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BIOCHEMICAL PROPERTIES OF ISOLATED YEAST FROM *RAPHIA HOOKERI* (RAPHIA PALM WINE AND *ELIASE GUINENSIS* (OIL PALM WINE)

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ABSTRACT

This study was conducted to comparatively evaluate the biochemical characteristics of industrial yeast and locally isolated yeast from palm wine. Palm wine samples were collected from Ikwuano/Isi-ala Ngwa areas of Abia state during the early hours of the morning. Samples collected were taken immediately to the laboratory for yeast isolation. Commercial yeast and isolated yeast were then tested for Ethanol tolerance, ethanol production capacity, sugar utilization, flocculence, and elevated temperature growth, dough leavening capacity, bread production and sensory evaluation. Results obtained were subjected to statistical analysis and data was presented in tables and figures. From the result, the isolated yeast samples had high ethanol tolerance at 14 % (v/v) ethanol while the commercial yeast sample used as control could not tolerate ethanol higher than 12 % (v/v). Also, the isolated yeast sample had the ability to produce approximately 5 % alcohol in a period of seven days. While the industrial yeast sample used as control was able to produce approximately 6 % alcohol within the same period of seven days. The result of sugar utilization test showed that yeast samples were able to assimilate Glucose, Sucrose, Maltose, Galactose and Raffinose and showed negative utilization of carbon for Lactose, Mannitol and Xylose sugars. All yeast samples showed flocculation abilities. Isolated yeast particularly the yeast isolated from the Raffia palm wine (Ngwo) showed good flocculation ability highly useful in breweries for brewing beer and wine. Test for the ability of yeast samples to grow at varying conditions, the isolated yeast was positive for growth in 3 % ethanol, 50 % glucose, temperatures of 30 °C and 35 °C and in 3 % sodium chloride while in 42 °C and 10 % NaCl isolated yeast was Negative.

Keywords: Morphology, Molecular, Biochemical, *Elaisesguinensis*, *Raphia hookeri.*

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INTRODUCTION

Palm wine is a whitish, effervescent, alcoholic beverage produced by the spontaneous yeast-lactic acid fermentation of the sugary sap of palm trees (Mavioga *et al.*, 2009). It can also be referred to as the collective name for a group of alcoholic beverages produced by the natural fermentation of the sap obtained from various tropical plants of the palmae family (Okafor, 1978). The sap is a sweet, clear colorless juice containing 10-20 % sugar (Nguyen *et al.*, 2014). Nguyen *et al* also said that the sap is an excellent substrate for microbial growth. When palm wine is fresh it is sweet and refreshing because of the presence of sucrose but within 24 hours the concentration of the sucrose falls to less than 50 % the initial amount (Bassir, 1962). The sweetness is lost due to the spontaneous fermentation of its sugars which happens in a 24 hour period (Obire, 2005).

Fermentation of the sap commences immediately by wild inoculum of yeast in the sap resulting into a milky-white product called palm wine that has increased microbial suspension caused by the prolific growth of the fermentation organism (Ikegwuand Iwouno, 2015)., thus the microorganisms in palm wine are alive when it is been consumed (Olawale *et al.*, 2010). In furtherance, the sugar in the sap is converted to ethanol (upto 4%) within an hour or two and subsequently to acetic acid leading to decreased acceptability and shortened shelf life (Nguyen *et al.*, 2014).

In Africa, the sap used to produce palm wine is most often taken from wild date palms such as silver date palm (*Phoenix sylvestris*), the Palmyra and the Jaggery palm (Caryota Urens) or from the oil palm such as the African oil palm (*Elaeis guineensis*) or from Raffia palms, Kithul palms or Nipa palms (Wikipedia encyclopedia, 2016). In Abia state Nigeria (Area of study) precisely, the sap used to make palm wine is taken from the oil palm tree (*Elaeis guineensis*) or from the Raffia palm tree (*Raffia* sp.) which is shorter and thus more accessible.

The unfermented *Raphia* palms ap contains 10–16.5 % (w/v) sugar (mainly in the form of sucrose) is fermented to ethanol and other minor constituents by a complex mixture of wild yeasts and bacteria. The naturally fermented Raphia palm wine contains about 5 to 6 % (v/v) ethanol (Nwokeke, 2001). Generally, palm wine is good for the body. According to Faparusi and Bassir (1972) and Oka for (1987), the bacteria that are most predominant in palm wine after fermentation are *Micrococcus*, *Leuconostoc*, *Lactobacillus* and *Acetobacter;* while the predominant yeasts usually identified are *Saccharomyces* and *Candida* spp.

