Journal of Medicinal Herbs
journal homepage:**www.jhd.iaushk.ac.ir**

Comparison of nutritional composition, HPLC characterization, antioxidants property and starch profile of *Sphenostylis stenocarpa* composite bread and wheat bread

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ARTICLE INFO

Type: *Original Research*
Topic: *Medicinal Plants*
Received *May 25th2022*
Accepted *August 29th2022*

Key words:
- ✓ *α-amylase*
- ✓ *bread*
- ✓ *Sphenostylis stenocarpa*
- ✓ *Wheat*
- ✓ *phenols*

ABSTRACT

Background & Aim: The use of composite flour and combined additives in wheat flour to improve their nutritional and health benefits have increased. This study focuses on the examination and comparison of the phenolic characterization, antioxidant properties, mineral content, starch profile, *in vitro* starch digestibility and *in vitro* α-amylase inhibition present in produced composite bread and wheat bread.

Experimental: *Sphenostylis stenocarp*a flour (SSF) and combined additives (dry gluten powder, fungal α-amylase and sodium stearoyl-2-lactylate) were incorporated into wheat flour to produce composite SSF bread. Wheat flour bread was prepared as a control.

Results: The HPLC result showed higher values of gallic acid (1806.68 µg/100 g), p-coumaric acid (104.49 µg/100 g) and quercetin (22054.67 µg/100 g) in SSF bread while sinapic acid (195.88 µg/100 g), caffeic acid (1372.90 µg/100 g), ferulic acid (535.79 µg/100 g) were higher in control bread. Ferric-reducing antioxidant properties and mineral contents (Zinc, Ca, Fe, K, Mg, Mn and copper) were higher in SSF in comparison to control bread ($P<0.05$). The SSF bread had higher resistant starch and slowly digestible starch values but decreased total starch and rapidly digestible starch values. The *in vitro* starch digestibility (IVSD) value was also 0.54 times lower in SSF compared to control bread. The α-amylase inhibitory potential of SSF bread (56.77%) was significantly higher ($P<0.05$) in comparison to control bread (29.96%). It could be concluded that the incorporation of *Sphenostylis stenocarp*a in baked products such as bread will be of high nutritional benefits to humans.

Recommended applications/industries: *Sphenostylis stenocarpa* is an underutilized bean that is rich in minerals, antioxidant properties and slow starch digestion potency which can be explored to prevent or manage the pathologic conditions that are related to sugar metabolisms. The utilization of underutilized *Sphenostylis stenocarpa* will go a long way in combating food insecurity.

1. Introduction

The conscious monitoring of the body's response to starch digestion after the consumption of carbohydrate-rich foods has been adopted as a means of managing and treating several sugar-related diseases. Diabetes, a

metabolic disorder has become a global burden as the data on the past and present status highlighted the incidence of its steady increase which is not only associated with developed countries but is also poised to aim at developing countries (Lu *et al.*, 2018). Classification of starch and its products are based on their digestibility which is tagged as rapidly digestible starch (RDS), slowly digestible starch (SDS) and resistant starch (RS) tend to influence the changes in blood glucose levels after eating a meal. Post-meal sugar control in diabetics is the top priority as far as health-related issues are concerned. Regular ingestion of rapidly digestible carbohydrates has been implicated to be the main cause of the development of hyperglycemia-induced metabolic diseases (Englyst *et al.*, 1996).

One of the enzymes linked to diabetes is α-amylase. Starch hydrolysis through the action of α-amylase to oligosaccharides (such as maltotriose) is the first step in the breakdown of starch in the digestive tract that leads to postprandial plasma glucose increase (Abhijit *et al.*, 2014). The inhibition of this enzyme has been suggested as one of the therapeutic means of managing diabetes. Synthetic drugs are currently still in use. However, their targets are non-specific on glycosidases which later produce some uncomfortable side effects after long-time use. This trend continues to fuel the zeal for further explorations of natural and effective hypoglycemic agents from dietary sources.

