EVALUATING THE POTENTIAL OF RUBBER SEED OIL FOR BIODIESEL FUEL PRODUCTION

M.Sc .THESIS

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APPROVAL SHEET 1

This is to certify that the thesis entitled "**Evaluating the Potential of Rubber Seed Oil for Biodiesel Fuel Production**" submitted in partial fulfilment of the requirements for the degree of master of science in Renewable Energy Utilization and Management, the graduate program of the Department of Environmental Science, and has been carried out by **Berhanu Sugebo Helallo** Id. Number GPREUMR/005/11, under my supervision. Therefore, I recommend that the student has fulfilled the requirements and hence hereby can submit the thesis to the department.

Dr. Zerihun Demrew	________________	______________
Name of Main Advisor	Signature	Date

_________________________	_________________	______________
Name of post graduate coordinator	Signature	Date

APPROVAL SHEET 2

We, the Undersigned, members of the board of examiners of the final open defense by **Berhanu Sugebo Helallo** have read and evaluated his thesis entitled "**Evaluating the Potential of Rubber Seed Oil for Biodiesel Fuel Production"** and examined the candidate. This is, therefore, to certify that the thesis has been accepted in partial fulfilment of the requirements for the degree of Master of Science in renewable energy utilization and management.

____________________	____________________	____________________
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SGD Approval	Signature	Date

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DECLARATION

I declare that this MSc. thesis entitled "**Evaluating the Potential of Rubber Seed Oil for Biodiesel Fuel Production**" is my original work and has not been submitted for a degree of award in any other university, and all sources of material used in this thesis have been duly acknowledged.

Berhanu Sugebo Helallo

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Name of student	Signature	Date

DEDICATION

Dedication of this research work is for my lovely parents Mr.Sugebo Helallo and Mrs.Worknesh Erigecho, who have gone to God seven years ago.

Lists of Acronyms & Abbreviations

AOC	Association of Official Analytical Chemists
ASTM	American Society for Testing and Materials
EN	European biodiesel standard
FAME	Fatty acid methyl ester
FFA	Free fatty acid
GC-MS	Gas chromatography mass spectrometry
Masl	Meter above sea level
Mixed	Rubber tree clone name
RT	Retention time
RSO	Rubber seed oil
S.K	Seed kernel to seed ratio
S.S	Seed shell to seed ratio
VIF	Variance inflations factor
v/v	Volume to volume ratio

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ABSTRACT

Energy from biofuel is one of attracting renewable energy sources to substitute fossil fuel. In Ethiopia there are potential of rubber plantations; however, even though the yield and quality of the rubber seed oil is high, little attention has been given for its use as a source for biodiesel production. The main aim of the present study was to determine physical properties of rubber seed, to optimize oil extraction from rubber seed and synthesize biodiesel from rubber seed oil. Seed oil was extracted by solvent extraction method and oil physicochemical properties were determined by Association of Official Analytical Chemists methods. Biodiesel was synthesised by two step acid-base catalyse transesterification method with 6:1 molar weight of methanol to oil, at 60^{o}C reaction temperature for ninety minutes. Biodiesel fatty acid composition was determined by Gas-chromatography mass spectrometry method. Data were analysed by design expert software central composite design of response surface methodology. The highest and the lowest oil yield obtained from rubber seed kernel were 61.3 and 3.09 weight %, respectively. Regarding the physicochemical properties of the rubber seed oil, it was 0.91g/cm^3 at 28^{o}C, 6.68mgKOH/g,113.02gI_2/100g, 7meq/Kg,215.51mgKOH/g and 914.65gm/mol for density, acid value, iodine value, peroxide value, saponification value and molecular mass of oil, respectively. Biodiesel yield obtained was 81.55wt%, and the biodiesel was composed of 82.45% unsaturated fatty acid and 15.71% saturated fatty acid. Biodiesel from rubber seed oil is one of the important multi-purpose sources to solve petroleum diesel scarcity and global warming issues coming from burning of petroleum diesel. In order to use the rubber seed oil for biodiesel production further research is required to enhance stability of the oil.

Key word: Biofuel, Fatty acid methyl ester, Rubber seed oil, Vegetable oil

1 Introduction

1.1Background

In recent years, renewable energy sources have been attracting the attention of public and policymakers, particularly to reduce natural degradation and greenhouse gas emission. Among renewable energy resources, biomass remains a key source of energy for developing country, particularly in the Sub-Saharan region. Biofuels are one of the important renewable energy source that draw increasing attention worldwide as substitutes for petroleum-derived transportation fuels to help address energy cost, energy security and global warming concerns associated with liquid fossil fuel (UNCTAD, 2008).

Biodiesel is a biofuel that can substitute petroleum diesel, which can be used in diesel engines alone or blending with diesel without or with minor engine modification (Van Gerpen *et al*., 2004). Biodiesel can be produced from various easily available bio-oils such as, castor oil, jatoropha oil, palm oil, rubber seed oil, waste cooking oil and animal fats (Dahiya, 2020; Knothe et al., 1997; Takase et al., 2015; Widayat and Suherman, 2012).

Rubber tree seed oil is one of the important potential sources for biodiesel with an average seed yield of 800-1200kg/ha/year and rubber tree can bear seed after four years of seedling planted and can bear seed for forty years (Bekele-Tesemma and Tengnäs, 2007; Zhu et al., 2014).Rubber seed is physically composed of nearly half of shell and oil content of the dried kernel is 40-50% which is comparable to the oil content of soybean and sunflower seed (Ahmad et al., 2014a; K.T et al., 2007; Rashid et al., 2008). Rubber seed oil is composed of 76.8% to 81.3% unsaturated fatty acid, that make better cold flow properties than other more saturated fatty acid chain composed bio-oils (Ahmad et al., 2014a; Bello and Otu, 2015).

In Ethiopia Guraferda District is one of the potential District for rubber tree plantation and in the District recently only public matured rubber tree plantation is more than 2774 hectares. Also in the District most local farmers are willing now to plant rubber tree for latex production (Dejene et al., 2018),that will be increased potential of rubber seed oil for biodiesel production. In Ethiopia rubber seed oil is not yet exploited or even not identified as energy sources, because of lack of awareness on available potential, short term unrealistic government strategy on biodiesel for transportation purpose and under development of biofuel sectors.

1.2 Statement of the problem

The non-renewable nature and environmental issues makes the fossil oil unsustainable energy source worldwide (Agarwal and Gupta, 2015; IEA, 2018; Sheehan et al., 1998). Unsustainable fossil fuel energy system is one of the reason for unstable price and foreign currency cries especially in developing countries (de Fraiture et al., 2008; Sheehan et al., 1998; Singh and Singh, 2010). Ethiopia is one of petroleum fuel importer with annual average imports about one billion litter's petroleum fuel (Minstry of water and energy, 2012). Reports indicate that 77% to 87% of export earnings of the country goes for petroleum fuel purchase which creates imbalance between import and export trade of the country (Alemu, 2013; Henok Zereu, 2016).Biodiesel from rubber seed oil can be used to minimize dependence on importing petroleum diesel.

Rubber tree (*Hevea brasiliensis*) seed oil is an alternative feedstock for biodiesel production, but the resource is underutilized (Widayat and Suherman, 2012; Zhu et al., 2014). Like other parts of the country, in Guraferda District, both public and private matured rubber tree plantations have seed potential for biodiesel production, but these resources are not properly utilized.

Rubber tree seed oil yield and their physicochemical properties are different at clone to clone and location to location based on planting pattern, agroecology of location and tree genetics (Asuquo et al., 2012; Eka et al., 2010; Zhu et al., 2014). In Ethiopia, although there are more than ten rubber tree clones that are adapted from different countries, studies on the clone's seed oil content, synthesis of biodiesel and its physicochemical properties are very limited. Hence, in present study conducted with the aim of evaluating the potential of rubber seed oil for biodiesel production.

1.3 Objective

1.3.1 General objective

The General objective of the present study was to evaluate the potential of the rubber seed oil for biodiesel production.

1.3.2 Specific objective

The specific objectives were:-

 To determine physical properties of rubber seed

 To optimize oil extraction from rubber seed

 To synthesize biodiesel from rubber tree seed oil and to investigate the physicochemical properties of the biodiesel

1.4 Research question

1 How much percentage of the rubber seed consist with kernel?

2 What are the factors that affect rubber seed oil yield and which level of the factors are optimum to extract oil from the rubber seed?

3 How much yield of biodiesel can obtain from the rubber seed oil and from which fatty acid chain is the biodiesel composed?

1.5 Significance of the study

The findings obtained in this study could be used to identify potential of rubber seed oil for biodiesel production and policymakers to consider the rubber seed oil as a potential source for biodiesel production to substitute petroleum diesel. Moreover, this study will also be useful for bio-energy researchers, higher educational institutions; stakeholder's those who involved on the rubber tree plantation.

1.6 Scope of the study

In the present study only mixed rubber tree clone seed that was planted separately from other clone was used. Also seed oil yield optimization, oil physicochemical properties, synthesis of biodiesel and evaluation of biodiesel physicochemical properties were done.

Limitation

Oil physicochemical and biodiesel properties could be different for each oil extracted treatment. Because of a budget and material limitation, characterization of the physicochemical properties of the oil and synthesis of the biodiesel was done for only those with highest oil yield.

2 Literature review

2.1 Rubber tree seed potential for biodiesel production

Rubber seed oil biodiesel can be used for combustion-engine purposes without further engine modification (Patil et al., 2015; Ulfah et al., 2018). An additional factor making crude rubber seed oil based biodiesel an attractive option is the fact that rubber plantations already exist, in particular area (Wagner et al., 2018). From a bio refinery perspective, the valorization of rubber seed by biodiesel production is highly relevant as it increases the economic attractiveness of the rubber plantations (Abduh et al., 2016).Rubber seed is one of oil rich seed source and seed kernel contains 40-50 % of oil (Gimbun et al., 2012; Meena Devi et al., 2015).In the world in many countries, especially in far East Asian countries the rubber seed oil is one of the future candidate alternative fuel (Gimbun et al., 2012; Meena Devi et al., 2015).

