Phenolic Compounds and Antioxidant Activity of Sorghum Grains of Varying Genotypes

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The effects of plant color, pericarp thickness, pigmented testa, and spreader genes on phenols and antioxidant activity levels of 13 sorghum genotypes were evaluated. Total phenols, condensed tannins, flavan-4-ols, and anthocyanins were measured. Antioxidant activity levels using the 2,2′-azinobis(3-ethyl-benzothiazoline-6-sulfonic acid) and 2,2-diphenyl-1-picrylhydrazyl assays were evaluated. Sorghums with a pigmented testa and spreader genes (B_1B_2S) had the highest levels of phenols and antioxidant activity. In addition, sorghums with purple/red plants (*PQ*) and thick pericarp (*z*) genes had increased levels of phenols and antioxidant activity. Sorghums with a black pericarp had higher levels of flavan-4-ols and anthocyanins than the other varieties. This suggests that genes for plant color, pericarp thickness, presence of a pigmented testa, and spreader genes increase phenols and antioxidant activity levels. This information can be useful in the production of sorghums with increased phenols and antioxidant activity levels.

KEYWORDS: *Sorghum bicolor* (L.) Moench; total phenols; flavan-4-ols; anthocyanins; antioxidant activity; ABTS; DPPH

INTRODUCTION

Sorghum [*Sorghum bicolor* (L.) Moench] is the fifth leading cereal crop in the world and is used primarily in Asia and Africa as a food crop (*1*). The United States, however, uses sorghum mainly as a feed grain. More recently, several additional potential health and pharmaceutical benefits of sorghum have been reported. These include slow digestibility, cholesterol lowering, cardiovascular disease reduction, and anticarcinogenic properties (*2*, *3*).

While all sorghums contain phenolic compounds, the amount present in any particular cultivar is influenced by its genotype and the environment in which it is grown. In addition, these same factors affect the color, appearance, and nutritional quality of the grain and its products (*4*). The pericarp color of the sorghum kernel is controlled by the *R* and *Y* genes, which interact epistatically to produce red, yellow, and white pericarp colors (*5*). A pericarp is white when the *Y* locus is homozygous recessive (*rryy* or *R_yy*); it is yellow in the presence of recessive alleles at the *R* locus and at least one dominant allele at the *Y* locus (*rrY_*). When both *R* and *Y* loci possess a dominant allele (*R_Y_*), the pericarp is red. The presence of a pigmented testa is controlled by the B_1 and B_2 genes, which interact in duplicate dominant epistasis ($B_1\_B_2\_$) to produce a pigmented testa. The spreader gene *S* controls the presence of pigments and tannins in the epicarp while pericarp thickness is controlled by the *Z*

gene with thin pericarp dominant to thick pericarp. Finally, secondary plant color is controlled by the *P* and *Q* genes, which interact epistatically to produce purple, red, and tan pigmented plants (*5*).

Sorghum phenols protect plants against insects and diseases (*4*), and they can also act as antioxidants in vitro (*6*, *7*). Free radicals play a role in diseases such as cancer, atherosclerosis, rheumatoid arthritis, inflammatory bowel disease, and cataracts (*8*), and phenolic compounds may decrease the risk of these diseases by lowering the amount of free radicals. Other roles of antioxidants include antifungal, antibacterial, and antiviral agents (*9*).

Because sorghum is a source of phenols that have varying antioxidant potential, it is necessary to isolate and characterize the phenolic compounds in sorghums to determine sources of compounds with unique attributes. Awika et al. (*7*) found that high tannin and sumac sorghum brans have higher oxygen radical absorbance capacity values than common fruits. Therefore, these sorghums are a good source of antioxidants, which have the potential to be used in the functional food/nutraceutical industry. To date, there is limited data on the antioxidant activity of different sorghum genotypes.

