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Native Yeasts, Better Wines: Palm-Wine Isolates as Scalable Starters for Clean, Reproducible Fruit Fermentation

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Abstract

Palm wine is a naturally fermented beverage that contains diverse yeast species important in food fermentation, with strain-specific properties influencing their applications. This study isolated and characterized yeasts from seven palm wine samples collected in Ibadan and assessed their potential in fruit wine production. A total of 101 isolates were obtained and phenotypically and biochemically characterized through sugar fermentation, urea hydrolysis, acid production, and acetic acid tolerance tests. Most isolates fermented glucose, fructose, and galactose but not maltose or mannitol. Screening for oenological properties identified strain PW10 as highly tolerant to ethanol (20%) and sugar (20%), making it suitable for watermelon must fermentation. Over a 3-day fermentation at 26–30 °C, pH declined, titratable acidity increased, specific gravity dropped from 1.055 to 1.006, and alcohol content reached 6.12%. Proximate analysis revealed significant changes in composition, microbial tests confirmed no contamination, and sensory evaluation showed good acceptability, demonstrating palm wine yeasts as promising starters for fruit wine production.

Keywords: *Palm Wine, Potatoes Dextrose Agar, Watermelon, Must, Fermentation, Yeast, Saccharomyces cerevisiae.*

I. INTRODUCTION

Palm wine is a spontaneously fermented beverage obtained from the sugary sap of oil palm (*Elaeis guineensis*) and raphia palm (*Raphia hookeri*), both in the *Palmae/Arecaceae* family (Kandiraju *et al*., 2025; Onyeukwu *et al*., 2024; Sanni and Lönner, 1993). It is widely produced and consumed across Nigeria and much of other sub-Saharan Africa, where it features prominently at ceremonies such as weddings and funerals (Adonu *et al*., 2025). Fresh, unfermented sap is clear, sweet, and colorless (Aparnna, *et al*., 2024; Amoa-Awua *et al*., 2007). Once tapped, however, the sap undergoes spontaneous fermentation, enabling the proliferation of yeasts that convert a nutrient-rich substrate predominantly sugars, into an alcoholic beverage (Onyeukwu *et al*., 2024; Nwachukwu *et al*., 2006).

The microbial composition of palm wine is shaped by palm species, geography, tapping methods, and handling/processing practices (Zhou *et al*., 2021; Naknean *et al*., 2010; Amoa-Awua *et al*., 2007). Broader environmental conditions, such as climate, temperature, sunlight, wind, and rainfall imprint a “terroir” on associated microbiomes and by extension, on the fermenting sap (Barata *et al*., 2012). The interactions between plants, soil and microbes further influence the starting inoculum and fermentation trajectory (Sumerta *et al*., 2024; Zhou *et al*., 2021). Within these consortia, yeasts are central: they drive alcoholic fermentation and contribute decisively to aroma, taste, and mouthfeel (Maicas, 2020; Noll, 2008; Owuama and Saunders, 1999). Yet quality is highly time-sensitive: within ~24 hours at ambient temperature, palm wine often loses its sweetness, becomes sour from over-fermentation, and the initially clear and colorless sap becomes milky-white (Khadka *et al*., 2024; Ezeronye and Okerentugba, 2000).

Biochemically, yeasts can generate energy anaerobically by converting sugars to ethanol and CO_2 (Ejimofor *et al*., 2023), while co-occurring bacteria produce lactic and acetic acids (Darko and Mills-Robertson, 2025; Santiago-Urbina and Ruiz-Terán, 2014; Stringini *et al*., 2009). Excess acetic acid accumulation is

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a principal driver of quality degradation and vinegar-like spoilage in palm wine (Oluwole *et al.*, 2023). Moreover, some yeast strains isolated during spontaneous fermentations can produce undesirable or harmful compounds, risks that may be amplified when such strains dominate the process and reduce the pH of the palm sap to 5 (Hai *et al.*, 2024; Oluwole *et al.*, 2023; Steensels *et al.*, 2014). It thereby erodes sensory acceptability and causes economic losses for vendors and food-service operators (Oluwole *et al.*, 2023; Bechem *et al.*, 2007).

Palm sap contains glucose, fructose, sucrose, maltose, and other carbohydrates; yeast strains differ in transport systems, invertase activity, and glycolytic control, leading to strain-specific preferences and kinetics that shape fermentation efficiency and final quality (Rodrigues *et al.*, 2006). Understanding which sugars are consumed, when, and by which microbes, helps explain the transition from pleasant sweetness and natural effervescence to excessive acidity and off-notes (Oluwole *et al.*, 2023). These insights also highlight opportunity. Yeasts from palm wine often tolerate high sucrose and rising ethanol, traits that could be exploited for controlled fermentations in tropical fruit substrates (Abdulkadir *et al.*, 2025; Bechem *et al.*, 2007). Indeed, palm-wine-derived isolates have produced acceptable fruit wines, suggesting technological potential beyond the traditional beverage (Ugbogu *et al.*, 2025; Chilaka *et al.*, 2010). Still, fermenting yeasts face multiple stresses, osmotic and oxidative stress, heat shock, ethanol toxicity, and nutrient limitations, that modulate viability and metabolism (Eigenfeld *et al.*, 2021; Salvadó *et al.*, 2008).

Despite extensive descriptive work, three limitations constrain progress from spontaneity to predictable, scalable production of palm wine yeast. First, most studies reveal their presence rather than functions, rarely linking community surveys to strain-level physiology under oenologically relevant stresses (high sugar, ethanol, low pH, acetic acid). Consequently, the functional roles of dominant yeasts in sugar-to-ethanol conversion and acidification control remain poorly quantified. Second, fermentation process performance is seldom captured with sufficient temporal resolution to connect substrate depletion (glucose, fructose, sucrose, maltose) to metabolite fluxes (ethanol, lactic and acetic acids) under relevant temperatures. This hinders prediction of when desirable freshness leads to spoilage. Third, there is no robust pathway for selecting palm-wine-derived starter cultures and translating screening results into pilot fermentations with explicit quality and safety profile.

To address these research gaps, we establish a strain-to-process framework that integrates ecology, physiology, and fermentation performance. Specifically, we isolated and phenotypically characterized yeasts from fresh palm wine, then we assessed the stress tolerance directly relevant to starter selection (high sugar and ethanol concentrations, growth at low pH, acetic-acid tolerance). Following these, the high-performing strains were molecularly characterized and validated in an experimental watermelon fruit must fermentation, monitoring time-resolved kinetics (specific gravity decline, ethanol formation, pH and titratable acidity), microbial safety, and sensory acceptability. By explicitly linking strain identity and stress physiology to process outcomes, our goal is to convert native yeast diversity into evidence-based starter options that improve quality, extend shelf life, and enhance economic resilience for fruit-wine production in tropical contexts.

II. MATERIALS AND METHODS

➢ *Sample Collection and Yeast Isolation*

Seven fresh palm-wine samples were collected in sterile plastic containers from different locations within Ibadan. Each container was labelled with the sampling location, date and time and immediately transported to the Microbiology laboratory, University of Ibadan. Before microbiological analysis, sample pH was measured with a calibrated pH meter. Yeasts were isolated by tenfold serial dilution. Briefly, 1 mL of thoroughly mixed sample was transferred into 9 mL of sterile distilled water and serially diluted to 10^{-7}. Aliquots (1 mL) from the 10^{-3}, 10^{-5}, and 10^{-7} dilutions were pour-plated using Potato Dextrose Agar (PDA) supplemented with chloramphenicol to suppress bacterial growth, then incubated at room temperature (28 ± 2 °C) for 48 h. Plates yielding 3–300 colonies were enumerated and morphologically distinct yeast colonies were selected and purified by streaking onto fresh PDA without chloramphenicol. Pure isolates were maintained by subculturing and stored on PDA slants at 4 °C for subsequent characterization (Abosede *et al.*, 2013).

