Case Study ISSN 2834-7854

Journal of Nutrition Food Science and Technology

# Determination of Optimum Conditions of pH and Temperature on the Saccharification of Cassava Starch by Amyloglucosidase

**George Ifeoluwa Pele[1], Esther Oluyinka Oladiti[2] and Amadu Kayode Salau[3]**

[1]*Department of Human Nutrition and Dietetics, Federal University of Health Sciences, P.M.B. 204 Ila- Orangun, Osun State, Nigeria.*

[2]*Department of Food Technology, Federal Polytechnic, P.M.B. 420, Offa, Kwara State, Nigeria.*

[3]*Department of Biochemistry, Federal University of Health Sciences, P.M.B. 204 Ila-Orangun, Osun State, Nigeria.*

***Corresponding author**

**George Ifeoluwa Pele,**
Department of Human Nutrition and Dietetics,
Federal University of Health Sciences,
P.M.B. 204 Ila- Orangun,
Osun State,
Nigeria

Submitted : 2 Oct 2023; Published : 10 Apr 2024

**Citation:** Pele, G. I. et al. (2024). Determination of Optimum Conditions of pH and Temperature on the Saccharification of Cassava Starch by Amyloglucosidase. J N food sci tech, 5(2):1-6. DOI : https://doi.org/10.47485/2834-7854.1039

**Abstract**

*Saccharification is the process of breaking complex carbohydrate into its monosaccharide components. The present study however was carried out to determine the optimum conditions of pH and temperature on the saccharification of cassava starch by amyloglucosidase. The optimum conditions of cassava starch hydrolysis was determined by using a pure culture of a thermostable amyloglucosidase for saccharification, and the activities of the enzyme determined at varying pH, temperature and time. Results showed that sample dry weight significantly decreased with respect to increased value of pH, temperature and time, while reducing sugar and dextrose equivalent significantly increased with respect to increased time. The optimal reducing sugar and dextrose equivalent were 74.23% and 96.41 DE, respectively at pH 4.5, 55 °C and 72 h. The glucose obtained from this process may serve as a substrate in fructose syrup production.*

**Keywords:** Amyloglucosidase, Cassava Starch, Dextrose Equivalent, Reducing Sugar.

## Introduction

Starch is a major and abundantly distributed polysaccharide produced by plants which are made up of two molecular weight polymers: amylose and amylopectin. While amylose is a linear chain of glucose residues linked by α-1, 4 glycosidic bonds, amylopectin is a branched polymer that is characterized with α-1, 4 and α-1, 6 glycosidic bonds (Riaz *et al.* 2012). Scientific study has established that heating starch with dilute sulphuric acid transformed it into a sugar, but Becks *et al.* (1995) has reported high yield and less complicated processes where amyloglucosidase preparation is found through which transglucosylase had been removed, thereby giving improved yields of dextrose (Raveendran *et al*. 2018). The disadvantages of using acid in the conversion process, however have been the requirement of the corrosion resistant materials that is able to withstand acidic state of the process, ability of the reaction to give rise to high colour and salt ash content after neutralization; the need for more energy for heating and the relative difficulty to control due to exothermic nature of the process. Amyloglucosidase is found to be an enzyme that has the capacity of hydrolyzing the α-1, 4 glycosidic bonds from the non-reducing ends of starch to produce glucose. It is also an exo-acting enzyme that is able to catalyse the production of β-D-glucose from the non-reducing ends of substrates that include starch, and maltooligosaccharides by consecutively hydrolysing α-1, 4 and α-1, 6 linkages (Sauer *et al.* 2000).

The process of converting starch to dextrose involves the systematic process of gelatinization followed by hydrolysis which is a complex biochemical reaction of breaking the glycosidic bond (Bello-Perez *et al*., 2002). Dextrose, maltose, isomaltose and dextrin are normally produced by saccharifying enzymatic reaction and the proportions of each constituent usually varies with the conditions of production and with the enzyme or combination of enzymes used (Fagain, 2003). The gelatinization, which is essential for effective hydrolysis can be achieved by heating a 30-40% weight suspension of the starch at a pH range between 4.0 - 5.5 but with an amylase enzyme obtained from *Rhizopus niveous* with the optimum pH of 4.4 and 4.8 and the batch adjusted to pH 5.0.