Nigeria is one of the developing countries with high demand for carbohydrate staples like yam, cassava and rice with less consumption of protein alongside the starch-oriented meals due to their high cost while the worst effect is experienced by the low-income earners (Asogwa and Onweluzo, 2010). Earlier research has proven that starches from legumes have low digestibility than those observed in cereals which if adopted can promote slow, moderate and low post-prandial glucose and insulin responses (Botham *et al.*, 1995). Legumes are cultivated in various geographical zones for different importance attached to their phytochemicals, nutrients and some health benefits linked to mineral contents (Oagile *et al.*, 2007). The traditional preparation of bean flour to paste as an alternative for its maximum use (Ofuya *et al.*, 1991) can be said to have taken a new dimension as bean flour is presently being incorporated in wheat flour to augment nutritional values in baked products.

Sphenostylis stenocarpa is a leguminous plant commonly called the African yam bean. It is one of the neglected and underutilized plants. *Sphenostylis stenocarpa* like other researched conventional beans (such as cowpea, pigeon pea and soybean) is rich with minerals (Nwokolo, 1987) while some bioactive compounds of its hydrolysate such as polyphenols have been suggested to exert antioxidant properties (Ajibola *et al.*, 2011). The earlier findings about bean starch have elated the interest of food scientists because they mostly contain resistant starch that characterizes their incomplete digestion and absorption in the small intestine to exert physiological functions similar to dietary fiber (Botham *et al.*, 1995).

Bread is a globally consumed baked product valued for its taste and aroma. However, the high consumption of conventional bread with low nutritional value has been characterized by adverse effects on glucose response that is prone to dietary hyperglycemia (Liu, 2002). The increasing demand for therapeutic dietary interventions through the use of plant natural's products is focused to improve diabetes management and also prevent further complications (Kikuzaki *et al.*, 2002). Going forward, one of the several avenues adopted is to partially supplement wheat flour with the nutritional indigenous plant to produce an improved wheat-based product (Lu *et al.*, 2018). Nevertheless, the use of additives is also very common to improve the characteristics of wheat-legume composite bread that will appeal to consumers (Katina *et al.*, 2006). This, in addition to the imperative need of improving overall nutrition in diets, will consequently elevate the status of food security.

Despite the potential of *Sphenostylis stenocarpa*, the crop is grossly underutilized, thereby limiting its contribution to food security because of some information that is still pending about its nutritional qualities and health benefit potentials. Based on the paucity of data on the studies of *Sphenostylis stenocarpa* composite bread biofunctional properties, this present study, therefore, sought to produce wheat bread (as control), additives enhanced *Sphenostylis stenocarpa* composite bread and compare their relationship on phenolic acids contents (using HPLC), ferric reducing antioxidant property (FRAP), mineral contents, starch profile, *in vitro* starch digestibility (IVSD) and *in vitro* α-amylase inhibitory activity.

2. Materials and Methods

Sphenostylis stenocarpa seed was purchased from a local market in Akungba Akoko, Ondo State. Nigeria. The bean seed was identified and authenticated with designated Voucher Number- 257 as that of *Sphenostylis stenocarpa* (Hochst ex A.Rich) in the herbarium of Plant Science and Biotechnology, Adekunle Ajasin University, Akungba Akoko.

The sample was picked to separate dirt and other unwanted substances then milled into flour in a hammer mill 3,100 (Perten Instruments AB, Huddinge, Sweden), sifted through a 250-µm sieve and then stored in an air-tight plastic until further use. Commercial wheat flour and ingredients were purchased from flour shop in Mysore, India.

2.1. Preparation of bread

Bread formulation (Table 1) contained wheat flour, SSF and combined additives (dry gluten powder, fungal α-amylase from *Aspergillus oryzae* and sodium stearoyl-2-lactylate in combination). The choice of the level of 15 g of SSF, additives and outcome of the sensory evaluation was based on the preliminary studies (Shodehinde *et al.*, 2021). The ingredients were mixed in a Hobart mixer (Model N-50, Hobart, GmbH, Offenburg, Germany) with a flat blade for 4 min at 61 rpm. The dough was fermented in a chamber maintained at 30 ^{o}C and 75% RH for 90 min, removed from the fermenter for 25 min, molded, proofed for 55 min and baked in an oven for 25 min at 220 ^{o}C.

Table 1. Ingredients for the formulation of dough.

Ingredients	Blends	
	Control	SSF
Wheat flour (g)	100.0	85.0
SSF (g)	-	15
Yeast (g)	2.0	2.0
Fat (g)	1.0	1.0
Salt (g)	1.0	1.0
Sugar (g)	2.5	2.5
Water (mL)	Optimum water absorption as determined with the farinograph	
DGP (g)	-	5.0
Fα-A (g)	-	0.002
SSL (g)		0.5

SSF: *Sphenostylis stenocarpa* flour; DGP: dry gluten powder; Fα-A: fungal α-amylase from *Aspergillus oryzae*; SSL: sodium stearoyl-2-lactylate. Optimum water absorption was measured by farinograph.

