Volume 10, Issue 7, July – 2025 International Journal of Innovative Science and Research Technology
ISSN No:-2456-2165 https://doi.org/10.38124/ijisrt/25jul914

Evaluation of Bioethanol Quality Produced by Sweet Sorghum Stalks in Uganda

Atem David[1]; Lating[1]; Betty Nabuuma[1]; Steven[4]; Alexander Bombom[2]; Wembazi Enock[3]; Babirye Fatumah[3]; Nuwamanya Ephrai[3]; Samuel Mulondo[2]; Byansi Anjero[2,*]

[1]Makerere University College of Engineering, Art and Technology
[2]National Livestock Resources Research Institute, National Agricultural Research Organization.
[3]National Crops Resources Research Institute, National Agricultural Research Organization
[4]University of South Florida, Tampa

Corresponding Author: Byansi Anjero[2,*]

Publication Date: 2025/08/11

Abstract: Sweet sorghum (*Sorghum bicolor*) is a drought-tolerant plant with high sugar content and low input requirements, making it a promising candidate for bioethanol production. This study aimed to assess bioethanol production from local sweet sorghum varieties in Uganda. Six varieties with brix content ranging from 10% to 25% were screened, and juice was extracted and fermented using the SC yeast strain for 10 days, followed by fractional distillation to produce bioethanol. The feedstock was characterized by protein, reducing sugars, and carbohydrate content. Using response surface methodology (RSM) and a central composite design, 20 experimental runs were conducted to optimize yeast loading, reaction time, and agitation rate. The optimal parameters identified were 25 g of yeast loading, 10 days of reaction time, and an agitation rate of 100 rpm. The resulting bioethanol concentrations from the sweet sorghum juice ranged from 56 % v/v to 90% v/v, while concentrations from the bagasse ranged from 15% v/v to 40% v/v. The higher heating value (HHV) of the bioethanol produced varied from 12.46 MJ/kg to 16.79 MJ/kg. Quality assessments using bomb calorimetry, density pycnometry, and fire and flash point tests revealed HHVs between 13.06 MJ/kg and 20.31 MJ/kg, juice densities of 0.82 g/cm³ to 0.87 g/cm³, bagasse densities of 0.90 g/cm³ to 0.96 g/cm³, and flash points ranging from 17.8°C to 23.0°C for juice and 25.5°C to 45.3°C for bagasse. This study demonstrates that local sweet sorghum stalks have significant potential for bioethanol production, offering a sustainable alternative to fossil fuels in developing countries.

How to Cite: Atem David; Lating; Betty Nabuuma; Steven; Alexander Bombom; Wembazi Enock; Babirye Fatumah; Nuwamanya Ephrai; Samuel Mulondo; Byansi Anjero (2025) Evaluation of Bioethanol Quality Produced by Sweet Sorghum Stalks in Uganda. *International Journal of Innovative Science and Research Technology*, 10(7), 3404-3415. https://doi.org/10.38124/ijisrt/25jul914

I. INTRODUCTION

Bioethanol has emerged as a promising alternative energy source in response to the declining availability of fossil fuels, a growing global population, and advancements in mobility technologies that utilize bioethanol as fuel. Projections indicate that non-renewable resources will be depleted within the next 50 years, while synthetic ethanol production contributes to global warming (Holechek, 2022). As a renewable energy source, bioethanol can help mitigate greenhouse gas emissions and reduce reliance on fossil fuels. Sweet sorghum, a C4 crop, presents significant advantages over traditional feedstocks due to its high sugar content, water efficiency, and adaptability to marginal lands. The combustion of fossil fuels is responsible for approximately 90% of global warming, leading to extreme weather events such as floods and droughts (Jeswani et al., 2020). Greenhouse gases, including carbon dioxide (CO_2), methane (CH_4), ozone (O_3), and nitrous oxide (N_2O)—absorb infrared radiation and contribute to climate change (Dahman et al., 2019). Considering these challenges, biofuels such as bioethanol, biodiesel, and biohydrogen have garnered considerable interest. Bioethanol is primarily produced through the fermentation of sugars or starches derived from various biomass sources. However, the use of food crops like corn and cassava for bioethanol production raises concerns about food security (Byun & Han, 2021). Sweet sorghum stalks represent a valuable waste material rich in fermentable sugars such as sucrose, glucose, and fructose, with sugar content varying by variety (Kasegn, 2023). These stalks typically contain between 8°Bx to 20°Bx (Adinurani et al., 2018), making them suitable candidates for bioethanol production (Cristina et al., 2017). Sweet sorghum not only offers high biomass yields but also exhibits rapid growth and

ISSN No:-2456-2165 https://doi.org/10.38124/ijisrt/25jul914

lower contamination risks during juice extraction compared to other biomass sources (López-Sandin et al., 2021). This study aims to explore the potential of sweet sorghum stalks as a sustainable feedstock for bioethanol production, addressing both environmental concerns and energy needs while minimizing competition with food sources.

II. MATERIALS AND METHODS

➢ *Plant Material*

Sorghum stalks from six distinct varieties (P11, P13, B1P9, P12, B7P1, and P1) were selected based on their Brix content, which ranged from 10% to 25%. These varieties were sourced from a total of sixty varieties cultivated at the National Livestock Resources Research Institute in Nakyesasa. To determine their Brix content, an automatic hand refractometer was utilized for screening directly in the field. After selection, the sorghum stalks were harvested using a panga (machete), leaves removed and subsequently transported to the Bioanalytical Laboratory at the National Crop Resources Research Institute for further analysis.

The Stems were weighed, kept for 48 hours at room temperature to concentrate the sugars, then pressed using a motorized cane juice extractor. The juice was filtered with fine sieve and muslin cloth to remove any sediments and then tested for brix values again before storing at 4c for further processing.

➢ *Bio-Ethanol Production*

The juice was fermented by adding 1g of fermenting yeast of the SC strain to 1g yeast nutrient to 1 litre of the juice in absence of oxygen then left to stand for 10 days. 5g of bentonite was added to 1 litre to aid in the clearing of the fermented product. The cleared product was filtered using filtration pads of the vine brite type then fractionally distilled for a number successive times to obtain bio ethanol for further analysis.

➢ *Sorghum Feedstock Characterization*

The samples treated were analyzed for protein, total carbohydrate and reducing sugar. The protein test was done using Bradford reagent. 0.1ml of the sample and 5 ml of distilled water were boiled at 80c for 30 minutes. On cooling 3ml of Bradford reagent was added to 0.1 ml of the solution then absorbance read at 595nm.Total carbohydrate was determined by adding 0.1g of the sample to 5 ml of 10% sulphuric acid, incubated at 80c for 30 minutes in a water bath. On cooling 0.5ml of supernatant was mixed with 5% phenol, 1 ml of distilled water,1ml of sulphuric acid then absorbance read at 490nm. Reducing sugar was done by boiling 0.1g of the sample in 2ml of distilled water, 30 minutes then added 1 ml of distilled water,0.5 ml of 5% phenol solution and 1ml of concentrated hydrochloric acid to 0.5 ml of the boiled solution. On cooling the absorbance was read at 490nm.

➢ *Optimization of Bioethanol Production*

To optimize the production of high-quality bioethanol, we employed Response Surface Methodology (RSM). This statistical technique is particularly useful for exploring the relationships between multiple input variables and their effects on desired outcomes. In this study, we focused on three critical input variables: yeast loading, reaction time and agitation rate. We utilized a three-factor Central Composite Design (CCD) to systematically investigate the interactions between these variables. CCD allows for the development of predictive models that correlate input variables with response outcomes, specifically: bioethanol concentration and high heating value.

➢ *Experimental Design*

The experimental setup consisted of 20 randomized runs which were generated using Design-Expert software (version X.X). This design included factorial points, axial Points and replicates at center points to ensure reliability and accuracy in the results. The specific calculations for the design were derived from established equations relevant to CCD, ensuring a robust and statistically sound experimental framework.

$$N = 2^n + 2\mathrm{n} + n_c$$

Where N was the total number of experiments performed.

