# Preservative Effect of Lime (Citrus Aurantiifolia) Juice on the Physicochemical and Microbiological Quality of Kunu During Ambient Storage

**Ohaegbu, Chinedu Godspower[1]*, Onwuakor, Chijioke Ethel[1], Osakuade, Felicia Oluwatoyin[2], NGENE, Anayochukwu Chibuike[1], UMEH, Michael Ndubuisi[1]**

**[1]Department of Microbiology, College of Natural Science, Michael Okpara University of Agriculture, Umudike, Abia State, Nigeria.**

**[2]Department of Microbiology, Faculty of Science, University of Port Harcourt, Choba, Rivers State.**

***Corresponding Author**

**DOI: https://doi.org/10.51244/IJRSI.2026.1310000050**

**Received: 08 October 2026; Accepted: 13 October 2026; Published: 31 October 2026**

## ABSTRACT

Background: Kunu, a cereal-based beverage drunk across Nigeria, normally spoils within a day or two at room temperature. We asked whether freshly squeezed lime (*Citrus aurantiifolia*) juice, added at 2.5, 5.0, 7.5 and 10.0% (v/v), could slow that deterioration. Methods: We prepared sorghum kunu by the traditional method, treated it, and held it at ambient temperature for four days (96 h). We recorded pH, total titratable acidity (TTA), ethanol, total heterotrophic bacterial count (THBC), total fungal count (TFC), coliform count, and the occurrence of fungal genera on Day 0 and daily thereafter. Results: In the untreated control, pH fell from 4.18 to 2.92, TTA rose from 0.28 to 1.04% and ethanol from 0.18 to 4.16%. Lime juice slowed all three changes in a dose-dependent manner: at 10.0%, the four-day pH fall was about 70% smaller than in the control and the ethanol gain about 54% smaller. Ethanol formation plateaued at 5.0% and above, whereas detections of the four fungal genera continued to fall, from 80% of possible detections in the control to 35% at 10.0%. *Penicillium* was not recovered at 5.0% or above. Coliforms were undetectable at Day 0 in the 7.5 and 10.0% samples and in every sample from Day 1. Bacterial counts were lower than the control on Days 1 and 2 but higher on Days 3 and 4, because the control eventually acidified enough to suppress them. Conclusion: Lime juice therefore appears to preserve kunu mainly by restraining fermentation and fungal growth, not by killing bacteria. A dose of 5.0–10.0% (v/v) is a reasonable range to take forward to sensory testing.

**Keywords:** lime juice; natural preservative; shelf life; fermentation; spoilage fungi

## INTRODUCTION

Walk through any motor park or market in Nigeria, and you'll see someone selling kunu from a cooler or a row of bottles. It is cheap, filling, lightly spiced and cold, and for millions of people it is the everyday drink of choice. Kunu belongs to the wider family of indigenous fermented foods made from locally available grains, tubers, and nuts that improve quality, extend shelf life, and make food safer than its raw materials [1,2] . Kunu zaki, the millet-based type, is one of the best-known [1]. It is increasingly described as a probiotic cereal beverage that can add variety to the everyday diet [3].

Kunu is made from millet, sorghum, maize, rice or acha, used singly or blended. Ginger, cloves and peppers give it its kick, and sugar or honey softens it. The grain usually ferments spontaneously, and lactic acid bacteria such as Lactobacillus, Streptococcus, and *Leuconostoc* are among the organisms associated with Nigerian fermented foods [1]. Lactic acid bacteria and yeasts work together during these fermentations, and their

combined activity shapes the product's flavour and character [4]. Kunu supplies carbohydrates, vitamins and minerals but little protein.

The same properties that make kunu appealing also make it fragile. It is a wet product with about 86% moisture in market samples from Benin City, and it carries a lively microbial population [5]. Spontaneous fermentation, small-scale production and variable hygiene leave it open to contamination. Market samples were heavily contaminated, and recovered organisms included Bacillus, *Staphylococcus aureus*, Pseudomonas, *Escherichia coli*, Mucor, and *Fusarium* [5]. Poor processing and weak production standards are recurring food safety concerns across African fermented foods [6]. Without preservation, kunu lasts a day or two at room temperature [7]. For vendors, that means daily losses, and for consumers, it can mean real health risks.

Refrigeration is unreliable where power supply is inconsistent, and many producers cannot afford it. Synthetic preservatives raise safety and consumer-acceptance concerns, which is why interest in plant-derived alternatives keeps growing. Lime (*Citrus aurantiifolia*) is an attractive candidate. It is acidic, lowering a drink's pH, and contains flavonoids, coumarins, and other compounds with antimicrobial activity. A recent lime peel extract inhibited *Staphylococcus aureus, Salmonella enterica, Listeria monocytogenes,* and *Bacillus cereus* at 5-10 mg/mL [8]. Citrus essential oils, including lime, have extended fruit shelf life without altering organoleptic properties [9]. Citrus peel extracts have also suppressed yeast growth and kept a locally produced fruit juice stable for up to three weeks [10]. In kunu itself, 1% lime and lemon essential oil extended shelf life to seven days at ambient temperature [7].

Much of this evidence comes from peel extracts and essential oils rather than the juice, which is the form producers can actually get and use. There is also a trade-off. Too little lime may not protect the drink, and too much can flatten the flavour and drive away the people it is made for. This study therefore compared kunu preserved with different lime juice concentrations to find the level that best protects the drink without compromising its quality. It determined the microbial load of the preserved kunu during storage, evaluated its pH and titratable acidity over the same period, and identified the lime juice concentration that best preserves it while keeping sensory quality acceptable.

## MATERIALS AND METHODS

### Materials and study site

We purchased sorghum (guinea corn) grains from traders at Ahiaeke Ndume Daily Market and took them to the laboratory for analysis.