➢ *Morphological Characteristics*

Following Mamun-Or-Rashid *et al.*, 2022, yeast isolates were evaluated for colony and growth characteristics on solid medium. Cultural assessments on agar plates documented: (i) texture (mucoid, fluid/viscous, or butyrous); (ii) elevation (flat or raised); (iii) pigmentation (e.g., cream, orange, red); (iv) surface appearance (glistening or dull; smooth, rough, or sectored); and (v) colony form and margin (entire, undulating, lobate, or filamentous). Features of each yeast isolates were recorded to support further identification.

➢ *Biochemical and Physiological Characterisation of Yeasts*

- *Fermentation of Carbohydrates (Sugars) and Gas Production*

Carbohydrate utilization by yeast isolates was assessed in yeast-fermentation broth with inverted Durham tubes following Kurtzman *et al.* (2011) with some modifications. Briefly, 3.75 g peptone and 5.0 g yeast extract were dissolved in 250 mL distilled water. Phenol red was added until bright red, and the base medium was divided into five flasks. To each flask, 0.5 g of carbohydrate (glucose, fructose, sucrose, maltose, or lactose; 1% w/v) was added. A 5 mL portions were dispensed into test tubes, each fitted aseptically with a sterile inverted Durham tube, then autoclaved at 121 °C for 15 min. After cooling, tubes were inoculated with 24 hours-old yeast cultures, with uninoculated tubes as negative controls, and incubated at 30 °C for up to 5 days. Fermentation was recorded as positive upon a phenol-red change from red to yellow (acid formation) and/or gas accumulation in the Durham tube (CO_2 production).

- *Urea Hydrolysis*

Urease activity was assessed using Christensen's urea agar following standard procedures (Christensen, 1946; Ebabhi *et al.*, 2013). The basal medium was prepared by dissolving 1 g peptone, 5 g NaCl, 2 g KH_2PO_4 and 12 µg phenol red in 1 L demineralized water. The pH was adjusted to 6.8, 20 g agar was added, and 4.5 mL of the medium was dispensed into 16-mm plugged glass tubes. The medium was autoclaved at 121 °C for 15 min and cooled to ~50 °C. Then, 0.5 mL of a filter-sterilized 20% (w/v) urea solution was aseptically added, mixed and the tubes slanted to solidify. Cells from 24 hours-old yeast cultures were streaked onto the slants and a urea-free basal medium served as the negative control. Cultures were incubated at 25 °C and inspected daily for up to 4 days. Urease production was recorded as positive upon development of a deep pink color in the urea medium but not in the control.

- *Gelatin Liquefaction*

Gelatinase activity was evaluated by monitoring gelatin liquefaction following Molnárová et al., 2014). A 5% (w/v) gelatin medium supplemented with yeast extract (2% w/v) was prepared in a sterile flask, homogenized, dispensed into slant bottles, and sterilized by autoclaving at 121 °C for 15 min. After cooling, bottles were aseptically inoculated with 24-h yeast cultures and incubated at room temperature for 7 days, with periodic inspection for loss of gel integrity; liquefaction of the medium was recorded as a positive result.

- *Acid Production from Glucose*

Acid production from glucose was assessed on Custer's chalk agar following Kurtzman *et al.* (2011). The medium contained 50 g glucose, 5 g finely powdered $CaCO_3$, 5 g yeast extract, and 20 g agar dissolved in 1L demineralized water. The medium was sterilized at 121 °C for 15 min. After autoclaving, the medium was gently agitated to resuspend the chalk, poured into the petri dishes, and allowed to gel rapidly to prevent sedimentation. Yeast isolates were streaked onto the set medium and incubated at 25 °C. The plates were examined periodically for up to 2 weeks. Acid production was recorded positive when a distinct zone of clearing formed around growth, indicating dissolution of $CaCO_3$ by organic acids produced from glucose.

- *Tolerance of Yeasts to 1% Acetic Acid*

Acetic acid tolerance was assessed on 1% (v/v) acetic acid agar following Kurtzman *et al.* (2011). For each 100 mL, 1 g tryptone, 10 g glucose, 1 g yeast extract, and 2 g agar were dissolved in demineralized water, autoclaved at 121 °C for 15 min, and cooled to ~50 °C. Exactly 1 mL glacial acetic acid was then added, thoroughly mixed, and poured into petri dishes. A loopful of standardized yeast cell suspension was streaked onto the plates, which were incubated at 25 °C and examined after 3 and 6 days for colony development. Growth on the acetic acid medium was recorded as evidence of acetic acid tolerance.

- *Tolerance to Low pH*

Survival of each yeast strain at low pH was determined by using the method of Akhtar *et al.*, (2021) with some modifications. The pH of sterile YPD broth was adjusted to 3.0 and 2.0 with 3mol/L HCl. The broth was inoculated with 1% (v/v) of 18hrs old broth culture of test yeast strains and incubated at 37ºC for 24 hours. The growth of each yeast isolates was confirmed by measuring the absorbance of grown culture at 600nm using a spectrophotometer. The non-inoculated YPD broth (pH 2 and pH 3) were calibrated as the control blanks respectively (Akhtar *et al.*, 2021).

- *Tolerance to Different Concentrations of Ethanol and Glucose*

The tolerance of the isolates to different ethanol and glucose concentrations were evaluated using acidified Yeast Peptone Dextrose (YPD; pH 3.5) according to the method described by Olee *et al.*, (2022). For the medium, 10 mL aliquots of YPD were supplemented with absolute ethanol to final concentrations of 5, 10, 15, or 20% (v/v) and glucose concentration of 10, 15, 20% (w/v). Each tube contained an inverted Durham tube. The medium was inoculated with 1% (v/v) of the freshly grown culture and incubated at 25 °C for 24–72 h alongside uninoculated controls. Tubes were inspected for CO_2 production (gas in Durham tubes) and growth. The tolerance was quantified by spectrophotometric measurement at 600 nm (OD_{600}) relative to the controls.

- *Molecular Characterization: Genomic DNA Extraction*

Yeast isolates exhibiting the highest tolerance to ethanol and glucose were selected for molecular characterization. Cells from overnight broth cultures were pelleted using a superspeed centrifuge (Sorvall Lynx 6000, Thermo Scientific Waltham, MA, USA) at 10,000 rpm for 15 minutes. The cells were washed with sterile distilled water, and resuspended in 400 µL extraction buffer (1 mol L^{-1} sorbitol, 100 mmol L^{-1} sodium citrate, 60 mmol L^{-1} EDTA, pH 7.0) containing lyticase (0.3 mg mL^{-1}) and β-mercaptoethanol (8 µL mL^{-1}) (Fietto et al., 2004). Suspensions were incubated in a Clifton water bath (Model S/W97719, Weston-super-Mare, UK) at 37 °C for 3 h to generate spheroplasts, after which one volume of lysis buffer (2% SDS in 50 mmol L^{-1} Tris, 10 mmol L^{-1} EDTA, pH 8.0) was added, mixed gently, and incubated at room temperature for 10 minutes. Sodium chloride (200 µL; 5 mol L^{-1}) was then added, and the mixture was held on ice for 2 h. Cellular debris was removed by centrifugation at 13,000 rpm for 10 min; the supernatant was transferred and extracted with phenol:chloroform:isoamyl alcohol (25:24:1) to deproteinize (Antia et al., 2018). The aqueous phase was recovered, and DNA was precipitated with two volumes of absolute ethanol, collected by centrifugation at 13,000 rpm, for 15 minutes. The precipitate was washed with ice-cold 70% ethanol, air-dried, and dissolved in 60 µL sterile distilled water for downstream molecular analyses.

