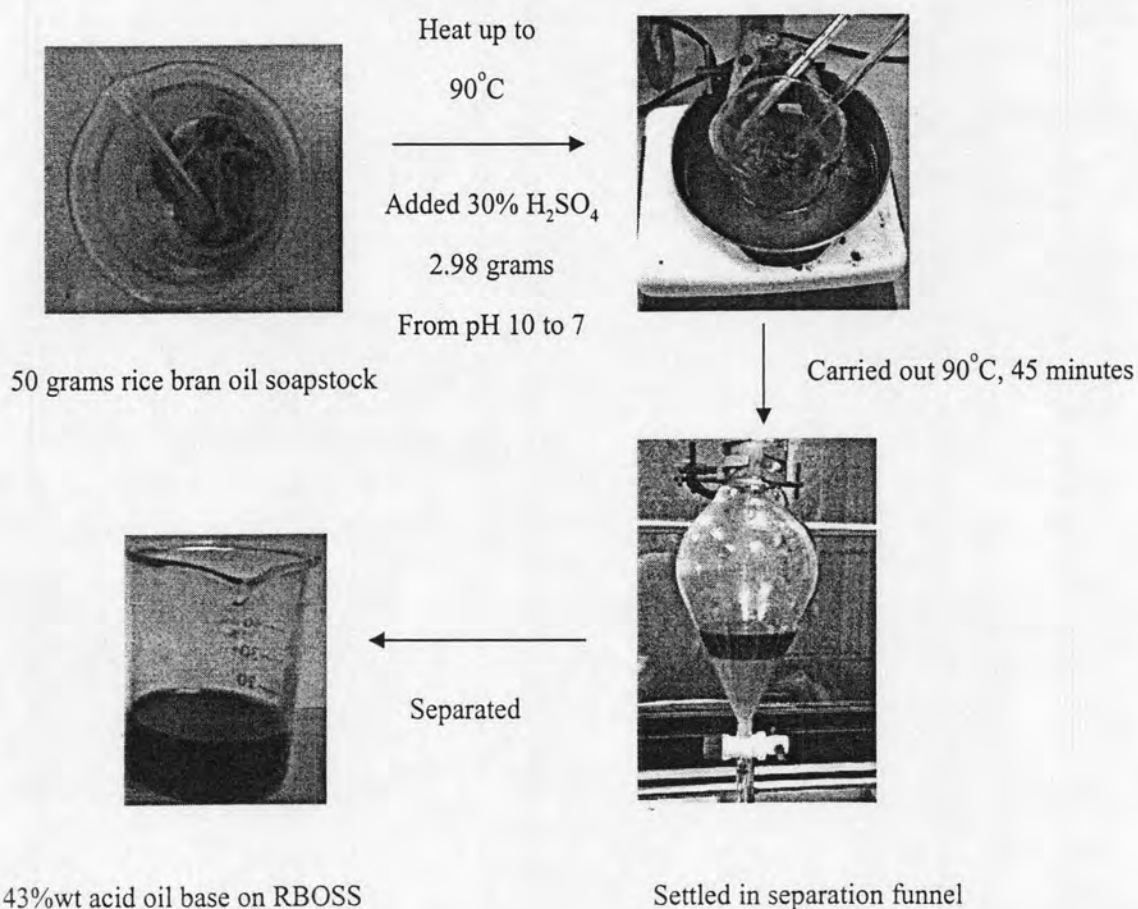


CHAPTER IV

RESULTS AND DISCUSSIONS

4.1 Neutralization of rice bran oil soapstock (RBOSS)

Soapstock, a by-product of edible oil refining, is substantially less expensive than edible grade refined oils. When rice bran oil soapstock was used as substrate in enzyme-catalyzed esterification, biodiesel was obtained only in 36%. After the soapstock was dried in desiccator, the result showed it was contain high percentage of moisture (50%). RBOSS was neutralized by addition of sulfuric acid to give acid oil 43%. The acid oil obtained from the neutralization was subjected to esterification with molar ratio of methanol to FFA was 2:1 in presence of Novozyme 435 10%wt of acid oil in reaction temperature 30°C and reaction time 2 hrs. The biodiesel was obtained in 80% with 73% of fatty acid methyl ester (FAME).



Scheme 4.6 Neutralization of Rice bran oil soapstock

4.2 Chemical properties of acid oil and rice fatty acid

4.2.1 Fatty acid compositions were determined by EN14103

Percentages of FFA of acid oil and rice fatty acid were determined by EN14103 and RBOSS was neutralized in laboratory analyzed by using gas liquid chromatography, Lepage & Roy 1984. (See details in Appendix A).

a) RBOSS was neutralized in laboratory: the percentages of saturated fatty acids, myristic acid, palmitic acid, stearic acid, arachidic acid, behenic acid and ligoceric acid were 0.303, 18.922, 1.837, 0.633, 0.229 and 0.378% respectively. The total of saturated fatty acids was 22.332%. The percentages of unsaturated fatty acids, palmitoleic acid, oleic acid, linolenic acid, α -linolenic acid, cis-11-eicosenoic acid were 0.164, 40.703, 35.139, 1.314 and 0.349% respectively. The total of unsaturated fatty acids was 77.669%.

b) Acid oil: the percentages of saturated fatty acids, myristic acid, palmitic acid, stearic acid, arachidic acid, behenic acid and ligoceric acid were 0.455, 19.533, 2.089, 0.889, 0.234 and 0.432% respectively. The total of saturated fatty acids was 23.632%. The percentages of unsaturated fatty acids, oleic acid, linolenic acid, α -linolenic acid, cis-11-eicosenoic acid were 42.245, 33.211, 1.047 and 0.311% respectively. The total of unsaturated fatty acids was 76.823%.

c) Rice fatty acid: the percentages of saturated fatty acids, myristic acid, palmitic acid, stearic acid, arachidic acid, behenic acid and ligoceric acid were 1.379, 22.853, 2.544, 1.876, 0.456 and 0.521% respectively. The total of saturated fatty acids was 29.629%. The percentages of unsaturated fatty acids, oleic acid, linolenic acid, α -linolenic acid, cis-11-eicosenoic acid were 38.983, 31.246, 1.173 and 0.348% respectively. The total of unsaturated fatty acids was 71.75%.

It can be seen that the fatty acid compositions of those samples above were not much different. The acid oil from Surin bran oil company was used in this study instead of neutralizing RBOSS to acid oil in laboratory.

The calculated average molecular weight FFA of acid oil and rice fatty acid were 278.61 g/mole and 280.41 g/mole respectively. The calculated average molecular weight TG of acid oil and rice fatty acid were 873.83 g/mole and 879.23 g/mole respectively. (See details in Appendix B)

4.2.2 Acid value content was determined by ASTM D664

Acid values in acid oil and rice fatty acid were 146.2 mgKOH/g and 132.4 mgKOH/g respectively. The calculated acid values to percentage of FFA in acid oil and rice fatty acid were 73.47% and 66.53% respectively (See details in Appendix C). Iodine value and moisture content of them from certificate of analysis of Surin rice bran oil company (See details in Appendix A) are shown in Table 4.8.

