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Ethanol Production from Lignocellulosic Materials by Fermentation Process Using Yeast

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ABSTRACT: Rapid industrialization and growing population result to high demand for energy. Depletion and rise in price of petroleum as well as environmental pollution necessitates the need for alternative source of fuel, hence bioethanol production. Rice bran (*Oryza sativa*), Corn bran (*Zea mays*) and Sorghum bran (*Sorghum guinense*) and saw dusts of *Khaya senegalensis* (Red wood), *Terminalia superba* (Black wood), *Gmelina arborea* (White wood), were used for the study. The yeasts used for the study were isolated from fermented beverages (Sorghum beer, Millet beverage and Palm wine). The results of the lignocellulosic biomass of white saw dust, red saw dust, black saw dust, rice bran, corn bran and sorghum bran revealed cellulose components as 77.78%, 75.55%, 68.59%, 64.83%, 54.82% and 55.14% respectively. A total of 25 yeasts were isolates and identified using API 20C AUX strip. The yeast isolates, K2, B5, B7 and P1 had the highest ethanol tolerance value of 14%. The results showed that the ethanol-producing ability of the yeast isolates ranged from 4.1% to 10.3%. Fourier Transform Infrared Spectrophotometer (FTIR) and Gas Chromatography and Mass Spectrometry (GC-MS) analyses showed that ethanol is the main compound produced by yeasts from the lignocellulosic materials. This study revealed that *Saccharomyces cerevisiae* isolated from palm wine (P1) is best in ethanol production and tolerance, and this high prolific strain can be exploited or engineered for ethanol production. Therefore, Lignocellulosic biomasses are recommended as raw materials for producing ethanol, which is a promising alternative energy source as against the depleting petroleum.

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The demand for energy is increasing due to growing population and industrialization, implying that energy shortage will be a global problem in the nearest future. Bioethanol has emerged as a favourable alternative for petroleum-based liquid fuels. The world production of bioethanol increased from 50 million cubic metres in 2007 to over 100 million cubic metres in 2012 (Kang *et al*., 2014). The use of ethanol in automobiles as an alternative fuel presents a viable option for improving energy security and reducing greenhouse emissions (Wyman, 1990). This has attracted worldwide attention to its production on a large scale while enhancing the economic status of a country (Cardona and Sanchez, 2007). Nearly 73% of bioethanol are produced globally (Balat *et al*., 2008). Ethanol produced from lignocellulosic materials is called second generation bioethanol and regarded as a carbon neutral fuel. Wood is one of the most important and adequate source of the lignocellulosic materials used for ethanol production (Okuda *et al*., 2007). Lignocelluloses in nature are derived from wood, grass, agricultural residues, forestry wastes and municipal solid wastes. They consist of three major components of polymers: cellulose, hemicelluloses and lignin. In addition, small amounts of other materials such as ash, proteins, pectin can be found in lignocellulosic residues in different degrees based on the sources (Saha, 2003). The plant biomass of the lignocellulose comprises on an average 23% lignin, 40% cellulose, and 37% hemicelluloses by dry weight (Sa-Pereira *et al*., 2003). This biomass has been recognized as a major renewable energy source to supplement declining fossil fuel sources of energy, and it is expected to play a crucial role in the world's future energy supplements (Amiri *et al*., 2014). More so, due to the rise in petroleum prices and environmental problems resulting from greenhouse gas emissions, the demands for traditional fossil fuels in recent years have increased drastically and there has been increasing interest towards an alternative sustainable energy resource such as bioethanol (Zhang *et al*., 2010). The transportation sector is unfortunately heavily dependent on crude oil as the only source of raw materials and the world is faced with a progressive

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depletion of crude oil and traditional fossils fuel. Being an oxygenated fuel when blended with gasoline, ethanol can be widely used for transportation purpose across the globe (Prasad *et al., 2*007). A way of addressing this problem could be through the usage of lignocellulosic materials to produce ethanol which will significantly lower the emission of exhaust gases that will result in clean and eco-friendly environment. More importantly, lignocellulosic materials do not compete with food crops and are less expensive than conventional agricultural feed stocks (Prasad *et al*., 2007). Ethanol as an alternative fuel can be obtained from forestry, agricultural, industrial and urban residues.

