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## Effects of Yeast Concentration and Total Soluble Solids on the Quality of Wine Produced from Pineapple

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## Authors' contributions

This work was carried out in collaboration among all authors. Author ECU designed the study, wrote the first draft of the manuscript. Author CNI wrote the protocol and performed the statistical analysis of the study. Author JOI managed the analysis of the study. Author CUO managed the literature searches. All authors read and approved the final manuscript.

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**Original Research Article** 

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## ABSTRACT

This research was carried out to determine the effects of yeast concentration and total soluble solids on the quality of wine produced from pineapple. The experiment was a Response Surface Methodology in the form of Rotatable Central Composite Design (RCCD). Ripe pineapple fruit (*Ananas comosus*) was processed using pressure extraction to obtain the juice. The juice was divided into thirteen (13) portions. Each portion's total soluble solids was adjusted using sugar syrup and then pitched with specified yeast concentration. All the thirteen (13) samples were fermented for 168 h at 25±3 °C and aged for seven weeks. The wine was analyzed for pH, titratable acidity, ash content, final total soluble solids and alcohol content using standard methods. The sensory attributes (colour, aroma, taste, mouth-feel, texture and general acceptability) of the wines were analyzed by a twenty-five member panelist using 9-point hedonic scale. The results of the physicochemical analysis of the pineapple wines showed that pH , titratable acidity (tartaric acid) content,

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alcohol and final total soluble solids ranged from 3.58 - 4.58, 0.33 - 0.59 %, 4.33 - 10.66 %v/v, 3.35 - 5.65 Brix respectively. The ash content ranged from 0.20 - 0.50 %. The mean sensory scores for colour, taste, aroma, texture, mouth-feel and general acceptance ranged from 3.28 - 7.96, 4.08 - 6.68, 4.04 - 5.96, 4.16 - 5.32, 4.00 - 5.84 and 4.04 - 6.72 respectively. The mouthfeel of the pineapple wine samples did not differ significantly ( $P \ge .05$ ). Taste and alcohol exhibited significant model (P < .05), and were fitted into regression models. Pineapple has been found suitable for wine production. Using *Saccharomyces cerevisiae var cerevisiae* concentration of 6 %v/v and total soluble solids of 25 Brix is suitable for production of pineapple wine and should be adopted.

Keywords: Pineapple wine; total soluble solids; yeast concentration; Response Surface Methodology (RSM).

## 1. INTRODUCTION

Wine is an alcoholic beverage made from juice of squeezed fruits. Wines earliest recorded presence was from Georgia, Iran and Sicily [1,2]. Fermentation action of yeast creates wine from fruits; a specific quality of wine may be adopted by fermenting with a known strain of yeast [3]. Fruit juice such as grape, pineapple, mango, paw-paw, lemon and watermelon can be used in the production of wine due to their sugar content; such wines thus produced bear the name of their fruit or fruit mixtures [4,5].

In Nigeria, pineapple (*Ananas comosus*) are produced in large quantities and can be a commodity with value added to avoid wastage due to poor handling methods, as Madrid and Felice [6] reported that between 4000 and 6000 tons of pineapple fruits produced each year end up as waste. Reduction of wastage of pineapple can be utilized by value addition through production of pineapple wine [7].

Pineapple is suitable for winemaking due to their considerable sugar contents [4,8]. Pineapple fruit contains 81.2% - 86.2% moisture, 13 - 19% total solids of which sucrose, glucose, and fructose are the main components, 2 - 3% fibre, a rich source of vitamin A, vitamin C, vitamin B<sub>6</sub>, minerals like calcium, phosphorus and iron which make it a good detoxifier. Also, it has sufficient levels of nitrogen sources to support yeast growth during fermentation without need for addition of further yeast nutrients [9]. Pineapple produces low alcohol wine with good sensory properties [8,7], and invariably, are of huge demand as Saliba et al. [10] stated that the taste of low alcohol wines is a major factor for huge demands for low alcohol wines.

In the production of pineapple wines different factors have been considered; use of

combination of palm wine yeast and baker's yeast was reported by Mbajiuka et al. [11], use of single and mixed starter cultures of different organism was reported by Chanprasartsuk et al. [12]. Umeh et al. [13] varied the process factors using RSM to produce pineapple wine but did not evaluate the sensory properties. The use of pineapple to produce wine using its innate microorganism was reported by Idise, [14]. Qi et al. [8] produced pineapple wine using process factors that were not varied but instead used specified factor levels. Also Balamze and Wambete [7], Ribeiro et al. [15] produced pineapple wine from pineapple peels and use of sugarcane syrup respectively; their sensory properties were determined but the process factors were not varied nor subjected to RSM.

This research produced wine using pineapple by varying the total soluble solids and yeast concentration at optimum fermentation time and fermentation temperature, in view to determine ideal combination of factor levels suitable for production of good quality pineapple wine using Response Surface Methodology (RSM).

Response Surface Methodology (RSM) is a collection of mathematical and statistical tools useful for developing, modeling, optimizing and improving processes [16]. RSM was introduced by Box and Wilson [17]. It is used to examine and optimize the operational variables for model experimental design, development modeling), (regression test variables. optimization conditions and to study interactions between multiple variables with fewer experimental trials. RSM solves the problems of one factor at a time approach which needs a large number of experiments and often the models are very complicated to describe the experimental observation and are also time consuming and neglects merging interactions of each factor and relationship between factors

[18,19]. RSM helps to develop a suitable experimental design that integrates all the independent variables and uses the data input from the experiment to finally come up with a set of equations that can give theoretical value of an output (response), this output are obtained from a well designed regression analysis that are gotten from the controlled values of independent variables. Thereafter, the dependent (response) variables can be estimated or predicted based on the new values of the independent variables [20]. The earlier use of RSM involved experimental runs that were reduced enormously compared to the numbers of runs which were determined using full factorial design. Therefore it has been used in many researches including Food Technology where the technical steps can be found in concise forms [21,22].

### 2. MATERIALS AND METHODS

## 2.1 Sources of Materials

Materials used for this study include pineapple (*Ananas comosus*), sugar syrups and genetically modified alcohol tolerant *Saccharomyces cerevisiae var cerevisiae* wine yeast. The major

raw material which is the pineapple was sourced from Duru's pineapple farm in Umuagwo, Nigeria and was cultivated and harvested after fifteen months of growth. Other raw materials used include granulated sugar for production of sugar syrup and was bought from Relief market, Onitsha - Nigeria.

