

CHAPTER II

THEORETICAL BACKGROUND AND LITERATURE REVIEW

2.1 Vegetable oils

Vegetable oils are triglycerides which are esters of one glycerol with three long-chain acids (which can be different types), commonly called fatty acids. The major component of vegetable oils is triglycerides. Vegetable oils comprise 90 to 98% triglycerides and small amounts of mono- and diglycerides. They contain free fatty acids (generally 1 to 5%) and traces of water (Srivastava and Prasad, 2000). The general formation of triglyceride is shown in Figure 2.1.

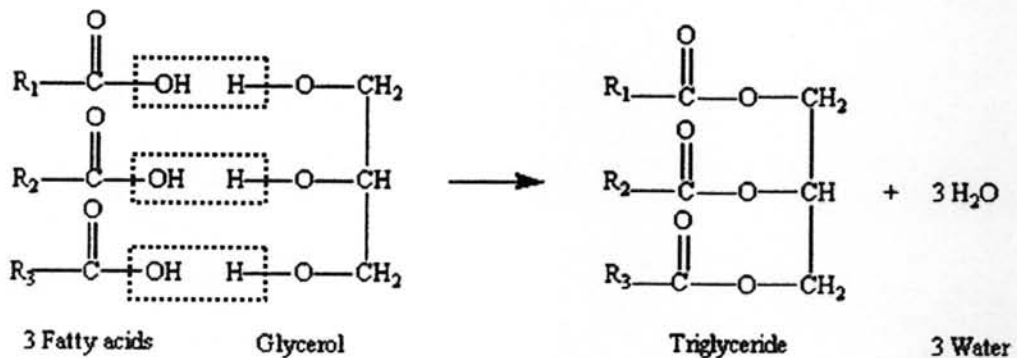


Figure 2.1 Formation of triglyceride.

In Figure 2.1, R_1 , R_2 , and R_3 represent a chain of carbon atoms with hydrogen atoms attached. The differences between R_1 , R_2 , and R_3 result from differences of fatty acids which glycerol backbone will be combined. Fatty acids vary in their carbon chain length and in the number of double bonds, which leads to different characteristics of vegetable oil. The common fatty acids found in vegetable oils are stearic, palmitic, oleic, linoleic and linolenic. The names and chemical structures of common fatty acids are shown in Table 2.1. The compositions of fatty acid in vegetable oils are summarized in Table 2.2 (Srivastava and Prasad, 2000). Some properties of the vegetable oils are shown in Table 2.3 (Marchetti *et al.*, 2005).

Table 2.1 Chemical structure of common fatty acids (Srivastava and Prasad, 2000)

Fatty acid	Systematic name	Structure ^a	Formula
Lauric	Dodecanoic	12:0	C ₁₂ H ₂₄ O ₂
Myristic	Tetradecanoic	14:0	C ₁₄ H ₂₈ O ₂
Palmitic	Hexadecanoic	16:0	C ₁₆ H ₃₂ O ₂
Stearic	Octadecanoic	18:0	C ₁₈ H ₃₆ O ₂
Arachidic	Eicosanoic	20:0	C ₂₀ H ₄₀ O ₂
Behenic	Docosanoic	22:0	C ₂₂ H ₄₄ O ₂
Lignoceric	Tetracosanoic	24:0	C ₂₄ H ₄₈ O ₂
Oleic	cis-9-Octadecenoic	18:1	C ₁₈ H ₃₄ O ₂
Linoleic	cis-9,cis-12-Octadecadienoic	18:2	C ₁₈ H ₃₂ O ₂
Linolenic	cis-9,cis-12,cis-15-Octadecatrienoic	18:3	C ₁₈ H ₃₀ O ₂
Erucic	cis-13-Docosenoic	22:1	C ₂₂ H ₄₂ O ₂

^axx:y indicates x carbons in fatty acid chain with y double bonds

Table 2.2 Fatty acid composition of vegetable oils (Srivastava and Prasad, 2000)

Vegetable oil	Fatty acid composition, wt. %									
	14:0	16:0	18:0	20:0	22:0	24:0	18:1	22:1	18:2	18:3
Corn	0	12	2	Tr	0	0	25	0	6	Tr
Cottonseed	0	28	1	0	0	0	13	0	58	0
Crambe	0	2	1	2	1	1	19	59	9	7
Linseed	0	5	2	0	0	0	20	0	18	55
Peanut	0	11	2	1	2	1	48	0	32	1
Rapeseed	0	3	1	0	0	0	64	0	22	8
Safflower	0	9	2	0	0	0	12	0	78	0
H.O. Safflower	Tr	5	2	Tr	0	0	79	0	13	0
Sesame	0	13	4	0	0	0	53	0	30	0
Soya bean	0	12	3	0	0	0	23	0	55	6
Sunflower	0	6	3	0	0	0	17	0	74	0

^aTr = traces.