Therefore, the objective of the study was to produce ethanol from lignocellulosic materials by fermentation process using yeast.

MATERIALS AND METHODS

Sample Collection and Processing: Lignocellulosic materials used in this study include: Rice bran (*Oryza sativa*), Corn bran (*Zea mays*), Sorghum bran (*Sorghum guinense*) and Saw dusts of Oswalee (red wood) (*Khaya senegalensis*), Afara (black wood) (*Terminalia superba*), Obeche (white wood) (*Gmelina arborea*). The samples were air-dried at room temperature for two weeks to remove moisture and were pulverized in order to reduce the particles. The pretreated samples were packaged in clean sealed plastic containers, labeled appropriately and stored at room temperature for further use.

The Proximate Composition of the Lignocellulosic Materials: Each of the pulverized samples that has been processed and sieved was analyzed proximately using the AOAC (1990) method, for dry matter, ash content, crude fiber and crude protein.

Determination of Dry Matter Content: Six clean crucibles were dried to a constant weight in an oven at 105°C, cooled in a desiccator and weighed (W_1). Two grams (2g) of each sample was weighed into the crucibles (W_2) and dried in the oven. The crucible and its contents were cooled to room temperature in a desiccator and reweighed. The procedure continued until a constant weight was obtained (W_3). The percentage moisture content was calculated thus:

$$\%\ \text{moisture content} = \frac{W_2 - W_3}{W_2 - W_1} \times 100$$

Determination of Ash Content: Two grams of the pulverized sample was weighed (W_2) into a previously weighed, clean crucible (W_1). The sample was then ignited and cooled in a desiccator before being taken to the furnace. After maintaining the sample at 550°C in a muffle furnace for eight hours, the crucible and its residual ash were removed from the furnace and then allowed to cool to room temperature in a desiccator and weight (W_3). The ash content was calculated thus:

$$\%\ \text{ash} = \frac{W_2 - W_3}{W_2 - W_1} \times 100$$

Determination of Crude Fibre Content: Two grams of the sample was weighed into a 500ml round bottom flask, then 100ml of 0.023M sulphuric acid solution was added and the mixture boiled under reflux for 30 minutes. The hot solution was quickly filtered under suction. The residues were transferred into the flask and 100ml of 0.312M sodium hydroxide solution was added and the mixture boiled again under reflux for 30 minutes and filtered under suction. The insoluble residue was washed until it was base free and dried to a constant weight in an oven set at 100°C, cooled in a desiccator and weighed C_2. The residues were incinerated in a muffle furnace at 550°C for 2 hours, cooled on a desiccator and reweighed C_3. The crude fiber content was calculated as:

$$\%\ \text{crude fiber} = \frac{C_2 - C_3}{W} \times 100$$

Determination of Crude Protein: Two grams of the sample were weighed into a 100ml Kjeldahl digestion flask and twenty five millilitres (25ml) of concentrated sulphuric acid were added into the flask. Thereafter, the content in the Kjeldahl digestion flask was heated slowly at first in a Kjeldahl digestion heating unit until fretting subsided and then more vigorously until the green digest was obtained. Then 100ml of 40% sodium hydroxide solution was added. The solution was steam-distilled and the liberated ammonia was trapped in a 250ml conical flask containing 10ml of 4% Boric acid and a drop of mixed indicator (methyl red and methyl blue in a ratio of 2:1). The content of the conical flask was titrated with 0.1M hydrochloric acid (HCl) and end point was indicated by a change from greenish to pink colour. The volume of the acid used for each distillate as well as the blank was noted.

Determination of Fractional Composition of Cellulose, Hemicellulose and Lignin: The different components present in lignocellulosic materials used in this study were determined by sequential chemical extraction of the substrates using Harper and Lyach (1981) method. One gram (1g) each of the dried samples (dried at 60°C for 16 hours) was weighed into 150ml conical flasks, and then hot-water and hot-ethanol soluble fractions were determined.

