



OPTIMUM HYDROLYSIS CONDITIONS OF CASSAVA STARCH FOR GLUCOSE PRODUCTION

A. Ayodeji Ayoola*

A. Opeyemi Adeeyo**

C. Vincent Efevbokhan***

D. Adeola Olasimbo****

Abstract: *Acid and enzymatic hydrolysis of cassava starch to glucose (fermentable sugar) were investigated and compared. And the effects of acid concentration, pH, temperature and time on the yield of glucose were studied. Experiments were carried out at a temperature range of (60 – 100)⁰C between 30 minutes and 4 hours. (0.2 – 1.0)M strength of H₂SO₄ acid was used and pH values range of 4 – 7 was considered during enzymatic hydrolysis. The study revealed that maximum concentration of glucose was obtained at 100⁰C using 1.0M H₂SO₄ acid for 4 hours during acid hydrolysis. At pH of 4, temperature of 60⁰C and 4 hours of operation, highest concentration of glucose was obtained during enzymatic hydrolysis. Enzymatic hydrolysis produced higher yield of glucose when compared to that obtained from acid hydrolysis.*

Keywords: *Cassava starch, hydrolysis, α- amylase, glucoamylase, glucose.*

*Lecturer II in Chemical Engineering Department, Covenant University, Nigeria.

**Lecturer II in Chemical Engineering Department, Covenant University, Nigeria.

***Lecturer II in Chemical Engineering Department, Covenant University, Nigeria.

****Undergraduate student in Chemical Engineering Department, Covenant University, Nigeria.



1. INTRODUCTION

Drop in the World reserves of petroleum has sensitized the world of the danger embedded in total dependency on fossil fuel, as the main source of energy in the world of geometric increase in energy demand. In many countries, Nigeria inclusive, energy consumption is based on imported refined fossil fuel. But there is need for alternative sources of energy which can successively compete with fossil fuel, in terms of cost and quality. Energy obtained from feedstock (such as sorghum, maize, cassava etc.) is such a good alternative, if efficiently harnessed [9].

Cassava (*Manihot esculenta*) is a very promising feedstock for glucose production (an energy source) and a good glucose production technology results in energy generation [4], [2], [11]. The energy in cassava is preserved in a form of carbohydrate, mainly as starch, the greatest component of dry matter in fresh roots [8]. Starches are generally insoluble in water at room temperature. There are two types of linkage in starchy structures: α -1,4 and α -1,6, linkages. Amylose, a kind of starch, is an unbranched, single chain polymer containing 500 to 2000 glucose subunits with only α -1,4 glycosidic links [6]. The presence of α -1,6 glycosidic linkages in some starchy materials results in a branched glucose polymer called amylopectin [1]. The breaking down of the α -1,4 and α -1,6 linkages to small units of glucose (monosaccharide) is made possible by the actions of α -amylase and glucoamylase (enzymes) respectively [13].

The two commonly used technologies in the conversion of starch to glucose are acid and enzymatic hydrolyses. Acid concentration, operating temperature and duration of hydrolysis play significant roles in determining both the quantity and quality of glucose, during acid hydrolysis [12]. During enzymatic hydrolysis, enzymes break both the α -1,4 and α -1,6 molecular bonds of starch to more simplified smaller units of monomers [6]. α -amylase splits α -1,4 bonds in amylose and amylopectin. It is an endo-acting enzyme and its action is often considered to be random. The α -1,6 glycosidic bonds are not hydrolyzed. The properties as well as the action of α -amylase depend on the microorganisms or plants from which it is derived. However, α -amylases rapidly decrease the viscosity of starch solutions [8]. Glucoamylase is an exo-acting enzyme, hydrolyzing α -1,4 and α -1,6, glycosidic linkages in amylose and amylopectin. The rates of hydrolysis depend on the molecular size and structure of the substrates [8].



