

**OPTIMIZING BIO-ETHANOL PRODUCTION FROM SWEET SORGHUM STALK
JUICE USING *Saccharomyces cerevisiae*, FINGER MILLET MALT AND SORGHUM
MALT BY TAGUCHI METHOD**

**BY
OKOTH DOLPHENE ADHIAMBO**

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DECLARATION

Candidate's declaration

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Okoth Dolphene Adhiambo

MSC/SC/00002/021

Sign..... Date.....

Supervisor's declaration

This thesis has been submitted with our approval as University supervisors.

Prof. Kowenje Chrispin

Department of Chemistry

School of Physical and Biological Sciences

Maseno University

Sign..... Date.....

Prof. Miruka Onyango David

Department of Zoology

School of Physical and Biological Sciences

Maseno University

Sign..... Date.....

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DEDICATION

To my parents Basil Okoth Ndheho and Gaudensia Adongo Okoth, my spouse Ezekiel Samwel Malugah Otieno and my loving sons Mactain James, Spencer Franklyne and James Tobias Otieno Mallugah because their love always kept me going.

ABSTRACT

Bio-ethanol is a viable alternative source of energy because it is renewable and environmentally friendly. However, the cost of its production remains prohibitive due to the high cost of feedstock, more so, food insecurity is caused if food crops are used. Studies on the use of sweet sorghum stalk juice for bio-ethanol production are ongoing due to its adaptability to different climatic and environmental conditions coupled with its high ability to accumulate high concentration of fermentable sugars within its stalk. Most of these fermentations are carried out using *Saccharomyces cerevisiae* as the main yeast which is obtained industrially. However, due to high cost of producing industrial yeast for bio-ethanol production, there is need to establish an alternative source of yeast that would be of low cost but of good quality and efficient in bio-ethanol production. The use of fossil fuels causes environmental pollution, adverse human health effects and high cost of production therefore there is an urgent need to produce bio-ethanol which is a cleaner alternative source of fuel. The objectives of this study were to find: the best sweet sorghum variety with the highest °Brix, optimum bio-ethanol production temperature, pH, yeast to substrate ratio and reaction time, compare the effectiveness of *Saccharomyces cerevisiae*, finger millet malt and sorghum malt as sources of enzyme for bio-ethanol production and finally characterize the bio-ethanol produced in terms of calorific value, pH, density and flame test. Five sweet sorghum varieties namely: IESV 92001 DL (V1), NTJ (V2), 15233 IESV (V3), 92008 DJ (V4) and IESV 92028 DL (V5) were planted at Jaramogi Oginga Odinga University of Science and Technology experimental farm. °Brix content of their stalk juice was determined using a digital refractometer (Model MA871, Milwaukee Co. Ltd., Romania) at the 11th to 16th week after sowing. The highest °Brix for all the genotypes, indicated by ANOVA, was registered at the 15th week where V1 had the highest °Brix of 22.07 ($P \leq 0.05$). It was then harvested for bio-ethanol production. Fermentation factors were optimized using $L_{16} (4^4)$ Taguchi approach. The optimal conditions were temperature of 30 °C, 36 hours, pH 5 and yeast to substrate ratio of 5 g/L using *Saccharomyces cerevisiae* while optimal conditions of pH 5, temperature of 35 °C, 48 hours and yeast to substrate ratio of 5 g/L on using sorghum malt finally with finger millet malt the optimal conditions were yeast to substrate ratio of 5 g/L, pH 5, 48 hours and temperature of 30 °C. Kinetics of the fermentation reaction for V_{max} and K_m were 0.35 g/L/h and 12.56 g/L respectively using finger millet malt, while a V_{max} and K_m of 0.34 g/L/h and 14.09 g/L obtained for sorghum malt and a V_{max} and K_m of 0.69 g/L/h and 13.96 g/L with *Saccharomyces cerevisiae*. Fermentation of sweet sorghum stalk juice with the 3 sources of enzyme followed Michaelis Menten model. Both the optimized and kinetic parameters were within reported literature values and therefore results show that finger millet malt has a greater potential, as a substitute yeast source in application in bio-ethanol production industries. In terms of characterization of the bio-ethanol produced the calorific value, pH, density and flame test were found to be 8740 ± 29 kcal/kg, 6.3 ± 0.2 , 0.895 ± 0.076 g/cm³ and a blue flame produced respectively. The bio-ethanol produced burnt with a hot blue flame hence a viable alternative fuel for domestic cooking. This information is important to policy makers in designing ways that can help implement it as a clean source of cooking fuel and at the same time create employment to the people living in the rural areas.

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LIST OF ABBREVIATIONS AND ACRONYMS

ANOVA	:	Analysis of Variance
ICRISAT	:	International Crops Research Institute for Semi-Arid Tropics
JOUST	:	Jaramogi Oginga Odinga University of Science and Technology
UN-SDG	:	United Nations Sustainable Development Goals
GDP	:	Gross Domestic Product
%	:	Percent
°Brix	:	Soluble sugars per 100 g of juice
°C	:	Degree celcius
C₇H₁₇	:	Gasoline
C₂H₅OH	:	Ethanol
CO	:	Carbon (ii) oxide gas
CO₂	:	Carbon (iv) oxide gas
COVID-19	:	Coronavirus disease 2019
DAP	:	Diammonium Phosphate
EU	:	European Union
FAO	:	Food and Agriculture Organization
g	:	Gram
GDP	:	Gross Domestic Product
g/L	:	Gram per litre
g/L.h	:	Gram per litre per hour
h	:	Hour
kcal/kg	:	Kilocalories per Kilogram

kg	:	Kilogram
kg/m³	:	Kilogram per metre cubic
K_m	:	Michaelis-Menten constant
L	:	Litre
LSD	:	Least significant difference
mcΔT	:	Heat change
MgSO₄.7H₂O	:	Hydrated magnesium sulphate heptahydrate
MJ/kg	:	Megajoule per kilogram
ml	:	Mililitre
mm	:	Milimetre
mol	:	mole
mol/L	:	mole per litre
MTBE	:	Methyl tertiary butyl ether
(NH₄)₃PO₄	:	Ammonium phosphate
NO₂	:	Nitrogen (iv) oxide gas
pH	:	Potential of hydrogen
R²	:	Coefficient of determination
RCBD	:	Randomized complete block design
RFA	:	Renewable fuels association
S/N	:	Signal to noise ratio
USA	:	United States of America
UN-SDG	:	United Nations Sustainable Development Goals
V1	:	IESV920001DL
V2	:	NTJ

V3	:	15233IESV
V4	:	92008DJ
V5	:	IESV92028 DL
v/v	:	Volume per volume
V_{max}	:	Maximum rate of reaction at infinite substrate concentration
[S]	:	Substrate concentration
E	:	Enzyme
E₀	:	Total enzyme concentration
ES	:	Enzyme substrate complex
P	:	Product
V₀	:	Velocity of fermentation reaction
K_{cat}	:	Turnover number
H⁺	:	Hydrogen ions
NRF	:	National Research Foundation
kW	:	Kilowatts
MW	:	Megawatts

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CHAPTER ONE

INTRODUCTION

1.1 Background of the Study

There is an increased demand for energy in the world caused by increase in human population which is projected to exceed 9 billion by 2050 (Sydney *et al.*, 2019). This has led to advancements in technology and industrial developments in order to maintain and improve on the supply of goods and services. About 80 % of the current energy used is sourced from natural gas, coal and oil which are non-renewable fossil fuels (Jurgen Scheffran, 2020). Fossil fuels have adverse impacts to 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 (Perera, 2018). For example, human exposure to nitrogen dioxide (NO₂) and particulate matter from fossil fuel emission cause respiratory tract illness, asthma and decreased lung function. Exposure to hydrogen sulphide (H₂S) for a long period of time causes loss of appetite, headache, poor memory, irritability, fatigue, dizziness and miscarriages in pregnant women (Oguntoke & Adeyemi, 2016).

Besides, there has been an upward trend in the prices of crude oil in the recent past, especially in the regions known to produce oil in bulk. There is a strong negative correlation between increase in oil prices and other traded assets. 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 (Ready, 2016). 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 (Firemichael *et al.*, 2020). According to Schmidt *et al.*, (2012) 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. All these are geared towards the achievement of sustainable growth and improvement of the quality of the environment (Tiba & Belaid, 2020). The 7th goal of the 2030 United Nations Sustainable Development Goals (UN-SDG) puts a lot of emphasis on the use of renewable resources to achieve sustainable development for the benefit of the current and future generations. A lot of stress is put on access to affordable, reliable, modern and sustainable energy by everyone. To achieve reduced carbon intensity in the environment, countries should embrace the use of renewable energy more than non-renewable energy. This can help in the reduction of greenhouse gas emission (Güney, 2019).

Although research studies on renewable energy has tremendously increased in the recent years, it is only in the past few years that the production and consumption of renewable energy have become significant. For example, renewable sources of energy made up 7 % of the United Kingdom's primary energy in 2018 compared to just 1 % about 10 years ago (Sydney *et al.*, 2019). However, in comparison to other energy sources, the current world production and consumption of renewable energy is still too low though it is anticipated to increase in the coming years as reported by Hirani *et al.*, (2018), courtesy of significant technological improvements in the current production methods. According to Mulak & Ogbonna, (2016), the African continent has the least growth in both production and consumption of renewable energy, for instance, there is no large-scale ethanol industry in the continent apart from medium scale ones in South Africa.

The unavailability of cheaper sources of energy together with the adverse effects of fossil fuels on the environment and living organisms is a key problem in the economic development, especially for a developing country like Kenya. 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 a viable alternative source of renewable energy. According to Khan *et al.*, (2015), production of bio-ethanol using food-based biomass shows a negative impact on 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 in sorghum stalk juice is sucrose (**1**) with the following structure; (Shi *et al.*, 2019).

Figure 1.1: Chemical structure of sucrose

Bio-ethanol production from sweet sorghum is appropriate in developing economies since the crop can be cultivated with cheap labour leading to generation of agricultural income to the poor rural areas making the process cost effective, efficient, renewable and sustainable (Baeyens *et al.*, 2015). However, determination of the best sorghum variety that can produce the highest amount of bio-ethanol cannot be done by mere 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. According to Teixeira *et al.*, (2017), maturity of the stem is determined through the measurement of soluble solid contents (°Brix) within the third middle part of the stem. The higher the °Brix the higher the content of total sugars required for fermentation in the presence of

yeasts. Umakanth *et al.*, (2018) observed that the concentration of fermentable sugars in the stalk juice of sweet sorghum ranges from 12-23 °Brix.

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, Imtiaz *et al.*, (2013), reported that the temperature ranges at which most fermentation occurs is between 30-36 °C with a requirement of a control within ± 0.5 °C. However, it is observed that in order to achieve efficient ethanol fermentation, parameters like pH, substrate concentration and temperature should be controlled (Lin *et al.*, 2014). As in their study, they 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 (Luo *et al.*, 2014). 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 and sorghum malt as sources of enzyme is missing and therefore, there is need for research to advance the fermentation efficiency using the “wild” yeast sources. In this study, finger millet malt and sorghum malt are referred to as “wild” yeast sources. Wild yeasts from finger millet malt and sorghum malt are naturally occurring microorganisms that play a role in traditional fermentation processes. These wild yeasts coexist with other microbes like bacteria and molds, and their presence in these malts is often leveraged in indigenous fermentation practices for brewing, baking and other forms of food and beverage production.

There are several methods of optimization; using Minitab 18.1.0.0, Origin Lab 9.80.200 and Image J software such as Taguchi method, response surface, factorial design and mixture. Taguchi had been successfully used to optimize pH, urea, ammonium sulphate and amount of molasses in the fermentation of molasses using *Saccharomyces cerevisiae* (Darvishi &

Moghaddami, 2019). However, literature on optimization of fermentation of sweet sorghum stalk juice using finger millet malt and sorghum 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, sorghum malt and *Saccharomyces cerevisiae* by Taguchi method since it involves fewer experimental trials.

A major drawback in Africa against the development of ethanol fuel industries is the high cost of enzymes despite being relatively cheap in most developed countries; in Africa they remain expensive since there are no industries that produce them locally. This therefore means that they must be imported at relatively high costs from other countries (Mulak & Ogbonna, 2016). Due to their composition, the industrial yeasts are easily deactivated at high temperatures experienced in most African countries coupled with fluctuations in electrical power supply which make the enzyme storage very difficult. The major source of enzyme normally used for industrial brewing is barley malt. However, barley cannot be cultivated in most tropical countries since it is a temperate crop. Therefore, there is need to establish alternative sources of enzyme that are locally available which can make ethanol production cheap and efficient.

Finger millet is a self-pollinated crop that belongs to the family *Poaceae* and genus *Eleusine*. It is cultivated annually mostly by the poor people in Asia and Africa. Finger millet has extremely small kernel which makes it less susceptible to insect attack and can be stored for more than 5 years in drought prone areas (Ceasar *et al.*, 2018). Sorghum is mostly grown in high temperature areas with little rainfall. The crop also thrives best in shallow to medium deep or light to medium-textured soils. Of all the sorghum grown and harvested all over the world, Africa accounts for 61 % while Asia accounts for 22 %. It is consumed as grain and also prepared into

different variety of some other products of food which include alcoholic beverages, weaning meals, porridges and bread (Mundia *et al.*, 2019). Malted sorghum provides a good source of hydrolytic enzymes normally used in brewing (Amadi *et al.*, 2022). For decades, the indigenous breweries in Eastern Africa have sustainably utilized the finger millet malt as yeast source. 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 (Taylor & Duodu, 2017).

The performance of enzymes from yeasts is evaluated by obtaining their Michaelis-Menten V_{max} and K_m . V_{max} is the maximum rate of reaction or velocity achieved when the binding sites for the enzyme are fully saturated or occupied at a hypothetical unlimited substrate supply. On the other hand, K_m is a measure of the affinity of an enzyme for its substrate (tendency to bind to its substrate). A higher K_m shows that the enzyme does not bind efficiently with the substrate (Cho & Lim, 2018). In this study, the kinetic parameters of fermentation of sweet sorghum stalk juice using finger millet malt and sorghum malt was determined and compared to those of the most commonly used *Saccharomyces cerevisiae* yeast.

The vision of the Kenya government bio-fuel policy is to increase access to energy through sustainable bio-fuel production and reduce dependence on fossil fuel. In addition, there is a strategy to produce sustainable bio-energy for all bio-energy users. This places Kenya firmly towards achieving 100 % access to bio-energy for all by 2030 (Kiprop *et al.*, 2018). There is also a commitment to meet clean cooking in Kenya (Karanja & Gasparatos, 2019). Access to clean cooking can reduce the time that Kenyan women spend collecting fuel and cooking since they are the main beneficiaries, end-users and agents of the change. It will also reduce the hazardous health effects caused by indoor pollution (Christley *et al.*, 2021). It is therefore important to

determine the calorific value, pH, density and flame test of the bio-ethanol produced through fermentation of sweet sorghum stalk juice as suggested by Kiprop *et al.*, (2018) to help determine if it is a suitable fuel for domestic cooking.

1.2 Statement of the Problem

Continued use of fossil fuels leads to emission of toxic air pollutants that cause hazardous health effects and emission of carbon (iv) oxide gas that is a major greenhouse gas which causes climate change. The combination of hazardous health effects together with climate change leads to high hospital expenditure. In addition, increase in oil prices lowers the GDP of different economies. Therefore, there is need for production and use of bio-ethanol since it is renewable and environmentally friendly alternative fuel. Even though bio-ethanol is an environmentally friendly source of energy that is renewable, using sweet sorghum juice requires that an ideal time for harvesting must be determined to ensure that maximum sugar content is obtained and the grains are also harvested to be used as food. Therefore, there is need to periodically determine the °Brix of the juice as the crop grows to ascertain the stage with the highest sugar concentration. The common micro-organisms used in the production of ethanol are yeasts like *Saccharomyces cerevisiae* which is commercially obtained. A good yeast must be able to ferment a wide variety of sugars with high ethanol productivity and it should also be able to tolerate high amount of ethanol in the broth. In fermentation using yeasts, there are some challenges which inhibit ethanol production, for example, inadequate production technologies, high concentration of ethanol in the broth, high temperature and the ability to ferment pentose sugars. Therefore, there is need to optimize the fermentation conditions which include time, temperature, pH and yeast to substrate ratio to obtain the highest possible volume of ethanol. Lack of efficient yeasts which can generate higher yield of ethanol compared to the commercial

yeast which is obtained industrially make the cost of bio-ethanol production high. The cost of production of bio-ethanol can be reduced if wild yeast sources which occur naturally, obtained locally and are cheap are used for bio-ethanol production. When yeasts that produce low ethanol yield are used, the process become inefficient and economically unstable. There is need to ascertain if the wild yeast sources which are cheap and locally available can be efficient and effective as the commercial yeast. Using bio-ethanol as a cooking fuel reduces household air pollution normally associated with the use of firewood, charcoal and kerosene cook stoves in poorly ventilated kitchens which causes respiratory and cardiovascular diseases. The suitability of the bio-ethanol produced as a fuel that can be used for domestic cooking should be determined since it burns with a flue less flame suitable for human health.

