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Optimization of bio-ethanol production from water yam using response surface methodology

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Abstract
The negative effects of fossil fuels usage have provided the pedigree to consider other alternative energy sources. Bio-fuel offers option as alternative energy source due to its environmental friendliness, its sustainability and renewability. Traditional crops used as dedicated energy crops for bio-ethanol production such as grains and tubers seems to have violated the technical and ecological reliance standard in terms of sustainability, by interfering with food chain. The objective of this study is to investigate the potentials of water yam for bio-fuels production in terms of: characterization of the crop, optimizing the enzymatic hydrolysis for ethanol production, produce ethanol from the starch. The starch of the crop was extracted by wet milling method. Reducing sugar yield was optimized using the Central Composite Design (CCD) and the result analyzed using Design Expert 8.0.7.1 Trial Version; where time, enzyme concentration, water quantity and temperature are the variables. *Saccharomyces cerevisiae* yeast was used for starch fermentation. Distillation of fermentation wort was done using distillation apparatus. Starch yield from the crop was 41.65%. Optimal reducing sugar yield of 122.56mg/mg was obtained from the crop at the temperature of 70ºC, 0.3g/g enzyme concentration, 3.00ml/g water quantity and at the time of 3hrs. Results from the study showed that water yam is a good primary feedstock for bio-ethanol production.

Keywords: water yam, alternative energy, bio-ethanol, optimization, reducing sugar yield, traditional crops

Introduction

Supplies of fossil fuels like all other natural resources are limited (Aneke, 2012. and Chukwuma, *et al*., 2012) [1, 5]. They are also non-renewable, and are bound to be depleted sometime in future. More so, rising cost of fossil-based petroleum products have made the products unaffordable to the rural dwellers that constitute about 70% of the population. Fortunately, most parts of Nigeria are suitable for energy crop cultivation and so the country cannot afford to be left behind in the recent quest for renewable sources of energy.

Presently, most of the energy crops currently exploited for bio fuels production such as tubers and grains compete with human and animal food (According to Chukwuma, *et al.*, 2012). [6]. But numerous feedstock abound locally which would not necessarily compete with staple food, and some of them have not been employed in bio-ethanol production (Umeghalu, 2016) [12]. Therefore, research on the fermentability of these locally available feed stocks in bio-ethanol production could provide more choices when availability of materials is limited.

Bio-ethanol

Ethanol is also known as pure alcohol, ethyl alcohol or bio-ethanol. It is a colourless, flammable and volatile liquid with strong odour. The melting point of ethanol is 114.1°C, whereas it boils at 78.5°C. Due to the low freezing point of ethanol, it has been used in thermometers for temperatures below –40°C, and in automobile radiators as antifreeze (Onyeike *et al*., 1995) [10]. The properties of ethanol are given in Table 1. The chemical formula of ethanol is C_2H_5OH.

Bio-ethanol has become an attractive fuel because it is renewable and oxygenated (Pfaller *et al*., 2008) [8]. Oxygenated ethanol reduces the emission of carbon dioxide and aromatic compounds. Ethanol is also non-toxic and is a non-contaminant to water sources and can be used for cooking, heating and lighting appliances. In some cases, ethanol can be used in modified appliances designed for conventional fuels. In other cases, appliances designed specifically for ethanol fuel are required. Bio–ethanol is being used purely or blended with gasoline for transportation in Brazil and in some states of the United States of America (Balat *et al*., 2008) [4]. Aside from being used as fuel, ethanol has other applications in various industries such as: in the personal care products, cleaning agents, pharmaceuticals, and production of beverages.

Table 1: Properties of ethanol

Description	Values
Chemical Formula	C_2H_5OH
Molecular weight (g/mol)	46
Density at 20°C (kg/m³)	789
Calorific value (MJ/kg)	26.9
Calorific value of stoichiometric mixture (MJ/m³)	3.85
Heat of evaporation (kJ/kg)	840
Temperature of self-ignition (K)	665

Stoichiometric air/fuel ratio (kg air/kg fuel)	9
Lower flammability (λ_1)	2.06
Higher flammability (λ_h)	0.3
Kinematic viscosity at 40°C (mm²/s)	1.4
Motor octane number /research octane number	89/107
Cetane number	8
Flame temperature (K)	2235
Molecular composition (by mass)	
C (%)	52.2
H (%)	13

Source: Ofuefule *et al*., [9].

