

Technical aspects of biodiesel production by transesterification—a review

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Abstract

Biodiesel is gaining more and more importance as an attractive fuel due to the depleting fossil fuel resources. Chemically biodiesel is monoalkyl esters of long chain fatty acids derived from renewable feed stock like vegetable oils and animal fats. It is produced by transesterification in which, oil or fat is reacted with a monohydric alcohol in presence of a catalyst. The process of transesterification is affected by the mode of reaction condition, molar ratio of alcohol to oil, type of alcohol, type and amount of catalysts, reaction time and temperature and purity of reactants. In the present paper various methods of preparation of biodiesel with different combination of oil and catalysts have been described. The technical tools and processes for monitoring the transesterification reactions like TLC, GC, HPLC, GPC, ¹H NMR and NIR have also been summarized. In addition, fuel properties and specifications provided by different countries are discussed.

Keywords: Transesterification; Vegetable oil; Biodiesel; Fatty acid alkyl esters; Reaction condition; Analytical methods

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1. Introduction

1.1. Background

Majority of the worlds energy needs are supplied through petrochemical sources, coal and natural gases, with the exception of hydroelectricity and nuclear energy, of all, these sources are finite and at current usage rates will be consumed shortly [1]. Diesel fuels have an essential function in the industrial economy of a developing country and used for transport of industrial and agricultural goods and operation of diesel tractor and pump sets in agricultural sector. Economic growth is always accompanied by commensurate increase in the transport. The high energy demand in the industrialized world as well as in the domestic sector, and pollution problems caused due to the widespread use of fossil fuels make it increasingly necessary to develop the renewable energy sources of limitless duration and smaller environmental impact than the traditional one. This has stimulated recent interest in alternative sources for petroleum-based fuels. An alternative fuel must be technically feasible, economically competitive, environmentally acceptable, and readily available. One possible alternative to fossil fuel is the use of oils of plant origin like vegetable oils and tree borne oil seeds. This alternative diesel fuel can be termed as

91 biodiesel. This fuel is biodegradable and non-toxic and has low emission profiles as
92 compared to petroleum diesel. Usage of biodiesel will allow a balance to be sought
93 between agriculture, economic development and the environment.

94 Chemically the oils/fats consist of triglyceride molecules of three long chain fatty acids
95 that are ester bonded to a single glycerol molecule. These fatty acids differ by the length of
96 carbon chains, the number, orientation and position of double bonds in these chains. Thus
97 biodiesel refers to lower alkyl esters of long chain fatty acids, which are synthesized either
98 by transesterification with lower alcohols or by esterification of fatty acids.

100 101 1.2. Vegetable oil as fuel

102
103 The use of vegetable oils as alternative fuels has been around for 100 years when the
104 inventor of the diesel engine Rudolph Diesel first tested peanut oil, in his compression
105 ignition engine [2]. He said, "The use of vegetable oils for engine fuels may seem
106 insignificant today. But such oils may in course of time be as important as petroleum and
107 the coal tar products of the present time". However, due to cheap petroleum products, such
non-conventional fuels never took off.

108
109 Vegetable oils occupy a prominent position in the development of alternative fuels
although, there have been many problems associated with using it directly in diesel engine
(especially in direct injection engine). These include;

- 110
111 1. Coking and trumpet formation on the injectors to such an extent that fuel atomization
112 does not occur properly or even prevented as a result of plugged orifices,
- 113
114 2. Carbon deposits,
- 115
116 3. Oil ring sticking,
- 117
118 4. Thickening or gelling of the lubricating oil as a result of contamination by vegetable
oils, and
- 119
120 5. Lubricating problems.

121
122 Other disadvantages to the use of vegetable oils and especially animal fats are the high
123 viscosity (about 11-17 times higher than diesel fuel), lower volatilities that causes the
124 formation of deposits in engines due to incomplete combustion and incorrect vaporization
125 characteristics. These problems are associated with large triglyceride molecule and its
126 higher molecular mass and avoided by modifying the engine less or more according to the
127 conditions of use and the oil involved. The modified engines built by Elsbett in Germany
and Malaysia and Diesel Morten und Geraetebau GmbH (DMS) in Germany and in USA
show a good performance when fuelled with vegetable oils of different composition and
grades [1].

130 131 1.3. Biodiesel

132
133 The plant oils usually contain free fatty acids, phospholipids, sterols, water, odourants
134 and other impurities. Because of these, the oil cannot be used as fuel directly. To overcome
135 these problems the oil requires slight chemical modification mainly transesterification,

136 pyrolysis and emulsification. Among these, the transesterification is the key and foremost
 137 important step to produce the cleaner and environmentally safe fuel from vegetable oils.

138 Biodiesel is the monoalkyl esters of long chain fatty acids derived from renewable feed
 139 stocks, such as vegetable oil or animal fats, for use in compression ignition engine.
 140 Biodiesel, which is considered as a possible substitute of conventional diesel fuel is
 141 commonly, composed of fatty acid methyl esters that can be prepared from triglycerides in
 142 vegetable oils by transesterification with methanol. The resulting biodiesel is quite similar
 143 to conventional diesel fuel in its main characteristics.

146 2. Transesterification

147
 148 Transesterification or alcoholysis is the displacement of alcohol from an ester by
 149 another in a process similar to hydrolysis, except than alcohol is used instead of water [1].
 150 This process has been widely used to reduce the high viscosity of triglycerides. The
 151 transesterification reaction is represented by the general equation as Fig. 1.

152 If methanol is used in this process it is called methanolysis. Methanolysis of
 153 triglyceride is represented in Fig. 2.

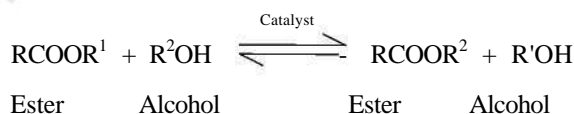
154 Transesterification is one of the reversible reactions and proceeds essentially by mixing
 155 the reactants. However, the presence of a catalyst (a strong acid or base) accelerates the
 156 conversion.

159 3. Transesterification kinetics and mechanism

160
 161 Transesterification of triglycerides produce fatty acid alkyl esters and glycerol. The
 162 glycerol layer settles down at the bottom of the reaction vessel. Diglycerides and
 163 monoglycerides are the intermediates in this process. The mechanism of transesterification
 164 is described in Fig. 3.

165 The step wise reactions are reversible and a little excess of alcohol is used to shift the
 166 equilibrium towards the formation of esters. In presence of excess alcohol, the forward
 167 reaction is pseudo-first order and the reverse reaction is found to be second order. It was
 168 also observed that transesterification is faster when catalyzed by alkali [3].

