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Sustainable Production of Bioethanol by *Zymomonas mobilis* and *Saccharomyces cerevisiae* using Rice Husk and Groundnut Shell as Substrates

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Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

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ABSTRACT

Background of Study: Plant waste such as rice husk and groundnut shell are generated in large amounts, these waste presents a tremendous pollution to the environment. Worldwide, these wastes are often simply dumped into landfills and oceans or used as animal feeds. The recovery of food processing wastes as renewable energy sources represents a sustainable option for the substitution of fossil energy in order to minimize environmental damages and to meet energy demands of the growing population.
Aim: To produce bioethanol from rice husk and groundnut shell using local strains of *Zymomonas mobilis* and *Saccharomyces cerevisiae*.
Place and Duration of Study: Conducted at the Microbiology Laboratory of Abubakar Tafawa Balewa University Bauchi, Bauchi state, Nigeria, between April to June, 2021.
Methods: Groundnut shell and Rice husk were collected from local milling center. The wastes were

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powdered, sieved and used as carbon source. Proximate composition of the subsrate was done and the total carbohydrate was determined by difference. The sum of the percentage moisture, ash, crude lipid, crude protein and crude fibre was subtracted from 100. *Zymomonas mobilis* and *Saccharomyces cerevisiae* were isolated from rotten sweet oranges and locally fermented beverage ('kunun-zaki') respectively by growing them on Malt Yeast Peptone Glucose Agar (MYPGA) after which they were further screened for their ability to tolerate ethanol and they serve as organisms for fermentation. The enzyme α- amylase was used for hydrolysis. The fermented substrates were distilled at 78oC and the distillate was collected as bioethanol in a conical flask. UV-VIS spectrophotometer was used to determine the absorbance of each concentration (0, 0.2, 0.4, 0.6 and 0.8cm3) of reducing sugar content of the hydrolysates and the bioethanol produced by developing a standard curve at a wavelength of 491nm and 588nm respectively. The concentration of reducing sugar and bioethanol was determined using a reference line from the Standard curve.

Results: Proximate analysis done shows that rice husk have 70.09% carbohydrates while groundnut shell has 65.09% carbohydrates. Groundnut shell yielded the highest reducing sugar of 5.096%. Rice husk yielded the lowest quantity of reducing sugar with a total yield of 2.962%. Maximum concentration of bioethanol of 0.971% was produced from the combination of *Saccharomyces cerevisiae* and *Zymomonas mobilis* from groundnut shell. The lowest concentration of 0.121% of bioethanol was produced when *Saccharomyces cerevisiae* was used on rice husk hydrolysates. The synergistic relationship of *Saccharomyces cerevisiae* and *Zymomonas mobilis* yielded the maximum bioethanol when compared with the yield obtained when the organisms were used singly. *Zymomonas mobilis* produced highest bioethanol content when the organisms are used single.

Conclusion: This study demonstrates the potentiality of local strains of *Saccharomyces cerevisiae* and *Zymomonas mobilis* isolated from rotten sweet orange and locally fermented beverage ('kunun-zaki') to produce bioethanol by fermenting the rice husk and groundnut shell hydrolysates.

Keywords: Bioethanol; groundnut shell; rice husk; sweet oranges; Saccharomyces cerevisiae and Zymomonas mobilis.

1. NTRODUCTION

Scientific developments have presented mankind with different ways to utilize resources to improve the quality of life. A development is 'sustainable' if it "meets the needs of the present without compromising the ability of the future generations to satisfy their own needs" [1]. Preference of unsustainable alternatives, along with the ever-rising world population has resulted in depletion of resources. The world population reached 7.3 billion in 2015, and projected to increase by 33% to reach 9.7 billion in 2050, and by 53% to cross 11.2 billion in 2100 [2]. In the current time, the importance of alternative energy sources has become even more necessary not only due to the continuous depletion of limited fossil fuel stocks but also for safe and better environment [3]. To meet the energy demand of such a growing population has been earmarked as one of the major challenges facing humanity [4]. Biofuels (Bioethanol, Biodiesel, and Biogas) are fuels produced from biomass (a biodegradable material) for heating, electricity generation and transport purposes etc [5]. Biofuel synthesis is an acceptable techniques for producing valuable products through biological process, using microorganisms as the biocatalysts, bacteria and yeast are the most promising group capable of fermenting difference substrates for high yield bioethanol production under laboratory condition.

