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Research Article

Effect of natural hydrocolloids addition on the physicochemical, antioxidant, and digestive-enzyme-inhibitory properties of *Sorghum bicolor* flour

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Abstract Studies have shown that adding modified food hydrocolloids as gluten replacement in gluten-free products could be associated with an increase in cost of production and loss of some health-benefitting qualities. In this study, the effects of adding two natural hydrocolloid sources—*Brachystegia eurycoma* (BE) and *Detarium microcarpum* (DM)—and a modified hydrocolloid, sodium carboxymethyl cellulose (SCMC), on the physicochemical, antioxidant, and digestive-enzyme-inhibitory properties (including pancreatic lipase, α-amylase, and α-glucosidase) of *Sorghum bicolor* (SB) flour were evaluated. Each of BE, DM and SCMC was blended with SB at 2 and 4% proportions. The blends' peak and final viscosities increased, while their starch content decreased significantly with an increasing BE and DM addition. Blends of SB with either BE or DM had higher polyphenolics (total phenol, tannins, and total flavonoids) levels, and stronger antioxidant and digestive-enzyme-inhibitory activities than the blend of SB with SCMC. Among the blends, SB+BE had the highest polyphenolics level and the most potent antioxidant and digestive-enzyme-inhibitory capacity. The blends' pasting attributes were significantly correlated with the polyphenolic's levels. The blends' polyphenolics levels were also correlated with their antioxidant and digestive-enzyme-inhibitory capacities. Hence, blending SB with either BE or DM may be a low-cost approach for developing a gluten-free flour, while retaining its antioxidant and digestive-enzyme-inhibitory qualities.

Keywords antioxidant activity, digestive enzyme inhibition, gluten-free flour, hydrocolloids, pasting properties

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1. Introduction

Most baked food products, including bread and biscuits, are made from processed wheat flour, the gold standard for baking (Irondi et al., 2023). Gluten in wheat flour gives its dough a visco-elastic quality desirable for a good-quality baked product (Imam et al., 2024; Mir et al., 2016). However, such products are characterized by low health-benefiting bioactive compounds and essential nutrients contents, as well as high calorie. This is attributed to the removal of wheat bran, where most of the bioactive constituents are deposited, before milling the starch-rich endosperm into processed flour (Irondi et al., 2022a; Parenti et al., 2020). On the other hand,

consumers' awareness of healthy food choices, including baked food products, has increased in recent time. This has led to the development of gluten-free (GF) products for both celiac patients and non-celiac individuals. For the celiac patients, consuming GF products and abstaining from gluten-containing products are the only sustainable and effective treatment options available to them. For the latter group, the desire for a healthy food pattern is their motivating factor. However, developing GF baked products with comparable qualities to those made from wheat has been a huge challenge to the food industry, partly because of the poor visco-elastic quality of GF cereal flours (Imam et al., 2024).

Several GF flours, including cassava (Gallagher et al., 2003), sorghum (Ofosu et al., 2020; Ratnavathi et al., 2016), and millets (Annor et al., 2017) are being used for the formulation of GF products. However, such GF products often possess a low quality due to lack of gluten (Arendt et al., 2002). In a quest to develop GF products acceptable to consumers, studies (Filipcev et al., 2021; Mir et al., 2016) have focused on replacing gluten with modified food hydrocolloids, capable of mimicking the visco-elastic feature of gluten. Modified food hydrocolloids, such as methylcellulose and sodium carboxymethylcellulose, are food hydrocolloids produced either by selective chemical modification and derivatization (Milani and Maleki, 2012) or through physical and enzymatic treatments of natural hydrocolloid (Seisun and Zalesny, 2021). However, these modified hydrocolloids increase production cost, as they are complex and expensive to manufacture (Ohimain, 2014). Consequently, some recent studies, such as Filipcev et al. (2021) and Irondi et al. (2021b), have shown that natural hydrocolloid may be a viable alternative to the modified hydrocolloids as gluten replacement. Natural food hydrocolloids can improve the physicochemical, sensory and nutraceutical qualities of GF products (Irondi et al., 2023). Some natural food hydrocolloid sources reported in the literature include okra powder (Tufaro et al., 2022), cress seed gum (Naji-Tabasi and Mohebbi, 2015), *Brachystegia eurycoma, Detarium microcarpum* (Irondi et al., 2021b) and psyllium (Filipcev et al., 2021).

Brachystegia eurycoma (BE) and *Detarium microcarpum* (DM) are underutilized legumes (Igwenyi and Akubugwo, 2010) that are gaining popularity in food applications owing to their availability, nutritional, physicochemical, hydrocolloid and nutraceutical qualities (Irondi et al., 2021a; Irondi et al., 2023; Mbaeyi-Nwaoha et al., 2017; Nwakaudu et al., 2017). For instance, Irondi et al. (2022b) formulated GF bread from the blend of pearl millet and DM, in which the DM served as a natural hydrocolloid substitute for gluten. DM seed flour also served as a source of hydrocolloid in a multi-purpose natural additive blend for instant bio-yoghurts formulation (Irondi et al., 2024). Furthermore, in a recent report Abdulrazaaq et al. (2024) formulated biofortified yellow maize-based GF cookies by incorporating BE flour as a rich natural hydrocolloid source replacing gluten. There is also a report on GF cookies formulated with sorghum and Turkish bean flours, containing cress seed gum as a hydrocolloid (Shahzad et al., 2021). However, the effect of adding DM and BE, as natural hydrocolloids-rich sources, on *Sorghum bicolor* (SB) GF flour's physicochemical, antioxidant and digestive-enzyme-inhibitory properties of has not been reported.

