Research Article

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High-throughput micro plate assays for screening flavonoid content and DPPH-scavenging activity in sorghum bran and flour‡

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Abstract

BACKGROUND: Sorghum possesses phenolic compounds that are health-promoting constituents of the grain. There are approximately 40 000 sorghum accessions, many of which have not been evaluated for the grain's health-promoting potential. Conventional methods for measuring total phenolic content, flavonoid content and 2,2-diphenyl-1-picrylhydrazyl (DPPH)-scavenging capacity are time-consuming and labour-intensive, resulting in low overall throughput. The objective of this study was to develop a high-throughput screening assay for large sorghum sample sets to determine flavonoid and phenolic content and to modify existing DPPH and total phenolic assays.

RESULTS: The 96-well assays exhibited a correlation of >0.9 with the conventional assays. The 96-well assays allowed for up to 64 samples to be run per day compared with 20–24 samples (depending on the test) for the conventional methods. The 96-well assays had excellent accuracy (97.65–106.16% recovery), precision (1.06–8.28% coefficient of variation (CV)) and reproducibility (1.32–2.13% CV inter-day and 1.36–2.09% CV intra-day).

CONCLUSION: The high-throughput 96-well plate method proved to be as robust and reproducible as the conventional method for determining total phenolic content, flavonoid content and DPPH-scavenging capacity in either sorghum bran or flour. Published 2012 by John Wiley & Sons, Ltd.

Keywords: 96-well plate; sorghum; phenolic compound; flavonoid; DPPH; antioxidant

INTRODUCTION

Sorghum (*Sorghum bicolor* (L.) Moench) is a drought-resistant and heat-tolerant crop that serves as a food staple in many arid regions of the world. Sorghum is void of gluten and may be used in many food products for the coeliac market. Another beneficial trait of sorghum grain is that it contains high levels of diverse and unique phenolic compounds (phenolic acids, flavonoids, anthocyanins, tannins) that may be a good source of natural antioxidants.[1–3] Thus sorghum flour, bran and extracts have potential applications as health-promoting ingredients in functional foods. Many sorghum lines possess more phenolic compounds with higher antioxidant capacity than those found in grapes, blueberries, pomegranates and other similar fruits identified and sold as health-promoting/nutraceutical foods.[4] Globally, there are 40 000 sorghum accessions,[5] most of which have not been characterised for the presence of phenolic compounds with human health benefits. A population subset of approximately 300 sorghum lines that represent sorghum's genetic diversity has been assembled, but the evaluation of phenolic compounds has not been systematically assessed.[6] Faced with the prospect of screening hundreds of samples grown over multiple years and locations, simpler and less time-consuming methods would be advantageous for breeders to use in their routine screening.

The conventional methods for measuring total phenolic content, flavonoid content and 2,2-diphenyl-1-picrylhydrazyl (DPPH)-scavenging capacity are time-consuming and labour-intensive, resulting in low overall throughput. An alternative is to use the 96-well microtitre assay that has been employed for decades in the pharmaceutical industry for drug screening.[7] More recently, the microplate assay has been used in estimating total polyphenol content and DPPH-scavenging capacity in seaweed,[8] plant tissue[9] and wheat extracts.[10] There are no high-throughput assays reported in the literature for measuring any of the phenolic compounds specifically from sorghum and their potential health benefits.

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† *Names are necessary to report factually on available data; however, the USDA neither guarantees nor warrants the standard of the product, and use of the name by the USDA implies no approval of the product to the exclusion of others that may also be suitable. The USDA is an equal opportunity provider and employer.*

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The objective of this study was to develop an exclusive diagnostic tool for efficient screening of large sample sets such as the sorghum diversity panel for health-promoting compounds. This was accomplished by developing a new high-throughput method for flavonoid determination and modifying current 96-well microtitre methods used in other food sources for total phenolic content and DPPH-scavenging activity assays. The 96-well assay was compared with conventional methods.

