World Journal of Biology Pharmacy and Health Sciences
eISSN: 2582-5542
Cross Ref DOI: 10.30574/wjbphs
Journal homepage: https://wjbphs.com/

(RESEARCH ARTICLE)

Check for updates

Isolation of spoilage microorganisms and evaluation of yeast inhibitory activity in selected traditional beverages in Sokoto State, Nigeria

Sadiya Sambo [1,*], Balkisu Isah Muhammad [2] and Ibrahim Haruna Aliyu [3]

[1] *Department of Biology, Shehu Shagari College of Education, Sokoto, Nigeria.*
[2] *Department of Integrated Science, Shehu Shagari College of Education, Sokoto, Nigeria.*
[3] *Department of Applied Biology, Umaru Ali Shinkafi Polytechnic, Sokoto, Nigeria.*

World Journal of Biology Pharmacy and Health Sciences, 2026, 25(03), 266-271

Publication history: Received on 29 January 2026; revised on 09 March 2026; accepted on 10 March 2026

Article DOI: https://doi.org/10.30574/wjbphs.2026.25.3.0136

Abstract

Traditional beverages that include *Kunun aya*, *Kunun zaki*, *Zobo*, *Ginger* drink, and *Fura* are widely consumed in Sokoto State, Nigeria. Their high-water activity, manual handling, and lack of preservatives render them susceptible to microbial spoilage. *Saccharomyces cerevisiae* has potential as a natural biocontrol agent against spoilage organisms. This study aimed to isolate and identify common spoilage microorganisms in selected traditional beverages and evaluate the inhibitory activity of *S. cerevisiae* against them. A total of 200 samples (40 per beverage type) were collected from households, market vendors, and small-scale processors in Four (4) selected Local government Areas of Sokoto State. Total bacterial and fungal counts were determined using standard plate count techniques, and isolates were identified phenotypically. Yeast inhibitory activity was assessed via agar well diffusion, co-culture broth assays, cell-free supernatants, and in-matrix challenge tests. Data were analysed using ANOVA ($p < 0.05$). Bacterial loads ranged from 3.51 to 5.61 $\log_{10}$ CFU/mL, with Fura and Kunun Zaki exhibiting the highest contamination, whereas Zobo exhibited the lowest. Fungal loads ranged from 2.41 to 4.01 $\log_{10}$ CFU/mL, with Fura having the highest levels. Predominant spoilage organisms included *Bacillus spp.*, *Staphylococcus aureus*, *E. coli*, *Enterobacter spp.*, *Aspergillus niger*, and *Candida spp*. *S. cerevisiae* significantly inhibited these organisms, producing inhibition zones of 8.5–14.2 mm and reducing bacterial counts by 2.6–3.5 $\log_{10}$ CFU/mL within 48 hours. *Fura* and *Kunun zaki* are highly susceptible to microbial contamination, whereas Zobo is more microbiologically stable. *S. cerevisiae* effectively inhibited spoilage organisms, demonstrating its potential as a natural biocontrol agent to enhance the safety and shelf-life of traditional beverages. Vendors and households should adopt strict hygiene practices during beverage preparation, including cleaning of utensils and containers, to reduce initial microbial loads.

Keywords: Traditional beverages; Spoilage; S. *cerevisiae*; Biocontrol and Food safety

1. Introduction

Traditional beverages such as *kunun zaki, kunun aya, zobo*, and *fura* are integral to the culinary and cultural landscape of Sokoto State, Nigeria. These drinks are valued not only for their affordability and accessibility but also for their rich nutritional composition, including carbohydrates, proteins, vitamins, antioxidants, and minerals [1 & 2]. They serve as important sources of dietary energy and are commonly consumed by both rural and urban populations as refreshing alternatives to carbonated drinks.

However, these beverages are highly susceptible to microbial spoilage due to intrinsic and extrinsic factors, including high water activity, neutral to slightly acidic pH, and the absence of chemical preservatives. Furthermore, traditional preparation methods, often involving manual handling, the use of unpasteurized ingredients, and storage in non-sterile containers, contribute to microbial contamination and proliferation [3 & 4]. Spoilage is typically caused by bacteria,

* Corresponding author: Sadiya Sambo.

moulds, and yeasts, leading to changes in taste, odour, texture, and, more importantly, potential health hazards from pathogenic organisms.

