The production of bioethanol from mango (*Mangifera indica*) peel and plantain (*Musa paradisiaca*) peel via water pretreatment, dilute acid hydrolysis and fermentation supported by *Saccharomyces cerevisiae*

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Abstract

There is an urgent serious need to further curb global warming, considering its ongoing catastrophic effects. One way to do this is to use greener/fuels such as bioethanol, to eventually substitute fossil fuels. Bio-ethanol is a cleaner burning fuel, than fossil fuel and will pump less carbon dioxide into the atmosphere. It is one form of renewable energy sources. Bio-ethanol can be obtained via the fermentation of sugar rich sources, such as fruits or pre-treatment, followed by acid hydrolysis of lignocellulosic material and subsequent fermentation of the hydrolyzates. In this research we have explored, the use of mango (*Mangifera indica*) and plantain peel (*Musa paradisiaca*) as our ethanol feedstock, using *Sacchromyces cerivisae* for the fermentation phase. The pre-treatment phase involved the use of water under a pressurized atmosphere, whereas the acid hydrolysis was accomplished using 10% H_2SO_4. The % yield of ethanol was found to range from 1.033 ± 0.158 %. to 1.1 ± 0.2 v/v for M *Mangifera indica* and *Musa paradisiaca* respectively. This research provides a pathway for environmental management of lignocellulosic waste and the provision for renewable energy.

Keywords: global warming, catastrophic effects, bioethanol, fossil fuels, lignocellulosic material, (*Mangifera indica*) and plantain peel (*Musa paradisiaca*)

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1.0. INTRODUCTION

Energy consumption linked to the transportation sector has had significant contributions to world problems such as global warming, climate change, energy shortages, and health conditions related to air pollution[1]. Moreover, due to the increased consumption of conventional fossil fuels and their unpredictable change in prices there is an urgent need to develop an alternative renewable source of energy like bioethanol. Fossil oil is associated with global warming, climate change, and several other energy and security problems. Its use was projected to peak about 2007 and the supply is then expected to be extremely limited in 40-50 years[2].

Bioethanol has similar properties to gasoline in terms of high octane content, high flame speed, stoichiometric air-fuel ratio, and low heating value[3] . Its use decreases the consumption of crude oil and reduces the emissions of air pollutants (CO_2, NO_2, and SO_4) released in the atmosphere as a result of fossil fuel combustion[4]. Bioethanol (C_2H_6O) is a colorless, flammable, volatile liquid with a molar mass of 46.07 g/mole, a density of 0.789 g/cm^3, a melting point of −114 °C, and a boiling point of 78.37 °C [1] . It is widely used as a solvent, a fuel, and as a raw material for the production of other useful chemicals that have wide applications in the industry[1]. Bioethanol is a feasible substitute for a fossil fuel because of its superior environmental benefits over the fossil fuel it displaces (gasoline) and it is economically competitive with gasoline. Ethanol doesn't have significant environmental impact as fossil fuel combustion [3]. It has low air polluting effect and low atmospheric photochemical reactivity, further reducing impact on the ozone layer[5] It contributes little net CO_2 accumulation to the atmosphere and thus should curb global warming[5-8].

Bio-ethanol is also producible in sufficient quantities that can make a meaningful impact on energy demands, and also provides a net energy gain over the energy sources used to produce the fuel [3]. It's a renewable source of energy. Ethanol can be used in three primary ways as biofuel, namely, E10 which is a blend of 10% ethanol and 90% unleaded gasoline, a component of reformulated gasoline both directly and or as ethyl tertiary butyl ether (ETBE) and as E85 which is 85% ethanol and 15% unleaded gasoline. When mixed with unleaded gasoline, ethanol increases octane levels, decreases exhaust emissions and extends the supply of gasoline[9].

The production of bioethanol from food crops like corn and sugarcane could lead to food versus fuel controversies[10] . Therefore, there is a need to explore the use of other lignocellulosic biomass such as, fruit wastes or vegetable wastes which are consumed in abundance. The

utilization of fruit and vegetable wastes to generate bioethanol would help in not only solving the problem of energy security but this may also help in solving the problems of climate change and waste management.

Lignocellulosic biomass include fruit and vegetable wastes, forestry waste, agroresidues, Municipal Solid Waste (MSW) etc. can be used to produce bioethanol [11] . The main components of the lignocellulosic materials are cellulose (30 % to 50 % dry wt.), hemicellulose (20 % to 40 % dry wt.), and lignin (10 % to 20 % dry wt.)[12] . Table 1.0. reflects the composition of lignocellulosic material encountered in the most common sources of biomass.