Determination of Total Carbohydrate Content of the Lignocellulosic Materials: The determination of total carbohydrates by Phenol-Sulphuric Acid Method was adopted as described by Salehi *et al.* (2013). A homogeneous suspension of the pulverized samples were made by weighing 0.005g each of the sample into 10ml of 1N NaOH solution and were heated to about 90°C in order to make fine suspension. Total carbohydrates in unknown samples were then determined from the prepared suspensions.

Isolation of Yeast from Fermented Beverages: For screening of yeasts, three different types of fermented beverages (Sorghum beer, Millet beverage and Palm wine) were randomly purchased from well-known market and at the rural seller shops in Kaduna, Kaduna State. Yeast Isolates were obtained using dilution plate count techniques. Adopting the method of Martini *et al*. (1996), serial dilution of the samples was prepared using sterile distilled water as diluents. The pure colonies were transferred onto Yeast Extract Peptone Glucose (YEPG) agar slants for subsequent identification and stored at refrigeration temperature of about 4°C to 10°C.

Yeasts Identification Test with API 20C AUX: The isolates obtained were identified using API 20C AUX a standardized system (BioMerieux, France), containing identification numeric profile catalogue for yeasts. Yeast isolates grown between 18 to 24 hours was aseptically picked and emulsified in 2ml sterile saline solution, whose turbidity was adjusted to equivalent of 2 McFarland. Hundred microlitres (100ul) of this suspension was transferred to each cupule (well) of the identification strip. The dehydrated composition of the API 20C AUX strip is given in the list of tests: D-glucose (GLU), glycerol (GLY), calcium 2-keto-gluconase (2KG), L-Arabinose (ARA), D – Xylose (XYL), Adonitol (ADO), Xylitol (XLT), D – Galactose (GAL), Inositol (INO), D-Sorbitol (SOR), Methyl-α-D-Glucopyranoside (MDG), N-acetylglucosamine (NAG), D-cellobiose (CEL), D-Lactose (LAC), D-Maltose (MAL), D-saccharose (SAC), D-Trehalose (TRE), D-Melezitose (MLZ), and D-Raffinose (RAF).

Ethanol Tolerance: The yeast isolates were screened for its efficiency in ethanol; the tolerance of each isolate was studied by allowing the yeast to grow in liquid YEPG medium as described by Osho *et al*. (2010). The prepared medium was dispensed into (150ml) Erlenmeyer flasks, and sterilized by autoclaving at 121°C for 15 minutes. The medium in the flasks were allowed to cool to a temperature of about 45°C and absolute ethanol was added in varying percentage concentrations of 6%, 8%, 10%, 12%, 14%, 16%.

Acid Hydrolysis of Lignocellulosic Substrates: Adopting the method of Dawson (2007), the experimental procedure for each of the substrate was carried out in triplicates. Five grams of each samples was separately mixed with 100ml of 1M H_2SO_4 in 250ml Erlenmeyer flasks and were left to soak for 24 hours. The samples were then autoclaved at 121°C for 15 minutes.

Determination of Ethanol-Producing Ability of Yeast Isolates: Quantitative estimation of ethanol produced from the samples was determined by the method of Salehi *et al.* (2013). The yeast strains were tested for their ethanol production efficiency in 250ml Erlenmeyer flasks which contained the hydrolyzed substrate and 50ml of YEPG broth. A loopful of each of the 24 hours yeast isolate was aseptically inoculated separately into the flasks and was incubated at room temperature of about 28°C±2 for 72 hrs. The fermented culture media were aseptically poured into centrifuge tubes and were centrifuged at 10000rpm for 10 minutes in a centrifuge machine so as to remove the yeast cells and other solids present. The ethanol content was determined in the supernatant. Optical density (O.D) was measured at 620nm on UV-Visible spectrophotometer. The blank was prepared in the same manner without ethanol. Ethanol production was assayed by comparing with standard ethanol curve.