Addressing microbial spoilage is essential not only to extend the shelf life of these products but also to safeguard public health, reduce food waste, and improve consumer trust in locally produced beverages. While synthetic preservatives have been used to control spoilage, their potential side effects, rising consumer preference for natural products, and regulatory concerns necessitate exploring safer, natural alternatives. Yeasts, particularly Saccharomyces cerevisiae, have attracted attention as promising biocontrol agents in food systems owing to their Generally Recognized as Safe (GRAS) status and multifaceted antimicrobial mechanisms. These include the production of ethanol, organic acids, fatty acids, volatile compounds, and killer toxins that selectively inhibit spoilage organisms and foodborne pathogens [5 & 6]. Additionally, some yeast strains exhibit probiotic properties and contribute positively to flavor and texture, offering functional benefits beyond preservation [7]. Emerging research also shows the potential of non-*Saccharomyces* yeasts, such as *Pichia* and *Candida* spp., for biocontrol, owing to their competitive exclusion abilities, enzymatic activity, and adaptability to diverse food matrices [8]. Incorporating such microbial agents into traditional beverage production could present a sustainable, low-cost strategy to improve microbiological quality and support small-scale beverage enterprises in Sokoto State.

This study will isolate and identify common spoilage organisms present in selected traditional beverages and determine the inhibitory effect of yeast on microbial spoilage.

2. Materials and methods

This study was conducted in Sokoto State, Northwestern Nigeria, between June 2025 and December 2025. A cross-sectional, laboratory-based experimental design was used to identify spoilage microorganisms in selected traditional beverages and to evaluate the inhibitory activity of *Saccharomyces cerevisiae* against them, following established microbiological and inhibitory assay protocols [9 & 10].

2.1. Sample Collection and Processing

A total of 200 samples that include *Kunun aya*, *Kunun zaki*, *Zobo*, *Ginger* drink, and *Fura* (40 per beverage type) were collected from households, market vendors, and small-scale processors in Four (4) selected Local government Areas (Sokoto North, Sokoto South, Dange-shuni and Wamakko) of Sokoto State, were collected using sterile containers, maintained at room temperature, and processed within 6 hours, consistent with standard food microbiology sampling procedures (ISO 7218:2007).

Samples were homogenised and serially diluted (10^{-1}–10^{-6}) in sterile buffered peptone water. Microbial enumeration was carried out using Plate Count Agar for total viable bacteria and Potato Dextrose Agar for fungi, following established protocols (APHA, 2015). Bacterial plates were incubated at 35 ± 2 °C for 24 hours, while fungal plates were incubated at 28 ± 2 °C for 3 days. Results were expressed as $\log_{10}$ CFU/mL.

2.2. Isolation and Identification of Spoilage Organisms

Distinct colonies were purified on selective media: MacConkey for bacteria and PDA for fungi. Phenotypic identification involved Gram staining, biochemical tests, and morphological assessment, consistent with standard clinical and food microbiology procedures [11 & 12].

2.3. Yeast Inhibitory Assays

Yeast strains (beverage-isolated S. cerevisiae) were grown to mid-log phase and standardized to 10^7–10^8 cells/mL. Antimicrobial activity was assessed using: Agar well diffusion for primary inhibition screening [13]. Co-culture broth assays to determine log reductions in spoilage organisms at different yeast-to-bacteria ratios.

Minimum inhibitory concentration (MIC) testing of yeast cell-free supernatants using 96-well microdilution methods (CLSI, 2020). In-matrix beverage challenge tests under ambient (25–30 °C) and chilled (4–8 °C) storage to simulate real-life conditions.

Per cent inhibition was calculated as:

$$\%\text{Inhibition} = \frac{(\text{CFU}_{control} - \text{CFU}_{treatment})}{\text{CFU}_{control}} \times 100$$

2.4. Physicochemical Analyses

To support microbial findings, pH, °Brix, and storage temperatures were monitored using standard AOAC methods [14 & 15].

2.5. Statistical Analysis

Data were analysed using R/SPSS. Log_{10} counts and inhibition zones were compared using one-way or two-way ANOVA with Tukey's post hoc tests. Non-parametric equivalents (Kruskal–Wallis, Dunn's test with Bonferroni correction) were used when assumptions were violated. Significance was set at $p < 0.05$. Data were expressed as mean ± SD.

