CHAPTER II

THEORY AND LITERATURE REVIEWS

2.1 Rice bran and rice bran oil

Bran is the hard outer layer of grain along with germ. Rice bran is a by-product of the rice milling process (the conversion of brown rice to white rice), and it contains various antioxidants that impart beneficial effects on human health.

Rice bran oil is the oil extracted from the germ and inner husk of rice. It is notable for its very high smoke point of 490°F (254°C) and its mild flavor, making it suitable for high-temperature cooking methods such as stir frying and deep frying [12].It is popular as a cooking oil in several Asian countries. Rice bran oil contains a range of fats, with 47% of its fats monounsaturated, 33% polyunsaturated, and 20% saturated [13]. The fatty acid composition of rice bran oil and world production rice are shown in Table 2.1 and Table 2.2 respectively.

Table 2.1 Percentage of fatty acid in rice bran oil [13]

Fatty acid	Percentage
Palmitic (16:0)	15.0%
Stearic (18:0)	1.9%
Oleic (18:1)	42.5%
Linoleic (18:2)	39.1%
Linolenic (18:3)	1.1%
Arachidic (20:0)	0.5%
Behenic (22:0)	0.2%

Table 2.2 World production rice in 2003/2004 year [14]

Consumption of rice by country—2003/2004 (million metric ton)	
China	135
India	85
Indonesia	37
Bangladesh	26
Vietnam	18
Thailand	10
Myanmar	10
Philippines	9.7
Japan	8.7
Brazil	8.1
South Korea	5.0
United States	3.9

The structure of the rice kernel is shown in Figure 2.1. The bran fraction, which includes the germ or embryo in most commercial milling operations, represents only about 8% of paddy weight but contains about three-fourths of the total oil [15]. Containing about 15-20% oil (the same general range of soybean), rice bran is commercially feasible for oil extraction.

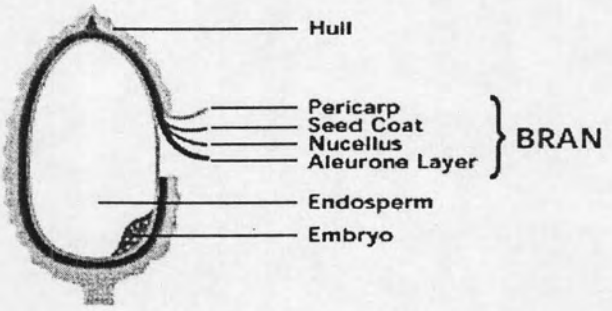


Figure 2.1 The structure of the rice kernel [16]

Rice bran oil differs from other vegetable oils due to its outstanding characteristic in helping lower the bad chloresterol (Low Density Lipoprotien Cholesterol : LDL-C) while increase or maintain the good cholesterol (High Density Lipoprotien Cholesterol : HDL-C). Rice bran oil is also high in natural antioxidants including Vitamin E and gamma-Oryzanol [17]. Structures of Vitamin E and gamma-Oryzanol are shown in Figure 2.2.

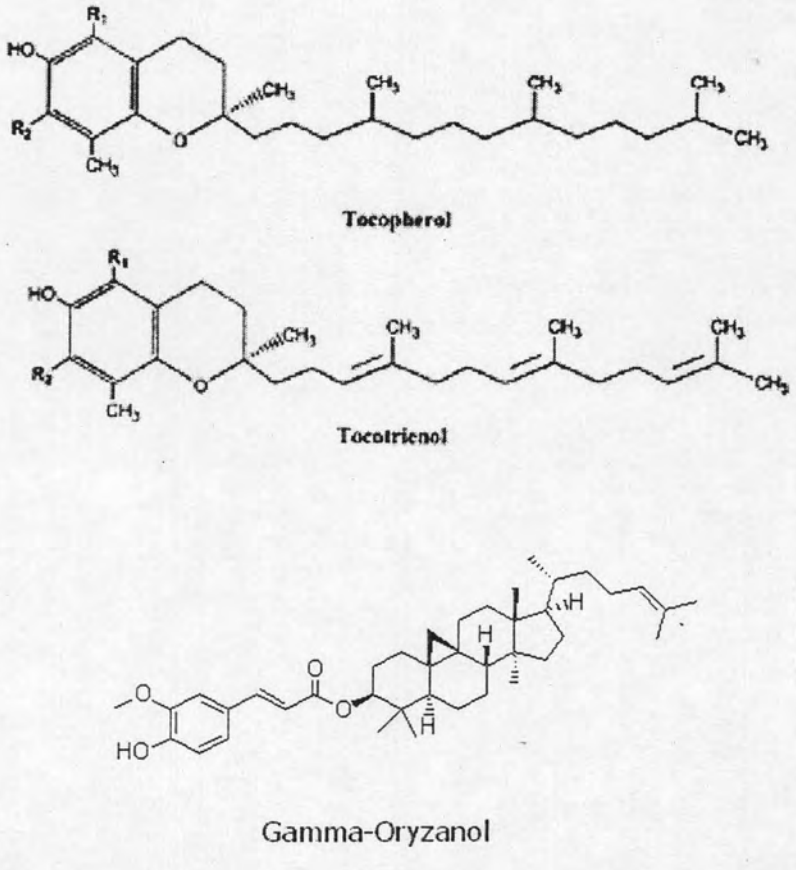
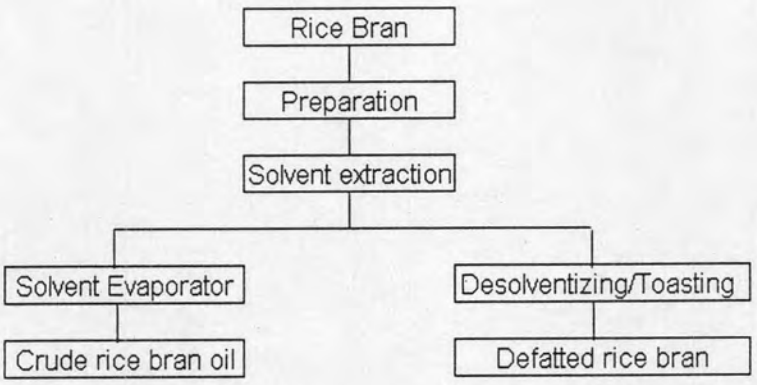


Figure 2.2 Structures of tocopherol, tocotrienol and gamma-oryzanol

2.2 Edible oil production and purification

2.2.1 Extraction process

Extraction of oil from the vegetable materials in which they occur is accomplished by pressing or by solvent extraction. Both processes are widely used. Extraction by pressing, vegetable seeds must be finely ground. The ground material is adjusted to certain moisture content and warmed or cooked in a steam-jacketed vessel. For solvent extraction the seeds are ground in such a way as to procedure the flake Sather than very fine particles. The flaked material is then extracted in suitable equipment by means of a low boiling point solvent [18]. Rice bran solvent extraction diagram is shown in Scheme 2.1.