### 2.2 Experimental Design

The experiment was designed using statistical software (MINITAB version 11.0). The design of the experiment was a response surface methodology (Rotatable Central Composite Design) that has two independent factors (yeast concentration ' $x_1$ ' and total soluble solids ' $x_2$ '). The design was generated after choosing the range for the independent variables as depicted in Table 1.

The experimental design (Table 1) shows the design key for the independent variables (yeast concentration and total soluble solids) and their factor levels, while Table 2 shows the sample runs in coded and actual values with factors like fermentation temperature  $(25\pm3^{\circ}C)$  and fermentation time (168 h) kept constant.

Table 1. Experimental design key for the independent variables (coded and actual)

			Levels		
	-α	-1	0	+1	+α
yeast concentration (%v/v) [x <sub>1</sub> ]	2	4	6	8	10
total soluble solids ( <sup>°</sup> Brix) [x <sub>2</sub> ]	15	18	20	22	25

## Table 2. Experimental design showing the samples, coded and actual values of the independent factors

	Coded	Values	Actual	Values
Samples	Yeast concentration	Total soluble Solids	Yeast concentration	Total soluble Solids
	(%v/v)	( <sup>°</sup> Brix)	(%v/v)	( <sup>°</sup> Brix)
	[X <sub>1</sub> ]	[X <sub>2</sub> ]	[X <sub>1</sub> ]	[X <sub>2</sub> ]
1	+1	+1	8	22
2	+α	0	10	20
3	0	0	6	20
4	0	-α	6	15
5	0	0	6	20
6	0	+α	6	25
7	0	0	6	20
8	-α	0	2	20
9	-1	+1	4	22
10	0	0	6	20
11	+1	-1	8	18
12	-1	-1	4	18
13	0	0	6	20

#### 2.3 Methods

#### 2.3.1 Preparation of pineapple wine

Mature-ripe pineapples of high quality were chosen from the lots. The pineapple headstock were cut-out and the pineapple washed and cleaned with potable water and left to dry. The pineapple was subsequently washed with 0.1% w/v sodium metabisulphite and was size reduced by cutting into pieces. The pieces were macerated and sparged to collect enough pineapple juice which was filtered into a clean stainless bowl that has been washed with 0.1% sodium metabisulphite solution using a cheese

cloth. The pineapple must (juice) was pasteurized at 70±5 °C for 30 min and was poured into a sterile container, covered and allowed to cool to 45±5°C. The pineapple must Brix value was adjusted to desired Brix values using the sugar syrup with hand refractometer. The pineapple must was inoculated with the produced yeast starter and was allowed to ferment for 7 days (168 h) at 25±3°C. After fermentation, the wine was siphoned off from the fermenter, raked after 3 days into a sterile container and allowed to age for 7 weeks with 2 weeks periodic raking into sterile containers for proper clarification before undergoing physicochemical and organoleptic analysis.

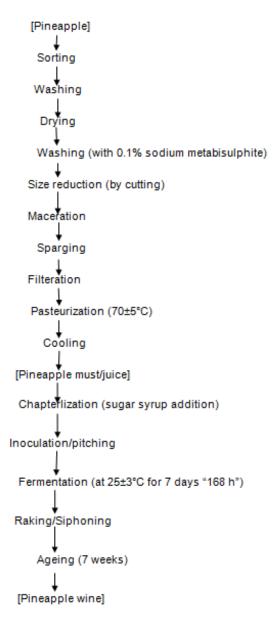


Fig. 1. Flowchart for the production of pineapple wine

## 2.3.2 Preparation of yeast starter culture medium

Pineapple must was filtered using a cheese cloth and poured into a 2000 ml sterile conical flask. A 0.2 ml of yeast food was added to the pineapple must and was sterilized by boiling for 30 min, and plugged with cotton wool and aluminium foil to cool to pitching temperature (45±5 °C). Eleven grams (11 g) of dry yeast (genetically modified alcohol tolerant S. cerevisiae var. cerevisiae) was first rehydrated (activated) in a 250 ml sterile beaker containing a 100 ml distilled water at 45±5 °C, 20 g of dissolved granulated sugar, 2 ml of normal saline and was swirled gently. The rehydrated yeast in 250 ml beaker was covered with aluminium foil and allowed to stand for 15 min. The prepared rehydrated yeast was poured into 2000 ml conical flask containing sterile pineapple juice and was shaked for 4-5 min. It was allowed to sit in a cool, dark place for 48 h with intermittent shaking/swirling using an orbital shaker at 50 rpm before inoculation into the pineapple must samples.

## 2.4 Analysis

# 2.4.1 Yeast cell enumeration and viability estimation of culture/starter medium

The viability of the yeast was determined using the methylene blue staining method as described in EBC Analytica Microbiologica [23]. The method provides the percentage of the yeast cells in the medium (Starter) that are viable. Methylene blue weighing 0.01 g was dissolved in 10 ml distilled water and 2 g of sodium citrate dehydrate added to the solution. A 1 ml of yeast sample from the medium (starter/culture) was diluted in 9 ml of distilled water. Solutions of the methylene blue and diluted yeast cells were placed on a glass slide under a cover slid and was mounted on a microscope for examination using 100× magnification. The viability was determined as the percentage of unstained cells (live cells), where the stained cells (dead cells) took up the methylene blue stain.

% Viability = <u>Total counted cells - Total counted of dead cells × 100</u> Total counted cells

The yeast cell density/concentration in the medium was counted as the number of yeast cell per ml.

Yeast cells/ml = Number of cells in grid (25 squares) × Dilution factor ×  $10^4$ 

Where,

Dilution factor (Df) =  $10^2 = 100$ 

# 2.4.2 Physicochemical analysis and ash content of the pineapple wine samples

The pH, total titratable acidity (%tartaric acid), alcohol content, ash content and total soluble solid (<sup>°</sup>Brix) of the pineapple wines were determined using standard methods [24,25] and the use of hand refractometer respectively. All experiments were carried out three times and the mean  $\pm$  standard deviation value of experiments presented.

# 2.4.3 Sensory evaluation of the pineapple wine samples

Sensory evaluation was carried out on the samples using a nine point hedonic scale as described by lwe [26]. Twenty-five (25) trained panelists who were instructed on what to do with the sensory evaluation forms presented to them as regards the pineapple wine samples. They judged each of the samples based on colour/appearance, aroma, taste. texture. mouthfeel and general acceptability. The panelists were advised to rinse their mouths with water before testing each sample and grade the samples based on the ratings in the nine point's hedonic scale, where 1 = disliked extremely and 9 = liked extremely. All sensory evaluation procedures were done in a well illuminated sensory evaluation room at standard room temperature and were done categorically in six sessions as regards to the six sensory attributes. The wine samples were presented in wine glasses.

