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# GLUCOSE SYRUP PRODUCTION FROM COCOYAM *(COLOCASIA ESCLUENTA)* TUBERS USING *ASPERGILLUS NIGER*

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**ABSTRACT**
*Glucose syrup is a highly valued liquid sweetener and a commercially important product. It is widely used by the beverage, confectionery, food and pharmaceutical industries. However, glucose syrup production by extraction of starch from cocoyam involves grating of raw material, settling, sieving, slurry decantation and finally drying the starch. Therefore, in order to simplify these processes, the present work aimed at one-pot glucose syrup production from cocoyam tuberby Aspergillus niger. The effects of various process parametres such as cell concentration, substrate concentration, pH and temperature were investigated. It was found from this study, that, an increase in substrate concentration was accompanied with a proportionate increase in glucose concentration up to* 20.1 *g/L and decrease in glucose yield, falling from 88 to 49%. This could be as a result inhibition, generally lower substrate concentrations are more suitable in order to avoid it. In addition, an increase in cell concentration resulted to increase in both the concentration and yield of glucose.The application of Aspergillus niger was found to directly hydrolysed cocoyam tubers (without starch extraction) to glucose atbest operating temperature of 45$^{o}$C and pH of 4.5 within the range of time investigated.*
***Keywords:*** *Cocoyam tubers, Aspergillus niger, direct conversion technique and glucose syrup.*

## INTRODUCTION

There has been a great emphasis on plant biomass as a source of fermentable sugars. Starch is considered as one of the world most abundant polymer, believed to be the renewable energy source that can provide sugars, liquid fuel and other bulk chemicals on a sustainable basis (Gomez *et al*., 2008). Nigeria has shown a record of reliance on imported glucose for industrial uses. In the last decade data from the Nigerian Sugar Development Council (NSDC) as at 2016 showed that a yearly average of $5.1 m was spent on glucose importation (Brandspur, 2018). However, the United States Department of Agriculture (USDA) forecasts glucose consumption in Nigeria to increase from 70,000 tonnes/year in 2016/17 to about 80,000 tonnes/year in 2017/18 (Brandspur, 2018). Nevertheless, local production improvement is expected as a result of government support through the CBN's Anchor Borrowers Program (ABP) and forex stability to facilitate importation of machinery necessary to further buoy local production, hence, bright prospects for glucose syrup production and milling in Nigeria (Brandspur, 2018).

Tubers are root crops of the tropical countries (FAO, 2006). Although, their primary use is, as food crops, the crops are widely used for the production of starch and of late, their role has been increasingly recognized as industrial crops for the production of bioethanol, glucose, high fructose syrup (HFS) etc. (Baskar *et al*., 2008). 85% glucose syrup production is from corn (Johnson *et al*., 2009), so with increased food and energy source demand, it becomes necessary to search for alternative and renewable substrates, in which cocoyam and sweet potato are potential raw materials (Johnson *et al*., 2009). The global cocoyam production was estimated to be about 10 million tonnes per year (FAO, 2015). Nigeria has been recognized as the largest contributor, with an average annual production rate of 4.28 million tonnes (FAO, 2015).

However, thetubers are conventionally converted to starch through time and labour intensive process, which is then converted to glucose and HFS. The possibility of using cassava chips, instead of cassava starch, for economic production of glucose and HFS was investigated by Ghildyal (2009), who reported that the cost of conversion of the tubers to starch led to the high cost of glucose and HFS production. The effect of *Aspergillus niger* on cassava starch hydrolysis for glucose production was reported by Osaribie (2013), who found that the best activity of *Aspergillus niger* occurred at pH 4.8 and 60°C for a yield of 74%. Johnson and padmaja (2013) compared the production of glucose syrup from tuber starches. The study showed that the glucose yield from arrowroot, cassava, *Curcuma*, *Dioscorea* sweet potato were superior to corn and cocoyam (*Xanthosoma).* But traditional method of extracting starch,prior to hydrolysis was used.Johnson *et al*. (2009) studied the comparative production of glucose and high fructose syrup from cassava and sweet potato roots by direct conversion techniques from six treatment systems, in order to simplify the cost-intensive steps, however expensive enzymes were used.