1.3 Objectives of the Study

1.3.1 General Objective

To produce bio-ethanol for domestic cooking from selected sweet sorghum variety stalk juice by optimizing temperature, pH, time and yeast to substrate ratio using finger millet malt, sorghum malt and *Saccharomyces cerevisiae* as sources of enzyme.

1.3.2 Specific Objectives

- i. To determine the best sorghum variety by quantifying the °Brix of the sweet sorghum juice at different stages of their growth.
- ii. To optimize production conditions; pH, temperature, time and yeast to substrate ratio in bio-ethanol production for the best variety from (i) above using *Saccharomyces cerevisiae*, finger millet malt and sorghum malt.
- iii. To compare the effectiveness of the yeast from finger millet malt, sorghum malt and *Saccharomyces cerevisiae* as sources of enzyme for bio-ethanol production.

- iv. To evaluate the suitability of the bio-ethanol produced using the best enzyme source in (iii) above as a potential fuel for domestic cooking by determining its calorific value, pH, density and flame test.

1.4 Null Hypotheses

- i. H₀1: There is no significant difference in °Brix by stage of growth of selected sweet sorghum varieties.
- ii. H₀2: Changes in temperature, pH, time and yeast to substrate ratio have no significant effect on fermentation of sweet sorghum stalk juice.
- iii. H₀3: There is no significant difference in the activity of enzymes from finger millet malt, sorghum malt and *Saccharomyces cerevisiae* in the fermentation of sweet sorghum stalk juice.
- iv. H₀4: Bio-ethanol produced is not a suitable fuel for domestic use.

1.5 Justification of the Study

Cooking energy is relevant to development. The main cooking fuel especially in rural areas is wood, charcoal and kerosene. Ethanol is a cleaner substitute that if not produced and used, the 5th millennium development goal that is geared towards improvement of maternal health will not be achieved. Secondly, cooking using wood fuels and other solid-biomass in poorly ventilated kitchens causes respiratory diseases lowering the quality of life and greenhouse gas emissions leading to climate change. To reduce the hazardous health effects and environmental pollution caused by fossil fuels, there is an urgent need to produce bio-ethanol since it is renewable and environmentally friendly source of energy. A promising raw material that can be used in its production is sweet sorghum due to its wide adaptability to drought, high soil salinity and ability to accumulate high levels of extractable sugar in the stalks. Sugar concentration within the stalks vary with age and variety of the sorghum. To ensure that the juice obtained from the stalks

contain high concentration of fermentable sugars, the °Brix of the stalk juice should be determined regularly to help in the selection of a variety with the highest sugar content in the juice and also determine the stage of growth where the sugar content is highest. The yield of bio-ethanol from sweet sorghum stalk juice using other yeasts like *Saccharomyces cerevisiae* has been found to depend on factors of production like temperature, pH, fermentation time and yeast to substrate ratio. The use of wild yeast sources demand optimization to determine the best possible conditions under which the unknown enzyme can work. It is also necessary to investigate the fermentation abilities of potential wild yeast sources in comparison to *Saccharomyces cerevisiae* which is commonly used commercial yeast. In addition, determination of pH, calorific value, density and flame test of the bio-ethanol produced is very important to help verify if it is suitable for domestic cooking. This will help reduce the indoor air pollution that causes respiratory diseases especially in this era of COVID-19.

1.6 Significance of the Study

The use of sweet sorghum stalk juice for bio-ethanol production gives a solution to the problem of food versus fuel conflict since it involves the use of waste for cheaper production. The cost of production of bio-ethanol is dependent on the raw materials used. This can be reduced by using locally available finger millet malt and sweet sorghum stalk juice derived from the agricultural waste. This does not only reduce the production cost but at the same time helps in tackling the disposal problem. Sweet sorghum is a potential renewable energy crop that is viable for bio-ethanol production due to its high photosynthetic efficiency and ability to grow in diverse climatic conditions. Production of bio-ethanol helps develop a cleaner environment hence enables the achievement of sustainable development. Availability of household clean cooking fuel which is renewable and can be produced locally is a major step towards raising the quality

of life especially of the rural people. In addition, the use of bio-ethanol for domestic cooking helps in creation of employment boosts rural agriculture and helps in conservation of forest from wood fuel exploitation.

CHAPTER TWO

LITERATURE REVIEW

2.1 Introduction

Increased industrialization and use of automobiles has led to an increased demand for energy. About 80 % of the total energy utilized is fossil fuels where the transport sector alone consumes around 50 % in a year (Zheng, 2017). These fossil fuels are non-renewable; their sources are also being depleted at a very fast rate once they are exploited. In addition, continual use of fossil fuels leads to emission of greenhouse gases that cause negative effects like global warming as a result of climate change leading to rise in sea levels. These emissions outrightly show that the use of fossil fuels is a threat to long term sustainable growth. The increased demand for energy results in the increase of prices of crude oil which directly affect the global economy (Chatha, 2020). All these have led to an urgent need to find a cost effective, viable, replaceable, efficient and sustainable sources of fuel that are environmentally friendly.

A better alternative to replace fossil fuels is bio-fuel with bio-ethanol a viable alternative since it is carbon neutral given that the amount of carbon it emits on burning is equal to the amount the crop had previously absorbed during photosynthesis. The use of bio-ethanol reduces the carbon dioxide load in the atmosphere since the carbon dioxide produced when it is burnt is utilized by plants during photosynthesis (Yang *et al.*, 2020). Sustainable development can be achieved by controlling climate change in both developed and developing nations. This is only possible if there is adequate production and consumption of renewable energy which will decrease overall energy consumption (Zafar *et al.*, 2020). Sweet sorghum which produces sugar juice and grains that can be used as human food and animal feed together with lignocellulosic biomass is considered a bioenergy crop because it has high concentration of fermentable sugars in the stem. Sweet sorghum stalk juice can also be processed into granulated sugars or used as substrate for

hydrogen and methane production as illustrated by Antonopoulou *et al.*, (2008). After juice extraction from the stalk, a dry fibrous lignocellulosic material which is bagasse remains. This can be fed to animals in form of fodder or it can be used for paper manufacturing, cellulosic ethanol or used in generation of compost manure used to improve soil fertility. In addition, sweet sorghum is a suitable bioenergy crop due to its high adaptation to the existing agricultural infrastructure, it has high productivity and high ability to tolerate stress (Appiah-Nkansah *et al.*, 2019).

2.2 First Generation Bio-fuels

Bio-fuels are forms of renewable energy that are obtained from biomaterials like cassava, wheat, sugar beet, corn, sugarcane and other cereals. They can also be produced from crop residues like rice straw, corn stover, corn cobs, rice husk and wheat straw. Waste biomass like food waste and livestock waste can also be used to generate biofuels. Biofuels exist in either gaseous forms for example biogas or liquid forms like bio-ethanol and bio-diesel (Hirani *et al.*, 2018).

2.3 Biogas

The main constituents of biogas are methane (50-75 %) and carbon dioxide at a range of (25-50 %) together with small amounts of water vapour and other gases (Plugge, 2017). Micro-organisms decompose complex organic matter through anaerobic digestion process producing biogas whose energy content is directly linked to the methane content. It is a reliable renewable source of energy that can be a source of electricity and heat which can be stored easily. The use of biogas can help reduce the use of fossil fuels hence assist in reduction of carbon dioxide emissions. Micro-organisms involved include methanogenic archaea, hydrolytic bacteria, fermenting bacteria and organic acid-oxidizing bacteria which degrade organic matter through a series of biochemical conversions leading to production of biogas (Tekelamanot, 2018).

These digesters are normally connected to gas-fired engines for heat and power generation with electrical capacity that lies between tens of kilowatts (kW) up to a few megawatts (MW). The amount of heat generated can be used to meet the local heat demand in the farms or used externally. When purified properly to remove traces of water, carbon dioxide and hydrogen sulphide it can be upgraded to bio-methane which is injected into natural gas network or used in transport vehicles (Scarlat *et al.*, 2018).

2.4 Bio-ethanol

It is a renewable source of energy that is easily produced from sources rich in carbohydrates making it a promising alternative to fossil fuels. Countries like Canada, USA, China, Brazil and several EU member states have shown high interest on bio-ethanol production to help reduce the over dependence on fossil fuels. Global bio-ethanol production has increased tremendously in the past decade with highest amount of ethanol being produced by United States (Zabed *et al.*, 2017). According to Engebretson & Diamond, (2019), the Renewable Fuels Association (RFA) outlines steady increase in production with an upward trend from 3.4 billion gallons in 2005 to 36 billion gallons of ethanol in 2022. Ethanol (C_2H_5OH) is a better energy compared to gasoline (C_7H_{17}). Amount of energy given out by one liter of ethanol is about 66 % of the energy provided by the same amount of gasoline, however, ethanol has a higher octane number (106-110) than gasoline (91-96). This helps in improving the performance of gasoline when blended with ethanol. The high octane number of ethanol makes it to burn faster with a higher compression ratio hence reducing the rate of engine knock (Zabed *et al.*, 2017). Bio-ethanol contains 34.7 % oxygen hence it has about 15 % higher combustion efficiency than gasoline which does not have oxygen thereby having a lower combustion efficiency. Bio-ethanol has negligible amount of sulphur therefore when mixed with gasoline decreases emission of sulphur

which is a carcinogen and contributes to acid rain compared to gasoline. Bio-ethanol can also be used to substitute methyl tertiary butyl ether (MTBE) used as an octane enhancer for gasoline which reduces production of carbon monoxide (CO) and carbon dioxide (CO₂). MTBE when spilled or emitted through exhaust systems into the environment find their way into surface and ground water, contaminating drinking water hence causing severe detrimental effect to human health (Niphadkar *et al.*, 2018).

2.4.1 Sources of Bio-ethanol

Bio-ethanol renewable sources include starch, sugars, lignocellulosic biomass and algae. The ethanol obtained from sugars and starch is referred to as the first generation bio-ethanol, ethanol produced from lignocellulosic biomass is second generation bio-ethanol while that prepared from algae is called third generation bio-ethanol. However, third generation bio-ethanol production is still under laboratory research. From sugar sources, ethanol is obtained through fermentation of the extracted sugar. The starchy crops need to undergo hydrolysis which enables conversion of starch into glucose since yeast such as *Saccharomyces cerevisiae* cannot convert starch directly to ethanol due to absence of the required enzymes in starch (Ray *et al.*, 2019). On the other hand, lignocellulosic biomass must be pretreated before hydrolysis in order to alter cellulose structures for enzyme accessibility (Pandiyan *et al.*, 2019).

2.4.1.1 Lignocellulosic Sources

Lignocellulosic sources can be classified in four groups based on where they are obtained which can be: forest residues, crop residues, municipal solid waste and waste paper. Lignocellulosic biomass is made up of three main components which include cellulose (6), hemicellulose (7) and lignin (8) (Abo *et al.*, 2019) as shown:

Figure 2.1: Chemical structure of cellulose

Figure 2.2: Chemical structure of hemicellulose

Figure 2.3: Chemical structure of lignin

To obtain ethanol from lignocellulosic biomass, it must first be pre-treated before it is enzymatically hydrolyzed to produce the fermentable sugars. The pre-treatment methods include application of liquid hot water, dilute acid or even dilute alkali. A major limitation with the pre-treatment methods is that they generate degrading products which inhibit enzyme activities, they

can also affect the microbial cell, sugar consumption thereby interfering with the ethanol yield (Yu *et al.*, 2019). It is evident that it is very difficult to convert lignocelulosics to bio-ethanol since the biomass is generally resistant to breakdown, the sugars produced as a product of the breakdown need to find the right organisms to enable sufficient fermentation. In addition, Balat, (2011) also reported that the cost of collecting and storing low density lignocellulosic materials is high.

2.4.1.2 Starch Sources

The sources include raw corn, sorghum gains, wheat, cassava and sweet potato tubers. The general starch (9) structure adopted from Saggi & Dey, (2019) is as shown below:

Figure 2.4: Chemical Structure of Starch

These sources are widely used for bio-ethanol production since they are readily available and can be stored for a long period of time and when used, high ethanol yields can be obtained. Starch must first be broken down through a process known as hydrolysis to obtain fermentable sugars. The two most commonly used hydrolysis methods are enzymatic and acidic hydrolysis. Acidic hydrolysis is further divided into concentrated acid and dilute acid hydrolysis. Hydrolysis in dilute acid is carried out at higher temperatures using an acid with a low concentration whereas hydrolysis in concentrated acid is carried out at lower temperatures using an acid with high

concentration. Dilute acid hydrolysis generates a large amount of inhibitors compared to concentrated acid hydrolysis, however, it is the most commonly used hydrolysis method. The major limitations of acid hydrolysis include the difficulty of acid recovery and difficulty in recycling process which results in the increase of the cost of production. Enzymatic hydrolysis on the other hand requires endo- β -1,4-glucanase enzyme, cello-bio hydrolases and β -glucosidase enzymes (Mohd Azhar *et al.*, 2017). The factors that affect the efficiency of enzymatic hydrolysis include, pH, temperature, time and enzyme to substrate ratio. One major limitation of using enzyme in hydrolysis is that they are too expensive for ethanol production from biomass in an economical way. The ethanol yield obtained from the crops depend on their conversion efficiency. One of the major cereal crop widely used for bio-ethanol production is corn which is normally used in commercial scale.

2.4.1.2.1 Corn

The most utilized feedstock for ethanol production in North America and Europe is starch from corn. The grains must first be milled then taken through hydrolysis process. Milling can be done through dry-grinding which is done at 67 % or through wet-grinding done at 33 %. The dry-grind plants are the most preferred since the cost of their capital is less as compared to wet-grind plants. In hydrolysis, starch is wholly broken down to glucose through the help of two enzymes that is: alpha-amylase and amyloglucosidase. This should be done before fermentation by yeast since the process yields glucose monomers which can be fermented easily (Mussatto *et al.*, 2010).

2.4.1.2.2 Sweet Potato

This is a crop grown perennially at a very low production cost compared to other conventional crops. The crop is resistant to pests and helps in the prevention of soil erosion since it is a cover

crop. Sweet potato grows easily and has high resistance to drought (Gad Loebenstein, 2009). In addition, when the crop matures it can be harvested manually which leads to job creation to the locals.

The major energy source in sweet potato is in the form of starch hence making it a promising complementary alternative raw material for bio-ethanol production. The process of bio-ethanol production from sweet potatoes involve three steps namely: hydrolysis, fermentation and distillation. Hydrolysis process helps in breaking down amylose molecules by forming dextrans which finally forms glucose. During fermentation, the simple sugars are transformed into ethanol where high yield of ethanol is only obtained by optimization of the relevant pre-treatments and fermentation conditions. Ethanol is obtained from the wet residue through distillation process (Virgínio e Silva *et al.*, 2018). Even though bio-ethanol can be obtained from sweet potatoes, maintaining production sustainability is a huge challenge since after harvesting few plant parts are returned to the soil to help maintain sustainability. To add to that, under subsistence circumstances, sweet potato is an excellent source of food (Widodo *et al.*, 2015).

2.4.1.2.3 Cassava

Cassava is a crop that can grow in tropical and subtropical climate. It can tolerate semi-arid conditions and can also grow in a wide range of soils. It is an important tuber crop that is ranked as the 6th food crop after rice, wheat, corn, potatoes and barley (Zabed *et al.*, 2017). It is an excellent non-grain crop whose root tubers are rich in starch with abundant cellulose and hemicellulose. To obtain ethanol from cellulose it must undergo saccharification followed by fermentation and the product obtained must then be distilled before it is finally dehydrated. One major disadvantage of using cassava for bio-ethanol production is that it needs thorough pre-treatments before hydrolysis is carried out. The pre-treatment helps in breaking down the cross

linked cellulose hemicellulose and lignin it has lignin that prevent adequate hydrolysis by the enzymes this results in utilization on high energy during the distillation process with low ethanol concentration generated. To add to that, for sufficient fermentation to take place, additional nutrients must be added. This generally shows that ethanol production from cassava is more costly as compared to ethanol production from sweet sorghum stalk juice (Lyu *et al.*, 2020). More so, cassava waste waters from the industries have high amount of suspended solids and high cyanide content that contaminate nearby drinking water and produce odours that pollute the environment as fermentation occurs. A major socio-economic challenge of cassava is the potential for food versus fuel conflicts since it is a major food for rural and urban centers (Ohimain, 2012).

2.4.1.3 Sugar Sources

Energy producing crops that are used as sugar sources for bio-ethanol production include sweet sorghum, sugar cane, sugar beet, fruits like; apple, water melon, grapes and dates. Some of the sugar refinery wastes like beet molasses and cane molasses can also be used. The use of these sugar crops in the production of bio-ethanol is advantageous since they have high sugar yield and low conversion costs while the only limiting factor of these crops is their seasonal availability (Sydney *et al.*, 2019).