2.2. Preparation of samples for HPLC

Ten milligrams (10 mg) of powdered bread sample was dissolved in 10 mL of methanol to get the final concentration of 1mg/mL. The solutions were filtered using a 0.45µm syringe filter (Millipore) and then degassed by ultrasonic bath before HPLC analysis. Identification of phenolic acids was done by comparison of the retention time of the phenolic acid standards and samples spiked with phenolic acid standards. Chromatographic analysis was performed with the use of liquid chromatographic system, which consisted of Prominence Liquid Chromatographic Shimadzu instrument with UV- Detector-SPD- 20A. The separation was carried out on Ascentis RP Amide (15 cm x 4.6 mm ID, 5 µm particles) reversed phase column and C18 column was used with mobile phases of 1% acetic acid (A) and 1% methanol (B). Phenolic acid was separated using a 30-min linear solvent gradient at a flow rate of 1.0 mL/min. The injection volume was 10 µL. The solvent gradient used was done according to Boligon *et al.*, (2010). Standards stock solutions were prepared in the HPLC mobile phases at a concentration range of 0.030–0.500 mg/mL and the wavelength was 280 for gallic acid, caffeic acid p-coumaric acid, ferulic acid and sinapic acid, and 320 for quercetin. Chromatographic peaks were confirmed by comparing their retention time with those of reference standards and diode array detection spectra (200-500 nm).

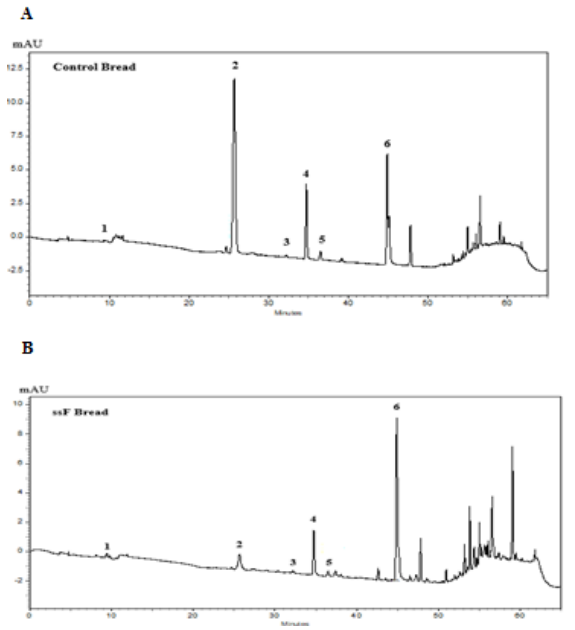

Figure 1. The identified peaks of phenolic acids and flavonoid on high-performance liquid chromatography profile for control (A) and ssF (B) bread. Peak 1 (gallic acid), peak 2 (caffeic acid), peak 3 (p-coumaric acid), peak 4 (ferulic acid), peak 5 (sinapic acid) and peak 6 (quercetin).

2.3. Aqueous extract preparation

The extraction of the bread samples was carried out according to the method adopted by Shodehinde and Oboh (2013).

2.4. Ferric-reducing antioxidant property (FRAP)

This antioxidant assay was determined by adding 1mL of 0.2 M phosphate buffer pH 6.6 and 1 mL of 1 % potassium ferricyanide to 2.5 mL of powdered bread extract, then incubated in the water bath at 50 °C for 20 min then allowed to cool. Afterward, 2.5 mL of 10 % trichloroacetic acid was added to stop the reaction and was centrifuged for 10 min. 2.5 mL of aliquots were pipetted out and 2.5 mL of distilled water and 0.5 mL of 0.1 % ferric chloride solution were added. The color changed to green. The mixture was allowed to stand for 10 min at room temperature and absorbance was measured at 593 nm using a spectrophotometer UV-visible Jasco V-630 instrument. Quercetin was used as standard (Patel *et al.*, 2012).

