Optimization of Process Conditions for Alcoholic Wine Production from Pineapple Using RSM

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***Abstract–* Process factors for the production of alcoholic wine from pineapple fruits were successfully optimized using central composite design with the aim of minimizing the final sugar concentration of the wine. The design involved four numeric factors; initial sugar concentration, pH, fermentation time and yeast concentration. All the factors were considered significant by ANOVA except pH. Quadratic model was developed and validated to explain the fermentation process. Numerical optimization of the process factors was achieved with the target of minimizing the final sugar concentration. The optimum conditions obtained were; yeast concentration of 7.46g/l, pH of 5.43, initial sugar concentration of 23%brix and fermentation time of 11days with predicted final sugar concentrations of 7.778% brix at 9.8 desirability.**

***Keywords–* ANOVA, Central Composite Design, Fermentation, Optimization and Pineapple**

I. INTRODUCTION

Although grapes are the main raw material used for wine production, there is an increasing interest in the search of other fruits, such as apricot, apple and palm sap, suitable for wine making. In countries where grapes are not abundantly available, local fruits that are cheap and readily available are used as an alternative [1]-[2]. Grapes and apples have been widely applied to ferment beverages [3] the use of other fruits, such as orange [4] cacao [5], mango [6], gabiroba [7], cajá [8], kiwi [9], in the production of wine has been recently demonstrated. Generally, fruits contain quantities of sugar that can be used by yeast during the fermentation process. In addition to the inherent characteristics of fruit (pH values, sugar contents and nitrogen contents), other factors must be taken into account during fruit wine production. The initial sugar concentrations, fermentation temperatures, SO_2 concentrations and specific yeast strains are key factors in determining successful fermentative processes of fruit wine [10]. Since the beginning of the 1980s, the use of *Saccharomyces cerevisiae* yeast starters has been extensively applied in the industrial and homemade beverage production processes. Currently, most of the wine production processes rely on *S. cerevisiae* strains that allow rapid and reliable fermentations, reduce the risk of sluggish or stuck fermentations and prevent microbial contaminations [11].

Pineapple is grown in Nigeria and so many other countries of the world. It is processed and utilized in different ways depending on the available technology and this tropically abundant plant has not attracted much research interest to stimulate its economic, medicinal and nutritional values to residents.

A bibliographical search on the current production levels of pineapple fruits in parts of Enugu state, Nigeria reveals that about 100,000 metric tonnes annually come from only three local government areas on subsistence basis [12]. This is an encouraging and sustainable level of production. A negligible portion of the fruits is consumed by the farmers or sold in the local markets but the major portion is wasted, as a result of the virtual absence of storage capacities. Optimal processing of pineapple therefore presents an economically beneficial option which would offer an intervention for improved technology driven by the need to reduce cost indices for processing.

Optimization of process condition is one of the most critical stages in the development of an efficient and economic bio-process. The classical method of studying a variable at a time can be effective in some cases, but it is useful to consider the combined effects of the entire factors involved [13]. The conventional one-factor-at -a-time approach of optimization is not only tiresome but also ignores to merge interactions of each factor. One of the most common optimization techniques used in the last two decades is the response surface methodology (RSM).

RSM is a powerful mathematical model with a collection of statistical techniques by which interaction between multiple variables can be identified with fewer experimental trials. It is used to examine and optimize the operational variables for experimental design, model development, test variables and optimization conditions.

The major problems of one factor at a time approach are that they need a large number of experiments and often the models are very complicated to describe the experimental observation [13].

II. MATERIALS AND METHODS

A) Materials

The pineapple fruits, fresh, ripe and matured were purchased from a local market at Abakpa Nike Enugu, Enugu State Nigeria.

Commercial bakers' yeast Saccharomyces cerevisiae was obtained from De-cliff integrated Main market Enugu, Enugu State Nigeria.

B) Experimental Method

Production of alcoholic wine

The pineapple fruits were pulped in a blender to a slurry consistency. Sodium metabisulphite solution 5.6 % (5 mL per 4.5 L of slurry) was added to the slurry and blended for 10 minutes. Fruit juice was stirred and sieved from the pulp using screens of 120 µm and 100 µm aperture. The juice was reconstituted; 1.5 litres of fruit juice with 4 litres of distilled water and ameliorated to 25% sugar content with sucrose and 0.01755% w/v ascorbic acid. This formed what is known as 'must'. The 'must' was then sieved through a standard sieve mesh no. 35 or cheese cloth and transferred into a 5 L fermentation tank inoculated with a known quantity of baker's yeast and allowed to ferment at room temperature for specified time intervals. The experimental work was strictly carried out based on the Central Composite Design (CCD) matrix as depicted in Table 2.

