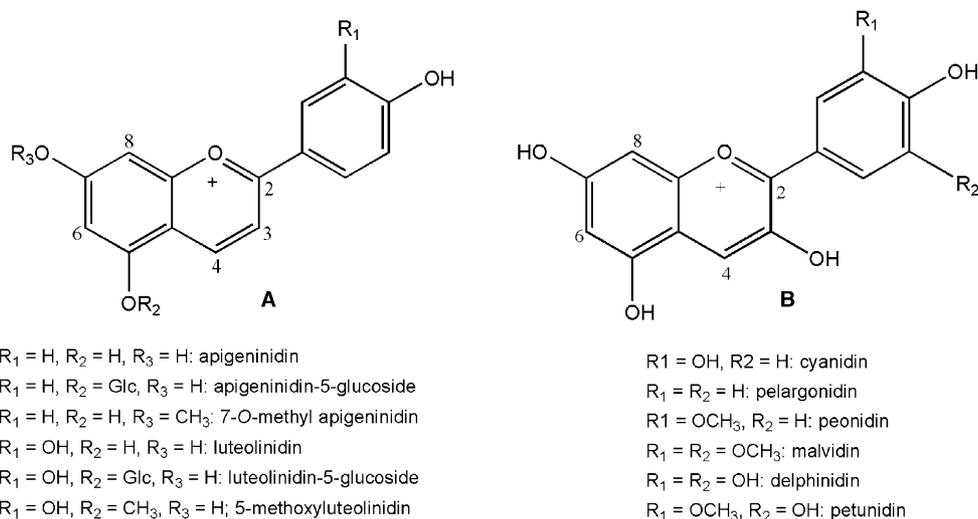


Fig. 1. Chemical structure of some common anthocyanidins compared to 3-deoxyanthocyanidins, luteolinidin and apigeninidin.

widely distributed anthocyanidins in that they lack oxygen at the C-3 position (Fig. 1). These 3-deoxyanthocyanidins were reportedly very stable in acidic solutions relative to the anthocyanidins commonly found in fruits and vegetables (Sweeny & Iacobucci, 1981). The lack of oxygen at C-3 is believed to improve their stability. This points to the potential advantage of sorghum over fruits and vegetables as a viable commercial source of anthocyanins.

To effectively characterize and quantify the sorghum anthocyanins, it is important to extract them in an efficient manner in which their original form is preserved as much as possible. The efficiency of several solvents to extract anthocyanins and other phenols from fruits, vegetables (Garcia-Viguera, Zafrilla, & Tomas-Barberan, 1998; Kallithraka, Garcia-Viguera, Bridle, & Bakker, 1995; Lu & Foo, 2001) and cereals (Gous, 1989; Hahn, 1984) have been reported. However, there is no agreement on which solvent extracts anthocyanins better. The solvents that stand out as most efficient are acidified methanol and aqueous acetone (70%). Consequently these two solvents were compared in this study in terms of their extracting power on black sorghum anthocyanins.

The objectives of this study were to assess the anthocyanin content and composition of a black sorghum (Tx430) variety over several seasons, and determine how the seasonal variations affect its antioxidant activity.

## 2. Materials and methods

### 2.1. Samples

Black Tx430 sorghum was grown in College Station, TX between 1998 and 2002. Samples from all 5 years

were kept at  $<-20^\circ\text{C}$  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 15% for each sample. Brans and grain samples were ground through a UDY mill (1 mm mesh) before extraction and analysis. All analyses were conducted in triplicate.

Anthocyanin standards were obtained from Chroma-Dex 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. The diammonium salt of 2,2'-azinobis (3-ethyl-benzothiazoline-6-sulfonic acid) (ABTS) was purchased from Sigma (St. Louis, MO); and Trolox obtained from Acros Organics, Morris Plains, NJ.

### 2.2. Sample extraction

Two extraction solvents were used: 1% HCl in methanol and 70% aqueous acetone. Extraction procedure involved the addition of 10 ml solvent to 0.5 g sample in 50 ml centrifuge tubes and shaking the samples for 2 h at low speed in an Eberbach shaker (Eberbach Corp., MI). Samples were then stored at  $-20^\circ\text{C}$  in the dark overnight to allow for maximum diffusion of phenolics from the cellular matrix. Samples were then equilibrated to room temperature and 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 mixed and stored at  $<-20^\circ\text{C}$  in the dark until analyzed.

