

## LIPASE-CATALYZED TRANSESTERIFICATION OF PALM KERNEL OIL WITH DIALKYL CARBONATES

<sup>1</sup>Tjahjono Herawan and <sup>2</sup>M. Rüsç Gen. Klaas

<sup>1</sup>Product Development and Quality Research Group, Indonesian Oil Palm Research Institute (IOPRI), Jl. B. Katamso Nr. 51 Medan 20158-Indonesia

<sup>2</sup>Department of Technology, University of Applied Sciences Emden-Leer, 26723 Emden-Germany

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### ABSTRACT

Lipase-catalyzed transesterifications-especially in a solvent-free medium-are important for industrial applications because such systems 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 also occurs when ethyl or methyl acetate are used as acyl acceptors. The main problems of lipase-catalyzed organic solvent-free alcoholysis are first, the solubility of the plant oil in the substrate or solvent and second, the fact that transesterification is an equilibrium reaction. Dialkyl carbonates, versatile compounds due to their chemical reactivity and physical properties, may provide an alternative to solve both problems. Using dialkyl carbonates transesterification is not an equilibrium reaction, because the intermediate compound immediately decomposes to carbon dioxide and an alcohol. Moreover, dialkyl carbonates (especially dimethyl carbonate) are cheap and widely available. For single step lipase-catalyzed transesterification of palm kernel oil, dialkyl carbonates (in this case dimethyl and diethyl carbonate) gave better yields compared to those of short chain alcohols. The rate of ester formation with dialkyl carbonates as substrate was about 6-7 times higher than that obtained with short chain alcohols. The formation of esters was gradually increased by a higher enzyme amount from 5-20% (w/w of oil) for 8 h reaction time. However from the economic point of view, an enzyme amount of 10% on the weight base of oil was proposed for further reaction. Generally, the highest ester formation was observed when a temperature of 60°C was used. However, in the case of dimethyl carbonate little difference was observed at reaction temperatures of 60 and 70°C and the reactions proceeded nearly identically. The esters formation increased drastically up to more than 70% when water was added up to 0.2%. However, ester formation extremely decreased when more than 0.4% water was added. Formation of ester was lower when a hydrocarbon solvent was added to the system compared to that of when dialkyl carbonates were used as substrate and solvent. *Candida antarctica* in the immobilized was found to be effective for transesterifying palm kernel oil with short-chain dialkyl carbonates with or without any added organic solvents.

**Keywords:** Lipase-Catalyzed Transesterification, Esters Formation Increased, Short-Chain Dialkyl Carbonates, Added Organic Solvents, Agriculture Lipid Feedstocks, Decomposes Immediately

### 1. 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 processes (Nelson *et al.*, 1996; Otera and Nishikido, 2009; Drauz *et al.*, 2012). The most interesting research today is focused on the

**Corresponding Author:** TjahjonoHerawan, Product Development and Quality Research Group Indonesian Oil Palm Research Institute (IOPRI), Jl. B. Katamso Nr. 51Medan 20158-Indonesia

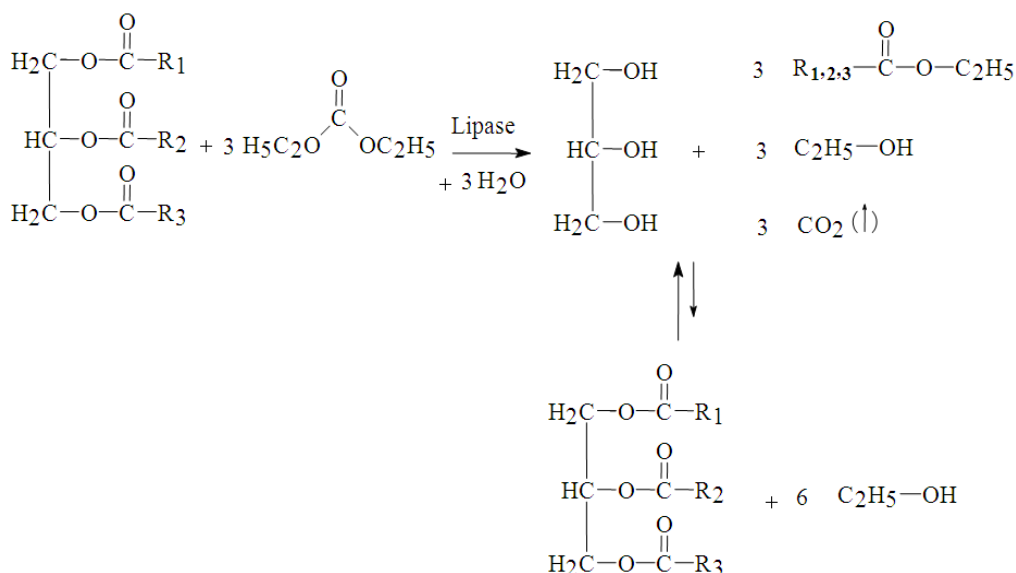
utilization of enzymes, normally lipases, for catalysing the synthesis of simple esters of vegetable oils or other agriculture lipid feedstocks with or without the presence of organic solvents (Iso *et al.*, 2001; Soumanou and Bornscheuer, 2003). Lipase-catalyzed transesterification in a solvent-free medium would be important for industrial applications because such a system would have an enormous advantage by avoiding the problems of separation, toxicity and flammability of organic solvents. However, the organic solvent-free alcoholysis, especially methanolysis, does not give high conversions. The same problem also occurs when ethyl or methyl acetate are used as acyl acceptors.

