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Research Article

Assessing the Nutritional and Functional Profiles of Formulated Kodo Millet Sourdough Bread

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Abstract

This study developed and evaluated a kodo millet flour (*Paspalum scrobiculatum*) sourdough bread formulation incorporating 15% (w/w) kodo millet in a wheat flour base. A mixed lactic acid bacteria starter culture was prepared over seven days at 25°C using a 1:4:4 (starter: water: flour) daily feeding regime and validated by the float test. The bread was evaluated for proximate composition (IS 12711; AOAC Official Method 2001.11), mineral profile by Inductively Coupled Plasma Optical Emission Spectrometry (ICP-OES), total phenolic content, estimated glycemic index, textural properties by texture profile analysis (TPA) on a TA.HD Plus analyser, sensory acceptability using a 9-point hedonic scale with 40 panelists over three consecutive days, microbial counts (ISO 4833:2003; ISO 21527-1:2008; ISO 15214:1998), and volatile compound profile by Solid Phase Micro Extraction gas chromatography–mass spectrometry (SPME-GC-MS; PerkinElmer Clarus SQ8C). Statistical analysis employed one-way analysis of variance (ANOVA) followed by Tukey's b post-hoc test (IBM SPSS Statistics Version 2022; $p < 0.05$; $n = 3$). The kodo millet bread showed significantly enhanced protein (8.36 ± 0.54 g/100 g), carbohydrate (52.00 ± 0.20 g/100 g), and calorific value (247.19 ± 1.64 kcal/100 g) compared to the control, which was made using all-purpose flour (APF). ICP-OES revealed 1.3×, 2.9×, and 2.1× higher magnesium, iron, and zinc respectively, attributable to kodo millet's inherent mineral density and lactic acid bacteria-mediated phytate degradation. The estimated glycemic index was reduced by 17.5% (50.18 to 41.39) in the kodo millet formulation relative to the control, classifying both as low-GI foods. The lactic acid bacteria-dominated starter culture achieved 6.41 ± 0.06 log CFU/g. SPME-GC-MS identified linoleic acid (9.4%), 3-methyl-2-butenoic acid cyclobutyl ester (17.1%), and fermentation-derived esters as the dominant volatile constituents. Sensory scores were acceptable (overall acceptability 6.4/9). The study confirms kodo millet sourdough bread as a nutritionally superior functional food aligned with sustainable agriculture and consumer health trends.

Keywords: Kodo Millet; Sourdough Bread; Lactic Acid Bacteria; Fermentation; GC-MS; Texture Profile Analysis; ICP-OES; *Paspalum scrobiculatum*

Abbreviations

The following abbreviations are used in this article. Note: To avoid ambiguity with Total Phenolic Content (TPC, Sections 2.6 and 3.4), the aerobic microbial plate count (Section 2.10 and Table 9) is denoted APC (Aerobic Plate Count) throughout, in place of the original 'TPC' label used for that assay.

LAB: Lactic Acid Bacteria; GI: Glycemic Index; eGI: Estimated Glycemic Index; TPC: Total Phenolic Content; APC: Aerobic Plate Count; YGC: Yeast and Mould Count (Yeast extract Glucose Chloramphenicol agar); MRS: de Man, Rogosa and Sharpe agar (used for lactic acid bacteria enumeration); ICP-OES: Inductively Coupled Plasma Optical Emission Spectrometry; SPME-GC-MS: Solid Phase Micro Extraction Gas Chromatography–Mass Spectrometry; TPA: Texture Profile Analysis; ANOVA: Analysis of Variance; FSSAI: Food Safety and Standards Authority of India; APF: All-Purpose Flour; KMF: Kodo Millet Flour; RDA: Recommended Dietary Allowance; EPS: Exopolysaccharides; FODMAP: Fermentable Oligosaccharides, Disaccharides, Monosaccharides and Polyols; GAE: Gallic Acid Equivalents; IBS: Irritable Bowel Syndrome; CFU: Colony Forming Units; PCA: Plate Count Agar; HI: Hydrolysis Index; AUC: Area Under the Curve; GABA: Gamma-Aminobutyric Acid; w/w: Weight by Weight.

Introduction

Sourdough bread is the product of spontaneous co-fermentation of flour and water by indigenous wild yeasts and lactic acid bacteria (LAB), a biological process that confers a characteristic acidic flavour and improved crumb texture while simultaneously extending shelf life and enhancing nutritional digestibility relative to baker's yeast-leavened counterparts [1]. Globally, the sourdough market was valued at US$3.30 billion in 2023 and is projected to grow at a compound annual growth rate (CAGR) of 7.2% through 2030, reflecting rising consumer demand for clean-label and preservative-free bakery products [2]. In India, urban health-conscious consumers are increasingly driving adoption of artisanal sourdough, with growing interest in natural, preservative-free foods across major cities including those in Tamil Nadu and Kerala.

The nutritional superiority of sourdough over conventional yeast-leavened bread is well established. LAB-driven acidification during sourdough fermentation activates endogenous cereal phytases and microbial phytases that collectively hydrolyse up to 90% of phytic acid, substantially elevating the bioaccessibility of iron, zinc, and magnesium [3]. The resulting glycemic index (GI 53–68) is markedly lower than that of conventional bread (GI 70+), a reduction driven by organic acid-mediated slowing of starch digestion kinetics [4]. Prolonged fermentation reduces FODMAP content by 30–50% through microbial carbohydrate catabolism, alleviating gastrointestinal symptoms in IBS-affected individuals [5], while generating bioactive peptides, prebiotic oligosaccharides, and phenolic antioxidants such as ferulic acid [6]. Natural acidification to pH 3.8–4.5 extends the shelf life of sourdough bread to 5–7 days in the absence of synthetic preservatives, compared to 2–3 days for conventionally leavened products [7]. The sourdough microbial consortium, predominantly lactic acid bacteria, acts in concert to improve dough rheology, develop aroma complexity, and strengthen crumb architecture through the coordinated production of organic acids, exopolysaccharides (EPS), and antimicrobial metabolites [8,9].