For HPLC analysis of anthocyanins, C-18 Sep-Pak preparative columns were initially compared with direct sample injection. However, samples processed through

the Sep-Pak columns resulted in significantly smaller anthocyanins peaks on HPLC than directly injected samples, with no significant gain in peak resolutions. Hence, all samples were subsequently directly injected (after filtration through 0.45  $\mu\text{m}$  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).

### 2.3. Analytical procedures

Samples were analyzed for phenols using the modified Folin–Ciocalteu method of Kaluza, McGrath, Roberts, and Schroder (1980). The pH differential method as reported by Fuleki and Francis (1968) and Wrolstad (1976) was used for quantitative determination with minor modifications. Each 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) or pH 4.5 buffer (400 ml of 1 N sodium acetate, 240 ml of 1 N HCl, and 360 ml distilled water) solutions, respectively. The absorbance was measured by scanning through a UV/VIS spectrophotometer (Cary 300 Bio, Varian Co., Walnut Creek, CA) from 210–750 nm. Total anthocyanin pigments were determined from absorbance in pH 1.0 buffer, while monomeric anthocyanins were determined from the differences between absorbance in pH 1.0 and 4.5 buffers. Extinction coefficients for anthocyanin standards were determined using the formula described by Fuleki and Francis (1968).

The ABTS method was used for antioxidant activity assay. The  $\text{ABTS}^{\cdot+}$  was generated from ABTS salt by reacting 3 mM of  $\text{K}_2\text{S}_2\text{O}_8$  with 8 mM ABTS salt in distilled, deionized water for 16 h at room temperature in the dark. The  $\text{ABTS}^{\cdot+}$  solution was then diluted with absolute ethanol or a pH 7.4 phosphate buffer (50:42.5:9.5; water:0.2 M  $\text{Na}_2\text{HPO}_4$ :0.2 M  $\text{NaH}_2\text{PO}_4$ ) solution containing 150 mM NaCl (PBS) to obtain an initial absorbance of 1.5 at 730 nm. Fresh  $\text{ABTS}^{\cdot+}$  solution was prepared for each day of analysis. Trolox standard was dissolved in methanol. Samples and standards (100  $\mu\text{m}$ ) were reacted with the  $\text{ABTS}^{\cdot+}$  solution (2900  $\mu\text{m}$ ) for 30 min (30 min was determined to be the required for complete reaction time for the sorghum antioxidants with  $\text{ABTS}^{\cdot+}$ ).

### 2.4. HPLC analysis

Waters system (Millford, MA) was used for HPLC analysis. The system included a Waters 600 pump with a Waters 600E control system, Waters 996 PDA detector, and Waters 717 autosampler. Data collection/manipulation was via Waters Millennium software. The profile was modified from Wang, Kalt, and Sporns (2000). Separation was on a reversed phase Waters Spherisorb

ODS-2.5  $\mu\text{m}$  (250  $\times$  4.6 mm) column. Flow rate was 0.5 ml/min; injection volume, 20  $\mu\text{l}$ ; column temperature, 35  $^\circ\text{C}$ ; detection, 210–600 nm. The mobile phase was (A) 10% formic acid in water, and (B) acetonitrile:water:formic acid (5:4:1). Gradient was; 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.

## 3. Results and discussion

### 3.1. Anthocyanin extraction and quantification

Acidified methanol resulted in significantly higher values for the mono and total anthocyanins than aqueous acetone (Table 1). The total anthocyanins extracted by acidified methanol extracts were on average 59% higher than aqueous acetone extracts. Several authors reported that aqueous acetone was better than various alcoholic solvents for fruit procyanidins, anthocyanins and other phenols (Garcia-Viguera et al., 1998; Kallithraka et al., 1995). However, more recently Lu and Foo (2001) observed significant anthocyanin interaction with aqueous acetone to form pyrano-anthocyanidins which significantly lowered quantities of detectable anthocyanins. This reaction was significantly affected by time of anthocyanin–acetone interaction and temperature. The authors did not observe such anthocyanin–solvent reactions in acidified methanol, and subsequently proposed acidified methanol as a better solvent.

