



Statistical Investigation on the Hydrolysis and Fermentation Processes of Cassava Peels in the Production of Bioethanol

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Abstract: There are several types of experiments which require statistical investigation. These are characterized by the nature of treatments under investigation and also the nature of comparison required among them so as to meet the objectives of the experiment. To achieve this, cassava peels was collected from Kasuwa Gwari market Minna, Niger state dried and taken for hydrolysis and fermentation processes. Temperature, acid concentration, cassava biomass ratio, ph and time were varied to get the optimum yield of reducing sugar. Curve fitting and a two-way analysis of variance were used in analyzing the data. Most of the results from the experiment follows quadratic model. Furthermore, time and temperature were very significant in both hydrolysis and fermentation processes. We therefore concluded that for hydrolysis process yield is optimum at 110°C and 30mins, while for fermentation process yield is optimum at 35°C and at 6 days and 7 days respectively.

Keywords: Statistical Investigation, Hydrolysis, Fermentation, Processes, Cassava, Production, Bioethanol

1. Introduction

In African, Cassava is the third largest source of carbohydrate in food for human consumption in the world [1]. That is why cassava (also known as *Manihot esculenta crantz*) is highly cultivated in African and in Nigeria in particular. In addition, cassava roots plays important role in African diet and are processed using simple methods. For instance, in Nigeria cassava can be processed to produce Gari, Fufu and Lafun floor [1]. Since cassava is in abundant in Nigeria, many times the cassava peels are wasted or converted to animal feeds.

Cassava peels contains high level of hydrogen cyanide, this toxic compound is remove by drying the peel under the sun in order to make it suitable for animal feeds [2]. Also researchers have found that cassava peel has some element of Bioethanol inherent in it.

Bioethanol is being considered as a potential liquid fuel due to the limited amount of natural resources [3]. And such

bioethanol can be found in non-food waste, such as cassava peel. But this present work focused on statistical investigation of the processing of producing bioethanol from cassava peel.

There are several types of experiments which require statistical investigation. These are characterized by the nature of treatments under investigation and also the nature of comparison required among them so as to meet the objectives of the experiment [4].

Curve fitting is the process of constructing a curve, or mathematical function, that has the best fit to a series of data points, possibly subject to constraints. Curve fitting can involve either interpolation, where an exact fit to the data is required, or smoothing, in which a "smooth" function is constructed that approximately fits the data. Fitted curves can be used as an aid for data visualization, to infer values of a function where no data are available, and to summarize the

relationships among two or more variables [5]. In addition, Curve fitting, also known as regression analysis, is used to find the "best fit" line or curve for a series of data points. Most of the time, the curve fit will produce an equation that can be used to find points anywhere along the curve [6].

On the other hand, the classical two-way analysis of variance (ANOVA) model is where one factor is the main focus of the study (which will be referred to as the main treatment factor) and the other factor is not of primary interest such as a block effect (which will be referred to as the secondary factor) [7-8].

In this present work, curve fitting Techniques and Two-way Analysis of variance will be applied to data collected during the hydrolysis and fermentation processes of the cassava peels in the production of Bioethanol.

2. Literature Review

[1] examined the ethanol production by *Saccharomyces cerevisiae* from cassava peel hydrolysate. Their result revealed that the cassava peel hydrolysate with *Saccharomyces cerevisiae* resulted in maximal ethanol production after three days.

[3] carried out a comparative study of bioethanol production from cassava peels by monoculture and co-culture of yeast. Their result revealed that cassava peel can produce high yields of ethanol.

[9] investigated the ethanol production capabilities of axenic cultures of *Saccharomyces cerevisiae* and *Escherichia coli* from cassava waste water. The study revealed that the isolates had the ability of ethanol production from cassava waste water.

[10] they studied the feasibility of using non-food parts of cassava for energy production. They found that the potential use of cassava peel can lead to the production of ethanol.