The aim of this study is to compare the two methods of acid hydrolysis and enzymatic hydrolysis for the conversion of cassava starch to glucose (fermentable sugar). Also to observe the effects of pH and temperature on the action of the enzymes involved during enzymatic hydrolysis. Also, to establish the effects of variation in acid concentration, temperature and time of operation on glucose obtained during acid hydrolysis.

2. MATERIALS AND METHODS

Fresh *Manihot esculenta* was obtained from a local market at Oshodi, Lagos State, Nigeria. α -amylase and amyloglucosidase were obtained from the culture collection unit of the Department of Biotechnology, Federal Institute of Industrial Research, Oshodi (FIIRO) Lagos, Nigeria. The reagents used during the course of this study include Sulphuric acid (H_2SO_4) solution, Sodium hydroxide (NaOH) solution, Anhydrous D-Glucose, Distilled water, 3,5-Dinitrosalicylic acid (DNSA) and Potassium Sodium Tartrate (Rochelle Salt).

Preparation of Cassava Starch: The tubers were peeled and washed. The tubers were grated and then soaked in water for three days. After which it was pressed using a muslin cloth to extract starch content. The starch was allowed to settle down and water was decanted and dry white powder of starch was obtained using a dryer.

Acid Hydrolysis: 50g of cassava starch was hydrolyzed by dispersing in 150ml of H_2SO_4 with solution strength ranging from 0.2M – 1.0M. The slurries obtained were kept in water bath set at different temperatures between 60°C and 100°C within the time range of 30mins and 4hrs. After the specified time, 50ml of 0.1M NaOH was used to neutralize the activity of any trace of H_2SO_4 that may be present. The sugar brix and concentration of clear glucose syrup obtained were determined using DNSA reagent (Ranken method 1984), with spectrophotometer operated at 540nm wavelength.

Enzymatic Hydrolysis: Enzymatic hydrolysis of cassava starch was done by dispersing 50g starch sample each in 150ml of distilled water. The slurry obtained was gelatinized at 80°C for 30mins in a water bath. The slurry was then transferred to water bath at 90°C, 2.0ml of alpha amylase (Termanyl) was added to the slurry and allowed to liquefy for 1 hr. The liquefied starch was cooled at 60°C, pH range of 4.0 – 7.0 was observed. 2.0ml of glucoamylase enzyme was added to each of the liquefied slurries and then maintained at different temperature between 60°C and 100 °C, between 30mins and 4hrs for



saccharification to take place. Sugar brix and glucose content of the glucose syrup obtained were determined.

3. RESULTS AND DISCUSSION

The following are the results obtained during acid and enzymatic hydrolysis.

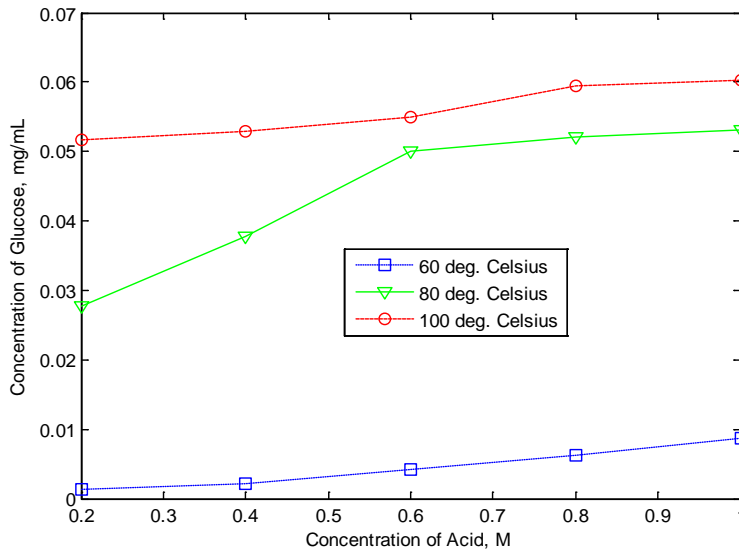


Figure 1: Graphs of concentration of glucose obtained during 30 minutes of acid hydrolysis at different temperatures.