169 The mechanism of alkali-catalyzed transesterification is described in Fig. 4. The first
 170 step involves the attack of the alkoxide ion to the carbonyl carbon of the triglyceride
 171 molecule, which results in the formation of a tetrahedral intermediate. The reaction of this
 172 intermediate with an alcohol produces the alkoxide ion in the second step. In the last step
 173 the rearrangement of the tetrahedral intermediate gives rise to an ester and a diglyceride
 174 [4].



175
 176
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 179
 180
 Fig. 1. General equation of transesterification.

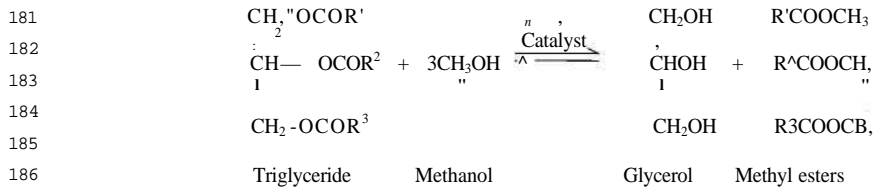


Fig. 2. General equation for transesterification of triglycerides.

Transesterification can be catalyzed by Bronsted acids, preferably by sulfonic and sulfuric acids. These catalysts give very high yields in alkyl esters but these reactions are slow, requiring typically temperature above 100 °C and more than 3 h to complete the conversion [5]. The mechanism of acid catalyzed transesterification of vegetable oil (for a monoglyceride) is shown in Fig. 5. However, it can be extended to di- and tri-glycerides. The protonation of carbonyl group of the ester leads to the carbocation, which after a nucleophilic attack of the alcohol produces a tetrahedral intermediate. This intermediate eliminates glycerol to form a new ester and to regenerate the catalyst. (Fig. 6).

The methanolysis of soybean oil in the presence of 1 % H₂SO₄ with an alcohol/oil molar ratio 30:1 was studied. At a reaction temperature of 65 °C the conversion was observed to be completed in 20 h, while butanolysis at 117 °C and ethanolysis at 78 °C using the same quantities of catalyst and alcohol, take 3 and 18 h, respectively [3].

4. Variables affecting transesterification reaction

The process of transesterification is affected by various factors depending upon the reaction condition used. The effects of these factors are described below.

4.1. Effect of free fatty acid and moisture

The free fatty acid and moisture content are key parameters for determining the viability of the vegetable oil transesterification process. To carry the base catalyzed reaction to completion; an free fatty acid (FFA) value lower than 3% is needed. The higher the acidity of the oil, smaller is the conversion efficiency. Both, excess as well as insufficient amount of catalyst may cause soap formation [6].

Ma et al. [7] studied the transesterification of beef tallow catalyzed by NaOH in presence of free fatty acids and water. Without adding FFA and water, the apparent yield of beef tallow methyl esters (BTME) was highest. When 0.6% of FFA was added, the apparent yield of BTME reached the lowest, less than 5%, with any level of water added.

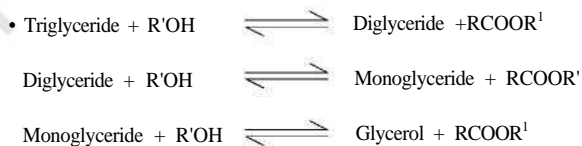


Fig. 3. General equation for transesterification of triglycerides.

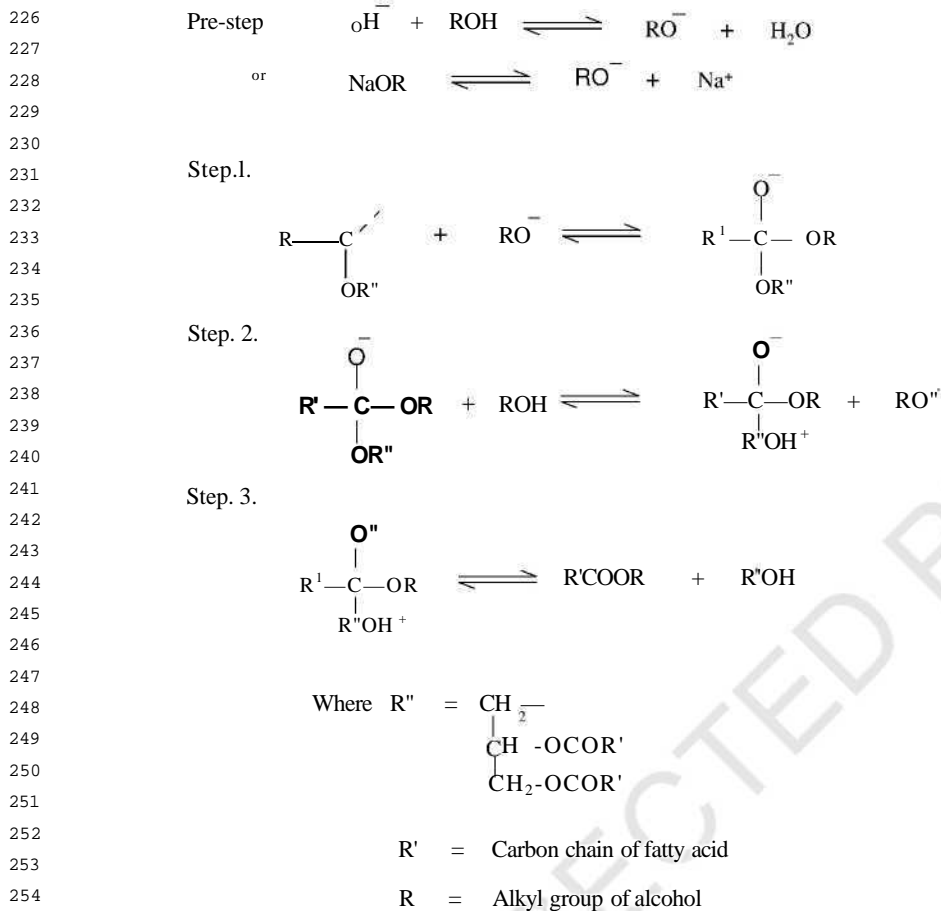


Fig. 4. Mechanism of base catalyzed transesterification.

258 The products were solid at room temperature, similar to the original beef tallow. When
 259 0.9% of water was added, without addition of FFA, the apparent yield was about 17%. If
 260 the low qualities of beef tallow or vegetable oil with high FFA are used to make biodiesel
 261 fuel, they must be refined by saponification using NaOH solution to remove free fatty
 262 acids. Conversely, the acid catalyzed process can also be used for esterification of these
 263 free fatty acids.

