

Physicochemical and Biochemical Characterisation of Ackee Apple Pod as a Potential Feedstock for Anaerobic Digestion

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Abstract

The growing global demand for energy for domestic and industrial applications has intensified reliance on fossil fuels, resulting in increased anthropogenic emissions, environmental degradation, global warming, and pollution. This study evaluated the potential of ackee apple pod as a substrate for biogas production through physiochemical and biochemical characterisation. Fresh ackee apple pods were collected from Odo-Oba Village, Ogo-Oluwa Local Government Area, Ogbomoso, Nigeria, mechanically processed into powder, and characterised using standard analytical techniques. Dry Matter (DM), Organic Dry Matter (ODM), Organic Carbon (OC), and Organic Nitrogen (ON) were determined following APHA standard methods, while morphological structure, elemental composition, functional groups, and microbial characteristics were analysed using Scanning Electron Microscopy coupled with Energy Dispersive X-ray spectroscopy (SEM/EDX), Fourier Transform Infrared Spectroscopy (FTIR), and Bergey's Manual of Determinative Bacteriology. The results showed DM and ODM values of 94% and 86%, respectively, with OC, ON, C/N ratio, and gross calorific value of 16.85%, 0.35%, 24:1, and 19.3 MJ kg⁻¹ respectively. SEM analysis revealed a rough, highly porous surface with a particle size of 2.69 mm, height of 1.98 mm, and bulk density of 2.15 g cm⁻³, while EDX identified essential elements including Silicon Si (24.07%), Aluminum Al (6.70%), Calcium Ca (5.70%), and Magnesium Mg (2.82%). FTIR confirmed the presence of cellulose, hemicellulose, and lignin, and the predominant anaerobic bacteria were *Bacillus polymyxa* and *Escherichia coli*. These physicochemical, structural, and microbial characteristics indicate that ackee apple pod is a promising lignocellulosic feedstock for efficient biogas production through anaerobic digestion.

Keywords: Ackee apple pod, Dry Matter, Organic Dry Matter, *Bacillus Polymyxa*, *Escherichia coli*, Carbon-Nitrogen ratio

Introduction

Energy is fundamental to domestic and industrial development. Fossil fuel combustion is a conventional process whose by-products result in adverse effects on the climatic conditions, health hazards and degradation of natural resources. Therefore, alternative energy sources are needed in order to minimize the negative effects of conventional energy production. One of such is biogas (Samani *et al.*, 2017).

Ackee apple (*Blighia sapida*) fruit waste, especially the fibrous pods ("rinds" and seeds left after the edible arils are removed), has hardly been characterised or exploited for bioenergy. Peer-reviewed studies on its use in anaerobic digestion (AD) or other bioenergy processes are scarce. Available data show that ackee seeds/pods are high in cellulose and carbohydrates but contain low protein and high fiber, leading to a moderate or high C:N ratio, favorable for anaerobic digestion, (AD) but when too high, needs to be controlled. Critically, the pods and seeds harbor the toxin hypoglycin A (at levels often >1000 ppm in unripe fruit), which poses serious health and microbial-inhibition concerns.

Recent research has mostly focused on biodiesel and pyrolysis/biochar from ackee seed oil, not on AD. Studies have revealed that enzyme-transesterification of ackee seed oil yields ~96% of biodiesel while pyrolysis of ackee seedpods yields a high-surface-area biochar. However, AD trials on ackee waste have not been reported. Key barriers include toxin hazards, seasonal availability (ackee fruits only ripen seasonally), collection logistics, and lack of awareness/standards. To advance the value this agricultural residues, thorough characterisation through proximate analysis, ultimate analyses and toxin assays is

required while a small-scale Biochemical Methane Potential (BMP) trial using standard protocols (mesophilic inoculum, Substrate/Inoculum (S/I) ratios ~1–2, co-digestion with nitrogen-rich waste, etc.) may be carried out to further valorise this agricultural residue. Pretreatment such as milling, thermal or alkaline treatment may be needed and monitoring of hypoglycin degradation will be required. A prioritised research plan would systematically fill data gaps (ackee apple pod composition, AD kinetics) and evaluate valorisation routes (AD → biogas, pyrolysis → biochar, biodiesel, and others), thereby enabling eventual integration of this residue into waste-to-energy systems.