Bioethanol can be produced from any biological feedstock's that contains appreciable amount of sugar/carbohydrate or materials that can be converted into sugar such as starch or cellulose. Bioethanol from renewable resources has been of interest in recent decades as an alternative fuel to the current fossil fuels. Lignocelluloses biomass like wood and agricultural crops residue, e.g., straw and sugar beet pulp are potential raw materials for producing several high-value products like fuel ethanol and biodiesel [6]. Steps involved in bioethanol production include pre-treatment, hydrolysis, fermentation and distillation [7,8]. Different feedstocks across the world are being investigated, including crops such as rice and sugar beets. The current production of bioethanol is, however, not enough to replace a substantial part of the one trillion gallons of fossil-based fuel consumed globally each year [9]. For a large production of bioethanol; it is convenient to use cheaper and abundant substrates always. So by using waste

products from forestry, agriculture and industries, the cost of feedstocks may be reduced; if we consider producing ethanol from feedstocks such as maize, sugarcane, sweet potatoes, rice pulps etc; which constitutes a larger percentage of the production cost [10]. For the reduction of food competition, it is necessary to use lignocelluloses which is considered as an alternative and attractive feedstock for the production of ethanol due to its availability in large quantities and affordability [11].

Many microorganisms are being developed for biofuel production, but all have certain limitations as economical production strains, such as industrial robustness, substrate utilization, productivity and yield. Yeast strains are among the current leading industrial biocatalyst microorganisms for fuel production [12]. The yeast, *Saccharomyces cerevisiae* is widely used in ethanol fermentation industry owing to its efficient conversion of sugars to ethanol. It can tolerate wide range of pH, with acidic pH as optimum, which protects contamination. It can also tolerate ethanol better than other ethanol producers. It is also GRAS (generally regarded as safe) for human consumption [13]. *Zymomonas mobilis* is a natural ethanol producer and has many desirable industrial biocatalyst characteristics, such as high specific productivity, high alcohol tolerance, a broad pH range for production (pH 3.5–7.5), and the generally regarded as safe status [14,15,16,17,3,18].

Plant waste such as rice husk and groundnut shell are generated in large amounts, these waste presents a tremendous pollution to the environment. Worldwide, these wastes are often simply dumped into landfills and oceans or used as animal feeds. The recovery of food processing wastes as renewable energy sources represents a sustainable option for the substitution of fossil energy in order to decrease expected environmental damages and to meet energy demands of the growing population.

2. MATERIALS AND METHODS

2.1 Collection and Processing of Samples

The agrowastes (groundnut shell and rice husk) were collected from local milling centers in Yelwa area of Bauchi metropolis of Bauchi state. Twenty grams of each agro-waste sample was collected in clean polythene bags and transported immediately to the laboratory. The wastes were powdered using pestle and mortar, sieved with a mesh size of 0.5mm and used as carbon source. Samples of rotten oranges and locally fermented beverage (*kunu-zaki*) were collected from minor refuse dumps at the Muda Lawan orange market of Bauchi metropolis for the isolation of *Zymomonas mobilis* and *Saccharomyces cerevisiae*. The rotten orange samples were placed in clean sterile polythene bags and locally fermented beverage collected in sterile container and transported immediately to the laboratory for analysis, as described by reference [19].

2.2 Isolation and Characterization of *Zymomonas mobilis*

The isolation of *Zymomonas mobilis* was carried out in accordance to the method described by Obire [20]. Five rotten sweet oranges (*Citrus sinensis*) samples coded CS-1, CS-2, CS-3, CS-4 and CS-5 were washed and then squeezed to obtain the juice separately in a sterile container. The juice was serially diluted from tube 1 (101) to tube 5 (105). Then 0.1 ml aliquot from each of the 105 tubes was plated onto the MYPGA (malt yeast peptone glucose agar) medium using spread plate techniques. Each medium was treated with actidione (cycloheximide) to inhibit yeast growth. The plates were incubated in an anaerobic jar in which Gas pack sachet was placed to exhaust the oxygen in the jar and incubated at 37^{o}C for 2 days. Colonies suspected to be those of *Zymomonas* were characterized on the basis of their cultural and morphological characteristics. The isolates were purified by streaking on freshly prepared media and incubated for 2 days at 37 ^{o}C in an anaerobic jar. The ability of *Zymomonas mobilis* to ferment various carbohydrates using glucose, fructose, sucrose, maltose, lactose and arabinose was determined by growing the isolates in liquid standard medium (Yeast glucose broth pH 6.8) containing 1 % (w/v) of the particular carbohydrate. Durham tubes were inverted into culture tubes for gas collection. The tubes will be incubated at 37^{o}C for 24 hours. Uninoculated broths will be used as control.