Design of Experiment (DOE)

Design of Experiment (DOE) is a computer-enhanced systematic approach to experimentation that considers all factors involved simultaneously (Anthony, 1996) [2]. DOE is concerned with the planning and conduct of experiments to analyze the resulting data so that a valid and objective conclusion is obtained. DOE fits response data to mathematical equations. Collectively, these equations serve as models to predict what will happen for any given combination of values, and its techniques enable designers to determine simultaneously the individual and interactive effects of many factors that could affect the output results in any design. It also provides a full insight of interaction between design elements.

Response Surface Methodology (RSM)

RSM was used in the design of experiment. Response Surface Methodology is an empirical statistical technique employed for multiple regression analysis by using quantitative data obtained from properly designed experiments to solve multivariate equations and graphically represented as response surface. It is also a collection of mathematical and statistical techniques used for the modeling and analysis of problems in which a response of interest is influenced by several variables and the objective is to optimize the response (output variable) which is influenced by several independent variables (input variables or factors) which can be used in three ways:

1. To describe how the test variables affect the response.
2. To determine the inter-relationship among the test variables on the response.
3. To describe the combined effects of all the test variables on the response.

Here, the inputs are called factors or variables and the outputs represent the response that generates the system under the causal action of the factors.

Central Composite Design (CCD)

The Central Composite Design (CCD) was used to study the effects of the variables towards their responses and subsequently in the optimization studies. This method is suitable for fitting a quadratic surface and it helps to optimize the effective parameters with a minimum number of experiments, as well as to analyze the interaction between the parameters. In order to describe the effects of temperature, water quantity, enzyme concentration and time on the reducing sugar yield were conducted based on the CCD. The coded values of the process parameters were determined by the following Equation 1:

$$x_i = \frac{X_i - X_o}{\Delta X} \qquad (1)$$

Where:
x_i – coded value of the i[th] variable,
X_i – uncoded value of the i[th] test variable and
X_o – uncoded value of the i[th] test variable at center point.
The Central Composite Design (CCD) of experiment in terms of coded values of the experimental design and range and levels of individual variables are given in Tables 2 and 3.
A statistical program package, Design Expert 8.0.7.1 was used for regression analysis of the data obtained and to estimate the coefficient of the regression equation. The equation was validated by the statistical tests called the ANOVA analysis. The significance of each term in the equation is to estimate the goodness of fit in each case. Response surface was drawn to determine the individual and interactive effects of the test variable on the reducing sugar yield.
The optimal values of the test variables were first obtained in coded units as shown in Table 2 below and then converted to the un-coded units.

Table 2: Central Composite Design of experiment in terms of coded values

Run	Time	Enzyme conc.	Water qty.	Temperature
1	-	-	-	-
2	+	-	-	-
3	-	+	-	-
4	+	+	-	-
5	-	-	+	-
6	+	-	+	-
7	-	+	+	-
8	+	+	+	-
9	-	-	-	+
10	+	-	-	+
11	-	+	-	+
12	+	+	-	+
13	-	-	+	+

14	+	-	+	+
15	-	+	+	+
16	+	+	+	+
17	-α	0	0	0
18	+α	0	0	0
19	0	-α	0	0
20	0	+α	0	0
21	0	0	-α	0
22	0	0	+α	0
23	0	0	0	-α
24	0	0	0	+α
25	0	0	0	0
26	0	0	0	0
27	0	0	0	0
28	0	0	0	0
29	0	0	0	0
30	0	0	0	0

The high (+) and low (-) values for optimal reducing sugar yield were time (2 and 4hours), enzyme concentration (0.1 and 0.3g) and water quantity (2.0gr and 4.0gr) temperature (40 and 70ºC). The alpha (α) value used was 1.5. The factor levels of the variables are given in Table 3 while the Central Composite Design for reducing sugar yield terms of real values is shown in Table 3.

Table 3: Factor levels of independent variables for reducing sugar yield.

Independent Factors	Low level (-)	Medium level (0)	High level (+)
Time (hrs)	2	3	4
Enzyme conc. (g/g).	0.1	0.2	0.3
Water quantity (ml/g)	2	2.5	3
Temp, ºC	40	55	70

Saccharification process for the starch

Hydrolysis of water yam starch using Design of Experiment (DOE)

Hydrolysis of the starch for optimization of reducing sugar yield was carried out using Response Surface Methodology (RSM) and Central Composite Design (CCD).

