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Extraction of bioethanol from cassava root and cocoyam using saccharomyces cerevisiae strain with differing H_2SO_4 concentrations

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Abstract: Bioethanol (CH_3CH_2OH), or ethyl alcohol, is a renewable biofuel produced by fermentation of various agricultural products. Cassava and cocoyam tubers were utilized as starting materials to extract ethanol. The two raw materials were separately weighed, peeled, washed in separate bowls, ground into a paste, and then mixed with distilled water. The samples were decanted and pressed with a cloth to remove the remaining water. The two samples were dried for 24 hours and then pre-treated in an autoclaved chamber for 15 minutes at 120^0C. The two samples were hydrolyzed in five different concentrations of dilute H_2SO_4 and then placed in a water bath for 5 hours, followed by adding NaOH to reduce the pH of the hydrolyzed starch solution. A culture solution (S. Cerevisiae) and broth media were separately prepared. The culture solution was added to the media solutions and incubated for 72 hours at 30^0C and 200 rpm. The samples were conditioned in a shaking incubator and fermented for 7 days. The samples were distilled in a 500 mL round bottom flask at 78^0C for 3 hours to extract ethanol from the solution. This procedure was done for cassava and cocoyam samples one at a time. The ethanol from cassava and cocoyam at different acid concentrations was measured and recorded. The results show that the ethanol yield from cassava at different acid concentrations was greater than that of cocoyam.

Keywords: Cassava, cocoyam, ethanol, starch, sulphuric acid, fermentation.

1. Introduction

The primary energy sources for most industries during the 20th century are fossil fuels (petroleum, coal, and natural gas) [1, 2]. They are still globally considered the most important feedstock for energy production, but they are now becoming scarce and no longer considered sustainable [3-5]. Ethanol is a biofuel with the chemical formula C_2H_5OH. Using starch materials to extract bioethanol was first discovered in the United States of America in the 20th century [6-10]. Nowadays, bioethanol is an alternative energy source to other fuels because it is an environmentally friendly renewable resource that contributes to the reduction of greenhouse gas emissions that affect climate change [6, 8, 11-14]. It also helps to mitigate the adverse global environmental effects of crude oil. In 2019, it was reported that about 110 billion liters of bioethanol were mainly extracted from corn and sugarcane crops, with about 59.8 billion liters produced from corn in the United States of America [15-19].

The first-generation feedstock is the most abundant renewable carbon source, which quickly digests and converts to biofuels compared to cellulose, and the second-generation feedstock is [20-25]. Fuel or energy crops such as maize, cassava and cassava products, wheat crops, waste straw, guinea corn husk, rice husk, millet husk, sawdust, sorghum plant, sugar cane, and sweet potato, etc are carbohydrate sources utilized to extract the ethanol [26-28]. Ethanol can be extracted from a variety of agricultural materials (feedstock). The average annual yield of ethanol from several feedstocks is cassava (31.25 metric ton/ha), sugar beet (56.00 metric ton/ha), sweet potato (30.00 metric ton/ha), wheat (9.00metric ton/ha), sugar cane (62.50metric ton/ha), rice (7.31metric ton/ha), sorghum (6.35 metric ton/ha), and starchy corn (6.00 metric ton/ha)[16]. Cassava, cocoyam, and other carbohydrates are also used to produce adhesives, cosmetics, pharmaceuticals, coatings, and dispersants for medicines, biomass fuels, paint fillers, etc [29, 30]. Fermentation is widely used in laboratory and industrial processes to transform complex sugars and carbohydrates into simple sugars in the presence of enzymes ($C_6H_{12}O_6 \xrightarrow{\text{s. cerevisiae}} C_2H_5OH_{(aq)} + CO_{2(g)}$). The microbiological process in which carbohydrate compounds are broken down into ethanol and carbon dioxide in the presence of an

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enzyme is referred to as fermentation. Ethanol is a biofuel with a high-octane number that is used as an octane enhancer to replace lead in petrol and is now principally utilized as a petrol substitute in vehicles [31-36] . Ethanol can also be produced by the chemical reaction of ethylene and steam [37].