2.2 Physical properties of rubber seed

Rubber seed is physically composed of kernel and shell. The seed contain approximately about 40% kernel with 20-25% moisture (Gimbun *et al*., 2012; Onoji *et al*., 2019). Fresh rubber seed contain about 16.57% moisture, and high moisture content promotes rapid deterioration of seeds (Widayat and Suherman, 2012).

2.3 Effect of kernel size on the oil yield.

Previously study on effect of kernel size on oil yield with various seed size ranges (150 μm , 300 and 400 μm), the maximum oil yield of 96% was reported at smallest mesh sizes of 150 μm (Abdulkadir et al., 2015).In addition, on other study evaluated kernel size effect on oil yield with five-particle sizes of (0.5, 1, 1.5, 2, and 2.5 mm),the highest oil yield was reported from kernel particle size of 0.5mm (Oniya et al., 2017). Smaller particle size has a large number of surface areas as well as an increased number of broken cells resulting in a high oil concentration (Abdulkadir et al., 2015; Oniya et al., 2017).

2.4 Rubber seed oil extraction

The basic aim of seed extraction is to separate fat from protein in order to obtain high-quality oil and produce high extraction yields (Atabani et al., 2012; Bhuiya et al., 2016; Gonfa Keneni and Mario Marchetti, 2017). In practice, extracting vegetable oil depends on the physical and chemical characteristics of the raw feedstock (Gonfa Keneni and Mario Marchetti, 2017).There are three major extraction methods: these are chemical, mechanical and enzymatic extractions (Atabani et al., 2012; Bhuiya et al., 2016; Gonfa Keneni and Mario Marchetti, 2017).

2.4.1 Chemical Extraction

It is a process based on placing grounded oilseeds in contact with a solvent that allows dissolution of the oil (Bhuiya et al., 2016). The efficiency of the process depends on the preparation of oilseed, temperature, operation mode and the nature of the solvent (Atabani et al., 2013, 2012).Practically, the choice of the solvent relies on two major considerations: oil solubility and the related utilization cost (Chaker Ncibi and Sillanpaa, 2014).Commonly used solvents for oil extraction include n-hexane, white spirit, trichloroethylene, carbon sulphide and some bio-solvents (Chaker Ncibi and Sillanpaa, 2014). Currently, n-hexane is widely used as solvent for the extraction of vegetable oil (Chaker Ncibi and Sillanpaa, 2014).In addition, chemical extraction could enhance oil recovery (Chhetri et al., 2008), solvent extraction on avocado seed provide 15% more oil yield than mechanical extraction (Rachimoellah et al., 2009) .

In addition, extraction temperature, seed to solvent ratio, extraction time and seed particle size affects rubber seed oil yield (Atabani et al., 2013, 2012). Also oil yield increased initially with increasing temperature and then subsequently decreased with further increase in temperature (Bashir et al., 2014; Oniya et al., 2017). The optimum oil yield from rubber seed was at oil extraction temperature of 65 °C (Bashir et al., 2014) and 68.13ºC (Oniya et al., 2017). The ratio of seed to solvent (hexane) in the range of 1:3 up to 1:6 was tested, the proper mass ratio which gave higher yield (40%) is 1:5, (50 g seeds with 250 ml n-hexane) (Bashir et al., 2014). Also on other study of *Hura Crepitans L* seed oil extraction is reported the oil yield of 63.4 wt% by reaction variables of seed/solvent ratio of 1:20, extraction temperature of 68.13 ^{o}C and for five hour extractions (Oniya *et al*., 2017).On other hand, other author reported, oil yield increased with increasing solid to solvent ratio up to 1:15 (Mohd-Setapar et al., 2014).

2.4.2 Mechanical Extraction

Mechanical extraction is widely used process with a pressure-based method that preserves the characteristics of the oil seed and the residual product (Gonfa Keneni and Mario Marchetti, 2017). The seeds are fed into an extruder to compresses it. Seed crushing action is often use before reaching extruder in case of seed with high oil content. The press type depends on the raw material use for extraction (Chaker Ncibi and Sillanpaa, 2014).

2.5 Rubber seed oil physicochemical properties

The rubber seed oil is stable at higher temperature (Aravind et al., 2015; Bello and Otu, 2015; Reshad et al., 2015). Bello and Out, (2015) studied on effect of temperature on rubber seed oil physicochemical properties, reported that rubber seed oil is sensitive to heat above 110 ^{o}C and turns to latex. Also Aravind et al. (2015) studied on thermogravimetry analysis (TGA) on rubber seed oil under an oxygen environment in the temperature range 0−500 °C, reported that rubber seed oil to be thermally stable up to 250 °C and gradual degradation occurs after 300 °C. Reshad et al. (2015) reported rubber seed oil is negligible weight loss (<0.2%) at 90 °C due to the presence of moisture (free water), but they were thermally stable up to 250 °C. Other physicochemical properties of rubber seed oil is given in Table 1.

Table 1: Previously studies on physicochemical properties of rubber seed oil

No	**Characteristics (unit)**	**Authors'**					
		(Asuquo *et al.*, 2012)	(Singh *et al.*, 2016)	(Ebewele et al., 2010)	(Bashir et al., 2014)	(Onoji et al., 2019)	(Aravind *et al.*, 2015; Reshad *et al.*, 2015)
1	Colour	Dark brown		Dark brown	Dark brown	Dark brown	Golden yellow, light/ dark brown
2	Moisture content (w %)		0.37	NA	0		
3	Density (g/cm^3)	NA	NA	NA	0.897@ 20°C	0.886 @ 25°C	0.857–0.943 @ 25°C
4	Specific gravity	0.92 @ 20 °C	0.91	0.943 @ 20 °C	0.873 @ 20 °C	0.909 @ 15 °C	0.91 @ 15 °C
5	Acid value (Mg KOH/g)	1.68	83.76	37.96	34	18.20	1.68–42.41
6	Free fatty acid (%FFA as oleic acid)	0.84	41.64	18.98	17	9.1	0.84-21.2
7	Peroxide value (Meq/Kg)	NA	3.4	NA	NA	10.46	1.6–16
8	Saponification value (mg KOH/g oil)	193.61	NA	226.02	179.6	195.30	183.91–235.28
9	Iodine value (g I2/100 g oil)	NA	NA	NA	NA	137.02	113–146
10	Kinematic viscosity @ 40°C(mm^2/s)	NA	40.86	NA	NA	40.18	6–66
11	PH value	NA	NA	NA	NA	6	6
12	Calorific value (MJ/Kg)		39.37	NA	NA	39.71	NA

Where, NA: is not available

2.5.1 Rubber seed oil fatty acid profile

In addition, rubber seed oil contains nearly 19% of palmitic and 3.87 % to 8.4% of stearic acid both of which are saturated, and 23.7% to 39% of oleic, 37.7% to 41% of linoleic and 15% to 16% of linolenic acid, in order that 76.8% to 81.3% of rubber seed oil is composed of unsaturated fatty acid, that make better cold flow properties of rubber seed oil than saturated oil (Ahmad et al., 2014a; Bello and Otu, 2015; Pianthong and Thaiyasuit, 2011).

2.6 Oil refining

Rubber seed oil has undesirable substances such as phosphatides, free fatty acids, waxes and colorants (Ulfah et al., 2018). Also impurities in oil can alter oil storage life and hamper further processing (Ulfah et al., 2018). Homogeneous acid esterification is an important method to reduce high free fatty acid (Pianthong and Thaiyasuit, 2011; A. Ramadhas et al., 2005; Van Gerpen et al., 2004; Widayat et al., 2013). High free fatty acid content in oil react with the base catalyst can produce soap and it is undesirable product in transesterification (Koh and Mohd. Ghazi, 2011).Amount of catalyst use for esterification is based on amount of available free fatty acid in oil (Pianthong and Thaiyasuit, 2011; Van Gerpen et al., 2004) and amount of catalyst has an effect on oil refining for biodiesel production. Sulfuric acid (H_2SO_4) is most commonly use catalyst for oil refining (Pianthong and Thaiyasuit, 2011; A. Ramadhas et al., 2005; Van Gerpen et al., 2004; Widayat et al., 2013).

2.7 methods of biodiesel production

There are different methods for modification of crude oils to reduce viscosities and to produce a better quality of biodiesel, these are:-blending of crude oils, micro-emulsification, pyrolysis and transesterification (Koh and Mohd. Ghazi, 2011; Singh and Singh, 2010).

2.7.1 Blending of crude oils or dilution

Crude vegetable oils can be mixed directly or diluted with diesel fuel to improve the viscosity so as to solve the problems associated with the use of pure vegetable oils with high viscosities in compression ignition engines (Singh and Singh, 2010). Also a blend of 20% vegetable oil and 80% diesel fuel was successfully reported (Singh and Singh, 2010).

2.7.2 Micro-emulsification

Micro-emulsion is clear, stable isotropic fluids with three components: an oil phase, an aqueous phase and a surfactant (Koh and Mohd. Ghazi, 2011).The binary compound could contain salts or alternative ingredients, but oil contains a posh mixture of various hydrocarbons and olefins (Koh and Mohd. Ghazi, 2011).Also, micro-emulsions with butanol, hexanol and octanol can meet the maximum viscosity limitation for diesel engines (Md. Abdullah et al., 2010) .

2.7.3 Pyrolysis

Pyrolysis is the method of conversion of substance into another by suggest of warmth or heat with the help of the catalyst within the absence of air or oxygen (Md. Abdullah et al., 2010).Vegetable oils and animal fats can be pyrolysis to produce biodiesel (Md. Abdullah et al., 2010).

