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Optimisation of Combined Acid and Enzymatic Hydrolysis of Cocoyam Starch to Produce Fermentable Hydrolysate

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ABSTRACT

Dilute acid hydrolysis and enzymatic hydrolysis were sequentially combined for the purpose of producing fermentable hydrolysate from cocoyam starch. A three variable Box Behnken design was used to study the effect of temperature, time and acid concentration for the acid hydrolysis step while for the enzymatic hydrolysis step, the variables optimised were temperature, time and pH. A total of 17 individual experiments were generated for each step of the hydrolysis and were used to develop regression models for each step. The regression models developed to represent the acid and enzymatic hydrolysis steps were statistically significant ($p<0.05$) and did not show lack of fit ($R^2>0.9$). For the acid hydrolysis step, the regression model predicted the maximum sugar concentration to be 79.81 g/L at optimum temperature 100°C, time 11.66 min and acid concentration 1.5% w/w. For the enzymatic hydrolysis step, the regression model predicted the maximum sugar concentration to be 93.44 g/L at optimum temperature 58ºC, time 55 min and pH of 5.5.

Keywords: Acid hydrolysis, box Behnken design, cocoyam, enzymatic hydrolysis, reducing sugar

INTRODUCTION

Root and tuber crops are among the most important group of food crops in many tropical African countries. In Nigeria for instance, cassava (Manihot esculenta) is the most important of these crops in terms of total production, importance and economic value (Okoye *et al*., 2008). Cocoyam (*Colocasia esculenta*), which belongs to the Araceae family, ranks third after cassava and yam (Onyenweaku & Okoye, 2007). According to a report by Ogunniyi (2008), Nigeria is the world's largest producer of cocoyam, accounting for about 40% of total world output as recorded by the Food and Agriculture Organisation in 2007 (FAO, 2007). Despite this, Nigeria and other developing nations are beset by the problem of lack of proper storage facilities for these tubers and as such, a large number of these tubers in the order of millions of tons are destroyed through pest infestation,

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deterioration, physical damage to the tubers, pilfering etc. (Omemu *et al*., 2005). In order to recover the losses resulting from this wastage, it is important to expand the usage range of these tubers with particular focus on converting them into value-added products.

The cocoyam tuber is rich in carbohydrate, containing about 77.9% starch (Akpata & Babalola, 2012). This starch can be hydrolysed to sugar syrups, which are employed by the food industry to make sweet drinks and juices (Betiku & Adesina, 2013). It can also be fermented by a suitable microorganism to produce organic acids such as citric acid, gluconic acid, oxalic acid and bioalcohols such as bioethanol and biobutanol (Kunamneni & Singh, 2005; Amenaghawon & Aisien, 2012). Conventionally, starch is converted to reducing sugars via dilute acid catalysed hydrolysis (Anozie & Aderibigbe, 2011; Amenaghawon *et al*., 2013a). It typically involves the use of dilute acids such as sulphuric acid, hydrochloric acid, phosphoric acid and nitric acid to hydrolyse the starch by cleaving the α and β linkages in the starch molecule (Najafpour *et al*., 2007). Nevertheless, the conversion of starch to glucose is never complete; hence, acid hydrolysis is typically combined with enzymatic hydrolysis in a sequential manner to obtain improved yield of reducing sugars (Woiciechowski *et al*., 2002; Amenaghawon *et al*., 2013b).

The conditions of hydrolysis such as temperature, time, pH etc. influence the yield of reducing sugars. Very little work has been done on the hydrolysis of cocoyam starch to produce hydrolysates (Omemu *et al*., 2005; Ajao *et al*., 2009; Braide & Nwaoguikpe, 2011). To the author's knowledge, no attempt has been made to sequentially combine acid hydrolysis with enzymatic hydrolysis to produce fermentable hydrolysate from cocoyam starch. In addition, none of these studies attempted to optimise the conditions of hydrolysis using statistically designed experiments for response surface methodology (RSM). It is important to optimise the variables that could influence the hydrolysis process in order to obtain the maximum yield of sugars during hydrolysis. RSM is a comprehensive experimental design and statistical modelling tool that is utilised for the optimisation of multivariable processes (Box & Wilson, 1951). Its main advantage is the ability to minimise the number of experimental runs needed to be conducted in order to obtain statistically acceptable results (Betiku *et al*., 2013).