Serra Leone is one of the bioethanol-producing countries in West Africa where sugar cane is utilized as the primary feedstock for ethanol extraction. Other energy-producing crops such as cassava, cocoyam, rice, and maize are produced in large quantities. According to FAO, 4, 761,385.00 tons of cassava, 2,576 tons of cocoyam, 919,785 tons of rice, and 25,000 tons of maize were produced in Sierra Leone in 2017, 2018 (cocoyam &rice), and 2019, respectively. Rice is a staple food in Serra Leone, and its production quantity is insufficient to feed the population of about 7.65 million people as of the 2018 national census. Even the waste from the rice is too small to produce enough bioethanol. Therefore, cassava and cocoyam with huge production quantities, as mentioned above, were utilized for this research. Only a smaller number of the Sierra Leone population feeds on cassava and cocoyam, and most of this produce often ends up in dustbins as waste. The researchers are therefore tempted to investigate the commercial viability of these agricultural energy crops in a comparative experimental analysis of ethanol production.

In many developing nations, cassava is a popular tropical-grown crop that significantly contributes to food security and income generation for millions of people [38] in sub-Saharan Africa. Some local traditional foods, such as gari, foofoo, etc in West Africa are cassava products. Ethanol extraction from molasses is done in many countries, but cassava is more beneficial for ethanol production [39-42]. Cassava cultivation in Sierra Leone is seasonal, adapts to growing conditions, requires minimum inputs, and is easy to harvest. In Brazil, dried cassava bagasse production in 2014 was estimated to be 3.5 million tons and can produce an additional 866.6 million liters of bioethanol, with a subsequent increase of 3.04% (28.48 billion) liters [43-46].

Cocoyam belongs to the Araceae family and is widely cultivated for its edible corms, petioles, and leaves [47-49]. The plant is a food staple in most African, South Asian, and Oceanic cultures and is believed to have been one of the earliest cultivated plants [50-54]. Cocoyam is utilized in this work in a comparable study with cassava for bioethanol production because of its wide usage, abundance, and little information on bioethanol production in biotechnology. The main objective of this research is to do a laboratory analysis of bioethanol yield extracted from cassava and cocoyam tubers grown in Sierra Leone using saccharomyces cerevisiae and to evaluate which crop has an energy resource advantage over the other.

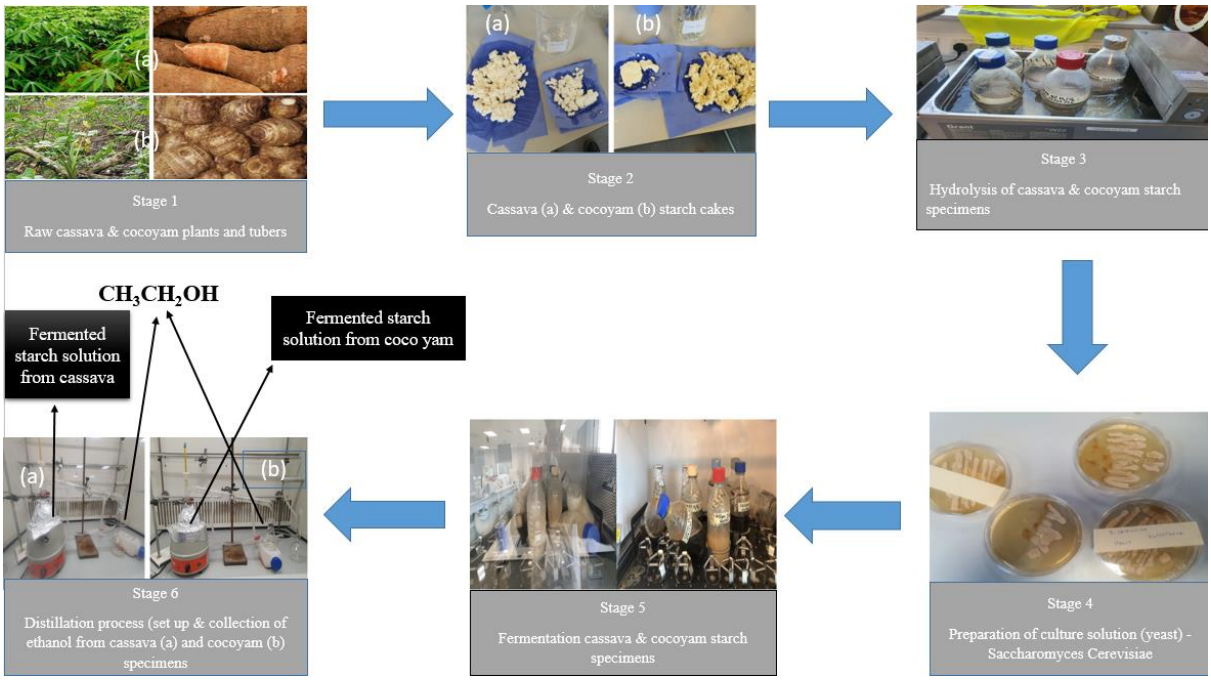


Figure 1. Stages of ethanol production from cassava & cocoyam tubers

The processing and procedures of laboratory production of ethanol from cassava and cocoyam (Figure 1) were done in the following stages:

1.1 Sample collection and preparation

Fresh cassava and coco yam tubers were washed and dried for 5 days before being packaged and sent to

Ireland. 2kg each of cassava and cocoyam was peeled off, washed in separate bowls, ground into powder using an electronic blender, and then mixed with distilled water. The pulp of the two samples was sieved in different bowls using screen mesh and suspended over water. This was done to remove fibrous and other unwanted materials in the starch pulp. The two samples were left for 24 hours for the milky substance to settle before decantation. The white milky substance (starch) at the bottom of each was pressed with a white cloth to remove the remaining water. The samples were then dried in an oven for 24 hours at a temperature of 40^0C and kept in a cupboard for further laboratory use.

1.2 Pretreatment of the cassava and cocoyam starch

50g of starch produced from the cassava and cocoyam was measured pre-treated. The measured samples were placed in reagent bottles capped with aluminum foil and filled with 500 mL of distilled water. The samples were autoclaved for 15 minutes at a temperature of 120^0C. The reagent bottles were removed after autoclaving, allowed to cool for 30 minutes, and labeled with the starch name and percentage of acid used.

1.3 Dilute acid hydrolysis of the starch samples

The starch samples were hydrolyzed by preparing five (5) different concentrations of dilute H_2SO_4 from 150 mL of H_2O and 150 mL H_2SO_4 as follows: 2%(v/v) diluted sulphuric acid (20mL H_2SO_4 in 500 mL starch solution), 4%(v/v) diluted sulphuric acid (40mL H_2SO_4 into 500 mL starch solution), 6%(v/v) diluted sulphuric acid (60mL H_2So_4 into 500mL starch solution), 8%(v/v) diluted sulphuric acid (80 mL H_2SO_4 into 500mL starch solution) and 10%(v/v) diluted sulphuric acid (100mL H_2SO_4 into 500mL starch solution). To break the bonds of the starch molecules, all five samples were pre-treated by placing them in a water bath for 4 hours. This was immediately followed by adding NaOH to the pre-treated solution to reduce the pH of the acidic starch solution to about 5.5 to enable the survival of the micro-organisms.

$$(C_6H_6O_5)_n \xrightarrow[dilute\ H_2SO_4]{+H_2O} (CH_2O)_n$$
Reducing sugar

1.4 Growing of the culture (yeast)

This stage involves the preparation of a culture solution (yeast) in which 0.2g of yeast extract, 1 g peptone, 10g dextrose, and 1g Agar were mixed with 100 mL distilled water in a 250 mL conical flask. The solution was autoclaved for 15 minutes at 120^0C, poured into five dishes, and cooled for 24 hours at room temperature. The dishes or plates were placed in a shaking incubator for 72 hours at 30oC and 200rpm to form Saccharomyces Cerevisiae strains. The strains were inoculated inside fume hoods to avoid contamination that would kill the fungi.

1.5 Preparation of broth media

The next stage is broth media preparation. This was done by combining 30g of dextrose sugar, 0.6g yeast, 3g peptone, and 3g MgSO4.7H2O in a conical flask and mixed with 300 mL of distilled water. This combination was repeated three times to prepare 4 bottles of media solutions. Two of the four media solutions were utilized for cassava starch, and the other two for cocoyam starch. The four conical flasks were covered with aluminum foil and autoclaved for 15 minutes at 120^0C in an autoclave chamber. The solution was left to cool down to room temperature. Saccharomyces Cerevisiae strains were added to each of the four media solutions using specially designated rods inside fume hoods and then placed into a shaking incubator at 30°C and 200 rpm for 72h.

1.6 Starch fermentation

This stage is the second to last stage that involves the fermentation process. The conical flasks of the hydrolyzed cassava and cocoyam starch solutions were placed in Fume hoods and then mixed with 25 mL of the prepared culture media (Saccharomyces Cerevisiae) at 30^0C and pH range 5.0 – 5.5. The mixtures were then subsequently conditioned for 30 minutes at 30°C at a rotation between 120rpm and 200rpm in the shaking incubator and left to ferment for 7 days.