### Preparation of kunu

We used the traditional method to prepare kunu [3]. We sorted the grains to remove stones and other extraneous matter, then soaked them overnight in clean water at room temperature (30 ± 2 °C) to soften them and start lactic fermentation. Ginger, cloves and a little sweet potato were added to the soaked grains, and the mixture was wet-milled in a clean laboratory blender with just enough water to give a smooth slurry. The slurry was sieved through clean muslin to remove chaff. A portion of the sieved slurry was gelatinised by boiling to the desired consistency, and boiled water was added with vigorous stirring for 5–10 min until a smooth paste formed. The hot paste was poured back into the remaining two-thirds of the cold slurry and stirred well; the paste's heat provided a mild, traditional form of pasteurisation. Sugar was added, and the kunu was sieved again. It was then divided into equal portions for the control and lime treatments and dispensed into clean, sterile, labelled plastic containers for the storage study.

### Lime juice treatment and storage

Freshly squeezed lime juice was added to the kunu portions to give 2.5, 5.0, 7.5 and 10.0% (v/v). A portion without lime juice served as the control. All samples were kept at ambient room temperature for 96 h [11]. They

ISSN No. 2321-2705 | DOI: 10.51244/IJRSI |Volume XIII Issue X October 2026

were analysed at Day 0, immediately after treatment, and then every 24 h until Day 4, so that each parameter was measured at five time points [12].

**pH**

Dilute a 10 mL sample with water to 25 mL in a clean, dry glass beaker. Immerse the electrode of a calibrated pH meter in the dilution and record the value once the reading is steady [13].

**Total titratable acidity**

Place 10 mL of sample in a clean, dry conical flask and mix with an equal volume of distilled water. Add three drops of phenolphthalein indicator, then titrate against 0.1 N NaOH to a pink end point that persists for at least 15 s [13, 14]. Total titratable acidity (TTA), expressed as per cent lactic acid, was calculated as:

$$TTA\ (\%) = (T \times N \times 0.09 \times 100) / V$$

where T is the titre (mL of NaOH), N is the normality of the NaOH, 0.09 is the milliequivalent weight of lactic acid (g/meq), and V is the volume of sample titrated (mL).

**Ethanol**

Ethanol was estimated with an alcohol hydrometer. Twenty millilitres of sample were made up to 100 mL with distilled water in a clean measuring cylinder standing on a level bench. The hydrometer was gently lowered into the cylinder and left to come to rest; the percentage was read from the stem and multiplied by the dilution factor (5) [14].

**Sensory analysis**

The sensory attributes were evaluated as described by Larmond [15].

**Microbiological analysis**

We added 1 mL of kunu to 9 mL of sterile distilled water in a sterile test tube, mixed, and serially diluted [16]. From suitable dilutions, we spread 0.1 mL onto nutrient agar (total heterotrophic bacteria), MacConkey agar (coliforms), and potato dextrose agar (fungi) using a sterile bent glass rod. Each analysis was done in triplicate. We incubated nutrient agar and MacConkey agar plates at 37 °C for 24 h, and potato dextrose agar plates at room temperature for 5–7 days. We counted colonies and expressed results as colony-forming units per millilitre (CFU/mL). We identified fungal isolates by colony colour, colony morphology, and mycelial growth patterns [17]. We recorded whether each genus was present each day.

**Data handling**

Results are expressed as mean ± standard deviation (SD) of triplicate determinations. For each parameter and storage day, we compared treatments using one-way analysis of variance (ANOVA), and we separated means with Duncan's multiple range test at the 5% level ($P < 0.05$). Microbial counts were log10-transformed before analysis; means and SDs in Table 1 are reported on the original CFU/mL scale. Within a column of Table 1, means that share a letter do not differ significantly; letters are shown only where the ANOVA was significant ($P < 0.05$), and values without letters were not significantly different. To summarise the rate of change, we fitted an ordinary least-squares line of pH, TTA, and ethanol against storage day to each replicate series (five points per line), and compared the slopes by ANOVA and Duncan's test. Restraint of fermentation was calculated for each replicate as the percentage reduction in its Day 0 to Day 4 change relative to the mean control change. The fungal detection index was calculated from presence/absence data (Table 2) as the number of genus-by-day detections as a percentage of the 20 possible; it is a single derived value per treatment and has no SD or test. We estimated times to reach pH 3.5 and 2% ethanol by linear interpolation between daily readings, using them only as reference points, not regulatory limits. Pooled correlations across the 25 treatment-by-day means describe only how closely the indices moved together, because these observations are not independent.

ISSN No. 2321-2705 | DOI: 10.51244/IJRSI |Volume XIII Issue X October 2026

# RESULTS

## Physicochemical changes during storage

Treatments differed significantly (ANOVA, $P < 0.05$) in pH on every day, in TTA on Days 0 and 2 to 4, and in ethanol on Days 1 to 4 (Table 1). Ethanol did not differ at Day 0 ($P = 0.27$), and TTA did not differ at Day 1 ($P = 0.14$). From Day 1 onward, the control had a significantly lower pH than every lime-treated sample. On Day 4, pH was 2.92 in the control and 3.36, 3.66, 3.66, and 3.74 at 2.5, 5.0, 7.5, and 10.0% lime juice, respectively; the 5.0% and 7.5% samples did not differ, while 2.5% was lower and 10.0% was higher than both. TTA on Day 4 ranged from 1.04% in the control to 0.54% at 10.0%, and the 5.0 and 7.5% samples were again not significantly different (0.60%). Ethanol on Day 4 was 4.16% in the control, 2.33% at 2.5%, 2.04% at both 5.0 and 7.5%, and 1.99% at 10.0%; all lime treatments were significantly below the control, and 10.0% was significantly below 5.0 and 7.5%, although the difference was only 0.05 percentage points.

Total heterotrophic bacterial counts were significantly lower in all treated samples than in the control on Days 1 and 2 and significantly higher on Days 3 and 4 (Table 1). At Day 0, the counts did not differ significantly ($P = 0.054$). Coliform counts at Day 0 decreased significantly with dose (control > 2.5% > 5.0% > 7.5% = 10.0%, the last two being undetectable). Total fungal counts on Days 3 and 4 were significantly lower in the 5.0 to 10.0% samples than in the control; on Day 4, the 5.0 and 7.5% samples ($5.30 \times 10^3$ CFU/mL each) did not differ, and 10.0% was slightly lower ($5.14 \times 10^3$ CFU/mL).