Detailed analyses of ackee pod themselves are lacking, but data on seeds and arils (which constitute the pods when fruit is processed) give clues. Edible arils (“ackee apple”) are ~73% water, ~14% fat, ~9% protein and <1% carbohydrate – they are nutrient-dense Adeosun *et al.* (2024). In contrast, the ackee apple seed are much dryer and fibrous. one study has shown that ripe ackee seeds has ~9% moisture, ~18% fat, ~11.7% protein and ~49% carbohydrate while unripe seeds had ~7% moisture, higher fat (22.5%) but lower protein (9.6%) and ~42% carbs (Inyinbor *et al.*, 2024). Ogunkunle and Olusanya (2026) optimized biodiesel from *Blighia sapida* seed oil, reporting it as a “viable option for biodiesel production”, while Inyinbor *et al.*, (2024) produced biochar from ackee seedpods. Their report revealed that ackee apple pod has highly porous biochar (BET ~1122 m²/g) which are used to adsorb chloroquine phosphate from water. The study confirmed that ackee pod biomass can yield high-quality carbon materials. These works, however, focused on adsorption applications, not energy. Likewise, Adeosun *et al.* (2024) characterised ackee seed oil and discovered that its properties are close to biodiesel but there is no data regarding the characterisation of the ackee apple pod to establish its promising properties for anaerobic digestion.

Ackee apple is an exotic fruit widely consumed in the Caribbean countries. In Jamaica, ackee plant bears fruit twice a year from January through March and June through August (Bello *et al.* 2021). Ackee (*Blighia sapida*) is an underutilised tree crop native to West Africa and widespread in tropical and subtropical environments. It is commonly known as ‘*Isin*’ among the Yoruba tribes of Nigeria and is a wild food plant that belongs to the family *Sapindaceae*. The fruit is like a capsule comprising three fleshy valves that split open when mature and ripe, displaying glossy black elongated seeds with cream-coloured arils at their base. Ackee apple fruit produces solid waste such as peel/skin and seeds. The seed comprises approximately 15–30% of the total weight of the ackee fruit, while the fleshy pod accounts for the remaining 70–85% (Inyinbor *et al.*, 2024). Following processing and consumption, large volumes of these residues are often disposed of indiscriminately, contributing to environmental pollution through the generation of unpleasant odour and the creation of conditions that favour the proliferation of disease-causing microorganisms. Consequently, the effective utilisation of these residues for value-added applications, such as biogas production, offers a sustainable approach to waste management and renewable energy generation.

In an attempt to place a value on this waste and eradicate this problem, the seeds were used as an adsorbent to remove Congo Red from aqueous solutions. Boey *et al.* (2012) investigated the ackee apple seed only, as an adsorbent in removing Congo Red dye and established its highest adsorption efficiency as an adsorbent of Congo Red dye from aqueous solution. Jimoh *et al.* (2012) also studied the use of *Blighia sapida* pod in their work and established that it is a low-cost adsorbent to remove Lead, Pb(II), Cadmium, Cd(II) and Cobalt, Co(II) ions from aqueous solutions. Ekue *et al.* (2010) submitted that the different parts of ackee apple tree cure over twenty- two diseases and this is gaining popularity in West Africa while Aladekoyi *et al.* (2019) and Omosuli (2014) reported that edible oil can be extracted from an ackee apple seed. The conversion of these ackee apple pods into useful biofuel has not gained popularity among researchers in recent times. Hence, this study assessed the potential of ackee apple pod as a feedstock for biogas production through physicochemical and biochemical characterisation.

Materials and Methods

Material procurement and processing

The raw ackee apple pods were freshly harvested from local farms in Odo-oba village, Ogo-Oluwa Local Government Area of Ogbomoso, Nigeria, situated at geographical coordinate 8° 8' 0" North, 4° 16' 0" East. Upon collection, the pods were thoroughly washed with water to remove dirt and grit. The washed samples were sun-dried for a period of seven days to achieve significant moisture reduction. The dried ackee apple pod residues were milled using a Janke and Kunkel hammer mill and subsequently sieved using ASTM standard laboratory sieve No 35. The fraction passing through a 0.5 mm sieve was collected and used for subsequent analyses. This size range was selected according to the standard of the biomass preparation principles described in ASTM E1757 (2019). This is to maximise the substrate surface area, thereby enhancing microbial accessibility and promoting efficient biodegradation of the organic constituents. The processed samples were then subjected to physicochemical characterisation using a Hitachi SU 3500 Scanning Electron Microscope coupled with Energy Dispersive X-ray Spectroscopy (SEM/EDX) and a Shimadzu FTIR-8400S Spectrometer for Fourier Transform Infrared (FTIR) analysis.