1..7 Ethanol distillation

The final processing stage is the laboratory distillation process of ethanol. The distillation process was set up using the following laboratory apparatus: 500 mL round bottom flask, thermometer, retort stand, burner, conical flask, water tap, and condenser. The sample prepared from cassava was first distilled. Different concentrations of the cassava solution were first transferred in a separate 500 mL round bottom flask, and each was heated at a temperature of 78^0C for 3 hours to extract/separate the ethanol from the solution. The same procedure was repeated for the cocoyam specimen to extract the ethanol from the solution. At the end of the distillation process, the amount of ethanol produced from the cassava and cocoyam starch solutions at different concentrations of dilute sulphuric acid was measured, and the results were recorded for analysis.

2. Results and Discussion

2.1 The role of the acid during hydrolysis

The cassava and cocoyam were ground to powder to enable an increase in lignin degradation during the pretreatment process. The powdered form also increases. Also, the powdered form increases the reaction between the dilute sulphuric acid and the samples, which augments the fermentation process. Cassava and cocoyam contain a carbohydrate build-

up material called lingo-cellulosic, mainly made of hemicellulose and cellulose, and lignin, a non-carbohydrate material. Lignin is reduced before the fermentation process of lignin [55]. Both samples were pre-treated by a physical method in which the samples were autoclaved for 15 minutes at 120^0C. This method offers significant pH alterations. Five concentrations (2%, 4%, 6%, 8%, and 10%) of sulphuric acid were used to hydrolyze the pre-treated samples to produce the reducing sugar (glucose). The samples were hydrolyzed for 3 hours in a shaking water bath. There was no time variation between the two samples, but comparative results showed that the sugar that reduced the most was produced by cassava starch compared to cocoyam using the spectrophotometric method (Table 1).

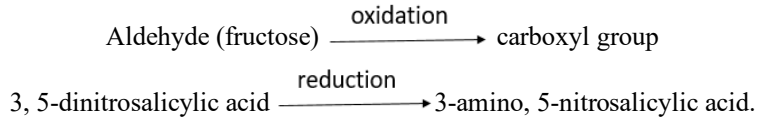
It has been reported that the reduction of sugar varies according to plant parts, confirming that the reduction of sugar produced from the tubers of cassava and cocoyam is more significant than that of their stems and peels [56-60]. It is always necessary to consider the feedstock type employed in experimenting with bioethanol production. The type of feedstock determines the quantity of reducing sugars for ethanol production. The capacity of a feedstock to produce a significant amount of glucose using dilute hydrolysis is a critical alternative for producing biomass fuel [61-63]. Other factors that may also be considered are the environmental and physiological conditions under which these plants are grown.

2.2 The effect of the yeast (s. cerevisiae) in the production of ethanol from cassava and cocoyam starch

In this experimental investigation, it was essential to determine whether the amount of yeast utilized affects the fermentation process and ethanol production. It was observed that yeast plays a significant role during fermentation by metabolizing glucose into crude ethanol. It was further noted that the yeast concentration does not have an effect on increasing ethanol production but on the time of fermentation. Many researchers have reported that yeast concentration in bioethanol production from cassava peel does not improve ethanol production but affects the time of fermentation.

2.3 Measurement of sugar content in cassava and coco yam solution

The amount of reducing sugar in both cassava and cocoyam samples was measured using the dinitro salicylic colorimetric method as stated below:



1. Prepared 10 mL solution (10mL distilled water,1g NaOH, 1g Dinitro salicylic acid)
2. Heated the solution for 15min and allowed to cool down
3. The DNS reagent solution containing 0.01g - 0.12g sugar absorbance was first measured as the reference point using a spectrophotometer.
4. 2 mL of the DNS reagent was added to 2 mL of cassava and cocoa yam samples, and the absorbencies were measured using a spectrophotometer.
5. The absorbance readings from the cassava and coco yam solution were compared to the absorbance of the DNS reference to determine the amount of reducing sugar present (Table 1).

Table 1. Amount of sugar content present in the samples.

Sugar content (g) absorbance		Reference samples absorbance (DNS)			
Cassava	Cocoyam	Sugar (g)	absorbance	Sugar (g)	absorbance
1.090	0.702	0.01	0.690	0.07	0.864
1.080	0.778	0.02	0.736	0.08	0.895
1.312	0.927	0.03	0.787	0.09	0.940
1.521	1.092	0.04	0.850	0.1	0.988
> 2.5	1.155	0.05	>2.5	0.12	1.134

2.4 Ethanol production using 2%, 4%, 6%, 8% and 10% of dilute sulphuric acid for cassava

Different concentrations of dilute sulphuric acid with equal volumes of water and cassava were used to know whether increased concentration is a critical factor in ethanol production. It was observed that an increase in concentration increases the yield of ethanol in the distillation process, as detailed in Table 2.

Table 2. Ethanol production using 2%, 4%, 6%, 8% and 10% dilute H_2SO_4 for cassava.

