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TRANSESTERIFICATION OF PALM KERNEL OIL WI TH DIALKYL CARBONATES USING LIP ASE AS BIOCATALYST

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ABSTRACT

For single step lipase-catalyzed transesterification of palm kernel oil, diakyl carbonates (in this case dimethyl and diethyl carbonate) gave a better yield compare to that of short chain alcohols. The rate of ester formation with dialkyl carbonates as substrate was about 6 – 7 times than that obtained with short chain alcohols. The formation of ester was gradually increase with enzyme amount from 5% - 20% (w/w of oil) for 8 hours reaction. For the economic point of view, enzyme amount of 10% on the weight base of oil was proposed for further reaction. Generally, the highest ester formation was observed when temperature of 60 °C was used. However, in the case of dimethyl carbonate that little difference was observed at reaction temperature of 60 and 70 °C, and the reaction proceeded nearly identical. The esters formation increase drastically up to more than 70% when water was added up to 0.2 %. However, ester formation extremely decreases when water was added more than 0.4. The formation of ester was lower when hydrocarbon solvent was added to the system compare to that of when dialkyl carbonates was used as substrate and solvent.

Key words: *palm kernel oils, dialkyl carbonates, lipase, transesterification*

ABSTRAK

Dengan katalisa lipase langkah tunggal transesterifikasi minyak inti sawit dengan diakil karbonat (dalam hal ini dimetil dan dietil karbonat) memberikan hasil yang lebih baik dibandingkan dengan alkohol rantai pendek. Tingkat pembentukan ester dari diakil karbonat sebagai substrat adalah sekitar 6-7 kali lebih tinggi dibandingkan dari penggunaan alcohol rantai pendek. Pembentukan ester meningkat dengan meningkatnya jumlah enzim dari 5-20% (w/w minyak) dalam 8 jam reaksi. Dari sudut pandang ekonomi, penggunaan enzim sebesar 10% dari total berat minyak dianjurkan untuk reaksi selanjutnya. Pada umumnya pembentukan ester terbanyak adalah pada suhu 60 °C. Namun, pada dimetil karbonat dijumpai sedikit perbedaan antara suhu reaksi 60 °C dan 70 °C dan jalannya reaksi hampir identik. Pembentukan ester meningkat drastis sampai melebihi 70% bila air ditambahkan sampai 0,2%. Namun,

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pembentikan ester menurun secara ekstrim bila air ditambahkan melebihi 0,4%. Pembentukan ester akan rendah bila pelarut hidrokarbon ditambahkan ke sistem dibandingkan jika diakil karbonat yang dignakn sebagai substrat dan pelarut.

Kata kunci: *minyak inti sawit, diakil karbonat, lipase, transesterifikasi*

INTRODUCTION

The transesterification of vegetable oils and animal fats to form esters has received considerable attention for several years. Various routes have been proposed for both chemical and enzymatic process *(4,6,7,13,14,15,16)*. The most interesting research to day is focused on the utilization of enzymes, normally lipases, for catalysing the synthesis of simple esters of vegetable oils or other agriculture lipid feedstock with or without the presence of organic solvents *(9,18)*. Lipase-catalyzed transesterification in a solvent-free medium is important in industrial application because such a system would have an enormous advantage by avoiding the problem of separation, toxicity, and flammability of organic solvents. However, the organic solvent-free alcoholysis, especially methanolysis, does not give high conversions. The same problem was also found when ethyl or methyl acetate was used as acyl acceptors.

Among several sources of lipases screened, only lipase from *Candida antarctica* lipase B (Novozym 435 and Candida B. Silica) was the most effective for transesterifying palm kernel oil with dialkyl carbonates both in the presence of isohexane as solvent or dialkyl carbonates as substrate and solvent *(12)*. Therefore, for further experiment, only Novozym 435 was used as lipase source.

Some key aspects of lipase-catalyzed esters synthesis have been investigated and reported such as the source of alkyl donor *(5,11,14)*, the role of water *(3,10,20)*, the effect of temperature and pressure condition *(1,9)*, and the effect of an organic solvents *(17)*. Some of these key aspects were also investigated in the transesterification of palm kernel oil with dialkyl carbonates catalyzed by Novozym 435.

MATERIALS AND METHOD

Material

Oilseed material. Palm Kernel was obtained from Indonesian Oil Palm Research Institute (IOPRI), Medan – Indonesia. The seed was milled using a mechanical grinder before use.

Chemicals. Dimethyl carbonate, diethyl carbonate, methanol, ethanol and isohexane were for analysis grade and purchased from Merck (Germany). All solvents used were percolated prior by Puralox SCCa 150/145 N before used. N-Methyl-N-trimethylsilylheptaflour(o)butyramide (MSHFBA) was used for sylilation and obtained from Macherey-Nagel (Germany).

Method

To 5 ml closed reaction tube containing several amount of palm kernel oil, was added several amount of dialkyl carbonates and Novozym 435. The mixture was stirred and heated to 60 °C for 24 hours. Samples were withdrawn at specified interval during the 24 hours reaction period. Samples were filtered (by Syringe filters; porosity 0.45 µm, 4-mm Nylon) to remove the enzyme, and the excess of alkyl source was then evaporated. Filtrates were stored at –20 °C before analyzed further.

