Optimizing the Fermentation of Banana (*Musa acuminate*) and Soursop (*Annona muricate*) Juice for Table Wine Production: A Response Surface Approach.

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Abstract

The objective of this work was to optimize the mixture components of banana, soursop juice and sugar in the process conditions of time and inoculum size, as it affects pH and specific gravity in wine production, using combine optimal design in response surface methodology (RSM). High alcohol tolerant yeast was isolated from raffia palm wine, characterized and used for the fermentation process. The fitted regression model for the response variables were significant (p<0.05). Pure error sum of squares for pH and specific gravity (SG) were respectively 0.0677 and 0.0018, with non-significant lack-of-fit (p>0.05). Coefficient of variation (CV) values were 2.90 and 2.17% for pH and SG, respectively. Coefficient of determination (R^2) for both models were significantly high, with values of 99.41 and 97.84 %, respectively for pH and specific gravity. Adequate precession values were significantly high (19.08 and 8.92). Predicted optimum condition and desirability index was 0.856, obtained respectively at 58.3, 20.0 and 21.7 % banana, soursop and sugar mix, and 2.70 % inoculum size fermented for 7 days. This optimum condition is recommended for wine production from fruit tropical juices.

Keywords: *Fermentation, Banana, Soursop, Wine, Optimization*

Introduction

Wine is any alcoholic beverage from juices of different fruits; thus, any fermented alcoholic fruit juice can be referred to as wine. Different fruits and strains of yeasts produce different types of wine, these variations result from the complex interactions between the biochemical components of the particular fruit, the reactions involved in fermentation and the overall production process (Ohoke and Igwebike, 2017). Historically, wine is the product of fermentation of grape species *Vitis vinifera*. The high sugar content of most *V. vinifera* varieties at maturity is the major factor in their selection for use in much of the world's wine production. Their natural sugar content provides the necessary material for fermentation.

Over the years, grape wine has dominated the wine market, except in those areas where cultivation of grapes is limited by climatic conditions. In such areas continuous efforts have been made to produce wine by fermenting other fruit juice (Ogodo *et al.*,2015). Blends of banana and soursop juices can be optimized for table wine production.

Banana (*Musa acuminata*) are potential substrates for wine production as a result of their sugar content and tropical flavor. It is a seasonal and highly perishable fruit but has a lot of nutritional benefits, thus demands in the market are high. It is known to be rich not only in carbohydrates, dietary fibres, high concentration of potassium, calcium, magnesium, and significant amount of sugar, but is also rich in many health-promoting bioactive phytochemicals (FAO, 2010).
Soursop (*Annona muricata*) fruit is a genus of tropical fruit trees belonging to the family *Annonaceae*; the trees are widespread in the tropics and frost-free subtropics of the world including Nigeria and other West African countries. The fruit pulp consists of white fibrous juicy segments surrounding an elongated receptacle. Juices produced from Soursop fruits pulp have increasingly gained global importance due to their characteristic unique flavor and colour. Soursop is the most versatile fruit for industrial purpose because it does not oxidize easily and there is a large recovery of pulp from the fruit during processing (Swami *et al.*, 2014).
Two important variables that determine the quality of a good wine are pH and specific gravity.
According to Fleet (2013), pH directly affects wine stability. This may be as a result of the fact that at a pH close to neutrality (7.0), most microorganisms such as bacterial and molds including some yeasts become more active for fermentation and subsequent spoilage of wine, while pH below 3.5 eliminates most of the microbes and favours only a few of the microorganisms for fermentation. Specific gravity refers to the ratio of the density of a liquid to the density of water (Okeke *et al*., 2015). It also indicates amounts of fermentable sugar or possible alcohol percentage in the must or wine.
Response Surface Methodology (RSM) has been used by different authors for optimization of processes. RSM relates product properties by using regression equations that describe interrelations between input variables and product properties. RSM can be used to reduce the number of experimental runs without affecting the accuracy of results while determining the interactive effect of different variables on the responses. It is different from the procedure that involves the isolation of test variables and changing one parameter at a time (Montgomery, 2005). RSM is an essential tool for designing, formulating, developing, and analyzing new scientific studies and product models. These models can then be used to calculate all combinations of variables and their effects within the test range. RSM has been widely applied in food processing. Some reported cases include, extrusion cooking of blends of soy flour and sweet potato flour (Iwe *et al*., 2001), extrusion of African breadfruit mixtures (Nwabueze, 2010), to mention a few. There is the Need to develop a suitable model that will optimize the fermentation process variables, the fruit mixture components and physicochemical properties of the fermented wine. The objective of this study was to optimize the mixture components of banana, soursop juice and sugar in the process conditions of time and inoculums size, as it affects pH and specific gravity in production of wine from blends of banana and soursop juices using combine optimal design in response surface methodology (RSM).

2.0 Material and Methods

2.1 Materials

Mature soursop (*Annona muricata L.*) and banana (*Musa acuminata*) fruits were purchased from Mile 1 Market in Port Harcourt, Rivers State, Nigeria. Fresh palm wine from raffia palm (*Raphia farinifera*) were collected from tapped source in Rivers State, Nigeria into sterile calabash and

transported in ice pack thermo-flask to the Department of Food Science and Technology, Microbiology Laboratory, Rivers State University for isolation of yeast.
Sugar, cheese cloth, wine vat was purchased from Spar super store, Port Harcourt, Rivers State.

2.2 Preparation of "mixed must"(Juice Extraction)

Using the method of Okeke *et al*. (2015) with modification.
Six kilograms each of "soursop and banana" fruit were weighed, washed, peeled, sliced, rewashed, with the removal of the seeds for soursop and then reweighed. The fruit was then blended with a sterile blender into puree and filtered. Three hundred (300mL) milliliters of distilled water were first added to the blender to avoid friction in the blender and then another 300mLwas added to extract the "must" The slurry was diluted in a ratio of 1:1 (water and pulp) blanched at 80 ℃ for 5 min in a water bath and sieved with a muslin cloth of pore size 0.8mm to obtain the filtrate "Must".The overall water added during the blending was 6000mls for each of the fruits. The blend ratio of banana (30 to 90%), soursop (0 to 40%), sugar (10 to 30%) (chaptalization) with palm wine yeast with inoculums size of 2 to 10mls/100mls of "Must". Fermentation was done for 7 to 14 days, based on design provision as seen in Tables 1 and 2.

2.3 Yeast Isolation and Inoculum preparation

Hundred (100mL) milliliters of each must (soursop and banana) were mixed in a 500ml conical flask. Ammonium sulphate and potassium di-hydrogen phosphate (0.12%) was added as yeast nutrient. The mixture was autoclaved at 121℃ for 15 minutes. Three loopfuls of the stock culture from an SDA slant was transferred into the 200ml standard "must" in a conical flask and incubated in a rotary shaker at 37 ^{0}Cfor 48 h as described by Okeke *et al.,* (2015). All procedures were done under aseptic condition.

