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Nutritional, Functional, and Anti-Hypercholesterolemic Properties of Sourdough Breads From Unripe Plantain, Tiger Nut, and Fenugreek Flours

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ABSTRACT

The increasing demand for functional bakery items, as well as interest in nutrient-dense health-promoting flour alternatives, has fueled the development of composite flour systems for bread production. This study looked at the nutritional composition, functional characteristics, and anti-hypercholesterolemic potential of sourdough breads made using mixes of unripe plantain (*Musa paradisiaca*), tiger nut (*Cyperus esculentus*), and fenugreek (*Trigonella foenum-graecum*) flours. The composite flour blends exhibited significant differences ($p < 0.05$) in pasting characteristics, with the 50% unripe plantain +39.4% tiger nut +10.6% fenugreek (50UP + 39.4TN + 10.6FG) blend showing the highest peak viscosity (2593.20 RVU). Blends containing more than 60% unripe plantain demonstrated superior stability and setback ratios, indicating better thermal and retrogradation behavior. Sourdough breads produced from these blends showed enhanced textural qualities compared with 100% wheat bread, including improved springiness (0.74–0.76 cm) and reduced firmness, suggesting a softer and more desirable crumb structure. Nutritional studies found that enriched breads contained increased quantities of dietary fiber, vital minerals, and bioactive components. Furthermore, feeding trials revealed that composite-flour sourdough breads helped to lower serum cholesterol markers, highlighting their anti-hypercholesterolemic properties. Overall, these data show that integrating unripe plantain, tiger nut, and fenugreek flours considerably improves the functional and health-promoting properties of sourdough bread.

1 | Introduction

Sourdough fermentation, which employs a complex consortium of naturally occurring microorganisms (primarily lactic acid bacteria and wild yeasts), has sparked widespread scientific interest due to its ability to improve both the nutritional profile of bread and its physicochemical behavior during baking (Lau et al. 2021). In contrast to traditional fermentation with *Saccharomyces cerevisiae*, this approach relies on a spontaneous slow-rising mechanism that affects the dough's biochemical environment, potentially boosting digestibility, increasing nutrient bioavailability, and adjusting textural qualities (Maicas 2020). Organic acids created during fermentation, such as lactic and acetic acids, reduce the dough's pH, influence starch gelatinization, and reinforce gluten networks; together, these biochemical changes improve flavor complexity, structural integrity, and shelf life (You et al. 2025). Sourdough's distinct scent and flavor are also ascribed to carbon dioxide produced during yeast metabolism, which forms the dough's porous crumb structure (Pérez-Alvarado et al. 2022). Aside from these technological advantages, sourdough fermentation modifies ingredient metabolism in ways that may affect physiological processes such as fat use. As a result, sourdough-based meals are increasingly being evaluated as part of dietary interventions

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to improve cardiovascular function or manage dyslipidemia. Sourdough fermentation-induced nutritional changes may assist people with hypercholesterolemia, a disorder defined by high levels of circulating cholesterol, particularly low-density lipoprotein cholesterol (LDL-C). Hypercholesterolemia continues to be a major global contributor to atherosclerosis risk, affected by genetic predisposition and saturated fat and cholesterol-rich diets (Diamond et al. 2021). Accumulation and oxidative alteration of LDL-C inside artery walls cause inflammatory reactions, plaque formation, and vascular constriction, increasing the risk of ischemic events (Maiolino et al. 2013). Concurrent oxidative stress and pro-inflammatory signaling aggravate endothelial injury, emphasizing the need for dietary options that modify lipid metabolism and minimize cellular stress (Altanam et al. 2025). Sourdough-based diets may help reduce cholesterol risk by modulating glycemic responses, altering starch hydrolysis, and increasing nutritional bioavailability. Incorporating nutrient-dense elements into sourdough formulations may result in breads with increased sensory quality and possibly cardioprotective properties.

Creating these working sourdough breads means picking ingredients meticulously, especially those known to improve health and boost nutrition. Fenugreek (*Trigonella foenum-graecum*), part of the bean family, has long been used in folk healing and food-based wellness aids. People commonly link it to better blood sugar control, heart health, liver function, plus improved circulation. Its seeds pack plenty of natural substances—such as saponins, alkaloids, polyphenols, steroids, flavonoids, fats, and proteins—that give rise to many body-supporting actions (Akhtar et al. 2025). Some of these substances help lower cholesterol, fight oxidative stress, or reduce inflammation—so fenugreek works well in foods designed to aid fat processing (Faisal et al. 2024). Tiger nut (*Cyperus esculentus*), much like other plant-based ingredients, brings solid nutrition and potential health perks when added to sourdough. Grown in many regions of Africa, southern Europe, and the Middle East, along with some areas in the Americas, these small tubers are eaten straight from the ground, dried out, or turned into drinks such as horchata (Bazine and Arslanoğlu 2020). Packed inside them are natural compounds such as sterols, alkaloids, tannins, and saponins, plus vitamins E and C, alongside various flavonoids and phenolic acids. Because of those active elements, the tuber shows strong antioxidant effects, helps reduce inflammation, fights microbes, and offers healing benefits—making it useful in everyday foods meant to boost wellness and lower illness risk (Abdullahi et al. 2022). Tiger nuts pack plenty of potassium, magnesium, iron, or calcium—helping feed good microbes such as lactic acid bacteria (LAB) and yeast during fermentation. Using tiger nut flour in sourdough may boost minerals while lifting antioxidant levels along with flavor depth.

Plantain (*Musa paradisiaca*) works well as a useful addition to sourdough mixes. Although common in West and Central Africa, this fruit offers plenty of carbs plus decent levels of fiber and key nutrients such as potassium or magnesium (Anajekwu et al. 2020). Plantain has been linked to multiple health benefits (Imam and Akter 2011). Fenugreek, tiger nut, and plantain bring extra nutrients to sourdough bread—using them during fermentation can boost flavor and texture while possibly helping people dealing with high cholesterol or heart issues. Therefore, this study seeks to produce sourdough breads from plantain and tiger nut flours enriched with fenugreek flour and assess the functional and structural properties of the sourdough breads as well as their effect on lipid profile in high fat-fed rats.

2 | Material and Methods

2.1 | Sample Collection, Preparation, and Chemical

Unripe plantain fruits, tiger nut tuber and Fenugreek were sourced from the General Market, Akure, South West, Nigeria. The unripe plantain was peeled, sliced and dried before grinding to powder. The tiger nuts and fenugreek were washed, dried, and ground to fine powder. The flour blends were used to formulate the sour dough bread as shown in Table 1 based on the ratios generated using response surface methodology (RSM). Response surface methodology (RSM) was employed to generate a 16-run experimental design (Table 1). A customized optimal mixture design was adopted to investigate the synergistic interactions among three formulation components: unripe plantain (50%–70%), tiger nut (20%–40%), and fenugreek seeds (5%–15%). The mixture design approach was selected due to its suitability for systems in which the response is governed by the relative proportions of components rather than their absolute quantities. This approach enables the imposition of compositional constraints while ensuring efficient and robust estimation of model parameters. The design matrix comprised 16 experimental runs, generated using Design-Expert version 13.0 (Stat-Ease Inc. Minneapolis, MN, USA). Of these, six runs were designated as model points for parameter estimation, five as lack-of-fit points to assess model adequacy, and five as replicate points to evaluate experimental error and ensure the reliability and reproducibility of the results.

TABLE 1 | Optimal mixture experiment for composite blend (unripe plantain, tiger nut, and fenugreek) formulation.

Run	A: unripe plantain	B: tiger nut	C: fenugreek
1	70.00	24.50	5.50
2	50.00	39.38	10.63
3	54.94	30.06	15.00
4	60.61	29.99	9.40
5	55.85	39.15	5.00
6	55.26	34.81	9.93
7	50.09	34.91	15.00
8	60.61	29.99	9.40
9	60.65	24.35	15.00
10	67.00	20.00	13.00
11	67.00	20.00	13.00
12	60.54	34.46	5.00
13	50.00	39.38	10.63
14	65.80	29.20	5.00
15	70.00	24.50	5.50
16	60.61	29.99	9.40

Chemicals such as N-nitro-L-arginine methyl ester (L-NAME) were obtained from Sigma-Aldrich Inc. (St. Louis, MO, USA).