[11] considered enzymatic production of ethanol from cassava starch using two strains of *Saccharomyces cerevisiae*. The yield of ethanol was found to vary but the highest ethanol concentration obtained was 5.3% at 10% initial sugar concentration, which gave a sugar conversion efficiency of 37.3%.

[12] examined the enzymatic production of bioethanol from cassava and sweet potato peels using two groups of organisms. The study revealed that bioethanol can be produced from cassava and sweet potato peels.

[13] they studied producing fermentable sugars by pretreatment and hydrolysis of cassava peels using *Aspergillus niger* and the crude enzymes. They reported the potentials of cassava peels in reducing sugar production.

[14] studied to determine the optimum concentration of H_2SO_4 and optimum time in the hydrolysis process and determine the optimum time in the fermentation time. The result revealed that the optimum production was 0.5M, 100°C, 4 days and produced 3.58% v/v bioethanol.

[15] considered the production of bioethanol as an

alternative source of fuel using cassava and yam peels as raw materials. The study revealed that bioethanol can be produced from cassava and yam peels with maximum yield from cassava peels.

[2] studied the ethanol production from cassava waste (pulp and peel) using alcohol tolerant yeast isolated from palm wine. The study revealed that ethanol produced from cassava pulp is higher than ethanol produced from cassava peel.

[16] investigated the potential of bioethanol production from cassava peels using different microbial inoculants simultaneously. The yield reported in the study competes favourably with those reported from cassava peels, potato peels and millet husks using other inoculant treatments by other researcher.

3. Model specification

3.1. Curve Fitting Techniques (Least Squares Curve Fits)

Least Squares is a method of curve fitting that has been popular for a long time. Least Squares minimizes the square of the error between the original data and the values predicted by the equation. While this technique may not be the most statistically robust method of fitting a function to a data set, it has the advantage of being relatively simple (in terms of required computing power) and of being well understood [17].

The following curve fits were adopted in this research work and the R-square criterion and the significance of the parameters will be used to choose the best fit that well describes the process. The curve fits will only be stated. They are found in SPSS 13.0 for windows. The following are the models for curve fitting:

(i). Linear: $Y = b_0 + b_1t$

(ii). Quadratic: $Y = b_0 + b_1t + b_2t^2$

(iii). Compound: $\ln Y = \ln b_0 + \ln b_1t$

(iv). Growth: $\ln Y = b_0 + b_1t$

(v). Logarithmic: $Y = b_0 + b_1 \ln t$

(vi). Cubic: $Y = b_0 + b_1t + b_2t^2 + b_3t^3$

(vii). S: $\ln Y = b_0 + b_1(1/t)$

(viii). Exponential: $\ln Y = \ln b_0 + b_1t$

(ix). Inverse: $Y = b_0 + b_1(1/t)$

(x). Power: $\ln Y = \ln b_0 + b_1 \ln t$

(xi). Logistic: $\ln(1/Y - 1/u) = \ln b_0 + \ln b_1t$ where u is the upper boundary value.

3.2. Two Factor Without Interaction Analysis of Variance (Two-Way ANOVA)

If there is no interaction between the two factors, then one can fit the model $Y_{ijk} = \mu + \alpha_i + \beta_j + \epsilon_{ijk}$ Where μ = the overall mean; α_i = the main effect of the i^{th} level of Factor A;

β_j = the main effect of the j^{th} level of Factor B; ϵ_{ijk} = the random error associated with Y_{ijk} [18].

Table 1. ANOVA table for the Two-way analysis of Variance.

Source of variance	df	Sum of squares	Mean squares	F
Blocks	b-1	$\sum B_i^2/t-G^2/N=SS_B$	$SS_B/(b-1)=MS_B$	MS_B/MS_E
Treatment	t-1	$\sum T_j^2/t-G^2/N=SS_T$	$SS_T/(t-1)=MS_T$	MS_T/MS_E
Error	(b-1)(t-1)	By Substraction= SS_E	$SS_E/(b-1)(t-1)=MS_E$	
Total	bt-1			

We reject H_0 if $F_{cal} > F_{tab}$. The Duncan Multiple Range Test (DMRT) was use for Post ANOVA comparison [19] & [20].

