JOURNAL OF AGRICULTURAL AND FOOD CHEMISTRY

ARTICLE

pubs.acs.org/JAFC

Uncommonly High Levels of 3-Deoxyanthocyanidins and Antioxidant Capacity in the Leaf Sheaths of Dye Sorghum

A. P. Polycarpe Kayodé,*[,†] M. J. Rob Nout,[‡] Anita R. Linnemann,[§] Joseph D. Hounhouigan,[†] Emmerich Berghofer,[∥] and Susanne Siebenhandl-Ehn[∥]

[†]Département de Nutrition et Sciences Alimentaires, Université d'Abomey-Calavi, 01 BP 526 Cotonou, Benin

[‡]Laboratory of Food Microbiology and [§]Product Design and Quality Management Group, Wageningen University, Post Office Box 8129, 6700 EV Wageningen, The Netherlands

[∥]Department of Food Science and Technology, University of Natural Resources and Life Sciences, Muthgasse 18, 1190 Vienna, Austria

ABSTRACT: Extracts from leaf sheaths of farmers' varieties of dye sorghum cultivated and used in Benin as a source of biocolorings were analyzed for their anthocyanidin and phenolic contents, as well as their antioxidant capacity. The aim was to identify and quantify the types of anthocyanin and phenolic acids. The total anthocyanin content of the leaf sheaths ranged from 13.7 to 35.5 mg of cyanidin 3-glucoside equivalent/g of dry matter (DM), with an average of 27.0 mg/g. The total anthocyanin content is 90 times higher than levels usually reported in fruits and vegetables. Anthocyanin consisted essentially of apigeninidin and luteolinidin, two 3-deoxyanthocyanidins with many applications in food, beverage, and pharmaceutical industries. The apigeninidin content of the leaf sheaths was 30 times higher than that in cereal bran and ranged from 14.7 to 45.8 mg/g, with an average of 31.3 mg/g. The amount of luteolinidin ranged from 0.4 to 2.4 mg/g, with a mean of 1.2 mg/g. The total phenolic content expressed as gallic acid equivalent averaged 95.5 mg/g. The free phenolic acids identified were benzoic acid, *p*-coumaric acid, and *o*-coumaric acid at amounts of 801.4, 681.6, and 67.9 μg/g, respectively. The leaf sheaths of dye sorghum have an antioxidant capacity [3.8−5.6 mmol of Trolox equivalent (TE)/g of DM] much higher than that reported for cereal bran and fruits and vegetables.

KEYWORDS: Dye sorghum, apigeninidin, luteolinidin, 3-deoxyanthocyanidins, biocolorant, antioxidant capacity

INTRODUCTION

The increasing awareness of environmental pollution and toxicity associated with the use of synthetic dyes has led to a revival of interest in plant-derived dyes[1] and a demand by consumers for safe natural foods. Food industries use coloring agents to produce foods with various organoleptic and therapeutic attributes. Some of these coloring compounds are synthetic, while others are natural, such as cochenille (E-120), beetroot red (E-162), and anthocyanins (E-163). These natural colorants are safe and have attractive bright colors. In addition, certain functional properties, i.e., antioxidant activities and a reduction of chronic diseases, related to natural pigments make them of particular interest to processors and consumers. A challenge to the food industry is to replace synthetic dyes by natural ones.[2] Considerable research is currently directed toward the identification and characterization of pigments from plant sources, such as vegetables,[3] fruits, and flowers.

Sorghum spp. are cereal crops, which are important as a food crop in Africa.[4] However, not all sorghum is mainly used for food; in Benin, some farmers' varieties, referred to as dye sorghum, are primarily grown for their pigments that are concentrated in the leaf sheaths adjacent to the stem of the plant.[5] Morphologically, dye sorghum [*Sorghum bicolor* (L.) Moench] resembles grain sorghum; the main difference is the ability to produce red pigments in the leaf sheaths. Dye sorghum is in the field from May to December. Harvesting of the leaf sheaths starts early, viz., 2 months after planting, and is performed weekly until the plant reaches the flowering stage and the production of the leaf sheath stops. As much as 1.5 tons of leaf sheaths can be harvested per hectare under traditional farming conditions. The production of dye sorghum is primarily performed by rural women, who derive a direct benefit from the commercialization of the leaf sheaths. Dye sorghum has been used in Benin and other African countries for centuries. The watery dye extracted from the leaf sheaths is used as biocolorings for foods (e.g., local cheese and porridge), leather, wickerwork, and ornamental calebashes, as lick stones for cattle, and in traditional medicine. The derived food products have a reddish, bright, and attractive color, which is highly appreciated by consumers. In addition, local people use dye sorghum to prepare a red infusion to treat anemia and menstrual disorders.

Sorghum is known to synthesize a unique class of flavonoid phytoalexins, 3-deoxyanthocyanidins, during defense responses.[6,7] However, whether the concentration of pigments in the leaf sheaths of dye sorghum is also a defense response has not yet been established. Sorghum is the only plant known to contain significant quantities of luteolinidin and apigeninidin (both 3-deoxyanthocyanidins), which are not commonly found in higher plants. Commonly, anthocyanins are isolated from either the grain or its pericarp. Nip and Burns[8,9] isolated apigeninidin and luteolinidin from the seeds of red and white sorghum varieties. Kouda-Bonafos et al.[10] found apigeninidin in the sheaths of *Sorghum caudatum*, and both 3-deoxyanthocyanidins are also reported as the major anthocyanidins from black sorghum

Received: October 11, 2010
Accepted: December 22, 2010
Revised: December 18, 2010
Published: January 25, 2011

