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Evaluation of the Effect of Germination on Phenolic Compounds and Antioxidant Activities in Sorghum Varieties

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The screening of 50 sorghum varieties showed that, on average, germination did not affect the content in total phenolic compounds but decreased the content of proanthocyanidins, 3-deoxyanthocyanidins, and flavan-4-ols. Independent of germination, there are intervarietal differences in antioxidant activities among sorghum varieties. Phenolic compounds and antioxidant activities were more positively correlated in ungerminated varieties than in germinated ones. Sorghum grains with pigmented testa layer, chestnut color glumes, and red plants had higher contents, larger diversity of phenolic compounds, and higher antioxidant activities than other sorghums. Some red sorghum varieties had higher antioxidant activities (30−80 μmol of Trolox equiv/g) than several sources of natural antioxidants from plant foods. Among varieties used for "tô", "dolo", couscous, and porridge preparation, the "dolo" (local beer) varieties had the highest average content and diversity in phenolic compounds as well as the highest antioxidant activities. The biochemical markers determined are useful indicators for the selection of sorghum varieties for food and agronomic properties.

KEYWORDS: Sorghum; germination; antioxidant; proanthocyanidins; 3-deoxyanthocyanidins; apigeninidins; flavan-4-ols

INTRODUCTION

Sorghum [*Sorghum bicolor* (L.) Moench] is one of the most important cereal crops in the world with a current annual production over 60 million tons, of which U.S.A. and Africa produce 20 and 40%, respectively (*1*). Sorghum is the staple food in several countries, notably in Africa (*1*−*4*). In West Africa, ungerminated sorghum grains are generally used for the preparation of "tô" (thick porridge) and couscous (granulated food). Malted sorghum is often used in the production of local beer ("dolo"), infant food (thin porridge), and nonfermented beverages.

Sorghum is a cereal reported to contain simple phenols, hydroxybenzoic acids, hydroxycinnamic acids (with ferulic acid being the most abundant), anthocyanins, proanthocyanidins (PAs), and several other flavonoids (*3*, *5*−*11*). The 3-deoxyanthocyanidins (3-DAs), namely, apigeninidins and luteolinidins, are particularly abundant in sorghum grain but rare or absent in other plants (*3*, *6*−*8*). 3-DAs are of interest because they are more stable in organic solvents as well as in acidic solutions than anthocyanidins commonly found in fruits, vegetables, and other cereals (*6*−*8*). An agronomic interest exists in sorghum grains containing flavan-4-ols, such as leucoapigeninidin (apiforol) and leucoluteolinidin (luteoforol), because they may confer a high resistance to the grain to molding (*9*−*11*). A new interest in flavan-4-ols is linked to their anticarcinogenic activity (*12*).

Nowadays, phenolic compounds are generally regarded as desirable components of human food, because of their antioxidant activity. Therefore, they are considered to be of nutraceutical importance (*3*, *13*, *14*). Even for proanthocyanidins (condensed tannins), their earlier classification as essentially antinutritional factors, throughout formation of complexes with proteins and carbohydrates, could be balanced against their potential to serve as biological antioxidants (*15*). All phenolic compounds are able to scavenge free radicals through electron-donating properties, generating a relatively stable phenoxyl radical or nonradical species (*13*, *14*, *16*, *17*). Some phenolic compounds protect against neurological disorders and exert anticarcinogenic, antimutagenic, and cardioprotective effects linked to their free-radical scavenging activities (*13*, *14*, *16*, *17*).

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Research on discovering naturally occurring antioxidants for use in foods or medicinal materials to replace synthetic antioxidants has attracted much attention (*13*, *14*). Recently, Awika et al. (*18*) reported methods to determine antioxidant activities of sorghums, their brans, and baked and extruded products. For red sorghum, the bran had 3−5 times more antioxidant activity than the whole grain (*18*). These antioxidant activities were higher than those of blueberries (*Vaccinium* species), one of the most important natural sources of antioxidants (*18*). In a previous study on 50 sorghum varieties (*19*), we showed that the varieties are highly diverse in their contents in phenolic compounds and oxidative enzymes. Although phenolic antioxidant activities are detected in sorghum (*18*), it is not known whether germination affects the content of these phenolic compounds and their antioxidant activities.

Germination is an essential step in the preparation of sorghum grains for several products. In one study, a severalfold increase in total phenolic compounds and PAs after germination of sorghum was reported (*20*). In other studies, both an increase or a decrease of total phenolic compounds and PAs upon germination has been observed (*21*−*23*). However, these studies were performed with only a limited number of sorghum varieties. In the present work, the effect of germination on phenolic compounds and antioxidant activities in 50 sorghum varieties is studied and correlations between these properties and local food applications are determined.

MATERIALS AND METHODS

Chemicals and Reagents. 2,2′-Azino-bis(3-ethylbenzthiazoline-6-sulfonic acid) (ABTS), 6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid (Trolox), Folin−Ciocalteu reagent for phenol, and ascorbic acid (vitamin C) were from Sigma−Aldrich. Gallic acid (3,4,5-trihydroxybenzoic acid) was from Aldrich. Sorghum apigeninidin, isolated and characterized by Kouda-Bonafos et al. (*8*), was a gift of Dr. Eloi Palé from the Laboratory of Natural Substances, University of Ouagadougou, Burkina Faso. Cyanidin chloride was from Extrasynthèse (Lyon, France). Apple cider procyanidin oligomers (average degree of polymerization of 7.4) were kindly provided by Stephanie Prigent (Wageningen University, Wageningen, The Netherlands) and Dr. Catherine M. G. C. Renard (INRA, Rennes, France). These procyanidins were purified by RP-HPLC and characterized by thiolysis-HPLC as described by Guyot et al. (*24*, *25*). All other chemicals were of analytical grade.

