Bull. Chem. Soc. Ethiop. **2024**, 38(1), 55-67. ISSN 1011-3924

Printed in Ethiopia
DOI: https://dx.doi.org/10.4314/bcse.v38i1.5 Online ISSN 1726-801X

OPTIMIZATION OF PARAMETERS FOR BIO-ETHANOL PRODUCTION FROM SWEET SORGHUM (*Sorghum bicolor* (L.) Moench) STALK JUICE AND FINGER MILLET MALT USING TAGUCHI METHOD

Dolphene Okoth[1], Stephen Otieno[1], Francis Kiema[1], David Onyango[2] and Chrispin Kowenje[1*]

[1]Department of Chemistry, Maseno University, Maseno, Kenya
[2]Department of Zoology, Maseno University, Maseno, Kenya

(Received May 22, 2023; Revised October 29, 2023; Accepted November 2, 2023)

ABSTRACT. Bio-ethanol is a promising renewable energy but its production is expensive from high cost of feedstocks. In this study, sweet sorghum (*Sorghum bicolor* (L.) *Moench*) stalk juice was investigated for bio-ethanol production. Most reports on bio-ethanol productions use commercial *Saccharomyces cerevisiae* as yeast. However, this study used finger millet (*Eleusine coracana*) malt with already high adaptation to local conditions and high economic viability as it is being utilized by the indigenous communities. Five sweet sorghum varieties of IESV-92001-DL (V1), NTJ (V2), 15233-IESV (V3), 92008-DJ (V4) and IESV-92028-DL (V5) were planted at 0°3'45.4644" North, 34°17'16.1052" South, in Kenya. °Brix content of juice was determined at 11th to 16th weeks after sowing. Highest °Brix for all varieties were at 15th week where V1 was highest at 22.07. V1 was then harvested for the juice. Factors affecting fermentation; temperature, time, pH and yeast to substrate ratio were optimized using Taguchi method and were obtained as 30 °C, 48 hours, pH 5 and 5 g/L, respectively. Kinetics parameters of V_{max} and K_m were 0.35 g/L/h and 12.56 g/L, respectively. The optimized and kinetic parameters were within literature values and therefore finger millet malt has a great potential, as a substitute yeast source, in commercial bio-ethanol production.

KEY WORDS: Bio-ethanol, Sweet sorghum juice, Taguchi method

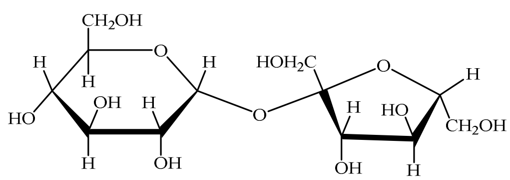
INTRODUCTION

There is an increased global demand for energy caused by increase in human population which is projected to exceed 9 billion by 2050 [1]. The increase in energy demand has led to advancements in technology and industrial developments in order to maintain and improve on the supply of goods and services for human needs. About 80% of the world's current energy usage is sourced from natural gas, coal and oil which are non-renewable fossil fuels [2]. Fossil fuels have adverse impacts on the environment and have caused an increase in the global total mortality rate and a decrease in the mean life expectancy arising from chronic diseases attributable to emissions during utilization [3]. Besides, currently there has been an upward trend in the prices of crude oil, especially in the regions known to produce oil in bulk. The upward changes in market oil prices are often quickly reflected in consumer prices and can therefore have adverse effects on stifling global growth and development [4]. Overdependence on non-renewable energy sources highly affects the well-being of the current and future generations in terms of environmental pollution, resource depletion leading to unsustainable development. Production and consumption of renewable energy can help in the reduction of greenhouse gas emissions and depletion of the ozone layer and hence help in addressing the climate change issue. This is geared towards improvement of the quality of the environment and achievement of sustainable growth and development [5]. However, in comparison to other energy sources, the current world production and consumption of renewable energy is still too low though it is projected to increase in the coming years courtesy of technological improvement in the current production methods [6]. According to Mulak and Ogbonna [7], the African continent has the least growth in both

*Corresponding author. E-mail: ckowenje@maseno.ac.ke

production and consumption of renewable energy; in fact, there is no large-scale ethanol industry in the continent apart from medium scale ones in South Africa [7].

The unavailability of cheaper sources of energy together with the adverse effects of fossil fuels on both the environment and living organisms is a key problem in the economic development, especially for developing regions in Africa and the rest of developing world. It is therefore important that green, renewable and sustainable energy is produced as there are adequate resources that can be utilized. Bio-fuels are promising renewable energy sources. Among the bio-fuels, bio-ethanol is an outstanding alternative source of renewable energy [8]. According to Khan *et al.* [9] production of bio-ethanol using human food-based biomass shows a negative impact on both the agricultural sector and food security. Thus, sweet sorghum stalk juice is a promising raw material for bio-ethanol production because it can be obtained readily and cheaply as a side-product from the sweet sorghum crop after harvesting the grains. In most parts of developing world, stalks are left in the fields as agricultural wastes despite them having a high sugar content that would be utilized for bio-ethanol production. The main sugar content in sorghum stalk juice is sucrose (**1**) with the following structure [10]:



1

Figure 1. Chemical structure of sucrose [10].

Bio-ethanol production from sweet sorghum is appropriate in developing economies since the crop can be cultivated with cheap labor leading to generation of additional agricultural income to the poor rural areas making the process cost effective, efficient, renewable and sustainable [11]. However, determination of the best sorghum variety that can produce the highest amount of bio-ethanol cannot be done by merely physical observation of the crop and hence there is need for a proper method to determine best varieties and the most appropriate time to carry out harvesting. Normally, the maturity of the stem is determined through the measurement of soluble solid contents (ºBrix) within the third middle part of the stem [12]. The higher the ºBrix the higher the content of total sugars required for fermentation in the presence of yeasts. Umakanth *et al.* [13] observed that the concentration of fermentable sugars in the stalk juice of sweet sorghum ranges from 12 to 23 ºBrix.