Irrespective of the source of starch used, the process involves two main steps; liquefaction, where the enzyme α-amylase partially hydrolyzes starch to maltodextrins, and saccharification where the low dextrose equivalent (D. E) syrup is completely converted to glucose by glucoamylase (Johnson *et al*., 2009). The extent of the hydrolysis process depends on many factors, such as type of catalyst used, production conditions and/or production technology.

Cocoyam (*Colocasiaescluenta*) have been shown to contain high starch fractions, few works reported its actual utilization in glucose production using *Aspergillus niger*. Hence, glucose syrup production from cocoyam chips (without starch extraction, contrary tothe conventional

method), using *Aspergillus niger*is the main focus of this work.

## MATERIALS AND METHODS

### Materials

Cocoyam was procured from Sabo market, Sabon Gari, Zaria Local Government Area, Kaduna State. It was identified, according to Coursey (2008), as *Colocasia esculenta*, voucher number: UNH No. 379, by the Institute of Tuber Research, Umudike, Ikwuano Local Government, Abia State.

### Methods

#### Organisms isolation and cultures

*Aspergillus niger* was isolated from soil samples and identified in the laboratory of the Department of Micro-Biology, Ahmadu Bello University Zaria, according to Gilman (1971). 1.0 gram of soil sample was taken from top 20 cm of the soil, dissolved in 10 mL of distilled water and ten-fold serial dilutions were made, then 1.0 mL of the 3rd step (0.0001 M) was transferred to petri plates containing potato dextrose agar (PDA). After inoculating the samples, the culture plates were incubated for 5 days at 30°C. After growth, the fungal colonies were further sub-cultured into a freshly prepared PDA plates and a pure isolate was obtained. Finally 'Haemocytometer Method' was employed to determine viability of the cell.

#### Preparation of wet Slurry

The cocoyam tubers were washed free of dirt, hand-peeled and was sliced to pieces. The pieces were immediately blended with a buffer solution (pH 4.5), according to Osaribie (2013). The slurry was made up to a concentration of 10% (w/v) using distilled water.

#### Enzymatic hydrolysis to glucose

Cocoyamtubers slurry 10% (w/v) that was prepared previously was kept in a thermostatic water bath at 90°C with stirring for about 5 min to attain equilibrium, this enlarges starch granules for easier attack by the enzymes. The resulting gelatinized syrup was cooled under running tap water, then 10% v/v (84.7% viable) of previously produced *Aspergillus niger cells* was added and incubated at 45°C for 16 h under constant agitation (Osaribie, 2013).

#### Determination of glucose concentration

A standard glucose curve was determined as shown in Figure 1, according to Aderemi (2008). A stock solution of D – glucose with concentration, 1.0 mg/ml was prepared. After which different dilutions were prepared by adding distilled water. 1.0 ml of each dilution was transferred to different test tubes and 1.0 ml of Di-nirosalicylic acid reagent was added to each of them. Each test tube was placed in a water bath (90$^{o}$C) for 5 minutes and cooled in cold water. Then the absorbance was read at a wavelength of 540 nm. The graph of absorbance against concentration of standard glucose was plotted. This was used in calculating the concentration of the samples obtained.

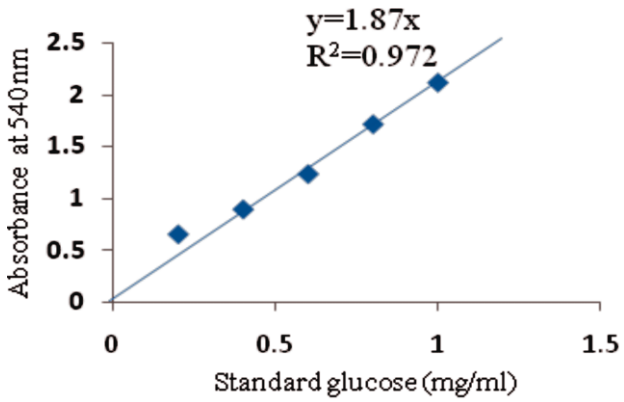


Figure 1: Calibration curve

To measure the concentration of glucose in the samples of hydrolysed cocoyam slurry, 1.0 ml of each sample was transferred into different test tubes. 1.0 ml of DNSA reagent was added to each sample and the tubes were placed into a water bath of 90$^{o}$C and heated for 5 minutes for colour change. They are then cooled. The colour change was measured with the aid of spectrophotometer at a wave length of 540 nm and the absorbance was read, finally these absorbances were converted to concentration.

