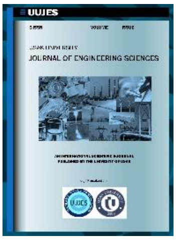

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Research article

DESIGN OF EXPERIMENT FOR ETHANOL USING STATISTICAL ANALYSIS FOR MANUFACTURING OF METHYLATED SPIRIT

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Abstract

Methylated spirit is a useful domestic and industrial product which serves as very important item at home, Industries, Schools and Hospitals. This study is focused on the production of high quality methylated spirit using palm wine as the primary raw material. The effects of fermentation period on alcohol content and characteristics of the palm wine was examined to ascertain the quality and effectiveness of the methylated spirit produced. Split-split plot design statistical model was employed at different stages of production such as conversion, fermentation, distillation, filtration and dehydration. Effective steps were taken to monitor temperature, yeast, density, percentage of alcohol and time which are the major factors that affect fermentation of palm wine. It was observed that during fermentation, there was increase in the temperature indicating possible evolution of heat, thereby, making the fermentation process exothermic. Percentage of ethanol obtained from the first, second and third distillation processes were 25%, 65% and 90%. It was observed that the longer the fermentation time, the lesser the percentage content of alcohol in the solution, which must undergo re-distillation in order to obtain higher percentage of alcohol (ethanol).

Keywords: *Fermentation; methylated spirit; distillates; alcohol; statistical model.*

1. Introduction

The need for biofuel has expanded since the 1992 Kyoto Protocol where many industrialized nations consented to lessen their carbon dioxide discharge and ozone depleting substance generation. Biofuel are not only environmental friendly, but have the potentials to reduce dependence on fossil fuels [1]. A standout amongst the most promising biofuels is ethanol which can be gotten from starch-based crops and sugar based

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crops such as cassava and sweet potato peel [2], banana, plantain and pineapple peels [3], Sweet Sorghum [4], Date palm [5], Maize cob and Groundnut shells [6], as well as lignocellulosic materials [7] etc.

Production of ethanol using the conventional feedstocks such as sugar based agricultural crops will in the long run result in high competition with the food consumed by humans as well as animal. To avoid this, it is important to explore various alternative feedstocks. Palm wine is the fermented palm sap obtained by tapping of palms (family Palmea). Two sources of palmwine in Nigeria are the fermented sap of oil palm tree (Elaeis guineensis, Jacq) and the Raphia palm tree (Raphia hookeri). Raphia palms inhabit swampy regions or areas of wet soil and usually yield more sap than oil palms although Raphia palms can only be tapped once in its life time because its terminal florescent is destroyed during tapping, E. guineensis, otherwise known as oil palm is the specie of Elaeis genus found in Nigeria [8, 9]. It is non-alcoholic at the original state prior to tapping, but few seconds after it has been tapped out becomes alcoholic. During fermentation, sugar content in the palm-sap are metabolized to alcohol and organic acids which result in the sap losing its sweetness [10]. The type of bacteria present depends on the stage of fermentation and the composition of the sap [11, 12]. Ethanol is a volatile, flammable, colorless liquid with a slight chemical odour. It is used as an antiseptic, a solvent, a fuel, perfumes and medicines etc.

