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Optimization of the liquefaction steps of breadfruit starch hydrolysis by alpha-amylase using a statistical approach

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ABSTRACT

Statistical approach was used to optimize the liquefaction steps of breadfruit starch hydrolysis by alpha-amylase. The optimum condition of breadfruit starch hydrolysis was determined using 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 ^{0}C) and 3-time ranges (40, 50 and 60 min) were employed for liquefaction and the data obtained were subjected to multiple regression and the degree of correlation evaluated. The results showed that the p-values of the model terms for sample dry weight, reducing sugar and dextrose equivalent were significant ($p < 0.05$), however the R2 which was found to be 90.7% for sample dry weight proved suitable for adequate representation of the actual relationship between the selected variables whereas the R2 for reducing sugar and dextrose equivalent which were 43.0 and 32.8% respectively were too low for adequate representation of the actual relationship between the selected variables. The optimal reducing sugar and dextrose equivalent were 14.88% and 12.30 DE, respectively at pH 6.5, 70 ^{0}C and 60 min. The model established the actual relationship between the actual variables of the liquefaction and the predictable values of the process while the maltodextrin obtained from the optimized process may serve as a substrate to initiate a saccharification process in the production of glucose syrup.

KEYWORDS

dextrose equivalent; maltodextrin; multiple regression; reducing sugar; sample dry weight

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INTRODUCTION

Liquefaction is a biochemical hydrolytic reaction involving alpha-amylase, an endoenzyme to break α (1, 4) glucosidic bonds in random along the chain [1]. The breaking down of starch to its constituents dextrins, maltose and glucose can be done by mineral acid catalysis but varying quantities of gentiobiose, levulinic acid and furfural derivatives and other degradation productions may interfere with the hydrolytic reaction and this can lead to reversion or condensation products while the conversion is pushed towards completion [2]. 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 [3]. The introduction of alpha-amylase in the breaking down of starch component has been found to show more specificity in its biochemical reaction and fewer several side reactions leading therefore to higher yields. The use of maize in the manufacturing of maltodextrin has been greatly explored, however efforts towards producing from other sources of starch, such as amaranth, cassava, potato, plantain and sorghum must be encouraged [4].

Breadfruit (*Artocarpus altilis*) is native to many parts of West and Tropical Africa and is a traditional staple crop grown for its starchy fruit throughout Oceania [5]. 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 [6]. Breadfruit is primarily used for its nutritious, starchy fruit with rich source of carbohydrates, calcium and phosphorus, minerals and vitamins [7]. The objective of the present study therefore was to optimize the liquefaction steps of breadfruit hydrolysis by alpha-amylase using a statistical approach.

MATERIALS AND METHODS

Materials

Matured breadfruit (*Artocarpus altilis*) 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^{0}C) was obtained from the International Brewery, Ilesa, Nigeria.

Production of Starch

Breadfruit starch was produced by using the method described by the International Institute of Tropical Agriculture [8]. The fresh breadfruits were manually peeled, washed with tap water and wet milled by a hammer mill. The mash obtained was solubilised with distilled water and filtered by a muslin cloth. The filtrate was allowed to settle (3 h) and the supernatant decanted, while the starch was dewatered 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.

Description 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.

Production of Maltodextrin

- ***Characterization 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,

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

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 ^{0}C) and 3-time ranges (40, 50 and 60 min) were employed for liquefaction.

- ***Determination of enzyme activity in alpha-amylase***

The hydrolysis of breadfruit starch by α-amylase was carried out by the method described by Betiku and Ajala (2010) [9]. Starch was suspended in distilled water 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 ^{0}C for 10 min. The gelatinized starch was cooled to 65, 70 and 75 ^{0}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 ^{0}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.

Determination of physicochemical properties of maltodextrin

- ***Determination of reducing sugar***

The reducing sugar was determined by dinitrosalicylic acid (DNS) method described by Miller (1972) [10] 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 while1 ml of Rochelle salt was added.The intensity or absorbance of the red coloured solution was read at 540 nm using UV-Visible Spectrophotometer (AJ-1C03) whileseries 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.

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

- ***Determination of sample dry weight***

Sample dry weight was determined by weighing two (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 ^{0}C and allowed to dry for 3 h after which the samples were transferred into a desiccator with the aid of a laboratory tong and allowed to cool for 30 minutes. After cooling in the desiccator, 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.

- ***Determination of dextrose equivalent (DE)***

Dextrose equivalent (DE) was determined by the expression described by Betiku *et al*. (2013) [11], which was calculated as the ratio of reducing sugar expressed as glucose to the sample dry weight.