- *PCR Amplification and Sequencing of the rDNA Internal Transcribed Spacer Region (ITS)*

The primer used to amplify the rDNA ITS was ITS1 (CCG GAT CCG TAG GTG AAC CTG CGG) and ITS4 (CGG GAT CCT CCG CTT ATT GAT ATG) as described by White *et al*., (1990). The amplification reaction was done in a 50-µL volume containing 20 pmol of each primer, 300 ng of genomic DNA template, 0.25 mmol/L each dNTP, 1.5 mmol/L MgCl and 0.5 U of Taq polymerase. The reactions were run for 34 cycles with denaturation of 94 °C for 45 seconds, annealing at 60 °C for 1 minute and extension at 72 °C for 5 minutes. Amplified products from PCRs were sequenced using automated sequencer (Chromous Biotech, Bengaluru, India). The sequence similarity search was done for the rDNA sequences using online search tool called Basic Local Alignment Search Tool (BLAST; http://www.ncbi.nlm.nih.gov/blast/). The unknown organism was identified using the maximum aligned sequence through the BLAST search (Antia *et al*., 2018).

- *Analysis of Sequence*

Isolates sharing identical restriction patterns were classified into groups and one or two samples were chosen as representative of each group for sequence analysis of the D1/D2 domains of the 26S rRNA gene. Amplification of the D1/D2 domains of 26S rRNA was carried out using NLI (5'GCATATCAATAAGCGGAGGAAAAG-3') and NL4 (5'-GGTCCGTGTTTCAAGACGG-3') primers (Invitrogen, Milan, Italy), according to Kurtzman and Robnett (1998) and the resulting products were sequenced. The sequences obtained in FASTA format were compared with those deposited at the National Center for Biotechnology Information (NCBI), using BLAST to determine their closest known relatives. In order to confirm the identification of isolates at the species level, the sequences of the D1/D2 domain of the 26S rRNA gene were further investigated. To this purpose, the multi-sequence alignments among our sequences and those of type strains of their closest relatives were performed using ClustalW (Bioedit v. 7.0.9). The number of nucleotide differences between D1/D2 sequences of our isolates and those of their closest relative were also analyzed.

- *Preparation of Watermelon Must*

Watermelon fruits were cleaned by washing the rinds thoroughly with soapy water and rinsing with distilled water to remove external contaminants. Each fruit was quartered longitudinally with a sterile knife, seeds were removed, and the edible mesocarp was aseptically sliced, and homogenized in an electric blender. The slurry was filtered through sterile muslin to obtain a clear, pink juice (must), which was analyzed for proximate composition and physicochemical parameters. The must was then pasteurized at 63 °C for 20 minutes (Eziaghighala *et al*., 2010) and cooled to room temperature prior to inoculation.

- *Development of the Inoculum for Must Fermentation*

The inoculum medium was prepared using the following formulation; glucose, 150g; yeast extract, 2g; peptone water, 2g; malt extract, 3g; $MgSO_4.7H_2O$, 1g; KH_2SO4, 1g; NH_4SO_4, 4g; NaCl, 1g; Ferrous Sulphate, 1g in 1litre of water. Exactly 10mL of the medium was dispensed into sterile universal bottle and was autoclaved. The selected isolates from Palm wine (PW10) were inoculated into 10mL of the inoculum medium and allowed to stay for 48 hours. Exactly 1.5mL of the medium was dissolved in 50mL of distilled water (Adedeji and Oluwalana, 2013).

- *Fermentation of the Fruit Must*

Fermentation vessels were washed with detergent, rinsed with sterile distilled water, and air-dried. One liter of the pasteurized watermelon must was aseptically transferred into each fermenter, 0.3 g sodium metabisulfite was added, and fitted with a mercury-in-glass bulb thermometer through the top cover. A 3 mL inoculum standardized to 0.5 McFarland was added, and the contents were mixed to ensure uniform dispersion. Fermentation proceeded at room temperature with twice-daily aeration by stirring to encourage yeast multiplication. Baseline measurements of specific gravity (SG), pH, temperature, titratable acidity (%TA), and yeast count were recorded prior to inoculation and subsequently at 12 hours intervals for 72 hours. After 3 days, fermentation was terminated; the wine was clarified by sieving to remove suspended solids, bottled, pasteurized, and analyzed for alcohol content (Adedeji and Oluwalana, 2013).

- *Determination of pH*

pH was measured with a standardized pH meter following AOAC (2005). Briefly, 10 mL of the sample was transferred to a sterile beaker, and the meter was calibrated immediately prior to use. The electrode was rinsed with distilled water, blotted dry, immersed in the sample, and the stabilized pH value recorded.

- *Estimation of Titratable Acidity (TA)*

Standard method of Lareo *et al*. (2013) was used to measure the titratable acidity. 5 mL of the wine sample was homogenized in 20 mL of distilled water and filtered through Whatman No.1 filter paper. Few drops of Phenolphthalein were added as indicator to 20 mL of the filtrate and titrated against 0.05 M NaOH. Titratable acidity was calculated using the equation below.

$$TA(\%\ w/v\ as\ lactic\ acid) = \frac{(M_{NaOH}\ \times V_{NaOH}\ \times 0.09 \times 100)}{Volume\ of\ sample\ (mL)}$$

M_{NaOH} = Molarity of NaOH used; V_{NaOH} = Volume of NaOH used; 0.09 = Equivalent weight of lactic acid

- *Determination of Specific Gravity*

A 50 mL specific-gravity bottle was washed with distilled water, oven-dried at 50 °C, cooled, and weighed (W_1). It was then filled with deionized water, wiped, and weighed (W_2). After rinsing twice with 10 mL of must, the bottle was filled to the brim with must, wiped, and weighed (W_3) (Adedeji and Oluwalana, 2013). The same procedure was repeated for the wine.

$$S.G = \frac{W3 - W1}{W2 - W1} = \frac{S}{W}$$

- *Proximate Analysis of Must and Wine*

Proximate composition (moisture, ash, crude protein, fat, total carbohydrate, and fiber) of the must and finished wine was determined according to AOAC (2016) official methods. Analyses were performed in triplicate, and results were reported as mean ± SD.

- *Microbial Analysis of the Wine*

- *Enumeration of Total Yeast Colony Count*

Potato dextrose agar supplemented with 50 mg/L chloramphenicol was used for selective enumeration of yeast. Serial dilution of the wine was carried out and inoculated using pour plate techniques. The inoculated plates were incubated at room temperature. Colonies from the plates of 10^{-6} dilution were counted after ~72 hours (Steger and Lambrechts, 2010; Mohammed, 2014).

- *Enumeration of Bacteria*

The enumeration of bacteria was done using nutrient agar. The wine was serially diluted and inoculated using pour plate methods. The plates were incubated at 37 °C for 24 hours. Colonies from the plates were observed (Adedeji and Oluwalana, 2013).

- *Enumeration Total Coliform Count of Bacteria*

MacConkey broth was used for the enumeration of coliform bacteria by the multiple tube technique. The homogenized fruit wine sample was serially diluted (10^{-1}, 10^{-2}, 10^{-3} and 10^{-4}) with 0.1% peptone water. Exactly 25 mL from each dilution was aseptically inoculated in 225 mL MacConkey broth in standard test tube and incubated for 48 hours at 37 °C (Adedeji and Oluwalana, 2013).

- *Sensory Evaluation of the Produced Wine*

Sensory evaluation of the watermelon wine was carried out by 21 panellists. Sensory attributes that were evaluated include taste, aroma, colour, clarity and overall acceptability using seven-point hedonic scales with 7 indicating extremely like and 1 indicating extremely dislike (Adedeji and Oluwalana, 2013).

- *Statistical Analysis*

Statistical Package for Social Sciences (SPSS) was used to analyze all the data. One-way analysis of variance (ANOVA) was used to compare the means.

III. RESULT

- *Morphological Characteristics of Yeast Isolates Obtained from Palm Wine*

A total of 101 yeast isolates were obtained from seven palm wine samples purchased from different locations within Ibadan. Table 1 shows the pH of the samples; sample from Arulogun (SAR) had the least acidic pH of 4.0 while sample from Oke Ado (SOA) had pH of 3.1. Also the total yeast counts range of each sample on PDA were recorded. Sample from Igbo-oloyin (SIO) had the highest yeast count of 16.8×10^6 CFU/mL while sample from Oke Ado (SOA) had the lowest yeast count of 1.0×10^6 CFU/mL.