#### Downstream recovery

During glucose syrup purification, the slurry was filtered with the aid of a vacuum pump machine. The water soluble glucose passed through the filter as product, while the protein, fiber, fats, ash, and other insolubles were removed from the resulting glucose solution.This process produced a batch of solid cake filtrate material with water content. Additional glucose was recovered by washing the filtrate with fresh water. Hence the glucose solution contained high amounts of water, which needed to be concentrated. The glucose syrup of this work which has boiling point of 100.3 $^{o}$C was finally concentrated in the oven at 90$^{o}$C for 30 min.

## RESULTS AND DISCUSSION

### Macroscopic Observation of *Aspergillusniger*

The growth of *Aspergillus niger*was monitored on a PDAS at 30$^{o}$C. The isolate grew rapidly on the PDAS, producing white floccose mycelia which turned yellow first and then black with profuse production of black conidia (spores). The reverse colour changed from white to cream, and then pale yellow and growth produce radial fissures in the agar as shown in Plate 1.

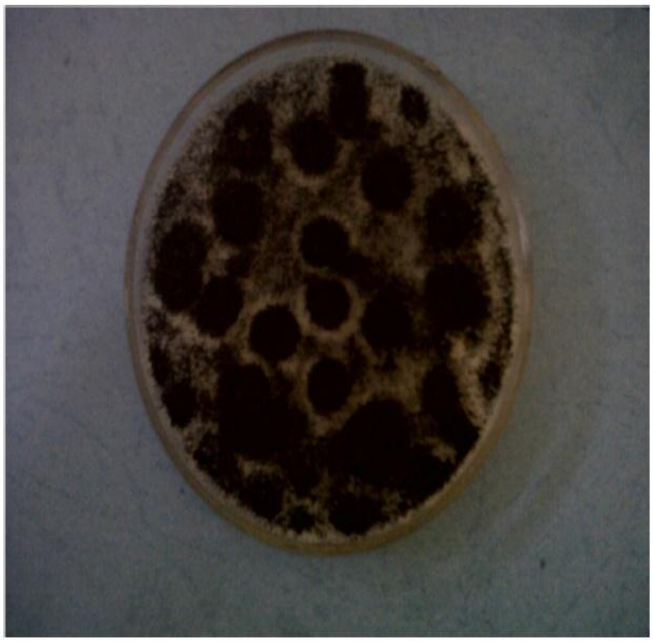

Plate 1: Photomicrograph of matured colonies of the isolate

**Microscopic observation of *Aspergilusniger* (morphology)**
The morphological characteristics of *Aspergillus niger* was observed as shown in Plate 2 based on unique characteristics of the organism such as the conidial head, presence of hyphae, conidia ornamentation, conidia and conodiophores. Confirmation of the fungi (*A. niger*) was made following a close comparison with the standard on Plate 3.