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

- ***Statistical Analysis***

The data obtained from the experiment were subjected to completely randomize experimental design and statistical analysis using Microsoft excel version 2010, SPSS version 20 and Mini Tab version 17. To correlate the response variable to the independent variables, multiple regressions was used to fit the coefficient of the polynomial model of the response in each case. The quality of the fit of the model was evaluated using test of significance and analysis of variance (ANOVA).

RESULTS AND DISCUSSION

Optimization of the Liquefaction Steps of Breadfruit Starch Hydrolysis

Evaluation and determination of the coefficient of the full regression model equation and the statistical significance of liquefaction steps of breadfruit starch hydrolysis are shown in Table 1. The results revealed that the p-values of the model terms for sample dry weight were significant ($p < 0.05$) but that the percentage reducing sugar and dextrose equivalent were not significant ($p > 0.05$). It further expressed the regression coefficient in term of relationship between the dependent and independent variables which was used to generate the equation model for prediction. Table 2 shows the analysis of variance of the regression equation model, the model fisher F-test of sample dry weight, 74.38; reducing sugar, 5.78; dextrose equivalent, 3.74 with the reducing sugar and dextrose equivalent showing low probability values whereas Sample dry weight demonstrated a high significance of the regression model (Kunammeni and Singh, 2005) [12]. The goodness of fit was checked by the coefficient of determination (R2), such that Guan and Yao (2008) [13] reported that R2 should be at least 0.80 for the good fit of a model, however for the liquefaction steps, the R2 was found to be 90.7% for sample dry weight, 43.0% for reducing sugar and 32.8% for dextrose equivalent. However, while R2 adjusted was found to be 89.4% for sample dry weight, 35.6% for reducing sugar and 24.0% for dextrose equivalent, the model of sample dry weight proved suitable for adequate representation of the actual relationship between the selected variables (Vazquez et al., 2009) [14]; the value of the R2 obtained in the model of sample dry weight showed a high consistency between the observed values and the predicted values, while the R2 model of reducing sugar and dextrose equivalent showed an inconsistency between the observed values and the predicted values (Guan and Yao, 2008). The inconsistency observed in the model of the reducing sugar and dextrose equivalent could be due to sample size of the experimental data which was not adequate for the R2 to be at least 0.80 for the good fit of a model.

TABLE 1: Test of Significance of Every Regression Coefficient for the Liquefaction Steps of Breadfruit Starch Hydrolysis.

Predictor	Coeff	SE Coef	T	P
Sample Dry Weight (g)				
Constant	0.33354	0.01410	23.66	0.000
pH	-0.009111	0.001427	-6.39	0.000
Temperature^{o}C	-0.0016667	0.0001427	-11.68	0.000
Time (min)	-0.00048333	0.00007134	-6.78	0.000
Percentage Reducing Sugar				
Constant	29.757	7.721	3.85	0.001
pH	-1.3213	0.7812	1.69	0.104
Temperature ^{o}C	-0.22422	0.07812	-2.87	0.009
Time (min)	0.09770	0.03906	2.50	0.020
Dextrose Equivalent				
Constant	11.367	6.493	1.75	0.093
pH	-0.4660	0.6570	-0.71	0.485
Temperature ^{o}C	-0.07929	0.06570	-1.21	0.240
Time (min)	0.09989	0.03285	3.04	0.006

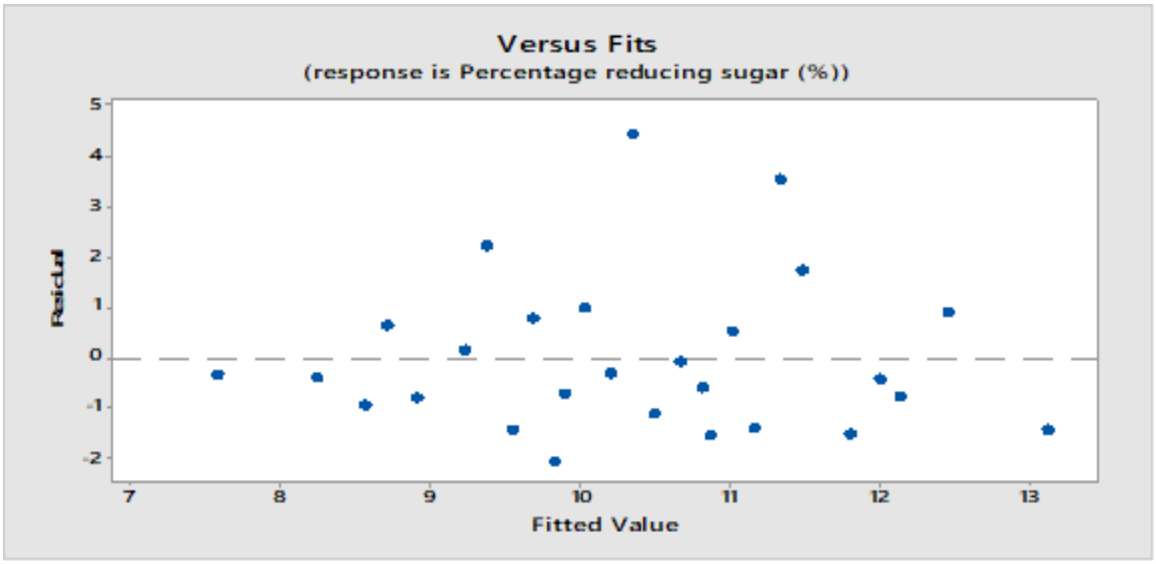
TABLE 2: Analysis of Variance (ANOVA) of the Regression Equation for the Liquefaction Steps of Breadfruit Starch Hydrolysis.

