Chemical and Enzymatic transesterification of rubber seed oil – A comparative study

Eldhose K Joy¹, Jilse Sebastian², Eldose K K³, Jithesh S R⁴

¹,³,⁴ Department of mechanical Engineering, Adi Shankara Institute of Engineering and Technology, Kalady.
² Department of mechanical Engineering, St. Joseph College of Engineering and technology, Pala.

Email:eldhosekj.me@adishankara.ac.in, jilsesebastian@gmail.com,eldhosekk.me@adishankara.ac.in, jithesh.me@adishankara.ac.in,

Abstract. Biodiesel produced from a wide variety of vegetable oils is expected to replace diesel fuel. Conventional diesel engines are the major sources of expelling the particulate matter, soot, smoke and oxides of nitrogen to the environment which are harmful. Biodiesel is an attractive alternative fuel to replace petroleum derived diesel because it is environment friendly and can be produced from edible or non-edible vegetable oils. The conflict between food and fuel led to the identification of more than fifty non-edible oil sources for biodiesel production. Utilization of locally available non-edible oil sources for production of biodiesel reduces the cost of fuel and increases production interest. Rubber seed oil is a non-edible type vegetable oil, identified as a potential feedstock for biodiesel production.

This paper comprises the synthesis of biodiesel from high free fatty acid rubber seed oil through transesterification by chemical and enzymatic methods. The transesterification of rubber seed oil with chemical catalyst and enzyme catalyst were investigated in the work. The variables affecting the reaction such as molar ratio, catalyst concentration, time and temperature were analysed. Kinetic study was carried out to find the reaction constants and activation energy of both chemical and enzymatic transesterification reactions. The properties of fuel such as kinematic viscosity, calorific value and specific gravity were found out and compared with the properties of diesel. Base catalyst concentration was varied from 0.5wt% to 2wt% for alkaline transesterification process and the result revealed that maximum yield of 90% was obtained with a base catalyst concentration of 1.5wt% with 1:9 molar ratio at 55± 3 °C. The best condition arise in enzymatic reaction was 10wt% enzyme and 1:4 methanol ratio with 5wt% addition of water as the solvent.

1. Introduction

Energy security is the constant availability and supply of affordable energy for consumers and industry. Risks to energy security include disruptions to the supply of imported fossil fuels, limited availability of fuel, and energy price hikes. Besides these, the greenhouse gas emissions get along with petroleum fuels creates many environmental problems. These issues look forward for a better replacement of fossil fuels. Many countries have already implemented deriving biofuels from locally available sources and using them as alternatives to petroleum products. Vegetable oils are promising alternatives to diesel since their properties are close to diesel. Since edible oils are used as food, non-edible oil sources (more than fifty are identified) are preferred for the application. In India, there are a variety of non-edible oils that can be potential sources for biodiesel production [1].
The high demand for diesel in the industrialized world and pollution problems caused by its wide use make it necessary to develop safe renewable energy sources with a smaller environmental impact than the conventional fuel sources. Biologically derived fuels are renewable, sustainable and expandable to meet the growing demand. They have the potential to be domestically and globally available for energy security [2]. Biodiesel is free from sulphur and its combustion will reduce the number of serious pollutants. Thus, the need for development of biodiesel as an alternative fuel for the diesel engines could reduce the harmful emissions without compromising on the performance. A major economic challenge for the biodiesel industry is the high cost of refined vegetable oils which constitutes between 70 and 85% of the overall production cost. In order to reduce the cost of biodiesel and the social impact, alternative feedstock that are readily available in large quantities and at low cost must be considered. One of these ways is to use non-edible vegetable oils for biodiesel production. An important technological challenge in biodiesel production is the development of heterogeneous catalysts and enzyme catalysts. The use of heterogeneous catalysts would result in simpler and cheaper separation processes, a reduced water effluent load, and reduced capital and energy costs. Enzyme catalysis proceeds without the generation of by-products, with easy recovery of biodiesel product, mild reaction conditions, and is insensitive to FFAs. The catalyst can be reused, thus contributing to reduced energy consumption and waste generation compared to the conventional base-catalyzed chemical process. Rubber seed oil is a promising non-edible oil being explored as an alternative fuel resource for diesel engine. The large availability of rubber seed from the existing rubber plantations in India and South East Asia region can potentially reduce the price of biodiesel production [3].

The high viscosity and free fatty acid of rubber seed oil are incompatible to use in diesel engines. The commonly employed methods, to reduce the viscosity of the vegetable oils are blending with diesel, emulsification, pyrolysis, cracking and transesterification. Among this transesterification is the most common method for biodiesel production [4]. High acid value feedstock’s follows acid pretreatment and alkaline esterification process [5]. A two-step transesterification process is developed to convert the high FFA oils to its mono-esters. The first step, acid catalyzed esterification reduces the FFA content of the oil from 17% to less than 2%. The factors affecting the process, the conversion efficiency and FFA reduction were studied. The maximum conversion efficiency reported at 0.5wt% acid catalyst and 9:1 molar ratio with temperature range of 45±5 ° C. Alkaline transesterification process was carried out at 1:6 molar ratio giving a maximum yield 98% with 0.5wt% NaOH as base catalyst. The oil used in this study was the filtered rubber seed oil of 17% FFA [6].