2.2 | Tiger Nut Flour Processing

Tiger nut flour was produced following the procedure described by Oladele and Aina (2007), with minor adjustments. The dried tiger nuts were first sorted to eliminate impurities such as stones, pebbles, and foreign seeds, then washed with tap water, drained, and dried in an oven (Model DHG RS 485, China) at 40°C for 24 h to achieve a moisture content of approximately 13%. The dried nuts were subsequently milled and passed through a 600-μm mesh sieve. The resulting flour was packaged in sealed polythene bags prior to analysis.

2.3 | Plantain Flour Processing

Plantain flour was produced using the procedure outlined by Kure et al. (1998). The plantain fingers were detached from the bunches, washed thoroughly, manually peeled, and sliced into 5-mm pieces using a slicer. The slices were dried in a conventional oven (Model DHG RS 485, China) at 60°C until a constant weight was achieved. The dried pieces were milled and sieved through a 0.25-mm mesh, then packaged in low-density polyethylene bags, sealed, and stored at 4°C for later use.

2.4 | Fenugreek Flour Preparation

Fenugreek seeds (*Trigonella foenum-graecum L.*) were processed into flour following the modified methods described by Pandey and Awasthi (2015), Mature healthy seeds were manually sorted to remove stones, damaged seeds, and other extraneous materials. The cleaned seeds were washed thoroughly under running water and subsequently air-dried to remove surface moisture. The seeds were soaked in distilled water for 12 hours at ambient temperature (25°C–27°C) to soften the seed coat and reduce bitterness, as described by Pandey and Awasthi (2015). After soaking, the water was decanted, and the seeds were drained and oven-dried (Model DHG RS 485, China) at 50°C to a constant weight. The dried seeds were milled using a laboratory hammer mill to obtain fine flour. The milled product was sieved through a 60-mesh stainless steel sieve to ensure uniform particle size. The resulting fenugreek flour was packed in airtight polyethylene bags and stored at room temperature (25°C–28°C) until further analysis.

2.5 | Production of Sourdough Bread From Blends of Unripe Plantain, Tiger Nut, and Fenugreek Flours

Sourdough bread was produced from composite flours of unripe plantain, tiger nut, and fenugreek using a modified procedure based on Adepehin et al. (2022). Prior to formulation of composite blends, all flours were re-sieved through a uniform mesh size (250 μm) to ensure consistency in particle size distribution and facilitate homogeneous mixing. The flour blends were formulated as follows: 70% unripe plantain (UP), 24.5% tiger nut (TN), and 5.5% fenugreek (FG): 70%UP + 24.5%TN + 5.5%FG; 50%UP + 39.38%TN + 10.63%FG; 60.61%UP + 29.99%TN + 9.40%FG; 55.85%UP + 39.15%TN + 5%FG; 60.54%UP + 34.46%TN + 5%FG and a 100% wheat flour (100%WF) formulation served as the control (Table 2). Each blend was prepared by accurately weighing the component flours to the specified proportions. The composite flours were then combined with 5 mL olive oil, 2 g hydroxypropyl methylcellulose (HPMC) as a hydrocolloid, 30 mL of a pre-prepared starter culture, and 15 mL water to obtain a moderately stiff and extensible dough. The doughs were molded and transferred into baking pans lightly greased with olive oil to prevent adhesion, followed by proofing at ambient conditions for 1 h. Subsequently, the doughs were baked in a preheated oven at 200°C for 30 min until a characteristic golden-brown crust developed. The loaves were removed from the oven, cooled at room temperature, and stored in airtight containers prior to further analyses. The control sample (100% WF) was prepared without the addition of starter culture and HPMC.

2.6 | Texture Analysis of Sourdough Bread Samples

Texture Profile Analysis (TPA) was used to assess the texture of bread crumbs using standard methods outlined by

TABLE 2 | Sourdough bread sample formulation.

Formulations	Unripe plantain flour	Tiger nut flour	Fenugreek flour	Wheat flour	Total (%)
UP70.00 + TG 24.50 + FG 5.50	70.00	24.50	5.50	—	100
UP 50.00 + TG 39.38 + FG 10.63	50.00	39.38	10.38	—	100
UP 60.61 + TG 29.99 + FG 9.40	60.61	29.99	9.40	—	100
UP 55.85 + TG 39.15 + FG 5.00	55.85	39.15	5.00	—	100
UP 60.54 + TG 34.46 + FG 5.00	60.54	34.46	5.00	—	100
100WF	—	—	—	100	100

Abbreviations: FG, fenugreek seed powder; TN, tiger nut flour; UP, unripe plantain flour; WF, wheat flour.
70UP + 24.5TN + 5.5FG—70% UP + 24.5% TN + 10.6% FG.
50UP + 39.4TN + 10.6FG—50% UP + 39.4% TN + 10.6% FG.
60.6UP + 30TN + 9.4FG—60.6% UP + 30% TN + 9.4% FG.
55.9UP + 39.2TN + 5FG—55.9% UP + 39.2% TN + 5% FG.
60.5UP + 34.5TN + 5FG—60.5% UP + 34.5% TN + 5% FG.
100WF—100% WF.

Szczesniak (1963) and Bourne (1978), (2002). After allowing the loaf slices to reach room temperature (22 ± 2°C) for an hour, cylindrical crumb cores measuring 20 mm in height and 25 mm in diameter were removed from the center using a cork borer and trimmed to a consistent height. A texture analyzer equipped with a 36 mm cylindrical probe was used for TPA. Using a 5 g trigger force, samples underwent a two-cycle compression test to 50% deformation at pre-test, test, and dc.; post-test speeds of 2.0, 1.0, and 1.0 mm•s^{-1}, respectively. According to Bourne (2002), hardness, cohesiveness, springiness, gumminess, chewiness, and resilience were computed from the force–time curves. For each treatment, at least 9–12 independent cores were examined, and the findings were reported as mean ± SD. One-way ANOVA was used for statistical comparisons ($\alpha = 0.05$).

2.7 | Microstructure Analysis of Bread Samples

Using light microscopy (LM) and scanning electron microscopy (SEM) in accordance with standard procedures, the microstructure of bread samples was investigated. Each loaf was cut into representative crumb sections, which were then fixed in 10% neutral buffered formalin for 24 h at 4°C, dehydrated using graded ethanol, cleared in xylene, and embedded in paraffin. To improve starch and cell wall polysaccharide visibility, thin sections (5–10 μm) were obtained using a rotary microtome, mounted on glass slides, and stained with periodic acid–Schiff (PAS) reagent. A bright-field light microscope was used to take micrographs at different magnifications (× 4–× 40) (adapted from Sanderson 2020). Crumb cubes (≈ 5–10 mm^3) were fixed for 2–4 h at 4°C in 2.5% glutaraldehyde in 0.1 M phosphate buffer (pH 7.2) for SEM. They were then cleaned and post-fixed in 1% osmium tetroxide. To maintain structural integrity, samples were dried using a critical-point dryer after being dehydrated in a graded ethanol series. After being mounted on aluminum stubs and sputter-coated with gold-palladium, dried specimens were imaged at 1–5 kV using a secondary electron detector (according to Autio and Laurikainen 1997; Scanlon and Zghal 2001). ImageJ was used for image analysis in order to measure wall thickness, porosity, cell density, and crumb cell size.