Distillation and Quantification of the Distilled Ethanol: The fermented samples were collected and were distilled using a laboratory fractional distillation unit. The sample was separately poured into the distillation flask and heated until boiling point temperature of ethanol (78.5°C) was reached on the thermometer attached to the setup. The distillate (ethanol) was collected in the receiving chamber by condensation process, then the volume of distillate collected for each sample was measured and stored in a screw cap container and they were labeled appropriately. The quantity of ethanol produced in g/L was calculated by multiplying the volume of the distillate collected at 78°C by the density (0.8033g/ml) of ethanol and ethanol content recovered after distillation was assayed (Nzelibe and Okafoagu, 2007).

Ethanol Analysis by Fourier Transform Infrared Spectrometer (FTIR): FTIR was carried out for detection of changes in functional groups that occurred in distillate (ethanol) produced from lignocellulosic substrates. The FTIR spectra were analyzed and recorded based on their absorption band mode of

infrared wave lengths to detect the functional groups of the distillates using (Shimadzu FTIR-8400S) of the National Research Institute for Chemical Technology, Zaria. The spectra identified were numbers. The wave numbers were saved and printed (Geethu *et al.,* 2014).

Ethanol Analysis by Gas Chromatography-Mass Spectrometry (GC-MS): The components present in the distilled ethanol were further estimated using GC-MS analytical technique. The analysis was performed using GC-MS Shimadzu 8400S system of the National Research Institute for Chemical Technology Laboratory Zaria. One microliter (1µ) of the sample was transferred to auto sampler vial and was taken into the analyzer to run the sample. Thereafter a library search was conducted to ascertain the possible functional groups present (Geethu *et al.,* 2014).

Statistical Analysis: Data were analyzed using descriptive statistics, one way Analysis of variance (ANOVA) at 95% probability level of Significance. Duncan's Multiple Range Test was used to compare the different means (groups).

RESULTS AND DISCUSSION

Proximate Composition and Total Carbohydrate Content of the Lignocellulosic Biomass: Proximate percentage values of dry matter (DM) in substrates are recorded as 96.60%, 96.12%, 95.86%, 95.09%, 93.73%, and 92.76% (Fig. 1). There is no substantial variation of dry matter among the substrates, the highest been 96.60 (rice bran) while the least is 92.76 (corn bran). Also the ash content in the substrates recorded as follows: 17.72%, 5.62%, 3.53%, 1.63% and 1.07%. Substrates of white saw dust and sorghum bran have the same percentage values of 1.63. The content of ash recorded is 17.72% for Rice bran and it showed the highest. The crude fibre (CF) content of the substrates show the percentage values of 75.56%, 68 39%, 63.29%, 28.37%, 5.17%, and 1.87%. The highest percentage value of 75.56% was found in white saw dust. Likewise, crude protein (CP) has percentage values ranged from 1.94% to 12.19%. The highest percentage value of CP is found in corn bran. The results of the lignocelluloses biomass of white saw dust, red saw dust, black saw dust, rice bran, corn bran and sorghum bran revealed cellulose components as 77.78%, 75.55%, 68.59%, 64.83%, 54.82% and 55.14% respectively; hemicellulose as 6.80%, 4.03%, 6.70%, 8.62%, 9.04% and 1.34% respectively; and lignin constituted 1.80%, 2.47%, 2.39%, 1.45%, 2.12% and 0.85% respectively (Fig. 2). This study revealed the carbohydrate content of 77.6%, 65.4%. 54.0%, 51.8%, 43.3% and 35.7% for red sawdust, black sawdust, corn bran, white sawdust, rice bran and sorghum bran respectively (Fig. 3).