Rubber seed oil has high FFA content of 45% was investigated by Junaid Ahammed et al. [7]. A methanol ratio of 50%v/v of rubber seed oil and 10%v/v of sulphuric acid was found as the optimum conditions for the reduction of acid value. The maximum conversion of methyl esters (biodiesel) was reported as 96.8% at 6:1 molar ratio, 1% catalyst concentration, 55 ° C reaction temperature and 67.5 minutes reaction time. Satyanarayana and Muraleedharan employed two step pretreatment processes for the production of methyl ester from rubber seed oil having acid value of 48 (24% FFA). The methanol ratio of 75%v/v of rubber seed oil with 1%v/v concentrated sulphuric acid in two steps with one hour reaction reduced an acid value to 1.72mgKOH/g. The base catalyst used in this study was KOH and a yield of 98% was reported after 40 minutes reaction time at 55±5 ° C. A two-step pretreatment process (acid esterification) was developed in this study for the reduction of acid value in an effective range [8]. The production of biodiesel using a biocatalyst eliminates the disadvantages of the alkali process by producing product of very high purity and less or no downstream processing [9]. Several literatures are available on the enzymatic biodiesel production by Candida Antarctica lipase [10-13]. A number of studies are reported on various enzymes and their reaction condition for biodiesel production. However, only few studies are related with rubber seed oil.

2. Characteristics of Rubber seed oil
Selection of vegetable oil used for biodiesel production is one major task to overcome the high cost of refined oils, problems related to food and fuel, treatment of feed stocks etc. Nowadays, researchers are looking into the locally available non edible sources for the fuel production in order to overcome the cost and other issues. In Kerala, rubber tree is a major plantation crop which accounts about 92% of India’s rubber production. Use of rubber seed oil for biodiesel production gives an added benefit to the farmers apart from the natural rubber latex as the main product.
Commercially available rubber seed oil has dark brown in colour [8], and high acid value. The fatty acid composition of rubber seed oil consists of 18.9% saturation comprising of palmitic and stearic acids and 80.5% unsaturation comprising mainly of oleic, linoleic and linolenic acids. Saturated fatty acid methyl esters increase the cloud point, cetane number and improve stability whereas more poly-unsaturation reduces the cloud point and cetane number and stability [6]. The fatty acid composition of rubber seed oil was analysed by GC MS technique in Care Keralam Ltd., Koratty. The molecular formula of rubber seed oil is determined as C_{18}H_{32}O_{32} and molecular weight is about 278. Table 2.1 shows the results of GCMS analysis

**Table 2.1. Fatty acid composition of rubber seed oil**

| Fatty acid       | Composition (%) |
|------------------|-----------------|
| Saturated        |                 |
| 1. Palmitic acid C_{16:0} | 10.2           |
| 2. Stearic acid C_{18:0}  | 8.7            |
| Unsaturated      |                 |
| 3. Oleic acid C_{18:1}   | 24.6           |
| 4. Linoleic acid C_{18:2} | 39.6           |
| 5. Linolenic acid C_{18:3} | 16.3           |
| Others           | 0.6             |

The GC MS detailed chromatogram of rubber seed oil was as shown in Fig. 2.1. The various properties of rubber seed oil was found out by different test methods. The major properties of RSO for the present work are compared with the previous studies and described in Table 2.2.

![Fig. 2.1. GC MS chromatogram of rubber seed oil (1) Linolenic acid (2) Palmitic acid (3) Linoleic acid (4) Oleic acid (5) Stearic acid and 1, 2-Cyclononadiene](image_url)
Table 2. Property comparison of rubber seed oil with previous studies

| Property                  | Ramadhas et al. [6] | Satyanarayana and Muraleedharan [8] | Present work |
|---------------------------|----------------------|-------------------------------------|--------------|
| Specific gravity          | 0.91                 | 0.883                               | 0.91         |
| Viscosity (mm²/s) at 40 °C| 66.2                 | 34.69                               | 59.10        |
| Flash point (°C)          | 198                  | 237                                 | 210          |
| Calorific Value (MJ/kg)   | 37.5                 | 38.44                               | 37.8         |
| Acid value (mgKOH/g)      | 34                   | 48                                  | 82           |

3. Experimental section

3.1. Materials and Equipment

The feed stock used in this work is rubber seed oil (RSO). The chemicals and other reagents used in the preparation of biodiesel are sulphuric acid, KOH, methanol (99.8% pure), toluene, ethanol, n-hexane, t butanol, de-ionised water for enzymatic method. The enzyme used in the present work is Candida Antarctica lipase B 10000 immobilized. The equipment and instruments used for the production process and analysis of biodiesel from rubber seed oil are Magnetic stirrer with hot plate, Incubated shaker, Centrifuge, Weighing balance, Micro pipette, Brookfield viscometer, Bomb calorimeter and Pensky Martin apparatus.