Kodo millet (Paspalum scrobiculatum) is an ancient drought-tolerant cereal grain cultivated predominantly across India and sub-Saharan Africa, increasingly recognised as a functional food ingredient owing to its high dietary fibre (8–11%), resistant starch (5–7%), essential minerals (iron, calcium, magnesium), and antioxidant polyphenols comprising flavonoids and ferulic acid [10]. Its inherently gluten-free nature, combined with its nutraceutical profile encompassing anti-inflammatory polyphenols and cholesterol-modulating properties, positions it as a candidate ingredient for dietary management of diabetes, obesity, and cardiovascular conditions [11]. Sourdough fermentation further amplifies its nutritional value: specific LAB strains including *Lactobacillus plantarum* degrade 60–70% of phytates, while the flour's inherent polyphenols show 40–50% increased antioxidant activity during fermentation. Despite its nutritional potential, kodo millet's absence of gluten necessitates specialised processing approaches, such as the use of LAB-produced exopolysaccharides as natural binders.

In terms of food safety, sourdough bread production in India must comply with Food Safety and Standards Authority of India (FSSAI) regulations mandating absence of *Salmonella* in 25 g samples, yeast/mould counts ≤100 CFU/g, pH 3.8–4.5, and aflatoxin B1 ≤5 µg/kg. Clear labelling, including 'no baker's yeast' declarations, is required for authentic sourdough products, and

revised 2023 guidelines mandate heavy metal testing from high-risk agricultural regions [12,13].

The objectives of this study were: (i) to styandardize kodo millet flour-incorporated sourdough bread formulation using a mixed lactic acid bacteria starter culture; (ii) to study the impact of the starter culture on volatile compounds in the sourdough crumb by GC-MS; and (iii) to analyse the physicochemical properties, textural characteristics, sensory acceptability, and microbial safety of the developed product.

Materials and Methods

Materials

Kodo millet flour, wheat flour, and all-purpose flour were procured from a local departmental store in Coimbatore, Tamil Nadu, India. This study was conducted at the Centre for Post Harvest Technology, Agricultural Engineering College and Research Institute, Tamil Nadu Agricultural University (TNAU), Coimbatore in 2025. Analytical-grade chemicals including sulfuric acid, petroleum ether, and sodium hydroxide were sourced from local chemical suppliers. Microbial growth media – Plate Count Agar (PCA), Yeast Extract Glucose Chloramphenicol Agar (YGC), and de Man, Rogosa and Sharpe (MRS) Agar – were used for microbial analysis. Low Density Poly Ethylene (LDPE) packaging was used for shelf life studies. All chemicals and reagents were of analytical reagent grade.

Preparation of starter culture (Levain)

The sourdough starter was prepared by combining water, all-purpose flour, and wheat flour in a 2:1:1 ratio (840 g water, 420 g all-purpose flour, 420 g wheat flour) (Table 1). The mixture was transferred to a sterilized glass bottle and fermented at 25°C for 24 hours. After confirming bubble formation, half the mixture was re-fed daily at a 1:4:4 ratio (starter:water:flour) for seven days. On day seven, the starter was assessed using the float test. The final starter exhibited a bubbly texture, tangy aroma, and thick pourable consistency with visible gas pockets, confirming vigorous microbial activity.

Preparation of Kodo millet sourdough bread

Kodo millet flour was incorporated at 5%, 10%, and 15% (w/w); 15% substitution was selected for standardisation based

Ingredient	Weight (g)
Wheat flour	420
All-purpose flour	420
Water	840

Table 1: Ingredients for starter culture (Levain) preparation.

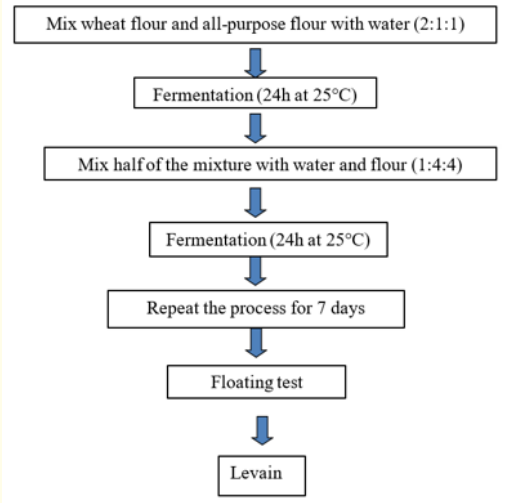

Figure 1: Preparation of starter culture (Levain) – flowchart.

Figure 2: Starter culture (Levain) on day seven.

on superior nutritional and rheological performance. The final formulation comprised 212 g wheat flour, 38 g kodo millet flour (15% w/w), 175 g water, 50 g active starter, and 5 g salt (Table 2). The preparation procedure comprised: (i) combining water with levain until fully mixed; (ii) incorporating both flours thoroughly; (iii) resting covered for 30–45 min; (iv) adding salt by surface-folding and pinching to ensure thorough distribution; (v) four stretch-and-fold cycles at 30-minute intervals to develop gluten structure; (vi) bulk fermentation for one hour with 20–30% volume increase; (vii) pre-shaping followed by 5–10 min bench rest; (viii) final shaping and cold retard proof at refrigeration temperature for 1–2 hours; and (ix) baking at 200°C steam-covered for 30 min, then uncovered for 30 min. Dough temperature was maintained at 24.4–27.7°C throughout fermentation.

