JIMMA UNIVERSITY

JIMMA INSTITUTE OF TECHNOLOGY (JIT)

SCHOOL OF CHEMICAL ENGINEERING

PROCESS ENGINEERING STREAM

OPTIMIZATION OF THE TOTAL REDUCING SUGARS YIELD FROM DILUTE ACID HYDROLYSIS AND BIO ETHANOL PRODUCTION FROM MORINGA OLEIFERA SEEDS HUSK

By: Fayruza Jemal

January, 2020.

Jimma, Ehiopia

JIMMA UNIVERSITY

JIMMA INSTITUTE OF TECHNOLOGY

SCHOOL OF CHEMICAL ENGINEERING

PROCESS ENGINEERING STREAM

OPTIMIZATION OF THE TOTAL REDUCING SUGARS YIELD FROM DILUTE ACID HYDROLYSIS AND BIO ETHANOL PRODUCTION FROM MORINGA OLEIFERA SEEDS HUSK

A THESIS SUBMITTED TO THE JIMMA UNIVERSITY, JIMMA INSTITUTE OF TECHNOLOGY, SCHOOL OF CHEMICAL ENGINEERING IN PARTIAL FULFILLMENT OF THE REQUIREMENT OF DEGREE OF MASTERS OF SCIENCE IN CHEMICAL ENGINEERING UNDER PROCESS ENGINEERING STREAM.

By: Fayruza Jemal

Advisor: Dr. K. Ramachandran

Co-advisor: Mr. Abowerk Wagank

January, 2020

Jimma, Ethiopia

JIMMA UNIVERSITY

JIMMA INSTITUTE OF TECHNOLOGY

SCHOOL OF CHEMICAL ENGINEERIN

This is to certify that the thesis prepared by **Fayruza Jemal**, entitled: "**optimization of the total reducing sugars yield from dilute acid hydrolysis and bioethanol production from moringa oleifera seeds husk**" and submitted in partial fulfillment of the requirement for the degree of Master of Science (Chemical Engineering) complies with the regulations of the University and meets the accepted standards with respect to originality and quality. .

Signed by the Examining Committee:

Dr. Eng. Hundessa Demsash		
External Examiner	Signature	Date
Mr. yigezu Mokonun		
Internal Examiner	Signature	Date
Mr. Abrha Gebremeskel		
School or Center of Chairperson	Signature	Date
Mr. Samuel Gesesse		
Program Coordinator	Signature	Date
Mr. Ermias Girma		
Dean, School of Chemical Engineering	Signature	Date

Declaration

I declare that thesis for M.Sc. Degree at Jimma University, here by submitted by me, is my original work and have not previously been submitted for the degree at this or any other university, and that all resources of materials used in this thesis have been duly acknowledged.

Name: Fayruza Jemal

Signature: _____

Date of submission:_____

This thesis has been submitted for examination with my approval as University Advisor and Co-advisor.

Name: Dr. K. Ramachandran

Signature: _____

Date: _____

Name: Mr. Abowerk Wagank

Signature: _____

Date: _____

Abstract

The objective of this thesis is bioethanol production from moringa oleifera seeds husk, which is in effects to minimize energy cost and substituting non-renewable energy by renewable resources. Moringa oleifera is a plant with various benefits to mankind from its root until leaves, from food to biofuel applications, all parts are useful. Moringa oleifera seeds husk, which is an agricultural waste with no appreciable value to industries and competitive use as a food.

This study involved the optimization of the total reducing sugars yield from dilute acid hydrolysis and bioethanol production from moringa oleifera seeds husk. The production process was carried out in four main steps, pretreatment, hydrolysis, fermentation and distillation. The husk was hydrolysis using acid hydrolysis which is dilute sulfuric acid and fermented using saccharomyces cerevisiae yeast. The experiment was designed by Response Surface Methodology (RSM) using central composite design (CCD) to investigate the effect of acid concentration (2-3%), reaction time (20-30min) and temperature (120-140ºC) of hydrolysis parameters using Design expert® version 11 software. The optimum combination of temperature, time and acid concentration was determined. High yield of total reducing sugar 44.83% (average) at the optimum parameters, temperature of 130ºC, 25 min reaction time, and 2.5% acid concentration. This process investigates the parameters which produce optimum total reducing sugar yield. Acid concentration, temperature and time have a statically significant effect on the yield with p-value 0.0029, 0.0007 and 0.0394 respectively. However, high acid concentration, temperature as well as increasing hydrolysis time causes a decline in the total reducing sugar yield. The statistical analysis also showed that the total reducing sugar yield of (44.98 %) and 3.71 ml/40g bioethanol yield was obtained at optimization variables of 2.72 % acid concentrations, 132.38 º C temperatures, and at a time 27.18 minutes. From this, it can conclude that a good agreement with the observed values of the total reducing sugar yield (44.83). Chemical characterization of the bioethanol produced was performed by FTIR. The result shows that, the ethanol produced contains O-H, C-O,- CH_2 and CH_3 functional groups which indicates the presence of ethanol in the product.

KEYWORDS: - bioethanol, moringa oleifera seeds husk, fermentation, saccharomyces cerevisiae, hydrolysis, total reducing sugar yield.

Acknowledgement

First of all, I would like to thank the Almighty Allah for giving me strength and patience for the successful accomplishment of this study.

Next, I would like to express my deepest gratitude to my advisor Dr. Ramachandran kasirajan for his guidance, advice, patience and encouragement in this work. My deepest respect and appreciation goes to my Co-advisor Mr. Abowerk Wagank for his conceptual orientation and framework. I would like to thank all the administrative and technical staffs of Debreziet agricultural research centre (Bishoftu) for providing the raw material and for giving me the relevant information about the raw material. I would like also to thank Mr. Asefa Germa for his help by collecting the raw material.

I am strongly indebted to AAU, Chemical and bio engineering laboratory to all technical staffs, and also chemistry Departments laboratory staffs for their technical guidance and assistance, especially to Mr. Samsom, Mr. Hintisaselasa Sefu and Ms. Hanna for providing all the necessary support during my study.

To kore, my loving husband. I owe you a very special thank you for all of your love, support and encouragement.

I must express my very profound gratitude to my parents and friends for providing me with unfailing support and continuous encouragement throughout my years of study and through the process of researching and writing this thesis. This accomplishment would not have been possible without them. Thank you. Finally, I offer my regards and blessing to all of those who supported me in any respect during the completion of the thesis.

Contents

List of tables

List of figures

List of Abbreviation

AAU	Addis Ababa university
AFEX	Ammonia fibber explosion
ANOVA	Analysis of variance
ASTM	American Society for Testing and Materials
CCD	Central Composite Design
CI	Confidence Interval
Co_2	Carbon Dioxide
C.V	Coefficient of Variance
DF	Degree of Freedom
Dp	Degree of Polymerization
FT-IR	Fourier Transform Infrared Spectroscopy
H_2SO_4	Sulphuric Acid
MOSH	Moringa oliefera Seeds Husk
Mpa	Mega Pascal
NaOH	Sodium hydroxide
pH	potential of hydrogen
rpm	revolution per minute
R^2	Regression Coefficient
RSM	Response Surface Methodology

TRS	Total Reducing Sugar
SNNP	Southern Nation and Nationality and People
Us	United State
UV-VIS	UV-visible Spectrophotometer
V/v	Volume per volume

1. INTRODUCTION

1.1 Background of the Study

Fossil resources are still primary energy and chemical sources; around 75 per cent is used for heat and energy production, about 20 per cent as fuel, and just a few per cent for the production of chemicals and materials. A small number of countries process the major reserve of fossil fuels, which additionally increase unsustainability of their production, increases greenhouse gas emission arises from fossil fuel combination and land use change as a result of human activates, and consequently results in an acceleration of the global warming crisis (Bušić et al., 2018)

Agricultural raw material and biomass can be utilized to produce renewable energy in the form of liquid or gaseous fuel which can reduce CO_2 emission on combustion unlike fossil fuels which can cause greenhouse effect. The climate change and the consequent need to diminish greenhouse gases emissions is a global concern. Because of that, it has been encouraged to use bioethanol as a gasoline replacement or as an additive (Balat Mustafa, Balat Havva, & Öz, 2008). Bioethanol may also be used as raw material for the production of different chemicals, thus driving a full renewable chemical industry. In most development countries, governments stimulate the use of renewable energies and resources with the following major goals: to secure to energy, to mitigate climate change, to maintain agricultural activates, and to ensure food safety (Bušić et al., 2018).

Bioethanol production has been investigated recently as this fuel is efficient, biodegradable, environmentally friendly and cost effective alternative for the conventional fossil fuel. Bioethanol derived from lignocellulosic material is a potential renewable energy source. There are countless lignocellulosic materials that have been used in recent years to produce bioethanol such as sugarcane bagasse, paper and pulp, cassava waste, saw dust and corn (Pandey and Soccol, 2000). Therefore, lignocellulosic biomass (second generation) represents an alternative feedstock for bioethanol production due to its low cost, availability, wide distribution and it is not competitive with food and feed crops (Bušić et al., 2018). Apart from that bio ethanol is one of biomass products. Bio ethanol is nowadays producing from edible sources such as sugar cane and corn which compete with human food (Hill, Nelson, and Tiffany, 2006). In this direction, it is a challenge for bio ethanol production to use feedstock that would not compete with human

food. The potential of moringa oliefera seeds husk for the production of bio ethanol which completely would not compete with human food.

Most important of *moringa oliefera* are medicinal and nutritional plant species. *Moringa oleifera*, also known as Aleko (Konso), Shiferaw (Amharic). It is deciduous tree that reaches a height of up to 10m, usually smaller, pale feathery foliage. *Moringa oleifera* originates from India and is introduced to Ethiopia long ago. The tree is now naturalized in many parts, mainly in the southern Ethiopia (SNNE). Konso people plant Moringa oleifera around their homesteads and also in the terraced fields (Padayachee and Baijnath, 2012). Moringa is most commonly found in southern Ethiopia, but today it is widely cultivated in all parts. It is considered one of the world's most useful trees, as almost every part of moringa tree can be used for food or has some other beneficial properties (Hamza et al., 2017).

The seeds of moringa are one of the best natural coagulants for water treatments in developing countries. Oilseeds for edible oil production, Moringa oilseeds are more advantageous in terms of oil content, costs and agronomic properties. Furthermore, moringa seeds oil can be used as potential sustainable feedstock for biodiesel production (Hamza et al., 2017).

Moringa oleifera is a tropical plant belongs to the family of Moringaceae. It also have fourteen different species (El-hack et al., 2018). The husks, generated during de-husking of the seeds for obtaining the kerneals, generally have low economic value and it is mainly disposed (Hamza et al., 2017). Production of bio ethanol from Moringa oleifera seeds husk using acid hydrolysis and fermenting using Saccharomyces cerevisiae yeast. Saccharomyces cerevisiae is commonly known as baker's yeast (Wang and Chen, 2006). In this study, the optimization of dilute acid hydrolysis of MOSH was studied. The objective of this study was to optimize the effect of acid concentration, temperature, and hydrolysis time levels. Using central composite design of experiments, a mathematical correlation between acid concentration, temperature, and time was developed to obtain maximum Total reducing sugar yield.

1.2 Statement of the problem

The commercial production of fuel ethanol in the world relies mainly on the fermentation of sugar and starch, but production of ethanol from such first generation feedstock is often viewed as competing with food production and increasing prices of food. So the productions of bioethanol or biofuel from agricultural wastes and *Moringa oleifera* seeds husk was expected to cure the concern associate with food security.

The major problem with bio ethanol production is the availability of raw materials for the production and also the production cost. Lignocellulosic biomass is the most promising feedstock considering its great availability and low cost. Therefore, sugar based materials was used as the feedstock.

Moringa oleifera seeds husk, which is an agricultural waste with no appreciable value to industries and competitive use as a food. Such presents high concentration of carbohydrates, thus it can be viewed the potential of as a feedstock for bio ethanol production. It was good to introduce too theirs the advantages and uses of husk instead of being thrown away. Thus, *moringa oleifera* seeds husk was used for the production of second generation biofuel. However, this study includes; optimization of hydrolysis condition for bioethanol production to obtain optimum point in order to get maximum amount of total reducing sugar yield during ethanol production from *moringa oliefera* seeds husk.

A few researchers have done bioethanol production from moringa olifera seeds husk and hydrolysis using Sodium hydroxide without optimizing hydrolysis conditions using dilute acid and characterizing the product properties. However, this study includes; optimization of dilute acid hydrolysis conditions and product characterization to obtain optimal point in order to get maximum amount of yield during ethanol production.

1.3 Objectives

1.3.1 General Objective

The general objective of this study was optimization of the total reducing sugars yield from dilute acid hydrolysis and bioethanol production from moringa oleifera seeds husk.

1.3.2 Specific Objectives

Specific objectives of the study were the following:-

- To characterize the composition of moringa oleifera seeds husk.
- To investigate the effect of process variables (acid concentration, time and temperature) in the hydrolysis process.
- To specify the optimum operating conditions for founding the maximum yield of TRS.
- To characterize bioethanol by FTIR.

1.4 Significance of the Study

The main importance of this study was to enhance the importance of *moringa olifera* seeds husk through the production of bioethanol through dilute acid hydrolysis. This study also performed preliminary analysis of as in put for ethanol production and great significance in terms of assuring the production of an alternative form of energy using the potential of *moringa oleifera* seeds husk. This study also highly contributes in the substitute fossil fuel by biofuel. Fossil fuels increases emission of greenhouse to the atmosphere gasses and causes global warming. As a renewable and non-food competitive feedstock raw material is desirable for the production of alternative fuel such as bioethanol. Therefore, MOSH is one of such renewable and non-food competitive raw material. This study also revealed for researchers to see this very cheap, safe and highly available plant use for other investigation. Bioethanol production from MOSH is considered a second generation biofuel process since it has no direct conflict with human food, as the case of first generation biofuels produced from agricultural crops, such as corn, sugar cane and soybean oil.

1.5 Scope of the study

The thesis work was generally covered collection and characterization of *Moringa oleifera* seeds husk, extraction were done by acid hydrolysis (dilute acid) to break it down to simple sugar and the simple sugar was fermented using *Saccharomyces cerevisiae* yeast to convert in to ethanol. To study the effect of temperature, acid concentration and reaction time in the hydrolysis process on the yield of total reducing sugar. The characterization of produced bioethanol using FTIR.

2. LITERATURE REVIEW

2.1 Introduction-what is Bioethanol

Ethanol obtained from biomass waste materials or renewable sources is called as bioethanol. It can be used as a fuel, chemical feedstock, and solvent in various industries. It has certain advantages as petroleum substitutes with alcohol. It can be produce from different renewable resources; alcohol as fuel burns clear than petroleum this aspect is environmentally more acceptable. It is biodegradable, less toxic than fossil fuels (Domínguez-bocanegra, Rosa, Torres-muñoz, Antonio, & Aguilar, 2015). Bioethanol, known as ethyl alcohol, grain alcohol or ETOH, has the chemical formula CH_3-CH_2-OH. It is a liquid biofuels which can be produced from different biomass feed stocks. Bioethanol can be used as an appropriate mixed fuel in gasoline engines because of its high octant number, and the high heat of vaporization obstructs self-ignition in diesel engines (Mahapatra & Manian, 2016).

Table 2.1 properties of Ethanol

	Ethanol
Molecular formula	C_2H_5OH
Appearance	Colorless liquid
Flash point	55^oF (12.6ºC)
Density	0.789kg/liter
Specific gravity	0.79
Vapour density	1.49
Vapour pressure	440mmHg
Boiling point	173ºF
Conductivity	Yes
Viscosity	1200mpa.s (20ºC) or 1.2
Molecular mass	46.07g/mol

(Member, Afolabi, & Ogochukwu, 2015)

Table 2.1 shows the chemical properties of bioethanol. Ethanol is a clear color less, flammable solvent with a boiling point of 78.5ºC also known as ethyl alcohol. Also has a vapor density of 1.49-1.59, which indicates that it is heavier than air. Ethanol specific gravity is 0.79, which indicates it is lighter than water but since it is water soluble it will thoroughly mix with water. The flash point varies with fuel volatility but is not related to engine performance. Also is lowest temperature to which a fuel must be heated to produce an ignitable vapor air mixture above the liquid fuels when exposed to an open flame. At temperature above the flash point, not enough fuel evaporates to form a combustible mixture. Density is an important parameter for ethanol fuel injection systems. The value of density must be maintained within the tolerable limits to allow optimal air to fuel ratios for complete combustion. High density bio ethanol can lead to incomplete combustion and particulate matter emission. It is important to state that the slight disparity in density observed can be strongly attributed to differences in feedstock used, fermentation process employed and presence of impurities (Flores, 2018).

2.2 Feedstock's for bioethanol production

Biofuels originated from plant oils, sugar beets, cereals, organic wastes and the processing of biomass. Biological feedstocks that contain appreciable amounts of sugar or materials that can be converted into sugar, however, the starch or cellulose can be fermented to produce bioethanol to be used in gasoline engines (Balat Mustafa et al., 2008). Bioethanol feedstock can be classified into the following: (a) sucrose containing feedstock, like sugar beet, sweet sorghum and sugar cane, (b) starchy (e.g. wheat, corn and barley), and (c) lignocellulosic biomass (e.g. wood, straw, and grasses) (Balat Mustafa et al., 2008).

2.2.1 Lignocelluloses biomass

Lignocellulosic biomass are complex biological materials that contains agricultural residues (corn, wheat straw, sugar baggas, rice husk, corn cob, corn fiber, cotton stalks), office weast paper, industrial cardboard and forestry products (Kumar, Ashish, et al., 2009). These resources are abundant and extensively accustomed, with a yearly supply. Available lignocellulosic sources are exploited, often in non-food manufacturing, such as the paper and pulp industries (Kumar, Ashish, et al., 2009). Because this biomass are outside the human food chain, lignocellulosis are relatively low cost feedstocks that is an ideal source of sugars for ethanol fuel and valuable commodities production via the development of lignocelluloses based bio refineries (Kumar & Singh, 2009). The main products of bio refineries are biofuels, biomaterials

and biochemical. Among those, bioethanol and furan biofuel are mentioned as the most important (Kucharska, Rybarczyk, Hołowacz, & Łukajtis, 2018). Lignocellulosic materials possess a great potential to be a good feedstock to produce biofuels as well as valuable chemicals. Pre-treatment is an indispensable step of lignocellulosic biomass processing due to the complex structure and disobedient nature of such a feedstock (Kucharska et al., 2018).

2.2.1.1 Composition of Lignocelluloses Biomass

Lignocellulosic biomass contains a mixture of carbohydrate polymers and promising substrate for bioethanol production, as it is unlikely to become depleted permanent damage (Sun & Cheng, 2002). The composition of lignocellulosic material depends on its species, variety, growth conditions and maturity. Ethanol yield and conversion productivity depend on the type of biomass, requiring a high content of cellulose and hemicellulose and low lignin content (Woiciechowski et al., 2016). Other factors which affect ethanol yield include the development of efficient technologies and the selection of appropriate or potential recombinant or non-recombinant microorganisms (Hill et al., 2006). It is widely believed that the structure of lignocellulose is resistant to degradation due to its compositional heterogeneity, consisting of cellulose, hemicelluloses, ash, extractive and lignin (Zaldivar, 2001). The elements of plant cell walls are connected strongly through covalent and hydrogen bonds. These bonds make lignocellulosic material resistant to different methods of pre-treatment (Ruel, Nishiyama, & Joseleau, 2012). Cellulose with hemicellulose forms a holocellulose, which comprises more than half of the entire dry biomass (Zhao, Zhang, & Liu, 2012). Lignocellulosic materials mainly consist of plant cell walls. The plant cell walls are composed of energy rich polymers such as cellulose, hemicellulose and lignin (Johansson, 2013).

Cellulose

Cellulose is an unbranched crystalline structured polymer composing the cell walls of plants as well as bacteria, fungi and algae (Kucharska et al., 2018). Cellulose is surrounded by lignin. In terms of chemical structure, it is unbranched linear polymer. The length of cellulose molecules or polymers is determined by the number of glucan units in the polymer, as the amount of polymerization. The amount of polymerization of cellulose depended on the type of plant and estimated about glucan unites. Properties; cellulose depends on its chain length or degree of

polymerization, the number of glucose unites that make up one polymer molecule. The following are basic properties of cellulose (Taherzadeh & Karimi, 2007):

- It is tasteless and odourless,
- It is insoluble in water and most organic solvents,
- It is hydrophilic,
- It is biodegradable,
- It can be broken down chemically into glucose units by treating,
- Cellulose is hard to digest because it has beta 1.4 glycosidic linkages.

The long-chain cellulose polymers are linked together by hydrogen and van der Walls bonds, which cause the cellulose to be packed into micro fibrils. By forming these hydrogen bounds, the chains tend to arrange in parallel and form a crystalline structure. Therefore, cellulose microfibrils have both highly crystalline regions (around 2/3 of the total cellulose) and less-ordered amorphous regions. More ordered or crystalline cellulose is less soluble and less degradable (Mussatto & Teixeira, 2010).