3.2. Acid value estimation of rubber seed oil

The primary part of biodiesel preparation using vegetable oils is determination of acid value or FFA content in the oil. It is very essential to estimate the base catalyst amount or to decide whether the feed stock requires any type of pre-treatment. The acid value of the collected rubber seed oil was found out by standard titration (AOCS) method [14]. 5 g of rubber seed oil was well mixed with 125 ml solution (50% isopropanol and 50% toluene) titrated against 0.1N KOH solution using Phenolphthalein as the colour indicator.

Acid value can be calculated using the equation

\[ AV = \frac{(A-B) \times 56.1 \times N}{W} \]  \hspace{1cm} (1)

Where, A= number of ml of KOH to neutralize sample
B= number of ml of KOH to neutralize blank
N= normality of KOH (0.1N in this case)
W= weight of RSO sample.

The acid value (eqn. 1) of rubber seed oil was found to be 82 (FFA of 41%) and the direct alkali transesterification will not convert the high FFA into methyl ester. Rubber seed oil was directly used in this work without any type of pre-treatment process because the enzymes are capable of transesterify any range of FFA and the comparison will be easier with chemical and enzymatic method.
3.3 Acid Esterification (Two step pretreatment process) of rubber seed oil

If the acid value of the oils or fats is very high, one-step esterification pretreatment is insufficient to reduce the FFA content effectively because of the large quantity of water produced during the reaction. The acid catalyst generally used for esterification reaction is Sulphuric acid (H2SO4). For effective alkaline esterification the acid value of the untreated feed stock oil is expected to be below 3%. The initial step was to reduce the fatty acid content of RSO. The acid catalysed esterification was considered in this work for the FFA reduction of the feed stock. Five sets of experiments were conducted with varying oil to alcohol molar ratio and acid catalyst concentration for the reduction of FFA content. In each run 200 ml of rubber seed oil was filled in the flask of 500 ml capacity and preheated to 50 °C. Pre-determined amount of methanol was then added to the oil and stirring was started. After a few minutes acid catalyst was added to the mixture and stirring continued. Different methanol to oil molar ratios of 0.20, 0.40, 0.50, 0.60, and 0.65 were used in the first step for a reaction duration of 1 h keeping temperature 45±5 °C throughout and the acid catalyst was 1% (v/v). The mixture was then poured into the separating funnel and kept for separation. Water and methanol mixture along with sulphuric acid content moved to top. There was a layer of residue settled at the bottom containing colouring pigments, gum forming agents, wax etc.

The second step was carried out with molar ratios 0.20, 0.35, 0.30, 0.20, and 0.20, respectively, keeping other parameters as same as in the first step. Among the results obtained, an overall molar value of 80% v/v methanol to oil and 2% v/v of H2SO4 are found to give the best results for 2 h reaction duration and at constant temperature 45±5 °C. A three-step reaction was also employed with lower methanol ratio, and it was observed that the increase in methanol ratio caused a significant reduction in acid value. The residue and methanol water mixture were separated after the reaction. The residue was observed as settled at the bottom and methanol water mixture as top layer for 20%, 40%, 60% molar ratios and both layers at the top for 50% and 65% molar ratios. Investigations were also carried out with varying acid catalyst concentration with optimum alcohol to oil molar ratio. It was found that there is no significant reduction of FFA value by increasing the acid catalyst concentration. Table 3.1 describes the experimental data obtained in acid esterification stage and the final FFA reduction. A favourable molar ratio of 80% and acid catalyst amount of 2% were observed with two step acid esterification.