Corn starch has been a major industrial raw material for glucose and fructose syrup production in United States and in many other parts of the world (Cabello,1999), but the limiting availability of corn starch in most developing countries has necessitated the essence of sourcing starch from other plant materials. Enzymatic hydrolysis from many plants such as cassava, corn, wheat, potato etc. have however been widely reported (Betiku and Ajala, 2010).

Cassava (*Manihot esculenta* Crantz), which have been found as an essential plant crop in the tropical regions (Aderibigbe *et al.,* 2012), comprises mostly the carbohydrates fraction which is starch and makes up to 20-35% of the fresh tuber. Adesanya et al. (2012) have reported that cassava is relatively rich in calcium and ascorbic acid (vitamin C) and appears to contain nutritionally significant amounts of thiamine, riboflavin and niacin. Other carbohydrate constituents found are fructose, dextrose and dextrin, although study has not clearly shown whether these are actually present in the live root or formed after harvesting as breakdown products from starch and sucrose (Aderibigbe *et al.,* 2012). Maniot has high tolerance to drought because it has the capacity to survive during the dry season, most especially when soil moisture is low and humidity is high. It has also found to survive low soil quality or nutrient as it thrives better in poor soils than any other major plant materials (Oluwole *et al.*, 2007).

The objective of the present study therefore was to determine the optimal conditions of pH and Temperature on the saccharification of cassava starch by amyloglucosidase.

## Materials and Methods

### Materials

Maltodextrin which has an optimal reducing sugar and dextrose equivalent of 17.84% and 14.74 DE, respectively at pH 6.5, 70 °C and 60 min was obtained from previous liquefaction process of cassava starch by alpha-amylase (Pele et al., 2018). Pure culture of thermostable amyloglucosidase (from *Aspergillus niger*; pH 4.5; temperature, 60 °C) was obtained from Federal Institute of Industrial Research, Oshodi (FIIRO), Nigeria. Rochelle salt and Dinitrosalicylic acid (DNS) were obtained from Pascal Store, Akure, Nigeria

### Description of Fermentor

A prototype of a fermentor was designed and fabricated to combine with thermostatic water bath (DK-600 SANFA Electrical thermostatic water bath boiler model) for liquefaction and saccharification as shown in Figure 1. The fermentor uses variable motor Gear: GIFA Transmission Bologna Italy, Type (TIPO): (Var 10/0) Code (Condice): AC3999 Motor.

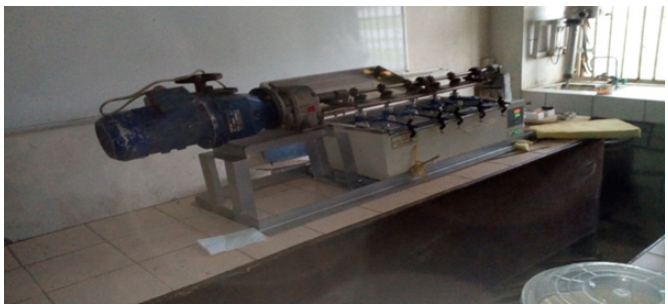


**Figure 1:** The fermentor used for liquefaction and saccharification

Note: (Motor) Kw: 0.75; Poles: 4; Rpm min–rpm max: 350–1750; Type: mas 20P; Code: 29602117; Mount POS: 2.5.4. Bonfiglioli Riduttori, Italy.

