Novel *Vigna subterranea* (L.) Verdc Soluble Dietary Fibre-Starch Nanocomposite: Functional and Antioxidant Characteristics

Running head: Bambara Groundnut Nanocomposite Functional and Antioxidant Characteristics

Yvonne Maphosa[1*], Victoria Adaora Jideani[1] and Daniel Imwansi Ikhu-Omoregbe[2]

[1]Department of Food Science and Technology, Cape Peninsula University of Technology, P.O Box 1906, 7535 Bellville, South Africa
[2]Department of Chemical Engineering, Cape Peninsula University of Technology, P.O Box 1906, 7535 Bellville, South Africa

SUMMARY

Research background. Bambara groundnut (BGN) (*Vigna subterranea* [L.] Verdc.) is a source of soluble dietary fibre (SDF) and starch, making it a great source of the two polysaccharides. Bambara groundnut soluble dietary fibre (BGN-SDF) is rich in bioactive compounds, namely, uronic acids (11.8 %) and hydrolysable polyphenols (20 mg Gallic acid equivalent (GAE)/g), with crucial physiological and functional benefits. The industrial use of native starch is limited because of the inherent undesirable attributes that render it unstable. The aim of this study was to characterise the antioxidant, functional and physicochemical properties of Bambara groundnut starch-soluble dietary fibre nanocomposite (STASOL).

Experimental approach. The pasting properties by rapid visco analysis (RVA), chemical composition following AOAC procedures, hydration properties, oil binding capacity (OBC), emulsion activity index (EAI), emulsion stability index (ESI) and antioxidant properties of Bambara groundnut starch (BGNS), BGN-SDF and STASOL were studied.

Results and conclusions. BGN-SDF and STASOL did not exhibit typical pasting properties. STASOL was high in carbohydrates (78.69 %) and proteins (6.96 %), low in fat (0.84 %) and had a

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*Corresponding author:
Phone: +27833395263
E-mail: jideaniv@cput.ac.za

considerable amount of ash (4.88 %). BGNS, BGN-SDF and STASOL showed significant ($p < 0.001$) differences in solubility. The EAI of BGNS, BGN-SDF and STASOL were 23.25, 85.71 and 90.65 %, respectively, and the ESI of BGNS, BGN-SDF and STASOL were 23.33, 87.13 and 87.49 %, respectively. The three biopolymers differed significantly ($p < 0.001$) in all colour characteristics; lightness (L^*), redness/greenness (a^*), yellowness/blueness (b^*), chroma and hue angles. The polyphenolic content of BGNS, BGN-SDF and STASOL was 0.10, 6.59 and 0.46 mg GAE/g, respectively, and their ferric reducing antioxidant power (FRAP) values were 1.16, 4.77 and 1.45 µmol ascorbic acid equivalents (AAE)/g, respectively. The phenolic compounds, chlorogenic acid (18 mg/g), monocrotaline (20 mg/g), luteolin 7-O-(6"-malonylglucoside) (4 mg/g) and casuarine 6-alpha-D-glucoside (27 mg/g) were present in BGN-SDF but absent in BGNS and STASOL. STASOL possesses desirable physicochemical and antioxidant properties, making it suitable as an ingredient in various food systems.

Novelty and scientific contribution. Nanocomposites have the potential to revolutionise the food industry but their study as food ingredients is very limited. Furthermore, nothing is known about the physicochemical, functional and antioxidant characteristics of STASOL. Investigating these properties of STASOL will address this knowledge gap.

Keywords: bambara groundnut; starch; dietary fibre; nanocomposite; antioxidants; pasting properties

INTRODUCTION

The demand for innovative food products formulated with natural components has driven the constant search for new biopolymer complexes with improved properties. The increasing demand for such complexes, both by consumers and the food industry, has led to the utilisation of biopolymers from underutilised and orphan crops, such as Bambara groundnut (BGN) (*1*). Starch and dietary fibres from different sources have been studied and applied in the production of various food products (*2*). Starch and starch-based composites immensely contribute to the functional properties of food products (*3*). The major functional properties of starches and dietary fibres include thickening, swelling, gelation, water absorption, water binding, foaming, emulsification and bulk density (*4*). Bambara groundnut starch and soluble dietary fibres have been reported to possess good functional properties and their complexes have improved pasting, thickening, viscoelastic and thermal properties than individual starches (*5*).

Previous studies have reported the effect of complexing starch with other biopolymers on the functionality, antioxidant composition and overall behaviour of the formed complexes. Such studies reported on composites made from potato starch and waxy maize starch (*6*), waxy rice starch and

non-waxy starch (*7*), lima bean starch and cassava starch (*8*) as well as potato starch and maize starch (*9*). It is important to study the functional and physicochemical properties of food components as they affect sensory characteristics as well as the behaviour of food during formulation, processing, storage and distribution (*1,2*). Knowledge of the functional and antioxidant properties of biopolymer composites enables their maximal utilisation and application in food systems for human nutrition and health (*5*).

The nanocomposite used in this study was a starch-soluble dietary fibre nanocomposite (STASOL), produced from the chemical complexing of BGN soluble dietary fibre (BGN-SDF) and BGN starch (BGNS). STASOL was reported to have an average particle size of 74.01 nm which qualifies it as a nanocomposite (nano=<100 nm) (*10*). The employment of STASOL in a food system would produce a relatively "green" product as all components used were natural. The objective of this study was to characterise the antioxidant, functional and physicochemical properties of STASOL.

MATERIALS AND METHODS

Source of materials

Analytical grade chemicals from Sigma-Aldrich (Johannesburg, South Africa) were used in this study. Equipment and apparatus from Food Science and Technology, Chemical Engineering and Oxidative Stress Research Centre of the Cape Peninsula University of Technology (Cape Town, South Africa) were used. STASOL is a BGN starch-soluble dietary fibre nanocomposite made from the chemical complexing of BGN-SDF and BGNS. The STASOL used in this study was produced following the method described by Maphosa *et al*. (*10*). In a 100 mL Schott bottle, 15 g BGNS, 37.5 mL deionised water, 16.5 % H_2O_2 (120 v) and 0.1 % ascorbic acid were mixed then incubated at 90 °C for 45 min in a temperature-controlled water bath (Ecobath, LaboTec, Midrand, South Africa). The mixture was cooled to room temperature before precipitating with 40 mL absolute ethanol drop wise with continuous agitation in a sonicator (Sonicator Heat Systems-Ultrasonics, Inc., Model W-225R, Krugersdorp, South Africa) for 10 min at high speed. The mixture was then centrifuged (Jouan MR1812 Thermo Electron Corporation, ThermoFisher Scientific, Massachusetts, USA) at 6 000 rpm for 5 min and the supernatant was discarded. To obtain regenerated BGNS particles as well as remove H_2O_2 and ascorbic acid, the residue was rinsed three times with 40 mL absolute ethanol. BGNS (15 g) and BGN-SDF (1.95 g) were dissolved in 40 and 15 mL ethanol:water (1:1 v/v), respectively. The two solutions were mixed to obtain a molecular dispersion of BGNS and BGN-SDF and left to react for 24 h, on a magnetic stirrer (Dragon Lab, MS-H-Pro, Beijing, China), in a dark fume hood. The resulting solution was freeze-dried (VirTis Genesis 25EL, Warminster, Pennsylvania, USA).).