There have been numerous studies that report transesterification reactions using lipases with and without organic solvents. Nelson *et al.* (1996) report lipase-catalyzed production of methyl and ethyl ester from various sources of oil in hexane. Shimada *et al.* (1999) also report continuous conversion of vegetable oils to methyl esters using lipases as biocatalyst. Both of those groups used alcohols as raw material. The disadvantage for technical applications of this method is, that is an equilibrium reaction. Based on the transesterification of a fatty acids with an dialkyl carbonates which was first reported by Pioch *et al.* (1991), Warwel *et al.* (1999) carried out lipase-catalyzed conversion of plant oils with dialkyl carbonates which is not

equilibrium reaction, because the intermediate compound (carbonic acid monoacyl ester) decomposes immediately to carbon dioxide and an alcohol (**Fig. 1**). By using high oleic sunflower oil and dimethyl carbonate (150 mmol plant oil/l solvent = 15% w/w, 3 g of Novozyme ® 435), after 72 hat 50°C, Warwel *et al.* (1999) obtained about 80% methyl esters. Based on this result, lipase-catalyzed transesterification using dialkyl carbonates, especially dimethyl or diethyl carbonate, may provide a general alternative for the transesterification of sensitive substrates such as tung oil, calendula oil or fish oil.

The method has later been further developed to reactive extraction, i.e., the simultaneous extraction and transesterification of triglycerols (Klaas and Warwel, 2001) and applied for the extraction of particular fatty acid esters (Klaas and Meuer, 2004).

Some key aspects of lipase-catalyzed esters synthesis have been investigated and reported: The source of alkyl donor (Mittelbach, 1990, Mittelbach *et al.*, 1995; Linko *et al.*, 1994; Brunet *et al.*, 1999), the role of water (Leitgeb and Knez, 1990; Jeong and Lee, 1997; Wehtje and Adlercreutz, 1997), the effect of temperature and pressure conditions (Iso *et al.*, 2001; Athawale *et al.*, 2003) and the effect of organic solvents (Amaya *et al.*, 1995). Some of these key aspects were also investigated in the transesterification of palm kernel oil with dialkyl carbonates catalyzed by Novozyme ® 435.



**Fig. 1.** Transesterification of a triglyceride with dialkyl carbonates

## 2. MATERIALS AND METHODS

Palm kernel oil was obtained from Indonesian Oil Palm Research Institute (IOPRI), Medan-Indonesia. Dimethyl carbonate, diethyl carbonate, methanol, ethanol and isohexane were analysis grade and purchased from Merck (Darmstadt, Germany). All solvents used were percolated through Puralox SCCa 150/145 N before use. N-Methyl-N-trimethylsilylheptafluor(o)butyramide (MSHFBA) was used for silylation and was obtained from Macherey-Nagel (Düren, Germany).

### 2.1. Screening of Commercially Available Lipases for their Abilities to Transesterify the Triglyceride of Palm Kernel Oil

In a 5 mL closed reaction tube containing 200.60 mg (0.286 mmol) of palm kernel oil in 0.8 mL isohexane, 3 mole equivalents of dialkyl carbonates or alcohols and the appropriate amount of enzyme were added. The reaction mixture was stirred for 24 h at 45°C in a heating block. The same method was also used without any added isohexane as solvent.

### 2.2. Transesterification of Palm Kernel Oil with Dimethyl and Diethyl Carbonate Catalyzed by Novozyme® 435

In a 5 mL closed reaction tube containing various amounts of palm kernel oil, various amounts of dialkyl carbonates and Novozyme® 435 were added. The mixture was stirred and heated to 60°C for 24 h. Samples were withdrawn at specified intervals during the 24 h 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 further analysis.

### 2.3. 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<sup>-1</sup>, airflow was 450 mL min<sup>-1</sup> and make up flow (Nitrogen) was 45.0 mL min<sup>-1</sup>. 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 min constant) to 420°C at 10°C/min and was held at 420°C for 6 min. All sample were silylated by N-

methyl-N-trimethylsilylheptafluor(o)butyramide (MSHFBA) containing methyl-imidazole (50 µL mL<sup>-1</sup> MSHFBA) for about 30 min and afterwards the excess of MSHFBA was evaporated, the sample was dissolved in dichloromethane and directly injected to GC. Heptadecanoic acid methyl ester was used as internal standard.