III. RESULT AND DISCUSSION

A) Optimization using CCD

The optimization of process factors for the maximization of alcohol content of alcoholic wine or minimization of the final sugar content of the alcoholic wine from pineapple fruit was achieved using central composite design which is a type of response surface methodology. The CCD had full factorial cores encompassing four numeric factors. The numeric factors had low and high factorial levels, low and high axial levels and center points. The factors and levels used for the CCD is shown on the Table 1, while the design matrix with the response data is shown on Table 2.

B) Analysis of variance (ANOVA)

ANOVA was used to inspect the selected model and to assess the significance of experimental results. It tests the model, linear terms, quadratic terms and interaction terms. Any of the terms was removed from the model when it has insignificant p-value or was included to support model hierarchy.

TABLE 1:
FACTORS AND LEVELS FOR CENTRAL COMPOSITE DESIGN (CCD)

FACTORS	UNITS	-α	-1	0	+1	+α
Yeast Concentration	g/L	3	5	7	9	11
pH	_	4.25	5	5.75	6.5	7.25
Initial Sugar Concentration	% Brix	15	20	25	30	35
Fermentation time	Days	4	7	10	13	16

TABLE 2:
DESIGN MATRIX FOR CCD

Std Order	Run order	yeast conc (g/l)	pH	Sugar conc (% brix)	Ferm Final time(days)	Sugar conc (%brix)
3	1	5.00	6.50	20.00	7.00	17
15	2	5.00	6.50	30.00	13.00	9.6
27	3	7.00	5.75	25.00	10.00	6.2
23	4	7.00	5.75	25.00	4.00	6.4
11	5	5.00	6.50	20.00	13.00	16.2
28	6	7.00	5.75	25.00	10.00	10
7	7	5.00	6.50	30.00	7.00	6.8
9	8	5.00	5.00	20.00	13.00	10
26	9	7.00	5.75	25.00	10.00	6
2	10	9.00	5.00	20.00	7.00	10
1	11	5.00	5.00	20.00	7.00	8
13	12	5.00	5.00	30.00	13.00	16.2
30	13	7.00	5.75	25.00	10.00	8
10	14	9.00	5.00	20.00	13.00	9
12	15	9.00	6.50	20.00	13.00	9
20	16	7.00	7.25	25.00	10.00	10
24	17	7.00	5.75	25.00	16.00	10
21	18	7.00	5.75	15.00	10.00	9.8
5	19	5.00	5.00	30.00	7.00	10
25	20	7.00	5.75	25.00	10.00	5
8	21	9.00	6.50	30.00	7.00	6.6
16	22	9.00	6.50	30.00	13.00	6.4
29	23	7.00	5.75	25.00	10.00	6
6	24	9.00	5.00	30.00	7.00	9
4	25	9.00	6.50	20.00	7.00	16.2
19	26	7.00	4.25	25.00	10.00	9.6
17	27	3.00	5.75	25.00	10.00	19
14	28	9.00	5.00	30.00	13.00	16.8
22	29	7.00	5.75	35.00	10.00	5
18	30	11.00	5.75	25.00	10.00	12

TABLE 3:
ANOVA TABLE

Source	Sum of Squares	df	Mean Square	F Value	P-value Prob>F
Model	388.67	9			<0.001
A-Yeast					
Concentration	25.63	1	25.63	8.21	0.0096
B-pH	6.667E-003	1	6.667E-003	2.372E-003	0.9617
C-initial Sugar conc.	23.21				0.0130
			23.21	7.44	
		1			
D- Fermentation time					
B.C					
BD	11.76				0.0664
			11.76	3.77	
CD	121.00	1			<0.001
			121.00	38.78	
A^2	26.01	1			0.009
			26.01	8.34	
B^2	34.81	1			0.0033
			34.81	11.16	
Residual	138.06	1			<0.0001
			138.06	44.25	
Lack of fit	17.22	1			0.0292
			17.22	5.52	
Pure error	62.40	1			
			3.12		
Cor total	45.87	20			0.5903
			3.06	0.92	
	16.53	15			
			3.31		
	451.08	5			
			451.08		
		29			