2.3 Isolation and Characterization of *Saccharomyces cerevisiae*

The isolation of *Saccharomyces cerevisiae* was carried out in accordance to the method described by Brooks [21]. *Saccharomyces cerevisiae* that was used in the research was

isolated from a local fermented beverage ("kunu-zaki") from five different sellers and were allowed to ferment for 2 days. The locally fermented beverage were coded KZ-1, KZ-2, KZ-3, KZ-4 and KZ-5 respectively in a sterile container. Aliquot of 0.1ml of 10-5 serial dilution of each of the locally fermented beverage was spread on the surface of a solidified Malt Yeast Peptone Glucose (MYPG) agar plate and was incubated for 48h at 30^{o}C. Colonies suspected to be *Saccharomyces cerevisiae* based on their colonial characteristics were sub-cultured on sterile MYPG slants. The ability of isolates to ferment glucose, fructose, sucrose, maltose, lactose, mannitol, galactose and arabinose was also tested.

2.4 Preparation of Standard Inoculum

This was carried out in accordance to the method described by [22]. A loopful of cells of *Zymomonas mobilis* and *Saccharomyces cerevisiae* was respectively inoculated into 100ml of standard broth medium and malt extract broth respectively. The broth containing *Zymomonas mobilis* was incubated at ambient temperature for 2 days in anaerobic gas jar while broth that contained Saccharomyces cerevisiae was incubated for 4 days. At the end of appropriate incubation period, cells were harvested by centrifugation at 4000rpm for 30 minutes using 800D centrifuge. Harvested cells were re-suspended in 100ml sterile physiological saline and respective total viable counts were performed. During this process the cultures were subjected to ten-fold serial dilution up to dilution factor of 10-8. An amount (0.1ml) was inoculated by pour plate technique into appropriate media and incubated appropriately. The dilution that produced 100 – 200 colonies were chosen and served as standard inoculum for preliminary screening for ethanol tolerance.

2.5 Screening of *Zymomonas mobilis* and *Saccharomyces cerevisiae* Isolates for Ethanol Tolerance

The method of Obire [20] was adopted for the determination of tolerance to ethanol by the test isolates. Ethanol concentrations of 1, 5, 10 and 20 (%v/v) were prepared using sterile distilled water. One milliliter of each standardized inoculum was aseptically introduced into nine milliliters of various ethanol concentration contained in test tubes. Incubation followed at ambient temperature and anaerobically for both *Zymomonas mobilis* and *Saccharomyces cerevisiae*. Controls contained the appropriate test organism and distilled water only. At the end of 24h incubation duration, 0.1ml were aseptically withdrawn and plated onto appropriate freshly prepared agar medium using the pour plate technique [23]. Incubation under appropriate cultural conditions as described previously for *Zymomonas mobilis* and *Saccharomyces cerevisiae,* followed immediately. At the end of which colony counts were performed and percent log survival determined by the method of Williamson *et al.* [24] and Log survival greater or equal to 70% were regarded as tolerant.

$$\% \text{ log survival} = (\log C / \log c) \times 100$$

Where, C = count in each ethanol concentration
c = count in control.

2.6 Proximate Analysis of Substrate

Proximate composition is the term usually used in the field of feed/food and means the 6 components of moisture, crude protein, ether extract, crude fiber, crude ash and nitrogen free extracts, which are expressed as the content (%) in the feed, respectively. The measured values of these 6 components in feed are important factors to understand the nature and the properties of the subject feed. The method described by Rabah [25] was used to determine the proximate composition of the substrate.