Yeast is unicellular fungi. The precise classification uses the characteristics of the cell, ascospore and colony. Physiological characteristics are also used to identify species. One of the more well-known characteristics is the ability to ferment sugars for the production of ethanol. Budding

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yeasts are true fungi of the phylum *Ascomycetes*, class *Hemiascomycetes*. The true yeasts are separated into one main order *Saccharomycetales* (Roger, 2004). Yeasts are characterized by a wide dispersion of natural habitats. Commonly on plant leaves and flowers, soil and salt water. Yeasts are also found on the skin surfaces and in the intestinal tracts of warm-blooded animals, where they may live symbiotically or as parasites (Roger, 2004). *Saccharomyces cerevisiae* yeast is unicellular fungi that divide asexually by budding or fission and whose individual cell size with a large diameter of 5 – 10 μm and a small diameter of 1 – 7 μm. The cells of *S. cerevisiae* are pigmented, where cream color may be visualized in surface-grown colonies (Walker and White, 2011). Yeast cell is completely deferred than bacterial cell in both structure and function. *S. cerevisiae* has an extensive history of uses in the area of food processing. Identification and characterization of yeast species may be according to a number of criteria such as cell morphology (e.g., mode of cell division and spore shape), physiology (e.g., sugar fermentation tests), immunology (e.g. immune fluorescence), and molecular biology (e.g., DNA re-association, ribosomal DNA phylogeny, karyo typing, random amplified polymorphic DNA (RAPD), DNA base composition and hybridization, and amplified fragment length polymorphism (AFLP) of D1/D2 domain sequences of 26S rDNA). Molecular sequence analyses are being increasingly used by yeast taxonomists to categorize new species (Walker, 2009). Among yeast, *S. cerevisiaeis* are industrially important due to its ability to convert sugars (i.e., glucose, maltose) into ethanol and carbon dioxide (baking, brewing, distillery, liquid fuel industries). *S. cerevisiae* breaks down glucose through aerobic respiration in presence of oxygen. If oxygen is absent, the yeast will then go through anaerobic fermentation. The net result of this process is two adenosine triphosphate molecules, in addition to two by products; carbon dioxide and ethanol.

The type or nature of yeasts with leavening activity from palm wine samples from Abia State (Nigerian eastern region) may have peculiar characteristic information on physiological studies of such indigenous yeasts from Ikwuano/Ngwa and environs is not available; this information is required for domesticating such yeasts for industrial use.

The conventional Yeast has a low alcohol tolerance level at about 10 - 12 % (Oke and Ijebor, 1997). No Studies have shown that when the tolerance level of yeasts sourced locally, its capacity to simultaneously convert glucose and xylose to ethanol. The ability to effectively ferment xylose to ethanol increases the yield of ethanol to about 40 % (Ho *et al*, 2004). This shows that the alcohol tolerance level and fermentative ability of yeasts from Palm wine can be greatly enhanced and employed in the global fermentation alcohol production.

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This study is aimed at isolating and characterizing of fermentation base yeast from Oil Palm wine and Raffia palm wine, and the determination of the biochemical and morphological characteristics of the isolated yeast.

MATERIALS AND METHODS

Samples Collection

Palm wine

Samples of freshly taped Palm wine was purchased from different locations in Ikwuano and Ngwa area in Abia State, South East Nigeria. Samples were set up on the day of purchase for physico-chemical and microbiological analysis while the rest were stored at 4 ″C for further use.

Sample Preparation

Sorghum Starch Preparation Two (2 Kg) of sprouted sorghum sweet grains or guinea corn (*Sorghum guineese*) was washed, dried and milled into flour of particle size less than 2.0 mm sieve. It was then stored on a sterile container until when needed for analysis of ethanol fermentation. The palm wine was divided into three, two liters each for after 24 hours, after 48 hours, and after 72 hours fermentation base yeast isolation. According to the information in the Catalogue, the brewer's yeasts for fermentation of sugars from the sorghum and as a result ethyl alcohol up to 8 g.kg -1 was obtained (Nikolova *et al.*, 2008)

The Baker's yeast was stored in a cool dry place until when needed for analysis.

