JOURNAL OF AGRICULTURAL AND FOOD CHEMISTRY

Screening Methods To Measure Antioxidant Activity of Sorghum (*Sorghum bicolor*) and Sorghum Products

JOSEPH M. AWIKA,*,† LLOYD W. ROONEY,† XIANLI WU,‡ RONALD L. PRIOR,‡ AND LUIS CISNEROS-ZEVALLOS§

Cereal Quality Lab, Soil & Crop Sciences Department, and Horticulture Department, Texas A&M University, College Station, Texas 77843, and Arkansas Children's Nutrition Center, ARS, USDA, Little Rock, Arkansas 72202

Specialty sorghums, their brans, and baked and extruded products were analyzed for antioxidant activity using three methods: oxygen radical absorbance capacity (ORAC), 2,2′-azinobis (3-ethylbenzothiazoline-6-sulfonic acid) (ABTS), and 2,2-diphenyl-1-picrylhydrazyl (DPPH). All sorghum samples were also analyzed for phenolic contents. Both ABTS and DPPH correlated highly with ORAC ($R^2 = 0.99$ and 0.97, respectively, $n = 18$). Phenol contents of the sorghums correlated highly with their antioxidant activity measured by the three methods ($R^2 \geq 0.96$). The ABTS and DPPH methods, which are more cost effective and simpler, were demonstrated to have similar predictive power as ORAC on sorghum antioxidant activity. There is a need to standardize these methods to allow for data comparisons across laboratories.

KEYWORDS: Sorghum; antioxidant; phenols; ORAC; ABTS; DPPH

INTRODUCTION

Sorghum is a major cereal food crop used in many parts of the world. Specialty sorghum hybrids contain high levels of diverse phenolic compounds that may provide health benefits. High levels of polyflavanols (procyanidins) (*1*−*3*), anthocyanins (*4*, *5*), phenolic acids (*6*, *7*), and other antioxidant compounds have been reported in sorghums. Sorghums could thus be an important source of ingredients for use in functional foods and other applications. However, data are hard to find on antioxidant activities of the specialty sorghums and/or their products. Such information is critical if sorghum is to become a competitive source of the phytonutrients. A quick, reliable, and cost effective method is necessary for screening sorghum samples for antioxidant activity.

Numerous methods are used to evaluate antioxidant activities of natural compounds in foods or biological systems with varying results. Two free radicals that are commonly used to assess antioxidant activity in vitro are 2,2′-azinobis (3-ethylbenzothiazoline-6-sulfonic acid) ($ABTS^{\bullet+}$) and 2,2-diphenyl-1-picrylhydrazyl ($DPPH^{\bullet}$). However, both of these radicals are foreign to biological systems. The ABTS assay measures the relative ability of antioxidant to scavenge the $ABTS^{\bullet+}$ generated in aqueous phase, as compared with a Trolox (water soluble vitamin E analogue) standard. The $ABTS^{\bullet+}$ is generated by reacting a strong oxidizing agent (e.g., potassium permanganate or potassium persulfate) with the ABTS salt. The reduction of the blue-green $ABTS^{\bullet+}$ radical by hydrogen-donating antioxidant is measured by the suppression of its characteristic long wave absorption spectrum (*8*). The method is usually expressed as Trolox equivalent antioxidant capacity (TEAC). The method is rapid and can be used over a wide range of pH values (*9*, *10*), in both aqueous and organic solvent systems. It also has good repeatability and is simple to perform; hence, it is widely reported. The method, however, has not been correlated with biological effects; hence, its actual relevance to in vivo antioxidant efficacy is unknown.

The $DPPH^{\bullet}$ is a stable free radical with an absorption band at 515 nm. It loses this absorption when reduced by an antioxidant or a free radical species. The $DPPH^{\bullet}$ method is widely used to determine antiradical/antioxidant activity of purified phenolic compounds as well as natural plant extracts (*11*−*16*). Bondet et al. (*13*) found that most phenolic antioxidants react slowly with $DPPH^{\bullet}$, reaching a steady state in 1−6 h or longer. This suggests that antioxidant activity using $DPPH^{\bullet}$ should be evaluated over time. The method also has good repeatability and is used frequently. However, like ABTS, it has limited, if any, relevance to biological systems. Also, color interference of $DPPH^{\bullet}$ with samples that contain anthocyanins leads to underestimation of antioxidant activity (*17*).