Finger millet (*Eleusine coracana*) is a grass crop that produce seeds which are harvested for human food and animal feed. It is grown majorly in the semi-arid tropics of Asia and Africa [14]. In addition, traditional malt manufacturers report that finger millet malt is superior to sorghum malt in terms of fermentation and flavor [15].

Previous studies on the factors affecting fermentation of sweet sorghum stalk juice show that pH, temperature, time and yeast to substrate ratio affect fermentation. Notably, the temperature ranges at which most fermentation occur is between 30 and 36 °C with a requirement of a control within ±0.5 °C [16]. However, it is observed that in order to achieve efficient ethanol fermentation, parameters like pH, substrate concentration and temperature should be controlled [17]. As per their study, Lin *et al.* [17] reported that the optimum pH of between 4-5 was best for fermentation. Another study observed that maximum production of ethanol could be obtained with a yeast to substrate ratio of 1 g:1000 mL [18]. All the cited fermentation procedures were carried out using *Saccharomyces cerevisiae* as a source of enzyme, however, optimization of fermentation parameters using finger millet malt as a source of enzyme is missing and therefore, there was need for research to advance the fermentation efficiency using the "wild" yeast. For decades, the indigenous breweries in Eastern Africa have sustainably utilized the finger millet malt as yeast source. Taguchi is a fractional factorial design of experiment based on orthogonal arrays that helps in the evaluation of maximum number of effects from a minimum number of experimental runs while allowing for differences in the number of factor levels [19]. The design is preferred over one factor at a time traditional technique for optimization that involves numerous trials hence take a lot of time and resources while not allowing the study of interaction between various variables. In Taguchi experimental design, data is evaluated using signal to noise (S/N) ratio and analysis of variance (ANOVA) with simultaneous evaluation of the significance of the factors in terms of their contribution to the response values [20]. Taguchi had been successfully used to optimize pH, urea, ammonium sulfate and amount of molasses in the fermentation of molasses using *Saccharomyces cerevisiae* [21]. However, literature on optimization of fermentation of sweet sorghum stalk juice using finger millet malt by Taguchi method is not available. Therefore, the objective of this work was to optimize temperature, pH, fermentation time and yeast to substrate ratio in the fermentation of sweet sorghum stalk juice using finger millet malt by Taguchi method. Based on this method, the number of moles of ethanol produced after the experiments were taken as response. In this work, the larger the better characteristic was considered in order to maximize the fermentation process. Therefore, the equation proposed by Taguchi for (S/N) ratio calculation was adopted from [22] as shown in the equation 1:

$$\frac{S}{N} = -10 \log\left(\frac{1}{r} \sum_{i=1}^{n} \frac{1}{R_i^2}\right) \tag{1}$$

where R_i is the percentage of ethanol produced, r represents the number of replications of the experiment in this study (each experiment was repeated three times), while n is the total number of experiments. The performance of an enzyme from yeasts is evaluated by obtaining their Michaeli-Mentene's V_{max} and K_m where higher relative V_{max} imply higher amount of enzyme required for a maximum reaction velocity while higher K_m shows that the enzyme does not bind efficiently with the substrate [23].

The above introduction makes finger millet malt a promising alternative source of fermentation enzyme since it is a good source of hydrolytic enzymes normally used in brewing [15]. Using the malt in bio-ethanol production reduces the cost of production. In addition, using grains produced by local farmers instead of importing the sources of yeast is beneficial for economic development in developing countries [24]. To add to that, there is a strategy to produce sustainable bio-energy for all bio-energy users with a commitment to meet clean cooking [25]. Access to clean cooking can reduce the time that women and girl child spend collecting fuel and subsequent cooking [26]. To actualize this, the current research focused on optimization of sweet sorghum (*Sorghum bicolor* (L.) *Moench*) stalk juice and finger millet malt fermentation parameters using Taguchi experimental design.

EXPERIMENTAL

Chemicals and reagents

Five sweet sorghum varieties used in this study were: IESV 92001 DL (V1), NTJ (V2), 15233 IESV (V3), 92008 DJ (V4) and IESV 92028 DL (V5). They were sourced from International Crops Research Institute for the Semi-Arid Tropics (ICRISAT), Nairobi Office-Kenya. Finger millet malt was prepared according to Amadou *et al.* [27] through traditional malting processes. Chemicals used in this work were of analytical grade and sourced from Sigma-Aldrich.

Sweet sorghum stalk juice

The five sweet sorghum varieties were evaluated under agronomic trials at a farm whose geographical co-ordinates are 0° 3' 45.4644" North, 34° 17' 16.1052" South. The type of soils at the farm had a pH of 5.2 with an annual rainfall of 1630 mm and a mean temperature of 25 - 27 °C. Planting of the five varieties was done during the short rainy season of August-December 2022 and on the 11th week after sowing, harvesting was done manually where leaves and panicles were removed. °Brix was determined using a digital refractometer (Model MA871, Milwaukee Co. Ltd., Romania). Calibration was done using distilled water thereafter 2 drops of the juice were put on the prism of the refractometer and readings taken in triplicates [12]. This procedure was repeated after every seven days up to week 16. V1 had the highest °Brix at week 15. The V1 was therefore bulked the following season and the juice extracted, at the 15th week, from the cut stalks using electrical stalk juice crushers.