### Production of Substrate

The Substrate was produced by preparing a cassava starch suspension of 10% (w/v) with distilled water, to make 10% slurry (Pele et al., 2018). Usually 10 g of starch was weighed into 100 ml distilled water to make slurry. The solution of 40 ppm $Ca^{2+}$ was added for stability of the enzyme. The pH was adjusted to 6.0, 6.5 and 7.0 with citrate-phosphate buffer. Gelatinization was done by heating the mixture to 97 °C and was held at this temperature for 10 min. The gelatinized starch was cooled to 65, 70 and 75°C. Liquefaction was carried out by adding 2% (w/v) of alpha-amylase for 40, 50 and 60 min at the temperatures. The fermentor was clamped with the thermostatic water-bath to maintain at 50 rpm; samples were however withdrawn at regular time intervals to follow the kinetics. The enzyme activity was stopped by heating the mixture to 97 °C for 15 to 20 min and centrifuged (80-2 Centrifuge Med-Lab Scientific Company England) at 2500 rpm for 10 min to obtain the supernatant. The procedures described above were done in triplicates; standard curve of glucose production was prepared to determine the optimum condition of liquefaction for cassava starch which is used as the substrate for saccharification process.

### Characterization of Amyloglucosidase

The optimum condition of cassava starch hydrolysis was determined using pure culture of thermostable amyloglucosidase for saccharification. The activity of the enzyme was determined at varying pH, temperature and time. A 3 x 3 x 12 completely randomized experimental design comprising 3 pH values (pH 4.0, 4.5 and 5.0); 3 temperatures (50, 55 and 60 °C) and 12 time ranges (6, 12, 18, 24, 30, 36, 42, 48, 54, 60, 66 and 72 h) were employed for saccharification.

### Determination of Enzyme Activity in Amyloglucosidase

The optimum samples from the liquefaction process above were brought down to temperatures of 50, 55 and 60°C and adjusted to pH values of 4.0, 4.5 and 5.0 with dilute hydrochloric acid, respectively. Saccharification process was carried out by adding 2% (w/v) of amyloglucosidase and reaction periods 6, 12, 18, 24, 30, 36, 42, 48, 54, 60, 66, and 72 h were allowed at the temperatures and pH values. The fermentor was also clamped with the thermostatic water-bath to maintain at 50 rpm; samples were withdrawn at regular intervals. The enzyme activity was stopped by heating the mixture to 97 °C for 15 to 20 min and centrifuged at 2500 rpm to obtain the supernatant for analyses. The procedures described above were done in triplicates; standard curve of glucose production was prepared to determine the optimum condition of saccharification for cassava starch hydrolysis.

### Determination of Physicochemical Properties of Glucose Syrup

#### Determination of reducing sugar

The reducing sugar content of the syrup samples was determined by DNS method described by Miller (1972) with the addition of Rochelle salt. The reducing sugar was determined by adding 3 ml of DNS solution to 1 ml of hydrolysed starch (supernatant) in a test tube and boiled for 10 min. This was

allowed to cool partially and 1 ml of Rochelle salt was added, while this was allowed to completely cool down before the intensity or absorbance of the red coloured solution was read at 540 nm using UV-Visible Spectrophotometer (AJ-1C03). Series of standard glucose (0-500 mg/l) were run and a standard graph was plotted to calculate the reducing sugar. Percentage reducing sugar was calculated by the percentage of the ratio of the amount of reducing sugar in the glucose syrup to the amount of starch slurry for the hydrolysis.

$$Reducing\ Sugar\ (mg/ml) = \frac{\text{Conc.obt (mg/l)}\ X\ vol.of\ extract X\ dil.factor\ (if\ any)}{Sample\ wt\ X\ vol\ of\ aliquot\ analysed} \quad (1)$$

## Determination of sample dry weight of glucose samples

Two (2) grams of each of the samples was weighed out with the aid of an analytical balance into dried, cooled and weighed dish in each case. The samples in the dishes were then put into a Genlab moisture extraction oven set at 105 ºC and allowed to dry for 3 h after which the samples were then transferred into a dessicator with the aid of a laboratory tong and then allowed to cool for 30 mins. After cooling in the dessicator, they were weighed again and their respective weights recorded accordingly. The above processes were repeated for each sample until a constant weight was obtained in each case. The difference in weight was calculated as the sample dry weight (AOAC, 2005).