2.7.4 Transesterification

Transesterification is the chemical reaction that involves triglycerides and alcohol in the presence of a catalyst to form esters and glycerol (Freedman et al., 1984; Van Gerpen et al., 2004). Transesterification involving three consecutive reversible reactions, they are the conversion of triglycerides to diglycerides, followed by the conversion of diglycerides to monoglycerides (Van Gerpen et al., 2004). There are several methods proposed for biodiesel production of which the transesterification of vegetable oil is the most preferable because of its renewability and sustainability (Koh and Mohd. Ghazi, 2011).

2.8 Effect of reaction temperature and time on biodiesel

Yield of biodiesel increase with increasing reaction time (Meher et al., 2006). Biodiesel yield highly increase in first fifteen minutes of reaction, while after one hour of reaction biodiesel yield is constant (Freedman et al., 1984; Ma et al., 1999; Meher et al., 2006). Reaction temperature on biodiesel production is one of the important factor, above and below optimum level of temperature on biodiesel production affect yield (Ma et al., 1999). Reaction temperature was based on type of alcohol use for biodiesel production and optimum level reaction temperature of 60^oC,75^oC, 114^oC for methanol, ethanol and butanol respectively (Freedman et al., 1984).Other authors' reported optimum level of temperature for biodiesel production using methanol was at 60^oC ,while at 45^oC biodiesel yield was slightly lower (Ma et al., 1999; Meher et al., 2006).Also other authors' reported that higher reaction temperature can decrease biodiesel yield, because of high reaction temperature accelerates saponification of oil (J.M. et al., 2010; Shereena and Thangaraj, 2009).

2.9 Types of alcohol use for biodiesel production

Alcohol can be used for biodiesel production is with short chain, including methanol, ethanol, butanol and amylic alcohol (Freedman et al., 1984; Van Gerpen et al., 2004). Because of their low cost and high boiling properties methanol (CH3OH) and ethanol (C2H5OH) are most frequently use alcohols for biodiesel production (Dahiya, 2020). In addition, methanol is most well-liked than fermentation alcohol because of its higher reaction speed and excess methanol could also be recovered at an occasional cost (Shereena and Thangaraj, 2009). According to Meher *et al.* (2006), the production of biodiesel by using ethanol with alkali-catalyzed transesterification is more difficult than using methanol.

2.10 Effect of oil to alcohol ratio on biodiesel properties

Based on the stoichiometry of the transesterification reaction, at least three mols of alcohol are required for transesterification of one mol oil to three mols of ester and one mol of glycerol (Dahiya, 2020; Knothe et al., 1997; Van Gerpen et al., 2004). The optimum ratio of methanol to oil for biodiesel production is 6:1, below 6:1 methanol to oil ratio is incomplete reaction (Meher et al., 2006; Pianthong and Thaiyasuit, 2011). Above 6:1 methanol to oil ratio has no effect on biodiesel yield and biodiesel physicochemical properties, but excess alcohol will make difficult to separate biodiesel from glycerol (Pianthong and Thaiyasuit, 2011; Srivastava and Prasad, 2000; Tomasevic and Siler-Marinkovic, 2003).

2.11 Types of catalysts use for biodiesel production

The catalysts that are used for biodiesel production can be classified as: homogeneous (liquid phase), heterogeneous (solid phase) and enzymes (Aransiola *et al*., 2014; Guo and Fang, 2011).

2.11.1 Biodiesel production using homogenous catalyst

Homogeneous catalysts use for biodiesel production is either acid or base, and usually in liquid form (Aransiola et al., 2014). Homogeneous alkali catalysts can convert triglycerides to their corresponding biodiesel with high yield, less time and low cost (Guo and Fang, 2011),but separating catalyst from the product mixture for recycling is technically difficult (Aransiola et al., 2014; Guo and Fang, 2011). In addition, after reaction catalyst should be neutralized or removed with a large amount of hot water, which produce a large amount of industrial waste water (Guo and Fang, 2011).Also homogeneous acid-catalyzed reaction is about 4000 times slower than the homogeneous base-catalyzed reaction (Lotero et al., 2005).

2.11.2 Biodiesel production using heterogeneous catalyst

Solid acid and base catalysts are developed to overcome the limitation of homogeneous catalysts (Aransiola *et al*., 2014). Also solid base catalyst is the ease of separation from the reaction mixture using only simple filtration and easily generated leading to cheaper operations (Georgogianni et al., 2009).Heterogeneous acid catalysts have ability to catalyse both esterification and transesterification simultaneously with fewer environmental problems from low cost feedstock with free fatty acid content greater than 6% (Agarwal and Gupta, 2015; Zhang, et al., 2011).On other hand, heterogeneous acid catalyse is slow reaction rate, require higher temperature for reaction and higher alcohol to oil ratio that increase capital cost for biodiesel production (Di Serio et al., 2008). Also, a long reaction time makes the process impractical and uneconomical (Arbain and Salimon, 2011).

2.11.3 Enzyme-catalyzed biodiesel production

Lipases as enzyme-catalysts for biodiesel production are environmentally friendly approach to solve energy problems (Aransiola *et al*., 2014). However, there are different challenges using enzyme-catalysed for biodiesel production, such as feedstock pre-treatment, high-energy requirements, catalyst removal problems and waste water treatment (Aransiola *et al*., 2014).

2.12 Effect of catalyst concentration on biodiesel production

Type and amount of catalyst use for biodiesel production is very important on product quantity and quality (Agarwal and Gupta, 2015). Previously study on transesterification of rape seed oil with 0.7%KOH by weight oil, reported highest yield of 97.6% (J.M. et al., 2010). Also on other study reported concentration of NaOH in the range of 1.0–1.4%

(w/w) provided biodiesel yield of 90–98 weight% from Jatropha oil (Agarwal and Gupta, 2015). Other authors' also reported that the optimum amount of KOH as catalyst of reaction is 1.5% by weight of oil (Pianthong and Thaiyasuit, 2011). Using catalyst above optimum level reduce biodiesel yield by gels formation and make difficult to separate biodiesel from glycerol (Freedman et al., 1984).On other hand, some researchers have reported lower catalyst concentrations such as 0.5% (Parla et al., 2009) , 0.7% or 0.8% NaOH (Md. Abdullah et al., 2010).

2.13 Yield of biodiesel from the rubber seed oil

Biodiesel yield from rubber seed oil is different based on method used for biodiesel production and purification level of the oil (Van Gerpen *et al*., 2004). Some authors reported biodiesel yield of 90-96.8% from rubber seed oil (Ahmad *et al*., 2014a; Pianthong and Thaiyasuit, 2011). But other author reported 83.06% of biodiesel yield (Onoji *et al.*, 2017).

2.14 Physicochemical properties of biodiesel from the rubber seed oil

Physicochemical properties of biodiesel from the rubber seed oil study by different authors is given in table 2.

Table 2: Previously studies on physicochemical properties of biodiesel from the rubber seed oil

No	**Characteristics (Unit)**	**Authors'**			
		(Bello and Otu, 2015)	(Ahmad *et al*., 2014a, 2014b)	(Gimbun *et al*., 2012)	(A. Ramadhas *et al*., 2005)
1	Moisture content (Wt%)	0.000264	NA	NA	NA
2	Density(g/cm^3)	0.8773 @ 25^{o}C	0.887 @ 25^{o}C	NA	NA
3	Specific gravity	5.77	NA	0.87	0.87
4	PH	7.90	NA	NA	NA
5	Kinematic viscosity@40^{o}C (mm^2/s)	5.77	3.9	4.64	5.81
6	Acid value (Mg KOH/g	0.448	0.42	0.07	0.8
7	Iodine value(g I_2/100 g oil)	101.01	NA	NA	NA
8	Peroxide value (Meq/Kg)	29.00	NA	NA	NA
9	Saponification value (mg KOH/g oil)	193.5	NA	NA	NA
10	Ester content (m/m)	NA	96.6	NA	NA
11	Calorific value (MJ/kg)	40.3	39.70	39.37	36.5
12	Ash content (Wt%)	0.04	NA	NA	NA
13	Cetane numer	52.56	53	NA	NA
14	Pour point (°C)	NA	-2	NA	NA
15	Flash point (°C)	NA	NA	154.6	130

Where, NA is not available

Rubber tree seed oil is one of the potential and suitable feedstock for biodiesel production, but the resources are not utilized as a potential for biodiesel production. Rubber seed oil has high free fatty acid content, in order to that acid esterification is required to remove high free fatty acid content and other impurities. Homogeneous base catalyst transesterification method is the most common method for biodiesel production and during biodiesel production process alcohol to oil ratio, catalyst ratio, reaction temperature and reaction time affects the quality and quantity of biodiesel yield. However, many researchers have done research on rubber seed oil biodiesel production, but there is research gap on rubbers seed oil content determination, optimization of rubber seed oil extraction, determination of oil physicochemical properties, synthesis of biodiesel and evaluating it’s physicochemical properties with location.

3 Material and methods

3.1 Seed collection

The rubber tree seed was collected from Ethiopian chemical corporation rubber tree plantation development project plantation site, located in South western part of Ethiopia, Bench Maji Zone (Guraferda District). The District is located about 630 km Southwest of Addis Abba. The rubber tree development project plantation site is located at geographical location of 06^{o}56’42’’ North latitude and 035^{o} 22’04’’longitude and the elevation on plantation site is 991 to 1000 masl. Soil type of the site is sandy loam and soil PH is 4.5. The yearly average rain fall of the site is 1500 to 1800mm and daily average temperature of the site is 24^{o}C. The rubber seed was collected from rubber tree clone name called Mixed that are in three separate plantation compartments. Age of trees from which the seeds collected were nearly thirty years. For the present study, 20 kg of fully ripen rubber seed was collected from plantation stand. Map of the area in which rubber tree seed was collected is given in Fig 1.

