# Characterisation and Identification of Yeasts Associated With Indigenously Brewed *Burukutu* Drink Produced from Different Sorghum Varieties

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**ARTICLE HISTORY**
Received Date: 13th May, 2025
Accepted Date: 23rd July, 2025

http: www.njphr.nspri.gov.ng
ISSN: 2630-7022

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**CONFLICT OF INTEREST:** None
**ETHICAL APPROVAL:** Not Applicable

This is a publication of the Nigerian Stored Products Research Institute (NSPRI)

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## Abstract

*Burukutu is a traditional African alcoholic beverage produced by spontaneous fermentation of malted red or white sorghum. Its unstandardised production often results in variability in microbial composition and product quality. This study investigated the microbial and fermentation profiles of burukutu prepared from both sorghum varieties, with emphasis on yeast population dynamics and acidification patterns. Laboratory-scale fermentation and analyses of locally brewed samples were conducted, with pH monitored over 48 hours. Yeasts were isolated and identified using morphological and microscopic features, alongside carbon assimilation profiles via the API 20C AUX system. Fermentation was characterised by a progressive decline in pH, confirming active microbial metabolism. Seven yeast species were identified: Saccharomyces carlsbergensis, Schizosaccharomyces pombe, Saccharomyces cerevisiae, Candida tropicalis, Candida auringiensis, Candida krusei, and Candida utilis. Among these, S. cerevisiae was predominant (36% of isolates), particularly in traditional samples. Its broad sugar assimilation and strong fermentative capacity underscore its central role in burukutu production. The dominant genera were Saccharomyces and Candida, consistent across both red and white sorghum fermentations. Comparative analysis revealed similar microbial and acidification profiles for the two sorghum varieties, indicating their equal suitability as raw materials. Notably, laboratory-prepared burukutu displayed more consistent yeast populations, suggesting that improved hygienic practices can enhance product quality and reduce microbial variability compared to traditional brewing.Overall, this study highlights the microbial ecology of burukutu fermentation, confirming the predominance of S. cerevisiae and underscoring the potential for process standardisation to improve quality and reproducibility in this indigenous beverage.*

**Keywords:**
API 20C AUX, *Burukutu*, Fermentation, *Saccharomyces cerevisiae*, Sorghum.

## Introduction

*Burukutu* is a traditional African alcoholic beverage produced mainly from sorghum through spontaneous fermentation. It is widely consumed in West African countries, particularly in Nigeria, Ghana, and the Benin Republic, due to its affordability, nutritional richness, and ability to serve as both food and drink (Fadahunsi et al., 2013; Djameh, 2021). The beverage is opaque with a vinegar-like taste. It provides essential nutrients such as dietary proteins, carbohydrates, B-complex vitamins, vitamin E, minerals, dietary fibre, and essential amino acids, although it is limited in cystine and tryptophan (Yusuf et al., 2024). In addition, *burukutu* contains bioactive phenolic compounds, including tannins, flavonoids, and anthocyanins, which contribute to its functional and health-promoting properties (Coulibaly et al., 2020). These attributes have made *burukutu* a culturally significant beverage and a potential source of nutraceutical value. The production of *burukutu* involves steeping, germination, mashing, spontaneous fermentation, and maturation of sorghum grains, primarily Sorghum bicolour and Sorghum vulgare (Kolawole et al., 2007; Chikodili et al., 2015). However, because the process relies on uncontrolled natural fermentation, the microbial community is highly diverse, with predominant contributions from yeasts belonging to

**How to cite:**
Abel, O. O., Echendu, A. M., Oyelakin, M. O., Balogun, D. A., Ajala, O. V., & Asaoye V. O. (2025). Characterisation and Identification of Yeasts Associated With Indigenously Brewed *Burukutu* Drink Produced from Different Sorghum Varieties. *Nigerian Journal of Post-Harvest Research*, 3(2), 38-44.

