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Two-Step Enzymatic Hydrolysis Optimization of Citric Acid Production: *Musa paradisiacal* Peels

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Abstract In this work, a two-step enzymatic hydrolysis of ripe *Musa paradisiaca* peels was optimized. The effects of time, temperature and pH on glucose concentration were examined. Box-behnken experimental design was adopted and a total of seventeen (17) runs were generated for both liquefaction and saccharification steps. For the liquefaction step, an ANOVA test showed the quadratic model achieved to be significant ($p < 0.05$). The statistical model projected the maximum glucose concentration of 8.421 (v/v) at a temperature of 50 °C, pH of 5.5 and time of 52 min, respectively. The predicted yield was validated in triplicate and an average optimum yield of 8.322 (v/v) was achieved. A quadratic model was also achieved for the saccharification step and the model was also significant ($p < 0.05$). The statistical model for the second step (saccharification),projected the maximum glucose concentration of 13.2994 (v/v)at a temperature of 40°C, pH of 5.5 and time of 20 min, respectively. The predicted yield was also validated in triplicate and an average optimum yield of 12.89 (v/v) was achieved. A statistical model predicted the highest citric acid concentration of 19.13 (g/l), at the following optimized conditions: DAHP of 3.5 (g/l), PHDP of 2.5 (g/l) and Time of 8 days. The predicted yield was also validated in triplicate and an average optimum yield of 19.02 (g/l) was achieved.

Keywords liquefaction, hydrolysis, saccharification, optimization, plantain peels, statistical analysis

1. Introduction

Plantain belongs to the genus *Musa* of the family *musaceae.* It is an evergreen herbaceous tropical plant that can be considered a giant herb. The external-trunk is a pseudostem formed by the concentric assemblement of leaf sheaths; the true stem which forms the complex inflorescence (fruit bearing part) is a subterranean organ which extends upward at the core of the pseudostem [1]. All edible plantain cultivars are derived from two wild species, *M. acuminate* and *M. balbisiana*. These wild species are classified on the basis of the proportion of the genetic constitution contributed by each parental source. Plantain is a staple crop and an important dietary source of carbohydrate in Nigeria and in the humid tropical zones of Africa, Asia and South America [2]. Plantain is rich in vitamins A, C and B group as well as minerals such as calcium and iron [2-3]. Plantain provides between 9% and 35% of the total calories in the diets of more approximately 14 million people in Sub Sahara Africa. Therefore, plantain is grown by many farmers for consumption and as a source of income. According to Baiyeri and Ajayi [4],

plantain is a starchy food consumed by about 70 million people in different parts of the world in different ways. It can be fried, baked, boiled, roasted; eaten alone or with other food like rice, beans, pap and so on. Plantain is considered a delicacy which is well accepted and enjoyed by many at meal time, including children and adults. Plantains are not only most economic source of dietary energy in terms of cost per area cultivated or weight harvested but also a useful source of carotene, vitamin A, potassium and Iron which are essential for healthy living [5]. Plantain flour is used in bakery industries due to the ban on wheat importation by Federal Government of Nigeria [6]. Plantain is an excellent food for young children and elderly people due to its easy digestibility, and lot of medicinal values. It is used in treatment of ulcer, diarrhoea, throat infection, asthma, low libido in men. Pectin also known as plantain fibre has been found effective in treatment of colon cancer and the plantain flour is a medically recommended diet for diabetic patients [7].

However, in most industries, restaurants and homes where plantain is eaten or treated into more value added products, the peel is mainly treated as a waste product and poses disposal problems. Hence, to solve the problem of disposal so as to help keep the surroundings clean and also find a cheap substitute carbon source for cultivation of many microorganisms, this peel can be hydrolysed to fermentable sugars, which can be converted to valuable products like citric acid, gluconic acid, oxalic acid, and ethanol, among others.