Materials and Methods

The water yam tubers used for this study were locally purchased from Eke Ojoto Market in Idemili South Local Government Area of Anambra State of Nigeria. The malted barley was used as the source of enzyme and the chemicals utilized for the reducing sugar measurements were procured from a chemical dealer at Head Bridge Market in Onitsha. The chemicals were used without further purification. The materials were taken to Springboard Laboratory at Udoka Housing Estate Awka, Anambra State for analysis.

Equipments utilized include Thomas Willey laboratory mill model ‘4’ equipped with 0.25mm sieve, Heating mantle (1 liter capacity, Sunbim, India), hot plate stoves, digital weighing balance (Ohaus, Adventurer, model- AR 3130), digital pH meter (Jenway 3510), hydrometer and specific gravity bottles.

Extraction of starch

Starch from the water yam was extracted using wet milling method according to Kunle *et al.* (2003) [7] as follows: the tubers all together measuring (10.76 kg) were washed and peeled to remove the epidermis. The peeled bulk was thoroughly washed with clean water, cut to pieces before slicing into smaller sizes to enhance milling with a mechanical grinder to break down the plant cells, thereby releasing the starch granules. The resultant paste was sieved with 0.25mm mesh to extract the starch using some quantity of water. The water from the resulting starch suspension was removed by allowing the starch to sediment by gravity and decanting of the water.

The starch sediment was squeezed in a clean muslin bag to remove the water. It was then allowed to sun dry for a period of 7days. This ensured thorough drying of the starch. The starch which was in cake form was dry milled with an electronic blender, which reduced it to a very fine powdery starch. The resulting starch flour was sieved through 150 µm vibration screens, weighed and stored dry for subsequent use.

Gelatinization process of water yam starch

Four sets of 100 g of water yam starch were weighed out accurately and added to 100 ml, 200 ml, 300 ml and 400 ml of distilled water (representing slurry concentrations of 1.0, 2.0, 3.0 and 4.0 ml/g) respectively. Each of the mixtures was contained in a 1000 ml beaker. The mixtures were thoroughly stirred to form a homogenous mixture and then put in a water bath on an electric hot plate. The temperature of the water bath was maintained at a constant boiling range. The starch-water mixtures were stirred continuously while monitoring the temperature for the first ten minutes. After that, they were occasionally stirred until a gel of moderate viscosity was formed the temperature ranges of the gel formation were noted. The formation of the gel ended the gelatinization process. The process was replicated and the mixtures set to cool down for further conversion.

Optimization of reducing sugar yield

The optimization process of the reducing sugar yield (RSY) was done using the Central Composite Design (CCD) for the processes of saccharification and hydrolysis Four important factors which are temperature, time, enzyme concentration and water quantity were used as the independent variables where their combined effects were examined while the yield of the reducing sugar was the dependent variable or the response. This was done to determine the best conditions for optimum yield of the reducing sugar. Using the CCD involves varying the independent variables at three different levels (-1, 0, +1). In this work, a set of 30 experiments were performed consisting of 16 core points, 8 star like points and 6 centre points or null points. The distance of the star like point α used was 1.5. The experiments were performed in random to avoid systematic error.

Statistical analysis of the optimization process of water yam

The Experimental and Predicted values of reducing sugar yield for water yam is shown in Table 4, as well as the combined effects of time (A), enzyme concentration (B), water quantity (C) and temperature (D). The highest reducing sugar yield was 122.56mg/mg occurring at a temperature of 70ºC, at the time of 2hrs, enzyme concentration of 0.10g/g and water quantity of 2ml/g. Design Expert 8.0.7.1 trial version was used to analyze the result.

Results and Discussions

The results of the predicted and experimental values of reducing sugar yields are shown in Table 4 below.

