Faculty of Natural and Applied Sciences Journal of Scientific Innovations
Print ISSN: 2814-0877 e-ISSN: 2814-0923
www.fnasjournals.com
Volume 5; Issue 3; March 2024; Page No. 79-86.

Evaluation of Optimal pH and Temperature Conditions for Saccharification of Breadfruit Starch by Amyloglucosidase

[1]Pele, G.I., [2]Pele, E.O., [3]Adepeju, A.B. & [4]Ajala, E.O.
[1]Department of Human Nutrition and Dietetics, Federal University of Health Sciences, Ila-Orangun, Nigeria
[2]Department of Food Technology, Federal Polytechnic, Offa, Nigeria
[3]Department of Food Science and Technology, Federal University of Oye-Ekiti, Nigeria
[4]Department of Chemical Engineering, University of Ilorin, Nigeria.

***Corresponding author email:** ife_pele@ymail.com

Abstract
Given the complex nature of the metabolic reactions involved in breaking down starch polymers into monosaccharides, identifying the most conducive conditions for this process was crucial. The current study aimed to determine the optimal pH and temperature ranges for the enzymatic saccharification of breadfruit starch by amyloglucosidase. Utilizing a pure culture of thermostable amyloglucosidase, the study investigated the conditions that best facilitate the hydrolysis of breadfruit starch. Through systematic experimentation, the ideal pH and temperature parameters for maximizing the saccharification process were elucidated, providing valuable insights into the enzymatic conversion of starch into fermentable sugars. Subsequently, the enzyme's activity was assessed across various pH values, temperatures, and durations. Findings revealed a notable increase in reducing sugar and dextrose equivalent levels over time, alongside a significant decline in sample dry weight in response to higher pH, temperature, and duration values. Notably, at pH 4 and 60°C for 72 hours, optimal levels of reducing sugar (71.71%) and dextrose equivalent (93.13 DE) were observed. These results underscore the importance of pH and temperature regulation in optimizing enzymatic saccharification processes, offering valuable insights into enhancing the efficiency of breadfruit starch hydrolysis.

Keywords: Amyloglucosidase, Breadfruit Starch, Dextrose Equivalent, Reducing Sugar

Introduction

Starch constitutes over half of the carbohydrates consumed by humans, serving as the primary carbohydrate source in the diet (George, 2004). It comprises two main polymers, namely amylose and amylopectin, with natural starches typically containing 10% to 30% amylose and 70% to 90% amylopectin (Bello-Perez et al., 2002). Amylose is a linear polysaccharide composed solely of D-glucose units linked by α-1,4-glycosidic bonds, contributing to its structural and functional properties in various food products. According to Aboje (2007), amylopectin is a polysaccharide characterized by a branched chain structure predominantly composed of α-1,4-glycosidic bonds linking glucose units. However, occasional α-1,6-glycosidic linkages lead to branching within the molecule. Starch, as highlighted by Ayoola et al. (2013), plays a pivotal role in synthesizing various valuable products such as organic acids, amino acids, glucose-fructose syrups, maltose, and glucose. These products hold significant importance in both the food and pharmaceutical industries. Alpha-amylase, as noted by Bello-Perez et al. (2002), is a complex biochemical enzyme involved in the hydrolysis of starch. Acting as an endo-acting enzyme, it catalyzes the breakdown of α-1,4-glycosidic bonds as well as certain branched α-1,6-glycosidic bonds within the inner chains of starch molecules. The primary products of starch hydrolysis, as noted by Betiku et al. (2013), predominantly consist of maltose, smaller oligosaccharides, and dextrin. In contrast, amyloglucosidase, as described by Aboje (2007), acts as an exo-acting enzyme, primarily targeting α-1,4-glycosidic bonds at the non-reducing ends of starch chains. This enzymatic activity results in the production of glucose molecules through hydrolysis. The acid conversion process, as highlighted by Betiku et al. (2013), presents several challenges, including the requirement for corrosion-resistant materials capable of withstanding the acidic environment, the generation of high colour and salt ash content post-neutralization, increased energy consumption for heating, and difficulties in process control due to its exothermic nature.

Cite this article as:
Pele, G.I., Pele, E.O, Adepeju, A.B., & Ajala, E.O. (2024). Evaluation of optimal pH and temperature conditions for saccharification of breadfruit starch by amyloglucosidase. *FNAS Journal of Scientific Innovations*, 5(3), 79-86.

Starch can undergo conversion to oligosaccharides and glucose through acid, acid-enzyme, and enzyme processes. The enzymatic process, as described by Fagain (2003), involves high-energy liquefaction and saccharification primarily utilizing high-temperature hydrolysis by alpha-amylase and amyloglucosidase. While corn serves as the primary raw material for glucose and fructose syrup production, Ayoola et al. (2013) note that other plant sources such as cassava, wheat, potatoes, etc., are also utilized. This diversity in raw materials is attributed to the scarcity of corn in many impoverished nations.