**Table 1.** Changes in physicochemical and microbiological characteristics of kunu treated with lime juice during ambient storage

| Treatment | Parameter | Day 0 | Day 1 | Day 2 | Day 3 | Day 4 |
|---|---|---|---|---|---|---|
| Control (0%) | pH | $4.18 \pm 0.02^{a}$ | $3.68 \pm 0.01^{c}$ | $3.35 \pm 0.01^{d}$ | $3.16 \pm 0.02^{d}$ | $2.92 \pm 0.02^{d}$ |
| | TTA (%) | $0.28 \pm 0.01^{b}$ | $0.35 \pm 0.03$ | $0.66 \pm 0.00^{a}$ | $0.88 \pm 0.01^{a}$ | $1.04 \pm 0.01^{a}$ |
| | Ethanol (%) | $0.18 \pm 0.01$ | $1.90 \pm 0.01^{a}$ | $2.44 \pm 0.01^{a}$ | $2.96 \pm 0.02^{a}$ | $4.16 \pm 0.01^{a}$ |
| | THBC (CFU/mL) | $2.76 \pm 0.01 \times 10^3$ | $4.38 \pm 0.02^{a} \times 10^3$ | $6.53 \pm 0.02^{a} \times 10^3$ | $4.52 \pm 0.01^{d} \times 10^3$ | $3.05 \pm 0.01^{d} \times 10^3$ |
| | TFC (CFU/mL) | $1.18 \pm 0.01^{d} \times 10^1$ | $4.80 \pm 0.02^{c} \times 10^2$ | $1.90 \pm 0.01^{b} \times 10^3$ | $6.80 \pm 0.01^{a} \times 10^3$ | $7.20 \pm 0.01^{a} \times 10^3$ |
| | TCC (CFU/mL) | $13.0 \pm 0.2^{a}$ | NIL | NIL | NIL | NIL |
| 2.5% lime juice | pH | $4.16 \pm 0.01^{ab}$ | $4.08 \pm 0.01^{b}$ | $3.84 \pm 0.01^{c}$ | $3.55 \pm 0.01^{c}$ | $3.36 \pm 0.02^{c}$ |
| | TTA (%) | $0.28 \pm 0.02^{b}$ | $0.31 \pm 0.02$ | $0.55 \pm 0.01^{b}$ | $0.63 \pm 0.01^{b}$ | $0.76 \pm 0.01^{b}$ |
| | Ethanol (%) | $0.16 \pm 0.01$ | $0.88 \pm 0.02^{b}$ | $1.26 \pm 0.01^{b}$ | $1.82 \pm 0.02^{b}$ | $2.33 \pm 0.01^{b}$ |
| | THBC (CFU/mL) | $2.75 \pm 0.02 \times 10^3$ | $3.88 \pm 0.01^{b} \times 10^3$ | $6.02 \pm 0.01^{b} \times 10^3$ | $6.11 \pm 0.01^{a} \times 10^3$ | $5.82 \pm 0.02^{a} \times 10^3$ |
| | TFC (CFU/mL) | $1.80 \pm 0.02^{b} \times 10^2$ | $3.82 \pm 0.01^{e} \times 10^2$ | $1.88 \pm 0.01^{c} \times 10^3$ | $5.76 \pm 0.01^{b} \times 10^3$ | $9.50 \pm 0.01^{d} \times 10^2$ |
| | TCC (CFU/mL) | $11.0 \pm 0.1^{b}$ | NIL | NIL | NIL | NIL |
| 5.0% lime juice | pH | $4.14 \pm 0.01^{bc}$ | $4.12 \pm 0.00^{a}$ | $3.93 \pm 0.01^{b}$ | $3.83 \pm 0.01^{b}$ | $3.66 \pm 0.01^{b}$ |
| | TTA (%) | $0.31 \pm 0.01^{a}$ | $0.33 \pm 0.01$ | $0.48 \pm 0.01^{c}$ | $0.61 \pm 0.02^{b}$ | $0.60 \pm 0.02^{c}$ |
| | Ethanol (%) | $0.16 \pm 0.01$ | $0.82 \pm 0.02^{c}$ | $1.19 \pm 0.02^{c}$ | $1.37 \pm 0.02^{c}$ | $2.04 \pm 0.01^{c}$ |
| | THBC (CFU/mL) | $2.75 \pm 0.01 \times 10^3$ | $3.77 \pm 0.01^{c} \times 10^3$ | $5.80 \pm 0.01^{c} \times 10^3$ | $5.91 \pm 0.01^{b} \times 10^3$ | $5.71 \pm 0.01^{c} \times 10^3$ |
| | TFC (CFU/mL) | $1.77 \pm 0.01^{a} \times 10^3$ | $3.91 \pm 0.01^{a} \times 10^3$ | $1.84 \pm 0.01^{d} \times 10^3$ | $4.30 \pm 0.01^{c} \times 10^3$ | $5.30 \pm 0.02^{b} \times 10^3$ |
| | TCC (CFU/mL) | $10.1 \pm 0.1^{c}$ | NIL | NIL | NIL | NIL |
| 7.5% lime juice | pH | $4.14 \pm 0.01^{bc}$ | $4.12 \pm 0.02^{a}$ | $3.98 \pm 0.01^{a}$ | $3.84 \pm 0.01^{b}$ | $3.66 \pm 0.01^{b}$ |
| | TTA (%) | $0.31 \pm 0.01^{a}$ | $0.33 \pm 0.01$ | $0.43 \pm 0.01^{d}$ | $0.55 \pm 0.01^{c}$ | $0.60 \pm 0.02^{c}$ |
| | Ethanol (%) | $0.16 \pm 0.02$ | $0.62 \pm 0.02^{d}$ | $1.12 \pm 0.01^{d}$ | $1.28 \pm 0.01^{d}$ | $2.04 \pm 0.01^{c}$ |
| | THBC (CFU/mL) | $2.73 \pm 0.01 \times 10^3$ | $3.75 \pm 0.01^{c} \times 10^3$ | $5.66 \pm 0.01^{d} \times 10^3$ | $5.66 \pm 0.01^{c} \times 10^3$ | $5.71 \pm 0.01^{c} \times 10^3$ |
| | TFC (CFU/mL) | $1.75 \pm 0.01^{c} \times 10^2$ | $3.91 \pm 0.01^{d} \times 10^2$ | $1.84 \pm 0.01^{d} \times 10^3$ | $4.96 \pm 0.02^{c} \times 10^3$ | $5.30 \pm 0.01^{b} \times 10^3$ |