The oxygen radical absorbance capacity (ORAC) method developed by Cao et al. (*18*) measures the ability of antioxidants to protect protein from damage by free radicals. In this assay, different generators are used to produce different radicals. Usually, three radicals are generated as follows: peroxyl radical ($ROO^{\bullet}$), hydroxyl radical ($OH^{\bullet}$), and Cu^{2+}, a transition metal. This is important since measured antioxidant activity of biologi-

* To whom correspondence should be addressed. Tel: 979-845-2925. Fax: 979-845-0456. E-mail: jawika@tamu.edu.
† Soil & Crop Sciences Department, Texas A&M University, College Station.
‡ USDA.
§ Horticulture Department, Texas A&M University.

10.1021/jf034790i CCC: $25.00
Published on Web 10/01/2003

cal samples depends on which free radical or oxidant is used in the assay (*19*). The method, however, recently adopted the ROO• as standard radical since it is the most common in biological systems (*20*). The target protein (until recently) was β-phycoerythrin (β-PE), whose loss of fluorescence was an indication of the extent of damage from its reaction with peroxyl radical. However, results with this method had poor repeatability, which was attributed to the protein interacting with sample polyphenols, among other factors. Ou et al. (*21*) adopted a new fluorescent substance (fluorescein) to replace β-PE as a probe. Data on the modified method normally give values that are 2−3 times higher than with the β-PE. A major advantage of ORAC is that the method is automated and largely standardized; hence, values can be easily compared across laboratories. Also, the ORAC method is reported to mimic antioxidant activity of phenols in biological systems better than other methods since it uses biologically relevant free radicals and integrates both time and degree of activity of antioxidants (*19*, *20*, *22*). However, the method often requires the use of expensive equipment.

The objective of this study was to establish a suitable rapid method for estimating antioxidant activity of sorghum and sorghum products.

MATERIALS AND METHODS

Materials. Sorghum samples included brown sorghums: ATx623 × SC103 grown in College Station, TX, in 1997 (SC103); Hi tannin sorghum grown in College Station, TX, in 2001 (HT01); Sumac grown in Vega, TX, in 1999 (SU99); Sumac from Coffee Seed, Hereford, TX, in 1999 (SH99); and Sumac grown in 2002 (SU02). Black Tx430 sorghums grown in College Station in 1998−2002 (BK98−BK02); a red sorghum, Tx2911, grown in College Station, TX, in 2000 (RD00); and a white sorghum ATx631 × Tx436 (WS01) were also used. All samples were decorticated using a PRL dehuller (Nutama Machine Co., Saskatoon, Canada) to obtain bran. Bran yields were 12% for brown, 15% for black and red, and 12% for white sorghums. Samples were stored at −20 °C until analyzed.

Processed sorghum products included cookies (50% bran), bread (30% bran), and whole sorghum extrudates. Breads were made as described by Gordon (*23*), and cookies were made as described by Mitre-Dieste et al. (*24*).

Grains were extruded whole through a friction type Maddox single screw extruder, model MX-3001 (Maddox Metal Works, Inc., Dallas, TX). Screw speed was 300 rpm, die diameter was 6.125 in, and sample moisture was 11.5−12.5% (nontempered).

Disodium fluorescein and Trolox were obtained from Aldrich (Milwaukee, WI). ABTS was obtained from Sigma (St. Louis, MO), 2,2′-azobis (2-amidinopropane)dihydrochloride (AAPH) was from Wako Chemicals (Richmond, VA), and DPPH was acquired from Acros Organics (Morris Plains, NJ).

Sample Extraction. All samples were ground through a cyclotec mill (UDY Corp., Fort Collins, CO) (1 mm mesh) prior to extraction. Aqueous acetone (70%) was used as a solvent. The extraction procedure for ABTS and DPPH assays involved addition of 10 mL of solvent to a 0.5 g sample in 50 mL centrifuge tubes and shaking of the samples for 2 h at low speed in an Eberbach shaker (Eberbach Corp., MI). Samples were then stored at −20 °C in the dark overnight to allow for maximum diffusion of phenolics from the cellular matrix. Samples were then equilibrated to room temperature and centrifuged at 2790*g* for 10 min in a Sorvall SS-34 rotor and centrifuge (Du Pont Instruments, Wilmington, DE) and decanted. Each sample residue was rinsed with two additional 10 mL volumes of solvent with shaking for 5 min, centrifuging at 2790*g* for 10 min as above, and decanting in each case. The three aliquots were mixed and stored at −20 °C in the dark until analyzed.