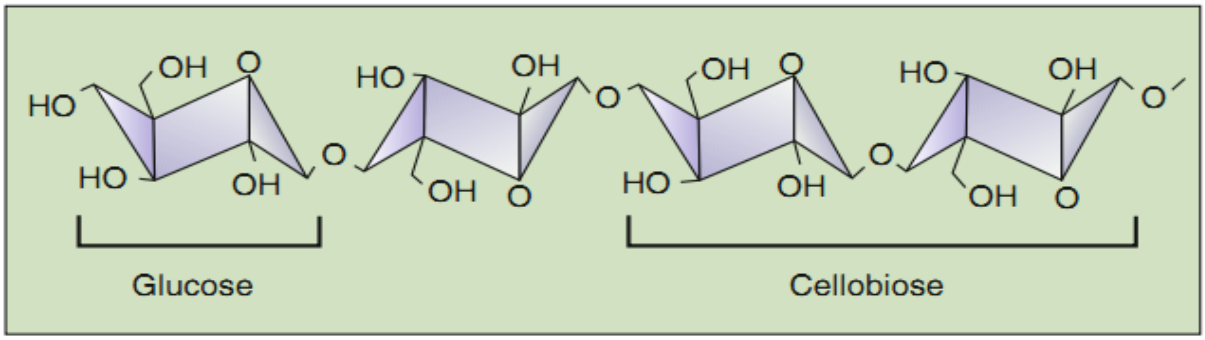


Figure 2.1 Molecular chains of structure of cellulose (Chen., 2014)

Hemicelluloses

Hemicelluloses is a complex, highly branched polysaccharide, which is found associated with cellulose and it's commonly about 30 percent of biomass weight. Unlike cellulose, hemicelluloses polymers are chemically heterogeneous, have lower degrees of polymerization and are mostly amorphous (Modenbach, 2013).

Hemicelluloses is a hetero polymer of short and branched chain sugars. Hemicelluloses are made up of different sugar units. Apart from monosaccharide's, there are sugar acids called uronic acids in the hemicelluloses fraction (Saha, Nichols, Qureshi, & Cotta, 2011).

Hemicelluloses is a branched heteropolymer composed of hexoses, pentoses, D-glucuronic acid and acetylated cultivation place and season (Kucharska et al., 2018).

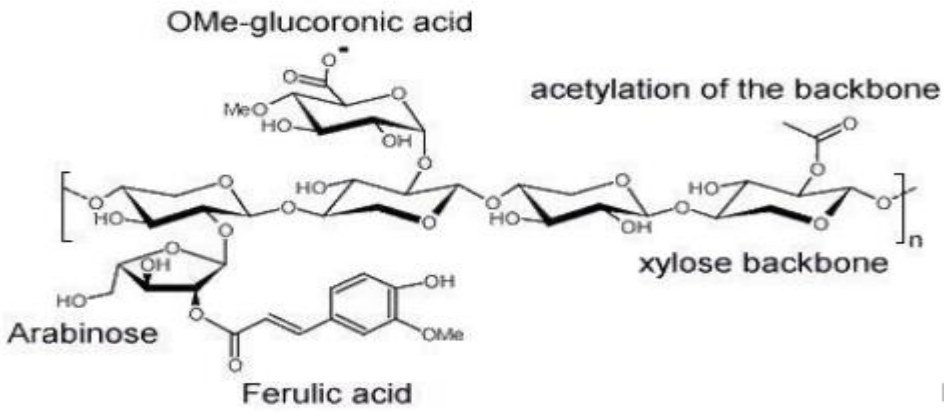


Figure 2.2 Basic structural composition of a hemicellulose (Modenbach, 2013)

Lignin

Lignin is a very complex molecule consists of phenyl propane units in a three dimensional structure and is considered a heterogeneous polymer (Chemical Composition and Structure of Natural Lignocellulose, 2014).Lignin is particularly difficult to biodegrade as it is not a desirable component in plant cell walls. Its disobedient character makes this three dimensional polymer molecule a physical obstacle to the action of enzymes. It is the most common aromatic polymer and is considered the glue that holds plants together (Hill et al., 2013).

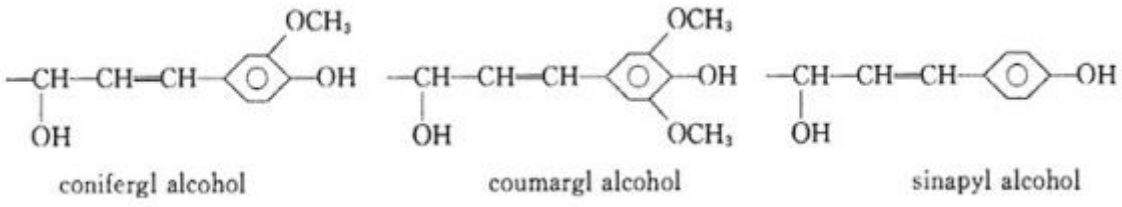


Figure 2.3 Basic structure unit of lignin (Chen., 2014)

Extractives

Extractives that are soluble in neutral organic solvents or water and wood compounds, and also usually represents a minor fraction of lignocelluloses materials. Extractives contain a large number of both lipophilic and hydrophilic constituents. It is can be classified in four groups Phenolic constituents, Inorganic components, Fates and waxes and Terpenoids and steroids (Taherzadeh and Karimi, 2007).

The amount of carbohydrate polymers and lignin vary from one plant species to another. In addition, the ratios between various constituents in a single plant may also vary with age, stage of growth, and other conditions. However, cellulose is usually the dominant structural polysaccharide of plant cell walls (35-50%), followed by hemicelluloses (20-35%) and lignin (10-20%) (Mussatto and Teixeira, 2010).

Table 2.2 Average values of the main components of lignocellulose wastes

Lignocellulosic Material	Cellulose (wt %)	Hemicellulose (wt %)	Lignin (wt %)
Wheat straw	32.90	24.00	8.90
Sunflower stalk	42.10	29.70	13.40
Sugarcane bagasse	40.00	27.00	10.00
Barely straw	33.80	21.90	13.80
Soya stalk	34.50	24.80	19.80
Corn cobs	33.70	31.90	6.10
Corn stalks	35.00	16.80	7.00
Oat straw	39.40	27.10	17.50
Rice straw	36.20	19.00	9.90
Rye straw	37.60	30.50	19.00
Cotton stalks	58.50	14.40	21.50

(Mussatto and Teixeira, 2010)

2.2.2.1 Moringa Oleifera Seeds Husk for Bioethanol Production

Moringa oleifera

Moringa oleifera is the most well known of the 13 species of trees and shrubs in the genus moringa. It is commonly known by regional names such as drumstick tree, murungai, kaai, and Benz olive: it is a rapidly growing tree that is widely cultivated and has now become naturalized in Afghanistan, Florida West Africa (Padayachee and Baijnath, 2012). Among 13 Moringa species, 5 of them were found in Ethiopia. These species of *Moringa* are widely distributed in the tropical regions. However, *M. oleifera* and *M. stenoplata* are the most well-known and documented compared to the other *Morigaceae* members (Hamza et al., 2017).

Geographical Distribution of Moringa Species

Moringa trees are highly distributed in the belt of tropics. Among 13 *Moringa* species, these *Moringa stenoplata* and *Moringa oleifera* are the most well-known species which often referred to as the Africa and Indian *Moringa* trees respectively. There are well established documents about these two *Moringa* species due to their extraordinary nutritional and medicinal properties in their ecological areas (Hamza et al., 2017). Moringa oleifera has a wide geographic range, growing from the Far East like chine along the southern Asia to England and around the western Africa and Australia. However, unlike, *M. oleifera, M. stenoplata* has limited geographical distribution which is native to Ethiopia and Kenya (Hamza et al., 2017).

Uses of different parts of Moringa Oleifera

Almost all parts of the plant which includes root, bark, seed, flowers, pods, seed oil, leaf, resin have potential food, agriculture and industrial uses. There are works which emphasize on considering *Moringa oleifera* seeds for bioethanol production as it has a high content of cellulose. The husk hydrolyzed using NaOH and fermented using saccharomyces cerevisiae produced considerable bioethanol. Bioethanol is an effective alternative of conventional fossil fuels in terms of cost, biodegradability and effects on environment (Sahay and Srinivasamurthy, 2017).

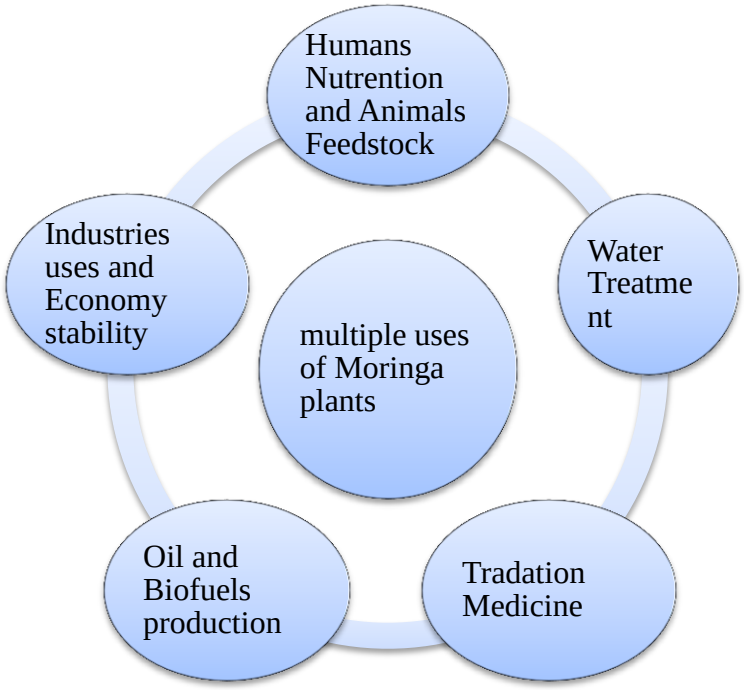


Figure 2.4 Multiple Uses of Moringa Trees (Hamza et al., 2017)

Previous research revealed that the husks of *Moringa* can be considered as potential substrates for ethanol production due to its high cellulose content which is approximately 30% (Martı and Domı, 2010). The investigation revealed that the husks of neem and *moringa* can be considered potential substrates for ethanol production due to their high cellulose content. The high cellulose content of neem and *moringa* suggests that these materials could be considered as sources of glucose for fermentative productions such as ethanol and lactic acid among others (Martı et al., 2010). The chemical composition of the husks aims to assess their potential for ethanol production. The moringa oleifera seeds husk is dried in the oven for 24 hours at temperature 80ºC to remove the moisture (Eman and Halim, 2014).

According to Ali (2017) Bioethanol produced from *Moringa oleifera* seeds husk by hydrolyzed using NaOH and fermented using *Saccharomyces cerevisiae* yeast. The highest amount of the ethanol yield was 29.69 g/L at 3 hours fermentation at temperature of 32°C, agitation speed of 180 rpm, pH 4.5 and yeast concentration of 1 g/L. This literature showed that *Moringa oleifera* seeds husk can be considered to produce bioethanol (E. N. Ali and Kemat, 2017). Moringa seeds husk is considered as one of the prospective and renewable biomass materials to produce bioethanol. The chemical composition of MOSH as described in the literature is variable containing mainly hemicellulose and cellulose (Abdullah and Yusoff, 2017) and has the

potential to serve as a low cost feedstock for the production of ethanol. The proximate analysis and composition of moringa oliefera seeds husk is presented in Table 2.3 below.

Table 2.3 Proximate analysis of *Moringa oleifera* seeds husk and composition

Parameters	Contents (%)	Content (%)
Moisture content	13.10±1.07	8.8±0.01
Ash	28.62±1.70	7.0±0.01
Extractive	12.28±0.30	-
Cellulose	28.66±0.50	50
Hemicellulose	17.34±0.19	-

(Abdullah et al., 2017) and (Thomas, 2016)

2.3 Bio ethanol Production Process

Production of ethanol from lignocellulosic

Lignocellulose consists of three main components: cellulose, lignin and hemicellulose. The first two being composed of chains of sugar molecules. Therefore, the chains can be hydrolysed to produce monomeric sugars, some of which can be fermented using ordinary baker's yeast (Galbe and Zacchi, 2002). Ethanol can be produced from lignocellulosic materials in have different ways. All processes comprise the same main components: hydrolysis of the hemicelluloses and the cellulose to monomer sugars, fermentation and product recovery. The main difference between the process alternatives is the hydrolysis steps, which can be performed by dilute acid, concentrated acid (Galbe et al., 2002a).

The production of fuel ethanol from lignocelluloses biomass and biological conversion comprises of six main steps. The basic process steps in producing ethanol from biomass are as follow (Alvira and Negro, 2010).

- Size reduction and pre-treatment
- Hydrolysis
- Fermentation
- Distillation and dewatering of the ethanol
- Denaturing of the ethanol or dehydration

2.3.1 Pre-treatment

The first step in bioconversion of lignocellosic to bioethanol is size reduction and pre-treatment (Balat Mustafa et al., 2008). The goal of the pre-treatment process is to break down the lignin structure and disrupt the crystalline structure of cellulose, so that the acids or enzymes can easily access and hydrolyze the cellulose. Pre-treatment can be the most expensive process in biomass to fuels conversion but it has great potential for improvements in efficiency and lowering of costs through further research and development. Pretreatment is an important tool for biomass to bio fuels conversion processes (Kumar, Ashish, et al., 2009).

A successful pre-treatment must meet the following requirements (Balat Mustafa et al., 2008).

i. Improve formation of sugar or the ability to subsequently form sugars by hydrolysis,
ii. Avoid degradation or loss of carbohydrate,
iii. Avoid formation of by-products inhibitory to subsequent hydrolysis and fermentation processes, and be cost effective.

Pre-treatment can be carried out in different ways such as mechanical pretreatment, steam explosion, ammonia fibber explosion, supercritical CO_2 treatment, alkali or acid pretreatment, ozone pre-treatment and biological pretreatment (Balat Mustafa et al., 2008).

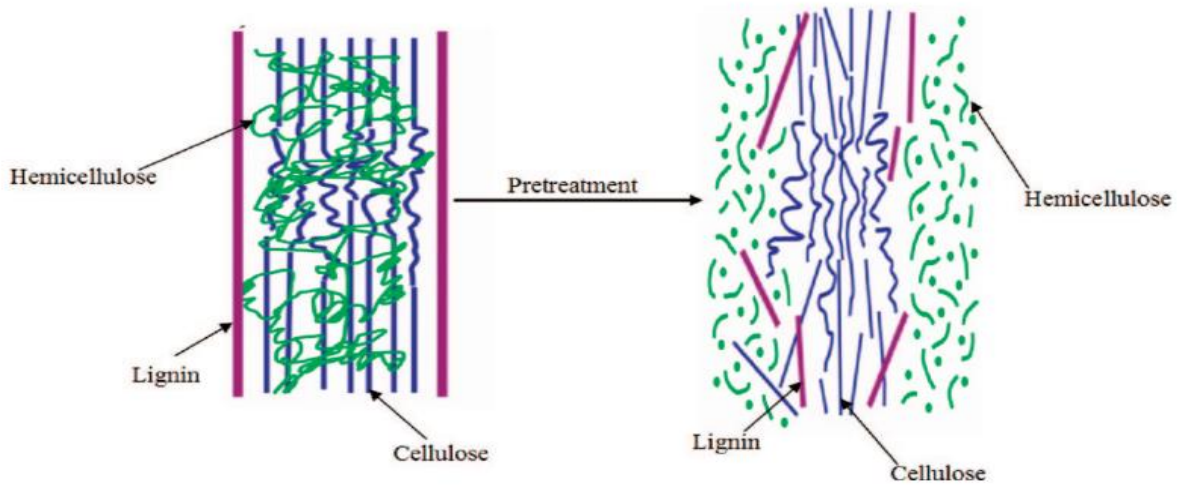


Figure 2.4 Pre-treatment in the conversion of biomass to fuel (Zhang & Shahbazi, 2011)

2.3.1.1 Physical pre-treatment

Biomass size reduction

Various mechanical size reduction methods are employed to increase the digestibility of lignocellulosic biomass such as chipping, shredding, grinding and milling. These pretreatment methods are decrease the cellulose crstallinity and the degree of polymerization as well as increase the specific surface area (TAYYAB et al., 2017). The energy demand for mechanical combination of lignocellulosic materials dependes on the agricultural biomass features and final particle size. Milling before pretreatment have the following advantages, low consumption of milling energy and no production of fermentation inhibitors (TAYYAB et al., 2017).

2.3.1.2 Physicochemical pretreatment

Ammonia fibre explosion (AFEX)

Ammonia fibre explosion is a physicochemical pre-treatment process in which lignocellulosic biomass is exposed to liquid ammonia at high temperature and pressure for a period of time, and then the pressure suddenly reduced. The AFEX process is very similar to steam explosion. In a typical AFEX process, the dosage of liquid ammonia is 1-2 kg of ammonia/kg of dry biomass, the temperature is 90 ºC, and the residence time is 30 min. AFEX pre-treatment can significantly improve the fermentation rate of various herbaceous crops and grasses. The AFEX technology has been used for the pre-treatment of many lignocellulose biomass including alfalfa, wheat straw and wheat chaff (Kumar, Parveen, et al., 2009).

Steam explosion

Steam explosion is the most commonly used method for the pretreatment of lignocellulosic materials. In this method, biomass is treated with high-pressure saturated steam and then the pressure is suddenly reduced, which makes the materials undergo an explosive decompression (Kumar, Parveen, et al., 2009). Steam explosion is typically initiated at a temperature of 160-260ºC (corresponding pressure, 0.69-4.83 MPa) for several seconds to a few minutes before the material is exposed to atmospheric pressure (Kumar, Parveen, et al., 2009).

Liquid hot water

Liquid hot water, also known as hot compressed water is same like stream pretreatment method. However, as its name indicates, water is used at high pressure up to5 Mpa and high temperature 170-230 ^{o}C instead of steam (TAYYAB et al., 2017).

2.3.1.3 Chemical pre-treatment

Alkaline pre-treatment

Alkaline pre-treatment processes utilize lower temperatures and pressures compared to other pretreatment technologies. Alkaline pretreatment time may be carried out at ambient conditions, but pretreatment tie is measured in terms of hours or days rather than minutes or seconds. The characteristic of alkaline pretreatment reactions (Balat Mustafa et al., 2008). NaOH treatment causes lignocellulosic biomass to swelling, leading to an increase in the internal surface area, a decrease in the degree of crystallinity, and disruption of the lignin structure (Balat Mustafa et al., 2008). Alkali reagents are used that are hydroxyl derivatives of sodium, calcium to these hydroxyl derivatives. However, with the alkali pre-treatment, the solubility of hemicellulose and cellulose is little as compared to acid pretreatment (Tayyab et al., 2017).

Ozonolysis pretreatment

Ozone treatment is one way of reducing the lignin content of lignocellulosic wastes. These results in an increase of the digestibility of the tested material and unlike other chemical treatments, it does not produce toxic residues. Ozone can be used to degrade lignin and hemicellulose in many lignocellulosic materials such as bagasse, wheat straw, peanut, pine, and cotton straw and popular sawdust. The degradation is mainly limited to lignin. Hemicelluloses is slightly affected, but cellulose is not (Kumar, Parveen, et al., 2009). Ozonolysis pretreatment has the following advantages: (a) it does not produce toxic residues for the downstream processes, (b) it effectively removes lignin; and (c) the reactions are carried out at room temperature and pressure. However, a large amount of ozone is required, which can make the process expensive (Kumar, Parveen, et al., 2009).

Dilute-acid pre-treatment

Acid pretreatment firstly developed in Germany in 1898. In this method concentrated and dilute mineral acids like sulfuric acid are used in order to break down hemicelluloses into monomeric sugars and simultaneously removing part of the lignin. Dilute acid hydrolysis is widely used

due to the high reaction time that can be achieved with hemicellulose which significantly improve the availability of the cellulose fraction for hydrolysis (Balat Mustafa et al., 2008).

The reaction is arrived out between 121-220^{o}C under pressure; however temperature (~121^{o}C) are optimal to reduce the formation of inhibitors. The reaction time dependent on the temperature used. This method needs a small amount of water since a small amount of energy is required to get an optimum temperature. Advantages of this method are: (a) High yield of hemicelluloses sugar, (b) Remove of lignin and hemicelluloses in this method increases exposing of cellulose to enzyme, (c) Remove of heavy metals in the raw materials. Some disadvantages of this method are: (a) Neutralization of acids is necessary,(b) Degradation of hemicelluloses sugar, (c) production of inhibitors like acetic acid and furfural, (d) High cost of reactor due to high pressure and temperature and resistance to low pH (Sanchez et al., 2004).

2.3.1.4 Biological pretreatment

Most pre-treatment technologies require expensive instruments or equipment that has high energy requirements, depending on the process. In particular, physical and thermochemical process require abundant energy for biomass conversion. Biological pretreatment employs wood degrading microorganisms, including white, brown, and soft rot fungi, and bacteria to modify the chemical composition and/or structure of the lignocellulosic biomass so that the modified biomass is more amenable to enzyme digestion. Most biological pretreatment so far has focused on the degradation of lignin in lingocellulosic biomass. However, degradation of lignin usually accompanies the loss of cellulose and hemicellulose. In order to reduce and eliminate the sugar loss during biological pretreatment, the microbial strains should have low cellulose activity. White rot fungi are the most widely studied for biological pretreatment since they can degrade lignin more effectively and more specifically. Biological pretreatment appears to be a promising technique and has very clear advantages, including no chemical requirement, low energy input, mild environmental conditions, and an environmentally friendly working manner. However, biological pretreatment is very slow (taking from weeks to a year) and requires careful control of growth conditions and a large amount of space to carry out. In addition, most lignolytic microorganisms solubilize or consume not only lignin but also hemicellulose and cellulose (Kumar, Parveen, et al., 2009).

