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Isolation, Characterization, and Fermentation Assessment of Indigenous Yeasts from Selected Traditional Nigerian Alcoholic Beverages (Burukutu, Pito and Palm Wine)

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ABSTRACT

Traditional fermented beverages are important sources of indigenous microorganisms, particularly yeasts, which contribute to fermentation processes, product characteristics, and the development of locally valuable fermented products. This study evaluated indigenous yeasts from burukutu, pito and palm wine and assessed their fermentation performance in beverages and malt extract. Yeasts were isolated and characterized from three independent sources of burukutu, pito and palm wine using cultural, microscopic and biochemical methods, followed by controlled fermentation and physicochemical analyses. Palm wine had the highest yeast count (2.65×10^8 CFU/mL), followed by pito (7.20×10^7 CFU/mL) and burukutu (1.98×10^7 CFU/mL); differences were significant ($p < 0.001$). All isolates were provisionally identified as *Saccharomyces* spp., with no significant association between beverage source and biochemical traits. Beverage pH ranged from 3.54 to 3.77, without significant differences, while malt extract was less acidic (pH 5.01). Indigenous yeast populations increased from 7.44–7.52 $\log_{10}$ CFU/mL initially to 8.45–8.63 at 48 hours, and declined slightly by 72 hours. Brewer's yeast reached 8.61–8.72 $\log_{10}$ CFU/mL at 48 hours. Specific gravity decreased from 1.03–1.05 to 1.01–1.02, while pH declined during fermentation, indicating substrate conversion and acidification. Uninoculated sterilized controls showed no detectable yeast growth and stable specific gravity. The findings identify palm wine as a promising source of indigenous yeasts and demonstrate fermentation potential across fermentation media, thus highlighting their potential for beverage production and supporting further molecular identification and industrial application studies.

Keywords: Indigenous Yeasts, Traditional Fermented Beverages, Yeast Characterization, Fermentation.

Introduction

Traditional fermented alcoholic beverages constitute an important component of Nigeria's food heritage, providing locally produced drinks associated with cultural practices, indigenous raw materials and small-scale processing systems (Obafemi et al., 2024). Among these beverages, burukutu, pito and palm wine are widely recognized for their spontaneous or semi-controlled fermentation processes and distinctive sensory characteristics (Raheem et al., 2023). Burukutu and pito are predominantly cereal-based beverages prepared from malted grains such as sorghum, while palm wine is obtained from the sap of various palm species and undergoes rapid spontaneous fermentation after collection. Recent research has continued to demonstrate the diverse microbial communities associated with these beverages and their potential as reservoirs of useful fermentative microorganisms (Abel et al., 2025; Oduokpaha and Afolabi, 2025).

Yeasts are important microorganisms in alcoholic fermentation because of their ability to convert fermentable carbohydrates into ethanol, carbon dioxide and a wide range of secondary metabolites that contribute to beverage characteristics (He et al., 2023). Although *Saccharomyces cerevisiae* is widely employed as a conventional brewing organism, indigenous fermentation environments can harbour yeast populations with physiological characteristics adapted to local substrates and environmental conditions (Páez-Lerma et al., 2024). Investigations of Nigerian traditional beverages have reported *Saccharomyces* and other yeast genera, demonstrating that indigenous alcoholic beverages may provide sources of strains with potential technological applications (Oduokpaha and Afolabi, 2025). In burukutu, for example, recent research identified several yeast species, with *S. cerevisiae* occurring prominently among the recovered isolates (Abel et al., 2025).

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The search for indigenous fermentative yeasts is relevant to brewing because yeast performance depends on characteristics that extend beyond abundance or morphological identification. Important properties include carbohydrate assimilation, maltose utilization, growth rate, fermentation kinetics, ethanol tolerance, attenuation, flocculation, viability and production of desirable or undesirable metabolites. Ogu et al. (2025) demonstrated that a *S. cerevisiae* isolate obtained from burukutu exhibited several characteristics associated with brewing performance, including substantial flocculation and viability, supporting the possibility that indigenous beverages can serve as sources of candidate brewing yeasts. Similarly, recent screening of yeasts from Nigerian indigenous alcoholic beverages has identified multiple genera and species with potentially useful physiological properties (Oduokpaha and Afolabi, 2025).

Despite these findings, the presence of yeasts in a fermented beverage does not necessarily indicate suitability for brewing. Traditional beverages contain complex microbial communities, and yeast populations may vary according to raw materials, fermentation practices, temperature, acidity, nutrient availability and microbial succession. Consequently, isolation and characterization are necessary to distinguish potentially useful strains from organisms that merely occur as transient members of the fermentation microbiota.

Phenotypic and biochemical characterization provides an initial basis for differentiating isolates, while fermentation assessment can reveal their ability to grow under relevant substrate conditions. Studies of burukutu have similarly demonstrated that differences in fermentation conditions can influence yeast populations and acidification patterns (Abel et al., 2025).

