FLAVONOID COMPOSITION AND ANTIOXIDANT ACTIVITY OF PIGMENTED SORGHUMS OF VARYING GENOTYPES

A Dissertation

by

LINDA DYKES

Submitted to the Office of Graduate Studies of
Texas A&M University
in partial fulfillment of the requirements for the degree of

DOCTOR OF PHILOSOPHY

May 2008

Major Subject: Food Science and Technology

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Approved by:

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ABSTRACT

Flavonoid Composition and Antioxidant Activity of Pigmented Sorghums of Varying Genotypes. (May 2008)

Linda Dykes, B.S., University of Mary Hardin-Baylor

Chair of Advisory Committee: Dr. Lloyd W. Rooney

A wide variety of sorghum genotypes with a pigmented pericarp were grown in varying environments and were analyzed for total phenols, condensed tannins, flavan-4-ols, and *in vitro* antioxidant activity. In addition, sorghum flavonoids were separated, characterized, and quantified using HPLC-PDA and LC-MS. Total phenols and *in vitro* antioxidant activity increased when sorghums had a pigmented testa causing the presence of condensed tannins. Flavan-4-ol levels were highest in sorghums with a black pericarp (5.8-16.1 abs/mL/g), followed by those with a red pericarp (1.1-9.2 abs/mL/g).

Sorghums with a black pericarp had the highest 3-deoxyanthocyanin levels (173-1054 μg/g) and these were increased when the grain had minimal weathering and was darkest in color. Sorghums with a lemon-yellow pericarp had the highest flavanone levels (134-1780 μg/g) with eriodictyol being the main flavanone. Flavanone levels were increased when the grain was bright yellow with minimum weathering and were high compared to those found in common sources (106-574 μg/g, fresh wts.). No flavonoids were predominant in

sorghums with a red pericarp. Flavonoid composition varied when all sorghums were grouped by secondary plant color. Sorghums with tan secondary plant color, including those with a white pericarp, had higher levels of flavones (19-386 μg/g) than those with red/purple secondary plant color (0-57 μg/g). On the other hand, 3-deoxyanthocyanin levels were higher in red/purple plant sorghums (8-1054 μg/g) than in tan plant sorghums (0-12 μg/g). Among red/purple plant sorghums, lemon-yellow pericarp sorghums had the highest levels of flavones (20-67 μg/g). Environment and weathering had an effect on flavonoid levels. The 3-deoxyanthocyanins were reduced for sorghums grown in a dry environment (i.e. Lubbock, TX) and flavonoid levels were increased in grains with minimum weathering or molding.

This study reports that all sorghums, including those with a white pericarp, have flavonoids and their levels and compositions are affected by the genotype. This information will be helpful for plant breeders, food scientists, and the pharmaceutical/nutraceutical industries in selecting sorghums with desired healthy components.

ACKNOWLEDGMENTS

I would like to thank my committee chair, Dr. Lloyd Rooney, for giving me this opportunity and for his guidance and support. Special thanks go to Dr. Ralph Waniska (deceased) for his help and guidance throughout the course of this research. Many thanks to Dr. Rosemary Walzem and Dr. Luis Cisneros-Zevallos for serving on my committee and for giving me their advice. I also would like to thank Dr. William Rooney for serving on my committee and for helping me understand sorghum genetics.

Special thanks to Dr. Gary Peterson from the Texas Agricultural Experiment Station for providing the samples from Lubbock. I would also like to thank Dr. Larry Seitz (USDA-ARS-GMPRC) for his help and advice on the 3-deoxyanthocyanin analysis. Many thanks to Dr. Steve Talcott for giving me access to his LC-MS. Also, many thanks to various student workers who have helped with the sample preparation and the members of CQL lab for their support.

I also would like to thank my husband, James, for all his patience, encouragement, and support throughout the course of my graduate study.

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CHAPTER I

INTRODUCTION

Sorghum is the fifth leading cereal crop in the world and it is used primarily in Asia and Africa as a food crop (Gous 1989; Anglani 1998). The Western Hemisphere uses sorghum mainly for feed. It is grown in arid and semi-arid regions and is one of the most drought-resistant cereal crops (Elkin et al 1996). All sorghums contain phenols, which affect the color, appearance and nutritional quality of the grain (Hahn et al 1984). The phenolic compounds in sorghum are classified into three classes: phenolic acids, flavonoids, and condensed tannins.

Black sorghums contain a significant amount of the rare 3-deoxyanthocyanins (Awika et al 2004a, b); the lack of the hydroxyl group at the C-3 position renders them more stable than the common anthocyanins (Sweeney et al. 1983). Due to increasing concerns over the use of synthetic colorants in foods/beverages, sorghum 3-deoxyanthocyanins could have great potential as natural food colorants. In addition, these compounds have antioxidant activity *in vitro* (Awika et al 2003b; Shih et al 2007).

Consumers have an increased interest in antioxidants found in plants because of the chemoprotection they are thought to provide. Sorghum contains

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This dissertation follows the style of Cereal Chemistry.

phenolic compounds in the bran layer that protect plants against insects and diseases (Hahn and Rooney 1986; Awika et al 2005). These non-nutrient compounds also act as antioxidants *in vitro* (Hagerman et al 1998; Awika et al 2003b) and some are more potent than vitamins found in plants (Rhodes and Price 1997). For example, caffeic acid, a phenolic acid found in sorghum (Hahn 1984), has a higher antioxidant activity than vitamin C, which protects against degenerative diseases in which reactive oxygen species (i.e. superoxide anion, hydroxyl radicals, and peroxy radicals) are involved (Rhodes and Price 1997, Harborne and Williams 2000). Free radicals play a role in diseases such as cancer, atherosclerosis, rheumatoid arthritis, inflammatory bowel disease and cataracts (Parr and Bolwell 2000). Antioxidants may decrease the risk of these diseases by lowering the amount of free radicals. Other roles of antioxidants include antifungal, antibacterial, and antiviral agents (Harborne and Williams 2000).

Since 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. Phenolic acids were reported in sorghums of varying genotypes (Hahn et al 1983). However, studies on sorghum flavonoids have been limited to identification and characterization (Nip and Burns 1969, 1971; Kambal and Bate-Smith 1976; Gujer et al 1986; Pale et al 1997; Wu and Prior 2005); information on the quantification of these compounds is limited. Awika et al (2004a, b) reported

data on luteolinidin and apigeninidin for a limited number of sorghum brans; several 3-deoxyanthocyanins were quantified but not identified. Using HPLC-PDA and LC-MS, Seitz (2004) identified and quantified six 3-deoxyanthocyanins in nine sorghum samples with a white or red pericarp with different secondary plant colors. The two flavones, luteolin and apigenin, were also quantified, but data on luteolin was unavailable for sorghums with purple secondary plant color due to interference with other compounds (Seitz 2004).

Phenols in sorghums are affected by their genotypes. For example, sorghums with $B_1\_B_2$ genes for the presence of a pigmented testa increase phenol levels since these genotypes contain condensed tannins. Pericarp color may be a reliable indicator for the type and levels of flavonoids present. For example, a black sorghum contains higher levels of 3-deoxyanthocyanins than a sorghum with a red pericarp (Awika et al 2004a, b). Sorghums come in a wide variety of pericarp colors to include white, lemon-yellow, red, brown, and black. Seitz (2004) reported that secondary plant color may affect the type of flavonoids present. Sorghums with tan and purple secondary plant color had high levels of flavones and 3-deoxyanthocyanins, respectively. However, in sorghums with purple secondary plant color, flavone levels were underestimated since only apigenin was quantified.

Flavanones were identified in a previous study but description of the sorghum sample was lacking (Gujer et al 1986). There is a necessity to determine whether the genes that control pericarp color and secondary plant

color affect flavonoid composition. Since environment also has an effect on phenol levels (Chaves et al 1997; Connor et al 2002; Lee et al 2005; Downey et al 2006), there is also a necessity to determine which environments are best to produce sorghums with maximum levels of the desired compounds. This information will help plant breeders, food scientists, and the pharmaceutical/cosmoceutical industries select cultivars with maximum levels of these compounds for the production of functional foods and for pharmarceutical/cosmoceutical applications.

The objectives of this research were:

- To evaluate total phenols, condensed tannins, flavan-4-ols, anthocyanins, and *in vitro* antioxidant activity levels in sorghum genotypes;
- To characterize and quantify flavonoids using HPLC-PDA and LC-MS and to compare flavonoid composition in sorghum genotypes;
- To determine which environment maximizes levels of desired flavonoids and to determine whether environment changes flavonoid composition.

CHAPTER II

LITERATURE REVIEW*

Overview of Sorghum Genetics and Kernel Structure Relevant to Tannins and Phenols

Sorghum genetics relevant to tannins and phenols were reviewed by Rooney and Miller (1982). The pericarp color of the sorghum kernel is controlled by the *R* and *Y* genes. A pericarp is white when *Y* is homozygous recessive (*rryy* or *R_yy*), whereas a yellow pericarp has recessive *R* and dominant *Y* genes (*rrY_*). When both *R* and *Y* genes are dominant, the pericarp is red. Black pericarp sorghums are also genetically red; their pericarp turns black when the grain is exposed to sunlight. The intensifier gene *I* affects the intensity of the pericarp color especially in red sorghums. Pericarp thickness is controlled by the *Z* gene. A pericarp is thick when the genes are homozygous recessive (*zz*) and thin when it is dominant (*ZZ*). Sorghums with a thick pericarp have starch granules in the mesocarp; sorghums with a thin pericarp do not contain starch granules in that area (Earp and Rooney 1982; Earp et al. 2004b). Secondary plant color is controlled by the *P* and *Q* genes. Plants with *PPqq* and *PPQQ* genes produce red- and purple-pigmented plants, respectively, while the *ppqq*

*Portions of this chapter were reprinted with permission from "Sorghum and millet phenols and antioxidant" by Dykes, L. and Rooney, L.W., 2006, J. Cereal Sci., Vol. 46, Pages 236-251, Copyright (2006) by Elsevier. DOI: 10.1016/j.jcs.2006.06.007.

and *ppQQ* genes produce tan-pigmented plants. All of the aforementioned genes affect phenol content.

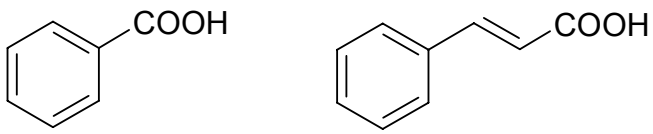
Pericarp color is not a reliable indicator of tannins in sorghums. Boren and Waniska (1992) investigated tannin content in a wide variety of sorghums varying in pericarp color. They showed that pericarp color and its intensity was not a good indicator of tannin content. It is erroneously believed that all sorghums with a red/brown pericarp contain tannins. Sorghums with white, yellow, red, or brown color pericarp may or may not have tannins depending upon the presence of a pigmented testa, which is controlled by B_1 and B_2 genes. Sorghums with a pigmented testa require dominant genes ($B_1\_B_2\_$). The spreader gene *S* controls the presence of brown pigments, possibly tannins in the epicarp and endocarp when a pigmented testa is present. (Blakely et al 1979). Testa color is controlled by the *Tp* gene. A testa is purple when *Tp* is homozygous recessive (*tptp*) and brown when it is dominant (*Tp_*). The levels of condensed tannins are highest in sorghums containing dominant $B_1\_B_2\_SS$ genes; these sorghums have high bird and mold resistance.

Sorghum varieties are divided into three groups based upon their genetics and chemical analyses (Rooney and Miller 1982). Type I sorghums ($b_1b_1B_2\_$, $B_1\_b_2b_2$, $b_1b_1b_2b_2$) do not have a pigmented testa, and contain low levels of phenols and no tannins. Types II and III both have a pigmented testa and contain tannins. The tannins in Type II sorghums ($B_1\_B_2\_ss$) are extracted with acidified methanol (1% HCl methanol) while the tannins in Type III

sorghums (*B1_B2_S_*) are extracted with either methanol or acidified methanol when using the vanillin/HCl assay. Earp et al (2004a) showed that the tannins in the Type II sorghums are deposited differently in the testa layer. In the grain of Type II sorghums, tannins are deposited in vesicles within the testa layer, whereas the tannins in type III are deposited along the cell walls of the testa and some are present in the pericarp. This may explain why acid is required to disrupt the structure of the vesicles, which releases tannins in Type II sorghums (Earp et al 2004a).

Sorghum Phenolic Acids

All sorghums contain phenolic acids, which are located in the pericarp, testa, aleurone layer, and endosperm (Hahn et al 1984). Phenolic acids consist of two classes: hydroxybenzoic and hydroxycinnamic acids (Fig. 1).



Benzoic Acid Cinnamic Acid

Fig. 1. Basic structure of phenolic acids.

Hydroxybenzoic acids are directly derived from benzoic acid and include gallic, *p*-hydroxybenzoic, vanillic, syringic, and protocatechuic acids, among others.

The hydroxycinnamic acids have a C_6-C_3 structure and include coumaric, caffeic, ferulic, and sinapic acids. The phenolic acids reported in sorghum are listed in Table 1.

TABLE 1

Phenolic Acids Detected in Sorghum

Phenolic Acid	References
Hydroxybenzoic Acids	
Gallic	Hahn et al (1983)
Protocatechuic	Hahn et al (1983)
p-Hydroxybenzoic	Hahn et al (1983)
Gentisic	Waniska et al (1989)
Salicylic	Waniska et al (1989)
Vanillic	Hahn et al (1983)
Syringic	Waniska et al (1989)
Hydroxycinnamic Acids:	
Ferulic	Hahn et al (1983)
Caffeic	Hahn et al (1983)
p-Coumaric	Hahn et al (1983)
Cinnamic	Hahn et al (1989)
Sinapic	Waniska et al (1989)

Hahn et al (1983) identified free and bound phenolic acids in sorghum (Table 2). Free and bound phenolic acids are extracted in methanol and in boiling 2*N* HCl respectively. Free phenolic acids are found in the outer layers of the kernel (pericarp, testa, and aleurone), whereas the bound phenolic acids are associated with the cell walls (Hahn et al 1984). According to Hahn et al (1983), the phenolic acids in sorghum are present mostly in bound form with ferulic acid

TABLE 2

Free and Bound Phenolic Acid Composition of Some Sorghum Varieties[a]

	Sorghum Varieties							
	White (CS3541)		Lemon Yellow (SC0748)		Red (SC0630)		Brown (SC0719)	
Phenolic Acid	**Free**	**Bound**	**Free**	**Bound**	**Free**	**Bound**	**Free**	**Bound**
Gallic	--[b]	19.7	ND[c]	13.2	--	46.0	--	26.1
Protocatechuic	7.4	133.9	11.0	11.5	13.0	83.0	8.0	15.8
p-Hydroxybenzoic	4.0	11.4	10.1	23.7	6.7	16.0	9.3	24.2
Vanillic	8.3	ND	15.5	--	7.7	19.2	23.3	27.4
Caffeic	3.4	22.2	6.0	44.6	4.1	48.0	8.7	26.8
p-Coumaric	45.7	138.5	109.1	123.0	13.5	72.5	6.4	79.9
Ferulic	45.4	297.2	74.0	213.0	8.9	95.7	26.0	91.9
Cinnamic	9.4	--	4.7	--	10.7	--	--	19.7

Ref.: Data from Hahn et al (1983).
[a]Values are expressed as µg/g, dry weight basis.
[b]Value not available.
[c]ND = Not detected.

being dominant (24-47%). In addition, gallic acid is found only in bound form (12.9-46.0 µg/g, dry wt), whereas cinnamic acid is found only in free form (2.0-10.7 µg/g, dry wt.) with the exception of one variety (SC0719, red pericarp with pigmented testa), which is also reported to contain that compound in bound form only (19.7 µg/g, dry wt.) (Hahn et al 1983). The other phenolic acids are present in free (54.1-230.4 µg/g, dry wt.) and bound (276.7-622.9 µg/g, dry wt.) forms.

Sorghum Flavonoids

Flavonoids have the C_6-C_3-C_6 skeleton and these include anthocyanins, flavones, flavonols, flavanones, and flavanols (Fig. 2). The biosynthetic pathways of the major sorghum flavonoids are illustrated in Fig. 3.
Many sorghum flavonoids have been isolated and identified over the years (Table 3). The major class of flavonoids studied in sorghum are the anthocyanins. In general, this class of compounds contributes the blues, purples, and reds in plants. The six common anthocyanidins are cyanidin, delphinidin, malvidin, pelargonidin, petunidin, and peonidin (Fig. 4). Unlike these common anthocyanins, sorghum anthocyanins are unique since they do not contain the hydroxyl group in the 3-position of the C-ring (Fig. 4) and thus are called 3-deoxyanthocyanins. This unique feature increases their stability at high pH compared to the common anthocyanins (Sweeney and Iacobucci 1983; Mazza and Brouillard 1987; Gous 1989; Awika et al 2004a, b), which confer to these compounds the potential to serve as natural food colorants. They are also

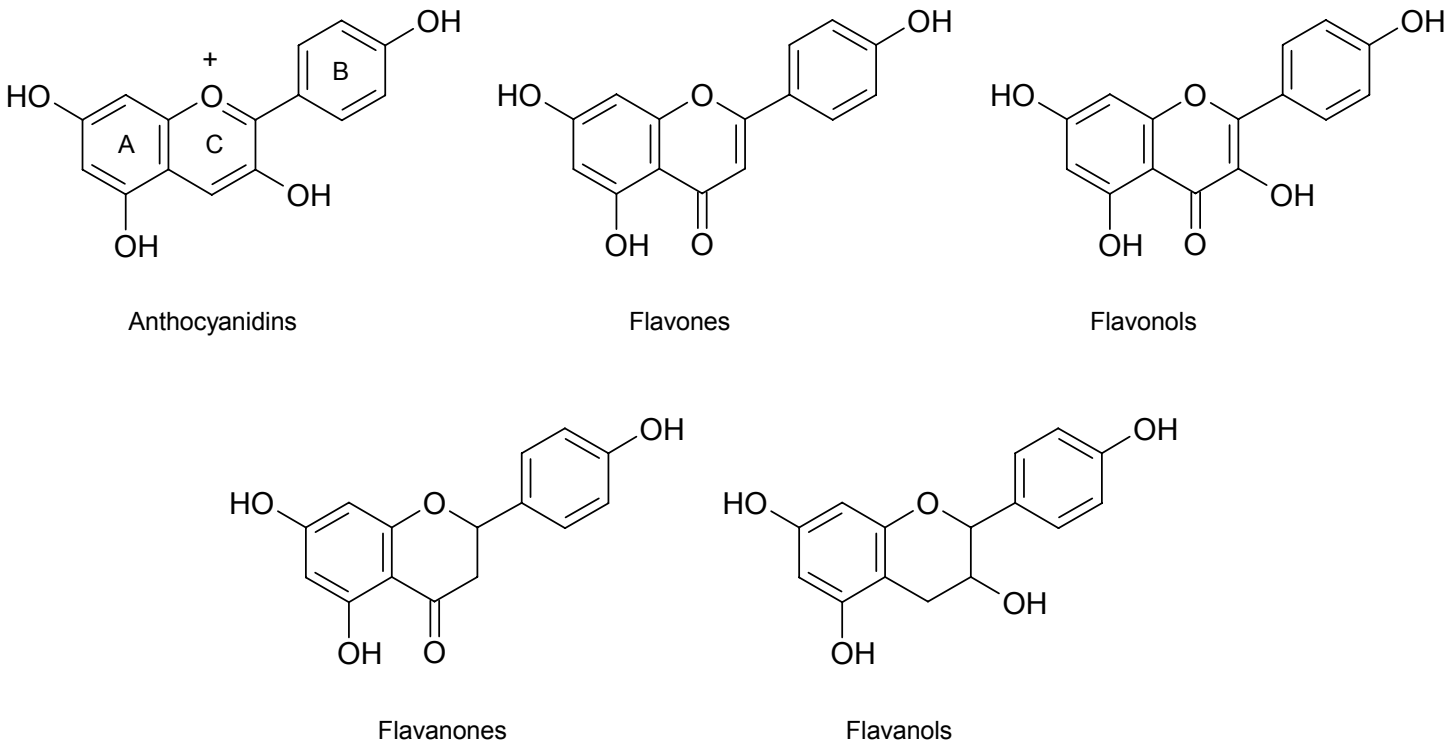


Fig. 2. Chemical structures of flavonoids.

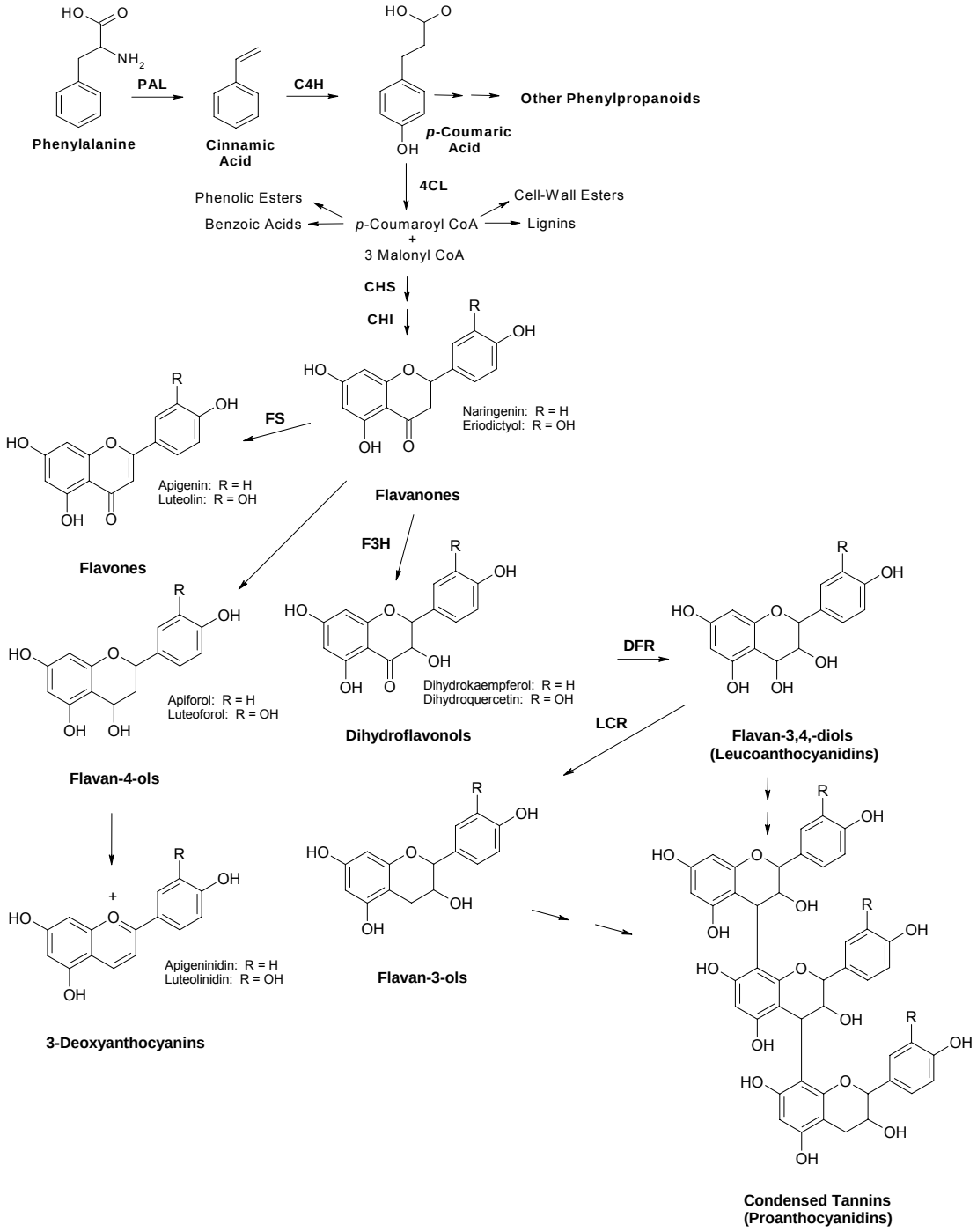


Fig. 3. The biosynthetic pathways for the major sorghum flavonoids. C4H = Cinnamic acid 4-hydroxylase; 4CL = 4-Hydroxycinnamic acid:CoA ligase; CHS = Chalcone synthase; CHI = Chalcone isomerase; FS = Flavone synthase; DFR = Dihydroflavonol 4-reductase; LCR = Leucoanthocyanidin reductase. *Refs.* Lo et al (1998); Winkel-Shirley (2001).

TABLE 3

Flavonoids Reported in Sorghum

Compound	References
Anthocyanins:	
Apigeninidin	Nip and Burns (1971); Gous (1989)
Apigeninidin 5-glucoside	Nip and Burns (1969, 1971); Wu and Prior (2005)
Luteolinidin	Nip and Burns (1971); Gous (1989)
5-Methoxyluteolinidin	Seitz (2004); Wu and Prior (2005)
5-Methoxyluteolinidin 7-glucoside	Wu and Prior (2005)
7-Methoxyapigeninidin	Pale et al. (1997); Seitz (2004); Wu and Prior (2005)
7-Methoxyapigeninidin 5-glucoside	Wu and Prior (2005)
Luteolinidin 5-glucoside	Nip and Burns (1971); Wu and Prior (2005)
5-Methoxyapigeninidin	Seitz (2004)
7-Methoxyluteolinidin	Seitz (2004)
Flavan-4-ols:	
Luteoforol	Bate-Smith (1969)
Apiforol	Watterson and Butler (1983)
Flavones:	
Apigenin	Gujer et al. (1986); Seitz (2004)
Luteolin	Seitz (2004)
Flavanones:	
Eriodictyol	Yasumatsu et al 1965; Kambal and Bate-Smith (1976)
Eriodictyol 5-glucoside	Gujer et al. (1986)
Naringenin	Gujer et al. (1986)
Flavonols:	
Kaempferol 3-rutinoside-7-glucuronide	Nip and Burns (1969)
Dihydroflavonols:	
Taxifolin	Gujer et al. (1986)
Taxifolin 7-glucoside	Gujer et al. (1986)

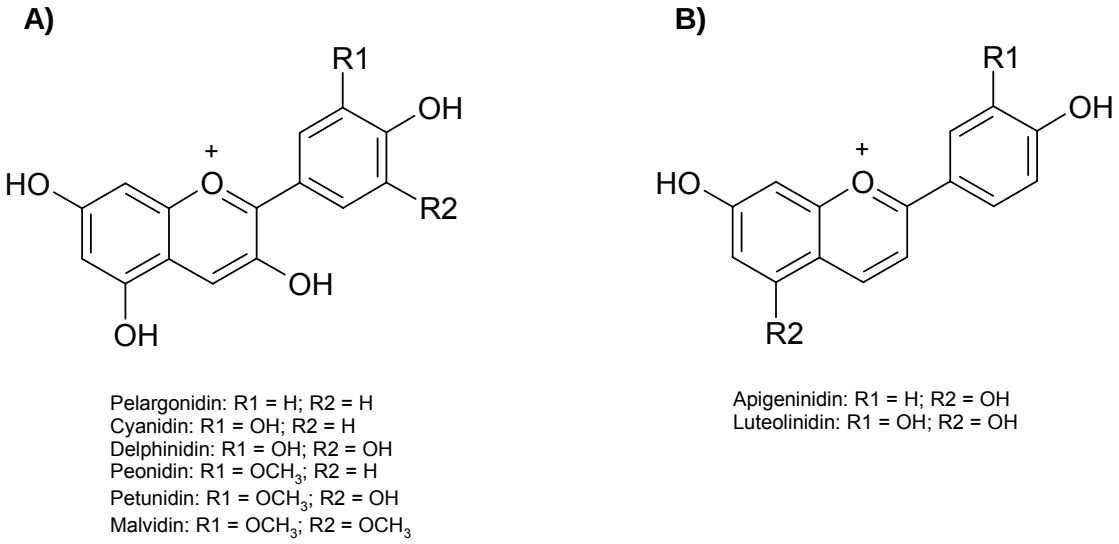


Fig. 4. Chemical structure of A) the six common anthocyanins and B) the 3-deoxyanthocyanins.

phytoalexins since they are produced as a response to mold invasion or other stresses in sorghum (Lo et al 1999; Waniska and Rooney 2000; Seitz 2004). Seitz (2004) reported these compounds were more prevalent in purple plant than in tan plant sorghum hybrids. The two common sorghum 3-deoxyanthocyanidins are the yellow apigeninidin and the orange luteolinidin (Nip and Burns 1971; Gous 1989; Awika et al 2004a, b; Wu and Prior 2005). Other 3-deoxyanthocyanins identified in sorghum grains include apigeninidin 5-glucoside (Nip and Burns 1969, 1971; Wu and Prior 2005), luteolinidin 5-glucoside (Nip and Burns 1971; Wu and Prior 2005), 5-methoxyluteolinidin (Seitz 2004; Wu and Prior 2005), 5-methoxyluteolinidin 7-glucoside (Wu and Prior 2005), 7-methoxyapigeninidin (Pale et al 1997; Seitz 2004; Wu and Prior 2005), 7-methoxyapigeninidin 5-glucoside (Wu and Prior 2005), 5-methoxyapigeninidin (Seitz 2004), and 7-methoxyluteolinidin (Seitz 2004).

Sorghums with a black pericarp have the highest levels of 3-deoxyanthocyanins (Gous 1989; Awika et al 2004a, b), which are concentrated in the bran (Awika et al 2005). Using the pH differential method of Fuleki and Francis (1968), Awika et al (2004b) reported the anthocyanin content of a black sorghum bran was 3-4 times higher than the whole grain and had at least twice the levels of anthocyanins (10.1 mg/g) compared to red (3.6 mg/g) and brown (3.6 mg/g) sorghum brans. In addition, Awika et al (2004a, b) reported luteolinidin and apigeninidin represented 36-50% of the total anthocyanin content in black (Tx430 Black) and brown (Hi Tannin) sorghum brans using

HPLC (Table 4). They also reported that apigeninidin represented 19% of the total anthocyanin in a red sorghum (Tx2911) and detected trace amounts of luteolinidin. These data suggest that black sorghum bran is a major source of 3-deoxyanthocyanins for potential use in natural food colorants.

TABLE 4

3-Deoxyanthocyanin levels[a] in Pigmented Sorghum Brans Measured by HPLC

	Luteolinidin	Apigeninidin	Total[b]
Black 1999	1.8	0.4	6.1
Black 2001	1.3	1.4	5.4
Black 2002	1.5	1.3	6.0
Red	tr[c]	0.3	1.6
SC103 (brown)	0.3	0.4	1.6
Sumac (brown)	0.7	0.6	2.8
CSC3*R28 (brown)	0.4	0.6	2.0
CV%[d]	1.4	1.4	1.4

[a]Values are expressed as mg/g luteolinidin equivalents (dry basis).
[b]Includes unidentified anthocyanin peaks.
[c]tr = trace.
[d]Coefficient of variation (percent relative standard deviation of each sample within column).
Ref: Data from Awika et al. (2004b).

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 sorghum anthocyanidins (Wharton and Nicholson 2000). Flavan-4-ols may play an important role in mold resistance, as several studies report a correlation between flavan-4-ols concentration and mold resistance in sorghums (Jambunathan et al 1990, 1991; Melake-Berhan et al

1996; Menkir et al 1996; Audilakshmi et al 1999). However, the concentration of flavan-4-ols has been ineffective in selecting sorghums that have resistance to molds.

Flavan-4-ol levels vary among sorghum genotypes. Gous (1989) reported that black pericarp sorghums have the highest levels of flavan-4-ols (11.8-13.6 abs/mL/g) compared to red pericarp sorghums (8.7-9.0 abs/mL/g). Dicko et al (2005) reported that red-plant sorghums with a red pericarp and pigmented glumes generally have higher levels of flavan-4-ols (0.20-0.42%, w/w, cyanidin, dry wt.) than the other varieties studied.

Other flavonoids isolated and identified in sorghum grains include the flavones, apigenin and luteolin, which are predominant in tan plant sorghum hybrids (Seitz 2004). The flavanones eriodictyol (Yasumatsu et al 1965; Kambal and Bate-Smith 1976) and eriodictyol 5-glucoside (Gujer et al 1986) have been reported. Naringenin was also reported (Gujer et al 1986) and was found as a major peak in the red sorghum Tx2911 (Awika 2003). The flavonol kaempferol 3-rutinoside-7-glucuronide (Nip and Burns 1969) and the dihydroflavonols taxifolin (Gujer et al 1986), and taxifolin 7-glucoside (Gujer et al 1986) have also been isolated.

Methods of Analysis of Sorghum Phenols and Antioxidant Activity

Many methods have been used to measure phenolic compounds and these have been reviewed in several papers (Shahidi and Naczk 1995; Hagerman et al 1997; Rohr et al 2000; Schofield et al 2001; Naczk and Shahidi

2004). Only major methods used in the analysis of sorghum phenols are discussed.

***Conventional Methods*.** Colorimetric methods give an estimate of phenol content and are rapid and economical to perform compared to other methods requiring expensive equipment. Total phenol content has been measured using the Folin-Ciocalteu assay (Singleton and Rossi 1965; Kaluza et al 1980) or the Prussian Blue assay (Price and Butler 1977). These methods are based on oxidation-reduction reactions, are not specific to a class of phenols, and suffer from interference by the amino acid tyrosine (Ring 1984; Hahn and Rooney 1986) and non-phenolics such as ascorbic acid (Hagerman et al 1997). The Folin-Denis assay and the ferric ammonium citrate of the International Organization for Standardization (ISO) have been used to measure total phenols and tannins in sorghum using tannic acid as the standard (Bate-Smith and Rasper 1969; Maxson and Rooney 1972; Beta et al 1999). Values were given in tannic acid equivalents, which led some scientists to erroneously conclude that tannin sorghums contain tannic acid/hydrolysable tannins.

Condensed tannins are measured using the vanillin/HCl or the butanol/HCl assays. The modified vanillin/HCl method of Price et al (1978) involves the condensation of the aromatic aldehyde vanillin (4-hydroxy-3-methoxy benzaldehyde) with monomeric flavanols and their oligomers to form a red adduct that absorbs at 500 nm. Type I sorghums give low tannin values due to the interference of other non-tannin phenolics (Table 5) (Waniska and Rooney

Table 5

Tannin Levels of Type I, II, and III Sorghums

	Tannins (Vanillin/HCl)[a]	References
Type I ($b_1b_1B_2\_$; $B_1\_b_2b_2$):		
02CA4796	0.1[b]	Dykes et al. (2005)
77CS2	1.8[b]	Boren and Waniska (1992)
BTx3197	0.5[b]	Boren and Waniska (1992)
BTx378	1.4[b]	Boren and Waniska (1992)
Combine Shallu	0.6[b]	Boren and Waniska (1992)
CS3541 x SC630-11E	0.7[b]	Boren and Waniska (1992)
TX2911	0.3[b]	Dykes et al. (2005)
TX430 Black	0.0	Dykes et al. (2005)
Type II ($B_1\_B_2\_ss$):		
(Combine Shallu x Hegari)OP5	15.5	Boren and Waniska (1992)
Feterita SA664-7-2	10.5	Boren and Waniska (1992)
Hegari	6.6	Boren and Waniska (1992)
(Hegari x Dobbs)OP5	8.9	Boren and Waniska (1992)
IRAT202	13.0	Boren and Waniska (1992)
Koro Kolla	12.8	Boren and Waniska (1992)
TAM-2566	11.4	Boren and Waniska (1992)
SC109-14E	6.4	Boren and Waniska (1992)
SC719-14E	10.9	Dykes et al. (2005)
Type III ($B_1\_B_2\_SS$):		
Dobbs	20.1	Boren and Waniska (1992)
SC103-12E	11.2	Boren and Waniska (1992)
SC103 x SC748 (dark)	15.5	Dykes et al. (2005)
SC156-14E	36.7	Boren and Waniska (1992)
SC574-14E	18.3	Boren and Waniska (1992)
PI Black Tall	11.0	Dykes et al. (2005)
Hi Tannin	35.3	Awika (2003)
Sumac	50.2	Awika (2003)
NK121	16.6	Unpublished data
NK121A	26.0	Unpublished data
NK180	18.2	Unpublished data
NK8810	47.7	Unpublished data
NK8830	56.3	Unpublished data
XM217	35.1	Unpublished data

[a]Values are expressed as mg catechin equivalents/g.
[b]Value is below detection limit.

2000). Sorghums that do not have a pigmented testa contain non-tannin phenolics that react with the reagents and give some “tannin values” that are not really tannins (Earp et al 1981; Hahn and Rooney 1986). These values are widely reported as tannins in the literature and give rise to the myth that all sorghums have tannins (Rooney 2005). Thus, significant confusion exists. The vanillin/HCl assay does not measure tannin content accurately. The lack of an appropriate standard for condensed tannins is a major problem due to the heterogeneity of these compounds (Schofield et al 2001). Attempts to prepare a pure tannin standard from sorghum or other tannin-containing material (i.e. quebracho) are difficult and time-consuming (Hagerman and Butler 1980; Butler 1989). The standard mostly used for the vanillin/HCl assay is catechin but it gives values that are unrealistically high (Rohr et al 2000; Schofield et al. 2001). In sorghum, the tannins reside mainly in the pigmented testa, which is only a portion of the outer covering that comprises approximately 5-6% (dry weight) of the kernel. Thus, catechin equivalent values of 5-6% (Table 5) are much too high. Therefore, tannin values from this assay are only relative indices of tannin content among samples. The butanol/HCl assay also measures tannin content and it involves the depolymerization of condensed tannins in boiling acidic butanol to yield anthocyanidins (Porter et al. 1986).

Flavan-4-ols are measured by a modification of the butanol/HCl assay as described by Butler (1982) or by Gous (1989). Total anthocyanin content is determined using the pH differential method of Fuleki and Francis (1968).

In vitro antioxidant activity in sorghums had been measured using the 2,2'-azinobis(3-ethyl-benzothiazoline-6-sulfonic acid (ABTS), 2,2-diphenyl-1-picrylhydrazyl (DPPH), and oxygen radical absorbance capacity (ORAC) methods (Awika et al 2003b, 2004a, b; Kamath et al 2004; Dicko et al 2005). A summary of each method is given in Table 6. These methods are useful at assessing antioxidant activity potential but they cannot predict how the compounds will act in the body. Among cereals, sorghums with a pigmented testa had the highest antioxidant activity potential followed by Chinese black rice and sorghums with a pigmented pericarp (Dykes and Rooney 2006).

***Instrumental Methods*.** Reversed-phase high-performance liquid chromatography (RP-HPLC) with UV-Vis or photodiode array (PDA) detection is the best method to separate, identify, and quantify sorghum phenolic acids and flavonoids (Hahn et al 1983; Gujer et al 1986; Awika et al 2004a, b). Mobile phases are usually binary, which consists of a polar solvent (i.e. aqueous formic or acetic acid) and a less polar solvent (i.e. methanol or acetonitrile) (Merken and Beecher 2000). RP-HPLC has been used to measure condensed tannins, but separation could only be accomplished on tannins up to tetramers and these were not separated according to their degree of polymerization (Prior and Gu 2005). Normal-phase HPLC with fluorescence detection allows the separation and quantification of tannins according to their degree of polymerization (Hammerstone et al 1999; Gu et al 2002; Awika et al 2003a). Tannins up to decamers were successfully separated; tannins with higher degrees of

Table 6

Common Methods Used to Measure Antioxidant Capacity in Sorghum

Method	Reaction Mechanism	Radical	Quantitation	Advantages	Disadvantages
ABTS	Single electron transfer	$ABTS^{\bullet+}$	Measures the decrease of $ABTS^{\bullet+}$ absorbance in the presence of antioxidants Trolox used as the standards and the results are expressed as trolox equivalents	Method can be performed at a wide pH range Radical is soluble in both aqueous and organic solvents Reaction occurs within 30 min Does not require expensive instrumentation; a UV-Vis spectrophotometer is used	Radical does not exist in biological systems Radical used is not commercially available and thus has to be generated by reacting ABTS with a strong oxidizing agent (i.e. potassium persulfate), which may take a long time (16 hrs for potassium persulfate) Radical is not stable for long periods of time
DPPH	Single electron transfer	$DPPH^{\bullet}$	Measures the decrease of $DPPH^{\bullet}$ absorbance in the presence of antioxidants Results are expressed as EC_{50} (sample concentration that causes initial $DPPH^{\bullet}$ concentration to decrease by 50%); results are also expressed as trolox equivalents	Radical used is commercially available and thus does not need to be generated Radical used is stable Does not require expensive instrumentation; a UV-Vis spectrophotometer is used	Long analysis time (up to 8 hours for sorghum) Radical used does not exist in biological systems Interference can occur by antioxidants (i.e. anthocyanins) having spectra that overlap with DPPH at 515 nm Method sensitive to low pH Antioxidants may react slowly or not all with $DPPH^{\bullet}$ due to steric hindrance Reducing agents decolorize $DPPH^{\bullet}$
ORAC	Hydrogen atom transfer	$ROO^{\bullet}$	Peroxyl radical reacts with a fluorescent probe (B-phycoerythrin, fluorescin, dichloroflueorescin) to produce a non-fluorescent product that is measured by fluorescence. Antioxidant capacity is determined by the decreased rate and amount of product formed over time Trolox used as the standard and results are expressed as trolox equivalents	Radical exists in biological systems Detects both hydrophilic and lipophilic antioxidants Method is readily automated	Expensive instrumentation Long analysis time (around 1 hr.) Uses of B-phycoerythrin as the fluorescent probe causes high variability of results and causes false low ORAC values Reaction temperature-sensitive; temperature variation causes low reproducibility of results

Refs: Awika et al 2003b; Prior et al 2005.

polymerization were shown as one single peak (Gu et al 2002; Awika et al 2003a).

Sorghum phenols are difficult to identify and characterize due to lack of standards. Methods used to determine structural characterization of these compounds include mass spectrometry, ^{1}H and ^{13}C nuclear magnetic resonance spectrometry, and infrared spectroscopy (Gujer et al. 1986; Watanabe 1999; Krueger et al. 2003; Wu and Prior 2005).

CHAPTER III

MATERIALS AND METHODS

Samples

For the red pericarp sorghum study, thirteen sorghum varieties were grown in a sorghum breeding nursery in College Station, TX in 2003, 2004, and 2005. A tan plant white-pericarp sorghum free of evident pigments (02CA4796) was used as the control. For the lemon-yellow pericarp sorghum study, twelve sorghum varieties were grown in Lubbock, TX in 2005 and in College Station, TX in 2006. Dorado, a tan plant white-pericarp sorghum, was used as the control. For the black pericarp sorghum study, eight sorghum varieties with black pericarps were grown in College Station in 2006; Tx430 Black was used as the control. Tx430 Black, Black PI Tall, Hyb116, Hyb117, and Hyb118 were then grown in College Station, TX and Corpus Christi, TX in 2007. Tx430 Black, Hyb116, and Hyb118 were also grown in Puerto Rico in 2007. The samples' genetic and physical descriptions are summarized in Tables 7-9. The line designations for all germplasms were given by breeders in the Texas Agricultural Experiment Station (TAES) Sorghum Improvement Program; the genotype of each line was based on observations made by TAES sorghum breeders. All sorghum samples were collected at maturity; they were air-dried, manually cleaned, and all glumes were removed from the kernels.

TABLE 7

Genotypes and Physical Characteristics of Varieties Used for the Red Pericarp Sorghum Study

Variety	Genotype	Plant Color	Pigmented Testa	Pericarp	Kernel Appearance
02CA4796	*$b_1b_1B_2B_2$RRyyZZppQQ*	Tan	Absent	White, thin	Pearly, white
B.01336	*$b_1b_1B_2B_2$RRYYZZppQQ*	Tan	Absent	Red, thin	Pearly, yellowish-red
99GWO92	*$b_1b_1B_2B_2$RRYYZZppQQ*	Tan	Absent	Red, thin	Pearly, orange-brown
98BRON1555	*$b_1b_1B_2B_2$RRYYZZppQQ*	Tan	Absent	Red, thin	Pearly, yellowish-red
99LGWO50	*$b_1b_1B_2B_2$RRYYZZppQQ*	Tan	Absent	Red, thick	Chalky, red
98CA4779	*$b_1b_1B_2B_2$RRYYZZPPQQ*	Purple	Absent	Red, thin	Pearly, orange
B.9904	*$b_1b_1B_2B_2$RRYYZZPPqq*	Red	Absent	Red, thin	Pearly, brown
SC103 x SC748 (light)	*$b_1b_1B_2B_2$RRYYZZPPQQ*	Purple	Absent	Red, thin	Pearly, yellowish-brown
Tx2911	*$b_1b_1B_2B_2$RRYYzzPPqq*	Red	Absent	Red, thick	Chalky, red
Tx430 Black	*$b_1b_1B_2B_2$RRYYzzPPQQ*	Purple	Absent	Red, thick	Chalky, mostly black
SC719-11E	*$B_1B_1B_2B_2$RRYYzzPPqq*	Red	Present	Red, thick	Chalky, reddish-orange
SC103 x SC748 (dark)	*$B_1B_1B_2B_2$RRYYzzPPQQ*	Purple	Present	Red, thick	Chalky, dark brownish-red
Black PI Tall	*$B_1B_1B_2$B2RRYYzzPPQQ*	Purple	Present	Red, thick	Chalky, black

TABLE 8

Genotypes and Physical Characteristics of Varieties Used for the Lemon-Yellow Pericarp Sorghum Study

Variety	Genotype	Plant Color	Pigmented Testa	Pericarp	Kernel Appearance
Dorado	*$b_1b_1B_2B_2$RRyyZZppQQ*	Tan	Absent	White, thin	Pearly, white
BRON176	*$b_1b_1B_2B_2$rrYYZZppQQ*	Tan	Absent	Yellow, thin	Pearly, lemon-yellow
B35	*$b_1b_1B_2B_2$rrYYzzPPQQ*	Purple	Absent	Yellow, thick	Pearly, light yellow
EBA3	*$b_1b_1B_2B_2$rrYYzzPPqq*	Red	Absent	Yellow, thick	Chalky, lemon-yellow
SC979	*$b_1b_1B_2B_2$rrYYzzPPQQ*	Purple	Absent	Yellow, thick	Chalky, lemon-yellow
SC35-14E	*$b_1b_1B_2B_2$rrYYZZPPQQ*	Purple	Absent	Yellow, thin	Pearly, lemon-yellow
SC1038	*$b_1b_1B_2B_2$rrYYzzPPQQ*	Purple	Absent	Yellow, thick	Chalky, brownish yellow
New B Line	*$b_1b_1B_2B_2$rrYYzzPPQQ*	Purple	Absent	Yellow, thick	Chalky, brownish yellow
SC748	*$b_1b_1B_2B_2$rrYYZZPPQQ*	Purple	Absent	Yellow, thin	Pearly, lemon-yellow
BTx399	*$b_1b_1B_2B_2$RRYYzzPPQQ*	Purple	Absent	Red, thick	Chalky, light red
Tx2911	*$b_1b_1B_2B_2$RRYYzzPPqq*	Red	Absent	Red, thick	Chalky, red
SC1155	*$B_1B_1B_2B_2$RRYYzzPPQQ*	Purple	Present	Red, thick	Chalky, dark brown

TABLE 9

Genotypes and Physical Characteristics of Varieties Used for the Black Pericarp Sorghum Study

Variety	Genotype	Plant Color	Pigmented Testa	Pericarp	Kernel Appearance
Tx430 Black	$b_1b_1B_2B_2RRYYzzPPQQ$	Purple	Absent	Red, thick	Chalky, black
Shawaya Black	$b_1b_1B_2B_2RRYYzzPPQQ$	Purple	Absent	Red, thick	Chalky, black
Black PI Tall	$B_1B_1B_2B_2RRYYzzPPQQ$	Purple	Present	Red, thick	Chalky, black
Hyb107	$B_1B_1B_2B_2RRYYzzPPQQ$	Purple	Present	Red, thick	Chalky, mostly black
Hyb115	$B_1B_1B_2B_2RRYYzzPPQQ$	Purple	Present	Red, thick	Chalky, mostly black
Hyb116	$B_1B_1B_2B_2RRYYzzPPQQ$	Purple	Present	Red, thick	Chalky, mostly black
Hyb117	$B_1B_1B_2B_2RRYYzzPPQQ$	Purple	Present	Red, thick	Chalky, mostly black
Hyb118	$B_1B_1B_2B_2RRYYzzPPQQ$	Red-Purple	Present	Red, thick	Chalky, dark brown with black spots

Standards and Reagents

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). Caffeic acid, ferulic acid, and naringenin were obtained from Sigma-Aldrich (St. Louis, MO). Apigenin and luteolin were obtained from Indofine Chemical Co., Inc. (Hillsborough, NJ). Eriodictyol, luteolinidin chloride, and apigeninidin chloride were obtained from ALSACHIM (Strasbourg, France) and 7-methoxyapigeninidin chloride was obtained from ChromaDex (Santa Ana, CA). *Sec*-butanol was reagent grade and all other solvents were HPLC grade.

Pericarp Color Determination

Color measurements of whole grains were obtained using a Minolta CR-310 Colorimeter (Osaka, Japan). Measurements were expressed as Commission Internationale de l'Eclairage *L**, *a**, and *b** (CIELAB) (Commission Internationale de l'Éclairage 1986).

Extraction for Colorimetric Assays

Samples were ground for 2 minutes using a Cuisinart DCG-20 coffee grinder (East Windsor, NJ) prior to analysis. For all assays with the exception of the DPPH assay, samples (0.1-0.5 g) were extracted in 25 mL 1% HCl/methanol (v/v) for two hours while shaking at low speed using an Eberbach shaker

(Eberbach Corp., MI). For the butanol assay, samples were extracted in 10 mL of 1% HCl in methanol (v/v). For the DPPH assay, samples (0.2-0.5 g) were extracted in aqueous 70% acetone (v/v) for two hours while shaking at low speed. All extracts were then centrifuged at 2790*g* for 10 minutes 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 hours.

Extraction for HPLC and LC-MS Analyses

Ground samples (1 g) were extracted in 10 mL of 1% HCl/methanol (v/v) for two hours in a shaker. The extracts were centrifuged at 2790*g* for 10 minutes and then decanted. For the lemon-yellow and black pericarp sorghum studies, a second set of extracts were prepared for flavanone analysis. Samples (1g) were extracted in 10 mL of 1% HCl/methanol (v/v) for two hours in a shaker. Each supernatant was then transferred to glass tubes, sealed, and placed in a water bath for 90 minutes at 80ºC. All extracts were immediately filtered using a 0.45 µm nylon membrane filter (Whatman Inc., Maidstone, UK) prior to HPLC and LC-MS analyses.

Colorimetric Assays

Total phenols of the acidified methanol extracts were measured using the modified Folin-Ciocalteu method of Kaluza et al (1980). One aliquot of the extract (0.1 mL) was dissolved in 1.1 mL of water and 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 minutes 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 (1978). Flavan-4-ol content was measured using the modified method of Govindarajan and Mathew (1965) as described by Gous (1989). 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 hour at room temperature and the absorbance was read at 550 nm. Anthocyanin content was measured using the method of Fuleki and Francis (1968). Briefly, one aliquot of each sample was diluted two-fold using the extraction solvent and was left to stand for 2 hours at room temperature in the dark. Absorbance was read at 485 nm. Antioxidant activity of sorghum extracts were measured *in vitro* by the DPPH and ABTS assays as described by Awika et al (2003b).