2.5. Mineral analysis

Minerals in bread samples were determined according to standard procedure AOAC (2000). A measured weight of each sample (5 g) was burnt to ashes in a muffle furnace at 550 °C. The resulting ash was dissolved in l0 mL of 2M HCl solution and diluted to l00 mL in a volumetric flask using distilled water, and then filtered. The filtrate was used for the mineral analysis (Zinc, Calcium, Magnesium, Potassium, Sodium and Iron, Manganese and Copper). Aliquots were analyzed using Microwave Plasma Atomic Emission Spectroscopy (MP-AES). The 2,000 mg/L ionization buffer solution was constantly mixed with the sample stream immediately before entering the double-pass cyclonic spray chamber held at room temperature using a simple mixing tee. The 6-point calibration curves were established between 0 and 1mg/L for the elements to account for the sample dilution and matrix interferences. Each element was analyzed in triplicate with replicates of 3 s read time per element. All the glassware used were washed and soaked in 10% HNO_3 and finally washed using milli-Q water.

2.6. Determination of starch profile

In vitro rapidly digestible starch (RDS), slowly digestible starch (SDS), resistant starch (RS) and total starch (TS) were analyzed using the method of Englyst *et al*. (1992). To convert glucose to starch, 0.9 was used as a conversion factor (Englyst *et al.*,1992). Each sample was analyzed in triplicates.

2.7. In vitro starch digestibility (IVSD) assay

Freeze-dried and ground bread sample (50 mg) were dispersed in 4 mL of sodium acetate buffer (pH 4.6, 0.4 M) containing amyloglucosidase and was incubated in the water bath for 30 min at 60 °C. The enzyme was inactivated by placing the tubes in a boiling water bath (100 °C) for 15 min. The tubes were allowed to assume room temperature and then centrifuged at 5,000 rpm for 10 min. The supernatant was measured for its glucose content using a glucose oxidase-peroxidase (GOD-POD) kit (Auto span, Span Diagnostics Limited, India). Absorption was measured at 505 nm and the glucose concentration was converted into starch content using a 0.9 factor (Englyst *et al.*, 1992).

2.8. In vitro α-amylase inhibitory assay

The 500 µL of bread sample extract was incubated with 500 µL of α-amylase solution. The 2 units/mL was obtained by dissolving 0.001 g of α-amylase in 100 mL of 0.02 M sodium phosphate buffer pH 6.9 with 6.7 mM sodium chloride) at room temperature (32 °C). After incubation, 500 µL of 1 % starch solution (dissolving 1 g of potato starch in 100 mL of distilled water with boiling and stirring for 15 min) was added and incubated at room temperature (32 °C) for about 10 min. Dinitrosalicylic acid (DNSA) reagent (1 mL) was added to stop the reaction and then incubated in a hot water bath (85 °C) for 5 min. The reaction mixture color then changed to orange-red and was allowed to cool to room temperature. Absorbance was measured at 540 nm in a Jasco V-630 spectrophotometer (Worthington, 1993).

2.9. Statistical analysis

The results of the three replicates were pooled and expressed as mean ± standard error (S.E.). Student t-test was carried out (Zar, 1984). The significant level was established at $P<0.05$.

3. Results and discussion

3.1. High-performance liquid chromatography (HPLC)

Phenolic acids continue to gain attention as they are repeatedly identified as effective natural antioxidants in plants. The high-performance liquid chromatography–

diode array detector analysis is one of the methods developed to characterize and quantify the presence of phenolics in plants (Dimitrios, 2006). There was a significant difference (P<0.05) in the results of HPLC analysis for control and SSF bread as presented in Table 2.

The HPLC readings revealed the presence of gallic acid (249.95 and 1806.68 µg/100 g), caffeic acid (1372.90 and 127.59 µg/100 g), p-coumaric acid (102.05 and 104.49 µg/100 g), ferulic acid (535.79 and 303.73 µg/100 g), sinapic acid (195.88 and 104.39 µg/100 g) and quercetin (18921.55 and 22054.67 µg/100 g) in control and SSF bread, respectively. However, SSF bread had a higher concentration of gallic acid, p-coumaric acid and quercetin while sinapic acid, caffeic acid and ferulic acid were higher in control bread.

