

Effect of Acid and Alkali Pretreatments on the Structural and Compositional Properties of Cassava Peels

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Abstract— The effect of pretreatment of cassava peels with Sulfuric acid, Ethanoic acid and Sodium hydroxide on its structural and compositional properties was studied. The structural properties were monitored using Fourier Transform Infrared Spectroscopy (FTIR) and Scanning electron microscopy (SEM). The compositional properties were determined using gravimetric method. The FTIR result indicated appearance of more chemical groups, depicting removal of lignin and hemicellulose barriers in all the pretreatment methods applied. The surface morphology of the CP, as observed by SEM showed disintegration of particles in the pretreated samples, corroborating the FTIR results, indicative of a breakdown in the lignin and hemicellulose barriers. Results of the compositional properties of untreated CP for lignin, hemicellulose, and cellulose contents were: 16.36%, 21.37% and 62.28% respectively. Similarly for water pretreated CP; 16.93%, 37.76% and 31.50%. Results for Sulfuric acid pretreated CP showed lignin; 24.62%, hemicellulose; 41.50% and cellulose; 42.36%. Results for Ethanoic acid pretreated CP, in same order showed 27.83%, 39.45% and 30.65%. Sodium Hydroxide pretreatment yielded; 18.19%, 44.34% and 32.23% respectively. Highest reducing sugar content of 190.04 mg/mL was observed in H₂SO₄ pretreated CP, followed by NaOH; 132.7 mg/mL, CH₃COOH; 51.5 mg/mL and water pretreated CP with 23.15 mg/mL.

Key Words: Pretreatment, Cassava Peels, Structural Character, Compositional Properties

1 INTRODUCTION

Today, the world is searching for alternative and cleaner energy sources amidst the growing concern of the environmental impact of greenhouse gas emissions, energy security, with an increasing demand for fuel. Among all alternatives so far, ethanol has proved as a promising biofuel with various advantages [7].

The production of ethanol from starch hydrolysis has become popular in recent years. Lignocellulosic feedstock represents an extraordinarily large amount of renewable bio-resource available in surplus on earth and is a suitable raw material for vast number of applications for human sustainability. The main composition of lignocellulosic feedstocks is cellulose, hemicellulose, and lignin. [3]. In many enzyme-based biomass conversion processes to ethanol or other chemicals, a thermochemical pretreatment step is required to disrupt the lignocellulosic structure of biomass, and partially solubilize polysaccharides [4]. Pretreatment improves the susceptibility of holocellulosic polysaccharides to enzymatic hydrolysis. A major reduction in cost of pretreatment would have a significant impact on the overall cost of ethanol production.

Pretreatment processes are mainly involved in effective separation of these complex interlinked fractions and increase the accessibility of each individual component, thereby becoming an essential step in a broad range of applications particularly for biomass valorization. However, a major hurdle is the removal of sturdy and rugged lignin component which is highly resistant to solubilization and is also a major inhibitor for hydrolysis of cellulose and hemicellulose. Some of the major factors are the recalcitrance of the plant cell wall due to integral structural complexity of

lignocellulosic fractions and strong hindrance from the inhibitors and byproducts that are generated during pretreatment [3]. This has led to extensive research in the development of various pretreatment processes. The major pretreatment methods include physical, chemical, and biological approaches. The selection of pretreatment process depends exclusively on the application. As compared to the conventional single pretreatment process, integrated processes combining two or more pretreatment techniques is beneficial in reducing the number of process operational steps besides minimizing the production of undesirable inhibitors.

This work seeks to evaluate the effect alkali, organic and inorganic acid pretreatments of lignocellulosic waste (cassava peels) on its structural and compositional properties, as a pre-step towards bioethanol production.

2 MATERIALS AND METHOD

2.1 Collection of Samples

Freshly harvested cassava root tubers (*Mannihot esculenta Crantz*) used for this study were purchased from a local farmer in Egono, Etsako West Local Government Area of Edo State, Nigeria. They were identified in the Department of Agricultural Technology, Auchi Polytechnic, Auchi

2.2 Processing of Cassava Peels

The cassava root tubers were washed thoroughly with tap water and peeled. The peels were again washed and air-dried for 48 hr and milled into flour using a disc-attrition mill. The resulting flour was then sieved (using a 250µm sieve) to obtain uniform particle size. The sieved flour and residue were packed in clean plastic containers for further analysis.