Pasting properties of Bambara groundnut starch, soluble dietary fibre and starch-soluble dietary fibre nanocomposite

Pasting properties were analysed using a Rapid Visco Analyser (RVA, 4500 Perten Instruments, Queensland, Australia) following the method of Gulu (*1*). A 3 g sample (14 % moisture) in 25 g deionised water was subjected to a heating and cooling cycle while continuously under shear. The samples were held at 50 °C for 1 min then heated at a ramp of 6 °C/min from 50 to 95 °C. Thereafter, the temperature was decreased to 50 °C at a ramp of 6 °C/min and held for 5 min. The pasting time, peak viscosity, pasting temperature, breakdown viscosity, cold paste viscosity and setback viscosity were obtained from the RVA curves and viscosities were expressed in Pa·s.

Colour characteristics of Bambara groundnut starch, soluble dietary fibre and starch-soluble dietary fibre nanocomposite

Colour characteristics of BGNS, BGN-SDF and STASOL were determined using a spectrophotometer (Spectrophotometer, model CM-5, Konica Minolta Sensing inc, Sakai, Japan) set at standard observer 10° and D65 following a method of Murevanhema and Jideani (*11*). The spectrophotometer was calibrated with a black tile (*L**=5.49, *a**=7.08, *b**=4.66) and a white tile (*L**=93.41, *a**=1.18, *b**=0.75) followed by zero calibration (*4*). Enough samples to completely cover the bottom of the glass sample holders (diameter 30 mm) were used. Colour parameters were assessed using *L**C*h* and CIE-*L**a*b* colour space systems. Colour differences (Δ*E*) amongst the samples were calculated using the colour difference equation:

$$[\Delta E = (\Delta L^{*2} + \Delta a^{*2} + \Delta b^{*2})^{1/2}] \hspace{2cm} /1/$$

where: *L** is lightness, *a** is redness/greenness and *b** is yellowness/blueness (*12*).

Chemical analysis of Bambara groundnut starch, soluble dietary fibre and starch-soluble dietary fibre nanocomposite

Chemical profiling was carried out according to AOAC (*13*) procedures of total fat (Gas Chromatography), protein (Leco, TruSpec® N, California, USA), moisture (Moisture analyser, Denver Instrument 1R-30, California, USA), ash (muffle furnace), minerals (Inductively coupled plasma-optical emission spectrometry) and carbohydrates (calculated by difference). Energy (kJ) was calculated using the corresponding values of fat (37 kJ/g), protein (17 kJ/g) and carbohydrates (17 kJ/g) (*13*).

Hydration properties of Bambara groundnut starch, soluble dietary fibre and starch-soluble dietary fibre nanocomposite

Water absorption capacity (WAC) and solubility index (SI) were determined according to methods described by Lai *et al.* (*14*). To determine WAC, 0.2 g samples were weighed into centrifuge tubes and mixed with 9.8 g of deionized water. The suspensions were heated at 70 °C for 10 min in a shaking water bath (Ecobath, LaboTec, Midrand, South Africa) then transferred to a boiling water bath for another 10 min. The suspensions were cooled then centrifuged at 40 000 × *g* at 5 °C for 30 min (Jouan MR1812 Thermo Electron Corporation, ThermoFisher Scientific, Massachusetts, USA). WAC was determined as the ratio of the mass of swollen samples after centrifugation (g) to their dry mass (g).

To determine SI, the supernatant from WAC analysis was decanted into tared evaporating crucible dishes and dried overnight at 110 °C. Solubility index was then calculated as the ratio of the mass of solids after drying (g) to the initial mass of the dry sample (g).

Oil binding capacity of Bambara groundnut starch, soluble dietary fibre and starch-soluble dietary fibre nanocomposite

The method of Maphosa and Jideani (*4*) was applied in the determination of the oil binding capacities (OBC) of BGNS, BGN-SDF and STASOL. Samples (1 g) were mixed with 5 g of canola oil in 50 mL centrifuge tubes. The mixtures were vortexed for 30 s at 5 min intervals for 30 min, then centrifuged (Jouan MR1812 Thermo Electron Corporation, ThermoFisher Scientific, Massachusetts, USA) at 1600 × *g* for 25 min at 23 °C. After centrifugation, the free oil was decanted and weighed. The difference between the original mass of oil (5 g) and the mass of the decanted oil was considered as retained oil. OBC was calculated as the mass of oil retained divided by the original mass of the sample.

Emulsion activity and stability of Bambara groundnut starch, soluble dietary fibre and starch-soluble dietary fibre nanocomposite

Emulsion activity index (EAI) and emulsion stability index (ESI) were determined following the methods of Chove *et al.* (*15*). Samples (1 g) were dissolved in 10 mL deionised water and homogenised (Ultra Turrax T-25 Homogeniser, IKA, Staufen, Germany) at 4500 × *g* for 2 min. Orange oil (10 mL) was added and the solution homogenised at 6000 × *g* for 1 min then centrifuged again at 1200 × *g* for 5 min. To calculate EAI, the emulsion volume was measured as the mL of emulsified layer per entire layer in the centrifuge tube and expressed as a percentage (%). To calculate ESI, the emulsion was heated at 80 °C for 30 min. After cooling at ambient temperature, the emulsion was centrifuged at 1200 × *g* for 5 min and ESI was calculated as the height of the emulsified layer after heating divided by the height of total content before heating.

Total phenolics content and antioxidant activity of Bambara groundnut starch, soluble dietary fibre and starch-soluble dietary fibre nanocomposite

Total phenolic compounds were determined following the method of Maphosa and Jideani (*4*). Samples (250 mg) were mixed with 10 mL of distilled water and 1 mL of H_2SO_4 in 14 mL centrifuge tubes then incubated at 80 °C for 20 h before centrifuging (4000 × *g*, 5 min, 21 °C). The supernatant was diluted tenfold and analysed in a 96 well plate using the Folin-Ciocalteu assay by mixing 25 µL of a sample with 125 µL of 0.2 M Folin-Ciocalteu and 100 µL of 7.5 % (w/v) Na_2CO_3 solution. The mixtures were left to stand for 2 h in the dark. Absorbance was then measured using a spectrophotometer (Multiskan Spectrum, ThermoFisher Scientific, Massachusetts, USA) at a wavelength of 765 nm using a gallic acid standard calibration curve. The results were expressed as mg/g gallic acid equivalents (GAE) of dry extract.

Ferric reducing antioxidant power (FRAP) assay was conducted following the method of Maphosa and Jideani (*4*) with Vitamin C as a standard. Samples (250 mg) were diluted tenfold then mixed with 0.3 Ml of FRAP reagent (30 Ml acetate buffer, 3 Ml $FeCl_3$, 3 Ml TPTZ and 6 Ml of H_2O). The mixture was poured into a 96 well plate, left to stand for 30 min and then read in a spectrophotometer (Multiskan spectrum, ThermoFisher Scientific, Massachusetts, USA) at a wavelength of 593 nm.