Therefore, this study focuses on the isolation and characterization of indigenous yeasts associated with burukutu, pito and palm wine and the assessment of their fermentation behaviour. The study evaluates yeast abundance, colonial and microscopic characteristics, biochemical properties, physicochemical conditions and growth during fermentation in indigenous beverages and malt extract. The findings should improve understanding of yeast ecology in Nigerian traditional alcoholic beverages and provide evidence of their value as sources of fermentative yeasts for brewing applications.

Recent evidence that indigenous Nigerian beverages can yield technologically relevant yeasts further supports the importance of investigating these locally available microbial resources (Oduokpaha and Afolabi, 2025; Ogu et al., 2025).

Materials and Methods

Study design

The study was conducted in Benin City, Edo State, Nigeria, using burukutu, pito and palm wine obtained from selected traditional beverage producers. The investigation employed a comparative laboratory experimental design to isolate, characterize and assess the fermentation potential of indigenous yeasts associated with the three beverages.

Three independent sources were sampled for each beverage. Approximately 1.5 L of each beverage was aseptically collected into sterile, labelled, screw-capped bottles. Samples were transported to the laboratory in an insulated container containing ice packs, stored at approximately 4°C and analysed within 24 h of collection.

Physicochemical analysis

Physicochemical properties of the beverages were evaluated by determining pH (ISO, 2008) and specific gravity - ASTM D891-18 (ASTM, 2018). The pH was measured using a calibrated digital pH meter standardized with pH 4.00 and 7.00 buffer solutions. Specific gravity was determined using a triple-scale hydrometer, with readings taken at the lower meniscus after the hydrometer had stabilized.

Isolation and enumeration of yeast

Yeasts were isolated and enumerated using the serial dilution and spread-plate technique (Beuchat, 1992). One millilitre of each beverage was transferred into 9 mL sterile peptone water, followed by ten-fold serial dilution to 10^{-6}. Appropriate dilutions were spread on potato dextrose agar supplemented with 0.005% chloramphenicol and incubated at approximately 30°C for 48–72 h.

Characteristic yeast colonies were counted and populations expressed as CFU/mL. Representative colonies were repeatedly streaked on fresh PDA to obtain pure cultures, which were maintained on PDA slants at approximately 4°C.

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Microscopic and cultural characterizations

The purified isolates were characterized microscopically, culturally and biochemically (Nout et al., 2007). Microscopic examination using lactophenol cotton blue assessed cell shape, arrangement and budding pattern, while cultural characterization considered colony size, shape, colour, elevation, margin, surface texture and pigmentation.

Biochemical characterization

Biochemical characterization included catalase and urease tests. Carbohydrate fermentation was assessed using glucose, fructose, sucrose and maltose incorporated into phenol red potato dextrose broth containing Durham tubes. Acid production and gas formation were recorded as indicators of fermentation activity (Krieg and Holt, 1984).

Fermentation Potentials in Traditional and Model Brewing Worts

Fermentation potential was evaluated using traditional brewing worts prepared from burukutu, pito and palm wine and a standardized model wort prepared from commercial malt extract at 12% w/v (Khadka et al., 2024). The substrates were characterized for pH and specific gravity and sterilized at 121°C for 15 min before aseptic cooling. Yeast isolates were propagated in potato dextrose broth for 24–48 h and standardized to approximately 5×10^8 CFU/mL. Five millilitres of each suspension were inoculated into 245 mL of sterile wort to obtain an approximate initial concentration of 10^7 CFU/mL. A commercial *Saccharomyces cerevisiae* preparation served as the reference yeast, while uninoculated wort served as the negative control. Fermentations were conducted at approximately 30°C for 72 h with agitation at 150 rpm. Samples collected at 0, 24, 48 and 72 h were analysed for pH, specific gravity and viable yeast population.

Results

Table 1 presents the yeast populations recovered from the three traditional Nigerian alcoholic beverages varied considerably. Yeast populations varied significantly among the beverages. Burukutu recorded the lowest count ($1.98 \times 10^7 \pm 5.51 \times 10^5$ CFU/mL; 7.30 ± 0.01 $\log_{10}$ CFU/mL), followed by Pito ($7.20 \times 10^7 \pm 4.00 \times 10^6$ CFU/mL; 7.86 ± 0.02 $\log_{10}$ CFU/mL). Palm wine had the highest population ($2.65 \times 10^8 \pm 5.00 \times 10^6$ CFU/mL; 8.42 ± 0.01 $\log_{10}$ CFU/mL). The minimum and maximum counts followed the same pattern, increasing from $1.94–2.04 \times 10^7$ CFU/mL in burukutu, to $6.80–7.60 \times 10^7$CFU/mL in pito and $2.60–2.70 \times 10^8$ CFU/mL in palm wine. One-way analysis of variance demonstrated a statistically significant difference in mean yeast counts among the three beverages ($F_{2,6} = 3635.91$, $p < 0.001$). Tukey's honestly significant difference (HSD) test showed that all pairwise comparisons were significant: burukutu versus pito ($p < 0.001$), burukutu versus palm wine ($p < 0.001$), and pito versus palm wine ($p < 0.001$). Accordingly, the order of mean yeast abundance was palm wine > pito > burukutu.