Analysis

Lipid composition was analyzed by Gas Chromatography instrument (HP6890) equipped with a FID Detector and a high temperature HT5 AQ (SGE), 12 m x 0.22 mm i.d column. The film thickness was 0.1 µm. Hydrogen flow was 40.0 ml/min, airflow was 450 ml/min and make up flow (Nitrogen) was 45.0 ml/min. The injector temperature was 400 °C, injector splitting was 10:1 and detector temperature was 420 °C. The oven temperature was programmed from 70 °C (2 minute) to 420 °C at 10 °C/minute and was held at 420 °C for 6 minute. All sample were syllilated prior by N-methyl-N-trimethylsilylheptaFluor-(o)Butyramide (MSHFBA) containing methyl-imidazole (50 µl / ml MSHFBA) for about 30 minute and after the excess of MSHFBA evaporated, sample was dissolved in dichloromethane and directly inject to GC. Heptadecanoic methyl ester was used as internal standard.

RESULTS AND DISCUSSI ON

Effect of Alkyl Sources on the Esters formation

The type of alkyl source plays an important role in the reaction kinetic when enzymes are used as catalyst. Four alkyl donors were studied on the lipase-catalyzed transesterification with palm kernel oil. Transesterification with 1:3 (palm kernel oil/alkyl source) molar ratio was performed without any added solvent. The result show that among the alkyl donor used, the highest rate of ester formation was obtained using dialkyl carbonates as alkyl sources.

As shown in Figure 1 below, by using short chain alcohols such as methanol and ethanol as alkyl source, the rate of esters formation was low. After 8 hours reaction, the formation of methyl and ethyl esters was only 11% and 12% respectively. The esters formation was still low and only slightly increases, 13% and 19% respectively, when reaction was extended up to 24 hours. Event though reaction was prolonged up to 48 hours, the esters formation was not more than 30%.

The result reported here agrees with those of Shimada *et al. (17)* who found that a single addition of methanol to solvent-free alcoholysis reaction catalyzed by SP435 (*Candida antarctica* lipase supported on a macro porous acrylic resin) resulted in low conversion to methyl esters *(8)*. Similar result also reported by Hsu *et al.* who found that the immobilized SP435 was less-active biocatalyst and gave low conversion to ester (20 – 30% after 48 h of reaction) on

the transesterification of restaurant grease with ethanol *(3)*. As well known, the lipase-catalyzed transesterification of triglyceride with an alcohol is an equilibrium reaction and when short chain alcohol was used as a substrate, it did not dissolve well with palm kernel oil and the reaction did not take place homogenously. Some papers reported that an organic solvent was required for this reaction *(9,15)*.

Different result was found when dimethyl and diethyl carbonate were used as alkyl donor. As shown in Figure 1, the esters formation rate with dialkyl carbonates about 6 – 7 times higher than that of with short chain alcohols. The formation of methyl and ethyl esters were about 40% after 1 hour, and reached up to 76% and 73% respectively after 8 hours. The esters formation was than increases up to 83% and 81% respectively when reaction was prolonged up to 24 hours. The highest of esters formation could be explained because when dialkyl carbonates are used as a substrate the reaction is not equilibrium, because intermediate compound was decomposed immediately to carbon dioxide and alcohol. In the same condition palm kernel oil was also dissolve well in dialkyl carbonates.

It can be seen that for single step lipase-catalyzed transesterification of vegetable oils (especially palm kernel oil), diakyl carbonates (in this case dimethyl and diethyl carbonate) is a better substrate compare to that of short chain alcohols.

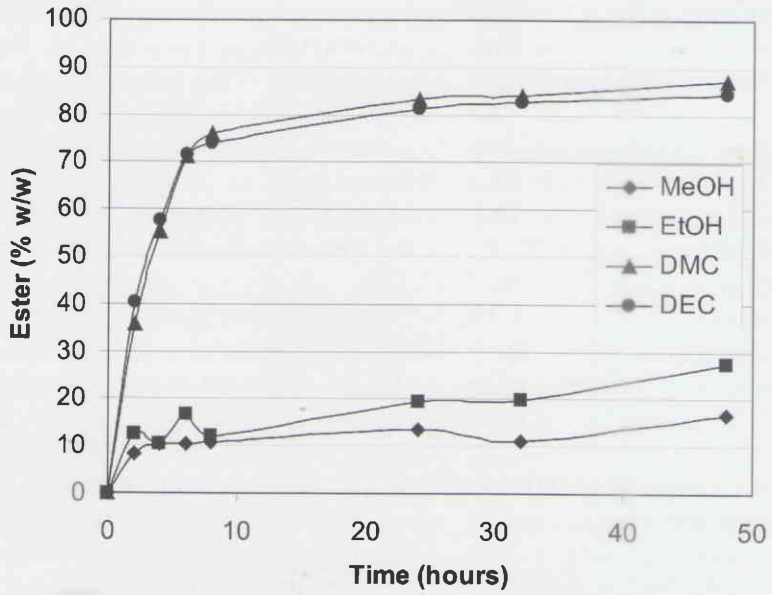

Figure 1. Time Course of Transesterification of Palm Kernel Oil with:
υ Methanol, ν Ethanol, λ Dimethyl Carbonate, σ Diethyl Carbonate, Without Organic Solvent at 60 °C .

Effect of Substrate Molar Ratio on the Esters Formation

To ensure that the reaction proceeds rapidly and completely, the enzyme should not be inhibited by substrate concentration. Various substrate molar ratios were performed to test its effect on the formation of esters.

As shown in Figure 2, by using dimethyl carbonate as substrate, the esters formation was low when molar ratio of dimethyl carbonate/palm kernel oil lower than three (mol/mol) was used. This could be explained because a quantity of dimethyl carbonate was not enough for complete reaction. The esters formation was increase with increasing of molar ratio. The highest esters formation was obtained when molar ratio of dimethyl carbonate/palm kernel oil was 20 mol/mol (82.45%). However, the ester formation was decrease when the molar ratio of substrate was increased more than100 mol/mol.

