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Application of Wild Yeasts (*Pichia kudriavzevii* and *Saccharomyces pastorianus*) Isolated from Palm Wine and Banana for Bread Baking

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ABSTRACT

Baker's yeast is utilized for manufacturing of bread throughout the globe at an industrial scale and is totally imported from developed countries. Therefore, the aim of this study was the isolation and use of wide yeast (*Pichia kudriavzevii* and *Saccharomyces pastorianus*) for bread baking. Banana (*Musa acuminate and Musa paradisiaca*) and freshly tapped Palm wine from *Raphia raphia* and *Elaensis guineensis* were used for the isolation of the wild yeast, using enrichment media and their potential in bread production was evaluated by Dough Raising Capacity (DRC). The commercial yeast, *Saccharomyces cerevisiae* was used as control. Yeast isolates were identified based on microscopic, macroscopic and molecular methods. Proximate analyses of the baked bread were carried out using standard procedures. The results obtained were analyzed statistically using SPSS version 25. The yeast counts ranged from 1.65 x 10^3 to 1.84 x 10^3 cfu/ml for palm wine and 8.4 x 10^3 to 9.3 x 10^3 cfu/g for the banana. The screened yeast isolates were further identified using molecular techniques as *Pichia pastoris* EF116884.1, *Pichia kudriavzevii* LC413230.1, *Saccharomyces pastorianus* D89889.1. *Saccharomyces cerevisiae* LC576598.1.1. The results of the proximate composition show that carbohydrate content ranged from 54.1 to 59. 6%, crude protein ranged from 7.6 to 8.7%, moisture content ranged from 19.6 to 24.8%, while fat content ranged from 7.8 12%. *Saccharomyces pastorianus* produced the highest carbohydrate content of 59.6% than the other isolates. The results showed that the bread baked with wild yeasts and commercial baker's yeast, that there was significant difference between the bread types with regards to moisture content, fat, protein, carbohydrate between the bread baked with *Pichia* species and the control but no significant difference between *Saccharomyces pastorianus* isolated from palm wine. The *Saccharomyces pastorianus* had 92.59% DRC while *Pichia kudriavzevii* had 74.01%, when compared to the commercial yeast with dough raising capacity of 100%. Hence, the results of the DRC are as follows: *Saccharomyces cerevisiae* > *Saccharomyces pastorianus* > *Pichia kudriavzevii* > *Pichia pastoris.* This study has shown that wild yeasts are a potential sources of bakers' yeasts, especially *Saccharomyces pastorianus* (with DRC of 92.59%) isolated from palm wine is recommended for use in bread making as an alternative to imported baker's yeast.

Keywords: Wild yeast, palm wine, banana, bread, *Pichia kudriavzevii*, *Saccharomyces pastorianus*.

Introduction

Bread is a staple food consumed worldwide, and the quality of bread greatly depends on the fermentation process. Traditionally, *Saccharomyces cerevisiae* has been the predominant yeast strain used in bread making. However, recent research has explored the potential of wild yeasts, especially *Pichia kudriavzevii* and *Saccharomyces pastorianus*, isolated from agro-produce, as alternative fermentation agents for bread baking (Warringer *et al.*, 2011). The exploration of alternative yeast strains for bread making is motivated by the need for diversity in flavour profiles, improved nutritional content, and increased resilience to environmental stress.

Wild yeasts, such as *Pichia kudriavzevii* and *Saccharomyces pastorianus*, have been isolated from various agro-produce sources, showcasing their potential for use in the bakery industry. Understanding the characteristics of these wild yeasts and their impact on bread quality can provide valuable insights into diversifying and enhancing the bread-making process (Young *et al.*, 2007). *Pichia kudriavzevii*, also known as *Candida krusei*, is a non-conventional yeast species that has been identified in various fermentative environments (Warringer *et al.*, 2011). It possesses unique metabolic traits, such as the ability to metabolize different sugars, high ethanol tolerance, and resistance to acidic conditions.

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These characteristics make *Pichia kudriavzevii* an intriguing candidate for bread fermentation, potentially imparting distinct flavours and enhancing the fermentation process (Figoni, 2011).

Saccharomyces pastorianus, commonly associated with lager beer production, is another wild yeast species that has been considered for bread making (Bellon *et al.,* 2013). Its lowers fermentation temperature range and ability to ferment maltose and can contribute to different flavour profiles in bread. Exploring the application of *Saccharomyces pastorianus* in bread fermentation may offer a unique combination of characteristics not achievable with traditional baker's yeast (Warringer *et al.,* 2011). There are several types of yeasts but the important ones for the bakery industry are those belonging to the genus *Saccharomyces cerevisiae,* which means "sugar eating yeast", which infer that Yeast has been used by man to make bread and alcohol for thousands of years (Boboye *et al.,* 2008). Evidence of this has been found in ancient Babylonian wall carvings and Egyptian hieroglyphics dating back to 2000BC (Warringer *et al.,* 2011). The leavening of bread was considered an art, as the ancient people do not understand the process of leavening bread when a piece of old over-fermented dough full of yeast cells were mixed in with fresh dough and the resulting bread was more palatable than the unleavened bread they had been used to (Rochelle, 2001). In 1676, Anton Van Leeuwenhoek, when looking through a microscope, identified that yeast was a cell and that different types of yeast cells could be used for brewing beer or making wine (Rochelle, 2001). In early days of bread production, a piece of dough from yesterday's bake was kept and added to the new day's dough because it was found that the resulting dough was more consistent and fermented faster. The old piece of dough is called the starter or 'leaven' (Rochelle, 2001).

Bananas are tropical fruits that are not only a tasty and wholesome snack but also a multipurpose agricultural tool with numerous uses. In addition to being eaten raw, bananas are utilized in a variety of industrial and culinary applications. They are a great source of potassium, vitamin C, vitamin B6, and dietary fibre, among other important nutrients. As a result, they are a beneficial addition to the diet that supports heart health, digestion, and general wellbeing. They also encourage the growth of some yeast that is helpful for baking bread (Mohapatra & Sutar 2013).

