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Research Article

Utilization of Agrowastes for Bioethanol Production using Separate Hydrolysis and Fermentation

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ABSTRACT
This study investigates the potential of water yam and white yam peels as sustainable substrates for bioethanol production. *Aspergillus niger* and *Saccharomyces cerevisiae* were isolated and identified using microbiological and molecular methods. *Aspergillus niger* was used to carry out enzymatic hydrolysis on the substrates and *Saccharomyces cerevisiae* was subsequently used for fermentation. The physical properties of the bioethanol distillates, including boiling point, density, and specific gravity, were evaluated. Proximate analysis and nutrient content across the substrates were determined. Fermentation was carried out at varying temperatures and pH levels to determine the optimal yield parameters. Gas Chromatography-Mass Spectrometry (GC-MS) and Fourier Transform Infrared Spectroscopy (FTIR) were employed to assess the quality and structural properties of the bioethanol produced. The results showed that water yam peels had higher moisture, fibre, and protein content than white yam peels ($P < 0.05$). The yield from white yams was 4.93% (v/v) compared to 4.43% (v/v) from water yams. The highest reducing sugar and ethanol yields were obtained from white yam peels, yielding a reducing sugar concentration of 1.86 g/L and an ethanol yield of 4.73 ± 0.21% (v/v). In contrast, water yam peels yielded 1.66 g/L of reducing sugar and 4.23 ± 0.31% (v/v) ethanol. These results demonstrate the feasibility of utilizing water yam and white yam peels as locally sourced raw materials for bioethanol production, contributing to biofuel development in regions with abundant yam resources.

Keywords: *Aspergillus niger*; Bioethanol; Fermentation; Proximate Analysis; *Saccharomyces cerevisiae;* Yam Peels

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INTRODUCTION

One of the primary drivers of biofuel production is the growing concern over global climate change (Rial, 2024). Traditional fossil fuels (coal, oil, and natural gas) are significant contributors to greenhouse gas emissions, primarily carbon dioxide (CO_2), which traps heat in the atmosphere and contributes to global warming (Bilgili *et al.,* 2024). Biofuels, derived from organic materials such as plants and algae, can help reduce net CO_2 emissions compared to fossil fuels

(Chougale *et al.,* 2024). This is because biofuels are considered part of the natural carbon cycle, where plants absorb CO_2 as they grow and release it when burned, creating a closed loop with a lower long-term impact (Suryawan and Lim, 2024). These factors have led to a significantly heightened interest in biofuel development as the global demand for sustainable, renewable energy continues to grow (Jolayemi *et al*., 2023). Many countries, especially those that are net importers of petroleum, are vulnerable to the geopolitical risks and price volatility associated with fossil fuel dependence (Puyo *et al.,* 2024). Biofuels provide an opportunity for countries to diversify their energy sources and reduce reliance on fossil fuel (Damian *et al.,* 2024). This is crucial for enhancing national energy security, ensuring a more stable and locally controlled energy supply.

Nigeria produces about 61 million tons of solid waste annually, but only a small portion of this waste is collected (Odejobi *et al.,* 2024). While first-generation biofuels (e.g., ethanol from corn or biodiesel from vegetable oils) have been the focus of early biofuel efforts, second-generation biofuels are made from non-food feedstocks like agricultural residues or municipal solid waste (Chauhan *et al.,* 2024). These fuels promise greater sustainability, as they do not compete with food production. Third-generation biofuels focus on other bio-based sources, which can offer even higher yields and energy efficiency (Padder *et al.,* 2024). Therefore, using agricultural wastes like yam peels and other lignocellulosic feedstocks for bioethanol production is essential for addressing environmental pollution, combating climate change, and creating alternative energy sources.

It is estimated that rice husk and some peels of some root crops yields and average of 7.6% v/v ethanol concentration, although the former was more promising (Odeh *et al.,* 2024). While rice husk offers superior yield and lower energy requirements, other food-based wastes showed profitability, thereby reducing production costs and enhancing sustainability. For example, it was reported that a cassava-based bioethanol plant required a total capital investment (TCI) of approximately $110 million, with an annual operating cost (AOC) of around $88 million. Despite the high TCI, the plant achieved a return on investment (ROI) of 64.41%, indicating economic viability (Sanni *et al.,* 2022).

The most significant species of *Dioscorea* in West Africa is *Dioscorea rotundata*, also known as white yam, guinea yam, or African yam, due to its commercial importance and applications (Odeh *et al.,* 2024). According to Sanni *et al.* (2022), this monocotyledonous perennial herb is grown for its starchy tuber. Two of the main food crops cultivated by small-scale farmers in the humid/sub-humid tropics are *D. rotundata* (white yam) and *D. alata* (water yam), providing a steady source of carbohydrates for the population (Ahmadu *et al.,* 2018). The latter is a staple crop that feeds most of Nigeria's population and is mostly grown in the North-central region. Because of its versatility in processing into various powder forms for both human and animal consumption, it is widely used by Nigerians (Kolo *et al.,* 2023).

Dioscorea alata (water yam) is a significant crop that was introduced to West Africa from Asia in the sixteenth century. Compared to the favoured native species, *D. rotundata*, it has a greater multiplication ratio, higher tuber production, and superior storability, making it potentially more sustainable for increasing wealth and food security in West Africa (Baah *et al.,* 2009). Its flesh, which is often softer than that of other species, is associated with its extended shelf life (Dufie *et al.,* 2013). Due to its high starch content, water yam is processed and used as a flavouring in products such as ice cream, milk, tarts, Swiss rolls, cookies, and other foods. In traditional medicine, water yam has been used to treat tumors, gonorrhoea, fever, and swollen hemorrhoids (Banjo *et al.,* 2019).

This research aims to determine the suitability of white yam *(Dioscorea rotundata*) and water yam (*Dioscorea alata*) peels for bioethanol production using *Aspergillus niger* and *Saccharomyces cerevisiae* as precursors. Conventional methods of bioethanol production were employed mainly due to the availability of resources during the course of this study.

MATERIALS AND METHODS

Sample Collection and Preparation

White yam (*D. rotundata*) and water yam (*D. alata*) were purchased from Kara market Sokoto. The yams were identified and authenticated by the Herbarium unit, Department of Plant Science, Usmanu Danfodiyo University Sokoto and assigned Voucher numbers UDUH/ANS/0952 and UDUH/ANS/0953 to Water yam (*D. alata*) and White yam (*D. rotundata*) respectively. The yam peels were washed under running tap water to remove sand and other impurities and then sundried for two weeks. Pulverization of the peels was carried out using mortar and pestle. The ground biomass was subsequently sieved to obtain a homogenous fine powder, which was then transferred into plastic

containers, the samples were properly labeled and preserved for further use (Baki *et al.,* 2020).

Proximate Analysis

Proximate analysis was used to determine the moisture, ash, fat, crude protein, crude fibre and carbohydrate contents of both water and white yam peels (AOAC, 2010).