The apparent solvent participation in new product formations with extracted anthocyanins make a direct comparison of actual extraction power of aqueous acetone versus acidified methanol on sorghum anthocyanins difficult. However, since acidified methanol preserves the extracted anthocyanins in their original form better, it should be the solvent of choice for quantification and analysis of anthocyanins. Additional data based on the antioxidant activities of the extracts, and their HPLC profiles may give insight on actual extraction efficiency of the two solvents. This data is discussed in subsequent sections.

Sorghum brans had on average three to four times the levels of anthocyanins in grains (Table 1). Sorghum anthocyanins are readily concentrated by decortication. Monomeric anthocyanins contributed an average of 30–50% of the anthocyanins in the sorghums. This implies the larger part of sorghum anthocyanins are polymerized or complexed with other compounds and are not readily separated by the extraction conditions used. Such complexed anthocyanins usually have better color stability in solution than their monomeric constituents

Table 1  
Anthocyanin levels<sup>A</sup> in black sorghum brans extracted with Me-HCl and Ac-aq

Solvent Sample	Monomeric				Total			
	Grain		Bran		Grain		Bran	
	Methanol <sup>B</sup>	Acetone <sup>C</sup>						
1998	0.3	nd <sup>D</sup>	2.3	0.3	1.0	0.6	5.3	2.1
1999	1.5	0.5	4.6	1.7	2.7	1.1	10.4	4.8
2000	0.4	nd	1.7	0.2	1.4	0.6	4.7	1.0
2001	1.0	0.5	4.2	1.5	2.3	1.4	8.9	4.0
2002	1.2	0.4	3.7	1.7	2.8	1.1	11.0	5.2
Mean	0.9a	0.5b	3.3a	1.1b	2.0a	1.0b	8.1a	3.4b
LSD	0.1	0.2	0.2	0.4	0.1	0.2	0.3	0.4

<sup>A</sup> mg/g Luteolinidin equivalents (DM basis), determined pH differential method. Samples were extracted for 2 h at room temperature and equilibrated for 24 h at  $-20^{\circ}\text{C}$  before analysis.

<sup>B</sup> Acidified methanol (1% HCl).

<sup>C</sup> 70% Aqueous acetone.

<sup>D</sup> nd, Not detected at significant levels. All comparisons between the two solvents were significant at ( $\alpha = 0.05$ ).

(Brouillard, Wigand, Dangles, & Cheminat, 1991; Francis, 1989; Timberlake & Bridle, 1980).

Seasonal variations in anthocyanin contents were significant. However, since no specific environmental parameters were tested or controlled for, the factors that contributed to the observed variability cannot be pinpointed. However, the 1998 and 2000 grains (which had the lowest anthocyanin contents) had wrinkled, poorly formed seeds. This suggests significant environmental stress during seed development. Such stress may have negatively affected anthocyanin biosynthesis. For example, Dube, Bharti, and Laloraya (1992) showed that ionic stress may inhibit light-induced anthocyanin synthesis in sorghum. Sene, Dore, and Gallet (2001) also reported that nitrogen nutrition and environmental factors that promote growth and grain yield also enhance phenol synthesis in sorghum. It is important to conduct additional studies to determine which specific environmental factors influence the anthocyanin contents of the sorghum. This will help maximize anthocyanin production in sorghum.

### 3.2. HPLC analysis

The absorption characteristics and other properties of the anthocyanin standards analysed are shown in Table 2. The 3-deoxyanthocyanidins, luteolinidin and apigeninidin had absorption maximas that were particularly different from those of the other anthocyanidins. These two anthocyanidins are structurally different from the rest of the anthocyanidins which are commonly found in fruits and vegetables since they lack an oxygen molecule at the C-3 position (Fig. 1). Their absorption maximas of 468 nm (apigeninidin), and 482 nm (luteolinidin) in pH 1 buffer solution made them appear yellow and orange, respectively. This was in contrast with the other anthocyanidins which were all reddish at pH 1. At near neutral pH (in methanol), the 3-deoxyanthocyanidins appeared yellowish orange, and reddish orange for the apigeninidin and luteolinidin, respectively. The rest of the anthocyanins ranged from red to dark blue in color at neutral pH.

