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Effect of lactic acid bacteria fermentation and tigernut supplementation on the nutritional quality and microbial safety of *Kunun zaki* beverage

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Abstract: *Kunun zaki* is a traditional cereal-based fermented beverage widely consumed in West Africa; however, its nutritional quality and microbiological stability are often limited due to spontaneous fermentation. This study investigated the effects of controlled fermentation using defined lactic acid bacteria (LAB; *Lactobacillus acidophilus* and *Lactobacillus plantarum*) combined with graded tigernut supplementation (10–40%) on the amino acid composition and microbial quality of sorghum-based *kunun zaki* beverage. Five formulations were produced: a spontaneously fermented 100% sorghum (control) and four LAB-inoculated sorghum-tigernut blends. Amino acid profiles were determined and microbiological quality was assessed during 72 h of storage. Controlled LAB fermentation significantly improved the essential amino acid profile of *kunun zaki*, with notable increases in lysine, isoleucine, and valine in tigernut-enriched formulations ($p < 0.05$), indicating enhanced protein quality. Tigernut supplementation contributed to compositional complementation, further increasing essential amino acid density without disrupting the EAA/NEAA balance. LAB-fermented samples exhibited sustained LAB dominance (>5.5 $\log_{10}$ CFU/mL), absence or minimal detection of coliforms and fungi and improved microbial stability compared with the control during storage. Among the formulations, the 70% sorghum: 30% tigernut blend demonstrated the most balanced nutritional and microbiological profile. These findings demonstrate that integration of defined LAB starter cultures with optimized tigernut inclusion represents an effective biotechnology strategy for enhancing the nutritional value, microbial safety and storage stability of *kunun zaki*, thereby supporting its development as an improved functional beverage with commercialization potential.

Keywords: *Kunun zaki*, lactic acid bacteria, tigernut, amino acid

1 Introduction

Kunun zaki is an energy- dense beverage normally prepared from germinated cereals and is widely consumed in Nigeria, especially in the Northern part. *Kunun zaki* has thirst quenching properties and is therefore extensively consumed during the dry season, although it is consumed throughout the year (Adelekan et al., 2013). Millet, sorghum, and maize grains are the three principal cereals from which *kunun zaki* can be produced (Adeleke et al., 2004). It is usually flavored with such spices as ginger, black pepper, and tamarind for improvement in its taste and aroma, and also to serve as purgative and cure for flatulent conditions. It is a considerably cheap beverage drink because of the ingredients used for preparation, and this makes the product readily available (Makinde & Oyeleke, 2012). One of the most important cereals for the production of *kunun-zaki* is sorghum.

Sorghum is a major cereal crop in the semi-arid regions of Africa and Asia where it is used in the preparation of several traditional foods. It is more heat and drought resistant than most other cereals (Chavan et al., 2009). Sorghum is rich in fiber and minerals, apart from having a sufficient quantity of carbohydrates (72%), proteins (11.6%) and fat (1.9%). Starch is the major constituent of the grain. The protein in sorghum contains albumin globulin (15%), prolamin (26%) and glutelin (44%) (Chavan & Patil, 2010). Sorghum has good nutritional composition similar to rice and wheat in some aspects (Shobha et al., 2008). The grains contain high fiber and non-starchy polysaccharides and starch with

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some unique characteristics. There is a considerable variation in sorghum for levels of proteins, lysine, lipids, carbohydrates, fiber, calcium, phosphorus, iron, thiamine and niacin (Chavan et al., 2009).

Tigernut (*Cyperus esculentus L.*) is an underutilized tuber of family *Cyperaceae*. It is a tuber that grow freely and is consumed widely in Nigeria (Abaejoh et al., 2006). It has different names in Nigeria, as "Aya" in Hausa, "Imumu" in Yoruba and "Aki Hausa" in Igbo (Ogbuele et al., 2022). The tiger nut milk was been described as a functional beverage due to its high energy value (Abaejoh et al., 2006). Tiger nut is also rich in mineral content such as sodium, calcium, potassium, magnesium, zinc and traces of copper (Udeozor, 2012). The dietary fibre content of tiger nut is effective in the treatment and prevention of diseases such as colon cancer, coronary heart diseases, obesity, diabetes and gastro-intestinal disorders (Udeozor, 2012). It is thought that composting sorghum grain with tiger nut in *kunun zaki* fermented beverage production will enhance the nutritive, functional and bioactive properties.