Expt. 1	Amount of H_2SO_4	Amount of water (mL)	Weight of cassava (g)	Ethanol produced (mL)
	20ml (2%)	500	50	29
	40ml (4%)	500	50	31
	60ml (6%)	500	50	33
	80ml (8%)	500	50	34
	100ml (10%)	500	50	37.5

Expt. 2	Amount of H_2SO_4	Amount of water (mL)	Weight of cassava (g)	Ethanol produced (mL)
	20ml (2%)	500	50	28
	40ml (4%)	500	50	32
	60ml (6%)	500	50	35
	80ml (8%)	500	50	36
	100ml (10%)	500	50	37

The quantity of ethanol produced with 2%, 4%, 6%, 8%, and 10% dilute H_2SO_4 was 29 mL, 31mL, 33 mL, 34 mL, and 37.5 mL, respectively, for experiment 1 and 28mL, 32mL, 35mL, 36mL, and 37 mL for the Experiment 2. It was observed that the yield of ethanol increased with concentration (Figures 2 & 3).

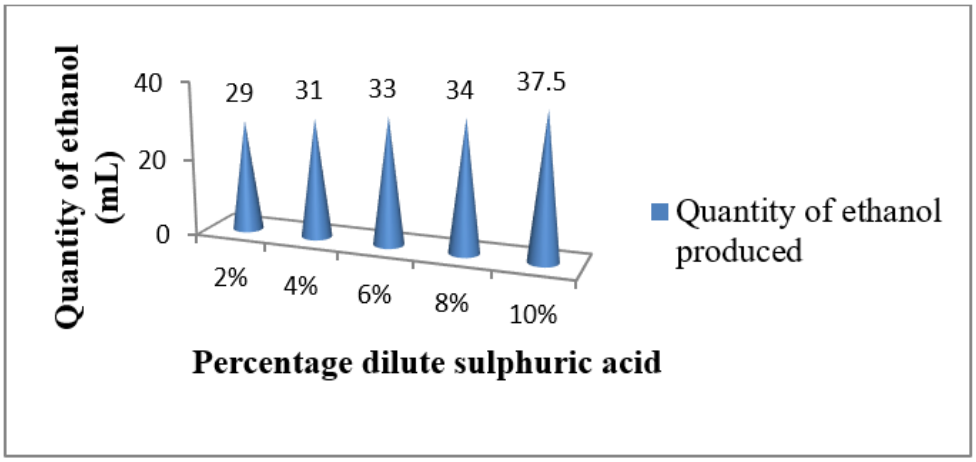


Figure 2. Ethanol yield with 2%, 4%, 6%, 8%, and 10% of dilute sulphuric acid for cassava in Experiment 1

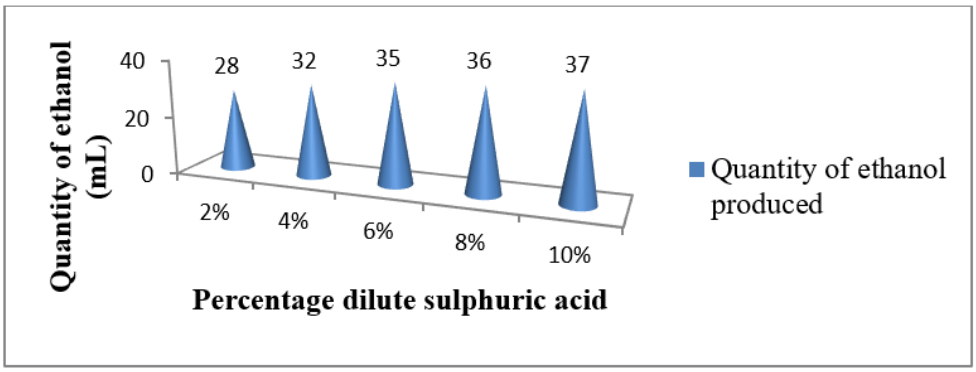


Figure 3. Ethanol yield with 2%, 4%, 6%, 8%, and 10% of dilute sulphuric acid for cassava in Experiment 2

Table 3. The mean of ethanol produced from cassava in experiments 1 and 2.