2.8 | Pasting Analysis of Flours

Rapid Visco Analyzer (RVA) from New Point Scientific was used for the analysis. Approximately 3.5 g of the flour samples were placed into the RVA canister, followed by the addition of 25 mL of distilled water. Before inserting the canister into the RVA machine, the mixture was thoroughly mixed. For 2 minutes, the slurry was heated to 50°C. The heating and cooling were carried out at a constant rate of 11.2°C per minute. Various viscosity parameters, including peak viscosity, trough, breakdown, final viscosity, setback, peak time, and pasting temperature, were recorded from the pasting profile using the Thermocline for Windows software connected to a computer. The stability ratio and setback ratio were calculated from the RVA parameters as follows: Stability ratio (SR) = Trough viscosity/Peak viscosity, whereas Setback ratio (SBR) = Setback viscosity/Peak viscosity, where peak viscosity represents the maximum viscosity during heating, trough viscosity is the minimum viscosity after peak during holding at high temperature, and setback viscosity is calculated as the difference between final viscosity and trough viscosity.

2.9 | Determination of Total Phenol Content of Sourdough Bread Samples

Total phenol content of the sourdough bread samples was assessed using the method described by Singleton et al. (1999). Appropriate dilutions of the extracts were treated with 2.5 mL of 10% (v/v) Folin–Ciocalteu reagent, followed by the addition of 2.0 mL of 7.5% sodium carbonate to neutralize the mixture. The samples were then incubated at 45°C for 40 min, after which the absorbance was read at 765 nm using a spectrophotometer. The total phenolic content was expressed as gallic acid equivalents (GAE).

2.10 | Determination of Total Flavonoid Content of Sourdough Bread Samples

Total flavonoid content was evaluated using a slightly modified procedure described by Meda et al. (2005). A 0.5 mL portion of the appropriately diluted sample was combined with 0.5 mL of methanol, 50 μL of 10% $AlCl_3$, 50 μL of 1 M potassium acetate, and 1.4 mL of water. The mixture was left to stand at room temperature for 30 min, after which its absorbance was measured at 415 nm. The flavonoid concentration was then calculated, and the non-flavonoid polyphenol fraction was obtained by subtracting the total flavonoid content from the total phenolic content.

2.11 | Vitamin C Content of Sourdough Bread Samples

Vitamin C was analyzed titrimetrically using 2,6-dichlorophenolindophenol as described by Barros et al. (2007). Results were expressed as mg/g dry weight.

2.12 | Determination of Amylose and Amylopectin Content of Sourdough Bread Samples

The amylopectin content in the sourdough bread samples was measured following the method outlined by Juliano (1971). The amylose content was calculated as the remainder from 100%, according to the procedures described by Williams et al. (1958) and Juliano (1971).

2.13 | Determination of Glycemic Indices of Sourdough Bread Samples

The glycemic indices of the bread samples were assessed using a modified procedure based on Goni et al. (1997). AJY 20002 electronic balance was used to weigh 50 mg of each sample into a beaker. The samples were mixed with 5 mL of a gastric

solution containing piglet pepsin (HCl–KCl buffer, pH 1.5) and incubated in a shaking water bath at 40°C for 60 min. After incubation, the mixture was diluted with 0.05 M phosphate buffer (pH 6.9), followed by the addition of 2.5 mL of α-amylase from porcine pancreas (Type VIB, 10 units/mg). After that, the mixture was incubated at 37°C. Test tubes were filled with 200 μL aliquots at intervals of 30 min (0, 30, 60, 90, 120, 150, and 180 min). After 15 min of boiling, 500 μL of sodium acetate buffer (pH 4.75) and 5 μL of *S. cerevisiae* α-glucosidase (Type I, 10 units/mg protein) were added, and the mixture was then incubated for 45 min at 60°C. After adding 200 μL of DNSA solution, the mixture was heated to 100°C for 5 minutes, diluted with 2.0 μL of distilled water, and centrifuged for 5 minutes at 3000 rpm. At 540 nm, the resultant supernatant's absorbance was measured. Finally, the glycemic index was calculated by dividing the total area under the curve (AUC) of each sample by that of the glucose standard and multiplying by 100.

2.14 | Animal Grouping and Treatments

Male wistar rats (180–200 g) were obtained from the Animal House of the Department of Biochemistry, Federal University of Technology, Akure, Nigeria. The animals were maintained under controlled conditions (22°C–25°C, 12-h light/dark cycle) with unrestricted access to food and water. Ethical approval for their use was granted by the School Animal Care and Use Committee (FUTA/ETH/25/274), and all procedures followed the NIH Guide for the Care and Use of Laboratory Animals and the ARRIVE guidelines. The rats were allowed to acclimate for 2 weeks before the experiment began. After acclimatization, they were randomly divided into eight groups ($n = 6$) as follows: Group 1: Standard rat diet (Basal); Group 2: L-NAME (40 mg/kg) + high fat diet (L-NAME + HFD); Group 3: L-NAME + high fat diet + Lisinopril (10mg/kg) (L-NAME + HFD + Lis); Group 4: L-NAME + 70UP + 24.5TN + 5.5FG; L-NAME + 50UP + 39.4TN + 10.6FG; Group 6: L-NAME + 60.6UP + 30TN + 9.4FG; Group 7: L-NAME + 55.9% UP + 39.2% TN + 5% FG; Group 8: L-NAME + 60.5UP + 34.5TN + 5FG; and Group 9: 100WF. The rats were maintained on the diet for 14 days. The experimental groups were administered L-NAME (a potent competitive inhibitor of nitric oxide synthase which suppresses nitric oxide production from L-arginine and modulating vascular tone, endothelial function, and associated pathophysiological processes). L-NAME was administered at a daily oral dose of 40 mg/kg body weight, alongside a high-fat diet (HFD). Conversely, the normal and control groups received water orally throughout the duration of the study. The standard diet consisted of a commercially prepared laboratory rat chow containing approximately 20% protein, 65% carbohydrate, and 5% fat (w/w). The high-fat diet was formulated by fortifying the standard chow with a defined mixture of animal fat and vegetable oil, yielding approximately 45%–60% of total energy from fat. The experimental bread samples were oven-dried, ground into a fine powder, and freshly reconstituted in distilled water prior to administration. A predetermined dose (g/kg body weight) was administered once daily as a single bolus via direct hand-feeding to ensure complete intake by each rat. Twenty-four hours after the final administration, the animals were sacrificed by cervical dislocation.

2.14.1 | Body Mass Index (BMI) and Weight Gain

Body weights were measured at the start of the experiment and then recorded weekly. The percentage increase in weight was computed at the conclusion of the study. The body mass index (BMI) was obtained by dividing the body weight (g) by the square of the naso-anal length (cm^2).

2.14.2 | Preparation of Tissue Homogenates

At the end of the feeding trial, the animals were euthanized by cervical dislocation. The kidney, heart, and penile tissues were promptly removed, washed with ice-cold saline, and homogenized in 50 mM Tris–HCl buffer (pH 7.4) using a Teflon–glass homogenizer at a buffer-to-tissue ratio of 3:1. The resulting homogenates were centrifuged at 4000 rpm for 15 min, and the supernatants were collected for biochemical analyses. Blood was obtained via cardiac puncture into EDTA-coated tubes and centrifuged at 10,000 × g for 15 min to separate the plasma.

2.14.3 | Biochemical Analysis

2.14.3.1 | Determination of Plasma Lipid Profile. Blood samples were collected by cardiac puncture after overnight fasting. Plasma was separated by centrifugation (4000 × g, 10 min). Total cholesterol, triglycerides, HDL, and LDL were determined using Randox kits according to the manufacturer's instructions. LDL was calculated using the Friedewald equation (Friedewald et al. 1972). Atherogenic index was computed as log (TG/HDL-C).

2.15 | Statistical Analysis

All data were expressed as mean ± standard deviation (SD) of triplicate determinations. Statistical comparisons among groups were made using one-way analysis of variance (ANOVA) followed by Tukey's post hoc test. Differences were considered significant at $p < 0.05$. GraphPad Prism version 8.0.2 (GraphPad Software, San Diego, CA, USA) was used for all statistical analyses and graphical representations.

