



Modelling of the Liquefaction Steps of Cassava Hydrolysis by Alpha-amylase using a Statistical Approach

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ABSTRACT

Alpha amylase is an enzyme that has the ability to break down the hydrogen bonds that exist between molecules of starchy compounds. The enzymatic hydrolysis of cassava starch was evaluated where a suitable medium was prepared for the enzyme to work. The experiment was done at different degrees of pH values and temperatures to assess the optimal conditions for the enzyme to work. The experiment was done at degrees of 3 pH values (pH 6.0, 6.5 and 7.0); 3 temperatures (65, 70 and 75 °C) and 3 times range (40, 50 and 60 min). The results showed that the p-value of the model terms for sample dry weight was significant whereas the p-values of the model terms for reducing sugar and dextrose equivalent were not significant. The R² was found to be 94.5% 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 21.6 and 26.8% respectively were too low for adequate representation of the actual relationship between the selected variables. The optimal reducing sugar and dextrose equivalent were 17.84% and 14.74 DE, respectively at pH 6.5, 70 °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 modelled process may serve as a substrate to initiate a saccharification process in the production of glucose syrup.

Key words: Cassava; Manihot esculenta Crantz ; α -amylase; amyolytic analysis, dextrose equivalent

INTRODUCTION

The industrial revolution and the increase in the need for fuel rates led to the necessity of using plant sources to obtain high levels of fuel. This prompted scientists to search for natural alternative sources to obtain sources of fuel [1].

There are traditional methods of obtaining glucose from multi-dextrose, such as using mineral acids, but using this method requires energy levels to extract a pure extract of glucose. This led scientists to the use of biochemical catalysts such as the enzyme alpha-amylase, which is characterized by being available in all living organisms and easy to extract from them, as it contributes to the production of a large amount of glucose at the lowest possible costs [2].

Amylase is one of the most important enzymes used in industry, as it constitutes 25% of industrial enzymes. Amylase is extracted from multiple sources including plant, animal and microbial sources. Microbial amylase is used in industry and is better than the chemical decomposition of starch. With scientific advances, alpha amylase has been used in many medical, clinical and food industries. The alpha amylase enzyme consists of 512 amino acids in a chain of 57.6 kDa that is organized in a three-dimensional form that binds to Ca^{2+} molecules [3]

Alpha-amylase is a chemical catalyst that breaks down starch into multidextrose by breaking down (1, 4) glycosidic bonds. It is known that multidextrose is composed of units of glucose ranging in number between 3-17 units of glucose that are linked by bonds of type (1, 4) glycosidic bonds, and are classified by Dextrose Equivalent (DE), which ranges between 3 and 20 [4]

Also, the alpha-amylase enzyme has the ability to break down corn starch to produce a large amount of glucose syrup and multidextrose [5]

Starch has nutritional and industrial importance for humans, as it undergoes chemical and enzymatic processes to obtain multiple products such as starch hydrolyte, glucose syrup, fructose, and maltodextrin. Starch is extracted from wheat, corn, potato, and cassava [6]

Starch is a glucose polymer bound to a glycosidic bond; there are two types of amylose and amylopectin. Amylose is a polymer of 6000 units of glucose linked by α -1, 4 glycosidic bonds, while amylopectin consists of 10-60 units of glucose linked by type bonds. α -1,6 [7]

The most important of these plant sources is cassava (*Manihot esculenta* Crantz), which is a plant known to contain a stock of starch used to produce a large amount of glucose syrup. For instance it is considered one of the most important crops spread in tropical regions; it does not have any damage to the environment in terms of soil and air, in addition to being tolerant of harsh conditions of drought, humidity, soil poverty, and salinity. The plant is spread in Asia, Europe and North Africa. Whereas, using amylolytic analysis, 80% of glucose syrup was extracted from cassava starch [8]. Cassava's ability to withstand harsh climatic conditions and its ability to rapidly hydrolyse have made it a substrate for chemical transformation and its use in the biofuel industry. Cassava has an important role in the food industry, as it is used in the manufacture of starch and yeasts [9].

The purpose of the study is to model the liquefaction of cassava hydrolysis using alpha-amylase in arithmetical attitude.

MATERIALS AND METHODS

Materials

Cassava (*Manihot esculenta*) (TME 419) was used from the International Institute for Tropical Agriculture Ibadan, Nigeria. For alpha-amylase, it was obtained in pure form from International Brewery, Ilesa, Nigeria. Where isolate from *Bacillus amyloliquefaciens* and conditions were controlled from pH 6.4-6.5 and temperature at 80-88°C.