The percentage of FFA in feedstock showed there were low grade feedstock. Biodiesel which produced from low grade feedstock, the pretreatment by acid or enzyme catalyst in the first step was necessary for preventing the formation of the soap when base catalyst was used in transesterification process.

The iodine value in sample is the mass of iodine in grams that is consumed by 100 grams of a substance. The iodine value is the determination of saturation of fatty acids as double bonds in fatty acids also react with iodine compounds. The higher the iodine number, the more unsaturated fatty acid bonds are present in a fat. And saponification value represents the number of milligrams of potassium hydroxide or sodium hydroxide required to saponify 1 gram of fat under the conditions specified. It is a measure of the average molecular weight (or chain length) of all the fatty acids present.

Table 4.8 Iodine value, saponification value and moisture content in acid oil and rice fatty acid

| | Acid oil | Rice fatty acid |
|-------------------------------------|----------|-----------------|
| Iodine value (Wijs) | 93.04 | 92.87 |
| Saponification value (meg/g) | - | 187.7 |
| moisture content (%) | 1.43 | 0.57 |

4.3 Biodiesel production from RBOSS using enzyme catalyzed process

The methyl ester produced from RBOSS by enzyme catalyzed process with 2:1 molar ratio of methanol to FFA. The reaction was carried out at 60°C for 3 hrs. This reaction gave low percentage of conversion (53%) because it contained high water content, providing non completed esterification of FFA (reversible reaction of methyl ester to FFA and methanol). Moreover, because RBOSS contains 43% of glycerides, phosphoglycerides and FFA; therefore, this reaction provided low percentage of product (only 36%). This is because the percentage of product could not be more than that of FFA in RBOSS.

4.4 Biodiesel production from acid oil using Novozyme 435

The effect of different process parameters such as reaction temperature, molar ratio of methanol to FFA and amount of catalyst on conversion of FFA to FAME was evaluated and presented below.

4.4.1 Optimization of process parameter

4.4.1.1 Effect of reaction temperature

The effect of temperature on conversion-of FFA to FAME was investigated at 30°C, 40°C and 50°C using 2:1 molar ratio of methanol and acid oil, and 10% Novozyme 435 (based on weight of acid oil). The reactions were monitored by TLC and ¹H-NMR analysis. The TLC result is shown in Figure 4.10. At 30°C and 40°C,

TLC analysis showed that the FFA were converted rapidly to FAME within 2 hrs while the conversion was relatively constant after 2 hrs. For the reaction at 50°C, small amount of FFA was converted constantly to FAME after 15 minutes.

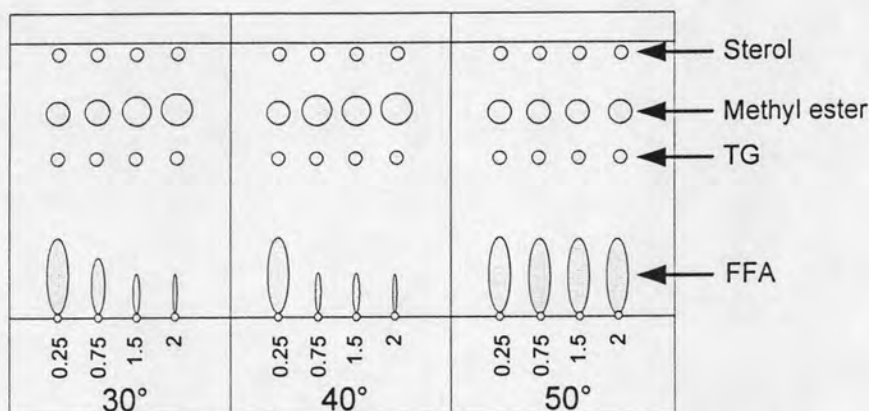


Figure 4.10 TLC for monitoring the methyl ester at reaction time 0.25, 0.75, 1.5 and 2 hrs of effect of reaction temperature at 30°C, 40°C and 50°C

From $^1\text{H-NMR}$ analysis in Table 4.9, the results were plotted as shown in Figure 4.11. At different temperature, highest percentage of FAME (73.26%) was obtained at 30°C after the reaction time for 2 hrs. The reaction progressed rapidly during the first 2 hrs similar to TLC analysis at 30°C and 40°C and the percentage of FAME obtained from reaction temperature at 40°C was lower than at 30°C about 5%. At 50°C the percentage of FAME was obtained in about 40% although the reaction was continued for 24 hrs.

Table 4.9 The effect of temperature on esterification of the acid oil by $^1\text{H-NMR}$ analysis

| T(°C) | Time (hour) | | | | | | | | | | | |
|-------|-------------|-------|-------|-------|-------|-------|-------|-------|-------|-------|-------|-------|
| | 0.25 | 0.5 | 0.75 | 1 | 1.5 | 2 | 2.5 | 3 | 4 | 6 | 8 | 24 |
| 30 | 37.88 | 44.44 | 55.1 | 62.89 | 62.89 | 73.26 | 73.91 | 72.46 | 72.46 | 71.68 | 73.56 | 73.26 |
| 40 | 35.27 | 45.98 | 59.00 | 60.06 | 56.02 | 69.44 | 68.73 | 67.96 | 70.00 | 69.97 | 69.95 | 68.70 |
| 50 | 35.46 | 38.1 | 38.99 | 40.65 | 38.54 | 38.54 | 39.92 | 37.88 | 38.31 | 42.46 | 38.54 | 39.92 |

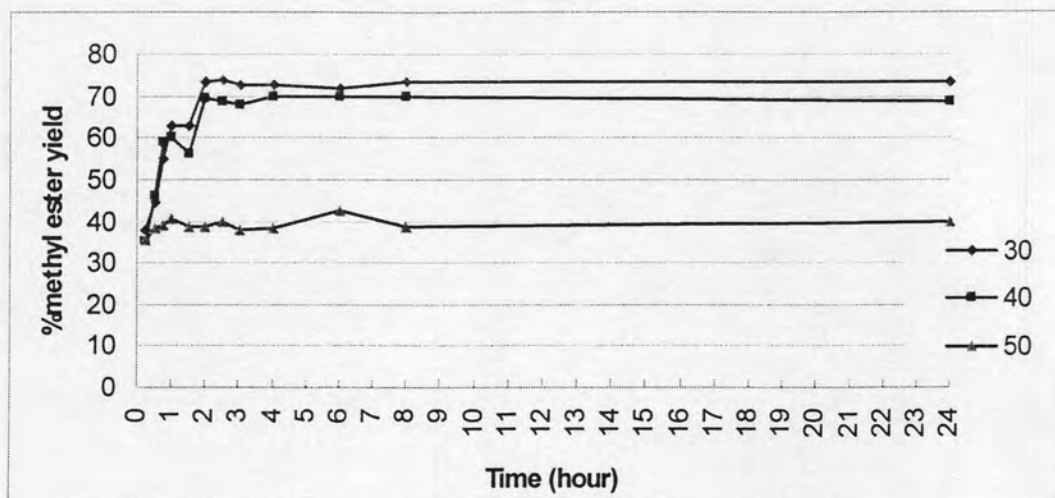


Figure 4.11 %Methyl ester yield versus time at reaction temperature 30°C, 40°C and 50°C during 24 hrs

The ester contents decreased when increasing temperature and higher temperature gave a faster transformation but too high temperature was lead to enzyme denaturing [8]. Therefore, the optimal reaction temperature is 30°C.

