ENZYMATIC HYDROLYSIS OPTIMIZATION OF SWEET POTATO (*Ipomoea batatas*) PEEL USING A STATISTICAL APPROACH

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Abstract - In this work, two-step enzymatic hydrolysis of sweet potato peel was optimized. The effects of time, enzyme dose and temperature on glucose concentration were investigated. The Box-Behnken design was applied and a total of 17 experimental runs were generated for each step. For the liquefaction step, an ANOVA test showed the quadratic model obtained to be significant ($p < 0.05$). The statistical model predicted the maximum glucose concentration to be 126.66 g/L at a temperature of 56.4 °C, α-amylase dose 1% (v/v) and time 60 min. A quadratic model was also obtained for the saccharification step and the model was also significant ($p < 0.05$). The statistical model for the second step predicted the maximum glucose concentration to be 178.39 g/L, established at the temperature of 45 °C, glucoamylase dose 1% (v/v) and time 60 min. The optimized liquefaction and saccharification conditions were validated with the actual glucose concentrations of 126.03 and 176.89 g/L, respectively.
Keywords: Hydrolysis; Enzymes; Optimization; Response surface methodology; Sweet potato peel.

INTRODUCTION

Ipomoea batatas L., commonly known as sweet potato, is a dicotyledonous plant which belongs to the family Convolvulaceae, ranked as the seventh most important food crop in global production. In most industries, restaurants and homes where sweet potatoes are eaten or processed into more value-added products, the peel is essentially treated as a waste product and poses disposal problems. Hence, to help keep the environment clean and also find a cheap alternative carbon source for cultivation of many microorganisms, this peel can be hydrolyzed to fermentable sugars, which can be converted to valuable products like citric acid, gluconic acid, oxalic acid, and ethanol, among others. Conventionally, starch is hydrolyzed by acid catalysis, but the method has now largely been replaced by enzymatic processes because it required the use of

corrosion-resistant materials, gave rise to high colour and salt ash content (after neutralization), used more energy for heating and was relatively difficult to control (Chaplin and Bucke, 1990). The two steps involved in enzymatic process are liquefaction and saccharification (Blanco *et al.*, 2000). In the first step, the enzyme α-amylase cleaves 1,4-glycosidic bonds to yield shorter chains of soluble dextrins. In the second step, the enzyme glucoamylase attacks 1,4 terminal bonds in the degraded molecules to release one glucose unit at a time. However, the degradation of starch to glucose is never complete, so the end product is typically a mixture of glucose, maltose and longer-chain-length sugars. The values of optimum time, pH, temperature and other operating conditions differ between the two steps (Gerhartsz, 1990).

Previous studies on the acid hydrolysis of sweet potato include the works of Azhar and Hamdy

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(1981a) and Kim and Hamdy (1985), while other studies on enzymatic hydrolysis include Azhar and Hamdy (1981b), Sawai *et al*. (2004) and Shariffa *et al*. (2009) to mention a few. However, all of these studies were carried out on the starch from the pulp of the sweet potato. In addition, none of the reported work made use of experimental design such as Response Surface Methodology (RSM). RSM, originally described by Box and Wilson (1951), is a useful optimization tool which has been applied in research to study the effect of individual variables and their interactions on response variables. Besides the fact that RSM is a less laborious and more cost effective method for gathering research results than the classical methods, its main advantage is the ability to reduced the number of experimental runs needed to provide sufficient information for statistically acceptable results. RSM has been applied to many area of research, but only a few have reported its application to enzymatic hydrolysis of starch. These include the work of Kunamneni and Singh (2005) on maize and Vázquez *et al*. (2009) on potato (*Solanum tuberosum*).

In this work, sweet potato peel was converted into fermentable sugars in a two-step enzymatic hydrolysis. To optimize the process, RSM was applied to determine the effects of three-level-three-factor and their reciprocal interactions on the glucose released both at the liquefaction and saccharification steps.

