



Extruded wholegrain sorghum porridges fortified with baobab fruit and moringa leaves display bioactive phenolics-related health-promoting properties

John Lubaale^a, June C. Serem^b, Megan J. Bester^b, M. Naushad Emmambux^a, Kwaku G. Duodu^{a,*}

^a Department of Consumer and Food Sciences, University of Pretoria, Private Bag X20, Hatfield, 0028, Pretoria, South Africa

^b Department of Anatomy, University of Pretoria, Private Bag X20, Hatfield, 0028, Pretoria, South Africa

ARTICLE INFO

Keywords:

Extrusion
sorghum
Wholegrain
Food-to-food fortification
Antioxidants
Anti-inflammatory
Antidiabetic
Anti-lipogenic

ABSTRACT

Background and objectives: Food-to-food fortification (FtFF) is gaining traction as a strategy to enhance nutritional value of cereal-based foods. Sorghum, a major food crop for millions in the semi-arid tropics lends itself to such FtFF efforts. Such food-to-food fortified (FtF fortified) foods also contain bioactive phenolics with health-promoting properties in relation to potential protection against diet-related non-communicable diseases (NCDs) whose prevalence is increasing in sub-Saharan Africa. In this study, the effects of extrusion cooking of sorghum-based porridges FtF fortified with baobab fruit powder and moringa leaf powder on antioxidant, anti-inflammatory, antidiabetic and anti-lipogenic properties were determined.

Findings: FtFF porridges showed higher phenolic content (phenolic acids and their esters, flavonoids and their glycosides) and greater radical scavenging properties and reduction in advanced glycation end products (AGEs) compared to unfortified porridges. Extruded instant porridges had lower phenolic content, radical scavenging properties and showed less reduction in AGEs compared to conventionally wet-cooked porridges. All porridges exerted antioxidant effects in Caco-2 cells and FtFF inhibited nitric oxide (NO) formation in RAW 264.7 cells. Extracts from all porridge samples exhibited prevention and reduction of adipocyte formation in 3 T3-L1 cells, indicating anti-lipogenic effects.

Conclusion: FtFF (with moringa and baobab) and extrusion cooking can be used to produce instant porridges from wholegrain sorghums with targeted health-promoting properties to address rising non-communicable diseases in sub-Saharan Africa.

Significance and novelty: This study highlights the potential of FtFF with tropical plant foodstuffs to improve health-promoting properties of cereal wholegrain-based starchy staple foods.

1. Introduction

Sorghum (*Sorghum bicolor* (L.) Moench) is a drought-tolerant cereal food crop consumption in the dry, semi-arid regions of the world which include much of sub-Saharan Africa [1,2]. It is also a good source of bioactive phenolic compounds that are being increasingly recognised for their potential health-promoting properties in providing protection against diet-related non-communicable diseases (NCDs) [3]. Currently, there is a growing burden of diet-related non-communicable diseases (NCDs) in developing countries. In 2018, NCDs were responsible for nearly 74% of deaths globally, accounting for >52% of the deaths in

Africa, with rising numbers in low-income countries [4]. NCDs stem primarily from reactive oxygen and nitrogen species as biomarkers of oxidative stress and inflammation [5].

Food-to-food fortification (FtFF) is gaining in focus and increasingly being applied to foods based on cereals such as sorghum to address micronutrient deficiencies using micronutrient-rich foodstuffs (such as moringa) and foods rich in mineral bioaccessibility enhancers (such as baobab fruit pulp) [6–9]. These foodstuffs used for FtFF are also rich in bioactive phenolic compounds [10,11] just as sorghum.

Instant porridges are popular among consumers in urban and pre-urban communities in sub-Saharan Africa mainly due to the

* Corresponding author.

E-mail address: gyebi.duodu@up.ac.za (K.G. Duodu).

<https://doi.org/10.1016/j.nfs.2024.100187>

Received 3 December 2023; Received in revised form 9 July 2024; Accepted 18 July 2024

Available online 20 July 2024

2352-3646/© 2024 The Authors. Published by Elsevier GmbH on behalf of Society of Nutrition and Food Science e.V. This is an open access article under the CC BY-NC-ND license (<http://creativecommons.org/licenses/by-nc-nd/4.0/>).

convenience they offer. Such porridges are produced using extrusion cooking technology. Typical extrusion conditions such as elevated temperatures, pressure and friction during extrusion cooking, can have various effects on bioactive phenolics [12,13] which can in turn affect the health-promoting properties of the resultant products.

Although the primary objective of FtFF is to enhance the nutritional quality of cereal-based staple foods, such FtF fortified foods could also have health-promoting properties in terms of offering protection against NCDs. This is due to contribution of bioactive health-promoting phytochemicals from the cereal staple and the plant foodstuff used for FtFF. In this research, the effects of FtFF of sorghum with moringa leaf and baobab fruit pulp, as well as the effect of extrusion cooking on bioactive phenolics and health-promoting properties of the FtF fortified sorghum-based porridges were determined. The information gained from this research could contribute to broader application of FtFF in enhancing the health-promoting properties of cereal-based foods.

2. Materials and methods

2.1. Materials

Whole grain red non-tannin sorghum was obtained from a local farmer in Mpumalanga, South Africa. Clean sorghum grains were milled into flour using a laboratory hammer mill fitted with a 500 µm mesh size screen. Baobab fruit powder was from Nautica Organic Trading, Durban, South Africa and moringa leaf powder was obtained from Supa Nutri (Pty) Ltd., Cape Town, South Africa.

Human colon adenocarcinoma (Caco-2) cell line, adult murine macrophage (RAW 264.7) cells and murine fibroblast cell line (3 T3-L1) were obtained from Cellonex, South Africa. All chemicals were obtained from Merck, South Africa.

2.1.1. Formulations and preparation of conventionally cooked and instant porridges

The following formulations were used for FtFF:

- A. Wholegrain sorghum flour+corn starch (85:15)
- B. Wholegrain sorghum flour+ferrous sulphate+corn starch (85:0.02:14.98) as a conventional iron fortification standard
- C. Wholegrain sorghum flour+baobab fruit pulp powder+corn starch (85:6:9), with baobab added before processing.
- D. Wholegrain sorghum flour+baobab fruit pulp powder+corn starch (85:6:9), with baobab added after processing.
- E. Wholegrain sorghum+moringa leaf powder+corn starch (85:6:9).
- F. Wholegrain sorghum+moringa leaf powder+baobab fruit pulp powder+corn starch (85:6:6:3), with baobab added before processing.
- G. Wholegrain sorghum+moringa leaf powder+baobab fruit pulp powder+corn starch (85:6:6:3), with baobab added after processing.

The given values for each formulation represent percentage proportions by weight. The purpose for inclusion of corn starch was as a filler to attain a constant final percentage weight for all formulations.

Conventionally cooked porridges were prepared as described by Lubaale et al. [14]. A slurry of sorghum-based flour in distilled water was prepared (3 g of flour to 10 g water). The slurry was heated to boiling temperature and maintained with constant stirring for 15 min to form the conventionally cooked porridge. The porridge was left to cool at ambient temperature, placed in plastic containers, frozen to -20°C and freeze-dried. Freeze-dried porridge flour was crushed to a particle size that passes through a 500 µm opening screen before further analyses. The pre-cooked porridge flour was stored at 4°C in double-sealed, airtight plastic bags.

Instant sorghum-based porridges produced using extrusion cooking were prepared as described by Lubaale et al. [14]. A co-rotating twin-screw extrusion cooker model TX 32 (CFAM Technologies,

Potchefstroom-South Africa) (Length/Diameter = 21.5:1) was used. Porridge formulations prepared as above were extruded separately. The barrel, which comprised five heating zones towards the die was set at 60/70/80/140/140 °C respectively. Water was fed into the system at a dosing rate of 3 L/h (to obtain a final moisture content of 20% calculated based on the moisture content of the flours) and the feed rate was 10 kg/h. A die opening of 3 mm was used and the screw speed was maintained at 250 rpm. Extrudates were collected three times after every 30 min to produce triplicates. They were dried immediately in a forced draught oven at 90°C for 5 min to a moisture content below 10%. The cooled extrudates were milled using an air-cooled analytical mill to a maximum particle size of 500 µm. The milled extrudates were stored at 4°C in double-sealed, airtight plastic bags.

2.1.2. Preparation of phenolic extracts

Phenolic extracts for the Folin-Ciocalteu and in vitro radical scavenging assays were prepared as described by Apea-Bah et al. [15]. Approximately 1 g of each dry sorghum-based porridge sample was extracted in duplicate using 10 mL acidified methanol (1% (v/v) conc HCl in methanol) by magnetic stirring for 2 h. The suspension was centrifuged at 1650 g for 10 min at 4°C and the supernatant was collected. The residue was similarly re-extracted twice each with 10 mL acidified methanol for 30 min. The supernatants were then pooled together and stored at -20°C in the dark before analysis.

Extracts for liquid chromatography-mass spectrometry (LC-MS) analysis were prepared as described by Nderitu et al. [16]. Approximately 5 g of each sorghum-based porridge flour was extracted using 10 mL 1% (v/v) HCl in methanol for 2 h with vortexing after every 5 min. The suspension was centrifuged at 1650 g for 10 min at 4°C and the supernatant was collected and filtered through 0.45 µm Acrodisc PSF syringe filters (Pall Life Sciences, Ann Arbor MI, USA) into 1.5 mL amber vials ahead of chromatographic analysis.