## 2.5 Statistical Analysis of Data

The data were analysed using statistical softwares like the MINITAB (version 11), IBM Statistical Package for Social Sciences (SPSS) (version 20). The IBM SPSS was used for ANOVA and Duncan multiple range test while the Minitab was used for the regression analysis, generation of response surface plots and contour plots where the response variable is significant ( $P \le .05$ ).

## 3. RESULTS AND DISCUSSION

The result of inoculums size before pitching was found to be 90.1% viable and at  $2.2 \times 10^9$  cells/ml as listed in Table 3.

Yeast culture	Number of colourle ss yeast cells	Number of Dark blue stained yeast cells	Total number of cells	Dilu tion fact or	consta nt	Viabili ty	Yeast concentration / Density
	(cells/gri d)	(cells/grid)	(cells/gr id)	(df )		(%)	(cells/ml)
Saccharomyc es cerevisiae var cerevisiae (wine yeast)	1981	217	2198	10 <sup>2</sup>	10 <sup>4</sup>	90.1%	2.2 × 10 <sup>9</sup>

Table 3. Viability test and yeast density estimation

Table 4. Physico-chemical composition of the pineapple wines

S/N	Sample	рН	Alcohol	Total Titratable Acidity	Total soluble solid	Ash Content
	(Y : T)		(%v/v)	(% tartaric acid)	(⁰Brix)	(%)
1	(8:22)	3.66±0.01 <sup>ª</sup>	7.66±0.57 <sup>de</sup>	0.48±0.01 <sup>bc</sup>	4.65±0.07 <sup>9</sup>	0.45±0.70 <sup>bcd</sup>
2	(10:20)	3.89±0.02 <sup>b</sup>	6.00±0.00 <sup>bc</sup>	0.58±0.01 <sup>e</sup>	4.10±0.00 <sup>d</sup>	0.35±0.07 <sup>abc</sup>
3	(6:20)	3.96±0.01 <sup>b</sup>	6.66±0.57 <sup>cd</sup>	0.46±0.01 <sup>b</sup>	4.20±0.00 <sup>de</sup>	0.30±0.14 <sup>ab</sup>
4	(6:15)	4.58±0.18 <sup>°</sup>	4.33±0.57 <sup>a</sup>	0.33±0.01 <sup>a</sup>	3.35±0.07 <sup>a</sup>	0.20±0.00 <sup>a</sup>
5	(6:20)	3.93±0.41 <sup>b</sup>	7.00±0.00 <sup>de</sup>	0.46±0.01 <sup>b</sup>	4.50±0.00 <sup>f</sup>	0.30±0.00 <sup>ab</sup>
6	(6:25)	3.58±0.01 <sup>ª</sup>	10.66±0.57 <sup>f</sup>	0.48±0.05 <sup>bc</sup>	5.65±0.07 <sup>y</sup>	0.50±0.00 <sup>cd</sup>
7	(6:20)	3.96±0.01 <sup>b</sup>	6.33±0.57 <sup>bc</sup>	0.46±0.02 <sup>b</sup>	4.50±0.00 <sup>f</sup>	0.25±0.07 <sup>a</sup>
8	(2:20)	3.92±0.01 <sup>b</sup>	5.66±0.57 <sup>bc</sup>	0.51±0.01 <sup>cd</sup>	4.30±0.00 <sup>e</sup>	0.20±0.00 <sup>a</sup>
9	(4:22)	3.61±0.01 <sup>ª</sup>	8.00±0.00 <sup>e</sup>	0.52±0.00 <sup>d</sup>	5.05±0.07 <sup>h</sup>	0.30±0.00 <sup>ab</sup>
10	(6:20)	3.97±0.02 <sup>b</sup>	6.33±1.15 <sup>bc</sup>	0.46±0.02 <sup>b</sup>	4.45±0.07 <sup>f</sup>	0.30±1.41 <sup>ab</sup>
11	(8:18)	3.88±0.00 <sup>b</sup>	5.33±0.57 <sup>b</sup>	0.54±0.01 <sup>d</sup>	3.45±0.07 <sup>a</sup>	0.35±0.07 <sup>abc</sup>
12	(4:18)	3.95±0.00 <sup>b</sup>	6.33±0.57 <sup>bc</sup>	0.59±0.02 <sup>e</sup>	3.70±0.00 <sup>b</sup>	0.50±0.14 <sup>cd</sup>
13	(6:20)	3.95±0.03 <sup>b</sup>	6.66±0.57 <sup>cd</sup>	0.45±0.01 <sup>b</sup>	3.95±0.07 <sup>c</sup>	0.35±0.07 <sup>abc</sup>

\*Values are mean ± standard deviation of three replicates

\*Values in the same column bearing different superscript differed significantly ( $P \le .05$ )

\* Y = yeast concentration (% v/v)

\* T = total soluble solids (<sup>°</sup>Brix)

In order to inoculate at appropriate rate, two parameters are measured accurately, *viz* – number of yeast cell and the viability of the yeast [27]. At viability greater than 90%, yeast cells are good for pitching. Erten et al. [28] and Ribeiro et al. [15] stated that increased yeast inoculum size above  $10^6$  cells/ml,  $10^7$  cells/ml,  $10^8$  cells/ml increases chances of having reduced ethyl acetate, acetic acid content of the wine and also, an improved sensory qualities.

#### 3.1 Physico-chemical Composition

The result of the physico-chemical composition of the pineapple wine is presented in Table 4.

#### 3.1.1 pH

pH is the measure of acidity or basicity of a solution. It was tested to determine if yeast

concentration and total soluble solid have effects on wine pH. The pH of the pineapple wines ranged from 3.55 – 4.58. From the result in Table 4 it can be deduced that the pH of wines are dependent on the total soluble solids of the pineapple must. Sample 6(6:25) and 4(6:15) had the least and the highest pH value; both are inoculated with 6%v/v of yeast and their total soluble solids differed as sample 6 had 25 Brix total soluble solid and sample 4 had 15 °Brix total soluble solids of must respectively, therefore, an increase in total soluble solids of must resulted to a more acidic pH value. The pH 3.5 is regarded as an ideal wine pH [29,30,31,32,8], however, sample 6(6:25) had pH of 3.58 therefore the higher the brix value of pineapple juice the lower the pH. It is also evident that different yeast strains, combination of strains also influences the pH of pineapple wines [12]. Fermentation time affects pH of wines; pH decreases as

fermentation proceeds and it is desirable as it inhibits growth of microorganisms [33,11].