Beans are richly endowed with many bioactive compounds and polyphenols that are capable of hindering the formation of free radicals (McDougall, 2017). Sinapic acid, ferulic acid and caffeic acid belong to the class of hydroxycinnamic acids. They act by donating their phenoxy hydrogen atom to neutralize the production of phenoxy radicals and to restore the equilibrium to the body system. Their health benefits have been unveiled in the protection against inflammation, oxidative stress and diabetes (Kikuzaki *et al.*, 2002). Their transfer of hydrogen and electron in biochemical reactions has been reported to involve the collaborations of ferulic acid and caffeic acid along with its biosynthetic precursor (p-coumaric acid) to exert the total radical-trapping antioxidative effect (Gorinstein *et al.*, 2008). Further actions of caffeic acid extend to forming a complex with Fe^{2+} to prevent its oxidation to Fe^{3+}, hence, preventing lipid peroxidation. Gallic acid also follows the same trend by acting as a potent active compound against the oxidation of oil in food and *in vivo* as well (Hsieh *et al.*, 2004). This present research exhibited higher content of gallic acid (1806.68 µg/100 g) in SSF bread.

Table 2. Phenolic contents of control and SSF bread

Phenolic acids	Control		SSF	
	RT	Conc. (µg/100g)	RT	Conc. (µg/100g)
Sinapic acid	36.523	195.88±0.02^{b}	36.512	104.39±0.02^{a}
Gallic acid	9.365	249.95±0.01^{a}	9.408	1806.68±0.01^{b}
Caffeic acid	25.696	1372.90±0.02^{b}	25.675	127.59±0.03^{a}
p-Coumaric acid	32.224	102.05±0.01^{a}	32.192	104.49±0.02^{b}
Quercetin	44.885	18921.55±0.02^{a}	44.885	22054.67±0.02^{b}
Ferulic acid	34.741	535.79±0.01^{b}	34.741	303.73±0.01^{a}

SSF: *Sphenostylis stenocarpa* flour; Conc.: concentration; RT; retention time; values are expressed as mean ± standard deviations (SD) of three determinations; values in the row with the same superscript letters are not significantly different from each other at P<0.05.

Flavonoids are versatile groups of polyphenolic compounds with much nutraceutical value. Many studies on flavonoids have shifted towards quercetin as an antioxidative flavonol in the human diet that inhibits α-amylase activity to lower postprandial blood glucose as well as increase insulin sensitivity (Schroeter *et al.*, 2002). In this experiment, the concentration of quercetin as a flavonoid was significantly higher in SSF bread (22054.67 µg/100 g) compared to control bread (18921.55 µg/100 g). The observed flavonoid increase in SSF bread agrees with earlier reports of wheat flour fortification with other non-wheat plant sources such as ginger powder (Balestra *et al.*, 2011) and turmeric powder (Lim *et al.*, 2011) to produce bread with improved antioxidant properties.

3.2.Ferric-reducing antioxidant property (FRAP)

The ferric-reducing antioxidant property of bread samples to reduce Fe (III) to Fe (II) was tested and presented in Table 3. The reported FRAP result followed the trend observed in quercetin value as SSF bread (0.42 mg/gQE) had higher (P<0.05) reducing properties compared to control bread (0.27 mg/gQE). Ferric redox activities of phenols majorly ferric reductones allow them to act as ferric reducing agents thereby reducing cellular oxidative stress by acting as a free radical scavenger (Hsu *et al.*, 2003). The increased FRAP activity in SSF bread corroborates the report of Tapas *et al.* (2008) that many of the antioxidant attributes conferred on phenolics have been linked to their interactions with functional groups present in

flavonoids. Maritim *et al*. (2003) also reported that the disequilibrium between the free radical generating and free-radical scavenging abilities in diabetic patients' cells favors an increase in free radical production to exacerbate the eventual reduction of antioxidants which initiate the progression of diabetes-associated complications.

Table 3. FRAP, starch profile, IVSD, α-amylase inhibitory activity of control and SSF bread.

Parameters	Bread	
	Control	SSF
FRAP (mg/g QE)	0.27±0.03[a]	0.42±0.09[b]
RDS (%)	34.68±0.05[b]	18.69±0.02[a]
SDS (%)	12.75±0.02[a]	23.94±0.04[b]
RS (%)	12.26±0.03[a]	20.80±0.02[b]
TS (%)	58.79±0.03[b]	56.82±0.01[a]
IVSD (g/100g)	3.11±0.03[b]	1.69±0.03[a]
α-amylase inhibition (%)	29.96±0.02[a]	56.77±0.09[b]

SSF: *Sphenostylis stenocarpa* flour; FRAP: ferric reducing antioxidant property; IVSD (*in vitro* starch digestibility); starch profile (RDS: rapidly digestible starch; SDS: slowly digestible starch; RS: resistant starch; TS: total starch). Values in the row with the same superscript letters are not significantly different from each other at P<0.05; values are means of three replicates ± standard deviation. QE; quercetin.