Determination of glucose in Bambara groundnut starch, soluble dietary fibre and starch-soluble dietary fibre nanocomposite

Samples (0.1 g) and 5 mL of 2 M trifluoroacetic acid were accurately weighed into 10 mL headspace tubes and hydrolysed at 110 °C for 12 h. The mixtures were then diluted with water:acetonitrile water (0.2 % triethylamine and 0.2 % NH_4OH in water) and acetonitrile (0.2 % triethylamine and 0.1 % 2-propanol and 0.2 % NH4OH in acetonitrile). The diluted samples were transferred into glass vials and analysed by UPLC-ELSD with a Waters Acquity BEH Amide 2.1 × 100 mm, 1.7 µm column (Waters, Milford, MA, USA). An injection volume of 5 µL was used with a column temperature of 50 ºC, seal wash of 5 min, run time of 10 min and a pressure gradient of $0-1.03421 \times 10^8$ Pa/min.

Phenolic profiling of Bambara groundnut starch, soluble dietary fibre and starch-soluble dietary fibre nanocomposite

Samples (2 g) and 15 mL of 50 % methanol/1 % formic acid were accurately weighed into 50 mL centrifuge tubes with screw-caps then vortexed for 1 min, followed by extraction in an ultrasonic bath for 1 h. Samples (2 mL) were then centrifuged at 14000 rpm for 5 min and the supernatants were

transferred into 1.5 mL glass vials using Liquid Chromatography-Mass Spectrometry (LCMS). A Waters Synapt G2 Quadrupole time-of-flight (QTOF) mass spectrometer (MS) (Waters, Milford, MA, USA) connected to a Waters Acquity ultra-performance liquid chromatography (UPLC) (Waters, Milford, MA, USA) was used for high-resolution UPLC-MS analysis. The column eluate first passed through a Photodiode Array (PDA) detector prior to flowing through the mass spectrometer, allowing concurrent collection of UV and MS spectra. Electrospray ionisation was used in negative mode with a cone voltage of 15 V, desolvation temperature of 275 °C, desolvation gas at 650 L/h, and the rest of the MS settings optimised for best resolution and sensitivity. Data were acquired by scanning from 150 to 1500 m/z in resolution mode as well as in MSE mode. In MSE mode two channels of MS data were acquired; one at low collision energy (4 V) and the second using a collision energy ramp (40-100 V) to obtain fragmentation data as well. Leucine enkephalin was used as a reference mass and the instrument was calibrated with sodium formate. Separation was achieved on a Waters HSS T3, 2.1 × 100 mm, 1.7 µm column and the mobile phase was made up of 0.1 % formic acid (solvent A) and acetonitrile containing 0.1 % formic acid (solvent B). The parameters used were: injection volume (2 µL), flow rate (0.3 mL/min) and column temperature (55 °C). The gradient began at 100 % solvent A for 1 min, then went to 28 % solvent B over 22 min, then changed to 40 % solvent B over 50 s and a wash step of 1.5 min at 100 % solvent B, and finally re-equilibrated to initial conditions for 4 min. A range of catechin standards were injected from 0.5 to 100 mg/L and used to establish a calibration curve against which compounds were quantified. Data were processed using MSDIAL and MSFINDER (RIKEN Center for Sustainable Resource Science: Metabolome Informatics Research Team, Kanagawa, Japan).

Data analysis

For statistical analysis, IBM Statistical Package for the Social Science (SPSS) version 25 was used (*16*). All experiments were carried out in triplicate. Data were expressed as mean±standard deviation. The results were subjected to multivariate analysis of variance (MANOVA) to establish differences between treatments. Duncan's multiple range test was used to separate means where a significant ($p \leq 0.05$) difference existed.

RESULTS AND DISCUSSION

Pasting properties of Bambara groundnut starch, soluble dietary fibre and starch-soluble dietary fibre nanocomposite by rapid visco analyser

The pasting characteristics of BGNS, BGN-SDF and STASOL are presented in Table 1. Pasting is a term used to describe the changes in the structure of starch molecules post gelatinisation

(*17*). It describes the changes in starch such as further swelling of granules, leaching of molecular components from the granules and disruption of granules. Several studies have reported the pasting properties of BGN starch (*1,2,5,17*). As expected, BGN-SDF and STASOL did not exhibit typical pasting properties since pasting is a characteristic typical of starch molecules.

Peak viscosity

Peak viscosity refers to the highest viscosity of starch obtained during gelatinisation (*18*). It is indicative of the strength of starch pastes and their water holding capacities (*19,20*). The peak viscosities of BGNS, BGN-SDF and STASOL were 4.875, 0.15 and 0.435 Pa·s and all three differed significantly (p<0.001). Peak viscosity is an indication of structural damage due to factors such as temperature and shear, with high values indicating more extensive damage (*2,21*).

STASOL had a significantly (p<0.001) lower peak viscosity than BGNS and BGN-SDF therefore it would be expected to maintain its structure at relatively high temperatures. This was confirmed by thermal studies (*10*) where the structural degradation of STASOL occurred at 293.14 °C (STASOL). The structural damage of BGNS at a lower temperature of 77.19 °C (*10*) was due to its ability to gelatinise (*22*). Starch is composed of repeating glucose units, dietary fibre is a mixture of chemically complex non-starch polysaccharides and STASOL is a mixture of BGNS and BGN-SDF suggesting that new functional groups were introduced during the chemical grafting process (*10*). The significantly (p<0.001) reduced peak viscosity of STASOL could be attributed to several factors such as the disruption of starch granules during the chemical grafting process resulting in loss of gelatinisation ability as well as the simplicity of the starch molecule compared to the other two compounds, hence allowing it to swell unrestrictedly (*2*). Furthermore, the difference in the microstructure and morphology of BGNS and STASOL were discussed by Maphosa *et al.* (*10*) and BGNS granules were reported to be spherical with smooth surfaces while STASOL was irregular and polygonal. These differences were attributed to the disruption of BGNS bonds during chemical grafting as well as the formation of new bonds when BGN-SDF was added in the formation of STASOL. Since peak viscosity is also an indication of water binding capabilities of starch (*21*); the higher peak viscosity of BGNS means that in comparison to BGN-SDF and STASOL, BGNS granules would swell more easily when heated and form a thicker paste.

Gulu (*1*) reported a similar trend in the peak viscosity of native and chemically modified starches reporting a reduction in their peak viscosities from 4.876 to 0.435 Pa·s, respectively. Oyeyinka *et al.* (*5*) reported a lower peak viscosity of 3.293 Pa·s for BGNS. The difference in the reported peak viscosity could be due to the fact that starches from different BGN varieties were studied. Starch from the black-eye variety was used in this study while starch from the cream variety

was used by Oyeyinka *et al.* (*5*). Lower starch peak viscosities were reported as 1.335, 2.152 and 4.145 Pa·s for wheat (*19*), barley (*23*) and potato (*24*), respectively. Differences in peak viscosities of starches can be attributed to source of starch, differences in amylose and amylopectin contents, strength of amylose-amylose and amylose-amylopectin chain interactions as well as the molecular structures of amylose and amylopectin (*19*).