Table 1. Dimension Characteristics of Leaf Sheaths of Dye Sorghum Analysed

sample origin	weight (g)[a]	length (cm)	basal width (cm)	middle width (cm)	terminal width (cm)
Parakou (n = 7)[b]	1.60 ± 0.44	25.66 ± 2.68	2.02 ± 0.50	1.48 ± 0.43	1.08 ± 0.43
Ketou (n = 10)	2.63 ± 0.54	26.69 ± 4.37	2.02 ± 0.56	1.40 ± 0.46	1.17 ± 0.31
Toucountouna (n = 5)	0.76 ± 0.55	22.28 ± 2.72	1.88 ± 0.82	1.38 ± 0.48	0.94 ± 0.27
Boukoumbe (n = 5)	0.90 ± 0.42	22.18 ± 3.32	1.96 ± 0.71	1.20 ± 0.24	0.80 ± 0.14
Paouingnan (n = 9)	2.54 ± 1.19	26.74 ± 3.56	1.70 ± 0.55	1.32 ± 0.63	1.03 ± 0.49
Dassa (n = 6)	2.05 ± 1.23	27.00 ± 2.19	1.63 ± 0.41	1.23 ± 0.27	0.90 ± 0.34
mean	1.75	25.09	1.87	1.34	0.99
CV[c]	45.9	9.02	8.95	7.98	13.5

[a] Mean ± standard deviation. [b] n = number of leaf sheath measured. [c] CV = coefficient of variation.

Table 2. Total Anthocyanin and 3-Deoxyanthocyanidin Contents of Leaf Sheaths of Dye Sorghum[a]

	spectrophotometry	HPLC		
sample origin	ACY (mg of C-3-glc/g)[b]	apigeninidin (mg/g)	luteolinidin (mg/g)	malvidin (mg/g)
Parakou	26.05 ± 0.35 c	28.83 ± 1.79 b	1.07 ± 0.10 c	0.79 ± 0.06 c
Ketou	35.51 ± 0.14 e	40.30 ± 0.87 c	2.35 ± 0.06 f	1.03 ± 0.04 d
Toucountouna	13.72 ± 0.28 a	16.67 ± 0.23 a	0.62 ± 0.01 b	0.59 ± 0.01 b
Boukoumbe	34.33 ± 0.28 d	45.76 ± 0.93 d	1.66 ± 0.02 e	0.59 ± 0.01 b
Paouingnan	18.57 ± 0.14 b	14.72 ± 0.11 a	0.43 ± 0.04 a	0.57 ± 0.00 a
Dassa	34.18 ± 0.85 d	41.46 ± 0.58 c	1.24 ± 0.02 d	0.84 ± 0.03 a
mean	27.06	31.29	1.23	0.70
CV[c]	32.53	40.68	54.79	31.67

[a] Results are based on DM. [b] Mean ± standard deviation; means with the same letter are not significantly different according to the LSD at the 0.05 level. [c] CV = coefficient of variation.

varieties.[11,12] The 3-deoxyanthocyanidins offer many applications in food, beverage, and pharmaceutical industries.[13] They are recognized as health-promoting phytochemicals; e.g., it has been demonstrated that they are more cytotoxic on human cancer cells than the 3-hydroxylated anthocyanidin analogues. At 200 μM, luteolinidin reduced the viability of HL-60 and HepG2 cells by 90 and 50%, respectively.[14] Recently Yang et al.[15] demonstrated that the sorghum 3-deoxyanthocyanidins possess strong phase-II enzyme inducer activity and cancer cell growth inhibition properties. However, information on contents and characteristics of pigments from dye sorghum grown in West Africa is scarce.

In the present study, we assessed the anthocyanin and phenolic contents in extracts from leaf sheaths of dye sorghum and their antioxidant capacity. In addition, the anthocyanidins and phenolic acids were characterized by high-performance liquid chromatography (HPLC).

MATERIALS AND METHODS

Chemicals and Reagents. 6-Hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid (Trolox), 2,2′-azinobis(3-ethylbenzothiazoline-6-sulfonic acid)diammonium salt (ABTS), 2,4,6-tripyridyl-*s*-triazine (TPTZ), 2,2′-diphenyl-1-picrylhydrazyl (DPPH), trifluoroacetic acid, potassium persulfate, sodium hydroxide, Folin−Ciocalteu reagent, and phenolic acid standards (gallic acid, protocatechuic acid, 4-OH-benzoic acid, vanillic acid, caffeic acid, *p*-coumaric acid, *o*-coumaric acid, and *trans*-ferulic acid) were purchased from Sigma-Aldrich (Vienna, Austria), and anthocyanidin standards (apigeninidin, cyanidin 3-glucoside, cyanidin 3-galactoside, cyanidin, delphinidin, luteolinidin, malvidin, peonidin 3-glucoside, pelargonidin, and peonidin) were purchased from Extrasynthese (Genay, France). Acetonitrile was obtained from VWR (Vienna, Austria). Ethyl acetate and methanol were purchased from Roth (Graz, Austria). All chemicals and solvents used in the study were of HPLC grade.

Plant Materials. Leaf sheaths of varieties of dye sorghum from farmers were collected from fields in six communities in Benin, i.e., Parakou (latitude, 11° 15′ N; longitude, 2° 23′ E), Ketou (latitude, 7° 22′ N; longitude, 2° 36′ E), Toucountouna (latitude, 10° 27′ N; longitude, 1° 22′ E), Boukoumbe (latitude, 10° 10′ N; longitude, 1° 6′ E), Paouingnan (latitude, 7° 34′ N; longitude, 2° 2′ E), and Dassa-Zounmè (latitude, 7° 55′ N; longitude, 1° 58′ E). The leaf sheaths were from dye sorghum grown in 2008 under natural conditions, characteristic of the Guinea Savannah climate of West Africa. The annual rainfall in the region varies from 1000 to 1300 mm, and the average yearly temperature is 26.5 °C.[16] The soil is a tropical ferruginous type.[17] After harvesting, the leaf sheaths were dried to a moisture content of 7−9% (w/w). The leaf sheaths were ground to powder using an Ultra Centrifugal Mill (Retsch GmbH, Haan, Germany) with a 0.1 mm sieve and, subsequently, stored at −20 °C until analysis.

