Investigation of the Bread Baking Potentials of Some *Ascosporogenous* Yeast Strains Isolated from Palmwine

Nnenna. E. Efuribe [1], Ngozi. Uchechukwu [2], Olubunmi. P Ibidapo [1], Chukwudum A. Anyiam [3, 4], Ngozika. C. Ivo [6] and Cynthia. C. Anyiam [5]
1. Food Technology Department, Federal Institute of Industrial Research Oshodi, Lagos, Nigeria
2. Food Science and Technology Department, Michael Okpara University of Agriculture, Umudike, Nigeria
3. Food Technology Department, Akanu Ibiam Federal Polytechnic Unwana, Afikpo, Ebonyi State
4. Biology Department Morgan State University, Baltimore, USA.
5. Science Laboratory Technology Department, Akanu Ibiam Federal Polytechnic Unwana, Afikpo, Ebonyi State.
6. Department of Food Science and Technology, Ebonyi State University, Nigeria
Corresponding Author: efuribennenna0791@gmail.com

Abstract

Yeast is the primary organism responsible for the leavening of doughs. In this study, yeast samples were isolated from palm wine samples collected from Nkpa in Abia State. The samples were cultured on malt extract agar (MEA) medium at room temperature of 28^{o}C to isolate three *ascosporogenous* yeast strains (*Saccharomyces fragilis, Saccharomyces cerevisiae, Pichia spp*) from the culture by using identification techniques such as their morphological, fermentative and microscopic properties. The isolated yeast, the combination of the strains and the commercial baker's yeast as the control were each used to produce eight different bread samples and their physical, sensory properties and correlation were examined. Amongst the bread samples examined for physical properties, sample D (Baker's yeast) had the highest value in bread volume followed by *Saccharomyces cerevisiae* while *Pichia spp* had the least performance. Likewise, significant differences ($p < 0.05$) occurred in specific volumes, which ranged from 1.91 – 3.94. The loaf weight ranged from 276.5- 320.1g. The result of the sensory attributes of the bread samples revealed that the taste value ranged from (4.3 -8.5), texture (3.5 -8.0), aroma (3.5 -8.0), crust colour (4.4 – 8.0), and general acceptability (4.7 -7.9). The general acceptability was significantly ($p < 0.01$) found to be positively correlated with bread volume ($r = 0.96$), taste ($r = 0.90$), crust colour ($r = 0.86$) and negatively correlated with the loaf weight ($r = -0.86$). Therefore, the data shows that some of the isolated yeast strains and the combination of strains could be valuable in the leavening of doughs.

Keywords: Palm-wine, Ascosporogenous yeasts, Bread, Physical properties, Sensory properties
DOI: 10.7176/FSQM/121-07
Publication date: June 30th 2023

1. Introduction

Bread is one of the common foods consumed by man for years and in many countries, it is considered a staple food especially in Nigeria. According to a 2016 KPMG report, Nigeria's bread sub-sector is developing at a quicker rate, with small and medium-scale bakers accounting for 72 percent of the $621 million business (Anudu, 2017). The basic concepts of bread making traditionally involves mixing wheat flour, yeast, salt and water, proofing and baking. The universal acceptance of bread has placed it in high importance, due to its role in global nutrition (Cauvain, 2004). It is rich in carbohydrates and usually low in fat. According to Lopez *et al.* (2001), bread contains valuable amounts of protein, dietary fibre, vitamins, some minerals and antioxidants needed for the well-being of the body. Hence, it has become a useful medium through which vital nutrients such as protein, vitamin, fibre and minerals can be delivered to both children and adults.

However, over the years, different society that consume bread have modified their bread-making to fit their culinary lifestyles, prevalent raw materials and the tradition of the society where it is being consumed. The global consumption of bread has increased over the years due to factors such as urbanization, rise in income, and the population that has resulted in increased demand for wheat flour, sugar and yeast. In Nigeria, bread has become a common food that is consumed anytime in the day because it is ready to eat and convenient.

Palm wine is a natural alcoholic beverage produced by tapping the inflorescence from oil palm (*Elaeis guineensis*) and raffia palm (*Raphia hookeri*) trees. The freshly tapped colourless palm wine is usually sweet to drink but loses its sweetness after some hours due to fermentation by yeast and bacterial microflora (Ezeronye, 2003). It is a popular drink in many tropical countries and is known for its distinctive taste, flavor, and alcohol content. Likewise, several researchers who studied the microbiology of palm wine gotten from different locations reported that it contains diverse bacteria and yeasts that have fermentative and oxidative abilities (Okechukwu *et al.,* 2019; Nester *et al.,* 2004).