Scheme 2.1 Extraction process of rice bran diagram [19]

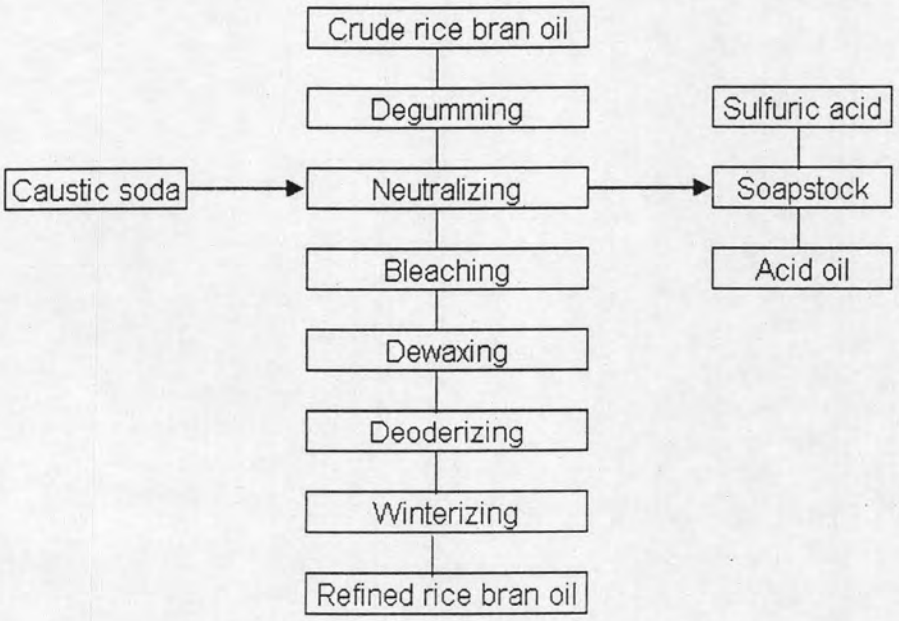
2.2.2 Refining process

Crude rice bran oil input refining process to be edible oil, gum, free fatty acid, color pigment, odor- volatile matter and wax have to be removed from rice bran oil. Once the oil has been extracted from the source of oil, it is necessary to remove impurities from the oil. The oil is essentially a pure triglyceride, is needed in order to produce high quality of oil.

The traditional caustic refining process has been known and used for over a century. It has proved to be both effective and reliable, but has relatively high refining losses and produces large quantities of effluent. In more recent times physical refining has become favored because of its lower running costs and low effluent generation.

a) Conventional alkali refining process

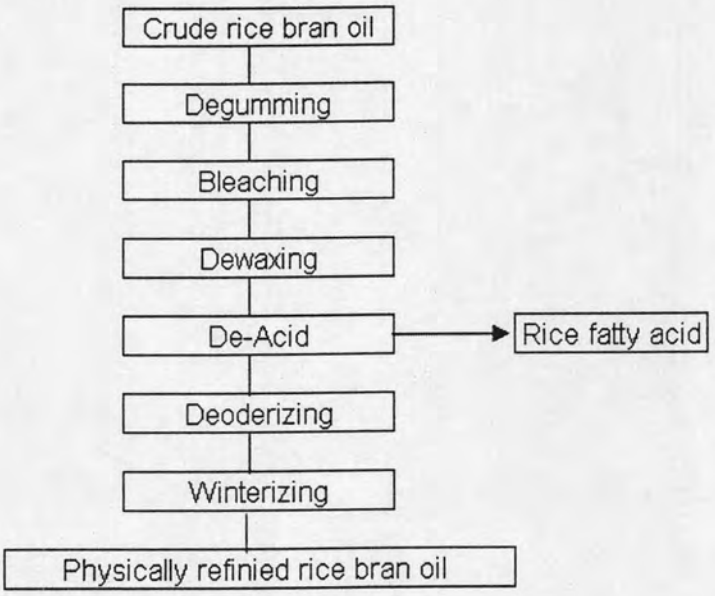
Free fatty acid in crude rice bran oil is removed by neutralizing the oil with caustic soda then becomes soapstock. In the same time, gamma oryzanol, tocopherol, tocotrienol and some color pigments have also been removed from rice bran oil. Refined rice bran oil from this process has lighter colour, less gamma-oryzanol, less tocopherol, less tocotrienol. Rice bran oil conventional alkali refining diagram is shown in Scheme 2.2.



Scheme 2.2 Rice bran oil conventional alkali refining process [19]

b) Physical refining process

With this process, free fatty acid in crude rice bran oil is removed by heat and vacuum in de-acid process and let most of gamma oryzanol, tocopherol and tocotrienol remain in rice bran oil. Rice bran oil physical refining diagram is shown in Scheme 2.3.



Scheme 2.3 Rice bran oil physical refining process [19]

c) Step of refining vegetable oil [20]

(1) Degumming

Gums in vegetable oil need to be removed to avoid colour and taste reversion during subsequent refining steps. Stage phosphoric acid treatment and a stage hot water treatment followed by continuous removal of the hydrated gums in a degumming centrifuge. The process is applied to many oils that contain phospholipids in significant amounts. Since the separated phospholipids are rather waxy or gummy solids, the term degumming was quite naturally applied to the separation. The aqueous phase can be removed from the lipids, and phospholipids can be removed from the oil.

(2) Neutralizing

The neutralization step is necessary to remove free fatty acids from the oil. This can be done in one of two ways: Alkali (Chemical) or Steam Stripping (Physical) means.

(a) Alkali/Chemical method: caustic soda (alkali) is mixed in the proper amounts and the aqueous solution is removed, leaving the neutral oil behind.

(b) Steam stripping: This is done under vacuum, to remove moisture, free fatty acids, odor bodies, and other impurities from the oil. As it is performed under vacuum conditions, the oil can be kept at a low temperature, preserving its chemical structure by not subjecting it to temperatures in which undesirable dehydration reactions can occur.

