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Optimization of Process Variables for Bioethanol Production using Saccharomyces Cerevisiae

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Abstract- ***This study explores the production and optimization of bioethanol from palm wine via enzymatic fermentation using Saccharomyces cerevisiae. Palm wine, rich in fermentable sugars, served as a low-cost renewable feedstock. Fermentation was facilitated by yeast enzymes (invertase and zymase), which hydrolyzed sucrose into glucose and fructose, subsequently converted to ethanol and carbon dioxide. The effects of yeast dosage, fermentation temperature, and time on ethanol yield and concentration were systematically investigated through 20 experimental runs, with ethanol quantified by acid dichromate oxidation and titrimetric back titration. Ethanol concentrations ranged from 1.47 to 6.80 mol/L, with yields between 8.61% and 39.56%. Optimal conditions 3.24% yeast, 51.15°C, and 55 minutes produced the highest yields. Physicochemical analysis (specific gravity, viscosity, flash point, refractive index, cloud point, and pour point) confirmed ethanol quality, though slight deviations indicated minor impurities such as water, glycerol, or residual sugars. FTIR spectroscopy verified ethanol identity, with characteristic O–H, C–H, and C–O peaks, while minor signals suggested traces of by-products. Optimization using Response Surface Methodology (RSM) with a Central Composite Design (CCD) demonstrated excellent predictive accuracy (R^2 = 0.9973, Adjusted R^2 = 0.9949, Predicted R^2 = 0.9683). The model predicted a maximum yield of 40.81% v/v, closely matching experimental validation (40.03% v/v, 1.91% deviation). These findings confirm palm wine as a promising substrate for efficient bioethanol production, offering a sustainable, locally adaptable pathway for renewable energy development.***

Keywords: Palm wine, enzymatic fermentation, Saccharomyces cerevisiae, Response Surface Methodology.

I. INTRODUCTION

The global demand for renewable and sustainable energy sources has become increasingly urgent in response to rising energy consumption, climate change, and the depletion of fossil fuel reserves. Biofuels, particularly bioethanol, have attracted significant attention as potential alternatives to fossil fuels due to their renewable nature, cleaner combustion profile, and compatibility with existing fuel infrastructure (Demirbas, 2009; Balat & Balat, 2010). Bioethanol is primarily produced through the fermentation of sugars and starches by microorganisms, most commonly yeast (Saccharomyces cerevisiae) (Lin et al., 2012). It is already blended with gasoline in several countries to reduce greenhouse gas emissions and improve energy security (Naik *et al*., 2010). While bioethanol production from crops such as sugarcane, corn, and cassava has been well established, concerns about food security, land use competition, and sustainability have created the need to explore alternative non-food feedstocks (Nigam & Singh, 2011). Agro-industrial residues, fruit wastes, lignocellulosic biomass, and locally available natural resources are being investigated as cost-effective and sustainable feedstocks for ethanol production. Among these, palm wine, which is a traditional beverage widely consumed in tropical and sub-tropical regions, presents an under-explored but highly promising substrate (Eze & Uzoechi, 2017).

Palm wine is a naturally fermented sugary sap tapped from various species of palm trees. It contains a rich composition of fermentable sugars such as sucrose, glucose, and fructose, along with amino acids, vitamins, and minerals that support microbial growth (Obire, 2005). Its high sugar content makes it a natural candidate for ethanol production without the extensive pre-treatment required for lignocellulosic materials. Moreover, palm wine is abundantly available in West and Central Africa, parts of Asia, and South America, making it a regionally adaptable feedstock for decentralized biofuel production (Ezeronye, 2004). Despite its potential, systematic scientific studies on bioethanol production from palm wine remain limited. Most research has focused on conventional feedstocks such as sugarcane, molasses, and corn, which have established industrial processes (Bai *et al*., 2008). Palm wine differs significantly in its composition and fermentation dynamics, which warrants detailed investigation. Specifically, the natural microbial flora present in palm wine often results in uncontrolled fermentation that reduces ethanol yield and leads to by-product formation such

as organic acids and esters (Ogbulie *et al*., 2007). Standardizing the fermentation process using a controlled inoculation of Saccharomyces cerevisiae offers a strategy to improve ethanol productivity and reproducibility. Optimization of fermentation parameters is a critical step in enhancing ethanol yield and concentration. Factors such as yeast dosage, fermentation temperature, and time significantly influence ethanol production efficiency (Lin & Tanaka, 2006). Excessive yeast dosage may lead to nutrient competition and ethanol inhibition, while insufficient dosage may prolong fermentation. Similarly, temperature affects yeast metabolism, where elevated temperatures can denature enzymes while sub-optimal temperatures slow down fermentation. Time must also be carefully managed, as extended fermentation may reduce ethanol concentration due to microbial metabolism of ethanol into secondary products (Ezeronye, 2004). Therefore, a scientific evaluation of these process parameters is essential to maximize productivity.

