Dough Rising Capacity of a Novel Yeast *Yarrowia Phangngaensis* Isolated from Palmwine as Bakery Yeast Option

Eleanya, Lilian Chinyere*[1], Igboanugo, Edwin Uchechukwu[2], Dibua, Nwamaka Anthonia[1], Anagor, Ifeoma Sandra[3], Ezeume, Eucharia Nkiruka[1], Chukwura, Eric Nnamdi[1] and Okpalla, Jude[1]

[1]Chukwuemeka Odumegwu Ojukwu University, Uli, Anambra State Nigeria,

[2]Legacy University, Okija, Anambra State, Nigeria,

[3]Nnamdi Azikiwe University, Nnewi campus, Nigeria

DOI: https://doi.org/10.51584/IJRIAS.2025.100500074

Received: 25 April 2025; Accepted: 11 May 2025; Published: 14 June 2025

ABSTRACT

The ability of yeast rise dough, enhance texture and sensory properties are highly valued by bakers. The aim of this study was to evaluate dough rising capacity of a novel yeast *Yarrowia phangngaensis* isolated from palmwine as bakery yeast option. Palmwine samples were collected from different parts of Anambra State. Thirty-five yeast isolate were screened for bakery yeast potentials, selected yeasts were subjected to stress tolerance, optimization of cultural conditions, pathogenicity test, dough leavening capacity, sensory evaluation and toxicology study. The results show that the most suitable yeast was *Yarrowia phangngaensis.* The optimum growth temperature of the test organisms were 28^0C and 30^0C for commercial yeast (CY) and *Yarrowia phangngaensis* (YP) respectively. Optimum growth time and pH was 72h and 5 respectively for both organisms. Stress tolerance test shows that both yeasts grew optimally at 2% salt concentration. The pathogenicity test on the isolate was negative and the dough leavening capacity of the isolate was appreciable. Maximum Dough Rising Capacity (DRC) of 5.69 and 5 was recorded at 150 h for CY and YP respectively. Statistically, CY shows strong positive correlation while YP shows moderate positive correlation between DRC and time. Albino rats used for toxicology study remained normal till the end of the observation period. *YP* can be utilized for industrial use.

Keywords: Dough leavening capacity, Novel yeast, *Yarrowia phangngaensis,* Palmwine

INTRODUCTION

People have taken good advantage of yeasts in bakery industry for bread, beverage, bioethanol production, single-cell-protein and glycerol production. They are easily genetically manipulated with *Saccharomyces cerevisiae* and *Candida albicans* being among the most exploited (Camila de Souza *et al*., 2019). The major functions of yeast in dough fermentation is to it produce carbon dioxide in reasonable quantities to leaven the dough; to improve texture of the bread when properly baked as well as to enhance sensory properties of the bread. In addition to producing carbon dioxide, the lactic acid-forming bacteria produce acids also (Mahoukoucd *et al*., 2019).

According to Michael *et al*., 2023 research has drifted from the conventional yeast *Saccharomyces cerevisae* to screening non conventional yeasts. This is due to its limitations to certain stress conditions, while strains with better properties for industrial applications are sought. Selection of strain is an important but overlooked area in bioprocess development, as selecting an organism that will yield the desired product may minimize strain engineering interventions, simplify downstream separation, limit product toxicity, and improve overall process metrics. Example of these important yeasts are *Yarrowia lipolytica* , *Kluyveromyces marxianus* and *Pichia kudriavzevii (Michael et al*., 2023).

Yarrowia phangngaensis is another promising, yet under utilized yeast which has shown prospects in the food

ISSN No. 2454-6194 | DOI: 10.51584/IJRIAS |Volume X Issue V May 2025

industry especially bakery industry as observed in Eleanya *et al*., 2025 and Nwagu *et al*., 2019.The ability of yeast to breakdown sugar in the dough under conditions of high environmental stress is essentially important to the baker. The aim of this study was therefore to isolate and investigate the dough leavening capacity of *Yarrowia Phangngaensis* isolated from palmwine.

MATERIALS AND METHODS

Sources and Collection of Samples

Fresh samples of wine obtained from oil palm (*Elaensisguineensis*) and raffia palm were purchased from palm wine tapers. Samples were bought 5 from palm wine tappers from the three senatorial zones in Anambra State namely Ihiala and Umunze (Anambra south), Atani and Igbariam (Anambra north), Mgbakwu and Ugbenu (Anambra central) of Anambra state of Nigeria. The palm wine samples were transported to the microbiology laboratory, of Chukwuemeka Odumegwu University, Uli, Anambra State, Nigeria for analysis within 3 h of collectionin an ice packed container.

Isolation of Yeasts

Palm wine samples were serially diluted, after which an aliquot (0.1 mL) of the broth was transferred to yeast extract peptone dextrose agar plates (YEPDA) supplemented with chloramphenicol and evenly spread using a sterile 52 glass rod. Plates were also incubated as described by Nnodim *et al*. (2021) for 30^0C for 48 hours. Different colonies were randomly chosen and transferred to YEPDA plates for further study.

Screening for Bakery Yeast Potentials

All selected isolates were tested for bakery yeast potentials which include sugar fermentation test, urea hydrolysis, hydrogen sulphide production and degree of flocculation.

Sugar fermentation test

This experiment was conducted in the same manner as described by Sonia *et al*. (2019). The isolates were tested to determine their capacity to ferment glucose, sucrose, maltose, fructose, and lactose. Isolates were inoculated into a test tubes containing inverted durham tube and peptone water containing1 gm of the sugar and a drop of bromocresol purple indicator. They were incubated for 48hours at 30^0C, change in color (purple to yellow) or otherwise, of the indicator as well as liberation and trapping of gas trapped in the durham tube indicate the result of each test, the presence of gas was taken as evidence of a reasonably high rate of fermentative activity.

Urea hydrolysis

The method described by Olowoniibi (2017) was used for this test. Urea broth was prepared by combining 9.68 grams of urea broth (difco) and 250 milliliters of distilled water, which was then filtered and sterilized. Aseptically, 0.5 ml of the solution was carefully transferred into sterile, empty test tubes. Each individual isolate was introduced into its designated tube, which was sterilized using a flame, and incubated for 24 hours at a temperature of 37 degrees celsius. The broth was monitored for a color change from yellow to a vibrant shade of pink.

Hydrogen sulfide production test

Bacto bismuth sulfite agar dehydrated - BSA (difco) was utilized for the selection of the H_2Sproducingyeast isolates, as outlined by Tseganye *et al*. (2018). All glassware used was properly sterilized at 160 ^{0}C for 1 h using a hot air oven. Twenty grams of BSA was dissolved in 500 milliliters of distilled water. The medium was heated gently with frequent agitation until it started to boil and simmer for 30 seconds to dissolve the agar. The medium was sterilized at 121°C for 15 minutes, then cooled to 50°C and poured into plates. The dish was partially covered to allow the medium to solidify. The yeasts were introduced and allowed to grow for 48

ISSN No. 2454-6194 | DOI: 10.51584/IJRIAS |Volume X Issue V May 2025

hours at a temperature of 30^0C. The non-sulfide producing strains resulted in white colonies, while the H_2S producers exhibited a range of colony colors, varying from light brown to black, depending on the intensity of production.