***Sorghum bicolor* L. (Moench) Varieties.** Sorghum varieties were grown during the rainy season of 2002 at the experimental station of Saria, in Burkina Faso (West Africa). Growth conditions were as described by Trouche et al. (*26*, *27*). The varieties were chosen according to criteria described previously (*19*). Information as to which varieties were "good" or "poor" for the preparation of specific dishes was collected from sorghum breeders familiar with the preference of the local population after a participatory survey (*27*). For convenience, the sorghum varieties were classified in alphabetic order of their name followed by Arabic numbers from 1 to 50 preceded by V (**Table 1**). Mature grains (>60 days after anthesis) were harvested, surface-sterilized, and germinated as described previously (*19*, *21*). Prior to germination, grains were steeped in water in the dark at 20−25 °C, for 16 h. Germination was performed at 27 ± 2 °C for 72 h. The appearance of primary shoots and roots was observed in all of the varieties at the end of germination. Germinated and ungerminated sorghum grains were dried, ground, and stored as described previously (*19*). The moisture contents of the flours as determined by heating in oven at 105 °C, for 5−6 h, were 5−7% (w/w).

Total Phenolic Compound Extraction and Assay. Sorghum phenolic compounds were extracted from sorghum flour as described previously (*19*). The total phenolic extract was analyzed directly or kept in the dark, at −30 °C for less than 48 h, to avoid oxidation. The total phenolic compound content was determined using a miniaturized Folin−Ciocalteu method (*19*). Results were expressed as gallic acid equivalents per gram of flour (w/w, dry matter basis). The same extract was used for quantification of PAs, 3-DAs, and flavan-4-ols.

Proanthocyanidins (PAs) and Flavan-4-ols Assay. The total phenolic extract was assayed for PAs and flavan-4-ols essentially as described by Melake-Berhan et al. (*11*) with miniaturization to adapt the assay to a 96-well plate format as follows. To determine flavan-4-ols, 50 μL of the extract was added to 700 μL of reagent A (30%, v/v, 12 N HCl in butan-1-ol) or to 700 μL of reagent B (15%, v/v, 0.1 N acetic acid; 15%, v/v, methanol; and 70%, v/v, butan-1-ol). The sample in reagent A was mixed by vortex and left at 25 °C, for 1 h, to allow the formation of anthocyanidin pigments derived from flavan-4-ols (*11*). Aliquots of the mixture (150 μL) were put in duplicate in a 96-multiwell plate, and the absorbance read at 550 nm to quantify anthocyanidins formed from flavan-4-ols (*11*). Cyanidin was used as a standard to estimate the total amount of the anthocyanidins derived from flavan-4-ols.

For PA quantification, the remaining sample in the tube with reagent A was further heated at 100 °C, for 2 h. Under these conditions, PAs are converted to anthocyanidins and the unstable pigments formed from flavan-4-ols are destroyed (*11*). After cooling, 200 μL of the sample was put in duplicate in a 96-multiwell plate and the absorbances of anthocyanidin compounds derived from PAs were read at 550 nm (*11*). Sample mixtures with reagent B, which were not heated, served as blanks for the quantification of both PAs and flavan-4-ols. Apple procyanidins with an average DP ≈ 7.4, treated as indicated above, were used as a standard for sorghum PA quantification (**Figure 1**).

3-Deoxyanthocyanidins (3-DAs) Assay. For direct spectrophotometric quantification of 3-DAs, 50 μL of the total phenolic extract was mixed with 150 μL of methanol and the absorbances were read at 475 nm (*11*). Sorghum apigeninidin (*8*) was used as a standard.

Determination of Antioxidant Activity. The antioxidant activity of sorghum phenolic compounds was determined both by the ability to scavenge 2,2′-azino-bis(3-ethylbenzthiazoline-6-sulfonic acid) radical ($ABTS^{\cdot+}$) (*28*) and by reduction of phosphomolybdenum Mo^{VI} to Mo^{V} (*16*, *29*). Calibration curves were made using Trolox and vitamin C. Trolox antioxidant equivalents (TAEs) and vitamin C antioxidant equivalents (VCAEs) were expressed on a weight basis (μmol/g of flour, dry matter).

Statistical Analysis. All spectrophotometric assays were carried out in 96-well microtiter plates (Nunclon, Denmark) using a multiwell plate reader (*μQuant* Bio-Tek Instrument, Inc.) on-line interfaced with a personal computer. The absorbances and slopes of absorbances (OD/min) were automatically recorded using KC junior software version 1.31.5 (Bio-Tek Instrument, Inc.). All assays were carried out at least in duplicate. Mean values, standard deviations, and standard errors are reported. Significant differences in mean performance for each composition among sorghum varieties were tested by Student's *t* test; $p < 0.05$ implies significance. Pearson linear correlation coefficients were used to assess relationships among biochemical constituents.