4.4.1.2 Effect of molar ratio of methanol to FFA

The effect of molar ratio of methanol to FFA on conversion of FFA to FAME was investigated at 1:1, 2:1 and 3:1 using an optimal reaction temperature 30°C, and 10% Novozyme 435 (based on weight of acid oil) and the reactions were monitored by TLC and $^1\text{H-NMR}$ analysis. The TLC result is shown in Figure 4.12. Using molar ratio of methanol to FFA at 1:1, 2:1 and 3:1, TLC analysis showed that the FFA were converted rapidly to FAME within 2 hrs while the conversion was relatively constant after 2 hrs for 2:1 and 3:1 ratios. For 1:1 molar ratio of methanol to FFA at the reaction time for 3 hrs and 4 hrs, the character FFA was occurred again from reversible reaction and FFA were converted to FAME was decreased from 2 hrs.

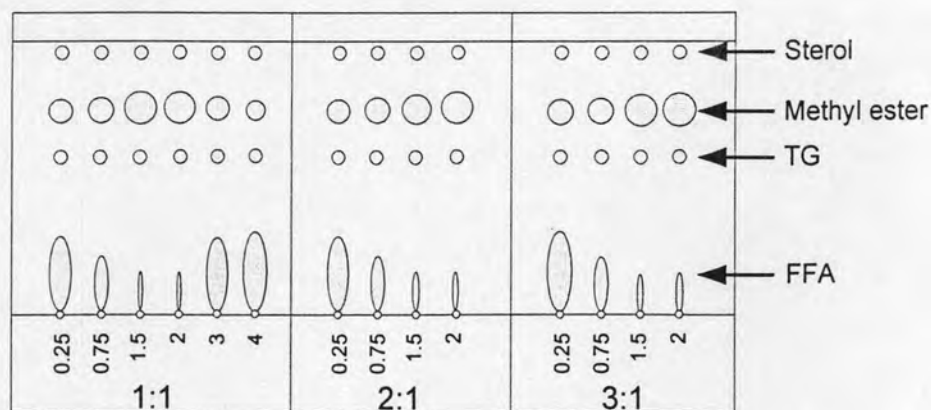


Figure 4.12 TLC for monitoring the methyl ester at reaction time 0.25, 0.75, 1.5 and 2 hrs of effect of molar ratio of methanol to FFA at 1:1, 2:1 and 3:1

Additionally the results also showed that the optimal reaction temperature was 30°C , an important variable which affected % conversion of methyl ester was the molar ratio of methanol to FFA. After esterification was occurred for 2 hrs at 1:1 molar ratio this gave the highest percentage of conversion but it decreased to 35.09% and 27.66% at 3 and 4 hrs, respectively (Table 4.10 and Figure 4.13). This study showed that one molar equivalent of methanol to FFA was less capability compared with the excess methanol to protect irreversible reaction, hydrolysis which converted methyl ester to FFA and methanol. The excess methanol more than one molar equivalent of methanol was protected reversible reaction, but more excess methanol caused enzyme deactivated. However, the 2:1 and 3:1 molar ratio of methanol to FFA were not considered as excess methanol caused enzyme deactivated and the percentage of conversion in 2 hrs were 73.26% and 72.73%, respectively. The efficiency of conversion between this two molar ratio were not different but the quantity of methanol used in molar ratio 2:1 was less than that of 3:1. So, The optimal molar ratio of methanol to FFA was 2:1 which caused decreasing in production cost and separated methanol.

Table 4.10 The effect of molar ratio of methanol to FFA on esterification of the acid oil by $^1\text{H-NMR}$ analysis

| Mole ratio | Time (hour) | | | | | | | | | | | |
|------------|-------------|-------|-------|-------|-------|-------|-------|-------|-------|-------|-------|-------|
| | 0.25 | 0.5 | 0.75 | 1 | 1.5 | 2 | 2.5 | 3 | 4 | 6 | 8 | 24 |
| 1: 1 | 39.92 | 64.1 | 63.49 | 69.44 | 70.17 | 73.75 | 70.92 | 35.09 | 27.66 | 64.72 | 70.92 | 69.44 |
| 2: 1 | 37.88 | 44.44 | 55.1 | 62.89 | 67.89 | 73.26 | 73.91 | 72.46 | 72.46 | 71.68 | 73.56 | 73.26 |
| 3: 1 | 28.25 | 40.16 | 53.33 | 64.72 | 66.26 | 72.73 | 72.73 | 72.46 | 74.19 | 72.55 | 73.26 | 70.43 |

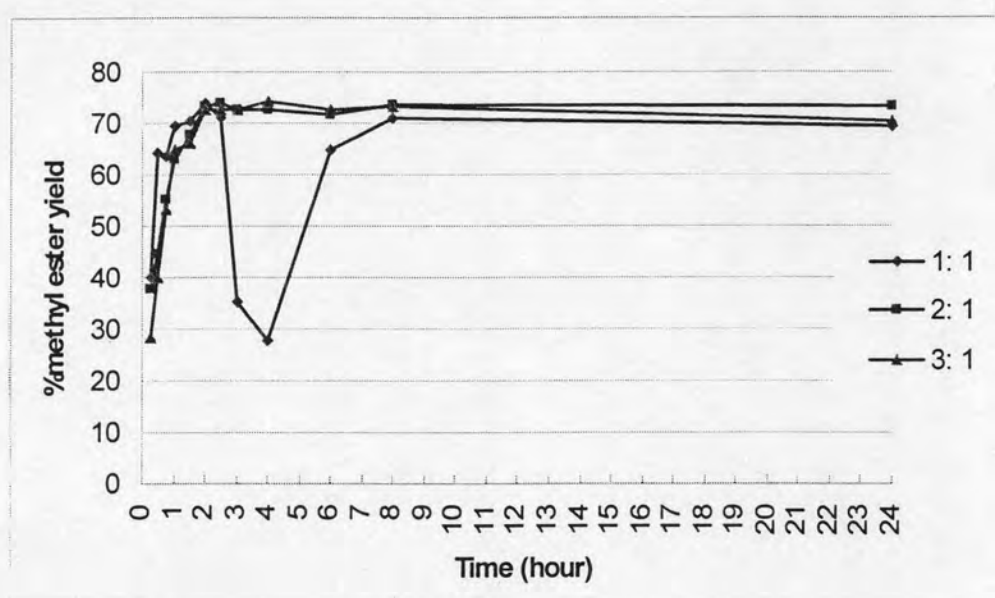


Figure 4.13 %Methyl ester yield versus time at molar ratios of methanol to FFA 1:1, 2:1 and 3:1 during 24 hrs

4.4.1.3 Effect of amount of Novozyme 435

The effect of amount of Novozyme 435 on conversion of FFA to FAME was investigated at 5%, 7.5% and 10% using an optimal reaction temperature 30°C , and 2:1 molar ratio of methanol to FFA and the reactions were monitored by TLC and $^1\text{H-NMR}$ analysis. The TLC result is shown in Figure 4.14. At 10% of Novozyme 435, TLC analysis showed that the FFA were converted rapidly to FAME within 2 hrs and the conversion was relatively constant after 2 hrs. At 7.5% Novozyme 435 showed slower conversion (8 hrs) of FFA to FAME than 10% (2 hrs) and the conversion was relatively constant after 8 hrs. At 5% Novozyme 435 showed the slowest conversion of

FFA to FAME. FFA still remained in acid oil although the reaction time passed to 24 hrs.

