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Enzymatic Synthesis of Castor Oil Hexyl Ester in Liquid Carbon dioxide Medium and its Potential Application as Biolubricant

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A novel green method for production of alkyls esters has been discussed in this paper. Liquid carbon dioxide (CO_2) can be used as reaction medium and having advantages of green solvent. Enzymatic esterification of castor oil has been carried at 1:3 stoichiometric amount of castor oil to alcohol in presence of immobilized lipase Lipozyme TL IM. The progress of the reaction has been monitored time to time and the maximum yield of 88.3% has been observed within 5 h for hexyl esters. In this process, liquid CO_2 has acted as solvent in the reaction system and has increased the mutual solubility between alcohol and castor oil. The produced hexyl esters have been evaluated for tribological properties.

Keywords: Immobilized lipase, Liquid CO_2, Hexyl esters, Transesterification, Biolubricants

1 Introduction

The enzymatic transesterification has attracted lot of attention in recent years, due to green processes such as enzyme catalysis in non aqueous media like sub and supercritical carbon dioxide. Supercritical fluids (SCF) have been considered as efficient alternative to organic solvents. SCF have shown to be excellent in solubilisation of the hydrophobic lipid substances, and in reversal of hydrolysis reactions in favour of synthesis[1]. Liquid CO_2 has been used as an excellent medium for enzymatic transesterification. The inhibition of enzyme has shown to be greater in n-hexane medium than in SC-CO_2 when the esterification of oleic acid has been carried out in ethanol. Enzyme catalysts have been more environmentally benign than chemical catalysts. Among the various catalysts which have been used for transesterification, enzymatic alcoholysis has become more prominent. Enzymatic transesterification has become more appealing and cost-effective approach as the reaction has been operative at moderate conditions, easy availability of feedstock and smooth separation of glycerol and product[2]. Lipases have significant catalytic performance and stability in non-aqueous environments, according to Gog et al. (2012), and their specificity, regioselectivity, and enantioselectivity has been used advantageously for a range of organic synthesis applications[3]. Liquid CO_2 and SC-CO_2 have several advantages, including the ability to replace organic solvents, better diffusivity, and reduced viscosity, all of which improve reaction kinetics. The easily tunable solvation capacity of liquid CO_2 and SC-CO_2 has allowed for easy separation of substrates from products and catalyst, whereas product purification in conventional method has proved to be expensive and time-consuming, rendering it unsuitable for commercial usage. CO_2 has been easily removed from the product due to its high volatility, ensuring a solvent-free reaction[4-5], which is essential for the industrial manufacturing of alkyl esters as biolubricants. Methanol has long been used to make enzymatic methyl esters, although it inhibits enzyme activity when compared to other higher alcohols, resulting in reduced enzymatic performance[6]. India has been the major importer of vegetable oil after China to fetch the food security issues so, there have been restrictions on the use of edible oils such as soybean, rapeseed, sunflower, palm, and peanut[7]. Vegetable oil seeds such as Jatropha, Karanja and Castor being non-edible can be utilized to make alkyl esters in developing countries

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*Corresponding author (E-mail: subhalaxmipradhan@gmail.com) like India[8]. India has been the world's leading producer of castor seed following China and Brazil and a largest exporter, with production of about 2,339,000 MT in 2011[9]. India has accounted for around 80% of worldwide production of castor but just 15% of it has been used for domestic consumption, so there is a lot of potential for using castor oil as a feed for making alkyl esters. Alkyl esters having large number of C-atoms have been valuable compounds found in foods, cosmetics, metalworking fluids, rolling oils, industrial oil, and lubricants. Over the last decade, the requirement of long-chain fatty acid esters has steadily increased[4]. According to Sharma et al. (2006), chemical alteration of soybean oil's fatty chains has improved lubricity as well as physical features like pour point and cloud point [10]. The hydroxyl and ester groups of the lubricant provide active oxygen sites that bind to the metal surface when it has been greased. Dalai et al. (2006) has investigated the performance of alkyl esters and found that an ester made from canola oil and a mixture of methanol and ethanol performed as an effective bioadditive, lowering scar diameter by 16 % and has increased the lubricating properties of base fuel by 20 % [11]. Alkyl esters of Castor and Lesquerella oil have increased the lubricating property of low-sulfur petro-diesels at lower bending levels than non-hydroxylated vegetable oil esters [12-13]. Hincapie et al. (2011) suggested that Castor oil methyl esters have an extraordinarily high viscosity compared to typical methyl esters, therefore they can be employed as an bioadditive in current internal combustion (IC) engines[14]. However, as compared to other vegetable oils, castor contains ricinoleic acid being a unsaturated hydroxyl fatty acid, has boosted lubricity, making it a great contender as a diesel fuel additive. Sulphur level of petro diesel must be less than 0.005 percent, according to Bharat IV norms, due to severe government regulations. Low-sulfur diesel fuel has a lower lubricating capacity which causes damage to the fuel injection system and other engine components. Blending of lower amounts of alkyl esters, especially from hydroxylated vegetable oils like castor oil in petro-diesel have preserved engine lubricity[7]. Transesterification is the alcoholysis of triglycerides in the existence of a catalyst to synthesize fatty alkyl esters and glycerol. It is a process analogous to hydrolysis, involves removal of alcohol from one triglyceride ester by another alcohol.