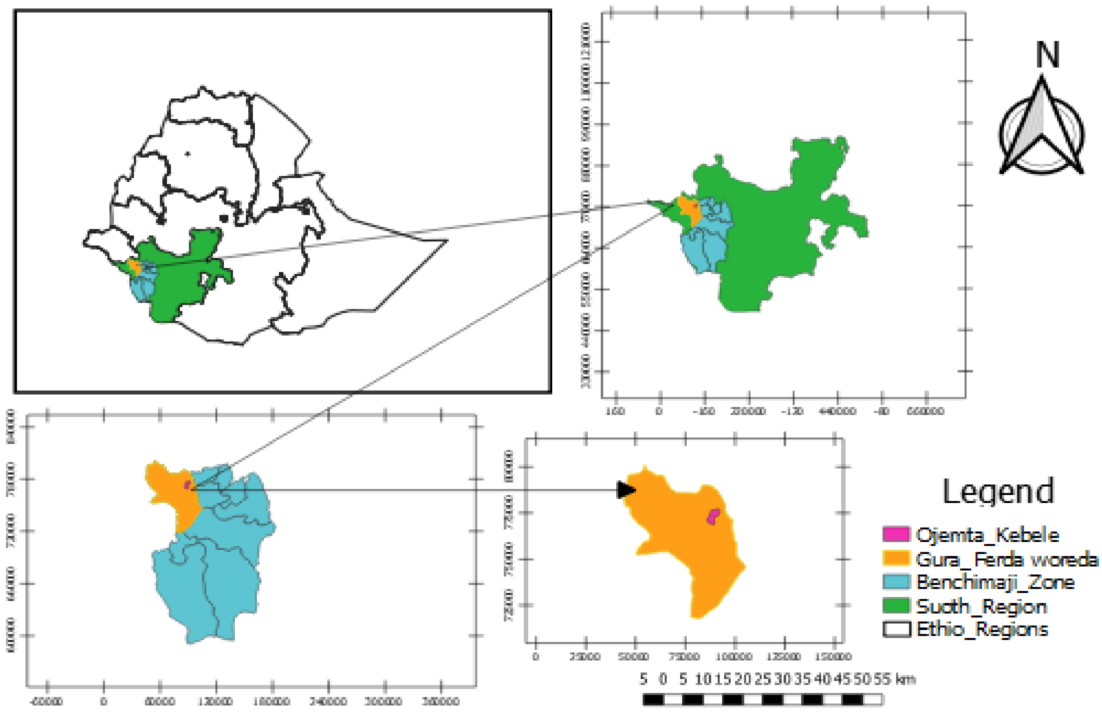


Figure 1: Map of the rubber tree seed collected area

Oil extraction from rubber tree seed, biodiesel synthesis and physicochemical properties analysis were done in the laboratory of Wondo Genet College of Forestry and Natural Resource. Fig 2 showed stepwise procedures from seed collection until physicochemical analysis of biodiesel.

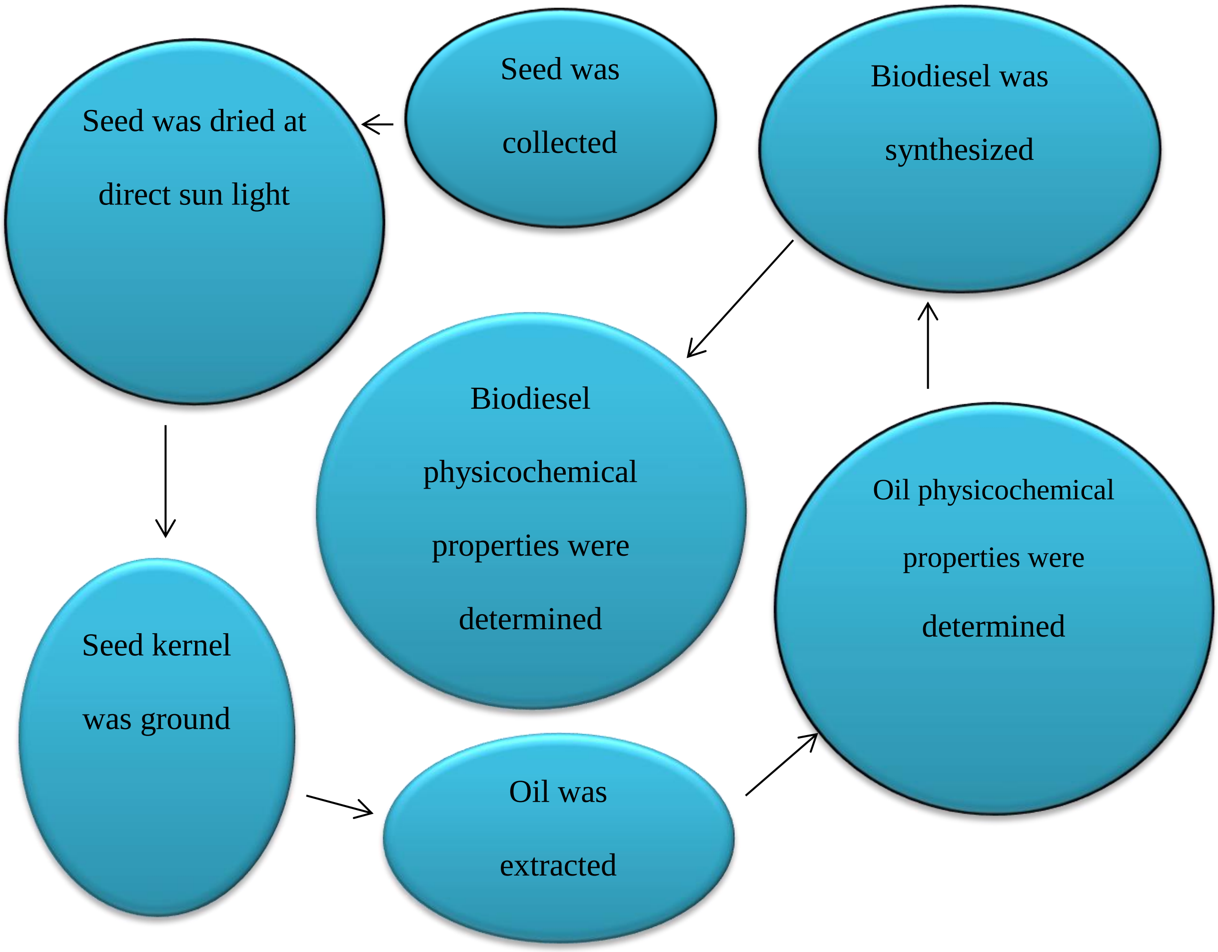


Figure 2: The general schematic representation of work

3.2 Seed moisture content determination

Immediately after the seed was collected from the field, fresh sample seed weight of 15.4gm was measured on sensitive balance and dried under the oven at 105°C for 24 hours. Then the sample dry weight was measured after cooling for 24 hours. Moisture content of fresh seed was determined using equation (3.1). Moisture content of fresh seed was determined with three times replication.

$$M.C\ (\%) = \frac{Fresh\ weight\ (g) - dry\ weight\ (g)}{Fresh\ weight\ \ (g)}\ x\ 100 \text{ equation ------- (3.1)}$$

3.3 Seed preparations for oil extraction

Seed was dried on direct sun light for three weeks and wet base moisture content of seed at extraction time was 13%.Seed shell was removed manually using the pestles and mortar. The ratio of seed kernel to seed and seed shell to seed were determined using equation 3.2 and 3.3 respectively. Moisture content of kernel was determined with sample weight of 10.66gm kernel dried in the oven at 105^{o}C for 5 hours. Moisture content of seed kernel was determined using equation (3.1) and during oil extraction seed kernel moisture content was 6.5%. The dried kernel was ground by coffee grinder and sieved with sieve size of 0.5mm to remove impurities and the kernel has equal particle size (≤ 0.5mm).

S.K (%)= $\frac{kernel\ weight(g)}{seed\ weight\ (g)} \times 100$ ----------equation (3.2)

S.S (%) = $\frac{Seed\ shell(g)}{seed\ weight\ (g)} \times 100$ -------------------------equation (3.3)

3.4 Oil extractions from seed kernel

Oil was extracted by solvent extraction method using soxhelt apparatus with water bath. Parameters selected for optimization of oil yield were solvent (n-hexane) to seed kernel powder weight ratio, extraction time and extraction temperature. The n-hexane to seed kernel powder ratio were (2.3:1-10.7:1ml/g), extraction temperature of (48-107^{o}C) atmospheric pressure (atm) for (4-9hours) continuous extractions. Optimizations for oil yield extractions were done by combination of three independent variables. The detail of the oil yield optimization method is given in Table 3.

Table 3: Parameters for rubber seed oil yield optimization

Factor	Name	Units	Type	Minimum	Maximum	Coded Low	Coded High	Mean	Std. Dev.
A	N-hexane to seed kernel powder ratio	ml/g	Numeric	2.30	10.70	-1 ↔ 4.00	+1 ↔ 9.00	6.50	2.12
B	Extraction temperature	Degree celsius	Numeric	48	107	-1 ↔ 60.00	+1 ↔ 95.00	77.50	14.84
C	Extraction time	Hour	Numeric	4	9	-1 ↔ 5.00	+1 ↔ 8.00	6.50	1.27

After each extraction treatment n-hexane was recovered by using fractional distillation method. Also oil was oven dried at least for thirty minutes at 70^oC to remove the remaining n-hexane from extracted oil. The oil was centrifuged at 400 rotations per minutes (rpm) for ten minutes to remove impurities from oil and pure oil was stored separately for each treatment in refrigerator at 4^oC. The percentage of oil yield was determined using equation (3.4) and the oil yield was analysed using Design-expert software trail version 12 of Response surface methodology of central composite design (CCD) and oil yield was predicted by multiple linear regression model.