## Determination of dextrose equivalent (DE)

Dextrose equivalent (DE) was determined by the expression described by Betiku et al. (2013). Dextrose equivalent was calculated as the ratio of reducing sugar expressed as glucose to the sample dry weight.

$$DE = \frac{\text{Reducing sugar expressed as glucose}}{Sample\ dry\ weight} X\ 100 \quad (2)$$

## Statistical Analyses

Data obtained from the experiment were subjected to completely randomized experimental design and statistical analysis using Microsoft excel version 2010, SPSS version 20 and Mini Tab version 17.

## Results and Discussion

Figure 2 showed the glucose syrup produced from the saccharification process of cassava starch with amyloglucosidase. Figure 3 showed the effect of pH 4 on amyloglucosidase and glucose produced at different temperatures. The result showed that at 50 ºC, reducing sugar of the glucose syrup ranged from 18.55 – 50.25%; sample dry weight, 0.083 – 0.113 g; dextrose equivalent, 16.42 – 60.57 DE, of 6 – 72 h. Results showed a significant increase in reducing sugar and dextrose equivalent, a significant decrease in sample dry weight was however observed in the saccharification process that terminated at 72 h. At 55 ºC, the result showed that reducing sugar ranged from 23.24 – 61.44%; sample dry weight, 0.082 – 0.109 g; dextrose equivalent, 21.32 – 74 DE, at 6 – 72 h. Results also showed a significant increase in reducing sugar and dextrose equivalent, however a significant decrease was observed in the sample dry weight in the process that terminated at 72 h. At 60ºC, the results showed that reducing sugar ranged from 21.44 – 73.87%; sample dry weight, 0.08 – 0.105g; dextrose equivalent, 20.42 – 92.34 DE, of 6 – 72 h. Results showed a significant increase in reducing sugar and dextrose equivalent, a significant decrease was however observed in the sample dry weight in the process that terminated at 72 h. Figure 4 showed the effect of pH 4.5 on amyloglucosidase and glucose produced at different temperatures. The result showed that at 50 ºC, reducing sugar ranged from 20.90 – 50.09%; sample dry weight, 0.081 – 0.11 g; dextrose equivalent, 19.00 – 61.83 DE, of 6 – 72 h.

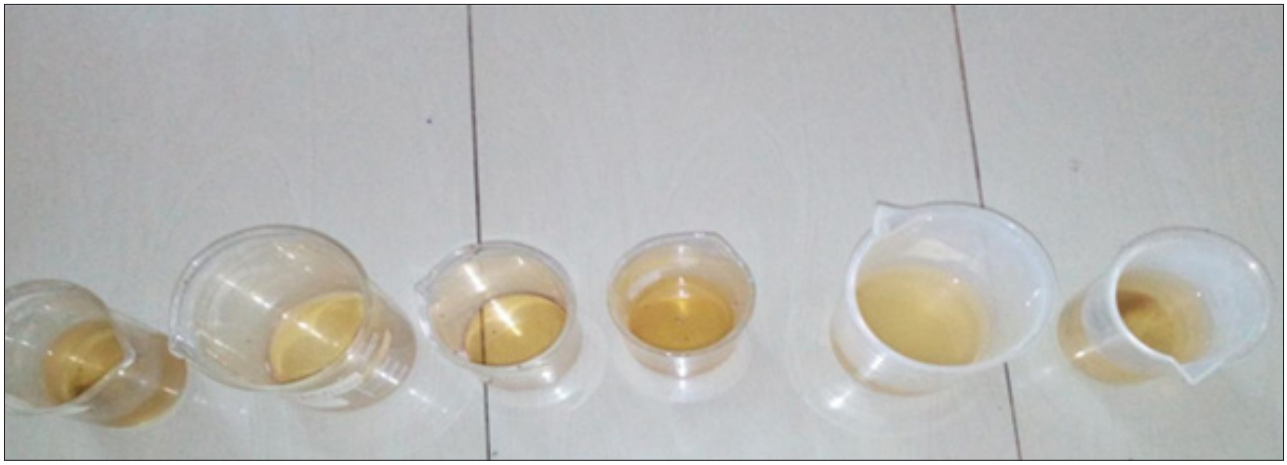
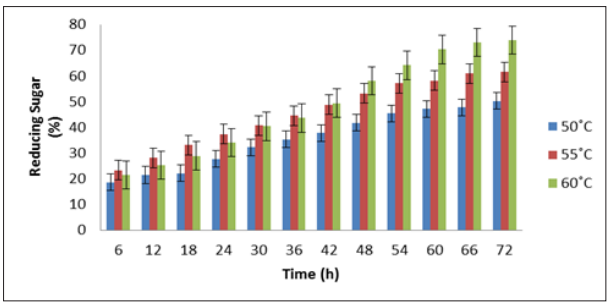
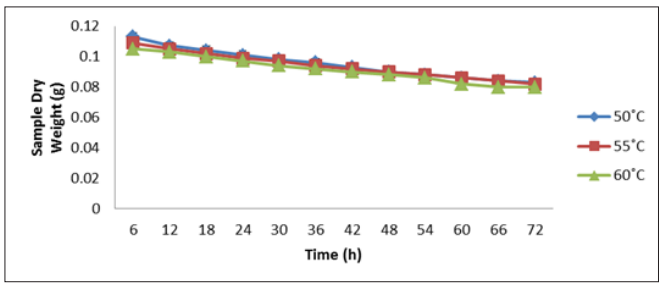