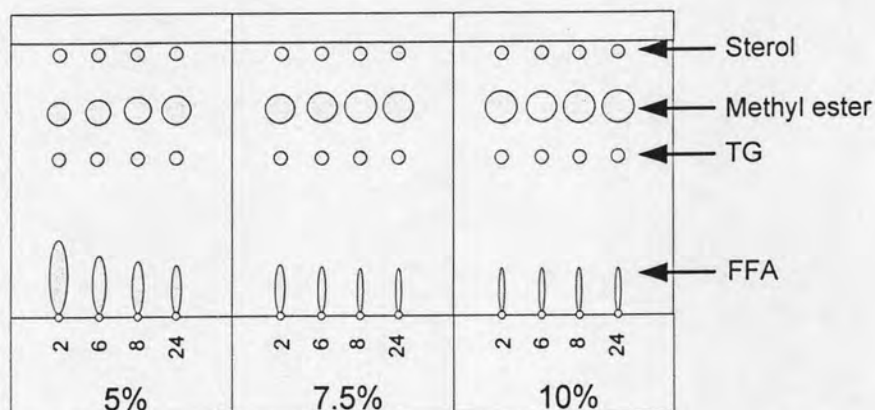


Figure 4.14 TLC for monitoring the methyl ester at reaction time 0.25, 0.75, 1.5 and 24 hrs of effect of amount of enzyme at 5%, 7.5% and 10%

From ^1H NMR analysis in Table 4.11 the results were plotted as shown in Figure 4.15. At 10% Novozyme 435 with reaction to equilibrium 2 hrs and 7.5% with reaction to equilibrium 8 hrs gave 73.26% and 72.15% of conversion to methyl ester, respectively. At 5% Novozyme 435 can not reached equilibrium although time passed to 24 hrs (still remained FFA in acid oil) and gave 65.38% of conversion to methyl ester. From the result showed that the percentage of conversion was proportionally to amount of enzyme and amount of enzyme related with production cost. Therefore, this study used 10%Novozyme 435 as optimal condition.

Table 4.11 The effect of amount of enzyme on on esterification of the acid oil by ^1H -NMR analysis

| %Enzyme | Time (hour) | | | | | | | | |
|---------|-------------|-------|-------|-------|-------|-------|-------|-------|-------|
| | 0.25 | 0.75 | 1 | 2 | 3 | 4 | 6 | 8 | 24 |
| 5% | 27.66 | 33.5 | 37.24 | 47.62 | 51.68 | 53.05 | 60.78 | 62.22 | 65.38 |
| 7.50% | 32.68 | 52.49 | 54.2 | 66.01 | 65.99 | 68.23 | 69.14 | 72.15 | 71.79 |
| 10% | 37.88 | 55.1 | 62.89 | 73.26 | 72.46 | 72.46 | 71.68 | 73.56 | 73.26 |

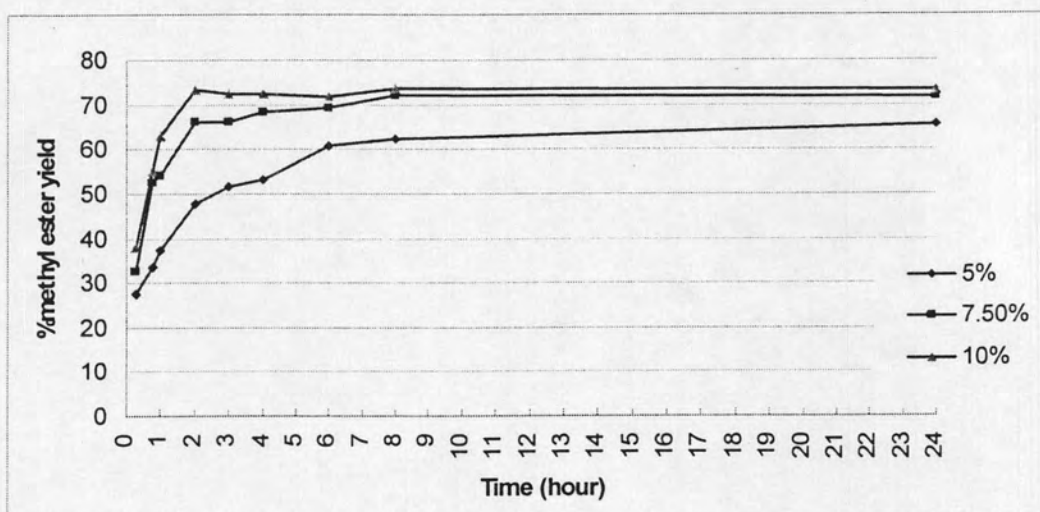


Figure 4.15 %Methyl ester yield versus time at amount of enzyme 5%, 7.5% and 10% during 24 hrs

4.4.2 The transesterification of remained triglyceride in methyl ester of acid oil by Novozyme 435

The chromatogram of $^1\text{H-NMR}$ analysis was shown in Figure 4.16. Proton peak of glyceryl moiety was appeared at 4.2-4.4 chemical shift although reaction time of transesterification by enzyme catalyzed process longer to 30 hrs. Thus, the transesterification of remained triglyceride in acid oil by normally using base catalyzed process.

