ADDIS ABABA UNIVERSITY

ADDIS ABABA INSITUTE OF TECHNOLOGY (AAiT)

SCHOOL OF CHEMICAL AND BIO- ENGINEERING

PROCESSES ENGINEERING STREAM

PRODUCTION AND CHARACTERIZATION OF BIODIESEL FROM PUMPKIN SEED OIL

A Thesis submitted to Addis Ababa Institute of Technology, in Partial fulfillment of the requirement of degree of Master of Science in Chemical Engineering (Process Engineering Stream)

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This is to certify that the thesis prepared by Fkre Goitom, entitled **production and characterization of biodiesel from pumpkin seed oil** submitted to the school of chemical and Bio engineering in partial fulfillment of the requirements for the degree of Master of Science complies with the regulations of the university and meets the accepted standards with respect to originality and quality.

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DEDICATION

This thesis work is dedicated to God Almighty for his guidance and protection for the countless sacrifices made for my sake. This work also dedicated to my parents. I hereby declare that the thesis is based on my original work except for flotation's and citations which have been duly acknowledged. I also declare that it has not been previously or currently submitted for any other department at Addis Ababa University or other institute.

Fkre Goitom

Date: June 12, 2019

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First of all I would like to thank God for giving me health and patience to accomplish this thesis work successfully and giving me strength on difficult times.

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Acronyms and Notations

A	Reaction temperature
AB	Reaction temperature and MeOH to oil ratio
ABC	Temperature, MeOH to oil ratio and amount of catalyst
AC	Temperature and amount of catalyst
ANOVA	Analysis of variance
ASTM	American Standards Tests and Materials
AV	Acid Value
B	MeOH to oil ratio
BC	Amount of catalyst and MeOH to oil ratio
C	Amount of catalyst
EN	European Normalization (European Standards)
FFA	Free fatty acid
M	Molarity
MeOH	Methanol alcohol
N	Normality
V/V	Volume per Volume
W/W	Weight per Weight
CCD	Central Composite Design
FAME	Fatty acid methyl esters
Rpm	Revolution per minute

Abstract

World’s energy needs are largely met by use of non-renewable resources such as petrochemicals, natural gas and coal. Increasing energy demands, environmental concerns and depletion of fossil fuel reserves has led to the search for alternate fuels.

This work was done with the aim of producing biodiesel from pumpkin seed oil by using methanol with alkali catalyst, sodium hydroxide. The effects of catalyst amount from 0.22g to 0.99g, molar ratio of methanol to oil from 6:1 to 12:1 and reaction temperature from 30℃ to 75℃ on biodiesel yield was investigated. Pumpkin seed oil was extracted using solvent extraction (using hexane as a solvent).

The extracted oil was purified through degumming, neutralization, washing and drying sequentially. Acid value, amount of free fatty acid, saponification value, kinematic viscosity, higher heating value, iodine value and flash point of the extracted oil were determined. Biodiesel was produced from pumpkin seed oil using anhydrous methanol 99% (w/w) and sodium hydroxide catalyst 98.6% (w/w).

The experimental design was done by using the Design Expert 7.0.0 software three levels; three factor Central Composite Design with full type in the optimization study, requiring 20 experiments. To determine the effect of temperature, amount of catalyst and molar ratio of alcohol to oil experiments were done in the ranges of 30℃to 60℃, 0.22g to 0.99g and 6:1 to 12:1 subsequently. The maximum biodiesel yield was 88.7 % (w/w) at 45℃, 9:1 molar ratio of alcohol to oil and 0.99g amount of sodium hydroxide catalyst. In contrast, the minimum biodiesel yield was 38% (w/w) at 60℃, 12:1 molar ratio of alcohol to oil and 1.76g mass of catalyst. The viscosity, density, flash point, acid value, saponification value, higher heating value, iodine value Cetane number, moisture content and ash content of the produced biodiesel were determined. These properties were matched with ASTM and EN standards specifications

1 INTRODUCTION

1.1 Background

World’s energy needs are largely met by use of non-renewable resources such as petrochemicals, natural gas and coal. Increasing energy demands, environmental concerns and depletion of fossil fuel reserves have led to the search for alternate fuels. An alternative fuel must be technically feasible, economically competitive, environmentally acceptable and readily available. Renewable sources such as vegetable oils and their derivatives have gained importance as alternative fuels for diesel engines (Cervero, Coca, & Luque, 2008). Vegetable oils and animal fats are mainly triglyceride molecules, in which three fatty acid groups are attached to one glycerol molecule. The biodiesel can be produced from these triglycerides by process of transesterification. Biodiesel is a liquid biofuel obtained by chemical processes from vegetable oils or animal fats and an alcohol that can be used in diesel engines, alone or blended with diesel oil (Fukuda, Kondo, & Noda, 2004).

There are different types of feed stocks that are used for the production of biodiesel. These includes linseed oil, palm seed oil ,waste cooked vegetable oil, sunflower seed oil, cotton seed oil, Jatropha seed oil, pumpkin seed oil and animal fats. Oilseed plants are used for the production of biodiesel through the process called transesterification reaction which is a process by which alcohol reacts with vegetable oil in the presence of catalyst.

The majority of biodiesel is produced today through the base catalyzed transesterification reaction because it involves low temperature and pressure processing, high conversion rates, no intermediate steps, and lower costs of processing materials. If a methanol molecules contact a fatty acid molecule, they will bond and form biodiesel molecule. The hydroxyl group from the catalyst stabilizes the glycerin (Agarwal & Bajaj, 2009).

Triglycerides are major components of vegetable oils and animal fats. Chemically, triglycerides are esters of fatty acids with glycerol. Fatty acid ethyl esters are mostly involved because ethanol is the cheapest alcohol, but other alcohols, namely methanol, may be employed as well.

In this way, highly viscous triglycerides are converted in long chain monoesters presenting much lower viscosity and better combustion properties to enhance the burning. Homogeneous or heterogeneous catalysis are used to enhance the reaction rate. Since different fatty acid has different physical and chemical properties, the fatty acid content is the most important parameter influencing the corresponding properties of a vegetable oil or animal fat (Alexander & Sorin Bildea, 2008).

Pumpkin, belonging to the genus *Cucurbita* and family *Cucurbitaceae* refers to any one of the species *Cucurbita moschata*, *Cucurbita mixta*, *Cucurbita maxima*, and *Cucurbita pepo*. Pumpkin (*C. pepo*) is mostly used to refer to cultivars with round fruits which are used in the mature state for cooking or feeding livestock. Pumpkin is among the economically most important vegetable crops worldwide and is grown in both temperate and tropical regions (Kwiri et al., 2014).

The seeds of pumpkin (Cucurbita sp.) are generally considered to be agro-industrial wastes and discarded. In some parts of the world, the seeds are consumed raw, roasted or cooked, but only at the domestic scale(Patel, 2013). In recent years, the acceptance of fatty acid methyl esters (biodiesel) as a substitute to petroleum diesel has rapidly grown in Greece (Cervero et al., 2008). The raw materials for biodiesel production in this country mainly include traditional seed oils (cotton seed oil, sunflower oil, soybean oil and rapeseed oil) and used frying oils. In the search for new low-cost alternative feed stocks for biodiesel production, a study done by researchers at National Technical University of Athens, Athens, Greece emphasizes the evaluation of pumpkin (Cucurbita pepo Linn.) seed oil (Schinas et al., 2009).

Based on the literature pumpkin seed serves as a vital source of biodiesel in Ethiopia (in our country) since it is locally available raw material for biodiesel production. There is no commercial application of pumpkin seed. Hence this research studies, the profile of pumpkin seed oil and its production to fatty acid methyl esters via a base-catalyzed transesterification were investigated.

1.2 Problem of the statement

At present, due to industrial revolution and high population growth, the demand of fossil fuel is increasing rapidly. To substitute the fossil fuel demand by renewable fuel, biodiesel production from vegetable oils is one alternative for energy demand and biodiesel has recently attracted much attention in all over the world because of its availability, renewability, non-toxicity, its less emission of carbon dioxide gas and its biodegradability. Hence, assessing sustainable and renewable energy alternatives is indispensable at present, not only to combat the fuel supply uncertainty and price fluctuations, but also becoming global concerns.

Biodiesel is a cleaner burning fuel that is renewable and can be substituted for petroleum diesel fuel; so the production of biodiesel from vegetable oil plays a vital role over the fossil fuels. Diesel made from fossil fuels has serious effects to human health. Each country has a responsibility to seek for environmentally and kindly energy sources that are advocating to the global activities to reduce greenhouse gases and air pollutions. The pollution is likely to cause global warming which will also damage our planet later on (Surma, 2008).

On the other hand petroleum products which are used mainly at urban household center are entirely an imported commodity. Demand for these products is rising rapidly increasing due to in scarcity of fuel wood and the change of life style of the people. The rise in demand is accompanied by a much faster growth in the import bill because of rising petroleum prices and products. Supply security of petroleum fuels is essential to ensure continuous economic growth. Reduction of the petroleum import bill will enable the government to allocate more of its foreign exchange earnings to other development investments and expenditures.

A number of countries have successfully applied the use of biodiesel made from plant seed oils or animal fats, purely or by blending with petroleum diesel, and developed appropriate technologies to exploit the resources specific to their agro-climatic conditions. Ethiopia, endowed with diverse ecological varieties that have an ample potential for biofuel-production (both alcohols and biodiesel), is not benefited from its wealth (Birhanu & Ayalew, 2011). However there are different vegetable seeds such as pumpkin for instance this seed is grown in a well manner but the Ethiopian farmers does not consider it as an important vegetable. If an industrial application such as biodiesel production introduced in our country the farmers can pay attention for this vegetable there by the farmers can benefited from this seed. After the flesh part of pumpkin is used for cooking the seed is regarded as a waste traditionally in most rural areas if this is introduced in to an industrial application in addition to the renewable and locally available source of energy it can create a job opportunity.

Therefore, this laboratory scale study helps for the development of clean, new, renewable energies and locally available, particularly biodiesel production from pumpkin seed oil, as an alternative energy solution to hydrocarbons, in response to the imported petro diesel which has an adverse effects on the economies of the country. By extracting crude oil from Pumpkin seed in a large scale, Ethiopia (our country), will save its foreign expenditure, create job opportunities, establish local industrial base, increase its economic growth and create clean environment. With that Ethiopia will be in a position to use biodiesel for energy use in the future from Pumpkin seeds oil.

1.3 Objectives of the study

1.3.1 General objective

Production and characterization of biodiesel from Pumpkin seed oil by base catalyzed trans-esterification processes.

1.3.2 Specific Objectives

- To determine the percentage (yield) of the extracted pumpkin seed oil
- To investigate the physiochemical properties of the extracted pumpkin seed oil
- To determine physical and chemical properties of biodiesel produced from Pumpkin seed oil
- To investigate the effects of amount of alkali catalyst and reaction temperature on biodiesel yield
- To identify the effect of molar ratio of alcohol to oil on chemical reaction of biodiesel production

1.4 Significance of the study

Biodiesels developed from vegetable oil reduces environmental pollution, greenhouse emission and it promotes a sustainable development because of its non-toxicity, renewability, and biodegradability. The production of biodiesel from locally available resource is reliable, renewable and domestically distributed; this reduces dependency on imported petroleum diesel and energy crisis. The biodiesel production from Pumpkin seed will build an agriculture based industry with potential for development of agri-businesses and agro-industries.

Improve the economic development of the country and helps on finding different alternative biofuels so as to increase the farmer's income by creating job opportunity around the rural areas and this motivates agricultural and sustainable development. This could be increases opportunities for agricultural diversifications which enhances doing of a research on another oil plants and seeds for biodiesel production. Thus, providing both locally and global environmental benefits by reducing the emissions of pollutants and greenhouse gases.

1.5 Scope of the study

The area of the study work covers pumpkin seed preparation prior to start the main production process. Oil extraction and study of physicochemical properties of the purified oil was done accordingly using the given methods in order to get the required product otherwise if the oil does not characterized it could not be proceed in to the main production process. A detail study of methods employed in production of biodiesel that is transesterification process; for this study the base catalyzed transesterification technique of biodiesel production was studied specifically to obtain biodiesel from the purified oil. Finally the methods of characterization and analyzing of the produced biodiesel was studied.

2 LITERATURE REVIEW

2.1 Introduction

Biofuel comprises of liquid or gaseous fuels used basically for the transport sector, which are primarily produced from biomass. They include ethanol, methanol, biodiesel, hydrogen and methane, respectively. Liquid biofuels to fuel vehicles, engines while gaseous fuels cells for electricity generation. An estimated 54 billion liters of biofuels was recorded in 2007 as a result of increased world production and these gains meant biofuels accounted for 1.5% of global supply of liquid fuels, up 0.25 % from the previous year. First generation biofuels include two main types (Babajide, Petrik, & Ameer, 2005):

- Bioethanol produced from plant sugars of biofuel crops, such as sugarcane and maize, which ferments to produce ethanol. The ethanol produced can be further blended with 5–10% gasoline fuel for use in normal cars; however, higher percentages of ethanol are needed for specially adapted cars
- Biodiesel synthesized from a chemical reaction between triglycerides and an alcohol. The use of triglycerides without any modification directly in modified diesel engines cut biodiesel processing costs and eliminates excessive glycerol by-product from the process.

ASTM International (originally known as the American Society for Testing and Materials) defines biodiesel as a mixture of long-chain monoalkylic esters from fatty acids obtained from renewable resources, to be used in diesel engines. Blends with diesel fuel are indicated as ‘‘Bx’’, where ‘‘x’’ is the percentage of biodiesel in the blend. For instance, ‘‘B5’’ indicates a blend with 5% biodiesel and 95% diesel fuel; in consequence, B100 indicates pure biodiesel (Fukuda et al., 2004).

2.2 Biodiesel as a source of fuel

Biodiesel is a liquid fuel produced from a variety of natural oils, such as vegetables oils, animal fats, or even waste cooking oils. It contains monoalkylic esters with hydrocarbon chains in the range of 14 -22 carbon atoms, capable of combusting properly in conventional diesel engines(Vicente Crespo, Martínez Rodríguez, & Aracil Mira, 2001).

Today, biodiesel is produced by transesterification of fatty acids in vegetable oils, using an alkaline catalyst (sodium or potassium hydroxide at 80 °C), and a reaction that proceeds with a very high yield. However, the process requires a complex downstream by-product separation and some wastewater problems may arise. The glycerol generated as a by-product is accompanied by the alkali employed as a catalyst, and has to be treated either as a waste stream or purified for further applications. Moreover, free fatty acids and water present in the vegetable oil form the corresponding salts and are extracted with the wash water along with small amounts of glycerol, methanol and catalyst. The alkaline water stream waste requires appropriate treatment. A simple life-cycle analysis shows that the conversion of vegetable oils to biodiesel requires a substantial amount of energy, because of the high temperature at which the reaction is carried out(Van Gerpen, 2006).

2.3 Specification and standardizations of Biodiesel

Discussions about vegetable oil as a fuel for diesel engines first started after the energy crisis in 1973 resulting from the need to secure the energy supply of agriculture. After various tests with blends of fossil diesel fuel and vegetable oil the transesterification of vegetable oil into methyl ester was first considered by Austrian research institutes. Between 1980 and 1990 several research projects were carried out to investigate the production and utilization of rape oil methyl ester as a diesel fuel primarily for tractors (Prankl & Mittelbach, 2004).

European biodiesel specification (EN 14214) is an international standard for biodiesel, which was approved by CEN (European Committee for Standardization). It was set up to control biodiesel quality within the EU and has been used as a guideline for many countries. This standard is mostly based on biodiesel produced from rapeseed and combination of oils that together provides similar characteristics to rapeseed oil. ASTM D-6751 is the standard that covers pure biodiesel (B100), for blending level up to 20% by volume in the US. It was setting up by the American Society for Testing and Materials (ASTM), which is a standard development organization. As this standard was designed for neutral process and feedstock such as soybean that different from EN 14214 specification, some properties of ASTM D-6751 are slightly different from the European specification (Goosen & Vora, 2007).

Biodiesel standards are in place in a number of countries in an effort to ensure that only high-quality biodiesel reaches the marketplace. The two most important fuel standards, ASTM D6751 in the United States and EN 14214 (European Committee for Standardization, CEN) in the European Union, are shown in table 2.1 (Moser, 2009).

Table 2.1 European Committee for Standardization EN 14214 biodiesel fuel standard

Property	Test method(s)	Limits	Units
Density, 15°C	EN ISO 3675, EN ISO 12185	860–900	kg/m^3
Kinematic viscosity, 40°C	EN ISO 3104, ISO 3105	3.5–5.0	mm^2/S
Flash point	EN ISO 3679	120 min	°C
Sulfur content	EN ISO 20846, EN ISO 20884	10.0 max	mg/kg
Carbon residue (10% distillation residue)	EN ISO 10370	0.30 max	% (mol/mol)
Cetane number	EN ISO 5165	51 min	
Sulfated ash	ISO 3987	0.02 max	% (mol/mol)
Water content	EN ISO 12937	500 max	mg/kg
Total contamination	EN 12662	24 max	mg/kg
Copper strip corrosion (3 h, 50°C)	EN ISO 2160	1	Degree of corrosion
Oxidation stability, 110°C	EN 14112	6.0 min	H
Acid value	EN 14104	0.50 max	mg KOH/g
Iodine value	EN 14111	120 max	g I2/100 g
Linolenic acid content	EN 14103	12.0 max	% (mol/mol)
Polyunsaturated (≥4 double bonds) methyl esters	EN 14103	1 max	% (mol/mol)
Methanol content	EN 14110	0.20 max	% (mol/mol)
Free glycerol	EN 14105 EN 14106	0.020 max	% (mol/mol)
Total glycerol	EN 14105	0.25 max	% (mol/mol)
Group I metals (Na,	EN 14108 EN 14109	5.0 max	mg/kg

K)			
Group II metals (Ca, Mg)	EN 14538	5.0 max	mg/kg
Phosphorous content	EN 14107	10.0 max	mg/kg

2.4 Biodiesel production processes

The plant oils usually contain free fatty acids, phospholipids, sterols, water, odourants and other impurities. Because of these, the oil cannot be used as fuel directly. To overcome these problems the oil requires slight chemical modification mainly transesterification, pyrolysis and emulsification. Among these, the transesterification is the key and foremost important step to produce the cleaner and environmentally safe fuel from vegetable oils (Meher & Naik, 2006).

2.4.1 Transesterification process

Transesterification process is a set of three consecutive chemical reactions between esters generally and mainly triglycerides and simple alcohols. The reaction is usually very slow to be of any industrial value and as such catalysts, which can be acidic, basic, organic or enzymatic, are usually used to achieve reasonable conversion at short time. Also, since the reaction is reversible as 1 mole of the triglyceride reacts with 3 moles of the alcohol, as shown in Figure 2.1, removal of one of the products or an increase in the mole ratio of one of the reactants can be used to drive the reaction to the right (alkyl ester formation). Usually, the methanol to oil/triglyceride mole ratio is made greater than 3:1; 6:1 (Paul Madus Ejikeme, Anthony, Egbounu, Anyaogu, & Eze, 2011).

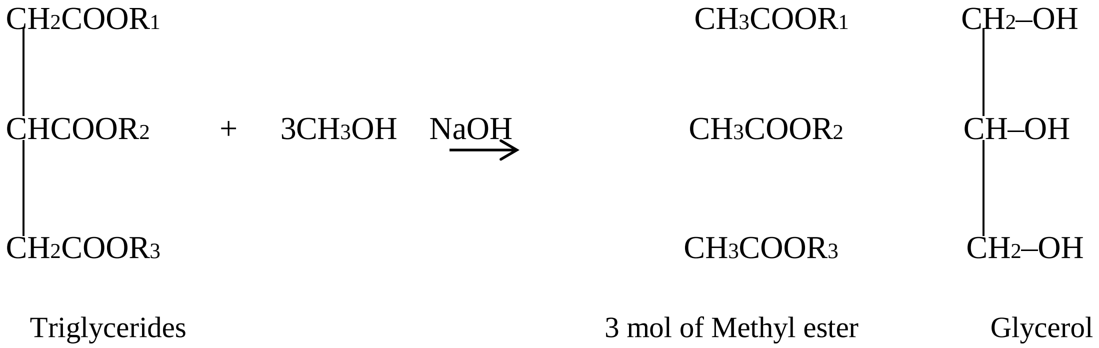


Figure 2.1 Typical transesterification reaction equations

Among the alcohols that can be used in the transesterification process are methanol, ethanol, propanol and butanol, Methanol and ethanol are used most frequently, especially methanol because of its low cost and its physical and chemical advantages (polar and shortest chain alcohol). It can quickly react with triglycerides and NaOH is easily dissolved in it. In this case, the reaction is referred to as methanolysis. The stoichiometry of methanolysis reaction requires 3 mol of methanol and 1 mol of triglyceride to give 3 mol of fatty acid methyl ester and 1 mol of glycerol. This reaction, in turn, consists of three consecutive reversible reactions with intermediate formation of diglycerides and monoglycerides. After the reaction, the glycerol is separated by settling or centrifuging and the layer obtained is purified to be used in its traditional applications (the pharmaceutical, cosmetics and food industries) or in its recently developed applications (animal feed, carbon feedstock in fermentations, polymers, surfactants, intermediates and lubricants) (Claude, 1999).

2.4.1.1 Transesterification using basic catalyst

The transesterification reaction is catalyzed by alkaline metal hydroxides or alkoxides, as well as sodium or potassium carbonates. The alkaline catalysts give good performance when raw materials with high quality (FFA < 1 wt% and moisture < 0.5 wt%) are used. The reaction is carried out at a temperature of 60–65 °C under atmospheric pressure with an excess amount of alcohol, usually methanol. The molar ratio of alcohol to oil is often 6:1 or more. It often takes several hours to complete the reaction when alkaline hydroxides such as NaOH or KOH are used.

