

Research Article

Enzymatic Interesterification of Crude Palm Oil with Methyl acetate: Effect of Pre-treatment, Enzyme Dosage and Its Stability

Muhammad Zarin Amin Zainal¹, Harumi Veny^{1,2,*}, Fazlena Hamzah^{1,2},
Miradatul Najwa Muhammad Rodhi^{1,2}, Andri Cahyo Kumoro³, Ratna Dewi Kusumaningtyas⁴,
Haniif Prasetiawan⁴, Dhoni Hartanto⁴

¹School of Chemical Engineering, College of Engineering, Universiti Teknologi MARA, Shah Alam 40450, Selangor, Malaysia.

²Biocatalysis & Biobased Material Tech (BBMT), Research Initiative Group (RIG), College of Engineering, Universiti Teknologi MARA, Shah Alam 40450, Selangor, Malaysia

³Department of Chemical Engineering, Universitas Diponegoro, Semarang 50275, Central Java, Indonesia.

⁴Chemical Engineering Department, Faculty of Engineering, Universitas Negeri Semarang, Kampus Sekaran, Gunungpati, Semarang 50229, Central Java, Indonesia.

Received: 26th March 2023; Revised: 10th July 2023; Accepted: 12th July 2023

Available online: 18th July 2023; Published regularly: August 2023



Abstract

In the present study, biodiesel was produced via the enzymatic interesterification of Crude Palm Oil (CPO) with methyl acetate within ultrasonic condition. In contrast to alcohol, methyl acetate as an acyl acceptor does not inhibit lipase activity and can create triacetin as a useful byproduct. In this work, Immobilized lipase from *Candida Antarctica A* (CaLA) was utilized as biocatalyst and the effect of using non-pretreated CPO and pre-treated CPO as feedstock were explored. The pre-treatment of CPO involves degumming with acid, washing with water, and bleaching. The enzymatic interesterification was conducted in three-neck flasks using an ultrasonic water bath at 45°C. Few parameter effects on biodiesel production were also investigated, including the effect of molar ratio of CPO to methyl acetate, the effect amount of lipase, and the reusability of immobilized lipase (CaLA) in the interesterification reaction. The highest average Biodiesel yield of 80.6% was obtained from pretreated CPO at a molar ratio of 1:9 with 100 mg (1% w/w) of Immobilized CaLA, after three hours of reaction. Further research on the reusability of immobilized CaLA revealed that the yield of biodiesel reduced significantly after the second run. The results of the present study also demonstrated that Immobilized CaLA performed well at low concentrations but had low stability, with productivity decreasing to 92% upon reuse after the initial run. In order to make Immobilized lipase economically viable, further research must be conducted to overcome its low stability in the reaction.

Copyright © 2023 by Authors, Published by BCREC Group. This is an open access article under the CC BY-SA License (<https://creativecommons.org/licenses/by-sa/4.0>).

Keywords: Pretreatment; Crude Palm Oil; Biodiesel; *Candida Antarctica Lipase A*; Stability

How to Cite: M.Z.A. Zainal, H. Veny, F. Hamzah, M.N.M. Rodhi, A.C. Kumoro, R.D. Kusumaningtyas, H. Prasetiawan, D. Hartanto (2023). Enzymatic Interesterification of Crude Palm Oil with Methyl acetate: Effect of Pre-treatment, Enzyme Dosage and Its Stability. *Bulletin of Chemical Reaction Engineering & Catalysis*, 18(2), 294-302 (doi: 10.9767/bcrec.17763)

Permalink/DOI: <https://doi.org/10.9767/bcrec.17763>

1. Introduction

Nowadays, the use of fuel is shifting towards renewable energy supplies due to various disad-

vantages of fossil fuels, such as not being environmentally friendly, increased release of carbon dioxide, which has resulted in rising global temperatures and climate change. This is when biodiesel comes in useful. Biodiesel is a renewable and biodegradable alternative fuel.

* Corresponding Author.

Email: harumi2244@uitm.edu.my (H.Veny)

Telp: +60-5543-6511

The oils contain triglyceride, a molecule that is an ester produced from three fatty acids and glycerol. Triglyceride from vegetable oil interacts with alcohol (acyl acceptor) with the aid of a catalyst to form biodiesel (alkyl ester) and glycerol (by-product). This is referred to as the transesterification process. Crude palm oil (CPO) was chosen for our study because Malaysia is one of the leading exporters and producers of palm oil [1]. Unlike other oils, Crude Palm oil offers several advantages, including perennial crops that are continuous and uninterrupted, a high output yield, and a plantation that requires less fertilizer, water, and pesticides [2].

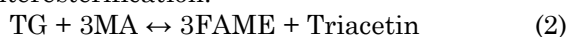
Many key aspects influence biodiesel manufacturing, such as conversion efficiency and production cost, as well as the molar ratio of feedstock (oil) to alcohol (acyl acceptor) [3]. In terms of stoichiometry, the molar ratio of oil to alcohol in a transesterification reaction is at 1:3. Higher molar ratios may be required for reversible reactions to progress, improve reactant contact, increase the miscibility of the triglyceride with the alcohol molecule, and further disrupt the glycerin-fatty acid bonds [4,5]. As a result, greater alkyl ester conversion occurs more quickly at higher molar ratios [6]. However, this is only the case up to a specific molar ratio, after which the yield will not rise [7,8]. A high molar ratio of oil to alcohol can inactivate the enzyme activity and reduce the biodiesel yield when using an enzyme or lipase as a biocatalyst [8–10]. The most typical byproduct of this process is glycerol. Glycerol's limited solubility in biodiesel is also an issue. During the process, glycerol deposits accumulate, covering the immobilized catalyst and limiting the enzyme's activity [11].