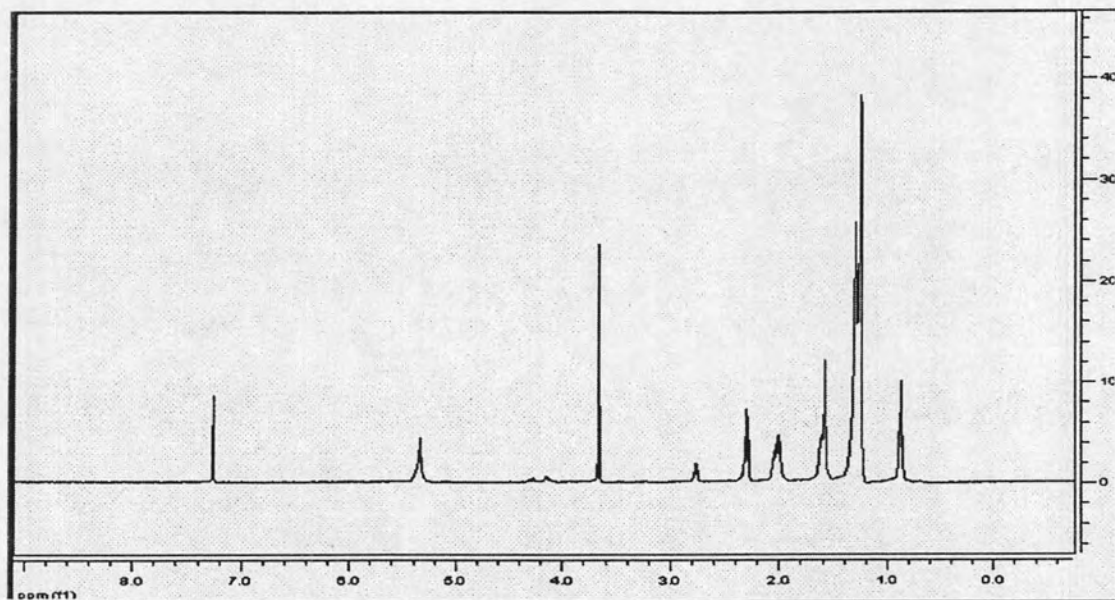


Figure 4.16 Chromatogram of ^1H NMR analysis of remained triglyceride in acid oil by enzymatic transesterification

4.5 Biodiesel production using enzyme and base catalyzed process

4.5.1 First step: enzyme catalyzed esterification

The optimal condition of esterification from acid oil gave 73.26% conversion to methyl ester which used in transesterification in section 4.5.2.

4.5.2 Second step: base catalyzed transesterification

This study determined the effect of molar ratio of methanol to TG in transesterification using ^1H -NMR analysis and acid value analysis in Table 4.12. The transesterification process was carried out in five differences of methanol to TG ratios.

Table 4.12 Molar ratio converts acid oil as methyl ester by base catalyzed process (0.8% NaOH, 65°C and 1 hr)

| Molar ratio of Methanol: TG | %FFA | Acid value | %Methyl ester yield |
|-----------------------------|------|------------|---------------------|
| 3:1 | 4.59 | 9.13 | 83.26 |
| 5:1 | 0.76 | 1.52 | 91.82 |
| 7:1 | 0.71 | 1.41 | 92.52 |
| 9:1 | 0.94 | 1.87 | 92.52 |
| 11:1 | 0.87 | 1.73 | 92.47 |

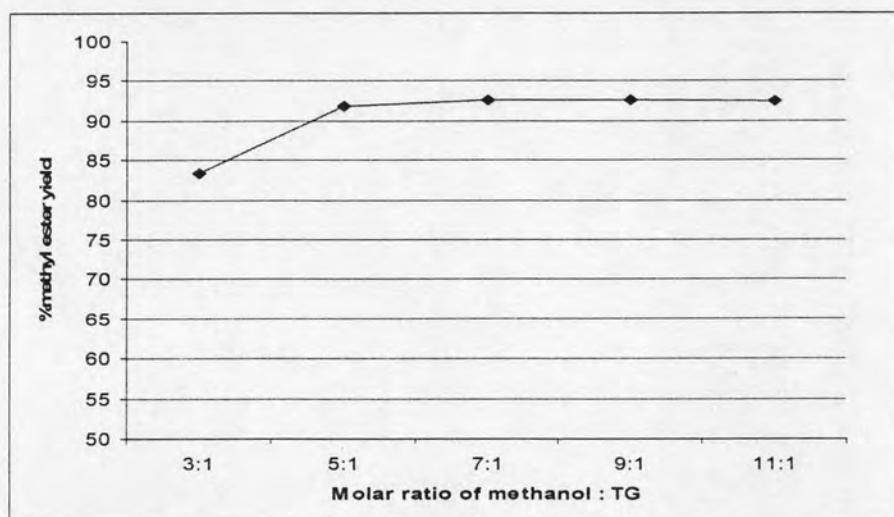


Figure 4.17 The effect of molar ratio on %methyl ester yield (NaOH catalyst)

From Table 4.12, the high conversion was obtained by 5:1 molar ratio of methanol to TG (91.82%), acid value from first step was reduced to 0.76 mgKOH/g corresponding to 1.52% FFA. The amount of methanol required for transesterification is analyzed in terms of the molar ratio. Stoichiometrically, the methanol to TG molar ratio required 3:1. But, practically this is not sufficient to complete the reaction. Higher molar ratio is required to drive the reaction to complete at a faster rate. It is observed that lower molar ratios requires the longer period of reaction. The effect of molar ratio on conversion efficiency is shown in Figure 4.17. It has been seen that yield of the

process increases when increasing of molar ratio. The higher ester yield are obtained by the molar ratio of 5:1, 7:1, 9:1 and 11:1 with non-significant difference (about 92%), with further increasing in molar ratio, the conversion efficiency more or less remains the same. The excess of methanol in the ester decreases the flash point of the biodiesel and could interfere by the separation of ester product. The optimal molar ratio of methanol to TG was 5:1.

4.5.3 Biodiesel production from rice fatty acid using enzyme and base catalyzed process

In Table 4.13 and Table 4.14, they are shown the investigation of trial batch that compares between acid oil and rice fatty acid in optimal condition. The optimal reaction temperature is 30°C, the molar ratio of methanol to FFA is 1:2 and 10% Novozyme 435 reaction time is 2 hrs bases on acid oil and rice fatty acid respectively. The percentage of conversion of acid oil was 74.01% and rice fatty acid was 68.79%. The percentage of conversion of rice fatty acid less than acid oil because it related with initial FFA in starting material.

Table 4.13 First step esterification by enzyme catalyst of acid oil and rice fatty acid

| %Conversion | Time (hour) | | | | | |
|-----------------|-------------|-------|-------|-------|-------|-------|
| | 0.25 | 0.75 | 1 | 2 | 2.5 | 3 |
| Rice fatty acid | 35.13 | 52.41 | 59.89 | 68.79 | 68.03 | 68.19 |
| Acid oil | 37.88 | 55.1 | 62.89 | 74.01 | 72.58 | 73.15 |



Table 4.14 Second step transesterification by base catalyst of acid oil and rice fatty acid

| | Molar ratio of methanol: TG | %Conversion | %FFA | Acid value |
|-----------------|-----------------------------|-------------|------|------------|
| Rice fatty acid | 5:1 | 92.26 | 0.63 | 1.25 |
| Acid oil | 5:1 | 91.31 | 0.77 | 1.54 |