Alkaline alkoxides, e.g., sodium alkoxide, are the most reactive catalysts because the yield of FAME that can be attained is higher than 98% in a short reaction time of 30 min. alkaline hydroxides are cheaper than the alkaline alkoxides, but less active. The yield of FAME can be improved by simply increasing the amount of the alkaline hydroxides by one or two mol% to oil, and thus they are a good alternative to the alkaline alkoxides. Sivakumar *et al.* were produce biodiesel from raw material dairy waste scum and the FAME yield reached 96.7% under the optimal conditions: KOH 1.2 wt.%; molar ratio of methanol to oil 6:1; reaction temperature 75 °C; reaction time 30 min at 350 rpm (Thanh, Okitsu, Boi, & Maeda, 2012).

In consequence, with vegetable oils or fats containing low FFA and water, the base-catalyst transesterification is much faster than the acid-catalyst transesterification and is most commonly used commercially on the industrial scale.

The base-catalyzed transesterification of vegetable oils proceeds faster than the acid-catalyzed reaction. Due to this reason, together with the fact that the alkaline catalysts are less corrosive than acidic compounds, industrial processes usually favor base catalysts, such as alkaline metal alkoxides and hydroxide as well as sodium or potassium carbonates. The mechanism of the base-catalyzed transesterification of vegetable oils is shown in figure 2.2. The first step (Eq. 1) is the reaction of the base with the alcohol, producing an alkoxide and the protonated catalyst. The nucleophilic attack of the alkoxide at the carbonyl group of the triglyceride generates a tetrahedral intermediate (Eq.2) from which the alkyl ester and the corresponding anion of the diglycerides are formed (Eq. 3). The latter deprotonates the catalyst, thus regenerating the active species (Eq. 4), which is now able to react with a second molecule of the alcohol, starting another catalytic cycle. Diglycerides and monoglycerides are converted by the same mechanism to a mixture of alkyl esters and glycerol (Ulf Schuchardt, Sercheli, & Matheus, 1998).

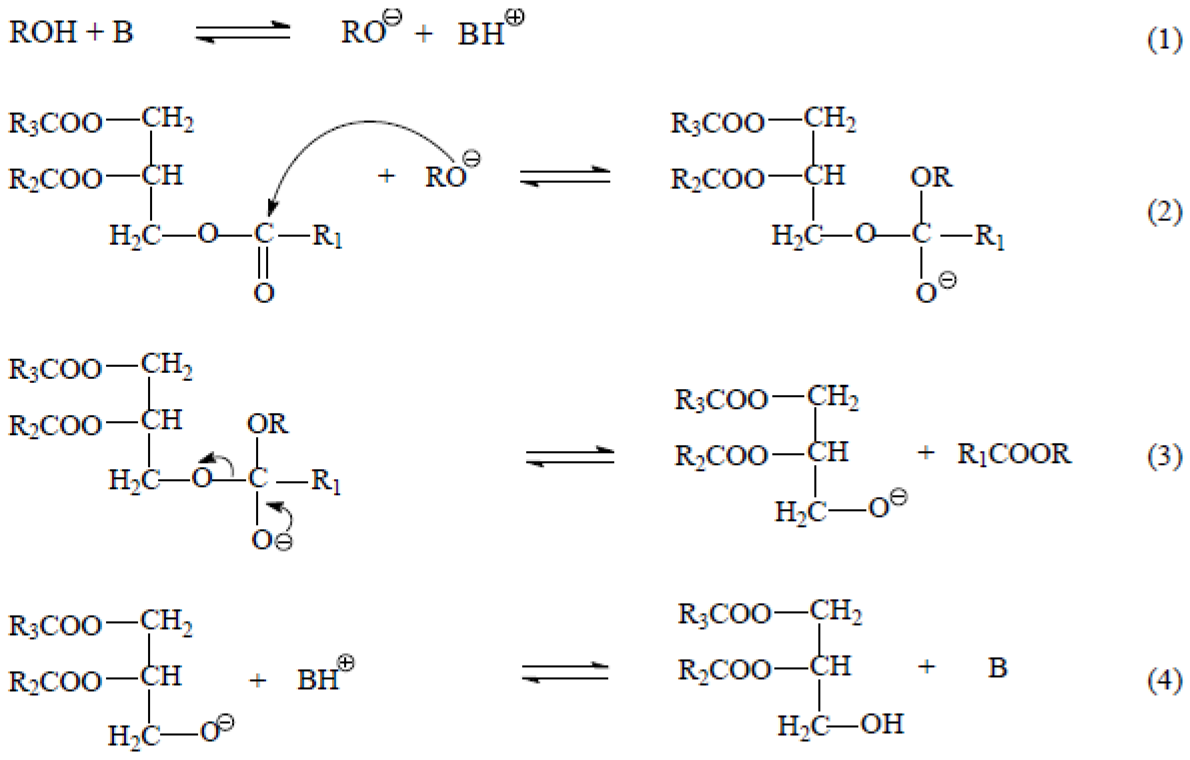
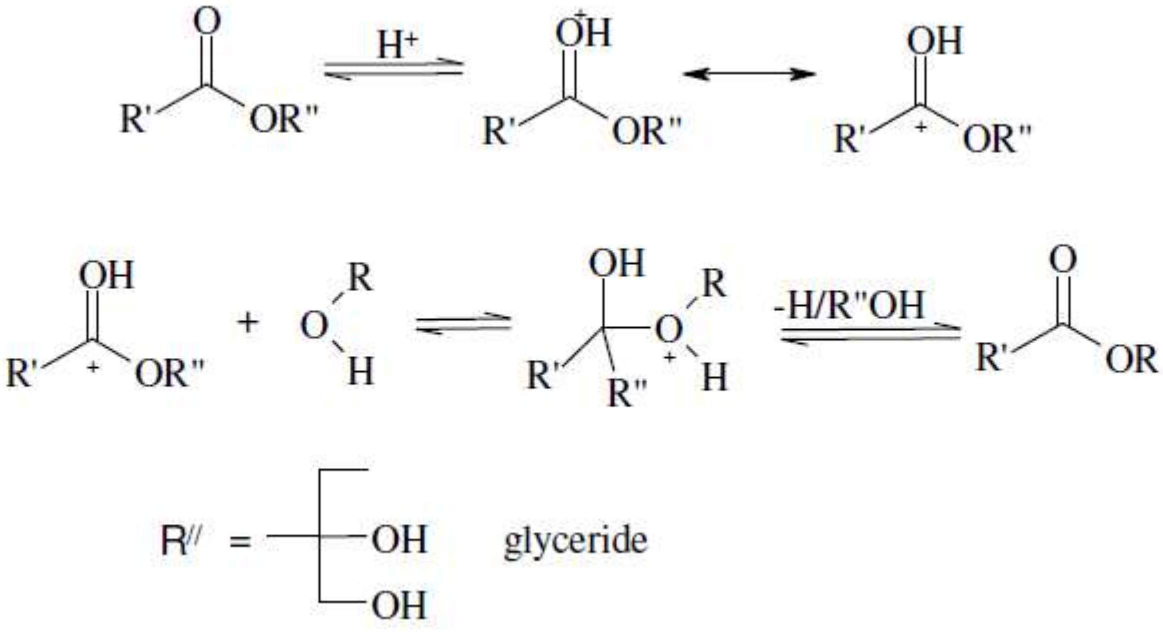


Figure 2.2 Mechanism of the base-catalyzed transesterification of vegetable oils

2.4.1.2 Transesterification using acidic catalyst

With starting raw materials containing a high amount of FFA such as waste cooking, *Jatropha curcas*, rubber, tobacco oils, etc., an acid-catalyst, usually a strong acid such as sulfuric, hydrochloric or phosphoric acid, is more favorable than base-catalyst because the reaction does not form soap. However, the acid-catalyst is very sensitive to the water content of the raw materials. It was reported that a small amount of water, *i.e.*, 0.1 wt.% in the reaction mixture affected the FAME yield of the of vegetable oil with methanol (Thanh et al., 2012).

The mechanism of the acid-catalyzed transesterification of vegetable oils is as shown in figure 2.3 for a monoglyceride. The protonation of the carbonyl group of the ester leads to the carbocation which after a nucleophilic attack of the alcohol produces the tetrahedral intermediate. This in turn eliminates glycerol to form the new ester, and to regenerate the catalyst. The mechanism can be extended to *di*- and triglycerides48. Carboxylic acids can be formed by reaction of the carbocation with water present in the reaction mixture. This suggests that an acid-catalyzed transesterification should be carried out in the absence of water, in order to avoid the competitive formation of carboxylic acids, which reduce the yield of alkyl esters (P M Ejikeme et al., 2010).



Where: $R^{/}$ = carbon chain of fatty acid, R = alkyl group of the alcohol

Figure 2.3 Mechanism of the acid-catalyzed transesterification of vegetable oils

2.4.1.3 Enzyme-Catalyst Transesterification

The use of lipases as enzyme-catalysts for biodiesel production is also increasingly interesting. The main purpose is to overcome the issues involving recovery and treatment of the by-products that requires complex processing apparatus. The main drawback of the enzyme-catalyzed process is the high cost of the lipases. In order to reduce the cost, enzyme immobilization has been studied for ease of recovery and reuse. Additionally, inactivation of the enzyme that leads to decrease of yields is mostly restricted by the low solubility of the enzyme in methanol (Thanh et al., 2012).

Due to their ready availability and the ease with which they can be handled, hydrolytic enzymes have been widely applied in organic synthesis. They do not require any coenzymes, are reasonably stable, and often tolerate organic solvent (P M Ejikeme et al., 2010).

2.4.1.4 Transesterification using heterogeneous catalyst

Heterogeneous catalysts such as amorphous zirconia, titanium-, aluminum-, and potassium-doped zirconias have also become popular for catalyzing the transesterification of vegetable oils. It has been reported that the conversion to methyl ester reaches 87% with the potassium-loaded alumina catalyst, when a mixture with a molar ratio of methanol to oil of 15:1 is refluxed for a reaction time of 7 h (Xie, W., Peng, H., Chen, 2006). However, the catalytic activities of most of them are not much greater than that of a homogeneous catalyst such as KOH. Furthermore, there is little information regarding their catalytic durability. Research is still in progress in order to solve the problem encountered in this process such as exhaustion of catalyst and to achieve higher conversions (Furuta, A., Matsuhashi, H., Aratab, 2006).

2.4.2 Factors affecting transesterification process

2.4.2.1 The effects of moisture and free fatty acids

In alkali-catalyzed transesterification, both fatty acids and alcohols have to be anhydrous, because water displaces the equilibrium reaction towards saponification, generating soap and reducing the final yield, consuming catalyst and incr**e**asing the viscosity and gel formation, which results in a more difficult glycerol separation (Freedman, Pryde, & Mounts, 1984).

Several studies have revealed that in order to achieve high conversions the maximum free fatty acid content must be at most 0.5%.This parameter is, nevertheless, less critical than the water content. Free fatty acid reduces the yield since the free fatty acids consume the alkali catalyst, because they take part in the neutralization reaction. Ethyl ester synthesis presents additional difficulties due to the formation of emulsions during the ethanolysis. Both methanol and ethanol are partially immiscible with the oil (ethanol being more soluble than methanol), therefore vigorous stirring is needed to enhance mass transfer (Cervero et al., 2008).

2.4.2.2 The effect of oil to alcohol molar ratio

One of the most important variables affecting the yield of ester is the molar ratio of alcohol to triglyceride. The stoichiometric ratio for transesterification requires three moles of alcohol and one mole of triglyceride to yield three moles of fatty acid alkyl esters and one mole of glycerol. However, transesterification is an equilibrium reaction in which an excess of alcohol is required to drive the reaction to the right (Refaat, 2010). However, an excessive amount of alcohol makes the recovery of the glycerol difficult, so that the ideal alcohol/oil ratio has to be established empirically (Schuchardt, Serchelia, & Vargas, 1998). For maximum conversion to the ester, a molar ratio of 6:1 is the most acceptable.

The reaction stoichiometry involves three moles of alcohol for each mole of triglyceride reacted to obtain three moles of ester and one mol of glycerol. Being an equilibrium reaction, an alcohol excess displaces the reaction to ester formation and higher conversions are reached. Nevertheless, a large excess of alcohol makes glycerol separation difficult due to the increasing solubility of glycerol in the alcohol. Moreover, when the glycerol is kept in the solution, it displaces the equilibrium to the left hand side, decreasing the ester yield. Nevertheless, yield and optimum rate depend on the oil used. For example, soybean, sunflower, peanut and cotton seed oils have an optimum ratio of 6:1This ratio is used at industrial scale in order to obtain high yields, almost 98% (Cervero et al., 2008).

2.4.2.3 The effect of catalyst

Among the most commonly used alkaline catalysts in the biodiesel industry are potassium hydroxide (KOH) and sodium hydroxide (NaOH) flakes which are inexpensive and easy to handle in transportation and storage. They are preferred by small producers. Alkyl oxide solutions of sodium methoxide or potassium methoxide in methanol, which are now commercially available, are the preferred catalysts for large continuous flow production processes (Refaat, 2010).

Catalyst concentration is closely related to the free acidity of the oil. When there is a large free fatty acid content, the addition of more potassium hydroxide, or any other alkaline catalyst, compensates this acidity and avoids catalyst deactivation (Freedman, Butterfield, & Pryde, 1986).

The type of catalyst is a key factor generally, alkaline catalysts lead to much faster rates than acid catalysts, and hence they are the most often used, except if free fatty acid content is high. Sodium methoxyde is more effective than sodium hydroxide, since the reaction between NaOH and the alcohol generates a small amount of water which decreases catalytic efficiency Product profile also depends greatly on the raw material and the catalyst. For example, tallow and waste cooking oils produce fuel with better ignition properties if amines (diethylamine) are used instead of sodium methoxyde. However, because of the low cost of sodium methoxyde, it is most commonly employed (Cervero et al., 2008).

2.4.2.4 The effect of reaction time

The completion of the basic-catalyzed transesterification process depends on reaction time. Most investigators have observed an optimum reaction time around 1 h. It was reported that excess reaction time does not increase the conversion but favors the backward reaction (hydrolysis of esters) which results in a reduction of product yield (Refaat, 2010). The transesterification increases with the reaction time. Using peanut, cottonseed, sunflower and soybean oils with methanol in a ratio methanol/oil of 6:1 at 60° C and NaOH as catalyst, and yield of 80% biodiesel was obtained after 1 min for soybean and sunflower oils. After 1 h the conversion was 93-98% for all four oils (Ma & Hanna, 1999).

In the transterification of palm oil with methanol at 50° C using 1% KOH as catalyst a conversion of 80% was observed after 8 min. and about 90% after 1 h. The formation of methyl esters is high in the first minutes. Then the rate diminished and finally reaches equilibrium after 1 h (Darnoko & Cheryan, 2000).

2.4.2.5 The effect of reaction temperature

Transesterification can be conducted at various temperatures ranging from room temperature to the boiling point of the alcohol employed (68^{o}C in case of methanol) so that the reactor does not need to be pressurized (van Gerpen, 2005). Thus, the usual temperature used during transesterification in most literature is 60-65 °C. When the reaction temperature closes or exceeds the boiling point of methanol (68 °C), the methanol will vaporize and form a large number of bubbles which may inhibit the reaction (Refaat, 2010).

2.5 Properties of Biodiesels

2.5.1 Cetane Number

Cetane number (CN) is a measure of a fuel's auto ignition quality characteristics. Since Biodiesel is largely composed of long-chain hydrocarbon groups (with virtually no branching or aromatic structures) it typically has a higher CN than petroleum diesel .The CN of pure FAME molecules increases with chain length, but this effect is masked when considering complex mixtures of FAME fuels (Saxena, Jawale, & Joshipura, 2013).

Cetane number is a measure of the self-ignition quality of the fuel. Higher Cetane numbers indicate shorter times between the injection of the fuel and its ignition. Higher numbers have been associated with reduced engine roughness and with lower starting temperatures for engines. No. 2 diesel fuel usually has a Cetane rating between 45 and 50 while vegetable oil is 35 to 45. Biodiesel is usually 50 to 60. The ignition quality affects engine performance, cold starting, warm up and engine combustion roughness.

Cetane rating is related to the volatility of the fuel where more volatile fuels have higher ratings. A high Cetane fuel also may lead to incomplete combustion and smoke if the fuel ignites too soon by not allowing enough time for the fuel to mix with air for complete combustion. Fuels with low Cetane Numbers will result in difficult starting, noise and exhaust smoke. In general, diesel engines will operate better on fuels with Cetane Numbers above 50. Cetane numbers measure the ignition of diesel, much like octane numbers measure the ignition of gasoline. These numbers represent the measure of a fuel's willingness to ignite. Biodiesel has a higher Cetane number than that of diesel, largely because of its higher oxygen content (Jackson & King, 1996).

2.5.2 Acid Number

The acid number of biodiesel should be low to ensure no residual free fatty acids or processing acids are present in the fuel. The presence of excess acids can lead to corrosion and deposits in fuel systems (Alleman, Fouts, & Chupka, 2013). Acid value or neutralization number is a measure of free fatty acids contained in a fresh fuel sample and of free fatty acids and acids from degradation in aged samples. If mineral acids are used in the production process, their presence as acids in the finished fuels is also measured with the acid number.

It is expressed in mg KOH required to neutralize 1g of biodiesel. It is influenced on the one hand by the type of feedstock used for fuel production and its degree of refinement. Acidity can on the other hand be generated during the production process. The parameter characterizes the degree of fuel ageing during storage, as it increases gradually due to degradation of biodiesel. High fuel acidity has been discussed in the context of corrosion and the formation of deposits within the engine which is why it is limited in the biodiesel specifications of the three regions. It has been shown that free fatty acids as weak carboxylic acids pose far lower risks than strong mineral acids (Ferrari, Leticia, Pighinelli, & Park, 2011).

2.5.3 Cloud Point, Pour Point and Cloud filter plugging point

Two important parameters for low-temperature applications of a fuel are cloud point (CP) and pour point (PP). Cloud point is defined as the temperature below which wax in diesel or bio wax in biodiesels form a cloudy appearance. The presence of solidified waxes thickens the oil and clogs fuel filters and injectors in engines. Pour point is the temperature at which the amount of wax out of solution is sufficient to gel the fuel. Biodiesel has a higher CP and PP compared to conventional diesel. Cold filter plugging point (CFPP) is the lowest temperature, expressed in 1°C, at which a given volume of diesel type of fuel still passes through a standardized filtration device in a specified time when cooled under certain conditions. It is important as in cold temperature countries, a high CFPP will clog up vehicle engines more easily (Saxena et al., 2013).

2.5.4 Viscosity

Viscosity refers to the thickness of the oil (flow properties), and is determined by measuring the amount of time taken for a given measure of oil to pass through an orifice of a specified size. Viscosity affects injector lubrication and fuel atomization. Fuels with low viscosity may not provide sufficient lubrication for the precision fit of fuel injection pumps, resulting in leakage or increased wear. Fuel atomization is also affected by fuel viscosity. Diesel fuels with high viscosity tend to form larger droplets on injection, which can cause poor combustion, increased exhaust smoke and emissions. The viscosity of biodiesel and biodiesel blends also increases more rapidly than diesel as temperature is decreased. Certain impurities also tend to significantly increase the viscosity of biodiesel (Allen, Watts, Ackman, & Pegg, 1999).

2.5.5 Iodine Value

Iodine number is a measure of the total unsaturation within a mixture of fatty acids, and is expressed in grams of iodine which react with 100 grams of biodiesel. Engine manufacturers have argued that fuels with higher iodine number tend to polymerize and form deposits on injector nozzles, piston rings and piston ring grooves when heated. Moreover, unsaturated esters introduced into the engine oil are suspected of forming high-molecular compounds which negatively affect the lubricating quality, resulting in engine damage. However, the results of various engine tests indicate that polymerization reactions appear to a significant extent only in fatty acid esters containing three or more double bonds. Three or more-fold unsaturated esters only constitute a minor share in the fatty acid pattern of various promising seed oils, which are excluded as feedstock according to some regional standards due to their high iodine value. Some biodiesel experts have suggested limiting the content of linolenic acid methyl esters and polyunsaturated biodiesel rather than the total degree of unsaturation as it is expressed by the iodine value (Ferrari et al., 2011).

2.5.6 Total Fatty Acids

Total Fatty Acid is the analysis of the amount of fatty acids present in the biodiesel. It is an indication of the conversion efficiency of the original feedstock. Gas Chromatographic Analysis of Fatty Acids indicates the composition of each of the biodiesels. The largest fraction of fatty acids for each of the biodiesels is a potential indication of the rest of the properties. The methyl esters follow some patterns. The soy, canola, and two yellow grease ME are mostly oleic and linoleic acid (C18, one and two double carbon bonds), while the two largest components of the lard, edible tallow, and inedible tallow ME are oleic and palmitic acid (C18, one C:C bond, and C16, saturated). Free glycerin and total glycerin (by the Christina Planc method) is a good indicator as to the cleanup of the fuel, or of the degree of completion during the reaction. As such, it should remain in the specifications for biodiesel (Neff, 2000).

2.5.7 Flash Point (FP)

The volatility of biodiesel is extremely low, and properly produced biodiesel presents minimal risk for handling and safety. To ensure safe handling of biodiesel, a minimum flash point of 130°C is set to limit residual methanol. The flash point temperature of diesel fuel is the minimum temperature at which the fuel will ignite (flash) on application of an ignition source. Flash point varies inversely with the fuel's volatility. Minimum flash point temperatures (standard) are required for proper safety and handling of diesel fuel. Biodiesel and diesel have a common boiling point, but biodiesel has a higher flash point because biodiesel has a high number of FAMEs, which are generally not volatile. Thus, biodiesel is to handle at higher temperatures than diesel (Alleman et al., 2013).