Trough viscosity

Trough viscosity is also known as hot paste viscosity and it describes the rate of breakdown in viscosity to equilibrium. A low trough viscosity indicates higher paste stability. The trough viscosity of BGNS (1.984 Pa·s) was significantly ($p=0.012$) higher than that of BGN-SDF (0.143 Pa·s) and STASOL (0.412 Pa·s). Gulu (*1*) reported a similar trend observed in this study where chemical modification of BGNS using hydrogen peroxide and ascorbic acid resulted in a decrease in trough viscosity. Gulu (*1*) reported a trough viscosity of 0.088 Pa·s for chemically modified BGNS while a trough viscosity of 0.143 Pa·s was reported in this study. The differences in results indicate that grafting catechin onto BGNS as was done by Gulu (*1*) resulted in the formation of a more robust complex compared to grafting BGN-SDF onto BGNS to form STASOL. Trough viscosity is dependent on temperature, degree of shear stress applied to a mixture as well as the nature of the material of interest. A higher trough viscosity (3.013 Pa·s) for BGNS was reported by Gulu (*1*). The difference in the results could be due to that starch from a mixture of different varieties of BGN seeds was studied by Gulu (*1*) while starch from the black-eye variety was evaluated in the present study.

Trough viscosities of 0.560 Pa·s for wheat starch (*19*), 1.423 cPa·s for barley starch (*23*), 2.351 Pa·s for potato starch (*24*) and 5.280 Pa·s for barley starch (*25*) were reported. These are starches commonly used in the food industry. Their trough viscosities were very high indicating that they would breakdown when exposed to elevated temperatures and form unstable pastes. As such, more stable composites such as STASOL with low trough viscosities (0.412 Pa·s) would be desirable replacements. The relatively low trough viscosity of STASOL suggests that the nanocomposite would find use in high temperature food systems such as baking and high shear processes such as those involved in the production of emulsions and dough.

Final viscosity

The final viscosities of BGNS, BGN-SDF and STASOL were 4.621, 0.271 and 0.537 Pa·s, respectively. There was no difference in the final viscosities of BGN-SDF and STASOL while both were significantly ($p<0.001$) lower than that of BGNS. Final viscosity gives an indication of the starch's ability to form a gel or viscous paste after cooking and cooling (*26*). It is also used to determine starch

quality and stability of the cooked starch paste in food products. According to Falade *et al.* (*21*), final viscosity plays a vital role in the rigidity and stability of the swollen granule structure. Higher final viscosities of 4.867, 4.876, 4.989 and 5.591 Pa·s were reported for rice starch (*27*), BGNS (*1*), quinoa starch (*27*) and barley starch (*25*), respectively. The lower final viscosity of STASOL compared to these starches was due to the loss of crystallinity during the grafting process. This was confirmed by crystallinity studies using powder X-ray diffraction (*10*) where native BGNS was described as crystalline in nature while STASOL was amorphous. The transformation from crystalline to amorphous nature of legume starch as a result of chemical treatment has been widely reported (*22*). The lower final viscosities of BGN-SDF and STASOL would result in the formation of more stable food systems. Therefore, these two biopolymers would make suitable alternatives to modified starches.

Breakdown viscosity

The breakdown viscosities of BGNS, BGN-SDF and STASOL were 2.890, 0.007 and 0.024 Pa·s. Both BGN-SDF and STASOL had significantly (p=0.003) lower breakdown viscosities than BGNS. Breakdown viscosity is an indication of the starch organisation structure and measures the susceptibility of starch granules to disintegration (*26*). A higher breakdown viscosity means the material has low heat and shear resistance (*21*). BGNS had the highest breakdown viscosity and therefore would have low thermal stability in comparison to BGN-SDF and STASOL (*20*). This was in agreement with thermal studies (*10*). BGNS had a higher breakdown viscosity than potato starch (2.195 Pa·s), wheat starch (0.775 Pa·s) and barley starch (1.938 Pa·s) (*1,24,25*). Therefore, the modification of native BGNS was necessary to improve its robustness. The low breakdown viscosity of STASOL would render the nanocomposite stable at high processing temperatures due to its high thermal stability making it a suitable ingredient for food products that are cooked at elevated temperatures and undergo extensive mixing in the preparation stage.

Setback viscosity

The setback viscosities of BGNS, BGN-SDF and STASOL were 2.637, 0.128 and 0.122 Pa·s, respectively (Table 1). Both BGN-SDF and STASOL had significantly higher (p<0.001) setback viscosities than BGNS. Setback viscosity refers to the tendency of starch to retrograde and undergo syneresis (*1*). After gelatinisation, amylopectin recrystallises and transforms from an amorphous state to a more crystalline state causing the starch pastes to thicken and form stiff gels (*28*). Setback viscosity is an indication of gel stability (*18*). When starch pastes cool down there is re-association between amylose and amylopectin molecules which results in the formation of a gel structure and consequently, increased viscosity (*21,26*). A high setback viscosity, as exhibited by BGNS, indicates

a high tendency towards retrogradation and syneresis (*20*). Bambara groundnut SDF and STASOL are complex polymers with hydrogen and covalent bonds which inhibit the re-association of bonds following heating. The lower setback viscosity of STASOL compared to BGNS could be attributed to the introduction of BGN-SDF to BGNS during the manufacturing process resulting in the formation of new functional groups as shown by FTIR studies (*10*). These functional groups indicate the formation of new bonds which restrict the re-association of starch molecules after cooling. This further proved that BGN-SDF was successfully grafted onto BGNS, reinforced the matrix and improved its characteristics. Gulu (*1*) reported the setback viscosity of BGNS as 1.957 Pa·s and established that inclusion complexes stabilise BGN starches. The differences in setback viscosities among studies could be attributed to starch extraction methods applied, machines used as well as variety of BGN tested.

Pasting temperature

When starch granules are exposed to elevated temperatures in the presence of water, they absorb water, swell and eventually rupture. The temperature at which they begin swelling is known as the pasting temperature (*27*). Pasting temperature gives an indication of the minimum temperature required to initiate starch gelatinisation (*26*). The pasting temperatures of BGNS (77.9 °C), BGN-SDF (96.7 °C) and STASOL (87.4 °C) were significantly ($p<0.001$) different. The results of the thermal properties of BGNS (*10*) were in fair agreement with the pasting properties of BGNS where initial peak thermal degradation due to gelatinisation of native BGNS was reported as 77.19 °C. Higher pasting temperatures of 84 and 83.2 °C were reported by Adebowale *et al.* (*2*) and Afolabi (*22*), respectively for BGNS. Pasting temperatures higher than those of BGNS but lower than those of STASOL and BGN-SDF were reported as 82.0 and 82.2 °C for Mucuna bean starch (*2*) and barley starch (23), respectively. The pasting temperature of starch observed in this study is similar to that of Sirivongpaisal (*17*) and Gulu (*1*) who reported the pasting temperature of BGNS as 77.7 and 79.90 °C, respectively.

Pasting temperatures of BGNS range between 77 and 84 °C depending on the source and variety of seeds (*5,17*). The pasting temperature of BGNS in this study was within this range. This range is higher than that of potato starch (66.2-68.6 °C) and comparable to that of corn starch (77−88 °C) (*29*). The relatively high pasting temperature of pulses compared to cereal starches could be accredited to their high amylose contents (*30*). A higher pasting temperature is an indication of thermal stability as more energy is required to rupture the granules. The higher pasting temperature of STASOL was a confirmation of the presence of stronger bonds which required more energy to disrupt.

