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Extraction and Optimization of Oil from Moringa Oleifera Seed as an Alternative Feedstock for the Production of Biodiesel

A.S. Abdulkareem[1], H. Uthman[2], A.S. Afolabi[1] and O.L. Awenebe[3]

[1]*Department of Civil and Chemical Engineering, College of Science, Engineering and Technology, University of South Africa, Johannesburg,*
[2]*Membrane Research Unit (MRU), Block L-01, Universiti Teknologi Malaysia (UTM), International Campus, Jalan Semarak, 54100 WP, Kuala Lampur*
[3]*Department of Chemical Engineering, School of Engineering and Engineering Technology, Federal University of Technology, Minna,*
[1]*South Africa*
[2]*Malaysia*
[3]*Niger State Nigeria*

1. Introduction

Energy production for industrial and domestic purpose has primarily been based upon the combustion of fossil fuels, such as oil and coal and it has been reported that these resources are finite and pose significant environmental impact from their combustion (Carraretto et al., 2004; Abdulkareem & Odigure, 2002; Odigure & Abdulkareem, 2001). It has been predicted that coal will be a viable energy resource for 90-200years, while the world oil supply is reaching its peak due to over dependence on oil consumption (Odigure et al., 2003). This was blame on the inability of energy sector to balance the oil supply with the increasing demand by various sectors including domestic consumption (Abdulkareem, 2005). Like any other commodity, fossil fuel price is also influence by shortage or oversupply and it has been reported that the change in demand as well as supply by the OPEC and non OPEC nations will greatly affects the price of the oil for many years (Abdulkareem, 2005). In other to meet up with the energy demand worldwide, government and oil sector embarks on the programme of new oil discoveries, and it has been reported that searching for new oil is a loss to the companies. For instance, about ten major oil companies spent $8 billion on searching for new oil; results of their search only produce commercial discoveries of oil worth approximately $4 billion. Consequently, the oil companies now consider searching for new oil not economical and unable to replace their rapidly depleting resources (Abdulkareem & Odigure, 2006; Abdulkareem & Odigure, 2010; Ahmmad et al., 2011; Udaeta et al., 2007). Apart from the price instability of the fossil fuel which is the major sources of energy, environmental pollution is also a major problem emanated from over dependence on fossil fuel. Combustion of fossil fuel is harmful to human health and the environment, and there is an increasing campaign for cleaner burning

fuel in order to safeguard the environment and protect man from the inhalation of genotoxic substances (Perez-Roa et al., 2006; Adeniyi et al., 2007; Abdulkareem et al., 2010). For instance, the exhaust from petroleum products, especially diesel is known to be toxic and carcinogenous in nature, since they contain polycyclic aromatic hydrocarbons (Ahmmad et al., 2011). Though, there is no energy source that is completely environmentally safe, hence it is important to use the available energy sources wisely to minimize environmental hazard and optimize the efficiency with which it is produced (Bernard & Wolfgang, 2009; Abdulkareem et al., 2011). The environmental impacts of using fossil fuel and other non-renewable fuels, such as coal and uranium, present a major obstacle to the continued use of such resources to meet our energy needs. The conventional petroleum-based fuels such as gasoline or diesel, as well as natural gas and coal, all contain carbon. When these fuels are burnt, their carbon recombines with oxygen from the air to form carbon dioxide which is the primary greenhouse gas that causes global warming. In the same vein, combustion of fossil fuels at the high temperature and pressures reached inside an internal combustion engine (what powers most vehicles) or in an electric power plant produces other toxic emissions (Abdulkareem et al., 2011). Carbon monoxide, oxides of nitrogen, oxides of sulphur, volatile organic chemicals, and fine particles are all components of air pollution attributable to the refining and combustion of fossil fuels. When released into the atmosphere, many of these compounds cause acid rain or react with sunlight to create ground level smog. Vast ecosystem damage, increase lung disease and cancer are the ultimate price inhabitant pay for consuming these fossil fuels (Abdulkareem & Odigure, 2006). The concern for price instability due to over dependence on the fossil and increasingly awareness on the environmental impact of combustion of fossil fuel have called for the alternative source of energy and proper utilization of existing energy sources. Biofuel which is described as the natural and renewable domestic fuel are now considered promising and economical alternative and sustainable energy sources (Shireen & Debabrate, 2008; Khunrong et al., 2011; Eevere et al., 2011). Biodiesel made from vegetable oil, has been reported to burns clearly, which result in a significant reduction for the types of pollutants that contribute to smog and global warming. Some of the advantages of the biodiesel over the fossil fuel diesel include cost of production, it has been reported that once the technology of biofuel is readily available, the cost of biofuel will be much less than that of fossil fuel (Ahmmad et al., 2011; Durosoy et al., 2011). This was blame on the fact that increases in word population will lead to an increment in demand for oil which will results in increase in price beyond the expectation level (Nashawi et al., 2010). While fossil fuel is described as limited resources since it is produce from a specific material, biofuel can be manufactured from a variety of material (Vera & Langlois, 2007). Other problem associated with fossil fuel is the non-biodegradability; the situation that makes it difficult to clean when spillage is experienced. While the biofuel are easily biodegradable and safer to handle than the fossil fuel, this makes spill of biofuel to be less hazardous and less expensive to clean up. Biodiesel also had a high flash point which makes it less explosive at moderate temperature, hence biodiesel is safer to transport and store (Hamamci et al., 2011). There is also wide spread of efforts to investigate an economical additives that can be blended with biodiesel to enhace its application in a colder climates which is the major detractor at moment. On the suitability of the biodiesel in a diesel engine, it has been reported that the first diesel engine which was invented by Rudolf Diesel in 1892, was originally designed to run on unrefined biodiesel.This provide an indiaction that diesel engine can easily adapt to biofuel with little

 modification (Agarwal and Das, 2001). Biodiesel is therefore, produced by the process known as transesterification, which is a chemical reaction for conversion of oil to biodiesel (El-Sabagh et al., 2011; Adeniyi et al., 2007). In this process the oil is chemically reacted with alcohol like methanol or ethanol in the presence of catalyst like sodium hydroxide or potassium hydroxide. After the chemical reaction, various components of the oil break down to form new compounds known as triglycerides. The triglycerides are converted into alkyl esters, which is the chemical name of biodiesel. If methanol is used in the chemical reaction, methyl esters are formed, while when ethanol is used, ethyl esters are formed. Both of these compounds are biodiesel fuels with different chemical combination (Silas, 2008). While the glycerin, that has been separated during transesterification process is released as a by-product of the chemical reaction. Glycerin will either sink to the bottom of reaction vessel or come to the surface depending on its phase. It can be easily separated by the centrifuges. This whole process is called transesterification. The feedstock (oil) therefore play a major role in the production of biodiesel (Ayhan, 2008).