2.6.1 Determination of moisture content

The method described by Rabah [25] was adopted, a clean crucible was dried to a constant weight in air oven at 110°C, cooled in a desiccator and Weighed (W1). Two grams of finely ground sample was accurately weighed into the previously labeled crucible and reweighed (W2). The crucible containing the sample was dried in an oven to constant Weight (W3). The percentage moisture content was calculated thus:

$$\% \, Moisture \; content = \frac{W2 - W3 \times 100}{W2 - W1} \text{ [25]}$$

2.6.2 Determination of ash content

The method described by Rabah [25] was used. The porcelain crucible was dried in an oven at 100°C for 10 min, cooled in a desiccator and Weighed (W1). Two grams of the finely ground

sample was placed into a previously weighed porcelain crucible and reweighed (W2), it was first ignited and then transferred into a furnace which was set at 550°C. The sample was left in the furnace for eight hours to ensure proper ashing. The crucible containing the ash was then removed; cooled in a desiccator and Weighed (W3). The percentage ash content was calculated as follows:

$$\%\ Ash\ Content\ = \frac{W3 - W1 \times 100}{W2 - W1}$$ [25]

2.6.3 Determination of crude lipid content by soxhlet method

The method described by Rabah [25] was adopted. A clean, dried 500 cm^3 round bottom flask containing few anti-bumping granules was Weighed (W1) with 300 cm^3 petroleum ether (40-60°C) for extraction poured into the flask filled with soxhlet extraction unit. The extractor thimble weighing twenty grams was fixed into the Soxhlet unit. The round bottom flask and a condenser were connected to the Soxhlet extractor and cold water circulation was connected/put on. The heating mantle was switched on and the heating rate adjusted until the solvent was refluxing at a steady rate. Extraction was carried out for 6 h. The solvent was recovered and the oil dried in an oven set at 70°C for 1 h. The round bottom flask and oil was then Weighed (W2). The lipid content was calculated thus:

$$\%\ Crude\ Lipid\ content\ = \frac{W2 - W1}{Weight\ of\ Sample} \times\ 100$$ [25]

2.6.4 Determination of crude fibre

The sample (2 g) was weighed into a round bottom flask, 100 cm^3 0.25 M sulphuric acid solution was added and the mixture boiled under reflux for 30 min. The hot solution was quickly filtered under suction. The insoluble matter was washed several times with hot water until it was acid free. It was quantitatively transferred into the flask and 100 cm^3 of hot 0.31 M, Sodium Hydroxide solution was added, the mixture boiled under reflux for 30 min and filtered under suction. The residue was washed with boiling water until it was base free, dried to constant weight in an oven at 100°C, cooled in a desiccator and weighed (C1). The weighed sample (C1) was then incinerated in a muffle furnace at 550°C for 2 h, cooled in a desiccator and reweighed (C2).

Calculation: The loss in weight on incineration = C1-C2

$$\%\ Crude\ fibre\ = \frac{C1 - C2}{Weight\ of\ original\ sample} \times 100$$ [25]

2.6.5 Determination of nitrogen and crude protein

The ground defatted sample (91.5 g) in an ashless filter study was dropped into a 300 cm^3 Kjeldahl flask. The flask was then transferred to the Kjeldahl digestion apparatus. The sample was digested unit a clear green colour was obtained. The digest was cooled and diluted with 100 cm^3 with distilled water.

2.6.5.1 Distillation of the digest

Into 500 cm^3 Kjeldahl flask containing anti-bumping chips and 40 cm^3 of 40% NaOH was slowly added to the flask containing mixture of 50 cm^3 2% boric acid and 3 drops of mixed indicator was used to trap the ammonia being liberated. The conical flask and the Kjeldahl flask were then placed on Kjeldahl distillation apparatus with the tubes inserted into the conical flask, heat was applied to distill out the NH_3 evolved with the distillate collected into the boric acid solution. The distillate was then titrated with 0.1M HCl.

Calculation:

$$\%\ \mathrm{N2}\ = \frac{14\ \times\ \mathrm{M}\ \times\ \mathrm{Vt}\ \times\ \mathrm{V100}}{\mathrm{Weight\ of\ sample\ (mg)}\ \times\ \mathrm{Va}}$$

% Crude Protein = % N_2 (Nitrogen) × 6.35
where, M = Actual Molarity of Acid
V = Titre Value (Volume) of HCl used
Vt = Total volume of diluted digest
Va = Aliquot volume distilled [25]

2.6.5.2 Determination of carbohydrate by (difference)

The total carbohydrate was determined by difference. The sum of the percentage moisture, ash, crude lipid, crude protein and crude fibre was subtracted from 100.