The role of palm wine in our society cannot be overemphasized as it serves different roles which could be religious, medicinal/nutritious and socioeconomic importance. Nutritionally, the sugar content which comprises mainly of sucrose is about 10 to 12%, protein 0.36%, vitamin C 10 to 19mg/100mL, vitamin B_{12} about 160µg/ml, and also probiotics which are of immense benefit to human health (Heller, 2001; Ezereonye, 2004).

Yeasts are unicellular fungi, belonging to ascomycetes, and have a single nucleus capable of reproducing asexually through budding and by fission occasionally or sexually by the formation of spores (Walker, 2009). They are living organism rich in protein and vitamin B. They thrive under warmth, albumen, water, sugars and nitrogenous material to grow (The Artisan, The Yeast Treatise, 2002). Yeasts are isolated from sugar-rich materials such as grapes, berries, apples, peaches and from plant saps or cacti (El-Ghwas *et al*., 2021). They are undoubtedly one of the first domesticated organisms. It was reported that after Louis Pasteur identified and isolated yeasts, the industrial manufacturing and commercial use of yeasts began at the end of the nineteenth century. As such, humans have over the years utilized yeast in alcoholic beverage as well as leavening of bread. It has also been used in producing other types of fermented products such as cheese, soy sauce production, vinegar, sourdoughs, vegetable products and even fermented meat. The role of yeast cannot be overemphasized as it also serves as a source of high nutritional value proteins, enzymes, and vitamins, with uses in the health food sector as nutritional supplements, food additives, conditioners, and flavoring agents, microbiological media as well as animal feeding as a protein source (*Bekatorou et al., 2006;* Otero *et al*., 2011*)*.

The role yeast plays in food industries cannot be overemphasized, as it ferments products thereby generating carbon dioxide (CO_2) and ethanol vital in the development of flavour and synthesis of alcohol (Akbar *et al*., 2012). Baker's yeast usually used for baking are carefully selected strains of *Saccharomyces cerevisiae* which react with the sugars and the starch present in the dough to produce carbon dioxide and alcohol and which leads to the rise of the dough (Olowonibi, 2017). Apart from the 2 major metabolites produced during fermentation, metabolites like organic acids, aromatic compounds and glycerol are also produced which impact positively on both the bread-making process and the final quality of the produced bread (Cho and Peterson 2010; Jayaram *et al*., 2013).

Several researchers have reported that yeasts are important microorganisms majorly responsible for the fermentation process in palm wine. There are several different species of yeasts that have been isolated from palm wine, including both anamorphic and ascosporogenous yeasts. Ascosporogenous yeasts are yeasts that produce asci, which are specialized structures that contain spores. These yeasts are typically found in the Ascomycota phylum of fungi, and they are known for their ability to produce a wide range of enzymes and other compounds that are important in the fermentation process (Gnanamani and Jayaraman, 2008). Examples of ascoporogenous yeasts that have been isolated from palmwine include *Pichia kluyveri, Schizosaccharomyces pombe* and *Saccharomyes cerevisiae* (Chilaka *et al*., 2010)

Currently, only *Saccharomyces cerevisiae* strain is being used in the baking industry, while the potentials of other natural diverse yeasts strains remain underutilized. According to Wedral *et al.* (2010), many non-conventional yeasts produce unique aroma compounds that might be perceived as desirable in particular fermented products. Microorganisms such as yeast (*Candida, Pichia, Kluyveromyces, Saccharomyces* and *Torulopsis*) have over the years been utilized in human consumption. However, only few of these species are available for commercial utilization. Hence, this research is targeted to isolate, characterize and identify some spore-forming palm wine yeast strains and to evaluate the bread-making potential of the single pure strains and their combination effect.

2 Materials and Methods

2.1 Source of Raw Materials

The fresh palm wine used for this experiment was collected from local palm wine tappers from Nkpa village in Abia State Nigeria, using clean sterile containers with perforated screw caps. The samples were cooled using an ice-block in a cooler to minimize the rate of fermentation while being transported to the laboratory for analysis. The All-purpose flour, baker's yeast, salt, sugar, fat etc. were purchased from Umuahia main market in Abia state.

2.2 Isolation of Yeasts

The method described by Mahoukoucd *et al*. (2019) was adopted in the isolation of yeast from palm wine. The fresh palm wine was thoroughly shaken, thereafter, under aseptic condition, one ml (1ml) of the palm wine sample was diluted serially up to six-fold using distilled water. One ml out of the diluents was plated out using the pour plate method on malt extract agar for yeasts. The plates were duplicated and incubated for 48 -72hours at room temperature of about 28^0C. After two days, the colonies of yeast which grew on the plates were randomly picked and streaked on freshly prepared malt extract to obtain pure cultures. The obtained pure cultures were sub-cultured in a fresh malt extract agar in slants and incubated for further use in biochemical tests.