3.3. Starch profile

The results of starch digestibility fractions: rapidly digestible starch (RDS), slowly digestible starch (SDS), total starch (TS) and resistant starch (RS) determination are presented in Table 3. The results depicted SSF bread with a reduced RDS value of 18.69% in contrast to the reported value for control bread which had a higher significant value of 34.68% (P<0.05). This showed that the rapid starch digestibility (RDS) which represents the hydrolysis of starch chains by enzyme digestion during the first 20 minutes was lowered in SSF bread by 1.86 times in comparison to the control. The slowly digestible starch (SDS) and resistant starch (RS) values recorded for SSF bread (23.94 and 20.80%) were also significantly higher (P<0.05) than the observed control bread values (12.75 and 12.26%) respectively. An earlier report on SDS had presented it as the most considered dietary starch with substantive health benefits in the management of starch-related pathology (Ambigaipalan *et al.*, 2011). The higher content of RS in *Sphenostylis stenocarpa* could be adopted for incorporation into functional food production. High RS content in food augments the healthy state of probiotic microorganisms' growth that directly influences an increase in mineral absorption and maintenance of glucose-related conditions (Hoover *et al.*, 2010). However, the recorded total starch (TS) content for SSF bread (56.82%) was lower (P<0.05) than that of control bread (58.79%). According to Hoover *et al*. (2010), the comparison of the starch in native legumes is lesser than that of cereals. This present report is in agreement with earlier findings that beans mostly contain resistant starch that characterizes their incomplete digestion and absorption in the small intestine to exert physiological functions similar to dietary fiber (Botham *et al.*, 1995).

3.4. In-Vitro starch digestibility (IVSD)

In vitro starch digestibility measures the quality of carbohydrates present in foods with their behavior *in vivo* (Englyst *et al.*, 1996). This is achieved by mimicking the physiological processes taking place in the mouth, stomach and small intestine. This method is time and cost-effective and can be considered in place of glycemic index analysis (Dona *et al.*, 2010). Poor starch digestion control is one of the factors considered to predispose an individual to hyperglycemic conditions and diabetic patients to long-term complications. The present research revealed a marked significant increase (P<0.05) in control bread (3.11 g/100 g) contrary to the decrease in SSF bread (1.69 g/100 g) (Table 3). The reported low IVSD value in SSF bread in this present study agrees with the earlier research that starches from pulses have low digestibility and low release of post-prandial glucose (Botham *et al.*, 1995).

3.5. α-amylase inhibition

Alpha amylase is an endo-hydrolase enzyme that is considered to be primarily responsible for starch hydrolysis (Abhijit *et al.*, 2014). The examined inhibitory effect of control and SSF bread samples on α-amylase are displayed in (Table 3). There was a higher value of α-amylase inhibition in SSF bread (56.77%) compared to control bread (29.96%). The α-amylase inhibition is considered the first step of starch hydrolysis and will form part of accumulating basis for the hypothesized mechanism of controlling glucose response (Abhijit *et al.*, 2014). In line with the long-time recognition of polyphenols as good inhibitors of carbohydrate hydrolyzing enzymes, quercetin has been reported to be involved in the activation of hexokinase and glucokinase during glycolysis (Vessal *et al.*, 2003). Earlier work on phenols has also proven that flavonoids

participate actively in mechanisms that are not related to them directly as antioxidants (Domitrios, 2006). The function of flavonoids to act as signaling molecules (Schroeter *et al.*, 2002) is also in agreement with their revealed dual effect on insulin secretagogue through insulin signal transduction by acting as an anti-hyperglycemic and an insulin-mimetic agent (Cazarolli *et al.*, 2009).