2.3 Pretreatment of Samples

The samples were subjected to organic acid, inorganic acid and alkali pretreatments. For organic and inorganic acid pretreatment, ethanoic (CH_3COOH) and Sulfuric acids (H_2SO_4) were used, while Sodium hydroxide was used for the alkali pretreatment. 100 g of processed cassava peels was mixed with 300 mL of a 2 % solution of the pretreatment chemicals, respectively in a 500 mL conical flask and autoclaved at 121°C for 1 hr [8]. Same process was repeated with the CP in 300 mL of distilled water as control. The set up was done in triplicate.

2.4 Scanning Electron Microscopy (SEM) of Samples

The cassava peel sample (CP) was loaded onto the SEM's sample stage. As the sample chamber reached vacuum, the electron gun was aligned in the system to the proper location. A beam of high-energy electrons were shot, which travel through a combination of lenses and apertures and eventually hit the sample creating a 3D image, viewed from a NavCam.

2.5 Fourier Transform Infrared Spectroscopy (FTIR) Analysis of Samples

Infrared radiation of about $10,000$ to 100 cm^{-1} was sent through the sample, with some radiation absorbed and some passed through. The absorbed radiation converted into rotational and/or vibrational energy by the sample molecules. The resulting signal at the detector presents as a spectrum, typically from 4000 cm^{-1} to 400 cm^{-1} , representing a molecular fingerprint of the sample. A change in the characteristic pattern of absorption bands clearly indicates a change in the composition of the material or the presence of contamination.

2.6 Determination of Lignin content.

The dried extracted raw biomass (0.3 g) was weighed and put in a glass test tube. 3 mL of 72 % H_2SO_4 was added, and the sample was kept at room temperature for 2 h with carefully shaking at 30 min intervals to allow for complete hydrolysis. After the initial hydrolysis, 84 mL of distilled water was added. The tube was put in an autoclave for 1 h at 121°C , for further hydrolysis. The slurry was then cooled at room temperature. The hydrolysates were filtered through vacuum using a filtering crucible. The acid insoluble lignin was determined by drying the residues at 105°C and accounting for ash by incinerating the hydrolyzed samples at 575°C in a muffle furnace. The acid soluble lignin fraction was determined by measuring the absorbance of the acid hydrolyzed samples at 320 nm. The lignin content was

calculated as the summation of acid insoluble lignin and acid soluble lignin [2].

2.7 Determination of Hemicellulose content

The dried extracted biomass (1 g) was transferred into a 250 mL conical flask. 150 mL of 500 mol/m³ NaOH was added. The mixture was boiled for 3.5 h with distilled water. It was filtered after cooling through vacuum filtration and washed until neutral pH. The residue was dried to a constant weight at 105°C in a convection oven. The difference between the sample weight before and after this treatment was recorded as the hemicellulose content (%w/w) of dry biomass [2]

2.8 Extractives

The dried cassava peel (2.5 g) was put into a cellulose thimble. With the Soxhlet extractor set up, 150 mL of acetone was used as solvent for extraction. Residence times for the boiling and rising stages was carefully adjusted to 70°C and 25 min respectively on the heating mantle for a 4 h run period. After extraction, the sample was air dried at room temperature for few minutes. Constant weight of the extracted material was achieved in a convection oven at 105°C . The %(w/w) of the extractives content was evaluated as the difference in weight between the raw extractive-laden CPF and extractive-free CPF [2].

2.9 Determination of Cellulose content

The cellulose content (%w/w) was calculated by difference, assuming that extractives, hemicellulose, lignin, ash, and cellulose are the only components of the entire biomass [2].

