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Influence Of pH And Temperature On The Activities Of Alpha-Amylase In Maltodextrin Production From Breadfruit Starch

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Abstract:*Maltodextrin is a starch-derived food additive used to add bulk and sweetness to packaged foods. The study was carried out toinvestigatethe influence of pH and temperature on the activities of alpha-amylase in maltodextrin production from breadfruit starch.The optimum condition of breadfruit starch hydrolysis was determined by pure culture of a thermostable alpha-amylase for liquefaction, and the activity of the enzyme determined at varying pH, temperature and time. A 3 x 3 x 3 completely randomized experimental design comprising 3 pH values (pH 6.0, 6.5 and 7.0); 3 temperatures (65, 70 and 75 ^{o}C) and 3 time ranges (40, 50 and 60 min) were employed for liquefaction, while the sample dry weight, reducing sugar and dextrose equivalent were used to determine the quality attributes of the maltodextrin produced. 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 14.88% and 12.30 DE, respectively at pH 6.5, 70^{o}C and 60 min.Maltodextrin produced in this research may initiate saccharification reaction in the production of glucose syrup.*

Keywords*:Alpha-amylase,breadfruit starch, hydrolysis,liquefaction, maltodextrin.*

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I. Introduction

Maltodextrin is a white hygroscopic spray-dried powder, consisting of D-glucose units connected in chains of variable length which are primarily linked together by α (1-4) glycosidic bond [1]. Maltodextrin is produced from the biochemical reaction of products consisting D-glucose units linked primarily by α (1, 4) bonds with dextrose equivalent (DE) lesser than 20[2]. The conversion of starch to maltodextrin is done by mineral acid catalysis. However the disadvantages of this method include the requirement of the corrosion resistant materials, giving rise to high colour and salt ash content after neutralisation; the need for more energy for heating and therelative difficulty to control [3]. Scientific developmenthas also led to obtaining enzymes that now allow the controlled production of a variety ofmaltodextrin.Alpha-amylase is an endoenzyme which hydrolyses α (1, 4)glucosidic bonds in random along the chain [4]; It also liquefies amylopectin to oligosaccharides containing 2 to 6 glucose units that can lead to a rapid decrease in viscosity[5]; such that a mixture of amylose and amylopectin can be hydrolysed into a mixture of dextrins, maltose and glucose[6].Traditionally, maize has been used in the manufacturing of maltodextrins, efforts have recently been geared towards the production from other sources of starch, such as amaranth, cassava, potato, plantain and sorghum [7].Breadfruit (*Artocarpusaltilis*) is native to many parts of West and Tropical Africa. It grows commonly in evergreen and deciduous forests, often by streams but may sometimes be planted in Nigeria where it is very common in the Western and Eastern States [8].Reports have shown that most works done on breadfruit have been on the nutritive value or physicochemical properties of the fruit [9]. The objective of the present study therefore was to evaluate the influence of pH and temperature on the quality of maltodextrin produced from breadfruit starch by alpha-amylase.

II. Materials And Methods

2.1 Materials

Matured breadfruit(Artocarpusaltilis) was obtained from a local farm in Ife, Nigeria and pure culture of thermostable alpha-amylase (AmylicTx: isolated from *Bacillus amyloliquefaciens*; pH 6.4-6.5; temperature, 80-88^{o}C) was obtained from International Brewery, Ilesa, Nigeria.

2.2 Production of Starch

Breadfruit starch was produced by using the method described by the International Institute of Tropical Agriculture [10]. The fresh breadfruits were manually peeled, washed with tap water and wet milled by a hammer mill. The mash obtained was solubilizedwith distilled water and filtered by a muslin cloth. The filtrate was allowed to settle (3 h) and the supernatant decanted, while the starch wasdewatered by squeezing in the muslin cloth and dried separately in a vacuum oven at 40 ^{0}C to a constant weight for 8 h. The dried starch was milled using a double disc attrition mill and sieved. The starch product was packaged in an air tight polyethylene bag place in plastic containers and stored at room temperature for further use.