Minerals, vitamins, and natural sugars can be found in palm wine. In comparison to other alcoholic beverages, it has low alcohol content. Bananas and palm wine are two examples of agricultural products, ranging from their use as staple foods to their contribution to cultural customs and industrial uses. Comprehending the diverse aspects of these agricultural products is imperative for implementing sustainable farming methods and realizing their complete capabilities (Amadi *et al.,* 2014).

Today, baker's yeast is utilized for manufacturing bread throughout the globe at an industrial scale and is wholly imported from developed countries (Blakely, 2004). Using wild yeasts *Pichia kudriavzevii* and *Saccharomyces pastorianus* isolated from agricultural products may offer a promising path toward bread-baking innovation. Nevertheless, there is still a lack of information regarding the precise effects of these wild yeasts on the critical factors affecting bread quality, despite the growing interest in varying yeast strains for fermentation, hence this study was aimed to isolate and use *Pichia kudriavzevii* and *Saccharomyces pastorianus* for bread baking.

Materials and Methods

Sample Collection

Palm Wine

Freshly tapped palm wine from *Raphia raphia* (Raphia palm tree) and *Elaensis guineensis* (oil palm tree) were purchased, put into a sterile plastic container at the point of tapping from traditional Palm wine Tappers in Omouko community, Aluu, Ikwerre Local Government Area of Rivers State, Nigeria. The samples were collected and transported to the Microbiology laboratory of Rivers State University, in coolers equipped with ice packs immediately for the isolation of the wild yeast (Ibekwe *et al*., 2006; Chijioke and Ukaegbu- Obi, 2016).

Unspoiled ripped banana used for the isolation of the wild yeast was purchased from Mile 3 Market, Diobu, Port Harcourt. The samples were transported to the Microbiology laboratory of Rivers State University Laboratory for analyses, immediately after purchase, in a thermos box containing an ice pack. The other baking items such as: sugar, flour, commercial yeast, salt, fat were all purchased from the same market.

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Isolation of Yeast Species

The isolation of Yeast species from palm wine and banana were achieved according to Lodder and Kreger,(2014), and Kurtzman *et al.*(2011), where the samples were allowed to ferment at 30ºC for 72 hours, then used for isolation.

The palm wine sample was serially diluted using peptone water to 10^{-2} and inoculated on the Yeast Peptone Dextrose Agar (YPDA), while solid sample (banana) was homogenized in 1% peptone given (1/10 w/v), diluted to 10^{-2} and inoculated on the YPDA.

Yeast peptone dextrose agar composed of 10 g/L of yeast extract, 10 g/L of peptone water, 10 g/L of dextrose, 20 g/L of agar and added chloramphenicol (30 mg/L) was used to prevent bacterial contamination, and then the plates were incubated at 28±2ºC for 48 to 72 hours (Kurtzman *et al.*, 2011).

Purification and Maintenance of Yeast Species

Purification of yeast colonies were achieved by streaking a colony on a freshly prepared Yeast peptone dextrose agar plate. The cell/colony morphologies of the purified yeasts isolates were examined macroscopically and under microscope. Macroscopically, colonial appearance on the agar, colour, margin and shape.

The isolates were identified microscopically, by viewing under the Microscope at x40 magnification after staining with lactophenol cotton blue. Yeast cultures were maintained on the YPD agar slants at 4ºC for short period storage.

In addition, equal volumes of propagated yeast cultures and 10% glycerol was mixed well and refrigerated at 4ºC for preservation (Kurtzman *et al.*, 2011).

Identification of Yeast Strains

Selected yeast isolates were identified based on their morphological and biochemical properties according to Kurtzman *et al.* (2011), the isolates were further identified using molecular techniques carried out according to Nisiotou *et al.* (2007). These involve the extraction of DNA, amplified, sequencing, and analysis of evolutionary relationship (Nisiotou *et al.*, 2007).

Screening of the Yeast Isolates

Nitrate Reduction Test

This test was carried out to identify yeast species that can assimilate and reduce nitrate. A well isolated colony was inoculated in nitrate broth (peptone 10 g, KNO_3 10 g in 1000 ml distilled water and sterilized). It was incubated at 30^{O}C for 48 hours. After incubation, 5 drops of both reactive 1 (α-naphthylamine 1 g, distilled water 22 ml, heat solution, filter, and then adding acetic acid 1 ml) and reactive 2 (sulphanilic acid 0.5 g, diluted acetic acid 150 ml) were added in the tube. The appearance of red colour will be observed after 5–10 minutes (Prescott *et al.*, 2005).

Lactose Utilization Test

Lactose uutilization test is a biochemical assay used to determine the ability of yeast species to utilize lactose (a disaccharide sugar) as a carbon source. Yeast cells were grown at 30^{O}C for 3 days in Yeast Fermentation Broth (YFB) (peptone 7.5 g/L, yeast extract 4.5 g/L; 1 ml of 1.6% (w/v), bromothymol blue as an indicator), with autoclaved 6% (w/v) lactose. The Durham tubes were also placed into the media to trap the carbon dioxide released. The medium, changing from green to yellow indicates that the yeast used up the lactose as carbon source (Cheesbrough, 2006).

Stress Exclusion Test

The Stress Exclusion Test (SET) is a biochemical assay used to evaluate the ability of yeast species to survive and grow under various stress conditions. In this test, the isolates were subsequently grown under different stress conditions that mimicked the various stresses. Firstly, the isolates were grown onto Yeast Peptone Glucose (YPG) medium and incubated at 30^{O}C for 3 days. From that, a single colony was transferred and grown on Yeast Peptone Glucose (YPG) medium and incubated at 37^{O}C for another 3 days. Again, a colony was selected and sub-cultured on YPG 8% (v/v) ethanol and incubated at 30^{O}C for 3 days. A single isolated colony was further sub-cultured on YPG supplemented with 20% (w/v) glucose and incubated under the same conditions. Finally, yeast cells were transferred on YP medium supplemented with 2% (w/v) sucrose and 8% (v/v) ethanol and incubated under the same conditions (Cheesbrough, 2006).