(3) Bleaching

Bleaching earth (adsorbent natural or activated earth, mixed with activated carbon if necessary): high-activity clay is added into oil, mix and heat mixture of oil and clay to make the small particle of pigment absorbed on the crystal of clay, at last filtering the oil, perfect mechanism, liable performance, well configured equipment. Bleaching results in the removal of coloring materials, phospholipids and oxidation products.

(4) Dewaxing

Dewaxing process includes slow chilling of the oil to temperatures sufficient to crystallize the waxy components from the crude oil, preferably under gentle agitation. The crystallized components are then generally removed by a cold filtration step. Dewaxing can improve oil transparency and brightness.

(5) Deodorizing

Deodorization results in the removal of odor from the oil. Most heat of bleached oil is recovered by heat exchangers, the bleached oil is heated to the process temperature by mineral oil or high pressure steam and then the oil enters into the combined deodorizer, the deodorizer is a combined type: the upper is packing structure, which is used to remove odor components like FFA, the lower is plate type used for heat bleaching and making product quality more consistent. Oil coming from the deodorizer is cooled and stored after series of heat exchange; volatile like FFA is collected and stored as by products.

(6) Winterizing

For refining products to be bottled as edible oils (e.g. rice bran oil, sunflower seed oil or corn oil) winterization is required to achieve the necessary cold stability. Winterization prevents crystallization and clouding of the waxes contained in the oil at ambient temperature. It is often desirable to remove the traces of waxes (*e.g.,* cuticle wax from seed coats) and the higher-melting glycerides from fats. Waxes can generally be removed by rapid chilling and filtering. Separation of high-melting glycerides, or stearine, usually requires very slow cooling in order to form crystals that are large enough to be removed by filtration or centrifuging.

2.3 Biodiesel

Biodiesel (fatty acid alkyl esters or FAAEs) has been a subject of importance because energy reserves are limited and the environmental pressure of exhausted gases from fossil fuels in increasing. Biodiesel has drawn attention as a non-toxic, biodegradable and renewable source of energy. Moreover, biodiesel shows quite lower exhaust emissions of particulate matter and green house gases such as CO, CO_2 and SO_x. Biodiesel is produced by esterification of fatty acids or transesterification of oils and fats with short chain alcohols. Methanol is mostly used because of its lower cost

compared with other alcohols, so FAAEs mainly refer to fatty acid methyl esters (FAMEs) [21].

Biodiesel can be produced from any material that contains fatty acids, be they linked to other molecules or present as free fatty acids. Thus various vegetable fats and oils, animal fats, waste greases, and edible oil processing wastes can be used as feedstocks for biodiesel production. The choice of feedstock is based on such variables as local availability, cost, government support and performance as a fuel. A variety of different types of reaction configurations can be employed in biodiesel synthesis, and may involve inorganic acid, inorganic base or enzymatic catalysis, biphasic or monophasic reaction systems, and ambient or elevated pressures and temperatures [22].

2.3.1 Biodiesel properties [23]

Biodiesel is made up of fourteen different types of fatty acids, which are transformed into fatty acid methyl esters (FAME) by transesterification. Different fractions of each type of FAME present in various feedstocks influence some properties of fuels. Table 2.3 shows some of the properties defined in the ASTM standards for diesel and biodiesel. These properties are described in the remainder of this section, and will be referred to later in this report.

Table 2.3 Comparison of fuel properties between diesel and biodiesel

Fuel Property	Diesel	Biodiesel
Fuel Standard	ASTM D975	ASTM PS 121
Fuel composition	C1O-C21 HC	C12-C22 FAME
Lower Heating Value, Btu/gal	131,295	117,093
Viscosity, @ 40° C	1.3-4.1	1.9-6.0
Specific Gravity kg/l @ 60° F	0.85	0.88
Density, lb/gal @ 15° C	7.079	7.328
Water, ppm by wt	161	0.05% max
Carbon, wt %	87	77
Hydrogen, wt %	13	12
Oxygen, by dif, wt %	0	11
Sulfur, wt %	0.05 max	0.0-0.0024
Boiling Point (°C)	188-343	182-338
Flash Point (°C)	60-80	100-170
Cloud Point (°C)	-15 to 5	-3 to 12
Pour Point (°C)	-35 to -15	-15 to 10
Cetane Number	40-55	48-65
Stoichiometric Air/Fuel Ratio wt./wt.	15	13.8
BOCLE Scuff, grams	3,600	>7,000

2.4 Biodiesel catalyst

Catalyst is another substance than reactants products added to a reaction system to alter the speed of a chemical reaction approaching a chemical equilibrium. Another reason for using a catalyst is that it promotes the production of a selected product. It changes the activation energy, E_a, of a reaction by providing an alternate pathway for the reaction. The *rate* and rate constant k of a reaction are related to E_a in the following ways:

$$Rate = k * \text{function of concentration}$$

$$k = A \exp\left(-E_a / RT\right)$$

Where A is a constant related to collision rates. Thus, a change in E_a changes the rate of a reaction.

2.4.1 Biodiesel acid catalyst [7]

Acids used for transesterification include sulfuric, phosphoric, hydrochloric, and organic sulfonic acids. Although transesterification by acid catalysis is much slower than that by alkali catalysis, acid-catalyzed transesterification is more suitable for glycerides that have relatively high free fatty acid contents and more water. Have a reported that it was necessary to perform transesterification under an acidic condition when the oil component was a low grade material such as sulphur olive oil. In general, the ethyl esters of monounsaturated or short-chain fatty acids with 2% sulfuric acid should make good alternative fuels.

2.4.2 Biodiesel base catalyst [7]

Alkalis used for transesterification include NaOH, KOH, carbonates, and alkoxides such as sodium methoxide, sodium ethoxide, sodium propoxide, and sodium butoxide. Alkali-catalyzed transesterification proceeds approximately 4000 times faster than that catalyzed by the same amount of an acidic catalyst, and is thus most

often used commercially. For alkali catalyzed transesteritication, the glycerides and alcohol must be substantially anhydrous because water causes a partial reaction change to saponification, which produces soap. The soap consumes the catalyst and reduces the catalytic efficiency, as well as causing an increase in viscosity, the formation of gels, and difficulty in achieving separation of glycerol. The free fatty acid content of the refined oil should be as low as possible, below 0.5%, also stressed the importance of oils being dry and free of free fatty acids. Ester yields were significantly reduced if the reactants did not meet these requirements; sodium hydroxide or sodium metboxide reacted with moisture and carbon dioxide in the air, diminishing their effectiveness.

