AMERICAN JOURNAL OF SMART TECHNOLOGY AND SOLUTIONS (AJSTS)

VOLUME 2 ISSUE 2 (2023)

PUBLISHED BY
E-PALLI PUBLISHERS, DELAWARE, USA

American Journal of Smart Technology and Solutions (AJSTS)

Volume 2 Issue 2, Year 2023
ISSN: 2837-0295 (Online)
DOI: https://doi.org/10.54536/ajsts.v2i2.1763
https://journals.e-palli.com/home/index.php/ajsts

Investigation into Optimal Conditions for Enzymatic Hydrolysis of Cassava Starch to Glucose by Amylase from Rice

Olosunde Adebisi William[1*], Onumadu Kelechi Selina[1], Antia Okon Orua[1]

Article Information

Received: June 18, 2023

Accepted: July 23, 2023

Published: July 30, 2023

Keywords

Amylase, Cassava, Glucose, Hydrolysis, Rice

ABSTRACT

The challenge of finding locally available materials in abundance to meet up with the increase in demand for glucose syrup necessitated this study. Enzymatic hydrolysis of cassava starch to glucose using glucose amylase sourced from rice was conducted. A-3 factor with 6 levels factorial viz. substrate concentration (0.5, 1.0, 1.5, 2.0, 2.5 and 3.0% w/v), pH (4, 5, 6, 7, 8 and 9) and temperature (30, 40, 50, 60, 70 and 80 °C) experiment were employed. Rice malt was prepared and enzyme activated. Starch (substrate), buffer solutions, standard glucose solution and its calibration curve were also prepared. Starch was hydrolyzed by α-amylase and tested for presence of reducing sugar using Benedict solution. Time course of the reaction was studied and enzyme activity determined. It was observed that as reaction time increased (t), amount of glucose produced [P] initially increased but soon recorded infinitesimal increase and later assumed constant. The effects of substrate concentration, pH and temperature were found to be essential on glucose production. Statistical analysis on the effect of substrate concentration [S], reaction time and their interactions showed significant impact at probability level of (p) = 0.05.

INTRODUCTION

Unmodified starches have diverse functional properties depending on the source of the crop. Several starch products may be made from these unmodified starches which are regarded as primary resources. The native starch has restricted applications. This is because it has high predisposition to high syneresis, retrogradation, risky processing factors such as temperature, pH, etc (Omojola *et al.*, 2011). The modification of native starch may go a long way in curbing the limitations. This could be attained through esterification, etherification, enzymatic or acid hydrolysis, cross linking and grafting of starch. Starches possess permeable surfaces. Cassava starch has smooth surfaces which are difficult to degrade than those of corn starch (Franco *et al.*, 1988; Jane, 2006). Starch structures are composed of two linkages: α-(1-4) and α-(1-6) linkages. Hydrolysis of starch involves the process of digestion in which enzyme hydrolysis in the digestion system break down the polymer to individual basic glucose units. Various industries extensively use starch hydrolysis in the production of several bio products. Many low molecular mass products such as sugar, brewing, spirits and textile are made by some food processing and other industries from starch. Starch hydrolysis is presently carried out using acid and enzymatic hydrolyses (Adenise *et al.*, 2002; Odebunmo and Owalude, 2005). Milder conditions such as normal pressure, lower temperature (up to 10 0C), and medium pH of 6 to 8 are used for enzymatic hydrolysis (Kolusheva and Marinova, 2007). Enzymatic hydrolysis is considered to have a high reaction rate in terms of its potency to denature detergents, solvents and proteolythic enzymes; and lower reaction medium viscosity at higher temperatures, etc. It is often done using α-amylase which may be got from diverse sources, while β-amylase is rarely employed (Eric, 2017). The source, in which the inner part of its chain composed of polysaccharide molecules, is always attacked by bacterial α-amylase enzymes. The destruction of spiral polysaccharide chain which produces 3 to 10 units of sugar is aided by the action of starch amylose which leads to the disappearance of a typical blue colour when stained with iodine (Pontoh and Low, 1995). For the purpose of hydrolysis of starch to glucose, various grains and cereals like rice, maize, sorghum and wheat could be used as enzyme sources. Recent report from the Western Press has that this simple technology currently being used for making simple sugars from cassava starch (Tello *et al.*, 1993). A study by Hammond and Ayernor (2000) gave maximum yield of sugars when starches obtained from various types of cereal malts were hydrolyzed. Many factors such as size of granules, source of starch, crystallinity, starch components extension of association, amylase and amylopectin reaction rates, type of polymorphism (A, B and C), enzyme type, complex of amylose lipid, and conditions of hydrolysis (concentrations, pH and temperature) may contribute to variations in the enzymatic vulnerabilities of starches (Hoover and Zhou, 2003; Li, 2004; Tester *et al.*, 2006). Simple enzymatic reactions must take into account the factors which may affect the rate of reactions. These factors include pH, temperature, concentration of reactant, enzyme concentration, inhabitation by products, etc (WCB, 2020). Enzyme deactivation cannot be over looked on either kinetic studies or reactor engineering. In Nigeria, because of high exchange rate of naira currency to dollar it is difficult to meet the importation of certain raw materials such as enzyme (gluco amylase). Therefore, there is need to carry out more researches on the use of rice seedlings as source of amylase to

[1] Department of Agricultural and Food Engineering, Faculty of Engineering, University of Uyo, Uyo, P. M. B. 1017, Akwa Ibom State, Nigeria
[*] Corresponding author's e-mail: williamolosunde@uniuyo.edu.ng

hydrolyze starch for glucose production (Bailey and Ollis, 1986; Onyenekwe, 2013).