2.10 Determination of Reducing Sugar content.

Estimation was done for cassava peel flour (CPF) pretreated with Hydrochloric acid, Ethanoic acid and Sodium hydroxide to determine the reducing concentration of sugar in the different extracts. DNS Method was followed. Standard graphs have been plotted by using Glucose solution ($200\mu\text{g}/\text{mL}$ of working standard). To the above samples 2 mL of DNS reagent was added into a lightly capped test tube. The mixture was heated at 90°C for 15 minutes to develop the red brown color. Thereafter 16 mL of distilled water was added to stabilize the color. After cooling it to room temperature in a cold water bath, absorbance was measured with a spectrophotometer at 540nm. The above procedure was repeated for 1.0 mL of extract and 1.0mL of water was taken for unknown estimation [10]. The standard graph for the estimation of reducing sugar have been plotted by using glucose as working standard solution ($200\mu\text{g}/\text{mL}$). The sugar content of sample extracts was calculated by comparing their optical density values at A 540 with the standard graph. The individual values were taken in triplicates.

3 Results

The FTIR results of the cassava peels (CP) pretreated with Sulfuric acid, Ethanoic acid and Sodium hydroxide are shown in figures 1-4.

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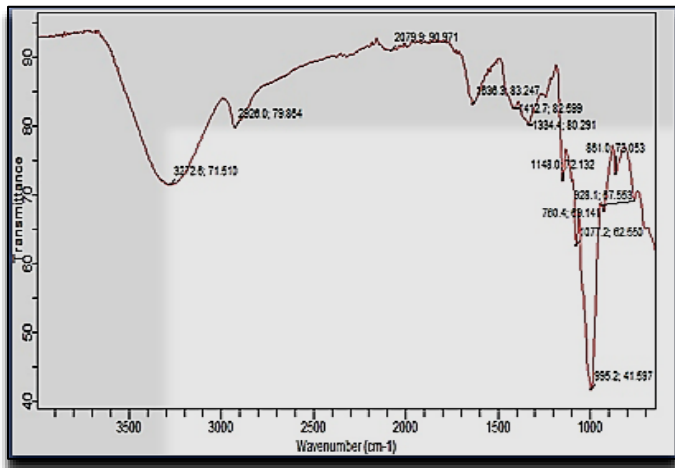