2.3.2 Hydrolysis

Hydrolysis is a process where carbohydrate polymers are converted to simple fermentable sugars. This is facilitated through the pre-treatment process, which changes the structure of biomass, thus allow the enzymes or chemicals to enter the fibre (Alfani, Gallifuoco, Saporosi, Spera, & Cantarella, 2000). Hydrolysis is essential before fermentation to release the fermentable sugars.

Cellulose Hydrolysis → Glucose

Hemicelluloses Hydrolysis → pentose and Hexoses

The hydrolysis can be carried out in different ways, either chemically (dilute and concentrated acid) or enzymatic hydrolysis (Galbe & Zacchi, 2002b)

2.3.2.1Enzymatic hydrolysis

The second basic method of hydrolysis is enzymatic hydrolysis. Enzymes are naturally occurring plant proteins that cause certain chemical reactions to occur. Enzymatic hydrolysis is not commercialized yet but is recognized to be the most promising hydrolysis technology.

A reduction of the cost of ethanol production can be achieved by reducing the cost of either the raw materials or the cellulose enzymes. Reducing the cost of cellulose enzyme production is a key issue in the enzymatic hydrolysis of lignocellulosic materials (DEMIRBAS, 2006).

2.3.2.2 Dilute acid hydrolysis

The combination of acid and high temperature and pressure dictate special reactor materials, which can make the reactor expensive. The first reaction converts the cellulosic materials to sugar and the second reaction converts the sugars to other chemicals. Unfortunately, the conditions that cause the first reaction to occur also are the right conditions for the second to occur (DEMIRBAS, 2006).

The principle of this technique is to degrade apply temperature and pressure in order to soften lignocellulosic providing better penetration of the acid, and then carbohydrate part of wood into monosaccharide. The main purpose of this process is to degraded cellulose in to its monomer in the optimal condition of temperature, acid concentration and reaction time. Nowadays, most of

dilute acid hydrolysis are performed in a batch mode with a few minutes of retention time (Badger, 2002).

Despite all of the benefits of sulfuric acid hydrolysis, some limitations take place including high corrosion rates and expensive construction materials. Also, liquors have to be neutralized prior to fermentation of sugars, thus gypsum is formed. The large amounts of gypsum negatively influence the downstream process, and also results in a low-value by product stream. Thus, the treatment entails considerable expenses, which limits wide commercial implementation in comparison with other possible methods of hydrolysis (Kumar & Singh, 2009).

Acid hydrolysis is direct hydrolysis method used for biomass conversion. A single stage with dilute acid less than 5 per cent acid concentration is typically used rather than concentrated acid. High temperature is needed to achieve a maximum conversion for a short reaction time (Andrew, Henrietta, Elvis, E, & O, 2014). Dilute acid hydrolysis is carried out using mineral acids such as H_2SO_4 or HCl, at temperature between 120-200 ºC, time (1-30 minute), (0.5-4.4 %) (Palmqvist, 2000).

On the dilute acid hydrolysis of different lignocellulosic materials have defined optimal process conditions, temperature (80-200 ^{O}C), sulfuric acid concentration 0.25-8wt% and reaction time (10-2000 minute) (Gladyshko, 2011). Sulfuric acid is a commonly used acid due to low cost, non volatileness, productive and affordable corrosion strength (Dias et al., 2009).

In hemicelluloses hydrolysis process, under moderate temperature used (120-160 ^{O}C) and the use of dilute acid concentration (1-4%), has proven to be adequate for hemicelluloses hydrolysis, promoting little sugar decomposition (Mussatto & Teixeira, 2010).

2.3.2.3 Concentrated acid hydrolysis

Concentrated acid process provides a complete and rapid conversion of cellulose to glucose and hemicelluloses to 5-carbon sugars with little degradation. Cellulosic materials hydrolysis by concentrated sulfuric or hydrochloric acids is a relatively old process. The concentrated acid process uses relatively mild temperature and the only pressures involves are those created by pumping materials from vessels to vessel. This method uses concentrated sulfuric acid followed by a dilution with water to dissolve and hydrolyze or convert the substrate into sugar. The concentrated acid process uses 75 percent sulfuric acid at 40-50 ^{o}C for 2-4 hour in a reactor. The

low temperature and pressure will lead to minimization of the sugar degradation (Gladyshko, 2011).

Table 2.4 Comparison between Dilute and Concentrated acid Hydrolysis

Hydrolysis method	Advantages	Disadvantages
Dilute acid process	-Low acid consumption -Short residence time	-operating at high temperature -Low sugar yield -Equipment corrosion -Formation of undesirable by products
Concentrated acid process	-Operated at low temperature -High sugar yield	-High acid consumption -Equipment corrosion -High energy consumption for acid recovery -Long reaction time(e.g 2-6hr)

(Gladyshko, 2011)

2.3.3 Fermentation

Microorganisms were used for the fermenting conversion of monomeric sugars to ethanol. Different organisms can be used for the conversion such as bacteria, yeast and fungi, however the most used microorganism for fermenting in industries process are the *Saccharomyces cerevisiae* (baker's yeast), which has proved to be very robust and well suited to the fermentation of lignocellulosic hydrolysates under anaerobic condition (Galbe et al., 2002a).

The fermentation process was done in closed conical flasks at temperature 30-40°C. The optimum fermentation temperature for free cells of *Saccharomyces cerevisiae* is near 30 ºC, too high temperature kills yeast, and low temperature slows down yeast activity. Agitation rate 150-200 rpm, with different yeast concentration. The process was continued for about 2-3 days (Zabed et al., 2014).

2.3.4 Distillation

Distillation was one of the steps of purification. Distillation is the method used to separate two liquid based on their different boiling point. However, several distillations were required to

achieve high purification. Separation was carried out by simple distillation at temperature 78 ^{0}C and time for 2 hour (Onuki, Leeuwen, Jenks, & Grewell, 2008). This is done so that the ethanol fuel can be collected because the ethanol produced during fermentation was continued in a mixture of water and unfermented spent materials. The distillation was carried out to firs separate the liquid from the spent materials and then to concentrate the alcohol produced (Sources and Jimoh, 2012).

2.3.5 Dehydration

Ethanol from distillation process was sent to the molecular sieves for further dehydration to produce 99.9 per cent (v/v) ethanol. After distillation about 5% of water remains in ethanol. This water is a big problem for fuel ethanol because the presence amount of water is enhances the molecular polarity of ethanol for example ethanol and gasoline is mixed, they separate into two phase, ethanol phase and gasoline phase. It is easy to imagine that this inhomogeneous fuel is not acceptable (Onuki, 2007).

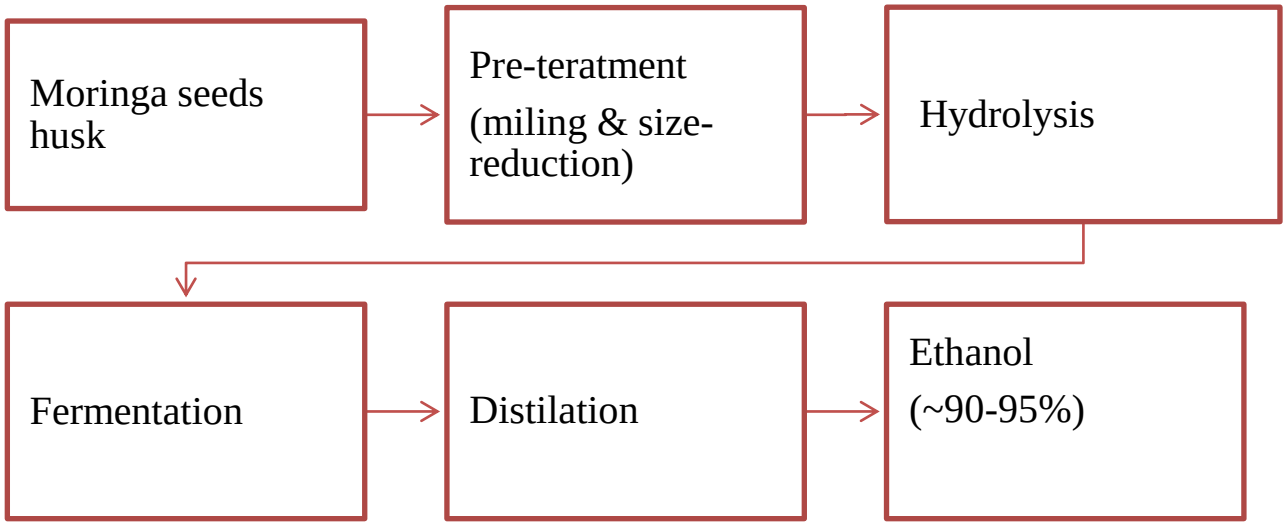


Figure 2.5 Lignocelluloses ethanol production process (Braide, Kanu, Oranusi, & Adeleye, 2016)

3. MATERIAL AND METHODS

The experimental work was done in laboratory of Addis Ababa Institute of Technology, School of Chemical and Bio-Engineering, Addis Ababa Ethiopia.

3.1 Materials and Equipment's

The following equipment's and chemicals were used during the experiment.

Chemicals: Sulphuric Acid (98% H_2SO4) was used as a hydrolysis, Sodium Hydroxide (NaOH) was used to adjust pH of soluble cellulose and hemicelluloses before fermentation, Dextrose sugar, Yeast extract, Urea and peptone was used as nutrients in media preparation, baker's yeast (Saccharomyces cerevisiae), Distilled water and Quantitative Benedict reagent solution.

Equipment's: Electronics balance to weigh samples, Graduated cylinders of different volumes for volume measurement, Shaking Incubator to shake sample and its additives after hydrolysis and before fermentation and fermentation, pH- Meter (Jenway, model 3505) to measure the pH of hydrolyzate before fermentation , muffle furnace to measure ash content of the sample, Aluminium foil, Distillation and fermentation set ups to distil and ferment respectively, Oven to dry sample, grinder to grind samples, Vacuum filter, test tubes and sieves, Vertical Autoclave for sterilization and hydrolysis, UV-Spectrophotometer and Fourier Transform Infrared spectroscopy (FTIR) to characterize the final product.

3.2 Methods

3.2.1 Characterization of Moringa Oleifera Seed Husk

Experiments were conducted to determine the moisture content and the ash content of oven dried biomass samples ground to particle size below 2.0 mm.

3.2.1.1 Proximate analysis

The properties of the sample conducted according to ASTM D 4442 for moisture content (MC) and ASTM E1755-01, Laboratory Analytical Procedure (LAP) NREL/TP-510-42622 for the ash content was determined using the following methods (Abdullah et al., 2017).

Moisture content

1 g of sample was taken in a pan weighed on a balance and placed inside a hot air oven at a temperature of 103°C for an hour. The sample was weighed at regular intervals and once the weight observed was constant, it was cooled to room temperature in desiccators. The moisture content was determined using (Materials, 2016).

$$M = \frac{W-F}{F} \times 100 \quad \text{.....} \quad (3.1)$$

Where M = moisture content in %, W = initial weight, F = final weight after draying.

Ash content

A crucible was weighed empty, and then 1 g samples were put in it. The sample and crucible were placed in muffle furnace at 575℃ for 3 hours. The crucible was removed from furnace and place in desiccators to cool for 30 minute, and then was reweighed.

$$\text{Ash } \% = \frac{W1-W2}{W3-w2} \quad \text{.....} \quad (3.2)$$

Where: W_1= weight of crucible with ash

W_2= weight of crucible

W_3 = Oven dried weight of sample

3.2.1.2 Chemical Compositional of Moringa Oleifera Seeds Husk

For the chemical compositional gravimetric method was used, because it is an economically viable and suitable method. Gravimetric analysis describes a set of method for the quantitative determination of a sample based on the mass of a solid. It does not require expensive equipment (Ayeni, Adeeyo, Oresegun, & Oladimeji, 2015).

Determination of extractive

7 g of oven dried sample was loaded into the extraction thimble. With the Soxhlet extractor set up, 150 ml of acetone was used as solvent for extraction. Residence times for the boiling and rising stages was carefully adjusted to 70ºC and 25 min respectively on the heating mental for 3 hours run period. After extraction, the sample was air dried at room temperature for few minutes. Extracted samples placed in an oven with temperature adjusted at 105°C for 20 minutes in a hot air oven until a constant dry weight was obtained.

Extractives (%) = $\frac{w1-w2}{w1} \times 100$ …… (3.3)

Where w_1= Initial weight and w_2= Weight of dry residue

Determination of lignin content

0.3 g of dried extracted seeds husk free sample was weighed placed in glass test tubes and 3 ml of 72% sulfuric acid was added. The sample was kept at room temperature for 2 hour with carefully shaking at 30 min intervals to allow for complete hydrolysis. After the initial hydrolysis, 84 ml of distilled water was added. After that, autoclave for 1 hour at 121^{O}C. The sample was cooled at room temperature. After that, the insoluble material (lignin) was filter by vacuum filtration. The lignin was washed until became acid free (with in hot water) then it was dried at 105ºC and weighed.

Lignin (%) = $\frac{O-E}{O} \times 100$ ……..…….....……. (3.4)

Where, O = oven dries sample and E= extracted residue

Determination of hemicelluloses content

For the estimation of hemicelluloses present in the biomass, 1g of sample from dried extractive was taken and 10 ml of 0.5 mol of NaOH solution was added to it. Then the solution was kept in a boiling water bath for 3 hour at 80°C. Then it was washed with distilled water until its pH was neutral. The NaOH solution of 0.5 mol was prepared by dissolving 20g of NaOH in 1 litre of distilled water.

Hemicelluloses = W_2-W_1 ……....…. (3.5)

Where, W_1, weight final and W_2, weight initial

Determination of cellulose content

The cellulose content (%w/w), was calculated by difference, assuming that extractives, hemicelluloses, lignin, ash, and cellulose are the only components of the entire biomass.

$100 = W_C + W_H + W_E + W_L + W_A$

$W_C = 100 - W_H + W_E + W_L + W_A$ ……... (3.6)

Where:

W_C, W_H, W_E, W_L, W_A are cellulose, hemicelluloses, extractive, lignin, and ash content respectively.

3.2.2 Experimental Set up and Procedures

The study was intended at investigating of acid hydrolysis parameters in the production of ethanol from *Moringa oleifera* seeds husk.

The following were the basic steps for the production of ethanol alcohol

- Sample collection.
- Size reduction to make the *Moringa oleifera* seeds husk agreeable to hydrolysis.
- Hydrolysis to break down the molecules of hemicelluloses and cellulose in to monomeric sugars.
- Fermentation of the resulting sugar solution.
- Distillation to produce alcohol.

3.2.2.1 Raw material preparation

First the raw material moringa oleifera pods was collected from Debre zeit agricultural research centre in August 2019, Bishoftu, Oromia, Ethiopia, after that the *Moringa oleifera* seeds was extracted from the pods and the seeds were manually separated to get the husk for experimental work. The husk was sun dried for three days to remove moisture. The dried sample was milled and sieved the oversize in to appropriate size which is below 2mm. The milled sample was sterilized at 121ºC for 15 min and stored at less than 4 º C refrigerators.

3.2.2.2 Dilute acid hydrolysis

The cellulose molecules which are composed of long chains are broken down to simple sugar, before it is fermented for alcohol production. Even though there are many types of hydrolysis, dilute acid hydrolysis is an easy and productive process and the amount of alcohol produced in case of acid hydrolysis is more than that of alkaline hydrolysis. The main purpose of this process was to degraded cellulose in to its monomeric sugar. Literature works on the dilute acid

hydrolysis of different lignocellulosic biomass have defined optimal process conditions for temperature, dilute acid concentration and reaction time as follows:

- Temperature 80-200 ºC,
- Sulphuric acid concentration 0.5-8%,
- Reaction time 10-2000 min (Gladyshko, 2011).

So, for this experiment the dilute acid hydrolysis started by adding 2%, 2.5%, and 3% diluted sulphuric acid to the distil water and the moringa seeds husk sample was added to each of the solution prepared. Then, the moringa oleifera husks were hydrolysing in the autoclave. The three parameter, were applied to hydrolysis step of the experimentation. The hydrolysis experiment for ethanol production and optimization were conducted in a completely randomized design using Design expert® version 11 software. 40 g of ground moringa oleifera seeds husk was used for each experiment and the factors for hydrolysis were hydrolysis temperature (120, 130 and 140 ^{O}C), time (20, 25 and 30 minute), and acid concentration (2. 2.5 and 3 % (v/v)) was used throughout the experiment.

Procedures for acid hydrolysis

- 2%, 2.5% and 3% dilute sulphuric acid was added to the non-soluble component obtained from pre-treatment steps in the order of experimental design.
- The moringa seeds husk was then hydrolyzed in the autoclave at a temperature of 120, 130 and 140ºC and at a time of 20, 25 and 30 min.
- After hydrolysis, the solid part was separated from the liquid in the hydroyzate by vacuum filtration (to remove the non-fermentable lignin portion).
- After the solid part was separated, it was washed by distilled water in order to extract all soluble sugars from the solid moringa oleifera seeds husk material.
- The filtrated hydrolyzed was neutralized with 10 M NaOH until the pH became in a range of 4.0-5.0. The pH value of filtrates was adjust to around 4.0-5.0 using NaOH (Zabed et al., 2014).

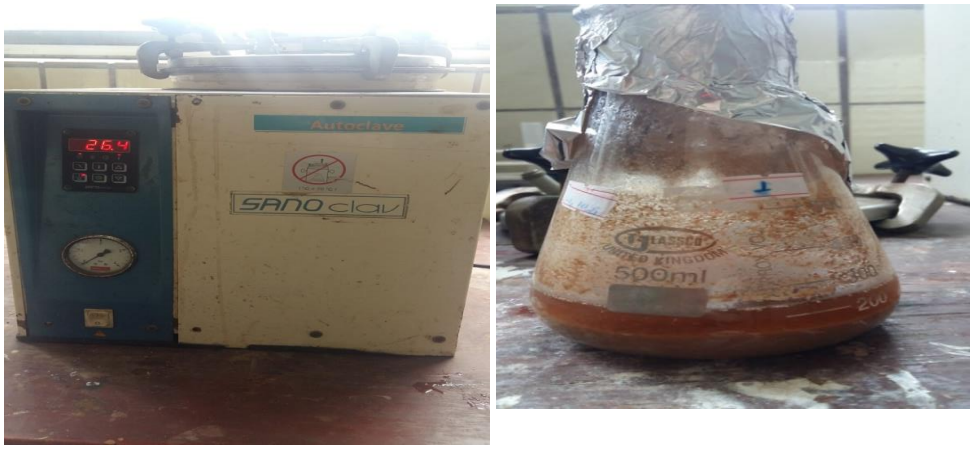


Figure 3.1 a, Hydrolysis in autoclave reactor b, Sample after hydrolysis

Filtration

The lignin and degraded cellulose which is called monomeric sugar was separated by using vacuum filtration unit. Then filtrate or sugar solution was neutralize and introduced into fermentation. The lignin which obtained this filtration was dried and weight before use for another purpose.

Figure 3.2 Vacuum filtrations unit

3.3 Measurement of Total reducing sugar content

Sugar content determination

In this case the reducing sugars are determined and cheeked by Benedict's solution.

The concentration of total reducing sugar (TRS) content of hydrolyzate which obtained from hydrolysis was determined using digital spectrophotometer by measuring absorbance Vs. Sugar concentration at 540nm wave length. The materials used in standard glucose, Benedict's solution, Test tubes, Water bath, and UV-spectrophotometer.

Benedict's solution and standard glucose solution was for assays to plot the calibration curve. Benedict's solution is designed to detect the presence of reducing sugars. In hot alkaline solution, reducing sugars reduce the blue Cooper (II) oxide ions to brick red Cooper (I) oxide precipitate. As the reaction proceeds, the color of the reaction mixture changes progressively from blue to green, yellow, orange, and red. When the conditions are carefully controlled, the colour developed and the amount of precipitate formed depends upon the amount of reducing sugars present. Hence, in most conditions, a sufficiently good estimation of the concentration of glucose-equivalent reducing sugars present in a sample can be obtained. The Copper (II) ions in the benedict's solution are reduced to copper (I) ions, which causes the colour change.

Calibration plot for glucose standard

- Standard glucose dilution series solution was prepared at different concentration of 10, 8, 6, 4, 2, 1, 0% and other six test tubes was prepared with 5ml of distill water for each test tubes..
- Pipette 0.5 mL from each of the dilution series into labelled test tubes, each containing 5 mL of Bendicate's solution, and mixed by shaking.
- All the labelled test tubes were heated at 90ºC water bath for 5 minutes.
- After that, the test tubes were removed from the water bath and filtered using filter paper to remove red precipitate formed when reducing sugar in the sample reacted with Benedict reagent.
- After filtered the precipitate, the absorbance was measured using spectrophotometer at 540nm.

- Calibration curve was plotted to show the % of absorbance of blue light by the standard glucose solution.

Determination of the total reducing sugar after hydrolysis

- Pipette 0.5 mL of each of the samples into labelled test tubes, each containing 5 mL of Benedict's solution and mixed by shaking.
- All the labelled test tubes were heated at 90ºC water bath for 5 minutes.
- The test tubes were removed from the water bath and filtered using filter paper to remove red precipitate formed when reducing sugar in the sample reacted with benedict reagent.
- After filtered the precipitate, the absorbance was measured using spectrophotometer at 540nm.
- The concentration of sugar in each samples were read from the calibration curve of the standard glucose solution.