Fruit and vegetable wastes are rich in cellulose and hemicellulose and have low lignin contents which makes them interesting for bioethanol production. The use of these lignocellulosic wastes for bioethanol production is a recent alternative with great promise and still under research. It is an efficient, cost-effective, and a food security-wise alternative[10] . Cellulose and hemicellulose fractions of lignocellulosic biomass are polymers of sugars that can be potential sources of fermentable sugars used for the production of bioethanol.

Table 1.0. Showing the Composition of Lignocellulose in Several Sources on a Dry basis[12] (Sun and Cheng, 2002 [12]

Lignocellulosic materials	Cellulose (%)	Hemicellulose (%)	Lignin (%)
Hardwood stems	40-55	24-40	18-25
Softwood stems	45-50	25-35	25-35
Nut shells	25-30	25-30	30-40
Corn cobs	45	35	15
Grasses	25-40	35-50	10-30
Paper	85-99	0	0-15
Wheat straw	30	50	15
Sorted refuse	60	20	20
Leaves	15-20	80-85	0
Cotton seed hairs	80-95	5-20	0
Newspaper	40-55	25-40	18-30

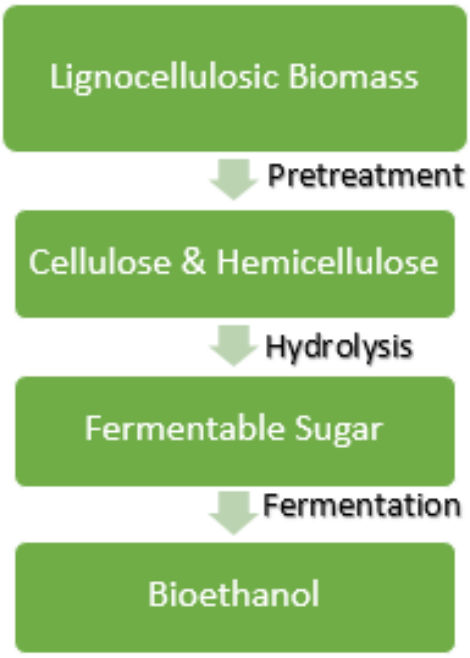
Waste paper from chemical pulps	60-70	10-20	5-10
Primary wastewater solids	8-15	NA	24-29
Swine waste	6.0	28	NA
Solid cattle manure	1.6-4.7	1.4-3.3	2.7-5.7
Coastal Bermuda grass	25	35.7	6.4
Switchgrass	45	31.4	12.0

Cellulose $(C_6H_{10}O_5)_n$ is a homogeneous polymer of high molecular weight, consisting of a linear chain of several hundred C-beta linked D-glucose units which can appear as a highly crystalline material. Hemicellulose is a branched heterogeneous polymer consisting of hexose sugars (D-glucose, D-mannose, and D-galactose) and pentose sugars (D-xylose and L-arabinose)[13] Both the cellulose and hemicellulose fractions of lignocellulosic biomass are potential sources of fermentable sugars used for the production of bioethanol. The hydrolysis of cellulose produces glucose which can then be converted to useful biochemical substances like bioethanol through biological processes[13].

Hemicellulose is insoluble in water at low temperature. However, its hydrolysis starts at a temperature lower than cellulose making it soluble at a higher temperature. Hemicellulose is more readily hydrolyzed to simple fermentable sugars compared to cellulose because of its branched, amorphous nature[14] . Lignin $[C_9H_{10}O_3\,(OCH_3)_{0.9–1.7}]_n$ is the most complex natural polymer that is amorphous and three-dimensional with phenylpropane units as the main building blocks. The most commonly encountered monomers in lignin are p-coumaryl alcohol, coniferyl alcohol, and sinapyl alcohol joined together by a set of linkages to create a matrix[15] . Lignin offers useful opportunities to obtain high-value products, such as carbon fibers, emulsifiers, dispersants, etc. However, `it is among the obstacles to the fermentation of lignocellulosic biomass because it is unaffected by chemical and biological degradation, hence it affects the quality of bioethanol production[16] .

The basic steps involved in the conversion of lignocellulosic biomass to ethanol are illustrated below in Fig. 1.0.

Fig.1.0. The basic steps involved in the conversion of lignocellulosic material to ethanol.