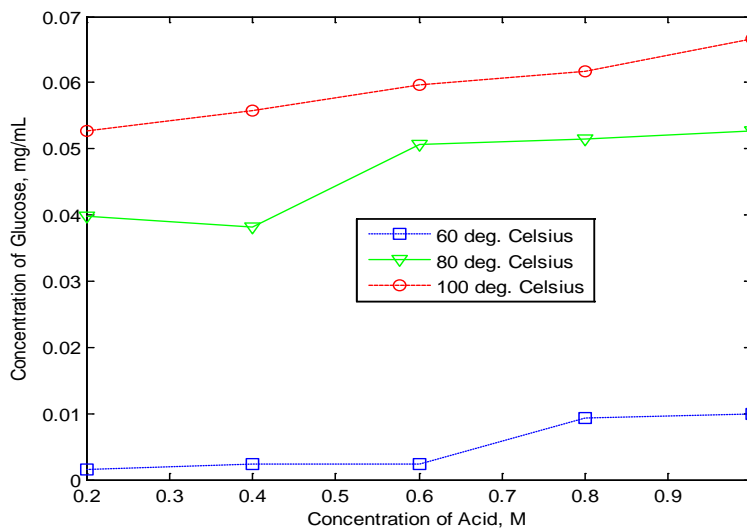


Figure 2: Graphs of concentration of glucose obtained during 1 hour acid hydrolysis at different temperatures.

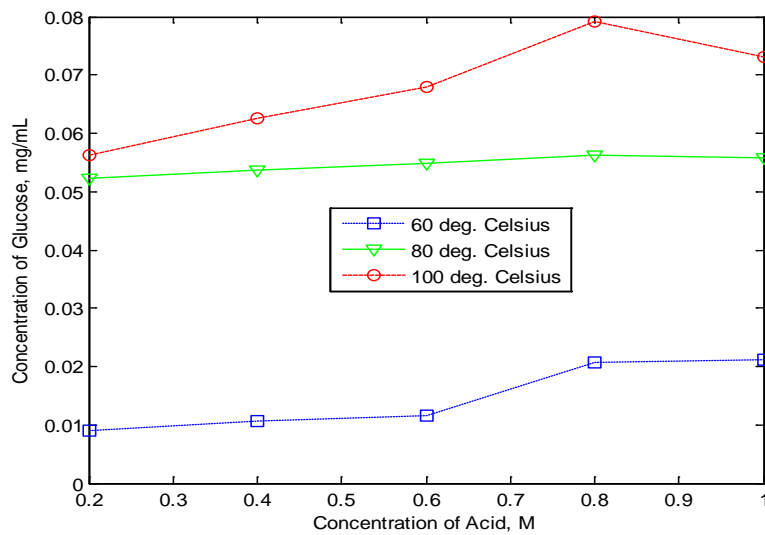


Figure 3: Graphs of concentration of glucose obtained during 2 hours acid hydrolysis at different temperatures.