RESULTS AND DISCUSSION

Effect of Germination on Phenolic Compounds in Sorghum Varieties. It is important to note that the levels of phenolic compounds within varieties found are of more interest for intervarietal comparison than as absolute levels because of the lack of universal assays and standards and because of differences in reactivity among phenolic compounds within a particular class. The apple procyanidins (DP ≈ 7.4) used as a standard could underestimate the level of PAs with a low degree of polymerization or overestimate the level of PAs with a high degree of polymerization (*24*). The wavelength (475 nm) used to measure the amount of 3-DAs may mostly quantify apigeninidins over the other 3-DAs such as luteolinidins (*11*). The used assays may not reveal all of the complexity of the chemistry of phenolic compounds that occurs in the plant tissue during germination, but they are useful in assessing the effect of germination on the selected classes of phenolic compounds.

Although grown under the same conditions, the sorghum grains screened varied significantly in their levels of total

Table 1. Comparison of Phenolics Composition of Ungerminated (g–) and Germinated (g+) Sorghum Varieties

code	name	color of grain/ glumes/plant	testa[e]	main food property	total phenolics (%)[a]		PAs (%)[b]		3-DAs (%)[c]		flavan-4-ols (%)[d]	
					g–	g+	g–	g+	g–	g+	g–	g+
V1	Ajabsido	W/R/R	+	good for "tô"	0.58	0.96	0.11	0.19	0.04	0.05	nd[f]	nd
V2	BF 88-2/31-1	W/P/tan	–	poor for "tô"	0.85	0.60	nd	nd	nd	nd	0.17	nd
V3	BF 88-2/31-3	W/R/tan	–	good for couscous	0.72	0.69	nd	nd	nd	nd	nd	nd
V4	BF 89-18/139-1-1	W/P/tan	–	good for "tô"	0.66	0.41	nd	nd	nd	nd	nd	nd
V5	Cauga 108-15	W/P/R	–	good for "tô"	0.72	0.63	0.17	0.18	0.04	0.02	nd	nd
V6	Cauga 22-20	W/P/R	+	good for "tô"	1.38	1.22	1.50	1.21	0.02	0.02	0.22	0.13
V7	CE 180-33	W/R/tan	+	good for couscous	0.59	0.87	0.11	0.06	nd	nd	nd	nd
V8	CEF 322/53-1-1	W/P/R	–	good for "tô"	0.71	0.67	0.07	nd	nd	nd	nd	nd
V9	CEF 395/9-2-3	W/P/tan	–	good for "tô"	0.87	0.91	nd	nd	nd	nd	nd	nd
V10	CEF 396/12-3-1	W/P/R	–	good for "tô"	0.68	0.88	0.06	0.17	0.02	0.02	nd	nd
V11	CEM 326/11-5-1-1	W/P/tan	–	good for "tô"	0.69	0.73	nd	nd	nd	nd	nd	nd
V12	CGM 1/19-1-1	W/P/R	–	good for "tô"	0.64	0.81	0.06	0.07	nd	0.02	nd	nd
V13	CGM 19/9-1-1	W/B/R	–	good for "tô"	0.61	0.82	nd	nd	nd	0.02	nd	nd
V14	CGM 19/9-1-2	W/P/R	–	good for"dolo"	0.55	0.81	0.08	nd	nd	nd	nd	nd
V15	CK 60	W/P/R	–	good for "tô"	0.76	1.22	0.24	0.28	0.04	0.07	nd	nd
V16	F2-20	W/R/tan	–	good for couscous	0.55	0.86	nd	nd	0.02	nd	nd	nd
V17	Farkakofsi 781	R/B/R	+	good for "dolo"	1.28	1.47	0.66	0.72	0.14	0.12	0.38	0.30
V18	Framida	R/C/R	+	good for "dolo"	1.74	1.85	1.13	0.58	0.03	0.05	0.37	0.29
V19	G 1296	R/R/R	+	good for "dolo"	3.01	2.95	2.18	1.85	0.42	0.24	0.42	0.37
V20	G 1414	W/P/R	–	good for "tô"	0.71	0.84	0.08	0.15	nd	nd	nd	nd
V21	G 1636	W/P/tan	–	poor for "tô"	0.76	0.74	0.07	0.08	nd	nd	nd	nd
V22	ICSV 1002	W/P/tan	–	good for "tô"	0.82	0.51	nd	nd	0.02	nd	nd	nd
V23	ICSV 1049	W/P/tan	–	good for porridge	0.64	0.78	nd	nd	nd	nd	nd	nd
V24	ICSV 745	W/P/tan	–	poor for "tô"	0.66	0.46	nd	nd	nd	nd	nd	nd
V25	IRAT 10	W/B/R	–	good for "tô"	0.82	0.72	0.06	0.06	nd	nd	nd	nd
V26	IRAT 174	W/C/R	–	good for "tô"	0.92	0.75	0.09	0.08	0.06	0.04	nd	nd
V27	IRAT 202	W/R/tan	+	good for couscous	1.20	1.22	0.62	0.33	nd	nd	nd	nd
V28	IRAT 204	W/P/tan	–	good for couscous	0.96	0.61	nd	nd	nd	nd	nd	nd
V29	IRAT 277	W/R/tan	–	poor for "tô"	0.87	0.79	nd	nd	nd	nd	nd	nd
V30	IRAT 9	R/C/R	+	good for "dolo"	1.50	1.18	0.90	0.54	0.07	0.03	0.41	0.38
V31	IS 15401	W/P/R	–	good for couscous	0.65	0.63	0.09	nd	nd	nd	nd	nd
V32	Kaapelga	W/P/tan	–	good for "tô"	0.73	0.83	nd	nd	nd	nd	nd	nd
V33	Kapla-57	R/P/R	+	good for "dolo"	0.60	0.97	0.53	0.41	nd	nd	nd	nd
V34	Kokologho	W/B/R	+	poor for "dolo"	0.81	1.02	0.66	0.41	0.02	0.06	0.19	nd
V35	90L1235	W/B/R	–	good for couscous	1.28	0.97	0.53	0.40	0.07	0.07	0.19	nd
V36	Magadji 1-509	R/B/R	–	good for "dolo"	0.70	0.99	0.07	0.11	nd	nd	nd	nd
V37	Nafo-Natogué 775	R/B/R	+	good for "dolo"	1.47	1.75	1.16	1.17	0.18	0.17	0.34	0.24
V38	Nazongala tan	W/B/tan	–	good for porridge	0.66	1.16	0.09	0.13	0.04	0.06	nd	nd
V39	Nongomsoba	W/B/tan	–	good for porridge	0.82	0.76	nd	0.08	nd	nd	nd	nd
V40	S 29	W/R/R	–	good for "tô"	0.72	1.01	0.07	0.12	nd	nd	nd	nd
V41	Sariaso 10	W/R/R	–	good for porridge	0.73	0.70	0.08	nd	nd	nd	0.23	0.22
V42	Sariaso 11	W/P/tan	–	good for "tô"	0.74	0.55	nd	nd	nd	nd	nd	nd
V43	Sariaso 12	W/B/R	–	good for "tô"	0.83	1.05	0.17	0.14	0.05	0.03	0.18	0.13
V44	Sariaso 14	W/P/tan	–	good for porridge	0.45	0.63	nd	nd	nd	nd	nd	nd
V45	Sariaso 9	W/B/R	–	good for "tô"	0.98	0.77	0.26	0.17	0.06	0.04	0.22	0.14
V46	Segaolane	W/P/R	–	poor for "tô"	0.63	0.71	0.12	0.06	nd	nd	nd	nd
V47	SRN 39	Y/P/tan	–	poor for "dolo"	1.10	0.96	0.17	0.09	0.06	0.03	0.19	0.12
V48	Tiamassie 289	W/B/R	+	poor for "tô"	0.58	0.61	0.06	0.06	0.03	0.02	nd	nd
V49	Tx 7000	W/P/R	–	poor for "tô"	0.46	0.81	0.08	0.07	nd	nd	nd	nd
V50	Zugilga	R/B/R	+	good for "dolo"	1.71	1.01	0.91	0.42	0.05	0.02	0.20	0.18
mean value ($n = 50$)					0.88	0.92	0.26	0.21	0.03	0.02	0.07	0.05
SE ($n = 2$)					0.05	0.05	0.01	0.01	0.002	0.002	0.003	0.003