The choice of cassava (*Manihot esculenta Crantz*) as a substrate source in this study is its abundance in Nigeria (Adejumo and Ola, 2010; Balagopalan, 2002; Osipina and Wheatley, 2005). Due to the increase in demand for glucose syrup and high cost of importing them, there is need to fully utilize and diversify cassava crop. More so, cassava and rice are viewed as rich sources of sugar and hydrolytic enzyme (gluco amylase) respectively; may be utilized as raw materials in the production of glucose as well as other industrial productions.

METHODS

Reagents and Equipment

The chemicals used were analytical grade from Merck, England; BDH, England and Sigma, USA. The equipment employed was spectrophotometer.

Sourcing of Cassava and Rice Paddy

Cassava (Manihot esculenta) roots, TMS 30572 tubers were purchased at Research Institute, Umudike, Abia State while rice paddy was sourced at Afikpo, Ebonyi State.

Preparation of Rice Malt, Enzyme Activation and Buffer Solutions

The rice paddy was soaked in water, drained and kept inside a container for 2 days (48 hours). It was spread on shallow bed (ridge) and allowed to germinate in the dark. Wet rice malt was harvested, cleaned, sun dried; and ground into high diastatic powder (Onyenekwe, 2013). 2g of the powder was suspended in 100 ml of distilled water at 600C for ten (10) minutes to activate enzyme amylase in the powder. The supernatant was discarded leaving the cells in the solution (Onyenekwe, 2013).

The following buffer solutions were prepared using mixing adjusters and salt solutions coupled with addition of distilled water to make up to 200 ml (AnalChem-Resources, 2023):

pH 4: 0.1 M potassium hydrogen phthalate (100 ml) + 0.1 M HCl (0.2 ml)

pH 5: 0.1 M potassium hydrogen phthalate (100 ml) + 0.1 M NaOH (45.2 ml)

pH 6: 0.1 M potassium hydrogen phosphate (KH_2P0_4) [100 ml] + 0.1 M NaOH (11.2 ml)

pH 7: 0.1 M potassium hydrogen phosphate (KH_2P0_4) [100 ml] + 0.1M NaOH (58.2 ml)

pH 8: 0.1 M tris aminomethane (100 ml) + 0.1 M HCl (58.4 ml)

pH 9: 0.1 M tris aminomethane (100 ml) + 0.1 M HCl (11.4 ml)

Preparation of Starch, Substrate and Standard Curve of Glucose D Concentration

Cassava roots (30 kg) were peeled, washed in water and grated with a commercial grater. The pulp was screened using 25 mm aperture mesh and later suspended in water. The supernatant was decanted after allowing the pulp to sediment for about 6 hours. The white starch cake was obtained and sun dried for about 72 hours (3 days) (Oyewole and Obieze, 1995).

Cassava starch concentrations of 0.5 intervals were prepared up to 3.0% (w/v) using each buffer solution obtained in Section 2.3. Each starch (substrate) concentration was gelatinized in water bath at 80 °C for 10 minutes (Nam, 2023).

A stock solution of 0.1% (w/v) was prepared by dissolving 1.0 g of glucose D in 1000 ml of distilled water. The stock solution was then used to prepare glucose concentration of 50 ppm interval up to 300 ppm (Rebecca *et al.*, 2016). For each glucose concentration, about 2 ml of Dinitrosylic acid (DNS) reagent was added and then warmed in water bath at 800C for 10 minutes to develop colour for spectrophotometer reading.

A blank solution of distilled water and DNS was prepared and used to calibrate spectrophotometer to be used in absorbance readings of glucose D concentration (Nam, 2023). The results (spectrophotometer readings) were recorded. A standard glucose curve was then produced.

Determination of Alpha Amylase Activity: Enzyme Assay

The activity of enzyme (rice amylase from malted rice) was determined according to Silva *et al.* (2008). The rice enzyme was activated by incubating 3 g of the enzyme (ground malted rice) suspended in 10 ml of distilled water at 50 °C for 10 minutes. The supernatant discarded leaving the cells in solution. Enzyme solution (6 ml) was mixed with phosphate buffer (4 ml) at pH of 5.0 and 10 ml of starch solution 2% (w/v). The mixture was incubated at 40 °C for 10 minutes. Then, the reaction was discontinued after addition of 2 ml of 0.1 M HCl and colour developed by adding 0.5 ml iodine reagent. After cooling to room temperature, the amount of glucose produced was found by measuring the solution absorbance at 540 nm using Spectrophotometer. However, 1.0 mg of glucose solution reacting with coloured reagent produced an absorbance of 1.0 under the same condition. One unit of enzyme is referred to as the quantity of enzyme which produced 1.0 mg equivalent of glucose per minute under the assay condition (Edu-Enzyme, 2018; Jasco International, 2019).

