



Identification and Quantification of Phenolic Compounds and Bioactive Properties of Sorghum-cowpea-based Food Subjected to an *In vitro* Digestion Model

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Authors' contributions

This work was carried out in collaboration between both authors. Author GD headed the project. Author OEA conducted the analysis and interpretation and drafted the manuscript. Author GD was involved in the early work and assisted with the interpretation of the results. Author OEA was in charge of data management. Both authors were involved in the critical revision of the manuscript for important intellectual content. Both authors read and approved the final manuscript.

Article Information

DOI: 10.9734/EJNFS/2017/20310

Original Research Article

Received 22nd July 2015
Accepted 11th May 2016
Published 5th July 2017

ABSTRACT

This work identified and quantified some phenolics compound in sorghum-cowpea porridge after subjection to *in vitro* digestion. Flours, porridge and digested porridge were analysed for total phenolics, total flavonoids, ABTS-radical scavenging capacity and specific phenolic acids and flavonoids. Total phenolics and flavonoid content of the gastric (346.1 µg CE/g; 35.2 µg CE/g) and intestinal phase digest (1389.8 µg CE/g; 142.6 µg CE/g) were lower than the composite flour (2720.1 µg CE/g; 220.9 µg CE/g) and while its porridge were (1218.4 µg CE/g; 173.8 µg CE/g). The ABTS-radical scavenging capacity of all samples ranged from 21.0 – 507.3 µg TE/g. The gastric phase and intestinal phase digests maintained 4.7% and 58.3% of the radical scavenging capacity of the porridge. Catechin and gallic acid were lower in the intestinal digest (2760.0 µg/g; 226.7 µg/g) than the undigested porridge (4188.3 µg/g; 193.9 µg/g). Sorghum-cowpea composite porridge contains phenolic antioxidants even after gastric and intestinal digestion with potential to significantly impact human health.

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Keywords: Sorghum; cowpea; *in vitro* digestion; antioxidant; phenolics acids; flavonoids.

1. INTRODUCTION

Many studies have established a positive association between the consumption of antioxidant-rich foods and their possible role in preventing chronic degenerative diseases. This is probably due to the fact that antioxidants; when present at low concentrations relative to an oxidizable substrate, significantly delay, retard or inhibit oxidation of that substrate [1]. Plant foods are identified as sources of a wide variety of dietary antioxidants, such as vitamins C and E, carotenoids, flavonoids and other phenolic compounds. Phenolic antioxidants in particular are compounds that act as terminators for free radicals [2]. Free radicals have been reported to be the cause of several diseases such as liver cirrhosis, atherosclerosis, cancer and diabetes and compounds that can scavenge free radicals therefore have great potential in ameliorating these disease processes [3-6]. Thus, antioxidants can play an important role to protect the human body against damage by reactive oxygen species [7].

Reactive oxygen species (ROS) which are the natural by-products of mitochondrial respiration and other cellular processes, have high chemical reactivity. Therefore, when they occur in excess of normal needs in the cell, they may damage the cell's structural and functional integrity. They usually do this, either by directly modifying cellular DNA, proteins, and lipids, or by initiating chain reactions that can bring about extensive oxidative damage to these critical molecules [8-10]. Beneficial effects of ROS in low concentration involve defence against microbial pathogens [11]. In healthy individuals, the generation of ROS is well balanced by the counter balancing act of antioxidant defences. However, if there is an imbalance between the ROS produced and antioxidant status of an individual, a process referred to as oxidative stress occurs [10,12].