Response Surface Methodology (RSM) has emerged as a powerful statistical tool for modeling and optimizing bioprocesses. It enables the evaluation of multiple interacting variables simultaneously and identifies optimal operating conditions with fewer experimental runs compared to traditional one variable at a time approaches (Montgomery, 2017). The Central Composite Design (CCD), a variant of RSM, is particularly effective for developing quadratic models that capture non-linear relationships between variables. Previous studies have successfully applied RSM in optimizing bioethanol production from cassava, sweet sorghum, and molasses (Ghosh & Hallenbeck, 2010; Onilude et al., 2012). However, limited studies have applied this approach to palm wine fermentation, leaving a significant knowledge gap. Beyond yield, the quality of ethanol must also be verified before considering industrial applications. Physicochemical characterization, including specific gravity, viscosity, flash point, cloud point, and refractive index, provides insights into the fuel properties and purity of the ethanol produced. Spectroscopic techniques such as Fourier Transform Infrared (FTIR) spectroscopy further confirm the presence of characteristic ethanol functional groups and detect impurities or fermentation by-products (Silverstein *et al.*, 2014). Together, these analyses ensure that the bioethanol produced meets the required standards for blending with gasoline or use in other industrial processes.

Given the global emphasis on sustainable energy transitions and the need for region-specific solutions, palm wine bioethanol production presents both an opportunity and a challenge. Its availability in rural and semi-urban regions offers potential for decentralized, small-to-medium-scale biofuel facilities that could alleviate energy poverty in developing countries (Akinbomi *et al.*, 2014). Moreover, integrating local resources into renewable energy production reduces dependence on imported fossil fuels and contributes to climate change mitigation. Therefore, this study aims to investigate the production and optimization of bioethanol from palm wine using enzymatic fermentation with Saccharomyces cerevisiae. Specifically, the study evaluates the effect of yeast dosage, temperature, and fermentation time on ethanol yield and concentration, applies RSM for optimization, and characterizes the ethanol produced through physicochemical and FTIR analysis. The findings of this research contribute to the development of sustainable, cost-effective, and regionally adaptable bioethanol production systems that align with the broader goals of renewable energy and environmental sustainability.

II. MATERIALS AND METHODS

2.1 Materials

2.1.1 Feedstock

Fresh palm wine was collected from local palm wine tappers in Agbani, Enugu State. The samples were transported in airtight containers to the laboratory to minimize uncontrolled fermentation and stored at 4 °C until use. The palm wine was used within 24 hours of collection to ensure sugar integrity and prevent microbial spoilage. Prior to fermentation, the palm wine was filtered using muslin cloth to remove suspended solids and impurities.

2.1.2 Microorganism

The yeast strain used was Saccharomyces cerevisiae, this strain was chosen due to its proven ethanol-producing capability, tolerance to osmotic and thermal stress, and high ethanol productivity under controlled fermentation conditions. The yeast culture was maintained on yeast extract peptone dextrose (YPD) agar slants at 4 °C and sub-cultured every two weeks to ensure viability.

2.1.3 Chemicals and Reagents

All chemicals used were of analytical grade. Acid dichromate reagent was prepared for ethanol determination through oxidation, while sodium thiosulfate and potassium iodide were used for back titration. Distilled water was used throughout the experimental procedures.

2.2 Experimental Design

A total of 20 experimental runs were conducted based on a Central Composite Design (CCD) of Response Surface Methodology (RSM). Three independent variables yeast dosage (wt%), fermentation temperature (°C), and fermentation time (minutes) were varied across five levels (−α, −1, 0, +1, +α). Ethanol yield and concentration were taken as response variables. The CCD approach enabled the construction of a second-order polynomial model for prediction and optimization while reducing the number of experiments compared to a full factorial design. Experimental runs were randomized to minimize systematic errors. Each run was performed in triplicate, and the mean values were used for statistical analysis.

2.3 Fermentation Process

2.3.1 Inoculum Preparation

A loopful of Saccharomyces cerevisiae was aseptically transferred from the YPD agar slant into 100 ml of sterile YPD broth and incubated at 30 °C for 24 hours in a rotary shaker at 150 rpm. The activated inoculum was standardized to an optical density (OD600) of 0.8 before being introduced into the fermentation medium.

2.3.2 Fermentation Setup

For each experimental run, 250 ml of palm wine was measured into 500 ml Erlenmeyer flasks, supplemented with yeast at the designated dosage (wt%), and incubated at the set fermentation temperature and time. The flasks were fitted with fermentation locks to allow the escape of carbon dioxide while preventing oxygen entry. At the end of each run, the fermented broth was distilled at 78 °C to separate ethanol from the fermentation medium.

