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Research Article

Bio-accessibility of nutritional and antioxidant contents of naturally fermented *Sphenostylis stenocarpa* (African yam bean) during gastrointestinal digestion

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ABSTRACT

Background: *Sphenostylis stenocarpa* (African yam bean, commonly called Otili) is an underutilized legume valued for its protein, carbohydrate, mineral, and phytochemical richness, but limited information exists on its digestibility and antioxidant bio accessibility. **Objective:** This study examined the nutritional and antioxidant properties of fermented flour (DF) and shaft fractions (NDF) of *S. stenocarpa* under simulated gastrointestinal digestion. **Materials and Methods:** All reagents used in this study were of analytical grade and suitable for biochemical and physiological experiments. Bean shaft and flour samples underwent *in vitro* digestion, the release and stability of both nutritional and anti-nutritional factors were assessed across the oral, gastric, and intestinal phases. **Results:** Findings revealed that fermented flour exhibited greater digestibility and higher bio accessible levels of phenolics (546.65 ± 0.02 mg/g) and flavonoids (332.33 ± 0.03 mg/g) compared with the shaft fraction, which retained many compounds bound to fiber. Sugar composition differed between fractions: reducing sugars were most abundant in digestible fractions at the colon (7.29 ± 0.03 mg/g) and intestine (6.88 ± 0.02 mg/g), whereas glucose (1.15 ± 0.02 mg/g) and fructose (1.40 ± 0.02 mg/g) dominated in non-digestible fractions at the intestine. Sucrose appeared mainly in non-digestible fractions at the colon (0.04 ± 0.02 mg/g) and intestine (0.01 ± 0.01 mg/g). The shaft fraction contributed higher ciceritol content (3.98 ± 0.03 mg/g) compared with the flour fraction. **Conclusion:** In conclusion, fermentation enhanced the release of soluble antioxidants and sugars in flour fractions, while shafts served as a reservoir of bound polyphenols and oligosaccharides that may provide delayed colonic benefits. These findings establish *S. stenocarpa* as a valuable functional food and demonstrate that fermentation improves its nutritional and health-promoting potential.

Keywords: *Sphenostylis stenocarpa*, African yam bean, Fermentation, Bioaccessibility, Antioxidant activity, Gastrointestinal digestion.

INTRODUCTION

Sphenostylis stenocarpa, commonly known as *Otili*, is a leguminous plant that was originally domesticated by pre-Columbian civilizations. Two primary gene pools, Andean and Mesoamerican, have been identified in this species, both of which can be distinguished in cultivated and wild germplasms [1]. Owing to its strong adaptability and high nutritional value, this crop has gained global significance, with beans now representing the world's leading legume, recording an estimated 28.3 million tons of production in 2022 [2].

In our previous studies, we evaluated the functional and nutritional properties of selected Nigerian wild-type beans, including *S. stenocarpa L*., and compared them with commonly consumed varieties. The findings revealed a rich nutritional profile, highlighting the abundance of health-promoting nutrients in the edible portions of *S. stenocarpa* L. Consequently, it was recommended as a valuable dietary component due to its high nutrient content [3-8]. Edible beans include *S. stenocarpa* (kidney, black, pinto, navy beans), but yet underutilized. Despite its therapeutic significance, this species remains underexploited, largely due to limited research and low commercial awareness. However, growing interest in neglected and underutilized species (NUS) has renewed attention to the pharmacological potentials of African yam bean [7-10]. The utilization of *S. stenocarpa* has been limited by its hard-to-cook nature and the presence of antinutritional factors [11,12]. In Nigeria, the beans are typically consumed after prolonged cooking, often following overnight soaking, a process that requires significant fuel and results in substantial nutrient loss. In response to the growing demand for affordable and accessible plant-based proteins to meet nutritional needs, several efforts have been made to enhance its dietary use. For instance, the dehulling process has been shown to reduce cooking time and improve the acceptability of pigeon pea [13,14].

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Phytochemical studies of *S. stenocarpa* have identified a wide array of bioactive compounds, including flavonoids, saponins, alkaloids, tannins, terpenoids, and phenolic acids [15]. These findings are consistent with our earlier research comparing the phytochemical and antinutritional profiles of selected wild and cultivated beans in Nigeria, which showed that the wild-type *S. stenocarpa* competes favorably with the common edible bean Phaseolus vulgaris in terms of bioactive compound content [3,4].

These secondary metabolites are largely responsible for the antioxidant, antimicrobial, and anti-inflammatory properties of the plant. Notably, methanolic extracts of *S. stenocarpa* seeds contain high concentrations of rutin, quercetin, gallic acid, and catechin, compounds recognized for their radical-scavenging and anti-aging activities [16]. Furthermore, alkaloid-rich fractions of the plant have exhibited mild cytotoxic effects against certain cancer cell lines *in vitro*, indicating potential anticancer benefits [17].