Assessment of Glucose Production from Starch using Enzymatic Hydrolysis

Phosphate buffer (0.2 M pH 6.0) was used to disperse 15% w/v starch, with bacterial α -amylase solution (0.2% w/v) (3 ml). Exactly 1 ml aliquot sodium azide solution (10% w/v) was incubated at 37 °C for 48 hours in an orbital shaker. The quantity of reducing sugar was found after solids were decanted, and the aliquots of the supernatant removed at 6, 9, 24, 30 and 48 hours. At the expiration of incubation period of 48 hours, the dispersed enzyme was deactivated by the addition of 0.1 N HCl to reach pH of 3.0. This was followed by 15 minutes stirring. The resultant solution was neutralized with 0.1 N NaOH and

centrifuged at 2100 rpm for 20 minutes. Distilled water and ethanol were used to wash the hydrolyzed residues through filtration. The residues were dried using a hot air oven at 40 °C (Franco and Ciacco, 1992). Thereafter, Benedict test for the presence of reducing sugar was then carried out according to AOAC (1998) and Geetha (2012) methods.

Experimental Design

The experimental design was three (3) factors (substrate concentration, pH and temperature) as variables at 6 levels as adopted by Khan (2013). The levels selected for the glucose production were as follows: substrate concentration, Sc (0.5, 1.0, 1.5, 2.0, 2.5 and 3.0% w/v), pH (4, 5, 6, 7, 8 and 9) and temperature, tp (30, 40, 50, 60, 70 and 80° C) (Torreggiani and Bertolo, 2001; Rosa and Giroux, 2001; Nieto *et al.*, 2001; Ozen *et al.*, 2002; Jain and Verma, 2003). The experiment was done in replicate.

Experimental Procedure

The optimal values of the variables for the production of glucose were obtained based on the following experiment:

Effects of pH on Concentration of Glucose (Sugar) Produced

10 ml of each of the six (6) pH level (4, 5, 6, 7, 8 and 9) was added separately to six (6) different test tubes containing 0.5% w/v of substrate prepared and gelatinized at 80 °C and cooled. They were positioned in water bath at 30 °C. About 4 ml of activated enzyme was then added to each of the six (6) contents of the test tubes. This was allowed to hydrolyze for 10 minutes. About 2 ml aliquot was used to prepare enzyme assay which was measured using spectrophotometer. Exactly 2 ml DNS reagent was added to discontinue the reaction and then heated to develop colour. This was cooled in cold water and their various absorbance readings with spectrophotometer at 540 nm were taken, recorded and tabulated. However, effects of pH on concentration of glucose (sugar) produced was evaluated. Statistical analysis analyzed was carried out using Analysis of Variance (ANOVA) at 5% level of probability embedded in Statistical Package for Social Scientists [SPSS] Version 20.

Effect of Temperature and Substrate Concentrations on Concentration of Glucose (Sugar) Produced

The process described in Section 2.8 (a) was carried out at 40, 50, 60, 70 and 80 °C. Also, the substrate concentrations of 0.1, 1.5, 2.0, 2.5 and 3.0% (w/v) were used separately. Their absorbance's readings were recorded and tabulated.

Evaluation of Optimal Parameters Required to Produce Glucose during Hydrolysis

The optimal parameters (pH, temperature and substrate concentration) were found based on the optimum concentration of sugar obtained.

Time Course of Reaction to Produce Glucose

The optimal parameters were then used to study the time course of the reaction to produce glucose. Spectrophotometer reading before and after dilution, and the corresponding mean amount of glucose produced after conversion were noted. These data were used to plot several curves of glucose concentrations produced against reaction times.

RESULTS

Standard Glucose Calibration Curve and Test for Presence of Reducing Sugar

The plot of spectrophotometer reading against glucose concentration is presented in Figure 1.

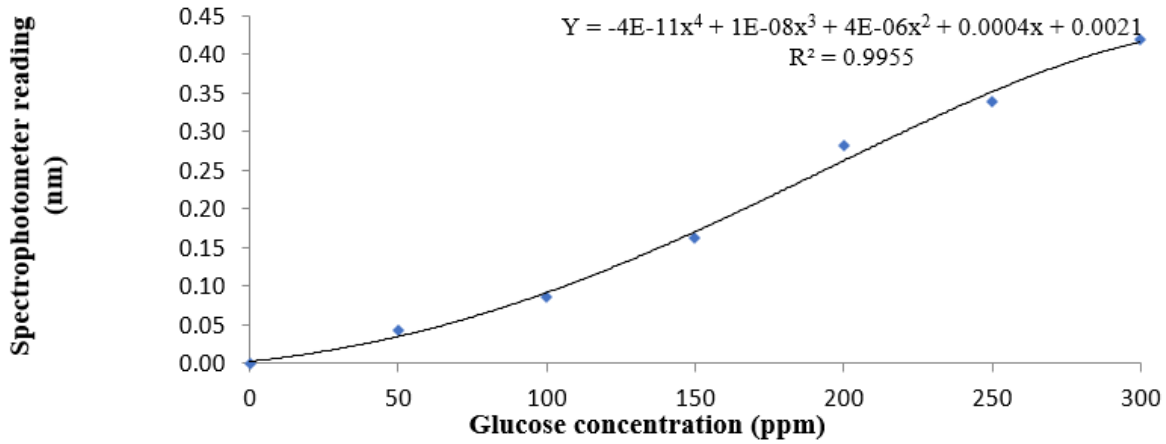

Figure 1: Standard glucose calibration curve.

The data for standard glucose calibration curve gave Equation 1.

$Y = (-4.0 \times 10^{-11}. G^4) + (1 \times 10^{-8}.G^3) + (4 \times 10^{-6}. G^2) + (0.0004 \times G) + 0.0021$ (1)

Where, 1 ppm = 1 mg.L-1 = 0.001 g.L-1, G = glucose concentration (ppm) as independent variable and Y = diluted value of spectrophotometer reading (nm) as dependent variable. The plot of spectrophotometer reading (nm) against glucose concentration is seen to be a polynomial function. From Figure 1, as the

spectrophotometer reading increased, the glucose concentration also increased which is an indication of a strong direct relationship with coefficient of determination (R^2) of 0.9955. Similar trend was reported by Sciencell (2019), Megazyme (2019) and Tunde (2020) with R^2 of 0.9962, 0.9990 and 0.9889, respectively.