2.4.3 Biodiesel enzyme catalyst [7]

Although chemical transesterification using an alkali-catalysis process gives high conversion levels of triglycerides to their corresponding methyl esters in short reaction times, the reaction has several drawbacks: it is energy intensive, recovery of glycerol is difficult, the acidic or alkaline catalyst has to be removed from the product, alkaline wastewater requires treatment, and free fatty acids and water interfere with the reaction. Both extracellular and intracellular lipases are also able to effectively catalyze the transesteritication of triglycerides in either aqueous or nonaqueous systems is shown in Table 2.4, enzymatic transesterification methods can overcome the problems mentioned above. In particular, it should be noted that the by-product, glycerol, can be easily recovered without any complex process, and also that free fatty acids contained in waste oils and fats can be completely converted to methyl esters. On the other hand, in general the production cost of a lipase catalyst is significantly greater than that of an alkaline one.

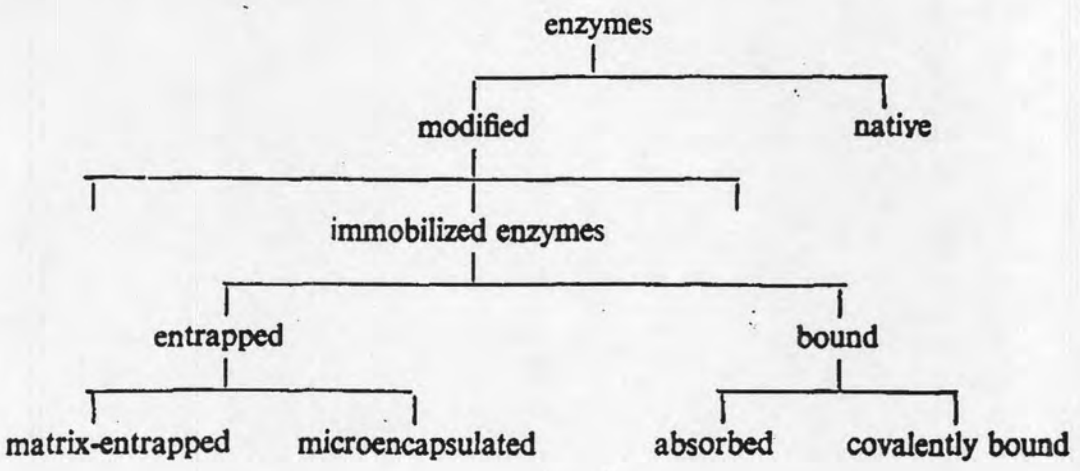
Table 2.4 Comparison between alkali-catalysis and lipase-catalysis methods for biodiesel fuel production

	Alkali-catalysis process	**Lipase-catalysis process**
Reaction temperature	60-70°C	30-40°C
Free fatty acids in raw materials	Saponified products	Methyl esters
Water in raw materials	Interference with the reaction	No influence
Yield of methyl esters	Normal	Higher
Recovery of glycerol	Difficult	Easy
Purification of methyl esters	Repeated washing	None
Production cost of catalyst	Cheap	Relatively expensive

2.4 Immobilized enzyme [24]

Immobilized enzymes are defined as "enzymes physically confined or localized in a certain defined region of space with retention of their catalytic activities, and which can be used repeatedly and continuously". Accordingly, enzymes modified to a water-insoluble form by suitable techniques satisfy this definition of immobilized enzymes. In addition, when an enzyme reaction using a substrate of high molecular weight is carried out in a reactor equipped with a semipermeable ultrafiltration membrane, a reaction product of low molecular weight can be removed continuously through the membrane without leakage of the enzyme from the reactor. This also can be considered as a kind of immobilized enzyme system.

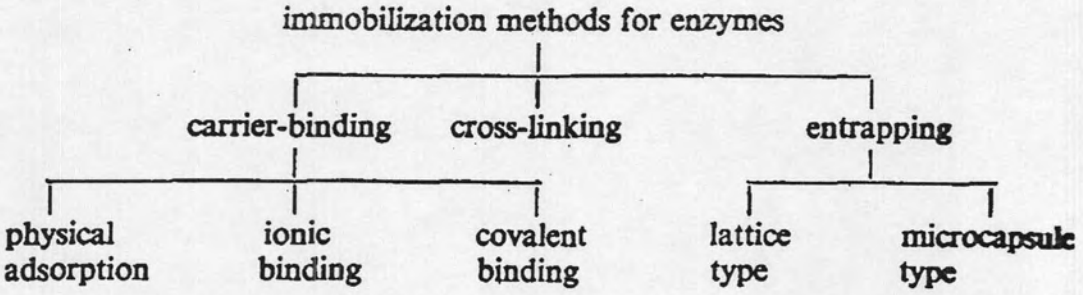
A classification of immobilized enzymes was proposed is shown in Scheme 2.4 Enzymes are first classified into native enzymes and modified enzymes. Immobilized enzymes belong to the latter type, which also includes chemically modified soluble enzymes and biologically, i.e., genetically, modified enzymes.



Scheme 2.4 Classification of immobilized enzymes

Thus, for practical use as catalysts, enzymes in the following three forms can be considered: 1) soluble form, 2) soluble immobilized form, and 3) insoluble immobilized form. For the latter two forms, the term "immobilized enzyme" is more suitable than "insoluble enzyme".

Immobilization of enzymes is classified into "carrier-binding", "cross-linking" and "entrapping" types is shown in Scheme 2.5 and Figure 2.3. Carrier-binding is subdivided into "physical adsorption", "ionic binding" and "covalent binding", while entrapping is divided into "lattice type" and "microcapsule type". As described later, this classification is reasonable and convenient. Except for the case using an ultrafiltration membrane, immobilized enzymes are apparently insoluble in water, and the enzyme reaction is carried out in a heterogenous medium.