The amount of total reducing carbohydrate present in the sample solution was calculating using the standard graph.

$Y = mx + b$ ……..... (3.7)

Where: y is absorbance

X is concentration

M is the slop and b is the intercept

Con.of unknown sample= $\frac{\text{(absorb. Of unknown sample) – (y-intercept)}}{\text{Slope}}$ ……..……. (3.8)

Reducing sugar yield (%w/w) which served as the following equation (Albarico et al., 2017)

$$\text{Yield of TRS (\%)} = \frac{\text{glucose of unknown sample}}{\text{gram of sample used}} \times \text{volume of hydrolyzate} \times 100 \quad \ldots\ldots\ldots\ldots \quad (3.9)$$

After calculating the concentration and yield of TRS of each unknown samples, the result with maximum TRS yield was selected for further process instead of using all in the next steps that consume more time and due to in availability of gas chromatography to measure the amount of

ethanol instead I was select the maximum result from hydrolysis step and that result was fermented to produce bioethanol. Experiment run 20 was selected in which maximum yield was registered in this experiment.

PH adjustment

Before addition of any microorganism to the above prepared samples, pH of these samples has to be adjusted. Otherwise the micro-organism will die in hyper acidic or basic state. A pH of around 4.0-5.0 was maintained. Pretreated and hydrolyzed solutions were mixed, shaken substrate primarily checked for pH using a digital pH meter. Since, the mixed sample was more acidic media, and then it would maintain the pH by adding sodium hydroxide solution.

Procedures in pH adjustment

- First the pH meter was calibrated by buffer solution.
- The hydrolayzate solution is acidic, so it needs highly basic solution to bring the pH in the range of 4.0-5.0.
- Sodium hydroxide solution was added drop wise to the others flask with constant stirring until the pH reaches to a range of 4.0-5.0. Then the pH was maintained 4.5 and it was stored until the next procedure.

3.2.1.3 Fermentation process

Microorganisms used for fermentation

In a fermentation process, the choice of the most optimum microorganisms and fermentation media was very important for high yield of product. The media and added chemicals as a nutrient was with the proportion of liquid/solid ration was 1:10 w/v or g/ml.

Media preparation

For preparing 100 ml media, the following were added.

Sugar dextrose 2 g, yeast extracts 1 g, Urea 1 g, and peptone 2 g. All were dissolved in 100ml of distil water in a conical flask. To the 100ml media prepared 0.5g of yeast, *Saccharomyces cerevisiae* was added in a 250ml conical flask and covered with the help of aluminium foil.

Procedures in media preparation

- The media was sterilized at 121ºC for 15 min and 0.5g of *Saccharomyces cerevisiae* were added into 100ml prepared at 250 mL conical flask.
- The conical flask were properly covered with aluminium foil and placed to a shaker incubator for 24 hours at a temperature of 30ºC and 20.

Figure 3.3 Cultured media and sterilized, sample and cultured media

Procedure for fermentation

- The hydrolyzates sample was conditioned at temperature of 30ºC. This temperature is favourable for the fermentation by which *Saccharomyces cerevisiae.*
- The pH of the sample was adjusted using 10M NaOH to make solution pH from (4.0-5.0) to establish a favourable condition for Saccharomyces cerevisiae.
- The hydrolyzate sample with 10% inoculum was placed into shaker incubator at 30ºC and 175 rpm for 3 days.
- After finishing fermentation time the sample were taken out and distilled.

3.2.1.4 Distillation

Distillation is the final step in the production of ethanol. It is a purification step. Distillation is the method used to separate two liquid based on their different boiling point. However, several distillations were required to achieve high purification. Separation was carried out by simple distillation at temperature 78 ^{0}C and time for 3 hour.

Figure 3.4 Distillation setup

Yield of ethanol

99.9% bioethanol yield from fermented sample was determined as follows;

Volume of Ethanol yield (V_{AE}) = $\frac{VHE*\rho HE*XE}{\rho AE}$……..…….. (3.10)

Where

V_{HE} = volume of hydrous ethanol formed.

V_{AE} = volume of anhydrous ethanol formed

ρ_{AE} = density of anhydrous ethanol which is 0.789g/ml

ρ_{HE} = density of anhydrous ethanol which is the sample

X_E = mass fraction of ethanol (% alcohol by weight)

3.4 FT-IR Determination of MOSH Bioethanol

The functional groups of MOSH Bioethanol were determined by using prinks Elmer spectrum 65 FTIR with the help of IR correlation charts in college of Natural science Addis Ababa University, 4kilo campus. The IR spectrum was reported by % transmittance. The wave number region for the analysis was 4000-40cm^{-1}(in the mid-infrared range). FTIR analysis was used to, identify and characterization the ethanol produced.

3.5 Experimental Design

Data analysis for this study was carried out by DESIGN EXPERT software using central composite design to evaluate the effect of the process variables: temperature (120 ºC, 130 ºC and 140ºC), reaction time (20 min, 25 min and 30 min) and acid concentration (2, 2.5 and 3% v/v). The response variable was total reducing sugar content after hydrolysis. This design of the experiment helps us to optimize process parameters using Response Surface Methodology (RSM). Response surface methodology was used to understand the effect of the factors for the optimization of the TRS yield from the dilute acid hydrolysis and bioethanol production from moringa oliefera seeds husk for the different hydrolysis variables on the glucose yield. Significance of the result was set from analysis of variance (ANOVA).

Table 3.1 Levels for the experimental design of dilute acid hydrolysis

Factor name	Unit	Coded levels		
		Min(-1)	centre point(0)	Max(1)
Acid concentration	%	2	2.5	3
Temperature	°C	120	130	140
Time	Min	20	25	30

4. RESULTS AND DISCUSSION

In this section raw material and product analysis results are presented. The experiment outcomes of those particular results were measured in the hydrolysis of cellulose to know the yield of sugar concentration. There were 20 experiments conducting by varying hydrolysis time, hydrolysis temperature and acid concentration. The amount of products obtained for each sample in the hydrolysis was measured and recorded, to select the optimum value for further process like fermentation, and distillation to obtain final product bioethanol and finally characterization of the product were analysing using FTIR and the results are shown as follows.

4.1 Characterization of Moringa oleifera Seeds Husk

4.1.1 Proximate Analysis

Table 4.1 The results of proximate analysis of the moringa seeds husk sample

Chemical composition	Weight percentage (w/w %)
Moisture	6.99
Ash	3.85
Extractive	14.57
Cellulose	38.28
Hemicellulose	14.3
Lignin	29

Literature (Abdullah, 2017) data for moringa olifera seeds husk of chemical compositions analysis of cellulose 28.66%, 17.34 % hemicellulose, 12.28% extractive, and 13.1% moisture. (Martin, 2010) also reported, 29.1% cellulose, lignin below 5% and 4.1% ash. The results from this study are in a comparable range with literatures values as reported by the a forementioned researchers. Therefore, the determination of hemicellulose and cellulose can be applied to quantify the theoretical production of bioethanol. However, yeast which is saccharomyces cerevisiae only converts glucose.

4.2 Determinations the Sugar Content of Moringa Oliefera Seeds Husk

4.2.1 Total reducing sugar content after hydrolysis

Total reducing sugar of the hydrolyzate sample was determined using calibration curve.

Preparation of standard glucose solution: preparation of standard solution is important to determining the slop and intercept of the standard one which are important in determining of the concentration of the unknown samples. The preparation step is identified in part three.

Table 4.2 concentration of standard glucose and its absorbance

Std. Glucose concentration (g/ml)	Absorbance
0	0.162
0.01	0.151
0.02	0.132
0.04	0.112
0.06	0.084
0.08	0.057
0.1	0.035

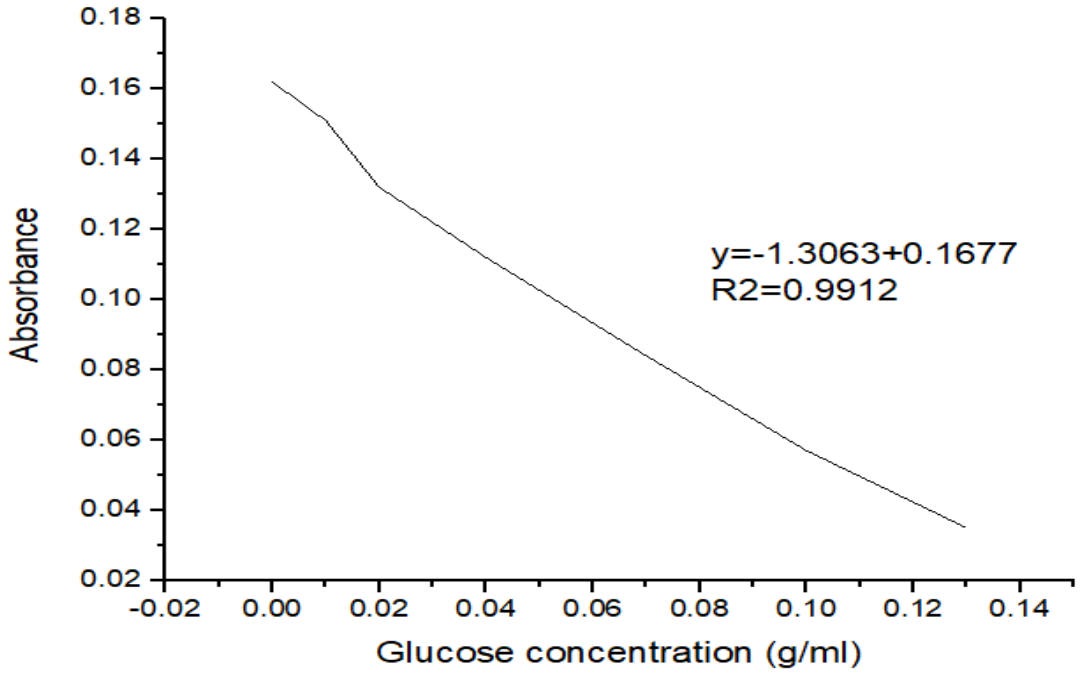


Figure 4.1 Calibration curve of glucose standard glucose concentration

The concentration of unknown sugar samples were determined from a standard curve of glucose using equation 3.8 and the yield of TRS after hydrolysis were determined using equation 3.9. ($y= -1.3063x+0.1677$; $R^2=0.9912$). The volume of hydrolyzate per sample was 400ml.

Table 4.3 The Yield of total reducing sugar (TRS) of samples

Run no.	Factor 1: Temp.(ºc)	Factor 2: Time (min)	Factor 3: Acid con. (%)	Absorbance	Glucose Con. of unknown sample (%m/v)	Yield of TRS after hydrolysis (%w/w)
1	130.00	25.00	3.34	0.097	5.41	43.6
2	120.00	20.00	2.00	0.117	4.88	36.95
3	130.00	25.00	2.50	0.105	3.89	44.75
4	130.00	25.00	2.50	0.114	5.56	44.78
5	140.00	20.00	3.00	0.123	4.1	36.36
6	130.00	16.59	2.50	0.095	5.33	37.58
7	130.00	25.00	2.50	0.096	5.49	43..25
8	130.00	25.00	2.50	0.095	5.56	44.76
9	120.00	30.00	2.00	0.135	2.5	34.1
10	146.82	25.00	2.50	0.113	4.19	35.39
11	140.00	30.00	2.00	0.11	5.49	43.67
12	130.00	25.00	1.66	0.096	4.41	40.3
13	113.18	25.00	2.50	0.12	3.65	30.75
14	130.00	25.00	2.50	0.084	6.4	44.89
15	140.00	20.00	2.00	0.086	6.25	35.36
16	140.00	30.00	3.00	0.096	5.56	43.89
17	120.00	30.00	3.00	0.117	3.88	36.18
18	120.00	20.00	3.00	0.11	5.64	42.23
19	140.00	33.41	2.50	0.101	5.1	38.55
20	130.00	25	2.50	0.084	5.64	44.98

The highest yield of total reducing sugar 44.83 %w/w (average) was obtained at 130ºC temperature, 25 min hydrolysis time and 2.5 % acid concentration (at run 3, 4, 8, 14 and 20) but the minimum yield (30.75%w/w) was obtained at 113.18ºC temperature, 25 min hydrolysis time and 2.5 % acid concentration as shown in the table 4.3 above. Because at low hydrolysis temperature, cellulose cannot convert into glucose (Balat, 2011).

According to Abdullah, 2017, the high cellulose content of moringa oliefera seeds husk suggests that this fermentative production such as ethanol, 46% by dry mass of moringa oleifera seeds husk is cellulose and hemicellulose. From this total carbohydrate (cellulose and hemicellulose) 97.46% was converted into monomeric sugar in this current investigation (Abdullah et al., 2017).

From the experiment work experiment run 20 gives maximum TRS yield (44.98%) and that yield was selected for further analysis for the production of ethanol. This sample was ferment in the fermenter and separate to produced ethanol using distillation at 78 ºC temperature and 3hrs time.

Volume of Ethanol yield (V_{AE}) = $\frac{VHE*\rho HE*XE}{\rho AE} = \frac{58.63ml*0.9987g/ml*0.05}{0.789g/ml} = 3.71ml$

The maximum sugar content 44.98%w/w and volume of hydrous ethanol after fermentation 58.63 ml, and density of hydrous ethanol 0.9987 (@ 0.9987 % alcohol by weight are 5% from Perry chemical engineering handbook chapter 2,Page 113 (Perry, Perry, Green, & Maloney, n.d.)) and the yield of ethanol was 3.71 ml/40g dry MOSH were obtained at run 20 (temperature 130ºC, 25min and 2.5 % acid concentration). After fermentation 56% sugar conversion was achieved. This indicates conversion of sugar into ethanol is small. The reason for this result is the drawback of *saccharomyces cerevisiae* which cannot able to produce ethanol from 5-carbon sugars (Galbe and Zacchi, 2002a). In contrast to this study, Ali et al.(2017) obtained that by hydrolyzed using NaOH and fermented using *Saccharomyces cerevisiae* yeast ethanol yield was 29.69 g/L (E. N. Ali and Kemat, 2017). This difference may be due to the different method of hydrolysis.

4.2.2 Statistical Analysis of the Experimental Results

4.2.2.1 Analysis of variance (ANOVA)

The analysis of variance of the quadratic regression model was a significant model, from evident of Fisher's F test with a very low probability value [(p-model > F) = 0.00001]. F-value is a test for comparing model variance with residuals (error) variance. If the variances are closed to the same, the ratio was closed to one and it is calculated by Model Mean Square divided by Residual Mean Square. Here F-value of 43.14 implies the model is significant. There is only a

0.01% chance that a "Model F-Value" this large could occur due to personal error or disturbance. From table 4.4 it was observed that the Values of "prob > F" less than 0.0500 indicates model terms are significant. In this case A, B, C, A^2, B^2, and AB are significant model terms. Values greater than 0.1000 indicates the model terms are not significant. The coefficient for the linear effect of temperature, time and acid concentration was highly significant. It was also observed that is an interaction effect between temperature and time.

The lack of fit F-value of 3.51 implies the lack of fit is not significant relative to the pure error. There is a 9.71% chance that a lack of fit F-value this large could occur due to noise. Lack of fit not significant is good.

Table 4.4 Analysis of variance (ANOVA) for Response Surface Quadratic Model

Source	Sum of Squares	DF	Mean square	F Value	p-value Prob>F
Model	372.36	9	41.37	43.14	<0.0001*
A	22.74	1	22.74	23.71	0.0007
B	5.38	1	5.38	5.61	0.0394
C	14.62	1	14.62	15.24	0.0029
A^2	206.22	1	206.22	216.70	<0.0001
B^2	58.91	1	58.62	61.60	<0.0001
C^2	6.06	1	5.96	6.27	0.0307
AB	76.51	1	76.51	80.40	<0.0001
AC	4.71	1	4.71	4.95	0.0510
BC	1.98	1	1.98	2.06	0.1813
Residual	9.52	10	0.95		
Lack of Fit	7.46	5	1.49	3.51	0.0971**
Pure Error	2.13	5	0.43		
Cor Total	381.96	19			
Significant (*)and not significant (**)					

The regression coefficient and the corresponding 95% CI (Confidence Interval) High and Low were presented in table 4.5 below. If zero was in the range High and Low 95% confidence interval, the factors has no effect. From the 95% CI High and Low values of each model term, it could be concluded that the regression coefficients of temperature, time, acid concentration and the interaction terms of temperature and time have highly significant effect in glucose production.

Table 4.5 Regression coefficient and the corresponding 95% CI High and Low

Factor	Coefficient Estimate	DF	Standard Error	95% Low	95% High	VIF
Intercept	44.51	1	0.40	43.62	45.40	
A-Temperature	1.29	1	0.26	0.70	1.88	1.00
B-Time	0.63	1	0.26	0.039	1.22	1.00
C-Acid concentration	1.03	1	0.26	0.45	1.62	1.00
A^2	-3.78	1	0.26	-4.36	-3.21	1.02
B^2	-2.02	1	0.26	-2.59	-1.44	1.02
C^2	-0.64	1	0.26	-1.22	-0.071	1.02
AB	3.09	1	0.35	2.32	3.86	1.00
AC	-0.77	1	0.35	-1.54	3.990E-003	1.00
BC	-0.50	1	0.35	-1.27	0.27	1.00

The following second order polynomial model derived to explain the yield of TRS of ethanol produced from MOSH.

The coded equation is useful for identifying the relative impact of the factors by comparing the factor coefficients. The final model equation in terms of coded factor was presented by equation 4.1.

Yield of TRS after hydrolysis = -547.67995 + 8.86391 * A -3.38390 * B + 39.86391 * C – 0.037828 * A^2 – 0.080671 * B^2 -2.57293 * C^2 +0.061850 * A *B – 0.15350 * A * C -0.19900 * B * C …… (4.1)

Where the coded values of the independent variables are;

A = temperature

B = time

C = acid concentration

Table 4.6 Model adequacy measures

Std. Dev.	0.98	R-Squared	0.9749
Mean	40.12	Adjusted R-Squared	0.9523
C.V.	2.44	Predicted R-Squared	0.8412
PRESS	60.65	Adeq Precision	18.625

The regression coefficient (R^2) quantitative evaluates the correlation between the experimental data and the predicted response. Coefficient of determination (R^2) was found to be 0.9749, which indicates that 97.49% of the experiment data were relevant and only 2.51% of the total variation was not explained by the model. In general, a high value of R^2 indicates that there is good fit between the predicted data and actual data. The "Predicted R-Squared" of 0.8412 is as closed to the "Adjusted R-Squared" of the 0.9523 is less than 0.2 difference as one might expect. The difference between Adjusted R-Squared and Predicted R-Squared is 0.111 (i.e. they are reasonably close to each other). This indicates a close fit of the model to the actual response data. "Adeq Precision" measures the signal to noise ratio due to the random error. A ratio greater than 4 is desirable. Here the ratio of 18.605 indicates an adequate signal. Therefore, the model can be used to navigate the design space.

Diagnostic plot

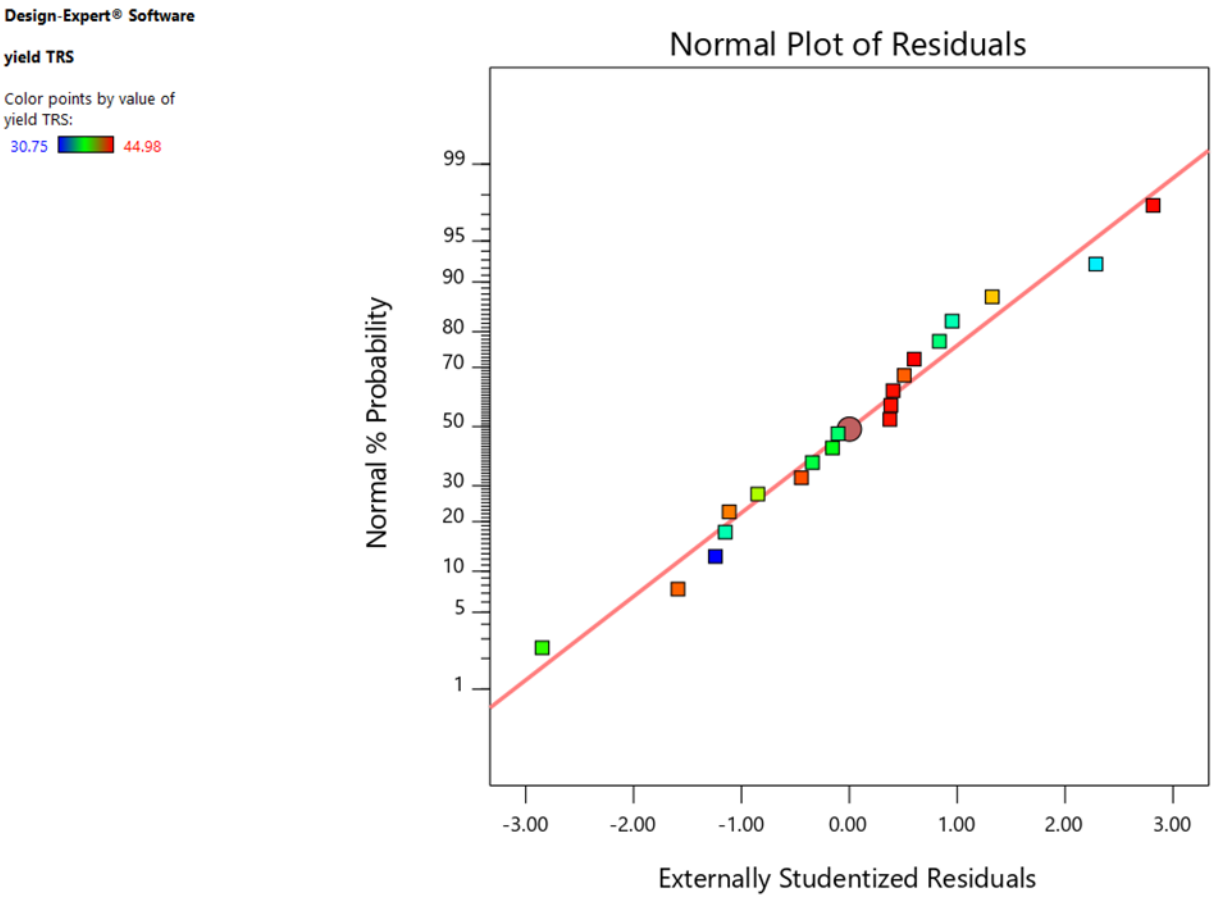


Figure 4.2 Normal plots of residuals

From the plot as shown above, the normal probability plot indicates the residuals following by the normal % probability distribution, in the case of this experimental data the points in the plots shows fitted to the straight line in the figure 4.2 i.e. the error distribution is approximately normal. This shows that the quadratic polynomial model satisfies the assumptions analysis of variance (ANOVA). The figure 4.2 indicates the residuals following a normal distribution, in which case the points follow a straight line.