2.3Description of Fermentor

A stainless steel fermentor, which was designed and fabricated to be used with a thermostatic water bath (DK-600 SANFA Electrical thermostatic water bath boiler model) for liquefaction is shown in Figure 1. The fermentor comprises Variable motor Gear: GIFA Transmission Bologna Italy, Type (TIPO): (Var 10/0) Code (Condice): AC3999 Motor (motore) Kw: 0.75 Poles: 4Rpm min – rpm max: 350–1750 Type: mas 20P; code: 29602117; Mount POS: 2.5.4. BonfiglioliRiduttori, Italy.

2.4Production of Maltodextrin via Enzyme Liquefaction

2.4.1Characterization of alpha-amylase

Breadfruit starch was hydrolysed by pure culture of thermostable alpha-amylase to determine the optimum condition. The activities of the enzyme were determined at varying pH, temperature and time. A 3 x 3 x 3 completely randomized experimental design comprising 3 pH values (pH 6.0, 6.5 and 7.0); 3 temperatures (65, 70 and 75 °C) and 3 time ranges (40, 50 and 60 min) were employed for liquefaction.

2.4.2 Determination of enzyme activity in alpha-amylase

The hydrolysis of breadfruit starch by α-amylase was carried out by the method described by [11]. Starch was suspended in distilled water at 10% (w/v) to make 10% slurry; such that 10 g of starch was weighed into 100 ml of distilled water to make slurry and solution of 40 ppm Ca^{2+} added for stability of the enzyme. The pH was adjusted to 6.0, 6.5 and 7.0 with Citrate-phosphate buffer, respectively. Gelatinization was done by increasing the temperature of the mixture to 97 °C for 10 min. The gelatinized starch was cooled to 65, 70 and 75 °C, respectively and the liquefaction was carried out by adding 2% (w/v) of alpha-amylase and held in the fermentor which was clamped with the thermostatic water-bath to maintain at 50 rpm for 40, 50 and 60 min. Samples were however withdrawn at regular time intervals to follow the kinetics and the enzyme activity was stopped by heating the mixture to 97 °C for 15 to 20 min while the samples were further centrifuged (80-2 Centrifuge Med-Lab Scientific Company England) at 2500 rpm for 10 min 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 liquefaction for breadfruit.

2.5 Determination of physicochemical properties of maltodextrin

2.5.1 Determination of reducing sugar

The reducing sugar was determined by dinitrosalicylic acid (DNS) method described by [12] with the addition of Rochelle salt. The reducing sugar was determined by adding 3 ml of DNS solution to 1ml of the sample in a test tube and boiled for 10 min. This was allowed to cool while 1 ml of Rochelle salt was added. The intensity or absorbance of the red coloured solution was read at 540 nm using UV-Visible Spectrophotometer

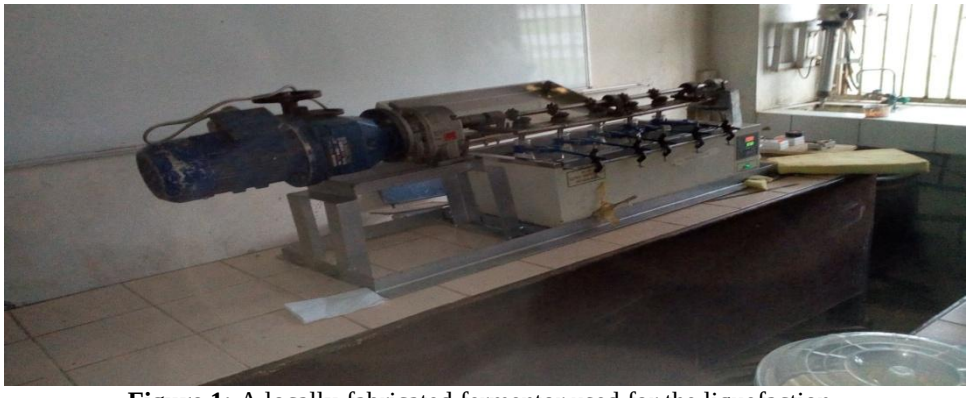

Figure 1: A locally-fabricated fermentor used for the liquefaction.