3 | Results and Discussion

3.1 | Texture Properties

Texture and functionality data are shown in Table 3. The highest breaking force (680.5 kg), was observed in 70UP + 24.5TN + 5.5FG and was not significantly different from 100WF (control) suggesting enhanced structural strength in the sourdough composite bread, whereas the lowest was recorded in the 60.6UP + 30TN + 9.4FG bread (605.4 kg). Enhanced structural integrity displayed by 70UP + 24.5TN + 5.5FG might be attributed to synergistic interaction of more resistant starch of the plantain, dietary fibers, proteins, and sourdough fermentation effects, which together compensate for the absence of gluten. Also, the highest firmness value (4.90 kgf) observed in

TABLE 3 | Texture and functional properties of sourdough bread samples.

Sample	Textural properties					Functional property
	Breaking force (kg)	Breaking strain (m)	Firmness (kgf)	Springiness (cm)	Resilience	Swelling power (mg/L)
70UP + 25.5TN + 5.5FG	680.5 ± 30.2^{a}	9.50 ± 0.24^{a}	4.45 ± 0.00^{b}	0.524 ± 0.10^{b}	0.325 ± 0.10^{b}	25.5 ± 0.4^{b}
50UP + 39.4TN + 10.6FG	620.6 ± 46.6^{b}	9.60 ± 0.42^{a}	4.60 ± 0.10^{b}	0.763 ± 0.10^{a}	0.450 ± 0.20^{a}	26.2 ± 1.5^{ab}
60.6UP + 30TN + 9.4FG	605.4 ± 30.4^{b}	9.40 ± 0.44^{a}	4.90 ± 0.00^{a}	0.750 ± 0.20^{a}	0.272 ± 0.20^{b}	25.5 ± 0.4^{b}
55.9UP + 39.2TN + 5FG	630.5 ± 40.7^{b}	8.50 ± 0.27^{b}	4.90 ± 0.10^{a}	0.740 ± 0.20^{a}	0.455 ± 0.00^{a}	25.7 ± 0.6^{b}
60.5UP + 34.5TN + 5FG	620.2 ± 35.2^{b}	9.22 ± 0.26^{a}	3.93 ± 0.00^{c}	0.450 ± 0.10^{c}	0.324 ± 0.10^{b}	24.5 ± 0.3^{c}
100WF	675.2 ± 20.4^{a}	9.20 ± 0.40^{a}	4.90 ± 0.00^{a}	0.650 ± 0.10^{b}	0.256 ± 0.10^{b}	26.5 ± 0.6^{a}

Note: Values represent mean ± SD. Different alphabetical superscripts in the same column are significantly different at $p < 0.05$.
Abbreviations: FG, fenugreek seed powder; TN, tiger nut flour; UP, unripe plantain flour; WF, wheat flour.
70UP + 24.5TN + 5.5FG—70% UP + 24.5% TN + 10.6% FG.
50UP + 39.4TN + 10.6FG—50% UP + 39.4% TN + 10.6% FG.
60.6UP + 30TN + 9.4FG—60.6% UP + 30% TN + 9.4% FG.
55.9UP + 39.2TN + 5FG—55.9% UP + 39.2% TN + 5% FG.
60.5UP + 34.5TN + 5FG—60.5% UP + 34.5% TN + 5% FG.
100WF—100% WF.

60.6UP + 30TN + 9.4FG, 55.9UP + 39.2TN + 5FG and 100WF, implies that the firmness of these bread samples compared favorably with the control (100WF). This observation might be attributed to the presence of hydrocolloid (HPMC) in the sourdough gluten free bread making it mimic 100WF closely. Springiness is a quality linked to the elasticity of the bread and depicts freshness; high values (0.740–0.763 cm) observed in 55.9UP + 39.2TN + 5FG, 60.6UP + 30TN + 9.4FG and 50UP + 39.4TN + 10.6FG are appraised as desirable in comparison to 100WF (0.650cm). Notably, the 50UP + 39.4TN + 10.6FG bread with the highest springiness (0.763 cm) also exhibited the highest resilience (0.45) compared to wheat bread (100WF). High resilience is associated with reduced crumbling during slicing, resulting in improved eating quality and minimized product waste.

As seen in this study, all these breads made with composite sourdough formulations exhibited textural and functional properties that are on par with or superior to those of traditional wheat bread (100WF) which might be attributed to the complementary functionality of plantain starch, tiger nut fiber–lipid interactions, fenugreek galactomannan gums, and sourdough-induced biochemical modifications, which collectively create a cohesive, resilient crumb structure despite the absence of gluten. One of the main findings of the study was the notable rise in the amount of bioactive chemicals in the composite sourdough breads.

3.2 | Microstructure

Microstructural analysis (Figure 1) showed that the gluten-free sourdough composite breads exhibited denser starch granules with less surface disruption, indicating that the starch-dominated networks showed controlled gelatinization, granule fusion, and hydrocolloid-mediated water binding, which compensate for the absence of gluten. Whereas wheat bread displayed larger more fragmented granules indicative of uncontrolled gelatinization and faster digestibility. As observed, optimized proportions of unripe plantain resistant starch, tiger nut fiber–lipid components, and fenugreek galactomannan especially in 50UP + 39.4TN + 10.6FG promoted the development of a compact continuous starch matrix, resulting in enhanced structural integrity, increased resilience, and reduced crumb fragmentation as earlier discussed.

3.3 | Pasting Properties of the Flour Blends

The pasting characteristics of the flour blends are presented in Table 4. The starch's capacity to swell freely prior to physical breakdown is known as its peak viscosity (Adegunwa et al. 2017). Peak viscosity was highest in the 50UP + 39.4TN + 10.6FG formulation (2593.20 RVU), indicating strong starch granule swelling and potential thickening ability. In contrast, the 60.6UP + 30TN + 9.4FG bread exhibited the lowest peak viscosity (1940.33 RVU). The increased peak viscosity especially in 50UP + 39.4TN + 10.6FG might be attributed to the synergistic effect of unripe plantain and fenugreek in the flour blends. Unripe plantain is rich in resistant starch, capable of forming strong viscous gels, similarly, galactomannan gum, the soluble fiber in fenugreek is a powerful thickening agent, capable of increasing peak viscosity in flour paste (Sakhare and Prabhasankar 2017; Xie et al. 2023). Stability ratios were generally high across all formulations, with values above 0.7, suggesting good resistance to shear stress during heating. The 55.9UP + 39.2TN + 5FG and 60.5UP + 34.5TN + 5FG samples showed the best stability profiles (0.98 and 0.97, respectively). Inferring that, these flours might produce more stable pastes; an essential quality for preserving bread texture during baking as reported by Adegunwa et al. (2017).

The setback ratio indicates the inclination of the starch to retrogradation after cooking, the setback ratios of the flour blends were not significantly different *($p > 0.05$)* from each other as values ranged from 1.40–1.67 RVU, implying a good stability against bread staling. The observation suggests better texture retention in cooked products which might be attributed