Well-known as a GF grain, SB is a staple crop of east Africa origin (Pérez et al., 2010), consumed by millions of people in Africa, Latin America, and Asia (Correia et al., 2010). It was ranked the sixth most highly cultivated and important cereals globally and the second in Africa (Zhao et al., 2019). SB flour serves as a functional constituent in many food products, including snacks, pasta, bread and noodles (Batariuc et al., 2021). It has been reported to be nutrients- and bioactive compounds-rich, possessing some health-promoting qualities, such as antioxidant, anti-hypertension, anti-cancer, anti-obesity, anti-diabetes and some disease-linked enzymes inhibitory activities (Cardoso et al., 2014; Irondi et al., 2019a; Wu et al., 2013). With these reported health benefits of SB, it is imperative to improve its flour quality for application in GF products' formulation. Flour, as an intermediate product, has a longer shelf life, stable nutritional value, and can easily be processed to different finish products (Antarlina et al., 2021). Hence, this study was designed to evaluate the effect adding natural hydrocolloids on SB flour's physicochemical, antioxidant and digestive-enzyme-inhibitory qualities.

2. Materials and methods

2.1. Materials

Two natural hydrocolloids sources BE and DM, red SB grains and modified hydrocolloid (sodium carboxymethylcellulose, SCMC) were bought at Ipata market in Ilorin metropolis, Kwara State, Nigeria. The BE and DM seeds samples were subjected to oven-drying at 45℃ for a week to ease their dehulling. Thereafter, SB and the dehulled BE and DM seeds

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were sorted and milled separately to produce their native flour sample. Each flour sample was then packed airtight in a sample container. Analytical grades of Sigma chemicals (St. Louis, Missouri, USA) were used in the different experiments conducted in this study.

2.2. Formulation of gluten-free flour blends with Sorghum bicolor and natural hydrocolloids

Six flour blends, including 98% SB+2% BE, 96% SB+4% BE, 98% SB+2% DM, 96% SF+4% DM, 98% SB+2% SCMC and 96% SB+4% SCMC, were formulated by separately mixing SB with 2% and 4% of one of BE, DM and SCMC. BE and DM were added as natural hydrocolloid sources, while SCMC, a modified hydrocolloid was added as a control. The blends were separately packed in air-tight sample containers for analysis.

2.3. Analysis of physicochemical properties of flour samples

2.3.1. Starch content analysis

Based on the protocol outlined by Elemosho et al. (2020), a mixture, comprising flour (0.02 g), 80% ethanol (1 mL), distilled water (2 mL), and hot 80% ethanol (10 mL) in a centrifuge tube, was subjected to a 10-min centrifugation at 2,000 rpm. After decanting the supernatant, the residue in the tube was hydrolyzed with perchloric acid (7.5 mL) at ambient temperature for 1 h. Thereafter, the resulting hydrolysate was diluted with distilled H_2O to 25 mL and the mixture was filtered through a Whatman filter paper (No. 1). A portion (0.05 mL) of the filtrate, 0.5 mL of 5% phenol solution, and 2.5 mL concentrated H_2SO_4 were mixed in a test tube. After cooling to ambient temperature, the absorbance was measured at 490 nm using a UV/visible spectrophotometer (Lasany, LI-722, UK) and the starch level of the flour samples was calculated from a D-glucose (10-100 mg D-glucose/mL) standard curve.

2.3.2. Analysis of amylose and amylopectin levels

In this assay, previously reported by Elemosho et al. (2020), 0.1 g of flour sample, 1 mL of ethanol (95%) and 9 mL of 1 M NaOH were mixed in a 50-mL capacity centrifuge tube. The starch in the mixture was gelatinized by heating the mixture at 100℃ in a water bath for 10 min. Following cooling to ambient temperature, the gelatinized sample (0.05 mL) was mixed with distilled H_2O (0.45 mL), 1 M acetic acid (0.1 mL) and iodine (0.2 mL) solutions. Next, the mixture was diluted to 10 mL with distilled H_2O and incubated for 20 min at ambient temperature, after which the absorbance reading was taken at 620 nm using a UV/visible spectrophotometer (Lasany, LI-722, UK). Finally, the sample's amylose level was calculated based on an amylose standard (10-100 mg corn starch amylose/mL).

Amylopectin level of the sample was calculated thus:

Amylopectin level (in percent)
= 100 - amylose level (in percent)

2.3.3. Analysis of pasting properties

To analyze the samples' pasting properties, 3 g of flour, suspended in 25 mL of distilled H_2O in an aluminum canister, was loaded on a Rapid Visco Analyzer (RVA) machine (model: RVA-4, Perten Scientific, Springfield, Illinois, USA) as reported by (Kareem et al., 2023). The RVA was connected to a personal computer with Thermocline software (Newport Scientific, USA). The samples' pasting properties were then analyzed using a standard analytical program of the RVA at a constant stirring (160 RPM). During the analysis, the samples' pasting properties were recorded with the aid of the Thermocline software.