For ORAC analysis, samples were extracted in two stages to obtain lipophilic and hydrophilic antioxidant constituents. Hexane:dichloromethane (1:1) (HD) was initially used for the lipophilic constituents, followed by acetone:water (70:30) for the hydrophilic constituents. Twenty milliliters of the HD solvent was added to a 0.5 g sample and extracted in a Dionex ASE 200 accelerated solvent extraction system (Dionex, Sunnyvale, CA) for 15 min. The residues were reextracted with the acetone:water solvent. The HD extracts were evaporated at room temperature (23 °C) under vacuum to dryness and then redissolved in 25 mL of acetone:water (70:30).

Table 1. Antioxidant Activities (μmol TE/g Sample, DM Basis) of Sorghum and Sorghum Products Measured by Three Methods

sample	ORAC-L[a]	ORAC-H[b]	ORAC[c]	ABTS[d]	DPPH[e]	phenols[f]
white grain	1	21	22	6	6	1
white extrudate	2	24	26	7	6	1
white bran			64	28	21	5
red grain	4	136	140	53	28	5
red bran	7	704	710	230	71	20
black (Bk) 2001 grain	14	205	219	57	41	6
Bk 2001 extrudate	4	90	94	37	32	5
Bk 2001 bran	38	970	1008	250	184	26
Bk 1999 bran bread	5	86	92	45	28	5
Bk 1999 bran cookie	18	153	170	90	51	9
Hi tannin grain	14	440	454	108	118	13
Hi tannin extrudate	3	282	286	90	74	6
Hi tannin bran			2400	512	495	55
sumac (SU99) grain	15	853	868	226	202	23
SU99 bran	25	3099	3124	768	716	66
SU99 30% bran bread	4	251	254	108	78	8
SU99 50% bran cookie	9	315	324	130	106	14
CV%	6.3	6.8	6.8	3.5	5.3	6.0

[a] Lipophilic ORAC (from HD extracts). [b] Hydrophilic ORAC (from aqueous acetone extracts). [c] Total ORAC (hydrophilic + lipophilic). [d] ABTS activity was measured after 30 min of reaction in pH 7.4 phosphate buffer, saline. [e] DPPH activity was measured after 8 h of reaction in methanol. [f] mg GAE/g (Folin–Ciocalteu method). Fluorescein was used as a probe for the ORAC assay.

Analytical Procedures. Phenol contents of aqueous acetone extracts were quantified using the modified Folin−Ciocalteu method of Kaluza et al. (*25*). The DPPH method of Brand-Williams et al. (*11*) was modified for this assay. To determine reaction kinetics for sorghum extracts, reactions were initially monitored over a 24 h period, with readings recorded every 30 min for the first 2 h, and every 2 h for the next 10 h, and every 6 h thereafter. Most of the samples tested showed residual reactivity even after 24 h. However, after 8 h, change in activity was very minimal for most of the samples. Hence, 8 h was used as the standard reaction time. The DPPH• was dissolved in methanol and kept at −20 °C in the dark prior to use. Sample extracts (150 μL) were reacted with 2850 μL of the DPPH solution for 8 h with shaking. Trolox was used as a standard.

For $ABTS^{\bullet+}$ generation from ABTS salt, 3 mM of $K_2S_2O_8$ was reacted with 8 mM ABTS salt in distilled, deionized water for 16 h at room temperature in the dark. The $ABTS^{\bullet+}$ solution was then diluted with pH 7.4 phosphate buffer solution containing 150 mM NaCl (PBS) to obtain an initial absorbance of 1.5 at 730 nm. Fresh $ABTS^{\bullet+}$ solution was prepared for each analysis. Reaction kinetics was determined over a 2 h period with readings every 15 min. Reactions were complete in 30 min. Samples and standards (100 μm) were reacted with the $ABTS^{\bullet+}$ solution (2900 μm) for 30 min. Trolox was used as a standard.

The ORAC assays were performed as detailed by Ou et al. (*21*) using an automated Fluostar Optima plate reader (BMG Technologies, Offenburg, Germany). Analyses were conducted in pH 7.0 phosphate buffer at 37 °C. Peroxyl radical was generated using AAPH, and fluorescein was used as the substrate. Fluorescence conditions were as follows: excitation, 485 nm; emission, 520 nm. All analyses except ORAC were conducted in triplicates. ORAC analyses were conducted in duplicates.