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Temperature Tolerance Test

Temperature Tolerance Test is a laboratory assay used to evaluate the ability of yeast species to grow and survive at various temperatures with an aim to identify yeast species with enhanced temperature resistance. Yeast isolates were cultured on Yeast Peptone Glucose (YPG) agar and incubated at 25^{O}C, 30^{O}C, 37^{O}C, and 45^{O}C for 72 hours. Growth was observed and analysed (Kurtzman *et al.*, 2011).

Carbohydrate Utilization Test

Carbohydrate uutilization test is a biochemical assay used to determine the ability of yeast species to utilize various carbohydrates as carbon sources in order to identify yeast species that can assimilate and metabolize specific carbohydrates. The carbohydrate utilization test was performed using broth (peptone: 10g; NaCl: 5g; phenol red: 0.018g; distilled water: 1000 ml; carbohydrate: 10 g) along with inverted Durham tubes in the broth. The carbohydrates used were dextrose, fructose, lactose, galactose, maltose, and sucrose. The media were inoculated with yeast strains and incubated for 24 hours. Uninoculated tubes were used as control for each of the sugars. Colour change from red to yellow indicated the fermentation using carbon sources (Obire, 2005).

Hyperosmotic Tolerance Test

The hyperosmotic tolerance test is a laboratory assay used to evaluate the ability of yeast cells to survive and grow in environments with high osmotic pressure. Yeast isolates were cultured on YPD broth containing 30, 40, and 50% dextrose and incubated at 30^{O}C for 48 hours. The cell density of different yeast isolates in response to high dextrose concentration was taken (Cauvian and Young, 2007).

Ethanol Tolerance Test

Yeast isolates were screened for their potential to withstand varying concentrations of ethanol and capacity to produce such concentrations. Medium without ethanol serves as control. The isolates were grown in Yeast Peptone Glucose (YPG) broth containing 3 different concentrations of ethanol, that is, 10%, 15%, and 20% (v/v), respectively, and incubated at 37^{O}C for 72 hours (Cauvian and Young, 2007).

After incubation, the yeast growths were measured by turbidiometry using a spectrophotometer at a wavelength of 600nm, measuring both the initial and final turbidity.

Cultivation and Dough Leavening Potential of the Yeast Isolates

The yeasts isolated were cultured separately at 25^{o}C ± 2^{o}C in peptone broth medium containing 20% (w/v) glucose and lactic acid at concentration of 0.2% in 1 litre conical flask equipped with air locks. The set-up was agitated continuously for 72 hours in rotary shaker regulated at 150 rpm. After incubation, the biomass concentrates for each yeast species were obtained by centrifuging in a centrifuge machine at 12,168 rpm for ten minutes (10minutes). The yeast concentrates were washed sufficiently with sterile distilled water after which they were resuspended in sterile distilled water. The same procedure was repeated for the commercial yeasts (Saf-instant) used as control too (Cauvian and Young, 2007).

Determination of Fermentative Ability of the Yeasts

All the yeast isolates were used to ferment dough in order to test their fermentative ability. Samples of dough were prepared as described by Cauvian and Young, (2007). Each dough sample contained yeast isolate (2g) bread flour (90g), salt half teaspoon, water 50ml, sugar (9g) and butter (9g). All the ingredients were properly mixed. Commercial yeast (Saf- instant) were used separately as positive control yeasts to ferment the dough. The dough rising capacity (DRC) of the various Yeast isolates were calculated using the formula

$$DRC = \frac{V_2 - V_1}{V_1} \text{ x}100$$

Where V_1 = initial volume,
V_2 = final volume

Kneading of Dough

Dough was obtained by kneading with kneading machine, using each yeast sample and the dough were cut into equal sizes of 50g, moulded manually into cylindrical shape, the dough were left to stand at room temperature for 3hours before baking in a cooker oven at 200^{o}C for 20minutes in an aluminum baking pan.

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Proximate Analyses

The proximate composition analysis which includes moisture content, fat, Crude Protein, Ash, Carbohydrate were carried out using standard analytical methods in the Department Food Science and Technology Rivers State University, on the bread baked with duplicate determination on all the parameters and averages taken (Cauvian and Young, 2007).

Statistical Analyses

The data were analysed using descriptive statistical tools in the form of mean, standard deviation and percentages.

The null hypothesis was tested with the aid of some selected inferential statistical tools such as ANOVA and T. test, while a turkey post hoc test was used to determine the pair of means that were significantly different.

Results

The results of the fungal counts from the fresh palm wine obtained from two different fresh palm wine ranged from 1.65 x 10^3 CFU/ml to 1.84 x 10^3 CFU/ml. The counts expressed in $\log_{10}$ CFU/ml are presented in Figures 1. Results showed that *Elaeis guineensis* has the highest fungal counts than *Raphia hookeri*. Fungal counts of the two different species of banana (*Musa acuminata* and *Musa paradisiaca*) ranged from 8.4 x 10^3 to 9.3 x 10^3CFU /g. The media used for the isolation and identification of the yeast from the natural environment was a selective enrichment media has ability to isolate microbes, especially yeast from natural environment, and enrichment culture is used in the laboratory for the isolation of the yeast (Bitrus *et al.*, 2020).

The results of the morphological characteristics of the isolates are shown in Table 1. The yeast *Saccharomyces* sp, *Pichia* sp, and *Pichia* sp were isolated from the palm wine and banana samples.