2.4. Preparation of flour extract

As earlier reported by Elemosho et al. (2021), the flour sample (0.3 g) was soaked for 24 h in methanol (15 mL) in an airtight 50-mL capacity centrifuge tube. The mixture was shaken intermittently for the first 1 h at ambient temperature. Afterwards, the mixture was filtered using Whatman filter paper (No. 1) and the supernatant (henceforth called extract) was collected and utilized for the polyphenolic's contents, antioxidant and digestive-enzyme-inhibitory activity assays.

2.5. Analysis of bioactive compound contents (total phenolics, tannins, and total flavonoids)

2.5.1. Quantification of total phenolics and tannins levels

As previously described by Singleton et al. (1999), the flour samples' total phenolics level was quantified using Folin-Ciocalteu reagent. Under ambient laboratory conditions, the extract (300 μL), Folin-Ciocalteu reagent (1.5 mL), 7.5% (w/v) Na_2CO_3 solution (1.2 mL) and distilled H_2O (7 mL)

were mixed in a test tube. Following a 30 min incubation of the resulting mixture at an ambient temperature in the dark, the absorbance reading was taken at 765 nm using a UV/visible spectrophotometer (Lasany, LI-722, UK). The flours' total phenolics level was calculated based on (10-100 mg/mL) gallic acid (GA) calibration curve and reported in GA equivalent in mg/g flour sample (GAE mg/g).

Tannins level in the flour was analyzed by adopting the procedure earlier outlined by Olatoye et al. (2023). Accordingly, the flour's extract (0.1 mL), distilled H_2O (7.5 mL), Folin-Denis reagent (0.5 mL) and 35% (w/v) Na_2CO_3 solution (1 mL) were mixed in a test tube. After a 30 min incubation of the mixture at ambient temperature, its absorbance reading was taken at 760 nm and tannins level was calculated based a calibration curve prepared using tannic acid (10-100 mg/mL).

2.5.2. Quantification of total flavonoids content

Based on Kareem et al. (2023), the flour's extract (0.5 mL), absolute methanol (1.5 mL), 10% $AlCl_3$ (0.1 mL), 1 M CH_3COOK (0.1 mL) and distilled H_2O (2.8 mL) were mixed in a test tube. After a 30 min incubation of the mixture at ambient temperature, its absorbance reading was taken at 514 nm using a UV/visible spectrophotometer (Lasany, LI-722, UK). Thereafter, the flour's total flavonoids level was calculated based on a calibration curve prepared using quercetin standard (10-100 mg/mL).

2.6. Analysis of antioxidant activity

2.6.1. Reducing power of flour samples

Following an established procedure (Elemosho et al., 2021), the reducing power of the flour's extract was assayed. The extract (2.5 mL), 200 mM sodium phosphate buffer (2.5 mL; pH 6.6), and 1% KFeCN (2.5 mL) were mixed in a test tube. After a 20 min incubation at 50℃, 10% (w/v) trichloroacetic acid (2.5 mL) was added to it. A portion (2.5 mL) of the mixture was diluted with distilled H_2O (2.5 mL) and mixed with 0.1% (w/v) $FeCl_3$ solution (1 mL). Subsequently, absorbance reading of the mixture was taken at 700 nm using a UV/visible spectrophotometer (Lasany, LI-722, UK), after incubating it (at ambient temperature) for 30 min. The reducing power of the flour's extract was calculated based on a GA (10-100 mg/mL) standard curve.

2.6.2. ABTS [2, 2-azinobis (3-ethyl-benzothiazoline-6-sulfonic acid) radical cation] scavenging capacity

A protocol previously described by Olatoye et al. (2023) was followed to analyze the flour's ABTS radical cation-scavenging capacity. Twenty milliliters of ABTS reagent was first generated by mixing 10 mL aqueous solution of each of 0.007 M ABTS and 0.00245 M $K_2S_2O_8$ at ambient temperature for 16 h in the dark. The ABTS reagent was then diluted using 95% ethanol to adjust its absorbance to 0.7±0.02 at a wavelength of 734 nm. Afterwards, 2,000 µL of ABTS reagent was mixed with 200 µL of the flour's extract and subjected to a 15 min incubation at ambient temperature. The sample's absorbance reading was measured at 734 nm using a UV/visible spectrophotometer (Lasany, LI-722, UK) and its capacity to scavenge ABTS was calculated based on a Trolox (1-10 mM) standard curve.

2.6.3. DPPH-scavenging activity of flour samples

To assay for the flour sample's DPPH radical-scavenging capacity, the procedure documented by Alamu et al. (2021) was adopted. Different concentrations of each sample's extract (1,000 µL) and 3,000 µL of 0.06 mM DPPH radical solution (in methanol) was subjected to a 30 min incubation in the dark at ambient temperature. Finally, the absorbance reading was measured at 517 nm using a UV/visible spectrophotometer (Lasany, LI-722, UK) and the sample's DPPH radical-scavenging activity, expressed as SC_{50} (extract concentration scavenging DPPH* by 50%), was calculated.