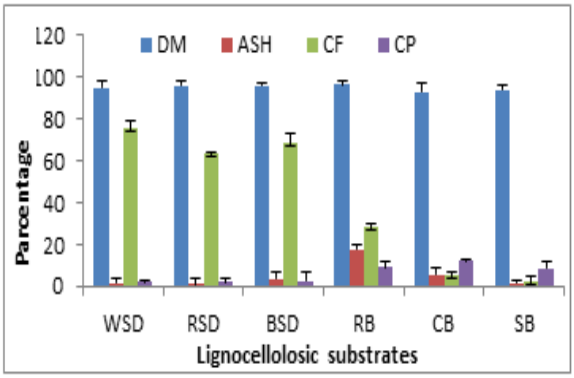

Fig.1. Proximate composition of lignocellulosic substrates. WSD: White saw dust; DM: Dry Matter, RSD: Red saw dust, ASH: Ash content, BSD: Black saw dust, CF: Crude fiber, RB: Rice bran, CP: Crude Protein, CB: Corn bran, SB: Sorghum bran

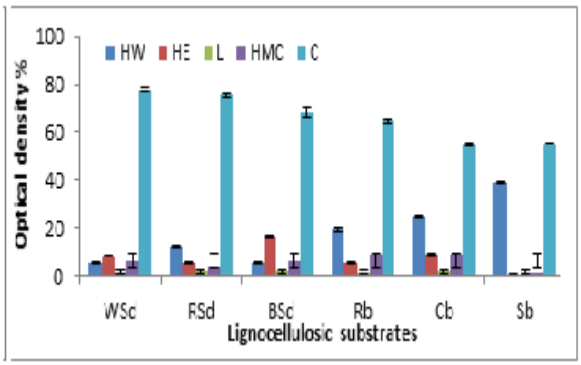

Fig. 2 Chemical Composition of lignocelluloses. WSd: White saw dust, HW: Hot water fractions, RSd: Red saw dust, HE: Hot ethanol fractions, BSd: Black saw dust, L: Lignin fractions, Rb: Rice bran, HMC: Hemicellulose fractions, Cb: Corn bran, C: Cellulose fractions, Sb: Sorghum bran

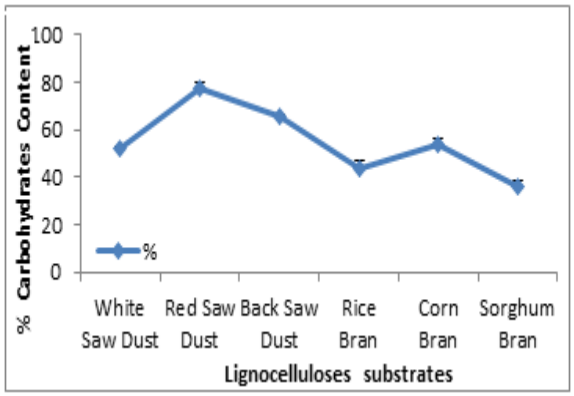

Fig. 3: Percentage total carbohydrate contents of the lignocellulosic biomass

Ash which is a constituent of the lignocellulosic biomasses is an impurity that will not burn. For this reason, biomasses with low ash content are better suited for pyrolysis than biomasses with high ash content. The low values of the ash content generated in this study are in line with the findings of Schild *et al*. (2010) and Salehi *et al*. (2013). According to Kim

et al. (2001), the combustion value and efficacy of biofuel depend on the low ash content. Combustion is another way of converting organic matters with oxygen to produce primarily carbon dioxide and water. The ash content of any biomass has a significant influence on the heat transfer to the surface of the biomass as well as the diffusion of the oxygen to the biomass surface during combustion (Kim *et al*. 2001; Mitchual *et al*. 2014). The crude protein and fibre contents in all the residues suggest that, this could be mainly attributed to hydroxyl proline-rich glycoprotein. As reported by Bartolome and Ruperez, (1995) and Smith *et al*. (1995), the glycoproteins in the shell of fruits are immersed in the primary cell wall forming a network of microfibrils with the cellulose. The results of the substrates component in terms of cellulose, hemicellulose and lignin are similar to the findings of Hu (2006), Shulga *et al*. (2007), Song *et al*. (2012) and Amiri *et al*. (2014). However variations in the biomass compositions may be attributed to different agronomic and cultural practices adopted for growth of the plants and different method employed for the analysis.