Because cultivar affects phenols content, the antioxidant potentials of sorghum grains with clearly identified genotypes were analyzed for total phenols, condensed tannins, flavan-4-ols, anthocyanins, and antioxidant activity. This manuscript reports on the content of these compounds in sorghum grains

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Table 1. Genotypes and Physical Characteristics of Sorghum Varieties

sample	line designation	genotype	plant color	pigmented testa	pericarp	grain appearance
1	02CA4796	$b_1b_1B_2B_2ssRRyyZZPPqq$	tan	absent	white, thin	pearly, white
2	B.01336	$b_1b_1B_2B_2ssRRYYZZPPqq$	tan	absent	red, thin	pearly, yellowish red
3	99GWO92	$b_1b_1B_2B_2ssRRYYZZPPqq$	tan	absent	red, thin	pearly, orange-brown
4	98BRON155	$b_1b_1B_2B_2ssRRYYZZPPqq$	tan	absent	red, thin	pearly, yellowish red
5	98CA4779	$b_1b_1B_2B_2ssRRYYZZPPQQ$	purple	absent	red, thin	pearly, orange
6	B.9904	$b_1b_1B_2B_2ssRRYYZZPPQQ$	red	absent	red, thin	pearly, brown
7	SC103-12 × SC748-5E (light)	$b_1b_1B_2B_2ssRRYYZZPPQQ$	purple	absent	red, thin	pearly, yellowish-brown
8	Tx2911	$b_1b_1B_2B_2ssRRYYZzPPQQ$	red	absent	red, thick	chalky, red
9	99LGWO50	$b_1b_1B_2B_2ssRRYYzzPPQQ$	red	absent	red, thick	chalky, red
10	Tx430 Black	$b_1b_1B_2B_2ssRRYYzzPPQQ$	purple	absent	red, thick	black
11	SC719-11E	$B_1B_1B_2B_2ssRRYYzzPPQQ$	red	present	red, thick	chalky, red
12	SC103-12 × SC748-5E (dark)	$B_1B_1B_2B_2SSRRYYzzPPQQ$	purple	present	red, thick	chalky, dark brownish-red
13	Black PI Tall	$B_1B_1B_2B_2SSRRYYzzPPQQ$	purple	present	red, thick	black

that vary in pericarp color, mesocarp thickness, and the presence and intensity of the pigmented testa layer.

MATERIALS AND METHODS

Materials. Thirteen sorghum varieties were grown in a sorghum breeding nursery in College Station, TX, in 2003; their designations and genetic and physical descriptions are summarized in **Table 1**. The line designations for the germplasm were given by breeders in the Texas Agricultural Experiment Station (TAES) Sorghum Improvement Program, and the genotype of each line was based on observations made by TAES sorghum breeders. Sample 1, a tan plant white pericarp sorghum free of evident pigments, was used as the control. All sorghum samples were collected at maturity; they were air-dried and manually cleaned, and all glumes were removed from the grains.

Gallic acid, catechin hydrate, and 2,2′-azinobis(3-ethyl-benzothiazoline-6-sulfonic acid) (ABTS) were obtained from Sigma (St. Louis, MO). The 2,2-diphenyl-1-picrylhydrazyl (DPPH) was obtained from Acros Organics (Morris Plains, NJ), and Trolox was obtained from Aldrich (Milwaukee, WI). All solvents were reagent grade.

Sample Preparation. Samples were ground for 1 min using a Braun KSM2 coffee grinder (Gillette Co., MA) prior to analysis. For all assays with the exception of the DPPH assay, samples (0.1−0.5 g) were extracted in 25 mL of 1% HCl/methanol (v/v) for 2 h while shaking at low speed using an Eberbach shaker (Eberbach Corp., MI). For the DPPH assay, samples (0.2−0.5 g) were extracted in aqueous 70% acetone (v/v) for 2 h while shaking at low speed. The extracts were then centrifuged at 2790g for 15 min in a Sorvall SS-34 centrifuge (DuPont Instruments, Wilmington, DE) and were decanted. To avoid oxidation, extracts were stored in the dark at −20 °C and analyses were performed within 24 h.