Citric acid among the entire product that can be derived from plantain peel is an essential multifunctional weak organic acid, which has wide range of household and industrial applications. The global market for Citric Acid is expanding every year at the rate of 5% per year, and its current production is about 1.7 million tons per year as estimated by Business Communications Co. The search for economical substrates as an alternative to high cost substrates is vital to reduce the production cost of Citric Acid. Meanwhile, interest has been placed on the use of agricultural and industrial wastes for Citric Acid production. In a search for amplified production of citric acid due to its significance to the food and pharmaceutical industries, emphasis is now moved to its production from cheap and readily available raw materials.

Therefore, this research work aim at production of citric acid from ripe plantain peels (RPP) through a two-step enzymatic hydrolysis. To determine the predicted yields, compare the error values, analyse the various variable factors responsible foroptimum yield and increasing the process efficiency in the two stepsenzymatic hydrolysis, response surface methodology was adopted.

2. Materials and Methods

2.1 Materials

2.1.1 Ripe plantain peels starch preparation

Ripe plantain peels were obtained from Port Harcourt, Rivers State, Nigeria. The peels were washed in clean water, chopped to smaller sizes and oven dried at 60 ^{o}C for 6 h. The dried peels were grind manually to make flour, sieved and the fine starch powder was collected and stored for further used. Alpha-amylase (E.C.3.2.1.1) from bacterium source (*Bacillus licheniformis*) and *glucoamylase* (E.C.3.2.1.3) from *Aspergillus niger* used in this study were both obtained from Champion Breweries, UyoAkwaIbom State, Nigeria. All chemicals used were of analytical grade and needed no further purification.

2.2 Methods

2.2.1 Experimental design and statistical analysis

To design the experiment for the two steps enzymatic hydrolysis of plantain peel powder, three factors were considered: X_1, temperature; X_2, time; and X_3, pH (Table 1). For citric acid production, selected factors such as DAHP: X_1, PHDP: X_2,and Time: X_3were chosen (Table 2). Since 2^3 full factorial design and central composite design result in 20 experimental runs [8]. In order to reduce the number of experimental runs, Box-Behnken Design (BBD) was employed which generated 17 experimental runs used to study the effects of selected factors on the yields. For the coefficient of the quadratic model of the response fitting, multiple regressions model was adopted using Statistical software 10 version 15.5 (Stat Inc., Tulsa, OK, USA). Regression analysis and test of significance

are the computational intensive process that is best carried out via statistical software; hence the quality of the fitted of the model was evaluated using test of significance and regression analysis of variance (ANOVA) via equation 1.

Table 1: Variable factors and their levels for Liquefaction and Saccharification Stage

Variables	Symbols	Coded variables level		
		Liquefaction		
		-1	0	1
Temperature (^{o}C)	X_1	50	60	70
Time (min)	X_2	50	55	60
pH	X_3	5.5	6.0	6.5
		Saccharification		
Temperature (^{o}C)	X_1	40	50	60
Time (min)	X_2	20	40	60
pH	X_3	5.5	6.0	6.5

$$\beta_F = \emptyset_0 + \sum_{i=1}^{k} \emptyset_i Y_i + \sum_{i=1}^{k} \emptyset_{ii} Y_i^2 + \sum_{i<j}^{k} \emptyset_{ij} Y_i Y_j + \varepsilon \quad (1)$$

Where, β_F is the response, $\emptyset_0$ is the intercept term, $\emptyset_i, \emptyset_{ii}, \emptyset_{ij}$ are the coefficient terms for linear (Y_i), quadratic (Y_i^2) and interaction ($Y_i Y_j$), X_i is the selected factors, i= 1, 2, 3,ε is the random error.

2.2.2. Model checking and evaluation

The important part of any regression analysis is to determine whether or not the standard assumptions of the simple linear regression model are satisfied [8]. The interactive relationship between the yields (reducing sugar/citric acid conc.) and variable factors (X_1, X_2, X_3)are assumed to be in form equation 1. To estimate the model precision and model estimation capabilities, the coefficient of determination (R^2) and adjusted coefficient of determination ($\bar{R}^2$) were determined by estimating these parameters using Eqns. (2) and (3) to compute $R^2$and$\bar{R}^2$, respectively.

$$R^2 = 1 - \sum_{i=1}^{n} \frac{(\rho_{i,cal} - \rho_{i,exp})^2}{(\rho_{avg,exp} - \rho_{i,exp})^2} \quad (2)$$

$$\bar{R}^2 = 1 - (1 - R^2)\left[\frac{n-1}{n-(k+1)}\right] \quad (3)$$

where n is the number of observed data/sample size, $\rho_{i,exp}$ is the experimental value, $\rho_{i,cal}$ is the calculated value and $\rho_{avg,exp}$ is the average experimental value, k is the number of independent variables in the regression analysis.