*Saccharomyces*, *Candida*, *Schizosaccharomyces*, and *Geotrichum* (Umeh et al., 2019; Sawadogo-Lingani et al., 2021). The metabolic activity of these yeasts determines the alcohol content, flavour development, and overall quality of the beverage. In particular, *Saccharomyces cerevisiae* has been consistently reported as the key fermenter, driving ethanol production and improving organoleptic properties (Umeh et al., 2019). Despite the nutritional and cultural importance of *burukutu*, its production remains artisanal, leading to inconsistencies in quality, safety, and shelf life. Identifying and characterising the indigenous yeast strains responsible for fermentation is therefore essential. Such knowledge not only provides insights into the microbial ecology of *burukutu* but also presents opportunities for the development of defined starter cultures that could standardise production, enhance product safety, and open new pathways for biotechnological applications (Ofosu et al., 2020; Khalid et al., 2022).

This study was therefore designed to isolate, characterise, and identify the predominant yeasts associated with *burukutu* production. By understanding their fermentation potential and contribution to beverage quality, this work provided a scientific basis for improving traditional brewing practices and supports the development of controlled fermentation strategies for *burukutu*.

## Materials and Methods

### Collection of samples

Fresh locally prepared burukutu (200 mL) sample was obtained from Odogbo barracks, Ojo, Ibadan, Oyo state and collected in a sterile bottle for immediate analysis in the laboratory. The red and white varieties of sorghum grains (500 g each) used for the laboratory preparation of *burukutu* were purchased from Bodija market, Ibadan, Oyo state, in sterile polyethene bags. The grains were thoroughly cleaned to remove dust and other extraneous materials before use.

### Laboratory production of *burukutu*

*Burukutu* was produced in the laboratory following the method described by Chinedu et al. (2010) with slight modifications. The sorghum grain varieties were individually sorted, dehulled, and soaked with clean tap water in a plastic container for 24 h at 30 ˚C without changing the water. The soaked grains were washed, and excess water drained. The drained grains were then spread uniformly on a moist jute bag. Clean banana leaves were used to cover the grains on the jute bag to reduce dehydration. The grains were kept moist by spraying with clean tap water every morning before they were turned over at intervals. Grain germination continued for 96 h at 30 ˚C. The germinated or malted sorghum grains were sun-dried in line with rural practices. The dried malted grains were milled into flour and sieved (0.2 mm pore size) to obtain fine particles. The flour obtained was stored in a sterile polyethene bag at ambient temperature before analysis (Chinedu et al., 2010). An adjunct, in the form of *garri*, was mixed with the ground malted sorghum flour and warm water (45 ˚C) in the ratio 1:2:6 of *garri*: malt: water, respectively. The mixture was left for 48 h to ferment. The fermented mixture was boiled at 100 ˚C for 4 h, and then the drink matured for another 48 h. The fermented drink was analysed after cooling.

### Isolation of yeasts from *burukutu* samples

Yeasts were isolated from the locally and laboratory-prepared *burukutu* samples. The samples were analysed every 12 h for 48 h and were serially diluted ($10^{-1}$ to $10^{-6}$) after continuous shaking. One millilitre of each sample was suspended in test tubes filled with 9 mL of sterile distilled water each. One millilitre each of dilutions $10^{-3}$ and $10^{-4}$ was dispensed into sterile Petri dishes following the pour plate method, after which about 20 mL of cooled (45 ˚C), sterilised molten malt extract agar (MEA) having streptomycin (50 mg/L) was introduced aseptically. The plates were left to set before incubation (30 ˚C for 48 h). The emerging colonies following incubation in an oven (MIN0/6, Genlab, UK) were subcultured on solidified plates of malt extract agar with an inoculating needle under aseptic conditions to obtain pure culture (Coulibaly et al., 2022).

### Characterisation and identification of yeast cells

Viable, rejuvenated yeast cells of 3-day-old isolates were observed macroscopically in culture plates. The viable yeast cells were morphologically examined based on colour, surface, shape, elevation, edge, bud, and ascospore formation. Microscopically observations were made by taking a loopful of yeast isolate mixed with a bit of sterile distilled water on the glass slide. After that, it is levelled and covered using a cover slip. The smears were observed under a light microscope at 1000× magnification (Terryana et al., 2022). The yeast isolates were characterised to species level using the API 20 C AUX kit (API Laboratory Products Ltd) (Jimoh et al., 2012).