Seer et al. [13] conducted an experiment on ethanol production using mixed cassava and durian seeds through fermentation by Saccharomyces cerevisiae yeast. In flask-scale fermentation using the mixed cassava-durian seeds, it was observed that maximum ethanol yield of 45.9% as well as ethanol concentration of 24.92 g/L were achieved at pH 5.0, temperature 35oC and 50:50 volume ratio of hydrolyzed cassava to durian seeds for a batch period of 48 hours. Examining the ethanol, glucose and biomass concentration level in a lab-scale bioreactor for the fermentation process using the same materials under flask-scale optimum conditions, ethanol yield of 35.7% as well as ethanol concentration level of 14.61 g/L were obtained over a period of 46 hours, where the glucose was almost fully consumed. The rate of ethanol yield can be improved by controlling a number of operating parameters other than increasing the initial substrate concentration. One of these parameters is the range of pH in which the fermentation process occurs [14]. Investigation carried out by Lin et al. [15] revealed that ethanol production under fixed glucose concentration can strongly be affected by pH of different ranges. Therefore, pH range of 4.0-5.0 is considered to be optimum for ethanol production. Ocloo and Ayernor [16] conducted an experiment to produce alcohol from cassava flour hydrolysate (CFH) with standard glucose and sucrose solutions used as controls. The conversion efficiency of sugar to alcohol, rate of fermentation and types of alcohol produced were determined. Results revealed that the maximum carbon dioxide evolved during fermentation was 8.57g recorded by CFH. The conversion efficiency of sugars to alcohol was 248.4, 99.51 and 95.37% for CFH, standard glucose and sucrose solutions respectively. Alcohol produced was mainly ethanol with traces of methanol.

Tulashie et al. [17] conducted an experiment to Determine and quantify methanol and ethanol concentration in alcoholic drinks and some local fermented food products. The results showed that some amounts of methanol between the ranges of “not detectable” to 0.161% were found in most alcoholic drinks investigated, but were however below the minimum oral lethal dose 0.3–1 g/kg (20 to 60 g or 25-75 ml/person in a 60 kg adult). No amount of methanol was indicated in the food products but contained small quantities of ethanol between the ranges of 0.006-0.140% which are not harmful to the body. The concentration of ethanol in most of the alcoholic drinks was below the suggested lethal dose of 5 to 8 g/kg for a 60 kg adult, 300 g (384 ml) of ethanol.

Ordonez et al. [18] developed a Direct Methanol Fuel Cell (DMFC) model, which incorporated an optimization approach, based on statistical Design of Experiment (DoE). For the output voltage model, all included effects were statistically significant at 1.5% level which resulted in R2 of 0.992 and a predicted R2 of 0.973. The results provided by the Central Composite Design were very close to the actual values, thus validating the model. Yakubu et al. [19] carried out an investigation to determine the disinfectant effect of Methylated spirit (95% methanol and 5% ethanol) as a teat dip against Listeria species. The high prevalence of Listeria monocytogenes in raw milk samples collected without disinfection and its absence in samples collected after disinfection suggests that the organisms are sensitive to Methylated spirit.

Studies carried out by Menezes et al. [20] to analyse the use of vinasse from cachaça as an ingredient of the fermentation medium for the production of spirit revealed that, vinasse addition do not affect the fermentation, distillation and chemical-sensorial quality of the beverage. Therefore, vinasse addition could be an alternative use for the residue. Spaho [21] employed two different types of distillation equipment for the production of fruit spirits: copper Charentais alembic and batch distillation columns. Alembic stills yielded better aroma and more characteristic fruit distillates but are slow and require more labour. Column still cleans the distillate giving a decent aroma and higher concentration of alcohol. In this paper, split-split plot design model was employed in the analysis the process of bioethanol production from palm wine.

2. Materials and Methods

The materials and methods employed in this study is discussed as follows:

2.1. Materials

Palm wine, condenser, water hose, retort stands and climbs, heating mantle, refractometer, light ray and cleaning reagent, receiving flask, Bunsen burner, thermometer, analytical balance.

2.2. Methods

The palm wine sample were collected from a palm wine taper in Okada town in Ovia North East local Government area of Edo State in Nigeria. The palm wine sample were collected with 2 liters of plastic container and then stored in a cool environment of 40^{o}C to avoid change in concentration of the palm wine samples. The palm wine samples were transported to the laboratory of chemistry Department in the University of Benin, Benin City for analysis of the samples. Others investigation were obtained from the chemistry laboratory of the same university. Saccharomyces Cerevisiae was introduced for conversion of the palm wine into ethanol. The methyl alcohol (methanol) was obtained in the Chemistry laboratory of the same University.