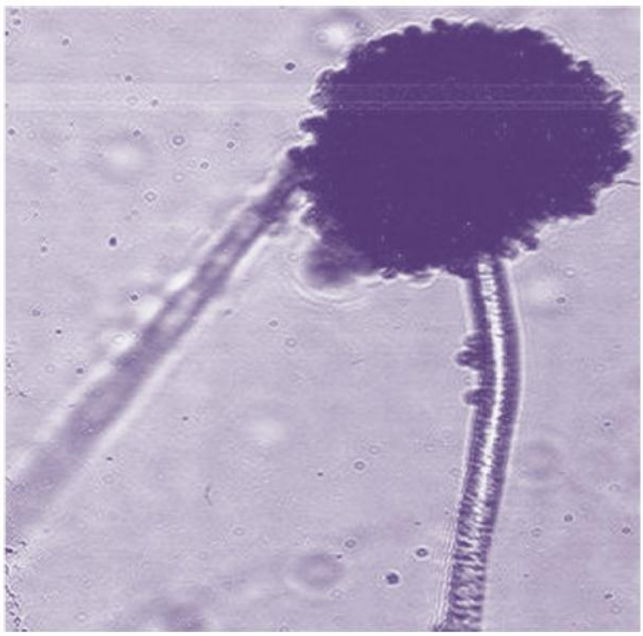

Plate 2: Photomicrograph of A. niger (*400) lactophenol cotton blue staining

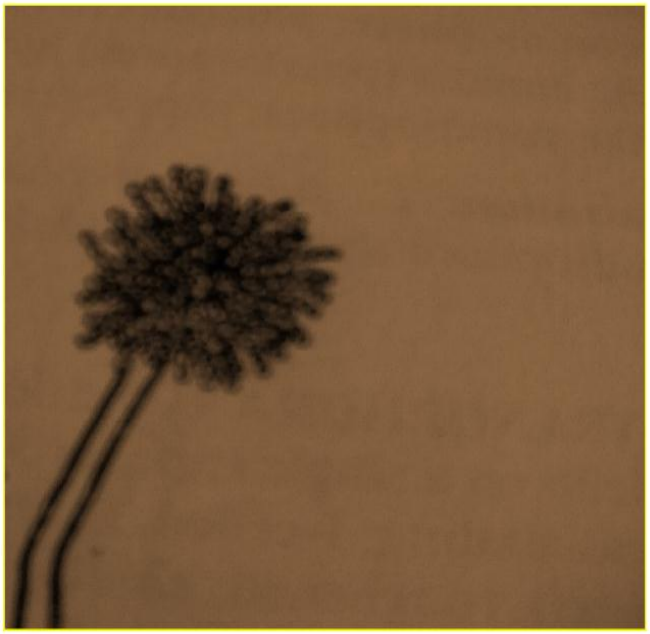

Plate 3: Standard structure of A. niger (Barnet and hunter, 2006)

**Cell Viability**
The live cell calculated was 2,837,500 per mL compared to the dead cell of about 512, 000 per mL, which showed high viability. The cells used are 84.7% viable as shown in Table 1. Viability is the total number of live cell compared to the total number of cell count (live and dead).

Table 1: Summary of the cell count of *A. niger*

| Method | Live cell per mL | Dead cell per mL | Viability |
|---|---|---|---|
| Hemocytometer | 2,837,500 | 512,000 | 84.7% |

**Proximate Analysis of Cocoyam (AOAC, 2010)**
The biochemical composition of the fresh cocoyam tuber is given in Table 2. Starch was the second major component, and it was found to be (22.24% (w/w)). While the third major compound was crude protein and it was found to be (5.69% (w/w)). The values were consistent with the report of Coursey (2008). Except for the cruide protein, the variation in absolute values may be attributed to maturity of the tubers processed, the variety, processing conditions etc.

Table 2: Cocoyam tuber proximate analysis

| Composition | Value % (wet weight base) | Literature value %* |
|---|---|---|
| Moisture | 65.35±0.21 | 63-85 |
| Carbohydrate | 22.24±0.11 | 13-29 |
| Fibre | 2.5±0.12 | |
| Protein | 5.69±0.18 | 1.4-3.0 |
| Oil | 0.17±0.71 | 0.6-1.3 |
| Ash | 4.05±0.59 | |

*Source: Coursey, (2008).

Table 3 shows anti-nutritional composition of the fresh cocoyam - hydrogen cyanide recorded as 8.3 ppm. After hydrolysis of the cocoyam slurry, the hydrogen cyanide found in the glucose syrup was 0.46 ppm as indicated in Table 3. It is interesting to note that hydrolysis alone may be satisfactory for cyanide removal. Johnson and Padmaja (2013) reported that more than 99% of 77 ppm hydrocyanic acid of cassava residue was destroyed during saccharification.