2.2. Analyses

2.2.1. Total phenolic content (TPC), in vitro radical scavenging assays and anti-glycation assay

TPC and in vitro ABTS radical scavenging were conducted as described by Apea-Bah et al. [17]. In each well of a 96-well microplate, 18.2 µL volume of the sample extract or catechin standard solution (0–0.5 mg/mL) was reacted with 36.4 µL of 10% Folin-Ciocalteu reagent (diluted with distilled water) and 145.4 µL of 700 mM sodium carbonate. The reaction mixture was incubated for 2 h in the dark after which absorbance was read at 750 nm using an Omega FluoSTAR microplate reader (BMG Labtechnologies, Ortenberg, Germany). Total phenolic content was calculated with the aid of the catechin standard calibration curve and expressed as milligrams of catechin equivalents per gram (mg CE/g) dry weight basis.

For ABTS radical scavenging, the extracts were diluted depending on their concentration with acidified methanol (1% (v/v) conc HCl in methanol). The ABTS radical cation stock solution was prepared by reacting equal volumes of 7 mM ABTS salt with 2.54 mM potassium persulphate in distilled water for 12–16 h at room temperature in the dark. A working solution was prepared by diluting the ABTS mother solution with 0.2 M phosphate-buffered saline at pH (7.4) in the ratio of 1:29. In each well of a 96-well microplate, 10 µL of the diluted sample extract extracts or Trolox standards (0–600 mM prepared in acidified methanol) were mixed with 190 µL of the working solution and incubated in the dark for 30 min at room temperature (20°C). The absorbance was read at 750 nm using the Omega FluoSTAR microplate reader (BMG Labtechnologies, Ortenberg, Germany). With the aid of a Trolox standard calibration curve, the ABTS radical scavenging capacity was calculated and expressed as micromole Trolox equivalent per gram sample (µmol TE/g) dry weight basis.

Oxygen radical absorbance capacity (ORAC) were conducted as described previously [18]. The extracts were diluted 10 times with 0.1 M

phosphate-buffered saline (PBS), pH 7.4. A 165 μL volume of 8.8 nM disodium fluorescein working solution and 25 μL of 0.24 M aqueous 2,2'-Azobis(2-amidinopropane) dihydrochloride (AAPH) were added to 10 μL of each diluted extract in a 96-microplate well. The reaction mixtures were shaken to mix well and incubated at 37 °C, while measuring their fluorescence decay every minute for 2 h at 485 nm excitation and 520 nm emission wavelengths, using an Omega FluoSTAR microplate reader (BMG Labtech, Ortenberg, Germany). The ORAC values of the samples were calculated using the net area under the fluorescence decay curves and expressed as mmol TE/g flour, dry weight basis.

The anti-glycation assay for anti-diabetic properties was performed as described by Siddiqui et al. [19]. A 50 μL volume of diluted 1% conc. Hydrochloric acid-methanolic extracts of sorghum-based porridges (diluted in PBS to yield 500 $\mu\text{g}/\text{mL}$ in the well) were transferred into a 96-well opaque fluorescence plate, followed by the addition of 50 μL of bovine serum albumin (BSA, 40 mg/mL, final concentration 10 mg/mL) and 50 μL methylglyoxal (MGO, 56 mM, final concentration 14 mM). Thereafter, 50 μL of 0.1 M PBS pH 7.4 was added to the plate and incubated at 37 °C for 7 days. After incubation, fluorescence was measured at an emission of 330 nm and excitation of 420 nm. The positive control contained 50 μL BSA, 50 μL MGO and 100 μL buffer while the negative control consisted of 50 μL BSA and 150 μL buffer. Sample controls consisted of 50 μL sample, 50 μL MGO, and 100 μL buffer (no BSA). The % advanced glycation end-product (AGE) formation relative to the 100% AGE formation by BSA and MGO alone was calculated.

2.2.2. Characterisation and quantification of phenolic compounds using LCMS

This was done using a Waters Acquity Ultra-Performance Liquid Chromatograph (UPLC) with a binary pump system (Waters, Milford, MA, USA) coupled to a Waters Synapt G2 system comprising a Quadrupole-Time of Flight Mass Spectrometer (QToF-MS) (Waters, Milford, MA, USA) using an electrospray ionization (ESI) source as described [15]. An incorporated photodiode array (PDA) detector (Waters, Milford, MA, USA) was set to monitor phenolic compounds at the wavelength range of 230–500 nm. Separation was done on a Waters BEH C18 (100 \times 2.1 mm, 1.7 μm) reverse phase column. The mobile phase consisted of 0.1% (v/v) aqueous formic acid (solvent A) and 0.1% (v/v) aqueous formic acid in acetonitrile (solvent B). Gradient elution of phenolic compounds was conducted as follows: 100% A (0–22 min); 72% A (22–22.5 min); 60% A (22.5–23 min); 0% A (23–24.5); 100% A (24.5–26). The injection volume was 3 μL , and the flow rate was 0.3 mL/min. Ionization was in the negative mode with a capillary voltage of 2.5 kV and a cone voltage of 25 V. Identification of phenolic compounds in the sample extracts was done by comparing the retention times, mass and UV-visible spectral data of the peaks which were observed in this study with those of pure authentic phenolic compound standards and with what has been reported in the literature. Integrated peak areas of phenolic compounds in extracts were compared with those of standards to quantify the phenolic compounds. Leucine enkephalin (molecular weight 555 Da) was used as lock mass. Data collection was done using MassLynx v. 4.1 software (Waters, Milford, MA, USA).

2.2.3. Culturing of cell lines (Caco-2, 3 T3-L1 and RAW 264.7)

Cell culturing was done as described by Viadel et al. [20] with Dulbecco's minimum essential media with Earle's salts, L-glutamine, sodium bicarbonate and sodium pyruvate supplemented with 10% v/v foetal bovine serum, 1% v/v non-essential amino acids and 1% v/v antibiotic-antimycotic solution. Cell monolayers were washed three times with buffer solution, and the cells were detached either using tryple express (Caco-2 and 3 T3-L1 cells) or with a cell scraper (RAW 264.7 cells) prior to the analyses.

2.2.4. Cellular antioxidant activity (CAA)

CAA was determined by monitoring the change in fluorescence in

Caco-2 cells upon treatment with extracts from the porridge samples due to conversion of dichlorofluorescein diacetate (DCFH-DA) to dichlorofluorescein (DCF) as described by Blasa et al. [21]. Caco-2 cells were plated in a 96-well plate at a concentration of 10×10^4 cells/mL (1×10^4 cells/100 μL) and incubated at 37 °C for 24 h. Following incubation, 50 μL of 75 μM DCFH-DA was added to each well to a final concentration of 25 μM . After an incubation period of 1 h at 37 °C, the medium was removed, and the cells were gently rinsed once with PBS. Immediately, a 50 μL volume of diluted 1% conc. Hydrochloric acid-methanolic extracts of sorghum-based porridges (diluted in PBS to yield 50 $\mu\text{g}/\text{mL}$ in the well) were added, thereafter 50 μL of 4.9 μM 2,2'-azobis(2-amidinopropane) dihydrochloride (AAPH) was added. The blank control was Caco-2 cells exposed to DCFH-DA and PBS, and the positive control was cells exposed to DCFH-DA and AAPH. The change in fluorescence was measured every 2 min for 60 min at excitation and emission wavelengths of 485 nm and 520 nm, respectively.

2.2.5. Anti-inflammatory activity by nitric oxide (NO) scavenging in RAW 264.7 cells

This was done by determining % NO production as reflected by absorbance at 570 nm of the reaction mixture of RAW 264.7 cells treated with extracts from the porridge samples relative to when the RAW 264.7 cells were exposed to only lipopolysaccharide (LPS) (100% NO produced) as described by Malan et al. [22]. Cells were grown until confluent at 37 °C and 5% CO₂ in DMEM supplemented with 10% foetal bovine serum (FBS) and 1% antibiotics. When confluent, cells were serum starved for 24 h. After 24 h 80 μL cells (final concentration 1×10^6 cells/mL) were combined with 10 μL of diluted 1% conc. Hydrochloric acid-methanolic extracts of sorghum-based porridges (diluted in PBS to yield 50 $\mu\text{g}/\text{mL}$ in the well) and 10 μL LPS (LPS, final concentration 100 ng/mL) and further incubated for 24 h. After 24 h, 50 μL of the cell supernatant was assayed for nitric oxide (NO) production using 50 μL Griess reagent (0.1% N-1-naphthyl ethylenediamine dihydrochloride, 1% sulphanilamide in 2.5% phosphoric acid) and the absorbance was read at 570 nm. Results were reported as % NO production, compared with RAW 264.7 cells exposed to only LPS (100% NO produced). Cell viability was determined with the crystal violet assay.