The α-amylase was found to hydrolyze the starch by giving a brick red colouration when applying Benedict test. This was an indication of the presence of glucose obtained. Similar studies were conducted by UkEassys (2018) and Cochran *et al.* (2008), and they also had the same result from the hydrolysis of starch using amylase enzyme.

Effect of pH, Temperature, and Substrate Concentrations on Glucose Concentration Produced for 10 Minutes of Reaction Time

The effect of pH, temperature and substrate concentrations on concentration of glucose produced was studied for 10 minutes of reaction time. Based on the data generated, the plots of glucose concentration against temperature at various pH values and substrate concentrations are presented in Figures 2 to 5, while that of glucose concentrate produced against pH values at various temperatures and substrate concentration are shown from Figures 6 to 9.

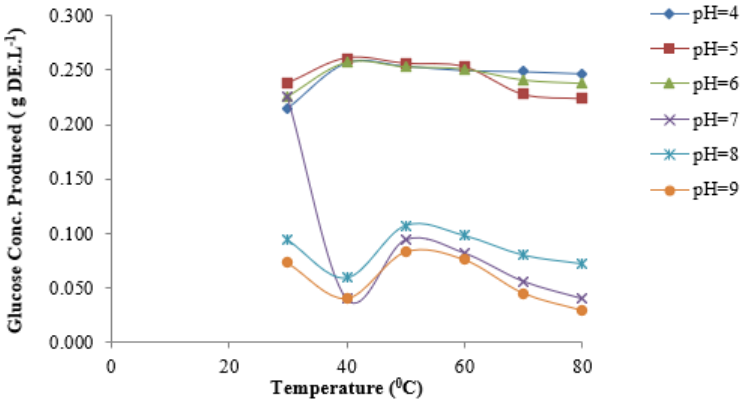

Figure 2: Plot of glucose conc. produced against temperature at various pH and constant substrate concentration of 0.5 %w/v.

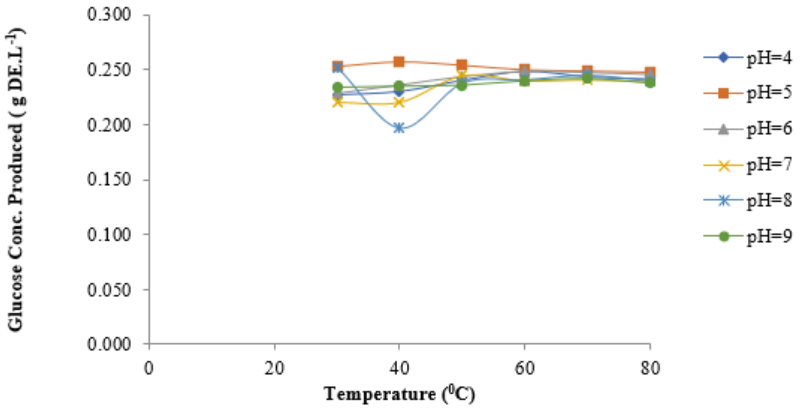

Figure 3: Plot of glucose conc. produced against temperature at various pH and constant substrate concentration of 2.0 %w/v.

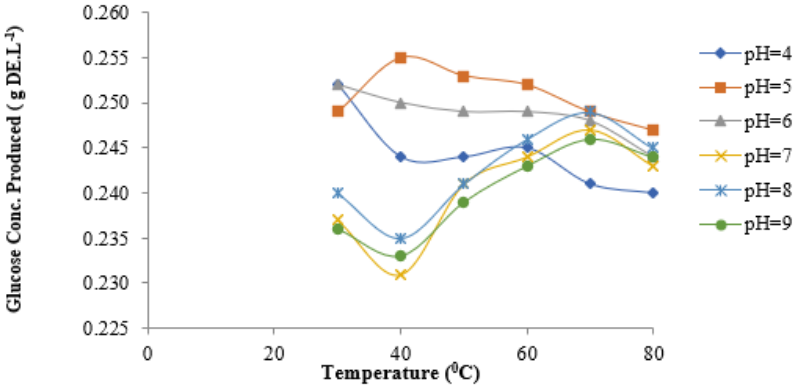

Figure 4: Plot of glucose conc. produced against temperature at various pH and constant substrate concentration of 2.5 %w/v.

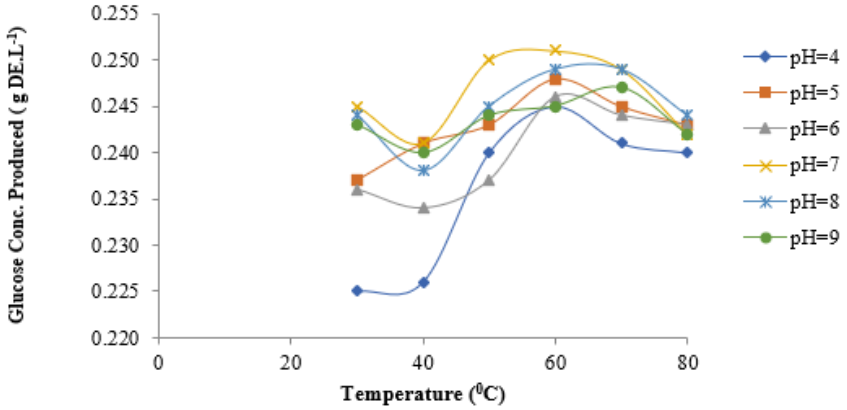

Figure 5: Plot of glucose conc. produced against temperature at various pH and constant substrate concentration of 3.0 %w/v.