Table 3: Anti-nutritional profile

| Anti-nutritional | Value (ppm) fresh cocoyam | Value (ppm) glucose syrup |
|---|---|---|
| Hydrogen cyanide | 8.3±0.77 | 0.46 ± 0.01 |

**Enzymatic Hydrolysis**

**Effect of reaction time on glucose yield**

Cocoyam slurry was incubated with solution of *Aspergillusniger* for 72 hours. The formation of glucose was monitored using the DNS method at different times. Figure 2 shows the glucose yield. The glucose yield of (88-89%) was achieved at 14-16 hours of reaction.There after no significant difference was observed. Silver *et al*. (2009) had reported maximum conversion rate of 90% after 24 hours of reaction, however, pure starch and refined enzymes were used.

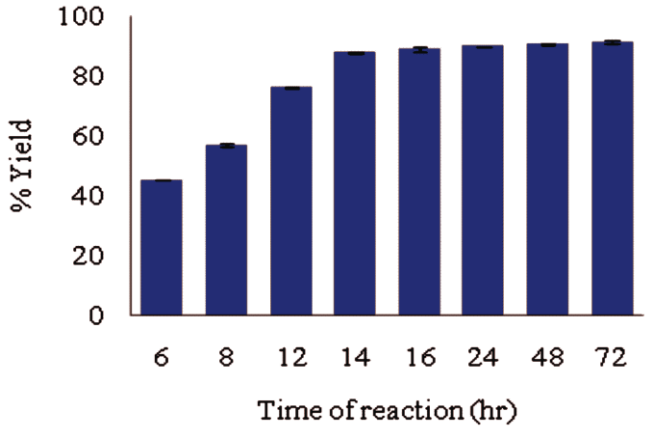


Figure 2: Effect of time of reaction on glucose yield*.

*Hydrolysis condition: Substrate concentration 10% (w/v), cell concentration 10 v/v %, pH 4.5, and temperature 45°C.

**Effect of cell loading on the glucose yield**

The effect of cell loading on glucose production was studied using cocoyam slurry as substrate by carrying out the experiment at different cell concentrations ranging from 2.0 to 10.0% v/v, keeping the other hydrolysis conditions constant. The result is shown in Figure 3. From the figure, it was found that increase in cell concentration was accompanied by a proportionate increase in glucose concentration within the range of cell concentration investigated.

The increase in glucose concentration observed may be as a result of the continuous secretion of enzymes by the cell into solution for the hydrolysis process. Similar profiles were reported by Aderemi *et al*. (2008) as well as Baskar *et al*. (2008) for hydrolysis of rice straw and cassava starch respectively using *Aspergillus niger*.

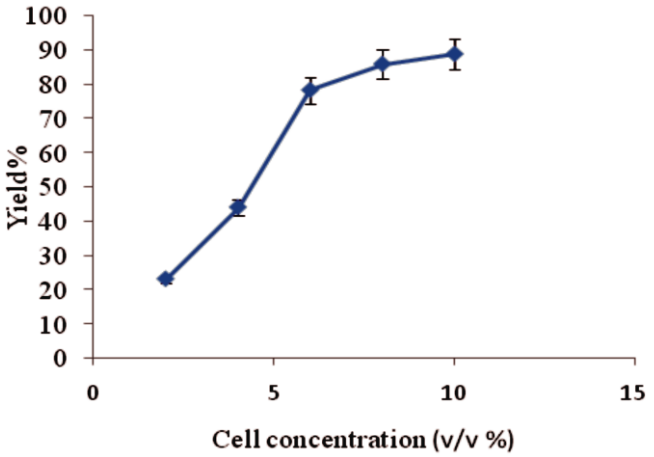


Figure 3: Effect of cell concentration on the glucose yield*.

*Hydrolysis condition: Substrate concentration 10% (w/v), reaction time 16 hour, pH 4.5, and temperature 45°C.