HPLC Analysis

Extracts were analyzed on an Alliance 2695 system (Waters Corp., Milford, MA) connected to a Waters 996 photodiode array detector (PDA). Sorghum phenolics were separated using a Luna C18 column (150 mm x 4.6 mm i.d., 5 µm) from Phenomenex (Torrance, CA). Column temperature was maintained at 35 ºC. Injection volume was 20 µL. The mobile phase consisted of 4% formic acid in water (v/v) (Solvent A) and acetonitrile (Solvent B). The solvent flow rate was 1.0 mL/min. The 3-deoxyanthocyanins were separated

using the following gradient: 0-20 min., 12-20% B; 20-40 min., 20-50% B; 40-50 min., 50% B. Flavones and flavanones were separated using the following gradient: 0-45 min., 15-41% B; 45-50 min., 41% B. The 3-deoxyanthocyanins, flavones, and flavanones were detected at 485 nm, 340 nm, and 280 nm respectively. Identification of sorghum flavonoids was determined based on commercial standards' retention times, UV-Vis spectra, and LC-MS data. Quantification of each compound was accomplished by comparing peak areas with that of a standard curve of each authentic standard. Molecular weight correction factors (Chandra et al 2001; Wu et al 2006) were used to quantify 5-methoxyluteolinidin and 7-methoxyapigeninidin. Data was collected and processed using the Empower software version 1.0 (Waters Corp., Milford, MA).

LC-MS-ESI Analysis

Structural information on 5-methoxyluteolinidin, eriodictyol glucoside, and naringenin glucoside was obtained by LC-MS analysis, which was performed on a Thermo Finnigan LCQ Deca XP Max MS^n ion trap mass spectrometer equipped with an ESI ion source (ThermoFisher, San Jose, CA). Separation was conducted using the same Luna C18 column mentioned earlier. Column temperature was maintained at 20ºC. The mobile phase consisted of 0.5% formic acid in water (v/v) (Solvent A) and 0.5% formic acid in acetonitrile (v/v) (Solvent B). The solvent flow rate was 0.4 mL/min. The compounds were separated using the following gradient: 0-5 min., 15-40% B; 5-15 min., 40-85% B. Electrospray ionization was conducted in the negative (flavanone glucosides)

and positive (3-deoxyanthocyanin) modes under the following conditions: sheath gas (N_2), 40 units/min; auxiliary gas (N_2), 5 units/ min; spray voltage 3.5 kV; capillary temperature, 250ºC; capillary voltage, -29 V; tube lens offset, -60 V.

Statistical Analysis

All values are expressed as means ± standard deviation for three replicates. One-way ANOVA was used to determine significant differences between phenols, antioxidant activity, and pericarp color. Pearson correlation was used to determine relationships between sorghum phenols and antioxidant activity and between sorghum phenols, antioxidant activity, and pericarp color. Genetics x environment (G x E) interaction was analyzed using a general linear modeling option. Statistical analysis was done using SPSS version 15.0 (SPSS Inc., Chicago, IL).

CHAPTER IV

FLAVONOID PROFILE OF RED SORGHUMS*

Sorghum Grain Characteristics

In 2003, Tx430 Black and Black PI Tall had the lowest *L** values (32.6-34.6), which means they were the darkest in color while 02CA4796 had the highest *L** value (62.2) (Table 10). This was expected since the grains of Tx430 Black and Black PI Tall were black while those of 02CA4796 were white. All samples had positive *a** values, which means they were more red than green. With the exception of 02CA4796, the *a** value increased as the *L** value increased. Tx430 Black 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 Black PI Tall, which was completely black. The *b** value was also positive for all samples, which means they were more yellow than blue; the *L** value increased as the *b** value increased.

When comparing grains grown in 2003 with those grown in 2004 and 2005, sorghums in 2003 had the least weathering whereas those harvested in 2005 had the most weathering, especially for Tx430 Black and Black PI Tall

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TABLE 10

CIELAB *L, *a**, *b** Values of Sorghums Grown in College Station, TX 2003-2005**

Variety	Year	*L**	*a**	*b**
02CA4796	2003	62.24 ± 0.13	3.80 ± 0.10	19.20 ± 0.18
	2004	62.94 ± 0.77	3.79 ± 0.31	19.36 ± 0.40
	2005	59.33 ± 0.08	4.66 ± 0.03	19.91 ± 0.04
B.01336	2003	42.64 ± 0.45	12.58 ± 0.18	13.44 ± 0.24
	2004	42.07 ± 0.25	12.23 ± 0.18	12.84 ± 0.07
	2005	42.06 ± 0.25	13.32 ± 0.09	13.28 ± 0.19
99GWO92	2003	41.96 ± 0.09	13.02 ± 0.31	13.73 ± 0.25
	2004	41.14 ± 0.26	11.78 ± 0.03	12.06 ± 0.26
	2005	40.56 ± 0.25	12.21 ± 0.13	11.40 ± 0.27
98BRON155	2003	46.45 ± 0.14	12.38 ± 0.34	18.04 ± 0.17
	2004	46.05 ± 0.30	12.67 ± 0.21	17.21 ± 0.26
	2005	44.98 ± 0.42	12.08 ± 0.19	16.26 ± 0.34
99LGWO50	2003	39.14 ± 0.23	16.17 ± 0.11	10.86 ± 0.28
	2004	37.62 ± 0.13	14.04 ± 0.17	9.29 ± 0.07
	2005	37.74 ± 0.31	11.95 ± 0.22	7.80 ± 0.38
98CA4779	2003	44.82 ± 0.08	16.36 ± 0.13	17.73 ± 0.13
	2004	45.06 ± 0.17	16.96 ± 0.09	18.29 ± 0.30
	2005	44.67 ± 0.07	15.44 ± 0.06	16.65 ± 0.06
B.9904	2003	41.78 ± 0.06	12.76 ± 0.56	12.30 ± 0.16
	2004	40.71 ± 0.21	13.51 ± 0.24	12.11 ± 0.16
	2005	39.00 ± 0.10	11.93 ± 0.13	9.60 ± 0.17
SC103 x SC748 (light)	2003	43.24 ± 0.14	12.52 ± 0.16	14.69 ± 0.17
	2004	41.76 ± 0.12	13.06 ± 0.26	13.57 ± 0.13
	2005	40.83 ± 0.23	11.54 ± 0.13	12.05 ± 0.27

TABLE 10 - Continued

Variety	Year	*L**	*a**	*b**
Tx2911	2003	40.34 ± 0.14	17.87 ± 0.01	13.01 ± 0.01
	2004	40.62 ± 0.08	18.20 ± 0.27	13.45 ± 0.20
	2005	38.46 ± 0.06	16.90 ± 0.26	10.67 ± 0.21
Tx430 Black	2003	34.62 ± 0.20	3.53 ± 0.03	3.00 ± 0.07
	2004	35.95 ± 0.05	5.53 ± 0.14	4.75 ± 0.19
	2005	34.84 ± 0.29	3.73 ± 0.22	2.37 ± 0.20
SC719-11E	2003	41.70 ± 0.15	18.26 ± 0.13	14.90 ± 0.12
	2004	41.32 ± 0.11	18.38 ± 0.42	15.50 ± 0.16
	2005	41.27 ± 0.17	17.47 ± 0.06	14.02 ± 0.20
SC103 x SC748 (dark)	2003	36.48 ± 0.22	9.93 ± 0.10	7.39 ± 0.13
	2004	36.12 ± 0.11	9.82 ± 0.12	6.34 ± 0.11
	2005	34.78 ± 0.07	8.27 ± 0.12	4.88 ± 0.09
Black PI Tall	2003	32.61 ± 0.07	2.29 ± 0.09	1.41 ± 0.06
	2004	31.48 ± 0.26	2.60 ± 0.10	0.93 ± 0.12
	2005	34.24 ± 0.28	4.01 ± 0.02	1.99 ± 0.10

Fig. 5. Sorghum varieties grown in College Station, TX 2003.

Fig. 6. Sorghum varieties grown in College Station, TX 2004.

Fig. 7. Sorghums varieties grown in College Station, TX 2005.

(Figs. 5-7). The elevated *L** value for Black PI Tall in 2005 (34.2) showed that it was less black. Some variation was observed in the *L**, *a**, and *b** values in 2003-2005 (Table 10); however, the same aforementioned trends were observed. Black sorghums had the lowest *L** values while those of 02CA4796 were highest. All *a** and *b** values were positive. With the exception of 02CA4796, the *a** and *b** values increased as the *L** value increased.

Evaluation of Sorghum Total Phenols

Plant color affected total phenol content (Fig. 8). Sorghum grains grown on plants with red/purple secondary plant color, with the exception of 98CA4778 and B.9904, had higher levels of total phenol (3.1-8.9 mg GAE/g) than those from tan plants with a thin pericarp (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 red/purple plants had total phenol levels similar to those from tan plants. This agrees with the results of Beta et al (1999) 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 (8.8-8.9 mg GAE/g).

Evaluation of Sorghum Condensed Tannins

Only three varieties (SC719-11E, SC103 x SC748-E (dark), Black PI Tall) had a pigmented testa and had significant amounts of condensed tannins (Fig.

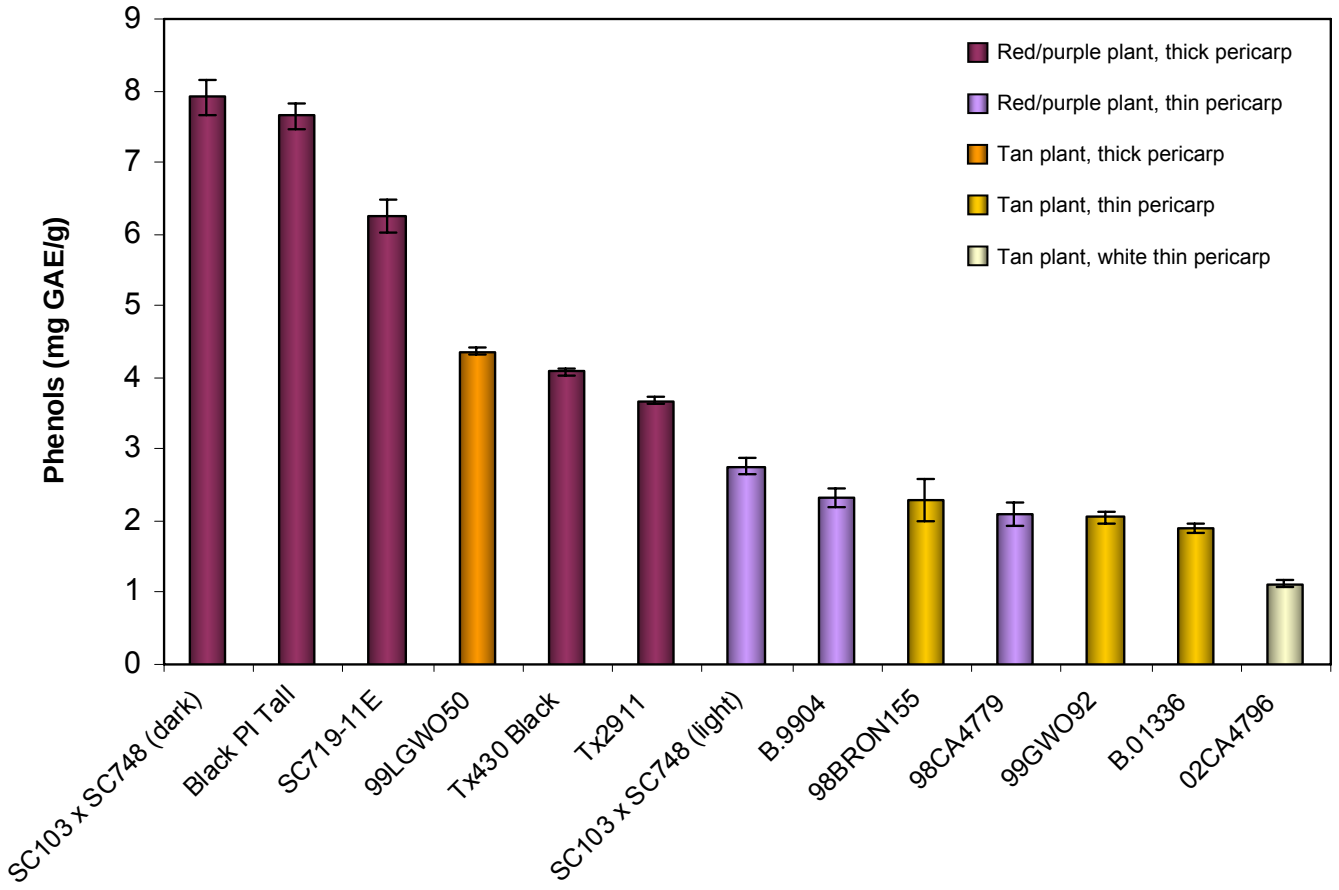

Fig. 8. Total phenol levels in sorghums grown in College Station, TX 2003.

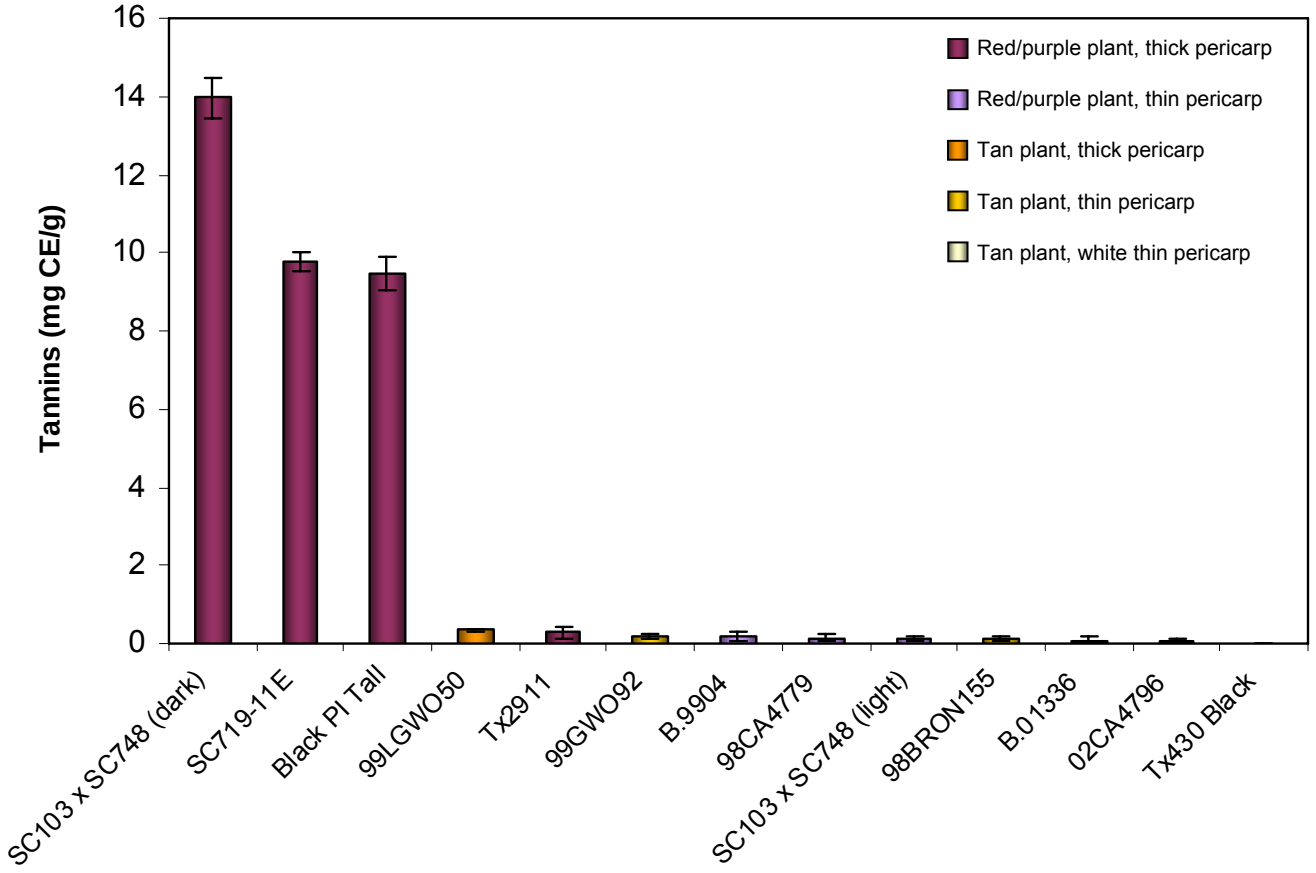

Fig. 9. Condensed tannin levels in sorghums grown in College Station, TX 2003.

9). 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 (Hahn and Rooney 1986). SC719-11E and Black PI Tall had similar levels of condensed tannins (11.9-12.0 mg CE/g) while sample SC103 x SC748 (dark) contained 15.5 mg CE/g. This was not expected since Black PI Tall has a dominant spreader gene *S*. Sorghums with a dominant spreader gene usually have higher tannins levels than those with a recessive gene (Hahn and Rooney 1986).

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 3-deoxyanthocyanidins in sorghums (Wharton and Nicholson 2000). In addition to the possibility of reducing mold damage in sorghums (Jambunathan et al 1990; Jambunathan et al 1991; Menkir et al 1996), 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 with a thin pericarp were lowest (2.3-2.7 abs/mL/g), followed by red/purple plant sorghums with a thin pericarp (3.0-3.6 abs/mL/g) (Fig. 10). Red/purple 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 11) suggests total phenols are contributed mostly by flavan-4-ols in red pericarp

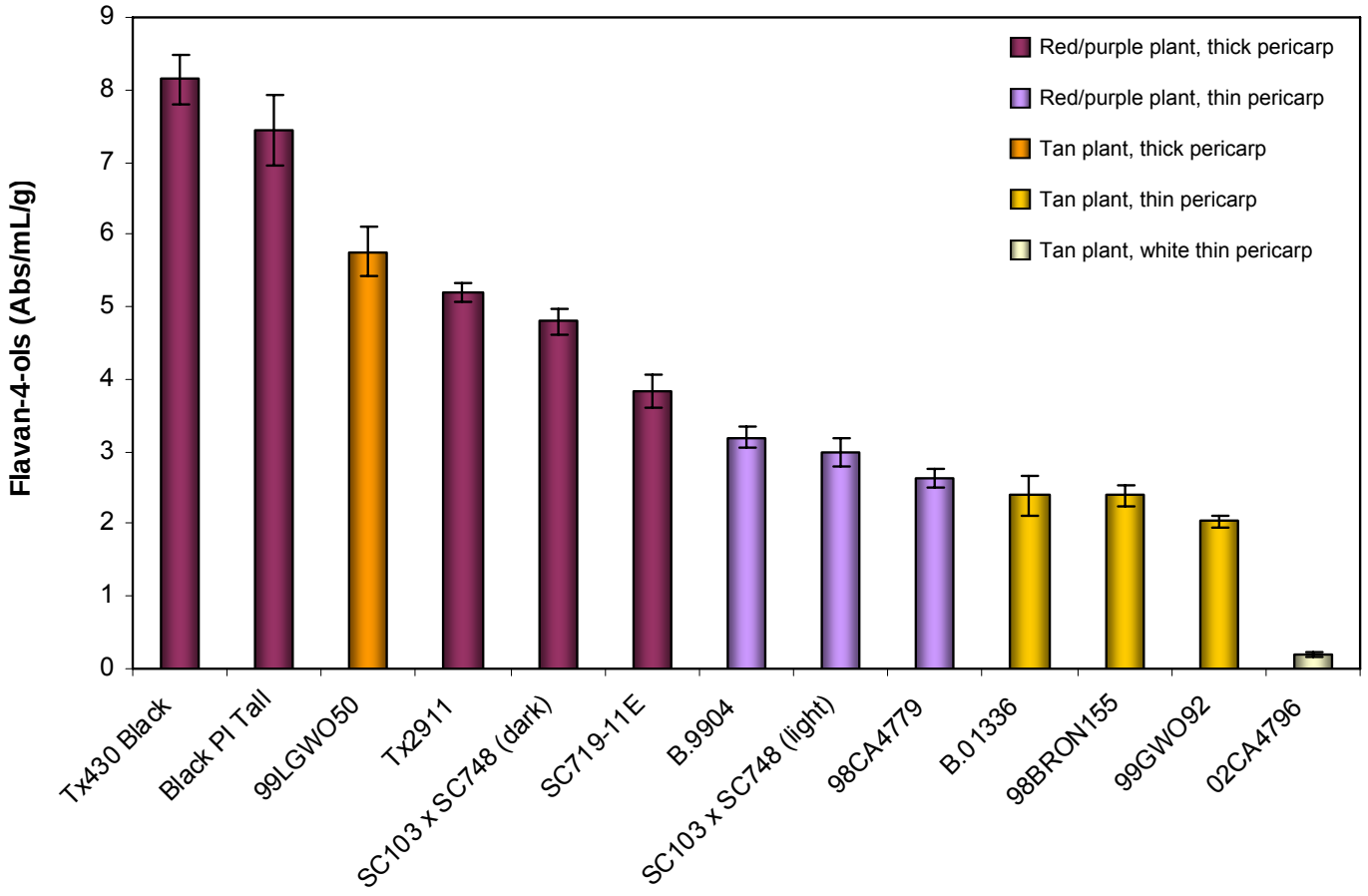

Fig. 10. Flavan-4-ol levels in sorghums grown in College Station, TX 2003.

Table 11

Pearson's Correlation Coefficients of Sorghum Phenols and Antioxidant Activity

	All Sorghum Varieties				Non-tannin Sorghum Varieties			
	FL-4-OL	**ANTH**	**ABTS**	**DPPH**	**FL-4-OL**	**ANTH**	**ABTS**	**DPPH**
PHE	0.70[b]	0.55	0.99[a]	0.98[a]	0.94[a]	0.65[c]	0.97[a]	0.97[a]
FL-4-OL		0.87[a]	0.65[c]	0.62[c]		0.85[b]	0.88[a]	0.96[a]
ANTH			0.47	0.50			0.56	0.74[c]
ABTS				0.97[a]				0.94[a]

PHE = Total phenols; FL-4-OL = Flavan-4-ol; ANTH = Anthocyanins.
[a], [b], [c] indicate $p < 0.001$, $p < 0.01$, and $p < 0.05$, respectively.

sorghums. This was especially true among non-tannin sorghums, which showed a much stronger correlation ($r = 0.94$, $p < 0.001$) (Table 11).

Evaluation of Sorghum Anthocyanins

The most common anthocyanins in sorghums are the 3-deoxyanthocyanidins, which include the orange luteolinidin and the yellow apigeninidin (Nip and Burns 1969; Nip and Burns 1971; Gous 1989; Awika et al 2004a, b; Rooney and Awika 2005). Both compounds have good potential for use as natural colorants due to their pH stability (Gous 1989; Awika et al 2004b). The levels of anthocyanins in the sorghums evaluated had the same pattern as found for the flavan-4-ols (Fig. 11). Sorghums with a black pericarp (Tx430 Black and Black PI Tall) had the highest levels of anthocyanins, followed by the other varieties with red/purple plants and a thick pericarp. Sorghums with a black pericarp are genetically red but when maturing in the presence of sunlight, their pericarp turns black. A correlation between flavan-4-ol and anthocyanin content ($r = 0.87$, $p < 0.001$) (Table 11) was observed. The same was observed among non-tannin varieties ($r = 0.85$, $p < 0.001$). No significant correlation was found between total phenols and anthocyanin content, which was previously reported by Awika (2000).

Correlations Between Pericarp Color and Sorghum Phenols

There were some significant correlations between pericarp color and sorghum phenols. The negative correlation between the L^* value and total

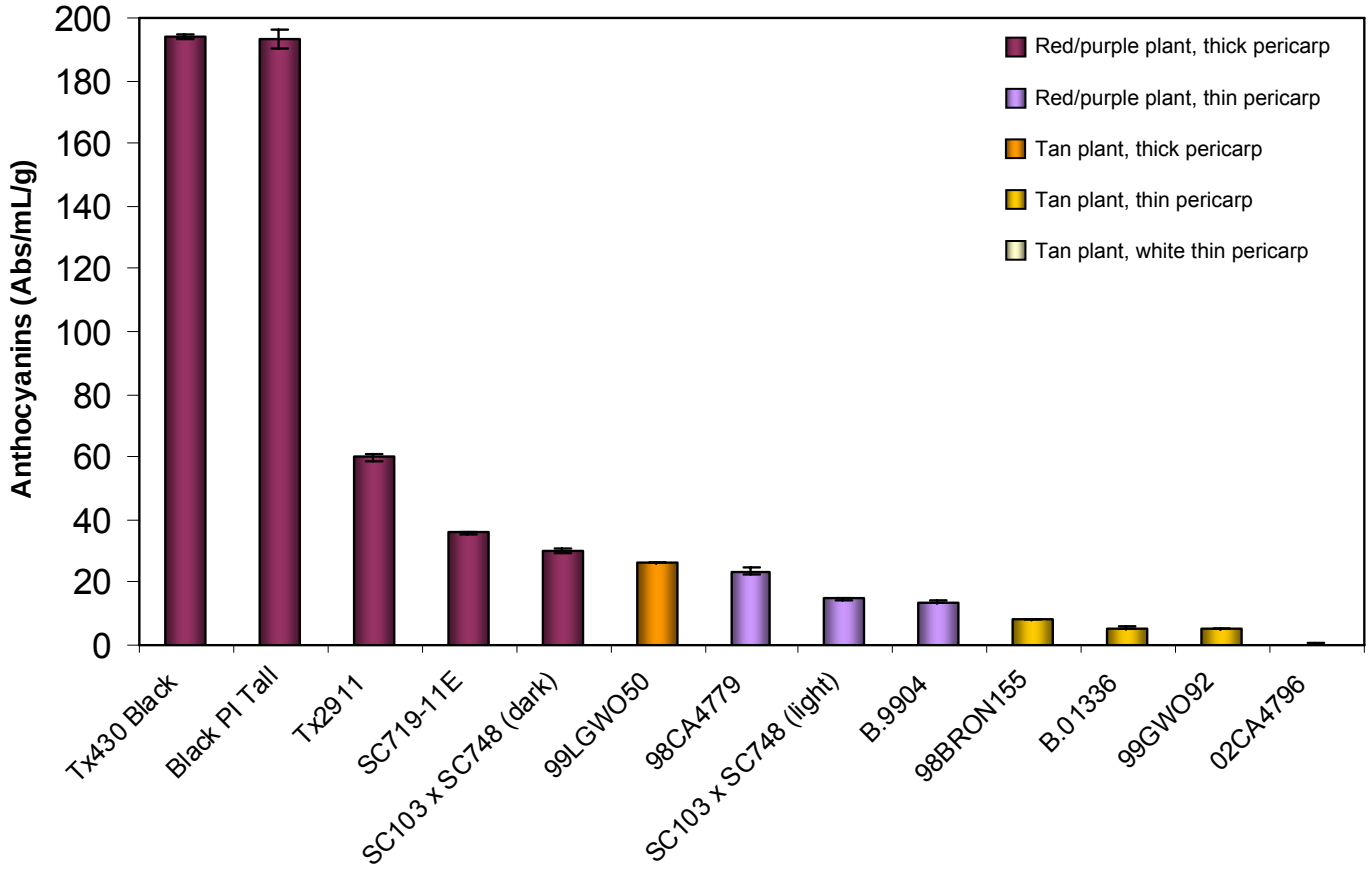

Fig. 11. Anthocyanin levels in sorghums grown in College Station, TX 2003.

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 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 (1989), 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 (1989). No significant correlations were found between the a^* value and total phenols.

Evaluation of Sorghum Antioxidant Activity

Sorghums with dominant B_1B_2 genes had the highest antioxidant activity, especially the varieties with the dominant *S* gene (Fig. 12). The antioxidant activity came mainly from condensed tannins, which have demonstrated higher antioxidant activity *in vitro* than other phenolic compounds (Hagerman et al 1998; Amarowicz et al 2003).

Plant color and pericarp thickness affected antioxidant activity. Sorghums from red/purple plants had higher antioxidant activity than those from tan plants with a thin pericarp. From the red/purple plant category, sorghum grains with a thick pericarp had higher antioxidant activity than those with a thin pericarp. This confirms that antioxidant activity comes mainly from the pericarp, which is rich in phenols (Awika et al 2005). A strong correlation between total phenols

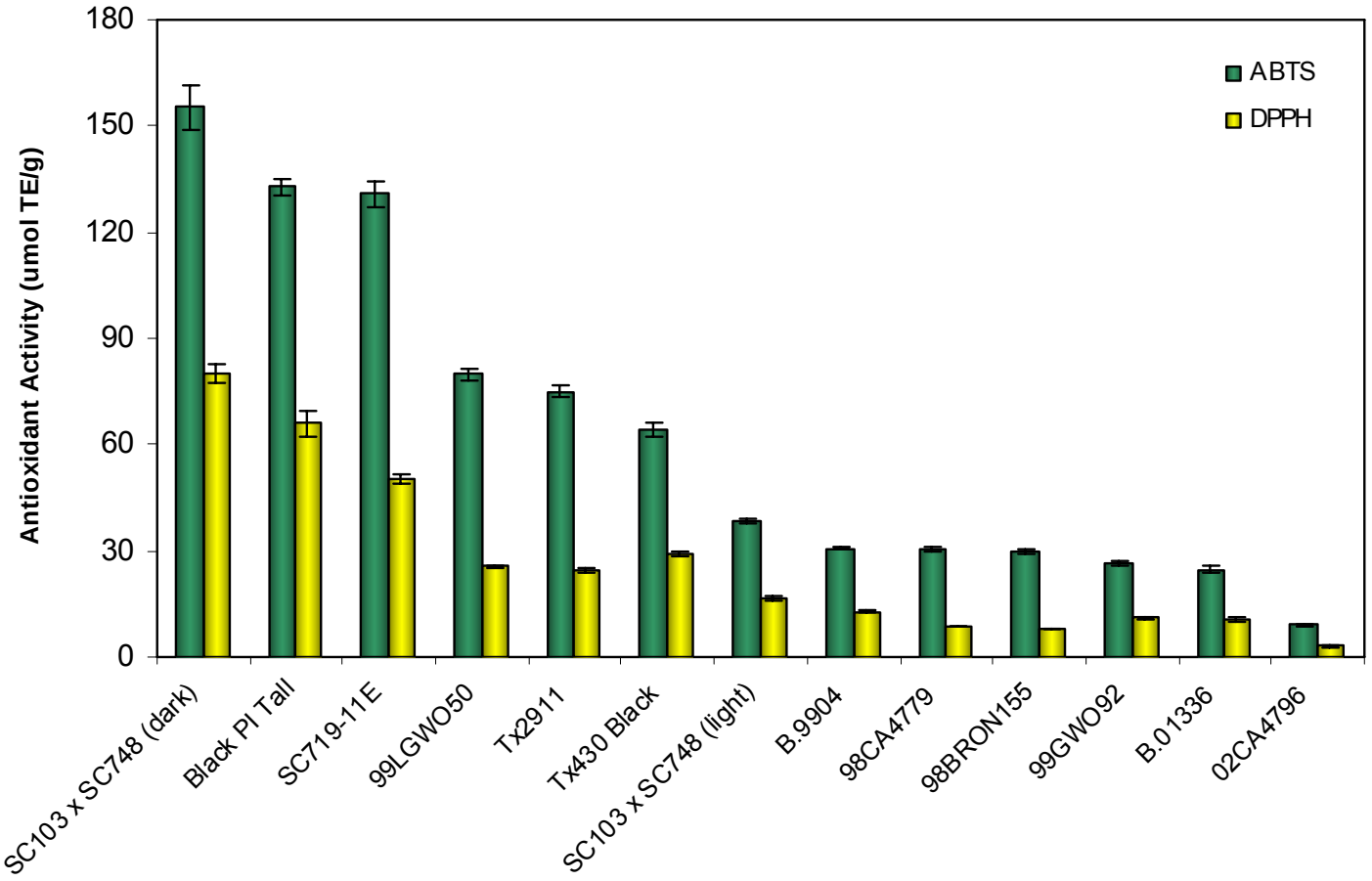

Fig. 12. Antioxidant activity potential of sorghums grown in College Station, TX 2003.

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 (Earp et al 2004b) and are more susceptible to molds and weathering (Beta et al 1999) causing the production of phytoalexins such as 3-deoxyanthocyanins (Lo et al 1999; Seitz 2004). This study shows sorghum grains with *PPqq* or *PPQQ* genes (red/purple plant) and recessive *z* (thick pericarp) genes increase antioxidant activity. This type of information is important for the selection of sorghums used for functional foods.

The strong correlations between total phenols and antioxidant activity could be due to the presence of tannins in SC719-11E, SC103 x SC748 (dark), and Black PI Tall, which increased the correlations. However, when those samples 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 11), which indicated other phenolic compounds such as flavan-4-ols or anthocyanins were 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 11). However, when determining the correlations without the tannin sorghums, the correlation between antioxidant activity and flavan-4-ols increased. No correlation was found between anthocyanins and

antioxidant activity among the non-tannin sorghums.

Among the non-tannin sorghum samples, Tx2911, 99LGWO50, and Tx430 Black had the highest antioxidant activity (Fig. 12). Interestingly, Tx2911 and 99LGWO50 had higher antioxidant activity than Tx430 Black despite the fact the grains of Tx2911 and 99LGWO50 were bright red while those of Tx430 Black were black. These results suggest the intensity of redness of non-tannin sorghum grains cannot predict their antioxidant activity potential. Tx2911 and 99LGWO50 may contain compounds that increase the antioxidant activity.

DPPH values were lower than the ABTS values for all samples. This could be due to the different extraction solvents used for both assays. Aqueous acetone and acidified methanol were used as extraction solvents for the DPPH and ABTS assays, respectively. Acidified methanol could not be used for the DPPH assay since the method is sensitive at low pH (Awika et al 2003b). In addition, pigments, such as anthocyanins, cause interference leading to underestimation of antioxidant activity when using the DPPH assay (Arnao 2000; Awika et al 2003b). However, there was a strong correlation between ABTS and DPPH ($r = 0.97$, $p < 0.001$) (Table 11), which was also observed in previous studies (Awika et al 2003b; De Beer et al 2003).

Evaluation of Sorghum 3-Deoxyanthocyanins

Four major 3-deoxyanthocyanins were detected: luteolinidin (LUT), apigeninidin (AP), 5-methoxyluteolinidin (5-MeO-LUT), and 7-methoxyapigeninidin (7-MeO-AP) and their retention times were 8.2, 11.1, 11.8,

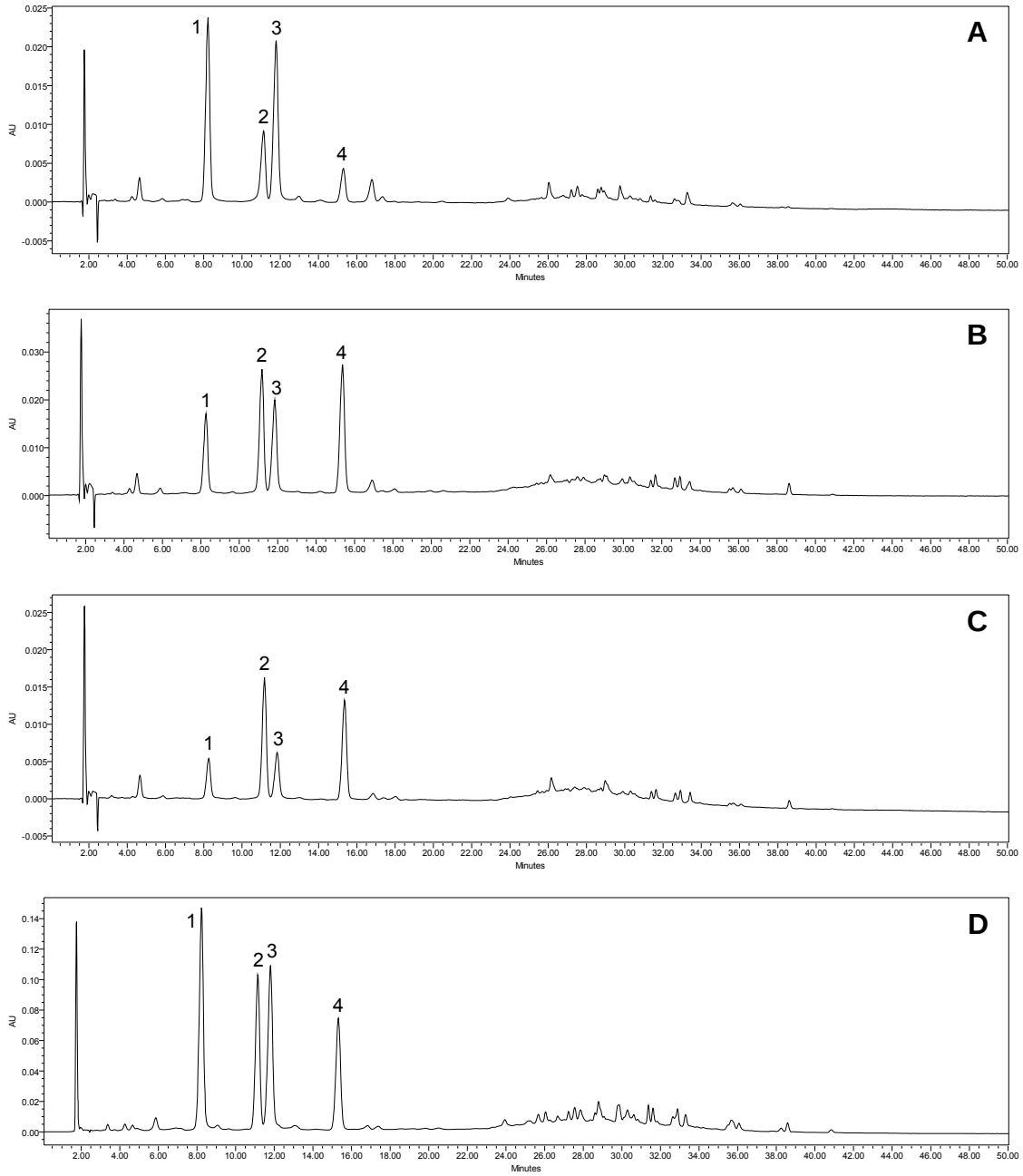

Fig. 13. HPLC chromatograms of 3-deoxyanthocyanins of sorghums grown in College Station, TX 2003. A) 98CA4778; B) Tx2911; C) SC719-11E; D) Tx430 Black. 1 = Luteolinidin; 2 = Apigeninidin; 3 = 5-Methoxyluteolinidin; 4 = 7-Methoxyapigeninidin. PDA = 485 nm.

and 15.3 minutes respectively (Fig. 13). These compounds were identified using commercial standards with the exception of 5-MeO-LUT, which was identified based on its UV spectra (λ_{max} = 485 nm) and LC-MS data ($[M]^-$, *m/z* 283; MS/MS, *m/z* 269). The common anthocyanins (i.e. cyanidin) were not detected. Sorghums with red/purple secondary plant color had the highest levels of 3-deoxyanthocyanins (32-680 µg/g) with the black sorghums (Tx430 Black and Black PI Tall) having the highest amounts (Fig. 14). The presence of a pigmented testa did not increase the levels of 3-deoxyanthocyanins.

A strong correlation between the 3-deoxyanthocyanin levels measured by the colorimetric method of Fuleki and Francis (1968) and by HPLC (r = 0.99, p < 0.001) was observed. This suggested that the anthocyanins measured using the colorimetric method consisted mostly of the four 3-deoxyanthocyanins detected by HPLC. The strong correlation also suggested that the colorimetric method is a quick, inexpensive, and reliable method to screen 3-deoxyanthocyanin levels in sorghums.

The 3-deoxyanthocyanin profiles varied among samples (Fig. 15). AP and 7-MeO-AP were the predominant 3-deoxyanthocyanins in red plant sorghums (B.9904, Tx2911, and SC719-11E), which accounted for 67-89% of the total. On the other hand, all purple plant sorghums, with the exception of Tx430 Black, had a higher proportion of the LUT compounds, which accounted for 61-75% of the total. The 3-deoxyanthocyanins in Tx430 Black were almost evenly distributed with LUT and 5-MeO-LUT accounting for 52% of the total.

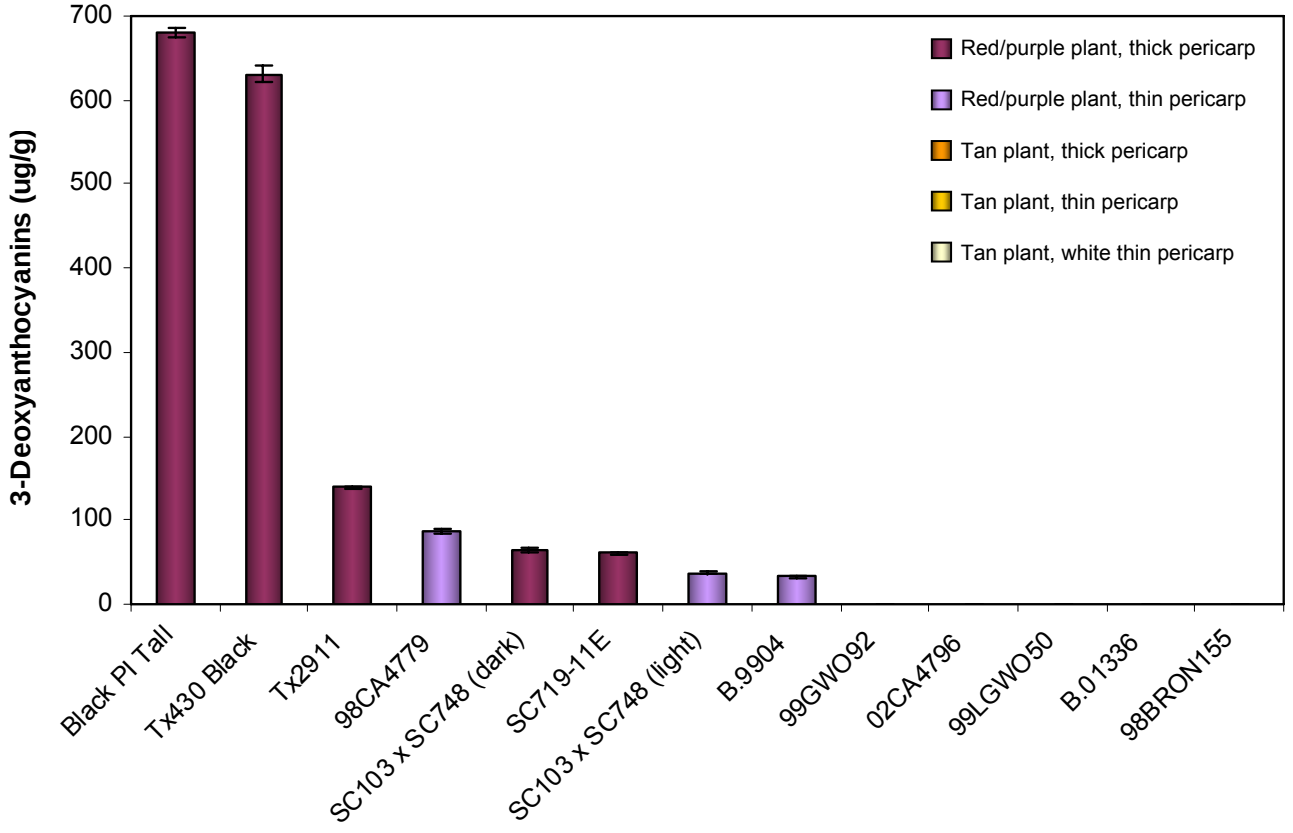

Fig. 14. 3-Deoxyanthocyanin levels in sorghums grown in College Station, TX 2003.

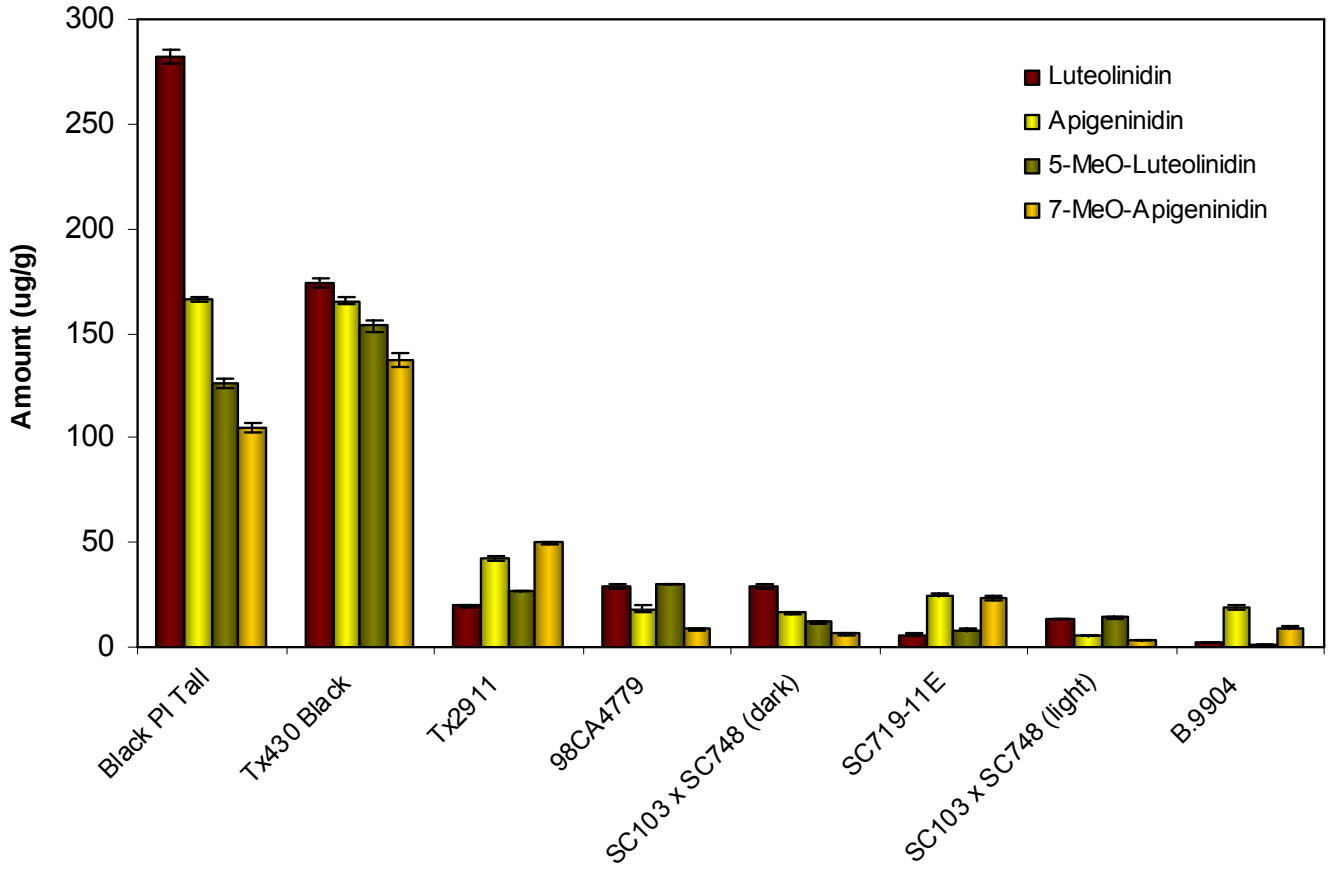

Fig. 15. 3-Deoxyanthocyanin profile of sorghums grown in College Station, TX 2003.

These results suggest that 3-deoxyanthocyanin profile can be predicted by secondary plant color and not by pericarp color.

It had been reported that these compounds are concentrated in the pericarp (Awika et al 2004a, b; Seitz 2004). When looking at the samples, red/purple plant sorghums had purple specks or stains while tan plant sorghums did not (Fig. 5). These stains could be the source of the extractable 3-deoxyanthocyanins. However, due to their high levels of 3-deoxyanthocyanins, it was unclear whether these compounds were produced as phytoalexins in the black sorghums. It is believed that these compounds were produced when the grain was exposed to sunlight. To test this hypothesis, panicles of the Tx430 Black were covered with a brown paper bag from anthesis to physical maturity during the crop year of 2004. When the bags were removed, the grains of

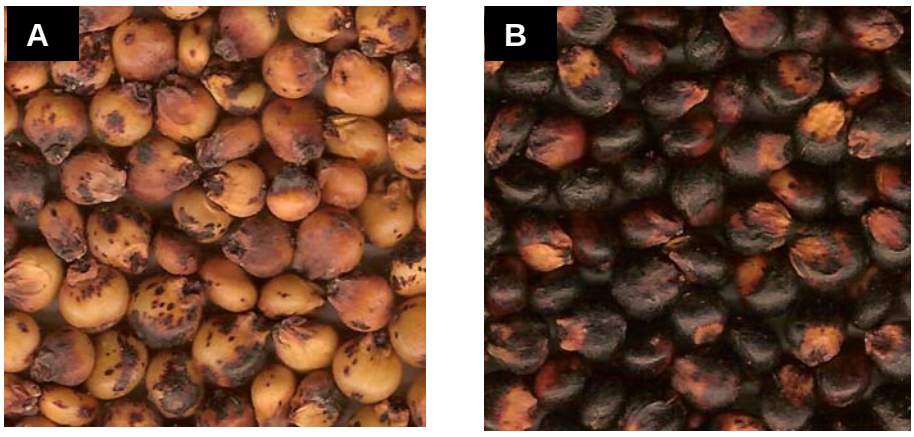

Fig. 16. Tx430 Black grains from panicles that were A) covered and B) uncovered.

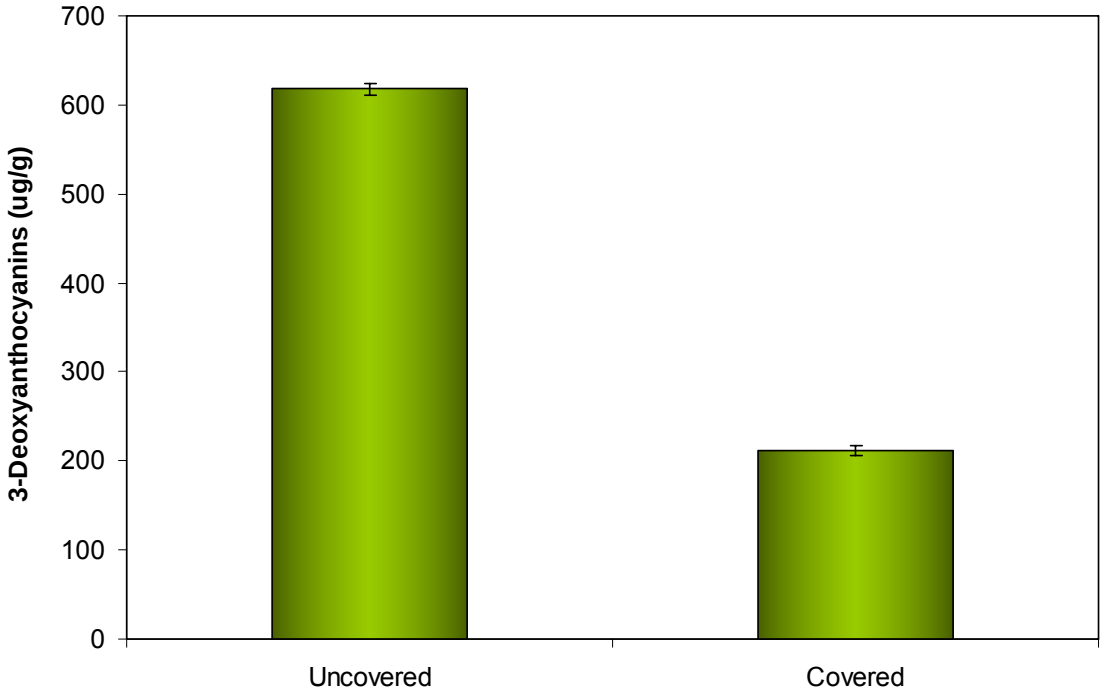

Fig. 17. 3-Deoxyanthocyanin levels of Tx430 Black (C.S. 2004) from uncovered and covered panicles.