2.5.8 Density

The air–fuel ratio and energy content within the combustion chamber are influenced by fuel density. In general, densities of Biodiesel fuels are slightly higher than those of petroleum diesel, and increasing the level of Biodiesel blends increases the blend's density. FAME density is strongly affected by the degree of unsaturation, with higher unsaturation leading to increased density. It has been reported that Biodiesel density is also affected by chain length, with higher chain length leading to lower fuel density (Saxena et al., 2013).

2.5.9 Higher heating value

Higher heating value is the amount of heat produced by the complete combustion of a unit quantity of fuel, this property is obtained when all products of the combustion are cooled down to the temperature before the combustion and the water vapor formed during combustion is condensed. Higher heating value of biodiesel is approximately 12% less than that of petro-diesel (39.57–41.33MJ/ kg compared to 46 MJ/kg). The higher heating value of a fuel increases with increasing carbon number in fuel molecules and also increases as the ratio of carbon and hydrogen to oxygen and nitrogen increases. In regard to the prediction, the estimated high calorific values are in a very close range, with a minimum and maximum of 39.70 MJ/kg and 39.77 MJ/kg, respectively (Ramírez-verduzco, Rodríguez-rodríguez, & Jaramillo-jacob, 2012).

2.5.10 Carbon residue

Carbon residue is defined as the amount of carbonaceous matter left after evaporation and pyrolysis of a fuel sample under specific conditions. Although this residue is not solely composed of carbon, the term carbon residue is found in all three standards because it has long been commonly used. The parameter serves as a measure for the tendency of a fuel sample to produce deposits on injector tips and inside the combustion chamber when used as automotive fuel. It is considered as one of the most important biodiesel quality criteria, as it is linked with many other parameters. So for biodiesel, carbon residue correlates with the respective amounts of glycerides, free fatty acids, soaps and remaining catalyst or contaminants (Ferrari et al., 2011).

2.5.11 Water content and sediment

The Brazilian and American standards combine water content and sediment in a single parameter, whereas the European standard treats water as a separate parameter with the sediment being treated by the Total Contamination property. Water is introduced into biodiesel during the final washing step of the production process and has to be reduced by drying. However, even very low water contents achieved directly after production do not guarantee that biodiesel fuels will still meet the specifications during combustion. As biodiesel is hygroscopic, it can absorb water in a concentration of up to 1000 ppm during storage. Once the solubility limit is exceeded (at about 1500 ppm of water in fuels containing 0.2% of methanol), water separates inside the storage tank and collects at the bottom Free water promotes biological growth, so that sludge and slime formation thus induced may cause blockage of fuel filters and fuel lines. Moreover, high water contents are also associated with hydrolysis reactions, partly converting biodiesel to free fatty acids, also linked to fuel filter blocking. Finally, corrosion of chromium and zinc parts within the engine and injection systems have been reported (Ferrari et al., 2011).

2.6 Description of Pumpkin

2.6.1 Definition of Pumpkin

Pumpkins (Cucurbit sp.) belonging to the Cucurbitaceae family are grown widely around the world as a vegetable (Figure 2.4 A). In the USA, they are vastly used for Halloween carvings and thanksgiving feasts. Most of the crops are processed into canned pumpkins or pie mix. However, the plentiful flat, oval seeds are generally discarded as agricultural residues (Figure 2.4 B). The seeds are uniquely flavoured with nutty taste and are consumed as roasted, salted snack in some parts of Canada, Mexico, USA, Europe and China. The seeds of pumpkin (Cucurbita sp.) are generally considered to be agro-industrial wastes and discarded. In some parts of the world, the seeds are consumed raw, roasted or cooked, but only at the domestic scale (Patel, 2013).

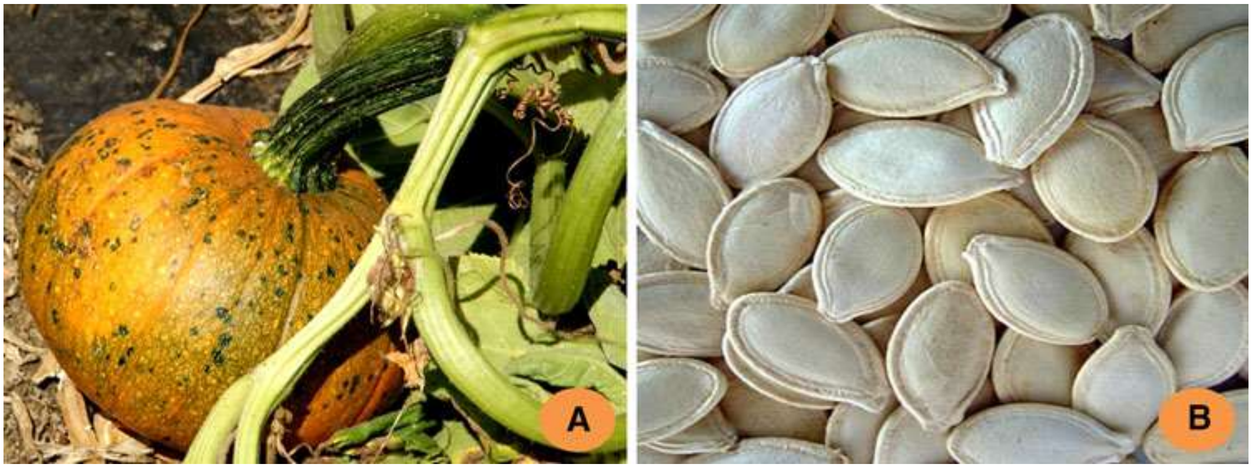


Figure 2.4 A. ripe pumpkin in vine, B. the seeds

2.6.2 Basic Growth Characteristics of pumpkin

Cucurbits are very sensitive to low temperatures because growth ceases at 6 to 7°C. In oil pumpkins temperatures between 2 and 4°C lasting longer than 3 days can reduce seedling growth and potential yield by more than half. At a temperature lower than 8°C seed germination can be reduced and temperatures of –1°C can be lethal. The plants require high levels of sunlight and a warm climate. Although pumpkins are quite resistant to drought, yield is significantly reduced after drought periods (Bavec, Mlakar, Rozman, & Bavec, 2007).

2.6.3 Soil and fertilization

Many soils are suitable for oil pumpkins, but the most successful production can be obtained in light sandy-loam soil. Appropriate soil PH must be higher than 6. In case of lower pH calcification needs to be applied at least one month before sowing or seedling planting. The soil needs to be airy, rich in humus and organic compounds with many available nutrients for the plants. Plowing depth is adjusted to the depth of the common tillage layer but should go no deeper than 25 cm. Compact soils and soils that do not have enough drainage are unsuitable for pumpkin production. Dry soil is harrowed in spring, as soon as conditions allow preserving as much moisture as possible. Shallow spring plowing and harrowing are recommended only if soil is too compact (Bavec et al., 2007)

2.6.4 Harvesting, storage, and processing

Fruit should be completely mature to achieve the highest oil content (Figure 2.5). Mature fruits are yellow with exception of the shady side, where they have yellow and green stripes. Leaves are yellow at this stage. With mature fruits the innermost fruit tissue can be easily removed from the seeds, either by hand or machine. Seed is dried at 40 to 60°C until it reaches the final moisture content of 8% to 10%. The physical quality of pumpkin seed oil was unaffected by heating temperature and duration of heating pretreatments (D. Joshi, Das, & Mukherjee, 1993).

A B

Figure 2.5 A. Oil seed pumpkins plants at the stage of the first brached primary stems; B. Mature fruits of oil seed pumpkins (identification growth stage)

2.6.5 Biodiesel from matured pumpkin seed

The advantage of pumpkin oil over sunflower oil, soybean oil and rapeseed oil would lie in the oil price. The reason of this price variation is possible due to the fact that pumpkin seed oil is not widely used as edible oil. New low- cost oil crops are needed to produce economical oils suitable for. One of the possible alternative oil crops for the Mediterranean area is pumpkin seed (Cucurbita pepo L.). The process flow diagram of biodiesel from pumpkin seed oil is figured out in figure 2.6 (Schinas et al., 2009).

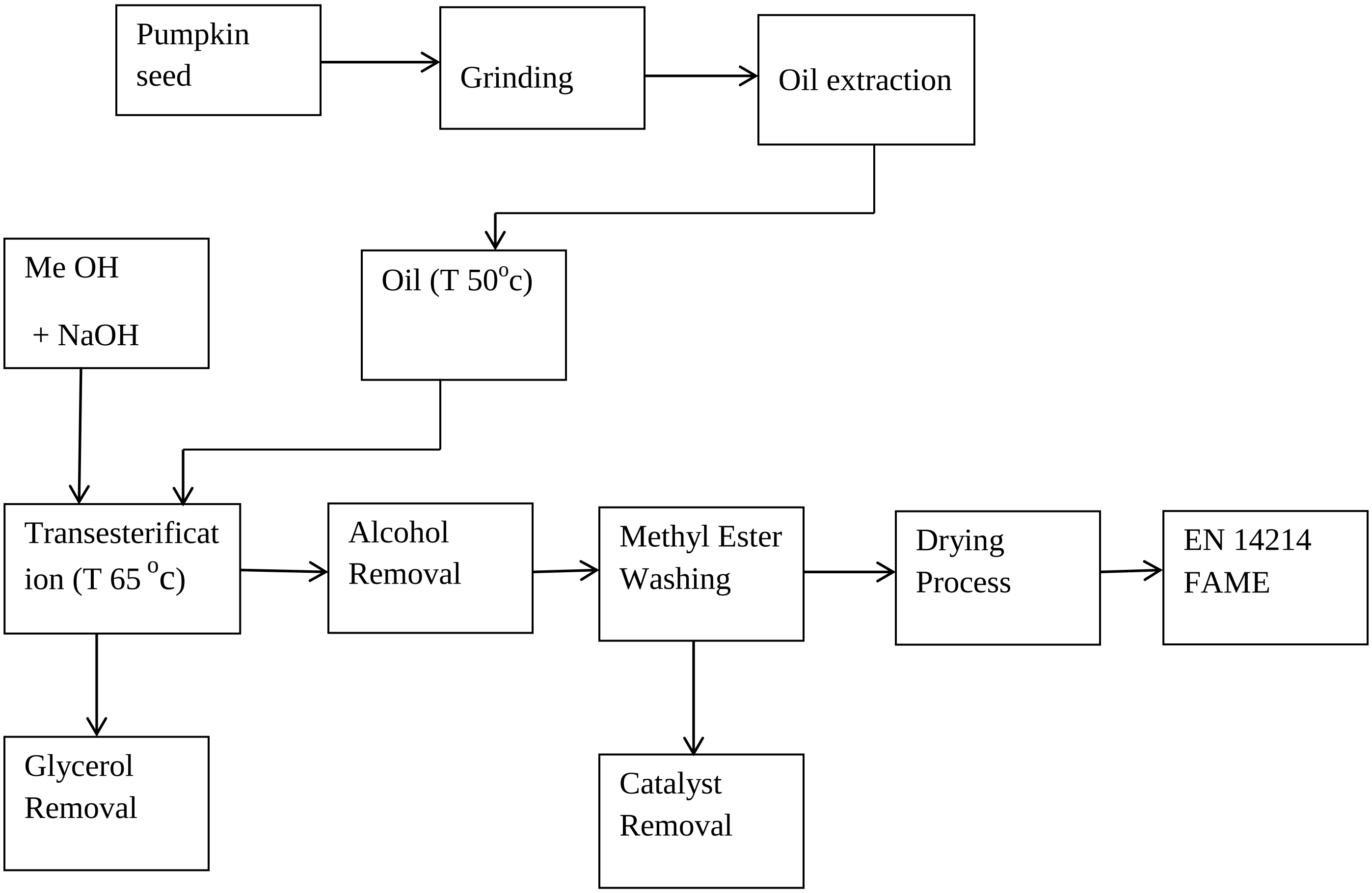


Figure 2.6 Pumpkin oil extraction and transesterification process flow diagram

2.7 Oil Extraction Techniques

Fatty materials processing is somehow different, varying with its nature and oil content. Thus, for centuries, various methods were adopted for oil extraction from oilseeds. The purpose of those extraction methods was to optimize the process by collecting the maximum quantity of the existing oil in oilseeds with the minimum costs. Currently, worldwide there are four basic methods for obtaining vegetable oil: chemical extraction, supercritical fluid extraction, steam distillation and mechanical extraction (Mariana, Ungureanu, Biris, & Voicu, 2013).

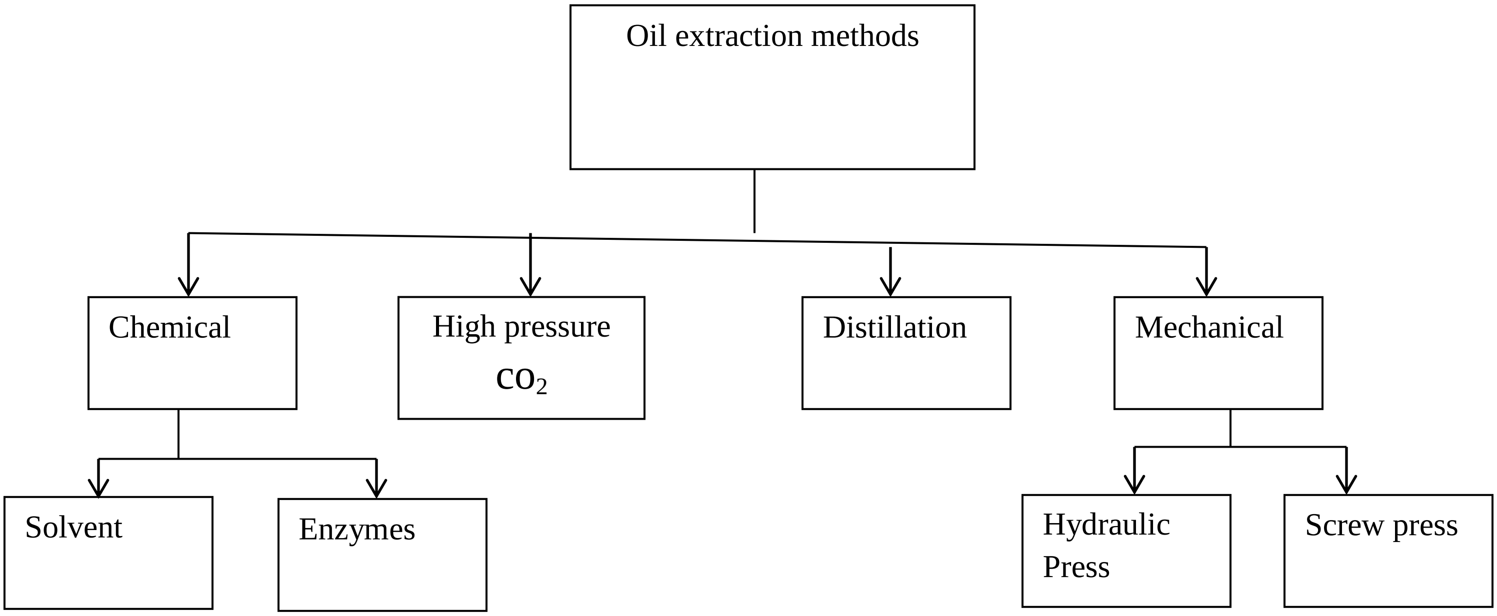


Figure 2.7 Basic methods for oil extraction

2.7.1 Mechanical extraction

The technique for oil extraction by mechanical presses is the most conventional one among the other methods. In this method, either a manual ram press or an engine driven screw press was used. The mechanical oil extraction technique was conducted using a minimum 40 screw press at Centre for Plant and Water science (CPWS) in Central Queensland University (CQU) as shown in Figure2. 8. Properly dried and treated beauty leaf kernel samples were used to extract oil by this method. It was found to be very difficult to process kernel by using this screw press. Three samples of whole and three samples of grated kernels were used to perform the experiment. Seed kernel samples were ground using a blender machine. Operator was required constantly to attend and operate the machine. It has been observed that the rate of oil production was very slow, typically taking couple of hours to process just one sample. It could be noted that grated kernels provided higher oil yields than the whole kernels (Bhuiya, Rasul, Khan, Ashwath, & Azad, 2015).

Figure 2.8 Mechanical oil extractions through a screw press

Through pressing, oil is separated from the oleaginous material (solid-liquid mixture) under the action of compressive external forces that arise in special machines called presses. This method ensures extraction of a non-contaminated, protein-rich low fat cake at relatively low-cost. The disadvantage of this method is that the mechanical presses do not have high extraction efficiencies, about 8-14% of the available oil remain in the press cake (Mariana et al., 2013).

2.7.2 solvent Extraction

The solvent extraction method recovers almost all the oils and leaves behind only 0.5% to 0.7% residual oil in the raw material. The solvent extraction method can be applied to any low oil content materials. It can also be used for pre-pressed oil cakes obtained from high oil content materials. Because, of the high percentage of recovered oil. Extraction refers to an operation in which one or more components of a liquid or a solid phase are transferred to another liquid phase. Extraction utilizes the differences in the solubility's of components. In liquid-liquid extraction or solvent extraction, a solute in a liquid solution is removed by contacting with another liquid solvent, which is relatively immiscible with the solution (Chavan & Bhagat, 2011).

Solvent extraction as employed in the United States is the most efficient means of deriving oil from the seeds. Generally, 9 – 12% more oil with fewer impurities can be extracted from the seeds by solvent extraction than by mechanical pressing. Further, a minimum of heat is involved in solvent extraction, so the oil produced is of better quality. This process is energy conserving as compared with mechanical extraction (Nadu, 2008).

The Soxhlet extractor is placed onto a flask containing extraction solvent. The Soxhlet is than equipped with a condenser. The solvent is heated to reflux. The solvent hexane forms vapors, which travels up a distillation arm, and floods into the chamber housing the thimble of solid. The condenser ensures that any solvent vapor that cools drips down into the chamber housing the solid material. The chamber containing the solid material slowly fills with warm solvent. Some of the desired compound will then dissolve in the warm hexane. When the Soxhlet chamber is almost full, the chamber is automatically emptied by the siphon side arm, with hexane running back to the distillation flask. Solvent extraction, shown in the figure below, is the process in which constituent is removed from a solid by means of a liquid solvent. It is also called leaching. The chemical extraction using n-hexane method results in the highest oil yield which makes it the most common method (Chavan & Bhagat, 2011).

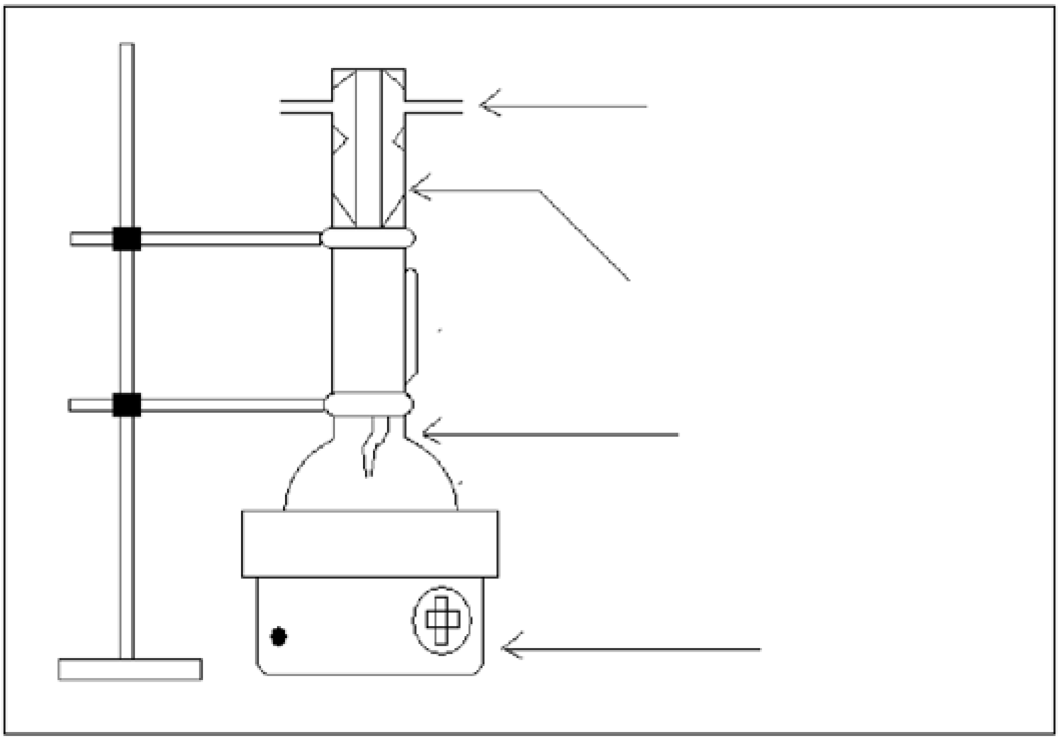


Figure 2.9 Extraction of oil using solvent extraction

2.7.3 Enzymatic oil extraction

This method is implemented by big vegetable oil companies because the process produces many high value products. The first step necessary is the cooking of the seeds and after that, the cooked seeds are put into water. The following step is the enzymes adding which digest the solid material. At the end, the separation of the residual enzymes and oil are made using a liquid-liquid centrifuge (Sari, 2006).

2.7.3 Fatty acid composition of pumpkin seed oil

Table 2.2 Fatty acid composition of pumpkin seed oil

Pumpkin seed oil fatty acid type	Pumpkin seed oil fatty acid composition
Palmitic (C16:0)	15.97-16.36
Stearic (C18:0)	4.68-5.24
Oleic (C18:1)	44.11-44.74
Linoleic (C18:2)	34.77-35.72
Arachidic (C20:0)	0.41-0.81

(Source: Leila Rezig, Moncef Chouaib 2012)

2.8 Design of Experiments

Experiment can be defined as a test or series of tests in which purposeful changes are made to the input variables of a process or system so that we may observe and identify the reasons for changes that may be observed in the output response.