Table 2.3 Properties of the vegetable oils

Vegetable oil	Kinematics viscosity (mm ² /s)	Cetane number	Cloud point (°C)	Pour point (°C)	Flash point (°C)	Density (kg/l)	Lower heating value (MJ/kg)
Peanut	4.9	54	5	-	176	0.883	33.6
Soya bean	4.5	45	1	-7	178	0.885	33.5
Babassu	3.6	63	4	-	127	0.875	31.8
Palm	5.7	62	13	-	164	0.880	33.5
Sunflower	4.6	49	1	-	183	0.860	33.5
Tallow	-	-	12	9	96	-	-
Diesel	3.06	50	-	-16	76	0.855	43.8
20% bio-diesel blend	3.2	51	-	-16	128	0.859	43.2

2.2 Derivatives of Triglycerides as Diesel Fuels

There are many problems associated with the use of vegetable oils (triglycerides) as fuels for diesel engines, such as high viscosity, low volatility and polyunsaturated fatty acids. The properties and performance of vegetable oil derivatives can be improved by using many ways to change their properties, but there are four major ways as follows:

2.2.1 Pyrolysis

Pyrolysis refers to a chemical change caused by the application of thermal energy under the absence of air or oxygen (Fangrui and Hanna, 1999). Many investigators have studied the pyrolysis of triglycerides to obtain products suitable for diesel engines. Those studies include the effect of temperature on the type of products obtained, the use of catalysts, largely metallic salts, to obtain paraffins and olefins similar to those present in petroleum-based diesel fuels, and the characterization of the thermal decomposition products. The thermal decomposition of triglycerides

produces the compounds of classes including alkanes, alkenes, alkadienes, aromatics and carboxylic acids. Different types of vegetable oils produce large differences in the composition of the thermally decomposed oil (Srivastava and Prasad, 2000). The selectivities of cracking products as a function of pyrolysis temperature are shown in Table 2.4 (Fangrui and Hanna, 1999).

Table 2.4 Selectivities of cracking products as a function of pyrolysis temperature

	Selectivity (molar % of carbon atoms cracked)						
	550°C	600°C	650°C	700°C	750°C	800°C	850°C
C ₁ -C ₄ cut	10.0	18.6	28.2	38.7	35.1	45.1	66.1
C ₅ -C ₉ cut	36.0	19.6	17.6	13.2	17.5	12.6	3.6
C ₁₀ -C ₁₄ cut	3.0	3.5	3.5	2.7	1.7	1.0	0.3
C ₁₅ -C ₁₈ cut	0.9	0.7	0.3	1.1	0.3	0.2	0.3
Aromatics	5.2	2.0	2.7	3.9	7.2	11.6	8.9
C _{3:1} -C _{8:1} esters	8.5	16.6	10.3	7.2	5.9	4.1	0.9
C _{9:1} -C _{16:1} esters	2.3	3.2	3.4	2.3	0.9	0.5	0.3
Saturated esters	2.0	1.2	1.6	2.4	3.7	3.1	2.6
CO	0.5	1.2	1.3	2.3	2.7	3.8	5.3
CO ₂	0.3	0.6	0.6	1.1	1.5	1.6	2.1
Coke	6.1	3.8	4.2	4.7	2.2	3.1	4.5
Other products	25.2	29.0	25.3	20.4	21.3	13.3	5.1
	Selectivity (molar % of hydrogen atoms cracked)						
H ₂	0.3	0.9	1.7	2.7	3.6	4.6	5.9

2.2.2 Microemulsification

Microemulsions are isotropic, clear, or translucent thermodynamically-stable dispersions of oil, water, surfactant, and often a small amphiphilic molecule, called a co-surfactant. The droplet diameters in microemulsions range from 10 to 100 nm. A microemulsion can be made of vegetable oils with an ester and dispersant (co-solvent), or of vegetable oils, an alcohol and a surfactant, with or

without diesel fuels. It was found that microemulsions with an immiscible liquid, such as methanol, and amphiphiles in vegetable oil are used to solve the problem of the high viscosity of vegetable oil (Srivastava and Prasad, 2000). It can perform nearly as well as diesel fuels.