Table 1 pH and Total Yeasts Count (CFU/mL) of Palm Wine

SAMPLES	pH	Total yeast count on PDA (CFU/mL) x10^6
SOA	3.1	3.0
SSO	3.2	9.3
SAR	4.0	8.0
SIO	3.8	16.8
SAK	3.5	4.0
SOJ	3.8	11.0
SOE	3.5	14.1

Key: SOA- sample from Oke Ado; SSO- sample from Sagbe Onibembe; SAR- sample from Arulogun; SIO- sample from Igbo-oloyin; SAK- sample from Akure; SOJ- sample from Ojoo; SOE- sample from Oje. PDA: Potato Dextrose Agar

The morphological characteristics of the yeast isolate from palm wine is shown in Table 2. The cellular features of the yeast isolates revealed that all the 101 yeasts isolates were circular in shape, cream in colour and all the 101 yeast isolates also had an entire margin. Seventy-one (71) yeast isolates had raised elevation while thirty (30) yeast isolates had flat elevation. Ninety-nine (99) yeast isolates had a smooth surface while only two yeast isolates had dull surface. The texture of the yeast isolates was either butyrous or moist.

Table 2 Morphological Characteristics of Yeast Isolated from Palm Wine

Shape	Colour	Surface	Elevation	Texture	Margin	Number of isolates	Percentage of occurrence (%)
Circular	Cream	Smooth	Raised	Moist	Entire	60	59.41
Circular	Cream	Smooth	Raised	Butyrous	Entire	9	8.91
Circular	Cream	Smooth	Flat	Moist	Entire	30	29.70
Circular	Cream	Dull	Raised	Butyrous	Entire	2	1.98

➢ *Biochemical and Physiological Characteristics of the Selected Yeast Isolates*

Haemolytic test was used to screen down the 101 yeast isolates. Seventeen were non-haemolytic (Gamma-haemolytic) while the other 84 were either alpha or beta haemolytic. The seventeen non-haemolytic yeast isolates were selected and used for further experiment.

The result of the biochemical and physiological characteristics of the yeast isolates are shown in Table 3. It was observed that all the seventeen yeast isolates (e.g. PW64, PW57, PW41, PW10) fermented and produced gas from fructose, galactose and glucose but did not ferment and produce gas from sorbitol, mannitol, maltose, sorbose, inositol and arabinose. Ten yeast isolates (e.g. PW64, PW10, PW18) fermented and produced gas from sucrose. Ten yeasts isolates (e.g. PW64, PW57, PW32, PW10) were able to grow at 1% acetic acid while the other seven (e.g. PW76, PW78, PW9) did not grow after a week of incubation. The urease test showed that eleven yeast isolates (e.g. PW64, PW76, PW32, PW10) could not hydrolyze urea after 7 days of incubation while 6 yeast isolates (e.g. PW81, PW57, PW41) hydrolyzed urea. The 17 yeast isolates were also subjected to acid production from glucose test, Isolate code PW9 had the highest clear zone of about 13mm in diameter. The result of the acid production from glucose test of the 17 yeast isolates was represented with bar chart and shown in Figure 1. The seventeen selected yeast isolates were negative for gelatin liquefaction and Dnase test.

Table 3 Biochemical and Physiological Characteristics of Selected Yeast Isolated

Isolate code	**Glucose**	**Fructose**	**Sorbose**	**Lactose**	**Sorbitol**	**Maltose**	**Sucrose**	**Galactose**	**Urease**	**Acid production from glucose**	**1% Acetic acid**	**Probable organism**
PW64	A/G	A/G	-	-	-	-	A/G	A/G	-	+	+	*Saccharomyces* sp.
PW76	A/G	A/G	-	-	-	-	-	A/G	-	+	-	*Saccharomyces* sp.
PW78	A/G	A/G	-	-	-	-	-	A/G	-	+	-	*Saccharomyces* sp.
PW81	A/G	A/G	-	-	-	-	A/nG	A/G	+	+	-	*Candida sp.*
PW80	A/G	A/G	-	-	-	-	-	A/G	+	+	-	*Candida sp.*
PW75	A/G	A/G	-	-	-	-	-	A/G	+	+	-	*Candida sp.*
PW69	A/G	A/G	-	-	-	-	-	A/G	-	+	-	*Saccharomyces* sp.
PW57	A/G	A/G	-	-	-	-	A/G	A/G	+	+	+	*Candida sp.*
PW41	A/G	A/G	-	-	-	-	A/G	A/G	+	+	+	*Candida sp.*
PW39	-	-	-	-	-	-	-	-	-	+	+	*Saccharomyces* sp.
PW32	A/G	A/G	-	-	-	-	A/G	A/G	-	+	+	*Saccharomyces* sp.
PW22	A/G	A/G	-	-	-	-	A/G	A/G	-	+	+	*Saccharomyces* sp.
PW26	A/G	A/G	-	-	-	-	A/G	A/G	-	+	+	*Saccharomyces* sp.
PW9	A/G	A/G	-	-	-	-	A/G	A/G	-	+	-	*Saccharomyces* sp.
PW17	A/G	A/G	-	-	-	-	A/G	A/G	+	+	+	*Candida sp.*
PW10	A/G	A/G	-	-	-	-	A/G	A/G	-	+	+	*Saccharomyces* sp.
PW18	A/G	A/G	-	-	-	-	A/G	A/G	-	+	+	*Saccharomyces* sp.

Key: - = negative; + = positive; A/G = Acid and Gas production; A/nG = Acid and no Gas production