Biodiesel as an alternative sources of energy, can be produced from biological or biomass material including corn, cellulose, sugar cane, edible oils such as vegetable oil, soybean, palm oil etc. Biodiesel Fuel can also be produced from a variety of non edible oil including rapeseed, mustard flax, sunflower, canola, hemp, Jatropha and waste vegetable oils (Aghan, 2005; Adeniyi et al., 2007; Helwani et al., 2009; Hossain and Boyce, 2009; Abdulkareem et al., 2010). This fuel sources are said to reduce engine wear and produce less harmful emissions. In different part of the world, depending on availability, biodiesel has been produced from different plants. This was first done as an academic exercise, but today commercialization of this production process and product is on-going. For instance Freedman et al, (1984) reported the use of fish oil, soy oil, rape seed oil, cotton seed oil, sun flower, safflower, peanut and line seed oil for the production of methyl esters. According to Barminas et al, (2001) categories of suitable agricultural products for bio-fuel production include seeds, nuts, fruits, leaves, and root and stem etc. It has also been reported that algae farming provide yields 4-5 times more biodiesel per acre than crops like soybeans, but the technology of producing biodiesel from algae is very expensive hence the possibility of achieving commercialization of biodiesel from algae might not happen any moment soon. The European standard specification EN 14214 in 2004, therefore defined biodiesel as fatty acid methyl esters (FAME) from any kind of feedstock (Canakci, 2007; Chhetri *et al*., 2008; Refaat *et al*., 2008). Therefore, from an economic point of view; the production of biodiesel is very feedstock sensitive. The cost of biodiesel has been estimated based on assumption regarding production volume, feedstock and chemical technology (Canakci and van Gerpen, 2001; Zhang *et al*., 2003b; Kulkarni and Dalai, 2006) and feedstock cost comprises a very substantial portion of overall biodiesel cost. Though, the production from various feed stocks had been investigated, however, each of the feedstock has its own problems. For instance, the production of biofuel from waste oil has been described to be economical and readily available, however the inherent difficulties of processing and gathering remain the major factor that militate against its usage as a feedstock in the production of biodiesel (Whang et al 2003(a&b)). Edible sources like soybeans, sunflower seeds and cotton seeds etc. which are easily available and can be gathered and processed easily need to be closely monitored and controlled in other not to create larger global problems of deforestation, hunger and poverty while trying to solve the problem of energy crises. Critics of biofuel fear that the uses of food (edible oil) as a feed feedstock in the production of biodiesel could result into food crisis. It has been reported that to produce 5% of the total diesel consumption in United State of America

from biodiesel, will require approximately 60 % of the crop produced in United State, the action consider being unethical by the critics. Despite the wide acceptance of biofuel as alternative energy source to supplement or replace fossil fuel. The latest research and development in the production of biodiesel is aimed at producing the biofuel from non edible oil like Jatropha Curcas, sunflower etc. Employing non edible oil as a feedstock in the production of biodiesel will actually help in solving the strain relationship between the energy sectors and critics of production of biodiesel from edible oil which was considered unethical. This present study is therefore focus on the optimization of extraction of oil from moringa oleifera seed kernel which is non edible oil especially in Nigeria where it is abundantly available, as an alternative feedstock in the production of biodiesel. Moringa Oleifera (Zogale) seed kernel contains about 45% oil by weight. The oil can be use for cosmetic making, lubrication and consumption. Beside its industrial use as a fine lubricant, the fatty acid profile of the oil with its high content of oleic acid make it an oil with potential for further industrial application (Michelle, 1994). Moringa oleifera seeds are available in abudant in Minna and utilizing the oil from the seed to produced biodiesel will not constituite any enviromental hazard.

1.1 War of energy and food security

Food security refers to the availability of food and one's access to it, a household is therefore, considered food-secure when its occupants do not live in hunger or fear of starvation. According to FAO, 2008, food security exists when all people, at all times, have physical, social and economic access to sufficient, safe and nutritious food to meet their dietary needs and food preferences for an active and healthy life. Hence, the stages of food insecurity range from food secure situations to full-scale famine. Generally, the food insecurity can be categorized as either chronic or transitory. In chronic food insecurity situation, the societies exposed to a high degree of vulnerability to famine and hunger. The situation is similar to undernourishment and is related to poverty, which is existing mainly in poor countries (Ayalew, 1988). Many countries experience perpetual food shortages and distribution problems, which resulted in chronic and often widespread hunger amongst significant numbers of people. Worldwide around 852 million people are chronically hungry due to extreme poverty, while up to 2 billion people lack food security intermittently due to varying degrees of poverty (FAO, 2003). According to CNN report (2009), six million children die of hunger every year - 17,000 every day. It has been reported that as of late 2007, export restrictions and panic buying, US Dollar depreciation, (HM Government, 2010) increased farming for use in biofuel (Smith and Edwards, 2011), world oil prices at more than $100 a barrel (The Monitor's view, 2008), global population growth, (Randerson, 2008) climate change (Vidal, 2007), loss of agricultural land to residential and industrial development (Dancy, 2008) and growing consumer demand in China and India (Walt, 2008) are claimed to have pushed up the price of grains (Brown, 2008). The issue of food security is therefore a complex objective pursued with shelter, safety, health and self-esteem in a world where individual households, face diverse complex and different livelihood opportunities (Smit *et al.*, 1993). Hence, the proper understanding of food security requires explicit recognition of complexity and diversity, and that it necessarily privileges the subjective perceptions of the food insecure themselves (World Bank, 1986).

Energy security is a term used to describe an association between national security and the availability of natural resources for energy consumption. Though, access to cheap energy has become essential to the functioning of modern economies, however, the uneven

distribution of energy supplies among countries has led to significant vulnerabilities. It has been reported that energy security resulted into the political instability of several energy producing countries, the manipulation of energy supplies, the competition over energy sources, attacks on supply infrastructure, as well as accidents, natural disasters, the funding to foreign dictators, rising terrorism, and dominant countries reliance to the foreign oil supply (Wesley, 2007). The world wide over dependence on the oil and the peaking limits of oil production, it is obvious that economics and societies will begin to feel the decline in the resources that we have dependent upon. Hence, the issue of energy security has become one of the leading issues in the world today as oil and other resources have become as vital to the world's people. The looming end of the era of cheap oil is going to put the energy security of most industrialized countries in jeopardy and the same is true in the case of food since many of the imports which are not sustainable even at this very moment, may become far too expensive to afford. It is therefore, important that both of these issues are extremely serious and need to be dealt with and addressed immediately.