Optimization of the bio-ethanol production parameters

The fermentation parameters were optimized using Taguchi experimental design (Minitab 18.1.0.0 software). This is a statistical experimental design approach for multifactor process optimization that helps in the optimization of the medium for bio-ethanol production by reducing the number of experimental runs and time [21]. Factors affecting fermentation process are as shown in Table 1.

Table 1. Experimental factors and their levels.

Factors	Levels			
	Low		High	
Temperature (°C)	30	35	40	45
pH	4	5	6	7
Time (h)	36	48	60	72
Yeast: Amount of sugar (g/50 mL)	0.05	0.15	0.25	0.35

Fermentation

Fermentation was carried out using the following experimental runs generated from Taguchi experimental design as shown in Table 2.

Table 2. Combinations of the experimental runs.

Exp. No.	Temperature (°C)	pH	Time (h)	Yeast: Amount of sugar (g/50 mL)
1	30	4	36	0.05
2	30	5	48	0.15
3	30	6	60	0.25
4	30	7	72	0.35
5	35	4	48	0.25
6	35	5	36	0.35
7	35	6	72	0.05
8	35	7	60	0.15
9	40	4	60	0.35
10	40	5	72	0.25
11	40	6	36	0.15
12	40	7	48	0.05
13	45	4	72	0.15
14	45	5	60	0.05
15	45	6	48	0.35
16	45	7	36	0.25

Then, 50 mL of the juice was put in 100 mL conical flasks and the pH of the juice adjusted to pH 4, pH 5, pH 6 and pH 7, one value at a time according to Table 2, using 0.5 M sodium hydroxide solution or 0.5 M dilute sulfuric (VI) acid. Yeast was added in different quantities which was 0.05 g, 0.15 g, 0.25 g and 0.35 g into different conical flasks containing the sweet sorghum juice according to the specifications in each experimental run (Table 2). 0.005 g/100 mL of ammonium phosphate [$(NH_4)_3PO_4$] and 0.001 g/100 mL magnesium sulfate penthydrate ($MgSO_4.7H_2O$) was added as nutrients into the flasks [28]. The samples were then placed in a water bath at different temperatures which were 30, 35, 40 and 45 °C and the duration of fermentation was varied at 36, 48, 60 and 72 hours to allow fermentation to take place. The amount of bio-ethanol produced from each experimental run was determined according to [29]. Response from each combination was analyzed statistically using Minitab 18.1.0.0 software that gave the optimum fermentation conditions required for the final bulk ethanol production.

Kinetics of the reaction

In this study, the kinetics were determined using the amount of product; ethanol, since it was a measurable reaction parameter. The experimental results were adjusted by converting the Michaelis-Menten equation 2 to linearized form as proposed by Lineweaver and Burk to obtain equation 3:

$$V_o = \frac{V_{max}[S]}{K_m + [S]} \tag{2}$$

$$\frac{1}{V} = \frac{K_m}{V_{max}} \times \frac{1}{[S]} + \frac{1}{V_{max}} \tag{3}$$

where V_o = the rate of the enzymatic reaction, V_{max} = the maximum possible rate of the reaction for a given total enzyme concentration, K_m = the Michaelis-Menten constant, and [S] = the substrate concentration. By plotting 1/V against 1/[S] (see equation 3) make it possible to obtain a straight line whose slope is equivalent to K_m/V_{max} with a y-intercept corresponding to $1/V_{max}$ [30].

RESULTS AND DISCUSSION

Quantification of the °Brix content of the Sweet Sorghum Stalk Juice

In this study, the immediate post-anthesis week was week 11 after the five sweet sorghum varieties which included: IESV 92001 DL (V1), NTJ (V2), 15233 IESV (V3), 92008 DJ (V4) and IESV 92028 DL (V5) were planted. V3 had a significantly higher °Brix of 10.07 ($p \leq 0.05$) followed by V4 with 8.27 °Brix ($p \leq 0.05$) as shown in Figure 2. There was no significant difference in the °Brix of V2 ($p \leq 0.05$) and V5 ($p \leq 0.05$) while V1 exhibited 6.67 °Brix ($p \leq 0.05$) which was the least °Brix value. Thereafter, all the cultivars accumulated approximately 2 °Brix after every seven days with the median value of 14.13 °Brix. The optimum °Brix was obtained on week 15 after which there was a sharp decline at week 16 with the median value of decrease of 5.5 °Brix. V1 had the highest brix content of 22.07 on week 15, while V4 had the least brix content of 15.30.

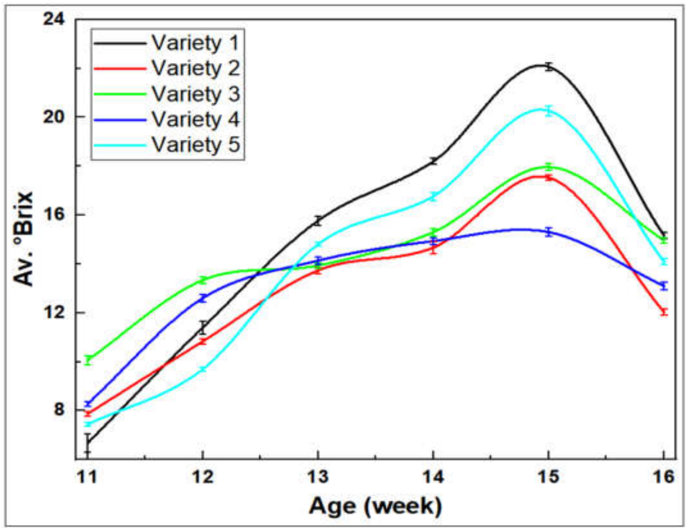


Figure 2. Trend of °Brix content per variety with age.