EXPERIMENTAL

Materials

Ten hybrids from tannin and non-tannin lines were selected from a genetically diverse set of sorghum (USDA-ARS, Manhattan, KS, USA) with a range of phenolic content. A Udy mill (Udy Corp., Fort Collins, CO, USA) was used to grind the sorghum grain into flour that could be passed through a 0.5 mm screen. Sorghum bran samples were prepared by grain decortication using a tangential abrasive dehulling device (Venebles Machine Works, Saskatoon, Canada) equipped with an 80-grit abrasive disc. Sorghum samples were designated as flours 1–5 and brans 1–5. The intent was to establish high-throughput screening methods applicable to both sorghum flour and bran for their bioactive components.

Chemicals

6-Hydroxy-2,5,7,8-tetramethylchromane-2-carboxylic acid (Trolox), gallic acid, sodium nitrite ($NaNO_2$), aluminium chloride ($AlCl_3$), DPPH, fluorescein disodium and 2,2′-azobis(2-amidinopropane) dihydrochloride (AAPH) were purchased from Sigma-Aldrich (St Louis, MO, USA). (+)-Catechin and Folin–Ciocalteu (F–C) reagent were obtained from Fluka (Milwaukee, WI, USA). Acetone, methanol, sodium phosphate dibasic, sodium phosphate monobasic and sodium carbonate (Na_2CO_3) were purchased from Thermo Fisher Scientific (Waltham, MA, USA).

Instrumentation

An Ultraspec 3000 UV–visible spectrophotometer (GE Healthcare Bio-Sciences Corp., Piscataway, NJ, USA) was used to measure the absorbance of samples obtained with conventional methods. A Synergy 2 microplate reader equipped with Gen5™data analysis software (Biotek Instruments Inc., Winooski, VT, USA) was used to read the 96-well plates.

Extraction

Sorghum samples (0.3 g) were suspended in 10 mL of 750 mL L^{-1} acetone and shaken for 2 h on a MaxQ 2500 shaker (Thermo Fisher Scientific). The extracts (sample + solvent) were stored at −20 °C overnight to allow the phenolics to completely diffuse in the solvent and enhance extraction. The next day the extracts were centrifuged at 2900 × *g* for 10 min and the supernatant was decanted. The residues were re-extracted with 10 mL of 750 mL L^{-1} acetone, shaken for 10 min and centrifuged at 2900 × *g* for 10 min, then the supernatant was added to the initial extract to obtain a total volume of 20 mL. Three replicates were performed on each of the ten sorghum hybrids for a total of 30 extracts. Four aliquots or subsamples were analysed, resulting in 120 measurements.

Conventional DPPH method

The DPPH radical-scavenging capacity assay was performed as described by Thaipong *et al.*[11] with some modifications. A solution of 0.1 mmol L^{-1} DPPH in 800 mL L^{-1} methanol was prepared daily. A 0.1 mL acetone extract was allowed to react with 2.9 mL of DPPH solution in the dark for 24 h at ambient temperature. The solution absorbance (*A*) at 517 nm was measured. A standard curve was created using 50–100 µmol L^{-1} Trolox solutions (average $R^2 = 0.9975$). Acetone was used as the control, and all solutions were measured against an 800 mL L^{-1} methanol blank. The % DPPH quenched was determined according to the equation

$$\%\ \text{DPPH quenched} = [1 - (A_{\text{sample}} - A_{\text{blank}})/(A_{\text{control}} - A_{\text{blank}})] \times 100 \quad (1)$$

The % DPPH quenched for each standard was plotted against the concentration of Trolox standards, and the antioxidant capacity of the samples was calculated from the linear equation. Antioxidant activity was expressed as µmol L^{-1} Trolox equivalent (TE) g^{-1} sorghum flour. Samples with DPPH values outside the linear range were diluted.