Both *in vitro* and *in vivo* studies have supported the diverse biological activities of *S. stenocarpa*. In our study, rats fed with *S. stenocarpa* diets displayed greater resistance to colon cancer cell proliferation induced by dextran sulfate, compared to those fed with other common edible beans [18]. Additionally, we observed that specific microorganisms facilitate the degradation of indigestible bean fractions (husks), leading to the increased production of short- and medium-chain fatty acids (SCFAs and MCFAs). This provides evidence that the consumption of underutilized beans such as *S. stenocarpa* contributes to enhanced colon health and overall gut function [6-8,19-21]. Extracts from *S. stenocarpa* seeds have demonstrated antimicrobial activity against both Gram-positive and Gram-negative bacterial strains, including *Staphylococcus aureus* and *Escherichia coli* [9]. We have also deduced that gut microbiota played a vital role in the metabolic breakdown of Indigestible and Soluble fraction of Carbohydrates derived from *S. stenocarpa*. Major role was played by *Proteus vulgaris* in the fermentative process of the indigestible fraction while *Esherichia coli* played the key role in the fermentative process of soluble fraction of carbohydrates in the bean samples. This provided evidence that consumption of *S. stenocarpa* have potential and crucial effect on health especially in non-communicable disease such as hypertension and diabetes [10,22].

The global food industry has fully embraced fermentation as a low-cost, eco-friendly, and health-promoting process [24]. By leveraging fermentation, food processors and researchers are able to enrich the nutrient profile of foods, improve sensory acceptability, and mitigate the impact of harmful compounds naturally present in raw agricultural materials. Moreover, fermented foods often contain probiotics beneficial microorganisms that support gut health, modulate the immune system, and may contribute to the prevention of certain non-communicable diseases [25,26]. Among local farming communities, *S. stenocarpa* is more than a food crop it is a multipurpose plant utilized for both nutritional and medicinal purposes [27]. The tubers, although less studied, have also shown evidence of containing oligosaccharides and non-starch polysaccharides, which may contribute to prebiotic effects and gastrointestinal health thus playing an important clinical role in the human nutrition with reduced risk of chronic disease and could be an exceptionally cost effectual approach for improving health [23,28,29].

As posited earlier Beans are widely consumed throughout the world, rich in phytochemicals and other bioactive constituents; however, research about in digestion impacts on the changes of bioactive compounds release and related antioxidant potential in different beans is limited. Sequel to our previous studies, this present work aimed to assess flour and shafts of *S. stenocarpa* during gastrointestinal digestion, its digestibility, bio-accessibility of nutritional and its antioxidants contents at the intestine. Hence the proposed study focuses on prebiotic tendency of this under-utilized Nigerian bean and their possible inclusion to our diet.

MATERIALS AND METHODS

Preparation of Beans

The beans seed, *Otili*, was purchased from an open local market at Oja-Oba, Ado-Ekiti, Ekiti State, Nigeria. These were identified and authenticated by the Chief Botanist of Plant Science at our institution and deposited in the University Herbarium (UHAE 2025035). They were thoroughly picked to remove the dirt and was then soaked for 24-48 hours for fermentation to occur. The natural fermentation was brought to a halt after 48hours, and the beans were dehulled and the shaft was separated from the beans and was dried separately. After two weeks of the air-drying process, the beans samples and the shaft were pulverized separately using a blender.

Preparation of Buffer

Krebs' Ringer buffer solution was freshly prepared at pH 6.8 according to standard procedures. The buffer was composed of 118 mM NaCl, 4.7 mM KCl, 1.2 mM $MgSO_4$, 1.2 mM KH_2PO_4, 25 mM $NaHCO_3$, 11 mM glucose, and 2.5 mM $CaCl_2$. For each 100 ml of solution, 0.690 g NaCl, 0.035 g KCl, 0.0144 g $MgSO_4$, 0.0163 g KH_2PO_4, 0.210 g $NaHCO_3$, 0.198 g glucose, and 0.277 g $CaCl_2$ were weighed and dissolved in distilled water. The solution was adjusted to pH 6.8 using a calibrated pH meter. To simulate intestinal fluid, 0.420 g of bile bovine and 500 mg of probiotics (Inulin, *Lactobacillus acidophilus, L. casei, L. plantarum, L. rhamnosus, Bifidobacterium breve, B. longum*) were incorporated into 100 ml of the Krebs' Ringer buffer solution, with continuous stirring until a homogenous solution was obtained. A total of 700 ml of the buffer was prepared for use in the study and stored under refrigeration until required.

Proximate analysis and Anti-nutritional contents

Nutrient such as protein, carbohydrates, fat, fibre and anti-nutritional contents such as phytate, trypsin inhibitors on dry pulverized bean before and after fermentation were analyzed according to the standard AOAC methods [2005] as modified by Awoyinka *et al.,* [3].

Experimental Animals

Following ethical approval from the Experimental Animal Research Ethics Committee, Ekiti State University (ORD/ETHICS/AD/156); Six healthy adult Wistar rats of both sexes, weighing between 150 g and 200 g, were obtained from the animal house of the Department of Medical Biochemistry. The animals were housed in clean polypropylene cages lined with wood shavings and kept under controlled environmental conditions of 12-hour light and 12-hour dark cycle, temperature of 22 ± 2 °C, and relative humidity of 50–60%. They were fed standard laboratory rat pellets and provided water ad libitum. The animals were allowed to acclimatize to the laboratory environment for two weeks prior to the commencement of the experiment to minimize stress and ensure physiological stability. All animal handling and experimental procedures were carried out in accordance with the guidelines of the institutional Animal Ethics Committee.

In vitro Gastrointestinal Digestion

The *in vitro* digestion simulation of African yam beans through the mouth, stomach, and small intestine phases was performed following the method described by Campos-Vega *et al.* [30], with slight modifications. A schematic overview of the *in vitro* gastrointestinal digestion and intestinal absorption model employed in this study is presented in Figure 1.