Preparation of Extracts. Samples were extracted in either methanol/HCl (85:15, v/v) for anthocyanin determination or methanol/H_2O (85:15, v/v) for the determination of total phenolics, phenolic acids, and antioxidant capacity. A total of 30 mg of each sample was extracted at room temperature with 10 mL of solvent under agitation using a magnetic stirrer for 30 min. The mixtures were centrifuged at 2500*g* for 10 min, and the supernatants were collected. The residues were re-extracted twice under the same conditions, resulting in 30 mL of crude extract. All extracts were used as they were after centrifugation for various analyses.

Total Anthocyanin Determination (ACY). ACY was calculated as described by Abdel-Aal and Hucl[18] using cyanidin 3-glucoside as the standard pigment. The absorbance of the pooled extracts was measured after centrifugation at 525 nm against a reagent blank. ACY was expressed as milligrams of cyanidin 3-glucoside equivalent per 1 g of powder based on dry matter (DM).

Total Phenolic Determination (TPC). Total phenolics were measured following the method by Singleton and Rossi[19] modified as follows: To 300 μL of extract, 4.2 mL of distilled water, 0.75 mL of Folin−Ciocalteu reagent (Merck, Germany), and 0.75 mL of sodium carbonate solution (200 g L^{-1}) were added. After incubation for 30 min, the optical density was measured at 760 nm against a blank. Gallic acid was used as the standard, and the results were expressed as gallic acid equivalent (GAE) per gram of sample DM.

HPLC Determination of Anthocyanidins and Phenolic Acids. The profile of anthocyanidins and corresponding glycosides and phenolic compounds was determined using a HPLC system (Shimadzu, Korneuburg, Austria) consisting of a SPD-M10AVP photodiode array detector, a chromatogram integrator, a LC-10ADVP pump, and an online degasser. Data signals were processed on a personal computer (PC) running the LC solution Multi software (Shimadzu, Korneuburg, Austria). Analytical separation of anthocyanidins and phenolic acids was carried out using a Phenomenex Luna 250 × 4.6 mm, 5 μm (HPLC Services, Breitenfurt, Austria) column.

For anthocyanidins, the solvent was removed from the extracts at 40 °C using a rotary evaporator and the resulting residues were suspended in 2 mL of methanol. Dissolved anthocyanidin concentrates were passed through a 0.45 μm polytetrafluoroethylene (PTFE) filter, and a 20 μL aliquot of the sample solution was injected. Elution for anthocyanidins was executed under gradient conditions with (A) 4.5% formic acid in water and (B) acetonitrile. The solvent gradient was programmed as follows: 10% B at 0 min, increasing to 12% within 9 min, to 13% within the next 7.5 min, 25% within the next 13.5 min, 90% within the next 15 min, holding at 90% for 5 min, followed by a decrease to 10% within the next 5 min, before equilibration at 10%. The solvent flow rate was set at 0.8 mL/min, and the chromatogram was recorded at 520 nm (anthocyanidins and glycosides) and 480 nm (deoxyanthocyanidins) at 35 °C. Peak areas were used for all calculations. Identification of compounds was performed by comparing the retention time and the ultraviolet (UV) spectra to those of pure substances. Diode array detector (DAD) response was linear for all anthocyanidins and anthocyanins within the calibration range of 0.05−40.0 μg/mL, with correlation coefficients exceeding 0.999. Coefficients of variation for sample replicates were consistently below 10%.

Phenolic acids were quantified as described by Siebenhandl et al.[20] A total of 20 μL of sample was injected into the column and eluted under gradient conditions performed with (A) 0.05% trifluoroacetic acid in water and (B) 0.05% trifluoroacetic acid in acetonitrile. The solvent gradient was programmed as follows: at 0 min, 10% B; increasing from 3 to 15 min, 15% B; 25 min, 20% B; 30 min, 40% B; 36−40 min, 80% B; decreasing thereafter within the next 4 min, 10% B; and equilibrated before the next injection. The flow rate was 1.0 mL/min. Analyte detection was at 260 nm for 4-OH-benzoic and vanillic acids, at 270 nm for gallic and *o*-coumaric acids, and at 280 nm for caffeic, *p*-coumaric, and *trans*-ferulic acids. DAD response was linear for all phenolic acids within the calibration range of 0.06−125.0 μg/mL, with correlation coefficients exceeding 0.999. Phenolic acids in the samples were identified by comparing their relative retention times and UV spectra to authentic compounds. Coefficients of variation for sample replicates were consistently below 10%.

Determination of Antioxidant Capacity. Extracts obtained with aqueous methanol were analyzed for their total antioxidant capacity by the ABTS radical cation scavenging assay [Trolox equivalent antioxidant capacity (TEAC)] and the ferric reducing antioxidant power (FRAP) assay. Moreover, the 2,2′-diphenyl-1-picrylhydrazyl (DPPH) radical-scavenging ability of sample extracts was evaluated.

The TEAC assay was analyzed following a modified method by Pellegrini et al.[21] and Moore et al.[22] A stable stock solution of ABTS radical cation was produced by reacting a 7 mmol/L aqueous solution of ABTS with 2.45 mmol/L potassium persulfate (final concentration) and allowing the mixture to stand in the dark at room temperature for 12−16 h before use. On the day of analysis, an ABTS radical cation working solution was obtained by diluting the stock solution in ethanol to an absorbance of 0.70 ± 0.02 AU (Absorbance Unit) at 734 nm. A total of 100 μL of extract was mixed with 1.25 mL of the ABTS working solution, and absorbance was read at 734 nm after a 1 min reaction time. Results were expressed as TEAC in millimoles of Trolox per gram based on DM.