2.2.3. Two-step enzymatic starch hydrolysis

The method adopted by Betiku *et al*. [9] was used with little modification. In the first step (liquefactionstep),the fine starch powder was made into slurry by adding appropriate quantity of distilled water to make 25 % (w/v) slurry. A solution of 40 ppm Ca^{2+} was used to make the slurry for the stability of the enzymes. The pH of the slurry was tested, and adjusted to a known pH using citrate-phosphate buffer. To gelatinize the solution, the mixture was heated to97^{o}Cfor 10 min, after which 1% (v/v) of α - amylase was added for liquefaction to take place, and the temperature was reduced toa known temperature for duration of a known time for enzyme activity. The enzyme activity was stopped by increasing the temperature to 97^{o}C for another 10 min. The final mixture was then centrifuged at 10000 rpm for 10 min and the supernatant obtained after centrifugation was analyzed for reducing sugar concentration. This experiment was repeated at varying temperature, reaction time and pH as design by response surface design software.

For the second step (saccharification step), the liquefied starch at the established optimum conditions was later subjected to saccharification optimization studies as designed in Table 1. The pH of the mixtures was adjusted to a known pH with citrate-phosphate buffer. Gelatinization was done by heating the mixture to a temperature of 97^{o}C for 10 min, 1% (v/v) of gluco-amylase was added for saccharification to take place and the temperature was

maintained at known temperature for 10 min. The enzyme activity was stopped by heating the mixture to 97^{o}C for 10 min. The final mixture was then centrifuged at 10000 rpm for 10 min and the supernatant was analyzed to get the reducing sugar concentration. This experiment was repeated at varying temperature, reaction time and pH as design by response surface design software.

2.2.3.1 Determination of reducing sugar

To determine the yield of the reducing sugar, Brix method was adopted and equation 3 was used to estimate the yield of sugar obtained. After which, the volume of a reference amount of the sample was divided by the total volume of the sample solution. The result obtained was multiplied by the weight of the reference sample volume.

The Brix value (ω) obtained was divided by the total volume of the sample and multiplied by the total volume of sugar solution gotten.

$$\omega = 261.3\left(1 - \frac{1}{\text{א}}\right) \quad (4)$$

whereא is the specific gravity.

2.2.4. Citric acid production

2.2.4.1 Medium preparation for citric acid production

The method used by Sankpal *et al*. [10] was adopted for the medium preparation with little modification. The fermentation medium used for this study weremade of carbon source(ripe plantain peel hydrolysis: RPPH), di-ammonium hydrogen phosphate $(NH_4)_2HPO_4$, (DAHP), potassium dihydrogen phosphate (KH_2PO_4), (PDHP) and $MgSO_4.7H_2O$ (0.10 g/l). All media and flasks were sterilized using an autoclave at 121 ^{o}C for 15 min to avoid contamination.

2.2.4.2 Submerged fermentation studies for citric acid production

Seventeen (17) 250 ml Erlenmeyer flasks containing the medium were autoclaved at 121 ^{o}C for 15 min and then allowed to cool. The pH was adjusted using 2 M sodium hydroxide/1 N hydrogen chloride solutions to make the inoculum. The flasks were then covered with cotton plug, placed on an incubator shaker operated at 200 rpm for proper fermentation to take place.