N is the number of process variables

Nc is the number of replicates at the center point

III. STATISTICS

➢ *Model*

The mathematical relationships between the input variables and the responses were modeled using a polynomial in equation below.

$$Y = b_0 + \sum_{i=1}^{n} b_i X_i + \sum b_{ii} X^2{}_i + \sum_{i=1}^{n-1} \sum_{j=i+1}^{n} b_{ij} X_i X_j + \mathcal{E}$$

Y was the predicted response (bioethanol yield, concentration)
X_i and X_j were the independent variables (yeast loading, fermentation/reaction time, agitation rate)
b_0 was the constant coefficient
b_i was the linear coefficient
b_{ii} was the quadratic coefficient
b_{ij} was the interaction coefficient,
Ɛ was the error of the model.

➢ *Development and Model Validation*

Design-Expert software was employed to identify optimal combinations of factors that meet the specified requirements for both response variables and input factors in bioethanol production. Through this software, we developed response surface models that predict the outcomes based on varying conditions. To validate the developed models, we conducted experiments under the identified optimal production conditions. The experimental results were then compared with the predicted values generated by the model to assess accuracy and reliability. This comparison is crucial for confirming that the models can effectively predict bioethanol yield and quality.

ISSN No:-2456-2165 https://doi.org/10.38124/ijisrt/25jul914

➢ *Analysis of Production Conditions*

The impact of production conditions specifically yeast loading, reaction time, and agitation rate on both the yield and quality of bioethanol was evaluated using surface plots. These visual representations allowed us to analyze the relationships between the input variables and their effects on bioethanol production.

➢ *Characterization of Bioethanol Quality*

Additionally, we characterized the produced bioethanol by examining key quality parameters, including Higher Heating Value (HHV), Density, Concentration, Fire Point and Flash Point.

➢ *Calorific Heating Value*

This was determined using a bomb calorimeter Model: C2000 basic, IKA Co., Germany) following the ASTM D5865 – 13 standards. An oxygen bomb calorimeter was used in determining the high heating value **(HHV)**. A small mass of ethanol was burnt in the presence of oxygen inside a sealed container and the heat released from combustion was measured.

➢ *Density*

The density was determined the mass of an empty pycnometer (Density bottle), using the Digital scale (Model: 500G/0.01G, China). The mass of pycnometer filled with distilled water was measured and recorded, dried using the hot air blower (Model: Bosch GHG500-2).The bioethanol was loaded into pycnometer, weighed to obtain the mass (M) using an electronic scale and the volume (V). The density (D) was calculated using

$$d = \frac{M}{V}$$

➢ *Fire and Flash Point*

This was determined using a test cup.10ml of the test sample of bioethanol was filled into a test cup. The temperature of the test specimen increased rapidly initially, then at a slower constant rate as the flash point was approached. At specific intervals, a test flame was passed across the cup and the flash point determined.

➢ *Data Analysis*

Data collected from the bioethanol production experiments, alongside the results from Response Surface Methodology (RSM), were thoroughly analyzed to elucidate the relationships among the variables. This analysis provided insights into how each factor influences bioethanol yield.

IV. RESULTS

The Juice Brix values and their corresponding volume of juice The sweet sorghum characterization was carried out by testing the sugar (glucose) content. The juice brix values ranged from 10.00 – 14.00 ^{0}Brix with their corresponding juice volumes ranged from 2.00 – 7.50 cm^3/kg as shown in Table 1

Table 1 Sugar Contents Obtained by Refractometer in Brix.

Coded Sample Name	Volume of Juice in cm^3/kg	Glucose Concentration in ^{0}Brix
P_{11}	5.00	14.00
P_{13}	6.00	12.50
B_1P_9	2.00	12.50
P_{12}	6.00	12.00
B_7P_1	2.50	10.50
P_1	7.50	10.00

➢ *Optimum Conditions for Bioethanol Production*

The study determined the optimum conditions for yeast loading, reaction/fermentation time, and agitation rate in relation to the responses of bioethanol concentration and higher heating value (HHV). A total of 20 experimental runs were conducted, as detailed in Table 2 where Yeast Loading varied from 0 to 25 g, Reaction Time ranged from 5 to 12 days and agitation rate adjusted between 33 and 117 RPM. The resulting bioethanol concentration ranged from 51.60% to 70%, while the HHV values were observed between 12.46 MJ/kg and 16.79 MJ/kg. These findings highlight the effective range of conditions for maximizing bioethanol yield.

Table 2 Central Composite Design and the Experimental Results

		Factor 1	Factor 2	Factor 3	Response 1	Response 2
STD	Run	A: Yeast loading	B: Reaction time	C: Agitation rate	Bioethanol concentration	HHV
		Grams	Days	RPM	%	MJ/kg
18	1	15	8	75	52.11	13.93
9	2	0	8	75	43.91	12.61
14	3	15	8	117	53.91	14.21
20	4	15	8	75	55.67	14.50
16	5	15	8	75	51.6	13.84
2	6	25	5	50	57.8	14.84
4	7	25	10	50	67.15	16.34
11	8	15	3	75	47.25	13.15

ISSN No:-2456-2165 https://doi.org/10.38124/ijisrt/25jul914

17	9	15	8	75	49.98	13.58
5	10	5	5	100	42.98	12.46
8	11	25	10	100	66.35	16.21
3	12	5	10	50	51.60	13.84
12	13	15	12	75	59.61	15.13
15	14	15	8	75	54.00	14.23
19	15	15	8	75	53.64	14.17
10	16	32	8	75	70.00	16.79
7	17	5	10	100	52.28	13.95
1	18	5	5	50	42.98	12.46
6	19	25	5	100	60.12	15.21
13	20	15	8	33	52.36	13.97

➢ *NOVA Analysis of Reduced Quadratic Models*

The Analysis of Variance (ANOVA) results for the reduced quadratic models assessing bioethanol concentration and higher heating value (HHV) are presented in Table 3.

- *Bioethanol Concentration*

For the response variable of bioethanol concentration, the F-values for the factors A (Yeast/Enzyme Loading) and B (Reaction Time) were recorded as 188.14 and 415.76, respectively. The p-value for both yeast loading and reaction time was found to be less than 0.0001, indicating a highly significant effect on bioethanol concentration. Additionally, the values for A^2, Residual, and Lack of Fit were 14.42, 1.88, and 0.2224, respectively. The corresponding F-value and p-value for these terms were 0.0016 and 0.9823, respectively, as illustrated in Table 3a.

- *Higher Heating Value (HHV)*

Similarly, for the response variable of HHV, the F-values for A (Yeast/Enzyme Loading) and B (Reaction Time) were again 188.14 and 415.76, respectively, with a p-value of less than 0.0001 for both factors, confirming their significant impact on HHV. The values for A^2, Residual, and Lack of Fit were recorded as 14.42, 0.0484, and 0.2224, respectively. The F-value and p-value for these terms were also consistent at 0.0016 and 0.9823, as shown in Table 3b.

Table 3 Anova for Responses Reduced Quadratic Models for Bioethanol Concentration (a) and HHV(b)

Source	Sum of Squares	*Df*	Mean Square	*F*-value	*p*-value	Remarks
a)						
Model	1063.55	3	354.52	188.14	< 0.0001	significant
A-Enzyme loading	783.42	1	783.42	415.76	< 0.0001	significant
B-Reaction time	215.89	1	215.89	114.57	< 0.0001	significant
A^2	27.17	1	27.17	14.42	0.0016	significant
Residual	30.15	16	1.88			
Lack of Fit	9.90	11	0.9004	0.2224	0.9823	not significant
Pure Error	20.24	5	4.05			
Cor Total	1093.70	19				
b)						
Model	27.34	3	9.11	188.14	< 0.0001	significant
A-Enzyme loading	20.14	1	20.14	415.76	< 0.0001	significant
B-Reaction time	5.55	1	5.55	114.57	< 0.0001	significant
A^2	0.6984	1	0.6984	14.42	0.0016	significant
Residual	0.7751	16	0.0484			
Lack of Fit	0.2546	11	0.0231	0.2224	0.9823	not significant
Pure Error	0.5205	5	0.1041			
Cor Total	28.12	19				

➢ *Goodness of Fit for Developed Models*

The goodness of fit for the developed models was assessed using several statistical metrics, including standard deviation (SD), coefficient of variation (CV), R^2 value, adjusted R^2 value, predicted R^2 value, and adequate precision. These metrics are summarized in Table 4.