Isolation of Yeast Strains

The yeast strains were isolated from the sap of palm wine for 24, 48 and 72 hours after harvest using the techniques of Teramoto *et al.* (2005) with some modification. This involved using a medium containing potato dextrose agar (Oxoid), 3.5 % (w/v), streptomycin 1.0 % (w/v), and sodium propionate, 0.5 % (w/v). The use of streptomycin and sodium propionate were to discourage the growth of bacteria and molds respectively. Serial dilution of the various samples of palm wine sap will be made. The stock culture was prepared by taking 50 ml of the palm wine sap sample, and mixed it with 450 ml of sterile peptone water. Each sample was serially diluted (10 fold dilution of 1:10, 1:100, 91 1:1000, 1:10,000) in sterile peptone water and 0.1 ml of each dilution was plated out in duplicate on potato dextrose agar (PDA). The medium was prepared according to specification by the manufacturers (Oxoid). The duplicate plate was incubated at ambient temperature (26 – 28 ^{0}C) for 48-72 h. The discrete isolated colonies (pure cultures) were picked out, purified by re-streaking on PDA, and maintained/ stocked on slants of the same medium at 5 ^{0}C in the refrigerator.

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Biochemical characterization of yeast species

Carbon assimilation test

The carbon assimilation activities of yeast species on carbohydrates will be carried out by the use of Analytical Profile Index (API 20 C) kit method as described by Espinel-Ingroff *et al.*, (1998) and Moghaddas *et al.* (1999). The API 20 C AUX strips (Biomerieux, Lyon, France) comprises of Dglucose, glycerol, 2-keto-gluconate, L-arabinose, D-xylose, adonitol, xylitol, D-galactose , Inositol, D-sorbitol, Methyl-αD-glucopyranosides, N-acetyl-glucosamine, D-celloboise, D- 93 trehalose, D-melezitose and D-raffinose respectively, API C medium, API C strips, API NaCl 0.85% medium, Incubation box (Tray and lid) and the result sheet. The API 20 C AUX strip consists of 20 cupules containing dehydrated substrates which enable the performance of the 19 assimilation tests as stated above. The cupules are inoculated with a semi-solid minimal medium. The yeasts will only grow if they are capable of utilizing each substrate as the sole carbon source. The reactions are read by comparing them to growth controls. Identification is obtained by referring to the analytical profile index or using the identification software (Biomerieux).

API technique

An ampule of API NaCl 0.85 % medium (2 ml) was inoculated with a 24 h old yeast culture and the turbidity of suspension was compared with McFarland standard no. 2. One- hundred microliter (100 µl)(0.1 ml) of the previous suspension was transferred into an ampule of API C medium and homogenized gently using sterile micropipette to avoid bubble formation. Five milliliters (5) of distilled water was dispensed into the honey-combed wells of the incubation box tray in order to create humid atmosphere. The API strips with 20 cupules containing 19 dehydrated substrates and 1 negative control was placed in the tray and filled with the suspension obtained in ampule of API C medium avoiding the formation of bubbles by placing the tip of the micropipette against the side of the cupule. Precautions were taken not to overfill or under-fill the cupules, the surfaces were either flat or slightly convex in order to avoid wrong results. The tray was covered with the lid and incubated at 29 ^{0}C ± 2 ^{0}C for 48 - 72 h according to the manufacturer's manual. The reactions were read by comparing the growth in each cupule with the control. Cupules more turbid than the control indicated a positive reaction meaning that the yeast was capable of utilizing the substrate as the sole carbon source. The reactions were recorded in the result sheet provided and the numerical profile constructed from the reaction patterns was used for identification of the yeast species by referring to Analytical Profile Index.

Fermentative ability of the yeast strains

The following parameters were used to characterize the yeast for attributes important for ethanol production and dough-leavening capacity.

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Flocculence test for yeasts

The flocculating ability of the yeast strains were tested by using a method described by Ameh *et al.* (1990) with some modifications. The test yeast was grown on PDA for 48 h harvested by centrifugation at 1500 rpm for 3 minutes, and washed twice with sterile distilled water. The washed yeast cells (3.0×10^9 cell/ml) were suspended in 10ml of sterile distilled water and 1 ml of acetate buffer (pH 4.5) was added and mixed thoroughly with the suspension and allowed to stand for 10 minutes. The amount of sediment formed after 10 minutes indicates whether or not the yeast was flocculent (0.5 - 1.0 ml flocculent; 0.0 - 0.4 non-flocculent), using calibrated centrifuged tubes.

