

Full Length Research Paper

Analysis of the suitability of yam, potato and cassava root peels for bioethanol production using *Saccharomyces cerevisiae*

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The feasibility of bio-ethanol production from yam, potato and cassava root peels was investigated. Slurry of each peel was saccharified using acid, commercially available α -amylase and *Aspergillus niger* which was screened and certified for amylase production. Subsequent fermentation by *Saccharomyces cerevisiae* followed at room temperature for 72 hours. Composition analysis revealed that yam, potato and cassava root peels contained 46, 96 and 114.5 (mg/g) starch respectively. Glucose yield obtained after hydrolysis of yam peels using amylolytic fungi, enzyme and acid were 51.6, 48.7 and 78.1 (mg/g) respectively. Similarly, glucose levels of the respective potato peel hydrolysates were 160.8, 90.7 and 248.3 (mg/g). Saccharification of cassava root peels resulted into glucose concentrations of 210.4 mg/g, 110.4 mg/g and 250.0mg/g in amylolytic fungi, enzyme, and acid hydrolysates respectively. The ethanol yield after fermentation of yam peels hydrolysed using amylolytic fungi, enzyme and acid were 1.68, 0.56 and 2.7 (%v/w) respectively. Ethanol yield from potato peels were 4.02%v/w (amylolytic fungi hydrolysate), 1.94% v/w (enzyme hydrolysate) and 9.38% v/w (acid hydrolysate). Fermentation of the respective cassava peel hydrolysates resulted in 10.5%v/w, 4.07% v/w and 17.52% v/w ethanol. Results obtained suggested that increasing the fermentation duration did not result in a corresponding increase in ethanol production hence maximum ethanol yield in various hydrolysates was at the 24th hour of fermentation following the order acid hydrolysates > amylolytic fungi hydrolysates > enzyme hydrolysates. Also, in terms of substrate yield, highest ethanol production was from cassava root peels, followed by potato peels while yam peels yielded the least ethanol concentration. This research therefore, indicates that these peels could serve as cheap sources of glucose which can be fermented locally for bio-ethanol production especially in areas where they are in abundance.

Keywords: Amylase, Hydrolysates, Bio-ethanol, *Saccharomyces cerevisiae* and Yam Potato and Cassava peels.

INTRODUCTION

There is an increasing demand for ethanol production through fermentation processes all over the globe as oil reserves are dwindling and prices are soaring (Brooks, 2008). The high prices have led to energy crises in both developing and developed countries that are oil dependent. According to Naylor *et al.*, 2007, "biofuels will remain a critical energy development target in many parts of the world if petroleum prices exceed US \$ 55 – 60 per barrel."

At the global level, Brazil has been the world leading producer of biofuel and recently, Nigeria joined the league of biofuel users with the aim of generating wealth (Aisien *et al.*, 2010). The design had generally been production from sugarcane and cassava. Klass (1998) categorized cassava alongside with sweet potato and yam as main starches that serve as staple foods for people through the world's hot and humid regions. These plants are so proficient at supplying essential calories that they are considered the quintessential subsistent crops. However, the success of these starch crops as staple foods limits their potential development and general economic growth, for instance, cassava which

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has become an important biofuel crop is a crop crucial for food security especially in Nigeria. The implication of this is that threats to food security exist in the face of growing fuel ethanol demand. Perhaps, the diversion of food resource to biofuel production may to a large extent have fuelled the current food crises worldwide (Srinorakutara *et al.*, 2008).

It therefore becomes imperative that the searchlight be turned at present to the use of non-food starchy items for the production of fuel ethanol. Hence this research is aimed at evaluating the suitability of using the peels of yam, potato and cassava roots for bio-ethanol production as well as assessing the performance of various substrate pre-treatment processes for the conversion of starch into fermentable sugar and subsequent development of the most effective for optimal ethanol production.

MATERIALS AND METHODS

Source of Substrate/Test Organism

Yam, potato and cassava root tubers were harvested from their respective farm sites located in Abraka, Delta State, Nigeria. The fresh and healthy tubers were transported to the laboratory within two hours of harvest, washed with sterile distilled water and peeled with a pre-sterilized knife. The various peels were ground separately using a milling machine to obtain stock samples.

Dehydrated industrial *Saccharomyces cerevisiae* (Brewers' yeast) was obtained from Nigeria Breweries PLC, Benin, Edo State, Nigeria. Activation of the yeast was done by introducing 20g of the dehydrated yeast into 100ml of distilled water that contained two cubes of table sugar. This was shaken at 150rpm for 10 minutes.. This served as stock culture for further test.