Flocculation test

The method described by Kumari *et al*. (2019) was carried out for this test. The yeast isolates were introduced into 5 ml of sterile yeast broth and incubated at 30 degrees Celsius for 72 hours. After incubation, the tubes were stirred to assess the level of flocculation. After agitation, the culture supernatant was carefully separated and the adherence of the yeast sediment to the test tube was visually observed and documented.

Characterization of Selected Isolates

Two of the best-performing isolates were selected for characterization based on the results of the baking yeast screening tests. They were characterized using molecular method.

Molecular characterization

Genomic DNA was extracted from the samples using the Quick-DNA^{TM} bacterial/fungal kit (Zymo Research, Catalogue No. D6005). The ITS target region was amplified using OneTaq Quick load 2X Master Mix (NEB, CATALOGUE No. M0486) with the primers presented in Table 1. The PCR product were run on a gel and EXOSAP method was used for enzymatic clean up. The extracted fragments were sequenced in the forward and reverse direction (Nimagen, BrilliantDye^{TM} Terminator Cycle Sequencing kit V3.I, BRD3-100/1000) and purified fragments were analyzed on the ABI 3500xl Genetic analyzer (Applied Biosystems, ThermoFisher Scientific) for each reaction for every sample, as listed in section 1. DNASTAR was used to analyze the ab1 files generated by the ABI 3500XL Genetic Analyzer and results were obtained by a BLAST search (NCBI).

Optimization of cultivation conditions of the selected yeast isolates

The selected isolates (5 and 12) were predicted as *Yarrowia phangngaensis YP.* Based on the percentage ID of isolates isolate 5 was chosen for optimization of its cultivation conditions.

Optimum time test

The method described by Karki *et al*. (2017) was adopted for this study. The ability of the yeast to grow at varying time interval was studied by plating the yeast isolates onto YEPD broth and incubated at 30^0C at different times, 24, 48, 72, and 96 h. Growth was measured at optical density 600 using spectrophotometer. The culture medium was used as blank.

Optimum temperature test

The method described by Karki *et al*. (2017) was adopted for this study. The yeasts capability of adapting to different temperatures was studied by inoculating the yeast isolates onto YEPD broth and grown at 4 different temperatures (24, 28, 30, and 37^0C) for 72 h. Result was read as described above.

Optimum pH test

The method described by Karki *et al*. (2017) was adopted for this study. The ability of the yeast to grow at different pH was studied by inoculating the yeast isolates onto YEPD broth and incubated at 4 different pH (3, 5, 7, and 10) at 30^0C for 72 h. Result was read as described above.

Stress Tolerance Tests on the Selected Yeast Isolate

The yeast isolate selected based on its baking potentials was subjected to the following stress tolerance tests in comparison with commercial baker's yeast:

ISSN No. 2454-6194 | DOI: 10.51584/IJRIAS |Volume X Issue V May 2025

Ethanol tolerance test

The yeast's ability to thrive in higher ethanol concentrations was determined as described by Tseganye*et al*. (2018). The yeast isolates were grown on YEPD broth containing different concentrations of ethanol, 9%, 11% and 15% (v/v), respectively and incubated at 30°C for 72 hours.Result was read as described above.

Hyperosmotic tolerance test

The test was carried out as described by Karki *et al*. (2017). Yeast isolates were cultured on YEPD broth containing 25, 35 and 45% dextrose and incubated at 30°C for 72 h. The cell density of the isolates in response to dextrose concentration was taken. Result was read as described above.

Sodium chloride (NaCl) tolerance test

The method of Liu *et al.* (2023) was adopted for sodium chloride tolerance test. Yeast isolates were cultured on YEPD broth containing different concentrations of sodium chloride (2, 3 and 5%) and incubated at 30°C for 72 h. The cell density of the isolates in response to salt concentration was taken. Result was read as described above.

Pathogenicity Test

***In vitro* pathogenicity test**

Blood hemolysis

The test was conducted using the method outlined by Dessalegn and Andualem (2023). Twenty-four-hour old yeast culture was streaked on a blood agar base supplemented with 5% sheep blood and incubated at 37°C for 48 h. The hemolytic activity of yeast isolate was evaluated after incubation by observing partial hydrolysis of red blood cells and the production of a green zone (α-hemolysis), total hydrolysis of red blood cells producing a clear zone around the streaked isolate cultures (β-hemolysis) or no hydrolysis of red blood cell, no formation of green and clear halo zone, growth normally (γ-hemolysis).

Indian ink inclusion test

Following the methods of Behera *et al*. (2013), 50µL of 24 h yeast culture of the different test organism was inoculated separately into Tryptic Soy Broth (TSB) containing 10% 0.45 µL filtered Indian ink. The cultured broth was incubated at 37 ^{0}C and observed at 10-12 h for visible Indian ink degradation as evidence of capsular virulence.

Yeast Leavening Activity

Preparation of Yeast Propagation Medium

The method of Ezemba *et al*. (2022) was modified and applied for preparation of yeast propagation medium. It consists of nutrient broth supplemented with 2% (w/v) of glucose which was dispensed (25ml) into fourteen 100ml Erlenmeyer flasks; they were plugged with cotton wool and foil and autoclaved at 121˚C for 15 minutes.

Propagation of yeast isolates

A modified method of Ezemba *et al.* (2022) was applied for propagation of yeast isolates. A loopful of each yeast isolates were separately inoculated into each of the 100ml Erlenmeyer flasks containing the propagation medium and were incubated in an orbital shaker at 150 rpm for 24 h. After 24 h, the entire cultures were transferred into another set of freshly prepared propagation medium (75 mL) in 250mL conical flasks and were further incubated for another 72 h on the shaker. After propagation, the biomass was recovered by centrifugation (4000 rpm, 10 min.) in an Eppendorf centrifuge (model 5804 R).

ISSN No. 2454-6194 | DOI: 10.51584/IJRIAS |Volume X Issue V May 2025

Dough preparation

The method described by Gabreslassie *et al*. (2019) was modified and applied in this study for dough preparation. Dough was prepared with wheat flour (25g), harvested yeast culture (2 g), table sugar (0.5 g). These ingredients were properly kneaded with distilled water (20 mL) and transfered into 100 mL measuring cylinders and covered with aluminium foil. The measuringcylinder was smeared with vegetable oil before transferring the dough. Commercial yeast (SkF- instant yeast) was used separately as a positive control to ferment the dough. The dough samples were left to ferment at 30 ^{0}C for 3h (180 minutes).