Predicted R – square = 0.6538, Adeq. Precisim = 13.317

R-square = 0.8617, Adj R – Square = 0.7994

From ANOVA Table 3, it was seen that F-value was significant. There was only 0.01% chance that a "model F-value" this large could occur due to noise. Values of Prob> F less than 0.0500 indicated that the model terms were significant, values greater than 0.100 indicated the model terms were not significant. In the study, the linear effect of sugar concentration, linear effect of fermentation time, interaction effect of yeast concentration and pH, interaction effect of pH and sugar concentration, interaction effect of sugar concentration and fermentation time, quadratic effect of yeast concentration and fermentation time, quadratic effect of pH were significant. The lack of fit F value of 0.92 implied the lack of fit was not significant relative to pure error. There was 59.03% chance that a lack of fit F-value this large could occur due to noise.

Other statistical tools that were used to assess the selected model were; R-Squared, predicted R-squared and adjusted R-squared. From the results, it can be seen that the R-squared value was high and the adjusted R-squared was in good agreement with predicted R-squared. The adequate precision measured the signal to noise ratio, a ratio of 13.31 obtained was high indicating an adequate signal.

C) Model equations

The model equation was presented on both actual and coded form. Both forms represent the mathematical form of the alcoholic wine production. The coded form can only be used for response prediction because it removes the factor's unit of measures. The actual form cannot be used because it has been scaled to accommodate their different units of measures. The model equation is good enough to help one move in the proper direction, but not to make exact prediction particularly outside the actual experimental region [14].

Final model equation in coded form:

Final sugar conc (% Brix) = + 7.61 – 103A – 0.017B – 0.98C + 0.70D – 2.95BC – 1.27BD + 1.48CD + 2.20A^2 + 0.78B^2 (1)

Final model equation in actual form:

Final sugar conc (%Brix) = - 26.75582 – 8.22760 yeast conc (gl) + 8.06944PH + 3.03667 initial sugar conce (% Brix) + 1.3333 fermentation time (days) – 0.7333pH. initial sugar conc (%Brix) – 0.56667pH. fermentation time (days) + 0.098333 initial sugar conc (% brix). Fermentation time (days) + 0.55078 yeast conc $(g/l)^2$ + 1.38333 pH^2. (2)

The effect of the process factors gave overall intercept of 7.61. Each factor adjusted the response by the value of its coefficient. Yeast concentration adjusted the intercept negatively by 1.03 while its quadratic effect increased the slope by 2.20. pH which was considered insignificant by ANOVA but was included to support model hierarchy adjusted the intercept negatively by 0.017, while its interaction with initial concentration decreased the slope by 2.75. Its interaction with fermentation time reduced its sensitivity by 1.27, while its quadratic term increased the slope by 0.78. Initial concentration adjusted the intercept negatively by 0.98, but its interaction with pH decreased the slope by 2.75. Fermentation time increased the sensitivity of the response by 0.70 while its interaction with pH decreased it by 1.27.

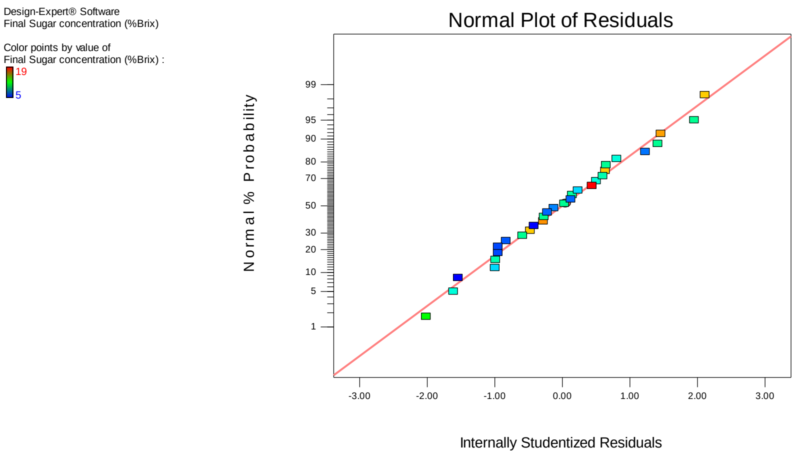
D) Model validation

It is good to check the model for adequacy in predicting the response. The best way of doing it is to generate residuals and observe its behaviors. For statistical purposes, ANOVA assumed that residuals were independent of each other and were distributed according to a normal distribution with constant variance. Table 4 shows the generated residuals with the predicted and actual values according to their standard order.