| Set no. | No of reaction steps | Methanol ratio (% v/v) | Acid Qty H2SO4 (% v/v) | Reaction time (min) | Temperature (°C) | Titration End point value (ml) | % FFA |
|--------|----------------------|------------------------|------------------------|--------------------|-----------------|-------------------------------|-------|
| 1      | 1st step             | 20                     | 1                      | 60                 | 45±5            | 29                            | 16.29 |
|        | 2nd step             | 20                     | 1                      | 60                 | 45±5            | 15                            | 8.41  |
|        | 3rd step             | 20                     | 1                      | 60                 | 45±5            | 5                             | 2.8   |
| 2      | 1st step             | 40                     | 1                      | 60                 | 45±5            | 20                            | 11.22 |
|        | 2nd step             | 30                     | 1                      | 60                 | 45±5            | 9                             | 5.04  |
|        | 3rd step             | 20                     | 1                      | 60                 | 45±5            | 3                             | 1.68  |
| 3      | 1st step             | 50                     | 1                      | 60                 | 45±5            | 14                            | 7.85  |
|        | 2nd step             | 30                     | 1                      | 60                 | 45±5            | 3.65                          | 2.03  |
| 4      | 1st step             | 60                     | 1                      | 60                 | 45±5            | 16                            | 8.97  |
|        | 2nd step             | 20                     | 1                      | 60                 | 45±5            | 4                             | 2.44  |
| 5      | 1st step             | 65                     | 1                      | 60                 | 45±5            | 15                            | 8.41  |
|        | 2nd step             | 20                     | 1                      | 60                 | 45±5            | 4.5                           | 2.52  |
3.4 Alkaline transesterification of rubber seed oil
Alkaline transesterification was carried out after the two step pretreatment process. The base catalyst used was KOH (85% assay) and molar ratio of oil to ethanol was always kept as 1:9 in every experiment. The yield obtained was investigated by varying the base catalyst concentration from 0.5wt% to 2wt%. The results showed an increase in FAME yield with increase in catalyst concentration. Properly mixed solution of KOH and methanol of required quantity was added to 150 ml of treated and preheated oil (50 °C) along with stirring. Maximum yield of 90% was obtained with 45 ml methanol and 2.04 g KOH (1.5wt %) catalyst. The temperature was maintained at 55±3 °C throughout the experiment for 40 minutes reaction time. After the completion of the reaction, products were allowed to settle for 24 hours. Glycerin was removed when it was settled at the bottom. The top layer (biodiesel) was purified by washing 3-5 times with demineralised water at room temperature.

4. Enzymatic transesterification
Enzymes are biological catalysts which accelerate the rate of a chemical reaction without undergoing a permanent change in the structure. Enzymes have been used in many industrial applications like reported in the production of detergents, textiles, starch hydrolysis and, genetic engineering, rare sugars, leather, pulp and lipase-based reactions. Enzyme catalyzed transesterification reaction or hydrolysis is used in biofuel production, enzyme based esterification etc. The microbes that are suggested for biodiesel production include Aspergillusniger Candida cylindracea, Candida rugosa, Chromo-bacteriumviscosum, Pseudomonas cepacia, Pseudomonas fluorescens, Rhizopusoryzae etc. Among these Candida antarctica displayed high activity in methanolysis and ethanolysis.

4.1 Selection of enzyme
Most of the literatures recommended the use of Candida antarctica lipase B (Novozym 435) for the transesterification of vegetable oils like soybean, rapeseed, sunflower etc. Most of the literatures recommended the use of Candida antarctica lipase B (Novozym 435) for the transesterification of vegetable oils like soybean, rapeseed, sunflower etc. Vorgelegt Von [15] studied different lipase abilities to transesterify the rubber seed oil with dimethyl carbonate and identified that Candida antarctica lipase B (Novozym 435) is the best member for rubber seed oil transesterification.

![Fig. 4.1. Lipase abilities to transesterify RSO with Dimethyl carbonate](image)

Based on the literature, the enzyme used in this work was selected as Candida antarctica lipase B 10000 immobilized and the enzyme sample supplied from Fermeta Biotec Ltd., Thane, India.
4.2 Experimental Methodology

Five sets of experiments were carried out to study the yield variation of biodiesel with different molar ratio, enzyme weight, solvent addition and time. A 10 ml of rubber seed oil was used in every experimental runs. The oil was poured into the clean and dried conical flask of 100 ml working volume. The enzyme was weighed according to the wt% of oil in each set and added to the rubber seed oil. Methanol was measured by micropipette and added to the oil in stepwise at equal time interval.

The reaction mixture with methanol, oil and enzyme were shaken in incubated shaker at 170-200 rpm and 37 °C. The methanol and solvents were added in three stages at equal time interval. The experimental runs for each set of enzymatic transesterifications of rubber seed oil with different conditions are shown in Table 4.2.

| Set No. | Oil Qty (ml) | Enzyme weight to oil (wt%) | Molar ratio (% v/v) | Solvent Addition (wt %) | Steps for addition | Time in hour | Temperature (°C) | Shaker Speed in rpm |
|---------|-------------|----------------------------|---------------------|-------------------------|-------------------|--------------|------------------|-------------------|
| I       | 10          | 10                         | 2                   | No                      | 3                 | 24           | 37               | 200               |
| II      | 10          | 5                          | 3                   | No                      | 3                 | 24           | 37               | 170               |
| III     | 10          | 5                          | 4                   | No                      | 3                 | 24           | 37               | 170               |
| IV      | 10          | 5                          | 5                   | No                      | 3                 | 24           | 37               | 170               |
| V       | 10          | 5                          | 4                   | No                      | 3                 | 24           | 37               | 170               |
| VI      | 10          | 5                          | 5                   | No                      | 3                 | 24           | 37               | 170               |