264 The starting materials used for base catalyzed alcoholysis should meet certain
 265 specifications. The triglycerides should have lower acid value and all material should be
 266 substantially anhydrous. The addition of more sodium hydroxide catalyst compensates for
 267 higher acidity, but the resulting soap causes an increase in viscosity or formation of gels
 268 that interferes in the reaction as well as with separation of glycerol [8]. When the reaction
 269 conditions do not meet the above requirements, ester yields are significantly reduced. The
 270 methoxide and hydroxide of sodium or potassium should be maintained in anhydrous

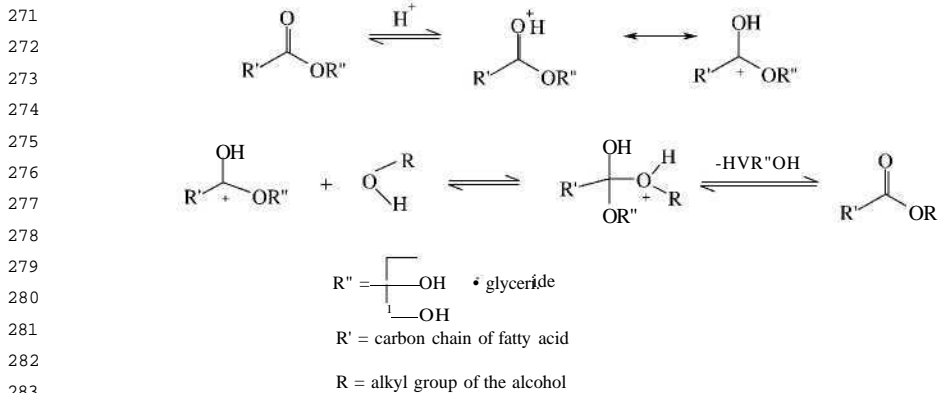


Fig. 5. Mechanism of acid catalyzed transesterification.

286 state. Prolonged contact with air will diminish the effectiveness of these catalysts through
 287 interaction with moisture and carbon dioxide.

288 Most of the biodiesel is currently made from edible oils by using methanol and alkaline
 289 catalyst. However, there are large amounts of low cost oils and fats that could be converted
 290 to biodiesel. The problems with processing these low cost oils and fats is that they often
 291 contain large amounts of free fatty acids that cannot be converted to biodiesel using
 292 alkaline catalyst. Therefore, two-step esterification process is required for these feed
 293 stocks. Initially the FFA of these can be converted to fatty acid methyl esters by an acid
 294 catalyzed pretreatment and in the second step transesterification is completed by using
 295 alkaline catalyst to complete the reaction [9]. Initial process development was performed
 296 with synthetic mixture containing 20 and 40% free fatty acid prepared by using palmitic
 297 acid. Process parameters such as molar ratio of alcohol to oil, type of alcohol, amount of
 298 acid catalyst, reaction time, free fatty acid level were investigated to determine the best
 299 strategy for converting the free fatty acids to usable esters. The work showed that the acid
 300 level of the high free fatty acids feed stocks could be reduced to less than 1% with a two-
 301 step pretreatment reaction. The reaction mixture was allowed to settle between steps so
 302 that the water containing phase could be removed. The two-step pretreatment reaction was
 303 demonstrated with actual feedstocks, including yellow grease with 12% free fatty acid
 304

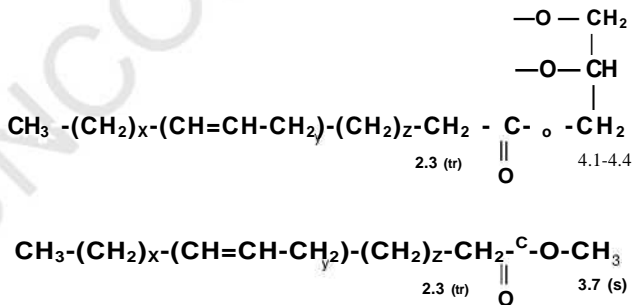


Fig. 6. Assignment of chemical shifts of protons in transesterification reaction.

316 and brown grease with 33% free fatty acids. After reducing the acid levels of these
317 feedstocks to less than 1%, the transesterification reaction was completed with an alkaline
318 catalyst to produce fuel grade biodiesel.

319 Turck et al. [10] have investigated the negative influence of base catalyzed
320 transesterification of triglycerides containing substantial amount of free fatty acid. Free
321 fatty acids react with the basic catalyst added for the reaction and give rise to soap, as a
322 result of which, one part of the catalyst is neutralized and is therefore no longer available
323 for transesterification. These high FFA content oils/fats are processed with an immiscible
324 basic glycerol phase so as to neutralize the free fatty acids and cause them to pass over into
325 the glycerol phase by means of monovalent alcohols. The triglycerides are subjected to
326 transesterification, using a base as catalyst, to form fatty acid alkyl esters, characterized in
327 that after its separation, the basic glycerol phase produced during transesterification of the
328 triglycerides is used for processing the oils/fats for removal of free fatty acids. The
329 minimum amount of catalyst required for this process was calculated, relative to 1000 g of
330 the oil to be processed, as a function of the acid value and the mean molar mass of the
331 oil/fat.

332 4.2. Catalyst type and concentration

333 Catalysts used for the transesterification of triglycerides are classified as alkali, acid,
334 enzyme or heterogeneous catalysts, among which alkali catalysts like sodium hydroxide,
335 sodium methoxide, potassium hydroxide, potassium methoxide are more effective [4]. If
336 the oil has high free fatty acid content and more water, acid catalyzed transesterification is
337 suitable. The acids could be sulfuric acid, phosphoric acid, hydrochloric acid or organic
338 sulfonic acid. Methanolysis of beef tallow was studied with catalysts NaOH and NaOMe.
339 Comparing the two catalysts, NaOH was significantly better than NaOMe [7]. The
340 catalysts NaOH and NaOMe reached their maximum activity at 0.3 and 0.5% w/w of the
341 beef tallow, respectively. Sodium methoxide causes formation of several by-products
342 mainly sodium salts, which are to be treated as waste. In addition, high quality oil is
343 required with this catalyst [11]. This was different from the previous reports [3] in which
344 ester conversion at the 6:1 molar ratio of alcohol/oil for 1% NaOH and 0.5% NaOMe were
345 almost the same after 60 min. Part of the difference may be attributed to the differences in
346 the reaction system used.

347 As a catalyst in the process of alkaline methanolysis, mostly sodium hydroxide or
348 potassium hydroxide have been used, both in concentration from 0.4 to 2% w/w of oil.
349 Refined and crude oils with 1% either sodium hydroxide or potassium hydroxide catalyst
350 resulted successful conversion. Methanolysis of soybean oil with the catalyst 1%
351 potassium hydroxide has given the best yields and viscosities of the esters [12].