Analytical Procedures. Color measurements were obtained using a Minolta CR-310 Colorimeter (Osaka, Japan). Measurements were expressed as Commission Internationale de l'Eclairage L^*, a^*, and b^* (CIELAB) (10). Total phenols of the acidified methanol extracts were measured using the modified Folin−Ciocalteu method of Kaluza et al. (11). One aliquot of the extract (0.1 mL) was diluted with 1.1 mL of water and was then reacted with 0.4 mL of Folin reagent and 0.9 mL of 0.5 M ethanolamine. The reaction was allowed to stand for 20 min at room temperature, and the absorbance was read at 600 nm. Condensed tannins were measured using the modified vanillin/HCl assay as described by Price et al. (12). Flavan-4-ol content was measured using the modified method of Govindarajan and Mathew (13). One aliquot (1 mL) of the extract was reacted with 5 mL of HCl−butanol reagent, which was prepared by dissolving 0.0616 g of $FeSO_4 \cdot 7H_2O$ in 5% HCl in *sec*-butanol (v/v). The reaction was allowed to stand for 1 h at room temperature, and the absorbance was read at 550 nm. Anthocyanin content was measured using the method of Fuleki and Francis (14). One aliquot of each sample was diluted 2-fold using the extraction solvent and was left to stand for 2 h at room temperature in the dark. Absorbance was read at 485 nm (luteolinidin) and at 465 nm

Table 2. Cielab L^*, a^*, and b^* Values of Sorghum Grains (Mean ± SD, $n = 3$)

sample	L^*	a^*	b^*
1	62.24 ± 0.13	3.80 ± 0.10	19.20 ± 0.18
2	42.64 ± 0.45	12.58 ± 0.18	13.44 ± 0.24
3	41.96 ± 0.09	13.02 ± 0.31	13.73 ± 0.25
4	46.45 ± 0.14	12.38 ± 0.34	18.04 ± 0.17
5	44.82 ± 0.08	16.36 ± 0.13	17.73 ± 0.13
6	41.78 ± 0.06	12.76 ± 0.06	12.30 ± 0.16
7	43.24 ± 0.14	12.52 ± 0.16	14.69 ± 0.17
8	40.34 ± 0.14	17.87 ± 0.01	13.01 ± 0.01
9	39.14 ± 0.23	16.17 ± 0.11	10.86 ± 0.28
10	34.62 ± 0.20	3.53 ± 0.03	3.00 ± 0.07
11	41.70 ± 0.15	18.26 ± 0.13	14.90 ± 0.12
12	36.48 ± 0.22	9.93 ± 0.10	7.39 ± 0.13
13	32.61 ± 0.07	2.29 ± 0.09	1.41 ± 0.06

(apigeninidin). Antioxidant activities of sorghum extracts were assessed in vitro by the ABTS and DPPH assays as described by Awika et al. (7).

Statistical Analysis. All values are expressed as means ± standard deviation (SD) for three replicates. Mean values of sorghum phenols and antioxidant activity levels are presented in graphs in descending order. The Pearson correlation was used to determine relationships between sorghum phenols and antioxidant activity and between sorghum phenols and grain color. Statistical analysis was done using SPSS version 11.0 (SPSS Inc., Chicago, IL).

RESULTS AND DISCUSSION

Sorghum Grain Characteristics. Samples 10 and 13 had the lowest L^* values (32.6−34.6), which means that they were the darkest in color while sample 1 had the highest L^* value (62.2) (**Table 2**). This was expected since the grains of samples 10 and 13 have a black pericarp while those of sample 1 have a white pericarp and are grown on plants with a tan secondary plant color. All samples had positive a^* values, which means that they were more red than green. With the exception of sample 1, the a^* value increased as the L^* value increased. Sample 10 had a higher a^* value than was expected since the hilar area, which was covered by the glume during its development, was light red, which affected the redness value. This was not observed with sample 13, which was completely black. The b^* value was also positive for all samples, which means that they were more yellow than blue; the L^* value increased as the b^* value increased.

Evaluation of Sorghum Total Phenols. Plant color affected total phenol content (**Figure 1**). Sorghum grains grown on plants

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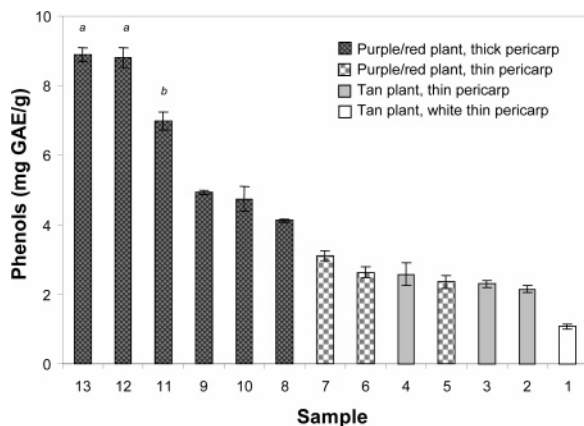*J. Agric. Food Chem.,* Vol. 53, No. 17, 2005 **6815**