2.2.4.3Analysis of citric acid concentration

To analysis the citric acid concentration, pyridine-acetic anhydride spectrophotometric method was used [11]. 1.3 ml of pyridine was added to 1 ml of filtered sample and then shakes briskly for homogeneous mixture. 5.7 ml of acetic anhydride was added to the mixture and was immediately heated to a temperature of 32 ^{o}C for 30 min in water bath. The resulting mixture was standardized with a known citric acid-water mixture and the absorbance of the mixture was read at 420 nm against the blank solution before the estimation of citric acid concentration. These procedures were repeated at different DAHP (X_1), PDHP (X_2) and time (X_3) (Table 2).

Table 2: Variable factors and their levels for citric acid concentration

Variables	**Symbols**	**Coded variables level**		
		-1	0	1
DAHP (g/l)	X_1	1.0	3.5	6.0
PDHP (g/l)	X_2	1.0	2.5	4.0
Time (days)	X_3	1	8	15

3. Results and Discussion

3.1 Optimization of liquefaction step RPPH

Displayed in Table 3aare the actual variables and the reducing sugar yield. Observation from the table showed that the highest reducing sugar obtained to be 8.2421%(v/v) at X_1 = 50^{o}C, X_2 = 50 min and X_3 = 6.0. However, statistical analysis predicted 8.3560% (w/w) at X_1 = 42 ^{o}C, X_2 = 50 min and X_3 = 5.8.

Table 3a: Experimental Data for Reducing Sugar from Liquefaction

Std	Temp (°C)	Time (min)	pH	Reducing sugar (v/v)
1	50	50	6	8.2421
2	70	50	6	4.5342
3	50	60	6	6.4905
4	70	60	6	5.1937
5	50	55	5.5	7.0947
6	70	55	5.5	7.3838
7	50	55	6.5	7.1332
8	70	55	6.5	5.9926
9	60	50	5.5	4.1369
10	60	60	5.5	6.7613
11	60	50	6.5	5.7222
12	60	60	6.5	5.7286
13	60	55	6	7.3036
14	60	55	6	7.3989
15	60	55	6	7.4222
16	60	55	6	6.2844
17	60	55	6	7.3036

The predicted yield was validated in triplicates, an average of yield of reducing sugar of8.2143% (v/v) was obtained, and this value is well within the range predicted by the model. Table 3b shows the results of the ANOVA test of significance for every regression coefficient. Observation from the table showed some $p < 0.05$ with significant F-value when tested using Fischer's and null-hypothesis tests. Since a higher F-value implies a good fit of model [8].

The use of R^2 to measure how much variability in the observed response value can be explained by the experimental factors and their interactions was adopted. The R^2 value must be between 0 and 100% for significant model time, however, a good fit of the model is better with 80% R^2. The results in Table 3c indicated an R^2 value of 82.50% for a good fit model.

The mathematical relationship between the response and the factors are expressed in equation 5. All negative signs reduce the response variable while the positive signs add increased the response. The final equation in terms of coded factors for the Box-Behnken design model is expressed as:

$$Y = 7.14 - 0.73X_1 + 0.19X_2 - 0.10X_3 + 0.60X_1X_2 - 0.36X_1X_3 - 0.65X_2X_3 + 0.14X_1^2 - 1.17X_2^2 - 0.38X_3^2 \quad (5)$$

Table 3b: Test of Significance for Every Regression Coefficient and ANOVA

Source	Sum of Squares	df	Mean Square	F Value	P-value
X1	4.290	1	4.290	5.69	0.0485
X2	0.300	1	0.300	0.39	0.5507
X3	0.080	1	0.080	0.11	0.7540
X1X2	1.450	1	1.450	1.93	0.2075
X1X3	0.510	1	0.510	0.68	0.4374
X2X3	1.710	1	1.710	2.27	0.1753
X12	0.086	1	0.086	0.11	0.7449
X22	5.770	1	5.770	7.66	0.0278
X32	0.620	1	0.620	0.83	0.3934
Model	14.97	9	1.66	2.21	0.1546
Residual	5.27	7	0.75		
Lack of Fit	4.34	3	1.45	6.21	0.0550
Pure Error	0.93	4	0.23		
Cor Total	20.24	16			

The interactive effects of variables are best explained by plotting the surface and 3-dimensionalplots of the variables against the response. The surface plots representing pH and time produced a perfect interaction than that produced between time and temperature or pH and time. This showed that pH and time are the most significant factors at the liquefaction stage (Fig. 1).