Tx430 Black were red (Fig. 16). Grains from the uncovered panicles had three times more 3-deoxyanthocyanins than those that were left covered (Fig. 17), which suggested these compounds were produced while the panicles were exposed to sunlight.

Evaluation of Sorghum Flavones

The two flavones detected in the varieties were luteolin and apigenin (Fig. 18). The separation method efficiently separated luteolin even in red/purple plant sorghums, which was not achieved by Seitz (2004) due to interference with

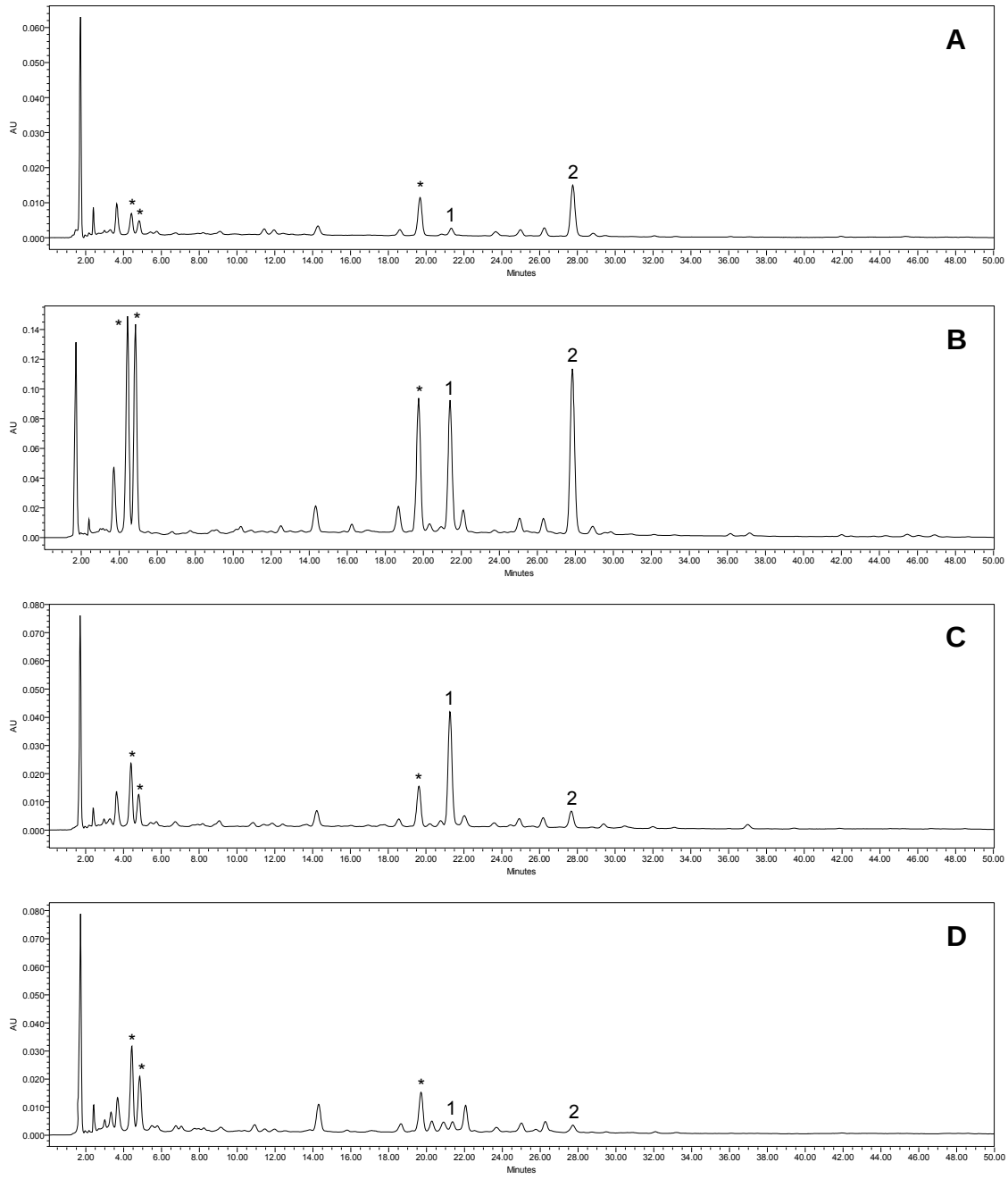

Fig. 18. HPLC chromatograms of A) 02CA4796; B) 99LGWO50; C) 99GWO92; D) 98CA4779. 1 = Luteolin; 2 = Apigenin; * = Identified as cinnamic acids (i.e. caffeic and ferulic acids) on the basis of their UV spectra. PDA = 340 nm.

other phenolics. Three major peaks at 4.4, 4.8, and 19.8 minutes were identified as cinnamic acids (i.e. caffeic and ferulic acids) on the basis of their UV spectra (shoulder 245 nm; λ_{max} = 321-327 nm). Red sorghums with tan secondary plant color had higher flavone levels (60-386 µg/g) than red/purple plant sorghums (0-41 µg/g) (Fig. 19). Flavones were not detected in SC719-11E. The elevated flavone levels for 99LGWO50 could explain its elevated total phenols and antioxidant activity levels (Figs. 8, 12). Among the red/purple plant sorghums, black sorghums had the highest flavone levels.

Information on the cause of the presence of these compounds is limited. Since luteolin and apigenin are yellow and pale yellow respectively, it was difficult to conclude whether these compounds contributed to pericarp color as it was observed for the 3-deoxyanthocyanins in black sorghums. These compounds are concentrated in the pericarp and are reported as phytoalexins (Seitz 2004). There is the possibility that these compounds were leached from the glumes to the grain however this was not supported by Seitz (2004). Further work is needed in this area.

Flavone profiles varied among the samples. Tx2911 and 98CA4779 had only luteolin (Fig. 20). Apigenin was the main flavone in 02CA4796 and B.01336, which accounted for 85-91% of the total. Luteolin and apigenin were almost evenly distributed for B.9904 and 99LGWO50. On the other hand, luteolin was the main flavone in the remaining samples which accounted for (76-100%) of the total. Flavone profile was not affected by secondary plant color.

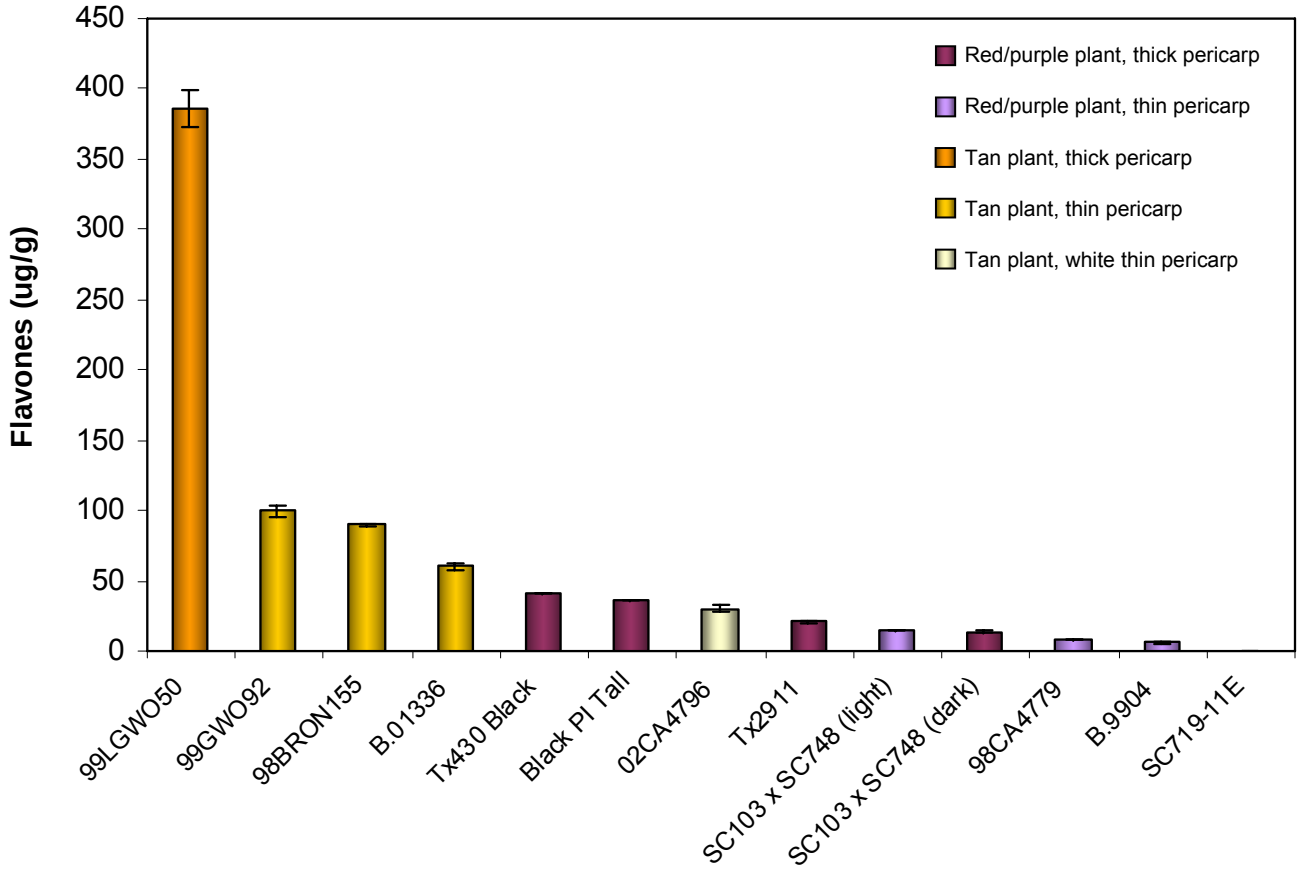

Fig. 19. Flavone levels in sorghums grown in College Station, TX 2003.

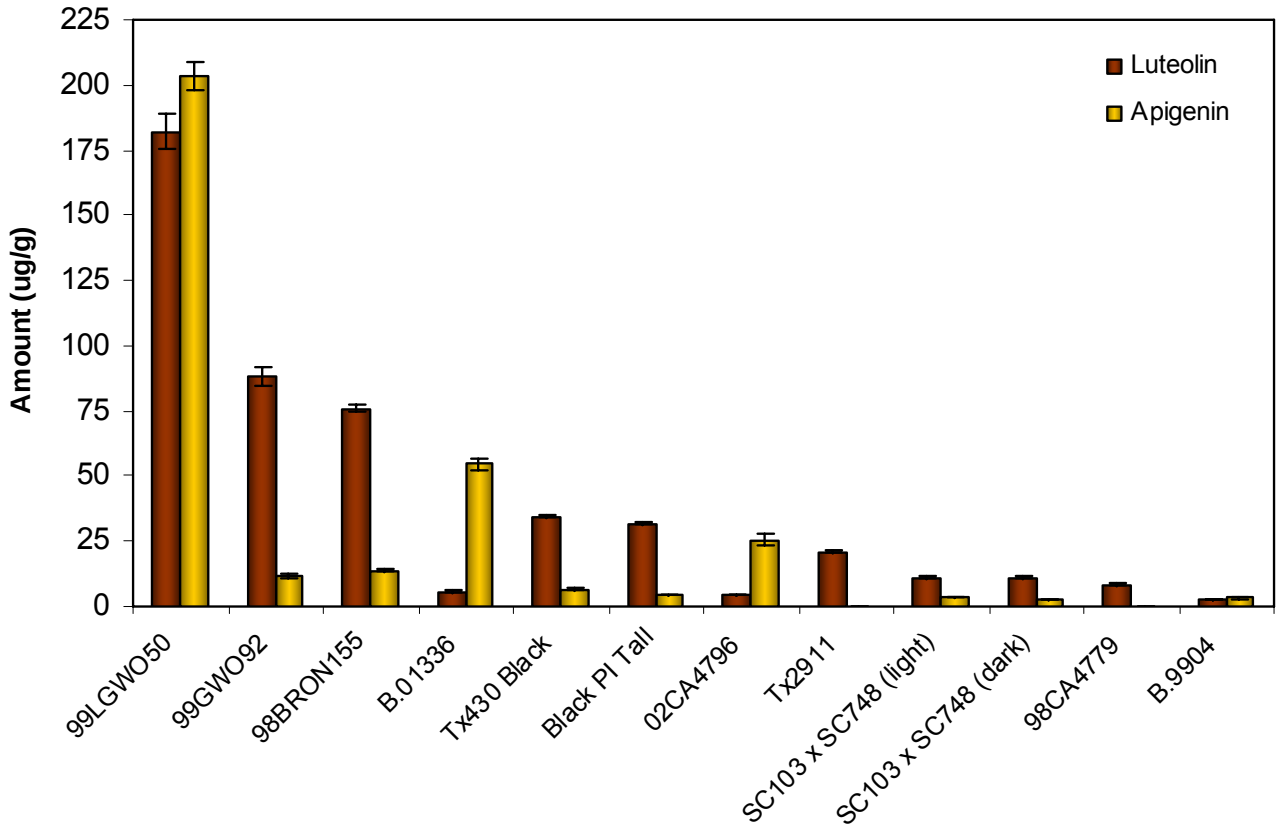

Fig. 20. Flavone profile of sorghums grown in College Station, TX 2003.

The results showed that tan plant sorghums are a source of flavones, which have potential health applications. These compounds have anticancer, anti-inflammatory, antiallergic, and analgesic properties (Block et al 1998; Hirano et al 2004; Horinaka et al 2005; Matsui et al 2005; Cherng et al 2007; Ziyan et al 2007). They are also effective as vascular relaxation agents and can be used for the treatment of corneal neovascularization (Block et al 1998; Xu et al 2007).

Evaluation of Sorghum Flavanones

Flavanone levels ranged from 0-48 µg/g with BRON155 having the highest levels (Fig 21). These compounds were not detected in 02CA4796, Tx430 Black, and Black PI Tall. Secondary plant color did not have an effect on flavanone levels.

Flavanone profile varied among the samples. Naringenin was the only flavanone detected in some sorghums (i.e. 98BRON155, Tx2911, 99GWO92) (Fig. 22). Naringenin was not detected as the major peak (Fig. 23), which contradicts the findings by Awika (2003). Both eriodictyol and naringenin were detected in some samples (i.e. B.01336, SC103 x SC748 (light), B.9904) and their proportions varied. Eriodictyol was the predominant flavanone in B.9904 and B.01336, which accounted for 54-57% of the total. Naringenin was the main flavanone in SC103 x SC748 (light) and SC103 x SC748 (dark) which accounted for 69-80% of the total.

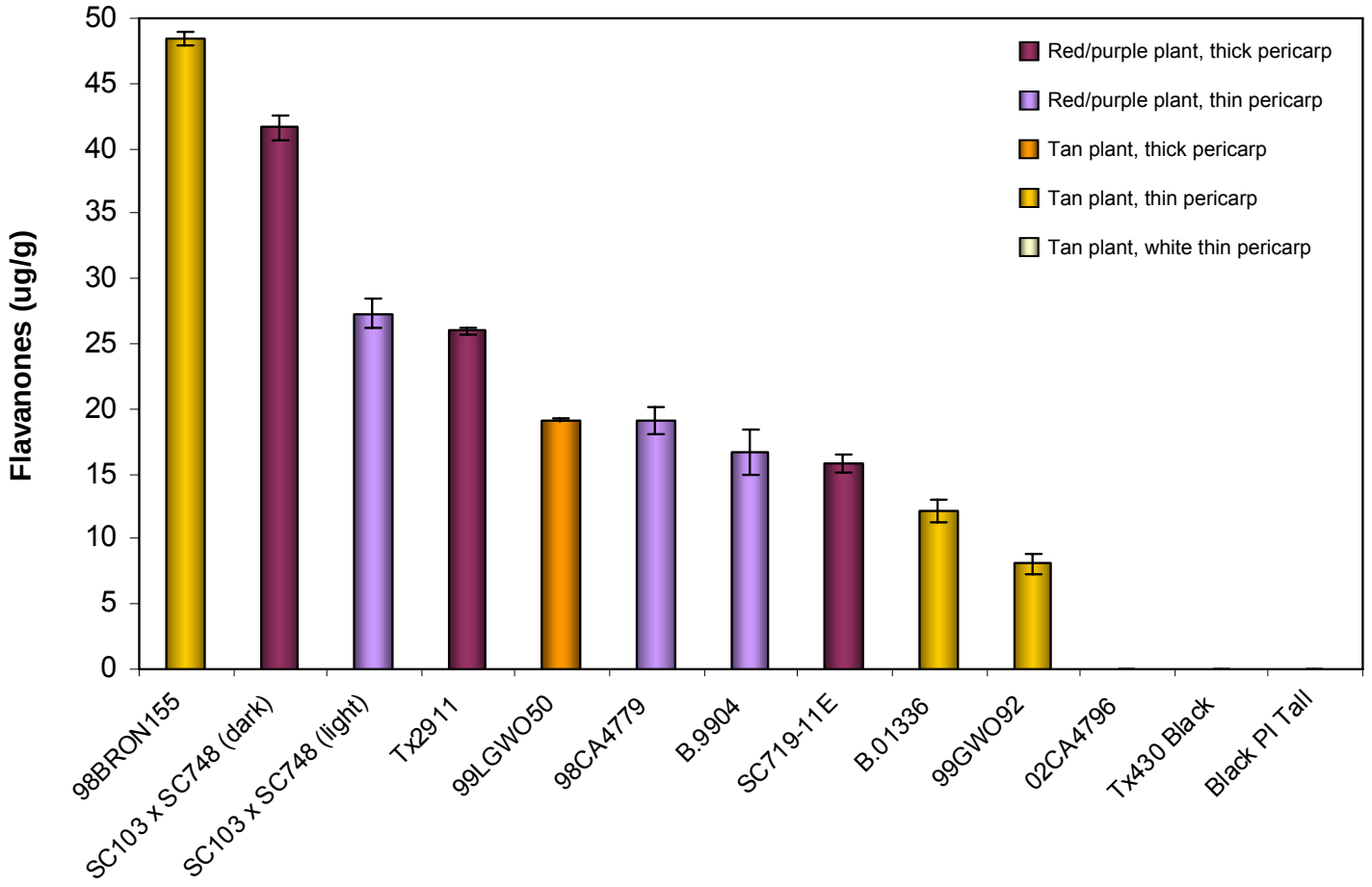

Fig. 21. Flavanone levels in sorghums grown in College Station, TX 2003.

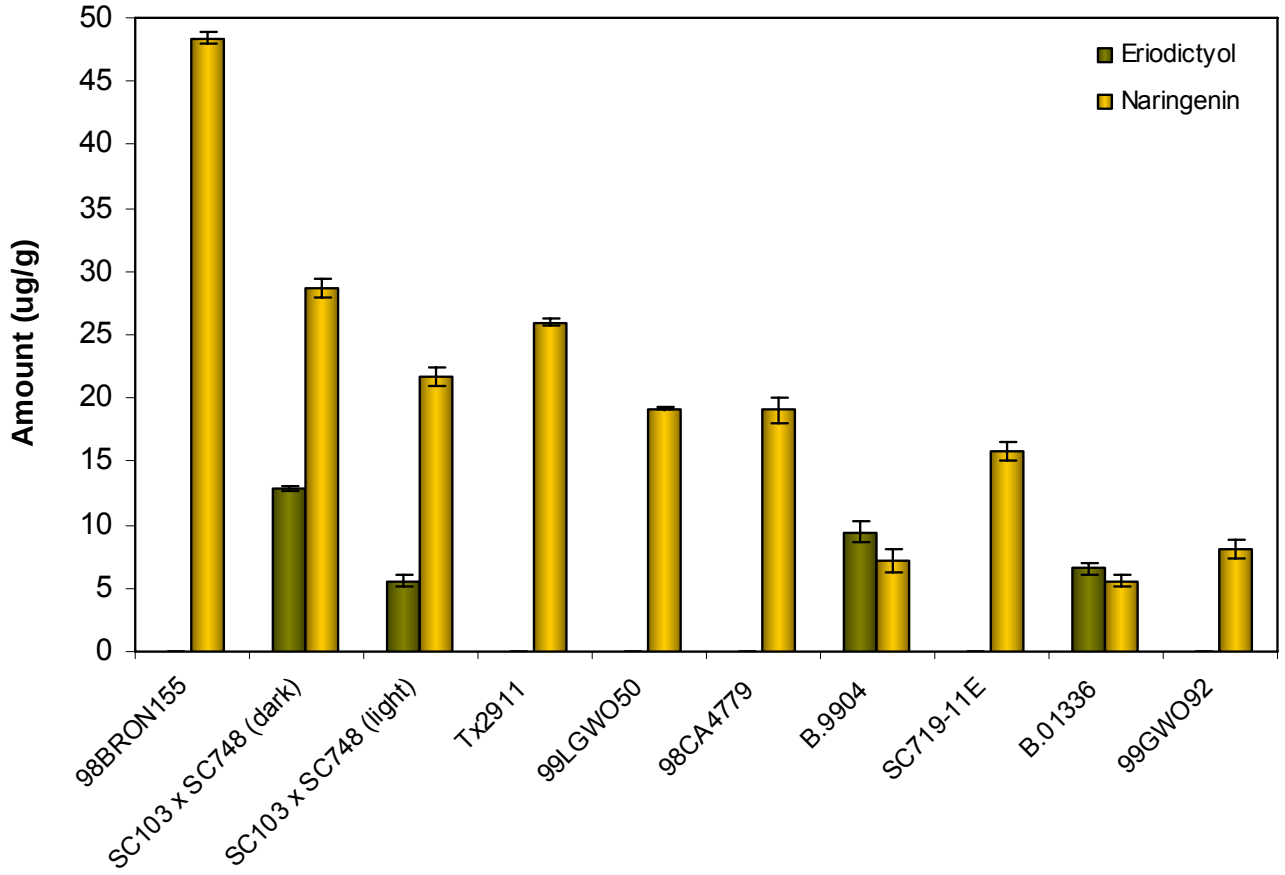

Fig. 22. Flavanone profile of sorghums grown in College Station, TX 2003.

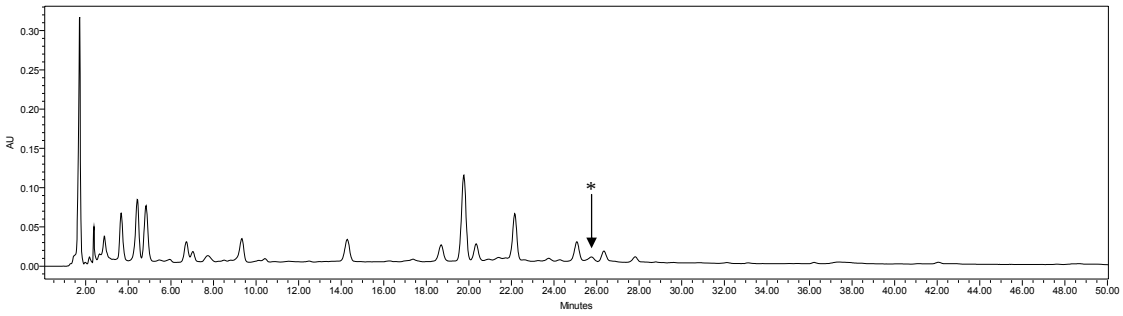

Fig. 23. HPLC chromatogram of Tx2911. *Naringenin. PDA = 280 nm.

Effect of Environment on Phenols and Antioxidant Activity Levels

The phenol and antioxidant activity levels of varieties grown in College Station, TX in 2003-2005 were compared. Panicles started to flower in May-June and the samples were collected in late August. Monthly rainfalls and temperatures are reported in Table 12. Temperatures did not vary among the years; however, rainfall pattern did. In 2003, rainfall occurred throughout the sorghum development. In 2004, rainfall was heaviest during panicle anthesis and development of the grain (May-June). In 2005, on the other hand, rainfall was heaviest when the grain reached maturity (July-August).

Even though sorghums were grown in the same location, variation in phenol and antioxidant activity levels was observed in 2003-2005. In general, sorghums grown in 2004 had higher condensed tannin, flavan-4-ol, and anthocyanin levels compared to those grown in 2003 and 2005 (Table 13). Only one sample, 99LGWO50, had higher total phenols, flavan-4-ols, and anthocyanins in 2003. SC103 x SC748 (light) from 2005 showed detectable

TABLE 12

Monthly Rainfalls and Temperatures for College Station, TX 2003-2005

		May	June	July	August
2003	Rainfall (in.)	0.6	6.6	4.1	4.5
	Temperature (ºF)	78.7	81.1	83.0	84.8
2004	Rainfall (in.)	7.8	11.8	2.3	2.5
	Temperature (ºF)	76.3	80.5	83.0	82.3
2005	Rainfall (in.)	2.5	0.5	4.6	3.3
	Temperature (ºF)	74.9	83.9	84.8	84.5

Data obtained from the National Weather Service Forecast Office, 2007.

condensed tannins (4.4 mg CE/g), which explains its elevated total phenol and antioxidant levels. Since that sample was a hybrid, two possibilities could explain the presence of condensed tannins. First, some panicles collected had the pigmented testa and thus increased the catechin equivalent value. Second, the seeds that were planted were from the 2004 crop year, and when planted, grains with a pigmented testa were developed.

Environment did have an effect on 3-deoxyanthocyanin levels. Sorghums from 2003 and 2004 had higher levels of 3-deoxyanthocyanins than those from 2005 (Table 14). In 2005, the 3-deoxyanthocyanin levels for Tx430 and Black PI Tall were 523 µg/g and 304 µg/g, respectively, which were lower than the 2003 and 2004 samples. When looking at the grains from 2005, the black sorghums had severe weathering causing the kernels to have a grayish-brown appearance (Fig 7). As reported in Table 12, the heaviest rainfall in the summer of 2005

Table 13

Phenol and Antioxidant Activity Levels in Sorghum Varieties Grown in College Station, TX 2003-2005

Variety	Year	Total Phenols (mg GAE/g)[a]	Tannins (mg CE/g)[b, c]	Flavan-4-ols (Abs/mL/g)	Anthocyanins (Abs/mL/g)	ABTS (umol TE/g)[d]	DPPH (umol TE/g)
02CA4796	2003	0.92 ± 0.08	0.05 ± 0.05	0.19 ± 0.03	0.33 ± 0.08	8.96 ± 0.44	3.23 ± 0.33
	2004	1.45 ± 0.02	0.14 ± 0.08	0.17 ± 0.01	0.77 ± 0.10	11.68 ± 0.64	5.30 ± 0.18
	2005	1.51 ± 0.08	0.07 ± 0.03	0.27 ± 0.01	1.79 ± 0.09	12.11 ± 0.47	4.77 ± 0.12
B.01336	2003	1.90 ± 0.08	0.09 ± 0.08	2.39 ± 0.27	5.61 ± 0.20	24.77 ± 0.93	10.51 ± 0.56
	2004	2.51 ± 0.02	0.36 ± 0.05	2.52 ± 0.05	6.81 ± 0.06	26.48 ± 0.75	13.69 ± 0.20
	2005	2.42 ± 0.05	0.29 ± 0.15	1.99 ± 0.05	5.40 ± 0.20	22.26 ± 0.75	11.07 ± 0.25
99GWO92	2003	2.05 ± 0.09	0.19 ± 0.06	2.04 ± 0.08	5.30 ± 0.22	26.40 ± 0.90	11.00 ± 0.26
	2004	3.08 ± 0.17	0.36 ± 0.05	2.93 ± 0.04	9.93 ± 0.21	34.14 ± 0.53	14.18 ± 0.41
	2005	2.89 ± 0.15	0.47 ± 0.16	2.85 ± 0.11	7.78 ± 0.21	30.19 ± 1.23	13.63 ± 0.13
98BRON155	2003	2.29 ± 0.29	0.12 ± 0.08	2.39 ± 0.13	8.30 ± 0.14	29.58 ± 0.54	7.93 ± 0.09
	2004	3.08 ± 0.06	0.44 ± 0.06	3.79 ± 0.04	12.53 ± 0.23	37.42 ± 0.49	9.56 ± 0.30
	2005	3.27 ± 0.04	0.68 ± 0.10	3.85 ± 0.05	12.16 ± 0.44	39.69 ± 1.17	10.81 ± 0.14
99LGWO50	2003	4.36 ± 0.05	0.36 ± 0.03	5.76 ± 0.34	26.33 ± 0.14	79.91 ± 1.52	25.55 ± 0.38
	2004	3.26 ± 0.05	0.44 ± 0.03	2.97 ± 0.04	10.07 ± 0.15	31.43 ± 1.36	16.04 ± 0.58
	2005	4.05 ± 0.05	0.84 ± 0.08	4.42 ± 0.08	14.23 ± 0.66	48.66 ± 0.53	20.92 ± 0.79
98CA4779	2003	2.09 ± 0.16	0.14 ± 0.11	2.63 ± 0.13	23.66 ± 0.88	30.47 ± 0.39	8.58 ± 0.11
	2004	2.78 ± 0.00	0.41 ± 0.09	3.41 ± 0.02	23.79 ± 1.09	30.11 ± 0.25	8.71 ± 0.48
	2005	2.93 ± 0.09	0.64 ± 0.10	3.63 ± 0.03	19.01 ± 0.15	35.48 ± 0.69	8.46 ± 0.19
B.9904	2003	2.33 ± 0.13	0.17 ± 0.13	3.20 ± 0.15	13.63 ± 0.46	30.59 ± 0.24	12.82 ± 0.45
	2004	2.97 ± 0.08	0.56 ± 0.09	3.87 ± 0.07	16.16 ± 0.25	32.73 ± 1.57	13.18 ± 0.63
	2005	3.04 ± 0.11	0.56 ± 0.05	3.15 ± 0.09	12.66 ± 0.71	28.73 ± 1.14	13.36 ± 0.52
SC103 x SC748 (light)	2003	2.76 ± 0.12	0.12 ± 0.08	2.99 ± 0.19	14.92 ± 0.30	38.43 ± 0.80	16.48 ± 0.46
	2004	3.69 ± 0.04	0.44 ± 0.06	3.99 ± 0.04	16.40 ± 0.32	44.37 ± 0.52	17.50 ± 0.70
	2005	5.38 ± 0.15	4.37 ± 0.27	3.82 ± 0.07	15.19 ± 0.53	63.05 ± 1.09	29.72 ± 1.28

Table 13 – Continued

Variety	Year	Total Phenols (mg GAE/g)	Tannins (mg CE/g)	Flavan-4-ols (Abs/mL/g)	Anthocyanins (Abs/mL/g)	ABTS (umol TE/g)	DPPH (umol TE/g)
Tx2911	2003	3.68 ± 0.40	0.30 ± 0.15	5.19 ± 0.13	59.79 ± 1.12	74.98 ± 1.47	24.68 ± 0.53
	2004	4.32 ± 0.04	1.19 ± 0.10	9.02 ± 0.12	57.95 ± 0.54	66.05 ± 0.42	14.37 ± 0.39
	2005	5.27 ± 0.22	1.19 ± 0.13	8.12 ± 0.27	46.48 ± 0.70	71.18 ± 0.86	23.97 ± 0.30
Tx430 Black	2003	4.14 ± 0.31	ND[e]	8.14 ± 0.33	193.70 ± 0.75	64.39 ± 1.89	29.19 ± 0.85
	2004	5.12 ± 0.09	0.86 ± 0.20	9.84 ± 0.25	152.31 ± 5.01	64.35 ± 1.02	62.74 ± 1.53
	2005	5.83 ± 0.13	1.10 ± 0.30	10.34 ± 0.05	174.25 ± 2.39	66.69 ± 0.64	35.02 ± 2.29
SC719-11E	2003	6.24 ± 0.22	9.77 ± 0.26	3.84 ± 0.23	35.73 ± 0.70	130.81 ± 3.65	50.43 ± 1.45
	2004	9.57 ± 0.30	18.37 ± 0.33	4.24 ± 0.04	50.28 ± 0.70	137.72 ± 4.91	75.21 ± 4.51
	2005	8.14 ± 0.24	10.84 ± 0.23	3.79 ± 0.11	32.92 ± 0.21	106.47 ± 3.26	56.87 ± 2.73
SC103 x SC748 (dark)	2003	7.91 ± 0.25	13.96 ± 0.51	4.79 ± 0.18	30.06 ± 0.66	155.21 ± 6.13	80.07 ± 2.79
	2004	10.18 ± 0.11	16.03 ± 0.69	4.87 ± 0.03	39.45 ± 0.23	125.78 ± 4.14	88.09 ± 2.61
	2005	9.19 ± 0.17	12.18 ± 0.51	4.58 ± 0.09	36.69 ± 0.60	123.66 ± 5.99	77.21 ± 4.65
Black PI Tall	2003	7.49 ± 0.37	9.44 ± 0.42	7.45 ± 0.48	192.89 ± 3.03	132.81 ± 2.48	65.96 ± 3.48
	2004	11.45 ± 0.14	12.87 ± 0.48	12.40 ± 0.11	229.64 ± 4.49	151.83 ± 1.66	89.65 ± 0.97
	2005	9.63 ± 0.08	11.75 ± 0.71	10.08 ± 0.04	144.11 ± 3.79	142.53 ± 1.02	83.03 ± 4.07

[a]GAE = Gallic acid equivalents.
[b]CE = Catechin equivalents.
[c]Varieties with values lower than 2.00 are considered tannin-free.
[d]TE = Trolox equivalents.
[e]ND = Not detected.

Table 14

3-Deoxyanthocyanin Levels in Sorghum Varieties Grown in College Station, TX 2003-2005[a]

Variety	Year	LUT[b]	AP[c]	5-MeO-LUT[d]	7-MeO-AP[e]	Total
02CA4796	2003	ND[f]	0.38 ± 0.09	ND	0.36 ± 0.06	0.74 ± 0.04
	2004	0.22 ± 0.03	ND	ND	ND	0.22 ± 0.03
	2005	ND	ND	ND	ND	ND
B.01336	2003	ND	ND	ND	ND	ND
	2004	0.36 ± 0.07	1.01 ± 0.13	0.12 ± 0.10	0.92 ± 0.10	2.41 ± 0.07
	2005	ND	0.75 ± 0.02	ND	0.37 ± 0.34	1.12 ± 0.36
99GWO92	2003	0.48 ± 0.08	ND	0.32 ± 0.18	ND	0.80 ± 0.26
	2004	4.07 ± 0.27	2.39 ± 0.19	3.31 ± 0.16	2.03 ± 0.10	11.80 ± 0.67
	2005	ND	ND	ND	ND	ND
98BRON155	2003	ND	ND	ND	ND	ND
	2004	0.87 ± 0.02	2.64 ± 0.54	1.06 ± 0.18	0.63 ± 0.10	5.20 ± 0.70
	2005	0.27 ± 0.04	1.73 ± 0.42	0.39 ± 0.13	ND	2.39 ± 0.47
99LGWO50	2003	0.40 ± 0.04	ND	ND	ND	0.40 ± 0.04
	2004	0.71 ± 0.11	0.99 ± 0.23	0.32 ± 0.10	0.93 ± 0.31	2.95 ± 0.46
	2005	ND	ND	ND	ND	ND
98CA4779	2003	29.17 ± 0.87	18.23 ± 1.54	30.21 ± 0.39	8.55 ± 0.21	86.16 ± 2.99
	2004	16.76 ± 0.45	16.83 ± 0.74	10.93 ± 0.30	6.07 ± 0.26	50.59 ± 0.85
	2005	8.19 ± 0.47	9.23 ± 0.25	4.65 ± 0.11	2.10 ± 0.08	24.17 ± 0.41
B.9904	2003	2.04 ± 0.14	19.39 ± 1.06	1.58 ± 0.09	9.40 ± 0.30	32.41 ± 1.40
	2004	1.88 ± 0.02	17.08 ± 0.86	1.47 ± 0.09	8.51 ± 0.52	28.94 ± 1.45
	2005	2.21 ± 0.15	12.86 ± 0.08	2.27 ± 0.18	7.12 ± 0.10	24.46 ± 0.44
SC103 x SC748 (light)	2003	13.52 ± 0.17	5.71 ± 0.23	14.12 ± 0.48	3.54 ± 0.09	36.89 ± 0.92
	2004	13.26 ± 0.27	6.22 ± 0.21	14.13 ± 0.57	4.54 ± 0.12	38.15 ± 1.03
	2005	5.27 ± 0.22	3.39 ± 0.19	7.34 ± 0.38	2.48 ± 0.33	18.48 ± 1.06

Table 14 - Continued

Variety	Year	LUT	AP	5-MeO-LUT	7-MeO-AP	Total
Tx2911	2003	19.97 ± 0.51	42.36 ± 1.27	26.97 ± 0.16	49.81 ± 0.61	139.11 ± 1.50
	2004	11.22 ± 0.31	46.77 ± 1.59	11.51 ± 0.59	41.08 ± 1.25	110.58 ± 0.35
	2005	10.93 ± 0.32	44.56 ± 1.09	16.40 ± 0.63	32.75 ± 0.68	104.64 ± 2.56
Tx430 Black	2003	174.16 ± 2.00	165.41 ± 1.96	153.45 ± 3.05	137.38 ± 3.10	630.40 ± 10.03
	2004	188.58 ± 2.71	162.74 ± 0.65	146.62 ± 2.69	119.46 ± 0.45	617.40 ± 6.19
	2005	149.79 ± 1.66	118.64 ± 3.18	148.89 ± 1.32	105.97 ± 1.38	523.29 ± 6.97
SC719-11E	2003	6.10 ± 0.21	24.95 ± 0.94	8.20 ± 0.32	23.12 ± 1.15	62.37 ± 2.59
	2004	9.22 ± 0.04	45.14 ± 0.42	9.29 ± 0.14	38.38 ± 0.49	102.03 ± 0.82
	2005	4.01 ± 0.27	20.32 ± 0.87	3.63 ± 0.34	11.50 ± 0.63	39.46 ± 2.02
SC103 x SC748 (dark)	2003	29.14 ± 0.84	16.37 ± 0.74	11.89 ± 0.66	6.33 ± 0.30	63.73 ± 2.26
	2004	52.30 ± 1.15	34.99 ± 1.86	8.38 ± 0.13	5.72 ± 0.50	101.39 ± 3.34
	2005	40.70 ± 0.94	21.72 ± 0.62	20.66 ± 0.24	7.96 ± 0.04	91.04 ± 1.72
Black PI Tall	2003	282.56 ± 3.29	166.19 ± 1.24	126.01 ± 1.87	104.93 ± 1.81	679.69 ± 6.15
	2004	264.90 ± 8.84	214.79 ± 8.25	106.00 ± 1.62	98.24 ± 2.61	683.93 ± 20.47
	2005	110.76 ± 0.83	71.98 ± 1.67	72.66 ± 0.74	48.52 ± 0.66	303.92 ± 3.45

[a]Levels are expressed as µg/g.
[b]LUT = Luteolinidin.
[c]AP = Apigeninidin.
[d]5-MeO-LUT = 5-Methoxyluteolinidin.
[e]7-MeO-AP = 7-Methoxyapigeninidin.
[f]ND = Not detected.

occurred in the later stage of the sorghum development. Black sorghums turned black 21 days after anthesis (DAA). The heavy rainfall occurred when the pericarp was already black and thus caused molding of the grain. In 2004, on the other hand, the heavy rainfall occurred when the grain was at its early development; no severe weathering was observed (Fig. 6). In 2003, rainfall occurred throughout the grain development and no severe weathering was observed (Fig. 5). It is possible that grains are more prone to produce phytoalexins at early stages of development as a means of defense against physical stresses. On the other hand, if the grain is not attacked at its early stage of development, then it is not adapted to produce these compounds throughout its development. When reaching its later stage of development and it is exposed to physical stress (i.e. heavy rainfall and high temperature), it produces less phytoalexins and thus grain damage occurs. Further work is needed to confirm this hypothesis.

The general linear model showed a genotype x environment interaction, which means that environment did have an effect on 3-deoxyanthocyanin levels ($p < 0.001$) (Table 15). The 3-deoxyanthocyanin profiles of tan plant sorghums differed when grown in different years whereas those of the red/purple plant sorghums did not.

Flavone levels were consistently highest in tan plant sorghums (Table 16) over the three crop years. There was a sample x environment interaction ($p < 0.001$) (Table 17), which showed that environment had an effect on flavone

Table 15

Genotype (Variety) x Environment (Year) Interaction of 3-Deoxyanthocyanins in Sorghums Grown in College Station, TX 2003-2005

Source	Type III Sum of Squares	df	Mean Square	F	Sig.	Partial Eta Squared
Corrected Model	4873113.371[a]	38	128239.826	6999.177	.000	1.000
Intercept	1642802.712	1	1642802.712	89662.221	.000	.999
Variety	4549710.486	12	379142.541	20693.150	.000	1.000
Year	57530.192	2	28765.096	1569.965	.000	.976
Variety * Year	265872.693	24	11078.029	604.626	.000	.995
Error	1429.126	78	18.322			
Total	6517345.209	117				
Corrected Total	4874542.497	116				

[a]R Squared = 1.000 (Adjusted R Squared = 1.000)

levels. However, based on the information from Table 12, it was difficult to explain what could be the cause since there was no trend as was observed for the 3-deoxyanthocyanins. For example, flavone levels were highest in 2003 for 99LGOW50 (386 µg/g) and Tx2911 (21 µg/g) while levels were highest in 2004 for B.01336 (91 µg/g) and 99GWO92 (153 µg/g). Flavone levels were highest in 2005 for 02CA4796 (62 µg/g) and 98BRON155 (104 µg/g). Flavone levels for Black PI Tall in 2005 were three times less (10 µg/g) than those in previous years (32-36 µg/g). The luteolin level for that sample in 2005 was also three times less (10 µg/g) than in previous years (27-32 µg/g) and apigenin was not detected. The proportions of luteolin and apigenin did not change for most samples during 2003-2005. A small amount of luteolin was found in 98CA4779 in 2003 whereas no flavone levels were detected in 2004 and 2005. Apigenin was detected in SC719-11E in the 2004 crop year. The luteolin and apigenin

Table 16

Flavone Levels in Sorghum Varieties Grown in College Station, TX 2003-2005[a]

Variety	Year	Luteolin	Apigenin	Total
02CA4796	2003	4.49 ± 0.17	25.44 ± 2.24	29.93 ± 2.42
	2004	6.92 ± 0.29	37.42 ± 0.39	44.34 ± 0.54
	2005	12.55 ± 0.40	50.14 ± 2.59	62.69 ± 2.99
B.01336	2003	5.60 ± 0.27	54.51 ± 1.86	60.11 ± 2.02
	2004	7.31 ± 0.10	83.47 ± 0.13	90.78 ± 0.18
	2005	3.38 ± 0.15	22.66 ± 0.70	26.04 ± 0.80
99GWO92	2003	88.10 ± 3.67	11.70 ± 0.65	99.80 ± 4.26
	2004	122.90 ± 2.32	29.71 ± 0.38	152.61 ± 2.69
	2005	72.16 ± 0.66	15.49 ± 0.06	87.65 ± 0.71
98BRON155	2003	75.73 ± 1.42	13.89 ± 0.15	89.62 ± 1.43
	2004	73.78 ± 1.00	22.48 ± 0.16	96.26 ± 1.08
	2005	92.81 ± 1.94	10.89 ± 0.25	103.70 ± 1.90
99LGWO50	2003	182.23 ± 7.00	203.68 ± 5.50	385.91 ± 12.48
	2004	39.01 ± 1.58	101.53 ± 5.79	140.54 ± 7.31
	2005	57.81 ± 0.91	68.04 ± 1.65	125.85 ± 2.49
98CA4779	2003	8.43 ± 0.16	ND[b]	8.43 ± 0.16
	2004	ND	ND	ND
	2005	ND	ND	ND
B.9904	2003	2.63 ± 0.10	3.16 ± 0.07	5.79 ± 0.14
	2004	2.98 ± 0.06	3.83 ± 0.05	6.81 ± 0.11
	2005	1.76 ± 0.06	1.55 ± 0.08	3.31 ± 0.08
SC103 x SC748 (light)	2003	11.18 ± 0.19	3.45 ± 0.09	14.63 ± 0.29
	2004	10.11 ± 0.32	3.62 ± 0.02	13.73 ± 0.30
	2005	3.37 ± 0.16	1.15 ± 0.02	4.52 ± 0.18

Table 16 - Continued

Variety	Year	Luteolin	Apigenin	Total
Tx2911	2003	20.79 ± 0.37	ND	20.79 ± 0.37
	2004	9.08 ± 0.09	ND	9.08 ± 0.09
	2005	8.84 ± 0.19	ND	8.84 ± 0.19
Tx430 Black	2003	34.23 ± 0.45	6.73 ± 0.08	40.96 ± 0.48
	2004	29.79 ± 0.47	7.12 ± 0.17	36.91 ± 0.64
	2005	29.58 ± 1.23	4.39 ± 0.16	33.97 ± 1.37
SC719-11E	2003	ND	ND	ND
	2004	ND	10.64 ± 0.22	10.64 ± 0.22
	2005	ND	ND	ND
SC103 x SC748 (dark)	2003	11.02 ± 0.26	2.81 ± 0.21	13.83 ± 0.46
	2004	12.92 ± 0.09	3.37 ± 0.03	16.29 ± 0.10
	2005	7.93 ± 0.36	2.34 ± 0.25	10.27 ± 0.61
Black PI Tall	2003	31.62 ± 0.49	4.63 ± 0.08	36.25 ± 0.50
	2004	27.11 ± 0.68	5.13 ± 0.16	32.24 ± 0.83
	2005	10.29 ± 0.33	ND	10.29 ± 0.33

[a]Levels are expressed as µg/g.
[b]ND = Not detected.

Table 17

Genotype (Variety) x Environment (Year) Interaction of Flavones in Sorghums Grown in College Station, TX 2003-2005

Source	Type III Sum of Squares	df	Mean Square	F	Sig.	Partial Eta Squared
Corrected Model	556466.481[a]	38	14643.855	2094.192	.000	.999
Intercept	287531.381	1	287531.381	41119.363	.000	.998
Variety	410878.361	12	34239.863	4896.583	.000	.999
Year	12495.839	2	6247.919	893.504	.000	.958
Variety * Year	133092.281	24	5545.512	793.054	.000	.996
Error	545.423	78	6.993			
Total	844543.285	117				
Corrected Total	557011.904	116				

[a]R Squared = .999 (Adjusted R Squared = .999)

proportions for 99LGWO50 of 2004 were different from those of 2003 and 2005. In 2004, apigenin accounted for 72% of the total whereas in 2003 and 2005, it was accounted for 53-54% of the total.

Environment affected flavanone levels ($p < 0.001$) (Table 18) although the environmental factor(s) were not evident. Flavanone levels were highest in samples grown in 2004 and 2005 (Table 19). Two samples (99LGWO50 and SC103 x SC745 (light)) had higher flavanone levels in 2003 than in the following years with levels of 19 and 27 µg/g, respectively. Flavanones were not detected in 02CA4796 for all three crop years. Eriodictyol was detected in B.01336, B.9904, and SC103 x SC748 (dark) in 2003-2005. Naringenin was consistently detected in the remaining samples from all three crop years with the exception of Tx430 Black in 2003 and Black PI Tall in 2003 and 2004. The naringenin proportion was consistent throughout the three crop years for SC103 x SC748

Table 18

Genotype (Variety) x Environment (Year) Interaction of Flavanones in Sorghums Grown in College Station, TX 2003-2005

Source	Type III Sum of Squares	df	Mean Square	F	Sig.	Partial Eta Squared
Corrected Model	26412.686[a]	38	695.071	1173.964	.000	.998
Intercept	46830.645	1	46830.645	79096.291	.000	.999
Variety	19382.564	12	1615.214	2728.073	.000	.998
Year	1374.800	2	687.400	1161.009	.000	.968
Variety * Year	5655.322	24	235.638	397.990	.000	.992
Error	46.182	78	.592			
Total	73289.513	117				
Corrected Total	26458.868	116				

[a]R Squared = .998 (Adjusted R Squared = .997)

(dark), which accounted for 69-75% of the total.

This study showed that sorghum genetics affected phenol content, profile, and antioxidant activity of sorghums with the *R_Y_* genes for red/black pericarp. The colorimetric methods showed that phenol and antioxidant activity levels were increased when sorghums had red/purple secondary plant color, a black or dark red pericarp, and a pigmented testa. Flavone levels were increased when sorghums had the gene for tan secondary plant color while 3-deoxyanthocyanin levels were increased when sorghums had the red/purple secondary plant color gene. Environment affected sorghum phenolic levels. These findings provide useful guidelines to produce sorghums with the greatest antioxidant activity levels and desired compounds, which are potentially quite important sources of healthy components in foods.

Table 19

Flavanone Levels in Sorghum Varieties Grown in College Station, TX 2003-2005[a]

Variety	Year	Eriodictyol	Naringenin	Total
02CA4796	2003	ND[b]	ND	ND
	2004	ND	ND	ND
	2005	ND	ND	ND
B.01336	2003	6.53 ± 0.38	5.57 ± 0.51	12.10 ± 0.88
	2004	8.13 ± 0.23	5.84 ± 0.08	13.97 ± 0.29
	2005	2.74 ± 0.16	2.64 ± 0.18	5.38 ± 0.34
99GWO92	2003	ND	8.08 ± 0.77	8.08 ± 0.77
	2004	ND	9.16 ± 0.28	9.16 ± 0.28
	2005	4.03 ± 0.33	6.71 ± 0.22	10.74 ± 0.55
98BRON155	2003	ND	48.40 ± 0.49	48.40 ± 0.49
	2004	ND	49.30 ± 1.99	49.30 ± 1.99
	2005	ND	22.14 ± 0.52	22.14 ± 0.52
99LGWO50	2003	ND	19.16 ± 0.13	19.16 ± 0.13
	2004	ND	7.91 ± 0.33	7.91 ± 0.33
	2005	ND	11.41 ± 0.13	11.41 ± 0.13
98CA4779	2003	ND	19.10 ± 1.02	19.10 ± 1.02
	2004	ND	37.01 ± 1.23	37.01 ± 1.23
	2005	ND	32.40 ± 0.93	32.40 ± 0.93
B.9904	2003	9.45 ± 0.82	7.17 ± 0.88	16.62 ± 1.70
	2004	17.15 ± 0.82	18.69 ± 0.66	35.84 ± 1.48
	2005	8.96 ± 0.49	9.97 ± 0.18	18.93 ± 0.66
SC103 x SC748 (light)	2003	5.56 ± 0.46	21.75 ± 0.71	27.31 ± 1.16
	2004	ND	22.19 ± 0.58	22.19 ± 0.58
	2005	4.70 ± 0.32	12.87 ± 0.27	17.57 ± 0.54

Table 19 - Continued

Variety	Year	Eriodictyol	Naringenin	Total
Tx2911	2003	ND	25.97 ± 0.32	25.97 ± 0.32
	2004	ND	28.44 ± 0.37	28.44 ± 0.37
	2005	ND	43.70 ± 0.69	43.70 ± 0.69
Tx430 Black	2003	ND	ND	ND
	2004	10.62 ± 0.16	9.33 ± 0.10	19.95 ± 0.22
	2005	7.75 ± 0.61	6.53 ± 0.04	14.28 ± 0.58
SC719-11E	2003	ND	15.80 ± 0.72	15.80 ± 0.72
	2004	ND	26.32 ± 0.47	26.32 ± 0.47
	2005	ND	20.69 ± 0.74	20.69 ± 0.74
SC103 x SC748 (dark)	2003	12.86 ± 0.23	28.76 ± 0.74	41.62 ± 0.93
	2004	15.83 ± 0.44	47.34 ± 0.80	63.17 ± 1.23
	2005	6.83 ± 0.07	15.92 ± 0.37	22.75 ± 0.31
Black PI Tall	2003	ND	ND	ND
	2004	9.55 ± 0.84	ND	9.55 ± 0.84
	2005	3.33 ± 0.24	ND	3.33 ± 0.24

[a]Levels are expressed as µg/g.
[b]ND = Not detected.