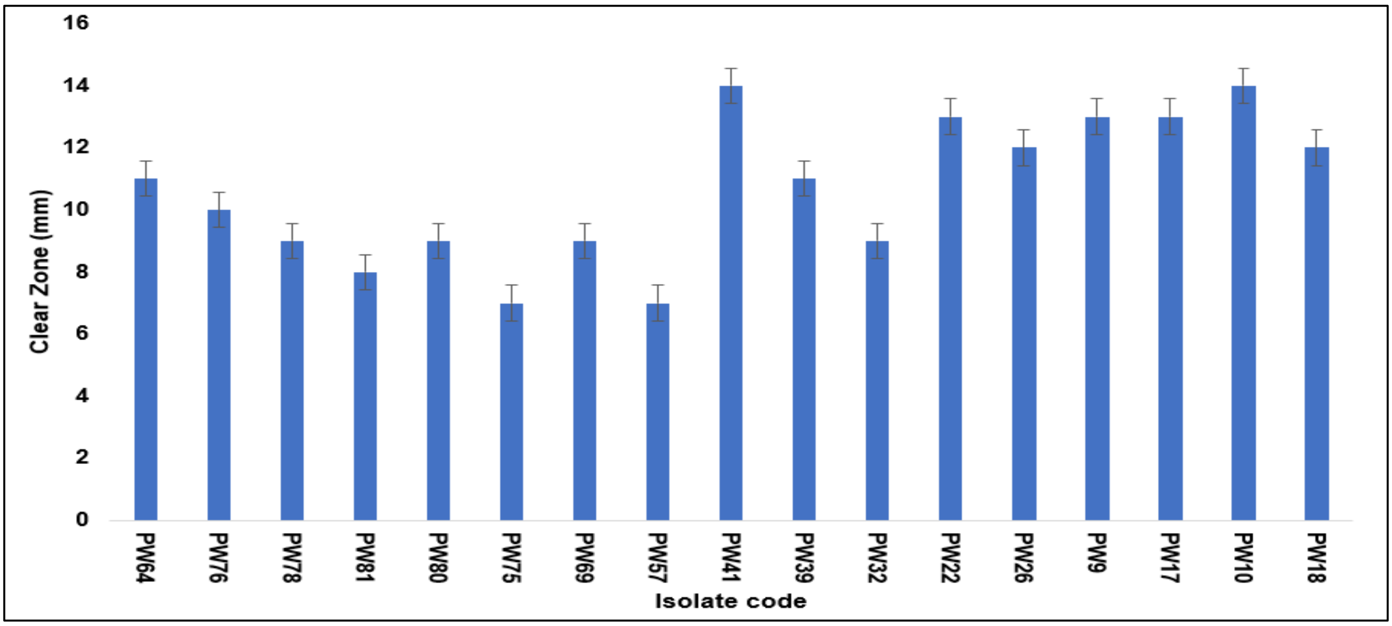

Fig 1 Acid Production from Glucose by Selected Yeast Isolates

- *Stress Tolerance Pattern of the Selected Yeast Isolates*

The seventeen isolates selected were further subjected to different stress tolerances and their growth indexes were determined by measuring their optical density using spectrophotometer. All the yeast isolates were able to tolerate low pH 3 but none tolerated the lower pH 2. the result of the yeasts tolerance to pH 2and 3 is represented with bar chart and shown in Figure 2. All the yeast isolates were able to tolerate ethanol at 5% and 10% concentrations giving high tolerances at 5% while no yeast isolate showed growth in 20% ethanol. All the selected yeast isolates grew on all percentages of the glucose concentrations (10% and 20%). The result of the ethanol concentrations tolerances is represented in bar charts and shown in Figure 3 while the yeast isolates tolerance to different glucose concentrations is shown in Figure 4.

Table 4 Physiochemical Properties of the Watermelon Must and Wines

Parameters	Specific gravity	Alcohol %
MUST	1.055	0
WINE	1.006	6.12

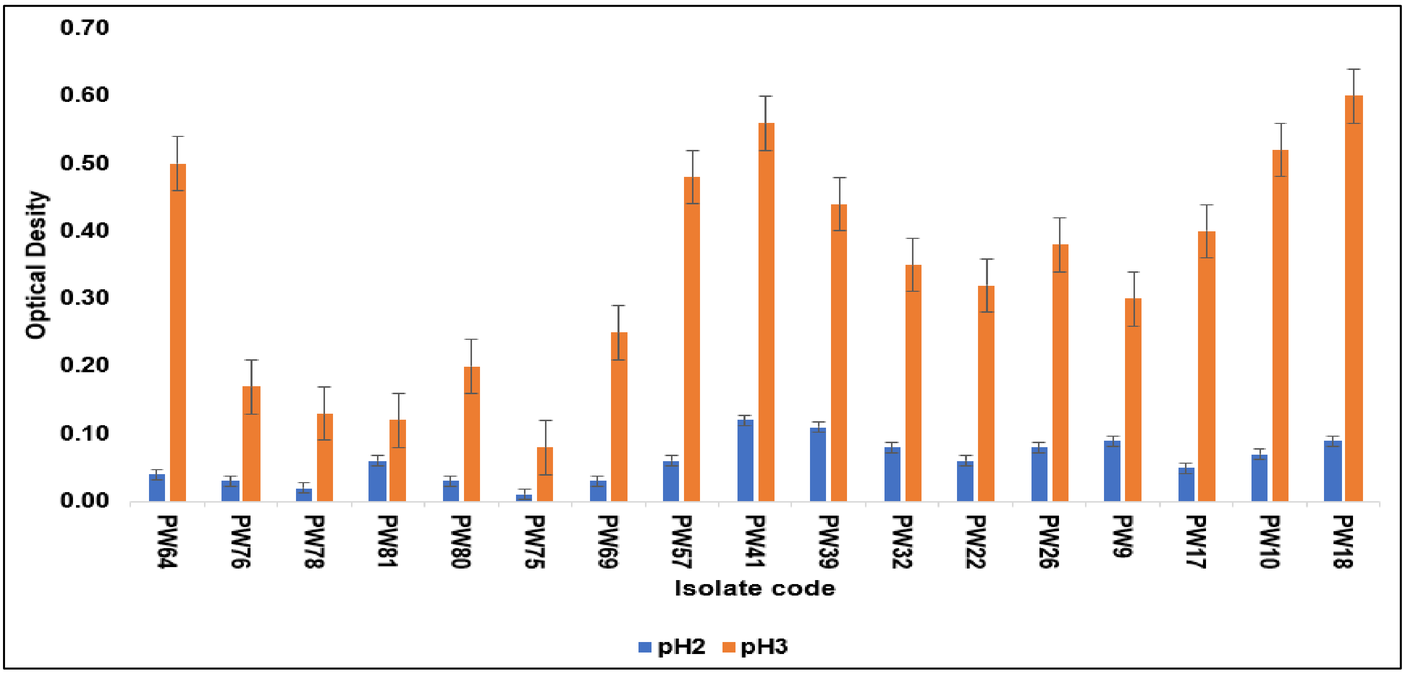

Fig 2 Tolerance of the Selected Yeast Isolates to Low pH at 24hours

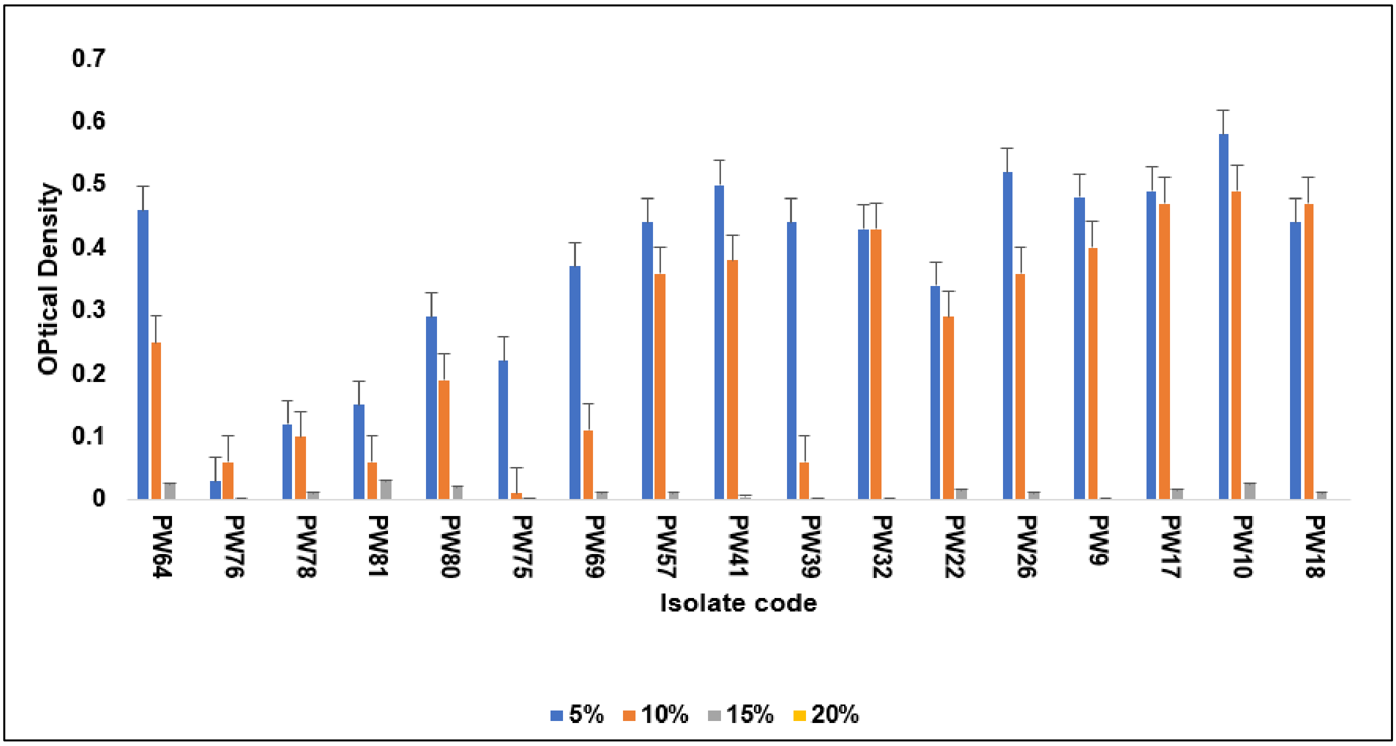

Fig 3 Tolerance of the Selected Yeast Isolates to Different Concentration of Ethanol