Production of Starch

The starch was extracted from cassava in five steps, which were peeled and washed with tap water. It was ground and then mixed with distilled water and the mixture was purified with a muslin cloth. The suspension was left for 3 hours, then the supernatant was discarded, the residue was washed with distilled water and the water was removed with muslin cloth. To obtain an amount of pure starch, the precipitate was dried using an in a vacuum oven at 40 ° C for 8 hours. Grinding the dry starch was packed in polyethylene packaging and kept at room temperature until use [10]

Production of Maltodextrin

Characterization of alpha-amylase

The hydrolysis of cassava starch was carried out using the alpha-amylase enzyme under optimal conditions in which the enzyme reached its maximum activity. The change in pH was studied using 3 measures (6, 6.5, 7), as well as at different temperatures (65, 70, 75 ° C), and at different times (40, 50, 60 minutes).

Determination of enzyme activity in alpha-amylase

The hydrolysis of cassava starch was performed using alpha-amylase by Betiku and Ajala (2010) [11]. Where 10 grams of starch were placed in 100 ml of distilled water to make 10% slurry, 40 ppm Ca²⁺ + was added to the solution to ensure the stability of the enzyme. Citrate-phosphate buffer was used to stabilize the pH at 6, 6.5, and 7. The starch was gelatinized by raising the temperature of the solution to 97 ° C for 10 minutes. The mixture was gradually cooled to 65, 70 and 75 C, and the liquefaction was done by adding 2% (w / v) of alpha amylase and it was placed in a fermenter installed with a thermal water bath to maintain the temperature at 50 ° C at different times 40, 50, 60 Samples were obtained at different times and separated in a centrifuge at 2500 rpm for 10 minutes using (80-2 Centrifuge Med-Lab Scientific Company England). These steps were repeated three times, and a glucose curve was worked out to determine the optimal conditions for maximum production amount of glucose.

Determination of physicochemical properties of maltodextrin

Determination of reducing sugar

Reducing sugar was quantified using dinitrosalicylic acid (DNS) Miller (1972) [12]. This was done by adding 3 ml of DNS to 1 ml of sample in a test tube and boiled for 10 minutes. The solution was left to cool and then 1 ml of 1 ml of Rochelle salt was added. The resulting color intensity was measured at 540 nm using a UV-Visible Spectrophotometer (AJ-1C03). The percentage of reducing sugar was measured by the percentage of the amount of reducing sugar in glucose to the amount of starch slurry.

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

Determination of sample dry weight

The dry weight of the sample was determined by weighing 2 grams of the sample in dry, cooled and weighed dishes. The samples were placed in a Genlab moisture extraction oven at a temperature of 105 ° C for 3 hours to get rid of moisture. The dishes were transferred to the desiccator to cool for 30 minutes. After the cooling process, the dishes are weighed and the weights are written. These steps are repeated until constant weights are obtained.

Determination of dextrose equivalent (DE)

Dextrose equivalent (DE) was measured using the method of Betiku et al (2013) [13], according to the following equation:

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

Statistical Analysis

The statistical results were studied using SPSS20 program. Where the double regression of the variables was studied using ANOVA, in addition to studying the effect of the fixed variable on other variables.

RESULTS AND DISCUSSION

Modelling of the Liquefaction Steps of Cassava Starch Hydrolysis

Evaluation and determination of the coefficient of the full regression model equation and the statistical significance of liquefaction steps of cassava starch hydrolysis are presented 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 ($p > 0.05$).

Table 2 shows the analysis of variance of the regression equation model, the model fisher F-test of sample dry weight, 132.94; reducing sugar, 2.12; dextrose equivalent, 2.80 with low probability values [($p_{\text{model}} > F$) = 0.000] demonstrated a high significance of the regression model (Kunamneni and Singh, 2005) [14]. The goodness of fit was checked by the coefficient of determination (R^2), such that Guan and Yao (2008) [15] reported that R^2 should be at least 0.80 for the good fit of a model, however for the liquefaction steps, the R^2 was found to be 94.5% for sample dry weight, 21.6% for reducing sugar and 26.8% for dextrose equivalent. However, adjusted R^2 was found to be 93.8% for sample dry weight, 11.4% for reducing sugar and 17.2% for dextrose equivalent, the model of sample dry weight proved suitable for adequate representation of the actual relationship between the selected variables [14]; the value of the R^2 obtained in the model of sample dry weight showed high consistency between the observed values and the predicted values, while the R^2 model of reducing sugar and dextrose equivalent showed an inconsistency between the observed values and the predicted values [15].

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 R^2 to be at least 0.80 for the good fit of a model.