Food security is probably on a more serious level than energy security even though both have to be considered side by side. The high prices of food today are mostly driven by elevating demand due to rapid population growth among other conventional factors such as urbanization and industrialization, economic growth and food consumption, and land use changes and water scarcity (Khan *et al.*, 2009). Today there are almost 219,000 additional people to feed at the global table every night, this unfortunate scenario is the consequenc of the current competition between food and energy. Most developed nations are now converting food products into energy sources, for instance, the United States is now converting massive quantities of grains into fuel for cars, even with increasing grain consumption. This massive capacity to convert grain into fuel means that the price of grain is now tied to the price of oil. The same phenomenon is also happening in Brazil, where distills ethanol from sugar cane, ranks second in production after the United States, while the European Union's goal of getting 10 percent of its transport energy from renewable, mostly biofuel, by 2020 is also diverting land from food crops (Eaves and Eaves, 2007).

1.2 Application of optimization in solvent extraction

Principles of optimization find applications in the fields of science, engineering, and business. Optimization is therefore concerned with selecting the best among the entire set by efficient quantitative methods (Onifade, 2002). It has been reported that the recent development in chemical and process engineering industry has undergone significant changes during the past few years due to the increased cost of energy, increasingly stringent environmental regulations, and global competition in product pricing and quality (Onifade, 2002). One of the most important engineering tools for addressing these issues is optimization of the technique involved. Effective optimization techniques are now available in software for personal computers, a capability that did not exist some years ago. To achieve effective application of optimization in the chemical and process industries, there is the need for proper understanding of both the theory and application by engineers and scientists who find optimization as the decision making process which is the exasperating and difficult. In this present study, optimization technique is employ to determine the best conditions at which oil can be extracted from moringa oleifera seed by solvent extraction method.

The process of solvent extraction of oil , includes diffusion of a solvent into oil-bearing cells of vegetable oil seeds resulting in a solution of the oil in solvent. Various solvents can be used for extraction. However, after extensive research and consideration of various factors, such as commercial economics, edibility of the various products obtained from extraction, physical properties of the solvent especially its low boiling point, volatility, toxicity, viscosity etc. Hence, the selection of the equipment for an extraction process is influenced by the factors, which are responsible for limiting the extraction rate. Thus if the diffusion of the solute through the pores of the residual solid is the controlling factors, the material should be of small size so that the distance the solute has to travel is small. On the other hand, if the diffusion of the solute from the surface of the particle to the bulk of the solution is the controlling factor, then a high degree of agitation is required for optimum leaching of the seed, thus particle size, temperature, agitation and solvent are the major factors influencing solvent extraction techniques. These factors are therefore combined during experimental design which resulted into what is described as factorial experimental design for the purpose of optimizing the process and to investigate the interaction between the various factors that influence the rate of extraction.

The method of factorial experimental design forces data to be orthogonal which allows one to determine the relative importance of each input variable and thus to develop a parametric model that includes only the most important variables and effects. It also represents efficient method of experimentation to determine the best operating condition for each variable factor that influences the process. In factorial experimental design, experiments are systematically planned and conducted in such a way that all the variable factors are changed simultaneously rather than one at a time, for the purpose of reducing the number of experiments. Due to the orthogonal nature of a factorial design method, statistical tests are effective in discriminating among the effects of different natural variations such as the unit operations, operators, batches and other environmental factors. The standard factorial design therefore requires 2^k tests, where k represents the number of input variables to be investigated. It is also important for the user of factorial design to decide the extent to which each of the variable input should be changed from its original value. To take this decision, the user needs to take into account the sensitivity of the process response to a change in a given input variable, as well as the typical operating range of the process. The experimental design range should therefore be chosen in other to avoid the possibility of the response of the resulting measurements not to generate errors that are far greater than the typical real values. To achieve this in experimental factorial design, variance (ANOVA) or regression analysis can be use to analyzed the experimental results effectively, which is relatively easy to determine the major effect of a variable factor. The factorial design therefore,becomes an important tool in the solvent extraction techniques.

2. Moringa oleifera

Moringa oleifera popularly called Zogale in the northern part of Nigeria is the most widely cultivated variety of the genus Moringa and belong to the family of Moringaceae. Moringa oleifera is a nutritious vegetable tree with a variety of potential uses. The moringa oleifera tree shown in Figure 2.1 is slender with drooping branch that grows to approximately 10 m in height. To maintain the pod and leaves within the arms reach, the tree is normally cut to one meter or less.

Fig. 2.1. Moringa oleifera tree

The Moringa tree grows mainly in semi-arid tropical and subtropical areas, but grows best in dry sandy soil; it tolerates poor soil, including coastal areas. It is a fast-growing, drought-resistant tree that is native to the southern foothills of the Himalayas, and possibly Africa and the Middle East. The tree has its origin from the Southern Indian State of Tamilnadu. Today, it is widely cultivated in Africa, Central and South America, Sri Lanka, India, Mexico, Malaysia and the Philippines. It grows up to 4m in height and develops to flowering and fruiting within one year of its cultivation. Moringa oleifera is considered as one of the world's most useful trees, this is because almost every part of the tree can be used for food, or has some other beneficial property. Hence it is commonly called the *'Wonder Tree'*. In the tropics, it is used as foliage for livestock. The immature green pods, called "drumsticks" are probably the most valued and widely used part of the tree. They are commonly consumed in India, and are generally prepared in a similar fashion to green beans and have a slight asparagus taste (Rajangam *et al, 2000*).

The Moringa seeds (Figure 2.2) yield 38–45% edible oil (called Ben oil, from the high concentration of Behenic acid contained in the oil) that can be used in cooking, cosmetics and lubrication. Unfortunately, the oil from moringa oleifera seed is not popular edible oil in Nigeria; hence extraction of oil for the purpose of biodiesel production will not pose any food shortage threat which is a major factor against the production of biodiesel from vegetable oil.

Fig. 2.2. Moringa oleifera (a) Dried pods (b) Seed kernel with husks (c) seed kernel without husk

The refined oil is clear, odourless and resists rancidity like any other botanical oil. The seed cake remaining after the oil extraction can be used as fertilizer or as flocculants to treat turbid water. The leaves are highly nutritious, being a significant source of beta-carotene, Vitamin C, protein, iron and potassium; it is consumed mostly among the Hausas in Northern Nigeria. In addition to being used fresh as a substitute for spinach, the leaves are commonly dried and processed into powder and used in soups and sauces.

3. Methodology

3.1 Material and equipment
This study focus on the extraction of oil from moringa oleifera seed by means of solvent extraction and production of bio-ethanol from rice husk using alkali as the hydrolising agent and zymomonas for fermentation. The entire chemicals used in this study are of analytical grade (98-99.5%). They include hexane, ethanol, iodine, sodium hydroxide, calcium oxide, potasium iodide and potassium hydroxide. The equipments used are mortar and pestle, sieve, electronic weighing balance, thimble, measuring cylinder, stop watch, pH meter,

electric oven and distillation column. The moringa oleifera seeds and rice husk used in this study were collected in Bosso Estate, Minna, Niger State, Nigeria.