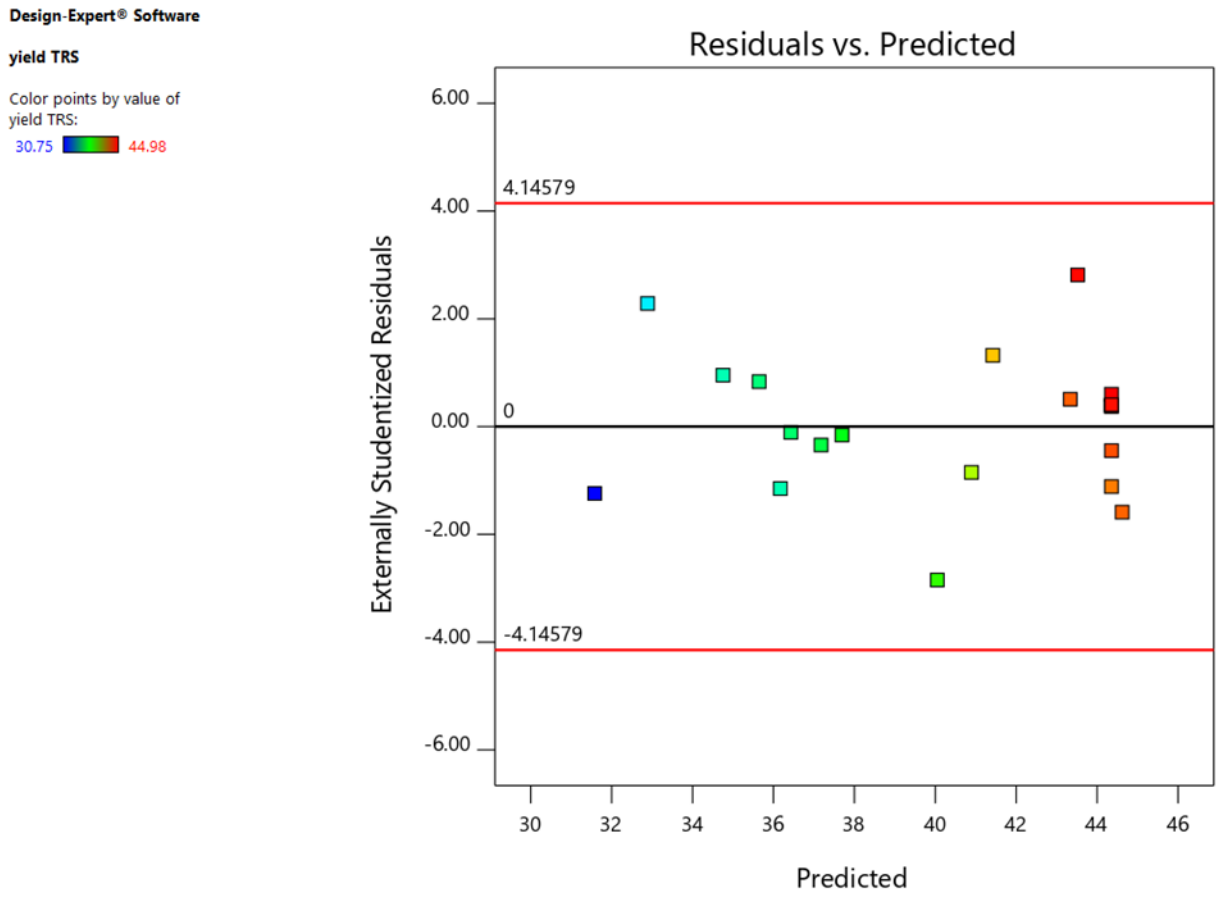


Figure 4.3 Plot of residuals versus model predicted values

If the model is correct and the assumption are satisfied, the residuals should be structure less; in particular, they should be unrelated to any other variable including the predicted response. A simple check is to plot the residuals verses the fitted (predicted) values. A plot of the residuals versus the rising predicted response values tests the assumption of constant variance. The plot figure 4.3 shows random scatter which justifying no need for an alternation to minimize personal error.

4.3 Determination of the Optimum Operating Conditions

The effects of the operating conditions on the TRS yield were investigated and the optimal values were determined in this study.

4.3.1 Effect of temperature on the TRS yield

The resulting plot of temperature versus the TRS yield, when Acid concentration and hydrolysis time were actual factors, was depicted in figure 4.4 below. From the plot as temperature increase from 120-130 ºC, TRS yield increase to 44.89 % by weight. Beyond 130ºC, decrease the yield was observed which is due to further conversion of other by product. Because at high temperature cellulose is degraded into not fermentable product such as 5-Methylhydroxy furfural (Gao, Kumar, & Wyman, 2014).

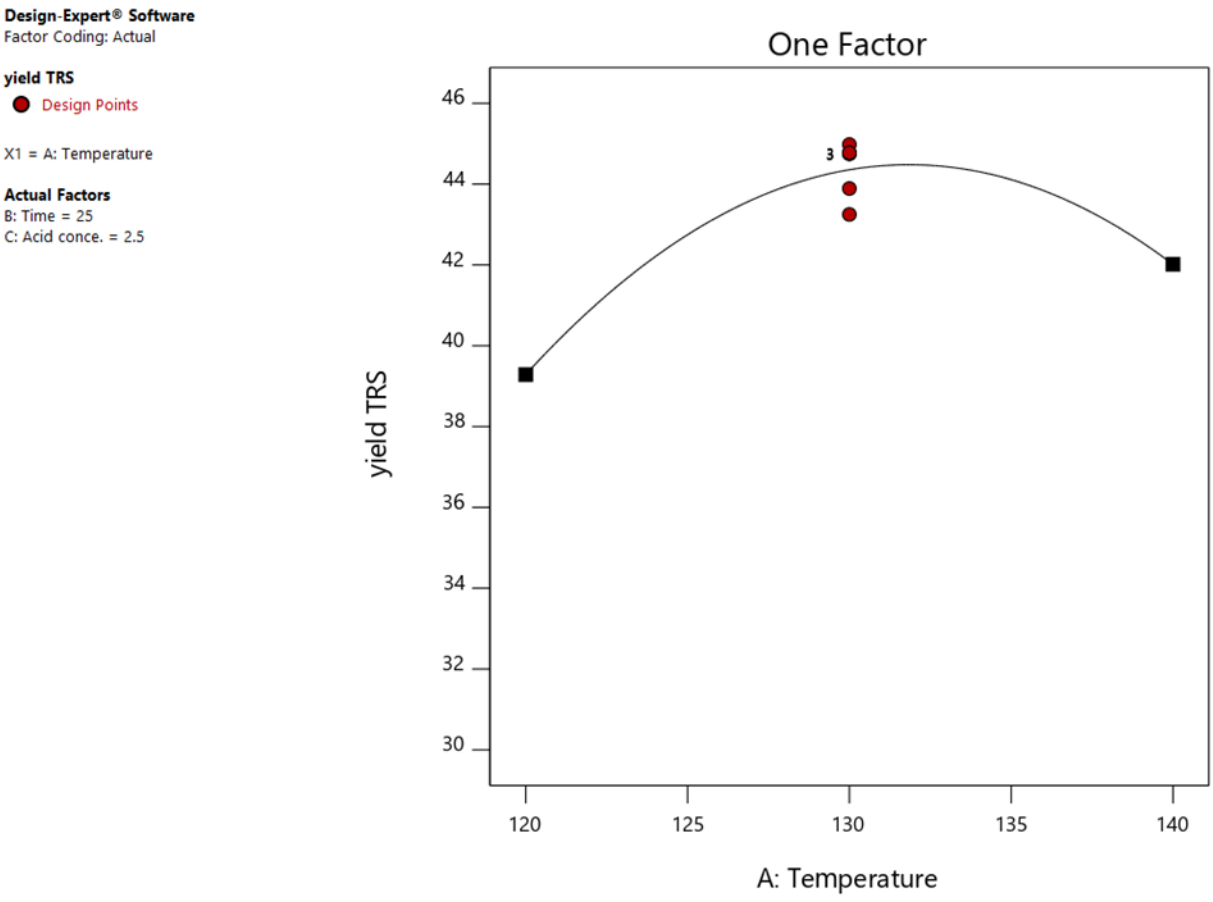


Figure 4.4 Effect of hydrolysis temperature on the yield of TRS

4.3.2 Effect of time on the TRS yield

The resulting plot of time versus the total reducing sugar yield, when Acid concentration and hydrolysis temperature were actual factors, was depicted in Figure 4.5 below. The yield of TRS is slightly affected by hydrolysis time, as the hydrolysis time increase from 20 to 25 minute the yield of TRS increase. Beyond 25 min hydrolysis time the yield of TRS slightly decreases.

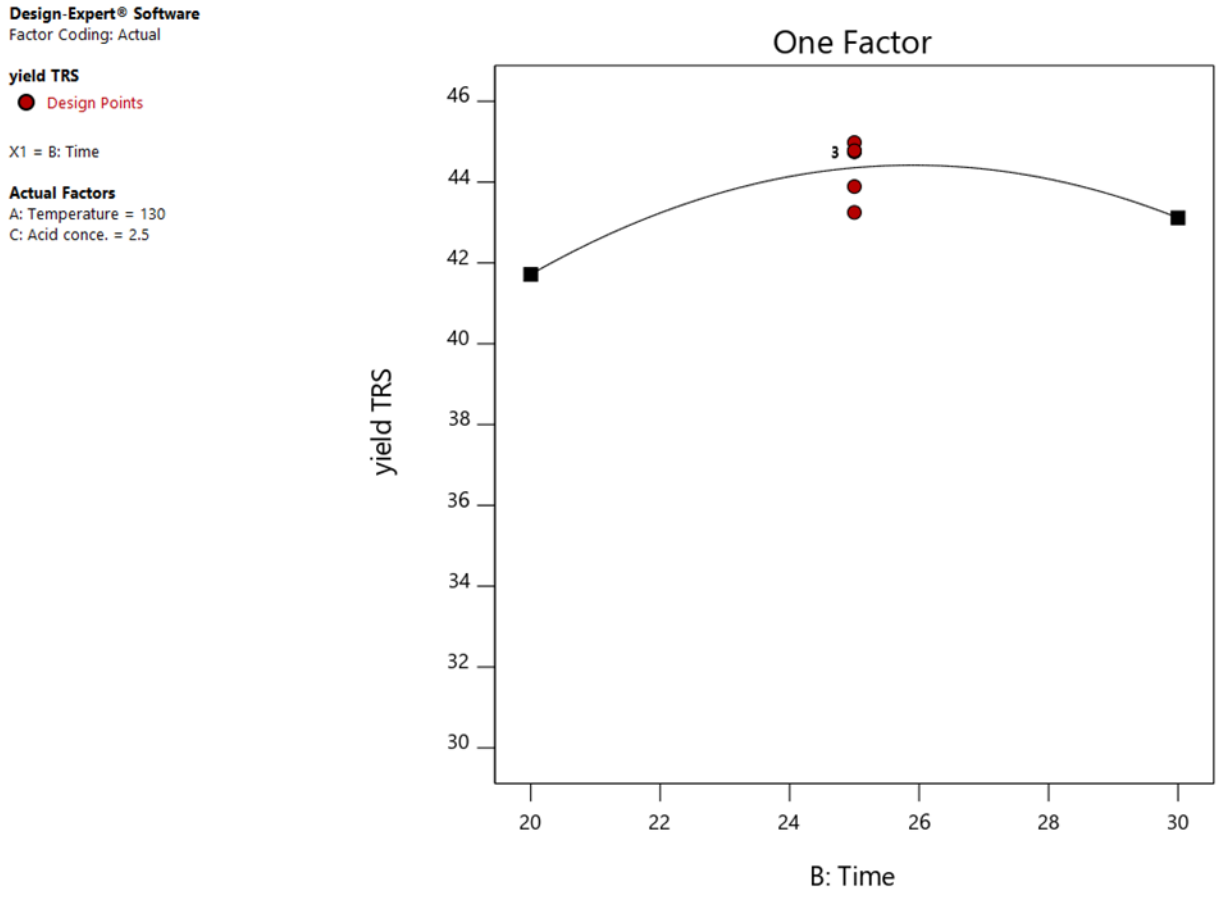


Figure 4.5 Effect of hydrolysis time on the yield of TRS

4.3.3 Effect of acid concentration

The resulting plot of acid concentration versus the TRS yield, when temperature and hydrolysis time were actual factors, was depicted in figure 4.6 below. As shown from the plot increasing acid concentration from 2% to 2.5 %, beyond 2.5 % the TRS yield slightly decreased, because high concentration of acid may decompose the sugar and producing inhibitor molecules for the fermentation process (Karimi, 2015) .

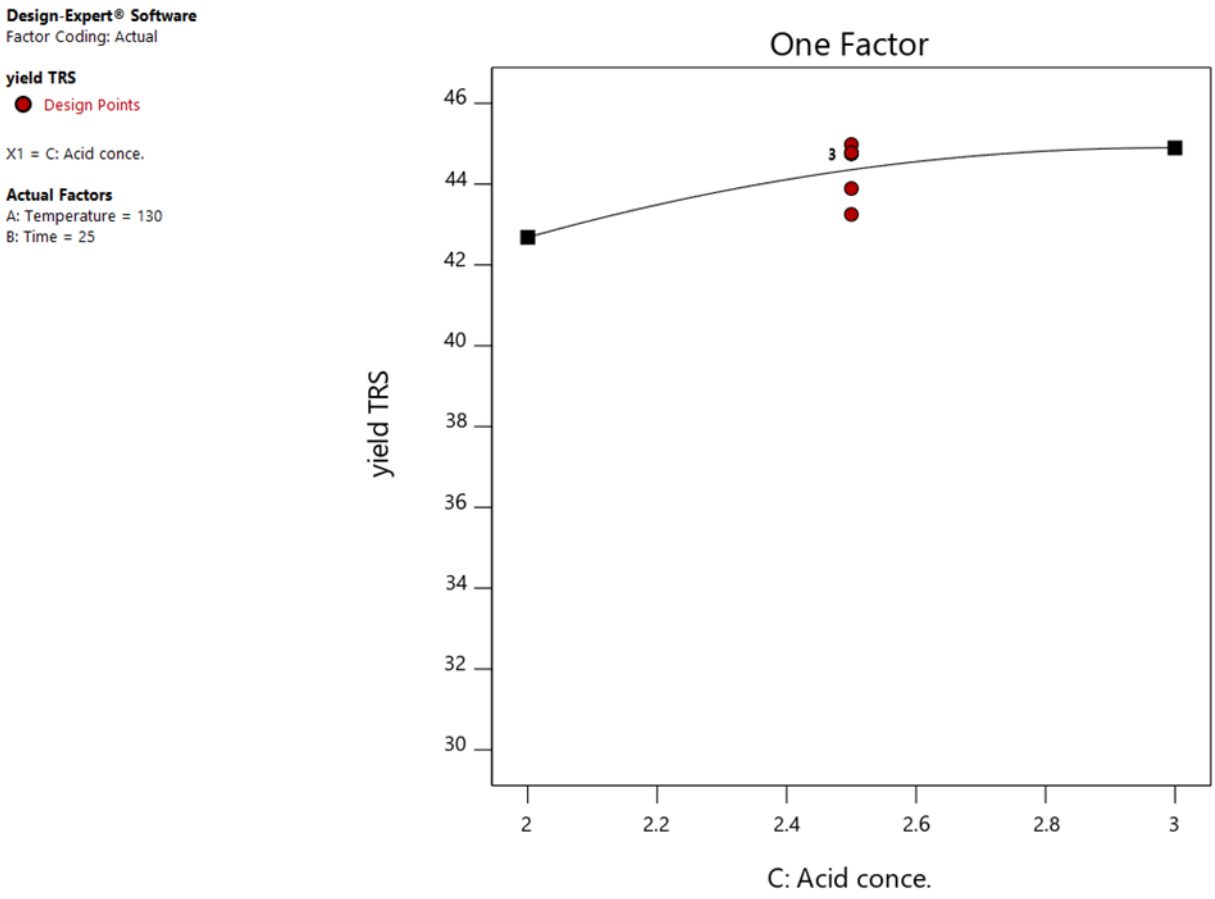


Figure 4.6 Effect of acid concentration on the yield of TRS

4.3.4 Effect of Experimental variables on acid hydrolysis

The best way of showing the effects of this parameter for the yield of total reducing sugar are to generate response surface plots of the equation. The three dimensional i.e. interactions, contours and response surface effect were plotted in the figure below.

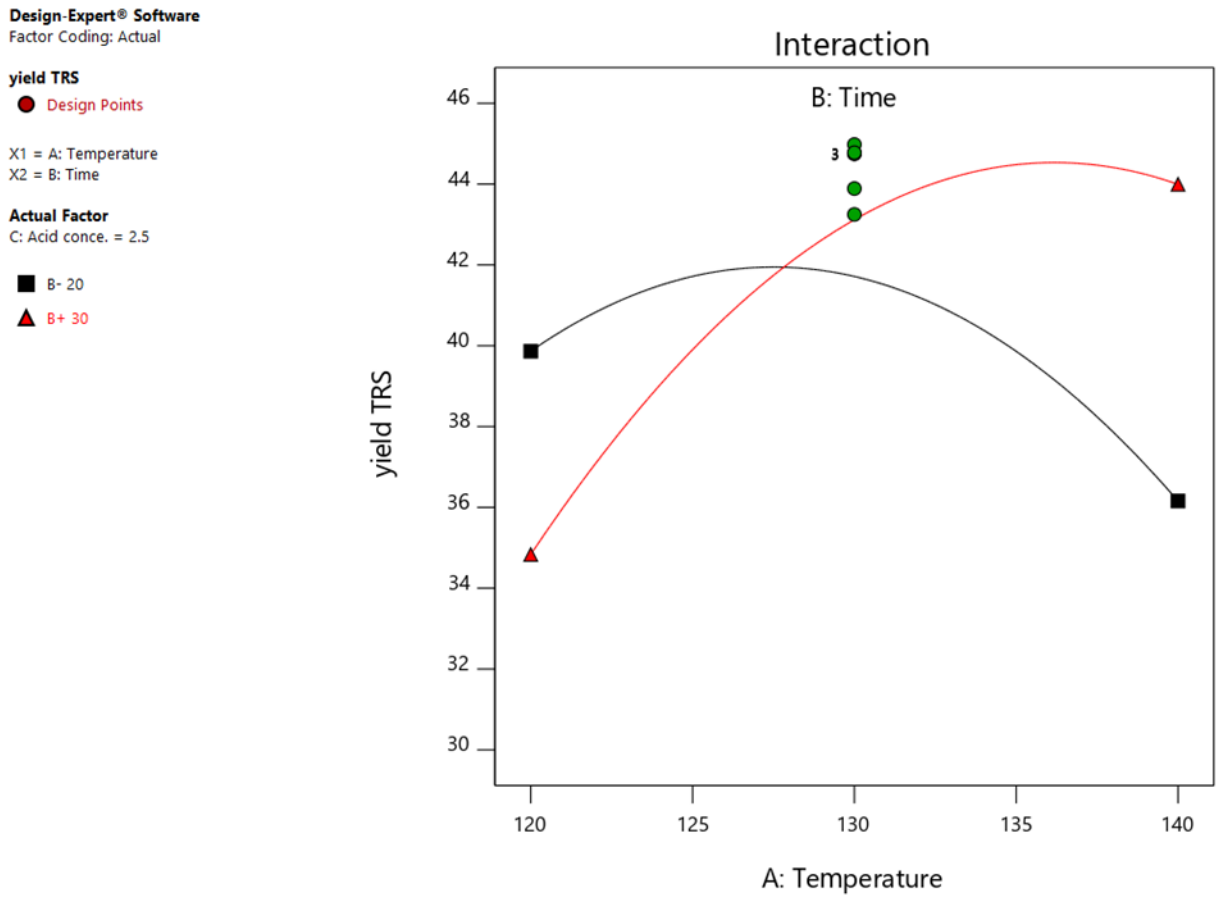


Figure 4.7 Interaction between temperature and time on the yield of TRS, when acid concentration at the centre point

Figure 4.7, 4.8 and 4.9 shows the effect of the interactions between hydrolysis temperature and time on total reducing sugars yield and the response surface and contour plots of the quadratic model. It was reported that Response surface plots provide a method to predict the yield of TRS for different parameter values of the tested variables and the interaction plots help in identification of the type of interaction between these variables. The axes of contour plot are the experimental variables and the area the axes are termed the response surface.