Determination of dough rising capacity

The method of Jiya *et al*. (2020) was applied for dough rising determination by measuring the volume increment at every 30 min interval and the results compared with the commercial baker's yeast. All dough samples were covered using aluminum foil to maintain an anaerobic environment. The initial and final dough volumes were recorded from the graduations on the measuring cylinder and the net increase in volume was calculated. The dough raising capacity (DRC) was computed as:

DRC = [(V2 – V1)/V1] x 100

Where V1 = Initial volume, V2 = Final volume

Dough Baking

Dough made from CY and YP were baked in hot air oven at 180 ^{0}C for 30min as described by Karki *et al.* (2017).

Analysis of Baked dough

The baked dough was subjected to various dough sensory properties as described by Nwakamma *et al*. (2015). Properties such as volume, crust, colour, internal colour, structure texture, flavor/aroma, crumb clarity and elasticity were tested by 5 enlightened judges. Five-point grades was used in the analysis starting with excellent=5, Very good=4, Good=3, Satisfactory=2, Poor=1.

Toxicological Studies

Bread baked with the test organism as the leavening agent was tested for toxicity using albino rats.

Animals and treatment

The method described by Olatoye and Arueya (2018) was modified and adopted for animal treatment. Total number of twelve (12) albino rats (*Rattus novergicus*) weighing between (50 and 55 g), obtained from the Animal House, Department of Clinical pharmacy, University of Nigeria, Nsukka were used. They were acclimatized for 7 days to well-ventilated room at temperature 30 ± 4°C and relative humidity of 60%. They were kept in cages, fed *adlibitum* with standard rat feed and clean water. All animal experiments were conducted in compliance with NIH guidelines for care and use of laboratory animal as reported by Akah *et al*., (2009). The study was conducted at Department of Microbiology, Chukwuemeka Odumegwu Ojukwu University, Uli, Anambra State, Nigeria.

Animal handling

The method described by Olatoye and Arueya (2018) was modified and adopted for animal handling. Rats were randomly grouped into three groups (four animals each) and separately housed after adaptation period. Groups were provided with diets as shown in Table 1 and water offered to them *adlibitum*. All animals were inspected daily for appearance of signs of toxicity and possible deaths. The same level of hygiene was maintained throughout the 14 days experimental period. The period of observation was adopted from Okereke *et al*., 2023.

ISSN No. 2454-6194 | DOI: 10.51584/IJRIAS |Volume X Issue V May 2025

Experimental feed formulation

Hundred percent bread (BF) and Rat feed (RF) were used as bases for diets BF1 and BF2, respectively (Table 1). BF1 and BF2, respectively were formulated from (45:55) and (65:35) combinations of BF and RF. Diet RF0 was the normal (control) diet purchase from commercial producer (Chukun broiler feeds)

Table 1: Animal grouping and diets consumed

Group	RF0 (I)	BF1 (II)	BF2 (III)
BF: RF	00:100	45: 55	65: 35

RF= Rat Feed; BF= Bread feed;

Data Analysis

The statistical methods applied in this work are correlation and regression analysis performed using SPSS version 22.

RESULTS

Screening for baking yeast potentials

All fungi isolates were screened for baking yeast potentials. They include; sugar fermentation test, H_2S production, degree of flocculation and urea hydrolysis. The isolates produced varying results as expressed in Table 2. Isolates 5 and 12 utilized almost all the sugars, produced no H_2S within the period of 48 h incubation and show reasonable degree of flocculation.

Characterization selected yeast isolates

Isolates 5 and 12 were chosen for further analysis. Table 3 shows their molecular identity. Both were identified as *Yarrowia phangngaensis.*

Table 2: Screening for baking yeast potentials

Isolate	Sugar					H_2S Production	Degree of Flocculation	Urea hydrolysis
	Glucose	Sucrose	Fructose	Maltose	Lactose			
3	++	++	++	++	+++	+	+	+
4	++++	++	+++	+++	+++	++	++	+
5	+	++++	++++	+	+	-	+++	+
7	-	++	+	+	+	+	+	+
8	+	++	+	+	+	++	++	+
10	+++	++	-	+++	+	+	++	+
11	+	++	+	+	++	++	++	+
12	++	++++	+	++	++	-	+	+
13	++	-	+	-	-	++	+	+
15	+	++	++	++	-	++	++	+
16	+	++	+	+	-	++	++	+
17	-	+	+	+	-	-	++	+
18	+	++	+	++	-	++	+	++

ISSN No. 2454-6194 | DOI: 10.51584/IJRIAS |Volume X Issue V May 2025

20	++++	-	+	++	-	++	+	+
21	+	++	++	-	-	++	+++	+
22	++	-	+	-	-	++	++	-
25	++	+	++++	+++	-	+	++	+
RP5	++++	+++	++	+++	-	+	+++	++
RP6	++	+	++++	+++	-	+	++	+
RP8	++++	-	+	++	++++	+	+	-
RP10	-	++	++	+	+	+	++	+
KP1	+	+	+++	++	+	++	+	+
KP3	++	+	++++	+++	-	+	++	+
KP4	+	+	++	+	+	-	+	+
U2	++	+	++	++	+	++	+	+
U3	++	-	++	++	++	++	++	+
11a	-	-	+	-	++	+	++	+
4b	-	-	-	-	+	+	+	+
4*	-	++++	+	+	-	++	+	+
13b	+	++	+	+	+	+	++	+
1*	+	-	+	-	-	+	+	+
2*	+	-	++++	-	+	++	+	+
3*	+	+	++++	-	+	+	+	+
4**	+	+++	-	-	+	+	+	+
5*	+++	-	+	+	++	-	++	+
Control	++	++	++	++	+	+	++	+

- = Not utilized; += low; ++=moderate ; +++=intense ++++= more intense

Table 3: Molecular identification of selected yeast isolates

Isolate	Percentage ID	Predicted Organism	Genbank accession
5	98.73%	*Yarrowiaphangngaensis*	MH79381.1
12	98.09%	*Yarrowiaphangngaensis*	MH79381.1

Optimization of cultural conditions

After molecular characterization it was observed that isolates 5 and 12 were same organism (*Yarrowia phangngaensis*). Further analysis was carried out using isolate 5 against control (commercial yeast). The Commercial yeast is denoted as CY while *Yarrowia phangngaensis*is denoted as YP. The optimum growth temperature of the test organisms is 28^0C and 30^0C for CY and YP respectively as shown in figure 1. Statistically, there was weak negative correlation between growth density and temperature. As the temperature increased growth density decreased.

Figure 2 shows the optimum growth time of the isolates. Both organisms grew optimally after 72 h. The optical density of the broth culture for both isolates rose 24 h to 72 h and a decline was observed after 72 h

ISSN No. 2454-6194 | DOI: 10.51584/IJRIAS |Volume X Issue V May 2025

through 96 h. There was weak positive correlation between the two variables. Increase in growth density was proportional with increase in time.

Figure 3 shows the optimum growth pH for the isolates. Both organisms grew optimally at pH 5. CY adapted better to pH 5 than YP with optical densities of 0.9 and 0.7 respectively. Statistically, there was weak positive correlation between the variables.