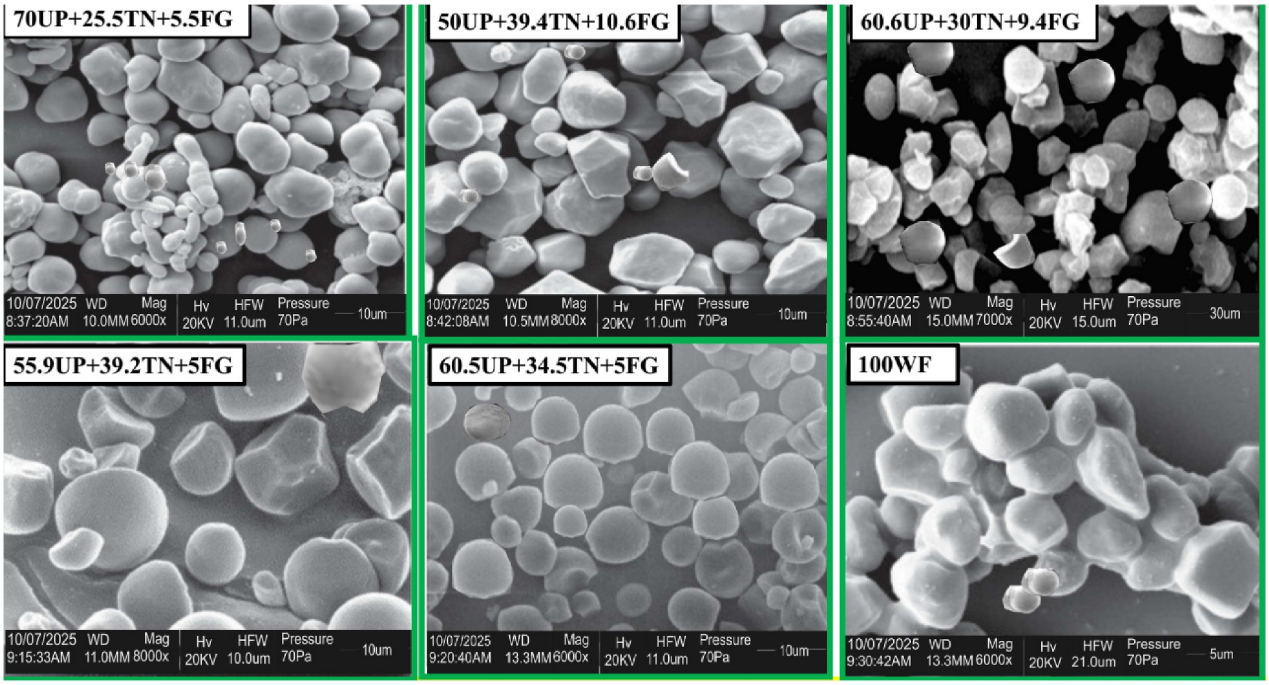


FIGURE 1 | Starch microstructure of sourdough bread samples. FG, fenugreek seed powder; TN, tiger nut flour; UP, unripe plantain flour; WF, wheat flour. 70UP + 24.5TN + 5.5FG—70% UP + 24.5% TN + 10.6% FG. 50UP + 39.4TN + 10.6FG—50% UP + 39.4% TN + 10.6% FG. 60.6UP + 30TN + 9.4FG—60.6% UP + 30% TN + 9.4% FG. 55.9UP + 39.2TN + 5FG—55.9% UP + 39.2% TN + 5% FG. 60.5UP + 34.5TN + 5FG—60.5% UP + 34.5% TN + 5% FG. 100WF—100% WF.

to the blending of high-amylose starches (unripe plantain) with moderate (tiger nut)—to-low-amylose starches (fenugreek) or attributed to the combination of ingredients (fats/proteins) that are capable of inhibiting staling/retrogradation. The impact of compositing flours with different amylose levels on the behavior of starch during gelatinization and retrogradation is reflected in the flour formulations' pasting properties.

3.4 | Phytochemical Composition

The total phenol, flavonoid, and vitamin C content of the sourdough breads are summarized in Table 5. All composite breads significantly outperformed the 100WF control in bioactive content. The 50UP + 39.4TN + 10.6FG formulation exhibited the highest phenolic (6.75 mg/g) and flavonoid (3.22 mg/g) concentrations, whereas the 70UP + 24.5TN + 5.5FG sample was particularly rich in vitamin C (0.87 mg/g). These results demonstrate that the incorporation of unripe plantain, tiger nut, and fenugreek enriched the breads with natural antioxidants. The addition of unripe plantains, tiger nuts, and fenugreek significantly increased the amounts of total phenolic, flavonoid, and vitamin C compounds when compared to the wheat control. The formulation with the highest phenolic and flavonoid contents was 50UP + 39.4TN + 10.6FG, whereas the sample with the highest vitamin C concentration was 70UP + 24.5TN + 5.5FG. Because tiger nuts, fenugreek, and unripe plantains each contain unique bioactive compounds, their combination in sourdough breads offers a synergistic antioxidant potential. Monounsaturated fatty acids, tocopherols, and flavonoids all of which have antioxidant qualities are abundant in tiger nuts (Yu et al. 2022).

Fenugreek seeds contain saponins, polyphenols, and vitamins A, B1, and C, hence they have hypoglycemic and antioxidant effects by improving insulin sensitivity, slowing the absorption of glucose, and shielding tissues from oxidative damage (Hamden et al. 2013). The dietary fiber, resistant starch, and polyphenols found in unripe plantains help with digestion and have extra antioxidant benefits. These components work in concert to increase the bread's overall antioxidant capacity, which may help it manage blood glucose levels and oxidative stress in a synergistic way (Akhtar et al. 2025). Fermentation may have further enhanced the release and bioavailability of these bioactives by breaking down complex matrices and liberating bound phenolics (Kumar et al. 2025; Akhtar et al. 2025). Sourdough bread's higher phenolic and flavonoid content is important because these substances scavenge free radicals, lowers oxidative stress, regulates lipid metabolism, and enhances vascular function (Gabriele et al. 2023). The antioxidant capacity of the breads might be significantly enhanced by phenolic compounds, whose concentrations often increase during mixing, fermentation, and baking processes, with particularly high accumulation in the crust. In addition, vitamin C, owing to its strong reducing power and antioxidant activity, contributes to improved redox stability within the bread matrix. In addition to its antioxidant properties, vitamin C improves dough elasticity, strengthens the formation of gluten networks, and improves gas retention during fermentation (Al-Faraji et al. 2019). As a result, the sourdough breads have better rise and crumb structure and a lighter airier texture.

When taken as a whole, these effects indicate that bread enhanced with phenolics, flavonoids, and vitamin C has better antioxidant properties that promote health as well as better

TABLE 4 | Pasting properties of flour blends.

Sample	Pasting time (min)	Pasting temp (°C)	Peak viscosity (RVU)	Holding viscosity (RVU)	Final viscosity (RVU)	Breakdown (RVU)	Stability ratio	Setback ratio
70UP + 25.5TN + 5.5FG	5.03 ± 0.05^{b}	74.19 ± 0.38^{b}	2400.21 ± 25.01^{b}	2050.30 ± 25.60^{a}	3260.57 ± 21.81^{a}	356.88 ± 8.34^{e}	0.79 ± 0.03^{c}	1.67 ± 6.01^{a}
50UP + 39.4TN + 10.6FG	4.12 ± 0.02^{c}	73.55 ± 0.07^{c}	2593.20 ± 7.06^{a}	1702.00 ± 5.61^{c}	2760.00 ± 20.80^{b}	890.30 ± 8.35^{b}	0.70 ± 0.03^{c}	1.57 ± 6.01^{ab}
60.6UP + 30TN + 9.4FG	5.20 ± 0.08^{b}	73.43 ± 0.06^{c}	1940.33 ± 8.48^{e}	1620.67 ± 5.76^{c}	2688.17 ± 7.96^{c}	320.23 ± 14.37^{e}	0.97 ± 0.03^{a}	1.40 ± 0.08^{b}
55.9UP + 39.2TN + 5FG	5.00 ± 0.03^{b}	75.80 ± 0.04^{a}	2245.63 ± 3.54^{c}	1570.29 ± 6.46^{c}	2449.29 ± 4.04^{e}	505.33 ± 5.20^{d}	0.98 ± 0.05^{a}	1.50 ± 3.96^{ab}
60.5UP + 34.5TN + 5FG	5.05 ± 0.04^{b}	74.22 ± 0.06^{b}	2220.53 ± 2.83^{c}	1690.67 ± 1.59^{c}	2720.97 ± 1.58^{c}	485.36 ± 4.07^{c}	0.97 ± 0.05^{a}	1.61 ± 1.23^{ab}
100WF	5.18 ± 0.04^{b}	73.67 ± 0.33^{bc}	2175.63 ± 8.47^{d}	1638.06 ± 23.06^{c}	2460.06 ± 6.71^{e}	547.28 ± 12.89^{c}	0.97 ± 0.03^{a}	1.65 ± 3.96^{a}

Note: Values represent mean ± SD. Different alphabetical superscripts in the same column are significantly different at $p < 0.05$.
Abbreviations: FG, fenugreek seed powder; TN, tiger nut flour; UP, unripe plantain flour; WF, wheat flour.
70UP + 24.5TN + 5.5FG—70% UP + 24.5% TN + 10.6% FG.
50UP + 39.4TN + 10.6FG—50% UP + 39.4% TN + 10.6% FG.
60.6UP + 30TN + 9.4FG—60.6% UP + 30% TN + 9.4% FG.
55.9UP + 39.2TN + 5FG—55.9% UP + 39.2% TN + 5% FG.
60.5UP + 34.5TN + 5FG—60.5% UP + 34.5% TN + 5% FG.
100WF—100% WF.

dough handling and final product quality with increased texture and volume (Jia et al. 2022). As a result, the composite sourdough breads are functional foods with natural antioxidant potential that may reduce oxidative stress-related metabolic diseases when regularly consumed.