4. Materials and Methods

Cassava peel waste was collected from Kasuwa Gwari market Minna Niger State. The sample was air dried for three days before taking it to the National Cereals Research Institute, Badeggi. Bida, Niger State. The dried samples of cassava peel were milled to make it ready for further analysis. Temperature, Acid Concentration, Substrate concentration and time were varied for hydrolysis process. Also pH, Temperature, Yeast

Concentration, Glucose concentration and time were varied for fermentation process. Lastly time with temperature were varied for hydrolysis and fermentation processes.

5. Analysis and Results

MINITAB and SPSS 13.0 version software were used for the analysis. We present the results in the following headings.

5.1. Hydrolysis Process

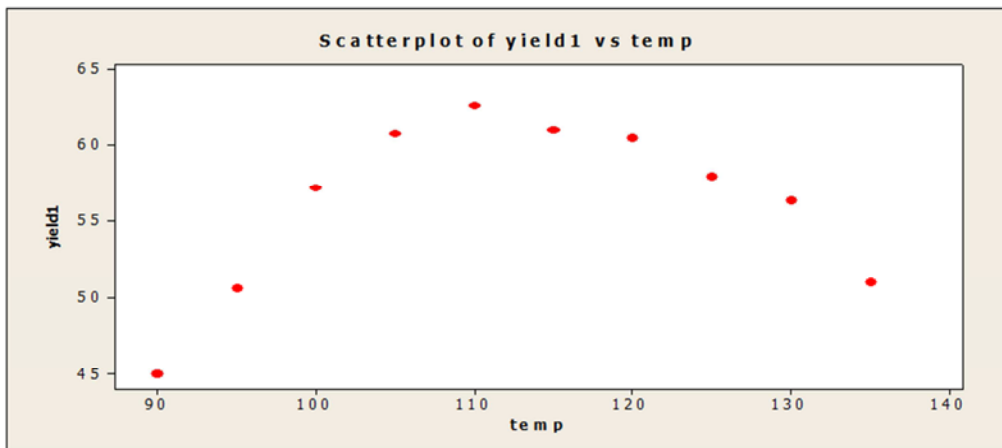


Figure 1. Temperature variation with glucose yield.

$$\text{Yield} = -302.942 + 6.372\text{temp} + 0.028\text{temp}^2 \quad R^2 = 0.971$$

t-values (-12.549) (14.680) (-14.434)

Interpretation: The relationship is explained by a quadratic model

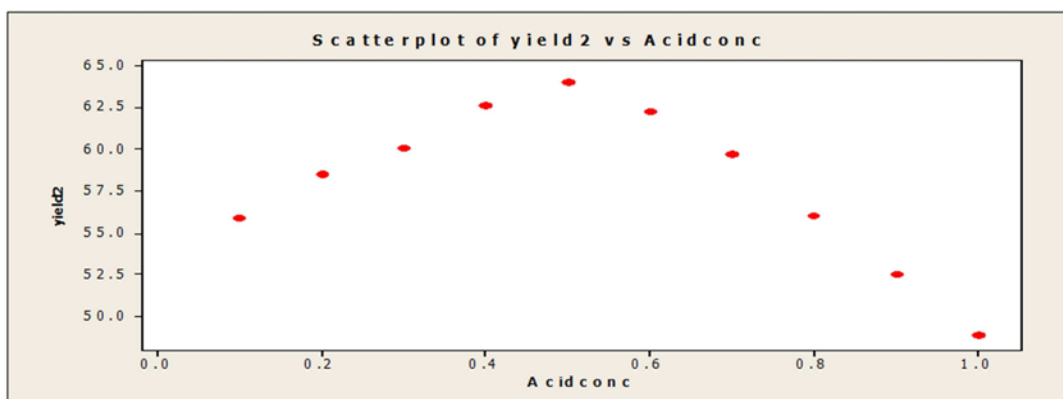


Figure 2. Acid concentration variation with yield.