Fermentation is the oldest form of food processing techniques. The fermentation process may last for 12–72 h. Brief fermentation, involving mainly lactic acid bacteria and yeast, usually occurs during steeping of the grains in water over 8–48 h (Gaffa & Ayo, 2002). Lactic acid bacteria (LAB) are generally regarded as safe (Elyas et al., 2015). They play an important function in the majority of food fermentations and preservations and an extensive diversity of strains are routinely used as starter cultures in the manufacture of bakery products, dairy, meat and vegetable (Gemechu, 2015). It is further used as starters in fermented dough, alcohol beverages, probiotic animal feeds and lactic acid fermentation of sorghum and maize-based cereals used as infant weaning foods (Wakil & Onilude, 2011).

Sorghum is mostly used in the production of *kunun zaki* in Nigeria, however the *kunun zaki* produced with sorghum is associated with low shelf life, low protein and other essential nutrients. As a result of the low nutritional value of this beverage, research efforts are required to ensure its improvement in nutritional quality. A good approach could be the exploitation of tiger nuts in complementing nutritional deficiency that is associated with *kunun* zaki. Although previous studies have reported the fermentation of cereal-based beverages using lactic acid bacteria and the nutritional potential of tigernut-based drinks separately (Obadina et al., 2013; Adebayo & Arinola, 2010), limited information exists on the combined application of defined LAB starter cultures and graded tigernut supplementation for improving the amino acid profile and microbial stability of sorghum-based *kunun zaki*. Most traditional *kunun zaki* production relies on spontaneous fermentation, which results in inconsistent quality, short shelf life, and variable nutritional composition (Gaffa & Ayo, 2002).

Furthermore, systematic evaluation of essential amino acid enhancement and microbial safety during controlled storage of LAB-fermented sorghum–tigernut blends remains underexplored. Therefore, the novelty of this study lies in the integration of defined starter cultures (*Lactobacillus acidophilus* and *Lactobacillus plantarum*) with graded tigernut inclusion (10–40%) to evaluate their synergistic effects on essential amino acid composition, microbial safety, and storage stability of *kunun zaki* beverage.

The graded tigernut inclusion levels (10–40%) were selected based on previous studies reporting acceptable sensory and nutritional performance of cereal–tigernut blends within this range (Adebayo & Arinola, 2010; Adeyuitan, 2011). Lower substitution levels (<10%) may produce minimal nutritional impact, whereas higher inclusion levels (>40%) may adversely affect viscosity and sensory properties. Therefore, graded inclusion was employed to determine the optimal balance between nutritional enhancement and product stability.

2 Materials and Methods

2.1 Source of Raw Materials

Sorghum, tiger-nut and spices were bought from Ubani Umuahia main market, Abia State. The lactic acid bacteria *Lactobacillus plantarium* and *acidophilus* were isolated from *kunun zaki* beverage.

2.2 Preparation of Lactic Acid Bacteria Inoculated Sorghum-Tigernut *Kunun Zaki* Beverage

The beverage was prepared according to the method described by Bolarinwa et al. (2015) with slight modifications. Sorghum grains (700 g) and tigernuts (400 g) were manually cleaned, weighed, and washed. Each was soaked separately in potable water for 12 h. After soaking, the grains and tigernuts were combined in different proportions and wet-milled into a slurry. Two-thirds of the slurry (2000 mL) was mixed with 2500 mL of boiling water and stirred continuously to form a gel. The mixture was allowed to cool for 3 h. The remaining one-third of the slurry (1000 mL) was then added to the gel, after which the mixture was cooled to 35°C for lactic acid bacteria (LAB) inoculation. Pure cultures of *Lactobacillus plantarum* and *Lactobacillus acidophilus* were individually activated and cultivated in MRS broth at 37°C for 18 h. The cultures were centrifuged to obtain the cell biomass, washed in sterile saline, and standardized to ~10^8 CFU/mL based on OD_{600} and confirmed by viable counts on MRS agar. The cooled *kunun zaki* slurry was inoculated with 1% (v/v) of the LAB suspension (i.e., 1 mL inoculum per 100 mL slurry). Fermentation was carried out at 37°C for 10 h, which falls within the optimum growth temperature range (30–37°C) for *L. plantarum* and *L. acidophilus*, ensuring efficient acid production and controlled fermentation.

After the fermentation, the beverage was sweetened with sucrose and flavored with selected spices (ginger) prior to analysis.