2.2.6. Anti-lipogenic activity by lipid droplet reduction in 3 T3-L1 cells

Lipid droplet reduction and inhibition in 3 T3-L1 cells were performed according to Ibrahim et al. [23]. Briefly, 3 T3-L1 pre-adipocyte cells were maintained in DMEM containing 10% FBS and 1% antibiotic solution (DMEM/FBS). Confluent cells were plated at a concentration of $1 \times 10^3/100$ μL in a 96-well plate and grown for 3 days until confluent. For the lipid droplet reduction assay, cells were differentiated for 14 days with differentiation medium (DM) 1 (DMEM/FCS containing final concentrations of 10 $\mu\text{g}/\text{mL}$ insulin, 25 mM IBMX, 50 μM dexamethasone, and 100 μM rosiglitazone) changed on days 4 and 7, and then with DM 2 (DMEM/FCS + final concentration of 10 $\mu\text{g}/\text{mL}$ insulin) on day 10. On day 14, cells were replenished with 90 μL DMEM/FCS only and exposed to 10 μL of the sample.

Staining with Oil red O dye was used to determine lipid content. The cultures after differentiation and exposure to sorghum-based porridge extracts were fixed with 2% formaldehyde for 30 min at 37 °C. The formaldehyde was then removed, the plates were dried and 100 μL ORO solution (5% w/v in 60% isopropanol, then further diluted 1.7 \times in ddH₂O) was added for 1 h. The plate was then rinsed with water and left to dry. Phase contrast images were taken, before dye extraction with 100 μL of a 60% isopropanol solution for 5 min. Absorbance of the Oil red O dye upon being taken up by cells was measured at 405 nm and the data was reported as % lipid present relative to unexposed differentiated 3 T3-L1 cells (100% lipid formation).

2.3. Statistical analyses

LCMS analysis for quantification of phenolic compounds was

Table 1

Retention time, UV–visible absorption maxima and mass spectral characteristics of phenolic compounds found in extracts of cooked sorghum (Sorg), Baobab fruit pulp (Bao), moringa (Mor), extruded sorghum (ESA) extruded sorghum fortified with baobab (EBA), extruded sorghum fortified with moringa (EMA) and extruded sorghum fortified with moringa and baobab (EGA).

t_R (min)	λ_{max} (nm)	[M- H] ⁻ (<i>m/z</i>)	MS/MS fragments (Intensity, %)	Proposed compounds	Peak	Sorg	Bao	Mor	ESA	EBA	EMA	EGA
Hydroxybenzoic acid derivatives												
10.85	254, 253	137	137 (67), 93 (100)	4-Hydroxybenzoic acid	6	+	+	+	+	+	+	+
8.08	294, 259, 230	153	153 (42), 109 (100)	Protocatechuic acid	2	+	+	+	+	+	+	+
15.27	297, 263	167	167 (17), 123 (1), 108 (100)	Vanillic acid	20	+	+	+	+	+	+	+
6.48	272, 230	169	169 (72), 125 (100), 107 (3)	Gallic acid	1	+	+	+	+	+	+	+
19.56	230	197	197 (77), 153 (45)	Syringic acid	32	+	+	+	+	+	+	+
Hydroxycinnamic acid derivatives												
15.29	310, 230	163	163 (25), 147 (10), 145 (53), 119 (76)	p-Coumaric acid	21	+	-	+	-	-	+	+
10.16	272, 230	179	179 (42), 164 (1), 161 (1), 135 (100)	Caffeic acid	5	+	-	+	+	+	+	+
18.48	324, 244	193	133 (100), 161 (15)	Ferulic acid	28	+	-	+	+	+	+	+
17.33	328, 325	223	223 (38), 208 (21), 179 (9), 164 (40)	Sinapic acid	27	+	+	-	-	+	+	+
Phenolic esters												
15.51	230	237	237 (10), 163 (12)	p-Coumaroyl glycerol	23	+	-	-	+	+	+	+
14.25	288, 230	253	253 (40), 179 (11), 161 (100)	Caffeoylglycerol	15	+	-	+	+	+	+	+
24.86	230	255	255 (100), 179 (7)	Dihydrocaffeoylglycerol	46	+	+	+	+	+	+	+
11.07	309, 230	337	337 (26), 191 (29), 173 (13), 163 (100)	3-p-coumaroylquinic acid	7	-	-	+	-	-	+	+
13.84	309, 230	337	337 (20), 191 (9), 173 (100), 163 (12)	4-p-coumaroylquinic acid	14	-	-	+	-	-	+	+
9.43	325, 230	353	353 (54), 191 (100), 179 (53)	3-Caffeoyl-quinic acid	3	+	+	+	+	+	+	+
11.91	325, 230	353	353 (70), 191 (45), 179 (63)	4-Caffeoyl-quinic acid	10	+	+	+	+	+	+	+
12.32	325, 230	367	367 (26), 193 (100), 191 (24), 173 (20)	3-Feruloylquinic acid	11	-	-	+	-	-	+	+
14.91	325, 230	367	367 (24), 193 (17), 191 (6), 173 (100)	4-Feruloylquinic acid	19	-	-	+	-	-	+	+
9.73	230	399	399 (31), 253 (2), 179 (50), 135 (81)	Coumaroyl-caffeoyl-glycerol	4	-	-	+	-	-	-	-
18.77	230	415	415 (13), 253 (40), 179 (11), 161 (55)	Dicafeoylglycerol	30	+	-	-	+	+	+	+
14.72	230	468	468 (14), 179 (14), 161 (73)	Dicafeoyl spermidine	17	+	-	-	+	+	+	+
Flavonols												
19.78	365, 266, 234	285	285 (100), 257 (2), 243 (1), 241 (1), 151 (5)	Kaempferol	33	+	+	+	+	+	+	+
23.90	363, 230	301	301 (100), 179 (22), 151 (69), 121 (15), 107 (18)	Quercetin	41	+	+	+	+	+	+	+
20.41	350, 265, 230	317	317 (100), 289 (4), 179 (55), 107 (19)	Myricetin	36	+	+	-	+	+	+	+
Flavonol glycosides												
19.53	350, 265, 230	447	447 (100), 285 (27), 151 (2)	Kaempferol glycoside	31	+	+	+	+	+	+	+
14.67	230	449	449 (10), 285 (65), 151 (100)	Dihydrokaempferol glycoside	16	+	-	+	+	+	+	+
22.85	230, 251	463	463 (100), 301 (41), 179 (3), 121 (1)	Quercetin glycoside	38	+	+	+	+	+	+	+
20.04	352, 230	477	477 (60), 301 (75), 229 (7), 179 (11), 121 (4)	Quercetin glucuronide	35	-	-	+	-	-	+	+
17.31	354, 255	609	609 (100), 301 (75), 179 (4), 121 (9)	Rutin	26	+	+	+	+	+	+	+
Flavan-3-ols												
11.36	274, 230	289	289 (100), 245 (36), 203 (46), 179 (14)	Catechin	9	+	+	-	+	+	+	+
13.48	274, 230	289	289 (100), 245 (36), 203 (46), 179 (13)	Epicatechin	13	+	+	-	+	+	+	+
Flavan-3-ol glycosides												
15.43	272	451	451 (6), 289 (43), 245 (5), 203 (10), 179 (9)	Epicatechin glycoside	22	-	+	+	-	+	+	+
Flavanones												
24.19	288, 234	271	271 (100), 177 (10), 151 (47), 119 (59)	Naringenin	42	+	-	-	+	+	+	+
23.05	289, 230	287	287 (2), 151 (29), 135 (100), 125 (5)	Eriodictyol	39	+	-	-	+	+	+	+
24.27	288, 234	301	301 (100), 151 (67), 135 (4), 125 (6)	Hesperetin	44	+	+	+	+	+	+	+
Flavanone glycosides												
17.04	324, 229	433	433 (33), 271 (100), 177 (19), 151 (92), 119 (35)	Naringenin glycoside	25	+	+	-	-	-	-	-
19.98	230	449	449 (15), 287 (34), 151 (100), 135 (67), 125 (16)	Eriodictyol glycoside	34	+	-	-	+	+	+	+
21.72	283, 230	579	579 (100), 459 (11), 271 (51), 177 (4), 151 (54), 119 (15)	Naringin	37	+	+	+	+	+	+	+

(continued on next page)

Table 1 (continued)

t_R (min)	λ_{max} (nm)	[M- H] ⁻ (<i>m/z</i>)	MS/MS fragments (Intensity, %)	Proposed compounds	Peak	Sorg	Bao	Mor	ESA	EBA	EMA	EGA
Flavones												
24.24	346, 334, 266, 230	269	269 (100), 151 (11), 117 (21)	Apigenin	43	+	-	-	+	+	+	+
23.64	340, 266, 230	285	285 (100), 151 (1), 133 (24)	Luteolin	40	+	+	+	+	+	+	+
Flavone glycosides												
24.41	230, 646	431	431 (78), 269 (4), 151 (11), 117 (6)	Vitexin	45	+	+	+	+	+	+	+
18.60	350, 265	447	447 (100), 285 (100), 151 (1)	Luteolin glycoside	29	+	+	+	+	+	+	+
16.04	232, 530	563	563 (100), 269 (38), 151 (8), 117 (61)	Glucosyl-arabinosyl apigenin	24	+	+	+	+	+	+	+
Proanthocyanidins												
11.33	279, 233	577	577 (26), 451 (45), 425 (20), 289 (100), 125 (91)	Procyanidin dimer	8	-	+	-	-	-	-	-
12.50	279, 234, 230	865	865 (18), 577 (29), 451 (9), 425 (21), 289 (100)	Procyanidin trimer	12	-	+	-	-	-	-	-
14.81	274, 233	1154	1154 [9], 865 (19), 577 (28), 425 (10), 289 (100)	Procyanidin tetramer	18	-	+	-	-	-	-	-

conducted on two samples analysed independently in triplicate ($n = 6$). All other experiments were conducted on three samples analysed independently in triplicate ($n = 9$). One-way analysis of variance was used to determine the differences in sample parameters. Fisher's LSD test at a 0.05 level of significance was applied. Statistica 10 (StatSoft Inc., Tulsa, OK, USA) was used.