Expts. 1 & 2 mean	Amount of H_2SO_4	Amount of water (mL)	Weight of cassava (g)	Ethanol produced (mL)
	20ml (2%)	500	50	28.5
	40ml (4%)	500	50	31.5
	60ml (6%)	500	50	34
	80ml (8%)	500	50	35
	100ml (10%)	500	50	37.3

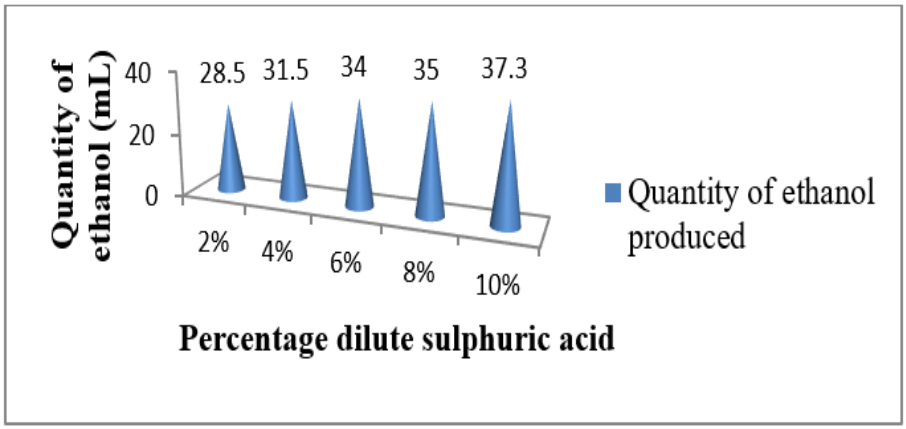


Figure 4. The mean of Ethanol yield with 2%, 4%, 6%, 8% and 10% of dilute sulphuric acid for cassava in Experiment 1 and 2

Figure 4 and Table 3 above illustrates the mean values of ethanol produced from cassava in experiments 1 and 2. Both experiments showed that ethanol production increased with an increase in concentration. This confirmation aligns with reports of other researchers involving ethanol production from similar raw materials reducing sugar.

2.5 Ethanol production using 2%, 4%, 6%, 8% and 10% of dilute sulphuric acid for cocoyam

Also, when 2%, 4%, 6%, 8%, and 10% dilute sulphuric acid was used for 50g of cocoyam starch, the yield increased with concentration as in the case of cocoyam starch but was not as much as that of cassava. Many studies have shown that increased concentration positively affects ethanol production. Table 4 illustrates the different concentrations of acid used.

Table 4. Ethanol production using 2%, 4%, 6%, 8% and 10% dilute H_2SO_4 for cocoyam.

Expt. 1	Amount of H_2SO_4	Amount of water (mL)	Weight of cassava (g)	Ethanol produced (mL)
	20ml (2%)	500	50	19
	40ml (4%)	500	50	24
	60ml (6%)	500	50	26
	80ml (8%)	500	50	28
	100ml (10%)	500	50	29

Expt. 2	Amount of H_2SO_4	Amount of water (mL)	Weight of cassava (g)	Ethanol produced (mL)
	20ml (2%)	500	50	17
	40ml (4%)	500	50	22
	60ml (6%)	500	50	25
	80ml (8%)	500	50	27
	100ml (10%)	500	50	29

The quantity of ethanol produced with 2%, 4%, 6%, 8%, and 10% dilute H2SO4 was 19mL, 24 mL, 26 mL, 28 mL, and 29 mL, respectively for experiment 1 and 17mL, 22mL, 25mL, 27mL, and 29mL for the experiment 2. This also showed clearly that the yield of ethanol from cocoyam increased with concentration. (Figures 5 & 6 and Table 5). The mean of ethanol yield for cocoyam in experiments 1 & 2 is shown in Figure 7 below.

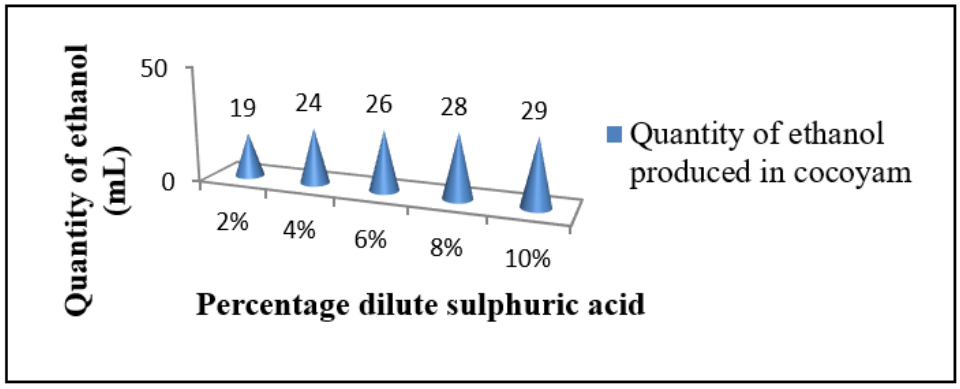


Figure 5. Ethanol yield with 2%, 4%, 6%, 8%, and 10% of dilute sulphuric acid for cocoyam in Experiment 1