The results indicate that the rate of accumulation of sugar increased towards maturity (Figure 2). Generally, there was a steady increase in °Brix as the sweet sorghum matured from the 11th week with the maximum °Brix occurring at week 15 followed by a significant drop at week 16. The low °Brix content at week 11 could be due to no accumulation of sugar in the stem between germination and anthesis, that is, the growth stage [31]. At this particular stage the invertase enzyme catalyze the conversion of sucrose obtained from photosynthesis into glucose and fructose for respiratory energy and cell wall synthesis in young and rapidly growing internodes [32]. The steady increase in the °Brix of the stalk juice after anthesis to an optimum level at week 15 could further be attributed to the fact that at this stage the activities of invertase (sugar-degrading enzymes) are reduced leading to accumulation of sucrose in the stem while the final drop after maximum is caused by reduced photosynthesis and remobilization of carbohydrates from the stalks to the grains in the final grain filling stage sink in stems [32].

At week 11 variety V3 had a significantly higher °Brix of 10.07 ($p \leq 0.05$). A study carried by Davila-Gomez *et al.* [33] reported 8 °Brix as the average of all the sweet sorghum varieties in the first week post-anthesis, this was lower than 10.07 °Brix exhibited by V3 in this study with the least significant difference (LSD) values calculated at 0.05 probability level. The 15th week after sowing was the stage that had the optimum sugar concentration which was within a range of 15.30 °Brix to 22.07 °Brix ($p \leq 0.05$) with a median value of 17.97 °Brix. These results are in the same range with those obtained by Nazli [34] after a study of six improved sweet sorghum varieties which were Icsv 93046, Top 76-6, Gulseker, Dale, Icsv 700 and M81-E the °Brix reported was in the range of 13.3 to 22.9. It is worth noting that among the five varieties that were used in this study; variety V1 depicted the highest °Brix of 22.07 ($p \leq 0.05$) at the fifteenth week of growth (115 days after sowing). This was higher than the °Brix obtained by Teixeira *et al.* [12] which was 16 °Brix at the hard dough stage (125 days after sowing). It is obvious that the physiological processes for this development depend on agronomic factors (like availability of water, nutrient content, temperature) that support productivity of the crop. According to, sugar related traits of sweet sorghum depends directly on the genetic and genotype interaction with the environment. There was a significant drop in sugar concentration in all the five varieties at the 16th week of their growth which was in agreement with the results obtained by Teixeira *et al.* [12]

and Appiah-Nkansah *et al.* [35]. Their study indicated that sugar concentration in sweet sorghum reaches the peak when it approaches the physiological maturity of the grains which is normally followed by a decline since the plants start re-allocating sugars to the seeds for new vegetative growth. In addition, Burks *et al.* [36] also stated that to ensure maximum sugar yield is obtained in sweet sorghum, the optimum harvest time should generally be at 30 days after anthesis. That is in agreement with the results of this study since the maximum sugar concentration was at 115 days after planting which occurred 28 days after anthesis. The outcomes of this study are in agreement with earlier reports by Gutjahr *et al.* [31] who observed that increase in sucrose concentration within the stems of sweet sorghum occurred slightly before panicle initiation stage to the middle of the grain filling stage with the optimum occurring at the hard dough stage, (125 days after planting), followed by a statistically significant decrease in the total sugar concentration.

Optimum fermentation conditions for bio-ethanol production

The factors that affect bio-ethanol productivity that included pH, temperature, catalyst load and time taken for the fermentation reaction to occur were controlled in order to obtain high yields of bio-ethanol. The $L_{16}(4^4)$ experimental design matrix used in the optimization of fermentation of sweet sorghum stalk juice using finger millet malt and response values (bio-ethanol yield), predicted bio-ethanol yield, S/N ratio are shown in Table 3

Table 3. The $L_{16}(4^4)$ experimental design matrix with bio-ethanol yield, standard deviation values, S/N ratio, and predicted yield.

Experiment trial	Reaction temp (°C)	pH	Reaction time (h)	Yeast:Substrate (g/50 mL)	Avg. bioethanol yield (mol)	Standard deviation ($\times 10^{-3}$)	S/N Ratio	Predicted bioethanol yield (mol)
1	30	4	36	0.05	0.0081	0. 903	-41.985	0.0110
2	30	5	48	0.15	0.0167	3.126	-35.839	0.0162
3	30	6	60	0.25	0.0194	1.367	-34.285	0.0177
4	30	7	72	0.35	0.0191	1.095	-34.401	0.0184
5	35	4	48	0.25	0.0126	3.561	-38.832	0.0119
6	35	5	36	0.35	0.0202	2.231	-34.016	0.0184
7	35	6	72	0.05	0.0103	1.014	-39.836	0.0098
8	35	7	60	0.15	0.0044	1.007	-47.573	0.0073
9	40	4	60	0.35	0.0107	0.441	-39.462	0.0102
10	40	5	72	0.25	0.0103	0.723	-39.431	0.0137
11	40	6	36	0.15	0.0085	1.530	-41.650	0.0078
12	40	7	48	0.05	0.0064	1.229	-44.145	0.0047
13	45	4	72	0.15	0.0007	0.077	-62.785	0.0009
14	45	5	60	0.05	0.0025	0.113	-51.900	0.0018
15	45	6	48	0.35	0.0048	0.325	-46.393	0.0078
16	45	7	36	0.25	0.0042	0.180	-47.631	0.0037

The 1st 5 columns of Table 3 represent the sixteen experimental runs generated by Taguchi design. The main aim of this study was to produce the highest amount of bio-ethanol possible, therefore, the S/N ratio with "Larger is Better" characteristic was used with the obtained response from the experimental trials which was the average bio-ethanol yield. The linear regression equation for the model developed correlated with the predicted response of all the process variables [37].