3.5 | Starch Composition

As shown in Table 5, amylose content ranged from 21.99% in the 100WF bread to 29.23% (50UP + 39.4TN + 10.6FG). Correspondingly, amylopectin was highest in wheat bread (78.01%) and lowest in the 50UP + 39.4TN + 10.6FG formulation (70.77%). The starch composition provided evidence of nutritional improvement, higher percentage of amylose as observed in composite sourdough breads implies reduced starch digestibility due to its linear chain structure, which prevents enzymatic hydrolysis and promotes a delayed release of glucose (Tan et al. 2025). Conversely, the branching structure of amylopectin encourages higher postprandial glycemia and faster digestion (Devi et al. 2024). The amylose-to-amylopectin ratio strongly dictates starch gelatinization, network formation, and crumb stability in gluten-free bread. The higher amylose-to-amylopectin ratios observed in composite breads (up to 0.41) suggest slower starch digestibility compared with wheat bread (0.28), this might be ascribed to the optimal amount of fenugreek in the bread sample (10.6) compared to other composite sourdough bread. Fenugreek galactomannan (gum) regulates water availability and reduces starch gelatinization by binding water, which improves texture and slows digestion (Xie et al. 2023).

3.6 | Glycemic Response

Figure 2 shows that the glycemic index (GI) was markedly lower in all composite breads compared to wheat bread. Among them, the 50UP + 39.4TN + 10.6FG and 55.9UP + 39.2TN + 5FG samples produced the most favorable GI values of 35.23 and 38.48, consistent with their higher amylose content and bioactive profiles. The lower glycemic index of the composite loaves as shown in Figure 3, reflects both structural and compositional changes caused by sourdough fermentation and ingredient selection. Organic acids produced during sourdough fermentation, slows down starch digestion and delay gastric emptying, convert starches into resistant starch and degrade phytic acid, prompting a slower more gradual sugar release and lower blood sugar spikes compared to regular bread. Such characteristics would be extremely beneficial for functional foods that focus on glycemic management (Demirkesen-Bicak et al. 2021), particularly for people with diabetes or metabolic syndrome. The feeding experiment provided physiological evidence of the metabolic effects of the composite breads.

3.7 | Effects on Weight Gain and BMI in Rats

The percentage weight gain and BMI of L-NAME + HFD-induced hypertensive rats are shown in Figure 3. Rats in the HFD + L-NAME group exhibited significant increases in both

TABLE 5 | Total phenol, flavonoid, vitamin C, amylose, and amylopectin contents of sourdough bread samples.

Sample	Total phenol (mg/g)	Total flavonoid (mg/g)	Vitamin C (mg/g)	Amylose (%)	Amylopectin (%)	Amylose: Amylopectin ratio
70UP + 25.5TN + 5.5FG	5.01 ± 0.02^{b}	2.41 ± 0.01^{d}	0.87 ± 0.02^{a}	24.37 ± 0.11^{c}	75.63 ± 0.10^{c}	0.32
50UP + 39.4TN + 10.6FG	6.75 ± 0.01^{a}	3.22 ± 0.04^{a}	0.81 ± 0.03^{b}	29.23 ± 0.12^{a}	70.77 ± 0.11^{e}	0.41
60.6UP + 30TN + 9.4FG	6.16 ± 0.03^{a}	2.89 ± 0.03^{b}	0.83 ± 0.03^{b}	26.38 ± 0.11^{b}	73.62 ± 0.11^{d}	0.35
55.9UP + 39.2TN + 5FG	6.18 ± 0.03^{a}	2.88 ± 0.02^{b}	0.81 ± 0.01^{b}	27.32 ± 0.14^{b}	72.68 ± 0.13^{d}	0.37
60.5UP + 34.5TN + 5FG	6.12 ± 0.02^{a}	2.84 ± 0.03^{b}	0.82 ± 0.03^{b}	25.34 ± 0.13^{c}	74.66 ± 0.11^{c}	0.33
100WF	1.50 ± 0.02^{c}	0.91 ± 0.03^{e}	0.42 ± 0.02^{c}	21.99 ± 0.12^{d}	78.01 ± 0.14^{a}	0.28

Note: Values represent mean ± SD. Different alphabetical superscripts in the same column are significantly different at $p < 0.05$.
Abbreviations: FG, fenugreek seed powder; TN, tiger nut flour; UP, unripe plantain flour; WF, wheat flour.
70UP + 24.5TN + 5.5FG—70% UP + 24.5% TN + 10.6% FG.
50UP + 39.4TN + 10.6FG—50% UP + 39.4% TN + 10.6% FG.
60.6UP + 30TN + 9.4FG—60.6% UP + 30% TN + 9.4% FG.
55.9UP + 39.2TN + 5FG—55.9% UP + 39.2% TN + 5% FG.
60.5UP + 34.5TN + 5FG—60.5% UP + 34.5% TN + 5% FG.
100WF—100% WF.

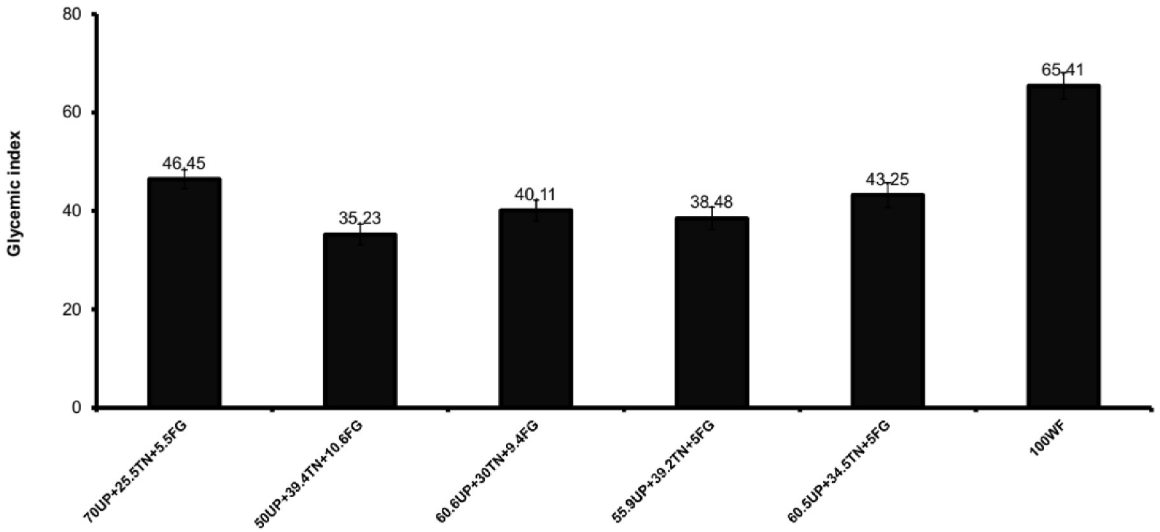


FIGURE 2 | Glycemic indices of sourdough bread samples. FG, fenugreek seed powder; TN, tiger nut flour; UP, unripe plantain flour; WF, wheat flour. 70UP + 24.5TN + 5.5FG—70% UP + 24.5% TN + 10.6% FG. 50UP + 39.4TN + 10.6FG—50% UP + 39.4% TN + 10.6% FG. 60.6UP + 30TN + 9.4FG—60.6% UP + 30% TN + 9.4% FG. 55.9UP + 39.2TN + 5FG—55.9% UP + 39.2% TN + 5% FG. 60.5UP + 34.5TN + 5FG—60.5% UP + 34.5% TN + 5% FG. 100WF—100% WF.