Ethanol tolerance test for yeasts

The ethanol tolerance was tested by qualitative and quantitative method. The qualitative test was carried out by using the method described by (Aguilera *et al.*, 2006). This was based on visual assessment of turbidity and sediment in a tube of basal medium containing graded concentrations (8, 10, 12, 14, 16, 18, 20 % (v/v) of absolute ethanol inoculated with fresh yeast cells at 3×10^9 cell/ml. The assessment was made after 48 h of incubation at room temperature (26 - 28 ^{0}C). The quantitative test was described as stated by Nigam *et al.* (1998). It was based on viable counts 95 determined at selected intervals of incubation time (0, 24, 48 hours) at room temperature (26 ^{0}C–28 ^{0}C). The medium used was made up of 2 % (w/v) glucose (Analar), 0.3% yeast extract (Oxoid), 0.5 % of peptone (Oxoid), 1.5 % agar (Oxoid) and 10 % (v/v) absolute ethanol. Percent viability was calculated for each yeast strain after the respective incubation period.

Sugar utilization test for yeasts

The isolated strains were tested for their ability to ferment other sugars apart from glucose. The basal medium contained 0.2 % yeast extract (Oxoid), and 1.0 % oxidative fermentative medium (O/F). This was dispensed in 8ml amounts each into test tubes containing Durham tubes and sterilized by autoclaving at 121 ^{0}C for 15 minutes. The carbon sources that were used included membrane filter-sterilized 6.0 % (w/v) solution of glucose, maltose, sucrose, galactose, lactose 96 and 12 % (w/v) raffinose. A standard suspension (0.1 ml) of washed yeast cells (3.0 X 109 cells/ml) were introduced into each of the test tubes containing the 8ml basal medium and 2ml of each of the various carbon sources. The test tubes were incubated at room temperature for 48 hours. A basal medium without any of the sugar were also inoculated with the test organisms to serve as control. After 48 hours the test tubes were observed for gas production and colour change, which indicated fermentative ability of the yeast strains (Amehet *al.*, 1990; Oke and Ijebor, 1997).

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Growth in 3 % ethanol test for yeasts

The inhibition of yeast growth by 3% ethanol or utilization of ethanol as a sole carbon source was tested using the method of Akinyemi, (1990). Aliquots (0.1) ml of 24 h washed yeast suspension (3.0 X 10^9 cells/ml) of wild, mutant and standard strains were inoculated in sterile normal saline (NaCl) 0.85 % (w/v) and 1.0 % (w/v) yeast extract (Oxoid) broth media containing 3 % of ethanol in each case. An inoculated sterile medium without ethanol was used as control. The tubes were incubated for 48 -72 h at room temperature (26-28 ^{0}C). Occurrence of turbidity or sedimentation was taken to indicate growth in ethanol.

Growth in 50 % glucose test for yeasts

The yeast isolates were grown on a basal agar medium of 50 % (w/v) glucose, 1.0 % (w/v) yeast extract (Oxoid), 0.75 % (w/v) peptone (LABM) and 2 % (w/v) agar (Oxoid), incubated at room temperature (26^{0}C-28 ^{0}C) and examined for growth within four to seven days.

Growth at elevated temperatures test for yeasts

The following temperatures, 30^{0}C, 35^{0}C, and 42 ^{0}C, were chosen and used for the observation of growth of the yeast isolates. Yeast strains were streaked on potatoes dextrose agar plates and 97 incubated at the different temperatures indicated above. Observation for growth was made after 48 - 72 hours incubations.

Growth in 3 % and 10 % sodium chloride test for yeasts

The medium that was used to observe growth on sodium chloride (NaCl) was composed of 3 % and 10 % (w/v) sodium chloride, also 3.0 % PDA, 1.0 % (w/v) yeast extract (Oxoid), 0.75 % (w/v) peptone and 2.0 % (w/v) agar (Oxoid) was added to make up the medium. The sterile medium was dispensed into sterile Petri dishes and allowed to set. Yeast strains were streaked on agar surface and examined for growth after four to seven days of incubation at room temperature (26 ^{0}C -28 ^{0}C).

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RESULTS

Table 1: Biochemical and Sugar assimilation test of the yeast isolated from palm wine

	Nkwu	Ngwo	Control
Lactose	- - (-AG)	- - (-AG)	- - (-AG)
Glucose	++ (AG)	++ (AG)	++ (AG)
Sucrose	+ + (AG)	++ (AG)	++ (AG)
Maltose	+ + (AG)	++ (AG)	++ (AG)
Mannitol	- - (-AG)	- - (-AG)	- - (-AG)
Xylose	-- (-AG)	- - (-AG)	- - (-AG)
Galatctose	++(AG)	++ (AG)	++ (AG)
Raffinose	++ (AG)	++ (AG)	++ (AG)
Macroscopic	Creamy colored mould colonies on PDA plates	Creamy colored mould colonies on PDA plates	
Microscopic	Oval shaped budded cells appearing bluish in color.	Oval shaped budded cells appearing bluish in color.	