Figure 1. Total phenol levels of sorghum grains with red pericarp (GAE, gallic acid equivalents). Superscripts *a* and *b* represent varieties with B_1B_2SS and B_1B_2ss genes, respectively.
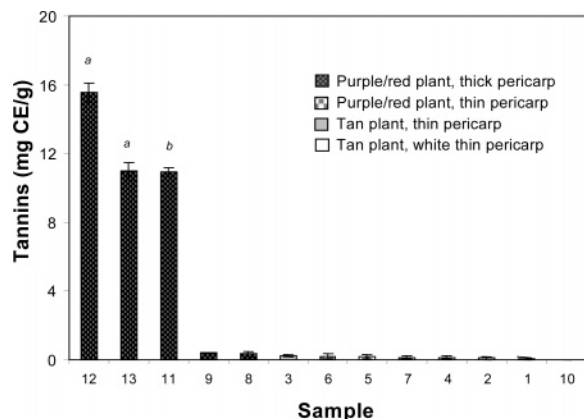

Figure 2. Condensed tannin levels of sorghum grains with red pericarp (CE, catechin equivalents). Superscripts *a* and *b* represent varieties with B_1B_2SS and B_1B_2ss genes, respectively.

Figure 3. Flavan-4-ol levels of sorghum grains with red pericarp. Superscripts *a* and *b* represent varieties with B_1B_2SS and B_1B_2ss genes, respectively.

with purple/red secondary plant color, with the exception of samples 5 and 6, had higher levels of total phenol [3.1−8.9 mg gallic acid equivalents (GAE)/g] than those from tan plants (2.1−2.6 mg GAE/g). Sorghums with a thick pericarp had higher total phenols (4.1−8.9 mg GAE/g) than those with a thin pericarp (2.1−3.1 mg GAE/g). However, sorghums with a thin pericarp from purple/red plants had total phenol levels similar to those from tan plants. This agrees with the results of Beta et al. (*15*) who found a positive relationship between pericarp thickness and total phenols. The presence of the pigmented testa gene B_1B_2 and the spreader gene S increased total phenols. Grains with B_1B_2S genes had the highest levels of total phenols (8.80−8.89 mg GAE/g).

Evaluation of Sorghum Condensed Tannins. Only three varieties (samples 11−13) contained a pigmented testa and had significant amounts of condensed tannins (**Figure 2**). Sorghums without a pigmented testa did not show any significant quantities of condensed tannins. The low levels of absorbance were due to other phenolic compounds that react with vanillin (*16*). Samples 11 and 13 had similar levels of condensed tannins [11.9−12.0 mg catechins equivalents (CE)/g] while sample 12 contained 15.5 mg CE/g. This was not expected since sample 13 has a dominant spreader gene S. Sorghums with a dominant spreader gene usually have higher tannin levels than those with a recessive gene (*16*).

Evaluation of Sorghum Flavan-4-ols. Red pericarp sorghums have flavan-4-ol compounds, such as luteoforol and apiforol, which are produced from flavanones (i.e., naringenin
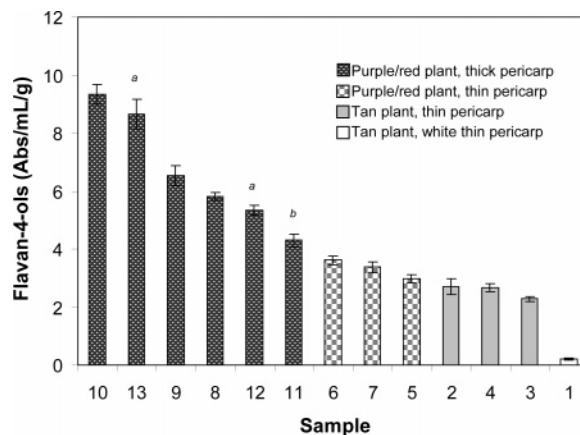
and eriodictyol) and may be precursors of anthocyanidins in sorghums (*17*). In addition to the possibility of reducing mold damage in sorghums (*18−20*), these compounds may act as antioxidants and have health benefits; however, evidence on their health-related benefits is lacking. Flavan-4-ols in tan plant sorghums were lowest (2.3−2.7 abs/mL/g), followed by purple/red plant sorghums with a thin pericarp (3.0−3.6 abs/mL/g) (**Figure 3**). Purple/red plant sorghums with a thick pericarp had the highest levels of flavan-4-ols (4.3−9.3 abs/mL/g), especially those with a black pericarp. A positive correlation between total phenols and flavan-4-ols ($r = 0.70$, $p < 0.01$) (**Table 3**) suggests that total phenols are contributed mostly by flavan-4-ols in red pericarp sorghums. This was especially true among nontannin sorghums, which showed a much stronger correlation ($r = 0.94$, $p < 0.001$) (**Table 3**).