MATERIALS AND METHODS

Sweet Potato Peel Preparation

Freshly harvested mature sweet potatoes were obtained from a local market in Ogbomoso, Oyo State, Nigeria. The sweet potatoes were washed in clean water to remove dirt and adhering latex. They were manually peeled, the weight of the peel measured and recorded followed by drying to constant weight using a plate dryer and milled to flour.

Enzymatic Studies

For this work, a two-step enzymatic hydrolysis method was adopted. In the first step (liquefaction), partially purified bacterial alpha amylase (6.4 units/ml) was used, while for the second step (saccharification), partially purified fungal glucoamylase (789.6 units/ml) was employed. The enzymes were purchased from the Federal Institute of Industrial Research Oshodi (Nigeria).

Experimental Design

For the liquefaction of the sweet potato peel starch, the Box-Behnken experimental design was employed for the optimization. A three-level-three-factor design was applied, which generated 17 experimental runs. This included 6 factorial points, 6 axial points and 5 central points to provide information regarding the interior of the experimental region, making it possible to evaluate the curvature effect. Selected hydrolysis variables for the liquefaction of the sweet potato peel starch were time (X_1), α-amylase dose (X_2) and temperature (X_3). The coded independent variables levels are depicted in Table 1, while Table 2 shows the 17 experimental runs generated in terms of coded variables, the observed glucose concentrations, the predicted glucose concentrations and the residual values. Also, for the saccharification step, the Box-Behnken experimental design was applied to generate 17 experimental runs with selected variables of time (X_1), glucoamylase dose (X_2) and temperature (X_3). The coded independent variables levels are shown in Table 3 and Table 4 displays the 17 experimental runs generated in terms of coded variables, the observed glucose concentrations, the predicted glucose concentrations and the residual values.

Two-Step Enzymatic Peel Starch Hydrolysis

For the hydrolysis investigation, the method of Betiku and Ajala (2010) was adopted. The sweet potato peel flour obtained was made into a starch slurry by adding appropriate quantity of water. In order to make a 27.5% (w/v) slurry, 10 g of flour was weighed into 20 mL of a 26 ppm $CaCl_2$ solution. The pH was adjusted to 6.5 with citrate-phosphate buffer and the solution was made up to 40 mL. The slurried starch was gelatinized by heating the mixture to 97 °C for 5 min followed by optimization studies of the liquefaction step based on Box-Behnken design as described in Table 2. The liquefied peel starch at the established optimal condition was later subjected to saccharification optimization studies as well, following the Box Behnken design in Table 3. The enzyme activities were stopped by heating the mixture to boil. The final mixtures were centrifuged at 10,000 rpm for 15 min and the supernatants were analyzed for reducing sugar.

Table 1: Factors and their levels for the Box-Behnken design (liquefaction step).

Variable	Symbol	Coded variable levels		
		-1	**0**	**+1**
Time (min)	X_1	20	40	60
α-Amylase dose % (v/v)	X_2	0.5	0.75	1
Temperature (°C)	X_3	50	60	70

Table 2: Liquefaction data for the observed glucose concentration, predicted glucose concentration and residual values.

Run	X_1 (min)	X_2 % (v/v)	X_3 (°C)	Observed value (g/L)	Predicted value (g/L)	Residual
1	-1	-1	0	63.00	63.56	-0.56
2	1	-1	0	76.42	76.28	0.14
3	-1	1	0	55.00	55.14	-0.14
4	1	1	0	125.83	125.27	0.56
5	-1	0	-1	18.22	17.57	0.65
6	1	0	-1	74.42	74.47	-0.05
7	-1	0	1	26.00	25.95	0.05
8	1	0	1	51.26	51.91	-0.65
9	0	-1	-1	43.23	43.32	-0.09
10	0	1	-1	55.45	55.96	-0.51
11	0	-1	1	29.10	28.59	0.51
12	0	1	1	56.61	56.52	0.09
13	0	0	0	28.24	28.54	-0.30
14	0	0	0	29.00	28.54	0.46
15	0	0	0	28.24	28.54	-0.30
16	0	0	0	28.24	28.54	-0.30
17	0	0	0	29.00	28.54	0.46

Table 3: Factors and their levels for the Box-Behnken design (saccharification step).