## 3. RESULTS

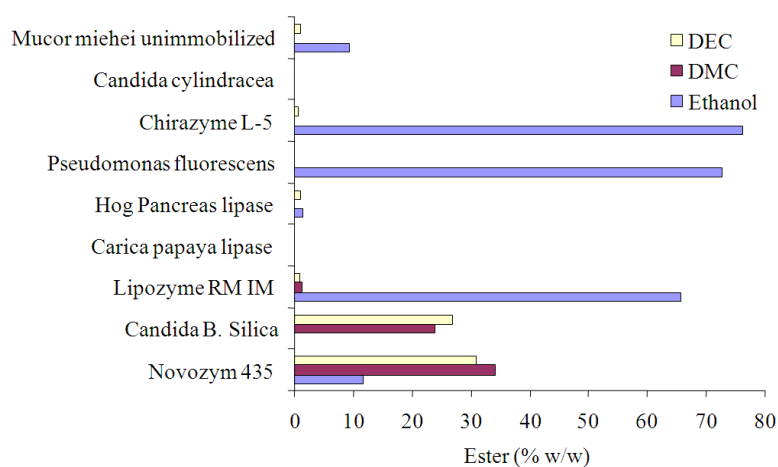
### 3.1. Screening of Commercially Available Lipases for their Abilities to Transesterify the Triglyceride of Palm Kernel Oil

Several commercially available lipases were screened for their ability to transesterify palm kernel oil with dimethyl carbonate, diethyl carbonate and short chain alcohols such as methanol and ethanol. Reactions were done in isohexane at 45°C without any addition of water to find the most active lipases for transesterification.

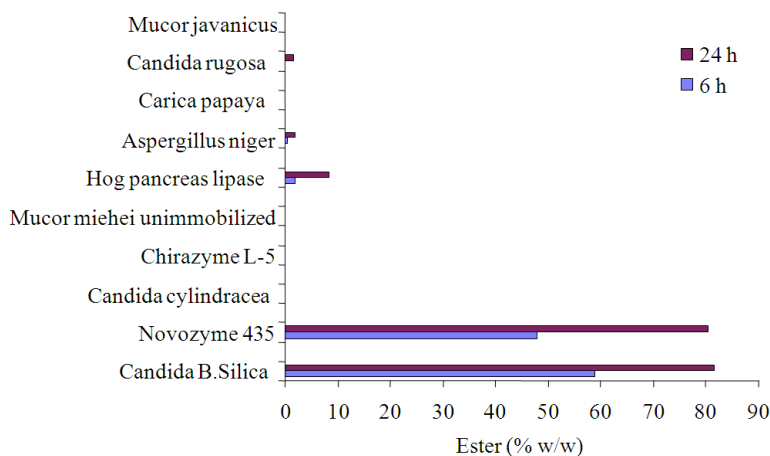
The results show that among of several lipases tested, only the lipase preparations from *Candida antarctica* lipase B (Novozyme® 435 and Candida B. on silica) were found to be most effective for transesterifying palm kernel oil with dimethyl or diethyl carbonate (34 and 31% yield respectively).

Even though the lipase from *Candida antarctica* lipase A (Chirazyme L-5), *Pseudomonas fluorescens* and *Mucor miehei* (Lipozyme® RM IM and its unimmobilized form) were found effective for transesterifying palm kernel oil with ethanol to the corresponding ethyl esters derivatives (76, 73, 66 and 9% yield respectively), these lipase were not effective for transesterifying palm kernel oil either with diethyl carbonate (0.71, 0.24, 0.93 and 1.05% respectively) or dimethyl carbonate as shown in **Fig. 2**. Other lipases that were screened in this experiment, such as Carica papaya, Hog pancreas lipase and *Candida cylindracea* were not effective to transesterify palm kernel oil either with dialkyl carbonates or short chain alcohols, as shown in **Fig. 2**.

Several commercially available lipases were also tested for their ability to transesterify palm kernel oil with dimethyl carbonate in isohexane at an elevated temperature of 60°C. Again, among several lipases tested the lipases from *Candida antarctica* (Novozyme® 435 and Candida B. Silica) were found most effective as shown in **Fig. 3**. The result also shows that at 60°C, the methyl ester formation was higher than at 45°C as done in the first experiment described above.



**Fig. 2.** Lipases' abilities to transesterify the triglyceride of palm kernel oil with Dimethyl Carbonate (DMC), Diethyl Carbonate (DEC) and ethanol at 45°C in Isohexane after 8 H



**Fig. 3.** Lipases' abilities to transesterify the triglyceride of palm kernel oil with dimethyl carbonate in isohexane at 60°C after 6 and 24 h

A series of commercially available lipases was screened for their ability to transesterify palm kernel oil with dimethyl carbonate without another organic solvent at 60°C. In this experiment, dimethyl carbonate at the same time acted both as substrate and solvent. The result shows that the lipase preparations from *Candida antarctica* (Novozyme® 435 and *Candida B. Silica*) was the most effective for transesterifying palm kernel oil with dimethyl carbonate as shown in **Fig. 4**. Ester formation in the transesterification of palm kernel oil with dimethyl carbonate without another organic solvent was higher than that in the transesterification of palm kernel oil with dimethyl carbonate in isohexane.

### 3.2. Transesterification of Palm Kernel Oil with Dimethyl and Diethyl Carbonate Catalyzed by Novozyme® 435

#### 3.2.1. Effect of the Alkyl Sources on the Esters Formation

The type of alkyl source plays an important role in the reaction kinetics 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 results show that among the alkyl donor

used, the highest rate of ester formation was obtained using dialkyl carbonates as alkyl sources.

As shown in **Fig. 5**, using short chain alcohols such as methanol and ethanol as alkyl source, the rate of esters formation was low. After 8 h reaction time the formation of methyl and ethyl esters was only 11 and 12% respectively. The ester yields increased only slightly to 13 and 19% respectively, when the reaction time was extended to 24 h. Even after 48 h the ester formation was not more than 30%.