Sorghum (*Sorghum bicolor* (L.) Moench, due to its unique property as a drought-tolerant crop, is adjudged as a very important cereal food in Africa. It has a variety of phytochemicals that have been shown to have antioxidant activity [13, 14,15,16]. When consumed regularly in diet, this diversity of phytochemicals in sorghum has a potential to significantly impact human health. Although, it is consumed majorly in the form of porridge in Southern Africa and serves as a

source of energy for many people living in this region [17], it however, contains a low protein content and quality which is critically required in many developing countries, where human diets consist mainly of cereals [18]. In view of this, [19] developed a protein-rich composite sorghum-cowpea instant porridge by extrusion cooking process and reported that composite of 50% sorghum and 50% cowpeas extruded at 130°C was the most similar to a commercial instant maize-soya composite porridge in terms of composition and functional properties. Cowpea on the other hand, apart from been a good source of protein, has also been reported to possess antioxidant property [20-22]. Therefore, it may be hypothesized that the combination of sorghum and cowpea in a composite food will further provide complementary phenolic compounds from the two components with potential synergistic antioxidant effects.

Furthermore, phenolic compounds need be released from the food matrix (bioaccessibility) and modified in the gastrointestinal tract before becoming bioavailable [23]. Studies on the bioaccessibility of phenolic compounds from solid matrices are important because not all the compounds present in the food matrix, but only those released in the first tract of the alimentary canal, are really bioaccessible in the gut and, therefore, potentially bioavailable. Most of the food antioxidant data usually refer to those analyzed in aqueous and organic extracts of foods. Usually, the amount that is bioaccessible may differ quantitatively and qualitatively from those extracted with chemical methods, which means that the most bioaccessible phenolics are not necessarily those present at higher concentrations in the food [24]. The overall bioavailability determination process includes gastrointestinal digestion, absorption and metabolism. Thus, *in vitro* digestion models have been developed as an alternative approach to animal and human studies as they are considered simple, cheap and reproducible tools to assess the digestive stability of different food constituents [25]. Generally, sorghum and cowpea are consumed in cooked/processed form. Presently, it appears nothing is known about the phenolic profile of sorghum - cowpea composite food after subjection to simulated *in vitro* digestion process. Therefore, the purpose of this work is to determine the phenolic profile of sorghum-cowpea based food on subjection to simulated *in vitro* digestion and the potential

health benefits of the food in terms of antioxidant properties.

2. MATERIALS AND METHODS

2.1 Chemicals

Phenolic acids (ferulic, gallic, *p*-coumaric, syringic, 4-hydroxybenzoic, synapic, caffeic, protocatechuic, transcinamic and vanillic acid) and flavonoid (tannic acid, catechin, epicatechin, quercetin, hesperidin, naringin and fisetin) standards and other chemicals used in this study were purchased from merck, South Africa.

2.2 Sample preparation

2.2.1 Flour sample preparation

Sorghum - *Sorghum bicolor* (orbit variety – 2 kg) and cowpea - *Vigna unguiculata* (bechuana white variety – 1.5 kg) were cleaned and milled into flour with hammer mill (500 μ m sieve). The milled sorghum and cowpea fractions were mixed thoroughly in 70:30 proportions respectively.

2.2.2 Preparation of Porridge

The mixed proportion of sorghum/cowpea (70:30) was used to prepare porridge according to the method of [26] which served as sorghum-cowpea-based food. Briefly, the cooking process involved mixing of 200 ml of cold water with 80g of flour to make slurry. The slurry was then gradually added to 600 ml of boiling water in a 2 L stainless steel saucepan while stirring continuously to avoid lump formation. The porridge was left to simmer on low heat (hot plate) for 20 min while stirring at every 5 min. A portion of the sorghum-cowpea composite porridge was freeze-dried while the remaining portion was taken through *in vitro* gastrointestinal digestion as described below, after which the gastric digest and intestinal digest samples were freeze-dried. The extracts from cowpea, sorghum, sorghum-cowpea mix and all the freeze-dried samples were assayed for antioxidant capacities and phenolic profile. All samples preparation were done in replicate.