Isolation and Identification of Microorganisms

Isolation and Identification of *Aspergillus niger*

Aspergillus niger was identified using both microbiological and molecular methods. A soil sample was collected from the biological garden, Usmanu Danfodiyo University, Sokoto. One gram (1g) of the soil sample was transferred into 10 mL of water to make a suspension. The suspension was serially diluted to 10^{-1}, 10^{-2} and 10^{-3} concentrations. From the third test tube, 0.5 mL soil suspension was inoculated in duplicate plates of Potato Dextrose Agar (PDA). A sterile glass rod was used to spread the suspensions in the entire surface of the PDA plates. Masking tape was used to seal the lid of the petri dish. All the inoculated plates were incubated at room temperature (28℃±2) in the laboratory for 5 days. The number of fungal growths showing blackish colouration were counted and sub-cultured separately onto fresh PDA plates to obtain pure fungal growths (Gwandu *et al.,* 2021). Identification of isolates was done macroscopically and microscopically according to the method described by Mukhtar *et al.* (2019). Macroscopically, colony characteristics such as appearance, change in medium colour and growth rate were observed on the petri dishes. A small portion of fungi from 5-day-old cultures was inoculated aseptically on a clean glass slide using a sterile inoculating loop for microscopic identification. A drop of lactophenol cotton blue was added, the slide was covered with a cover slip and examined using a light microscope at x40 objective lens. The conidia and conidiophore shapes were noted. These features were matched with standards described by Barnett and Hunter (1972).

Isolation and Identification of *Saccharomyces cerevisiae*

The palm wine sample was serially diluted. An Aliquot of 0.1 mL of 10^{-5} serial dilution of the palm wine was spread on the surface of solidified Sabouraud Dextrose Agar (SDA) plate for 48 hours at 30℃. Colonies suspected to be *Saccharomyces cerevisiae* based on their colonial characteristics were subcultured on sterile SDA slants (Danmadami *et al.,* 2017). A smear of the isolate was examined microscopically (x100) after staining with methylene blue dye.

Molecular Identification of Isolates

Fungal DNA Extraction

Fungal isolates were grown on PDA for 7 days at 25ºC. A small lump of mycelia from the pure culture was taken and transferred with a sterile needle into a 1.5 mL Eppendorf tube containing a lysis buffer (400 mM Tris-HCL (pH 8), 60 mM EDTA-pH 8.0, 150 mM NaCl, and 1% sodium dodecyl sulphate). After quickly vortexing the tube to disrupt the mycelia, it was left at room temperature for 10 min. 150 µL of potassium acetate was added into the Eppendorf tube and this was followed by brief vortexing. The tube and contents were finally centrifuged at >13,000 x g for 1 min. The supernatant was transferred to a second 1.5 mL Eppendorf tube and centrifuged again, following the previously described procedure. The resulting supernatant was transferred into a new 1.5 mL Eppendorf tube and an equal volume of isopropyl alcohol was added. The tube was thoroughly mixed by inversion and centrifuged at >13,000 x g for 2 min, and the supernatant was discarded. Following a 300 µL wash in 70% ethanol, the resulting DNA pellet was spun for one minute at 10,000 rpm, and the supernatant was discarded. The DNA pellet was air-dried and dissolved in 50 µL of 1xTris- EDTA (Bechem and Afanga, 2017).

PCR Amplification

The ITS1-2 region of the isolates was amplified using the fungal universal primer pair ITS4, forward: (5′-TCCTCCGCTTATTGATATGS-3′) and ITS5, reverse: (5′-GGAAGTAAAAGTCGTAACAAGG3′). The PCR cocktail was made up of 1.0 µL of DMSO, 1.0 µL of 2.5 mM DNTPs, 1.0 µL of 25 mM $MgCl_2$ (Promega), 0.1µL of Taq polymerase, 1.0µL of 10X PCR buffer, 1.0 µL of each primer (concentration of 5 µM), and 13.4 µL of nuclease-free water, making a total volume of 25 µL. In a thermal cycler (GeneAmp® 9700 PCR System, Applied Biosystems, California, USA), amplifications were carried out using the following protocol: 5 min of initial denaturation at 94°C, 30 s of annealing at 54°C, 45 s of elongation at 72°C, and 7 min of final extension at 72°C (Iyanyi and Ataga, 2020).

Gel Electrophoresis

Gel electrophoresis was used to separate the amplified products on a 1.0% agarose gel that had been stained with ethidium bromide. After dissolving 1 g of Agarose gel powder in 100 mL of 1X Tris Acetate EDTA (TAE), the suspension was boiled in a microwave for 5 min. After allowing the molten agarose to cool at 60°C and stained with 5 µl of ethidium bromide, a fluorescent dye that absorbs

ultraviolet (UV) light and emits visible orange fluorescence. The molten agarose was poured into the casting tray after inserting a comb into its slots. To form the wells, the gel was left to solidify for 20 min. The gel was then transferred to the electrophoresis tank holding 1X TAE after the comb was gently removed. After adding the 100 bp DNA ladder to well 1, samples and the negative control were distributed into the designated wells using 10X blue gel loading dye, which gives the samples colour and density to make it easier to load into wells. The electrophoresis was run for 45 min at 100 v. After completion, the gel was transferred to an ultraviolet trans-illuminator for the visualization of the DNA bands, and a photograph of the gel image was taken using a gel documentation system equipped with a UV-sensitive camera. The mobility of a 100 bp molecular weight ladder, run alongside the experimental samples, was used to estimate the sizes of the PCR products (Mohammad, 2021).

Purification of amplified product

After gel electrophoresis, the PCR products were purified through ethanol precipitation to eliminate any leftover reagents. Specifically, about 40 µL of the amplified product was transferred into a sterile 1.5 mL Eppendorf tube, where 7.6 µL of 3M sodium acetate and 240 µL of 95% ethanol were added. The mixture was thoroughly vortexed and then incubated at -20°C for a minimum of 30 minutes. The samples were subsequently centrifuged at 13,000 × g for 10 minutes at 4°C. Following the removal of the supernatant, the resulting pellet was washed with 150 µL of 70% ethanol, mixed well, and centrifuged again at 7,500 × g for 15 minutes at 4°C. The supernatant was discarded, and the tubes were turned upside down on tissue to air-dry in a fume hood for 10–15 minutes. The pellet was ultimately resuspended in 20 µL of sterile distilled water and kept at -20°C. Purification was verified through 1.5% agarose gel electrophoresis (110V, 1 hour), and DNA quantification was performed using a NanoDrop 2000c spectrophotometer (Thermo Fisher Scientific, USA) (Mohammad, 2021).

Sequencing

Sequencing was done at Inqaba Biotec West Africa Company (Ibadan, Nigeria), and the sequences obtained were blasted on the National Centre for Biotechnology Information (NCBI) database.

Bioethanol Production

Inoculum Preparation

Aspergillus niger inoculum was prepared from a 5-day-old, fully sporulated culture grown on Potato Dextrose Agar. Spores were harvested using sterile distilled water supplemented with 0.1% (v/v) Tween 80, followed by vortexing to ensure homogenization. Using a haemocytometer, the spore concentration was adjusted to 2.5 x 10^6 spores/mL, and 0.5 mL of this suspension was inoculated into flasks containing the substrates (Alabdalall *et al.,* 2023). Separately, a 24-hour-old yeast culture was grown in Yeast extract Peptone Dextrose (YEPD) broth at 30°C and 200 rpm. A 1 mL aliquot of this culture was transferred into 200 mL of fresh YEPD broth for further cultivation. Cells were harvested by centrifugation (3000 rpm, 5 min), and the resulting pellets were washed three times with sterile distilled water. Finally, the yeast suspension was standardized to a 0.5 McFarland standard (approximately 1.5 x 10^8 cells/mL), and 10 mL of this inoculum was added to the hydrolyzed samples (Olawale *et al.,* 2021).