2.4 Determination of bioethanol concentration

Method used by previous author (Tripathi, 2018) was employed in the determination of bio-ethanol concentration by titration. The following solutions were used; acid dichromate solution (0.01 mol/L in 5.0 mol/L sulphuric acid), starch indicator solution (1.0% solution), sodium thiosulphate (0.03 mol/L) and potassium iodide solution (1.2 mol/L). Sample solution was diluted in 1:20 (10 ml in 200 ml) with distilled water. To a 250 ml conical flask, 10 ml of acid dichromate solution was added and then the flask was sealed with rubber stopper. 1 ml of sample solution was poured into 5ml beaker with the aid of pipette and then three samples was prepared accordingly. The 5 ml beaker was suspended over the acid dichromate solution. The flask was incubated overnight at 25-30 °C. Then after the incubation, the flask was kept at room temperature and then the stopper was loose and then the 5 ml beaker was discarded. The walls was rinsed with distilled water; about 100 ml of distilled water and 1 ml of potassium iodide solution was added to it and then it was slightly placed in vortex position to mix. 3 blank titrations of same was prepared by adding 10 ml of acid dichromate solution and 100 ml of distilled water and 1 ml potassium iodide solution and they was mixed. Sodium thiosulphate solution was filled in the burette and then titrated against each solution. 1 ml of starch solution was added till when the colour of the solution change to yellow and then it was titrated until the blue colour disappeared. Titrimetric method used by previous research report (Tripathi, 2018) was employed in the determining the ethanol concentration. Ethanol concentration (mol/l) was calculated using stoichiometric relationships.

2.4.2 Yield Calculation

Ethanol yield was expressed as percentage volume per volume (% v/v) of ethanol produced relative to the total fermentation volume. Yield values were calculated for each experimental run and compared across different fermentation conditions.

2.5 Determination of Physico-chemical Properties of the Bio-ethanol

The bio-ethanol was distilled from palm wine and characterized to ascertain its properties in terms of specific gravity, flash and smoke points, viscosity, refractive index, cloud, and pour points.

Specific gravity: The Specific gravity bottle method was used for the determination of the specific gravity. A clean empty bottle was weighed on an electronic balance and the weight (W_1) recorded. It was then filled with the sample and weighed (W_2). All the determinations were at room temperature. The

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volume (V) of the specific gravity bottle was recorded.

$$\text{Specific gravity} = \frac{W_2 - W_1}{V} \quad (1)$$

Flash and smoke point: The flash points of the samples were determined. A Pensky-Martins Flash point (closed) apparatus was used to measure the flash point of the samples. The sample was filled in the test cup up to the specified level and was heated and stirred at a slow and constant rate. At every 10℃ temperature rise, flame was introduced for a moment with the help of a shutter. The temperature at which a flash appeared in the form of sound and light was recorded as the flash point.

Viscosity: This was done with a digital viscometer made by Searchtech instruments, England. The appropriate spindle was selected and fixed on the instrument. The spindle was inserted in the sample to be analyzed till the level mark on the spindle reached the surface of the sample. Enter button on the instrument was pressed and the viscosity of the sample was displayed on the screen.

Refractive index: Abbe refractometer -bench type (Model: WAY-2S, made by Searchtech Instruments) was used to determine the refractive index of the bioethanol. The power switch was pressed on, and the illuminating lamp came up, and the display showed 0000. A drop of the sample was introduced on the working surface of the lower refracting prism. The rotating arm and the collecting lens cone of the light-gathering illuminating units were rotated to make the light-intake surface of the upper light-intake prism evenly illuminated. The field of view was observed through the eyepiece, and the adjustable hand wheel was rotated to make the line dividing the dark and light areas fall in the cross line. The dispersion correction hand wheel was rotated to get a good contrast between the light and dark areas and minimum dispersion. The read button was then pressed, and the refractive index was displayed on the screen.

Cloud and pour points: The Cloud and Pour point of the sample was determined using the Cloud and Pour point apparatus. The apparatus mainly consists of 12cm high glass tubes of 3cm diameter. These tubes are enclosed in an air jacket, which was filled with a freezing mixture of crushed ice and sodium chloride crystals. The glass tube containing the fuel sample was removed from the jacket at every 10 ^{o}C interval as the temperature fell, and was inspected for cloud/pour point. The point at which a haze was first seen at the bottom of the sample was taken as the cloud point. The pour point was taken to be the temperature 10 ^{o}C above the temperature at which no motion of the fuel was observed for five seconds on tilting the tube to a horizontal position.

2.6 Fourier Transform Infrared (FTIR) Analysis
FTIR spectroscopy was conducted to confirm the chemical identity of the ethanol produced. Samples were analysed using an FTIR spectrophotometer over a wavelength range of 4000–400 cm^{-1} at a resolution of 4 cm^{-1}. Characteristic absorption peaks corresponding to functional groups such as O–H, C–H, and C–O were used to verify ethanol formation. The spectra of raw palm wine and fermented ethanol were compared to assess compositional changes.

2.7 Statistical Analysis
Experimental data were analysed using Design-Expert software. The quadratic polynomial model was fitted to the response variables, and the adequacy of the model was assessed using analysis of variance (ANOVA), coefficient of determination (R^2), adjusted R^2, and predicted R^2 values. Significance was determined at a 95 % confidence level ($p < 0.05$). Three-dimensional response surface plots were generated to visualize the effects of the independent variables and their interactions on ethanol yield and concentration.

III. RESULTS

3.1 Ethanol Concentration and Yield
The fermentation of palm wine under different conditions demonstrated wide variability in ethanol concentration and yield, confirming the significant influence of process parameters. Across the 20 experimental runs, ethanol concentration ranged between 1.47 and 6.80 mol/l, while yields spanned 8.61% to 39.56% v/v. Runs performed near the central points of the design consistently recorded higher yields, whereas those at extreme combinations of high temperature and prolonged fermentation exhibited reduced productivity.