Five (5) formulations were prepared comprising a 100% sorghum (control) produced using traditional spontaneous fermentation without the addition of tiger nut or LAB starter cultures. The control underwent identical soaking, milling, heating and storage conditions as the experimental

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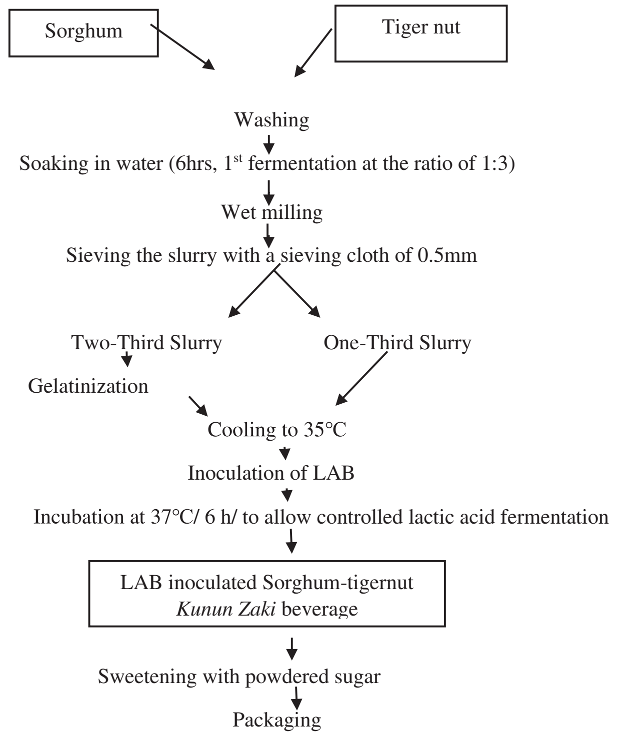

Figure 1. Flow chart for the production of Inoculated sorghum-tigernut *kunun zaki* beverage

samples. The remaining formulations consisted of sorghum–tigernut blends containing 10%, 20%, 30%, and 40% tigernut. The substitution range (10–40%) was selected to evaluate graded nutritional enhancement while maintaining acceptable physicochemical properties of the beverage. The production process of *Kunun-zaki* is illustrated in Figure 1.

2.3 Determination of Amino Acid Profile

The amino acid composition of the samples was determined using an Applied Biosystems PTH Amino Acid Analyzer (Benitze, 1989). Samples were dried to constant weight and defatted using chloroform/methanol (2:1, v/v) in a Soxhlet apparatus for 15 h (AOAC, 2006).

Defatted samples (~4–5 g) were hydrolyzed with 6 N HCl at 105 ± 5°C for 22 h under nitrogen to prevent oxidation of labile amino acids. Hydrolysates were filtered, evaporated under vacuum, and reconstituted in acetate buffer (pH 2.0).

Tryptophan, which is destroyed under acid hydrolysis, was determined separately using alkaline hydrolysis with 4.2 M NaOH at 105°C for 4 h (Maria et al., 2004) Hydrolysates were neutralized, evaporated under vacuum, and reconstituted in borate buffer (pH 9.0).

Norleucine was added as an internal standard prior to injection. Aliquots (60 µL) were injected into the amino acid analyzer, and amino acids were separated via ion-exchange chromatography. Quantification was performed using external standards, and results were expressed as g/100 g protein.

2.4 Microbial Analysis of the Inoculated *Kunun Zaki* Beverage During Storage

The samples were packaged and stored at room temperature for 3 days and collected daily for microbial analysis. A ten-fold serial dilution was prepared for the enumeration of microorganism present in the samples. Five grams of samples were blended for 2 min in 45 mL sterile normal saline using a stomacher Laboratory Blender (Model 500 JAC, London, UK). Aseptically, 1 mL from the original suspension was transferred using a sterile pipette into a sterile test tube containing 9 mL of sterile peptone normal saline water (0.9%), achieving a 10 dilution. Subsequent ten-fold serial dilutions were then prepared. Bacterial colonies exhibiting distinct cultural characteristics were randomly selected and sub-cultured onto fresh nutrient agar using the streaking method to obtain pure cultures. The identification of bacterial species isolated from *kunun zaki* beverages was confirmed based on their morplogical and biochemical characteristics on various differential and selective media, including Enterococcus selective differential media (Merck, Germany), Mannitol salt agar (Titan Biotech Ltd, India), Eosin methylene blue agar (Oxoid), Cystine-lactose-electrolyte-deficient agar (K.L.E.D: Oxoid, UK) and Centrimide agar (Oxoid) (Olaoye & Dodd, 2010).