Table 1: Yeast counts in the indigenous beverages

Beverages	Minimum yeast count (CFU/mL)	Maximum yeast count (CFU/mL)	Mean ± SD (n = 3) (CFU/mL)	Mean ± SD (n = 3) (Log_{10} CFU/mL)
Burukutu	19400000	20400000	19766666.67 ± 550757.05	7.30 ± 0.01
Pito	68000000	76000000	72000000 ± 4000000	7.86 ± 0.02
Palm wine	260000000	270000000	265000000 ± 5000000	8.42 ± 0.01

n: number of beverage samples; SD: standard deviation; values are reported as mean ± standard deviation.

Nine yeast isolates, three from each beverage that was examined and characterized were all provisionally identified as *Saccharomyces* spp. Six isolates (66.7%) produced cream colonies, while three (33.3%) were white. Circular, smooth colonies predominated (66.7%), with 77.8% showing raised elevation and 66.7% having entire margins. Microscopically, 66.7% displayed oval cells and 33.3% spherical cells. All isolates exhibited multilateral budding, while three (33.3%) produced pseudohyphae. Overall, the cultural and microscopic features supported their preliminary classification as *Saccharomyces* spp.

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The same biochemical pattern occurred across all three beverage sources, with no beverage-specific profile observed. Chi-square analysis showed no significant association between beverage source and urease activity (χ^2 = 0.000, df = 2, p = 1.000) or maltose utilization (χ^2 = 0.000, df = 2, p = 1.000).

The physicochemical properties of the three traditional alcoholic beverages and the malt extract are presented in Table 2. Among the beverages, burukutu recorded the lowest mean pH (3.54 ± 0.19), followed by pito (3.63 ± 0.22), whereas palm wine had the highest mean pH (3.77 ± 0.14). The malt extract was less acidic than the fermented beverages, with a mean pH of 5.01 ± 0.09.

The observed pH ranges were 3.35–3.73 for burukutu, 3.41–3.85 for pito, 3.63–3.91 for palm wine and 4.94–5.11 for malt extract. One-way analysis of variance conducted on the three beverage groups did not reveal a statistically significant difference in mean pH ($F_{2,6}$ = 1.16, p = 0.375). Malt extract was treated as the fermentation substrate/reference material rather than included in this beverage-to-beverage comparison. Specific gravity was comparatively uniform within each reported group. Burukutu and palm wine both had a mean specific gravity of 1.03 ± 0.00, pito recorded 1.04 ± 0.00, and malt extract had the highest value at 1.05 ± 0.00.

Table 2: Physicochemical characteristics of indigenous beverages and malt extract used for fermentation

Beverage	Parameter					
	pH			Specific Gravity		
	Minimum	Maximum	Mean ± SD (n = 3)	Minimum	Maximum	Mean ± SD (n = 3)
Burukutu	3.35	3.73	3.54 ± 0.19	1.03	1.03	1.03 ± 0.00
Pito	3.41	3.85	3.63 ± 0.22	1.04	1.04	1.04 ± 0.00
Palmwine	3.63	3.91	3.77 ± 0.14	1.03	1.03	1.03 ± 0.00
Malt	4.94	5.11	5.01 ± 0.09	1.05	1.05	1.05 ± 0.00

Key: n = number of beverage samples; SD = standard deviation

The fermentation potentials of selected indigenous yeast isolate in traditional and model brewing worts, as presented in Tables 3A–C, and commercial yeasts (Tables 4A – C.) were evaluated using two categories of brewing substrates. Traditional substrates were prepared from burukutu, pito and palm wine to represent the indigenous beverages from which the yeast isolates had been recovered. A standardized model wort was also prepared from commercial malt extract to provide a consistent basis for comparison. The traditional substrates reflected the natural fermentation environments associated with the respective yeast isolates, whereas the malt-extract wort provided a more controlled brewing medium. This approach enabled the fermentation characteristics of the selected yeasts to be assessed across both indigenous and standardized brewing substrates under comparative conditions.

Yeast populations increased in all six fermentation treatments with indigenous yeast during the first 48 h, after which a decline was recorded at 72 h (Table 3A). At the beginning of fermentation, counts ranged from 7.44 ± 0.02 to 7.52 ± 0.02 $\log_{10}$ CFU/mL. By 24 h, the populations had increased to between 7.98 ± 0.02 and 8.16 ± 0.02 $\log_{10}$ CFU/mL.