[a] Gallic acid equivalents (%, w/w, dry matter basis). [b] PAs = proanthocyanidins (%, w/w, procyanidins DP ≈ 7.4, dry matter basis). [c] 3-DAs = 3-deoxyanthocyanidins (%, w/w, apigeninidin). [d] Anthocyanidin equivalents derived from flavan-4-ols (%, w/w, cyanidin, dry matter basis). [e] Pigmented testa layer present (+) or absent (–). [f] Abbreviations: W = white, R = red, P = pale, Y = yellow, C = chestnut, B = black, nd = not detected, g– = ungerminated sorghum, and g+ = germinated sorghum.

phenolic compounds, PAs, 3-DAs, and flavan-4-ols (**Table 1**). The average total phenolics content of sorghum varieties used in this study (harvested in 2002) was on average 40% higher than that of the same sorghum varieties harvested in 1998 (*19*). This increase in total phenolic content was observed in 80% of the varieties. However, in some varieties, the total phenolic content decreased by a factor of 1.2−2.5. Nevertheless, varieties containing the highest amounts of total phenolic compounds (V18 and V19) remained the same over the years. These results indicate that there is an interseasonal variation of the total phenolic compound content among sorghum varieties. This is in agreement with the observation that the environment may affect the biosynthesis of phenolic compounds in sorghum (*30*). Germination affected the content in phenolics according to variety (**Table 1**). Nwanguma et al. (*20*) found an increase of severalfold in total phenolic compounds in all screened four sorghum varieties after germination. In contrast, the results of the present study showed that on average germination does not generally affect the total phenolic compound content in sorghum. However, in line with previous observations (*21*−*23*), depending on variety, sorghum total phenolic compounds decreased or increased upon germination. The presence of PAs (condensed