352 Attempts have been made to use basic alkaline-earth metal compounds in the
353 transesterification of rapeseed oil for production of fatty acid methyl esters. The reaction
354 proceeds if methoxide ions are present in the reaction medium. The alkaline-earth metal
355 hydroxides, alkoxides and oxides catalyzed reaction proceeds slowly as the reaction
356 mixture constitutes a three-phase system oil-methanol-catalyst, which for diffusion reason
357 inhibits the reaction [13]. The catalytic activity of magnesium oxide, calcium hydroxide,
358 calcium oxide, calcium methoxide, barium hydroxide, and for comparison, sodium
359
360

361 hydroxide during the transesterification of rapeseed oil was investigated. Sodium
362 hydroxide exhibited the highest catalytic activity in this process. The degree to which
363 the substrates were reacted reached 85% after 30 min of the process and 95% after 1.5 h,
364 which represented a close value to the equilibrium. Barium hydroxide was slightly less
365 active with a conversion of 75% after 30 min. Calcium methoxide was medially active.
366 The degree to which the substrates were reacted was 55% after 30 min. Eighty percents
367 after 1 h and state of reaction equilibrium (93%) was reached after 2.5 h. The rate of
368 reaction was slowest when catalyzed by CaO. Magnesium oxide and calcium hydroxide
369 showed no catalytic activity in rapeseed oil methanolysis.

370 Acid catalyzed transesterification was studied with waste vegetable oil. The reaction
371 was conducted at four different catalyst concentrations, 0.5, 1.0, 1.5 and 2.25 M HCl in
372 presence of 100% excess alcohol and the result was compared with 2.25 M H₂SO₄ and the
373 decrease in viscosity was observed. H₂SO₄ has superior catalytic activity in the range of
374 1.5-2.25 M concentration [14].

375 Although chemical transesterification using an alkaline catalysis process gives high
376 conversion levels of triglycerides to their corresponding methyl esters in short reaction
377 times, the reaction has several drawbacks: it is energy intensive, recovery of glycerol
378 is difficult, the acidic or alkaline catalyst has to be removed from the product, alkaline
379 waste water require treatment, and free fatty acid and water interfere the reaction.
380 Enzymatic catalysts like lipases are able to effectively catalyze the transesterification
381 of triglycerides in either aqueous or non-aqueous systems, which can overcome the
382 problems mentioned above [15]. In particular, the by-products, glycerol can be easily
383 removed without any complex process, and also that free fatty acids contained in
384 waste oils and fats can be completely converted to alkyl esters. On the other hand, in
385 general the production cost of a lipase catalyst is significantly greater than that of an
386 alkaline one.

387 4.3. Molar ratio of alcohol to oil and type of alcohol

389 One of the most important variables affecting the yield of ester is the molar ratio of
390 alcohol to triglyceride. The stoichiometric ratio for transesterification requires three moles
391 of alcohol and one mole of triglyceride to yield three moles of fatty acid alkyl esters and
392 one mole of glycerol. However, transesterification is an equilibrium reaction in which a
393 large excess of alcohol is required to drive the reaction to the right. For maximum
394 conversion to the ester, a molar ratio of 6:1 should be used. The molar ratio has no effect on
395 acid, peroxide, saponification and iodine value of methyl esters [12]. However, the high
396 molar ratio of alcohol to vegetable oil interferes with the separation of glycerin because
397 there is an increase in solubility. When glycerin remains in solution, it helps drive the
398 equilibrium to back to the left, lowering the yield of esters. The transesterification of
399 Cynara oil with ethanol was studied at molar ratios between 3:1 and 15:1. The ester yield
400 increased as the molar ratio increased up to a value of 12:1. The best results were for molar
401 ratios between 9:1 and 12:1. For molar ratios less than 6:1, the reaction was incomplete.
402 For a molar ratio of 15:1 the separation of glycerin is difficult and the apparent yield of
403 esters decreased because a part of the glycerol remains in the biodiesel phase. Therefore,
404 molar ratio 9:1 seems to be the most appropriate [16].
405

406 The base catalyzed formation of ethyl ester is difficult compared to the formation of
407 methyl esters. Specifically the formation of stable emulsion during ethanolysis is a
408 problem. Methanol and ethanol are not miscible with triglycerides at ambient temperature,
409 and the reaction mixtures are usually mechanically stirred to enhance mass transfer.
410 During the course of reaction, emulsions usually form. In the case of methanolysis, these
411 emulsions quickly and easily break down to form a lower glycerol rich layer and upper
412 methyl ester rich layer. In ethanolysis, these emulsions are more stable and severely
413 complicate the separation and purification of esters [17]. The emulsions are caused in part
414 by formation of the intermediates monoglycerides and diglycerides, which have both polar
415 hydroxyl groups and non-polar hydrocarbon chains. These intermediates are strong-
416 surface active agents. In the process of alcoholysis, the catalyst, either sodium hydroxide
417 or potassium hydroxide is dissolved in polar alcohol phase, in which triglycerides must
418 transfer in order to react. The reaction is initially mass-transfer controlled and does not
419 conform to expected homogeneous kinetics. When the concentrations of these
420 intermediates reach a critical level, emulsions form. The larger non-polar group in
421 ethanol, relative to methanol, is assumed to be the critical factor in stabilizing the
422 emulsions. However, the concentration of mono- and di-glycerides are very low, then the
423 emulsions become unstable. This emphasizes the necessity for the reaction to be as
424 complete as possible, thereby reducing the concentrations of mono- and di-glycerides.

425 426 4.4. *Effect of reaction time and temperature*

All
428 The conversion rate increases with reaction time. Freedman et al. [8] transesterified
429 peanut, cotton-seed, sunflower and soybean oil under the condition of methanol-oil molar
430 ratio 6:1, 0.5% sodium methoxide catalyst and 60 8C. An approximate yield of 80% was
431 observed after 1 min for soybean and sunflower oils. After 1 h, the conversion was almost
432 the same for all four oils (93-98%). Ma et al. [18] studied the effect of reaction time on
433 transesterification of beef tallow with methanol. The reaction was very slow during the
434 first minute due to mixing and dispersion of methanol into beef tallow. From one to 5 min,
435 the reaction proceeds very fast. The production of beef tallow methyl esters reached the
436 maximum value at about 15 min.

437 Transesterification can occur at different temperatures, depending on the oil used. For
438 the transesterification of refined oil with methanol (6:1) and 1% NaOH, the reaction was
439 studied with three different temperatures [8]. After 0.1 h, ester yields were 94, 87 and 64%
440 for 60, 45 and 32 8C, respectively. After 1 h, ester formation was identical for 60 and 45 8C
441 runs and only slightly lower for the 32 8C run. Temperature clearly influenced the reaction
442 rate and yield of esters [4].