In this work, cocoyam starch was converted into reducing sugars in a sequentially combined two-step acid and enzymatic hydrolysis. To optimise the process, RSM was applied to determine the effects of three factors (temperature, time and acid concentration) over three levels and their interactions on the amount of reducing sugar released during the acid hydrolysis step. For the enzymatic hydrolysis step, the factors considered were temperature, time and pH.

MATERIALS AND METHODS

Cocoyam Starch Preparation

Cocoyam tubers were obtained from the Faculty of Agriculture model farm in the University of Benin, Benin City, Edo State, Nigeria. The tubers were washed in clean water to remove the adhering dirt, after which they were peeled manually and crushed using a roller mill. The crushed pulp was sieved with a sieve of Teflon cloth. The starch obtained was allowed to settle for about 12 hours. It was decanted and the starch cake obtained was oven dried. The dried starch was then packed in a container for storage (Betiku & Adesina, 2013).

Enzymes

Amyloglucosidase obtained from the biotechnology division of the Federal Institute of Industrial Research Oshodi (FIIRO), Lagos, Nigeria was used for the enzymatic hydrolysis step.

Dilute Acid Hydrolysis

Dilute acid hydrolysis of the prepared cocoyam starch was carried out in a 500 mL Duran round-bottom flask using dilute hydrochloric acid concentration in the range 0.5-1.5% w/w and 10 g of cocoyam starch. The operating conditions of the hydrolysis reaction were as follows: temperature (80-100°C) and time (5-15 min). The mixture of starch in acid was heated rapidly to the set point temperature and upon completion of the hydrolysis reaction, the reaction vessel was removed from the thermostat heating mantle and cooled under running tap water. Once cooled, the liquid content of the round-bottom flask was filtered using a Whatman No. 4 filter paper to obtain a clear hydrolysate that was subsequently analysed for fermentable sugars.

Enzymatic Hydrolysis

The hydrolysate resulting from the acid hydrolysis step at the optimised conditions was subjected to enzyme treatment. The pH was adjusted to the appropriate level using a citrate-phosphate buffer as required. Amyloglucosidase of concentration 1% (v/v) was added for the enzymatic hydrolysis to take place. After the hydrolysis reaction, the enzyme was deactivated by heating the mixture to 100°C for 10 min. The final mixture was centrifuged at 10,000 rpm for 10 min and the supernatant was analysed for reducing sugar.

Analytical Methods

The reducing sugars recovered from the cocoyam starch during hydrolysis were quantified using the method of Miller (1959). To 1 mL of the supernatant, 3 mL of dinitrosalicylic acid (DNS) reagent was added in the test tube and the mixture was boiled for 5 min. It was subsequently cooled and diluted appropriately, after which the absorbance was measured at a wavelength of 540 nm using the UV-Visible Spectrophotometer (PG Instruments model T70).

Experimental Design

TABLE 1 : Box Behnken Design for Acid Hydrolysis Step

Independent Variable	Symbols	Coded and Actual Levels		
		-1	0	+1
Temperature (°C)	X_1	80	90	100
Time (min)	X_2	5	10	15
Acid Concentration (w/w %)	X_3	0.5	1.0	1.5

TABLE 2 : Box Behnken Design for Enzymatic Hydrolysis Step

Independent Variable	Symbols	Coded and Actual Levels		
		-1	0	+1
Temperature (°C)	X_1	55	60	65
Time (min)	X_2	55	60	65
pH (-)	X_3	5.5	6.0	6.5

A three variable Box Behnken design (BBD) for response surface methodology was used to develop a statistical model for both hydrolysis steps. The range of the variables that were optimised for both steps are shown in Tables 1 and 2. The experimental design made up of 17 runs for each step was developed using Design Expert® 7.0.0 (Stat-ease, Inc. Minneapolis, USA).

The coded and actual values of the independent variables were calculated as follows

$$x_i = \frac{X_i - X_o}{\Delta X_i} \qquad [1]$$

where x_i and X_i are the coded and actual values of the independent variable, respectively. X_o is the actual value of the independent variable at the centre point and ΔX_i is the step change in the actual value of the independent variable. The following generalised second-order polynomial equation was used to estimate the response of the dependent variable:

$$Y_i = b_o + \sum b_i X_j + \sum b_{ij} X_i X_j + \sum b_{ii} X_i^2 + e_i \qquad [2]$$

where Y_i is the dependent variable or predicted response, X_i and X_j are the independent variables, b_o is the offset term, b_i and b_{ij} are the single and interaction effect coefficients and ei is the error term.