Fig. 1: FTIR: Untreated CP

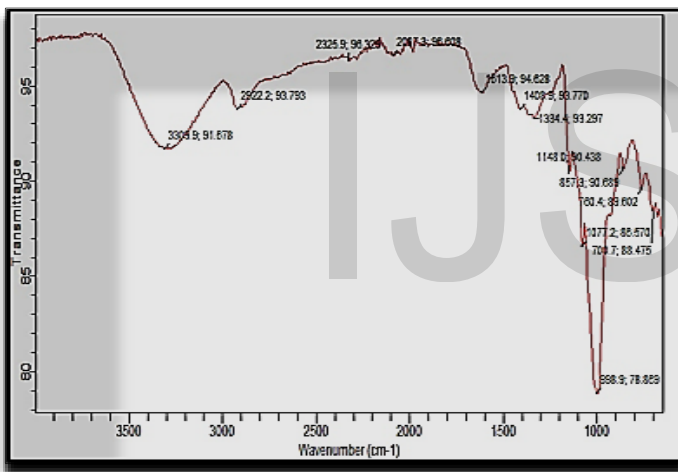


Fig. 2: CP pretreated with CH₃COOH

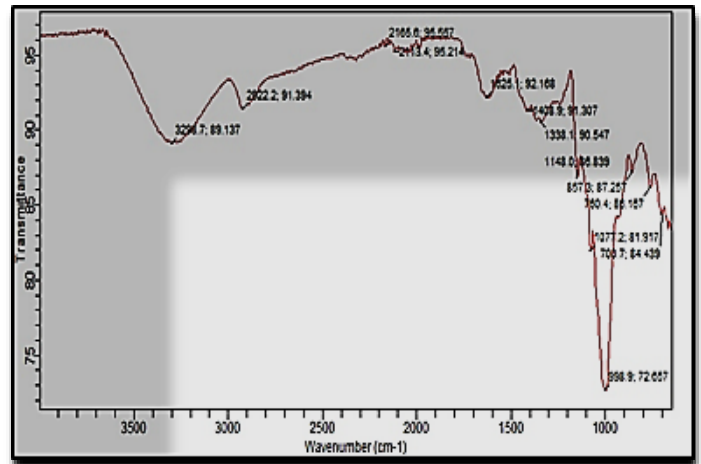


Fig. 3: CP pretreated with H₂SO₄

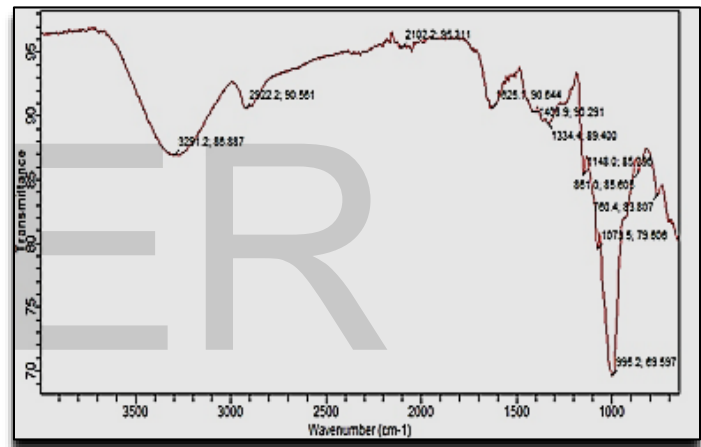


Fig. 4: CP pretreated with NaOH

Similarly, the SEM results of CPF pretreated with Sulfuric acid, Ethanoic acid and Sodium hydroxide are shown in plates 1-4