The current study has focused on the enzymatic synthesis of castor oil hexyl ester (COHE) in a liquid CO_2 (liquid vapour equilibrium) medium and optimization of the reaction conditions tuned to obtain the best yield of COHE. The kinetics of the reaction and the efficacy of the enzyme for high FFA oil are examined. Lubricity study has conducted at various blending levels with Low lubricating diesel fuel (LLDF) to investigate biolubricant potential of COHE.

2 Material and Methods

2.1 Reagents and materials

Oil was extracted from castor seeds collected from Gujarat, western part of India. Reagents like analytical grade methanol, (P99%) were obtained from Merck and utilised without purification. Lipase (immobilized) from *Thermomyces lanuginosus (TL IM)* were obtained as generous gift from Novozyme A/S (Bagsvaerd, Denmark). Carbon dioxide (99.98% purity) was procured from Laser gases, New Delhi, India. Standard methyl esters were obtained from Sigma Aldrich for comparative analysis and quantification of reaction product.

2.2 Extraction of castor oil and physiochemical characterization

The oil was extracted from the grinded seeds by using methanol as extraction solvent in Soxhlet apparatus. After extraction methanol was evaporated by rotavapour; the remaining oil was weighed after cooling. The properties such as density, viscosity, acid value, iodine value, saponification value and unsaponifiable matter of the oil were measured following standard protocol. Transmethylating agent was used to convert the oil to fatty acid methyl esters. Fatty acid content of the oil was measured using GC (Nucon5765) having flame ionisation detector (Nucon Engineers, Delhi, India) and a capillary column BPX-70, 60 m x 0.25 mm x 0.25 µm (Nucon Engineers, Delhi, India) (SGE, India). Column temperature was set to rise at a rate of 4°C/min from 180°C to 240°C and temperature of the detector and injector were fixed to 240°C and 230°C, respectively. Nitrogen (40 pressure) with a flow rate of 45.0 mL/min was utilised as the carrier gas, while air and hydrogen flow rates were 30 mL/min and 300 mL/min, respectively. Pure fatty acid methyl esters (FAMEs) of Sigma Aldrich (USA) make run in GC, and corresponding fatty acids of sample were identified by matching the retention periods of pure FAMEs. The peak regions were used to calculate the percentage fatty acid compositions.

2.3 Liquid CO_2 mediated enzymatic esterification

Enzyme catalyst was used to transesterify castor oil and hexanol in liquid CO_2 medium to produce castor oil hexyl ester (COHE). Fig. 1 shows the schematic unit of high pressure stainless steel vessel for liquid CO_2 [15]. The high-pressure section of the device was a stainless steel (German code 1.4571) having cylindrical autoclave (inner diameter 6.3 cm, outside diameter 8.0 cm, height 34.0 cm, volume 935 cm^3) with bottom and top closures. A sapphire glass was installed in the top closure to allow viewing of CO_2 condensation. For actual visual inspections, light from a tiny lamp was sent through the glass. The cooling finger and high-pressure tubes connecting a gauge and a valve were likewise incorporated within the top lid. The valve is used for loading and discharging of the CO_2.The content of CO_2 in the autoclave was around 180 g. The inner reaction vessel was a cylindrical glass container. The autoclave is heated with a water bath at 50°C for the refluxing of liquid CO_2. The cooling water at a temperature of about 10°C was circulated through the cooling finger for condensation of CO_2. Transesterification of castor oil (20 g) with methanol was carried out in a glass container in presence of Lipozyme TL IM. The product was analyzed by HPLC.