2.2.3 Dilution or Blending

The dilution of vegetable oils can be accomplished with such materials as diesel fuels, a solvent or ethanol (Srivastava and Prasad, 2000). For example, the dilution of sunflower oil with diesel fuels in the ratio of 1:3 by volume has been studied in engine tests. The viscosity of this blend was 4.88 cSt at 40°C. Although the dilution method gives proper properties of vegetable oils for use in direct injection diesel engines, the blend can not be recommended for long-term use because they have many negative effects on diesel engines, such as severe injector nozzle coking and sticking. This fuel produces heavy carbon deposit on the tulips of the intake valves and shows considerable top ring wear.

2.2.4 Transesterification

Transesterification, or alcoholysis, is the reaction of a fat or oil with an alcohol to form esters and glycerol. A catalyst (a strong acid or base) is usually used to improve the reaction rate and yield. This process has been widely used to reduce the high viscosity of triglycerides (Meher *et al.*, 2006). The transesterification reaction is represented by the general equation given in Figure 2.2. The alcohols used in the transesterification process are methanol, ethanol, propanol, butanol and amyl alcohol. Methanol and ethanol are used most frequently, especially methanol, because of its low cost and physical and chemical advantages (polar and shortest chain alcohol). It can quickly react with triglycerides, and NaOH is easily dissolved in it. Because transesterification is a reversible reaction and proceeds essentially by mixing the reactants, excess alcohol is required to shift the equilibrium to the products side (Fangrui and Hanna, 1999). To complete the transesterification stoichiometrically, a 3:1 molar ratio of alcohol to triglycerides is needed. In practice, the ratio needs to be higher to drive the equilibrium to a maximum ester yield. If methanol is used in this process, it is called methanolysis, as presented in Figure 2.3.

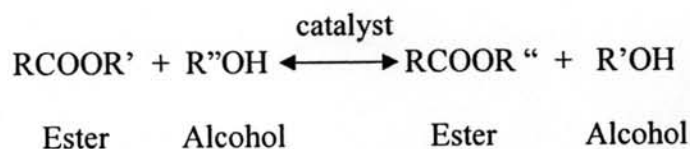


Figure 2.2 General equation for a transesterification reaction.

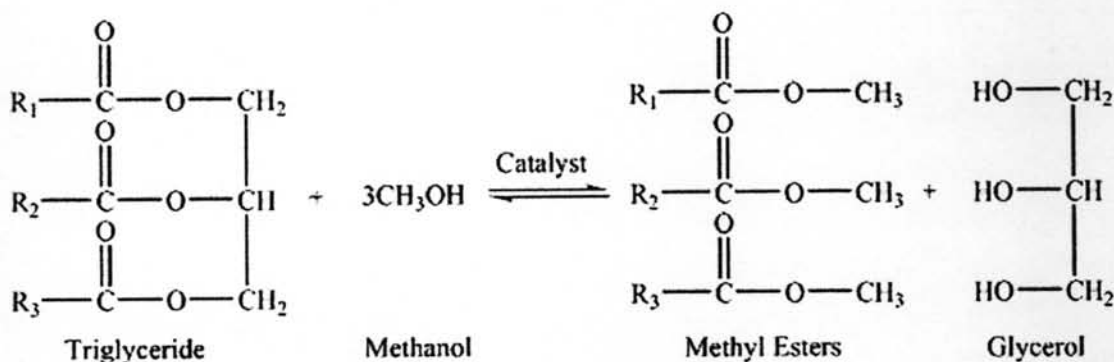


Figure 2.3 Methanolysis of triglyceride reaction.

2.3 Synthesis of Ester

In chemistry, esters are a class of organic compounds which, unlike many organics, have pleasant odors. In fact, many of the artificial flavors used in food products are esters. Esters not only occur naturally but can also be synthesized in the lab. The ester is formed when dehydration occurs. The alcohol and acid react to form the ester and a molecule of water. The $-\text{OH}$ of carboxyl group (acid) has been replaced by OR' of alcohol. The general formula of esters is shown in Figure 2.4.

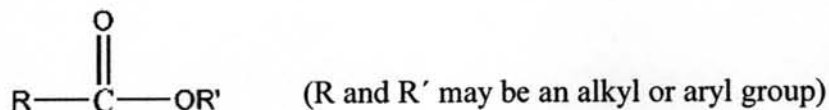


Figure 2.4 General formula of esters.

Esters are usually prepared by the most common reaction, esterification, as shown in Figure 2.5.