3.2 The 2^k factorial experimental design

When several factors are of interest in an experiment a factorial method of analysis is used in order to study the effect of individual factor and its interaction with other factors to economize the experimental resources (Azeez, 2005;Zhang and Huang, 2011; Wang et al., 2011). In this study, three factors namely temperature, particle size and resident time are of interest while agitation was kept constant. This gives rise to three-factor factorial experiment; the factors are tested at high and low levels. When three factors are tested at two levels as applicable in this study, it is denoted by 2^3 factorial; thus there exist eight (2^3) treatment combinations as shown in Table 3.1. The table indicates how the individual effect and interactions are calculated. It was assumed that A,B and C are the fixed factors where there are 'a' levels of A, 'b' levels of B and 'c' levels of C arranged in the factorial experiment. Generally there will be abc…..n total observations if there are n replicates of the complete experiment. The analysis variance is shown in Table 3.2.

Treatment combination	Factorial Effect							
I	I	A	B	C	AB	AC	BC	ABC
A	+	-	-	-	+	+	+	-
B	+	+	-	-	-	-	+	+
Ab	+	-	+	-	-	+	-	+
C	+	-	-	+	+	-	-	+
Ac	+	+	-	+	-	+	-	-
Bc	+	-	+	+	-	-	+	-
Abc	+	+	+	+	+	+	+	+

Table 3.1. Design matrix for a 2^3 Factorial Design

Consider a three factors experiment, with underlying model as shown in Equation1, before the model equation can be fitted, it is important to conduct some statistical tests such as G-test, T-test and F-test, which involves calculation of these statistical parameters with the aid of certain formulae shown in Equations 2-4 and compare them with those given in the statistical tables. G-test is used to check if the output has the maximum accuracy of replication. T-test is used to check the significance of regression coefficient, and F-test is used to test for the adequacy of the model. Equations 2-4 represent the formulae to calculate G-test, T-test and F-test respectively.

Sources of Variation	Sum of Squares	Degree of Freedom	Mean Square	Expected Mean Squares	F_o
A	SS_A	$(a-1)$	MS_A	$\delta^2 + (bcn \Sigma \tau_i^2)/ (a-1)$	MS_A/MS_E
B	SS_B	$(b-1)$	MS_B	$\delta^2 + (can \Sigma \beta_j^2)/(b-1)$	MS_B/MS_E
C	SS_C	$(c-1)$	MS_C	$\delta^2 + (abn \Sigma \gamma_k^2)/(c-1)$	MS_C/MS_E
AB	SS_{AB}	$(a-1)(b-1)$	MS_{AB}	$\delta^2+(cn\Sigma\Sigma(\tau\beta)^2_{ij})/(a-1)(b-1)$	MS_{AB}/MS_E
AC	SS_{AC}	$(a-1)(c-1)$	MS_{AC}	$\delta^2+(bn\Sigma\Sigma(\tau\gamma)^2_{ik})/(a-1)(c-1)$	MS_{AC}/MS_E
BC	SS_{BC}	$(b-1)(c-1)$	MS_{BC}	$\delta^2+(an\Sigma\Sigma(\beta\gamma)^2_{jk})/(b-1)(c-1)$	MS_{BC}/MS_E
ABC	SS_{ABC}	$(a-1)(b-1)(c-1)$	MS_{ABC}	$\delta^2+(n\Sigma\Sigma\Sigma(\tau\beta\gamma)^2_{ijk})/(a-1)(b-1)(c-1)$	MS_{ABC}/MS_E
Error	SS_E	$abc(n-1)$	MS_E	δ^2	
Total	SS_T				

Table 3.2. Variance (ANOVA) analysis

$$Y_{ijkl} = \mu + \tau_i + \beta_j + \gamma_k + (\tau\beta)_{ij} + (\tau\gamma)_{ik} + (\beta\gamma)_{jk} + (\tau\beta v)_{ijk} + E_{ijk} \qquad (1)$$

$i = 1,2,---a$
$j = 1,2,---b$
$k = 1,2,---c$
$l = 1,2,---n$
Where μ is the overall mean effect,

     τ_i is the effect of the ith level of factor A

     β_j is the effect of jth level of factor B

     γ_k is the effect of kth level of factor C

     $(\tau\beta)_{ij}$ is the effect of the interaction between A and C

     $(\beta\gamma)_{ik}$ is the effect of the interaction between B and C

     $(\tau\beta v)_{ijk}$ is the effect of the interaction between A, B and C

E_{ijkl} is the random error component having a normal distribution with zero and variance δ^2

$$G_{-cal} = \frac{Su^2_{max}}{\Sigma\, Su^2} \qquad (2)$$

$$T_{-cal} = \frac{|b_j|}{Sb} \qquad (3)$$

$$Sb = \frac{\sqrt{(Su^2)}}{\sqrt{(N.r)}}$$

$$F_{-cal} = \frac{S_a^2 d}{su^2} \qquad (4)$$

$$S_a^2 d = \frac{r}{N-\lambda}\Sigma(Y - Y_{cal})^2$$

$$Su^2 = \frac{1}{r-1}\Sigma(Y_r - Y_i)$$

Where

S^2_ad = the dispersion of adequacy
Su^2 = sum of dispersion
bj = coefficient of equation variable
λ = insignificant coefficient = 2
r = number of replicates for a particular run = 2
N = number of runs =8
Y = experimental yield
Y_{cal} = response yield calculated using the appropriate model equation
Y_r = response yield of a replicate
Y_i = average response yield of the replicate for a run

3.3 Production of bio-ethanol from rice husk

Prior to the production of bio-ethanol, the rice was treated to confirm the presence of starch. Paddy rice was milled sieved and the residue was collected and weighed. $2cm^3$ of sample was measured from the bulk sample and transferred into the test tube. Potassium iodide reagent was then added drop wise into the sample in the test tube and stirred until colour was changed from yellow to black, which confirm the presence of starch. 500g of the husk was collected and soaked in $750cm^3$ of water for a period of 24 hours after which it was filtered with the aid of a filter cloth, $600cm^3$ of the filtrate was collected and made up to $1000cm^3$ with boiled water, the mixture was stirred continuously to avoid formation of lumps, it was then allowed to cool and on cooling, a thick-jelly mass was formed, gelatinized mixture was then poured into a $2000cm^3$ flask for hydrolysis. $200cm^3$ of 0.5m potassium hydroxide was added to the sample and immersed in the water bath for hydrolysis and the temperature was maintained at 75°C for 60 minutes. $100cm^3$ of 50% ethanoic acid was then added to serve as a terminator of the hydrolysis reaction after which the mixture was set aside to cool. $4cm^3$ of hydrolyzed sample, , few drops of Fehling's solution was added in a conical flask and heated, colour change was observed and recorded, sample changes to brick red precipitate, which confirm the presence of simple sugars.