Yeast dosage, fermentation temperature, and time were all found to play critical roles. Increasing yeast dosage initially enhanced ethanol formation by accelerating sugar hydrolysis and fermentation, but

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beyond an optimum level, yield declined due to ethanol inhibition and nutrient competition. Similarly, ethanol yield increased with temperature up to an optimum (51 °C), after which further increases reduced yeast viability. Fermentation time also followed a non-linear trend: yields rose rapidly within the first 50–55 minutes but plateaued thereafter, with extended runs showing slight decreases, likely due to ethanol re-consumption or secondary metabolite production.

The highest yield was achieved at approximately 3.24% yeast concentration, 51.15 °C, and 55 minutes, producing ethanol at 40.03% v/v. This aligns with the predicted maximum from the RSM model (40.81% v/v), validating the optimization approach.

3.2 Response Surface Methodology (RSM) Optimization

The quadratic polynomial model generated through RSM effectively described the relationship between the variables and the responses. Analysis of variance (ANOVA) confirmed the model's statistical significance with a p-value < 0.0001, and diagnostic metrics indicated excellent predictive ability (R^2 = 0.9973, Adjusted R^2 = 0.9949, Predicted R^2 = 0.9683). Response surface plots revealed the interactive effects of the three process variables. Yeast dosage and temperature exerted the most significant influences on ethanol yield, while fermentation time had a lesser but still notable effect. Ethanol yield increased non-linearly with yeast dosage and temperature until reaching a peak, beyond which efficiency declined. The close agreement between predicted and experimental results (only 1.91% deviation) demonstrates the robustness of the model and confirms its utility in guiding industrial-scale optimization.

3.3 FTIR Spectroscopy Analysis

Fourier Transform Infrared (FTIR) spectroscopy was employed to confirm the chemical identity of the fermentation products. The spectrum of raw palm wine exhibited broad absorption bands attributed to alcohols, phenols, water, and carboxylic acids, indicating the presence of diverse organic compounds. In contrast, the spectrum of the fermented ethanol revealed sharp and distinct peaks characteristic of ethanol: O–H stretching around 3300 cm^{-1}, C–H stretching near 2900 cm^{-1}, and C–O stretching in the region of 1050–1100 cm^{-1}. The presence of these peaks confirmed the successful conversion of sugars to ethanol. Only minor traces of unconverted compounds or fermentation by-products were detected, consistent with the deviations observed in physicochemical properties. These results validate the fermentation process both qualitatively and quantitatively, ensuring that the ethanol produced is chemically comparable to conventional bioethanol.

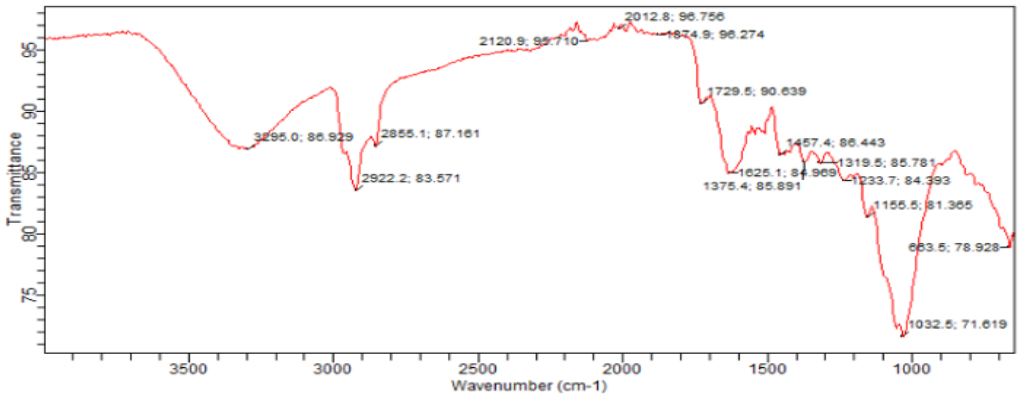

Figure 1a: FTIR spectrum of the Palm wine

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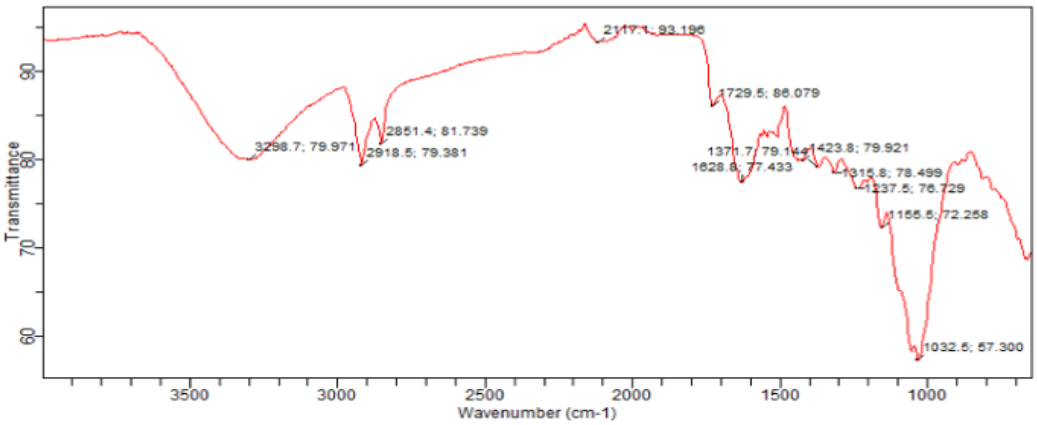

