

Sustainable Production, Physicochemical and GC-MS Characterisation of Bioethanol from Cassava Peels

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ABSTRACT

The increasing demand for renewable energy sources has intensified research for sustainable biofuels derived from agro-wastes. This study investigated the production and characterisation of bioethanol from cassava (*Manihot esculenta*) peels as an alternative and eco-friendly feedstock. The cassava peels were hydrolysed with 0.5 M H₂SO₄, adjusting the pH to 6 and 8, followed by fermentation using *Saccharomyces cerevisiae* for six days. Fermentation efficiency was gauged by monitoring total soluble solids (%Brix), which notably decreased from 28.50% to 7.80%, showcasing effective sugar conversion into ethanol. Distillation occurred at 78 °C, and the bioethanol underwent characterisation via physicochemical methods and gas chromatography-mass spectrometry (GC-MS). The density, specific gravity, and refractive index of the bioethanol produced at a pH of 6 were 0.79 g/cm³, 0.61, and 1.342, respectively, while 0.94 g/cm³, 0.71, and 1.345, respectively, were observed at a pH of 8. The study found that cassava peels are a viable feedstock for bioethanol production, particularly at an acidic pH level (pH 6), aligning with ASTM specifications. GC-MS analysis revealed ethanol as the sole compound at this pH, while pH 8 showed additional by-products. This research supports agro-waste valorisation and aligns with global sustainability objectives in renewable energy development.

Keywords: Bioethanol, Biomass conversion, Cassava peels, Fermentation, Sustainable biofuel,

INTRODUCTION

There is a need to address the world's energy problem and inadequate power supply, especially as human population growth increases energy consumption [1]. More than 85% of the world's energy comes from fossil fuels, which have adverse environmental impacts, including carbon dioxide emissions that contribute to global warming [2]. To meet the growing energy demand and conserve petroleum reserves, the search for low-cost, efficient, effective, universally accessible, sustainable, non-toxic, highly biodegradable, low-emission, carbon monoxide-free, and environmentally friendly alternative energy sources is of great importance [3].

Biofuel can improve social, economic, and environmental sustainability while reducing dependence on fossil fuels [4]. Biofuels are viewed as a means to improve agricultural economies, reduce reliance on imported petroleum, and lower greenhouse gas emissions [2]. The major biofuels include bioethanol or biodiesel, which are good forms of renewable energy [5]. In recent times, bioethanol has been one of the most attractive biofuels as an alternative to fossil products. This has led nations such as the United States, Brazil, China, Canada, India, Thailand, Argentina, and several EU members to commit to reducing their dependence on fossil fuels to increase bioethanol production [6]. According to estimates, bioethanol can reduce greenhouse gas emissions by 30-85% compared with petrol, depending on the feedstock used. Greenhouse gas emissions can be reduced by 60 - 80% with an 85% ethanol, 15% petrol blend. Emissions can be reduced by up to 8% when a 10% and 90% petrol are combined [7].

Bioethanol has applications in the transportation, beverage, cosmetic and pharmaceutical industries, as well as in the production of electricity [8]. In transportation, it is an alternative to fossil-based transportation fuels because of its higher flammability limits, higher octane number, higher heat of vaporisation, and higher flame speeds, which enables a shorter burn time and a higher compression ratio, all of which ultimately lead to an advantage in theoretical efficiency over petrol in an internal combustion engine [9].

Appropriate feedstock for the production of bioethanol has been a challenge for industrial-scale production because of increasing concern for food security [6]. Consequently, plant biomass comprising lignocellulose has attracted attention due to its sustainability and the ability to be hydrolysed into fermentable sugars [10-11]. In spite of the possibilities of producing bioethanol from agricultural wastes like orange and cassava peels, Nigeria currently imports ethanol to meet local demand [2].

This research investigated the production of bioethanol from cassava peels as a sustainable feedstock for ethanol production, focusing on the effect of pH differences on the production, physicochemical and GC-MS properties rather than the yield and fermentation efficiency reported by most works in the literature.