Table 2  
Molar properties and antioxidant activity of anthocyanin standards

Standard	$\lambda_{\text{max}}$ (pH 1) <sup>a</sup>	$\epsilon$ (pH 1) <sup>b</sup>	Antioxidant activity <sup>c</sup>	# of OH groups
Luteolinidin <sup>d</sup>	482	31,700 $\pm$ 1100	4.81	4
Apigeninidin <sup>d</sup>	468	30,400 $\pm$ 500	4.20	3
Peonidin	516	27,200 $\pm$ 4100	3.62	4
Pelargonidin	506	28,100 $\pm$ 200	3.33	4
Pelargonidin-3,5-diglycoside	498	28,800 $\pm$ 1100	2.88	2
Cyanidin	516	24,800 $\pm$ 60	5.35	5
Cyanidin-3-glucoside	512	28,600 $\pm$ 800	4.60	4
Cyanidin-3,5-diglycoside	510	33,900 $\pm$ 1700	6.34	3
Cyanidin-3-rutinoside	514	26,100 $\pm$ 1700	4.20	4

<sup>a</sup> Maximum absorption wavelength in pH 1 buffer.

<sup>b</sup> Extinction coefficient in pH 1 buffer.

<sup>c</sup> mol Trolox/mol, ABTS method.

<sup>d</sup> Compounds identified in sorghum. LSD for antioxidant activity = 0.11 ( $\alpha > 0.05$ ).

The lack of oxygen on C-3 of the 3-deoxyanthocyanidins is thought to accord them greater stability in solution compared to the other anthocyanidins (Iacobucci & Sweeny, 1983; Sweeny & Iacobucci, 1981; Timberlake & Bridle, 1980). For example, Timberlake and Bridle (1980) reported that apigeninidin was stable in pH 2.8 solution for up to 1 year at room temperature and laboratory light, whereas cyanidin degraded within a few hours under similar conditions. Hence the 3-deoxyanthocyanidins may have an advantage over the other anthocyanidins in food applications.

The HPLC profiles for the sorghums and their brans were all similar, hence only one sample (2001 bran) profile is shown for illustration purposes (Figs. 2 and 3). Among the anthocyanin standards used, only the 3-deoxyanthocyanidins (apigeninidin and luteolinidin) were identified in acidified methanol sorghum extracts (Fig. 2 and Table 3). Other HPLC peaks, including one major, and five minor ones from the black sorghums, could not be identified due to lack of appropriate standards. The unknown peaks 2 and 3 (Fig. 2) had spectral characteristics similar to apigeninidin. They were tentatively identified as glycosides of apigeninidin, based on their elution times and spectral characteristics. The other unidentified peaks were all spectrally similar to luteolinidin, suggesting they may be structurally related.

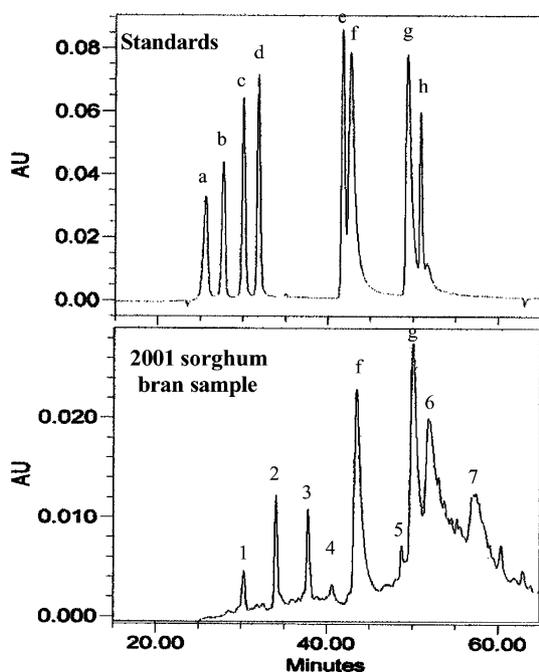


Fig. 2. HPLC profiles of acidified methanol (1% HCl) anthocyanin extracts from 2001 black sorghum bran. Detection was at 480 nm: (a) cyanidin-3,5-diglucoside; (b) pelargonidin-3,5-diglucoside; (c) cyanidin-3-glucoside; (d) cyanidin-3-rutinoside; (e) cyanidin; (f) luteolinidin; (g) apigeninidin; (h) peonidin. Peaks 2 and 3 were tentatively identified as glycosides of apigeninidin. Peaks 1 and 4–7 were spectrally similar, and possibly related to luteolinidin. Seventy percent of aqueous acetone extracts did not give significant peaks at this wavelength.