2.7. In vitro analysis of enzyme inhibition

2.7.1. Pancreatic lipase inhibition

To analyze for the sample's capacity to inhibit pancreatic lipase, the procedure documented by Eom et al. (2013) was followed. Enzyme solution comprised 30 µL of 10 units of porcine pancreatic lipase (EC 3.1.1.3, Sigma) in morpholine propane sulphonic acid (10 mM), EDTA (1 mM, pH 6.8), and 850 µL of Tris buffer [containing Tris-HCL (100 mM) and $CaCl_2$ (5 mM), pH 7.0]. *P*-nitrophenyl butyrate served as a substrate. The enzyme (880 µL) was incubated (37℃, 10 min) with 100 µL of the sample's extract (or orlistat, a reference inhibitor) at varied concentrations in test tubes. 20 microliter of 10 mM *p*-nitrophenly butyrate solution in dimethyl formamide was added to each test tube to initiate pancreatic lipase-catalyzed hydrolysis of the *p*-nitrophenly

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butyrate, while incubating (37°C, 20 min). After this, the absorbance readings, obtained at 405 nm using a UV/visible spectrophotometer (Lasany, LI-722, UK), were used to calculate the pancreatic lipase inhibition by the sample, in terms of IC_{50} (that is, sample extract's concentration inhibiting the enzyme activity by 50%).

2.7.2. α-Amylase inhibition

Adopting a protocol documented by Kareem et al. (2022), alpha-amylase inhibitory assay was performed by incubating (37°C, 10 min) a mixture of 0.5 mL of the sample's extract (or acarbose, a reference inhibitor) and 0.5 mL of 0.5 mg/mL porcine pancreatic α-amylase solution (EC 3.2.1.1, Sigma) contained in 0.02 M sodium phosphate buffer (pH 6.9, with 0.006 M NaCl) in a test tube. After adding 0.5 mL of 1% (w/v) starch solution (in 0.02 M sodium phosphate buffer), the mixture was further incubated (37°C, 15 min). Next, alpha-amylase-catalyzed starch hydrolysis was terminated by adding 1 mL of 3,5-dinitrosalicyclic acid reagent (comprising 3,5-dinitrosalicyclic acid (1%) and sodium potassium tartrate (12%) in 0.4 mol/L sodium hydroxide). The mixture in each test tube was subjected to a 5 min heating in a boiling water bath, allowed to cool to ambient temperature, before diluting with distilled H_2O (10 mL). Finally, absorbance readings were taken at 540 nm using a UV/visible spectrophotometer (Lasany, LI-722, UK) and the sample's alpha-amylase inhibitory capacity was calculated in terms of IC_{50}.

2.7.3. α-Glucosidase inhibition assay

In this assay, as previously reported (Kareem et al., 2022), 0.05 mL of flour's extract (or acarbose, a reference inhibitor) and 0.05 mL of α-glucosidase (EC 3.2.1.20, 5 units, Sigma) from *Bacillus stearothermophilus* were mixed in a test tube and subjected to a 10 min incubation at 37°C. To this mixture, 0.1 mL of 3 mmol/L *p*-nitrophenyl glucopyranoside (PNPG, in 0.02 M phosphate buffer, with a pH of 6.9) was added. Next, the mixture was subjected to a 20 min incubation at 37°C, during which the PNPG was hydrolyzed by α-glucosidase. Thereafter, 2 mL of Na_2CO_3 (0.1 M) was dispensed into the mixture to halt PNPG hydrolysis. Alpha-glucosidase inhibition, expressed in terms of IC_{50}, was calculated from the absorbance reading, taken at 400 nm.

2.8. Statistical analysis of data

Results obtained for the various assays (in triplicate) were analyzed using one-way analysis of variance (ANOVA). Comparison of different treatments' mean values was performed by Duncan's multiple range test at $p<0.05$, using Statistical Package for Social Sciences software (version 17, IBM Corporation, USA). Pearson correlation analysis was also carried out on the result.

3. Results and discussion

3.1. Starch, amylose and amylopectin contents of samples

Table 1 contains the starch, amylose and amylopectin levels of the samples. Among the flours, SB had the highest ($p<0.05$) starch level (79.93±0.29%). Generally, adding hydrocolloids resulted in a significant decrease ($p<0.05$) in SB's starch level, with 96% SB+4% BE having the lowest starch level. The amylose contents of SB, 98% SB+2% BE and 98% SB+

Table 1. Starch, amylose and amylopectin contents in *S. bicolor* flour blended with hydrocolloids

Sample	Starch (%)	Amylose (%)	Amylopectin (%)
SB[1)]	79.93±0.29[2)a3)]	17.57±0.07[a]	82.43±0.07[c]
98% SB+2% BE	75.78±0.43[b]	16.79±0.21[a]	83.20±0.21[bc]
96% SB+4% BE	61.51±0.29[f]	16.17±0.27[b]	83.83±0.27[ab]
98% SB+2% DM	69.07±0.34[d]	16.27±0.13[b]	83.72±1.34[ab]
96% SF+4% DM	64.58±0.14[e]	15.52±0.40[b]	84.48±0.40[a]
98% SB+2% SCMC	69.97±0.04[c]	17.49±0.27[a]	82.51±0.27[c]
96% SB+4% SCMC	63.95±028[e]	16.07±0.41[b]	83.93±0.41[ab]

[1)]SB, wholemeal *Sorghum bicolor* flour; BE, *Brachytegia eurycoma* flour; DM, *Detarium microcarpum* flour; SCMC, sodium carboxymethyl cellulose.
[2)]All values are mean±SD (n=3).
[3)]Values with different lowercase superscript letters in the same column differ significantly ($p<0.05$).