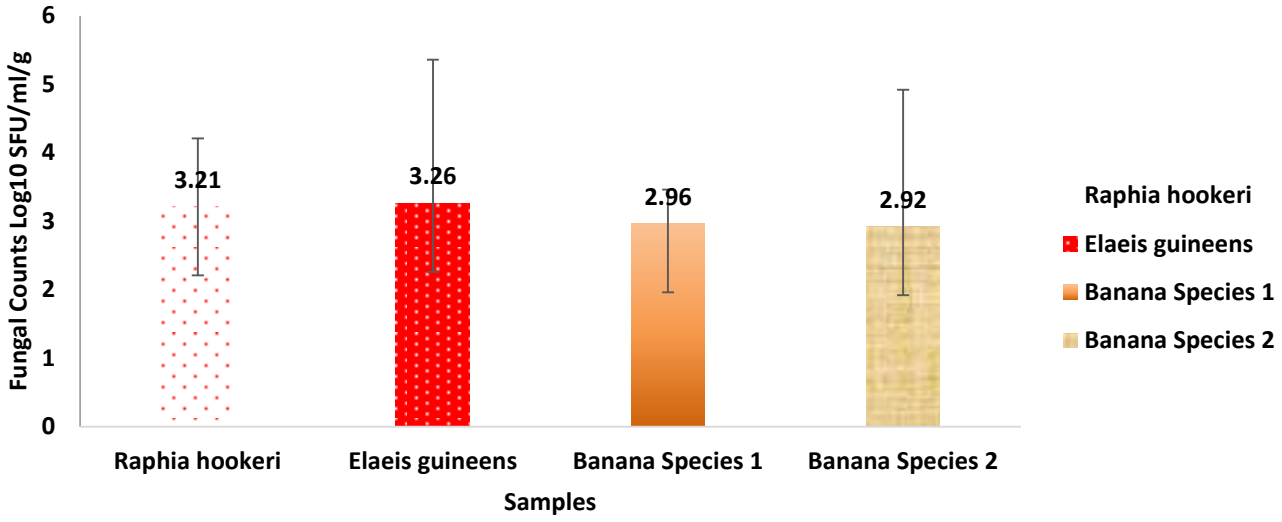

Figure 1: Mean Fungal Counts from the Agro Products (Palm wine and Banana)

Table 1: Macroscopic and Microscopic Characterization of the Fungal Isolates

Isolate Code	Macroscopy	Microscopy	Probable Identity
PW1	Creamy, Round, smooth, and entire, Umbonate	Oval, spherical, or ellipsoidal, 3-5 µm in diameter Budding	*Saccharomyce* sp
BB1	White, Round, smooth, entire and flat	Elongated, Cell size: 2-5 µm in length, 1-3 µm in width, Cell wall: Thin, and Multilateral budding	*Pichia* sp
PW2	White, translucent colony	Oval, ellipsoidal,Multilateral budding	*Saccharomyces* sp
PW3	Creamy, Soft, smooth, and slightly shiny and Convex	Oval, ellipsoidal, or elongateda, budding and septate	*Pichia* sp

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The results of the screening of yeast isolates to high level of pH tolerance, alcohol tolerance, stress exclusion, hyperosmotic tolerance, and were positive, as presented in Table 2.

While the result of the dough rising capacity of yeast isolates from different sources and the net increase in volumes of dough inoculated with yeast isolates are presented in Table 3.

Table 2: Screening of the Yeasts Isolates for Fermentation Potentials

Parameter	**Cont.**	**Iso1 (pw)**	**Iso2 (pw)**	**Iso3 (pw)**	**Iso4 (pw)**	**Iso5 (pw)**	**Iso1 (ba)**	**Iso2 (ba)**	**Iso3 (ba)**
pH tolerance	+	+	-	+	-	+	-	-	+
Alcohol tolerance	+	+	-	+	-	+	-	-	+
Stress Exclusion	+	+	-	+	-	+	-	+	+
Hyperosmotic tolerance	+	+	-	+	-	+	-	+	+
Mannitol	-	+	-	-	-	-	-	-	-
Fructose	+	+	-	+	-	+	-	-	+
Galactose	+	+	-	+	-	+	_	-	+
Maltose	+	+	-	+	-	+	-	-	+
Sucrose	+	-	-	+	+	+	+	-	+
Sorbitol	-	-	-	-	-	-	-	-	-
Isolate	*Saccharomyces cerevisiae*	*Pichia pastoris*	*Saccharomyces pastorianus*	*Saccharomyces pastorianus*	*Pichia pastoris*	*Pichia kudriavzevii*	*Pichia pastoris*	*Saccharomyces pastorianus*	*Pichia kudriavzevii*

Table 3: Dough Rising Capacity of Yeast Isolates from Different Sources

	Yeast Isolate And Sources			
Dough Volume (cm)	**Control *Saccharomyces cerevisiae***	**ISO 1 (PW) *Pichia pastoris***	**ISO 3 (BA) *Pichia kudriavzevii***	**ISO 5(PW) *Saccharomyces pastorianus***
Initial volume at 0 min	3.0	3.0	3.0	3.0
Final volume at 3 hours	9.5	6.6	7.8	9.0
Dough rise (V2-V1)	6.5	3.6	4.8	6.0
DRC	216	120	160	200

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The molecular identification of the isolates is shown in Figure 2. It shows the level of relatedness of the isolates when the sequence was compared to their nearest neighbour in the Gene bank. Isolate PW1 identified as *Saccharomyces* sp was identified as *Saccharomyces cerevisiae* with accession number LC576598.1 with 98% relatedness, ISO 5 morphologically identified as *Pichia* sp was now identified as *Pichia kudriavzevii* with accession number of LC413230.1 and has 100% relatedness. Isolate PW2 initially identified as *Saccharomyces* sp, was confirmed to be *Saccharomyces pastorianus* D89889.1, with 75% relatedness. And isolate ISI, *Pichia* sp is was identified as *Pichia pastoris* EF116884.1with 100% relatedness. Figure 2 shows the phylogenic tree showing the evolutionary distance among the yeast isolates.