96-Well plate DPPH method

Because no 96-well assay exists for DPPH determination in sorghum, a modification of the 96-well plate assay described by Fukumoto and Mazza[12] was employed. DPPH solution (150 mmol L^{-1}) was prepared daily, and 200 µL of this solution was added to all wells except the blank test wells. Sample, control or standard solutions (25 µL) were added as depicted in Fig. 1. The solutions were mixed, covered and allowed to react in the dark for 6 h, after which the absorbance at 517 nm was read. Preliminary testing indicated that some of the sorghum extracts took as long as 6 h to fully react with DPPH and reach steady state. Therefore the method was modified to allow sufficient time to complete the reaction without substantial loss of the solution due to evaporation. In contrast, substantial evaporative loss was observed during the 24 h reaction time used in the conventional method. Trolox was used as a standard at 50–500 µmol L^{-1} to generate a calibration curve (average $R^2 = 0.9997$, Fig. 2(a)).

Conventional total flavonoid method

Total flavonoid concentration was quantified using the spectrophotometric method described by Jia *et al.*[13] Acetone extract (0.25 mL), 1 mL of distilled deionised water (DDW) and 0.075 mL of 50 g L^{-1} $NaNO_2$ were combined and vortexed for 5 min. Next, 0.15 mL of 100 g L^{-1} $AlCl_3$ was added to the solution. The solution was left to stand for 6 min, after which 0.5 mL of 1 mol L^{-1} NaOH and 0.5 mL of DDW were added. The solution was centrifuged (3220 × *g* for 5 min at room temperature) to void the precipitate, and the absorbance at 510 nm was measured against the reagent blank. Each sample and standard was run in duplicate. Total flavonoid concentration was expressed as mg catechin equivalent (CE) g^{-1} sample. Catechin was used as a standard at 5–250 µg mL^{-1} to produce a calibration curve (average $R^2 = 0.9996$).

96-Well plate total flavonoid method

Currently the only means to evaluate total flavonoids in sorghum is by the conventional method. The proposed high-throughput technique allows for faster reading of the absorbance and

	1	2	3	4	5	6	7	8	9	10	11	12
A	BLNK	STD2	STD4	CTRL	S1	S2	S3	S4	S5	S6	S7	S8
B	BLNK	STD2	STD4	CTRL	S1	S2	S3	S4	S5	S6	S7	S8
C	BLNK	STD2	STD4	CTRL	S1	S2	S3	S4	S5	S6	S7	S8
D	BLNK	STD2	STD4	CTRL	S1*	S2*	S3*	S4*	S5*	S6*	S7*	S8*
E	STD1	STD3	STD5	S9	S10	S11	S12	S13	S14	S15	S16	BLNK
F	STD1	STD3	STD5	S9	S10	S11	S12	S13	S14	S15	S16	BLNK
G	STD1	STD3	STD5	S9	S10	S11	S12	S13	S14	S15	S16	BLNK
H	STD1	STD3	STD5	S9*	S10*	S11*	S12*	S13*	S14*	S15*	S16*	BLNK

Figure 1. Template for 96-well assays. The control was used only for the DPPH assay and contained 25 µL of acetone added to the DPPH solution. *For the total phenolic assay, these wells contained the sample control.