Table 3c: Regression coefficients and significant of Response Surface Quadratics

Factor	Coefficient Estimate	df	Standard Error	95% CI Low	95% CI High	VIF
Intercept	7.14	1	0.39	6.22	8.06	
X_1	-0.73	1	0.31	-1.46	-6.314E-003	1.00
X_2	0.19	1	0.31	-0.53	0.92	1.00
X_3	-0.10	1	0.31	-0.83	0.63	1.00
X_1X_2	0.60	1	0.43	-0.42	1.63	1.00
X_1X_3	-0.36	1	0.43	-1.38	0.67	1.00
X_2X_3	-0.65	1	0.43	-1.68	0.37	1.00
X_1^2	0.14	1	0.42	-0.86	1.14	1.01
X_2^2	-1.17	1	0.42	-2.17	-0.17	1.01
X_3^2	-0.38	1	0.42	-1.38	0.62	1.01
R^2 = 92.50%						

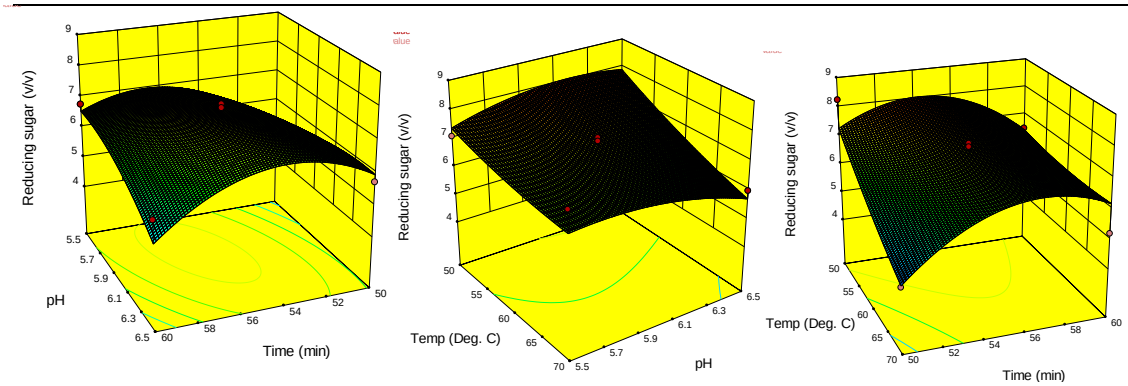

Figure 1: Surface plots for liquefaction step of ripe plantain peels

3.2. Optimization of Saccharification step of Plantain Peels hydrolysis

In this study, the optimum condition obtainedfor liquefaction stage was employed to carryout the reducing sugar content of the hydrolysate. Seventeen experimental runs were also generated using three variable factors namely: temperature (X_1), pH (X_2) and time (X_3).

Table 4a shows the results of the ANOVA test of significance for every regression coefficient. Observation from the table showed some $p < 0.05$ with significant F-value when tested using Fischer's and null-hypothesis tests. Since a higher F-value implies a good fit of model [8].

The use of R^2 to measure how much variability in the observed response value can be explained by the experimental factors and their interactions was adopted. The R^2 value must be between 0 and 100% for significant model time, however, a good fit of the model is better with 80%R^2. The results in Table 4b indicated an R^2 value of 98.70% for a good fit model.