443 444 4.5. *Mixing intensity*

446 Mixing is very important in the transesterification reaction, as oils or fats are
447 immiscible with sodium hydroxide-methanol solution. Once the two phases are mixed and
448 the reaction is started, stirring is no longer needed. Initially the effect of mixing on
449 transesterification of beef tallow was studied by Ma et al. [18]. No reaction was observed
450 without mixing and when NaOH-MeOH was added to the melted beef tallow in the reactor

451 while stirring, stirring speed was insignificant. Reaction time was the controlling factor in
452 determining the yield of methyl esters. This suggested that the stirring speeds investigated
453 exceeded the threshold requirement of mixing.

454

455 4.6. Effect of using organic cosolvents

456

457 The methoxide base catalyzed methanolysis of soybean oil at 40 °C (methanol-oil
458 molar ratio 6:1) shows that to form methyl esters proceeds approximately more slowly
459 than butanolysis at 30 °C. This is interpreted to be the result of a two phase reaction in
460 which methanolysis occurs only in the methanol phase. Low oil concentration in methanol
461 causes the slow reaction rate; a slow dissolving rate of the oil in methanol causes an
462 initiation period. Intermediate mono- and di-glycerides preferentially remain in the
463 methanol, and react further, thus explaining the deviation from second order kinetics. The
464 same explanations apply for hydroxide ion catalyzed methanolysis.

465 In order to conduct the reaction in a single phase, cosolvents like tetrahydrofuran, 1,4-
466 dioxane and diethyl ether were tested. Although, there are other cosolvents, initial study
467 was conducted with tetrahydrofuran. At the 6:1 methanol-oil molar ratio the addition of
468 1.25 volume of tetrahydrofuran per volume of methanol produces an oil dominant one
469 phase system in which methanolysis speeds up dramatically and occurs as fast as
470 butanolysis. In particular, THF is chosen because its boiling point of 67 °C is only two
471 degrees higher than that of methanol. Therefore at the end of the reaction the unreacted
472 methanol and THF can be co-distilled and recycled [19].

473 Using tetrahydrofuran, transesterification of soybean oil was carried out with methanol
474 at different concentrations of sodium hydroxide. The ester contents after 1 min for 1.1, 1.3,
475 1.4 and 2.0% sodium hydroxide were 82.5, 85, 87 and 96.2%, respectively. Results
476 indicated that the hydroxide concentration could be increased up to 1.3 wt%, resulting in
477 95% methyl ester after 15 min. [20]. Similarly for transesterification of coconut oil using
478 THF/MeOH volume ratio 0.87 with 1% NaOH catalyst, the conversion was 99% in 1 min.

479 A single-phase process for the esterification of a mixture of fatty acids and triglycerides
480 were investigated. The process comprises forming a single-phase solution of fatty acids
481 and triglyceride in an alcohol selected from methanol and ethanol, the ratio of said alcohol
482 to triglyceride being 15:1-35:1. The solution further comprises a cosolvent in an amount
483 to form the single phase. In a first step, an acid catalyst for the esterification of fatty acid is
484 added. After a period of time, the acid catalyst is neutralized and a base catalyst for the
485 transesterification of triglycerides is added. After a further period of time, esters are
486 separated from the solution [21].

487 An improved process was investigated for methanolysis and ethanolysis of fatty acid
488 glycerides such as those found in naturally occurring fats and oils derived from plant and
489 animals. The processes comprise solubilizing oil or fat in methanol or ethanol by addition
490 of a cosolvent in order to form a one-phase reaction mixture, and adding an esterification
491 catalyst. The processes proceed quickly, usually in less than 20 min, at ambient
492 temperatures, atmospheric pressure and without agitation. The co-solvent increases the
493 rate of reaction by making the oil soluble in methanol, thus increasing contact of the
494 reactants. The lower alkyl fatty acid monoesters produced by the process can be used as
495 biofuels and are suitable as diesel fuel replacements or additives [22].

5. Transesterification under different conditions

Palm oil was transmethylated continuously at 70 °C in an organic solvent with sodium methoxide as a catalyst. The optimum ratio of toluene to palm oil is 1:1 (v/v). When the methanol to oil molar ratio was 13:1, transmethylation was 96% complete within 60 s. At higher molar ratio (17:1), transmethylation was 99% complete in 15 s. For lower molar ratios of methanol to oil (9:1 and 5.8:1), yields of palm oil methyl esters were 84 and 58%, respectively. Benzene was a good solvent for transmethylation, but the yield of palm oil methyl ester was slightly lower than toluene. Tetrahydrofuran did not accelerate transmethylation [23].

The direct methanolysis of triglycerides in flowing supercritical carbon dioxide by an immobilized lipase is described. The reaction system consists of two syringe pumps for substrate addition and another two syringe pumps for delivering CO₂ at 24.1 MPa. Corn oil was pumped into the carbon dioxide stream at a rate of 4 mL/min and methanol is pumped at 5 mL/min to yield fatty acid methyl ester 98%. Direct methanolysis of soy flakes gives FAME at similar yield. This combined extraction is performed at 17.2 MPa and 50 °C [24].

Transesterification reaction of rapeseed oil in supercritical methanol was investigated without using the catalyst. An experiment has been carried out in the batch-type reaction vessel preheated at 350 and 400 °C and at a pressure of 45-65 MPa, and with a molar ratio of 1:42 of the rapeseed oil to methanol. It was consequently demonstrated that, in a preheating temperature of 350 °C, 450 s of supercritical treatment of methanol was sufficient to convert the rapeseed oil to methyl esters and that, although the prepared methyl esters were basically the same as those of the common method with a basic catalyst, the yield of methyl esters by the former was found to be higher than that by the later. In addition, it was found that this new supercritical methanol process requires the shorter reaction time and simpler purification procedure because of the unused catalyst [25].

Commercially available crude oils and fats contain considerable amounts of free fatty acids. These free fatty acids are known to react with the alkaline catalyst and form saponified products during transesterification reactions for biodiesel fuel production in the conventional commercial process. Purification of the products therefore becomes necessary after the reaction. In addition, it causes a longer production process and increases the production costs. For this aspect, supercritical methanol method without using any catalyst is evaluated in this work for the reaction of free fatty acids. As a result, complete conversion is achieved for saturated fatty acids to methyl esters at temperatures above 400 °C, whereas for unsaturated fatty acids, lower temperature of 350 °C is appropriate, and higher temperature resulted in a degradation of the products. Consequently, a conversion of free fatty acids to methyl esters is highest, over 95%, when treated at 350 °C. Fortunately, this temperature treatment is also most appropriate for transesterification of triglycerides. Thus, the overall conversion process of rapeseed oil to methyl esters is concluded to be adequate at 350 °C. This finding supports the superiority of supercritical methanol process on biodiesel fuel production, compared with the conventional method, in which the production process becomes much simpler and increases the total yield due to methyl esters produced from free fatty acids [26].