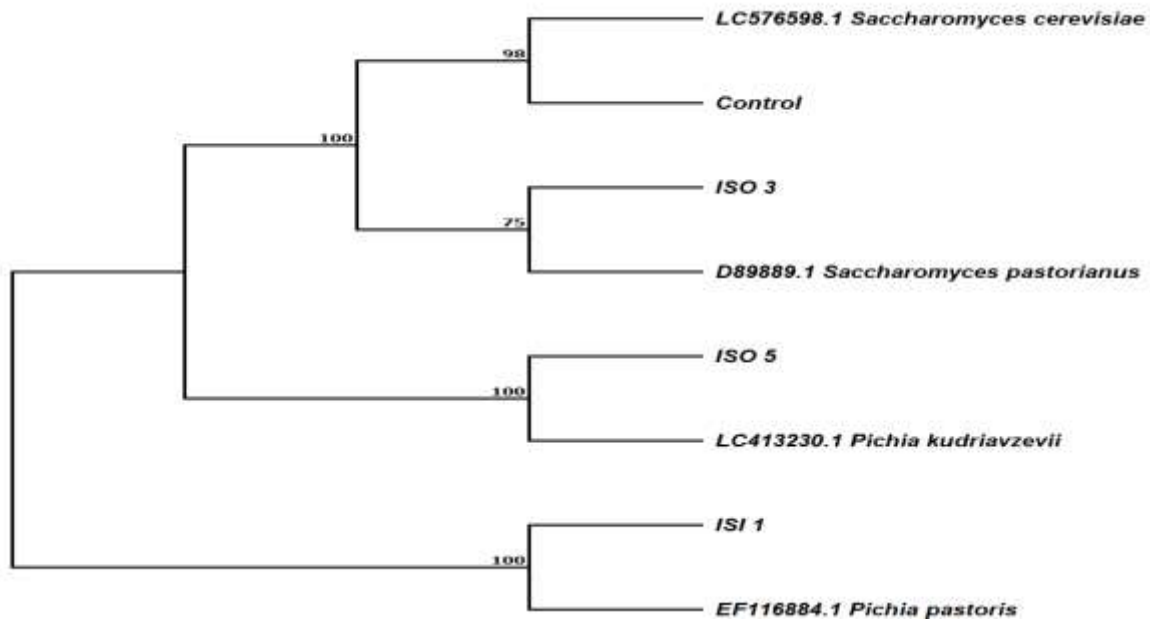

Figure 2: Phylogenetic Tree Showing the Evolutionary Distance

The results of the proximate composition of the baked bread using the various yeasts isolates are presented in Figures 3 to 8. Figure 3 shows the moisture content of the baked bread which ranged from 19.6 to 24.8%, bread that was dough with *Saccharomyces pastorianus* recorded the lowest moisture content (19.6 %) followed by *Saccharomyces cerevisiae* (Control), which has a moisture content of 22.1% while *Pichia kudriavzevii*, and *Pichia pastoris* recorded 24.3 and 24.8% , respectively. Figure 4 shows the fat content of the baked bread which ranges from 7.8% to 12.9%, bread that was dough with *Saccharomyces cerevisiae* (Control), recorded the highest fat content of 13.2%, followed by *Saccharomyces pastorianus* which had a fat content of 7% and *Pichia pastor* recorded 7.8% while the lowest fat content was in bread dough with *Pichia kudriavzevii*, which recorded 7.6%.

Figure 5 shows the ash content of the baked bread which ranged from 7.8% to 12.9%, bread that was dough with *Saccharomyces cerevisiae* (Control) had the lowest ash content while bread dough with *Pichia* kudriavzevii, recorded the highest percentage. Crude Protein content ranged from 7.6 to 8.7% with control having the lowest while bread dough with *Pichia kudriavzevii* recorded the highest quantity of the crude protein as presented in Figure 6. Crude fiber ranged from 3.1 to 4.0 % with control having the lowest value of 3.1%, while bread dough with *Pichia kudriavzevii* recorded the highest value of 4.0%, of the crude protein, as presented in Figure 7. Carbohydrate Content of the baked Bread ranged from 54.1 to 59.6% with control having the lowest value of 54.1%, while bread dough with *Saccharomyces pastorianus* recorded the highest content of 59.6% of the carbohydrate as presented in Figure 8.

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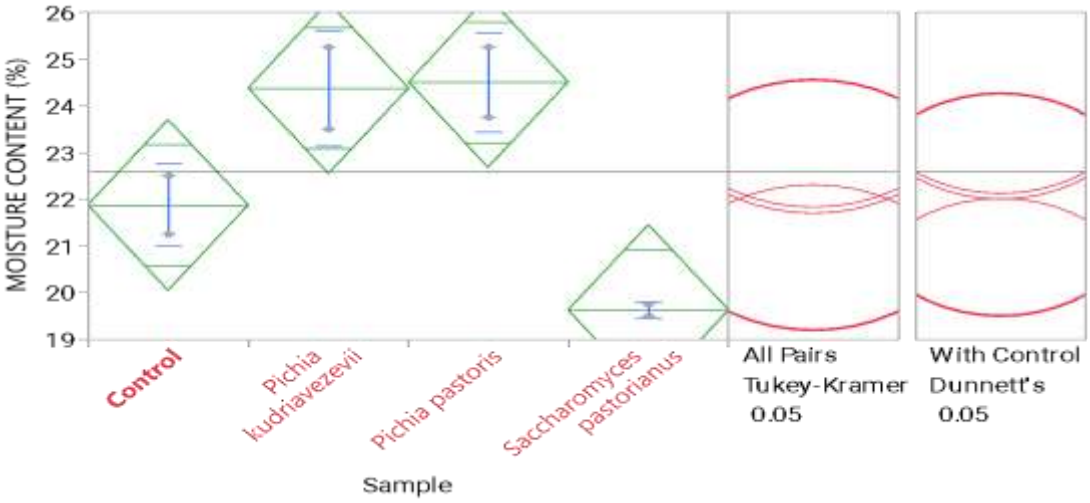