The production of fermentable sugars is usually approached in two steps namely pretreatment and hydrolysis[1] . Pretreatment is the first step of the process by which the cellulose and hemicellulose polymers are made accessible for further conversion. In this step, the hydrolysis of hemicellulose under mild conditions occurs, as well as the separation of the lignin fraction. However, the cellulose fraction is more resistant and therefore requires more rigorous treatment. The second step involves the enzymatic or acidic hydrolysis of cellulose, using cellulase enzyme cocktails or an acidic medium respectively. There are two types of acid hydrolysis: dilute and concentrated. Dilute acid hydrolysis is done at higher temperatures, utilizing a low acid concentration, while concentrated acid hydrolysis is carried out at a lower temperature using a high acid concentration[17]. Following the production of fermentable sugars from the hydrolysis of the cellulose and hemicellulose fraction of lignocellulosic biomass, the fermentation process is used to produce bioethanol. In this process, *Saccharomyces cerevisiae*, more commonly known as "baker's yeast consumes the simple sugars and produces bioethanol, along with carbon dioxide (CO_2) [1] . as shown in Fig. 2.0.

Fermentation is the process of energy production in a cell in an anerobic environment with the lack of an external electron acceptor[18]. Sugars are the common substrate of fermentation and

the products include ethanol, lactic acid and hydrogen. In some instances, compounds such as butyric acid and acetone are produced [18].

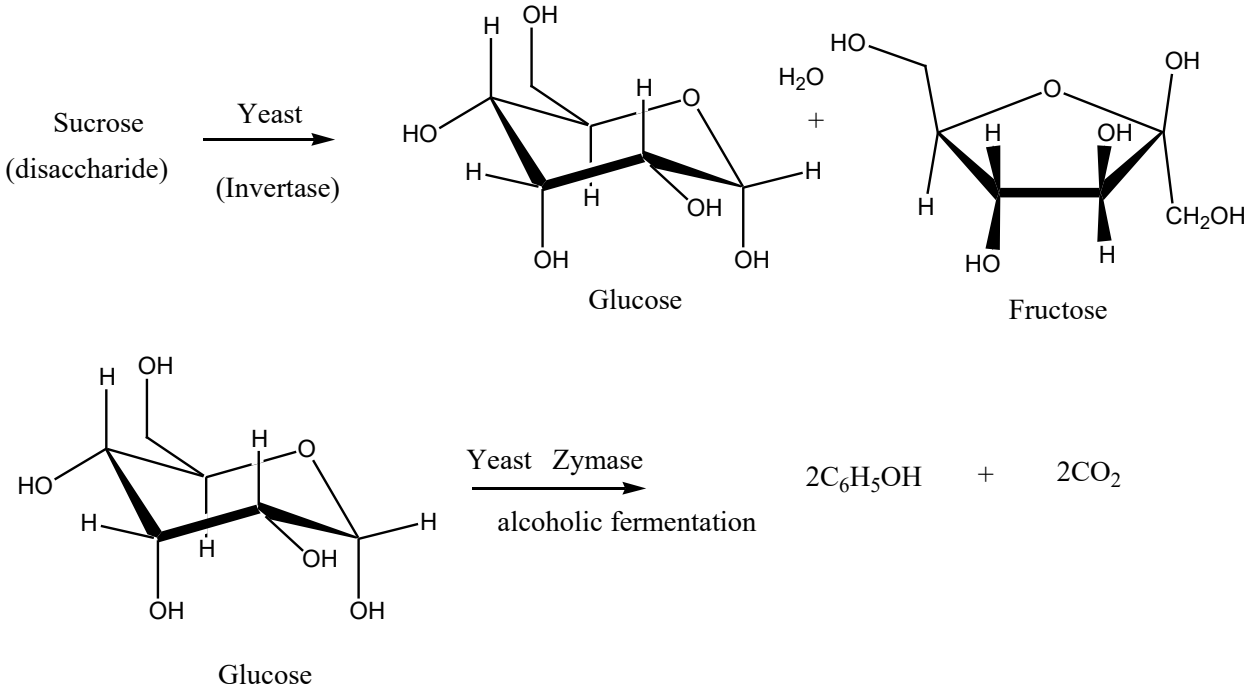


Fig. 2.0

The fermentation process begins with the yeast breaking down the different forms of sugar in any fermenting matrix. *Saccharomyces cerevisiae* contains two enzymes that is very important for the yeast enzyme activity in the fermentation process. These two enzymes are called Invertase and Zymase and they functions are similar but somewhat prerequisite to each other. Invertase aids in converting any sucrose sugar that is present in any biomass that is used in fermentation to glucose and fructose while zymase aids in the conversion of glucose to ethanol [18]., Fig. 1.0.

During Fermentation, starch is first hydrolysed to maltose by the action of the enzyme diastase. This enzyme is obtained from germinating barley seeds or malt. Maltose is converted to glucose by the enzyme maltase.