RESULTS AND DISCUSSION

Comparison of ORAC with ABTS and DPPH Methods. Because the lipophilic ORAC values were relatively low (**Table 1**), total ORAC values (lipophilic + hydrophilic) are used in

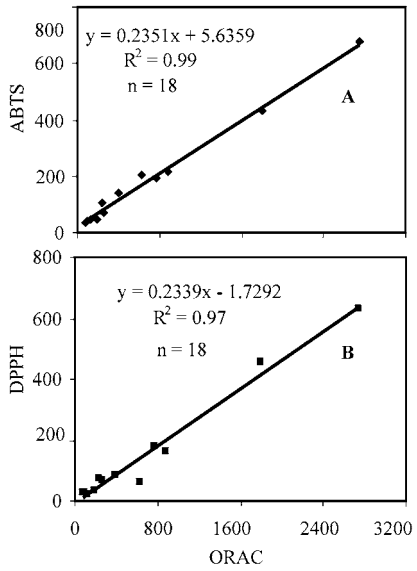

Figure 1. Correlations between ORAC and ABTS (**A**) and ORAC and DPPH (**B**) values for sorghum and sorghum products. Fluorescein was used as a probe for the ORAC assay. Results are expressed as μmol Trolox equivalents per gram, dry weight basis.

the comparative discussion. The ORAC values were generally 3−4 times higher than the ABTS or DPPH values (**Table 1**). However, because individual antioxidant molecules are more efficient at quenching certain radicals than others (*26*), the relative rank in activity of different samples across methods is more relevant than absolute values for comparing activities. Both ABTS and DPPH correlated highly with the ORAC method (R^2 = 0.99 and R^2 = 0.97, respectively, n = 18) (**Figure 1**), demonstrating that the sorghums had comparable activities in all three systems. Proteggente et al. (*27*) found good correlations among ORAC, ferric-reducing antioxidant power (FRAP), and ABTS methods for antioxidant activity among different fruits and vegetables.

Recently, Ou et al. (*22*) conducted a comprehensive study comparing the ORAC to the FRAP of different vegetables. They observed no correlation between the two methods among most of the vegetables that they tested and concluded that ORAC was a better indicator of antioxidant activity of vegetables than FRAP, based on the reaction mechanisms involved. However, as the authors observed, the low pH (3.6) used for the FRAP assay vs the ORAC assay (pH 7.4) as well as color interference with vegetable extracts in the FRAP assay would seriously compromise antioxidant activities measured among different samples. Additionally, the authors used β-PE as the probe for the ORAC assay. This particular protein was reported to give poor reliability due to significant lot variability, lack of photostability, and interaction with polyphenols due to nonspecific protein binding (*21*). In this study, fluorescein was used as a probe for the ORAC assay as recommended by Ou et al. (*21*), thus eliminating some of the problems reported for β-PE. Also, the ABTS and DPPH analyses were conducted at pH levels comparable to that for the ORAC assay, hence reducing the pH effect. In addition, ABTS absorbance was measured at 730 nm, a wavelength that was far removed from the absorbance bands of sorghum extracts, thus eliminating color interference in this assay.

The more common, low cost ABTS and DPPH methods are good predictors of ORAC activity of sorghums. This is important given that the validity of ABTS and DPPH methods has been questioned since they use oxidants (free radicals) that

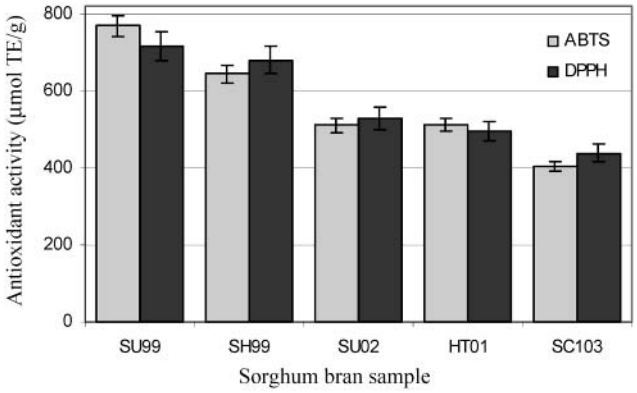

Figure 2. Comparison of ABTS and DPPH values of brown sorghum brans. DPPH values were determined after 8 h of reaction in methanol. ABTS values were determined after 30 min of reaction in pH 7.4 phosphate buffer, saline. Error bars represent standard deviations. TE = trolox equivalents.