++ (AG): Positive fermentation, - - (-AG): Negative Fermentation, -AG: Acid/Gas production,

AG: No Acid/Gas Production

Biochemical and Sugar assimilation test of the yeast isolated from palm wine

Table 1 above shows carbon utilization of yeast isolated from palm wine. From the result, in lactose, the carbon utilization was negative for Nkwu, Ngwo and control samples. For glucose, sucrose, and maltose, carbon utilization test for glucose yielded positive for both Nkwu, Ngwo and control respectively. Also for Mannitol and Xylose sugars, carbon utilization test for palm wine samples and control yielded negative respectively. Galactose and Raffinose equally yielded positive for carbon utilization of yeast isolated for Nkwu, Ngwo and control respectively.

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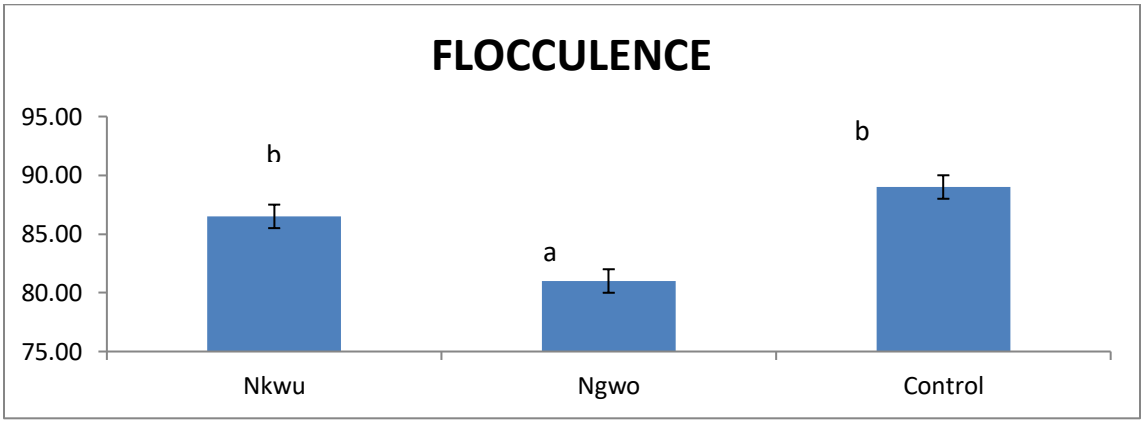
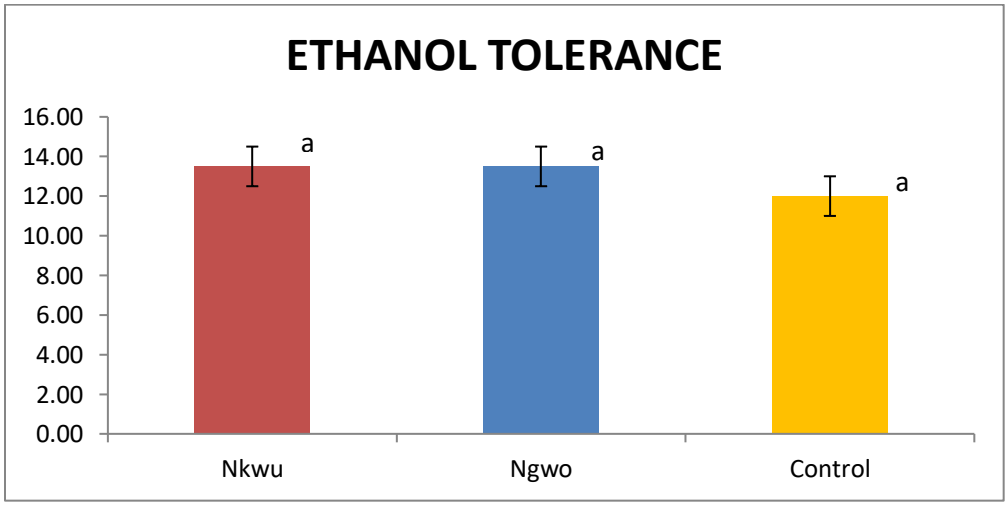


Means with same superscript alphabet are not significantly different (p≥0.05).