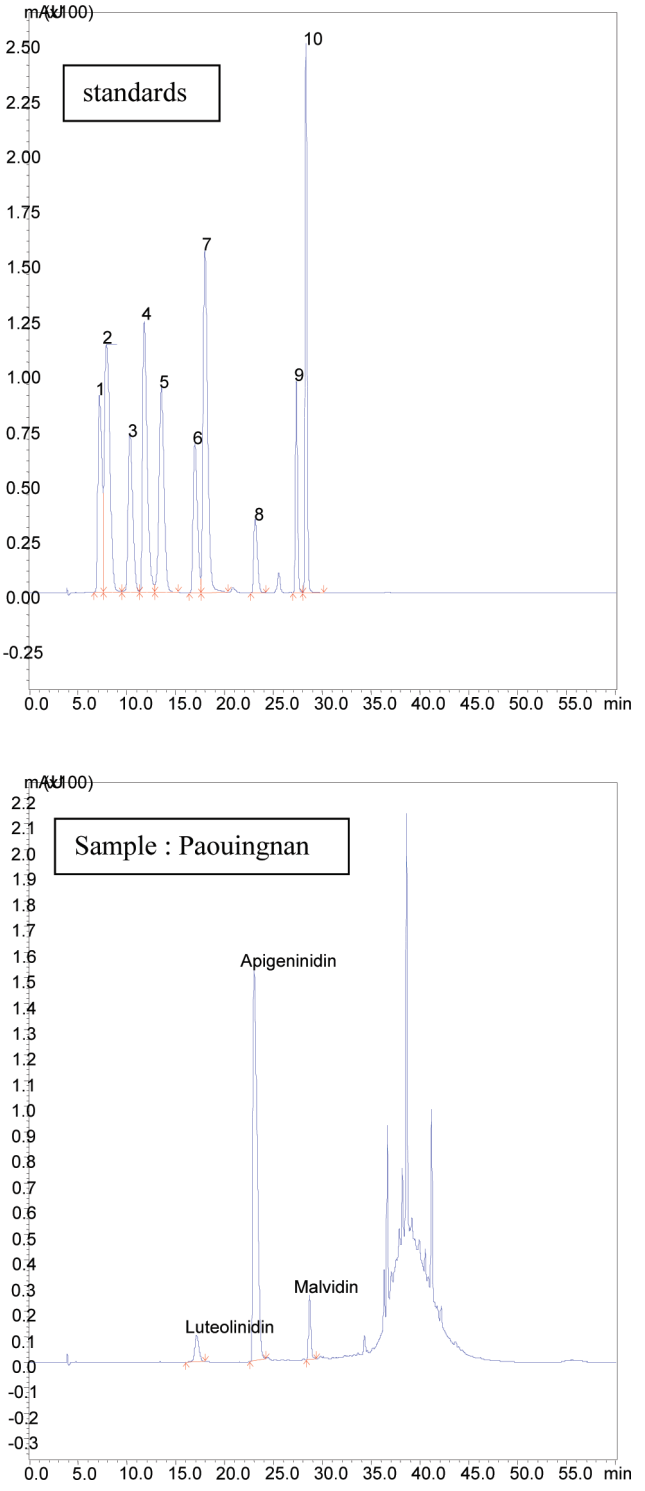

Figure 1. HPLC profiles of anthocyanidins in extract from leaf sheaths of dye sorghum: (1) cyanidin-galactoside, (2) cyanidin-glucoside, (3) pelargonidin-glucoside, (4) delphinidin, (5) peonidin-glucoside, (6) luteolinidin, (7) malvidin-glucoside, (8) apigeninidin, (9) cyanidin, and (10) malvidin.

Table 3. Total Phenolic and Phenolic Acid Contents of Leaf Sheaths of Dye Sorghum[a]

	spectrophotometry	HPLC		
sample origin	TPC (mg of GAE/g)[b]	OH-benzoic acid (μg/g)	*p*-coumaric acid (μg/g)	*o*-coumaric acid (μg/g)
Parakou	100.11 ± 6.91 c	802.27 ± 3.18 a	772.76 ± 5.98 bc	67.86 ± 1.65 c
Ketou	105.19 ± 5.08 c	694.84 ± 2.86 c	773.26 ± 0.06 bc	94.44 ± 3.11 d
Toucountouna	65.43 ± 1.79 a	604.95 ± 10.52 b	594.75 ± 6.64 ab	32.73 ± 2.82 a
Boukoumbe	134.97 ± 13.69 d	1555.39 ± 18.48 f	833.62 ± 7.20 b	134.90 ± 7.05 e
Paouingnan	71.11 ± 3.69 ab	770.05 ± 13.71 d	512.12 ± 79.99 a	35.07 ± 2.09 ab
Dassa	96.28 ± 22.59 bc	381.01 ± 2.32 a	603.01 ± 181.17 ab	42.59 ± 3.27 b
mean	95.51	801.42	681.59	67.93
CV[c]	26.67	47.48	19.97	56.80

[a] Results are based on DM. [b] Mean ± standard deviation; means with the same letter are not significantly different according to the LSD at the 0.05 level. [c] CV = coefficient of variation.