Completely different results were now found when dimethyl and diethyl carbonate were used as alkyl donors. As shown in **Fig. 5**, the ester formation rates with dialkyl carbonates were about 6-7 times higher than that with short chain alcohols. The formation of methyl and ethyl esters were about 40% after 1 h and reached up to 76 and 73% respectively after 8 h. Then, the ester formation increased slowly to 83 and 81% respectively when reaction was prolonged to 24 h.

### 3.3. Effect of the Substrate Molar Ratio on the Esters Formation

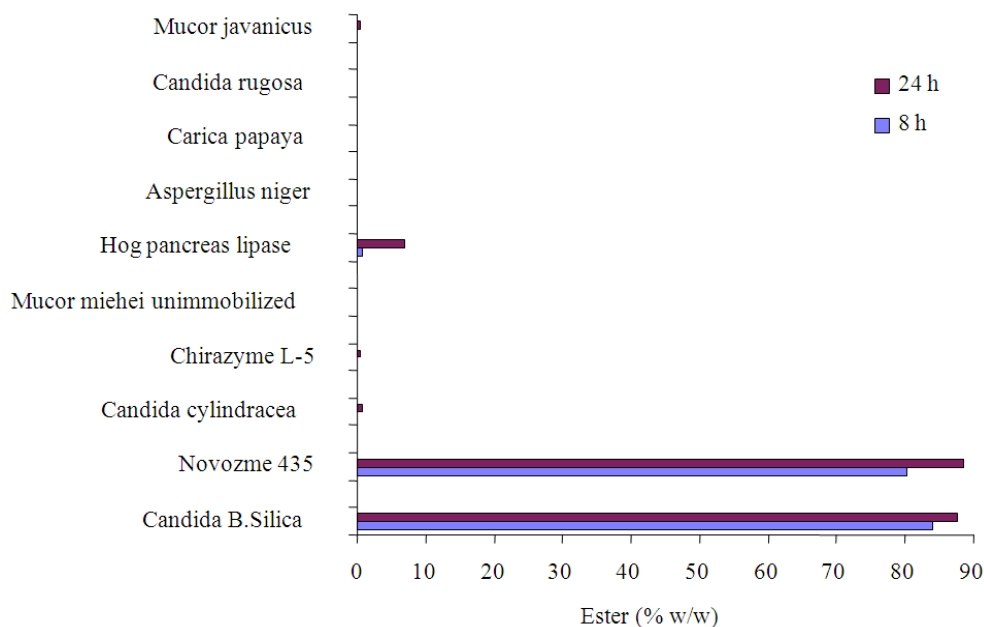
To ensure that the reaction proceeds rapidly and completely, the enzyme should not be inhibited by substrate concentration. Various substrate molar ratios

were tested to understand their effect on the formation of esters.

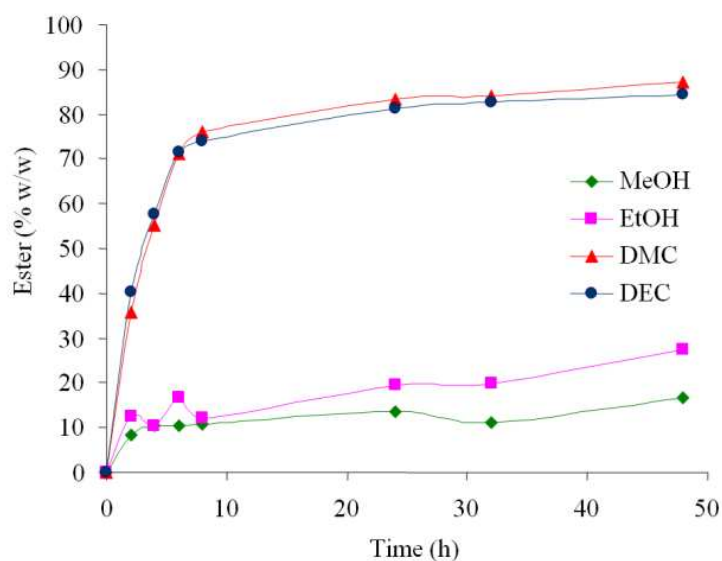
As shown in **Fig. 6**, using dimethyl carbonate as substrate, the ester formation was low when a molar ratio of dimethyl carbonate/palm kernel oil lower than three (mol/mol) was used, because then the amount of dimethyl carbonate is not sufficient for a complete reaction. The ester formation increased with increasing molar ratio. The highest ester formation was obtained when the molar ratio of dimethyl carbonate/palm kernel oil was 20 mol<sup>-1</sup> mol (82%). However, the ester formation decreased when the molar ratio of substrate was higher than 100 mol<sup>-1</sup> mol (dissolution effect).

As shown in **Fig. 7**, using diethyl carbonate as solvent the highest ester formation was obtained with a molar ratio of diethyl carbonate/palm kernel oil of three mol/mol, i.e., the stoichiometric amount (79% for 8 hours reaction). The formation of esters decreased with an increase in the molar ratio excess.