Evaluation of Sorghum Anthocyanins. The most common anthocyanins in sorghums are the 3-deoxyanthocyanidins, which include orange luteolinidin and yellow apigeninidin (*2, 3, 21−24*). Both compounds have good potential for use as natural colorants due to their pH stability (*23, 24*). The levels of anthocyanins in the sorghums evaluated had the same pattern as found for the flavan-4-ols (**Figure 4**). Sorghums with a black pericarp (samples 10 and 13) contained the highest levels of anthocyanins, followed by those from purple plants with a thick pericarp. Sorghums with a black pericarp are genetically red, but when maturing in the presence of sunlight, their pericarp turns black. In general, the varieties had higher levels of luteolinidin than apigeninidin; these compounds were strongly correlated with each other ($r = 0.99$, $p < 0.001$) as found in previous studies (*20, 25*). Characterization of these compounds using high-performance liquid chromatography is necessary to determine which ones are predominant in these genotypes. A correlation between flavan-4-ol and anthocyanin contents ($r = 0.87$, $p < 0.001$) (**Table 3**) was observed. The same was observed among nontannin varieties especially between flavan-4-ols and apigeninidin ($r = 0.86$, $p < 0.001$). No significant correlation was found between total phenols and anthocyanin content, which was previously reported by Awika (*26*).

Correlations between Pericarp Color and Sorghum Phenols. There were some significant correlations between pericarp color and sorghum phenols (**Table 4**). The negative correlation between the L^* value and total phenols ($r = -0.69$, $p < 0.01$) suggests that darker grains contain higher levels of phenolic compounds. A stronger negative correlation between the L^* value and flavan-4-ol content ($r = -0.84$, $p < 0.001$) was observed, suggesting that dark pigments in the pericarp increase

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Table 3. Pearson's Correlation Coefficients among Sorghum Phenols and Antioxidant Activity[a]

	all sorghum varieties					nontannin sorghum varieties				
	FL-4-OL	LUT	APIG	ABTS	DPPH	FL-4-OL	LUT	APIG	ABTS	DPPH
PHE	0.70 b	0.55	0.56 c	0.99 a	0.98 a	0.94 a	0.65 c	0.67 c	0.97 a	0.97 a
FL-4-OL		0.87 a	0.87 a	0.65 c	0.62 c		0.85 b	0.86 a	0.88 a	0.96 a
LUT			0.99 a	0.47	0.50			1.00 a	0.56	0.74 c
APIG				0.48	0.51				0.58	0.75 c
ABTS					0.97 a					0.94 a

[a] PHE = total phenols; FL-4-OL = flavan-4-ol; LUT = luteolinidin; and APIG = apigeninidin. Letters a, b, and c indicate $p < 0.001$, $p < 0.01$, and $p < 0.05$, respectively.
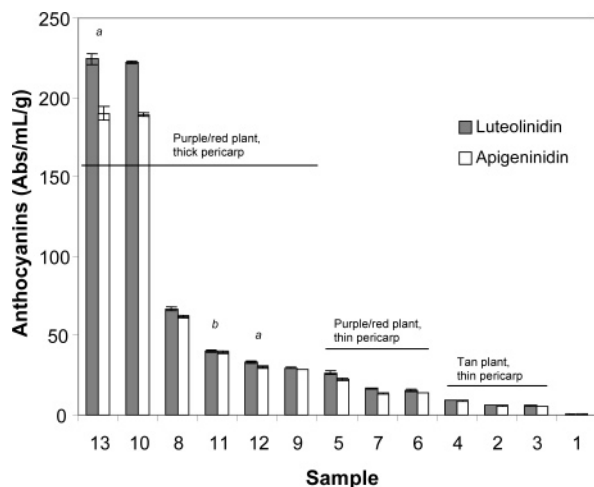