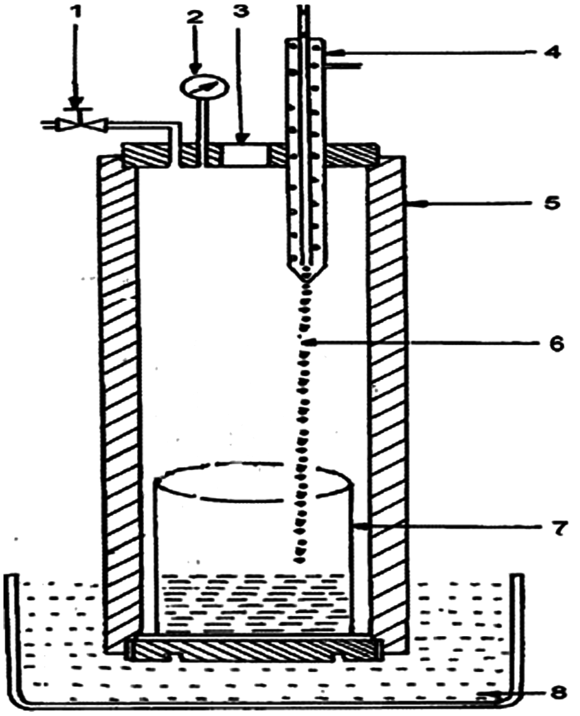


Fig. 1 — High Pressure liquid CO_2 apparatus, where (1) high pressure valve, (2) pressure gauge, (3) sapphire window, (4) cooling finger, (5) cylinder, (6) condensed CO_2, (7) glass vessel serving as reservoir, and (8) water bath.

Reactants, Castor oil (20g) and hexanol with Lipozyme TL IM was taken into high pressure autoclave, and the pressure of 65 bar CO_2 is applied from dip tube CO_2 cylinder. The reaction was conducted for 6 hours at the required temperature, with cooling water circulated via the autoclave's cooling figure. The CO_2 pressure was released via a valve after 6 hours of reaction time. The reaction mixture was removed and centrifuged at 4000 revolutions per minute. A vacuum rotary evaporator was used to extract the surplus alcohol in the result mixture, and the upper layer of hexyl esters was washed with hot distilled water and separated. The residual moisture was evaporated by drying the ester layer at 80 degrees Celsius in a rotavapour and then passing it over anhydrous Na_2SO_4. Several experiments were carried out for enzymatic esterification in liquid CO_2 medium, and reaction parameters were optimised.

2.4 Analysis of COHE by HPLC

High-pressure liquid chromatography (Waters 600 HPLC system) was used to analyse castor oil hexyl esters, with an RP C18 column (250 mm x 4 mm i.d.), Waters 2414 Refractive Index detector, Waters 600 HPLC quaternary pump, Waters inline degasser, and empower software. The flow rate was 1 mL/min and the column temperature was kept at 40°C. The carrier solvent was methanol, which was filtered and degassed using a Waters inline degasser. Before HPLC analysis, the transesterification products were diluted up to 20 times in HPLC grade methanol and filtered using a 2 m PTFE syringe filter.

2.5 Lubricity study of COHE

The lubricity of hexyl esters was investigated using a high frequency reciprocating rig (HFRR) as a neat fluid and additives/blends with low lubricity diesel fluid (model v 2.11-D1377; PCS, U.K.). Wear scar diameter (wsd), coefficient of friction (CoF), and film thickness are the three key characteristics that determine the lubricating value of any liquid fuel. HFRR was calibrated before the test using high lubricity reference fluid A and low lubricity reference fluid B as indicated by ASTM D 6079-4 procedure. The lubricating properties of COHE were studied alone and as an additive to poor lubricity diesel fuel after the instrument were calibrated. In an HFRR lubricity test, alkyl esters sample (2ml) was deposited in the test reservoir of an HFRR and kept at 60 ± 0.2°C according to the ASTM D 6079-4 procedure. Once the reservoir temperature was regulated, a vibrator arm with a horizontally non-rotating steel ball and a 200 g weight was lowered until it came into contact with a test disc totally submerged in the liquid[16]. The test ball oscillated at a constant frequency (50 Hz) and stroke length (10.02 mm). The testing ball was detached from the vibrator arm after 75 minutes and washed in a 50:50 mixture of iso-octane and 2-propanol to remove all metal particles from the steel ball. The scar on the ball's surface was observed using a microscope, image was captured and scar diameter is measured. The size of the spot was used to determine the liquid's lubricating properties. The HFRR test conditions are listed in Table 1.

Table 1 — Test conditions of HFRR

Fluid Volume	2 ± 0.2 ml
Stroke length	1 ± 0.02 mm
Frequency	50 ± 1 Hz
Fluid Temperature	60°C
Relative Humidity	>30%
Applied Load	200±1 g
Test duration	75± 0.1 min
Bath surface area	6±1 cm^2

Table 2 — Characteristics and fatty acid composition of castor oil

Properties	Castor Oil	Fatty acid	Amount (%)
Specific gravity at 20°C (g/cm^3)	0.961	Ricinoleic acid	87.0
Acid value (mg KOH/g oil)	0.91	Linoleic acid	5.0
Iodine value (g I_2/100 g oil)	89	Oleic acid	3.4
Saponification value (mg KOH/g oil)	185	Palmitic acid	1.3
Unsaponifiable matter (% w/w)	0.94	Stearic acid	1.5
Viscosity (cp) at 40 °C	241	Linolenic acid	0.6