Table 4: Experimental and Predicted values of reducing sugar yield for water yam

Std	Factor 1 A: time hrs	Factor 2 B: enzyme conc. g/g	Factor 3 C:water qty. ml/g	Factor 4 D: temp. ^{0}C	Experimental value	Predicted value
1	2.50	0.20	2.50	55.00	75.23	81.82
2	2.50	0.20	3.25	55.00	83.45	79.82
3	2.50	0.20	1.75	55.00	75.23	72.61
4	2.00	0.30	2.00	40.00	85.78	89.74
5	2.00	0.10	4.00	40.00	78.34	90.26
6	3.00	0.10	4.00	40.00	90.36	87.95
7	2.00	0.30	4.00	40.00	98.76	98.14
8	3.00	0.30	4.00	40.00	110.23	114.97
9	2.00	0.10	2.00	70.00	118.23	119.75
10	3.00	0.10	2.00	70.00	120.14	125.81
11	2.00	0.30	2.00	70.00	86.23	93.69
12	3.00	0.30	2.00	70.00	122.56	120.90
13	2.00	0.10	4.00	70.00	93.21	94.30
14	3.00	0.10	4.00	70.00	89.17	97.05
15	2.00	0.30	4.00	70.00	73.45	82.34
16	3.00	0.30	4.00	70.00	105.77	104.23
17	1.75	0.20	3.00	55.00	101.34	83.77
18	3.25	0.20	3.00	55.00	99.45	98.69
19	2.50	0.05	3.00	55.00	97.11	83.27
20	2.50	0.35	3.00	55.00	86.22	81.74
21	2.50	0.20	1.50	55.00	93.11	90.16
22	2.50	0.20	4.50	55.00	103.11	87.73
23	2.50	0.20	3.00	32.50	74.89	67.52
24	2.50	0.20	3.00	77.50	101.12	90.16
25	2.50	0.20	3.00	55.00	63.13	70.49
26	2.50	0.20	3.00	55.00	75.12	70.49
27	2.50	0.20	3.00	55.00	63.13	70.49
28	2.50	0.20	3.00	55.00	63.13	70.49
29	2.50	0.20	3.00	55.00	63.13	70.49
30	2.50	0.20	3.00	55.00	63.13	70.49

Table 5: Summary of P-values for percentage reducing sugar yield for water yam.

Sequential R^2	Lack of Fit R^2	Adjusted	Predicted	Source	p-value
Linear	0.2631	0.0034	0.0522	-0.1219	
2FI	0.2293	0.0039	0.1549	-0.1646	
Quadratic	0.0020	0.0241	0.6323	0.1080	Suggested
Cubic	0.0020	0.0043	0.5207	-10.6982	Alias

Table 6: Lack of Fit Test for percentage reducing sugar yield for water yam.

Source	Sum of Squares df		Mean F Square Value		p-value	Prob > F
Linear	7443.51	20	372.18	15.30	0.0034	
2FI	5004.68	14	357.48	14.70	0.0039	
Quadratic	1639.15	10	163.92	6.74	0.0241	**Suggested Aliased**
Cubic	949.64	2	474.82	19.52	0.0043	
Pure Error	121.61	5	24.32			

Table 7: Model Summary Statistics for reducing sugar yield for water yam

Source	Std. Dev.	R-Squared	Adjusted R-Squared	Predicted R-Squared	PRESS	
Linear	17.40	0.1829	0.0522	-0.1219	10386.85	
2FI	16.43	0.4463	0.1549	-0.1646	10782.82	
Quadratic	10.83	0.9178	0.7323	0.1080	8259.05	Suggested
Cubic	12.37	0.8843	0.5207	-10.6982	1.083E+005	Aliased

ANOVA analysis for reducing sugar yield for water yam

Design Expert 8.0.7.1 trial version was used to analyze the results. The summary of P-values indicates that a quadratic model fitted the ANOVA analysis and hence it was suggested. The linear and 2FI models were not suggested. The Cubic model is always aliased because the CCD does not contain enough runs to support a full cubic model.

The Adjusted $R^2 = 0.9208$ obtained in the study was in close agreement with the Predicted $R^2 = 0.8323$. The adequate precision obtained in the study is 7.609. Adequate Precision measures the signal to noise ratio and compares the range of the predicted value at the design points to the average prediction error. Adequate precision ratio above 4 indicates adequate model efficacy. Hence, the adequate precision ratios of 7.609 obtained in this study indicates adequate model efficacy. Also, a PRESS value of 8416.05 indicates an adequate signal implying that the model can be used to navigate the design space.