Variable	Symbol	Coded variable levels		
		-1	**0**	**+1**
Time (min)	X_1	10	35	60
Glucoamylase dose % (v/v)	X_2	0.5	0.75	1
Temperature (°C)	X_3	40	50	60

Reducing Sugar Assay

The DNS method described by Miller (1959) and modified DNS Reagent were used to determine the reducing sugar produced, which was expressed as glucose in this work. To 1 ml of the supernatant, 3 ml of the DNS solution was added to the test tube and was boiled for 15 min, cooled and diluted appropriately after which their absorbance were measured at a wavelength of 540 nm using a UV-Visible Spectrophotometer (Libra 21 Model). Dextrose equivalent (DE) was calculated as follows:

$$DE = \left(\frac{\text{Reducing sugar expressed as glucose}}{\text{Sample dry weight}} \right) \times 100 \qquad (1)$$

Statistical Data Analysis

The data obtained from both the liquefaction and saccharification steps were analyzed statistically using response surface methodology, in order to fit the second-order mathematical model generated by the Design-Expert software version 8.0.3.1 (Stat-Ease Inc., Minneapolis, USA). To correlate the response variable to the independent variables, multiple regression 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). The fitted second-order mathematical model is described in Eq. (2).

$$Y = b_0 + \sum_{i=1}^{k} b_i X_i + \sum_{i=1}^{k} b_{ii} X_i^2 + \sum_{i<j}^{k} b_{ij} X_i X_j + e \qquad (2)$$

where Y is the response factor (glucose concentration), b_o is the intercept value, b_i (i = 1, 2, …, k) is the first order model coefficient, b_{ij} is the interaction effect, and b_{ii} represents the quadratic coefficients of X_i, and e is the random error.

RESULTS AND DISCUSSION

Optimization of the Liquefaction Step of Sweet Potato Peel Starch Hydrolysis

In order to find a useful means of disposing of sweet potato peel, optimization of the two-step enzymatic hydrolysis of the peel to glucose-rich syrup, needed by food and pharmaceutical industries, was carried out. The glucose-rich syrup obtained could also serve as an alternative carbon source for cultivation of many microorganisms for production of such products as citric acid, gluconic acid, oxalic acid, and ethanol, among others. Results of the liquefaction step as designed by Box Behnken are presented in Table 3, which contained the coded factors considered in this study with observed values, predicted values as well as the residual values obtained.

Design Expert 8.0.3.1 software (Trial version) was employed to evaluate and determine the coefficients of the full regression model equation and their statistical significance. Table 5 shows the results of test of significance for every regression coefficient. The results revealed that the p-values of the model terms were significant, i.e., $p < 0.05$. In this case, the three linear terms (X_1, X_2, X_3), three cross-products (X_1X_2, X_1X_3, X_2X_3) and the three quadratic terms (X_1^2, X_2^2 and X_3^2) were all markedly significant model terms at 95% confidence level. Table 6 shows the analysis of variance of the regression equation model.

Table 4: Saccharification data for the observed glucose concentration, predicted glucose concentration and residual values.

Run	X_1 (min)	X_2 %(v/v)	X_3 (°C)	Observed value (g/L)	Predicted value (g/L)	Residual
1	-1	-1	0	130.51	132.89	-2.38
2	1	-1	0	132.29	131.78	0.51
3	-1	1	0	102.43	102.94	-0.51
4	1	1	0	174.36	171.98	2.38
5	-1	0	-1	101.85	99.54	2.31
6	1	0	-1	141.00	141.58	-0.58
7	-1	0	1	69.23	68.65	0.58
8	1	0	1	92.21	94.52	-2.31
9	0	-1	-1	134.00	133.93	0.07
10	0	1	-1	140.10	141.90	-1.80
11	0	-1	1	99.60	97.80	1.80
12	0	1	1	100.00	100.07	-0.07
13	0	0	0	131.46	133.09	-1.63
14	0	0	0	135.48	133.09	2.39
15	0	0	0	133.67	133.09	0.58
16	0	0	0	132.29	133.09	-0.08
17	0	0	0	132.53	133.09	-0.56

Table 5: Test of significance for every regression coefficient for the liquefaction step.