3 Results and Discussion

3.1 Characterization of castor oil

The extraction study revealed that seed has 48.25 % oil content. Castor oil contained 85–90 % ricinoleic acid which is soluble in alcohols, so extraction using methyl alcohol as solvent yielded higher amount of oil in comparison to hexane as extracting solvent. Table 1 shows the physicochemical parameters of the oil. Table 2 displays the fatty acid content of the oil and the oil contains 0.45 % FFA (free fatty acid). Figures 2 and 3 showed gas chromatograms and HPLC chromatograms of castor oil alkyl ester respectively. The primary components of the oil were $C_{18:1}$ hydroxy fatty acid (87%), $C_{18:2}$ fatty acid (5%), and $C_{18:1}$ fatty acid (3.4%). In minor amounts, $C_{16:0}$ fatty acid (1.3%), $C_{18:0}$ fatty acid (1.5%) and $C_{18:3}$ fatty acid (0.6%) were present. The findings were comparable to those of other studies.

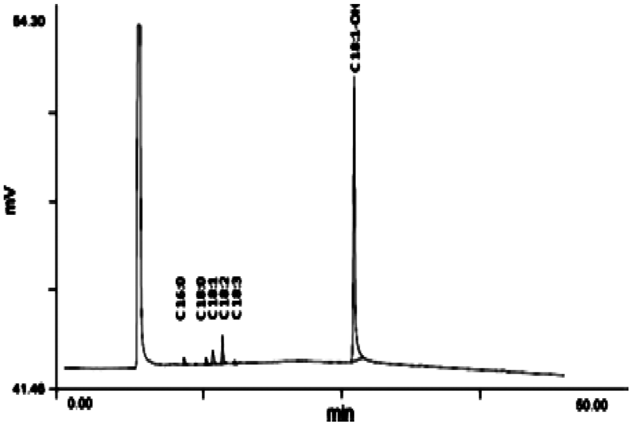


Fig. 2 — Gas chromatogram of fatty acid methyl ester of castor oil.

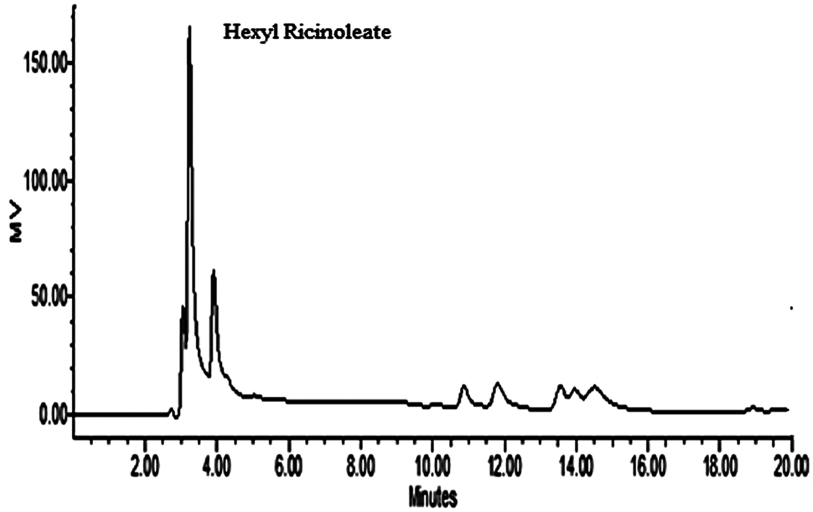


Fig. 3 — HPLC chromatogram of castor oil hexyl ester (COHE).

3.2 Transesterification of castor oil

Castor oil was transesterified in a high-pressure autoclave in liquid CO_2-mediated reactions with hexanol, catalysed by Lipozyme TL IM at 60 bar and 40°C. Several studies were carried out at temperatures ranging from 35-55°C to optimize the reaction parameters of liquid CO_2 mediated enzymatic transesterification. The cooling figure in the high pressure autoclave was created in such a way that cooling water was cycled through it. Liquid CO_2 is an excellent medium for vegetable oil, the reaction mixture can form a single phase at considerably lower temperatures[17]. As a result, a substantially low molar proportion of alcohol to oil was required, resulting in higher yield of COHE.

3.2.1 Effect of oil to hexanol molar ratio

In transesterification reaction, the impact of alcohol to oil molar proportion is important parameter and greatly influences the yield of the alkyl esters. The transesterification reaction was carried out with different molar proportion of oil to hexanol such as, 1:3, 1:6, 1:9 with other reaction parameters as CO_2 Pr. =65 bar, reaction temperature = 60°C, catalyst loading 10% of oil wt, mixing speed 50 rpm. The yields of COHE vs. time at varied mole proportion of oil to hexanol are shown in Fig. 4 and observed that yield of COHE at 1:3, 1:6, 1:9 molar ratios of oil/hexanol were 88.3%, 74.6% and 68.3% respectively. Addition of liquid CO_2 to hexanol increased polarity of reacting medium and improved the solubility of oil in hexanol. Too high content of alcohol inhibited the enzyme activity and dried out the enzyme. From Fig. 4 it is concluded that the enzymatic transesterification in liquid CO_2 gave better yield of COHE in stoichiometric molar ratio and the yield decreased with increasing the molar ratio. The transesterification reaction was faster in liquid CO_2 medium as compared to reactions in organic solvents and solvent free process.