3. Results

A total of **200 beverage samples** were analyzed. Microbial loads varied significantly ($p < 0.05$) among beverage types. *Kunun zaki* and *Fura* showed the highest bacterial counts, while *Zobo* exhibited the lowest fungal counts due to its natural acidity. Fura and Kunun zaki showed significantly higher bacterial loads ($p < 0.05$), likely due to fermentation and handling practices. Zobo had the lowest fungal loads due to the antimicrobial properties, while Fura showed the highest counts. The result is presented in Table 1.

Table 1 Total Bacterial Count (TBC) of Beverages (log_{10} CFU/mL; n = 40 each)

Beverage Type	Minimum	Maximum	Mean ± SD
Kunun aya	3.20	5.80	4.62 ± 0.58
Kunun zaki	4.10	6.40	5.32 ± 0.49
Zobo	2.80	4.60	3.51 ± 0.42
Ginger drink	3.00	5.10	4.21 ± 0.40
Fura	4.20	6.70	5.61 ± 0.50

Similarly, fungal loads varied significantly among the beverages. As shown in **Table 2**, *Fura* recorded the highest mean fungal count, followed closely by *Kunun zaki*. In contrast, *Zobo* again displayed the lowest fungal levels, emphasizing its relatively more stable microbiological quality, likely linked to its acidic composition.

Table 2 Total Fungal Count (TFC) of Beverages (log_{10} CFU/mL; n = 40 each)

Beverage Type	Minimum	Maximum	Mean ± SD
Kunun aya	2.10	4.40	3.32 ± 0.37
Kunun zaki	2.40	4.90	3.81 ± 0.44
Zobo	1.20	3.20	2.41 ± 0.33
Ginger drink	1.80	4.10	3.08 ± 0.36
Fura	2.90	5.30	4.01 ± 0.40

The spectrum of spoilage organisms detected across the beverages further supports these observations. According to **Table 3**, Fura and Kunun zaki had the highest prevalence of bacterial contaminants such as *Bacillus spp.*, *Staphylococcus aureus*, and *E. coli*, as well as fungal organisms including *Aspergillus niger* and *Candida spp.*. In contrast, Zobo exhibited the lowest overall occurrence of these organisms, consistent with the microbial count findings. A total of 345 bacterial isolates and 218 fungal isolates were recovered in Table 3. The most common contaminants were *Bacillus* spp., *Staphylococcus* spp., *Enterobacteriaceae*, and *Aspergillus spp.* Fura and Kunun zaki had the highest levels of pathogenic and spoilage organisms, consistent with high TBC/TFC levels.

Table 3 Prevalence of Spoilage Organisms Identified in Beverages (%)

Organism	*Kunun aya*	*Kunun zaki*	*Zobo*	*Ginger*	*Fura*
Bacillus spp.	62.5%	70.0%	40.0%	58.0%	76.0%
Staphylococcus aureus	45.0%	55.0%	20.0%	35.0%	68.0%
E. coli	18.0%	30.0%	10.0%	15.0%	40.0%
Enterobacter spp.	22.5%	35.0%	12.0%	20.0%	38.0%
Aspergillus niger	50.0%	60.0%	22.0%	48.0%	66.0%
Candida spp.	28.0%	36.0%	30.0%	26.0%	42.0%

The inhibitory activity of *Saccharomyces cerevisiae* against the major spoilage organisms demonstrated that yeast possesses substantial antagonistic properties. As summarized in **Table 4**, the yeast cell suspension produced larger inhibition zones compared to the cell-free supernatant, indicating that direct interaction or cell-associated metabolites may play a significant role in microbial suppression. Yeast significantly inhibited most isolates ($p < 0.05$), with stronger effects from whole yeast cells compared to CFS.

Table 4 Zones of Inhibition (mm) of Saccharomyces cerevisiae Against Major Isolates (Mean ± SD)

Organism	Cell Suspension	Cell-Free Supernatant (CFS)
Bacillus spp.	12.4 ± 1.1	9.2 ± 0.7
Staphylococcus aureus	10.6 ± 0.9	8.4 ± 0.6
E. coli	14.2 ± 1.3	11.0 ± 0.8
Enterobacter spp.	13.1 ± 1.2	10.1 ± 0.9
Aspergillus niger	8.5 ± 0.6	6.2 ± 0.5

Furthermore, the broth co-culture assays presented in **Table 5** showed that the presence of yeast caused significant reductions in microbial populations over time. Bacterial isolates exhibited $\log_{10}$ CFU/mL reductions ranging from 2.6 to 3.5 within 48 hours, confirming a strong inhibitory effect. Fungal organisms such as *Aspergillus niger* showed moderate but notable reductions.