Figure 3: Moisture Content of the Baked Bread using Different Yeast Species

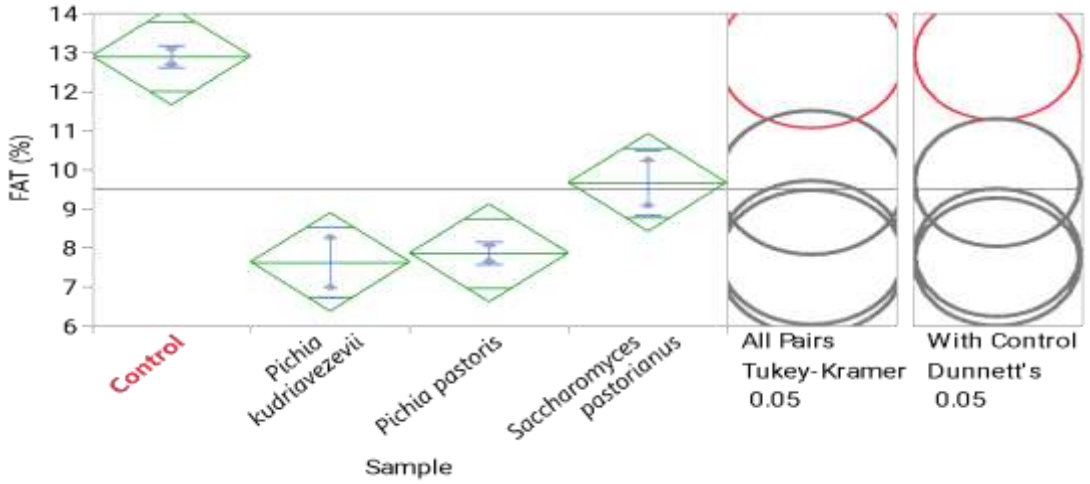

Figure 4: Fat content of the Baked Bread Using Different Yeast Species

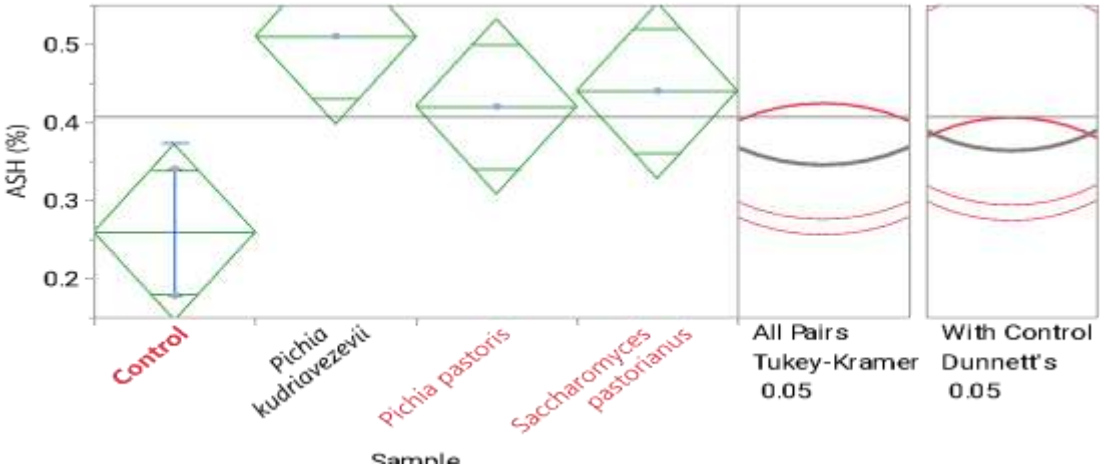

Figure 5: Ash content of the Baked Bread Using Different Yeast Species

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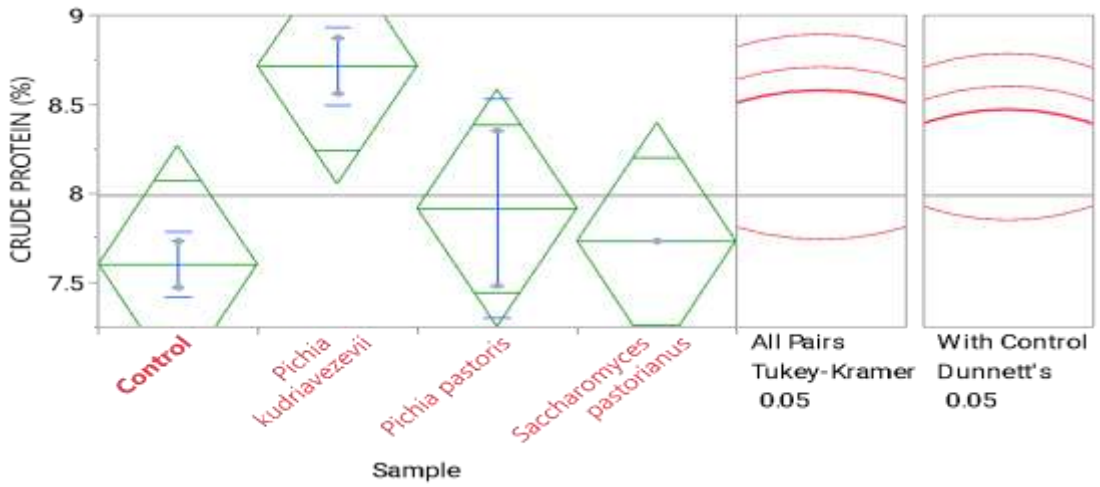