2.8.1 Central composite design

A central composite design (CCD) of experiments, originally developed by Box and Wilson (1951), is one the most efficient class of designs capable of generating a response surface. Several factors at several levels can generate a response surface. The minimum number of levels and factors is three for a second order model. CCD are useful for sequential experimentation in which the cube portion is used to allow estimation of the first order effects and the later addition of the star points permits second order terms to be added to the model and estimated. Systematic errors were avoided by randomized order of experimental runs. For each numerical factors three variables with 6 center points and 14 un centered points total 20 experiments were done, replicates of factorial points 1, replicates of axial or star points is also 1 with alpha=1, face centered. As calculated from the following equation:

$$N = 2^n + 2 * n + c \qquad 2.1$$

Where:

N= the total number of experiments required
n= the number of factors
c=center points

$$N = 2^3 + 2 * 3 + 6 = 20 \text{ Experiments}$$

The experimental plan was made using the CCD and the responses measured, yield of fatty acid methyl esters (FAME), for transesterification. A three-level-three-factor CCD was employed in the optimization study, requiring 20 experiments. The methanol-to-oil molar ratio, catalyst concentration, and reaction temperature were the independent variables selected to optimize the conditions for FAME production. The experiments were carried out in randomized order and data was statistically analyzed by the Design Expert software version 7.0.0 (STAT-EASE Inc., Minneapolis, USA).

3 MATERIALS AND METHODS

The experimental work has been done in the laboratory of school of Chemical and Bio-Engineering, Addis Ababa Institute of Technology; Addis Ababa University, Addis Ababa, Ethiopia.

3.1 Materials

The major raw materials used during the experiment are pumpkin seed oil, analytical grade (AG) methanol and NaOH as a homogeneous catalyst. The oil was obtained by using solvent extraction method in a soxhlet apparatus. Hexane was used as a solvent. The chemicals were purchased and obtained from RANCHEM INDUSTRY AND TRADING, at Charkos Kfleketea in Addis Ababa City and the experimental laboratory was done at the school of Chemical and Bio-Engineering, Addis Ababa Institute of Technology (AAIT), Addis Ababa, Ethiopia. The chemicals and materials are listed in (Table 3.1).

Table 3.1 list of materials and chemicals used during the work time

Materials/Equipment's	**Purpose**
Oven	to dry the sample
Miller	To grind or mill the sample
Soxhlet	to extract oil
Filter paper	to put the sample in to the Soxhlet
Rotary Evaporator	to separate hexane from oil
Density bottle	to measure density
Vibro viscometer	to measure viscosity
Thermometer	to measure temperature
Bunsen burner	to measure flash point
Digital balances	to weigh the samples
Graduated cylinder	to measure different volumes
Separating funnel	to separate biodiesel from glycerol
PH meter	Measure PH

Chemicals	**Purpose**
Hexane	to extract oil
Phosphoric acid and sodium hydroxide	For Oil degumming and neutralization
Potassium hydroxide, ethanol, Phenolphthalein and distilled water.	To determine the Acid value
Potassium hydroxide, hydrochloric acid, ethanol alcohol, Phenolphthalein and distilled water	To determine Saponification value
Methanol and sodium hydroxide	For Biodiesel production
Phosphoric acid	To Neutralization of excess catalyst

3.2 Experimental Method

3.2.1 Pumpkin seed preparation

Pumpkin fruit of (Cucurbita pepo) was purchased from the shop in Addis Ababa, Ethiopia. Pumpkin fruit was undergo various processing in the course of its preparation for extraction. Pumpkin fruit shells were carefully cut open to expose the seeds which were embedded in an orange –yellow fibrous material. The seeds were removed from the fruit and thoroughly washed with distilled water to remove other component of pumpkin and impurities. After that Pumpkin seeds was cleaned manually in order to remove foreign material and impurities and then dehulled manually to remove the outer seed coat and subsequently dried in an electric oven at 105^0C until almost a relatively constant weight was obtained. Figure 3.1 shows peeled pumpkin seed while the details of figure Pumpkin seed preparation were listed in appendix F.

The ratio of peeled seed weight to the raw seed weight (W) was calculated using equation 3.1.

$$W = \frac{\text{mass of the peeled pumpkin seed}}{\text{total mass of pumpkin seed}} \qquad (3.1)$$

3.2.2 Determination of moisture content of Pumpkin seed kernel

The pumpkin seed kernels were cut into smaller chips to ease moisture removal. The crucible was weighed with and without the amount of peeled seeds. The pumpkin seed oil was dried in an electric oven at 105^0C. Then the sample was removed each 2h from the oven and placed in the desiccator for 30 minutes to cool and re-weighed. Finally, the weight was taken and compared with the initially recorded weight. This was taken in triplicate.

The percentage moisture content was then calculated using the formula in equation (4.1). The moisture content of the peeled seed with triplicated test was analyzed.

3.2.3 Oil extraction

Experimental work was conducted using soxhlet equipment by solvent extraction process. The solvent used during extraction was hexane. Initially the raw material pumpkin seed was prepared and cleaned well and then dried in order to remove the moisture contents. After the moisture removed the pumpkin seeds were grinded and sieved in order to get good surface area or particle size and dried well again in order to get high yield. The grounded pumpkin seed samples were placed in a filter paper.

The filter paper was then placed in an extraction chamber which is being suspended above a flask containing the solvent and below a condenser. Heat is being applied to the flask and the solvent evaporates and moves to the condenser where it is converted into liquid that trickles in to the extraction chamber containing the sample. The extraction chamber was made in such a way that when the solvent surrounding the sample exceeds a certain level it overflows and trickles back down in to the boiling flask. Finally, the oil and solvent was separated through rotary evaporator at the end of the extraction process.

100g of milled and sieved seed with a particle size 2mm was loaded into the soxhlet that had 600ml of hexane. The extraction was carried out at 70^oC for 9 hour in a water bath per each run. The experimental set up for oil extraction was shown in appendix F.

3.2.4 Determination of the percentage of seed oil extracted

100g of the sample was placed in the filter paper and 600ml of hexane was poured into 1000ml round bottom flask. The apparatus was heated at 70^0C and allowed for 9 h of continuous extraction using soxhlet apparatus. At the end the solvent was distilled off using rotary evaporator set at a temperature of the boiling point of hexane. The percentage of oil extracted was determined using equation (4.3).

3.2.5 Pumpkin seed oil refining

1 Degumming

It is used to remove phosphorus compounds of crude oil using a phosphoric acid and hot distilled water. Distilled water 3% (v/v) of oil at 70℃ and 1.5% phosphoric acid (v/v) of oil were mixed with the oil which was heated at 70℃. The mixtures were stirred at speed of 200 rpm for 1 hour at a temperature of 70℃. The impurities were separated using a centrifuge at a speed of 800 rpm for 20 minutes.

2 Neutralization: After determining the free fatty acid (FFA) of oil, the free fatty acid was neutralized by 0.05N of NaOH. Neutralization was done by heating the oil at 70℃. The mixture of oil and NaOH solution were stirred at 200 rpm at a temperature of 70℃ for 1 hour. The mixture was washed with a distilled water to remove a trace NaOH and produced soap.

3 Washing About 20% hot distill water was added to the neutralized pumpkin seed oil and stirred vigorously for 3-5 minutes. The mixture was poured in to a separatory funnel (decanter) and the water was drained off. Finally, the oil was dried in oven at 105 ℃ for 6h to remove the water present.

3.2.6 Characterization of extracted oil

Saponification value and acid tests were done based on experimental procedures of (A. Joshi, Singhal, & K.R., 2001) and (Odoom & Edusei, 1999).

Acid value of oil

Acid value (AV) of the oil was determined using the method described by (A. Joshi et al., 2001) For determining acid value, firstly, a titration solution of 0.1N of KOH in distilled water was prepared. Subsequently, 4g of oil was added to the 250ml conical flask and heated at 70℃ for 3 minutes. Then, 20ml of anhydrous ethanol (99.5%w/w) and 5 drops of phenolphthalein were added into the titration beaker with sample oil. Then oil sample was mixed with 20ml of ethanol and 5 drops of phenolphthalein. Finally, titration solution, 0.1N of KOH was being added 1 drop at a time until the first color change was observed.

Once the color change was observed, the titration volume (ml) was recorded and titration was stopped. The titration volume recorded (ml) was used to calculate the acid value the result was shown in equation (4.4).

Saponification value of oil

Saponification value (SV) of the oil was determined using the method described by (Odoom & Edusei, 1999).

To determine saponification value of the oil, initially, 0.5mol/l potassium hydroxide in anhydrous ethanol (99.5%w/w) and 0.5mol/l hydrochloric acid solution in distilled water was prepared. Then, a conical 2 g sample was placed in a 250ml flask. Next, 25ml of 0.5mol/l potassium hydroxide in ethanol solution was added. The flask was heated at a temperature of 70℃ and shaken when adjusting the heat so that backflow ethanol did not reach the top of cooling pipe. After that, the sample was heated for 30 minutes and it was cooled immediately. 5 drops of phenophtaline was added as an indicator. Finally, the sample was titrated with 0.5mol/l hydrochloric acid solution before the test liquid solidified. The titration was stopped and the value was recorded when the color change was observed. A blank level test was also performed in parallel using all above procedures without the oil sample addition.

Density of oil

Specific gravity of oil was determined using density bottle method. A washed, dried and weighed density bottle was filled with the oil sample and placed in a water bath at temperature of 15 °C to be attainable. The weight of the oil was recorded after which the bottle was emptied, washed and dried again before refilling with water maintained at a temperature of 15 °C.

The weight of water at this temperature was recorded and the specific gravity was calculated to determine the density of oil.

Kinematic viscosity of oil

Vibro viscometer was used to determine a viscosity of the oil. The sample was kept in the water bath heated by thermostat until it reaches the 40^0C that was the kept constant temperature of water bath (equilibrium temperature**.** After maintaining the equilibrium temperature**,** the vibro viscometer tip was inserted in the sample and the reading was taken from the controller. This was done in triplicate and the average dynamic viscosity was recorded.

Higher heating value of oil (HHV)

Heating Value or Heat of combustion is the amount of heating energy released by the combustion of a unit value of fuels. Calorific value (energy content or heat of combustion) of oil was determined using a correlation developed according to the relation between viscosity and HHV in MJ/Kg (Demirbas, 2008):
For vegetable oils:

$$\mathrm{HHV} = 0.0317 * \mathrm{VS} + 38.053 \qquad (3.2)$$

Iodine value of oil (IV)

Iodine value is the measure of the degree of unsaturation of a particular oil or fuel. It was determined using empirical equations given in equation 3.3 developed by (Demirbag, 1998).

$$\mathrm{HHV} = 49.43 - 0.041 * \mathrm{SV} - 0.015 * \mathrm{IV} \qquad (3.3a)$$

Solving for IV from equation (3.3a)

$$\mathrm{IV} = \frac{-\mathrm{HHV} + 49.43 - 0.041 * \mathrm{SV}}{0.015} \qquad (3.3b)$$

Flash point, moisture content and ash content of oil

Flash point

A flash point of the Pumpkin seed oil was determined using an open cup method. A cup was filled with the oil up to a mark (about 75 ml) and was heated by a Bunsen burner. A hand thermometer was inserted into the cup to read the temperature. A small open flame was maintained from an external supply of natural gas. Periodically, the flame was passed over the surface of the oil. When the flash temperature was reached, the surface of the oil caught with fire. The temperature at this moment was noted and reported as flash point temperature.

Moisture content of oil

The empty dish was weighed with and without the amount of pumpkin seed oil and dried in an oven at 105℃ for 7hr, weighing each 2hr till almost constant weight is obtained and finally the weight was taken and compared with the initially recorded weight. The percentage weight in the pumpkin seed oil was calculated using the formula:

$$\text{Moisture content\%}\left(\frac{w}{w}\right) = \frac{w_1 - w_2}{w_1} * 100 \qquad (3.4)$$

Where:

w_1 = Original weight of the sample before drying

w_2= Weight of the sample after drying

Ash content of oil

Ash content of oil was determined using a furnace. A 20 g of oil was added in a burning cup. Then, the sample was placed in a furnace. A furnace was located at a temperature of 500℃ for 1 hour and after burning the residue sample was weighted and ash content was calculated using the equation 4.8.

3.2.6 Feed Material Requirement for Biodiesel Production

For every run 50ml of purified Pumpkin seed oil was used. Hence, the amount of methanol and catalyst was calculated as follows using the process parameters. The amount of methanol required when the molar ratio of methanol to oil ratio was 6:1 ratio:

$$\frac{\text{n Methanol}}{\text{n Oil}} = 6$$

Substituting mass for mole;

$$\frac{\text{Given mass of Methanol/molecular weight of Methanol}}{\text{Given mas of Oil/molecular weight of Oil}} = 6 \qquad (3.5)$$

Where:

Molecular mass of Methanol is=32g/mol

Average weight of vegetable oil = 3*molecular weight of fatty acid

The molecular weight of fatty acid of pumpkin seed oil is 289.56g/mol. Thus: average molecular mass of Pumpkin seed oil=868.68g/mol; and for the experiment 50ml of pumpkin seed oil was used, and density of oil was 917.8 kg/m^3. Therefore given mass of oil is;

$$\text{Given mass of oil} = \text{density of oil} * \text{volume of oil}$$

$$\text{Given mass of oil} = 917.8\text{Kg/m}^3 * 50 * 10^{-6}\text{m}^3$$

Given mass of oil =45.89g; substituting in to equation 3.5 to calculate mass of methanol:

$$\frac{\text{Given mass of Methanol}/32\text{g/mol}}{45.89\text{g}/868.68\text{g/mol}} = 6$$

Thus, given mass of methanol=10.14 g. To get the volume of methanol used for 50ml of oil; given mass of methanol=density of methanol*volume of methanol

Substituting; density of methanol=791Kg/m^3 and given mass of methanol=10.14g

10.14g=791g/l*volume of methanol Thus, volume of methanol=12.8ml.

3.2.8 Processes of biodiesel production

Transesterification reaction is the most commonly used method of producing biodiesel. Transesterification is the reaction of a triglyceride such as vegetable oil with an alcohol, usually methanol, in the presence of an acidic or alkaline catalyst to produce fatty acid esters and glycerol. A base-catalyzed transesterification process is normally adopted for biodiesel production because alkaline metal alkoxides such as sodium methoxide and hydroxides are more effective than acid catalysts (Asakuma, Kawanami, Maeda, Kuramochi, & Fukui, 2011).

3.2.8.1 Transterification procedure (making biodiesel)

Biodiesel production process by transterification reaction was made using three main components thus are: pumpkin seed oil, alcohol (methanol), and a base catalyst (NaOH) have been studied in the laboratory experiments. A 500ml glass three neck flask reactor equipped with magnetic stirrer, electric thermostat, and con-denser was used in all experiments. The reactor connected to oil bath heated with electric thermostat, which was capable of controlling the temperature. The procedures for the biodiesel production by transterification reaction were:

A. Mixing of the methanol and the catalyst in 250 ml beaker using the mixing ratio of methanol and catalyst concentration respectively. A quantity of methanol was poured in a beaker and the sodium hydroxide pellet was placed in the weighing balance to get exactly weight and mix with methanol to 40^0C (in a water bath) and stirred manually until the catalyst is completely dissolved in methanol. The methanol and sodium hydroxide solution was poured in the warm pumpkin seed oil in a 500 ml three neck flask and stirred vigorously using a magnetic stirrer at 500 rpm for 2 hours. The mixture was then allowed to settle for 24 hours in a separating funnel. After settling, the upper layer which was biodiesel and the lower layer (i.e. glycerol, soap and other residual) were separated. The quantity of biodiesel collected was measured and recorded in each run after purification.

B. Washing biodiesel. After the neutralization of biodiesel by phosphoric acid this reduces the amount of catalyst present, warm distilled water was used to wash the biodiesel to remove any impurities like, excess methanol (after the distillation process), glycerol and soap that remain in the funnel.

C. The washed sample was dried by placing it on a hot plate (oven) to evaporate the excess water in the biodiesel.

D. The quantity of biodiesel was measured and collected in the sample holder and recorded for each sample run.

Note: The above procedures were repeated by varying the mole ratio of methanol to pumpkin seed oil, catalyst concentration, and reaction temperature by the design experimental run.

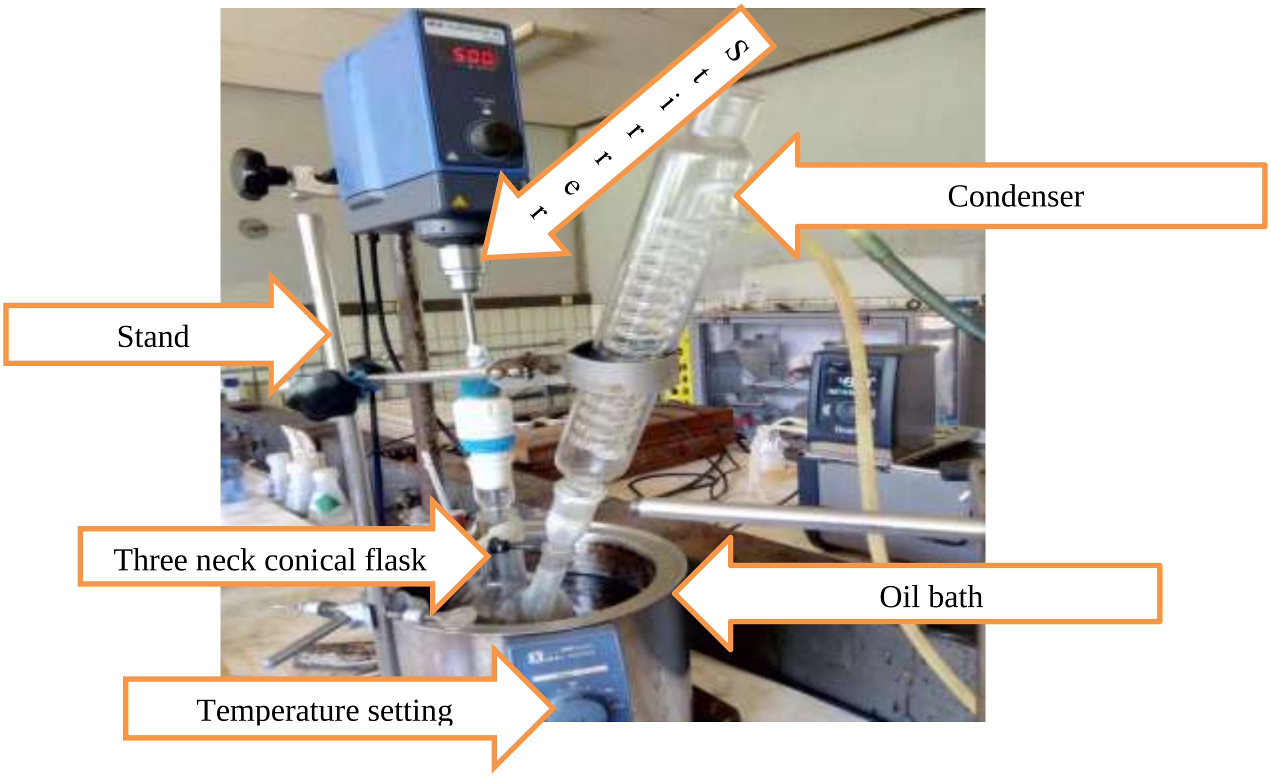


Figure 3.1 Experimental set up for biodiesel production

3.2.8.2 Separation process

After the reaction was completed the separation process is one of the most crucial parts of biodiesel production. The product from the transesterification process is normally composed of biodiesel, glycerin, alcohol, catalyst and unreacted glycerides. The properties of the fuel are strongly influence by the purity of the biodiesel product. Normally, the biodiesel was separated from by-product glycerin using a simple gravitational settling (density difference) method and left for 24 hours. After 24 hours two different layers were seen in the separating funnel. The above biodiesel layer was separated for further purified and the lower glycerol layer was decanted off.

3.2.8.3 Washing and Drying

Once phase separation has been achieved, the purification of the ester phase is necessary to ensure that the biodiesel meet specifications of ASTM and EN. After the phase separation of glycerol, the biodiesel still has an excessive amount of soaps, aggressive pH, catalyst, FFA, methanol, glycerides and other impurities. The biodiesel was washed with warm distilled water to remove any contaminants include (primarily) excess alcohol, excess catalyst, soap formation of glycerin.

The biodiesel was washed with warm distilled water about 50^0C, 50% (related to the biodiesel volume), of water and 3% of phosphoric acid (related with water volume) were added to the biodiesel in order to extract contaminants passed through the esters to allow soluble material, excess catalyst and other impurities to stick to the water and be settled to the bottom of the vessel. The purpose of adding phosphoric acid was to neutralize biodiesel there by it was reduce the amount of water adding and energy required during evaporation (Abbaszadeh, Ghobadian, Najaf, & Yusaf, 2014). The mix was allowed to separate, forming a top biodiesel layer and a bottom aqueous layer, due to difference in densities and immiscibility. After complete separation, the aqueous layer was removed and the washing process repeated until the aqueous layer shows no contamination and the pH of the biodiesel becomes relatively neutral.

The last step was drying process. The biodiesel washing process sometimes leaves the biodiesel looking a bit cloudy. This means there is still a little water in it. Drying biodiesel requires a little more than heating up the final biodiesel at 65^0C, for 15 - 20 minutes until all any remaining moisture (water) evaporate and removed from the sample. After the biodiesel washed and dried, the yield of biodiesel production was analyzed.

The fuel cannot be called biodiesel until it meets the standard specifications of ASTM D 6751or EN 14214. Therefore, the purification step is essential. Untreated biodiesel contains impurities such as free glycerin, soaps, metals, methanol, free fatty acids, catalyst, water and glycerides. The high impurities level can reduce engine life. Table 3.4 shows each impurity effects on biodiesel (Duran, Berrones, Eapen, & Sebastián, 2014).