| | | | | | | |
|---|---|---|---|---|---|---|
| | TCC (CFU/mL) | NIL | NIL | NIL | NIL | NIL |
| 10.0% lime juice | pH | $4.12 \pm 0.01^{c}$ | $4.06 \pm 0.01^{b}$ | $3.98 \pm 0.01^{a}$ | $3.88 \pm 0.02^{a}$ | $3.74 \pm 0.01^{a}$ |
| | TTA (%) | $0.33 \pm 0.01^{a}$ | $0.34 \pm 0.00$ | $0.43 \pm 0.01^{d}$ | $0.46 \pm 0.01^{d}$ | $0.54 \pm 0.01^{d}$ |
| | Ethanol (%) | $0.16 \pm 0.01$ | $0.55 \pm 0.01^{e}$ | $1.12 \pm 0.01^{d}$ | $1.24 \pm 0.02^{e}$ | $1.99 \pm 0.01^{d}$ |
| | THBC (CFU/mL) | $2.73 \pm 0.01 \times 10^{3}$ | $3.65 \pm 0.01^{d} \times 10^{3}$ | $5.58 \pm 0.01^{e} \times 10^{3}$ | $5.67 \pm 0.02^{e} \times 10^{3}$ | $5.75 \pm 0.01^{b} \times 10^{3}$ |
| | TFC (CFU/mL) | $1.78 \pm 0.01^{b} \times 10^{2}$ | $3.88 \pm 0.01^{b} \times 10^{3}$ | $4.90 \pm 0.02^{a} \times 10^{3}$ | $4.92 \pm 0.01^{d} \times 10^{3}$ | $5.14 \pm 0.01^{c} \times 10^{3}$ |
| | TCC (CFU/mL) | NIL | NIL | NIL | NIL | NIL |

Values are the mean ± SD of triplicate determinations. Within each parameter and day (column), means followed by different letters differ significantly (Duncan's multiple range test, $P < 0.05$); letters are omitted where ANOVA was not significant. Letters are assigned alphabetically from the highest mean. TCC values are $\times 10^{0}$ CFU/mL and were compared at Day 0 only. TTA, total titratable acidity (as lactic acid); THBC, total heterotrophic bacterial count; TFC, total fungal count; TCC, total coliform count; NIL, no detectable growth.

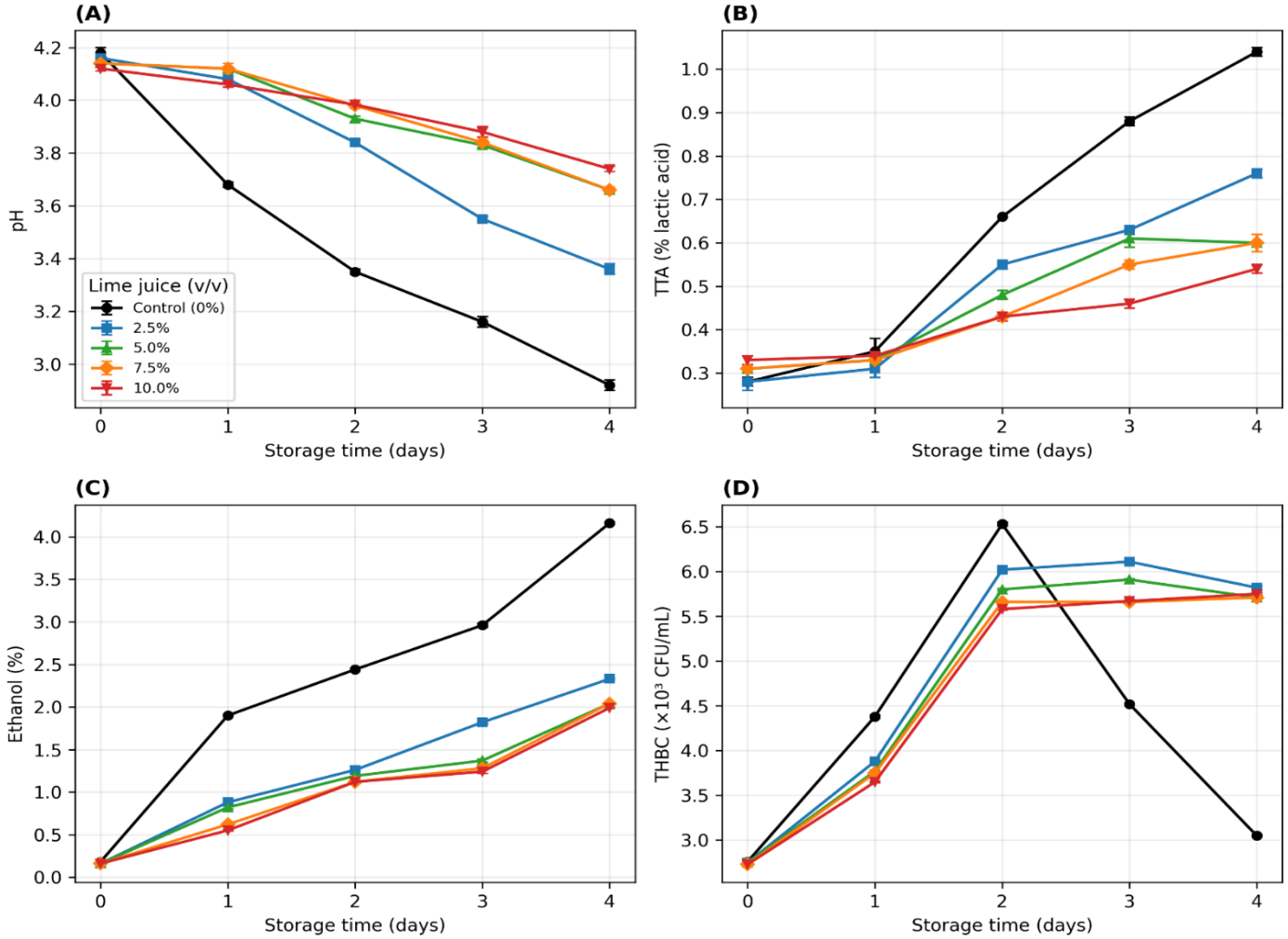



**Figure 1.** (A) pH, (B) total titratable acidity, (C) ethanol and (D) total heterotrophic bacterial count (THBC) of kunu containing 0–10.0% (v/v) lime juice over four days at ambient temperature.