CHAPTER V

FLAVONOID PROFILE OF LEMON-YELLOW SORGHUMS

Sorghum Grain Characteristics

White pericarp sorghums with a yellow endosperm are often called yellow sorghums due to their appearance. However, these sorghums are not genetically yellow in terms of pericarp color and therefore much confusion exists. A lemon-yellow sorghum has a bright yellow pericarp once it reaches maturity. As the grains become more mature and are subjected to weathering, they turn light brown, tan or bronze.

Among the varieties grown in Lubbock, TX, SC1155 had the lowest *L** value (36.9) while Dorado had the highest *L** value (65.0) (Table 20). This was expected since the grains of SC1155 had a dark brown pericarp while those of Dorado had a white pericarp (Fig. 24). Dorado, which is grown on plants with tan secondary plant color, was free of pigments (Fig. 24). All samples had positive *a** values, which means they were more red than green. The *a** values for the red pericarp sorghums were, in general, higher (7.6-18.8) than the other sorghums (3.4-7.8). The *b** value was positive for all samples, which means that they were more yellow than blue; the *L** values increased as the *b** values increased with the exception of Dorado and B35.

The twelve samples were also grown in College Station, TX. At the time of harvest, no grains were available for B35 and SC35-14E since the panicles

Table 20

CIELAB *L, *a**, *b** Values of Sorghums Grown in Lubbock, TX 2005 and in College Station, TX 2006**

Variety	Location[a]	*L**	*a**	*b**
Dorado	LB 2005	65.01 ± 0.30	3.39 ± 0.10	18.47 ± 0.32
	CS 2006	62.58 ± 0.32	3.46 ± 0.27	19.00 ± 0.04
BRON176	LB 2005	51.40 ± 0.25	6.75 ± 0.11	21.88 ± 0.19
	CS 2006	44.38 ± 0.38	6.72 ± 0.04	14.81 ± 0.32
B35	LB 2005	51.32 ± 0.15	6.82 ± 0.15	19.28 ± 0.08
EBA3	LB 2005	56.02 ± 0.18	6.67 ± 0.11	24.21 ± 0.24
	CS 2006	52.19 ± 0.15	7.84 ± 0.08	23.31 ± 0.22
SC979	LB 2005	49.46 ± 0.05	7.76 ± 0.12	20.67 ± 0.11
	CS 2006	44.80 ± 0.05	8.16 ± 0.13	17.72 ± 0.02
SC35-14E	LB 2005	52.20 ± 0.12	5.55 ± 0.08	21.33 ± 0.10
SC1038	LB 2005	45.88 ± 0.18	6.80 ± 0.10	16.84 ± 0.14
	CS 2006	42.72 ± 0.28	6.47 ± 0.12	11.97 ± 0.28
New B Line	LB 2005	49.09 ± 0.29	7.13 ± 0.07	18.94 ± 0.15
	CS 2006	48.27 ± 0.78	6.56 ± 0.16	18.64 ± 0.71
SC748	LB 2005	50.76 ± 0.10	7.21 ± 0.09	20.88 ± 0.21
	CS 2006	46.05 ± 0.16	7.78 ± 0.09	17.63 ± 0.15
BTx399	LB 2005	53.73 ± 0.22	11.42 ± 0.15	21.12 ± 0.04
	CS 2006	49.61 ± 0.36	11.37 ± 0.17	19.13 ± 0.23
Tx2911	LB 2005	41.98 ± 0.24	18.82 ± 0.08	15.34 ± 0.33
	CS 2006	39.15 ± 0.10	17.95 ± 0.22	12.89 ± 0.28
SC1155	LB 2005	36.93 ± 0.17	7.63 ± 0.11	6.85 ± 0.13
	CS 2006	36.19 ± 0.17	7.99 ± 0.19	6.61 ± 0.12

[a]LB = Lubbock; CS = College Station.

Fig. 24. Sorghum varieties grown in Lubbock, TX 2005.

had been attacked by sorghum midge [*Stenodiplosis sorghicola* (Coquillett)]. The adult sorghum midge lays its eggs between the glumes of the flowering spikelets. The sorghum midge larva feeds on fertilized ovaries causing the prevention of sorghum kernel development (Teetes and Pendleton 2000). Overall, grains grown in Lubbock were brighter in color and had less weathering than those grown in College Station (Fig. 24, 25). This was due to Lubbock's drier environment with less rainfall compared to College Station. During the period of sorghum development, total rainfall for Lubbock in 2005 and College Station in 2006 were 7 and 14 inches, respectively (National Weather Service Forecast Office 2007). All sorghums grown in College Station had lower *L** values than those from Lubbock which confirmed that the former were darker in color.

Evaluation of Sorghum Total Phenols

Dorado had the lowest total phenol levels (1.4 mg GAE/g) while SC1155 had the highest levels (11.9 mg GAE/g) (Fig. 26). The high levels for SC1155 was due to the presence of condensed tannins as was confirmed by the vanillin/HCl assay, which gave a value of 25.1 mg CE/g (Fig. 27). Total phenol levels for red and lemon-yellow sorghums ranged from 2.0-11.9 mg GAE/g and 2.3-3.4 mg GAE/g, respectively. Secondary plant color did not have an effect on total phenols. BRON176, a tan plant sorghum, had the third highest total phenol levels among lemon-yellow sorghums.

Fig. 25. Sorghum varieties grown in College Station, TX 2006.

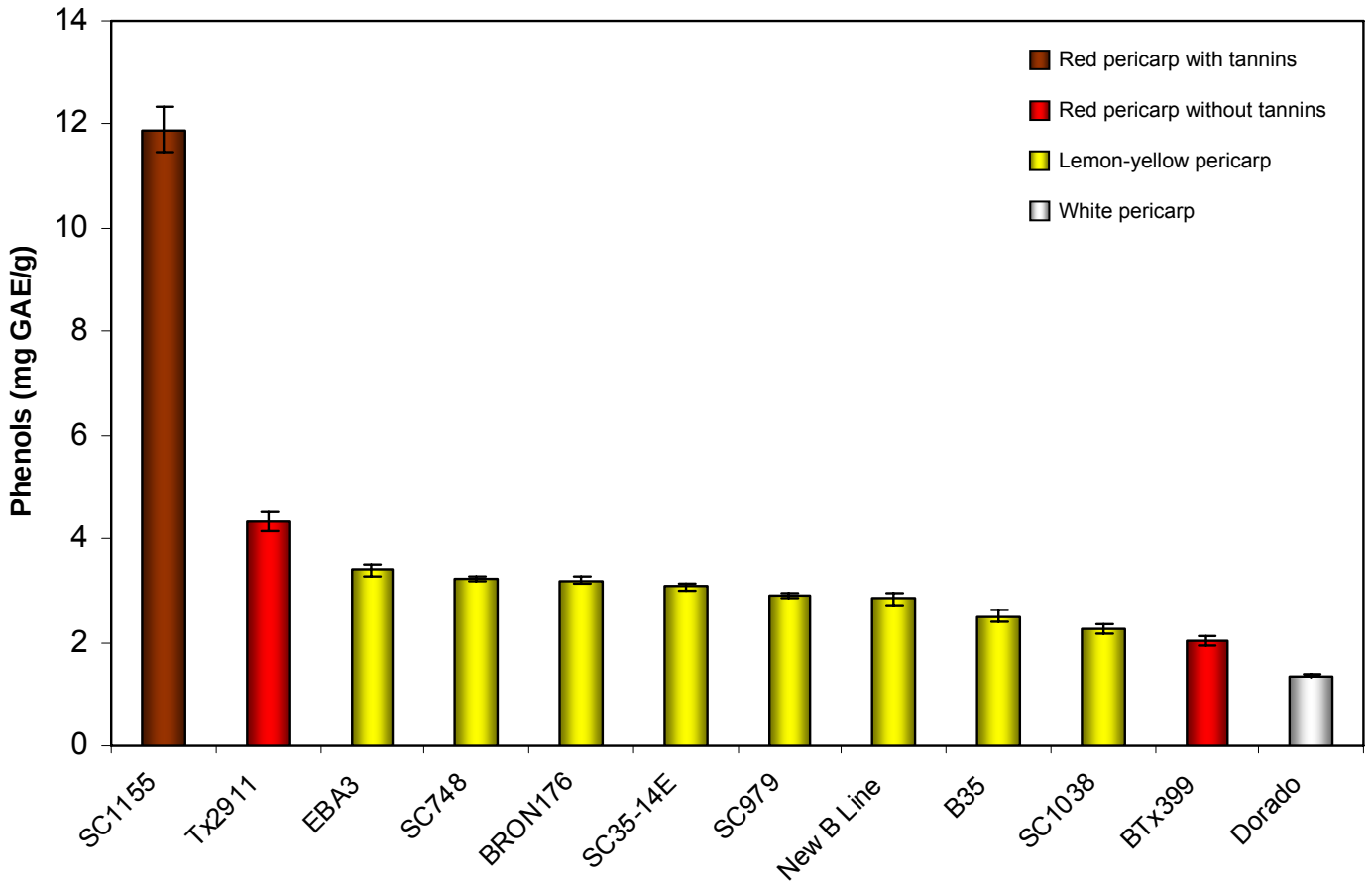

Fig. 26. Total phenol levels in sorghums grown in Lubbock, TX 2005.

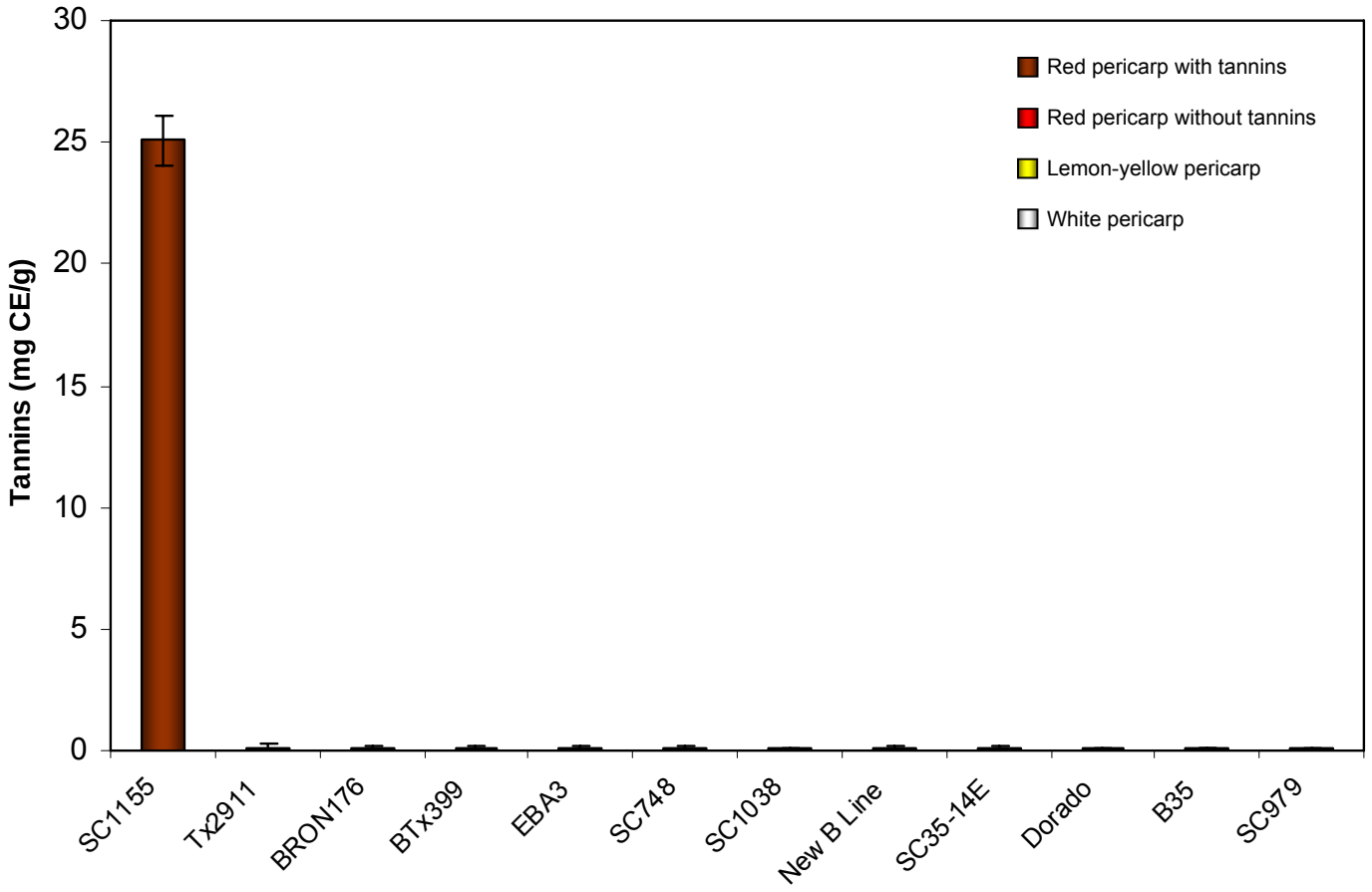

Fig. 27. Condensed tannin levels in sorghums grown in Lubbock, TX 2005.

Evaluation of Sorghum Flavan-4-ols

Red pericarp sorghums had the highest flavan-4-ol levels (1.1-4.5 abs/mL/g) followed by lemon-yellow (0.3-0.5 abs/mL/g) and white pericarp (0.2 abs/mL/g) sorghums (Fig. 28). These results were in agreement with Menkir et al (1996) who found higher flavan-4-ol levels in red sorghums. Tan plant sorghums (Dorado and BRON176) had the lowest flavan-4-ol levels (0.2-0.3 abs/mL/g). The low flavan-4-ol levels in the lemon-yellow sorghums could increase mold damage in those genotypes (Jambunathan et al 1990, 1991; Menkir et al 1996); however, further studies are needed to confirm this.

Evaluation of Sorghum Anthocyanins

Using the colorimetric method of Fuleki and Francis (1968), sorghums with a red pericarp had the highest anthocyanin levels (14.7-28.9 abs/mL/g) with the exception of BTx399, which had levels (5.5 abs/mL/g) comparable to the lemon-yellow sorghums (1.6-11.4 abs/mL/g) (Fig. 29). The two tan plant sorghums (Dorado and BRON176) had the lowest anthocyanin levels (0.6-1.6 abs/mL/g). These results suggested that red/purple plant sorghums produced higher levels of anthocyanins, which was consistent with the results found in the previous chapter. A strong correlation between flavan-4-ols and anthocyanins was found ($r = 0.93$, $p < 0.001$).

Evaluation of Sorghum Antioxidant Activity

SC1155 had the highest antioxidant activity (Fig. 30). This was due to the

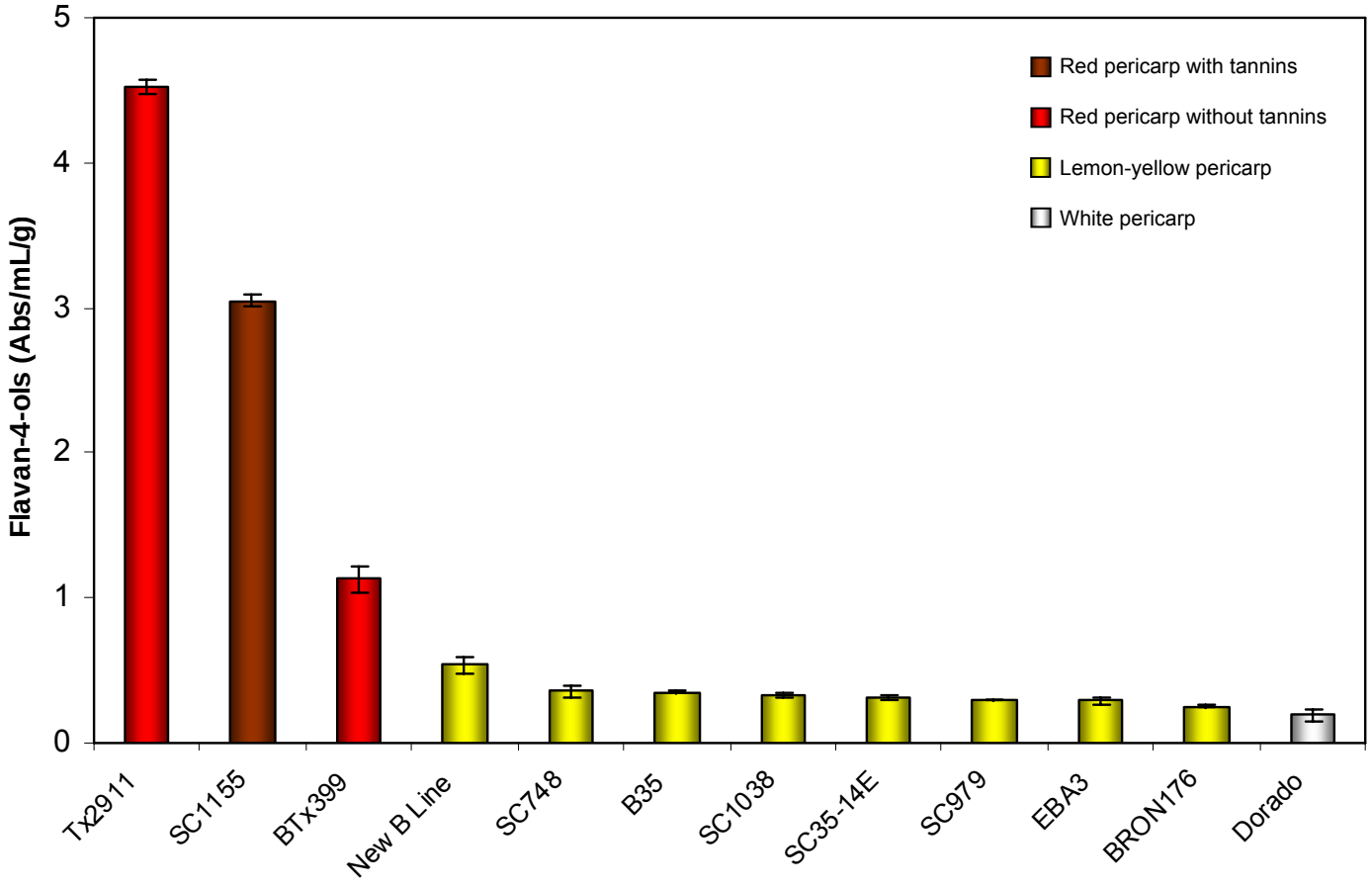

Fig. 28. Flavan-4-ol levels in sorghums grown in Lubbock, TX 2005.

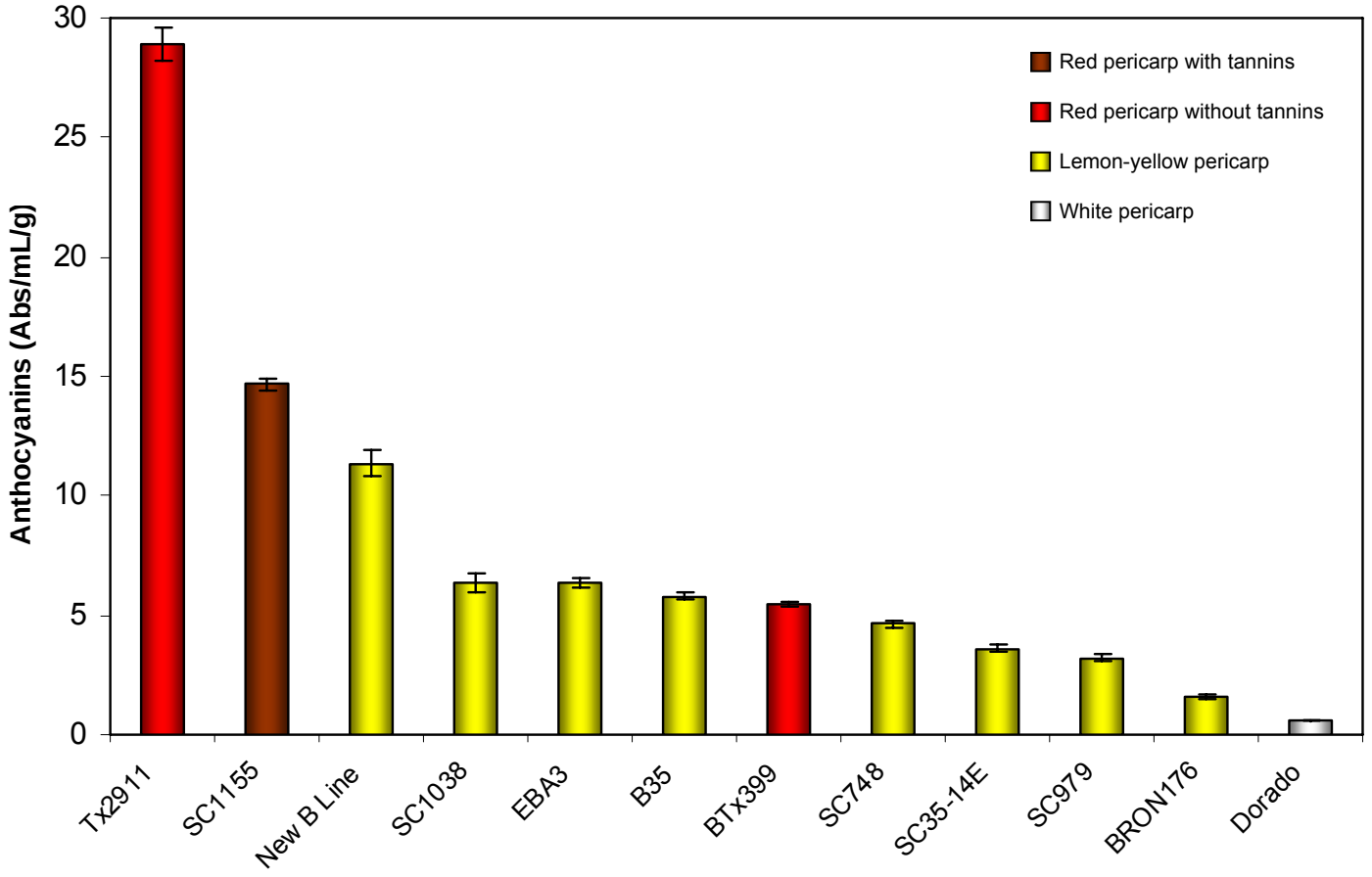

Fig. 29. Anthocyanin levels in sorghums grown in Lubbock, TX 2005.

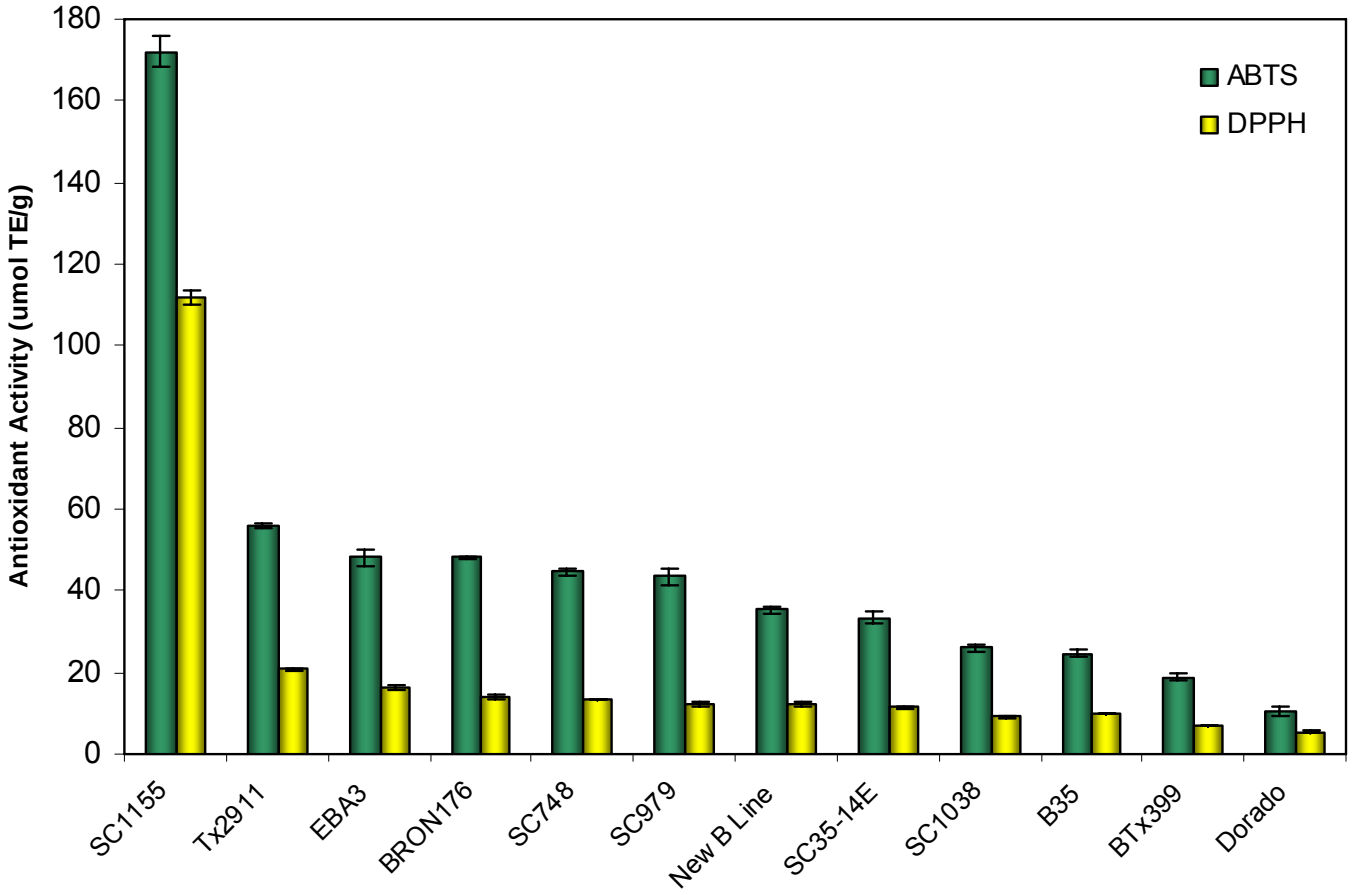

Fig. 30. Antioxidant activity potential of sorghums grown in Lubbock, TX 2005.

presence of condensed tannins, which have shown higher antioxidant activity *in vitro* than other phenolic compounds (Hagerman et al 1998; Amarowics et al 2003). ABTS and DPPH values for SC1155 were 172.1 and 111.8 µmol TE/g respectively. The antioxidant activity levels from the ABTS assay for lemon-yellow and red sorghums were 24.6-48.2 and 18.7-172.1 µmol TE/g, respectively. From the DPPH assay, antioxidant levels for lemon-yellow and red sorghums were 9.1-16.4 and 7.1-111.8 µmol TE/g respectively. Secondary plant color did not have an effect on antioxidant activity; however, the ANOVA analysis showed that pericarp color had a significant effect on antioxidant activity ($p \leq 0.001$). A strong correlation between total phenols and antioxidant activity was observed (total phenols vs ABTS, $r = 0.99$; total phenols vs DPPH, $r = 0.99$), which indicated that antioxidant activity was contributed by phenolic compounds. This strong correlation could be due to SC1155, which had condensed tannins and therefore increased the correlations. When that variety was removed from the analysis, strong correlations were still observed (total phenols vs ABTS, $r = 0.94$; total phenols vs DPPH, $r = 0.97$), which indicated that the antioxidant activity was contributed by non-tannin phenols such as phenolic acids and flavonoids.

Evaluation of Sorghum 3-Deoxyanthocyanins

The four major 3-deoxyanthocyanins detected were luteolinidin (LUT), apigeninidin (AP), 5-methoxyluteolinidin (5-MeO-LUT), and 7-apigeninidin (7-MeO-AP) (Fig. 31); the common anthocyanins (i.e. cyanidin) were not detected.

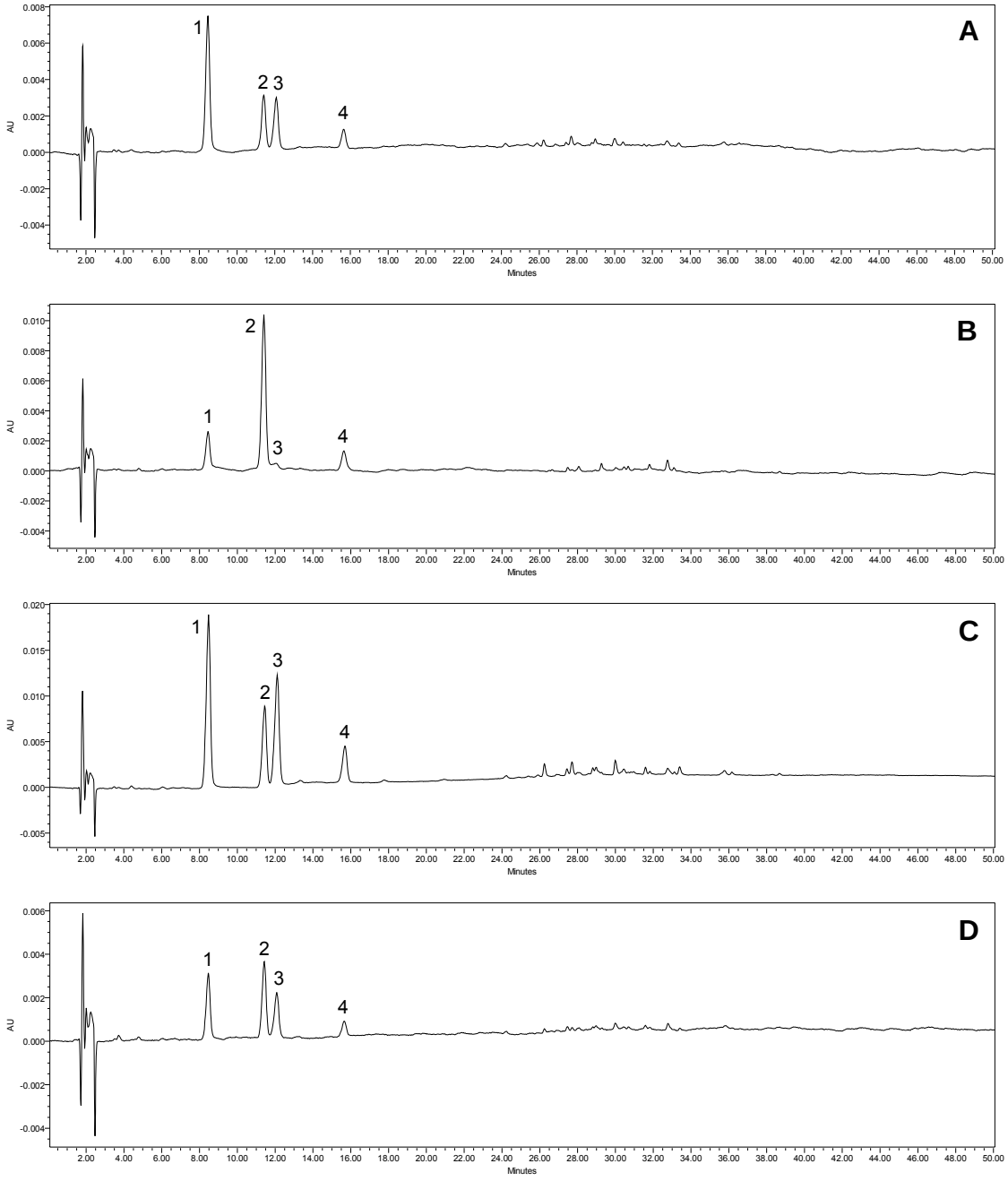

Fig. 31. HPLC chromatograms of 3-deoxyanthocyanins in lemon-yellow sorghums grown in Lubbock, TX 2005. A) B35; B) EBA3; C) New B Line; D) SC748. 1 = Luteolinidin; 2 = Apigeninidin; 3 = 5-Methoxyluteolinidin; 4 = 7-Methoxyapigeninidin. PDA = 485 nm.

Red/purple plant sorghums had higher 3-deoxyanthocyanin levels (8-62 µg/g) than tan plant sorghums (1 µg/g) with New B Line having the highest levels among the varieties (Fig. 32). This confirmed that tan plant sorghums produce low levels of these compounds as found in the previous chapter. Pericarp color had no effect on the 3-deoxyanthocyanin levels since there were no significant differences between red and lemon-yellow sorghums ($p > 0.05$). The presence of a pigmented testa did not increase 3-deoxyanthocyanin levels.

Two possibilities could explain the presence of these compounds. The 3-deoxyanthocyanins are phytoalexins that are formed once the grain undergoes fungal attack (Lo et al 1996; Nicholson and Wood 2001; Seitz 2004) or other damages. Red/purple plant sorghums have red/purple “stains” or “spots” on the pericarp; tan plant sorghums do not have these stains (Fig. 24). For the lemon-yellow sorghums, the extractable 3-deoxyanthocyanins could be coming from these “stains”. Another possibility is that these compounds were coming from the red/purple glumes, which were attached to the grains during their development. These compounds could have leached to the pericarp as seen in EBA3 where red pigmentation was observed on the hilar area (Fig. 24). It was observed in our laboratory that purple glumes of black sorghum (Tx430 Black) are a rich source of 3-deoxyanthocyanins [(8000 µg/g), unpublished data]. This phenomenon could have contributed to the detection of these compounds; however, this was not supported by Seitz (2004).

The 3-deoxyanthocyanin profile varied among samples (Fig. 33). Red

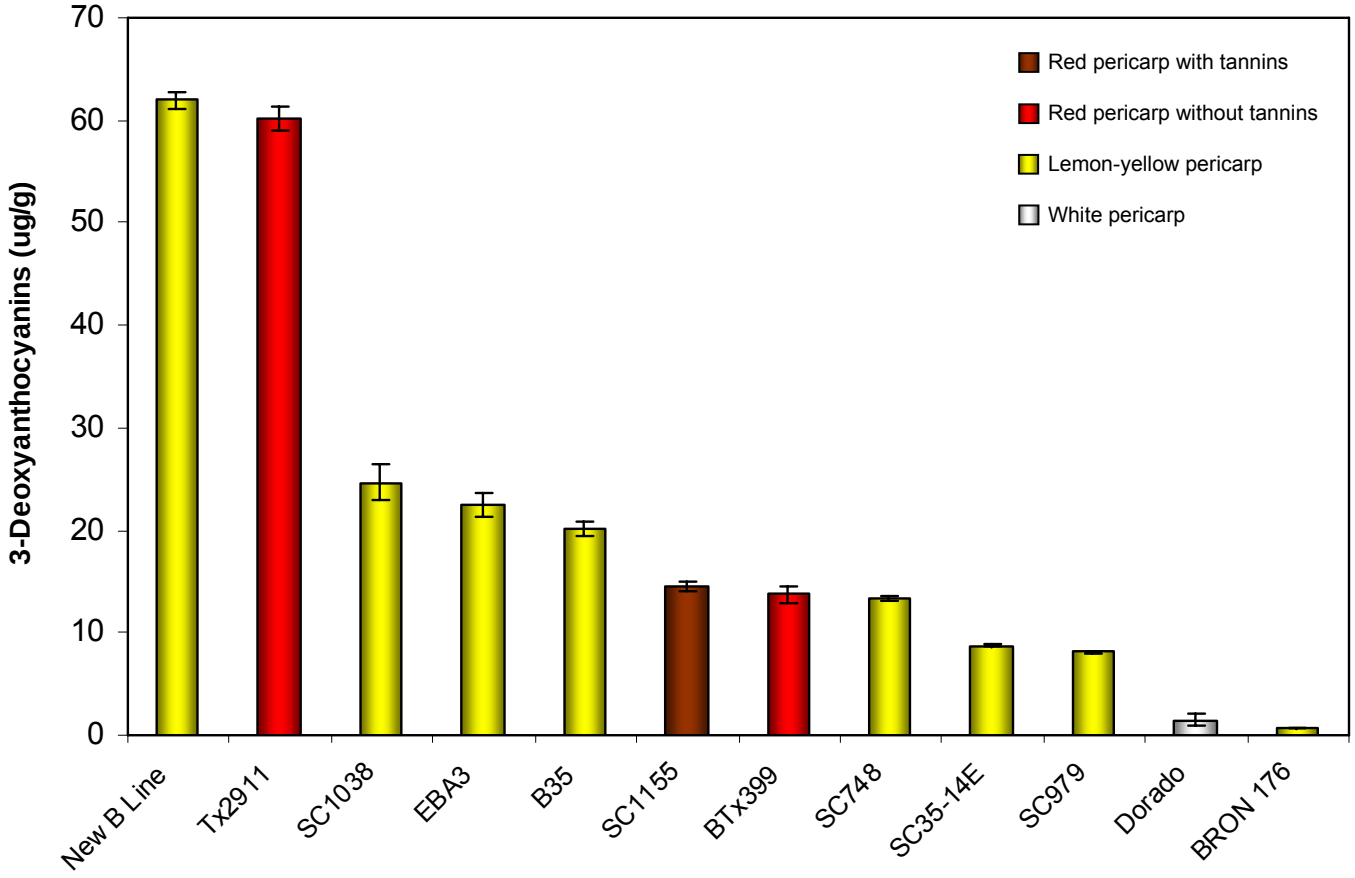

Fig. 32. 3-Deoxyanthocyanin levels in sorghums grown in Lubbock, TX 2005.

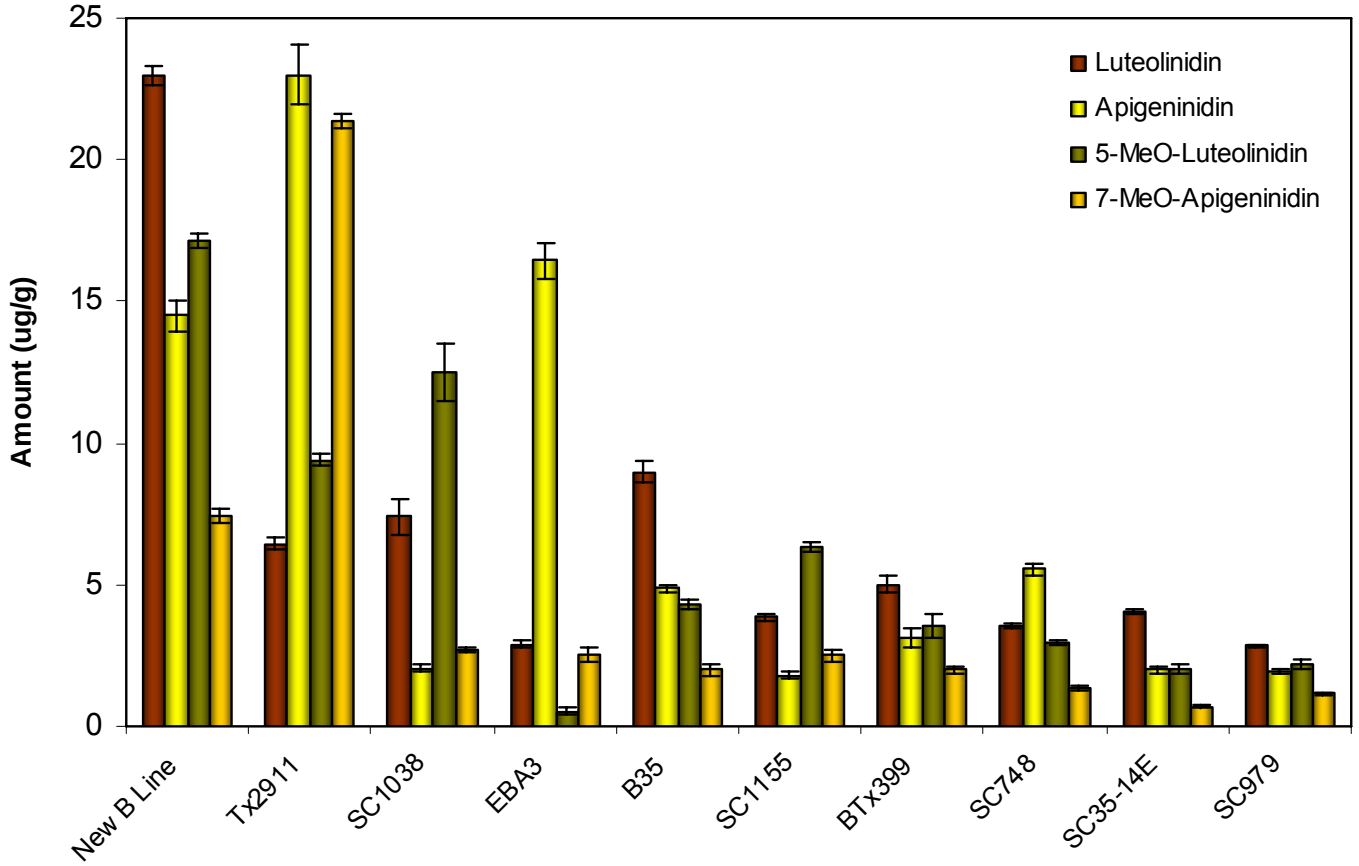

Fig. 33. 3-Deoxyanthocyanin profile of sorghums grown in Lubbock, TX 2005.

plant sorghums (EBA3 and Tx2911) had high proportions of AP and 7-MeO-AP, which accounted for 74-85% of their total. The yellow AP was the main 3-deoxyanthocyanin in EBA3 which accounted for 73% of the total. All purple plant sorghums, with the exception of SC748, had high proportions of LUT and 5-MeO-LUT, which accounted for 62-81% of their total. For SC748, only 48% of the total was accounted by the two compounds. These results suggested that secondary plant color had an effect on 3-deoxyanthocyanin composition as observed in the previous chapter.

Evaluation of Sorghum Flavones

Two flavones were detected in the samples: luteolin and apigenin (Fig. 34). Lemon-yellow sorghums had higher levels of flavones (34-268 µg/g) than those from white/red pericarp sorghums (4-19 µg/g) (Fig. 35) suggesting that pericarp color had an effect on flavone levels. Unsurprisingly, BRON176 had the highest flavone levels (268 µg/g) since it was a tan plant sorghum, which further supports the findings reported in the previous chapter.

Information on the cause of the presence of these compounds is limited. Since luteolin and apigenin are yellow and pale yellow respectively, it was difficult to visually see them on the grain. Seitz (2004) reported that these compounds are concentrated on the pericarp and it was suggested that these compounds are phytoalexins. There is the possibility that these compounds are leached from the glumes to the grain however it was not supported by Seitz (2004). Apigenin was the predominant flavone in BRON176 and Dorado, which

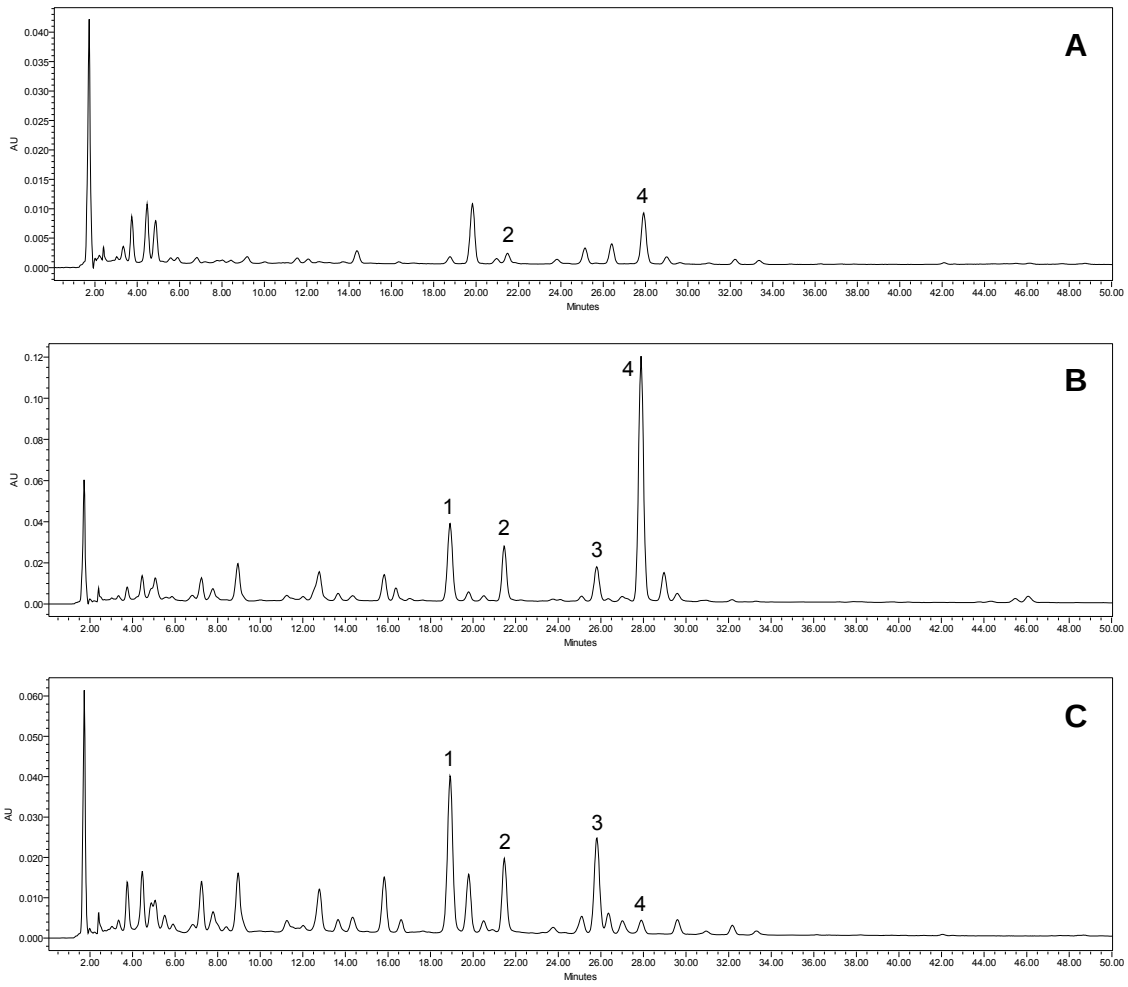

Fig. 34. HPLC chromatograms of flavonoids in A) Dorado; B) BRON176; C) SC748 before acid hydrolysis. 1 = Eriodictyol; 2 = Luteolin; 3 = Naringenin; 4 = Apigenin. PDA = 340 nm.

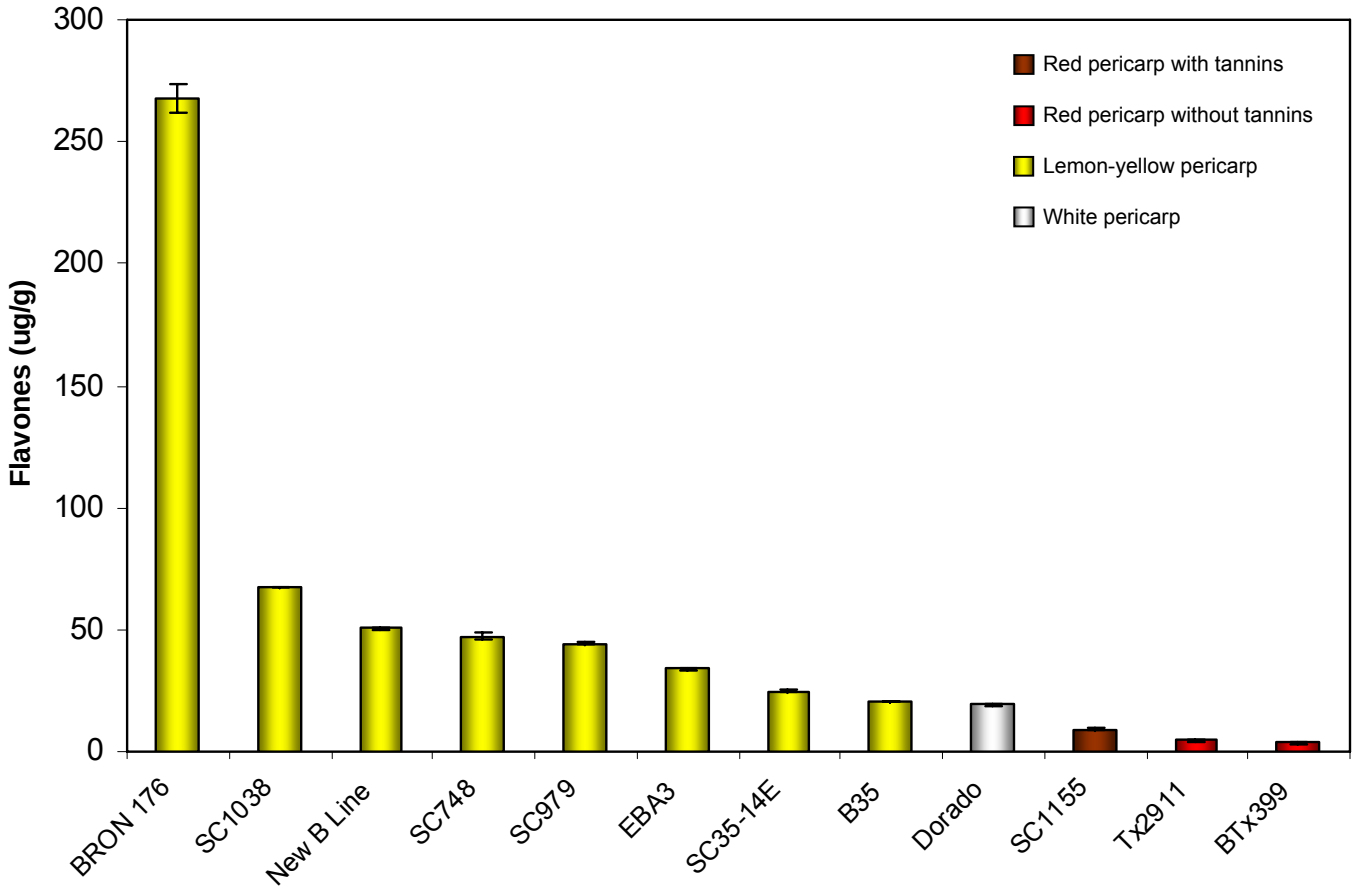

Fig. 35. Flavone levels in sorghums grown in Lubbock, TX 2005.

accounted for 80% of the total (Fig. 36). On the other hand, luteolin was the main flavone in red/purple plant varieties, which accounted for 77-100% of the total. SC1038 had the highest luteolin levels (65 μg/g) while apigenin was not detected in Tx2911.

Evaluation of Sorghum Flavanones

Flavanones were first extracted using the same extraction method as for the other flavonoid analysis. However, for the lemon-yellow sorghums, it was observed that the flavanones, eriodictyol and naringenin, were synthesized as the extracts were sitting in queue to be analyzed. It was therefore necessary to identify the original compounds that were turning into flavanones. The first possibility is that the two starting materials could have been eriodictyol and naringenin chalcones which, in the presence of HCl, turned into eriodictyol and naringenin, respectively (Gujer et al 1986; Tomás-Barberán and Clifford 2000). The second possibility is that the two starting materials were flavanone glucosides which lost their glucose moiety while being exposed to the acidic extraction solvent.