Ingredient	Weight (%)
Wheat flour	44.17
Kodo millet flour	7.92
Water	36.46
Starter (Levain)	10.42
Salt	1.04

Table 2: Final ingredients for 15% kodo millet sourdough bread formulation.

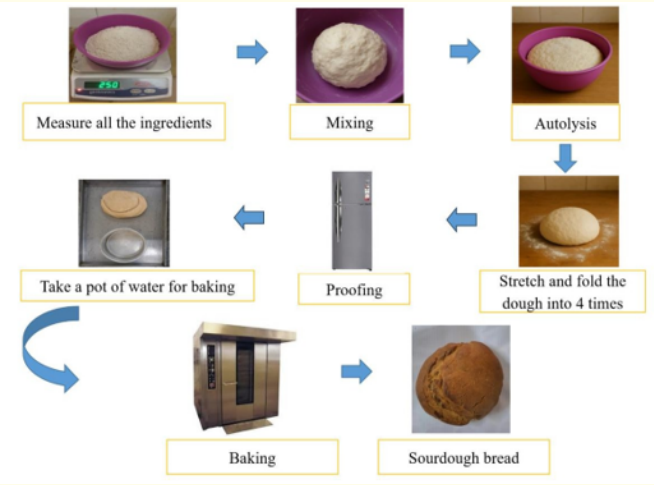

Figure 3: Preparation flowchart for kodo millet sourdough bread.

Figure 4: Final kodo millet sourdough bread.

Proximate analysis

Proximate composition was determined in triplicate. Moisture content was determined gravimetrically by oven-drying at 105 ± 2°C to constant weight (IS 12711). Ash content was determined by incineration at 550 ± 25°C for 4–6 h in a muffle furnace (IS 12711). Crude protein was determined by the Dumas combustion method (AOAC Official Method 2001.11) using a nitrogen-to-protein conversion factor of 6.25. Fat content was determined by Soxhlet extraction with petroleum ether (40–60°C) for 6 h (IS 12711). Crude fibre was determined by sequential acid–alkali digestion: approximately 2 g of defatted sample was refluxed with 0.255 N H_2SO_4 and 0.313 N NaOH for 30 min each, filtered through a pre-weighed sintered glass crucible, dried at 105°C, and ashed at 550°C (IS 12711). Carbohydrate content was estimated by difference as per IS 1656: Carbohydrate (%) = 100 − [%Moisture + %Protein + %Fat + %Ash + %Crude Fibre]. Calorific value was calculated by the (DGHS Lab Manual 2005) method (4 kcal/g for carbohydrate and protein; 9 kcal/g for fat) and expressed as kcal/100 g.

Mineral analysis

Mineral composition including magnesium (Mg), zinc (Zn), potassium (K), iron (Fe), and calcium (Ca) was determined by ICP-OES. Samples were acid-digested prior to analysis. All mineral concentrations are reported as mg/100 g (mean ± SD, n = 3).

Tannin content and total phenolic content (TPC) determination

Total phenolic content, inclusive of tannin fractions, was determined using the Folin-Ciocalteu colorimetric method [14]. A methanolic extract was prepared from each bread sample, and an aliquot was reacted with Folin-Ciocalteu reagent under alkaline

conditions (sodium carbonate solution). Following incubation in the dark, absorbance was measured at 760 nm against a gallic acid standard curve. Results were expressed as milligrams of Gallic Acid Equivalents per gram of sample (mg GAE/g), with all determinations performed in triplicate (n = 3).

Estimated Glycemic determination

The estimated glycemic index (eGI) of each bread sample was determined using an *in vitro* starch hydrolysis procedure adapted from Goñi., *et al*. [15]. Approximately 44 mg of homogenized bread crumb was weighed into a 30 mL Erlenmeyer flask and cooked in an autoclave at 120°C for 30 minutes with 4 mL of distilled water. After cooling, 10 mL of HCl-KCl buffer (pH 1.5) was added and the sample was homogenized for 2 minutes using an Ultra Turrax T18 homogeniser (IKA India Pvt. Ltd., India). A 0.2 mL aliquot of pepsin solution (22 mg porcine pepsin in 10 mL HCl-KCl buffer) was then added, and the mixture was incubated at 40°C for 60 minutes in a shaking water bath (Reico Equipment and Instruments Pvt. Ltd., India). Tris-Maleate buffer (pH 6.9) was subsequently added to bring the volume to 25 mL, followed by 5 mL of α-amylase solution (2.6 IU α-amylase in Tris-Maleate buffer) to hydrolyse the starch fraction. Samples were incubated at 37°C in a shaking water bath, and 0.1 mL aliquots were withdrawn at 30-minute intervals from 0 to 180 minutes, immediately heated in boiling water for 5 minutes to inactivate the enzyme. Each aliquot was then incubated at 60°C for 45 minutes with 3 mL of 0.4 M sodium acetate buffer (pH 4.75) and 60 µL of amyloglucosidase (*Aspergillus niger*) to convert the hydrolysed starch to glucose. Glucose concentration was determined using Benedict's method, and total starch was calculated by multiplying glucose concentration by a conversion factor of 9.5. Each sample was analysed in triplicate. The percentage of starch hydrolysed at each time point (0–180 min) was fitted to the non-linear model $C = C\infty(1 - e^{-kt})$, where C is the percentage hydrolysed at time t, C∞ is the percentage hydrolysed at 180 minutes, and k is the kinetic constant. The area under the hydrolysis curve (AUC) was calculated as $AUC = C\infty(tf - t0) - (C\infty/k)[1 - \exp(-k(tf - t0))]$, where t0 and tf are the initial (0 min) and final (180 min) time points. The Hydrolysis Index (HI) was obtained by dividing each sample's AUC by that of a white bread reference. The eGI was then calculated as $eGI = 39.71 + (0.549 \times HI)$.