(AJ-1C03)while series of standard glucose (0 – 500mg/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.

$$\text{ReducingSugar (mg/ml)} = \frac{\text{Conc. obt (mg/l) Xvol. ofextractXdil. factor (ifany)}}{\text{SamplewtXvolofaliquotanalysed}}$$

2.5.2 Determination of sample dry weight

Sample dry weight was determined by weighingtwo (2) grams of the samples into dried, cooled and weighed dishes. The samples in the dishes were then put into a Genlab moisture extraction oven set at 105°C and allowed to dry for 3 h after which the samples were transferred into a dessicator with the aid of a laboratory tong and allowed to cool for 30 min. After cooling in the dessicator, they were weighed and their respective weights recorded accordingly. The above processes were carried out repeatedly for each sample until a constant weight was obtained in each case. The difference in weight was calculated as the sample dry weight.

2.5.3 Determination of dextrose equivalent (DE)

Dextrose equivalent (DE) was determined by the expression described by [13], which was calculated as the ratio of reducing sugar expressed as glucose to the sample dry weight.

$$\text{DE} = \frac{\text{Reducing sugar expressed as glucose}}{\text{Sampledryweight}} \text{X } 100$$

2.5.4 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.

III. Results And Discussion

3.1 Effect of pH and Temperature on the Kinetics of Liquefaction and Maltodextrin Produced from Breadfruit by Alpha-amylase

The results of the effect of pH 6 on the kinetics of liquefaction and maltodextrin produced from breadfruit starch are shown in Figures 2a, b and c. At pH 6, liquefaction of breadfruit starch at 65 ^{0}C showed that reducing sugar were 9.75, 11.37 and 11.68%, respectively at 40, 50 and 60 min of liquefaction time, a significant increase with respect toliquefaction time was observed. The results showed that samples dry weight were 0.152, 0.147 and 0.143 g, respectively at 40, 50 and 60 min of liquefaction time indicating a gradual decrease as liquefaction time increased. Dextrose equivalent were 6.41, 7.73 and 8.17 DE, respectively at 40, 50 and 60 min of liquefaction time, results showed a significant increase in respect of time. The results of the effect of pH 6 on the liquefaction of breadfruit at 70 °C showed that reducing sugar were 11.03, 11.53and 11.59%, respectively at 40, 50 and 60 min of liquefaction time indicating a significant increase in respect of time.The results also showed that sample dry weight were 0.140, 0.136 and 0.133 g, respectively at 40, 50 and 60 min of liquefaction time with a significant decrease in respect of increase in liquefaction time. Dextrose equivalent were 7.88, 8.48 and 8.71 DE, respectively at 40, 50 and 60 min of liquefaction time indicating a significant increase as the liquefaction time increased. The results of the effect of pH 6 on the liquefaction of breadfruit at 75 °C showed that the reducing sugar were 8.13, 9.19 and 9.33%, respectively at 40, 50 and 60 min of liquefaction time, a significant increase was observed in respect of liquefaction time. The results also showed that sample dry weight were 0.134, 0.131 and 0.129 g, respectively at 40, 50 and 60 min of liquefaction time, however results showed that a significant decrease was observed as the liquefaction time increased. Dextrose equivalent were 6.06, 7.01 and 7.24 DE, respectively at 40, 50 and 60 min of liquefaction time indicating a significant increase as the liquefaction time increased. The results of the effect of pH 6.5 on the kinetics of liquefaction and maltodextrin produced from breadfruit starch are shown in Figures 3a, b and c. At pH 6.5, liquefaction of breadfruit starch at 65 ^{0}C showed that reducing sugar were 9.39, 13.21 and 13.35%, respectively at 40, 50 and 60 min of liquefaction time. While an increase was observed in respect of time, there was no significant increase at 50 and 60 min. The results also showed that sample dry weight were 0.150, 0.143 and 0.141 g, respectively at 40, 50 and 60 min of liquefaction time with a significant decrease as the liquefaction time increased.Dextrose equivalent were 6.26, 9.24 and 9.47 DE, respectively at 40, 50 and 60 min of liquefaction time, a gradual increase in respect of time was observed, but there was no significant increase at 50 and 60 min of liquefaction time. The results of the effect of pH 6.5 on the liquefaction of breadfruit starch at 70 °C showed that reducing sugar were 11.60, 14.79 and 14.88%, respectively at 40, 50 and 60 min of liquefaction