MATERIALS AND METHODS

Sample collection and pretreatment

Fresh cassava peels were collected from local cassava processors in Makurdi, Benue State, using polythene bags, washed with clean water and sun-dried for 14 days. The dried samples were reduced to powder using a wooden mortar and pestle and stored in drying conditions before use, after being sieved with a 2.2 mm sieve mesh to enhance their surface area and make the cellulose easily accessible for hydrolysis.

Dilute acid hydrolysis

A quantity of 200 g of each powdered sample (cassava peels) was weighed and placed into two conical flasks. About 500 mL of 0.5 M H_2SO_4 was added to each of the conical flasks containing the dried samples. The flasks were covered with cotton wool and wrapped with aluminium foil to prevent air exposure, and then heated at 100 °C for 1 h using a hot plate [12].

pH adjustment

The pH of the samples was adjusted with 0.5 M NaOH to a pH of 6 and 8 (slightly acidic and slightly basic) to neutralise acidity using a digital pH meter (pHep^R) before the addition of yeast to the above hydrolysed samples. The contents were filtered through Whatman No. 1 filter paper after being allowed to cool as described by [12].

Yeast activation

Baker's yeast (*Saccharomyces cerevisiae*) was activated as described by Ezejiofor et al. [13], with little modifications. Exactly 15 g of the yeast was dissolved in 100 mL of warm water and continuously stirred for 1 min. The mixture was kept for 10 min, after which visible bubble formation was observed on the surface, indicating full reactivation of the yeast.

Fermentation process

The activated yeast (*Saccharomyces cerevisiae*) was added to the conical flask containing samples of cassava peels, shaken thoroughly, left at room temperature, and allowed to ferment for 6 days. The flasks were shaken at 24 h intervals to ensure a homogeneous solution and an even distribution of the organisms in the substrate mixture [13].

Distillation process

This was carried out using a distillation apparatus. The fermented liquid was poured into the distillation flask and placed on a heating mantle attached to a distillation column, which was enclosed in running tap water. Another flask was fixed to the other end of the distillation column to collect the distillate at 78 °C. This was done for each of the fermented broths [14].

Determination of the refractive index of the bioethanol samples

The Abbe 60/DR refractometer was used to calculate the bioethanol's refractive index. The refractometer's glass slide was filled with a few drops of the sample. The dark area observed was adjusted using the refractometer's eyepiece to align with the cross's junction. The scale's pointer indicated the refractive index when there was no parallax error. This was repeated, and the refractive index was determined by noting and recording the mean value [15].

Determination of the density and specific gravity of the bioethanol samples

The density of the bioethanol samples was determined using a 25 mL density bottle. The density bottle was washed and dried, after which its mass was measured and recorded as M_1 . The empty density bottle was filled with the bioethanol sample, weighed and recorded as M_2 [16]. The density was calculated using Equation (i)

$$\text{Density} = \frac{m_2 - m_1}{v} \quad (i)$$

Where; M_1 = Mass of density bottle, M_2 = Mass of density bottle + Bioethanol sample, V = Volume of density bottle (25 mL)

The specific gravity of the bioethanol was determined from the expression in equation (ii)

$$\text{Specific gravity} = \frac{\text{Density of ethanol}}{\text{density of water}} \quad (ii)$$

Determination of % Brix of the hydrolysate before and after fermentation

The % Brix of the hydrolysate was tested using the Abbe 60/DR refractometer. A few drops of the samples were applied to the prism of the refractometer, and the Brix rating was read by looking through the viewer where the colour changed, and the value was recorded [17].