weight gain and BMI relative to the basal diet group. Lisinopril treatment attenuated these increases. Similarly, rats fed composite sourdough breads showed reduced weight gain and BMI compared to the hypertensive control. The occurrence of serious weight gain in this experimental model was evidenced by significant increases in body weight gain and body mass index (BMI) in rats fed a high-fat diet (HFD) in combination with L-NAME administration, this agrees with Arika et al. (2019) who also induced obesity with HFD. Rats fed a high-fat diet exhibit marked increases in body weight, and fat pad weight in comparison to controls, according to study (Marques et al. 2015). The nitric oxide synthase inhibitor L-NAME is added, which exacerbates the vascular and metabolic dysfunction that is commonly linked to obesity (Nascimento et al. 2011). In order to validate the establishment of obesity in these rats, this model is frequently used to replicate pathophysiological changes associated with obesity, such as increased fat mass, endothelial dysfunction, and altered vascular responses. However, compared to the HFD + L-NAME control, rats fed with the composite sourdough breads showed less weight gain and a lower BMI. The greatest effects were seen in animals fed the 60.5UP + 34.5TN + 5FG formulation. These results imply that eating the composite breads may alter lipid utilization and energy balance.

The bioactive components of fenugreek, tiger nuts, and unripe plantains are probably responsible for the increased satiety and decreased adiposity associated with their consumption. Rich in resistant starch and dietary fiber, unripe plantains slow down digestion and increase insulin sensitivity, which lowers total caloric intake and increases feelings of fullness (Garcia-Valle et al. 2019). By enhancing lipid metabolism and having anti-inflammatory and antioxidant properties, tiger nuts and fenugreek contain lipid-lowering bioactive substances such as

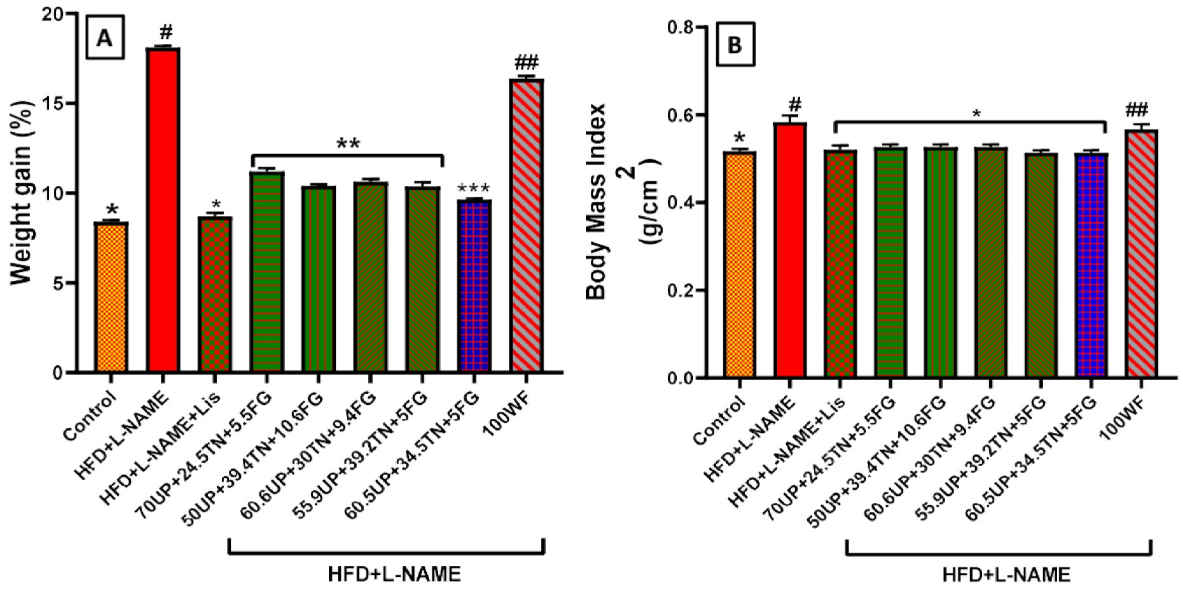


FIGURE 3 | Effect of formulated sourdough bread samples on (A) percentage weight gain and (B) Body mass index (BMI) of L-NAME + high fat diet (HFD)-induced hypertensive rats. FG, fenugreek seed powder; TN, tiger nut flour; UP, unripe plantain flour; WF, wheat flour. 70UP + 24.5TN + 5.5FG—70% UP + 24.5% TN + 10.6% FG. 50UP + 39.4TN + 10.6FG—50% UP + 39.4% TN + 10.6% FG. 60.6UP + 30TN + 9.4FG −60.6% UP + 30% TN + 9.4% FG. 55.9UP + 39.2TN + 5FG—55.9% UP + 39.2% TN + 5% FG. 60.5UP + 34.5TN + 5FG—60.5% UP + 34.5% TN + 5% FG. 100WF—100% WF. Lis—Lisinopril.

flavonoids and saponins that lower blood lipid levels and adiposity (Askarpour et al. 2020; Ogunlade 2015). Foods high in fiber prolong feelings of fullness and slow down the emptying of the stomach, which causes people to eat less (Akhlaghi 2022). The composite sourdough breads' potential function in managing obesity is suggested by their capacity to prevent excessive weight gain. Given the rising prevalence of obesity and related comorbidities worldwide, this feature is especially beneficial for the design of functional foods. Accordingly, the results establish the formulated sourdough breads as nutritionally sound choices that can support appropriate body weight control without sacrificing flavor. An important part of this study was evaluating the rats' lipid metabolism in relation to the bread samples.

3.8 | Effects on Plasma Lipid Profile

Plasma lipid parameters are shown in Figure 4. Total cholesterol, LDL-cholesterol, triglycerides, and atherogenic index were all significantly elevated in the HFD + L-NAME group compared with the basal group, confirming diet-induced dyslipidemia. Lisinopril markedly improved lipid profiles, whereas composite sourdough breads also produced substantial reductions. The 60.5UP + 34.5TN + 5FG formulation was the most effective, lowering total cholesterol, LDL, and triglycerides while significantly increasing HDL-cholesterol. Other formulations also demonstrated notable lipid-lowering effects compared to 100WF. The results clearly demonstrated that dyslipidemia, characterized by elevated levels of total cholesterol, triglycerides, and LDL cholesterol and decreased HDL cholesterol, was brought on by the high-fat diet and L-NAME treatment (Nascimento et al. 2011). These alterations are consistent with the abnormalities in lipid metabolism that underlie hypertension and atherosclerosis. Remarkably, the lipid abnormalities of rats fed composite sourdough loaves significantly improved. This outcome could be the result of the combined actions of the bioactive components in the three flours (Jikah and Christopher 2024; Shodehinde and Oboh 2013; Abdullahi et al. 2022). Fenugreek contains soluble fibers and saponins that are known to bind bile acids and promote cholesterol excretion (Amit et al. 2023). Tiger nuts provide healthy unsaturated fats and phytosterols that control lipid metabolism (Abdullahi et al. 2022), whereas unripe plantains provide resistant starch and phenolics that improve hepatic lipid management (Adekiya et al. 2018).