RESULTS AND DISCUSSION

Statistical Modelling of the Acid Hydrolysis Step

Table 3 shows the Box-Behnken design matrix for the acid hydrolysis step. The response variable was chosen as the reducing sugar concentration. Equation (3) is the quadratic regression model in terms of actual variables that was obtained after applying a multiple regression analysis to the experimental data presented in Table 3.

$$Y = 87.81 + 0.53X_1 - 2.42X_2 - 55.98X_3 + 0.076X_1X_2 + 0.84X_1X_3 + 0.67X_2X_3 - 0.012X_1^2 - 0.26X_2^2 - 10.32X_3^2 \qquad [3]$$

The values of total sugar concentration as predicted by Equation (3) are also presented in Table 3. The results of the analysis of variance (ANOVA) carried out to determine the fit of the regression model are presented in Tables 4 and 5.

TABLE 3 : Box Behnken Design Matrix for the Optimisation of the Acid Hydrolysis Step

Run No	Factors						Response	
	Coded Values			Actual Values			Sugar Concentration (g/L)	
	X_1	X_2	X_3	X_1	X_2	X_3	Observed	Predicted
1	-1	0	-1	80	10	0.5	72.06	72.32
2	-1	1	0	80	15	1.0	61.47	62.21
3	1	1	0	100	15	1.0	69.25	70.51
4	0	0	0	90	10	1.0	75.22	75.22
5	0	1	1	90	15	1.5	70.64	69.64
6	1	0	-1	100	10	0.5	64.85	64.59
7	1	0	1	100	10	1.5	79.33	79.07
8	0	-1	-1	90	5	0.5	64.74	65.74
9	0	0	0	90	10	1.0	75.22	75.22
10	-1	-1	0	80	5	1.0	73.20	71.94
11	0	0	0	90	10	1.0	75.22	75.22
12	-1	0	1	80	10	1.5	69.66	69.92
13	0	0	0	90	10	1.0	75.22	75.22
14	0	-1	1	90	5	1.5	67.44	68.44
15	0	0	0	90	10	1.0	75.22	75.22
16	0	1	-1	90	15	0.5	61.25	60.25
17	1	-1	0	100	5	1.0	65.80	65.06

TABLE 4 : Analysis of Variance (ANOVA) of Regression Model for the Acid Hydrolysis Step

Sources	Sum of Squares	df	Mean Squares	F value	p value
Model	456.08	9	50.68	41.63	< 0.0001
X_1	1.01	1	1.01	0.83	0.3930
X_2	9.18	1	9.18	7.54	0.0287
X_3	73.02	1	73.02	59.99	0.0001
X_1X_2	57.61	1	57.61	47.33	0.0002
X_1X_3	71.23	1	71.23	58.52	0.0001
X_2X_3	11.19	1	11.19	9.19	0.0191
X_1^2	5.73	1	5.73	4.70	0.0667
X_2^2	184.73	1	184.73	151.76	< 0.0001
X_3^2	28.00	1	28.00	23.00	0.0020
Residual	8.52	7	1.22		
Lack of Fit	5.52	3	1.84	2.45	0.2034
Pure Error	3.00	4	0.75		
Cor Total	464.60	16			

TABLE 5 : Statistical Information for ANOVA for the Acid Hydrolysis Step

Source	Response Value
R_2	0.98
CV %	1.57
Adeq. Precision	22.24

The model F value of 41.63 and very low p value (<0.0001) showed that the model was significant. The lack-of-fit F value of 2.45 implies that there was insignificant lack of fit. The coefficient of variation (CV) obtained was 1.57% (Table 5). The coefficient of variation gives a measure of the degree of precision with which the treatments were carried out; a low value of CV typically implies that the treatments were carried out with high precision and reliability (XuJie & Wei, 2008). The adequate precision value of 22.24 indicates an adequate signal, and shows that the model can be used to navigate the design space (Cao *et al*., 2009). An R^2 value of 0.98 indicates that 98% of the variability in the response could be explained by the regression model (Amenaghawon *et al*., 2014). The R^2 value indicates the degree to which the model was able to predict the response. Qi *et al*. (2009) reported that the closer the R^2 value is to unity, the better the model can predict the response.