The measured volume of yeast used is 5 liters and methyl alcohol is 2cm^3 during the investigation. Palm wine was gotten from a palm tree where the sap is collected by a tapper. Typically the sap is collected from the cut flower of the palm tree. A container precisely a gourd or a bottle is fastened to the flower stump to collect the sap. The white liquid which is initially collected tends to be very sweet and contains trace of alcohol; the tapper collects a sticky white liquid from the head of the tall tree.

At the initial stage when the palm wine was on zero percent alcohol, analytical balance was used to check the percentage of alcohol and the Refractometer was also used to know the refractive index through which the level of alcohol was determined. Thereafter, the yeast content was measured according to the level of palm wine poured into it, to facilitate the fermentation process. The percentage and level of alcohol was constantly checked until the 3rd day (72 hrs), which is the suitable time for fermentation. A filter paper was used to filter out the yeast and other suspended particles. This was done before the distillation process. The fractional distillation column was properly fixed, while the fermented palm wine was poured into the distillation flask in order to distil, thus separating alcohol from water. The distillation process was repeated four times in order to get the ethanol of higher purity which is that area of concentration. Table 1 represents the physical properties of pure ethanol while Figure 1 illustrates the distillation process of ethanol.

Table 1 Selected physical properties of pure ethanol

Property	Value of Ethanol
Autoignition temperature	390-430 °C
Normal boiling point at 760mm Hg	78.32 °C
Change in boiling point, dt/dp at 760mm Hg,	0.033 °C
Coefficient of expansion	0.0011 °C-1
Critical temperature	243.1 °C
Critical pressure	63.0 atm
Critical volume	0.161 L/Mol
Density	0.7893 g/ml
Dielectric constant at 200C explosive limits in air vol.	1.35 × 10-9 %
Flash point (tag open cup)	60.0
Freezing point at 760mm Hg	-114.1 °C
Heat of combustion at 250 °C	328 Kcal/g mol
Heat of formation at 25°C	166.36 Kcal/g mol
Heat of vaporization boiling point	9.30 Kcal/g mol
Heat of fusion freezing point	-1.187 Kcal/g mol
Heat capacity at 25°C	0.574 cal/g
Refractive index at 760mm Hg and 20°C	-1.36143
Surface tension at 20°C	222 dryness/cm
Thermal conductivity at 200 °C Kcal/hr m^2	0.15 °C
Viscosity at 25°C	1.078 Cp
Boiling point	78.50 °C
Melting point	-117.30 °C

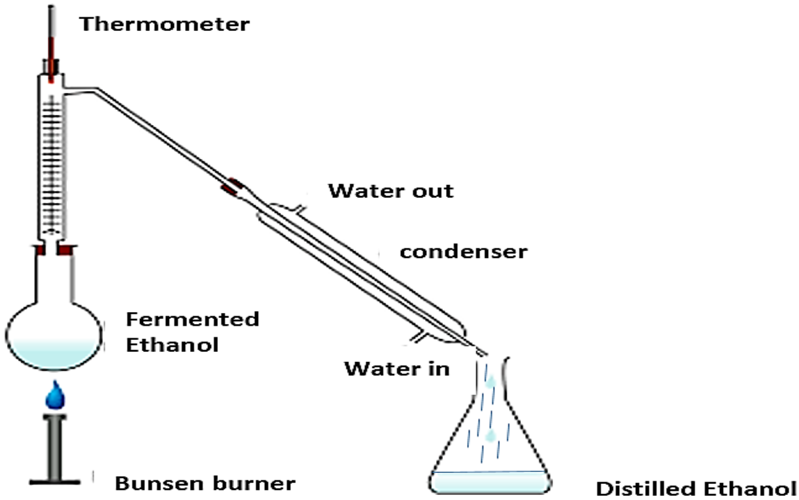

Fig. 1 Distillation process of ethanol

3. Statistical Models Employed

The statistical model employed in this study is called a split-split plot design. The split-split plot design is an extension of the split plot design to contain a third factor, making it one factor in the main-plot, another factor in the sub-plot and a third factor in the sub-plot. In other words, it is ideal to consider a third factor or more in order to have substantial evidence on how different factors interact with one another. The linear statistical model for the split-split plot is expressed as follows:

$$X_{ijkl} = \mu + \alpha_i + \beta_j + \gamma_k + \delta_l + (\alpha\beta)_{ij} + (\alpha\gamma)_{ik} + (\alpha\delta)_{il} + (\beta\gamma)_{jk} + (\beta\delta)_{jl} + (\gamma\delta)_{kl} +$$

$$(\alpha\beta\gamma)_{ijk} + (\alpha\beta\delta)_{ijl} + (\alpha\gamma\delta)_{ikl} + (\beta\gamma\delta)_{jkl} + (\alpha\beta\gamma\delta)_{ijkl} + \varepsilon_{ijk(l)} \begin{cases} i = 1,\ 2, 3 \ldots \mathrm{I} \\ \mathrm{j} = 1, 2, 3, 4 \ldots \mathrm{J} \\ \mathrm{k} = 1, 2, \ldots \mathrm{K} \\ \mathrm{l} = 1, 2, 3 \ldots \mathrm{L} \end{cases} \quad (1)$$

Where, I = 3 Parameters Measured, j = 4 Days of fermentation, k = 2 Raw materials, 1 = 3 variation in yeast content

$$SS_T = \sum_{i=1}^{I=3}\sum_{j=1}^{J=4}\sum_{k=1}^{K=2}\sum_{l=1}^{L=3} X_{ijkl}^2 - \frac{X_{....}^2}{IJKL} \quad (2)$$

$$= \left[\left(0.39^2 + 0.40^2 + 0.41^2 + \ldots + 0.30^2 + 0.28^2 + 0.27^2\right) - \frac{44.93^2}{3 \times 4 \times 2 \times 3} \right]$$

$$= 37.75 - 28.04 = 9.71$$

$$SS_A = \sum_{i=1}^{I=3} \frac{X_{i...}^2}{JKL} - \frac{X_{....}^2}{IJKL} \tag{3}$$

$$= \left[\left(\frac{7.99^2 + 21.26^2 + 15.68^2}{4 \times 2 \times 3}\right) - 28.04\right]$$

$$= 31.74 - 28.04 = 3.70$$

$$SS_B = \sum_{j=1}^{J=4} \frac{X_{.j..}^2}{IKL} - \frac{X_{....}^2}{IJKL} \tag{4}$$

$$= \left[\left(\frac{12.97^2 + 11.63^2 + 10.80^2 + 9.53^2}{3 \times 2 \times 3}\right) - 28.04\right]$$

$$= 28.38 - 28.04 = 0.34$$

$$SS_C = \sum_{k=1}^{K=2} \frac{X_{..k.}^2}{IJL} - \frac{X_{....}^2}{IJKL} \tag{5}$$

$$= \left[\left(\frac{28.55^2 + 16.38^2}{3 \times 4 \times 3}\right) - 28.04\right]$$

$$= 30.09 - 28.04$$

$$= 2.05$$

$$SS_{AB} = \sum_{i=1}^{I=3}\sum_{j=1}^{J=4} \frac{X_{ij..}^2}{KL} - \sum_{i=1}^{I=3} \frac{X_{i...}^2}{JKL} - \sum_{j=1}^{J=4} \frac{X_{.j..}^2}{IKL} + \frac{X_{....}^2}{IJKL} \tag{6}$$

$$= \left[\left(\frac{1.82^2 + 1.96^2 + 2.06^2 + ... + 3.98^2 + 3.91^2 + 3.67^2}{2 \times 3}\right) - 31.74 - 28.38 + 28.04\right]$$