Further increases were observed at 48 h, when counts ranged from 8.45 ± 0.02 to 8.63 ± 0.02 $\log_{10}$ CFU/mL. The highest recorded population was 8.63 ± 0.02 $\log_{10}$ CFU/mL in malt extract inoculated with pito-recovered yeast. At 72 h, counts decreased in all treatments, ranging from 8.35 ± 0.02 to 8.54 ± 0.02 $\log_{10}$ CFU/mL. At 48 h, malt extract inoculated with pito-recovered yeast recorded the highest count (8.63 ± 0.02 $\log_{10}$ CFU/mL), followed by malt extract inoculated with palm-wine-recovered yeast (8.56 ± 0.02 $\log_{10}$ CFU/mL) and malt extract inoculated with burukutu-recovered yeast (8.57 ± 0.03 $\log_{10}$ CFU/mL). The corresponding indigenous beverage fermentations recorded 8.45 ± 0.02, 8.53 ± 0.03 and 8.46 ± 0.03 $\log_{10}$ CFU/mL for palm wine, pito and burukutu, respectively. Thus, the malt extract treatments generally supported slightly higher populations than their corresponding beverage-based treatments.

Specific gravity declined progressively in all six fermentation treatments over the 72-h monitoring period (Table 3B). At 0 h, values ranged from 1.03 ± 0.00 in the palm wine and burukutu systems to 1.05 ± 0.00 in all three malt extract systems.

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Table 3A: Yeast Counts during the Fermentation of Alcoholic Beverages with Indigenous Yeast

Fermentation Period (hour)	Yeast count during the fermentation (Log_{10} Mean ± SD CFU/mL) n = 3					
	Palmwine with yeast recovered from palm wine	Malt extract with yeast recovered from palm wine	Pito Extract with Pito-Recovered Yeast	Malt Extract with Pito-Recovered Yeast	Burukutu with Yeast Recovered from Burukutu	Malt Extract with Yeast Recovered from Burukutu
0	7.44 ± 0.02	7.44 ± 0.02	7.52 ± 0.02	7.52 ± 0.02	7.44 ± 0.03	7.44 ± 0.03
24	7.98 ± 0.02	8.06 ± 0.03	8.07 ± 0.03	8.16 ± 0.02	7.99 ± 0.03	8.08 ± 0.02
48	8.45 ± 0.02	8.56 ± 0.02	8.53 ± 0.03	8.63 ± 0.02	8.46 ± 0.03	8.57 ± 0.03
72	8.35 ± 0.02	8.47 ± 0.02	8.43 ± 0.04	8.54 ± 0.02	8.37 ± 0.03	8.48 ± 0.03

Table 3B: Specific Gravity during the Fermentation of Alcoholic Beverages with Indigenous Yeast

Fermentation Period (h)	Specific Gravity during the fermentation, n = 3					
	Palmwine with yeast recovered from palm wine	Malt extract with yeast recovered from palm wine	Pito Extract with Pito-Recovered Yeast	Malt Extract with Pito-Recovered Yeast	Burukutu with Yeast Recovered from Burukutu	Malt Extract with Yeast Recovered from Burukutu
0	1.03 ± 0.00	1.05 ± 0.00	1.04 ± 0.00	1.05 ± 0.00	1.03 ± 0.00	1.05 ± 0.00
24	1.02 ± 0.00	1.04 ± 0.00	1.03 ± 0.00	1.04 ± 0.00	1.02 ± 0.00	1.04 ± 0.00
48	1.02 ± 0.00	1.03 ± 0.00	1.03 ± 0.00	1.03 ± 0.00	1.02 ± 0.00	1.03 ± 0.00
72	1.01 ± 0.00	1.02 ± 0.00	1.02 ± 0.00	1.02 ± 0.00	1.01 ± 0.00	1.02 ± 0.00

Table 3C: pH during the Fermentation of Alcoholic Beverages with Indigenous Yeast

Fermentation Period (h)	pH during the fermentation, n = 3					
	Palmwine with yeast recovered from palm wine	Malt extract with yeast recovered from palm wine	Pito Extract with Pito-Recovered Yeast	Malt Extract with Pito-Recovered Yeast	Burukutu with Yeast Recovered from Burukutu	Malt Extract with Yeast Recovered from Burukutu
0	3.77 ± 0.14	5.00 ± 0.09	3.63 ± 0.22	5.01 ± 0.09	3.54 ± 0.19	5.01 ± 0.09
24	3.61 ± 0.14	4.71 ± 0.10	3.47 ± 0.20	4.69 ± 0.09	3.39 ± 0.19	4.70 ± 0.10
48	3.45 ± 0.14	4.40 ± 0.09	3.30 ± 0.18	4.36 ± 0.08	3.23 ± 0.18	4.38 ± 0.08
72	3.32 ± 0.13	4.18 ± 0.09	3.16 ± 0.16	4.12 ± 0.07	3.09 ± 0.16	4.15 ± 0.07