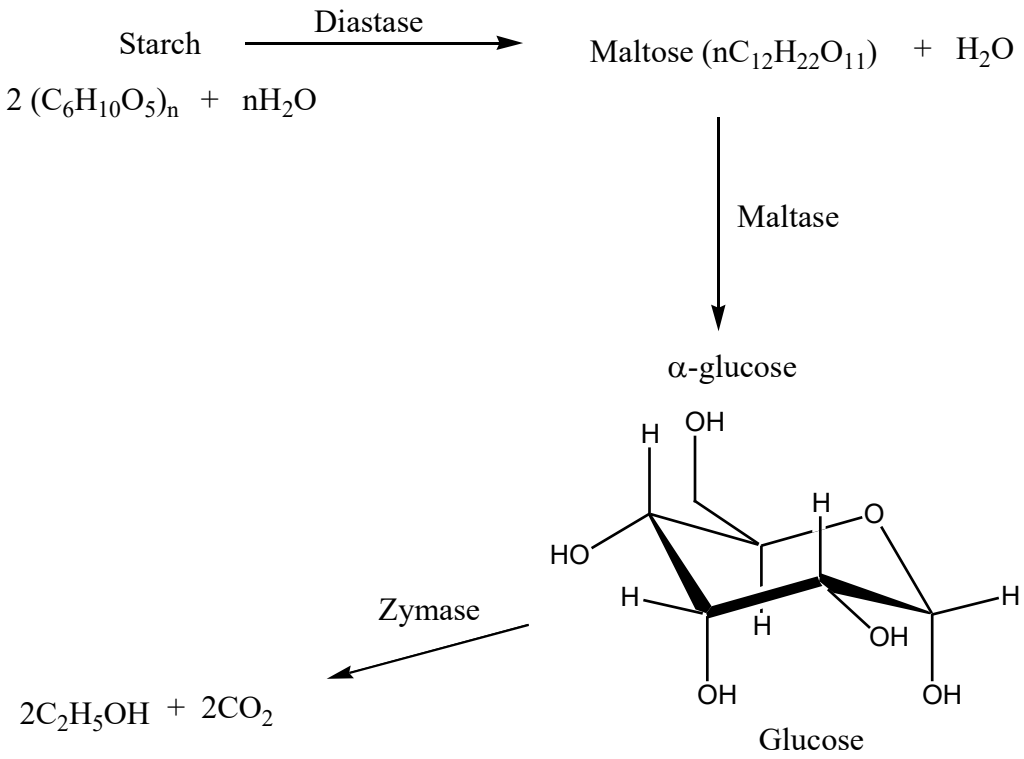


Fig. 3.0

maltase. Glucose is then fermented to ethanol via the enzyme zymase [19], Fig. 3.0. Once the sugars are broken down into monosaccharides, the yeast can now use them. *Saccharomyces cerevisiae* is able to perform both aerobic and anaerobic respiration.

Plantain peel and mango peel are lignocellulosic agricultural wastes that have the potential to produce bioethanol as a renewable form of energy Thus, the objectives of the research were

(1) to investigate the production of bioethanol from plantain (*Musa* × *paradisiaca)* peel and mango (*Mangifera indica*) peel via water pretreatment, dilute H_2SO_4 hydrolysis of their lignocellulosic content and fermentation supported by *Saccharomyces cerevisiae.*

(2) To compare the % yield of ethanol from the two lignocellulosic feed stock.

This research was focused on converting the lignocellulosic content of these wastes into fermentable sugar for bioethanol production in a readily available, cost-effective and environmentally sustainable way. These two feedstock were selected as the lignocellulosic

biomass due to their abundance, low cost and accessibility in Guyana. Their use also solve the problem of waste management[10] and reduce government expenditure on fossil fuel. It was hypothesized that: There is a significant difference between the mean concentrations of bioethanol produced by mango peel and plantain peel after fermentation (Ha) or there is no significant difference between the mean concentrations of bioethanol produced by mango peel and plantain peel after fermentation (Ho).

Bioethanol production from acid pretreated water hyacinth by separate hydrolysis and fermentation has been reported [20] . . The study evaluated water hyacinth as a feedstock for bio-ethanol production. Various acids were used for pre-treatment. However, it was found that H_2SO_4 was the most effective. Structural changes in the matrix prior and after pre-treatment were evaluated via SEM, FTIR and XRD analysis. Bioethanol was obtained with a percentage yield of 0.292% w/v.

Bio-ethanol production from rice & wheat husks after acid hydrolysis & yeast fermentation is noted [21] . The objective of the research was to produce bio-ethanol from rice & wheat husks via fermentation process and to determine the effect of temperature on bio-ethanol yield. H_2SO_4 was used for the pre-treatment process. The highest ethanolic concentrations were obtained at a temperature of 35°C and pH 6.0.