The coefficient of regression R^2 was used to validate the fitness of the model equation. For percentage reducing sugar yield for water yam, the R^2 has a high value of 0.9254 showing that 92.54% of the variability in the response can be explained by the model. This implies that the prediction of experimental data is quite satisfactory. The quadratic model equation obtained is:

$$Y_{Reducing\ sugar} = +70.49 +4.97A -0.51B -0.81C +7.55D +4.79AB -0.077AC +1.27AD +4.27BC -4.96BD -9.22CD +9.22A^2 +5.34B^2 +8.20C^2 +3.71D^2 \ldots\ldots \quad (2)$$

Comparing with the P-values, when in a regression equation, an independent variable having a positive sign indicates that an increase in the variable will cause an increase in the response while a negative sign will result to decrease in the response. Also if the values of $P < 0.05$, it is an indication that the model term is significant. In this study, P value it was found that, among the test variables used in the study of the optimization of reducing sugar yield, that by eliminating the insignificant terms, the final model equation becomes as expressed in Equation 3 below.

$$Y_{Reducing\ sugar} = 70.49 +4.97A +7.55D +4.79AB +1.27AD +4.27BC +9.22A^2 +5.34B^2 +8.20C^2 +3.71D^2 \ldots\ldots \quad (3)$$

Normal Plot of Residuals

Normal Plot of Residuals was used to check whether the points follow a straight line in which it will be concluded that the residuals follow a normal distribution. This will indicate a good relationship between experimental and predicted values.

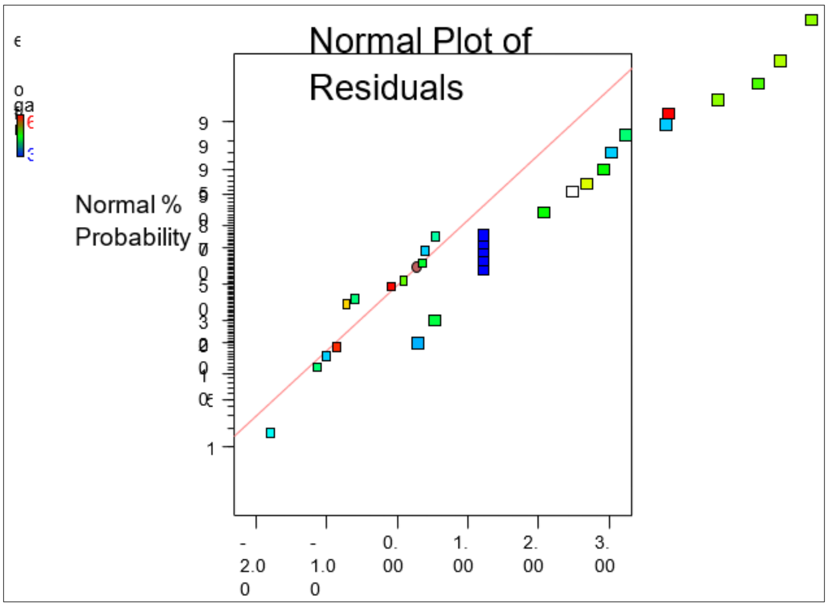

Fig 1: Normal plot of Residual for reducing sugar yield for water yam starch

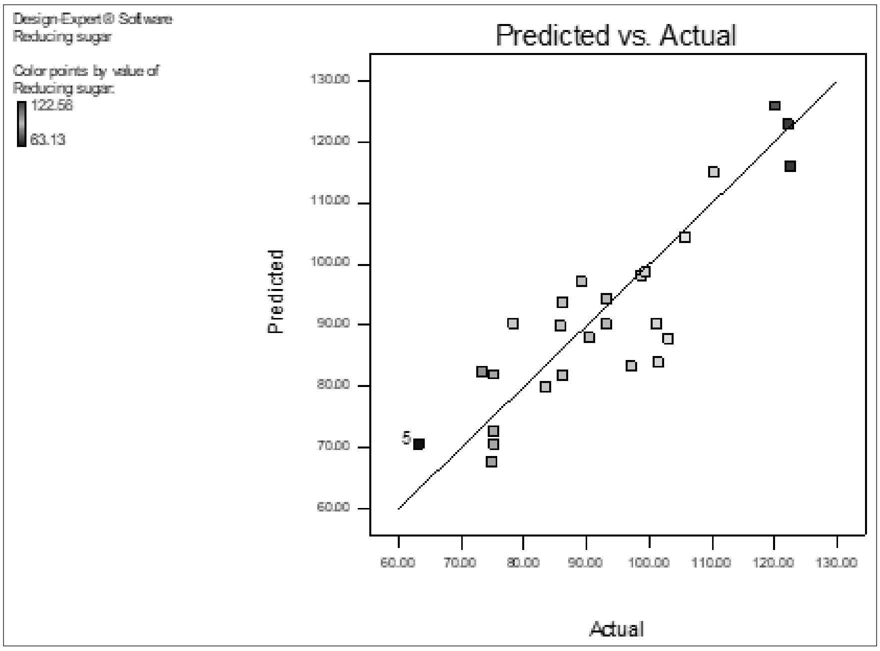

Fig 2: Plot of Predicted vs Actual for reducing sugar yield for water yam.