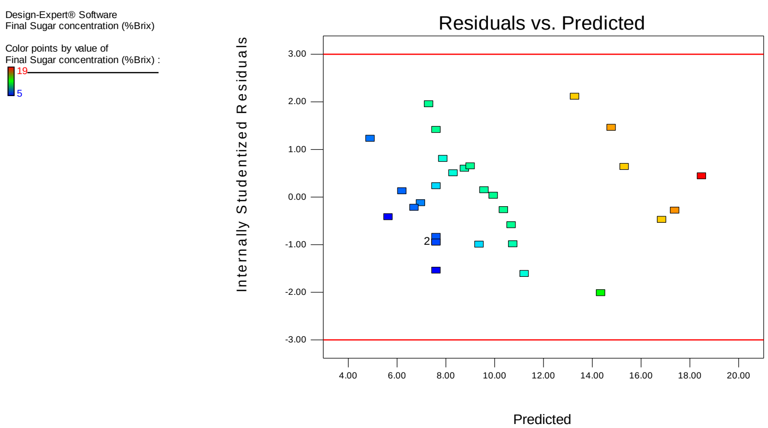
To test the generated residuals for conformation with ANOVA assumptions, diagnostic plots were used. The diagnostic plots used were normal plots of residuals, Residual vs. predicted values, residual vs. run and predicted versus actual values.

TABLE 4:
RESIDUALS WITH THE ACTUAL AND PREDICTED VALUES

Standard order	Actual values	Predicted values	Residuals
1	8.00	9.37	-1.37
2	10.00	7.31	2.69
3	17.00	17.39	-0.39
4	16.20	15.32	0.88
5	10.00	9.96	0.044
6	9.00	7.89	1.11
7	6.80	6.97	-0.17
8	6.60	4.91	1.69
9	10.00	10.37	-0.37
10	9.00	8.31	0.69
11	16.20	13.29	2.91
12	9.00	11.22	-2.22
13	16.20	16.86	-0.66
14	16.80	14.79	2.01
15	9.60	8.77	0.83
16	6.40	6.71	-0.31
17	19.00	18.49	0.51
18	12.00	14.35	-2.35
19	9.60	10.75	-1.15
20	10.00	10.69	-0.69
21	9.80	9.58	0.22
22	5.00	5.64	-0.64
23	6.40	6.21	0.19
24	10.00	9.01	0.99
25	5.00	7.61	-2.61
26	6.00	7.61	-1.61
27	6.20	7.61	-1.41
28	10.00	7.61	2.39
29	6.00	7.61	-1.61
30	8.00	7.61	0.39



(a)



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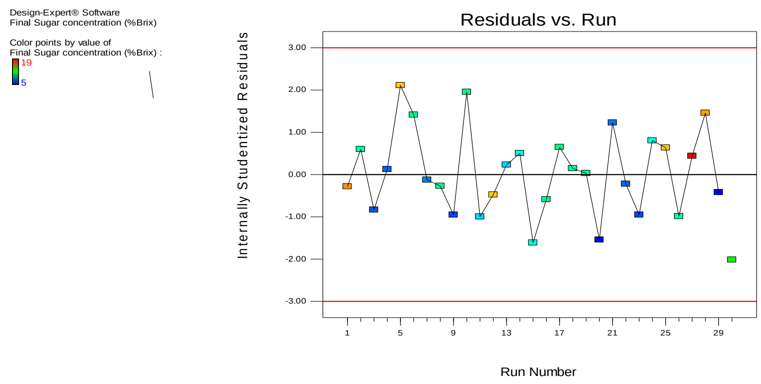

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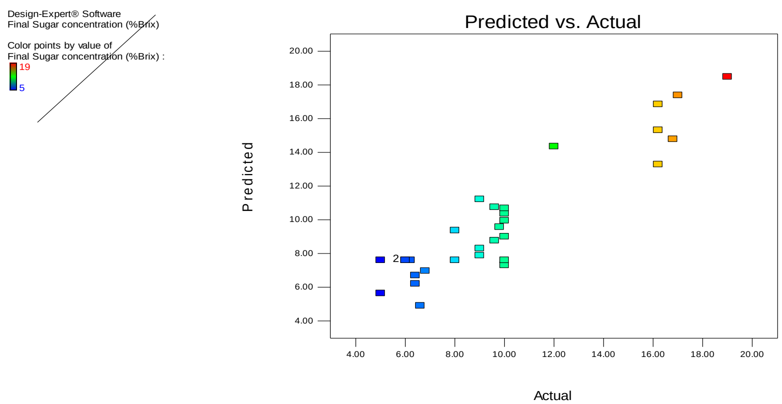