541 6. Analytical monitoring of the reaction

542
543 Various analytical methods were developed for analyzing mixtures containing fatty
544 acid esters and mono-, di-, and tri-glycerides obtained by the transesterification of
545 vegetable oils. Analyses were performed by thin layer chromatography/flame ionization
546 detection (TLC/FID) with an Iatroscan TH-10 instrument. Steryl alcohol served as an
547 internal standard. From plots of areas and weight ratios of methyl linoleate, and mono-, di-
548 and tri-linolein, linear equations were developed from which response factors were
549 calculated. Hydrogen flow rate and developing solvent strongly influenced resolution and
550 base line stability. Variation in scan speed affected compactness of burning and response
551 factors, but not measured composition. A computerized procedure for data analysis were
552 developed so that 30 samples can be completed in 2-3 h. The relative standard deviations
553 were 1-2% for major components in the range of 90-100% range and 6-83% for minor
554 components in the 1-2% range [27].

556 6.1. Gas chromatographic method

557
558 A gas chromatographic method for the simultaneous determination of glycerol, mono-,
559 di-, and tri-glycerides in vegetable oil methyl esters has been developed. Trimethylsilyla-
560 tion of glycerol, mono-, and di-glycerides, followed by GC using a 10 m capillary coated
561 with a 0.1, mm film of DB-5 allows the determination of all analytes in a single GC run.

562 In principle, glycerol, mono-, di-, and tri-glycerides can be analyzed on highly inert
563 columns coated with apolar stationary phases without derivatization. The inertness of the
564 column, required to obtain good peak shapes and satisfactory recovery, cannot be easily
565 maintained in routine analysis. Trimethylsilylation of the free hydroxyl groups of glycerol,
566 mono- and di-glycerides, however, ensures excellent peak shapes, good recoveries and
567 low detection limits and enormously improves the ruggedness of the procedure. For
568 complete silylation of glycerol and partial glycerides, the conditions of the derivatization
569 reaction have to be controlled carefully. Extensive studies on the silylation of partial
570 glycerides showed that silylation can be obtained under the following conditions; (i) bis-
571 trimethylsilyl trifluoroacetamide (BSTFA) as silylating agent, addition of pyridine or
572 dimethylformamide and heating to 70 8C for 15 min; (ii) BSTFAC1% trimethylchlor-
573 osilane as silylating agent, addition of pyridine and a reaction time of 15 min at room
574 temperature; (iii) N-methyl N-trimethylsilyl-trifluoroacetamide (MSTFA) as silylating
575 agent, addition of pyridine and reaction time of 15 min at room temperature; (iv) MSTFA
576 as silylating agent and heating to 70 8C for 15 min. The internal standard 1,2,4-butanetriol
577 serves as a very sensitive indicator of incomplete derivatization [28]. In case of insufficient
578 silylation, the peak of 1,2,4-butanetriol appears splitted and drastically reduced in height.

579 Mittelbach [29] have prepared the sample by mixing 100-500 µl rapeseed methyl ester
580 (depending upon glycerol content) with 100 µl of 1,4-butanediol standard solution in N,N-
581 dimethylformamide (approximately 0.1 mg 1,4-butanediol/ml) and 600-200 µl of DMF.
582 Finally 200 µl of BSTFA is added to reach the final volume of 1 ml, the mixture was
583 shaken vigorously and 2 µl aliquots were injected in the GC after at least 10 min. It is
584 necessary to use fresh BSTFA reagent to guarantee quantitative derivatization. The GC
585 capillary column used was Fused silica, 60 m ! 0.25 mm, 0.25 µm DB 5 (JandW Scientific

586 Inc.) where as Boocock [21] has used the DB-1 fused silica capillary column of dimension
587 2 m!0.25 mm i.d. coated with 0.25 mm film of 100% polymethyl siloxane.

588 Most reports on the use of GC for biodiesel analysis employ flame-ionization detectors
589 (FID), although the use of mass spectrometric detector (MSD) would eliminate any
590 ambiguities about the nature of the eluting materials since mass spectra unique to
591 individual compounds would be obtained [30]

592

593 6.2. High performance liquid chromatography method

594

595 A general advantage of HPLC compared to GC is that time and reagent consuming
596 derivatization are not necessary, which reduces analysis time. The first literature on HPLC
597 method describes the determination of overall content of mono-, di- and tri-glycerides in
598 fatty acid methyl esters by isocratic liquid chromatography using a density detector. The
599 separation was achieved by coupling a cyano-modified silica column with two GPC
600 columns; chloroform with an ethanol content of 0.6% is used as an eluent. This system
601 allowed for the detection of mono-, di- and tri-glycerides as well as methyl esters as
602 classes of compounds. The system was useful for the study of degree of conversion of the
603 transesterification reaction [31].

604 HPLC with pulsed amperometric detection (the detection limit is usually 10-100 times
605 lower than for amperometric detection and the detection limit is 1 mg/g) was used to
606 determine the amount of free glycerol in vegetable oil esters. The HPLC-PAD method has
607 proved to be simple, rapid and accurate [32]. The major advantage of this method is its
608 high sensitivity. The simultaneous detection of residual alcohol is also possible with this
609 technique [30].

610 In an extensive study [33] reverse phase high performance liquid chromatography (RP-
611 HPLC) was used for the determination of compounds occurring during the production of
612 biodiesel from rapeseed oil with various detection methods: UV detection at 205 nm,
613 evaporative light scattering detection (ELSD) and atmospheric pressure chemical
614 ionization mass spectrometry (APCI-MS) in positive ion mode. Two gradient solvent
615 systems were used: one consisting of methanol (A) with 2-propanol-hexane (5:4, v/v) from
616 100% A to 50:50 A:B—a non-aqueous reverse phase system and the other consisting of
617 water (A), acetonitrile (B) and 5:4 2-propanol-hexane (C) with two linear gradient steps
618 (30% AC 70% B in 0 min, 100% B in 10 min, 50% B C 50% C in 20 min followed by
619 isocratic elution with 50% B C 50% C for last 5 min). The first solvent system was used for
620 rapid quantitative determination of the yield of the transesterification reaction of rapeseed
621 oil with methanol by comparing the sum of the areas of the peaks of methyl esters and
622 triglycerides. The sincerity and linearity of each detection method varied with the
623 individual triglycerides. ELSD and APCI-MS have decreased sensitivity with increasing
624 number of double bonds in the fatty acid methyl esters. However, the sensitivity of UV
625 detection is also different for individual triglycerides. APCI-MS is stated to be the most
626 appropriate detection method for the analysis of rapeseed oil biodiesel.