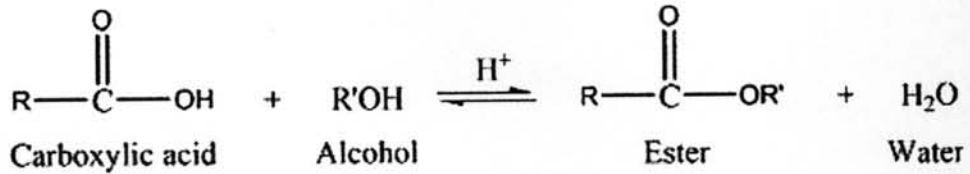


Figure 2.5 Esterification reaction.

2.4 Biodiesel

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

The properties of biodiesel and diesel fuels are compared in Table 2.5. Biodiesel fuels produced from various vegetable oils have viscosities close to those of diesel. Their volumetric heating values are a little lower, but they have high cetane numbers and flash points. Since the characteristics of biodiesel are generally similar to those of diesel, the former is a strong candidate to replace diesel if the need arises.

There are many advantages for using biodiesel, such as it being a renewable resource, its limitation on greenhouse gas emissions because of the closed CO₂ cycle, lower sulfur content, biodegradability, use without engine modification, good engine performance, improved combustion because of its oxygen content, low toxicity, good lubrication properties, and finally its ability to be blended in any proportion with regular petroleum-based diesel fuels.

Vegetable oils usually contain free fatty acids, phospholipids, sterols, water, odorants and other impurities. Because of these, the oil cannot be used as a fuel directly. To overcome these problems, the oil requires slight chemical modification, mainly through pyrolysis, dilution, microemulsion, and transesterification, as previously described. Among these, the transesterification is the key and important step to produce cleaner and environmentally safe fuel from vegetable oils (Meher *et al.*, 2006).

Table 2.5 Comparison of some properties of biodiesel and diesel fuel (Fukuda *et al.*, 2001)

Vegetable oil methyl ester	Kinematic viscosity (mm ² /s)	Cetane number	Lower heating value (MJ/l)	Cloud point (°C)	Flash point (°C)	Density (g/l)	Sulfur (wt%)
Peanut	4.9 (37.8°C)	54	33.6	5	176	0.883	-
Soybean	4.5 (37.8°C)	45	33.5	1	178	0.885	-
Babassu	3.6 (37.8°C)	63	31.8	4	127	0.879	-
Plam	5.7 (37.8°C)	62	33.5	13	164	0.880	-
Sunflower	4.6 (37.8°C)	49	33.5	1	183	0.860	-
Rapeseed	4.2 (40°C)	51-59.7	32.8	-	-	0.882	-
Used rapeseed	9.48 (30°C)	53	36.7	-	192	0.895	0.002
Used corn oil	6.23 (37.0°C)	63.9	42.3	-	166	0.884	0.0013
Diesel fuel	12-3.5 (40°C)	51	35.5	-	-	0.830-0.840	-
JIS-2D (gas oil)	2.8 (30°C)	58	42.7	-	59	0.833	0.05

2.5 Transesterification of Vegetable Oils

In the transesterification of vegetable oils, a triglyceride reacts with an alcohol in the presence of a strong acid or base, producing a mixture of fatty acids,

alkyl esters, and glycerol. The stoichiometric reaction requires 1 molecule of a triglyceride and 3 molecules of the alcohol. The overall transesterification reaction is shown in Figure 2.6. However, an excess of alcohol is used to increase the yields of the alkyl esters and to allow its phase separation from the glycerol formed (Schuchardt *et al.*, 1998).

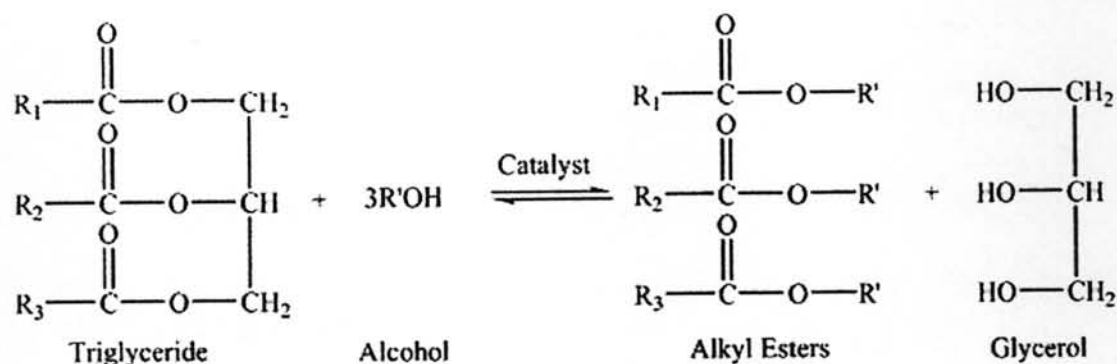


Figure 2.6 Overall transesterification reaction.