2.3 *In vitro* digestion

In vitro digestion models are employed to study the structural changes, digestibility, and release of food components under simulated gastrointestinal conditions [27]. Generally, *in vitro*

digestion models give a useful alternative to animal and human models by rapid screening of food ingredients. Ideally, *in vitro* digestion method should provide accurate results in a short time [28]. The *in vitro* digestion for this study was carried out on the porridge sample following the procedure of [29] with a little modification. About 10 ml of simulated gastric juice (NaOH/Pepsin/HCl) was added to 1 g flour equivalent of sorghum-cowpea composite porridge in 50 ml tube; this was mixed gently, flushed with nitrogen and sealed. Incubation of the tubes was done for 1 h at 37°C in a shaking water bath. After 1 h, about 2.5 ml from the tube was transferred into 15 ml polypropylene tubes, flushed with nitrogen and kept on ice (this is the gastric digest sample), while digestion was continued with the sample in the 50 ml tube as stated below.

Gastric phase was terminated by adding 1 N NaHCO₃ to increase the pH to 6. About 0.5 ml porcine pancreatin stock and 0.5 ml bile extract stock was added; while the pH adjusted to 6.9 with 1 N NaOH and the volume was increased to 15 ml with distilled water. The sample was flushed with nitrogen, sealed and incubated for 2 hours in the shaking water bath at 37°C. During the 2-h incubation, the 2.5 ml aliquot from the gastric phase was centrifuged (3500g at 4°C) in an Eppendorf centrifuge for 35 min. Aliquot of the clear supernatant was then transferred to clearly labelled Eppendorf tubes, flushed with nitrogen and frozen immediately at -80°C. At the end of the 2-h incubation, the same was done with the digester in the 50 ml tubes. The simulated gastric and small intestine conditioned samples were then freeze dried, packaged in clearly labelled plastic bag and extracted when required for antioxidant activities and phenolic profiling. All preparations were done in replicate.

2.4 Preparation of Sample Extract

Eighty percent methanol (4:1 of methanol and water) was used as the extracting solvent for determination of total phenolic content and antioxidant activity of the samples. 1 g of the flour or freeze-dried sample was mixed with 10 ml of 80% methanol. This was incubated for 1 hour at 37°C in a shaking water bath and thereafter centrifuged (3500 g at 4°C) in 460 R centrifuge (Labotech T., Johannesburg, South Africa) for 35 min. The supernatant was then stored in a glass bottle covered with aluminium foil and kept in a cold room (4°C) until analysed [30].

2.5 Determination of the Total Phenolic Content

Determination of total phenolic content of the samples extracts was determined according to the method of [31]. Briefly, 0.5 ml Folin-Ciocalteu reagent was added to the methanolic extract (0.5 ml) of each sample placed in separate tubes and vortexed for 1 min. After 3 min of incubation, 10 ml of sodium carbonate (Na_2CO_3) solution (75 g/L) and 5 ml distilled water were added, mixed and incubated for 1 h at room temperature in the dark. The absorbance of the resulting solutions was read at 760 nm using a Lambda EZ150 spectrophotometer (Perkin Elmer, USA). Catechin/gallic was used as a standard and the results were expressed as catechin equivalents (CE, mg catechin equivalents/g of dry weight extract sample) on dry matter basis.

2.6 Determination of the Total Flavonoid Content

The total phenolic content was measured with an aluminium chloride colorimetric method and using standard. 1 ml extract of samples or a standard solution of catechin (20, 40, 60, 80 and 100 $\mu\text{g/ml}$) was added to 4 ml of distilled deionized water (dd H_2O), after which 0.3 ml 5% NaNO_2 was added. After 5 min, 0.3 ml 10% AlCl_3 was added and at the 6th min, 2 ml of 1 M NaOH was added and the total volume made up to 10 ml with dd H_2O . The solution was mixed well and the absorbance was measured against reagent blank at 510 nm with Lambda EZ150 spectrophotometer (Perkin Elmer, USA). The data of the total flavonoid contents of the samples were expressed as micrograms of catechin equivalent per gram of dry weight extract ($\mu\text{g CE/g}$).