### pH determination of *burukutu* samples

The pH of locally made *burukutu* (LMB), laboratory-prepared *burukutu* from red sorghum (LBRS), and laboratory-prepared *burukutu* from white sorghum (LBWS)samples were determined with the aid of a digital pH meter (Model: Jenway 3310).

## Results and Discussion

### Isolation of yeast strains from *burukutu* samples

The frequency and percentage occurrence of different yeast strains isolated from *burukutu* samples fermented under laboratory and local conditions using red and white sorghum are represented in Table 1. 75 isolates were identified, comprising seven yeast species most predominantly *Saccharomyces cerevisiae*, accounting for 36% of the total isolates. Its dominance is due to its sugar metabolism and ethanol production efficiency, making it the principal fermenter in many traditional alcoholic beverages (Zhang et al., 2023). *Candida krusei* had a high occurrence of 22.67%, highlighting its ability to tolerate acidic and alcoholic environments. The relatively high frequency of this species suggests its contributory role in *burukutu* fermentation, possibly influencing flavour and aroma. *Candida tropicalis* (10.67%) and *Schizosaccharomyces pombe* (9.33%) occurred at moderate levels.

In comparison, *Candida utilis* (6.67%) was less common, absent in locally brewed red and white sorghum samples but present in laboratory-brewed samples. The least represented yeast was *Candida auringiensis* (2.67%), indicating it plays only a minor role in fermentation. Generally, the predominance of *Saccharomyces* and *Candida* genera corroborates previous findings (Umeh et al., 2022; Ogu et al., 2025), confirming them as the dominant contributors to *burukutu* fermentation. The variation in species distribution between laboratory and local brewing reflects the impact of fermentation environment and hygienic practices on microbial ecology (Jimoh et al., 2012; Falegan & Akoja, 2014).

The morphological and microscopic features of the yeast strains isolated from *burukutu* are outlined in Table 2. The isolates displayed differences in shape, colour, colony elevation, surface texture, edge characteristics, bud formation, and ascospore production, which guided their identification. *Saccharomyces cerevisiae* and *S. carlsbergensis* were identified as spherical, cream-colored yeasts with smooth, raised colonies. Both formed ascospores, with *S. cerevisiae* showing single budding and *S. carlsbergensis* exhibiting multipolar budding. *Schizosaccharomyces pombe* was distinct, forming spherical white colonies that were flat, smooth with an entire edge, and a bipolar budding. Candida species exhibited greater morphological diversity. *C. utilis* possessed an ellipsoidal, flat, smooth, multipolar budding, *C. krusei* gave a spherical, flat, dry surface with an undulating edge, *C. auringiensis* was spherical, raised, smooth, with a single budding, while *C. tropicalis* presented an elongated, flat, smooth morphology with single budding.

These observations emphasised the overlapped morphology among the isolated yeasts, hence the need for confirmatory biochemical identification, such as the API 20C AUX system employed (Jimoh et al., 2012; Devadas et al., 2017). The consistent identification of *Saccharomyces spp*. and *Candida spp*. aligns with their established roles as dominant fermenters in traditional sorghum-based beverages.

**Table 1. Frequency of occurrence of yeasts strains in burukutu beverage**

| Yeasts Isolates | LMB | LBRS | LBWS | Frequency of occurrence | Percentage Occurrence (%) |
|---|---|---|---|---|---|
| *Saccharomyces cerevisiae* | 12 | 8 | 7 | 27 | 36 |
| *Saccharomyces carlsbiensis* | 3 | 2 | 4 | 9 | 12 |
| *Schizosaccharomyces pombe* | - | 5 | 2 | 7 | 9.33 |
| *Candida utilis* | 5 | - | - | 5 | 6.67 |
| *Candida krusei* | 5 | 7 | 5 | 17 | 22.67 |
| *Candida auringiensis* | - | 1 | 1 | 2 | 2.67 |
| *Candida tropicalis* | - | 3 | 5 | 8 | 10.67 |
| Total number of isolates | 25 | 26 | 24 | 75 | 100 |