2.4 Experimental Design

Combined Optimal (I-Optimal) design in response surface methodology (RSM) was used, no block with forty-six runs generated, as shown in the design Matrix (Table 1). The mixture components (A, B and C) were coded low and high, with values ranging from 0.3 to 0.9, 0 to 0.4 and 0.1 to 0.3 for Banana, Soursop and sugar, respectively, as shown in the mixture component table (table 1). Dependent variables such as pH and specific gravity (SG) of the juice blends were evaluated as responses. Process factors were yeast (2 - 10%) and time (7 - 14 days) represented by D and E respectively (table 2). All experiments were performed in triplicates. statistical design, analyses and optimization was performed using design expert (Stat-Ease Inc., Minneapolis, MN, USA) software version 11 (Stat-Ease, 2018).

Table 1 Mixture Components

Component	Name	Units	Type	Minimum	Maximum	Coded Low	Coded High	Mean	Std. Dev.
A	BANANA		Mixture	0.3	0.9	+0 ↔ 0.3	+1 ↔ 0.9	0.5933	0.1728
B	SOUSOP		Mixture	0	0.4	+0 ↔ 0	+0.666667 ↔ 0.4	0.2056	0.1488
C	SUGAR		Mixture	0.1	0.3	+0 ↔ 0.1	+0.333333 ↔ 0.3	0.2011	0.0778
				Total =	1.0000	**L_Pseudo Coding**			

Table 2 Process Factors

Factor	Name	Units	Type	Minimum	Maximum	Coded Low	Coded High	Mean	Std. Dev.
D	YEAST	%	Numeric	2.00	10.00	-1 ↔ 2.00	+1 ↔ 10.00	6.23	2.93
E	TIME	days	Numeric	7.00	14.00	-1 ↔ 7.00	+1 ↔ 14.00	10.61	2.59

Table 3 Combined Optimal Design Matrix and Experimental Values of the Responses

Run	Component 1 A: BANANA	Component 2 B: SOUSOP	Component 3 C: SUGAR	Factor 4 D: YEAST (%)	Factor 5 E: TIME (days)	pH	SG
1	0.41	0.29	0.30	3.20	14.00	3.22	1.13
2	0.81	0.00	0.19	2.00	9.66	3.7	1.13
3	0.50	0.40	0.10	10.00	8.05	4.14	1.08
4	0.38	0.32	0.30	10.00	14.00	3.16	1.08
5	0.64	0.17	0.18	4.80	7.00	4.2	1.13
6	0.57	0.24	0.19	6.62	10.89	3.77	1.01
7	0.49	0.40	0.11	2.00	9.63	4.25	1.11
8	0.80	0.10	0.10	2.00	9.70	4.41	1.09
9	0.50	0.40	0.10	6.60	11.12	4.59	1.17
10	0.56	0.24	0.21	2.00	9.59	3.2	1.07
11	0.54	0.36	0.10	4.80	7.00	4.18	1.08
12	0.39	0.40	0.21	3.24	14.00	3.65	1.06
13	0.55	0.15	0.30	6.52	10.85	3.64	1.01
14	0.82	0.08	0.10	10.00	7.88	3.32	1.03
15	0.81	0.09	0.10	6.60	10.82	3.23	1.01
16	0.33	0.40	0.27	6.56	11.03	3.71	1.01
17	0.73	0.00	0.27	4.88	7.00	4.84	1.15
18	0.63	0.16	0.21	10.00	14.00	4.24	1.1
19	0.30	0.40	0.30	6.60	14.00	3.18	1.07
20	0.57	0.24	0.19	6.62	10.89	3.77	1.01
21	0.60	0.30	0.10	10.00	14.00	3.07	1.09
22	0.90	0.00	0.10	3.60	14.00	3.55	1
23	0.37	0.40	0.23	4.80	7.00	4.04	1.16

24	0.70	0.00	0.30	9.60	13.51	3.36	1.05
25	0.64	0.17	0.18	4.80	7.00	4.2	1.19
26	0.72	0.00	0.29	3.56	14.00	3.34	1
27	0.89	0.00	0.12	9.84	13.65	3.31	1.02
28	0.78	0.00	0.22	6.52	10.85	3.16	1.01
29	0.30	0.40	0.30	2.00	9.59	3.37	1.08
30	0.51	0.19	0.30	7.16	8.33	4.44	1.17
31	0.65	0.16	0.19	3.20	14.00	3.5	1
32	0.79	0.00	0.21	10.00	8.02	3.12	1.07
33	0.30	0.40	0.30	10.00	11.06	4.07	1.01
34	0.73	0.17	0.10	10.00	10.89	4.71	1.03
35	0.78	0.00	0.22	6.52	10.85	3.22	1.01
36	0.56	0.24	0.21	2.00	9.59	3.14	1.07
37	0.43	0.27	0.30	4.76	7.00	4.26	1.12
38	0.57	0.24	0.19	10.00	7.88	4.2	1.13
39	0.58	0.32	0.10	3.16	14.00	3.42	1
40	0.73	0.17	0.10	6.67	14.00	3.67	1
41	0.90	0.00	0.10	4.84	7.00	2.93	1
42	0.59	0.11	0.30	10.00	8.02	4.2	1.07
43	0.57	0.24	0.19	6.62	10.89	3.46	1.01
44	0.32	0.40	0.29	10.00	8.05	3.31	1.16
45	0.41	0.40	0.19	10.00	14.00	4.14	1.01
46	0.63	0.07	0.30	2.00	9.66	3.14	1.13

The experimental data was fitted to a quadratic model to express the response variables as a function of the independent variables using equation (1).

$$Y = \beta_o + \beta_1 A + \beta_2 B + \beta_3 C + \beta_{12} AB + \beta_{13} AC + \beta_{23} BC + \beta_{11} A^2 + \beta_{22} B^2 + \beta_{33} C^2 \quad (1)$$

Where Y is the desired value of response; A, B, C are independent variables; β_o is the constant; β_1, β_2, β_3 are coefficients of linear regression; β_{12}, β_{13}, β_{23} are coefficients of interaction regression and β_{11}, β_{22} and β_{33} are coefficients of quadratic regression. The model lack of fit was evaluated, and the model adequacies assessed using coefficient of determination (R^2), adjusted R^2, predicted R^2, adequate precision and coefficient of variation (CV). Analysis of variance (ANOVA) Tables were generated and the effect and regression coefficients of individual linear, quadratic and interaction terms were determined. The significance of all terms in the polynomial were judged statistically at 5% level of probability ($p<0.05$) (Iwe *et al*., 2023).