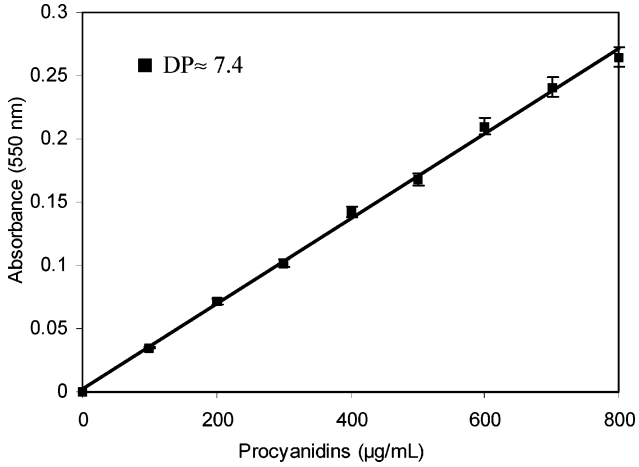

Figure 1. Calibration curve of procyanidins of DP ≈ 7.4. The phenolic extract (50 μL) was incubated with 700 μL of reagent A (30%, v/v, 12 N HCl in butan-1-ol) at 100 °C for 2 h. The blank was prepared by mixing 50 μL of the sample with 700 μL of reagent B (15%, v/v, 0.1 N acetic acid; 15%, v/v, methanol; and 70%, v/v, butan-1-ol) without heating. Vertical bars indicate the average standard error for each experiment.

tannins) in sorghum varieties was previously (*19*) assessed using the ferric ammonium citrate (International Standard Organization method). In the present, it was confirmed using the acid−butanol assay that the majority (more than 80%) of the screened sorghums from Burkina Faso are not high tannins (<0.5%, w/w) containing sorghums. This is observed for worldwide sorghums (*1*, *20*, *22*, *31*). Some of the screened varieties (32%) were PA free. Overall, the PA content decreased after germination. The decrease in extractable PAs after germination could be due to leaching of water-soluble PAs, which are located in the pericarp and testa (*3*, *31*, *32*), or due to formation of insoluble complexes with proteins (*28*). Quantifying PA content in two sorghum varieties, Iwuoha and Aina (*23*), also found a decrease in PA content after germination. This indicates that the effect of germination on the total phenolic compound content is dependent on variety.

Among the varieties, 42% contained 3-DAs before and after germination. On average, germination did not induce the synthesis of 3-DAs among varieties but decreased their content. Variety V19 displayed a higher content of 3-DAs (0.42%, w/w of apigeninidin equivalent) than sorghum variety Monome Kaya found by Sérémé et al. (*33*). This sorghum variety Monome Kaya was determined to have the highest content (0.25%, w/w of apigeninidin) among 30 sorghum varieties from Burkina Faso, which were screened for apigeninidin (*33*). Because 3-DAs are anthocyanidins with industrial importance in food, varieties V19 and V37, which were found to be rich in these compounds in this study, can be considered as important sources. The apigeninidin content is also an indicator of grain resistance to fungi such as *Colletrichum graminicola*, *Fusarium oxysporum*, *Gibberelle zeae*, and *Gliocladium roseum* (*9*). The screening for 3-DAs is, therefore, of great interest for breeders.

Only 28% of varieties displayed detectable amounts in flavan-4-ols. After flavan-4-ol contents were screened in 22 sorghum varieties, Audilakshmi et al. (*10*) detected these compounds in only four varieties (18%). This supports that flavan-4-ols are quite rare in sorghum. On average, germination reduced the content in flavan-4-ols by 33% in varieties containing those compounds and even led to the complete disappearance in some varieties (V2, V34, and V35). The decrease of flavan-4-ol content in all varieties upon germination may be related to their conversion into other flavonoids (anthocyanidins and flavan-3-ols), which is stimulated by enzymes that are produced during germination and that are involved in the biosynthesis of the latter compounds (*5*).

Effect of Germination on Antioxidant Activities in Sorghum Varieties. Because the ranking obtained by the comparison of antioxidant activity of plant food is dependent on the assays used (*17*), the evaluation of the antioxidant activities of sorghum phenolic compounds was performed in this study using two different methods. While the ABTS assay monitored the capacity of phenolic compounds to scavenge free radicals (*28*), the phosphomolybdenum measured their reducing power (*29*). Phenolic extracts of nongerminated sorghum varieties evaluated using the ABTS assay showed antioxidant activities ranging from 16 to 80 μmol of TAE/g or 6 to 28 μmol of VCAE/g (parts **A** and **B** of **Figure 2**). The evaluation of the antioxidant activity using the phosphomolybdenum assay gave values ranging from 17 to 85 μmol of TAE/g or 9 to 96 μmol of VCAE/g. The mean value of antioxidant activities in sorghum varieties before germination was 42 μmol of TAE/g or 15 μmol of VCAE/g, with the ABTS assay, and 45 μmol of TAE/g or 24 μmol of VCAE/g with the phosphomolybdenum assay. Antioxidant activities changed differently in varieties upon germination, but on average, they were not affected by germination. The intervarietal differences of the antioxidant activities of sorghum varieties could be related to the difference in their total phenolic compound content and to the diversity of phenolic compounds. Indeed, several studies of structurally related phenolic compounds have revealed differences in their antioxidant capacity (*34*, *35*). The antioxidant activity of phenolic compounds depends on the number of conjugated unsaturated bonds, the redox properties, and the presence of vicinal hydroxyls in the aromatic ring (*16*, *34*, *35*).