RSO yield (wt %) = $\frac{Weight\ of\ extracted\ oil\ (g)}{Weight\ of\ rubber\ seed\ powder\ used\ (g)} \times 100$ --equation (3.4).

3.5 Determination of oil physicochemical properties

The physicochemical properties analysis of the oil and synthesis of biodiesel were done for the highest oil yield (weight %). Physicochemical properties of oil were determined with three times replication for each characteristic. Standardized methods used to determine the oil physicochemical properties is given in Table 4.

Table 4: Standardized methods for determination of oil physicochemical properties

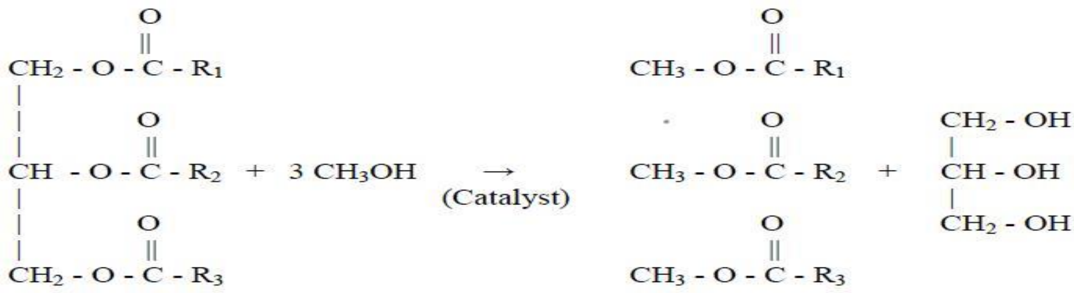
No	Properties	Methods	References
1	Density	Pycnometer	(Association of Official Analytical Chemists and Helrich, 1990; Firestone, 1994)
2	Acid value	Titration method	
3	Free fatty acid	calculated	
4	Peroxide value	Titration method	("AOAC 965.33 Peroxide Value of Oils and Fats.pdf | Chemical Substances | Food Ingredients," n.d.)
5	Saponification value	Titration method	(Association of Official Analytical Chemists and Horwitz, 2000)
6	Iodine value	Wijs method	(Firestone, 1994)
7	PH value	Digital PH meter	
8	Molecular mass of oil	GC-MS (calculated from biodiesel fatty acid composition)	

3.6 Synthesis of biodiesel from the rubber seed oil

Biodiesel was synthesised by two step acid- base catalysed process. The first step was esterification of oil with acid (sulphuric acid) to minimize free fatty acid of oil that makes soap during alkaline transesterification. On first step 200g of rubber seed oil was esterified with 6:1 molar weight of methanol to oil (42gm of methanol was used to esterify 200gm oil) based on 914.65 g/mol of molecular mass of rubber seed oil, with 5% v/v sulphuric acid dilution of 1.5% by weight of oil (3gm of H_2SO_4 dilution), at 60^{o}C with magnetic string bar of 600rpm for 90 minutes (Pianthong and Thaiyasuit, 2011; A. Ramadhas et al., 2005).Biodiesel from the purified oil was synthesised by transesterification method (Dahiya, 2020; Freedman et al., 1984; Van Gerpen et al., 2004).

$$RCOOR' + R''OH \overset{cat}{\Leftrightarrow} R'OH + RCOOR''$$

RCOOR′ indicates an ester, R″OH an alcohol, R′OH another alcohol (glycerol), RCOOR″ an ester mixture and cat is a catalyst.



A (Triglyceride) B (Alcohol) C (Methyl ester) D (Glycerol)

Where, R_1, R_2 and R_3 are long hydrocarbon chains, sometimes called fatty acid chains.

Figure 3: General transesterification chemical reaction for biodiesel production

In present study biodiesel was synthesized with 6:1molar weight of methanol to oil (44.02 gram of oil with 9.24gm of methanol), 1% of NaOH (0.44gm) by weight of oil as catalyst of reactions, at 60^{o}C with magnetic string bar of 600 rpm for 90 minutes. After completion of reaction, biodiesel was transferred to separating funnel and left for twenty four hours for proper sedimentation. Then the glycerol was drained from separating funnel. Biodiesel was washed with equal volumes of warm water (50^{o}C) until clear colour layer formed between biodiesel and water. Finally, biodiesel was separated from water using separating funnel and excess methanol from biodiesel was removed by fractional distillation. Glacial acetic acid was poured to biodiesel until PH of biodiesel became neutral. Biodiesel was synthesised with three times replication. Yield of biodiesel was calculated using equation (3.5).

Yield of biodiesel (weight %) = $\frac{Weight\ of\ \ biodiesel\ (g)}{Weight\ of\ refined\ oil\ used\ (g)}\ x100$ ---- equation (3.5)

3.7 Determination of fatty acid composition of biodiesel

Gas Chromatography analysis of biodiesel was performed with 7890B Gas Chromatography in JIJE Analytical Testing Service Laboratory. Sample analysis was carried out split mode of column DB-5MS, 30 m × 0.25 mm x 0.25µm. Samples were injected by a sampler injector volume of 1μL and inlet temperature of 250 °C and helium used as carrier gas with column flow of 1ml/min. Oven temperature of 100 °C was increased by 15 °C/min to 160 °C, by 3^{o}C/min to 185 °C for 20 min, at 5 °C/min to 220^{o}C, by 10 °C/min to 270 °C. The data were obtained using 5977B MS experimental conditions of ionization mode of EI, EMV mode of gain factor 1, transfer line temperature of 230 °C, quad temp of 150°C, solvent delay of 3 minutes and acquisition mode of scan 45–500 amu.

3.8 Determination of physicochemical properties of biodiesel

Physicochemical properties of biodiesel like density, specific gravity, acid value, free fatty acid value, iodine value, peroxide value and saponification value were determined using the same method used for determination of oil physicochemical properties (Table 4). Calorific value of the biodiesel was determined by adiabatic calorie metter and gravimetric method and ash content of biodiesel was determined using method of (Association of Official Analytical Chemists and Helrich, 1990; Firestone, 1994). Kinematic viscosity of biodiesel was determined using empirical formula.

3.8.1 Determination of kinematic viscosity of biodiesel

Kinematic viscosity of biodiesel at 40^{o}C was determined using empirical formula suggested (Huang *et al*., 2020).The formula was determined by relating saturated and unsaturated fatty acid composition of biodiesel.

Kv =$e^{A-Bt+Ct^2}$(3.98$_{X1}$ +3.73$_{X2}$ +7.54$_{X3}$+3.19$_{X4}$+3.02$_{X5}$) -------------------equation (3.6)

Where,

Kv = kinematic viscosity (mm^2. s^{-1})

A, B and C are the parameters whose values were estimated using the algorithm based on the biodiesel experimental viscosity data at different temperatures (0.98, 0.0312 and 1.18 × 10^{-4} were A, B and C respectively). X is the concentration of biodiesel (%) and number 1, 2, 3, 4, and 5 indicate stearic methyl ester, palmitic methyl ester, oleic methyl ester, linoleic methyl ester, and linolenic methyl ester, respectively and t is the temperature (°C).

3.9 Evaluation of biodiesel physicochemical properties with biodiesel standards

Biodiesel standards are different in country to country, but for this study American society for testing and materials (ASTM 6751) biodiesel standard was used to compare biodiesel fuel properties with specified standards.

3.10 Data analyses

Data were analyzed using Design-expert software trail version 12 at 95% confidence interval level and Microsoft excels software.

4 Results and discussion

The result and discussion section presents all the results of the finding, discuss and generalizes in comparison with the reported results in related investigations. This section begins with the physical properties of rubber seed and oil extracted from seed and discuss how rubber seed oil could be used for biodiesel production. The biodiesel produced and its physical and chemical properties are presented in the subsequent sections. The comparison of the findings are presented and discussed in comparison with American society for testing and materials biodiesel standard. Discussions were made based on the results. Additionally, the main findings in the present study were compared with the findings of other similar studies whether the results supported by or contradicting with and the possible reasons for their difference.

4.1 Physical properties of rubber seed

4.1.1 Moisture content of seed and seed kernel

Fully matured rubber tree seeds were collected from the rubber tree, packed and transported to the laboratory for physicochemical analysis. The findings on moisture content of the sample fresh rubber seed and seed kernel is given in Table 5. The finding on fresh seed moisture content was in line with (Widayat and Suherman, 2012),but finding was in contrast with (Gimbun *et al*., 2012; Onoji *et al*., 2019).

Table 5: Moisture content of fresh seed and seed kernel

Biomass	Fresh weight (gm)	Dry weight (gm)	Difference (gm)	Moisture content (%)
Seed	15.4	12.84	2.56	16.81
Seed kernel	10.66	10.01	0.65	6.09

4.1.2 Rubber seed physical composition

Rubber seed is physically composed of the kernel and shell. The result showed that, nearly half of the rubber seed constitutes the shell (Fig 4). The finding was in contrast with (Gimbun *et al*., 2012; Onoji *et al*., 2019).In present study rubber seed kernel composition was more than 11% of authors report.