➢ *Bioethanol Concentration*

For the model predicting bioethanol concentration, the goodness of fit metrics were as follows: SD: 1.37, CV: 2.53%, R^2 Value: 0.96724, adjusted R^2 Value: 0.9673, predicted R^2 Value: 0.9613, Adequate Precision: 44.8661.These values indicate a strong fit of the model to the observed data, with a high R^2 value suggesting that approximately 96.7% of the variability in bioethanol concentration can be explained by the model.

➢ *Higher Heating Value (HHV)*

Similarly, for the model predicting higher heating value (HHV), the goodness of fit metrics was SD: 0.2201, CV: 1.54%, R^2 Value: 0.96724, Adjusted R^2 Value: 0.9673, Predicted R^2 Value: 0.9613, Adequate Precision: 44.8661.

ISSN No:-2456-2165 https://doi.org/10.38124/ijisrt/25jul914

Like the bioethanol concentration model, these results reveal a robust fit, with an R^2 value indicating that about 96.7% of the variability in HHV is accounted for by the mode.

Table 4 Model Summary Showing Goodness of Fit of the Developed

Statistical Parameter	Bioethanol Concentration	HHV
Standard Deviation	1.37	0.2201
Mean	54.26	14.27
Coefficient of Variation (%)	2.53	1.54
R^2 value	0.9724	0.9724
Adjusted R^2 value	0.9673	0.9673
Predicted R^2 value	0.9613	0.9613
Adequate precision	44.8661	44.8661

➢ *Normal Distribution Residue*

The analysis of residuals for bioethanol concentration (Figure 1a) and higher heating value (HHV) (Figure 1b) demonstrates that the external studentized residuals are normally distributed around the central line. This indicates that the residuals do not show significant deviations from normality, which is essential for validating the regression models used in this optimization study. Furthermore, the comparison of predicted versus actual values (Figures 1c and 1d) reveals a close alignment in their distribution patterns, highlighting a strong correlation and confirming that the models effectively capture the underlying relationships in the data. Additionally, the analysis of residuals shows a random distribution pattern for both bioethanol concentration (Figure 1a) and HHV (Figure 1b). The predicted residuals are well scattered within acceptable margins, indicating no outliers or factors outside the expected range. This randomness further supports a robust model fit and validates the optimization experiment, confirming that the values involved are accurately modeled without significant deviations.

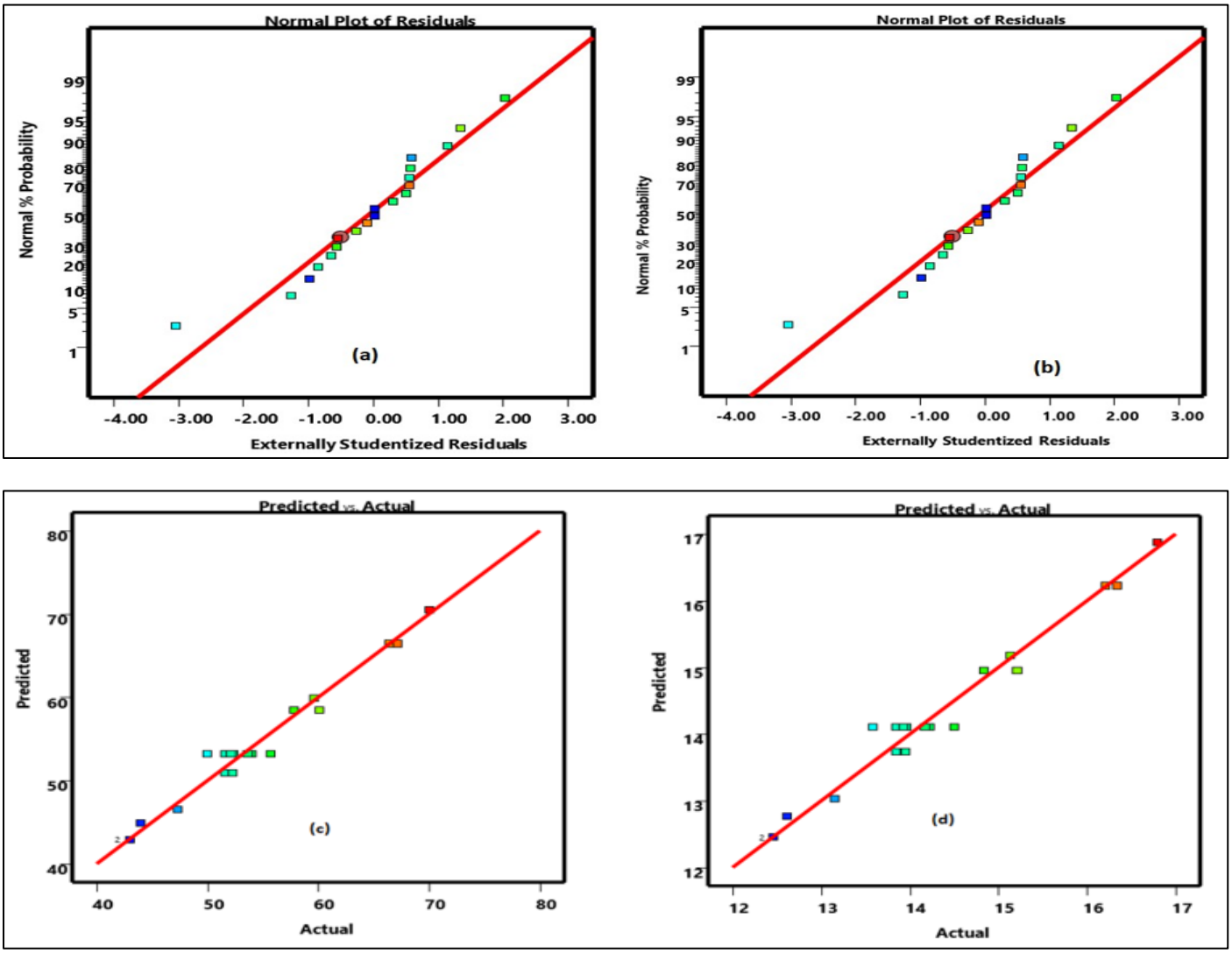


Fig 2 Normal Probability Plot of Residues for (a) Bioethanol Concentration, (b) HHV; Predicted Values Versus Experimental Values for (c) Bioethanol Concentration, (d) HHV