GC-MS analysis of the bioethanol samples

Gas chromatography and mass spectrometry analysis was performed. The equipment used included an Agilent Technologies 6890N GC system coupled with a 5973-mass selective detector and a 7683B series injector. Helium served as the carrier gas at a flow rate of 1.2

pmL/min, with an injection volume of 1 nanolitre (nL). The inlet temperature was set to 230 °C, while the oven temperature began at 50 °C for 5 min, then increased to 300 °C at 10 °C/min and was maintained for an additional 15 min. The total runtime was 15 min. The mass spectrometry transfer line was kept at 250 °C, with the source at 230 °C and the MS gauged at 150 °C. Electron ionisation mode at 70 eV was utilised for ionisation, and total ion count (TIC) assisted in compound identification and quantification by comparing the spectrum with the NIST02 Reference Spectra Library. Data analysis and peak area measurements were processed using Agilent software [18].

RESULTS AND DISCUSSION

The results of the physicochemical properties of bioethanol produced from cassava peels under different pH values are presented in Table 1.

Table 1: Physicochemical parameters of bioethanol samples produced from cassava peels at varying pH

Sample	pH	Parameters	Value	ASTM standard
A	6	Density (g/cm ³)	0.79 ± 0.00	0.750-850 [19]
A	6	Specific gravity	0.61 ± 0.00	0.789-0.801 [20]
A	6	Refractive Index	1.342 ± 0.00	1.3568-1.444 [21]
B	8	Density (g/cm ³)	0.94 ± 0.02	0.750-850 [19]
B	8	Specific gravity	0.71 ± 0.01	0.789-0.801 [20]
B	8	Refractive Index	1.345 ± 0.00	1.3568-1.444 [21]
Cassava peel	-	% Brix before fermentation	28.50	-
Cassava peel	-	% Brix after fermentation	7.80	-

Odongo et al. [19], Harrison et al., [20], Rabiou et al. [2021]

Physicochemical properties of the bioethanol

The density of bioethanol produced in this research was 0.79 g/cm³ and 0.94 g/cm³ at pH levels of 6 and 8, respectively, as shown in Table 1. The result showed that the density of the bioethanol produced in the acidic range (pH of 6) was less than the density at the basic range (pH of 8) and fell in the range specified by the ASTM (0.750-850 g/cm³) for bioethanol. In contrast, the ethanol in the basic range exceeds the ASTM recommendations. The density of the bioethanol produced at the acidic range was less than the range of values of densities (0.871-0.893 g/cm³) reported by Isah et al. [12] on bioethanol produced from cassava peel and sugarcane bagasse. The result from the acid range was also in the range of values (0.784 - 0.789 g/cm³) reported by Bashir et al. [22] on the bioethanol produced from yam peels. However, the

values obtained in the basic condition were higher than the values reported by these authors. Higher density implies higher moisture content and low alcohol yield [23]. Therefore, the bioethanol produced under acidic conditions can be said to be of better yield.

The specific gravity of bioethanol produced, as shown in Table 1 at a pH of 6 was 0.61, and at a pH of 8 was 0.71, respectively. Though the specific gravity is less than the ASTM specification for bioethanol (0.789-0.801), the value at a pH of 8 is higher than the value at a pH of 6. The results are lower than the 0.8779 reported by Odongo et al. (2024) for cassava peels and the 0.90 reported by Palacios-Velásquez et al. [24] for fermented carrot. The specific gravity of bioethanol shows that the products have less water content. The higher the specific gravity, the higher the water content [20]. These results suggest a lower water content, preferred for combustion engines.

The refractive indices of bioethanol samples, as shown in Table 1. were 1.342 and 1.345 at pH of 6 and 8, respectively. The values are lower than the ASTM specification for bioethanol (1.3568-1.444) and also lower compared with 1.362 reported by Egbosiuba et al. [25] on bioethanol produced from cassava peels, but higher than the values (1.2804-1.3030) reported on bioethanol produced from rice and corn straws by Rabiou et al. [21]. Refractive index indicates the amount of solute present in the ethanol sample and the concentration of ethanol [26]. The lower refractive index of the ethanol produced at the acidic pH value may indicate the presence of fewer dissolved solids or impurities, which is good for the safety of the engines in which it may be used.