Source	Sum of squares	df	Mean square	F-Value	p-Value
X_1	3432.48	1	3432.48	8753.30	<0.0001
X_2	822.96	1	822.96	2098.67	<0.0001
X_3	100.47	1	100.47	256.20	<0.0001
$X_1 X_2$	823.98	1	823.98	2101.26	<0.0001
$X_1 X_3$	239.32	1	239.32	610.30	<0.0001
$X_2 X_3$	58.45	1	58.45	149.05	<0.0001
X_1^2	2414.77	1	2414.77	6157.99	<0.0001
X_2^2	3200.56	1	3200.56	8161.88	<0.0001
X_3^2	422.49	1	422.49	1077.40	<0.0001

Table 6: Analysis of variance (ANOVA) of the regression equation for the liquefaction step.

Source	Sum of squares	df	Mean Square	F-value	p-Value
Model	11618.83	9	1290.98	3292.19	<0.0001
Residual	2.74	7	0.39		
Lack of Fit	2.05	3	0.68	3.95	0.109
Pure Error	0.69	4	0.17		
Cor Total	11621.57	16			
			R^2=99.98%,	R^2 (adj) = 99.95%	

The model Fisher *F-test* of 3292.19 with low probability value [(p_{model} > F) = 0.0001] demonstrated a high significance for the regression model (Kunamneni and Singh, 2005). The goodness of fit was checked by the coefficient of determination (R^2). Guan and Yao (2008) reported that R^2 should be at least 0.80 for the good fit of a model. For the liquefaction step, the R^2 was 99.98% while the adjusted R^2 was found to be 99.95% and all p-value coefficients were less than 0.0001, which implied that the model proved suitable for the adequate representation of the actual relationship between the selected variables (Vázquez *et al.*, 2009). These values indicated that the regression is statistically significant; only 0.02% of the total variation was not explained by this regression model. Furthermore, the value of the R^2 obtained in this work showed a high consistency between the observed values and the predicted values. The lack-of-fit term of 0.109 was not significant relative to the pure error (Vázquez *et al.*, 2009). In this case, a non-significant lack of fit

is good (Vázquez *et al.*, 2009). Therefore, it could be used for theoretical prediction of the liquefaction of sweet potato peel starch. The final regression equation in terms of coded factors for the Box-Behnken response surface quadratic model for the liquefaction step is described in Eq. (3).

$$Y = 28.54 + 20.71X_1 + 10.14X_2 - 3.54X_3$$
$$+ 14.35X_1X_2 - 7.74X_1X_3 + 3.82X_2X_3 \qquad (3)$$
$$+ 23.95X_1^2 + 27.57X_2^2 - 10.02X_3^2$$

where Y = reducing sugar produced (expressed as glucose) in g/L.

The low values of standard error observed in the intercept and all the model terms showed that the regression model fits the data well and the prediction was good (Table 7). The variance inflation factor (VIF) obtained in this work revealed that the center points are orthogonal to all other factors in the model (Table 7).

Table 7: Regression coefficients and significance of response surface quadratic for the liquefaction step.

Factor	Coefficient estimate	df	Standard error	95%CI low	95%CI high	VIF
Intercept	28.54	1	0.28	27.88	29.21	-
X_1	20.71	1	0.22	20.19	21.24	1.00
X_2	10.14	1	0.22	9.62	10.67	1.00
X_3	-3.54	1	0.22	-4.07	-3.02	1.00
X_1X_2	14.35	1	0.31	13.61	15.09	1.00
X_1X_3	-7.74	1	0.31	-8.48	-6.99	1.00
X_2X_3	3.82	1	0.31	3.08	4.56	1.00
X_1^2	23.95	1	0.31	23.23	24.67	1.01
X_2^2	27.57	1	0.31	26.85	28.29	1.01
X_3^2	-10.02	1	0.31	-10.74	-9.30	1.01