Textural properties

Texture Profile Analysis (TPA) was conducted using a TA.HD Plus texture analyser fitted with a P/36R probe (36 mm cylindrical aluminium probe) at 1 mm/s test speed and 50% strain. Uniform bread slices (25 mm thickness) were equilibrated at 25 ± 1°C for 60 min prior to testing. Parameters measured included compression force (g), hardness (N), compression area (N·mm), springiness (mm), cohesiveness, gumminess (Hardness × Cohesiveness, N), chewiness (Hardness × Cohesiveness × Springiness, mJ), and resilience. A minimum of five replicates were analysed per batch.

Sensory evaluation

Sensory evaluation was conducted with 40 untrained panelists (ages 25–50) using a 9-point hedonic scale (1 = dislike extremely; 9 = like extremely) over three consecutive days to assess consistency [16]. Freshly prepared samples at room temperature were evaluated under controlled lighting for appearance, colour, flavour, texture, taste, and overall acceptability.

Microbial analysis

Aerobic Plate Count (APC) was determined on PCA at 30°C for 72 h per ISO 4833:2003 [17]. Yeast and mould count was determined on YGC agar at 25 ± 1°C for 5–7 days per ISO 21527-1:2008 [18]. LAB enumeration was performed on MRS agar supplemented with 0.1% cycloheximide under anaerobic conditions at 30 ± 1°C for 72 h per ISO 15214:1998 [19]. Results are expressed as log CFU/g. Only plates with 30–300 colonies (bacteria) or 15–150 colonies (yeast/mould) were accepted for enumeration.

GC-MS analysis of volatile compounds

Volatile compound profiling was performed by SPME-GC-MS on a PerkinElmer Clarus SQ8C instrument. Compounds were identified by comparison of mass spectra against the NIST Mass Spectral Library and quantified as relative peak area (%).

Statistical analysis

All experiments were conducted in triplicate (n = 3) and results are expressed as mean ± standard error (SE) or mean ± standard deviation (SD), as indicated. Statistical analysis employed one-way analysis of variance (ANOVA) followed by Tukey's b post-hoc test (IBM SPSS Statistics, Version 2022; $p < 0.05$). Values with different superscript letters within a column were considered significantly different at $p < 0.05$, with differing superscript letters indicating statistically significant pairwise differences.

Results and Discussion

Starter culture development

The sourdough starter culture was successfully established over seven days of daily feeding at a 1:4:4 ratio. By day seven, the culture exhibited a bubbly texture, slightly tangy aroma, visible gas pockets, and a significantly reduced pH, all indicators of a healthy, active microbial population dominated by lactic acid bacteria. The culture passed the float test, confirming adequate leavening potential for bread production. This spontaneous fermentation approach is consistent with classical sourdough development, in which lactic acid bacteria progressively dominate the microbial ecosystem through competitive acidification, creating the stable fermentation environment required for consistent bread quality [9].

Proximate composition

The proximate composition of the control all-purpose flour (APF) sourdough bread and the 15% kodo millet-incorporated sourdough bread is presented in Table 3. Significant differences were observed across all parameters between the two formulations.

S. No.	Parameter	Control Sourdough Bread	15% Kodo Millet Sourdough Bread
1	Moisture (g/100 g)	44.10 ± 0.18	35.40 ± 0.19
2	Carbohydrates (g/100 g)	46.14 ± 0.18	52.00 ± 0.20
3	Protein (g/100 g)	6.72 ± 0.17	8.36 ± 0.54
4	Fat (g/100 g)	0.46 ± 0.07	0.82 ± 0.03
5	Ash (g/100 g)	2.42 ± 0.08	3.48 ± 0.20
6	Crude Fibre (g/100 g)	0.84 ± 0.07	0.54 ± 0.06
7	Calorific Value (kcal/100 g)	216.52 ± 1.60	247.19 ± 1.64

Table 3: Proximate composition of control and 15% kodo millet sourdough bread (mean ± SE, n = 3).

Values are mean ± SE, n = 3.

Moisture content was significantly lower in the kodo millet bread (35.40 ± 0.19 g/100 g) compared to the all-purpose flour control (44.10 ± 0.18 g/100 g). This reduction arises because kodo millet's high insoluble fibre content reduces water-binding capacity relative to the gluten-starch network of all-purpose flour, consistent with the hydrophobic character of millet fibres and their physical interference with the continuous water-absorbing gluten matrix [20]. The lower moisture may extend shelf life through reduced water activity, though texture adjustments may be required to prevent excessive crumb dryness. Comparable moisture reductions with millet incorporation have been reported for bread made from kodo, little and foxtail millets [21], for finger millet-substituted wheat bread, where moisture content fell significantly with increasing substitution level [22], and for wheat–millet–sorghum composite bread, where moisture declined across all substitution levels relative to the wheat-only control [23].

Carbohydrate content increased from 46.14 ± 0.18% in the APF control to 52.00 ± 0.20% in the kodo millet variant. This increase is primarily attributable to kodo millet's high starch content (60–70%), substantially greater than that of all-purpose flour [20]. Importantly, the nature of carbohydrates shifts towards nutritionally superior components: kodo millet contributes complex dietary fibres and resistant starch rather than rapidly digestible simple starches, contributing to a lower glycemic response despite elevated total carbohydrate values [4].