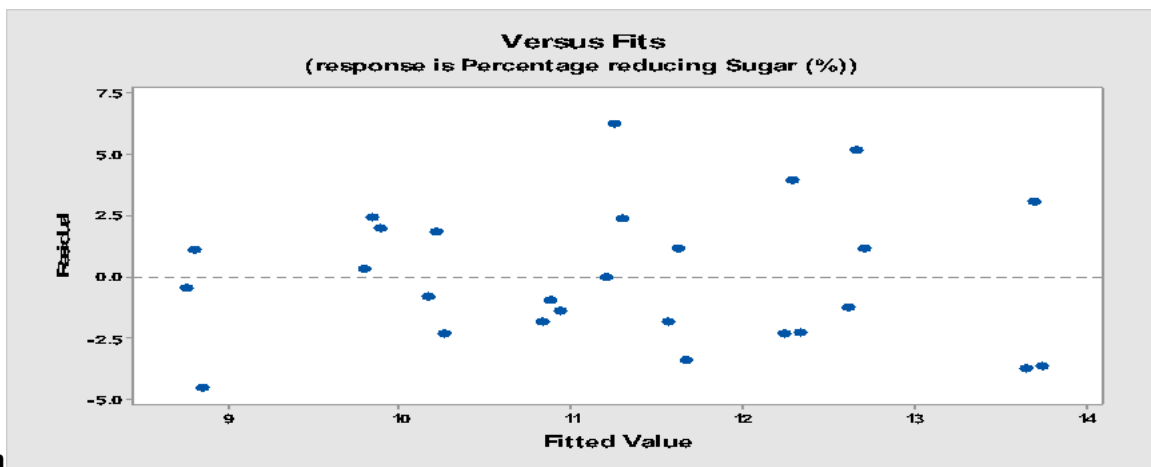
The regression models of the effect of pH, temperature and time on the liquefaction steps of cassava hydrolysis are shown on Figures 1a, b and c. The graph showed the regression equation and fitness of the plot which expressed the residual on the vertical axis and fitted value on the horizontal axis, where residual value is the difference between the observed value and predicted value. Percentage reducing sugar showed a weak correlation between the model's predictions and its actual results where the plots are observed to be unevenly distributed vertically and the residual plots are not randomly dispersed around the horizontal axis, however non-linear model coefficient of determination showed that a unit increase in pH will reduce the percentage reducing sugar by 10%; a unit increase in temperature will reduce the reducing sugar by 20.8% whereas a unit increase in liquefaction time will increase the reducing sugar by 14.1%. 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 0.58%; a unit increase in temperature will reduce sample dry weight by 0.08% and a unit increase in time will reduce the sample dry weight by 0.06%. 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 increase the dextrose equivalent by 29%; a unit increase in temperature will reduce dextrose equivalent by 11% while a unit increase in time will increase the dextrose equivalent by 15%.

Table 1. Test of Significance of Every Regression Coefficient for the Liquefaction Steps of Cassava Hydrolysis

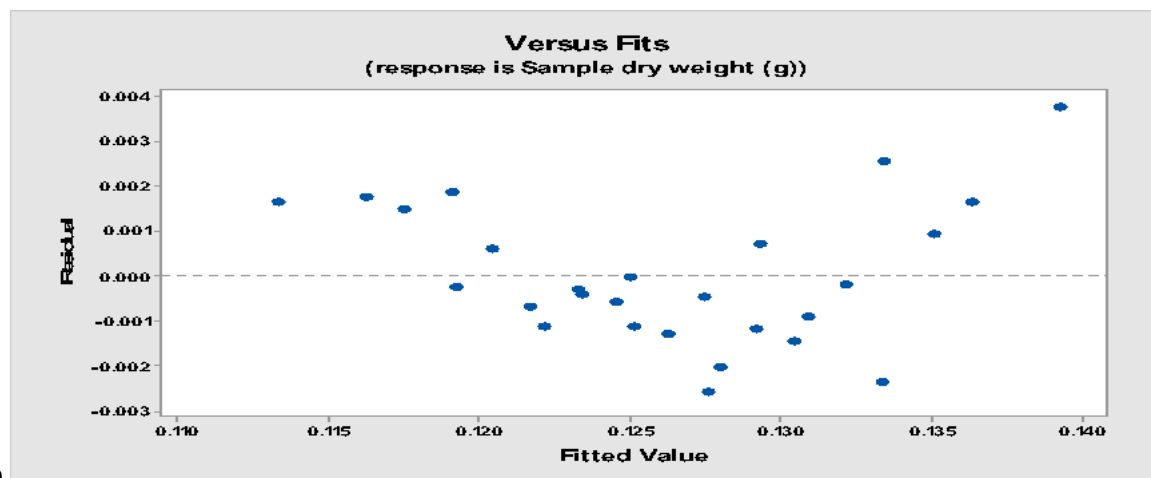
Predictor	Coeff	SE Coeff	T	P
Sample Dry Weight (g)				
Constant	0.251630	0.007691	32.72	0.000
pH	-0.0057778	0.0007782	-7.42	0.000
Temperature °C	-0.00083333	0.00007782	-10.71	0.000
Time (min)	-0.00058889	0.00003891	-15.13	0.000
Percentage Reducing Sugar				
Constant	19.42	13.76	1.41	0.172
pH	-0.100	1.392	-0.07	0.943
Temperature °C	-0.2082	0.1392	-1.50	0.148
Time (min)	0.14114	0.06961	2.03	0.054
Dextrose Equivalent				
Constant	7.40	11.04	0.67	0.510
pH	0.286	1.117	0.26	0.800
Temperature °C	-0.1124	0.1117	-1.01	0.325
Time (min)	0.15119	0.05584	2.71	0.013