Table 4. Antioxidant Capacities in the Leaf Sheaths of Dye Sorghum[a]

sample origin	TEAC (mmol/g)[b]	FRAP (mmol/g)	DPPH (% remaining)
Parakou	5.55 ± 0.78 b	0.65 ± 0.01 c	84.49 ± 6.19 a
Ketou	4.47 ± 0.02 ab	0.81 ± 0.00 d	86.85 ± 0.49 a
Toucountouna	3.93 ± 0.23 a	0.49 ± 0.016 b	78.14 ± 5.76 a
Boukoumbe	5.58 ± 0.97 b	1.06 ± 0.06 e	80.23 ± 1.18 a
Paouingnan	3.76 ± 0.66 a	0.35 ± 0.02 a	81.46 ± 9.72 a
Dassa	4.53 ± 0.48 ab	0.78 ± 0.03 d	86.19 ± 1.42 a
mean	4.64	0.69	82.89
CV[c]	18.74	35.31	6.21

[a] Results are based on DM. [b] Mean ± standard deviation; means with the same letter are not significantly different according to the LSD at the 0.05 level. [c] Coefficient of variation.

The FRAP assay is based on the reduction of the Fe^{3+}−TPTZ complex to the ferrous form at low pH. This reduction is monitored by measuring the absorption change at 595 nm.[23] Briefly, 0.2 mL of sample extract was mixed with 1.3 mL of the FRAP reagent. Absorption was measured at 595 nm in a spectrophotometer (U-1100 type, Hitachi, Japan) after 30 min of incubation at 37 °C. The FRAP reagent was prepared daily and consisted of 0.3 M acetate buffer (pH 3.6), 10 mM TPTZ in 40 mM HCl, and 20 mM $FeCl_3$ in a ratio of 10:1:1 (v/v/v). FRAP values were obtained by comparing the absorption change in the test mixture to doses obtained from increasing concentrations of Fe^{3+} and expressed as millimoles of Fe^{2+} equivalents per gram of leaf sheaths based on DM.

DPPH radical-scavenging activity of sample extracts was determined according to the method reported by Brand-Williams et al.[24] The reaction mixture consisting of 1.5 mL of DPPH working solution (4.73 mg of DPPH in 100 mL of HPLC-grade ethanol) and 300 μL sample extract was shaken and incubated for 40 min in the dark at room temperature. The absorbance was measured at 515 nm against a blank, using a UV−vis spectrophotometer (Hitachi, Japan). DPPH free radical-scavenging ability was calculated using the following formula:

$$\text{scavenging ability (\%)} = [\text{absorbance}_{515\text{ nm of control}} - \text{absorbance}_{515\text{ nm of sample}}/\text{absorbance of control}] \times 100$$

Statistical Analysis. Data were reported as the mean ± standard deviation. The data were analyzed using the statistical program SPSS 11.0 (SPSS, Chicago, IL), and the one-way analysis of variation (ANOVA) model was used applying the least significant difference (LSD) test to evaluate significant difference among means.

RESULTS AND DISCUSSION

The weights of the leaf sheaths collected in six farming communities of Benin ranged from 0.8 to 2.6 g, with a mean of 1.75 g (Table 1), and their lengths ranged from 22.1 to 27 cm, with an average of 25 cm. The mean values of the basal, middle, and terminal width are 1.9, 1.3, and 1 cm, respectively.

Anthocyanidin Content. The anthocyanin contents, expressed as cyanidin 3-glucoside, estimated by spectrophotometry were on average 18.5% lower than the HPLC data (Table 2). The reason for this underestimation lies in the different absorption maxima of anthocyanidins and deoxyanthocyanidins, which are above 500 nm at pH 1 for anthocyanidins and their glycosides and around 470 nm for 3-deoxyanthocyanidins.[25] However, both methods generally correlated well (r = 0.961 and 0.852) for ACY and apigeninidin or luteolinidin contents at $p \leq 0.001$, respectively. The ACY of leaf sheaths ranged from 13.7 to 35.5 mg of C-3-glc/g, with an average value of 27.1 mg/g. Analytical separation of anthocyanidins on the HPLC system allowed us to detect apigeninidin, luteolinidin, and malvidin as the major anthocyanidin compounds of the leaf sheaths of dye sorghum (Figure 1). The level of apigeninidin ranged from 14.7 to 45.8 mg/g, with an average value of 31.3 mg/g. The luteolinidin amount ranged from 0.43 to 2.35 mg/g, while the malvidin content averaged 0.70 mg/g. Clearly, we observed uncommonly high levels of 3-deoxyanthocyanindins in the leaf sheaths of dye sorghum. Particularly, the level of apigeninidin was 30 times higher than that in cereal bran, and the ACY is 90 times higher than levels usually reported in fruits and vegetables (Table 5).

With respect to origin, leaf sheaths from Ketou showed the highest content of ACY, luteolinidin, and malvidin. Apigeninidin was highest in the sample originating from Boukoumbe, followed by the samples from Dassa and Ketou. Apparently, the concentration of 3-anthocyanindins is linked to the genetic makeup of the crop and/or the environmental conditions. This result is corroborated by Ortega-Regules et al.,[26] who indicate that the level and stability of these phenolic substances may vary according to the genetic and environmental conditions. Likewise, a study by Dykes et al.[27] revealed that the phenolic concentration in the sorghum grain is genetically determined. Thus, there might be a possibility to increase the amount of these phytochemicals through breeding and crop management. Further studies are needed to determine the contribution of the individual factors to the amount of anthocyanidins in the leaf sheaths of dye sorghum.