The graphical representations of the regression equation for the optimization of the liquefaction step are displayed as three dimensional (3-D) surfaces in Figure 1(a)-(c). Figure 1a shows the response surface plot representing the effect of time, α-amylase dose and their reciprocal interaction on hydrolysis yield while keeping temperature constant. The results showed that a linear relationship exists between both time and α-amylase dose. As both the time and α-amylase dose increase, the glucose concentration also increases. The curvature nature of the surface plot in Figure 1a indicates a mutual interaction between α-amylase and time (Yuan *et al.*, 2008). The response surface plot representing the effect of temperature, time and their reciprocal interaction on glucose concentration while keeping α-amylase constant is presented in Figure 1(b). The results showed that increasing hydrolysis temperature does not favour glucose accumulation. Figure 1c describes the response surface plot representing the effect of

temperature, α-amylase dose and their reciprocal interaction on glucose concentration while hydrolysis time was held constant. The results revealed that high temperature and high α-amylase dose favour glucose accumulation.

The optimal values of the independent variables selected for the liquefaction step were obtained by solving the regression equation (Eq. (3)) using the Design-Expert software. The optimal condition for this step was estimated as $X_1 = 60$ min, $X_2 = 1\%$ (v/v) and $X_3 = 56.4$ °C. The predicted glucose concentration under the above condition was $Y = 126.66$ g/L. To verify the prediction of the model, the optimal condition was applied to three independent replicates and the average glucose concentration obtained was 126.03 g/L (dextrose equivalent of 50.4), which is well within the estimated value of the model equation. Valetudie *et al.* (1993) reported dextrose equivalent of 25 when bacterial α-amylase was used for 24 h to hydrolyze sweet potato starch.
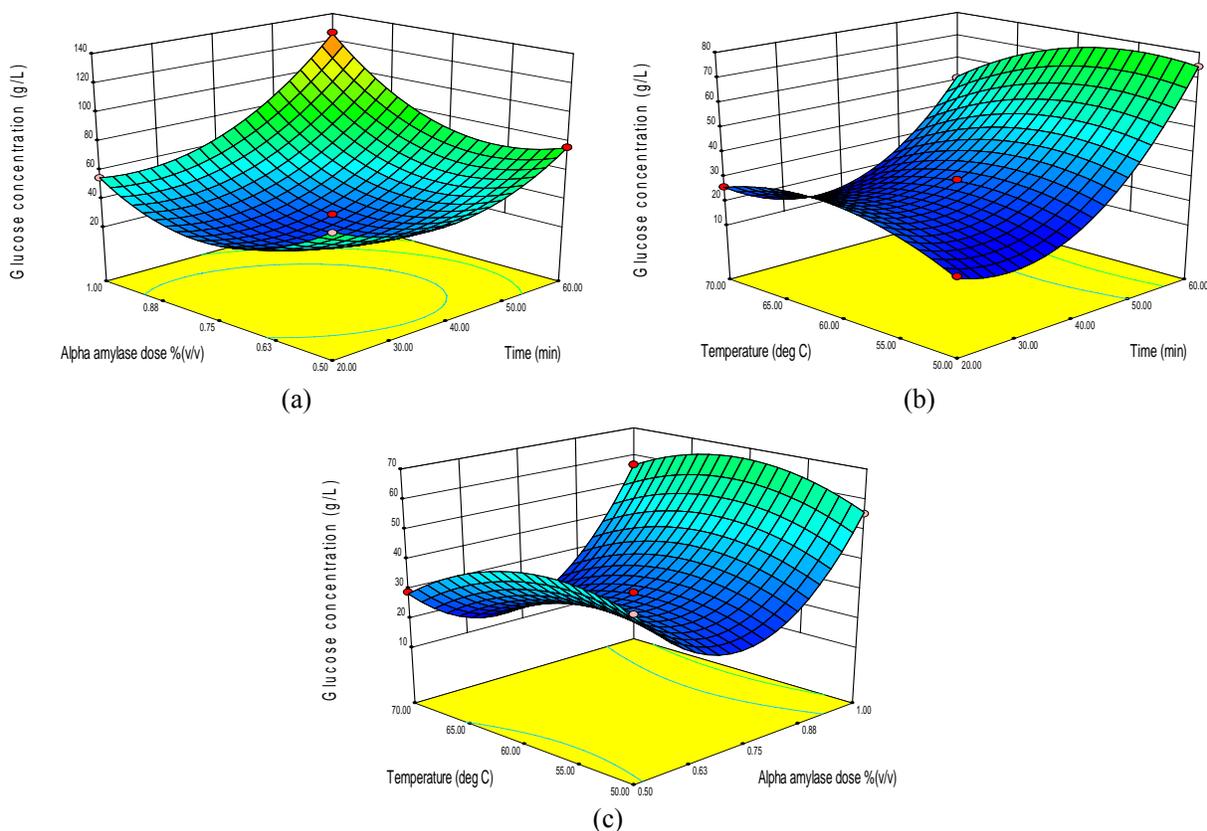