Briefly, four volunteers who had provided written consent participated in the study. Each participant refrained from eating for 90 minutes before the trial. They were instructed to chew 1 g of African yam beans for 15 seconds, performing 15 chewing motions, without brushing their teeth beforehand. The chewed sample was then collected in a glass containing 5 mL of distilled water. Participants rinsed their mouths for 60 seconds with an additional 5 mL of distilled water, which was also transferred into the same glass. After the procedure, all suspensions from the participants were pooled into a single beaker. A blank sample was prepared in the same way, using only distilled water without the beans.

From both the sample and blank, 10 mL aliquots were taken to represent the oral phase, and their pH was adjusted to 2 using 2 N HCl. Subsequently, pepsin (0.055 g, ≥2500 U mg^{-1}; Sigma-Aldrich) dissolved in 0.94 mL of 20 mM HCl was added. The mixtures were then incubated for 2 hours at 37 °C in a shaking water bath (80 cycles per minute) to simulate the gastric digestion phase.

For the intestinal phase, an intestinal extract was prepared by dissolving 3 mg of bovine bile (Sigma-Aldrich) and 500 mg of probiotics (Inulin, *Lactobacillus acidophilus*, *L. casei*, *L. plantarum*, *L. rhamnosus*, *Bifidobacterium breve*, *B. longum*) in 5 mL of Krebs–Ringer buffer solution (118 mM NaCl, 4.7 mM KCl, 1.2 mM $MgSO_4$, 1.2 mM KH_2PO_4, 25 mM $NaHCO_3$, 11 mM glucose, and 2.5 mM $CaCl_2$; pH 6.8). The reagents were equilibrated to pH 7.4 and prepared 30 minutes before use. The intestinal extract was then added to both the sample and blank from the gastric phase to obtain a 15 mL suspension.

To simulate intestinal absorption, everted gut sacs were prepared from male Wistar rats (250-300 g; n = 6 per experiment). The animals were fasted for 16 hours, anesthetized with pentobarbital (60 mg kg^{-1}, intraperitoneally), and euthanized. A midline abdominal incision was made to expose the small intestine, and a 20-25 cm segment of the proximal jejunum was excised. The section was rinsed with CO_2-gassed Krebs-Ringer buffer at 37 °C to remove residual contents. The intestine was then cut into 6 cm segments and everted over a glass rod. Each segment was tied at one end, filled with 1 mL of Krebs-Ringer buffer, and sealed at the other end to form a closed sac, which was kept immersed in buffer to prevent tissue deterioration.

The everted sacs were placed in glass tubes containing the intestinal extract and the samples derived from the gastric digestion phase. They were incubated at 37 °C for 2 hours under continuous shaking (80 cycles per minute). Samples were withdrawn at 15, 30, and 60 minutes. The material remaining outside the sacs after incubation was termed the non-digestible fraction (NDF), whereas the content inside the sacs was referred to as the digestible fraction (DF). All experiments were conducted in duplicate for both blank and experimental samples.

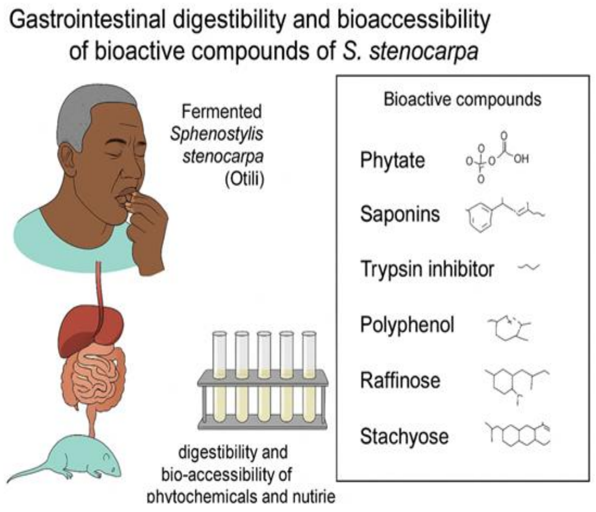

Figure 1: Schematic representation of the *in vitro* gastrointestinal digestion and intestinal absorption model used for *S. stenocarpa*

Bio-accessibility Analysis of Phytochemicals and Sugars

The Phytochemicals including phenolic compounds, flavonoids, alkaloids and tannins were extracted and determined from the fermented bean samples before and after gastrointestinal digestion according to the method of Harborne [31] as modified by Awoyinka *et al.,* [4], total phenolic, total flavonoid were calculated as gallic acid, quercetin equivalent respectively. However, the method of Sofowora, [32] as modified by Awoyinka *et al.,* [4] was used to determine the alkaloids and tannins respectively. While determination of sugars (total sugar and reducing sugar) was carried out using the Layne-Enyon method as described in AOAC [33,63]. The non-reducing sugar-Raffinose, Verbascose, Stachyose, Ciceritol and reducing sugar content- Glucose, Fructose, Sucrose, Maltose were assessed for their bio-accessibility during gastrointestinal digestion on the bean seed and been shaft respectively.

Statistical Analysis

A one-way analysis of variance (ANOVA) was conducted using GraphPad Prism version 10.2 (SAS Institute, Cary, NC, USA). Post-hoc comparisons were carried out with the Tukey-Kramer test to determine statistically significant differences among groups, with significance accepted at $p < 0.05$.