3.3.1 Fermentation of hydrolysed rice husk

Zymomonas mobilis "Local strain" was isolated from palm wine using standard solid medium. Media constituents include 5.0g of yeast extract, 20g of agar and $1000cm^3$ of distilled water with pH 6.8. Medium was treated with actidione (cycloheximide) to inhibit *Zymomonas mobilis* growth before autoclaving at 121°C for 15 minutes. *Zymomonas mobilis* was then inoculated into the medium and incubated an aerobically at 3°C for 24 hours. Working close to the flame (creating aseptic environment), *Zymomonas mobilis* was introduced into the conical flask containing the substrate, the flask were then shaken (agitation process) and the mouths of the conical flasks were flamed before corking back and incubating at room temperature, they were shaken at various intervals in order to produce a homogenous paste and even distribution of the organisms in the substrates. After fermentation process, the substrates were then filtered using filter cloth and collected in a conical flask, in order to separate the desired product (the filtrate) from the residue. The filtrates were then distilled at 78.3°C using alcohol distillation apparatus, round bottom flask containing the filtrate was placed in the heating mantle and the mouth fixed to the condenser, a beaker for distillate collection was placed at the end of the set up, rubber pipes

or hose were connected to the condenser to supply water from the tap for cooling the condenser to supply water from the tap for cooling the condenser and letting out water out of the condenser simultaneously. Temperature on the heating mantle was set to the standard temperature for the production of ethanol which is 78.3oC, as the filtrate was heated, the vapour rose and entered into the condenser, tap water was passed into and out of the condenser using the rubber pipes and this condenses the vapor from the heated filtrate, condensed vapour was collected into the beaker at the other end of the distillation set up as the distillate (bio-ethanol), this process was repeated for other samples. The distillate was further purified by the use of calcium oxide (lime), a basic oxide, when added to the ethanol, absorbed the water to form calcium hydroxide, an alkaline solution; calcium hydroxide formed was separated from ethanol by further distillation which leaves absolute ethanol. One cm^3 of alcohol was treated with iodine and sodium hydroxide, the colour change was observed and recorded, yellow precipitate was formed, which confirm that ethanol is present. The produced bio-ethanol was characterized to determine the density, flash point, pour point.

4. Results and discussion of results

4.1 Results and statistical analysis of experimental results

Tables 4.1 and 4.2 present the results on the extraction of oil from moringa oleifera seed with hexane and ethanol as the solvent respectively at different temperature, particle size and resident time. Results obtained as presented indicate that there are thirty two experimental runs with two replicates each for sixteen samples. It can be seen from the results that the extraction time, temperature, particle size and type of extraction solvent affects the rate of extraction of oil from oleifera moringa seed. Ethanol plays a major role in the production of biodiesel from oil, achieve a suistainable production of biodiesel therefore, it is important to employ a cheap and suistanable method of ethanol production. In this work the production of bioethanol from agricultural waste (rice waste) was also conducted and the results obtained are presented in Tables 4.3 and 4.4.

Results presented reveals that at the extraction conditions combination with all samples at low levels, the oil yield was 37.78% and 37.35% for the replicate using n-hexane. Ethanol yielded 19.90% and 20.25% for the replicate. While For treatment combination where the temperature was high (65oC) while particle size and extraction time were low (500µm and 6hr respectively) n-hexane yielded 38.58% and 38.37% for the replicate. Ethanol at a high temperature of 75oC and particle size (500µm and extraction time of 6hrs yielded 20.82% and 21.23% for the replicate. Similarly For treatment combination where the temperature was low (55oC) while particle size high (710µm) and extraction time low (6hr) n-hexane yielded 43.17% and 43.26% for the replicate. Ethanol at a low temperature of 65oC, particle size high (710µm) and extraction time low (6hrs) yielded 38.71% and 38.65% for the replicate. While for extraction combination where the temperature was (55oC) and particle size (500µm) are low, extraction time was high (7hrs) n-hexane yielded 42.22% and 41.98% for the replicate. Ethanol at a low temperature and particle size (65oC and 500µm respectively) and extraction time high (7hrs) yielded 22.16% and 21.96% for the replicate. Also for the extraction conditions combination where the temperature and particle size were high (65oC and 710µm respectively) and extraction time were low (6hr) n-hexane yielded 43.01% and 42.95% for the replicate. Ethanol at a high temperature and particle size of (75oC and 710µm respectively) and low extraction time of 6hrs yielded 35.32% and 35.68% for the replicate. Results

presented also shows that, for the extraction combination where the temperature was high (65°C), low particle size (500µm) and high extraction time (7hrs) n-hexane yielded 42.81% and 42.25% for the replicate. Ethanol at a high temperature of 75°C, low particle size (500µm) and high extraction time of 7hrs yielded 26.67% and 26.14% for the replicate. It could be observed from the Tables of result that, for treatment combination where the temperature was low (55°C) while particle size and extraction time were high (710µm and 7hrs respectively) n-hexane yielded 41.38% and 41.35% for the replicate. Ethanol at a low temperature of 65°C, high particle size and extraction time (710µm and 7hrs respectively) yielded 28.84% and 28.24% for the replicate. Finally, for extraction condition combination where all the parameters are high temperature (65°C), particle size and extraction time were (710µm and 7hr respectively) n-hexane yielded 42.03% and 42.52% for the replicate. Ethanol at a high temperature of 75°C, particle size and extraction time (710µm and 7hrs respectively) yielded 24.75% and 25.03% for the replicate

S/N	wt of oil extracted (g)	% wt of oil extracted	Temp (°C)	Particle Size (µm)	Resident Time (hr)
Solvent = Hexane					
1	4.22	42.03	65	710	7
2	4.15	41.38	55	710	7
3	4.23	42.22	55	500	7
4	4.29	42.81	65	500	7
5	3.87	38.58	65	500	6
6	3.79	37.78	55	500	6
7	4.31	43.01	65	710	6
8	4.33	43.17	55	710	6
Solvent = Ethanol					
1	2.48	24.75	75	710	7
2	3.76	28.84	65	710	7
3	2.22	22.16	65	500	7
4	2.67	26.67	75	500	7
5	2.09	20.82	75	500	6
6	2.00	19.90	65	500	6
7	3.55	35.32	75	710	6
8	3.89	38.71	65	710	6

Table 4.1. Oil yield at various conditions from the first run with hexane and ethanol as the solvent