The introduction of new functional groups as well as the disappearance and shifting of functional groups in STASOL in FTIR studies confirmed the formation of a new, stronger complex (*10*).

Peak time

Peak time is the time when peak viscosity is reached (*31*). The peak times of BGNS, BGN-SDF and STASOL were 4.78, 6.87 and 6.97 min, respectively (Table 1). The peak times of all three biopolymers were significantly ($p < 0.001$) different. A similar peak time of 4.70 min for BGNS was reported by Gulu (*1*). Complexing increases the time required to reach peak viscosity (*1*) because more time is needed to break down the structure of the material under study. Hence, STASOL (a biopolymer nanocomposite) and BGN-SDF (a complex polysaccharide) exhibited higher peak times that BGNS (a simpler polysaccharide). A comparable peak time of 4.33 min for potato starch was reported by Kaur *et al.* (*24*). Potato starch is commonly used as a thickener in soups, sauces, custards and puddings (*32*) hence suggesting that BGNS could be employed for similar use in these systems.

Pasting studies showed that the pasting properties of BGNS would render it unsuitable for use as an ingredient in many food systems as it would breakdown at temperatures below 100 °C, gelatinise, retrograde and be prone to syneresis. Hence the modification of BGNS by grafting it with BGN-SDF to form STASOL was justified. STASOL has improved properties and would be useful as an ingredient in various food systems as it withstands high shear rates and temperatures. STASOL exhibited a similar behaviour to some starches commonly used in the food industry, suggesting that it would behave in a similar desirable manner.

Colour characteristics of Bambara groundnut starch, soluble dietary fibre and starch-soluble dietary fibre nanocomposite

The lightness (*L**), redness/greenness (*a**), yellowness/blueness (*b**), chroma and hue angles were 87.94, 1.33, 12.43, 12.50 and 83.87° (BGNS), 76.12, 2.21, 18.86, 18.96 and 83.32° (BGN-SDF) as well as 89.16, 0.4, 14.61, 14.62 and 88.42° (STASOL), respectively (Table 2). The three biopolymers differed significantly ($p < 0.001$) in all colour characteristics. The relatively higher degree of lightness observed in STASOL can be attributed to the bleaching properties of H_2O_2 used in the formulation of the nanocomposite. All the polymers had positive *a** and *b** values indicating that they were more associated with redness and yellowness, respectively.

The redness and yellowness of BGN-SDFs relate to phenolics and other antioxidant-possessing chemicals such as anthocyanins, cyanidins as well as a wide range of isoflavones and phenolic acids (*11*). The lightness of biopolymers destined for food use is of importance in the final products as it determines how much the original colour of the food system will be affected (*32,33*). As

such, STASOL can be used as an ingredient in food systems without negatively impacting their colour. It was observed that lightness increased with increasing hue and this was in agreement with Maphosa and Jideani (*4*). Maphosa and Jideani (*4*) studied BGN-SDF from the black-eye variety and reported the colour attributes as *L** (73.0), *a** (1.7), *b** (13.8), chroma (13.9) and hue angle (83.1°). These were comparable with the colour attributes reported in this study, *L** (76.1), *a** (2.2), *b** (18.9), chroma (19.0) and hue (83.9°).

The colour differences among the biopolymers are presented in Table 2. A colour difference (Δ*E*) of 1 is known as a just-noticeable difference (JND) and is the threshold at which trained observers notice the difference between two colours (*34*). The difference between two colours can be noticeable but still considered acceptable. A colour difference between 4 and 8 is acceptable. Above 8, the colour difference is deemed unacceptable and likely to be rejected by consumers (*35*). The colour differences between BGNS and STASOL, BGNS and BGN-SDF, and BGN-SDF and STASOL were 6.60, 13.48 and 14.22, respectively. There was a noticeable difference in colour among the three biopolymers. The difference between BGNS and STASOL (Δ*E*=6.60) was considered acceptable as it falls in the region 4−8 (*4*). BGNS-BGN-SDF and BGN-SDF-STASOL had ΔE above 8, meaning their colour differences would be very apparent if they were used interchangeably.

Chemical composition of Bambara groundnut starch, soluble dietary fibre and starch-soluble dietary fibre nanocomposite

The chemical composition of BGNS, BGN-SDF and STASOL is given in Table 3.

Bambara groundnut starch and BGN-SDF did not differ significantly in their moisture (p=0.620) and fat (p=0.116) content while both had a significantly (p<0.001) higher moisture content and a significantly (p<0.001) lower fat content than STASOL. All three biopolymers differed significantly (p<0.001) in energy, protein and carbohydrate composition. Bambara groundnut SDF and STASOL were not significantly (p=0.217) different in their ash content and both had a significantly (p<0.001) higher ash content than BGNS.

Ash is an indication of the mineral content of samples and the high values obtained in BGN-SDF and STASOL suggested that they may contribute micro and macro elements to food systems (*17,34*). The moisture content of all three biopolymers in this study was similar to that of cassava starches (8.21−12.39 %) reported by Agyepong and Barimah (*34*). Lower ash values (0.38-0.98 %) were reported for cassava modified starches (*34*). These were considerably lower than those of STASOL and this could be because the modification of BGNS in the formation of STASOL involved the introduction of a new biopolymer (BGN-SDF) which has a relatively high ash content (4.90 %). Other starches are commonly modified using methods that involve the use of enzymes, heat and

chemicals without the introduction of a second nutritional compound hence the ash content is solely an indication of the minerals present in the starch. As such STASOL would make a better fortifier of minerals in food products. The mineral composition of BGNS, BGN-SDF and STASOL determined using ICP-OES is presented in Fig 1.

Minerals are of importance in the physiological functioning of the human body. BGNS contained the highest amount of potassium (15.69 mg/L) and zinc (0.72 mg/L) but the lowest amount of sodium (1.42 mg/L) and magnesium (4.39 mg/L). BGN-SDF had the highest amount of zinc (0.34 mg/L) sodium (1.67 mg/L), magnesium (14.66 mg/L) and iron (1.41 mg/L). STASOL had the lowest amount of iron (0.98 mg/L) but a high amount of potassium (11.64 mg/L) and magnesium (10.47 mg/L). The total mineral content of the biopolymers determined using ICP-OES agreed with the nutritional studies, with BGN-SDF having the highest ash content and BGNS the least (Table 3). Bambara groundnut SDF had the highest amount of ash (4.90 g) and total mineral content (31.55 mg/L) while BGNS had the lowest amount of ash (0.07 g) and total mineral content (23.25 mg/L). This was because the minerals in STASOL were from the BGNS and BGN-SDF used in the production. Generally, the values for most of the minerals in this study were less than those reported for BGN seeds (*2,36*). This was expected as only components of BGN were analysed in this study while whole seeds were reported by other researchers.

Hydration and oil binding properties of BGNS, BGN-SDF and STASOL

The solubility index (SI), water absorption capacity (WAC) and oil binding capacity (OBC) of BGNS, BGN-SDF and STASOL are given in Table 4.