Acid hydrolysis of sawdust waste into bio-ethanol has received attention. The accumulation of saw dust is polluting the environment. One way to remove saw dust to use saw dust as a feed stock for bioethanol production. Authors use the pre-treatment, acid hydrolysis, fermentation route to produce bio-ethanol. For acid hydrolysis, H_2SO_4 and HCl at 0.6M, 6M, 11M and stock concentrations were used. Fermentation was conducted in a continuous stir tank reactor (CSTR) using *Saccharomyces cerevisiae*). H_2SO_4 produced a glucose yield of 92.9% and ethanol 80.9%[22]. . There are an increasing number of articles on bio-ethanol production from lignocellulosic material [23-40] , demonstrating intense research in their area.

2.0. METHODOLOGY

2.1. Description of the study area

Lignocellulosic residues of mango and plantain peels were obtained from mangoes and plantains bought at Stabroek Market in Georgetown, Guyana. The experimental aspect of this

project was conducted in the alcohol analysis laboratory of the Food and Drug Department of the Ministry of Public Health.

2.2. Research design

The research design that employed in this study is the experimental research design conducted using the scientific approach. This research design relies on statistical analysis to prove or disprove a hypothesis, making it the most accurate form of research. The experiment was carried out using the completely randomized experimental design to compare the two the yield of bioethanol produced from the two feedstock materials. A simplified statistical analysis, the 2-sample T-test was used to analyse the data. The 2 sample T-test helps to determine whether the difference observed in the two samples is due to natural variation or real difference.

2.3. Sample Collection and Preparation

Mangoes and plantains were obtained, washed and their outer coats were removed and cut into smaller pieces. For each sample, 500g of the chopped peel was weighed and blended in a food processor.

2.4. Water Pre-treatment

Pre-treatment was done to reduce the strength, compactness, and crystalline nature of cellulose aiding in the hydrolysis of lignocellulosic biomass to simple sugar. 500g of peel and 3000ml of water (6:1 water to fibre ratio) were added to a large pressure cooker and cooked for three hours. The sample was allowed to cool and later filtered. The residue was allowed to air dry and the filtrate was discarded.

2.5. Acid Hydrolysis

Hydrolysis was done to further degrade the polysaccharides present in the pre-treated plantain peel and mango peel fibres into fermentable reducing and non-reducing simple sugars. Under the fume hood, 200g of peel fibre and 1,125.5ml of 10% H_2SO_4 (6:1 acid to fibre ratio) were added to a large beaker and mixed well. The mixture was then cooked in an autoclave at 120°C for four hours. The sample was allowed to cool and later filtered. The residue was discarded and the filtrate was stored in a cool place.

2.6. Fermentable Sugars Assay

Reducing sugar assay was carried out using Benedict's test to confirm the presence of reducing sugars prior to the fermentation process. 1ml of the sample and 2 ml of benedict's reagent were added to a test tube and heated in a hot bath. The colour change was observed and recorded.

The content of the fermentable non reducing sugar (sucrose) was measured by adding one drop of the sample at 20°C to a digital refractometer recording the percentage (%) brix.

2.7. Fermentation

Fermentation was the final stage of bioethanol production. *Saccharomyces cerevisiae* (baker's yeast) was used to convert the simple fermentable sugars produced during hydrolysis into ethanol. The pH of the acid hydrolysate sample was adjusted to a pH level between 4.0 and 4.5 using concentrated KOH. 400ml of a 12 % mixture of deionized water and *Saccharomyces cerevisiae* along with 400ml of the sample were added to a fermentation vessel and left to ferment for 72 hours. Fermentation was carried out in triplicates along with a controlled experiment. After fermentation the samples were centrifuged and distilled. The percentage brix of the samples after fermentation was measured using the refractometer and the results were recorded.

2.8. Ethanol Analysis

Ethanol analysis was carried out using the density meter to determine the concentration of ethanol by volume produced from the plantain peel and mango peel samples. The distilled samples were tested at 20^{o}C to determine the percentage of ethanol content (v/v) using a hand-held density meter. The results were recorded.

NB: This procedure was carried out for both the plantain peel and mango peel samples

3.0. RESULTS

Table 2. Showing the Results for the Benedict's Test for Reducing Sugars for the Acid Hydrolysate Samples of Plantain Peel and Mango Peel

Mango Peel Acid Hydrolysate	**Plantain Peel Acid Hydrolysate**
Positive (brown precipitate)	Positive (brown precipitate)
	

Table 3. Showing the Percentage Brix Content of Mango Peels Acid Hydrolysate Samples Before Fermentation

Sample	A	B	C	Average
% Brix	10.5	10.5	10.5	**10.5 ±0**

Table 4. Showing the Percentage Brix Content of Plantain Peel Acid Hydrolysate Samples Before Fermentation