Figure 1: illustration of flocculence properties of yeast isolated from palm wine Illustration of flocculence properties of yeast isolated from palm wine

Figure 1 above shows the flocculence ability of yeasts from palm wine. From the result as illustrated above, Nkwu showed 86 %, Ngwo showed 82%, and control showed 90% flocculence ability respectively. This shows that Nkwu has high flocculation ability, Ngwo has a low or moderate flocculation ability, while the control sample, shows a very high or increased flocculation ability. Although *saccharomyces cerevisiae* is present in both Nkwu and Ngwo, different flocculence levels can be observed in both samples. While Nkwu had high flocculation ability, Ngwo had moderate flocculation ability.



Means with same superscript alphabet are not significantly different (p≥0.05).

Figure 2: Illustration showing ethanol tolerance of yeast from palm wine.

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Illustration showing ethanol tolerance of yeast from palm wine.

Figure 2 above illustrates the ethanol tolerance of yeast from palm wine. From the result, both Nkwu and Ngwo showed ethanol tolerance below 14.00 % while control showed ethanol tolerance at 12.00 %. Therefore, yeast cells of *saccharomyces cerevisiae* present in the Nkwu and Ngwo is highly ethanol tolerant. This shows that the yeast cells in *S. cerevisiae* have evolved to become more resilient to environmental stress as opposed to the Control sample which was poorly adapted to environmental stress induced by ethanol concentrations which inhibit cell growth and viability, limiting fermentation productivity and ethanol yield.

Table 2: Growth of isolated yeast cells in Elevated temperature

Sample code	Growth in 3% ethanol	50% glucose	30°c	35°c	42°c	37%NaCl	10%NaCl
Ngwo	+	+	+	+	-	+	-
Nkwu	+	+	+	+	-	+	-

+ = positive, - = Negative

Growth of isolated yeast cells in Elevated temperature

Table 2 above shows the growth of isolated yeast cells in Elevated temperature. From the result, for growth in 3 % ethanol test for yeast, the isolated yeast cells in Ngwo was positive, while the isolated yeast cells in Nkwu were also positive. In 50 % glucose test for yeast, the isolated yeast cell from both Nkwu and Ngwo were positive. Also, when incubated at temperature of 30°c, isolated yeast cells for both Nkwu and Ngwo were positive. The yeast cells were further incubated at temperature 35 °C and both yeast cell samples Nkwu and Ngwo respectively were positive. At temperature of 42 °C isolated samples from both Nkwu and Ngwo were negative. For growth in 37 % NaCl test for yeasts, isolated yeast cells from both Nkwu and Ngwo were positive. Finally, for growth in 10 % NaCl test for four (4) yeasts, the yeast cells isolated from Nkwu were negative while the yeast cells isolated from Ngwo were likewise negative.

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Table 3: Ethanol concentration of yeast fermentation medium

	SOURCE			
Days	Control	Ngwo	Nkwu	**Total**
Day 1	2.93±0.11	1.98±0.11	2.18±0.11	**2.36[a]**
Day4	4.08±0.04	3.33±0.11	3.70±0.00	**3.70[b]**
Day 7	5.55±0.21	4.90±0.00	5.10±0.00	**5.18[c]**
Total	**4.18±1.18[c]**	**3.40±1.31[a]**	**3.66±1.31[b]**	**3.75**

Means ± standard deviation values with different superscript alphabet are significantly different ($p \leq 0.05$)

Ethanol concentration of yeast fermentation medium

Table 3 above, shows the ethanol concentration from yeast fermentation medium. From the result, in the day1, control yeast sample produced an ethanol concentration of 2.93 %, Ngwo produced 1.98 % while Nkwu produced an ethanol concentration of 2.18 %. Four days after inoculation, the control yeast sample produced ethanol concentration of 4.08 %, yeast isolated from Ngwo produced 3.33 %. While yeast isolated from Nkwu produced 3.70 %. at the 7th day after yeast inoculation, control sample produced 5.55 %, Ngwo produced 4.90 % and Nkwu produced 5.10 % ethanol respectively. Effect of difference in the sources of yeast was significant ($p \leq 0.05$). Similarly, effect of change in time (days) was significant ($p \leq 0.05$) on the ethanol production levels.