Calculation:

% Total carbohydrate = 100 - (% moisture + % Ash + % fat + % Protein + % Fibre) [25]

2.7 Sample Hydrolysis

Hydrolysis was carried out to recover fermentable sugars used to produce the bioethanol from the pretreated substrate. The powdered rice husk and groundnut shell samples was hydrolyzed using the enzyme α- amylase. Four 500 ml capacity flasks were used for enzymatic hydrolysis of the agrowastes. The Conical fasks were plugged with cotton wool and aluminum foil and then sterilized at 121^{o}C for 30min. The hydrolysis was performed at 50 mM acetate buffer (pH 5.0) at 50^{o}C for 72hrs with continuous stirring in an orbital shaker and hydrolysates recovered using Whatman filter paper No.1. The filtrate was then used for the determination of reducing sugar and fermentation.

2.8 Determination of Reducing Sugar

The amounts of sugar in the hydrolysed samples were determined by the use of the spectrophotometer at the wavelength of 491nm as described by Rabah et al. [19]. Whereby 2cm^3 of the samples collected were each treated with 2cm^3 of DNS solution and heated in a water bath for 15 minutes to develop the reddish brown colouration and thus, equalled with 1cm^3 of 40% rochellet salt solution and allowed to cooled; then, measured at 491nm using the spectrophotometer.

A 0, 0.2, 0.4, 0.6 and 0.8cm^3 from the glucose Standard solution were pipette in a test tube each. To each of the test tubes 2cm^3 of DNS solution was added. The volumes were then diluted by adding 2, 1.8, 1.6, 1.4 and 1.2cm^3 of distilled water respectively and placed in a hot boiling water bath for 10minutes (to develop red brown colour). Furthermore, 1cm^3 of 40% rochellet salt solution was added (to stabilize the colour) and then allowed to cooled at room temperature. The absorbance was measured at 491nm with a UV-VIS spectrophotometer. The concentration of reducing sugar was determined using a reference line from the Standard curve.

2.9 Fermentation

The fermentation of the hydrolysed samples was carried out in accordance with the methods described by Brooks [26] and Oyeleke [27]. Ten milliliters (10 ml) of the rice husks hydrolysates was dispensed into twelve different 100 ml capacity conical flasks. Each conical flask was replicated three times. The flasks were then covered with cotton wool, wrapped in aluminium foil and autoclaved at 121^{o}C for 15 minutes. The tubes were allowed to cool at room temperature and aseptically inoculated with the fermentative organisms. Conical flask A is inoculated with *Saccharomyces cerevisiae*; B inoculated with *Zymomonas mobilis* and C with *Saccharomyces cerevisiae* and *Zymomonas mobilis*. All the flasks were incubated anaerobically at 37^{o}C. The same procedure was repeated for the groundnut shell hydrolysates. The hydrolysates were then distillated according to standard method. The fermented substrates will be transferred into respective flasks and will be fixed to the distillation apparatus. The apparatus will be then heated up to 80^{o}C and the distillate will be collected as ethanol in a conical flask and stored in a closed lid reagent bottle.

2.10 Distillation

The fermented substrates were transferred into respective flasks and fixed to the distillation apparatus. The apparatus was then heated up to 78^{o}C and the distillate was collected as ethanol in a conical flask and stored in a closed lid reagent bottle.

2.11 Determination of Concentration of Bioethanol Produced

Determination of concentration of bioethanol produced was carried out using the method described by Oyeleke and Jibrin [28]. A 0, 0.2, 0.4, 0.6 and 0.8% of alcohol solution was prepared by pipetting 1cm^3 of 1% alcohol Standard solution in a test tube each and 2cm^3 of chromium reagent solution was added. The volumes were then diluted by adding 2, 1.8, 1.6, 1.4 and 1.2cm^3 of distilled water respectively and placed in a hot boiling water bath for 10minutes (to develop leaf green colouration). Water content from the bioethanol was removed by azeotrophic distillation. It involves addition of benzene to the mixture which forms a heterogeneous azeotrophic mixture of water and benzene which when condensed becomes a two-phased liquid. To each of the varying ethanol concentrations 2 mls of chromium reagent was added and allowed to stand for an hour for colour development. The absorbance of each concentration was measured at 588 nm using UV-VIS spectrophotometer and the readings used to develop standard ethanol curve.