$$= 32.44 - 31.74 - 28.38 + 28.04$$

$$= 0.36$$

$$SS_{AC} = \sum_{i=1}^{I=3}\sum_{k=1}^{K=2} \frac{X_{i.k.}^2}{JL} - \sum_{i=1}^{I=3} \frac{X_{i...}^2}{JKL} - \sum_{k=1}^{K=2} \frac{X_{..k.}^2}{IJL} + \frac{X_{....}^2}{IJKL} \tag{7}$$

$$= \left[\left(\frac{4.70^2 + 3.29^2 + 12.33^2 + 8.93^2 + 11.52^2 + 4.16^2}{4 \times 3}\right) - 31.74 - 30.09 + 28.04\right]$$

$$= 34.56 - 31.74 - 30.09 + 28.04 = 0.77$$

$$SS_{BC} = \sum_{j=1}^{J=4}\sum_{k=1}^{K=2}\frac{X^2_{.jk.}}{IL} - \sum_{j=1}^{J=4}\frac{X^2_{.j..}}{IKL} - \sum_{k=1}^{K=2}\frac{X^2_{..k.}}{IJL} + \frac{X^2_{....}}{IJKL} \quad (8)$$

$$= \left[\left(\frac{4.47^2 + 4.25^2 + 4.10^2 + 3.56^2}{3\times 3}\right) - 28.38 - 30.09 + 28.04\right]$$

$$= 7.503 - 28.38 - 30.09 + 28.04 = -22.93$$

$$SS_{ABC} = \sum_{i=1}^{I=3}\sum_{j=1}^{J=4}\sum_{k=1}^{K=2}\frac{X^2_{ijk.}}{L} - \sum_{i=1}^{I=3}\sum_{j=1}^{J=4}\frac{X^2_{ij..}}{KL} - \sum_{j=1}^{J=4}\sum_{k=1}^{K=2}\frac{X^2_{.jk.}}{IL} + \sum_{j=1}^{J=4}\frac{X^2_{.j..}}{IKL} \quad (9)$$

$$= \left[\left(\frac{1.20^2 + 1.25^2 + 1.14^2 + ... + 1.07^2 + 1.06^2 + 0.85^2}{3}\right) - 32.44 - 7.50 + 28.38\right]$$

$$= 35.58 - 32.44 - 7.50 + 28.38 = 24.02$$

$$SS_E = SS_T - SS_A - SS_B - SS_C - SS_{AB} - SS_{AC} - SS_{BC} - SS_{ABC}$$
$$= 9.71 - 3.70 - 0.34 - 2.05 - 0.36 - 0.77 + 22.93 - 24.02$$
$$= 1.4 \quad (10)$$