The breadfruit tree, *Artocarpus altilis*, holds significant value for its nutritious fruit, widely regarded as a staple food in many Pacific Islands (Soetan et al., 2010). Appiah and Ellis (2011) note that ripe breadfruits offer versatile culinary options, including consumption raw or cooked, steamed, fried, processed into flour for baking, roasted, freeze-dried, or traditionally fermented. According to Nwabueze and Uchendu (2011), breadfruit stands out for its gluten-free composition, low fat and cholesterol content, and high levels of complex carbohydrates. Its nutritional profile has led to investigations into its potential as an ingredient in chick feed, despite findings by the same authors indicating lower weight gain compared to cassava or yam, despite its higher consumption. Breadfruit is distinguished by its moderate glycemic index, offering a milder impact on blood sugar levels compared to white potatoes, white rice, and white bread. Additionally, it boasts low-fat content and moderate levels of essential vitamins and minerals (Betiku & Ajala, 2010). Mature breadfruit, with starch constituting over 60% of its total carbohydrate content, serves as a substantial source of carbohydrates (Soetan et al., 2010). The primary objective of the current study, however, was to investigate the optimal pH and temperature conditions for the saccharification of breadfruit starch using amyloglucosidase. This research endeavours to shed light on the enzymatic process involved in breaking down breadfruit starch into fermentable sugars, contributing to a better understanding of its utilization in various industries.

Materials and Methods

In the process of liquefying breadfruit starch, alpha-amylase was utilized, leading to the production of maltodextrin. Pele et al. (2018) reported that under specific conditions of pH 6.5, 70°C temperature, and 60 minutes duration, maltodextrin exhibited an ideal reducing sugar content of 14.88% and dextrose equivalent of 12.30 DE. For the enzymatic saccharification process, a pure culture of thermostable amyloglucosidase sourced from Aspergillus niger was provided by the Federal Institute of Industrial Research, Oshodi (FIIRO) in Nigeria. This enzyme functions optimally at pH 4.5 and a temperature of 60°C. Chemicals such as dinitrosalicylic acid (DNS) and Rochelle salt were procured from Pascal Store in Akure, Nigeria, to facilitate the analytical processes involved in the study. As shown in Figure 1, a prototype fermentor was meticulously designed and constructed to integrate seamlessly with a thermostatic water bath (DK-600 SANFA Electrical thermostatic water bath boiler model) for the saccharification and liquefaction processes. The fermentor's motor is variable, employing a Type (TIPO) Var 10/0 gear from GIFA Transmission in Bologna, Italy, with the specific code AC3999 Motor (Condice). This setup ensures precise control and efficient operation during the enzymatic processes involved in saccharification and liquefaction, enhancing the reliability and reproducibility of experimental results.

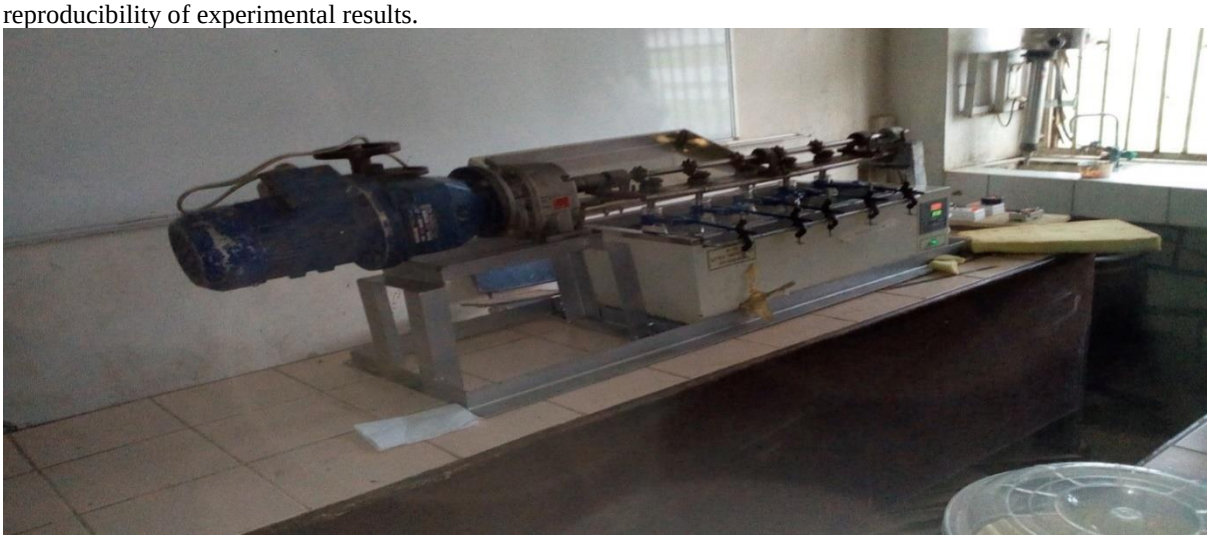

Figure 1: The fermentor used for liquefaction and saccharification

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.