Screening / Selection of amylolytic fungal strain

One gram each of yam, potato and cassava peel slurry were aseptically withdrawn and diluted using the ten-fold serial dilution technique after which, fungal isolates were obtained using the pour plate method as described by Akerele (1990). Twenty fungal isolates were randomly picked and screened for amylase production by inoculating into starch agar plate surface which contained 20g cassava starch, 3g yeast extract, 5g (NH₄)₂SO₄, 0.5g MgSO₄·7H₂O and 4g KH₂PO₄. Incubation followed for four days at ambient temperature (26). Amylolytic zones corresponding to enzymatic degradation of starch were detected by staining the plates with iodine solution. Isolate that produced the largest zone of clearance was selected, identified according to criteria of Barnett and Hunter, 1972 and used for saccharification of substrates' starch.

Substrate Saccharification using Amylolytic fungus

The selected amylolytic fungal strain (*Aspergillus niger*) was harvested and introduced into 9ml sterile distilled water to fungal load of 1.00×10^9 spores/ml. This was then inoculated into 100g of various substrates (treated and untreated). The various substrates slurries were distributed in duplicates into 500ml capacity Erlenmeyer flask in 100g amounts and treatment was by steaming a set at 100°C for 30minutes. The second unsteamed or untreated set served as control.

The inoculated samples, were incubated at ambient temperature for 7 days. However, at intervals of 1, 3, 5 and 7 days, samples were collected for analysis of glucose. This also determined the effect of incubation duration and nature of substrate on saccharification.

Enzymatic hydrolysis

Commercially available α - amylase was added to 100g of each substrate contained in 500ml Erlenmeyer flask, after steaming at 100 °C for 30minutes and subsequent cooling. The enzyme substrate mixture was mixed thoroughly on a shaker at 400rpm for 15minutes at a pH 5.5 after which glucose concentration was evaluated.

Acid hydrolysis

Distilled water (20ml) was added to 100g of various substrates, each contained in 500ml Erlenmeyer flask. Thorough mixing followed and samples were hydrolyzed with 0.6M H₂SO₄ at 90°C in a water bath for 30minutes. At the end of hydrolysis the pH of each hydrolysate was brought up to 5.5 by the addition of NaOH and phosphate buffer.

Fermentation of Hydrolysate

Activated *S. cerevisiae* was aseptically inoculated into various saccharified substrates. Fermentation proceeded with shaking at 120rpm for 72 hours at room temperature. During fermentation, samples were taken at intervals of 24hours each for analyses of pH, reducing sugar and ethanol. Ethanol determination was done using an alcohol meter.

RESULTS AND DISCUSSIONS

Twenty fungal isolates were isolated from the yam, potato and cassava root peel substrates out of which fifteen isolates grew on starch agar plates giving various sizes of zones of clearance at the performance of iodine test as illustrated in Table 1. Efficiency of the isolate in starch

Table 1. Screening test for starch hydrolysis ability of fungal isolates

Source of isolate	Isolate No	Zone of Clearance (mm)	Culture Type
Yam peels	1	2.0	Mould
Yam peels	2	-	Yeast
Yam peels	3	2.8	Mould
Cassava peels	7	18	Mould
Cassava peels	8	3.0	Mould
Cassava peels	9	1.0	Yeast
Cassava peels	10	1.8	Mould
Cassava peels	11	0.5	Yeast
Cassava peels	12	11.6	Mould
Cassava peels	13	6.4	Mould
Potato peels	14	8.0	Mould
Potato peels	16	-	Yeast
Potato peels	17	1.0	Mould
Potato peels	18	0.4	Mould
Potato peels	19	13.6	Mould

Table 2. Effect of starch hydrolysis method on sugar recovery from various substrates

Substrate	Initial starch concentration (mg/g)	Reducing sugar concentration (mg/g)		
		Amylolytic hydrolysis	Enzymatic hydrolysis	Acid hydrolysis
Yam peel	46	51.6	48.7	78.1
Potato peel	96	160.8	90.7	248.3
Cassava peel	114.5	210.4	110.4	250.0

hydrolysis was determined by the size of zone of clearance. Identification protocol indicated that the isolate with the highest efficiency in amylase production (zone of clearance = 18mm), was *Aspergillus niger*. Based on the cultural characteristics of the various isolates obtained, it was deduced that several genera of moulds and yeast were present in the various substrates (also shown in Table 1). The occurrence of isolates that produced large diameters of zones of clearance is suggestive that these substrates could serve as sources of potential amylase producing fungi. Similar observations have been made by Arotupin (2007) and Akponah (2011).