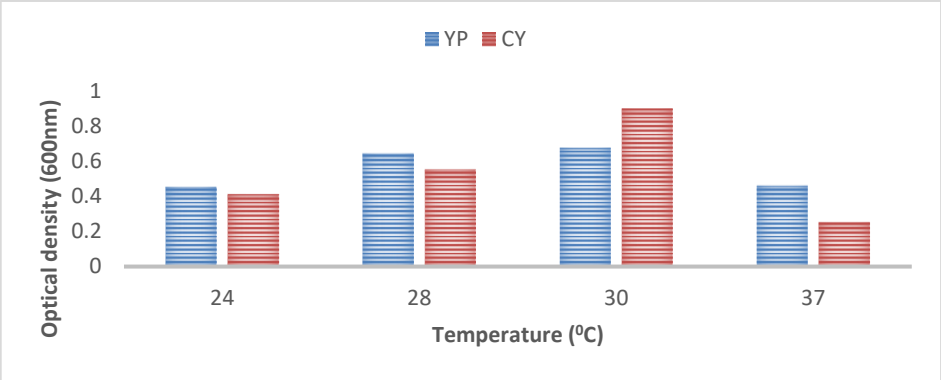

Figure 1: Optimum growth temperature of YP and CY

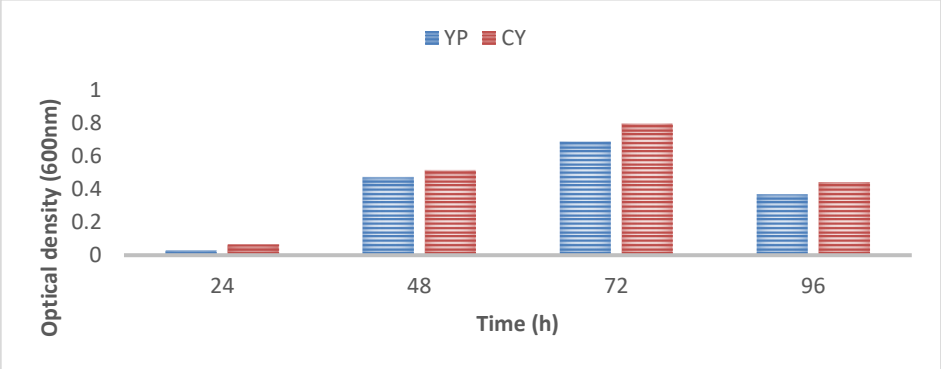

Figure 2: Optimum growth time of YP and CY

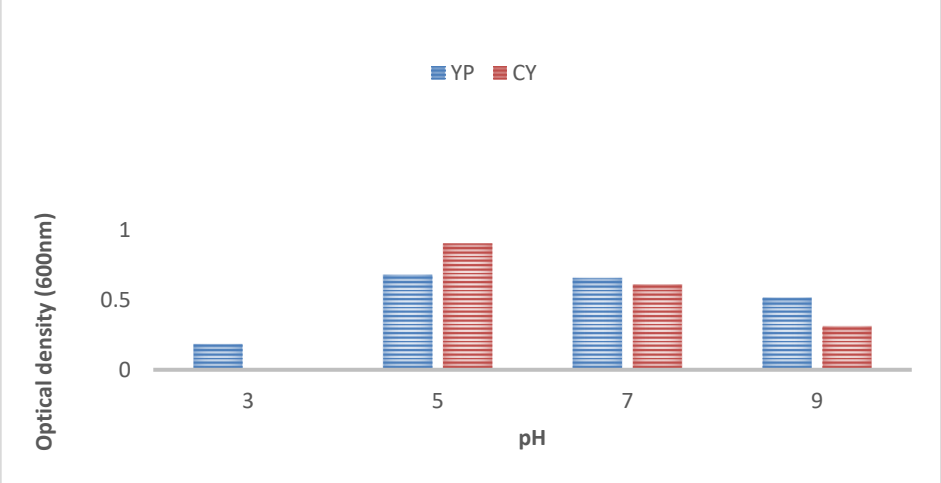

Figure 3: Optimum growth pH of YP and CY

Stress tolerance of selected isolate

Figure 4 shows the Sodium chloride tolerance of CY and YP. Both tolerated all the salt concentrations they were exposed to, but grew optimally at the least salt concentration of 2%. Their tolerance decreased with increase in salt concentration. YP showed higher tolerance to salt than CY. Statistically, there is very strong negative correlation between salt concentration and growth density.

ISSN No. 2454-6194 | DOI: 10.51584/IJRIAS |Volume X Issue V May 2025

Figure 5 shows the ethanol tolerance of CY and YP. Both tolerated all the ethanol concentrations they were exposed to, but optimally growth was observed at 10% concentration. Their tolerance increased from 5% to 10% and fall in optical density was observed at 15% concentration. YP has higher tolerance to ethanol than CY. Statistically, there was no correlation between the variables, this result showed there was no correlation between ethanol concentration and growth density.

Figure 6 shows the hyper-osmotic tolerance of CY and YP. Both tolerated all the glucose concentrations they were exposed to. YP showed optimum growth at 45% glucose concentration, optical density increased with increase in glucose concentration. CY showed optimum growth at 45% glucose concentration, optical density increased from 25% to 45% glucose concentration. YP tolerated higher glucose concentration than CY. CY showed weak correlation while YP showed very strong positive correlation. Growth tends to increase with increase in glucose concentration.