3. Results and discussion

3.1. Effect of FtFF and extrusion on phenolic compounds

Table 1 shows the chromatographic and mass spectral data of the phenolic compounds identified in the sorghum-based porridges and the baobab fruit and moringa leaf powders used as fortificants. The phenolic compounds were identified based on comparison with appropriate standards and their fragmentation patterns obtained from the literature. The phenolic compounds consisted of various phenolic acids and their derivatives, flavonoids and their glycosides as well as proanthocyanidins which were only present in baobab fruit pulp.

Table 2 shows the effect of FtFF and extrusion on the concentration of phenolic compounds identified in the sorghum-based porridges. The plant foodstuffs used as fortificants contained high levels of some of the phenolic compounds. For example, in comparison with sorghum and baobab, moringa leaf powder contained the highest levels of total phenolic acids, total phenolic acid esters and total flavonoid glycosides. Baobab fruit pulp contained the highest levels of flavonoid aglycones. Therefore, although the proportion of baobab fruit pulp and moringa leaf powder in the FtF fortified porridges were at a level of 6%, they contributed significantly to the concentration of phenolic compounds in the porridges.

All the extruded instant sorghum porridges had decreased concentration of phenolic compounds (total phenolic acids, total phenolic acid esters, total flavonoid aglycones, and total flavonoid glycosides) compared to conventionally cooked sorghum porridge (Table 2). Similar reductions in levels of phenolic compounds during extrusion cooking have been reported in sorghum [2,13,24], faba bean and kidney bean [25]. Extrusion cooking involves application of high temperature and pressure as well as high moisture conditions. It is suggested that such conditions could lead to reactions such as decarboxylation and polymerisation of phenolic compounds [26,27] as well as binding of phenolic compounds to other chemical components of food material being extruded for example flavonoids can bind to proteins [28] resulting in their reduced extractability. As mentioned earlier, proanthocyanidins were only identified in baobab fruit pulp but were absent

in all samples fortified with baobab fruit pulp possibly due to their breakdown following extrusion cooking.

3.2. Total phenolic content (TPC) and antioxidant properties

Table 3 shows the TPC and in vitro radical scavenging properties of the sorghum-based porridges. The TPC of sorghum (404 mg CE/100 g) was within the range (100–1300 mg CE/100 g) reported by Kruger et al. [29]. Baobab fruit pulp had higher TPC (4792 mg CE/100 g) than moringa leaf powder and plain sorghum. The TPC of moringa leaf powder (4300 mg CE/100 g or 43 mg CE/g) was within the range reported by Leone et al. [30] (29–53 mg CE/g). Of the three raw materials (plain sorghum and the plant foodstuffs), moringa leaf powder had the highest ABTS radical scavenging activity and ORAC, and both baobab fruit pulp and moringa leaf powder had higher TPC and radical scavenging activity than plain sorghum.

As would be expected, FtFF of the sorghum flour with baobab, moringa and a combination of the two increased TPC (by 12% - 64%), ABTS radical scavenging (by 31% - 177%) and ORAC (by 27% - 189%) compared to sorghum flour (Table 3). Sorghum-based porridges fortified with a combination of baobab fruit pulp and moringa leaf powder (whether the sorghum was added before or after processing) showed the largest increases in TPC and radical scavenging properties. This may be indicative of potential synergistic effects of the different phenolic compounds in the baobab fruit pulp and moringa leaf powder in exerting antioxidant properties [31].

Extrusion cooking in general lowered the TPC and radical scavenging properties. Extruded instant porridges had lower TPC (by 11% - 44%), lower ABTS radical scavenging activity (by 14% - 42%) and lower ORAC (by 11% - 48%) compared to conventionally wet cooked porridges (Table). This agrees with the observed decrease in concentration of phenolic compounds during extrusion cooking reported in Table 2. As mentioned earlier, this could be due to various reactions that occur during the extrusion cooking process which would have led to either degradation of phenolic compounds or reduced their extractability.

The ability of extracts from the porridges to exert antioxidant activity in Caco-2 cells was also determined as an indicator of potential to protect against oxidative stress (Fig. 1). Extracts from all the sorghum-based porridges (conventionally cooked and instant) exhibited high CAA of at least 83%. Extracts from instant sorghum-based porridges fortified with baobab alone or baobab with moringa had higher CAA than those fortified with moringa alone. Apart from the extract from extruded sorghum FtF fortified with moringa, extracts from all the other extruded samples had higher CAA (by up to 11.4%) than the extract from cooked

Table 2
Effect of food-to-food fortification and extrusion on the concentration of phenolic compounds (µg/g) in wholegrain sorghum-based porridges.