Methods of characterisation

Proximate analysis of the ackee apple pod

Proximate analysis of the ackee apple pod was performed at the Department of Animal Production and Health Laboratory, Ladoke Akintola University of Technology, Ogbomoso, Nigeria, using standardised APHA (2017) procedures, using the average values of three replicates. The analysis encompassed the determination of moisture content, ash content, volatile matter, organic matter, and fixed carbon fractions. Accurate measurements of sample and water masses were obtained using a top-loading balance. In addition, the nutritional constituents of the agricultural residue, including crude protein, crude fat, moisture, ash, and carbohydrates, were quantified through a combination of solvent extraction techniques and the Kjeldahl method for nitrogen determination.

The Dry Matter (DM) and Organic Dry Matter (ODM) contents in the ackee apple pod were measured according to Standard Methods APHA (2017). The total solids of substrates in a digester are a combination of organic and inorganic matter in the feedstock (Adams and Happiness 2010). To measure total Dry Matter (DM), an empty crucible or moisture dish is weighed, W_1 (g), then a fresh amount of a feedstock was added to the empty crucible and weighed as W_2 (g). This was dried in an oven, maintained at 105 °C until a constant weight was obtained. It was then cooled in a desiccator and the dried sample was weighed W_3 (g). The percentage total Dry Matter (DM) was calculated using equation 1 (APHA 2017; VDI 4630 2016).

$$\%DM = \frac{W_3 - W_1}{W_2 - W_1} \times 100 \quad 1$$

Where: W_1 = weight of empty crucible (g), W_2 = weight of crucible + wet sample (g), W_3 = weight of crucible + oven-dried sample (g). Moisture content was estimated by equation 2, given as:

$$\text{Moisture Content (\%)} = 100 - \text{DM (\%)} \quad 2$$

Organic Dry Matter is the combustible organic fraction remaining after subtracting the ash content from the Dry Matter. The process of measuring the Organic Dry Matter of an organic substrate involves using the oven-dried sample obtained from DM determination. The oven dried sample was placed in a muffle furnace at 550 °C for 2 – 4 hours to form ash, and then cooled in a desiccator. The residue (ash) was weighed W_4 (g). The ash content was calculated according to equation 3

$$\text{Ash (\%DM)} = \frac{W_4 - W_1}{W_3 - W_1} \times 100 \quad 3$$

Where W_4 = weight of crucible + ash after ignition (g)

The Organic Dry Matter (ODM) was therefore calculated directly according to equation 4 (APHA 2017; VDI 4630 2016)

$$\text{ODM (\%DM)} = \frac{W_3 - W_4}{W_3 - W_1} \times 100 \quad 4$$

The ultimate analysis of the ackee apple pod

The ultimate analysis of the ackee apple pod was carried out in the laboratory using standard APHA analytical procedures (APHA, 2017) and the average value of three replicates was used. The analysis focused on quantifying the major elemental constituents, namely carbon, hydrogen, oxygen, nitrogen, sulphur, and other associated elements. Atomic Absorption Spectrophotometry (AAS) was utilised for elemental determination in the liquid sample extracts. The technique operates on the principle that free atoms absorb light at element-specific wavelengths, typically between 190 and 900 nm, thereby enabling accurate quantification of the elemental concentrations present in the ackee apple pod samples. The Carbon (C), Hydrogen (H), and Nitrogen (N) contents were determined experimentally using a CHNS (Carbon-Hydrogen-Nitrogen-Sulphur) elemental analyzer, Oxygen (O) was determined by equation 5 where sulphur is negligible:

$$\%O = 100 - (\%C + \%H + \%N + \%Ash) \quad 5$$

This work estimated the gross calorific value (Higher Heating Value) of the ackee apple pod directly from estimation from proximate analysis using Parikh equation 6, (Parikh, *et. al.*, 2005). The calorific value obtained provides an indication of the energy potential of the biomass and its suitability as a feedstock for bioenergy production.

$$HHV = 0.3536(FC) + 0.1559(ODM) - 0.0078(A) \quad 6$$

Where, FC = fixed carbon, ODM = Organic Dry Matter and A = Ash content.