Identification of Yeast Isolates with API 20C AUX Strip: A total of 25 yeast isolates were recovered from the YEPG medium culture of fermented beverages and were identified with high degree of certainty. Using the API 20C AUX system strips (bio Merieux, France) for the identification of yeast isolates, sixteen isolates were identified (Table 1).

Table 1 Biochemical characterization of microbial isolates from Kunu zaki, brukutu and palm wine juice using strip of API 20C AUX database catalogue.

GLU	GLY	2KG	ARA	XYL	ADO	XLT	GAL	INO	SOR	MDG	NAG	CEL	LAC	MAL	SAC	TRE	MLZ	RAF	Hyphae/ Pseudo-Hyphae	API Profile Coding	IDENTITY
+	+	-	-	-	-	-	+	-	-	-	-	-	-	+	-	V	V	+	+	60400	*Saccharomyces cerevisiae 1*
+	-	-	-	-	-	-	+	V	+	-	-	-	-	+	+	-	-	-	+	2042034	*Saccharomyces cerevisiae 2*
+	+	V	V	V	V	-	-	V	-	-	V	-	-	-	+	+	-	-	+	6000064	*Sporobolomyces strain*
+	+	-	-	+	-	-	V	-	+	-	V	-	V	-	-	-	-	-	+	6442004	*Geotrichum klebahniidum*
+	+	+	V	+	-	V	+	-	+	+	+	+	+	+	+	+	+	V	+	6556775	*Candida famata*
+	+	+	V	+	-	-	-	-	-	-	+	-	-	-	+	+	-	-	+	6500164	*Rhodotorula minuta*
+	+	V	V	V	-	V	+	V	V	+	+	V	V	+	+	+	+	+	+	6044177	*Saccharomyces cerevisiae 3*
+	+	+	V	+	V	V	+	V	-	+	+	+	+	+	+	+	+	+	+	6544777	*Trichosporon mucoides*
+	+	+	V	+	+	V	+	V	+	+	+	V	V	+	+	+	+	V	+	6556175	*Candida parapsilosis*
+	V	+	+	+	V	+	+	V	+	+	+	+	+	+	+	+	+	+	+	2766777	*Cryptococcus laurentii*
+	+	-	-	-	-	-	V	-	+	+	V	V	V	+	-	+	+	V	+	6006055	*Candida pelliculosa*
+	+	+	V	+	+	V	+	+	V	+	+	+	V	+	+	+	+	V	+	6556375	*Candida Lusitaniae 1*
+	V	+	V	V	V	V	+	V	V	V	V	V	V	V	+	+	V	+	+	2140066	*Candida colliculosa*
+	+	V	V	+	-	-	+	V	+	V	V	-	-	-	-	-	V	V	+	6442004	*Geotrichum candidum*
+	+	+	V	+	+	V	V	V	V	V	+	-	-	+	+	+	+	V	+	6510175	*Candida Lusitaniae 2*
+	+	+	V	+	+	-	V	V	V	V	+	-	-	+	+	+	+	V	+	6510175	*Candida Lusitaniae 3*

(+ = Carbon assimilated (turbid): - = Carbon not assimilated (non-turbid); V (Variation) = +/-) , GLU = D- glucose; MDG = methyl-αD – glucopyranosides; GLY = Glycerol; NAG = N – acetyl-glucosamine; 2KG = 2-keto-gluconate; CEL = D- cellobiose; ARA = L-arabinose; MAL = D-Maltose; XYL = D-xylose; SAC = D-Saccharose (sucrose); ADO =Adonitol; TRE = D-Trehalose; XLT = Xylitol; MLZ = D- Melezitose; GAL = D – Galactose; RAF = D – Raffinose; INO = Inositol; LAC = D-Lactose; SOR = D-Sorbitol.