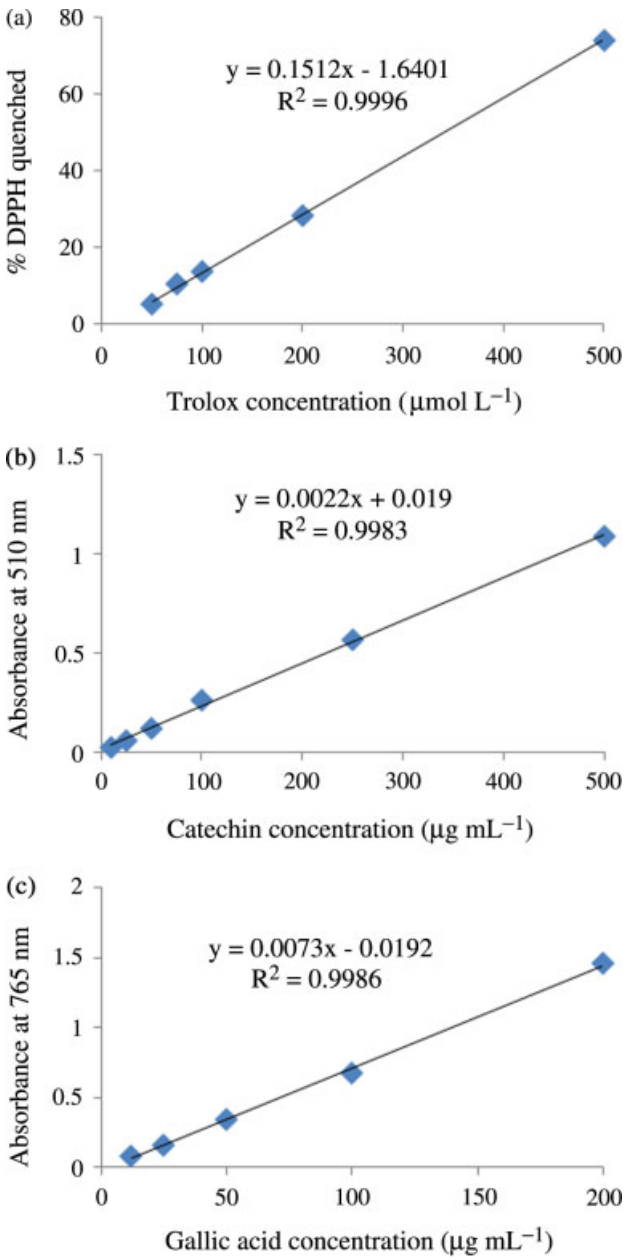

Figure 2. Representative standard curves for 96-well assays: (a) DPPH-scavenging activity; (b) flavonoid content; (c) total phenolic content.

reduction in solvents required for the assay. This is convenient when large sample sets need to be analysed. Distilled water (100 µL) was added to each of the 96 wells, followed by 10 µL of 50 g L^{-1} $NaNO_2$ and 25 µL of standard or sample solution. After 5 min, 15 µL of 100 g L^{-1} $AlCl_3$ was added to the mixture; 6 min later, 50 µL of 1 mol L^{-1} NaOH and 50 µL of distilled water were added. The plate was shaken for 30 s in the plate reader prior to absorbance measurement at 510 nm. All samples and standards were measured against an acetone reagent blank according to the template depicted in Fig. 1. Catechin was used as a standard at 5–250 µg mL^{-1} to generate a calibration curve (average $R^2 = 0.9990$, Fig. 2(b)).

Conventional total phenolic method

Total phenolic content was included in this research as the assay is a standard for measuring phenolics and is often used to optimise experimental conditions. Total phenolic concentration was analysed using the method described by Singleton and Rossi[14] with modifications. The acetone extract or standard (0.1 mL) was mixed with 1 mL of DDW and 0.1 mL of F–C reagent. After allowing the solution to react for approximately 6 min, 0.8 mL of 75 g L^{-1} Na_2CO_3 was added. The solution was placed in the dark for 90 min at room temperature to allow for colour development, then the absorbance at 760 nm was read against the reagent blank. Gallic acid was used as a standard at 0–300 µg mL^{-1} to produce a calibration curve (average $R^2 = 0.9990$). Each sample extract and standard was run in duplicate. Total phenolic concentration was expressed as mg gallic acid equivalent (GAE) g^{-1} dried sample.