Scheme 2.5 Classification of immobilization methods for enzyme

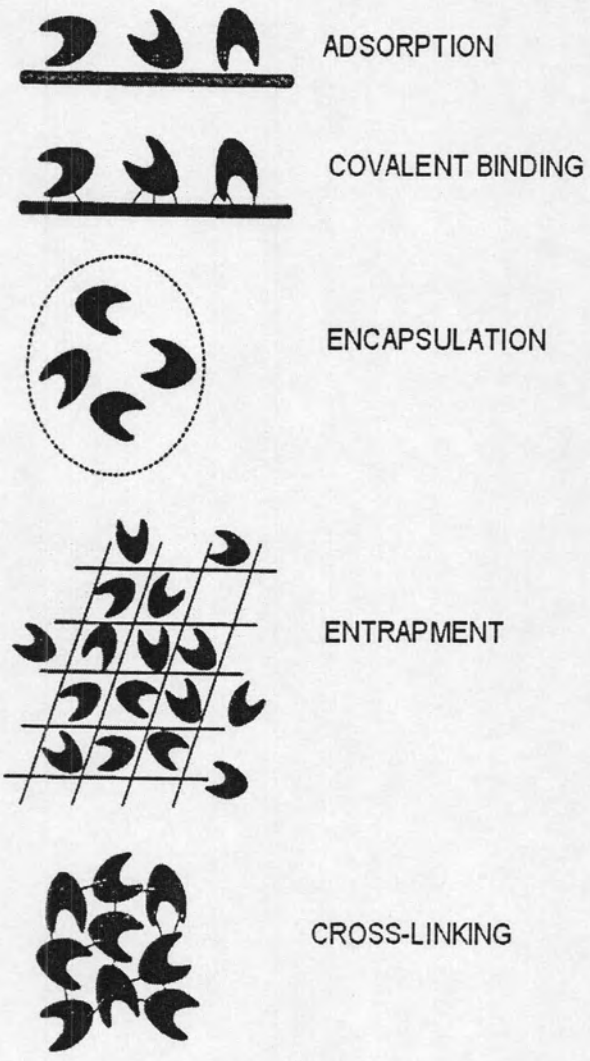


Figure 2.3 Principal method of immobilization

2.5 Biodiesel process [26]

There are several generally accepted ways to make biodiesel. There processes are as follows;

2.5.1 Direct use and blending

The direct use of vegetable oils in diesel engines is problematic and has many inherent failings. It has only been researched extensively for the past couple of decades, but has been experimented with for almost a hundred years. Although some diesel engines can run pure vegetable oils engines, turbocharged direct injection engines such as trucks are prone to many problems. Energy consumption, with the use of pure vegetable oils, was found to be similar to that of diesel fuel. For short term use ratios of 1:10 to 2:10 oil to diesel have been found to be successful.

2.5.2 Viscosity

Table 2.5 Comparison of typical properties of diesel, canola oil and biodiesel

	Diesel	Canola	Biodiesel
Density (kg/L)	0.835	0.922	0.88
Gross calorific value (MJ/L)	38.3	36.9	33.3
Viscosity (mm^2/s @ 37.8°C)	3.86	37	4.7
C:H:O (ratio)	3.59	3.26	2.38
Sulphur (%)	0.15	0.0012	<0.01

The properties of canola oil and diesel show in Table 2.5 are very similar, except a significant difference in viscosity, with canola oil having twelve times the viscosity of diesel. Even after heating to around 80°C it is still six times as viscous as diesel. This leads to problems with flow of oils from the fuel tank to the engine, lockages in filters and subsequent engine power losses. Even if preheating is used to lower the viscosity, difficulties may still be encountered with starting due to the temperatures required for oils to give off ignitable vapours. Further, engines can suffer coking and gumming which leads to sticking of piston rings due to multibonded compounds undergoing pyrolyses. Polyunsaturated fatty acids also undergo oxidation

in storage causing gum formation and at high temperatures where complex oxidative and thermal polymerisation can occur.

2.5.3 Microemulsions

Microemulsions are defined as a colloidal equilibrium dispersions of optically isotropic fluid microstructures, with dimensions generally in the 1-150 nm range. These are formed spontaneously from two normally immiscible liquids and one or more ionic or non-ionic amphophile. A microemulsion is designed to tackle the problem of the high viscosity of pure vegetable oils by reducing the viscosity of oils with solvents such as simple alcohols.

The performances of ionic and non-ionic microemulsions where found to be similar to diesel fuel, over short term testing. They also achieved good spray characteristics, with explosive vaporisation which improved the combustion characteristics. In longer term testing no significant deterioration in performance was observed, however significant injector needle sticking, carbon deposits, incomplete combustion and increasing viscosity of lubricating oils.

2.5.4 Thermal cracking

Pyrolysis is the conversion of one substance into another by means of applying heat i.e. heating in the absence of air or oxygen with temperatures ranging from 450°C –850°C. In some situations this is with the aid of a catalyst leading to the cleavage of chemical bonds to yield smaller molecules.

Unlike direct blending, fats can be pyrolyised successfully to produce many smaller chain compounds. The pyrolysis of fats has been investigated for over a hundred years, especially in countries where there is a shortage of petroleum deposits. Typical catalyst that can be employed in pyrolysis are SiO_2 and Al_2O_3. The ratios of light to heavy compounds are temperature and time dependent. Typical breakdown of compounds found from pyrolysis of safflower and soybean oil, are listed in Table 2.6.

Table 2.6 Compositional data of pyrolysis of oils

	Percent by weight	
	*HO Safflower	Soybean
Alkanes	40.9	29.9
Alkenes	22	24.9
Alkadienes	13	10.9
Aromatics	2.2	1.9
Unresolved unsaturates	10.1	5.1
Carboxylic acids	16.1	9.6
Unidentified	12.7	12.6

*HO High Oleic Safflower oil

The equipment for pyrolysis or thermal cracking is expensive for modest throughputs. Although, the products are chemically similar to pyrochemically based diesel, oxygen removal from the process decreases the products benefits of being an oxygenated fuel. This decreases its environmental benefits and generally produces more fuel similar in properties of gasoline than diesel, with the addition of some low value materials.

2.5.5 Transesterification

Transesterification is the reaction of a lipid with an alcohol to form esters and a byproduct, glycerol. It is in principle the action of one alcohol displacing another from an ester, the term alcoholysis (cleavage by an alcohol).The reaction, as shown in Figure 2.4 is reversible and thus an excess of alcohol is usually used to force the equilibrium to the product side. The stoichiometry for the reaction is 3:1 alcohol to lipids; however in practice this is usually increased to 6:1 to increase product yield. A catalyst is usually used to speed up the reaction and may be basic, acid or enzymatic in nature. The alkalis that are generally used include NaOH, KOH, carbonates and corresponding sodium and potassium alkoxides such as sodium methoxide, ethoxide, propoxide and butoxide. Sodium hydroxide is the most common alkali catalyst that is used, due to economical reasons and availability. Alkalicatalysed reactions are used more often commercially than acid catalysts, as the reactions are faster.

Only simple alcohols can be used in transesterification such as, methanol, ethanol, propanol, butanol and amyl alcohol. Methanol is most often used for commercial and process reasons related to its physical and chemical nature (shortest chain alcohol and is polar). However ethanol is becoming more popular as it is a renewable resource and does not raise the same toxicity concerns as methanol. The type of catalyst, the reaction conditions and the concentration of impurities in a transesterification reaction determine the path that the reaction follows.