ISSN No:-2456-2165 https://doi.org/10.38124/ijisrt/25jul914

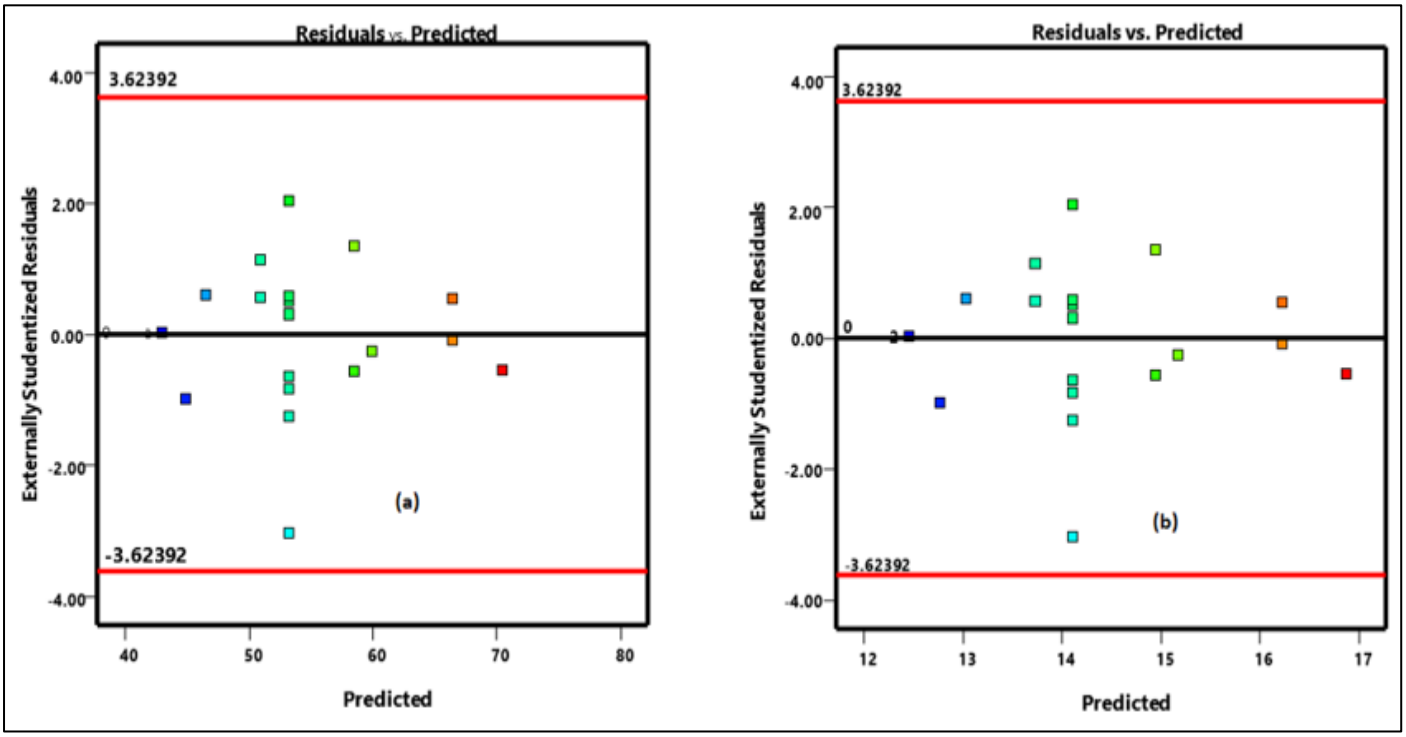
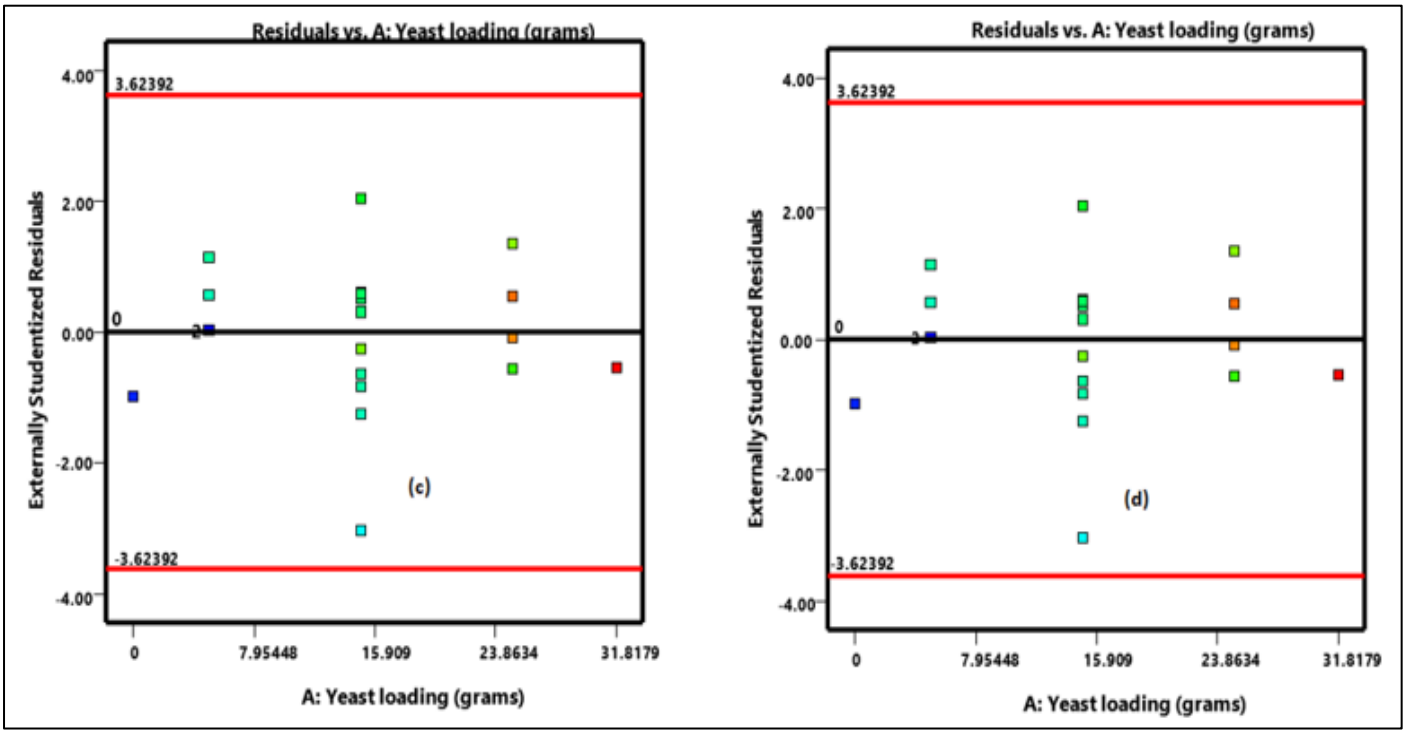


Fig 2 Random Distribution Plot of Residues for (a) Bioethanol Concentration, (b) HHV; Predicted Values Versus Experimental Values for Bioethanol Concentration, and HHV

➢ *Residual Analysis*

The plots of residuals versus run for bioethanol concentration (a) and HHV (b), as well as residuals versus experimental factors for bioethanol concentration (c) and HHV (d), demonstrate that all points are distributed within the acceptable margin of -3.62392 to 3.62392 (Figures 3a-d). This indicates a good distribution pattern, with no outliers, confirming the robustness of the experimental fits and parameters.



ISSN No:-2456-2165 https://doi.org/10.38124/ijisrt/25jul914

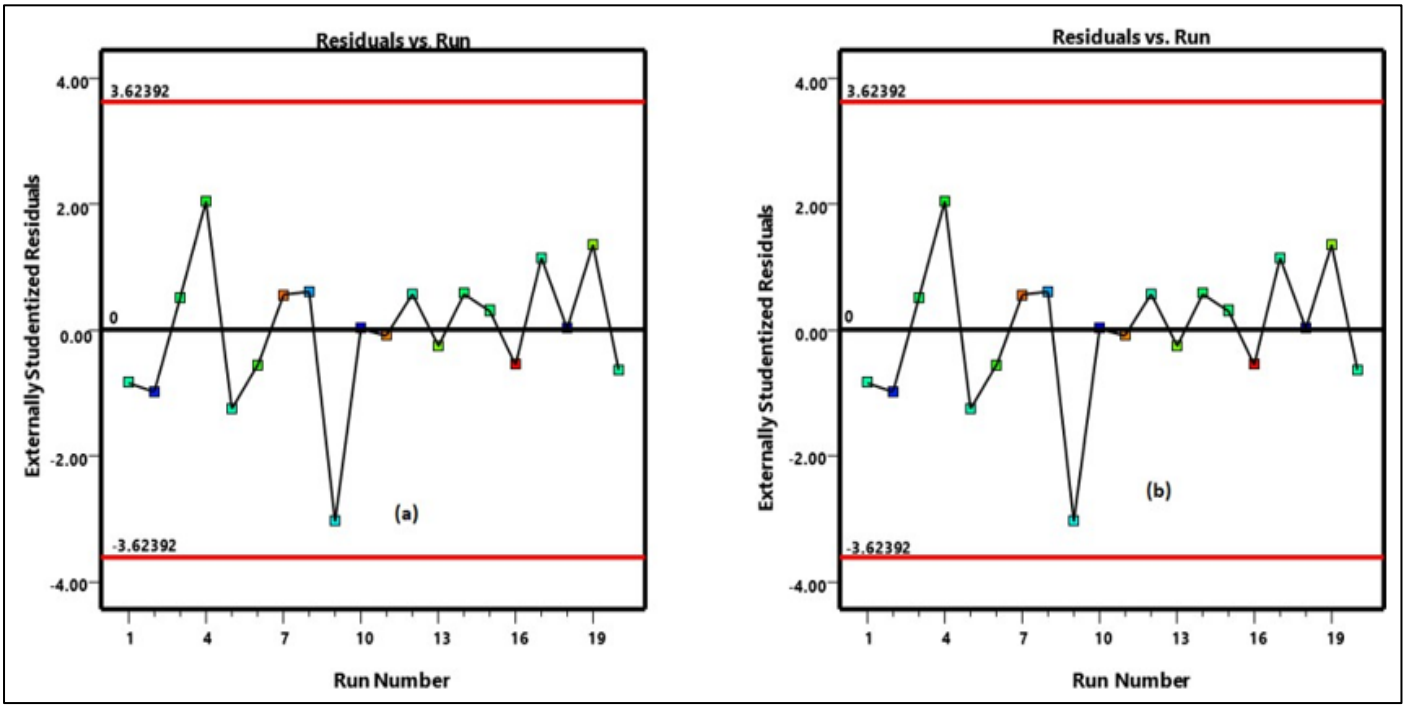


Fig 3 Plot of Residues for (a) Bioethanol Concentration, (b) HHV; Residues Versus Experimental Factors for (c) Bioethanol Concentration, (d) HHV