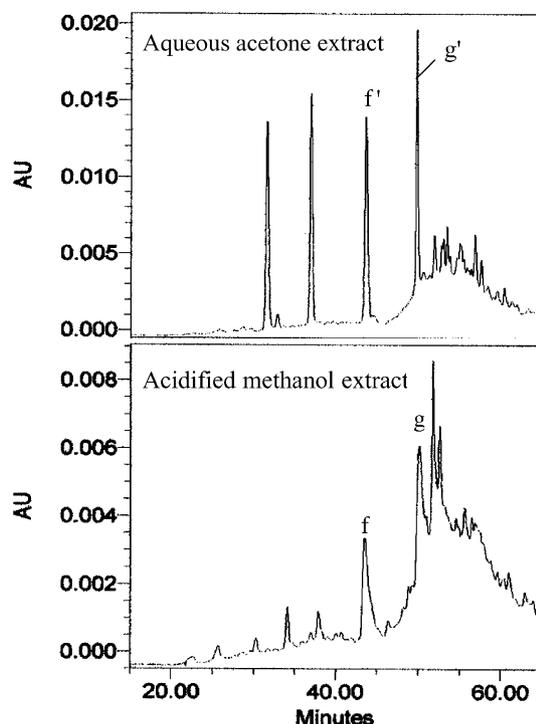


Fig. 3. HPLC anthocyanin profiles of 2001 black sorghum bran extracts monitored at 375 nm. Note the increased intensity of resolved peaks for 70% aqueous acetone extracts relative to acidified methanol (1% HCl) extracts at this wavelength. Peaks 'f' and 'g' correspond to acetone-modified luteolinidin and apigeninidin, respectively (see Fig. 4). Peaks 'f' and 'g' are luteolinidin and apigeninidin, respectively. Samples were extracted for 2 h and equilibrated at  $-20^{\circ}\text{C}$  for at least 24 h before analysis.

Aqueous acetone extracts produced very low peaks at the 480 nm wavelength used for anthocyanin detection. However, the aqueous acetone extracts had four major distinct peaks, 2 of which had elution characteristics corresponding to luteolinidin and apigeninidin, but with absorption maximas at between 370 and 380 nm, instead of the typical 470–490 nm for these anthocyanidins in the HPLC solvents (Figs. 3 and 4). The typical absorption peaks for luteolinidin and apigeninidin were still present but at much reduced intensity (Fig. 4). Apparently, the anthocyanin molecules undergo significant structural modification in aqueous acetone, a phenomenon that did not occur in acidified methanol. This observation agrees with that of Lu and Foo (2001), who were able to confirm formation of pyrano-anthocyanins from anthocyanins through oxidative addition mediated by acetone. These compounds may be responsible for the observed spectra of the aqueous acetone extracts. Thus aqueous acetone is not an appropriate solvent for extracting sorghum anthocyanins for HPLC characterization.

Apigeninidin and luteolinidin represented an average of 49% of the total peak areas, whereas the major unknown peak (peak number 6) represented about 25% of

Table 3  
Anthocyanin contents of black sorghum brans determined by HPLC

Year	Luteo <sup>a</sup>	Apig <sup>b</sup>	1 <sup>c</sup>	2 <sup>d</sup>	3 <sup>d</sup>	4 <sup>c</sup>	5 <sup>c</sup>	6 <sup>c</sup>	7 <sup>c</sup>	Total
1998	0.89	0.63	0.10	0.19	0.20	Trace	0.04	0.47	0.26	2.78
1999	1.77	0.43	0.08	0.19	0.14	Trace	0.22	3.09	0.21	6.12
2000	0.53	0.57	0.10	0.21	0.14	0.10	Trace	0.15	Trace	1.79
2001	1.34	1.42	0.10	0.26	0.29	0.05	Trace	1.05	0.58	5.35
2002	1.49	1.26	0.27	0.25	0.23	Trace	0.11	1.55	0.63	5.96
Mean	1.20	0.86	0.13	0.22	0.20	0.03	0.07	1.26	0.34	4.40
CV	1.4	1.8	0.9	1.5	1.2	0.8	0.9	1.4	4.5	2.3

All unknowns and totals expressed as luteolinidin equivalents.