2.4.1.3.1 Fruits

Several fruits that are considered waste fruits which are discarded at harvest or during marketing might be because of their physical appearance or low quality can be fermented into bio-ethanol since they are rich in soluble fermentable sugars. Collection of the juice from fruits can at times be easier than the extraction of juice from sugar cane, however, fresh juice cannot be used for bio-ethanol production since it is meant for human consumption. For example, date palms which

grow mostly in arid and semi-arid areas and have high tolerance to environmental stresses, contain large amounts of sugar hence considered one of the important natural sugar resources are normally directly consumed as food. The date syrups contain minerals, vitamin, monosaccharides (glucose and fructose) and some small amount of sucrose (Ahmad *et al.*, 2021). The sugar from dates can be fermented by *Saccharomyces cerevisiae* to produce ethanol. Juice from the spoiled date fruits can be used as a substrate for ethanol, butanol and acetone production. Waste from fruits result from inappropriate harvest time, inefficient grading and packaging coupled with incorrect harvest practices. When fruits are harvested earlier than they should, their value and quality decreases and they can end up being exposed to pathogens due to increased moisture content. Birds may also invade the fruits if harvested late reducing the market appeal of the fruits. Some wastes are also obtained through the use of unsuitable tools during harvesting which cause damages to the fruits (Taghizadeh-alisaraei *et al.*, 2019).

2.4.1.3.2 Molasses

Molasses are obtained as by-products of sugar extraction and are used in animal nutrition. They are normally fed to ruminants as energy giving source since they can be consumed easily due to their sweet taste. The sugar contents in molasses vary depending on the processes that are in sugar extraction or the composition of the starting materials. Cane and beet molasses are good sources of fermentable sugars which can be used for bio-ethanol production (Palmonari *et al.*, 2020).

2.4.1.3.3 Sugar Beet

Sugar beet belong to the family of *amaranthaceae* and order caryophyllales with a C3 system of photosynthesis. It is cultivated in countries like United states of America, China, Poland, Russian Federation, Ukraine, Turkey, France, Germany, United Kingdom and Egypt (FAO 2019).

According to Zicari *et al.*, (2019), sugar beet contributes nearly 30 % to the world's sugar production. The crop is rich in sucrose and also resistance to water and salt stress. It takes a duration of five to six months from planting to reach its maturity stage when it can be harvested. The main producer of sugar beet is Europe where the crop competes favourably with sugarcane for the production of crystal sugar and ethanol. It is one of the most frequently cultivated plants used in manufacture of sugar. It is also used to produce the second largest amount of sugar in the world after sugarcane as reported by the Food and Agricultural Organization (FAO) of the United Nations (Borysiuk *et al.*, 2019). Through genetic and agro-technological improvements, generation of varieties that can grow favourably in tropical countries has been carried out (Kumar *et al.*, 2021). In addition, this crop can also grow well in cooler climatic conditions where the survival for sugarcane is minimal. It can take 5-9 months to be harvested depending on the soil type and the environmental conditions (Zicari *et al.*, 2019).

2.4.1.3.4 Sugarcane

Sugarcane is an agricultural crop that is normally grown in tropical and subtropical countries normally for valuable industrial products like sugar, waxes, bio-fibers and biofuels. The juice obtained from the stalks are the main feedstock in Brazil for the production of bio-ethanol, whereas molasses is the major feedstock for ethanol in India. The concentration of sugar in sugarcane plants depend on the time of harvest, variety and also the maturity of the plant (Shabbir *et al.*, 2021). The cane juice has a variety of organic minerals and nutrients suitable for bio-ethanol production. The production procedure for bio-ethanol from sugarcane stalk juice is through cleaning and cutting of the harvested cane followed by extraction and concentration of the juice which is fermented and the ethanol produced distilled and dehydrated (Wu *et al.*, 2021). Cleaning of the harvested cane is normally done through the technology for dry cleaning which

has been adopted by most industries. This prevents dirt from entering the industrial process taking into consideration that the cost of sugar lost due to dirt should be higher than the cost of electricity used in the dry cleaning (Eliseu Nicula de Castro *et al.*, 2019).

2.4.1.3.5 Sweet Sorghum

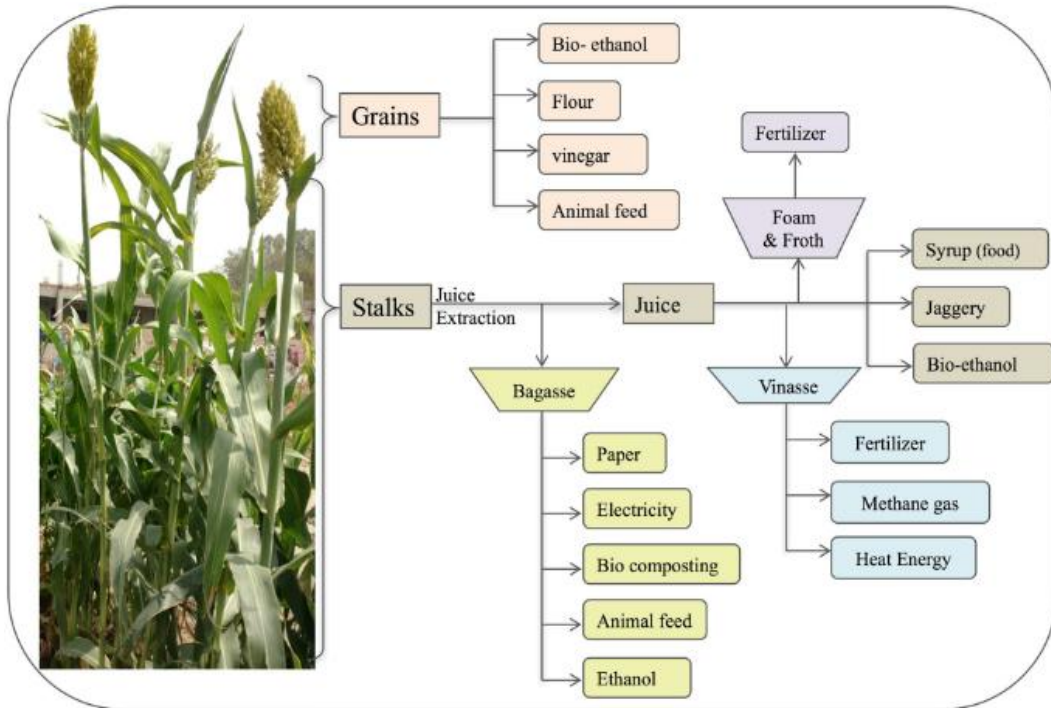


Figure 2.5: Sweet sorghum plant and illustration of its uses (Mathur *et al.*, 2017).

Sweet sorghum is a grass plant whose stalk contains sugar-rich juice that is similar to that of sugarcane. The juice consists of a mixture glucose, fructose and sucrose whose ratio vary from one variety to another. In addition to the stalk juice, the crops also produce grains, therefore offers a solution to the debate of food versus fuel since the grains are used for human consumption or as animal feed (Umakanth *et al.*, 2018). The juice can be extracted from the stalks and the sugars directly fermented to produce bio-ethanol. Fermentation reaction is a two-step mechanism where the first step involves breaking down of sucrose (1) to glucose (2) and fructose (3) as shown in equation (1) (Kehlbeck *et al.*, 2014).

The second step involves the conversion of the glucose (2) obtained into ethanol (4) and carbon (iv) oxide gas (5) as shown in equation (2)

(1)

(2)

The bagasse that remains after squeezing off the juice can be used to generate heat as fodder by burning or used to generate biogas through anaerobic digestion. Sorghum efficiently undertakes photosynthesis and can also utilize the soil nutrients. Under high temperatures of 25-30 °C the crop matures faster after a short period of time. It can withstand saline, water logging and drought conditions. Moreover, the utilization of radiation, water and nitrogen by sorghum is highly efficient as compared to sugar beet, corn and sugarcane. (Ratnavathi *et al.*, 2011). Sweet sorghum therefore is a suitable crop for bio-ethanol production because it requires minimal water and inputs as compared to sugarcane. Bio-ethanol from sweet sorghum can help reduce the over dependence on fossil fuels leading to reduction of green gas emissions. The reduction of CO₂ in the atmosphere when using sweet sorghum stalk juice for bio-ethanol production occurs in two main ways. To start with, during its growth through photosynthesis, it captures the atmospheric

CO₂ converting it to bio-mass. Secondly, when bio-ethanol is used as a renewable fuel, there is lower net CO₂ emissions because the CO₂ released during fermentation and combustion of bio-ethanol is offset by the CO₂ absorbed by the plant during growth. This creates a more sustainable, carbon-neutral energy cycle compared to fossil fuel use (Abdulkadeem *et al.*, 2022). Sweet sorghum being a multipurpose crop has a potential to improve the food security in Kenya by provision of food and feed (Oyier *et al.*, 2017). In developing countries like Kenya, growing of sugarcane as a cash crop for generating bio-ethanol complicates the food security situation since the cash crop competes unfavourably with the food crop for the arable land. Therefore, there is need to use a multipurpose crop like sweet sorghum for bio-ethanol production. Even though ethanol yield per unit weight of feedstock is lower for sweet sorghum juice when compared to sugarcane as reported by Abdulkadeem *et al.*, (2022), there is still a competitive cost advantage of using sweet sorghum for bio-ethanol production which includes low production cost and low water requirement.

2.5 °Brix of the Stem Juice

This is an analysis normally done to validate sampling strategies in relation to the ideal harvest time hence the °Brix should be determined in a reliable and proper way. The concentration of sugar which is represented by °Brix increases from the lower internodes with the highest concentration at the middle-third internodes and this trend decreases at the upper internodes. Since it is the middle-third portion of the stem that has the highest sugar concentration it is the part that is normally sampled for the °Brix determination (Teixeira *et al.*, 2017). The ideal harvest time corresponds to the time when the °Brix is highest since this outlines the time when the sugar concentration is highest in the juice. However, it is expected that when juice is squeezed out of the whole stalk the sugar concentration in the resultant juice will be lower than

that of the middle-third internodes because lower concentrations in the upper stem portions causes the dilution (Teixeira *et al.*, 2017). Since sweet sorghum is a multipurpose crop, it is therefore important to determine the best harvesting stage to ensure that the quality grains and fuel is obtained which is important to poor farmers who can use the grains as food source. The knowledge of the best harvesting stage can enable farmers know when to harvest their sorghum crop in relation to their economically important parts with the end result geared towards realization of maximum benefit (Oyier *et al.*, 2017).

2.6 Yeasts in Bio-ethanol Production

Yeasts are eukaryotic, single-celled natural living micro-organisms that belong to the fungus kingdom (Baghban *et al.*, 2019). They have the ability of reproducing by budding or fission forming spores that are not enclosed in a fruiting body. They can be isolated from aquatic, terrestrial and aerial environment. Pretscher *et al.*, (2018) reported that the major habitat of yeast is plants. The use of yeast in ethanol production reduces the cost of distillation which results in high ethanol yield. The most commonly used yeast is *Saccharomyces cerevisiae* in production of ethanol since it can withstand a wide pH range. When the temperature of the reaction is increased, the rate at which yeasts grow also increases until an optimum value is reached. Yeast strains that can withstand high temperature and ethanol concentration can be isolated from natural resources like plants, soil, water and animals. Yeast strains from natural sources are thermotolerant and ethanol-tolerant because through natural selection their cells adapt to their environment with time (Mohd Azhar *et al.*, 2017). In addition, wild-type yeasts could have more efficient potential to ferment sugars to ethanol than the commercial yeast strains as reported by Scordia *et al.*, (2012) in the fermentation of giant reed (*Arundo donax* L.) hemicelluloses using a native xylose fermenting yeast.

2.6.1 *Saccharomyces cerevisiae*

Saccharomyces cerevisiae has been used in alcohol production mainly in the wine and brewery industries since it keeps the cost of distillation low and gives a high yield of ethanol. However, increase in temperature, ethanol concentration, bacterial contamination and osmotic stress are the main reasons why *Saccharomyces cerevisiae* cannot survive during fermentation. Increase in temperature leads to an increase in the reaction, meaning that ethanol concentration also increases. This is not good for the growth of *Saccharomyces cerevisiae* since they cannot thrive in a media with very high levels of alcohol resulting to inhibition of ethanol production. The other limitation of *Saccharomyces cerevisiae* is that it cannot ferment pentoses only hexoses (Mohd Azhar *et al.*, 2017).

2.6.2 Finger Millet Malt and Sorghum Malt as Sources of Enzyme for Bio-ethanol Production

Finger millet (*Eleusine coracana*) and sorghum (*Sorghum bicolor*) are grass crops that produce seeds which are harvested for human food and animal feed. They are grown majorly in the semi-arid tropics of Asia and Africa (Ceasar *et al.*, 2018). In Kenya these crops are mainly grown in the warm, humid areas of Kericho, Kisii, Bungoma, Busia and Siaya. They are mainly used as major ingredients in the traditional manufacture of malt in East Africa.

Malting of finger millet and sorghum for use as a major source of hydrolytic enzymes (mainly amylases) that convert starch in the sweet sorghum stalk juice into fermentable sugars, for brewing purposes has received greater attention since time immemorial. For decades, the indigenous breweries in Eastern Africa have sustainably utilized the finger millet malt and sorghum malt as a yeast source. The use of malted cereals in brewing is advantageous since they provide amino acids required by yeast during fermentation, good buffering capacity and a balanced sugar profile (Amadi *et al.*, 2022). Finger millet malt contains various enzyme which

can aid in breaking down complex sugars and proteins in the sweet sorghum juice. This enzymatic activity is beneficial because it enhances fermentation efficiency, potentially reducing the need for additional enzyme or additives (Amadi *et al.*, 2022). The chemical composition of finger millet and sorghum have been shown to be similar and are therefore expected to exhibit similar malting performance when the brewing varieties of both cereals are carefully selected. This shows that these two grains share comparable proportions and types of key nutrients and biochemical compounds. These similarities can occur in their macronutrients (carbohydrates, proteins and fats), micronutrients (vitamins and minerals) and other bioactive compounds (like antioxidants). A study carried out by Van Khle *et al.*, (2001) on samples of dry yeast from sorghum beer indicated that they consisted of *Saccharomyces cerevisiae* strains almost exclusively. Lyumugabe *et al.*, (2014) also reported that *Saccharomyces cerevisiae* was the dominant yeast in all the stages of fermentation of *ikigage* (a Rwandese traditional sorghum beer). In addition, a wide variety of yeasts having varied characteristics were also isolated by Jane *et al.*, (2015) from a traditional opaque beer made of finger millet malt. The predominant yeast in the study was *Saccharomyces cerevisiae*. These studies show that the major yeast in sorghum malt and finger millet malt is *Saccharomyces cerevisiae*. However, malted finger millet is considered superior to malted sorghum by the local malt manufacturers who maintain that it has a better activity and flavour (Taylor, 2018). Among the millets, finger millet is superior and is ranked second after barley. This indicates that finger millet malt can be used to partly substitute barley which could greatly help in saving a substantial amount of foreign currency (Karki & Kharel, 2012). Commercial sale of finger millet by farmers will financially boost people living in rural areas who produce this crop (Usai *et al.*, 2013). Even though studies on malting of different varieties of millet and sorghum and the predominant yeast within their malts

have been conducted, information on the kinetic parameters of the fermentation of sweet sorghum stalk juice using finger millet malt and sorghum malt is scanty.

2.7 Factors Affecting Bio-ethanol Production

2.7.1 pH

The pH of the broth affects yeast growth, bacterial contamination, by-product formation and fermentation rate hence directly influences ethanol production. The ability of some nutrients to reach the cells is determined by the concentration of hydrogen ions (H^+) in the fermentation broth. The survival and growth of yeasts is directly influenced by pH as indicated by Lin *et al.*, (2014), who showed that when *Saccharomyces cerevisiae* is used, optimum fermentation pH range of 4.0-5.0 is required keeping in mind that beyond this range by-products like butyric acid and acetic acid may consume some of the substrate thereby reducing the efficiency of ethanol production. Wu *et al.*, (2017) also reported that a pH of 4.0 might maintain continuous ethanol production while a pH below 4.0 requires a longer incubation period without significantly reducing the concentration of ethanol. However, pH above 5.0 leads to substantial reduction of the amount of ethanol produced. Therefore, this work sought to establish the pH under which the three enzyme sources had their maximum activity.

2.7.2 Time

The growth of microorganisms is directly affected by fermentation time. Insufficient fermentation is caused by a shorter fermentation time due to inadequate growth of microorganisms. On the contrary, when fermentation takes a longer time, the microorganisms become intoxicated mostly in batch fermentation mode mainly caused by high concentration of ethanol in the fermentation broth. When fermentation is carried out within a longer time at a lower temperature, complete fermentation occurs. Wang *et al.*, (2008) stated that optimum ethanol production can be achieved when fermentation is allowed to take place for a long time at

a temperature below 34 °C. When time is increased at high temperatures ethanol production increases, but when temperature goes higher than 34 °C, ethanol production decreases. Since the duration of fermentation of sweet sorghum stalk juice directly influences the amount of bio-ethanol produced, there should be a balance between maximizing bio-ethanol production and maintaining the viability and health of the yeast cells. Therefore, this sought seeks to find the optimal fermentation period where the yeast cells were most efficient.