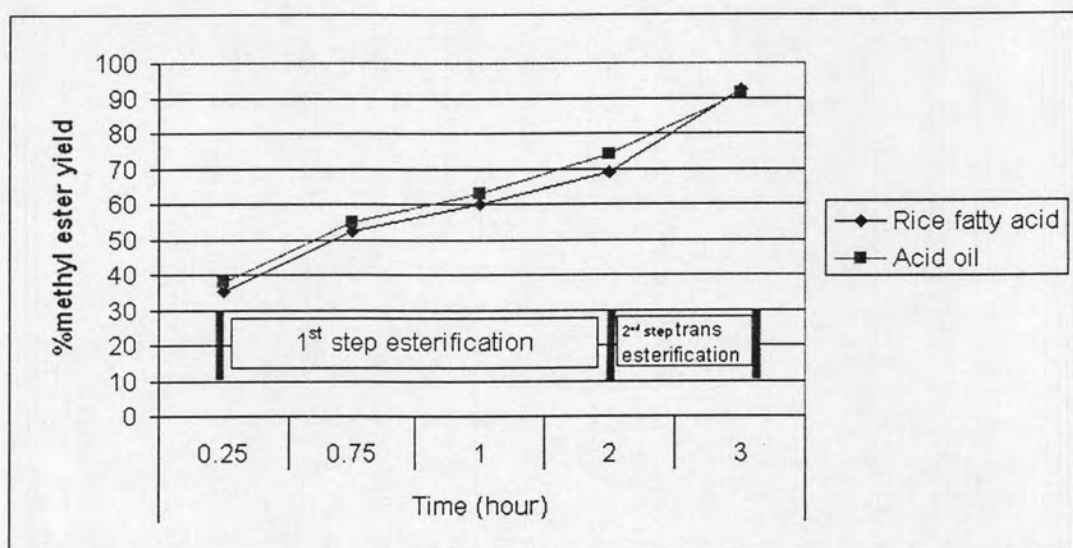


Figure 4.18 %Methyl ester yield versus time in optimal condition of 2 step enzyme-base catalyzed process of acid oil and rice fatty acid

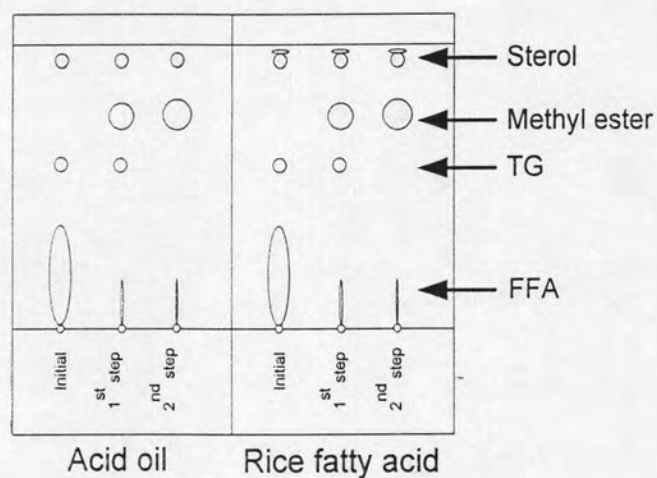


Figure 4.19 TLC for monitoring the methyl ester in optimal condition of 2 step enzyme-base catalyzed process of acid oil and rice fatty acid

From $^1\text{H-NMR}$ analysis and acid value analysis in Table 4.13 and Table 4.14 the result as shown in Figure 4.18 that showed trend of the ester yield in both substrates are concurrently in the first step of esterification and the second step of tranesterification. The TLC result is shown in Figure 4.19, the methyl ester occurred in first step and second step of both substrates. In the first step of acid oil there was a little remained FFA, biodiesel was occurred but triglycerides did not react with methanol to produced methyl ester. In the second step, all triglycerides react with methanol and changed to methyl ester because no spot of triglyceride appeared. The TLC result of rice fatty acid was the same as acid oil.

4.6 Biodiesel production from acid oil using acid and base catalyzed process

4.6.1 First step: acid catalyzed esterification

This study determined the effect of molar ratio of methanol to TG in esterification using $^1\text{H-NMR}$ analysis and acid value analysis in Table 4.15. The esterification process was carried out in six differences of methanol to TG ratios.

Table 4.15 The molar ratios convert acid oil as methyl ester by acid catalyzed process (1% Sulfuric acid, 65°C and 90 minutes) in first step

| Molar ratio of methanol: TG | %FFA | Acid value | %Methyl ester yield |
|-----------------------------|-------|------------|---------------------|
| 3:1 | 35.62 | 70.89 | 34.54 |
| 5:1 | 28.88 | 57.47 | 41.15 |
| 7:1 | 23.10 | 45.96 | 48.66 |
| 9:1 | 16.95 | 33.74 | 55.89 |
| 11:1 | 14.91 | 29.67 | 58.48 |
| 13:1 | 16.55 | 32.93 | 56.50 |

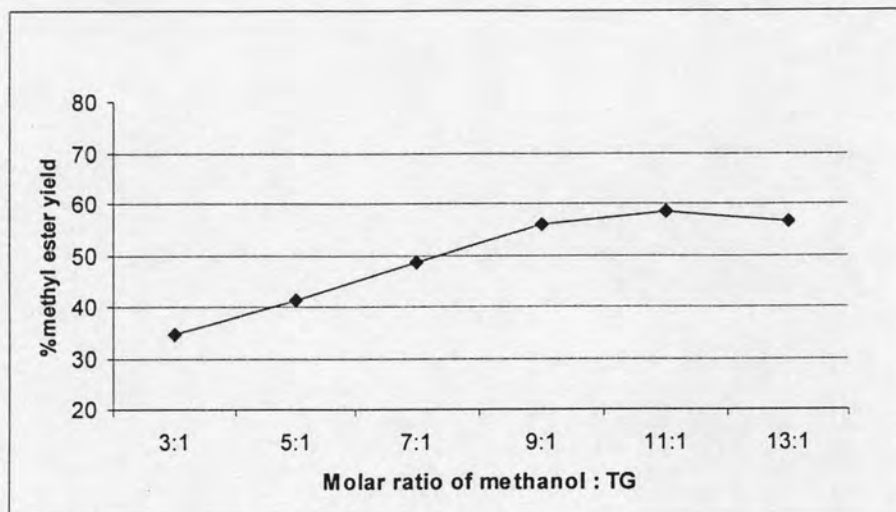


Figure 4.20 The effect of molar ratio on %methyl ester yield (H_2SO_4 catalyst) in first step esterification

From Table 4.15, the high conversion was obtained by 9:1 molar ratio of methanol to TG (55.89%), acid value from first step was reduced to 33.74 mgKOH/g corresponding to 16.95% FFA. stoichiometric ratio required 1:1 molar ratio of methanol to FFA, the esterification is commonly carried out with an extra amount of methanol in order to shift the equilibrium to the proposed product, methyl ester. But, practically this is not sufficient to complete the reaction. Higher molar ratio is required to drive the reaction to complete at a faster rate. It is observed that lower molar ratios requires the longer period of reaction. The effect of molar ratio on conversion efficiency is shown in Figure 4.20. It has been seen that yield of the process increases when increasing of molar ratio. The higher ester yield are obtained by the molar ratio of 9:1, 11:1 and 13:1 with non-significant difference (about 56%), with further increasing in molar ratio, the conversion efficiency more or less remains the same. The excess of methanol in the ester decreases the flash point of the biodiesel and could interfere by the separation of ester product. The optimal molar ratio of methanol to TG was 9:1.