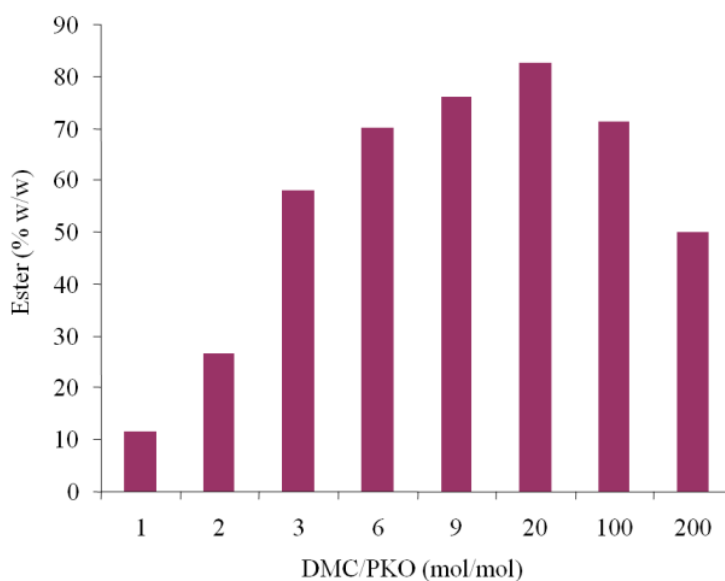
When the molar ratio of diethyl carbonate/palm kernel oil was 20 mol<sup>-1</sup>, triglyceride conversion was complete after 8 h, but the yield of ester was somewhat lower. Around 20% are converted to by-products (free fatty acids). The ester formation increased up to 93% with a corresponding decrease in free fatty acids when the reaction time was extended to 24 h.



**Fig. 4.** Lipases' abilities to transesterify the triglyceride of palm kernel oil with dimethyl carbonate at 60°C after 8 and 24 H



**Fig. 5.** Time course of transesterification of palm kernel oil with: ♦ Methanol, ■ Ethanol, ▲ Dimethyl Carbonate, ● Diethyl Carbonate, without organic solvent at 60°C



**Fig. 6.** Ester formation in the transesterification of palm kernel oil with dimethyl carbonate for various molar ratios of substrate at 50°C after 8 H

### 3.4. Effect of the Amount of Novozyme® 435 on the Ester Formation

The amount of enzyme spent is a crucial economical factor for any successful industrial application. Therefore, the effect of the amount of

enzyme on the transesterification of palm kernel oil with dialkyl carbonates was examined with various amounts of enzyme in the range from 5-20% on the weight base of oil at 60°C (**Fig. 8**).

There was a gradual increase in the formation of esters with increasing the enzyme amount from 5-12.5

% (w/w of oil) in the lipase-catalyzed transesterification of palm kernel oil with dimethyl carbonate after 8 h reaction time. When reaction time was prolonged to 24 h, under all condition used the formation of esters reached nearly the same value, which indicates that the reaction was nearly finished.

The similar result was also found when diethyl carbonate was used as alkyl source instead of dimethyl carbonate. The formation of ester gradually increased with

the enzyme amount from 5-20% (w/w of oil) after 8 h. Except for the lowest enzyme amount of 5% (w/w of oil), the formation of esters reached nearly in the same value when the reaction time was extended to 24 h (Fig. 9).

For economic reasons an enzyme amount of 10% on the weight base of oil was used for further reaction. Under this condition, enzyme activity was  $9.8 \mu\text{mol}^{-1} \text{min.g}$  (defined as the amount of substrate converted per gram of Novozyme ® 435 used).

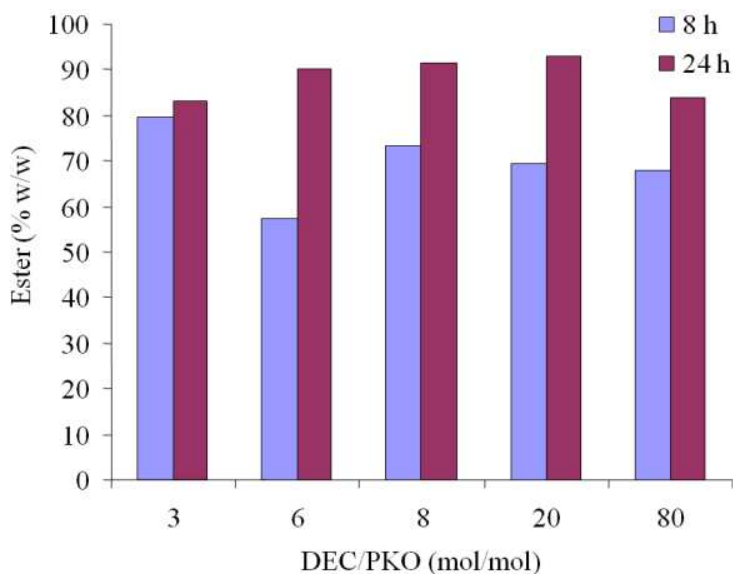


Fig. 7. Ester formation in the transesterification of palm kernel oil for various molar ratios of substrate / diethyl carbonate at 60°C