The conical flask was taken out from the shaker after the completion of reaction. Enzyme was separated out from the reaction mixture by filtration method. The remaining mixture was poured into centrifuge tube and separated by centrifuging the mixture at 5000 rpm for 15 minutes. Glycerol content settled at the bottom of the tube was separated by separating glass funnel. The separated mixture was heated to above 90 o C to remove unreacted methanol, water contents and any other volatile impurities. Viscosity of final mixture was measured by Brookfield viscometer. Enzyme after reaction was washed by distilled water and t butanol, dried and stored under the required temperature.
5. Results and discussion

5.1. Two-step acid pre-treatment of rubber seed oil

The reaction parameters of acid esterification have been optimized by performing five sets of experiments. Single step esterification reaction was not able to reduce the acid value. A best suited methanol ratio was obtained after two step pre-treatment process with 80% v/v methanol. There were a three-step reaction employed for lower methanol ratio, and the increase in methanol ratio caused a significant reduction in acid value.

5.1.1. Effect of molar ratio. The alcohol to oil molar ratio is an important parameter to determine the conversion efficiency and acid value reduction. The alcohol used in the present work is methanol which is a low-cost chemical. The amount of alcohol used in both acid esterification and alkaline transesterification plays a significant role in production cost of biodiesel. Experiments started from 20%v/v methanol and a better reduction in acid value was observed by increasing the percentage of oil-alcohol ratio. Use of excess alcohol was not given any significant reduction of acid value. The result obtained from pre-treatment process shows less oil-methanol ratio in comparison with the work done by Satyanarayana (Rubber seed oil having 24% FFA used 75% methanol in two step pre-treatment) [8].

![Fig. 5.1. FFA reduction with methanol ratio (After first step pre-treatment)](image)

A better acid value reduction (FFA of 2.03%) reached with total 2 % (v/v) acid catalyst in two step pre-treatment. The residue and methanol water mixture were separated after the reaction was observed on bottom and top, respectively at 20%, 40%, 60% and both were at the top in 50% and 65% molar ratios.

5.1.2. Effect of acid catalyst. Experiments were conducted with varying acid catalyst amount from 1% to 4% with favourable methanol ratio. Table 3.1 shows that the maximum conversion efficiency is obtained at 2 % (v/v) H$_2$SO$_4$. Junaid Ahammed et al. [7] conducted experiments with 10wt% H$_2$SO$_4$ in which 45% FFA rubber seed oil was reduced to 0.82% in single step acid treatment. A better acid value reduction (Fig. 6.2) of 4.06 (FFA of 2.03%) was obtained in the present work with total 2 % (v/v) acid catalyst in two step pre-treatments.
5.1.3. Conversion efficiency of treated oil. The maximum conversion efficiency was obtained at 1:12 molar ratio. The conversion efficiency with various methanol ratios and acid catalyst amount are plotted as shown in Figs. 6.3 and 6.4. The yield/conversion efficiency is calculated as:

\[
\text{Treated oil conversion efficiency} = \frac{\text{Amount of treated oil collected}}{\text{Amount of rubber seed oil taken}} \times 100
\]  

The reaction time in the investigation was 30 and 60 minutes, and observed that the latter was sufficient for completing the reaction in each step of acid esterification. Reaction temperature was always maintained at 45±5 °C. The increase in temperature above 55 °C led the product to become dark in colour and not suitable for further treatment. The mild temperature condition is observed as advantage to reduce the heat input for the reaction.
5.1.4. **Comparison of acid esterification with previous studies.** Comparison of variables (methanol ratio, acid catalyst amount, reaction time and reaction temperature) in acid esterification reaction with similar works available in literature [9, 18] is given in Table 5.1. The results show a lower acid catalyst amount and lower methanol ratio as compared with the previous studies in two step acid pre-treatment of high acid value rubber seed oil. The results obtained from the present study show the use of comparatively lower methanol ratio and significant reduction in acid catalyst amount.

| Variable                      | Satyanarayana and Muraleedharan (2009) | Junaid Ahmed et al. (2012) | Present study |
|-------------------------------|----------------------------------------|---------------------------|--------------|
| FFA value                     | 24%                                    | 42%                       | 41%          |
| Treatment steps               | Two steps                              | Single step               | Two steps    |
| Methanol ratio                | 75%                                    | 50%                       | 80%          |
| Acid catalyst amount          | 1%v/v                                  | 10wt%                     | 2%v/v        |
| Reaction time                 | 1 h                                    | 1.5 h                     | 2 h          |
| Optimum acid value            | < 2 mgKOH/g                            | 0.820 mgKOH/g             | 2.03 mgKOH/g |
| reduction                     |                                        |                           |              |
| Reaction temperature          | 63±2 °C                                | 45 °C                     | 45±5 °C      |

5.2. **Alkaline transesterification**  
The experiments were conducted to verify the base catalyst amount for maximum yield of biodiesel along with temperature and reaction time. In this study it was found that 55±3 °C was sufficient for completion of the reaction and the same temperature was maintained until the reaction was completed. The lower temperature increases the reaction time and higher temperature reduces the FAME yield. It has been observed that 40 minutes reaction duration was sufficient for alkaline transesterification of rubber seed oil. Results obtained from the reactions show that increase in reaction duration will not give high biodiesel yield.