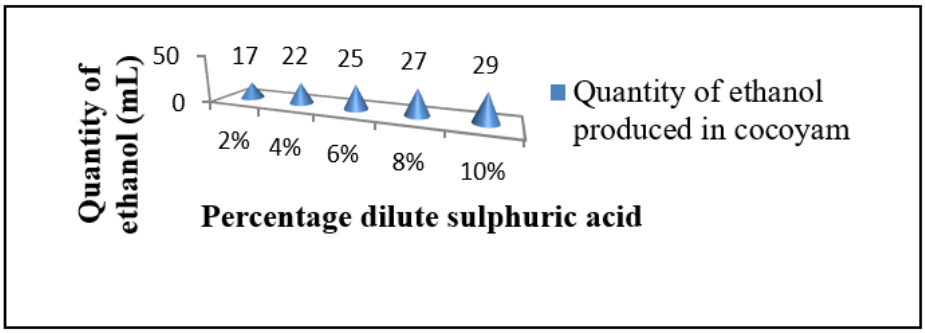


Figure 6. Ethanol yield with 2%, 4%, 6%, 8%, and 10% of dilute sulphuric acid for cocoyam in Experiment 2

Table 5. The mean of ethanol produced from Cassava and Cocoyam.

Amount of H_2SO_4	Mean of ethanol produced by cassava (mL)	Mean of Ethanol produced by cocoyam (mL)
20mL (2%)	28.5	18
40mL (4%)	31.5	23
60mL (6%)	34	25.5
80mL (8%)	35	27.5
100mL (10%)	37.3	29

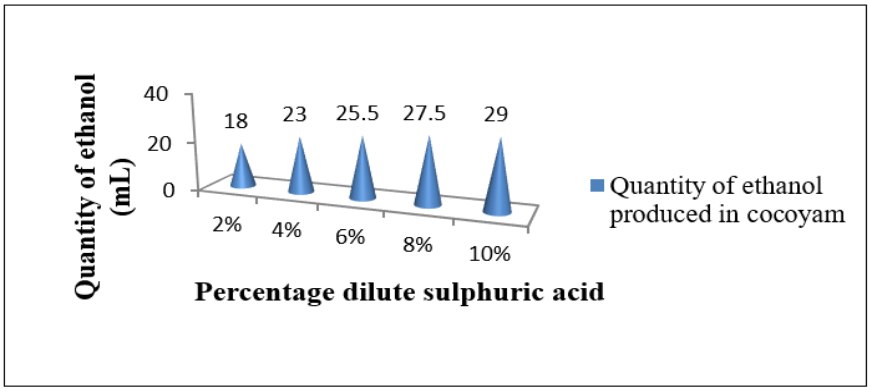


Figure 7. Mean of Ethanol yield with 2%, 4%, 6%, 8%, and 10% of dilute sulphuric acid for cocoyam in experiments 1 and

2.6 Comparison of different dilute H_2SO_4 concentrations with ethanol yield in the two food crops

Table 6 and Figure 8 shows a comparative analysis of how H_2SO_4 concentrations differ in ethanol yield for both cassava and cocoyam. It was observed that the ethanol yield in the different starch samples slightly differed at various acid concentrations. This indicates that both crops (cassava and cocoyam) share similar carbohydrate and sugar build-up, making them suitable raw materials for ethanol production.

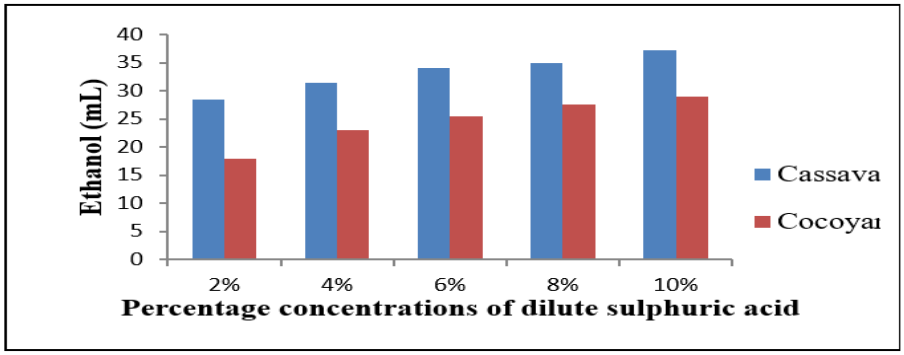


Figure 8. Comparative acid concentrations and ethanol yield in the two food crops

The amount of ethanol produced in this work in different percentage concentrations of dilute H_2SO_4 was compared with those reported in previous works of some researchers to confirm whether an increase in acid concentration does affect the amount of ethanol produced. Table 6 shows data obtained from different previous studies, which shows that an increase in acid concentration leads to an increase in ethanol production, which aligns with the data obtained in this research.