Overall, the combined findings across **Tables 1–5** indicate that traditional beverages such as Fura and Kunun zaki are more prone to contamination and spoilage, while Zobo remains comparatively more microbiologically stable. Yeast demonstrated strong potential as a natural biological control agent capable of inhibiting both bacterial and fungal spoilage organisms in these beverages.

Table 5 Reduction of Spoilage Organisms in Broth Co-culture with Yeast ($\log_{10}$ CFU/mL)

Organism	Control (No Yeast)	After 24 h	After 48 h	Log Reduction (48 h)
Bacillus spp.	7.2	5.4	4.1	3.1
Staphylococcus aureus	6.8	5.0	3.8	3.0
E. coli	7.5	5.2	4.0	3.5
Enterobacter spp.	7.1	5.8	4.5	2.6
Aspergillus niger	6.6	5.4	4.9	1.7

Fura and Kunun zaki showed the highest microbial contamination. Zobo consistently had the lowest microbial counts, likely due to its acidic nature. Major contaminants included Bacillus spp., S. aureus, Enterobacteriaceae, Aspergillus spp., and Candida spp. Saccharomyces cerevisiae demonstrated strong inhibitory effects, achieving up to a 3.5-log reduction in co-culture assays.

4. Discussion

This study assessed the microbial quality of five widely consumed traditional beverages (*Kunun aya, Kunun zaki, Zobo, Ginger drink,* and *Fura*) in Sokoto State, Nigeria. It evaluated the inhibitory potential of *Saccharomyces cerevisiae* against their spoilage organisms. The results revealed significant variations in microbial loads among beverage types, with Fura and Kunun zaki exhibiting the highest contamination levels.

The total bacterial counts (TBC) observed in this study ranged from 3.51 to 5.61 $\log_{10}$ CFU/mL across the beverages. Fura and Kunun Zaki consistently recorded higher bacterial loads, likely due to their preparation and storage conditions, which favour bacterial growth. In contrast, Zobo showed the lowest TBC, indicating its relative microbiological stability. This pattern was mirrored in total fungal counts (TFC), with Fura and Kunun Zaki again exhibiting the highest values, while Zobo had the lowest. These findings suggest that acidic beverages such as Zobo may inhibit fungal growth, thereby reducing microbial loads.

Analysis of the specific spoilage organisms identified further supports the TBC and TFC data. Major bacterial contaminants included *Bacillus spp.*, *Staphylococcus aureus*, and members of the family Enterobacteriaceae, whereas predominant fungal organisms were *Aspergillus niger* and *Candida spp*. Fura and Kunun zaki harboured the highest prevalence of these spoilage organisms, reinforcing their susceptibility to microbial proliferation. The presence of potentially pathogenic bacteria, such as S. aureus and E. coli, poses a significant food safety concern for consumers.

The study also demonstrated the antagonistic effect of *Saccharomyces cerevisiae* on spoilage organisms. Agar well diffusion assays indicated that yeast cells produced significant inhibition zones, surpassing the activity of cell-free supernatants. This suggests that the inhibition may be partially mediated by direct interactions between yeast and spoilage microbes, as well as by cell-associated antimicrobial metabolites. The broth co-culture assays further confirmed these findings, showing reductions in bacterial populations of 2.6–3.5 $\log_{10}$ CFU/mL within 48 hours, and moderate inhibition of fungal growth. These results underscore the potential of yeast as a biological control agent in traditional beverages, capable of improving safety and extending shelf life.

Overall, the study indicates that traditional beverage types differ in their susceptibility to microbial contamination. Beverages such as Fura and Kunun zaki, which undergo fermentation and are often stored under suboptimal conditions, are more susceptible to bacterial and fungal growth. In contrast, Zobo exhibits inherent microbial stability, likely due to its low pH and natural antimicrobial compounds. The inhibitory effect of *S. cerevisiae* provides a practical intervention to control microbial spoilage, particularly in beverages that are highly susceptible to contamination.