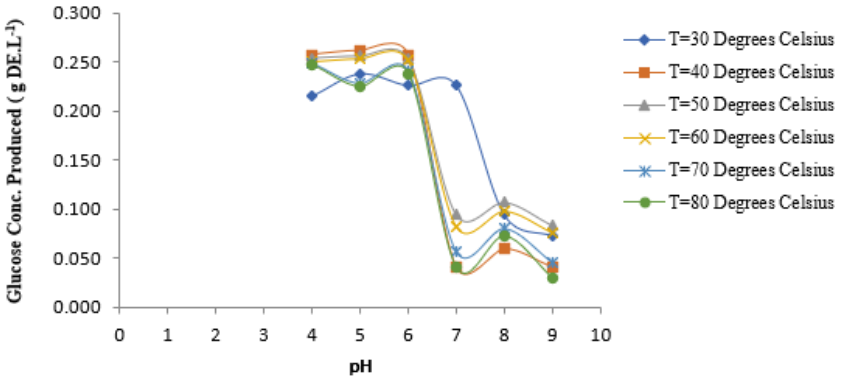

Figure 6: Plot of glucose conc. produced against pH at various temperatures and constant substrate concentration of 0.5 %w/v.

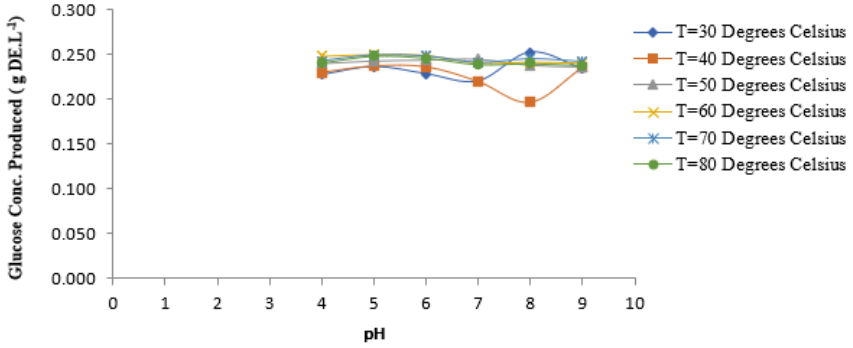

Figure 7: Plot of glucose conc. produced against pH at various temperatures and constant substrate concentration of 2.0 %w/v.

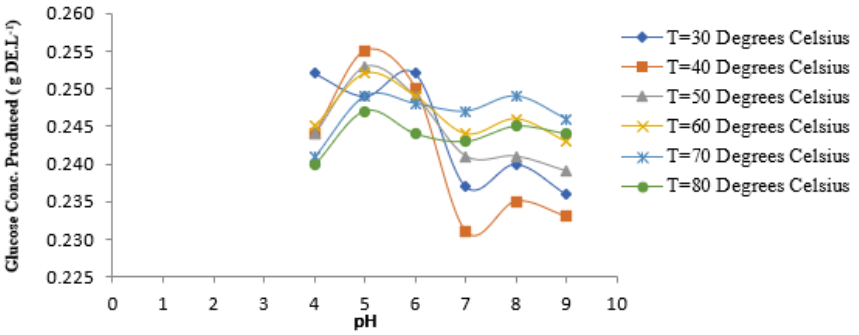

Figure 8: Plot of glucose conc. produced against pH at various temperatures and constant substrate concentration of 2.5 %w/v.

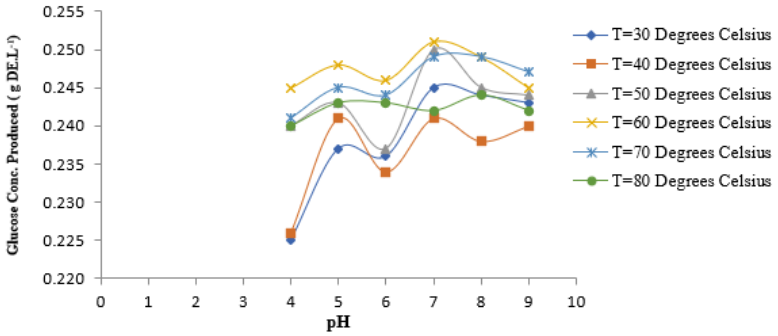

Figure 9: Plot of glucose conc. produced against pH at various temperatures and constant substrate concentration of 3.0 %w/v.

From Figures 2 to 9, the minimum glucose concentration produced (0.029 g DE. L^{-1}) was recorded when pH of 9, temperature of 800C and substrate concentration of 0.5% (w/v) were used, whereas the maximum glucose concentration (0.261 g DE. L^{-1}) was obtained, when pH of 5, temperature of 400C and substrate concentration of 0.5% (w/v) were used. Generally, lower pH favoured the higher amount of glucose produced while high pH values produced lesser amount of glucose. Increases in temperature, decreased the concentration of glucose produced. This implies that too high acidic or alkaline medium does not favour the activities of enzymes. However, different kinds of enzymes have specific range of conditions necessary for their optimal performances. At higher temperature, some enzymes might be denatured as they are made up of protein. Hence, pH of 5, temperature of 400C and substrate concentration of 0.5% (w/v) were considered as optimum condtions. These conditions were used in studing the time course of reaction or simply the production of glucose with time. In a study conducted by UkEssays (2018), the effects of pH (5 to 9) and temperature (300C to 900C) were conspicuously observed on the enzymatic hydrolysis of starch to glucose. Other researchers such as Alias-Rodinah (2009) and Karolina (2015) also reported the effect of pH, temperature, and substrate and enzyme concentrations on the production of glucose.