An additional indication of the composite breads' potential for cardioprotection is the decrease in the atherogenic index observed in animals fed with them, which suggests a decreased risk of cardiovascular disease, A powerful and trustworthy biomarker linked to cardiovascular diseases (CVD), such as atherosclerosis, coronary heart disease, and stroke, is the atherogenic index of plasma (AIP) (Fernández-Macías et al. 2019). It correlates with the size of lipoprotein particles and represents the equilibrium between atherogenic and protective lipoproteins. Because of a number of mechanisms, composite sourdough breads have been shown to have positive effects on hypertension and dyslipidemia (Tucker et al. 2010). Consuming sourdough is linked to higher HDL levels, which may indicate better reverse cholesterol transport and increased endothelial protection, both of which support cardiovascular health (Ribet et al. 2022). Although the blood pressure-lowering effects of composite sourdough breads are typically mild, the advantageous rearrangement in cholesterol transport dynamics supports their potential use as dietary interventions to treat dyslipidemia and hypertension. Hence, regular consumption may improve cardiovascular health through lipid modification, antioxidant defense, and blood pressure regulation, this is similar to the outcome of Tucker et al. (2010) who analyzed the effect of the consumption of whole wheat grain sourdough

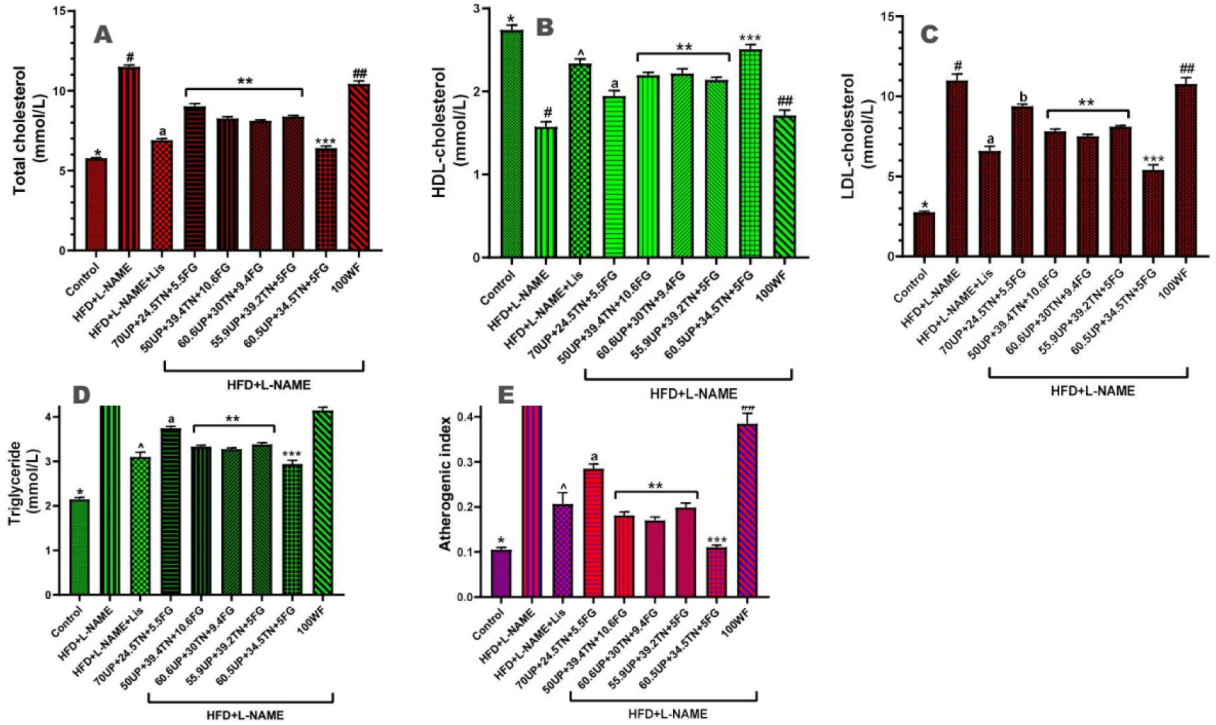


FIGURE 4 | Effect of formulated sourdough bread samples on (A) plasma total cholesterol. (B) HDL-cholesterol. (C) LDL-cholesterol. (D) Triglyceride. (E) Atherogenic index concentration of L-NAME + high fat diet (HFD)-induced hypertensive rats. FG, fenugreek seed powder; TN, tiger nut flour; UP, unripe plantain flour; WF, wheat flour. 70UP + 24.5TN + 5.5FG—70% UP + 24.5% TN + 10.6% FG, 50UP + 39.4TN + 10.6FG—50% UP + 39.4% TN + 10.6% FG, 60.6UP + 30TN + 9.4FG—60.6% UP + 30% TN + 9.4% FG, 55.9UP + 39.2TN + 5FG—55.9% UP + 39.2% TN + 5% FG, 60.5UP + 34.5TN + 5FG—60.5% UP + 34.5% TN + 5% FG, 100WF—100% WF, Lis—Lisinopril.

bread on serum lipids. The remarkable similarity between the lipid profiles of rats administered lisinopril and the best-performing bread formulation highlights the functional therapeutic potential of these formulations (Chandra et al. 2018).

All of the results demonstrated that sourdough breads enhanced with unripe plantains, tiger nuts, and fenugreek are not only more beneficial but also more nutrient-dense. From the perspective of food technology, the formulations showed remarkable stability, appealing textural characteristics, and excellent functional chemical retention. From a nutritional standpoint, they offered improved bioactive content, a lower glycemic response, and an elevated starch digestibility profile. From a physiological perspective, they changed body weight, lipid metabolism, and glycemic control in models of dyslipidemia. These many advantages show how fermentation biotechnology and ingredient functioning work together harmoniously. It is well established that sourdough fermentation increases the bioavailability of nutrients, lowers antinutritional factors, and produces bioactive peptides that may have health benefits (Graça et al. 2021). It enhanced the inherent functional potential of the plant-based flours utilized in this situation. Additionally, the observed decrease in lipid markers and glycemic index implies that these sourdough breads could help prevent or treat metabolic syndrome, which includes insulin resistance, obesity, hypertension, and dyslipidemia. In terms of physicochemical and physiological metrics. The formulation containing reasonable amounts of all three flours (50UP + 39.4TN + 10.6FG and 60.5UP + 34.5TN + 5FG) consistently performed well, suggesting that formulation balance is essential for maximizing nutritional result. Practically speaking, consumers who are concerned about their health and wish to reduce their intake of refined carbohydrates without sacrificing bread quality may find the composite sourdough breads to be helpful alternatives. Furthermore, the formulations promote food diversification and value addition in functional baked goods by providing a sustainable means of utilizing locally available nutrient-rich crops. These findings open the door for additional optimization, including human testing, shelf-life analysis, and sensory evaluation, to bolster the identified health-promoting properties.

4 | Conclusion

The study found that composite sourdough breads made with fenugreek, tiger nut, and unripe plantain flours exhibit enhanced nutritional, functional, and health-promoting properties compared to traditional wheat sourdough bread. The composite loaves demonstrated reduced starch digestibility, a moderated glycemic response, increased antioxidant potential, and improved textural characteristics. In addition, they exerted beneficial physiological effects on hypercholesterolemia risk markers, body weight, and lipid metabolism in rat models of dyslipidemia. Among the formulations evaluated, the 50UP + 39.4TN + 10.6FG and 60.5UP + 34.5TN + 5FG provided the most optimal balance between functionality and health benefits. However, a key limitation of the present study is the lack of direct quantification of antioxidant activity, which would have provided more robust validation of the observed antioxidant potential. Future studies will address this by

incorporating comprehensive antioxidant activity measurements into the experimental design. Overall, these findings highlight the potential of composite sourdough breads as innovative functional food products that can support cardiovascular and metabolic health through natural diet-based interventions.

Author Contributions

Mary T. Ademosun: conceptualization, investigation, formal analysis, writing – review and editing, writing – original draft, methodology, visualization. **Olufunmilayo S. Omoba:** conceptualization, supervision, writing – review and editing, methodology. **Ganiyu Oboh:** conceptualization, supervision, formal analysis, methodology, project administration. **Adedamola I. Akinyede:** supervision, writing – review and editing, methodology. **Rukayat O. Awolola:** formal analysis, methodology.

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The authors have nothing to report.

Ethics Statement

Ethical approval for animal use was obtained from the School Animal Care and Use Committee (FUTA/ETH/25/274), and all procedures complied with the NIH Guide for the Care and Use of Laboratory Animals and ARRIVE guidelines.

Consent

The authors have nothing to report.

Conflicts of Interest

The authors declare no conflicts of interest.

Data Availability Statement

All data and materials are available by direct request.

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