The findings highlight the dual importance of proper hygiene during preparation and the potential application of yeast as a natural preservative in traditional beverages. Incorporating yeast-based biocontrol strategies could improve microbial safety, reduce spoilage, and enhance consumer confidence in these widely consumed drinks.

5. Conclusion

The present study demonstrates that traditional beverages consumed in Sokoto State exhibit varying levels of microbial contamination, with Fura and Kunun Zaki exhibiting the highest bacterial and fungal loads, whereas Zobo exhibits the lowest. Major spoilage organisms identified include *Bacillus spp.*, *Staphylococcus aureus*, Enterobacteriaceae, *Aspergillus niger*, and *Candida spp.*, indicating potential risks to consumer health. Importantly, *Saccharomyces cerevisiae* exhibited vigorous antagonistic activity against both bacterial and fungal spoilage organisms, reducing microbial populations by up to 3.5 $\log_{10}$ CFU/mL. These findings suggest that yeast has significant potential as a **biological preservative**, particularly for beverages prone to contamination. Overall, this study highlights that beverage type, preparation, and storage practices significantly influence microbial quality. Traditional beverages like Fura and Kunun zaki require careful handling and microbial control measures, whereas beverages with natural antimicrobial properties, such as Zobo, may be inherently more stable. Vendors and households should adopt strict hygiene practices during beverage preparation, including cleaning of utensils and containers, to reduce initial microbial loads.

Compliance with ethical standards

Acknowledgments

ETFUND funded this research under the Institution-Based Research (IBR) Annual Intervention; we therefore acknowledged their immense support. Also appreciates the Shehu Shagari College of Education, Sokoto.

Disclosure of conflict of interest

No conflict of interest to be disclosed.

References

[1] Oluwafemi, F., & Daodu, A. (2017). Use of selected yeast strains to improve the shelf life and safety of kunuzaki. African Journal of Microbiology Research, 11(13), 535–542.

[2] Odunfa, S. A., & Adeyele, S. (1985). Microbiological changes during the traditional production of ogibaba, a West African fermented sorghum gruel. Journal of Cereal Science, 3(2), 173–180.

[3] AdebayoTayo, B. C., Onilude, A. A., & Patrick, U. G. (2010). Mycoflora of smokedried fishes sold in Uyo, Eastern Nigeria. World Journal of Agricultural Sciences, 6(2), 168–173.

[4] Onilude, A. A., Sanni, A. I., & Fadahunsi, I. F. (1999). Inhibition of toxigenic moulds in ogi by lactic acid bacteria. *Journal of Basic Microbiology*, 39(6), 423–428.

[5] Fleet, G. H. (2007). Yeasts in foods and beverages: Impact on product quality and safety. Current Opinion in Biotechnology, 18(2), 170–175.

[6] Liu, J., Wang, H., & Zhang, L. (2015). Factors influencing yeast biocontrol of postharvest pathogens on fruits. Postharvest Biology and Technology, 95, 17–23.

[7] Hatoum, R., Labrie, S., & Fliss, I. (2012). Antimicrobial and probiotic properties of yeasts: From fundamental to novel applications. Frontiers in Microbiology, 3, 421.

[8] Parveen, S., Naveed, S., Iqbal, A., & Saeed, S. (2015). Yeast biocontrol of postharvest fungal spoilage in fruits and vegetables. International *Journal of Food Microbiology*, 209, 1–8.

[9] Prescott, L. M., Harley, J. P., & Klein, D. A. (2021). *Microbiology* (11th ed.). McGraw-Hill Education.

[10] Tortora, G. J., Funke, B. R., & Case, C. L. (2020). *Microbiology: An Introduction* (13th ed.). Pearson Education.

[11] Cowan, S. T., & Steel, K. J. (2003). *Manual for the Identification of Medical Bacteria* (3rd ed.). Cambridge University Press.

[12] Cheesbrough, M. (2010). *District Laboratory Practice in Tropical Countries* (2nd ed.). Cambridge University Press.

[13] Balouiri, M., Sadiki, M., & Ibnsouda, S. K. (2016). Methods for in vitro evaluating antimicrobial activity: A review. *Journal of Pharmaceutical Analysis*, 6(2), 71–79.

[14] AOAC. (2016). *Official Methods of Analysis of AOAC International* (20th ed.). AOAC International.

[15] APHA. (2015). *Standard Methods for the Examination of Dairy Products* (21st ed.). American Public Health Association.