#### 3.1.2 Total titratable acidity

This measures the ionic strength of a solution and subsequently determines the rate of chemical reaction. Organic acids are the main acids in wines; they include tartaric acid, malic acid, citric acid, succinic acids and acetic acids etc. The total titratable acidity of the pineapple wine samples ranged from 0.33 - 0.59 (%tartaric acid). From the result (Table 4), it shows that veast concentration and total soluble solids have effects on titratable acidity of wines; as their increased levels lead to increased acidity vice versa while sample 4(6:15) had the least and sample 12(4:18) had the highest acidity levels. Yeast strains; both single and mixed strain have effects on wines acidity [12], 0.6 and 0.7% levels of acidity was reported by Chanprasartsuk et al. [12], however samples 2(10:20) and 12(4:18) had 0.58 and 0.59% level of acidity respectively. Percentage tartaric acid in wines has been reported to be around  $4.0 - 8.0 (g/dm^3)$  [34]. The acidity of the wine samples met the NAFDAC permissible levels of %tartaric acids in wines which ranged from 0.3-0.55% [35]. The reduced titratable acidity level could be due to yeast concentration level of 10<sup>9</sup> cells/ml as Erten et al. [28] and Ribeiro et al. [15] reported that increased yeast concentration reduces acids production.

#### 3.1.3 Alcohol

Alcohol happens to be the most abundant of the volatile compounds found in wines and it helps to improve the sensory properties and acceptability of wines [36]. Alcohol content of wines is dependent on the initial sugar content (<sup>B</sup>Brix) of juice/must [13]. From the result (Table 4), the alcohol content of the pineapple wines ranged from 4.33% -10.66%, samples 4 which had the least sugar content (<sup>B</sup>Brix) of 15 <sup>B</sup>Brix produced the least alcohol while sample 6(6:25) which had the highest sugar content of 25 <sup>°</sup>Brix produced the highest alcohol content; thus in tandem with the report of Umeh et al. [13]. Qi et al. [8]

reported that pineapple wine alcohol content of 10.2% v/v is acceptable and can be regarded as low alcohol wine, however sample а 6(6%v/v:25°Brix) produced 10.66%v/v alcohol and can be termed a low alcohol wine. It is recommended that consumption of small quantity of wines with low alcohol, once or twice a day is necessary for reduced risk of heart diseases, strokes, diabetes mellitus, metabolic syndrome and early death; also drinking more than standard drinking amount increases risk of heart diseases, strokes and cancer [37,38,39].

The %alcohol content of the pineapple wine samples was fitted into a regression model having a significant regression and a substantial  $R^2$  adj. value (Table 5 and 6) and therefore met the conditions for fitting a response into a model and also in terms of variable selections [40,41,42,43].

The quadratic model of the variables could be presented in equation 1

$$Y=\beta_{0} + \beta_{1}X_{1} + \beta_{2}X_{2} + \beta_{12}X_{1}X_{2} + \beta_{1}X_{1}^{2} + \beta_{2}X_{2}^{2} + e$$
(1)  
[44,45,46,43]

Where,

Y= Responses or the dependent variables considered

 $\beta_0$  = Constant

 $\beta_1$  = Coefficient of the yeast concentration (independent variable)

 $\beta_2$  = Coefficient of the total soluble solids"<sup>o</sup>Brix value"(independent variable)

 $X_1$  = Level of the yeast concentration (independent variable)

 $X_2$  = Level of the total soluble solids "<sup>o</sup>Brix value" (independent variable)

E = Estimated error

Alcohol being the response variable could be used to substitute for Y in Eq.1, as

$$\begin{array}{l} & & \textbf{Alcohol} = \beta_0 + \beta_1 X_1 + \beta_2 X_2 + \beta_{12} X_1 X_2 + \\ & & \beta_1 X_1^2 + \beta_2 X_2^2 + e \dots \end{array}$$

**Table 5. Estimated Regression Coefficients for Alcohol** 

Term	Coef	S	R-Sq	R-Sq(adj)
Constant	11.989	0.4168	95.6%	92.5%
Yeast co	-0.282			
Total so	-1.038			
Yeast co*Yeast co	-0.048			
Total so*Total so	0.035			
Total so*Total so	0.041			

Source	DF	Seq SS	Adj SS	Adj MS	F	Р
Regression	5	26.6449	26.6449	5.32898	30.68	0.000
Linear	2	23.8563	0.5450	0.27250	1.57	0.274
Square	2	2.6797	2.6797	1.33983	7.71	0.017
Interaction	1	0.1089	0.1089	0.10890	0.63	0.454
Residual Error	7	1.2159	1.2159	0.17370		
Lack-of-Fit	3	0.9030	0.9030	0.30099	3.85	0.113
Pure Error	4	0.3129	0.3129	0.07823		
Total	12	27.8608				

Table 6. Analysis of Variance for Alcohol

From the regression and ANOVA (Table 5 and 6), only the regression and the square terms exhibited *P*-values  $\leq$  .05. The P-value for lack of fit is 0.113, which is insignificant and is good, therefore two terms only should be used to fit the mathematical model for % alcohol, as shown in Eq.3

## **%Alcohol =** $11.98 - 0.048X_1^2 + 0.035X_2^2$ . (3)

In Eq.3.  $X_{1}^{2}$  (levels of yeast concentration) has – ve coefficient and  $X_{2}^{2}$  (levels of total soluble solids) has +ve coefficient. The negative sign implies that increasing the concentration of  $X_{1}$  (Yeast concentration) will decrease the % alcohol while the +ve coefficient for  $X_{2}$  implies that

increasing the concentration of  $X_2$  (Total soluble solids) will increase the % alcohol yield.

The 3D-surface plot (Fig. 2) of the % alcohol content of the wine; rather than showing the individual data points, the surface plots shows the relationship between the wines percentage (response variable) and the two alcohol independent variables (yeast concentration and total soluble solids). In the graph, the interaction effect of yeast concentration (%v/v) and total soluble solids (Brix) on % alcohol content can be estimated. Also, the contour plot (Fig. 3) of the response variable (% Alcohol) shows the relationship between % Alcohol, yeast concentration and total soluble solids.