2% SCMC were similar ($p>0.05$), but they were significantly higher ($p<0.05$) than the amylose contents of the rest samples. The decrease in the samples' amylose content due to hydrocolloids addition is consistent with a previous report in which adding *B. eurycoma* to high- quality cassava flour resulted in a decrease in amylose level (Irondi et al., 2019b) and whole millet flour (Irondi et al., 2021a). In conformity with earlier reports by Elemosho et al. (2020), Irondi et al. (2021a) and Yotsawimonwat et al. (2008), amylopectin was more predominated than amylose in the flours' starch. Amylose and amylopectin proportions in a starchy flour affect its functional properties, influencing its food, as well as industrial applications (Irondi et al., 2017a). It also impacts the starch digestibility and glycemic index (GI) of a starchy food, such that a high amylose and low amylopectin content result in a decreased starch digestibility/GI, as reported by Shanita et al. (2011) and Irondi et al. (2021b). Amylose's linear and more flexible structure confers on it a higher tendency to form double helices after cooking (retrogradation) than amylopectin, making it more resistant to hydrolysis by α-amylase than amorphous starch (Irondi et al., 2022b). Thus, SB with the least amylopectin level (82.43±0.07%) may possess a lower starch digestibility and GI than the rest flours.

3.2. Pasting properties of samples

The samples' pasting attributes are presented in Table 2. Pasting attributes represent the changes that starch in suspension undergoes, during a definite heating and cooling cycle under a shear force (Alcázar-Alay and Meireles, 2015; Irondi et al., 2017b; Liao and Wu, 2016). Generally, the samples' pasting attributes varied significantly ($p<0.05$). Adding hydrocolloids caused an increase in the peak viscosity and trough viscosity of the sorghum flour. Peak viscosity denotes a flour's ability to bind water, representing its starch granules' tendency to swell freely before breaking down physically (Alamu et al., 2017). As expected and consistent with previous reports (Irondi et al., 2022b), 96% SF+4% DM, having the least amylose level (Table 1), exhibited the highest level of peak viscosity (Table 2). In contrast, SB had the lowest peak viscosity. In this context, a flour sample having low amylose content swells easily, due to a weaker binding force existing in its starch granule, with an attendant increase in viscosity, when heating at a reduced temperature (Hoover et al., 1996). Similarly, a starchy sample containing more amylopectin (branched) than amylose (linear chain) attains a higher peak viscosity occasioned by its higher water absorption and retention propensity (Gayral et al., 2015). This may explain the higher peak viscosity obtained in 96% SF+4% DM.

Further, 96% SF+4% DM also had the highest final viscosity. Final viscosity denotes the viscosity change after a cooked starch is held at 50°C, representing its stability and the resistance of its paste to shear force during stirring (Elemosho et al., 2020; Irondi et al., 2019a). Among the pasting attributes, final viscosity is prominent as the routine attribute used to ascertain the food and industrial applications a given flour/starch is suitable for. This suggests that 96% SF+4% DM, with the highest final viscosity, may be more

Table 2. Pasting attributes of *S. bicolor* flour blended with hydrocolloids

Sample	Peak viscosity (RVU)	Trough viscosity (RVU)	Breakdown viscosity (RVU)	Final viscosity (RVU)	Set-back viscosity (RVU)	Peak time (min)	Pasting temp. (°C)
SB[1)]	63.78±0.16[2)f3)]	59.94±0.16^{g}	3.84±0.01^{a}	185.62±0.18^{d}	125.68±0.21^{b}	5.23±0.04^{g}	88.17±0.11^{c}
98% SB+2% BE	70.02±0.03^{d}	67.11±0.04^{e}	2.91±0.01^{b}	183.15±0.69^{e}	116.04±0.65^{e}	6.91±0.02^{a}	87.27±0.04^{d}
96% SB+4% BE	72.56±0.33^{c}	71.97±0.32^{d}	0.59±0.01^{d}	189.60±0.74^{c}	117.62±0.42^{d}	6.33±0.09^{d}	87.35±0.71^{d}
98% SB+2% DM	79.88±0.19^{b}	76.86±0.16^{b}	3.02±0.03^{b}	199.91±0.12^{b}	123.05±0.28^{c}	5.71±0.02^{f}	87.15±0.14^{d}
96% SF+4% DM	92.35±0.45^{a}	90.11±0.55^{a}	2.23±0.09^{c}	223.77±0.21^{a}	133.66±0.34^{a}	6.16±0.13^{e}	85.55±0.71^{e}
98% SB+2% SCMC	68.45±0.28^{e}	66.01±0.26^{f}	2.43±0.02^{c}	135.30±0.17^{f}	69.28±0.42^{f}	6.45±0.07^{c}	89.60±0.71^{b}
96% SB+4% SCMC	79.35±0.45^{b}	75.02±0.56^{c}	4.32±0.11^{a}	134.04±0.76^{f}	59.02±0.21^{g}	6.79±0.01^{b}	93.65±0.71^{a}

[1)]SB, wholemeal *Sorghum bicolor* flour; BE, *Brachytegia eurycoma* flour; DM, *Detarium microcarpum* flour; SCMC, sodium carboxymethyl cellulose.
[2)]All values are mean±SD (n=3).
[3)]Values with different lowercase superscript letters in the same column differ significantly ($p<0.05$).