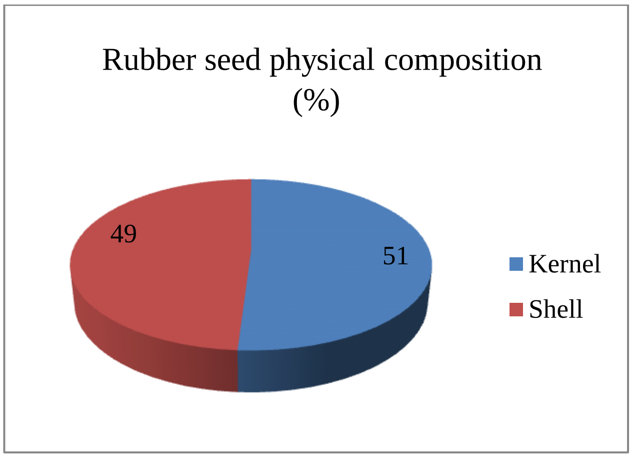


Figure 4: Physical composition of rubber seed

4.2 Rubber seed oil yield

The rubber seed oil yield were different for each of oil extraction treatment and individual independent variables showed effect on the dependent variable (oil yield).Both experimental and predicted result of the rubber seed oil yield is given in Table 6.

Table 6: Experimental and predicted oil yield from the rubber seed

Run order	Space Type	Factor 1	Factor 2	Factor 3	Responses	
		A:n-hexane to seed kernel powder ratio	**B:Extraction temperature**	**C:Extraction time**	**Experimental oil yield by seed powder weight**	**Predicted oil yield by seed powder weight**
		ml/gm	Degree celsius	Hour	(wt %)	(wt %)
1	Center	6.5:1	77.5	6.5	30	26.82
2	Factorial	9:1	95	5	55.69	41.78
3	Center	6.5:1	77.5	6.5	35	26.82
4	Axial	2.3:1	77.5	6.5	18.59	22.94
5	Factorial	4:1	95	5	47.46	37.16
6	Center	6.5:1	77.5	6.5	20.53	26.82
7	Center	6.5:1	77.5	6.5	23.94	26.82
8	Center	6.5:1	77.5	6.5	23.14	26.82
9	Factorial	4:1	95	8	50.3	42.48
10	Factorial	9:1	60	8	17.3	16.49
11	Axial	6.5:1	48	6.5	3.09	1.08
12	Axial	10.7:1	77.5	6.5	17.52	30.71
13	Factorial	9:1	95	8	61.3	47.10
14	Factorial	4:1	60	5	7.97	6.54
15	Factorial	9:1	60	5	17.88	11.16
16	Axial	6.5:1	107	6.5	33.13	52.57
17	Factorial	4:1	60	8	13.08	11.87
18	Axial	6.5:1	77.5	9	26.08	31.30
19	Axial	6.5:1	77.5	4	12.17	22.34
20	Center	6.5:1	77.5	6.5	22.3	26.82

The results indicated that, the highest oil yield of 61.3 wt% was obtained from extraction temperature of 95^{o}C, n-hexane to seed kernel powder ratio of 9:1ml/gm for eight hours continuous extraction. On the other hand, the oil yield was decreased to (33.13 wt%) and color of the oil was changed to darker at the highest extraction temperature of 107^{0}C, n-hexane to seed kernel powder ratio of 6.5:1ml/gm, for six- half hours continues extractions, because boiling of the solvent above optimum level could change physicochemical properties of the oil and solvent loss by evaporation. The lowest oil yield of (3.09 wt%) was obtained when the extraction temperature set at 48^{o}C, n-hexane to seed kernel powder ratio of 6.5:1ml/gm for six and half hours continuous extractions, because of boiling of the solvent below boiling point of the solvent can't boil to circulate in extraction unit to extract oil.

The rubber seed oil yield obtained in the present study was in line with the finding of (Ishak and Salimon, 2013; A. S. Ramadhas et al., 2005), but finding was in contrast with (Onoji et al., 2019).

4.3 Data assumptions to analysis of oil yield

Residual analysis is necessary to confirm that the assumptions for the analysis of variance (ANOVA) are met. Other diagnostic plots may provide information in some situations.

4.3.1 Data transformation

If the ratio of maximum data value to minimum data value greater than10, then data transformation is required. Data will be transformed to other form based on type of data characteristics and given lambda value of data. In the present study, the ratio of maximum datum value to minimum datum was 19.83, that indicated data to be transformed to other form to make data a theoretical distribution with finite mean and variance. In present study considering the lambda value of data and model type, data was transformed to square root form.

4.4 Statistical Analysis of factors that affect rubber seed oil yield

To determine the effect of each oil extraction factors on rubber seed oil yield, analysis of variance was used at 95% confidence interval and the response measured variable was oil yield. Effect of individual variables on oil yield is given in Table 7

Table 7: Analysis of variance (ANOVA) linear model for oil extraction

ANOVA for Response Surface Linear model						
Source	Sum of Squares	df	Mean Square	F-value	p-value	Remark
Model	34.68	3	11.56	16.55	< 0.0001	significant
A- n-hexane to seed kernel powder ratio	0.6798	1	0.6798	0.9731	0.3386	
B-Extraction temperature	32.82	1	32.82	46.97	< 0.0001	
C-Extraction time	1.18	1	1.18	1.69	0.2120	
Residual	11.18	16	0.6986			
Lack of Fit	9.79	11	0.8897	3.20	0.1048	not significant
Pure Error	1.39	5	0.2782			
Cor Total	45.85	19				

The model F-value of 16.55 implied that the model was significant. There is only a 0.01% chance that a "Model F-Value" large could occur due to error or disturbance. Probability value (p-value) less than 0.05 indicate model terms are significant and p-value greater than 0.05 indicates the model terms are not significant. In the present investigation, extraction temperature was significant model term (Table 7).

The "lack of fit F-value" of 3.20 implied that it was not significant relative to the pure error. There is a 10.48% chance that a "lack of fit F-value" large could occur due to noise. None significant lack of fit is good because if lack of fit is significant the model isn't fit for analysis (Table 7).

4.4.1 Model adequacy check

The model was tested for adequacy by analysis of variance. The regression model was found to be significant with correlation coefficient determination of R-Squared (R^2), adjusted R-Squared and predicted R-Squared having a value of 0.7562, 0.7105, and 0.5756 respectively.The qualities of the model developed are often evaluated from their coefficients of correlation. The value of R-squared for the developed model was 0.7562. It indicated that 75.62% of the overall variation within percentage oil yield of rubber seed is attributed to the experimental variables studied. The predicted R^2 of 0.5756 was reasonable in agreement with the adjusted R^2 of 0.7105; because the difference is less than 0.2. Adeq precision measures the signal to noise ratio. Adeq precision ratio greater than 4 is desirable. In the present study, adeq precision ratio of 13.9489 indicates the model was acceptable for analysis (Table 8).

Table 8: Fit statics table of response surface linear model for oil extraction

Std. Dev.	0.8358	R^2	0.7562
Mean	4.95	Adjusted R^2	0.7105
C.V. %	16.88	Predicted R^2	0.5756
		Adeq Precision	13.9489

4.4.2 Development of regression model equation

The coefficient estimated represents the expected change in response per unit change in factor value when all remaining factors are held constant. The intercept in an orthogonal design is that the overall average response of all the runs. The coefficients are adjustments around that average based on the factor settings. When the factors are orthogonal, the variance inflation factors (VIFs) are 1; VIFs greater than 1 indicate multi-collinearity, the higher the VIF the more severe the correlation of factors. The equation in terms of coded factors is often wont to make predictions about the response for given levels of each factor. The mathematical model that correlate rubber seed oil yield indicated in the present study showed that all independent variables has positive effect on the oil yield (Table 9).

The predicted model for a percentage of oil yields in terms of the coded factors is given in equation (4.1).

Square root of oil yield (wt%) = 4.95 +0.2231A +1.55 B+0.2941C +ϵ ----- equation (4.1)

Where,

A = N-hexane to seed kernel powder ratio

B = Extraction temperature at atmospheric pressure (^{o}C)

C = Extraction time (hour)

ϵ = Error term

Table 9: Regression coefficients of response surface linear model for oil extraction

Factor	Coefficient Estimate	df	Standard Error	95% CI Low	95% CI High	VIF
Intercept	4.95	1	0.1869	4.56	5.35	
A- n-hexane to seed kernel powder ratio	0.2231	1	0.2262	-0.2564	0.7026	1.0000
B-Extraction temperature	1.55	1	0.2262	1.07	2.03	1.0000
C-Extraction time	0.2941	1	0.2262	-0.1854	0.7735	1.0000

4.5 Physicochemical properties of the rubber seed oil

Rubber seed oil physicochemical properties were determined based on standardized methods of official oil analytical chemists. Physicochemical properties of the rubber seed oil is given in Table 10.

Table 10: Physicochemical properties of the rubber seed oil

No	Characteristics (unit)	Average experimental result	Other non-edible oil physicochemical properties	
			Jatoropha oil (Akbar *et al*., 2009)	Palm oil (Japir *et al*., 2017)
1	Colour	Golden yellowish	-	-
2	Density (g/cm^3)	0.91 at 28 °C	0.90 at 20°C	0.88 at 28°C
3	Acid value (Mg KOH/g)	6.68	-	≤ 10.95
4	Free fatty acid (Mg KOH/g)	3.35	2.23±0.02	≤ 5
5	Peroxide value (Meq/Kg)	7	1.93±0.012	-
6	Saponification value (Mg KOH/g)	215.51	193.55±0.61	200.6
7	Iodine value (g I_2/100 g)	113.02	103.62±0.07	50.4- 53.7
8	PH value	5	-	-
9	molecular mass of oil (g/mol)	914.65	872.07	847.78

4.5.1 Density of rubber seed oil

Density of the oil in the present study at 28°C was 0.91g/cm^3 and the finding was in line with (Bello and Otu, 2015).

4.5.2 Acid value of the rubber seed oil

Acid value of the rubber seed oil was 6.68MgKOH/g and the finding was in line with (Asuquo *et al*., 2012). The finding claimed that rubber seed oil requires small amount of acid catalyst to purify it and oil of high-quality feedstock to synthesis biodiesel.