➢ *Contour Plots of Reaction Time and Yeast Loading*

The contour plots illustrating the relationship between reaction time and yeast loading demonstrate their effects on bioethanol concentration and higher heating value (HHV), as shown in Figures 1.4a and 1.4b, respectively. In Figure 1.4a, the contour plot indicates that with a reaction time of less than 5 days and a yeast loading of 7 grams, the bioethanol concentration reaches 45%. However, as yeast loading increases from 7 grams to 25 grams and reaction time extends from 6 days to 10 days, there is a significant rise in bioethanol concentration, peaking at 66.46%. Similarly, Figure 1.4b shows that increasing both reaction time and yeast loading leads to enhanced bioethanol concentration, which corresponds to an increase in HHV from 13 MJ/kg to 16.23 MJ/kg. This trend underscores the positive impact of optimizing these parameters on both bioethanol yield and quality.

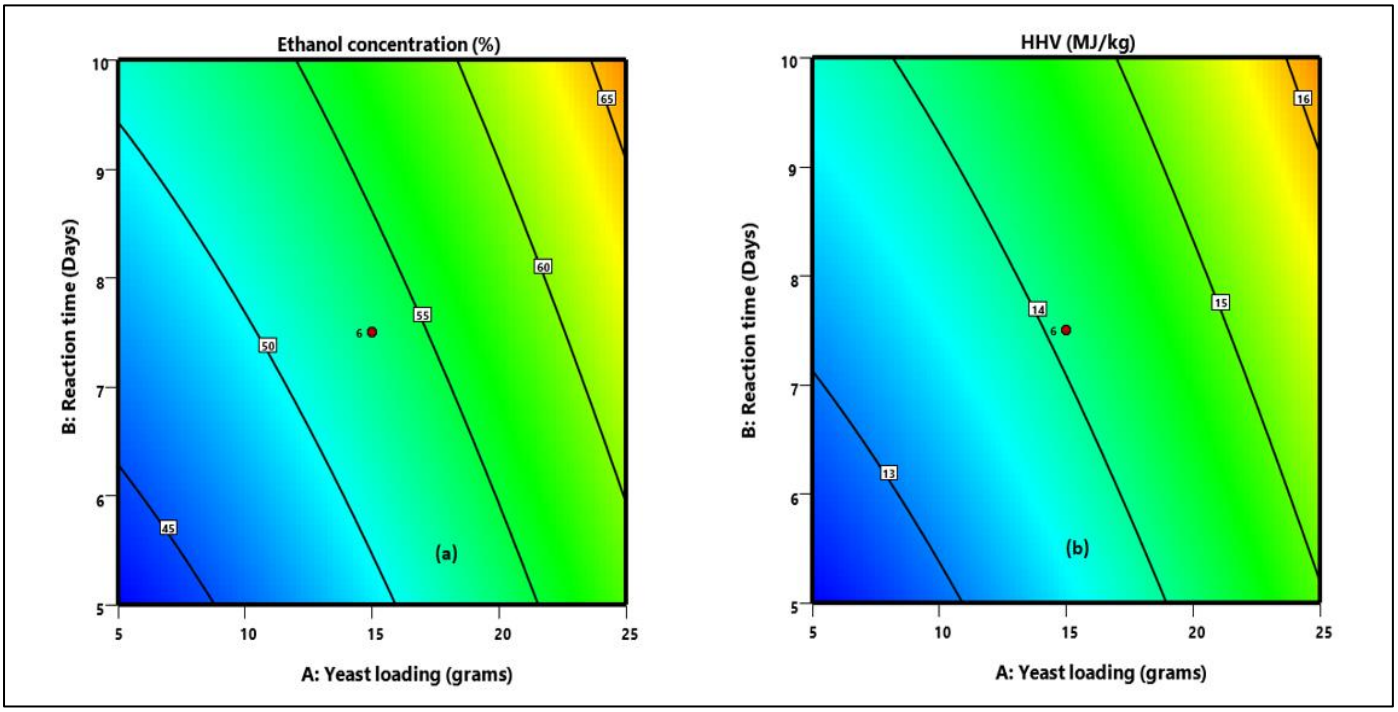


Fig 4 The Contour Plots of (a) Bioethanol Concentration, and (b) HHV. Effect of Yeast Loading, and Reaction Time on the Bioethanol Concentration and HHV

ISSN No:-2456-2165 https://doi.org/10.38124/ijisrt/25jul914

➢ *3D Surface Plots of Reaction Time and Yeast Loading*

The 3D surface plot in Figure 5 illustrates the effects of reaction time and yeast loading on bioethanol concentration. As yeast loading increases from 5 to 25 grams and reaction time extends from 5 to 10 days, there is a marked exponential increase in bioethanol concentration, rising from 10% to 66.46%. This indicates a strong dependence of bioethanol concentration on both parameters; as yeast loading and reaction time increase, bioethanol concentration also increases significantly, as demonstrated by the surface plot.

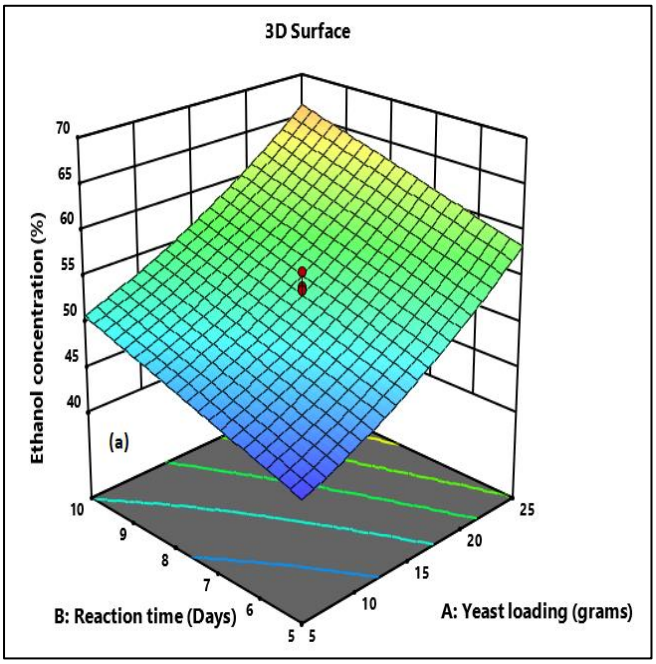


Fig 5 Three-Dimensional Response Surface Plots of Bioethanol Concentration. Effect of Yeast Loading, and Reaction Time on the Bioethanol Concentration.

➢ *3D Surface Plots of Reaction Time and Yeast Loading on Higher Heating Value*

Figure 6 presents the 3D surface plots depicting the effects of reaction time and yeast loading on the higher heating value (HHV) of bioethanol. The analysis reveals that as yeast loading increases from 5 to 25 grams and reaction time extends from 5 to 10 days, there is a pronounced exponential increase in HHV, ranging from 10 MJ/kg to 16.23 MJ/kg. This demonstrates that the HHV is significantly influenced by both yeast loading and reaction time, emphasizing the importance of optimizing these parameters to enhance the energy content of the produced bioethanol.