Units are mg/g DM basis.

<sup>a</sup> Luteolinidin.

<sup>b</sup> Apigeninidin.

<sup>c</sup> Unidentified, had spectral characteristics similar to luteolinidin.

<sup>d</sup> Tentatively identified as glycosides of apigeninidin. Samples were extracted with 1% HCl in methanol. CV, coefficient of variation.

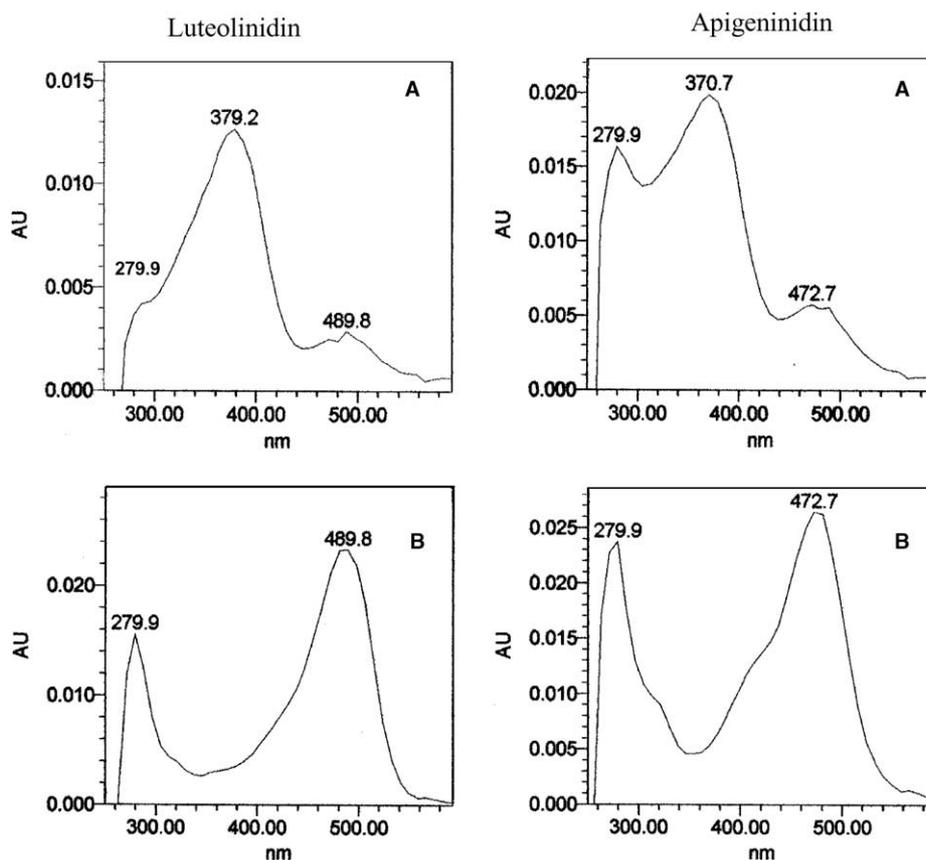


Fig. 4. Spectral characteristics of peaks corresponding to solvent modified (A) and unmodified (B) luteolinidin and apigeninidin isolated from 2001 black sorghum bran by HPLC. Spectra 'A' were obtained from 70% aqueous acetone extracts and 'B' from acidified methanol (1% HCl) extracts. Note the prominent presence of a third peak, not typical for anthocyanins, and the diminished intensity of the typical absorption maxima of the anthocyanidins in the aqueous acetone extracts. Samples were extracted for 2 h and equilibrated at  $<-20$  °C for at least 24 h before analysis.

the total black sorghum anthocyanins (Table 3 and Fig. 2). Gous (1989) also identified luteolinidin and apigeninidin as the major anthocyanins in a black sorghum variety. Glycosylated forms of these anthocyanins were also identified in sorghums by Nip and Burns (1969, 1971). Unlike most anthocyanins in fruits and

vegetable that are naturally found as glycosides, the 3-deoxyanthocyanidins do exist in nature as aglycones (Clifford, 2000; Stafford, 1965). Hence their abundance in the sorghum extracts may not necessarily mean degradation during extraction. The total anthocyanin contents measured by HPLC were strongly correlated with