Many types of alcohol can be used in the transesterification reaction, such as methanol, ethanol, propanol, butanol, and amyl alcohol. Methanol and ethanol are used most frequently, especially methanol, because of its lowest cost when compared to other alcohols, and because of its physical and chemical advantages (polar and shortest chain alcohol). It can quickly react with triglyceride, resulting in a higher conversion of alkyl esters (Fangrui and Hanna, 1999).

Transesterification consists of a sequence of three consecutive and reversible reactions. The step reactions are shown in Figure 2.7. In the first step, triglyceride is converted to diglyceride. In the second, diglyceride is converted to monoglyceride, and then finally, in the third step, monoglyceride is converted to glycerol. For each step, one molecule of methyl ester is liberated (Srivastava and Prasad, 2000).

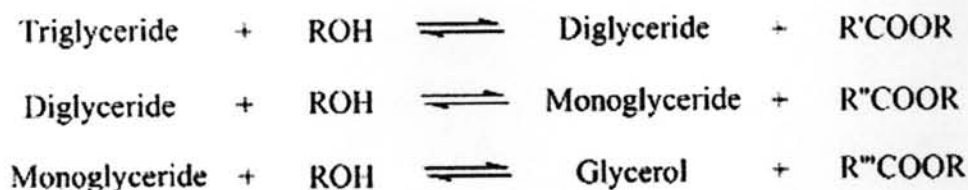


Figure 2.7 Transesterification reaction of vegetable oil (Meher *et al.*, 2006).

The transesterification reaction can be catalyzed by alkalis (basic), acid, or enzymes in order to increase the rate of reaction and yield of alkyl esters. Sodium hydroxide and potassium hydroxide are used most frequently as alkali catalysts. Sulfuric acid and hydrochloric acid are usually used as acid catalysts. Moreover, the transesterification reaction can be achieved without a catalyst, by the supercritical method.

A review done by Srivastava and Prasad (2000) summarizes the important variables that influence transesterification and ester conversion. These variables are reaction temperature, ratio of alcohol to oil, catalyst type and concentration, mixing intensity, and purity of reactants. The maximum yield of alkyl esters occurs at a temperature close to the boiling point of the alcohol used, at a molar ratio (alcohol to oil) of 6:1. They also pointed out that a basic homogeneous catalyst like sodium alkoxide is the most effective for transesterification. However, the presence of free fatty acids and water can cause some side reactions, thereby affecting the ester conversion.

2.5.1 Transesterification using Base Catalyst

Typically, the base-catalyzed transesterification of vegetable oils proceeds faster than the acid-catalyzed reaction. Because of this, together with the fact that the alkaline catalysts are less corrosive than acidic compounds, industrial processes usually prefer base catalysts, such as sodium hydroxide, potassium hydroxide and corresponding sodium and potassium alkoxide such as sodium methoxide and potassium methoxide. The mechanism for the base-catalyzed transesterification of vegetable oils is shown in Figure 2.8 (Schuchardt *et al.*, 1998). The first step is the reaction of the base with the alcohol, producing an alkoxide (the type of alkoxide

be dried (moisture level <0.06%) and free fatty acid content must be <0.05 (Zullai-kah *et al.*, 2005).

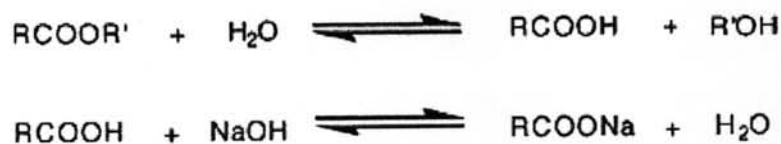


Figure 2.9 Saponification of fatty acid alkyl ester.

Foidl *et al.* (1996) prepared biodiesel from *Jatropha curcas L.* They found that 1850 g of pure methyl esters were obtained when using 2-step transesterification at 30°C with KOH as a catalyst. The ester fuel can be used directly in existing engines without modifications. It has high qualities and meets the existing standards for vegetable-oil-derived fuels.