RESULTS AND DISCUSSION

Effect of Fermentation on the nutritional and anti-nutritional contents of *Otili*

The proximate analysis of *S. stenocarpa* (*Otili*) showed that fermentation influenced its nutritional composition. As found in this Table 1, there was increase in moisture content from 7.10 ± 0.01% to 8.3 ± 0.02%; this reflects enhanced water-binding capacity during fermentation. This is often associated with partial hydrolysis of polysaccharides and proteins, which exposes hydrophilic sites and allows for greater hydration [34]. Increased moisture in fermented products may improve palatability but also raises concerns about storage stability, since higher water activity promotes microbial growth and reduces shelf life. Therefore, although fermentation improves functional hydration properties, subsequent drying or preservation steps may be necessary for long-term storage. The increase in crude fibre 6.10 ± 0.01% to 6.8 ± 0.05% suggests the liberation of structural carbohydrates such as cellulose, hemicellulose, and lignin. Fermentation is known to cause partial breakdown of non-starch polysaccharides, which not only modifies texture but also enhances the physiological role of fibre in improving bowel motility, regulating blood glucose levels, and reducing serum cholesterol [35].

Additionally, dietary fibre contributes to satiety, making fermented. *Otili* potentially valuable in the formulation of functional foods aimed at weight management and metabolic health. The slight decline in fat content from 2.84 ± 0.02% to 2.77 ± 0.01% is consistent with microbial utilization of lipids as energy substrates during metabolic processes. Lipolytic enzymes secreted by fermenting microorganisms hydrolyze triglycerides into free fatty acids, which are then metabolized [35,36]. While the reduction is small, it may be nutritionally relevant, as fermented products often display improved lipid digestibility and may contribute to reduced caloric density. Furthermore, fat reduction lowers the risk of oxidative rancidity, thereby improving product stability. Ash content increased from 4.20 ± 0.01% to 4.47 ± 0.02%, indicating higher mineral bioavailability. This effect can be attributed to the microbial degradation of chelating agents such as phytates and tannins during fermentation. These compounds normally bind divalent minerals such as calcium, iron, and zinc, rendering them unavailable for absorption. Their reduction through fermentation enhances the nutritional value of legumes, making minerals more accessible for human metabolism [37]. Thus, the increase in ash content reflects not only higher mineral recovery but also improved bioefficacy of essential micronutrients.

Protein content showed a notable increase from 31.5 ± 0.08% to 32.9 ± 0.02%. This aligns with previous studies that demonstrated enhanced protein concentration and quality after legume fermentation [38]. The increase may result from microbial synthesis of amino acids, reduction of carbohydrate fractions (which concentrates protein proportionally), and breakdown of protein inhibitors such as trypsin inhibitors. Fermentation also improves protein digestibility by denaturing complex storage proteins into peptides and amino acids, thereby enhancing nitrogen utilization in humans. Importantly, the improvement in protein quality positions fermented *Otili* as a potential alternative protein source in regions where animal protein is limited or unaffordable. Carbohydrate levels decreased slightly from 48.28 ± 0.04% to 47.01 ± 0.02%, reflecting microbial utilization of sugars as carbon sources during fermentation. This reduction may also indicate enzymatic hydrolysis of starch into simpler sugars, which are subsequently consumed by fermenting microbes. While total carbohydrate decreased, this process enhances digestibility and reduces the glycemic index of legumes, making them more suitable for individuals managing diabetes and metabolic disorders [39]. Additionally, the partial degradation of indigestible oligosaccharides may reduce flatulence and improve consumer acceptance.

The antinutritional analysis of *Sphenostylis stenocarpa* (*Otili*) also showed it is influenced by fermentation in this study. As seen in Table 2, the analysis of anti-nutritional factors in *Sphenostylis stenocarpa* (*Otili*) revealed substantial reductions across most compounds following fermentation. Specifically, saponins decreased from 0.583 ± 0.08 mg/g to 0.40 ± 0.01 mg/g, phytates dropped from 10.5 ± 0.1 mg/g to 6.41 ± 0.01 mg/g, tannins declined from 0.43 ± 0.02 mg/g to 0.298 ± 0.01 mg/g, and trypsin inhibitors reduced markedly from 19.92 ± 0.02 TIU/mg to 12.0 ± 0.00 TIU/mg. Interestingly, polyphenols increased slightly (0.30 ± 0.1 to 0.325 ± 0.01 mg/g).

Saponins are glycosides that can impart bitterness and have been associated with reduced palatability and potential gastrointestinal irritation when consumed in high concentrations. The reduction observed here corroborates findings by Egounlety and Aworh [37], who reported that microbial metabolism during fermentation, can hydrolyze saponins, thereby reducing their concentration. Lowering saponin content improves sensory acceptability and enhances the safety of legume-based foods, while small residual amounts may still provide beneficial cholesterol-lowering and anticancer effects [40,41]. Phytates are well-documented anti-nutrients that chelate essential minerals such as calcium, iron, and zinc, thereby limiting their bioavailability. The decrease from 10.5 to 6.41 mg/g demonstrates the role of endogenous phytase and microbial phytase enzymes during fermentation in hydrolyzing phytic acid into inositol and inorganic phosphate [42]. This enzymatic action is nutritionally significant, as reducing phytate enhances the absorption of critical micronutrients, an essential factor in combating iron and zinc deficiencies in populations dependent on legume-based diets. Tannins are polyphenolic compounds known to form complexes with proteins and digestive enzymes, thereby reducing protein digestibility. The reduction from 0.43 to 0.298 mg/g after fermentation is consistent with reports that fermentation degrades tannin-protein complexes and reduces tannin concentration [43,44]. While excessive tannin intake is detrimental, moderate levels may provide beneficial antioxidant and antimicrobial properties. Thus, the observed reduction optimizes the balance between reducing negative impacts on protein digestibility and retaining potential health-promoting effects.