Dilute acid pretreatment

Pretreatment of the yam peels was conducted following the method described by Baki *et al.* (2020). Specifically, 100 g each of pretreated white and water yam peels were weighed into two sets of separate 1dm^3 conical flasks. To each flask, 500 mL of dilute H_2SO_4 was added. The flasks were then sealed with cotton wool and aluminum foil before being autoclaved at 121℃ for 30 minutes. Following sterilization, the mixtures were filtered through Whatman No. 1 filter paper to isolate the solid residues. These residues were washed repeatedly until a neutral pH was achieved, then oven-dried at 100℃ and stored in plastic bags for further use.

Enzymatic Hydrolysis

Enzymatic hydrolysis was conducted according to the methodology described by Tambuwal *et al.,* (2018). For each substrate, white and water yam peels, ten grams (10 g) of pretreated material was distributed into five sets of 250 mL conical flasks. Each experimental set consisted of four flasks: three served as replicates for the treatment group, while the fourth remained uninoculated to serve as a negative control. The substrates were suspended in 100 mL of distilled water, and the flasks were sealed with cotton wool and aluminium foil. Following sterilization via autoclaving at 121°C for 15 minutes, the mixtures were allowed to cool and adjusted to the required volume with sterile distilled water. The flasks were inoculated with 0.5 mL of *Aspergillus niger* suspension, whereas the control flasks remained uninoculated. All samples were incubated at 37°C for 5 days with periodic agitation to ensure a homogeneous solution and uniform microbial distribution. Following incubation, the mixtures were filtered through Whatman No. 1 filter paper, and the

resulting hydrolysate was subsequently used for reducing sugar determination and fermentation.

Determination of Reducing Sugar

The reducing sugar concentration of the hydrolysates was determined using the dinitrosalicylic acid (DNS) method, as described by Miller (1959). One millilitre (1 mL) of the DNS reagent was added to 1 mL of each sample in appropriately labelled test tubes. The mixtures were heated in a boiling water bath for 10 minutes to facilitate the development of a red-brown colour. Subsequently, 1 mL of 40% potassium sodium tartrate (Rochelle salt) was added to stabilize the colour, followed by rapid cooling under running tap water. A blank was prepared using 1 mL of DNS reagent and 1 mL of distilled water. The absorbance (optical density) of the samples was measured against the blank using a UV-VIS spectrophotometer (Shimadzu UV-1650PC) at a wavelength of 540 nm. The concentration (g/L) of reducing sugars was calculated using a glucose standard curve.

Fermentation

The resulting hydrolysates (100 mL) were transferred into sets of 250 mL conical flasks, sealed with cotton wool and aluminium foil, and sterilized by autoclaving at 121°C for 15 minutes. After cooling to room temperature, the pH of the fermentation medium was adjusted to 5.0. Each flask was inoculated with 10 mL of a *Saccharomyces cerevisiae* suspension (1.5×10^8 cells/mL) (Zakpaa *et al.,* 2009). The flasks were then incubated under anaerobic conditions at 35°C for a total of 120 hours. At 24-hour intervals throughout the 5-day fermentation period, representative flasks were removed for analysis. The resulting fermented broth from each flask was subjected to fractional distillation to recover the bioethanol, following the procedure described by Oyeleke and Jibrin (2009).

$$Bioethanol\ yield\ (v/v) = \frac{\text{Volume of bioethanol produced}}{\text{Volume of sample used}} * 100$$

Distillation

The bioethanol was recovered from the fermented broth through fractional distillation, as described by Muhammad *et al.* (2023). The broth was transferred into a round-bottom flask, which was then connected to a distillation apparatus equipped with a Liebig condenser. To facilitate condensation, a continuous flow of cold tap water was maintained through the condenser jacket. A receiving conical flask was positioned at the outlet to collect the distillate. The round-bottom flask was heated using a heating mantle maintained at a constant temperature of 78°C, corresponding to the boiling point of ethanol. The volume of the resulting distillate was quantified using a graduated measuring cylinder.

Optimization of Fermentation Conditions

Effect of Temperature on Ethanol Production

To determine the optimal temperature for bioethanol yield, the fermentation process was conducted at various temperatures: 25, 30, 35, and 40°C. During these experiments, the pH of the substrates was maintained at a constant value of 5.0. Following the completion of the fermentation period, the amount of ethanol produced at each temperature was measured and recorded.

Effect of pH on Ethanol Production

To determine the optimal pH for bioethanol yield, the fermentation process was carried out at various initial pH levels: 4.0, 4.5, 5.0, and 5.5. Throughout these experiments, the incubation temperature was maintained at a constant 35°C. After the fermentation period, the amount of ethanol produced at each pH level was measured and recorded (Banjo *et al.,* 2019).

Physicochemical Characterization of Produced Bioethanol

Determination of Density and Specific Gravity

The density and specific gravity of the produced bioethanol were determined according to the method described by Bashir *et al*. (2022) using a pycnometer (specific gravity bottle). First, the mass of the empty, dry pycnometer was recorded (M_0). The bottle was then filled with the bioethanol sample, and any excess liquid was carefully removed. The mass of the filled pycnometer (M_1) was recorded, and the density was calculated using the following formula:

$$\rho = \frac{M_1 - M_0}{V}$$

where V represents the volume of the pycnometer. Subsequently, the pycnometer was cleaned and filled with distilled water, and its mass (M_w) was recorded. The specific gravity (SG) was then calculated as the ratio of the mass of the ethanol to the mass of an equal volume of distilled water:

$$SG = \frac{M_1 - M_0}{M_w - M_0}$$

Determination of Flammability

The flammability of the distilled bioethanol was evaluated as described by Salihu *et al.,* (2022). Approximately 10 mL of the sample was transferred into a spirit lamp and ignited. A sustained flame was used as a qualitative indicator of the sample's flammability and purity.

Determination of Boiling Point

The boiling point was determined using the method described by Bala *et al.* (2022). A 50 mL conical flask

containing the bioethanol sample was fitted with a cork and a calibrated thermometer. The flask was heated gradually, and the boiling point was recorded as the constant temperature reached when the liquid transitioned into the vapor phase.

pH Measurement

The pH of the bioethanol samples was measured using a digital pH meter (Bala *et al.,*2022). Prior to analysis, the instrument was calibrated using standard buffer solutions (pH 4.0 and 7.0). The electrode was then immersed into the sample, and the stabilized pH value was recorded.

Determination of Bioethanol and Volatile Compounds

Gas Chromatography-Mass Spectrometry (GC-MS) analysis was conducted at the Centre for Advanced Science Research and Analytical Services (CASRAS), Usmanu Danfodiyo University, Sokoto, Nigeria. The analysis utilized an Agilent Intuvo 9000 GC system coupled with a 5977B Mass Selective Detector (MSD) and a split/splitless injector.

A DB-5MS (5% phenyl-methylpolysiloxane) fused-silica capillary column (30 m length × 320 µm internal diameter × 0.25 µm film thickness) was used. Helium (99.999% purity) served as the carrier gas at a constant flow rate of 1.2 mL/min. The inlet temperature was maintained at 300°C, while the MS source and MS quad temperatures were set at 230°C and 150°C, respectively.