4.5.3 Free fatty acid of the rubber seed oil (FFA)

Free fatty acid of oil is related to acid value of oil and the finding in the present study indicated that the rubber seed oil require prior esterification, if the alkaline catalyst is used to synthesis biodiesel. In the present study free fatty acid level of the rubber seed oil was 3.35 Mg KOH/g and free fatty acid level was reduced to 1 Mg KOH/g by acid esterification before biodiesel was synthesised.

4.5.4 Potential of hydrogen (PH) of the rubber seed oil

PH of the rubber seed oil of the present study was five; the finding indicated that the rubber seed oil had less acidity. The finding showed that present finding was slightly acidic than the previously study of (Onoji *et al*., 2019). The weak acidity of oil in the present study may vary with the rubber seed oil physicochemical properties from other locations.

4.5.5 Iodine value of the rubber seed oil

Iodine value of the oil was 113.02gI_2/100g and the finding was in line with (Aravind et al., 2015). Higher iodine value of the oil indicates that the rubber seed oil is mostly composed of unsaturated fatty acid and oil has better cold flow properties than oil which mostly composed of saturated fatty acid (Table 10).

4.5.6 Peroxide value of rubber seed oil

Peroxide value of the oil in present study was 7meq/Kg, the finding indicated that the rubber seed oil can take moderate time for the development of rancidity.

4.5.7 Saponification value of rubber seed oil

The saponification value of the oil in the present study was 215.51mgKOH/g oil and the result was slightly higher than of the report (Bashir *et al*., 2014). The finding showed that the oil from rubber seed can be used for soap and cosmetic industries.

4.5.8 Molecular mass of rubber seed oil

Molecular mass of the rubber seed oil in the present study was 914.65g/mol. The result was in contrast with (Pianthong and Thaiyasuit, 2011).

Physicochemical properties of the rubber seed oil was comparable with physicochemical properties of the jatoropha and palm oil, except iodine value of the rubber seed oil was higher than palm oil and molecular mass of the rubber seed oil was higher than molecular mass of the palm and jatoropha oil (Table 10).

4.6 Biodiesel of rubber seed oil

The average biodiesel yield in the present study was 81.55 wt% of refined oil. The result was in line with (Onoji *et al*., 2017). However, the finding was lower than (Ahmad et al., 2014a; Pianthong and Thaiyasuit, 2011). The lower biodiesel yield in the present study may be attributed to variation in oil purification level or due to variation in transesterification processes.

4.7 Fatty acid compositions of biodiesel from rubber seed oil

Fatty acid compositions of the biodiesel produced from the rubber seed oil was determined by GC-MS and the values are available in Table 11.

Table 11: Fatty acid compositions of the biodiesel from the rubber seed oil

No	Name	Formula	Common acronym	RT	Area	% Area
1	Methyl tetradecanoate	C15H30O	C14:0	10.88	67735	0.10
2	7-Hexadecenoic acid, methyl ester, (Z)-	$C17H32O_2$	-	15.48	50551	0.08
3	Methyl palmitoleate	$C17H32O_2$	C16:1	15.63	70026	0.11
4	Hexadecanoic acid, methyl ester	$C17H34O_2$	C16:0	16.44	5616928	8.55
5	Heptadecanoic acid, methyl ester	$C18H36O_2$	-	20.99	49494	0.08
6	Linoleic acid, methyl ester	$C19H34O_2$	C18:2	25.05	22240609	33.86
7	Linolenic acid, methyl ester	$C19H32O_2$	C18:3	25.46	17538955	26.70
8	9-Octadecenoic acid (Z)-, methyl ester	$C19H36O_2$	C18:1	25.63	14378051	21.89
9	13-Octadecenoic acid, methyl ester	$C19H36O_2$	-	25.94	661452	1.01
10	Methyl stearate	$C19H38O_2$	C18:0	27.67	4701338	7.16
11	cis-11-Eicosenoic acid, methyl ester	$C21H40O_2$	C20:1	39.02	115536	0.18
12	Eicosanoic acid, methyl ester	$C21H42O_2$	C20:0	40.03	195640	0.30

Based on the investigation, the biodiesel from rubber seed oil was mostly composed of Linoleic acid, methyl ester, Linolenic acid, methyl ester, 9-Octadecenoic acid (Z)-, methyl ester, Hexadecanoic acid, methyl ester and Methyl stearate fatty acid (Table 11). Unsaturated and saturated fatty acid composition of biodiesel is given in Fig 5.

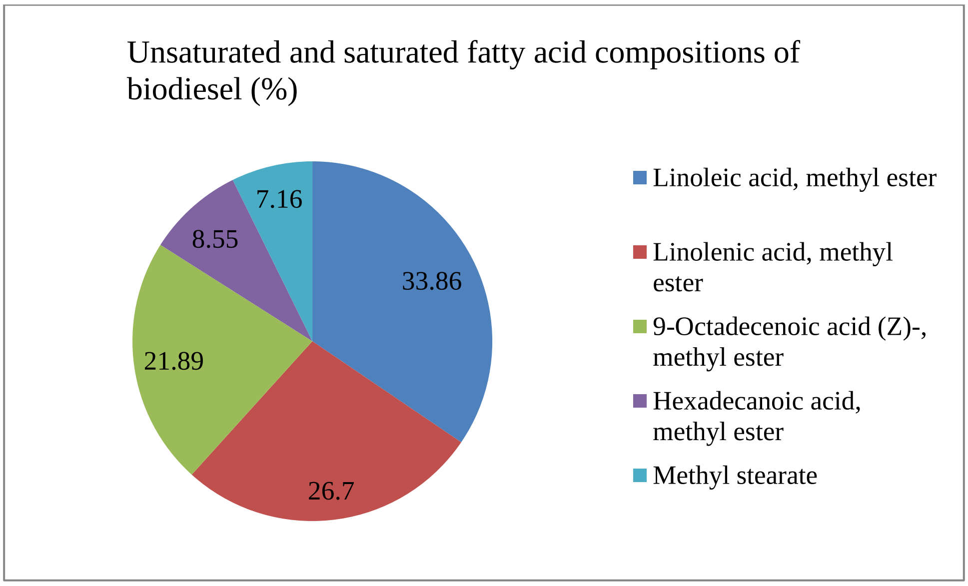


Figure 5: Unsaturated and saturated fatty acid compositions of biodiesel

Hexa decanoic acid, methyl ester, Eicosanoic acid, methyl ester and methyl stearate, have saturated fatty acid properties, while Linoleic acid, methyl ester, Linolenic acid, methyl ester and 9-Octadecenoic acid (Z)-, methyl ester have unsaturated fatty acid properties (Table 10). Nearly, 82.45% of biodiesel from rubber seed oil was composed of unsaturated fatty acid and 15.71% was composed of saturated fatty acid Fig 5.

The finding in the present study revealed that the fatty acid compositions of biodiesel was in line with (A. Ramadhas *et al*., 2005). The fatty acid compositions of biodiesel in the present investigation indicates that, biodiesel from rubber seed oil has better cold flow properties than biodiesel from higher saturated bio-oil, but less oxidation stability than biodiesel from higher saturated bio-oil.

4.8 Physicochemical properties of the biodiesel from rubber seed oil

After transesterification of the oil, the oil physicochemical properties were changed, that indicated the oil was effectively processed to biodiesel. Physicochemical properties of the biodiesel from rubber seed oil is given in Table 12.

Table 12: physicochemical properties of the biodiesel from rubber seed oil

No	Characteristics (unit)	Experimental result	ASTM 6751 (B100) min	max	Petroleum diesel (Demirbas, 2009)
1	Moisture content (%)	0.91	-	-	0.02-0.05
2	Density at 15^{o}C(g/cm^3)	0.86 at 27^{o}C	-	-	0.75-0.84 g/cm^3 @ 15^{o}C
3	PH	7.42	-	-	-
4	Kinematic viscosity@40^{o}C (mm^2/s)	2.81	1.9	6	1.9-4.1 mm^2/s @40^{o}C
5	Acid value (Mg KOH/g)	0.22	-	0.5	-
6	Iodine value (g I_2/100 g oil)	118.52	-	-	-
7	Peroxide value (Meq/Kg)	3.4	-	-	-
8	Saponification value (Mg KOH/g)	204.765	-	-	-
9	Calorific value (MJ/kg)	43.87	-	-	Higher heating value 46.48-45.62
10	Carbon residue (%)	0.998	-	0.01	0.008-0.01
11	Ester content (m/m)	204.54	-	-	-

Where, min= minimum, max = maximum, hyphen (-) not specified

4.8.1 Density of biodiesel

Density of the biodiesel at 27^{o}C was 0.86g/cm^3 and the finding was in line with (Ahmad *et al*., 2014b). Density of biodiesel from rubber seed oil was higher than density of petroleum diesel (Table 12).The finding indicated that blending of biodiesel with petroleum diesel is important to minimize biodiesel density to use in car engine.

4.8.2 Kinematic viscosity of biodiesel

The kinematic viscosity of biodiesel at 40^{o}C was 2.81mm^2/s. The kinematic viscosity of biodiesel in present study was in contrast with (Gimbun *et al*., 2012; A. Ramadhas *et al*., 2005). Kinematic viscosity of biodiesel in present study was lower than by 1.83-3mm^2/s of the authors report. The lower kinematic viscosity of biodiesel than the authors could be the variation in accuracy level of empirical formula used to predict kinematic viscosity of the biodiesel.

4.8.3 Acid value of biodiesel

In present study, acid value of the biodiesel was 0.22 Mg KOH/g and it was in line with (Ahmad et al., 2014a; Bello and Otu, 2015). Acid value of biodiesel in present study was acceptable range of ASTM 6751 biodiesel standard **(**Table 12).Lower acid value of the biodiesel in present study could be method of the oil esterification process, type and amount of catalyst used for esterification to minimize the free fatty acid value of the oil.