Ethanol Tolerance of the Yeast Isolates: The ethanol tolerance of the yeast isolates showed ranges from 6% to 14% (Table 2). The yeast Isolates K2, B5, B7 and P1 had the highest tolerance percentage value of 14% while the least tolerance percentage value of 0.6% were recorded for B8, P3, and P4 respectively. As concentration of ethanol increases in media, a reduction in growth is generally observed. Ethanol tolerance of isolates is taken at the very concentration of ethanol after which there was a sharp decrease in growth. Ethanol tolerance of yeast is important in fermentation because high production of ethanol requires yeast that can withstand high concentrations of ethanol. During production, this ethanol is known as an inhibitor of microbial growth. It damages mitochondrial DNA in yeast cells and causes inactivation of some enzymes, such as hexokinase and dehydrogenase (Ibeas and Jimenez, 1997). This is because plasma membranes of the unicellular organisms are in direct contact with the surrounding culture medium. It is likely that its characteristics will influence tolerance of cells to all kinds of changes occurring during fermentation (Herrera, 2001). Ethanol tolerance of seven yeast strains isolated from fruits by Tikka *et al*. (2013) reported maximum tolerance of 12% by one of the yeasts YDE. Also, this result is in agreement with the findings of Ergun and Ferda (2000), who reported to have obtained two yeast strains that tolerated 14% ethanol. The baker yeast was able to tolerate maximum of 12% ethanol, and beyond this concentration growth was decreased exponentially as shown by drops in optical density values. K1, K3, K5, K6, K9, B3 and P6 isolates tolerated ethanol concentration up to 10%. K4, K7, B4, P2, P5 and P7 isolates tolerated ethanol concentrations up to 8%.

Ethanol-Producing Ability of the Yeast Isolates: The results showed that the ethanol-producing ability of the yeast isolates ranged from 4.1% to 10.3% (Table 2). The highest percentage was observed in P1 and the least ethanol-producing percentage value of 4.1% was observed in K3. Ethanol production and recovery does not only depend on the substrates used but also depends on the efficiency of yeast strains to convert the reducing sugars to ethanol. According to Kurtzman (1998), all ethanol contained in alcoholic beverages is produced by means of fermentation induced by yeast. The diversity of yeast in indigenous fermented beverages utilized for the research showed that most traditional fermentation employ the whole range of natural microflora that could function under the varied environmental and non-sterile conditions presented by the different processes.

Table 2: Ethanol tolerance and Ethanol Production of Yeast levels of the identified yeast isolates

Yeast Isolates	Ethanol Tolerance (%) and ± S.D	Ethanol Production (%) and ± S.D
K1	10^{c} ± 0.1000	5.7^{i} ± 0.1789
K2	14^{a} ± 0.1340	6.0^{h} ± 0.2908
K3	10^{c} ± 0.1000	4.1 ± 0.3650
K4	08^{d} ± 0.2165	4.2 ± 0.2000
K5	10^{c} ± 0.1000	5.1 ± 0.1750
K6	10^{c} ± 0.1670	5.4^{j} ± 0.2800
K7	08^{d} ± 0.2500	7.4^{e} ± 0.2500
K8	12^{b} ± 0.3000	6.9^{g} ± 0.1000
K9	10^{c} ± 0.1000	6.2^{g} ± 0.3590
B1	12^{b} ± 0.3000	7.7^{d} ± 0.2001
B2	12^{b} ± 0.2570	6.2^{g} ± 0.1399
B3	10^{c} ± 0.1092	4.2 ± 0.2006
B4	08^{d} ± 0.3578	5.2 ± 0.3555
B5	14^{a} ± 0.1330	5.0 ± 0.2567
B6	12^{b} ± 0.1333	5.2 ± 0.3333
B7	14^{a} ± 0.2560	5.5^{j} ± 0.1000
B8	06 ± 0.1080	5.0 ± 0.3560
P1	14^{a} ± 0.3600	10.3^{a} ± 0.2890
P2	08^{d} ± 0.2350	8.5^{b} ± 0.3678
P3	06 ± 0.1950	8.2^{c} ± 0.2680
P4	06 ± 0.1007	7.5^{e} ± 0.9280
P5	08^{d} ± 0.2560	7.1^{f} ± 0.3560
P6	10^{c} ± 0.1006	4.2 ± 0.7779
P7	08^{d} ± 0.2580	4.4 ± 0.8260
P8	12^{b} ± 0.1592	6.0^{h} ± 0.7520
Baker Yeast	12^{b} ± 0.2560	6.2^{g} ± 0.5560
SE ±	0.297	6.258

Means of the same column having different superscript are significantly different (P<0.05) according to the Duncan Multiple Range Test.