time, while a gradual increase was observed in respect of time, results showed no significant difference at 50 and 60 min. The results showed that sample dry weight were 0.136, 0.128, and 0.121 g, respectively at 40, 50 and 60 min of liquefaction time with a significant decrease as the liquefaction time increased. Dextrose equivalent were 8.54, 11.56 and 12.30 DE, respectively at 40, 50 and 60 min of liquefaction time, a significant increase in respect of time was observed. The results of the effect of pH 6.5 on the liquefaction of breadfruit at 75 ºC showed that reducing sugar were 7.86, 9.39 and 9.90%, respectively at 40, 50 and 60 min of liquefaction

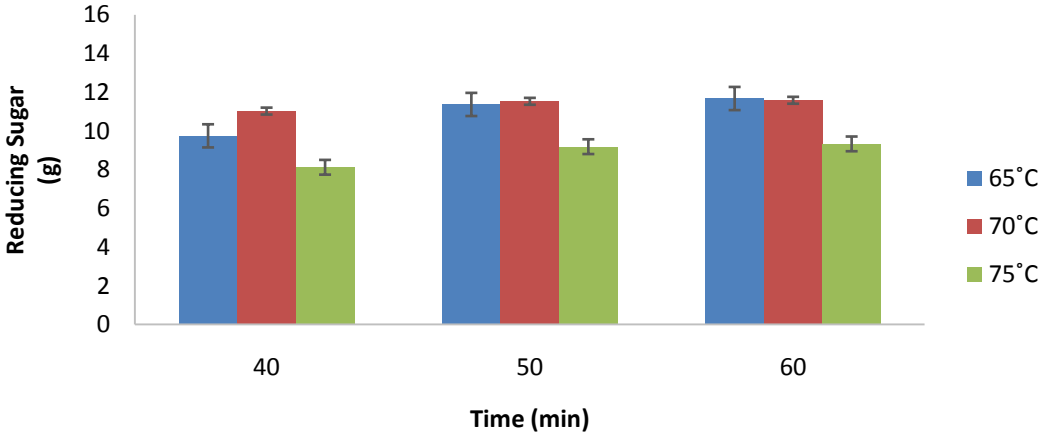

Figure 2a: Reducing sugar content during liquefaction of breadfruit at pH 6.

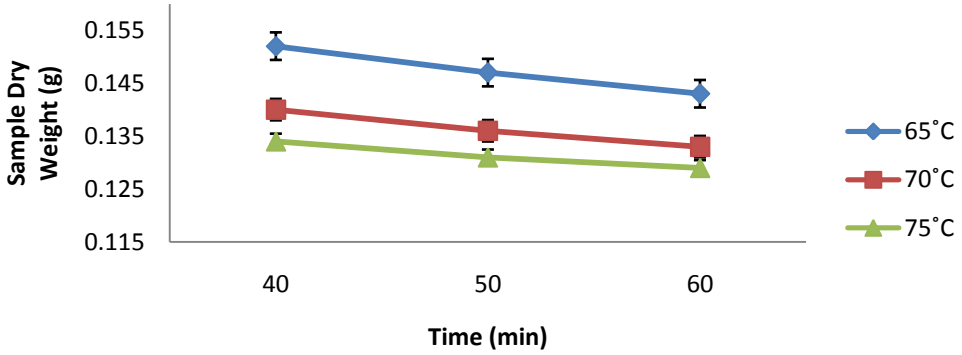

Figure 2b: Sample dry weight from liquefaction of breadfruit at pH 6.