To determine the cause of the flavanone syntheses, a ground sample (SC748) was extracted in methanol for two hours and the extract was injected into the HPLC. Two major peaks were found (Fig. 37 A) and their PDA spectra showed their lambda max was at 283-284 nm (Fig. 38), which were typical for flavanones (Merken and Beecher 2001). The possibility that the starting materials were chalcones was eliminated since these compounds absorb

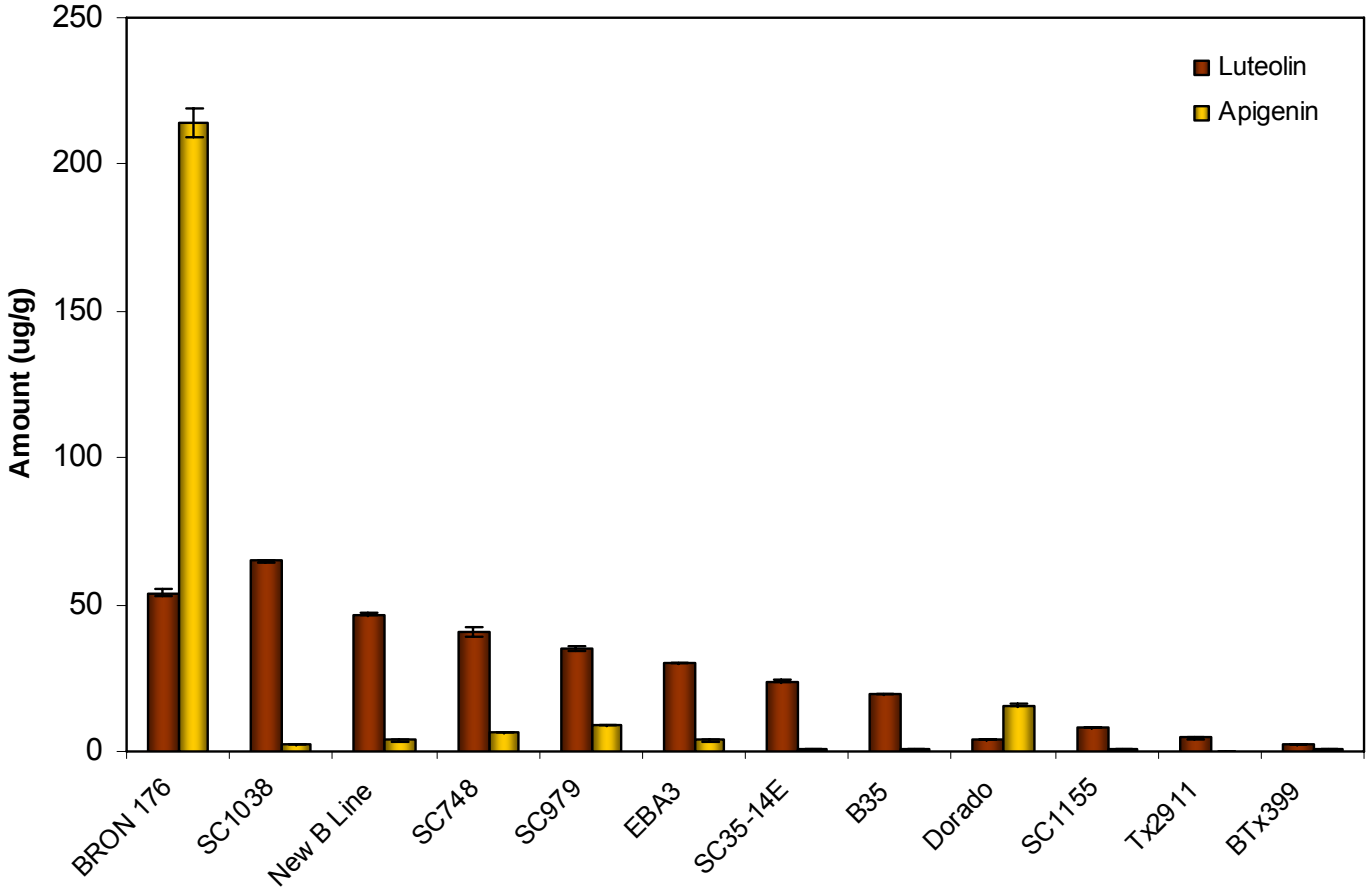

Fig. 36. Flavone profile of sorghums grown in Lubbock, TX 2005.

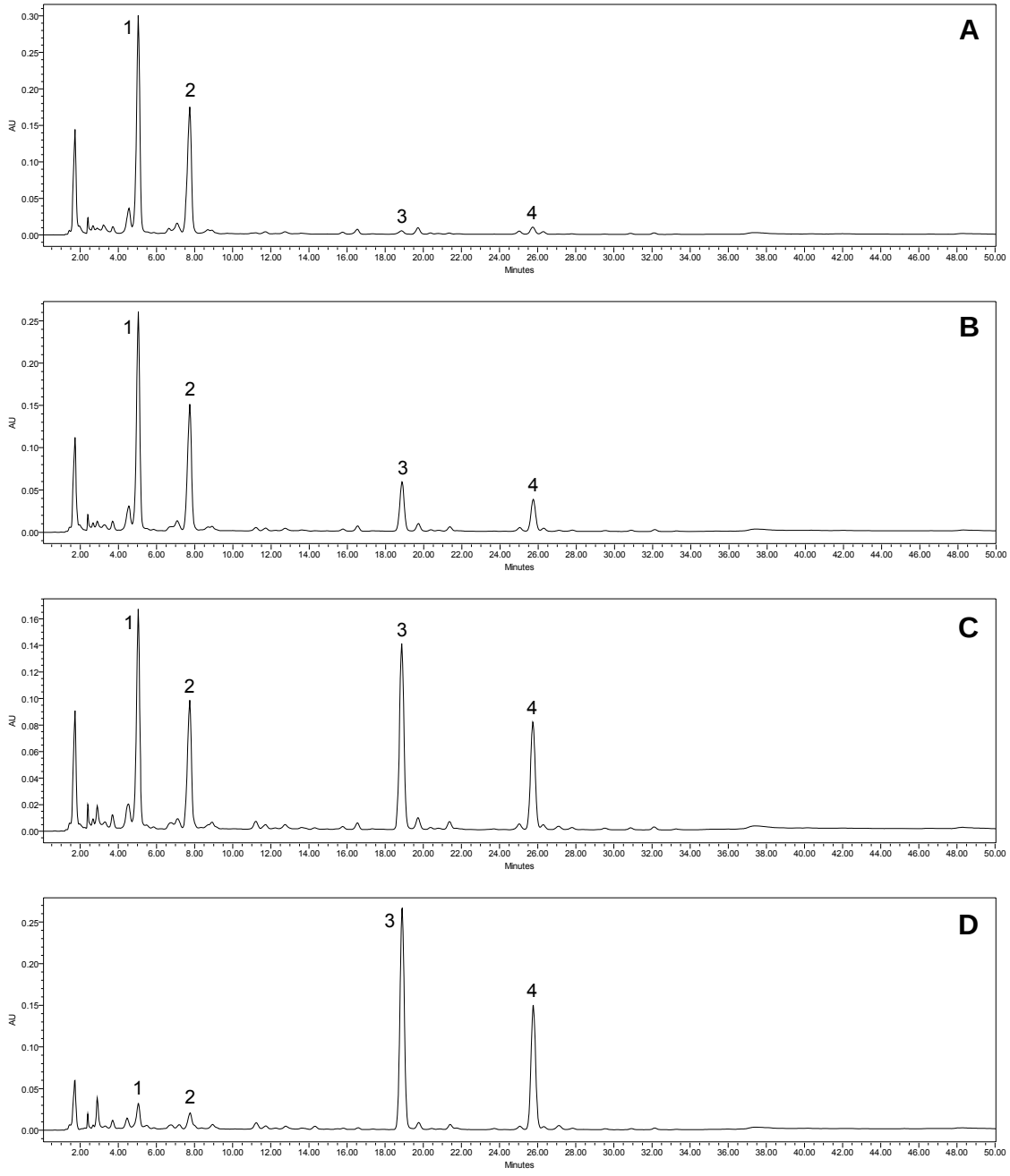

Fig. 37. Formation of eriodictyol and naringenin in SC748 after the addition of HCl in methanol after A) 0, B) 2, C) 6, D) 24 hrs. 1 = Eriodictyol glucoside; 2 = Naringenin glucoside; 3 = Eriodictyol; 4 = Naringenin. PDA = 280 nm.

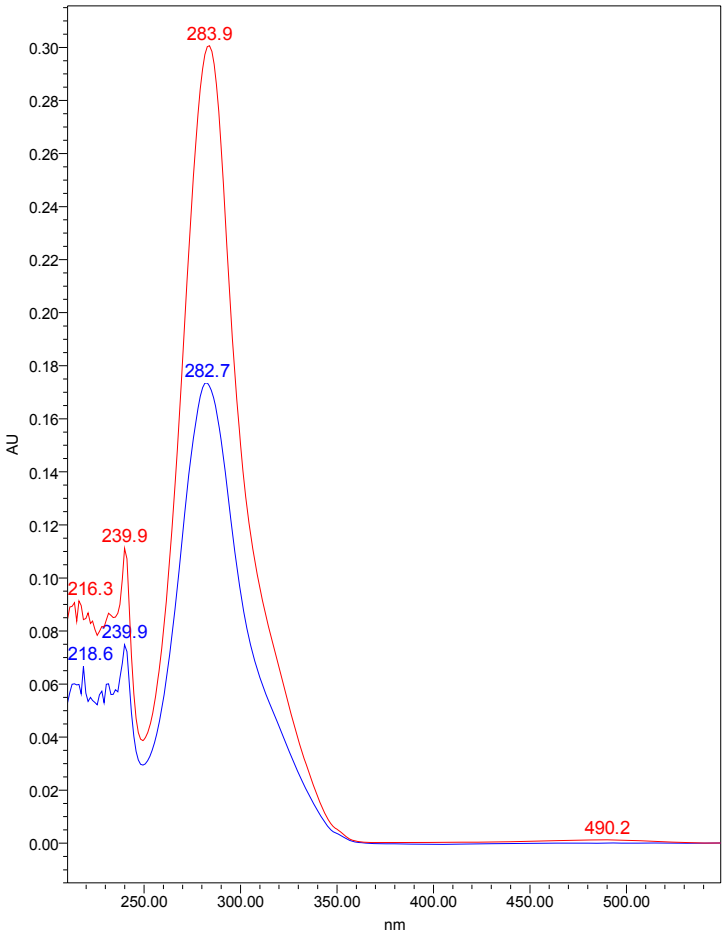

Fig. 38. PDA spectra of flavanone glucosides. (—— eriodictyol glucoside; —— naringenin glucoside).

strongly at 340-390 nm (Gujer et al 1986; Waterman and Mole 1994). Hydrochloric acid was then added to the extract so that its composition was 1% and the extract was injected into the HPLC at one-hour intervals for a total of 24 hours. It was observed that the peaks of the two flavanone glucosides decreased while the peaks of eriodictyol and naringenin increased over time (Fig. 37 B-D).

Characterization of the two compounds was achieved by LC-MS-ESI. Peak 1 (Fig. 37) was identified as eriodictyol glucoside ($[M]^-$, *m/z* 449; MS/MS, *m/z* 287). Peak 2 (Fig. 37) was identified as naringenin glucoside ($[M]^-$, *m/z* 433; MS/MS, *m/z* 271). Since both flavanone glucosides were still reacting to produce their aglycones even after 24 hours, it was necessary to end the reaction in a shorter period of time. The best method is to perform acid hydrolysis (Merken and Beecher 2001). The acidified methanol extract was boiled for 90 minutes at 80ºC to hydrolyze both eriodictyol and naringenin glucosides to their aglycones (Fig. 39).

Lemon-yellow sorghums had the highest flavanone levels (474-1780 µg/g) with EBA3 having the highest (Fig. 40). Red sorghums had flavanone levels of 68-238 µg/g. No flavanones were detected in the white pericarp sorghum. These compounds were concentrated in the pericarp since decorticating the grain to produce bran concentrated these compounds almost ten-fold (Fig. 41).

Eriodictyol was the predominant flavanone in the lemon-yellow sorghums,

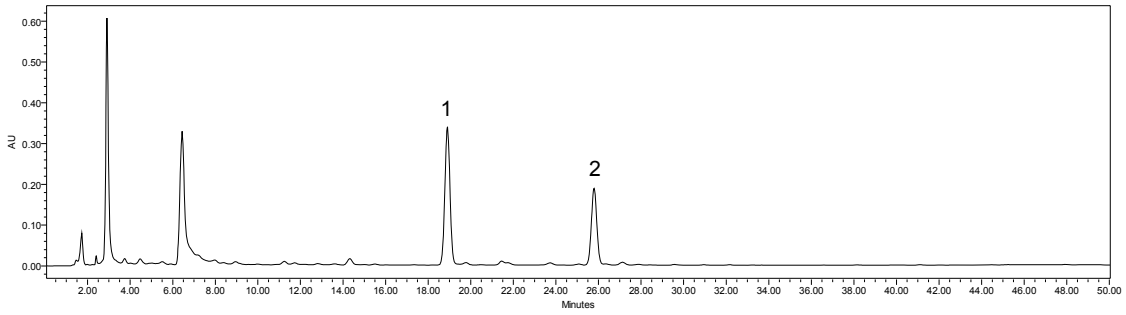

Fig. 39. HPLC chromatogram of SC748 after acid hydrolysis. 1 = Eriodictyol; 2 = Naringenin. PDA = 280 nm.

which accounted for 62-92% of the total (Fig. 42). EBA3 had the highest levels of eriodictyol (1504 µg/g) followed by BRON176 (987 µg/g) and SC35-14E (946 µg/g). In red pericarp sorghums, eriodictyol accounted for 0-69% with SC1155 having the highest levels (163 µg/g). Eriodictyol was not detected in Tx2911. SC748 had the highest level of naringenin (567 µg/g) followed by SC979 (479 µg/g) and BRON176 (441 µg/g) (Fig. 42).

Effect of Environment on Phenols and Antioxidant Activity Levels

Overall, samples from College Station had higher total phenol, condensed tannin, flavan-4-ol, and anthocyanin levels than those from Lubbock (Table 21). Only one sample from College Station (BRON176) had lower total phenol levels (3.2 mg GAE/g) than the one from Lubbock (2.9 mg GAE/g). Flavan-4-ol levels in Dorado, New B Line, and BRON176 did not differ among locations. The antioxidant activity levels of samples from College Station were mostly lower than those from Lubbock even though their total phenols were higher than those

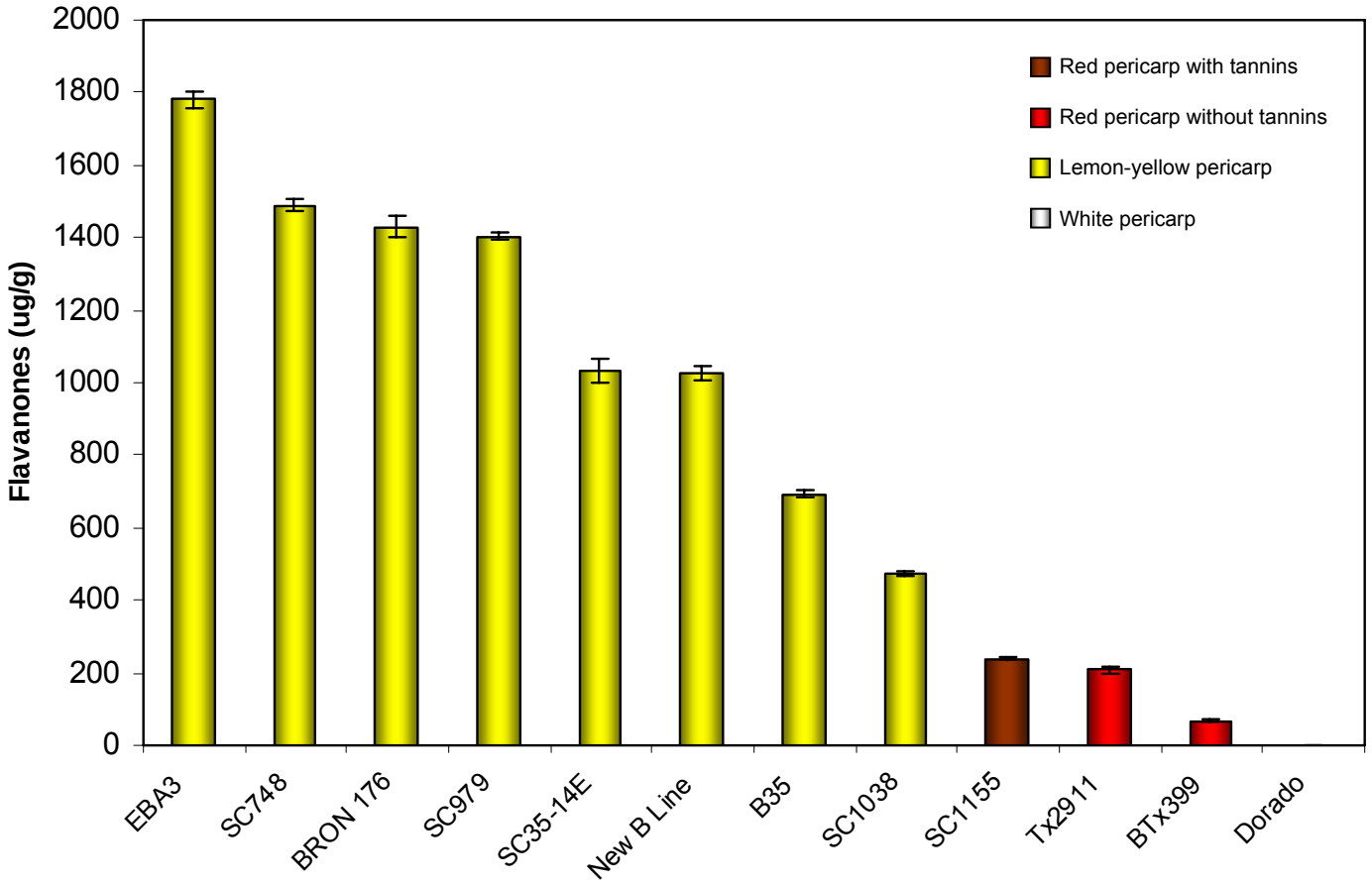

Fig. 40. Flavanone levels in sorghums grown in Lubbock, TX 2005.

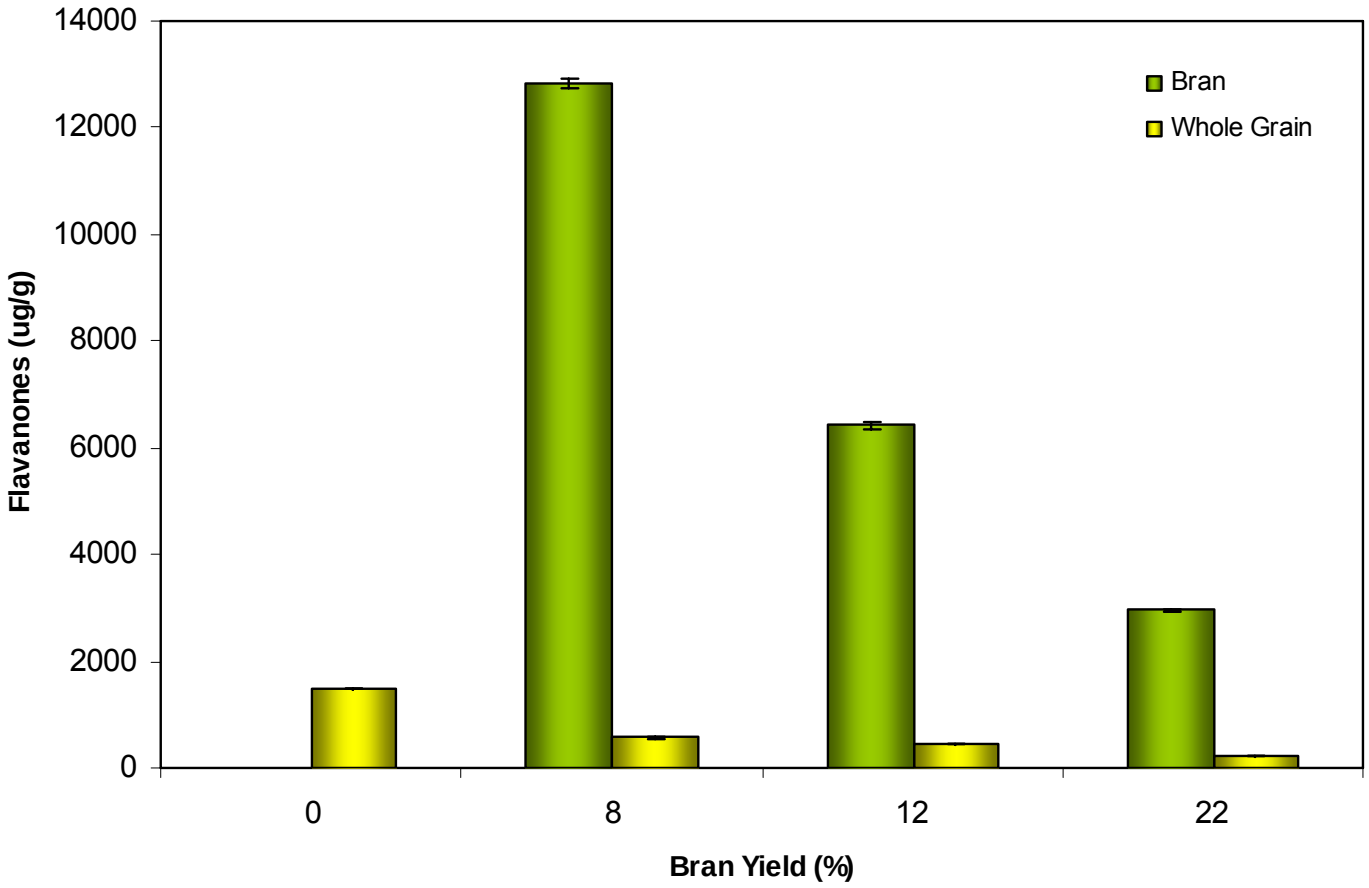

Fig. 41. Flavanone levels in decorticated fractions of SC748.

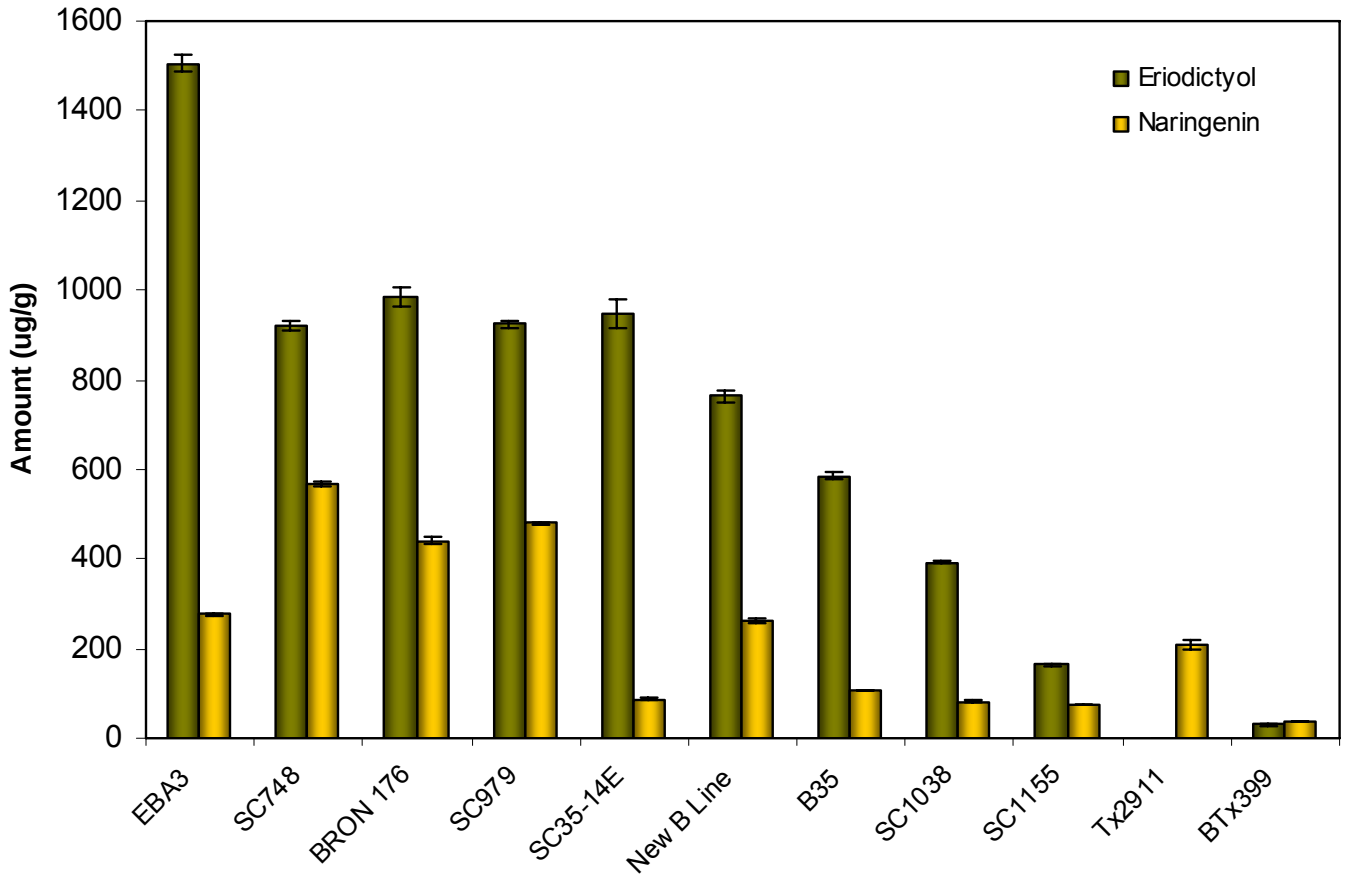

Fig. 42. Flavanone profile of sorghums grown in Lubbock, TX 2005.

Table 21

Phenol and Antioxidant Activity Levels in Sorghums Grown in Lubbock, TX 2005 and in College Station, TX 2006

Variety	Location[a]	Total Phenols (mg GAE/g)[b]	Tannins (mg CE/g)[c, d]	Flavan-4-ols (Abs/mL/g)	Anthocyanins (Abs/mL/g)	ABTS (umol TE/g)[e]	DPPH (umol TE/g)
Dorado	LB 2005	1.35 ± 0.02	0.06 ± 0.06	0.19 ± 0.04	0.58 ± 0.00	10.45 ± 1.04	5.48 ± 0.25
	CS 2006	1.65 ± 0.02	0.02 ± 0.03	0.18 ± 0.00	0.52 ± 0.12	9.67 ± 0.15	3.97 ± 0.11
BRON176	LB 2005	3.19 ± 0.09	0.10 ± 0.06	0.25 ± 0.01	1.55 ± 0.09	48.07 ± 0.48	13.90 ± 0.48
	CS 2006	2.91 ± 0.04	0.10 ± 0.00	0.28 ± 0.03	2.60 ± 0.12	33.89 ± 0.31	8.86 ± 0.13
EBA3	LB 2005	3.39 ± 0.11	0.08 ± 0.09	0.29 ± 0.02	6.34 ± 0.18	48.16 ± 1.90	16.36 ± 0.51
	CS 2006	3.69 ± 0.07	0.11 ± 0.07	0.40 ± 0.03	14.51 ± 0.97	39.16 ± 0.90	14.71 ± 0.30
SC979	LB 2005	2.90 ± 0.06	0.06 ± 0.06	0.30 ± 0.00	3.23 ± 0.16	43.45 ± 2.03	12.23 ± 0.60
	CS 2006	3.47 ± 0.08	0.06 ± 0.07	0.60 ± 0.03	14.35 ± 0.75	40.90 ± 0.51	13.46 ± 0.76
SC1038	LB 2005	2.27 ± 0.09	0.08 ± 0.03	0.33 ± 0.01	6.37 ± 0.37	26.08 ± 0.84	9.12 ± 0.45
	CS 2006	2.52 ± 0.03	0.07 ± 0.03	0.79 ± 0.03	22.41 ± 0.76	18.90 ± 0.87	8.11 ± 0.05
New B Line	LB 2005	2.84 ± 0.13	0.08 ± 0.09	0.54 ± 0.06	11.35 ± 0.53	35.31 ± 0.74	12.06 ± 0.48
	CS 2006	3.04 ± 0.11	0.12 ± 0.03	0.56 ± 0.03	12.16 ± 0.29	28.71 ± 0.83	9.86 ± 0.41
SC748	LB 2005	3.23 ± 0.06	0.07 ± 0.13	0.36 ± 0.03	4.64 ± 0.14	44.58 ± 0.84	13.31 ± 0.06
	CS 2006	3.62 ± 0.04	0.05 ± 0.09	0.50 ± 0.03	10.44 ± 0.86	42.92 ± 0.55	13.84 ± 0.44
BTx399	LB 2005	2.02 ± 0.10	0.08 ± 0.09	1.14 ± 0.09	5.48 ± 0.09	18.71 ± 0.81	7.07 ± 0.21
	CS 2006	2.52 ± 0.06	0.19 ± 0.00	1.82 ± 0.06	13.31 ± 0.58	20.14 ± 0.15	9.01 ± 0.20
Tx2911	LB 2005	4.33 ± 0.17	0.13 ± 0.12	4.52 ± 0.06	28.92 ± 0.71	55.90 ± 0.83	20.74 ± 0.49
	CS 2006	4.80 ± 0.09	0.85 ± 0.05	6.99 ± 0.16	61.99 ± 1.15	71.02 ± 0.80	23.02 ± 0.73
SC1155	LB 2005	11.90 ± 0.45	25.08 ± 0.99	3.05 ± 0.05	14.66 ± 0.28	172.13 ± 3.93	111.80 ± 1.76
	CS 2006	15.03 ± 0.30	33.64 ± 0.51	2.84 ± 0.06	23.64 ± 0.24	173.15 ± 6.27	144.69 ± 3.62

[a]LB = Lubbock; CS = College Station.
[b]GAE = Gallic acid equivalents.
[c]CE = Catechin equivalents.
[d]Varieties with values lower than 2.00 are considered tannin-free.
[e]TE = Trolox equivalents.

from Lubbock. After removing the tannin sorghum (SC1155), a strong correlation between total phenols and antioxidant activity was observed for the College Station samples (total phenols vs ABTS, $r = 0.97$; total phenols vs DPPH, $r = 0.98$).

Samples from College Station had higher 3-deoxyanthocyanin levels than those from Lubbock with the exception of Dorado and New B Line (Table 22). This was expected since Lubbock has a drier climate with less rainfall than College Station. The environmental conditions in College Station would render the grains more prone to mold damage and, as a result, increase the production of 3-deoxyanthocyanins. The general linear model showed a genotype x environment interaction ($p < 0.001$) (Table 23), which suggested that environment had an effect on 3-deoxyanthocyanins. The 3-deoxyanthocyanin proportions in the College Station samples did not differ from those of Lubbock except for Dorado, SC979, BTx399, and SC1155.

Environment also had an effect on flavone levels ($p < 0.001$) (Table 24) although no trend was shown (Table 25). Some samples from College Station (BRON176 and SC979) had higher flavone levels than those from Lubbock whereas the opposite was observed for other samples (i.e. SC1038, New B line). The flavone levels for SC748 did not vary among locations. Pericarp color did not have an effect on flavones ($p > 0.05$) as was observed in the samples from Lubbock. The flavone proportion did vary among locations however. Most samples from College Station had a lower proportion of luteolin than those from

Table 22

3-Deoxyanthocyanin Levels in Sorghums Grown in Lubbock, TX 2005 and in College Station, TX 2006[a]

Variety	Location[b]	LUT[c]	AP[d]	5-MeO-LUT[e]	7-MeO-AP[f]	Total
Dorado	LB 2005	0.46 ± 0.06	0.43 ± 0.15	0.41 ± 0.04	0.18 ± 0.31	1.48 ± 0.54
	CS 2006	ND[g]	0.34 ± 0.05	ND	0.27 ± 0.26	0.61 ± 0.30
BRON176	LB 2005	ND	ND	ND	0.63 ± 0.04	0.63 ± 0.04
	CS 2006	ND	ND	ND	0.75 ± 0.07	0.75 ± 0.07
EBA3	LB 2005	2.90 ± 0.14	16.44 ± 0.61	0.52 ± 0.13	2.56 ± 0.26	22.42 ± 1.12
	CS 2006	7.84 ± 0.03	58.11 ± 1.56	2.02 ± 0.30	6.76 ± 0.15	74.73 ± 1.70
SC979	LB 2005	2.83 ± 0.08	1.96 ± 0.10	2.17 ± 0.17	1.15 ± 0.07	8.11 ± 0.10
	CS 2006	17.57 ± 0.53	19.77 ± 1.69	12.82 ± 0.30	11.82 ± 0.35	61.98 ± 2.47
SC1038	LB 2005	7.40 ± 0.67	2.06 ± 0.14	12.52 ± 1.01	2.71 ± 0.06	24.69 ± 1.81
	CS 2006	35.93 ± 1.79	17.05 ± 0.59	41.49 ± 2.72	12.97 ± 0.53	107.44 ± 5.32
New B Line	LB 2005	22.94 ± 0.34	14.48 ± 0.56	17.11 ± 0.24	7.41 ± 0.25	61.94 ± 0.87
	CS 2006	14.62 ± 0.27	8.52 ± 0.23	10.63 ± 0.37	5.10 ± 0.19	38.87 ± 0.60
SC748	LB 2005	3.54 ± 0.06	5.54 ± 0.21	2.95 ± 0.07	1.36 ± 0.07	13.39 ± 0.23
	CS 2006	10.18 ± 0.32	16.57 ± 1.12	7.65 ± 0.08	4.46 ± 0.04	38.86 ± 1.21
BTx399	LB 2005	5.01 ± 0.31	3.14 ± 0.33	3.54 ± 0.39	2.01 ± 0.13	13.70 ± 0.90
	CS 2006	14.21 ± 0.64	6.26 ± 0.19	14.92 ± 0.43	4.57 ± 0.10	39.96 ± 1.23
Tx2911	LB 2005	6.42 ± 0.21	22.98 ± 1.06	9.39 ± 0.23	21.37 ± 0.26	60.16 ± 1.16
	CS 2006	20.23 ± 0.55	74.86 ± 2.20	24.87 ± 0.53	66.85 ± 0.79	186.81 ± 3.90
SC1155	LB 2005	3.85 ± 0.14	1.81 ± 0.11	6.33 ± 0.16	2.49 ± 0.21	14.48 ± 0.55
	CS 2006	14.30 ± 0.69	12.46 ± 0.60	15.73 ± 0.63	9.87 ± 0.52	52.36 ± 2.38

[a]Levels are expressed as µg/g.
[b]LB = Lubbock; CS = College Station.
[c]LUT = Luteolinidin.
[d]AP = Apigeninidin.
[e]5-MeO-LUT = 5-Methoxyluteolinidin.
[f]7-MeO-AP = 7-Methoxyapigeninidin.
[g]ND = Not detected.

TABLE 23

Genotype (Variety) x Environment (Location) Interaction of 3-Deoxyanthocyanins in Sorghums Grown in Lubbock, TX 2005 and in College Station, TX 2006

Source	Type III Sum of Squares	df	Mean Square	F	Sig.	Partial Eta Squared
Corrected Model	115626.399[a]	19	6085.600	1746.826	.000	.999
Intercept	101694.017	1	101694.017	29190.516	.000	.999
Variety	67883.677	9	7542.631	2165.056	.000	.998
Location	21810.360	1	21810.360	6260.503	.000	.994
Variety * Location	25932.362	9	2881.374	827.077	.000	.995
Error	139.352	40	3.484			
Total	217459.768	60				
Corrected Total	115765.751	59				

[a]R Squared = .999 (Adjusted R Squared = .998)

TABLE 24

Genotype (Variety) x Environment (Location) Interaction of Flavones in Sorghums Grown in Lubbock, TX 2005 and in College Station, TX 2006

Source	Type III Sum of Squares	df	Mean Square	F	Sig.	Partial Eta Squared
Corrected Model	461773.881[a]	19	24303.888	6761.389	.000	1.000
Intercept	232326.496	1	232326.496	64633.685	.000	.999
Variety	441395.367	9	49043.930	13644.117	.000	1.000
Location	3291.997	1	3291.997	915.840	.000	.958
Variety * Location	17086.517	9	1898.502	528.167	.000	.992
Error	143.780	40	3.595			
Total	694244.157	60				
Corrected Total	461917.661	59				

[a]R Squared = 1.000 (Adjusted R Squared = 1.000)

Table 25

Flavone Levels in Sorghums Grown in Lubbock, TX 2005 and in College Station, TX 2006[a]

Variety	Location[b]	Luteolin	Apigenin	Total
Dorado	LB 2005	3.83 ± 0.05	15.62 ± 0.44	19.45 ± 0.43
	CS 2006	9.12 ± 0.03	55.85 ± 1.64	64.97 ± 1.61
BRON176	LB 2005	54.00 ± 1.35	214.14 ± 4.65	268.14 ± 5.82
	CS 2006	74.88 ± 0.92	287.31 ± 4.92	362.19 ± 5.03
EBA3	LB 2005	30.19 ± 0.16	3.83 ± 0.42	34.02 ± 0.34
	CS 2006	25.68 ± 0.47	5.13 ± 0.05	30.81 ± 0.52
SC979	LB 2005	34.96 ± 0.52	9.32 ± 0.03	44.28 ± 0.54
	CS 2006	48.83 ± 0.59	8.38 ± 0.17	57.21 ± 0.74
SC1038	LB 2005	64.75 ± 0.35	2.48 ± 0.23	67.23 ± 0.26
	CS 2006	35.87 ± 0.57	5.55 ± 0.04	41.42 ± 0.58
New B Line	LB 2005	46.66 ± 0.39	3.68 ± 0.05	50.34 ± 0.42
	CS 2006	29.90 ± 0.49	2.66 ± 0.13	32.56 ± 0.45
SC748	LB 2005	40.83 ± 1.82	6.51 ± 0.06	47.34 ± 1.88
	CS 2006	38.29 ± 1.66	7.90 ± 0.20	46.19 ± 1.85
BTx399	LB 2005	2.70 ± 0.08	0.80 ± 0.03	3.50 ± 0.09
	CS 2006	6.59 ± 0.16	1.86 ± 0.19	8.45 ± 0.33
Tx2911	LB 2005	4.71 ± 0.32	ND[c]	4.71 ± 0.32
	CS 2006	19.85 ± 0.30	23.36 ± 0.52	43.21 ± 0.81
SC1155	LB 2005	8.09 ± 0.12	1.09 ± 0.05	9.18 ± 0.12
	CS 2006	8.13 ± 0.12	1.21 ± 0.07	9.34 ± 0.12

[a]Levels are expressed as µg/g.
[b]LB = Lubbock; CS = College Station.
[c]ND = Not detected.

Lubbock. Apigenin was detected in Tx2911 from College Station and its level was higher than luteolin.

Flavanones were also affected by environment ($p < 0.001$) (Table 26). Samples from College Station that were more weathered (i.e. BRON176 and SC1038) (Fig. 25) had lower flavanone levels than those from Lubbock (Table 27). This suggests the intensity of yellowness may have an effect on flavanone levels even though these compounds are colorless in nature. Among locations, flavanone levels and proportions did not change significantly for all samples with the exception of BRON176 from College Station, which had higher levels of naringenin than eriodictyol. More research is needed to determine the effect of environment on flavanone levels and to determine whether these compounds are phytoalexins.

TABLE 26

Genotype (Variety) x Environment (Location) Interaction of Flavanones in Sorghums Grown in Lubbock, TX 2005 and in College Station, TX 2006

Source	Type III Sum of Squares	df	Mean Square	F	Sig.	Partial Eta Squared
Corrected Model	24230993.020[a]	19	1275315.422	8088.995	.000	1.000
Intercept	32312306.394	1	32312306.394	204948.572	.000	1.000
Variety	22521715.013	9	2502412.779	15872.155	.000	1.000
Location	361052.203	1	361052.203	2290.060	.000	.983
Variety * Location	1348225.805	9	149802.867	950.161	.000	.995
Error	6306.422	40	157.661			
Total	56549605.837	60				
Corrected Total	24237299.443	59				

[a]R Squared = 1.000 (Adjusted R Squared = 1.000)

Table 27

Flavanone Levels in Sorghums Grown in Lubbock, TX 2005 and in College Station, TX 2006[a]

Variety	Location[b]	Eriodictyol	Naringenin	Total
Dorado	LB 2005	ND[c]	ND	ND
	CS 2006	ND	ND	ND
BRON176	LB 2005	986.81 ± 21.11	441.31 ± 8.69	1428.12 ± 29.73
	CS 2006	204.14 ± 5.46	274.20 ± 4.06	478.34 ± 9.01
EBA3	LB 2005	1504.01 ± 18.75	275.61 ± 3.76	1779.62 ± 22.45
	CS 2006	1235.07 ± 22.90	276.77 ± 3.94	1511.84 ± 26.78
SC979	LB 2005	924.15 ± 7.27	479.37 ± 2.88	1403.52 ± 10.09
	CS 2006	937.35 ± 7.17	480.26 ± 2.78	1417.61 ± 7.48
SC1038	LB 2005	391.80 ± 3.07	82.50 ± 0.88	474.30 ± 3.95
	CS 2006	110.02 ± 3.89	24.39 ± 0.79	134.41 ± 4.66
New B Line	LB 2005	762.92 ± 14.31	261.84 ± 4.76	1024.76 ± 19.04
	CS 2006	642.71 ± 4.68	226.33 ± 1.23	869.04 ± 5.36
SC748	LB 2005	921.48 ± 11.35	567.47 ± 3.16	1488.95 ± 14.50
	CS 2006	997.02 ± 2.73	639.00 ± 0.54	1636.02 ± 3.09
BTx399	LB 2005	30.52 ± 2.28	37.39 ± 2.02	67.91 ± 4.30
	CS 2006	37.55 ± 0.86	51.55 ± 1.30	89.10 ± 2.16
Tx2911	LB 2005	ND	209.35 ± 10.53	209.35 ± 10.53
	CS 2006	ND	240.58 ± 1.59	240.59 ± 1.58
SC1155	LB 2005	163.42 ± 2.75	74.31 ± 1.01	237.73 ± 3.75
	CS 2006	118.86 ± 2.59	66.99 ± 0.70	185.85 ± 3.18

[a]Levels are expressed as µg/g.
[b]LB = Lubbock; CS = College Station.
[c]ND = Not detected.

Comparison of Flavones and Flavanones in Sorghums with Common Sources

The high levels of flavones in tan plant sorghum such as BRON176 makes it comparable to those found in common sources. The main source of apigenin is parsley (Fig. 43). When comparing the mean level of apigenin in BRON176 to common sources, sorghum's biggest competitor is parsley and it ranks third as having the highest levels of apigenin (Fig. 43). The mean level of luteolin in BRON176 also makes it a good source (Fig. 44). Since these compounds are concentrated in the pericarp (Seitz 2004), flavone levels can be concentrated by decorticating the grain to produce bran.

Compared to common sources, lemon-yellow sorghums are also a major source of flavanones. The mean amount of eriodictyol in SC748 ranks first on an as-is-basis with other major sources (Fig. 45). The mean amount of naringenin from SC748 also ranks first among other high sources (Fig. 46). Decorticating sorghums also concentrates these compounds as aforementioned (Fig. 41) and can be used for nutraceutical/pharmaceutical applications. For example, eriodictyol has anti-inflammatory properties (Zhang et al. 2006), prevents pulmonary insufficiency (Aviado et al 1974), protects skin cells from UV-induced damage (Lee et al 2007), and decreases the development of age-related macular degeneration (Hanneken et al 2006). Eriodictyol also has food applications where it acts as a bitter-masking agent by decreasing the bitterness of caffeine (Ley et al 2005). Naringenin has cholesterol-lowering (Borradaile et

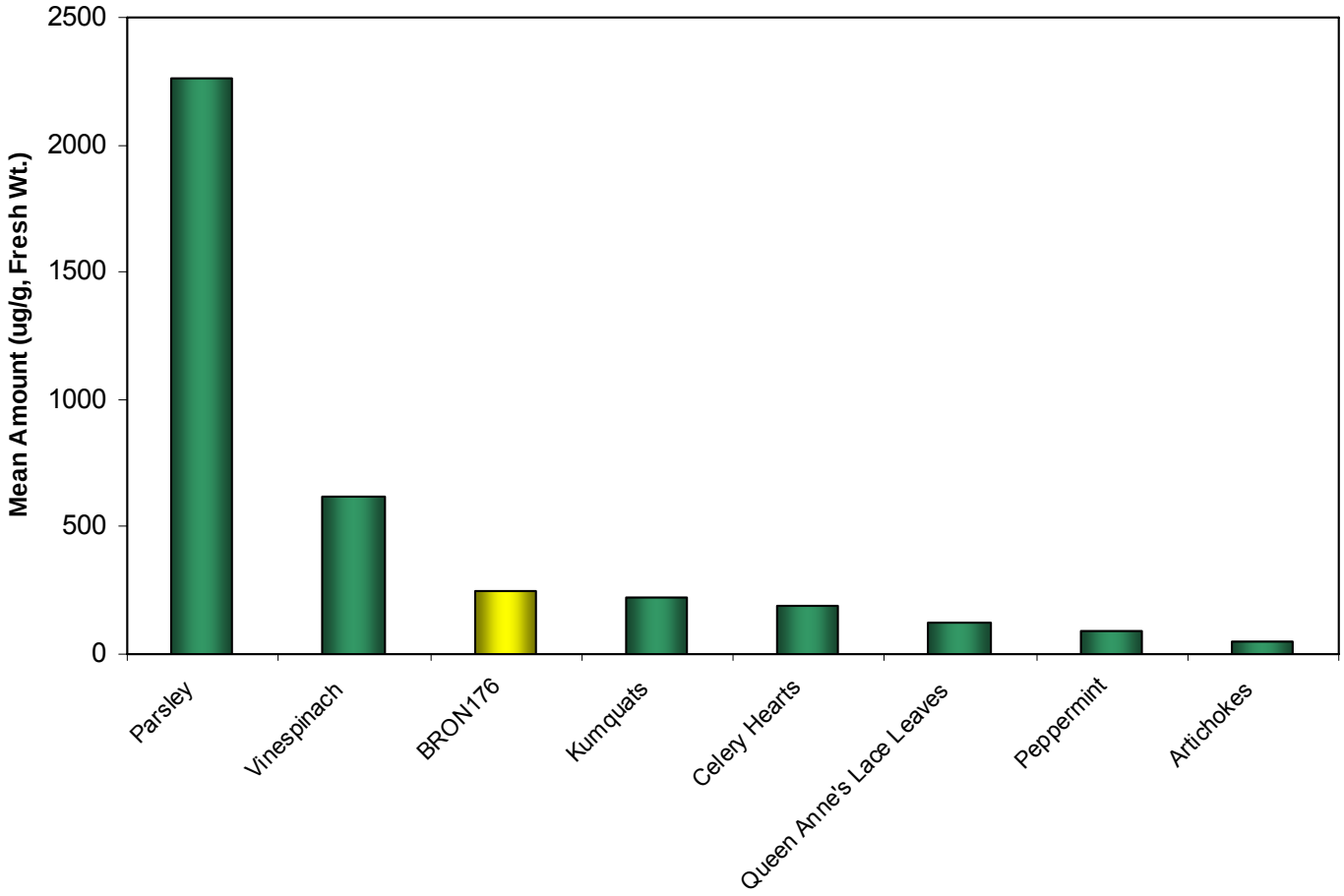

Fig. 43. Comparison of apigenin levels in BRON176 with common sources. (Data obtained from the USDA Database for the Flavonoid Content of Selected Foods, Release 2.1, 2007).

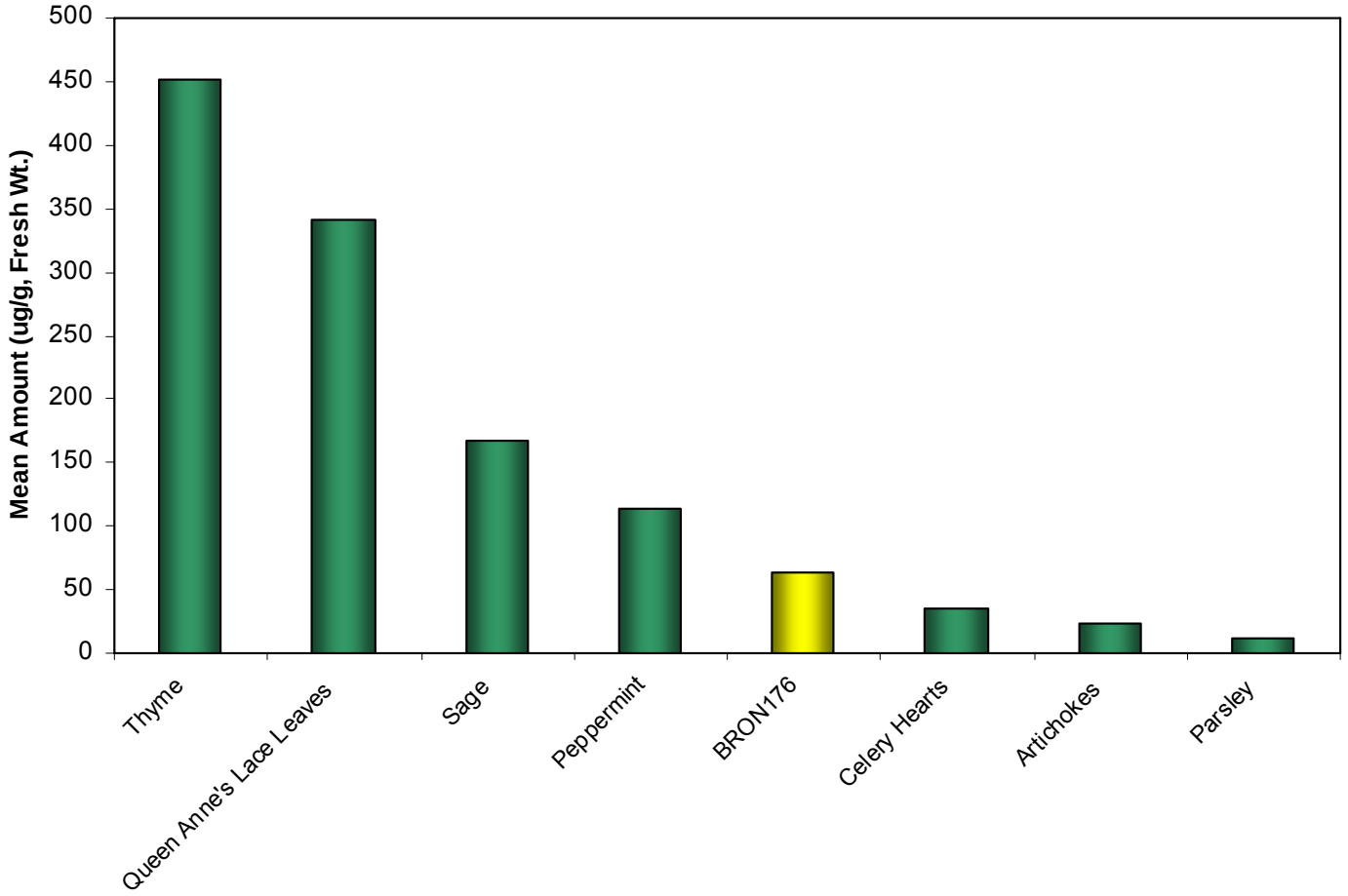

Fig. 44. Comparison of luteolin levels in BRON176 with common sources. (Data obtained from the USDA Database for the Flavonoid Content of Selected Foods, Release 2.1, 2007).