Validation of model predicted result
The derived model was validated by carrying out a statistical analysis (Correlation), comparison with standard model (Regression model) and deviational analysis.

i) Comparison with standard model (Regression Model)
The comparison of the Adjusted R^2 is 0.9208 with Predicted R^2 is 0.8323 shows that they are in close agreement with each other, which indicates that the derived model predictions are significantly reliable and hence valid considering the proximate agreement with the results from actual experiment and regression model.

ii) Comparison with Adequate Precision ratio
Adequate Precision ratio measures the signal to noise ratio and compares the range of the predicted values at the design points to the average prediction error. The value of adequate precision ratio obtained in this study is 7.609 which indicates adequate model efficacy.

iii) Comparison with coefficient of regression (R^2)
The coefficient of regression R^2 was used to validate the fitness of the model equation. Result from the study shows that the R^2 value for water yam was 0.9254. This shows that 92.54% of the variability in the response can be explained or accounted for by the model implying that the predictions of the experimental data are quite satisfactory.
3 D Surface plots for optimization of reducing sugar yield for water yam.

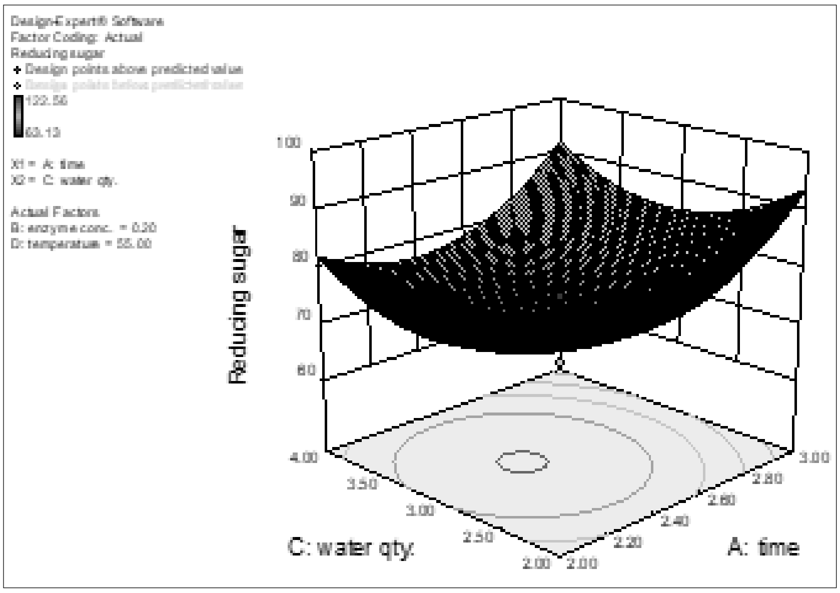

Fig 3: 3D Surface plot of reducing sugar yield showing combined effect of water quantity and enzyme on water yam starch.