2.5 Test for pH

pH of the wine sample was measured using a pre-calibrated digital pH meter (Hanna pH 211 micro processor pH meter). Before reading pH, each sample was agitated (using a magnetic stirrer) for 30s until a stable reading is measured. Between readings, the electrode was rinsed with distilled water for the accuracy of the measurement. Test was made in triplicates as described by Ogodo *et al*., (2015).

2.6 Determination of specific gravity

The specific gravity was determined using a 50ml pycnometer (AOAC, 2012). The bottle (50mL) was cleaned with distilled water, dried in an oven and cooled. The dried empty bottle was weighed, and the value recorded as W_1. Then the bottle was filled with distilled water and weighed, recorded as W_2. Again, the bottle was dried and filled with the wine sample, weighed and recorded as W_3. The specific gravity of the sample will be calculated as shown below;

Specific gravity = $\frac{w3-w1}{w2-w1}$

Where,
W1 = weight of empty pycnometer
W2 = weight of distilled water
W3 =weight of wine sample

3. Results and Discussion

3.1. Model Fitting

3.1.1 ANOVA, Fit Statistics and Estimated Regression Coefficient for pH of Fermented Banana, Soursop and Sugar Blends, Based on Response Surface Methodology.

The analysis of variance, estimated regression coefficient and fit statistics for pH of fermented banana, soursop and sugar blends using combine optimal Design (I-Optimal) based on Response surface methodology is presented in table 4 and equation 2. The model was significant with p-value of 0.0003 ($p<0.05$). Coefficient of determination (R^2), Adjusted R^2 and Predicted R^2 were respectively 0.9941, 0.9556 and -44.8965, while the coefficient of variability (C.V) was 2.90 %. CV of less than 10 % is desirable (Edem and Elijah, 2016). CV is a measure of deviation from the mean values, which shows the reliability of the experiment. It is the standard deviation expressed as a percentage of the mean. Calculated by dividing the Standard deviation by the Mean and multiplying by 100. CV also describes the extent to which the data were dispersed as well as the reproducibility and repeatability of the model (Firatiligil-Durmus and Evranus, 2010). Shishir *et al*. (2016) reported that a CV < 10% indicates better precision and reliability.

Table 4 ANOVA and Fit Statistics For pH

Source	Sum of Squares	Df	Mean Square	F-value	p-value
Model	11.62	39	0.298	25.81	0.0003
(1)Linear Mixture	0.6921	2	0.346	29.97	0.0008
AB	0.4715	1	0.4715	40.84	0.0007
AC	0.4069	1	0.4069	35.24	0.001
AD	0.5009	1	0.5009	43.38	0.0006
AE	0.178	1	0.178	15.42	0.0077
BC	0.5132	1	0.5132	44.45	0.0006
BD	0.4193	1	0.4193	36.31	0.0009
BE	0.0396	1	0.0396	3.43	**0.1136**
CD	0.1243	1	0.1243	10.76	0.0168
CE	0.0259	1	0.0259	2.24	**0.185**
ABC	0.4023	1	0.4023	34.84	0.0011

ABD	0.5349	1	0.5349	46.33	0.0005
ABE	0.027	1	0.027	2.34	**0.1767**
ACD	0.1458	1	0.1458	12.63	0.012
ACE	0.0449	1	0.0449	3.89	**0.0961**
ADE	0.2556	1	0.2556	22.14	0.0033
BCD	0.1261	1	0.1261	10.92	0.0163
BCE	0.0229	1	0.0229	1.99	**0.2085**
BDE	0.7619	1	0.7619	65.99	0.0002
CDE	0.0059	1	0.0059	0.5108	**0.5016**
AB(A-B)	1.3	1	1.3	112.27	< 0.0001
AC(A-C)	0.1945	1	0.1945	16.85	0.0063
BC(B-C)	0.7738	1	0.7738	67.01	0.0002
ABCD	0.1959	1	0.1959	16.97	0.0062
ABCE	0.0648	1	0.0648	5.61	**0.0556**
ABDE	0.8621	1	0.8621	74.66	0.0001
ACDE	0.0095	1	0.0095	0.8244	**0.3989**
BCDE	0.0098	1	0.0098	0.8477	**0.3927**
ABD(A-B)	0.8917	1	0.8917	77.23	0.0001
ABE(A-B)	0.2178	1	0.2178	18.87	0.0049
ACD(A-C)	0.2487	1	0.2487	21.54	0.0035
ACE(A-C)	0.1883	1	0.1883	16.31	0.0068
BCD(B-C)	0.0499	1	0.0499	4.32	**0.0828**
BCE(B-C)	0.0026	1	0.0026	0.2282	**0.6498**
ABCDE	0.0255	1	0.0255	2.21	**0.1875**
ABDE(A-B)	0.4715	1	0.4715	40.84	0.0007
ACDE(A-C)	0.0275	1	0.0275	2.39	**0.1734**
BCDE(B-C)	0.0215	1	0.0215	1.86	**0.2218**
Residual	0.0693	6	0.0115		
Lack of Fit	0.0016	1	0.0016	0.1189	**0.7443**
Pure Error	0.0677	5	0.0135		
Cor Total	11.69	45			

$P<0.05$ (significant), **$p>0.05$** (not significant)

R^2=0.9941**, Adj**. **R^2**=0.9556, **Pred. R^2**=-44.8965, **C.V** (%) =2.90, **Adeq. Prec**.=19.0835, **Mean**=3.71, **Std Dev.**=0.1075

The fitted regression model in terms of coded factors, excluding insignificant terms:

pH = +8.87A – 52.29B +727.29C +115.18AB -1165.39AC +20.38AD -1278.86BC -86.13BD -2457.52CD +935.81ABC +174.77ABD +4640.03ACD -22.21ADE +4528.89BCD +320.74BDE -127.79AB(A-B) +373.71AC(A-C) +1037.23BC(B-C) -5343.07ABCD -644.17ABDE -284.92ABD(A-B) +74.44ABE(A-B) -2699.60ACD(A-C) +531.45ACE(A-C) +667.00ABDE(A-B) (2)

The goodness-of-fit of the model was also ascertained by the coefficient of determination (R^2). It is a measure of the amount of variation around the mean explained by the model (Stat-Ease, 2018). Jusoh *et al*. (2013) reported that the best R^2 value for a good model fitting was estimated between

0.8 and 1.0. Consequently, R^2 of 0.9941 (99.41%) given for pH indicates good fit for the model. Adequate precision was 19.0835. Adequate precision measures the signal to noise ratio. It compares the range of the predicted values at the design points to the average prediction error. A ratio greater than 4 is desirable (Edem and Elijah, 2016; Stat-Ease, 2018). The ratio of 19.0835 given, indicates an adequate signal. The model can be used to navigate the design space. Negative predicted R^2 indicates more terms that were insignificant (Ghosh *et al*., 2012).
The estimated regression coefficient for pH showed that all linear mixture terms were significant ($p<0.05$). Mean pH was 3.71.