2.3 Cultural and Morphological Tests

The solid media was streaked on malt extract agar and was incubated for 2-3 days at room temperature in order to identify the cultural and morphological characteristics of the yeasts. Thereafter, the colour, edge of colonies, shape and colony size of the isolates were examined. For the microscopic view, a smear was prepared on each colony and stained for a minute with methylene blue, washed off in running water, air dried and the slide was first viewed with 40x objective lenses and thereafter the shape of the cell and type of the bud determined by oil immersion objective lens. The spore formation identification, the heat-fixed preparation was stained with 5% aqueous malachite green, counter-stained with 0.5% safranine solution thereafter viewed under the microscope. The vegetative cells stain red while ascospores stain green (Nwachukwu *et al.,* 2006).

2.4 Sugar Fermentation Test

This test was carried out as described by Kurtzman *et al.* (2011). The nutrient broth was prepared and 10ml of it was dispensed into the different test tubes labeled according to the yeast isolates and test sugars. A Durham tube was inserted in inverted position on each test tube at 121^0C for 15 minutes and allowed to cool. Thereafter, the broth in the tubes were respectively treated with these test sugars glucose, sucrose, lactose, maltose and galactose. Then the isolated test yeasts were aseptically inoculated into each labeled tube and then incubated for 48-72 hours. Thus, colour change and presence of air in the inverted durham tube (gas production) indicated a positive result for sugar fermentation by the yeast strain.

2.5 Production of Bread Samples

The ingredients were used to produce the bread samples using the straight dough method as described by FIIRO, (2008). The bread baking formula was flour (100%), water (55%), fat (4%), sugar (12%), salt (1.5%) and (1.5%) isolated yeasts was inoculated. All the dry ingredients with each isolated yeast were added in the mixer (Binatone, Model; HM-350S-15N, 210-Watt motor, manufactured in England) and mixed at low speed for 5 min and later water was added, then mixed for about 10 minutes to form a smooth soft dough. The formed dough was cut into 250 g sizes, then molded and placed into a greased baking pan to proof at room temperature for 2hr 30min and subsequently baked at 200°C for 25 minutes. The produced bread samples were allowed to cool and later used for further analysis.

2.6 Physicochemical Analysis of Bread Samples

The weight of the individual bread loaves was measured using a clean measuring scale. The millet displacement was adopted to determine the bread volume of the bread samples using the modified method described by Al-Saleh and Brennan, (2012). A container was used to measure the volume of millet grains. Thereafter, each bread loaf was placed inside the container of known volume, then the weighed millet grains were poured into the container to fill the top with the seeds. The extra millets were measured in a graduated cylinder and the volume noted. Afterwards, the specific volume was calculated using this formula

Specific volume (cm^3 /g) = $\frac{\text{loaf volume}}{loaf\ weight}$ … … … … … … … … … … … … (1)

2.7 Sensory Evaluation

The sensory evaluation was conducted using the method described by Iwe, (2002). Twenty member panelists were used to assess the quality attributes of the eight bread samples based on their sensory parameters such as taste, texture, aroma, crust colour and general acceptability using the 9-point hedonic scale where 9= Like extremely, 8=Like very much, 7=Like moderately, 6=Like slightly, 5=Neither like nor dislike, 4=Dislike slightly, 3=Dislike moderately, 2=Dislike very much and 1=Dislike extremely. The different eight bread samples were randomly coded and presented to each panelist. Water was also provided for rinsing of the mouth after each test.

2.8 Statistical Analysis

The one-way analysis of variance (ANOVA), Duncan's Multiple Range Test which was also used to separate the means at ($p < 0.05$) and Pearson correlations were statistically analyzed using Statistical Package for Social Scientists (SPSS) software (version 26.0).

3. Results and Discussion

Table 4.1 shows that the ascosporogenous yeast strains isolated from the palm wine samples are *Saccharomyces cerevisiae,* and *Pichia spp* and *Saccharomyces fragilis (kluyveromyces)* which were identified by their morphological and physiological characteristics.

The sample A cells were shiny, white to creamish in colour with round and oval shape. This isolated strain was characterized as *Saccharomyces fragilis* (*Kluyveromyces*) as it was positive to glucose, lactose, galactose, negative to sucrose, and maltose in the sugar fermentative test. This supports the findings of Hamidimotlagh *et al*., (2007), who reported that *S. fragilis (kluyveromyces)* ferments glucose and galactose completely. *Kluyveromyces* strains have the unique ability to assimilate and ferment lactose as they are used commercially for the production of biomass or alcohol from whey. However, Lucilia *et al*., (2010), reported that natural microorganisms, such as yeast *Kluyveromyces* do not ferment lactose efficiently.

The sample B cell features were round, creamy mucoid, and shiny with round and oval shapes. The sugar fermentation test of this yeast strain was positive for sucrose, maltose, glucose, galactose and lactose. Hence, it was revealed to be *Saccharomyces cerevisiae*. This is also in line with the findings of Obi *et al*., (2015) and Ebana e*t al*., (2018).