$$\text{Yield} = 51.127 + 49.036\text{Aidconc} - 52.121\text{Aidconc}^2 \quad R^2 = 0.974$$

t-values (49.443) (11.355) (-13.622)

Interpretation: The relationship is explained by a quadratic model

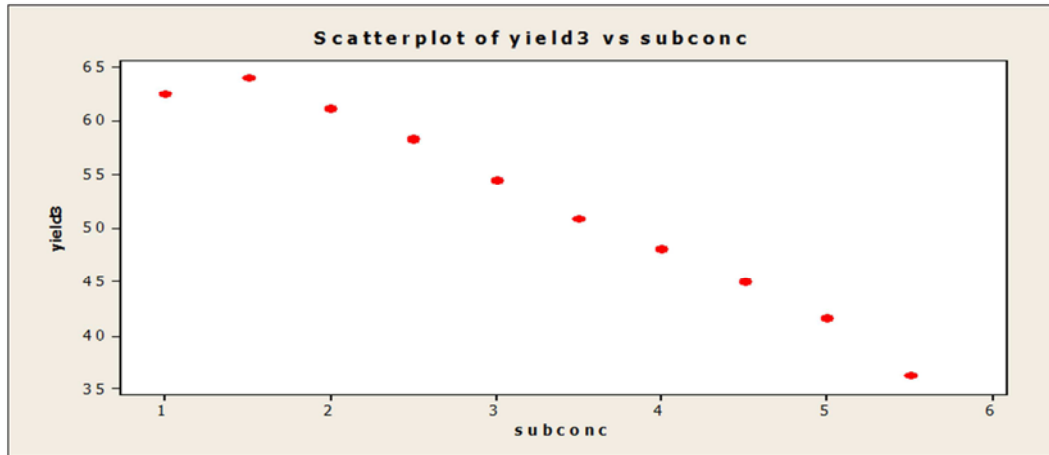


Figure 3. Substrate concentration variation with yield.

$$\text{Yield} = 72.190 - 6.154\text{subconc} \quad R^2 = 0.972$$

t-values (54.776) (16.591)

Interpretation: The relationship is explained by a Linear model

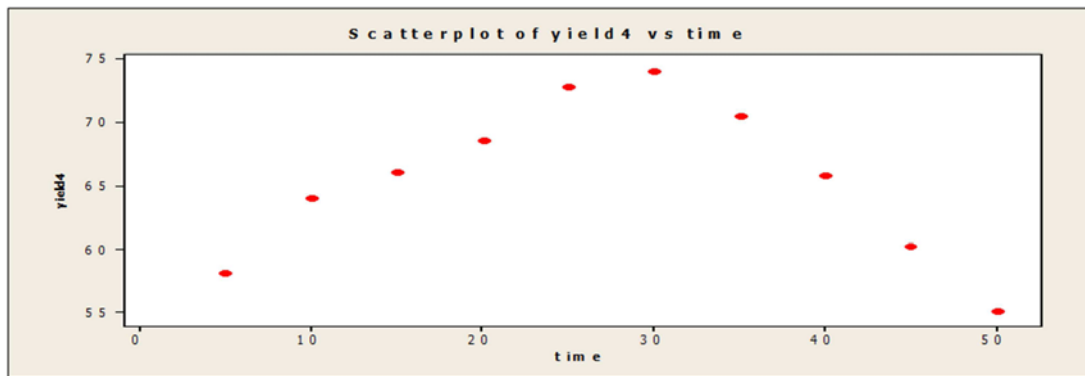


Figure 4. Time variation with yield.

$$\text{Yield} = 49.708 + 1.680\text{time} - 0.032\text{time}^2 \quad R^2 = 0.952$$

t-values (27.032) (10.939) (-11.618)