Then 5 mls of each bioethanol samples were put in test tubes and treated with 2 mls of the chromium reagent. The mixture was allowed to stand for an hour and the absorbance was measured as for standard curve. The concentration of bioethanol was determined using a reference line from the Standard curve on the graph.

2.12 Statistical Analysis

Statistical Analysis of Data Results obtained were presented in form of tables and were further subjected to descriptive statistical analysis using SPSS.

3. RESULTS AND DISCUSSION

3.1 Physical Characteristics of the Substrates (Rice husk and Groundnut Shell)

The detailed physical features of the plant waste in investigation are presented (Table 1). As such the characteristics includes: the colour appearance (of the waste material as collected from the field before pretreatment); the smell of the waste material for the analysis; the size (of the sample after grinding for the pretreatment) and then the weight of the sample.

Table 1. Physical characteristics of the rice husk and groundnut shell used in this study

Property	Rice Husk (RH)	Groundnut Shells (GS)
Colour	Pure brown	Dirty brown
Smell	Odourless	Oil rich smell
Size (mm)	3-5	3-5
Weight (g)	20	20

The study (Table 1) shows that rice husk is pure brown in colour while groundnut shell has a dirty brown colour. Rice husk is Odourless while groundnut have an oil rich smell. They both have a size of between 3-5mm after grinding (pretreatment) and the amount that was collected for both is 20g.

This study was focused on the production of bioethanol from rice husk and groundnut shell using local isolates from locally fermented beverage ("kunun-zaki") and rotten sweet oranges (*Citrus sinensis*). The findings of the study are presented and interpreted as follows:

3.2 Characteristics of Isolates from Rotten Sweet Oranges (*Citrus sinensis*)

In this study (Table 2) twenty one isolates were isolated from the 5 different samples of rotten sweet oranges. The highest isolates of 6 was gotten from CS-1 while CS-5 have the least isolates of 1.

Five rotten sweet oranges (*Citrus sinensis*) samples coded CS-1, CS-2, CS-3, CS-4 and CS-5 were used for the isolation of the suspected organism. The study (Table 2) shows that CS-1 has the highest number of 6 isolates making 28.6% from the total isolates gotten while CS-5 has the least isolates of 1 making 4.6% of the total isolates. The isolates were further confirmed after biochemical.

Table 2. Distribution of isolates from rotten sweet oranges (*Citrus sinensis*) samples

Sample	Frequency of isolates (n=21)	Percentage (%)
CS-1	06	28.6
CS-2	04	19.0
CS-3	05	23.8
CS-4	05	23.8
CS-5	01	4.6

CS= *Citrus sinensis*

3.3 *Zymomonas mobilis* Isolated from Rotten Sweet Oranges (*Citrus sinensis*) Samples

In this study (Table 3) sixteen isolates were confirmed to be *Zymomonas mobilis*. The highest isolates of 4 was gotten from CS-1 with 25.0% while CS-5 have the least isolates of 1 making 6.3% of the total isolates.

Table 3. Isolates confirmed to be *Zymomonas mobilis* from rotten sweet oranges (*Citrus sinensis*) samples

Sample	Frequency of isolates (n=16)	Percentage (%)
CS-1	04	25.0
CS-2	03	18.8
CS-3	04	25.0
CS-4	04	25.0
CS-5	01	6.3

CS= Citrus sinensis

The study shows that *Zymomonas mobilis* is seen to have brilliant white to cream colour, plumb white with round ends, Gram negative, motile, catalase positive, oxidase and urease negative, produces gas from glucose, fructose, and sucrose. These findings are in agreement with that of reference Obire [20] who reported the isolation of *Zymomonas mobilis* from fresh palm wine saps and Tambuwal et al. [29] also reported the isolation *Zymomonas mobilis* from rotten oranges.

3.4 Characteristics of Isolates from Locally Fermented Beverage ("kunun-zaki")

In this study (Table 4) twenty seven isolates were isolated from the 5 different samples of locally fermented beverage (kunun-zaki). The highest isolates of 7 was gotten from KZ-2 while KZ-3 have the least isolates of 3.