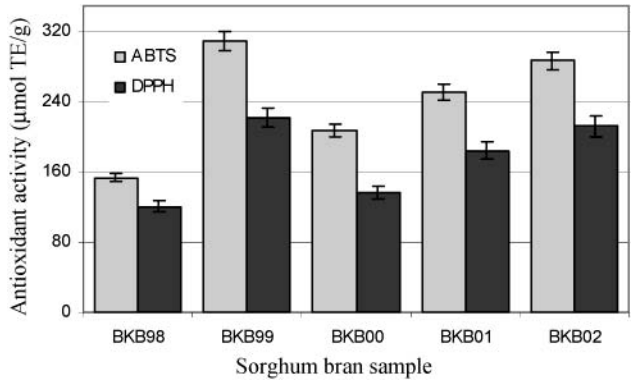

Figure 3. Comparison of ABTS and DPPH values of brans from black sorghums. DPPH values were determined after 8 h of reaction in methanol. ABTS values were determined after 30 min of reaction in pH 7.4 phosphate buffer, saline. Error bars represent standard deviations. TE = trolox equivalents.

are not necessarily prooxidants and are of no known biological value, unlike ORAC that uses oxidants that are actually prooxidants (e.g., ROO$^\bullet$, OH$^\bullet$) and are of pathological significance (*20*).

Comparison of ABTS and DPPH Antioxidant Methods on Sorghum Extracts. Having established correlations of the ABTS and DPPH methods with the ORAC, the ABTS and DPPH methods were further compared with additional samples to establish their consistency across different sorghum types. **Figures 2** and **3** compare ABTS and DPPH activities of brown and black sorghum brans, respectively. Among the brown sorghums (**Figure 2**), minimal differences were observed between the ABTS and the DPPH values. The methods were equally good at measuring antioxidant activity of brown sorghums.

Among the black sorghum brans, however, all ABTS values were significantly higher than the DPPH values (**Figure 3**). Anthocyanins are the major extractable phenols from black sorghums (*4*, *5*); hence, they contribute a major portion of the measured antioxidant activity of these sorghums. Because the sorghum anthocyanins absorbed maximally at 475−485 nm, color interference with the DPPH chromogen, which has an absorption maxima at 515 nm, likely resulted in the relatively lower measured activity. Arnao (*17*) reported similar color interference of DPPH with different plant materials. The ABTS chromogen, on the other hand, has several absorption bands

Table 2. Summary of Properties of the Different Antioxidant Methods

	merits	demerits
ORAC	uses biologically relevant free radicals	normally requires use of expensive equipment
	standardized: allows for data comparison across laboratories	data variability can be large across equipment
	integrates both degree and time of antioxidant reaction	pH sensitive
ABTS	inexpensive and easy to use	extra step to generate free radical from ABTS salt necessary
	stable to pH hence can be used to study pH effect on activity	generated free radical not stable for long periods of time
	fast reaction: total antioxidant value can be estimated	not standardized, hence hard to compare values across laboratories
	high correlation with ORAC	
DPPH	inexpensive and easy to use	slow reaction, hence difficult to obtain absolute antioxidant values
	stable free radical that is ready to use	color interference may lead to underestimation of activity
	high correlation with ORAC	sensitive to pH
		not standardized

between 380 and 850 nm (*11*, *17*); hence, an absorption band that is far removed from that of the sorghum samples (730 nm) was selected to eliminate color interference. Thus, the ABTS values for the black sorghums were more reliable measures of their antioxidant activities than the DPPH values.

For comparing different sorghum samples with different phenolic compositions, ABTS is a better choice than DPPH. The ABTS method has the extra flexibility in that it can be used at different pH levels (unlike DPPH, which is sensitive to acidic pH) and thus is useful when studying the effect of pH on antioxidant activity of various compounds (*10*, *28*). It is also useful for measuring antioxidant activity of samples extracted in acidic solvents. Additionally, ABTS is soluble in aqueous and organic solvents and is thus useful in assessing antioxidant activity of samples in different media and is currently most commonly used in simulated serum ionic potential solution (pH 7.4 phosphate buffer containing 150 mM NaCl) (PBS). Another advantage of $ABTS^{+\bullet}$ method was that samples reacted rapidly with ABTS in the aqueous buffer solution (PBS) reaching a steady state within 30 min. The $DPPH^{\bullet}$ reacted very slowly with the samples, approaching, but not reaching, steady state after 8 h. This slow reaction was also observed when ABTS was reacted with samples in alcohol (data not shown), implying that the reactivity of the antioxidants in sorghums with these free radicals is somehow slowed in alcoholic media. Brand-Williams et al. (*11*) reported similar slow reaction of most antioxidants that they tested with the DPPH.