Scanning Electron Microscopy (SEM)/Energy Dispersive X-ray Spectroscopy (EDX) analysis of the ackee apple pod

The SEM/EDX analysis of the ackee apple pod was done at National Geosciences Research Laboratory (NGRL) Kaduna, Nigeria, using Hitachi SU 3500 scanning electron microscope machine in three replicates, to determine their external morphology image, crystalline structure, orientation and elemental composition. The sample was prepared by cleaning its surface, rinsing it, dehydrating it, mounting it and coating it with a thin layer of approximately 20 nm of a gold. The major purpose of coating the sample was to increase its conductivity and to provide the build-up of high voltage charges on the specimen. On a two-side carbon tape was sprinkled 0.3 g of each sample and the carbon tape was fixed to the SEM stub. A stream of light was applied on the machine and this generated signals that produced a two-dimensional image. This showed microstructural morphology information in the SEM compartment. The EDX component was also attached to the SEM analysis and this determined the elemental composition of the samples.

Fourier Transform Infrared Spectroscopy (FTIR) of the ackee apple pod

The FTIR spectroscopy analysis of the ackee apple pod was carried out at the laboratory of the Department of Chemical Science, Redeemers University, Ede, Osun State, Nigeria, to identify the functional groups in the ackee apple pod. The analysis was conducted using a Shimadzu FTIR-8400S spectrometer in three replicates. The instrument operates with a mid-infrared range, typically between 4000 cm⁻¹ and 400 cm⁻¹, providing a comprehensive spectrum of the material's vibrational modes.

Biochemical and morphological characterisation of the ackee apple pod

Morphological and biochemical methods were used for the characterisation and identification of isolates of the ackee apple pod according to Bergy's Manual of Bacteriology (Whitman *et al.*, 2015). Four replicate isolates of the ackee apple pod were characterised to determine the anaerobic bacterial present in the ackee apple pod and their striving medium. Gram staining was done to classify the two main groups of bacteria (Gram positive and gram negative bacterial) because Gram-positive bacteria retain the crystal violet stain and appear purple while Gram-negative bacteria do not retain the primary stain but take up the counterstain and appear pink or red. Simmon citrate test was carried out to determine whether a microorganism can utilise citrate as its sole source of carbon and energy and their growth in citrate solution. Catalase test was done on the ackee apple pod to identify the presence of enzyme catalase which helps in speeding up the breaking down of hydrogen peroxide during the process of metabolism.

The oxidase test was performed on the isolate of the ackee apple pod to determine the ability of the isolated microorganisms to produce cytochrome c-oxidase, an enzyme involved in the electron transport chain during aerobic respiration. The test aided in the identification of bacteria species based on their respiratory enzyme systems. Sugar fermentation tests were also conducted to evaluate the ability of the microorganisms to ferment specific carbohydrates, including glucose, lactose, sucrose, fructose, and mannitol, as well as to determine the metabolic end-products generated during fermentation. In addition, starch hydrolysis tests were carried out to assess the production of amylase, an enzyme responsible for the degradation of starch into simpler sugars. Casein hydrolysis tests were performed to identify microorganisms capable of secreting extracellular proteolytic enzymes that hydrolyze casein into peptides and amino acids. Furthermore, gelatin hydrolysis tests were conducted to determine the production of gelatinase, an enzyme that breaks down gelatin into soluble peptides and amino acids. All tests were performed in accordance with the procedures outlined in Bergey’s Manual of Determinative Bacteriology (Whitman *et al.*, 2015).