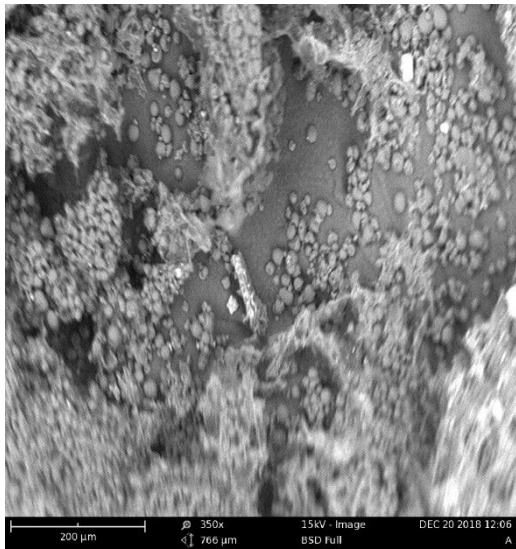


Plate 1: SEM (200μ) Untreated CP

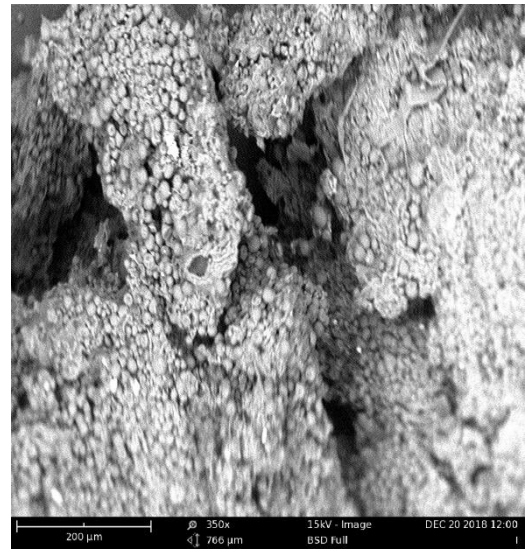


Plate 3: SEM (200μ) CP pretreated with H₂SO₄

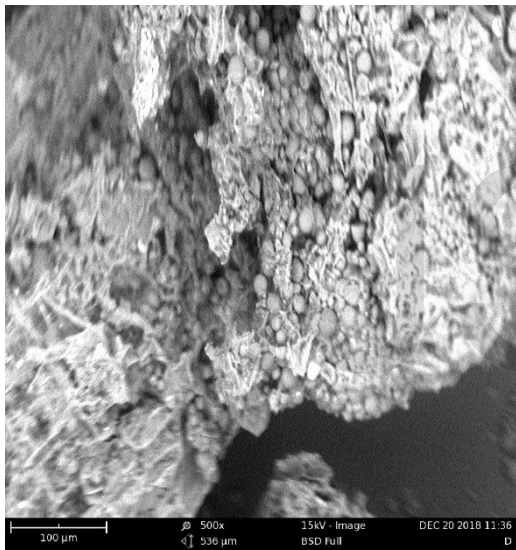


Plate 2: SEM (200μ) CP pretreated with Ethanoic acid



Plate 4: SEM (200μ) CP pretreated with NaOH

Table 1 and 2 show the results of the compositional property; in terms of contents of Lignin, Hemicellulose, Cellulose and reducing sugars, of CP pretreated with NaOH, H₂SO₄, CH₃COOH and water at 121°C for 1 hour

TABLE 1
COMPOSITION PROPERTY OF
UNTREATED CASSAVA PEELS

Parameter	Untreated CP	Pretreated With water
Lignin (%)	1636±0.15	1693± 0.02
Hemicellulose (%)	21.37±0.10	37.76± 0.15
Cellulose (%)	62.28±0.27	31.50± 0.13
Reducing Sugar (mg/mL)	2.34±0.01	23.15 ± 0.03

Results are means of triplicate treatment ± SD

TABLE 2
COMPOSITION PROPERTY OF CP
PRETREATED WITH ALKALI,

Parameter	Pretreated With Ethanoic acid	Pretreated With Sulfuric acid	Pretreated With Sodium hydroxide
Lignin (%)	27.83 ± 0.03	24.62 ±0.05	18.19±0.09
Hemicellulose (%)	39.45 ± 0.24	41.50 ± 0.20	44.34± 0.25
Cellulose (%)	30.65 ± 0.23	42.36 ± 0.19	32.23 ± 0.21
Reducing Sugar (mg/mL)	51.5 ± 0.55	190.04 ± 0.01	132.7 ± 0.07

Results are means of triplicate treatment ± SD

4 Discussion

FTIR results of untreated Cassava Peel (Untreated-CP) reveal a strong broad not too sharp absorption centered near 3272.6 cm⁻¹ indicating the presence of a hydroxyl group (O-H stretching). The absorbance at 2928.0 cm⁻¹ suggests the presence of C-H stretch probably from alkane group. The band at 1638.3 cm⁻¹ represent C=O stretching (amide I) while the peak that appear at 1334.4 cm⁻¹ is due to C-N stretch and 1412.7 cm⁻¹ is due to CH₂ wagging symmetric bending. The absorption band at 1148.0 cm⁻¹ represent the stretching band of C-O from C-OH group in starch. The peak at 1077.2 cm⁻¹ is as a result of C-H stretching of the amide group. However the band at 995.2 cm⁻¹ is due to C-O stretching and the peak at 861.0cm⁻¹ represents C-OH bending vibrations.

Ethanoic acid pretreated-CP gave a broad peak at 3305.9 cm⁻¹ due to O-H stretching. The absorption band at 2922.2 cm⁻¹ indicates a C-H group stretching, while the peak at 2335.9 cm⁻¹ suggest the presence of cyanide ions. The band at 1613.9 cm⁻¹ shows the presence of N-H bend (primary amine band). The peak at 1334.4 cm⁻¹ corresponds to C-N stretch. The absorption band at 1077.2 cm⁻¹ is attributed to C-N stretch and the peak at 998.9 cm⁻¹ corresponds to C-O stretching from C-OH group in the sample. The appearance of more chemical groups in the CH₃COOH pretreated CP in consistent with the work of [5] who noted that this is indicative of the removal of hemicellulose and lignin barrier in biomass samples. Hemicelluloses have branches with short lateral chains consisting of different sugars (hexoses and pentoses). It coats the cellulose micro-fibrils in the plant cell wall, forming a

physical barrier to accessing the sugar contents.