Subsequently, sample C cell also had round, white to creamy colonies with shapes that were round, oval and elated. It was only positive to glucose in the sugar fermentation test and this corroborates with the experimental result of Shshidan *et al*., (2011) which revealed that glucose was effective as a carbon source for both the expression and proliferation of *P. pastoris* cells.

Table 4.2 depicts the loaf weight, volume, and specific loaf volume of bread samples. There were significant differences ($p < 0.05$) among the loaf weight of the bread samples. The values ranged from 276.5 to 320.1g. The bread produced with Baker's yeast had the lowest score while the highest score was recorded in sample C (*Pichia specie*). However, the statistical analysis also revealed that there was no significant difference (P > 0.05) between bread samples A *(Saccharomyces fragilis)*, B *(Saccharomyces cerevisiae)* and D (Baker's yeast) with regard to their loaf weight.

The result of the bread volume ranged from 610 to 1090 cm^3 where sample C (*Pichia specie)* had the least value and sample D (Baker's yeast) had the highest value. The statistical result revealed there was no significant difference (P > 0.05) between sample A (*Saccharomyces fragilis*) and E. Among the isolated strains, sample B (*Saccharomyces cerevisiae)* had the highest value of 990cm^3 while among the combined strains, sample E (*Saccharomyces fragilis + Saccharomyces cerevisiae)* had the highest value of 920 cm^3, implying improved performance, this can be linked to the ability of these strains to ferment glucose and sucrose as evidenced in sugar fermentation test presented in Table 4.1. The high-volume value recorded in sample B indicates that *Saccharomyces cerevisiae* has great dough-raising capability as it is able to reduce glucose in anaerobic conditions thereby producing ethanol and carbon dioxide needed during the leavening process. The high volume of bread is a desirable quality index that influences consumers' acceptance of the product. According to Olubunmi *et al*., (2015), the bread volume, which is an external characteristic, is linked to the quantitative measurement of baking performance.

There was a significant difference (p < 0.05) in the specific volume of the bread samples and their values ranged from 1.91 to 3.94 cm3/g. The lowest value was observed in Sample C (*Pichia sp*) while the highest specific volume was recorded in sample D, which is the Commercial Baker's yeast, then followed by sample B, (isolated *Saccharomyces cerevisiae)* with a value of 3.56cm3/g. Among the combined strains, sample E (*Saccharomyces fragilis + Saccharomyces cerevisiae)* recorded the highest value of 3.23cm3/g.

The result of the sensory evaluation of bread samples produced from the isolated yeast strains and the combination of strains is shown in Table 4.3. Sensory attributes of foods such as aroma, taste colour and texture influence alimentary behavior. Hence, play major roles in the choice of foods chosen during a meal, ingestion, and also determine the level of satisfaction during consumption (Dias *et al.,* 2012). Significant differences (P < 0.05) were observed in the sensory parameters investigated, which include (taste, color, texture crumb, aroma and overall acceptability).

Taste is considered one of the main factors that influence the acceptance of any food. It also determines whether consumers enjoyed and accept the product. The taste of the bread samples ranged from 4.3-8.5, sample H (*S. cerevisiae* + *S. fragilis* + *Pichia specie*) was the least and sample D (*S. cerevisiae*), the control, was the most preferred. There was no significant difference (P >0.05) in the taste of bread samples A (*Saccharomyces fragilis*) and B (*Saccharomyces cerevisiae*).

Food texture can be likened to the physical characteristics of food samples such as density, viscosity and surface tension, which influence the sensation derived from receptors in the mouth during/after food consumption (Rustagi, 2020). There was a significant difference in the texture of the bread samples. It ranged from 3.5 -8.0. The crumb texture of sample D (Baker's yeast) had the highest score while the lowest reduction was observed in bread samples H (*S. cerevisiae* + *S. fragilis* + *Pichia specie*) compared with samples C, F, and G which also had low values but not significantly different. The decrease in the crumb textural quality observed in this study could be attributed to the inability of these strains of yeast to efficiently ferment the bread dough, which could have led to the poor formation of the bread network.

The aroma result of the bread samples revealed showed the scores ranged from 3.5 to 8.0 where sample C had the least value while sample D recorded the highest value. From the statistical result, it was observed that there was no significant difference among samples A, B and E (*S. fragilis, S. cerevisiae and S. fragilis* + *S. cerevisiae)*. Similarly, no significant variation was found between samples G and H. However, a significant difference (P < 0.05) was observed among samples C, D and F. The high aroma values from the yeast isolate indicate their ability to produce carbon dioxide, which leavens the dough and highly contributes to the aroma/flavour development during baking (Cofalec (2022) and Francisca *et al.,* (1999). Hence, a food with undesirable aroma will lead to the rejection of the foods by consumers while a desirable aroma will elicit high acceptance by consumers.