Although the antioxidant activities of phenolic compounds could be beneficial, the major nutritional concern is the ability of PAs to bind strongly to large proteins and to proline-rich proteins, thereby reducing their digestibility (*36*). PAs display free-radical scavenging activity even when forming complexes with proteins (*28*), and they may be degraded in the digestive system into low molecular weight monomers, which could be absorbed by the intestine (*3*). This supports the idea that the potential of PAs to diminish nutrient digestibility may be compensated by their *in vivo* antioxidant activity, even when bound to proteins (*15*).

Correlations Between Antioxidant Activities and Phenolic Compounds. In line with the report of Awika et al. (*18*), antioxidant activities were positively correlated with the level of total phenolic compounds, both before and after germination (**Table 2**). A relatively strong correlation was found between antioxidant activities and PAs when compared to those for 3-DAs and flavan-4-ols. The weaker correlation between antioxidant activity and phenolic compounds upon germination could be due to the synthesis of other antioxidant compounds such as vitamin C and tocopherols in sorghum during germination (*37*, *38*). Germination lowered the correlations between different phenolic compounds and antioxidant activities. The difference found between Trolox and vitamin C on scavenging the $ABTS^{\cdot+}$ radical is related to the difference in their mechanism of radical scavenging (*34*). Indeed, it has been found that α-tocopherol, an analogue of Trolox, is a much more effective chain-breaking antioxidant in scavenging lipid peroxyl radicals than vitamin C (*34*). On weight/weight basis, the total phenolic compounds (gallic acid equivalents) of whole sorghum grains have an antioxidant capacity comparable to that of pure sorghum PAs (*28*). This may explain the good correlation found