(a)

(b)

(c)

Figure 1(a)-(c): Surface plots for liquefaction of sweet potato peel.
(a) effect of α-amylase dose, time and their reciprocal interaction on glucose concentration.
(b) effect of temperature, time and their reciprocal interaction on glucose concentration.
(c) effect of temperature, α-amylase dose and their reciprocal interaction on glucose concentration.

Optimization of the Saccharification Step of Sweet Potato Peel Starch Hydrolysis

A saccharification step was required because a significant amount of starch was left unhydrolyzed at the liquefaction stage. Results of the optimization of the saccharification step are displayed in Table 4, which contains the coded factors together with observed values, predicted values as well as the residual values. Table 8 gives the results of the test of significance for every regression coefficient. It was observed that the p-values of the model terms were remarkably significant except for the cross-product of X_2X_3, which was not significant at 95% confidence level. Results of the analysis of variance of the regression equation model are shown in Table 9.

The model Fisher *F-test* of 193.04 with a low probability value [$(p_{model} > F) = 0.0001$] indicated a high significance for the regression model (Kunamneni and Singh, 2005). It exhibited a low standard deviation and very high R^2 values. The R^2 was 99.90%, the adjusted R^2 was found to be 99.61% and all *p*-value coefficients were significant except that of X_2X_3 (Table 8). This suggested that the model was suitable for the representation of the actual relationship between the selected factors (Vázquez *et al*., 2009). These values showed that this regression was statistically significant; only 0.1% of the total

variation was not explained by this regression model. In addition, the value of the R^2 obtained in this saccharification step implied a high consistency between the observed values and the predicted values. The lack-of-fit term of 0.103 was not significant relative to the pure error (Vázquez *et al*., 2009). In this case, a non-significant lack of fit is good (Vázquez *et al*., 2009). Hence, it could be used for theoretical prediction of the saccharification of liquefied sweet potato peel starch. The final regression equation in terms of coded factors for the Box-Behnken response surface quadratic model for the saccharification step is expressed in Eq. (4).

$$Y = 133.09 + 16.98X_1 + 2.56X_2 - 19.49X_3$$
$$+ 17.54X_1X_2 - 4.04X_1X_3 - 1.42X_2X_3 \quad (4)$$
$$- 7.77X_1^2 + 9.58X_2^2 - 24.24X_3^2$$

where Y = reducing sugar produced (expressed as glucose) in g/L.

The low values of the standard error of the intercept and all the model terms revealed that the regression model fits the data well and the prediction was good (Table 10). The variance inflation factor (VIF) obtained in this work revealed that the center points are orthogonal to all other factors in the model (Table 10).

Table 8: Test of significance for every regression coefficient for the saccharification step.