2.4.1 Total Fungal Count

Potato dextrose agar (PDA Oxoid, UK) supplemented with chloramphenicol (250 µg/1000 mL) was utilized to enumerate fungi. One mL of selected sample aliquot was inoculated onto sterile plates in duplicate and thoroughly mixed with about 20 mL of previously adjusted (45 ± 1°C). Cultures were incubated under aerobic conditions inside a dark cupboard at 25°C for five days. The various colonial morphologies observed on PDA agar were documented before transferring the cultures to a fresh chloramphenicol-PDA plate. A small fragment of each sub-cultured fungal colony was carefully placed on a sterile glass slide using sterile forceps, covered with a coverslip and then positioned inside a petri dish containing moistened cotton. The set up was incubated for three days at 25°C. After incubation, the coverslips were carefully removed with forceps, placed onto glass slides with lactophenol-cotton blue stain and examined under a microscope using immersion magnification (Olaoye & Dodd, 2010).

2.4.2 Total Bacteria Count

To assess the total bacteria count, the beverage samples were blended with sterile saline in a 1:9 (w/v) ratio using a stomacher for three minutes. Subsequently, serial 10-fold dilutions were prepared and spread onto plate count agar (Hi-Media). The inoculated plates were incubated at 37°C for 48 h, after which the bacteria colonies were enumerated and documented (Chen et al., 2022). deMan Rogosa Sharpe agar (MRS) was used for LAB.

2.4.3 Total Coliform Count

MacConkey Agar (Titan Biotech Ltd, India) was utilized to enumerate the total coliform bacteria present in the samples. The cultures were inoculated and once solidified, an additional 5 mL of MacConkey agar was layered over the plates to inhibit surface colony formation. Both the inoculated and control plates were then incubated at 37°C for 24 h (Khalid et al., 2020). Colonies exhibiting a deep pink coloration and measuring more than 0.5 mm in diameter were identified counted and recorded as the total coliform count. To assess the average microbial load in the samples from day 0 to day 3, the standard plate count method was applied (Andrew, 1994).

2.5 Data Analysis

Data of *kunun zaki* beverage were analyzed according to a completely randomized design with three replicates. Data were subjected to variance analyses and differences between means were evaluated by Duncan's multiple range test using SPSS version 22. Significant differences were expressed at ($P < 0.05$).

3 Results and Discussion

3.1 Amino Acid Profile of the *Kunun Zaki* Beverage Samples

The observed modification of amino acid composition in Table 1, reflects distinct yet complementary contributions of LAB fermentation and tigernut supplementation. LAB fermentation enhanced amino acid availability primarily through proteolytic degradation of sorghum storage proteins, notably kafirin, whose disulfide cross-linked structure limits digestibility. Microbial proteases likely disrupted these structural constraints, facilitating amino acid release and improving lysine availability, the first limiting amino acid in cereals (Adebo & Medina-Meza, 2020; Gänzle, 2014). Increased branched-chain amino acids further indicate aminotransferase-mediated metabolic remodeling during fermentation (AdeboS et al., 2019), while the reduction in tryptophan is consistent with selective microbial catabolism (Smid & Kleerebezem, 2014).

In contrast, tigernut supplementation exerted its effect predominantly through compositional complementation rather than biochemical transformation. The intrinsic amino acid profile of tigernut protein likely compensated for cereal deficiencies, thereby enhancing essential amino acid density without substantially altering the overall EAA/NEAA equilibrium. The stable EAA/NEAA ratio supports this interpretation, indicating improved amino acid enrichment without structural imbalance.

Overall, fermentation improved amino acid bioavailability through enzymatic modification, whereas tigernut inclusion enhanced compositional balance through substrate enrichment. The interaction of these mechanisms explains the improved protein quality observed in the fortified formulations.