2.7.3 Temperature

Yeast cells show lower specific growth rates at lower temperatures due to their low tolerance to ethanol. According to Lin *et al.*, (2014), the specific rate of growth of yeast cells occur when the temperature is between 30-45 °C. As the temperature increases, the maximum fermentation time reduces, however, the cells growth is inhibited at a much higher temperature of about 50 °C leading to reduced fermentation rate. The transport activity or saturation level of soluble compounds and solvents in the cells changes with high temperatures which might increase the accumulation of toxins including ethanol inside the cell. In addition, ribosomes and enzymes become denatured at high temperatures (Imtiaz *et al.*, 2013). As the temperature increases, the viability of yeast cells decreases mainly because at higher temperatures there is accumulation of intracellular ethanol which produces cell toxicity and alteration of membrane structure occurs which decreases their functionality. In addition, it is worth noting that lower temperatures can slow down fermentation but may result in a higher yield of ethanol and fewer by-products. However, some yeast strains are competitive over a large range of temperature than others as indicated by Poblet *et al.*, (2003). Therefore, fermentation temperature should be regulated carefully throughout the fermentation process as optimal temperature varies depending on the specific microorganism used and substrate being fermented. It is for these reasons that this work

sought to determine the optimal fermentation temperature of sweet sorghum stalk juice on using *Saccharomyces cerevisiae*, finger millet malt and sorghum malt.

2.7.4 Yeast to Substrate Concentration Ratio

The amount of yeast to substrate concentration affects bio-ethanol production. Higher yeast concentrations typically lead to faster fermentation rates and higher ethanol yields up to a certain point where substrate limitations or inhibition may occur (Jhariya *et al.*, 2021). Using too much yeast can also lead to increased competition for nutrients and substrate, potentially reducing ethanol production efficiency. A study by Luo *et al.*, (2014) also reported that maximum rate of ethanol production is achieved with a yeast to substrate ratio of 1 g/L. It is important to find a balance in this study to maximize ethanol yield while minimizing costs and potential side effects.

2.8 Fermentation Kinetic Parameters

The Michaelis-Menten equation is one of the best known models that helps in describing the enzyme kinetics. The hypothesis proposed a reaction where a substrate (S) reacts with an enzyme (E) to form an intermediary called enzyme-substrate (ES) complex, which in turn releases a product (P) regenerating the free enzyme or it can dissociate forming E+S as indicated in equation 3 (Bisswanger, 2017).

(3)

The equilibrium assumption is as outlined in equation (4)

(4)

$$\frac{[E][S]}{[ES]} = \frac{K_{-1}}{K_1} = K_d \quad (5)$$

Where K_d represents the dissociation constant. The second assumption is pseudo-steady-state hypothesis where the concentration of ES complex remains constant during the enzymatic reaction. This means that [ES] formation is equal to [ES] breakdown generated from equation (3) to form equation (6)

$$K_1[E][S] = K_{-1}[ES] + K_{cat}[ES] \quad (6)$$

$$K_1[E][S] = [ES](K_{-1} + K_{cat})$$

$$\frac{[E][S]}{[ES]} = \frac{K_{-1} + K_{cat}}{K_1} = K_m \quad (7)$$

K_m is Michaelis Menten constant which indicates the enzyme affinity for the substrate, low K_m imply high affinities.

$$V_o = \frac{d[P]}{dt} = K_{cat}[ES] \quad (8)$$

where V_o is the velocity of the reaction which is the rate of product formation per unit time. The velocity V_o depends on the breakdown of ES complex. The velocity of the enzymatic reaction becomes maximum when all the active sites of the enzymes are occupied by the substrate, that is $E = 0$ therefore $E_o = ES$ which implies that $V_o = K_{cat}[ES]$ becomes the maximum velocity (V_{max})

$$V_{max} = K_{cat}[E_o] \quad (9)$$

The relationship $E_o = E + ES$ is taken to substitute E in equation (7)

$$K_m = \frac{[E_o - ES][S]}{[ES]}$$

$$K_m = \frac{[E_0][S] - [ES][S]}{[ES]}$$

$$K_m = \frac{[E_0][S]}{[ES]} - \frac{[ES][S]}{[ES]}$$

$$K_m = \frac{[E_0][S]}{[ES]} - \frac{[S]}{1}$$

Since $E_0 = \frac{V_{max}}{K_{cat}}$ as indicated in equation (9)

$$K_m = \frac{V_{max}[S]}{K_{cat}[ES]} - \frac{[S]}{1} \text{ but } V_o = K_{cat}[ES] \text{ as indicated in equation (8)}$$

$$K_m = \frac{V_{max}[S]}{V_o} - \frac{[S]}{1} \text{ add [S] on both sides}$$

$$K_m + [S] = \frac{V_{max}[S]}{V_o} \text{ multiply both sides by } \frac{1}{V_{max}[S]}$$

$$\frac{1}{V_o} = \frac{K_m + [S]}{V_{max}[S]}$$

$$V_o = \frac{V_{max}[S]}{K_m + [S]} \quad (10)$$

This is the Michaelis-Menten equation that is used to determine K_m and V_{max} 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. However, maximum velocity (V_{max}) can only be achieved at infinite substrate concentration which is an asymptote. Therefore, to improve the accuracy of the (V_{max}) and K_m , the original nonlinear Michaelis-Menten equation is transformed to a linear Line Weaver-Burk equation (11)

$$\frac{1}{V} = \frac{K_m}{V_{max}} \times \frac{1}{[S]} + \frac{1}{V_{max}} \quad (11)$$

By plotting $1/V$ against $1/[S]$ (see equation 11) make it possible to obtain a straight line whose slope is equivalent to K_m/V_{max} with a y-intercept corresponding to $1/V_{max}$ (Jojoa-Unigarro & González-Martínez, 2022). The Michaelis-Menten constant (K_m) shows the affinity or strength of the binding between the substrate and the enzyme. The lower the K_m values the higher affinity of the enzyme towards the substrate, the more efficient the enzyme is at carrying out its functions at a lower substrate concentration.

2.9 Bio-ethanol as a Fuel for Domestic Cooking

Ethanol has been promoted as an alternative fuel for cooking which can replace charcoal and firewood since it can improve household cooking efficiency more so it has substantial health and environmental benefits. It is very efficient, clean and produces lower indoor air pollution as compared to firewood and charcoal (Mudombi *et al.*, 2018). According to Dioha *et al.*, (2012), the common liquid fuel used for domestic cooking is kerosene. This liquid fuel produces soot and greenhouse gases together with an unpleasant smell when blown out compared to ethanol that burns without producing smoke or an unpleasant smell when blown out. A global population of about 2.8 billion people have no access to clean cooking and still use sources of fuel that produce health hazards. A study carried out by Kiprop *et al.*, (2018) indicated that in sub-Saharan Africa 30 % of the population do not have access to clean cooking fuel. Based on the study, about 36 million Kenyans a majority of whom live in the rural areas still cook using firewood, charcoal and kerosene. Implementation of clean cooking creates employment opportunities to the youth and women in the rural areas This has a potential of reaching the poor in the rural areas since the sector requires lesser skilled work force that is locally available. Properties of ethanol such as calorific value, density, flame test and pH affect its efficiency. For example, determination of the pH of the ethanol produced gives an information on its acidity

hence shows whether it can be corrosive to the cooking appliances or not. Secondly, the amount of heat produced during the combustion process of a fuel which is known as its calorific value relates directly to the hotness of the flame produced (Ansar *et al.*, 2020).

There is little knowledge on the calorific value, pH, flame test and density of ethanol produced from sweet sorghum stalk juice. Therefore, there is need to determine these properties and compare with the approved properties of bio-ethanol shown in Table 2.1.

Table 2.1: Approved properties of bio-ethanol fuel

Properties	Values
Density (kg/m ³)	794
Flash point (°C)	13.0
Calorific value (MJ/kg)	30.0
Distillation temperature (°C)	75-80
pH	6.5-9.0

Adopted from (Nwufo *et al.*, 2016).

CHAPTER THREE

MATERIALS AND METHODS

3.1 Study Site

The experiment was carried out in Western part of Kenya at Jaramogi Oginga Odinga University of Science and Technology (Siaya town campus) experimental farm with geographical coordinates of 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 temperature range of 25-27 °C.

3.2 Sweet Sorghum Varieties

Five sweet sorghum varieties were used which included: IESV 92001 DL (V1), NTJ (V2), 15233 IESV (V3), 92008 DJ (V4) and IESV 92028 DL (V5). The five varieties were sourced from International Crops Research Institute for the Semi-Arid Tropics (ICRISAT), Nairobi Office-Kenya. These varieties had been identified by an ongoing project on which this study was superimposed. They were the only sweet sorghum varieties that were under development at that time from ICRISAT and had not been introduced to farmers, the main reason why they were represented using codes as indicated.

3.3 Plant Materials, Experimental Design and Juice Extraction

The selected 5 sweet sorghum varieties were planted during the short rainy season of September-December 2021. The experimental design was randomized complete block design (RCBD) with three replications of each variety planted in a plot size of 6 m long by 4.2 m wide (25.2 m²). The spacing was 60 cm by 20 cm. The five sweet sorghum varieties were randomly assigned to the experimental units within a block where each variety only appeared once in every block. Each block had a similar structure but the order of the treatments within the blocks was randomized to reduce bias (Shieh & Jan, 2004). Cultural practice such as application of Diammonium Phosphate (DAP) fertilizer on planting was also carried out. The recommended fertilizer

application rate used by the local farmers was implemented. Weeding and disease control were done to obtain maximum stalk yield. On the 11th week after planting, the immediate post-anthesis week, harvesting was done manually in triplicates, where the sweet sorghum plants were selected randomly, their leaves stripped and panicles cut, thereafter, the °Brix of the juice was determined. This procedure was repeated after every 7 days up to week 16. V1 had the highest °Brix at week 15. It was therefore bulked the following season and the juice extracted at the 15th week from the cut stalks using electrical stalk juice crushers and was held in a freezer until further analyses. *Saccharomyces cerevisiae* was obtained from a local market.

3.4 Determination of °Brix

°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 (Teixeira *et al.*, 2017).

3.5 Preparation of Finger Millet Malt and Sorghum Malt

The finger millet malt and sorghum malt were prepared according to Amadou *et al.*, (2011) through traditional malting processes that involved three main steps which were soaking, germination and drying. Soaking was done to help awaken the dormant grains by immersing them in water for 24 hours. The naturally existing enzymes in the grains were activated by the absorbed water which later stimulated the grains to develop new enzymes that helped in seed growth. Excess water was then drained from the soaked grains to prevent growth of fungus which could occur during germination if the grains were too wet. Secondly, the soaked grains were placed on green banana leaves inside an aerated container and kept for 96 hours at room temperature to allow the grains to enter the growth phase and develop the enzymes required for brewing. The green banana leaves ensured that the grains germinated in moist air which

promoted the development of hydrolytic enzyme normally inactive in the raw grains. Germinated grains were then sun dried to reduce moisture content and prevent further germination. The rootlets and shoots were then removed as suggested by Baranwal, (2017). Thereafter, the dry kernels were ground into homogenous powder to help allow increased surface area for the reaction.

3.6 Optimal Production of Bio-ethanol

The optimal fermentation parameters were achieved using a design of experiments where a scheme of experiments in different conditions was developed using Taguchi experimental design (Minitab 18.1.0.0 software).

3.6.1 Experimental Design Array

The Taguchi method 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 (Ghosh & Mondal, 2019). The design was preferred over one factor at a time traditional technique for optimization that involves numerous trials hence takes a lot of time and resources while not allowing the study of interaction between various variables (Dhawane *et al.*, 2016). The representation of Taguchi orthogonal array is $L_a(Q^b)$ where 'a' denotes the number of experimental runs, 'Q' is the number of levels and 'b' is the number of factors being optimized.

A four level ($L_{16}(4^4)$) Taguchi orthogonal array design of experiment with a total of 16 experimental runs was developed in Minitab 18.1.0.0 software. Table 3.1 show the levels of chosen independent factors. Levels of each factor are specific settings or conditions that are tested.

Table 3.1: Levels of chosen independent factors in the L₁₆ (4⁴) Taguchi design of experiments

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

The L₁₆(4⁴) experimental design matrix used in the optimization study is shown in 3.2.

Table 3.2: The L₁₆(4⁴) experimental design matrix

Exp. No.	Temperature (°C)	pH	Time (hrs)	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

3.6.2 Analysis of variance (ANOVA)

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 (Karmakar *et al.*, 2018).

The signal to noise ratio (S/N) was used to measure the quality characteristics deviating from the desired value. Based on the S/N ratio, it is possible to get the optimum level of the individual

process parameters providing the highest yield of bio-ethanol. In this study, “larger is better” S/N ratio, formula shown in equation (12), was selected to attain maximum yield of bio-ethanol.

$$S/N = -10 \times \log_{10} \left(\sum_{j=1}^N \frac{\left(\frac{1}{y_j^2}\right)}{n} \right) \quad (12)$$

where y_j is the mean value of response (bio-ethanol yield), j is the trial number and n is the number of repetitions of each experiment. The term $\left(\sum_{j=1}^N \frac{\left(\frac{1}{y_j^2}\right)}{n} \right)$ is the mean square deviation.

To identify the factor with the most significant effect on the bio-ethanol yield and the response magnitude, statistical analysis of variance (ANOVA) of the response data was used. The basic property in ANOVA is that the total variation is equal to the sum of the squares of the deviations (SS) of all the condition parameters and the error components (Kumar *et al.*, 2015). The percentage of contribution of the factors was evaluated using equation (13).

$$\text{Contribution factor (\%)} = \frac{\text{sum of squares of } f^{\text{th}} \text{ factor}}{\text{total sum of squares}} \times 100 \% \quad (13)$$

3.7 Fermentation

Fermentation was carried out using the 16 experimental runs generated from Taguchi experimental design shown in Table 3.2. In the process, 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 using 0.5 M sodium hydroxide solution or 0.5 M dilute sulphuric (vi) acid. Yeast source 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. A 0.005 g/100 mL of ammonium phosphate $[(\text{NH}_4)_3\text{PO}_4]$ and 0.001 g/100 mL magnesium sulphate penthydrate

(MgSO₄·7H₂O) were added as nutrients into the flasks (Ahmad *et al.*, 2018). The samples were then placed in a water bath at different temperatures which were 30 °C, 35 °C, 40 °C and 45 °C and the duration of fermentation was also varied at 36, 48, 60 and 72 hours to allow fermentation to take place. The volume of carbon (iv) oxide gas was measured in cm³ thereafter converted to moles. Finally, the moles of carbon (iv) oxide obtained was used to calculate number of moles of ethanol produced with the help of the mole ratio. Each experiment was repeated thrice in order to minimize error and a control which was fermentation at room temperature done in triplicates was subtracted from the experimental values. The response from each combination was analyzed statistically using Minitab 18.1.0.0 software. The optimum fermentation conditions obtained after using the 3 enzyme sources were used in the kinetic analysis to determine their efficiencies.

3.8 Determination of Fermentation Kinetic Parameters

In this study, the measurement of fermentation kinetic parameters was determined using the amount of product; ethanol, since it was a measurable reaction parameter. The substrate was loaded at varying concentrations with a constant enzyme source load to initiate the fermentation reaction and the amount of product at different time intervals recorded. Calculation of the initial velocity (V_o) values was done by graphically plotting amount of product versus time data using linear regression R (Cho & Lim, 2018). For each [S], the slope obtained from the regression with the largest R^2 value was selected to indicate the velocity of the reaction. After the V_o values were obtained, additional manipulation was performed where the reciprocal of V_o and [S] was determined. By plotting $1/V_o$ against $1/[S]$ according to Jojoa-Unigarro & González-Martínez, (2022), a straight line was obtained with a slope equal to K_m/V_{max} and an intercept corresponding to $1/V_{max}$ from this K_m and V_{max} were calculated. The source with the most efficient enzyme or

group of enzymes was used to produce bio-ethanol whose physico-chemical properties were determined.

3.9 Characterization of Bio-ethanol

The calorific value, pH, density and flame test of the bio-ethanol obtained were determined as follows:

3.9.1 Calorific Value

A 1 mL of bio-ethanol was put into a dry crucible then placed inside the bomb and a fuse placed in contact with ethanol. The bomb was filled with oxygen to a pressure of 25 kg/cm². The calorimeter was filled with water and the bomb placed inside it. The water was placed in such a way that the bomb was covered completely. The initial temperature of water was noted then the fuel was ignited by pressing the fire button. The water was stirred continuously until the end of the experiment where the final temperature was noted. The calorific value was calculated according to Ozyuguran *et al.*, (2018) as shown:

Heat change = $mc\Delta T$ where: m- mass of water

c- specific heat capacity of water

ΔT - change in temperature

3.9.2 Determination of pH Value

pH was measured using a digital pH meter (BANTI 901-UK pH) that was calibrated using deionized water and buffer tablets of pH values 4.0 and 7.0. After calibration, the electrode tip was rinsed in deionized water then dipped in the samples to enable pH measurement that were made after the readings became stable and done in triplicate (Efunwoye & Oluwole, 2019).

3.9.3 Density

Density was measured using a hydrometer which was dipped in the liquid bio-ethanol produced. Through this method, the hydrometer floats higher in liquids with high density and lower in liquids with low density as reported by Troncoso, (2021). This was done three times and the readings taken then average calculated.

3.9.4 Flame Test

A 2 cm³ of the bio-ethanol produced was placed in a watch glass then ignited according to Ansar *et al.*, (2020). The colour of the flame was recorded.

3.10 Statistical Analysis

One-way analysis of variance was used in the determination of significant differences of the °Brix within the selected varieties and stages of growth. The output of fermentation with the yeasts was organized and analysis of variance (ANOVA) was done using Minitab 18.1.0.0, Origin Lab 9.80.200 and Image J software.