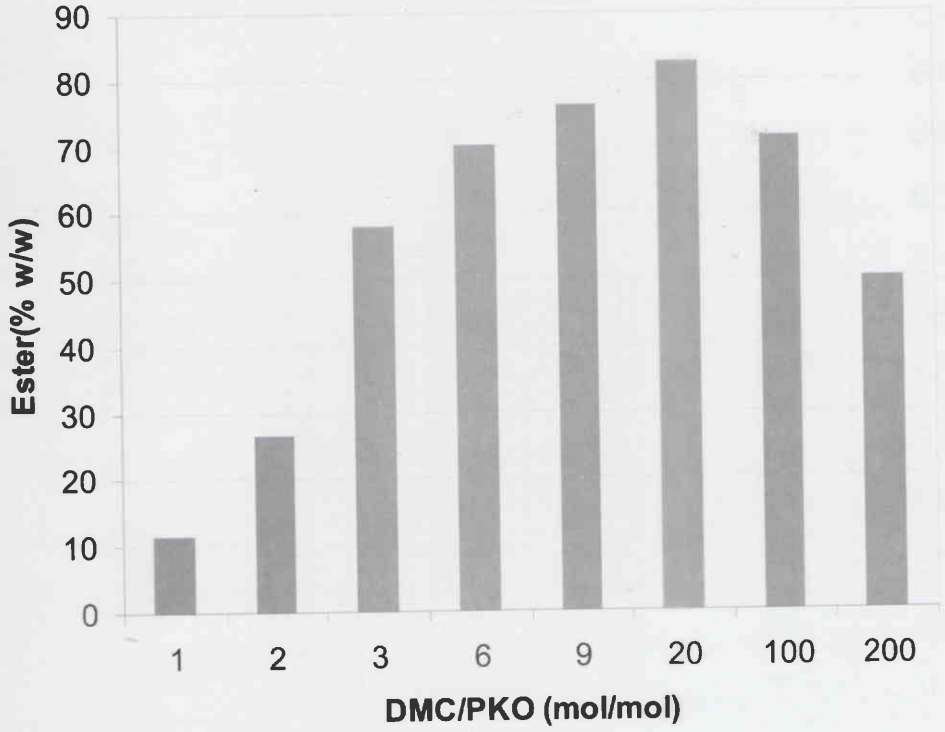

Figure 2. Ester Formation on the Transesterification of Palm Kernel Oil with Dimethyl Carbonate in Various Molar Ratios of Substrate at 50 °C for 8 Hours.

As shown in Figure 3, by using diethyl carbonate as solvent, the highest ester formation was obtained when molar ratio of diethyl carbonate/palm kernel oil of three mol/mol or stoichiometric amounts (79% for 8 hours reaction). The formation of esters decreased with an increase in the molar ratio excess.

When the molar ratio of diethyl carbonate/palm kernel oil was 20 mol/mol, event though all of the triglyceride seems to be converted completely, but the formation of ester lower than that of when molar ratio was three mol/mol or eight mol/mol. This could be because of highly content of 'other' compound (fatty acids) at product (around 20%). The ester formation increased up to 93% and its fatty acids content than decreased when reaction was prolonged up to 24 hours. This suggests that the water present in the enzyme preparation and in the system is a better nucleophile than diethyl carbonate, and therefore, fatty acids is produced first and is then esterified by the enzyme with diethyl carbonate acting as nucleophile.

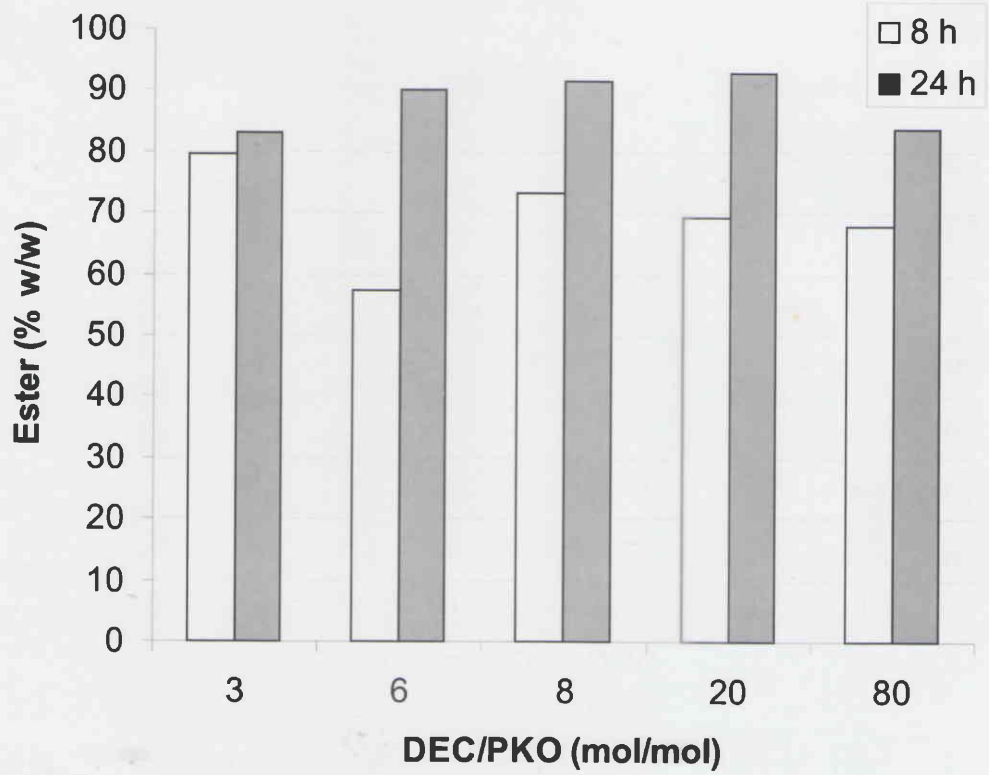

Figure 3. Ester Formation on the Transesterification of Palm Kernel Oil with Diethyl Carbonate in Various Molar Ratio of Substrate at 60 °C .