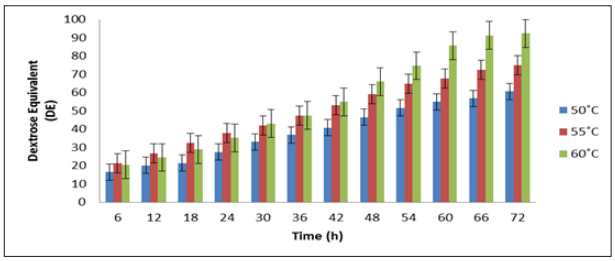
**Figure 2:** Glucose produced from the saccharification of previous maltodextrin from cassava starch by pure amyloglucosidase




(a)

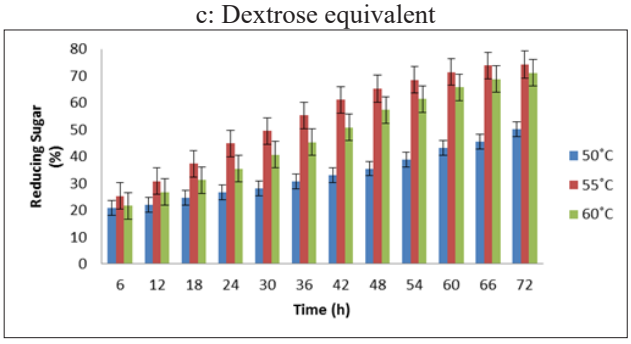



(b)

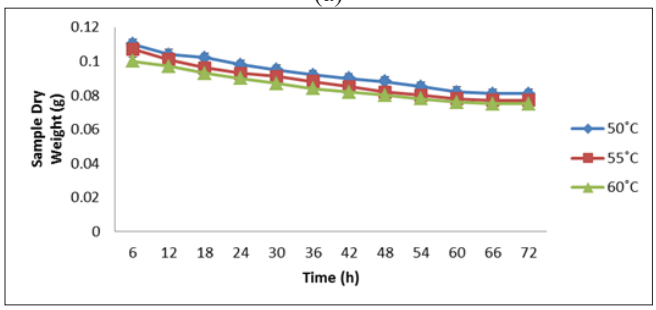



(c)