Interpretation: The relationship is explained by a quadratic model

5.2. Fermentation Process

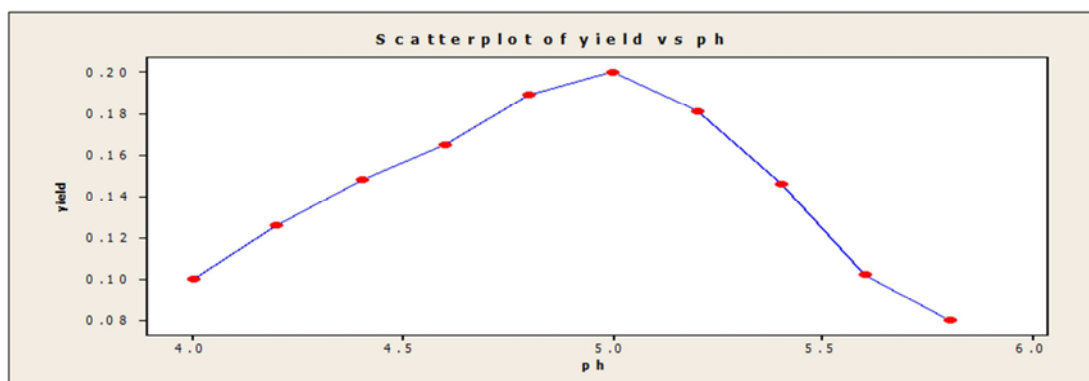


Figure 5. pH variation with yield.

$$\text{Yield} = -2.844 + 1.246\text{ph} - 0.128\text{ph}^2 \quad R^2 = 0.934$$

t-values (-9.223) (9.804) (-9.888)

Interpretation: The relationship is explained by a quadratic model

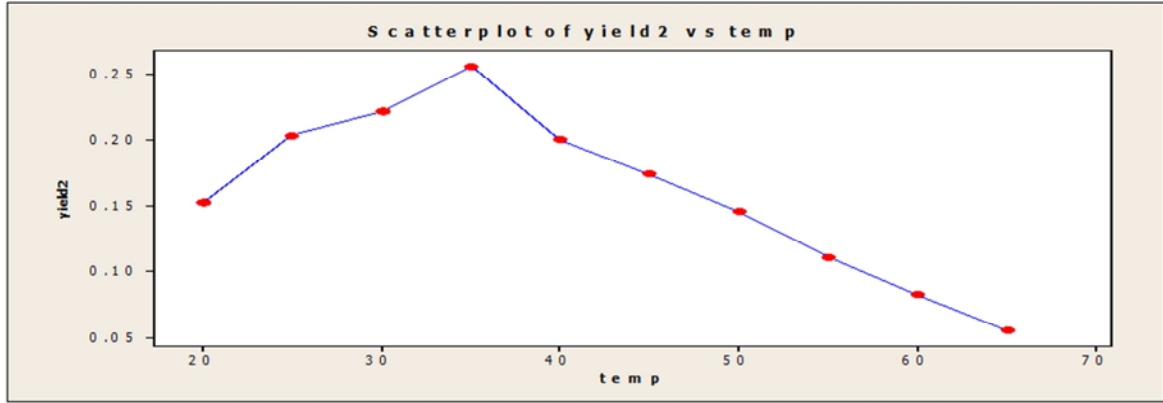


Figure 6. Temperature variation with yield.

Yield= $-0.496 + 0.053temp - 0.001temp^2 + 8.12 \times 10^{-6}temp^3$ $R^2=0.974$
 t-values (-4.259) (5.744) (-5.275) (4.499)
 Interpretation: The relationship is explained by a Cubic model