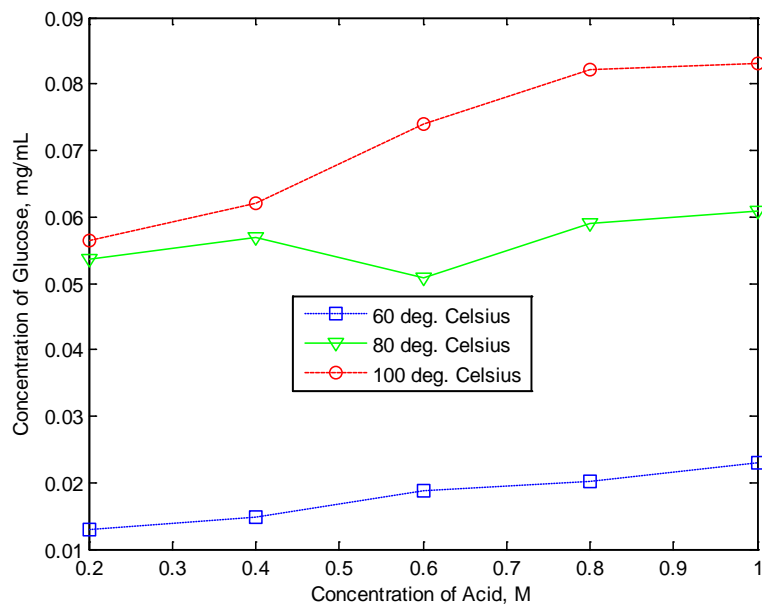


Figure 4: Graphs of concentration of glucose obtained during 4 hours acid hydrolysis at different temperatures.

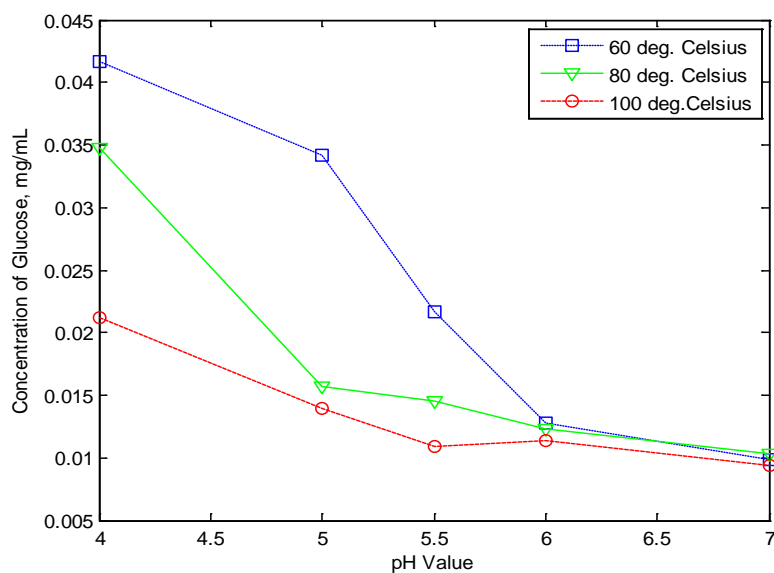


Figure 5: Graphs of glucose concentration produced and pH during 30 minutes enzymatic hydrolysis at various temperatures.

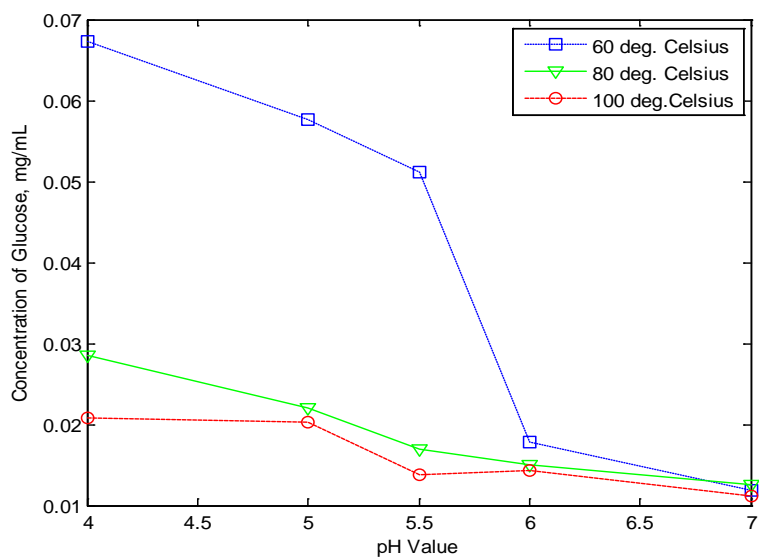


Figure 6: Graphs of glucose concentration produced and pH during 1 hour enzymatic hydrolysis at various temperatures.