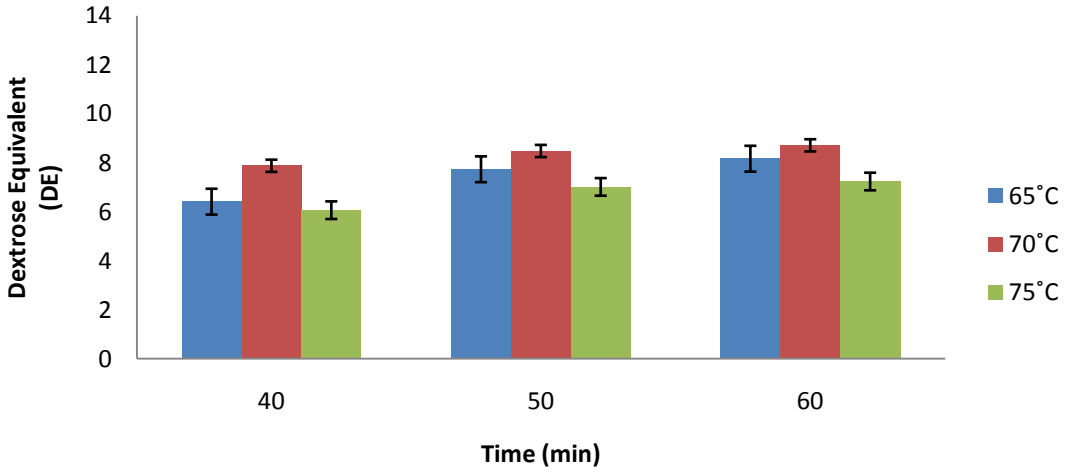

Figure 2c: Dextrose equivalent of syrup from liquefaction of breadfruit at pH 6.

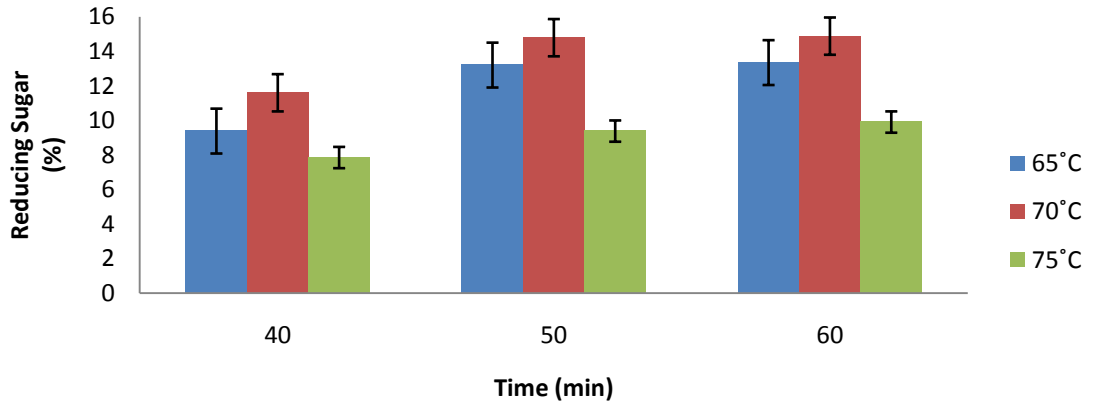

Figure 3a: Reducing sugar content during liquefaction of breadfruit at pH 6.5.