Table 4  
Anthocyanin content of sorghum brans relative to common fruits and vegetables

Commodity	Content <sup>a</sup> (mg/g)	Reference
Black sorghum bran	4.0–9.8	—
Blueberry	0.2–5.0	b,c
Cranberry	0.6–2.0	c
Black currant	0.3–4.0	b,c
Red grapes	0.3–7.5	d
Black raspberry	1.7–4.3	b,e
Red raspberry	0.1–0.6	b,e
Strawberry	0.2–0.9	c,f
Elderberry	2.0–10.0	g
Red cabbage	0.3–0.9	b,d

Sorghum samples extracted in 1% HCl in methanol.

<sup>a</sup> Fresh weight.

<sup>b</sup> Wang et al. (1997).

<sup>c</sup> Clifford (2000).

<sup>d</sup> Bridle and Timberlake (1997).

<sup>e</sup> Torre and Barrit (1997).

<sup>f</sup> Garcia-Viguera et al. (1998).

<sup>g</sup> Bronnun-Hansen et al. (1985).

those measured by the pH differential method ( $R^2 = 0.98$ ). The simpler pH differential method gives a good estimation of anthocyanin content of black sorghum.

### 3.3. Comparison of black sorghum with commercial anthocyanin sources

Black sorghum brans were a very good source of anthocyanins (4.0–9.8 mg/g) relative to the commercial sources currently available (0.2–10 mg/g) (Table 4). This coupled with the fact that they possess mostly the relatively stable 3-deoxyanthocyanidins gives them an edge over the fruits and vegetables as a source of natural anthocyanins. The sorghum has an additional advantage in terms of storage stability relative to the fruits and vegetables. Sorghum is harvested at low moisture (13–15%) compared to fruits and vegetables which normally have >80% moisture and require significant time and energy for drying to improve their storage stability. The black sorghum can be a competitive source of anthocyanins.

### 3.4. Antioxidant activity of black sorghum

Antioxidant activities of samples extracted in acidified methanol and aqueous acetone were compared (Fig. 5). Samples extracted in acidified methanol had significantly higher antioxidant activity than those extracted in aqueous acetone. This implies that the acidified methanol is a more powerful solvent than aqueous acetone at extracting black sorghum antioxidants.

The 3-deoxyanthocyanidins found in the black sorghum had antioxidant activities that were similar to the anthocyanins found in fruits and vegetables (Table 2).

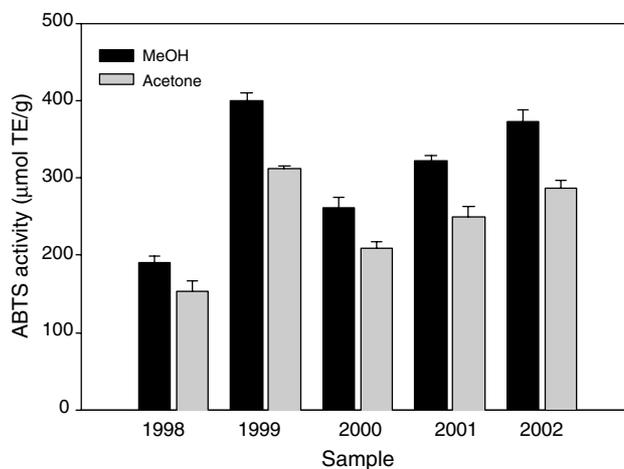


Fig. 5. Comparison of antioxidant activities of black sorghum brans extracted in acidified methanol (1% HCl) and 70% aqueous acetone. Samples were extracted for 2 h and equilibrated at  $<-20$  °C for at least 24 h before analysis. Differences within each sorghum were significant ( $\alpha = 0.05$ ).