CHAPTER FOUR

RESULTS AND DISCUSSION

4.1 Quantification of the °Brix 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 Table 4.1. 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. 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 as in Figure 4.1.

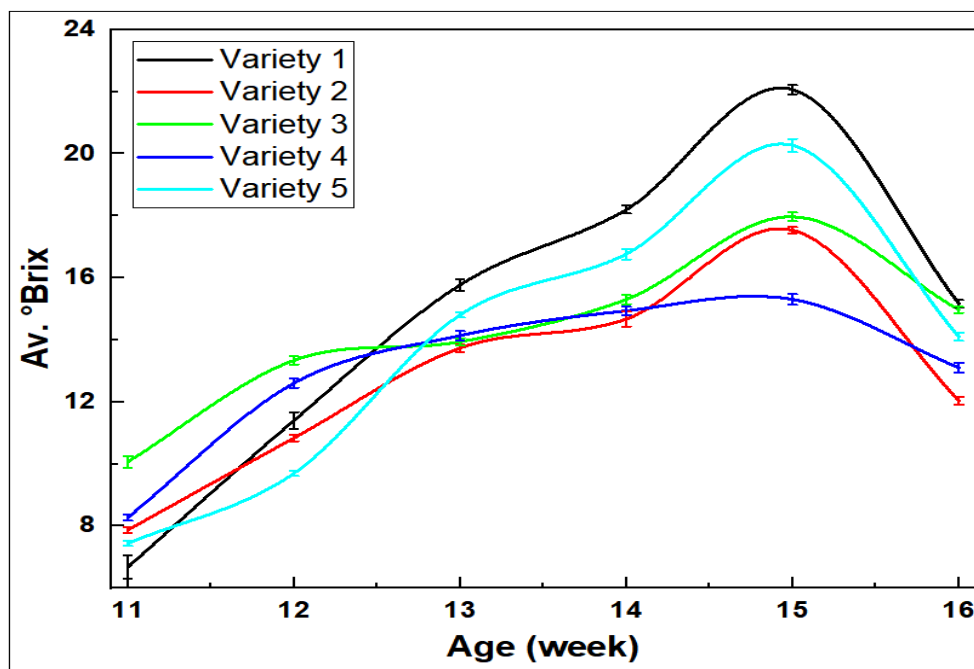


Figure 4.1: Trend of °Brix content per variety with age

Table 4.1: Mean °Brix comparison among five sweet sorghum varieties

Variety	Week 11	Week 12	Week 13	Week 14	Week 15	Week 16
V1	6.67c	11.4bc	15.77a	18.20a	22.07a	15.17a
V2	7.86bc	10.83cd	13.73a	14.67c	17.53b	12.03a
V3	10.07a	13.33a	13.93a	15.30bc	17.97b	14.97a
V4	8.27b	12.60ab	14.13a	14.93bc	15.30c	13.10a
V5	7.43bc	9.70d	14.80a	16.77ab	20.26a	14.10a
LSD (P≤0.05)	1.3993	1.4546	2.5124	1.9426	1.9125	3.6454
CV %	18.22	13.20	18.22	12.76	10.78	27.58
P-value	0.0003	<.0001	0.4819	0.0030	<.0001	0.3884

Values with different letter(s) within columns are statistically different according to LSD at (P≤0.05).

The results indicate that the rate of accumulation of sugar increased towards maturity as shown (Figure 4.1 and Table 4.1). 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 (Gutjahr *et al.*, 2013). At this particular stage the invertase enzyme catalyzes the conversion of sucrose obtained from photosynthesis into glucose and fructose for respiratory energy and cell wall synthesis in young and rapidly growing internode. 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 might be caused by reduced photosynthesis and remobilization of carbohydrates from the stalks to the grains in the final grain filling stage (Kawahigashi *et al.*, 2013).

At week 11 variety V3 had a significantly higher °Brix of 10.07 (P≤0.05). A study carried by Davila-Gomez *et al.*, (2011) 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, (2020) 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 15th week of growth (105 days after sowing). This was higher than the °Brix obtained by Teixeira *et al.*, (2017) 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 factors (like availability of water, nutrient content, temperature) that support productivity of the crop. According to Li *et al.*, (2019), 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.*, (2017) and Appiah-Nkansah *et al.*, (2019) who stated 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. Burks *et al.*, (2013) also stated that to ensure that maximum sugar yield is obtained in sweet sorghum, the optimum harvest time should generally be at 30 days after anthesis. This is in agreement with the results of this study since the maximum sugar concentration was at 105 days after planting which occurred 28 days after anthesis. The outcomes of this study are in line with earlier reports of Gutjahr *et al.*, (2013) who reported that increase in sucrose concentration within

the stems of sweet sorghum occurred slightly before panicle initiation stage. This continues 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.

4.2 Optimizing Fermentation Conditions for Sweet Sorghum Stalk Juice

The factors that affect ethanol productivity that included pH, temperature, yeast to substrate ratio and time taken for the fermentation reaction to occur were controlled in order to obtain high yields of bio-ethanol.

4.2.1 Optimizing Fermentation Conditions Using *Saccharomyces cerevisiae*

The $L_{16}(4^4)$ experimental design matrix used in the optimization of fermentation of sweet sorghum stalk juice using *Saccharomyces cerevisiae* and response values (bio-ethanol yield), predicted bio-ethanol yield, S/N ratio are shown in Table 4.2.

Table 4.2: The $L_{16}(4^4)$ experimental design matrix with bio-ethanol yield, standard deviation values and S/N ratio obtained using *Saccharomyces cerevisiae*

Experiment trial	Reaction temp (°C)	pH	Reaction time (h)	Yeast: Substrate (g/50 mL)	Avg. Bioethanol yield (mol)	Std Deviation ($\times 10^{-3}$)	S/N Ratio	Predicted Bioethanol yield (mol)
1	30	4	36	0.05	0.0069	0.4786	-43.309	0.0071
2	30	5	48	0.15	0.0122	2.0604	-38.510	0.0138
3	30	6	60	0.25	0.0178	1.1356	-35.016	0.0157
4	30	7	72	0.35	0.0141	0.8242	-37.075	0.0144
5	35	4	48	0.25	0.0100	0.8933	-40.091	0.0103
6	35	5	36	0.35	0.0176	1.5372	-35.172	0.0154
7	35	6	72	0.05	0.0049	0.8215	-46.389	0.0065
8	35	7	60	0.15	0.0135	1.3893	-37.461	0.0138
9	40	4	60	0.35	0.0044	0.8185	-47.540	0.0059
10	40	5	72	0.25	0.0062	0.7304	-44.285	0.0065
11	40	6	36	0.15	0.0048	0.2704	-46.382	0.0052
12	40	7	48	0.05	0.0041	0.8569	-48.160	0.0019
13	45	4	72	0.15	0.0005	0.0784	-65.522	0.0016
14	45	5	60	0.05	0.0013	0.0807	-58.070	0.0016
15	45	6	48	0.35	0.0036	0.4483	-48.882	0.0039
16	45	7	36	0.25	0.0035	0.2831	-49.133	0.0051

Values of responses represented in Table 4.2 were used to generate mean of means and the S/N ratio. The mean of means serves as a baseline performance level before any factor optimization. It represents the overall system's response without any factor adjustments. On the other hand, the S/N curves shown in Figure 4.2 are the graphical representation of variation in factor levels with change in responses.

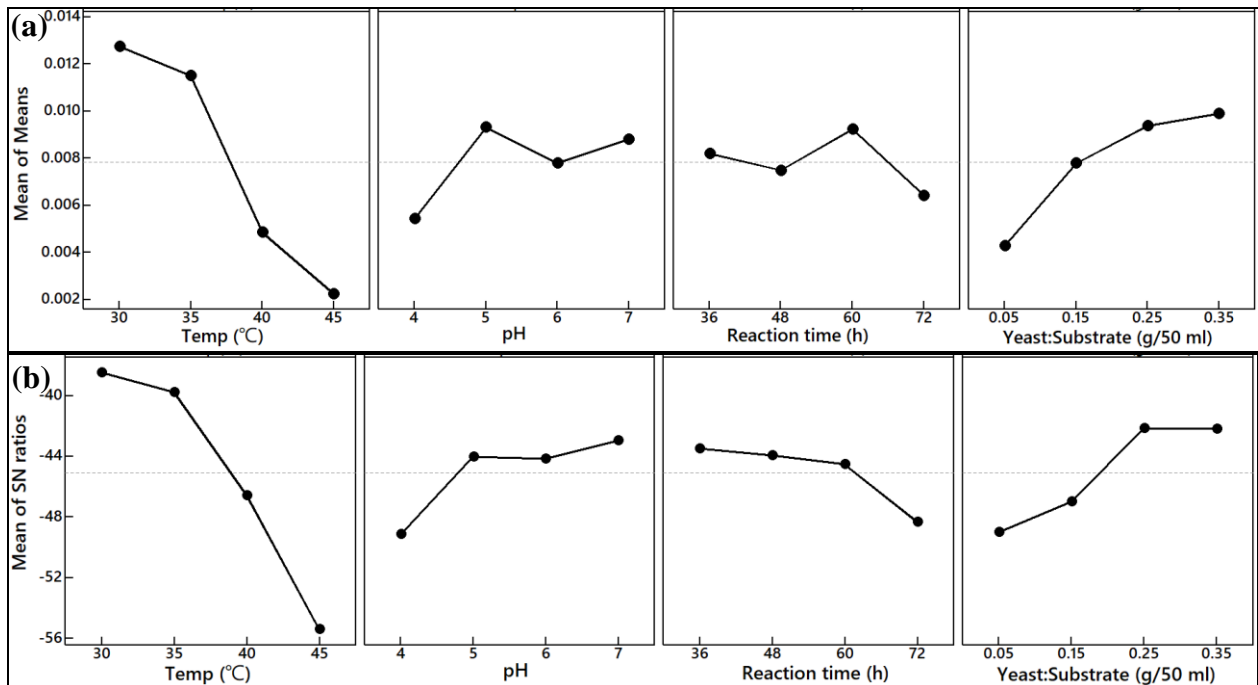


Figure 4.2: The main effects plot for data mean and S/N ratios for bio-ethanol yield using *Saccharomyces cerevisiae*

The main function of S/N ratio curves was to note the optimum levels of the fermentation parameters. In this study response was taken in one criterion “Larger is better” in which a higher S/N ratio corresponds to better quality so that the optimum level of process parameters is the level with the highest S/N ratio (Shehata & Abd, 2014). S/N ratio at four levels of a particular factor was taken, thereafter plots for means of S/N with respect to factor level were drawn. Figure 4.2 indicates that the factor that had the highest influence on sweet sorghum stalk juice fermentation using *Saccharomyces cerevisiae* was temperature since it had the highest mean. The highest yield was observed at a temperature of 30 °C. This was the optimum temperature as was illustrated also on Appendix 2. On the same note, optimum yeast to substrate ratio was 0.25 g to 50 mL of the substrate while pH 5 and fermentation duration of 36 hours were found to be the optimum pH and time respectively.

Ranking of the four fermentation parameters by the response of S/N ratios in Table 4.3 also shows that temperature had the highest influence on the fermentation followed by the amount of yeast to substrate ratio. At the third position was pH while reaction time was at the fourth position.

Table 4.3: Response Table for S/N Ratios on Using *Saccharomyces cerevisiae*

Level	Reaction temp (°C)	pH	Reaction time (h)	Yeast: Substrate (g/50 mL)
1	-38.48	-49.12	-43.50	-48.98
2	-39.78	-44.01	-43.91	-46.97
3	-46.59	-44.17	-44.52	-42.13
4	-55.40	-42.96	-48.32	-42.17
Delta	16.92	6.16	4.82	6.85
Rank	1	3	4	2

A plot of actual versus predicted yield of ethanol was used to test the accuracy of the model as shown in Figure 4.3. This is a plot of the experimental ethanol yield against the predicted yield. The closeness of the plotted points to the regression line coupled with a high R^2 values show that the model fits the experimental data well as reported by Berkane *et al.*, (2020) and further indicates that the actual response values agree well with the predicted response values. In addition, predicted R^2 was found to be 0.95 which is in reasonable agreement with the R^2 of 0.9999 and adjusted R^2 of 0.947. This gave a reconfirmation that there is a good agreement between the experimental and the theoretical values predicted by the model.

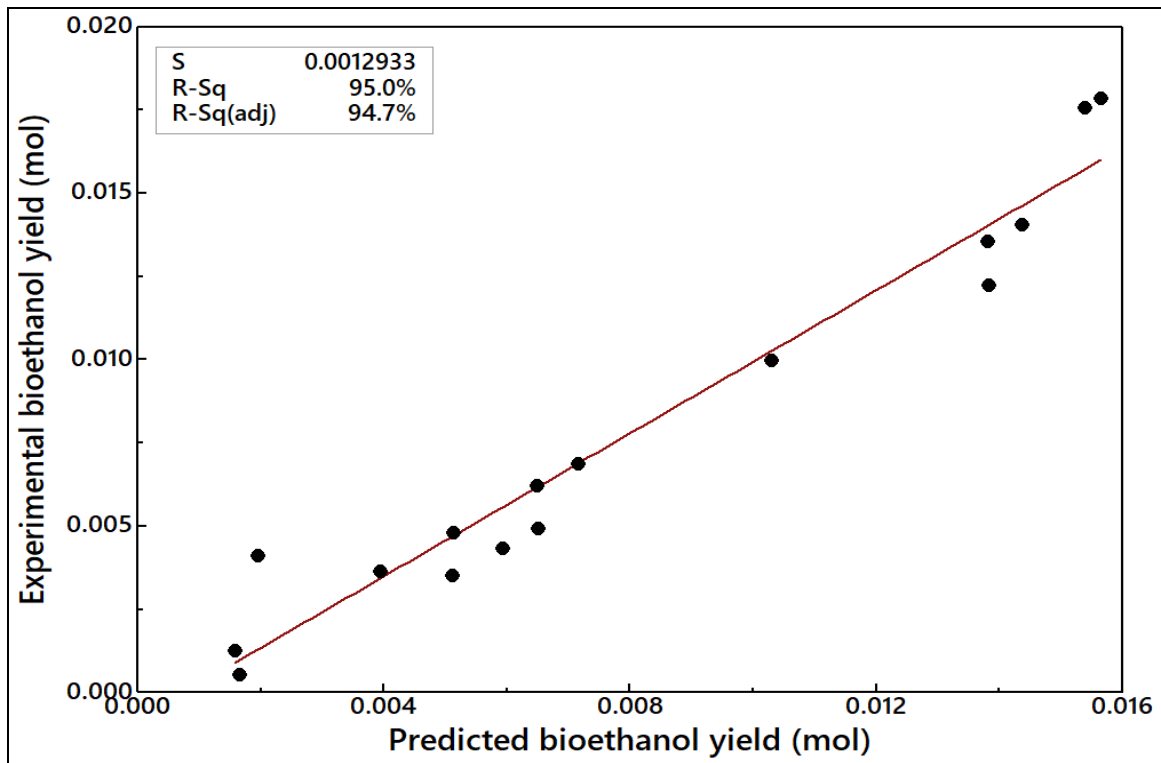


Figure 4.3: A plot of experimental bio-ethanol yield vs predicted bio-ethanol yield

4.2.1.1 Analysis of Variance (ANOVA) on Using *Saccharomyces cerevisiae*

Statistical analysis of variance (ANOVA) of bio-ethanol yield was conducted to find the fermentation parameters whose effects are statistically significant and their inputs on bio-ethanol yield on using *Saccharomyces cerevisiae* as the source of enzyme. The results of ANOVA in Table 4.4 show that the considered fermentation parameters were highly significant factors affecting bio-ethanol yield. From the Table 4.4 it was observed that temperature with contribution of 70.12 % was the most significant parameter influencing bio-ethanol yield followed by amount of yeast to substrate ratio with contributing percentage of 13.97 %. The other controlled parameters were pH and reaction time that recorded contributing percentages of 8.84 % and 5.69 %, respectively.

Table 4.4: Analysis of Variance for S/N ratios on Using *Saccharomyces cerevisiae*

Source	DF	Seq SS	Adj SS	Adj MS	F	P	%SS
Reaction temp (°C)	3	722.10	722.10	240.701	51.05	0.004	70.12
pH	3	91.07	91.07	30.358	6.44	0.080	8.84
Reaction time (h)	3	58.64	58.64	19.547	4.15	0.137	5.69
Yeast:Substrate (g/50 mL)	3	143.89	143.89	47.964	10.17	0.044	13.97
Residual Error	3	14.14	14.14	4.715			1.37
Total	15	1029.85					100.00

Even though the significance of each parameter was justified by the contribution factor, the Fitcher test (F-test) and probability value can still be used to reconfirm the significance of the process parameters. Devaiah *et al.*, (2018) stated that the change of the process parameter significantly affects the quality characteristics when F-value is high. Among the 4 controlled fermentation parameters temperature had the highest F-value of 51.05 and lowest p-value of 0.004. This reconfirmed that it was the most significant parameter that had the strongest effect on bio-ethanol yield.