The colours of food play significant role in the choice of food as it influences if a food will be tasted or eaten. According to Mareile *et al*., (2015), the reactions between the reducing sugars and amino acids result in Maillard reaction and caramelization, which are majorly responsible for the crust colour of bread loaves. There was significant difference (p < 0.05) in the scores of crusts colour of the bread samples. The scores ranged from 4.4 to 8.0. The lowest score was observed in sample C while sample B (*S. cerevisiae)* had the highest value. However, the crust colour of sample B was not significantly different (P > 0.05) from that of sample D (Baker's yeast) with value of 7.6. The lowest value of bread sample C in crust color indicates that it was not attractive. According to Crepaldi, (2006), the colour of a food awakens sensations, which defines actions and behaviors that leads to physical and psychological reactions.

The value of the general acceptability of the bread samples varied significantly from 4.7 to 7.9 where sample H (*S. cerevisiae* + *S. fragilis* + *Pichia specie*) had the lowest value and the highest value was observed in sample D (Baker's yeast). However, there was no significant difference between sample B (*S. cerevisiae*) and sample D

(Baker's yeast). The general acceptability of any product is highly dependent on the sensory attributes of the food such as taste, texture, aroma and appearance because they influence the consumers towards the food preferences and ultimately the satisfaction derived from the consumed food (Kostyra *et al.,* 2016). The Maillard reaction that occurred during baking of the bread samples also contributed to the improved colour, taste, appearance and aroma which consequently influenced the general acceptability of the bread samples.

In this study, the result of Pearson correlation in Table 4.4, indicates a negative correlation of specific volume with bread weight BW (r = -0.94) for the bread samples. It also indicates that the higher the bread weight, the lower the specific volume. The high bread weight observed in this study could be as a result of insufficient carbon dioxide and ethanol formation due to inability of the yeast strains to adequately ferment the substrate which subsequently has a negative effect on the bread volume (r = -0.93), crust colour (r = -0.79), taste (r = -0.72) and general acceptability (r = -0.86) respectively. Meanwhile, specific volume showed a strong positive correlation with the bread volume (r = 0.99), implying that higher specific volume is associated with high bread volume. Similar positive correlations were observed between specific volume and crust colour (r = 0.77), taste (r = 0.84), and general acceptability (r = 0.95). The high bread and specific volumes of the bread loaves may be attributed to formation of strong gluten network in the dough because of the yeast's fermentation of the flour, which led to retaining high carbon dioxide gas hence, the low weight of the bread. The bread volume also correlated positively with crust colour (r = 0.76), taste (r = 0.84) and general acceptability (r = 0.95). This is in line with Koletta *et al.,* (2014), who stated that volume is one of the most important technological characteristics of bread, because it affects both appearance and sensory acceptance.

The general acceptability was also found to be positively correlated with bread volume (r = 0.96), taste (r = 0.90) and crust colour (r = 0.86), indicating that bread volume, taste and crust colour determine the general acceptability of bread loaves. Colour is an indicative quality control parameter that influences the acceptance of any food. Hence, consumers expect certain foods to have particular colors and non-conformity from this will negatively affect the sales of such foods (Molnár, 2022). Meanwhile, general acceptability negatively correlated with bread weight (r = -0.86).

4. Conclusions

This study showed that ascosporogenous yeast strains such as *S. cerevisiae, S. fragilis and Pichia spp* were isolated yeast from Palm wine. The result of the baking performance revealed that *S. fragilis*, *S. cerevisiae*, as well as the combined yeast strains of sample E (*Saccharomyces fragilis* + *Saccharomyces cerevisiae*) compared favourably with commercial Baker's yeast in leavening the bread. Based on the correlation result, it was revealed that the high bread volume, specific volume and crust colour had a positive significance ($p < 0.05$) on the general acceptability of the bread samples. However, the high bread weight value correlated negatively with the bread's crust colour taste and general acceptability mainly due to the inability of the yeasts (C= *Pichia specie*, F= *Saccharomyces fragilis* + *Pichia specie,* G= *Saccharomyces cerevisiae* + *Pichia specie*, H= *S. cerevisiae* + *S. fragilis* + *Pichia specie)* to produce enough carbon dioxide CO_2 and other metabolites which impact the final appearance of the dough, volume, and texture as well as the taste of the bread samples. There is need to adopt technology that will refine the isolated wild yeasts into commercial yeasts as this will further improve their leavening performances.

References

Akbar Ali, Aamir Shehzad, Moazzam Rafiq Khan, Muhammad Asim Shabbir & Muhammad Rizwan Amjid (2012). Yeast, its types and role in fermentation during bread making process-A Review. *Pakistan Journal of Food Sciences*, Volume 22, Issue 3, Page(s): 171-179.