5.2.1. **Effect of base catalyst.** Base catalyst concentration was varied from 0.5wt% to 2wt% in the present study and the result revealed that maximum yield of 90% was obtained with a base catalyst concentration of 1.5wt% (refer Fig. 6.5). Emulsion was observed with lower catalyst concentrations (0.5 - 1wt %) due to insufficient catalyst amount to completely neutralize the entire FFA resulting in saponification reaction. The soap content in the biodiesel will form emulsion during water washing. The water reacted with soap and unreacted base catalyst form the emulsion. An excellent medium to break the emulsion is NaCl solution. Sodium chloride solution of 50% by volume was added to the emulsion and heated to 60 oC and stirred. It has been observed that the biodiesel was separated from the emulsion solution and coming out at the top.

**Fig. 5.5. Effect of catalyst concentration on conversion efficiency**
5.2.2. GC MS Analysis of RSO methyl ester. The GC MS analysis of the rubber seed oil methyl ester sample was done by using Flame Ionization Detector (FID) method. The detailed chromatogram of the analysis is shown in Fig. 5.6 The results show the content in the sample are all methyl esters of corresponding fatty acids of rubber seed oil. The complete ester conversion was achieved in chemical method. The maximum methyl ester conversion of Oleic acid and Linoleic acid is observed from the chromatogram peaks.

![Chromatogram](image)

**Fig. 5.6.** GC MS results of rubber seed oil methyl ester by chemical method

5.3. Enzymatic transesterification
Rubber seed oil was transesterified by Candida antarctica lipase B 10000 immobilized and the results obtained from the various experimental conditions are discussed below. The effect of molar ratio, enzyme weight, and solvent addition is discussed.

5.3.1 Variation of Kinematic viscosity. Six set of experiments were carried out with different enzyme weight and methanol ratio. Stepwise addition of methanol used in reaction helps to decrease the inhibition rate of reaction. The kinematic viscosity of final mixture separated by centrifuging and separating funnel is measured by Brookfield viscometer. The ASTM standard for the Kinematic viscosity at 40°C of biodiesel is 6 cSt.

**Table 5.2.** Experimental details and kinematic viscosity variation of enzymatic transesterification

| Set No. | Oil Qty (ml) | Enzyme weight to oil (%) | Molar ratio (v/v) | Solvent Addition (wt%) | Steps for addition | Time in hour | Temperature (°C) | Shaker Speed in rpm | Kinematic viscosity cSt (30°C) |
|---------|--------------|--------------------------|------------------|------------------------|-------------------|--------------|------------------|---------------------|-------------------------------|
| I       | 10           | 10                       | 2                | No                     | 3                 | 24           | 37               | 200                 | 14.33                         |
|         | 10           | 5                        | 3                | No                     | 3                 | 24           | 37               | 170                 | 25.56                         |
|         | 10           | 5                        | 5                | No                     | 3                 | 24           | 37               | 170                 | 24.33                         |
|         | 10           | 8                        | 4                | No                     | 3                 | 24           | 37               | 170                 | 25.44                         |
| II      | 10           | 10                       | 4                | No                     | 3                 | 24           | 37               | 170                 | 14.78                         |
|         | 10           | 12                       | 4                | No                     | 3                 | 24           | 37               | 170                 | 17.11                         |
|         | 10           | 10                       | 6                | 5 (water)              | 3                 | 24           | 37               | 170                 | 12.89                         |
| III     | 10           | 10                       | 5                | 0.75 (t butanol)       | 3                 | 24           | 37               | 170                 | 15.33                         |
|         | 10           | 10                       | 5                | No                     | 3                 | 24           | 37               | 170                 | 16.00                         |
|         | 10           | 10                       | 2                | 5 (Water)              | 3                 | 48           | 37               | 170                 | 14.78                         |
|         | 10           | 10                       | 3                | 5 (Water)              | 3                 | 48           | 37               | 170                 | 11.22                         |
|         | 10           | 10                       | 2                | 10 (Water)             | 3                 | 48           | 37               | 170                 | 13.33                         |
|         | 10           | 10                       | 3                | 10 (Water)             | 3                 | 48           | 37               | 170                 | 15.22                         |
Kinematic viscosity value of the separated mixture reduced to 9.09 cSt after 48 h reaction with water as the solvent medium. Lower enzyme concentration was not sufficient for the viscosity reduction and 10wt% of enzyme shows a better condition of reaction. Table 5.2 gives the details of kinematic viscosity value after each set of experiment with different reaction condition.

