

PRODUCTION OF BIOETHANOL FROM CASSAVA PEEL WASTE HYDROLYSATE USING SACCHAROMYCES CEREVISIAE: A SUSTAINABLE APPROACH FOR AGRICULTURAL WASTE VALORIZATION

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ABSTRACT

The increasing demand for renewable energy and sustainable waste management strategies has stimulated interest in converting agricultural residues into biofuels. Cassava processing generates massive volumes of peel waste that pose disposal challenges and environmental pollution risks in major producing regions such as Nigeria. Valorizing this lignocellulosic biomass into second-generation (2G) bioethanol offers a dual solution for waste management and renewable energy generation. This study investigated the production of bioethanol from cassava peel waste using dilute acid hydrolysis (1% v/v H₂SO₄ at 100°C for 60 min) and subsequent fermentation using *Saccharomyces cerevisiae* isolated from overripe banana (*Musa acuminata*) fruit surfaces. The isolate was characterized morphologically and screened for ethanol tolerance across 4%, 12%, and 20% v/v ethanol concentrations via spectrophotometric monitoring (OD₆₀₀). Detoxified hydrolysate (pH 5.0-6.0, neutralized with Ca(OH)₂) was fermented under anaerobic conditions at 30°C for 5-7 days. Simple distillation yielded 5.2 mL of bioethanol distillate. Fourier-Transform Infrared (FTIR) spectroscopy confirmed the functional group composition of the distillate, displaying strong characteristic bands at 3330 cm⁻¹ (O-H stretching), 2980 cm⁻¹ (aliphatic C-H stretching), 1640 cm⁻¹ (O-H bending), and 1085-1043 cm⁻¹ (C-O stretching fingerprint). These results demonstrate the technical feasibility of utilizing cassava peel waste as a low-cost feedstock for bioethanol production using wild-type *S. cerevisiae*.

Keywords: Bioethanol, Cassava peel, Dilute acid hydrolysis, *Saccharomyces cerevisiae*, FTIR spectroscopy

INTRODUCTION

Cassava (*Manihot esculenta* Crantz) is a primary staple crop cultivated extensively across tropical regions. However, its processing into food derivatives generates large quantities of solid peel waste, constituting 20%-35% of the total tuber weight (Olanbiwoninu & Odufa, 2012; Afolabi *et al.*, 2020). Open dumping of untreated peels leads to land degradation, noxious odors, leaching into surface and groundwater, and the release of toxic cyanogenic glucosides into local ecosystems (Afolabi & Ayodele, 2020; Obene & Obwola, 2025). Biomass residues such as cassava peels are rich in structural carbohydrates, containing approximately 37%-44% cellulose,

10%-37% hemicellulose, and 7%-10% lignin (Ali *et al.*, 2021). This carbohydrate profile positions cassava peel waste as an ideal, non-food-competing feedstock for second-generation (2G) bioethanol production (Efeovbokhan *et al.*, 2023; Lauwly *et al.*, 2022). Converting this waste into bioethanol addresses environmental pollution while supporting national energy security and rural economic diversification (Mustafa *et al.*, 2019; Olaniyan *et al.*, 2025).

The production process relies on an efficient pre-treatment step to disrupt the recalcitrant lignocellulosic matrix. Dilute acid hydrolysis using mineral acids such as sulfuric acid (H₂SO₄) cleaves β-1,4 glycosidic bonds within cellulose and hemicellulose, releasing fermentable monosaccharides (Ali *et al.*, 2021; Bezerra *et al.*, 2024). Downstream fermentation of these released hexose sugars requires robust microorganisms. *Saccharomyces cerevisiae* remains the preferred strain due to its rapid glycolytic conversion rate, industrial resilience, and high ethanol tolerance (Adegboye & Bello, 2022; Witantri *et al.*, 2017).

While the general potential of cassava peels has been recognized (Oladijo *et al.*, 2022), empirical evaluations integrating localized wild-type yeast isolation, ethanol stress tolerance screening, and spectroscopic end-product validation remain crucial for regional implementation. This study evaluates the feasibility of producing bioethanol from cassava peel waste collected in Kaduna, Nigeria, utilizing dilute acid hydrolysis and a locally isolated, ethanol-tolerant strain of *S. cerevisiae*.