Al-Saleh Abboud & Brennan Charles S (2012). Bread Wheat Quality: Some Physical, Chemical and Rheological Characteristics of Syrian and English Bread Wheat Samples. *Foods*, 1, 3-17; doi:10.3390/foods1010003.

Anudu Odinaka (2017). 72% of Nigeria's $621m bread industry dominated by small scale bakers.https://businessday.ng/real-sector/article/72-nigerians-621m-bread-industry-dominated-small-scale-bakers/. Accessed 19th June, 2023.

Bekatorou Argyro, Costas Psarianos & Athanasios A. Koutinas (2006). Production of Food Grade Yeasts. *Food Technol. Biotechnol. 44* (3) 407–415.

Cauvain, S.P., (2004). How much more bread research do we need. *Getreide technologie* 58, 364-366.

Chilaka, C. A., Uchechukwu, N., Obidiegwu, J. E. & Akpor, O. B. (2010): Evaluation of The Efficiency of Yeast isolates from palm wine in diverse fruit wine production. *African Journal of Food Science*. 4(12): 764 – 774.

Cho I. H & Peterson D. G. (2010). Chemistry of bread aroma: a review. *Food Sci Biotechnol* 19:575–82.

Cofalec, Utilization of yeast for baking. Retrieved from http://www.cofalec.com/theworld-of-yeast/yeast-characteristics/ on 19th December, 2022.

Crepaldi, L. A (2006). Influence of colors on the purchase decision: a study of consumer behavior in the ABC region of São Paulo. In: Brazilian Congress of Communication Sciences - Intercom, 29., Brasilia. Annals. *Brasília*, p. 1-11.

Dias Nayane Aparecida Araújo, Lara Suzana Borges, Miranda Lucilene Soares Miranda, Pires Ivy Scorzi Cazelli, Pires Christiano Vieira, & Halboth Nadia Veronica (2012). Influence of color on acceptance and identification of flavor of foods by adults**.** Ciênc. Tecnol. Aliment., Campinas, 32(2): 296-301.

Ebana, R.U.B, U. O. Edet, U. O, Anosike, I. K, Etok, C. A & Ekpenyong, V. B (2018). Bread Production from Different Flours Using Strains of Baker's and Palm Wine Yeasts. *Asian Food Science Journal 5(3): 1-10.*

El-Ghwas DE, Elkhateeb WA, Akram M & Daba GM (2021). Yeast as Biotechnological Tool in Food Industry. *Journal of Pharmaceutical Research*, 5(3): 000243.

Ezereonye O.U. (2004). Nutrient utilization profile of *Saccharomyces cerevisiae* from palmwine fruit fermentation. *Antonie van Leeuwenhoek;* 86(3): 235-240.

Ezeronye, O.U (2003) Hybridization of palm wine yeasts (*Saccharomyces cerevisae*) with brewer's yeast by protoplast fusion; *Journal of Biological Resource and Biotechnology;* 1:53-62.

FIIRO In: Federal Institute of Industrial Research, Oshodi, (FIIRO) Activities on Cassava Processing, 2008, pp 2-48.

Francisca, R. G., Pascual, S. & Jose, A.P. (1999). Engineering Baker's yeast: Room for improvement. *Trends in Biotechnology* (TIBTECH), 17: 237-244.

Gnanamani, A & Jayaraman, K (2008). Isolation, identification and characterization of yeasts from palmwine. *International Journal of Food Microbiology*, 123(2), 187-193.

Hamidimotlagh Rouhollah, Nahvi Iraj, Emtiazi Giti & Abedinifar Sorah (2007). Mixed sugar fermentation by *Pichia stipitis*, *Sacharomyces cerevisiaea,* and an isolated xylosefermenting *Kluyveromyces marxianus* and their cocultures. *African Journal of Biotechnology* Vol. 6 (9), pp. 1110-1114.

Heller K.J (2001). Probiotic bacteria in fermented foods: product characteristics and starter organisms. *Amer. J. Clin. Nutr;* 73: 374S-379S.

Iwe, M. O (2002). Sensory Methods and Analysis. Rejoint Communication Services, Ltd Enugu, Pp.71-75.

Jayaram VB, Cuyvers S, Lagrain B, Verstrepen KJ, Delcour JA & Courtin CM. (2013). Mapping of *Saccharomyces cerevisiae* metabolites in fermenting wheat straight-dough reveals succinic acid as pH-determining factor. *Food Chem* 136:301–8.

Koletta Pelagia, Irakli Maria, Papageorgiou Maria & Skendi Adriana (2014). Physicochemical and technological properties of highly enriched wheat breads with wholegrain non wheat flours. *Journal of Cereal Science* 60; 561 – 568.