**Effect of substrate concentration on the glucose yield**

Figure 5a shows result of investigation carried out at various initial substrate concentrations ranging from 4 to 30 % (w/v). It was observed that glucose concentration increased proportionally with the increase in initial substrate concentration. However, it was observed that after 10% substrate concentration, glucose yield experienced a reverse trend (Figure 5b), falling from a value of about 88.8 % conversion to 49% at 30% substrate concentration.This could be as a result of substrate inhibition, keeping cell concentration constant and increasing substrate concentration, the cells will become insufficient to hydrolyze all the substrate supplied, this phenomena causes the yield to drop. Similar results were reported from previous works on the effect of substrate on enzyme activity by Aderemi *et al*. (2008) *and* Lee (2002). Generally lower substrate concentrations are more suitable in order to avoid substrate inhibition. For example, Siti (2009) reported that when a 16% suspension of corn flour is hydrolyzed, the glucose yield was 76%, while when a 40% suspension is hydrolyzed the yield was only 50.2% .

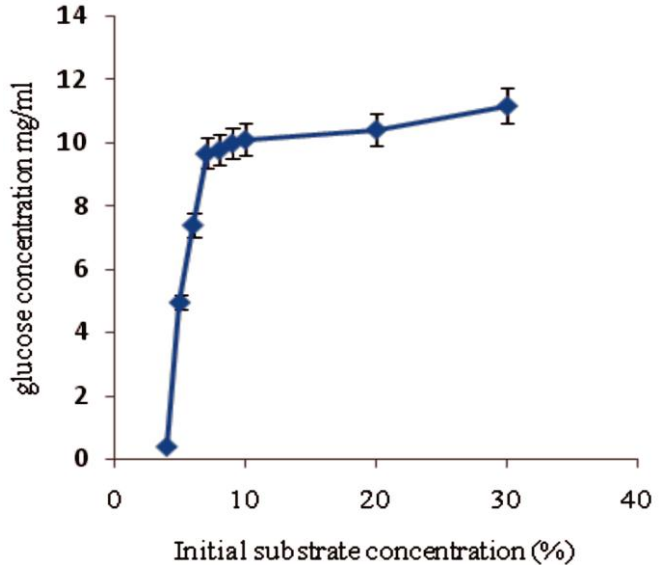


Figure 4a: Effect of substrate concentration on glucose yield*

*Hydrolysis condition: Reaction time 16 hour, cell load 10 mL, pH 4.5, and temperature 45°C.

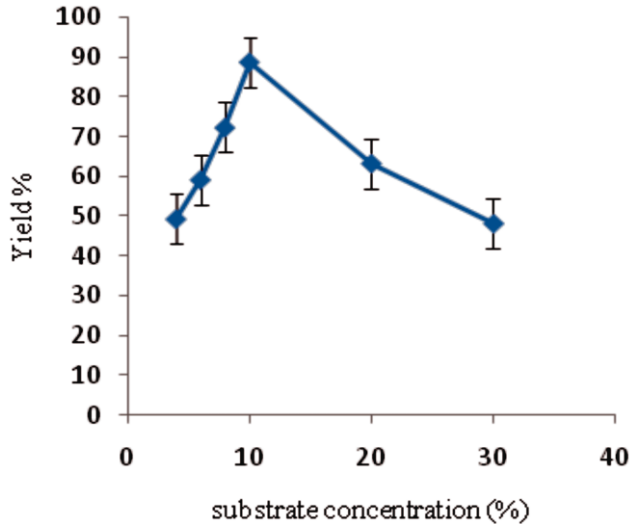


Figure 4b: Effect of substrate concentration on glucose yield*.

*Hydrolysis condition: Reaction time 16 hour, cell load 10 v/v %, pH 4.5, and temperature 45°C.

## CONCLUSIONS

Glucose syrup production by extraction of starch from cocoyam involves grating of raw material, settling, sieving, slurry decantation and finally drying the starch. These time and energy consuming steps were avoid in this work.The concept gives a possibility to carry out one-pot glucose syrup production using *Aspergillus niger*, the cells of *Aspergillus niger* was isolated from soil sample to obtained pure culture, then the viability of the cells was determined to be 84.7%. Operating temperature of 45°C, pH of 4.5, substrate concentration of 10% (w/v), cell concentrationof 10% (v/v) and reaction time of 16 h gave the highest glucose yield of 88%. Substrate concentrations, when in low range at a fixed cell concentration, favorably affect the glucose concentration. Cell concentration increases both glucose concentrationand yield.

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