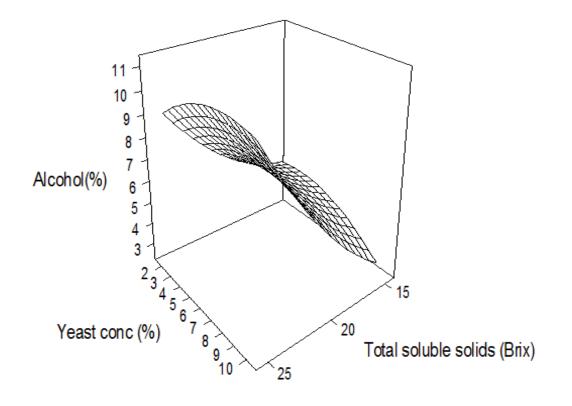


Fig. 2. 3D-surface plot of % alcohol content

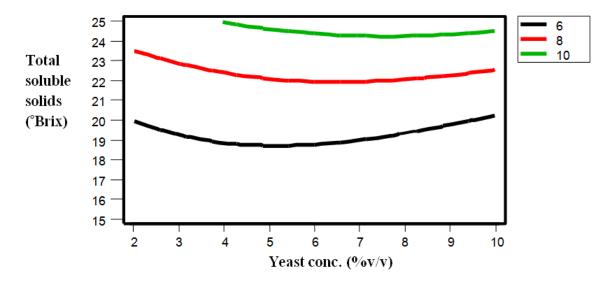


Fig. 3. Contour Plot for % alcohol content

#### 3.1.4 Total soluble solid of the wines (°Brix)

The total soluble solid reflects the aqueous solution's sugar content. One degree brix is 1g of sucrose in 100g of solution and reflects a proportion by weight strength of the solution. The periodic sharp decrease in the total soluble solids during the must fermentation can be attributed to the activities of the yeasts [47,12,8], and also the fermentation time [48]. The amount of total soluble solids (Brix) left after wine fermentation and ageing categorizes wines as being sweet or dry as above 3.0°Brix are termed sweet wines. From the result (Table 4), the total soluble solids of the pineapple wines ranged from 3.35 - 5.65Brix. Sample 11(8:18) had the least brix value having being fermented with high yeast concentration of 8%v/v and low total soluble solids of the pineapple must. Also the pineapple wine of sample 6(6:25) had the highest brix value having being fermented with a moderate yeast concentration (6%v/v) and 25 Brix total soluble solids of must and produced 5.65 Brix of pineapple wine, this is also in tandem with the report of Qi et al. [8] which produced 5.4 Brix pineapple wine, therefore the total soluble solids of the pineapple wines are dependent on the amount of yeast and brix value of their must respectively.

### 3.1.5 Ash content of the wine

Ash is the inorganic residue of a food left after the organic part of the food is burnt off. From the result(Table 4), the pineapple wine samples ash contents ranged from 0.20 - 0.50%, though the ash content of sample 6(6:25) is highest at 0.50% and sample 4(6:15) and 8(2:20) having the least ash content, this can invariably be linked to the total soluble solids of the pineapple must and wine. Ribeiro et al. [15] reported 0.36% ash content for pineapple wines, however samples 2(10:20), 3(6:20), 5(6:20), 9(4:22), 10(6:20), 11(8:18) and 13(6:20) has ash contents of 0.3%.

#### **3.2 Sensory Properties**

The results of the organoleptic properties are shown on Table 7.

# 3.2.1 Sensory evaluation of the pineapple wines samples

#### 3.2.1.1 General acceptance

The sensory attributes of wines determines its acceptance among consumers. From the result (Table 7), the general acceptance of the pineapple wine samples ranged from 4.04 - 6.72 mean test scores, sample 3(6:20) had the least acceptance which is slightly disliked while sample 6(6:25) had the highest acceptance and is liked slightly, this was also in accordance with the report of Ribeiro et al. [15], Balamze and Wambete, [7] which had 6.90 and 6.29 pineapple wine general acceptability and which happens to be slightly liked, judging from the 9 point hedonic scale. The pineapple wine of Sample 6(6:25) having the highest acceptance could be attributed to its alcohol content, aroma, Brix value and its taste score.

S/N	Sample	Colour	Taste	Aroma	Texture	Mouthfeel	General Acceptance
	(Y:T)						
1	(8:22)	4.00±2.19 <sup>tg</sup>	4.44±2.02 <sup>a</sup>	4.84±1.88 <sup>ab</sup>	4.36±2.07 <sup>a</sup>	4.16±2.42 <sup>a</sup>	4.76±2.31 <sup>a</sup>
2	(10:2)	6.36±1.97 <sup>abc</sup>	4.52±1.85 <sup>ab</sup>	5.12±2.57 <sup>ab</sup>	4.80±1.87 <sup>a</sup>	4.00±2.08 <sup>a</sup>	4.04±2.35 <sup>a</sup>
3	(6:20)	5.44±2.00 <sup>acde</sup>	4.60±2.41 <sup>ab</sup>	4.48±2.12 <sup>a</sup>	4.48±2.08 <sup>a</sup>	4.60±2.12 <sup>ab</sup>	4.08±2.43 <sup>a</sup>
4	(6:15)	7.96±1.01 <sup>y</sup>	4.32±2.01 <sup>a</sup>	4.04±1.94 <sup>a</sup>	4.56±2.48 <sup>a</sup>	5.00±2.16 <sup>ab</sup>	4.56±2.51 <sup>a</sup>
5	(6:20)	4.92±1.38 <sup>def</sup>	4.20±1.87 <sup>a</sup>	4.28±2.18 <sup>a</sup>	4.28±1.40 <sup>a</sup>	4.24±1.80 <sup>a</sup>	4.56±2.14 <sup>a</sup>
6	(6:25)	3.28±1.64 <sup>h</sup>	6.68±2.09 <sup>c</sup>	5.96±1.36 <sup>b</sup>	5.32±1.77 <sup>a</sup>	5.84±2.21 <sup>b</sup>	6.72±1.98 <sup>b</sup>
7	(6:20)	4.72±1.88 <sup>ef</sup>	4.64±2.11 <sup>ab</sup>	4.44±1.70 <sup>a</sup>	4.28±1.42 <sup>a</sup>	4.24±2.22 <sup>a</sup>	4.92±1.95 <sup>a</sup>
8	(2:20)	5.28±2.15 <sup>cde</sup>	4.28±2.26 <sup>a</sup>	5.44±1.85 <sup>ab</sup>	5.08±1.99 <sup>a</sup>	4.72±2.09 <sup>ab</sup>	5.28±2.38 <sup>a</sup>
9	(4:22)	6.24±1.47 <sup>abc</sup>	4.64±1.95 <sup>ab</sup>	5.40±2.06 <sup>ab</sup>	4.16±1.88 <sup>a</sup>	4.24±2.47 <sup>a</sup>	5.20±2.16 <sup>a</sup>
10	(6:20)	6.16±2.05 <sup>abc</sup>	4.72±1.79 <sup>ab</sup>	5.00±2.38 <sup>ab</sup>	4.96±1.96 <sup>a</sup>	4.56±2.20 <sup>ab</sup>	5.08±2.39 <sup>a</sup>
11	(8:18)	5.92±1.91 <sup>acd</sup>	5.16±2.56 <sup>ab</sup>	5.08±2.23 <sup>ab</sup>	4.72±2.07 <sup>a</sup>	4.92±2.41 <sup>ab</sup>	5.04±2.13 <sup>a</sup>
12	(4:18)	6.56±2.50 <sup>ab</sup>	4.08±2.15 <sup>a</sup>	4.76±2.38 <sup>ab</sup>	4.40±1.93 <sup>a</sup>	4.92±2.23 <sup>ab</sup>	5.40±2.27 <sup>a</sup>
13	(6:20)	6.56±1.52 <sup>ab</sup>	4.68±1.97 <sup>ab</sup>	4.76±2.29 <sup>ab</sup>	4.96±2.09 <sup>a</sup>	4.92±2.46 <sup>ab</sup>	5.00±2.53 <sup>a</sup>