Table 1.1A. Essential amino acid profile of the beverage samples

Amino acids	S0	STLPA 1	STLPA2	STLPA 3	STLPA 4
Arginine	3.16 ± 0.02^{c}	3.12 ± 0.09^{e}	3.17 ± 0.05^{b}	3.19 ± 0.10^{a}	3.14 ± 0.06^{d}
Histidine	1.62 ± 0.01^{b}	1.58 ± 0.11^{d}	1.59 ± 0.03^{c}	1.72 ± 0.07^{a}	1.62 ± 0.04^{b}
Isoleucine	3.20 ± 0.14^{d}	3.18 ± 0.09^{e}	3.23 ± 0.04^{b}	3.44 ± 0.12^{a}	3.22 ± 0.09^{c}
Leucine	6.24 ± 0.02^{c}	6.32 ± 0.03^{b}	6.39 ± 0.06^{a}	6.14 ± 0.22^{d}	6.21 ± 0.04^{c}
Lysine	1.57 ± 0.05^{b}	1.54 ± 0.08^{c}	1.53 ± 0.02^{d}	1.57 ± 0.08^{b}	1.79 ± 0.16^{a}
Methionine	1.28 ± 0.06^{b}	1.25 ± 0.09^{c}	1.22 ± 0.02^{d}	1.20 ± 0.06^{e}	1.32 ± 0.07^{a}
Phenylalanine	2.26 ± 0.08^{e}	2.37 ± 0.03^{b}	2.32 ± 0.03^{c}	2.27 ± 0.08^{d}	2.38 ± 0.14^{a}
Threonine	1.84 ± 0.06^{b}	1.94 ± 0.12^{a}	1.76 ± 0.06^{e}	1.80 ± 0.06^{d}	1.82 ± 0.11^{c}
Tryptophan	0.65 ± 0.16^{a}	0.53 ± 0.09^{c}	0.56 ± 0.07^{b}	0.50 ± 0.07^{e}	0.52 ± 0.05^{d}
Valine	3.42 ± 0.11^{c}	3.44 ± 0.09^{d}	3.38 ± 0.11^{b}	3.50 ± 0.06^{a}	3.39 ± 0.03^{e}
NON-ESSENTIAL					
Alanine	3.02 ± 0.05^{e}	3.20 ± 0.11^{b}	3.23 ± 0.09^{a}	3.19 ± 0.10^{c}	3.04 ± 0.02^{d}
Aspartic acid	4.54 ± 0.12^{c}	4.56 ± 0.14^{b}	4.45 ± 0.07^{d}	4.58 ± 0.04^{a}	4.50 ± 0.06^{e}
Cysteine	0.71 ± 0.03^{c}	0.77 ± 0.17^{a}	0.67 ± 0.02^{e}	0.76 ± 0.06^{b}	0.70 ± 0.05^{d}
Glutamic acid	7.26 ± 0.19^{e}	7.50 ± 0.06^{a}	7.28 ± 0.04^{d}	7.47 ± 0.02^{b}	7.36 ± 0.02^{c}
Glycine	0.71 ± 0.03^{c}	0.77 ± 0.17^{a}	0.67 ± 0.02^{e}	0.76 ± 0.06^{b}	0.70 ± 0.05^{d}
Proline	4.26 ± 0.06^{c}	4.40 ± 0.06^{a}	4.13 ± 0.05^{e}	4.34 ± 0.13^{b}	4.22 ± 0.07^{d}
Serine	2.41 ± 0.06^{b}	2.36 ± 0.13^{d}	2.39 ± 0.03^{c}	2.54 ± 0.08^{a}	2.41 ± 0.03^{b}
Tyrosine	2.04 ± 0.14^{d}	2.09 ± 0.02^{a}	2.03 ± 0.12^{e}	2.05 ± 0.07^{c}	2.08 ± 0.09^{b}

Note: Values show the mean of duplicate analysis and ± standard deviation. Figures with different superscripts (a–e) within the same row are significantly different ($p < 0.05$). **Keys:** S0 (100% Sorghum), STLPA1 (90% Sorghum: 10% Tigernut + *L. plantarium and L. acidophilus*), STLPA2 (80% Sorghum: 20% Tigernut + *L. plantarium and L. acidophilus*), STLPA3 (70% Sorghum: 30% Tigernut + *L. plantarium and L. acidophilus*), STLPA4 (60% Sorghum: 40% Tigernut + *L. plantarium and L. acidophilus*).