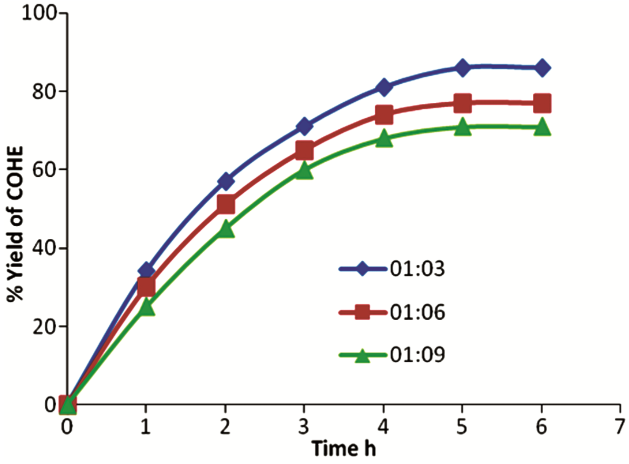


Fig. 4 — COHE yield versus reaction time for different substrate molar ratios. Reaction parameters are, CO_2 Pr. =65 Bar, reaction temperature = 60°C, catalyst loading 10% of oil wt, mixing speed 50 rpm.

3.2.2 Influence of Enzyme loading

Transesterification of castor oil with hexanol was carried out by loading of 5%, 10%, 15 % of Lipozyme TL IM (wt. of oil) at 60°C with oil: hexanol mole proportion of 1:3 and CO_2 pressure of 65 Bar. Figure 5 shows the yield of hexyl esters vs time for various enzyme loadings. The yield of hexyl esters with 5%, 10%, and 15% enzyme loading were 71%, 88.3% and 91.3% respectively. After 5 hours, the yield of the reaction at 15% enzyme concentration was 91.3 percent. Lipozyme TL IM was considered to be a promising enzyme for transesterification of castor oil with hexanol, resulting in greater alkyl ester yields. Also, as the enzyme loading increases, the yield of the product increased but, it was observed that at 15% enzyme loading the yield was not significantly increased as compared to yield at 10% enzymes. Thus 10% enzyme loading gave the optimum yield.

3.2.3 Effect of Temperature

Temperature is an important parameter in the enzymatic reactions, as the solubility of reactants in liquid CO_2 depends upon the temperature. Higher temperatures in enzymatic reactions normally results

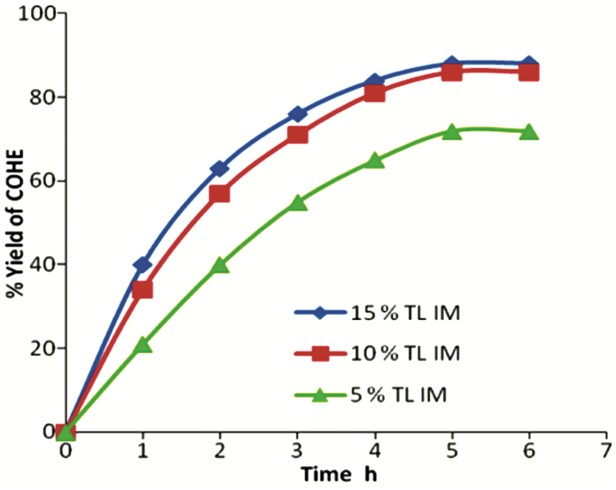


Fig. 5 — COHE yield versus reaction time for different enzyme concentration. Reaction parameters are, CO_2 Pr. = 65 Bar, reaction temperature = 60°C, hexanol to oil molar ratio = 3:1, mixing speed 50 rpm.

in faster kinetics as the diffusion rate increases, but too high temperatures cause thermal denaturation of the enzyme. Varying enzymes have different optimal reaction temperatures, which are influenced by the system's other reaction parameters. Lipozyme TL IM, the enzyme utilised in this study, was stable in liquid CO_2 at temperatures over 70°C. The enzymatic transesterification of castor oil was carried out at different temperatures i.e. 30°C, 40°C, 50°C, 60°C with other reaction parameters are CO_2 Pr. =65 Bar, hexanol to oil molar ratio = 3:1, catalyst loading 10% of oil wt, mixing speed 50 rpm. From Fig. 6 it is clear that increasing temperature has positive effect on yield of COHE. Temperatures above 70°C were avoided in this investigation due to the risk of enzyme denaturation. The maximum yield of 88.3% of COHE was obtained with the temperature of 60°C.