It can be seen that amount of dimethyl and diethyl carbonate gave a different effect on the esters formation. It could be because in lipase-catalyzed transesterification with dimethyl carbonate, activity of enzyme was inhibited by methanol that produced during reaction. The same problem was not happening when diethyl carbonate was used as substrate.

Effect of Amount of Novozym 435 on the Ester Formation

The amount of enzyme used is a crucial economical factor for successful industrial application. Therefore, the effect of amount of enzyme on the transesterification of palm kernel oil with dialkyl carbonates was examined with the various amount of enzyme range from 5% to 20% on the weight base of oil at 60 °C.

There was a gradual increase in the formation of esters with the enzyme amount from 5 % to 12 % (w/w of oil) on the lipase-catalyzed transesterification of palm kernel oil with dimethyl carbonate for 8 hours reaction. When reaction was prolonged up to 24 hours, the result show that in all condition used the formation of esters nearly at the same value, this indicate that reaction was nearly finish. Further increases in the quantity of the enzyme up to 15 % (w/w of oil) resulted slightly increase in the esters formation, as shown in Figure 4.

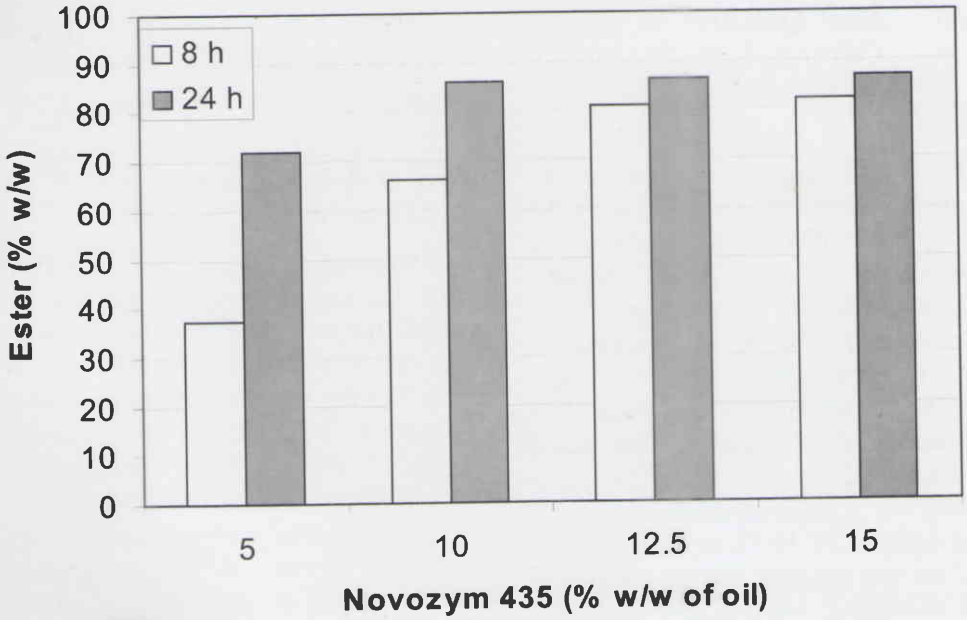

Figure 4. Ester Formation on the Transesterification Palm Kernel with Dimethyl Carbonate in the Presence of Various Concentrations of Novozym 435 at 60 °C.

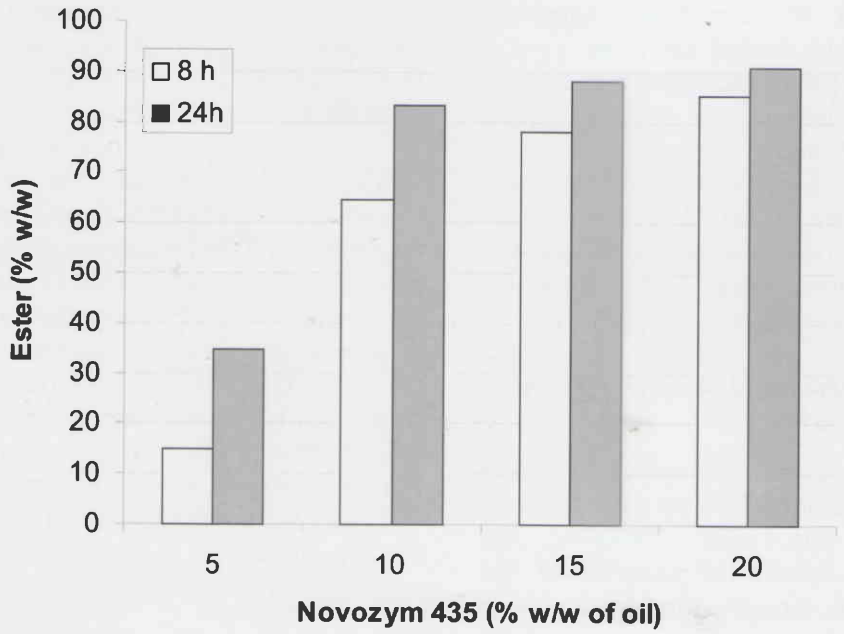

Figure 5. Ester Formation on the Transesterification Palm Kernel with Diethyl Carbonate in the Presence of Various Concentrations of Novozym 435 at 60 °C.