From Table 3, the main effects plot was generated by Minitab 18.1.0.0 software. The S/N ratio helps in determination of the deviation of the response from the desired value. This deviation in the process is lowered by maximizing the S/N ratio. The main effects plot for the means data and S/N ratios are as shown in Figure 3.

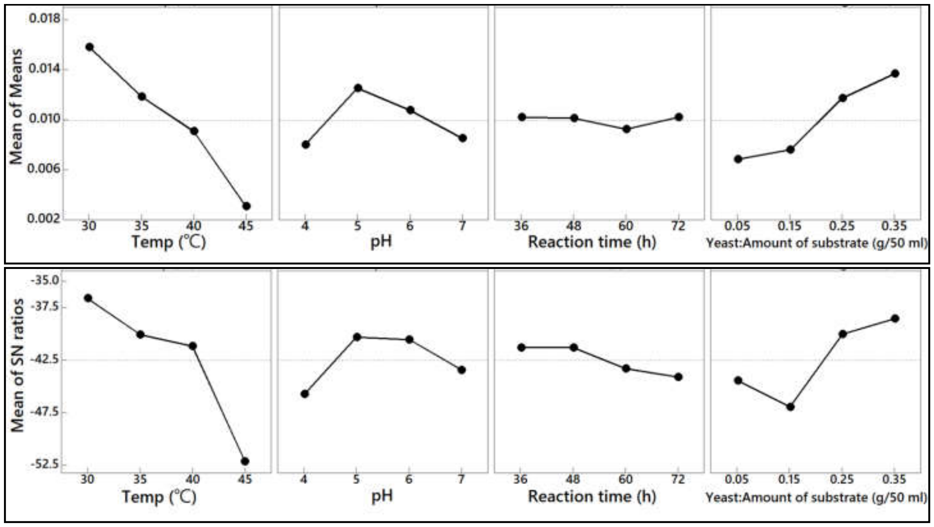


Figure 3. The main effects plot for data mean, and S/N ratios for the bioethanol yield.

From the means and S/N ratio shown in Figure 3, it is evident that temperature had the strongest influence on sweet sorghum stalk juice fermentation at 30 °C. A higher mean of this control factor indicates that it has a stronger effect on ethanol yield. The highest yield was observed at a temperature of 30 °C. It can also be seen that for the reaction temperature, the increase in ethanol yield was very steep as compared to other parameters where the extent of rise was lower. The ranking of parameters from the S/N ratios in Table 4 also show that temperature had the highest influence (at 30 °C) on the bio-ethanol yield followed by yeast to substrate ratio (at 0.25 g/50 mL), pH (at pH 5) and finally the reaction time (at 48 hours).

Table 4. Response table for signal to noise ratios.

Level	Reaction temp (°C)	pH	Reaction time (h)	Yeast:Substrate (g/50 mL)
1	-36.63	-45.77	-41.32	-44.47
2	-40.06	-40.30	-41.30	-46.96
3	-41.17	-40.54	-43.31	-40.04
4	-52.18	-43.44	-44.11	-38.57
Delta	15.55	5.47	2.81	8.39
Rank	1	3	4	2

The optimum conditions for the fermentation of sweet sorghum stalk juice using finger millet malt were found to be pH 5, yeast:substrate ratio of 0.25 g/50 mL, of the substrate, fermentation time of 48 hours and temperature of 30 °C. These results are in agreement with those obtained by Wu [38] where a pH 5.5 was reported to produce maximum ethanol during fermentation while a study by Lin *et al.* [17] stated that the highest ethanol production rate occurred at pH 5 and a temperature range of 30-45 °C. Another study reported that maximum ethanol production can be achieved by a yeast to substrate ratio of 1 g/L, temperature of 35 °C and fermentation time of 72 hours [18].

The accuracy of Taguchi model, for this work, was tested using a plot of actual or experimental versus predicted yields as shown in Figure 4. The closeness of the points to the straight regression line and high R^2 values show that the model fits the experimental data well [39]. The actual R^2 (0.921) and adjusted R^2 (0.915) are in reasonable agreement. It is evident that there is a good agreement between the experimental and the theoretical values predicted by the model and almost all the variations could be accounted for by the equation of the model [40].

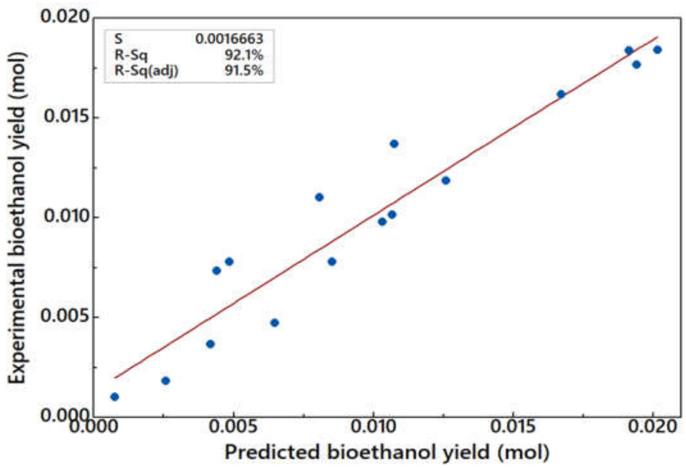


Figure 4. A plot of experimental bio-ethanol yield vs predicted bio-ethanol yield.