Optimisation of Dilute Acid Hydrolysis Step

The graphical representations of the regression equation for the optimisation of the acid hydrolysis step are displayed as three dimensional (3D) response surface curves in Figures 1 to 3. Fig.1 shows the effect of acid concentration and time on the total sugar concentration. For the range of time investigated, the total sugar concentration increased progressively with increase in acid concentration. This trend may be attributed to the catalytic activity of the acid. Since the hydrogen ions in a solution are responsible for the catalytic activity of the acid, increasing the acid concentration results in an increase in the number of hydrogen ions in the solution, which in turn results in a corresponding increase in the catalytic activity of the acid. Hence, the rate at which the glycosidic bonds are cleaved will increase, resulting in the formation of more fermentable sugars (Kumar *et al*., 2009). Similar observations have been reported by other researchers (Hu *et al*., 2010; Lenihan *et al*., 2010). The hydrolysis time showed a quadratic effect on the total sugar concentration as shown in Fig.1. For the range of acid concentrations investigated, intermediate levels of time were needed to obtain high fermentable sugar concentrations, and any further increase in time resulted in a decrease in sugar concentration. The decline might have resulted from the degradation of fermentable sugars to by-products such as furfural and hydroxyl methyl furfural (Najafpour *et al*., 2007).

The effect of temperature and time on the total sugar concentration is shown in Fig.2. At low values of hydrolysis time, the total sugar concentration increased with an increase in temperature. Although a similar trend was observed at high values of hydrolysis time, the rate of the hydrolysis reaction was, however, faster. This observation might be attributed to the increase in the rate of collision of the molecules of the reacting species during the reaction. Hence, the higher the temperature, the more frequently the molecules collided with each other, resulting in a reaction. With respect to time, a trend similar to that shown in Fig.1 was observed. Anozie

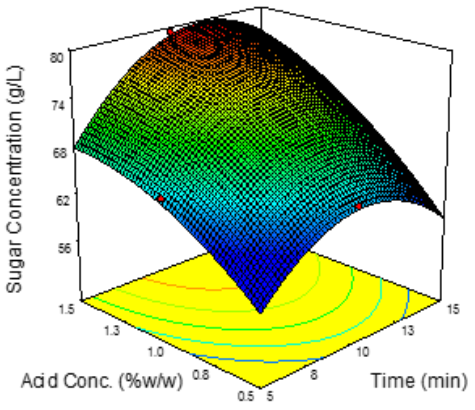

Fig.1: Effect of acid concentration and time on total sugar concentration for acid hydrolysis.

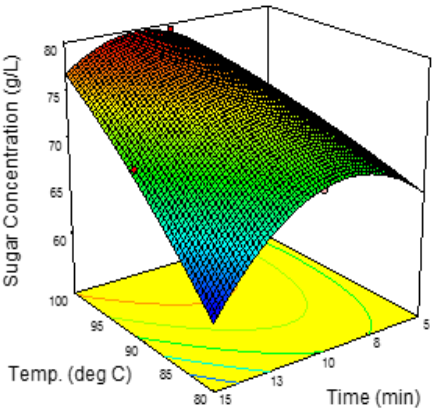

Fig.2: Effect of temperature and time on total sugar concentration for acid hydrolysis.

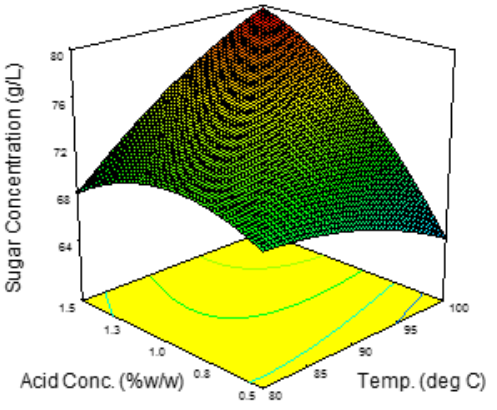

Fig.3: Effect of acid concentration and temperature on total sugar concentration for acid hydrolysis.

and Aderibigbe (2011) reported a similar trend for the optimisation of fermentable hydrolysate production from cassava starch using the response surface methodology. They observed that temperature had a positive influence on the hydrolysis reaction.