Figure 1b: FTIR spectrum of the bio-ethanol

3.4 Physicochemical Properties of Bioethanol

The physicochemical characterization of the ethanol samples (Table 1) further established their suitability for fuel applications. The specific gravity of 0.872 was close to standard ethanol values, confirming the density of the product. The viscosity of 1.70 mPa·s indicated favourable flow properties for handling and fuel injection systems. The flash point of 15.96 °C aligned with commercial ethanol specifications, reinforcing its safety and usability as a combustible fuel. The refractive index of 1.348 was consistent with ethanol-water mixtures, while the cloud point (19.90 °C) and pour point (4.92 °C) fell within acceptable thresholds for tropical and subtropical climates. Together, these values indicated high ethanol quality, although slight deviations suggested the presence of residual impurities such as glycerol or unfermented sugars. This observation reinforces the FTIR findings and highlights the need for further purification when higher fuel-grade ethanol is required.

Table 1: Characteristics of the bio-ethanol

Parameters	Values
Cloud point (ºC)	19.90
Flash point (ºC)	15.96
Pour point (ºC)	4.92
Refractive index	1.348
Specific gravity	0.872
Viscosity (mPas)	1.70

3.5 RSM Results

Equation in terms of coded factor:

The equation in terms of coded factors (Equation 2) was used to make predictions about the response for given levels of each factor. The coded equation is useful for identifying the relative impact of the factors by comparing the factor coefficients.

$$Bio - ethanol\ yield\ = +39.42 + 6.17A + 2.34B + 3.64C + 0.9275AB + 0.6875AC + 1.13BC - 11.38A^2 - 3.15B^2 - 6.41C^2 \quad (2)$$

Coefficients in terms of coded factors:

The coefficients estimate in Table 2 represents the expected change in response per unit change in factor value when all remaining factors are held constant. The intercept in an orthogonal design is the overall average response of all the runs. The coefficients are adjustments around that average based on the factor settings. When the factors are orthogonal the VIFs are 1; VIFs greater than 1 indicate multi-collinearity, the higher the VIF the more severe the correlation of factors. As a rough rule, VIFs less than 10 are tolerable.

Table 2: Coefficient terms of coded factors

Factor	Coefficient Estimate	df	Standard Error	95% CI Low	95% CI High	VIF
Intercept	39.42	1	0.2695	38.82	40.02	
A-Yeast dosage	6.17	1	0.2479	5.61	6.72	1.00
B-Temp.	2.34	1	0.2479	1.78	2.89	1.00
C-Time	3.64	1	0.2479	3.08	4.19	1.00

AB	0.9275	1	0.2772	0.3099	1.55	1.00
AC	0.6875	1	0.2772	0.0699	1.31	1.00
BC	1.13	1	0.2772	0.5099	1.75	1.00
A^2	-11.38	1	0.4728	-12.43	-10.33	1.82
B^2	-3.15	1	0.4728	-4.21	-2.10	1.82
C^2	-6.41	1	0.4728	-7.47	-5.36	1.82

3.6 Influence of Fermentation Variables on Ethanol Yield

The present study demonstrated that yeast dosage, fermentation temperature, and time significantly affects bioethanol production from palm wine. Ethanol concentrations ranged from 1.47 to 6.80 mol/l, while yields varied between 8.61% and 39.56%, with optimal production achieved at 3.24% yeast dosage, 51.15 °C, and 55 minutes as seen in Table 4. These findings confirm that the efficiency of enzymatic fermentation is highly dependent on balancing the physiological requirements of yeast with substrate availability and process conditions. The observed non-linear effects of yeast dosage are consistent with previous reports on cassava and molasses fermentation, where ethanol yield increased with yeast concentration up to an optimum, but declined at higher dosages due to ethanol inhibition and nutrient depletion. This suggests that excessive inoculum sizes not only increase production costs but may also compromise yield efficiency. Similarly, temperature optimization is crucial, while moderate heating accelerates metabolic activity, excessive temperatures denature enzymes such as zymase, thereby reducing fermentation efficiency. The identified optimum of 51 °C is relatively higher than typical fermentation temperatures (30–35 °C), suggesting that palm wine yeast systems may have adapted to more thermos-tolerant conditions. This thermo-tolerance offers a potential advantage in tropical regions where ambient temperatures are naturally high Azis and Fudholi, 2021).

Fermentation time also played a critical role. Ethanol yield increased rapidly within the first 50 minutes, followed by a plateau and a slight decline with prolonged fermentation. This trend reflects the classical batch fermentation profile, where sugar availability and ethanol accumulation govern yeast metabolism. Extended fermentation may result in ethanol re-consumption or by-product formation, as reported in studies on sorghum and sugarcane juice. Therefore, maintaining fermentation within the identified time window is essential for maximizing yield.