Results and Discussion

Proximate and ultimate characteristics of the ackee apple pod

The results of proximate and ultimate analysis of the ackee apple pod are shown in the Table 1. The Dry Matter and Organic Dry Matter of the ackee pod were 94% and 86%, respectively. These results are in agreement with the range reported by Ebana *et al.* (2015) and Fabunmi (2018), that, both Dry Matter and Organic Dry Matter for any biomass that will be suitable for biogas production should not be less than 75%. These high Dry Matter and Organic Dry Matter contained in the ackee apple pod agrees with the observation of Fajobi *et al.* (2022), who emphasized that the physicochemical characteristics of lignocellulosic biomass influence its biological conversion behaviour and that a suitable biomass for biogas production must possess relatively high organic matter for methane conversion bacterial activities. Similarly, the relatively high percentage of lignin content of 21.09% in the pod requires that catalyst could be employed to ease the decomposition of the organic matter, as the methanogenic bacteria find it difficult to quickly break down the residues and this may result in a long retention period for the anaerobic digestion process, according to Hamzah *et al.* (2023), and Okeke *et al.* 2024. The Carbon-Nitrogen ratio (C/N) of 24:1 obtained from the ultimate analysis revealed an excellent nutrient constituent for methane conversion bacteria for biogas generation. This agrees with the report of Panpong *et al.* (2014), Hagos *et al.* (2017) and Alqaralleh *et al.* (2017) because methane conversion microorganisms consume more carbon than nitrogen.

Scanning Electron Micrograph (SEM) and elemental composition of ackee apple pod

Plates 1(a and b): shows the SEM/EDX morphological structure of the ackee apple pod. The ackee apple pod showed a rough surface structure, more dispersed with smaller pores. Its particle size is 2.69 mm, height: 1.98 mm and bulk density: 2.15 g/cm³. The morphological characteristics observed in the ackee apple is highly dispersed porous structure with a rough irregular surface. This will increase the surface area for microbial activities and catalytic action. This supports the findings of Adaramaja *et al.* (2024) that the dispersed pores increase the surface area for microbial activities and catalytic action.

Table 1: Proximate and ultimate analysis of ackee apple pod

Proximate		Ultimate	
Parameter	%	Element	%
DM	94.00	Oxygen	22.46
ODM	86.00	Hydrogen	2.81
Ash content	8.00	Nitrogen	0.35
Crude fibre	20.00	Carbon	16.85
Lignin	21.09	C/N ratio	24:1
Moisture content	6.00	Calorific value (MJ/kg)	19.3
Fat content	2.00		
Crude protein	21.88		
Fixed Carbon	80.50		

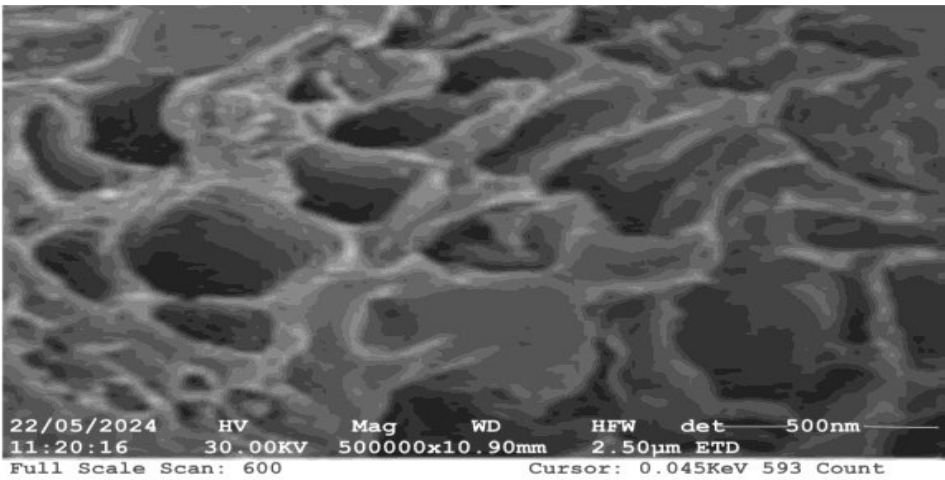


Plate 1a : SEM Image of ackee apple pod.