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Table 1.1B. Summary of amino acid distribution

Parameters	S0	STLPA 1	STLPA2	STLPA 3	STLPA 4
TEAA	25.24	25.27	25.15	25.33	25.41
TNEAA	24.95	25.65	24.85	25.69	25.01
Total AA	50.19	50.92	50.00	51.02	50.42
EAA/NEAA	1.01	0.99	1.01	0.99	1.02
%EAA	50.29	49.63	50.30	49.65	50.40

Note: TEAA: Total Essential Amino acids; TNEAA: Total Non-Essential Amino acids; Total AA: TEAA + TNEAA.

3.2 Microbial Quality of *Kunun Zaki* Beverage Samples

The microbial counts ($\log_{10}$CFU/mL) of *kunnu zaki* samples during 72 h are shown in Table 2. The result revealed that, there is a clear microbial succession characterized by early lactic acid bacteria (LAB) dominance and suppression of spoilage and indicator organisms.

Lactic acid bacteria counts were highest at 0 h, ranging from approximately 6.0–6.9 $\log_{10}$ CFU/mL, confirming successful inoculation and rapid establishment of the starter culture. LAB populations remained dominant throughout storage, with values generally maintained above 5.5 $\log_{10}$ CFU/mL even at 72 h. The sustained dominance of LAB is expected in controlled cereal fermentations, as LAB rapidly metabolize fermentable sugars to produce lactic acid, thereby lowering pH and creating an unfavorable environment for spoilage and pathogenic microorganisms. Similar LAB growth patterns have been reported in starter-culture fermented *kunu* by Gaffa and Ayo (2002); Akoma et al. (2006) where LAB populations ranged between 6–8 $\log_{10}$ CFU/mL during fermentation. Comparable findings were also observed in sorghum-based fermented beverages (Chelule et al., 2010), where LAB dominance contributed significantly to microbial safety and shelf stability. The slight reduction in LAB counts observed at 72 h in some treatments may be attributed to nutrient depletion, acid stress, and accumulation of metabolic by-products, which are known to limit LAB proliferation at later stages of fermentation (Holzapfel, 2002).

Table 2. Microbial load count of the beverage samples

Sample codes	Duration of Storage	TBC	Coliform	Fungal	LAB
A		ND	ND	ND	6.96 ± 0.03^{a}
B		ND	ND	ND	6.06 ± 0.02^{b}
C	0 h	ND	ND	ND	6.06 ± 0.02^{b}
D		ND	ND	ND	6.06 ± 0.02^{b}
E		ND	ND	ND	6.06 ± 0.02^{b}
A		ND	ND	ND	6.93 ± 0.04^{a}
B		ND	ND	ND	6.08 ± 0.03^{b}
C	24 h	ND	ND	ND	6.06 ± 0.03^{b}
D		ND	ND	ND	6.04 ± 0.02^{b}
E		ND	ND	ND	6.00 ± 0.02^{b}
A		6.88 ± 0.05^{a}	1.00 ± 0.00^{a}	2.70 ± 0.04^{a}	6.88 ± 0.05^{a}
B		7.00 ± 0.04^{b}	<1.00	2.30 ± 0.03^{b}	6.00 ± 0.03^{b}
C	48 h	6.95 ± 0.06^{ab}	<1.00	2.18 ± 0.02^{b}	5.98 ± 0.04^{b}
D		6.90 ± 0.05^{a}	1.00 ± 0.00^{a}	2.00 ± 0.03^{bc}	5.95 ± 0.02^{b}
E		6.78 ± 0.04^{c}	1.00 ± 0.00^{a}	<1.00	5.90 ± 0.03^{b}
A		6.78 ± 0.03^{a}	1.00 ± 0.00^{a}	2.48 ± 0.03^{a}	6.80 ± 0.04^{a}
B		6.90 ± 0.05^{b}	1.00 ± 0.00^{a}	2.00 ± 0.02^{b}	5.25 ± 0.03^{b}
C	72 h	6.85 ± 0.04^{ab}	1.00 ± 0.00^{a}	2.00 ± 0.03^{b}	5.90 ± 0.02^{b}
D		6.78 ± 0.03^{a}	1.00 ± 0.00^{a}	<1.00	5.85 ± 0.03^{b}
E		6.60 ± 0.04^{c}	1.00 ± 0.00^{a}	<1.00	5.78 ± 0.04^{b}

Note: Nutrient agar was used to enumerate the total bacterial load while the total fungal load was enumerated on SDA. The microbial load (bacterial/fungal) expressed in cfu/g was estimated using microbial load = reciprocal of dilution factor × number of colonies on plate. Where: Dilution factor (DF) = initial dilution used × subsequent dilution used for inoculation × volume of inoculum. A: SO; B: STLPA1; C: STLPA2; D: STLPA3; E: STLPA4; ND: Not detected (below detection limit of 10CFU/mL). TBC: Total bacterial count; LAB: Lactic acid bacteria. Values represent mean of triplicate determinations. Microbial counts were log transformed prior to statistical analysis. Means with different superscripts within the same column differ significantly ($p < 0.05$).