3.7 Model Robustness and Optimization

The statistical modelling performed using Response Surface Methodology (RSM) with Central Composite Design (CCD) produced a highly reliable quadratic model, with excellent agreement between predicted and experimental values (deviation of only 1.91%) (Table 5). The high coefficients of determination (R^2 = 0.9973, Adjusted R^2 = 0.9949, Predicted R^2 = 0.9683) seen in Table 2 reflect the accuracy of the model in capturing the interactive effects of yeast dosage, temperature, and time. These findings reinforce the suitability of RSM for bioethanol process optimization, as demonstrated in prior studies on substrates such as cassava, sweet sorghum, and molasses. However, the novelty of this study lies in its application to palm wine, an underexplored substrate. The close agreement between predicted and experimental values (Table 3) suggests that this modelling approach can serve as a valuable tool for scaling bioethanol production from palm wine in industrial contexts, reducing the reliance on costly trial-and-error experimentation.

Table 2: ANOVA for the quadratic model of bio-ethanol yield

Source	Sum of Squares	df	Mean Square	F-value	p-value	
Model	2265.29	9	251.70	409.51	< 0.0001	significant
A-Yeast dosage	380.20	1	380.20	618.57	< 0.0001	
B-Temp.	54.62	1	54.62	88.86	< 0.0001	
C-Time	132.13	1	132.13	214.98	< 0.0001	
AB	6.88	1	6.88	11.20	0.0074	
AC	3.78	1	3.78	6.15	0.0325	
BC	10.17	1	10.17	16.55	0.0023	

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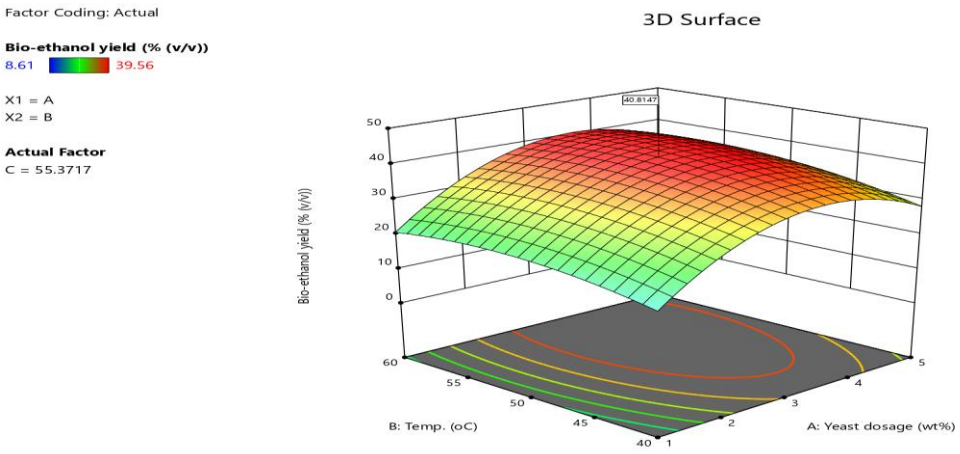
A^2	356.08	1	356.08	579.34	< 0.0001
B^2	27.36	1	27.36	44.51	< 0.0001
C^2	113.14	1	113.14	184.07	< 0.0001
Residual	6.15	10	0.6146		
Lack of Fit	6.15	5	1.23		
Pure Error	0.0000	5	0.0000		
Cor Total	2271.43	19			
Std. Dev.	0.7840		R^2		0.9973
Mean	28.95		Adjusted R^2		0.9949
C.V. %	2.71		Predicted R^2		0.9683
			Adeq Precision		54.7347

Table 3: RSM Table analysis

Run Order	Actual Value	Predicted Value	Residual	Leverage	Internally Studentized Residuals	Externally Studentized Residuals	Cook's Distance	Influence on Fitted Value DF FITS
1	36.73	36.64	0.0855	0.491	0.153	0.145	0.002	0.142
2	8.61	9.08	-0.4709	0.793	-1.321	-1.379	0.669	-2.700
3	39.56	39.42	0.1364	0.118	0.185	0.176	0.000	0.064
4	28.88	29.37	-0.4945	0.491	-0.884	-0.874	0.075	-0.858
5	18.34	18.18	0.1571	0.793	0.441	0.422	0.074	0.827
6	37.28	38.61	-1.33	0.491	-2.371	-3.401	0.542	-3.340
7	39.56	39.42	0.1364	0.118	0.185	0.176	0.000	0.064
8	39.56	39.42	0.1364	0.118	0.185	0.176	0.000	0.064
9	39.56	39.42	0.1364	0.118	0.185	0.176	0.000	0.064
10	39.56	39.42	0.1364	0.118	0.185	0.176	0.000	0.064
11	34.56	34.21	0.3495	0.491	0.625	0.605	0.038	0.594
12	10.64	9.64	0.9951	0.793	2.791	5.633	2.988	11.032
13	39.56	39.42	0.1364	0.118	0.185	0.176	0.000	0.064
14	33.93	33.36	0.5731	0.793	1.608	1.771	0.991	3.468
15	22.27	22.46	-0.1869	0.793	-0.524	-0.504	0.105	-0.987
16	17.74	17.79	-0.0549	0.793	-0.154	-0.146	0.009	-0.286
17	13.01	12.72	0.2891	0.793	0.811	0.796	0.252	1.559
18	34.85	33.93	0.9175	0.491	1.640	1.820	0.259	1.787
19	23.68	24.57	-0.8929	0.793	-2.504	-3.891	2.405	-7.619
20	21.12	21.88	-0.7585	0.491	-1.356	-1.424	0.177	-1.398