Solubility is defined by the IUPAC (*37*) as the analytical composition of a saturated solution expressed as a proportion of a designated solute in a designated solvent. A high SI positively affects the functional properties both in food systems and in the gastrointestinal canal (*37*). All biopolymers showed significant ($p<0.001$) differences in SI with native BGNS being practically insoluble in water (0.9 %). Native starch is insoluble in cold water because the amylose and amylopectin chains are arranged in a tight interlocking structure that becomes the hydrophobic crystalline-like starch granule (*1*). This was in agreement with the results of Agyepong and Barimah (*34*) who reported very low solubility of native cassava starch in the range 1.29−7.38 %. Furthermore, the researchers reported an increase in solubility of starch after modification. This observation was also made for STASOL in this study. The smaller average particle size (74.01 nm) of STASOL (*10*) translated to a larger surface area to volume ratio, hence increasing its interaction with water molecules. The high solubility of STASOL would make it a useful ingredient in food preparations such as soups, dairy products, beverages, coffee creamers and gravies (*38*).

Water absorption capacity (WAC) is a function of the water holding ability of a sample and it plays a significant role in food systems where the sample is employed as a bulking agent, stabiliser, thickener and anti-caking agent (*2*). The WAC of BGNS was 1.62 g/g while that of BGN-SDF and STASOL could not be determined as they were significantly soluble in water as shown in Table 4. A similar WAC of BGNS was reported by Sirivongpaisal (*17*) [1.67 g/g] while Gulu (*1*) reported a lower WAC (1.17 g/g) for BGNS. Differences could be attributed to the different BGN varieties used. Gulu (*1*) used mixed BGN seeds while only the black-eye variety was used in the production of STASOL. Adebowale *et al.* (*2*) reported a higher WAC (2.0 g/g) for BGNS, which could be attributed to the different extraction methods. Adebowale *et al.* (*2*) employed a wet milling method while a dry milling method was used in this study. The relatively lower WAC reported in this study suggested that BGNS could find use in food applications where moderate swelling is required (*35*).

The oil binding capacities (OBC) of BGNS, BGN-SDF and STASOL differed significantly (p<0.001) and were 1.13, 3.78 and 1.61 g/g, respectively. Oil binding capacity is the ability of lipids to form interactions with non-polar side chains of molecules (*35*) and is affected by factors such as particle size, surface area, charge and hydrophobicity (*2*). It works on the principle of physically entrapping oil through capillary attraction (*1*). It indicates the ability and extent to which biopolymers can stabilise high-fat food products and emulsions as well as act as fat binders and replacers in products such as meat (*39*). The OBC of native BGNS in this study (1.13 g/g) was lower than the WAC (1.62 g/g) which indicated a higher level of hydrophilicity compared to hydrophobicity. The low OBC of BGNS compared to STASOL and BGN-SDF could be attributed to the low levels of proteins (Table 3). The proteins present in STASOL and BGN-SDF may be hydrophobic hence would display superior lipid binding characteristics (*38*). The ability of biopolymers and biopolymer nanocomposites like STASOL to bind oil can be harnessed by the food industry in reducing fat losses during cooking as well as in foodstuffs such as meat, emulsions and baked goods by improving palatability, flavour retention and shelf life extension (*40*). Physiologically, the OBC of biopolymers would allow them to play a significant role in bile acid absorption and consequently cholesterol reduction (*35*).

Emulsion activity and stability indexes of BGNS, BGN-SDF and STASOL

Emulsion activity index (EAI) is the ability of a compound to participate in the formation and stabilisation of a newly created emulsion (*41*) while emulsion stability index (ESI) measures the ability of an emulsion to resist changes to its structure and maintain its physicochemical properties over time (*35*). The significantly (p<0.001) lower EAI and ESI of BGNS can be highly attributed to the insolubility and low WAC of BGNS (Table 4). Biopolymers find use as stabilisers in food emulsions. They accomplish this function by increasing the viscosity of the system thereby retarding oil droplet

migration (*42*). The EAI and ESI of STASOL and BGN-SDF suggest that they would effectively carry out these functions. To successfully increase the viscosity of a system, a polysaccharide needs to be adequately soluble in the aqueous phase. The low hydration properties of BGNS, therefore, hindered it from being a suitable emulsion stabiliser. As emulsions have a lipid component, a suitable stabiliser needs a suitable degree of hydrophobicity to adequately perform a stabilising function (*43*). BGN-SDF and STASOL both exhibited significantly ($p \leq 0.05$) higher hydration and OBCs (Table 4) thus making them good potential emulsion stabilisers.

Glucose content of Bambara groundnut starch, soluble dietary fibre and starch-soluble dietary fibre nanocomposite

Table 5 shows the glucose content of BGNS, BGN-SDF and STASOL. In Table 3, the carbohydrate content of BGNS was 86.8 %. This was in fair agreement with the results of this section. Starch is made up of repeating units of glucose (*44*) hence its hydrolysis yielded 87.9 % glucose, similar to its carbohydrate content.

STASOL had a higher carbohydrate content (78.7 %) as reported in Table 3 compared to its glucose content (63.4 %). Proportionately, the glucose content of STASOL should have been higher, however, some may have been lost during production. STASOL is a nanocomposite made from BGNS:BGN-SDF (15:1.95) hence the quantified glucose would be from the hydrolysis of BGNS and the difference would have been carbohydrates from BGN-SDF that were not made up of glucose units. No glucose was detected in BGN-SDF. This was in agreement with Maphosa (*4*) who reported an insignificant amount of glucose (<1 %) in BGN-SDFs.

Antioxidant properties of BGNS, BGN-SDF and STASOL

The presence of polyphenolic compounds (TPC) and ferric reducing antioxidant power (FRAP) in biopolymers is suggestive of the presence of antioxidant properties. The TPC and FRAP of BGNS, STASOL and BGN-SDF are presented in Table 5. All the biopolymers differed significantly ($p < 0.001$) in their TPC and FRAP. BGN-SDF is rich in antioxidant compounds while starches are generally low in active compounds (*4*). This validated the results obtained in this study whereby BGN-SDF had the highest amount of polyphenolic and ferric reducing compounds while BGNS had significantly ($p < 0.001$) low antioxidant capabilities. This proved that antioxidant compounds were successfully delivered from BGN-SDF to BGNS. However, since a low percentage (11.5 %) of BGN-SDF was incorporated into STASOL, the total antioxidant activity of STASOL was lower than of BGN-SDF.

Jayawardena *et al.* (*45*) reported the total phenolic content of 22 fruit juices in the range 0.24−0.39 mg GAE/g. These could be a result of the presence of common antioxidants such as vVitamin C, especially in the citrus juices. These results were lower than those reported for STASOL (0.46 mg GAE/g). Fruit juices are a reliable source of antioxidants, therefore STASOL having superior antioxidant properties is a positive characteristic.

The significantly ($p < 0.001$) higher phenolic content of STASOL compared to BGNS could also be attributed to the chemical modification process used in the production of STASOL. To expose the reactive starch functional groups, ascorbic acid was oxidised by hydrogen peroxide resulting in the formation of hydroxyl and ascorbate radical intermediates (*46*). These intermediates may have interfered with the hydrogen atom transfer mechanism of oxygen radical absorbance capacity (ORAC) assay thereby influencing the antioxidant mechanism leading to the higher antioxidant capacity of STASOL (*1*).