The oven temperature program began at 50°C for 2 minutes, increased to 250°C over 20 minutes, and finally reached 300°C at a rate of 20°C/min, where it was held for 3 minutes. Data were acquired using GCMSD/Enhanced MassHunter software and processed via GCMSD Data Analysis software integrated with the 2017 NIST Library. For each analysis, 1 µL of the sample extract was injected in splitless mode using an Agilent G4513A Automated Liquid Sampler (ALS).

Fourier Transform Infrared (FT-IR) Analysis

The produced bioethanol distillate was subjected to FT-IR analysis to identify functional groups and confirm the presence of ethanol. The analysis was conducted at the Centre for Advanced Science Research and Analytical Services (CASRAS), Usmanu Danfodiyo University, Sokoto, using an Agilent Cary 630 FT-IR spectrometer. The spectrometer utilized a high-emission infrared radiation base system integrated with a five-bounce diamond Attenuated Total Reflection (ATR) sampling accessory, featuring an internal reflection element (IRE) crystal. Data was acquired in transmittance mode, with signals collected over a wavenumber range of 4000–650 cm^{-1}. Sample spectra were generated and processed using MicroLab software.

Data Analysis

Statistical significance was determined using a one-way analysis of variance (ANOVA). Mean comparisons were performed using the Bonferroni post-hoc test, with significance level of $p < 0.05$.

RESULTS

Macroscopic and Microscopic Characteristics of Fungal Isolates

Macroscopic examination of *Aspergillus niger* on PDA plates showed the presence of black colonies with powdery texture. The microscopic examination showed the presence transparent conidiophore with large globose head as presented in Table 1. The starch hydrolysis of the isolates for determining starch hydrolysing potential of *Aspergillus niger* showed the highest zone of clearance, the highest zone of clearance obtained from the isolates was 47 mm.

Macroscopic examination of *Saccharomyces cerevisiae* growth showed cream-coloured colonies that were spherical in shape. The microscopic examination showed that the yeasts were round in shape with multilateral budding under the microscope using x100 magnification. Molecular identification of the isolates as presented in Table 2 indicate significant percentage query covered with *Aspergillus niger* and *Saccharomyces cerevisiae*. Phylogenetic tree analysis revealed close relationship with *Aspergillus* specie and some uncultured fungal species for the *Saccharomyces* identified in this study as indicated in Figures 1 and 2.

Temperature Tolerance Test

The yeasts isolates were incubated at varying temperatures of 25, 30, 35, 40, and 45℃. Growth was observed based on the turbidity of the medium. There was evidence of growth from 25 to 40℃ whilst absence of growth was observed at 45℃. The result of the temperature tolerance of the selected yeast isolated is presented in Table 3.

Ethanol Tolerance Test

The yeasts isolates were incubated at varying ethanol concentrations of 0, 5, 10, 15, 20, and 25%. Growth was observed at up to 15% ethanol concentration whereas no visible growth was observed at 20 and 25% ethanol concentrations respectively. The result of the ethanol tolerance of the selected yeast isolated is presented in Table 4.

Osmotolerance Test

The yeast isolates were incubated at 5, 10, 15, 20, 25 and 30% glucose concentrations to determine their glucose tolerance ability. Growth was observed based

on the turbidity of the medium using Spectrophotometer at 600nm. The highest optical density of the medium was observed at 20% which indicates the higher glucose concentration tolerable by the isolate. Glucose tolerance of the selected yeast isolate is presented in Table 5.

Sugar Fermentation Test

The yeast isolates were subjected to sugar fermentation using glucose, galactose, lactose, sucrose, maltose and fructose. The fermentation pattern of the yeast isolates is presented in Table 6.

Proximate Composition of the yam peels

The proximate composition analysis reveals significant variations in nutrient content across the three substrates as shown in Table 7. White yam peels are characterized by higher fat and protein levels but relatively lower fiber content. Water yam peels show the highest moisture and protein levels, as well as the highest fiber content but the lowest carbohydrate content. Statistically, the means with different superscripts indicate significant differences between the substrates for each parameter, suggesting distinct compositional profiles among them.

Bioethanol yield % (v/v) of White (WHT) and Water (WAT) Yam Peels % (v/v)

The results presented in Table 8 shows a significant increase in the mean values of white and water yam peels as the time intervals progress, particularly at higher substrate concentrations. White yam consistently has the highest values across all time points and concentrations, with a peak at 10.0g after 72 hours (4.73±0.21 %v/v) and water yam peels (4.23±0.31 %v/v) follow a similar trend. The differences between substrates (yam peels) marked by distinct superscripts (a, b, c) indicate statistically significant differences at the $p<0.05$ level. The control group consistently shows no bioethanol yield across time intervals, confirming that the bioethanol yields obtained are due to the fermentation activity of *Saccharomyces cerevisiae.* This analysis highlights that higher substrate concentrations and longer incubation times yielded higher bioethanol yield up to 72 hours before declining.

Determination of optimum Temperature

The fermentation temperature was varied at 25, 30, 35 and 40℃. The optimal temperature for bioethanol yield was observed at 30℃ for all the substrates. A decline in bioethanol yield was observed at 35 and 40℃ as presented in Table 9.

Determination of optimum pH

The optimal pH for bioethanol yield was pH 4.5. There was decline in bioethanol yield with increase in pH as presented in Table 10.

Determination of Physicochemical Properties of the Bioethanol Produced

Table 11 presents the physicochemical properties of the bioethanol produced including density, specific gravity, boiling point, pH, colour and flammability.

Volatile Compounds Present in the Bioethanol Produced from White and Water Yam peels

In Table 12, the volatile compounds present in the bioethanol produced from White yam peels were presented. Ethanol, Dimethyl ether and Formic acid were found to be present with peak areas (Figure 3) of 16.04, 10.85 and 1.92 respectively. Similarly, Table 13 presents the volatile compounds present in the bioethanol produced from Water yam peels. Ethanol, Dimethyl ether and 1-Propene, 1 methylthio-2-trifluoromethyl-1,3,3,3,-tetrafluoro were found to be present with peak areas (Figure 4) of 31.73, 13.06 and 2.37 respectively.

Functional Groups and Class of Compounds in the White and Water Yam Peels Distillate

In Table 14, functional groups in the White yam peels distillate were presented which include C-H (Alkene), C-O (Primary Alcohol), C=C (Alkene), C-H (Alkane) and O-H (Alcohol) functional groups. Table 15 presents the functional groups present in the Water yam peels distillates which include C-H (Alkene), (C-O) Primary Alcohol, C-O (Secondary Alcohol), C=C (Alkene), C-H (Alkane), O-H (Alcohol).