Cite this article as:
Pele, G.I., Pele, E.O, Adepeju, A.B., & Ajala, E.O. (2024). Evaluation of optimal pH and temperature conditions for saccharification of breadfruit starch by amyloglucosidase. *FNAS Journal of Scientific Innovations*, 5(3), 79-86.

To create a 10% slurry, a 10% (w/v) breadfruit starch suspension was meticulously prepared using distilled water, as outlined by Pele et al. (2018), serving as the substrate for subsequent enzymatic processes. Typically, 10 grams of starch were accurately weighed and combined with 100 millilitres of distilled water to form the slurry. To ensure enzyme stability, a 40 ppm Ca2+ solution was incorporated into the mixture. Furthermore, the pH was adjusted to 6.0, 6.5, and 7.0 using citrate-phosphate buffer. Subsequently, the mixture was heated to 97°C and maintained at this temperature for 10 minutes to facilitate gelatinization of the starch. Following gelatinization, the mixture was cooled sequentially to 65°C, 70°C, and 75°C. Liquefaction was initiated by adding 2% (w/v) of alpha-amylase at each respective temperature for durations of 40, 50, and 60 minutes, as specified in the experimental protocol. These meticulously controlled conditions ensured optimal enzymatic activity and facilitated the breakdown of starch into fermentable sugars. To maintain the fermentor's operation at a constant speed of 50 rpm, a thermostatic water bath was securely clamped over it. However, regular sampling was conducted to monitor the kinetics of the process. Following this, the mixture underwent heating at 97°C for a duration of 15 to 20 minutes to halt the enzyme activity effectively. Subsequently, centrifugation was performed using an 80-2 Centrifuge from Med-Lab Scientific Company, England, at 2500 rpm for 10 minutes to separate and extract the supernatant. This process was repeated in triplicate to ensure consistency and reliability of results. Furthermore, a standard curve was constructed for the synthesis of glucose, enabling the determination of optimal conditions for the liquefaction of breadfruit starch, which serves as the fundamental substrate for the subsequent saccharification process. This meticulous approach ensures accurate assessment and optimization of the enzymatic reactions involved in the conversion of starch to fermentable sugars.

A culture of thermostable amyloglucosidase was employed to explore the optimal conditions for saccharifying breadfruit starch. The enzyme's efficacy was evaluated across a spectrum of pH levels, temperatures, and durations. Saccharification experiments were conducted following a 3 x 3 x 12 completely randomized experimental design, encompassing 3 pH values (4.0, 4.5, and 5.0), 3 temperature settings (50°C, 55°C, and 60°C), and 12 different time intervals (ranging from 6 to 72 hours). This systematic approach allowed for comprehensive exploration and identification of the most favourable conditions for hydrolyzing breadfruit starch to facilitate saccharification.

The optimal samples obtained from the preceding liquefaction step were subsequently cooled to temperatures of 50°C, 55°C, and 60°C, and their pH levels were adjusted to 4.0, 4.5, and 5.0, respectively, using diluted hydrochloric acid. The saccharification process involved the addition of 2% (w/v) amyloglucosidase, with reaction times ranging from 6 to 72 hours and corresponding pH values. To maintain the fermentor's operation at a constant speed of 50 rpm, a thermostatic water bath was affixed onto it, and periodic samples were withdrawn for monitoring purposes. Following this, the mixture underwent heating at 97°C for 15 to 20 minutes to deactivate the enzyme activity effectively. Subsequently, the supernatant was obtained for analysis by subjecting the mixture to centrifugation at 2500 rpm. These procedures were conducted in triplicate to ensure the reliability and consistency of results. Moreover, a standard curve depicting glucose production was generated, enabling the determination of the optimal saccharification conditions for the hydrolysis of breadfruit starch. This methodological rigour ensures precise assessment and optimization of the saccharification process.

To determine the reduced sugar content of the syrup samples, the procedure outlined by Miller (1972) involving Rochelle salt and the DNS method was employed. This entailed combining 1 millilitre of hydrolyzed starch (supernatant) with 3 millilitres of DNS solution in a test tube, followed by boiling for 10 minutes. Subsequently, after allowing the solution to cool slightly, 1 cc of Rochelle salt was added, and sufficient time was provided for complete cooling. Once cooled, the UV-visible spectrophotometer (AJ-1C03) was utilized to measure the intensity or absorbance of the red solution at 540 nm. The quantification of reducing sugar was achieved by conducting a series of conventional glucose tests (ranging from 0 to 500 mg/l) and plotting the outcomes on a standard graph. The percentage of reducing sugar was calculated by determining the ratio of the amount of reducing sugar in the glucose syrup to the amount of starch slurry utilized for hydrolysis. This method facilitates the accurate assessment of the reducing sugar content in the syrup samples, essential for evaluating the efficiency of the saccharification process.