627 HPLC method of quantification of mono-, di-, tri-glycerides and esters using UV
628 detection method at the region of 205 nm was studied for methanolysis of rapeseed oils
629 [34]. The glass column 150!3 mm with pre-column 30!3 mm, both packed with C-18,
630 particles with diameter 7 mm and the mobile phase A (acetonitrile:water 80:20), B

631 (acetonitrile), C (hexane:2-propanol 40:50) with 0-2 min—100% A, 2-12 min—change
 632 to 100% B, 12-22 min—change to 50% B and 50% C, 22-29 min—change to 100% B,
 633 30-32 min—change to 100% of B, 32-33 min change to initial 100% A.

634 An HPLC equipped with a refractive index detector was use for the analysis biodiesel
 635 produced from soybean oil [35]. The HPLC mobile phase was consisted of acetone and
 636 acetonitrile. Mono-, di-, and tri-glycerides were quantified to study the reaction kinetics
 637 for transesterification.

638

639

6.3. Gel permeation chromatography method

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641

642 A method for simultaneous analysis of transesterification reaction products—
 643 monoglycerides, diglycerides, triglycerides, glycerol and methyl esters—was developed
 644 using gel permeation chromatography coupled with refractive index detector [36]. The
 645 mobile phase was HPLC grade tetrahydrofuran at a flow rate of 0.5 mL/min at room
 646 temperature and the sample injection size was 10 mL. Sample preparation involves only
 647 dilution and neutralization. For analysis, 300 mg of the sample was taken from
 648 transesterification reactor and neutralized by adding 5 ml HPLC grade tetrahydrofuran
 649 and one drop of 0.6 N HCl. The samples were then kept at 20 8C until analysis.
 650 Reproducibility of the method was good: analysis of palm oil transesterification products
 651 at different levels of conversion showed a relative standard deviation of 0.27-3.87%.
 652 Similarly GPC was used to evaluate the influence of different variables affecting the
 653 transesterification of rapeseed oil with anhydrous ethanol and sodium ethoxide as catalyst
 654 [37]. GPC has made the quantitation of ethyl esters, mono-, di- and tri-glycerides and
 glycerol possible.

655

656

6.4. ¹H NMR method

657

658

659 The first report on spectroscopic determination of the yield of transesterification
 660 reaction utilized ¹H NMR depicting its progressing spectrum [38]. The signal due to
 661 methylene protons adjacent to the ester group in triglycerides appear at 2.3 ppm and after
 662 the reaction the methoxy protons of the methyl esters appear at 3.7 ppm. These authors
 663 have used the areas of the signals of methylene and methoxy protons to monitor the yield
 664 of transesterification.

665 A simple equation is given by the authors:

666

667

668

669

670

$$C Z 100 ! \left(\frac{2A_{ME}}{3A_{CH_2}} \right)$$

671 where C, percentage conversion of triglycerides to corresponding methyl ester; A_{ME},
 672 integration value of the protons of the methyl esters (the strong singlet); and A_{CH₂},
 673 integration value of the methylene protons.

674 The factors 2 and 3 derived from the fact that the methylene carbon possesses two
 675 protons and the alcohol (methanol derived) carbon has three attached protons.

676 6.5. NIR spectroscopy

677
678 More recently, NIR spectroscopy has been used to monitor the transesterification
679 reaction [40]. The basis for quantitation of the turn over from triglyceride feedstock to
680 methyl ester product is differences in the NIR spectra of these classes of compounds. At
681 6005 cm^{K1} and 4425-4430 cm^{K1}, the methyl esters display peaks, while triglycerides
682 display only shoulders. Ethyl esters could be distinguished in a similar fashion. Using
683 quantitation software, it is possible to develop a method for quantifying the turnover of
684 triglycerides to methyl esters. The absorption at 6005 cm^{K1} gave better result than the one
685 at 4425 cm^{K1}. The mid range IR spectra of triglycerides and methyl esters of fatty acids
686 are almost identical and offer no possibility for distinguishing.

687 NIR spectra were obtained with the aid of a fibre-optic probe coupled to the
688 spectrometer, which render their acquisition particularly easy and time-efficient.

689 While the first NIR paper used a model system to describe monitoring of
690 transesterification and for developing quantitation methods, a second paper applied the
691 method to a transesterification reaction in progress on a 6L scale. Here spectroscopic results
692 were obtained not only by NIR but also by ¹H-NMR and NIR [30]. The results of both
693 spectroscopic methods, which can be correlated by simple equations, were in good
694 agreement. Two NMR approaches were used, one being the use of methyl esters protons
695 (peak at 3.6 ppm) and the protons on the carbons next to the glyceryl moiety (α-CH₂; peaks
696 at 2.3 ppm). The second approach was the use of methyl ester protons and the protons of the
697 glyceryl moiety (peaks at 4.1-4.4 ppm) in the triglycerides using the equation:

$$698 \quad C \sim 100 \times \frac{5 \times I_{ME}}{5 \times I_{ME} + 9 \times I_{TAG}} \quad 699$$

700 where C, percentage conversion of triglycerides to corresponding methyl ester; I_{ME},
701 integration value of the methyl esters peak; ITAG, integration value of the glyceridic peaks in
702 the triacylglycerol.
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7. Fuel properties and specification of biodiesel

708 Since biodiesel is produced in quite differently scaled plants from vegetable oils of
709 varying origin and quality, it was necessary to install a standardization of fuel quality to
710 guarantee engine performance without any difficulties. Austria was the first country in the
711 world to define and approve the standards for rapeseed oil methyl esters as diesel fuel. As
712 standardization is a prerequisite for successful market introduction and penetration of
713 biodiesel, standards or guidelines for the quality of biodiesel have also been defined in
714 other countries like Germany, Italy, France, the Czech Republic and in the United states.

715 The parameters, which define the quality of biodiesel, can be divided into two groups.
716 One group contains general parameters, which are also used for mineral oil based fuel, and
717 the other group especially describes the chemical composition and purity of fatty acid
718 alkyl esters [41]. Table 1 contains the general and Table 2 the vegetable oil specific
719 parameters and the corresponding value of fatty acid methyl esters according to standards
720 of above countries.