3.2.4 Effect of free fatty acids

Lipases are good catalyst for the esterification of fatty acids along with transesterification of triglycerides for the production of fatty acid alkyl esters[18]. One of the advantages of enzymes is that, it can be used for the transesterification of glycerides with high FFA content. The FFA content of castor oil was increased to 25% by adding ricinoleic acid to study its influence on enzymatic transesterification. The reaction was carried out with stoichiometric molar ratio of hexanol and oil with 10% of (wt/wt of oil) lipase TL IM in liquid CO_2 medium. The influence of FFA on the yield of hexyl esters of castor oil is shown in Fig. 7. With the increase in the FFA of the castor oil up to 25%, there was no significant change in the yield of hexyl esters. This was due to the fact that lipases simultaneously catalyze the esterification and transesterification of high FFA oils for the production of alkyl esters.

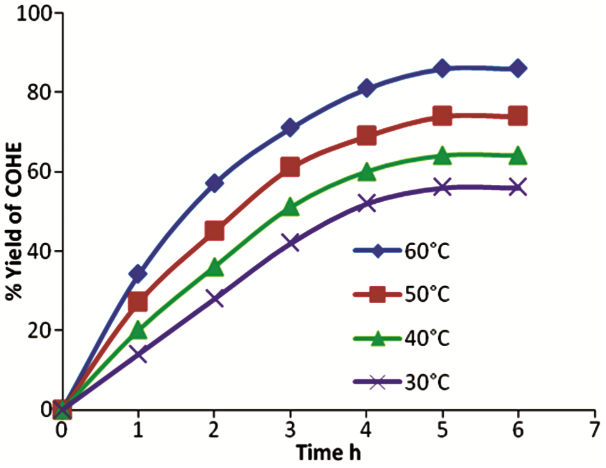


Fig. 6 — COHE yield versus reaction time for different temperatures. Reaction parameters are, CO_2 Pr. = 65 Bar, reaction temperature = 60°C, hexanol to oil molar ratio = 3:1, catalyst loading 10% of oil wt, mixing speed 50 rpm.

3.3 Lubricity studies

The lubricity of the fluid can be predicted by measuring the amount of wear and tear take place between moving metal parts in surrounding with the fluid. The low lubricating fluids leads to the wear and damages of moving parts, shortening the life of component. When compared to non-hydroxylated vegetable oil esters, esters of vegetable oil having hydroxyl group of fatty acids, such as castor oil, enhance lubricating property at low concentrations[12,19]. The HFRR method is simple to apply and can be used to pressurise and investigate the lubricity of volatile fuels that are gases in ambient circumstances[20]. A steel ball and a static steel disc were submerged in a tested sample at a temperature of 60°C (controlled to less than ± 1°C) in the HFRR test. The test ball oscillated at a steady frequency of 50Hz against the disc for 75 minutes. A HFRR test result was determined by measuring the wear scar diameter left on the ball after completing the test with a microscope. Low wear scar diameter, low CoF, and high film formation are all characteristics of an excellent lubricant. The test was carried out separately on low lubricity diesel fuel (LLDF) and hexyl esters (COHE), and castor oil esters were also utilised as a lubricity enhancer in low lubricity diesel fuel (LLDF). All fluids' variations in wsd, CoF, and film formation were documented. To verify data repeatability, the test was repeated three times. As shown in Fig. 8, the wear scar diameter of LLDF and COHE on HFRR was 671 µm and 174 µm, respectively. Wear scar diameters of COHE was quite low, thus castor oil hexyl esters have highest lubricity compared to LLDF.

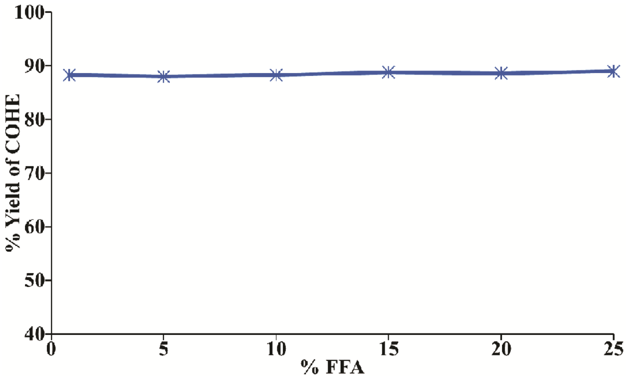
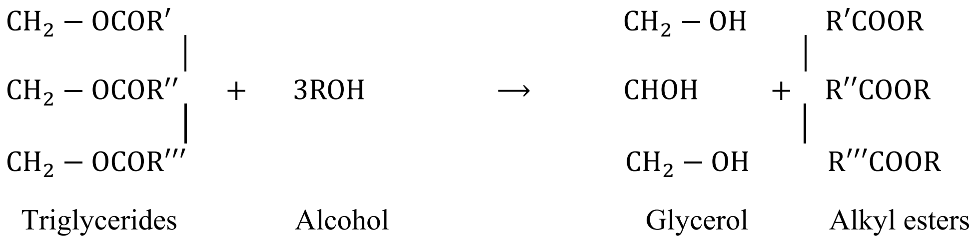