As we observed from figure 4.7 there is an interaction between time and temperature. Positive yield of TRS was observed at low temperature and low reaction time and also at high temperature and high time.

Figure (4.8) and (4.9) shows the response surface and contour plots respectively developed as a function of temperature and time, while the acid concentration was constant at 2.5%. It was observed that the yield of TRS after hydrolysis was more sensitive to temperature change, when the temperature changes from 120 to 130ºC the yield of TRS after hydrolysis reaches at the

peak and beyond 130º C the yield slightly decreased. The reason for this observation is due to the fact that when the cellulose expose to high temperature, the sugar which obtained from cellulose degraded into not fermentable product and gives low yield of TRS. The centre point of figure 4.8 reveals the optimal values of hydrolysis time and temperature that may be combined to obtain optimal yield of TRS. As observed from figure (4.5) there is an interaction between time and temperature at the given interval.

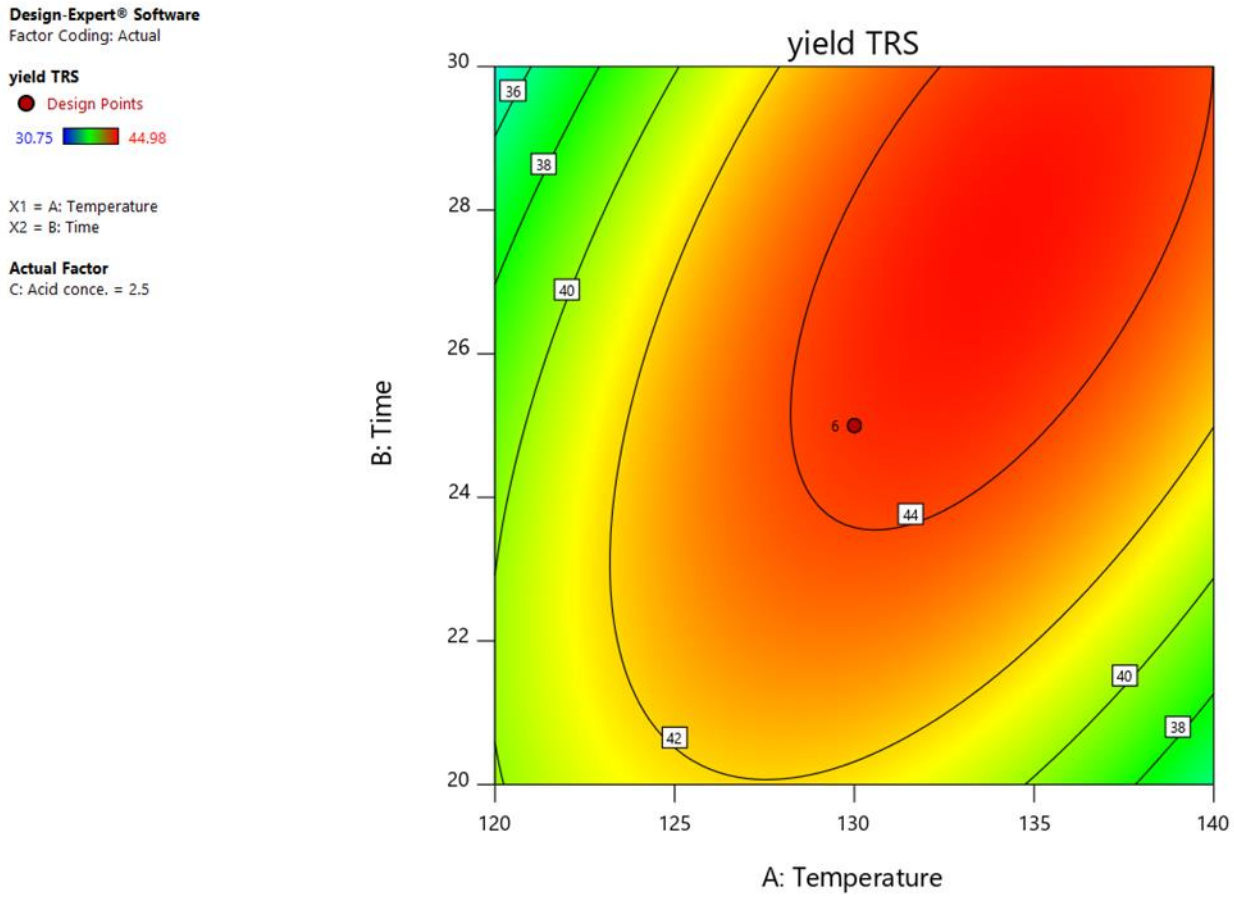


Figure 4.8 Contour plot of predicted TRS yield at fixed acid concentration

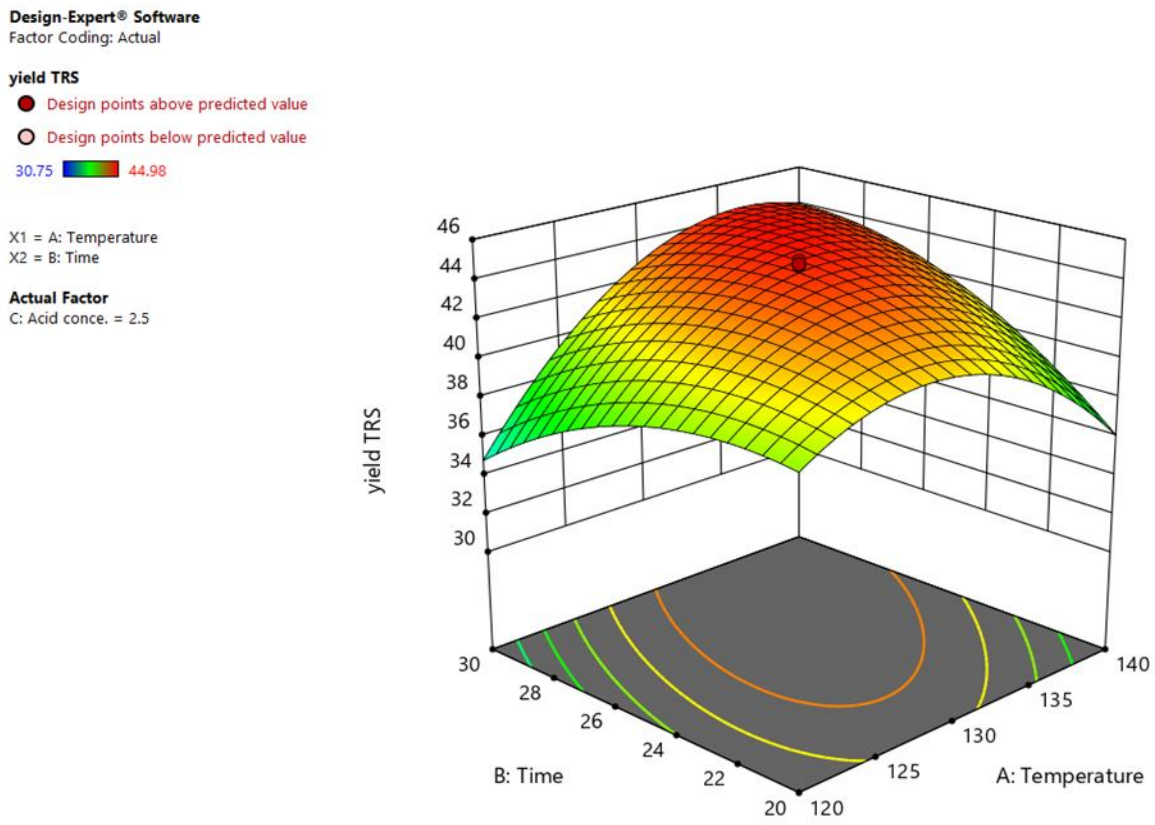


Figure 4.9 Response surfaces of predicted TRS at fixed acid concentration

These figure 4.9 shows above maximum value at the centre points (red colour) and its value decrease as its moves toward the centre (green colour). This shows hydrolysis time and temperature can highly affect to TRS yield and also it can suggest that based on the model developed there were well defined optimum operating conditions.

4.4 Optimization of operating process variables in hydrolysis process using RSM

Response surface methodology (RSM) is a collection of statistical design and mathematical optimization techniques useful for developing, improving and to optimize process and product designs (Carley, 2014). It also has important applications in the development, and formulation of new products, as well as in the improvement of existing product designs. The optimization of hydrolysis criteria for ethanol production from moringa oleifera seeds husk using dilute acid hydrolysis are summarized as follows:

Table 4.7: optimization criteria for optimum yield of TRS

Name	Goal	Lower Limit	Upper Limit
Acid concentration (%)	In range	2	3
Temperature ºC	In range	120	140
Reaction time (min)	In range	20	30
TRS (%w/w)	Maximum	30.75	44.98

Table 4.8 Optimum possible solutions

Number	Temperature	Time	Acid concen.	Yield of TRS	Desirability	
1	132.38	27.18	2.72	44.9822	1	Selected
2	132.62	26.88	2.67	45.003	1	
3	132.45	26.61	2.85	45.0135	1	
4	133.36	26.73	2.67	44.9955	1	
5	132.87	26.41	2.76	45.0258	1	
6	133.46	27.31	2.71	45.9834	1	
7	132.66	26.17	2.67	44.997	1	
8	132.87	27.00	2.71	45.0114	1	
9	132.84	26.87	2.76	45.0193	1	
10	133.29	27.04	2.75	45.0032	1	

The desirability lies between 0 and 1 and it represents the closeness of a response to its ideal value. If a response falls within the unacceptable intervals, the desirability is 0, and if a response falls within the ideal interval or the response reaches its ideal value, the desirability is 1. Based on the above analysis best local maximum for TRS yield 44.98% was found at temperature 132.38ºC, acid concentration 2.72% and 27.18 min and the value of desirability obtained was 100%.

4.5 Model Validation

According to the central composite design result using Design Expert 11 software, an experiment with hydrolysis temperature, acid concentration, and time were conducted in order to study the outcome or effect of the factorial. The experiment was carried out at the optimized conditions. As a result, the model was considered to be accurate and reliable for predicting the concentration of glucose from moringa oleifera seeds husk using dilute acid hydrolysis. The optimal values of variables were 2.72 % acid concentration, 132.38 ºC temperature and 27.18 minutes time (obtained from table 4.8). Total reducing sugar yield of 44.98 % obtained and was in good agreement with the predicted one. Therefore the model is considered to be accurate and reliable for predicting the yield of TRS.

4.6 Bioethanol Characterizations by FTIR

Alcohols have characteristic IR absorptions associated with the O-H, C-O, and the C-H stretching vibrations. When run as a liquid film the region 3570-3200cm-1 with a very intense and broad band indicated the O-H stretch of alcohols, while the region 1200-1700cm-1 confirms the C-O stretch. The bands at around 2935 and 2950 cm-1 where assigned as the symmetric stretching modes of the –CH2 and CH3 groups, respectively (Coates, 2006). This assures that the product obtained from moringa oleifera seeds husk is exactly ethanol due to the confirmation of these regions. In this study, viscosity, density and flash point of produced bioethanol were determined as shown in table 4.9 below.

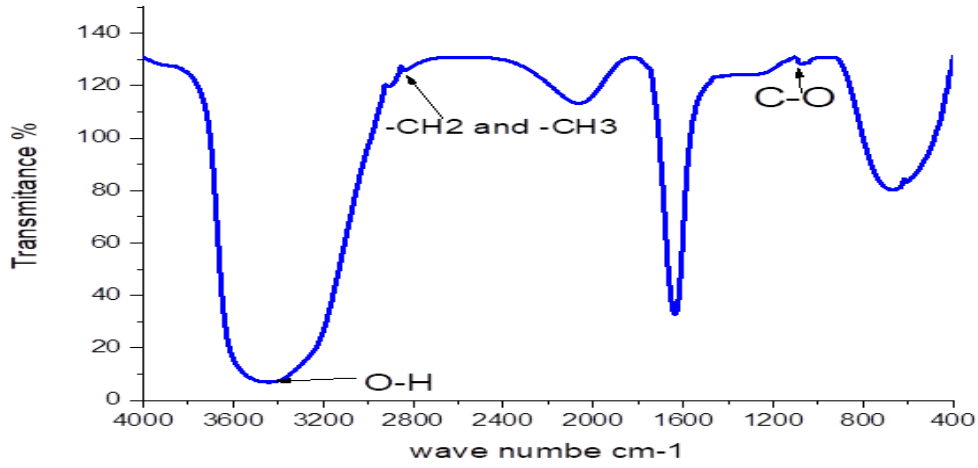


Figure 4.10 FTIR of the produced bioethanol from moringa oleifera seeds husk

Table 4.9 General properties of produced bioethanol and literature values

	Experimental values	Literatures
Appearance	Colourless liquid	Colourless liquid
Density	0.9987g/ml at 19.92 ºC	0.789kg/lit
Specific gravity	1.00006	0.79
Flash point	19ºC	18.60 ºC
Viscosity	1.23 @ 18.6 ºC	1.2

The fuel of the bioethanol produced was conducted and presented in table 4.9; the viscosity of the produced bioethanol is 1.23 which high compared to the set limit of 1.2. Viscosity determines the ease of flow of fuels through pipes. Also tested for the flash point of the produced bioethanol from moringa oiefera seeds husk. Flash point is described as the lowest temperature at which the fuel will ignite when exposed to an ignition source. The result as presented indicate the of the produced bioethanol was flash point 19 which is a bit higher compared to the literature values of 18.60, which implies that the produced bioethanol is slightly less flammable than the standard fuel. As shown the result the specific gravity of the produced bioethanol is 1.00006 which is slightly higher than recommended value 0.79.

5. CONCLUSION AND RECOMMENDATIONS

5.1 Conclusion

Due to the diminishing of fossil fuel resources, production of ethanol from lignocellulosic biomass has attained significance as a fuel for the future. This study examines the possibility of moringa oleifera seeds husk for ethanol production. As biofuels are very essential for the environment and the economy when they are produced from lignocellulosic biomass, selection of the cheap and appropriate raw material is big task. Bioethanol production from such lignocellulosic biomass was carried out in four main stages such as pre-treatment, hydrolysis, fermentation and distillations.

In this study dilute acid hydrolysis were used and the effect of the hydrolysis process variables (time, acid concentration and temperature) in the yield of total reducing sugar was investigated and optimized using response surface methodology. Based on analysis of variance (ANOVA) hydrolysis temperature, time, acid concentration and interaction between temperature and time have significant effect on the yield of total reducing sugar. Positive yield of TRS was obtained at a high acid concentration and low temperature as well as at high temperature and low acid concentration. As the result RSM optimization at 132.38ºC, 27.18 min and 2.72% hydrolysis temperature, time and acid concentration, respectively afforded 44.98 %w/w yield of TRS and 3.7ml/40g of moringa oleifera seeds husk yield of ethanol. All points were located near to the centre point of the design. Based on this study, it is evident that the chosen method of optimization was efficient, and reliable. From this result it can be concluded that moringa oleifera seeds husk has the potential to serve as a low cost feedstock for the production of ethanol and dilute acid hydrolysis process is very effective. The chemical properties of the bioethanol like density, viscosity, flash point and specific gravity were determined and obtained comparable results from literature. Chemical characterization of the bio-ethanol produced was performed using FTIR. From result, it was observed that the ethanol produced from moringa seeds husk contains O-H, C-O, -CH_2 and CH_3 functional groups; which confirm the presence of ethanol in the product.

5.2 Recommendation

Producing ethanol from renewable resources is becoming an important issue for the whole world. Therefore, the work needs to be continued for scaling up of ethanol production from moringa oleifera seeds husk. Future studies should include optimization of pretreatment process, optimization of fermentation process and optimization of distillation process variables to obtain maximum yield of bio-ethanol from moringa oleifera seeds husk. Finally, it is recommended that further researches should be done on bioethanol production from different types of moringa seeds husk such as moringa oleifera seeds husk and moringa sethnopelata seeds husk. Also recommended that further researchers should be done to compare between manual de husking and mechanical de husking of the moringa seeds husk.

REFERENCES

Abdullah, N. S., Hussin, M. H., Sharifuddin, S. S., Azroie, M., & Yusoff, M. (2017). *PREPARATION AND CHARACTERIZATION OF ACTIVATED CARBON FROM Moringa Oleifera SEED POD*. *29*(1), 7–11.

Albarico, J. S., Detras, M. C. M., Sanchez, P. R. P., Alfafara, C. G., Borines, M. G., Nayve, F. R. P., … Ventura, J. S. (2017). *Yield Optimization of Reducing Sugars from Acid Hydrolysis of Chlorella vulgaris Waste Biomass*. (2013).

Alfani, F., Gallifuoco, A., Saporosi, A., Spera, A., & Cantarella, M. (2000). *Comparison of SHF and SSF processes for the bioconversion of steam-exploded wheat straw*. 25–26.

Ali, E. N., & Kemat, S. Z. (2017). Bioethanol produced from Moringa oleifera seeds husk. *IOP Conference Series: Materials Science and Engineering*. https://doi.org/10.1088/1757-899X/206/1/012019

Ali, Eman N, & Halim, M. S. B. A. (2014). *Comparison of bioethanol yield produced from moringa oliefra seeds husk removed manually and mechanically* (pp. 1–6). pp. 1–6.

Alvira, P., Ballesteros, M., & Negro, M. J. (2010). Bioresource Technology Pretreatment technologies for an efficient bioethanol production process based on enzymatic hydrolysis : A review. *Bioresource Technology*, *101*(13), 4851–4861. https://doi.org/10.1016/j.biortech.2009.11.093

Andrew, A. N., Henrietta, I., Elvis, A., E, O. S., & O, O. C. (2014). *MODELLING AND OPTIMISATION OF DILUTE ACID HYDROLYSIS OF CORN STOVER USING BOX-BEHNKEN DESIGN*. *9*(4), 443–454.

Ayeni, A. O., Adeeyo, O. A., Oresegun, O. M., & Oladimeji, T. E. (2015). Compositional analysis of lignocellulosic materials: Evaluation of an economically viable method suitable for woody and non-woody biomass. *American Journal of Engineering Research*. Retrieved from www.ajer.org

Badger, P. C. (2002). *Ethanol From Cellulose : A General Review*. 17–21.

Balat, M. (2011). Production of bioethanol from lignocellulosic materials via the biochemical

pathway : A review. *Energy Conversion and Management*, *52*(2), 858–875. https://doi.org/10.1016/j.enconman.2010.08.013

Balat Mustafa, Balat Havva, & Öz, C. (2008). Progress in bioethanol processing. *Progress in Energy and Combustion Science*. https://doi.org/10.1016/j.pecs.2007.11.001

Braide, W., Kanu, I. A., Oranusi, U. S., & Adeleye, S. A. (2016). *PRODUCTION OF BIOETHANOL FROM AGRICULTURAL WASTE*. (December 2017), 372–386. https://doi.org/10.4314/jfas.v8i2.14

Bušić, A., Morzak, G., Belskaya, H., & Šantek, I. (2018). *Bioethanol Production from Renewable Raw Materials and Its Separation and Purification : A Review*. *9818*, 1–23. https://doi.org/10.17113/ftb.56.03.18.5546

Carley, K. M. (2014). *Response Surface Methodology*. (May).

Chemical Composition and Structure of Natural Lignocellulose. (2014). https://doi.org/10.1007/978-94-007-6898-7

Chen., H. (2014). *Chemical Composition and Structure of Natural Lignocellulose*. https://doi.org/10.1007/978-94-007-6898-7

Coates, J. (2006). *Interpretation of Infrared Spectra , A Practical Approach*. 1–23. https://doi.org/10.1002/9780470027318.a5606

DEMIRBAS, A. (2006). *Bioethanol from Cellulosic Materials : A Renewable Motor Fuel from Biomass*. (June 2013), 327–337. https://doi.org/10.1080/00908310390266643

Dias, M. O. S., Ensinas, A. V, Nebra, S. A., Maciel, R., Rossell, C. E. V, Regina, M., & Maciel, W. (2009). *Chemical Engineering Research and Design Production of bioethanol and other bio-based materials from sugarcane bagasse : Integration to conventional bioethanol production process*. *7*(October 2008), 1206–1216. https://doi.org/10.1016/j.cherd.2009.06.020

Domínguez-bocanegra, Rosa, A., Torres-muñoz, J., Antonio, O., & Aguilar, R. (2015). *Production of Bioethanol from agro-industrial wastes*. *149*, 85–89. https://doi.org/10.1016/j.fuel.2014.09.062

El-hack, M. E. A., Alagawany, M., Elrys, A. S., Desoky, E. M., Tolba, H. M. N., Elnahal, A. S. M., … Swelum, A. A. (2018). *Effect of Forage Moringa oleifera L . (moringa) on Animal Health and Nutrition and Its Beneficial Applications in Soil , Plants and Water Purification*. 1–22. https://doi.org/10.3390/agriculture8090145

Flores, G. (2018). *The characteristics of bioethanol fuel made of vegetable raw materials The characteristics of bioethanol fuel made of vegetable raw materials*. 1–7. https://doi.org/10.1088/1757-899X/296/1/012019

Galbe, A., & Zacchi. (2002a). *A review of the production of ethanol from softwood*. 618–628. https://doi.org/10.1007/s00253-002-1058-9

Galbe, A., & Zacchi, G. (2002b). *A review of the production of ethanol from softwood*. 618–619. https://doi.org/10.1007/s00253-002-1058-9

Gao, X., Kumar, R., & Wyman, C. E. (2014). *Fast Hemicellulose Quantification Via a Simple One-Step Acid Hydrolysis*. *111*(6), 1088–1096. https://doi.org/10.1002/bit.25174

Gladyshko, Y. (2011). *EXTRACTION OF HEMICELLULOSES BY ACID CATALYZED HYDROLYSIS*. 1–45.