On completion of this reaction, the product is poured into a separating funnel for separating the excess alcohol. The excess alcohol, with sulfuric acid and impurities moves to the top surface and is removed. The lower layer is separated for further processing (second step of esterification)

4.6.2 Second step: acid catalyzed esterification

This study determined the effect of molar ratio of methanol to TG in esterification using $^1\text{H-NMR}$ analysis and acid value analysis in Table 4.16. The esterification process was carried out in four differences of methanol to TG ratios.

Table 4.16 The molar ratios convert acid oil as methyl ester by acid catalyzed process (1% Sulfuric acid, 65°C and 90 minutes) in second step

| Molar ratio of methanol: TG | %FFA | Acid value | %Methyl ester yield |
|-----------------------------|------|------------|---------------------|
| 3:1 | 7.2 | 14.4 | 67.9 |
| 5:1 | 3.38 | 6.75 | 71.2 |
| 7:1 | 2.55 | 5.1 | 73.5 |
| 9:1 | 2.5 | 5.0 | 73.4 |

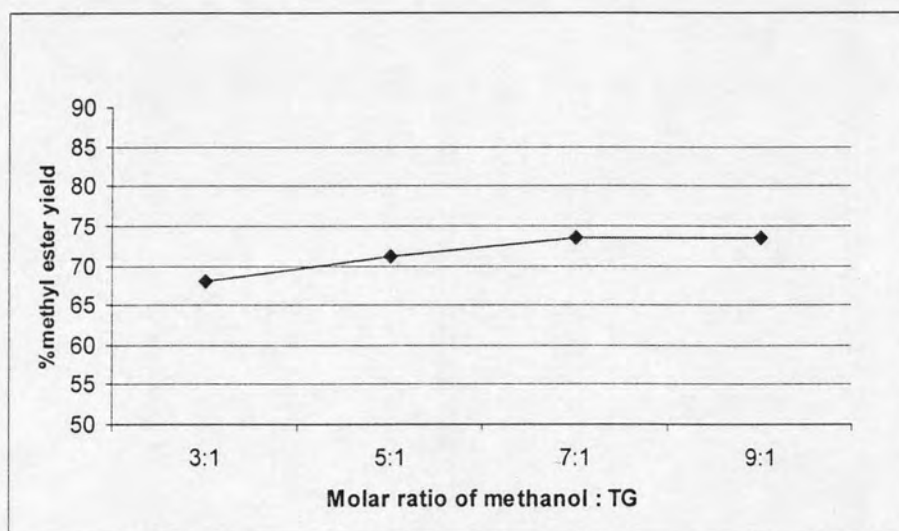


Figure 4.21 The effect of molar ratio on %methyl ester yield (H_2SO_4 catalyst) in second step esterification

From Table 4.16, the high conversion was obtained by 7:1 molar ratio of methanol to TG (73.5%), acid value from first step was reduced to 5.1 mgKOH/g corresponding to 2.55% FFA. stoichiometric ratio required 1:1 molar ratio of methanol to FFA, the esterification is commonly carried out with an extra amount of methanol in order to shift the equilibrium to the proposed product, methyl ester. But, practically this is not sufficient to complete the reaction. Higher molar ratio is required to drive the reaction to complete at a faster rate. It is observed that lower molar ratios requires the longer period of reaction. The effect of molar ratio on conversion efficiency is shown in Figure 4.20. It has been seen that yield of the process increases when increasing of molar ratio. The higher ester yield are obtained by the molar ratio of 7:1 and 9:1 with non-significant difference (about 73.5%), with further increasing in molar ratio, the conversion efficiency more or less remains the same. The excess of methanol in the ester decreases the flash point of the biodiesel and could interfere by the separation of ester product. The optimal molar ratio of methanol to TG was 7:1.

On completion of this reaction, the product is poured into a separating funnel for separating the excess alcohol. The excess alcohol with sulfuric acid and impurities moves to the top surface and is removed. The lower layer is separated for further processing (third step of transesterification).

4.6.3 Third step: base catalyzed transesterification

From 4.5.2 it was found that the transesterification at 65°C for 1 hr gave 92.45% of conversion when the ratio of methanol to TG was 5:1. This condition is similar to 4.5.2 that gave 91.32% of conversion as showed in Table 4.17.

Table 4.17 The molar ratios convert acid oil as methyl ester by base catalyzed process (0.8% NaOH, 65 °C, and 1 hr) in third step

| Molar ratio of TG: | | | |
|---------------------------|-------------|-------------------|--------------------|
| methanol | %FFA | Acid value | %Conversion |
| 5:1 | 0.845 | 1.7 | 92.45 |

4.6.4 Biodiesel production from rice fatty acid using acid and base catalyzed process

From table 4.18 , the investigation of trial batch compares between acid oil and rice fatty acid in optimal condition. The optimal reaction temperature is 65°C, the molar ratio of methanol to TG was 9:1 in the first step of acid catalyst and 7:1 in the second step of acid catalyst, 1% of sulfuric acid, the reaction time is 90 minutes and in the third step of transesterification optimal condition were 0.8% of NaOH, reaction temperature is 65°C, the molar ratio of methanol to TG 5:1 and the reaction time 1 hr between acid oil and rice fatty acid.

Table 4.18 The methyl ester produced by 3 step acid and base catalyzed process of acid oil and rice fatty acid

| Rice fatty acid | condition | Acid value | %FFA | %Conversion |
|------------------------|--------------------------------|-------------------|-------------|--------------------|
| First step | 65°C, 90 minutes, 1% H_2SO_4 | 37.4 | 18.9 | 49.42 |
| Second step | 65°C, 90 minutes, 1% H_2SO_4 | 3.81 | 1.92 | 68.15 |
| Third step | 65°C, 60 minutes, 0.8%NaOH | 1.7 | 0.854 | 92.05 |
| Acid oil | condition | Acid value | %FFA | %Conversion |
| First step | 65°C, 90 minutes, 1% H_2SO_4 | 31.49 | 15.83 | 57.14 |
| Second step | 65°C, 90 minutes, 1% H_2SO_4 | 4.00 | 2.01 | 74.12 |
| Third step | 65°C, 60 minutes, 0.8%NaOH | 1.95 | 0.980 | 92.93 |

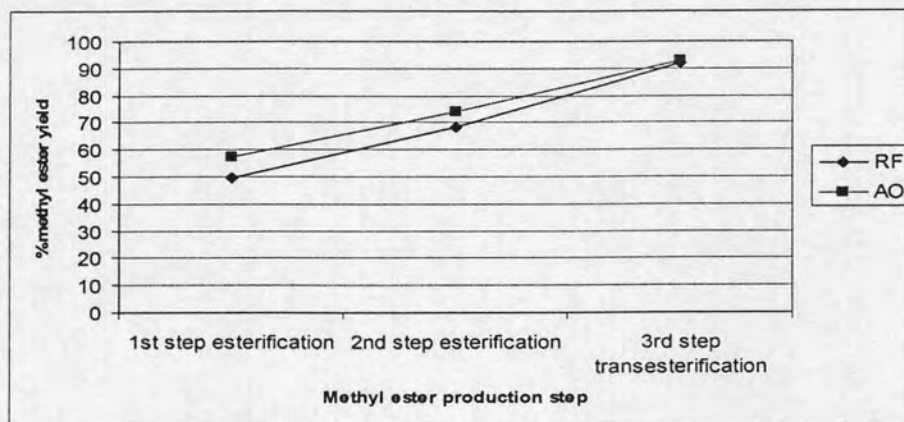


Figure 4.22 %Methyl ester yield versus time in optimal condition of 3 step acid-base catalyzed process of acid oil and rice fatty acid