3.1.2 ANOVA, Fit Statistics and Estimated Regression Coefficient for Specific Gravity (SG) of Fermented Banana, Soursop and Sugar Blends, Based on Response Surface Methodology.

From table 5, the model for SG was significant with p-value of 0.0107 ($p<0.05$). Coefficient of determination (R^2) was 0.9784 (97.84%), this showed good fit for the model, implying that 97.84% of variation in SG was explained by the quadratic model. Adjusted R^2 and Predicted R^2 were respectively 0.838 and -3177.1866, while the coefficient of variability (C.V) and adequate precision were respectively 2.17 % and 8.9161. CV < 10% indicates better precision and reliability (Shishir *et al*., 2016). The Lack of Fit F-value of 3.95 implies the Lack of Fit is not significant relative to the pure error. There is a 10.35% chance that a Lack of Fit F-value this large could occur due to noise. Non-significant lack of fit is good.
The estimated regression coefficient for Specific gravity showed that all linear mixture terms were significant ($p<0.05$). Mean specific gravity was 1.07 with Std, deviation 0.0232. The model equation is good enough to help one move in the proper direction, not to make exact prediction particularly outside the actual experimental region (Ejikeme *et al.,* 2004).

Table 5 ANOVAand Fit Statistics for Specific Gravity (SG)

Source	Sum of Squares	df	Mean Square	F-value	p-value
Model	0.146	39	0.0037	6.97	0.0107
(1)Linear Mixture	0.0134	2	0.0067	12.44	0.0073
AB	0.0202	1	0.0202	37.53	0.0009
AC	0.0306	1	0.0306	57.06	0.0003
AD	0.0003	1	0.0003	0.5253	**0.4959**
AE	0.0039	1	0.0039	7.25	**0.036**
BC	0.0298	1	0.0298	55.51	0.0003
BD	5.05E-06	1	5.05E-06	0.0094	**0.9259**
BE	0.0033	1	0.0033	6.09	0.0486
CD	0.0141	1	0.0141	26.31	0.0022
CE	0.0048	1	0.0048	9.02	0.0239
ABC	0.0287	1	0.0287	53.47	0.0003
ABD	0.0007	1	0.0007	1.25	**0.307**
ABE	0.0037	1	0.0037	6.95	0.0387
ACD	0.0147	1	0.0147	27.29	0.002
ACE	0.0048	1	0.0048	8.93	0.0244

ADE	0.0027	1	0.0027	5.1	**0.0648**
BCD	0.0144	1	0.0144	26.75	0.0021
BCE	0.0051	1	0.0051	9.53	0.0215
BDE	0.0017	1	0.0017	3.21	**0.1233**
CDE	0.0141	1	0.0141	26.19	0.0022
AB(A-B)	0.0228	1	0.0228	42.44	0.0006
AC(A-C)	0.0295	1	0.0295	54.92	0.0003
BC(B-C)	0.0278	1	0.0278	51.81	0.0004
ABCD	0.0163	1	0.0163	30.27	0.0015
ABCE	0.0055	1	0.0055	10.24	0.0186
ABDE	0.0036	1	0.0036	6.77	0.0405
ACDE	0.0135	1	0.0135	25.2	0.0024
BCDE	0.0133	1	0.0133	24.83	0.0025
ABD(A-B)	0.0018	1	0.0018	3.38	**0.1158**
ABE(A-B)	0.0045	1	0.0045	8.39	0.0275
ACD(A-C)	0.0166	1	0.0166	30.86	0.0014
ACE(A-C)	0.0036	1	0.0036	6.67	0.0416
BCD(B-C)	0.0122	1	0.0122	22.71	0.0031
BCE(B-C)	0.0053	1	0.0053	9.88	0.02
ABCDE	0.0114	1	0.0114	21.29	0.0036
ABDE(A-B)	0.0002	1	0.0002	0.2913	**0.6088**
ACDE(A-C)	0.0119	1	0.0119	22.16	0.0033
BCDE(B-C)	0.0215	1	0.0215	40.02	0.0007
Residual	0.0032	6	0.0005		
Lack of Fit	0.0014	1	0.0014	3.95	**0.1035**
Pure Error	0.0018	5	0.0004		
Cor Total	0.1492	45			

$P<0.05$ (significant), **$p>0.05$** (not significant)
R^2=0.9784, **Adj**. **R^2**=0.838, **Pred. R^2**=-3177.1866, **C.V** (%) =2.17, **Adeq. Prec**.=8.9161, **Mean**=1.07, **Std Dev.**=0.0232

The fitted regression model in terms of coded factors, excluding insignificant terms:
SG = +1.14A -9.33B +165.11C +23.82AB -319.85AC +0.95AE -308.23BC +6.84 -828.64CD +134.56CE +250.05ABC -17.00ABE +1471.16ACD -222.37ACE +1528.98BCD -270.69BCE -1029.12CDE -16.95AB(A-B) +145.52AC(A-C) +196.71BC(B-C) -1539.35ABCD +255.63ABCE -41.85ABDE +1819.47ACDE +1806.51BCDE +10.70ABE(A-B) -696.88ACD(A-C) +73.32ACE(A-C) -863.29BCD(B-C) +143.07BCE(B-C) -1672.96ABCDE -870.64ACDE(A-C) -954.25BCDE(B-C) (3)

3.2 Effect of Mixture Components (Banana, Soursop, Sugar), Fermentation Time and yeast Concentration on the pH.

Result showed that A, C, AB, AD, ABC, ABD, ACD, CD, BDE, AC(A-C), BC(B-C), ABE(A-B), ACE(A-C) and ABDE(A-B) are significant model terms and synergistic to pH of the fermented fruits, thus increase in these linear and interactive terms will increase the pH of the fermented wine significantly (Stat-Ease, 2018). B, AC, BC, BD, CD, ADE, AB(A-B), ABCD, ABD(A-B) and

ACD(A-C) are also significant model terms, but antagonistic to the pH of fermented fruits, which indicates that an increase in their linear and interaction value will cause a significant decrease in pH of the ferment.
As shown in the 3D surface plot of Fig 1 and 2. Increased interaction effect of ABC and ABD, respectively increased the pH of the fermented wine. Increased interaction effect of BCD showed a synergistic effect on the pH (Fig. 3). Effects of interaction of banana, sugar and yeast concentration (ACD) on pH is presented in Fig. 3. pH is shown to decrease as interaction of banana, sugar and yeast (ACD) increased, probably due to increased fermentation and acid production. Low pH and high acidity are known to give fermentation yeast comparative advantage in natural environments (Kiin-Kabari *et al*., 2019). A similar observation has been reported by Okeke *et al*., (2015) in their study on mixed fruits (pineapple and watermelon). Increased interaction of ACD also increased the pH of the fermented wine (Fig. 4). pH of the wine is considered as an important attribute that significantly enhances the organoleptic properties and provides suitable atmosphere for the yeast growth (Arroyo-López *et al*., 2009).