For alkali catalysed transesterification, water and FFA are not favourable to the reaction, so anhydrous triglycerides and alcohol are necessary to minimise the production of soap. Soap production decreases the amount of esters and renders the separation of glycerol and esters difficult. In current commercial processes using crude feed stock, excess alkali is added to remove all the FFAs. Transesterification of triglyceride with alcohol shown in Figure 2.4.

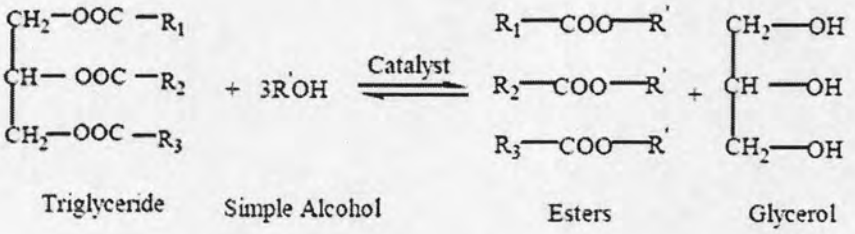


Figure 2.4 Transesterification of triglycerides with alcohol

2.5.6 Saponification

The production of soap sometimes called alkaline hydrolysis, converts triacylglycerols to glycerol and a mixture of salts of long-chain carboxylic acids. As can be seen from Figures 2.5 and 2.6, the reaction can be carried out with an ester (i.e. triglycerides) or with carboxylic acids (i.e. free fatty acids). However, the production of fatty acids is an intermediate step when triglycerides are directly used for saponification.

The commercial production of soap is usually conducted in two phases. The first phase is the conversion of lipids into FFAs by boiling with aqueous sodium hydroxide until hydrolysis is complete and then adding sodium chloride to precipitate the soap.

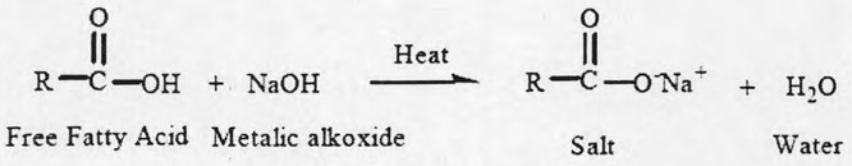


Figure 2.5 Saponification from free fatty acid

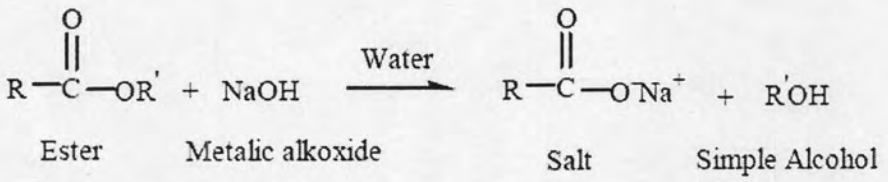


Figure 2.6 Saponification from ester

2.5.7 Esterification

The formation of esters occurs through a condensation reaction known as esterification. This requires two reactants, carboxylic acids (fatty acids) and alcohols. Esterification reactions are acid catalysed and proceed slowly in the absence of strong acids such as sulphuric acid, phosphoric acid, organic sulfonic acids and hydrochloric acid. The equation for an esterification reaction is shown in Figure 2.7.

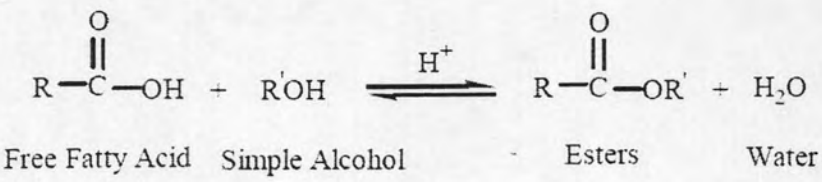


Figure 2.7 Esterification of free fatty acid

2.5.8 Hydrolysis

The hydrolysis of lipids forms a heterogenous reaction system made up of two liquid phases. The disperse aqueous phase consists of water and glycerol; the homogenous lipid phase consists of fatty acids and glycerides. The hydrolysis of glycerides takes place in the lipid phase in several stages via partial glycerides (diglycerides and monoglycerides). Acid catalysts are very effective at accelerating the hydrolysis reaction. However, at high temperatures substantial material corrosion occurs. Diabasic metal oxides have a higher activity than more strongly alkaline monobasic metal oxides. Zinc oxide in its soap form has been suggested to be the most active catalyst for hydrolysis reactions. The equation for hydrolysis reaction of triglyceride is shown in Figure 2.8.

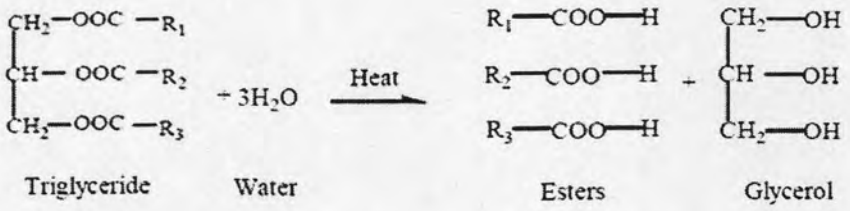


Figure 2.8 Hydrolysis of triglycerides

2.5.9 Aminolysis

Esters undergo nucleophilic substitution at their acyl carbon atoms when they are treated with primary or secondary amines. These reactions are slow but are synthetically useful. The equation for an aminolysis reaction of triglyceride is shown in Figure 2.9.

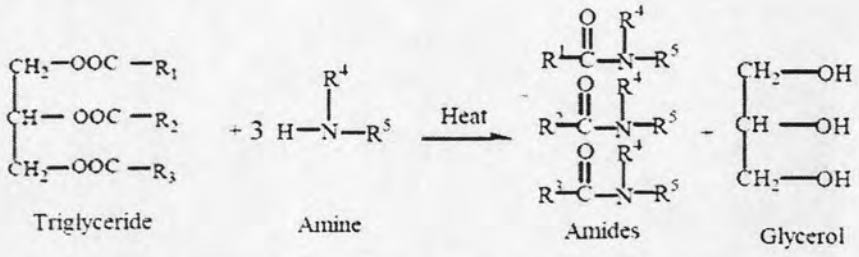


Figure 2.9 Aminolysis of triglycerides

2.5.10 Biocatalysts

Biocatalysts are usually lipases; however conditions need to be well controlled to maintain the activity of the catalyst. Hydrolytic enzymes are generally used as biocatalysts as they are ready available and are easily handled. They are stable, do not require co-enzymes and will often tolerate organic solvents. "Their potential for regioselective and especially for enantioseletive synthesis makes them valuable tools". Recent patents and articles have shown that reaction yields and times are still unfavourable compared to base-catalysed transesterification for commercial application.