Source	Sum of squares	df	Mean square	F-Value	p-Value
X_1	2306.56	1	2306.56	409.77	<0.0001
X_2	52.48	1	52.48	9.32	<0.0001
X_3	3038.49	1	3038.49	539.81	<0.0001
$X_1 X_2$	1230.26	1	1230.26	218.56	<0.0001
$X_1 X_3$	65.37	1	65.37	11.61	<0.0113
$X_2 X_3$	8.12	1	8.12	1.44	0.2687
X_1^2	254.23	1	254.23	45.17	0.0003
X_2^2	386.59	1	386.59	68.68	<0.0001
X_3^2	2474.62	1	2474.62	439.63	<0.0001

**Table 9: Analysis of variance (ANOVA) of regression equation for the saccharification step.**

Source	Sum of squares	df	Mean square	F-Value	p-Value
Model	9779.35	9	1086.59	193.04	<0.0001
Residual	39.40	7	5.63		
Lack of Fit	29.74	3	9.91	4.11	0.103
Pure Error	9.66	4	2.41		
Cor Total	9818.75	16			
			$R^2 = 99.90\%$,	R^2 (adj) = 99.61%	

Table 10: Regression coefficients and significance of the response surface quadratic for the saccharification step.

Factor	Coefficient estimate	df	Standard error	95%CI low	95%CI high	VIF
Intercept	133.09	1	1.06	130.58	135.59	-
X_1	16.98	1	0.84	15.00	18.96	1.00
X_2	2.56	1	0.84	0.58	4.54	1.00
X_3	-19.49	1	0.84	-21.47	-17.51	1.00
X_1X_2	17.54	1	1.19	14.73	20.34	1.00
X_1X_3	-4.04	1	1.19	-6.85	-1.24	1.00
X_2X_3	-1.42	1	1.19	-4.23	1.38	1.00
X_1^2	-7.77	1	1.16	-10.50	-5.04	1.01
X_2^2	9.58	1	1.16	6.85	12.32	1.01
X_3^2	-24.24	1	1.16	-26.98	-21.51	1.01

The graphical representations of the regression equation for the optimization of the saccharification step are displayed as three dimensional (3-D) surfaces in Figure 2(a)-(c). Figure 2(a) depicts the response surface plot representing the effect of time, glucoamylase dose and their reciprocal interaction on

glucose concentration while keeping temperature constant. It was observed that, as both the time and glucoamylase dose increase, the glucose concentration also increases, suggesting a linear relationship between the two variables. High glucoamylase dose and low saccharification time or vice versa did not

(a)

(b)