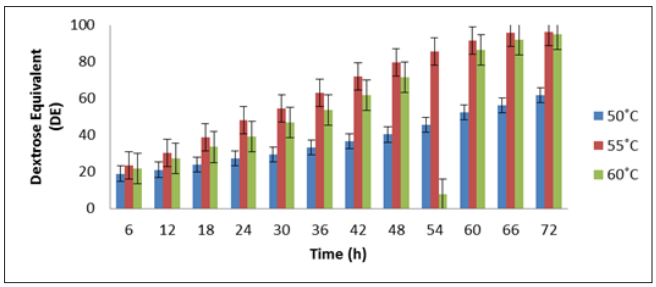
**Figure 3:** Effect of temperature on the saccharification of cassava starch at pH 4
a: Reducing sugar;
b: Sample dry weight;
c: Dextrose equivalent




(a)




(b)

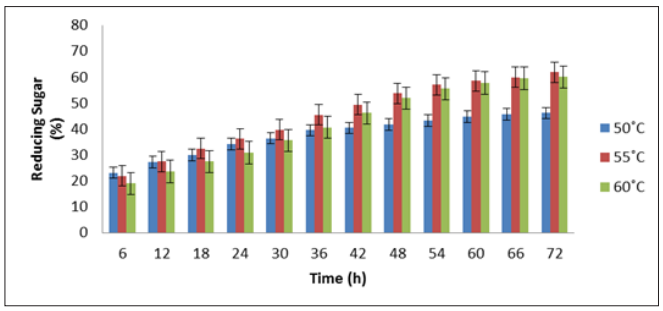



(c)

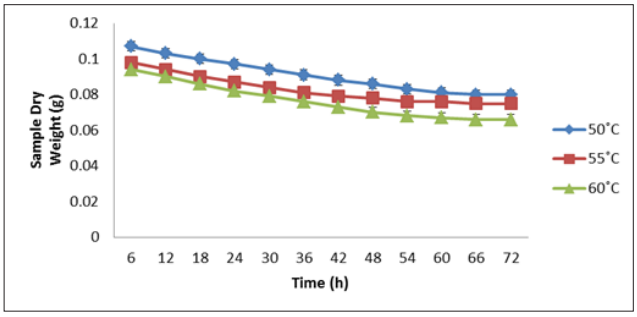
**Figure 4:** Effect of temperature on the saccharification of cassava starch at pH 4.5
a: Reducing sugar; b: Sample dry weight;
c: Dextrose equivalent

Results showed a significant increase in reducing sugar and dextrose equivalent, a significant decrease was observed in the sample dry weight in the process that terminated at 72 h. At 55 ºC, the result showed that reducing sugar ranged from 25 – 74.23%; sample dry weight, 0.077 – 0.107 g; dextrose equivalent, 23.58 – 96. 41 DE, from 6 to 72 h. Results also showed a significant increase in reducing sugar and dextrose equivalent, a significant decrease was however observed in the sample dry weight. At 60 ºC, the result showed that reducing sugar ranged from 21.62 – 71.17%; sample dry weight, 0.075 – 0.1 g; dextrose equivalent, 21.62 – 94.90 DE, 6 – 72 h. Results showed a significant increase in reducing sugar and dextrose equivalent, a significant decrease was however observed in the sample dry weight.

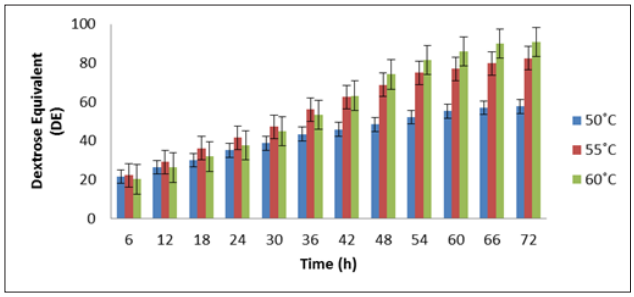
Figure 5 a, b and c showed the effect of pH 5 on amyloglucosidase and glucose produced at different temperatures. The result showed that at 50 ºC, reducing sugar ranged from 23.06 – 46.13%; sample dry weight, 0.08 – 0.107 g; dextrose equivalent, 21.55 – 57.65 DE, 6 – 72 h. Results showed a significant increase in reducing sugar and dextrose equivalent, however a significant decrease was observed in the sample dry weight. At 55 ºC, the result showed that reducing sugar ranged from 21.80 – 61.80%; sample dry weight, 0.075 – 0.098 g; dextrose equivalent, 22.25 – 82.40 DE, of 6 – 72 h. Results also showed a significant increase in reducing sugar and dextrose equivalent, however a significant decrease was observed in the sample dry weight. At 60 ºC, the result showed that reducing sugar ranged from 18.92 – 60%; sample dry weight, 0.066 – 0.094; dextrose equivalent, 20.13 – 90.90 DE, 6 – 72 h. Results showed a significant increase in reducing sugar and dextrose equivalent, however a significant decrease was observed in the sample dry weight in the saccharification process that terminated at 72 h.