Sample	A	B	C	Average
% Brix	11.5	11.5	11.5	**11.5 ±0**

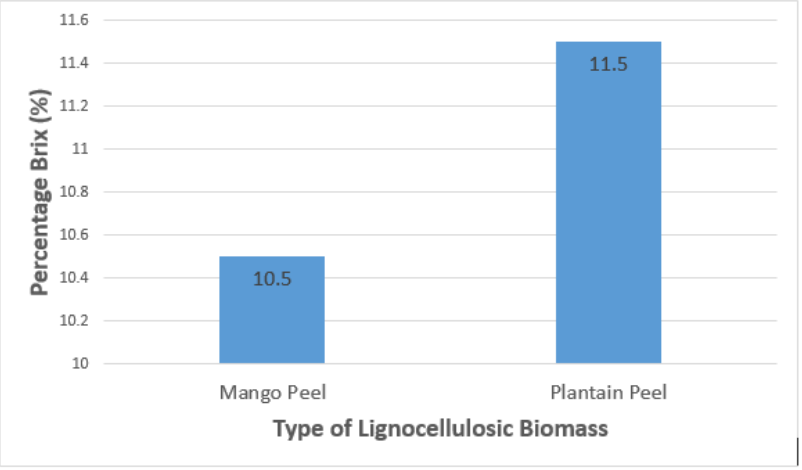


Figure 4.0. Bar Graph showing the Mean Percentage Brix Content of the Mango Peel versus the Plantain Peel Samples.

Table 5 Showing the pH of Mango Peel Acid Hydrolysate Samples Before Fermentation

Sample	A	B	C	Average
pH	4.11	4.11	4.11	**4.11 ±0**

Table 6 Showing the pH of Plantain Peel Acid Hydrolysate Samples Before Fermentation

Sample	A	B	C	Average
pH	4.26	4.26	4.26	**4.26±0**

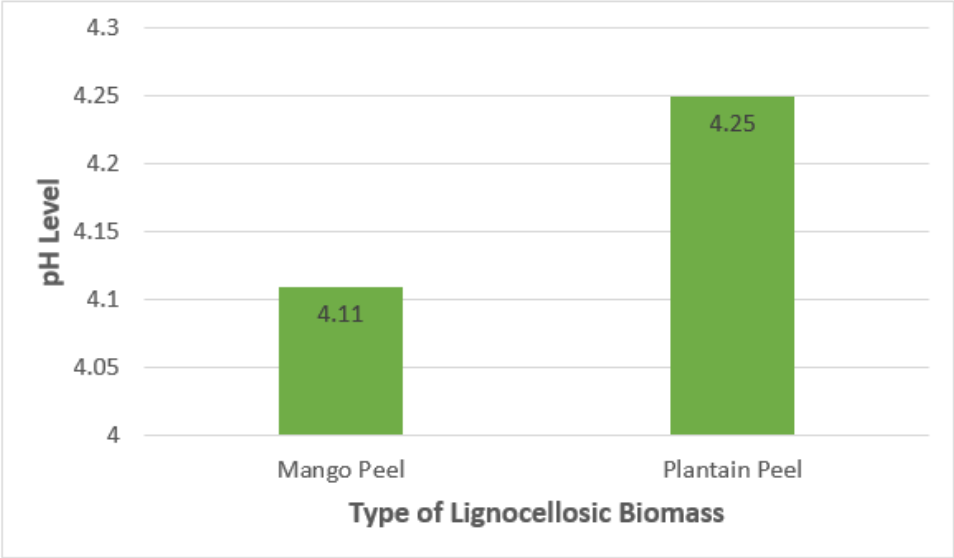


Figure 5.0. Bar Graph showing the Mean pH of the Manho Peel versus Plantain Peel samples before Fermentation.

Table 7 Showing the Percentage Alcohol Obtained from the Samples of Mango Peel

Sample	A	B	C	Average
% Alcohol (v/v)	1.0	0.9	1.2	**1.033 ± 0.152753**

Table 8 Showing the Percentage Alcohol Obtained from the Samples of Plantain Peel