Table 4. Distribution of isolates from locally fermented beverage ("kunun-zaki")

Sample	Frequency of isolates (n=27)	Percentage (%)
KZ-1	05	18.5
KZ-2	07	25.9
KZ-3	03	11.1
KZ-4	06	22.2
KZ-5	06	22.2

KZ= "kunun-zaki"

The locally fermented beverage ("kunun-zaki") were coded KZ-1, KZ-2, KZ-3, KZ-4 and KZ-5 were used for the isolation of the suspected organism. The study (table 4) shows that KZ-2 has the highest number of 7 isolates making 25.9% of the total isolates gotten while KZ-3 have the least isolates of 3 making 11.1% of total isolates. The isolates were further confirmed after biochemical.

3.5 *Saccharomyces cerevisiae* Isolated from Locally Fermeneted Beverage ("Kunun-zaki") Samples

In this study (Table 5) eighteen isolates were confirmed to be *Saccharomyces cerevisiae*. The highest isolates of 4 was gotten from KZ-1 with 22.2% while KZ-3, 4 and 5 have the least isolates of 16.7% each.

Saccharomyces cerevisiae are seen to be smooth creamish, white spherical shape, Gram-positive, non-motile, catalase positive, the organisms was able to ferment glucose, fructose, sucrose, maltose and galactose, producing acid and gas. The result is in agreement with that of Rabah et al*.* [19] who isolated *Saccharomyces cerevisiae* from palm wine [17] who isolated *Saccharomyces cerevisiae* from palm wine juice in sake-type fermentation, Moneke et al. [30] reported the isolation of *Saccharomyces cerevisiae* from orchard soil and Tambuwal *et al.* [29] reported the isolation of *Saccharomyces cerevisiae* from *Hibiscus sadriffa* (Zobo).

Table 5. Isolates confirmed to be *Saccharomyces cerevisiae* from locally fermented beverage ("Kunun-zaki") samples

Sample	Frequency of isolates (n=18)	% Isolates
KZ-1	04	22.2
KZ-2	05	27.8
KZ-3	03	16.7
KZ-4	03	16.7
KZ-5	03	16.7

KZ= "kunun-zaki"

3.6 Ethanol Tolerance of *Zymomonas mobilis* and *Saccharomyces cerevisiae* Isolates

The isolates obtained were screened for tolerance to the toxicity of ethanol at 1%, 5%, 10% and 20% concentrations and results obtained shows that both *Saccharomyces cerevisiae* and *Zymomonas mobilis* were able to tolerate ethanol at various concentrations and results presented (Table 6). In the result of the screening, percentage log survival that ranged from 70-100% was taken as tolerant.

The study shows that both isolates proofs to be ethanol tolerant from 1% to 5% (v/v). Only *Zymomonas mobilis* was tolerant to 10% (v/v) ethanol. This result is in agreement with the findings Akponah [22] who isolated *Saccharomyces cerevisiae* and *Zymomonas mobilis* from raffia palm (*Eleasis guineessi*) sap and further test their tolerance to ethanol. Ethanol tolerance by both *Saccharomyces cerevisiae* and *Zymomonas mobilis* isolates further informed the ability to use them in fermentation.

Table 6. Response of isolates to toxicity of ethanol

Ethanol concentration (% v/wt)				
Isolates	**1**	**5**	**10**	**20**
Z. moblis	+++	+++	+++	++
S. cerevisiae	+++	+++	+	_

+++ = ≥ 70% log survival
++ = 50 – 69% log survival
+ = 30 – 49 % log survival
- = ≤ 30 % log survival

3.7 Proximate Composition of Rice husk and Groundnut Shell

From the result of this research presented (Table 7) which include the 6 components of Moisture, Ash, Lipid, Protein, Fibre and Carbohydrate (%) in the substrate (Rice husk and Groundnut Shell), respectively. The measured values of these 6 components in feed are important factors to understand the nature and the properties of the subject feed.

Table 7. Proximate composition of rice husk and groundnut shell

Parameters	Rice husk (%)	Groundnut shell (%)
Moisture	1.9	2.9
Ash	14.8	15.8
Lipid	4.1	5.1
Protein	5.5	6.5
Fibre	2.8	3.8
Carbohydrate	70.9	65.9

RH = Rice husk, GS = Groundnut shell

The proximate composition of the agro-wastes (rice husk and groundnut shell) with relation to percentage occurrence of Moisture, Ash, Lipid, Protein, Fibre, and Carbohydrate are shown (table 7). Yoswathana et al. [31] stated that bioethanol can be produced from any biological feedstock's that contain appreciable amount of sugar/carbohydrate or materials that can be converted into sugar such as starch and cellulose. From the result obtained rice husk have 70.09% carbohydrate while groundnut shell has 65.9% carbohydrate. High carbohydrate composition above all other components is a proof of the potentiality of the substrate to be used in the production of bioethanol. Proximate composition of any substrate is dependent on factors such as; variety of substrate, soil chemistry, fertilizer used and geographical location of the lignocellulosic material planted.