3.6. Mineral contents

*Sphenostylis stenocar*pa is also rich in essential minerals that can boost health status. The results for mineral contents of control and SSF bread are presented in Table 4. There was a significant ($P<0.05$) difference in all the reported mineral contents of SSF bread compared to the control bread. The revealed values for Zinc (1.33 mg/100 g), Ca (2.35 mg/100 g), Fe (0.23 mg/g), K (25.40 mg/100 g), Mg (3.68 mg/100 g), Mn (0.38 mg/100 g) and copper (0.12 mg/100 g) in SSF bread are of higher values compared to the observed values of Zinc (0.18 mg/100 g), Ca (1.38 mg/100 g), Fe (0.12 mg/100 g), K (8.95 mg/100 g), Mg (1.85 mg/100 g), Mn (0.18 mg/100 g) and Copper (0.06 mg/100 g) in control bread. The values of elements in this present research are compared with some earlier reported values in other pulses (Nwokolo, 1987).

Table 4: Mineral contents of control and SSF bread

Minerals	Bread	
(mg/100g)	Control	SSF
Zn	0.18±0.03^a	1.33±0.01^b
Ca	1.38±0.02^a	2.35±0.03^b
Fe	0.12±0.01^a	0.23±0.03^b
K	8.95±0.02^a	25.40±0.03^b
Mg	1.85±0.01^a	3.68±0.02^b
Mn	0.18±0.01^a	0.38±0.01^b
Cu	0.06±0.01^a	0.12±0.02^b

SSF: *Sphenostylis stenocarpa* flour; values in the row with the same superscript letters are not significantly different from each other at $P<0.05$; values are means of three replicates ± standard deviation.

The interactions of macro elements (Mg, K and Ca) and trace elements (Zn, Cu and Fe) with various macromolecules have been reported to mediate various catalytic and regulatory functions (Aggett, 1985). The presence of magnesium and zinc has been reported to exert a reduction of blood glucose by interacting with insulin. The mechanism of action of these elements enhanced insulin activity by activating the insulin receptor sites and also serving as cofactors to initiate the enzyme signaling cascade involved in glucose metabolism. Epidemiological studies have revealed an inverse correlation between the intake of magnesium-rich food and the condition of diabetes as this had been described to prevent the risk condition from prediabetes to manifest diabetes (Ruel *et al.*, 2014). The function of Zn also extends to glycolysis by influencing glyceraldehyde-3-phosphate dehydrogenase and also protects the beta cell from disruption (Mooradian and Morley, 1987).

Calcium intake has been proven to alleviate osteoporosis (Cryer *et al.*, 1994). Apart from this, Calcium and cyclic AMP play important roles in the stimulation of insulin release (Dey *et al.*, 2002). Potassium is an essential macronutrient that partakes in the synthesis of amino acids and proteins. Its consumption has been linked to the prevention of one of the long-term complications of diabetes such as cardiovascular diseases (Norbiato *et al.*, 1984).

The condition of anemia is worst in developing countries which results from iron deficiency. Iron (Fe) occupies the central atom of hemoglobin to perform the role of transferring oxygen through red blood cells. Transferrin is a glycoprotein responsible for the transportation of Fe in the bloodstream. Concerning diabetes, the proper functioning of transferrin will become hampered thereby leading to an abnormal increase in serum ferritin levels. This condition was proposed as a surrogate marker of diabetes to predict disease onset (Sun *et al.*, 2013). Copper is needed in the human body system for the proper utilization of iron (Cooper *et al.*, 2011). Mineral elements are enveloped in various metabolic pathways to play active roles in maintaining their configurational coherences. Metallic ions, therefore, are very essential as a pre-requisite to forestalling the production of free radicals. The present study has reported mineral elements of appreciable quantities in SSF bread with 7.34, 1.70, 1.92, 2.84, 1.99, 2.11 and 2.00 times increase in Zn, Ca, Fe, K, Mg, Mn and Cu, respectively than control bread.

4. Conclusion

The results of present study have been able to elucidate the antioxidant and hypoglycemic properties of SSF composite bread in comparison to wheat bread. SSF composite bread exhibited higher values of SDS, RS, mineral contents, antioxidant properties and α-amylase inhibitory activity than those observed in wheat bread (control). In conclusion, efforts from both

government and private sectors are needed to create awareness that will facilitate the priority for the consumption of cereal-legume-based products as against cereal only and other refined foods to curb health-based challenges that are related to sugar metabolisms.

5. Acknowledgements

The Authors acknowledged TETFund Nigeria, Government of India and Director, CSIR-CFTRI, for extending facilities.

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