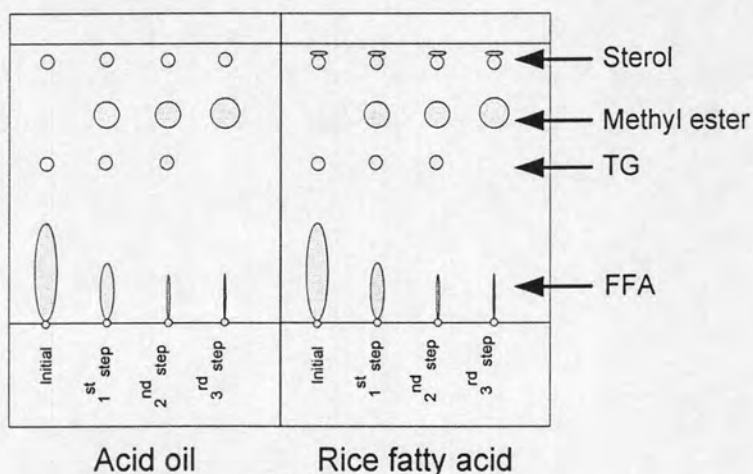


Figure 4.23 TLC for monitoring the methyl ester in optimal condition of 3 step acid-base catalyzed process of acid oil and rice fatty acid

The %methyl ester yield versus time in optimal condition of 3 step acid-base catalyzed processes is shown in Figure 4.22, trend of graph of the ester yield in both substrates are concurrently in the first step and second of esterification and the third step of transesterification. Figure 4.23 shows TLC for monitoring the methyl ester occurred in first step and second step of both substrates. In the first step of acid oil, esterification was not completed, so the FFA remained in methyl ester. Hence, esterification in second step reduced FFA from first step and biodiesel was occurred but triglycerides did not react with methanol to produced methyl ester. In the third

step, all triglycerides react with methanol and changed to methyl ester because no spot of triglyceride appeared. The TLC result of rice fatty acid was the same as acid oil.

4.7 Repeated use of Novozyme 435

To investigate the stability of Novozyme 435, the esterification was repeated every 2 hrs. The operational stability of the immobilized enzyme washed Novozyme 435 by *tert*-butanol and unwashed Novozyme 435 are shown in Table 4.19 and Figure 4.24. No evident decrease of the lipase activity was observed during the first nine cycles. The FAME yields of both methods were higher than 65%. So, Novozyme 435 could keep its activity for at least 9 cycles in the system.

Table 4.19 The operational stability of Novozyme 435

| Number of repeated time | %Methyl ester yield | | | | |
|--------------------------------|---------------------|-------|-------|-------|-------|
| | 1 | 3 | 5 | 7 | 9 |
| Washed by <i>tert</i> -butanol | 68.45 | 67.78 | 65.32 | 65.44 | 67.59 |
| unwashed | 68.08 | 65.44 | 65.43 | 68.52 | 67.90 |

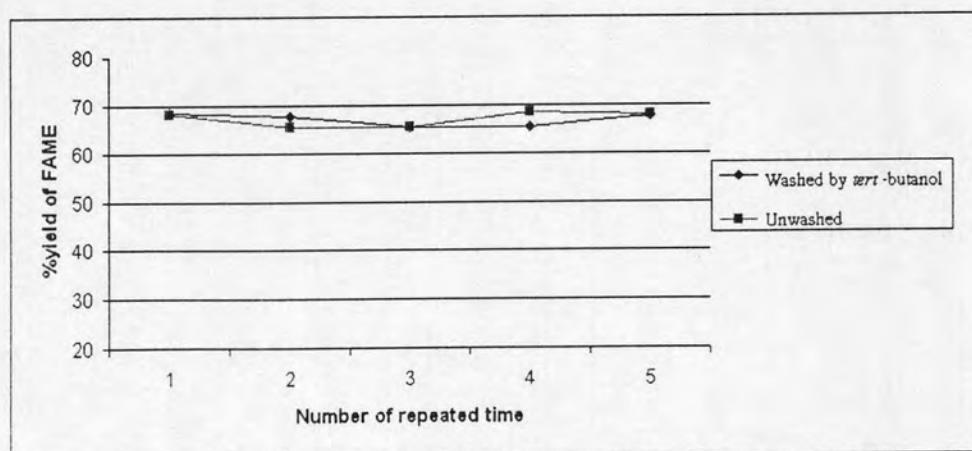


Figure 4.24 %Methyl ester yield versus number of repeated time of the operational stability of Novozyme 435

The operational stability of the immobilized lipase is an important parameter in an industrial process. It has been demonstrated that the cost of lipase accounts for a large part in the total cost of biodiesel production, and one of the main advantages of immobilized lipase is that it can be used repeatedly over an extended period of time.

4.8 Properties of biodiesel

The characteristics of biodiesel produced from acid oil and rice fatty acid by using enzyme and base catalyzed process and acid and base catalyzed process were comparable to that of standard biodiesel as shown in Table 4.20. The comparison of these properties with American standards for biodiesel (ASTM D6751-02) shows the specific gravity, flash point and oxidation stability are in range of fuel properties prescribed in standard biodiesel, except percentage of methyl ester. The purity of biodiesel samples is measured in terms of their methyl ester content (%wt). Methyl ester content of biodiesel samples increased with increasing catalyst concentration [36], if increased catalyst concentration not too high (might be associated with the formation of soaps which are known to frequently occur under homogeneous alkali catalyst), the percentage of methyl ester might be higher. The oxidation stability of biodiesel from rice fatty acid by enzyme and base catalyzed process higher than the standard biodiesel providing longer storage which oxidation reaction did not occur.

Table 4.20 Properties of biodiesel

| Fuel Property | Standard Biodiesel ASTM D6751-02 | Biodiesel from acid oil via enzyme- base | Biodiesel from rice fatty acid via enzyme- base | Biodiesel from acid oil via acid- base | Biodiesel from rice fatty acid via acid- base |
|---------------------------------|---|---|--|---|--|
| Methyl ester, %wt | >96.5 | 90.48 | 91.25 | 91.13 | 91.48 |
| Specific Gravity kg/l @ 15°C | 0.87-0.90 | 0.8894 | 0.8862 | 0.8894 | 0.8864 |
| Flash Point (°C) | 100-170 | 156 | 161 | 155 | 161 |
| Oxidation stability(hr) | >6 | 6 | 10 | - | - |