Protein content increased from 6.72 ± 0.17% in the APF control to 8.36 ± 0.54% in the kodo millet bread, consistent with kodo millet's documented protein content of 8–11% [10,24]. This enhancement occurs because kodo millet proteins, including prolamins and glutelins, supplement the protein contributed by wheat flour. Additionally, LAB proteolytic activity during fermentation partially hydrolyses storage proteins, potentially releasing bioactive peptides that improve the functional protein fraction [6]. This direction of change parallels other composite breads, where incorporation of an alternative grain flour increased crumb protein content relative to the refined-wheat control [25].

Fat content increased from 0.46 ± 0.07% in the APF control to 0.82 ± 0.03% in the kodo millet variant, consistent with kodo millet's documented fat content of 1.3–2.6% [26]. Kodo millet's lipid fraction, rich in unsaturated fatty acids including linoleic

acid, contributes to the higher fat content and is associated with improved flavour perception and mouthfeel. These lipids are consistent with the GC-MS-identified linoleic acid volatile profile.

Ash content increased from 2.42 ± 0.08% to 3.48 ± 0.20%, directly reflecting kodo millet's superior micronutrient content, including iron, calcium, and zinc [10,27]. Calorific value increased from 216.52 ± 1.60 to 247.19 ± 1.64 kcal/100 g, arising from the combined effect of higher carbohydrate and fat content. Crude fibre decreased from 0.84 ± 0.07% to 0.54 ± 0.06%, consistent with reports that LAB-driven fermentation lowers dietary fibre content through enzymatic breakdown of the fibre structure, which may reflect partial degradation of cell wall polysaccharide fractions during prolonged sourdough fermentation [28].

Mineral profile analysis

The ICP-OES mineral analysis revealed substantial nutritional advantages of the kodo millet bread over the APF control (Table 4).

S. No.	Mineral	Control Sourdough Bread	15% Kodo Millet Sourdough Bread
1	Magnesium (mg/100 g)	82.06 ± 2.4	108.92 ± 6.7
2	Zinc (mg/100 g)	4.52 ± 0.6	9.30 ± 1.4
3	Potassium (mg/100 g)	83.04 ± 6.2	103.85 ± 19.5
4	Iron (mg/100 g)	2.73 ± 1.2	7.78 ± 2.9
5	Calcium (mg/100 g)	26.00 ± 7.1	27.32 ± 10.3

Table 4: Mineral profile comparison – control vs. 15% kodo millet sourdough bread (mg/100 g, mean ± SD).

Values are mean ± SD from ICP-OES analysis.

The dramatic improvement in mineral content is attributable to two complementary mechanisms. First, kodo millet inherently contains substantially higher concentrations of these minerals compared to refined all-purpose flour, and their direct incorporation transfers this mineral density into the bread matrix [10,27]. Second, the sourdough fermentation process promotes mineral bioavailability through LAB-mediated phytate degradation: lactic acid bacteria strains produce phytases that hydrolyse phytic acid–mineral complexes, releasing previously bound minerals into a bioaccessible form [3]. A single 100 g serving of kodo millet sourdough bread provides approximately 26% of the daily magnesium requirement (RDA: 420 mg), 43% of iron (RDA: 18 mg), and 85% of zinc (RDA: 11 mg), compared to 19%, 15%, and 41% respectively from the all-purpose flour control bread [27]. Comparable mineral enrichment following incorporation of small millets and sorghum into wheat-based bread formulations has been reported elsewhere, with magnesium, calcium, iron and zinc levels all elevated over the wheat-only control [23].

Tannin content and total phenolic profile

The total phenolic content (TPC) of the bread formulations, expressed as milligrams of Gallic Acid Equivalents per gram (mg GAE/g), is presented in Table 5. The control sourdough bread exhibited the highest measurable phenolic content, at 1.80 mg GAE/g. The 15% kodo millet sourdough bread showed a marginal reduction to 1.75 mg GAE/g. The observed downward trend in TPC, despite the incorporation of the polyphenol-rich kodo millet flour, reflects the biochemical transformation of phenolic compounds during sourdough fermentation. LAB-produced esterases and phenolic acid decarboxylases convert bound ferulic acid and condensed tannins into lower-molecular-weight hydroxycinnamic acid derivatives that retain high biological antioxidant activity [11]. This biotransformation phenomenon is well-documented in millet fermentation systems and should not be interpreted as a net loss of antioxidant capacity, since the bioavailability of converted phenolic metabolites substantially exceeds that of the native bound forms [29]. A comparable reduction in measurable phenolic/antioxidant content despite the addition of a phenolic-rich grain has been reported for other composite breads, where total phenolic content and antioxidant activity declined with increasing flour substitution as a result of processing-related phenolic transformation [25].

Formulation	TPC (mg GAE/g)
Control sourdough bread	1.80
15% kodo millet sourdough bread	1.75

Table 5: Tannin/Total Phenolic Content of sourdough bread formulations (mg GAE/g).

Glycemic index

The glycemic index (GI) values of the bread formulations are presented in Table 6. Both formulations qualify as low-GI foods

(GI ≤ 55). The control sourdough bread achieved a GI of 50.18, attributable to the organic acid-mediated reduction in starch digestibility inherent to sourdough fermentation [4]. Substituting 15% wheat flour with kodo millet reduced the GI substantially, to 41.39, a 17.5% reduction, attributable to kodo millet's high dietary fibre, resistant starch, and polyphenol content acting as physical barriers around starch granules and competitively inhibiting digestive enzymes including α-amylase and α-glucosidase [30]. These GI values are significant for individuals with type 2 diabetes and metabolic syndrome [30]. This magnitude of reduction is consistent with a systematic review and meta-analysis of millets, which found that intermediate-glycemic-index millets – including kodo millet specifically – register 13–35% lower glycemic index values than high-GI cereal controls such as refined wheat [31].