## Table 7. Sensory evaluation result

\*Values are mean ± standard deviation of twenty five (25) replicates \*Values in the same column bearing different superscript differed significantly (P≤.05)

\* Y = yeast concentration (%v/v) \* T = total soluble solids ( Brix)

#### 3.2.1.2 Texture

The texture of the pineapple wine samples do not differ significantly ( $P \ge .05$ ) and ranged from 4.16 – 5.32.

## 3.2.1.3 Colour

The colours of wines are the most easily recognized sensory attributes of wines. The colours of the pineapple wine samples ranged from 3.28 - 7.96, sample 6(6:25) had the least score and sample 4(6:15) had the highest score, it can be deduced that total soluble solids and yeast activities in must can affect the colour of pineapple wines. According to Ribeiro et al. [15], the colour of pineapple wine had sensory score of 7.08, however sample 4(6:15) had score of 7.96 which is liked moderately judging from the 9-point hedonic scale. 6.56 Score was reported by Balamze and Wambete [7], however, samples 2(10:20), 9(4:22), 10(6:20), 12(4:18) and 13(6:20) had score range of 6.16 - 6.56 which are also liked slightly.

#### 3.2.1.4 Aroma

Yeast strains impart good sensory qualities like aroma to wines [49,50] and their perception involves interaction between large number of chemical compounds and sensory receptors [51]. From result (Table 7), the aroma of the pineapple wine samples ranged from 4.04 - 5.96, this shows that increased total soluble solids influences the aromas of pineapple wines as sample 4(6:15) had the least score and sample 6 had the highest score. Ribeiro et al.[15] reported score of 7.27 for pineapple wines, none of the samples had that aroma score which is liked moderately, however Balamze and Wambete, [7] reported aroma score of 5.89 for pineapple wines and samples 2(10:20), 6(6:25), 8(2:20), 9(4:22), 10(6:20) and 11(8:18) had scores ranging from 5.00 - 5.96 which are also neither liked nor disliked according to the 9-point hedonic scale.

#### 3.2.1.5 Mouthfeel

The range of score for mouthfeel is 4.00 - 5.84. Sample 2(10:20) had the lowest score and was disliked slightly, while sample 6(6:25) had the highest score and was neither liked nor disliked. Score of 5.61 has been reported by Balamze and wambete [7] as the best score for pineapple wine (from pineapple peel) mouthfeel and it was neither liked nor disliked, however sample 6(6:25) had score of 5.84 and was also neither liked nor disliked according to the 9-point hedonic scale. Also Ribeiro et al.[15] reported that the mouthfeel/viscosity of pineapple wine score is 7.81 which was liked moderately, however none of the samples met this mouthfeel/viscosity score but nonetheless sample 6(6:25) had the highest score.

#### 3.2.1.6 Taste

The responses for taste of the pineapple wine samples, as shown in the result (Table 7) depicts significant difference ( $P \leq .05$ ) among test samples. The sensory score for taste of the pineapple wine samples ranged from 4.08 -6.68, this also showed that varied yeast concentration and total soluble solids of pineapple must have effects on the taste of pineapple wines. Ribeiro et al. [15] reported a pineapple wine with taste score of 6.40; however sample 6 had a sensory score of 6.68 which also happened to be liked slightly. Also according to Balamze and Wambete, [7], 5.78 taste score was recorded for pineapple wines and pineapple wine sample 11(8:18) had score of 5.16 which is also neither liked nor disliked judging from the 9-point hedonic scale.

The taste was fitted into a regression model; having a significant regression and a substantial  $R^2$  adj. value (Tables 8 and 9) and therefore met the conditions for fitting a response into a model and also in terms of variable selections [40,41,42,43].

The quadratic model of the variables could be presented in equation 1

$$Y = \beta_0 + \beta_1 X_1 + \beta_2 X_2 + \beta_{12} X_1 X_2 + \beta_1 X_1^2 + \beta_2 X_2^2 + e...$$
(1)

[44,45,46,43]

Taste being the response variable could be used to substitute for Y in Eq.1, as

**Taste**= 
$$\beta_0 + \beta_1 X_1 + \beta_2 X_2 + \beta_{12} X_1 X_2 + \beta_1 X_1^2 + \beta_2 X_2^2 + e.$$
 (4)

From the regression and ANOVA of Taste (Tables 8 and 9), the terms that have P- value  $\leq$  .05 include the regression, the linear and the square terms. Therefore, only these terms could be fitted into mathematical model as shown in Eq. 5.

$$Taste = 6.2673 + 1.682X_1 - 0.8664X_2 - 0.0040X_1^2 + 0.0380X_2^2 \dots$$
(5)

Table 8. Estimated Regression Coefficients for Taste

Term	Coef	S	R-Sq	R-Sq(adj)
Constant	6.2637	0.4001	77.9%	62.2%
Yeast co	1.6882			
Total so	-0.8664			
Yeast co*Yeast co	-0.0040			
Total so*Total so	0.0380			
Yeast co*Total so	-0.0800			