The effect of acid concentration and temperature on the hydrolysis process is shown in Fig.3. At low values of temperature, the total sugar concentration increased albeit not very significantly with an increase in acid concentration. At high values of temperature, a similar trend was observed but the hydrolysis reaction was observed to be faster as seen in the rapid increase in the total sugar concentration. This observation might not be unconnected with the enhancement of the rate of the hydrolysis reaction at high temperatures. With respect to acid concentration, the rate of the reaction was observed to be higher at higher concentrations of acid and this could also be attributed to the enhancement of the catalytic activity of the acid as a result of the increase in the amount of hydrogen ions in solution.

The regression model (Equation 3) was optimised to determine the optimum values of acid concentration, hydrolysis temperature and time that resulted in the maximum sugar concentration. The result of optimisation by RSM indicated a maximum reducing sugar concentration of 79.81 g/L. The optimum conditions of hydrolysis that resulted in this value were as follows: acid concentration, 1.5% w/w; hydrolysis temperature, 100 °C and hydrolysis time, 11.66 min. To confirm the validity of the results predicted by the model, experiments were performed in triplicate at the established optimal hydrolysis conditions. The results obtained showed that the total sugar concentration (79.14 g/L) obtained was close to the predicted value (79.81 g/L). The excellent correlation between the predicted and measured values shows the validity of the response model. Similar results have been reported by previous researchers. Anozie and Aderibigbe (2011) investigated the application of dilute acid hydrolysis for the recovery of fermentable sugars from cassava starch. Their results showed the optimum value of temperature, time and agitation speed to be 80 °C, 60 min and 200 rpm respectively. Under these conditions, the maximum reducing sugar production was recorded as 46.12 g/L. In another study, Gaewchingduang and Pengthemkeerati (2010) reported optimal hydrolysis conditions (acid concentration, time and temperature) of 0.98 %, 60 min and 120°C respectively for the conversion of starch present in cassava bagasse to fermentable sugars. This work reports a higher sugar concentration at milder conditions. Mild conditions of hydrolysis are important in the sense that there is the possibility of degradation of sugars to inhibitory products such as furfural and hydroxymethylfurfural when the hydrolysis is carried out under harsh conditions (Najafpour *et al*., 2007).

Statistical Modelling of the Enzymatic Hydrolysis Step

Results of the enzymatic hydrolysis step are shown in Table 6, which contains the coded and actual values of the variables that were optimised. The Table contains experimental sugar concentrations as well as those predicted by the regression model (Equation 4).

$$Y = 1337.42 + 29.39X_1 - 32.60X_2 - 362.54X_3 - 0.060X_1X_2 + 3.95X_1X_3 + 6.69X_2X_3 - 0.41X_1^2 - 0.045X_2^2 - 24.00X_3^2 \quad [4]$$

Table 7 shows the analysis of variance of the regression model (Equation 4). The model F-value of 9.03 and a low p value of 0.0042 implied that the model was significant. The data obtained fit the quadratic model best with an R^2 value of 0.92, showing that the model proved suitable for the adequate representation of the actual relationship between the selected variables. The lack-of-fit p value of 0.1734 showed that the lack-of-fit of the model was not significant. According to Vázquez *et al*. (2009), a non-significant lack of fit is actually desirable. This shows that the model could be used in theoretical prediction of the enzymatic hydrolysis of cocoyam starch.

The low value of CV obtained (Table 8) shows that the treatments were carried out with high reliablility (XuJie & Wei, 2008). The model can be used to navigate the design space as evident from the Adeq. Precision value obtained (Cao *et al*., 2009).