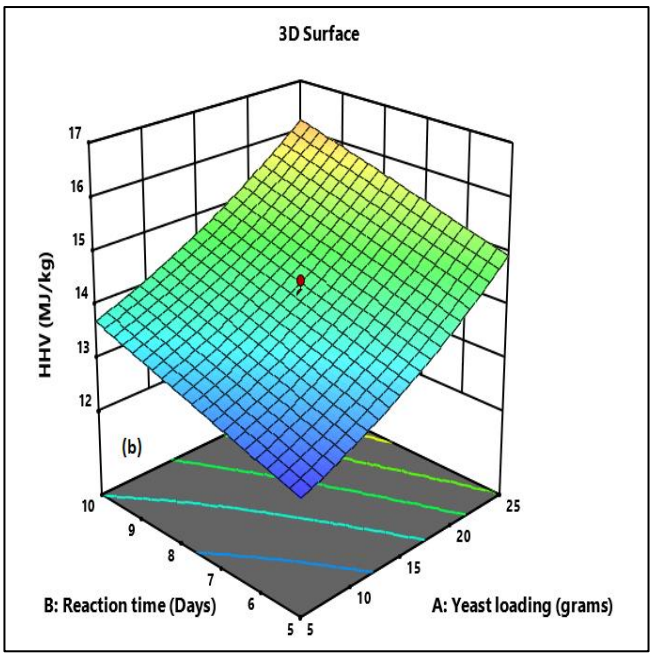


Fig 6 Three-Dimensional Response Surface Plots of High Heating Value. Effect of Yeast Loading, and Reaction Time on the High Heating Value.

➢ *Validation of Bioethanol Production Models*

The fermentation of sweet sorghum stalk juice and bagasse was conducted under optimized conditions, resulting in a bioethanol concentration of 66.46% and a higher heating value (HHV) of 16.23 MJ/kg, as predicted by the developed models (Table 6). To validate the predictive capability of these models, the experimental values were compared to the predicted values. For a reliable assessment, the mean experimental values should fall within the 95% prediction interval (PI), both lower and upper bounds. In this study, the mean experimental values for each response were found to lie within this range (Table 6), confirming the validity of the developed models. This alignment indicates that the models accurately predict bioethanol concentration and HHV, reinforcing their applicability in optimizing bioethanol production from sweet sorghum

Table 5 Validation Experiments for the Proposed Models

Analysis	Predicted Mean	Predicted Median	Std Dev	df	SE Pred	95% PI low	Data Mean	95% PI high
Bioethanol concentration	66.4607	66.4607	1.3727	2	1.15125	64.0201	66.535	68.9012
HHV	16.2263	16.2263	0.2201	2	0.184591	15.835	16.26	16.6176

➢ *Characteristics of Bioethanol from Juice Samples*

The analysis of various juice samples revealed a range of bioethanol concentrations, mean calorific values, juice densities, and flash points, as summarized in Table 7. Specifically, the bioethanol concentration varied from 56.80% to 90.20% v/v, the calorific value ranged from 13.06 to 20.31 MJ/kg, the juice density was between 0.82 and 0.87 g/cm³, and the flash point ranged from 17.80°C to 23. 30°C.The results indicate a clear relationship among these parameters: higher bioethanol concentrations correlate with increased calorific values and decreased densities and flash points. Conversely, as the juice bioethanol concentration decreases (from 96.00% to 56.80% v/v), the calorific value, density, and flash points increases (up to 23.30°C). This inverse relationship highlights the importance of optimizing bioethanol concentration to enhance fuel quality and safety characteristics.

ISSN No:-2456-2165 https://doi.org/10.38124/ijisrt/25jul914

Table 6 The Juice Bioethanol Concentration mean Calorific Value, Density and its Flash Points of Different Samples

Sample ID	Juice Concentration (%v/v)	Mean Calorific value MJ/Kg	Density of the juice Bioethanol (g/cm^3)	Flash and Fire Point (^oC)
Synthetic Ethanol	96	26.90	0.79	17.00
P13	90.20	20.31	0.82	17.80
P1	85.20	18.65	0.83	20.00
P12	85.20	18.59	0.83	20.00
P11	80.20	18.29	0.85	19.30
B7P1	75.40	14.75	0.85	22.30
B1P9	56.80	13.06	0.87	23.30

V. DISCUSSION

This study highlights the promising potential of sweet sorghum stalks as a feedstock for bioethanol production, achieving yields comparable to those obtained from traditional sources. The findings indicate that both pretreatment and fermentation conditions play crucial roles in influencing bioethanol yield and quality. The results underscore the importance of optimizing process parameters and selecting appropriate yeast strains to enhance large-scale bioethanol production from sweet sorghum. By fine-tuning these factors, it may be possible to maximize efficiency and improve the overall viability of sweet sorghum as a sustainable biofuel source.

➢ *Development of the Fermentation Parameters*

- *Analysis of Variance (ANOVA)*

The process parameters for bioethanol production from sweet sorghum, including yeast loading (A), fermentation reaction time (B), and agitation rate (C), are detailed in Tables 2, 3, 4, and 5, as well as in Figures 1a and b, 1.2, and 1.3. ANOVA was utilized to evaluate the statistical significance of the quadratic models, assess model fit, and determine the significance of the respective model coefficients. As shown in Table 1.4a and b, the model F-values for enzyme loading, reaction time, and agitation rate were recorded at 415.76, 114.57, and 14.42, respectively. These values indicate that the developed models are statistically significant ($P < 0.0001$), confirming their reliability for predicting bioethanol production outcomes.

The Model F-value of 188.14 indicates that the model is significant. According to statistical criteria, terms with p-values less than 5% are considered significant, while those greater than 10% are not. In this context, yeast loading (F-value 415.76), reaction time (F-value 114.57), and the quadratic term for agitation rate (F-value 14.42) emerged as essential factors in the response surface reduced quadratic model for bioethanol production. The main effect of agitation rate and its interactions with other parameters did not significantly impact bioethanol yield, as indicated by p-values exceeding 5%. These findings align with previous research (Saini et al., 2013), suggesting that the high concentrations and heating values of sweet sorghum biomass may contribute to this outcome. Additionally, similar studies have shown that once juice is extracted and fermented, agitation rate has minimal influence on bioethanol concentration and HHV (Pappu & Gummadi, 2016), and that yeast loading's effect on yield is limited at a constant agitation rate (Nuanpeng et al., 2023).

The significant terms for the response surface reduced quadratic model related to higher heating value (HHV) include yeast loading (A), fermentation reaction time (B), and the quadratic term for yeast loading (A^2) (Table .4a). Among these, yeast loading had the most substantial impact on bioethanol concentration, with an F-value of 415.76, followed by the quadratic effect of fermentation reaction time (F-value 114.57) and agitation rate (F-value 14.42).The goodness of fit for the developed models was assessed using standard deviation, coefficient of variation, R^2 values, adjusted R^2 values, predicted R^2 values, and adequate precision (Table 1.6). The small standard deviations indicate a good model fit, while coefficient of variation values below 10 suggest high reproducibility (Rajewski & Dobrzyńska-Inger, 2021). The high R^2 values demonstrate that the data align well with the models, with an R^2 of 0.972 for both bioethanol concentration and HHV. The response surface equations for bioethanol concentration (Y1) and HHV (Y2), after excluding insignificant terms, are presented in Equations 5-1 to 5-2.

$$Y_1 = +32.6 + 0.33A + 1.59B \quad (1)$$

$$Y_2 = +10.86 + 0.05A + 0.26B \quad (2)$$

The adjusted R^2 and predicted R^2 values for each response indicate minimal divergence from the straight line, suggesting a good model fit, as noted by Brakenhoff et al. (2022). Both bioethanol concentration and higher heating value (HHV) exhibited R^2 values of 0.972, indicating that the models effectively explain 97.2% of the variations in the responses related to the fermentation parameters investigated.