Initial starch concentrations in potato, yam and cassava peels were 96, 49 and 114.5 (mg/g) respectively. Saccharification of these respective substrates with amylolytic fungus resulted in 160.8, 51.6 and 210.4 (mg/g) glucose while acid hydrolysis resulted in 248.3, 78.1 and 250 (mg/g) glucose. Glucose yield when α -amylase (commercially available) was used for saccharification were 48.7mg/g (yam peels) 90mg/g (potato peels) and 110.4mg/g (cassava peels) as shown in Table 2. There was a significant difference at ($p < 0.05$) in glucose yield from various substrates. Also, the various

saccharification techniques tested resulted in a significant difference in glucose yield. The differences observed may be due to the differences in the nature of substrate used. Additionally, the recovery of reducing sugar concentration may also be directly proportional to the initial starch concentration present in the various substrates as shown in Table 2. These results are similar to those obtained by Srinorakutara *et al.* (2008) and Zakpa *et al.*, 2009. Also, Nadir *et al.* (1998) reported that substrates with smooth surfaces and fissures after liquefaction, yield higher glucose at the end of saccharification. Although, the microstructures of the substrates' granules were not viewed in this study, the results as presented in Table 2 indicates that cassava peels were more susceptible to both acidic and enzymatic attacks. The trend in the efficiency of methods used in saccharification was acid hydrolysis > amylolytic fungal hydrolysis > enzymatic hydrolysis. It is probable that acid hydrolysis resulted in the highest glucose yield due to the great random collision between the acid and the substrates. The higher values obtained from amylolytic fungus hydrolysis in comparison to enzymatic hydrolysis may be consequent upon the capability of the fungus to produce other enzymes such as glucoamylase and cellulase in addition

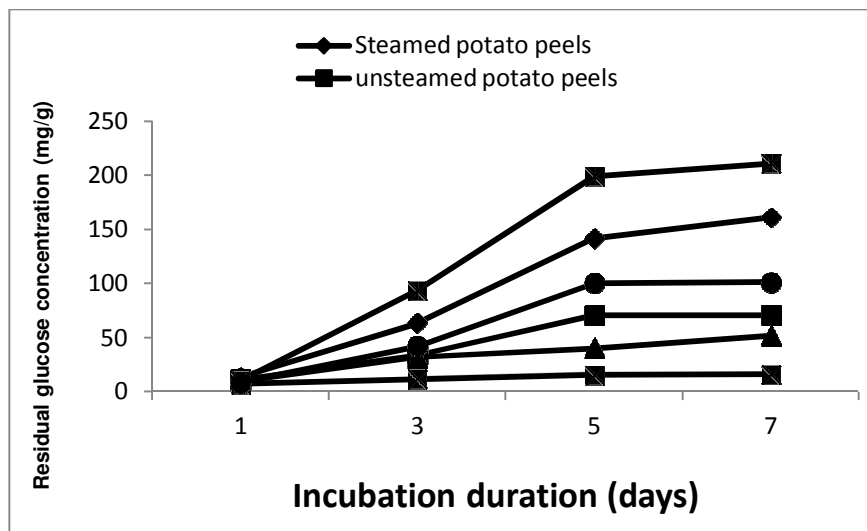


Figure 1. Effect of incubation duration and nature of substrate on starch saccharification by *Aspergillus niger*