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suitable than the rest flours for developing food products requiring starch of a high viscosity (Irondi et al., 2021a). Meanwhile, 98% SB+2% SCMC and 96% SB+4% SCMC (sorghum flour blended with 2 and 4% SCMC, respectively) had the least final viscosity, among all the flour samples. The results further show that 96% SF+4% DM had the lowest ($p<0.05$) pasting temperature, whereas blends with SCMC had the highest pasting temperature. Pasting temperature describes the least temperature a particular sample requires to be cooked (Offia-Olua, 2014). Thus, using 96% SF+4% DM for product development may involve a cheaper energy cost due to its low pasting temperature, in comparison with the rest flours, as suggested by a previous report (Elemosho et al., 2020).

3.3. Polyphenolics levels of samples

The flour samples' polyphenolic contents, including total phenolics, tannins and total flavonoids, are presented in Table 3. Generally, SB (100% sorghum flour) had the highest total phenolics (11.50±0.16 GAE mg/g), tannins (15.89±1.15 TAE mg/g) and total flavonoids (4.71±0.11 QE mg/g) levels. Blending with the hydrocolloids (BE, DM and SCMC) resulted in a significant ($p<0.05$) reduction in the samples' total phenolics, tannins and total flavonoids levels, as the proportion of each hydrocolloid increased. However, among the blends, polyphenolics levels were in the order of SB+BE > SB+DM > SB+SCMS, with 98% SB+2% BE having the highest total phenolics, tannins and total flavonoids levels. As earlier reported by Saberi et al. (2017), the observed decrease in the polyphenolics levels due to hydrocolloids addition may be attributed to bonding between hydroxyl groups of the hydrocolloids and phenolics, resulting in a decrease in total phenolics content and antioxidant activity.

Previous reports affirmed some health benefits of polyphenolic compounds, such as anti-inflammatory, anti-diabetic, antioxidant, anti-microbial, anti-obesity and anti-hypertensive effects. In food, polyphenolic compounds are known to prolong the food's shelf-life and maintain its nutritional value by preventing and/or stalling the oxidative degradation of nutrients (Alamu et al., 2021; Avila-Roman et al., 2021; Irondi et al., 2022b; Sethiya et al., 2014).

3.4. Antioxidant activity of samples

The ferric reducing power, DPPH radical and ABTS scavenging activity of the samples, representing their antioxidant activity, are presented in Table 4. Like the polyphenolic's levels, SB displayed a stronger ($p<0.05$) antioxidant activity (ferric reducing power, 16.42±0.13 GAE mg/g; ABTS scavenging activity, 2,160.33±2.67 TE μM/g; and $DPPH^{\bullet}$ SC_{50}, 227.10±0.47 mg/mL) than when blended with the hydrocolloids. However, the antioxidant activity of the blends was dependent on the hydrocolloid's proportion and in the same order (SB+BE > SB+DM > SB+SCMS) as observed for the polyphenolic's levels. Thus, 98% SB+2% BE consistently had the strongest antioxidant activity, while 96% SB+4% SCMS had the weakest. Polyphenolic compounds' antioxidant activity has been attributed to diverse mechanisms, including chain auto-oxidation reactions propagation disruption, lipid radicals' formation inhibition, transition metal ions chelation, and endogenous antioxidant enzymes activation. Other

Table 3. Polyphenolics contents in *S. bicolor* flour blended with hydrocolloids

Sample	Total phenols (GAE mg/g)	Tannins (TAE mg/g)	Total flavonoids (QE mg/g)
SB[1)]	11.50±0.16[2)a3)]	15.89±1.15[a]	4.71±0.11[a]
98% SB+2% BE	10.97±0.20[b]	14.71±0.58[b]	4.35±0.04[b]
96% SB+4% BE	9.33±0.09[d]	12.99±0.19[d]	4.01±0.01[d]
98% SB+2% DM	9.67±0.09[c]	13.82±0.29[c]	4.18±0.21[c]
96% SF+4% DM	8.88±0.11[e]	12.45±0.19[d]	3.97±0.21[d]
98% SB+2% SCMC	8.82±0.06[e]	12.53±0.19[e]	4.00±0.14[d]
96% SB+4% SCMC	7.39±0.09[f]	10.59±0.19[f]	3.84±0.28[e]

[1)]SB, wholemeal *Sorghum bicolor* flour; BE, *Brachytegia eurycoma* flour; DM, *Detarium microcarpum* flour; SCMC, sodium carboxymethyl cellulose.
[2)]All values are mean±SD (n=3).
[3)]Values with different lowercase superscript letters in the same column differ significantly ($p<0.05$).