Source	DF	SS	MS	F	P
Sample Dry Weight (g)					
Regression	3	0.00204406	0.00068135	74.38	0.000
Residual Error	23	0.00021069	0.00000916		
Total	26	0.00225474			
	S = 0.00302659	R-Sq = 90.7%	R-Sq(Adj) = 89.4%		
Percentage Reducing Sugar					
Regression	3	47.662	15.887	5.78	0.004
Residual Error	23	63.168	2.746		
Total	26	110.830			
	S = 1.65724	R-Sq = 43.0%	R-Sq(adj) = 35.6%		
Dextrose Equivalent					
Regression	3	21.768	7.256	3.74	0.025
Residual Error	23	44.682	1.943		
Total	26	66.450			
	S = 1.39380	R-Sq = 32.8%	R-Sq(adj) = 24.0%		

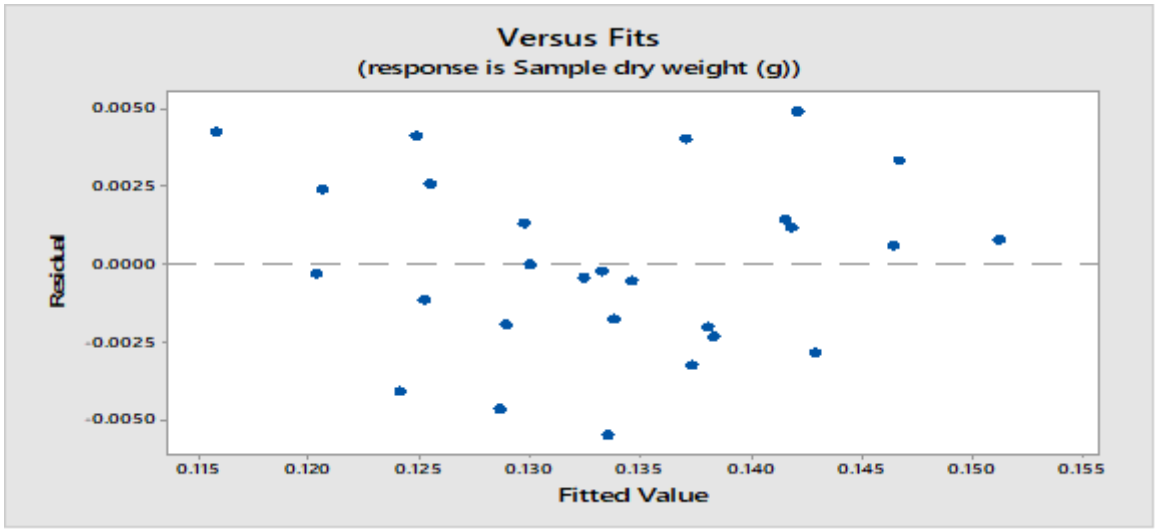
The regression models of the effect of pH, temperature and time on the liquefaction steps of breadfruit starch hydrolysis are shown in Figures 1a, b and c. The graph established the regression equation and fitness of the plot of the residual value on the vertical axis and fitted value on the horizontal axis. Percentage reducing sugar showed a weak correlation between the model's predictions and its actual results; the plots are observed to be unevenly distributed vertically and the residual plots are not randomly dispersed around the horizontal axis, though temperature and time are observed to be significant ($p < 0.05$), the non-linearity of the model may be due to the fact that pH is not significant, however the coefficient of determination of the model showed that a unit increase in pH will reduce the percentage reducing sugar by 132%; a unit increase in temperature will reduce the reducing sugar by 22.4% whereas a unit increase in time will increase the reducing sugar by 9.77%. Sample dry weight showed a strong correlation between the model's prediction and its actual results. The plots are observed to be pretty symmetrically distributed; the points in a residual plot are randomly dispersed around the horizontal axis where a linear regression model coefficient of determination is established. A unit increase in pH will reduce the sample dry weight by 0.91%; a unit increase in temperature will reduce sample dry weight by 0.17% and a unit increase in time will reduce the sample dry weight by 0.05%. Dextrose equivalent showed a weak correlation between the model's predictions and its actual results, though the time of liquefaction was observed to be significant ($p < 0.05$), the plots are unevenly distributed vertically and the residual plots are not randomly dispersed around the horizontal axis, however a non-linear model coefficient of determination showed that a unit increase in pH will reduce the dextrose equivalent by 46.6%; a unit increase in temperature will reduce dextrose equivalent by 7.93% while a unit increase in time will increase the dextrose equivalent by 9.99%.