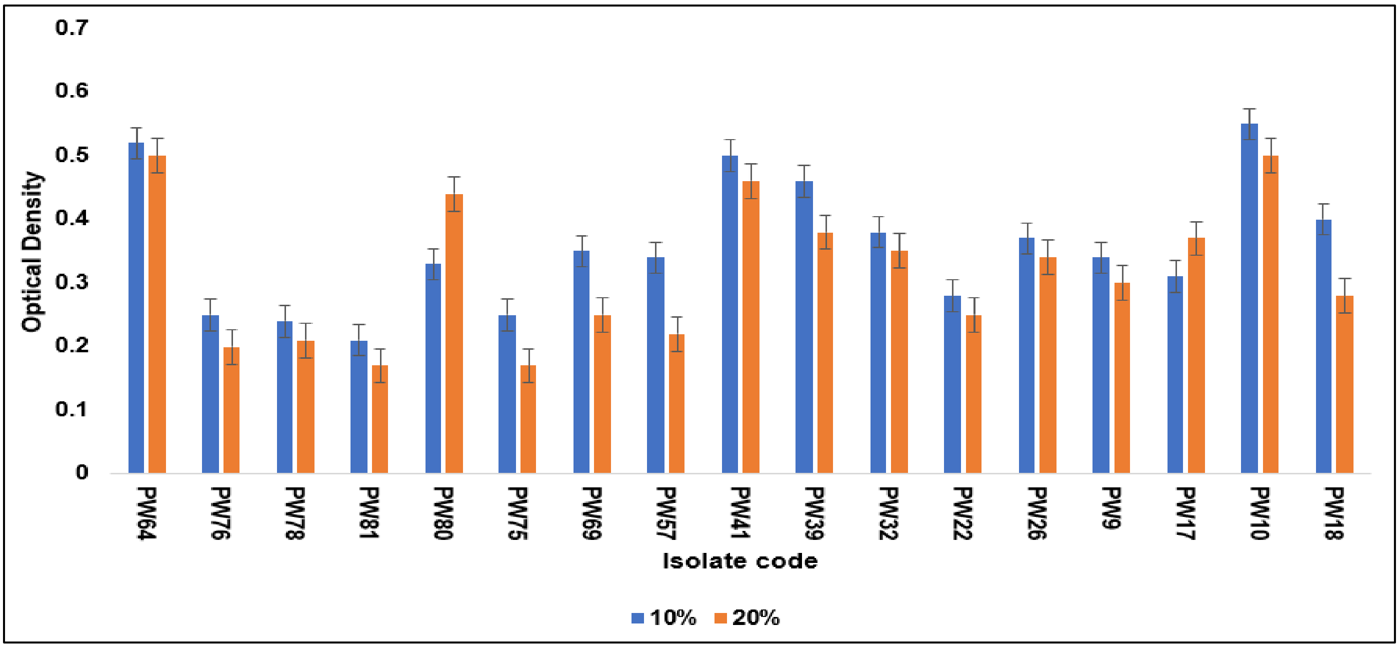

Fig 4 Tolerance of the Selected Yeast Isolates to Different Concentration of Glucose

The yeast isolate (molecularly characterized as *Saccharomyces cerevisiae*) with the best tolerance to the highest ethanol and sugar concentration was selected for the watermelon fruit fermentation.

- *Physiochemical Properties of the Must and Wine Produced*

Figure 5 showed the result of variation in pH of the wine during fermentation at intervals of 12 hours. The pH showed a gradual decline (5.0 to 4.3) up to the 72 hours of fermentation. Figure 6 shows the result of the variation in temperature of the wine during fermentation at interval of 12 hours. The temperature of wine increases within the first two days (from 26°C to 32°C) of fermentation and then declined (from 32°C to 30°C) towards the end of fermentation period. Figure 7 shows that the titratable acidity increased for the fruit wine produced using the yeast isolate obtained from palm wine. Table 4 shows that the specific gravity decreased from 1.055 – 1.006. Table 4 also shows the alcoholic content of the produced watermelon wine which increased from 0 to 6.12.

Table 5 Proximate Analysis of Must and Wines

Parameters	Moisture content (%)	Protein (%)	Fat (%)	Ash (%)	Fibre (%)	Carbohydrate (%)
MUST	93.81±0.12^a	4.23±0.25^a	0.00± 0.0^a	0.11±0.01^a	0.00±0.0^a	1.85±0.28^a
WINE	96.55±0.17^b	2.56±0.13^b	0.00±0.0^b	0.08±0.0^b	0.00±0.0^b	0.81±0.09^b

Keys: All values are the average value ± standard deviation of three replications. Different alphabets in lower case denote a significant difference ($p < 0.05$) between the samples.