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After 24 h, specific gravity had decreased to 1.02 ± 0.00 in the palm wine and burukutu treatments, 1.03 ± 0.00 in the pito extract treatment, and 1.04 ± 0.00 in the malt extract treatments. At 48 h, values ranged from 1.02 ± 0.00 to 1.03 ± 0.00. By 72 h, the palm wine and burukutu fermentations had reached 1.01 ± 0.00, while the other four treatments recorded 1.02 ± 0.00. The total reduction between 0 and 72 h was 0.02 specific-gravity units in the palm wine, pito and burukutu beverage-based treatments, and 0.03 units in the corresponding malt extract treatments. The malt extract systems therefore showed a slightly larger absolute decline over the monitoring period. Despite this, the malt extract treatments retained higher final specific gravity values (1.02) than the palm wine and burukutu beverage treatments (1.01).

The pH of all six fermentation systems decreased progressively over the 72-h observation period (Table 3C). At 0 h, the three indigenous beverage treatments had pH values between 3.54 ± 0.19 and 3.77 ± 0.14, whereas the malt extract treatments began at approximately pH 5.00–5.01. After 24 h, pH had fallen to 3.39–3.61 in the beverage-based systems and 4.69–4.71 in the malt extract systems. A further decline was observed at 48 h, when values ranged from 3.23 to 3.45 in the beverage treatments and 4.36–4.40 in malt extract. At 72 h, the lowest pH was recorded in burukutu fermented with its indigenous yeast (3.09 ± 0.16), while the corresponding malt extract treatment recorded pH 4.15 ± 0.07. The final pH values for palm wine and pito were 3.32 ± 0.13 and 3.16 ± 0.16, respectively, while their corresponding malt extract systems recorded 4.18 ± 0.09 and 4.12 ± 0.07. Across the 72-h period, pH decreased by 0.45 units in palm wine fermented with palm-wine-derived yeast, 0.47 units in pito fermented with pito-derived yeast and 0.45 units in burukutu fermented with burukutu-derived yeast.

In the malt extract systems, the corresponding reductions were 0.82, 0.89 and 0.86 pH units. Thus, the malt extract treatments showed larger numerical decreases in pH than the indigenous beverage treatments, although their final pH values remained higher.

Fermentation of alcoholic beverages with commercial Brewer's yeast revealed that yeast populations increased in all four fermentation systems during the first 48 h, followed by a modest decline at 72 h (Table 4A). At the beginning of fermentation, all treatments had the same reported mean population of 7.53 ± 0.05 $\log_{10}$ CFU/mL. After 24 h, counts increased to 8.13 ± 0.03 $\log_{10}$ CFU/mL in palm wine, 8.17 ± 0.03 $\log_{10}$ CFU/mL in pito extract, 8.15 ± 0.03 $\log_{10}$ CFU/mL in burukutu and 8.22 ± 0.02 $\log_{10}$ CFU/mL in malt extract. The populations continued to rise and reached their highest values at 48 h, ranging from 8.61 ± 0.03 to 8.72 ± 0.02 $\log_{10}$ CFU/mL.

Table 4A: Yeast Counts during the Fermentation of Alcoholic Beverages with Brewer's Yeast

Fermentation Period (hour)	Yeast count during the fermentation ($\log_{10}$ Mean ± SD CFU/mL) n = 3			
	Palmwine with Brewer's Yeast	Pito Extract with with Brewer's Yeast	Burukutu with Brewer's Yeast	Malt Extract with Brewer's Yeast
0	7.53 ± 0.05	7.53 ± 0.05	7.53 ± 0.05	7.53 ± 0.05
24	8.13 ± 0.03	8.17 ± 0.03	8.15 ± 0.03	8.22 ± 0.02
48	8.61 ± 0.03	8.66 ± 0.02	8.65 ± 0.02	8.72 ± 0.02
72	8.52 ± 0.03	8.57 ± 0.02	8.56 ± 0.02	8.63 ± 0.02

Malt extract recorded the highest count at this time, while palm wine had the lowest. At 72 h, the yeast populations declined slightly in all treatments, with counts of 8.52 ± 0.03 $\log_{10}$ CFU/mL in palm wine, 8.57 ± 0.02 $\log_{10}$ CFU/mL in pito extract, 8.56 ± 0.02 $\log_{10}$ CFU/mL in burukutu and 8.63 ± 0.02 $\log_{10}$ CFU/mL in malt extract. Despite the decline, all final counts remained above the initial population. The net increase from 0 to 72 h ranged from 0.99 to 1.10 $\log_{10}$ CFU/mL, with malt extract showing the greatest increase. Specific gravity decreased in all four fermentation systems during the 72-h observation period (Table 4B). At 0 h, the malt extract had the highest value (1.05 ± 0.00), followed by pito extract