Compound	Cooked Sorghum	Baobab	Moringa	Extruded Sorghum	Extruded sorghum + Baobab	Extruded sorghum + Moringa	Extruded sorghum + Moringa + Baobab
Phenolic acids							
p-Hydroxybenzoic acid	¹ 617 ^{c2} ± 66	86 ^a ± 21	371 ^b ± 12	340 ^b ± 29	405 ^b ± 20	341 ^b ± 44	371 ^b ± 39
Protocatechuic acid	121 ^c ± 13	599 ^e ± 9	271 ^d ± 11	28 ^a ± 5	50 ^b ± 3	72 ^b ± 4	86 ^{bc} ± 35
Vanillic acid	12 ^d ± 0	26 ^e ± 2	5 ^a ± 2	4 ^a ± 0	10 ^{cd} ± 1	6 ^{ab} ± 0	9 ^{bc} ± 1
Galic acid	7 ^a ± 1	42 ^c ± 2	110 ^d ± 7	2 ^a ± 0	13 ^{ab} ± 11	5 ^a ± 1	20 ^b ± 0
Syringic acid	21 ^b ± 0	33 ^c ± 4	51 ^d ± 6	1 ^a ± 0	6 ^a ± 0	4 ^a ± 2	22 ^b ± 3
p-Coumaric acid	181 ^c ± 20	³ ND	919 ^d ± 34	ND	ND	42 ^a ± 3	101 ^b ± 3
Caffeic acid	941 ^d ± 49	ND	647 ^c ± 7	263 ^b ± 25	285 ^b ± 5	256 ^b ± 13	305 ^b ± 8
Ferulic acid	92 ^e ± 0	ND	2 ^a ± 0	42 ^b ± 0	58 ^d ± 3	50 ^c ± 3	55 ^d ± 3
Sinapic acid	4 ^a ± 0	3 ^a ± 0	ND	ND	24 ^b ± 3	4 ^a ± 0	20 ^b ± 0
Total Phenolic acids	1996	789	2376	680	851	780	989
Phenolic acid esters							
p-Coumaroyl glycerol	1837 ^c ± 82	ND	ND	1491 ^b ± 59	1220 ^a ± 8	1186 ^a ± 34	1091 ^a ± 84
Caffeoyl glycerol	3104 ^e ± 79	ND	57 ^a ± 4	1743 ^d ± 97	1603 ^{cd} ± 27	1277 ^b ± 29	1470 ^c ± 6
Dihydrocaffeoyl glycerol	501 ^a ± 62	3008 ^b ± 91	2636 ^b ± 184	169 ^a ± 8	617 ^a ± 45	235 ^a ± 8	418 ^a ± 48
3-Coumaroyl quinic acid	ND	ND	5309 ^b ± 53	ND	ND	36 ^a ± 3	42 ^a ± 6
4-Coumaroyl quinic acid	ND	ND	2882 ^c ± 139	ND	ND	14 ^a ± 2	22 ^a ± 2
3-Caffeoyl quinic acid	57 ^a ± 19	93 ^a ± 4	22695 ^b ± 1350	12 ^a ± 1	9 ^a ± 1	64 ^a ± 4	136 ^a ± 12
4-Caffeoyl quinic acid	1594 ^a ± 155	2819 ^a ± 69	464493 ^b ± 4985	523 ^a ± 91	277 ^a ± 22	1708 ^a ± 78	1730 ^a ± 130
3-Feruloyl quinic acid	ND	ND	390 ^b ± 8	ND	ND	239 ^a ± 10	406 ^c ± 4
4-Feruloyl quinic acid	ND	ND	265 ^d ± 3	ND	ND	247 ^c ± 14	319 ^e ± 9
Coumaroyl caffeoyl glycerol	ND	ND	41751 ^a ± 2279	ND	ND	ND	ND
Dicafeoyl glycerol	3506 ^c ± 82	ND	ND	2253 ^a ± 41	2459 ^b ± 92	2292 ^a ± 20	2408 ^b ± 17
Dicafeoyl spermidine	663 ^c ± 58	ND	16 ^a ± 5	728 ^c ± 68	446 ^b ± 4	492 ^b ± 75	524 ^b ± 31
Total Phenolic acid esters	11,262	5920	540,494	6919	6631	7790	8566
Flavonoid aglycones							
Kaempferol	115 ^f ± 5	16 ^a ± 0	61 ^d ± 0	20 ^a ± 0	40 ^b ± 6	46 ^{bc} ± 4	53 ^c ± 1
Quercetin	15 ^b ± 0	17 ^b ± 0	83 ^d ± 1	11 ^a ± 0	11 ^a ± 0	17 ^b ± 1	36 ^c ± 0
Myricetin	5 ^d ± 0	9 ^f ± 0	ND	1 ^a ± 0	6 ^c ± 0	3 ^b ± 0	6 ^c ± 0
Catechin	7 ^b ± 1	309 ^d ± 6	ND	2 ^a ± 0	25 ^c ± 0	1 ^a ± 0	19 ^c ± 1
Epicatechin	145 ^a ± 8	3563 ^b ± 226	ND	20 ^a ± 4	133 ^a ± 17	3 ^a ± 1	128 ^a ± 13
Naringenin	71 ^d ± 6	ND	ND	33 ^{bc} ± 8	41 ^c ± 6	27 ^b ± 1	38 ^{bc} ± 6
Apigenin	29 ^d ± 1	ND	ND	11 ^b ± 1	19 ^c ± 2	10 ^b ± 0	27 ^d ± 5
Eriodictyol	119 ^e ± 3	ND	ND	52 ^c ± 4	82 ^d ± 5	6 ^a ± 0	28 ^b ± 0
Hesperitin	10 ^b ± 0	12 ^c ± 0	78 ^g ± 0	4 ^a ± 0	17 ^d ± 0	19 ^e ± 2	32 ^f ± 0
Luteolin	99 ^e ± 4	5 ^a ± 0	29 ^b ± 0	32 ^b ± 2	36 ^{bc} ± 6	41 ^{cd} ± 4	45 ^d ± 1
Total flavonoid aglycones	610	3931	251	186	410	173	406
Flavonoid glycosides							
Kaempferol glycoside	8 ^a ± 1	23 ^a ± 1	1425 ^c ± 42	5 ^a ± 1	7 ^a ± 0	213 ^b ± 11	244 ^b ± 17
Dihydrokaempferol glycoside	34 ^f ± 0	ND	8 ^b ± 1	15 ^c ± 2	11 ^{cd} ± 0	9 ^{bc} ± 0	13 ^{de} ± 2
Quercetin glucoside	24 ^a ± 4	23 ^a ± 0	1687 ^d ± 37	8 ^a ± 1	9 ^a ± 2	281 ^b ± 14	360 ^c ± 21
Rutin	60 ^{ab} ± 2	100 ^c ± 1	5604 ^e ± 12	22 ^a ± 1	29 ^{ab} ± 1	681 ^c ± 57	861 ^d ± 40
Epicatechin glucoside	ND	ND	293 ^e ± 3	ND	ND	5 ^b ± 1	9 ^c ± 0
Eriodictyol glucoside	96 ^b ± 4	ND	ND	36 ^a ± 2	36 ^a ± 2	35 ^a ± 2	38 ^a ± 3
Naringenin glucoside	53 ^c ± 2	22 ^b ± 2	ND	27 ^{bc} ± 2	30 ^{cd} ± 0	27 ^{bc} ± 2	36 ^d ± 6
Naringin	7 ^a ± 0	8 ^a ± 1	577 ^b ± 49	10 ^a ± 1	9 ^a ± 0	11 ^a ± 1	5 ^a ± 0
Vitexin	7 ^c ± 0	1 ^a ± 0	40 ^d ± 1	4 ^b ± 0	3 ^b ± 0	4 ^b ± 0	6 ^c ± 0
Luteolin glucoside	41 ^a ± 1	24 ^a ± 0	1261 ^c ± 23	41 ^a ± 0	36 ^a ± 2	190 ^b ± 9	290 ^b ± 0
Glucosyl-arabinosyl apigenin	23 ^c ± 1	1 ^a ± 0	10 ^d ± 0	6 ^{bc} ± 1	8 ^{cd} ± 0	5 ^b ± 0	6 ^{bc} ± 0
Total flavonoid glycosides	353	202	10,865	174	151	1461	1607
Proanthocyanidins							
Procyanidin dimer	ND	896 ^a ± 47	ND	ND	ND	ND	ND
Procyanidin trimer	ND	947 ^a ± 17	ND	ND	ND	ND	ND
Procyanidin tetramer	ND	2.02 ^a ± 0.09	ND	ND	ND	ND	ND
Total proanthocyanidins		1845.02					

¹ Values are the means±SD of two samples of each formulation analysed independently in triplicate (n = 6).

² Values within the same column followed by different letters are significantly different (p < 0.05).

³ ND = not detected.

Table 3

Effects of extrusion cooking and food-to-food fortification of sorghum with baobab fruit pulp and moringa leaf powder on total phenolic content (TPC, mgCE/100 g⁴), 2,2'-azino-bis(3-ethylbenzothiazoline-6-sulphonate) radical (ABTS, μMTE/100 g⁴) radical scavenging capacity and Oxygen Radical Absorbance Capacity (ORAC, μMTE/100 g⁴) of sorghum-based porridges.

		Plain sorghum	Baobab fruit pulp	Moringa leaf powder	Sorg+Bao*	Sorg+Bao**	Sorg+Mor	Sorg+Mor+Bao*	Sorg+Mor+Bao**
Conventionally cooked	TPC	12404 ^{d2} ± 19	4792 ⁱ ± 102	4300 ^h ± 167	465 ^e ± 45	452 ^e ± 20	563 ^f ± 49	664 ^g ± 51	547 ^f ± 33
	ABTS	2538 ^b ± 50	28648 ^k ± 512	37506 ^l ± 365	5271 ^h ± 416	4221 ^e ± 8	3334 ^d ± 201	8151 ^j ± 805	6155 ⁱ ± 34
Extrusion cooked	ORAC	90 ^{cd} ± 6	385 ^h ± 14	463 ^g ± 17	132 ^e ± 15	146 ^e ± 12	152 ^e ± 16	214 ^g ± 23	200 ^{fg} ± 14
	TPC	250 ^a ± 16	N/A ³	N/A	278 ^{ab} ± 28	401 ^d ± 12	316 ^c ± 17	405 ^{de} ± 50	536 ^f ± 29
	ABTS	2177 ^a ± 18	N/A	N/A	3044 ^c ± 40	4851 ^{fg} ± 198	3301 ^{cd} ± 393	5071 ^g ± 134	6030 ⁱ ± 211
	ORAC	62 ^{ab} ± 11	N/A	N/A	88 ^{de} ± 14	153 ^e ± 17	79 ^{bc} ± 16	132 ^e ± 45	179 ^f ± 12

¹ Values are the means ±1 Standard deviation of three samples of each plant food analysed independently in triplicate (n = 9).

² Means with different superscripts letters in a row differ significantly (p ≤ 0.05).

³ N/A- Not applicable.

⁴ mgCE- milligrams of catechin equivalents, μMTE- micro molar Trolox equivalents.

* Baobab added before processing.

** Baobab added after processing.

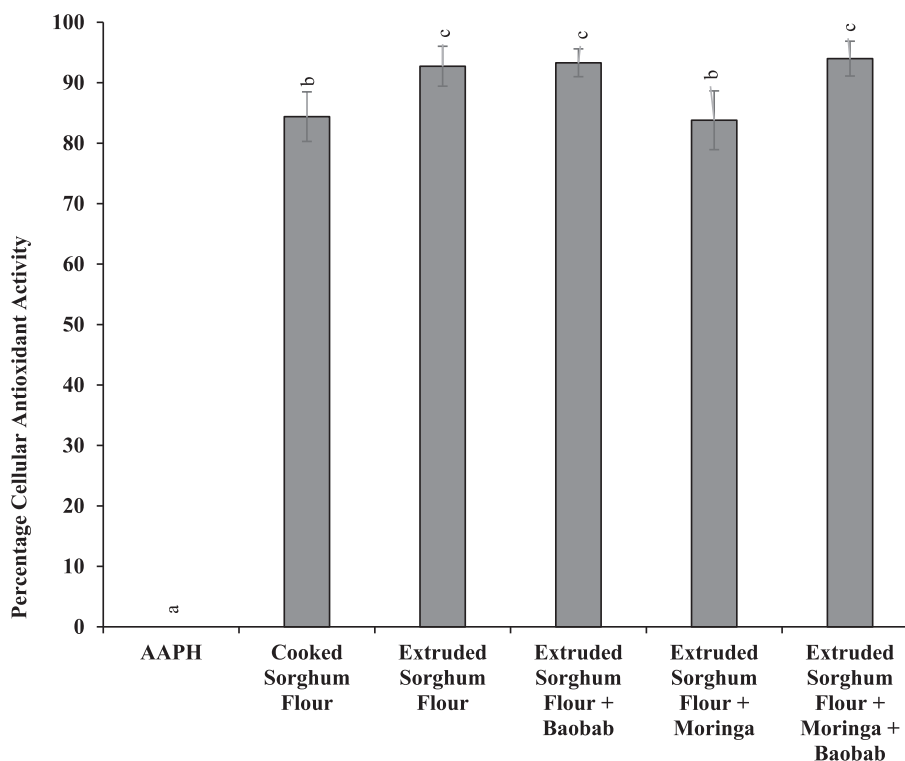


Fig. 1. Cellular antioxidant activity against AAPH-induced oxidative damage by extracts from sorghum-based porridge extracts in the Caco-2 cell line. Bars with different letters are significantly different (p ≤ 0.05). Error bars indicate standard deviation. Data are the mean of three samples analysed independently in triplicate (n = 9).