(a)




(b)




(c)

**Figure 5:** Effect of temperature on the saccharification of cassava starch at pH 5
a: Reducing sugar; b: Sample dry weight; c: Dextrose equivalent

The significance of reducing sugar, sample dry weight and dextrose equivalent were observed to affect the quality of the glucose produced. It was also observed that the sample dry weight decreases with respect to increase in pH, temperature and time which is significant to the quality of glucose produced. The optimal quality of glucose was produced at pH 4.5, 55 ºC and 72 h with 74.23% and 96.41 DE of reducing sugar and dextrose equivalent. The results are comparable with the reports of Feroza et al. (1998), where optimum enzyme concentration of 0.25% α-amylase and 0.15% amyloglucosidase were used, the results are in contrast with the reports of Ayoola et al. (2013) and Aderibigbe et al. (2007) where saccharification time was terminated at 4 and 2 h. The results obtained in this study was higher than the value obtained by Betiku and Ajala (2010), where Dextrose Equivalent (DE) of 90 was obtained after the enzymatic hydrolysis of breadfruit starch was completed.

## Conclusion

The present study has demonstrated the determination of optimum conditions of pH and temperature on the saccharification of cassava starch by amyloglucosidase. The results obtained in this research showed that the optimal reducing sugar and dextrose equivalent were 74.23% and 96.41 DE, respectively. The optimum pH, temperature and time of saccharification of cassava starch were pH 4.5, 55 ºC and 72 h, respectively. The glucose obtained in this work may serve as a substrate to initiate an isomerization reaction process in the production of fructose syrup.

## References


1. Riaz, M., Rashid, M. H., Sawyer, L., Akhtar, S., Javed, M. R., Nadeem, H., & Wear, M. (2012). Physio-chemical properties and kinetics of glucoamylase produced from deoxy-glucose resistant mutant of Aspergillus niger for soluble starch hydrolysis. *Food Chemistry, 130*(1), 24-30. DOI: https://doi.org/10.1016%2Fj.foodchem.2011.06.037
2. Becks, S., Bielawaski, C., Henton, D., Padala, R., Burrows, K., & Slaby, R. (1995). Application of a liquid stable amylase reagent on the ciba coming express clinical chemistry system. *Clinical Chemistry*, 41, 186-190. Retrieved from https://www.mutagens.co.in/jgb/vol.04/1S/12.pdf
3. Raveendran, S., Parameswaran, B., Ummalyma, S. B., Abraham, A., Mathew, A. K., Madhavan, A., Rebello, S., & Ashok-Pandey, A. (2018). Applications of microbial enzymes in food industry. *Food Tech Biotechnol, 56*(1), 16–30. DOI: https://doi.org/10.17113%2Fftb.56.01.18.5491
4. Sauer, J., Sigurskjold, B. W., Christensen, U., Frandsen, T. P., Mirgorodskaya, E., Harrison, M., Roepstorff, P., & Svensson, B. (2000). Glucoamylase: structure/function relationships, and protein engineering. *Biochimica et Biophys Acta, 1543*(2), 275–293. DOI: https://doi.org/10.1016/s0167-4838(00)00232-6
5. Bello-Perez, L. A., Sanchez-Hernandez, L., Moreno-Damian, E., & Toro-vazquez, J. (2002). Laboratory scale production of maltodextrins and glucose syrup from banana starch. Acta. *Cient. Venez, 53*(1), 1-9. Retrieved from https://www.researchgate.net/publication/11171762_Laboratory_scale_production_of_maltodextrins_and_glucose_syrup_from_banana_starch
6. Fagain, C. O. (2003). Enzymes stabilization-Recent experimental progress. *Journal of Enzyme Microbial Technology, 33*(2-3), 137-149. DOI: https://doi.org/10.1016/S0141-0229(03)00160-1
7. Cabello, C. (1999). Amylases using in glucose syrup production. In: Seminario Brasileiro De Technologia Enzimatica- Enzitec, 4 Rio de Janeiro Anais 1: 1-3
8. Betiku, E., Akindolani, O. O., & Ismaila, A. R. (2013). Enzymatic hydrolysis and optimization of sweet potato (Ipomoea batatas) peel using a statistical approach. *Braz. J. Chem. Eng, 30*(3), 467-476. DOI:http://dx.doi.org/10.1590/S0104-66322013000300005
9. Aderibigbe, R. U., Anozie, F. A., Adejumo, L. A., & Owolabi. (2012). Optimization of cassava starch hydrolysis by sorghum malt. *New Clues in Sciences*, 2, 50-58. Retrieved