(d)

Fig. 1: Diagnostic plots (a) Normal plot of residuals (b) Residual vs. predicted values (c) Residual vs. run (d) Predicted versus actual values

The normal probability plot in Fig. 1(a) indicates whether the residuals follow a normal distribution in which case the point will follow a straight line. From the graph, the points followed a straight line. The plot of residual vs. predicted value in Fig. 1(b) was used to test the assumption of constant variance. The plot should be a random scatter. The pattern shows that there was a constant range of residuals across the graph. Plot of residual vs. runs in Fig. 1(c) allows one to check for lurking variables that may have influenced the response during the experiment in which case, the plot shows a random scatter. The plot of predicted vs. actual values in Fig. 1(d) was used to detect a value or group of values that were not easily predicted by the model. The data should be split evenly by 45^0 line. The results of the diagnosis revealed no problem which shows that the model met the assumptions of ANOVA and can be used to navigate the design space.

D) Effect of yeast concentration

Yeast concentration was considered significant by ANOVA. It is very necessary for fermentation because it converts the sugar to ethanol. The concentration of yeast was studied at low level of 5g/l and high level of 11g/l. It can be seen from fig. 2, that there was a slight curvature at the mid points of the factor. It means that increase in yeast concentration decreased the final sugar concentration to a point that further increase increased the final sugar concentration. It is worthy to note that final sugar concentration has inverse relationship with ethanol concentration, therefore, as the yeast concentration was increased, the ethanol concentration increased to a point that further increase led to decrease in ethanol concentration.

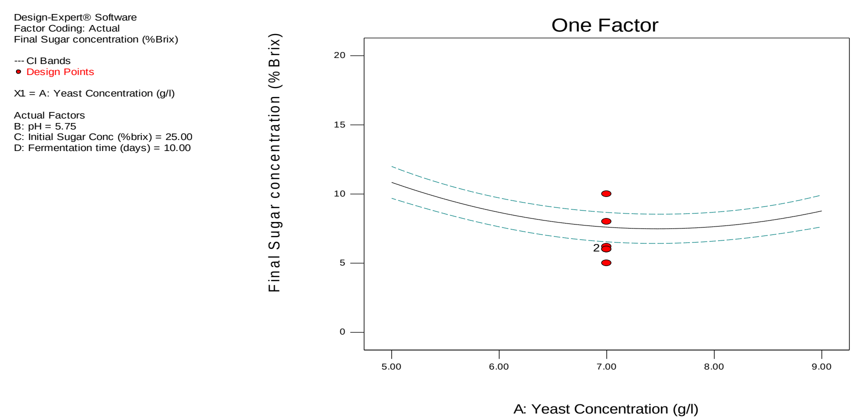


Fig. 2: Effect of yeast concentration

E) Effect of pH

pH is the measure of the acidity or basicity of an aqueous solution. It was studied because it affects the performance of yeast and consequently, the final sugar concentration. ANOVA confirmed pH to be insignificant within the ranges studied. This can be confirmed from the effect plot in Fig. 3. pH was included in the model to support model hierarchy since its interaction with other factors were significant.

F) Effect of initial sugar concentration

Sugar was the substrate for yeast; it utilizes the sugar for ethanol yield. It was considered significant by ANOVA and was studied at low level of 20.% brix and high level of 30% brix. The effect of initial sugar concentration is shown in Fig. 4 below. From the plot, it can be observed that the line graph had negative slope. This means that as the initial sugar Concentration was increased, the final sugar concentration decreased with attendant increase in ethanol content.