(unextruded) sorghum flour. Therefore, even though extrusion cooking reduced TPC and in vitro radical scavenging properties of the sorghum-based porridges, it seemed to enhance CAA in Caco-2 cells. The observed antioxidant effects of the extracts in Caco-2 cells can be attributed to the phenolic compounds identified in them. Extrusion reduced phenolic acid esters, flavonoid glycosides and proanthocyanidins in Ftf-fortified sorghum-based porridges while consistently increasing the free phenolic acids and flavonoid aglycones. Smaller phenolic compounds such as free phenolic acids have been argued to have greater cell permeability [32,33] and therefore able to more easily diffuse into Caco-2 cells than their more complex counterparts such as flavonoid glycosides and proanthocyanidins and thus exert greater CAA. The higher content of phenolic acid esters and aglycones in the Ftf-fortified and extruded

sorghum-based porridges could account for the higher CAA observed in these porridges. The role of phenolic compounds such as caffeic acid, gallic acid, ferulic acid, luteolin, quercetin, quercetin glycoside, myricetin, kaempferol, catechin and epicatechin (which were identified in this study) in exerting antioxidant activity in Caco-2 cells has been reported [34].

3.3. Anti-inflammatory properties

Nitric oxide (NO) is one of the products of molecular reaction pathways that are set in motion during inflammation. Thus, the ability of any agent to reduce production of NO can be used as an indicator of anti-inflammatory activity of that agent. The RAW 264.7 murine cell line is a

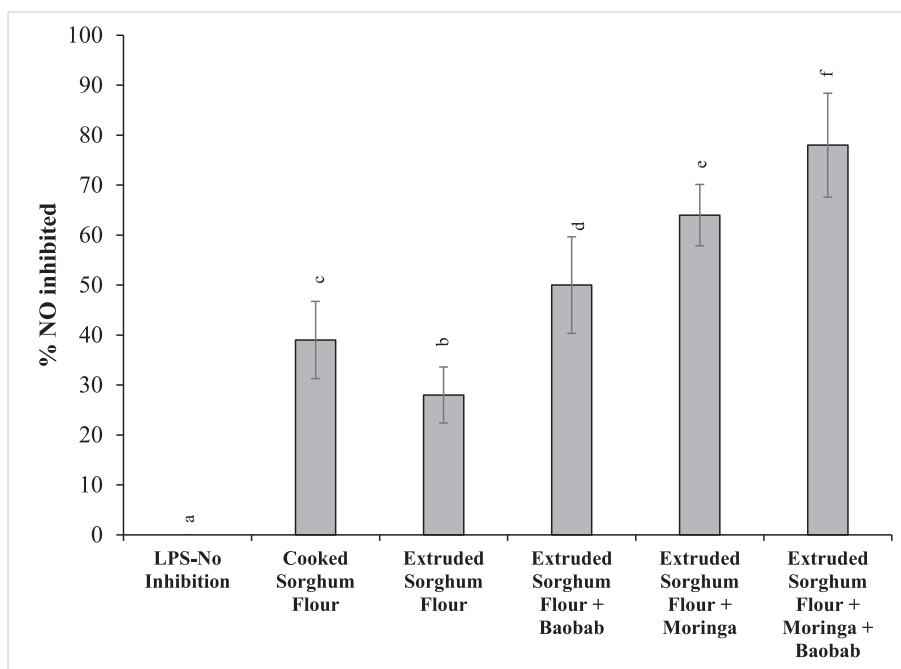


Fig. 2. Percentage inhibition of nitric oxide (NO) production in RAW 264.7 macrophages by extracts from sorghum-based porridges. Bars with different letters are significantly different ($p < 0.05$). Error bars indicate standard deviation. Data are the mean of three samples analysed independently in triplicate ($n = 9$).

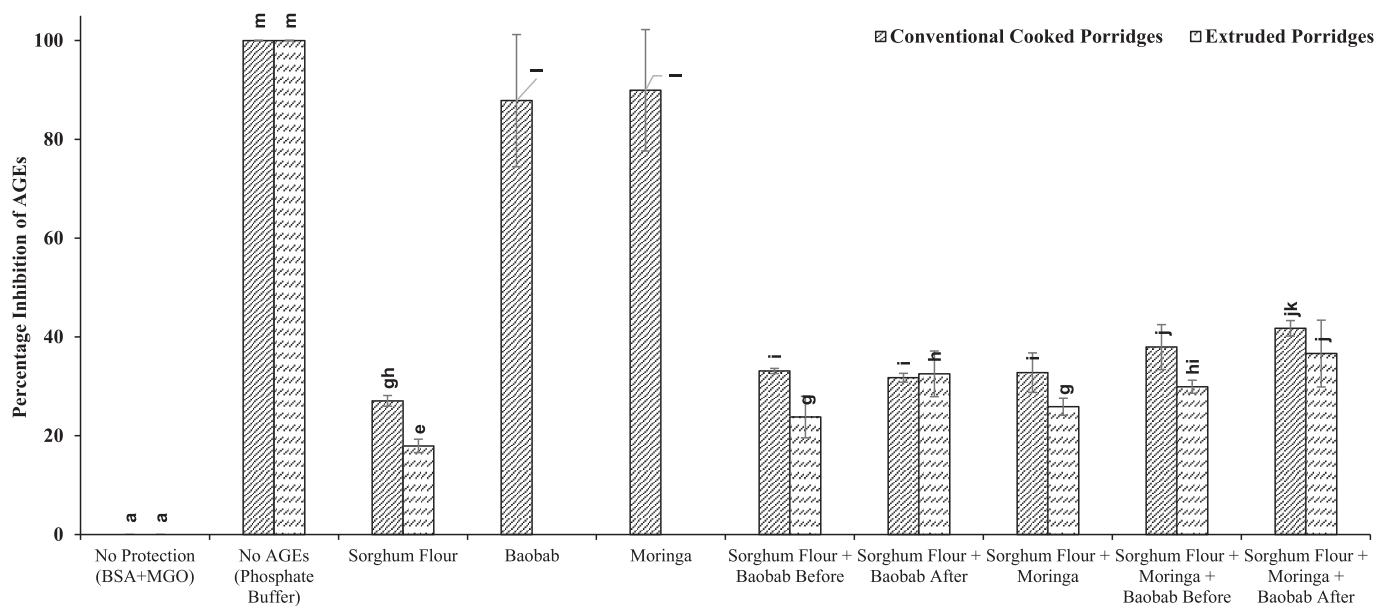


Fig. 3. Inhibitory effects of extracts from sorghum-based porridges against formation of advanced glycation end-products (AGEs) in a bovine serum albumin-methylglyoxal model system. Bars with different letters are significantly different ($p < 0.05$). Error bars indicate standard deviation. Data are the mean of three samples analysed independently in triplicate ($n = 9$). ¹Baobab before: Denotes addition of baobab before cooking; ²Baobab after: Denotes addition of baobab after cooking.

commonly used model for the study of anti-inflammatory properties [35], which is done by monitoring the production of NO by the cells.

Fig. 2 shows the percentage inhibition of NO production in RAW 264.7 macrophages by extracts from the sorghum-based porridges. Extracts from extruded instant sorghum porridges FtF fortified with baobab fruit pulp alone, moringa leaf powder alone or a combination of the two showed higher inhibition of NO production in RAW 264.7 cells compared to conventionally cooked and instant plain sorghum porridges. This was a clear indication of enhanced anti-inflammation properties by FtFF. Extracts from porridges FtF fortified with moringa

leaf powder showed significantly higher reduction in NO production than sorghum porridges FtF fortified with baobab fruit pulp. A combination of moringa leaf powder and baobab fruit pulp used in FtFF caused the largest reduction in NO production.