4. Results and Discussions

The results obtained by investigating the changes in the physical and chemical characteristics of the fermented palm wine and the ethanol product are discussed under the following sub-headings. The effect of fermentation period on properties of palm wine, the effect of fermentation period on alcohol content and the characteristics of products were examined. To achieve this, samples of the palm wine were collected and analyses on 24 hours basis were conducted. It was observed that there was foaming in the palm wine during fermentation and the foaming increased greatly during the first two days (48 hours) and decreased to none after 72 hours of fermentation. The foaming however is mainly caused by the evolution of CO_2 gas during fermentation process when the yeast breaks and settles out. Thus, the absence of foaming after 72 hours confirms a decrease in fermentation rate, but as fermentation period increased, concentration in terms of density and refractive index of the palm wine also increased which is in agreement with the findings of Ukpaka and Farrow [22], as indicated in Table 2, while the sugar content in the palm wine solution decreased as the fermentation time prolonged, the solution temperature varied within 28^{o}C and 31^{o}C as presented in Table 2. A rapid drop in the sugar content of the palm wine during the first few hours of fermentation which gradually reduced its value with significantly suppressed fermentation rate was observed. From Table 2 also, it is seen that the pH gradually decreased during the fermentation periods (of 24 hours, 48 hours, 72 hours and 77 hours) respectively as shown in Table 2. This correlates with the findings of Nguyen et al. [23]. This implies that acidity increased during the first 72 hours of fermentation before decreasing. This could be attributed to the fact that, some of the CO_2 gas evolved during the process went into solution forming a weak carbonic acid H_2CO_2 which increased the acidity of the solution and hereby decreasing the pH. The temperature of the fermented palm wine increased slightly for the first 72 hours then reduced slightly after 72 hours. Table 3 represents analysis of the raw Palm wine while Table 4 represents measured values of the filtered palm wine. Table 5-9 represents analysis of palm wine subjected to various distillation stages. The results presented in Table 5 and 6 indicates the first distillate characteristics of the functional parameters, which its values is given as 0.99g/ml for density 1.0 for specific gravity 1.35 for refractive index and 25% of ethanol produced. Similarly, considering the results obtained in Table 7 for second distillate indicates that volume of distillate is 7.5ml; volume of residue is 20ml, yield of distillate 35. 2% and volume of sample distillate is 40.5ml. Results presented in Table 8 illustrate the characteristics of the functional parameter obtained after the second distilled products were produced. The density has 0.87g/ml, specific gravity with 0.8986, refractive index with a value of 1.36 and percentage of ethanol produced was 65%. Finally, the results presented in Table 9 indicate 90% of ethanol obtained at third distillation process.

Table 2 Analysis of fermented palm wine fermentation time

Properties	Time (Hours)				
	0	24	48	72	77
Density g/ml	0.90	0.93	0.95	0.96	0.98
Refractive Index	1.35124	1.37024	1.41024	1.43608	1.45598
% of sugar w/w	12.253	11.896	11.767	9.601	9.587
Temperature ^oC	29	31	31	30	28
Ph	6.3	6.0	5.8	5.5	5.3

Table 3 Analysis of Raw Material (Palm wine)

Properties	Values
Total wt. of palm wine	4.00g
Total wt of palm wine filtered	289g
Total vol. of palm wine filtered	0.80g/ml
Density of filtered palm wine	295.95ml

Table 4 Measured values of the filtered palm wine

Properties	Values
Wt. of yeast	30.183g
Wt. of palm wine	289g
Wt of yeast filtered out	12.20%
Percentage composition of palm wine	14.0%
Percentage content of sugar in palm wine	12.25%
Percentage water content in palm wine	84%
Refractive index	1.35

Table 5 Values of first distillate (volume of sample distilled)

Properties	Values
Volume of distillate	30.5ml
Volume of residue	259ml
Yield of distillate	12.9%

Table 6. Analysis of first distillate

Properties	Values
Density g/ml	0.99
Specific gravity	1
Refractive index	1.35
% of ethanol	25%

Table 7. Values of second distillate

Properties	Values
Volume of distillate	7.5ml
Volume of residue	20ml
Yield of distillate	35.2%
Volume of sample distilled	40.5ml

Table 8. Analysis of second distillate

Properties	Values
Density g/ml	0.87 g/ml
Specific gravity	0.8986
Refractive index	1.36
% of ethanol	65%

Table 9. Value of third distillate

Properties	Values
% of ethanol	90%

After the completion of the fermentation process and the fermented palm wine was filtered, it was poured into the distillation column; however, during this period, it was observed that the resultant solution now has a stronger smell of alcohol as the distillation process was done repeatedly. From the distillation carried out, the alcohol concentration was found to be 95% after re-distillation. Low concentration of ethanol in distillate may be attributed to high water content as well as possible conversion of the ethanol into minor products such as aldehydes, acetic acid, esters, etc. Previous investigators has shown that fermentation of sugar will produce ethanol as main product as well as other products such as carbon dioxide, acids, aldehydes, ketones and esters, and that these minor products increases in quantity while the alcohol decreases with increase in fermentation time [24]. The Statistical Computation of the Palm wine Processing is indicated in Table 10.