- *Diagnostics and Adequacy Checking*

The adequacy of the fitted models was evaluated to confirm their validity, focusing on the random and normal distribution of residuals. This was assessed using normal probability plots of internally studentized residuals, predicted versus actual values, and externally studentized residuals versus predicted values (Figures 1.1a-d). The normal probability plots (Figures 1.a-b) indicate that the data points closely align with a straight line, supporting a normal distribution. The predicted versus actual value plots for bioethanol concentration and higher heating value (Figures 1.1c-d) show minimal divergence from this line, confirming that the response surface models accurately represent the relationships between experimental factors and response variables. Additionally, the plots of externally studentized

ISSN No:-2456-2165 https://doi.org/10.38124/ijisrt/25jul914

residuals versus predicted values for both bioethanol concentration (Figure 1.2a) and HHV (Figure 1.2b) exhibit no discernible patterns, with all points falling within the acceptable margin of -3.62392 to +3.62392 (Figures 1.2a-d). This suggests a random distribution of residuals, which is crucial for a robust model (Brakenhoff et al., 2022). The distribution of all tested parameters within the model's suggested margins indicates effective fitting and interdependence among factors (Figures 1.3c-d). While numerous parameters can influence bioethanol production, this study focused on three key factors due to their significant impact on final yield, which have been underexplored in previous research.

- *Response Surfaces: Bioethanol Concentration and HHV*

Figures 4a-b present contour and 3D response surfaces illustrating the effects of fermentation variables on bioethanol concentration and higher heating value (HHV). The plots reveal that the combined effect of yeast loading and reaction time (Figure 4a) has the most significant influence on both bioethanol concentration and HHV, followed by the interaction between yeast loading and HHV (Figure 4b). As yeast loading and reaction time increases, both bioethanol concentration and HHV rise. This is attributed to yeast consuming simple sugars like fructose and glucose in the juice or hydrolysate, which enhances bioethanol production until the sugars are depleted, leading to a stabilization or decline in concentration (Barcelos et al., 2016). Interestingly, agitation during the distillation process did not affect bioethanol concentration or HHV. As long as the flask rotates sufficiently, varying yeast loading and reaction time can ensure effective fermentation (Phukoetphim et al., 2017). The results indicate that most juice-to-bioethanol conversion occurs with 25 grams of yeast over 10 days; further increases in these parameters yield only marginal gains in concentration (Figure 4a). This finding aligns with previous research indicating that higher bioethanol concentrations correlate with optimized fermentation parameters, ultimately leading to increased HHV (Frankowski et al., 2022). Notably, Figure 4b shows that the agitation rate had no impact on bioethanol concentration, which is why it was excluded from the model figures. Similar observations have been reported by Azhar et al. (2017). Overall, this study suggests that high bioethanol concentrations can be achieved by fermenting sweet sorghum juice with minimal yeast addition over shorter fermentation periods.

- *Bioethanol Concentration*

Figure 5 illustrates the three-dimensional response surfaces depicting the effects of yeast loading and fermentation time on bioethanol concentration. The plots clearly demonstrate that the combined effect of yeast loading, and fermentation time significantly influences bioethanol concentration; as both parameters increase, so does concentration (Figure 5). Notably, the agitation rate had no impact on bioethanol concentration, which is why it was excluded from the model figures. This finding aligns with observations reported by Azhar et al. (2017). Overall, the results emphasize that optimizing yeast loading and fermentation time is crucial for enhancing bioethanol production.

- *High Heating Value*

Figure 6 illustrates the three-dimensional response surface depicting the interactive effects of fermentation variables on the higher heating value (HHV) of sweet sorghum bioethanol. The plots indicate that the interaction between yeast loading, and reaction time has the most significant impact on HHV. As yeast loading and reaction time increase, the HHV of the bioethanol also rises, while the agitation rate does not affect HHV.This increase in HHV is attributed to yeast's ability to metabolize sucrose in the biomass during fermentation, resulting in higher alcohol content and thus a higher HHV (Muhaji & Sutjahjo, 2018). Conversely, the interaction between reaction time and agitation rate showed no influence on HHV (Figure 6). To achieve a high HHV in sweet sorghum bioethanol production, it is recommended to ferment for shorter durations with lower yeast loading, depending on the juice volume. This approach positions sweet sorghum as a viable alternative for synthetic ethanol production.

- *Process Development and Validation*

Following numerical optimization using Design-Expert software, the optimal fermentation conditions for sweet sorghum stalk juice and bagasse were established at a yeast loading rate of 25 g and a fermentation reaction time of 10 days. Under these conditions, the predicted bioethanol concentration and higher heating value (HHV) were 66.46% and 16.23 MJ/kg, respectively (Table 6). To validate the predictive capability of the developed models, the fermentation was conducted under these optimal conditions, and the experimental values were compared to the predicted ones. For reliable model validation, mean experimental values should fall within the 95% prediction interval (PI). In this study, the mean experimental values for both responses were within this range (Table 6), confirming the models' validity. The deviations between actual and predicted values were minimal, at only 0.08% for bioethanol concentration and 0.03% for HHV, indicating excellent agreement between experimental and predicted outcomes. However, it is important to note that these results were obtained from laboratory-scale experiments, which may not directly translate to larger-scale bioethanol production. Despite this limitation, the bioethanol concentration and HHV achieved (66.46% and 16.23 MJ/kg) align well with those obtained through bomb calorimetry and distillation in this research, validating the effectiveness of the optimized fermentation process.

➢ *Essential Fuel Properties of the Produced Bioethanol*

- *Fire and Flash Point Test*

The flash point is a critical parameter for classifying flammable liquids, as outlined by the European Classification, Labelling and Packaging (CLP) regulations and transport of dangerous goods guidelines. For low-concentration flammable liquid aqueous solutions, flash points can be ambiguous, making their flammability uncertain (Janes & Chaineaux, 2014). In this study, flash and fire points were measured using the open cup method at CEDAT, where the test sample was heated with a temperature gun. The results indicated that higher bioethanol

ISSN No:-2456-2165 https://doi.org/10.38124/ijisrt/25jul914

concentrations correlate with lower flash and fire points. Specifically, the bioethanol concentration ranged from 56.8% to 90.2% v/v, with flash points between 17.8°C and 23.3°C (Table 7). As concentration increased, the flash point decreased, consistent with findings from Carareto et al. (2012), which state that lower concentrations yield higher flash points. Understanding the flash point is essential for safely handling flammable liquids and ensuring compliance with regulations. Bioethanol is highly flammable and exhibits low flash and fire points that vary with concentration; for instance, standard synthetic ethanol at 99.5% v/v has a flash point of 12°C (Janes & Chaineaux, 2014). Among the samples tested, sweet sorghum stalk variety P13 achieved the highest juice bioethanol concentration of 90.2% v/v, resulting in a flash point of 17.8°C due to its high brix content of 14%.

- *The Calorific Value of Different Sweet Sorghum Varieties*

The calorific value of sweet sorghum varies significantly based on bioethanol concentration, with values ranging from 12.95 MJ/kg to 20.20 MJ/kg. The variety P13 exhibited the highest calorific value at 20.20 MJ/kg, correlating with its bioethanol concentration of 90.2% v/v. As the concentration decreases, the calorific value also declines. These findings align with previous studies indicating that high bioethanol concentrations can be blended with fuels like gasoline, yielding calorific values between 26.90 MJ/kg and 27.33 MJ/kg for synthetic ethanol (Micic & Jotanovic, 2015).

The experimental calorific values for the local sweet sorghum varieties were comparable to those of synthetic ethanol at 96% v/v, which has a calorific value of approximately 26.9 MJ/kg (Assaye et al., 2021). Overall, this study reinforces the notion that higher bioethanol concentrations result in increased calorific values, particularly for the genotype P13, highlighting its potential as an effective biomass for energy production.

- *Densities of the Produced Bioethanol*

Density, defined as the mass per unit volume of a substance, is a critical characteristic for assessing bioethanol quality. The standard density of synthetic ethanol at 96% v/v is 0.789 g/cm³, which served as a control for comparing the densities of different sweet sorghum varieties. The densities of these varieties ranged from 0.82 g/cm³ to 0.87 g/cm³, with the genotype P13 exhibiting the lowest density of 0.82 g/cm³ at a bioethanol concentration of 90.2% v/v. As bioethanol concentration decreases, density increases, confirming that higher concentrations yield lower densities. For instance, P13's density of 0.82 g/cm³ corresponds to its high bioethanol concentration, while lower concentrations result in higher densities.