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

To determine the dry weight of each sample, two (2) grams of each sample were precisely measured using an analytical balance and placed into dried, cooled, and pre-weighed dishes. Subsequently, the samples were subjected to a three-hour drying period in a Genlab moisture extraction oven set to 105 °C. After drying, the samples were carefully

Cite this article as:
Pele, G.I., Pele, E.O, Adepeju, A.B., & Ajala, E.O. (2024). Evaluation of optimal pH and temperature conditions for saccharification of breadfruit starch by amyloglucosidase. *FNAS Journal of Scientific Innovations*, *5*(3), 79-86.

transferred—using laboratory tongs—into a desiccator and allowed to cool for thirty minutes. Following cooling, the samples were weighed once more, and their respective weights were recorded. This process was repeated until a consistent weight was achieved for each sample in every instance. The dry weight of each sample was then determined by computing the weight difference using established protocols outlined by AOAC (2005). This meticulous procedure ensures the accurate determination of the dry weight of each sample, which is essential for subsequent analyses and calculations in the study.

Betiku et al. (2013) outlined a method for calculating the dextrose equivalent (DE), which involves determining the ratio of reducing sugar, typically represented as glucose, to the sample dry weight. This ratio serves as the basis for computing the dextrose equivalent, providing valuable insight into the saccharification process and the resulting glucose content of the samples.

$$\mathrm{DE} = \frac{\text{Reducing sugar expressed as glucose}}{Sample\ dry\ weight}\ X\ 100 \qquad [2]$$

The data obtained from the experiment underwent statistical analysis using a completely randomized experimental design. Analysis was conducted using various software tools, including Microsoft Excel version 2010, SPSS version 20, and MiniTab version 17. These software packages facilitated comprehensive analysis and interpretation of the experimental results, allowing for robust conclusions to be drawn from the data.

Results

Figure 2 illustrates the production of glucose syrup through the saccharification of breadfruit starch using amyloglucosidase. In Figures 3.2a, 3.2b, and 3.2c, the impact of pH 4 on saccharification and glucose production in breadfruit is demonstrated. At a temperature of 50°C and pH 4, the reducing sugar content ranged from 17.30% to 48.83%, while the sample dry weight ranged from 0.096 g to 0.121 g, and the dextrose equivalent ranged from 14.30 to 50.86 DE throughout 6 to 72 hours. After 72 hours of saccharification, there was a notable decrease in sample dry weight, accompanied by a significant increase in reducing sugar and dextrose equivalent levels. The study investigated the impact of pH 4 on breadfruit saccharification at 55°C, revealing that over 6 to 72 hours, reducing sugar content ranged from 19.64% to 61.44%, sample dry weight from 0.093g to 0.119g, and dextrose equivalent from 16.50 to 66.06 DE. Notably, there was a substantial increase in reducing sugar and dextrose equivalent levels, although sample dry weight notably decreased. Similarly, the effects of pH 4 on breadfruit maltodextrin saccharification at 60°C were observed. Over 6 to 72 hours, reducing sugar ranged from 18.56% to 71.71%, sample dry weight from 0.077g to 0.105g, and dextrose equivalent from 17.68 to 93.14 DE. The results demonstrated significant increases in reducing sugar and dextrose equivalent levels, while sample dry weight notably decreased throughout the 72-hour process.

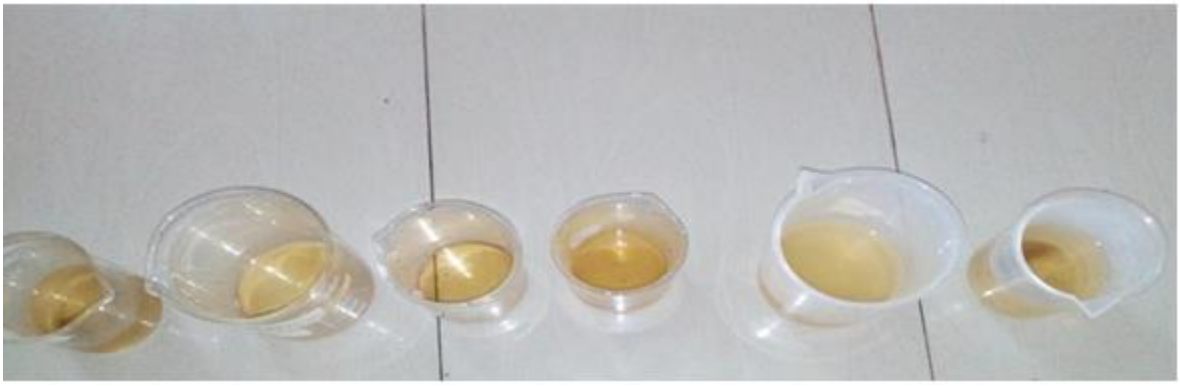

Figure 2. Glucose is produced from the saccharification of previous maltodextrin from breadfruit starch by pure amyloglucosidase.