Figure 4. Luteolinidin and apigeninidin levels of sorghum grains with red pericarp. Superscripts *a* and *b* represent varieties with B_1B_2SS and B_1B_2ss genes, respectively.

Table 4. Pearson's Correlation Coefficients among Sorghum Phenols and CIE *L**, *a**, and *b**[a]

	PHE	FL-4-OL	LUT	APIG
L*	−0.69 b	−0.84 a	−0.61 c	−0.62 c
a*	−0.15	−0.27	−0.59 c	−0.58 c
b*	−0.72 b	−0.90 a	−0.85 a	−0.85 a

[a] PHE = total phenols; FL-4-OL = flavan-4-ol; LUT = luteolinidin; and APIG = apigeninidin. Letters a, b, and c indicate $p < 0.001$, $p < 0.01$, and $p < 0.05$, respectively.

the levels of flavan-4-ols. A weak correlation between the *L** value and anthocyanin content was observed ($r = -0.61$, $p < 0.05$). This is in contrast to Gous (*23*), who observed a much stronger correlation between the *L** value and anthocyanin content ($r = -0.82$). The *b** value also correlated with flavan-4-ol ($r = -0.90$, $p < 0.001$) and anthocyanin ($r = -0.85$, $p < 0.001$) contents as found by Gous (*23*). No significant correlations were found between the *a** value and both total phenols and flavan-4-ols.

Evaluation of Antioxidant Activity in Sorghums. Sorghums with dominant B_1B_2 genes had the highest antioxidant activity, especially the varieties with the dominant *S* gene (**Figure 5**). The antioxidant activity came mainly from condensed tannins, which have demonstrated higher antioxidant activity in vitro than other phenolic compounds (*6, 27*).

Plant color and pericarp thickness affect antioxidant activity. Sorghums from purple/red plants had a higher antioxidant activity than those from tan plants. From the purple/red plant category, sorghum grains with a thick pericarp had a higher antioxidant activity than those with a thin pericarp. This confirms that the antioxidant activity comes mainly from the pericarp,
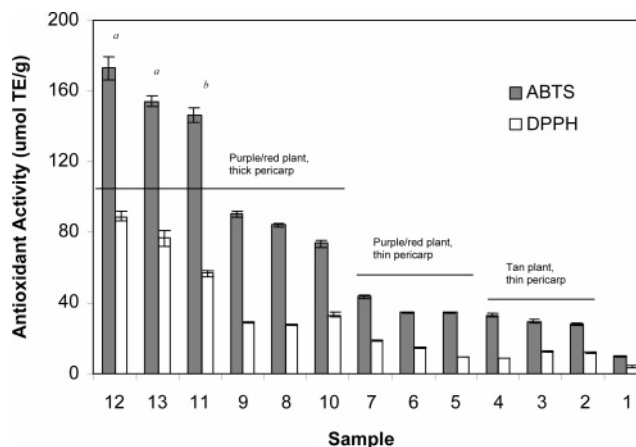

Figure 5. ABTS and DPPH values of sorghum grains with red pericarp (TE, Trolox equivalents). Superscripts *a* and *b* represent varieties with B_1B_2SS and B_1B_2ss genes, respectively.

which is rich in phenols (*26*). A strong correlation between total phenols and antioxidant activity was observed (total phenols vs ABTS, $r = 0.99$; total phenols vs DPPH, $r = 0.98$) indicating an association between pericarp thickness and antioxidant activity. Thick pericarp sorghum grains contain starch granules in the mesocarp (*28*) and are more susceptible to molds and weathering (*15*) causing the production of phytoalexins such as 3-deoxyanthocyanins (*29, 30*). This study shows that sorghum grains with dominant *PQ* (purple/red plant) and recessive *z* (thick pericarp) genes increase antioxidant activity. To our knowledge, this is the first time that this type of information is reported, which is important for the selection of sorghums used for functional foods.