Karmee *et al.* (2005) prepared biodiesel from the non-edible oil of *Pongamia pinnata* by transesterification of the crude oil with methanol in the presence of KOH as a catalyst. They found that the maximum conversion of 92% (oil to ester) was achieved using a 1:10 molar ratio of oil to methanol at 60°C. And if they used tetrahydrofuran (THF) as a co-solvent, the conversion was increased to 95%.

2.5.2 Transesterification using Acid Catalyst

Acid-catalyzed transesterification is suitable for starting vegetable oil, which has a high level of water and free fatty acid, but the rate of reaction is slower than the base-catalyzed transesterification. The mechanism of the acid-catalyzed transesterification of vegetable oils is shown in Figure 2.10. First is the protonation of the carbonyl group of the ester, which leads to the carbocation II which, after a nucleophilic attack of the alcohol, produces the tetrahedral intermediate III, which eliminates glycerol to form the new ester IV, and to regenerate the catalyst H^+ (Schuchardta *et al.*, 1998).

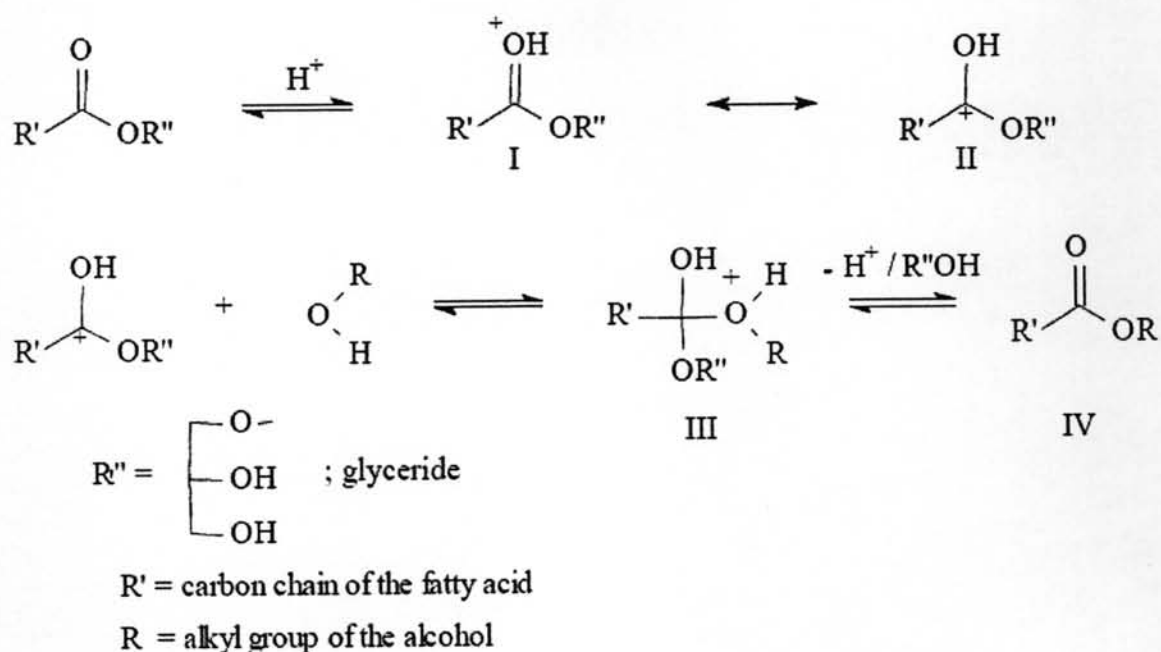


Figure 2.10 Mechanism of the acid-catalyzed transesterification of vegetable oils.

2.5.3 Transesterification using Lipase Catalyst

Lipase can also be used as a biocatalyst to catalyze the transesterification of vegetable oil. It is one type of enzyme that exists in vegetable oil and fat. Moreover, lipase can synthesize many types of esters, depending on the type of alcohol used in the reaction.

Salis *et al.* (2005) investigated *Candida antarctica B*, *Rizhomucor miehei*, and *Pseudomonas cepacia lipase* in transesterification of pure triolein. They found that *Pseudomonas cepacia lipase* seemed to be the most active enzyme, reaching 100% of conversion after 6 hours.

Noureddini *et al.* (2005) used nine different types of lipases as biocatalysts in the transesterification of soybean oil. They found that lipase PS from *Pseudomonas cepacia* resulted in the highest yield of alkyl esters.