S/N	wt of oil extracted (g)	% wt of oil extracted	Temp (ᵒC)	Particle Size (μm)	Resident Time (hr)
Solvent = Hexane					
1	4.27	42.52	65	710	7
2	4.14	41.35	55	710	7
3	4.21	41.98	55	500	7
4	4.23	42.25	65	500	7
5	3.83	38.15	65	500	6
6	3.70	36.92	55	500	6
7	4.30	42.95	65	710	6
8	4.34	43.26	55	710	6
Solvent = Ethanol					
1	2.57	25.65	75	710	7
2	3.71	27.05	65	710	7
3	2.35	23.45	65	500	7
4	2.77	27.65	75	500	7
5	2.18	21.74	75	500	6
6	2.44	20.34	65	500	6
7	3.45	34.28	75	710	6
8	3.92	38.96	65	710	6

Table 4.2. Oil yield at various conditions from the second run with hexane and ethanol as the solvent

Substrate	Volume of hydrolysate (cm³)	Volume of ethanol (cm3)	% Ethanol concentration
Rice husk	350	25	7.143
	300	35	11.667
	250	37.5	14.880
	150	43	28.667

Table 4.3. Ethanol production using Zymomonas mobilis from distillation process

Properties	Commercial grade ethanol	Bio-ethanol produced from rice husk
Appearance	Clear, colourless liquid	Clear, colourless liquid
Boiling point (ᵒC)	78.15	78.3
Density (g/cm³)	0.789	0.787
Viscosity	1.20	1.34
Flammability	Flammable	Flammable
Flash point (ᵒC)	13	14.5
Refractive index	1.3614-1.3618	1.3626

Table 4.4. Properties of produced ethanol compared to commercial ethanol

4.1.1 Statistical analysis of experimental results

Statistical analyses were conducted with the aim of developing a model to represent the relationship between the factors investigated and the yield of oil from the moringa oleifera seeds with hexane and ethanol as the extraction solvent. Table 4.4 shows an estimation of upper and lower levels of the three factors (temperature, particle size and time). While Tables 4.5 and 4.6 indicates factorial experimental design results with n-hexane and ethanol as the extraction solvent respectively.

The average effect of a factor which is described as the change in response produced by a change in the level of factor response produced by a change in the level of factor averaged over the levels of other factors. This has been calculated and subsequently tabulated in Table 4.7 for n-hexane and ethanol.

Level of Factor	Code	A Temperature (°C)		B Particle Size (μm)	C Time (hrs)
		Hexane	Ethanol		
High level	+1	65	75	710	7
Low level	-1	55	65	500	6

Table 4.4. Factors and their coded levels

Treatment combination		Design factor	First yield Y_1	Second yield Y_2	Average yield Y_{av}
Run		A B C			
1	I	-1 -1 -1	37.78	36.92	37.35
2	A	+1 -1 -1	38.58	38.15	38.37
3	B	-1 +1 -1	43.17	43.26	43.22
4	C	-1 -1 +1	42.22	41.98	42.10
5	Ab	+1 +1 -1	43.01	42.95	42.98
6	Ac	+1 -1 +1	42.81	42.25	42.53
7	Bc	-1 +1 +1	41.38	41.35	41.37
8	Abc	+1 +1 +1	42.03	42.52	42.28

Table 4.5. 2^3 Factorial experimental design results using n-hexane as extraction solvent

Treatment combination		Design factor	First yield Y_1	Second yield Y_2	Average yield Y_{av}
Run		A B C			
1	I	-1 -1 -1	19.90	20.25	20.08
2	A	+1 -1 -1	20.82	21.25	21.04
3	B	-1 +1 -1	38.71	38.65	38.65
4	C	-1 -1 +1	22.16	21.96	22.06
5	Ab	+1 +1 -1	35.32	35.68	35.50
6	Ac	+1 -1 +1	26.67	26.14	26.41
7	Bc	-1 +1 +1	28.84	28.24	28.54
8	Abc	+1 +1 +1	24.75	25.03	24.89

Table 4.6. 2^3 Factorial experimental design results using ethanol as extraction solvent

Factors and interactions	Main effects (n- hexane)	Main effects (Ethanol)
A	0.5300	-0.3813
B	2.4975	9.5213
C	1.5900	-3.3488
Ab	-0.1925	-3.0338
Ac	0.1400	0.7288
Bc	-2.8675	-7.0263
Abc	0.4325	-0.8750

Table 4.7. Effects and interactions for solvent extraction of oil using n-hexane and ethanol

Variance (ANOVA) analysis, which enables one to examine the magnitude and direction of the factors' effect and determine which variable are likely to be important was also conducted and the results are presented in Table 4.8 and 4.9 respectively for n-hexane and methanol as the extraction solvent. Variance analysis also helps to determine the statistical significance of the regression coefficients (β_i). The level of significance was assumed to be 5% ($\alpha = 0.05$), which implies that there are about five chances in hundred that reject the hypothesis when it should be accepted: i.e. 95% confidence that right decision is made. Therefore the critical value for each of the F-ratio F {α ,dfr, abc(n -1)}i.e. F(0.05,1,8 is equal to 5.32 from statistical table is equal to 5.32 from statistical table. The F-ratios were compared with this critical value (5.32) and the null hypothesis using Fcal > F(0.05,1,8) = 5.32. The magnitude of the effects when n-Hexane was used as the extraction solvent indicates that particle size (factor B) is dominant and has a high significant followed by the extraction time (factor C) and the effect of factor A, extraction temperature which is relatively low.

Sources of variation	Sum of square	Degree of freedom	Mean square	Expected mean square	F_o
A	1.1236	1	1.1236	39.5575	2.2940
B	22.5150	1	22.5150	41.4025	45.9677
C	10.1124	1	10.1124	40.6175	20.6460
Ab	0.1482	1	0.1482	41.9325	0.3026
Ac	0.0784	1	0.0784	41.1475	0.1601
Bc	32.8902	1	32.8902	42.9925	67.1503
Abc	0.7482	1	0.7482	43.5225	1.5276
Error	3.9182	8	0.4898		
Total		15			

Table 4.8. Analysis of variance (ANOVA) for the solvent extraction of oil using n-hexane

The magnitude of the effects when ethanol was used as the extraction solvent, clearly shows that particle size (factor B) is dominant and has a high significant followed by the interaction of factor A, extraction temperature and factor C, extraction time and the effect of factor A, extraction temperature which is relatively low. Presented in Tables 4.10 and 4.11 are the basic statistical test on the yield of oil from the moringa oleifera seed with n-hexane and ethanol as the extraction solvent respectively. While Table 4.12 present the statistical calculated values of G and F test.