TABLE 6: Box Behnken Design Matrix for the Optimisation of the Enzymatic Hydrolysis Step

Run No	Factors						Response	
	Coded Values			Actual Values			Sugar Concentration (g/L)	
	X_1	X_2	X_3	X_1	X_2	X_3	Observed	Predicted
1	-1	0	-1	80	10	0.5	72.06	72.32
2	-1	1	0	80	15	1.0	61.47	62.21
3	1	1	0	100	15	1.0	69.25	70.51
4	0	0	0	90	10	1.0	75.22	75.22
5	0	1	1	90	15	1.5	70.64	69.64
6	1	0	-1	100	10	0.5	64.85	64.59
7	1	0	1	100	10	1.5	79.33	79.07
8	0	-1	-1	90	5	0.5	64.74	65.74
9	0	0	0	90	10	1.0	75.22	75.22
10	-1	-1	0	80	5	1.0	73.20	71.94
11	0	0	0	90	10	1.0	75.22	75.22
12	-1	0	1	80	10	1.5	69.66	69.92
13	0	0	0	90	10	1.0	75.22	75.22
14	0	-1	1	90	5	1.5	67.44	68.44
15	0	0	0	90	10	1.0	75.22	75.22
16	0	1	-1	90	15	0.5	61.25	60.25
17	1	-1	0	100	5	1.0	65.80	65.06

TABLE 7: Analysis of Variance (ANOVA) of Regression Model for the Enzymatic Hydrolysis Step

Sources	Sum of Squares	df	Mean Squares	F value	p value
Model	2879.08	9	319.90	9.03	0.0042
X_1	6.44	1	6.44	0.18	0.6827
X_2	397.95	1	397.95	11.23	0.0122
X_3	308.68	1	308.68	8.71	0.0214
X_1X_2	8.88	1	8.88	0.25	0.6320
X_1X_3	389.47	1	389.47	10.99	0.0129
X_2X3	1118.44	1	1118.44	31.56	0.0008
X_1^2	450.94	1	450.94	12.72	0.0091
X_2^2	5.26	1	5.26	0.15	0.7114
X_3^2	151.60	1	151.60	4.28	0.0774
Residual	248.11	7	35.44		
Lack of Fit	248.00	3	82.67	2.79	0.1734
Pure Error	0.10	4	0.03		
Cor Total	3127.19	16			

TABLE 8: Statistical Information for ANOVA for the Enzymatic Hydrolysis Step

Source	Response Value
R^2	0.92
CV %	9.88
Adeq. Precision	11.91

Optimisation of the Enzymatic Hydrolysis Step

The graphical representations of the regression model for the optimisation of the enzymatic hydrolysis step are displayed as three-dimensional (3D) response surface curves in Fig.4 to Fig.6. Fig.4 shows the effect of time and temperature on the concentration of sugars produced during enzymatic hydrolysis. Within the range of temperature investigated, the total sugar concentration increased with increase in time. This suggests that the residual starch in the acid hydrolysate was being converted to more sugars. Increasing the hydrolysis temperature did not favour the formation of reducing sugars. As noted earlier, this might be attributed to the degradation of sugars to unwanted products (Najafpour *et al*., 2007).

The response surface plot representing the effect of pH and temperature on sugar concentration while keeping time constant is presented in Fig.5. The results showed that more sugars were produced at low pH values, indicating that acidic conditions favoured the production of fermentable sugars during enzymatic hydrolysis. This observation was recorded both at high and low temperatures. The pH of the solution is important to the biochemical functioning of the enzyme. Changes in pH could affect the configuration of an enzyme as well as the charge properties of the substrate. Extremely high or low pH values generally result in complete loss of activity for enzymes. The loss of activity results from the inability of the substrate to bind to the active site on the enzyme as a result of the change in its configuration. In general, enzymes typically have a pH optimum with the optimum differing from one enzyme to another. The results obtained are in agreement with the fact that amyloglucosidase has the most acidic optimum pH of all amylases. It is reportedly most active at a pH value of around 5.

Fig.6 shows the effect of pH and time on the hydrolysis process. In terms of pH, a trend similar to that presented in Fig.5 was observed. However, the effect of pH was not very significant at high values of hydrolysis time.