4.2.2 Optimizing Fermentation Conditions Using Finger Millet Malt

The responses obtained after performing the experiments of fermentation of sweet sorghum stalk juice using finger millet malt following the $L_{16}(4^4)$ experimental design matrix designed by Taguchi method are tabulated in Table 4.5. It had S/N ratio, response values (bio-ethanol yield) and predicted bio-ethanol yield, are shown in Table 4.5

Table 4.5: The $L_{16}(4^4)$ experimental design matrix with bio-ethanol yield, standard deviation values and S/N ratio obtained using Finger millet malt

Experiment trial	Reaction temp (°C)	pH	Reaction time (h)	Yeast: Substrate (g/50 ml)	Avg. Bioethanol yield (mol)	Std 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 represent the 16 experimental runs generated by the Taguchi experimental design while the 6th and 9th columns are for the response of experimental bio-ethanol yield generated in the laboratory and the bio-ethanol yield predicted by the model, respectively. This experimental design matrix was used to generate the main effects plot where the S/N ratio was used to determine the optimum fermentation conditions as in Figure 4.4.

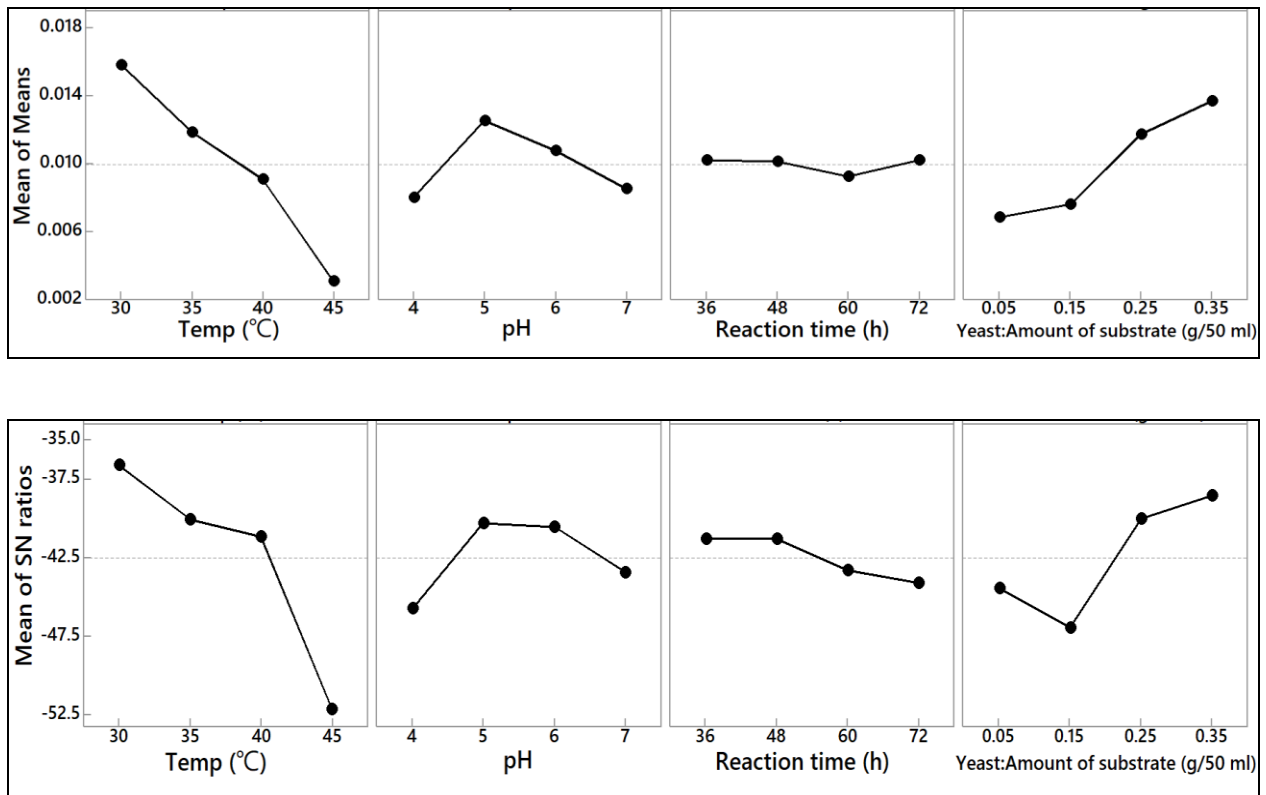


Figure 4.4: The main effects plot for data mean, and S/N ratios for the bio-ethanol yield

From the means and S/N ratio shown in Figure 4.4, it is evident that temperature had the strongest influence on sweet sorghum stalk juice fermentation. This was shown by a higher mean of S/N ratio under this control factor which indicated that it had 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 very low. The optimum conditions for the fermentation of sweet sorghum stalk juice using finger millet malt were found to be pH 5, malt to substrate ratio of 0.25 g to 50 mL of the substrate, fermentation time of 48 hours and temperature of 30 °C. Appendix 3 also illustrate that the optimum fermentation temperature was obtained to be 30 °C.

The ranking of parameters based on S/N ratios in Table 4.6 help in the identification of the factors with more significant influence on the fermentation reaction. The results show that

temperature had the highest influence on the bio-ethanol yield followed by yeast to substrate ratio, pH and finally the reaction time.

Table 4.6: Response Table for S/N Ratios on Using Finger Millet Malt

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 accuracy of the Taguchi model was tested using a plot of actual or experimental versus predicted yields of ethanol. This was done by a scatter plot of the actual yield values on the x-axis and the predicted yield values on the y-axis. The closeness of the plotted points to the regression line indicate a good predictive performance hence show the Taguchi model accurately predicted the yield under the tested conditions as illustrated in Figure 4.5. The actual R^2 and adjusted R^2 are statistical measures that were used to help assess the goodness of fit of the model to the experimental data. Since they were 0.921 and 0.915, respectively which were closer to 1 indicated a better fit. This showed that a larger percentage of variability in the response variable could be explained by the model and the capability of the model to predict the response was in acceptable range.

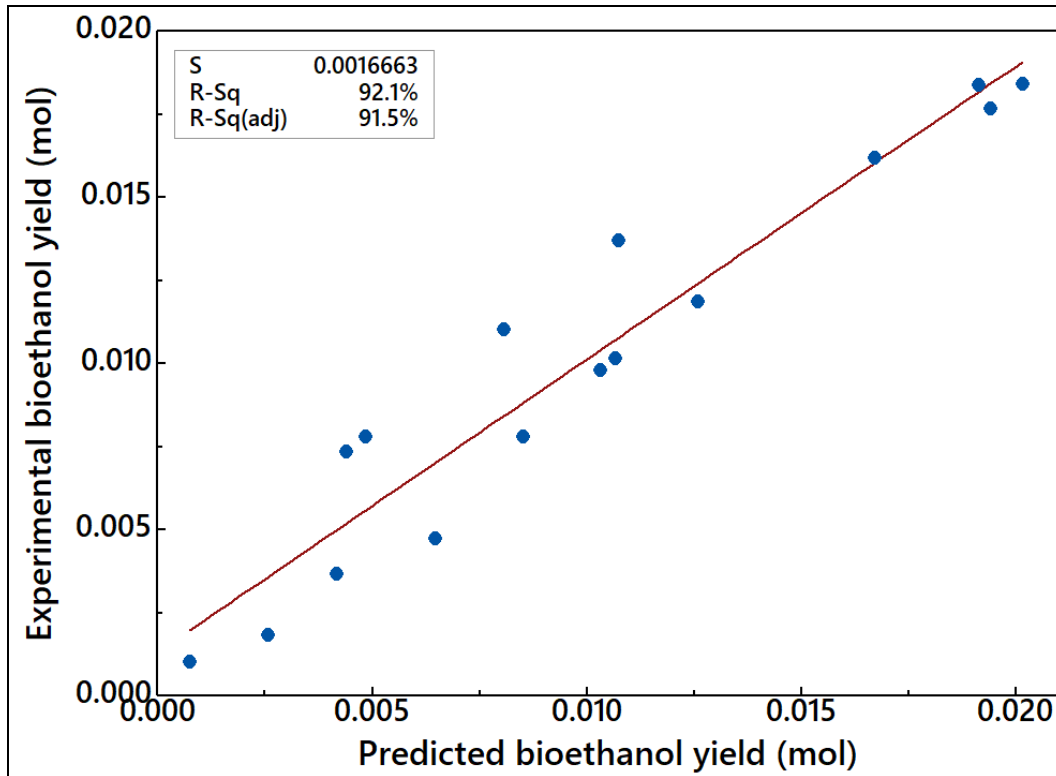


Figure 4.5: A plot of experimental bio-ethanol yield vs predicted bio-ethanol yield

4.2.2.1 Analysis of Variance (ANOVA) on Using Finger Millet Malt

Analysis of variance gives statistical significance of every fermentation parameter and their impacts on ethanol yield. The most significant parameter affecting bio-ethanol yield was recognized by the contribution factor for each parameter summarized in Table 4.7. From the Table it was observed that temperature with contribution of 63.22 % was the most significant parameter influencing bio-ethanol yield followed by malt to substrate ratio at 21.07 %, pH at 9.42 % and finally reaction time at 2.83 %. Amongst these four considered fermentation parameters, temperature had the highest F-value (18.27) and the lowest p-value (0.020). This further justifies that temperature was the most significant parameter in the fermentation of sweet sorghum stalk juice using finger millet malt.

Table 4.7: Analysis of Variance for S/N ratios on Using Finger Millet Malt

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

4.2.3 Optimizing Fermentation Conditions Using Sorghum Malt

On using sorghum malt as a source of enzyme in the fermentation of sweet sorghum stalk juice, Table 4.8 generated from Taguchi design show the $L_{16}(4^4)$ experimental design matrix used in the optimization of fermentation. It shows the experimental bio-ethanol yield (response values), S/N ratio and predicted bio-ethanol yield.

Table 4.8: The $L_{16}(4^4)$ experimental design matrix with bioethanol yield, standard deviation values and S/N ratio

Experiment trial	Reaction temp (°C)	pH	Reaction time (h)	Yeast:Substrate (g/50 ml)	Avg. Bioethanol yield (mol)	Std Deviation ($\times 10^{-3}$)	S/N Ratio	Predicted Bioethanol yield (mol)
1	30	4	36	0.05	0.0090	0.415	-40.972	0.0092
2	30	5	48	0.15	0.0124	0.082	-38.158	0.0112
3	30	6	60	0.25	0.0026	0.747	-52.594	0.0023
4	30	7	72	0.35	0.0017	0.494	-55.920	0.0030
5	35	4	48	0.25	0.0104	0.726	-39.694	0.0117
6	35	5	36	0.35	0.0144	1.260	-36.911	0.0141
7	35	6	72	0.05	0.0085	2.535	-42.454	0.0073
8	35	7	60	0.15	0.0042	0.185	-47.488	0.0044
9	40	4	60	0.35	0.0007	0.036	-62.981	0.0005
10	40	5	72	0.25	0.0051	0.375	-45.923	0.0053
11	40	6	36	0.15	0.0017	0.077	-55.223	0.0030
12	40	7	48	0.05	0.0048	0.651	-46.479	0.0046
13	45	4	72	0.15	0.0007	0.061	-63.540	0.0004
14	45	5	60	0.05	0.0024	0.258	-52.382	0.0037
15	45	6	48	0.35	0.0024	0.085	-52.579	0.0025
16	45	7	36	0.25	0.0047	0.243	-46.584	0.0035

The information in Table 4.8 (experimental design matrix) was used to determine optimum fermentation conditions through the generation of main effects plot as shown in Figure 4.6

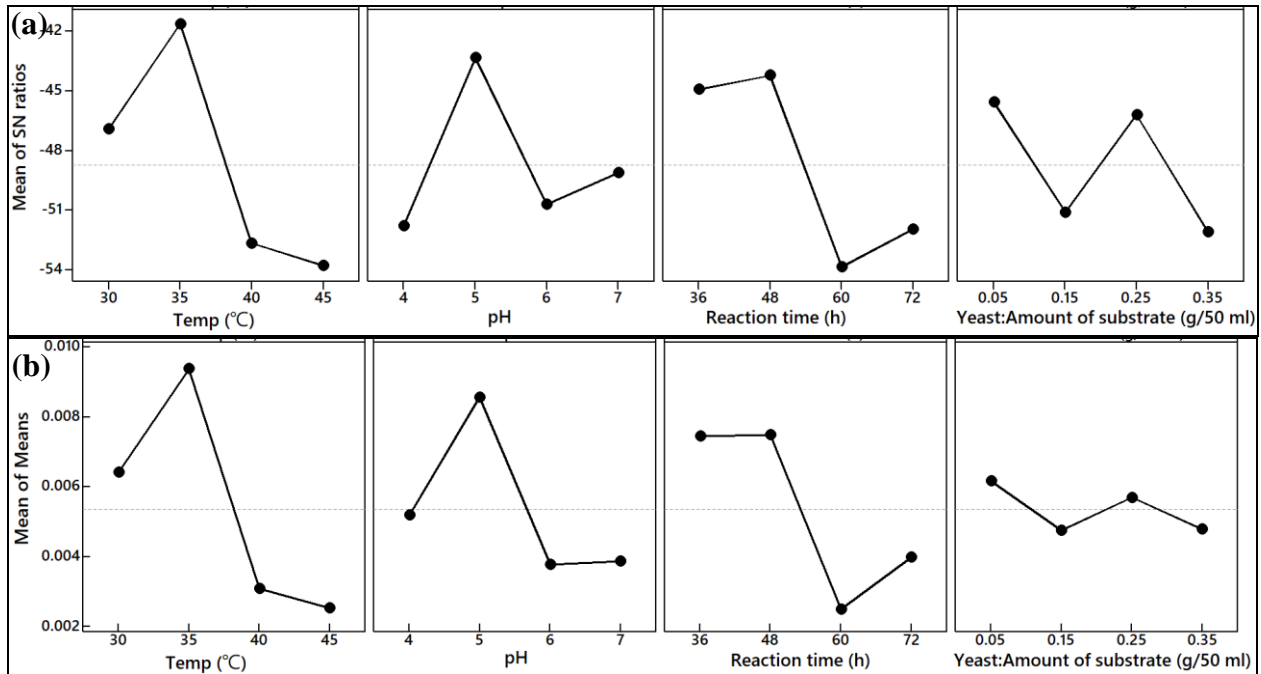


Figure 4.6: The main effects plot for (a) data mean, and (b). S/N ratios for the bio-ethanol yield

Temperature had the highest influence on the fermentation of sweet sorghum stalk juice using sorghum malt. This was shown by the high value of means depicted in the means of means and means of S/N ratios in Figure 4.6. The highest yield was exhibited at 35 °C. Therefore, 35°C was the optimum fermentation temperature while pH 5, fermentation duration of 48 hours and amount of malt to substrate ratio of 0.25 g to 50 mL of the substrate represented optimum fermentation pH, time and yeast to substrate ratio.

The influence of temperature is also shown to be highest in the ranking of parameters as indicated in Table 4.9. At the second position was fermentation time followed by pH and finally the amount of yeast to substrate ratio.

Table 4.9: Response Table for S/N Ratios on Using Sorghum malt

Level	Reaction temp (°C)	pH	Reaction time (h)	Yeast: Substrate (g/50 ml)
1	-46.91	-51.80	-44.92	-45.57
2	-41.64	-43.34	-44.23	-51.10
3	-52.65	-50.71	-53.86	-46.20
4	-53.77	-49.12	-51.96	-52.10
Delta	12.13	8.45	9.63	6.53
Rank	1	3	2	4

The accuracy of the model was tested by a plot of experimental bio-ethanol yield versus predicted yield. A high R^2 of 0.959 and $R^2(\text{adj})$ of 0.957 indicated that the model fitted the experimental data well as shown in Figure 4.7.

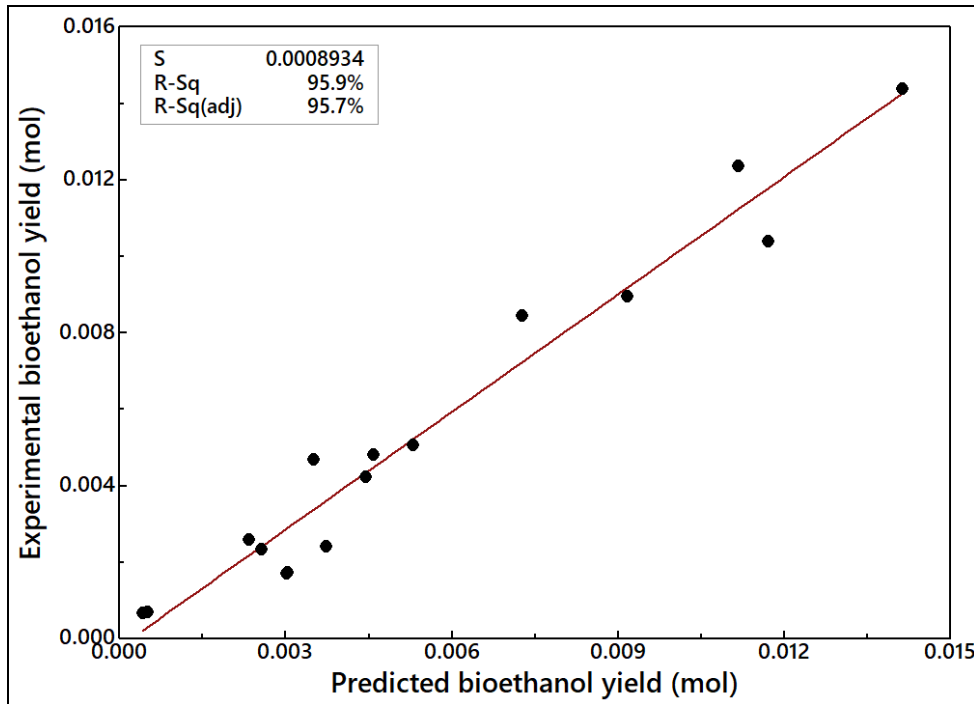


Figure 4.7: A plot of experimental bioethanol yield vs predicted bioethanol yield

4.2.3.1 Analysis of Variance (ANOVA) on Using Sorghum Malt

The analysis of variance (ANOVA) for the S/N ratios indicate the significance of every parameter and their input on the yield of bio-ethanol. Using the Fitcher test (F-test) the significance of each of the four controlled fermentation parameters was determined. When the experimental data fit well in the model then a high F-test value is obtained. From Table 4.10 the contribution from the fermentation temperature was more significant at 37.35 %. The contributions of time, pH, and amount of yeast to substrate ratio were 28.30 % 16.81 % 13.19 %, respectively. Temperature had the highest F- value of 8.59 and a corresponding low p-value of 0.055 confirming its highest significance among the four factors that were considered.