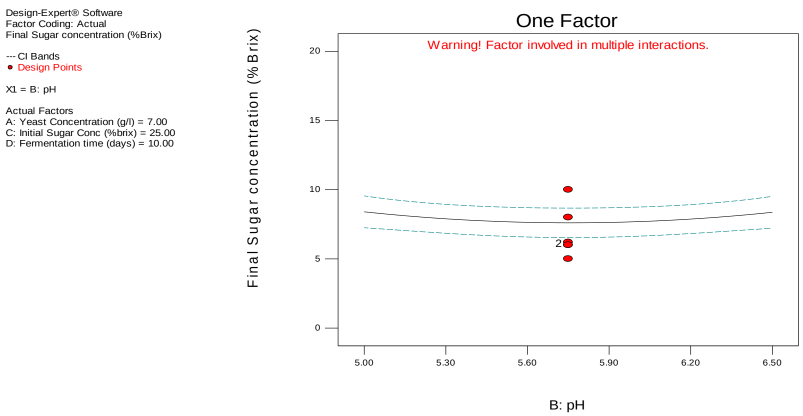


Fig. 3. Effect of pH

As the initial sugar concentration increases the yeast had more substrate to feed on with resultant increase in ethanol concentration.

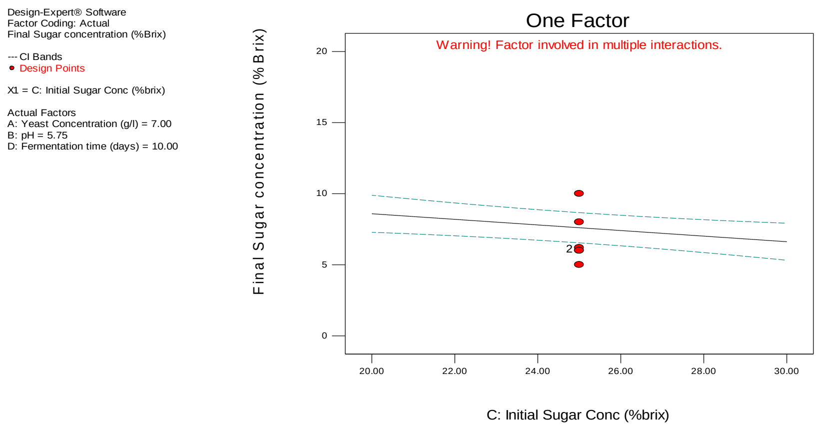


Fig. 4: Effect of initial sugar concentration

G) Effect of fermentation time

Time is needed for efficient conversion of sugar to ethanol by the yeast. Fermentation time was studied at how level of 7 days and high level of 13 days. The plot of effect of fermentation time is shown in Fig. 5.

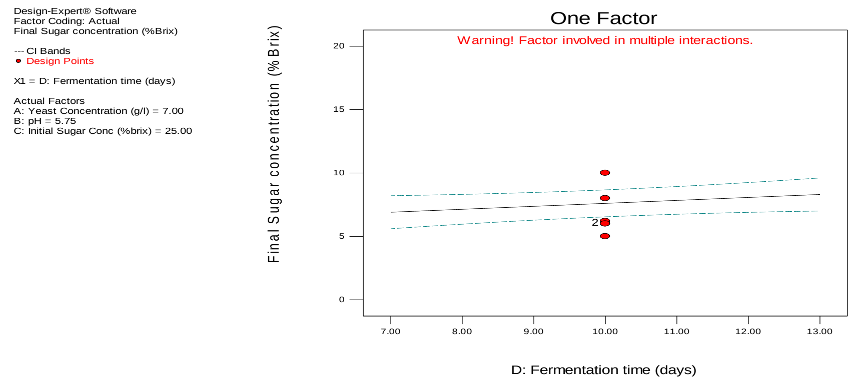
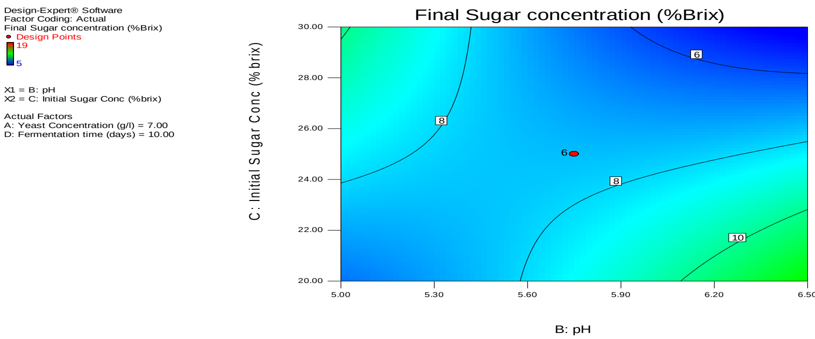