The surface morphology of the CH₃COOH pretreated CP as depicted in the SEM result, shows a disintegration of seed-like particles further depicting breakdown of lignin and hemicellulose barriers.

Similarly, the H₂SO₄-pretreated-CP showed a broad peak at 3298.7 cm⁻¹ indicative of O-H stretching, while the absorption band at 2922.2 cm⁻¹ indicates the presence of C-H group. The band at 1625.1 cm⁻¹ is due to asymmetrical CO₂⁻ stretching vibration (acid salt). The band at 1338.1 cm⁻¹ is due to weak O-H vibration while the peak at 857.3 cm⁻¹ is due to O-H bend. The absorption band at 1077.2 cm⁻¹ is due to C-N (alkyl amine) and the band at 998.9 cm⁻¹ is due to C-O stretching.

CP pretreated with NaOH displayed a broad not too sharp absorption at 3291.2 cm⁻¹ an indication of the presence of O-H stretch and the absorption peak at 2922.2 cm⁻¹ indicates C-H group stretch, probably from alkane. The absorption peak that appears at 1825.1 cm⁻¹ is due to C=O bond (carboxyl group). The peak at 861.0 cm⁻¹ t is due to O-H banding while the absorption band at 1073.5 cm⁻¹ t and 995.2 cm⁻¹ t are due to C-O stretching.

The occurrence of more chemical groups in the H₂SO₄ and NaOH pretreated CP, also is indicative of the disintegration of the lignin and hemicellulose barriers in the samples as observed in CH₃COOH pretreated CP.

The SEM results for H₂SO₄ pretreated CP gave a smooth surface appearance with pores and large cavities within the structure. NaOH pretreated CP gave a starch-like structure, appearing congeal with flake-like projections, and cavities within the structure. This is consistent with the findings of [1] who reported there was a physical change from non-pretreated lignocelluloses to alkali-pretreated lignocelluloses with the fiber bundles observed in lignocelluloses unloosened by the removal of lignin to change into the thin fibers in the alkali pretreated lignocelluloses. This was expected to increase the accessibility of enzyme to the cellulose by the alkali-pretreatment.

The compositional property of the un-treated CP samples showed a lignin composition of 16.36%, hemicellulose- 21.37%, cellulose- 62.28. In a related experiment, analysis of the untreated cassava peel, recorded hemicellulose composition of 29.81%, cellulose- 3.05% and lignin- 2.65% [6]. Results by other workers obtained cellulose- 5.40%, hemicellulose- 21.65% and lignin- 4.81%. The differences in the compositional character may be due to cassava variety being analyzed, age of cassava, as well as duration of storage before analysis. Cassava peel sample (CP) pretreated with water at 121°C for 1 hr. showed a lignin content of 16.93%, CP pretreated with ethanoic acid, Sulfuric acid and Sodium Hydroxide gave lignin contents of 27.88%, 24.62% and 18.19% respectively. The observed increase in lignin content may be due to the disruption of the

Lignocellulosic crystalline complex. Sun and coworkers studied the effectiveness of different alkaline solutions in delignification and dissolution of hemicellulose in wheat straw. They found that using 1.5% sodium hydroxide for 144 hours at 20°C, resulted in 60% release of lignin and 80% release of hemicellulose. Dilute acid and enzymatic saccharification procedures have been used for conversion of corn fiber to fermentable sugars [8]. They found that corn fiber pretreated with 0.5% H₂SO₄ at 121°C for 1 h facilitated commercial enzymes to highly hydrolyze remaining starch and hemicellulose components without generation of inhibitors such as furfural and hydroxymethyl furfural (HMF), which are generally considered inhibitors for fermentative microorganisms.