### Bacterial, fungal and coliform counts

THBC rose from about $2.7 \times 10^{3}$ CFU/mL at Day 0 in all treatments (Table 1; Fig. 1D). In the control, it peaked at $6.55 \times 10^{3}$ CFU/mL on Day 2 and then fell to $3.05 \times 10^{3}$ CFU/mL on Day 4. Treated samples were 8–17% below the control on Days 1 and 2, but they did not decline afterwards: counts stayed between 5.6 and $6.1 \times 10^{3}$ CFU/mL, which put them 25–35% above the control on Day 3 and 87–91% above it on Day 4. Differences among the four lime doses were small at every time point.

ISSN No. 2321-2705 | DOI: 10.51244/IJRSI |Volume XIII Issue X October 2026

Fungal counts rose with storage time in all samples. The control went from $1.18 \times 10^1$ to $7.20 \times 10^3$ CFU/mL. On Day 4, all treated samples were below the control by 26–29% at 5.0–10.0% lime juice ($5.14–5.30 \times 10^3$ CFU/mL).

Coliforms were detected only on Day 0, and their numbers decreased with dose: 1.30, 1.10, and 1.01 × 101 CFU/mL in the control, 2.5%, and 5.0% samples, respectively, and were absent at 7.5% or 10.0%. No coliforms were recovered from any sample from Day 1 onwards, including the control.

**Occurrence of fungal genera**

We isolated four genera: Aspergillus, Saccharomyces (yeast), Candida, and *Penicillium* (Table 2). In the control, *Aspergillus* and *Saccharomyces* were present on all five days (100% occurrence), *Candida* on four (80%) and *Penicillium* on two (40%). The 2.5% treatment showed a similar pattern, except for *Candida* (60%). At 5.0% and above, *Penicillium* was never recovered. *Aspergillus* occurred at 60% at 5.0 and 7.5% and 40% at 10.0%, and was absent from Day 2 onward. *Candida* occurred at 60%, 40%, and 20% at 5.0, 7.5, and 10.0, respectively. *Saccharomyces* was the most persistent genus, present on 80% of days at all three higher doses, and was recovered up to Day 3.

**Table 2.** Occurrence of fungal genera in kunu treated with lime juice during ambient storage

| Treatment | Storage day | Aspergillus | Saccharomyces | Candida | Penicillium |
|---|---|---|---|---|---|
| Control | 0 | + | + | + | + |
| | 1 | + | + | + | + |
| | 2 | + | + | + | − |
| | 3 | + | + | + | − |
| | 4 | + | + | − | − |
| | Occurrence (%) | 100 | 100 | 80 | 40 |
| 2.5% lime juice | 0 | + | + | + | + |
| | 1 | + | + | + | + |
| | 2 | + | + | + | − |
| | 3 | + | + | − | − |
| | 4 | + | + | − | − |
| | Occurrence (%) | 100 | 100 | 60 | 40 |
| 5.0% lime juice | 0 | + | + | + | − |
| | 1 | + | + | + | − |
| | 2 | + | + | + | − |
| | 3 | − | + | − | − |
| | 4 | − | − | − | − |
| | Occurrence (%) | 60 | 80 | 60 | 0 |
| 7.5% lime juice | 0 | + | + | + | − |
| | 1 | + | + | + | − |
| | 2 | + | + | − | − |
| | 3 | − | + | − | − |
| | 4 | − | − | − | − |
| | Occurrence (%) | 60 | 80 | 40 | 0 |
| 10.0% lime juice | 0 | + | + | + | − |
| | 1 | + | + | − | − |
| | 2 | − | + | − | − |
| | 3 | − | + | − | − |
| | 4 | − | − | − | − |
| | Occurrence (%) | 40 | 80 | 20 | 0 |

+, isolated; −, not detected. Occurrence (%) is the proportion of the five sampling days on which the genus was isolated.

### Rate of change and degree of restraint

The daily rates of pH fall, TTA rise and ethanol gain were all significantly lower in every lime treatment than in the control (Table 3). The pH and TTA slopes decreased significantly with dose up to 5.0%, and the 10.0% sample had the slowest rate. Restraint of the pH fall, TTA rise and ethanol gain was significantly greater at 5.0% than at 2.5% ($P < 0.05$) and was not different between 5.0 and 7.5%; it was significantly greater again at 10.0%, but the gain in ethanol restraint from 5.0 to 10.0% was only about 1.2 percentage points (52.8 to 54.0%).