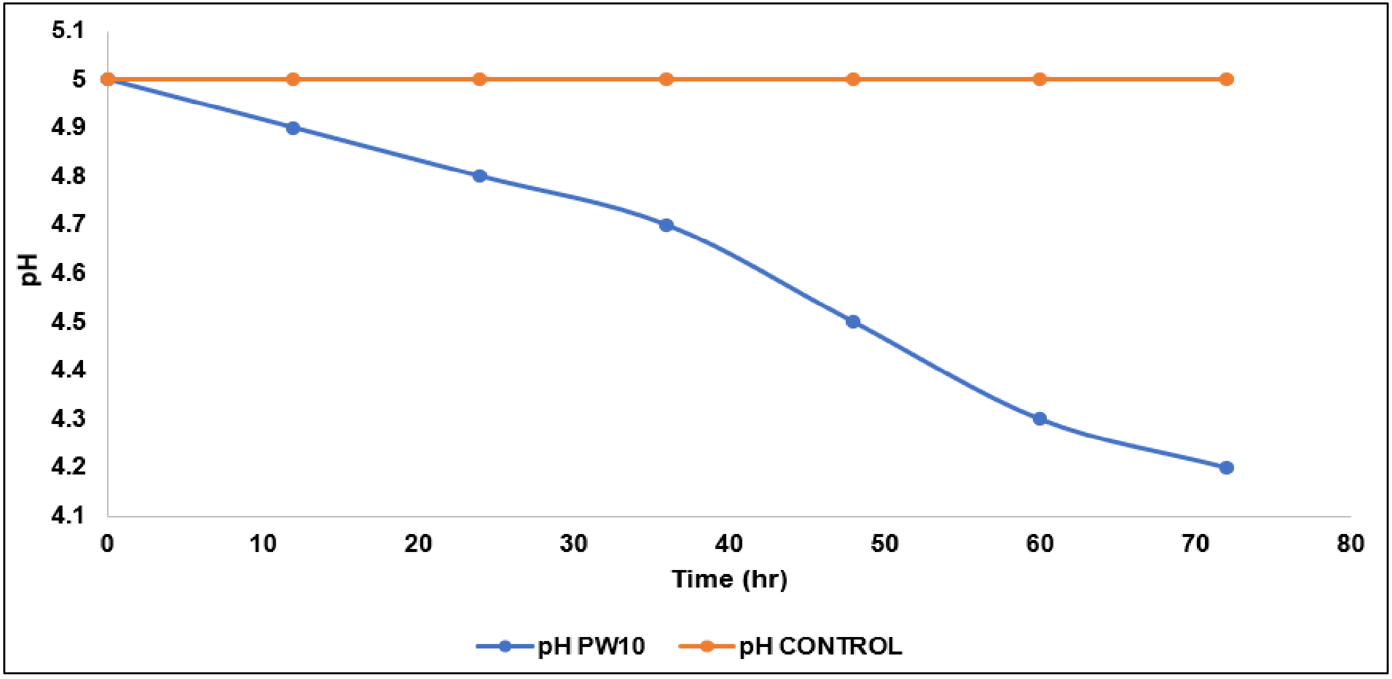

Fig 5 Variation in pH of the Wine During Fermentation with Selected Yeast

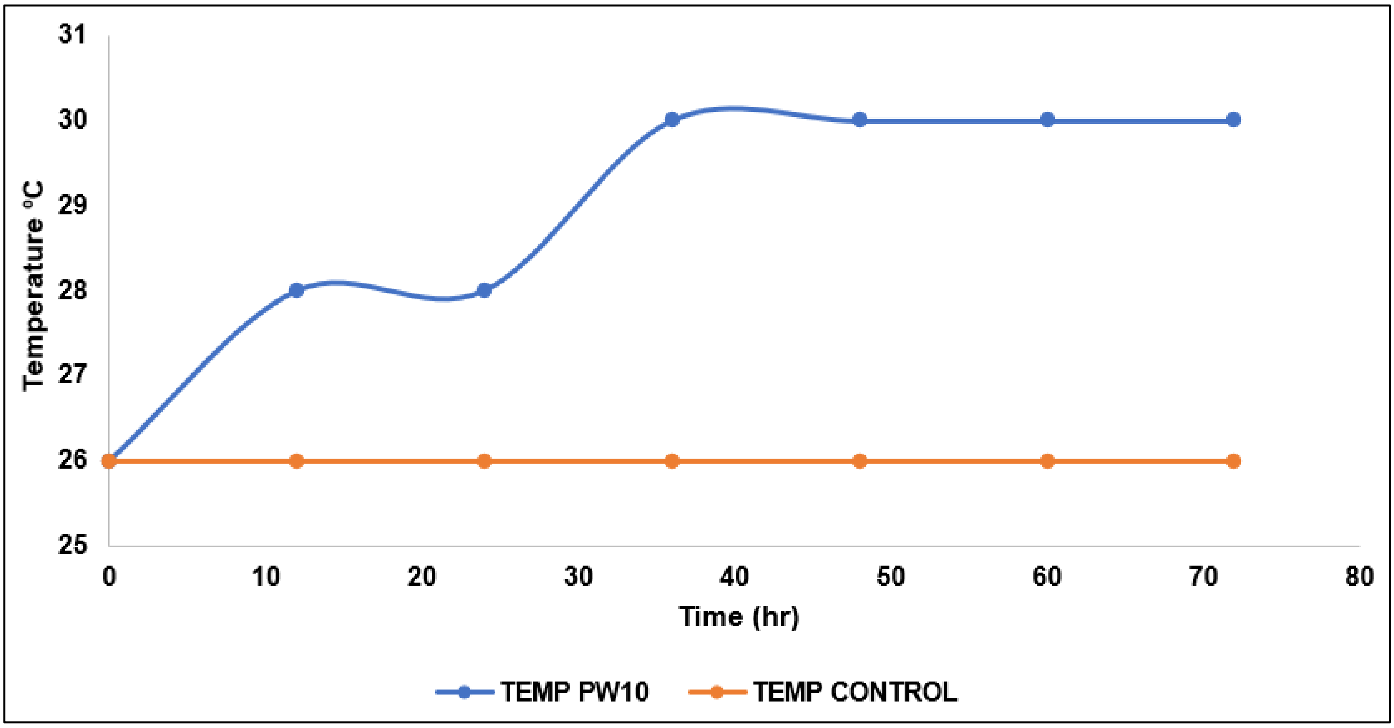

Fig 6 Variation in Temperature of the Wine During Fermentation with Selected Yeast

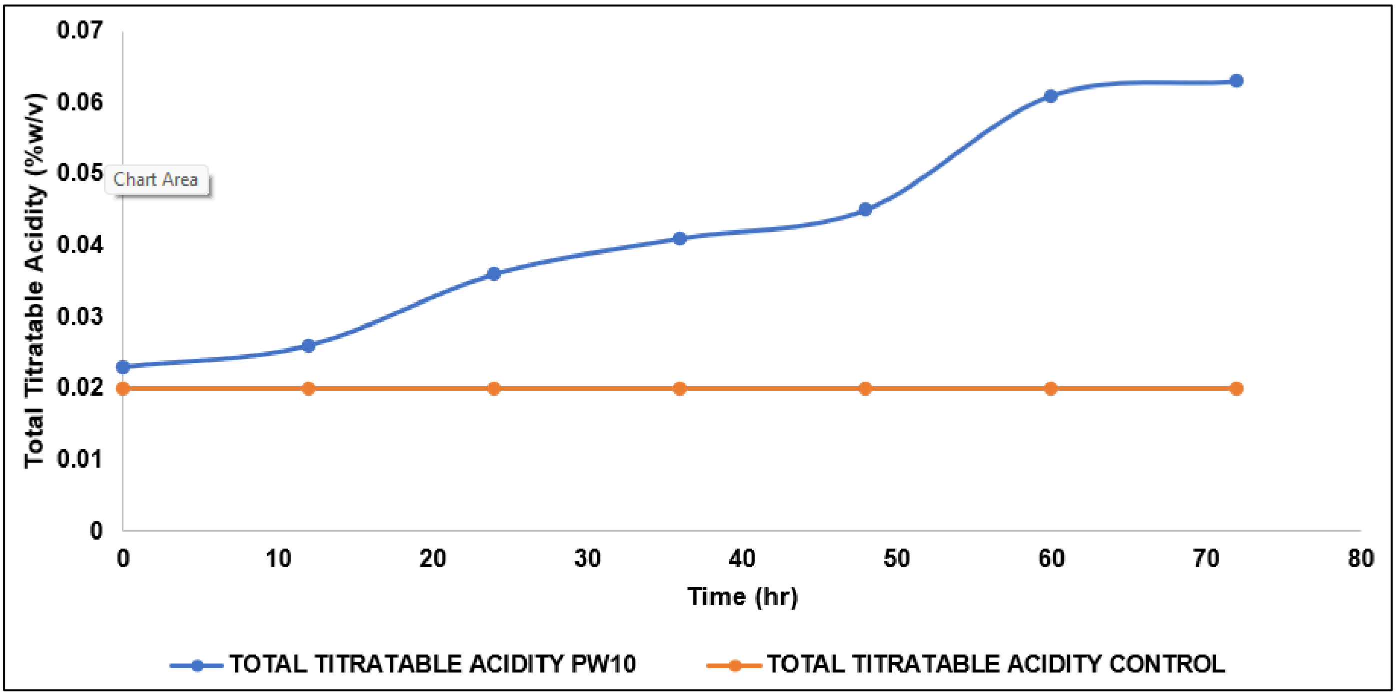

Fig 7 Variation in Total Titratable Acidity of the Wine During Fermentation

- *Proximate Analysis of Must and Wines*

Table 5 compared the proximate analysis of the watermelon must and the fruit wine produced. The percentage moisture content obtained from the watermelon must was 93.81% and an increase was observed in the produced wine which had 96.55%. The percentage protein content obtained from the watermelon must was 4.23% but there was a decrease in the produced wine which had 2.56%, percentage carbohydrate content of the must was 1.85% that of the produced wine was 0.81%, the percentage of ash content of the watermelon must was 0.11% while that of the produced wine had 0.08% showing no significant difference. Both the watermelon must, and the wine had no fat and fibre content.

- *Microbial Analysis of the Wine*

The results of microbial analysis were shown in Table 6. The result revealed that there was no bacterial and coliform growth before, during and after fermentation, while for the total viable yeast count, no count was recorded after pasteurization (at 0hr) before fermentation but at 12 hour – 60 hours the count increased from 3-8.1 10^{-7} CFU/mL and at 72 hours there was a decrease in the total yeast count of 7.5. A decrease was observed during last days of fermentation of watermelon wine.