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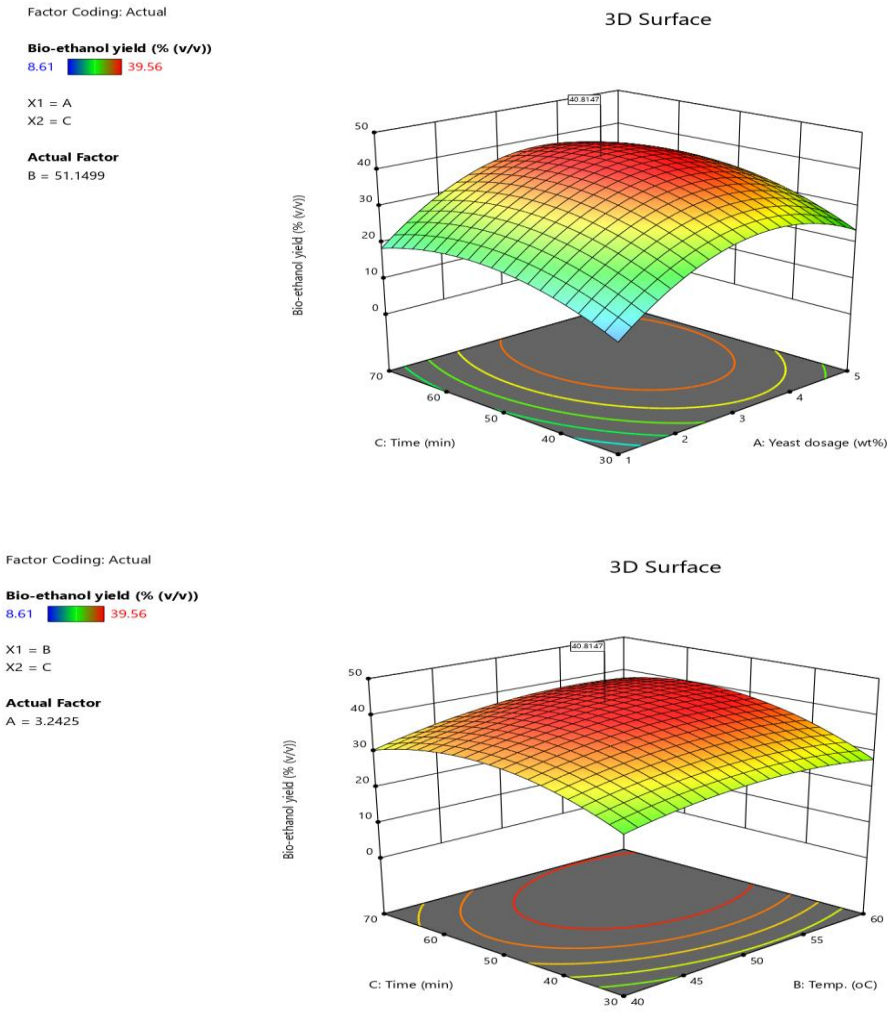

Figure Three Dimensional Representation

Table 4: Optimum result of the RSM

Yeast dosage (g)	Temp. (ºC)	Time (min)	Predicted or optimum bio-ethanol yield (%)
3.24	51.15	55.37	40.81

Table 5: Validation of the result

Yeast dosage (g)	Temp. (ºC)	Time (min)	Predicted bio-ethanol yield (%)	Experimental bio-ethanol yield (%)	Percentage deviation (%)
3.24	51.15	55.37	40.81	40.03	1.95

3.8 Quality and Purity of Produced Ethanol

Physicochemical characterization confirmed that the ethanol produced from palm wine is of comparable quality to commercial bioethanol. Specific gravity, viscosity, refractive index, and flash point values all fell within or near the standard ranges, indicating suitability for blending with gasoline and other industrial uses. Cloud point and pour point values were also acceptable for tropical climates, further emphasizing the regional applicability of palm wine ethanol. However, minor deviations were observed, particularly in flash point and refractive index, which suggest the presence of impurities such as residual sugars, glycerol, or water. This aligns with FTIR results, which detected minor peaks corresponding to unconverted compounds. Similar findings have been

reported in studies on fruit-based substrates such as pineapple waste and banana peel, where purification challenges limited ethanol purity. These results underscore the importance of integrating efficient distillation and purification methods, such as molecular sieves or pervaporation, to achieve fuel-grade ethanol. Nonetheless, the ethanol produced in this study is adequate for industrial and household applications where absolute purity is not required, thereby expanding its potential uses in local economies.