2.7 Determination of Antioxidant Activity

The 2,2'-azino-bis-3-ethylbenzthiazoline-6-sulphonic acid (ABTS) and 1,1-diphenyl-2-picrylhydrazyl (DPPH) assays were employed to determine the antioxidant activity of samples (undigested and digested). The ABTS assay is based on the generation of a blue/green ABTS⁺ that can be reduced by antioxidants and the DPPH assay is based on the reduction of the purple DPPH to 1,1-diphenyl-2-picryl hydrazine.

The free radical scavenging activity of the samples was determined using the Trolox Equivalent antioxidant capacity (TEAC) assay

(ABTS⁺ free radical scavenging) as described by [16] with slight modifications. Aliquots (0.1 ml) of samples (methanolic extracts of all the samples) or Trolox standard solution (prepared in methanol) was reacted with 2.9 ml ABTS⁺ radical cation working solution for 30 min. The absorbance of the standards and samples was measured at 734 nm using a Lambda EZ150 spectrophotometer (Perkin Elmer, USA). The results were expressed as μg Trolox equivalent/g sample extract, on dry matter basis.

The hydrogen donating or radical scavenging ability of the samples was also determined by using the stable DPPH method [32] and the results were expressed as % inhibition.

2.8 HPLC-MS/MS Analysis

HPLC analysis of phenolic compounds from all the extracted samples were performed on a Waters 2695 HPLC (Waters, Milford, MA) equipped with a Waters 996 photodiode array (PDA) and a reverse phase Kinetex C18, 2.6 μm , analytical column and an auto sampler (717 Plus, Waters) to inject 5 μL of sample. The phenolic acids were separated under Isocratic conditions using acetonitrile : water : acetic acid (87.5 : 12.5 : 0.1 v/v/v) as mobile phase at a flow rate of 0.4 mL/min, with 30 min running time. These phenolic acids were detected at 280 nm. The flavonoids on the other hand were separated under gradient elution using 0.1% formic acid in high purity water (solvent A) and acetonitrile (solvent B). The gradient set up was as follows: 98% A to 0% A (30 min); 0% A to 98% A (30 -32 min); 98% A (32 – 35 min). Phenolic acid and flavonoids quantification was based on the standard curves of the corresponding phenolic acids at a wavelength of 280 nm and flavonoids at wavelength of 280 or 380 nm. The peak area was used for calculations. Identification of phenolic acids and flavonoids were performed by comparison of the retention time to that of MS/MS spectra with external standards. MS/MS was conducted using a quadrupole time-of flight mass spectrometer (Q-TOF MS) (Micromass, Waters Corp., Milford, MA), C18: 1.7 μm , 2.1 x 100 mm column. Full mass spectra were acquired in the negative mode using cone and capillary voltages of 27 and 1.55 kV, respectively. Desolvation and cone gases (He) was set to flow at 900 and 35 l/h, respectively, while the desolvation temperature and the source temperatures was 300 and 130°C, respectively. MS/ MS spectra were acquired using collision energy of 25 V in the range of m/z 100–1500.

2.9 Statistical Analysis

All data were expressed as means \pm SD (standard deviation). Statistical analysis involved the use of the Statistical Analysis System software package. Analysis of variance was performed by ANOVA procedures. Significant differences between means were determined by Duncan's multiple range tests at a level of $P < 0.05$.