Kostyra E, Wasiak-Zys G, Rambuszek M & Waszkiewicz-Robak B. (2016). Determining the sensory characteristics, associated emotions and degree of liking of the visual attributes of smoked ham. A multifaceted study. *LWT-Food Science and Technology*; 65:246-253.

Kurtzman, C., Fell, J. W., Boekhout, T. & Robert, V. (2011): Methods for isolation, Phenotypic characterization and maintenance of Yeast. In: Kurtzman, C.P., Fell, J.W and Teun, B., Eds., The Yeasts, A Taxanomy , 5th Edition, *Elsevier Publishers*, Amsterdam, 87-110.

Lopez, H.W., Adam, A., Leenhardt, F., Scalbert, A &. Remesy, C., (2001). Control of the nutritional value of bread. Industries des Cereales 124, 15-20.

Lucília Domingues, Pedro M.R. Guimarães & Carla Oliveira (2010). Metabolic engineering of *Saccharomyces cerevisiae* for lactose/whey fermentation. *Bioengineered Bugs* 1:3, 164-171.

Mahoukoucd Sheti-Kouabo D.F, Goma-Tchimbakala J, Lebonguy A. A & 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,* Vol. 7, No. 6, 205-214

Mareile Heitmann, Emanuele Zannini & Elke K. Arendt (2015). Impact of different beer yeasts on wheat dough and bread quality parameters. *Journal of Cereal Science;* S0733-5210(15)00031-4 DOI: 10.1016/j.jcs.2015.02.008.

Molnár Pál J. (2022). Food Quality Indices. Food quality and standards in *Encyclopedia of Life Support Systems* (EOLSS) – Vol. II

Nester E.W., Anderson D.G., Roberts C.E., Pearsall N.N. & Nester M.T. (2004). Microbiology; A human Perspective. 4th ed., McGraw Hill, New York, USA. Pp 610-628

Nwachukwu, I. N., Ibekwe, V. I., Nwabueze, R. N. & Anyanwu B. N. (2006): Characterization of palm wine yeast isolates for Industrial utilization. *African Journal of Biotechnology*. 5(19): 1725 – 1728.

Obi C.N, Ogbulie, J.N & Nkwo, A.M (2015). Assessment of microbial growth and survival in fresh rafia palm-wine from Umuariaga community, Ikwuano L. G. A. Abia State, Nigeria. *International Journal of Current Microbiology and Applied Sciences 4(1): 484-494.*

Okechukwu, A.N, Chinwe I. S & Adeola E, (2019). Isolation and Identification of Yeast Cells from Palm Wine in Ado Ekiti Southwest Nigeria. *International Journal of Science and Research* (IJSR) ISSN: 2319-7064 SJIF: 7.583.

Olowonibi, O. O (2017). Isolation and Characterization of Palm Wine Strains of *Saccharomyces cerevisiae* Potentially Useful as Bakery Yeasts. *European Journal of Experimental Biology* ISSN2248-9215, Vol.7No.2: 11.

Olubunmi, I.P, Akinyele O. O, Babatunde K. S, Olusola, O. E, Adetokunbo, O. A, & Elemo. G. N (2015). Influence of Coconut Fibre Inclusion on Rheological Properties of Composite Wheat-Cassava Flour using the Mixolab. *Journal of Food and Nutrition Sciences,* 3(6), 229-235. https://doi.org/10.11648/j.jfns.20150306.15.

Otero A Miguel, Isabel Guerrero, Jorge R Wagner, Agustín J Cabello, Paula Sceni, Roxana García, Jorge Soriano, Araceli Tomasini, Gustavo Saura & Oscar Almazán (2011). Yeast and its derivatives as ingredients in the food industry*, Biotecnología Aplicada;* 28:272-275.

Rustagi Sarvesh (2020). Food Texture and Its Perception, Acceptance and Evaluation. *Biosciences Biotechnology Research Asia*, Vol. 17(3), p. 651-658.

Shahidan, N. H, Rahman R. N. Z. A., Leow T. C., Rosfarizan M, M. Basri M & Salleh A. B (2011). The effect of carbon sources on the expression level of thermostable L2 lipase in Pichia pastoris. *African Journal of Biotechnology* Vol. 10(62), pp. 13528-13535. DOI: 10.5897/AJB11.1550.

The Artisan, The Yeast Treatise, 2002, Yeast Production - General Discussion [online]. Available from: http://www.theartisan.net/yeast_treatise_framese t.htm [accessed on 4 September, 2022].

Walker G.M, (2009). Yeasts. University of Abertay Dundee, Dundee, Scotland. ©Elsevier Inc.

Wedral D, Shewfelt R & Frank J. (2010). The challenge of *Brettanomyces* in wine. *LWTĐ Food Science and Technology*; 43(10):1474±9.