Figure 6: Crude Protein of the Baked Bread Using Different Yeast Species

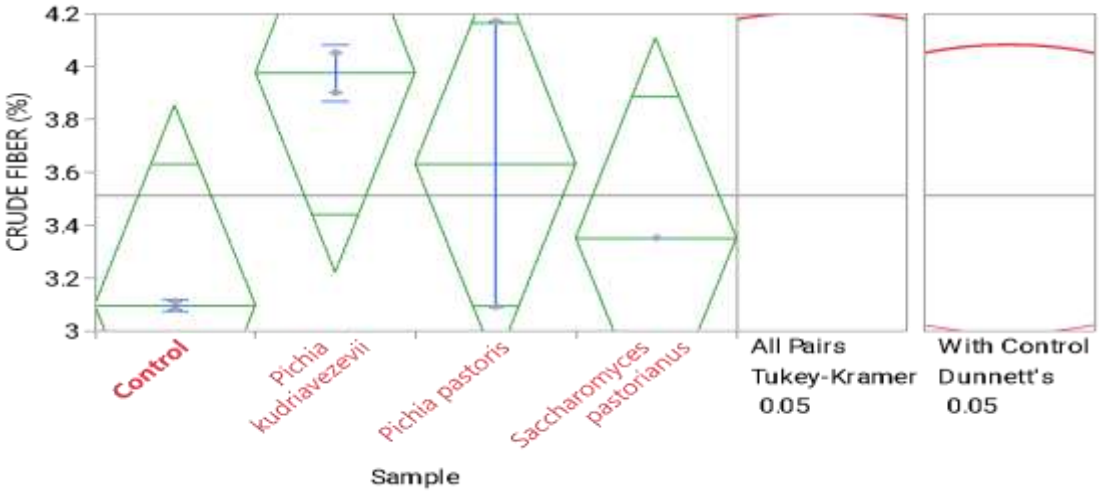

Figure 7: Crude Fibre content of the Baked Bread Using Different Yeast Species

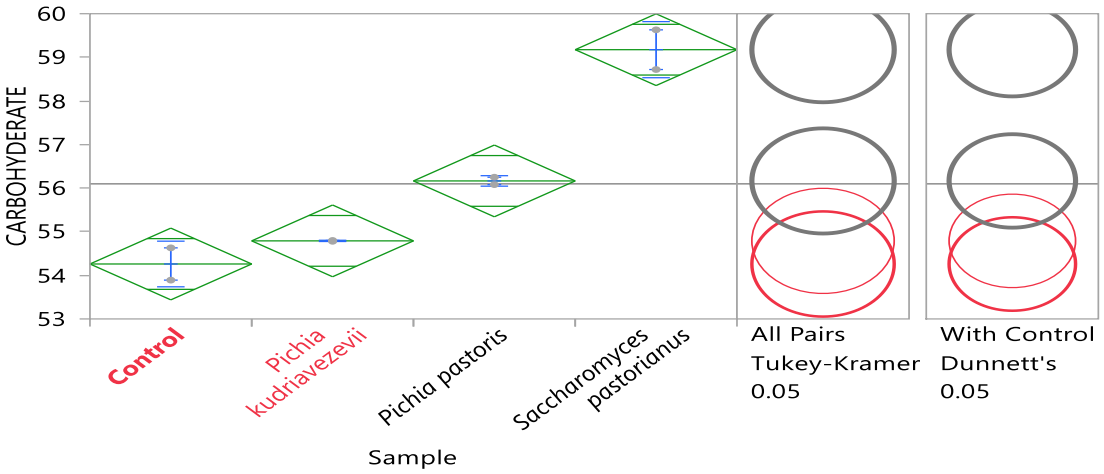

Figure 8: Carbohydrate Content of the Baked Bread using Different Yeast Species

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Discussion

This present study has revealed the fermentation potential and the Application of wild yeasts (*Pichia kudriavzevii* and *Saccharomyces pastorianus*) isolated from palm wine and banana for bread baking. The fungal counts enumerated in two different fresh palm wine of *Elaeis guineensis* (oil palm tree) and *Raphia raphia* (*Raphia* palm tree) revealed that palm wine from oil palm tree (*Elaeis guineensis*) recorded higher counts fungal colonies compared to *Raphia raphia* (Raphia palm tree). Similar findings have been reported by other investigators such as Santiago-Urbina *et al.,* (2013). The high counts obtained from *Elaeis guineensis*(oil palm tree) may be attributed to the nature of palm sap of the oil palm tree which has the ability to encourage the growth of more microorganisms (Amoa-Awua *et al.,* 2007). The results in the present study showed that the palm wine and banana samples harboured various strains of fungi which may be due to air borne fungal spores in the environment where the palm wine was tapped or as a result of contamination from the containers used for the tapping, collection and distribution of the wine. However, Obi *et al.,* (2015) also reported of similar high microbial counts which ranged from 1.35 x 10^3 CFU/ml to 1.74 x 10^3 CFU/ml in palm wine sample from Ikwuano Local Government area of Abia State.

The results of the morphological characteristics of the isolates are shown in Table 1. The yeast *Saccharomyces* sp, *Pichia* sp, and *Pichia* sp were isolated from the palm wine and banana samples. Odu *et al*., (2022) isolated *Saccharomyces* sp, and *Pichia* sp from Palm wine samples too. When these cells were viewed under the microscope they were observed to be oval or spherical. Some of the cells were even budded which is typical of yeast cells.

The yeasts (*Saccharomyces pastorianus, Pichia pastoris* and *Pichia kudriavzevii*), isolated from palm wine and banana as well as the commercial baker's yeasts (were screened for pH tolerance, Alcohol tolerance, Stress Exclusion, Hyperosmotic tolerance, Mannitol, Fructose, Galactose, Maltose, Sucrose and Sorbitol to determine their ability withstand and adapt to hash conditions and also their fermentative potentials. The results of the screening tests carried out on yeast strains indicated that all the isolates 1, 3 and 5 from the palm wine and isolate 3 from banana has high potential to ferment sugars such as Sucrose, Maltose, Fructose, and Mannitol but could not ferment Sorbitol and galactose. The same set of isolates also showed high level of pH tolerance, alcohol tolerance, stress exclusion, hyperosmotic tolerance, and were positive, as presented in Table 2.

The isolates identified showed similar properties to the commercial yeast and exhibited ability to ferment galactose, fructose, mannitol, fructose, maltose, sucrose, with colour change i. e. production of acid and gas production which shows the fermentative potential of the isolates.

The initial and final dough volumes were recorded from the graduated surface of the measuring cylinder and the net increase in volumes of dough inoculated with yeast isolates are presented in Table 3. *Saccharomyces cerevisiae* which was used as positive control had an initial volume of 3.0cm and a final volume of 9.5cm giving a dough rise of 6.5cm. Among the isolated Yeast species (wild yeast) dough rise ranged from 3.6 cm (*Pichia pastoris*) to 6.0cm (*Saccharomyces pastorianus).* *Pichia pastoris* (Isolate 1 from palm wine) had the lowest (3.6cm) dough rise after 180 minutes followed by *Pichia kudriavzevii* (isolate 3 from banana) which had 4.8cm dough rise while *Saccharomyces pastorianus* isolated from palm wine recorded the highest DRC of 6.0cm.