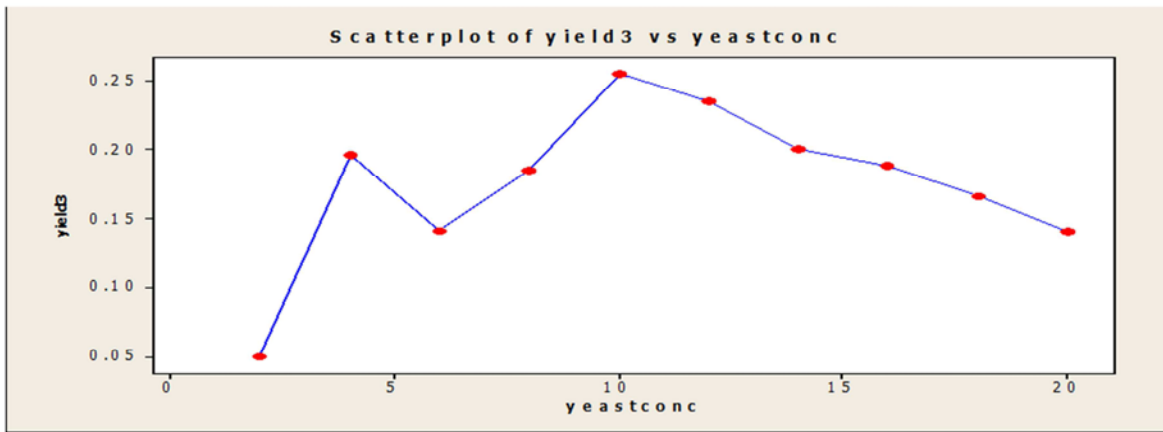


Figure 7. Yeast concentration variation with yield.

Yield= $0.015 + 0.036yeastconc - 0.001yeastconc^2$ $R^2=0.719$
 t-values (0.378) (4.209) (-4.002)
 Interpretation: The relationship is explained by a quadratic model

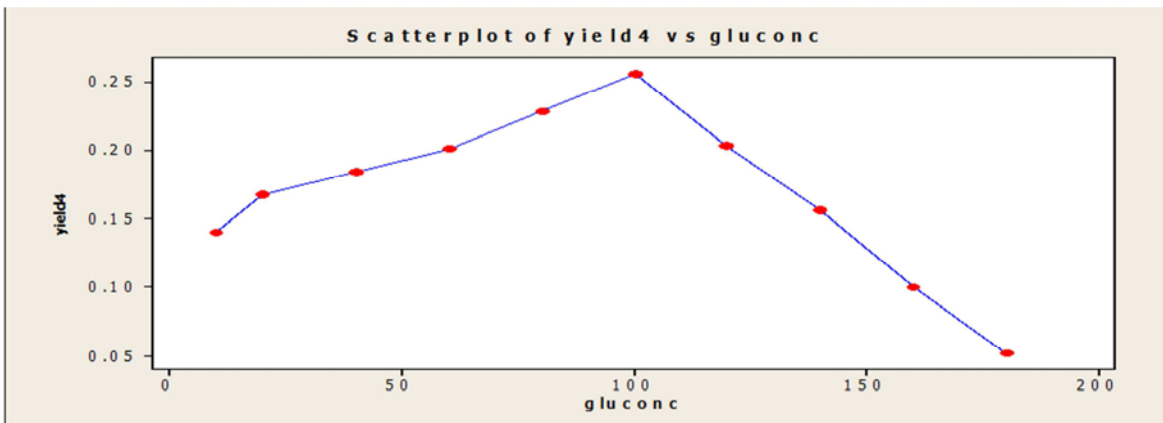


Figure 8. Glucose concentration variation with yield.

Yield= $0.0106 + 0.003gluconc - 1.9 \times 10^{-5}gluconc^2$ $R^2=0.939$
 t-values (7.013) (7.847) (-9.283)
 Interpretation: The relationship is explained by a quadratic model