Table 10 Statistical computation of the Palm wine processing

Sources of Variation	Sums of Squares	Degree of Freedom	Mean of Squares	Fisher's ratio Calculated (F_{cal})	Fisher's ratio Tabulated (F_{tab})	Decision
Parameter Measured (A)	3.70	(I -1) = 2	1.85	$\frac{MS_A}{MS_B} = 16.82$	9.55	$F_{cal} \succ F_{tab}$ Reject H_0
Days of Fermentation (B)	0.34	(J -1) = 3	0.11	$\frac{MS_B}{MS_E} = 3.67$	2.80	$F_{cal} \succ F_{tab}$ Reject H_0
Raw Material Type (C)	2.05	(K-1) = 1	2.05	$\frac{MS_C}{MS_{AC}} = 5.39$	18.51	$F_{cal} \prec F_{tab}$ Accept H_0
Days of Fermentation x Parameters measured (AB)	0.36	(I-1)(J-1) = 6	0.06	$\frac{MS_{AB}}{MS_{AC}} = 0.16$	19.33	$F_{cal} \prec F_{tab}$ Accept H_0
Raw materials x Parameter Measured (AC)	0.77	(I-1)(K-1) =2	0.38	$\frac{MS_{AC}}{MS_E} = 12.67$	3.19	$F_{cal} \succ F_{tab}$ Reject H_0
Days of Fermentation x Raw Material Type (BC)	-22.93	(J-1)(K-1) = 3	-7.64	$\frac{MS_{BC}}{MS_{ABC}} = -1.91$	4.76	$F_{cal} \prec F_{tab}$ Accept H_0
Parameter Measured x Days of Fermentation x Raw Material Type (ABC)	24.02	(I-1)(J-1)(K-1) = 6	4.00	$\frac{MS_{ABC}}{MS_E} = 133.33$	2.30	$F_{cal} \succ F_{tab}$ Reject H_0
Error	1.4	IJK(L-1) = 48	0.03			
Total	9.71					

In order to characterize the product obtained from the distillation process, samples were collected and analysed for density and refractive index. The results of value gotten are then related to the standard values of ethanol from text. This is done basically to proof that, the end product can liken to be the 95% ethanol or have characteristics that are related to that of ethanol. But be informed that at the end of the analysis, there are variations in values with that of the standards; this could be, attributed to the means or form of production, environmental conditions and generally, the refined nature of the product due to the local means of production [25].

4.1. Hypothesis Results

i. Examination of parameter measured (SS_A)
   $H_0: \alpha_i = 0 \qquad H_1: \alpha_i \neq 0$

Since $F_{cal} = 16.82 > F_{tab} = 9.55$, it is concluded that the experimental data provides paucity of evidence for the null hypothesis to be accepted. This implies that the parameter measured during the experimental runs do not significantly influence the reaction process.

ii. Examination of days of fermentation (SS_B)

$H_0^i : \beta_j = 0 \quad H_1^i : \beta_j \neq 0$

Since $F_{cal} = 3.67 > F_{tab} = 2.80$, the result suggests that the null hypothesis be rejected at α – value of 0.05. The conclusion therefore is that, there is differential treatment effect observed in the days of fermentation considered during the experiment.

iii. Examination of raw material type (SS_C)

$H_0^{ii} : \gamma_k = 0 \quad H_1^{ii} : \gamma_k \neq 0$

Since $F_{cal} = 5.39 < F_{tab} = 18.51$, the experimental data do not furnish enough evidence for the null hypothesis to be rejected, H_0'' at α – value of 0.05. It is therefore concluded that the type of raw materials employed do have significant influence on the methylated spirit produced.

iv. Examination of days of fermentation × parameters measured interaction (SS_{AB})