*Keys: LMB-Locally made burukutu; LBRS-Laboratory made burukutu with red sorghum; LBWS-Laboratory made burukutu with white sorghum*

**Table 2. Characteristics of yeast morphology isolated from burukutu**

| Shape | Colour | Elevation | Surface | Edge | Bud Formation | Ascospore Formation | Probable Isolate |
|---|---|---|---|---|---|---|---|
| Spherical | Cream | Raised | Shiny/Smooth | Entire | Singly Budding | + | *Saccharomyces cerevisiae* |
| Spherical | Cream | Raised | Shiny/Smooth | Entire | Multipolar Budding | + | *Saccharomyces carlsbiensis* |
| Spherical | White | Flat | Shiny/Smooth | Entire | Bipolar | + | *Schizosaccharo myces pombe* |
| Ellipsoidal | Cream | Flat | Smooth | Entire | Multipolar Budding | + | *Candida utilis* |
| Spherical | Cream | Flat | Dry/Smooth | Undulating | Multipolar Budding | + | *Candida krusei* |
| Spherical | Cream | Raised | Shiny/Smooth | Entire | Singly Budding | + | *Candida auringiensis* |
| Elongated | Cream | Flat | Shiny/Smooth | Entire | Singly Budding | + | *Candida tropicalis* |

**Yeast identification by carbon assimilation**

Table 3 presents the carbon assimilation profiles of the yeast strains isolated from *burukutu*, determined using the API 20C AUX kit. Carbon assimilation is a key physiological marker for distinguishing yeast species, since it reflects their ability to utilise sugars and related compounds as carbon and energy sources (Jimoh et al., 2012; Devadas et al., 2017). Most isolates, particularly *Saccharomyces cerevisiae*, *Schizosaccharomyces pombe*, *Candida tropicalis*, *Candida utilis*, and *S. carlsbergensis*, showed strong assimilation of D-glucose, confirming its role as the primary fermentable sugar during *burukutu* production. However, *Candida auringiensis* could not assimilate glucose, suggesting limited fermentative potential and supporting its low frequency of occurrence (2.67%) as observed in Table 1. Assimilation of galactose was generally positive across isolates except for *C. auringiensis* and C. *krusei*, which were negative, implying their inability to metabolise this sugar as a sole carbon source. Interestingly, *S. cerevisiae* was negative for xylose utilisation, a finding consistent with previous studies that attribute this to the absence of xylose reductase and xylitol dehydrogenase enzymes (Songdech et al., 2022). In contrast, S. carlsbergensis displayed xylose assimilation, suggesting diversity of metabolic activity even within the *Saccharomyces* genus. The ability of *S. cerevisiae* to utilise multiple sugars such as maltose, sucrose, and raffinose highlights its broad fermentative potential and explains its dominance in the yeast population. The more limited carbon assimilation by *Candida species* suggests they play secondary roles in fermentation, likely influencing flavour and aroma rather than driving alcohol production.

**pH determination of *burukutu* samples**

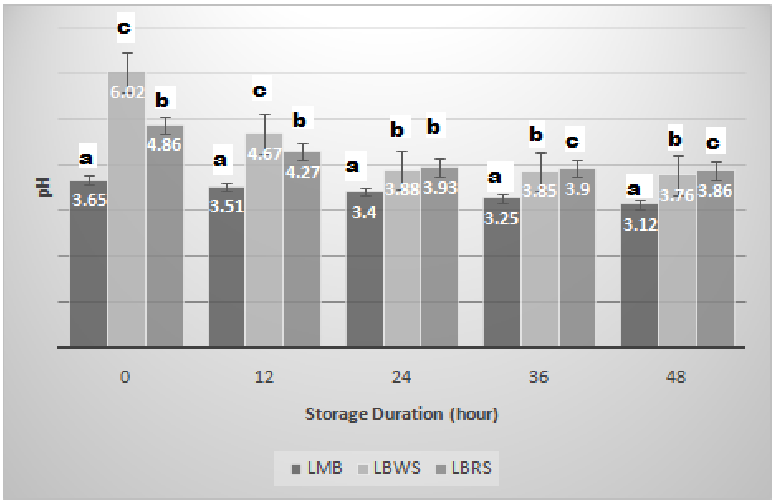
The pH variations in locally made *burukutu* (LMB) and laboratory-prepared *burukutu* from red sorghum (LBRS) and white sorghum (LBWS) over a 48-hour fermentation period are illustrated in Figure 1. There was a progressive decline in pH across all samples, signifying active microbial metabolism and acidification.