721 Table 1
722 General parameters of the quality of biodiesel

723 Parameters	Austria (ON)	Czech 724 republic 725 (CSN)	France (journal offi- cial)	Germany (DIN)	Italy (UNI)	USA (ASTM)
726 Density at 15 8C g/cm ³	0.85-0.89	0.87-0.89	0.87-0.89	0.875-0.89	0.86-0.90	—
727 Viscosity at 40 mm ² /s	3.5-5.0	3.5-5.0	3.5-5.0	3.5-5.0	3.5-5.0	1.9-6.0
728 Flash point (8C)	100	110	100	110	100	130
729 CFPP (8C)	0/K5	K5	-	0-10/K20	-	-
730 Pour point (°C)	-	-	K10	-	0/-5	-
731 Cetane 732 number	R49	R48	R49	≥49	—	R47
733 Neutraliz- 734 ation 735 number (mgKOH/g)	%0.8	%0.5	%0.5	%0.5	%0.5	%0.8
736 Conradson 737 carbon resi- 738 due (%)	0.05	0.05	—	0.05	—	0.05

745 Table 2
746 Vegetable oil specific parameters for the quality of biodiesel

747 Parameters	Austria (ON)	Czech 748 republic 749 (CSN)	France (journal offi- cial)	Germany (DIN)	Italy (UNI)	USA (ASTM)
750 Methanol/ 751 ethanol (% mass)	≤0.2	—	%0.1	%0.3	≤0.2	-
752 Ester content (% mass)	-	—	≥96.5	-	≥98	-
753 Monoglycer- 754 ide (% mass)	-	—	%0.8	%0.8	≤0.8	-
755 Diglyceride (% mass)	-	-	%0.2	%0.4	≤0.2	-
756 Triglyceride (% mass)	-	-	%0.2	%0.4	≤0.1	-
757 Free glycerol (% mass)	%0.02	%0.02	%0.02	%0.02	%0.05	%0.02
758 Total 759 glycerol (% mass)	%0.24	%0.24	%0.25	≤0.25	-	%0.24
760 Iodine num- 761 ber	≤120	-	≤ 115	≤ 115	-	-

766 Among the general parameters for biodiesel, the viscosity controls the characteristics of
767 the injection from the diesel injector. The viscosity of fatty acid methyl esters can go very
768 high levels and hence it is important to control it within an acceptable level to avoid
769 negative impacts on fuel injector system performance. Therefore, the viscosity
770 specifications proposed are nearly same as that of the diesel fuel.

771 Flash point of a fuel is the temperature at which it will ignite when exposed to a flame or
772 spark. The flash point of biodiesel is higher than the petrodiesel, which is safe for transport
773 purpose.

774 Cold filter plugging point (CFPP) of a fuel reflects its cold weather performance. At low
775 operating temperature fuel may thicken and might not flow properly affecting the
776 performance of fuel lines, fuel pumps and injectors. CFPP defines the fuels limit of
777 filterability, having a better correlation than cloud point for biodiesel as well as
778 petrodiesel. Normally either pour point or CFFP are specified. Pour point is the lowest
779 temperature at which the oil specimen can still be moved. French and Italian biodiesel
780 specifications specify pour point where as others specify CFPP.

781 Cetane number is indicative of its ignition characteristics. The cetane number measures
782 how easily ignition occurs and the smoothness of combustion. Higher the cetane number
783 better it is in its ignition properties. Cetane number affects a number of engine
784 performance parameters like combustion, stability, driveability, white smoke, noise and
785 emissions of CO and HC. Biodiesel has higher cetane number than conventional diesel
786 fuel, which results in higher combustion efficiency.

787 Neutralization number is specified to ensure proper ageing properties of the fuel and/or
788 a good manufacturing process. It reflects the presence of free fatty acids or acids used in
789 manufacture of biodiesel and also the degradation of biodiesel due to thermal effects.

790 Carbon residue of the fuel is indicative of carbon depositing tendencies of the fuel.
791 Conradsons Carbon Residue for biodiesel is more important than that in diesel fuel
792 because it shows a high correlation with presence of free fatty acids, glycerides, soaps,
793 polymers, higher unsaturated fatty acids and inorganic impurities.

794 The presence of high level of alcohol in biodiesel cause accelerated deterioration of
795 natural rubber seals and gaskets. Therefore control of alcohol content is required.
796 Biodiesel fuel is mainly consists of fatty acid alkyl esters and its quantities are specified
797 according to the specifications of various countries. The presence of mono- di- and tri-
798 glycerides cause engine problems like fuel filter plugging affecting the fuel properties and
799 are specified in most of the biodiesel standards.

800

801 7.1. Biodiesel storage stability

802

803 One of the main criteria for the quality of biodiesel is the storage stability. Vegetable oil
804 derivatives especially tend to deteriorate owing to hydrolytic and oxidative reactions.
805 Their degree of unsaturation makes them susceptible to thermal and/or oxidative
806 polymerization, which may lead to the formation of insoluble products that cause
807 problems within the fuel system, especially in the injection pump. The storage stability of
808 biodiesel prepared from rapeseed oil was investigated by Mittelbach and Gangl [42]. The
809 neutralization number and peroxide number of the biodiesel were observed on long-term
810 storage.

811 Bondioli et al. [43] have presented the results obtained from a long-term storage study
812 using eleven different biodiesel samples. These samples prepared from several feedstocks
813 using different manufacturing technologies, some containing an antioxidant additive, were
814 stored in 200 L drums. These were periodically monitored during the complete storage
815 period by analysis of fifteen different properties.

816 Several properties do not show any significant change during storage, while others such
817 as viscosity, peroxide value and more dramatically, Rancimat Induction Period
818 demonstrated changes related to the nature of the starting product.

819 A parallel test, carried out in simulated wrong storage conditions lead to some strong
820 changes in biodiesel composition and can be used as a guide for devising biodiesel
821 production set-up, storage and distribution chain.

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825 8. Conclusion

826

827 Biodiesel has become more attractive to replace petroleum fuel. As per the reported
828 literature, most of the transesterification studies have been done on edible oils like
829 rapeseed, soybean, sunflower, canola etc. by using methanol and NaOH/KOH as catalyst.
830 There are very few studies reported on non-edible oils, which are produced in the
831 wastelands/wild species like *Pongamia pinnata*, *Jatropha curcas*, *Simarouba glauca*, etc.
832 The alkaline catalyzed transesterification is the promising area of research for production
833 of biodiesel but for high free fatty acid content non-edible oils, it is one of the main
834 constrains. The use of heterogeneous catalysts is a new area in the development of suitable
835 catalysts in the transesterification of high FFA oil. Ethanolysis is one of the important
836 aspects for biodiesel production as ethanol is derived from renewable biomass. But, the
837 yield of fatty acid ethyl esters is less compared to methyl esters as well as separation of
838 glycerol is the main constrains in the process of ethanolysis. This process can be further
839 improved.

840 The quality of biodiesel is most important for engine part of view and various standards
841 have been specified to check the quality. As per the analytical method reported in
842 literature, high performance liquid chromatography method is suitable to analyze the
843 reaction intermediates and products of transesterification reaction.

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