Analysis of variance (ANOVA)

Analysis of variance gives statistical significance of every fermentation parameter and their impacts on ethanol yield. The significance of a particular experimental factor is shown by the Fischer's test (F-test). When the experimental data fits well with the model a high F-test value is obtained. The high F-test value is obtained when the probability value (p-value) is below 0.05 which shows that the high F-test value is caused by the well fitted experimental data to the model and not because of noise.

The results of ANOVA are shown in Table 5. The contribution from the reaction temperature is more significant at 63.22%, followed by yeast:substrate ratio at 21.07%, pH at 9.42% and finally reaction time at 2.83%. The reaction temperature had the highest F-value of 18.27 and the least p-value of 0.02 confirming its highest significance among the four factors that were considered in this study. The Taguchi model is therefore significant and can be used to optimize the fermentation of sweet sorghum stalk juice using finger millet malt for bio-ethanol production.

Table 5. Analysis of variance for S/N ratios.

Source	DF	Seq SS	Adj SS	Adj MS	F	P	%SS
Reaction temp (°C)	3	543.34	543.34	181.114	18.27	0.020	63.22
pH	3	80.96	80.96	26.987	2.72	0.216	9.42
Reaction time (h)	3	24.30	24.30	8.100	0.82	0.564	2.83
Yeast:Substrate (g/50 mL)	3	181.05	181.05	60.349	6.09	0.086	21.07
Residual error	3	29.74	29.74	9.914			3.46
Total	15	859.39					100

Kinetics of the reaction

The maximum enzymatic reaction velocity (V_{max}), depicts the point at which the enzyme shows the highest turnover. It reflects how fast an enzyme can catalyze a particular reaction. On the other hand, K_m is the affinity of an enzyme to a substrate, a lower K_m shows that the enzyme is efficient at carrying out its function at a lower substrate concentration. In Michaelis-Menten kinetics it is known that the velocity of the reaction increases linearly with the increase in substrate concentration up to a point where there is no change in velocity with increase in substrate concentration. The linear increase in velocity at the beginning of the reaction gives 1st order reaction kinetics. This is normally followed by a point where the reaction velocity is independent of the substrate concentration at the 0th order kinetics; the point with the maximum velocity (V_{max}) which is an asymptote [30]. This was the case observed in this study. Therefore, to improve the accuracy of the (V_{max}) and K_m, Lineweaver-Burk plot was used. The maximum reaction velocity V_{max} and Michaelis-Menten constant (K_m) of the fermentation reaction from the Lineweaver-Burk plot in Figure 5 were found to be 0.35 g/L/h and 12.56 g/L of the substrate, respectively. The correlation coefficient (R^2) obtained is close to 1, this shows that the kinetics of fermentation of sweet sorghum stalk juice using finger millet malt follow the Michaelis-Menten model. These results are in agreement with those obtained in a study by Igbokwe *et al.* [30] on the production of bio-ethanol from plantain peels using *Saccharomyces cerevisiae* where a V_{max} and K_m of 0.85 g/L/h and 16.2 g/L, respectively, was obtained. In addition, other workers obtained a V_{max} and K_m of 0.70 mol/L.s and 81.63 mol/L, respectively in the production of bio-ethanol from sugar molasses with *Saccharomyces cerevisiae* which is in the same range with this study [41].

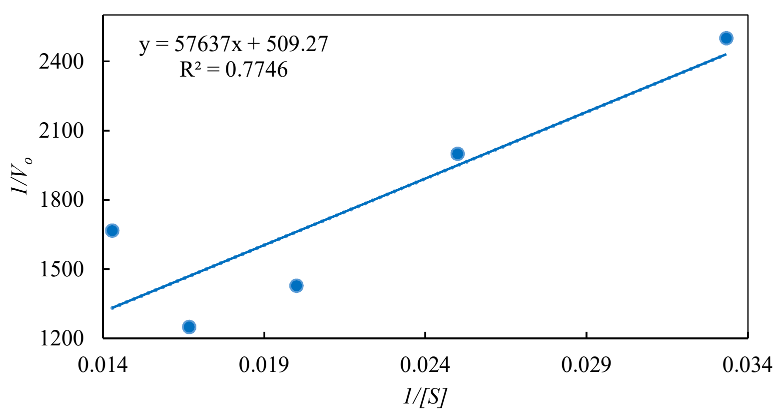


Figure 5. Lineweaver-Burk plot of $1/V_o$ against $1/[S]$ for the fermentation reaction.

This work obtained a K_m of 12.56 g/L on fermentation of sweet sorghum stalk juice using finger millet malt. This showed that the enzyme from finger millet malt is more efficient compared to the enzyme from *Saccharomyces cerevisiae* that had a K_m of 16.2 g/L in a study by Igbokwe *et al.* [30] on the production of bio-ethanol from plantain peels. A smaller V_{max} of 0.35 g/L/h obtained in this study compared to 0.85 g/L/h obtained in the study by Igbokwe *et al.* [30] indicate that only a small amount of substrate (which is industrially desirable) is needed for the reaction to reach its maximum velocity.

CONCLUSION

This study demonstrates that the sugar content within the stalk juices of sweet sorghum increases as the crop matures and reaches maximum and then dropped. Based on the results obtained, V1 from ICRISAT is the best sweet sorghum variety. It had a °Brix of 22.07 ($p \leq 0.05$) at 115 days after planting. The applied statistical tool, Taguchi method, proved to be efficient for optimization of bio-ethanol production through fermentation since the obtained results showed close agreement between the expected and obtained activity level. The optimum fermentation conditions for the sweet sorghum stalk juice using finger millet malt were obtained to be fermentation temperature of 30 °C, pH 5, yeast to substrate ratio of 5 g/L and fermentation time of 48 hours. The kinetics of the fermentation reaction were found as V_{max} of 0.35 g/L/hand K_m of 12.56 g/L. The values of V_{max} and K_m are indicative that finger millet malt is a viable yeast source for this work and that both the enzyme and the substrate have high affinity for one another.