from https://scholar.google.com/citations?view_op=view_citation&hl=en&user=cSSeeU0AAAAJ&citation_for_view=cSSeeU0AAAAJ:mVmsd5A6BfQC
10. Adesanya, S. L., Udio A. J. and Shuntogun, B. A. (2012). Studies on the carbohydrate content of breadfruit (Artocarpus commumis forest) from South Western Nigeria. *Journal of Starch and Starke*, 47: 287-294.
11. Aderibigbe, A. F., Anozie, A. N., Adejumo, L. A., & Owolabi, R. U. (2007). Kinetics study of the enzymatic hydrolysis of cassava Starch. *International Journal of Science and Engineering Investigations*, 1: 11.
12. Oluwole, O. B., Kosoko, S. B., Owolabi, S. O., Adeyoju, A. O., Bankole, A. O. Ozumba-Augusta, U. E., & Gloria, N. S. (2012). Development and production of high protein and energy density beverages from blends of maize, Sorghum and Soybeans for School Aged Children: Effect of Malting Period on Selected Proximate Parameters and Sensory Qualities of Developed Beverages, *International Journal of Applied Science and Technology, 2*(7), 285-292. Retrieved from https://fiiro.gov.ng/index.php/publications?task=download.send&id=82&catid=4&m=0
13. Pele, G. I., Bolade, M. K., Enujiugha, V. N., Sanni, M., & Ogunsua. A. O. (2018). Effect of pH and temperature on the activities of alpha-amylase in cassava starch liquefaction. *African Journal of Food Science and Technology, 9*(2), 37-42. DOI: http:/dx.doi.org/10.14303/ajfst.2018.233
14. Miller, G. L. (1972). Use of dinitrosalicylic acid reagent for determination of reducing sugar. *Anal. Chem, 31*(3), 426-428. DOI: https://doi.org/10.1021/ac60147a030
15. AOAC. (2005). Official Methods of Analysis. Association of Official Analytical Chemist. (17th ed.). USA. Retrieved from https://www.scirp.org/reference/referencespapers?referenceid=1477891
16. Feroza, B., Begum, S., & Hossain, M. (1998). Production of glucoamylase by Aspergillus niger in liquid culture and determination of its cultural condition. *Bangladesh Journal of Science and Industrial Resources, 33*(2), 309–311.
17. Ayoola, A. A., Adeeyo, A. O., Efeovbokhan, C. V., & Olasimbo, D. A. (2013). Optimum hydrolysis conditions of cassava starch for glucose production. Int. J. Adv. Res. IT Engin. 2(1), 1-4.