The similar result was also found when diethyl carbonate was used as alkyl source instead of dimethyl carbonate. The formation of ester was gradual increase with enzyme amount from 5% - 20% (w/w of oil) for 8 hours reaction. Except with enzyme amount of 5% (w/w of oil), the formation of esters is nearly in the same value when reaction was increased up to 24 hours (Figure 5).

From the economic point of view, enzyme amount of 10 % on the weight base of oil was used for further reaction. In this condition, enzyme activity was 9.8 µmol/min.g (enzyme activity was defined as amount of substrate converted per gram of Novozym 435 used).

Effect of Reaction Temperature on the Ester Formation

Temperature is one of important factor influencing enzyme activity. In general, the rate of a reaction increases with rise in temperature. However, by increasing the temperature, the mobility of protein segments in the enzyme increases while the strength of hydrophobic interaction decreases. Generally, above ambient temperature condition, up to 45 °C, a gradual decrease in the catalytic activity of the enzyme is observed *(19)*.

The effect of temperature on transesterification of palm kernel oil with dialkyl carbonates was examined at the

temperature range from 40 °C to 80 °C. As shown in Figure 3.10 and Table 3.10, by using dimethyl carbonate as alkyl source the highest ester formation was observed when temperature of 60 °C was used. In contrast to 40 and 50 °C, at 60 and 70 °C, little difference were observed, and the reaction proceeded nearly identically with about 70 and 68% of esters reached within 8 hours respectively. However, the esters formation decreased with temperature more than 70 °C.

Nevertheless, different result was found when diethyl carbonate was used as alkyl source instead of dimethyl carbonate. As shown in Figure 7, the formation of esters increased up to 64% when temperature was raised up to 60 °C. The ester formation decrease when temperature was raised up to 70 °C.

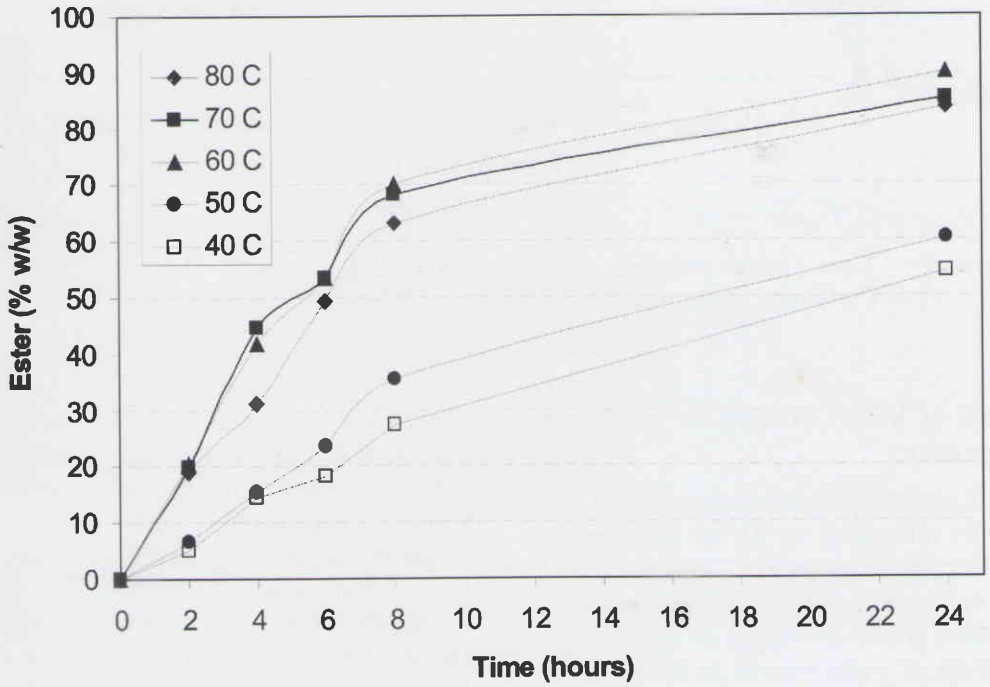

Figure 6. Time Course of Transesterification of Palm Kernel Oil with Diethyl Carbonate at Various Temperatures.

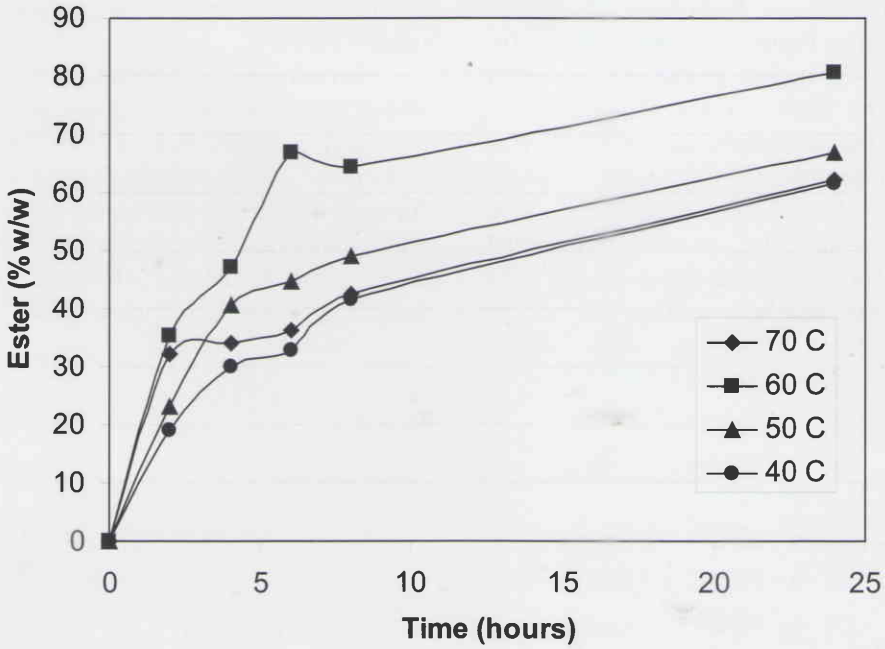

Figure 7. Time Course of Transesterification of Palm Kernel Oil with Diethyl Carbonate at Various Temperatures.