3.8 Reducing Sugar Content Result

In this study (Table 8), the highest yield of reducing sugar of 5.096% was obtained from groundnut shell, whereas 2.962% was obtained from rice husk.

The highest yield of reducing sugar was obtained from the groundnut shell when compared with that of rice husk. Lignin residues, acids or aldehydes accumulated in the fermentation medium of the rice husk which may inhibit enzymatic hydrolysis of the cellulose in the lignocellulose biomass which makes it to have low reducing sugar.

Table 8. Reducing Sugar Content of hydrolyzed rice husk and groundnut shell

Substrate	Total Sugar contents(%)	*Mean ± SD
Rice husk	2.962	0.246±0.051
Groundnut shell	5.096	0.42±0.073

**Values are mean ± standard deviation of three replications*

3.9 Bioethanol Concentration

The results of the concentration of the bioethanol produced from fermentation of the rice husk and groundnut shell using *Saccharomyces cerevisiae*, *Zymomonas mobilis* and a combination of *Saccharomyces cerevisiae* and *Zymomonas mobilis* is presented (Table 9). The highest concentration of bioethanol of 0.971 % was produced using a mixture of *Saccharomyces cerevisiae* and *Zymomonas mobilis* from groundnut shell, while the lowest concentration of 0.121 % was obtained when *Saccharomyces cerevisiae* was used alone with rice husk. The combination of *Saccharomyces cerevisiae* and *Zymomonas mobilis* yielded the highest ethanol yield as when compared with the yields obtained from the individual organisms.

Saccharomyces cerevisiae and *Zymomonas mobilis* were found to be successful in the production of bioethanol. The synergistic relation of *Saccharomyces cerevisiae* and *Zymomonas mobilis* yielded the highest bioethanol [32]. When the organism was used single *Zymomonas mobilis* yielded the highest bioethanol. The ability of *Zymomonas mobilis* to produce ethanol is due to the fact that it has the ability to degrade sugar

Table 9. Bioethanol produced from the substrate (%)

Substrates	Fermentative organism	Total (%)	*Mean±SD
Rice husk	*Saccharomyces cerevisiae*	0.121	0.010±.016
	Zymomonas mobilis	0.400	0.033±0.25
	S. cerevisiae + Z. mobilis	0.424	0.035±0.16
Groundnut Shell	*Saccharomyces cerevisiae*	0.202	0.017±0.25
	Zymomonas mobilis	0.883	0.074±0.29
	S. cerevisiae + Z. mobilis	0.971	0.081±0.10

*Values are mean ± Standard Deviations of three replications

using the Entner-doudoroff pathway as well as high tolerance ethanol [17]. The unique and dual presence of pyruvate decarboxylase and alcohol dehydrogenase in *Zymomonas mobilis* might have facilitated the rapid conversion of glucose to ethanol.

The highest yield of bioethanol in groundnut shell when compared to rice husk might be as a result of lignin residues, acids or aldehydes accumulated in the fermentation medium in rice husk which might inhibit enzymatic hydrolysis of cellulose in the lignocellulose biomass. Zakapa et al. [31] reported low bioethanol may be as a result of toxic compounds such as lignin residues, acids and aldehydes accumulated in the fermentation medium.

These findings are in agreement with the works of Epstein et al. [33] who reported a bioethanol yield of as low as 0.06% from apple and grape juice and Rabah et al. [19] also reported bioethanol yield as low as 0.105% from groundnut shell.

4. CONCLUSION

The presence of local strains of *Saccharomyces cerevisiae* and *Zymomonas mobilis* during the fermentation periods confirms that they grow in close association with the substrates (rice husk and groundnut shell) and produce extracellular enzymes responsible for fermentation of most legumes, cereals and cereals wastes. Rice husk and groundnut shell wastes can be exploited as cheap carbon sources for industrial production of bioethanol. However, there is the need to optimize the processes for higher yields of both reducing sugar and bioethanol.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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