Sources of variation	Sum of square	Degree of freedom	Mean square	Expected mean square	F_o
A	0.5814	1	0.5814	22.9526	1.0561
B	362.6206	1	362.6206	21.8374	658.9508
C	44.8578	1	44.8578	32.4602	81.5152
Ab	36.9372	1	36.9372	22.2200	67.1219
Ac	2.1246	1	2.1246	31.3450	3.8608
Bc	197.4756	1	197.4756	22.5700	358.8508
Abc	3.0625	1	3.0625	32.4602	5.5681
Error	0.5814	8	0.5503	32.0776	
Total		15			

Table 4.9. Analysis of variance (ANOVA) for the solvent extraction of oil using ethanol

	Y_{r1}	Y_{r2}	Y_T	Y_{av}	Y_{cal}	$(Y_{r1}-Y_{cal})^2$	$(Y_{r2}-Y_{av})^2$	$(Y_{r1}-Y_{av})^2$
1	42.03	42.52	84.55	42.28	39.03	9.0000	0.0576	0.0625
2	41.38	41.35	82.73	41.37	39.56	3.3124	0.0004	0.0001
3	42.22	41.98	84.20	42.10	41.40	0.6724	0.0144	0.0144
4	42.81	42.25	85.06	42.53	40.62	4.7961	0.0784	0.3136
5	38.58	38.15	76.73	38.37	41.93	11.2200	0.0484	0.0441
6	37.78	36.92	74.70	37.35	41.15	11.3600	0.1849	0.1849
7	43.01	42.95	85.96	42.98	42.99	0.0004	0.0009	0.0009
8	43.17	43.26	86.43	43.22	43.52	0.0081	0.0016	0.0025
Σ						40.3694	0.3866	0.6230

Table 4.10. Basic statistical test (n-Hexane)

	Y_{r1}	Y_{r2}	Y_T	Y_{av}	Y_{cal}	$(Y_{r1}-Y_{cal})^2$	$(Y_{r2}-Y_{av})^2$	$(Y_{r1}-Y_{av})^2$
1	24.75	25.65	50.40	25.20	22.95	3.2400	0.2025	0.2025
2	28.84	27.05	55.89	27.95	21.84	49.0000	0.8100	0.7921
3	22.16	23.45	45.62	22.81	32.46	106.0900	0.4096	0.4225
4	26.67	27.65	54.32	27.16	22.22	19.80000	0.2401	0.2401
5	20.85	21.74	42.56	21.28	31.35	0.8454	0.2116	0.2116
6	19.90	20.34	40.24	20.12	22.57	7.1289	0.0484	0.0484
7	35.32	34.28	69.60	34.80	32.46	8.1796	0.2704	0.2704
8	38.71	38.96	77.67	38.84	32.08	43.9569	0.0144	0.0169
Σ						238.24	2.2070	2.2405

Table 4.11. Basic statistical test (Ethanol)

Test	From Statistical Table	Calculated	
		n-Hexane	Ethanol
G-test	0.6800	0.6171	0.5003
F-test		34.8073	35.9825

Table 4.12. Statistical calculated values for G-test and F-test

Based on the statistical analysis of experimental results, the regression model for the 2^3 design analysis is therefore given by Equation 5, i.e.

$$Y = a_o + a_1 x_1 + a_2 x_2 + a_3 x_3 + a_{12} x_1 x_2 + a_{13} x_1 x_3 + a_{23} x_2 x_3 + a_{123} x_1 x_2 x_3 \qquad (5)$$

The Residual for 2^3 designs for the yield of oil from moringa oleifera seed kernel using n-Hexane can now be obtained by considering only the three largest effects, which are B, C and A. Equation 5 therefore reduced to;

$$Y = a_o + a_1 x_1 + a_2 x_2 + a_3 x_3 \qquad (6)$$

$$a_0 = \frac{1}{8} \sum (Y_i)$$

$$a_j = \frac{1}{8} \sum (Y_i S_i)$$

Where a_j is the coefficient of factor j and S_i is the sign of eight factor combinations from the design matrix table. Thus

$$Y = 41.275 + 0.265 X_1 + 1.1875 X_2 + 0.795 X_3 \qquad (7)$$

Similarly, the residual for 2^3 designs for the yield of oil from moringa oleifera seed with ethanol as the extraction solvent can be obtained by considering only the three largest main effects, which are B, AC and A. The regression equation can therefore reduced to

$$Y = a_o + a_1 x_1 + a_2 x_2 + a_{13} x_1 x_3 \qquad (8)$$

Thus

$$Y = 27.1488 - 0.1913 X_1 + 4.7538 X_2 + 0.3663 X_1 X_3 \qquad (9)$$

4.2 Discussion of results

The world is presently on the brinks of an environmental disaster owing to the build-up of harmful materials from the use of fossil oil as base oil for lubricants. Coupled with the prediction that the fossil oil will ultimately run out sometime in the future, there is therefore, the urgent need to source for replaceable and environmentally friendly base oil for lubricants. Biodiesels which is the product of transesterification of vegetables oil is considered as perfect alternative and sustainable energy sources, due to less emission and availability. The Promotion of Biomass faces an increasing rate of awareness, research and adoption. One way of increasing the adoption rate is to promote the utilization of the product from plants such as the leaves, fruits, stem, flowers and the roots of the trees. Presently, the alternative way of utilizing the fruit is to extract oil from the seeds, most of which are edible oil which is a source of concern. Despite the wide acceptance of biofuel as alternative energy to supplement or replace the fossil fuel, it will be wise to recognise the consequences of the new technology on the society. For instance, the production of biodiesel from edible oil could result in pressure on farmers, consequence of which is food shortage and environmental problem as a result of deforestation. Hence the need to produce the biodiesel from non-edible oil or from the sources that are not sources of production of edible

oil such as oil from moringa oleifera seed which is the focus of this study. In order to depend fully on less expensive materials for the production of biodiesel, this study also investigates production bio-ethanol from rice husk. The results of the effects of various parameters, such as extraction time, particle size, temperatures and types of extraction solvents influence the rate extraction of oil from moringa oleifera. In this study, effect of extraction on the yield of oil from moringa oleifera seed was investigated using n-hexane and ethanol as the extraction solvent. It has been reported that the entrainer chosen has to be a good selective solvent with sufficient low viscosity for it to circulate freely. A relatively pure solvent will initially increase the extraction rate, but as the extraction proceeds, the concentration of solute will increase and the extraction rate will progressively decrease, firstly because the concentration gradient will reduce and secondly, the solvent will generally become more viscous and thereby decreases its penetration power.