2.5.4 Supercritical Transesterification

Supercritical transesterification has two major steps for converting the vegetable oil to biodiesel which are transesterification of triglyceride and esterification of free fatty acid. These two reactions occur at the same time. Supercritical transesterification has many advantages, such as short reaction time, high yield of biodiesel, ease of separation of the biodiesel from glycerol, and no effect with water and free fatty acid in the starting vegetable oil. Although this process has many advantages, it is not commercialized now because it is very expensive due to the high pressure and temperature required for the process.

Although the transesterification of vegetable oil using homogeneous catalyst is an available commercial technology, it has major problems, such as the removal of the soluble catalyst, the formation of side products, and the unrecovery of catalyst. These hurdles have led to the study of other possible methods for biodiesel production.

2.5.5 Transesterification using Heterogeneous Catalyst

Nowadays, many researchers are attempting to use a heterogeneous catalyst for replacing a homogeneous catalyst because heterogeneous catalysts are easily separated out from the products, leading to a high yield of biodiesel and no waste from the washing step. Peterson and Scarrah (1984) discovered that the CaO.MgO catalyst has activity comparable to the sodium methoxide catalyst, with 95% methyl ester yield. However, this catalyst produces substantial amounts of saponified product, thereby reducing the effectiveness of this heterogeneous catalyst.

Gryglewicz (1999) found that the basic alkaline-earth metal compounds can be effectively used as a catalyst in the transesterification of rapeseed oil by methyl alcohol. The reaction increases in the order of $\text{CaO} < \text{Ca}(\text{CH}_3\text{O})_2 < \text{Ba}(\text{OH})_2$. In spite of its high activity, $\text{Ba}(\text{OH})_2$ is not recommended to be used because it is much more soluble in methanol than other alkaline earth metal compounds. Moreover, the soluble $\text{Ba}(\text{OH})_2$ compounds are highly toxic. Although calcium catalysts are less active than sodium hydroxide, they are much cheaper and lead to a decrease in the number of technical stages and the amount of unwanted waste products.

Leclerq *et al.* (2001) investigated the basic zeolites and solid catalysts in the transesterification of rapeseed oil. They found that magnesium oxide, with a high surface area over $300 \text{ m}^2/\text{g}$, is far more active than cesium-exchanged NaX faujasites and hydrotalcites.

Kim *et al.* (2004) investigated a Na/NaOH/ γ - Al_2O_3 heterogeneous catalyst and a NaOH homogeneous catalyst in the transesterification of vegetable oil. They found that the NaOH catalyst gave a higher yield of biodiesel than the Na/NaOH/ γ - Al_2O_3 catalyst. Moreover, they studied the effect of co-solvent and the optimum conditions. They found that if they used n-hexane as a co-solvent with the Na/NaOH/ γ - Al_2O_3 catalyst, the biodiesel production yield was increased by 10% at the optimum conditions.

Suppes *et al.* (2004) studied the transesterification of soybean oil with methanol at 60, 120, and 150°C in the presence of a series of NaX faujasite zeolite, ETS-10 zeolite, and metal catalysts. They found that the ETS-10 catalysts provided higher conversions than the Zeolite-X type catalysts. The increased conversions were attributed to the higher basicity of the ETS-10 zeolites and the larger pore structures improved intra-particle diffusion. Methyl ester yield increased with an increase in reaction temperature from 60 to 150°C.

Furuta *et al.* (2004) investigated the sulfated tin and zirconium oxides and tungstated zirconia as a solid superacid catalyst in the transesterification of soybean oil with methanol at 200–300°C and the esterification of n-octanoic acid with methanol at 175–200°C. They found that the tungstated zirconia–alumina gave a high performance in the conversions, over 90%.

Lopez *et al.* (2005) investigated the kinetics and selectivities of different solid catalysts for the transesterification of triacetin with methanol. Reaction was carried out at 60°C in a batch reactor with a variety of solid and liquid, acid and base catalysts. The homogeneous catalysts NaOH and H_2SO_4 were compared with Amberlyst-15, Nafion NR50, sulfated zirconia, and ETS-10 (Na, K) as a heterogeneous catalyst. They found that the reactivity of the acid catalysts was in the order of $\text{H}_2\text{SO}_4 > \text{Amberlyst-15} > \text{SZ} > \text{Nafion NR50} > \text{WZ} > \text{SPA} > \text{zeolite Hb} >$

ETS-10 (H), and the reactivity of the basic catalyst was in the order of $\text{NaOH} > \text{ETS-10 (H)} > \text{MgO}$.