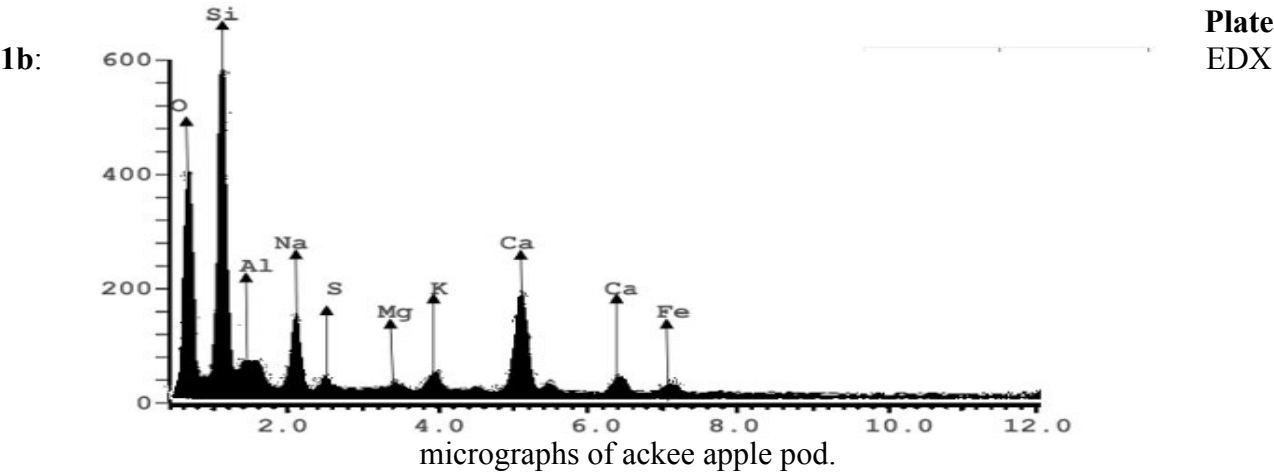


Table 2: shows the elemental composition of the ackee apple. The elemental compositions of the samples further established their suitability as biogas substrates. High oxygen content of 48.75% indicates the potential for aerobic pre-digestion. Additionally, the presence of essential macro-elements such as Silicon (Si), Calcium (Ca), and Magnesium (Mg) suggests these substrates support microbial metabolism and buffer the digester against acidification. The moderate level of Calcium aids in stabilising pH levels, a critical factor for optimal anaerobic digestion. This supports the finding of Bardi *et al.* (2023), Aworanti *et al.* (2023) and Geng *et al.*, (2024) that moderate Calcium content and other metals serves as essential cofactors for microbial enzymes and can significantly improve biogas and methane yields when supplied at appropriate concentrations.

The presence of Silicon present in agricultural residues will contribute to anaerobic digestion by influencing methanogenic activity and accelerating methane formation, (Braga *et al* 2024). Calcium has a more clearly established role in anaerobic digestion in that it is an important nutrient for microbial growth and can affect microbial aggregation, granulation, biomass structure and reactor stability (Salazar-Batres and Moreno-Andrade 2025). Magnesium is also important because it participates in microbial metabolism and enzyme-mediated biochemical reactions. In AD, adequate mineral availability helps maintain microbial growth and metabolic activity (Pires, *et al* 2025). Moreover, the presence of trace elements like Iron (Fe) and Phosphorus (P) plays a crucial role in enzyme activation and microbial growth, thus, these will enhance biogas production. Comparable observations were reported by Joo *et al.* (2018), who found that food waste containing essential metallic elements such as

iron (Fe), calcium (Ca), magnesium (Mg), zinc (Zn), and other trace metals produced higher biogas yields due to improved microbial metabolism and enzymatic activity during anaerobic digestion.

Table 2: Elemental composition of the ackee apple pod from EDX analysis

Element	Composition in % wt
Oxygen (O ₂)	48.75
Silicon (Si)	24.07
Aluminum (Al)	6.98
Magnesium (Mg)	2.82
Potassium (K)	1.31
Calcium (Ca)	5.7
Sodium (Na)	2.46
Sulphur (S)	1.53
Iron (Fe)	4.32
Phosphorus (P)	1.85
Chlorine (Cl)	0.21

Fourier Transform Infrared (FTIR) spectra of the ackee apple pod

Figures 1 shows FTIR spectra of the ackee apple pod (*Blighia sapida*). The FTIR analysis of the ackee apple pod, revealed a diverse range of functional groups that highlight its potential as a renewable energy source for biogas generation. The spectrum of the ackee apple pod displayed characteristic peaks indicative of cellulose, hemicellulose, and lignin, the primary components of plant biomass. The observed very strong broad band from wave number ~4000 – 3500 cm⁻¹ corresponds to O–H stretching (both free and H-bonded hydroxyls) and possibly N–H (amino/amide) groups. This reflects abundant hydroxyl groups in cellulose/hemicellulose (and phenolic –OH in lignin) (Olajesu and Abegunde 2026). Weaker features in 2950–2850 cm⁻¹ arise from aliphatic C–H stretching, while a peak around 1730–1650 cm⁻¹ is due to C=O stretching in carbonyls (esters/acetyls in hemicellulose and carboxylic acids). Aromatic skeletal C=C rings (from lignin) give bands near 1600–1500 cm⁻¹. Strong C–O–C and C–OH stretching bands from cellulose/hemicellulose appear in the “fingerprint” region (1200–900 cm⁻¹), especially ~1020–1100 cm⁻¹.