Fig. 5: Effect of Fermentation Time

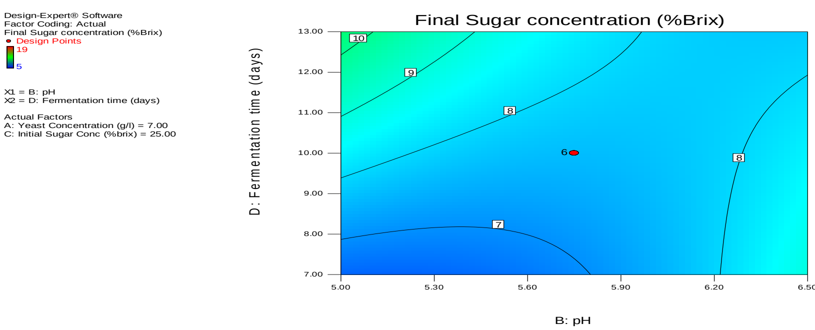
It can be seen from the plot that final sugar concentration increased with increase in fermentation time. This is possible because there exist maximum ethanol concentration that inhibits yeast activity. If fermentation time is increased beyond the optimum ethanol concentrations, the ethanol content decreases.

H) Contour plots

The contour plots are two dimensional plots of the response across the selected factors. The circular nature of some of the plots showed that the optimum values of the test variables cannot be easily obtained. It gives an idea of region of interest, where the optimum conditions could be found without specifying the conditions. The contour plot of interaction effect of initial sugar concentration and pH is shown in fig. 6a, the contour plot of interaction effect of fermentation time and pH as shown in fig. 6b, and the interaction effect of fermentation time and initial sugar concentration is shown in Fig. 6(c).



(a)



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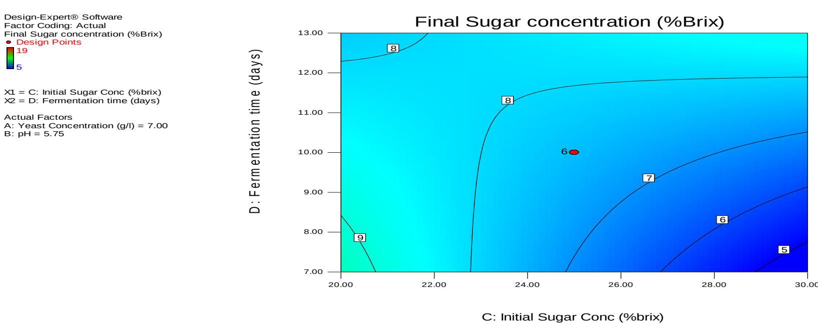


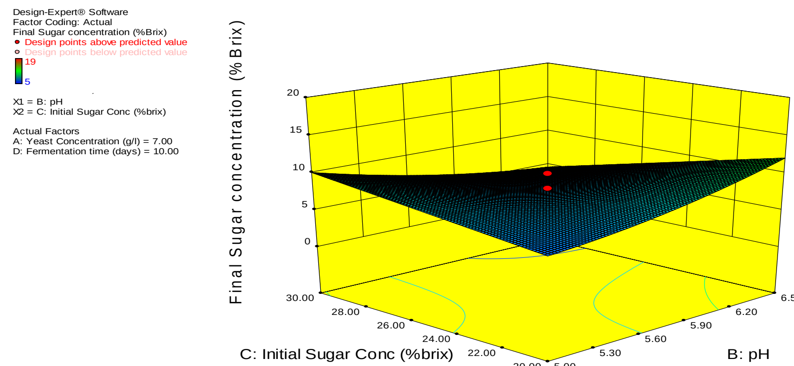
(c)

Fig. 6: Contour Plots of Interaction Effects (a)Initial concentration and pH (b) fermentation time and pH, (c) fermentation time and initial sugar concentration.