Trypsin inhibitors are protease inhibitors that limit protein digestion by interfering with pancreatic enzymes. The marked decline from 19.92 to 12.0 TIU/mg after fermentation indicates that microbial enzymes and acidic conditions during fermentation denature or hydrolyze trypsin inhibitors. Oliveira de Lima *et al.,* [45] observed similar results in legumes, reporting that fermentation effectively reduces protease inhibitors and thereby enhances protein digestibility. This improvement is crucial, given that legumes are a primary protein source in many African diets. Contrary to the reductions observed in other anti-nutritional compounds, polyphenols increased slightly following fermentation. This observation is consistent with the release of bound phenolic compounds from the food matrix during microbial hydrolysis of cell wall polysaccharides [46]. The increase in polyphenols is nutritionally beneficial, as these compounds are potent antioxidants with anti-inflammatory and cardio-protective effects [47]. Thus, fermentation not only reduces harmful anti-nutrients but may also enhance the concentration of bioactive compounds with health-promoting properties.

Effect of Fermentation on the antioxidant's contents of *Otili*

The antioxidant analysis of *S. stenocarpa* (*Otili*) also showed it is influenced by fermentation in this study (Table 3) The DPPH radical scavenging activity decreased slightly from 56.04 ± 2.15% to 53.18 ± 0.26%, suggesting a modest decline in overall antioxidant activity. Similarly, Vitamin E decreased significantly (0.985 ± 0.01 to 0.302 ± 0.01 mg/g), and Vitamin C declined from 6.83 ± 0.51 to 4.90 ± 0.23 mg/g. Interestingly, however, polyphenols increased slightly (0.30 ± 0.10 to 0.325 ± 0.01 mg/g), indicating a compositional shift in the antioxidant profile. The significant reductions in vitamins C and E can be explained by their sensitivity to oxidation and microbial metabolism during fermentation. Vitamin C is highly unstable and prone to oxidative degradation under conditions of heat, pH changes, and microbial enzymatic activity [48]. Vitamin E, a lipid-soluble antioxidant, is also susceptible to degradation in the presence of oxidative enzymes and microbial lipases. Similar declines in vitamin-based antioxidants have been documented in other fermented legumes such as soybeans and cowpeas [38]. While this reduction may decrease the contribution of these vitamins to antioxidant capacity, it highlights the need for optimized fermentation conditions to minimize nutrient losses. Contrary to the decline in vitamin antioxidants, the polyphenol content increased slightly after fermentation. This trend is consistent with the hypothesis that fermentation facilitates the release of bound phenolic compounds from the seed matrix through microbial hydrolysis of cell wall polysaccharides [46]. The release of these bioactive phenolics enhances the non-vitamin antioxidant pool, contributing to free radical scavenging, anti-inflammatory, and cardio-protective effects [47]. The slight increase suggests that fermentation can transform the antioxidant profile of *S. stenocarpa*, favoring polyphenolic contribution.

The modest reduction in DPPH radical scavenging activity (≈3%) despite vitamin losses can be attributed to compensatory effects from increased polyphenols. Polyphenols often contribute more significantly to radical scavenging than vitamins in plant-based foods [46]. Thus, while vitamin C and E declined, the enhanced availability of polyphenols may have helped sustain much of the antioxidant capacity. This finding is consistent with studies on fermented African locust beans (*Parkia biglobosa*), where fermentation reduced vitamin antioxidants but improved phenolic-related antioxidant activity [38]. The shift in antioxidant composition has important nutritional implications. Although fermentation reduces heat- and oxidation-sensitive vitamins, it simultaneously enriches phenolic antioxidants, which are more stable during storage and may provide long-term health benefits. This duality suggests that fermented *S. stenocarpa* products could serve as functional foods, providing bioactive compounds with antioxidant potential despite lower vitamin levels. Furthermore, the fermentation-induced release of phenolics may enhance the role of *S. stenocarpa*in mitigating oxidative stress and related chronic diseases.

Effect of *in vitro g*astrointestinal digestion on the sugars and phytochemical capacity of *Otili* seed and its shaft