The result of ethanol production is similar to the findings of Gupta *et al*. (2009) who reported less than 12.5% ethanol produced by *Saccharomyces cerevisiae*. Similarly, this agrees with the findings of Patil and Patil (2006) who reported 8.33% by *S. ellipsoideus* 101. The yeast isolates are found to have utilized some amounts of sugar during the batch fermentation period (72 hours) but not all isolates are efficient in ethanol production. Three yeast isolates are efficient ethanol producers: P1, P2 and P3. This finding proved that *Saccharomyces cerevisiae* is more efficient for ethanol production compared to other species (Ergun and Ferda, 2000). This is due to the fact that some species adopt different metabolic pathways by having special genes or enzymes such as invertase genes and invertase enzymes respectively for the conversion of sugars to ethanol or other metabolites (Fregonesi *et al.,* 2007). The average ethanol yield during substrate fermentation is very similar to that of Ezeogu and Emeruwa (1993) who reported 12.2% yield of ethanol for Sake-type fermentation using Nigerian palm wine *Saccharomyces cerevisiae*. However, the ethanol contents produced were also similar as compared to those produced by industrial yeast strains in Japan, in which the ethanol content was reported to be 17.0% to 19.0% as reported by Yoshizawa and Kishi (1994). Differences in the ethanol levels may be due to the variability in fermentative capacities of yeast strains.

Fourier Transform Infrared Spectrophotometer (FTIR) and Gas Chromatography and Mass Spectrometry (GC-MS) Analyses: The FTIR showed the presence of peaks range between 1697cm^{-1} and 1512cm^{-1}, 1813cm^{-1} and 1797cm^{-1}, 3286cm^{-1} and 2985cm^{-1}, 3456cm^{-1} and 3371cm^{-1} which suggests the presence of C=N, N-H bend, C=O stretch, R-CO-NH_2, O-H stretch, C-H stretch, H-C=O stretch, and R-C=C-H. The results suggest that all the functional groups present are majorly alcohol (O-H) at the absorption range. GC-MS revealed that the most common compounds detected after the fermentation by yeasts are as follows: 3-floro-B, 5-dihydroxy-N-methyl-benzeneethanamine, (R)-(-)-2-Amino-1-propanol. Other compounds detected are Methylhydrazine, 2-Aziridinylethylamine, Topotecan, 4-[2-(Methylaminol) ethyl-1,2-benzendiol and 2-Fonnylhistamine. Hydroxyl-urea was only detected in sorghum bran. Ethanol was majorly detected in all the tested lignocellulosic substrates. Ethanol is one of many kinds of alcohol. It is also known as ethyl alcohol and can be distinguished based on their molecular structure. The result of FTIR spectroscopic and GC-MS analyses are similar to the findings of Pankajkumar *et al.* (2014) who investigated structural changes in waste lignocellulosic material. According to Geethu *et al*. (2004), FTIR spectrum is able to predict the phytoconstituents in *Calotropis gigantea* produced after dye bioremediation under solid state fermentation.

*Conclusion***:** The study revealed that the lignocellulosic composition differed from one substrate to another. It is observed from this study that yeasts from fermented beverages that can be utilized

for bioethanol production, and that *Saccharomyces cerevisiae* isolated from palm wine is best in ethanol tolerance and ethanol production. Lignocellulosic biomasses are recommended as raw materials for producing ethanol, a promising alternative energy source as against the depleting crude oil. This study also recommends the application of biological engineering for increased ethanol production.

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