Generally, the two methods (ABTS and DPPH) correlated strongly with each other (**Figure 4**). Leong and Shui (*29*) found similarly high correlation ($R^2 = 0.90$) between ABTS values and DPPH values for crude extracts from different fruits. Hence, both methods could be equally useful for assessing antioxidant activities of natural extracts at physiological pH and where color interference is not significant.

The relative merits and demerits of the three antioxidant methods are summarized in **Table 2**. The ABTS method was more suitable for sorghums than the DPPH or ORAC methods. The cost of ORAC was the major disadvantage, but because it remains the only standardized in vitro method that uses biologically relevant free radicals, it will continue to be a useful tool for antioxidant activity assay and validation.

Correlations between Phenol Content and Antioxidant Activity of Sorghum. Phenol contents of the sorghums (Folin−Ciocalteu method) correlated strongly with their antioxidant activity measured by all three methods. The correlation coefficients were as follows: phenol vs ORAC, $R^2 = 0.96$; phenol vs ABTS, $R^2 = 0.97$ (**Figure 5**); phenol vs DPPH, $R^2 = 0.96$. This confirms that the phenols are largely responsible for the antioxidant activity of the sorghums. Several authors have reported similar correlations between phenols and antioxidant activity measured by various methods (*27*, *30*−*32*). The phenol contents of sorghum and sorghum products can be good predictors of their antioxidant activities.

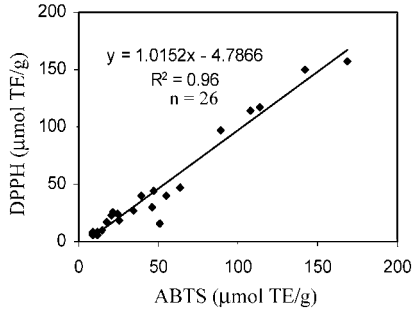

Figure 4. Correlation between ABTS and DPPH for sorghum and sorghum products. All values are expressed on a DM basis. DPPH values were determined after 8 h of reaction in methanol. ABTS values were determined after 30 min of reaction in pH 7.4 phosphate buffer, saline. TE = trolox equivalents.

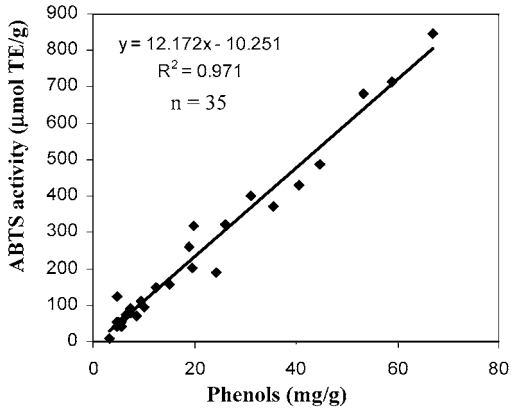

Figure 5. Correlation between ABTS activity and level of phenols in sorghum and sorghum products. TE = trolox equivalents.

Antioxidant Activity of Sorghums and Their Products. There were significant differences in the phenol levels and antioxidant activities of the sorghums and their products. The brown tannin-containing sorghums, Sumac and Hi tannin, and their products had consistently higher antioxidant activities than the other samples (**Table 1**). Tannins are known to have higher

antioxidant activity as compared to other phenols (*33*, *34*) and are likely responsible for the high activity in the brown sorghums. The black sorghums, which have no tannins but have high levels of anthocyanins (*4*, *5*), also had a relatively high antioxidant activity (**Table 1**, **Figure 3**). Anthocyanins are reported to contribute significant antioxidant activity in fruits (*28*). The white sorghum with no tannins or anthocyanins and very low phenol levels had the lowest antioxidant activity. In general, the pigmented sorghums had ORAC values (mg TE/g) of 140−870 in the grains and 710−3100 in the brans; blueberries, which are considered an excellent source of antioxidants, have ORAC values of 90−870 mg TE/g, estimated on DM basis (*35*).

The sorghum brans had 3−5 times the antioxidant activity in the grains (**Table 1**). Hence, there is a potential to easily obtain high antioxidant sorghum fractions that may be used in food and other applications. When processed into foods, most of the antioxidant activities of the raw sorghums were retained, 57−78% for baked and 70−100% for the extrudated products (**Table 1**). This implies that the sorghums can be processed into foods that are functional. We are conducting more analysis in this area to determine how different processing conditions affect the phytochemical components in the sorghum fractions and their antioxidant activity.