Sample	A	B	C	Average
% Alcohol (v/v)	0.9	1.1	1.3	**1.1 ± 0.2**

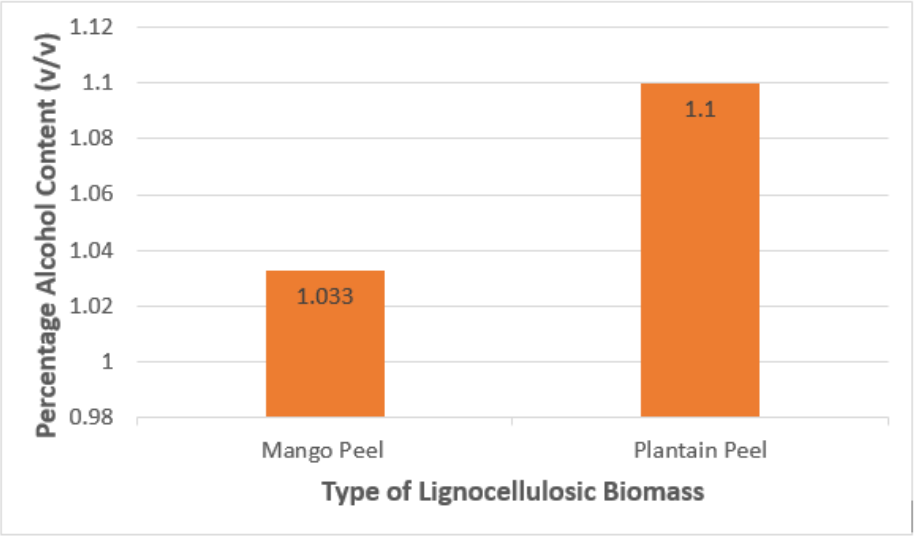


Fig 6.0. Bar Graph Showing the Mean Percentage of Ethanol Content of the Mango Peel Versus the Plantain Peel Samples.

Table 9 Showing the Percentage Brix Content of Plantain Peel Samples After Fermentation

Sample	A	B	C	Average
% Brix	9.0	8.8	8.8	**8.867 ± 0.11547**

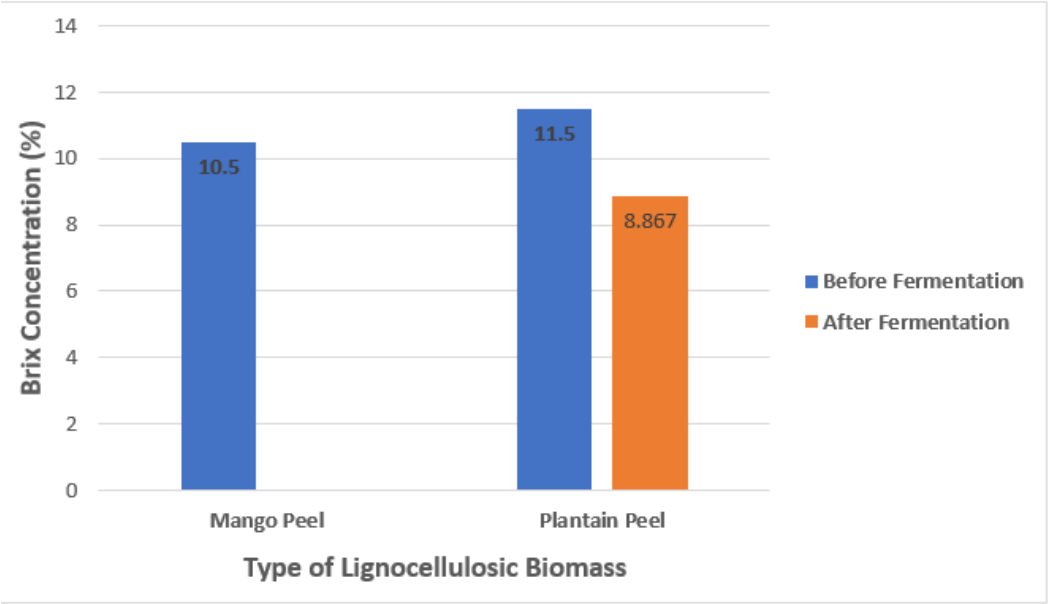


Fig. 7.0. Column Graph Comparing the Mean % Brix Concentrations of the Mango Peel Versus Plantain Peel Samples Before and After fermentation.

TREATMENT OF RESULTS

Statistical Analysis

2-Sample T-test to Compare the Mean Percentage (%) Bioethanol Produced from Mango Peel and Plantain Peel

Table 10.0 Showing the Review of the Data

Group	Plantain Peel	Mango Peel
Mean	1.03300000	1.10000000
SD	0.15275300	0.20000000
SEM	0.08819199	0.11547005
N	3	3

Table 11 Showing the Intermediate Values Used in Calculations

t-value	0.461
Degrees of freedom (df)	4
Standard error of difference	0.145

P value and statistical significance:

The two-tailed P value equals 0.6687

By conventional criteria, this difference is considered to be not statistically significant.