Xie *et al.* (2006) studied the effects of catalyst loading, oil to methanol ratio, reaction time, and temperature on the conversion of soybean oil. They used alumina loaded with potassium as a solid base catalyst in a heterogeneous system and found that the catalyst loaded KNO_3 of 35 wt.% on Al_2O_3 , after being calcined at 500°C for 5 hours, was the suitable catalyst. It had the highest basicity and gave the best catalytic activity for this reaction. The best conditions are a molar ratio of methanol to soybean oil of 15:1, at a reaction time of 7 hours, and a catalyst amount of 6.5%, showing the highest conversion of 87%.

Jitputti *et al.* (2006) investigated several acid and base solids, such as ZrO_2 , ZnO , $\text{SO}_4^{2-}/\text{SnO}_2$, $\text{SO}_4^{2-}/\text{ZrO}_2$, KNO_3/KL zeolite and $\text{KNO}_3/\text{ZrO}_2$ as heterogeneous catalysts for the transesterification of crude palm kernel oil (PKO) and crude coconut oil (CCO) with methanol. They found that ZnO and $\text{SO}_4^{2-}/\text{ZrO}_2$ exhibited the highest activity for both PKO and CCO transesterification. In the case of $\text{SO}_4^{2-}/\text{ZrO}_2$, only 1 wt% of this acid solid catalyst was needed to catalyze the reaction, and resulted in a fatty acid methyl ester content higher than 90%. Moreover, a study of the catalyst's recyclability indicated that the spent $\text{SO}_4^{2-}/\text{ZrO}$ cannot be directly reused for the transesterification. However, this spent catalyst can be easily regenerated and the same activity can be obtained.

2.6 Analysis of Methyl Esters

Many analytical methods were developed for analyzing mixtures containing fatty acid esters and mono-, di-, and tri-glycerides obtained by the transesterification of vegetable oils due to incomplete transesterification and insufficient purification. A sensitive and reliable method is needed to monitor the purity of the methyl esters, such as gas chromatography (GC) and High Performance Liquid Chromatography (HPLC).

In principle, glycerol, mono-, di- and tri-glycerides can be analyzed on a highly inert column coated with a polar stationary phase.

Mittelbach (1993) used a gas chromatograph (GC) equipped with a flame ionization detector. The GC capillary column used was fused silica, 60 m x 0.25 mm, 0.25 μm DB-5 (J and W Scientific Inc.). The sample was prepared by mixing 100–500 ml of rapeseed methyl ester (depending upon glycerol content) with 100 ml of 1,4-butanediol standard solution in N,N-dimethylformamide (approximately 0.1 mg 1,4-butanediol/ml) and 600–200 ml of N,N-dimethylformamide. Finally 200 ml of N,O-bis(trimethylsilyl) trifluoroacetamide is added to reach the final volume of 1 ml. The mixture was shaken vigorously and 2 ml aliquots were injected in the GC after at least 10 min.

Plank and Lorbeer (1995) used a gas chromatograph (GC) equipped with a flame ionization detector. A DB-5 (10 m x 0.32 mm I.D.) fused-silica capillary column coated with a 0.25 μm film was used. Samples (1 μl) were injected on-column by an AS 800 automatic sampler (Fisons Instruments) at an oven temperature of 50°C. After an isothermal period of 1 min, the GC oven was heated at 15°/min to 180°C, at 7°/min to 230°C, and ballistically to 370°C (holding for 10 min). Hydrogen was used as carrier gas at a flow rate of 3 ml/min (measured at 50°C). The detector temperature was 370°C. Nitrogen served as the detector make up gas at an inlet pressure of 0.5 bar. Run time was 30 min.

HPLC is a technique that has many advantages compared to GC, due to shorter analysis time and because reagent consuming derivatization is not necessary.

Exconde (2002) used an HPLC equipped with a refractive index detector to analyze the biodiesel products. A Zorbex Eclipse XDB-C18 column (4.6 mm x 250 mm x 5 μm) was used and the mobile phase was an acetone/acetonitrile mixture (50:50 vol/vol).

The composition of the biodiesel products was determined by HPLC using a Perkin-Elmer consisting of a model Series 200 LC-pump and a refractive index Series 200 detector. It was controlled by a PC with software (Perkin-Elmer Turbochrom Navigator) (Jitputti *et al.*, 2006). A Zorbax Eclipse XDB-C18 column (4.6mm x 250mm x 5 μm) was used and the mobile phase was an acetone/acetonitrile mixture (70:30 v/v) at a flowrate of 0.7 ml/min. The biodiesel samples were diluted with acetone (HPLC grade) and the injection volume was 20 μl .