The results of analysis yield a model equation presented in Equation 7, which was not only used to obtain the effect of one factor on the other but also their interaction. From the analysis it was discovered that when n-hexane was used as the extraction solvent, the effect of factor B (particle size), has the highest magnitude of 2.4975 followed by the effect of factor C (extraction time) with a magnitude of 1.5900, the effect of factor A, extraction temperature is relatively low with a magnitude value of 0.5300. A "– "in a 2^k model equation implies an inverse proportionality, while "+" implies a direct proportionality. This means that the extraction temperature, X_1, the particle size X_2 and the extraction time X_3 are all directly proportional to the oil yield (Averill and Kelton, 1996 and Onifade, 2001). Results obtained on the effect of extraction temperature on the yield of oil as expected indicate that rate of extraction of oil from moringa oleifera seed is increases with increase in temperature. Increase in temperature positively affects the diffusivity of the solvent into the inner part of the seed and consequently aid the solubility of the oil in the solvent which increase the rate of extraction of oil from the seed. Though results obtained shows that increase in temperature favoured the extortion of oil, care must be taken not to exceed the limit, which is the boiling point of the solvent n-hexane. Exceeding the boiling temperature of the solvent could result into the evaporation of the solvent consequence of which is the quick usage of the solvent, which is not economical. Also investigated in this research is the effect of particle size on the rate of extraction of oil from the seed and from the model equation, the result reveal that the oil yield is directly proportional to the particle size i.e. oil yield increases with a increase in the particle size. It has been reported that the size of particle could influence the extraction rate and the yield of oil in a number of ways. For instance, the smaller the size of the particle the higher the interfacial area between the solid and the solvent, the higher the rate of transfer of the solute (oil), and the smaller the distance the solute must be diffused within the solid particle, hence the higher the rate of extraction of oil. It is therefore desirable that the range of the particle size should be small so that each particle will require approximately the same time of extraction. It is therefore important that particles size are well selected not to exist a critical particle size at which oil yield will no longer be optimum. Since above the optimum particle size, there will be a reduction in the surface area of the oil molecules exposed to the solvent for dissolution. The effect of time on the extraction of oil from moringa oleifera seed was studied using n-hexane as the solvent. Results obtained as presented in Table 4.1 and the statistical model (Equation 7) indicates that the oil yield has a direct proportion effect with the extraction time. This means that increasing the extraction time will bring about a high yield of oil, however, there is the need to optimized extraction time to save cost of production of oil from the seed. This is

because higher extraction time above the optimum time cannot yield oil more than the maximum oil content in the seed kernel.

With ethanol as extraction solvent, the effect of factor B (particle size) has the highest dominance of 9.5213. The interaction of factor AC, temperature and extraction time has an effect of 0.7288, while temperature has the least effect of -0.3813. The 2^3 factorial analyses, give a model equation (Equation 8). From the statistical model, it can be seen that the extraction temperature, X_1, is inversely proportional to the oil yield, the particle size X_2 and the extraction time X_3 are all directly proportional to the oil yield. The inverse proportion effect of temperature on the extraction of oil from moringa oleifera seed is an indication that a range of 65 – 70°C is adequate to give a better yield of oil. Above this temperature range, the effect of temperature on the oil yield is negative. Thus a reduction in the extraction temperature from the maximum will result in an increase in the yield of oil. Results obtained also shows that the oil yield is directly proportional to the particle size. Hence the effect of particle size on oil yield increases with an increase in the particle size this is because greater surface area of the oil molecules exposed to solvent for dissolution. In the same vein, increase in the extraction time leads to increase in the yield of from moringa oleifera seed with ethanol as the solvent.

Production of ethanol from starch or sugar based feedstock is among man's earliest ventures into value added processing, while the basic steps remain the same, the process has been considerably refined in recent years, leading to a very efficient process. Bio-ethanol is an alcohol made by fermenting sugar components of biomass (Bailey and Ollis, 1986; Elba and Antenieta, 1996). Apart from food and pharmaceutical uses, bio-ethanol is finding alternative uses as motor fuel and fuel additive, ethanol as motor fuel is preferred to fossil fuel in that, it is environmentally friendly, comes from a renewable source and has a higher performance in engine (Eurasia, 2009).It can be mass-produced by fermentation of sugars or by hydration of ethylene from petroleum and other sources (Eurasia, 2009). Hence the need to produce bio-ethanol from relatively inexpensive and readily available raw materials like rice husks. In this study, rice husks were used to produce ethanol through hydrolysis and fermentation with Zymomonas mobilis. In the process of fermentation, the organism fermented the substrate (rice husk) to produce ethanol, Zymomonas mobilis possesses alcohol dehydrogenase (ADH) and pyruvate decarboxylase (PDC) which is key enzymes in ethanol fermentation from organic substrate as stated by Gunasegaram and Chandra (1998). Results obtained of bio-ethanol from rice husks as presented in Table 4.3 indicates that the volume of bio-ethanol is influence by the volume of hydrolysate. The maximum volume of bio-ethanol produced was 43cm^3 from 150 cm^3 of hydrolysate, while 25cm^3 of bio-ethanol was produced from 350cm^3 of the hydrolysate. The high yield of bio-ethanol from rice husk may be due to high carbohydrates contents of rice husk or the high ethanol tolerance of Zymomonas mobilis and the presence of alcohol dehydrogenase in Zymomonas mobilis which appears to facilitate ethanol formation even at high ethanol concentration. Presented in Table 4.2 are the properties of oil, such as viscosity, refractive index, density and flash point of the bio-ethanol produced from rice husk, which compared favorably with those of the commercially available methanol. The slight variation between the values of properties of bio-ethanol and that of the commercially available methanol can be attributed the sources of production and experimental methods employed. It can therefore be inferred that the bio-ethanol produced from rice husk cab be used as an alternative feedstock for the production of biodiesel base on the properties of bio-ethanol presented in table 4.2.

5. Conclusion

The need for alternative sources of energy other than fossil fuel gained momentum recently, and biofuel is considered perfect alternative sources of energy that is sustainable and reliable. However, the possibility of producing biofuel in commercial quantities is not certain; this is blame on the consequence effects of producing the biofuel from vegetable oil, as this can lead to food shortage. To achieve commercial availability of biofuel, it is therefore important to produce biofuel from non-edible oil or from the sources that are not popular sources of edible oil. To achieve commercial realisation of biodiesel production, this work focuses on the extraction and optimization of oil from moringa oleifera seed as an alternative feedstock for the production of biodiesel. Analysis of results indicates that when n-hexane was employed as the extraction solvent, the effect of particle size has the highest effect with magnitude of 2.50, followed by the extraction temperature with magnitude of 1.59, while the effects of extraction time was the lowest with the magnitude value of 0.53. With ethanol as the extraction solvent, particle size also has the highest dominance of 9.52, while the interaction of temperature and time has an effect of 0.73, while the extraction temperature was -0.3813. Based on these results it can be deduce that for an appreciable yield of oil to be achieved with ethanol as the solvent, the particle size and interaction of temperature and time are the factors which have high significance. Results obtained from the production of bio-ethanol from husk indicate that, it is possible to produced bio-ethanol from rice husk, which is also a major a feedstock in the production of biodiesel.

6. Acknowledgment

7. References

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