#### Table 9. Analysis of Variance for Taste

Source	DF	Seq SS	Adj SS	Adj MS	F	Р
Regression	5	3.9586	3.9586	0.79172	4.95	0.030
Linear	2	2.0736	1.5928	0.79641	4.97	0.045
Square	2	1.4754	1.4754	0.73769	4.61	0.053
Interaction	1	0.4096	0.4096	0.40960	2.56	0.154
Residual Error	7	1.1206	1.1206	0.16009		
Lack-of-Fit	3	0.9493	0.9493	0.31645	7.39	0.042
Pure Error	4	0.1713	0.1713	0.04282		
Total	12	5.0792				

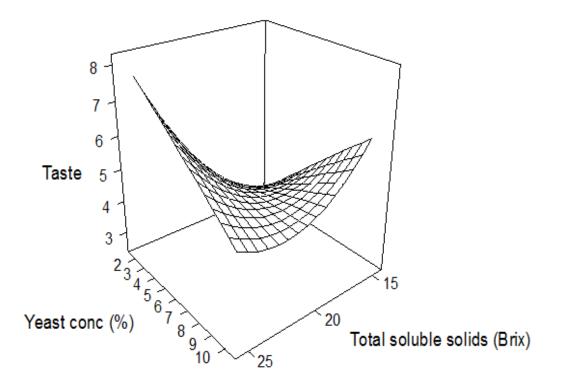


Fig. 4. 3D-Surface plot of taste

3D-surface plot (Fig. 4) shows the interaction effects of yeast concentration and total soluble solids on the taste of the pineapple. Also the contour plot (Fig. 5), shows the relationship between the two independent factors and the taste of the wine.

From the plot it can be deduced that the higher the total soluble solids  $(x_2)$ , the more acceptable the taste of the wine and vice versa.

The sensory attributes of pineapple wines can be attributed to the yeast concentration and total soluble solids of the pineapple must.

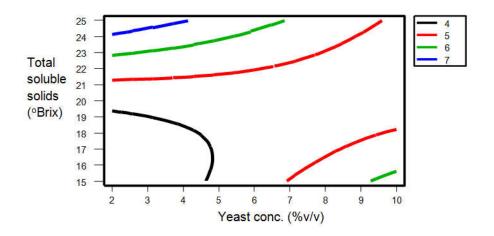


Fig. 5. Contour plot of taste

## 4. CONCLUSION

The study revealed that varying the amount of yeast concentration and total soluble solids had significant effects on the physico-chemical properties, sensory properties and the general acceptability of the pineapple wines. The study differed the process variables (yeast concentration and total soluble solids) using RSM in view of obtaining suitable process combinations for pineapple wine production. Employing an ideal yeast concentration and total soluble solids in the production of a high quality pineapple wine is recommended. The wine samples met some recommended standards in terms of physico-chemical properties like pH, titratable acidity, alcohol, total soluble solids, ash content and sensory attributes (colour, taste, texture. mouthfeel. and aroma. general acceptance). Sample (6%v/v 6 veast concentration and 25 Brix total soluble solids) is more acceptable in terms of physico-chemical composition and sensory attributes (general acceptance) and should be adopted in the fermentation of pineapple must into wine.

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## **COMPETING INTERESTS**

Authors have declared that no competing interests exist.

## REFERENCES

- 1. Spilling M, Wong W. Cultures of the world Georgia; 2008.
  - Available: ISBN 978-0-7614-3033-9.
- Ellsworth A. 7,000 year old wine jar. University of Pennsylvania museum of archeology and anthropology; 2012.
- Walker GM, Batt C, Tortorello ML. Microbiology of winemaking. In: Encyclopaedia of food microbiology;. eds. Elsevier science publishers: Boston, MA, USA; 2014.
- 4. Adaikan P, Ganesan AA. Mechanism of the oxytoxic activity of Comosus proteinases. Pharm. Biol. 2004;42(8):646-655.
- Robinson J. The oxford companion to wine. 3<sup>rd</sup> ed. Oxford university press; 2006. Available: ISBN 0-19-860990-6.
- Madrid RE, Felice CJ. Microbial Biomass Estimation. J. Microbiol. Methods. 2005;351:37-42.
- Balamze J, Wambete J. Production of good quality wine from single and mixture of fruit peels. AJFAND. 2017;17(1): 11822:11831.
- Qi N, Ma L, Li L, Gong X, Xe J. Production and quality evaluation of pineapple fruit wine. 10P Conf. Ser. Earth Environ. Sci. 2017;100:12028.

- Akamine EK. Postharvest control of endogenous brown spot in fresh Australian pineapples with heat. Hort. Sci. 1976;11:586-588.
- Saliba A, Ovington L, Moran CC. Consumer demand for low-alcohol wine in an Australian sample. Int. J. Wine Res. 2013;5:1-8.
- Mbajiuka CS, Obeagu EI, Agbor EC, Enya E. Alcohol and biomass production from pineapple juice using a combination of palm wine yeast and baker's yeast. Int. J. Life Sci. Biotechnol. Pharm. Res. 2014;3(4):2250-3137.
- 12. Chanprasartsuk O, Pheanudomkitlert K, Toonwai D. Pineapple wine fermentation with yeasts isolated from fruit as single and mixed starter cultures. As. J. Food Ag-Ind. 2012;5(02):104-111.
- Umeh JI, Ejikeme PCN, Egbuna SO. Optimization of process conditions for alcoholic wine production from pineapple using RSM. IJMSE. 2015;6(3):23-30.
- Idise OE. Studies of wine produced from pineapple (Ananascomosus). Int. J. Biotechnol. Mol. Biol. Res. 2012;3(1):1-7.
- Ribeiro LS, Duarte WF, Dias DR, Schwan RF. Fermented sugarcane and pineapple beverage produced using Saccharomyces cerevisiae and Non-saccharomyces yeast. J. inst. Brew. 2015;121:262-272. Available: http://doi.org/10.1002/jib.218
- Myers Raymond H, Montgomery DC. Response surface methodology; process and product optimization using designed experiment. A wiley-interscience publication; 2002.
- 17. Box GEP, Wilson KB. The experimental attainment of optimum conditions. J. R. Stat. Soc. 1951;13(1):1-45.
- Nadya H, Zainal S, Átikah O, Tengku-elide TZM.Optimization of ethanol fermentation from pinapple peel extract using response surface methodology (RSM). World Acad. Sci. Eng. Technol. 2012;6(12):1102-1108. Available: ISNI:0000000091950263.
- Ecksibi I, Haddar W, Ben-Ticha M, Gharbi R, Mhenni MF. Development and optimization of a non conventional extraction process of natural dye from olive solid waste using response surface methodology (RSM). Food Chem. 2014;161:345-352.
- Meilgaard BT, Civille M, Carr GV. Sensory evaluation techniques, 2<sup>nd</sup> ed., CRC press, Boca Raton, FL; 1991.