The mathematical relationship between the response and the factors are expressed in equation 6. All negative signs reduce the response variable while the positive signs add increased the response. The final equation in terms of coded factors for the Box-Behnken design model is expressed as (Table 4c):

$$Y = 4.72 + 0.15x_1 - 1.06x_2 - 0.34x_3 + 1.04x_1x_2 - 0.27x_1x_3 + 3.91x_2x_3 - 1.20x_1^2 + 3.97x_2^2 - 0.11x_3^2 \quad (6)$$

Observation from the surface plots representing the interaction between the pH and time also produced the perfect interaction among the selected variables, this showed that pH and time are the most significant factors at the saccharification stage. The surface plots of interactions between the pH and temperature as well as temperature and time produced the low sugar produced (Fig. 2). A statistical model predicted that the highest reducing sugar concentration would be 13.2994 (v/v), at the following optimized conditions: temperature of 40°C, pH of 5.5 and time of 20 minutes. The results of this study demonstrated that RSM with appropriate experimental design can be effectively applied to the optimization of the process variables during Saccharification stage.

Table 4a: Experimental Data for Reducing Sugar from Saccharification

Std run	Temp (°C)	Time (min)	pH	Actual values of Reducing Sugar	Predicted values of Reducing sugar
1	40	20	6	9.5603	9.44
2	60	20	6	7.5370	7.67
3	40	60	6	5.3820	5.24
4	60	60	6	7.5013	7.62
5	40	40	5.5	3.6000	3.32
6	60	40	5.5	4.7100	4.18
7	40	40	6.5	2.6600	3.19
8	60	40	6.5	2.6700	2.95
9	50	20	5.5	13.4900	13.89
10	50	60	5.5	3.5350	3.95
11	50	20	6.5	5.8124	5.40
12	50	60	6.5	11.4800	11.08
13	50	40	6	4.3500	4.72
14	50	40	6	4.9091	4.72
15	50	40	6	4.3300	4.72
16	50	40	6	5.0000	4.72
17	50	40	6	5.0000	4.72

Table 4b: Test of Significance for Every Regression Coefficient and ANOVA

Source	Sum of Squares	df	Mean Square	F Value	Prob> F
X_1	0.18	1	0.18	0.67	0.4401
X_2	9.03	1	9.03	32.74	0.0007
X_3	0.92	1	0.92	3.33	0.1106
X_1X_2	4.29	1	4.29	15.55	0.0056
X_1X_3	0.30	1	0.30	1.10	0.3299
X_2X_3	61.02	1	61.02	221.13	< 0.0001
X_1^2	6.02	1	6.02	21.83	0.0023
X_2^2	66.47	1	66.47	240.91	< 0.0001
X_3^2	0.053	1	0.053	0.19	0.6754
Model	146.34	9	16.26	58.93	< 0.0001
Residual	1.93	7	0.28		
Lack of Fit	1.45	3	0.48	4.01	0.1064
Pure Error	0.48	4	0.12		
Cor Total	148.27	16			

Table 4c: Regression Coefficients and significance response surface Quadratics

Factor	Coefficient timate	df	Standard Error	95% CI Low	95% CI High	VIF
Intercept	4.72	1	0.23	4.16	5.27	
X_1	0.15	1	0.19	-0.29	0.59	1.00
X_2	-1.06	1	0.19	-1.50	-0.62	1.00
X_3	-0.34	1	0.19	-0.78	0.10	1.00
X_1X_2	1.04	1	0.26	0.41	1.66	1.00
X_1X_3	-0.27	1	0.26	-0.90	0.35	1.00
X_2X_3	3.91	1	0.26	3.28	4.53	1.00
X_1^2	-1.20	1	0.26	-1.80	-0.59	1.01
X_2^2	3.97	1	0.26	3.37	4.58	1.01
X_3^2	-0.11	1	0.26	-0.72	0.49	1.01
R^2 = 98.70%						