Fig. 7 — Effect of FFA on lipase catalysed transesterification of castor oil in liquid CO_2 medium.



$$\begin{array}{l} CH_2-OCOR' \\ | \\ CH_2-OCOR'' \\ | \\ CH_2-OCOR''' \end{array} + 3ROH \longrightarrow \begin{array}{l} CH_2-OH \\ | \\ CHOH \\ | \\ CH_2-OH \end{array} + \begin{array}{l} R'COOR \\ R''COOR \\ R'''COOR \end{array}$$

Scheme 1 — Transesterification of triglycerides.

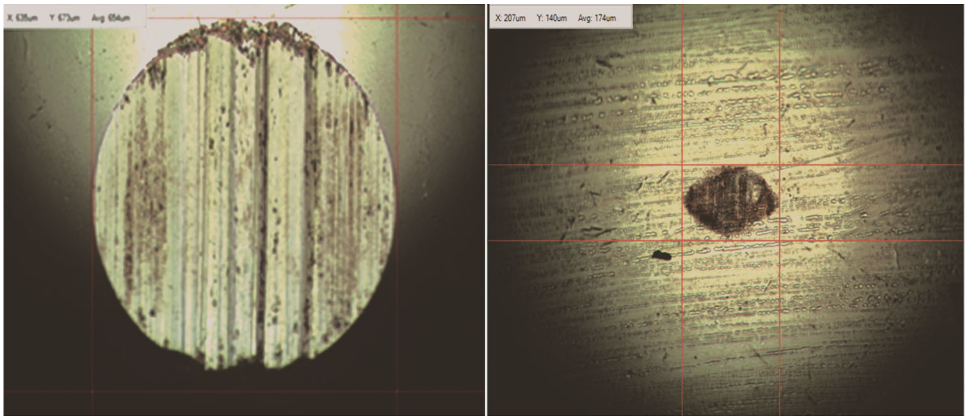


Fig. 8 — Wear scar images (a) LLDF, and (b) COHE.

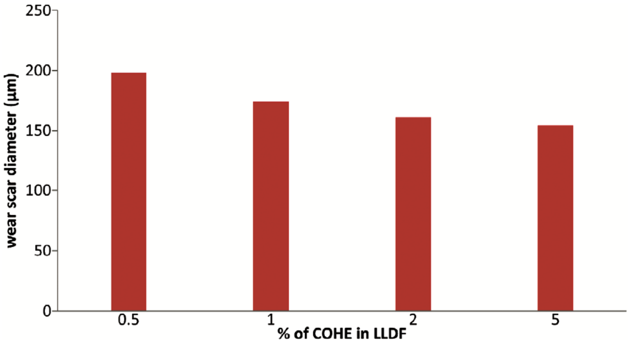


Fig. 9 — Wear scar diameters of COHE as additives in LLDF.

The efficacy of hexyl esters of castor oil as lubricity enhancers was investigated by mixing 0.5 percent, 1%, 2%, and 5% of the ester (v/v) with low lubricity diesel fuel (LLDF) and testing the blends on the HFRR. Figure 9 shows the effect of adding esters to LLDF at various concentrations on the wear scar diameter. The wear scar diameter of LLDF was initially 671 µm, but following the addition of alkyl esters, the wear scar diameter fell drastically as the concentration of alkyl esters increased. Wear scar diameter of LLDF with blend of 0.5%, 1%, 2%, and 5% hexyl esters in LLDF were 198, 174, 161, 154 µm respectively. Higher concentrations of esters resulted in more film formation, which means there is less contact between metals when they oscillate, resulting in less wear scarring and a lower coefficient of friction. The polar hydroxyl group present in castor oil enhances film formation over metal surfaces, hence castor oil hexyl ester has high lubricity. Based on the aforementioned study, it can be concluded that a small amount of COHE (2%) is necessary to reduce the wear scar diameter of LLDF by 75%.

3.4 Kinetics of transesterification in liquid CO_2 medium

As shown in scheme 1, the intermediate reactions i.e. TG⇄DG, DG⇄MG, MG⇄GL which is 3 step reaction can be simplified to the one overall reaction. It is assumed that the reaction follows first order kinetics by ignoring the intermediate reactions
First order Reaction rate can be written as