The strong correlations between total phenols and antioxidant activity could be due to samples 11−13, which contain condensed tannins and therefore increase the correlations. However, when samples 11−13 were removed from the set, strong correlations were still observed (total phenols vs ABTS, $r = 0.97$; total phenols vs DPPH, $r = 0.97$) (**Table 3**), which indicate other phenolic compounds such as flavan-4-ols or anthocyanins are contributing to the antioxidant activity in sorghums. These findings suggest that total phenol content is a good predictor of in vitro antioxidant activity. Among all sorghum varieties, a weak correlation was found between antioxidant activity and flavan-4-ols while no correlation was found between anthocyanins and antioxidant activity (**Table 3**). However, when determining the correlations without samples 11−13, the correlation between antioxidant activity and flavan-4-ols increased. A weak correlation was found between anthocyanins and antioxidant activity among the nontannin sorghums.

Among the nontannin sorghum samples, samples 8−10 had the highest antioxidant activity by the ABTS and the DPPH assays (**Figure 5**). Interestingly, samples 8 and 9 had a higher

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antioxidant activity than sample 10 despite the fact that the grains of sample 8 and 9 were bright red while those of sample 10 were black. These results suggest that the intensity of redness of nontannin sorghum grains cannot predict their antioxidant activity potential. Samples 8 and 9 may contain compounds that increase the antioxidant activity, and further analysis is needed.

DPPH values were lower than the ABTS values for all samples. Pigments, such as anthocyanins, cause interference leading to underestimation of antioxidant activity when using the DPPH assay (*7*, *31*). However, there is a strong correlation between ABTS and DPPH ($r = 0.97$, $p < 0.001$) (**Table 3**), which was also observed in previous studies (*7*, *32*).

This study shows that genetics affect phenol content and antioxidant activity in sorghums. The antioxidant activity potential increases in sorghums with the pigmented testa gene ($B_1\_B_2\_$) due to the presence of tannins.

Significant confusion exists regarding tannin sorghums. It is erroneously believed that tannin sorghums are toxic to humans and animals. Tannin sorghums have been utilized for centuries as human foods (i.e., breads, porridges, alcoholic beverages) in Africa and Asia and, in many cases, are preferred for certain products (*2*, *3*). Tannin sorghums are used as commercial livestock feeds, but animals fed tannin sorghums have significantly reduced feed efficiency and slightly lower weight gains as compared to animals fed nontannin sorghums (*2*−*4*). The decrease of feed efficiency depends on the species, methods of feeding, and other variables (*4*). The reduction of protein digestibility by tannin sorghums may not be entirely due to tannins. Elkin et al. (*33*) reported that tannin sorghums containing equivalent amount of tannins have different digestibilities. Tannin sorghums are not toxic to birds and animals, and they are not literally "bird resistant" or "bird proof." Birds prefer foods other than tannin sorghums, but they eat the bird resistant sorghums if they do not have an alternative food supply and they thrive on it (*4*).

Breads containing tannin sorghum bran with a natural dark brown color, whole grain taste, increased dietary fiber, and high antioxidant levels are comparable to commercial specialty breads (*34*, *35*). High tannin sorghum bran has excellent potential as an antioxidant in precooked and stored meat patties (*36*), and it can be used to delay oxidative damage due to high energy irradiation (*37*). Thus, tannin sorghums can and should be considered as a source of natural color, antioxidants, and dietary fiber.

In summary, the levels of phenols and antioxidant activity are highest when sorghums have secondary purple/red plant color; a black or dark red, thick pericarp; and a pigmented testa with a spreader gene (*S*). These findings provide useful guidelines to produce sorghums with the greatest antioxidant levels, which are potentially quite important sources of healthy component in foods.

ABBREVIATIONS USED

ABTS, 2,2′-azinobis(3-ethylbenzothiazoline-6-sulfonic acid); DPPH, 2,2-diphenyl-1-picrylhydrazyl; GAE, gallic acid equivalents; CE, catechins equivalents; TE, trolox equivalents.

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