5.3.2. Effect of methanol ratio. The methanol ratio of the reaction was varying from 2 to 6 with solvent free system and with solvent addition. The maximum viscosity reduction achieved is 10.89 cSt with solvent free system and 9.09 cSt with water as the solvent system.

![Product yield variation with methanol ratio with 5wt% enzyme](image)

**Fig. 5.7.** Product yield variation with methanol ratio with 5wt% enzyme

![Variation of Product yield with solvent medium and ethanol](image)

**Fig. 5.8.** Variation of Product yield with solvent medium and ethanol

The best solvent medium for enzyme transesterification is identified as water with methanol. The viscosity value obtained with t butanol mediated reaction is more than that of water-based reaction. The maximum yield of the final mixture obtained is 80% with methanol ratio of 3 in solvent free system and 85% with t butanol solvent system.

5.3.3. Effect of enzyme concentration. The variation of product yield and kinematic viscosity was analyzed with different enzyme concentrations. Enzyme weights were varying from 5 to 12 and a suitable wt% of enzyme is found as 10wt%. The maximum viscosity value was reduced to 9.09 cSt with 5wt% water and 10wt% enzyme. The kinematic viscosity value variation with different enzyme concentration is shown in Fig. 5.9
Fig. 5.9. Variation of Kinematic viscosity with enzyme weight
Maximum product yield was 80% with 5wt% of enzyme to the oil with the viscosity value of 19.71 cSt measured in that reaction. Figure 6.22 shows the product yield with different enzyme concentrations.

Fig. 5.10. Product yield variation with enzyme concentration without solvent

5.3.4. Effect of solvent addition. The addition of solvent to the reaction mixture will help the enzyme activity. The solvent prevents the inhibition rate by dissolving the alcohol and gives the flexibility to the enzyme.

Fig. 5.11. Kinematic viscosity variation with methanol ratio in water-based reaction
Water and t butanol are the solvents used in this work, the viscosity variation and product yield was measured. Water based reaction gives more viscosity reduction than t butanol. Water concentrations of 5wt% and 10wt% were analysed and the 5wt% water was found to be the optimum condition. Figure
6.23 shows the variation of kinematic viscosity at 5wt% water mediated reaction with different methanol ratio. The maximum viscosity reduction of 8.80 cSt was achieved at 5wt% water with 4 molar ratio of methanol to oil. The product yield with water based solvent reaction reached to 80% at 1:6 molar ratio. The excess alcohol ratio was not supporting to achieve a viscosity reduction during the reaction; it might inhibit the reaction rate.

The product yield variation with 5wt% water is shown in Fig. 5.12.

5.4 GC MS analysis of biodiesel by enzymatic method
GC MS analysis of rubber seed methyl ester by enzymatic method was done. The sample has lowest viscosity value obtained after the enzymatic transesterification with water as the solvent medium. The chromatogram of GC MS analysis is as shown in Fig. 5.13. Result shows the presence of Tetraoxatetradecan-1-ol and Isopropyl linoleate in the final mixture. The complete ester conversion was not taken place during enzymatic transesterification.

5.5 Separation of biodiesel in enzymatic method
The mixture of biodiesel and oil are obtained after enzymatic transesterification. The biodiesel is separated out from the mixture by 3/27 conversion process. It is a quantitative and qualitative test used in biodiesel industry. The test procedure is: 3 ml of sample is cooled to 20 °C and mixed with 27 ml methanol and allow settling. The basic chemistry behind this test is that biodiesel is fully dissolved in methanol, and oil and other unreacted compounds settle at the bottom of reaction funnel. The mixture after centrifuging is cooled to 20 °C and mixed with required amount of methanol and allowed to settle. Figure 6.25 shows the separation process, in which the oil and impurities are settled at the bottom separated by a separating funnel. The mixture of methanol and biodiesel are separated by the
distillation of methanol. The biodiesel collected after complete distillation is used to compare the properties with chemical method.

6. **Comparison of properties of rso methyl ester**

The various properties of rubber seed oil methyl ester produced by chemical and enzymatic transesterification were compared. Table 6.6 gives the property comparison of rubber seed methyl ester obtained from both the production method with ASTM biodiesel standards.

| Property                | Rubber seed oil | Biodiesel standard ASTM 6751 | Biodiesel (Chemical method) | Biodiesel (Enzymatic method) | Diesel |
|-------------------------|-----------------|-------------------------------|----------------------------|----------------------------|--------|
| Specific gravity        | 0.91            | 0.82-0.90                     | 0.87                       | 0.86                       | 0.835  |
| Kinematic viscosity at 40 °C (cSt) | 59.10          | 1.9-6.0                       | 4.78                       | 5.16                       | 4.5    |
| Calorific value (MJ/kg) | 37.8            | 35                            | 37.3                       | 36.2                       | 42     |
| Acid value (mgKOH/g)    | 82              | 0.5                           | 0.228                      | 0.32                       | < 0.5  |