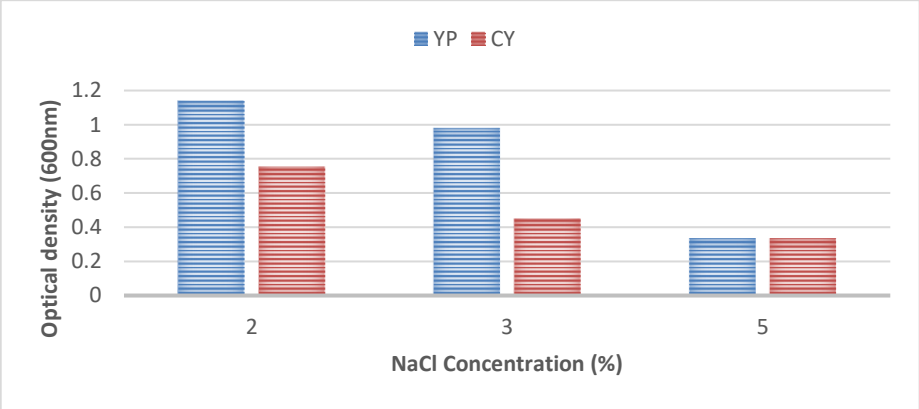

Figure 4: Nacl tolerance of YP and CY

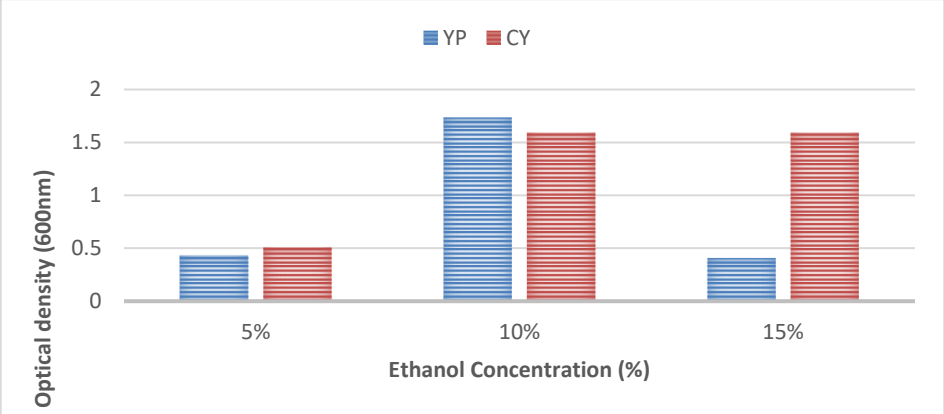

Figure 5: Ethanol tolerance of YP and CY

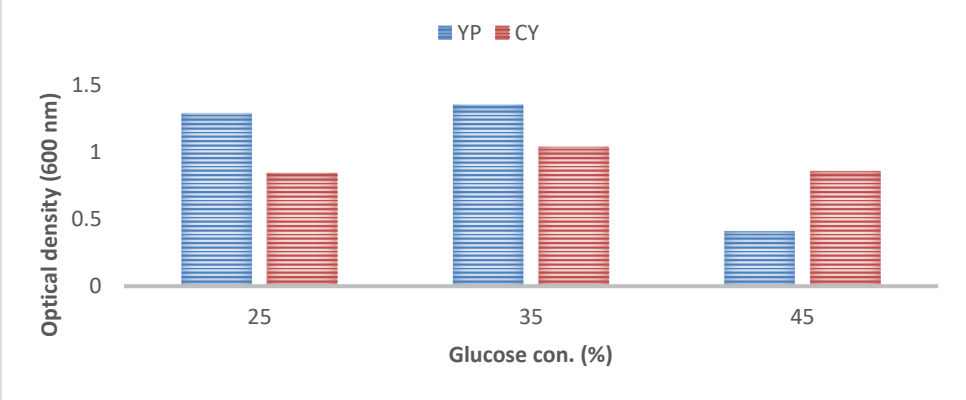

Figure 8: Hyper-osmotic tolerance of YP and CY

ISSN No. 2454-6194 | DOI: 10.51584/IJRIAS |Volume X Issue V May 2025

Pathogenicity test

Since these yeasts were isolated for prospective use in food, they were subjected to in vivo pathogenicity test. Both isolates were negative in all the tests, which includes; blood haemolysis test and Indian ink inclusion test. The outcome is as shown in Table 4.

Table 4: Pathogenicity test

Sample	BHT	InI
5	-	-
12	-	-
Control	-	-

Key

BHT= Blood haemolysis test;

InI= Indian ink inclusion test

Negative result, -

Positive result, +

Yeast leavening activity

Figure 7 shows dough leavening activity or dough rising capacity (DRC) of the yeast, CY and YP for 180 h. Maximum DRC of 5.69 and 5 was recorded at 150 h for CY and YP respectively. Statistically, CY shows strong positive correlation while YP shows moderate positive correlation between DRC and time.

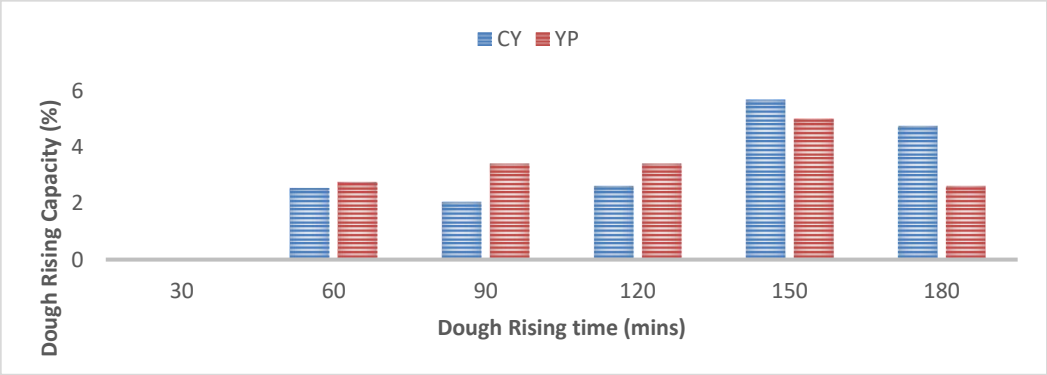

Figure 7: Dough Rising Capacity of CY and YP

Sensory evaluation of baked dough

Table 5 shows the mean sensory evaluation of the baked dough. CY show higher mean score in volume (4.6), internal colour (4.4), elasticity (4.8) while YP higher mean score in crust colour (5.0), texture (4.5), aroma (4.4).

Table 5: Mean sensory evaluation of the baked dough

Parameters	Yeast Isolates	
	CY	YP
Volume	4.6	4.5

ISSN No. 2454-6194 | DOI: 10.51584/IJRIAS |Volume X Issue V May 2025

Crust colour	4.8	5.0
Internal colour	4.4	4.2
Texture	4.0	4.5
Elasticity	4.8	4.4
Aroma	3.8	4.4

Toxicology study

Albino rats were fed with bread made with the test isolate, *YP*. No casualty was observed as recorded in Table 6.

Table 6: Doses of bread supplemented with rat feed administered to rats.

Dose (BF:RF)	Death	Observation
0:100	0/4	The rats remained normal
45:55	0/4	The rats remained normal
65:35	0/4	The rats remained normal

BF= Bread, RF= Rat Feed

DISCUSSION

Palm wine is a whitish, sparkling, alcoholic drink produced by the impulsive yeast-lactic acid fermentation of the sugary juice of palm trees. The juice of the tropical plants of the Arecaceae family, when fermented, produces palm wine. The southeastern part of Nigerian produces and consumes it in large quantities. It is rich in nutritionally essential components which include vitamins, amino acids, sugars, and proteins. These make this wine an authentic medium for the growth of a group of microbes, whose growth, causes a change in the physicochemical conditions of the wine, resulting in rivalry and successions of organisms (Oti *et al*., 2021).The native flora of palm wine is mostly influenced by the palm tree species and its geographical location (Djeni *et al*., 2020).

Palm wine samples were screened in the work for baker's yeast. They were serially diluted and plated on YEPD agar for 48 h. After incubation, yeast colonies were observed on the agar. All the isolates were screened for bakery yeast potentials; this includes sugar fermentation, urease hydrolysis, H_2S production and flocculation ability. Thirty-five isolates were examined in this work for their baking yeast potential, each showed varying degrees of its capability as presented in Table 2.