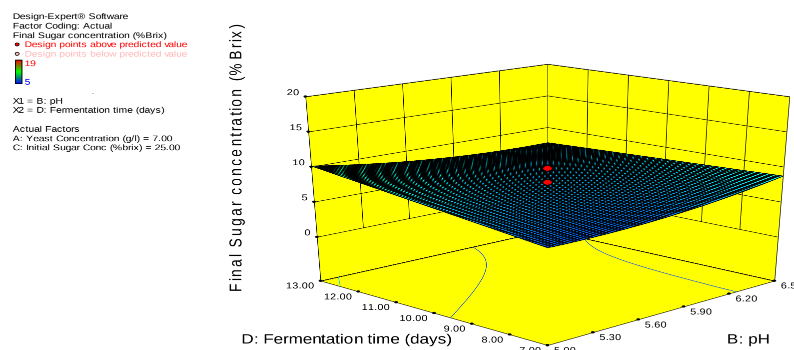
It can be seen from Fig. 6(a) that final sugar concentration decreases as initial sugar concentration decreases with decrease in pH. In Fig. 6(b), it was observed that final sugar concentration decreases as fermentation time decreased with decrease in pH. Fig. 6(c) shows that final sugar concentration decreased as initial sugar concentration increased with increase in fermentation time. The optimum conditions of the plots of Fig. 6 lie on the free spaces.

I) 3D surface plots

There dimensional plots of the factors are shown in Fig. 7. The slope of the curve gives an idea of the type of optimization involved. The 3D plot of interaction effect of initial sugar concentration and pH is shown in Fig. 7(a), the 3D plot of interaction effect of fermentation time and pH as shown in Fig. 7(b), and the interaction effect of fermentation time and initial sugar concentration is shown in Fig. 7(c).



(a)



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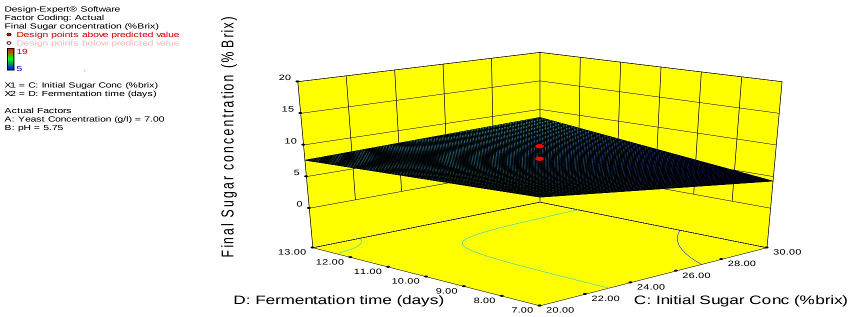

(c)

Fig. 7: 3D surface Plots of Interaction Effects (a) Initial concentration and pH (b) fermentation time and pH, (c) fermentation time and initial sugar concentration

The shape of the curves shows the directions for the maximization or minimization of the response. Fig. 7(a) shows that the final sugar concentration increased as the pH was decreased and initial sugar concentration increased. Fig. 7(b) shows that the final sugar concentration decreases with decrease in pH and decrease in fermentation time. Fig. 7(c) shows that final sugar concentration decreased with decrease in fermentation time and increase in initial sugar concentration.

J) Process optimization

The numerical type of optimization was used to minimize the response in order to obtain the optimum process conditions needed for maximal ethanol production in alcoholic wine. The target was to minimize the final sugar concentration which will definitely results to increase in ethanol concentration. The optimum conditions with highest desirability were selected. The optimum conditions are; yeast concentration of 7.46g/l, pH of 5.43, initial sugar concentration of 23%brix and fermentation time of 11days with predicted final sugar concentrations of 7.778% brix at 9.8 desirability.

IV. CONCLUSION

This study has proven viability of producing alcoholic wine from pineapple fruits. Optimization of the process factors for the minimization of the final sugar concentration of the alcoholic wine was successfully achieved using CCD. Four process factors were considered for this study; initial sugar concentration in %brix, pH, fermentation time in days and yeast concentration in g/l. It was found according to ANOVA that all the process factors except pH were significant; pH was included to support model hierarchy. ANOVA equally confirmed the interaction effect of yeast concentration and pH, interaction effect of pH and sugar concentration, interaction effect of sugar concentration and fermentation time, quadratic effect of yeast fermentation time, and quadratic effect of pH to be significant. Quadratic model was developed for the process and was validated.

Numerical optimization was done with the target of minimizing the final sugar concentration. The optimum conditions obtain were; yeast concentration of 7.46g/l, pH of 5.43, initial sugar concentration of 23%brix and fermentation time of 11days with predicted final sugar concentrations of 7.778% brix at 9.8 desirability.

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