2.5.11 Catalyst free

Transesterification will occur without the aid of a catalyst, however at temperatures below 300°C the rate is very low. It has been said that there are, from a broad perspective, two methods to producing biodiesel and that is with and without a catalyst.

2.5.12 Supercritical methanol

The study of the transesterification of rapeseed oil with supercritical methanol was found to be very effective and gave a conversion of >95% within 4min. A reaction temperature of 350°C, pressure of 30MPa and a ratio of 42:1 of methanol to rapeseed oil for 240s were found to be the best reaction conditions. The rate was substantially high from 300 to 500°C but at temperatures above 400°C it was found that thermal degradation takes place. Supercritical treatment of lipids with a suitable solvent such as methanol relies on the relationship between temperature, pressure and the thermophysical properties such as dielectric constant, viscosity, specific weight and polarity. A comparison of supercritical methanol production and alcoholysis is shown in Table 2.7.

Table 2.7 Comparison between productions of biodiesel

	Common Method	*SC MeOH method
Reaction Time	1-6h	0.067h
Reaction Condition	0.1MPa, 30-65°C	35MPa, 350°C
Catalyst	acid or alkali	none
Free Fatty Acids	Saponified products	methyl esters
Yield	97% (normal)	98.50%
Removal for Purification	methanol, catalyst and saponified products	methanol
Process	Detailed	Simple

*SC MeOH method - Supercritical Methanol

2.6 Soapstock

Soapstock, a by-product of the refining of edible vegetable oil process, contains lipids, acylglycerides, phophoglyceride, pigments, and water-soluble organic materials. Although soapstock has a relatively low value it may be readily converted into more valuable products by addition of strong mineral acids in a process known as acidulation. The conventional process for acidulation and recovery of lipids from soapstock requires the addition of large excesses of acid at high temperatures to recover the fatty acid rich oil (Acid oil).

2.7 Literature reviews

2.7.1 Biodiesel production by using immobilized lipase

In 1999 Watanabe and coworkers [10] studied stepwise ethanolysis of tuna oil using immobilized *Candida antractica* lipase. The first step was carried out at 40°C for 12 hrs in a mixture of tuna oil and 1/3 molar equivalent of ethanol with 4% immobilized lipase; the second step was performed for 36 hrs after adding 2/3 molar equivalent of ethanol. The three-step reaction was conducted as follows: the first step

was conducted under the same conditions as those in the two-step ethanolysis; in the second and third steps, l/3 molar equivalent of ethanol was added after 12 and 24 hrs, respectively; and in the third step, the mixture was shaken for 24 hrs. Both types of ethanolysis achieved the conversion of 95%. The two and three-step reactions maintained over 95% of the conversion for 70 d and over 100 d, respectively.

In 2002 Shimada and coworkers [11] studied enzymatic alcoholysis for biodiesel fuel production and application of the reaction to oil processing. Two-step batch methanolysis: the first-step reaction was conducted in the presence of 1/3 molar equivalent of MeOH for the stoichiometric amount, and the second-step reaction was performed by adding 2/3 molar equivalent of MeOH. If the immobilized carrier is destroyed by agitation in a reactor with impeller, three-step flow reaction will be available: the first-step substrates were waste oil and 1/3 molar equivalent of MeOH; the second-step, the first-step eluate and 1/3 molar equivalent of MeOH; the third-step, the second-step eluate and 1/3 molar equivalent of MeOH. The conversion of waste oil to biodiesel fuel reached >90% in the two reaction systems, and the lipase catalyst could be used for >100 days without decrease of the activity.

In 2005 Hass [2] studied improving the economics of biodiesel production through the use of low value lipids as feedstocks: vegetable oil soapstock. The most effective method involved the complete saponification of the soapstock followed by acidulation using methods similar to those presently employed in industry. This resulted in an acid oil with a free fatty acid (FFA) converted to methyl esters by acid-catalyzed esterification. Following a simple washing protocol, this preparation met the established specifications for biodiesel of the ASTM standard. Engine emissions and performance during operation on soy soapstock biodiesel were comparable to those on biodiesel from soy oil. An economic analysis suggested that the production cost of soapstock biodiesel would be approximately US$ 0.41/L, a 25% reduction relative to the estimated cost of biodiesel produced from soy oil.

In 2005 Noureddini and coworkers [27] studied immobilized *Pseudomonas cepacia* lipase for biodiesel fuel production from soy bean oil. Methanol, ethanol and nine lipases that were tested in the initial screening, lipase PS from *Pseudomonas cepacia* resulted in the highest yield of alkyl esters. Using the immobilized lipase PS, the effects of water and alcohol concentration, enzyme loading, enzyme thermal stability and temperature in the transesterification reaction were investigated. The optimal conditions for processing 10 g of soybean oil were: 35°C, 1:7.5 oil/methanol molar ratio, 0.5 g water and 475 mg lipase for the reactions with methanol, and 35°C, 1:15.2 oil/ethanol molar ratio, 0.3 g water, 475 mg lipase for the reactions with ethanol. Subject to the optimal conditions, methyl and ethyl esters formation of 67 and 65 mol% in 1 hr of reaction were obtained for the immobilized enzyme reactions. Upon the reaction with the immobilized lipase, the triglycerides reached negligible levels after the first 30 min of the reaction and the immobilized lipase was consistently more active than the free enzyme. The immobilized lipase also proved to be stable and lost little activity when was subjected to repeated uses.

In 2006 Nie and coworkers [28] studied lipase catalyzed methanolysis to produce biodiesel: optimization of the biodiesel production. The conversion ratio of salad oil to biodiesel could reach up to 96% with the optimal reaction conditions. Continuous reaction in a fixed bed reactor in three-step transesterification with methanol of oil was conducted by using a series of nine columns packed with immobilized *Candida* sp. 99–125 lipase. As substrate of the first reaction step, plant or waste oil was used together with 1/3 molar equivalent of methanol against total fatty acids in the oil. Mixtures of the first- and second-step eluates and 1/3 molar equivalent of methanol were used for the second- and third-reaction steps. A hydrocyclone was used in order to on-line separate the by-product glycerol after every 1/3 molar equivalent of methanol was added. Petroleum ether was used as solvent (3/2, v/v of oil) and the pump was operated with a flow rate of 15 L/h giving an annual throughput

of 100 t. The final conversion ratio of the FAME from plant oil and waste oil under the optimal condition was 90% and 92%, respectively. The life of the immobilized lipase was more than 10 days.