In this study, the sugar composition of the underutilized beans and its shaft during *ex-vivo* digestion process (Table 4). The reducing sugar content was significantly high in digestive fraction of *Otili* at the Colon (7.29 mg/g) closely followed by the digestive fraction of the bean at Intestine (6.88 mg/g). However, non-digestive fraction of the bean had the least reducing sugar the intestine with the value 1.29 mg/g. Glucose was markedly higher in NDF at the Intestine (1.15

mg/g) compared to the DF beans both at colon and the intestine. Fructose content was highest in NDF at Intestine (1.40 mg/g) and lowest in NDF at Colon (0.19 mg/g). Sucrose was only notable in NDF at Colon (0.04 mg/g) and NDF at Intestine (0.01 mg/g). These observations clearly indicated and support that bioaccesibility of reducing sugars occur at the intestine while only fructose appeared to be less absorbed at the intestine in the non-digestible fraction compared to others. High concentrations of reducing sugars in the digestible fraction are concordant with classical descriptions of carbohydrate digestion: α-amylase and brush-border hydrolases rapidly cleave starch and larger oligosaccharides into smaller, reducing sugars that become available for epithelial uptake [49,50]. *In vitro* digestion models typically show enrichment of the "bioaccessible" fraction (the portion available for absorption) with reducing sugars relative to the residual, non-absorbed material [49]. Thus, the elevated reducing-sugar values observed in DF-Colon and DF-Intestine are consistent with effective enzymatic hydrolysis of the *S. stenocarpa* carbohydrate fraction during the simulated digestive phase. The presence of comparatively higher glucose and fructose in the NDF at the intestinal sampling point is noteworthy and aligns with documented kinetic limitations in uptake processes. Parada and Aguilera [49] and Minekus *et al*., [50] emphasize that bio accessibility (release from the food matrix) and bioavailability (intestinal uptake and systemic availability) are discrete steps; rapid hydrolysis can produce monosaccharides faster than transport processes can remove them, causing transient luminal accumulation measurable in the non-absorbed fraction.

Additionally, residual matrix interactions (e.g., viscous polysaccharides or entrapped sugar pools) may impede immediate contact between liberated monosaccharides and epithelial transporters, leaving measurable amounts in the NDF [49]. Lajolo and Genovese [51] and Egounlety and Aworh [37] have also documented that anti-nutritional constituents (phytates, tannins, protease inhibitors) can modulate digestive enzyme kinetics and membrane function. Although fermentation reduces many ANFs, incomplete removal could still affect brush-border enzyme efficiency or transporter activity and thus contribute to residual mono-saccharides in the non-absorbed pool.

The greater accumulation of fructose relative to glucose in NDF-Intestine accords with known differences in enterocyte transport kinetics. Glucose uptake is largely driven by the sodium-coupled SGLT1 transporter and subsequently GLUT2; these systems can rapidly clear luminal glucose under many experimental conditions. Fructose primarily uses facilitated diffusion via GLUT5 (and to some extent GLUT2), which often displays saturation and inter-individual variability [49]. Consequently, fructose can appear to "lag" in uptake and persist longer in the lumen, a pattern that matches your measured fructose enrichment in the NDF intestinal sample. Sucrose is normally hydrolysed by sucrase-isomaltase at the brush border into glucose and fructose and is rarely found intact beyond the proximal small intestine unless sucrase activity is inhibited or overwhelmed [50]. The near-absence of sucrose in DF and only trace levels in NDF is therefore consistent with rapid sucrase activity during the intestinal phase or with prior hydrolysis during earlier digestive stages. Literature on fermented legumes indicates that microbial or endogenous enzymatic activity during processing may also pre-hydrolyse sucrose and oligosaccharides, further reducing intact sucrose in later digestion fractions [37,38].

The measurable sugars remaining in the non-digestible fraction represent substrates that would reach the colon *in vivo* and be available for microbial fermentation. Reddy *et al.* [39] and Patel and Goyal [52] review that residual oligosaccharides and resistant starches in legumes serve as prebiotic substrates, promoting short-chain fatty acid (SCFA) production (acetate, propionate, butyrate) with recognized benefits for colonic health and systemic metabolism.

Several studies of fermented legumes report reductions in readily fermentable oligosaccharides (raffinose, stachyose) and concomitant increases in monosaccharides or oligosaccharide redistribution, depending on the fermentative organism and processing conditions [37,38]. Where fermentation includes saccharolytic microbes or exogenous enzymes, partial hydrolysis of complex carbohydrates can increase the proportion of small sugars present during simulated digestion [38]. Substantial reducing sugars in DF coupled with residual sugars in NDF, parallel these observations and underscore that fermentation and the digestive process jointly determine the ultimate sugar distribution available for absorption and colonic fermentation.

From a nutritional perspective, the observed profile suggests that fermented *S. stenocarpa* supplies a substantial pool of bio accessible reducing sugars (potentially raising post-prandial glucose) while also delivering fermentable residues to the colon [39,52]. This duality has practical implications: processing strategies (e.g., controlling particle size, fermentation parameters, or combining with fibers) can be tailored to modulate glycaemic response while preserving prebiotic benefits [49]. The observed changes highlight the dual role of fermentation in reducing harmful anti-nutrients while enhancing functional bioactives. By lowering phytates, tannins, trypsin inhibitors, and saponins, fermentation significantly improves the digestibility and bioavailability of proteins and minerals, thereby addressing one of the major nutritional limitations of legumes. At the same time, the modest increase in polyphenols suggests enhanced antioxidant potential, positioning fermented *S. stenocarpa*as a functional food with added health benefits. Raffinose was highest in NDF at Colon (due to one extreme value) and lowest in DF at Intestine. Verbascose was generally low, but NDF at Colon recorded slightly higher values than the beans and NDF at Intestine. Stachyose was greatest in NDF at Colon (0.51 mg/g) and lowest in NDF at Intestine (0.37 mg/g), while DF at Intestine was higher than DF at Colon. Ciceritol was highest in NDF at Intestine (3.98 mg/g), followed by NDF at Colon (2.08 mg/g), DF at Intestine (1.86 mg/g), and lowest in DF at Colon (1.05 mg/g). The presence of raffinose, stachyose, and verbascose all members of the raffinose family oligosaccharides (RFOs) is consistent with prior reports that legumes are major sources of these α-galactosides [39,51]. These sugars are resistant to digestion in the upper gastrointestinal tract due to the absence of α-galactosidase in humans, and therefore reach the colon intact, where they undergo fermentation by the gut microbiota [52]. The relatively higher concentrations observed in the non-digestible fractions (NDF)**,** particularly at the colon stage, confirm their persistence through simulated gastrointestinal digestion and support their role as prebiotic substrates.