Table 2. Analysis of Variance (ANOVA) of the Regression Equation for the Liquefaction Steps of Cassava Hydrolysis

Source	DF	SS	MS	F	P
Sample Dry Weight (g)					
Regression	3	0.00108694	0.00036231	132.94	0.000
Residual Error	23	0.00006269	0.00000273		
Total	26	0.00114963			
	S = 0.00165089	R-Sq = 94.5%	R-Sq(Adj) = 93.8%		
Percentage Reducing Sugar					
Regression	3	55.410	18.470	2.12	0.126
Residual Error	23	200.629	8.723		
Total	26	256.040			
	S = 2.95348	R-Sq = 21.6%	R-Sq(adj) = 11.4%		
Dextrose Equivalent					
Regression	3	47.196	15.732	2.80	0.062
Residual Error	23	129.086	5.612		
Total	26	176.282			
	S = 2.36906	R-Sq = 26.8%	R-Sq(adj) = 17.2%		



a



b

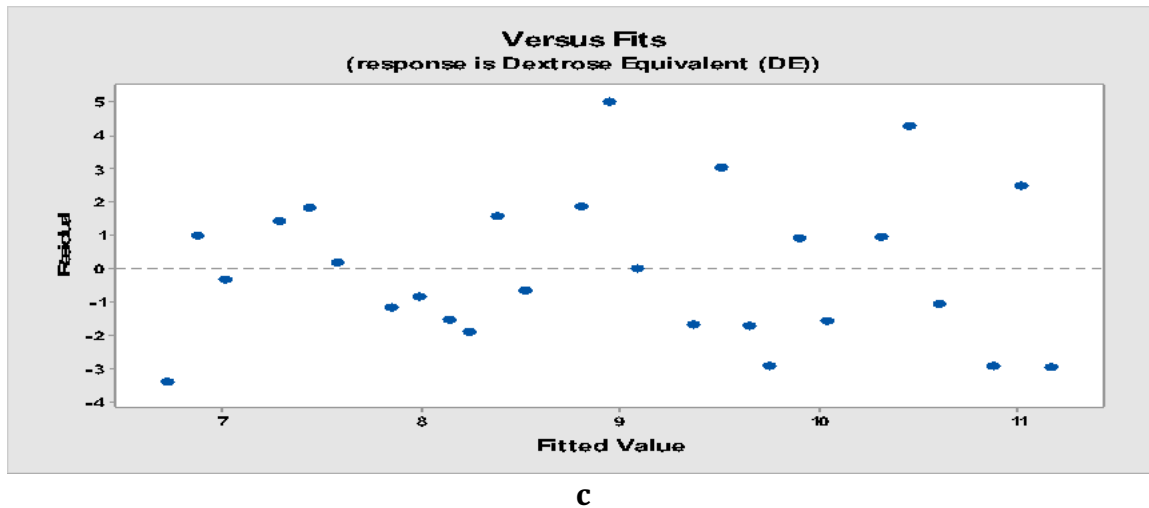


Figure 1: Fit regression model of liquefaction of cassava starch
a: Reducing sugar; **b:** Sample dry weight; **c:** Dextrose equivalent

The final regression equation in terms of coded factors for the response surface quadratic model for the liquefaction steps is described in equations:

Sample dry weight (g) = 0.252 - 0.00578 pH - 0.000833 Temperature ($^{\circ}$ C)

$$- 0.000589\text{Time (min)} \dots\dots\dots [\text{Eq. 1.1}]$$

Percentage reducing sugar (%) = 19.4 - 0.10 pH - 0.208 Temperature ($^{\circ}$ C)

$$+ 0.141 \text{Time (min)} \dots\dots\dots [\text{Eq. 1.2}]$$

Dextrose equivalent (DE) = 7.4 + 0.29 pH - 0.112 Temperature ($^{\circ}$ C)

$$+ 0.151 \text{Time (min)} \dots\dots\dots [\text{Eq. 1.3}]$$

CONCLUSION

Statistical Approach was successfully applied to the liquefaction steps of cassava 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 $^{\circ}$ 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. Where an efficient and simple method was designed to produce bioethanol from cassava starch due to the ease and speed of liquefaction.

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