Cite this article as:
Pele, G.I., Pele, E.O, Adepeju, A.B., & Ajala, E.O. (2024). Evaluation of optimal pH and temperature conditions for saccharification of breadfruit starch by amyloglucosidase. *FNAS Journal of Scientific Innovations*, *5*(3), 79-86.

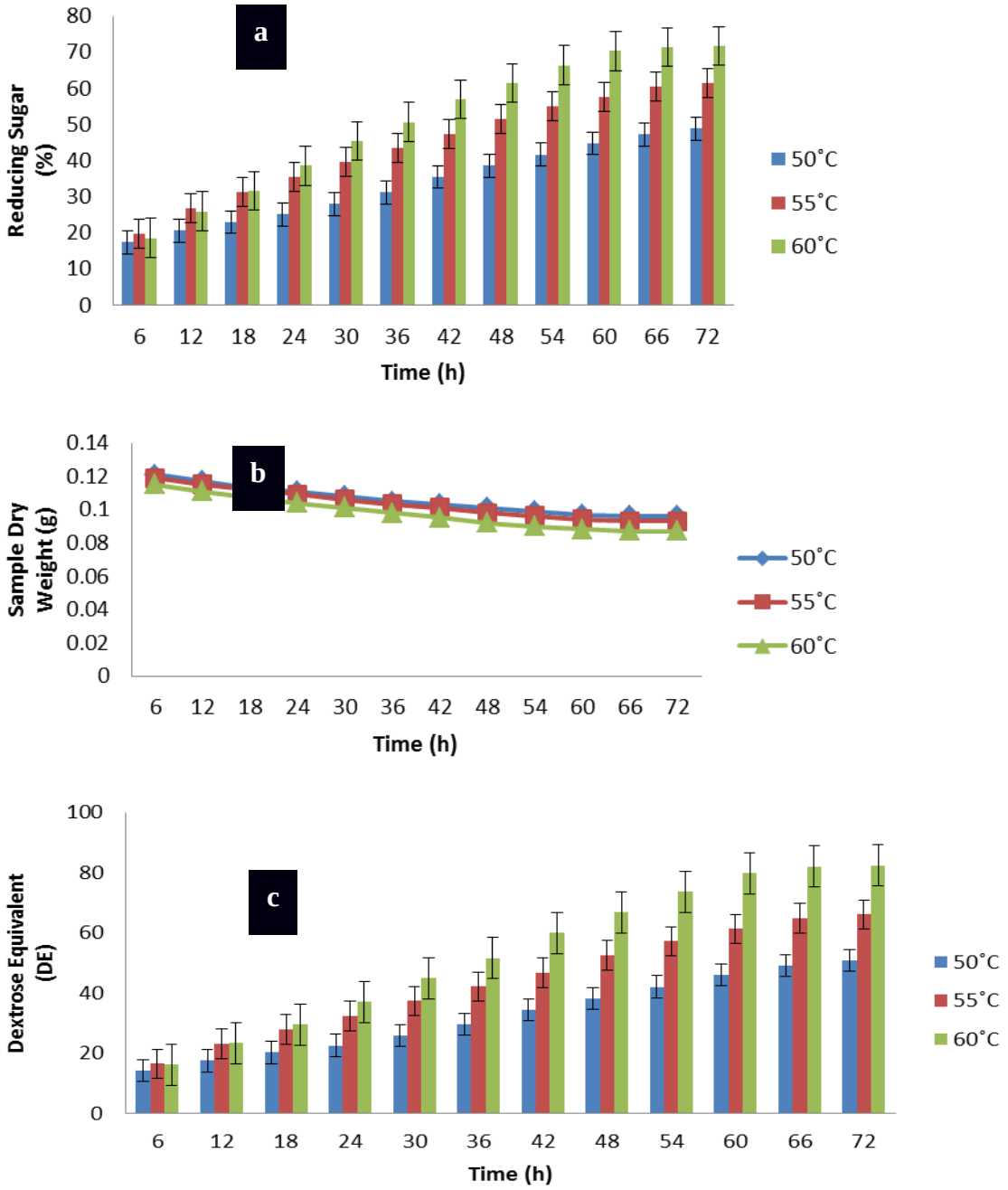

Figure 3: Effect of temperature on amyloglucosidase in breadfruit starch saccharification at pH 4

a: Reducing sugar; **b**: Sample dry weight; **c:** Dextrose equivalent

Figures 3a, b, and c illustrate the impact of pH 4.5 on breadfruit maltodextrin saccharification and glucose production. At 50°C and pH 4.5, over 6 to 72 hours, reducing sugar content ranged from 22.52% to 59.46%, sample dry weight from 0.087g to 0.115g, and dextrose equivalent from 19.60 to 68.34 DE. The findings revealed a notable increase in