The elevated stachyose content in NDF at the colon compared to intestine (0.51 vs. 0.37 mg/g) suggests incomplete degradation or selective retention in the non-digestible pool. Similar findings have been reported in cowpea and soybean, where stachyose was identified as the dominant RFO [53]. While traditionally considered "flatulence factors" due to microbial gas production during colonic fermentation, contemporary research highlights their prebiotic potential, particularly in stimulating bifidobacteria and lactobacilli growth [52]. The lower values in DF compared to NDF indicate that these sugars largely bypass small intestinal digestion, as expected. Verbascose levels were consistently low, echoing earlier studies that described it as the least abundant RFO in legumes [54]. Its slightly higher concentration in NDF at the colon relative to intestine further illustrates its resistance to enzymatic hydrolysis. Despite its low abundance, verbascose contributes synergistically to the fermentable carbohydrate pool that shapes colonic microbial ecology and short-chain fatty acid (SCFA) production. The distinct distribution of ciceritol with highest concentrations in NDF at the intestine (3.98 mg/g) suggests that unlike RFOs, this compound may be variably released from the food matrix or modified during fermentation. Ciceritol, a type of C-glycosylflavone-derived oligosaccharide, has been less frequently studied than classical RFOs, but emerging reports suggest antioxidant, anti-inflammatory, and prebiotic functions [55]. Its enrichment in the NDF fraction is consistent with incomplete absorption and potential colonic fermentation, indicating that ciceritol may serve as an additional bioactive carbohydrate in *S. stenocarpa*.

The persistence of RFOs and ciceritol in the non-digestible fractions highlights their dual nutritional significance. On one hand, they may contribute to flatulence and gastrointestinal discomfort, which is often cited as a reason for the underutilization of legumes [51]. On the other, they play a beneficial role as prebiotic substrates, supporting colonic health and systemic benefits via SCFA production [39,52]. Additionally, the fermentation of these oligosaccharides by gut microbiota has been linked to reduced risk of colorectal cancer, improved lipid metabolism, and modulation of immune function [54].

Table 5 shows the phytochemical composition of fermented beans and bean shafts. Total phenolic content (TPC) was highest in DF at Colon (546.65 mg/g), followed by DF at Intestine (518.30 mg/g), NDF at Colon (478.66 mg/g), and lowest in NDF at Intestine (449.03 mg/g). Total flavonoid content was also greatest in DF at Colon (332.33 mg/g), higher than DF at Intestine (310.92 mg/g), and much greater than NDF at Colon (214.26 mg/g) and NDF at Intestine (200.43 mg/g). Alkaloid levels were highest in DF at Colon (170.33 mg/g) and DF at Intestine (161.75 mg/g), compared to lower values in NDF at Intestine (118.55 mg/g) and NDF at Colon (107.02 mg/g). Tannin content was highest in DF at Colon (470.33 mg/g), slightly higher than DF at Intestine (461.75 mg/g), and lower in NDF at Intestine (485.12 mg/g) and NDF at Colon (450.16 mg/g). Fermentation appeared to improve the digestibility and availability of certain compounds in the flour fraction compared with the shaft fraction [26]. This may be due to the breakdown of cell wall components and reduction in anti-nutritional factors during fermentation, which enhances the release of phenolics, simple sugars, and other bioactive constituents. Previous studies have shown that microbial fermentation increases the concentration of low-molecular-weight phenolics, improving their solubility and potential absorption [56,57]. In contrast, the shaft fraction, which is richer in structural fibers and bound phenolics, may act as a reservoir of antioxidant fiber. These non-extractable polyphenols remain bound to the indigestible matrix and are less bioaccessible in the upper GI tract but may become available in the colon following microbial metabolism [58].

Bioaccessibility

The digestion and absorption of bioactive compounds are dynamic processes influenced by the physicochemical conditions of the gastrointestinal tract [59]. Although *in vitro* gastrointestinal models cannot fully reproduce the complexity of *in vivo* metabolism, they provide valuable insights into the potential digestibility and bioaccessibility of nutrients and antioxidants from plant-based foods [60]. In this study, the digestibility and bioaccessibility of fermented flour and shaft fractions of *S. stenocarpa* occurring during simulated gastrointestinal digestion was examined.