Table 3.2 Effects of impurities on biodiesel and engines

Impurity	Effect
water	Oxidation, corrosion and bacteriological growth (filter blockage)
Glycerol	Deposits in the injectors (carbon residue), high viscosity, crystallization, settling problems
Methanol	Low values of density and viscosity, low flash point (transport, storage and usage problems), corrosion of Al and Zn
Soap	Deposits in the injectors (carbon residue), filter blockage (sulphated ashes), engine weakening

3.2.9 Characterization of biodiesel

The production of biodiesels from pumpkin seed oil by base catalyzed transesterification reaction was characterized their physicochemical properties. The characterized physical and chemical properties were specific gravity, kinematic viscosity, higher heating value, acid value, saponification value, iodine value, flash point, Cetane number, ash content and moisture content. In addition to those properties the details of analysis of Fourier transforms infrared (FT-IR) of biodiesel produced from pumpkin seed oil was also discussed in appendix C.

3.2.9.1 Determination of Specific Gravity and density

The density of the biodiesel was determined by using density bottle. A clean and dry bottle was weighed (W0) and then the bottle was filled with the biodiesel and reweighed to give (W1). The biodiesel was substituted with water after washing and drying the bottle and reweighed to give (W2). The specific gravity of biodiesel was calculated using equation (4.10) and then the density of biodiesel was calculated using density of water.

Since density is strongly influenced by temperature, the quality standards state the determination of density at 15℃ using the same procedure for determining density of oil.

3.2.9.2 Kinematic Viscosity

Viscosity is the most important factor of fuels that affects the flow of the fuels i.e. its fluidity. The kinematic viscosity (ν) is defined as the resistance to flow of a liquid against gravity. The same procedure was used to determine the kinetic viscosity of the biodiesel as discussed on characterization of purified pumpkin seed oil. The kinematic viscosity biodiesel was shown in table 4.6.

3.2.9.3 Acid Value

The same procedure was used to determine the acid value of the biodiesel as the procedures explained for characterization of pumpkin seed oil acid value. The acid value of biodiesel was calculated using equation (4.12).

3.2.9.4 Saponification value

The same procedure was used to determine the saponification value of the biodiesel as the procedures explained for characterization of pumpkin seed oil saponification value.

3.2.9.5 Higher heating value (HHV)

Calorific value (energy content or heat of combustion) of biodiesel was determined using a correlation developed according to the relation between viscosity and HHV in MJ/Kg (Demirbas, 2008) using equation (4.13).

3.2.9.6 Iodine value (IV)

Iodine value is the measure of the degree of unsaturation of a particular oil or fuel. It was determined using empirical equations given in equation 3.6 developed by (Demirbag, 1998).

$$\mathrm{HHV} = 49.43 - 0.041 * \mathrm{SV} - 0.015 * \mathrm{IV} \qquad (3.6)$$

Solving for IV from equation 3.6 the iodine value of biodiesel was calculated using equation (4.14).

3.2.9.7 Detrmination of the Flash point (FP)

A flash point of the biodiesel was determined using an open cup method. A cup was filled with the biodiesel up to a mark (about 75 ml) and was heated by a Bunsen burner. A hand thermometer was inserted into the cup to read the temperature. A small open flame was maintained from an external supply of natural gas. Periodically, the flame was passed over the surface of the biodiesel. When the flash temperature was reached, the surface of the biodiesel caught with fire. The temperature at this moment was noted and reported as flash point temperature.

3.2.9.8 Determinations of moisture content and ash content

The procedures for determining the moisture content and ash content of were the same as the determinations moisture content and ash content of purified oil.

3.2.9.9 Cetane Number

Cetane number is a relative measure of the interval between the beginning of injection and auto ignition of the fuel. The Cetane of biodiesel was calculated using an empirical formula in equation (4.15).

3.3 Experimental work design

During this work the biodiesel was produced using purified pumpkin seed oil and Methanol with a homogeneous catalyst of sodium hydroxide. Experimental design was analyzed and done by the Design Expert 7.0 software application. Experimental design selected for this study is CCD and the output measured is biodiesel yield gained.

Process variables revised were reaction temperature, molar ratio of methanol to oil and weight or mass of catalyst. To get maximum conversion; reaction period and rotation speed was set at 2 hours and 500 rpm respectively and at constant atmospheric pressure. The operating limits of the biodiesel production process conditions are reasons to choose levels of the variables.

Three level three factors CCD was made use of in the optimization study, needing 20 experiments to be done. Catalyst concentration, Methanol to oil molar ratio and reaction temperature were the independent variables selected to optimize the conditions for biodiesel production by using sodium hydroxide as main catalyst for performing transesterification reaction.

Twenty experiments were done and the data was statistically analyzed by the Design Expert 7.0 software and to get suitable model for the percentage of fatty acid methyl ester as a function of the independent variables.

Table 3.3 Complete experimental design matrix of CCD

Variables	Factor Coding	Unit	Levels		
			-1	0	+1
Reaction Temperature	x1	℃	30	45	60
MeOH to Oil ratio	x2	-	6	9	12
Amount of Catalyst	x3	G	0.22	0.99	1.76

Table 3.3 indicates the complete experimental design matrix of CCD for the factorial design.

Order in which the runs were made randomized to avoid errors which are caused by systematic error.

Table 3.4 Experimental design matrix of CCD

Std	Block	Run	Coded factors			Actual factors			Actual Biodiesel yield %(w/w)
			x1	x2	x3	Reaction temperature (^OC)	MeOH to oil ratio (g)	Amount of catalyst (-)	
8	Block1	1	+1	+1	+1	60	12	1.76	
6	Block1	2	+1	-1	+1	60	6	1.76	
20	Block1	3	0	0	0	45	9	0.99	
19	Block1	4	0	0	0	45	9	0.99	
12	Block1	5	0	+1	0	45	12	0.99	
2	Block1	6	+1	-1	-1	60	6	0.22	
9	Block1	7	-1	0	0	30	9	0.99	
18	Block1	8	0	0	0	45	9	0.99	
14	Block1	9	0	0	+1	45	9	1.76	
7	Block1	10	-1	+1	+1	30	12	1.76	
5	Block1	11	-1	-1	+1	30	6	1.76	
4	Block1	12	+1	+1	-1	60	12	0.22	
11	Block1	13	0	-1	0	45	6	0.99	
1	Block1	14	-1	-1	-1	30	6	0.22	
16	Block1	15	0	0	0	45	9	0.99	
17	Block1	16	0	0	0	45	9	0.99	
15	Block1	17	0	0	0	45	9	0.99	
3	Block1	18	-1	+1	-1	30	12	0.22	
13	Block1	19	0	0	-1	45	9	0.22	
10	Block1	20	+1	0	0	60	9	0.99	

4 RESULTS AND DISCUSSIONS

4.1 The percentage of peeled and moisture content of Pumpkin seed

The total mass of peeled pumpkin seed was 3.7Kg and the total weight of the raw seed was 5Kg. There for the ratio of weight of the peeled seed to the total weight of the raw seed used (W) was calculated by substituting these values in to equation (3.1).

$$W = \frac{3.7}{5}$$

The result was: W=0.74

Therefore, the peeled seed weight was 74 % of raw seed weight.

The moisture content with trireplicated test was summarized in table 4.1.

$$\text{Moisture content} = \frac{\text{W1}-\text{W2}}{\text{W1}} \times 100\% \qquad (4.1)$$

Where: W 1 = Original weight of the sample before drying

W 2 = Weight of the sample after drying

Table 4.1 Moisture content of pumpkin seed kernel

Run	Sample weight in (g)			Moisture content (%w/w)	
	w1	w2	w1-w2	For each run	Average
1	10	9.28	0.72	7.2	7.33
2	10	9.17	0.83	8.3	
3	10	9.35	0.65	6.5	

Seeds which are dry will retain their viability for longer periods of storage in gene banks. It is recommended that, in general, seeds should be dried to between 3-8% moisture content for long-term storage (Nadu, 2008).

4.2 Percentage of extracted oil

3.5 Kg seeds were grounded in to almost 2mm particle size. 3 Kg of the powdered seed was used for oil extraction using soxhlet apparatus. After separation, about 1.5 liter of crude oil was obtained. Then, the density (ρ) of the crude oil was measured and the result was 917.8kg/m3.

The mass, m, of the oil extracted was calculated from the basic mass-density relationship given below in equation 4.2.

$$m = \rho_{oil} * V_{oil} \tag{4.2}$$

Substituting values,

m= 1.38 Kg of pumpkin seed oil was extracted. The oil content (oil yield) of the powdered seed was calculated by:

$$\text{Oil Yield} = \frac{\text{mass of crude oil extracted}}{\text{mass of total seed used}} * 100\% \tag{4.3}$$

Substituting values,

$$\text{Oil Yield} = \frac{1.38}{3} * 100\% = 46\%$$

The oil content of the seed calculated was 46.0%. This value was found to be in agreement with the previouse findings (Schinas et al., 2009).

4.3 Physicochemical properties of purified oil

4.3.1 Acid value of oil (AV)

For testing acid value, a mixture was kept until it changed to pink after 0.496ml of titration volume added.

Table 4.2 Determination of acid value of oil

Run Number	Titration volume, ml	Color change
1	0.51	colorless to pink
2	0.48	colorless to pink
3	0.5	colorless to pink
Average value	0.496	

$$\text{acid value}, \frac{\text{mgKOH}}{\text{gOil}} = 56.1 * \frac{\text{V} * \text{M}}{\text{m}} \quad (4.4)$$

Where: V =Volume of potassium hydroxide (ml), when the color change was observed

M=Concentration of potassium hydroxide, molarity of KOH

56.11 =Molecular weight of potassium hydroxide

m= mass of Oil

$$\text{Acid value}, \frac{\text{mgKOH}}{\text{gOil}} = 56.1\text{g/mol} * \frac{0.496\text{ml} * 0.1\text{mol/l}}{4\text{g}}$$

Acid value=0.69mgKOH/goil

Therefore, the acid value was 0.69 mg KOH/g of oil. And thus, the free fatty acid content of the oil is half of the acid value which is equal to 0.347 mgKOH /g of oil.

4.3.2 Saponification value of oil (SV)

First the blank level changed from pink to colorless at 16ml titration volume. The color at which the saponification test changed from pink color to colorless color was 2.7ml of titration volume. Titration results for saponification test were illustrated in Table 4.3.

Table 4. 3 Titration results for Saponification value test

Run Number	Titration volume, ml	Color change
1	3.1	Pink to colorless
2	2.4	Pink to colorless
3	2.5	Pink to colorless
4	2.67	Pink to colorless
Average value	2.7	

$$saponification\ \text{value} = \frac{56.1 * \text{N} * (\text{V2} - \text{V1})}{\text{m}} \quad (4.5)$$

Where: N = normality of HCl

V1= volume of HCl used in the test, (mL)

V2 = volume of HCl used in the blank, (mL)

m= weight of sample, (g)

$$saponification\ value = \frac{56.1\text{gKOH/mol} * 0.5\text{mol/l} * (16\text{ml} - 2.7\text{ml})}{2\text{g oil}}$$

Saponification value=186.5mgKOH/g Oil

4.3.3 Density of oil

$$\text{specific gravity (SG)} = \frac{w_1 - w_0}{w_2 - w_0} \quad (4.6)$$

Where: w_0=mass of empty density bottle

W_1 = mass of oil plus mass of density bottle

W_2 =mass of water plus mass of density bottle

w1= 24.42g, $w_{oil} = 70.90g$, and $w_{water} = 75.06g$

Substituting the values in to equation 4.6

$$SG = \frac{70.9 - 24.42}{75.06 - 24.42} = 0.9178$$

$$SG = \frac{\rho oil}{\rho w}$$

Where, ρ oil = Density of Pumpkin seed

ρw = Density of equal volume water =1g/ml

$$\rho_{oil} = SG * \rho_w$$

$$\rho_{oil} = 0.9178 * \frac{1g}{ml} = 0.9178g/ml$$

Substituting 1g/ml for water density and 0.9178 for oil specific gravity in to equation 3.8, density of oil was gained as 0.9178g/ml (917.8kg/m^3).Therefore the density of pumpkin seed oil is 9178kg/m3.

4.3.4 Kinematic viscosity of oil

The dynamic viscosity of oil was recorded as 32.12m.Pa.s at the temperature of 40℃ by using Vibro viscometer.

The kinematic viscosity (ν) of the oil was calculated from equation 4.7.

$$\nu = \frac{\mu}{\rho} \qquad (4.7)$$

Where, ν =kinematic viscosity, mm^2/s

μ =dynamic viscosity, mpa.sec

ρ= density, Kg/m^3

Inserting the value of 917.8 kg/m3 for density of oil and the value of 32.12m.Pa.s for dynamic viscosity of pumpkin seed oil in equation (4.7) gives kinematic viscosity of oil. Dynamic viscosity of oil=32.12mpa.s=$32.12*10^{-3}$ *Kg/m.s

$$\nu = \frac{32.12 * 10^{-3} Kg/m.s}{917.8 Kg/m^3}$$

Thus; kinematic viscosity (ν) of pumpkin seed oil is equal to ($0.03499*10^{-3} m^2/s$) *($\mathrm{mm}^2/10^{-6}\mathrm{m}^2$), $\nu = 34.99\mathrm{mm}^2/s$.

4.3.5 Higher heating value of oil

The calculated kinematic viscosity of pumpkin seed oil was 34.99mm^2 substituting this value into equation (3.2).

$$\mathrm{HHV} = 0.0317 * 34.99 + 38.053$$

HHV=39.1621MJ/Kg

PH value of oil the determination of pH value of pumpkin seed oil was determined to be 6.08; this shows the slightly acidic nature of the oil, it meets the standard value of (5.0-6.7).

4.3.6 Iodine value of oil

The iodine value of oil was determined using equation 3.3b

Where:

HHV=39.1621MJ/Kg

SV=186.5mgKOH/g Oil

$$\mathrm{IV} = \frac{-39.1621 + 49.43 - 0.041 * 186.5}{0.015}$$

Thus, IV (I_2/100g) =174.76

4.3.7 Flash point, moisture content and ash content of oil

Flash point of oil

For determining the flash point of oil open cup method test was used, thus flash point of the Pumpkin seed oil was 231℃. The temperature shows that the oil is appropriate for using and storing since its high flash point that opposes spontaneous combustion.

Moisture content

Initial mass of oil=41.00g

Final mass of oil=30.81g Inserting the above values in to equation (3.3);

$$\text{Moisture content\%}\left(\frac{\mathrm{w}}{\mathrm{w}}\right) = \frac{41 - 30.1}{41.00} * 100$$

Thus, the moisture content of oil was 0.26485% (w/w). This lower value of oil moisture helps to prevent formation of soap during transesterification reaction which causes difficulty of glycerol separation and reduction of biodiesel yield.

Ash content of oil

$$\text{Ash content\%}\left(\frac{w}{w}\right) = \frac{\text{final mass of oil after burning}*100\%}{\text{Initial mass of sample}} \qquad (4.8)$$

Initial mass of sample=20g

Final mass of sample=0.0089g Inserting values in equation (4.8) gives the ash content of oil.

$$\text{Ash content\%}\left(\frac{w}{w}\right)=\frac{0.0089\text{g}*100\%}{20\text{g}}=0.04$$

It shows that the oil was refined and was suitable for biodiesel production as it is small.

Table 4.4 Physicochemical properties of the pumpkin seed oil

Physicochemical properties	Units	Values
Density @ 15℃	Kg/m^3	917.8
Kinematics viscosity @ 40℃	mm^2/s	34.990
Acid value	mgKOH/g	0.690
Saponification value	mgKOH/g	186.5
Flash point	℃	231
Moisture content	% (w/w)	0.26485
Ash content	% (w/w)	0.040
Higher heating value	MJ/kg	39.1621
Iodine value	$I_2g/100g$	174.76

4.4 Physico-chemical properties of biodiesel

$$\text{Biodiesel Yield }\%\left(\frac{w}{w}\right)=\frac{\text{mass of biodiesel produced}}{\text{mass of oil feed}} \qquad (4.9)$$

Table 4.5 Number of runs selected for determination of physicochemical properties of the FAME and the yield of biodiesel gained

Run number	Actual factors			Biodiesel yield gained		
	Temperature (°C)	MeOH to oil ratio (-)	Amount of catalyst (g)	yield (ml)	ρ Kgm^{-3}	Yield %(w/w)
1	60	12	1.76	19.8	880	38.00
2	60	6	1.76	26.2	883	50.34
3	45	9	0.99	43.8	879	84.00
4	45	9	0.99	44.6	879	85.56
5	45	12	0.99	41.9	880	80.50
6	60	6	0.22	30.4	878	58.30
7	30	9	0.99	38.3	881	73.70
8	45	9	0.99	45.4	878	87.00
9	45	9	1.76	32.7	877	62.45
10	30	12	1.76	32.9	881	61.30
11	30	6	1.76	31.9	877	57.30
12	60	12	0.22	27.7	880	53.14
13	45	6	0.99	42.9	880	82.34
14	30	6	0.22	27.3	878	52.16
15	45	9	0.99	45.8	878	87.58
16	45	9	0.99	46.3	879	88.79
17	45	9	0.99	46.0	878	88.00
18	30	12	0.22	31.1	882	59.8
19	45	9	0.22	34.5	883	66.40
20	60	9	0.99	35.4	880	67.82

Physicochemical properties analyses were conducted based on eight experimental numbers. These experiment numbers were 1, 2, 4, 6, 13, 16, 17 and 20.

4.4.1 Specific gravity(SG) and density of biodiesel

Specific gravity of biodiesel was determined using density bottle method.

$$\text{specific gravity (SG)} = \frac{w_1 - w_0}{w_2 - w_0} \tag{4.10}$$

Where: w_0=mass of empty density bottle

W_1 = mass of biodiesel plus mass of density bottle

W_2 =mass of water plus mass of density bottle

The density of the biodiesel was found in the range value 877-883 shown in table 4.5.

The air fuel ratio and energy content within the combustion chamber are influenced by fuel density. The denser the biodiesel the higher the energy content per liter.

4.4.2 Kinematic viscosity of biodiesel

The dynamic viscosity (μ) of biodiesel was recorded for each selected number of runs at a temperature of 40℃ by using Vibro viscometer.

The kinematic viscosity (ν) of the biodiesel was calculated as

$$\text{kinematic viscosity of biodiesel} = \frac{\text{dynamic viscosity of biodiesel}}{\text{dinsity of biodiesel}}$$

Table 4.6 Viscosity and density of the biodiesel

Run	Temperature (^{O}C)	MeOH to oil ratio (-)	Amount of catalyst (g)	FAME (ml)	Dynamic viscosity @40 ^{O}C (m.Pa.s)	Density of biodiesel (Kg/m^3)	Kinematic viscosity of biodiesel (mm^2/s)
1	60	12	1.76	19.8	3.0	880	3.40
2	60	6	1.76	26.2	2.5	883	2.80
4	45	9	0.99	44.6	2.8	879	3.20
6	60	6	0.22	30.4	2.3	878	2.60
13	45	9	0.99	27.7	2.1	880	2.30
16	45	9	0.99	46.3	2.4	879	2.70
17	45	9	0.99	46.0	2.5	878	2.80
20	60	9	0.99	35.4	2.0	880	2.20

The viscosities of the biodiesel produced at lower temperature are higher than that of the corresponding experiments conducted with the same feed ratio but at higher temperature. This is due to effects of the operating parameters that affect the transesterification reaction.

4.4.3 Saponification value of biodiesel (SV)

Saponification value of biodiesel was determined by a titration procedure.
First the blank level changed from pink to colorless at 20ml titration volume.

$$\text{saponification value} = \frac{56.1 * \mathrm{N} * (\mathrm{V2} - \mathrm{V1})}{\mathrm{m}} \qquad (4.11)$$

Where: N = normality of HCl
V1= volume of HCl used in the test, (mL)
V2 = volume of HCl used in the blank, (mL)
m= weight of sample, (g)

Table 4.7 Saponification values of the biodiesel

Run	Temperature (^{O}C)	MeOH to oil ratio (-)	Amount of catalyst (g)	FAME (ml)	V1 (ml)	V2 (ml)	Saponification of biodiesel (mgKOH/g)
1	60	12	1.76	19.8	6.4	880	190.40
2	60	6	1.76	26.2	6.5	883	189.30
4	45	9	0.99	44.6	6.8	879	185.13
6	60	6	0.22	30.4	6.6	878	187.90
13	45	9	0.99	27.7	6.5	880	189.30
16	45	9	0.99	46.3	6.7	879	186.53
17	45	9	0.99	46.0	6.6	878	187.9
20	60	9	0.99	35.4	6.8	880	185.13

Saponification value of the biodiesel was from 185.13mgKOH/g to190.4 mgKOH/g. Saponification value shows the amount of biodiesel that changes to soap by KOH. Lower saponification value of the result is higher in quality of the product.

4.4.4 Acid value of biodiesel (AV)

Titration method was used to determine the acid value of biodiesel

$$\text{acid value,} \frac{\text{mgKOH}}{\text{gOil}} = 56.1 * \frac{\text{V}*\text{M}}{\text{m}} \qquad (4.12)$$

Where: V =Volume of potassium hydroxide (ml), when the color change was observed

M=Concentration of potassium hydroxide, molarity of KOH

56.11 =Molecular weight of potassium hydroxide

m= mass of biodiesel

Table 4.8 Biodiesel acid values

Run	Temperature (^{O}C)	MeOH to oil ratio (-)	Amount of catalyst (g)	FAME (ml)	Titration volume (ml)	Acid value (mgKOH/g)
1	60	12	1.76	19.8	0.50	0.70
2	60	6	1.76	26.2	0.48	0.67
4	45	9	0.99	44.6	0.40	0.56
6	60	6	0.22	30.4	0.46	0.64
13	45	9	0.99	27.7	0.49	0.68
16	45	9	0.99	46.3	0.42	0.59
17	45	9	0.99	46.0	0.45	0.63
20	60	9	0.99	35.4	0.40	0.56

Acid value of the biodiesel varied from 0.56mgKOH/g to 0.70mgKOH/g. The acid value is used to determine the amount of free fatty acid content in biodiesel. The lower acid value indicates that the quantity of free fatty acid in the biodiesel is also lower so higher in quality.