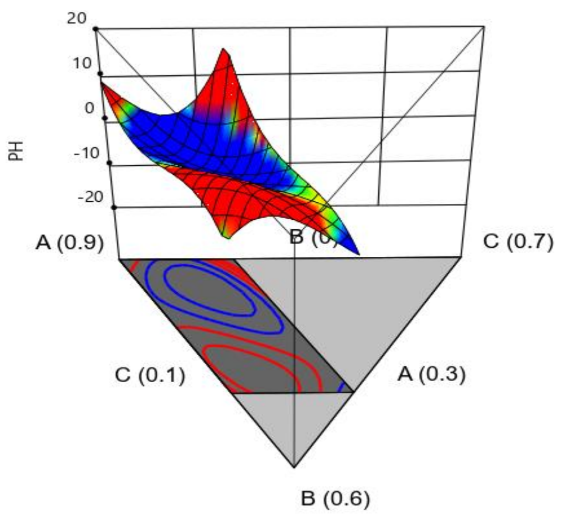

Fig1 Effects of interaction of mixture components on pH

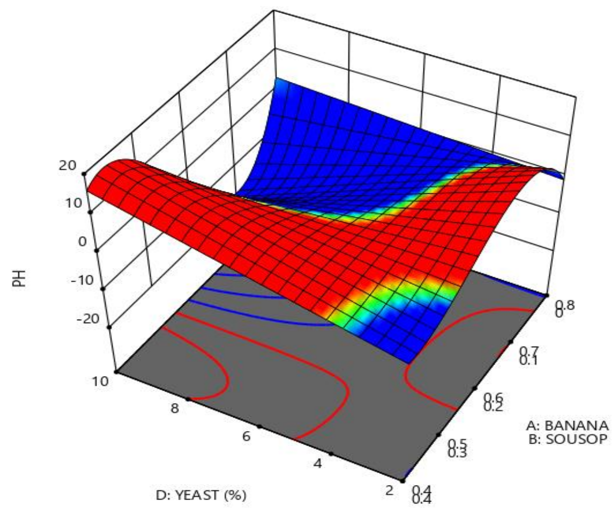

Figure 2 Effects of interaction of Banana, Soursop and Yeast concentration on pH

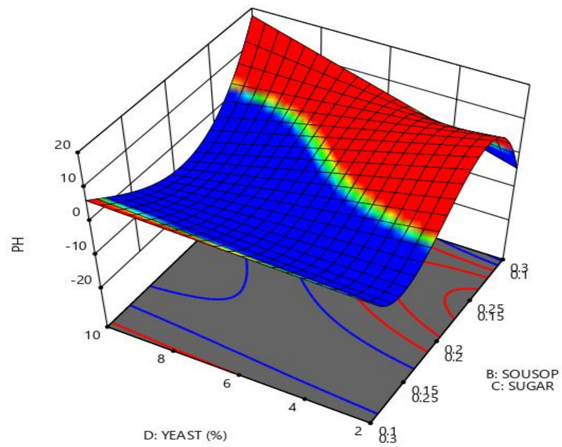

Figure 3 Effects of interaction of Soursop, Sugar and Yeast concentration on pH

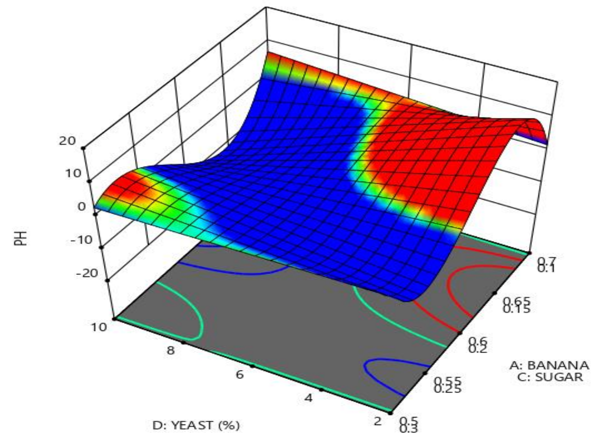

Figure 4 Effects of interaction of Banana, Sugar and Yeast concentration on pH

3.3 Effect of Mixture Components (Banana, Soursop, Sugar), Fermentation Time and yeast Concentration on The Specific Gravity (SG).

Increase in the interaction effect of banana, soursop and sugar only (Fig. 5), is shown to increase the specific gravity of the fermented wine. Result showed that A, C, AB, AE, CD, CE, ABC, ACD, BCD, AC(A-C), BC(B-C), ABCE, ACDE, BCDE, ABE(A-B), ACE(A-C) and BCE(B-C) are significant model terms and synergistic to Specific gravity of the fermented fruits. B, AC, BC, ABE, ACE, BCE, CDE, AB(A-B), ABCD, ABDE, ACD(A-C), BCD(B-C), ABCDE, ACDE(A-C) and BCDE(B-C) are also significant model terms, but antagonistic to the specific gravity of fermented fruits. A negative coefficient of the independent variables in the model represents antagonistic effects, while positive coefficient represents synergistic effects. The Effects of interaction of Banana, Soursop and fermentation time (ABE) has an antagonistic effect on the specific gravity (Fig. 6), this was probably due to longer fermentation time with increased alcohol production, leading to reduced specific gravity. Reduction in SG with increased fermentation time had earlier been reported (Kiin-Kabari *et al*., 2019). Increased interaction effect of ACD (Fig. 7) showed a synergistic effect on the specific gravity. Effects of interaction of banana, sugar and time (ACE) on specific gravity is presented in Fig.8 with a coefficient of the specific gravity is seen to decrease. (Stat-Ease, 2018). Specific gravity decreased probably due to conversion of sugar to alcohols since alcohols have less Specific gravity than sugar (Okafor *et al*., 2014).

Effects of interaction of soursop, sugar and yeast concentration (BCD) on specific gravity is presented in Fig 9. The specific gravity is seen to increase with increased interaction of BCD. Fig.10 3D surface plot represents interaction effects of soursop, sugar and fermentation time (BCE) on the specific gravity of fermented wine. SG is seen to increase with increase interaction of BCE.