The bio-accessibility of nutrients and antioxidant compounds such as phenolics, flavonoids, and tannins showed a general decline across the gastric and intestinal phases. This reduction could be attributed to pH variations, enzymatic hydrolysis, and oxidative degradation that occur during gastrointestinal transit [60]. Specifically, glycosylated phenolic compounds are often hydrolyzed or transformed into secondary metabolites under acidic and alkaline environments, leading to reduced recovery during *in vitro* digestion [61]. The structural complexity of phenolics in the African yam bean matrix may further limit their release, as interactions with dietary fibers, proteins, and polysaccharides can result in the formation of complexes that are less extractable under GI conditions [62]. In this study, *S. stenocarpa* flour demonstrated higher release of soluble antioxidants and nutrients in the gastric and intestinal phases, shafts may contribute to sustained antioxidant activity through delayed release in the colon [25]. Moreover, the incomplete release of antioxidants during GI digestion does not necessarily indicate a loss of functionality, as colonic fermentation may generate metabolites with distinct bioactivities, contributing to systemic health benefits.

It is important to situate these results within the known limitations of *in vitro* digestion and absorption models. Static or semi-dynamic systems replicate key physicochemical and enzymatic steps but cannot fully reproduce *in vivo* phenomena such as intestinal perfusion, hormonal regulation, transporter up-/down-regulation, and continuous luminal flow [50]. Consequently, measured sugar concentrations reflect the balance of hydrolysis and uptake within the constrained conditions of the assay; kinetic mismatches (rapid hydrolysis vs. limited transporter capacity) can produce transient luminal sugar levels that would differ quantitatively from an *in vivo* profile. Parada and Aguilera [49] advocate combining *in vitro* data with targeted *in vivo* or *ex vivo* transporter assays to resolve such kinetic ambiguities.

Table 1: Nutritional Factors of *Otili* before consumption

Otili	Moisture content	Crude fibre	Fat content	Ash	Protein	Carbohydrate
Dry pulverised	7.10±0.01	6.10±0.01	2.84±0.02	4.20±0.01	31.5±0.08	48.28±0.04
Fermented pulverised	8.3±0.02	6.8±0.05	2.77±0.01	4.47±0.02	2.9±0.02	47.01±0.02

Table 2: Anti- Nutritional Factors of *Otili* before consumption

Otili	Saponin	Phytate	Tannin	Trypsin	Polyphenol
Dry pulverised	0.583±0.08	10.5±0.1	0.43±0.02	19.92±0.02	0.3±0.01
Fermented pulverised	0.40±0.01	6.41±0.01	0.298±0.01	12.0±0.01	0.325±0.01

Table 3: Antioxidants Factors of *Otili* before consumption

Otili	DPPH	Vitamin E	Vitamin C
Dry pulverised	56.04±0.15	0.985±0.01	6.83±0.51
Fermented pulverised	53.18±0.26	0.0302±0.01	4.90±0.23

Table 4: Digestibility and Bioaccessibility of Sugar composition of *S. stenocarpa* beans and beans shaft

Property	DF at Colon	DF at Intestine	NDF at Colon	NDF at Intestine
Reducing Sugar (mg/g)	7.29 ± 0.03	6.88 ± 0.02	2.44 ± 0.04	1.29 ± 0.02
Glucose	0.20 ± 0.03	0.16 ± 0.01	0.15 ± 0.01	1.15 ± 0.02
Fructose	0.23 ± 0.04	0.25 ± 0.02	0.19 ± 0.01	1.40 ± 0.02

Sucrose	0.00 ± 0.00	0.00 ± 0.00	0.04 ± 0.02	0.01 ± 0.01
Maltose	ND	ND	ND	ND
Non-Reducing Sugar (mg/g)	4.54 ± 0.03	5.02 ± 0.03	4.00 ± 0.01	2.52 ± 0.02
Raffinose	0.09 ± 0.04	0.07 ± 0.01	5.23 ± 5.78	0.16 ± 0.02
Verbascose	0.06 ± 0.02	0.06 ± 0.02	0.10 ± 0.03	0.05 ± 0.01
Stachyose	0.20 ± 0.03	0.33 ± 0.03	0.51 ± 0.02	0.35 ± 0.03
Ciceritol	1.06 ± 0.02	1.88 ± 0.03	2.08 ± 0.02	3.98 ± 0.03

Data are presented as mean ± SD of triplicate readings. Key: DF- Digestible Fraction; NDF- Non Digestible Fraction

Table 5: Digestibility and Bioaccessibility of Phytochemical composition of *S. stenocarpa* beans and bean shafts

Property	DF at Colon	DF at Intestine	NDF at Colon	NDF at Intestine
Total Phenolic Content (TPC)	546.65 ± 0.02	518.30 ± 0.02	478.66 ± 0.03	449.03 ± 0.04
Total flavonoid content	332.33 ± 0.03	310.92 ± 0.02	214.26 ± 0.03	200.43 ± 0.03
Alkaloid	170.33 ± 0.04	161.75 ± 0.03	107.02 ± 0.02	118.55 ± 0.06
Tannin	470.33 ± 0.04	461.75 ± 0.03	450.49 ± 0.57	485.12 ± 0.99

Data are presented as mean±SD of triplicate readings.

CONCLUSION

This study has demonstrated that fermentation significantly improves the nutritional quality, digestibility, and bioaccessibility of *S. stenocarpa* (African yam bean). Through *in vitro* gastrointestinal digestion, the fermented flour and shaft fractions showed complementary nutritional and functional benefits, reinforcing the value of this underutilized legume as a sustainable food resource.

Authors' Contributions

The corresponding author, AOA designed and led the study and Authors OEO and OOE analyzed the data. Authors OTR, AFA and OFC. Authors AOA, OVO and OEO handled the literature searches. All authors read and approved the final manuscript.

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Conflict of interest

The authors declared no conflict of interest.

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