Palm wine contains a high level of sucrose (10-12%). This high sugar level favours the growth of yeasts. The yeast fermentative capacity test was used to check the fermentative ability of the test isolate. Yeasts bring about fermentation whenever it comes to a favorable environment i.e. with a sugar source. The main function of sugar is to provide food for the yeast. The various sugars used for the tests are the sugars found in dough and so the yeasts‟ ability to ferment them will show that they possess the various enzymes which ferment these sugars. The flour contains starch with the enzyme, amylase, which was produced during the milling process. Once water comes in contact with the flour, the action of the enzyme is activated which turns the starch into maltose. The enzymes possessed by the yeasts then come to play, first starting off with maltase which converts maltose to simple sugar like the glucose which is then acted upon by zymase to give carbon dioxide and alcohol. But if sucrose (table sugar) was added in the dough, the yeast then provides the enzyme, invertase, which breaks it down to glucose and fructose, then subsequently to carbon dioxide and alcohol (Ezemba *et al*., 2022). Many of the isolates obtained in this work exhibited high fermentative ability, as well as gas production as shown in Table 2. On this basis isolates 5, 12 and KP4 were chosen. They were able to use all the sugar which was ascertained by a change of colour from purple to yellow and also produced reasonable amount of

ISSN No. 2454-6194 | DOI: 10.51584/IJRIAS |Volume X Issue V May 2025

gas. The gas was trapped in a durham tube inverted inside the test tube.

All the isolates except 5 and 12 produced H_2S after 48 h while KP4 showed mild H_2S production. The control which is commercial yeast got from the market also was positive for H_2S production. Isolates 5 and12 produced no dark coloration while KP4 showed little. These three isolates exhibited unique properties which distinguished them from others, but the best two were picked for comparison with commercial yeast. They were different from the regular organisms as reported in other studies. The complexity and variation in species of yeasts isolated could be related to the fact that the yeast originate from different sources such as the tapping container (which may retain some yeasts) and from the tapping environment (Ezemba*et al*., 2022).

Flocculation abilities of the isolates were also tested as shown in Table 2. Yeast cells which had ability to flocculate caused by cell adhesion process were selected. The flocculation characteristic was determined by yeast cells sticking together and this helps easy separation from the broth medium. This phenomenon has an economic effect on the production of yeast biomass due to the fact that it can reduce the energy cost involved in biomass centrifugation. In addition, flocculation properties of yeast ensure a high cell density and large volume of harvested cells and also able to raise the ethanol productivity during the fermentation process. Initiation of flocculation ability of cells was observed at the moment the cells stop dividing because of nitrogen limitation. A shift in concentration of the limiting nutrient resulted in a corresponding shift in cell division and initiation of flocculence (Olowonibi 2017). Reasonable degree of flocculence was observed in isolates 5, 12 and KP4.

Almost all the isolates obtained in this work produced hydrogen suphide (H_2S) at varying degrees as observed in Table 2. Three isolates produced no H_2S while others produced either low, moderate or high H_2S. The control which was the commercial yeast produced H_2S moderately. Yeasts with elevated production of hydrogen sulfide are undesirable for bread making because it confers flavor and taste that compromise the quality of the bread (Umeh *et al*., 2019). The report of this work on H_2S production is in contrast with that of Umeh *et al*.(2019) and Okafor *et al*.(2022). Their controls were non hydrogen sulphide producers while Gebreslassie *et al*.(2019) discovered lowH_2S producer as control. Gebreslassie's result and the result from this work shows that some of the yeasts available in the market produces hydrogen sulphide which adversely could affect the end-product of a bakery. Carefully, isolates 5 and 12 were selected for further characterization.

Isolates 5 and 12 were characterized based on molecular characteristics and identified as *Yarrowia phangngaensis*(Table 3). The finding was in contrast with that of Ezemba *et al*.(2022); Olowonibi, 2017; Bolaniran *et al*.(2017) whose major isolates were *Sacharomyces cerevisae*. It agrees with the report of Nwagu *et al*. (2021) who isolated yeast from soil, fruit and wine samples and screened for the ability to synthesize lipase, cellulase, xylanase, pectinase, amylase and protease. Among other isolates *Yarrowiaphangngaensis* XB3 was most suitable for cassava-wheat composite bread.

In the Yarrowia clade the most popular one is *Yarrowia lipolytica. Yarrowia phangngensisis* is one of the species in the genus but under explored. Since this under explored organism was proposed for food production, it was pertinent to test it for pathogenicity. Table 4 shows the pathogenicity test results of the isolates. They were negative in all the tests ranging from blood haemolysis, Indian ink tests. The above certifies the organism as nonpathogenic. Based on the percentage ID's of 98.73% and 98.08 % shown in Table 3 for isolates 5 and 12 respectively, isolate 5 was selected for further analysis.

The metabolic and production efficiency of cells depends on many factors such as temperature, pH, incubation period, inoculums size, genetic background (Gebreslassie *et al*., 2019). The isolate, YP was exposed to different growth conditions (figures 1-3) to know which supports its growth best. It showed higher biomass at incubation time of 72 hours, temperature of 30^{o}C, and pH of 5while the commercial yeast (control) shared similar characteristics as regards this work. This result shows that the growth of the isolated yeasts (this study) is comparable to that of the commercial yeast strain. In contrast to this result, Nwagu *et al*., (2019) has found that the yeast grew maximally at 24^{o}C though it could withstand higher temperatures (28 and 37^{0}C) temperature at 48 hours of incubation period. The yeast strain in this study could tolerate temperature up to 40^{o}C including the control (figure 1). The ability of yeast to tolerate high temperature suggests that the isolates can withstand excess heat associated with fermentation process and therefore can be used to accomplish

ISSN No. 2454-6194 | DOI: 10.51584/IJRIAS |Volume X Issue V May 2025

fermentation at a wide range of temperature conditions. In agreement with this study, Gebreslassie *et al*. (2019); Umeh*et al*. (2019); Ezemba*et al*. (2022) have also reported that yeasts can grow at elevated temperatures of 40^{o}C though sparingly, but the optimal temperature is approximately 30^{o}C.

In the current study, a maximum biomass was obtained at 72 hours of incubation period as recorded in figure 2, but the biomass decreased with increasing incubation time. This is supported by the scientific fact that the stationary phase of yeast growth is a period of no growth, when metabolism slows and cell division is stopped due to nutrient deprivation, toxic metabolites and high temperatures which led cells to die and autolyse. In contrary to the present study, Mamun-Rashid et al. (2013) have stated that the highest biomass was recorded after 144 hours of incubation period. The difference in these results may be due to the genetic constituent of their cells and cultivation conditions.

The organism was tested for its stress tolerance in comparism with commercial yeast which served as control. Stress tolerance test as was carried out to ascertain if the selected strain can adapt to bread making conditions. Survival of baker's yeast under various stress conditions could provide useful information on its ability to grow and carry out fermentation as impaired yeast. The growth of impaired yeast during fermentation may not be optimal especially when it is exposed to several stresses such as osmotic and ethanol stress (Olowonibi, 2017). In this research, as indicated in figures 4-6, the yeast grew under stressful conditions (Olowonibi, 2017).