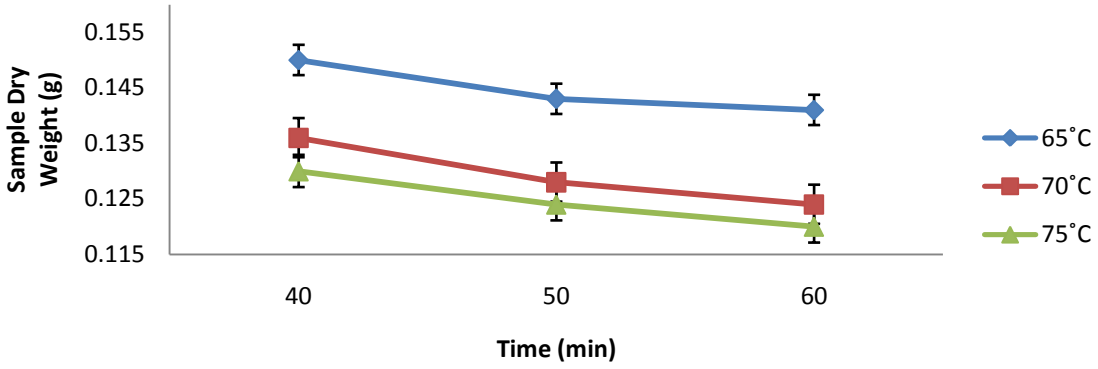

Figure 3b: Sample dry weight from liquefaction of breadfruit at pH 6.5.

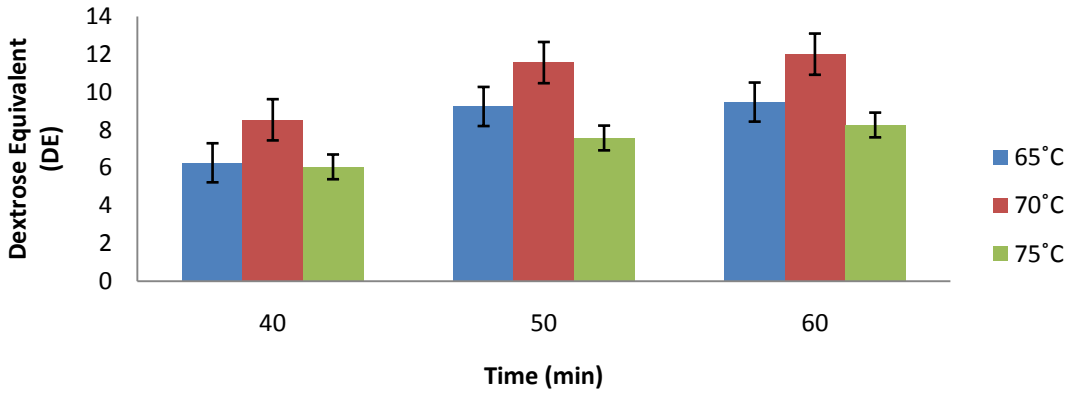

Figure 3c: Dextrose equivalent of syrup from liquefaction of breadfruit at pH 6.5.

time, an increasewas observed in respect of time, but there was no significant difference at 50 and 60 min. Results also showed that sample dry weight were 0.123, 0.114 and 0.106 g, respectively at 40, 50 and 60 min, a significant decrease was observed in respect of liquefaction time. Dextrose equivalent were 6.39, 8.24 and 9.35 DE, respectively at 40, 50 and 60 min of liquefaction time with a significant increase as the liquefaction time increased. The results of the effect of pH 7 on the kinetics of liquefaction and maltodextrin produced from breadfruit starch are shown in Figures 4a, b and c. At pH 7, liquefaction of breadfruit starch at 65 ºC showed that reducing sugar were 7.77, 10.22 and 10.27%, respectively at 40, 50 and 60 min of liquefaction time with a gradual increase in respect of time, but there was no significant difference at 50 and 60 min. The results showed that sample dry weight were 0.147, 0.134 and 0.132 g, respectively at 40, 50 and 60 min of liquefaction time, a gradual decrease was observed in respect of time, but there was no significant difference at 50 and 60 min. Dextrose equivalent were 5.28, 7.62 and 7.78 DE, respectively at 40, 50 and 60 min of liquefaction time, an