Formulation	Glycemic Index
Control sourdough bread	50.18
15% kodo millet sourdough bread	41.39

Table 6: Glycemic Index of sourdough bread formulations.

Textural properties

The TPA revealed notable differences between the two bread variants (Figure 5).

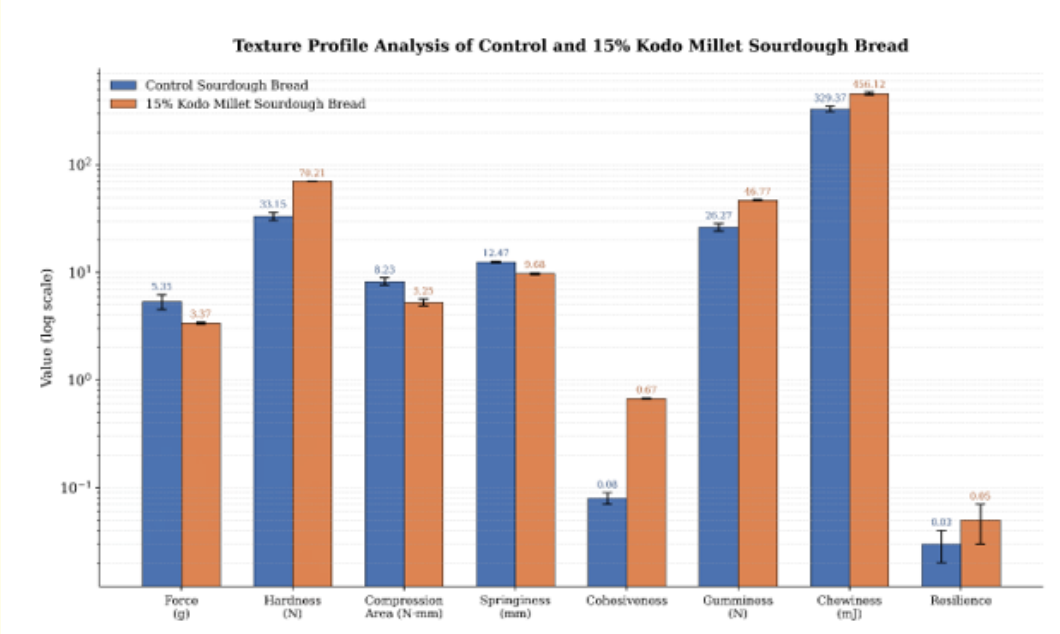

Figure 5: Texture Profile Analysis – control vs. 15% kodo millet sourdough bread.

Values are mean ± SE, n = 5. Hardness and gumminess are expressed in Newtons (N); springiness in mm; chewiness in mJ.

Compression force decreased in the kodo millet bread (3.37 ± 0.06 g vs. 5.35 ± 0.86 g for the control), suggesting that millet fibre–gluten interactions produced an initially softer surface structure. However, hardness increased dramatically (70.21 ± 0.19 N vs. 33.15 ± 2.92 N for the control), attributable to kodo millet's high amylose content and pronounced starch retrogradation during cooling, in which amylose chains reassociate into ordered crystalline structures creating a denser, harder crumb matrix [32]. Springiness declined (9.68 ± 0.22 mm vs. 12.47 ± 0.23 mm for the control) because kodo millet's gluten-free proteins lack

the elastic network that allows wheat-based crumbs to recover after deformation [33]. Cohesiveness improved (0.67 ± 0.01 vs. 0.08 ± 0.01 for the control), attributable to kodo millet's water-absorbing fibres creating a more uniformly bonded internal crumb structure. Gumminess increased (46.77 ± 0.66 N vs. 26.27 ± 2.10 N for the control) due to millet's particulate insoluble fibre content. Chewiness increased accordingly (456.12 ± 14.11 mJ vs. 329.37 ± 19.59 mJ), since chewiness is the product of hardness, cohesiveness, and springiness; the substantial gains in hardness and cohesiveness outweighed the moderate decline in springiness, driving net chewiness upward. Resilience slightly improved (0.05 ± 0.02 vs. 0.03 ± 0.01), consistent with starch gelatinisation-driven elastic recovery [34]. The direction of these textural shifts mirrors other millet- and grain-substituted breads, where hardness increased and springiness declined with cereal substitution [23], and where hardness, gumminess, cohesiveness and chewiness rose together with alternative-grain flour incorporation [25].

Sensory evaluation

Sensory scores across three consecutive evaluation days for both bread formulations are presented in Table 8. APF = All-Purpose Flour control; KMF = Kodo Millet Flour (15% substitution).