3. RESULTS AND DISCUSSION

3.1 Analysis of Phenolic Acids and Flavonoids by HPLC

The extracts from the cowpea, sorghum, sorghum-cowpea mixture, sorghum-cowpea porridge, gastric digest and intestinal digest (80% methanol) were analysed using HPLC at 280 nm for phenolic acid and 280 or 380nm for flavonoids. Nine phenolic acid compounds (gallic acid; protocatechuic acid; hydroxybenzoic acid; vanillic acid; caffeic/syringic acid co-eluting; *p*-coumaric acid; ferulic acid and sinapic acid) were detected from the extract by matching their retention times with those of standards. Peak assignment was confirmed by injection of standards. Nine flavonoid compounds (Quercetin dehydrate, Naringin, Fisetin, Kaempferol, Kaempferol 3- β -D- glucopyranoside, taxifolin, catechin, epicatechin and tannic acid) were also identified by comparison of their retention times and UV spectra with those of reference standards. To determine the content of the phenolic compounds, calibration curves were prepared in the range of 12.5 – 200 $\mu\text{g/mL}$. Absorbance at $\lambda = 280$ nm for phenolic acid and $\lambda = 280$ or $\lambda = 380$ nm for flavonoids increased linearly for all the standards. Fig. 1 shows the HPLC chromatogram of a sample extract after comparison with the peak of the mixtures of nine phenolic acids. Fig. 2 shows LC-MS chromatogram of mixtures of nine phenolic acids standards.

Table 1 shows the content of each phenolic compound in the extracts of all flour samples and the porridge before and after *in vitro* digestion. As seen from the table, cowpea flour has the highest catechin content of 8798.0 $\mu\text{g/g}$, while the composite flour is 3590.2 $\mu\text{g/g}$ and porridge has 4188.3 $\mu\text{g/g}$ which is about 26.4% increase when compared to its original composite flour. Gastric phase digest phase on the otherhand had the lowest catechin content of 1957.4 $\mu\text{g/g}$.

After porridge digestion, the gastric and the intestinal phase maintained 46.8% and 65.8% respectively of the catechin content. Generally, there was a reduction in the value of phenolic compounds analysed after preparation of the composite flour into porridge. Further reduction was observed after *in vitro* digestion with gastric phase having the lowest content. The percentage of the phenolic compounds retained by the porridge from the original composite flour ranged from 59.7% - 89.8% with naringin having the lowest and caffeic acid having the highest. Only five phenolic compounds (epicatechin, catechin, taxifolin, ferulic acid and *p*-coumaric acid) showed an increment within the range of 13.2% (epicatechin - lowest) and 129.1% (*p*-coumaric acid - highest) of the original composite flour after porridge preparation. It has been widely recognised that processing such as heating could lower the nutritional value of food due to nutrient degradation, however, the increments observed in the above five phenolic compounds may be due to the release of phytochemicals during cooking or structure modification of some bioactive compounds [33]. [34] had suggested that heat treatment might yield structural changes in food component that would end up with their higher bioaccessibility. [35] also reported that thermal processing disrupts cell membranes and cell walls of food material from the insoluble portion, which might increase bioaccessibility [36-37].

The gastric phase and the intestinal phase digest maintained a range of 6.2% - 64.9% and 23.6% - 116.8% respectively of the phenolic compounds of the porridge. Stability of phenolics of pomegranate was earlier reported by [38]. They reported 115% stability during gastric condition and 25% loss in pancreatic digestion. From this experiments, we could emphasize that chemical extraction could overestimate the potential bioaccessibility of phenolic compounds in sorghum-cowpea porridge. Many polyphenols data usually refer to those analyzed in aqueous or organic extracts, and many nutritional advises are based on these data. It would be more appropriate to give nutritional data about the effective quantity of compounds released from foods. Once released, polyphenols may exert their biological activity in the gastrointestinal tract and eventually be absorbed.

3.2 Antioxidant Activity Determination

Many spectrophotometric assays are currently employed to measure the antioxidant capacity of

biological samples, the most popular are 2,2'-azino-bis-3-ethylbenzthiazoline-6-sulphonic acid (ABTS) and 1,1-diphenyl-2-picrylhydrazyl (DPPH) assay [39]. Specifically, the ABTS assay is based on the generation of a blue/green $ABTS^+$ that can be reduced by antioxidants; the DPPH assay is based on the reduction of the

purple DPPH to 1,1-diphenyl-2-picryl hydrazine. These assays are quick and do not require sophisticated equipment, like fluorescence detector or GCMS, which make them suitable for analyses of multiple tissue samples. Antioxidant activities of the extracted samples were evaluated using these two methods.