4.4.5 Flash point of biodiesel

Flash point was determined by using an opened cup method. The biodiesel flash point value was shown in table 4.9.

Table 4.9 Flash point of biodiesel

Run	Temperature (^OC)	MeOH to oil ratio (-)	Amount of catalyst (g)	FAME (ml)	Flash point (^OC)
1	60	12	1.76	19.8	132
2	60	6	1.76	26.2	128
4	45	9	0.99	44.6	123
6	60	6	0.22	30.4	126
13	45	9	0.99	27.7	131
16	45	9	0.99	46.3	125
17	45	9	0.99	46.0	127
20	60	9	0.99	35.4	124

Flash point of the biodiesel was between124℃ to 132℃. Flash point shows the first temperature where biodiesel is going up in to flames. From Table 4.9, the flash point value of the biodiesel fulfills ASTM Standards. Therefore, the flash point of the biodiesel is good for handling, storage or transportation.

\

4.4.6 Higher heating value of biodiesel (HHV)

Calorific value (energy content or heat of combustion) of biodiesel was determined using a correlation developed according to the relation between viscosity and HHV in MJ/Kg (Demirbas, 2008):

$$\mathrm{HHV} = 0.4625 * \mathrm{VS} + 39.450 \tag{4.13}$$

Where:

VS= viscosity

Table 4.10 Higher heating value of biodiesel

Run	Temperature (^{O}C)	MeOH to oil ratio (-)	Amount of catalyst (g)	FAME (ml)	Dynamic viscosity @40^{O}C (m.Pa.s)	Kinematic viscosity of biodiesel (mm^2/s)	Higher heating value (MJ/Kg)
1	60	12	1.76	19.8	3.0	3.40	40.8
2	60	6	1.76	26.2	2.5	2.80	40.6
4	45	9	0.99	44.6	2.8	3.20	40.7
6	60	6	0.22	30.4	2.3	2.60	40.5
13	45	9	0.99	27.7	2.1	2.30	40.4
16	45	9	0.99	46.3	2.4	2.70	40.5
17	45	9	0.99	46.0	2.5	2.80	40.6
20	60	9	0.99	35.4	2.0	2.20	40.3

4.4.7 Iodine value of biodiesel

The iodine value of biodiesel was calculated using an emprical formula rearanging and solving it from equation (3.6)

$$\mathrm{IV} = \frac{-\mathrm{HHV} + 49.43 - 0.041 * \mathrm{SV}}{0.015} \qquad (4.14)$$

Where: HHV higher heating value of biodiesel and SV saponification of biodiesel respectivly.

Table 4.11 Iodine value of biodiesel

Run	Temperature (^{O}C)	MeOH to oil ratio (-)	Amount of catalyst (g)	FAME (ml)	Saponification of biodiesel (mgKOH/g)	Higher heating value (MJ/Kg)	Iodine value (I_2g/100g)
1	60	12	1.76	19.8	190.40	40.8	54.90
2	60	6	1.76	26.2	189.30	40.6	74.25
4	45	9	0.99	44.6	185.13	40.7	76.00
6	60	6	0.22	30.4	187.90	40.5	81.74
13	45	9	0.99	27.7	189.30	40.4	84.58
16	45	9	0.99	46.3	186.53	40.5	85.48
17	45	9	0.99	46.0	187.9	40.6	75.07
20	60	9	0.99	35.4	185.13	40.3	102.64

4.4.8 Moisture content and ash content of biodiesel

Determination of moisture contents and ash content of biodiesel was done in similar manner with that of oil in Sections 3.2.6.7.

$$\text{Moisture content}\%\left(\frac{\mathrm{w}}{\mathrm{w}}\right) = \frac{\mathrm{w}_1 - \mathrm{w}_2}{\mathrm{w}_1} * 100$$

Initial mass of biodiesel, w1

Final mass of biodiesel, w2

$$\text{Ash content\%}\left(\frac{w}{w}\right) = \frac{\text{final mass of biodiesel after burning} * 100\%}{\text{Initial mass of sample}}$$

Table 4.12 Moisture and ash contents of biodiesel

Run	Temperature (^{O}C)	MeOH to oil ratio (-)	Amount of catalyst (g)	FAME (ml)	Moisture content %(w/w)	Ash content %(w/w)
1	60	12	1.76	19.8	0.019	0.0200
2	60	6	1.76	26.2	0.016	0.0170
4	45	9	0.99	44.6	0.013	0.0800
6	60	6	0.22	30.4	0.017	0.0200
13	45	9	0.99	27.7	0.018	0.0160
16	45	9	0.99	46.3	0.014	0.0150
17	45	9	0.99	46.0	0.017	0.0196
20	60	9	0.99	35.4	0.015	0.0170

The determined moisture content was from 0.013% (w/w) to 0.019% (w/w) for biodiesel. If there is high moisture content in the biodiesel, it causes further oxidation due to microbial growth during storage which reduces the shelf life and the product quality.

The ash content of biodiesel was from 0.015% (w/w) to 0.020% (w/w). If high ash content is present, it shows that the biodiesel has solid materials that are resulted from catalysts during transesterification reaction or seed cake during oil extraction process. The moisture and ash contents are in the range of standards shown in table 4.14.

4.4.9 Cetane number (index)

The Cetane number (CN) was determined using empirical formula developed by Kalayasiri et al. (1996). The empirical formula used to determine the CN was:

$$\mathrm{CN} = 46.3 + \left(\frac{5458}{\mathrm{SV}}\right) - 0.225 * \mathrm{IV} \qquad (4.15)$$

Table 4.13 Cetane number of biodiesel

Run	Temperature (^{O}C)	MeOH to oil ratio (-)	Amount of catalyst (g)	FAME (ml)	Saponification of biodiesel (mgKOH/g)	Iodine value (I_2g/100g)	Cetane number (-)
1	60	12	1.76	19.8	190.40	54.90	62.6
2	60	6	1.76	26.2	189.30	74.25	58.40
4	45	9	0.99	44.6	185.13	76.00	58.68
6	60	6	0.22	30.4	187.90	81.74	56.95
13	45	9	0.99	27.7	189.30	84.58	56.10
16	45	9	0.99	46.3	186.53	85.48	56.32
17	45	9	0.99	46.0	187.9	75.07	58.45
20	60	9	0.99	35.4	185.13	102.64	52.68

4.5 Comparision of biodiesel physico-chemical properties against standards

Table 4.14 Comparison of biodiesel physicochemical properties against standards

Biodiesel properties	Measured values	USA and EU Standard
Density @15℃ Kg/m3	877-883	860-900 EN14214
Kinematic viscosity @40 ^{o}C (mm^{2}/s)	2.2-3.4	1.9-6.0 ASTM D 6751
Flash point(℃)	123-132	≥120 ASTM D 6751
Acid value(mgKOH/g biodisel)	0.56-0.07	≤0.8 ASTM D 6751
Sapofification value(mgKOH/g biodiesel)	185.13-190.4	-
Higher heating value (MJ/Kg)	40.3-40.8	-
Moisture content%(w/w)	0.013-0.019	≤0.03 EN 14214
Ash content%(w/w)	0.015-0.02	≤0.02 ASTM D 6751
Cetane number(-)	52.68-62.6	≥51 EN 14214

Iodine value (I_2g/100g)	54.9-102.64	≤120 EN 14214

Properties of biodiesel were under standard specification of ASTM and EN. Iodine value, higher heating value and Cetane number were done through empirical formulas. The lower density of fuel burns quickly and consumed immediately while higher density fuel burns for longer time.

4.6 Biodiesel yield analysis for One factor experimental design

4.6.1 Efect of reaction temprature on biodioesel production

Setting reaction time and mixing rate at 2 hours and 500 rpm, respectively for all the runs. The effect of temperature at 30℃, 45℃ and 60℃ on biodiesel yield for 0.99g mass of catalyst, 50 ml oil feed and 9:1 ratio of alcohol to oil is indicated in Table 4.15.

Table 4.15 Effect of temperature on biodiesel yield

Run number	Temperature (°C)	Biodiesel yield (%w/w)
1	30	73.7
2	45	85.56
3	60	67.82

Biodiesel yield was directly proportional to temperature from 30℃ to 55℃ and inversely proportional from 45℃ to 60℃. The yield obtained at 60℃ was small as the formation emulsion was facilitated. Thus, the maximum and minimum yield was obtained at a temperature of 45℃ and 60℃, correspondingly.

4.6.2 Effect of catalyst amount on biodiesel production

Setting reaction time and mixing rate at 2 hours and 500 rpm, respectively for all runs, the effect of catalyst at 0.22g, 0.99g and 1.76g of sodium hydroxide on biodiesel yield at 45℃ temperatures, 50ml oil feed and 9:1 ratio of alcohol was indicated in Table 4.16.

Table 4.16 Effect catalyst amount on biodiesel yield

Run number	Mass of catalyst (g)	Biodiesel yield (%w/w)
1	0.22	66.4
2	0.99	85.56
3	1.76	62.45

Biodiesel yield was directly proportional to catalyst amount from 0.22g to0.99g and inversely proportional from 0.99g to 1.76g. As the amount of catalyst increases further above the optimum amount the yield reduced as a result of formation of the soap and emulsion. Thus, biodiesel yield was achieved maximum for a catalyst amount of 0.99g while the minimum yield was obtained at 1.76g catalyst amount.

4.6.3 Effect of molar ratio of alcohol to oil on biodiesel production

Setting reaction time and mixing rate at 2 hours and 500 rpm, respectively for all runs, the effect of molar ratio of alcohol to oil of 6:1, 9:1and 12:1 for 0.99g mass of catalyst, 50ml oil feed and at a temperature of 45 ℃ on biodiesel production is shown in Table 4.17.

Table 4.17 Effect Molar ratio of alcohol to oil on biodiesel yield

Run number	Molar ratio of alcohol to oil	Biodiesel yield (%w/w)
1	6:1	82.34
2	9:1	87.0
3	12:1	80.5

4.7 Analysis on biodiesel production

Transesterification reaction was carried out at reflux of methanol, in 500ml glass three neck flask reactor equipped with magnetic stirrer, electric thermostat, and con-denser were used in all experiments. The statistical analysis of the biodiesel production was discussed below.

4.7.1 Statistical analysis on factors affecting biodiesel yield

Experimental design was selected for the statistical analysis of the study by selecting Central Composite Design (CCD) and the response measured is the yield of biodiesel or fatty acid methyl esters (FAME). The three transesterification process factors chosen to be studied were reaction temperature, ratio of methanol to oil and weight of catalyst. Regression analysis and analysis of variance (ANOVA) was done by using Design Expert 7.0.0 program. The software program was used to generate surface plots, using the fitted equation obtained from the regression analysis, keeping one of the independent variables constant.

Response of the transesterification process was used to develop a mathematical model that correlates the yield of biodiesel to the transesterification process variables studied. Design Expert software version 7.0.0 was used for the regression analysis of the experimental data and also for evaluation of the statistical significance of the equation developed.

The central composite design results and responses, and the statistical analysis of the ANOVA are given in Tables 4.18 and 4.19, respectively. The actual yield of biodiesel produced at different process parameters. The model was tested for adequacy by analysis of variance.

Table 4.18 Experimental analysis of biodiesel yield predicted verses actual value or diagnostic summery of the result from design expert 7.0

Standard Order	Actual Value	Predicted Value	Residual	Leverage	Internally Studentized Residual	Externally Studentized Residual	Influence on Fitted Value DFFITS	Cook's Distance	Run Order
1	52.16	50.97	1.19	0.793	0.995	0.995	1.948	0.380	14
2	58.30	58.35	-0.054	0.793	-0.045	-0.043	-0.084	0.001	6
3	59.80	59.42	0.38	0.793	0.320	0.305	0.598	0.039	18
4	53.14	52.23	0.91	0.793	0.755	0.738	1.445	0.219	12
5	57.30	57.02	0.28	0.793	0.230	0.219	0.428	0.020	11
6	50.34	49.54	0.80	0.793	0.665	0.645	1.264	0.170	2
7	61.30	60.06	1.24	0.793	1.030	1.034	* 2.02	0.407	10
8	38.00	38.01	-0.012	0.793	-0.010	-0.010	-0.019	0.000	1
9	73.70	76.79	-3.09	0.491	-1.642	-1.822	-1.789	0.260	7
10	67.82	69.46	-1.64	0.491	-0.870	-0.858	-0.843	0.073	20
11	82.34	84.55	-2.21	0.491	-1.176	-1.202	-1.180	0.133	13
12	80.50	83.01	-2.51	0.491	-1.335	-1.398	-1.372	0.172	5
13	66.40	68.83	-2.43	0.491	-1.291	-1.341	-1.317	0.161	19
14	62.45	64.75	-2.30	0.491	-1.221	-1.255	-1.233	0.144	9
15	88.00	85.25	2.75	0.118	1.112	1.127	0.412	0.017	17
16	87.58	85.25	2.33	0.118	0.942	0.936	0.343	0.012	15
17	88.79	85.25	3.54	0.118	1.431	1.522	0.557	0.027	16
18	87.00	85.25	1.75	0.118	0.708	0.689	0.252	0.007	8
19	85.56	85.25	0.31	0.118	0.127	0.120	0.044	0.000	4
20	84.00	85.25	-1.25	0.118	-0.503	-0.484	-0.177	0.003	3

* Exceeds limits

From Table 4.18, the maximum yield was 88.79% (w/w), at experiment number 16, while the minimum yield was 38% (w/w), at experiment number 1. Also from the table experiment numbers 3, 4, 8, 15, 16 and 17 were maximum amount of yield gained. Therefore it was concluded that the maximum amount of biodiesel yield was gained at 0.99g of catalyst, 9:1molar ratio of ethanol to oil and 45℃. The minimum yield was obtained at a temperature of 60℃, 1.76g amount of catalyst and 12:1 molar ratio of alcohol to oil, at experiment number 1.

4.7.1.1 Development of regression model equation

The model equation that correlates the response yield of pumpkin seed oil to biodiesel conversion using transesterification process variables in terms of coded value after excluding the insignificant terms was given below in equation 4.16.

Final Equation in terms of coded factors:

$$\text{Biodiesel yield} = +85.5 + 3.67 * \text{A} + 2.04 * \text{C} - 3.64 * \text{A} * \text{B} + 3.72 * \text{A} * \text{C} - 12.12 * \text{A}^2 - 18.46 * \text{C}^2 \qquad 4.16$$

Where: A= Reaction temperature

B= MeOH to oil ratio

C= Amount of catalyst

Table 4.19 ANOVA analysis of experimental result

source	Sum of squares	Df	Mean Squares	F value	P-value Prov>F	Remark
Model	4273.51	9	497.06	71.45	<0.0001	significant
A-Reaction temprature	134.4	1	134.4	19.32	0.0013	
B-MeOH to oil ratio	5.93	1	5.93	0.85	0.3776	
C-Amount of catalyst	41.66	1	41.66	5.99	0.0344	
AB	106.14	1	106.14	15.26	0.0029	
AC	110.56	1	110.56	15.89	0.0026	
BC	14.63	1	14.63	2.10	0.1776	

A^2	404.17	1	404.17	58.10	<0.0001	
B^2	5.89	1	5.89	0.85	0.3792	
C^2	936.94	1	936.94	134.69	<0.0001	
Residuals	69.56	8	6.96			
Lack of fit	34.14	3	7.83	3.51	0.0972	Not significant
Pure error	5.42	2	1.08			
Cor tatal	4343.07	19				

The Model F-value of 71.45 implies the model is significant. Values of "Prob > F" less than 0.0500 indicate model terms are significant. In this case A, C, AB, AC, A^2 and C^2 are significant model terms. Values greater than 0.1000 indicate the model terms are not significant. If there are many insignificant model terms not counting those required to support hierarchy.

R-Squared	0.9847
Adj R-Squared	0.9709
Pred R-Squared	0.9382
Adeq Precision	25.32

The "Pred R-Squared" of 0.9382 is in reasonable agreement with the "Adj R-Squared" of 0.9709. "Adeq Precision" measures the signal to noise ratio. A ratio greater than 4 is desirable. Therfore the ratio of 25.327 indicates an adequate signal. This model can be used to navigate the design space.

As shown in figure 4.1 below, 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, this shows that the quadratic polynomial model satisfies the assumptions analysis of variance (ANOVA) i.e. the error distribution is approximately normal.

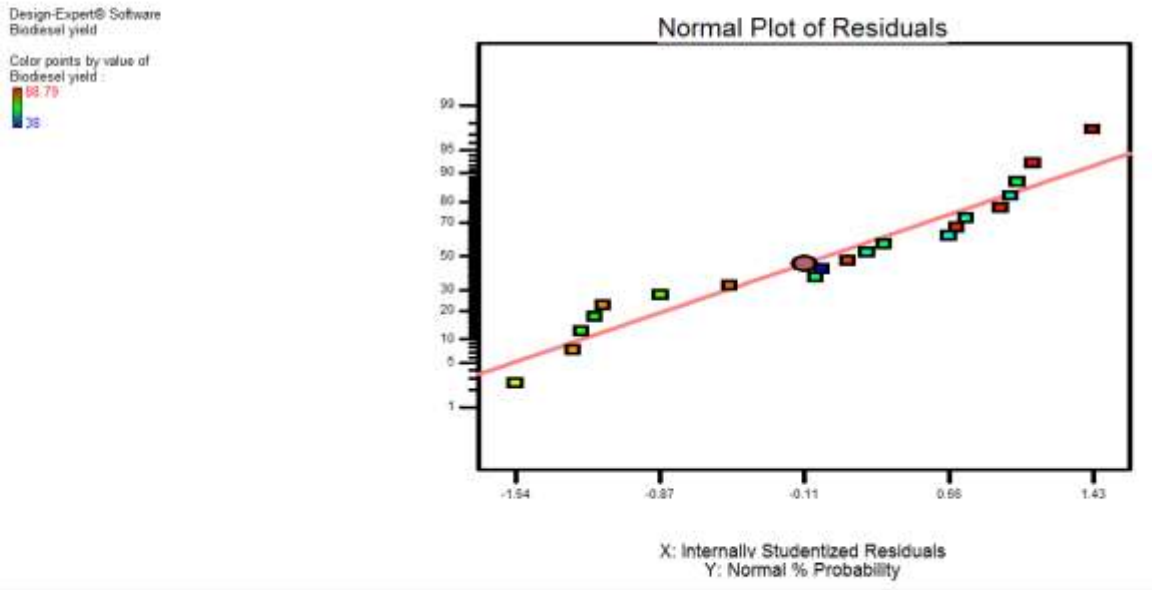


Figure 4.1 Normal plot of residuals

4.7.1.2 Effect of process parameters

Based on the analysis of variance, the transesterification reaction was significantly affected by various interactions between the process variables. This result demonstrated the advantage of using design of experiments in capturing the interaction between variables that affects the transesterification reaction. The effect could be due to the independent variable alone or due to their interaction. The individual and interaction effects of the variables were well discussed below:

Effect of individual parameter on biodiesel yield

Effect of catalyst concentration

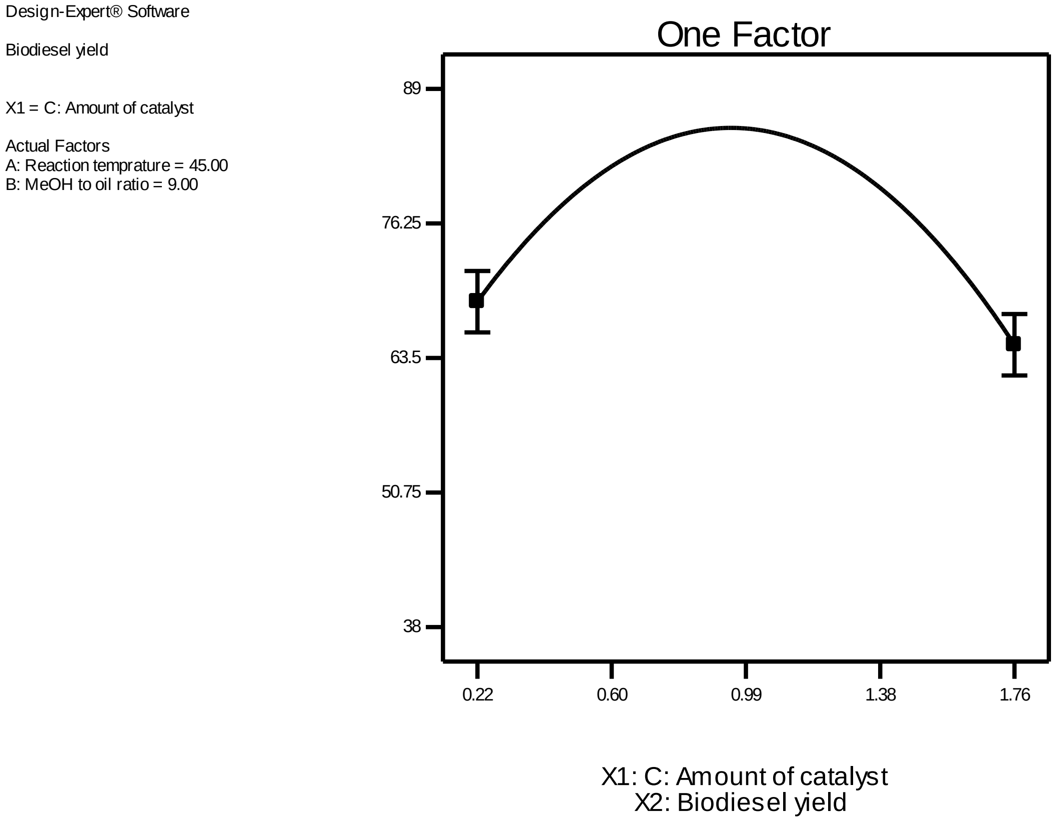


Figure 4.2 Effect of catalyst concentration on biodiesel yield

From Figure 4.1 it was observed that the catalyst concentration influenced the biodiesel yield in a positive manner up to a certain concentration. Beyond this concentration, the biodiesel yield decreased with increase in sodium hydroxide concentration. When the catalyst mount was improved, the interactive (active) site of the catalyst was increased; thus, the transesterification reaction was accelerated and biodiesel yield was increased up to the optimum point.