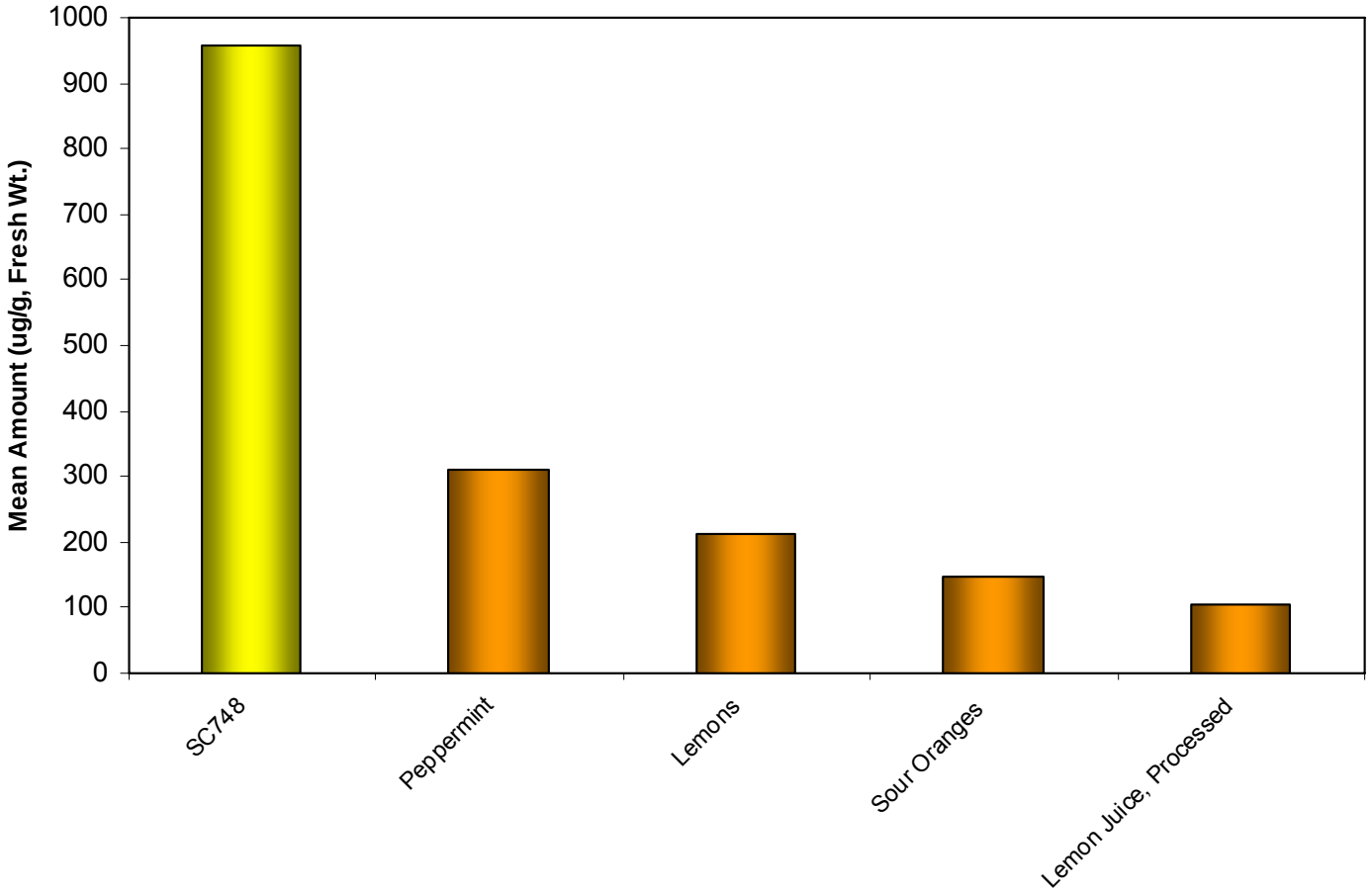

Fig. 45. Comparison of eriodictyol levels in SC748 with common sources. (Data obtained from the USDA Database for the Flavonoid Content of Selected Foods, Release 2.1, 2007).

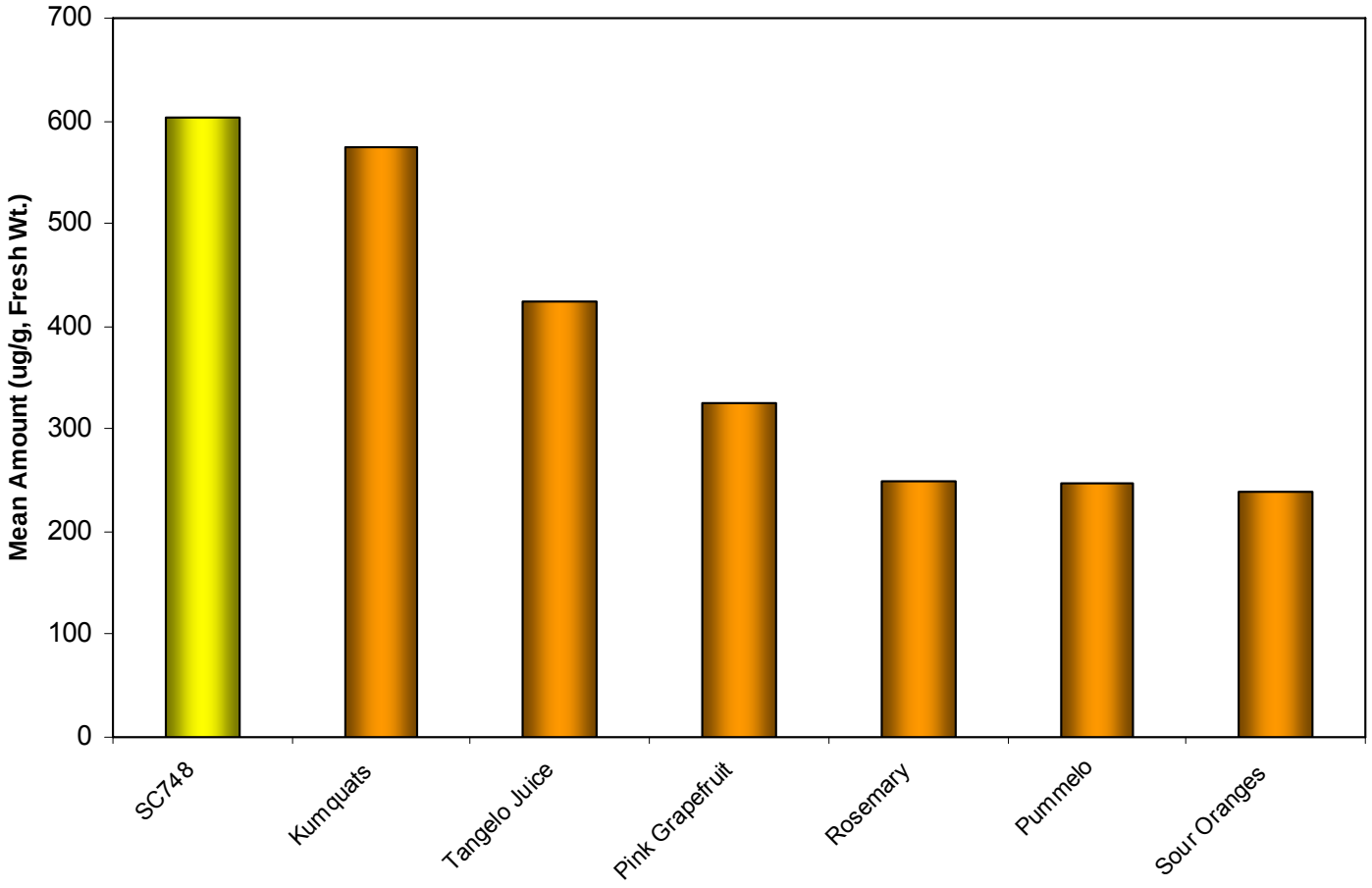

Fig. 46. Comparison of naringenin levels in SC748 with common sources. (Data obtained from the USDA Database for the Flavonoid Content of Selected Foods, Release 2.1, 2007).

al. 1999; Wilcox et al. 2001), anti-ulcer (Martin et al. 1993), and anti-inflammatory properties (Tsai et al. 1999). Naringenin has also been reported as a chemopreventive agent against neurodegenerative disease (Heo et al 2004) and as an expectorant (Lin et al 2007). Lemon-yellow sorghums as a source of flavanones have several advantages which are appealing to the food/nutraceutical producer. In contrast to other sources, sorghums are dry and are easy to store for long periods of time.

The results of this study showed that the flavonoid levels varied according to the sorghum genotype. Lemon-yellow sorghums are a major source of flavanones, which are the major flavonoids in those genotypes. This study also confirmed that the levels of 3-deoxyanthocyanins were increased when sorghums had red/purple secondary plant color whereas flavone levels were increased when sorghums had tan secondary plant color. The 3-deoxyanthocyanins levels decreased when sorghums were grown in a drier environment (i.e. Lubbock, TX). This information will be helpful for plant breeders to select and produce sorghums with desired healthy components.

CHAPTER VI

FLAVONOID PROFILE OF BLACK PERICARP SORGHUMS

Sorghum Grain Characteristics

Tx430, Shawaya Black, and Black PI Tall are three lines available. Unfortunately, their agronomic properties are undesirable. Tx430 Black has low yield whereas Shawaya Black and Black PI Tall are too tall (7-10 feet) to make it practical to harvest. Therefore, the Sorghum Breeding Program developed black hybrids with desirable agronomic properties. Phenols, antioxidant activity, and flavonoid composition of these hybrids were assessed and compared with the lines.

Pericarp colors are listed in Table 28. For samples grown in College Station 2006, Shawaya Black and Black PI Tall had the lowest *L** values (31.5-31.6), which means they were darkest in color. The hybrids had higher *a** and *b** values than the lines. Visually, the hybrids were less black than the lines (Fig. 47). Hyb118 was more dark brown than black in color (Fig. 47). All samples had positive *a** and *b** values, which means they were more red than green and more yellow than blue, respectively. In general, *L** values increased as the *a** and *b** values increased.

Samples were also grown in College Station, Corpus Christi, and Puerto Rico in 2007 (Figs. 48-50). The two hybrids from Puerto Rico were lighter in color and more red due to their higher *L**, *a**, and *b** values. The grains from

TABLE 28

CIELAB *L, *a**, *b** Values of Black Sorghums Grown in Different Environments**

Variety	Location[a]	*L**	*a**	*b**
Tx430 Black	CS 2006	34.23 ± 0.16	3.79 ± 0.10	2.84 ± 0.14
	CS 2007	35.27 ± 0.23	4.30 ± 0.23	3.73 ± 0.31
	CC 2007	35.18 ± 0.07	3.69 ± 0.11	2.47 ± 0.06
	PR 2007	34.44 ± 0.31	5.48 ± 0.29	4.10 ± 0.36
Shawaya Black	CS 2006	31.48 ± 0.10	2.46 ± 0.05	0.95 ± 0.04
Black PI Tall	CS 2006	31.55 ± 0.08	3.10 ± 0.04	1.29 ± 0.05
	CS 2007	32.10 ± 0.17	3.34 ± 0.19	1.09 ± 0.16
	CC 2007	33.90 ± 0.05	4.13 ± 0.04	2.17 ± 0.04
Hyb107	CS2006	34.69 ± 0.50	6.05 ± 0.13	4.68 ± 0.23
Hyb115	CS 2006	35.35 ± 0.20	6.22 ± 0.19	4.96 ± 0.13
Hyb116	CS 2006	35.87 ± 0.06	6.62 ± 0.18	5.70 ± 0.16
	CS 2007	35.52 ± 0.15	6.64 ± 0.08	5.33 ± 0.06
	CC 2007	35.78 ± 0.12	6.53 ± 0.31	4.53 ± 0.27
	PR 2007	37.86 ± 0.30	8.84 ± 0.19	8.32 ± 0.39
Hyb117	CS 2006	35.58 ± 0.25	6.05 ± 0.13	5.43 ± 0.24
	CS 2007	35.21 ± 0.24	6.47 ± 0.19	4.61 ± 0.13
	CC 2007	35.24 ± 0.13	5.98 ± 0.15	3.93 ± 0.05
Hyb118	CS 2006	34.19 ± 0.11	6.32 ± 0.19	3.73 ± 0.18
	CS 2007	35.07 ± 0.27	6.87 ± 0.03	4.33 ± 0.21
	CC 2007	34.89 ± 0.13	6.09 ± 0.07	3.71 ± 0.05
	PR 2007	35.85 ± 0.09	8.84 ± 0.19	6.40 ± 0.17

[a]CS = College Station; CC = Corpus Christi; PR = Puerto Rico.

Fig. 47. Black sorghum varieties grown in College Station, TX 2006.

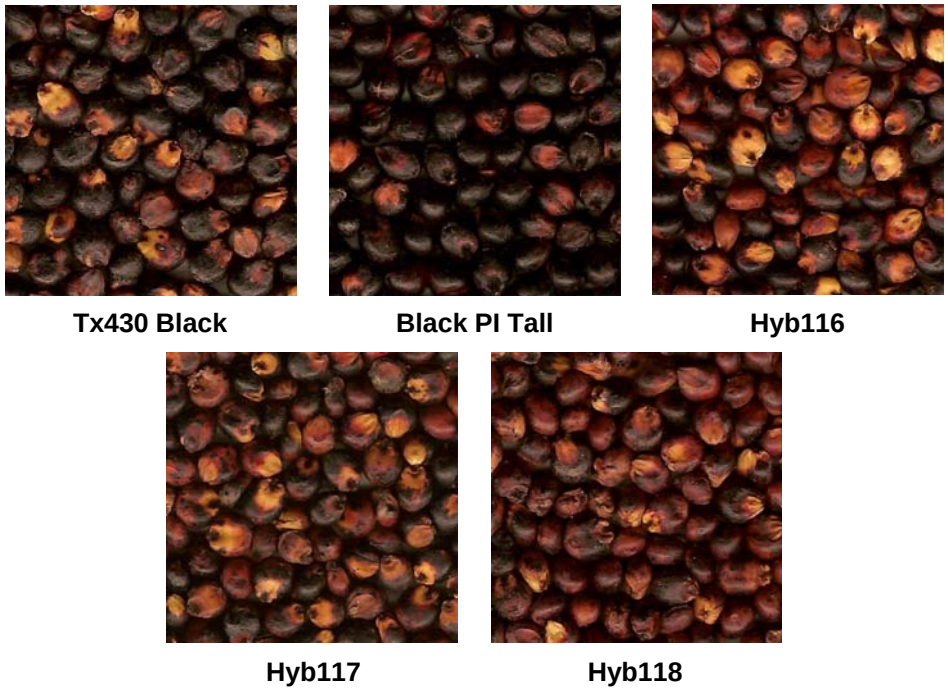

Fig. 48. Black sorghum varieties grown in College Station, TX 2007.

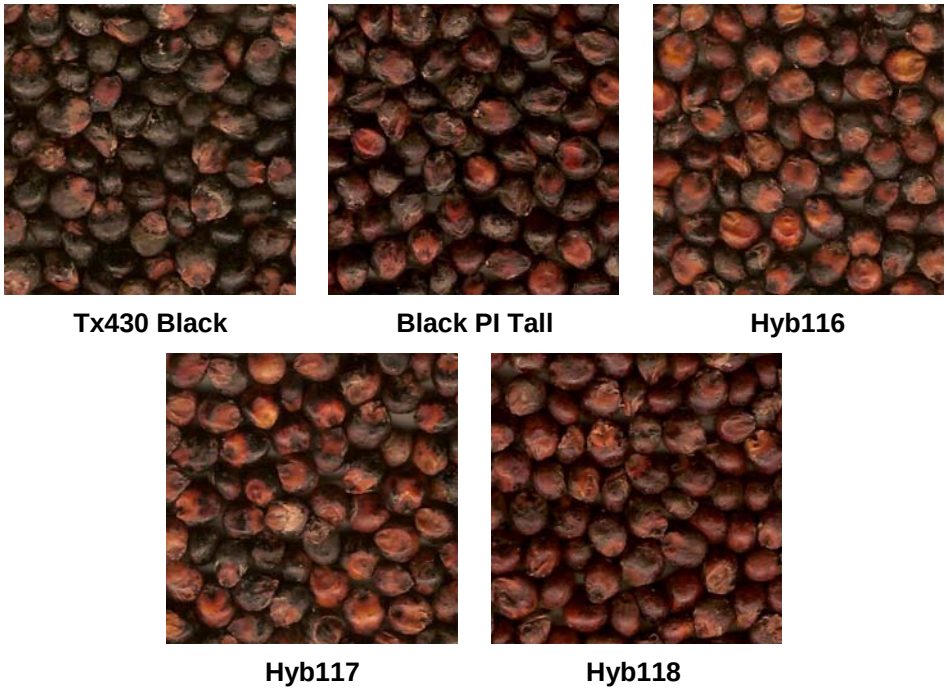

Fig. 49. Black sorghum varieties grown in Corpus Christi, TX 2007.

Fig. 50. Black sorghum varieties grown in Puerto Rico 2007.

Corpus Christi were darker but had more mold damage than the other samples due to heavy rainfall during their development.

Evaluation of Sorghum Total Phenols

Total phenol levels for the black sorghums grown in College Station in 2006 ranged from 5.3-19.8 mg GAE/g with Tx430 Black and Black PI Tall having the lowest and highest levels, respectively (Fig. 51). The total phenol level for Shawaya Black (7.4 mg GAE/g) agreed with the value (6.7 mg GAE/g) reported by Gous (1989). Hybrids had total phenol levels that ranged from 9.5-17.0 mg GAE/g.

Evaluation of Sorghum Condensed Tannins

Six varieties had a pigmented testa and had significant levels of condensed tannins. Black PI Tall had the highest levels (48.7 mg CE/g) (Fig. 52). Tx430 Black and Shawaya Black did not have condensed tannins. Gous (1989) also reported that Shawaya Black had no detectable tannins. As with other non-tannin sorghums, the low absorbance was due to other phenolic compounds that react with vanillin (Hahn and Rooney 1986). All hybrids contained condensed tannin levels, which ranged from 14.5-42.8 mg CE/g. Tannin detection was not expected since the hybrids were produced using two non-tannin sorghums. The presence of tannins in the hybrids could be that the parents had complementary $B_1\_B_2$ genes causing the presence of a pigmented testa when crossed. The tannin level for Hyb118 (42.8 mg CE/g) was

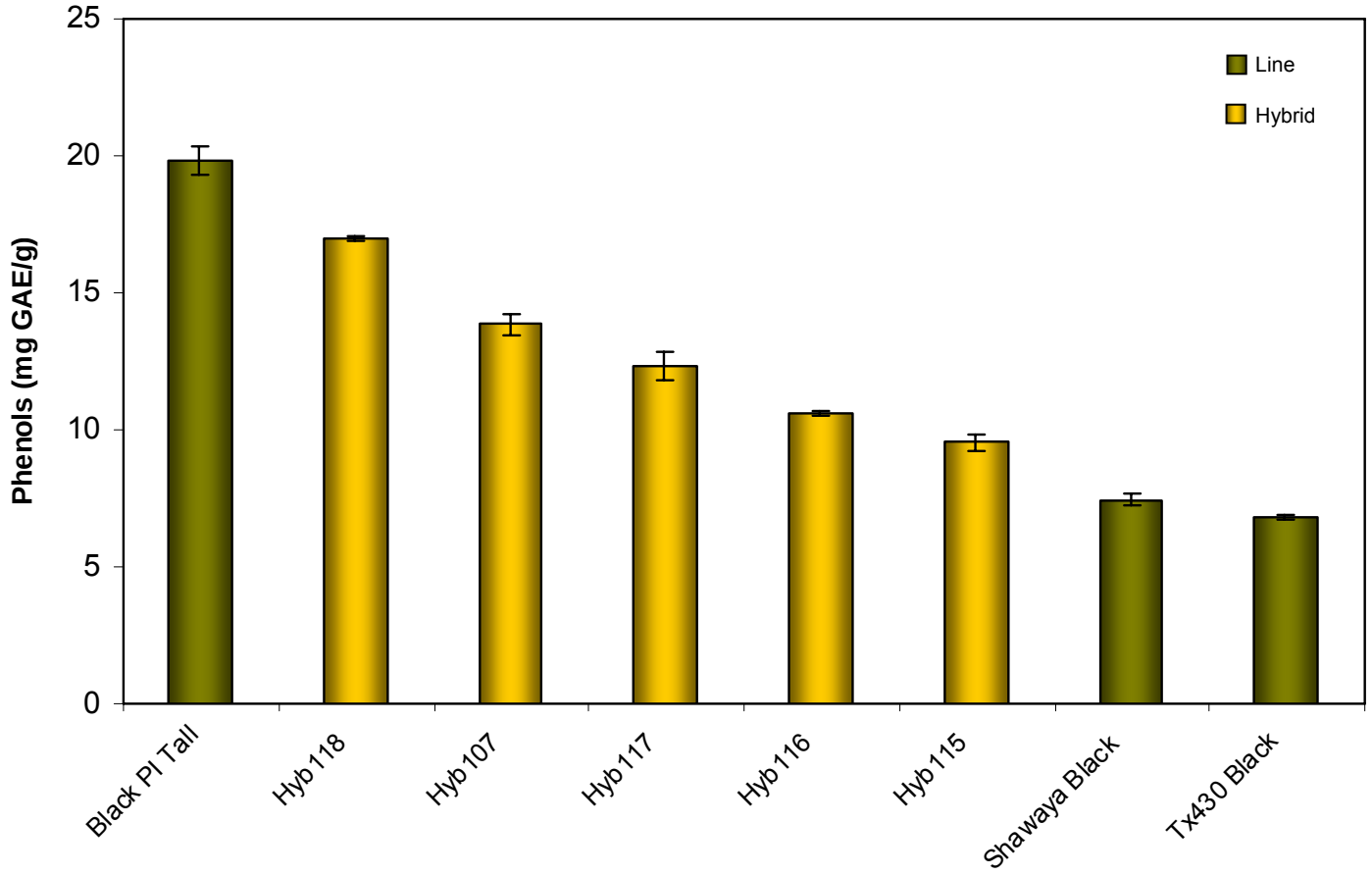

Fig. 51. Total phenol levels in black sorghums grown in College Station, TX 2006.

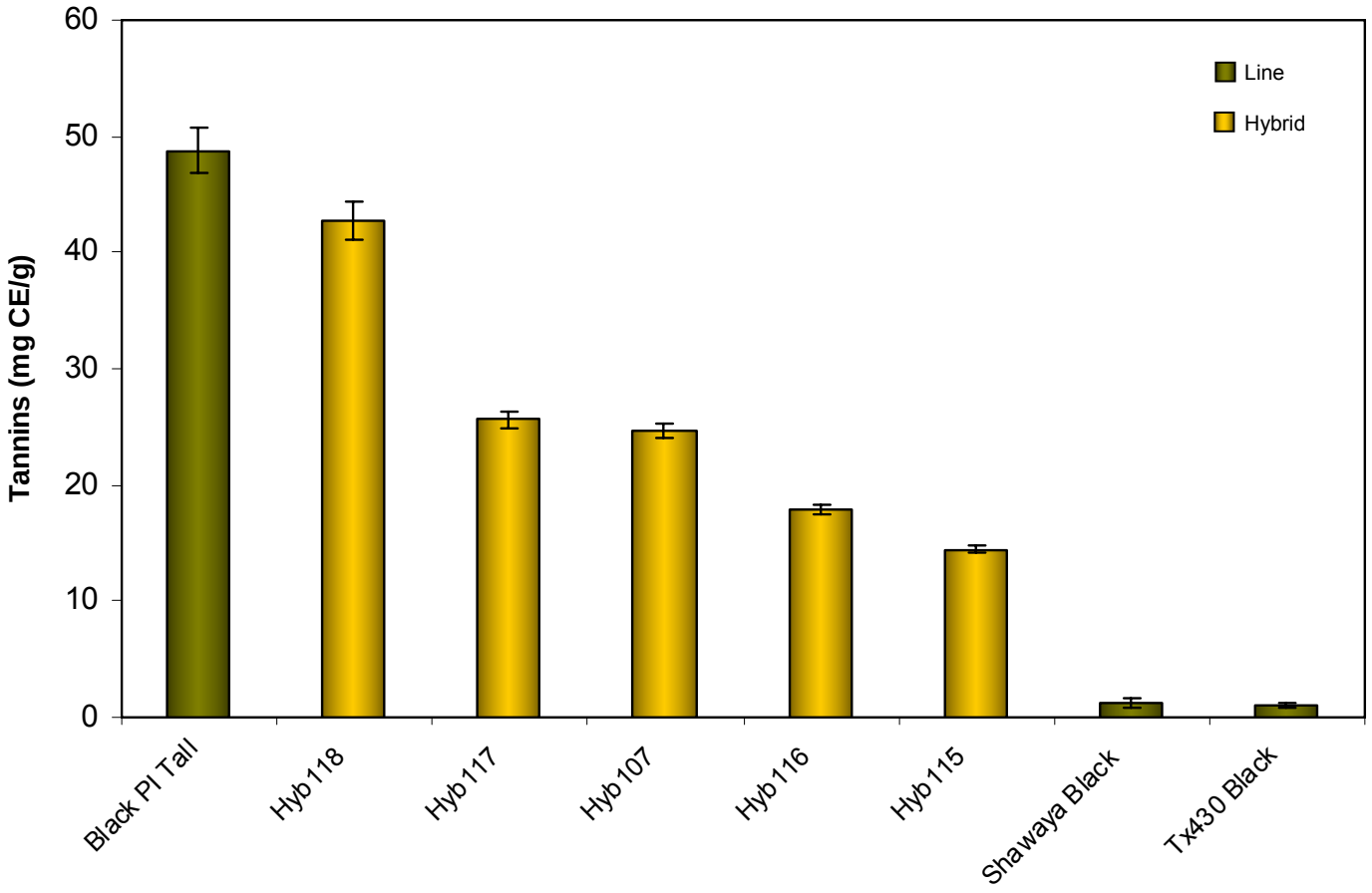

Fig. 52. Condensed tannin levels in black sorghums grown in College Station, TX 2006.

comparable to Sumac, a high tannin sorghum (Awika et al 2005). Hyb118 could be selected as a substitute for Sumac since the latter does not have good agronomic properties; it is very tall (7-10 ft) and has very small grains. Among tannin sorghums, a strong correlation between total phenols and condensed tannins ($r = 0.98$, $p < 0.001$) suggested that total phenol levels were contributed by condensed tannins.

Evaluation of Sorghum Flavan-4-ols

The sorghum lines had the highest flavan-4-ol levels (12.0-16.0 abs/mL/g) (Fig. 53). The flavan-4-ol level of Shawaya Black (15.6 abs/mL/g) agreed with the value (13.6 abs/mL g) found by Gous (1989). Flavan-4-ol levels in the hybrids were 8.8-11.2 abs/mL/g. A negative correlation between L^* and flavan-4-ols (-0.87, $p < 0.01$) suggested that dark pigments in the pericarp increase flavan-4-ol levels. The high flavan-4-ol levels also suggest the possibility of mold damage reduction in those genotypes (Jambunathan et al 1990, 1991; Menkir et al 1996).

Evaluation of Sorghum Anthocyanins

Anthocyanin levels followed the same pattern as found for flavan-4-ols. Sorghum lines had the highest anthocyanin levels (218.2-297.6 abs/mL/g) with Shawaya Black as having the highest (Fig. 54). The anthocyanin level for Shawaya Black could not be compared with the value (27.6 abs/mL/g) found by Gous (1989) since the former was measured using the colorimetric method of

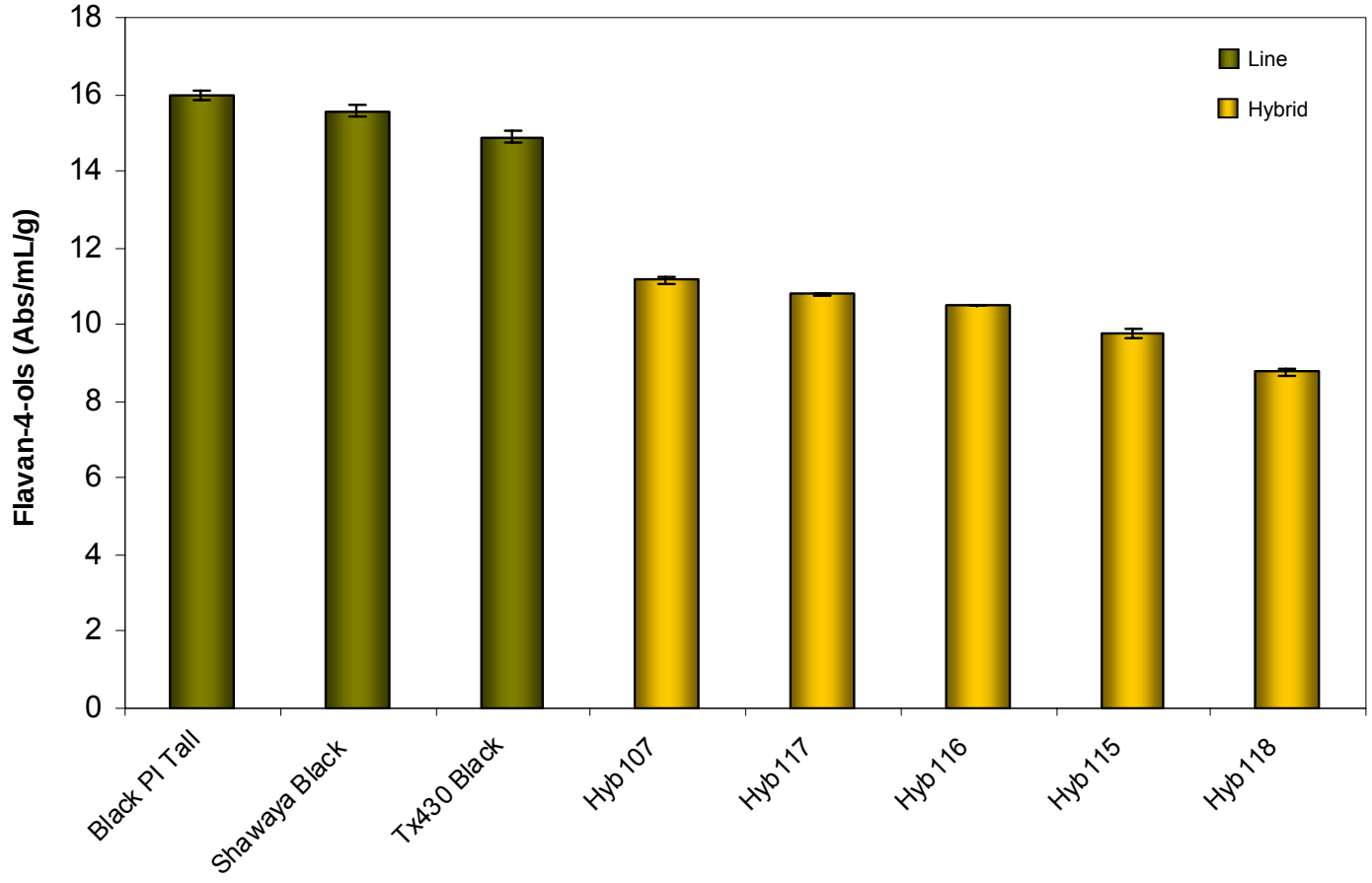

Fig. 53. Flavan-4-ol levels in black sorghums grown in College Station, TX 2006.

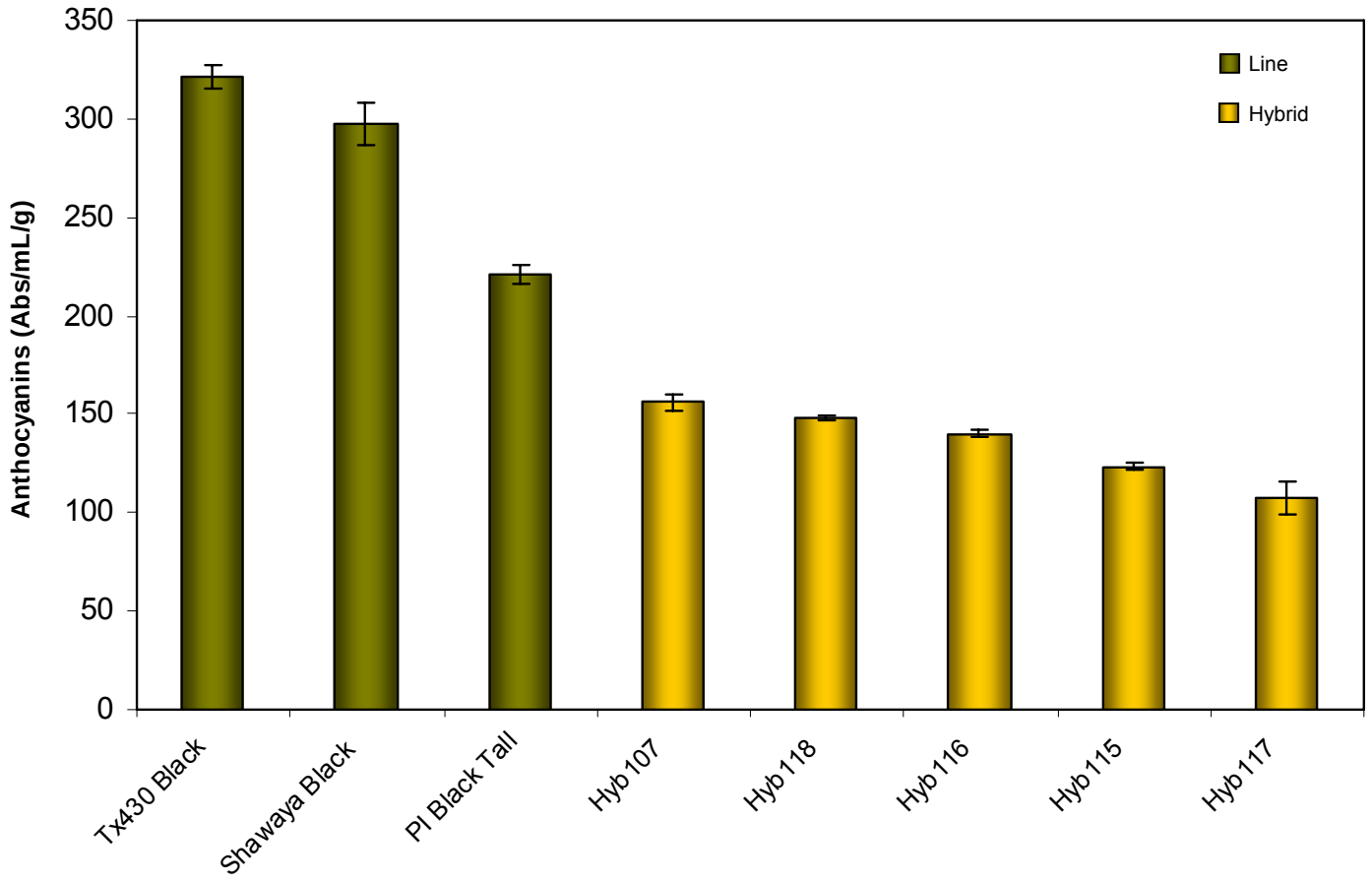

Fig. 54. Anthocyanin levels in black sorghums grown in College Station, TX 2006.

Fuleki and Francis (1968) while the latter was measured using the blank absorbances from the vanillin/HCl assay. Anthocyanin levels in the hybrids ranged from 123.6-155.9 abs/mL/g, which were lower than the lines. A correlation between flavan-4-ols and anthocyanins (r = 0.83, p < 0.001) suggested that anthocyanins were increased as flavan-4-ol increased. As found in Chapter IV, no significant correlation was found between total phenols and anthocyanins as previously reported by Awika (2000). A negative correlation between anthocyanin and L^* value (r = -0.87, p < 0.001) suggested that darker pericarps increased anthocyanin levels.

Evaluation of Sorghum Antioxidant Activity

Sorghums with the dominant $B_1\_B_2$ gene for the presence of a pigmented testa had the highest antioxidant activity levels (Fig. 55). The antioxidant activity came mainly from condensed tannins, which have higher antioxidant activity *in vitro* than other phenolic compounds (Hagerman et al 1998; Amarowicz et al 2003). ABTS values for non-tannin and tannin sorghums were 80.0-110.4 and 159.8-334.2 µmol TE/g, respectively. DPPH values were 32.4-45.1 and 85.5-177.2 µmol TE/g for non-tannin and tannin sorghums, respectively. A strong correlation between total phenols and antioxidant activity was observed (total phenols vs ABTS, r = 0.99; total phenols vs DPPH, r = 0.94), which means that antioxidant activity was contributed by sorghum phenols.

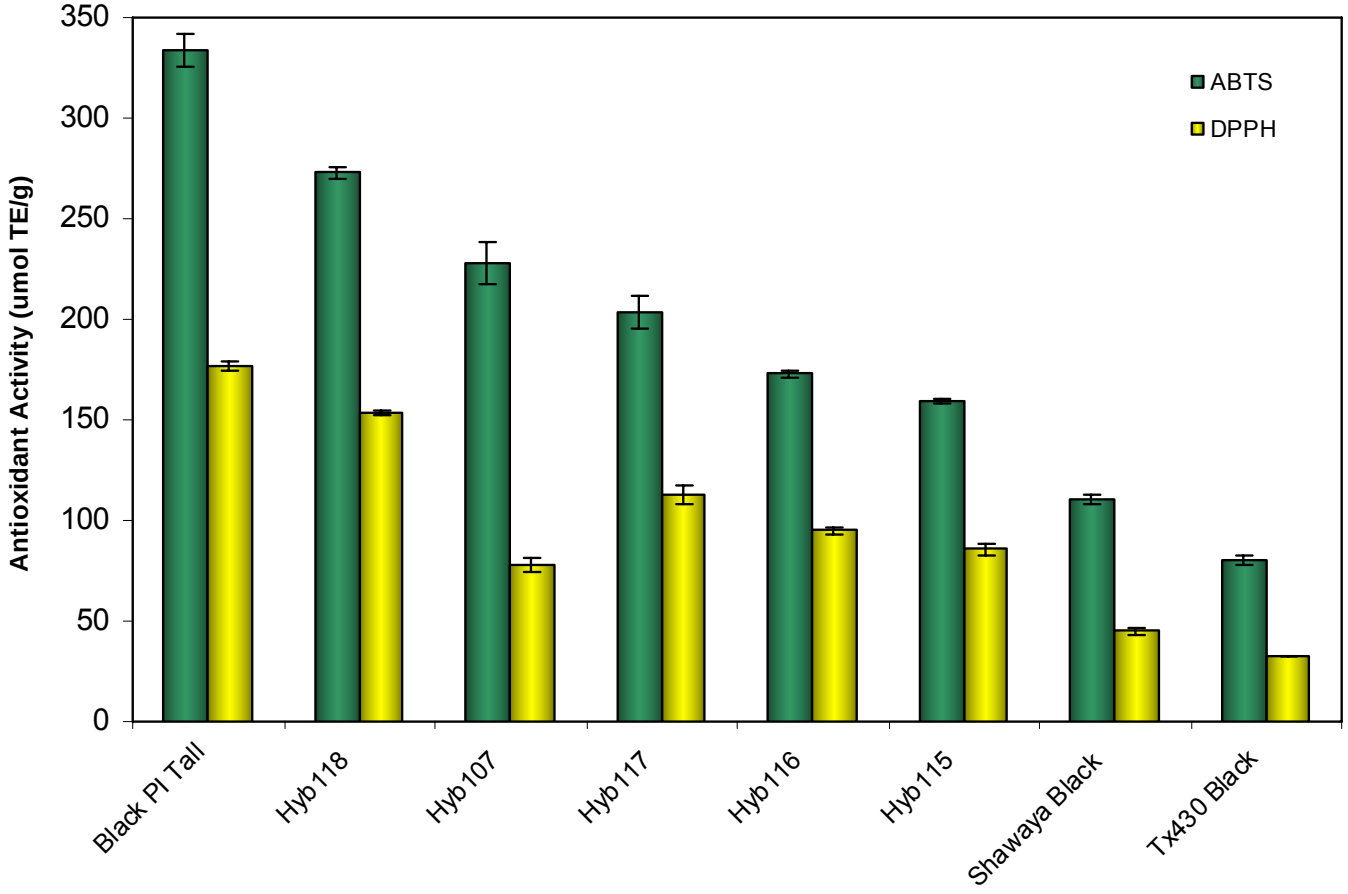

Fig. 55. Antioxidant activity potential of black sorghums grown in College Station, TX 2006.

Evaluation of Sorghum 3-Deoxyanthocyanins

Awika et al (2004a, b) identified and quantified luteolinidin and apigeninidin in the bran of Tx430 Black. Wu et al. (2005) identified several 3-deoxyanthocyanins in Tx430 Black, which included luteolinidin, apigeninidin, and their derivatives but their amounts were not reported. In this study, four major 3-deoxyanthocyanins were detected: luteolinidin (LUT); apigeninidin (AP); 5-methoxyluteolinidin (5-MeO-LUT); and 7-methoxyapigeninidin (7-MeO-AP) (Fig. 56). The two methylated 3-deoxyanthocyanins were also reported by Seitz (2004) and Wu et al (2005).

The sorghum lines had higher 3-deoxyanthocyanin levels (676-1054 µg/g) than the hybrids (329-485 µg/g) with Shawaya Black having the highest levels (Fig. 57). The presence of a pigmented testa did not increase 3-deoxyanthocyanin levels since all hybrids had the lowest levels of those compounds. The negative correlations between 3-deoxyanthocyanins and L^*, a^*, and b^* were r = -0.92, r = -0.94, and r = -0.94, respectively, which indicated that these compounds were contributed by pericarp color. The 3-deoxyanthocyanins decreased in lighter and redder grains. These results were expected since the hybrids were less black than the lines. Because these compounds are orange and yellow, it is unknown what compounds contributed the black color of the pericarp. After extracting whole grain flour, the residues were still black. Further work is needed to determine which solvents would efficiently extract the pigments and to determine which compounds are

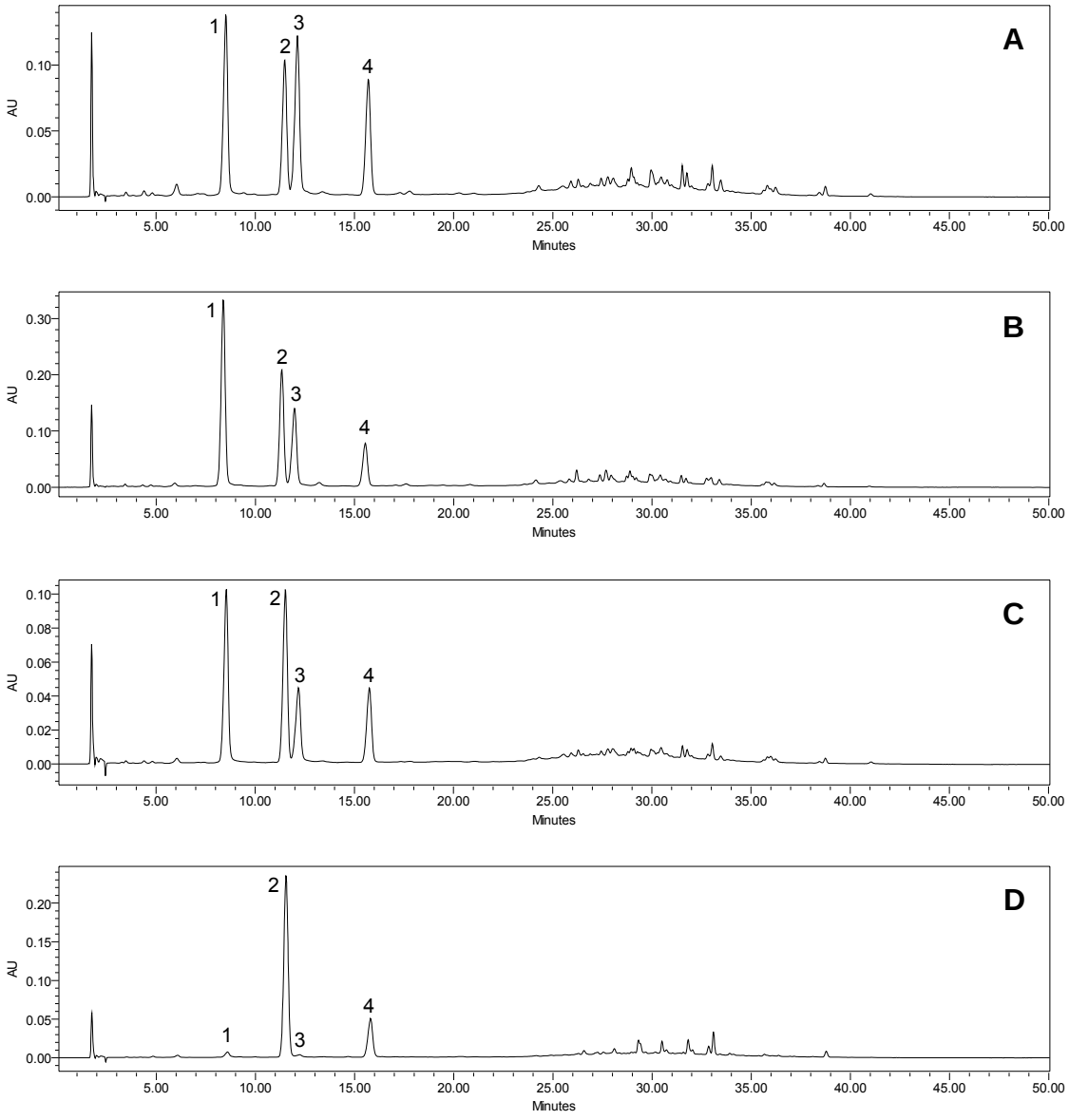

Fig. 56. HPLC chromatograms of 3-deoxyanthocyanins in black sorghums grown in College Station, TX 2006. A) Tx430 Black; B) Shawaya Black; C) Hyb116; D) Hyb118. 1 = Luteolinidin; 2= Apigeninidin; 3 = 5-Methoxyluteolinidin; 4 = 7-Methoxyapigeninidin. PDA = 485 nm.

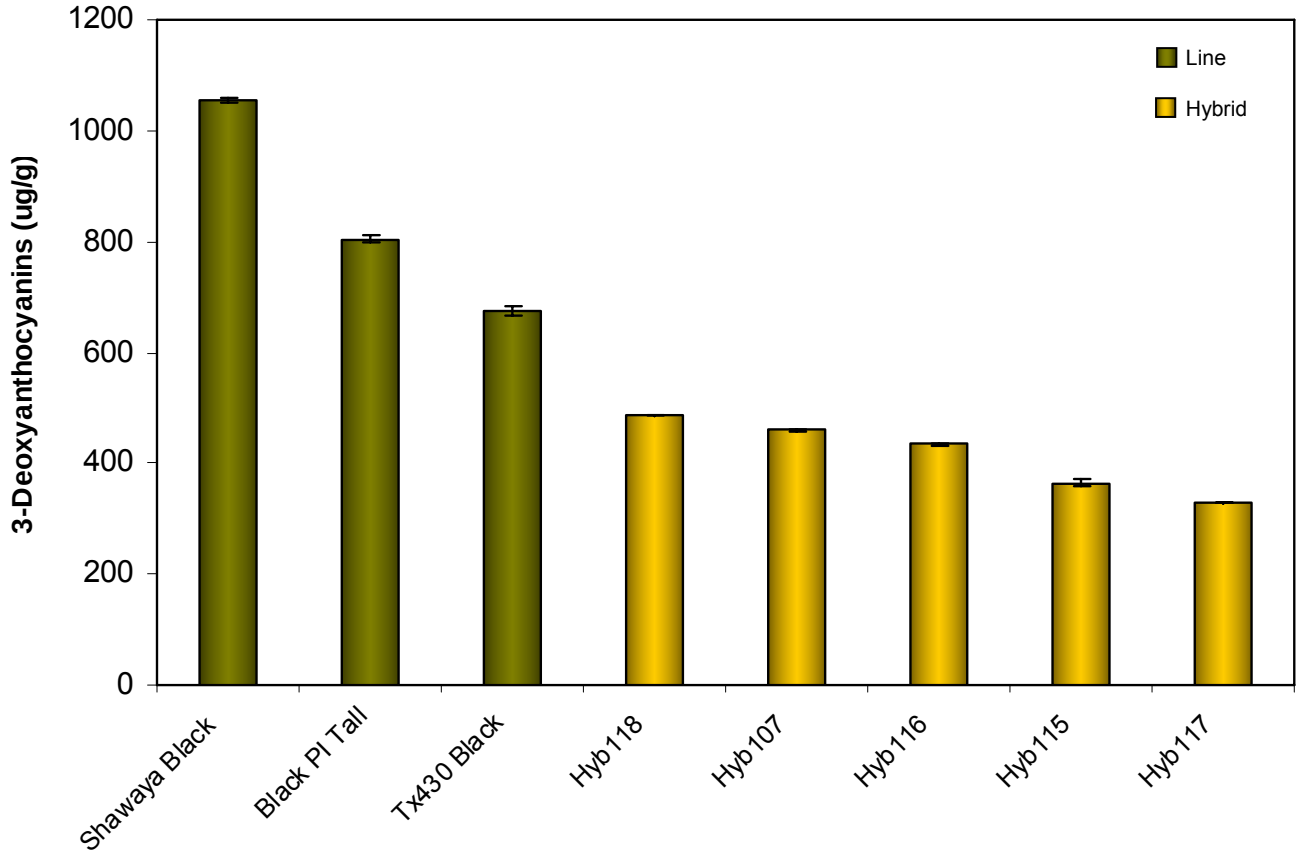

Fig. 57. 3-Deoxyanthocyanin levels in black sorghums grown in College Station, TX 2006.

contributing the black color of the pericarp.

A strong correlation between the 3-deoxyanthocyanins measured using the colorimetric and HPLC methods was also observed ($r = 0.99$, $p < 0.001$). This confirmed that the anthocyanins measured using the colorimetric method consisted mostly of the four 3-deoxyanthocyanins detected by HPLC. The strong correlations seen in this study and in Chapter IV confirmed that the colorimetric method is a quick, inexpensive, and reliable method to screen 3-deoxyanthocyanin levels in sorghums.

The 3-deoxyanthocyanin composition varied among samples (Fig. 58). Hyb118 consisted mainly of the yellow AP and 7-MeO-AP, which accounted for 96% of the total. The high proportion of AP makes this sample an attractive source of a natural yellow pigment. For the remaining samples, the 3-deoxyanthocyanin compositions for Shawaya Black and Black PI Tall were similar while those of the hybrids, with the exception of Hyb107, were similar. The 3-deoxyanthocyanin composition of Tx430 Black was evenly distributed while the other samples had a higher LUT and AP combined (58-69%) than their methylated derivatives. Shawaya Black and Black PI Tall had the highest proportion of LUT (37-39%) while the hybrids had the highest proportion of AP (30-37%).