The FRAP of BGNS, STASOL and BGN-SDF was 1.16, 1.45 and 4.77 µmol AAE/g, respectively (Table 4). All three biopolymers differed significantly ($p < 0.001$) in their ferric reducing capabilities. The FRAP assay is based on the reduction of ferric ions to ferrous ions by the tested compound and is often used to measure the antioxidant capacity of foods and beverages containing polyphenols (*47*). The polyphenolic content of STASOL suggested that it can be exploited as a novel antioxidant and would be of importance in protecting against superoxide radicals, free radicals and lipid peroxidation (*39*). STASOL would therefore find use in fatty food products to improve oxidative stability, thereby extending their shelf life (*39*). As a natural antioxidant, STASOL would be a suitable alternative for artificial antioxidants. Artificial antioxidants have been reported to be carcinogenic and teratogenic hence their use in food products is discouraged (*48*).

Polyphenolic compounds in Bambara groundnut starch, soluble dietary fibre and starch-soluble dietary fibre nanocomposite

Table 6 shows the phenolic content of BGNS, BGN-SDF and STASOL. All three biopolymers showed appreciable amounts of phenolics. The phenolic compounds chlorogenic acid (18 mg/g), monocrotaline (20 mg/g), luteolin 7-O-(6''-malonylglucoside) (4 mg/g) and casuarine 6-alpha-D-glucoside (27 mg/g) were present in BGN-SDF and absent in BGNS and STASOL while blumealactone C (7 mg/g) was present in BGNS and absent in BGN-SDF and STASOL. Of the three biopolymers, BGN-SDF had the highest quantity of phenolics compared to BGNS and STASOL, with high levels of (+)-sesamin (12 mg/g), 12,13-TriHOME (8602 mg/g), dronabinol (3389 mg/g), 9(S)-HPODE (50 mg/g), alpha-dimorphecolic acid (273 mg/g), dimethyltryptamine (7281 mg/g) and [1R-

(1alpha,4abeta,6alpha,8aalpha)]-1,2,4a,5,6,8a-Hexahydro-6-hydroxy-4,7-dimethyl-a-methylene-1-naphthaleneacetic acid methyl ester (7594 mg/g).

The polyphenolic compounds reported in this section were in agreement with the TPC and FRAP results (Table 5) where BGN-SDF had the highest amount of total polyphenols as well as the strongest ferric acid-reducing power. BGNS is generally low in antioxidant activity (*4*) and this was demonstrated by its relatively lower phenolic content (Table 6). The presence of phenolic compounds in STASOL demonstrated that active compounds were successfully delivered from BGN-SDF to BGNS. Therefore, it was concluded that STASOL possessed appreciable amounts of antioxidant compounds. However, the phenolic compounds furcatin, dimethyltryptamine, isatidine, casuarine and 6-alpha-D-glucoside [1R-(1alpha,4abeta,6alpha,8aalpha)]-1,2,4a,5,6,8a-Hexahydro-6-hydroxy-4,7-dimethyl-a-methylene-1-naphthaleneacetic acid methyl ester, luteolin 7-O-(6''-malonylglucoside) were present in high quantities in BGNS and BGN-SDF but were either not delivered at all to STASOL or were delivered in very low quantities. This was attributed to the low amount of BGN-SDF used in the formulation of STASOL. As such, future studies should look into using a higher amount of BGN-SDF to increase the antioxidant capacity of STASOL.

The findings of this study highlight the potential of BGN as an economic source of natural antioxidants for human consumption and could therefore open horizons to its industrial use in the development of functional food. The antioxidant activity of BGN seeds and constituents has been reported by several researchers (*5,11*). The antioxidant behaviour of phenolics arises from the ability of their hydroxyl groups to donate hydrogen as well as react with oxygen and nitrogen species, producing radical species in a termination reaction (*49*). Phenolic compounds such as those identified in BGNS, BGN-SDF and STASOL have been reported to possess many desirable characteristics such as anti-inflammatory, anti-microbial, anti-fungal, anti-allergic, anti-oxidant, anti-apoptotic, anti-tumour and estrogenic activity (*50*).

CONCLUSIONS

The grafting of BGN-SDF onto BGNS successfully mitigated the undesirable characteristics of both biopolymers in their native state while retaining their desirable characteristics. STASOL possesses desirable properties making it a suitable ingredient in various food products. The physicochemical, antioxidant and functional properties of STASOL make it a desirable emulsion stabilizer, thickening agent and fortifier for delivering active compounds, such as phenolics, to food systems. Unlike BGNS, STASOL did not exhibit typical pasting properties, thus confirming the formation of a new compound. The light colour of STASOL would allow it to be used as an ingredient

without negatively impacting the colour of food systems. Nanocomposites like STASOL have the potential to revolutionise the food industry.

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CONFLICT OF INTEREST

The authors declare no conflict of interest.

AUTHORS' CONTRIBUTION

Yvonne Maphosa conceived and designed the experiments, performed the experiments, analysed and interpreted the data as well as wrote the paper. Victoria Jideani conceived and designed the experiments, analysed and interpreted the data, as well as contributed reagents, materials and analysis tools. Daniel Ikhu-Omoregbe conceived and designed the experiments, interpreted the data as well as proof read the manuscript.

ORCID ID

Y. Maphosa https://orcid.org/0000-0001-9124-338X

V.A Jideani https://orcid.org/0000-0002-4550-6334

D.I. Ikhu-Omoregbe https://orcid.org/0000-0002-9308-9221

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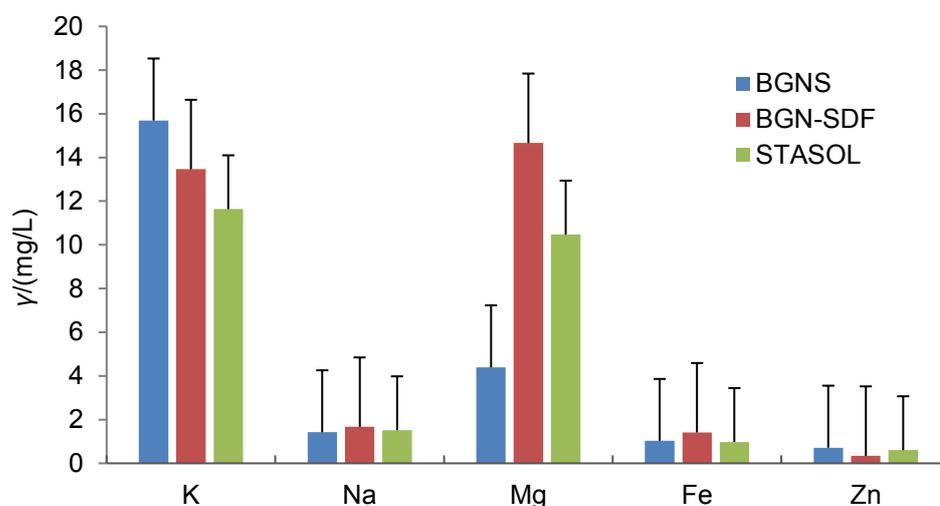https://doi:10.1016/B978-0-12-801734-0.00074-6