DISCUSSIONS

The production of alcoholic beverages from fermentable carbon sources by yeast is the oldest and most economically important of all biotechnologies. Yeast plays an important role in the production of all alcoholic beverages and the selection of suitable yeast strains is essential not only to maximize alcohol yield, but also to maintain beverage sensory quality. The yeast specie that dominates the production of alcoholic beverages worldwide is *saccharomyces cerevisiae*. The particular strains of this species employed in fermentation exert a profound influence on the flavor and aroma characteristic of different beverages (Graeme and Graham 2016). Palm wine contains a high level of sucrose and this high sugar level favors the growth of yeasts. A diverse yeast population was detected in oil palm wine and Raphia palm wine samples, with *saccharomyces cerevisiae* occurring in both samples. The presence of *saccharomyces cerevisiae* in palm wine fermentation has been reported by various authors who designated the species as responsible for the fermentation and aroma of the wine (Aidoo *et al* 2006, Amoa-Awua *et al* 2007; Stringiini *et al.,* 2009). The presence of *saccharomyces cerevisiae* in the samples indicates

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the importance of this species in fermentation of palm sap. In this study, the performances of the yeast cells were compared to that of the commercial yeast. In the presence of sugar and other essential nutrients such as Amino acids, minerals and vitamins, *saccharomyces cerevisiae* will conduct fermentative metabolism to ethanol and carbon dioxide. Therefore, yeasts are of vital importance in providing the alcohol content and sensory profiles of such beverages. The time of yeast fermentation may be greatly influenced by environmental factors. The characteristics of this yeast such as fermentative ability and dough leavening activities may differ from place to place. It was shown in this study that all yeast strains which utilized respective sugars also produced carbon dioxide. The carbon dioxide released during dough fermentation process is prominent as a leavening agent of dough. It is notable that yeast strains identified to be *saccharomyces cerevisiae* were able to assimilate many of the sugars tested. The leavening activities of various yeasts isolated from palm wine shows that the wine is a rich source of potential bakery yeast. Interestingly, they occurred in palm wine from all locations from which samples used in this study were obtained.

Flocculence:

Yeast flocculation is a spontaneous process of auto-immobilization; it is an off-cost, fast, easy and eco-friendly process of cell-broth separation process (Soares 2010). Flocculation properties of *S. cerevisiae* ensure a highcell density and large volume of harvested cells and also able to raise the ethanol productivity during fermentation process. In this study, the yeasts present in the palm wine expressed flocculation abilities with the oil palm wine (Nkwu) yeast isolate showing more flocculent ability than the Raphia palm wine (Ngwo). While Nkwu had high flocculation ability, Ngwo had moderate flocculation ability. The control had higher flocculation ability than the Nkwu and Ngwo respectively. This shows that although *S. cerevisiae* is present in both Nkwu and Ngwo, Nkwu has high flocculation *a*bility. This makes the palm wine yeast especially the yeast from Ngwo more desirable when compared to the industrial yeast used as control. In this study, the flocculation characteristic was determined by yeast cells sticking together and provides 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 energy cost involved in biomass centrifugation. This disagrees with the result of (Olabisi *et al.,* 2017) in his work on, isolation and characterization of palm wine strains of *S.* cerevisiae potentially useful as bakery yeast which showed that many samples showed negative response to flocculence test while few samples showed positive response to focculence test. They concluded that the result was because, initiation of flocculation ability of *S. cerevisiae* cells was observed at the moment the cells stop dividing because of nitrogen limitation and a shift in concentration of the limiting nutrient, resulted in a corresponding shift in cell division and initiation of flocculence.

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Ethanol tolerance:

This is the ability of yeast to continue fermentation in the presence of high alcohol concentrations (Thammasittirong, 2013). The *Saccharomyces* species in this present test had high ethanol tolerance between 13 % and 14 % v/v and were not inhibited by those levels of ethanol. Maximum yield of yeast growth was obtained at 14 % ethanol in the presence of glucose, yeast extract, peptone, agar and absolute ethanolas against the control sample whose growth was inhibited at 12 % (v/v) ethanol under similar conditions. Ethanol tolerance is a unique property of the yeast that makes it exploitable for industrial applications (Pataro *et al.*, 2000).Reported that most *Sacchromyces* specie strains isolated from conventional fermentation processes were physiologically adapted to extreme conditions. In this experiment, the highest concentration in which the commercial yeast strain used as control was able to grow was at 12 % (v/v). Those strains which were capable of growing in similar concentration were expected to have the ability to produce similar quality of bread. While, palm wine yeast strains were able to grow at the highest concentration of 14 % (v/v). This result agrees with the work of Olabisi(2017) who studied Isolation and characterization of palm wine strains of *Saccharomyces cerevisiae* potentially useful as Bakery yeasts. From their result, the *Saccharomyces cerevisiae* in the samples under test had high ethanol tolerance of 13 % (v/v) and were also not inhibited by that level of ethanol. The result of this experiment in respect to high ethanol tolerance agrees with the work of Ikenna *et al.* (2006) who studied the characterization of palm wine yeast isolates for industrial utilization. In their result, Raffia palm wine recorded high ethanol tolerance of 15 % and even up to 20 % (v/v) and oil palm recorded 13 % and 19 % (v/v) ethanol tolerance. This shows that *S. cerevisiae* yeast has high level of ethanol tolerance and may increase from one location to another.