Table 1: Morphological Characteristics of the Fungi

Macroscopic characteristics	Microscopic Characteristics	Inference
Black growth bordered by white mycelia.	Septate hyphae; large and pigmented dark brown conidial heads with spores spreading in radial columns.	*Aspergillus niger*
Cream coloured with smooth surface, circular yeast-like growth	Oval-shaped cells. Multilateral budding.	*Saccharomyces cerevisiae*

Table 2: Hit Similarity of the Selected Isolates from NCBI Genbank after BLAST

Organism	Sequence length (bp)	% Identity	Accession No.	Alignment Score	Query Coverage
Aspergillus niger (Sample A)	619	100	MT550026.1	≥200	99
Saccharomyces cerevisiae (Sample B)	799	96.6	MK973014.1	≥200	99

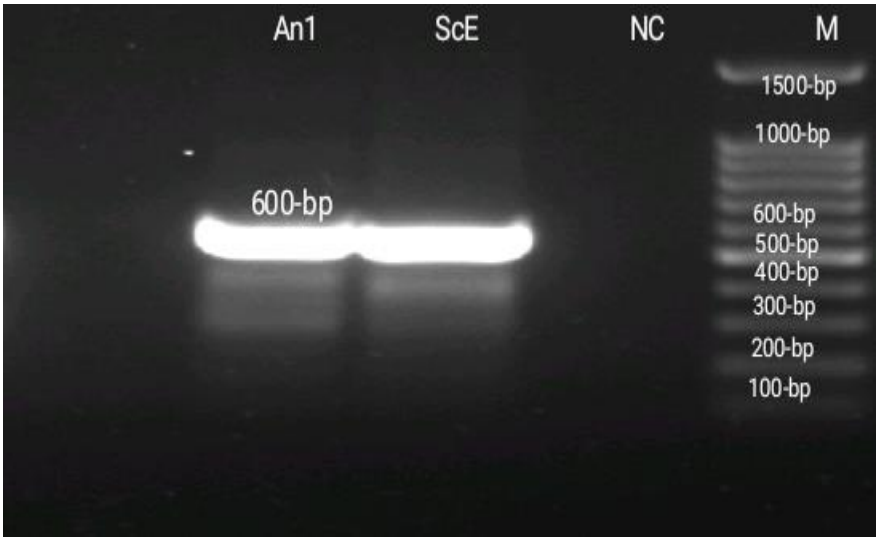

Figure 1: Gel image showing the PCR products after electrophoretic separation

Table 3: Temperature Tolerance of the Selected Yeast Isolate

Temperature (℃)	Growth
25	++
30	+++
35	+++
40	+
45	–

Key: Intensive growth = +++, Moderate growth = ++, Low growth = +, No growth = –

Table 4: Ethanol Tolerance of the Selected Yeast Isolate

Ethanol concentration (%)	Growth
0	+++
5	+++
10	++
15	+
20	–
25	–

Key: Intensive growth= +++, Moderate growth = ++, Low growth = +, No growth = –

Table 5: Glucose Tolerance of the Selected Yeast Isolate after 48 hours

Glucose concentration (%)	Optical Density (OD) at 600 nm
5	1.993
10	2.371
15	2.489
20	2.519
25	2.018
30	1.923

Key = Higher values indicate increased microbial growth

Table 6: Sugar fermentation pattern of the Yeast Isolates at 37℃ for 24 hours

Isolates	Glucose	Galactose	Lactose	Sucrose	Maltose	Fructose
Sc. A	+	+	-	+	+	+
Sc. B	+	+	-	+	+	+
Sc. C	+	+	-	+	+	+
Sc. D	+	-	-	+	-	+
Sc. E	+	+	-	+	+	+

Key: Positive (+) (fermentation occurred); Negative (-) No fermentation

Table 7: Proximate Analysis of White and Water Yam Peels

Parameters	White yam peels (%)	Water yam peels (%)
Moisture	9.39 ± 0.03^{b}	10.22 ± 0.02^{c}
Ash	2.39 ± 0.01^{b}	1.73 ± 0.03^{a}
Fat	4.21 ± 0.01^{b}	3.72 ± 0.01^{c}
Fibre	20.18 ± 0.03^{b}	23.38 ± 0.03^{c}
Protein	5.51 ± 0.02^{b}	6.32 ± 0.03^{c}
Carbohydrate	58.33 ± 0.04^{b}	54.62 ± 0.04^{a}

Note: Superscripts 'a', 'b' and 'c' indicate that the means with different letters are statistically different, while means with the same letters are not statistically different. Each value represents mean of three independent tests ± Standard Deviation

Table 8: Effect of Time on Bioethanol yield % (v/v)

	Duration (hours)				
Substrate (10g)	**24**	**48**	**72**	**96**	**120**
WHT	3.47±0.25	4.17±0.32	4.73±0.21	4.53±0.15	4.20±0.30
WAT	3.10 ± 0.30	3.70 ± 0.40	4.23 ± 0.31	3.93 ± 0.32	3.60 ± 0.36

Key: WHT = White yam peels, WAT = Water yam peels

Table 9: Effect of Temperature on Bioethanol Yield

Temperature (℃)	WHT %(v/v)	WAT % (v/v)
25	4.67 ± 0.23	4.07 ± 0.21
30	4.93 ± 0.45	4.43 ± 0.20
35	4.80 ± 0.21	4.27 ± 0.31
40	4.23 ± 0.29	3.90 ± 0.10

Key: WHT = White yam peels, WAT = Water yam peels

Table 10: Effect of pH on Bioethanol Yield

pH	WHT % (v/v)	WAT % (v/v)
4	4.50 ± 0.25	4.10 ± 0.20
4.5	4.70 ± 0.17	4.57 ± 0.10
5	4.33 ± 0.25	4.23 ± 0.32
5.5	4.13 ± 0.25	3.63 ± 0.15

Key: WHT = White yam peels, WAT = Water yam peels

Table 11: Physicochemical Properties of the Bioethanol Produced

Parameters	WHT	WAT
Density (g/cm^3)	0.921	0.871
Specific gravity	0.941	0.889
Boiling point (℃)	81	80
pH	5.3	5.6
Colour	Colourless	Colourless