96-Well plate total phenolic method

The method used was similar to that of Zhang *et al.*[8] To each of the 96 wells, 75 µL of DDW was added, followed by 25 µL of either sample or standard and 25 µL of F–C reagent (diluted 1 : 1 (v/v) with DDW). All reagents other than samples and standard were delivered through a repeating pipette. After the solutions had been mixed and left for 6 min, 100 µL of 75 g L^{-1} Na_2CO_3 was added to each well. The solutions were mixed again and the plates were covered and left in the dark for 90 min. The absorbance at 765 nm was measured with a spectrophotometric microplate reader (set to shake for 60 s before reading). An acetone reagent blank was prepared using the aforementioned procedure. Each standard and sample solution was analysed in triplicate, and the latter was assayed against a sample control (i.e. sample solution without F–C reagent and Na_2CO_3). Gallic acid was used as a

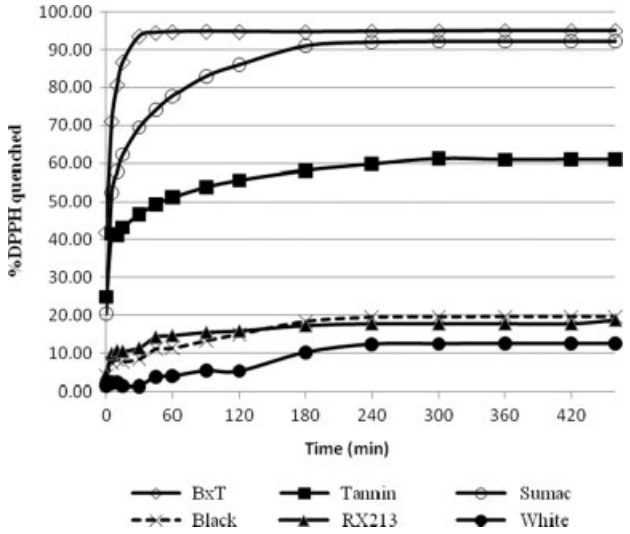

Figure 3. Determination of DPPH steady state for six sorghum extracts.

standard at 12.5–200 μg mL^{-1} to produce a calibration curve (average $R^2 = 0.9993$, Fig. 2(c)). Total phenolic concentration was expressed as mg GAE g^{-1} sorghum flour.

Accuracy, precision and reproducibility of 96-well assay

To evaluate the accuracy and precision of the 96-well assays, three replicates of a standard at three different concentrations (25, 50 and 100 μg mL^{-1}) were performed in triplicate. Ferulic acid was used as the standard for the total phenolic and DPPH assays, and catechin was used for the total flavonoid assay. Inter- and intra-day variations for the 96-well assays were tested using 100 μg mL^{-1} ferulic acid (total phenolics and DPPH) and 100 μg mL^{-1} catechin (total flavonoids). For all assays, five plates were run on a single day to determine inter-day reproducibility. Intra-day variation was determined by running one plate a day for five consecutive days. Ferulic acid (12.5–200 μg mL^{-1} for total phenolics and 25–500 μg mL^{-1} for DPPH) and catechin (25–500 μg mL^{-1} for total flavonoids) were used to generate standard curves.

RESULTS AND DISCUSSION

DPPH-scavenging capacity

The DPPH-scavenging capacity is a measure of the antioxidant capacity of the sample. In this assay, DPPH, a stable free radical, is reduced and loses its purple colour, turning yellow in the presence of oxidising compounds. The colour loss is proportional to the ability of the oxidising agent to donate an electron against nitrogen radicals and is measured as absorbance at 517 nm.[15,16] It is essential that the measurement is taken when the reaction between antioxidant and DPPH is complete, as determined by no further change in the absorbance of the solution (steady state). Figure 3 shows the different reaction rates of various sorghum extracts with DPPH. All sorghum samples tested in preliminary studies achieved a stable reading within 6 h. These data suggest that not all sorghums reach steady state at the same time. Awika *et al*.[2] reported that 8 h was required to reach steady state. The time required to complete the reaction did not result in substantial loss of the solution due to evaporation, as observed by the low coefficient of variation (CV). In contrast, if the plates were incubated for 24 h as in the conventional method, substantial evaporative loss was observed (data not reported).