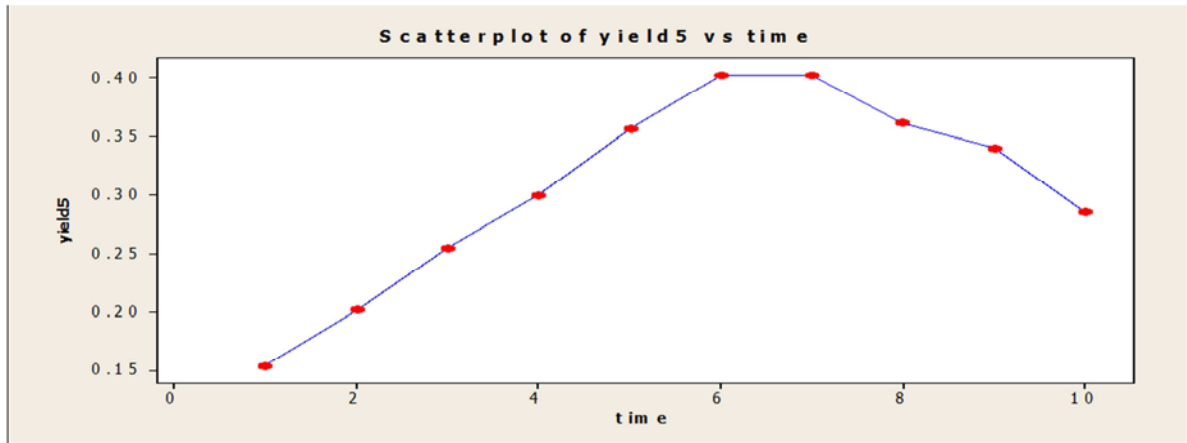


Figure 9. Time variation with yield.

$$\text{Yield} = 0.035 + 0.103\text{time} - 0.008\text{time}^2 \quad R^2=0.953$$

t-values (1.457) (10.249) (-8.646)

Interpretation: The relationship is explained by a quadratic model

5.3. Analysis of Variance for Time with Temperature Variation for Hydrolysis Process

The ANOVA table for time with temperature variation for hydrolysis process is presented in table 2 below, while the post ANOVA result for temperature and time are presented in tables 3 and 4 respectively.

Table 2. Analysis of Variance for Time with Temperature Variation for Hydrolysis Process.

Tests of Between-Subjects Effects					
Dependent Variable: yield					
Source	Type III sum of Squares	df	Mean Square	F	Sig
Corrected Model	8373.007 ^a	18	465.167	239.271	.000
Intercept	314743.440	1	314743.440	161896.4	.000
Temp	2886.488	9	320.721	164.971	.000
Time	5486.520	9	609.613	313.570	.000
Error	157.472	81	1.944		
Total	323273.920	100			
Corrected Total	8530.480	99			

^a R Squared=982(Adjusted R Squared=977)

Table 3. Post Hoc Tests for temperature.

Homogeneous Subsets								
yield								
Duncan ^{a b}								
temp	N	Subset						
		1	2	3	4	5	6	7
90.00	10	45.2200						
135.00	10		51.3700					
95.00	10		52.3900					
130.00	10			54.4600				
100.00	10				56.2400			
125.00	10				56.5100			
120.00	10					58.8600		
105.00	10					59.4400		
115.00	10						61.0500	
110.00	10							65.4800
Sig		1.000	.006	1.000	.666	.355	1.000	1.000

Means for groups in homogeneous are displayed

Based on Type III Sum of Squares

The error term is Mean Square (Error)=1.944

^a Uses Harmonic Mean Sample Size=10.000

^b Alpha=.05

Table 4. Post Hoc Tests for time.

Homogeneous Subsets										
yield										
Duncan ^{a,b}										
time	N	Subset								
		1	2	3	4	5	6	7	8	9
50.00	10	41.9900								
45.00	10		47.1200							
5.00	10			50.2100						
40.00	10				53.5300					
10.00	10					56.4500				
15.00	10						59.5700			
35.00	10						59.7500			
20.00	10							62.0400		
25.00	10								64.1900	
30.00	10									66.1700
Sig		1.000	1.000	1.000	1.000	1.000	774	1.000	1.000	1.000

Means for groups in homogeneous are displayed

Based on Type III Sum of Squares

The error term is Mean Square (Error)=1.944

^a Uses Harmonic Mean Sample Size=10.000

^b Alpha=05

Interpretation: Time and temperature were very significant in the analysis. The yield is optimum at 110°C and in 30mins.