Table 4.10: Analysis of Variance for S/N ratios on Using Finger Millet Malt

Source	DF	Seq SS	Adj SS	Adj MS	F	P	%SS
Reaction temp (°C)	3	377.67	377.67	125.89	8.59	0.055	37.35
pH	3	170.00	170.00	56.67	3.87	0.148	16.81
Reaction time (h)	3	286.11	286.11	95.37	6.51	0.079	28.30
Yeast:Substrate (g/50 mL)	3	133.39	133.39	44.46	3.03	0.193	13.19
Residual Error	3	43.97	43.97	14.66			4.35
Total	15	1011.14					100.00

Optimization of fermentation conditions is a simple and effective way to economically produce bio-ethanol. The Taguchi method is an effective method for optimization of ethanol production by reducing the number of experiments and time. The results obtained in this study strongly indicate that temperature had the greatest significant influence on the fermentation of sweet sorghum stalk juice using *Saccharomyces cerevisiae*, finger millet malt and sorghum malt. In order to convert the sweet sorghum stalk juice into bio-ethanol, the enzymes must collide with and bind to the active site of the substrate. Therefore, enough energy is necessary to make a good orientation of collision by increasing temperature. Consequently, 30 °C was the best

fermentation temperature condition on using both *Saccharomyces cerevisiae* and finger millet malt while the optimum fermentation temperature on using sorghum malt was 35 °C. By increasing temperature above the optimum value, the enzymes become denatured resulting in termination of bio-ethanol production. On the other hand, decreasing temperature below the optimum value can make the enzymes have less energy to collide with the substrate. This result was supported by Lin *et al.*, (2014) and Imtiaz *et al.*, (2013) who reported that optimum fermentation occurs within a temperature of 30-45 °C and 30-36 °C, respectively.

Optimum fermentation pH was 5 in all cases which is in agreement with the results of Wu *et al.*, (2017) together with Liu & Shen, (2008) who reported an optimal pH of 5.5 and 5.0 on acidogenic fermentation of fruit and vegetable waste and fermentation of sweet sorghum stalk juice using *Saccharomyces cerevisiae*, respectively. The effect of pH observed in the current study shows that the enzyme is active in the acidic range. Alcoholic fermentation in acidic conditions is important because the growth of harmful bacteria is stopped by acidic conditions and yeast growth is better under acidic conditions. A change in pH above or below the optimum pH will reduce the rate of enzyme reaction considerably. This is because the changes in pH lead to the breaking of the ionic bonds that hold the tertiary structure of the enzyme in place which gives effect to the total net charges of the enzymes. Therefore, the enzyme begins to lose its functional shape, particularly the shape of the active site, such that the substrate will no longer fit into it. This denatures the enzymes making them unable to catalyze chemical reactions. As a result, the rate of production of bio-ethanol decreases as the rate of fermentation also decreases. Furthermore, changes in pH may not only effect the shape of the enzyme, but it may also change the shape or change the properties of the substrate so that either the substrate cannot bind to the active site or it cannot undergo catalysis (Rosdee *et al.*, 2020).

On the other hand, the ratio of mass of source of enzyme to substrate was also obtained to be 5 g/L which was in line with the observed value of 3 g/L by Laopaiboon *et al.*, (2009) and 1 g/L reported by Luo *et al.*, (2014). In addition, optimal fermentation time on using *Saccharomyces cerevisiae* was found to be 36 hours while that of finger millet malt and sorghum malt was 48 hours. This points out that fermentation using the three enzyme sources in this study took a shorter duration compared to a fermentation duration reported by Luo *et al.*, (2014) and Lin *et al.*, (2014) of 72 hours.

4.3 Measurements of Kinetic Parameters

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 as indicated in Appendix 4. 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 (Igbokwe *et al.*, 2016). This was the case observed in this study. Since V_{\max} is an asymptote, to improve its accuracy and that of K_m , Lineweaver-Burk plot was used.

The maximum reaction velocity V_{\max} and Michaelis-Menten constant (K_m) of the fermentation reaction using the commercial yeast *Saccharomyces cerevisiae* from the Lineweaver-Burk plot as shown in Figure 4.8 were found to be 0.69 g/L/h and 13.96 g/L of the substrate, respectively.

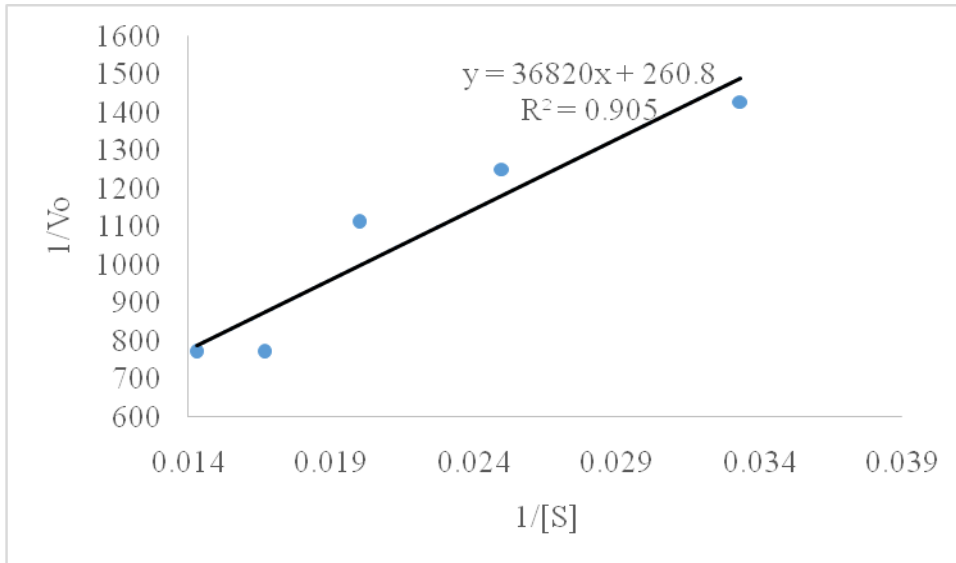


Figure 4.8: Lineweaver-Burk Plot of $1/V_o$ against $1/[S]$ for the Fermentation Reaction using *Saccharomyces cerevisiae*

On the other hand, the maximum reaction velocity V_{max} and Michaelis-Menten constant (K_m) of the fermentation reaction using finger millet malt as shown in Figure 4.9 were found to be 0.35 g/L/h and 12.56 g/L of the substrate, respectively.

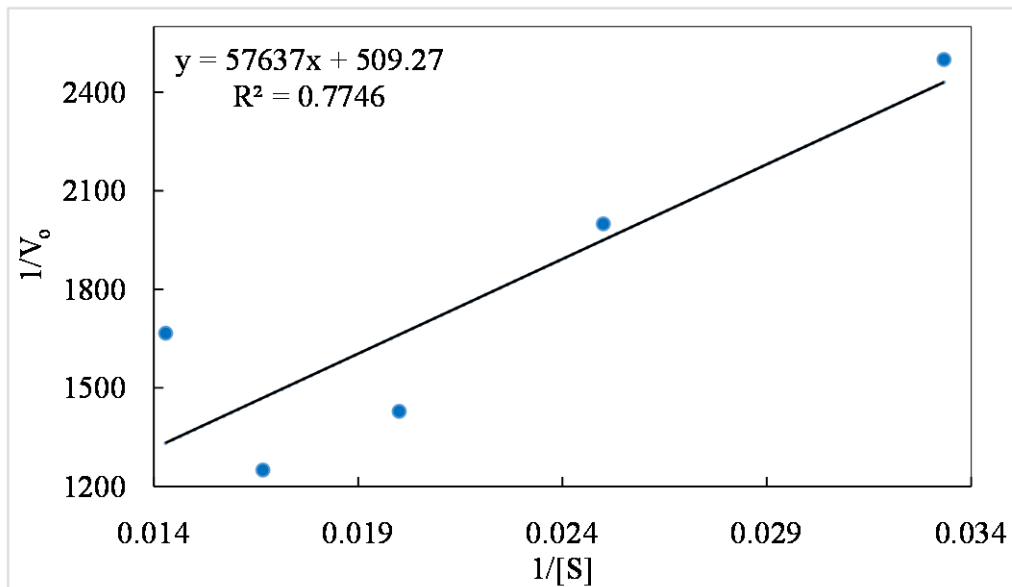


Figure 4.9: Lineweaver-Burk Plot of $1/V_o$ against $1/[S]$ for the Fermentation Reaction using finger millet malt

Finally, the V_{\max} and K_m obtained during fermentation using sorghum malt were obtained to be 0.34 g/L/h and 14.09 g/L respectively as shown in Figure 4.10.

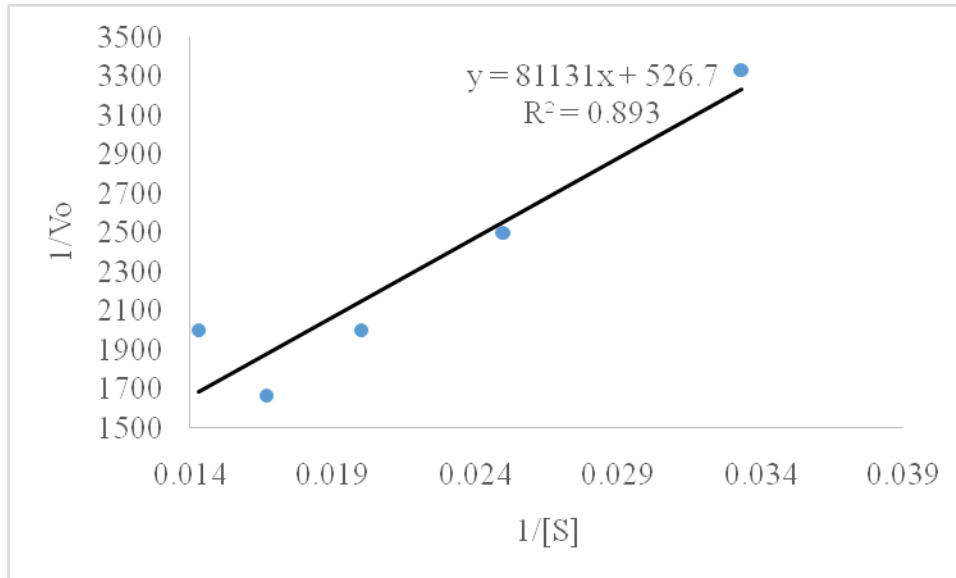


Figure 4.10: Lineweaver-Burk Plot of $1/V_o$ against $1/[S]$ for the Fermentation Reaction using Sorghum malt

Table 4.11 shows the kinetic parameters obtained from the graphs in Figure 4.8, 4.9 and 4.10. The adjustments were made using the Lineweaver-Burk linearization of the Michaelis-Menten model.

Table 4.11: Kinetic Parameters According to Lineweaver-Burk Linearization Method

Source of enzyme	Slope	Intercept	R^2	V_{\max} (g/L.h)	K_m (g/L)
<i>Saccharomyces cerevisiae</i>	36820	260.84	0.905	0.69	13.96
Finger millet malt	57637	509.27	0.7746	0.35	12.56
Sorghum malt	81131	526.7	0.8937	0.34	14.09

Finger millet malt presented the lowest K_m of 12.56 g/L followed by *Saccharomyces cerevisiae* that showed a K_m of 13.96 g/L. At the third position was sorghum malt that had a K_m of 14.09 g/L. The correlation coefficients (R^2) obtained with the three enzyme sources were close to 1, this showed that the kinetics of fermentation of sweet sorghum stalk juice using *Saccharomyces*

cerevisiae, finger millet malt and sorghum malt followed the Michaelis-Menten model. These results are in agreement with those obtained in a study by Igbokwe *et al.*, (2016) 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 was obtained respectively. 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 (Periyasamy *et al.*, 2009)

This work obtained a K_m of 12.56 g/L on fermentation of sweet sorghum stalk juice using finger millet malt (Table 4.11). To determine whether this K_m was statistically lower than that obtained on using *Saccharomyces cerevisiae* which was 13.96 g/L, a 1 tailed unpaired T-test was used and a $p = 0.014$ was obtained. This indicated that the K_m obtained on using finger millet malt was statistically lower than the K_m obtained on using *Saccharomyces cerevisiae*. Similarly, a 1 tailed unpaired T-test was used to compare the K_m of finger millet malt and sorghum malt and a $p = 0.006$ was obtained. This also indicated that K_m obtained on using finger millet malt was statistically lower than that obtained on using sorghum malt. K_m is an indicator of the affinity between the substrate and enzyme or a group of enzymes. The better the affinity, the lower the K_m value. Of all the 3 tested sources of enzymes in this study, finger millet malt produced the enzyme or group of enzymes that had the highest affinity towards sweet sorghum stalk juice. 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.*, (2016) on the production of bio-ethanol from plantain peels. A smaller V_{max} of 0.35 g/L/h obtained in this study when finger millet malt was used compared to 0.85 g/L/h obtained in the study by these researchers indicate that only a small amount of substrate (which is industrially desirable) is

needed for the reaction to reach its maximum velocity. In addition, these results showed that of the three enzyme sources that were used in this study, finger millet malt was superior to *Saccharomyces cerevisiae* and sorghum malt. The superiority of finger millet malt may be because of presence of nutrients, malt sugar and presence of metal ion-chelating activity caused by polyphenols. All these qualities help in keeping the yeast cells viable for a longer time during fermentation as was reported by Reddy & Reddy, (2006).

4.4 Characterization of the bio-ethanol produced in terms of calorific value, density, pH and flame test

Since finger millet malt was found to be effective and efficient compared to *Saccharomyces cerevisiae* and sorghum malt, it was used in bulk ethanol fermentation. The fermented liquid was distilled to remove yeast solids and excess water. In order to reduce the water content and obtain a good percentage of bio-ethanol, the distillate was re-distilled at a regulated temperature of (55-60) °C using rotary evaporator (rotavap) that helped to efficiently and gently remove the ethanol from the fermented mass. The calorific value was determined since it is a very important feature of a fuel which shows the amount of heat given out when a given amount of fuel undergoes combustion. It also indicates the available energy in a fuel (Innocent *et al.*, 2022). In this study, the calorific value of the bio-ethanol produced was 8740 kcal/kg. This was in close proximity to the findings of Flores, (2018) who reported a value of 8756 kcal/kg on the bio-ethanol produced after fermentation of *Saccharum officinarum linn.* Another study by Nwufo *et al.*, (2016) reported a lower calorific value of 7112.8 kcal/kg on fermentation of sugarcane juice under natural fermentation method that occurred after 12 days. However, the calorific value obtained from this study deviated from the standard value of 6380 kcal/kg as reported by Flores, (2018). These variations could be attributed to the chemical composition of the fuel samples.

Density of the bio-ethanol obtained was also determined since it helps in determining the power delivery of the fuel. The bio-ethanol produced had a density of 0.8954 g/cm^3 which was lower than the findings reported by (Uthman & Jimoh, 2015) which was 0.969 g/cm^3 after fermentation of corn cereals for 12 days. The density for the experimental bio-ethanol produced in this study falls within the ASTM E100 specification of $(0.789\text{-}0.801) \text{ g/cm}^3$ as reported by Harrison *et al.*, (2022).

It is also important to determine the pH since it gives the potential corrosiveness of the fuel which can cause damage to the inside of the furnace surface during the burning process. The pH of the bio-ethanol produced was 6.3 ± 0.2 which was within the standard recommended bio-ethanol pH which ranges between pH (6.5-9.0) as reported by Flores, (2018). It was also in close proximity to the findings of Harrison *et al.*, (2022) who obtained a pH of 7.2 on the bio-ethanol produced from maize cobs.

The bio-ethanol produced burnt with a blue flame as shown in Figure 4.11.