The yeast *Saccharomyces cerevisiae* (100%) showed the highest dough raising performance because the yeast showed the best dough leavener ability obtained in this study. Followed closely, was *Saccharomyces pastorianus (*92.59%) isolate 5 from palm wine, *Pichia kudriavzevii* (isolate 3 from banana, 74.07%) and *Pichia pastoris* (isolate 1 from palm wine, is 55.5%) in that order. That is *Saccharomyces cerevisiae* > *Saccharomyces pastorianus* >*Pichia kudriavzevii*> *Pichia pastoris.*

The sugar metabolism derived from flour and sucrose added, as ingredients to the dough may be attributed to the evolution of carbon dioxide by the yeast leading to dough expansion (Arendt *et al.* 2017). The yeast *Pichia pastoris* performed least in the dough raising test implying that they are poor dough leavening. The technological role of yeast in flour dough is a strong alcoholic fermentation with extensive carbon dioxide liberation. The gassing power of yeast depends on the zymase enzyme complex of the yeast cells with available fermentable carbohydrates (Stear, 2010).

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The difference in the gassing power produced by yeasts used in this work may be due to the various maltase and zymase activities of these yeasts. This study has showed that local isolates could be considered as a potential source of bakers' yeasts and is in conformity with the report of Yabaya and Jatau, (2009) whose work revealed that wild yeast, *Saccharomyces pastorianus* isolated from local beverages could be used in baking industries. The dough rising capacity of yeasts refers to their ability to produce carbon dioxide gas through fermentation, which causes the dough to rise (Stear, 2010). Yeasts are microorganisms that feed on sugars in the dough and produce carbon dioxide as a byproduct. The amount of carbon dioxide produced by the strains of yeasts has varying levels of rising capacity. Some strains are more efficient at producing carbon dioxide, resulting in a greater volume increase in the dough. This is desirable for certain types of bread, as it creates a lighter and fluffier texture. Factors that can influence the dough rising capacity of yeasts include temperature, moisture content, and the presence of nutrients. Yeasts thrive in warm and moist conditions, which promote their growth and fermentation activity. Adequate nutrients, such as sugars and minerals, are also essential for yeast metabolism and optimal rising capacity. Yeast-based doughs are typically given enough time to rise (3 hours) before baking (Yabaya and Jatau, 2009). During this rising period, the yeasts consume the sugars in the dough and produce carbon dioxide gas, causing the dough to expand. This process improves the, flavour, and overall quality of the baked product (Yabaya and Jatau, 2009).

The results of the proximate composition of the baked bread using the various yeasts isolates are presented in Figures 3 to 8. Figure 3 shows the moisture content of the baked bread which ranged from 19.6 to 24.8%, bread that was dough with *Saccharomyces pastorianus* recorded the lowest moisture content (19.6 %) followed by *Saccharomyces cerevisiae* (Control), which has a moisture content of 22.1% while *Pichia kudriavzevii*, and *Pichia pastoris* recorded 24.3 and 24.8% , respectively. Figure 4 shows the fat content of the baked bread which ranges from 7.8% to 12.9%, bread that was dough with *Saccharomyces cerevisiae* (Control), recorded the highest fat content of 13.2%, followed by *Saccharomyces pastorianus* which had a fat content of 7% and *Pichia pastor* recorded 7.8% while the lowest fat content was in bread dough with *Pichia kudriavzevii*, which recorded 7.6%.

Figure 5 shows the ash content of the baked bread which ranged from 7.8% to 12.9%, bread that was dough with *Saccharomyces cerevisiae* (Control) had the lowest ash content while bread dough with *Pichia* kudriavzevii, recorded the highest percentage. Crude Protein content ranged from 7.6 to 8.7% with control having the lowest while bread dough with *Pichia kudriavzevii* recorded the highest quantity of the crude protein as presented in Figure 6. Crude fiber ranged from 3.1 to 4.0 % with control having the lowest value of 3.1%, while bread dough with *Pichia kudriavzevii* recorded the highest value of 4.0%, of the crude protein, as presented in Figure 7. Carbohydrate Content of the baked Bread ranged from 54.1 to 59.6% with control having the lowest value of 54.1%, while bread dough with *Saccharomyces pastorianus* recorded the highest content of 59.6% of the carbohydrate as presented in Figure 8.

The results of the proximate composition of the baked bread with wild yeasts and commercial baker's yeasts showed that there was significant difference between the bread types with regards to moisture content, fat, protein, carbohydrate between the bread baked with *Pichia* species and the control. But no significant difference between *Saccharomyces pastorianus* isolated from palm wine. Generally, the study has revealed that the carbohydrate content of the four bread types compared favourably with the results of Omorodion *et al.,* (2019) where wheat flour was substituted by cassava flour. The results revealed a suspicion that the bakeries were using composite flour instead of refined wheat flour for the bread types. The results of the study corroborated very well with the study of Omorodion *et al.*, (2019) in all the parameters (crude protein, crude fat, crude fibre, ash, carbohydrate and the moisture content) assessed - showing that the yeast isolates used in the baking of the bread can compete with the commercial yeast.

In conclusion, this study has revealed the efficacy of different yeast strains for specific baking applications and optimize the dough fermentation process through the dough rising capacity of the yeast, and the volume increase of the dough after a rising time, height and structure of the final baked product. The wild yeast *Saccharomyces pastorianus* (92.59%) and *Pichia kudriavzevii* (74.07%) obtained in this study have sufficient potential to be produced commercially, because of their fermentative abilities and fast growing potential on yeast medium.

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They also demonstrated high potential to be in bread production especially *Saccharomyces pastorianus* isolated from palm wine which showed the highest dough rise capacity of 92.59% after 3 hours. It is very feasible to produce leavened bread using wild yeasts species *Saccharomyces pastorianus* and *Pichia kudriavzevii* obtained from agro-products in this study as the sole leavening agent for optimum loaf height and volume as they matched baker's yeast in their ability to utilize maltose and showed a high leavening capability similar with the commercial baker's yeast. This is possible as *Saccharomyces pastorianus* has 92.59% dough raising capacity while *Pichia kudriavzevii* has 74.07%.

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