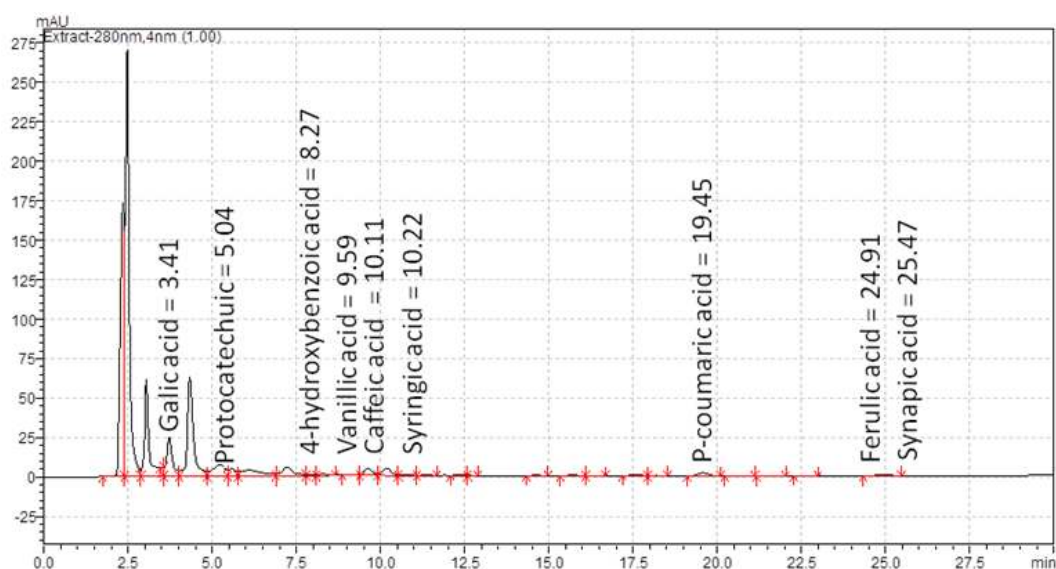


Fig. 1. Typical HPLC chromatogram of extract of intestinal digest of sorghum-cowpea porridge; injected volume 5 μ L

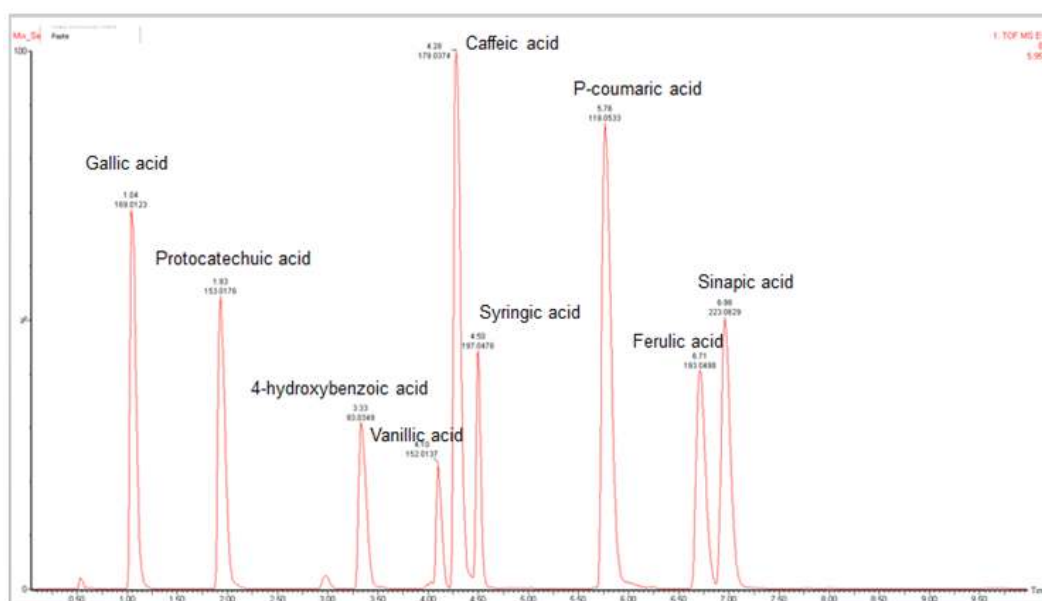


Fig. 2. LC-MS chromatogram of nine phenolic standards analysed with Acquity UPLC, C18: 1.7 μ m, 2.1 x 100 mm column