**Table 3.** Rates of change over four days, restraint of fermentation relative to the control, and fungal detection index

| Treatment | pH (per day) | TTA (% per day) | Ethanol (% per day) | Restraint of pH fall (%) | Restraint of TTA rise (%) | Restraint of ethanol gain (%) | Fungal index (%) |
|---|---|---|---|---|---|---|---|
| Control (0%) | -0.304 ± 0.007$^{d}$ | 0.205 ± 0.006$^{a}$ | 0.902 ± 0.001$^{a}$ | — | — | — | 80 |
| 2.5% lime juice | -0.213 ± 0.002$^{c}$ | 0.128 ± 0.001$^{b}$ | 0.528 ± 0.000$^{b}$ | 36.5 ± 0.8$^{c}$ | 36.8 ± 1.3$^{c}$ | 45.5 ± 0.0$^{c}$ | 75 |
| 5.0% lime juice | -0.125 ± 0.005$^{b}$ | 0.086 ± 0.005$^{c}$ | 0.431 ± 0.004$^{d}$ | 61.9 ± 1.6$^{b}$ | 61.8 ± 3.9$^{b}$ | 52.8 ± 0.5$^{b}$ | 50 |
| 7.5% lime juice | -0.124 ± 0.007$^{b}$ | 0.080 ± 0.002$^{c}$ | 0.442 ± 0.007$^{c}$ | 61.9 ± 1.6$^{b}$ | 61.8 ± 1.3$^{b}$ | 52.8 ± 0.8$^{b}$ | 45 |
| 10.0% lime juice | -0.094 ± 0.001$^{a}$ | 0.054 ± 0.003$^{d}$ | 0.435 ± 0.001$^{d}$ | 69.8 ± 0.0$^{a}$ | 72.4 ± 2.6$^{a}$ | 54.0 ± 0.0$^{a}$ | 35 |

Rates are least-squares slopes fitted to each replicate series (mean ± SD, n = 3). Restraint is the percentage reduction in the Day 0 to Day 4 change relative to the mean control change (mean ± SD, n = 3). Means in the same column followed by different letters differ significantly (Duncan's multiple range test, $P < 0.05$; letters assigned from the highest mean, so for pH slopes, which are negative, the least negative slope is $^{a}$). The fungal index is the percentage of the 20 possible genus-by-day detections in Table 2 and has no SD or test.

**Table. 4: Effect of lime juice on overall sensory acceptability of Kunu**

| Treatment | Day 0 | Day 1 | Day 2 | Day 3 | Day 4 |
|---|---|---|---|---|---|
| Control (0%) | 2.2 | 3.5 | 4.5 | 5.6 | 6.9 |
| 2.5% lime juice | 2.0 | 2.2 | 2.5 | 2.7 | 3.0 |
| 5.0% lime juice | 2.3 | 2.6 | 2.9 | 3.4 | 4.5 |
| 7.5% lime juice | 2.5 | 2.8 | 3.1 | 3.6 | 4.7 |
| 10.0% lime juice | 2.8 | 3.0 | 3.3 | 3.9 | 5.0 |

## DISCUSSION

### Lime juice slowed fermentation rather than simply adding acid

The usual expectation is that adding lime juice preserves food by lowering pH. Our data suggest a different story for kunu. At Day 0, the treated samples' pH was within 0.06 units of the control (4.12–4.16 versus 4.18 ± 0.02) because the beverage's starch and protein buffered the added acid. This small difference was statistically significant ($P < 0.01$), but it went in the opposite direction of what added acid would predict at later times and had no practical effect on starting acidity. The treatments then diverged: from Day 1 onward, pH differed significantly among treatments (ANOVA, $P < 0.001$ each day), and higher lime juice led to higher pH at Day 4 (Table 1). The control, with no added acid, had the lowest pH (2.92 ± 0.02), significantly lower than all lime treatments (3.36 ± 0.02, 3.66 ± 0.01, 3.66 ± 0.01, and 3.74 ± 0.01 at 2.5, 5.0, 7.5, and 10.0%). Across all treatment-by-day means, the fall in pH, rise in TTA, and rise in ethanol were tightly linked ($|r| \geq 0.93$), which reflects continued lactic and alcoholic fermentation. The most economical reading is that lime juice restrained that fermentation, so less acid and ethanol accumulated.

The rate data support this reading. The daily rates of pH fall, TTA rise and ethanol gain were significantly lower in every lime treatment than in the control (Duncan's test, $P < 0.05$; Table 3). For example, pH fell by 0.304 ± 0.007 units per day in the control but only 0.094 ± 0.001 units per day at 10.0%, and ethanol rose by 0.902 ± 0.001% per day in the control compared with 0.431–0.528% per day in the treated samples. On Day 4, the

control's ethanol (4.16 ± 0.01%) was significantly higher than in all treated samples (1.99–2.33%). This is consistent with Kolo et al. [18], who found that an acidifying additive improved the keeping quality of kunu, although their samples were refrigerated and their additive acted as a sweetener, so the comparison is only partial.

The restraint is dose-dependent but saturates early. Restraint of ethanol gain rose significantly from 45.5 ± 0.0% at 2.5% lime juice to 52.8 ± 0.5% at 5.0%, but the 5.0 and 7.5% treatments did not differ from each other (52.8 ± 0.8% at 7.5%), and 10.0% (54.0 ± 0.0%) was only about 1.2 percentage points higher. On Day 4, ethanol was identical in the 5.0% and 7.5% samples (2.04%) and was significantly, though only marginally, lower in the 10.0% sample (1.99%, a difference of 0.05 percentage points). Restraint of the pH fall and TTA rise continued to increase significantly at 10.0% (69.8 ± 0.0 and 72.4 ± 2.6%, against 61.9 and 61.8% at 5.0 and 7.5%), but the 5.0% and 7.5% treatments did not differ. Doubling the dose beyond 5.0% therefore offers little additional control over fermentation, and the small extra effect at 10.0%, though statistically detectable, must be weighed against its likely impact on flavour. These data do not indicate which lime juice constituents are responsible. Citric acid, other organic acids and phenolic compounds have all been proposed as antimicrobial components of citrus products [19], and in a hurdle framework [20] it is plausible that several act together. Testing would require a known composition and a comparison with an equivalent amount of citric acid alone, neither of which we had.