Figure 4.11: Flame produced on burning bio-ethanol

The blue flame indicated high ethanol levels which undergoes complete combustion. This result is in line with the observation made by Vita *et al.*, (2021) who also reported that the bio-ethanol produced through fermentation of cassava based industrial waste burnt with a blue flame. Another study by Ansar *et al.*, (2020) also carried out a flame test on the bio-ethanol produced in their study and reported a flame with a red yellow colour and pointed out that it signified low ethanol content.

CHAPTER FIVE

CONCLUSION, RECOMMENDATION AND SUGGESTIONS FOR FURTHER RESEARCH

5.1 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 drops. 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 105 days after planting. This was followed by V5 variety that had a °Brix of 20.26 ($P \leq 0.05$). At the third position was V3 genotype that had a °Brix of 17.97 ($P \leq 0.05$) while at position four was V2 variety with a °Brix of 17.53 ($P \leq 0.05$) and finally at the fifth position was V4 that had a °Brix of 15.30 ($P \leq 0.05$).

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 *Saccharomyces cerevisiae* were obtained to be fermentation temperature of 30 °C, pH 5, yeast to substrate ratio of 5 g/L and fermentation time of 36 hours. On the other hand, optimum amount of ethanol obtained on using finger millet malt were at a temperature of 30 °C, pH 5, malt to substrate ratio of 5 g/L and fermentation time of 48 hours. Lastly, with sorghum malt the optimum fermentation conditions were fermentation temperature of 35 °C, pH 5, malt to substrate ratio of 5 g/L and fermentation time of 48 hours.

The fermentation process followed the Michaelis-Menten model. The highest affinity (lower K_m) values was for finger millet malt and lower affinity (higher K_m) was for sorghum malt. Based on K_m obtained from Michaelis-Menten kinetic values, finger millet malt can be considered the best possible source of enzyme for the fermentation of sweet sorghum stalk juice. The fermentation

kinetics for finger millet malt were found as V_{\max} of 0.35 g/L/h and K_m of 12.56 g/L. The values of V_{\max} and K_m are indicative that finger millet malt is a viable enzyme source for this work and that both the enzyme and the substrate have high affinity for one another.

Bio-ethanol produced is a viable alternative fuel for domestic cooking since it produced 8740 kcal/kg of heat energy on combustion. It also burnt with a blue flame and had a pH of 6.3 showing that it is less corrosive. The density of the bio-ethanol produced in this study was 0.8954 g/cm³

5.2 Recommendation

Harvesting of the five sweet sorghum varieties under the agronomic conditions in this study should be done at the 15th week or 105 days after sowing. This was the stage where they all had maximum °Brix required for high yield of bio-ethanol.

The optimal fermentation conditions of sweet sorghum stalk juice using finger millet malt are temperature of 30 °C, pH 5, amount of malt to substrate ratio of 0.25 g/50 mL over a fermentation duration of 48 hours.

Finger millet malt should be used for fermentation of sweet sorghum stalk juice since the enzyme and the substrate have a high affinity for one another. There is also need to investigate the fermentation of sweet sorghum stalk juice with other wild and commercial yeasts.

Bio-ethanol produced through fermentation of sweet sorghum stalk juice using finger millet malt can be used for domestic cooking as an alternative fuel to kerosene and firewood.

5.3 Suggestions for further work

Given that this study was conducted in an experimental farm it is likely that the maturity and performance of these five genotypes in other location may differ. Therefore, it is essential to test these varieties in different environments to help determine their overall adaptability and stability.

Finger millet malt derived yeast characterization should be carried out in future to determine its morphological and surface characteristics.

The future studies should investigate the fermentation capabilities of other yeasts isolated from fruits, roots and barks of locally available plants since most of the yeast sources are used locally with minimal or no scientific findings.

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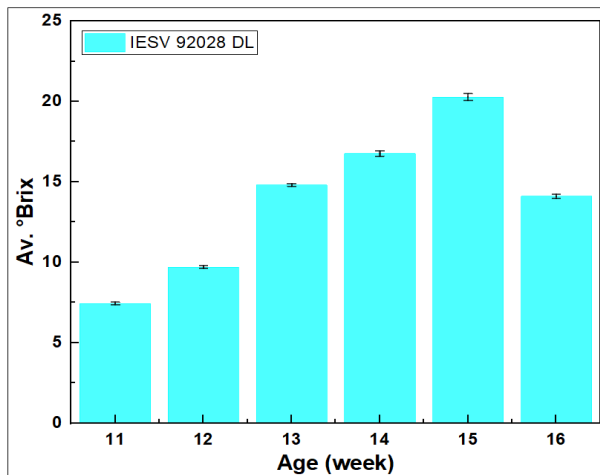
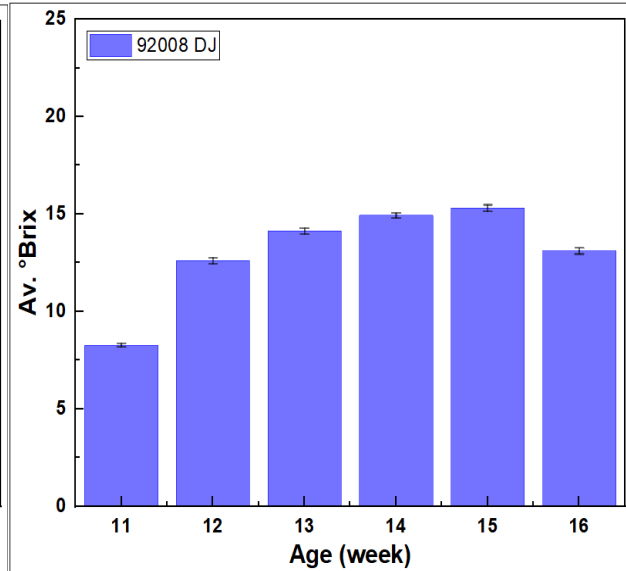
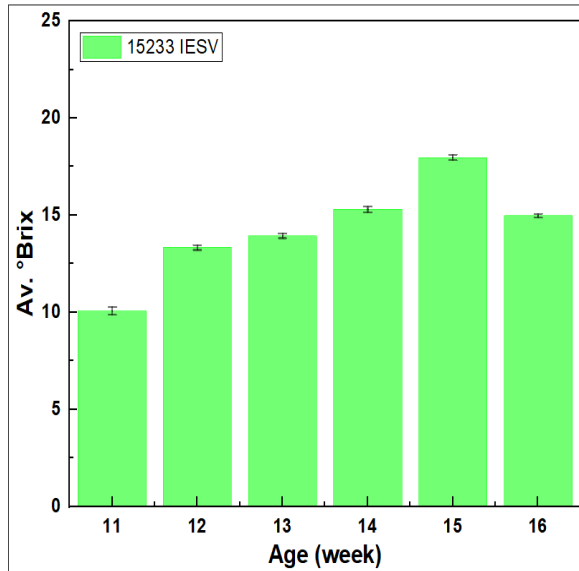
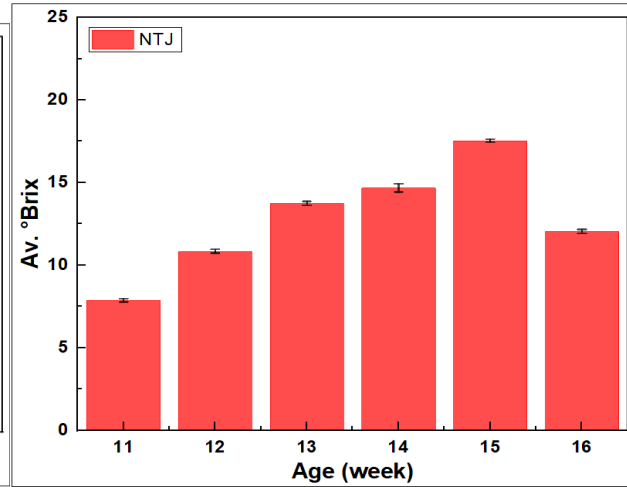
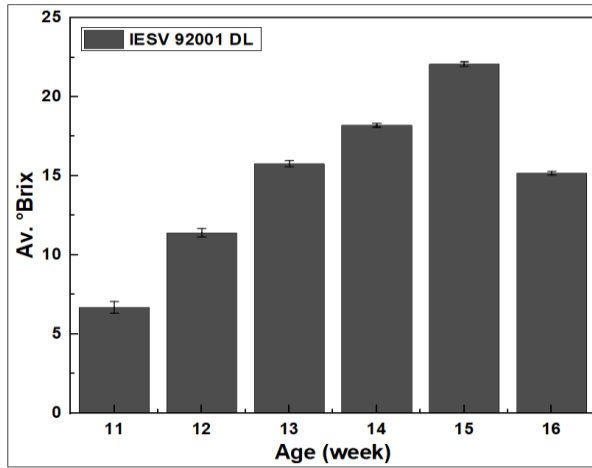
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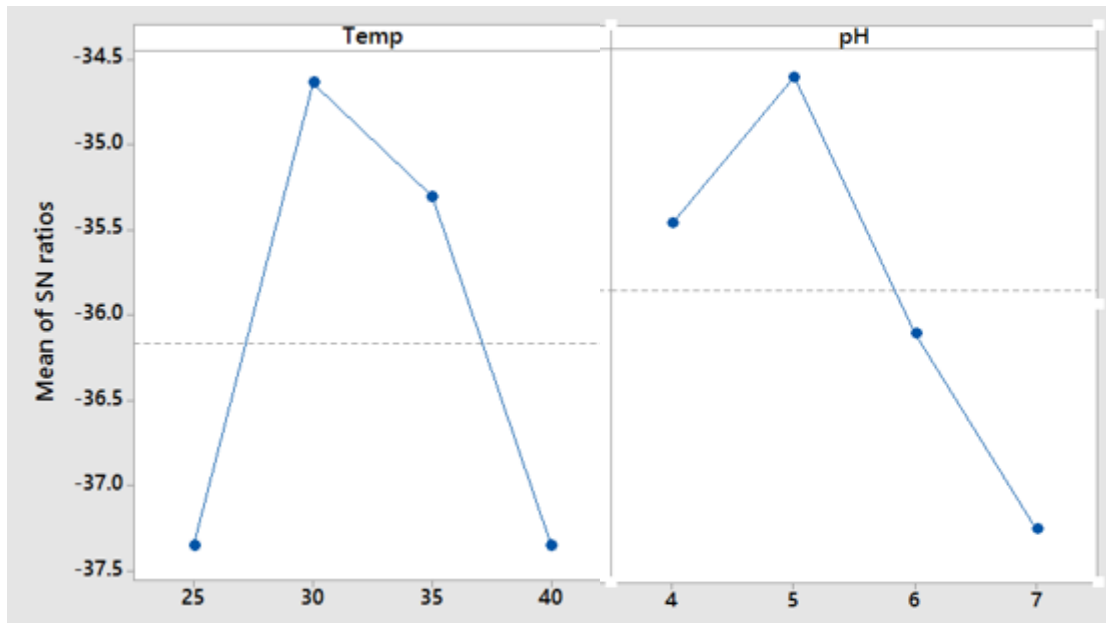
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APPENDICES

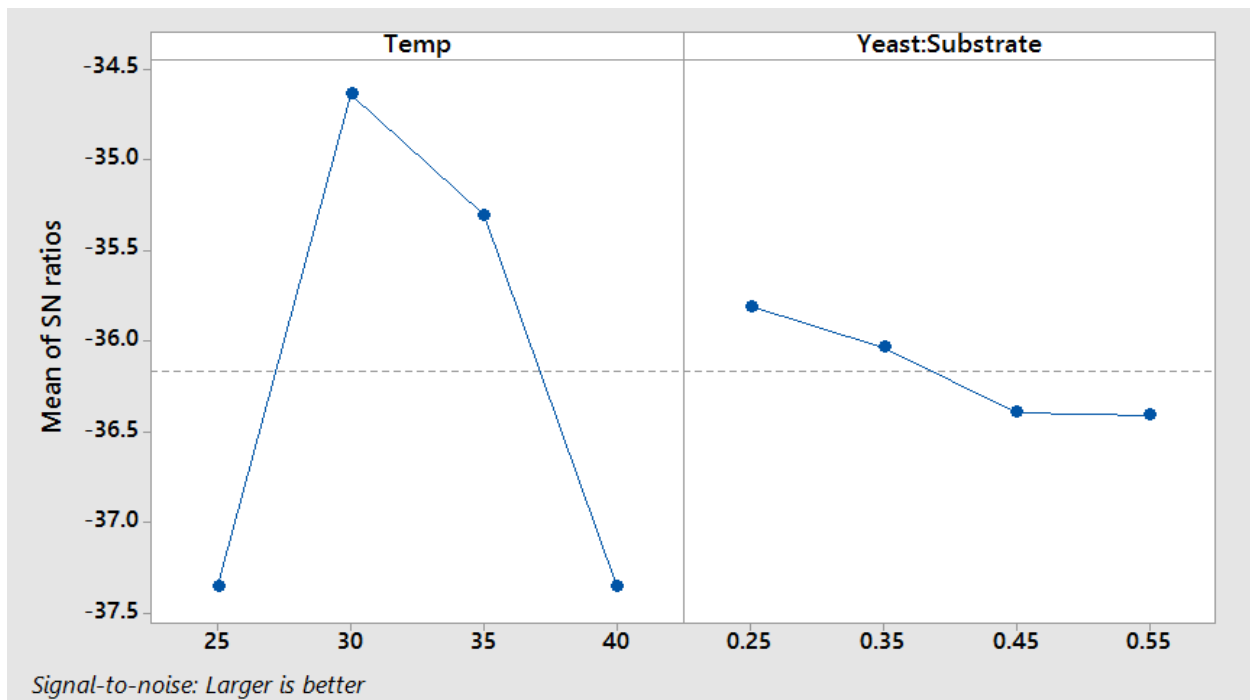
Appendix 1: Bar graphs for the trend of °Brix content per variety with age



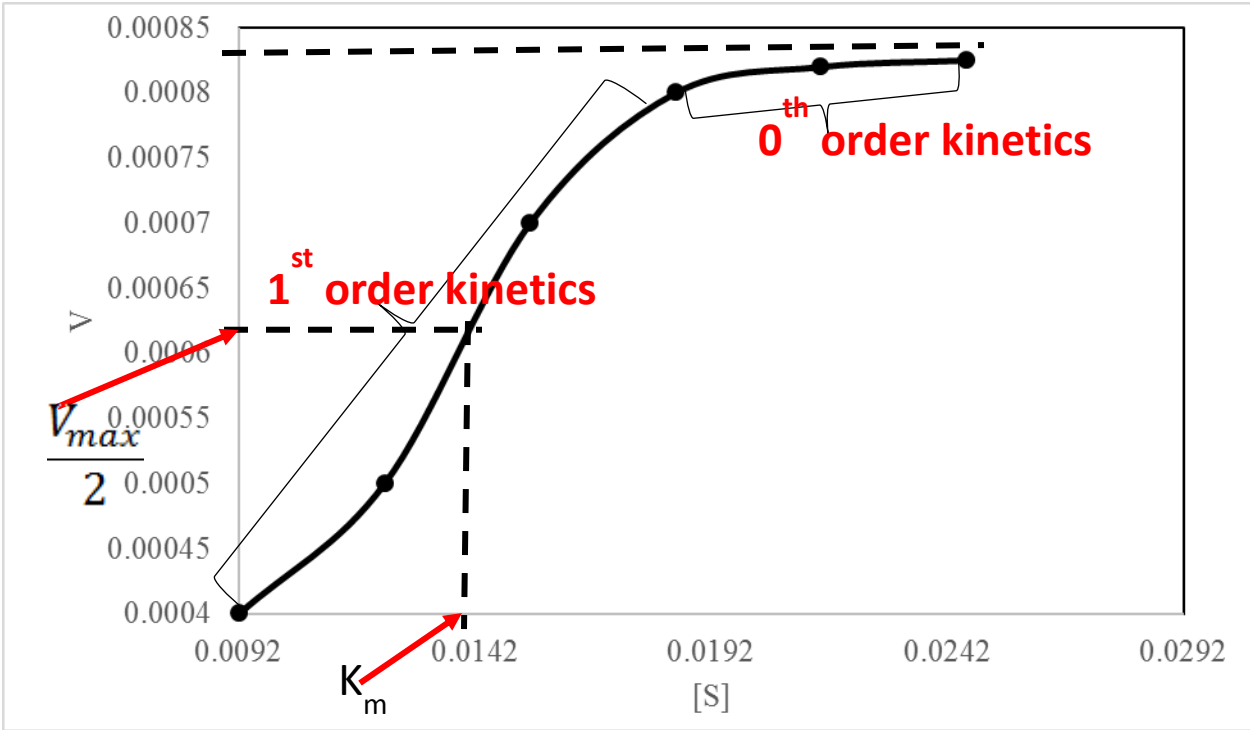
Appendix 2: Second optimization of temperature and pH on using *Saccharomyces cerevisiae*



Appendix 3: Second optimization of temperature and source of enzyme load on using finger millet malt



Appendix 4: Illustration of Michaelis-Menten enzyme kinetics graph



Appendix 5: Formulae for the calculation of juice and bio-ethanol yield

$$\% \text{ juice yield} = \frac{\textit{juice mass}}{\textit{mass of stalks}} \times 100$$

$$\text{Bio-ethanol yield} = \frac{\textit{produced bio-ethanol}}{\textit{total amount of juice}} \times 100$$