Table 1. Comparison of 96-well plate and conventional methods in determination of (a) DPPH-scavenging activity, (b) total flavonoid content and (c) total phenolic content in sorghum flour and bran samples

	96-Well plate method		Conventional method	
Sample	Value	% CV	Value	% CV
(a) DPPH-scavenging activity (μmol L^{-1} TE g^{-1})				
Flour 1	181.94 ± 6.17	3.39	213.44 ± 14.28	6.69
Flour 2	14.23 ± 0.90	6.35	15.72 ± 1.34	8.54
Flour 3	11.03 ± 2.00	18.14	11.22 ± 0.52	4.68
Flour 4	15.11 ± 0.11	0.72	15.47 ± 0.45	2.91
Flour 5	8.81 ± 0.39	4.45	8.96 ± 0.34	3.83
Bran 1	697.09 ± 10.25	1.47	855.63 ± 27.94	3.27
Bran 2	107.78 ± 7.94	7.37	103.04 ± 0.64	0.62
Bran 3	438.49 ± 27.02	6.16	553.78 ± 8.68	1.57
Bran 4	89.95 ± 6.93	7.71	88.22 ± 8.82	5.04
Bran 5	1883.33 ± 253.63	13.47	167.07 ± 22.45	1.34
(b) Total flavonoid content (mg CE g^{-1})				
Flour 1	7.26 ± 0.22	3.01	6.81 ± 0.61	9.03
Flour 2	1.00 ± 0.02	1.85	1.09 ± 0.11	9.77
Flour 3	0.87 ± 0.0.4	4.63	0.66 ± 0.03	5.23
Flour 4	1.03 ± 0.11	10.68	1.04 ± 0.18	17.31
Flour 5	0.69 ± 0.07	9.44	0.50 ± 0.06	12.72
Bran 1	51.01 ± 0.18	4.88	52.25 ± 2.14	4.10
Bran 2	5.51 ± 3.49	3.18	5.65 ± 0.11	1.95
Bran 3	27.87 ± 3.49	12.50	28.70 ± 1.05	3.66
Bran 4	4.30 ± 0.40	9.30	4.32 ± 0.40	9.18
Bran 5	77.32 ± 1.17	1.51	78.10 ± 3.14	4.02
(c) Total phenolic content (mg GAE g^{-1})				
Flour 1	18.10 ± 0.8	4.41	17.79 ± 0.84	4.71
Flour 2	3.54 ± 0.15	4.18	2.32 ± 0.52	5.72
Flour 3	2.49 ± 0.18	7.30	2.48 ± 0.04	1.76
Flour 4	2.55 ± 0.19	7.31	2.97 ± 0.01	0.29
Flour 5	2.48 ± 0.08	3.36	2.22 ± 0.06	2.75
Bran 1	77.46 ± 2.63	3.39	77.35 ± 0.98	1.27
Bran 2	12.03 ± 0.18	1.50	14.34 ± 0.42	2.93
Bran 3	57.25 ± 2.37	4.13	67.36 ± 3.20	4.75
Bran 4	10.76 ± 0.16	1.52	12.97 ± 1.08	8.33
Bran 5	124.85 ± 6.98	5.59	109.30 ± 3.58	3.27

Values are expressed as mean ± standard deviation ($n = 3$).

The DPPH-scavenging capacity of the sorghum flour and bran samples as determined by both methods is shown in Table 1(a) and was calculated based on % DPPH quenched. The correlation between the two assay methods was 0.997. The mean % CV for the conventional assay was 3.85, whereas it was 6.92 for the 96-well assay. These % CV values are similar to those in reported in the literature for the conventional method.[2]

In the high-throughput DPPH assay described by Cheng *et al*.[10] for wheat bran, the authors proposed that the antioxidant capacity can be calculated using the area under the curve obtained by reading the reacting solution kinetically over 40 min. The conditions described by Cheng *et al*. were not suitable for sorghum extracts, because the reaction was not completed after 40 min for many of the extracts. Furthermore, lengthy kinetic reads limit the amount of plates that can be read per day and consequently decrease the number of sorghum samples analysed. Therefore it would not serve as an efficient screening tool.