It is interesting to note that there was particularly high inhibition of NO production in RAW 264.7 cells whenever moringa leaf powder was used as a fortificant. The ability of phenolic acid esters (e.g. caffeoyl glycerols) to inhibit production of NO in RAW 264.7 cells has been reported [35,36]. It has also been proposed that reduction of NO production in RAW 264.7 cells is mainly attributed to the methyl ester of

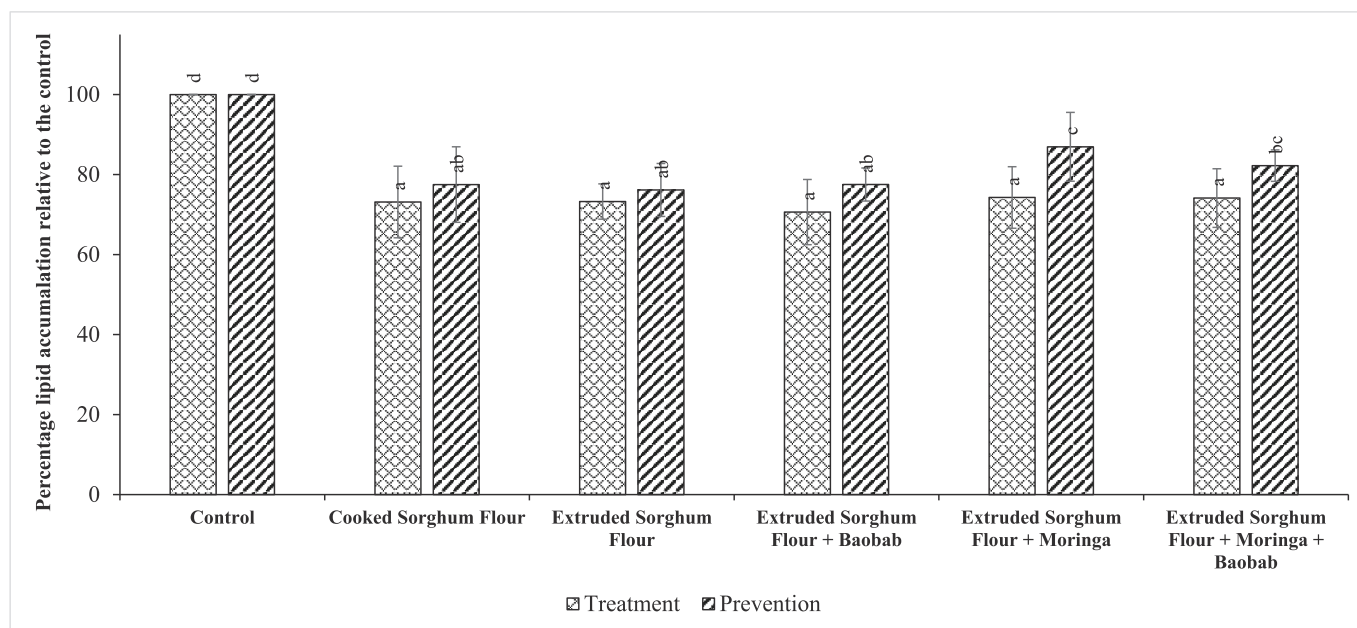


Fig. 4. Effect of extracts from sorghum-based porridges on percentage lipid accumulation in 3 T3-L1 cells. Bars with different letters are significantly different ($p < 0.05$). Error bars indicate standard deviation. Data are the mean of three samples analysed independently in triplicate ($n = 9$).

such phenolic acid esters and not merely by simple methylation or *O*-methyl substitution of the OH group in free phenolic acid derivatives [36]. In this regard, the very high levels of phenolic acid esters observed in this study (Table 2) is noteworthy.

3.4. Anti-diabetic properties

Protein glycation is a Maillard-type reaction between methylglyoxal (a highly reactive α -dicarbonyl compound) and proteins which leads to formation of advanced glycation end-products (AGEs) [37]. The occurrence of the protein glycation reaction in diabetics with hyperglycaemic conditions leads to production of AGEs. Therefore, inhibition of the formation of AGEs could be used as an indicator of anti-diabetic properties.

Fig. 3 shows the inhibitory effects of the extracts from the sorghum-based porridges against formation of AGEs. Extracts from plant foodstuff fortificants baobab fruit pulp and moringa leaf powder exhibited highest percentage inhibition of AGEs of >80%. This would be expected given their high contents of phenolic compounds (Table 2). Fig. 3 shows that FtFF enhanced percentage inhibition of AGEs. Extracts from sorghum porridges FtF fortified with baobab fruit pulp and moringa leaf powder showed higher inhibition of AGEs by 5–7% and 6–8% respectively compared to extracts from sorghum porridge alone. FtF fortification with both baobab fruit pulp and moringa leaf powder produced greater inhibition of AGEs compared to fortification with either baobab fruit pulp or moringa leaf powder alone.

These observations could be attributed to the increases in phenolic content and antioxidant activity following FtFF with baobab fruit pulp and moringa leaf powder (Table 2). The plant foodstuffs used in fortification also contained a much wider variety of phenolic compounds compared to the sorghum alone (Table 1). As a result, the FtF fortified porridges contained a greater complement of phenolic compounds which led to enhanced antioxidant activity and ability to inhibit formation of AGEs. Compounds with antioxidant activity have been reported to be useful in preventing diabetic complications through the reduction of AGEs formation by preventing oxidation of Amadori products and metal-catalyzed glucooxidation [38].

3.5. Anti-lipogenic properties

The 3 T3-L1 cell line is of murine origin and the cells are described as fibroblasts which can differentiate into adipocytes [39]. The differentiation process involves synthesis of triglycerides which accumulate as lipid droplets that are located in the cell cytoplasm. The amount and size distribution of the lipid droplets can be related to conditions such as obesity. The 3 T3-L1 cell line is therefore a useful model for the study of anti-lipogenic and anti-obesity properties.

In this study, extracts from the sorghum-based porridges were tested for their effect on accumulation of lipid droplets during 3 T3-L1 adipocyte differentiation. The extracts demonstrated the ability to prevent the formation of lipid droplets (prevention) and the ability to reduce lipid droplet size and/or density after formation (treatment). The percentage cellular lipid accumulation was then determined and is shown in Fig. 4. The percentage cellular lipid accumulation when treated with extracts from the porridges ranged from 70.62% to 86.94%. This shows that the extracts were able to prevent accumulation of lipid droplets in the cells by up to approximately 30% which indicates potential anti-lipogenic or anti-obesity properties.

Some of the phenolic compounds identified in the sorghum-based porridges have been reported to have an influence on lipid accumulation at different stages [40]. Such compounds include quercetin which was observed to reduce lipid accumulation during the whole period of incubation while apigenin and myricetin were active during the onset and completion of differentiation. Phenolic acids such as ferulic, gallic and vanillic acids were active in reducing lipid accumulation during the onset of differentiation and *p*-coumaric acid reduced lipid accumulation throughout the whole period of differentiation [40].

4. Conclusions

FtFF of wholegrain sorghum with baobab fruit pulp and moringa leaf powder either alone or in combination enhances phenolic content and health-promoting properties of their resultant porridges. The FtF fortified porridges showed enhanced radical scavenging properties and antioxidant activity in Caco-2 cells (protection against oxidative stress), inhibited NO formation in RAW 264.7 cells (anti-inflammatory properties), reduction in AGE (anti-diabetic properties) and reduction in lipid

droplet formation in 3 T3-L1 cells (anti-obesity effects). Although extrusion cooking to produce instant porridges reduced phenolic content, these porridges still exhibited health-promoting properties. Although FtFF is primarily applied to improve mineral nutritional quality of cereal-based foods, it can also contribute to enhanced health-promoting properties in terms of protection against diet-related non-communicable diseases. A combination of extrusion of wholegrain sorghum and FtFF with moringa leaves and baobab fruit pulp could be explored as techniques to address the rising occurrence of NCDs in sub-Saharan Africa.

CRedit authorship contribution statement

John Lubaale: Conceptualization, Data curation, Formal analysis, Investigation, Methodology, Writing – original draft, Writing – review & editing. **June C. Serem:** Formal analysis, Investigation, Methodology, Writing – review & editing. **Megan J. Bester:** Formal analysis, Investigation, Methodology, Writing – review & editing. **M. Naushad Emmambux:** Methodology, Supervision, Writing – review & editing. **Kwaku G. Duodu:** Conceptualization, Formal analysis, Funding acquisition, Investigation, Methodology, Project administration, Supervision, Writing – review & editing.

Declaration of competing interest

None to declare.

Acknowledgements

This publication was made possible through support provided by the U.S. Agency for International Development (USAID) Food Processing & Post Harvest Innovation Lab (FPLAID-OAA-L-14-00003). The opinions expressed herein are those of the authors and do not necessarily reflect the views of the USAID.