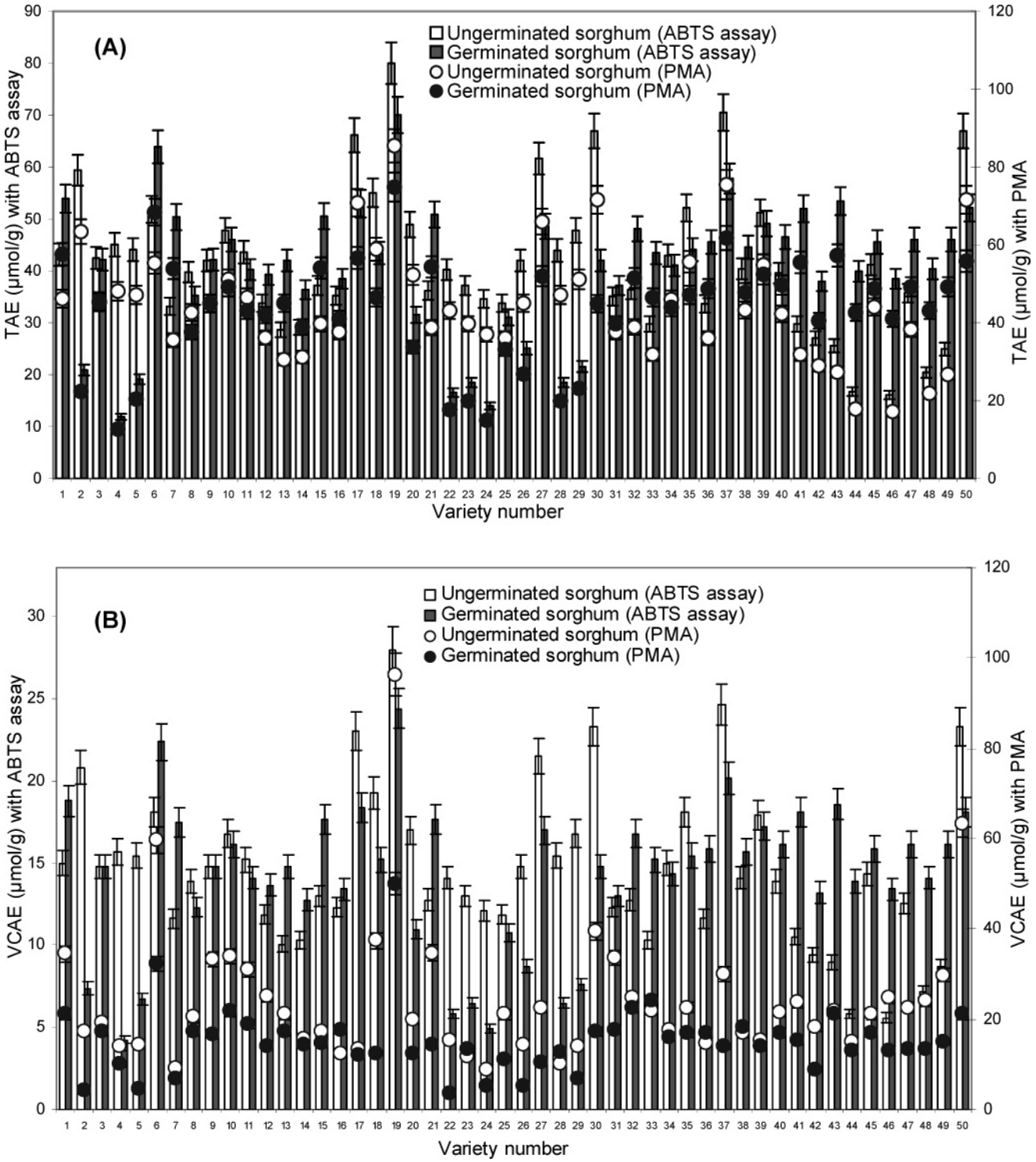

Figure 2. Comparison of the effect of germination on antioxidant activities of sorghum varieties. (A) Evaluation of the antioxidant activities as Trolox antioxidant equivalents (TAE). (B) Evaluation of the antioxidant activity as vitamin C antioxidant equivalents (VCAE). ABTS = (2,2′-azino-bis(3-ethylbenzthiazoline-6-sulfonic acid), and PMA = phosphomolybdenum assay. Vertical bars indicate the average standard error for each experiment.

between antioxidant activity and the content in PAs (**Table 2**). PAs are reported to have a higher antioxidant activity than other phenolic compounds because they have little or no pro-oxidant activity and because of the presence of *ortho*-hydroxyl groups in their structures (*15*). In addition, oxidized PAs undergo phenolic coupling reactions, generating hydroxyls, which govern their antioxidant activity and which are higher than the other phenolic compounds (*35*).

Phenolic Compounds and Antioxidant Activities of Sorghum Varieties Grouped According to Plant or Grain Properties. Independent of germination, red varieties contained on average significantly more total phenolic compounds, PAs, 3-DAs, and flavan-4-ols than white varieties (**Table 3**). Nevertheless, some white varieties (V6, V27, and V35) have a relatively high contents of total phenolic compounds (1.2−1.4%, w/w). Investigations carried out by others also showed phenolic content in white varieties to be as high as 3% (w/w) (*31*). All red varieties contained 3-DAs, whereas among white varieties, only 35% possessed trace amounts of 3-DAs (0−0.07%, apigeninidin equivalents), if no interference of other phenolics

Table 2. Pearson Correlation (*r* Values) Matrix between Phenolic Compounds and Their Antioxidant Activities in Ungerminated (g–) and Germinated (g+) Sorghum Varieties

	antioxidant activities[a]		total phenolics		PAs[b]		3-DAs[c]	
	g–	g+	g–	g+	g–	g+	g–	g+
total phenolics	0.78	0.64						
PAs	0.79	0.63	0.92	0.87				
3-DAs	0.66	0.56	0.80	0.86	0.68	0.78		
flavan-4-ols	0.67	0.07[d]	0.68	0.65	0.61	0.52[d]	0.60	0.57

[a] Trolox antioxidant equivalents using the ABTS (2,2′-azino-bis(3-ethylbenzthiazoline-6-sulfonic acid) assay. [b] PAs = proanthocyanidins. [c] 3-DAs = 3-deoxyanthocyanidins, g– = ungerminated sorghum, and g+ = germinated sorghum. [d] Not significant ($p < 0.05$); all others are significant.

in the assay is assumed. Red sorghum grains had on average significantly higher antioxidant activities (30−80 μmol of TAE/g) than white grains (16−62 μmol of TAE/g) both before and after germination. These results are in line with those of Awika et al. (*18*) who found the same trend for ungerminated sorghum grains. Sorghum grains with pigmented testa layer have higher levels of total phenolic compounds (2-fold), PAs (9−11-fold), 3-DAs (8−10-fold), and antioxidant activities (1.5-fold) than sorghum without pigmented testa. Sorghum grains covered with glumes possessing the chestnut color have a higher content in total phenolic compounds, PAs, 3-DAs, flavan-4-ols, and antioxidant activities than the other red, black and pale glume colors. Audilakshmi et al. (*10*) also found correlations between glume colors and the content of the grains in total phenols and flavan-4-ols. **Table 3** shows that the grains from red sorghum plants have higher phenolic compounds and antioxidant activities than grains from tan sorghum plants. The levels of phenolic compounds in plant tissues are not only dependent on the genotype, but they are also dependent on the biotic and abiotic stresses. Because the varieties were grown in the same environment and no visible effect of stress was observed, these correlations between the levels of phenolic compounds and the plant and grain characteristics may be essentially attributed to the genetic diversity of sorghum varieties (*10*, *32*, *39*).

Phenolic Compounds and Antioxidant Activities of Sorghum Varieties Grouped According to Food Properties. The groups of sorghum varieties used for specific local foods displayed significant differences in their contents of total phenolic compounds, PAs, 3-DAs, flavan-4-ols, and antioxidant activities before and after germination (**Table 3**). Varieties good for "dolo" had, before and after germination, on average the highest total phenolic content (1.40−1.44%, w/w), PAs (0.61−0.79%, w/w), 3-DAs (0.06−0.09%, w/w), flavan-4-ols (0.20−0.24%, w/w), and antioxidant activities (49−55 μmol of TAE/g) of all groups. To obtain the desired reddish or opaque color of "dolo", local manufacturers tend to use red varieties that contain, even after malting, a high content of colored polyphenols such as 3-DAs. In addition, the fact that varieties good for "dolo" had more flavan-4-ols and 3-DAs than varieties poor for "dolo" may be an advantage for grain-molding resistance (*9*−*11*) during the traditional malting of sorghum. The high antioxidant activities found in varieties used for "dolo" (49−55 μmol of TAE/g) indicate that "dolo" varieties contain antioxidant activity levels higher than red wines (9−12 μmol of TAE/g) (*17*). From our data, it can be inferred that the whole grains of (for example) the sorghum varieties V17, V19, V30, V37, and V50 have antioxidant activities (66−80 μmol of TAE/g) higher than most other plant foods (*17*), e.g., blackberry (20 μmol of TAE/g). Furthermore, it is important to stress that a high antioxidant activity is expected to be found in sorghum brans (*18*), which are commercially available in West Africa and are used as animal feed (*40*).