ACKNOWLEDGMENT

The authors are grateful to Royal Society-DFID Africa Capacity Building Initiative Program Grant Ref. AQ150029 (financial support to PhD student), NRF – 2nd Multidispilinary Research Grant NRF/2/MMC/135 (agronomic activities and part instruments), and OeAD c/o Africa-UniNet Project P055 (part instruments and analysis of samples) for funding this research. Special thanks to Dr. Bowa Kwach for his unconditional support and guidance offered in this work.

REFERENCES

1. Sydney, E.; Letti, L.; Karp, S.; Sydney, A.; Vandenberghe, L.; Carvalho, J.; Woiciechowski, A.; Medeiras, A.; Soccol, V.; Soccol, C. Current analysis and future perspective of reduction in worldwide greenhouse gases emissions by using first and second generation bioethanol in the transportation sector. *Bioresour. Technol. Reports*. **2019**, 7, 37-47.
2. Jurgen, S.; Miriam, F.; Rebecca, F. Economic growth and the global energy demand. *Green Energy Sustain.* **2020**, 6, 1-43.
3. Firemichael, D.; Hussen, A.; Abebe, W. Production and characterization of biodiesel and glycerine pellet from macroalgae strain: *Cladophora glomerata*. *Bull. Chem. Soc. Ethiop.* **2020**, 34, 249-258.
4. Ready, R.C. Oil prices and the stock market. *Rev. Financ.* **2016**, 71, 1-22.
5. Tiba, S.; Belaid, F. Modeling the nexus between sustainable development and renewable energy: the African perspectives. *J. Econ. Surv.* **2021**, 35, 307-329.
6. Hirani, A.H.; Javed, N.; Asif, M.; Basu, S.K.; Kumar, A.A. review on first- and second-generation biofuel productions. *Biofuels Greenh. Gas Mitig. Glob. Warm. Next Gener. Biofuels Role Biotechnol.* **2018**, 8, 141-154.
7. Mulak, N.; Ogbonna, J. Acha (*Digitaria exilis*) malt as a source of enzyme for bio-ethanol production from starchy materials. *Niger. J. Biotechnol.* **2016**, 30, 1-6.
8. Mohd, A.; Abdulla, R.; Jambo, S.; Marbaw, H.; Gansau, J.; Faik, A.; Rodrigues, K. Yeasts in sustainable bio-ethanol production: A review. *Biochem. Biophys. Reports* **2017**, 10, 52-61.
9. Khan, M.A.; Khaliq, S.; Shadiq, R. Investigation of waste banana peels and radish leaves for their bio-ethanol potential. *Bull. Chem. Soc. Ethiop.* **2015**, 29, 239-245.
10. Shi, Y.; Liu, G.; Wang, L.; Zhang, H. Heteroatom-doped porous carbons from sucrose and phytic acid for adsorptive desulfurization and sulfamethoxazole removal: A comparison between aqueous and non-aqueous adsorption. *J. Colloid Interface Sci.* **2019**, 557, 336-348.
11. Baeyens, J.; Kang, Q.; Appels, L.; Dewil, R.; Lv, Y.; Tan, T. Challenges and opportunities in improving the production of bio-ethanol. *Prog. Energy Combust. Sci.* **2015**, 47, 60-88.