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(1.04 ± 0.00), while palm wine and burukutu both recorded 1.03 ± 0.00. After 24 h, values had declined to 1.02 ± 0.00 in palm wine and burukutu, 1.03 ± 0.00 in pito extract and 1.04 ± 0.00 in malt extract. At 48 h, specific gravity further decreased to 1.01 ± 0.00 in palm wine, 1.02 ± 0.00 in pito extract and 1.02 ± 0.01 in burukutu, while malt extract recorded 1.03 ± 0.00. By 72 h, palm wine and burukutu had reached 1.01 ± 0.00, whereas pito extract and malt extract recorded 1.02 ± 0.01 and 1.02 ± 0.00, respectively. The total numerical reduction from 0 to 72 h was 0.02 specific-gravity units in palm wine and burukutu, 0.02 units in pito extract and 0.03 units in malt extract. Malt extract therefore showed the largest overall decrease, although it retained a higher final specific gravity than the palm wine and burukutu treatments. The pattern indicates a gradual change in liquid density during fermentation in each substrate.

The pH of all four fermentation media decreased progressively during the 72-hour fermentation period (Table 4C). In palm wine, pH declined from 3.77 ± 0.14 at 0 h to 3.16 ± 0.11 at 72 h. Pito extract showed a reduction from 3.55 ± 0.12 to 3.05 ± 0.08, while burukutu decreased from 3.54 ± 0.19 to 3.03 ± 0.14. Malt extract also became more acidic, with pH falling from 5.01 ± 0.09 to 4.02 ± 0.08. The total numerical decreases over the fermentation period were 0.61, 0.50, 0.51 and 0.99 pH units for palm wine, pito extract, burukutu and malt extract, respectively. The significant overall effects indicate that the pH profiles were not equivalent across the four media at any of the measured time points. The malt extract consistently recorded the highest pH, whereas burukutu and pito extract had the lowest final pH values.

Table 4B: Specific Gravity during the Fermentation of Alcoholic Beverages with Brewer's Yeast

Fermentation Period (hour)	Specific Gravity during the fermentation, n = 3			
	Palmwine with Brewer's Yeast	Pito Extract with with Brewer's Yeast	Burukutu with Brewer's Yeast	Malt Extract with Brewer's Yeast
0	1.03 ± 0.00	1.04 ± 0.00	1.03 ± 0.00	1.05 ± 0.00
24	1.02 ± 0.00	1.03 ± 0.00	1.02 ± 0.00	1.04 ± 0.00
48	1.01 ± 0.00	1.02 ± 0.00	1.02 ± 0.01	1.03 ± 0.00
72	1.01 ± 0.00	1.02 ± 0.01	1.01 ± 0.00	1.02 ± 0.00

Table 4C: pH during the Fermentation of Alcoholic Beverages with Brewer's Yeast

Fermentation Period (hour)	pH during the fermentation, n = 3			
	Palmwine with Brewer's Yeast	Pito Extract with with Brewer's Yeast	Burukutu with Brewer's Yeast	Malt Extract with Brewer's Yeast
0	3.77 ± 0.14	3.55 ± 0.12	3.54 ± 0.19	5.01 ± 0.09
24	3.54 ± 0.15	3.37 ± 0.11	3.35 ± 0.17	4.63 ± 0.09
48	3.31 ± 0.14	3.19 ± 0.10	3.17 ± 0.15	4.28 ± 0.08
72	3.16 ± 0.11	3.05 ± 0.08	3.03 ± 0.14	4.02 ± 0.08

The result of the fermentation of sterilized alcoholic beverages without yeast inoculation showed that No yeast colonies were recovered from any of the four sterilized, uninoculated fermentation media at the beginning of the experiment or after 24, 48 and 72 h of incubation (Table 5A). The yeast count remained 0.00 ± 0.00 CFU/mL in sterilized palm wine, pito, burukutu and malt extract throughout the observation period. Thus, none of the uninoculated media showed detectable yeast proliferation during the 72-h monitoring period. The specific gravity of the four sterilized, uninoculated fermentation media remained unchanged throughout the 72-h observation period (Table 5B). Sterilized palm wine and burukutu maintained a specific gravity of 1.03 ± 0.00 at 0, 24, 48 and 72 h.

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Pito extract remained at 1.04 ± 0.00, whereas malt extract consistently recorded the highest value of 1.05 ± 0.00. No numerical increase or decrease was observed in any of the media during incubation.