Figure 4 show tolerance of YP and CY to the varying concentrations of salt. All the yeast strains show tolerance to the varying concentrations of salt (2%, 3%, and 5%). The yeast could tolerate the different salt concentrations it was exposed to, this followed the trend reported by Olowonibi (2017) and Eshet *et al*.(2022). As the salt concentration increased its adaptability reduced. The significance of salt tolerance lies in the fact that in Nigeria, experience shows that some bakeries use salt instead of sugar because the former is cheaper and preferred by people with some health condition such as diabetes. Salt has several functions in baked goods. It modifies flavour, increases crust color and controls the rate of yeast fermentation and enzyme activity. Salt also strengthens gluten, making it more cohesive and less sticky. With salt present, gluten holds more water and carbon dioxide, allowing the dough to expand without tearing (Olowonibi 2017).

Since the yeast cells in bread making produce ethanol as secondary metabolite the ethanol stress tests were conducted to observe its tolerance to ethanol. A suitable concentration of alcohol is needed in bread making in order to achieve the preferred flavor. As shown in Figure 5, all yeast strains were able to grow in a medium containing 5%, 10%, and 15% (v/v) of ethanol. At 15% (v/v) of ethanol the optical density drastically reduced for both test organisms. This could be due to toxic effect of high concentration of alcohol on yeast, which inhibits the cells growth due to the destruction of the cell membrane as reported by Olowonibi (2017). Those strains which were capable of growing in similar concentration were expected to have ability to produce similar quality of bread as commercial strain.

Dough rising capacity (DRC) of YP was tested against CY. The dough with isolate incorporated in it was allowed to rise for 180 minutes. The DRC was noted at 30 minutes interval. This rising time gave room for yeast to act on the dough. It was observed in this work that the indigenous yeast isolated from palm wine YP had higher rising capacity than the controlCY as shown in figure 9 the maximum DRC (5.69) was recorded at 90 minutes for YP while CY was maximum at 120 minutes with DRC (5).This indicates that YP could breakdown the sugar in the dough faster than CY, therefore can be developed for use in large scale production. This can potentially increase the varieties of yeasts and ultimately decrease their importation at huge amount of foreign currencies.

YP and CY were used to bake bread to check their sensory properties on dough. The volume, crust colour, internal colour, texture, elasticity and aroma of baked dough were analyzed (table 5). As observed in this work YP has mean value of 4.5 for volume, crust colour, internal colour, texture, elasticity and aroma respectively. CY has mean value of 4.6 for volume, crust colour, internal colour, texture, elasticity and aroma respectively. Comparing the volume of the two loaves had same rating in volume. This shows that the yeast isolate increased the volume of the bread even more than the commercial yeast which is due to its ability to produce carbon dioxide and this agrees with Nwakamma *et al*. (2015) and Cofalec (2014) who stated that yeast

ISSN No. 2454-6194 | DOI: 10.51584/IJRIAS |Volume X Issue V May 2025

produces carbon dioxide that results in dough leavening and contributes to the flavor and crumb structure of bread. The crust color and internal color of the breads were checked to get the overall acceptability of the bread since it could dictate the yeast's interaction with the ingredients of the bread.

The bread ingredients are formulated to give bread its taste, structure, aroma, texture and nutrients. These ingredients include: flour, liquids, leavening agent, salt, sweeteners, fats or oil and additives (optional). (Nwakamma *et al*., 2015). YP gave the best results regarding crust colour, texture and aroma while CY performed better in internal colour and elasticity. The structures of the different breads were checked and bread 5 was significantly different from bread 1 and bread 6, as it gave a good bread structure because it produced enough CO_2 which helped in the development of the gluten network. As a result, the dough gets fatter and bigger, and rises, of course. Thus, when the dough is baked, you have a bold loaf, light and airy; when you cut it you can see all the tiny holes formed by the gas, so that it looks like a sponge.

The structure of bread depends on dough ingredients, yeast activity, fermentation temperature and gas bubble formation (Lassoued *et al*., 2007). This agrees with Chukwuka, (2013) that During dough fermentation, yeast produces secondary by products like the ketones, higher alcohols, organic acids, aldehydes and esters. Most of the alcohols are cooked off during baking. Then the others react with one another and other elements contained in the fermenting dough to form new and more complex flavored compound (Nwakamma *et al*., 2015). These parameters occur as a result of the yeasts ability to leaven the dough. These attributes are due to the ability of the yeast to produce CO_2 and the strength of the dough to hold the gases produced and ability to be stable enough to hold its shape and cell structure. It is therefore obvious that palm wine yeast isolate YP has the characteristics of a good baker's yeast and can be utilized for industrial production.

The aim of food toxicology study is to provide a scientific basis to ensure food safety. In the subfield of regulatory toxicology, the knowledge of food toxicology may be used as a scientific basis for food policy decisions, e.g., about maximum limits of certain compounds in foods, as well as a basis for enforcement action by authorities, i.e., taking unsafe foods from the market (Lachenmeier *et al*., 2022). It could be done using *in-vitro* or *in-vivo* methods. As regards this research *in-vivo* method was applied by feeding albino rats with the end product (bread). Toxicological studies carried out on albino rats showed that the bread produced using the test organism is safe for consumption as the rats remained normal without casualty for the 14-day period of observation as shown in table 6.The dosage fed the rats as shown in table 1 were similar to that of Olatoye and Arueya, (2018) on toxicology parameters of albino rat fed with extruded snack.Absence of casualty has been adopted by researchers as evidence for lack of adverse toxic effect (Okereke *et al*., 2023; Nmezi *et al*., 2022; Nalimu *et al*., 2022; Bulus *et al*., 2011).The isolate showed good prospects for use in the bakery industry on the basis of its dough rising capacity, sensory properties and safety of use.

CONCLUSION

Potential bakery yeast *YP* was isolated palm wine. It showed prospects for bakery use among others such as its sugar utilization, flocculation ability and inability to produce H_2S. Furthermore, it showed reasonable stress tolerance ability. Pathogenicity studies on the isolate and toxicity study of the end product (bread) shows it is safe for use in food. However, strain improvement may be required for more robust performance and application at industrial level.

REFERENCES

1. Aslankoohi E, Herrera-Malaver B, Rezaei MN, Steensels J, Courtin CM, Verstrepen KJ (2016) Non-Conventional Yeast Strains Increase the Aroma Complexity of Bread. PLoS ONE 11(10): e0165126. https://doi.org/10.1371/journal.pone.0165126
2. Bitrus, J., Amadi, O., Nwagu, T., Nnamchi, C. and Moneke, A. (2020) Application of wild yeast (Saccharomyces cerevisiae) isolates from palm wine and honey in baking of cassava/wheat composite bread. Food and Nutrition Sciences 11: 695-711. doi: 4236/fns.2020.117050.
3. Bolaniran, T., Arotupin, D. J., Afolami, O. I. and Fasoranti, O. F. (2017). Sensory Evaluation Assessment of Bread Produced with Composite Flour Fermented by Baker's Yeast in Akure, Nigeria.