$$r = -\frac{dTG}{dT} = \frac{k}{TG}$$

By integrating it gives,

$$\int_0^t TG = kt$$

Where k = rate constant and [TG, t] = concentration of TG at time t,

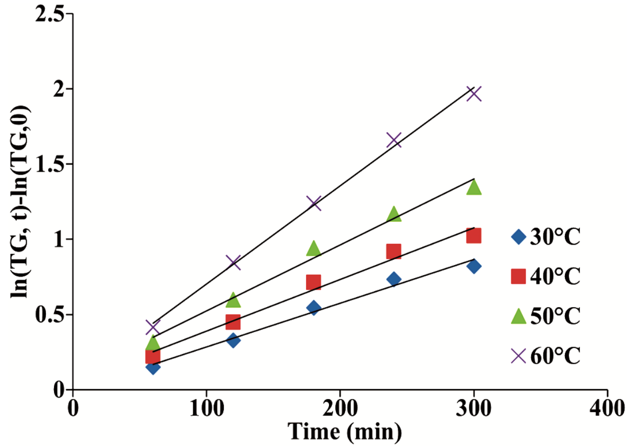


Fig. 10 — Prediction of {ln[TG, 0] − ln[TG, t]} as a function of reaction time t at different temperatures.

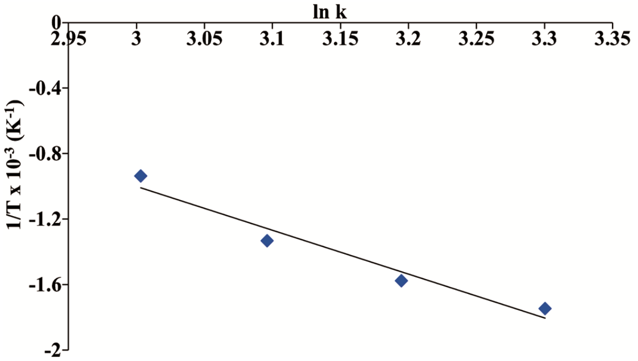


Fig. 11 — Prediction of ln k as a function of 1/T for enzymatic transesterification of castor oil in liquid CO_2 medium.

Table 3 — Rate constant for the enzymatic transesterification of castor oil in liquid CO_2

Temperature °C	**k (min^{-1})**	**R^2**
30	0.0029	0.9836
40	0.0034	0.9805
50	0.0044	0.9861
60	0.0065	0.997

The kinetics of castor oil and hexanol transesterification were studied at temperatures of 30, 40, 50, and 60°C. The other parameters were set at a 3:1 molar ratio of hexanol to oil, a CO_2 pressure of 65 bar, a reaction catalyst loading of 10% of the oil weight, and a mixing speed of 50 rpm. At various temperatures, the experimental data were fitted, and a graph of ln [TG, t] ln [TG,0] vs time t was displayed in Fig. 10. It had an excellent fit and a linear correlation, implying that castor oil transesterification in liquid CO_2 medium is a first order reaction. The activation energy (E_a) of the reaction was determined by plotting ln k vs 1/T at a temperature range of 30-60°C (Fig. 11). The rate constants for COHE formation ranged from 0.0029 to 0.0065 min^{-1}. In liquid CO_2 media, E_a for enzymatic transesterification of castor oil is 22.517 KJ/mol. Table 3 shows the rate constants and their corresponding correlation coefficients at various temperatures.

3.5. Reusability of Enzyme

The advantages of using immobilized enzyme for alkyl esters production is its reusability. The enzyme after transesterification loses their catalytic activity when by-product glycerol is absorbed on the surface of enzyme support[21]. In order to eliminate the effect of glycerol inhibition, 2-propanol was used to remove glycerol by washing the immobilized lipase during the repeated use[22]. In present study, the enzyme was washed after every experiment with 2-propanol and transesterification of castor oil was carried out by transferring the recovered enzyme to a fresh reactant mixture. The yield of hexyl esters remains unchanged with ~88% indicating no loss of catalytic activity of enzyme during transesterification.

4 Conclusion

Liquid carbon dioxide mediated enzymatic esterification of castor oil has been conducted to synthesize COHE and the parameters of reaction have been optimized. The effect of several parameters such as enzyme concentration, alcohol to oil molar ratio, reaction temperature, and the presence of free fatty acid on COHE yield has been examined. The most significant factors have been determined to be hexanol to oil molar ratio, enzyme concentration, and temperature, while the influence of free fatty acid has been minimal. The ideal parameters for COHE transesterification of castor oil are 3:1 molar ratio of hexanol to oil, a 10% enzyme concentration in the oil, and a reaction temperature of 60° C. As lipases simultaneously catalyse the esterification and transesterification of high FFA oils for the synthesis of alkyl esters, there is no substantial change in COHE yield for high FFA concentration. Lubricity study has showed that mixing 0.5 % COHE with LLDF reduced the wear scar diameter by 70%, indicating that it can be utilised as a bio additive with poor lubricating fuels. Enzymatic esterification in Liq. CO_2 medium follows first order kinetics, with rate constants ranging from 0.0029 to 0.0065 min^{-1} for the production of COHE. The enzyme has good reusability and catalytic activity, with a COHE yield of roughly 88% after the fifth use. All the authors have declared no conflict of interest.

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