Table 4.1 Characteristics of yeast strains isolated from palm wine

Yeast	Colony features	Cell morphology	Ascospores	Sugar fermentation					Probable identity
				1	2	3	4	5	
A	Round, shiny, white to creamish colour	Mainly oval, round	+ Unconjugated asci. (1-3) smooth ascospores	AG		AG		AG	*Saccharomyces fragilis (kluyveromyces)*
B	Round and creamy mucoid and shiny	Round and oval	Produced pores	AG	AG	AG	AG	AG	*Saccharomyces cerevisiae*
C	Round, white to creamy colonies	Round, oval and elongated	Produced spores, heart or Saturn shaped (1-4) spores	AG					*Pichia spp*

1 = Glucose

2 = Sucrose

3 = Lactose

4 = Maltose

5 = Galactose

- = Negative

+ = Positive

AG = Acid and gas

Table 4.2 Physical characteristics of yeast strains and baker's yeast in bread

Sample	Loaf weight (g)	Loaf volume (cm^3)	Specific volume (vol/wt) cm^3/g
A	279.4 ± 0.55^{e}	925.7 ± 7.57^{c}	3.31 ± 0.029^{c}
B	278.2 ± 0.00^{e}	990.0 ± 0.00^{b}	3.56 ± 0.00^{b}
C	320.1 ± 2.15^{a}	610.3 ± 2.51^{f}	1.91 ± 0.01^{g}
D	276.5 ± 5.75^{e}	1090 ± 7.00^{a}	3.94 ± 0.06^{a}
E	285.4 ± 2.62^{d}	920.7 ± 3.06^{c}	3.23 ± 0.02^{d}
F	305.4 ± 0.35^{b}	620.2 ± 0.20^{e}	2.03 ± 0.00^{f}
G	300.6 ± 1.54^{c}	630.4 ± 2.68^{d}	2.10 ± 0.01^{e}
H	305.9 ± 2.61^{b}	621.0 ± 4.58^{e}	2.03 ± 0.00^{f}

Means with different superscripts in the same column are significantly different (P <0.05)

A= *Saccharomyces fragilis*, B= *Saccharomyces cerevisiae*, C= *Pichia specie*, D= Baker's yeast, E= *Saccharomyces fragilis* + *Saccharomyces cerevisiae*, F= *Saccharomyces fragilis* + *Pichia specie*, G= *Saccharomyces cerevisiae* + *Pichia specie*, H= *S. cerevisiae* + *S. fragilis* + *Pichia specie*

Table 4.3: Sensory evaluation of the bread samples

Samples	Taste	Texture (crumb)	Aroma	Crust colour	General Acceptability
A	6.9 ± 0.57^{b}	5.4 ± 0.70^{c}	7.0 ± 0.67^{b}	6.0 ± 1.05^{b}	6.0 ± 1.49^{b}
B	6.9 ± 0.57^{b}	6.9 ± 0.57^{b}	6.3 ± 0.82^{b}	8.0 ± 0.67^{a}	7.0 ± 0.94^{a}
C	6.0 ± 0.47^{c}	4.5 ± 0.71^{d}	3.5 ± 0.71^{e}	4.4 ± 1.26^{c}	4.9 ± 1.29^{c}
D	8.5 ± 0.71^{a}	8.0 ± 0.47^{a}	8.0 ± 0.67^{a}	7.6 ± 1.07^{a}	7.9 ± 0.74^{a}
E	6.2 ± 0.63^{c}	5.5 ± 0.71^{c}	6.4 ± 0.84^{b}	5.2 ± 0.91^{bc}	6.2 ± 0.92^{b}
F	5.0 ± 0.67^{d}	4.5 ± 0.71^{d}	5.4 ± 0.97^{c}	5.2 ± 0.63^{bc}	5.0 ± 0.67^{c}
G	6.1 ± 0.57^{c}	4.4 ± 0.70^{d}	4.4 ± 0.70^{d}	6.2 ± 0.92^{b}	5.3 ± 0.82^{bc}
H	4.3 ± 0.67^{e}	3.5 ± 0.71e	4.3 ± 1.16^{d}	4.7 ± 1.49^{c}	4.7 ± 1.06^{c}

Means with different superscript in the same column are significantly different (P <0.05)

Table 4.4: Pearson Correlation Matrix of physical properties with sensory parameters of the bread samples produced from the isolated yeast strains

	SPV	BW	BV	CRC	TST	GNA
SPV	1.00					
BW	-.944**	1.00				
BV	.999**	-.931**	1.00			
CRC	.773**	-.789**	.759**	1.00		
TASTE	.835**	-.715**	.841**	.755**	1.00	
GNA	.952**	-.864**	.954**	.858**	.901**	1.00

**. Correlation is significant at the 0.01 level (2-tailed).

SPV: Specific volume, BW: Bread weight, BV: Bread volume, CRC: Crust colour, TST: Taste, GNA: General acceptability