Cassava peels pretreated with water gave hemicellulose content 37.76%, while CH₃COOH, H₂SO₄ and NaOH gave hemicellulose contents 39.54%, 41.50% and 44.34% respectively, indicating a release from the lignocellulosic structural barrier. There was also an increase in contents of reducing sugars, with H₂SO₄ pretreated CP having the highest reducing sugar content of 190.04 mg/mL followed by NaOH (132.7 mg/mL), CH₃COOH (51.5 mg/mL) and water (23.15 mg/mL). This is consistent with the work of [11] who observed that pretreatment with NaOH of 1% and 2% yielded significantly higher total reducing sugars than that with 3% NaOH due to higher lignin removal. Other workers also reported an increase in glucose yield obtained from cassava peel hydrolyzed with 2.5%, 5%, 7.5% and 10% phosphoric acid [6]. Results observed using 2.5% acid concentration, showed a gradual increase in glucose yield which peaked after 30 minutes of reaction time.

5 Conclusion

This study showed the impact of hot Ethanoic acid, Sulfuric acid and Sodium Hydroxide on the structural and compositional character of cassava peels and the corresponding reducing sugar content. The effect of Sulfuric acid pretreatment enhanced better hydrolysis of CP and gave higher reducing sugar content, making it a better pretreatment method in the conversion of lignocellulosic biomass to ethanol. Sodium Hydroxide pretreatment was also effective in the breaking down of the lignocellulosic barrier of CP, releasing a good percentage of reducing sugars. Ethanoic acid and hot water pretreatment, however were not as effective.

REFERENCES

[1] K.C. Anuj, and S. Silvio, "Sustainable Degradation of Lignocellulosic Biomass - Techniques, Applications and Commercialization". Publisher: In Tech, Croatia. 284pp.<http://dx.doi.org/10.5772/1490>

[2] O.A. Augustine, A.A. Opeyemi, M.O. Oyinlola, E.O. Temitayo E. O. "Compositional analysis of lignocellulosic materials: Evaluation of an economically viable method suitable for woody and non-woody biomass". *American Journal of Engineering Research* 2015 (AJER) Vol-4, Issue-4, pp-14-19. e-ISSN: 2320-0847 p-ISSN : 2320-0936

[3] A.K. Kumar, and S. Sharma, "Recent updates on different methods of pretreatment of lignocellulosic feedstocks: a review". *Bioresour Bioprocess* (2017) 4(1): 7-9

[4] A. Mohammed, S.B. Oyeleke, and E.C. Egwim, "Pretreatment and hydrolysis of cassava peels for fermentable sugar production". *Asian Journal of Biochemistry*. (2014) Vol. 9(1): 65-70

[5] D. Olga, "Investigation of lignin by FTIR Spectroscopy". *Macromolecular Symposia* 265(1): 61-68. DOI: 10.1002/masy.200850507.

[6] O.E. Onyelucheya, J.T. Nwabanne, C.M. Onyelucheya, O.E. Adeyemo, "Acid Hydrolysis of Cassava Peel". *International Journal Of Scientific & Technology Research*. (2016) Vol 5, ISSUE 01, 184-188.

[7] D. Pinaki W. Lhakpa and S. Joginder. "Simultaneous Saccharification and Fermentation (SSF), an Efficient Process for Bio-Ethanol Production": An Overview. *Biosciences Biotechnology Research Asia*, April 2015. Vol. 12(1), 87-100

[8] B.C. Saha, and M.A. Cotta, "Ethanol production from alkaline peroxide pretreated enzymatically saccharified wheat straw". *Biotechnology Prog* (2006) 22, 449-453.

[9] R. Sun, J.M. Lawther, and W.B. Banks, "Influence of alkaline pre-treatments on the cell wall components of wheat straw", *Industrial Crops and Products*, (1995) vol. 4, no. 2, pp. 127-145, 1995.

[10] G. Venkatachalapathy, R.K. Krishnappa and T.G. Sirangala "Estimation of sugar and bio ethanol from different decaying fruits extract". *Advances in Applied Science Research*, (2014) 5(1):106-110

[11] Z. Wang, D.R. Keshwani, A.P. Redding, and J.J.Cheng, "Sodium hydroxide pretreatment and enzymatic hydrolysis of coastal Bermuda grass". *Bioresource Technology* (2010); doi: 10.1016