Effect of reaction temperature

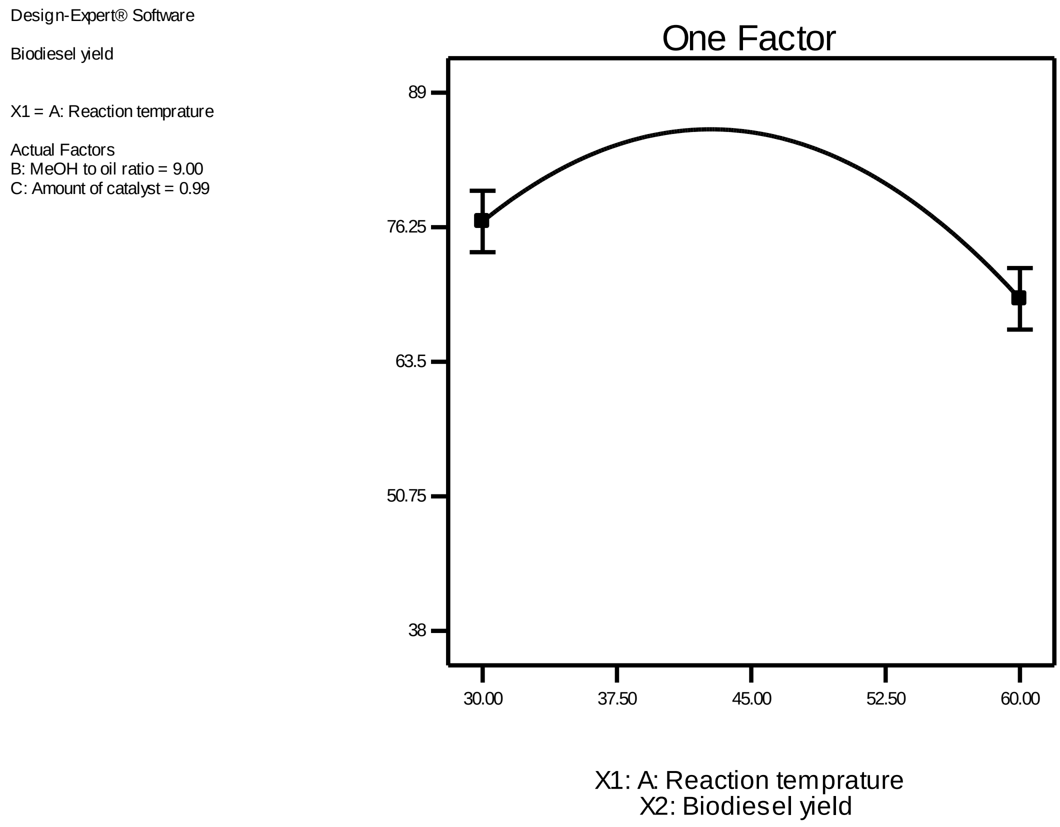


Figure 4.3 Effect of temperature on biodiesel yield

Temperature increase clearly influences the reaction rate and biodiesel yield in a positive manner. The temperature increase affected the biodiesel yield in a positive manner till 45°C and after that it decreased (Figure 4.2). The increase in the yield of FAME at the optimum reaction temperature is due to higher rate of reaction beyond 45°C the yield of biodiesel was decreased.

Effect of MeOH to oil ratio

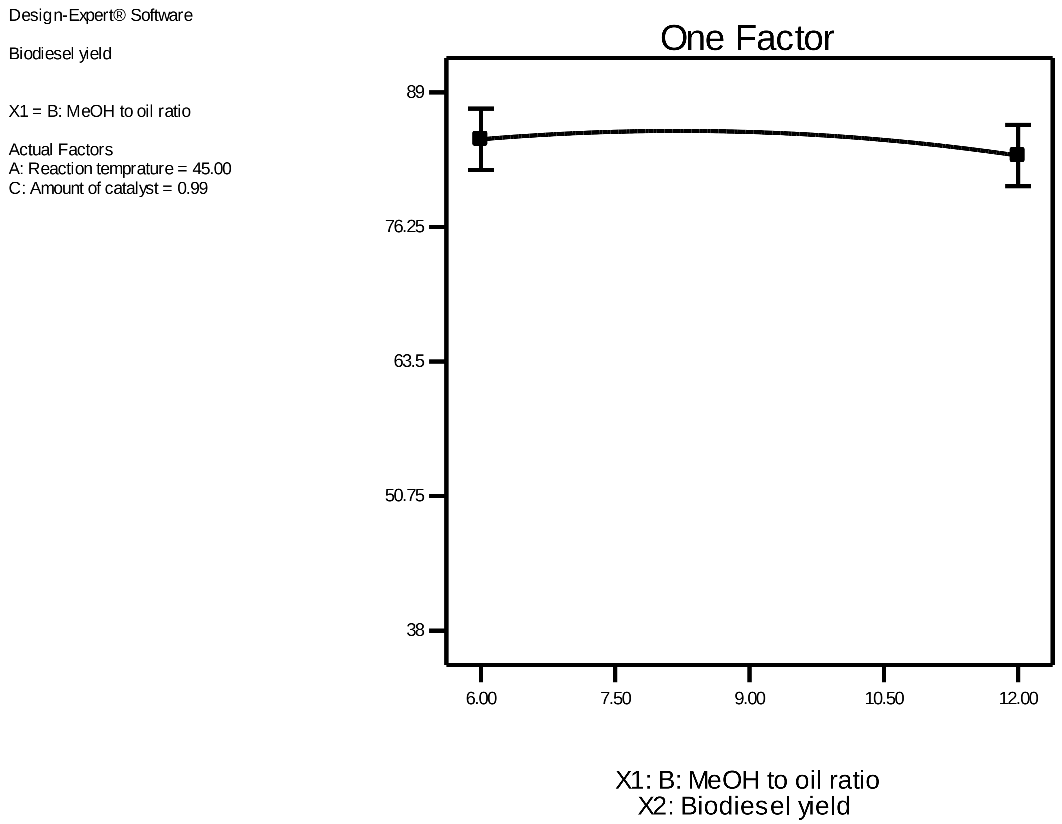


Figure 4.4 Effect of MeOH to oil ratio on biodiesel yield

The effect of alcohol to oil molar ratio on the biodiesel yield was almost constant with slightly decrease beyond the molar ratio of 9.00. This shows the biodiesel production was highly affected by reaction temperature and amount of the catalyst.

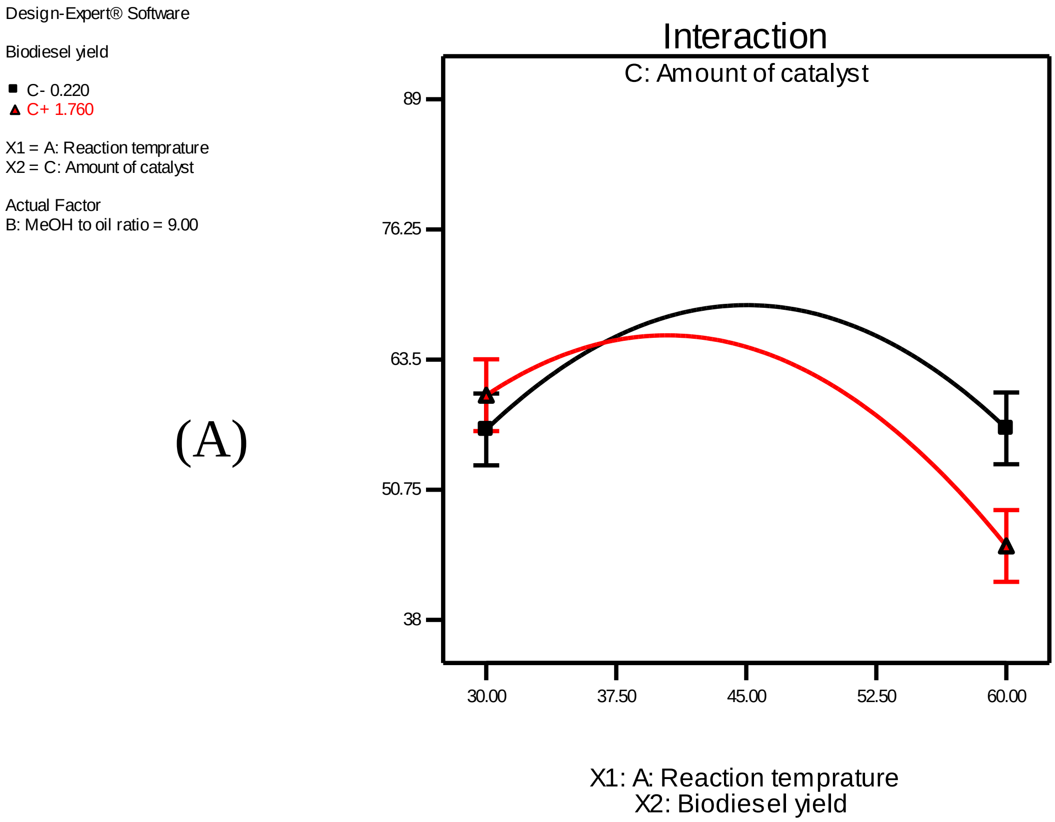
Effect of interaction between process variables

The process variables were found to have significant interaction effects. Figure 4.4, 4.6 and 4.8 shows the interaction between catalyst weight and reaction temperature, weight of catalyst and methanol to oil molar ratio, and reaction temperature with methanol to oil molar ratio, respectively, on the yield of biodiesel yield. Generally, an increase in reaction temperature is found to increase the yield of biodiesel up to some optimal value in all three cases.

Additionally it was observed that at lower range of reaction temperature, higher weight of catalyst and higher molar ratio of methanol to oil, always resulted in higher yield than when using lower weight of catalyst and lower ratio of methanol to oil.

Reactions which were carried out using lower ratio of reaction temperature and lower weight of catalyst which means below the optimum points the biodiesel yield is higher when compared to high amount of reaction temperature and weight of catalyst.

However, at higher range of reaction temperature, the observations showed that using a combination of both, higher weight of catalyst and higher molar ratio of methanol to oil is not beneficial in increasing the yield of biodiesel. This is probably because at these conditions, the higher reaction temperature is already sufficient to push the reaction forward. This phenomenon is further supported by the fact that reaction temperature is the most significant process variable that affects the yield of the biodiesel as indicated by graphs and from the results of ANOVA.



(A)

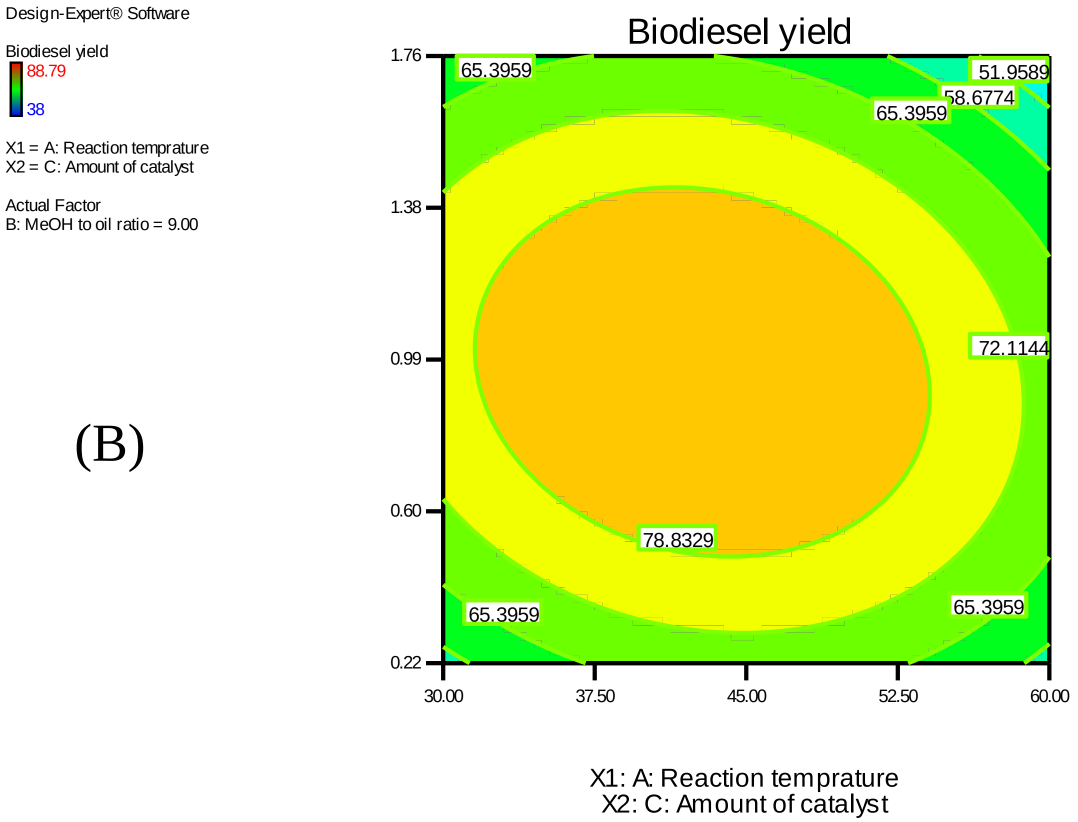


Figure 4.5 (A) interaction effect and (B) Contour plot of the interaction effect of reaction temperature and amount of catalyst versus biodiesel yield

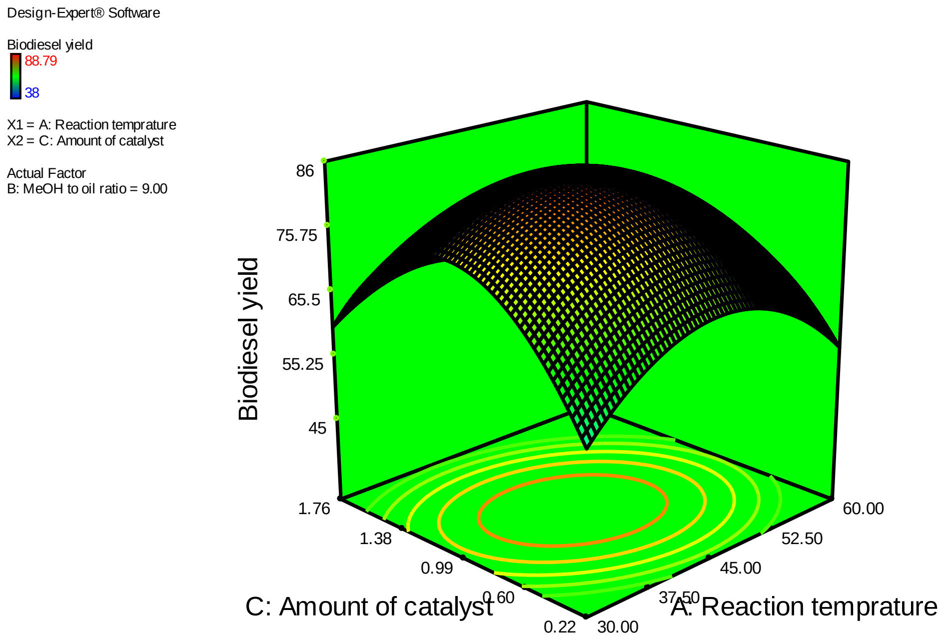
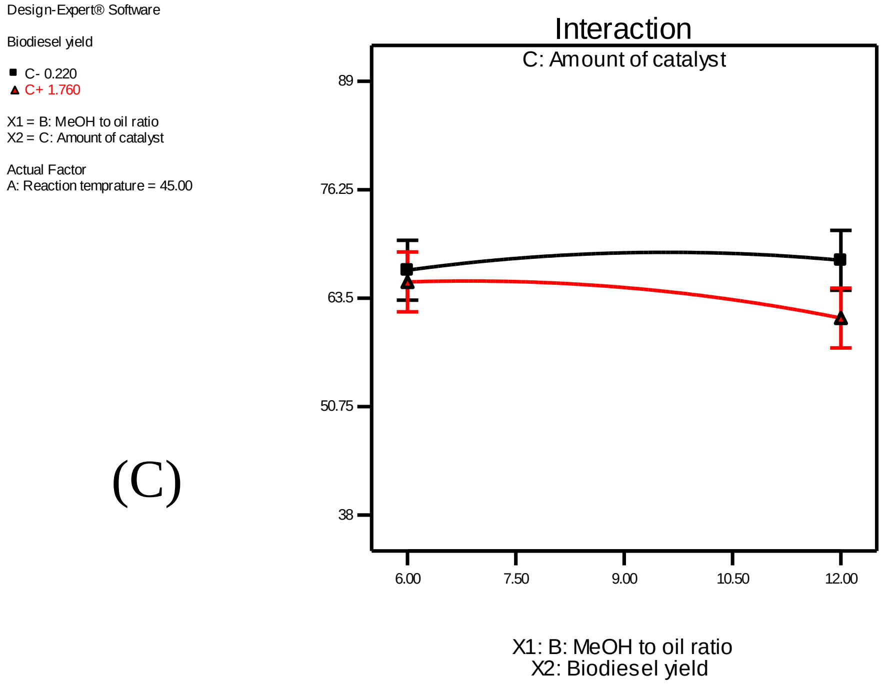


Figure 4.6 Surface plot of the interaction effect of reaction temperature and amount of catalyst versus biodiesel yield



(C)

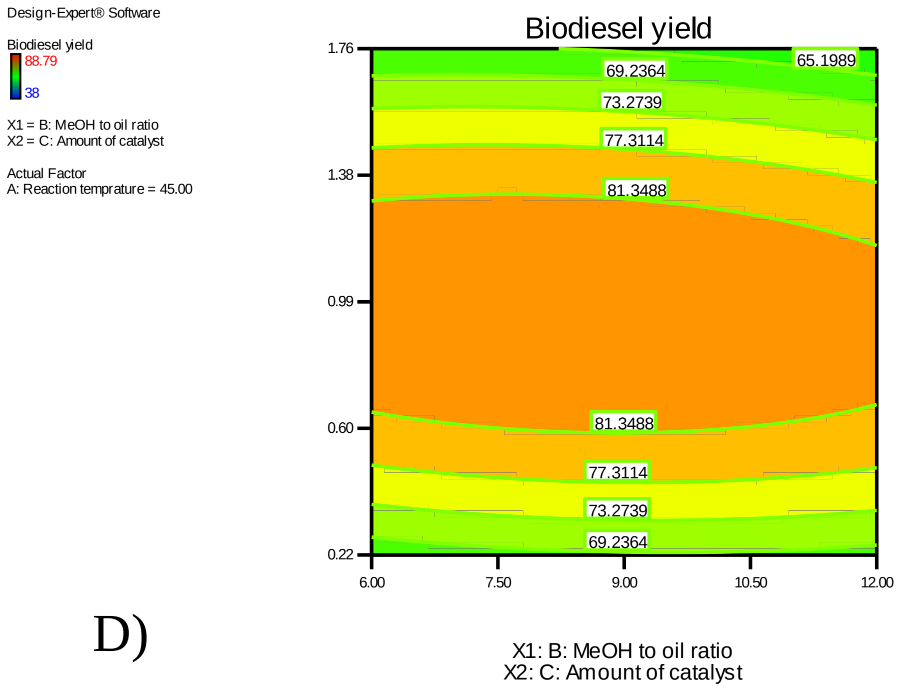


Figure 4.7 (C) interaction effect and (D) Contour plot of the interaction effect of MeOH to oil molar ratio and amount of catalyst versus biodiesel yield

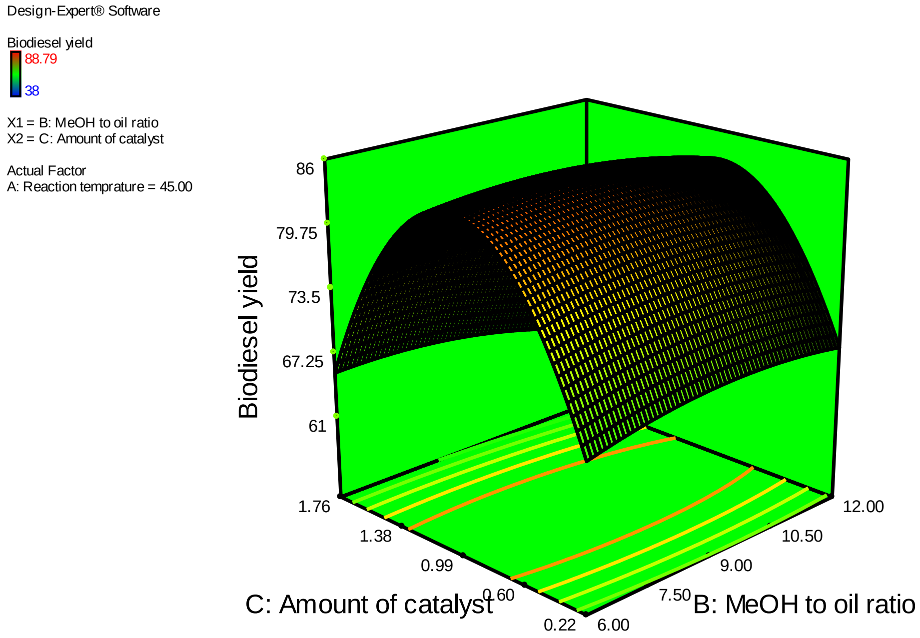


Figure 4.8 Surface plot of the interaction effect of MeOH to oil molar ratio and amount of catalyst versus biodiesel yield