Table 6. To Compare the effect of acid concentration of ethanol production for cassava and cocoyam with previous studies.

Studies	Acid Hydrolysis	Quantity of Materials used	Amount of Ethanol Produced	References
Research Findings	2% dilute H_2SO_4	50g of cassava	28.5mL	Main Study
	4% dilute H_2SO_4	50g of cassava	31.5mL	
	6% dilute H_2SO_4	50g of cassava	34mL	
	8% dilute H_2SO_4	50g of cassava	35mL	
	10% dilute H_2SO_4	50g of cassava	37.3mL	
Research Findings	2% dilute H_2SO_4	50g of cocoyam	18mL	
	4% dilute H_2SO_4	50g of cocoyam	23mL	
	6% dilute H_2SO_4	50g of cocoyam	25.5mL	Main study
	8% dilute H_2SO_4	50g of cocoyam	27.5mL	
	10% dilute H_2SO_4	50g of cocoyam	29mL	
Previous Studies	2% dilute H_2SO_4	20g of cocoyam	23.45mL	[64]
	6% dilute H_2SO_4	20g of cocoyam	29.80mL	
	10% dilute H_2SO_4	20g of cocoyam	37.35mL	
Previous studies	0.3M HCL	20g of cassava	25.64mL	[65]
	0.5 M HCL	20g of cassava	28.80mL	
	0.7M HCL	20g of cassava	32.74mL	
Previous studies	0.4 M H_2SO_4	20g of corncob	42%	[66]
	0.6M H_2SO_4	20g of corncob	47%	
	0.8M H_2SO_4	20g of corncob	50.5%	
	1M H_2SO_4	20g of corncob	55.5%	

3 Conclusion

Two raw materials (cassava and cocoyam) were utilized for the extraction of bioethanol due to their high carbohydrate (starch) contents obtained during the grinding, mashing, and sieving of the residue. The presence of the reducing sugar was confirmed after crushing the raw materials (cassava and cocoyam). Any agricultural product with a high carbohydrate

content can be a good starting material for ethanol production.

Analysis of research results shows that cassava produces more reducing sugar than cocoyam, indicating that raw materials with high carbohydrate contents during pretreatment are essential alternatives for bioethanol production. The samples were hydrolyzed using dilute sulphuric acid at various percentage concentrations of 2%, 4%, 6%, 8%, and 10%. The H_2SO_4 was utilized because it is cost-effective and offers a more effective result. The hydrolyzed samples were fermented to break down the bond between the glucose molecules in the feedstock to produce ethanol. Saccharomyces Cerevisiae was in the fermentation process as the most effective, commonly used, and easier culture solution. The cassava and cocoyam residues were separately analyzed for ethanol production when hydrolyzed in the same concentrations (2%, 4%, 6%, 8%, and 10%) of the H_2SO_4 acid. It was observed that the average ethanol yield from the cassava residue at the various acid concentrations was 28.5, 31.5, 34, 35, and 37.3 mL, while the average ethanol yield from cocoyam was 18, 23, 25.5, 27.5, and 29 mL. The ethanol yield from both raw materials (cassava and cocoyam) increases with an increase in the concentration of dilute sulphuric acid. Although both raw materials yielded significant amounts of ethanol, it was observed that the ethanol yield from cassava across all five concentrations of acid solutions was more significant compared to cocoyam. Unprecedentedly, no previous or past reports have ever outlined a comparative analysis of bioethanol extraction from cassava and cocoyam, making this work phenomenal and innovative. The hydrolysis in cassava was very fast due to its low fiber contents compared to cocoyam, which might also be another factor for the high yield of ethanol from cassava than cocoyam.

Using energy crops such as cassava and cocoyam for bioethanol production as an alternative fuel source reduces greenhouse gas emissions and is also a cheaper fuel source compared to the raw material of fossil fuel. The use of lower concentrations of dilute acid in the starch hydrolysis of these crops showed that they are excellent energy crops for bioethanol production. Based on the experimental data, both raw materials have significant ethanol yield but are more abundant in cassava than cocoyam.

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Conflicts of Interest: The authors declare no conflict of interest.

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