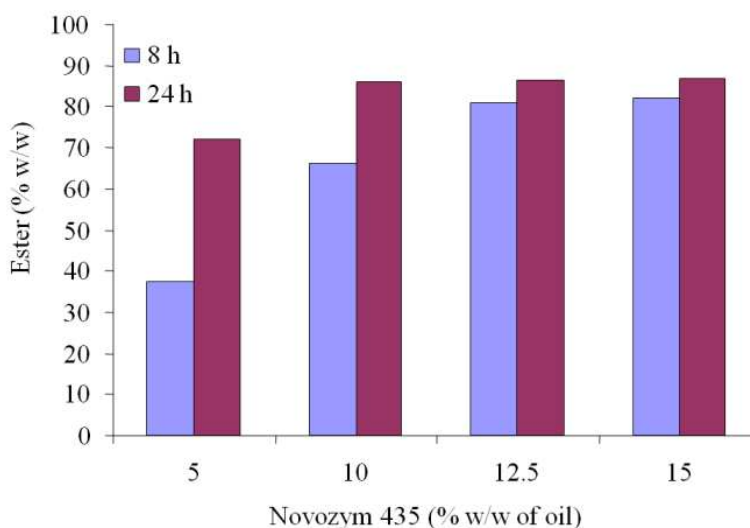
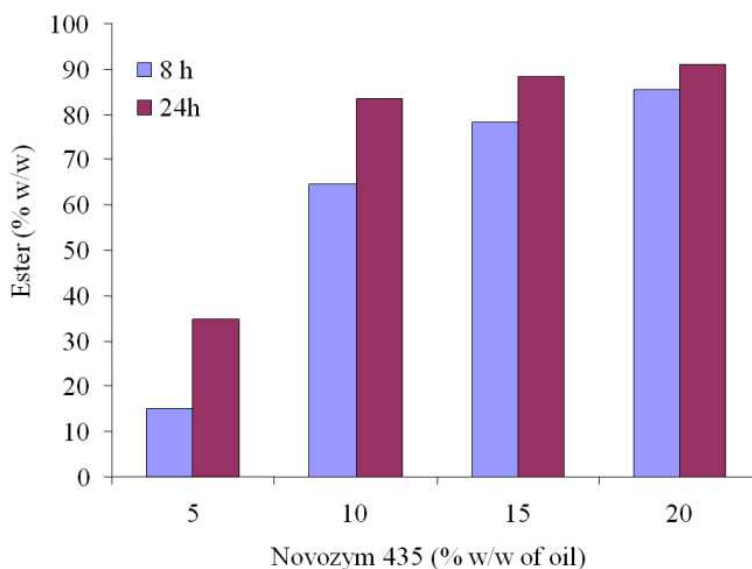
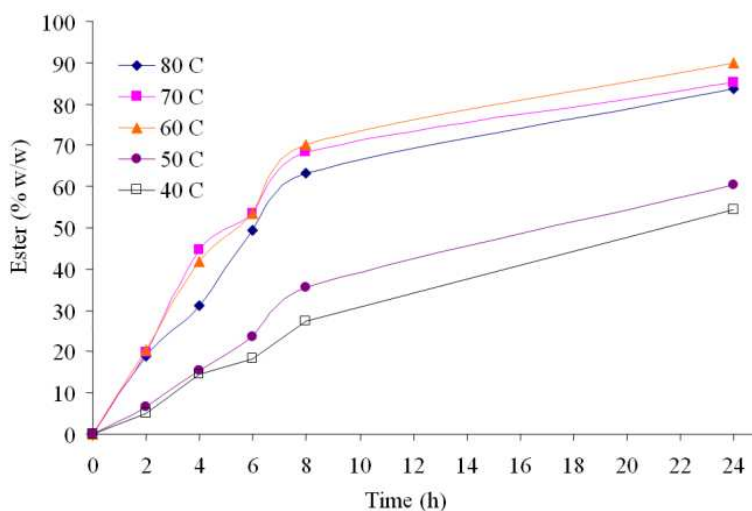


Fig. 8. Ester formation in the transesterification palm kernel Oil with dimethyl carbonate in the presence of various amounts of Novozyme ® 435 at 60°C



**Fig. 9.** Ester formation in the transesterification of palm kernel with diethyl carbonate in the presence of various amounts of Novozyme @ 435 at 60°C



**Fig. 10.** Time course of the transesterification of palm kernel oil with dimethyl carbonate at various

### 3.5. Effect of the Reaction Temperature on the Ester Formation

Temperature is one of the important factors influencing enzyme activity. In general, the rate of a reaction increases with a rise in temperature. However, increasing the temperature leads to an enhanced mobility of protein segments in the enzymes while the strength of hydrophobic interaction decreases (Athawale *et al.*, 2003).

The effect of the reaction temperature on the transesterification of palm kernel oil with dialkyl carbonates was examined in the temperature range of 40-80°C. As shown in **Fig. 10** using dimethyl carbonate as the alkyl source the highest ester formation was observed when a temperature of 60°C was used. The results for t 60 and 70°C were very similar and the reaction proceeded nearly identically with about 70 and 68% of esters reached within 8 h respectively. However, the esters formation decreased slightly at temperatures of more than 70°C.



Different results were found when diethyl carbonate was used as alkyl source. As shown in **Fig. 11**, the formation of esters increased up to 64% when the temperature was raised up to 60°C. But then the ester formation decreased when temperature was raised further to 70°C.