Effect of Water Addition on the Esters Formation

Enzyme catalysis in organic media is greatly influenced by the environment of the enzyme. The organic solvent as well as the water content in the reaction medium affects the rate of catalysis *(2,3)*. Amount of water present in the media is one of critical parameter. When using enzymes in organic media some water is necessary for enzyme activity. Water in such a system is distributed between enzyme, a possible carrier, solvent, substrate, and head space. As reported by Shah *et al.*, as the water level increases, it increases the enzyme flexibility and the expressed activity. After the optimum level of water, hydrolytic reaction become significant and transesterification yield is expected to go down.

In order to investigate the effect of the water content as a factor limiting the activity of the catalyst, various amount of water was added to the dialkyl carbonates before lipase-catalyzed transesterification of palm kernel oil with dialkyl carbonates was taken.

The result shows that the formation of esters is lowest when no added any water in the system, i.e. 13% for 8 hours reaction. The esters formation increase

drastically up to more than 70% when water was added up to 0.2 % on the volume base of dimethyl carbonate. The formation of ester was slightly changes when water was added about 0.3% - 0.5% (v/v). However, ester formation extremely decreases when water was added up to 1.0 % (v/v) (Figure 8).

Almost similar result was found when diethyl carbonate was used as substrate instead of dimethyl carbonate. As shown in Figure 9, without any added water the formation of ester only reached 43%. The highest ester formation, about 64%, was found when 0.1 % - 0.2 % (v/v) water was added to the diethyl carbonate. The formation of esters decrease when water was added more than 0.4 % (v/v).

Temperature combinations and water content that affected the ester formation is shown in Figure 10. The highest ester formation was obtained at water content of 0.2% and 60 °C.

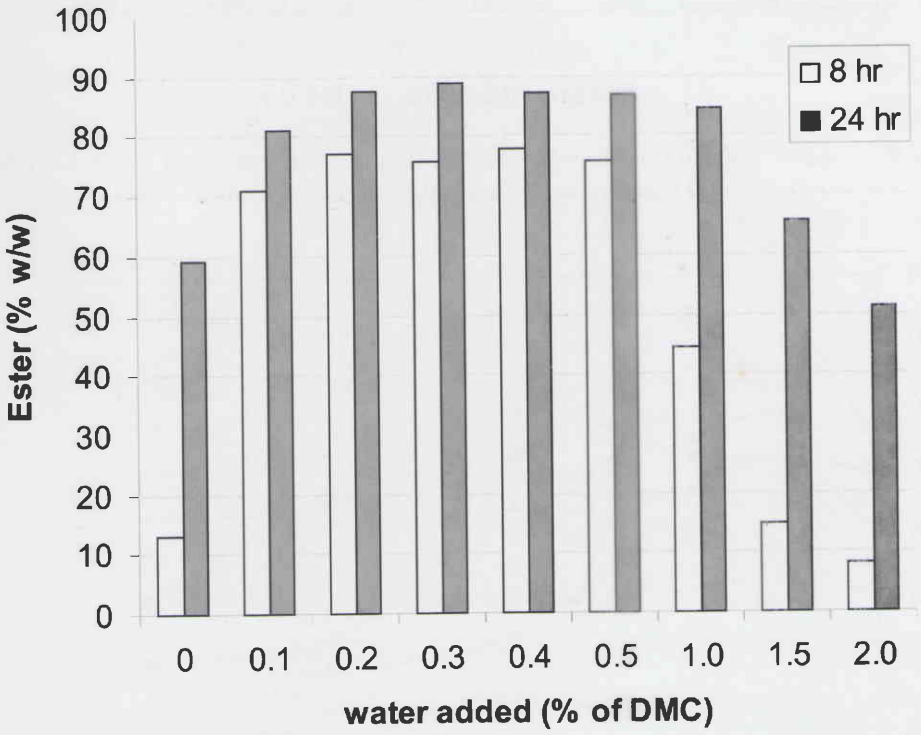

Figure 8. Ester Formation (% w/w) on the Transesterification of Palm Kernel Oil with Dimethyl Carbonate Containing Various Amount of Water.