This relationship aligns with findings from Khattab et al. (2012), which noted a density difference of 0.031 g/cm³ between bioethanol concentrations of 96% v/v and 90.2% v/v. The observed densities indicate that the quality of bioethanol produced from sweet sorghum is high; lower densities correspond to higher concentrations and better quality. This study further confirms that the density measurements meet market standards for bioethanol, demonstrating sweet sorghum's significant potential for bioethanol production.

VI. CONCLUSION

This study demonstrated that sweet sorghum could produce bioethanol for home cooking fuel at a lower cost than synthetic alternatives, offering an environmentally friendly and economically viable option. However, some varieties were found to be less economically viable for bioethanol production. Notably, four out of the six varieties studied (P13, P1, P12, P11) showed exceptional promise for bioethanol production.

➢ *Author's Contribution*

A.D.K Conceptualization, Methodology, Writing - Original Draft, Writing - Review & Editing); B.A; (Data Curation, Formal Analysis, Validation), E (Investigation, Resources, Writing - Review & Editing), L., S and B.N; (Supervision, Project Administration, Funding Acquisition).

- Funding
- Acknowledgements
- Conflicts of interest
- There was no conflict of interest

REFERENCES

[1]. Adinurani, P., Rahayu, S., Sulistiyo Budi, L., Nindita, A., Soni, P., & Mel, M. (2018). Biomass and Sugar Content of Some Varieties of Sorghum (Sorghum Bicolor L. Moench) on Dry Land Forest as Feedstock Bioethanol. MATEC Web of Conferences, 164, 1–5. https://doi.org/10.1051/matecconf/201816401035

[2]. Assaye, M., Tamirat, B., & Fekadu, B. (2021). Ethanol concentration and calorific value of some local distilled Ethiopian alcohol (Areki): an energy potential assessment Ethanol concentration and calorific value of some local distilled Ethiopian alcohol (Areki): an energy potential assessment. Cogent Engineering, 8(1). https://doi.org/10.1080/23311916.2021.1979444

[3]. Azhar, S. H., Abdulla, R., Jambo, S. A., Marbawi, H., Gansau, J. A., Mohd Faik, A. A., & Rodrigues, K. F. (2017). Yeasts in sustainable bioethanol production. Elsevier, 10(March), 52–61. https://doi.org/10.1016/j.bbrep.2017.03.003

[4]. Barcelos, C. A., Maeda, R. N., Maria, L., Anna, M. S., & Pereira, N. (2016). Biomass and Bioenergy Sweet sorghum as a whole-crop feedstock for ethanol production. Elsevier, 94, 46–56.

[5]. Brakenhoff, D. A., Vonk, M. A., Collenteur, R. A., & Baar, M. Van. (2022). Application of Time Series Analysis to Estimate Drawdown From Multiple Well Fields. Frontiers, 10(June), 1–13. https://doi.org/10.3389/feart.2022.907609

[6]. Byun, J., & Han, J. (2021). Environmental analysis of bioethanol production strategies from corn stover via enzymatic and nonenzymatic sugar production. Elsevier, 328(February), 124808. https://doi.org/10.1016/j.biortech.2021.124808

[7]. Carareto, N. D. D., Kimura, C. Y. C. S., Oliveira, E. C., Costa, M. C., & Meirelles, A. J. A. (2012). Flash points of mixtures containing ethyl esters or ethylic

ISSN No:-2456-2165 https://doi.org/10.38124/ijisrt/25jul914

biodiesel and ethanol. Elsevier, 96, 319–326. https://doi.org/10.1016/j.fuel.2012.01.025

[8]. Cristina, B., Gheorghe, M., & Maria, D. A. (2017). Evaluation of Sugar Content in Stem Juice From Sweet Sorghum Hybrids Grown in South West of Romania. Cadastre Series), Anglani 1998, 25–29.

[9]. Frankowski, J., Wawro, A., Batog, J., Szambelan, K., & Łacka, A. (2022). Bioethanol Production Efficiency from Sorghum Waste Biomass. Energies, 15(9), 1–11. https://doi.org/10.3390/en15093132

[10]. Holechek. (2022). A Global Assessment: Can Renewable Energy Replace Fossil Fuels by 2050? MDPI, 14(8), 1–22. https://doi.org/10.3390/su14084792

[11]. Janes, A., & Chaineaux, J. (2014). Experimental determination of flash points of flammable liquid aqueous solutions To cite this version : HAL Id : ineris-00976242 Experimental Determination of Flash Points of Flammable Liquid Aqueous Solutions. Hall Open Science.

[12]. Jeswani, H. K., Chilvers, A., & Azapagic, A. (2020). Environmental sustainability of biofuels: A review: Environmental sustainability of biofuels. Royal Academy of Engineering, 476(2243). https://doi.org/10.1098/rspa.2020.0351

[13]. Kasegn, M. M. (2023). Production of Bioethanol from Sweet Sorghum [Sorghum Bicolor L .] Juice Using Yeast Isolated from Fermented Sweet Sorghum Juice. Research Square, 1–12.

[14]. Khattab, I. S., Bandarkar, F., Amin, M., Fakhree, A., & Jouyban, A. (2012). Density , viscosity , and surface tension of water + ethanol mixtures from 293 to 323 K. Korean Journal of Chemical Engineering, 29(6), 812–817. https://doi.org/10.1007/s11814-011-0239-6

[15]. Kumari, M., & Gupta, S. K. (2019). Response surface methodological (RSM) approach for optimizing the removal of trihalomethanes (THMs) and its precursor's by surfactant modified magnetic nanoadsorbents (sMNP) - An endeavor to diminish probable cancer risk. Scientific Reports, 9(1), 1–11. https://doi.org/10.1038/s41598-019-54902-8

[16]. López-Sandin, I., Gutiérrez-Soto, G., Gutiérrez-Díez, A., Medina-Herrera, N., Gutiérrez-Castorena, E., Galicia-Juárez, M., & Zavala-García, F. (2021). Biomass and sugar production dynamics in sweet sorghum variety roger. Chilean Journal of Agricultural Research, 81(1), 92–101. https://doi.org/10.4067/S0718-58392021000100092

[17]. Micic, V., & Jotanovic, M. (2015). Bioethanol as fuel for internal combustion engines. Zastita Materijala, 56(4), 403–408. https://doi.org/10.5937/zasmat1504403m

[18]. Muhaji, & Sutjahjo, D. H. (2018). The characteristics of bioethanol fuel made of vegetable raw materials. IOP Conference Series: Materials Science and Engineering, 296(1). https://doi.org/10.1088/1757-899X/296/1/012019

[19]. Nuanpeng, S., Thanonkeo, S., Klanrit, P., & Yamada, M. (2023). Optimization Conditions for Ethanol Production from Sweet Sorghum Juice by Thermotolerant Yeast Saccharomyces cerevisiae : Using a Statistical Experimental Design. MDPI, 04, 1–15.

[20]. Pappu, J. S. M., & Gummadi, S. N. (2016). Multi response optimization for enhanced xylitol production by Debaryomyces nepalensis in bioreactor. Springer, 6(2), 1–10. https://doi.org/10.1007/s13205-016-0467-x

[21]. Phukoetphim, N., Salakkam, A., Laopaiboon, P., & Laopaiboon, L. (2017). Improvement of ethanol production from sweet sorghum juice under batch and fed-batch fermentations: Effects of sugar levels, nitrogen supplementation, and feeding regimes. Electronic Journal of Biotechnology, 26, 84–92. https://doi.org/10.1016/j.ejbt.2017.01.005

[22]. Rajewski, J., & Dobrzyńska-Inger, A. (2021). Application of response surface methodology (Rsm) for the optimization of chromium(iii) synergistic extraction by supported liquid membrane. MDPI, 11(11), 1–17. https://doi.org/10.3390/membranes11110854

[23]. Saini, J. K., Anurag, R. K., Arya, A., Kumbhar, B. K., & Tewari, L. (2013). Optimization of saccharification of sweet sorghum bagasse using response surface methodology. Industrial Crops and Products, 44, 211–219. https://doi.org/10.1016/j.indcrop.2012.11.011

[24]. Yaser Dahman, Kashif Syed, Sarkar Begum, Pallavi Roy, and B. M. D. (2019). Biofuels: Their characteristics and analysis. In Elsevier. Elsevier Ltd. https://doi.org/10.1016/B978-0-08-102426-3.00014-X