7. **Conclusion**

In this study, a comparison of biodiesel production by chemical and enzymatic method has been carried out. High free fatty acid rubber seed oil was used in this study without any kind of treatment and filtration. Experimental investigation was carried out for chemical transesterification, kinetic study and enzyme-based biodiesel production. The acid value reduction of rubber seed oil with high FFA and methyl ester production were investigated and found that direct alkaline transesterification cannot be used for high FFA feed stocks. Two stage acid esterification process was required to reduce FFA from 41% to 2.03% with less amount of acid catalyst. An overall molar value of 80% v/v methanol to oil and 2% v/v of H₂SO₄ are found to give the best results for 2 h reaction duration and at constant temperature 45±5 °C. Base catalyst concentration was varied from 0.5wt% to 2wt% for alkaline transesterification process and the result revealed that maximum yield of 90% was obtained with a base catalyst concentration of 1.5wt% with 1:9 molar ratio at 55± 3 °C for 40 minutes. The enzyme-based transesterification did not give a complete conversion of methyl ester. The best condition arise in enzymatic reaction was 10wt% enzyme and 1:4 methanol ratio with 5wt% addition of water as the solvent. The kinematic viscosity was reduced to 10.87 cSt after 30 h reaction with 37 °C in an incubated shaker at 170 rpm. The calorific value of methyl ester produced by enzymatic method (36.2 MJ/kg) is found to be less compared with chemical method (37.3 MJ/kg) and the acid value and viscosity have higher values in the product from enzymatic method.

**References**

[1] Kaul S, Kumar A, Bhatnagar A K, Goyal H B and Gupta A K. Biodiesel: A clean and sustainable fuel for future. Scientific strategies biofuels. All India seminar on national policy on nonedible oils as biofuels. SUTRA, IISc Bangalore, India

[2] Linghong Zhang, Chunbao Xu, Pascale Champagne. Overview of recent advances in thermochemical conversion of biomass. Energy conservation and management,(2010) Vol-51: 969-982

[3] Wendy Pei Qin Ng, Mook Tzeng Lim, Sohibatul Muizzah bt Mohamad Izhar, Hon Loong Lam, Suzana Yusup. Overview on economics and technology development of rubber seed utilisation in Southeast Asia. Clean Techn Environ Policy (2014) 16:439–453. DOI10.1007/s10098-013-0667-6More references
[4] A.S. Ramadhas, S. Jayaraj, C. Muraleedharan, Biodiesel production from high FFA rubber seed oil, Fuel 84 (2005) 335–340.
[5] Amit Sarin. Biodiesel Production and Properties. ISBN: 978-1-84973-470-7
[6] A.S. Ramadhas, S. Jayaraj, C. Muraleedharan, Biodiesel production from high FFA rubber seed oil, Fuel 84 (2005) 335–340
[7] Junaid Ahmad, Suzana Yusup, Awais Bokhari, Ruzaimah Nik, Mohammad Kamil. Study of fuel properties of rubber seed oil based biodiesel. Energy Conversion and Management 78 (2014) 266–275
[8] Satyanarayana M, Muraleedharan C. Biodiesel Production from Vegetable Oils: A Comparative Optimization Study, Journal of Bio based Materials and Bioenergy Vol. 3, 1–7, 2009. doi:10.1016/j.energy.2010.09.050
[9] Hideki Fukuda, Akihiko Kond, Hide Noda. Biodiesel Fuel Production by Transesterification of Oils. Journal of Bioscience and Bioengineering. Vol. 92, No. 5, 405%416.2001
[10] Mukesh Kumar Modi, J.R.C. Reddy, B.V.S.K. Rao, R.B.N. Prasad. Lipase-mediated conversion of vegetable oils into biodiesel using ethyl acetate as acyl acceptor. Bioresource Technology 98 (2007) 1260–1264
[11] Nie K, Xie F, Wang F, Tan T., 2006. Lipase catalyzed methanolysis to produce biodiesel: Optimization of the biodiesel production. J. Mol. Catal. B: Enzym. 43, 142–147
[12] Royon D, Daz M, Ellenrieder G, Locatelli S., 2007. Enzymatic production of Biodiesel from cotton seed oil using t-butanol as a solvent. Bioresour. Technol. 98, 648–653.
[13] Nevena Ognjanovic, Dejan Bezbradic, Zorica Knezevic Jugovic. Enzymatic conversion of sunflower oil to biodiesel in a solvent-free system: Process optimization and the immobilized system stability. Bioresource Technology 100 (2009) 5146–5154.
[14] J. Van Gerpen, B. Shanks, and R. Pruszko, D. Clements, G. Knothe. Biodiesel Production Technology August 2002–January 2004. July 2004 NREL/SR-510-36244.
[15] Vorgelegt Von. Lipase catalysed transesterification of plant oils with Dialkyl carbonates. Master of Science. Tjahjono Herawan, 29 November 2004