Hamza, T. A., & Azmach, N. N. (2017). *The miraculous moringa trees : From nutritional and medicinal point of views in tropical regions*. *5*(4), 151–162.

Hill, J., Nelson, E., Tilman, D., Polasky, S., & Tiffany, D. (2006). *Environmental , economic , and energetic costs and benefits of biodiesel and ethanol biofuels*.

Johansson, E. (2013). *Fermentation of lignocellulosic material : A study on bacterial contamination and yeast physiology*.

Karimi, M. J. A. T. (2015). *Acid-based hydrolysis processes for ethanol from lignocellulosic materials: a review*. *2*(2007), 472–499.

Kucharska, K., Rybarczyk, P., Hołowacz, I., & Łukajtis, R. (2018). *Pretreatment of Lignocellulosic Materials as Substrates for Fermentation Processes*. 1–32. https://doi.org/10.3390/molecules23112937

Kumar, Ashish, Singh, K, L., Ghosh, & Sanjoy. (2009). Bioresource Technology Bioconversion of lignocellulosic fraction of water-hyacinth (Eichhornia crassipes) hemicellulose acid hydrolysate to ethanol by Pichia stipitis. *Bioresource Technology*, *100*(13), 3293–3297. https://doi.org/10.1016/j.biortech.2009.02.023

Kumar, Parveen, Barrett, M, D., Delwiche, M. J., Stroeve, P., … Stroeve, P. (2009). *Methods for Pretreatment of Lignocellulosic Biomass for Efficient Hydrolysis and Biofuel Production Methods for Pretreatment of Lignocellulosic Biomass for Efficient Hydrolysis and Biofuel Production*. https://doi.org/10.1021/ie801542g

Kumar, S., & Singh, S. P. (2009). *Recent Advances in Production of Bioethanol from Lignocellulosic Biomass*. (4), 517–526. https://doi.org/10.1002/ceat.200800442

Mahapatra, S., & Manian, R. P. (2016). *Review Article Bioethanol from Lignocellulosic Feedstock : A Review*. *8*(9), 31–41.

Martı, C., Parajo, J. C., & Domı, H. (2010). *Fractional characterisation of jatropha , neem , moringa , trisperma , castor and candlenut seeds as potential feedstocks for biodiesel production in Cuba*. *34*, 533–538. https://doi.org/10.1016/j.biombioe.2009.12.019

Materials, W. (2016). *Standard Test Methods for Direct Moisture Content Measurement of Wood and*. *04*(Reapproved 2003), 1–6.

Member, A. A. S., Afolabi, A. S., & Ogochukwu, M. U. (2015). *Production and Characterization of Bioethanol from Sugarcane Bagasse as Alternative Energy Sources*. *II*, 1–5.

Modenbach, A. (2013). *Sodium hydroxide pretreatment of corn stover and subsequent enzymatic hydrolysis : An investigation of yields , kinetic modeling and glucose recovery*.

Mussatto, S. I., & Teixeira, J. A. (2010). *Lignocellulose as raw material in fermentation processes*. 897–907.

Onuki, S. (2007). *Bioethanol : Industrial production process and recent studies Shinnosuke Onuki*. 1–12.

Onuki, S., Leeuwen, J. Van, Jenks, W. S., & Grewell, D. A. (2008). *Ethanol production ,*

purification , and analysis techniques : a review.

Padayachee, B., & Baijnath, H. (2012). *An overview of the medicinal importance of Moringaceae*. *6*(48), 5831–5839. https://doi.org/10.5897/JMPR12.1187

Palmqvist, E. (2000). *Fermentation of lignocellulosic hydrolysates . II : inhibitors and mechanisms of inhibition*. *74*, 25–33.

Pandey, A., Soccol, C. R., Nigam, P., & Soccol, V. T. (2000). *Biotechnological potential of agro-industrial residues . I : sugarcane bagasse*. *74*.

Perry, S., Perry, R. H., Green, D. W., & Maloney, J. O. (n.d.). *CHEMICAL ENGINEERS ' HANDBOOK SEVENTH*.

Ruel, K., Nishiyama, Y., & Joseleau, J. (2012). Plant Science Crystalline and amorphous cellulose in the secondary walls of Arabidopsis. *Plant Science*, *193–194*, 48–61. https://doi.org/10.1016/j.plantsci.2012.05.008

Saha, B. C., Nichols, N. N., Qureshi, N., & Cotta, M. A. (2011). *Comparison of separate hydrolysis and fermentation and simultaneous saccharification and fermentation processes for ethanol production from wheat straw by recombinant Escherichia coli strain FBR5*. 865–874. https://doi.org/10.1007/s00253-011-3600-0

Sahay, S., Yadav, U., & Srinivasamurthy, S. (2017). *Potential of Moringa oleifera as a functional food ingredient : A review*. *2*(5), 31–37.

Sanchez, G., Pilcher, L., Roslander, C., Modig, T., Galbe, M., & Liden, G. (2004). *Dilute-acid hydrolysis for fermentation of the Bolivian straw material Paja Brava*. *93*, 249–256. https://doi.org/10.1016/j.biortech.2003.11.003

Sources, E., Recovery, P. A., Uthman, H., & Jimoh, A. (2012). *Energy Sources , Part A : Recovery , Utilization , and Environmental Effects The Production and Characterization of Ethanol Fuel from Agricultural Products as an Alternative Fuel for Gasoline*. (July 2016), 1–9. https://doi.org/10.1080/15567036.2010.494095

Sun, Y., & Cheng, J. (2002). *Hydrolysis of lignocellulosic materials for ethanol production : a review q*. *83*, 1–11.

Taherzadeh, M. J., & Karimi, K. (2007). *Acid-based hydrolysis processes for ethanol from lignocellulosic materials: A review*.

TAYYAB, M., NOMAN, A., ISLAM, W., WAHEED, S., … ALI, F. (2017). *BIOETHANOL PRODUCTION FROM LIGNOCELLULOSIC BIOMASS BY ENVIRONMENT-FRIENDLY PRETREATMENT METHODS : A REVIEW*. *16*(1), 225–249.

Thomas, A. O. (2016). *PROTEIN QUALITY AND PATHOLOGICAL EFFECTS OF Moringa oleifera (Syns Moringa pterygosperma Gaertn) SEED AND HUSK ON ALBINO RAT ORGANS*. *11*(5), 193–197.

Wang, J., & Chen, C. (2006). *Biosorption of heavy metals by Saccharomyces cerevisiae : A review*. *24*, 427–451. https://doi.org/10.1016/j.biotechadv.2006.03.001

Woiciechowski, A. L., Bianchi, A., Medeiros, P., Rodrigues, C., Porto, L., & Vandenberghe, D. S. (2016). *Feedstocks for Biofuels*. https://doi.org/10.1007/978-3-319-30205-8

Zabed, H., Faruq, G., Sahu, J. N., Azirun, M. S., Hashim, R., & Boyce, A. N. (2014). *Bioethanol Production from Fermentable Sugar Juice*. *2014*.

Zaldivar, M. J. (2001). *Fuel ethanol production from lignocellulose : a challenge for metabolic engineering and process integration*. 17–34. https://doi.org/10.1007/s002530100624

Zhang, B., & Shahbazi, A. (2011). *Recent Developments in Pretreatment Technologies for Production of*. *2*(2). https://doi.org/10.4172/2157-7463.1000108

Zhao, X., Zhang, L., & Liu, D. (2012). *Biomass recalcitrance . Part I : the chemical compositions and physical structures affecting the enzymatic hydrolysis of lignocellulose*. https://doi.org/10.1002/bbb

APPPENDICES

Appendix A: Laboratory equipment's and sample photos

A. Moringa oliefera tree

B.moringa oliefera pods

C. moringa oliefera seeds

Fig1: Dry moringa oleifera seeds husk

Fig 2**:** Grinding Machine

Fig 3: sieve analysis

Fig 4: powder sample

fig 5: water distiller unit

Fig 6: sulfuric acid

Fig 7: before hydrolysis

Fig 8: sample ready for hydrolysis

Fig 9: autoclave unit

Fig 10: hydrolyzate sample

Fig 11: vacuum filtration

Fig 12: filtered sample

Fig 13: Sterilization machine

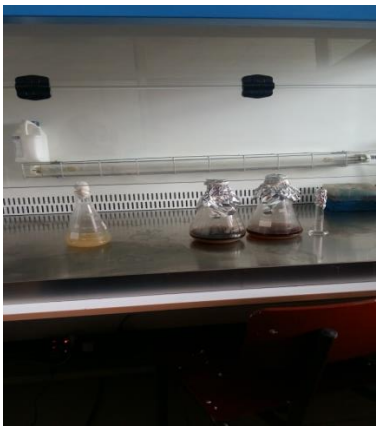


Fig 14: Media after sterilization

Fig 15: pH adjacent

Fig 16: Shaker incubator

Fig 17: Distillation set up

Fig 18: produced ethanol

Fig 19: Spectrophotometer

Fig 20: sample after mixing with benedicts

Fig 21: reaction of reducing sugar with benedicts solution at 90ºC water bath

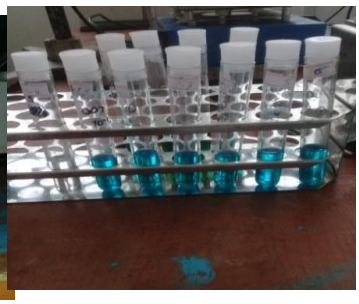


Fig 22: Hydrolyzate sample after reaction with benedict solution and Fig 23: sample of standard glucose after reaction with benedict solution, Fig 24: standard glucose with dedicates at 90 ºC water bath.

Fig 25: desiccator

Fig 26: muffle furnace

Fig 27: Density measurement

Fig 28: Viscometer

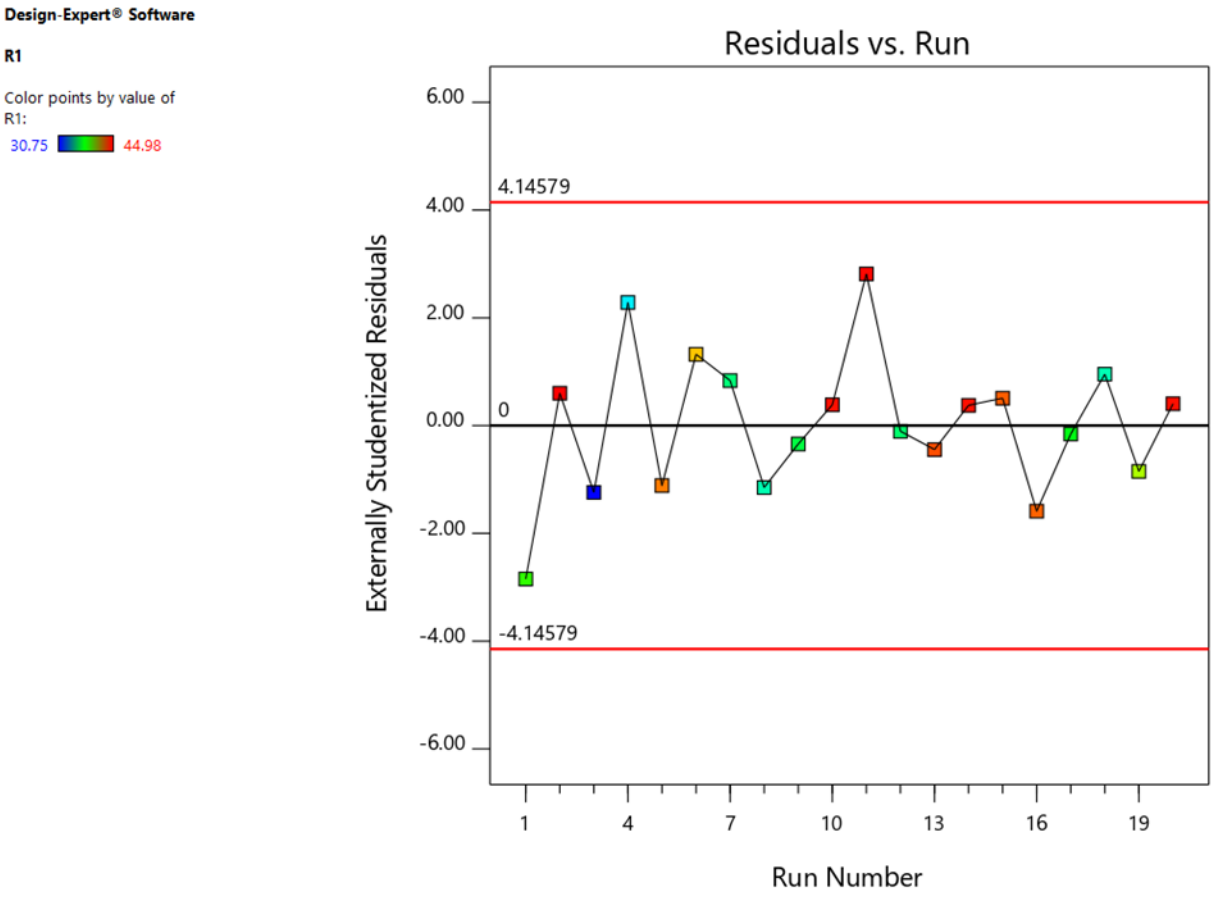
Appendix B: Diagnostics Case Statistics

Standard Order	Actual Value	Predicted Value	Residual	Leverage	Student Residual	Cook's Distance	Outlier t	Run Order
1	36.95	37.18	7.458E-003	0.670	0.013	0.026	0.013	2
2	35.36	34.75	0.49	0.670	0.865	0.186	0.853	15
3	34.10	32.89	1.09	0.670	1.941	0.745	2.332	9
4	43.67	43.34	0.36	0.670	0.642	0.057	0.622	11
5	42.23	41.42	0.69	0.670	1.223	0.331	1.258	18
6	36.36	36.40	-0.043	0.670	-0.076	0.002	-0.072	5
7	36.18	35.62	0.56	0.670	1.000	0.146	1.000	17
8	44.89	42.85	1.04	0.670	1.852	0.950	2.167	16
9	30.75	31.64	-0.83	0.607	-1.453	0.226	-1.241	13
10	35.39	35.98	-0.59	0.607	-0.965	0.144	-0.962	10
11	37.58	37.75	-0.17	0.607	-0.280	0.012	-0.266	6
12	38.55	39.86	-1.31	0.607	-2.139	0.707	-2.755	19
13	40.30	40.95	-0.65	0.607	-1.062	0.175	-1.070	12
14	43.60	44.43	-1.83	0.607	-1.356	0.284	-1.424	1
15	44.75	44.53	0.22	0.166	0.251	0.001	0.239	3
16	43.89	44.53	0.36	0.166	0.407	0.003	0.390	14
17	44.76	44.53	0.23	0.166	0.262	0.001	0.249	8
18	44.98	44.53	0.45	0.166	0.504	0.005	0.488	20
19	44.78	44.53	0.25	0.166	0.284	0.002	0.271	4
20	43.25	44.53	-1.28	0.166	-1.427	0.041	-1.517	7

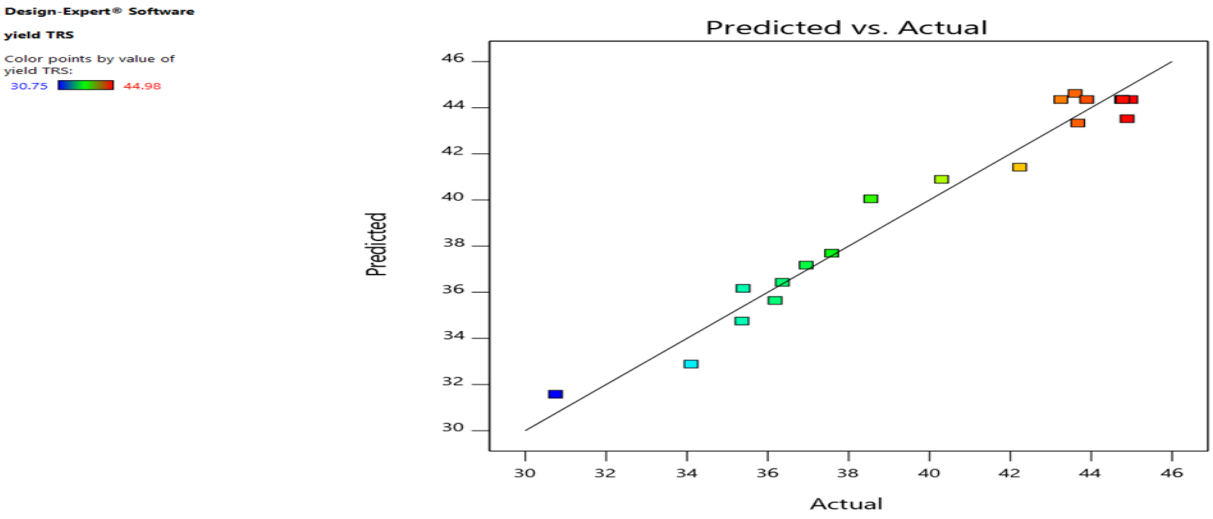
Appendix C: central composite design for hydrolysis

Run no.	Factor 1: Temp.(°c)	Factor 2: Time (min)	Factor 3: Acid con. (%)
1	130.00	25.00	3.34
2	120.00	20.00	2.00
3	130.00	25.00	2.50
4	130.00	25.00	2.50
5	140.00	20.00	3.00
6	130.00	16.59	2.50
7	130.00	25.00	2.50
8	130.00	25.00	2.50
9	120.00	30.00	2.00
10	146.82	25.00	2.50
11	140.00	30.00	2.00
12	130.00	25.00	1.66
13	113.18	25.00	2.50
14	130.00	25.00	2.50
15	140.00	20.00	2.00
16	140.00	30.00	3.00
17	120.00	30.00	3.00
18	120.00	20.00	3.00
19	130.00	33.41	2.50
20	130.00	25.00	2.50

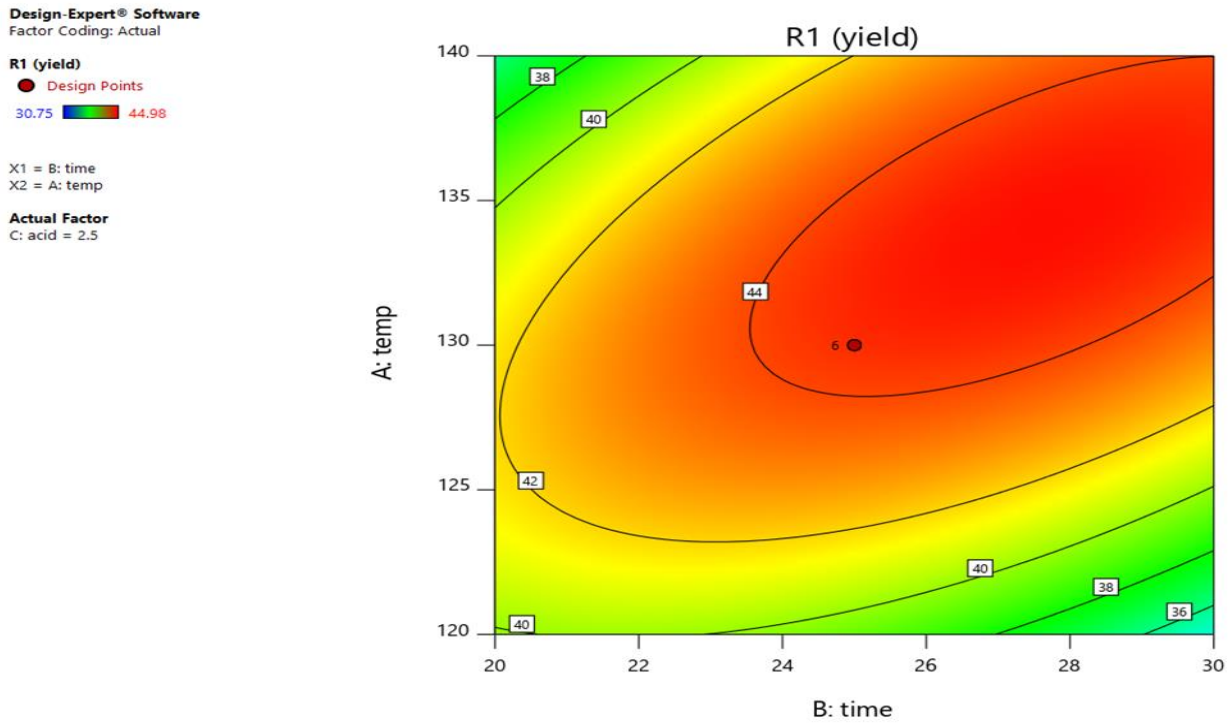
Appendix D: Residuals Vs. Run of yield of total reducing sugar after hydrolysis