Table 4. Antioxidant activity in *S. bicolor* flour blended with hydrocolloids

Parameter	Reducing power (GAE mg/g)	ABTS (TE μM/g)	DPPH SC_{50} (mg/mL)
SB[1)]	16.42±0.13[2)a3)]	2160.33±2.67[a]	227.10±0.47[e]
98% SB+2% BE	16.00±0.09[b]	2118.29±2.68[c]	236.50±1.03[d]
96% SB+4% BE	15.26±0.05[f]	2059.18±2.66[b]	245.02±0.76[c]
98% SB+2% DM	15.55±0.09[c]	2080.43±2.68[b]	243.60±1.63[c]
96% SF+4% DM	14.81±0.05[d]	2025.33±2.66[d]	253.01±0.82[ab]
98% SB+2% SCMC	14.87±0.09[d]	2055.82±5.35[e]	248.71±1.13[bc]
96% SB+4% SCMC	13.03±0.09[e]	1994.30±2.67[f]	259.06±1.85[a]

[1)]SB, wholemeal *Sorghum bicolor* flour; BE, *Brachytegia eurycoma* flour; DM, *Detarium microcarpum* flour; SCMC, sodium carboxymethyl cellulose.
[2)]All values are mean±SD (n=3).
[3)]Values with different lowercase superscript letters in the same column differ significantly ($p<0.05$).

mechanisms of polyphenolics' antioxidant activity are reduction of H_2O_2 to stable compounds, singlet oxygen suppression, mopping up of free radicals, and inhibition of endogenous pro-oxidative enzymes (Imam et al., 2024; Irondi et al., 2022a; Sęczyk et al. 2019).

3.5. In vitro inhibitory activity against digestive enzymes (pancreatic lipase, α-amylase, and α-glucosidase

To provide more insight into the possible health-benefiting effects of the flour samples, their inhibitory capacities on three digestive enzymes, including pancreatic lipase, α-amylase, and α-glucosidase) are presented in Table 5. Among the flour samples, the IC_{50} values of SB and 98% SB+2% BE against pancreatic lipase, α-amylase and alpha-glucosidase were comparable ($p>0.05$), indicating a similarity in their enzyme inhibitory capacity. The 96% SB+4% SCMC blend had the highest IC_{50} values against the three digestive enzymes tested, representing the weakest inhibitory capacity. However, the reference inhibitors of the tested enzymes (orlistat for pancreatic lipase and acarbose for alpha-amylase and alpha-glucosidase) had a stronger inhibitory effect on their respective enzymes, as indicated by their lower IC_{50} values.

The observed inhibitory effect of the flour samples on the three digestive enzymes in this study may be ascribed to their polyphenolics content. Polyphenolics have been extensively reported to inhibit the activity of digestive enzymes by different mechanisms. For instance, polyphenolics' high affinity for enzymes, via hydrophobic and hydrogen interactions, causes enzyme denaturation, resulting in their catalytic activity inhibition (Irondi et al., 2021a; Villiger et al., 2015).

Pancreatic lipase catalyzes the hydrolysis of dietary fats to fatty acids (Li et al., 2011). Inhibiting its activity retards dietary fats digestion and absorption, making it an index for assessing an anti-obesity agent's effectiveness (Sugiyama et al., 2007). Dietary starch, on the other hand, is broken down by α-amylase and α-glucosidase into absorbable monosaccharides that can be utilized for energy in the body (Etsassala et al., 2020). Their inhibition, therefore, stalls dietary starch hydrolysis, representing an important approach for ameliorating postprandial hyperglycemia (Li et al., 2022). Thus, the flour samples, especially SB and 98% SB+2% BE, may have potential for developing functional food products for ameliorating the elevated fatty acids and postprandial blood glucose levels associated with obesity and diabetes mellitus, respectively.

3.6. Correlations among pasting attributes and polyphenolic constituents in the flours

With the exception of pasting temperature and pasting time, all the pasting attributes showed a significant and negative correlation with the polyphenolics (Table 6). Pasting temperature showed a significant correlation with the polyphenolics (r=0.834, 0.838, 0.843 for total phenolics, tannins and total flavonoids, respectively), while pasting time had a significant negative correlation (r=-0.434) with total flavonoids only. Earlier, Ponjanta et al. (2016) also reported a significant negative correlation between total phenolics and peak viscosity, as well as a positive correlation between total phenolics and pasting temperature. Thus, increasing the polyphenolics level of the flour samples could lead to a decrease in their peak viscosity, trough viscosity, breakdown

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Table 5. Inhibitory activity of digestive enzymes (pancreatic lipase, α-amylase, and α-glucosidase) in *Sorghum bicolor* flour blended with hydrocolloids

Parameter	Pancreatic lipase IC_{50} (μg/mL)	Alpha-amylase IC_{50} (μg/mL)	Alpha-glucosidase IC_{50} (μg/mL)
SB[1)]	66.11±0.41[2)d3)]	122.83±0.35[d]	90.35±0.64[d]
98% SB+2% BE	67.08±0.42[cd]	124.88±037[cd]	93.84±0.28[cd]
96% SB+4% BE	69.43±0.30[bc]	126.32±0.18[bc]	95.94±0.15[bc]
98% SB+2% DM	69.01±0.60[c]	126.45±0.76[bc]	95.84±0.57[bc]
96% SF+4% DM	71.15±0.32[bc]	127.53±0.76[bc]	97.29±0.30[bc]
98% SB+2% SCMC	69.96±0.45[bc]	129.87±0.59[bc]	96.56±0.15[bc]
96% SB+4% SCMC	73.21±0.67[b]	135.28±0.86[b]	99.89±0.62[b]
Orlistat	0.48±0.02[a]	-	-
Acarbose	-	10.93±0.84[a]	19.37±0.96[a]

[1)]SB, wholemeal *Sorghum bicolor* flour; BE, *Brachytegia eurycoma* flour; DM, *Detarium microcarpum* flour; SCMC, sodium carboxymethyl cellulose.
[2)]All values are mean±SD (n=3).
[3)]Values with different lowercase superscript letters in the same column differ significantly ($p<0.05$).