increase was observed in respect of time, but there was no significant difference at 50 and 60 min. The results of the effect of pH 7 on the liquefaction of breadfruit at 70 °C showed that reducing sugar were 9.37, 10.47 and 10.60%, respectively at 40, 50 and 60 min of liquefaction time, an increase was observed in respect of time, but there was no significant difference at 50 and 60 min. Results showed that sample dry weight were 0.132, 0.127 and 0.12 g, respectively at 40, 50 and 60 min of liquefaction time with a gradual decrease as the liquefaction time increased. Dextrose equivalent were 7.10, 8.24 and 8.83 DE, respectively at 40, 50 and 60 min of liquefaction time, a significant increase in respect of liquefaction time was observed. The effect of pH 7 on the liquefaction of breadfruit at 75 °C showed that reducing sugar were 7.26, 7.62 and 8.13%, respectively at 40, 50 and 60 min of liquefaction time indicating an increase as the liquefaction time increased. The results showed that sample dry weight were 0.128, 0.116 and 0.111g, respectively at 40, 50 and 60 min of liquefaction time, results showed gradual decrease in respect of time, but there was no significant difference at 50 and 60 min. Dextrose equivalent were 5.67, 6.57 and 7.32 DE, respectively at 40, 50 and 60 min of liquefaction time, a significant increase in respect of liquefaction time was observed.

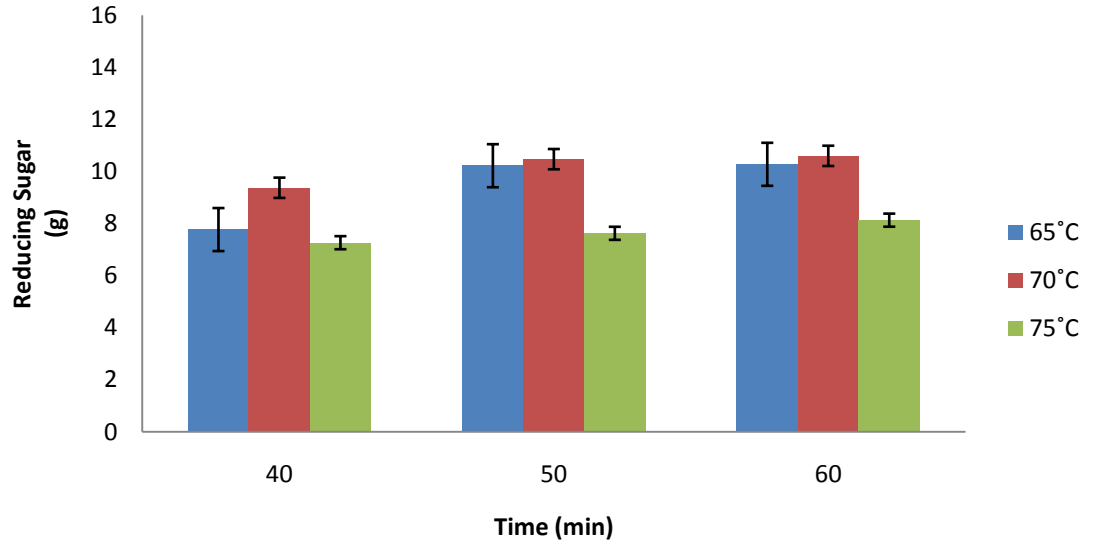

Figure 4a: Reducing sugar content during liquefaction of breadfruit at pH 7.