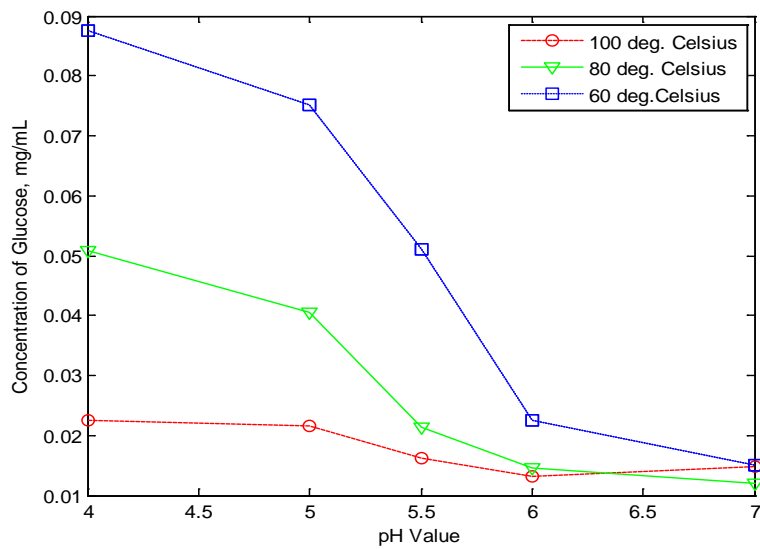


Figure 7: Graphs of glucose concentration produced and pH during 2 hours enzymatic hydrolysis at various temperatures.

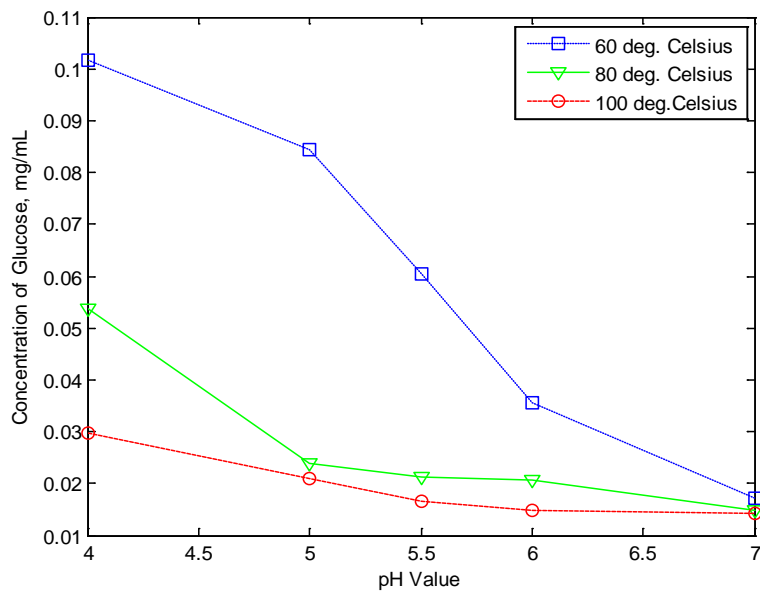


Figure 8: Graphs of glucose concentration produced and pH during 4 hours enzymatic hydrolysis at various temperatures.

Acid Hydrolysis

Highest glucose concentration was observed at 100⁰C while lowest concentration was obtained at 60⁰C, during the 30 minutes of operation (**Figure 1**). The results of the three graphs showed a progressive trend indicating that the 30 minutes of hydrolysis was not



sufficient to generate maximum glucose concentration. Similar result is observed at 1 hour of acid hydrolysis (**Figure 2**). **Figure 3** showed that at 2 hours of acid hydrolysis, maximum glucose concentration was obtained at 0.8M acid concentration and 100⁰C. That is, during acid hydrolysis, increase in the acid concentration, time and temperature favour increase in concentration of glucose produced (**Figure 4**). That is, the complete breaking of $\alpha - 1,4 -$ and $\alpha - 1,6 -$ glycosidic bonds to glucose (fermentable sugar) required 4 hours of operation at acid concentration of 1.0M at 100⁰C.