In summary, the ORAC method is generally regarded highly due to its use of biologically relevant free radicals and also integration of both degree and time of inhibition. However, it did not offer any advantage in terms of predicting the overall antioxidant activity of sorghums and sorghum products, when compared with the more common ABTS and DPPH methods. The ABTS method gave the most rapid and consistent results among diverse sorghum varieties. There is a need to establish standard of analysis so that values obtained in different laboratories can be easily compared. Meanwhile, the standardized ORAC procedure will remain an important method for comparing in vitro antioxidant activities across laboratories.

However, because the overall goal of antioxidant assay is to predict biological effectiveness of antioxidants, a method can only be truly appropriate if it correlates with the in vivo efficacy of the antioxidants. Because a complex mix of factors influence access and response of antioxidants to different reactive species (free radicals) in vivo, it is difficult to predict "actual overall antioxidant value" of samples based on any single in vitro assay. Additionally, antioxidant activity alone does not explain the potential effects of a compound in vivo since other properties such as modification of enzyme activity or cell signaling pathways are possible. It is hoped that as more epidemiological and specific biological data on antioxidants become available, a better insight will be gained on the relevance of the in vitro data.

LITERATURE CITED

(1) Hahn, D. H.; Rooney, L. W.; Earp, C. F. Tannins and phenols of sorghum. *Cereal Foods World* **1984**, *29*, 776−779.

(2) Jambunathan, R.; Butler, L. G.; Bandyopadhyay, R.; Mughogho, L. K. Polyphenol concentration in grain, leaf, and callus tissue of mold-susceptible and mold-resistant sorghum cultivars. *J. Agric. Food Chem.* **1986**, *34*, 425−430.

(3) Gu, L.; Kelm, M.; Hammerstone, J. F.; Beecher, G.; Cunnigham, D.; Vannozzi, S.; Prior, L. 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.* **2002**, *50*, 4852−4860.

(4) Awika, J. M. Ph.D. dissertation, Texas A&M University, College Station, TX, 2003.

(5) Gous, F. Ph.D. dissertation, Texas A&M University, College Station, TX, 1989.

(6) Hahn, D. H.; Faubion, J. M.; Rooney, L. W. Sorghum phenolic acids, their high performance liquid chromatography separation and their relation to fungal resistance. *Cereal Chem.* **1983**, *60*, 255−259.

(7) Waniska, R. D.; Poe, J. H.; Bandyopathyay, R. Effects of growth conditions on grain molding and phenols in sorghum caryopsis. *J. Cereal Sci.* **1989**, *10*, 217−225.

(8) Miller, N. J.; Rice-Evans, C. A. Factors influencing the antioxidant activity determined by the $ABTS^{+}$ radical cation assay. *Free Radical Res.* **1997**, *26*, 195−199.

(9) Arnao, M. B.; Cano, A.; Acosta, M. Methods to measure the antioxidant activity in plant material. A comparative discussion. *Free Radical Res.* **1999**, *31*, s89−s96.

(10) Lemanska, K.; Szymusiak, H.; Tyrakowska, B.; Zielinski, R.; Soffer, A. E. M. F.; Rietjiens, I. M. C. M. The influence of pH on the antioxidant properties and the mechanism of antioxidant action of hydroxyflavones. *Free Radical Biol. Med.* **2001**, *31*, 869−881.

(11) 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.

(12) Sripriya, G.; Chandrasekharan, K.; Murty, V. S.; Chandra, T. S. ESR spectroscopic studies on free radical quenching action of finger millet (*Eleusine coracana*). *Food Chem.* **1996**, *47*, 537−540.

(13) Bondet, V.; Brand-Williams, W.; Berset, C. Kinetics and mechanism of antioxidant activity using the DPPH•free radical method. *Lebensm. Wiss. Technol.* **1997**, *30*, 609−615.

(14) Mahinda, W.; Shahidi, F. Scavenging of reactive-oxygen species and DPPH free radicals by extracts of borage and evening primrose meals. *Food Chem.* **2000**, *70*, 17−26.

(15) Peyrat-Maillard, M. N.; Bonnely, S.; Berset, C. Determination of the antioxidant activity of phenolic compounds by coulometric detection. *Talanta* **2000**, *51*, 709−716.

(16) Fukumoto, L. R.; Mazza, G. Assessing antioxidant and prooxidant activities of phenolic compounds. *J. Agric. Food Chem.* **2000**, *48*, 3597−3604.

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

(18) Cao, G.; Alessio, H. M.; Culter, R. G. Oxygen-radical absorbance capacity assay for antioxidants. *Free Radical Biol. Med.* **1993**, *14*, 303−311.