### 3.6. Effect of Water Addition on Ester Formation

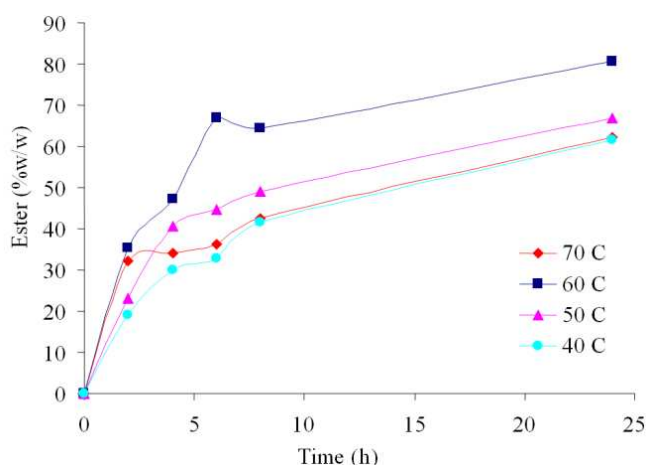
In order to investigate the effect of the water content as a factor limiting the activity of the catalyst, various amounts of water were added to the dialkyl carbonates before lipase-catalyzed transesterification of palm kernel oil with dialkyl carbonates was carried out.

The results show that the formation of esters was lowest when no water is added to the system, i.e., 13% after for 8 h reaction time. The ester formation increased

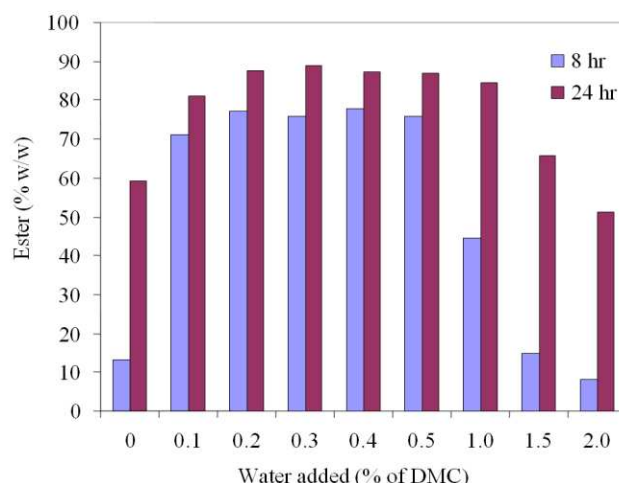
drastically up to more than 70% when 0.2% (on the volume base of dimethyl carbonate) water was added. The formation of ester was fairly constant in the range of 0.3-0.5% (v/v). However, ester formation extremely decreased when 1.0% (v/v) water was added (**Fig. 12**).

A similar result was found when diethyl carbonate was used as substrate instead of dimethyl carbonate. As shown in **Fig. 13**, 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 decreased when more than 0.4% (v/v) water was added.

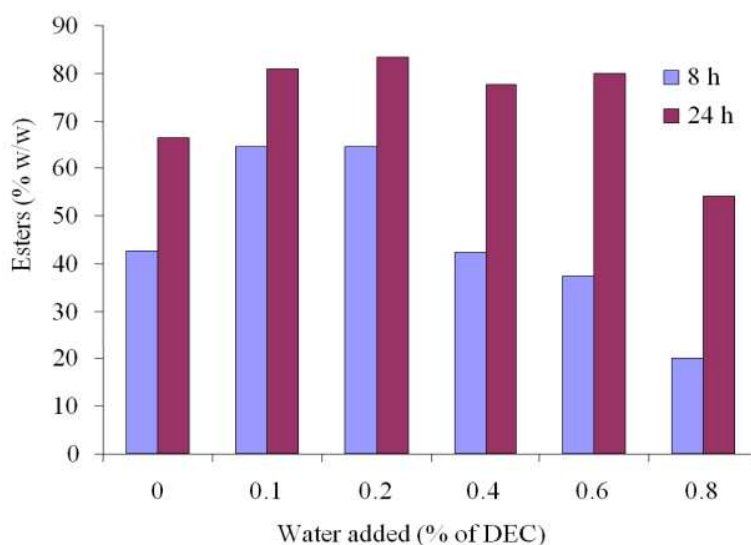
The combined effect of temperature and water content on the ester formation is shown in **Fig. 14**. The highest ester formation was obtained with a water content of 0.2% and at 60°C.



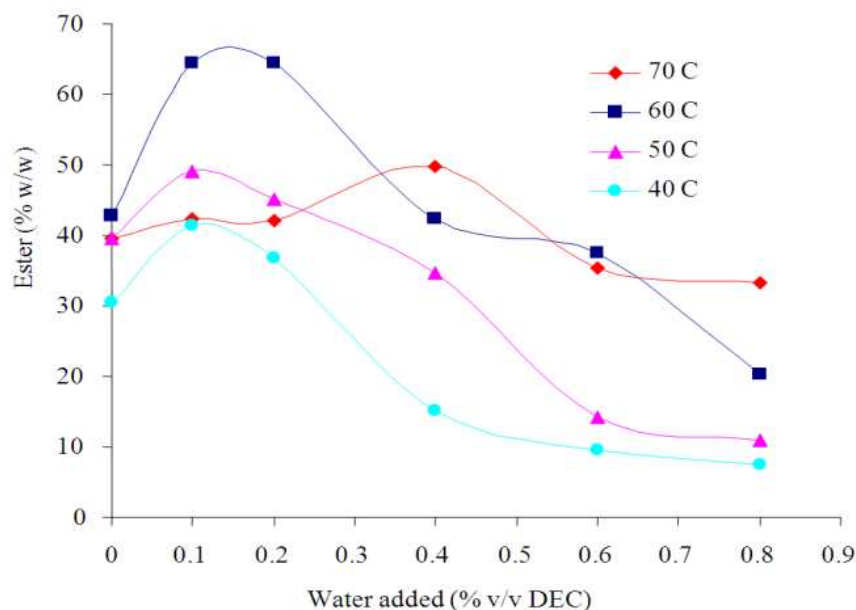
**Fig. 11.** Time course of the transesterification of palm kernel oil with diethyl carbonate at various temperatures