**Bacterial counts**

Lime juice did not consistently reduce bacterial numbers. Counts did not differ significantly among treatments at Day 0 ($P = 0.054$). Early in storage, the treated samples had significantly fewer bacteria than the control (8–17% lower on Days 1–2; $P < 0.001$), suggesting a modest inhibitory effect, with the lowest counts at 10.0% (3.65 ± 0.01 × $10^3$ CFU/mL on Day 1, against 4.38 ± 0.02 × $10^3$ in the control). However, the control counts fell after Day 2, presumably because its acidity (pH 3.16 on Day 3 and 2.92 on Day 4) became unfavourable for many bacteria, whereas the treated samples remained less acidic and maintained high counts. The pattern reversed significantly: on Day 3 every treated sample had a higher count than the control (5.66–6.11 versus 4.52 × $10^3$ CFU/mL; $P < 0.001$), and by Day 4 the treated samples carried nearly twice the bacterial load of the control (5.71–5.82 versus 3.05 × $10^3$ CFU/mL; $P < 0.001$). Differences among the four lime doses on Day 4 were statistically detectable but small (about 2%). This is why we avoid describing lime juice as bactericidal, or even broadly bacteriostatic. The counts here ($10^3$ CFU/mL) are also five orders of magnitude below those reported for market kunu in Calabar [21], as expected: our kunu was prepared under laboratory conditions, and market samples reflect the hygiene problems the authors describe. For the same reason, our counts are closer to the 1–8.8 × $10^3$ CFU/mL bacterial counts reported for laboratory-made kunu stored at 4 °C for five days [22], even though ours were held at room temperature.

**Coliforms**

Coliforms were found only at Day 0 and were already gone by Day 1 in every sample, treated or not. Lime juice therefore did not determine whether coliforms eventually disappeared; fermentative acidification did that within 24 h. Similar patterns have been reported elsewhere. In laboratory-produced kunun-zaki, Enterobacter and Shigella were present at the start of production while Bacillus and Lactobacillus persisted to the final stage, and no coliforms were detected in the finished products [23]. Sorghum kunun zaki fermented with defined lactic acid bacteria likewise showed absent or minimal coliforms throughout 72 h of storage [24].

Lime juice changed the starting point: coliform counts at Day 0 differed significantly among treatments ($P < 0.001$), falling with dose from 13.0 ± 0.2 CFU/mL in the control to 11.0 ± 0.1 and 10.1 ± 0.1 CFU/mL at 2.5 and 5.0%, with none detected at 7.5 or 10.0%. A direct effect is plausible, because lime juice inhibited Escherichia coli in disc diffusion tests [25]. However, the lowest concentration still active in that test was 12.5%, above our highest dose, and kunu's starch and protein buffer added acid. We also did not test the juice against coliforms in isolation, so the Day 0 effect remains an inference. Because many consumers drink kunu on the day it is made, that first day matters. We caution, though, that the Day 0 differences are small in absolute terms (about 10 CFU/mL). Although the replicate spread was narrow (SD 0.1–0.2 CFU/mL) and the differences were significant, they come from a single sampling day, so treat them as suggestive.

### Fungi

Fungi showed the clearest difference with lime juice beyond fermentation rate. Total fungal counts on Day 3 were significantly lower at every lime dose than in the control (4.30–5.76 versus 6.80 ± 0.01 × $10^3$ CFU/mL; $P < 0.001$), and on Day 4 they were 26–29% lower than the control at 5.0–10.0% (5.14–5.30 versus 7.20 ± 0.01 × $10^3$ CFU/mL; $P < 0.001$), with no significant difference between 5.0 and 7.5%. The fungal detection index dropped from 80% to 35% as the dose rose to 10.0%. Penicillium was never recovered at 5.0% or above, and Aspergillus was lost from Day 2 onward at 10.0%. Yeasts were the most tolerant group: Saccharomyces was isolated on 80% of days even at 10.0%, which fits yeasts' general tolerance to acid and likely explains the ethanol that still accumulated in treated samples. The same two genera, Aspergillus and Saccharomyces, along with Penicillium and Fusarium, were the fungi found in market kunu in Calabar, with Aspergillus the most frequent [21], so the spoilage fungi we targeted are the ones that matter in practice.

In the control, Candida and Penicillium also disappeared from the plates later in storage, when pH fell below about 3.4. Self-acidification therefore removes some fungi in unpreserved kunu too. Lime juice brought that loss forward in time and removed Penicillium altogether, but did not make kunu fungus-free: total fungal counts still reached about 5 × $10^3$ CFU/mL by Day 4 at 5.0–10.0%.

### Sensory evaluation

The sensory analysis showed that the treated samples were more acceptable than the untreated control sample. The high acceptability rate of the treated samples among the 10-member panel is attributed to the extra flavour added by the lime juice [26]. The kunu with 2.5% lime juice was more acceptable to the panellists, with acceptability decreasing with concentration, and it extended the shelf life by about four (4) days. However, Kunu with increasing concentration of lime showed apparent antimicrobial benefits, but consumer acceptability was lower, confirming the off-flavour that occurred at higher concentrations.

## CONCLUSION

Lime juice added at 2.5–10.0% (v/v) slowed the fall in pH, the rise in titratable acidity, and ethanol formation in kunu stored at room temperature for four days, and reduced spoilage fungi, notably *Penicillium* and *Aspergillus*. Fermentation restraint was greatest at 5.0%, whereas fungal occurrence continued to fall up to 10.0%. Lime juice did not reliably reduce bacterial counts beyond Day 2, and fermentation acidity cleared coliforms in all samples within 24 h; the dose effect on coliforms was limited to Day 0. We therefore regard lime juice as a partial, fermentation-restraining preservative for kunu, best used with good hygiene and cool storage. A dose of 5.0–10.0% (v/v) should be examined next in a sensory study, together with a longer storage trial and an equal-acidity citric acid control.

### Data Availability Statement

The data supporting the findings of this study are available from the corresponding author upon reasonable request. The data are not publicly available because they have not been deposited in a publicly accessible repository.

## REFERNCES

1. Obafemi, Y. D., Ajayi, A. A., Adebayo, H. A., Oyewole, O. A., & Olumuyiwa, E. O. (2024). The role of indigenous Nigerian fermented agrifoods in enhancing good health and well-being. Discover Food, 4, 133.
2. Obafemi, Y. D., Oranusi, S. U., Ajanaku, K. O., Akinduti, P. A., Leech, J., & Cotter, P. D. (2022). African fermented foods: Overview, emerging benefits, and novel approaches to microbiome profiling. npj Science of Food, 6, 15.
3. Ndukwe, J. K., Aduba, C. C., Ughamba, K. T., Chukwu, K. O., Eze, C. N., Nwaiwu, O., & Onyeaka, H. (2023). Diet diversification and priming with kunu: An indigenous probiotic cereal-based non-alcoholic beverage in Nigeria. Beverages, 9(1), 14.