$H_0^{iii} : (\alpha\beta)_{ij} = 0 \quad H_1^{iii} : (\alpha\beta)_{ij} \neq 0$

With $F_{cal} = 0.16 < F_{tab} = 19.33$, it is obvious from the result that the data do not show enough evidence for the null hypothesis to be reject, H_0'''. We however infer that there is a differential interaction treatment effect between the days of fermentation and parameters measured during the experimental runs.

v. Examination of raw materials × parameters measured interaction (SS_{AC})

$H_0^{iv} : (\alpha\gamma)_{ik} = 0 \quad H_1^{iv} : (\alpha\gamma)_{ik} \neq 0$

The calculated value $F_{cal} = 12.67 > F_{tab} = 3.19$ shows paucity of evidence for the null hypothesis to be accept, H_0^{iv}. It is therefore concluded that, there appears to be an interaction between the raw materials and the different parameters measured during the experiment.

vi. Examination of days of fermentation × raw materials type interaction (SS_{BC})

$H_0^v : (\beta\gamma)_{jk} = 0 \quad H_1^v : (\beta\gamma)_{jk} \neq 0$

The investigation points out that $F_{cal} < F_{tab}$. This implies that there is no sufficient proof to reject the null hypothesis, and therefore conclude that both days of fermentation and the type of materials employed produced significant differential treatment during the experiment.

vii. Examination of parameters measured × days of fermentation × raw material type interaction (SS_{ABC})

$H_0^{vi} : (\alpha\beta\gamma)_{ijk} = 0 \quad H_1^{vi} : (\alpha\beta\gamma)_{ijk} \neq 0$

Since $F_{cal} = 133.33 > F_{tab} = 2.30$, there is lack of sufficient evidence to accept the null hypothesis, H_0^{vi} of lack of differential treatment. It is therefore concluded that the type of raw materials used have differential treatment effects on the days of fermentation observed in the parameters measured.

5. Conclusion

From the experiment and analysis carried out in this study, putting all conditions in place, and comparing with known standards, it can therefore be concluded that methylated spirit produced from palm wine, conforms to accepted standards and can compete with methylated spirits found in the supermarkets and shops based on the following:

From experimental findings, ethanol can be manufactured from palm wine by bacteria or yeast fermentation that is top acting yeast. The palm wine used is composed approximately of 12.2% sugar and 84% water. The refractive index decreased with prolong fermentation up to 72 hours of completion. Evolution of carbon dioxide caused foaming on top of the fermented palm wine which increased with continued increase of carbon dioxide evolution. At the end of fermentation, it was observed that bottom yeast fluctuated and settled to the bottom of the fermented beer. From experimental results obtained, it can be said that prolonged fermentation would lead to decrease in the percentage yield of ethanol. This conclusion was drawn as a result of the renewed increase in the density, refractive index etc. of the fermented palm wine after 72 hours duration.

The chemical properties of the palm wine and fermented sample were not analyzed. It would have been better to carry out compositional analysis to determine certain contents such as Nitrogenous bodies, protein, fats etc. before and after the fermentation process. It is therefore recommended that this points be considered in future studies. This paper was not able to determine the properties and compositions of the distillation residue. It is therefore also recommended that future study be done in this area. The strength is legally estimated by Syke's hydrometer which was legalized in 1816 by 56 Geo. 111C. 40, but was not ascertained because such instruments are not found around. It is therefore recommended that while doing such analysis, all instruments should be available. The study did not take into consideration the possible percentage yield of alcohol after 72 hours, only reasonable productions were made based on some observations.

Therefore, further work on this area should experimentally determine the percentage yield of ethanol after 72 hours, may be up to 168 hours. Finally, since it has been experimentally proven that ethanol can be produced from the fermentation of crops/plants that contain sugar from carbohydrate, it is necessary to consider the production and preservation of such agricultural crops. It is sad to note that every year millions of tons of starchy contained crops are wasted and lost due to lack of good sufficient storage facilities, fertilizing facilities and land for such crops to be planted. Also in developing countries like Nigeria where storage facilities and technologies are inadequate, thorough work has to be done by government and individuals as well.

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