4.8.4 Peroxide value of biodiesel.

Peroxide value of biodiesel was 3.4Meq/kg. It indicates that the biodiesel from rubber seed oil can take moderate time for the development of rancidity.

4.8.5 Calorific value of biodiesel

Calorific value of the biodiesel in the present study was 43.87 MJ/kg. It was slightly higher than previous reports (Gimbun et al., 2012; A. Ramadhas et al., 2005). The finding on calorific value of biodiesel in the present study claimed that, calorific value of biodiesel from rubber seed oil is nearly related with calorific value of the petroleum diesel (Table 12).

4.8.6 Carbon residue

The carbon residue of biodiesel indicates the quantity of incombustible inorganic residue (mineral) resulting from complete combustion of biomass. In the present study the carbon residue of biodiesel from rubber seed oil was 0.998wt% (Table 12).Carbon residue of biodiesel in present study was not acceptable range of ASTM 6751 biodiesel standard. The high level carbon residue in present study could be biodiesel mix with glycerol.

4.9 Evaluation of the biodiesel physicochemical properties with biodiesel standards

Some of the fuel properties of biodiesel from rubber seed oil tested were comparable with ASTM6751 biodiesel standard as shown in Table 12.The comparison showed that the rubber seed oil biodiesel can be used as an alternative to diesel.

5 Summary and conclusion

Based on the findings from the of present study, nearly half of rubber seed consist with seed kernel and optimum factor level for rubber seed kernel oil extraction was 95^{o}C,with 9:1 n-hexane to seed kernel powder ratio for eight hours continuous extraction. Biodiesel yield obtained from rubber seed oil was 81.55% and nearly, 82.45% of biodiesel from rubber seed oil was composed of unsaturated fatty acid that provides better cold flow properties, but lower oxidative stability of biodiesel. Biodiesel from rubber seed oil is one of the important sources to solve petroleum diesel scarcity and global warming issues coming from burning of the petroleum diesel.

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APPENDICES

Appendix A: Experimental Result

Table A.1: Moisture content of the rubber seed

Bimass	Fresh weight (gm)	Dry weight (gm)	Difference (gm)	Moisture content (%)
Seed	15.4	12.51	2.89	18.76
	15.4	13.2	2.2	14.28
	15.4	12.72	2.68	17.4
Average				16.81

Table A.2: Rubber seed physical composition

Seed weight (gm)	Kernel weight (gm)	Percentage
52.80	27.18	51.47
52.80	27.31	51.32
52.80	26.81	50.77
Average		51.18

Table A.3: Rubber seed oil yield

Run	Replic ation	N-hexane volume(m1)	Seed kernel powder(gm)	Temperat ure (^{o}C)	Extraction time (h)	Oil extracted (gm)	Oil yield (wt%)
A	1	250	62.5	60	5	4.98	7.96
B	1	250	27.8	60	5	4.96	17.84
C	1	250	62.5	95	5	29.66	47.45
D	1	250	27.8	95	5	15.49	55.71
E	1	250	62.5	60	8	8.18	13.08
F	1	250	27.8	60	8	4.81	17.3
H	1	250	62.5	95	8	31.44	50.3
I	1	250	27.8	95	8	17.04	61.3
J	1	250	108.7	77.5	6.5	20.21	18.59
K	1	250	23.36	77.5	6.5	4.09	17.5
L	1	250	38.46	48	6.5	1.19	3.09
M	1	250	38.46	107	6.5	12.74	33.13
N	1	250	38.46	77.5	6.5	4.68	12.16
O	1	250	38.46	77.5	9	10.03	26.07
P1	1	250	38.46	77.5	6.5	9.46	24.5,
P2	1	250	38.46	77.5	6.5	9.21	23.94
P3	1	250	38.46	77.5	6.5	7.9	20.53
P4	1	250	38.46	77.5	6.5	13.5	35.1
P5	1	250	38.46	77.5	6.5	11.5	30
P6	1	250	38.46	77.5	6.5	8.9	23.14

Table A.4: Density of the rubber seed oil

Run	Temperature (^oC)	Volume of water (ml)	Volume of oil (ml)	Mass of water (gm)	Mass of oil (gm)	Density of oil (g/cm^3)
1	28	5ml	5ml	5.08	4.65	0.915
2	28	5ml	5ml	5.08	4.64	0.913
3	28	5ml	5ml	5.09	4.62	0.90
	Average					0.91

Table A.5: Acid value and free fatty acid of the rubber seed oil

Run	mass of oil (g)	Normality of KOH	Volume of KOH titrated (ml)	Acid value, (mg KOH/g)	Free fatty acid composition (mg KOH/g)
1	10	0.1	11.91	6.68	3.34
2	10	0.1	11.2	6.28	3.34
3	10	0.1	12.63	7.08	3.34
Average				6.68	3.34

Table A.6: Iodine value of the rubber seed oil

Run	mass of oil (g)	Normality of $Na_2S_2O_3$	Volume of $Na_2S_2O_3$ (ml) titrated	Blank value (ml)	Iodine value (gI_2/100g)
1	0.5	0.1	3.5	48.7	114.71
2	0.5	0.1	4	48.7	113.44
3	0.5	0.1	5	48.7	110.91
Average					113.02

Table A.7: Peroxide value of the rubber seed oil

Run	mass of oil (g)	Normality of $Na_2S_2O_3$	Volume of $Na_2S_2O_3$ (ml) titrated	Peroxide value (Meq/Kg)
1	5	0.1	3.5	7
2	5	0.1	3.4	6.8
3	5	0.1	3.6	7.2
Average				7

Table A.8: Saponification value of the rubber seed oil

Run	mass of oil (g)	Normality of HCI	Volume of HCI (ml) titrated	Blank value (ml)	Saponification value (mgKOH/g)
1	2	0.1	31.2	47	221.59
2	2	0.1	31.7	47	214.58
3	2	0.1	32	47	210.37
			Average		215.51

Table A .9: Biodiesel yield from the rubber seed oil

Run	Oil (g)	Methanol (g)	NaOH (g)	Biodiesel (g)	Biodiesel yield (wt%)
1	40	8.82	0.4	32.52	81.3
2	40	8.82	0.4	32.14	80.35
3	40	8.82	0.4	33.21	83.02
			Average		81.55

Table A. 10: Density of the biodiesel

Run	Temperature (^oC)	Volume of water (ml)	Volume of biodiesel (ml)	Mass of water (gm)	Mass of biodiesel (gm)	Density of oil (g/cm^3)
1	27	5ml	5ml	5.09	4.52	0.88
2	27	5ml	5ml	5.10	4.5	0.88
3	27	5ml	5ml	5.12	4.32	0.84
			Average			0.86

Table A.11: Acid value of the biodiesel

Run	mass of biodiesel (g)	Normality of KOH	Volume of KOH titrated (ml)	Acid value (mg KOH/g)	FFA composition
1	5	0.1	0.3	0.33	0.16
2	5	0.1	0.1	0.11	0.05
3	5	0.1	0.2	0.22	0.11
			Average	0.22	0.1

Table A.12: Peroxide value of the biodiesel

Run	mass of oil (g)	Normality of $Na_2S_2O_3$	Volume of $Na_2S_2O_3$ (ml) titrated	Peroxide value (Meq/Kg)
1	5	0.1	1.7	3.4
2	5	0.1	1.9	3.8
3	5	0.1	1.5	3.0
			Average	3.4

Table A.13: Iodine value of the biodiesel

Run	mass of biodiesel (g)	Normality of $Na_2S_2O_3$	Volume of $Na_2S_2O_3$ (ml) titrated	Blank value (ml)	Iodine value (gI_2/100g)
1	0.5	0.1	2.1	48.7	118.27
2	0.5	0.1	2.2	48.7	118.01
3	0.5	0.1	1.7	48.7	119.28
			Average		118.52

Table A.14: Saponification value of the biodiesel

Run	mass of oil (g)	Normality of HCI	Volume of HCI (ml) titrated	Blank value (ml)	Saponification value (mgKOH/g)
1	2	0.1	19.1	34	208.97
2	2	0.1	20.1	34	194.94
3	2	0.1	19.0	34	210.37
			Average		204.76

Appendix B: Calculations and Formulas

Acid value, mg KOH/ g of oil = $\frac{\text{ml alkali X N X 56.1}}{\text{Weight of sample}}$

Where, ml alkali= amount of KOH solution (ml) used, N=normality of KOH solution.

Iodine value = $\frac{[(B-S) X\ N X 12.69]}{\text{weight of sample}}$

Where, B= titration of blank (ml), S=titration of sample (ml), N= Normality of $Na_2S_2O_3$

Peroxide value (meq/Kg) = $\frac{\textbf{Titre X N X 100}}{\textbf{Weight of the sample}}$

Where,

Titre = ml of $Na_2S_2O_3$ used (blank corrected), N = Normality of $Na_2S_2O_3$ solution.

Saponification Value $\frac{(V_{blank} - V_{test})\ x\ normality\ of\ HCl\ x\ 56.1}{Weight\ of\ sample}$

Where,

B = Volume in ml of standard hydrochloric acid required for the blank.

S = Volume in ml of standard hydrochloric acid required for the sample

N = Normality of the standard hydrochloric acid and

W = Weight in gm of the oil taken for the test.

Appendix C: Photos During Laboratory Activities

(a) Hevea brasiliensis trees (b) seed drying

(c) Shelled seed (d) Ground seed kernel powder

Figure C-1: Rubber tree, seed and kernel powder

(a) Solvent method oil extraction (b) Fractional distillation to recovery n-hexane

(c) Oil from single extraction unit (d) Crude rubber seed oil

Figure C-2: Solvent method oil extractions procedure

(A) Oil transesterification (B) Biodiesel separation

(C) Washing with warm water (D) Biodiesel

Figure C-3: Process of biodiesel synthesis