The % Brix of the cassava peels before fermentation was 28.50% and 7.80% after fermentation, respectively. The Brix of the cassava peels was higher than the range of values (13.26-17.01%) reported by Kasegn et al. [17] on “*Nech Tinkish*” and “*Hawaye*” sweet sorghum for bioethanol production. Brix is a measure of dissolved sugar content in a liquid and is used to measure the sugar content of the feedstock to be used in the fermentation process [27]. These results indicate that the cassava peels have a large amount of sugar that can be converted into bioethanol, which contributes positively to the economy of the process. The reduction in the Brix from 28.50% to 7.80% is an indication that there was fermentation of the sugars present in the cassava

GC-MS analysis of the bioethanol produced from cassava peels

The results of the GC-MS chromatograms of the bioethanol prepared at a pH of 6 and 8 are presented on Figure 1 and Figure 2 respectively.

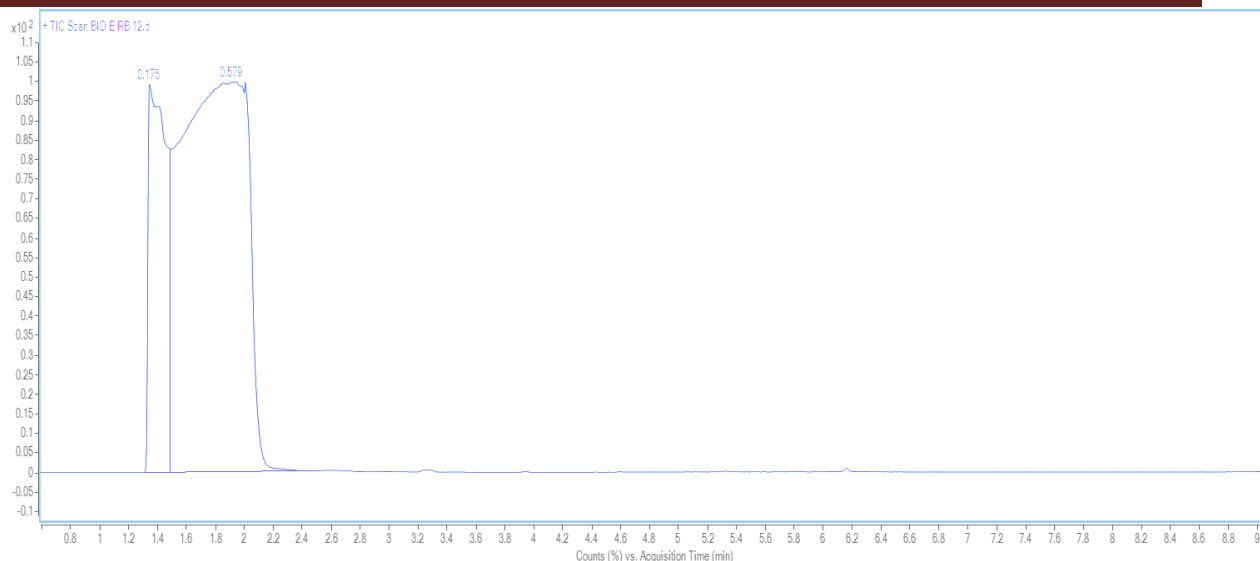


Figure 1: A GC-MS chromatogram of the sample produced from cassava peels at pH value of 6