Fig. 1. Mineral composition of BGNS, BGN-SDF and STASOL

Table 1. Pasting properties of BGNS, BGN-SDF and STASOL

	Peak Viscosity	Trough viscosity	Breakdown viscosity	Final viscosity	Set back	Peak time (min)	Pasting temperature (°C)
BGNS	4875 ± 219^a	1984 ± 944^a	2890 ± 1143^a	4621 ± 500^a	2637 ± 577^a	4.78 ± 0^a	77.9 ± 2^a
BGN-SDF	435 ± 16^b	412 ± 20^b	24 ± 4^b	537 ± 9^b	122 ± 9^b	7.00 ± 0^b	96.7 ± 3^b
STASOL	150 ± 10^c	143 ± 1^b	7 ± 1^b	271 ± 9^b	128 ± 0^b	6.87 ± 0^c	87.4 ± 0^c

Values are mean±standard deviation. Means within a column followed by different superscripts differ significantly [p≤0.05]. BGNS=Bambara groundnut starch; BGN-SDF=Bambara groundnut soluble dietary fibre; STASOL=Bambara groundnut starch-fibre nanocomposite.

Table 2. Colour attributes of BGNS, BGN-SDF and STASO

	L^*	a^*	b^*	C^*	$h°$	ΔE
BGNS	87.9 ± 0.0^a	1.3 ± 0.0^a	12.4 ± 0.0^a	12.5 ± 0.0^a	83.9 ± 0.0^a	
BGN-SDF	76.1 ± 0.0^b	2.2 ± 0.0^b	18.9 ± 0.0^b	19.0 ± 0.0^b	83.3 ± 0.0^b	
STASOL	89.2 ± 0.0^c	0.4 ± 0.0^c	14.6 ± 0.0^c	14.6 ± 0.0^c	88.4 ± 0.0^c	
BGNS – BGN-SDF						13.5
BGNS – STASOL						6.6
BGN-SDF – STASOL						14.2

Values are mean±standard deviation. Means within a column followed by different superscripts differ significantly [$p \leq 0.05$]; L^*: lightness; a^*: red/ green; b^*: yellow/blue; BGNS=Bambara groundnut starch; BGN-SDF=Bambara groundnut soluble dietary fibre; STASOL=Bambara groundnut starch-fibre nanocomposite

Table 3. Chemical composition of BGNS, BGN-SDF and STASOL

	Proximate (%)					Energy (kJ)
	Moisture	Ash	Protein	Fat	Carbohydrates	
BGNS	10.84 ± 0.0^a	0.07 ± 0.0^a	1.74 ± 0.4^a	0.56 ± 0.0^a	86.79 ± 0.3^a	1525.78 ± 0.7^a
BGN-SDF	10.82 ± 0.1^a	4.90 ± 0.1^b	15.54 ± 0.3^b	0.54 ± 0.0^a	68.20 ± 0.2^b	1443.47 ± 2.2^b
STASOL	8.63 ± 0.3^b	4.88 ± 0.1^b	6.96 ± 0.7^c	0.84 ± 0.0^b	78.69 ± 0.6^c	1487.08 ± 5.6^c

Values are mean±standard deviation. Means within a column followed by different superscripts differ significantly [$p \leq 0.05$; BGNS=Bambara groundnut starch; BGN-SDF=Bambara groundnut soluble dietary fibre; STASOL=Bambara groundnut starch-fibre nanocomposite

Table 4. Physicochemical properties of BGNS, BGN-SDF and STASOL

	Water absorption capacity (g/g)	Solubility index (%)	Oil binding capacity (g/g)	Emulsion activity (%)	Emulsion stability (%)
BGNS	1.6 ± 0.2^a	0.9 ± 0.2^a	1.13 ± 0.1^a	23.3 ± 32.0^a	23.3 ± 18.2^a
BGN-SDF	-	95.3 ± 0.0^b	3.78 ± 0.1^b	85.7 ± 6.0^b	87.1 ± 6.5^b
STASOL	-	93.3 ± 0.1^c	1.52 ± 0.1^c	90.7 ± 1.9^c	87.5 ± 3.3^c

Values are mean±standard deviation. Means within a column followed by different superscripts differ significantly [$p \leq 0.05$; BGNS=Bambara groundnut starch; BGN-SDF=Bambara groundnut soluble dietary fibre; STASOL=Bambara groundnut starch-fibre nanocomposite

Table 5. Antioxidant properties and glucose content of BGNS, BGN-SDF and STASOL

	Polyphenols (mg GAE/g)	FRAP (µmol AAE/g)	Glucose (%)
BGNS	0.10±0.01[a]	1.16±0.40[a]	87.9
BGN-SDF	6.59±0.177[b]	4.77±0.78[b]	ND
STASOL	0.46±0.048[c]	1.45±0.24[c]	63.4

Values are mean±standard deviation. Means within a column followed by different superscripts are significantly [$p≤0.05$] different; BGNS: Bambara groundnut starch; BGN-SDF: Bambara groundnut soluble dietary fibre; STASOL: Bambara groundnut starch-fibre nanocomposite. FRAP: Ferric reducing antioxidant power

Table 6. Phenolic composition of BGNS, BGN-SDF and STASOL

Phenolics	Phenolic group	Elution time (min)	BGNS (mg/g)	BGN-SDF (mg/g)	STASOL (mg/g)
Scoparone	Flavonoid	7.19	0	11	28
Chlorogenic acid	Phenol esters	7.60	0	18	0
Blumealactone C	Terpene lactone	7.71	7	0	0
(+)-Sesamin	Polyphenol	8.12	4	12	11
4-hydroxymethyl-2-methoxyphenyl-1-O-beta-D-apiofuranosyl-(1->6)-O-beta-D-glucopyranoside	Flavonone	8.40	0	10	6
Furcatin	Phenylpropene	8.72	4	6	0
Monocrotaline	Pyrrolizidine alkaloid	9.30	0	20	0
[1R-(1alpha,4abeta,6alpha,8aalpha)]-1,2,4a,5,6,8a-Hexahydro-6-hydroxy-4,7-dimethyl-a-methylene-1-naphthaleneacetic acid methyl ester	Sesquiterpenoid	9.99	237	7594	8
Luteolin 7-O-(6"-malonylglucoside)	Flavonoid	10.23	0	4	0
Dimethyltryptamine	Phenol amide	10.48	1446	7281	13
Isatidine	Pyrrolizidine alkaloid	10.63	8	27	0
Casuarine 6-alpha-D-glucoside	Pyrrolizidine alkaloid	10.95	0	27	0

9,12,13-TriHOME	Linoleic acid derivative	12.92	396	8602	3009
Dronabinol	Tetrahydrocannabinol	13.94	103	3389	643
9(S)-HPODE	Linoleic acid derivative	14.12	26	50	63
Alpha-dimorphecolic acid	Linoleic acid derivative	14.35	49	273	161

BGNS=Bambara groundnut starch; BGN-SDF=Bambara groundnut soluble dietary fibre; STASOL=Bambara groundnut starch-fibre nanocomposite