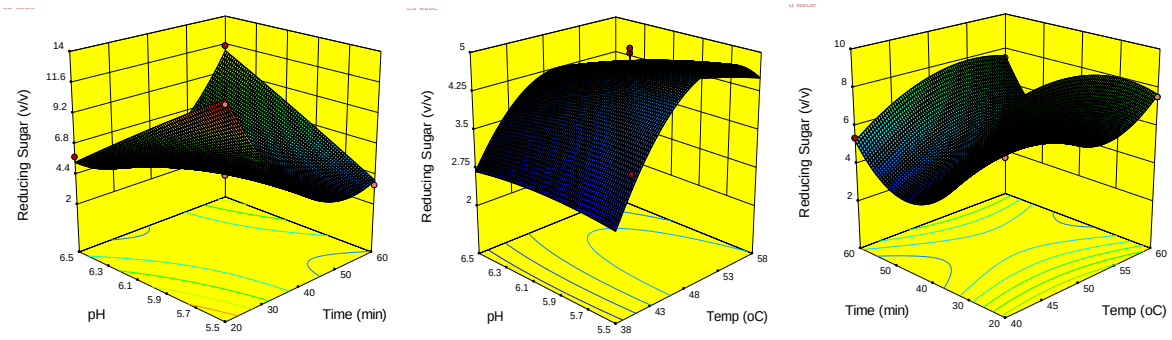

Figure 2: Surface plots for saccharification of liquefied ripe plantain peels starch

3.3. Fermentation of RPPH for Citric Acid production

The study investigated the possible use of ripe plantain peels hydrolyzate (RPPH) as the sole carbon source for the production of citric acid using *Aspergillus niger* under submerged fermentation. The experimental runs were generated by considering three variable factors namely: diammonium hydrogen phosphate (DAHP), potassium hydrogen diphosphate (PHDP) and time (min). Seventeen (17) experimental runs were also generated and were carried out. Table 5a showed the experimental and predicted values of Citric acid produced obtained. Table 5b showed the result of test of significance for every regression coefficient and ANOVA. The model F-value of 10.81 with low p-value implied that the model was significant. The coefficient of determination (R^2) was found to be 93.27%, which established that the model proved suitable for the adequate representation of the actual relationship among the selected factors. The low values of standard error observed in the intercept and all the models showed that the regression model fits the data well and the prediction was good. The final equation in terms of coded factors for the Box-Behnken design quadratic model is expressed in Eq. (7) as found in Table 5c.

$$Y = 19.09 - 0.31X_1 - 0.029X_2 + 0.30X_3 + 0.16X_1X_2 - 0.93X_1X_3 - 0.42X_2X_3 - 0.25X_1^2 - 0.83X_2^2 - 0.25X_3^2 \quad (7)$$

The coded equation is useful for identifying the relative impact of the factors by comparing the factor coefficients. The response surface plots representing the interactive effects between the time and DAHP, time and PHDP, and

DAHP and PHDP, are showed in Fig. 3. However, the perfect interaction between the time and PHDP produced the best result than that of interaction between time and DAHP and DAHP and PHDP. A statistical model predicted that the highest citric acid yield of 19.13 (g/l), at the following optimized conditions: DAHP of 3.5 (g/l), PHDP of 2.5 (g/l) and time of 8 days. This was validate in triplicate and an average mean of 19.02 (g/l) was obtained. This value was well in line with the predicted value.

Table 5a: Experimental data for citric acid concentration from fermentation

Run Order	DAHP (g/l)	PDHP (g/l)	Time (days)	Actual Value	Predicted Value	Residual
1	3.5	1.0	1	15.08	15.16	-0.079
2	1.0	4.0	8	18.02	18.12	-0.100
3	1.0	2.5	15	14.81	15.51	-0.700
4	3.5	2.5	8	19.05	19.09	-0.038
5	3.5	4.0	15	18.00	17.98	0.024
6	3.5	2.5	8	19.12	19.09	0.032
7	3.5	2.5	8	19.07	19.09	-0.018
8	6.0	2.5	15	17.20	16.60	0.600
9	1.0	2.5	1	16.21	15.51	0.700
10	6.0	2.5	1	16.73	16.75	-0.024
11	3.5	1.0	15	15.79	15.71	0.079
12	3.5	4.0	1	15.34	15.94	-0.600
13	6.0	4.0	8	18.45	17.83	0.620
14	6.0	1.0	8	17.67	17.57	0.100
15	1.0	1.0	8	17.88	18.50	-0.620
16	3.5	2.5	8	19.07	19.09	-0.018
17	3.5	2.5	8	19.13	19.09	0.042