Determination of Enzyme Activity

The outcome of enzyme activity is presented in Table 1.

Table 1: Activity of α-amylase from rice in the production of glucose from cassava starch.

Spectrophotometer Reading After Dilution [Absorbance] (nm)	Amount of Glucose Produced (g DE.L^{-1})	Molar Conc. of Glucose Produced (mol DE.L^{-1})	Enzyme Activity
0.349	0.248	0.04468	0.0203 U.L^{-1} or 0.0203 μ mol.min^{-1}.L

From Table 1, 0.248 g DE.L^{-1} of glucose was produced when the total volume of enzyme mix in assay of 22 ml was incubated for 10 minutes at 400C and pH of 5. However, the enzyme activity in this study was found to be 0.0203 U.L^{-1} or 0.0203 μ mol.min^{-1}.L. This means 0.0203 units of enzyme catalyzed the transformation of 1 μ mol of substrate into glucose in 1 minute under standard conditions. The low value of the enzyme activity might be as a result of change in optimal pH of the enzyme. This slows down the enzyme activity. However, high value might cause enzyme to denature (Cornish-Bowden, 1995; Jasco International, 2019).

Production of Glucose with Time

Time course of reaction was conducted using 40 ^{0}C and pH of 5.

Plots of several curves of glucose concentrations produced against reaction times is presented in Figure 10. In Figure 10, initially, the amount of glucose produced at reaction time, t = 0 minute was 0 g DE.L^{-1}. After 10 minutes, the amount of glucose produced were 0.150, 0.190, 0.194, 0.207, 0.207 and 0.227 g DE.L^{-1} in the six test tubes containing substrate concentrations of 0.5, 1.0, 1.5, 2.0, 2.5 and 3.0 % (w/v) respectively. The production within this period increased rapidly. Test tube I with 0.5 % (w/v) of substrate concentration showed a gradual increase in glucose concentration from 10 minutes to 50 minutes of reaction time. Beyond this period, there was a decrease in glucose concentration. Test tubes II and III with 1.0 and 1.5 % (w/v) of substrate concentrations, also recorded similar increase from 0 to 0.239 and 0.247 g DE.L^{-1} at 50 and 60 minutes, respectively.

Moreover, test tubes IV and V (contain with substrate concentrations of 2.0 and 2.5 % [w/v]), respectively; and had almost the same trend. A conspicuous increase in the amount of glucose produced was recorded between 0 – 20 minutes. This is, an increase from 0.0 to 0.246 g DE.L^{-1} for both samples. Test tube VI (with substrate concentration of 3.0 % [w/v]) recorded an increase in glucose concentration produced from zero to 0.253 g DE.L^{-1} within 40 minutes. Further increase in reaction time in both test tubes V and VI did not have any

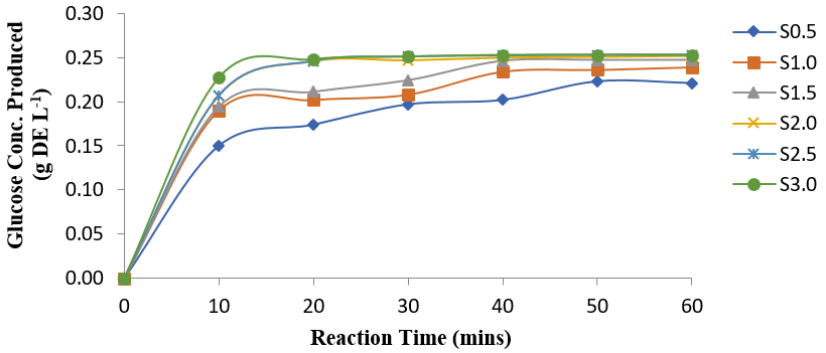

Figure 10: Plot of glucose concentration produced against reaction time.

observable increase in the amount of glucose produced. Generally, increase in substrate concentration yielded an increase the amount of glucose concentration produced. Virtually, unobservable increase or constant production might be due to that the fact the enzyme had reached its optimum activity and the substrate had been consumed completely. Therefore, maximum glucose concentration (0.253 g DE.L^{-1}) produced was found when 2.5% (w/v) substrate concentration at 50 minutes reaction time or 3.0% (w/v) substrate concentration at 40 minutes reaction time was employed.

Similar trends were observed by Cecil (1995) at optimum temperature of 500C, where glucose amylase was used to hydrolyze cassava starch / paddy rice to maltose. In addition, the variations of glucose concentration with time of reaction in this study were in accordance with the works done by many researchers (Cochran *et al.*, 2008; Tunde, 2020; Nor, 2009).

Effect of Substrate Concentration, Reaction Time and Substrate Concentration-Reaction Time Interaction on Glucose Produced at Constant pH = 5 and Temperature = 40 ^{0}C

The summary of ANOVA showing the effect of substrate concentration, reaction time and substrate concentration-reaction time interaction on glucose concentration produced at constant pH = 5 and temperature = 40 ^{0}C is presented in Table 2.