The pH dropped from 3.7 to 3.2 for LMB, while LBRS and LBWS also showed steady decreases, indicating that both red and white sorghum varieties supported comparable fermentation dynamics. The decline in pH aligns with reports by Bayoï & Etoa (2021) and Fadahunsi et al. (2013), who observed increased acidity during *burukutu* storage and fermentation. Acidification is primarily due to organic acid production and metabolic by-products of fermentative yeasts and bacteria (Fadahunsi et al., 2013). The reduction in pH is significant for microbial safety, inhibiting spoilage organisms and pathogens, thus extending shelf stability (Fadahunsi et al., 2013). Also, regarding fermentation efficiency, acidification reflects vigorous microbial activity, particularly by *Saccharomyces cerevisiae*, which dominated the fermentation system. Notably, the similarity in pH reduction between LBRS and LBWS suggests that red and white sorghum are equally suitable for *burukutu* brewing. However, the laboratory-prepared samples displayed slightly more consistent acidification, highlighting the role of controlled hygienic practices in improving fermentation outcomes compared to traditional methods (Bayoï & Etoa, 2021).

**Table 3. Identification of yeasts by carbon assimilation using API 20C AUX Kit**

| Carbon source | *S. cerevisiaece* | *Schizosaccharomyc pombe* | *Candida tropicalis* | *Candida auringien* | *Candida krusei* | *Candida utilis* | *Saccharomyces carlsbergiences* |
|---|---|---|---|---|---|---|---|
| D-glucose | + | + | + | - | + | + | + |
| Glycerol | + | + | + | - | + | - | + |
| Calcium2-keto-gluconate | - | - | + | + | + | - | + |
| L-arabinose | - | - | - | + | - | - | + |
| D-xylose | - | - | - | - | - | - | + |
| Adonitol | - | - | - | - | - | - | + |
| Xylitol | - | - | - | - | - | - | - |
| D-galactose | + | + | + | - | - | + | + |
| Inositol | - | - | - | - | - | - | - |
| D-sorbitol | - | - | + | - | - | - | + |
| Methyl-α-D-glucopyranoside | - | - | - | - | - | - | + |
| N-acetyl-glucosamine | + | + | + | - | + | - | + |
| D-celiobiose | - | - | + | - | - | - | + |
| D-lactose | - | - | - | - | - | - | - |
| D-maltose | + | + | - | + | - | - | + |
| D-saccharose | + | + | - | - | - | - | + |
| D-trehalose | - | - | - | - | + | - | + |
| D-melezitose | - | - | - | - | - | - | + |
| D-raffinose | - | - | - | - | + | - | + |

*Keys. (+) = Carbon assimilation (-) = No carbon assimilation*




Each bar represents the mean of triplicate determinations ± standard error. Mean values on the bars with different letters are significantly different ($P < 0.05$)

**Figure 1. Changes in the pH of LMB ( ), LBRS ( ), and LBWS ( ) from 0-48 h of storage**

## Conclusion

Yeast species were successfully isolated, characterised, and identified from the fermentation of *burukutu* produced from red and white sorghum grains using both traditional and laboratory methods. *Saccharomyces cerevisiae* occurred most frequently in all the samples among the seven yeast strains identified, with much abundance in the locally brewed drink. Similar strains of yeasts in the laboratory-prepared samples indicate that both sorghum varieties are suitable for *burukutu* production. Laboratory conditions limited the presence of undesired or opportunistic yeasts, indicating improved hygienic practice over the locally brewed alcoholic beverage. Therefore, the laboratory environment presented a reproducible alternative that retains the core microbial population of burukutu and enhances its safety and quality. These findings are a foundation for the standard procedure for scaling up *burukutu* production to an industrial level.

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