The FTIR wavenumber peak 3200–3600 cm⁻¹ (broad) showed a – O–H stretch. Free (non-H-bonded) OH appeared near ~3640 cm⁻¹, while strongly H-bonded OH appeared around 3300–3400 cm⁻¹. These arise from cellulose/hemicellulose hydroxyls, lignin phenolics and residual moisture. Broadening indicates extensive H-bonding in the native plant polymers. The band 3100–2800 cm⁻¹ showed – C–H stretching. Aliphatic C–H stretches of –CH₂– and –CH₃ (from cellulose, hemicellulose) occurred at ~2900–2850 cm⁻¹ while aromatic C–H stretches (~3030 cm⁻¹) may also appear if lignin has aromatic C–H. The band ~1730–1650 cm⁻¹ showed – C=O stretching. Ester/acetyl C=O in hemicellulose and carboxylic acids (including acetyl groups on Xylan) appeared at ~1730–1700 cm⁻¹, while conjugated carbonyls and lignin carbonyls appeared slightly lower (~1650–1680 cm⁻¹). These bands reflect acetyl esters and carboxylic acids in the biomass. The bands 1600–1500 cm⁻¹ showed – Aromatic C=C stretch. Characteristic lignin aromatic ring vibrations occur near 1595–1515 cm⁻¹ (Pires, *et al* 2025).

The bands 1500–1200 cm⁻¹ showed – C–H bending and C–O stretching. In-plane C–H bending from cellulose/hemicellulose occurs in 1420–1370 cm⁻¹, and C–O or aromatic skeletal vibrations contribute around 1440–1350 cm⁻¹. The band at ~1240 cm⁻¹ often corresponds to C–O–C stretching (acetyl esters) in hemicellulose (Tkachenko and Niedzielski, 2022) The bands 1200–900 cm⁻¹ (“fingerprint region”) showed – C–O and C–C stretching in polysaccharides. Strong peaks at ~1100–1030 cm⁻¹ arise from C–O–C and C–OH stretching in cellulose and hemicellulose. Lignin also contributes in this region (aromatic ring C–H out-of-plane bends ~1150 and ~830 cm⁻¹).

These bands confirm that the ackee pod is rich in cellulose, hemicellulose and lignin. Notably, the very broad –OH band (4000–3500 cm^{-1}) indicates abundant hydroxyls and hydrogen bonding (as expected in plant cell walls). This band's shape suggests mostly H-bonded –OH (broad, ~3300–3400 cm^{-1}) with possibly a shoulder or component of free –OH near 3600 cm^{-1} . A strong hydrophilic –OH band means the biomass is highly polar and water-interactive (Pires, *et al* 2025). In anaerobic digestion, this hydrophilicity tends to increase the accessibility of enzymes and microbes to the carbohydrates, facilitating hydrolysis. Conversely, if –OH groups are engaged in tight H-bonds, some accessibility is reduced. Phenolic –OH (from lignin) appear in this region too and correlate with lignin content.