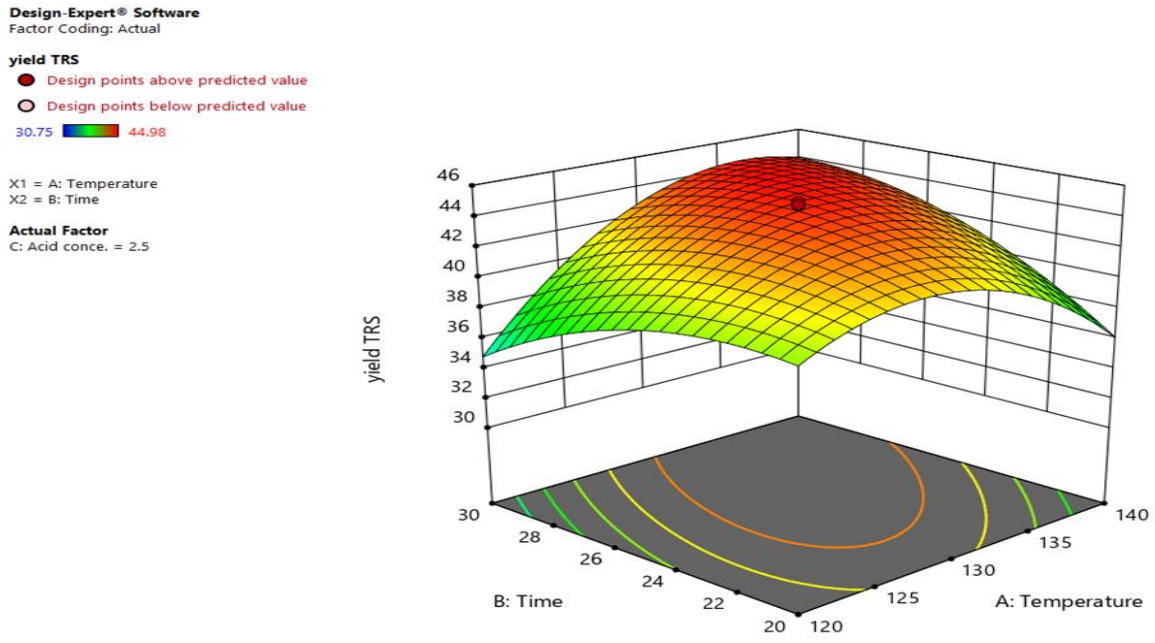
Appendix E: Actual values Vs. predicted value



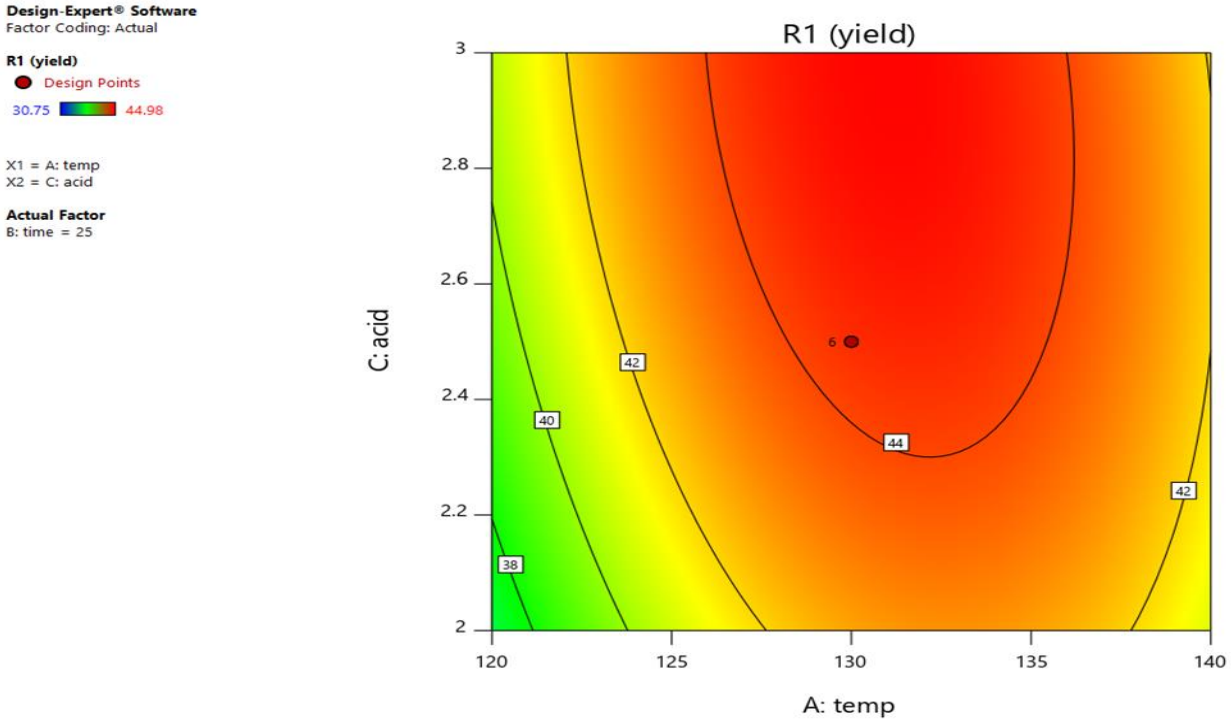
Appendix F: Response surface and contour plot of optimization yield of TRS



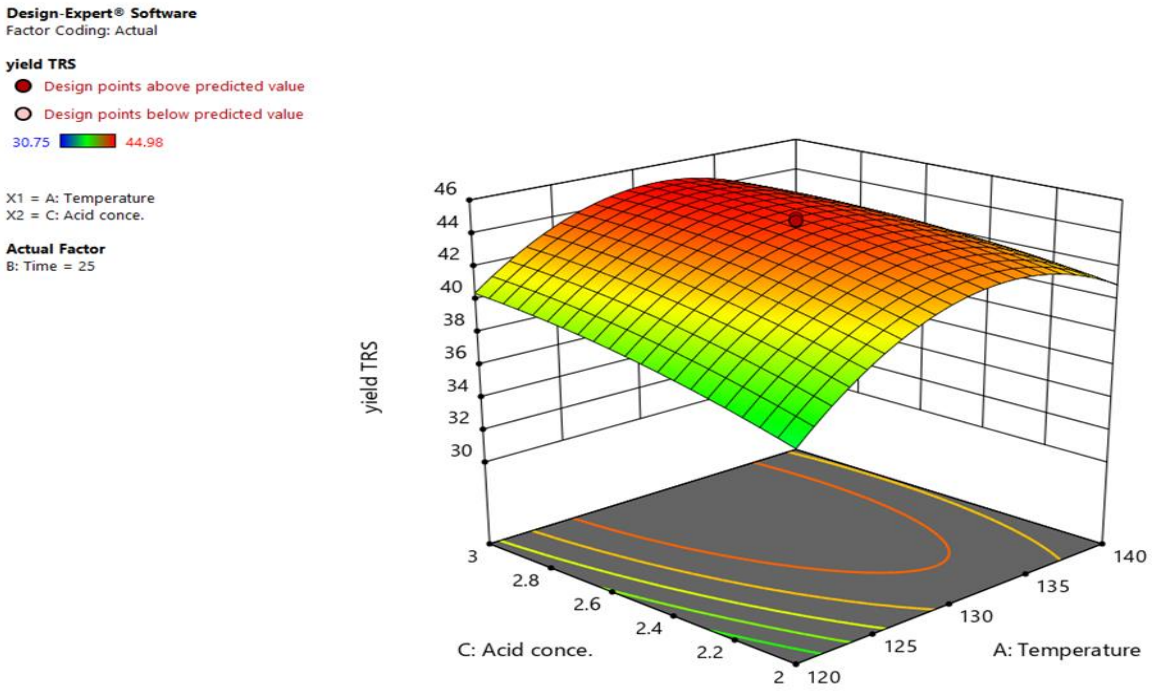
(1) Response surface plot of predicted TRS yield at constant acid concentration



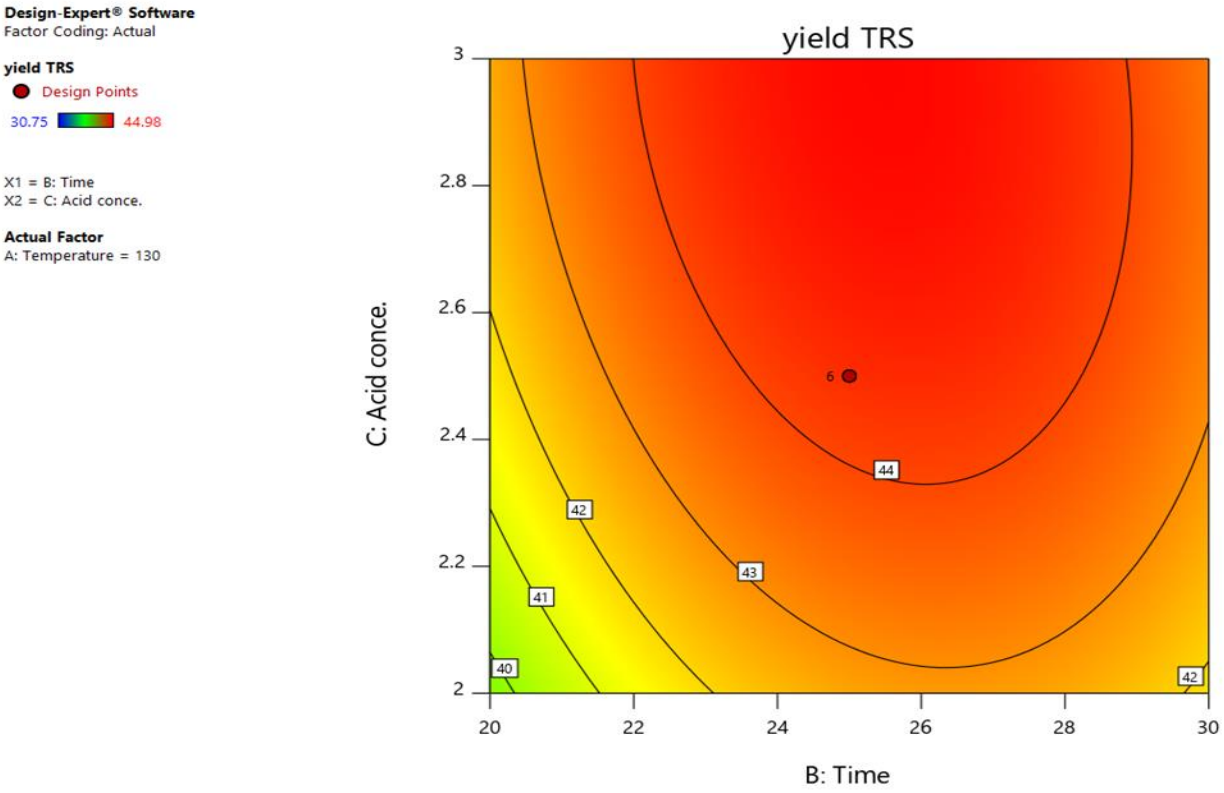
(2) (2) contour plot of predicted TRS yield at constant acid concentration



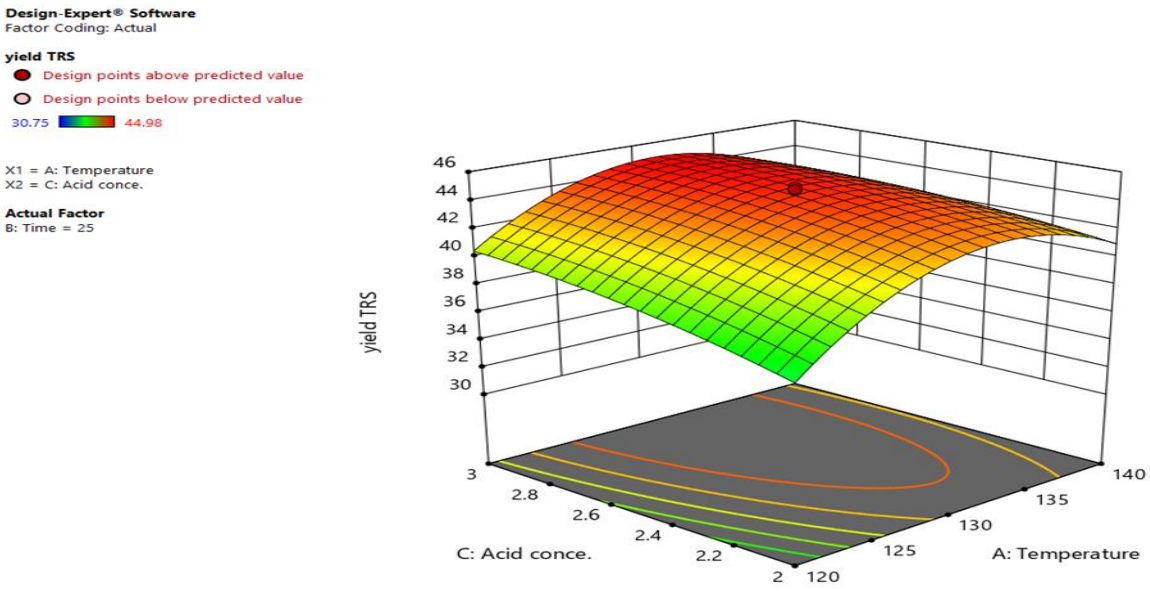
(3) contour plot of predicted TRS yield at constant time



(4) Response surface plot of predicted TRS at constant time



(4) Contour plot of predicted TRS yield at constant temperature



(5) Response surface plot of predicted TRS yield a constant time

Appendix G: Design summery

Response	Name	Obs	Minimum	Maximum	Trans	Model
Y_1	Absorbance	20	0.084	0.14	None	No
Y_2	Glu. con. Of unk. (%)	20	2.5	6.40	None	No
Y_3	Yield of TRS(%w/w)	20	30.75	44.98	None	Quadratic
Factor	Name	Type	Low Actual	High Actual	Low coded	High coded
A	Temperature	Numeric	120.00	140.00	-1.00	1.00
B	Time	Numeric	20.00	30.00	-1.00	1.00
C	Acid conce.	Numeric	2.00	3.00	-1.00	1.00

Appendix H: Density of mixture of C_2H_5OH and H_2O at 20ºC

TABLE 2-111 Densities of Mixtures of C_2H_5OH and H_2O at 20°C

g/mL

% alcohol by weight	Tenths of %										% alcohol by weight	Tenths of %									
	0	1	2	3	4	5	6	7	8	9		0	1	2	3	4	5	6	7	8	9
0	0.99823	804	785	766	748	729	710	692	673	655	50	0.91384	361	339	317	295	272	250	228	206	183
1	636	618	599	581	562	544	525	507	489	471	51	160	138	116	093	071	049	026	004	*981	*959
2	453	435	417	399	381	363	345	327	310	292	52	.90936	914	891	869	846	824	801	779	756	734
3	275	257	240	222	205	188	171	154	137	120	53	711	689	666	644	621	598	576	553	531	508
4	103	087	070	053	037	020	003	*987	*971	*954	54	485	463	440	417	395	372	349	327	304	281
5	.89938	922	906	890	874	859	843	827	811	796	55	258	236	213	190	167	145	122	099	076	054
6	780	765	749	734	718	703	688	673	658	642	56	031	008	*985	*962	*939	*917	*894	*871	*848	*825
7	627	612	597	582	567	553	538	523	508	493	57	.88803	780	757	734	711	688	665	643	620	597
8	478	463	449	434	419	404	389	374	360	345	58	574	551	528	505	482	459	436	413	390	367
9	331	316	301	287	273	258	244	229	215	201	59	344	321	298	275	252	229	206	183	160	137
10	187	172	158	144	130	117	103	089	075	061	60	113	090	067	044	021	*998	*975	*951	*928	*905
11	047	033	019	006	*992	*978	*964	*951	*937	*923	61	.88882	859	836	812	789	766	743	720	696	673
12	.87910	896	883	869	855	842	828	815	801	788	62	650	626	603	580	557	533	510	487	463	440
13	775	761	748	735	722	709	696	683	670	657	63	417	393	370	347	323	300	277	253	230	206
14	643	630	617	604	591	578	565	552	539	526	64	183	160	136	113	089	066	042	019	*995	*972
15	514	501	488	475	462	450	438	425	412	400	65	.87948	925	901	878	854	831	807	784	760	737
16	387	374	361	349	336	323	310	297	284	272	66	713	689	666	642	619	595	572	548	524	501
17	259	246	233	220	207	194	181	168	155	142	67	477	454	430	406	383	359	336	312	288	265
18	129	116	103	089	076	063	050	037	024	010	68	241	218	194	170	147	123	099	075	052	028
19	.86997	984	971	957	944	931	917	904	891	877	69	004	*981	*957	*933	*909	*885	*862	*838	*814	*790
20	864	850	837	823	810	796	783	769	756	742	70	.86766	742	718	694	671	647	623	599	575	551
21	729	716	702	688	675	661	647	634	620	606	71	527	503	479	455	431	407	383	359	335	311
22	592	578	564	551	537	523	509	495	481	467	72	287	263	239	215	191	167	143	119	095	071
23	453	439	425	411	396	382	368	354	340	326	73	047	022	*998	*974	*950	*926	*902	*878	*854	*830
24	312	297	283	269	254	240	225	211	196	182	74	.85806	781	757	733	709	685	661	636	612	588
25	168	153	139	124	109	094	080	065	050	035	75	564	540	515	491	467	443	419	394	370	346
26	020	005	*990	*975	*959	*944	*929	*914	*898	*883	76	322	297	273	249	225	200	176	152	128	103
27	.95867	851	836	820	805	789	773	757	742	726	77	079	055	031	006	*982	*958	*933	*909	*884	*860
28	710	694	678	662	646	630	613	597	581	565	78	.84835	811	787	762	738	713	689	664	640	615
29	548	532	516	499	483	466	450	433	416	400	79	590	566	541	517	492	467	443	418	393	369
30	382	365	349	332	315	298	281	264	247	230	80	344	319	294	270	245	220	196	171	146	121
31	213	195	178	161	143	126	108	091	074	056	81	096	072	047	022	*997	*972	*947	*923	*898	*873
32	038	020	003	*985	*967	*950	*932	*914	*896	*878	82	.83848	823	798	773	748	723	698	674	649	624
33	.94860	842	824	806	788	770	752	734	715	697	83	599	574	549	523	498	473	448	423	398	373
34	679	660	642	624	605	587	568	550	531	512	84	348	323	297	272	247	222	196	171	146	120
35	494	475	456	438	419	400	382	363	344	325	85	095	070	044	019	*994	*968	*943	*917	*892	*866
36	306	287	268	249	230	211	192	172	153	134	86	.82840	815	789	763	738	712	686	660	635	609
37	114	095	075	056	036	017	*997	*978	*958	*939	87	583	557	531	505	479	453	427	401	375	349
38	.93919	899	879	859	840	820	800	780	760	740	88	323	297	271	245	219	193	167	140	114	088
39	720	700	680	660	640	620	599	579	559	539	89	062	035	009	*983	*956	*930	*903	*877	*850	*824
40	518	498	478	458	437	417	396	376	356	335	90	.81797	770	744	717	690	664	637	610	583	556
41	314	294	273	253	232	212	191	170	149	129	91	529	502	475	448	421	394	366	339	312	285
42	107	086	065	044	023	002	*981	*960	*939	*918	92	257	230	203	175	148	120	093	066	038	010
43	.92897	876	855	834	812	791	770	749	728	707	93	.80983	955	928	900	872	844	817	789	761	733
44	685	664	642	621	600	579	557	536	515	493	94	705	677	649	621	593	565	537	509	480	452
45	472	450	429	408	386	365	343	322	300	279	95	424	395	367	338	310	281	253	224	195	166
46	257	236	214	193	171	150	128	106	085	063	96	138	109	080	051	022	*993	*963	*934	*905	*875
47	041	019	*997	*976	*954	*932	*910	*889	*867	*845	97	.79846	816	787	757	727	698	668	638	608	578
48	.91823	801	780	758	736	714	692	670	648	626	98	547	517	487	456	426	396	365	335	305	274
49	604	582	560	538	516	494	472	450	428	406	99	243	213	182	151	120	089	059	028	*997	*966
											100	.78834									

*Indicates change in the first two decimal places.

Appendix I: Model Summary Statistics

Source	**Std. Dev.**	**R-Squared**	**Adjusted R-Squared**	**Predicted R-Squared**	**PRESS**
Linear	4.60	0.1119	-0.0546	-0.4030	535.90
2FI	4.44	0.3297	0.0204	-0.3081	499.63
Quadratic	0.98	0.9749	0.9523	0.8412	60.65 Suggested
Cubic	1.13	0.9800	0.9366	-2.1925	1219.41 Aliased