Table 6 Microbial Analysis of the Wine

Time (hr)	Bacterial (CFU/ml)	Coliform (CFU/ml)	Total Yeast (x10^7CFU/mL)
0	-	-	-
12	-	-	3
24	-	-	4.7
36	-	-	5.6
48	-	-	6.8
60	-	-	8.1
72	-	-	7.5

- *Sensory Evaluation of the Wine Produced*

The sensory evaluation of the produced fruit wine is shown in Table 7, which showed the different views of the six panellists on the fruit wine produced. 67% evaluated the taste of the wine like moderately while 33% liked extremely, the aroma was either like moderately or like slightly while 67% moderately like the colour and 33% slightly like. The evaluation for the clarity of the wine ranges like moderately to neither like nor dislike.

Table 7 Sensory Evaluation of the Produced Wine

	LE	LM	LS	LD	DS	DM	DE
Taste	2(33%)	4(67%)	-	-	-	-	-
Aroma	-	3(50%)	3(50%)	-	-	-	-
Colour	2(33%)	4(67%)	-	-	-	-	-
Clarity	-	3(50%)	2(33%)	1(17%)	-	-	-
Overall acceptance	-	6(83%)	1(17%)	-	-	-	-

KEY: LE- Like Extremely, LM- Like Moderately, LS- Like Slightly, LD- Neither Like Nor Dislike, DS- Dislike Slightly, DM- Dislike Moderately, DE- Dislike Extremely.

IV. DISCUSSION

This study is aimed at the isolation, characterization, and evaluation of yeast strains obtained from palm wine samples collected from different locations in Ibadan, Nigeria, and their potential application in wine fermentation. The results reveal significant findings regarding the morphological, biochemical, physiological, and oenological properties of the yeast isolates, as well as their safety and performance in watermelon wine production. A total of 101 yeast isolates were obtained from the palm wine samples, with distinct morphological characteristics. The predominance of circular, cream-colored, and smooth-surfaced colonies aligns with features commonly associated with *Saccharomyces* and *Candida* species (Kurtzman *et al*., 2011). The observed variations in elevation and texture reflect the natural diversity of yeasts in traditional fermentation systems. The pH of the palm wine samples ranged from 3.1 to 4.0, which is consistent with previous studies on the acidic nature of palm wine reported by Adebiyi *et al*., (2018). The total yeast counts varied significantly among samples, with the highest count observed in the sample from Igbo-Oloyin (16.8 × 10^6 CFU/mL), suggesting favourable conditions for yeast proliferation in this environment.

The biochemical characterisation of the selected seventeen yeast isolates showed their ability to ferment common sugars such as glucose, fructose, and galactose, producing both acid and gas. This is a well-documented trait of fermentative yeasts like *Saccharomyces cerevisiae* and some *Candida* species by Fleet, (2003). The ability of 10 isolates to tolerate 1% acetic acid suggests their effectiveness under acidic conditions, an important trait for industrial fermentation processes reported by Palma *et al*., (2018).

The Oenological properties demonstrated the yeasts isolates' varying abilities to tolerate ethanol and high sugar concentrations. Notably, the two isolates (PW10, PW17) showing the highest optical densities at 5% and 10% ethanol, indicates their potential for high-alcohol fermentation. The ethanol tolerance of yeast is a distinctive characteristic that renders it beneficial for industrial applications as stated by Ukwuru and Awah, (2013). The selected yeast for wine fermentation shows the capability to grow in a medium with 15% (v/v) of ethanol. This indicates that this yeast strain can sustain metabolic activity in the fermentation medium and tolerate alcohol concentrations of up to 15% throughout the fermentation period. The yeast in the study showed a maximum ethanol tolerance of 15%, consistent with the findings of Ukwuru and Awah (2013), who reported a similar tolerance level in their research on the properties of palm wine yeast and its performance in winemaking. The ability of all isolates to grow at 10% and 20% glucose concentrations further supports their suitability for fermenting sugar-rich musts according to Pretorius, (2000).

The selected yeast isolate performed well in fermenting watermelon must, as evidenced by the gradual decline in pH and increase in titratable acidity over the fermentation period. These changes are characteristic of yeast which are able to convert sugars into ethanol and organic acids according to Chidi *et al*., 2018. The decrease in specific gravity from 1.055 to 1.006 and the production of 6.12% alcohol confirm successful fermentation and ethanol yield comparable to other fruit wines like apple and banana (Jackson, 2008) The alcohol content percentage does not agrees with the findings of Sandipan and Subhajit, (2011), who reported that wines that has 7 - 14% of alcohol are considered as table wine. The alcohol percentage generated from the fruit used for fermentation by the selected yeast strain exceeded 2%, comparable to moderate grape wine, as noted by Okeke *et al*., (2015).

Proximate analysis of the must and wine showed significant difference ($p < 0.05$) in the moisture, crude protein and carbohydrate content, reflecting metabolic activity and nutrient consumption during fermentation. except for percentage ash and fat which had no significant difference. It was observed that the moisture content of the fruit was high. This explains why it has a short shelf life under normal storage conditions and it is also highly perishable, Okeke *et al*., (2015) and Zainab *et al*., (2018) reported similar findings. The high moisture content of the wine makes it ideal as a refreshing and thirst-quenching product, which is a feature of good beverages. No amount of ash was obtained. This agrees with the reports by Inuwa *et al*., (2011) who found reported 0.5% of ash content. No fat content was obtained both in the watermelon fruit and wine. This is contrast to the work of Kantiyok *et al*., (2021) who reported a wine with a fat content of 7.96%. The no fat content in the wine, suggests that the wine could provide protection against excess body lipids (cholesterol) and it demonstrate the desirable nutritive quality of the fruit wine produced as reported by Awe *et al*., (2013). There was no fibre content in the must and the wine produced which agrees with the reports of Kantiyok *et al*., (2021); Zainab *et al*., (2018). The protein content of the watermelon fruit obtained was low, this was also reported by Praise *et al*., (2025) who recorded low protein content in the unboiled watermelon. The protein content of the fruit decreased after fermentation which agrees with report of Zainab *et al*., (2018) who also obtained a decrease in protein content after fermentation of the fruit. Low protein content of the wine is good for maintenance of cellular organization as reported by Awe *et al*., (2013). A decrease in the carbohydrate content of the wine was observed. This might be due to reduction in the sugar content as a result of the yeast cells quick and effective utilization of the sugar present in the must leading to the fermentation of the must. Awe and Nnadoze, (2015); Zainab *et al*., (2018) reported similar observations.

The absence of bacterial and coliform growth throughout the process reflects the hygienic quality of the fermentation and the fact that the must was pasteurized before fermentation. This indicates that the microbial contaminant in the wine can be eliminated by heat treatment. This is similar to the reports given by Adedeji and Oluwalana, (2013). The yeast viable count of this study indicated a significant increase in the number of yeast cells during the first few hours (54 hours) of fermentation, followed by a decrease, which is consistent with the findings of Zainab et al., (2018).

This increase in the number of yeast cell during the first few hours of fermentation can be attributed to the effective utilization of the available sugar component and the daily aeration of the fermenting must leading to their cell propagation and rapid multiplication. This agrees with the findings of Awe and Nnadoze, (2015). The decrease in the number of yeast cells in the fermenting must might be due to a significant decrease in the sugar content as a result of rapid and effective utilization of the sugar available in the must by the yeast cells, leading to the fermentation of the must, while an increase in the alcohol content recorded will also affect the growth rate of the yeast, this can be confirmed by Awe, (2011).

The sensory evaluation rates the wine produced acceptable particularly in terms of taste, aroma, and colour. The high overall acceptance rate (83%) highlights the potential of the isolated yeast strains to produce appealing and high-quality fruit wines. A similar observation was reported by Zainab *et al*., (2018).

V. CONCLUSION

The results of this study indicate that palm wine is a good source of a different strains of yeast, and several yeast strains demonstrated reasonable physiological and oenological properties. It also reveals that palm wine is a reliable source from which yeast strain, *Saccharomyces cerevisiae* with good industrial properties and functionalities. Our study suggests strong potential for using yeast isolated from palm wine in fruit wine production. These yeast strains support desirable fermentation and microbiological safety, which can help reduce wastage of seasonal fruits.

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