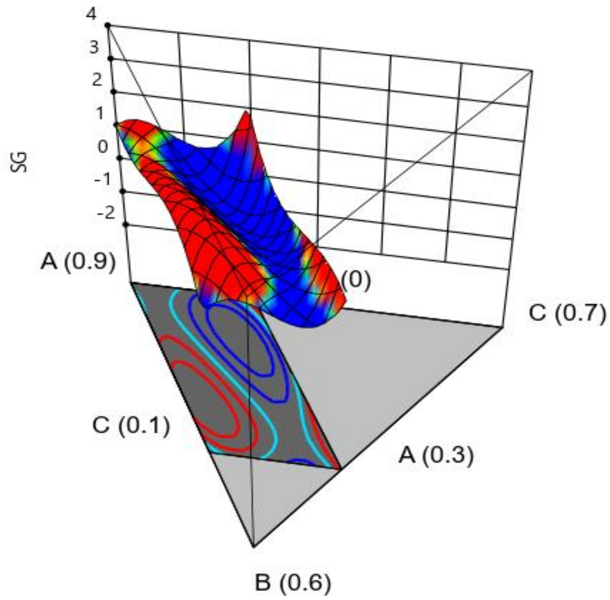

Fig 5 Effects of interaction of mixture components on Specific Gravity

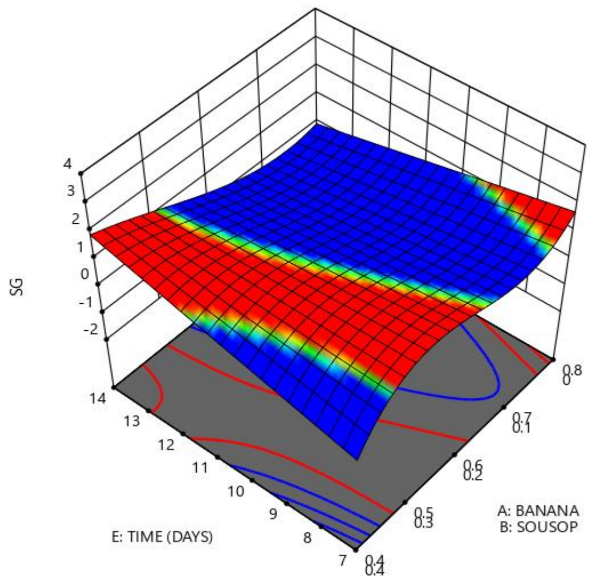

Fig. 6 Effects of interaction of Banana, Soursop and Time on Specific Gravity

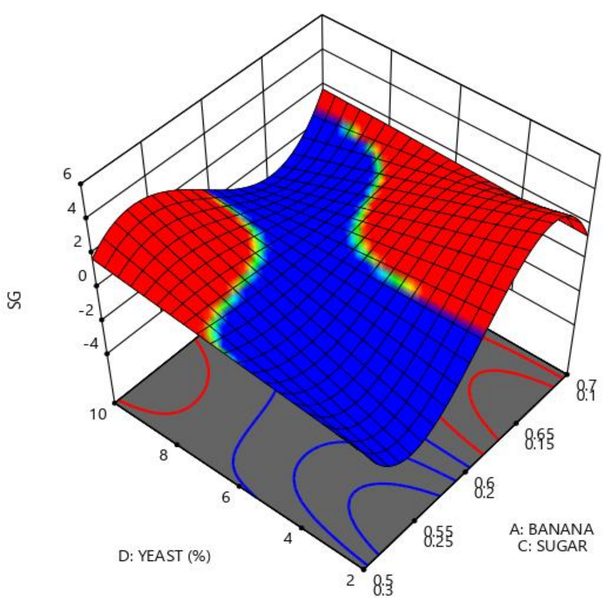

Fig 7 Effects of interaction of Banana, Sugar and Yeast on Specific Gravity

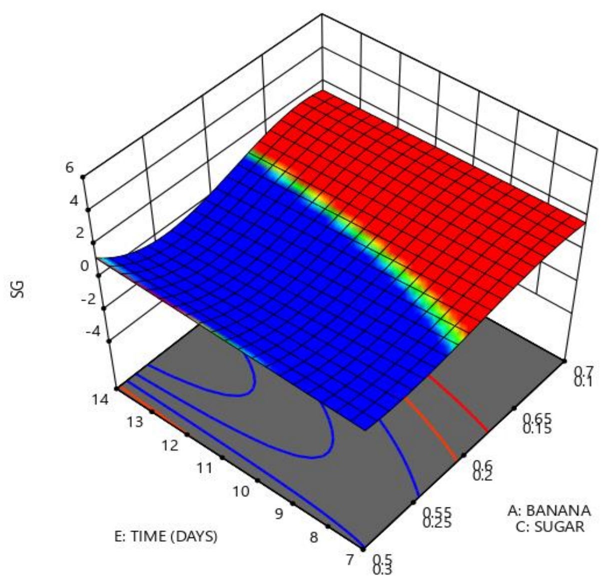

Fig 8 Effects of interaction of Banana, Sugar and Time on Specific Gravity

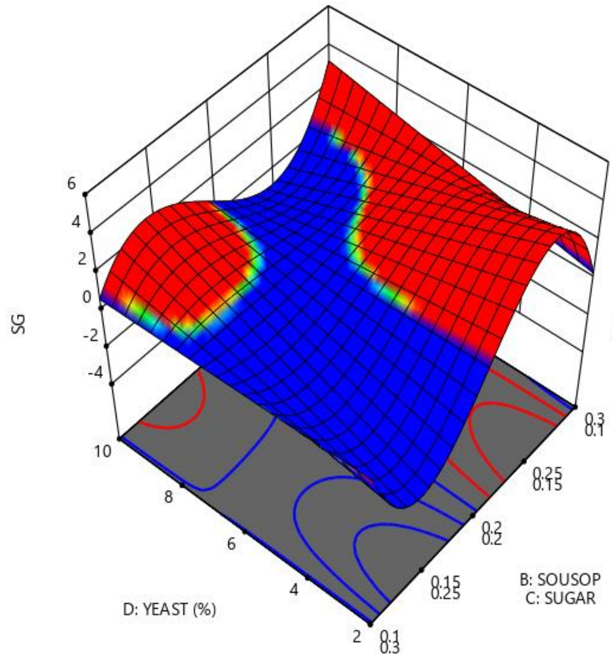

Fig 9 Effects of Interaction of Soursop, Sugar and Yeast on Specific Gravity

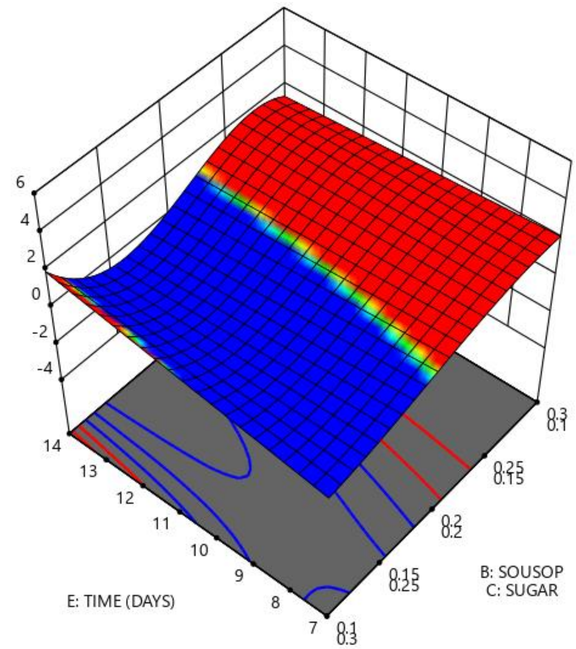

Fig 10 Effects of Interaction of Soursop, Sugar and Time on Specific Gravity

3.4 Actual and Predicted pH and SG

From the result in Table 6, actual pH based on experiment ranged from 2.93 at run 41 to 4.84 at run 17. Predicted pH also ranged from 2.93 (R41) to 4.84 (R17). Significant differences ($p<0.05$) were only noticed in runs 6, 20 and 43, based on residual values. The Table and plot of predicted vs. actual values is used to detect a value or group of values that are not easily predicted by the model (Umeh *et al*., 2017). With significantly low residue, it shows that the model met the assumptions of ANOVA and can be used to navigate the design space. Respond surface methodology has been proving to give significantly accurate predictions from actual imports. It has been used to reduce the number of experimental runs without affecting the accuracy of results while determining the interactive effect of different variables on the responses (Adeyanju *et al*., 2016). It is different from the procedure that involves the isolation of test variables and changing one variable at a time (Montgomery, 2005). RSM is an essential tool for designing, formulating, developing, and analyzing new scientific studies and product models (Nwabueze, 2010).
Actual specific gravity ranged from 1.00 at runs 22, 26 and 31 to 1.19 at run 25. Predicted specific gravity ranged from 0.999 (R22, 26, 31) to 1.17 (R30). No Significant difference ($p>0.05$) were noticed in the actual and predicted results for SG, based on residual values. The table and plot of predicted vs. actual values is used to detect a value or group of values that are not easily predicted by the model (Umeh *et al*., 2017). With significantly low residue, it shows that the model met the assumptions of ANOVA and can be used to navigate the design space.

Table 6 Actual and Predicted pH and Specific Gravity (SG)Based on RSM