Asian Journal of Advances in Agricultural Research 4(1): 1-9, 2017; Article no.AJAAR.36899 ISSN: 2456-8864

4. Camila de Souza Varize, Renata Maria Christofoleti-Furlan, Elisangela de Souza Miranda Muynarsk, Gilberto Vinícius de Melo Pereira, Lucas Dantas Lopes and Luiz Carlos Basso (2019). Biotechnological Applications of Nonconventional Yeasts. Yeasts in Biotechnology. DOI: 10.5772/intechopen.83035
5. Das, S. and Tamang, J.P. (2021). Changes in microbial communities and their predictive functionalities during fermentation of toddy, an alcoholic beverage of India. Microbiol Research 248:126769. doi: 10.1016/j.micres.2021.126769
6. Eleanya, Lilian Chinyere, Igboanugo, Edwin Uchechukwu, Dibua, Nwamaka Anthonia, Anagor, Ifeoma Sandra, Ezeumeh, Eucharia Nkiruka, Chukwura, E. Nnamdi and Okpalla, Jude (2025). Isolation and Screening For Non-Conventional Yeast With Bakery Potentials. International Journal of Research and Innovation in Applied sciences
7. Ribeiro, R.A., Bourbon-Melo, N. and Sá-Correia, I. (2022). The cell wall and the response and tolerance to stresses of biotechnological relevance in yeasts. Frontiers of Microbiology, 13:953479. doi: 10.3389/fmicb.2022.953479
8. Gebreslassie, E.B., Tefera, A.T., Muleta, D., Fantaye, S.K. and Wessel, G.M. (2019). Optimization of the cultivation conditions of indigenous wild yeasts and evaluation of their leavening capacity. International Journal of Sciences 8(6) DOI: 10.18483/ijSci.2043
9. Jiya, M. J., Damisa, D., Abdullahi, I.O. and Chukwu, V.C. (2020) Comparative studies of the dough raising capacity of local yeast strains isolated from different sources. Anchor University Journal of Science and Technology 1(1): 90 – 95
10. Oti , V. O., Okwulehie, I. C., NwankwoB. J. and Ogoke, H. I. (2021). Assessment of the Physico-Chemical Properties of Oil Palm Wine (Elaeis guineensis) and Raphia Palm Wine (Raphia vinifera). Nigeria Agricultural Journal, 52(3)
11. Olowonibi, O.O. (2017). Isolation and characterization of palm wine strains of Saccharomyces cerevisiae potentially useful as bakery yeasts. European Experimental Biolology 7:11. doi:10.21767/2248-9215.100011
12. Liu, T., Li, Y., Sadiq, F. A., Yang, H., Gu, J., Lee, Y. K., and He, G. (2018). Predominant yeasts in Chinese traditional sourdough and their influence on aroma formation in Chinese steamed bread. Food Chemistry 242: 404–411. doi: 10.1016/j.foodchem. 2017.09.081
13. Karki, T.B. and Timilsina, P.M., Yadav, A., Pandey, G.R., Joshi, Y., Bhujel, S., Adhikari, R. and Neupane, K. (2017). Selection and Characterization of Potential Baker's Yeast from Indigenous Resources of Nepal. Biotechnology Research International. Volume 2017 https://doi.org/10.1155/2017/1925820
14. Kumari, S., K.Jha, A. and Singh, A.K. (2019) Isolation and characterization of temperature and ethanol tolerant strain of Saccharomyces cerevisiae strains from naturally fermented juices. Biosciences Biotechnology Research Asia 16(1): 97-103. http://dx.doi.org/10.13005/bbra/27266
15. Ezemba, C. C., Onyemalu, V. T., Nwanya, A. C., Ogbonna, E., Ezemba, A. S., Anakwenze, V. N., Anaukwu, G. C., Okoye, P. O. and Odibo, F. J. C. (2022). Isolation and characterization of palm wine yeasts and a comparison of their dough-raising activity with baker's yeast. Asian Journal of Plant and Soil Sciences 7(1): 66-72, 2022
16. Mahoukoucd Sheti-Kouabo D.F, Goma-Tchimbakala J.,*, Lebonguy A. A.,and Limingui Polo C. (2019). Characterization of Saccharomyces cerevisiae Strains from Palm Wine for Baking Leaven Production in Republic of Congo American Journal of Food Science and Technology, 7(6): 205-214
17. Michael E. Pyne, James A. Bagley, Lauren Narcross, Kaspar Kevvai, Kealan Exley, Meghan Davies, Qingzhao Wang, Malcolm Whiteway and Vincent J. J. Martin (2023). Screening non-conventional yeasts for acid tolerance and engineering Pichia occidentalis for production of muconic acid. Nature Communications 14(5294)
18. Dessalegn, M., and Andualem, B. (2023). In vitro evaluation of probiotic properties of yeasts and lactic acid bacteria isolated from ersho samples collected from different parts of Ethiopia. International Journal of Food Properties, 26(2), 3048–3064. https://doi.org/10.1080/10942912.2023.2270177

ISSN No. 2454-6194 | DOI: 10.51584/IJRIAS |Volume X Issue V May 2025

19. Nwagu, T.N. T., Osilo, Arinze, M.N., Okpala, G.N., Amandi, O.C., Ndubuisi, I.A. and Agu, R. (2021). A novel strain of Yarrowia phangngaensis producing a multienzyme complex; a source of enzyme additives for baking high cassava-wheat composite bread. Food Biotechnology 35(2) 158-177 https:// doi.org/ 10.1080/08905436.2021.1910520
20. Umeh, S. O., Okpalla, J. and Okafor, J. N. C. (2019). Novel Sources of Saccharomyces Species as Leavening Agent in Bread Making. International Journal of Trend in Scientific Research and Development (IJTSRD) 3(2) 827-832
21. Zhou, N., Semumu, T. and Gamero, A. (2021). Non-Conventional Yeasts as Alternatives in Modern Baking for Improved Performance and Aroma Enhancement. Fermentation 7(3) 102; https://doi.org/10.3390/fermentation7030102
22. Kazeem K Olatoye and Gibson L Arueya (2017). Toxicological parameters of albino rats fed with extruded snacks from Aerial yam (Dioscoria bulbifera) and African breadfruit seed (Treculia africana). Food Science and Nuturition 6(1):94–100. doi: 10.1002/fsn3.533
23. Stanley Chukwuma Okereke, Valentine Chibuike Edom, Caleb Joel Nwaogwugwu, Aaron Chinomso Friday, Ifegwu Prince Oko, Iwuchukwu Bruno Obinna, George Ekechukwu, Udochukwu Stanley Alugbuo and Ugoaghalam Uche James (2023). Toxicological Assessment of Irvingia gabonensis Leaf Extracts in Albino Rats: A Comparative Study between Aqueous and Ethanol Extraction Methods. Advances in Biological Chemistry 13(4):143-170
24. Nwakanma, C., Unachukwu, N.M., Onah, P. and Enwa A.G. (2015). Isolation and Sensory Evaluation of Saccharomyces cerevisiae From Palm wine (Elaeis guinneensis) gotten from different sites in Enugu. European Journal of Biomedical and Pharmaceutical Sciences 2(7), 19-26.