Phenolic Acid Content. TPC of the different extracts was expressed as GAE per gram of DM. The highest TPC was found

Table 5. Comparison of Leaf Sheaths of Dye Sorghum to Other Dye Sources for Their Anthocyanin Contents and Antioxidant Properties

commodity	TPC (mg/g)	ACY (mg/g)	total antioxidant activity	reference
leaf sheaths of dye sorghum	65.4−135.0[a]	13.7−35.5[b]	FRAP: 350.0−1060.1 μmol/g of DM DPPH: 78.1−86.8% rem[c] TEAC: 3760.6−5580.9 μmol/g of DM	this work
black sorghum bran		4.7−11.0[d]	TEAC: 261.0−400.0 μmol/g of DM	Awika et al. (2004)
black rice	3.4−6.7[e]	1.1−2.6[b]	FRAP: 36.5−75.8 μmol/g of DM DPPH: 16.0−30.3% rem[c] TEAC: 49.8−120.3 μmol/g of DM	Sompong et al. (2011)
red rice	0.8−6.9[e]	0.0−0.01[b]	FRAP: 8.5−80.8 μmol/g of DM DPPH: 13.0−62.8% rem[c] TEAC: 20.8−122.9 μmol/g of DM	Sompong et al. (2011)
blueberry			FRAP: 116 μmol/g of FW	Dragović-Uzelac et al. (2009)
commercial	2.2[f]	1.7[g]	DPPH: 16 μmol of TE[h]/g of FW	Garzón et al. (2010)
wild type	7.6[f]	3.3[g]	TEAC: 45.5 μmol/g of FW	Denev et al. (2010)
sour cherry	2.6[f]	1.9[g]	DPPH: 17 μmol of TE/g of FW[h]	Dragović-Uzelac et al. (2009)
blackberry				
wild type		6.6−9.2[b]		Cuevas-Rodríguez et al. (2010)
commercial		3.4[b]		Cuevas-Rodríguez et al. (2010)
cultivar		0.16[b]		Denev et al. (2010)
black currant		0.28[b]		Denev et al. (2010)
red raspberry	1.49−3.48[f]	0.20[i]	FRAP: 19.8 μmol/g of FW TEAC: 21.5 μmol/g of FW	Çekiç and Özgen (2008)
strawberry	1.7−3.1[f]	0.10−0.30[j]	TEAC: 14.18 μmol/g of FW	Tulipani et al. (2008)
elderberry		2.0−10.0 wb 0.63[b]		Denev et al. (2010)
red cabbage	0.13−0.17[f]	0.60−0.85[g] 0.76[g]	TEAC: 10−12.5 μmol/g of FW	Podsędek et al. (2008) Scalzo et al. (2008)
red onion	15.56[a]	0.45[b]	DPPH: 41.32 μM TE/g of DM TEAC: 58.94 μM TE/g of DM	Gorinstein et al. (2009)

[a] mg of GAE/g of DM. [b] mg of C-3-glc/g of DM. [c] % remaining DPPH at t = 40 min. [d] mg of luteolinidin equivalents/g of DM. [e] mg of ferulic acid equivalent/g of DM. [f] mg of GAE/g of FW. [g] mg of C-3-glc/g of FW. [h] Trolox equivalent (TE). [i] mg of C-3-soph/g of FW. [j] ACY based on HPLC data, mg/g of FW.

in the sample from Boukoumbe, followed by Ketou and Parakou (Table 3), with 135, 105, and 100 mg of GAE/g of DM, respectively. Only hydroxybenzoic, *o*-coumaric, and *p*-coumaric acids were detected. However, leaf sheaths from Boukoumbe contained 4 times more 4-OH-benzoic acid than samples from Dassa, with 1555 versus 381 μg/g of DM. *p*-Coumaric acid was found as the second dominant phenolic acid, ranging from 512 to 834 μg/g, whereas *o*-coumaric acid was on average 1 magnitude lower. Co-pigmentation of deoxyanthocyanidins can improve the anthocyanidin stability and color intensity in moderately acidic environments as investigated by Awika.[28]

Antioxidant Capacity. The total antioxidant capacity describes the cumulative capacity of food components to scavenge free radicals, and high intakes of dietary TAC have been related to several health benefits in both cross-sectional and randomized intervention studies. In this context, methanolic extracts of leave sheaths were assessed for their antioxidant properties.

All samples showed very high antioxidant capacity (Table 4). The highest ABTS radical-scavenging ability was found in the leaf sheaths from Boukoumbe and Parakou, respectively, followed by leaf sheaths from Ketou and Dassa. FRAP values were, in general, lower than the respective TEAC values but followed the same order. Despite the variation in TEAC and FRAP values, no significant difference between the samples was found when DPPH was used. As described by Arnao,[29] color interference of the sorghum extracts might have led to great variations within the samples and masked the underlying differences. On the other hand, phenolic acids react differently with different radicals. Table 5 summarizes TPC, ACY, and antioxidant capacity of other anthocyanin-rich plant sources. The dye sorghum largely surpasses the other plant commodities for its TPC and anthocyanin content. It also ranks first for antioxidant capacity among other anthocyanin-rich plant species. Clearly, the dye sorghum showed a comparative advantage as a deoxyanthocyanidin and antioxidant source compared to cereal bran and fruits and vegetables.

Relationship between Anthocyanidin and Phenolic Acid Content and Antioxidant Capacity of Dye Sorghum. TPC correlated strongly with the ACY and the antioxidant capacity, particularly when measured with the FRAP assay (Table 6). ACY significantly correlated with levels of apigeninidin (r = 0.96; $p \leq 0.01$) and luteolinidin (r = 0.85; $p \leq 0.01$), while no significant relationship could be found between apigeninidin and luteolinidin, as previously reported in grain sorghum.[27] Correlation experiments to predict the antioxidant capacity have been performed by many others, with different observations; a high correlation was found between TEAC and TPC in strawberries (r = 0.95),[30] red cabbage (r = 0.98),[31] red raspberries (r = 0.74),[32] milling fractions of pigmented wheat and barley (r = 0.96),[20] and red and black rice varieties (r = 0.93).[33]

Table 6. Pearson Correlation Matrix between ACY and TPC and the Antioxidant Capacity

	ACY	TPC	TEAC	FRAP
TPC	0.796[a]			
TEAC	−0.087 ns[b]	0.719[a]		
FRAP	0.847[a]	0.890[a]	0.662[c]	
DPPH	0.454 ns[b]	0.156 ns[b]	−0.087 ns[b]	0.165 ns[b]

[a] Significant at the 0.01 level (two-tailed). [b] ns = not significant. [c] Significant at the 0.05 level (two-tailed).