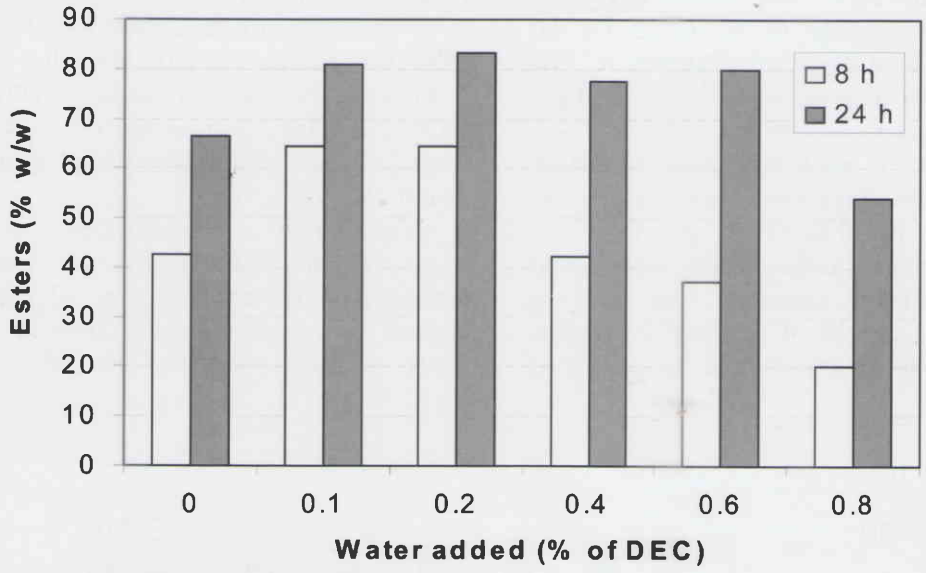

Figure 9. Ester Formation (% w/w) on the Transesterification of Palm Kernel Oil with Diethyl Carbonate Containing Various A mount of Water

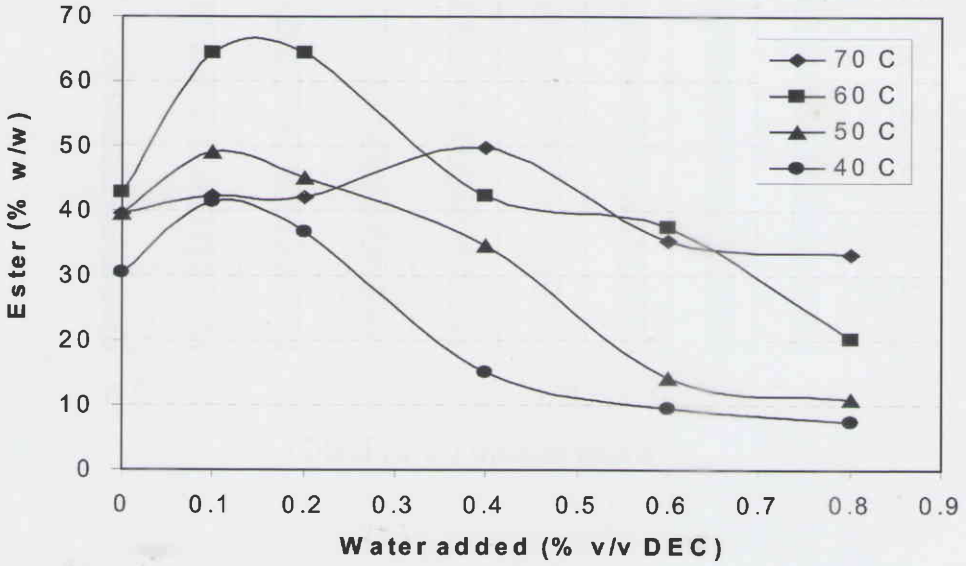

Figure 10. Ester Formation (% w/w) on the Transesterification of Palm Kernel Oil with Diethyl Carbonate Containing Various Amount of Water.

Effect of Repeated Used of Enzyme on the Ester Formation

The main advantage of immobilization of an enzyme is that an expensive enzyme can be repeatedly used. It was observed, how the reaction behavior changes when an immobilized enzyme is used repeatedly.

The change of enzymatic activity of Novozym 435 was examined at 60 °C. As shown in Figure 11, the formation of esters was decreased to about two-third when Novozyme 435 was used for second time, continue decreases when it was used for third and forth time. The decreasing of ester formation could be happen because of the decreasing of enzyme activity, because when an immobilized enzyme was used for the first time, some amount of enzyme was desorbed and cannot observed after further repeated use. Glycerol that produced during reaction and adsorbed onto the enzymatic support might also be leading to drastic decrease in enzyme activity.

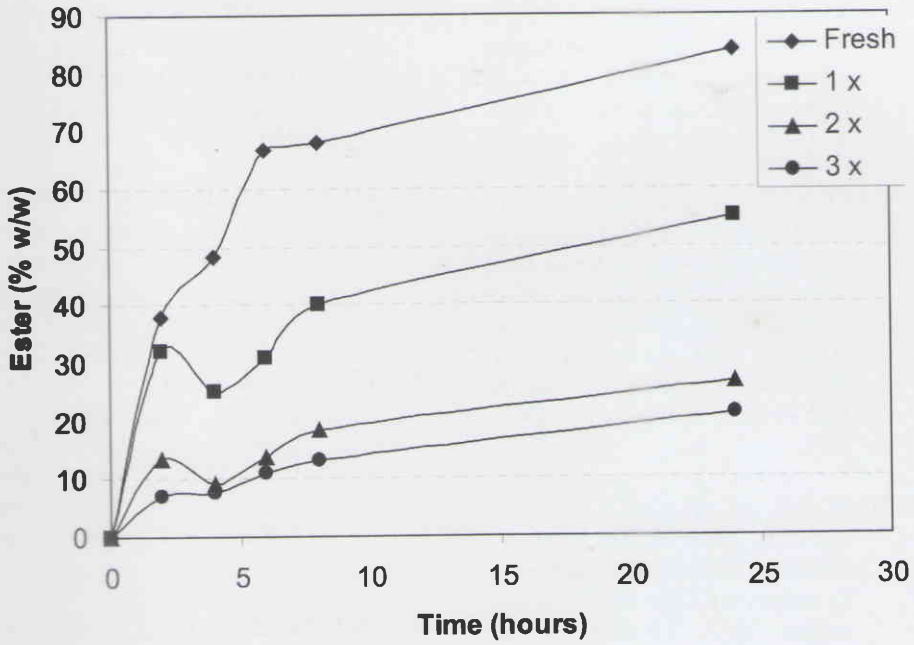

Figure 11. Time Course of Transesterification of Palm Kernel Oil with Diethyl Carbonate Using Reused Enzyme

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REFERENCES

1. AMAYA, C. L., D. STUBBS, and A. G MARANGONI. 1995. A New Assay for Lipase Activity in Organic Solvent. Lipase-Catalyzed Synthesis of Octyl-Linolenate in a Hexane Microaqueous Reaction System. Enzyme Microb. Technol. 17(2) : 131 – 135.