Germination did not significantly affect the content of PAs in groups of varieties both good and poor for tô. The group of varieties good for porridge had on average low contents of total phenolic compounds, PAs, DAs, and flavan-4-ols. Apparently, varieties with moderate or high PAs and colored flavonoid levels are already avoided for porridge preparation. The high PA contents in varieties may impair the nutritional quality of their final products with respect to the possible inhibitory effect of PAs on hydrolytic enzymes. Therefore, varieties good for porridge, which do not contain PAs after germination (V41 and V44), may be the best for the preparation of infant porridges

Table 3. Comparison of the Average Content of Phenolics and Antioxidant Activities in Groups of Ungerminated (g–) and Germinated (g+) Sorghum Varieties of Known Properties

		total phenolics (%)[a]		PAs (%)[b]		3-DAs (%)[c]		flavan-4-ols (%)[d]		antioxidant activities[e]	
	group properties	g–	g+	g–	g+	g–	g+	g–	g+	g–	g+
food	good for "tô" ($n = 20$)	0.78	0.81	0.15	0.14	0.02	0.02	0.03	0.02	40	39
	poor for "tô" ($n = 7$)	0.69	0.68	0.05	0.04	<0.01	<0.01	0.02	<0.01	34	33
	good for "dolo" ($n = 9$)	1.40	1.44	0.79	0.61	0.09	0.06	0.24	0.20	55	49
	poor for "dolo" ($n = 2$)	0.96	0.99	0.41	0.25	0.04	0.05	0.19	0.06	39	44
	good for couscous ($n = 7$)	0.85	0.84	0.19	0.11	<0.01	<0.01	0.03	<0.01	43	40
	good for porridge ($n = 5$)	0.66	0.81	0.04	0.04	<0.01	<0.01	0.05	0.04	35	41
	mean value ($n = 50$)	0.88	0.92	0.26	0.21	0.03	0.02	0.07	0.05	42	41
plant/grain	red grains ($n = 9$)	1.46	1.46	0.80	0.62	0.11	0.07	0.26	0.21	56	50
	white grains ($n = 41$)	0.76	0.80	0.13	0.11	<0.01	<0.01	0.03	<0.01	39	38
	grains without testa ($n = 37$)	0.75	0.78	0.07	0.07	<0.01	<0.01	0.03	0.02	38	37
	grains with testa ($n = 13$)	1.27	1.31	0.81	0.61	0.10	0.08	0.32	0.27	53	51
	glumes chestnut ($n = 3$)	1.39	1.26	0.71	0.40	0.05	0.04	0.26	0.22	55	37
	glumes red ($n = 9$)	1.00	1.12	0.35	0.28	0.05	0.03	0.07	0.07	46	47
	glumes black ($n = 13$)	0.97	1.01	0.36	0.30	0.05	0.05	0.13	0.08	44	46
	glumes pale ($n = 25$)	0.74	0.78	0.16	0.13	<0.01	<0.01	<0.01	<0.01	37	37
	red plants ($n = 30$)	0.96	1.03	0.41	0.32	0.04	0.04	0.11	0.08	43	44
	tan plants ($n = 20$)	0.77	0.75	0.05	0.04	<0.01	<0.01	<0.01	<0.01	41	35
	SE ($n = 2$)	0.05	0.05	0.01	0.01	0.002	0.002	0.003	0.003	2	2

[a] Gallic acid equivalents (%, w/w, dry matter basis). [b] PAs = proanthocyanidins (%, w/w of apple procyanidins DP ≈ 7.4, dry matter basis). [c] 3-DAs = 3-deoxyanthocyanidins (%, w/w, apigeninidin, dry matter basis). [d] Anthocyanidin equivalents derived from flavan-4-ol (%, w/w, cyanidin, dry matter basis). [e] Trolox antioxidant equivalents (μmol/g of flour, dry matter basis) using the ABTS (2,2′-azino-bis(3-ethylbenzthiazoline-6-sulfonic acid) assay. g– = ungerminated sorghum, and g+ = germinated sorghum.

from a nutritional standpoint. For infant porridges, the low PA content is presumably more desired than high antioxidant activity. Varieties good for couscous did not contain 3-DAs before and after germination, in line with the preparation of this dish for which a colorless final product is often preferred.

ABBREVIATIONS USED

ABTS, 2,2′-azino-bis(3-ethylbenzthiazoline-6-sulfonic acid); 3-DA, 3-deoxyanthocyanidin; DP, degree of polymerization; PA, proanthocyanidin; PMA, phosphomolybdenum assay; TAE, Trolox antioxidant equivalent; VCAE, vitamin C antioxidant equivalent.

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