TEAC and anthocyanins by HPLC ($r = 0.90$) correlated in a study of red cabbage,[31] and TEAC and total monomeric anthocyanidins ($r = 0.74$) correlated in a study of red raspberries,[32] whereas Tulipani et al.[30] found no correlation between ACY and TEAC ($r = -0.07$) in strawberries. Denev et al.[34] found that the antioxidant capacity correlated in general better with the polyphenol content than the anthocyanin content and was highest for TPC and FRAP ($r = 0.95$) when different berries were compared.

Pair-wise correlations between the different assays to measure the antioxidative capacity were found between TEAC and FRAP at $r = 0.81$ in red raspberries,[32] at $r = 0.95$ and $p \leq 0.001$ in strawberries,[30] at $r = 0.90$ and $p < 0.0001$ in milling fractions of pigmented wheat and barley,[20] and at $r = 0.93$ in red and black rice varieties,[33] where in addition to these findings, high negative correlation coefficients were obtained between DPPH and FRAP ($r = -0.81$) and between TEAC and DPPH ($r = -0.66$).

In conclusion, the analyzed dye sorghum contains a very high level of anthocyanidins, which mainly consists of apigeninidin and luteolinidin, two 3-deoxyanthocyanidins with high stability under processing conditions. These two pigments are not common in plant species. The crude extract from the leaf sheath also showed very high antioxidant capacity. The exploitation of the dye sorghum as a multipurpose crop to produce biocolorant and food grain could be envisaged. Research initiatives are being taken to generate more knowledge on this crop and to valorize it for the benefit of rural farmers in Benin.

AUTHOR INFORMATION

Corresponding Author

*Telephone: +229-97870734. E-mail: polykap@yahoo.fr.

Funding Sources

The International Foundation for Science (IFS), Stockholm, Sweden, is acknowledged for financial support to A. P. Polycarpe Kayodé (Grant IFS E/3736-2). Support provided by the Department of Food Science and Technology, University of Natural Resources and Life Sciences, Vienna, Austria, is gratefully acknowledged.

ACKNOWLEDGMENT

Thanks are due to Michael Prückler for laboratory assistance.

ABBREVATIONS USED

ABTS, 2,2′-azinobis(3-ethylbenzothiazoline-6-sulfonic acid); C-3-glc, cyanidin-3-glucoside; ACY, total anthocyanidin content; DM, dry matter; DPPH, 2,2′-diphenyl-1-picrylhydrazyl; FW, fresh weight; FRAP, ferric reducing antioxidant power; GAE, gallic acid equivalent; TAC, total antioxidant capacity; TEAC, Trolox antioxidant capacity assay; TPC, total phenolic content; TPTZ, 2,4,6-tripyridyl-*s*-triazine

REFERENCES

(1) Magda, T. R. Going back to natural red colors (red sorghum, cochineal, and *Monascus*). *Drink Technol. Mark.* **2003**, *7*, 1433–1594.

(2) Bao, J.; Cai, Y. M.; Sun, M.; Wang, G.; Corke, H. Anthocyanins, flavonols, and free radical scavenging activity of Chinese bayberry (*Myrica rubra*) extracts and their color properties and stability. *J. Agric. Food Chem.* **2005**, *53*, 2327–2332.

(3) Giusti, M.; Wrolstad, R.; Gloria, M. Anthocyanins from banana bracts as potential food colorants. *Food Chem.* **2001**, *73*, 327–332.

(4) Dicko, M. H.; Gruppen, H.; Traore, A. S.; Voragen, A. G. J.; Van Berkel, W. J. H. Sorghum grain as human food in Africa: Relevance of content of starch and amylase activities. *Afr. J. Biotechnol.* **2006**, *5*, 384–395.

(5) Boon, E. J. M. C.; Engels, F. M.; Struik, P. C.; Cone, J. W. Stem characteristics of two forage maize (*Zea mays* L.) cultivars varying in whole plant digestibility. II. Relation between *in vitro* rumen fermentation characteristics and anatomical and chemical features within a single internode. *NJAS—Wageningen J. Life Sci.* **2005**, *53*, 87–111.

(6) Lo, S. C. C.; Nicholson, R. L. Reduction of light-induced anthocyanin accumulation in inoculated sorghum mesocotyls: Implication for a compensatory role in the defense response. *Plant Physiol.* **1998**, *116*, 979–989.

(7) Lo, S. C. C.; de Verdier, K.; Nicholson, R. L. Accumulation of 3-deoxyanthocyanidin phytoalexins and resistance to *Colletotrichum sublineolum* in sorghum. *Physiol. Mol. Plant Pathol.* **1999**, *55*, 263–273.

(8) Nip, W. K.; Burns, E. E. Pigment characterization in grain sorghum. I. red varieties. *Cereal Chem.* **1969**, *46*, 490–495.

(9) Nip, W. K.; Burns, E. E. Pigment characterization in grain sorghum. II. White varieties. *Cereal Chem.* **1971**, *48*, 74–80.