Flavonoid content

To our knowledge, a high-throughput microtitre assay has not been developed for sorghum flavonoids using the $NaNO_2/AlCl_3/NaOH$ system, though anthocyanin data have been reported for sorghum.[3] The results of the flavonoid analysis are summarised in Table 1(b). The correlation between the two assay methods was 0.993. The average % CV for the conventional assay was 7.70, whereas it was 6.09 for the 96-well assay.

The flavonoid assay has been described by numerous authors in a wide variety of samples, including fruits, buckwheat, wheat and rice.[13,17–20] The aforementioned cited flavonoid method produces a red colour measured at 510 nm by reacting with the $NaNO_2/AlCl_3/NaOH$ system. Specifically, the flavonoid molecule is oxidised by $NaNO_2$, followed by nitrosylation and formation of a complex with the aluminium ion. In the presence of NaOH the complex will produce a red-coloured chelate.[21] A drawback to the above method is that not all flavonoids are measured because of the structural composition of the flavonoid molecule.[22,23] He *et al.*[22] described an assay for the measurement of total flavonoids, but it consists of several time-consuming steps and would be unsuitable as a quick screening tool for large sample sets.

Total polyphenol content

This is the first report of total phenolics being measured in sorghum bran and flour using the microplate assay. In general, the bran fractions possessed a higher content of total phenolics. This is consistent with research indicating that phenolic compounds are concentrated in the bran and germ fractions of sorghum that are removed during the milling of sorghum into flour.[1]

The results using the 96-well assay compared favourably with those using the conventional assay for total phenolic content (correlation 0.991) (Table 1(c)). The average % CV for the conventional method was 3.81 compared with 4.04 for the 96-well method. The 96-well method worked equally well for both bran and flour. The total phenol and antioxidant capacity values found in this study are comparable to those reported previously for sorghum using conventional assays.[1,2]

Preliminary research using the methods described by Zhang *et al.*[8] resulted in a vigorous bubbling reaction after addition of Na_2CO_3. This observation was not reported by the aforementioned researchers. The bubbles persisted until the end of the 90 min reaction time, and the reaction resulted in inconsistent absorbance values. Therefore in this study the method was modified to reduce the reaction between F–C reagent and Na_2CO_3. The F–C reagent was diluted twofold before being added to the wells. This reduced the amount of F–C reagent used by tenfold without affecting the reproducibility of the data.

Accuracy, reproducibility and precision of 96-well assay

The accuracy and precision of the high-throughput assay are shown in Table 2. Accuracy was calculated as described by Cheng *et al.*[10] and expressed as % recovery (% REC). It was the difference between the calculated value of the standard obtained after running the 96-well assay and the actual value of the prepared standard, expressed as a percentage. The % REC values ranged from 97.65 to 106.16 for the three concentrations of ferulic acid and catechin tested, suggesting that the 96-well assays can be reproduced with excellent accuracy. No edge effects were observed for the border wells in the microplate system. Similarly, the % CV values were <10% for all three concentrations tested, which is considered good precision. The % CV values for inter-day variability and intra-day reproducibility were <5% over 5 days and are shown in Table 3. Cheng *et al.*[10] reported similar DPPH accuracy and reproducibility values using 96-well plate assays for a ferulic acid standard. No data on the accuracy, precision or reproducibility of the F–C 96-well plate assay were found in the literature.