In 2007 Watanabe and coworkers [29] studied conversion of acid oil by-produced in vegetable oil refining to biodiesel fuel by immobilized *Candida antractica* lipase. The first-step reaction was conducted by shaking a mixture of 66 wt% acid oil (77.9 wt% FFAs, 10.8 wt% acylglycerols) and 34 wt% MeOH with 1 wt% immobilized lipase, to convert FFAs to their methyl esters. The second-step reaction was performed by shaking a mixture of 52.3 wt% dehydrated first-step product (79.7 wt% FAMEs, 9.7 wt% acylglycerols), 42.2 wt% rapeseed oil, and 5.5 wt% MeOH using 6 wt% immobilized lipase in the presence of additional 10 wt% glycerol, to convert acylglycerols to FAMEs. The resulting product was composed of 91.1 wt% FAMEs, 0.6 wt% FFAs, 0.8 wt% triacylglycerols, 2.3 wt% diacylglycerols, and 5.2 wt% other compounds. Even though each step of reaction was repeated every 24 hrs by transferring the immobilized lipase to the fresh substrate mixture, the composition was maintained for >100 cycles.

In 2008 Shao and coworkers [30] studied analysis of immobilized *Candida rugosa* lipase catalyzed preparation of biodiesel from rapeseed soapstock. Response surface methodology (RSM) was used to optimize the production of biodiesel. Methanol substrate molar ratio, enzyme amount, water content and reaction temperature were four important parameters employed. RSM analysis showed good correspondence between experimental and predicted values. It was found that the most effective parameter was water content, which was in good agreement with the experimental value. The coefficient of determination ($R2$) for the model was 92.86%. Probability value ($P < 0.0001$) demonstrated a very high significance for the regression model. Methyl conversion of 63.6% was obtained when optimum conditions of immobilized lipase catalyzed for biodiesel production were methanol substrate molar

ratio of 4:1, enzyme amount of 8%, water content of 6% and reaction temperature of 45°C. Methyl ester content was above 95% after molecular distillation process.

2.7.2 Biodiesel production from low grade feedstock

In 2005 Vilas Ghadge and Raheman [31] studied biodiesel production from mahua (*Madhucaindica*) oil having high free fatty acids. The high FFA level (19%) of mahua oil was reduced to less than 1% by a two-step pretreatment process. Each step was carried out with 0.30–0.35 v/v methanol-to-oil ratio in the presence of 1% v/v H_2SO_4 in 1 hr reaction at 60°C. After the reaction, the mixture was allowed to settle for an hour and methanol–water mixture that separated at the top was removed. The second step product at the bottom was transesterified using 0.25 v/v methanol and 0.7% w/v KOH. The fuel properties of mahua biodiesel were found to be comparable to those of diesel and conforming to both the American and European standards.

In 2006 Zheng and coworkers [32] studied acid-catalyzed production of biodiesel from waste frying oil. The reaction kinetics of acid-catalyzed transesterification in excess methanol was studied. Rate of mixing, feed composition (molar ratio oil: methanol: acid) and temperature were independent variables. There was no significant difference in the yield of FAME when the rate of mixing was in the turbulent range 100 to 600 rpm. The oil: methanol: acid molar ratios and the temperature were the most significant factors affecting the yield of FAME. At 70°C with oil: methanol:acid molar ratios of 1:245:3.8, and at 80 °C with oil: methanol: acid molar ratios in the range 1:74:1.9–1:245:3.8, the transesterification was essentially a pseudo-first-order reaction as a result of the large excess of methanol which drove the reaction to completion (99±1% at 4 hrs).

In 2006 Wang and coworkers [33] studied comparison of two difference processes to synthesize biodiesel by waste cooking oil. Waste cooking oil (WCO) samples with the acid value of 75.92±0.04 mgKOH/g mixed with methanol were

catalyzed under 95°C for various reaction time, followed by methanol recovery under vacuum (10±1 mmHg) at 50°C with a rotational evaporation. FAME analyzed by gas chromatography was obtained directly from sulfuric acid catalyzed reaction, whereas in the two-step method it was produced from ferric sulfate (2.0%) catalyzed reaction followed by alkali (1.0% KOH) transesterification. The conversion of free fatty acids of WCO into FAME in the two-step method was 97.22% at the reaction time of 4 hrs, mole ratio of methanol to TG of 10:1, compared in the acid method with 90%, 10 hrs, and 20:1, respectively, showing much higher catalyzed activity of ferric sulfate, advantages of no acidic waste water, high efficiency, low equipment cost, and easy recovery.

In 2007 Chongkhong and coworkers [34] studied Biodiesel production by esterification of palm fatty acid distillate. Batch esterifications of palm fatty acid distillate (PFAD) study the influence of: reaction temperatures of 70–100°C, molar ratios of methanol to PFAD of 0.4:1–12:1, quantity of catalysts of 0–5.502% (wt of sulfuric acid/wt of PFAD) and reaction times of 15–240 min. The optimum condition for the continuous esterification process was molar ratio of methanol to PFAD at 8:1, 1.834 wt% of H_2SO_4 at 70°C and 60 min. The amount of FFA was reduced from 93 wt% to less than 2 wt%. The FAME was purified by neutralization with 3M sodium hydroxide in water solution at 80°C, 15 min followed by transesterification process with 0.396M sodium hydroxide in methanol solution at a reaction temperature of 65°C, 15 min. The final FAME product met with the Thai biodiesel quality standard, and ASTM D6751-02.

In 2008 Phan A.N. and Phan T.M. [35] studied biodiesel production from waste cooking oils. The effects of methanol/waste cooking oils ratio, potassium hydroxide concentration and temperature on the biodiesel conversion were investigated. Biodiesel yield of 88–90% was obtained at the methanol/oil ratios of 7:1–8:1, temperatures of 30–50°C and 0.75 wt% KOH. Biodiesel and its blends with diesel

were characterized for their properties referring to a substitute for diesel fuel. The results showed that the biodiesel experienced a higher but much narrower boiling range than conventional diesel. Carbon residue content was up to 4 wt% blends with a percentage of the biodiesel below 30 vol% had their physical properties within EN14214 standard, which indicated that these could be used in engines without a major modification.