The final regression equation presented in Table 1 in terms of coded factors for the response surface quadratic model for the liquefaction steps at different pH, temperature (T), and time (t) is described in equation 1 – 3.

(a)



(b)



(c)

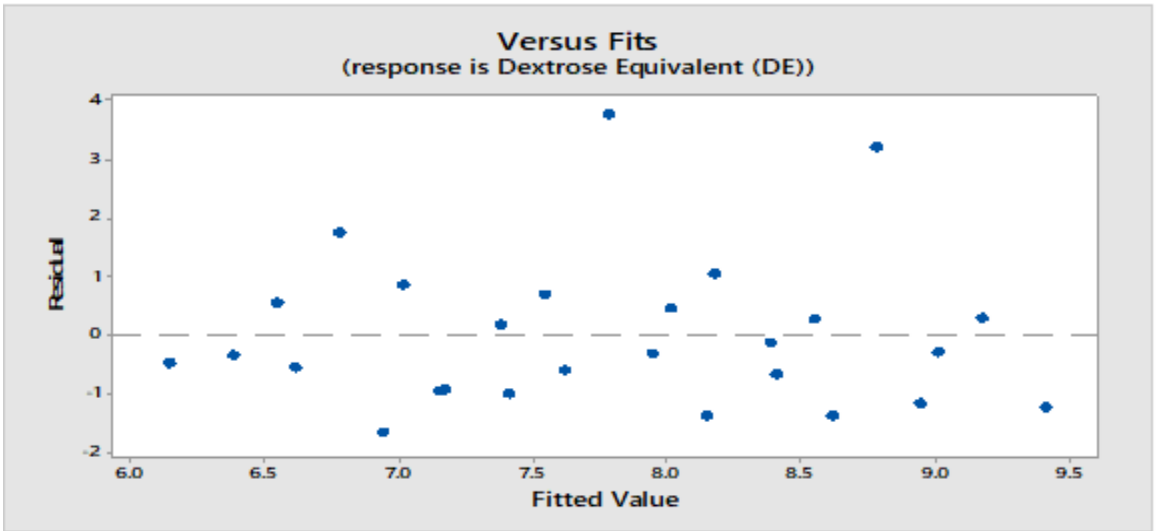


FIGURE 1: Fit regression model of liquefactionof breadfruit starch
(a) Reducing sugar; (b) Sample dry weight; (c) Dextrose equivalent

Sample Dry weight = 0.334 - 0.00911 pH - 0.00167 Temperature (^{0}C) - 0.000483 Time (min)…….. [Eq. 1]

Percentage Reducing Sugar = 29.8 - 1.32 pH - 0.224 Temperature (^{0}C) + 0.0977 Time (min)…….. [Eq. 2]

Dextrose Equivalent = 11.4 - 0.466 pH - 0.0793 Temperature (^{0}C) + 0.0999 Time (min)…….. [Eq. 3]

CONCLUSION

Multiple regression analysis was successfully applied to the liquefaction steps of breadfruit starch hydrolysis by alpha-amylase. The model terms of sample dry weight, reducing sugar and dextrose equivalent were significant ($p < 0.05$), however the R^2 for sample dry weight which was found to be 90.7% was found suitable for adequate representation of the actual relationship between the selected variables, whereas the R^2 for reducing sugar and dextrose equivalent which were found to be 43 and 32.8% respectively were too low for adequate representation of the actual relationship, though the model terms were significant. The optimal reducing sugar and dextrose equivalent were 14.88% and 12.30 DE, respectively at pH 6.5, 70 ^{0}C and 60 min; the model however established the actual relationship between the actual variables of the liquefaction and the predictable values of the process.

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