Run	A: BANANA	B: SOUSOP	C: SUGAR	D: YEAST	E: TIME	Act. pH	Pred pH	Residual	Act. SG	Pred SG	Residual
1	0.41	0.29	0.3	3.2	14	3.22	3.22	*-0.0023*	1.13	1.13	*0.0022*
2	0.81	0	0.19	2	9.66	3.7	3.69	*0.0068*	1.13	1.14	*-0.0064*
3	0.5	0.4	0.1	10	8.05	4.14	4.14	*-0.0026*	1.08	1.08	*0.0024*
4	0.38	0.32	0.3	10	14	3.16	3.16	*-0.0012*	1.08	1.08	*0.0011*
5	0.64	0.17	0.18	4.8	7	4.2	4.21	*-0.0092*	1.13	1.15	*-0.0213*
6	0.57	0.24	0.19	6.62	10.89	3.77	3.66	*0.1133*	1.01	1.02	*-0.0094*
7	0.49	0.4	0.11	2	9.63	4.25	4.25	*-0.0001*	1.11	1.11	*0.0001*
8	0.8	0.1	0.1	2	9.7	4.41	4.4	*0.0068*	1.09	1.1	*-0.0064*
9	0.5	0.4	0.1	6.6	11.12	4.59	4.58	*0.0139*	1.17	1.18	*-0.0131*
10	0.56	0.24	0.21	2	9.59	3.2	3.16	*0.0356*	1.07	1.08	*-0.0053*
11	0.54	0.36	0.1	4.8	7	4.18	4.18	*-0.0047*	1.08	1.08	*0.0044*
12	0.39	0.4	0.21	3.24	14	3.65	3.66	*-0.0056*	1.06	1.05	*0.0052*
13	0.55	0.15	0.3	6.52	10.85	3.64	3.64	*-0.005*	1.01	1.01	*0.0047*
14	0.82	0.08	0.1	10	7.88	3.32	3.32	*0.0031*	1.03	1.03	*-0.0029*
15	0.81	0.09	0.1	6.6	10.82	3.23	3.24	*-0.0096*	1.01	1	*0.009*
16	0.33	0.4	0.27	6.56	11.03	3.71	3.7	*0.0129*	1.01	1.02	*-0.0121*
17	0.73	0	0.27	4.88	7	4.84	4.84	*-0.0043*	1.15	1.15	*0.004*
18	0.63	0.16	0.21	10	14	4.24	4.24	*-0.0028*	1.1	1.1	*0.0026*
19	0.3	0.4	0.3	6.6	14	3.18	3.18	*-0.0016*	1.07	1.07	*0.0015*
20	0.57	0.24	0.19	6.62	10.89	3.77	3.66	*0.1133*	1.01	1.02	*-0.0094*
21	0.6	0.3	0.1	10	14	3.07	3.08	*-0.006*	1.09	1.08	*0.0057*
22	0.9	0	0.1	3.6	14	3.55	3.55	*-0.0006*	1	0.9994	*0.0006*
23	0.37	0.4	0.23	4.8	7	4.04	4.05	*-0.0059*	1.16	1.15	*0.0056*
24	0.7	0	0.3	9.6	13.51	3.36	3.36	*0.0019*	1.05	1.05	*-0.0017*
25	0.64	0.17	0.18	4.8	7	4.2	4.21	*-0.0092*	1.19	1.15	*0.0387*
26	0.72	0	0.29	3.56	14	3.34	3.34	*-0.0007*	1	0.9994	*0.0006*
27	0.89	0	0.12	9.84	13.65	3.31	3.31	*0.0015*	1.02	1.02	*-0.0014*
28	0.78	0	0.22	6.52	10.85	3.16	3.19	*-0.0337*	1.01	1.01	*0.0035*
29	0.3	0.4	0.3	2	9.59	3.37	3.37	*0.0009*	1.08	1.08	*-0.0008*
30	0.51	0.19	0.3	7.16	8.33	4.44	4.44	*0.0005*	1.17	1.17	*-0.0005*
31	0.65	0.16	0.19	3.2	14	3.5	3.51	*-0.0102*	1	0.9904	*0.0096*
32	0.79	0	0.21	10	8.02	3.12	3.12	*0.0042*	1.07	1.07	*-0.004*
33	0.3	0.4	0.3	10	11.06	4.07	4.07	*0.0002*	1.01	1.01	*-0.0002*
34	0.73	0.17	0.1	10	10.89	4.71	4.71	*0.0028*	1.03	1.03	*-0.0026*
35	0.78	0	0.22	6.52	10.85	3.22	3.19	*0.0263*	1.01	1.01	*0.0035*
36	0.56	0.24	0.21	2	9.59	3.14	3.16	*-0.0244*	1.07	1.08	*-0.0053*
37	0.43	0.27	0.3	4.76	7	4.26	4.26	*-0.0045*	1.12	1.12	*0.0043*
38	0.57	0.24	0.19	10	7.88	4.2	4.2	*-0.0019*	1.13	1.13	*0.0018*
39	0.58	0.32	0.1	3.16	14	3.42	3.43	*-0.0064*	1	0.994	*0.006*
40	0.73	0.17	0.1	6.67	14	3.67	3.67	*0.0045*	1	1	*-0.0043*