Growth at elevated temperature:

This is a temperature test for yeast cells to determine the highest temperature at which a yeast cell can maintain viability. If yeast cells are placed in a temperature too low or too high, the yeast cells grow much slower and finally ceases to grow. The selected yeast strains were also tested for their growth at high temperature. Most of the yeast strains could tolerate temperatures up to 35 °C but showed negative at temperature of 42 °C. therefore, it can be said that most yeasts isolated from different locations in Ikwuano and Isi-ala Ngwa were able to grow at high ethanol concentration (14 %) and at high temperature (35 °C). the yeasts also showed good growth at 3 % ethanol test and 50 % glucose test. In the test for 10 % NaCl, there was no growth but at 3 % NaCl growth was observed. This is in line with the result of Anthony *et al.* (1994) who reported on the development of baking yeast from Nigerian palm-wine yeast, in their result at 3 % NaCl, good growth was recorded, at 10% NaCl, there was growth in some culture while some recorded

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no growth and at 50 % glucose test, and considerable growth was recorded in the samples. This result is also in line with Nwakanma *et al.* (2015,) who at 30 ℃ recorded positive yeast growth in their work on Isolation and sensory evaluation of *S. cerevisiae* from palm wine gotten from different sites in Enugu. In similar way Ukponobong *et al.* (2017) reported also at 30 ℃ and 35 ℃ recorded intensive growth of yeast in their study of isolation and screening of yeast isolates indigenous palm wine for ethanol production.

Sugar test:

A sugar test was conducted, to determine carbon assimilation of the yeast on different sugars and its ability to cause rise in dough for baking. When tested in Lactose, Mannitol and Xylose sugars, test was negative while the test came out positive when the extracted yeast was tested in Glucose, Sucrose, Maltose, Galactose and Raffinose. The ability of yeast to assimilate Glucose, Sucrose, Maltose, Galactose and Raffinose confirms that *S.* cerevisiae isolated in this research as having the potentials to breakdown the aforementioned sugars. This result corresponded with the work of Engwa *et al.*, (2015) who studied the isolation and sensory evaluation of *saccharomyces serevisiae* gotten from different sites in Enugu. In his work, Fructose, Glucose, Sucrose and Maltose equally showed positive results for sugar test, indicating the presence of *S. cerevisiae*. In the works of Olabisi (2017) on isolation and characterization of palm wine strains of *saccharomyces cerevisiae* potentially useful as bakery yeast. In their result for sugar test, Glucose, Sucrose, Maltose, Galactose and Raffinose were also positive for carbon assimilation also in their findings, Xylose and Manitol were negative for carbon assimilation.

CONCLUSION

This work evaluated the biochemical characteristics of industrial yeast and yeast locally isolated from palm. Flocculence test for yeast showed that the isolated yeast strain met the industrial requirement for flocculent yeasts. The isolated yeast was able to withstand high ethanol tolerance and withstand high temperature. The ability of yeast to assimilate different sugar confirmed the presence of *S. cerevisiae* in the Carbon assimilation test.

RECOMMENDATION

This research was able to prove that yeast can be isolated from palm wine. Based on this, industries should endeavor to utilize local sources of yeast for their production given that this yeast was able to meet high quality standard for industrially produced yeast. Also, companies should draft policies which will encourage strictly isolation and utilization of locally sourced yeast for production of yeast base product. Further studies should examine possible storage means for yeast isolates of palm wine for higher commercial purposes, durability and

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enhancement. Lastly, further studies should attempt to expand locations from which palm wine is sampled and also try their DNA sequencing for a possible proof to strain differences.

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