(10) Kouda-Bonafos, M.; Czyzewska, E; Nacro, M.; Oehlschlager, A. C. Isolation of apigeninidin from leaf sheaths of sorghum caudatum. *J. Chem. Ecol.* **1994**, *20*, 2123–2125.

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

(12) Awika, J. M.; Rooney, L. W.; Waniska, R. D. Properties of 3-deoxyanthocyanins from sorghum. *J. Agric. Food Chem.* **2004**, *52*, 4388–4394.

(13) Awika, J. M.; Rooney, L. W.; Waniska, R. D. Anthocyanins from black sorghum and their antioxidant properties. *Food Chem.* **2004**, *90*, 293–301.

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

(15) Yang, L.; Browning, J. D.; Awika, J. M. Sorghum 3-deoxyanthocyanins possess strong phase II enzyme inducer activity and cancer cell growth inhibition properties. *J. Agric. Food Chem.* **2009**, *57*, 1797–1804.

(16) Saïdou, A.; Kuyper, T. W.; Kossou, D. K.; Tossou, R.; Richard, P. The sustainable soil fertility management in Benin: learning from farmers. *NJAS—Wageningen J. Life Sci.* **2004**, *52*, 349–368.

(17) Faure, P. *Notice explicative No. 66, carte pédologique de reconnaissance de la République Populaire du Bénin à 1/200.000, Feuille de Natitingou (6)-Porga (8)*; Travaux et documents de l'ORSTOM, Paris, France, 1977; p 68.

(18) Abdel-Aal, E.-S. M.; Hucl, P. A rapid method for quantifying total anthocyanins in blue aleurone and purple pericarp wheat. *Cereal Chem.* **1999**, *76*, 350–354.

(19) Singleton, V. L.; Rossi, J. A. Colorimetry of total phenolics with phosphomolybdic-phosphotungstic acid reagents. *Am. J. Enol. Vitic.* **1965**, *16*, 144–158.

(20) Siebenhandl, S.; Grausgruber, H.; Pellegrini, N.; Del Rio, D.; Fogliano, V.; Pernice, R.; Berghofer, E. Phytochemical profile of main antioxidants in different fractions of purple and blue wheat, and black barley. *J. Agric. Food Chem.* **2007**, *55*, 8541–8547.

(21) Pellegrini, N.; Del Rio, D.; Colombi, B.; Bianchi, M.; Brighenti, F. Application of the 2,2′-azobis(3-ethylenebenzothiazoline-6-sulfonic acid) radical cation assay to a flow injection system for the evaluation of antioxidant activity of some pure compounds and beverages. *J. Agric. Food Chem.* **2003**, *51*, 260–264.

(22) Moore, J.; Hao, Z.; Zhou, K.; Luther, M.; Costa, J.; Yu, L. L. Carotenoid, tocopherol, phenolic acid, and antioxidant properties of Maryland-grown soft wheat. *J. Agric. Food Chem.* **2005**, *53*, 6649–6657.

(23) Benzie, I. F. F.; Strain, J. J. Ferric reducing/antioxidant power assay: Direct measure of total antioxidant activity of biological fluids and modified version for simultaneous measurement of total antioxidant power and ascorbic acid concentration. *Methods Enzymol.* **1999**, *299*, 15–27.

(24) Brand-Williams, W.; Cuvelier, M. E.; Berset, C. Use of a free radical method to evaluate antioxidant activity. *Lebensm.-Wiss. Technol.* **1995**, *28*, 25–30.

(25) Fossen, T.; Cabrita, L.; Andersen, Ø. Colour and stability of pure anthocyanins influenced by pH including the alkaline region. *Food Chem.* **1998**, *63*, 435–440.

(26) Ortega-Regules, A.; Romero-Cascales, I.; López-Roca, J. M.; Ros-Garcia, J. M.; Gómez-Plaza, E. Anthocyanin fingerprint of grapes: Environmental and genetic variation. *J. Sci. Food Agric.* **2006**, *86*, 1460–1467.

(27) Dykes, L.; Rooney, L. W.; Waniska, R. D.; Rooney, W., L. Phenolic compounds and antioxidant activity of *Sorghum* grain of varying genotypes. *J. Agric. Food Chem.* **2005**, *53*, 6813–6818.

(28) Awika, J. M. Behaviour of 3-deoxyanthocyanidins in the presence of phenolic copigments. *Food Res. Int.* **2008**, *41*, 532–538.

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

(30) Tulipani, S.; Mezzetti, B.; Capocasa, F.; Bompadre, S.; Beekwilder, J.; Rice de Vos, C. H.; Capanoglu, E.; Bovy, A.; Battino, M. Antioxidants, phenolic compounds, and nutritional quality of different strawberry genotypes. *J. Agric. Food Chem.* **2008**, *56*, 696–704.

(31) Podsędek, A.; Sosnowska, D.; Redzynia, M.; Koziolkiewicz, M. Effect of domestic cooking on the red cabbage hydrophilic antioxidants. *Int. J. Food Sci. Technol.* **2008**, *43*, 1770–1777.

(32) Çekiç, Ç.; Özgen, M. Comparison of antioxidant capacity and phytochemical properties of wild and cultivated red raspberries (*Rubus idaeus* L.). *J. Food Compos. Anal.* **2010**, *23*, 540–544.

(33) Sompong, R.; Siebenhandl-Ehn, S; Linsberger-Martin, G.; Berghofer, E. Physicochemical and antioxidative properties of red and black rice varieties from Thailand, China and Sri Lanka. *Food Chem.* **2011**, *124*, 132–140.

(34) Denev, P.; Ciz, M.; Ambrozova, G.; Lojek, A.; Yanakieva, I.; Kratchanova, M. Solid-phase extraction of berries' anthocyanins and evaluation of their antioxidative properties. *Food Chem.* **2010**, *123*, 1055–1061.