The regression model for the enzymatic hydrolysis step (Equation 4) was optimised to determine the optimum values of hydrolysis temperature, time and pH that resulted in the maximum sugar concentration. The result of optimisation by RSM indicated a maximum reducing sugar concentration of 93.44 g/L. The optimum conditions of hydrolysis that resulted in this value were as follows: hydrolysis temperature, 58 °C; hydrolysis time, 55 min and pH, 5.5. To confirm the validity of the results predicted by the statistical model, experiments were performed in triplicate under the established optimal hydrolysis conditions. The results obtained showed that the total sugar concentration (93.14 g/L) obtained was close to the predicted value (93.44 g/L). The excellent correlation between the predicted and measured values shows the validity of the response model. Similar results have been obtained by other researchers. Sunaryanto *et al*. (2013) subjected sago starch to a combined process of acid and enzymatic

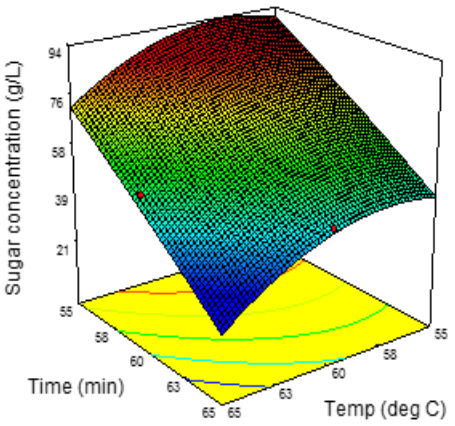

Fig.4: Effect of time and temperature on total sugar concentration for enzymatic hydrolysis.

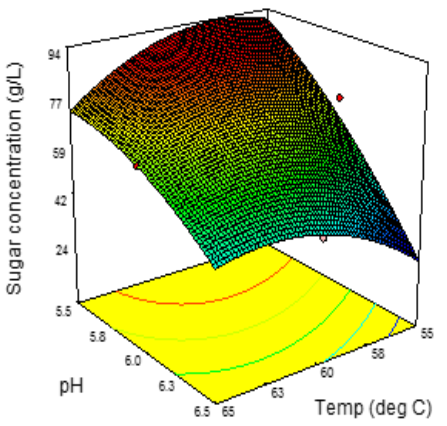

Fig.5: Effect of pH and temperature on total sugar concentration for enzymatic hydrolysis.

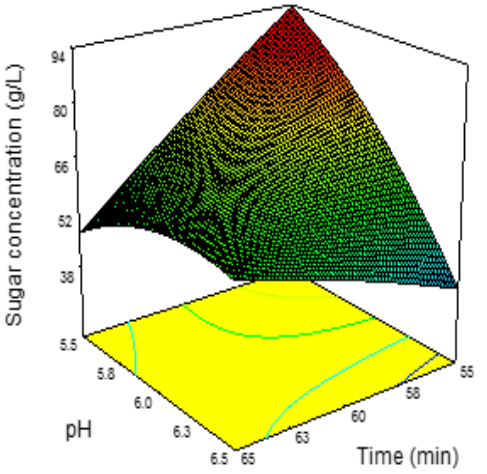

Fig.6: Effect of pH and time on total sugar concentration for enzymatic hydrolysis.

hydrolysis for the purpose of producing bioethanol. Their results showed that the maximum sugar concentration obtained for the acid hydrolysis step was 66 g/L while a value of 70 g/L was obtained for the enzymatic hydrolysis step. In another study, Betiku and Adesina (2013) reported optimum hydrolysis conditions (temperature, time and pH) of 61.05 °C, 55.02 min and 6.5 respectively for the enzymatic hydrolysis of potato starch. At these optimised conditions, the maximum sugar concentration was obtained as 172.23 g/L. The significantly higher amount of sugar recovered by Betiku and Adesina (2013) compared to the result obtained in this work could be attributed to the fact that they used twice the amount of starch used in this work.

CONCLUSION

The response surface methodology was successfully applied to the optimisation of sequentially combined acid and enzymatic hydrolysis of cocoyam starch. The regression models developed to represent the acid and enzymatic hydrolysis steps were statistically significant ($p<0.05$) and did not show lack-of-fit ($R^2>0.9$). The maximum reducing sugar concentration obtained for the acid hydrolysis step was 79.81 g/L at temperature 60 ºC, time 60 min and pH 6.5. For the enzymatic hydrolysis step, the maximum reducing sugar concentration obtained was 93.44 g/L at temperature 58 ºC, time 55 min and pH 5.5. The hydrolysate obtained in this work may be utilised as a carbon source for the biotechnological production of useful products like citric acid, gluconic acid, oxalic acid and ethanol.

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