- 21. Henika RG. Simple and effective system for use with response surface methodology. Cereal Science Today. 1972;17(10):309-334.
- 22. Henika RG. Use of response surface methodology on sensory evaluation. Food Technol. 1982;36:96-101.
- 23. EBC Analytica Microbiologica. Method 2.2.2.3. J. Inst. Brew. 1977;83:109-118.
- Association of Official Analytical Chemist (AOAC). Official methods of analysis of the Association of Analytical Chemists, 18<sup>th</sup> ed. AOAC, Washington D.C; 2005.
- 25. International Organization of Vine and Wine (OIV). Compendium of international methods of wine and must analysis. Paris, France; 2009.
- 26. Iwe MO. Handbook of sensory methods and analysis. Rejoint Communication Enugu; 2003.
- 27. Thornton R. Evaluation of yeast viability and concentration during wine fermentation using flow cytometry. BD Bioscience; 2002.

Available: www.bdbiosciences.com

- Erten H, Tangular H, Cabaroglu T, Canbas A. The influence of inoculum level on fermentation and flavor compounds of white wines made from cv. J. Inst. Brew. 2006;112(3):232-236.
- 29. Davis CR, Wibowo DJ, Lee TH, Fleet GH. Growth and metabolism of lactic acid bacteria during and after malolactic fermentation of wines at different pH. Appl. Environ. Microbiol. 1986;51:539-545.
- Ribereau-Gayon P, Dubourdien D, Doneche B, Lonvaud F. The microbiology of wine and vinification. Handbook of enology (1). John Wiley and Sons LTD, England; 2000.
- Yan HG, Zhang, Chen JH, Ding Z. Optimization of the alcoholic fermentation of blueberry juice by AS 2316 Saccharomyces cerevisiaewine yeast. Afr. J. Biotechnol. 2012;11(15):3623-3630.
- 32. Mundaragi A, Thangaduri D. Process optimization, physicochemical characterization and antioxidant potential of novel wine from an underutilized fruit Carissa spinarum. L.(apocynaece). Food Sci. Technol. Campinas. ISSN 0101-2061; 2017.

Available:http://dx.doi.org/10.1590/1678-457x.06417.

33. Ihekoronye AI, Ngoddy PO. Integrated Food Science and Technology for the Tropics. MacMillan Publishers, London; 1985.

- Rajkovic MB, Novakovic ID, Petrovic A. Determination of titratable acidity in white wine. J. Agric. Sci. 2007;52(2):169-184.
- 35. National Agency for Food and Drug Administration and Control (NAFDAC). Determination of Total acidity in alcoholic beverages. In: ABL/SOP/06; 2006.
- Goldner MC, Zamora MC, Di leo lira P, Giannin-oto H, Bandoni A. Effects of ethanol level in the perception of aroma attributes and the detection of volatile compounds in red wine. J. Sens. Stud. 2009;24:243-257.
- Jin M, Cai S, Guo J, Zhu Y, Li M, Yu Y, Zhang S, Chen K. Alcohol drinking and all cancer mortality: a meta-analysis. Ann. Oncol. 2013;24(3):807-816.
- O'keefeJH, Bhatti SK, Bajwa A, Dinicolantonio JJ, Lavie CJ. Alcohol and cardiovascular health: the dose makes the poison or the remedy. Mayo Clin. Proc. 2014;89(3):382-392.
- Shen J, Wilmot KA, Ghasemzadeh N, Molloy DL, Burkman G, Mekonnen G, Gongora MC, Quyyumi AA, Sperling LS. Mediterranean dietary patterns and cardiovascular health. Annu. Rev. Nutr. 2015;35:425-449.
- Chin WW. The partial least squares approach for structural equation modeling. In: G.A. marcoulides (Ed), methodology for business and management. Modern methods for business research pp.295-336. Mahwah, NJ, US: Lawrence Erlbaum Associates; 1998.
- Henseler J, Ringle C, Sinkovics R. The use of partial least squares path modeling in international marketing. In: Advances in International Marketing (AIM), 20,277-319. Emerald group publishing limited; 2009. Available:ISSN:1474-7979/doi:10.1108/S1474-7979/2009)0000020014.
- 42. Hair JF, Ringle CM, Sarstedt M. Partial least square structural equation modeling:

Rigorous Applications, Better results and higher acceptance. LRP. 2013;46(1-2):1-12.

- 43. Harrel<sub>jr</sub> FE. Regression modeling strategies with application to linear models, logistic and ordinal regression, and survival analysis. 2<sup>nd</sup>ed, Springer; 2015.
- 44. Williams JD. A regression approach to experimental design. J. Exp. Educ. 1970;39(2):88-90.
- 45. Kleijnen JPC, Standridge CR. Experimental design and regression analysis in simulation: An FMS case study. Eur. J. Oper. Res. 1988;33(3):257-261.
- 46. Atkinson AC, Donev AN, Tobias RD. Optimum experimental designs with SAS, Oxford University Press, Oxford; 2007.
- 47. Ribereau-Gayon P, Glories Y, Maujean A, Dubourdieu D. Alcohols and other volatile compounds, the chemistry of wine stabilization and treatments. Handbook of enology, 2<sup>nd</sup> edn, vol 2. John Wiley and Sons LTD, chichester. UK; 2006.
- Van Rooyen PC, Tromp A. The effect of fermentation time (as induced by fermentation and must conditions) on the chemical profile and quality of chin blanc. Wine. S. Afr. J. Enol. Vitic. 1982;3(2):75-80.
- Carrau F, Medina K, Farina L, Boido E, Della-cassa E. Effects of Saccharomyces cerevisiae inoculum size on wine fermentation aroma compounds and its relation with assimiliable nitrogen content. Int. J. Food Microbiol. 2010;143(1-2):81-85.
- 50. Del-Barrio-Gelan R, Caceres-Mella A, Medel-Maraboli M, Pena-Daira, A. Effects of selected Saccharomyces cerevisiaeyeats strains and different aging techniques on the polysaccharide and polyphenols composition and sensorial characteristics of cabernet-sauvignon red wines. J. Sci. Food Agr. 2014;95:2132-2144.
- 51. Styger G, Prior B, Bauer FF. Wine flavor and aroma. J. Ind. Microbiol. Biotechnol. 2011;38:1145-1159.

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