Table 2: Summary of ANOVA showing the effect of substrate concentration, reaction time and substrate concentration-reaction time interaction on the glucose produced.

From Table 2, since p-value [0.00] < 0.05 and coefficient of determination (R^2) = 1.00, F value is significant, which shows that the substrate concentration and reaction time had greater influence on glucose produced. More so, the resulting interaction between substrate concentration and reaction time had a significant impact on glucose produced. In a study conducted by Nor (2009), the effects of liquefaction temperature and saccharification pH on glucose production were very significant while the saccharification temperature and liquefaction pH, on the other hand did not influence the glucose production. The observed trend in the present study implies that these factors should not be rolled out when considering enzymatic hydrolysis of starch to produce glucose because they really influence the amount of glucose production.

Table 2: Summary of ANOVA showing the effect of substrate concentration, reaction time and substrate concentration-reaction time interaction on the glucose produced

Source of Variation	df	F	P-value @ 5%	Significant?
Substrate concentration	6	3365.130	0.000	Yes
Reaction time	5	74679.197	0.000	Yes
Substrate concentration * reaction time	30	199.227	0.000	Yes

R^2 = 1.000

CONCLUSION

Based on the outcome of the findings, the optimum conditions necessary for reasonable glucose production (0.253 g DE.L^{-1}) were temperature of 40 ^{0}C, pH of 5 and substrate concentration of 2.5% (w/v) for 50 minutes or substrate concentration of 3.0% (w/v) for 40 minutes. Alpha-amylase activity was found to be 0.0203 µ mol. min^{-1}.L. Analysis of Variance (ANOVA) results on the effect of substrate concentration, reaction time and their interaction at 40 ^{0}C and pH of 5 on glucose production showed a statistically significant impact since Pcal < Ptab at probability level (p) = 0.05. Furthermore, the use of rice amylase to produce glucose from cassava starch would complement increase in demand for glucose syrup production.

REFERENCES

Adejumo, B. A. and Ola, F. A. (2010). *The Effect of Cassava Effluent on the Chemical Composition of Agricultural Soil Engineering*. 2nd Edition. Academic Press Inc., New York, 250p.

Adenise, L.W., Saul, N. I., Ashock, P. and Carlos, S. (2002).

Acid and enzymatic hydrolysis to recover reducing sugars from cassava. Baggasse: An economic study. *Brazil Archive of Biotechnology ,45*(3), 1516-1522.

Alias-Rodianah, B. (2009). The Effect of Substrate and Enzyme Concentration to the Fructo- oligosaccharides (FOS) Production. *BEng Project*. Faculty of Chemical & Natural Resources Engineering, University Malaysia Pahang, Malaysia, 125p. (Thesis).

AnalChem-Resources (2023). Preparation of pHBuffersolutions http://delloyd.50megs.com/MOBILE/buffersolns.html (Online).

AOAC (Association of Official and Analytical Chemists) (1998). *Official Methods of Analysis*. 13th Edition, Association of Official and Analytical Chemists, Washington D.C. 876p.

Bailey, J. E. and Ollis, D. F. (1986). *Biochemical Engineering Fundamentals*. 2nd Edition. Mc Graw Hill, United Kingdom, 928p.

Balagopalan, C. (2002). Cassava utilization in food, feed and industry. In: R. J. Hillocks, J. M. Tresh and A.C. Belloti (Editors) Cassava: Biology, Production and Utilization. CIAT, Columbia, 301-318.

Cochran, B., Lunday, D. and Miskevich, F. (2008). Kinetic Analysis of Amylase Using Quantitative Benedict's and Iodine Starch Reagents. *Journal of Chemical Education, 85*(3), 401- 404.

Cornish- Bowden, A. (2014). *Fundamental of Enzymes Kinetics* Revised Edition, Elsevier Science, London, 173p.

Edu-Enzyme (2018). *Enzyme Activity Determination*. http://sites.psu.edu/kmf5429/ lab3 enzyme (Retrieved on 10th May, 2018).

Eric, B. (2017). Recent progress. *In: Ian, J. T.(Editor) Understanding Starch Structure. Agronomy, 2*(7), 56, https://doi.org/10.3390/agronomy7030056.

Franco, C. M. and Ciacco, C.F. (1992). Factors that affect the enzymatic degradation of natural starch granules: Effect of the size of the granules. *Starch Starke, 44* (11), 422-426.

Franco, C. M., Ciacco, C. F. and Tavares, D. Q. (1988). Studies on the susceptibility of granular cassava and corn starches to enzymatic attack. Part 2: Study of the granular structure of starch. *Starch-Starke, 40*(1), 29-32.

Geetha, D. K. (2012). *Practical Biochemistry*. Jaypee Brothers Medical Publishers Ltd, New Delhi: 4 - 7.

Hammond,T. K. and Ayernor, G. S. (2000). Combination of malted series and cassava starch in the production of sugar syrup. *Journal of the Ghana Science Association, 2*(1), 87-92.

Hoover, R. and Zhou,Y. (2003). Invitro and invivo hydrolysis of legume starches by alpha amylase and resistance starch for formation in legumes: A review. *Carbohydrate Polymers, 54*(4), 401-417.

Jain, S. K. and Verma, R. C. (2003). Osmotic dehydration: A new, promising and emerging industry. *Beverage Food World, 30*, 30–34.