Table 1. Compound detected in the extracts of flours, porridge and digested porridge ($\mu\text{g/g}$)

Compounds	Cowpea	Sorghum	Sorghum/ cowpea	Sorghum /cowpea porridge	Gastric digest	Intestinal digest
Gallic acid	378.0c	96.8ab	242.6bc	193.9ab	61.7a	226.7c
Protocatechuic acid	493.6d	132.4b	179.0c	122.7b	39.6a	92.2b
4-hydroxybenzoic acid	81.6d	74.5d	73.2d	59.6c	17.5a	42.2b
Vanillic acid	13.394a	150.604e	99.850d	77.035c	21.715a	44.0b
Caffeic acid	11.9c	42.6f	27.1e	18.1d	1.7a	6.3b
Syringic acid	920.4d	2011.8e	794.1cd	684.9c	402.7b	178.0a
p-coumaric acid	5.2b	13.3d	7.1bc	12.9d	1.7a	10.3cd
Ferulic acid	3.8b	16.1f	12.2d	13.9e	2.2a	9.5c
Quercetin dihydrate 380 nm	442.8c	606.3f	555.3e	489.6d	58.2a	218.1b
Hesperidin 380 nm	ND	ND	ND	ND	ND	ND
Naringin 280 nm	617.4c	690.5d	815.9e	486.0b	190.5a	558.9c
Fisetin 380 nm	403.3b	ND	ND	ND	ND	191.2a
Kaempferol 380 nm	ND	939.6e	719.2d	612.2c	38.2a	144.7b
Kaemferol glucoside 380 nm	184.8d	ND	68.7c	ND	9.4a	30.0b
Taxifolin 280 nm	430.3b	549.6c	632.0d	876.2e	290.9a	575.4c
Catechin 280 nm	8799.0d	3045.2ab	3590.2bc	4188.3c	1957.4a	2760.0ab
Epicatechin 280 nm	1545.2ab	2593.1ab	3324.9b	3417.9b	1049.9a	3007.7b
Tannic acid 280 nm	7031.9e	5368.3d	4935.1d	2935.0c	1942.1a	2420.9b

Values are means of two determinations

Values with the same letter in the same row are not significantly different ($P < 0.05$)

ND: Not detected

The percent inhibition of DPPH, Trolox equivalent antioxidant capacity (TEAC) and the total phenolic content for all the extracted samples is as shown in Table 2. Digestion *in vitro* with enzymatic extracts mimicking conditions in the gastrointestinal tract showed that the amount of antioxidants released from the sorghum-cowpea porridge was significantly different ($P < 0.05$) from the undigested porridge. The ABTS-radical scavenging capacity of all samples ranged from 21.0 – 507.3 $\mu\text{g TE/g}$ with gastric phase digest having the lowest and cowpea extract having the highest. The gastric phase and intestinal phase digests maintained 4.7% and 58.3% of the ABTS radical scavenging capacity of the porridge respectively. The percentage inhibition of DPPH for all the samples also ranged between 5.7%– 97.7% with gastric phase digest having the lowest and cowpea extract the highest. The gastric phase and intestinal phase digests again maintained 8.6% and 58.7% of the

DPPH radical scavenging capacity of the porridge respectively (Table 2).