Table 6. Correlations between pasting properties and polyphenolic constituents in *S. bicolor* flour blended with hydrocolloids

Parameter	Total phenolics	Tannins	Total flavonoids
Peak viscosity	-0.713[**1)]	-0.758[**]	-0.844[**]
Trough viscosity	-0.699[**]	-0.731[**]	-0.795[**]
Breakdown viscosity	-0.436[*2)]	-0.499[*]	-0.618[**]
Final viscosity	-0.608[**]	-0.659[**]	-0.766[**]
Setback viscosity	-0.443[*]	-0.513[*]	-0.664[**]
Pasting time	-0.388	-0.417	-0.434[*]
Pasting temperature	0.834[**]	0.838[**]	0.843[**]

[1)]**Significant correlation at $p<0.01$.
[2)]*Significant correlation at $p<0.05$.

viscosity, final viscosity and setback viscosity, while increasing their pasting temperature. This may have an important influence on the samples' applications in food industry.

3.7. Correlations among polyphenolic constituents and bioactivities of the flours

There was a strong correlation ($p<0.01$) between the polyphenolic's constituents and the antioxidant activity, as well as the digestive-enzyme-inhibitory capacity of the flour samples (Table 7). Each of total phenolics, tannins and total flavonoids correlated positively with the reducing power and ABTS-scavenging activity of the flour samples. However, they correlated negatively with the DPPH SC_{50} and digestive enzymes (pancreatic lipase, alpha-amylase and alpha-glucosidase) IC_{50} values of the samples. The strong positive correlation between the polyphenolics and each of reducing power and ABTS-scavenging capacity of the samples is consistent with previous reports affirming a strong positive correlation between polyphenolics and antioxidant activity (Khiya et al., 2021).

Similarly, the significant negative correlation ($p<0.01$) between the polyphenolics, DPPH SC_{50} and the digestive enzymes IC_{50} values of the samples further confirms the flour samples' free radical-scavenging capacity and digestive-enzyme-inhibitory property. This trend is consistent with the report of Elemosho et al. (2021) in which a negative correlation between polyphenolics level and each of DPPH

Table 7. Correlations between polyphenolics and antioxidant activity, as well as digestive enzymes inhibitory activity, in *S. bicolor* flour blended with hydrocolloids

Parameter	Reducing power	ABTS	DPPH SC_{50}	Pancreatic lipase IC_{50}	α-Amylase IC_{50}	α-Glucosidase IC_{50}
Total phenolics	0.982**[1)]	0.967**	-0.964**	-0.890**	-0.911**	-0.930**
Tannins	0.978**	0.980**	-0.958**	-0.873**	-0.894**	-0.915**
Total flavonoids	0.944**	0.983**	-0.918**	-0.808**	-0.830**	-0.857**

[1)]**Significant correlation at $p<0.01$.

SC_{50} and starch-hydrolyzing enzymes (α-amylase and α-glucosidase) IC_{50} indicated a strong free radical-scavenging and enzymes inhibitory activities. This is explained by the inverse relationship between DPPH SC_{50} and free radical-scavenging capacity, as well as between enzyme IC_{50} and enzymes inhibitory activity, where a lower value represents a stronger activity (Irondi et al., 2017a). Thus, an increase in the polyphenolics level of the samples could result in a stronger antioxidant and digestive-enzyme-inhibitory capacity, representing an enhanced health-promoting property. As earlier stated, polyphenolics exhibit antioxidant (Irondi et al., 2022b; Sęczyk et al. 2019) and digestive-enzyme-inhibitory (Irondi et al., 2021a; Liu and Xu, 2015) activities through different mechanisms.

4. Conclusions

In this study, SB blended with natural hydrocolloid flours BE and DM had a higher content of polyphenolics (total phenolics, tannins, and total flavonoids), and stronger antioxidant and digestive-enzyme-inhibitory capacities than the SB blended with a modified hydrocolloid SCMC. Among the blends, 98% SB+2% BE had the highest polyphenolics level and the most potent antioxidant and digestive-enzyme-inhibitory capacities. The peak viscosity and final viscosity of the blends increased as the BE and DM proportion increased. The peak viscosity, trough viscosity, breakdown viscosity, final viscosity, and setback viscosity correlated negatively, while the pasting temperature correlated positively with the flour samples' polyphenolics levels. Further, the polyphenolics levels correlated positively with the flour samples' antioxidant capacity and digestive-enzyme-inhibitory activity. Therefore, blending SB with either BE or DM may be a low-cost strategy for developing a gluten-free flour with improved antioxidant and digestive-enzyme-inhibitory properties.

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Conflict of interests

The authors declare no potential conflicts of interest.

Author contributions

Conceptualization: Irondi EA. Methodology: Irondi EA, Bankole AO, Olatoye KK. Formal analysis: Irondi EA, Bankole AO, Olatoye KK, Imam YT. Validation: Aliyu OM, Irondi EA. Writing - original draft: Bankole AO, Imam YT. Writing - review & editing: Irondi EA, Aliyu OM.

Ethics approval

This article does not require IRB/IACUC approval because there are no human and animal participants.

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