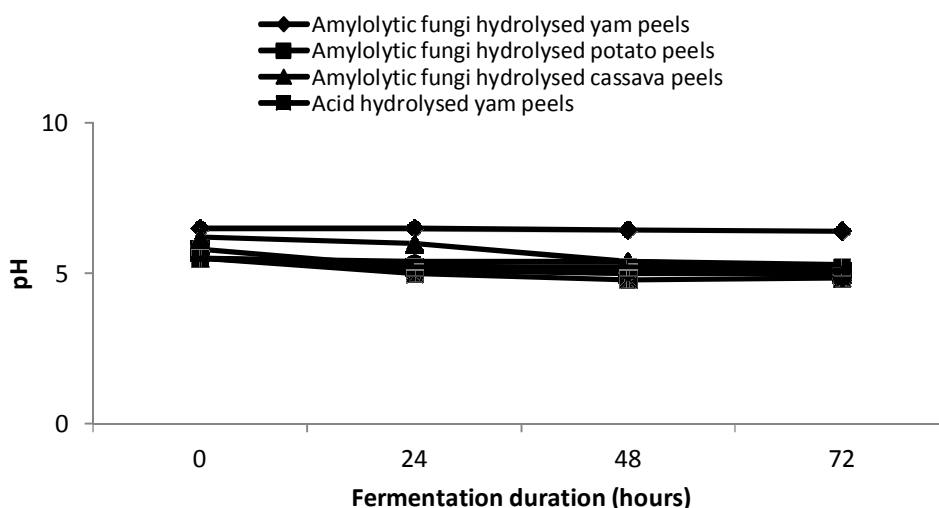


Figure 2. Changes in pH of various hydrolysates

to α - amylase as previously reported by Arotupin (2007) and Zakpa *et al.* (2009).

Results of experiments conducted to assess the effects of incubation duration as well as nature of substrate on starch conversion into glucose (saccharification) by amyolytic fungi are as shown in Figure 1. The rate of saccharification when *Aspergillus niger* was used increased with increase in incubation duration. Highest values were obtained on the 7th day of incubation which probably corresponds with the exponential growth phase of the isolate. The results is suggestive of the fact that hydrolysis of starch by the amyolytic fungus is dependent on the growth kinetics of the fungus. Therefore, in the use of amyolytic fungus for starch saccharification, it becomes imperative to monitor the process intermittently in order to decide the most appropriate time to halt the

reaction. Importantly, glucose obtained from the saccharification of steamed or treated substrates by *Aspergillus niger* were higher than from untreated substrates as indicated in Figure1 This tendency might be attributable to the high reduction in microbial load of the various peels by the treatment (steaming). This condition might have promoted the growth of the inoculated pure culture of *Aspergillus niger* by drastically reducing competition from bacterial and fungal contaminants. Furthermore, steaming could have reduced the molecular size of the substrates' starch, thus facilitating enzyme activity.

The changes in fermentation parameters which include pH, reducing sugar concentration and ethanol are presented in Figures 2, 3 and 4 respectively. It was observed that pH of all hydrolysate reduced gradually as