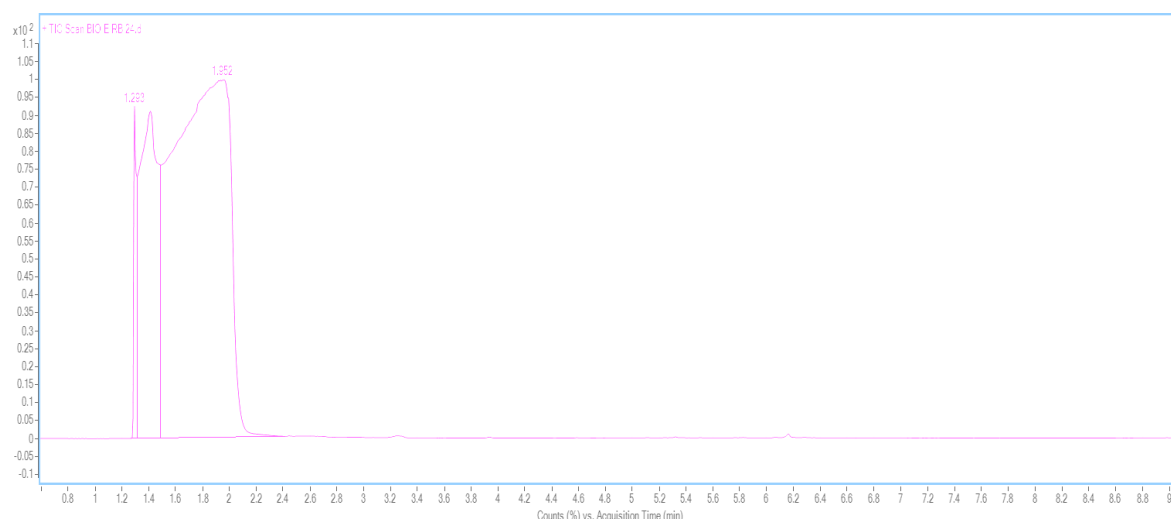


Figure 2: Chromatogram of the sample produced from cassava peels at a pH value of 8

The chromatogram of the bioethanol under acidic conditions had two peaks appearing at retention times of 1.34 and 1.91, respectively, with a molecular weight of 46.0 g/mol, implying the presence of ethanol as the only product of reaction, as seen in **Table 2** suggesting a high-purity ethanol with no or fewer metabolites.

Table 2: Chemical composition of bioethanol produced from cassava peels at pH value of 6

Peak number	Name	Formula	Retention time/min	Molecular weight/gmol ⁻¹	% Area
1	Ethanol	C ₂ H ₅ OH	1.34	46.00	26.71
2	Ethanol	C ₂ H ₅ OH	1.91	46.00	100.00

The chromatogram of the bioethanol produced under basic conditions (Figure 2) identified ethanol at a retention time of 1.29 min with a molecular weight of 46 g/mol and two other peaks for 1-Butanamine, 4-methoxy- and Undecanoic acid, 2,8-dimethyl-, methyl ester. The presence of these compounds indicates that the basic condition allowed the formation of other byproducts, as presented on Table 3.

Table 3: Chemical composition of the sample produced from cassava peels at pH value of 8

Peak number	Name	Formula	Retention time/min	Molecular weight/gmol ⁻¹	% Area
1	Ethanol	C ₂ H ₅ OH	1.29	46.00	4.31
2	1-Butanamine, 4-methoxy-	C ₅ H ₁₃ NO	1.41	103.00	29.29
3	Undecanoic acid, 2,8-dimethyl-, methyl ester	C ₁₄ H ₂₈ O ₂	1.95	228.00	100.00

CONCLUSION

The study investigated the production of bioethanol from cassava peels through dilute acid hydrolysis, utilising *Saccharomyces cerevisiae*-assisted fermentation and distillation. The physicochemical properties and GC-MS analysis showed that pH has a significant role in determining the quality of bioethanol produced. From the study, the bioethanol produced at the pH of 6 possessed acceptable physicochemical properties, including density, specific gravity and refractive index, than the bioethanol produced at a pH of 8, when compared to the ASTM standard. The analysis of Brix on the cassava before and after fermentation showed conversion of dissolved sugar to ethanol, which enhances the economy of the ethanol production process. The GC-MS analysis also revealed ethanol as a predominant compound at a pH of 6 than at a pH of 8. Therefore, the results indicated that cassava peels can be a sustainable alternative feedstock for the production of ethanol, especially in an acidic medium.

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