References

- J.M. Awika, L.W. Rooney, Sorghum phytochemicals and their potential impact on human health, *Phytochemistry* 65 (9) (2004) 1199–1221.
- N.R. Dlamini, J.R.N. Taylor, L.W. Rooney, The effect of sorghum type and processing on the antioxidant properties of African sorghum-based foods, *Food Chem.* 105 (4) (2007) 1412–1419.
- K.G. Duodu, J.M. Awika, Phytochemical-related health-promoting attributes of sorghum and millets, in: *Sorghum and Millets: Chemistry, Technology and Nutritional Attributes*, Elsevier, 2019, pp. 225–258.
- WHO, World Health Organization, World Health Statistics 2018: Monitoring Health for the SDGs, Sustainable Development Goals, World Health Organization Technical Report Series, Geneva, Switzerland, 2021.
- Neda Seyedsadjadi, Ross Grant, The potential benefit of monitoring oxidative stress and inflammation in the prevention of non-communicable diseases (NCDs), *Antioxidants* 10 (1) (2020) 15.
- O.Y. Adetola, J. Kruger, Z. White, J.R.N. Taylor, Comparison between food-to-food fortification of pearl millet porridge with moringa leaves and baobab fruit and with adding ascorbic and citric acid on iron, zinc and other mineral bioaccessibility, *LWT* 106 (2019) 92–97.
- O.Y. Adetola, J. Kruger, M.G. Ferruzzi, B.R. Hamaker, J.R.N. Taylor, Potential of moringa leaf and baobab fruit food-to-food fortification of wholegrain maize porridge to improve iron and zinc bioaccessibility, *Int. J. Food Sci. Nutr.* (2021) 1–13.
- R. Van der Merwe, J. Kruger, M.G. Ferruzzi, K.G. Duodu, J.R.N. Taylor, Improving iron and zinc bioaccessibility through food-to-food fortification of pearl millet with tropical plant foodstuffs (moringa leaf powder, roselle calyces and baobab fruit pulp), *J. Food Sci. Technol.* 56 (4) (2019) 2244–2256.
- J. Kruger, Potential of food-to-food fortification with cowpea leaves and orange-fleshed sweet potato, in combination with conventional fortification, to improve the cellular uptake of iron and zinc from ready-to-eat maize porridges, *Food Sci. Nutr.* 8 (7) (2020) 3190.
- B.B. Ismail, Y. Pu, M. Guo, X. Ma, D. Liu, LC-MS/QTOF identification of phytochemicals and the effects of solvents on phenolic constituents and antioxidant activity of baobab (*Adansonia digitata*) fruit pulp, *Food Chem.* 277 (2019) 279–288.
- P. Kashyap, S. Kumar, C.S. Riar, N. Jindal, P. Baniwal, R.P. Guiné, et al., Recent advances in drumstick (*Moringa oleifera*) leaves bioactive compounds: composition, health benefits, bioaccessibility, and dietary applications, *Antioxidants* 11 (2) (2022) 402, <https://doi.org/10.3390/antiox11020402>.
- J. Gu, A. Bk, H. Wu, P. Lu, M.A. Nawaz, C.J. Barrow, et al., Impact of processing and storage on protein digestibility and bioavailability of legumes, *Food Rev. Int.* (2022) 1–28.
- M. Adarkwah-Yiadom, K.G. Duodu, Effect of extrusion cooking and simulated in vitro gastrointestinal digestion on condensed tannins and radical scavenging activity of type II and type III whole grain sorghum, *Int. J. Food Sci. Technol.* 52 (10) (2017) 2282–2294.
- J. Lubaale, J.R.N. Taylor, M.N. Emmambux, K.G. Duodu, Extrusion cooking of food-to-food fortified wholegrain sorghum-based porridges enhances Caco-2 ferritin formation, *Cereal Chem.* 100 (2) (2023) 371–383.
- F.B. Apea-Bah, A. Minnaar, M.J. Bester, K.G. Duodu, Does a sorghum–cowpea composite porridge hold promise for contributing to alleviating oxidative stress? *Food Chem.* 157 (2014) 157–166.
- A.M. Nderitu, L. Dykes, J.M. Awika, A. Minnaar, K.G. Duodu, Phenolic composition and inhibitory effect against oxidative DNA damage of cooked cowpeas as affected by simulated in vitro gastrointestinal digestion, *Food Chem.* 141 (3) (2013) 1763–1771.
- F.B. Apea-Bah, A. Minnaar, M.J. Bester, K.G. Duodu, Sorghum–cowpea composite porridge as a functional food, part II: antioxidant properties as affected by simulated in vitro gastrointestinal digestion, *Food Chem.* 197 (2016) 307–315.
- J.C. Serem, M.J. Bester, Physicochemical properties, antioxidant activity and cellular protective effects of honeys from southern Africa, *Food Chem.* 133 (4) (2012) 1544–1550.
- M.A. Siddiqui, S. Rasheed, Q. Saquib, A.A. Al-Khedhairi, M.S. Al-Said, J. Musarrat, et al., In-vitro dual inhibition of protein glycation, and oxidation by some Arabian plants, *BMC Complement. Altern. Med.* 16 (1) (2016) 1–10.
- B. Viadel, S. Perales, R. Barberá, M.J. Lagarda, R. Farré, Ferritin synthesis by Caco-2 cells as an indicator of iron bioavailability: application to milk-based infant formulas, *Food Chem.* 102 (3) (2007) 925–931.
- M. Blasa, D. Angelino, L. Gennari, P. Ninfali, The cellular antioxidant activity in red blood cells (CAA-RBC): a new approach to bioavailability and synergy of phytochemicals and botanical extracts, *Food Chem.* 125 (2) (2011) 685–691.
- M. Malan, J.C. Serem, M.J. Bester, A.W. Neitz, A.R. Gaspar, Anti-inflammatory and anti-endotoxin properties of peptides derived from the carboxy-terminal region of a defensin from the tick *Ornithodoros savignyi*, *J. Pept. Sci.* 22 (1) (2016) 43–51.
- M.A. Ibrahim, J.C. Serem, M.J. Bester, A.W. Neitz, A.R. Gaspar, New antidiabetic targets of α -glucosidase inhibitory peptides, SVPA, SEPA, STYV and STY: inhibitory effects on dipeptidyl peptidase-IV and lipid accumulation in 3T3-L1 differentiated adipocytes with scavenging activities against methylglyoxal and reactive oxygen species, *Int. J. Pept. Res. Ther.* 26 (4) (2020) 1949–1963.
- J.M. Awika, L.W. Rooney, X. Wu, R.L. Prior, L. Cisneros-Zevallos, Screening methods to measure antioxidant activity of sorghum (*Sorghum bicolor*) and sorghum products, *J. Agric. Food Chem.* 51 (23) (2003) 6657–6662.
- R. Alonso, A. Aguirre, F. Marzo, Effects of extrusion and traditional processing methods on antinutrients and in vitro digestibility of protein and starch in faba and kidney beans, *Food Chem.* 68 (2) (2000) 159–165.
- C. Brennan, M. Brennan, E. Derbyshire, B.K. Tiwari, Effects of extrusion on the polyphenols, vitamins and antioxidant activity of foods, *Trends Food Sci. Technol.* 22 (10) (2011) 570–575.
- P. Sharma, H.S. Gujral, B. Singh, Antioxidant activity of barley as affected by extrusion cooking, *Food Chem.* 131 (4) (2012) 1406–1413.
- M.J. Arts, G.R. Haenen, L.C. Wilms, S.A. Beetstra, C.G. Heijnen, H.-P. Voss, et al., Interactions between flavonoids and proteins: effect on the total antioxidant capacity, *J. Agric. Food Chem.* 50 (5) (2002) 1184–1187.
- J. Kruger, A. Oelofse, J.R.N. Taylor, Effects of aqueous soaking on the phytate and mineral contents and phytate: mineral ratios of wholegrain normal sorghum and maize and low phytate sorghum, *Int. J. Food Sci. Nutr.* 65 (5) (2014) 539–546.
- A. Leone, A. Spada, A. Battezzati, A. Schiraldi, J. Aristil, S. Bertoli, Cultivation, genetic, ethnopharmacology, phytochemistry and pharmacology of *Moringa oleifera* leaves: an overview, *Int. J. Mol. Sci.* 16 (6) (2015) 12791–12835.
- D. Skroza, V. Šimat, L. Vrdoljak, N. Jolić, A. Skelin, M. Čagalj, et al., Investigation of antioxidant synergisms and antagonisms among phenolic acids in the model matrices using FRAP and ORAC methods, *Antioxidants* 11 (9) (2022) 1784, <https://doi.org/10.3390/antiox11091784>.
- H. Wan, D. Liu, X. Yu, H. Sun, Y. Li, A Caco-2 cell-based quantitative antioxidant activity assay for antioxidants, *Food Chem* 175 (2015) 601–608.
- Y. Murakami, A. Kawata, S. Suzuki, S. Fujisawa, Radical-scavenging and pro-/anti-inflammatory activity of tetracycline and related phenolic compounds with or without visible light irradiation. *In Vivo* 34 (1) (2020) 81–94.
- N.J. Yang, M.J. Hinner, Getting across the cell membrane: an overview for small molecules, peptides, and proteins, *Methods Mol. Biol.* 1266 (2015) 29–53.
- Y.Y. Choo, S. Lee, P.-H. Nguyen, W. Lee, M.-H. Woo, B.-S. Min, et al., Caffeoylglycolic acid methyl ester, a major constituent of sorghum, exhibits anti-inflammatory activity via the Nrf2/heme oxygenase-1 pathway, *RSC Adv* 5 (23) (2015) 17786–17796.
- P.-H. Nguyen, B.T. Zhao, J.H. Lee, Y.H. Kim, B.S. Min, M.H. Woo, Isolation of benzoic and cinnamic acid derivatives from the grains of *Sorghum bicolor* and their inhibition of lipopolysaccharide-induced nitric oxide production in RAW 264.7 cells, *Food Chem* 168 (2015) 512–519.
- X. Wu, V.M. Monnier, Enzymatic deglycation of proteins, *Arch. Biochem. Biophys.* 419 (1) (2003) 16–24.

- [38] F.A. Dil, Z. Ranjkesh, M.T. Goodarzi, A systematic review of antiglycation medicinal plants, *Diabetes Metab. Syndr. Clin. Res. Rev.* 13 (2) (2019) 1225–1229.
- [39] V. Rizzatti, F. Boschi, M. Pedrotti, E. Zoico, A. Sbarbati, M. Zamboni, Lipid droplets characterization in adipocyte differentiated 3T3-L1 cells: size and optical density distribution, *Eur. J. Histochem.* 57 (e24) (2013) 159–162.
- [40] P. Aranaz, D. Navarro-Herrera, M. Zabala, I. Miguélez, A. Romo-Hualde, M. López-Yoldi, et al., Phenolic compounds inhibit 3T3-L1 adipogenesis depending on the stage of differentiation and their binding affinity to PPAR γ , *Molecules* 24 (6) (2019) 1045, <https://doi.org/10.3390/molecules24061045>.