3.9 Comparison with Other Feedstocks

The maximum ethanol yield achieved in this study (40.03% v/v) compares favourably with values reported for other agricultural feedstocks. For instance, cassava-based fermentation typically yields 25–35% v/v ethanol (Do & Vu, 2024; Krajang et. al., 2024), while molasses yields range between 30–38% v/v under optimized conditions (Beigbeder etal., .2021; Hawaz etal., 2023). Similarly, fruit-based substrates such as pineapple waste (Kanthavelkumaran etal., 2023) and mango peel (Boyce, 2014) generally produce ethanol yields below 30% v/v. The higher yield from palm wine can be attributed to its naturally high sugar content, which reduces the need for extensive pre-treatment and enzymatic hydrolysis steps required for starch or lignocellulosic substrates. Moreover, the relatively short fermentation time (55 minutes) observed in this study is a significant improvement compared to conventional systems, which often require 24–72 hours. This reduction in processing time has direct implications for energy efficiency and cost reduction, positioning palm wine bioethanol as a highly competitive renewable fuel source.

3.10 Industrial and Sustainability Implications

The findings of this study have broader implications for biofuel development, particularly in tropical regions where palm wine is readily available. The optimized process demonstrated here requires modest yeast input, operates under relatively mild conditions, and achieves high yields in a short time frame, all of which reduce operational costs. This makes it suitable for small-to-medium-scale production units that could be established in rural and semi-urban areas. From a sustainability perspective, palm wine ethanol production offers a locally adaptable solution that reduces dependence on fossil fuels and contributes to energy security. By utilizing a resource that is already abundant and underutilized, communities can generate renewable energy with minimal environmental impact. Furthermore, the decentralized nature of palm wine production aligns with rural development initiatives, providing employment opportunities and reducing energy poverty.

3.11 Limitations and Future Directions

While the results of this study are promising, several limitations should be acknowledged. The presence of residual impurities highlights the need for improved distillation and purification steps to enhance ethanol purity for high-end applications. Additionally, the variability of palm wine composition, influenced by palm species, tapping methods, and storage conditions, may affect process reproducibility. Future research should focus on standardizing raw material quality, exploring continuous fermentation systems, and integrating advanced purification technologies. Further studies could also examine the potential for co-fermentation with other agro-industrial wastes to enhance yield and reduce substrate costs. Life cycle assessment (LCA) studies are recommended to evaluate the environmental footprint of palm wine ethanol compared to conventional fuels. Such efforts will strengthen the case for palm wine as a viable, scalable, and sustainable biofuel feedstock.

IV. CONCLUSION

This study has successfully demonstrated the technical feasibility and optimization of bioethanol production from palm wine through enzymatic fermentation using Saccharomyces cerevisiae. By systematically investigating the effects of yeast dosage, fermentation temperature, and fermentation time, the process was optimized using Response Surface Methodology (RSM) with a Central Composite Design (CCD). Ethanol yields varied between 8.61% and 39.56% v/v, with a maximum of 40.03% v/v achieved at 3.24% yeast dosage, 51.15 °C, and 55 minutes. The close agreement between experimental results and model predictions (deviation of only 1.91%) confirmed the robustness and predictive reliability of the optimization model. Fourier Transform Infrared (FTIR) spectroscopy verified the successful conversion of sugars to ethanol, with distinct peaks corresponding to O–H, C–H, and C–O stretching vibrations. Physicochemical characterization further confirmed that the ethanol produced exhibited properties

consistent with commercial ethanol, including specific gravity, viscosity, flash point, and refractive index. Minor deviations in some parameters, however, indicated the presence of residual impurities such as water, glycerol, or unfermented sugars, suggesting the need for further purification when fuel-grade ethanol is required.

Compared to other agro-based substrates such as cassava, molasses, and fruit wastes, palm wine demonstrated superior performance, particularly in yield and fermentation time. The ability to achieve high ethanol concentrations within less than an hour of fermentation represents a significant advancement in terms of process efficiency and energy savings. This positions palm wine-derived bioethanol as a competitive and sustainable alternative to conventional biofuel sources, especially in tropical regions where palm wine is abundant. The industrial and sustainability implications of this study are noteworthy. Palm wine-based bioethanol production offers a cost-effective and energy-efficient pathway that can be deployed in small-to-medium-scale facilities, particularly in rural and semi-urban communities. Such decentralized production systems could contribute to reducing energy poverty, creating employment opportunities, and lowering dependence on imported fossil fuels. Additionally, the use of locally available renewable resources aligns with global goals of climate change mitigation and sustainable energy development. Nevertheless, challenges remain. Variability in palm wine composition due to species, tapping methods, and storage conditions could affect process reproducibility. Moreover, achieving fuel-grade ethanol requires more advanced purification methods. Future work should focus on scale-up studies, continuous fermentation systems, and integration of purification technologies such as molecular sieves or pervaporation. Life cycle assessments and techno-economic analyses are also recommended to evaluate the broader environmental and economic impacts of palm wine ethanol production.

In conclusion, this research highlights palm wine as a promising, underutilized resource for sustainable bioethanol production. With process optimization, quality verification, and targeted scale-up, palm wine bioethanol could play a valuable role in the renewable energy mix of tropical regions, offering a locally adaptable solution that supports both environmental sustainability and energy security.

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