Total phenolics and flavonoid content were higher in the cowpea extract than all other samples analyzed. Generally, the gastric phase digest had the lowest value for all the analysis performed. For instance, the total phenolics content were significantly higher ($P < 0.05$) in the composite flour (2720.1 $\mu\text{g CE/g}$) and its porridge (1218.4 $\mu\text{g CE/g}$) than that from the gastric phase digest (346.1 $\mu\text{g CE/g}$). Intestinal phase digest (1389.8 $\mu\text{g CE/g}$) however, had lower total phenolics content than the composite flour (2720.1 $\mu\text{g CE/g}$) but higher than its porridge (1218.4 $\mu\text{g CE/g}$). Also, the flavonoid content for the gastric (35.2 $\mu\text{g CE/g}$) and intestinal phase digest (142.6 $\mu\text{g CE/g}$) were lower than the composite flour (220.9 $\mu\text{g CE/g}$) and its porridge (173.8 $\mu\text{g CE/g}$) (Table 2).

Table 2. Antioxidant activity and Total phenolic content of extracts from flour, porridge and digested porridge

Samples	Antioxidant activity (% inhibition of DPPH)	Antioxidant activity (Teac value)	Total Phenolic content ($\mu\text{g CE/g}$)	Flavonoid content ($\mu\text{g CE/g}$)
Cowpea	97.7f	507.3f	2856.8e	561.8e
Sorghum	30.9b	170.9b	4535.6f	147.4bc
Sorghum cowpea flour mix	58.7d	250.5c	2720.1d	220.9d
Sorghum-cowpea porridge	66.5e	447.6e	1218.4b	173.8c
Gastric digest	5.7a	21.0a	346.1a	35.1a
Intestinal digest	39.0c	260.9d	1389.8c	142.6b

Values are means of three determinations

Values with the same letter in the same column are not significantly different ($P < 0.05$)

The Trolox equivalent antioxidant activity (TEAC) of the antioxidant is defined as the concentration of Trolox solution (μg) with an equivalent antioxidant potential to 1.0 μg solution of the substance under investigation [43]

From this study, it is obvious that cooking into porridge had a positive impact on the antioxidant activity of the sorghum-cowpea composite flour. This may be due to the release of some phenolic compounds during cooking or structure modification of some bioactive compounds [33]. Although lower total phenolics and flavonoids contents were observed in the cooked porridge when compared with the composite flour, however, higher antioxidant activities were found in the former than the later. This suggested that different structure of the phenolic compounds might influence the antioxidant activities [33]. For example higher epicatechin, catechin, taxifolin, ferulic acid and p -coumaric acid (Table 1) detected in the porridge above its composite flour might be responsible for the high antioxidant activity. These phenolic compounds are easier to lose H atom that is able to scavenge the antioxidant assay. The antioxidant activity of the compound structure was reported to be dependable on the number of included active group (OH) and the position of the active groups. For instance, the ortho position was found to be more active, due to the ability to form intramolecular hydrogen bonding [40]. In the work of [41], boiling and oven steaming was reported to increase the total antioxidant capacity of fresh broccoli. Also, their work further, showed that boiling increased some of the phytochemical content in fresh broccoli and brussels sprout. Our *in vitro* study only represented a useful and simple approach to estimate the release of phenolic compounds from sorghum-cowpea porridge matrix during simulated digestion in the first track of alimentary canal. Phenolic compounds may have protective effects directly in the gastrointestinal tract by scavenging

reactive oxygen species [24] which its usually exposed to from diet and other activation of phagocytes in the gut [42]. Based on the higher antioxidant activity observed in the intestinal phase digest over that of gastric phase, it is speculated that the intestinal cells may be protected efficiently from oxidative stress by the released phenolics compounds with respect to gastric cells [24].

4. CONCLUSIONS

This study has been able to determine the effect of *in vitro* digestion of the antioxidant capacity of sorghum cowpea porridge and also to identify and quantify the phenolic compounds present in the digested and undigested samples through HPLC. Sorghum-cowpea composite porridge contains phenolic antioxidants even after gastric and intestinal digestion with potential to significantly impact human health.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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