Key: WHT: White yam peels WAT: Water yam peels

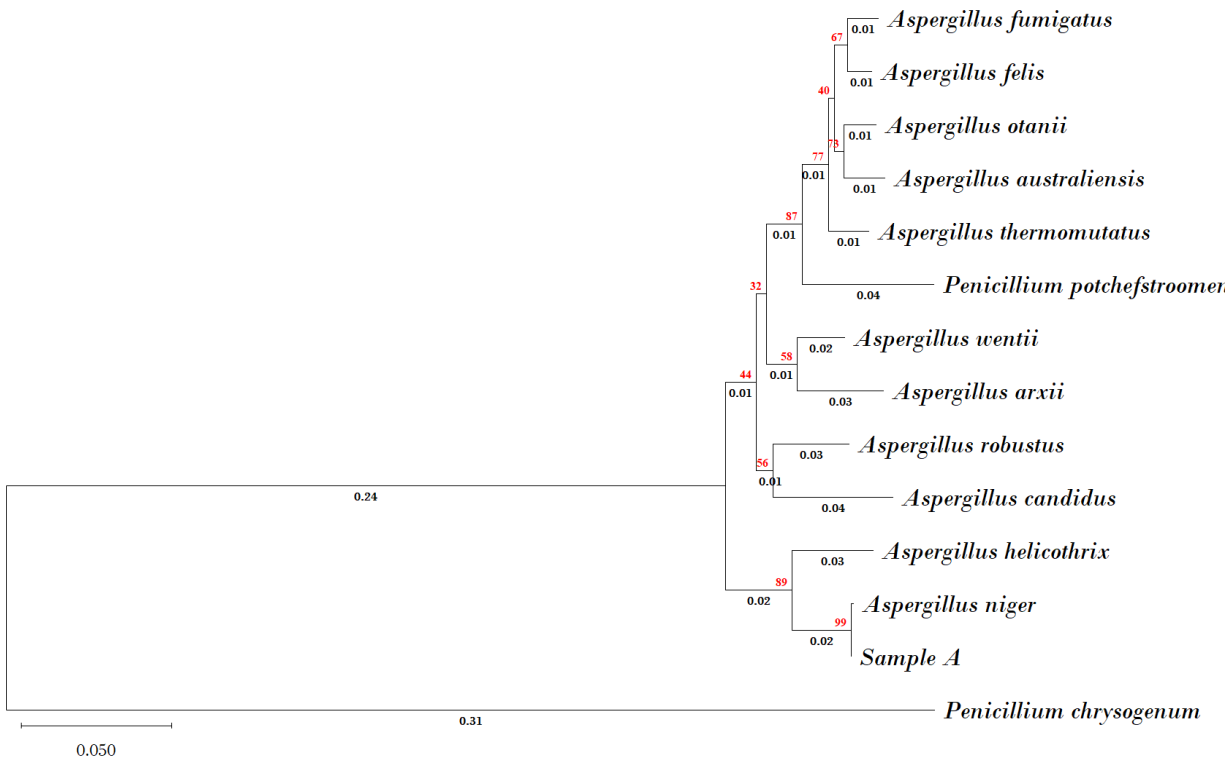

Figure 2: Phylogenetic tree of *Aspergillus niger* strain MT550026.1 (Sample A) using Neighbor Joining Method (Bootstrap values are in red)

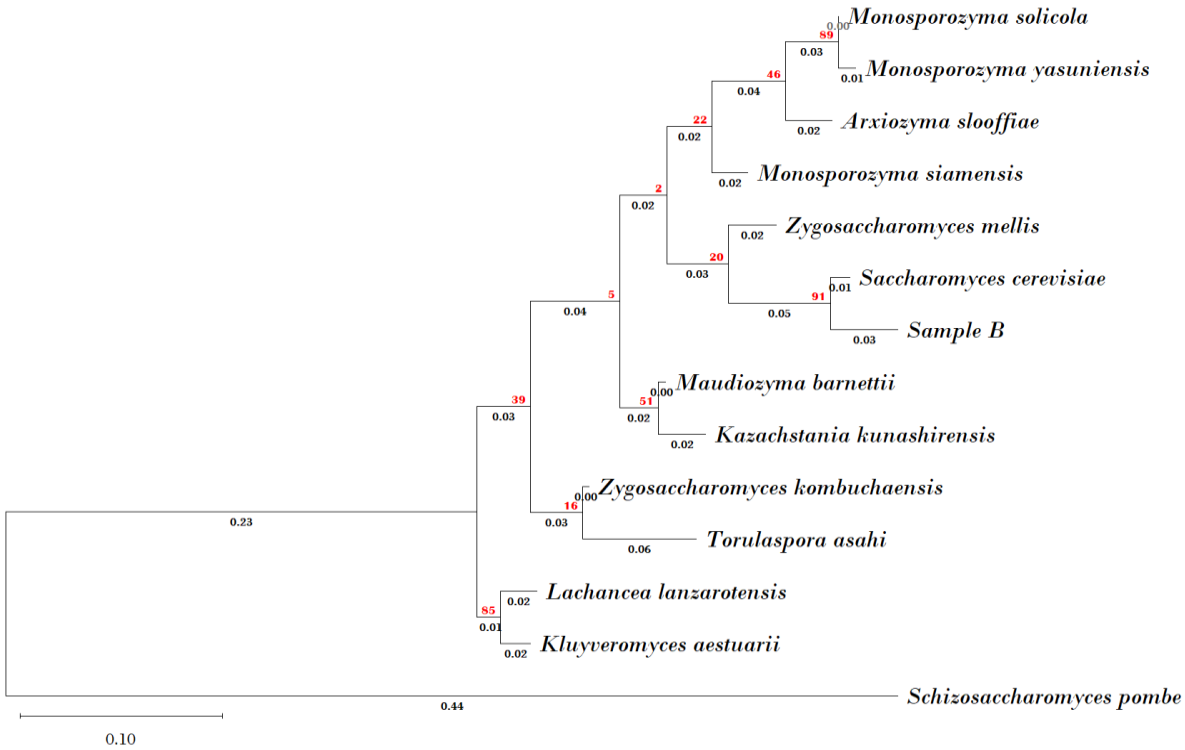

Figure 3: **Phylogenetic tree of *Saccharomyces cerevisiae* strain MK973014.1 (Sample B) using Neighbor Joining Method (Bootstrap values are in red)**

Table 12: Volatile Compounds in the Produced Bioethanol from White Yam Peels

Volatile compound	Retention Time	Peak Area (%)
Ethanol	11.152	16.04
Dimethyl Ether	10.665	10.85
Formic Acid	16.250	1.92

Table 13: Volatile Compounds in the Produced Bioethanol from Water Yam Peels

Volatile Compound	Retention time	Peak Area (%)
Ethanol	9.537	31.73
Dimethyl Ether	9.840	13.06
1-Propene, 1 methylthio-2-trifluoromethyl-1,3,3,3-tetrafluoro	16.447	2.37

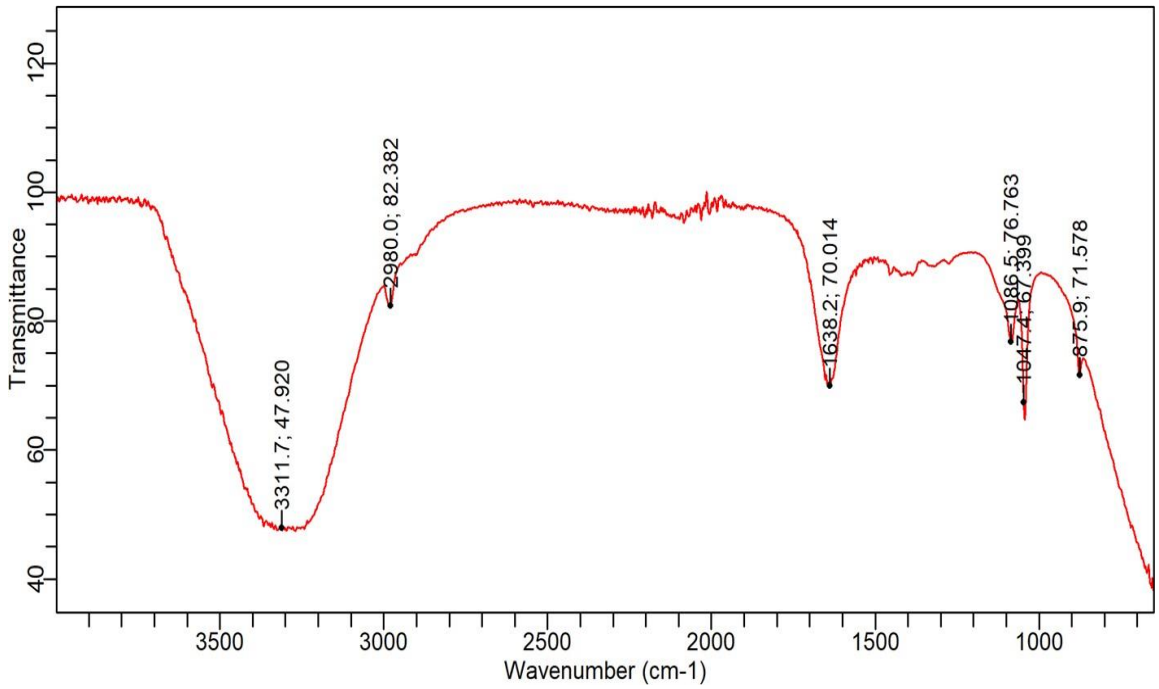

Figure 4: FT-IR Spectrum of White Yam Peels Distillate

Table 14: Functional Groups and Class of Compounds in the White Yam Peels Distillate