Cite this article as:
Pele, G.I., Pele, E.O, Adepeju, A.B., & Ajala, E.O. (2024). Evaluation of optimal pH and temperature conditions for saccharification of breadfruit starch by amyloglucosidase. *FNAS Journal of Scientific Innovations*, 5(3), 79-86.

reducing sugar and dextrose equivalent levels, albeit with a significant decrease in sample dry weight throughout the 72-hour saccharification process. The impact of pH 4.5 on breadfruit maltodextrin saccharification at 55°C revealed reduced sugar content ranging from 18.38% to 61.62%, sample dry weight from 0.08g to 0.11g, and dextrose equivalent from 16.71 to 77.03 DE over 6 to 72 hours. Similarly, at 60°C, over the same duration, reducing sugar ranged from 20.18% to 63.96%, sample dry weight from 0.072g to 0.1g, and dextrose equivalent from 20.18 to 88.84 DE. While there was a notable increase in reducing sugar and dextrose equivalent levels, the sample dry weight experienced a significant decline during the 72-hour saccharification process. Figures 4a, b, and c depict the impacts of pH 5 on the saccharification and glucose production from breadfruit maltodextrin. At 50°C and pH 5, saccharification resulted in reducing sugar levels ranging from 18.56% to 52.25%, sample dry weights of 0.081g to 0.11g, and dextrose equivalents of 16.87 to 64.51 DE over 6 to 72 hours.