MATERIALS AND METHODS

Sample Collection and Processing

Fresh cassava peels were collected from local processors in Kaduna Metropolis, Kaduna State, Nigeria. The raw peels were washed thoroughly under running tap water to remove soil particles and debris, sun-dried for 3-5 days to reach a constant dry weight, and milled into a fine powder using an electric laboratory mill. The pulverized substrate was stored in airtight containers at room temperature (25 – 28 °C).

Isolation and Phenotypic Identification of *S. cerevisiae*

Wild-type yeast strains were isolated from the outer surface of overripe banana (*Musa acuminata*) fruits. Fruit surfaces were

swabbed under aseptic conditions using sterile cotton swabs and streaked directly onto Sabouraud Dextrose Agar (SDA) plates. The plates were incubated at 30 °C for 48 hours. Distinct, creamy, smooth colonies were subcultured onto fresh SDA plates to obtain pure cultures and were stored on SDA slants at 4 °C. Identification was carried out through evaluation of macroscopic colony morphology and light microscopy (cell shape and budding pattern), comparing features with standard mycological keys (de Hoog *et al.*, 2021).

Acid Hydrolysis and Hydrolysate Detoxification

Saccharification was performed by mixing 20 g of cassava peel powder with 200 mL of 1% v/v dilute H₂SO₄ in a 250 mL conical flask. The mixture was heated at 100 °C for 60 minutes. After cooling, the mixture was filtered through Whatman No. 1 filter paper.

To eliminate residual acidity and mitigate microbial inhibitors (e.g., furfurals and hydroxymethylfurfurals), the hydrolysate was detoxified by overliming: Ca(OH)₂ was gradually added under constant stirring until the pH reached 5.0- 6.0. Precipitated calcium sulfate (CaSO₄) was removed by filtration, and the clear detoxified filtrate was stored at 4 °C.

Ethanol Stress Tolerance Screening

Yeast Extract Peptone Glucose (YEPG) broth formulations were prepared with ethanol concentrations of 4%, 12%, and 20% (v/v), along with an ethanol-free control. Test tubes were prepared according to the volumetric treatments in Table 1. Each tube was inoculated with 0.5 mL (5% v/v) of a standardized *S. cerevisiae* culture. Tubes were incubated at 30 °C for 48 hours. Microbial growth was monitored spectrophotometrically by measuring optical density at 600 nm (OD₆₀₀) at 0, 24, and 48 hours using a HACH spectrophotometer.

Table 1: Preparation of YEPG Broth Media with Varying Ethanol Concentrations for Stress Testing

Ethanol Conc. (% v/v)	YEPG Broth (mL)	Ethanol (mL)	Yeast inoculum (mL)	Total Volume (mL)
4%	9.6	0.4	0.5	10.5
12%	8.8	1.1	0.5	10.4
20%	8.0	2.0	0.5	10.5

Fermentation, Distillation, and FTIR Characterization

A 100 mL volume of detoxified hydrolysate was partitioned into 10 sterile test tubes (10 mL per tube) and inoculated with 1 mL of standardized yeast suspension (~3.0 × 10⁸ cells/mL, standardized to McFarland 1.0). The tubes were sealed and incubated under anaerobic conditions at 30 °C for 5 – 7 days. Fermentation progress was tracked visually via gas evolution and culture turbidity.

Following fermentation, the broth was pooled and subjected to simple thermal distillation. Distillate collected at around 78°C was retrieved, measured, and analyzed via Fourier-Transform Infrared (FTIR) spectroscopy (Agilent MicroLab PC, resolution 8 cm⁻¹, range 4000-650 cm⁻¹, 32 sample scans) to identify characteristic functional group absorption bands.

RESULTS

Identification and Ethanol Tolerance Profile of *S. cerevisiae*

The formulation of YEPG broth media for ethanol stress testing is summarized in Table 1. Macroscopic observation on SDA revealed creamy-white, smooth, round, convex colonies with entire margins. Microscopic examination confirmed oval-to-round cells occurring singly or in pairs, displaying active budding, matching the standard taxonomy of *S. cerevisiae* (Table 2).