Total viable bacterial counts were not detected at 0 and 24 h for all the samples, indicating strong microbial control immediately after processing and early fermentation stages. However, by 48 h and 72 h, TVC ranged between approximately 6.0–7.0 $\log_{10}$ CFU/mL. The increase in total viable counts at later storage stages is consistent with the natural microbial succession that occurs in fermented cereal beverages. While LAB constituted the dominant microbiota, minor populations of acid-tolerant microorganisms may have contributed to the total aerobic counts. Similar increases in total aerobic plate counts during storage of *kunu* and other cereal beverages have been reported by Adeyemi and Umar (1994), Amusa and Ashaye (2009). Importantly, the observed TVC values fall within ranges commonly reported for traditionally fermented non-alcoholic cereal beverages and are largely reflective of beneficial fermentative microbiota rather than pathogenic contamination.

Coliforms were either not detected or remained below the detection limit (<1.0 $\log_{10}$ CFU/mL) throughout storage, with only minimal counts observed at later stages. The near absence of coliform bacteria indicates good hygienic processing and effective acidification by LAB. Acid production during fermentation significantly reduces coliform survival due to pH reduction and production of antimicrobial metabolites such as organic acids and bacteriocins (Holzapfel, 2002). Previous studies on starter-controlled *kunu* fermentation have similarly reported rapid decline or complete suppression of coliforms within 24–48 h (Gaffa & Ayo, 2002). The results therefore confirm the microbiological safety advantage of starter culture fermentation over spontaneous fermentation.

Fungal counts were either not detected or remained very low (<2.5 $\log_{10}$ CFU/mL) across storage periods. The low fungal counts may be attributed to: Competitive inhibition by LAB, Reduced pH, Limited oxygen availability during storage. Although yeasts may contribute positively to flavor development in spontaneous fermentations, their excessive growth can reduce shelf stability. The relatively low fungal counts observed in this study align with findings reported for controlled cereal fermentations (Chelule et al., 2010).

Variations among sample (A–E) suggest that substrate composition influenced microbial dynamics. Treatments with higher tigernut inclusion showed slight differences in LAB persistence and total counts, possibly due to increased sugar availability from tigernut, which may enhance fermentative activity. Similar substrate-dependent microbial variations have been reported in fortified *kunu* formulations (Ayo et al., 2014), where compositional differences influenced LAB growth kinetics.

4 Conclusion

This study demonstrated that controlled fermentation using defined lactic acid bacteria (*Lactobacillus plantarum* and *Lactobacillus acidophilus*) combined with graded tigernut supplementation significantly improved the nutritional quality and microbial safety of sorghum-based *kunun zaki*. LAB fermentation enhanced essential amino acid availability, particularly lysine, isoleucine, and valine, through proteolytic modification of sorghum proteins, while tigernut inclusion contributed complementary amino acid enrichment.

Microbiologically, LAB-inoculated formulations maintained dominant and stable LAB populations throughout storage, with minimal or no detectable coliforms and fungi, confirming the preservative and safety advantages of controlled starter culture fermentation over spontaneous fermentation.

Among the treatments, the 70% sorghum: 30% tigernut formulation exhibited the most balanced amino acid profile and desirable microbial stability, indicating an optimal inclusion level for product enhancement. Overall, the integration of defined LAB starter cultures with optimized tigernut supplementation provides a practical and scalable approach for upgrading traditional *kunun zaki* into a nutritionally enhanced and microbiologically stable functional beverage suitable for commercialization.

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Author Contributions

S.C. conceived the study, conducted the experiments, analyzed the data, and drafted the manuscript. The supervisors (0.A, A.N) provided academic guidance, reviewed the methodology and results, and critically revised the manuscript. All authors read and approved the final manuscript.

Availability of Data and Materials

The authors declare that should any raw data files be needed about the further data of the study, they are available from the corresponding author upon reasonable request. Source data are provided with this paper.

Conflicts of Interest

The authors declare that they have no conflicts of interest.

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