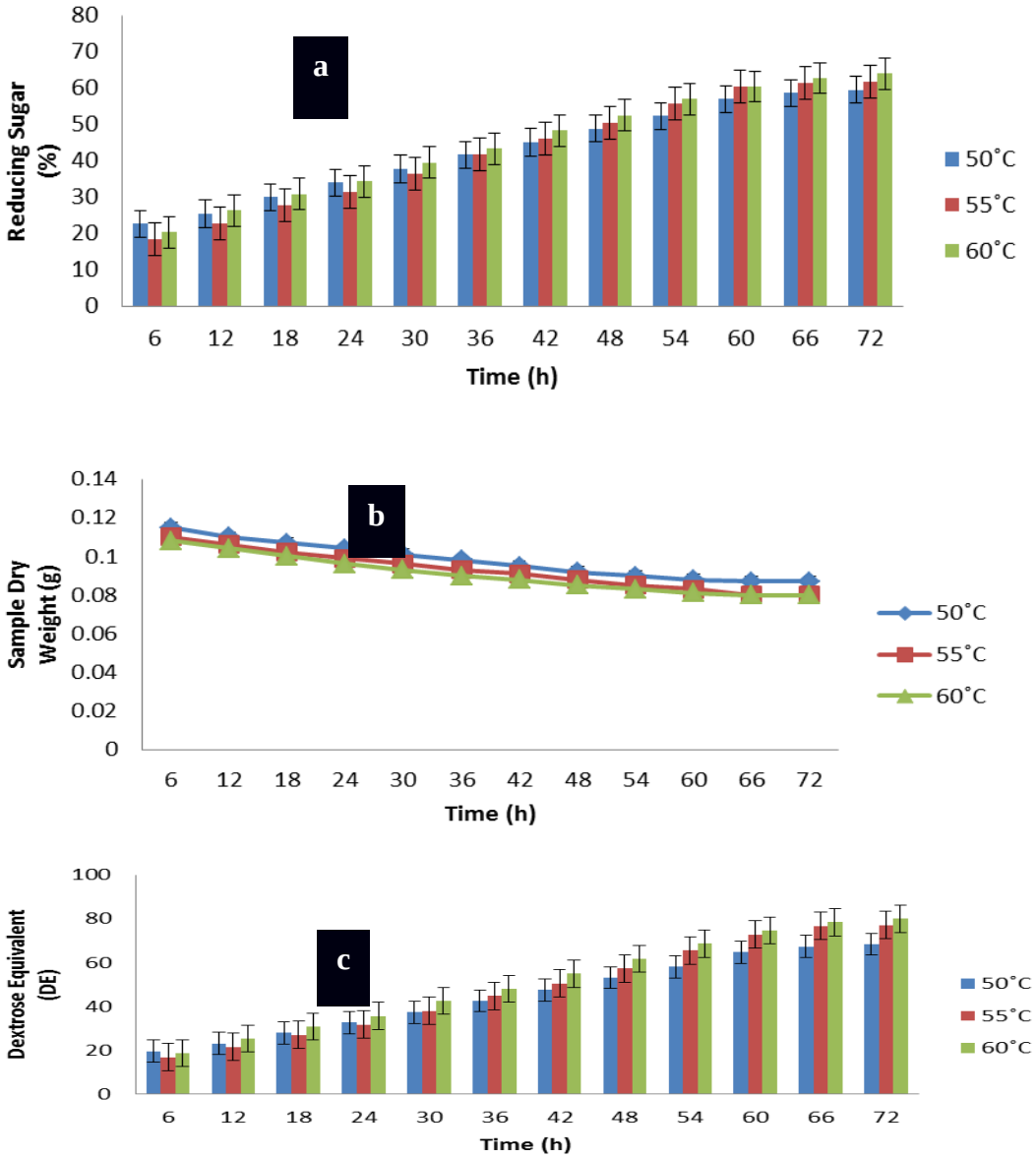

Figure 4: Effect of temperature on amyloglucosidase in breadfruit starch saccharification at pH 4.5

a: Reducing sugar; **b**: Sample dry weight; **c**: Dextrose equivalent

Cite this article as:
Pele, G.I., Pele, E.O, Adepeju, A.B., & Ajala, E.O. (2024). Evaluation of optimal pH and temperature conditions for saccharification of breadfruit starch by amyloglucosidase. *FNAS Journal of Scientific Innovations*, 5(3), 79-86.

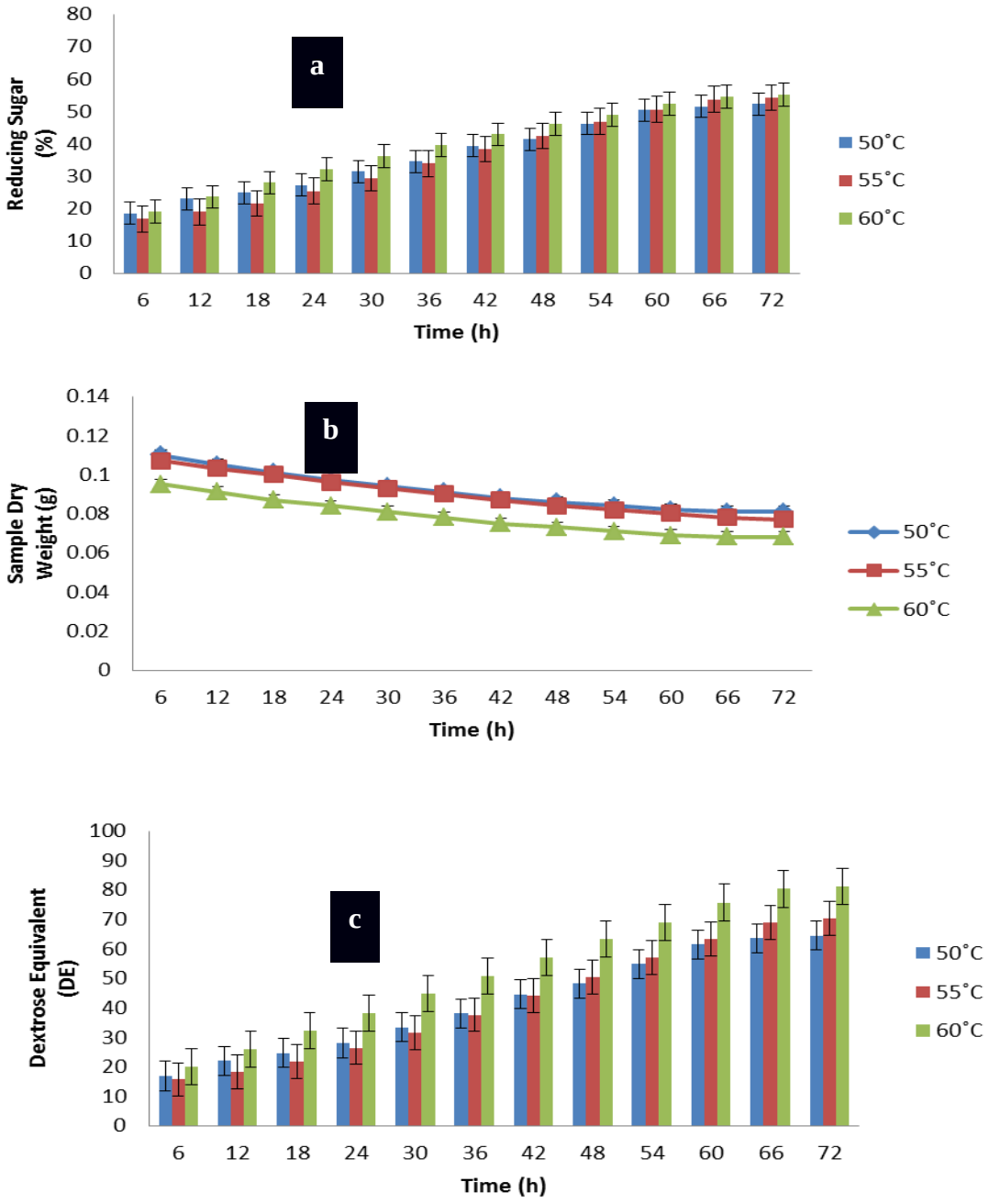

Figure 5: Effect of temperature on amyloglucosidase in breadfruit starch saccharification at pH 5
a: Reducing sugar; **b**: Sample dry weight; **c**: Dextrose equivalent