The pH values of the sterilized, uninoculated beverages showed only small fluctuations during the 72-h observation period, with no consistent progressive acidification across all four media (Table 5C). Palm wine remained relatively stable, changing from 3.92 ± 0.01 at 0 h to 3.93 ± 0.02 at 72 h. Pito extract also showed little variation, with values ranging from 3.86 ± 0.02 to 3.88 ± 0.03. Burukutu maintained the lowest pH throughout the experiment, fluctuating between 3.56 ± 0.012 and 3.61 ± 0.107. Malt extract had the highest initial pH (4.95 ± 0.06), increased slightly to 5.00 ± 0.03 at 24 h, and subsequently declined to 4.67 ± 0.59 at 72 h. One-way ANOVA based on the reported means and standard deviations indicated significant differences in pH among the four media at each sampling time: 0 h, $F(3, 8) = 685.48$, $p < 0.001$; 24 h, $F(3, 8) = 354.60$, $p < 0.001$; 48 h, $F(3, 8) = 1980.55$, $p < 0.001$; and 72 h, $F(3, 8) = 7.20$, $p = 0.012$. These overall tests indicate that the pH values differed among the beverage media at each time point. The consistently lower pH of burukutu and the higher pH of malt extract account for much of the separation among the treatment means.

Table 5A: Yeast counts for sterilized alcoholic beverages without yeast inoculation

Fermentation Period (hour)	Yeast count during the fermentation without yeast inoculation (Mean ± SD CFU/mL) n = 3			
	Sterilized Palmwine without Yeast Inoculation	Sterilized Pito without Yeast inoculation	Sterilized Burukutu without Yeast inoculation	Sterilized Malt Extract without Yeast inoculation
0	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
24	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
48	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
72	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00

Table 5B: Specific Gravity for sterilized alcoholic beverages without yeast inoculation

Fermentation Period (hour)	Specific Gravity during the fermentation without yeast inoculation, n = 3			
	Sterilized Palmwine without Yeast Inoculation	Sterilized Pito Extract without Yeast Inoculation	Sterilized Burukutu without Yeast Inoculation	Sterilized Malt Extract without Yeast Inoculation
0	1.03 ± 0.00	1.04 ± 0.00	1.03 ± 0.00	1.05 ± 0.00
24	1.03 ± 0.00	1.04 ± 0.00	1.03 ± 0.00	1.05 ± 0.00
48	1.03 ± 0.00	1.04 ± 0.00	1.03 ± 0.00	1.05 ± 0.00
72	1.03 ± 0.00	1.04 ± 0.00	1.03 ± 0.00	1.05 ± 0.00

Table 5C: pH for sterilized alcoholic beverages without yeast inoculation

Fermentation Period (hour)	pH during the fermentation without yeast inoculation, n = 3			
	Sterilized Palmwine without Yeast Inoculation	Sterilized Pito Extract without Yeast Inoculation	Sterilized Burukutu without Yeast Inoculation	Sterilized Malt Extract without Yeast Inoculation
0	3.92 ± 0.01	3.88 ± 0.03	3.59 ± 0.040	4.95 ± 0.06
24	3.91 ± 0.01	3.87 ± 0.02	3.61 ± 0.107	5.00 ± 0.03
48	3.93 ± 0.02	3.88 ± 0.03	3.56 ± 0.012	4.99 ± 0.03
72	3.93 ± 0.02	3.86 ± 0.02	3.60 ± 0.050	4.67 ± 0.59

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Discussion

This study examined culturable yeast populations in burukutu, pito and palm wine, compared their physicochemical characteristics, and assessed fermentation in media inoculated with indigenous or commercial brewer's yeast. The discussion integrates yeast enumeration, pH, specific gravity and uninoculated controls. Overall, the findings indicate that the beverages contained culturable yeasts, differed in initial acidity and density, and supported yeast growth during fermentation. The controls provided a baseline for distinguishing inoculation-associated changes from changes occurring during incubation.

Palm wine contained the highest mean culturable yeast population, followed by pito and burukutu. The differences among beverage means were statistically significant, showing that beverage type was associated with the number of yeasts recovered under the culture conditions. The high population in palm wine is plausible because palm sap contains fermentable sugars that support naturally occurring yeasts during tapping, collection and storage. Nigerian studies have recovered indigenous yeasts, including *Saccharomyces cerevisiae*, from palm wine and reported activities relevant to food fermentation (Osilo et al., 2023).

The three beverages were acidic, with mean pH values ranging from 3.54 to 3.77. Burukutu had the lowest mean and palm wine the highest, but the differences were not statistically significant. Madilo et al. (2024) describe the diversity of African cereal beverage production and the participation of lactic acid bacteria, *Saccharomyces* and *Candida* species. Osilo et al. (2023) reported a higher pH in freshly tapped Nigerian palm wine, illustrating the influence of sampling and fermentation stage. Initial specific gravity was highest in malt extract (1.05), followed by pito (1.04), while palm wine and burukutu each recorded 1.03. These differences likely reflect variations in soluble sugars and other dissolved constituents. It should be interpreted alongside pH, yeast counts, residual sugar and direct ethanol measurements.