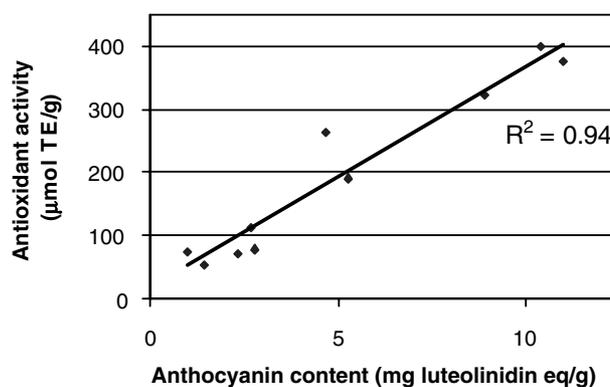


Fig. 6. Correlation between anthocyanin content and antioxidant activity of black sorghums and their brans. The ABTS method was used for antioxidant assay. Anthocyanins were determined by the different method. Acidified methanol (1% HCl) was the extracting solvent.

Additionally, a good correlation ( $R^2 = 0.94$ ) was observed between total anthocyanin levels and antioxidant activity of the sorghum samples (Fig. 6), confirming the major contribution of anthocyanins to black sorghum antioxidant activity.

The sorghum grains and brans had significantly higher phenols and antioxidant activity than other cereals (Table 5). Cereal brans from wheat, barley, buckwheat, and rice, among others, are promoted as good sources of antioxidants (Emmons & Peterson, 1999; Haber, 2002; Quettier-Deleu et al., 2000; Yu et al., 2002; Zielinski & Kozłowska, 2000). Such brans are sold in the market for use in fortified baked products. Black sorghum bran offers a major advantage in terms of antioxidant value per unit weight. The sorghum bran can be used as a high value source of antioxidants at lower quantities than other cereal brans, or used at similar quantities to

Table 5  
ABTS Antioxidant activity and phenol levels among black sorghums and other cereals

Sample	Activity ( $\mu\text{mol TE/g}$ )		Phenol (mg/g)	
	Grain	Bran	Grain	Bran
Black 1998	75	190	5.6	18.1
Black 1999	112	400	10.0	32.0
Black 2000	52	261	5.5	19.1
Black 2001	72	323	6.4	26.0
Black 2002	78	374	7.3	35.6
Mean	78	310	7.0	26.2
CV	2.8	3.5	4.0	5.0
Wheat	<0.1–2.0 <sup>a,b</sup>	28 <sup>c</sup> –34	0.4–0.9 <sup>b</sup>	3.0
Barley	9–11 <sup>a</sup>	–	–	–
Rye	4 <sup>a</sup>	–	–	–
Oats	3 <sup>a</sup>	5 <sup>c</sup>	0.2–0.3 <sup>d</sup>	0.2–0.3 <sup>d</sup>
Buckwheat	25 <sup>a</sup>	–	3.2 <sup>f</sup>	3.3 <sup>c</sup>

Sorghum samples extracted with 1% HCl in methanol.

<sup>a</sup> Zielinski and Kozłowska (2000).

<sup>b</sup> Yu et al. (2002).

<sup>c</sup> Haber (2002).

<sup>d</sup> Emmons and Peterson (1999).

<sup>e</sup> Quettier-Deleu et al. (2000).

<sup>f</sup> Estimated from Quettier-Deleu et al. (2000).

provide higher antioxidant activity in products. Additionally black sorghum bran imparts a natural dark appealing color normally associated with 'healthy' baked goods, and was shown to produce acceptable quality bread (Gordon, 2001) and cookies (Mitre-Dieste, Gordon, Awika, Suhendro, & Rooney, 2000) at levels of up to 15% and 50%, respectively. Since the black sorghum had no condensed tannins, any perceived nutritional concerns related to tannins are eliminated.

In summary, black sorghum brans were a very good source of anthocyanins (4.0–9.8 mg/g) compared to commercial sources currently available (0.2–10 mg/g). The black sorghum anthocyanins were composed largely of the 3-deoxyanthocyanidins, which are more stable than the anthocyanins mostly found in fruits and vegetables used currently as commercial sources of anthocyanins. This should make the sorghum anthocyanins competitive as a source of natural food color. The antioxidant activity of the sorghum anthocyanins were similar to those of the anthocyanins found in fruits and vegetables, hence they may offer similar health benefits. The black sorghum brans are superior to other cereal brans as a source of antioxidants. They may provide more health benefits when used in cereal based foods than the current commercial brans.

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