Table 5b: Test of Significance for Every Regression Coefficient and ANOVA

Source	Sum of Squares	df	Mean Square	F Value	Prob> F
X_1	0.75	1	0.75	2.10	0.1902
X_2	6.613E-003	1	6.613E-003	0.019	0.8955
X_3	0.74	1	0.74	2.09	0.1918
$X_1 X_2$	0.10	1	0.10	0.29	0.6086
$X_1 X_3$	3.44	1	3.44	9.65	0.0172
$X_2 X_3$	0.70	1	0.70	1.96	0.2047
X_1^2	0.26	1	0.26	0.73	0.4205
X_2^2	2.93	1	2.93	8.21	0.0241
X_3^2	24.28	1	24.28	68.11	< 0.0001
Model	34.70	9	3.86	10.81	0.0024
Residual	2.50	7	0.36		
Lack of Fit	2.49	3	0.83	680.57	< 0.0001
Pure Error	4.880E-003	4	1.220E-003		
Cor Total	37.19	16			

Table 5c: Regression Coefficients and significance response surface Quadratics

Factor	Coefficient Estimate	df	Standard Error	95% CI Low	95% CI High	VIF
Intercept	19.090	1	0.27	18.46	19.72	
X_1	-0.310	1	0.21	-0.81	0.19	1.00
X_2	-0.029	1	0.21	-0.53	0.47	1.00
X_3	0.300	1	0.21	-0.19	0.80	1.00
$X_1 X_2$	0.160	1	0.30	-0.55	0.87	1.00
$X_1 X_3$	-0.930	1	0.30	-1.63	-0.22	1.00
$X_2 X_3$	-0.420	1	0.30	-1.12	0.29	1.00
X_1^2	-0.250	1	0.29	-0.94	0.44	1.01
X_2^2	-0.830	1	0.29	-1.52	-0.15	1.01
X_3^2	-2.400	1	0.29	-3.09	-1.71	1.01

R^2 = 93.27%

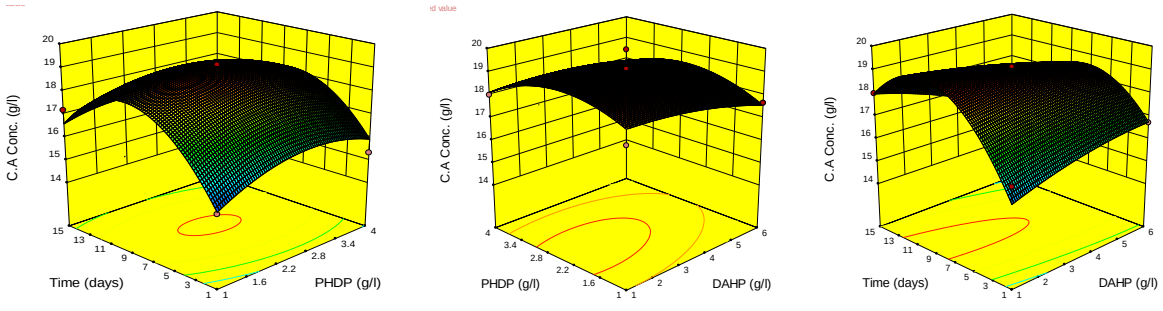

Figure 3: Surface plots for fermentation of ripe plantain peels starch

Conclusions

The utilization of waste ripe plantain peels for the production of citric acid will not only be a good way for controlling waste disposal in the environment but will also serve as economically valuable, because citric acid is a wanted product in the market, thus helping to grow the gross domestic product (GDP) of the nation.

Response surface methodology was successfully applied in the two step enzymatic hydrolysis of ripe plantain peels starch. The maximum RPPH concentration obtained for liquefaction step was 8.4241 (v/v) at temperature of 50°c, pH of 5.5 and time of 52 minutes. For saccharification step, the RPPH concentration increased to 13.2994 (v/v) at temperature of 40°C, pH of 5.5 and time of 20 minutes. The RPPH obtained was further used as feedstock for fermentation which was subsequently converted to citric acid with an optimum production yield of 19.02 (g/l) after 8 days of fermentation.

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