Wavenumber (cm^{-1})	Functional Group	Class of Compounds
875.9	C-H	Alkene
1047.4	C-O	Primary Alcohol
1086.5	C-O	Secondary Alcohol
1638.2	C=C	Alkene
2980.0	C-H	Alkene
3311.7	O-H	Alkene

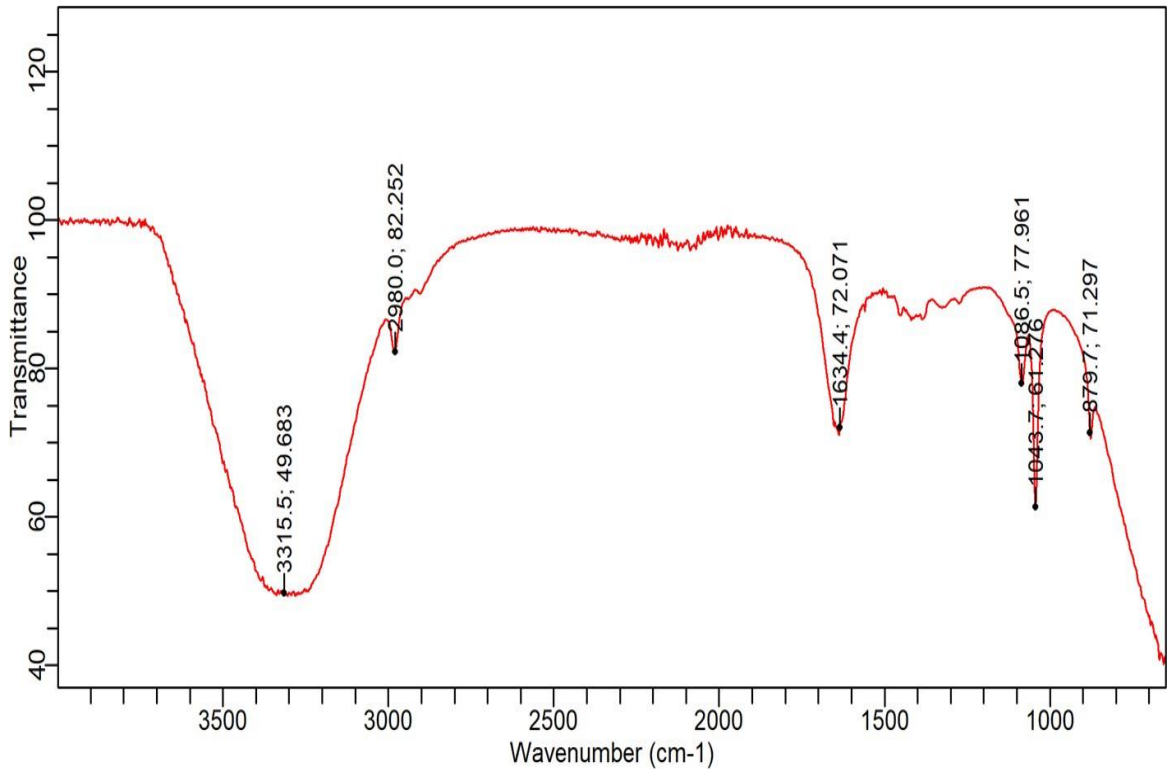

Figure 4: FT-IR Spectrum of Water Yam Peels Distillate

Table 15: Functional Groups and Class of Compounds in the Water Yam Peels Distillates

Wavenumber (cm^{-1})	Functional Group	Class of Compounds
879.7	C-H	Alkene
1043.7	C-O	Primary Alcohol
1086.5	C-O	Secondary Alcohol
1634.4	C=C	Alkene
2980.0	C-H	Alkane
3315.5	O-H	Alcohol

DISCUSSION

This study successfully identified strains of *Aspergillus niger* and *Saccharomyces cerevisiae* capable of efficient sugar fermentation and high stress tolerance. Both genera have been utilized in traditional fermentation for centuries, a history that has facilitated the long-term optimization of their metabolic properties. Consequently, they remain staple organisms in the food, beverage, pharmaceutical, and biofuel industries. A study by Kitsom-Hytey *et al.* (2024) reported the use of *A. niger* and *S. cerevisiae* in biofuel production, while Antia *et al.* (2018) and Odu *et al.* (2022) highlighted the broader application of yeasts in food product fermentation.

The fermentation patterns of the isolates, including their tolerance to ethanol, thermal stress, and high glucose concentrations, were evaluated to identify strains capable of withstanding the rigorous conditions of bioethanol production. While all isolates efficiently fermented glucose and sucrose, evidenced by the production of acid and gas, variations were observed in the utilization of maltose and galactose. Notably, none of the yeast isolates were capable of fermenting lactose. This observation is consistent with the findings of Olaniyi *et al.* (2019), who reported that *Saccharomyces cerevisiae* lacks the enzyme lactase (β-galactosidase) required for lactose hydrolysis, despite its proficiency in fermenting sugars such as glucose, fructose, maltose, and sucrose.

Ethanol tolerance is a critical parameter in industrial fermentation, as high concentrations of ethanol necessitate the use of robust yeast strains capable of

withstanding significant ethanol-induced stress (Bakare *et al.,* 2019). In this study, yeast growth decreased as ethanol concentrations increased. This inhibition is attributed to ethanol's role as a potent microbial inhibitor, which can damage mitochondrial DNA and inactivate key metabolic enzymes, including hexokinase and dehydrogenase (Vanthienen *et al.,* 2024). Furthermore, ethanol accumulation within the fermenter has been shown to suppress growth rates, ethanol productivity, cell viability, and substrate consumption (Sener *et al.,* 2007). Our yeast isolates exhibited tolerance up to 15% ethanol, aligning with the findings of Ali and Khan (2014) and Odu *et al.* (2022), who reported thresholds of 15% and 14%, respectively. Notably, other studies have identified highly robust strains from sources such as palm wine that can tolerate concentrations ranging from 15% to 20% (Olaniyi *et al.,* 2019).

The yeast isolates demonstrated osmotolerance at glucose concentrations up to 20%, consistent with the findings of Osho (2005). However, this threshold was lower than the 30% maximum sugar tolerance reported by Negera (2017) for *Saccharomyces cerevisiae*. Regarding thermal stability, yeast growth increased proportionally with temperature up to a specific point; however, exceeding 35 °C resulted in a significant decline in viability, with complete growth inhibition observed at 45 °C. Such robust strains, particularly those isolated from palm wine, have been widely exploited for industrial applications including baking, bioethanol, and single-cell protein production (Frances *et al.,* 2023).