(19) Cao, G.; Sofic, E.; Prior, R. L. Antioxidant capacity of tea and common vegetables. *J. Agric. Food Chem.* **1996**, *44*, 3426−3431.

(20) Cao, G.; Prior, R. L. Measurement of total antioxidant capacity in nutritional and clinical studies. In *Handbook of Antioxidants*, 2nd ed.; Cadenas, E., Packer, L., Eds.; Marcel Dekker: New York, 2001; pp 47−55.

(21) Ou, B.; Hampsch-Woodill, M.; Prior, R. L. Development and validation of an improved oxygen radical absorbance capacity assay using Fluorescein as the fluorescent probe. *J. Agric. Food Chem.* **2001**, *49*, 4916−4926.

(22) Ou, B.; Huang, D.; Hampsch-Woodill, M.; Flanagan, J.; Deemer, E. K. Analysis of antioxidant activities of common vegetables employing oxygen radical absorbance capacity (ORAC) and ferric reducing antioxidant power (FRAP) assays: a comparative study. *J. Agric. Food Chem.* **2002**, *50*, 3122−3128.

(23) Gordon, L. A. M.S. Thesis, Texas A&M University, College Station, TX, 2001.

(24) Mitre-Dieste, C. M.; Gordon, L. A.; Awika, J.; Suhendro, E. L.; Rooney, L. W. Cookies made with sorghum brans high in phenols and catechins. *AACC Annual Meeting Abstracts*, URL http://www.aaccnet.org/meetings/2000/abstracts/a00ma203.htm.

(25) Kaluza, W. Z.; McGrath, R. M.; Roberts, T. C.; Schroder, H. H. Separation of phenolics of *Sorghum bicolor* (L.) Moench grain. *J. Agric. Food Chem*. **1980**, *28*, 1191−1196.

(26) Lotito, S. B.; Actis-Goretta, L.; Renart, M. L.; Caligiuri, M.; Rein, D.; Schmitz, H. H.; Steinberg, F. M.; Keen, C. L.; Fraga, C. G. Influence of oligomer chain length on antioxidant activity of procyanidins. *Biochem. Biophys. Res. Commun*. **2000**, *276*, 945−951.

(27) Proteggente, A. R.; Pannala, A. S.; Paganga, G.; Van Buren, L.; Wagner, E.; Wiseman, S.; De Put, F.; Dacombe, C.; Rice-Evans, C. The antioxidant activity of regularly consumed fruit and vegetable reflects their phenolic and vitamin C composition. *Free Radical Res.* **2002**, *36*, 217−233.

(28) Tyrakowska, B.; Soffers, A. E. M. F.; Szymusiak, H.; Boeren, S.; Boersma, M. G.; Lemanska, K.; Vervoort, J.; Rietjems, I. M. C. M. TEAC antioxidant activity of 4-hydroxybenzoates. *Free Radical Biol. Med*. **1999**, *27*, 1427−1436.

(29) Leong, L. P.; Shui, G. An investigation of antioxidant capacity of fruits in Singapore markets. *Food Chem*. **2001**, *76*, 69−75.

(30) Frankel, E. N.; Waterhouse, A. L.; Teissedre, P. L. Principal phenolic phytochemicals in California wines and their antioxidant activity in inhibiting oxidation of human low-density lipoproteins. *J. Agric. Food Chem*. **1995**, *28*, 890−894.

(31) Simonetti, P.; Pietta, P.; Testolin, G. Polyphenol content and total antioxidant potential of selected Italian wines. *J. Agric. Food Chem*. **1997**, *45*, 1152−1155.

(32) De Beer, D.; Joubert, E.; Gelderblom, W. C. A.; Manley, M. Antioxidant activity of South African red and white cultivar wines: free radical scavenging. *J. Agric. Food Chem.* **2003**, *51*, 902−909.

(33) Bors, W.; Michel, C.; Stettmaier, K. Electron paramagnetic resonance studies of radical species of proanthocyanidins and gallate esters. *Arch. Biochem. Biophys.* **2000**, *374*, 347−355.

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

(35) Moyer, A. R.; Humer, K. E.; Finn, C. E.; Frei, B.; Wrolstad, R. E. Anthocyanins, phenolics, and antioxidant capacity in diverse small fruits: Vaccinium, Rubus, and Ribers. *J. Agric. Food Chem.* **2002**, *50*, 519−525.

Received for review July 16, 2003. Revised manuscript received August 29, 2003. Accepted September 2, 2003.

JF034790I