From the spectrophotometric growth kinetics (OD₆₀₀) under ethanol stress (Table 3), the isolate exhibited strong growth at 4 % v/v ethanol, increasing from an initial OD₆₀₀ of 0.210- 0.215 to 0.510- 0.525 at 48 hours. At 12 % v/v ethanol, growth was moderately suppressed (OD₆₀₀ = 0.360- 0.370 at 48 h), whereas 20 % v/v ethanol severely inhibited yeast propagation (OD₆₀₀ = 0.210- 0.215 at 48 h).

FTIR Spectroscopic Analysis of Produced Bioethanol

Distillation of 100 mL of fermented hydrolysate yielded 5.2 mL of clear bioethanol distillate. Infrared spectrum analysis revealed structural absorption bands confirming the presence of ethanol (Table 4). FTIR spectrum of bioethanol distilled from cassava peel hydrolysate (Figure 1) shows a broad O-H absorption band around 3330 cm⁻¹ and strong C-O stretching bands characteristic of ethanol.

Table 2: Morphological and Microscopic Characterization of *S. cerevisiae*

Macroscopic Characteristics	Microscopic Characteristic	Inferred Identity
Colonies are creamy white, smooth, round, and convex, with entire margins.	Cells are oval to round, occurring singly or in pairs, with active budding observed.	<i>Saccharomyces cerevisiae</i>

Table 3: Spectrophotometric Growth (OD₆₀₀) of *S. cerevisiae* under Varying Ethanol Concentrations

Ethanol Conc. (% v/v)	Incubation Time (h)	1 st OD ₆₀₀ Reading	2 nd OD ₆₀₀ Reading	Growth Observation
4%	0	0.210	0.215	Strong growth
	24	0.420	0.435	Strong growth
	48	0.510	0.525	Strong growth
12%	0	0.190	0.195	Growth slowed
	24	0.310	0.315	Growth slowed
	48	0.360	0.370	Growth slowed
20%	0	0.180	0.185	Minimal growth
	24	0.200	0.205	Minimal growth
	48	0.210	0.215	Minimal growth

Table 4: FTIR Functional Group Identification and Absorption Peak Assignments of Bioethanol Distillate

Frequency Range (cm ⁻¹)	Observed Peak (cm ⁻¹)	Peak Assignment Vibrational Mode	Functional Group	Biological/Chemical Significance
3300 - 3400	3330.00	O-H Stretching	Hydroxyl group (-OH)	Primary broad band characteristic of liquid alcohol
2950 - 3000	2980.00	Aliphatic C-H Stretching	Methyl/Methylene (-CH ₃ , -CH ₂ -)	Aliphatic hydrocarbon chain structure of ethanol
1600 - 1650	1640.00	O-H Bending	Water/Alcohol hydroxyl bending	Residual structural H ₂ O or hydroxyl interaction
1000 - 1100	1085 - 1043	C-O Stretching	Primary alcohol/ether/ester link	Fingerprint confirmation of primary alcohol (C-OH)