2. ANTHONSEN, T. and B. H. HOFF. 1998. Resolution of Derivatives of 1,2-Propanediol with Lipase B from *Candida antarctica*. Effect of Substrate, Medium, Water Activity, and Acyl Donor on Enantiometric Ratio. Chemistry and Physics of Lipids 93: 199 – 207.

3. ATHAWALE, V., N. MANJREKAR, and M. ATHAWALE. 2003b. Effect of Reaction Parameter on Synthesis of Citronellyl Methacrylate by Lipase-Catalyzed Trans-esterification. Biotechnol. Prog. 19: 298 – 302.

4. BASRI, M., A. C. HENG, C. N. A RAZAK, W. M. Z WAN YUNUS, M. AHMAD, R. N. A RAHMAN, K. AMPON, and A. B. SALLEH. 1997. Alcoholysis of Palm Oil Mid-Fraction by Lipase from *Rhizopus rhizopodiformis*. JAOCS 74(2): 113 – 116.

5. BRUNET, C., M. ZAREVUCKA, Z. WIMMER, and M. D. LEGOY. 1999. Lipase B from *Candida antarctica* Catalyses Enantioselective Transesterification Of 2-(4-Methoxybenzyl)-1-Cyclohexa-nols and 2-(4-Methoxybenzyl)-1-Cyclopentanols. Biotechnology Letters 21: 605 – 610.

6. FREEDMAN, B., E. H. PRYDE, and T. L. MOUNTS. 1984. Variables Affecting the Yields of Fatty Esters from Transesterified Vegetable Oils. JAOCS 61(10): 1638 – 1643.

7. HAAS, MICHAEL J. 2004. The Interplay Between Feedstock Quality and Esterification Technology in Biodiesel Production. Lipid Technology 16(1): 7 – 11.

8. HSU, A., K. JONES, T. A. FLOGIA, and W. N. MARMER. 2002. Immobilized Lipase-Catalyzed Production of Alkyl Esters of Restaurant Grease As Biodiesel. Biotechnol. Appl. Biochem. 36: 181 – 186

9. ISO, M., B. CHEN, M. EGUCHI, T. KUDO, and S. SHRESTHA. 2001. Production of Biodiesel Fuel from Triglycerides and Alcohol Using Immobilized Lipase. Journal Of Molecular Catalyst B: Enzymatic 16: 53 – 58

10. JEONG, J. C. and S. B. LEE. 1997. Enzymatic Esterification Reaction In Organic Media with Continuous Water Stripping: Effect of Water Content on Reaction Performance and Enzyme Agglomeration. Biotechnology Techniques 11(12): 853 – 858.

11. LEITGEB, M. and Z. KNEZ. 1990. The Influence of Water on the Synthesis of N-Butyl Oleate by Immobilized *Mucor miehei* Lipase. JAOCS 67(11): 775 – 778.

12. LINKO, Y. Y, M. LAMSA. A. HUHTALA and P. LINKO. 1994. Lipase-Catalyzed Transesterifica-tion of Rapeseed Oil and 2-Ethyl-1-Hexanol. JAOCS 71(12): 1411 – 1414.

13. MITTELBACH, M., A. SILBERHOLZ, and M. KONCAR. 1995. Novel Aspects Concerning Acid Catalyzed Alcoholysis of Triglycerides. Proceedings of the 21st World Congress of The International Society for Fat Research (ISF), The Hague (Netherland), Vol. 3: 497 – 499.

14. MITTELBACH, MARTIN. 1990. Lipase-Catalyzed Alcoholysis of Sun Flower Oil. JAOCS 67(3): 168 – 170.

15. NELSON, L. A., T. A. FOGLIA, and W.N. MARMER. 1996. Lipase-Catalyzed Production of Biodiesel. JAOCS 73(8): 1191 – 1195.

16. NIMCEVIC, D. R. PUNTIGAM, M. WOERGETTER, and J.R. GAPES. 2000. Preparation of Rapeseed Oil Esters of Lower Aliphatic Alco-hols. JAOCS 77(3): 275 – 280.

17. SHIMADA, Y., Y. WATANABE, T. SAMU-KAWA, A. SUGIHARA, H. NODA, H. FUKUDA, and Y. TOMINAGA. 1999. Conversion of Vegetable Oil to Biodiesel Using Immobilized *Candida antarctica* Lipase. JAOCS 76(7): 789 – 793.

18. SOUMANOU, M. M. and U. T. BORNSCHEUER. 2003. Lipase-catalyzed Alcoholysis of Vegetable Oils. Eur.J.Lipid Sci. Technol. 105 : 656 – 660.

19. TORRES, C. and C. OTERO. 1996. Influence of the Organic Solvent on the Activity in Water and the Conformation of *Candida rugosa* Lipase : Description of a Lipase-Activity Prereatment. Enzyme Microb. Technol. 19(12): 594-600.

20. WEHTJE, E. and P. ADLERCREUTZ. 1997. Lipases Have Similar Water Activity Profiles in Different Reactions. Biotechnology Letters 19(6): 537 – 540.