Evaluation of Sorghum Flavones

The two flavones detected in the black sorghums were luteolin and apigenin. Flavone levels were higher for the sorghum lines (35-56 μg/g) than

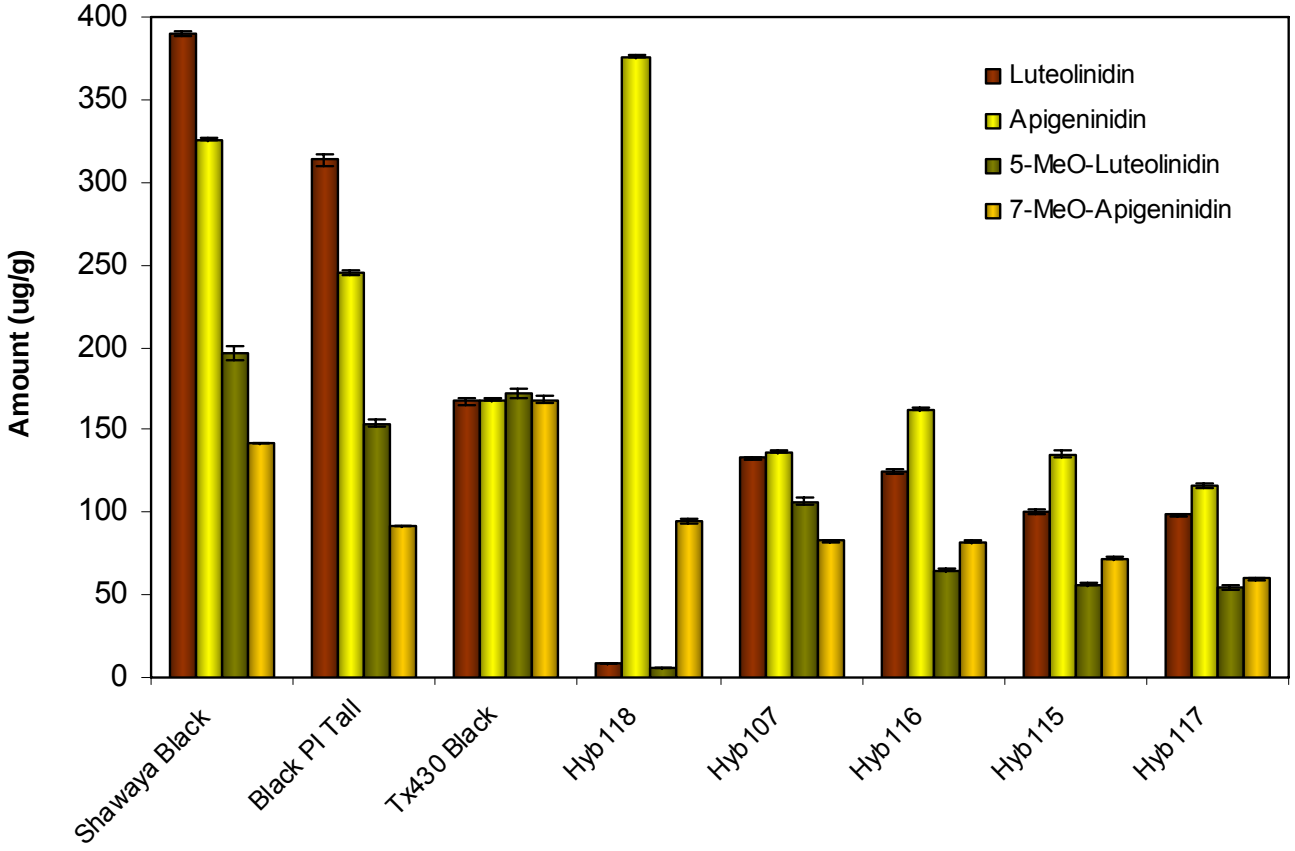

Fig. 58. 3-Deoxyanthocyanin profile of black sorghums grown in College Station, TX 2006.

the hybrids (18-24 µg/g) (Fig. 59). Shawaya Black and Hyb115 had the highest and lowest flavone levels, respectively. Comparing these levels with those of the 3-deoxyanthocyanins, flavones are minor flavonoids in black sorghums. A strong correlation between flavones and 3-deoxyanthocyanins (r = 0.96, p < 0.001) suggests that 3-deoxyanthocyanins are increased as flavones increased. This was not expected since no correlation was observed in the samples discussed in Chapter IV (r = -0.18, p > 0.05) and Chapter V (r = -0.25, p > 0.05). This strong correlation could only happen in black pericarp sorghums and further work is needed to confirm this.

Luteolin was the predominant flavone in all samples, which accounted for 81-88% with the exception of Hyb118 which had a higher proportion of apigenin (68%) (Fig. 60). The different flavone composition of Hyb118 could be because the sample is not a “black” pericarp sorghum. A larger number of black pericarp sorghums should be analyzed to confirm that the major flavone in those genotypes is luteolin.

Evaluation of Sorghum Flavanones

The flavanone levels in the black sorghums reported in Chapter IV were low (Fig. 21) and could be due to the incomplete reaction of flavanone glucosides to their aglycones; however, that finding was not known at the time of the study. It was difficult to determine whether the flavanones in the black sorghums were glucosides due to interference with other compounds. As a precaution, acid hydrolysis of the extracts was performed to convert flavanone

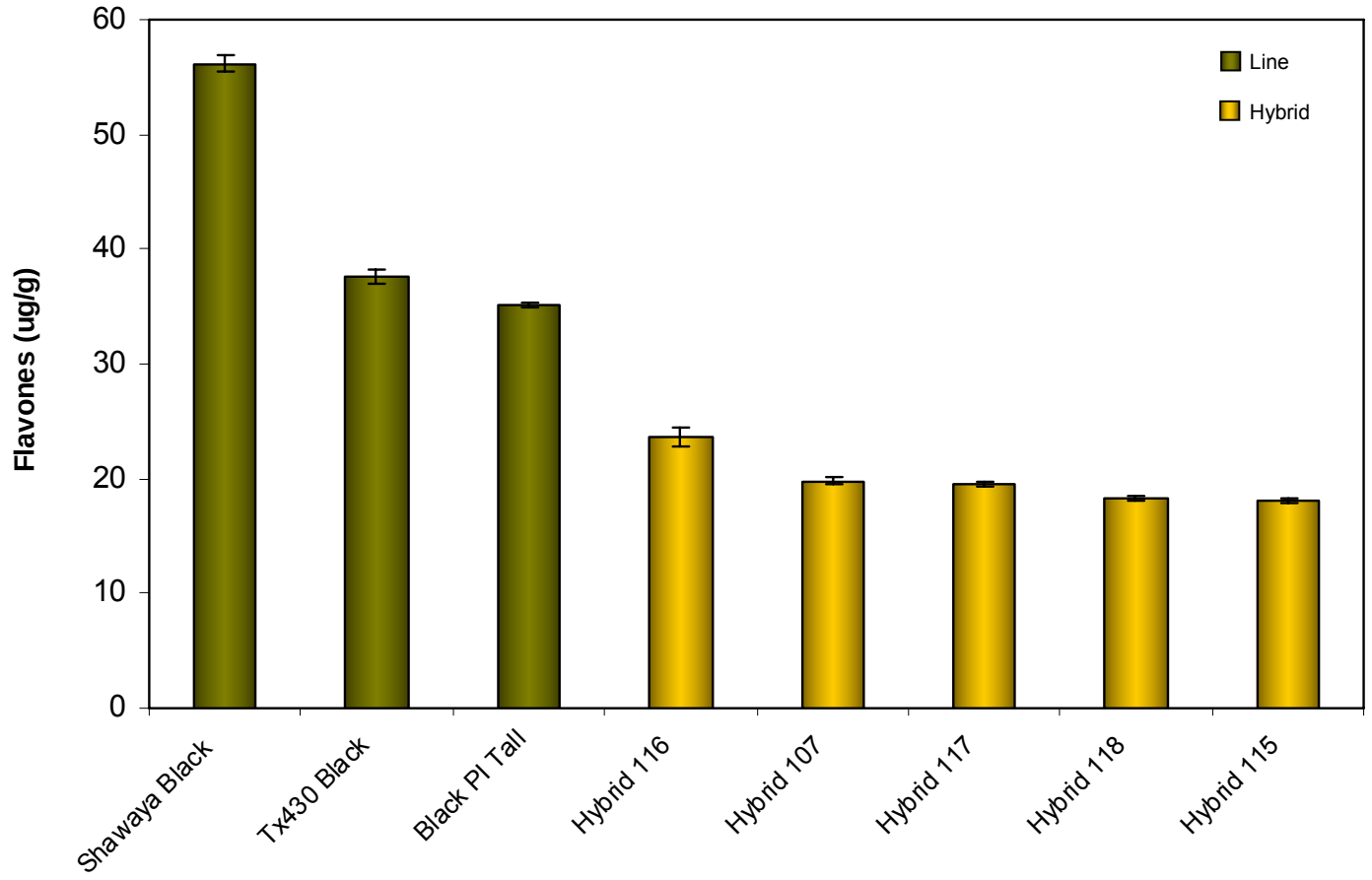

Fig. 59. Flavone levels in black sorghums grown in College Station, TX 2006.

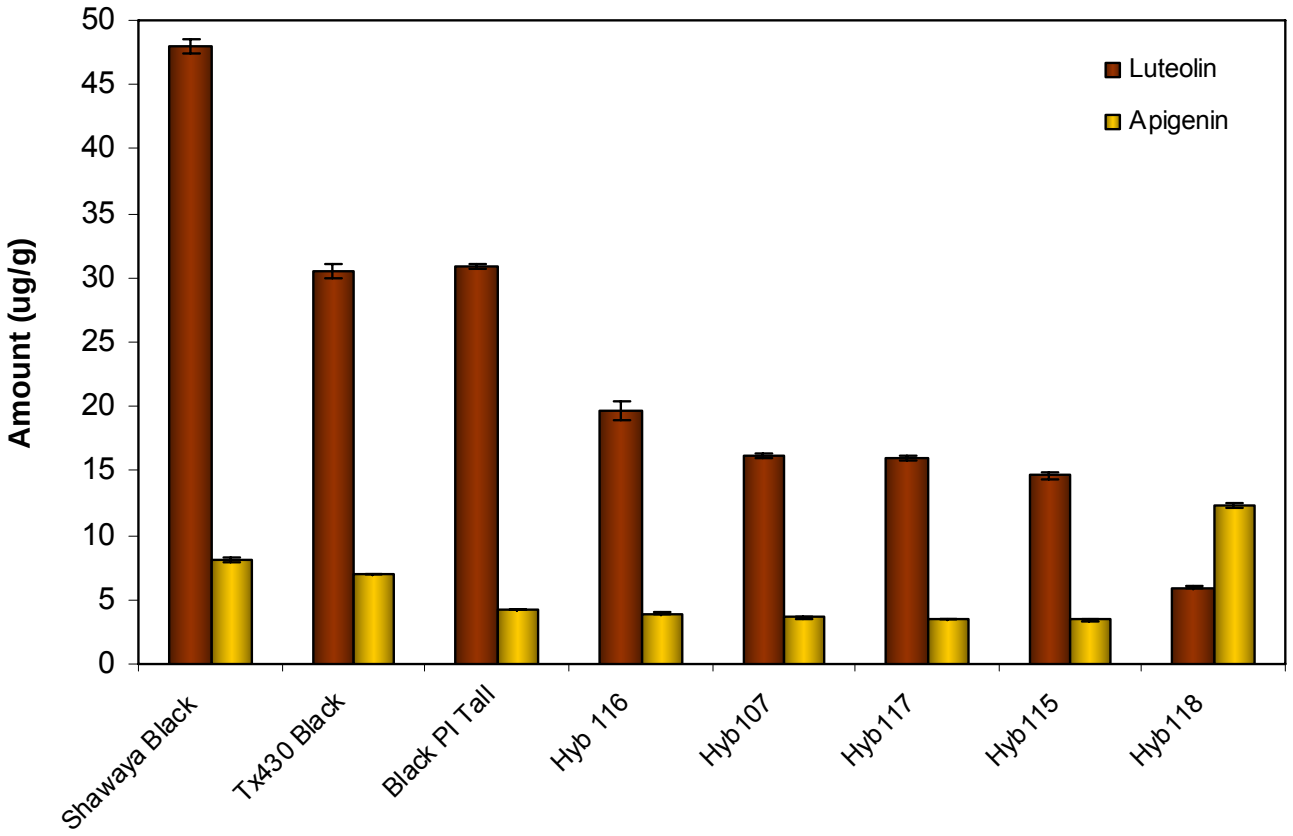

Fig. 60. Flavone profile of black sorghums grown in College Station, TX 2006.

glucosides, if any, to their aglycones as described in Chapter V. Two flavanones were identified: eriodictyol and naringenin. Flavanone levels in the samples were 89-119 µg/g with Hyb117 and Hyb116 having the highest and lowest levels, respectively (Fig. 61). Pericarp and secondary plant colors did not have an effect on flavanone levels. No significant correlations were found between flavanones and pericarp color nor were there any significant correlations between flavanones and other flavonoids.

Eriodictyol was the predominant flavanone in the black sorghums, which accounted for 55-61% of the total (Fig. 62). Hyb117 had the highest level of both eriodicytol (66 µg/g) and naringenin (53 µg/g).

Effect of Environment on Phenols and Antioxidant Activity Levels

Among locations, no trend was observed for total phenols, condensed tannins, or antioxidant activity. For example, total phenols (19.8 mg GAE/g) and condensed tannins (48.7 mg CE/g) in Black PI Tall were highest in College Station 2006 whereas those in Hyb118 were highest in Puerto Rico 2007 (Table 29). A strong correlation between total phenols and condensed tannins (r = 0.98, $p < 0.001$) indicated that total phenols were contributed by condensed tannins.

Overall, samples grown in College Station had higher flavan-4-ols and anthocyanins than those grown in Corpus Christi and Puerto Rico (Table 29). The lower anthocyanin levels in the samples from Puerto Rico and Corpus Christi could be due to pericarp color/appearance. The grains from Puerto Rico

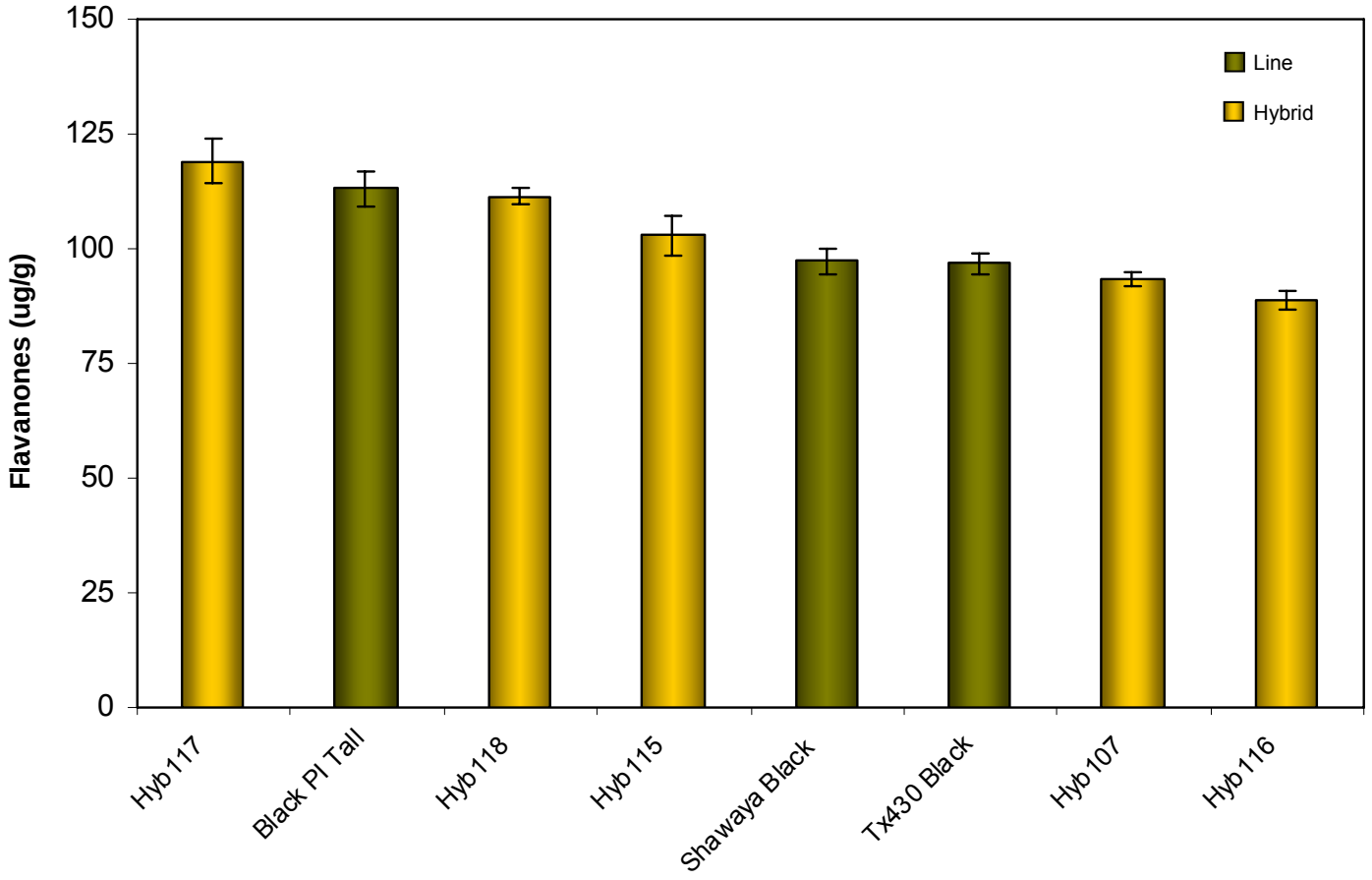

Fig. 61. Flavanone levels in black sorghums grown in College Station, TX 2006.

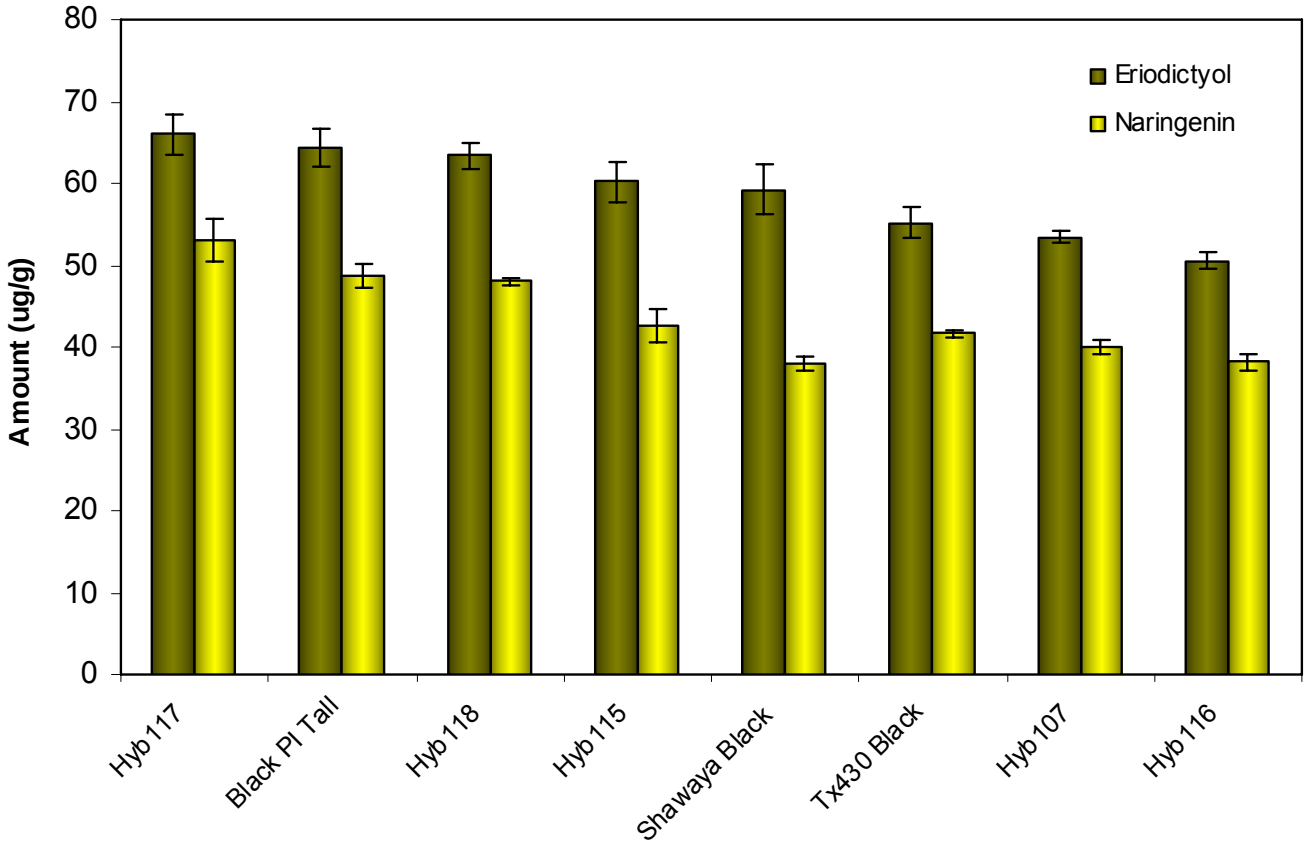

Fig. 62. Flavanone profile of black sorghums grown in College Station, TX 2006.

Table 29

Phenol and Antioxidant Activity Levels in Black Sorghums Grown in Different Environments

Variety	Location[a]	Total Phenols (mg GAE/g)[b]	Tannins (mg CE/g)[c, d]	Flavan-4-ols (Abs/mL/g)	Anthocyanins (Abs/mL/g)	ABTS (umol TE/g)[e]	DPPH (umol TE/g)
Tx430 Black	CS 2006	5.34 ± 0.11	0.70 ± 0.37	12.01 ± 0.07	218.15 ± 7.84	80.02 ± 2.52	32.42 ± 0.23
	CS 2007	5.66 ± 0.08	0.45 ± 0.45	13.37 ± 0.38	220.52 ± 1.60	95.62 ± 1.30	39.98 ± 0.88
	CC 2007	6.11 ± 0.14	1.85 ± 0.25	11.11 ± 0.12	175.57 ± 3.74	99.53 ± 1.76	33.55 ± 2.00
	PR 2007	4.58 ± 0.03	0.39 ± 0.33	9.42 ± 0.06	138.77 ± 1.33	77.21 ± 1.68	35.06 ± 0.35
Black PI Tall	CS 2006	19.81 ± 0.53	48.72 ± 1.96	15.97 ± 0.14	221.33 ± 4.62	334.19 ± 8.06	177.21 ± 2.34
	CS 2007	17.76 ± 0.13	37.57 ± 0.82	15.48 ± 0.17	205.54 ± 5.35	291.63 ± 7.50	169.03 ± 4.02
	CC 2007	16.76 ± 0.20	36.75 ± 1.12	11.23 ± 0.21	133.05 ± 1.53	268.07 ± 1.38	164.30 ± 1.99
Hyb116	CS 2006	10.61 ± 0.06	17.80 ± 0.42	10.50 ± 0.01	139.99 ± 1.64	172.95 ± 1.82	94.86 ± 1.53
	CS 2007	11.46 ± 0.16	18.68 ± 0.45	10.54 ± 0.19	137.67 ± 1.87	184.27 ± 1.15	99.93 ± 2.23
	CC 2007	11.93 ± 0.23	23.89 ± 0.99	6.62 ± 0.05	81.37 ± 4.05	184.46 ± 3.55	99.12 ± 2.26
	PR 2007	9.93 ± 0.23	16.94 ± 0.52	8.18 ± 0.08	76.63 ± 0.74	186.75 ± 8.63	85.65 ± 3.91
Hyb117	CS 2006	12.33 ± 0.50	25.61 ± 0.72	10.80 ± 0.04	108.07 ± 8.36	203.56 ± 8.19	112.77 ± 4.56
	CS 2007	15.36 ± 0.38	34.81 ± 1.26	10.47 ± 0.07	126.25 ± 1.11	242.64 ± 4.08	138.55 ± 0.57
	CC 2007	11.43 ± 0.08	21.71 ± 1.19	7.00 ± 0.08	87.72 ± 2.53	180.67 ± 1.37	97.36 ± 5.12
Hyb118	CS 2006	16.96 ± 0.10	42.78 ± 1.59	8.77 ± 0.09	148.17 ± 1.39	272.80 ± 2.50	152.98 ± 1.14
	CS 2007	19.05 ± 0.22	53.37 ± 0.85	8.66 ± 0.16	134.92 ± 1.29	311.07 ± 4.23	173.62 ± 7.23
	CC 2007	16.51 ± 0.27	39.19 ± 0.67	5.96 ± 0.19	93.62 ± 0.24	263.28 ± 4.87	152.58 ± 4.66
	PR 2007	19.25 ± 0.28	55.50 ± 0.64	6.84 ± 0.10	55.63 ± 3.60	186.75 ± 8.63	183.66 ± 9.64

[a]CS = College Station; CC = Corpus Christi; PR = Puerto Rico.
[b]GAE = Gallic acid equivalents.
[c]CE = Catechin equivalents.
[d]Varieties with values lower than 2.00 are considered tannin-free.
[e]TE = Trolox equivalents.

were not as black as the other samples since these were grown in the winter and thus had shorter exposure to sunlight. On the other hand, grains from Corpus Christi were grown in the summer but that location had high rainfall in the first week of July (13.9 in., National Weather Service Forecast Office, 2007), which caused the grains to undergo weathering and molding. As a result, anthocyanin levels decreased. A strong correlation between flavan-4-ols and anthocyanins was observed ($r = 0.88$, $p < 0.001$).

From the HPLC analysis, the 3-deoxyanthocyanin levels in the samples from College Station were higher than those from Corpus Christi and Puerto Rico (Table 30). This confirmed that the short sunlight exposure and the presence of molds on the grains from Puerto Rico and Corpus Christi, respectively, affected the levels of these compounds. The panicles in Corpus Christi reached anthesis early May and reached physical maturity at the end of May – beginning of June. Total rainfall in May and June was only 2.2 and 2.5 inches, respectively. As stated in Chapter IV, it is possible that the mold susceptibility of the grain could have been increased due to its incapability to produce 3-deoxyanthocyanins. If the grain had been exposed to low environmental stresses before reaching physical maturity as seen in Corpus Christi, then the grain would not trigger the gene to produce enough phytoalexins to minimize molding. As a result, 3-deoxyanthocyanin levels were 2-3 times lower than those from College Station. The general linear model showed a genotype x environment interaction ($p < 0.001$), which meant that

Table 30

3-Deoxyanthocyanin Levels in Black Sorghums Grown in Different Environments[a]

Variety	Location[b]	LUT[c]	AP[d]	5-MeO-LUT[e]	7-MeO-AP[f]	Total
Tx430 Black	CS 2006	167.31 ± 2.21	168.21 ± 1.08	172.39 ± 2.98	168.18 ± 1.82	676.09 ± 8.00
	CS 2007	250.73 ± 3.05	138.26 ± 1.79	254.74 ± 3.25	156.26 ± 2.11	799.99 ± 10.07
	CC 2007	104.74 ± 1.86	61.27 ± 1.60	141.82 ± 1.43	82.85 ± 1.03	390.68 ± 4.97
	PR 2007	241.48 ± 5.09	117.39 ± 0.77	144.98 ± 2.51	70.58 ± 0.82	574.43 ± 9.15
Black PI Tall	CS 2006	313.60 ± 3.55	245.29 ± 1.60	153.75 ± 2.45	91.75 ± 0.40	804.39 ± 7.01
	CS 2007	240.16 ± 1.49	164.97 ± 0.37	132.88 ± 0.82	67.48 ± 0.50	605.49 ± 2.14
	CC 2007	119.78 ± 2.05	90.15 ± 2.41	75.36 ± 0.58	32.60 ± 0.55	317.89 ± 5.33
Hyb116	CS 2006	124.13 ± 1.41	162.53 ± 1.23	64.86 ± 0.47	82.34 ± 0.91	433.86 ± 1.06
	CS 2007	176.24 ± 1.87	151.13 ± 2.18	101.56 ± 1.20	87.00 ± 1.06	515.93 ± 4.52
	CC 2007	49.25 ± 0.82	43.81 ± 0.98	46.83 ± 0.81	32.98 ± 0.47	172.87 ± 1.42
	PR 2007	125.35 ± 1.08	98.63 ± 0.60	47.11 ± 0.37	31.64 ± 0.15	302.73 ± 0.69
Hyb117	CS 2006	98.46 ± 0.67	116.22 ± 1.15	54.57 ± 1.10	59.80 ± 0.77	329.05 ± 1.88
	CS 2007	153.29 ± 3.45	110.71 ± 1.96	105.45 ± 2.07	76.64 ± 1.27	446.09 ± 8.70
	CC 2007	53.64 ± 0.34	42.59 ± 0.43	55.79 ± 0.25	38.88 ± 0.34	190.90 ± 1.15
Hyb118	CS 2006	9.11 ± 0.21	376.09 ± 0.62	5.51 ± 0.25	94.60 ± 0.88	485.31 ± 0.55
	CS 2007	20.44 ± 0.68	364.26 ± 3.71	18.97 ± 0.44	109.04 ± 1.69	512.71 ± 5.87
	CC 2007	10.36 ± 0.31	100.10 ± 0.75	12.99 ± 0.27	53.18 ± 0.23	176.63 ± 0.84
	PR 2007	4.70 ± 0.11	181.27 ± 2.05	2.93 ± 0.33	29.83 ± 0.73	218.73 ± 3.10

[a]Levels are expressed as µg/g.
[b]CS = College Station; CC = Corpus Christi; PR = Puerto Rico.
[c]LUT = Luteolinidin.
[d]AP = Apigeninidin.
[e]5-MeO-LUT = 5-Methoxyluteolinidin.
[f]7-MeO-AP = 7-Methoxyapigeninidin.

Table 31

Genotype (Variety) x Environment (Location) Interaction of 3-Deoxyanthocyanins in Black Sorghums Grown in Different Environments

Source	Type III Sum of Squares	df	Mean Square	F	Sig.	Partial Eta Squared
Corrected Model	3129997.222[a]	20	156499.861	5869.794	.000	1.000
Intercept	10326154.871	1	10326154.871	387300.045	.000	1.000
Variety	1698693.353	7	242670.479	9101.770	.000	.999
Location	1038755.473	3	346251.824	12986.765	.000	.999
Variety * Location	167875.816	10	16787.582	629.647	.000	.993
Error	1119.800	42	26.662			
Total	16940324.894	63				
Corrected Total	3131117.022	62				

[a]R Squared = 1.000 (Adjusted R Squared = .999)

environment had an effect on 3-deoxyanthocyanin levels (Table 31).

Environment also affected 3-deoxyanthocyanin composition. For example, LUT, AP, 5-MeO-LUT, and 7-MeO-AP of Tx430 Black from College Station 2006 were evenly distributed (Fig. 63). On the other hand, the combined proportions of LUT and 5-MeO-LUT for Tx430 Black from College Station, Corpus Christi, and Puerto Rico 2007 were 63, 63, and 67%, respectively. Another example is Hyb116. The sample harvested in College Station 2006 had a high LUT and AP composition (66%) with AP being dominant (Fig. 64). On the other hand, both samples from College Station and Puerto Rico 2007 had a high LUT and AP composition (63-74%) with LUT being dominant. The 3-deoxyanthocyanin composition of the sample grown in Corpus Christi was almost evenly distributed. The composition of Hyb118 was similar in all locations in terms of AP being the predominant 3-deoxyanthocyanin (57-83%)

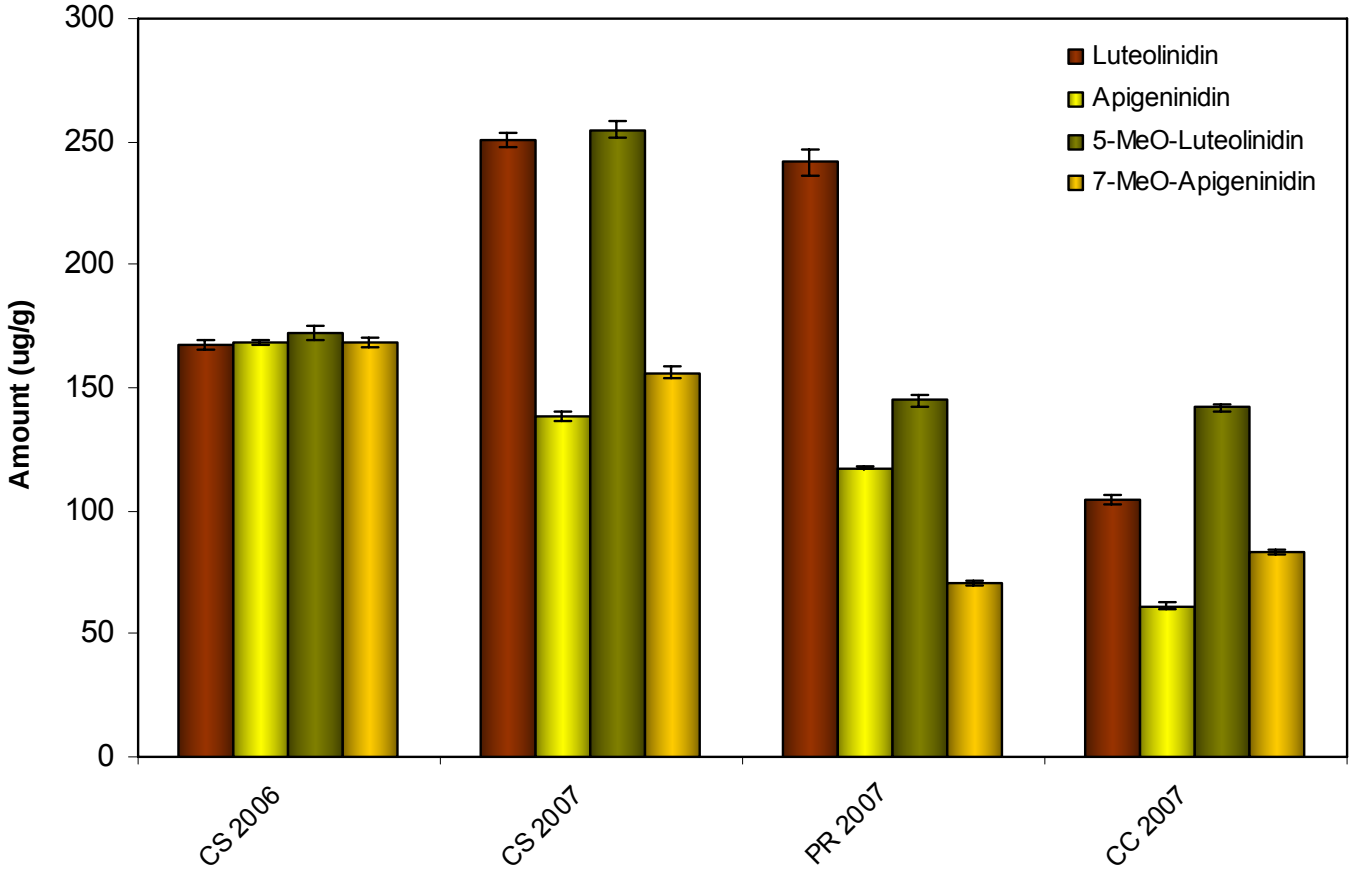

Fig. 63. 3-Deoxyanthocyanin profile of Tx430 Black grown in different environments. CS = College Station; PR = Puerto Rico, CC = Corpus Christi.

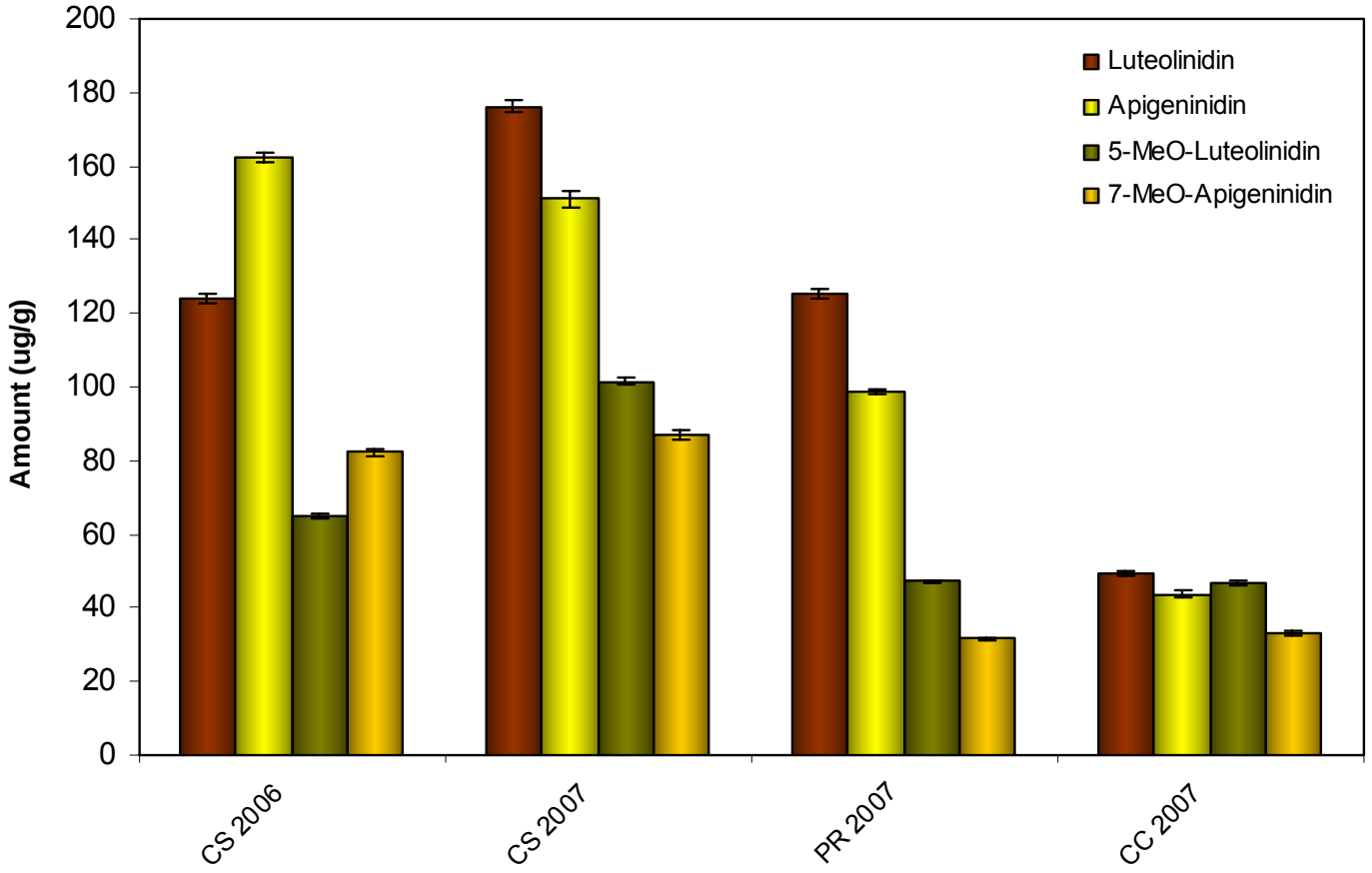

Fig. 64. 3-Deoxyanthocyanin profile of Hyb116 grown in different environments. CS = College Station; PR = Puerto Rico, CC = Corpus Christi.

(Fig. 65). The sample from Corpus Christi had the lowest AP composition but that could be due to the grain being damaged. These results confirmed that Hyb118 consistently has the yellow AP as the predominant 3-deoxyanthocyanin.

As observed for the 3-deoxyanthocyanins, samples from College Station had higher flavone levels than those from the other two locations (Table 32) and there was a genotype x environment interaction ($p < 0.001$, Table 33). Luteolin was consistently the predominant flavone (79-91%) in all samples with the exception of Hyb118. Apigenin was the predominant flavone in Hyb118 from College Station and Puerto Rico (56-68%) whereas luteolin was predominant in the sample from Corpus Christi (60%).

Samples from College Station and Puerto Rico generally had higher flavanone levels than those from Corpus Christi (Table 34) and there was a genotype x environment interaction ($p < 0.001$) (Table 35). These results suggested that molding decreased flavanone levels. Eriodictyol was the predominant flavanone (51-67%) for all samples with the exception of Hyb117 (50%) and Hyb118 (46%) from Corpus Christi. The flavanone distribution for the samples from Corpus Christi was, in general, evenly distributed.

This study showed that the main flavonoids in black pericarp sorghums are the 3-deoxyanthocyanins. These compounds are located in the pericarp and these are concentrated 3-4 times in the bran fraction (Awika et al 2004a, b). Sorghum is the only dietary source of those compounds (Wu et al 2005) and these have good potential to be utilized as natural food colorants due to their pH

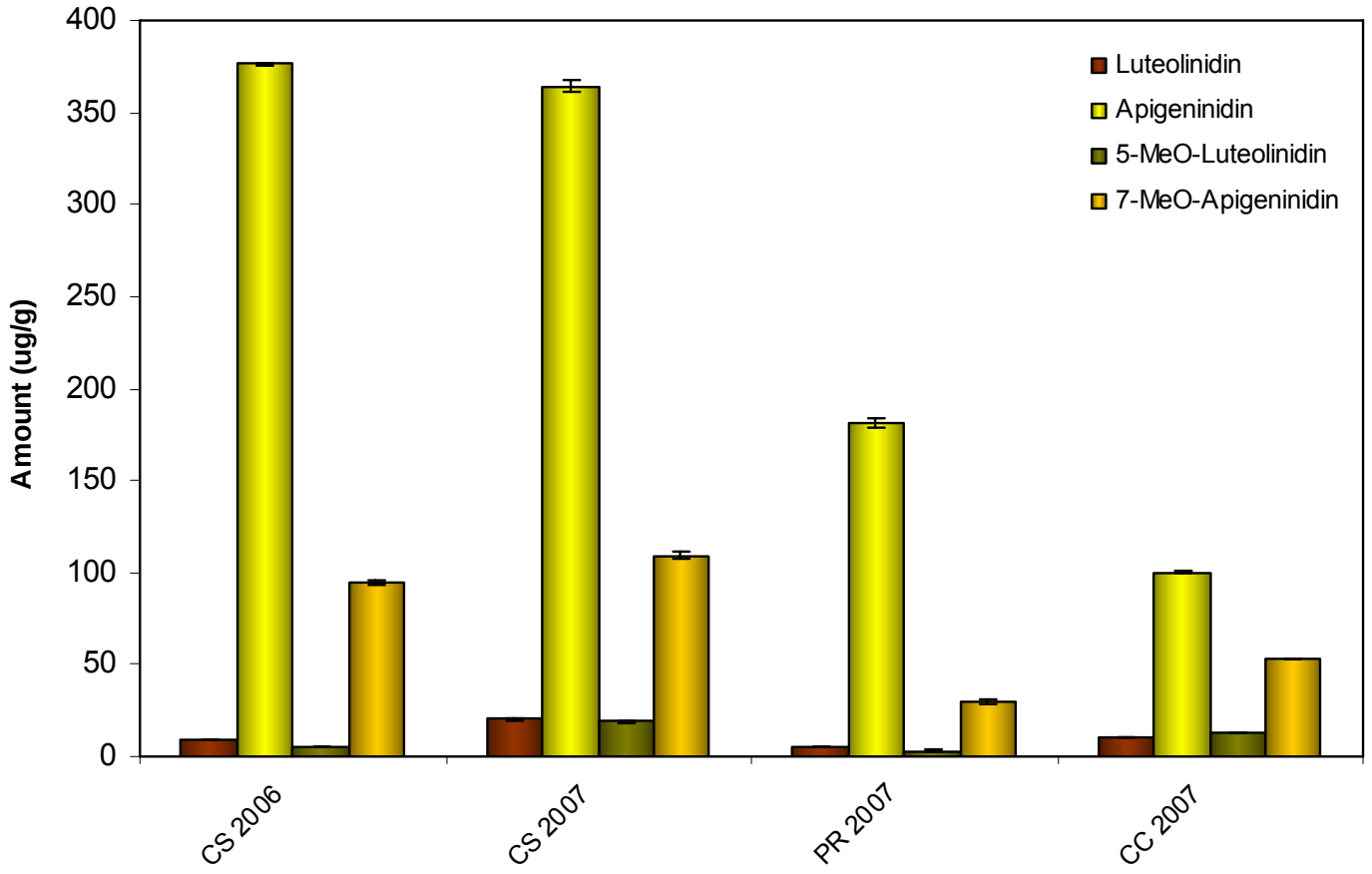

Fig. 65. 3-Deoxyanthocyanin profile of Hyb118 grown in different environments. CS = College Station; PR = Puerto Rico, CC = Corpus Christi.

Table 32

Flavone Levels in Black Sorghums Grown In Different Environments[a]

Variety	Location[b]	Luteolin	Apigenin	Total
Tx430 Black	CS 2006	30.56 ± 0.59	7.03 ± 0.02	37.59 ± 0.61
	CS 2007	37.20 ± 0.40	6.46 ± 0.05	43.66 ± 0.44
	CC 2007	13.76 ± 0.11	3.56 ± 0.11	17.32 ± 0.08
	PR 2007	33.81 ± 0.06	3.43 ± 0.04	37.24 ± 0.07
Black PI Tall	CS 2006	30.90 ± 0.22	4.23 ± 0.08	35.13 ± 0.30
	CS 2007	24.04 ± 0.26	3.40 ± 0.09	27.44 ± 0.25
	CC 2007	10.44 ± 0.07	2.13 ± 0.10	12.57 ± 0.13
Hyb116	CS 2006	19.62 ± 0.75	3.95 ± 0.09	23.57 ± 0.83
	CS 2007	25.00 ± 0.25	4.00 ± 0.07	29.00 ± 0.25
	CC 2007	4.83 ± 0.06	1.25 ± 0.08	6.08 ± 0.11
	PR 2007	19.51 ± 0.08	2.41 ± 0.02	21.92 ± 0.09
Hyb117	CS 2006	16.04 ± 0.19	3.48 ± 0.03	19.52 ± 0.16
	CS 2007	19.96 ± 0.52	3.06 ± 0.03	23.02 ± 0.55
	CC 2007	6.70 ± 0.16	1.61 ± 0.03	8.31 ± 0.19
Hyb118	CS 2006	5.93 ± 0.12	12.35 ± 0.19	18.28 ± 0.29
	CS 2007	8.38 ± 0.19	10.82 ± 0.05	19.20 ± 0.23
	CC 2007	3.46 ± 0.18	2.29 ± 0.09	5.75 ± 0.21
	PR 2007	3.55 ± 0.02	6.73 ± 0.04	10.28 ± 0.06

[a]Levels are expressed as µg/g.
[b]CS = College Station; CC = Corpus Christi; PR = Puerto Rico.

Table 33

Genotype (Variety) x Environment (Location) Interaction of Flavones in Black Sorghums Grown in Different Environments

Source	Type III Sum of Squares	df	Mean Square	F	Sig.	Partial Eta Squared
Corrected Model	9986.282[a]	20	499.314	3913.246	.000	.999
Intercept	26512.875	1	26512.875	207787.850	.000	1.000
Variety	5841.407	7	834.487	6540.076	.000	.999
Location	3134.290	3	1044.763	8188.064	.000	.998
Variety * Location	443.078	10	44.308	347.251	.000	.988
Error	5.359	42	.128			
Total	44269.338	63				
Corrected Total	9991.641	62				

[a]R Squared = .999 (Adjusted R Squared = .999)

(1-5), temperature, and water activity stabilities (Sweeny and Iacobucci 1983; Mazza and Brouillard 1987; Gous 1989; Awika et al 2004a, b; Cardenas-Hinojosa et al 2007; Njongmeta-Nenge et al 2007). The health benefits of those compounds are unknown. However, one study demonstrated that 3-deoxyanthocyanins have greater anticancer properties in human leukemia HL-60 and hepatoma hepG2 cell lines than the common anthocyanins (i.e. cyanidin, pelargonidin) (Shih et al 2007).

Environment did have an effect on flavonoid levels. The preliminary data suggested that long sunlight exposure and minimum weathering/molding increased 3-deoxyanthocyanin levels. These results provide useful guidelines to produce sorghums with the greatest levels of the rare 3- deoxyanthocyanins, which are potentially quite important sources of healthy components in foods and other applications.

Table 34

Flavanone Levels in Black Sorghums Grown in Different Environments[a]

Variety	Location[b]	Eriodictyol	Naringenin	Total
Tx430 Black	CS 2006	55.09 ± 1.89	41.67 ± 0.47	96.76 ± 2.25
	CS 2007	65.08 ± 1.01	41.99 ± 2.58	107.07 ± 3.50
	CC 2007	36.77 ± 0.45	30.04 ± 1.84	66.81 ± 2.16
	PR 2007	71.08 ± 1.58	35.14 ± 1.49	106.22 ± 3.05
Black PI Tall	CS 2006	64.36 ± 2.37	48.74 ± 1.52	113.10 ± 3.89
	CS 2007	65.14 ± 2.61	53.78 ± 2.61	118.92 ± 5.22
	CC 2007	40.48 ± 0.60	33.02 ± 0.26	73.50 ± 0.64
Hyb116	CS 2006	50.50 ± 1.06	38.16 ± 0.94	88.66 ± 2.00
	CS 2007	74.61 ± 5.13	44.43 ± 3.06	119.04 ± 8.14
	CC 2007	28.71 ± 0.48	28.03 ± 0.29	56.74 ± 0.76
	PR 2007	76.15 ± 3.32	44.97 ± 1.38	121.12 ± 4.68
Hyb117	CS 2006	65.98 ± 2.41	53.09 ± 2.58	119.07 ± 4.98
	CS 2007	76.86 ± 2.43	55.66 ± 3.08	132.52 ± 5.41
	CC 2007	28.77 ± 0.93	28.84 ± 0.80	57.61 ± 1.70
Hyb118	CS 2006	63.43 ± 1.61	48.02 ± 0.43	111.45 ± 1.96
	CS 2007	86.55 ± 3.48	64.52 ± 2.23	151.07 ± 5.65
	CC 2007	26.96 ± 1.65	31.81 ± 0.37	58.77 ± 1.91
	PR 2007	96.26 ± 3.42	59.61 ± 2.03	155.87 ± 5.45

[a]Levels are expressed as µg/g.
[b]CS = College Station; CC = Corpus Christi; PR = Puerto Rico.

Table 35

Genotype (Variety) x Environment (Location) Interaction of Flavanones in Black Sorghums Grown in Different Environments

Source	Type III Sum of Squares	df	Mean Square	F	Sig.	Partial Eta Squared
Corrected Model	47898.375[a]	20	2394.919	155.712	.000	.987
Intercept	523276.529	1	523276.529	34022.288	.000	.999
Variety	5700.554	7	814.365	52.948	.000	.898
Location	38310.020	3	12770.007	830.278	.000	.983
Variety * Location	4605.189	10	460.519	29.942	.000	.877
Error	645.977	42	15.380			
Total	707594.738	63				
Corrected Total	48544.352	62				

[a]R Squared = .987 (Adjusted R Squared = .980)

CHAPTER VII

SUMMARY

A wide variety of pigmented sorghum genotypes were evaluated for total phenols, condensed tannins, flavan-4-ols, and *in vitro* antioxidant activity. Total phenol and antioxidant activity levels were increased in tannin sorghums, which were also reported by Awika et al (2003b). Flavan-4-ol levels were highest in sorghums with a black pericarp, followed by those with a red pericarp (Fig. 66). These compounds could reduce mold damage in sorghums (Jambunathan et al 1990, 1991; Melake-Berhan et al 1996; Menkir et al 1996; Audilakshmi et al 1999). Information on the health benefits of flavan-4-ols are lacking.