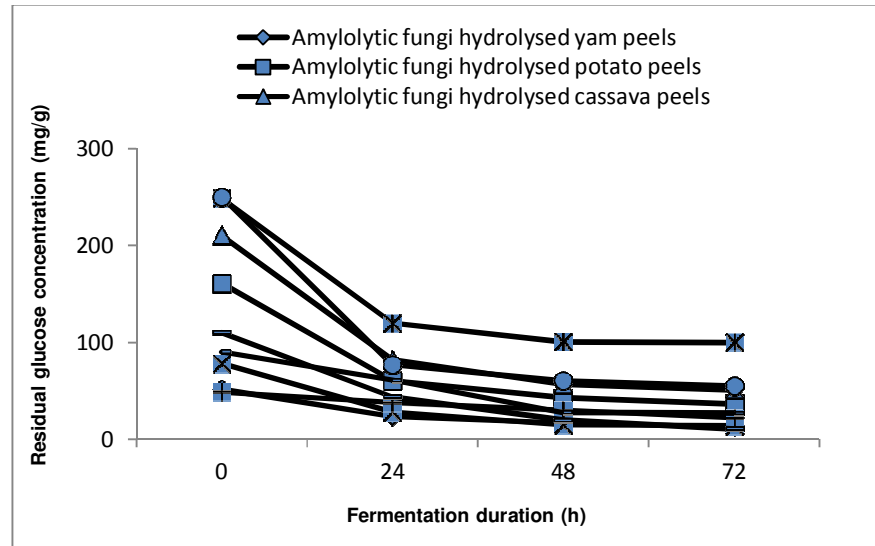


Figure 3. Changes in glucose concentration in various hydrolysates

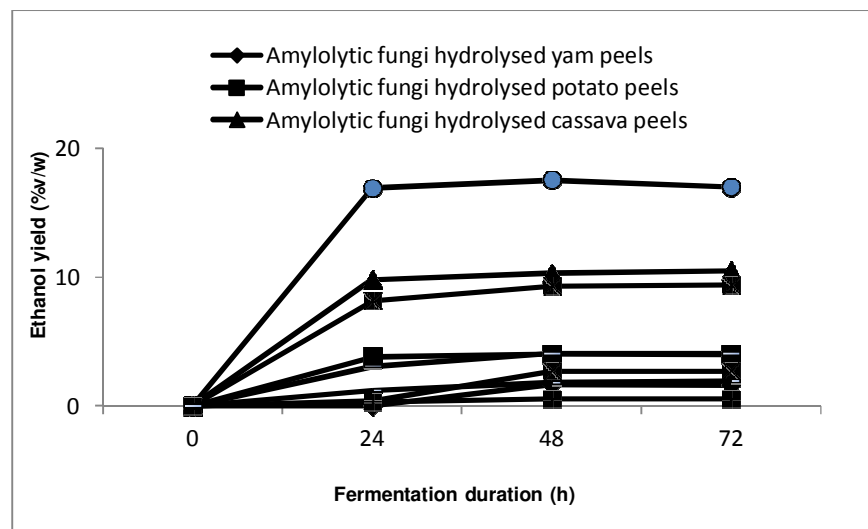


Figure 4. Changes in ethanol concentration in various hydrolysates

fermentation period increased. At Day 0, pH of yam peel hydrolysate obtained from acid, amylolytic fungi and enzyme hydrolyses were 5.5, 6.5 and 5.5 respectively. At the end of fermentation, pH in these hydrolysates dropped to 5.0, 6.4 and 5.2 respectively. Fermentation of potato peel hydrolysate obtained by acid, amylolytic fungi and enzymatic hydrolyses resulted in a drop in pH from 5.5 to 4.84, 5.8 to 5.0 and 5.5 to 5.3 respectively. A decrease in pH from 5.5 to 4.9 (acid hydrolysis), 6.2 to 5.2 (amylolytic fungi hydrolysis) and 5.5 to 5.1 (enzymatic hydrolysis) were recorded during the fermentation of cassava peels.

Glucose concentrations decreased progressively with increase in fermentation duration. Reductions in glucose

concentration at the end of the 72h fermentation of yam, potato and cassava peels hydrolyzed with acid were 64.1, 148.3 and 195 (mg/g) respectively. Residual glucose concentration at the end of fermentation of amylolytic fungi hydrolysates were 13.3mg/g (yam peels), 36.4mg/g (potato peels) and 50.8mg/g (cassava peels). Similarly, glucose concentrations in enzyme hydrolysates of yam, potato and cassava peels reduced from 48.7 to 21.9(mg/g), 90 to 27 (mg/g) and 110.4 to 10.8 (mg/g) respectively.

The trend in ethanol production was similar in all fermentors (Figure 4). Generally, ethanol concentration increased with increase in fermentation duration. However, the increase was steepest within the first 24

hours. This reduction in the rate of ethanol production may be related to the inhibitory activities of both the ethanol and other by-products in the fermentation medium. Maximum ethanol production over the 72h fermentation period were 2.7, 9.38 and 17.52 (%v/w) in acid hydrolysates of yam, potato and cassava peels respectively. The respective ethanol produced in the amyolytic fungus hydrolysate equivalents were 1.68% w/v (yam peels), 4.02% w/v (potato peels) and 10.5% w/v (cassava peels). Fermentation of enzyme hydrolysates of yam, potato and cassava peels by the test organism yielded 0.56, 1.94 and 4.07 (% v/w) respectively. There was significant difference in the ethanol yield from the various hydrolysates at $p < 0.05$. Ethanol yield followed the order acid hydrolysate > amyolytic fungi hydrolysate > enzyme hydrolysate. There were negative correlations between residual glucose concentration and ethanol yield by the test organism in various hydrolysates. It has been reported that final ethanol concentration obtained differ either in the type of pre-treatment given to substrate, substrate concentration, substrate detoxification method if any, fermentation strain, temperature or mode of operation (Olofssen *et al.*, 2008).

Although ethanol produced from acid hydrolysate of various substrate were higher than ethanol from amyolytic fungus hydrolysates, the study showed that ethanol yield from the later were appreciable and if the process is optimized, the method could be adopted as a cost-effective alternative in the pursuit of fuel ethanol production protocol. Also, results obtained revealed that ethanol production was highest from cassava peel hydrolysates followed by potato peels while yam peels yielded the least ethanol concentration. These peels could therefore serve as cheap sources of glucose which can be fermented locally for bio – ethanol production especially in areas where they are in abundance (for instance, cassava peels in the tropics and potato peels in the temperate region).

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