Research on palm wine and other fermented beverages similarly uses physicochemical indicators to monitor fermentation (Khadka et al., 2024). In the inoculated indigenous yeast fermentations, pH declined over 72 hours in all beverage and malt extract treatments. The traditional beverage media began at pH 3.54 to 3.77 and remained more acidic at the end, whereas malt extract began near pH 5.00 and showed a larger numerical decline. Significant differences among the six treatments at individual sampling times were mainly attributable to the contrast between acidic beverage matrices and less acidic malt extract. Tukey comparisons did not identify significant differences among the three beverage-based treatments or among the three malt treatments. Thus, the overall ANOVA does not mean that every treatment differed from every other treatment. Yeast strains can produce or consume organic acids depending on strain and conditions (Vion et al., 2024). Titratable acidity and organic-acid analysis would clarify the chemical basis of the pH changes. Brewer's yeast grew in all four fermentation media. Counts increased markedly during the first 48 hours and declined slightly by 72 hours, while remaining high. Malt extract supported the highest populations at later sampling times, reaching 8.72 $\log_{10}$ CFU/mL at 48 hours. Significant differences among media emerged at 24, 48 and 72 hours, whereas initial counts did not differ. Growth in pito and burukutu is consistent with their cereal-derived nutrients, while palm wine supplied a sugar-containing sap matrix. Malt extract's apparent advantage may relate to soluble carbohydrates and nutrients, although its actual sugar and assimilable nitrogen concentrations were not provided. Yeast growth depends on inoculum preparation, substrate composition and strain physiology (Ginovart et al., 2024; Elhalis, 2024). Ethanol, residual sugar and sensory measurements are needed to assess performance. Specific gravity decreased in the brewer's yeast treatments, consistent with conversion of fermentable sugars into ethanol and carbon dioxide. Malt extract began at 1.05 and showed the largest numerical decline, reaching 1.02 at 72 hours. Palm wine and burukutu reached 1.01, while pito and malt extract ended at 1.02. Measurement resolution, temperature, suspended particles and non-fermentable solids can influence readings. The slight decline in yeast counts after 48 hours alongside continued gravity reduction is not contradictory: cell growth and substrate conversion are different processes, and metabolism may continue after population growth slows. Studies of palm sap and cashew-apple wine also demonstrate the value of combining specific gravity with other physicochemical measures (Khadka et al., 2024).

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The sterilized, uninoculated controls provided an important reference. No culturable yeast was detected in any medium at 0, 24, 48 or 72 hours. Specific gravity remained constant at 1.03 in palm wine, 1.04 in pito, 1.03 in burukutu and 1.05 in malt extract. Control pH values fluctuated only slightly, without progressive acidification. These findings support the interpretation that yeast growth and associated fermentation changes in inoculated samples followed deliberate starter addition. However, zero plate counts mean that no culturable yeast was detected under the method's conditions and detection limit; they do not prove absolute sterility. Recovery depends on plated volume, medium, incubation conditions and possible cell injury. Identical gravity readings with zero reported standard deviations do not permit meaningful ANOVA, and all-zero yeast counts likewise cannot yield a valid conventional F-test.

Interpretation is limited by small sample sizes. Larger independent samples would improve generalization across producers, locations and seasons. Repeated-measures or mixed-effects analysis using replicate trajectories would better test time, substrate and interaction effects. The unusually large standard deviation for malt extract pH at 72 hours also requires verification. Further work should identify isolates and assess sugar use, ethanol tolerance, fermentation rate, alcohol yield, aroma, titratable acidity, residual sugars and sensory quality. Recent reviews emphasize that microbial composition, processing and controlled starters shape traditional beverage properties (Madilo et al., 2024; Hawaz et al., 2025; Kitessa, 2024). Overall, palm wine provided the highest culturable yeast population, the three beverages supported fermentation, and malt extract supported strong growth. The indigenous isolates warrant further characterization, but their technological value and safety must be established at strain level.These findings guide future work. Further work should validate these observations experimentally and independently using larger samples.

Conclusion

The study established that burukutu, pito and palm wine harbour yeasts, with palm wine recording the highest population (2.65×10^8 CFU/mL), followed by pito and burukutu. The nine recovered isolates were provisionally identified as *Saccharomyces* spp. based on cultural, microscopic and biochemical characteristics. Their populations increased during fermentation, reaching maximum levels at 48 hours before declining slightly by 72 hours. Decreases in specific gravity and pH indicated substrate utilization and changes in acidity. Malt extract supported yeast growth, while uninoculated controls showed no detectable yeast growth. The beverages therefore represent promising sources of indigenous yeasts for characterization and fermentation applications.

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