5.4. Analysis of Variance for Time with Temperature Variation for Fermentation Process

The ANOVA table for time with temperature variation for fermentation process is presented in table 4 below, while the post ANOVA result for temperature and time are presented in tables 5 and 6 respectively.

Table 5. Analysis of Variance for Time with Temperature Variation for Fermentation Process.

Tests of Between-Subjects Effects					
Dependent Variable: yield 1					
Source	Type III sum of Squares	df	Mean Square	F	Sig
Corrected Model	9794.198 ^a	18	544.122	135.303	.000
Intercept	37570.069	1	37570.069	9342.262	.000
Temp	6540.716	9	726.746	180.714	.000
Time	3253.482	9	361.498	89.891	.000
Error	325.743	81	4.022		
Total	47690.010	100			
Corrected Total	10119.941	99			

^a R Squared=.968(Adjusted R Squared=.961)

Table 6. Post Hoc Tests for temperature.

Homogeneous Subsets										
yield1										
Duncan ^{a,b}										
Time1	N	Subset								
		1	2	3	4	5	6	7	8	9
65.00	10	5.4600								
60.00	10		7.6000							
55.00	10			13.5200						
50.00	10				16.1100					
20.00	10					19.6500				
45.00	10						22.9300			
25.00	10						24.1400	24.1400		
30.00	10							25.9000		
40.00	10								27.9500	

Homogeneous Subsets										
yield1										
Duncan ^{a,b}										
Time1	N	Subset								
		1	2	3	4	5	6	7	8	9
35.00	10									30.5700
Sig		1.000	1.000	1.000	1.000	1.000	181	053	1.000	1.000

Means for groups in homogeneous are displayed

Based on Type III Sum of Squares

The error term is Mean Square (Error)=4.022

^a Uses Harmonic Mean Sample Size=10.000

^b Alpha=05

Table 7. Post Hoc Tests for time.

Homogeneous Subsets										
yield1										
Duncan ^{a,b}										
Time1	N	Subset								
		1	2	3	4	5	6	7	8	9
1.00	10	8.9200								
2.00	10		12.4600							
3.00	10			15.9200						
10.00	10			15.9200						
4.00	10				19.9000					
9.00	10				19.9000					
5.00	10					23.5900				
8.00	10					23.8600				
6.00	10								26.6500	
7.00	10								26.6500	
Sig		1.000	1.000	1.000	947	764			1.000	

Means for groups in homogeneous are displayed

Based on Type III Sum of Squares

The error term is Mean Square (Error)=4.022

^a Uses Harmonic Mean Sample Size=10.000

^b Alpha=05

Interpretation: Time and temperature were very significant in the analysis. The yield is optimum at 35°C and in 6 days and 7 days respectively.

6. Discussion of Results

Curve fitting technique and a Two factors analysis of variance (ANOVA) without interaction were used to analyze data collected during hydrolysis and fermentation processes in the production of Bioethanol from cassava peels. For hydrolysis process, temperature, Acid concentration, substrate concentration and time were varied with yield. Results revealed that fig. 1, 2 and 4 follows a quadratic model and fig. 3 was explained by linear model. While for fermentation process, pH, Temperature, Yeast Concentration, Glucose concentration and time were varied with yield. The analysis shows that figs. 5, 7 and 9 follows quadratic model and fig. 6 follows a cubic model. Time and temperature were very significant in the production of Bioethanol from cassava peels. For hydrolysis process, yield is optimum at 110°C and in 30mins, while for fermentation process yield is optimum at 35°C and in 6days and 7 days respectively.

7. Conclusion

This present work therefore concludes that linear, cubic and quadratic models can be used to predict yield for both hydrolysis and fermentation process. Bioethanol yield is optimum at 110°C and in 30mins for hydrolysis process, while yield is optimum at 35°C and in 6days and 7 days respectively for fermentation process.

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