4. Adesulu-Dahunsi, A. T., Dahunsi, S. O., & Olayanju, A. (2020). Synergistic microbial interactions between lactic acid bacteria and yeasts during production of Nigerian indigenous fermented foods and beverages. Food Control, 110, 106963.
5. Emmanuel-Akerele, H. A., & Edobor, E. O. (2021). Microbiological analysis of kunun-zaki: A fermented millet drink. Annals of Food Science and Technology, 22(2), 380–386.
6. Anyogu, A., Olukorede, A., Anumudu, C., Onyeaka, H., Areo, E., Adewale, O., Odimba, J. N., & Nwaiwu, O. (2021). Microorganisms and food safety risks associated with indigenous fermented foods from Africa. Food Control, 129, 108227.
7. Towobola, M. (2021). Production and preservation of kunun-zaki from millet and groundnut blend [Master's thesis, Landmark University].
8. Cloete, L., Venter, A., Emmambux, M. N., Ramful-Baboolall, D., Ramasawmy, B., Neetoo, S. H., Picot-Allain, C., & Duodu, K. G. (2025). Bioprospecting the Rodriguan lime (Citrus aurantifolia Swingle) as a novel source of antioxidants and antimicrobials for food application. International Journal of Food Science, 2025(1), 9985071.
9. Ajayi-Moses, O. B., Ogidi, C. O., & Akinyele, B. J. (2024). Preservation properties of citrus essential oils in fruits against food spoilage microorganisms. Contemporary Agriculture, 73(3–4), 145–156.
10. Ugwu, E. I., Nyam, M. A., & Ogbonna, A. I. (2021). Changes in the population of Schizosaccharomyces japonicus in Carissa edulis Vahl (simple-spined carissa) juice treated with extracts of Citrus aurantifolia Christm. (lime) and Citrus limon Burm F. (lemon) peels as natural preservatives during storage. Journal of Advances in Biology & Biotechnology, 24(5).
11. Beetseh, C.I., Yoo, M.A. and Achikpi, F. (2022). Effects of different preservative treatments on the sugar content of kunu-zaki in the University of Agriculture, Makurdi, Benue State, Nigeria. Journal of Environment and Earth Science, 12(3).
12. Ayomide F. Sowemimo, Abiola O. Obisesan, Funmilola A. Ayeni (2021). Evaluation of lactic acid bacteria viability and anti-diarrhoeagenic Escherichia coli activities of non-alcoholic fermented beverage 'Kunu'. Croatian Journal of Food Science and Technology, 13(1), 122–127.
13. AOAC International (2023). Official Methods of Analysis, 22nd ed. Oxford University Press / AOAC International.
14. Onwuka, G. I. (2005). Food analysis and instrumentation: Theory and practice. Naphthali Prints, Lagos.
15. Larmond, E. Laboratory methods for Sensory Evaluation of Foods. Canada Department of Agriculture, Ottawa. Canada. (1977) Pp. 12–88.
16. Salfinger, Y. & Tortorello, M.L. (eds.) (2015). Compendium of Methods for the Microbiological Examination of Foods, 5th ed. American Public Health Association, Washington, DC.
17. Watanabe, T. (2010). Pictorial Atlas of Soil and Seed Fungi: Morphologies of Cultured Fungi and Key to Species, 3rd ed. CRC Press, Boca Raton, FL.
18. Kolo, S. I., Anuonye, J. C., Chinma, C. E., & Makun, H. A. (2026). A Review on Quality Evaluation of Kunun-zaki in Nigeria. Nigerian Journal of Technological Development, 23(2), 37-57.
19. Sweet, R., Kroon, P. A., & Webber, M. A. (2024). Activity of antibacterial phytochemicals and their potential use as natural food preservatives. Critical Reviews in Food Science and Nutrition, 64(8), 2076-2087.
20. Wu, Y., & Wang, X. (2025). Hurdle technologies. In Antimicrobial Strategies in the Food System: Updates, Opportunities, Challenges (pp. 239–268). Cham: Springer Nature Switzerland.
21. Asuquo, N. E., Antai, S. P., & Tiku, D. R. (2017). Microbiological and nutritional quality characteristics of kunu drinks sold in Calabar metropolis. Advances in Biotechnology & Microbiology, 7(4), 555719.
22. Abiodun, O. A., Dauda, A. O., Adebisi, T. T., & Alonge, C. D. (2017). Physicochemical, microbial and sensory properties of kunu zaki beverage sweetened with black velvet tamarind (Dialium guineense). Croatian journal of food science and technology, 9(1), 46–56.
23. Egbere, J. O., Odu, C. E., Onyimba, I. A., Ngene, A. C., Yusuf, A. E., Ogaji, A. O., Dashen, M. M., Hemen, W. N., & Entonu, E. E. (2021). Microbiological and organoleptic quality characteristics of laboratory-produced date fruit-based kunun-zaki. European Journal of Biology and Biotechnology, 2(3), 97–104.

ISSN No. 2321-2705 | DOI: 10.51244/IJRSI |Volume XIII Issue X October 2026

24. Kalu, S. C., Olaoye, O. A., & Ukom, A. N. (2026). Effect of lactic acid bacteria fermentation and tigernut supplementation on the nutritional quality and microbial safety of kunun zaki beverage. Records of Agricultural and Food Chemistry, (1), 2.
25. Nerdy, N., Tarigan, P., Elysa, E., Lestari, P., & Nurmalisa, S. (2020). Antibacterial activity test of lime juice extract against Escherichia coli. Jurnal Penelitian Farmasi & Herbal, 3(1), 135–139.
26. Edward, K.C. & Ohaegbu, C.G. (2012). The effect of ginger and garlic on the microbial load and shelf life of Kunun-zaki. Journal of Applied Pharmaceutical Science 02 (05): 150-153