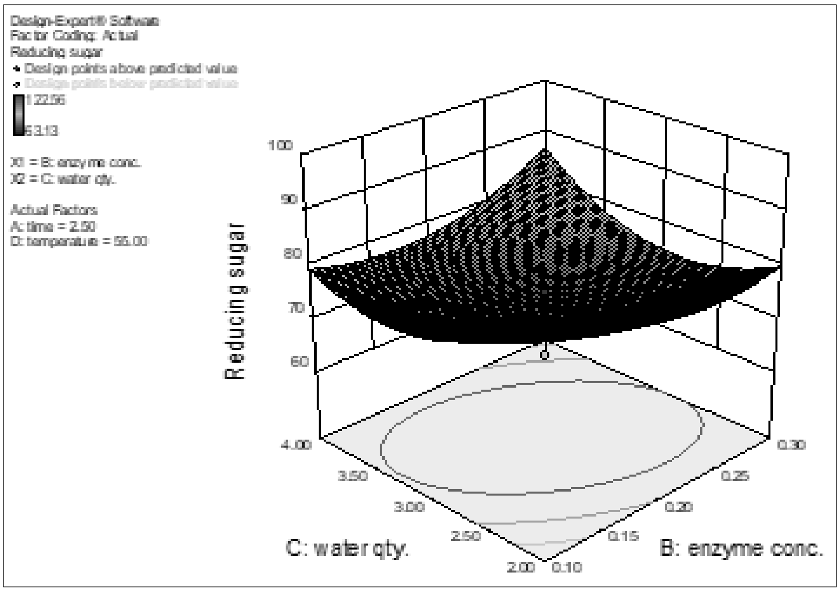

Fig. 4: 3D Surface plot of reducing sugar yield showing combined effect of water quantity and enzyme water yam starch

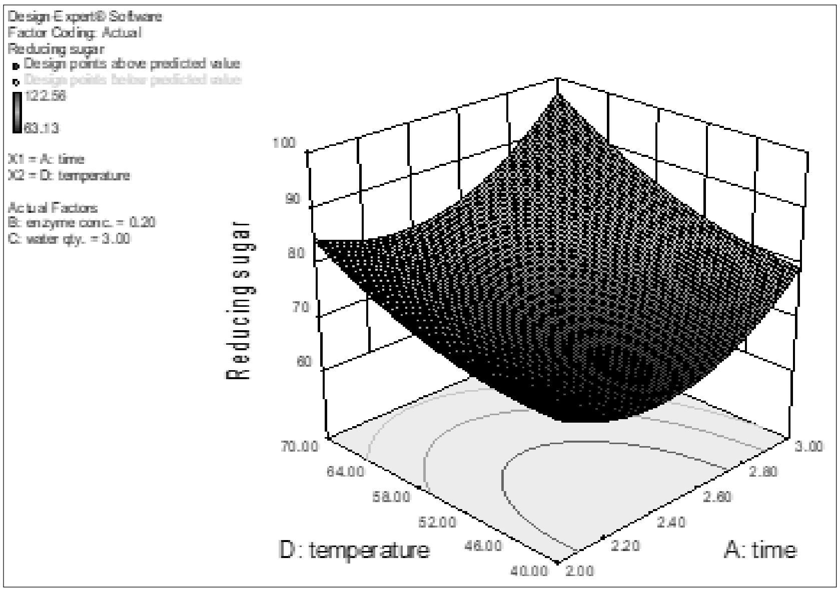

Fig 5: 3D Surface plot of reducing sugar yield showing combined effect of temperature and time water yam starch.

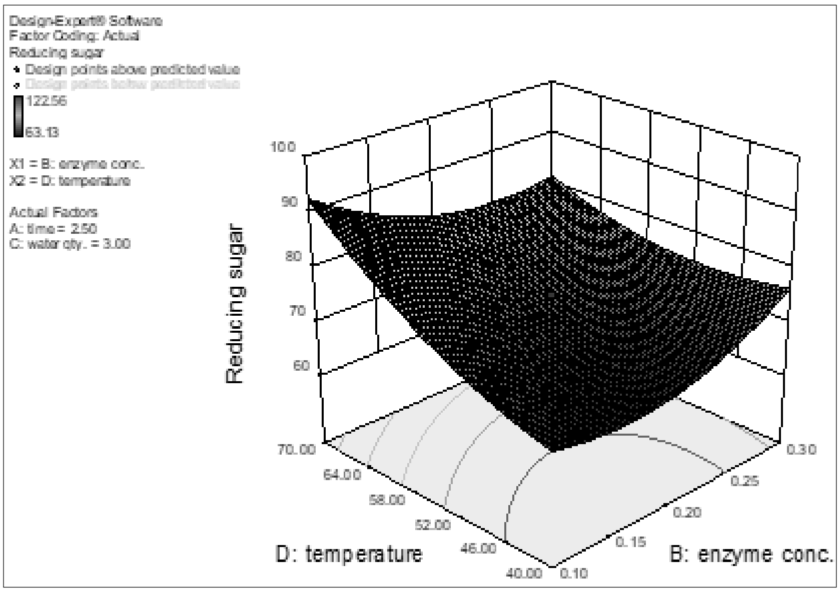

Fig 6: 3D Surface plot for reducing sugar yield showing combined effect of temperature and enzyme concentration on water yam starch.

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