**Fig. 12.** Ester formation (% w/w) in the transesterification of palm kernel oil with dimethyl carbonates containing various amounts of water



**Fig.13.** Ester formation (% w/w) in the transesterification of palm kernel oil with diethyl carbonates containing various amounts of water



**Fig. 14.** Ester formation (% w/w) on the transesterification of palm kernel oil with diethyl carbonates containing various amount of water

#### 4. DISCUSSION

The lipase B from *Candida antarcticawas* found to be effective for transesterifying palm kernel oil with dimethyl or diethyl carbonate. The ineffectiveness of lipases from *Candida antarctica* lipase A (Chirazyme L-5), *Pseudomonas fluorescens* and *Mucor miehei* (Lipozyme® RM IM and its unimmobilized form) to

transesterify palm kernel oil with dialkyl carbonates was only because dialkyl carbonates are poor substrates for these lipases and not because they are enzyme inhibitors'. This could be shown by transesterification of palm kernel oil with a mixture of ethanol and dimethyl carbonate (or a mixture of ethanol and diethyl carbonate) in the presence of isohexane as solvent and Lipozyme® RM IM as biocatalyst. Under this

conditions, ethyl ester yields were about 61 and 60% respectively. This value was not really different compared to that of ethyl esters produced only with ethanol as alkyl source (65%) (Herawan, 2004).

Among the alkyl donors used, the rate of ester formation using dimethyl or diethyl carbonates was much higher than that obtained with methanol and ethanol. The high ester formation are consistent because when dialkyl carbonates are used as a substrate the reaction is not an equilibrium, because the intermediate compound decomposes immediately to carbon dioxide and alcohol (Fig. 1). Furthermore, palm kernel oil dissolves well in dialkyl carbonates. These results agree with those of Shimada *et al.* (1999) who found that a single addition of methanol to solvent-free alcoholysis catalyzed by Novozyme ® 435 resulted in low conversion to methyl esters. Similar result were also reported by Hsu *et al.* (2002) who found that the same enzyme gave low conversion to esters (20-30% after 48 h of reaction) in the transesterification of restaurant grease with ethanol. So, it is well known, that the lipase-catalyzed transesterification of a triglyceride with an alcohol is an equilibrium reaction and when short chain alcohol are used as a substrate, the two components did not dissolve well and the reaction cannot take place homogeneously. Some papers reported that therefore an organic solvent is required for this reaction (Nelson *et al.*, 1996; Iso *et al.*, 2001). In conclusion for single step lipase-catalyzed transesterification of vegetable oils (like palm kernel oil), dialkyl carbonates (in this case dimethyl and diethyl carbonate) are much better substrates than short chain alcohols.

The formation of ester effected by molar ratios of substrate. Increasing molar ratio up to 20 mol mol<sup>-1</sup> will increase the ester formation. 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 are produced first and are then re-esterified by the enzyme with diethyl carbonate acting as nucleophile. It can be seen that dimethyl and diethyl carbonate behave differently. One reason might be that the coproduct methanol inhibits the enzyme activity whereas ethanol does not.

Enzyme catalysis in organic media is greatly influenced also by the environment of the enzyme. The organic solvent as well as the water content in the reaction medium affects the rate of catalysis (Torres and Otero, 1996; Wehtje and Adlercreutz, 1997). The amount of water present in the media is one of the critical parameters. When using enzymes in organic media some water is necessary for enzyme activity. Water in such a

system is distributed between the enzyme, a carrier if present, the solvent, the substrate and the head space (Anthonen and Hoff, 1998). As reported by Shah *et al.* (2004) as the water level increases, it increases the enzyme flexibility and the expressed activity. Above the optimum level of water, hydrolytic reactions become significant and transesterification yield is expected to go down (Shah *et al.*, 2004). The whole topic has been recently excellently reviewed by Serdakowski and Dordick (2008).

## 5. CONCLUSION

Among several lipases screened, only the lipase B from *Candida antarctica* in the immobilized forms, namely Novozyme ® 435 and Candida B. Silica, was found to be effective for transesterifying palm kernel oil with short-chain dialkyl carbonates in isohexane at 45°C. Novozyme ® 435 was also found to be the most effective lipase to transesterify palm kernel oil at 60°C without any added organic solvents.

For the single step lipase-catalyzed transesterification of palm kernel oil, dialkyl carbonates (in this case dimethyl and diethyl carbonate) gave much better yields compared to of short chain alcohols. Generally, the highest ester formation was observed at 60°C. The formation of esters was strongly influenced by the amount of water in the system.

## 6. ACKNOWLEDGMENT

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