Enzymatic Hydrolysis

In **Figure 5 – 8** above, during enzymatic hydrolysis, decrease in pH and temperature favour increase in concentration of glucose produced, while increase in time favour glucose production. Highest concentration of glucose was obtained at pH of 4.0, temperature of 60⁰C during 4 hours of operation. These results agreed with the findings of Teerapatr & et. al. [12].

4. CONCLUSION

During hydrolysis, highest concentration of glucose obtained was during 4 hours of operation using 1.0M H₂SO₄ acid concentration during acid hydrolysis. Enzymatic hydrolysis produced highest yield of glucose at pH of 4.0, temperature of 60⁰C during 4 hours of operation. And it was found that enzymatic hydrolysis produced higher concentration of glucose when compared to that obtained from acid hydrolysis. Further work can still be done on this study. These may be consideration of the effect of: (i) using HCl acid instead of H₂SO₄, (ii) increasing time of operation above 4 hours and (iii) decreasing the pH below 4.

BIBLIOGRAPHY

- [1] Amutha, R. and Gunasekaran, P. Production of ethanol from liquefied cassava starch using co-immobilised cells of *Zymomonas mobilis* and *Saccharomyces diastaticus*. J. Biosci. Bioeng., Vol. 92, p 560-564, 2001.
- [2] Bede N. O. Nutritional implications of projects giving high priority to the production of staples of low nutritive quality: The case for Cassava in the Humid Tropics of West Africa, International Institute of Tropical Agriculture, Ibadan, Nigeria. 2006.
- [3] Carl, A.B., Edward, R.A., and David, E.B. Fundamentals of Clinical Chemistry. Saunders, Imprint of Elsevier. Inc. 2008.



- [4] Demirbas, A. Biofuels sources, biofuel policy, biofuel economy and global biofuel projections. *Energy Convers. Manage.*, 49: 21 06-21 16. 2008.
- [5] Erhabor, P.O., Azaiki, S.S. and Ingawa, S.A. *Cassava: The White Gold*. Initiative Publication Company, Benin City, Edo State. 2008.
- [6] Kanlaya Y. and Jirasak K. A Study of Optimal Conditions for Reducing Sugars Production from Cassava Peels by Diluted Acid and Enzyme, (*Nat. Sci.*) Vol.38, p.29 – 35, 2004.
- [7] Klanarong, S. and Kuakoon P. *Cassava Ethanol Technology Growth in Thailand*. 2008.
- [8] Kosaric, N. and Vardar-Sukan, F.). Potential Source of Energy and Chemical Products. In: *The Biotechnology of Ethanol: Classical and Future Applications*, Roehr, M. (Ed.). Wiley-VCH, Weinheim. 2001.
- [9] Mousdale, D.M. *Biofuels: Biotechnology, Chemistry and Sustainable Development*. CRC Press, Taylor & Francis Group, U.S.A. 2008.
- [10] Ranken, M.D. *Food Industries Manual*, 21st Edition. Kaptain Szabo Publiser Washinton DC p138-139. 1984
- [11] Sriroth, K.K., Piyachomkwan, K., Amornitikul, N. T. and Buranares, I. A Study of Directions for the Developments of Ethanol Industry in Thailand, Ministry of Industry. Cassava and Starch Technology Research Unit. 2003.
- [12] Teerapatr, S., Cholada, S., Bongotrat, P., Wichien, K. and Sirintip, C. Utilization of Waste from Cassava Starch Plant for Ethanol Production. 2004.
- [13] Wong, Y.C., Wong, C.T., Onyiruka, S.O. and Akpanisi, L.E. *University Organic Chemistry, The Fundamentals*. Africana-FEP Publishers Limited. 2001.