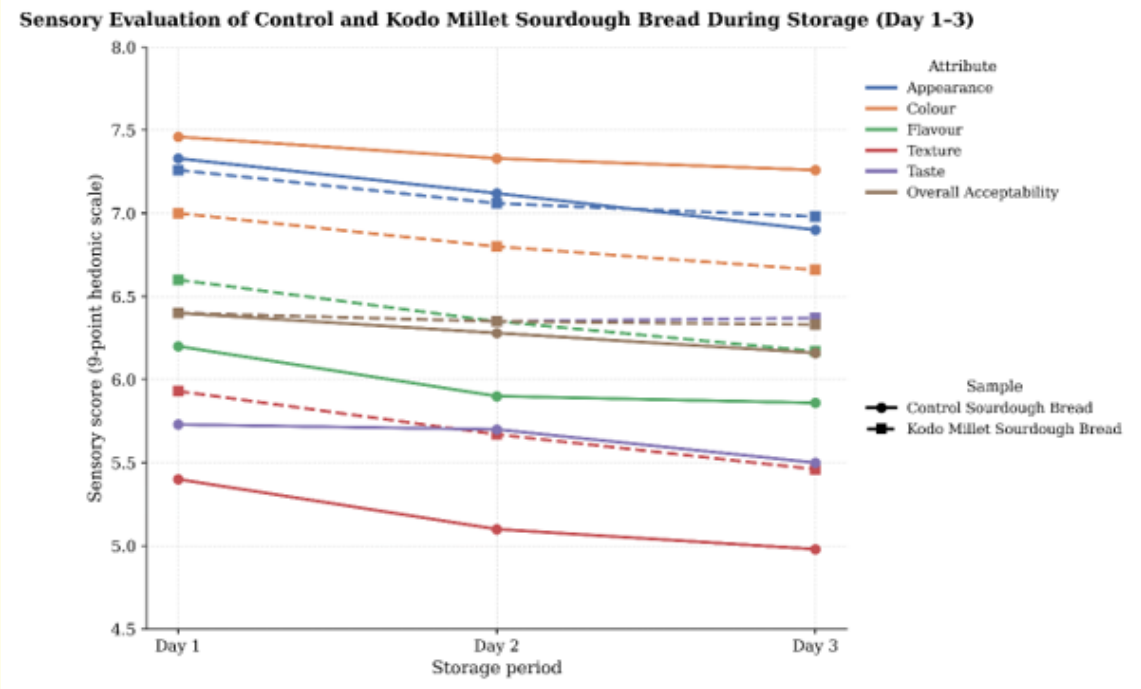

Figure 6: Sensory evaluation scores (9-point hedonic scale; control and kodo millet sourdough bread; n = 40 panelists).

Scores on 9-point hedonic scale: 1 = dislike extremely; 9 = like extremely [16].

The kodo millet bread demonstrated more balanced and consistent sensory performance across the three-day evaluation period. The APF control showed a progressive decline in most attributes from Day 1 to Day 3, while the kodo millet variant maintained more stable scores, particularly for taste (6.40–6.37 across three days) and overall acceptability (6.40–6.33). Flavour scores were higher in the kodo millet bread (6.60 vs. 6.20 on Day 1), indicating that sourdough fermentation effectively modulated kodo millet's natural astringency through microbial conversion of phenolic compounds into less bitter metabolites, while developing desirable nutty and umami notes through LAB-mediated amino acid catabolism and organic acid production [4]. Texture scores were consistently lower than other attributes in both variants, reflecting the textural challenge observed in the texture profile

analysis, particularly the elevated hardness. Despite this, overall acceptability scores were equivalent at Day 1 (6.40 for both) and remained stable for the kodo millet bread through Day 3 (6.33), demonstrating consistent consumer acceptance of the product profile.

Microbial analysis

Microbial counts across the starter culture, fermented dough, and final baked bread are presented in Table 7.

Sample	APC (log CFU/g)	YGC (log CFU/g)	MRS/LAB (log CFU/g)
Culture	6.43 ± 0.05a	2.69 ± 0.03c	6.41 ± 0.06a
Dough	5.25 ± 0.06b	4.12 ± 0.05a	5.21 ± 0.05b
Bread	6.42 ± 0.08a	3.33 ± 0.05b	3.34 ± 0.07c

Table 7: Microbial analysis of starter culture, dough, and sourdough bread (log CFU/g, mean ± SE, n = 3).

APC = Aerobic Plate Count (PCA, ISO 4833:2003 [17]); YGC = Yeast and mould count (YGC agar, ISO 21527-1:2008 [18]); MRS/LAB = Lactic acid bacteria count (MRS agar, ISO 15214:1998 [19]). Different superscripts within each column indicate significant differences (Tukey's b, $p < 0.05$).

APC results showed consistently high microbial loads in both the starter culture (6.43 ± 0.05 log CFU/g) and final bread (6.42 ± 0.08 log CFU/g), with a moderate reduction in the dough phase (5.25 ± 0.06 log CFU/g). The persistence of high APC through baking indicates the presence of heat-resistant strains and bacterial endospores that survive the baking process, consistent with thermal resistance patterns documented in traditional sourdough systems [9]. YGC counts peaked in the dough phase (4.12 ± 0.05 log CFU/g), reflecting active yeast proliferation during bulk fermentation when substrate availability is high. The subsequent decline in the final bread (3.33 ± 0.05 log CFU/g) reflects the thermal sensitivity of most yeast strains to baking temperatures [6]. LAB counts decreased substantially but incompletely, from 6.41 ± 0.06 log CFU/g in the culture to 3.34 ± 0.07 log CFU/g in the final bread, with active metabolic activity maintained at 5.21 ± 0.05 log CFU/g during dough fermentation. This partial LAB survival through baking may contribute to continued production of antimicrobial compounds during storage [9]. Final YGC counts (3.33 log CFU/g) approach the FSSAI limit of ≤3 log CFU/g, which is characteristic of traditional sourdough and is associated with competitive inhibition of spoilage organisms rather than a food safety concern [12]. This pattern of substantial but incomplete LAB and yeast attenuation during baking is consistent with reported sourdough systems, in which LAB counts have been shown to fall from approximately 8.8 to 4–5 log CFU/g and yeast counts from approximately 9 to 4 log CFU/g over the course of baking [35].

GC-MS analysis of volatile compounds

The SPME-GC-MS volatile profile of the kodo millet sourdough bread crumb identified a diverse range of fermentation-derived compounds. Major constituents are presented in Table 8.