Confidence interval:

The mean of Mango Peel Minus Plantain Peel equals -0.06700000

95% confidence interval of this difference: From -0.47040870 to 0.33640870

4.0. DISCUSSION

The result from the Benedict's test for reducing sugar is shown in **Table 2.0** and illustrate that the acid hydrolysate samples of both mango and plantain peels were brown signifying highest level of sugars. This brown colour is observed because the blue copper (II) ions present in the Benedict's reagent are reduced to copper (I) ions in the presence of sugars. The ions are precipitated as a reddish brown copper (I) oxide which is insoluble in water. The brix concentration, which indicates the sucrose (non-reducing fermentable sugar) content, was measured using a digital refractometer recorded. **Tables 3** and **4** show the percentage Brix content obtained for the acid hydrolysate samples of mango peel and plantain peel. **Figure 4.0** compares the mean percentage brix concentration for mango peel and plantain peel which were 10.5% and 11.5 % respectively. The plantain peel sample had a 1% greater yield of sucrose (11.5 ± 0.0) than the mango peel sample (10.50 ± 0.0). The exact quantitative amounts of total reducing sugar present in the samples were not measured. However, the fermentable sugar assay indicated that there was a relatively high percentage of fermentable sugars that could be used as substrates to proceed with fermentation.

Fermentation was the final stage of bioethanol production, utilizing *S. cerevisiae* to convert the fermentable sugars produced during hydrolysis into ethanol with the help of invertase and zymase enzymes present in *S. cerevisiae*. **Figure 5.0** shows the pH levels of the acid hydrolysate samples of mango and plantain peel which were 4.11 and 4.26 respectively. A pH level between 4.0 and 4.5 is an essential condition for the fermentation process utilizing *S. cerevisiae.* The low yield of ethanol obtained from the acid hydrolysate samples of mango peel and plantain peel were 1.033% and 1.1% respectively are shown in **Tables 7 and 8**. This was due to the fermentable sugars not been utilized. This is indicated by the Brix content shown in Table 9.0. Only a small percentage of the reducing sugar was utilized as judged by the average brix, 8.867 ± 0.12. **Figure 3** compares the mean percentage ethanol (v/v) content obtained from the mango and plantain peel samples. It shows that the plantain peel produced 0.067 of a percent ethanol more than the mango peel sample. **Figure 7.0** compares the mean brix concentration of plantain peel samples before and after fermentation. Unfortunately, this test was not carried for the mango peel samples after fermentation. This was done to obtain a rough estimate of how much sugar was used up during

fermentation. It indicates that all the fermentable sugars were not used up during fermentation despite the relatively high content of fermentable sugars.

The strain of *S. cerevisiae* that is usually employed in bioethanol production produces a large quantity of ethanol, and has the advantage over other organisms of resisting multiple inhibitors such as furans, phenolic compounds and organic acid. However, the strain of *Saccharomyces cerevisiae* (baker's Yeast) used is for fermentation in this study was not the best choice for optimum production of bioethanol. There is a great possibility that the *S. cerevisiae* used was inhibited by degradation by-products of acid hydrolysis of the lignocellulosic biomasses used. Therefore, there is a need to extend further research work that utilizes *S. cerevisiae* engineered to withstand inhibitions. Additionally, research can be conducted with the intention to determine the best conditions for the optimum production of ethanol utilizing the method employed in this study.

A two sample T-test was done to compare the mean percentage ethanol produced from mango peel and plantain peel. **Table 10.0 and Table 11.0** shows the statistical analysis of the data and. **Table 11.0** shows the intermediate values used in the calculations: t-value (0.461), df (4) and the standard error of difference (0.145). The P-value obtained was 0.6687 which is greater than 0.5 and suggest there is no significant difference between the mean percentage ethanol (v/v) produced by plantain peel and mango peel. Therefore, the null hypothesis was accepted and the alternative hypothesis was rejected.

5.0. CONCLUSION

This study investigated and compared the yield of bioethanol from plantain peel and mango peel via water pretreatment, dilute H_2SO_4 hydrolysis and fermentation supported by *Saccharomyces cerevisiae.* The water pretreatment and dilute H_2SO_4 hydrolysis of both samples gave relatively high yields of total reducing sugar- proven by the benedict's test of reducing sugars. The brix content obtained for mango peel and plantain peel were 10.5% and 11.5 % respectively which was an indication that the strain of *Saccharomyces cerevisiae* used was inhibited. Fermentation of the acidic hydrolysates of mango peel and plantain peel yielded low percentages of bioethanol -1.033% (v/v) and 1.1% (v/v) respectively. The low yield of bioethanol is an indication that the strain of

Saccharomyces cerevisiae used was not engineered to withstand inhibitions from degradation by-products produced during the acid hydrolysis of the lignocellulosic biomasses used. The two tailed P-Value of the 2- sample T-test was 0.6687 which indicated that there is no significant difference between the mean concentrations of bioethanol produced by mango peel and plantain peel after fermentation. Therefore, the null hypothesis was accepted and the alternate hypothesis was rejected.

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