(c)
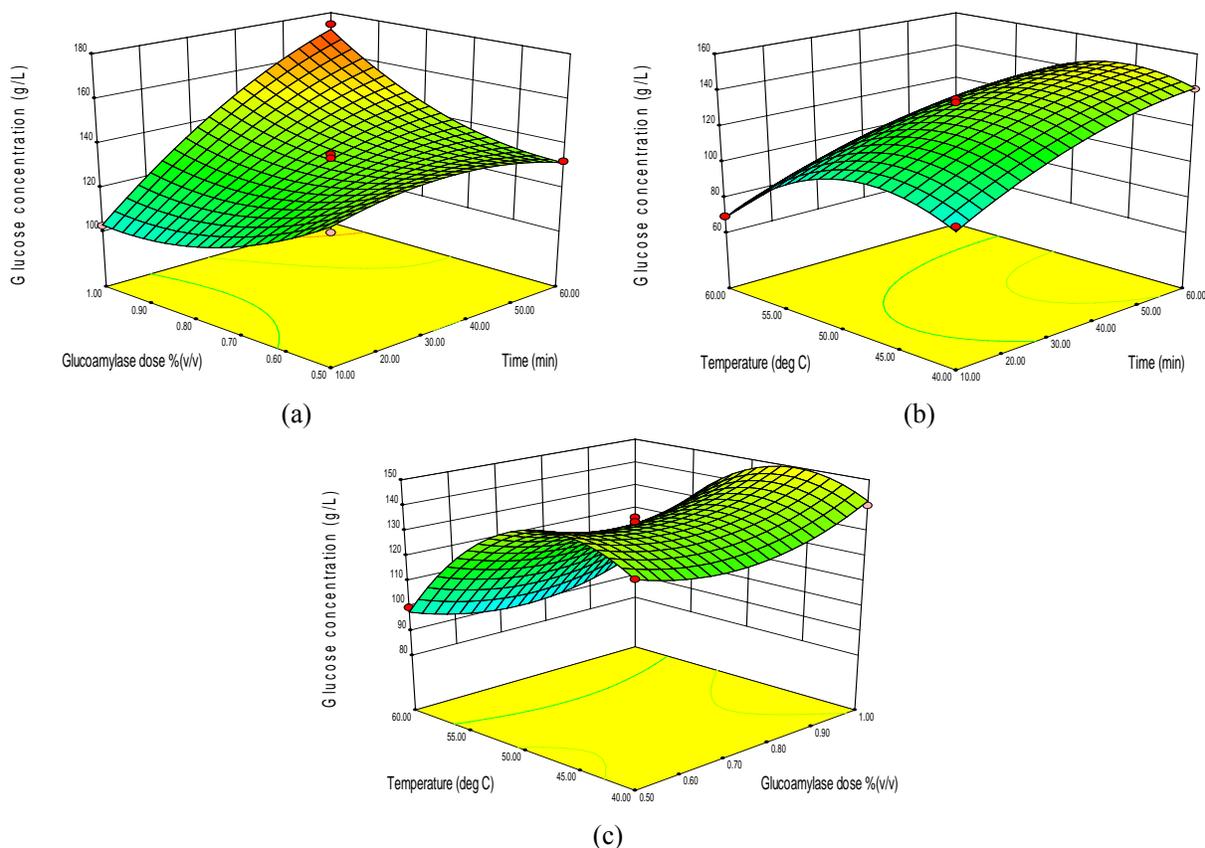
Figure 2(a)-(c): Surface plots for saccharification of sweet potato peel.
(a) effect of glucoamylase dose, time and their reciprocal interaction on glucose concentration.
(b) effect of temperature, time and their reciprocal interaction on glucose concentration.
(c) effect of temperature, glucoamylase dose and their reciprocal interaction on glucose concentration.

favour glucose concentration. The response surface plot representing the effect of temperature, time and their reciprocal interaction on glucose concentration while keeping glucoamylase dose constant are shown in Figure 2(b). Figure 2(c) displays the response surface plot representing the effect of temperature, glucoamylase dose and their reciprocal interaction on glucose concentration when hydrolysis time was held constant. The curvatures of the three dimensional surfaces in Figure 2(b) and (c) indicated mutual interactions between temperature and saccharification time and between temperature and glucoamylase dose (Yuan *et al.*, 2008).

The optimal values of the independent variables selected for the saccharification step were also obtained by solving the regression equation (Eq. (4)) using the Design-Expert software. The optimal condition for this step was established as $X_1 = 60$ min, $X_2 = 1\%$ (v/v) and $X_3 = 45$ °C. The predicted glucose concentration under the above set of conditions was $Y = 178.39$ g/L. To verify the prediction of the model, the optimal condition was applied to three independent replicates and the average glucose concentration obtained was 176.89 g/L (dextrose equivalent of 70.4), which is well within the predicted value of the model equation. The results of this work have shown that response surface methodology could be used to optimize the two-step enzymatic hydrolysis of sweet potato peel starch. Kim and Hamdy (1985) achieved a dextrose equivalent of 84.2 using acid hydrolysis of sweet potato starch. The results obtained in this study for sweet potato peel hydrolysis are therefore promising.

CONCLUSIONS

Response surface methodology was successfully applied to the two-step enzymatic hydrolysis of sweet potato peel. The maximum glucose concentration obtained for the liquefaction step was 126.66 g/L at time 60 min, α-amylase dose 1% (v/v) and temperature 56.4 °C, while the glucose concentration increased to 178.39 g/L at time 60 min, glucoamylase dose 1% (v/v) and temperature 45 °C. The optimized liquefaction and saccharification conditions were validated with actual glucose concentrations of 126.03 and 176.89 g/L, respectively. Hence, the glucose syrup obtained in this work may serve as the sole carbon source for feeding many microorganisms and subsequently converted to useful products like citric acid, gluconic acid, oxalic acid and ethanol, among others.

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