S. No.	Compound	Chemical Class	Relative Peak Area (%)
1	3-Methyl-2-butenoic acid, cyclobutyl ester	Ester	17.1
2	5-Dodecen-7-yne	Alkyne/hydrocarbon	16.1
3	Linoleic acid	Fatty acid	9.4
4	Dipropyl phthalate	Ester	8.8

Table 8: Major volatile compounds identified in kodo millet sourdough bread by SPME-GC-MS (relative peak area, %).

Compounds identified against NIST Mass Spectral Library; quantified as relative peak area (%).

The dominance of esters in the volatile profile arises from esterification reactions between organic acids produced by LAB and alcohols released by yeasts during the prolonged sourdough fermentation, producing characteristic fruity and complex aroma notes. This ester and hydrocarbon dominance is consistent with reported whole-grain sourdough volatile profiles, in which esters and hydrocarbons were identified among the major aroma compound classes generated during fermentation [36]. The presence of linoleic acid (9.4%) is consistent with kodo millet's lipid profile, which is rich in polyunsaturated fatty acids; its detection in the bread volatile fraction suggests thermal-oxidative release during baking. Millet-based sourdoughs have been reported to

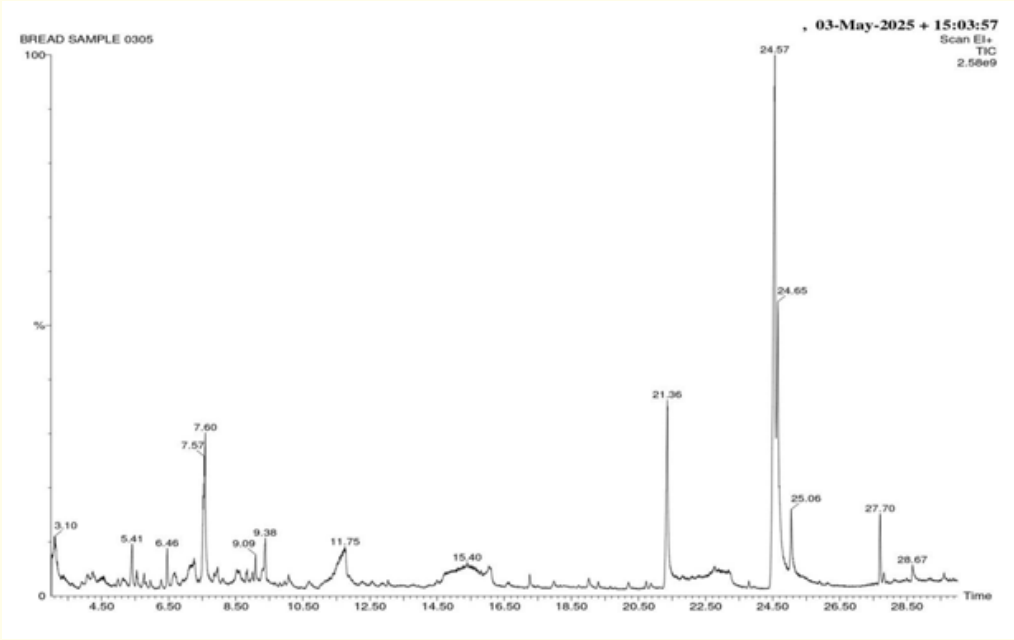

Figure 7: GC-MS chromatogram of volatile compounds in kodo millet sourdough bread crumb.

contain up to 2.5 times more flavonoids than wheat sourdough [10]. The gamma-aminobutyric acid (GABA) content in kodo millet sourdough has been estimated at 28.67 mg/100 g, exceeding wheat (15–20 mg/100 g) but remaining slightly below rye (30–35 mg/100 g), as documented in quinoa sourdough systems employing similar LAB consortia [37]. The identification of diverse ester classes confirms effective fermentation kinetics and validates the starter culture's contribution to the complex aroma profile of the kodo millet sourdough bread.

Conclusion

This study successfully demonstrated the production of a nutritionally superior sourdough bread incorporating kodo millet flour at 15% (w/w) substitution. The kodo millet variant exhibited significantly enhanced protein content (8.36 g/100 g), calorific value (247.19 kcal/100 g), and meaningfully improved mineral concentrations – magnesium (1.3× higher), iron (2.9× higher), zinc (2.1× higher), and potassium (1.3× higher) – compared to the all-purpose flour control. These improvements are attributable to the intrinsically superior mineral density of kodo millet and the phytate-degrading activity of the LAB-dominated starter culture.

The traditional sourdough fermentation process, validated by a robust starter culture dominated by LAB at 6.41 log CFU/g, effectively enhanced mineral bioavailability and generated a distinctive volatile compound profile characterised by linoleic acid (9.4%) and diverse fermentation-derived esters confirmed by SPME-GC-MS. Sensory evaluation confirmed consumer acceptability, with consistent overall scores of 6.4/9 over three evaluation days. While the increased hardness (70.21 N vs. 33.15 N in the control) represents the primary textural challenge associated with millet incorporation, arising from kodo millet's high amylose content and pronounced starch retrogradation, all other sensory attributes were well-accepted. Glycemic index analysis confirmed both formulations as low-GI foods, with kodo millet incorporation reducing GI by 17.5% (50.18 to 41.39), supporting suitability for diabetic and metabolic health-conscious consumers.

These findings confirm kodo millet sourdough bread as a nutritionally superior functional food aligned with sustainable agriculture and evolving consumer health preferences. Future research could explore hydrocolloid-based strategies to optimise crumb texture, assess long-term shelf life under different packaging conditions, and investigate the specific LAB strains driving fermentation to develop optimised starter cultures for industrial-scale production.

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