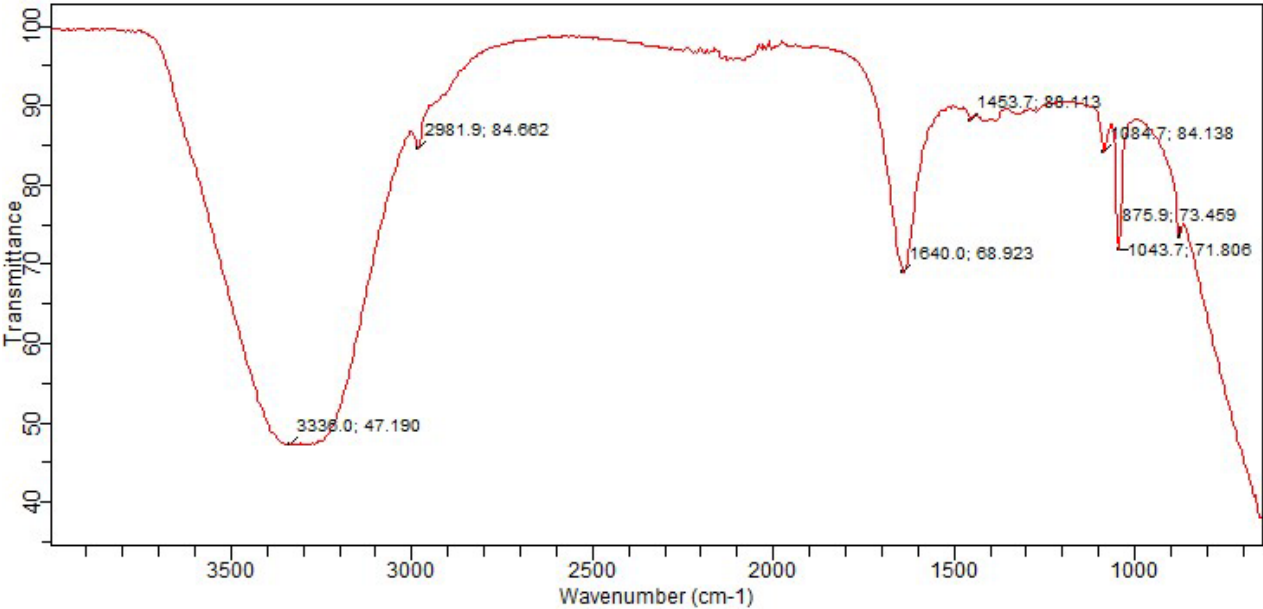


Figure 1: FTIR spectrum of bioethanol distilled from cassava peel hydrolysate

DISCUSSION

The utilization of cassava peel waste hydrolysate as a substrate for *S. cerevisiae* fermentation proved successful. Dilute sulfuric acid pre-treatment (1 % v/v) at 100 °C effectively depolymerized structural cellulose and hemicellulose into fermentable monosaccharides. This aligns with findings from Vargas-González *et al.* (2025) and Bezerra *et al.* (2024), which confirmed that dilute-acid treatments reduce biomass recalcitrance and liberate glucose suitable for microbial fermentation.

The isolation of *S. cerevisiae* from fruit surfaces verified that wild strains possess robust physiological characteristics. Screening for ethanol tolerance revealed that while 4 % v/v ethanol allowed strong growth (OD₆₀₀ > 0.500), higher levels (12 % and 20 % v/v) progressively restricted biomass production. Excessive ethanol concentrations disrupt plasma membrane fluidity, denature intracellular enzymes, and induce osmotic stress in yeast cells (Mustapha *et al.*, 2017; Sarkar *et al.*, 2012). Nevertheless, the strain's ability to maintain growth at 4 % - 12 % v/v ethanol confirms its suitability for converting low-to-moderate sugar streams into ethanol.

Spectroscopic validation using FTIR yielded functional group profiles that matched bioethanol standards (Balat, 2011; Aditiya *et*

al., 2016). The strong broad absorption band centered at 3330 cm⁻¹ corresponds to hydrogen-bonded O–H stretching vibrations of alcohols. Peaks at 2980 cm⁻¹ correspond to asymmetric and symmetric C–H stretching of methyl (-CH₃) and methylene (-CH₂-) groups. Strong absorption bands between 1085 cm⁻¹ and 1043 cm⁻¹ correspond to C–O stretching vibrations, which constitute the definitive fingerprint region for primary alcohols such as ethanol (Eze *et al.*, 2024).

These findings demonstrate that local agricultural residues, such as cassava peels, can be valorized through low-cost chemical pre-treatment and locally isolated microorganisms, thereby advancing waste management and sustainable bioenergy production (Afolabi & Ayodele, 2020; Olaniyan *et al.*, 2025). Despite the encouraging findings, optimization remains necessary. Future studies should evaluate sugar conversion efficiency, fermentation kinetics, and process economics at pilot and industrial scales. The application of genetically improved yeast strains and advanced pre-treatment technologies may further improve productivity and commercial viability.

Conclusion

This study demonstrates the successful conversion of cassava peel waste into bioethanol using dilute acid hydrolysis (1% v/v H₂SO₄) and fermentation by *Saccharomyces cerevisiae* isolated from over-ripe fruit. Spectrophotometric analysis confirmed yeast survival under moderate ethanol stress. In contrast, FTIR characterization confirmed that the recovered distillate (5.2 mL yield) was ethanol, as evidenced by functional group identification (O–H, C–H, and C–O bands). Converting cassava peel waste into bioethanol presents a viable strategy for agro-industrial waste management and sustainable energy production.

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