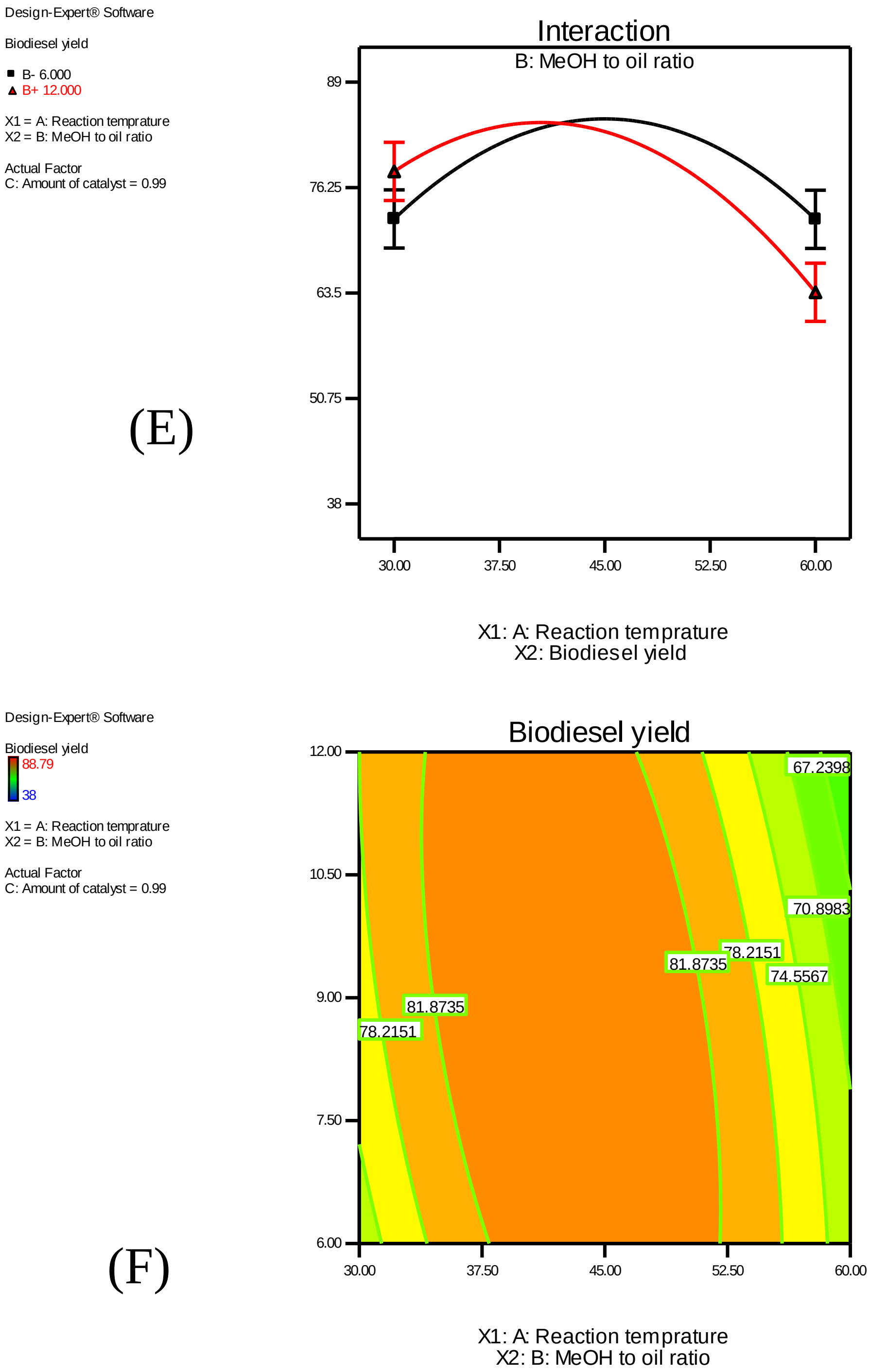


Figure 4.9 (E) Interaction effect and (F) Contour plot of the interaction effect of reaction temperature and MeOH to oil molar ratio versus on biodiesel yield

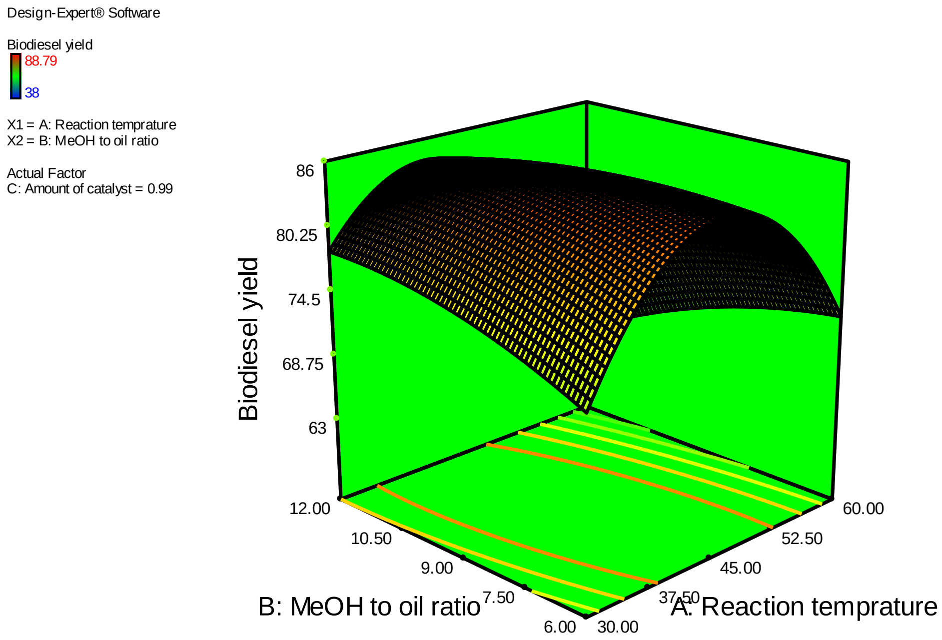


Figure 4.10 Contour plot of the interaction effect of reaction temperature and MeOH to oil molar ratio versus on biodiesel yield

Figure 4.6 show the interaction between methanol to oil molar ratio and amount of catalyst used, on the yield of biodiesel. From Figure 4.6, the percentage of biodiesel amount increased with increasing catalyst concentration at the medium value of methanol to oil molar ratio which had almost constant value until the optimum value of catalyst, beyond the optimum value of catalyst amount the yield decreased. From figure 4.8, the percentage of biodiesel amount increased with the increasing methanol to oil molar ratio for a low reaction temperature.

4.7.2 Optimization of Process Variables

The above results have shown that the three transesterification process variables and their interactions among the variables affecting the yield of biodiesel. Using the optimization function in Design Expert 7.0.0, it was predicted that at the following conditions 45^oC of reaction temperature, methanol to pumpkin seed oil molar ratio of 9:1 and 0.99g amount of catalyst, an optimum biodiesel yield of 88.79% was obtained. The optimization result showed that the same result as the ANOVA output. The ANOVA output shows that the transesterification process is highly and significantly affected by the temperature, mass of catalyst and the interaction between the temperature and the catalyst.

5 CONCLUSIONS AND RECOMMENDATIONS

5.1 Conclusions

Biodiesel was produced using sodium hydroxide and methanol alcohol catalyst at constant reaction time of 2 hours, mixing rate of 500 rpm and at atmospheric pressure. Production of biodiesel was performed by batch process system.

The physicochemical properties of the biodiesel were determined, within ASTM standard values. Hence, the produced biodiesel can be used as an engine fuel.

The effects of amount of sodium hydroxide catalyst, reaction temperature and molar ratio of alcohol to oil on biodiesel yield were determined. By using Design Expert 7.0.0 software three levels; three factor Central Composite Design with full type, when reaction temperature, catalyst amount and molar ratio of alcohol to oil were increased, the biodiesel yield increased until the optimal amount. However, further addition of these working variables during transesterification reaction results in reduction of biodiesel yield due to formation of emulsion which made difficulty in the separation of biodiesel from glycerol.

Additionally, it was observed that the biodiesel yield has a quadratic response with temperature and catalyst weight, almost linear response with alcohol to oil ratio. From these three parameters and their interaction effects, the highest effect on biodiesel yield was observed due to reaction temperature with both catalyst weight and alcohol to oil ratio while the effect of alcohol to oil ratio and catalyst weight was small relatively. The interaction effect of the three operating parameters was significantly detected on biodiesel yield. The maximum biodiesel yield was attained at a temperature of 45℃, 0.99g of NaOH catalyst amount and 9:1 molar ratio of alcohol to oil. In contrast, the average minimum biodiesel yield was at 60℃, 12:1 molar ratio of alcohol to oil and 1.76g amount of catalyst.

5.2 Recommendations

- The oil used for transesterification was extracted using hexane but, related to its cost it is better to use mechanical extraction method
- To reduce the amount of waste water that requires high amount of energy during drying heterogeneous catalyzed transesterification reaction should be explored
- Biodiesel production was done in batch process system; but, production should be conducted in continuous process system
- Efficiency of separation process should be improved
- The property of glycerol was not determined; but, glycerol has relevance in cosmetics and soap industries the property of glycerol should be analyzed and purified for further applications
- In order to reduce the cost of reagents the concept of recycle is important; the catalyst and excess alcohol should be reused

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Appendix

Appendix A: Molecular Mass of Triglyceride Calculation

1. R is the alkyl group of triglyceride components (palmitic, arachidic, stearic, oleic and linoleic)
2. Palmitic: R = - (CH2)14 – CH3, 16 carbons and 0 double bonds (16:0)
3. Stearic: R = - (CH2)16 – CH3, 18 carbons and 0 double bonds (18:0)
4. Oleic: R = - (CH2)7 CH=CH (CH2)7CH3, 18 carbons and 1 double bond (18:1)
5. Linoleic: : R = - (CH2)7 CH=CH-CH2-CH=CH (CH2)4CH3, 18 carbons and 2 double bonds (18:2)
6. Arachidic: R = - (CH2)18 – CH3, 20 carbons and 0 double bond (20:0)

Pumpkin seed oil fatty acid type	Pumpkin seed oil fatty acid composition
Palmitic (C16:0)	15.97-16.36
Stearic (C18:0)	4.68-5.24
Oleic (C18:1)	44.11-44.74
Linoleic (C18:2)	34.77-35.72
Arachidic (C20:0)	0.41-0.81

Molecular mass of Triglyceride=Molecular mass of arachidic acid + Molecular mass Palmitic Acid + Molecular mass Stearic Acid +Linoleic Acid +Oleic Acid

Molecular mass of triglyceride=312.538*0.0041+256.63*0.1597+284.477*0.068+282.468*0.4411+280.452*0.3477

Molecular mass of triglyceride=289.56g/mol

Molecular mass of pumpkin seed oil = 3*molecular mass of triglyceride

Molecular mass of pumpkin seed oil=868.68g/mol

Appendix B: Saponification value calculation

The saponification number was determined by using titration. The solution was prepared with the required concentration. Since the exact concentration was not known, we have to standardize the solution. Thus, primary and secondary standardization was used. Mass of KOH = N * equivalent weight * Volume of solution in liter =0.5mol/lit *56.1g/mol*1lit= 28.055gm.

Mass of HCL= N*equivalent weight*Volume of solution in liter=0.5*36.5*1 =18.25gm.

Volume of HCL=m/ρ=18.25/1.16 =15.73ml and 0.2gm of sodium hydroxide was dilute in 100ml distilled water was used as a primary standard with a known concentration which is 0.1 normality. 25ml solution of sodium hydroxide was taken and titrated with HCL solution to determine the concentration using 3 drops of methyl orange as indicator. The Volume was noted as the end point appears.

$$N_1 V_1 = N_2 V_2$$

$$N_2 = \frac{N_1 V_1}{V_2}$$

$$N_2 = \frac{0.1*25}{5.2} \quad \text{N HCL}= 0.4807$$

Similarly, HCL is used to standardize the ethanolic alcohol solution. Then 30ml of ethanolic KOH was titrated against HCL using 3 drop methyl orange as indicator to determine the exact concentration of ethanolic KOH. The volume of HCL was noted

$$N_2 = \frac{N_1 V_1}{V_2}$$

$$N_2 = \frac{32.1*0.4807}{28} \quad \text{N of ethanolic KOH} = 0.5510$$

2gm of oil was dissolved in 25ml ethanolic KOH solution and titrated with HCL. A blank titration was done in a similar way. In both cases, the volume of HCL was recorded.

Appendix C: Analysis of Fourier transforms infrared (FT-IR)

Comment;

FTIR for biodiesel from pumpkin seed oil

Date/Time; 09/05/20019 3:50 AM

No. of Scans: 2

Resolution; 4 (1/cm)

Apodization; Happ-Genzel

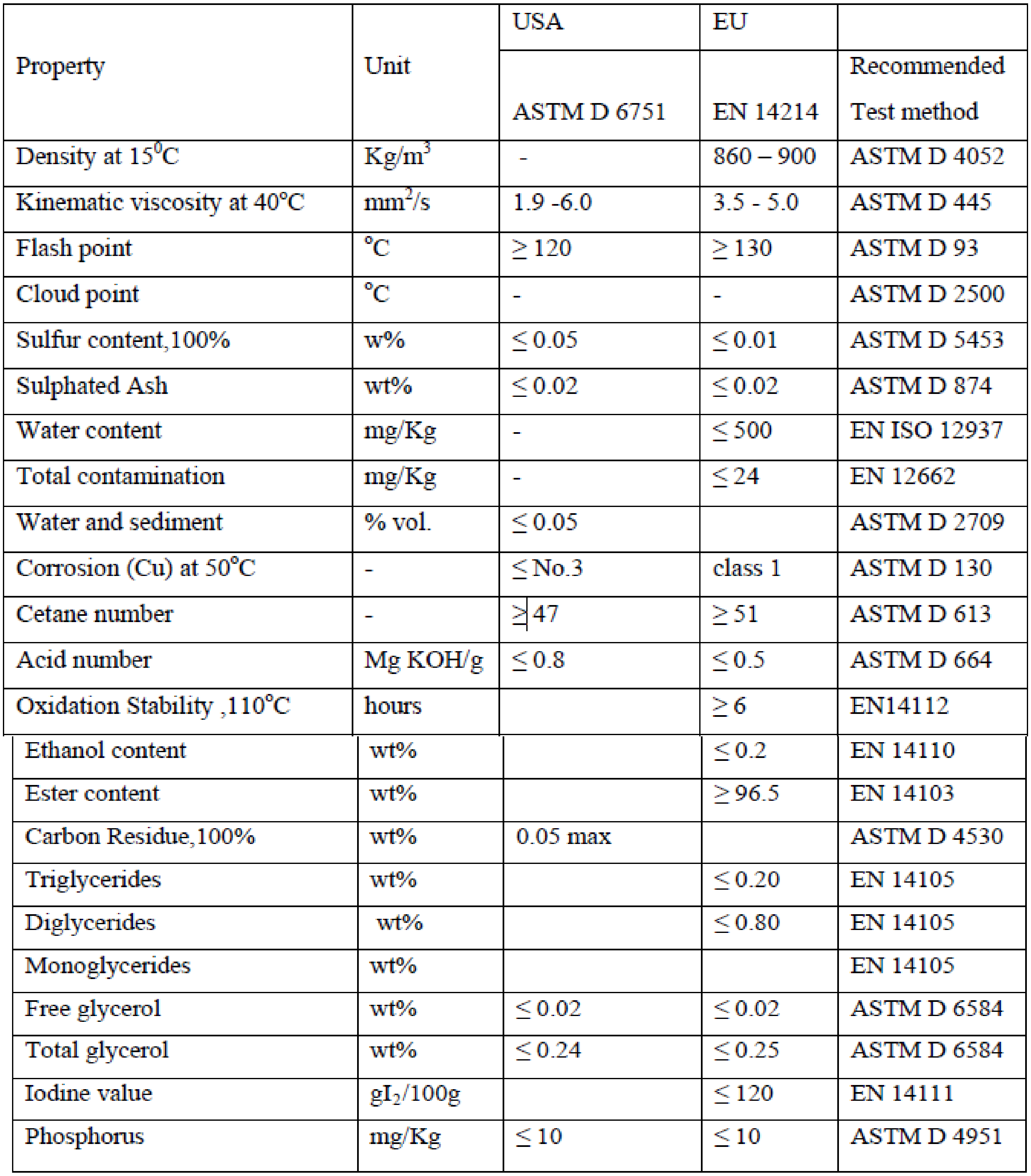
09/05/2019

APF

Pharmaceutical Factory S.C

An analysis of FT-IR spectrum showed in the above figure which was done in Adigrat, Addis Pharmaceutical factory. The biodiesel prepared from pumpkin seed oil was analyzed by FT- IR. The major change that takes place during the conversion from triglyceride oil to biodiesel was the gain of a CH3 carbon in the methyl ester (biodiesel) product, which is found in the range of 1438- 1459 cm-1. Biodiesel results in the formation of carbon hydrogen bonds at 2855-3008 cm-1 and ester functionality at 1738-1759 cm-1; thus pumpkin seed oil methyl ester particularly has ester functionality at 1754.15 cm-1.The carbon oxygen bonds at 1171-1197 cm-1. C= O carbonyl compound (aldehydes, acids, etc.) are the strong C=O stretching absorption bands in the region of 1870-1540 cm-1.If ester this band appears in the 1705 cm-1 and 1658 cm-1. C-O-C (Ethers), these stretching vibrations produce a strong band in the 1200-900 cm-1 region. C-H, adsorption bands as an example 2931 cm-1 and 2954cm-1 correspond to the asymmetric and symmetric vibration modes of methyl group, ethylene groups respectively, H2O the adsorption bands of water can be between 1800 -1200 cm-1.

Appendix E: Standard Specifications of Biodiesel: USA and European



Property	Unit	USA ASTM D 6751	EU EN 14214	Recommended Test method
Density at 15^{0}C	Kg/m^3	-	860 – 900	ASTM D 4052
Kinematic viscosity at 40°C	mm^2/s	1.9 -6.0	3.5 - 5.0	ASTM D 445
Flash point	°C	≥ 120	≥ 130	ASTM D 93
Cloud point	°C	-	-	ASTM D 2500
Sulfur content,100%	w%	≤ 0.05	≤ 0.01	ASTM D 5453
Sulphated Ash	wt%	≤ 0.02	≤ 0.02	ASTM D 874
Water content	mg/Kg	-	≤ 500	EN ISO 12937
Total contamination	mg/Kg	-	≤ 24	EN 12662
Water and sediment	% vol.	≤ 0.05		ASTM D 2709
Corrosion (Cu) at 50°C	-	≤ No.3	class 1	ASTM D 130
Cetane number	-	≥ 47	≥ 51	ASTM D 613
Acid number	Mg KOH/g	≤ 0.8	≤ 0.5	ASTM D 664
Oxidation Stability ,110°C	hours		≥ 6	EN14112
Ethanol content	wt%		≤ 0.2	EN 14110
Ester content	wt%		≥ 96.5	EN 14103
Carbon Residue,100%	wt%	0.05 max		ASTM D 4530
Triglycerides	wt%		≤ 0.20	EN 14105
Diglycerides	wt%		≤ 0.80	EN 14105
Monoglycerides	wt%			EN 14105
Free glycerol	wt%	≤ 0.02	≤ 0.02	ASTM D 6584
Total glycerol	wt%	≤ 0.24	≤ 0.25	ASTM D 6584
Iodine value	gI$_2$/100g		≤ 120	EN 14111
Phosphorus	mg/Kg	≤ 10	≤ 10	ASTM D 4951

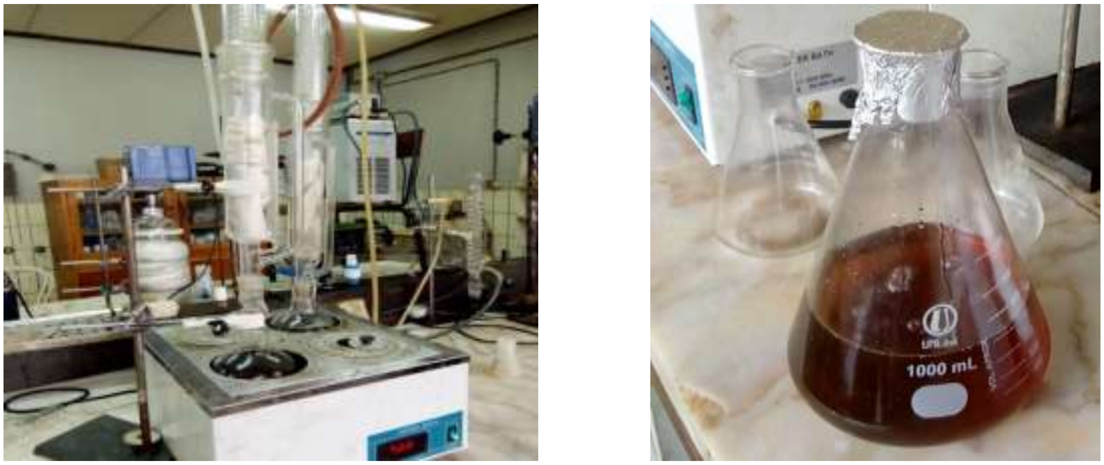
Source: Adopted from Biodiesel industries, Australia 2003

Appendix F: pictures of samples and some laboratory Equipment's


A B C

D E G

Pumpkin seed preparation: Matured pumpkin(A), half pumpkin(B), hulled pumpkin seed(C), peels of pumpkin seed(D) miller machine with size of 2mm (E) and sieved pumpkin seed meal with of 2mm (G) respectively.



A B

Experimental set up for oil extraction (A) and purified oil (B)

Batch reaction set up for biodiesel production

Glycerol separation

Washing of biodiesel

Purified biodiesel

Ash content determination