41	0.9	0	0.1	4.84	7	2.93	2.93	*-0.0021*	1	0.998	*0.002*
42	0.59	0.11	0.3	10	8.02	4.2	4.2	*0.0032*	1.07	1.07	*-0.0031*
43	0.57	0.24	0.19	6.62	10.89	3.46	3.66	*-0.1967*	1.01	1.02	*-0.0094*
44	0.32	0.4	0.29	10	8.05	3.31	3.31	*-0.0016*	1.16	1.16	*0.0015*
45	0.41	0.4	0.19	10	14	4.14	4.14	*-0.0049*	1.01	1.01	*0.0046*
46	0.63	0.07	0.3	2	9.66	3.14	3.13	*0.0061*	1.09	1.1	*-0.0058*

Respond surface methodology has been proving to give significantly accurate predictions from actual imports (Nwabueze, 2010). It has been used to reduce the number of experimental runs without affecting the accuracy of results while determining the interactive effect of different variables on the responses (Adeyanju *et al*., 2016). It is different from the procedure that involves the isolation of test variables and changing one variable at a time. RSM has been found to be an effective tool for prediction of process outcomes (Pishgar-Komleh *et al*., 2012).

3.5 Set Goals and Constraints for Numerical Optimization of The Fermentation mixture components, Process and Response Variables, Based on Response Surface Methodology.

From the constraints and goals set for the numerical optimization of the dependent and independent variables for the fermentation of banana, soursop and sugar mix, using combined optimal design based on Response Surface Methodology, banana was maximized, this was based on the high sugar content of banana and more sugar is needed for fermentation (Ashwini *et al*., 2021). Soursop was set in range, as to exploit the potentials of soursop pulp in wine making, as observed earlier by Okafor *et al*. (2014). sugar was maximized, to maximize the energy need of the inoculum and optimum alcohol production, as noted by earlier researchers (Mundaragi and Thangadurai, 2017). Yeast was set in range to determine the right inoculum size for optimum performance, as supported by Mundaragi and Thangadurai (2017). Fermentation time was minimized for time efficiency and energy economy. Response variables of pH and SG were minimized for good preservation and adequate alcohol production.

3.6 Predicted Optimum Condition and Desirability Index for The Fermentation of Banana/Soursop/Sugar mix, Based on Response Surface Methodology.

The optimum mixture components of banana, soursop and sugar were 58.3, 20.0 and 21.7 %, respectively, while the optimum process factors of yeast and fermentation time were 2.70 % and 7 days. The desirability index was 0.856.The desirability function approach is one of the most frequently used multi-response optimization techniques in practice. The desirability lies between 0 and 1 and it represents the closeness of a response to its ideal value. If a response falls within the unacceptable intervals, the desirability is 0, and if a response falls within the ideal intervals or the response reaches its ideal value, the desirability is 1(Iwe *et al*., 2023). Meanwhile, when a response falls within the tolerance intervals but not the ideal interval, or when it fails to reach its ideal value, the desirability lies between 0 and 1. The more closely the response approaches the ideal intervals or ideal values, the closer the desirability is to 1 (Raissi and Farsani, 2009).

Table 7 Predicted Optimum Condition and Desirability Index for The Fermentation of Banana/Soursop/Sugar mix, Based on Response Surface Methodology.

Number	BANANA (%)	SOUSOP (%)	SUGAR (%)	YEAST (%)	TIME (days)	PH	SG	Desirability
1	**58.3**	**20.0**	**21.7**	**2.70**	**7.00**	**2.93**	**1.000**	**0.856 Selected**
2	49.8	22.8	27.4	5.42	7.00	2.93	1.000	0.848
3	53.5	18.6	27.9	4.83	7.00	2.93	0.495	0.810
4	56.8	22.1	21.1	2.08	8.86	2.93	0.995	0.779
5	63.1	16.3	20.6	4.71	7.15	2.93	1.000	0.739

3.7 Validation of Optimization for The Fermentation of Banana, Soursop and Sugar Blends Based on Response Surface Methodology (RSM)

There were no significant differences ($p>0.05$) in the predicted and experimental values. The predicted optimal pH and specific gravity were estimated to be 2.93 and 1.00.
The result showed that there was no significant difference ($p>0.05$) on the corresponding experimental values between the predicted (simulated) and actual properties. This result attests to the effectiveness of this design for optimum and effective fermentation of banana, soursop and sugar blends. In general, the optimized values of mixture variables obtained from the predicted optimum condition and desirability index were different from data on RSM report for predicted and actual value Table. This is because the optimization has been carried out by software and the variable in range has been selected to obtain the optimum response, as supported by report from earlier researchers (Edem and Elijah, 2016).

Table 8 Validation of Optimization for The Fermentation of Banana, Soursop and Sugar Blends Based on Response Surface Methodology

	Values	
Responses	**Predicted**	**Experimental**
PH	2.93	3.2±0.28
SG	1.00	1.064±0.07

*Significant ($p<0.05$); using independent samples T-test

4. Conclusion

The fitted regression model for the response variables based on response surface methodology were significant ($p<0.05$) with coefficient of variation (CV) value of 2.90 and 2.17% and coefficient of determination (R^2) of 99.41 and 97.84 %, respectively for pH and Specific gravity. Adequate precession values were significantly high in both models. Lack-of-fit for the models were not significant ($p>0.05$). Predicted optimum condition and desirability index of 0.856 was obtained at 58.3, 20.0 and 21.7 % banana, soursop and sugar mix, respectively, with 2.70% yeast for 7 days. pH and Specific gravity obtained from the optimum fermentation condition was suitable for desired table wine.

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