12. Teixeira, T.P.M.; Pimentel, L.D.; Dias, L.A.S.; Parrella, R.A.C.; Paixão, M.Q.; Biesdorf, E.M. Redefinition of sweet sorghum harvest time: New approach for sampling and decision-making in field. *Ind. Crops Prod.* **2017**, 109, 579-586.
13. Umakanth, A.V.; Kumar, A. A.; Vermerris, W.; Tonapi; V.A. Sweet sorghum for biofuel industry. *Breed. Sorghum Divers. End Uses*. **2018**, 16, 255-270.
14. Ceasar, S.A.; Maharajan, T.; Krishna, T.P.A.; Ramakrishnan, M. Finger millet (*Eleusine coracana* (L.) *Gaertn*). improvement: Current status and future interventions of whole genome sequence. *Front. Plant Sci.* **2018**, 9, 1-16.
15. Taylor, J.R.N. Sorghum and millets. *Taxonomy, history, distribution, and production*. **2018**, 13, 1-12.
16. Imtiaz, U.; Assadzadeh, A.; Jamuar, S.S.; Sahu, J.N. Bioreactor temperature profile controller using inverse neural network (INN) for production of ethanol. *J. Process Control*. **2013**, 23, 731-742.
17. Lin, Y.; Zhang, W.; Sakakibara, C.K.; Tanaka, S.; Kong, H. Factors affecting ethanol fermentation using *Saccharomyces cerevisiae* BY4742. *Biomass and Bioenergy*. **2014**, 47, 395-401.
18. Luo, L.; Wang, L.; Shahbazi, A. Optimization of ethanol production from sweet sorghum (*Sorghum bicolor* (L.) *Moench*) juice using response surface methodology. *Biomass and Bioenergy*. **2014**, 67, 53-59.
19. Ghosh, S.B.; Mondal, N.K. Application of Taguchi method for optimizing the process parameters for the removal of fluoride by Al-impregnated Eucalyptus bark ash. *Environ. Nanotechnol. Monit. Manag.* **2019**, 11, 1-26.
20. Karmakar, B.; Dhawane, S.H.; Halder, G. Optimization of biodiesel production from castor oil by Taguchi design. *J. Environ. Chem. Eng*. **2018**, 6, 2684-2695.
21. Darvishi, F.; Moghaddami, N.A. Optimization of an industrial medium from molasses for bio-ethanol production using the Taguchi statistical experimental-design method. *Fermentation* **2019**, 5, 2-9.
22. Berkane, N.; Meziane, S.; Aziri, S. Optimization of Congo red removal from aqueous solution using Taguchi experimental design. *Sep. Sci. Technol.* **2020**, 55, 278-288.
23. Cho, Y.S.; Lim, H.S. Comparison of various estimation methods for the parameters of Michaelis-Menten equation based on in vitro elimination kinetic simulation data. *Transl. Clin. Pharmacol.* **2018**, 26, 39-47.
24. Taylor, J.R.N.; Duodu, K.G. *Sorghum and Millets: Grain-Quality Characteristics and Management of Quality Requirements*. **2017**, 13, 317-351.
25. Karanja, A.; Gasparatos, A. Adoption and impacts of clean bioenergy cookstoves in Kenya. *Renew. Sustain. Energy Rev.* **2018**, 102, 285-306.
26. Christley, E.; Ljungberg, H.; Ackom, E.; Fuso, N.F. Sustainable energy for slums? Using the sustainable development goals to guide energy access efforts in a Kenyan informal settlement. *Energy Res. Soc. Sci.* **2021**, 79, 102-176.
27. Amadou, I.; Gbadamosi, O.S.; Le, G.W. Millet-based traditional processed foods and beverages-a review. *Cereal Foods World*. **2011**, 56, 115-121.
28. Ahmad, D.R.; Ahmad, D.E.; Kaur, A.; Gupta, P.U. Sweet sorghum-a promising alternative feedstock for biofuel production. *Renew. Sustain. Energy Rev.* **2018**, 82, 4070-4090.
29. Nwufo, O.C.; Nwaiwu, C.F.; Igbokwe, J.O.; Nwafor, O.M.I. Production and characterisation of bio-ethanol from various Nigerian feedstocks. *Int. J. Ambient Energy* **2016**, 37, 469-473.
30. Igbokwe, P.K.; Idogwu, C.N.; Nwabanne, J.T. Enzymatic hydrolysis and fermentation of plantain peels: Optimization and kinetic studies. *Adv. Chem. Eng. Sci.* **2016**, 6, 216-235.
31. Gutjahr, S.; Clement-vidal, A.; Soutiras, A.; Sonderegger, N.; Braconnier, S.; Dingkuhn, M.; Luquet, D. Grain, sugar and biomass accumulation in photoperiod-sensitive sorghums (II). Biochemical processes at internode level and interaction with phenology. *Funct. Plant Biol.* **2013**, 40, 355-368.

32. Kawahigashi, H.; Kasuga, S.; Okuizumi, H.; Hiradate, S. Evaluation of brix and sugar content in stem juice from sorghum varieties. *Japanese Soc. Grassl. Sci.* **2013**, 59, 11-19.
33. Davila-Gomez, F.J.; Chuck-Hernandez, C.; Perez-Carrillo, E.; Rooney, W.L.; Serna-Saldivar, S.O. Evaluation of bio-ethanol production from five different varieties of sweet and forage sorghums (*Sorghum bicolor* (L.) *Moench*). *Ind. Crops Prod.* **2011**, 33, 611-616.
34. Nazli, R.I. Evaluation of different sweet sorghum cultivars for bioethanol yield potential and bagasse combustion characteristics in a semiarid Mediterranean environment. *Biomass Bioenergy* **2020**, 139, 105624.
35. Appiah-Nkansah, N.B.; Li, J.; Rooney, W.; Wang, D. A review of sweet sorghum as a viable renewable bioenergy crop and its techno-economic analysis. *Renew. Energy*. **2019**, 143, 1121-1132.
36. Burks, P.S.; Felderhoff, T.J.; Viator, H.P.; Rooney, W.L. The influence of hybrid maturity and planting date on sweet sorghum productivity during a harvest season. *Agron. J.* **2013**, 105, 263-267.
37. Dhawane, S.H.; Kumar, T.; Halder, G. Biodiesel synthesis from *Hevea brasiliensis* oil employing carbon supported heterogeneous catalyst: Optimization by Taguchi method. *Renew. Energy* **2016**, 89, 506-514.
38. Wu, Y.; Wan, C.; Zheng, M.; Zuo, J.; Wu, J.; Wang, K.; Yang, B. Effect of pH on ethanol-type acidogenic fermentation of fruit and vegetable waste. *Waste Manag.* **2017**, 60, 158-163.
39. Das, A.K.; Padhi, M.R.; Hansdah, D.; Panda, A.K. Optimization of engine parameters and ethanol fuel additive of a diesel engine fuelled with waste plastic oil blended diesel. *Process Integr. Optim. Sustain.* **2020**, 7, 1-15.
40. Chilakamarry, C.R.; Sakinah, A.M.M.; Zularism, A.W.; Khilji, I.A.; Kumarasamy, S. Glycerol waste to bio-ethanol: Optimization of fermentation parameters by the Taguchi method. *J. Chem.* **2022**, 2022, 1-11.
41. Periyasamy, S.; Venkatachalam, S.; Ramasamy, S.; Srinivasan, V. Production of bio-ethanol from sugar molasses using *Saccharomyces cerevisiae*. *Mod. Appl. Sci.* **2009**, 3, 32-37.