The effects of pH 5 on the saccharification of breadfruit maltodextrin at 55°C were investigated. Throughout 6 to 72 hours, the reducing sugar content ranged from 16.76% to 54.23%, the sample dry weight from 0.077g to 0.107g, and the dextrose equivalent from 15.66 to 70.43 DE. Notably, after the 72-hour saccharification process, there was a substantial decrease in sample dry weight, accompanied by a significant increase in reducing sugar and dextrose equivalent. The effects of pH 5 on the saccharification of breadfruit maltodextrin at 60°C were examined. Throughout 6 to 72 hours, the reducing sugar content ranged from 19.10% to 55.14%, the sample dry weight from 0.068g to 0.095g, and the dextrose equivalent from 20.10 to 81.08 DE. After 72 hours, the saccharification process concluded, revealing a notable decrease in sample dry weight alongside a significant increase in reducing sugar and dextrose equivalent. The optimal conditions for producing glucose from breadfruit were identified as pH 4, 60°C, and 72 hours,

Cite this article as:
Pele, G.I., Pele, E.O, Adepeju, A.B., & Ajala, E.O. (2024). Evaluation of optimal pH and temperature conditions for saccharification of breadfruit starch by amyloglucosidase. *FNAS Journal of Scientific Innovations*, 5(3), 79-86.

resulting in 71.71% reducing sugar, 0.077g sample dry weight, and 93.13 DE dextrose equivalent. Interestingly, consistent glucose quality was observed at 60, 66, and 72 hours. These outcomes surpass those reported by Betiku and Ajala (2010), possibly attributed to variations in pH, temperature, and saccharification duration.

Conclusion

The present study has elucidated the optimal pH and temperature conditions for the saccharification of breadfruit starch by amyloglucosidase. Notably, the highest observed reducing sugar content and dextrose equivalent were 71.71% and 93.13 DE, respectively. The ideal saccharification conditions were determined to be pH 4, 60°C, and 72 hours. These findings hold significant implications for further applications, such as utilizing the glucose produced as a substrate for isomerization to generate fructose syrup.

References

Aboje, A. (2007). Production and export of glucose from cassava starch. [Internet document] URL, 2007http://www.independentngonline.eom/news/l 90/article/l 8394/2007/01/Accessed on 2/27/2007

AOAC. (2005). Official Methods of Analysis. Association of Official Analytical Chemists. (17th ed.). USA.

Appiah, F. I., & Ellis, W. O. (2011). Proximate and mineral composition of Artocarpus altilis pulp flour as affected by fermentation. *Pakistan Journal of Nutrition, 10*(7), 653-657.

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*. 2:1-4

Bello-Perez, I. 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: 1-9.

Betiku, E. & Ajala, O. (2010) Enzymatic hydrolysis of breadfruit starch: a case study with utilization for gluconic acid production. *Ife Journal of Technology, 19*(2), 10-14

Betiku, E., Akindolani, O. O., & A. R. Ismaila, A. R.(2013). Enzymatic hydrolysis and optimizations of sweet potato (Ipomoea batatas) peel using a statistical approach. *Brazilian Journal of Chemical Engineering, 30*(3), 467 – 476.

Fagain, C. O. (2003). Enzymes stabilization-Recent experimental progress. *Journal of Enzyme Microbiology and Technology,* 33, 137-149.

George, A. B. (2004). Consumption of high-fructose corn syrup in beverages may play a role in the epidemic of obesity. *American Journal of Clinical Nutrition*, 79, 537-543

Miller, G. I. (1972). Use of dinitrosalicylic acid reagent for determination of reducing sugar.Analytical Chemistry, 31(3): 426-428.

Nwabueze, T. U., & Uchendu, C. B. (2011). African Breadfruit (Treculia africana) seed as an adjunct in Ethanol production. *European Journal of Food Research and Review*, *1*(1), 2011, 15-22.

Pele, G. I., Bolade, M. K., Enujiugha, V. N., Sanni, D. M. & Ogunsua. A. O. (2018). Influence of pH and temperature on the activities of alpha-amylase in maltodextrin production from breadfruit starch. *International Journal of Engineering Science Invention*, 7,10.

Soetan, K. O., Olaiya, C. O., & Oyewole, O. E. (2010). The importance of mineral element for humans, domestic animals and plants: A review. *African Journal of Food Science*, *4*(5), 200-222.