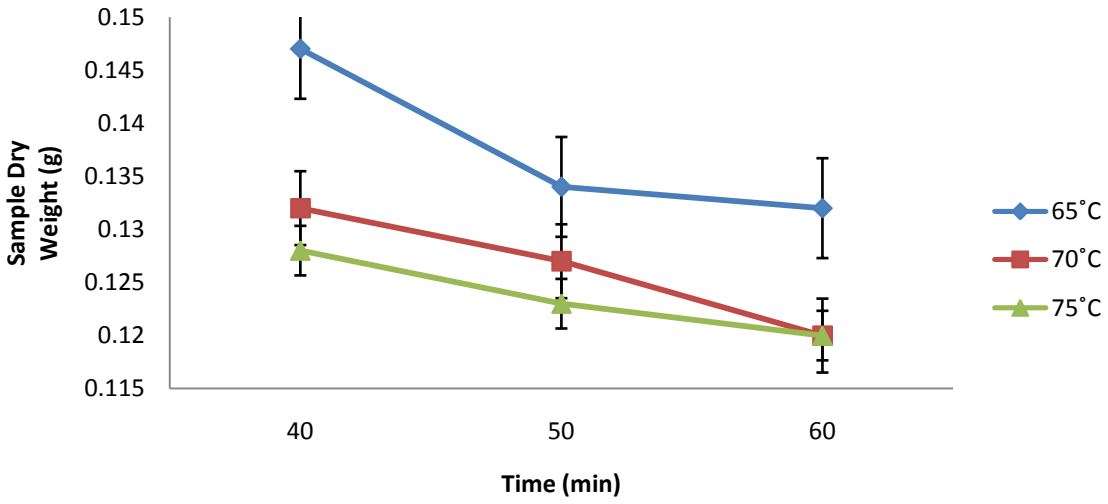

Figure 4b: Sample dry weight from liquefaction of breadfruit at pH 7.

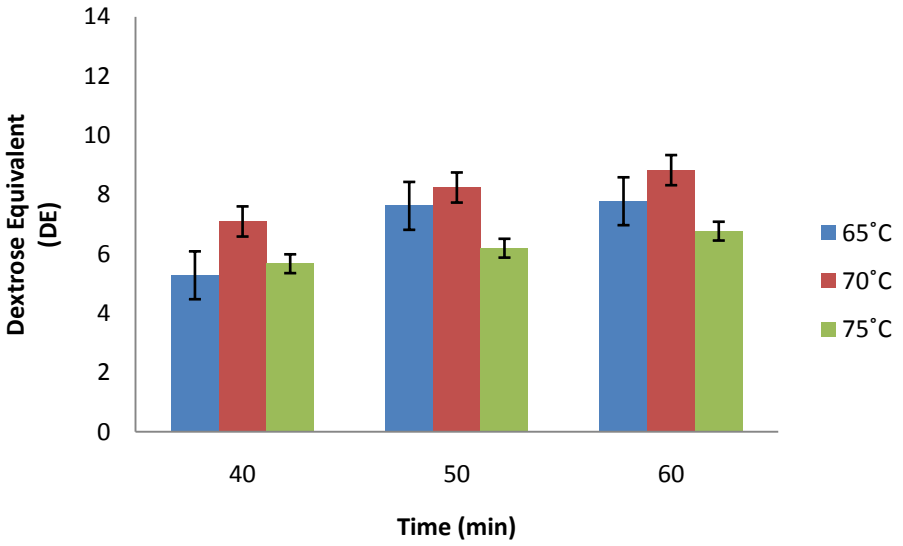

Figure 4c: Dextrose equivalent of syrup from liquefaction of breadfruit at pH 7.

The effect of temperature on the kinetics of liquefaction and maltodextrin produced from breadfruit starch showed that the highest level of reducing sugar and dextrose equivalent were produced at 70 ºC. It was also observed that sampledry weight decreased as the temperature, pH and time increased. Results however showed that the optimum pH, temperature and time of liquefaction of breadfruit starch hydrolysis were6.5, 70 ºC and 60 min, respectively. The results obtained from this work followed the kinetics without a decrease in reducing sugar and dextrose equivalent, respectively of time; however this is in contrast with the previous work of [11] whoattributed the reduction of dextrose equivalent, most especially at 60 min to the formation ofmaltulose (4-α-D-glucopyranosyl-D-fructose), which is resistant to hydrolysis by α- amylase as reported by [3].

IV. Conclusion

The present study has demonstrated the effect of pH and temperature on the maltodextrin produced from breadfruit starch by alpha-amylase. The results obtained in this research showed that the optimal reducing sugar and dextrose equivalent were 14.88% and 12.30 DE, respectively. However, the optimal pH, temperature and time of liquefaction of breadfruit starch hydrolysis were 6.5, 70 ºC and 60 min, respectively. The maltodextrin obtained in this work may serve as a substrate to initiate a saccharification reaction in the production of glucose syrup.

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