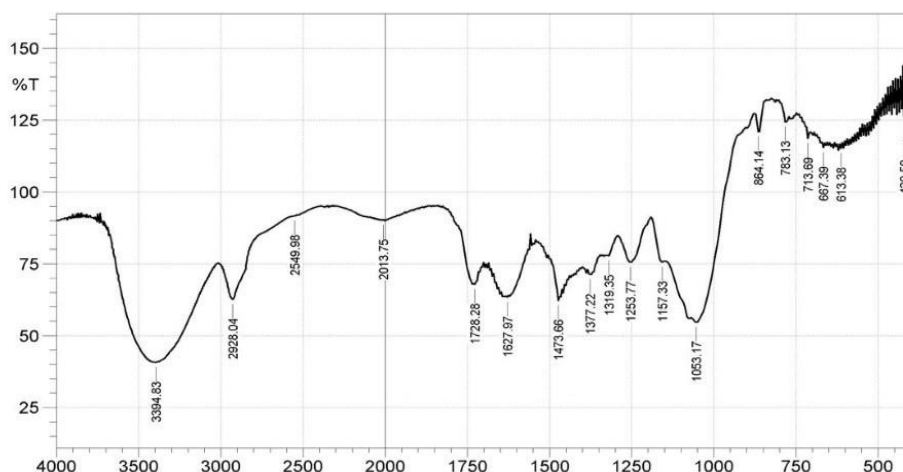


Figure 1: FTIR spectrum of ackee apple pod

Microbial characteristics of the substrates

Table 3 shows the result of the microbial characterisation of the ackee apple pod. The presence of “R” in the cell morphology of the agricultural residue indicates rod-shape bacteria, more formally called *Bacilli* and also established that those active substances present in the pod are not effective against or harmful to the growth of anaerobic bacteria for biogas production. *Bacillus Polymyxa* and *Escherichia coli* were predominant in ackee apple pod. These microorganisms grow effectively at mesophilic temperature and basic pH medium of 9.2. The starch hydrolysis showed it has insignificant level of starch. The micro-organism found presents were basically bacteria. *Bacillus Polymyxa* and *Escherichia coli* were predominant in ackee apple pod. These anaerobic bacteria were effective for methane conversion during anaerobic digestion. This is in agreement with the findings reported by Aworanti *et al.* (2017) in their work on biomethanisation of the mixture of cattle dung co-digested with pineapple peels.

The ackee apple pod shows positive motility for the microbes present, indicating that they do not have confined growth, as such, they can escape from any unsuitable environment to exploit new material and they have ability to sense a chemical gradient and direct movement accordingly. This helps the bacteria to pursue nutrients and to reach specific target.

Table 3: Morphological and microbial characterisation of substrate

Code	Gram Reacti on	Cell Morph ology	Catal ase	Oxidas e	Casein Hydrolys is	Gelatin Hydroly sis	Meth yl red	Voges Proska er	Nitrat e Redn	Growt h 60 ⁰	Growt h 30 ⁰	Coagulati on	Urease	Growth at pH 3.9	Growth at pH 9.2	Starch Hydroly ses	Growt h NaCl
AAP1	+	R	+	+	+	+	+	-	+	-	-	-	-	-	+	-	-
AAP2	+	R	+	+	+	+	+	-	+	-	-	-	-	-	+	-	-
AAP3	-	R	+	-	-	-	+	+	-	-	-	+	-	-	+	-	-
AAP4	-	R	+	-	-	-	+	+	-	-	-	+	-	-	+	-	-

Table 3 (continued). Morphological and microbial characterisation of the substrates

Code	Citrate Utilizati on	Mot ility	Indole test	Glucos e	Fruct ose	Malt ose	Lacto se	Sucro se	Galait ose	Xylos e	Arabi nose	Raffi nose	Rham nose	Dulaito l	Mannitol	Identity
AAP1	-	+	-	+G	-	+	+	+	+	+	+	-	-	-	+	<i>Bacillus Polymyza</i>
AAP2	-	+	-	+G	-	+	+	+	+	+	+	-	-	-	+	<i>Bacillus Polymyza</i>
AAP3	-	+	+	+	+	+	+	D	+	D	+	-	D	D	+	<i>Escherichia coli</i>
AAP4	-	+	+	+	+	+	+	D	+	D	+	-	D	D	+	<i>Escherichia coli</i>

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Conclusion

This study assessed the potential of ackee apple pod as a promising feedstock for biogas production through anaerobic digestion by physicochemical characterisation. The Ackee apple pod has shown a suitable high Dry Matter (DM), Organic Dry Matter (ODM), Carbon-Nitrogen ratio (C/N ratio) and a highly dispersed porous structure which will increase the surface area for microbial activities and catalytic action. The physicochemical and structural characteristics indicate that ackee apple pod is a promising candidate for anaerobic digestion, although experimental validation through biogas production trials is required. Also, these findings suggest that ackee apple pod is a promising agricultural residue for applications such as bioethanol production, biochar, or bioplastics, which capitalises on their lignocellulosic nature. *Bacillus Polymyxa* and *Escherichia coli* anaerobic bacteria were predominant in ackee apple pod and the two bacteria will thrive and grow well at a mesophilic temperature range of 25 – 45 and 25 – 40 °C, respectively. Future studies can determine biogas yield and methane content from anaerobic digestion of ackee apple pod.

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