Proximate analysis of the agricultural residues revealed that white yam peels contained a higher carbohydrate content compared to water yam peels. This disparity is likely due to the superior starch sequestration in white yam tubers, where the peel serves as both a protective barrier and a repository for residual storage carbohydrates (Nath and Dutta, 2024). These results correlate closely with carbohydrate values reported by Ossamulu *et al.* (2022), Awoyale *et al.* (2021) and Bashir *et al.* (2021) (56.27%, 51.5%, and 57.93%, respectively). Furthermore, the high carbohydrate and fiber concentrations observed in these peels align with the findings of Tambuwal *et al.* (2018), who characterized ideal bioethanol feedstocks as being predominantly sugary, starchy, and lignocellulosic.

Reducing sugar yields exhibited a positive correlation with substrate concentration. In this study, a substrate concentration of 10 g resulted in peak yields of 1.86 g/L for white yam peels and 1.66 g/L for water yam peels. These findings are consistent with the reports of Marwadati *et al.* (2019) and Permanasari *et al.* (2018), who observed that higher substrate concentrations (15% and 40%) facilitated maximum reducing sugar releases of 3.2 g/L and 191.60 g/L, respectively. At the 10 g substrate level, white yam peels produced 4.73 ± 0.21% (v/v) ethanol, while water yam peels yielded 4.23 ± 0.31% (v/v) ethanol. This enhanced productivity may be attributed to the availability of substrates readily hydrolyzed into fermentable sugars by the amylolytic activity of *Aspergillus niger*, which are subsequently converted to ethanol by the yeast (Mustafa *et al.,* 2019). These results correlate with Jimoh *et al*. (2009) and Ajay *et al.* (2014), who both reported increased ethanol yields at higher substrate concentrations, identifying optimal levels at 10% and 12%, respectively. In contrast, Marwadati *et al.* (2019) observed a divergent trend, where ethanol yield decreased at higher concentrations, reaching a maximum at only 3%.

Fermentation was conducted over a 120-hour (5-day) period, with ethanol yield initially increasing over time. This trend is likely attributable to the progressive proliferation of yeast cells and the subsequent utilization of available substrates. However, the yield began to decline after 72 hours, potentially due to a reduction in viable cell counts, diminished metabolic activity, or the depletion of fermentable substrates. These observations are consistent with Tsunatu *et al.* (2017) and Braide *et al.* (2016), who identified 72 hours as the optimal duration for bioethanol production.

The resulting distillates were clear and colourless. Boiling points were recorded at 81°C and 80°C for white and water yam peels distillates respectively, slightly exceeding the 78.5 °C boiling point of pure ethanol. This elevation may be due to the presence of residual impurities. Similarly, the recorded densities of 0.921 g/cm³ and 0.871 g/cm³ for white and water yam peels distillates respectively, reflect the influence of these impurities. Upon ignition, the distillates produced a characteristic blue flame and demonstrated complete miscibility in water. Specific gravity values were determined to be 0.941 and 0.889 for white and water yam distillates, respectively; the higher values observed in the white yam peels distillate suggest a relatively higher water content.

The influence of temperature and pH on bioethanol production was also evaluated. Peak ethanol concentrations were achieved at 30 °C, with white and water yam peels yielding 4.93% (v/v) and 4.43% (v/v), respectively. The fermentation process is highly temperature-sensitive, as specific starches may

undergo more efficient enzymatic conversion to fermentable sugars at optimal temperatures. These results align with Hadeel *et al.* (2011) and Altınışık *et al.,* (2024), who reported maximum bioethanol yields from various biomass feedstocks at similar temperatures. Conversely, ethanol yields declined at temperatures exceeding 30 °C, suggesting that thermal stress impedes yeast proliferation and induces enzyme denaturation, thereby reducing metabolic output (Abdulsalam *et al.,* 2024).

Furthermore, bioethanol yield increased as the pH was increased from 4.0 to 4.5, followed by a decline at higher pH levels. This suggests that the relevant enzymes exhibit maximum catalytic activity in mildly acidic environments. The reduced yield observed at pH 4.0 likely reflects suppressed yeast metabolic activity (Tsunatu *et al.,* 2017), despite the fact that the broader optimal pH range for yeast growth typically spans from 3.0 to 6.0 (Salihu *et al.,* 2024).

Gas Chromatography-Mass Spectrometry (GC-MS) was employed to characterize the end products of the fermentation process, confirming the presence of bioethanol across all samples. Fourier Transform Infrared Spectroscopy (FT-IR) analysis corroborated these findings, revealing characteristic absorption peaks at 1047.4 cm^{-1}, 1668 cm^{-1}, and 3198 cm^{-1}. These signals correspond to C–O stretching, C=C stretching, and O–H stretching vibrations, respectively, in both white and water yam peel distillates. Supplementary peaks identified between 1047.4 cm^{-1} and 1086.5 cm^{-1} (C–O stretch), 1638.2 cm^{-1} (C=C stretch), 2980.0 cm^{-1} (C–H stretch), and 3311.7 cm^{-1} (O–H stretch) further substantiate the presence of hydroxyl functional groups, confirming the presence of alcohol in the distillates.

Comparative studies regarding the bioethanol production potential of various feedstocks have yielded diverse results. Specifically, Isah *et al.* (2019) reported a higher ethanol yield from yam peels (146.33 g/L) compared to banana peels (133.53 g/L). Similar trends were observed by Omoruyi *et al.,* [66], who found that yam peels outperformed cassava peels in terms of ethanol yield. In contrast, Jolayemi *et al.* (2023) reported a relatively low ethanol yield of 1.10 mL from yam peels, which was lower than that of potato and cassava peels, though it surpassed the 0.98 mL yield recorded for plantain peels.

Furthermore, Sharma *et al.* (2025) achieved high bioethanol yields using pineapple leaf waste, a result potentially influenced by the substantial cellulose and hemicellulose content inherent in that specific biomass. Other significant lignocellulosic materials, including sugarcane bagasse and rice husks, have also demonstrated substantial potential for ethanol production Gani *et al.,* (2018), Audu *et al.,* (2023) and Parveen *et al.* (2025). However, it is important to note that these studies utilized different starter organisms, which likely contributed to the variations in reported yields across the literature.

CONCLUSION

Aspergillus niger and *Saccharomyces cerevisiae* were successfully isolated from soil and palm wine, then validated through molecular techniques. Proximate analysis confirmed that both white and water yam peels are carbohydrate-rich, making them viable lignocellulosic feedstocks. This study demonstrated that white yam peels possess a higher fermentation potential than water yam peels, with optimal bioethanol output achieved under moderate temperature and mildly acidic conditions over a fixed fermentation period. Consequently, yam peels serve as a sustainable, locally sourced material for waste valorisation and renewable energy production. To further advance this research, it is recommended that the process be evaluated in large-scale bioreactors to assess industrial feasibility. Additionally, investigating advanced pre-treatment methods and strain optimization techniques could further enhance sugar release and ethanol tolerance.

COMPETING INTERESTS

The authors declare that they have no competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

AUTHORS' CONTRIBUTIONS

Amina Bello (Conceptualization, design and laboratory work), Umar Balarabe Ibrahim (Conceptualization and supervision), Ahmad Ali Farouq & Aminu Yusuf Fardami (Data analysis), Ibrahim U. Karaye and (Interpretation and article drafting). All authors have read and approved the manuscript.

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