Flavonoid profile of all sorghums was also evaluated using HPLC-PDA. Sorghums with a black pericarp had the highest 3-deoxyanthocyanin levels (Fig. 67) and these were increased when the grain had minimal weathering and was darkest in color. These compounds are located in the pericarp and are phytoalexins (Lo et al 1999; Nicholson and Wood 2001; Awika et al 2004 a, b; Seitz 2004). Although black pericarp sorghums are genetically red, they should be reclassified into a new category based on their high levels of the rare 3-deoxyanthocyanins.

Sorghums with a lemon-yellow pericarp had the highest flavanone (eriodictyol and naringenin) levels (Fig. 68) and these were increased when the grain was brighter in yellow color and had minimum weathering. Compared to

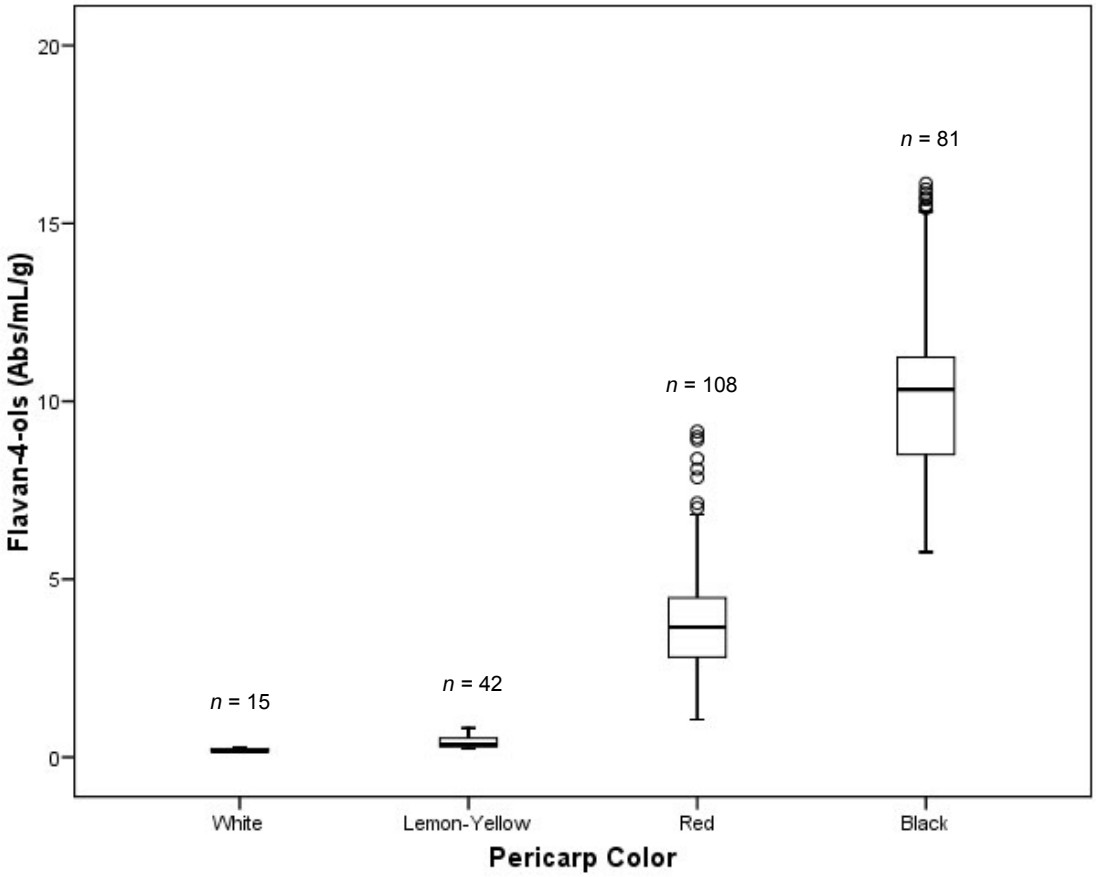

Fig. 66. Boxplot showing the distribution of flavan-4-ol levels in all sorghum varieties of varying pericarp color from all locations studied. The median is indicated by the black line within the box. The 25th and 75th percentiles are the lower and upper boundaries of the box, respectively. The open circles represent mild outliers. *n* = total number of measurements.

common sources (i.e. citrus fruits), these sorghums are a good source of eriodictyol and naringenin. For example, SC748 had higher eriodictyol (959 µg/g, fresh wt.) and naringenin levels (603 µg/g, fresh wt.) than the common sources with amounts of 106-309 and 238-574 µg/g (fresh wts.), respectively (Figs. 45, 46). Flavanone concentrations were increased almost ten-fold in the

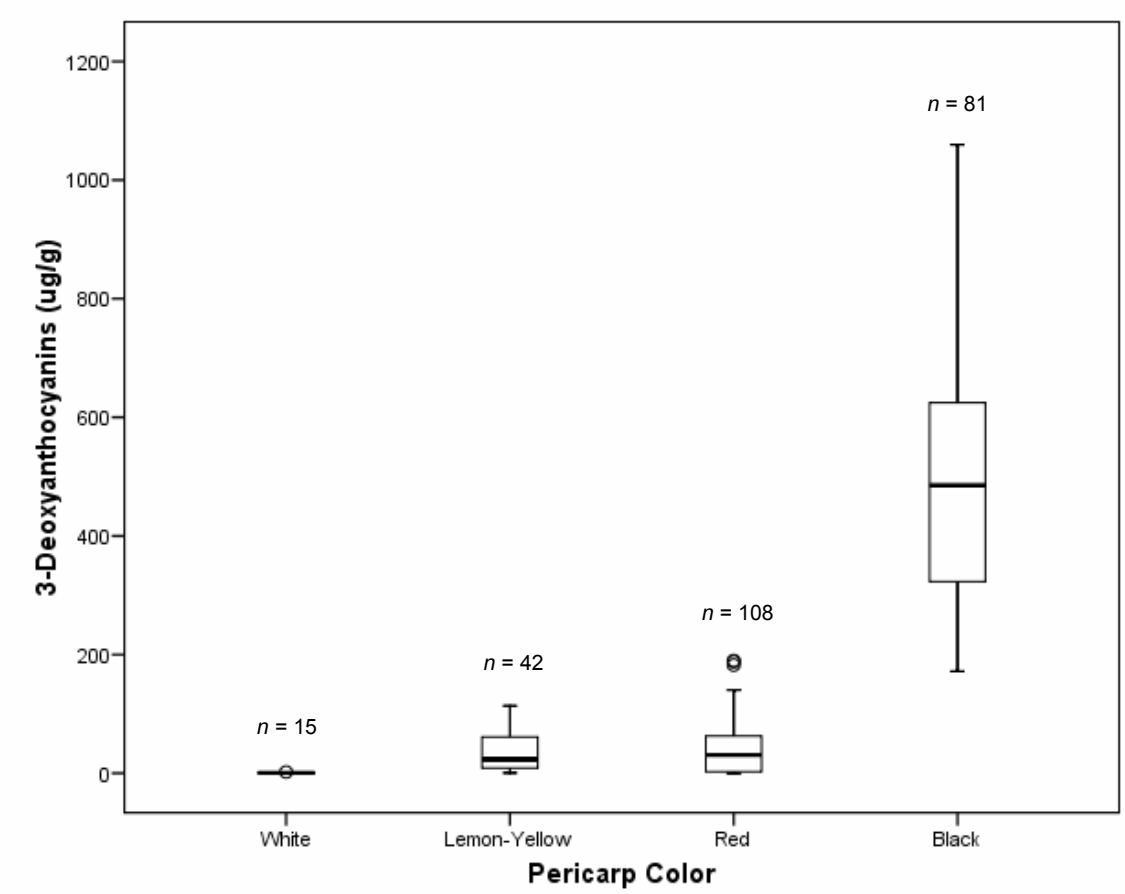

Fig. 67. Boxplot showing the distribution of 3-deoxyanthocyanin levels in all sorghum varieties of varying pericarp color from all locations studied. The median is indicated by the black line within the box. The 25th and 75th percentiles are the lower and upper boundaries of the box, respectively. The open circles represent mild outliers. *n* = total number of measurements.

bran fraction (Fig. 41). Studies are needed to determine the stability of sorghum flavanones during storage and processing. For example, it was reported by Patil et al (2004) that low doses of irradiation (≤ 200 Gy) coupled with 35 days of storage increased flavanone levels in early season grapefruits while higher doses (400-700 Gy) had negative effects. There is a need to determine the fate

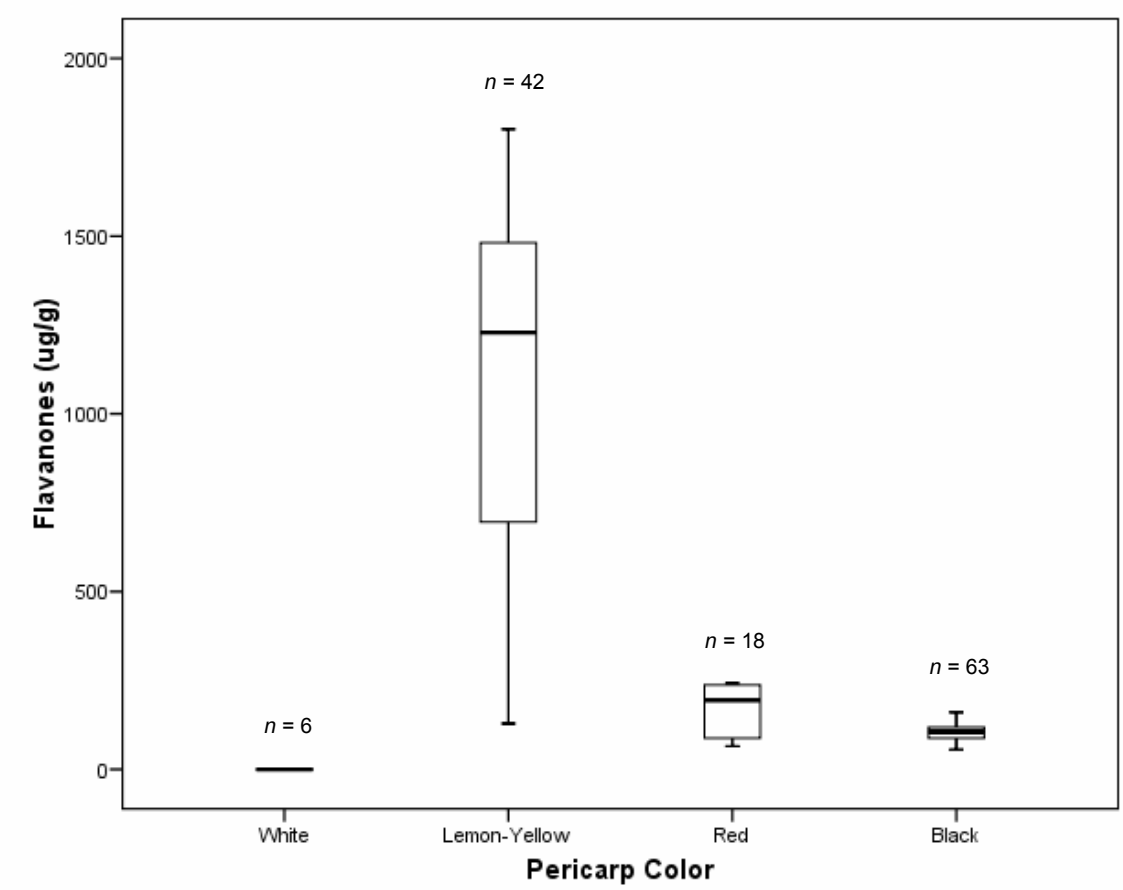

Fig. 68. Boxplot showing the distribution of flavanone levels in sorghum varieties of varying pericarp color. Flavanone levels were measured after acid hydrolysis. The median is indicated by the black center line within the box. The 25th and 75th percentiles are the lower and upper boundaries of the box, respectively. The *'s represent extreme outliers. *n* = total number of measurements.

of these compounds when lemon-yellow sorghums are processed into food products (i.e. bread, extrudates). Studies are also needed to determine the biological activity of sorghum flavanones in pure form and as part of a crude extract.

No flavonoids were predominant in sorghums with a red pericarp. However, flavonoid composition did vary when these sorghums were separated by secondary plant color. All sorghums with tan secondary plant color, including those with a white pericarp, had higher levels of flavones than those with

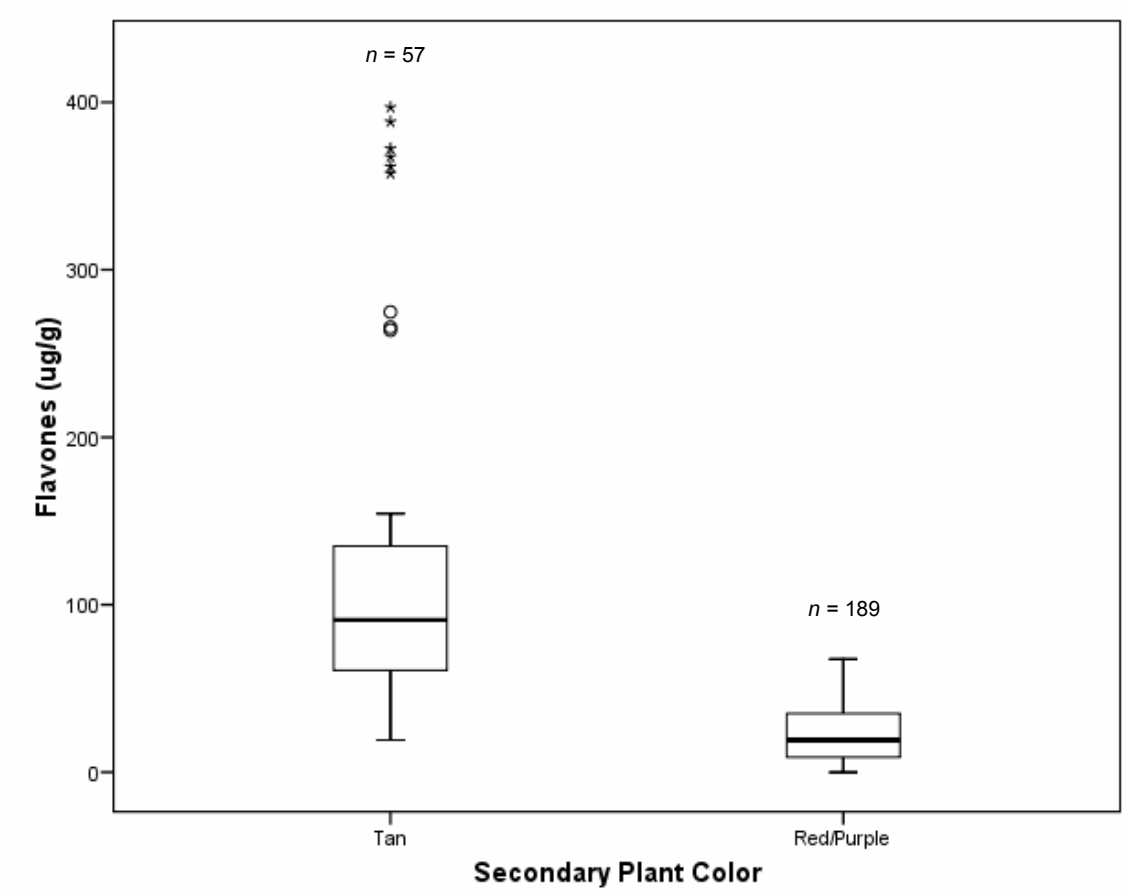

Fig. 69. Boxplot showing the distribution of flavone levels in all sorghum varieties with tan or red/purple secondary plant color from all locations studied. The median is indicated by the black center line within the box. The 25th and 75th percentiles are the lower and upper boundaries of the box, respectively. The open circles represent mild outliers. *n* = total number of measurements.

red/purple secondary plant color (Fig. 69). On the other hand, all red/purple plant sorghums had higher 3-deoxyanthocyanin levels than tan plant sorghums (Fig. 70). Among red/purple plant sorghums, sorghums with a lemon-yellow pericarp had the highest levels of flavones (Fig. 71). Flavonoid composition was

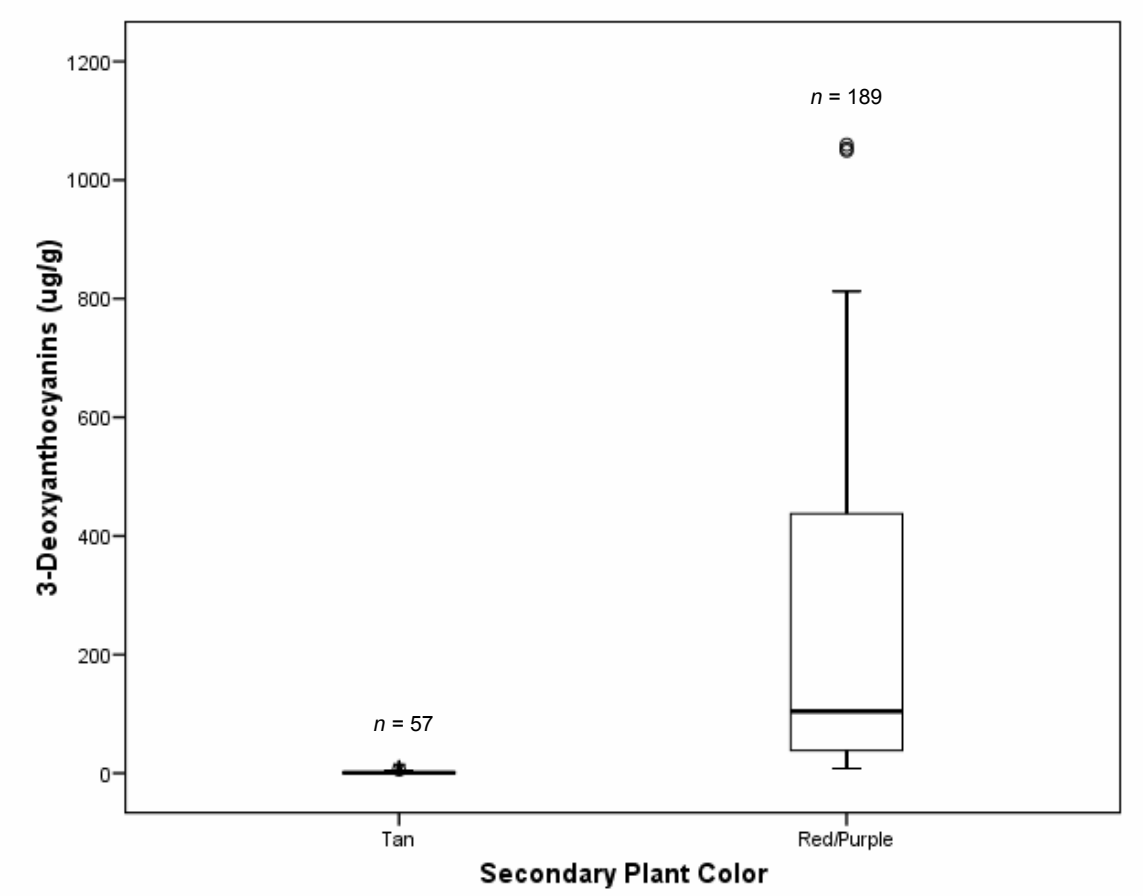

Fig. 70. Boxplot showing the distribution of 3-deoxyanthocyanin levels in all sorghum varieties with tan or red/purple secondary plant color from all locations studied. The median is indicated by the black center line within the box. The 25th and 75th percentiles are the lower and upper boundaries of the box, respectively. The open circles and *'s represent mild and extreme outliers, respectively. *n* = total number of measurements.

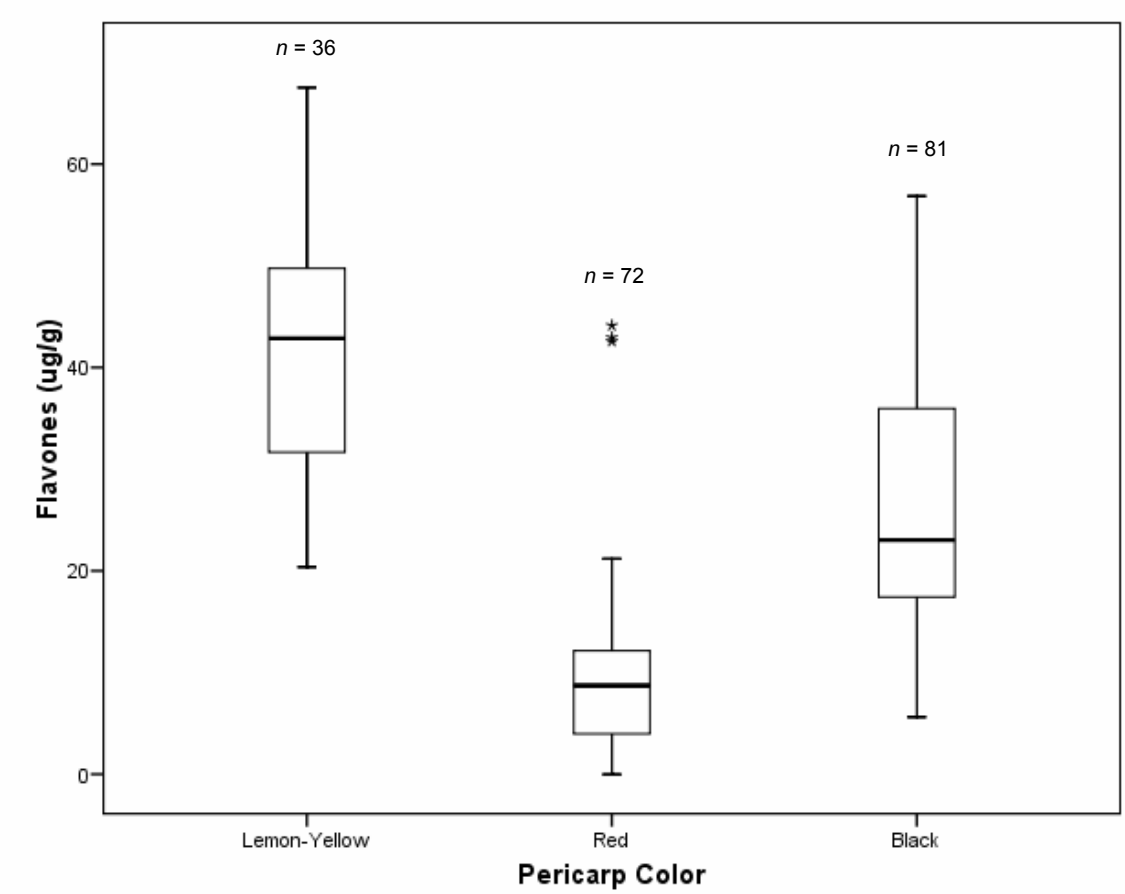

Fig. 71. Boxplot showing the distribution of flavone levels in all red/purple plant sorghum varieties of varying pericarp color from all locations studied. The median is indicated by the black center line within the box. The 25th and 75th percentiles are the lower and upper boundaries of the box, respectively. The open circles and *'s represent mild and extreme outliers, respectively. *n* = total number of measurements.

consistent in sorghums grown in different locations. Black sorghums grown in an environment that has long sunlight exposure had higher levels of 3-deoxyanthocyanins. Flavanones and 3-deoxyanthocyanins were increased when sorghums had minimal weathering or molding.

Further studies are needed to explain the differences in flavonoid composition among sorghum genotypes. This could be due to the overexpression of certain genes causing the increased production of specific compounds. For example, tan plant sorghums could have an overexpression of a flavone synthase gene that triggers high production of flavones from flavanones, which is not occurring in red/purple plant sorghums. The elevated levels of 3-deoxyanthocyanins in red/purple plant sorghums could be due to the overexpression of a gene that triggers high production of these compounds. Sorghums with a lemon-yellow pericarp could also have an overexpression of a chalcone synthase gene that triggers high production of flavanones. It was reported by Yu et al (2005) that the chalcone synthase gene, *SbCHS2*, is involved in the production of flavanones. However, they did not report this gene in the lemon-yellow SC748 sorghum. Instead, they reported that SC748 had the *SbSTS1* gene, which is a gene that triggers the production of stilbenes (i.e. resveratrol) by stilbene synthases. Stilbenes have not been reported in sorghums but studies are needed to determine their presence. Stilbenes are reported to have antioxidant, anticancer anti-inflammatory, and phytoestrogenic properties and they are believed to promote cardiovascular health (Tsai et al 1999; Cassidy et al 2000; Cornwell et al 2004; King et al 2006).

This study reports that all sorghums, including those with a white pericarp, have flavonoids and their levels and compositions are affected by the genotype. This information will be helpful for plant breeders, food scientists, and

the pharmaceutical/nutraceutical/cosmoceutical industries in selecting sorghums with desired healthy components. Sorghum as a source of flavonoid has several attractive advantages. Sorghum is (1) dry; (2) easy to store for long periods of time, and (3) easy to process into shelf-stable concentrates.

LITERATURE CITED

Amarowicz, R., Karamac, M., and Shahidi, F. 2003. Antioxidant activity of phenolic fractions of lentil (*Lens culinaris*). J. Food Lipids 10:1-10.

Anglani, C. 1998. Sorghum for human food: A review. Plant Foods Hum. Nutr. 52: 85-95.

Arnao, M.B. 2000. Some methodological problems in the determination of antioxidant activity using chromogen radicals: A practical case. Trends Food Sci. Technol. 11:419-421.

Audilakshmi, S., Stenhouse, J.W., Reddy, T.P., and Prasad, M.V.R. 1999. Grain mould resistance and associated characters of sorghum genotypes. Euphytica 107:91-103.

Aviado, D.M., Bacalzo, Jr., L.V., and Belej, M.A. 1974. Prevention of acute pulmonary insufficiency by eriodictyol. J. Pharmacol. Exp. Ther. 189:157-166.

Awika, J.M. 2000. Sorghum phenols as antioxidants. M.S. Thesis. Texas A&M University: College Station, TX.

Awika, J.M. 2003. Antioxidant properties of sorghum. Ph.D. Dissertation. Texas A&M University: College Station, TX.

Awika, J.M., Dykes, L., Gu, L., Rooney, L.W., and Prior, R.L. 2003a. Processing of sorghum (*Sorghum bicolor*) and sorghum products alters procyanidin oligomer and polymer distribution and content. J. Agric. Food Chem. 51:5516-5521.

Awika, J.M., McDonough, C.M., and Rooney, L.W. 2005. Decorticating sorghum to concentrate healthy phytochemicals. J. Agric. Food Chem. 53:6230-6234.

Awika, J.M., Rooney, L.W., and Waniska, R.D. 2004a. Anthocyanins from black sorghum and their antioxidant properties. Food Chem. 90:293-301.

Awika, J.M., Rooney, L.W., and Waniska, R.D. 2004b. Properties of 3-deoxyanthocyanins from sorghum. J. Agric. Food Chem. 52:4388-4394.

Awika, J.M., Rooney, L.W., Wu, X., Prior, R.L., and Cisneros-Zevallos, L. 2003b. Screening methods to measure antioxidant activity of sorghum (*Sorghum bicolor*) and sorghum products. J. Agric. Food Chem. 51:6657-6662.

Bate-Smith, E.C. 1969. Luteoforol (3',4,4',5,7-pentahydroxyflavan) in *Sorghum vulgare* L. Phytochem. 8:1803-1810.

Bate-Smith, E.C. and Rasper, V. 1969. Tannins of grain sorghum: Luteoforol (leucoluteolinidin), 3',4,4',5,7-pentahydroxyflavan. J. Food Sci. 34:203-209.

Beta, T., Rooney, L.W., Marovatsanga, L.T., and Taylor, J.R.N. 1999. Phenolic compounds and kernel characteristics of Zimbabwean sorghums. J. Sci. Food Agric. 79:1003-1010.

Blakeley, M.E., Rooney, L.W., Sullins, R.D., and Miller, F.R. 1979. Microscopy of the pericarp and the testa of different genotypes of sorghum. Crop Sci. 19:837-842.

Block, L.C., Santo, A.R.S., De Souza, M.M., Scheidt, C., Yunes, R.A., Santos, M.A., Monache, F.D., and Filho, V.C. 1998. Chemical and pharmacological examination of antinociceptive constituents of *Wedelia paludosa*. J. Ethnopharmacol. 61:85-89.

Boren, B. and Waniska, R.D. 1992. Sorghum seed color as an indicator of tannin content. J. Appl. Poultry Res. 1:117-121.

Borradaile, N.M. Carroll, K.K., and Kurowska, E.M. 1999. Regulation of HepG2 cell apolipoprotein B metabolism by the citrus flavanones hesperitin and naringenin. Lipids 34:591-598.

Butler, L.G. 1982. Relative degree of polymerization of sorghum tannin during seed development and maturation. J. Agric. Food Chem. 30:1090-1094.

Butler, L.G. 1989. Sorghum polyphenols. Pages 95-121 in: Toxicants of Plant Origin, Vol. IV, Phenolics. P.R. Cheeke, ed. CRC Press, Inc.: Boca Raton, FL.

Cardenas-Hinojosa, A.P., Njongmeta-Nenge L.A., Dykes, L., Cisneros-Zevallos, L., and Rooney, L.W. 2007. Concentration and temperature stability of anthocyanins in black sorghum. Cereal Foods World 52:A38.

Cassidy, A., Hanley, B., and Lamuela-Raventos, R.M. 2000. Isoflavones, lignans and stilbenes – Origins, metabolism and potential importance to human health. J. Sci. Food Agric. 80:1044-1062.

Chandra, A., Rana, J., and Li, Y. 2001. Identification, quantification and method validation of anthocyanins in botanical supplement raw materials by HPLC and HPLC-MS. J. Agric. Food Chem. 49:3515-3521.

Chaves, N., Escudero, J.C., and Gutierrez-Merino, C. 1997. Role of ecological variables in the seasonal variation of flavonoid content of *Cistus ladanifer* exudate. J. Chem. Ecol. 23:579-603.

Cherng, J.-M., Shieh, D.-E., Chiang, W., Chang, M.-Y., and Chiang, L.-C. 2007. Chemopreventive effects of minor dietary constituents in common foods on human cancer cells. Biosci. Biotechnol. Biochem. 71:1500-1504.

Commission Internationale de L'Éclairage. 1986. Colorimetry. Publication 15.2 Central Bureau of the CIE: Vienna, Austria.

Connor, A.M., Luby, J.J., and Tong, C.B.S. 2002. Genotypic and environmental variation in antioxidant activity, total phenolic content, and anthocyanin content among blueberry cultivars. J. Amer. Soc. Hort. Sci. 127:89-97.

Cornwell, T., Cohick, W., and Raskin, I. 2004. Dietary phytoestrogens and health. Phytochem. 65:995-1016.

De Beer, L, Joubert, E., Gelderblom, W.D.A., and Manley, M. 2003. Antioxidant activity of South African red and white cultivar wines: Free radical scavenging. J. Agric. Food Chem. 51:902-909.

Dicko, M.H., Gruppen, H., Traore, A.S., Van Berkel, W.J.H., and Voragen, A.G.J. 2005. Evaluation of the effect of germination on phenolic compounds and antioxidant activities in sorghum varieties. J. Agric. Food Chem. 53:2581-2588.

Downey, M.O., Dokoozlian, N.K., and Krstic, M.P. 2006. Cultural practice and environmental impacts on the flavonoid composition of grapes and wine: A review of recent research. Am. J. Enol. Vitic. 57:257-268.

Dykes, L. and Rooney, L.W. 2006. Phenolic compounds in cereal grains and their health benefits. Cereal Foods World 52:105-111.

Earp, C.F., Akingbala, J.O., Ring, S.H., and Rooney, L.W. 1981. Evaluation of several methods to determine tannins in sorghums with varying kernel characteristics. Cereal Chem. 58:234-238.

Earp, C.F., McDonough, C.M., Awika, J., and Rooney, L.W. 2004a. Testa development in the caryopsis of *Sorghum bicolor* (L.) Moench. J. Cereal Sci. 39:303-311.

Earp, C.F., McDonough, C.M., and Rooney, L.W. 2004b. Microscopy of pericarp development in the caryopsis of *Sorghum bicolor* (L.) Moench. J. Cereal Sci. 39:21-27.

Earp, C.F. and Rooney, L.W. 1982. Scanning electron microscopy of the pericarp and testa of several sorghum varieties. Food Microstructure 1:125-134.

Elkin, R.G., Freed, M.B., Hamaker, B.R., Zhang, Y., and Parsons, C.M. 1996. Condensed tannins are only partially responsible for variations in nutrient digestibilities of sorghum grain cultivars. J. Agric. Food Chem. 44:848-853.

Fuleki, T. and Francis, F.J. 1968. Quantitative methods for anthocyanins. 2. Determination of total anthocyanin and degradation index for cranberry juice. J. Food Sci. 33:78-83.

Gous, F. 1989. Tannins and phenols in black sorghum. Ph.D. Dissertation. Texas A&M University: College Station, TX.

Govindarajan, V.S. and Mathew, A.G. 1965. Anthocyanidins from leucoanthocyanidins. Phytochem. 4:985-988.

Gu, L., Kelm, M., Hammerstone, J.F., Beecher, G., Cunningham, D., Vannozzi, S., and Prior, R.L. 2002. Fractionation of polymeric procyanidins from lowbush blueberry and quantification of procyanidins in selected foods with an optimized normal-phase HPLC-MS fluorescent detection method. J. Agric. Food Chem. 50:4852-4860.

Gujer, R., Magnolato, D., and Self, R. 1986. Glucosylated flavonoids and other phenolic compounds from sorghum. Phytochem. 25:1431-1436.

Hagerman, A.E. and Butler, L.G. 1980. Condensed tannin purification and characterization of tannin-associated proteins. J. Agric. Food Chem. 28:947-952.

Hagerman, A.E., Riedl, K.M., Jones, G.A., Sovik, K.N., Ritchard, N.T., Hartzfeld, P.W., and Riechel, T.L. 1998. High molecular weight plant polyphenolics (tannins) as biological antioxidants. J. Agric. Food Chem. 46:1887-1892.

Hagerman, A.E., Zhao, Y., and Johnson, S. 1997. Methods for determination of condensed and hydrolysable tannins. Pages 209-222 in: Antinutrients and Phytochemicals in Food. F. Shahidi, ed. American Chemical Society: Washington, D.C.

Hahn, D.H., Faubion, J.M., and Rooney, L.W. 1983. Sorghum phenolic acids, their high performance liquid chromatography separation and their relation to fungal resistance. Cereal Chem. 60:255-259.

Hahn, D.H. and Rooney, L.W. 1986. Effect of genotype on tannins and phenols of sorghum. Cereal Chem. 63:4-8.

Hahn, D.H., Rooney, L.W., and Earp, C.F. 1984. Tannins and phenols of sorghum. Cereal Foods World 29:776-779.

Hammerstone, J.F., Lazarus, S.A., Mitchel, A.E., Rucker, R., and Schmitz, H.H. 1999. Identification of procyanidins in cocoa (*Theobroma cocoa*) and chocolate using high-performance liquid chromatography/mass spectrophotometry. J. Agric. Food Chem. 47:490-496.

Hanneken, A., Lin, F.-F., Johnson, J., and Maher, P. 2006. Flavonoids protect human retinal pigment epithelial cells from oxidative-stress-induced death. Invest. Ophthalmol. Vis. Sci. 47:3164-3177.

Harborne, J.B. and Williams, C.A. 2000. Advances in flavonoids research since 1992. Phytochem. 55:481-504.

Heo, H.J., Kim, D.-O., Shin, S.C., Kim, M.J., Kim, B.G., and Shin, D.-H. 2004. Effect of antioxidant flavanone, naringenin, from *Citrus junos* on neuroprotection. J. Agric. Food Chem. 52:1520-1525.

Hirano, T., Higa, S., Arimitsu, J., Naka, T., Shima, Y., Ohshima, S., Fujimoto, M., Yamadori, T., Kawase, I., and Tanaka, T. 2004. Flavonoids such as luteolin, fisetin, and apigenin are inhibitors of interleukin-4 and interleukin-13 production by activated human basophils. Int. Arch. Allergy Immunol. 134:135-140.

Horinaka, M., Yoshida, T., Shiraishi, T., Nakata, S., Wakada, M., Nakanishi, R., Nishino, H., Matsui, H., and Sakai, T. 2005. Luteolin induces apoptosis via death receptor 5 upregulation in human malignant tumor cells. Oncogene 24:7180-7189.

Jambunathan, R., Kherdekar, M.S., and Bandyopadhyay, R. 1990. Flavan-4-ols concentration in mold-susceptible and mold-resistant sorghum at different stages of grain development. J. Agric. Food Chem. 38:545-548.

Jambunathan, R., Kherdekar, M.S., and Vaidya, P. 1991. Ergosterol concentration in mold-susceptible and mold-resistant sorghum at different stages of grain development and its relationship to flavan-4-ols. J. Agric. Food Chem. 39:1866-1870.

Kaluza, W.Z., McGrath, R.M., Roberts, T.C., and Schröder, H.H. 1980. Separation of phenolics of *Sorghum bicolor* (L.) Moench grain. J. Agric. Food Chem. 28:1191-1196.

Kamath, V.G., Chandrashekar, A., and Rajini, P.S. 2004. Antiradical properties of sorghum (*Sorghum bicolor* L. Moench) flour extracts. J. Cereal Sci. 40:283-288.

Kambal, A.E. and Bate-Smith, E.C. 1976. Genetic and biochemical study on pericarp pigments in a cross between two cultivars of grain sorghum, *Sorghum bicolor*. Heredity 37:413-416.

King, R.E., Bomser, J.A., and Min, D.B. 2006. Bioactivity of resveratrol. Compr. Rev. Food Sci. Food Saf. 5:65-70.

Krueger, C.G., Vestling, M.M., and Reed, J.D. 2003. Matrix-assisted laser desorption/ionization time-of-flight mass spectrometry of heteropolyflavan-3-ols and glucosylated heteropolyflavans in sorghum (*Sorghum bicolor* (L.) Moench). J. Agric. Food Chem. 51:538-543.

Lee, E.-R., Kim, J.-H., Kang, Y.-J., and Cho, S.-G. 2007. The anti-apoptotic and anti-oxidant effect of eriodictyol on UV-induced apoptosis in keratinocytes. Biol. Pharm. Bull. 30:32-37.

Lee, J.J., Crosby, K.M, Pike, L.M., Yoo, K.S., and Leskovar, D.I. 2005. Impact of genetic and environmental variation on development of flavonoids and carotenoids in pepper (*Capsicum* spp.). Sci. Hort. 106:341-352.

Ley, J.P., Krammer, G., Reinders, G., Gatfield, I.L., and Bertram, H.-J. 2005. Evaluation of bitter masking flavanones from Herba Santa (*Eriodictyon californicum* (H. & A.) Torr., Hydrophyllaceae). J. Agric. Food Chem. 53:6061-6066.

Lin, B.-Q., Li, P.-B., Wang, Y.-G., Peng, W., Wu, Z., Su, W.-W., and Ji, H. 2007. The expectorant activity of naringenin. Pulm. Pharmacol. Ther. doi: 10.1016/j.pupt.2007.05.001 (in press).

Lo, S.C.C, De Verdier, K., and Nicholson, R.L. 1999. Accumulation of 3-deoxyanthocyanidin phytoalexins and resistance to *Colletotrichum sublineolum* in sorghum. Phys. Mol. Plant Path. 55:263-273.

Lo, S.C.C. and Nicholson, R.L. 1998. Reduction of light-induced anthocyanin accumulation in inoculated sorghum mesocotyls. Plant Phys. 116:979-989.

Lo, S.-C., Weiergang, I., Bonham, C., Hipskind, J., Wood, K., and Nicholson, R.L. 1996. Phytoalexin accumulation in sorghum: Identification of a methyl ether of luteolinidin. Physiol. Mol. Plant Path. 49:21-31.

Martin, M.J., Motilva, V., and Alarcón de la Lastra, C. 1993. Quercetin and naringenin: Effects on ulcer formation and gastric secretion in rats. Phytother. Res. 7:150-153.

Matsui, J., Kiyokawa, N., Takenouchi, H., Taguchi, T., Suzuki, K., Shiozawa, Y., Saito, M., Tang, W.-R., Katagiri, Y.U., Okita, H., and Fujimoto, J. 2005. Dietary bioflavonoids induce apoptosis in human leukemia cells. Leuk. Res. 29:573-581.

Maxson, E.D. and Rooney, L.W. 1972. Evaluation of methods for tannin analysis in sorghum grain. Cereal Chem. 49:719-729.

Mazza, G. and Brouillard, R. 1987. Color stability and structural transformations of cyaniding 3,5-diglucoside and four 3-deoxyanthocyanins in aqueous solutions. J. Agric. Food Chem. 35:422-426.

Melake-Berhan, A., Butler, L.G., Ejeta, G., and Menkir, A. 1996. Grain mold resistance and polyphenol accumulation in sorghum. J. Agric. Food Chem. 44:2428-2434.

Menkir, A., Ejeta, G., Butler, L., and Melakeberhan, A. 1996. Physical and chemical kernel properties associated with resistance to grain mold in sorghum. Cereal Chem. 73:613-617.

Merken, H.M. and Beecher, G.R. 2001. Measurement of food flavonoids by high-performance liquid chromatography: A review. J. Agric. Food Chem. 48:577-599.

Naczk, M. and Shahidi, F. 2004. Extraction and analysis of phenolics in food. J. Chromatogr. A 1054:95-111.

National Weather Service Forecast. 2007. Temperature and rainfall data. URL: http://www.nws.noaa.gov.

Nicholson, R.L. and Wood, K.V. 2001. Phytoalexins and secondary products, where are they and how can we measure them? Physiol. Mol. Plant Path. 59:63-69.

Nip, W.K. and Burns, E.E. 1969. Pigment characterization in grain sorghum. I. Red varieties. Cereal Chem. 46:490-495.

Nip, W.K. and Burns, E.E. 1971. Pigment characterization in grain sorghum. I. White varieties. Cereal Chem. 48:74-80.

Njongmeta-Nenge, L.A., Cardenas-Hinojosa, AP, Dykes L., Cisneros-Zevallos L., and Rooney, L.W. 2007. Stability of colored compounds from black sorghum: Effects of pH and water activity. Cereal Foods World 52:A54.

Pale, E., Kouda-Bonafos, M., Mouhoussine, N., Vanhaelen, M., Vanhaelen-Fastre, R., and Ottinger, R. 1997. 7-*O*-Methylapigeninidin, an anthocyanindin from *Sorghum Caudatum*. Phytochem. 45:1091-1092.

Parr, A.J. and Bolwell, G.P. 2000. Phenols in the plant and in man. The potential for possible nutritional enhancement of the diet by modifying the phenols content and profile. J. Sci. Food Agric. 80:985-1012.

Patil, B.S., Vanamala, J., and Hallman, G. 2004. Irradiation and storage influence on bioactive components and quality of early and late season "Rio Red" grapefruit (*Citrus paradis* Macf.). Postharvest Biol. Technol. 34:53-64.

Porter, L.J., Hrstich, L.N., and Chan, B.G. 1986. The conversion of procyanidins and prodelphinidins to cyanidin and delphinidin. Phytochem. 25:223-230.

Price, M.L. and Butler, L.G. 1977. Rapid visual estimation and spectrophotometric determination of tannin content of sorghum grain. J. Agric. Food Chem. 25:1268-1273.

Price, M.L., Van Scoyoc, S., and Butler, L.G. 1978. A critical evaluation of the vanillin reaction as an assay for tannin in sorghum grain. J. Agric. Food Chem. 26:1214-1218.

Prior, R.L. and Gu, L. 2005. Occurrence and biological significance of proanthocyanidins in the American diet. Phytochem. 66:2264-2280.

Prior, R.L., Wu, X., and Schaich, K. 2005. Standardized methods for the determination of antioxidant capacity and phenolics in foods and dietary supplements. J. Agric. Food Chem. 53:4290-4302

Rhodes, M.J.C. and Price, K.R. 1997. Identification and analysis of plant phenolic antioxidants. Eur. J. Cancer Prev. 6:518-521.

Ring, A.S. 1984. Phenolic compounds in sorghum from boot stage to post harvest maturity by Folin-Ciocalteau and high-pressure liquid chromatography methods. M.S. Thesis. Texas A&M University: College Station, TX.

Rohr, G.E., Meier, B., and Sticher, O. 2000. Analysis of procyanidins. Stud. Nat. Prod. Chem. 21:497-570.

Rooney, L.W. 2005. Ten myths about tannins in sorghums. International Sorghum and Millets Newsletter 46:3-5.

Rooney, L.W. and Awika, J.M. 2005. Specialty sorghums for healthful foods. Pages 283-312 in: Specialty Grains for Food and Feed. E. Abdel-Aal and P. Wood, eds. American Association of Cereal Chemists, Inc.: St. Paul, MN.

Rooney, L.W. and Miller, F.R. 1982. Variation in the structure and kernel characteristics of sorghum. Pages 143-162 in: Internation Symposium on Sorghum Grain Quality. L.W. Rooney, D.S. Murty, and J.V. Mertin, eds. ICRISAT: Patacheru, AP, India

Schofield, P., Mbugua, D.M., and Pell, A.N. 2001. Analysis of condensed tannins: A review. Anim. Feed Sci. Technol. 91:21-40.

Seitz, L.M. 2004. Effect of plant-type (purple vs. tan) and mold invasion on concentrations of 3-deoxyanthocyanidins in sorghum grain. AACC Annual Meeting Abstracts. URL: http://www.aaccnet.org/meetings/2004/abstracts/a04ma384.htm.

Shahidi, F. and Naczk, M. 1995. Food Phenolics. Technomic Publishing Co., Inc.: Lancaster, PA.

Shih, C.-H., Siu, S.-O., Ng, R., Wong, E., Chiu, L.C.M., Chu, I.K., and Lo, C. 2007. Quantitative analysis of anticancer 3-deoxyanthocyanidins in infected sorghum seedlings. J. Agric. Food Chem. 55:254-259.

Singleton, V.L. and Rossi, J.A. 1965. Colorimetry of total phenols with phosphomolybdic phosphotungstic acid reagents. Am. J. Enol. Vitic. 16:144-158.

Sweeny, J.G. and Iacobucci, G.A. 1983. Effect of substitution on the stability of 3-deoxyanthocyanidins in aqueous solutions. J. Agric. Food Chem. 31:531-533.

Teetes, G.L. and Pendleton, B.B. 2000. Insect pests of sorghum. Pages 443-495 in: Sorghum: Origin, history, technology, and production. C.W. Smith and R.A. Frederiksen, eds. John Wiley and Sons, Inc.: New York.

Tomás-Barberán, F.A. and Clifford, M.N. 2000. Flavanones, chalcones and dihydrochalcones – nature, occurrence and detary burden. J. Sci Food Agric. 80:10731080.

Tsai, S.-H., Lin-Shiau, S.-Y., and Lin, J.-K. 1999. Suppression of nitric oxide synthase and the down-regulation of the activation of NFκB in macrophages by resveratrol. Brit. J. Pharmacol. 126:673-680.

United States Department of Agriculture. 2007. USDA Database for the Flavonoid Content of Selected Foods. Release 2.1. URL: http://www.ars.usda.gov/Services/docs.htm?docid=6231.

Waniska, R.D., Poe, J.H., and Bandyopadhyay, R. 1989. Effects of growth conditions on grain molding and phenols in sorghum caryopsis. J. Cereal Sci. 10:217-255.

Waniska, R.D. and Rooney, L.W. 2000. Structure and chemistry of the sorghum caryopsis. Pages 649-688 in: Sorghum: Origin, history, technology, and production. C.W. Smith and R.A. Frederiksen, eds. John Wiley and Sons, Inc.: New York.

Watanabe, M. 1999. Antioxidative phenolic compounds from Japanese barnyard millet (*Echinochloa utilis*) grains. J. Agric. Food Chem. 47:4500-4505.

Waterman, P.G. and Mole, S. 1994. Structure elucidation of phenolics. Pages 168-198 in: Analysis of Phenolic Plant Metabolites. J.H. Lawton and G.E. Likens, eds. Blackwell Scientific Publications: Oxford, U.K.

Watterson, J.J. and Butler, L.G. 1983. Occurrence of an unusual leucoanthocyanidin and absence of proanthocyanidins in sorghum leaves. J. Agric. Food Chem. 47:4500-4505.

Wharton, P.S. and Nicholson, R.L. 2000. Temporal synthesis and radiolabelling of the sorghum 3-deoxyanthocyanidin phytoalexins and the anthocyanin, cyanidin3-dimalonyl glucoside. New Phytol. 145:457-469.

Wilcox, L.J., Borradaile, N.M., de Dreu, L.E., and Huff, M.W. 2001. Secretion of hepatocyte apoB is inhibited by the flavonoids, naringenin and hesperitin, via reduced activity and expression of ACAT2 and MTP. J. Lipids Res. 42:725-734.

Winkel-Shirley, B. 2001. Flavonoid biosynthesis. A Colorful model for genetics, biochemistry, cell biology, and biotechnology. Plant Physiol. 126:485-493.

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

Wu, X. and Prior, R.L. 2005. Identification and characterization of anthocyanins by high-performance liquid chromatography-electrospray ionization-tandem mass spectrometry in common foods in the United States: vegetables, nuts, and grains. J. Agric. Food Chem. 53:3101-3113.

Xu, Y.C., Leung, S.W.S., Yeung, D.K.Y., Hu, L.H., Chen, G.H., Che, C.M., and Man, R.Y.K. 2007. Structure-activity relationships of flavonoids for vascular relaxation in porcine coronary artery. Phytochem. 68:1179-1188.

Yasumatsu, K., Nakayama, T.O.M, and Chichester, C.O. 1965. Flavonoids of sorghum. J. Food Sci. 30:663-667.

Yu, C.K.Y, Springob, K., Schmidt, J., Nicholson, R.L., Chu, I.K., Yip, W.K., and Lo, C. 2005. A stilbene synthase gene (*SbSTS1*) is involved in host and nonhost defense responses in sorghum. Plant Phys. 138:393-401.

Zhang, X.F., Hung, T.M., Phuong, P.T., Ngoc, T.M., Min, B.-S., Song, K.-S., Seong, Y.H., and Bae, K. 2006. Anti-inflammatory activity of flavonoids from *Populus davidiana*. Arch. Pharm. Res. 29:1102-1108.

Ziyan, L., Yongmei, Z., Nan, Z., Ning, T., and Baolin, L. 2007. Evaluation of the anti-inflammatory activity of luteolin in experimental animal models. Planta Med. 73:221-226.

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Dykes, L., Rooney, L.W. 2006. Sorghum and millet phenols and antioxidants. J. Cereal Sci. 44:236-251.

Dykes, L., Rooney, W.L., Waniska, R.D., and Rooney, L.W. 2005. Phenolic compounds and antioxidant activity of sorghum genotypes. J. Agric. Food Chem. 53:6813-6818.

Awika, J.M., Dykes, L., Gu, L., Rooney, L.W., and Prior, R.L. 2003. Processing of sorghum (*Sorghum bicolor*) and sorghum products alters procyanidin oligomer and polymer distribution and content. J. Agric. Food Chem. 51:5516-5521.