Table 2. Accuracy of 96-well assays in determining total phenolic, DPPH and total flavonoid contents using ferulic acid and catechin standards

Assay	Std 1 25 μg mL^{-1}	Std 2 50 μg mL^{-1}	Std 3 100 μg mL^{-1}
Total phenolics[a]			
Calculated μg mL^{-1}	26.33 ± 2.18	52.46 ± 3.22	106.16 ± 1.83
% REC	105.32	104.93	106.16
% CV	8.28	6.15	1.72
DPPH[a]			
Calculated μg mL^{-1}	24.76 ± 1.42	48.83 ± 0.72	100.46 ± 2.69
% REC	99.05	97.65	100.46
% CV	5.73	1.46	2.68
Total flavonoids[b]			
Calculated μg mL^{-1}	25.69 ± 0.17	51.70 ± 1.11	102.65 ± 2.19
% REC	102.76	103.40	102.65
% CV	1.06	2.15	2.13

Values are expressed as mean ± standard deviation of three replicates, with four wells per concentration.
[a] Ferulic acid was used for the total phenolic and DPPH assays.
[b] Catechin was used for the total flavonoid assay.

Table 3. Reproducibility of 96-well assays in determining total phenolic, DPPH and total flavonoid contents using ferulic acid and epicatechin standards

	Inter-day variability		Intra-day variability	
Assay	Mean	% CV	Mean	% CV
Total phenolics GAE[a]	86.09 ± 1.83	2.13	84.71 ± 1.15	1.36
DPPH μmol L^{-1} TE[a]	407.72 ± 5.40	1.32	418.37 ± 6.97	1.66
Total flavonoids CE[b]	127.90 ± 1.70	1.33	126.69 ± 2.65	2.09

Values are expressed as mean ± standard deviation ($n = 5$).
[a] Ferulic acid at a concentration of 100 μg mL^{-1} was used for the total phenolic and DPPH assays.
[b] Epicatechin at a concentration of 100 μg mL^{-1} was used for the total flavonoid assay.

General observations: conventional *versus* 96-well assays

The 96-well assays used less solution and were faster than the conventional assays. The 96-well assay reduced the time and manpower needed to transfer the reactant solutions to the cuvettes in order to be manually read in the spectrophotometer, and allowed for more replicates to be run on the same extracts than was possible with the conventional methods. In the high-throughput assay, each measurement is an average of nine (for the F–C assay, 3 replicates × 3 subsamples) or 12 (for the flavonoid and DPPH assays, 3 replicates × 4 subsamples) measurements. Conversely, for the conventional method, each measurement is an average of six measurements (3 replicates × 2 subsamples).

The need for numerous cuvettes, transfer pipettes and test tubes was eliminated. Instead, the only consumables used were pipette tips and the 96-well plates. The amount of reagents required was decreased. In the conventional DPPH assay a total of 5.8 mL of DPPH was used per extract, whereas only 800 µL of DPPH was required per extract for the 96-well assay. An additional advantage of using a plate reader was that all data could be automatically analysed by the data analysis software. Each time a plate is run, a standard curve is automatically generated by the software, and the concentrations of the extracts are calculated. This process offers significant time and labour savings as well as potentially a reduction in error because of the nearly simultaneous measurement of sample and standards. The % CV values were slightly higher for the 96-well assays than for the traditional methods, but they were all under 10%. However, the savings on time, the amount of samples that can be run per day and the reduced amount of solvent due to use of the 96-well assays (225 µL) more than compensated for the slightly higher variability. The 96-well assays will allow for analysis of up to 16 sorghum extracts per plate. Four plates can be run without significant time constraints, allowing for up to 64 extracts to be run comfortably within a day; in comparison, 20–24 samples (depending on the test) per day can be run by the conventional methods.

CONCLUSION

The high-throughput 96-well plate method proved to be as robust, reproducible, efficient, accurate and precise as the conventional method for determining total phenolic content, partial flavonoid content and DPPH-scavenging capacity in either sorghum bran or flour. The high-throughput microtitre plate assays developed are usable for routine screening of a large number of samples in early generation breeding programmes.

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