Jane, J. (2006). Current understanding on starch granule structure. *Journal of Applied Glycoscience, 53*(3), 205-213.

Jasco International (2019). Application Note: Measurement of Enzyme Activity - Alkaline Phosphatase Activity. http://www.jasco corporation.com (Retrieved on August, 2019).

Karolina, A. B. (2015). Kinetic Modelling of Enzymatic Starch Hydrolysis. PhD Thesis, Wageningen University, Wageningen, 160p. (Thesis).

Khan, S. (2013). Saying Meritocracy and Doing Privilege: *The Sociological Quarterly*. http://www.wileyonlinelibrary.com (Retrieved on 25th August, 2019).

Kolusheva, T. and Marinova, A. (2007). A study of the optimal conditions for starch hydrolysis through thermostable α-amylase. *Journal of the University of Chemical Technology and Metallurgy, 42*(1), 93-96.

Li, J. H. (2004). Starch from hull-less barley: In-vitro susceptibility of waxy normal and high amylase starches towards hydrolysis by alpha amylases and amyloglucosidase. *Food Chemistry, 84*(4), 621-634.

Megazyme (2019). *D-glucose Assay Procedure*. www.megazyme.com (Retrieved on 19th April 2020).

Nam, S. W. (2023). Starch Hydrolysis by Amylase: Experiment NO. 5 https://user.eng.umd.edu/~nsw/ench485/lab5.htm (Retrieved on 7th June 2023). (Online).

Nieto, A. B., Salvatori, D. M., Castro, M. A. and Alzamora, S. M. (2004). Structural changes in apple tissue during glucose and sucrose osmotic dehydration: shrinkage, porosity, density and microscopic features. *Journal of Food Engineering, 61*(2), 269 – 278.

Nor, S. (2009). *Effects of pH and Temperature on Glucose Production from Tapioca Starch Using Enzymatic Hydrolysis: A Statistical Approach*. BEng. Faculty of Chemical and Natural Resources Engineering University Malaysia, Pahang, Malaysia, 24p. (Thesis).

Odebunmi, E. O. and Owalude, S. O. (2005). Kinetics and mechanism of oxidation of sugars by chromium (VI) in perchloric acid medium. *Journal Chemical Society of Nigeria, 30*(2), 187-191.

Omojola, M.O., Manu, N. I., Thomas, S. A. (2011). Effect of acid hydrolysis on the physicochemical properties of colar starch. *African Journal of Pure and Applied Chemistry, 5* (9), 307-315.

Onyenekwe, P.C. (2013). *Glucose Syrup Production*. RMRDC Proceedings Series 025: Adoption of Improved Processing Equipment for Cassava Cluster Development in CRCRI Adopted Villages, Abuja, Nigeria, 123p. (Proceedings).

Osipina, B. and Wheatley, C. (2005). *Processing of Cassava Tubers, Meals and Chips*. http://www.fao.org (Retrieved on 24th July 2019). (Proceedings).

Oyewole, O.B. and Obieze, N. (1995). Processing and characteristics of tapioca meal from cassava. *Tropical Science, 35*, 401-404.

Ozen, B. F., Dock, L. L. Ozdemir, M. and Floros, J. D. (2002). Processing factors affecting the osmotic

dehydration of diced green peppers. *International Journal of Food Science and Technology, 37*, 497–502.

Ponto, J. and Low, N.H. (1995). Glucose syrup production from Indonesian palm and cassava starch. *Food Research International, 28*(4), 379-385.

Rebecca, V. A., Wout, B. and Ruben, V. (2016). Saccharification protocol for small-scale lignocellulosic biomass samples to test processing of cellulose into glucose. *Bioprotocol, 6*(1), 1 -10 https://www.researchgate.net/publication/308753308 (Retrieved 13th March, 2018). (Online).

Rosa, M. D. and Giroux, F. (2001) Osmotic treatments and problems related to the solution management. *Journal of Food Engineering, 49*, 223 – 236.

Sciencell (2019). Glucose Assay. Science Cell Research Laboratories. (Book). www.sciencellonline.com (Retrieved 11th April 2020).

Silva, R. N., Fabio, P. Q., Valdirene, N. M., Eduardo, R. A., Ciencia, T. A. (2008). Production of glucose and fructose syrups from cassava (Manihot esculanta Crantz) starch using enzymes produced by microorganisms isolated from Brazilian Cerrado soil. The *Journal of Biological Chemistry, 30*.

Tello, P.G., Rubio, F.C., Alameda, E. J. and Rodriguez, R.S. (1993). Kinetics of the hydrolysis of soluble starch by glucoamylase. *International Chemical Engineering, 33* (3), 450- 455.

Tester, R.F., Qi, X. and Karkalas, J. (2006). Hydrolysis of native starches with amylase. *Animal Feed Science and Technology, 130,* 39-54.

Torreggiani, D. and Bertolo, G. (2001). Osmotic pretreatments in food processing: Chemical, physical and structurals effects. *Journal of Food Engineering, 49*, 247-253.

Tunde, A. A. (2020). Production of glucose from hydrolysis of potato starch. *World Scientific News, 145,* 128-143.

Uk Essays (2018). *Starch Hydrolysis by Amylase: An Experiment* https://www.ukessays.com/essays/biology/the-starch-hydrolysis-of-amylase (Retrieved 11th April 2020).

WBC (Worthington Biochemical Corporation) (2020). Introduction to Enzymes. http://www.worthington-biochem.com/introbiochem/enzymeconc.html (Retrieved on 29th April 2022).