Instead of alcohol, an alternate acyl acceptor, such as methyl acetate, is introduced with triacetin as a byproduct. Methyl acetate has no adverse effect on immobilized lipase. The process of converting triglycerides and methyl acetate into biodiesel and triacetin is known as interesterification [12]. Equations (1) and (2) below illustrate how the stoichiometric reactions of transesterification and interesterification differ from one another.

Transesterification:



Interesterification:



where, TG stands for triglyceride (from any vegetable oil & fats), MeOH for methanol, MA

for methyl acetate, FAME for biodiesel, and Gly for glycerol.

Stoichiometrically both reactions are identical, the difference only in the acyl acceptor (methanol & methyl acetate) and by products (glycerol & triacetin). Many studies on Biodiesel production from transesterification with methyl acetate have been reported by Ognjanovic *et al.* [13], where they investigated the effect of methanol and methyl acetate on immobilized lipase, Novozyme 435. The result was nearly 100% conversion with no inactivation of the immobilized lipase.

Triacetin, a by product in this study, is primarily utilized as a plasticizer and gelatinizing agent in polymers and explosives, as well as an additive in tobacco, pharmaceutical compounds, and cosmetics [14]. It can be produced as by product from Biodiesel synthesis and from acetylating the glycerol with acetic acid [12,15]. In addition to being a compound of greater value, triacetin has the benefit of being soluble in biodiesel, allowing it to be incorporated into its formulation [12]. According to recent research, triacetin also can be added to biodiesel formulations up to 10% by weight and still meet Biodiesel quality standards [14].

Lipase has drawbacks such as high cost and deactivation due to high alcohol concentrations or extreme temperatures. Deactivation can reduce the effectiveness of enzymes. As a result, the enzyme cannot be reused, making industrial application technically challenging [16,17]. To reuse the enzyme, the lipase enzyme's stability must be altered. The ability of an enzyme to maintain its active structural shape in the presence of disruptive stimuli, such as high-temperature influence, is referred to as "enzyme stability" [18]. Immobilization of enzyme is one method. The goal of immobilisation is to create a biocatalyst that is stable and can be reused multiple times [19,20]. This method must take into account relative enzymatic activity, immobilisation procedure cost, enzyme deactivation, lipase loading, reagent toxicity, and final catalyst properties [21].

Temperature, reaction time, solvent, and agitation method are all factors that can affect the stability of immobilized enzyme. Previous research on enzyme stability in the interesterification of CPO from Shamel *et al.* [22] found that the stability of immobilized lipases in transesterification within ultrasonic was reduced to 70% after one hour when the temperature rose to 70 °C. As a result, the ideal temperature should be lower. The optimal temperature range is 30 to 50 °C [1]. Hence, in this study, the chosen temperature of 45 °C is with-

in the range. Many advantages have been demonstrated by the use of organic solvent tolerant lipase in organic media, including increased activity and stability, regiospecificity and stereoselectivity, higher substrate solubility, ease of product recovery, and the ability to shift the reaction equilibrium towards the synthetic direction [23]. However, the solvent used must be carefully chosen, either to dissolve the oil and alcohol/methyl acetate or to act as a washing agent after each run, as the solvent may deactivate the lipase. One example is the use of hexane in the reaction to improve oil and alcohol homogeneity but may deactivate the enzyme when used in transesterification [24].

Ultrasonics were used to aid the transesterification process in this study. The most notable advantages of this ultrasonic assistant are high yields, gentle response conditions, and quick reaction times [25,26]. Furthermore, the cavitation effect increases the surface area between the reactant and the reactant, replenishing the surface of the solid reactant or catalyst and accelerating dissolution [27,28]. Prolonged exposure to ultrasonic waves, on the other hand, can harm the enzyme's catalytic function. Few studies on biodiesel production via enzymatic interesterification with ultrasonic assistance have been published. One of them is Batistella *et al.* [29] research, which reported achieving 90% yields in the transesterification of soybean oil with ethanol using ultrasonic-assisted lipase *Lipozyme RM IM* and Novozym 435 in a four-hour reaction.

Biodiesel is produced in this study by intertransesterifying Crude Palm Oil (CPO) with immobilized lipase from *Candida antarctica* A (CaLA). Several parameters were investigated, including the effect of CPO pretreatment on yield, the effect of molar ratio and enzyme concentration, and the stability of immobilized li-

pase. The findings of this study are expected to help researchers better understand the optimal conditions and stability of immobilized lipase in a transesterification reaction using CPO as the feedstock.

2. Materials and Methods

2.1 Materials

The CPO in this investigation was courtesy of a generous donation from one of Malaysia's biodiesel companies. Phosphoric acid (H_3PO_4) with 85% purity, which was used for acid degumming in the pretreatment process, hexane butanol was supplied by R&M Chemicals Malaysia. Activated granulated charcoal of the grade AR was produced by Riendemann Schmidt was employed to bleach the oil. Methyl acetate, 99% purity, FAME Standard C4-C24 and Immobilized lipase A from *C. antarctica* (CaLA) were all purchased from Sigma-Aldrich.

2.2 Methods

2.2.1 Pre-Treatment of CPO

The CPO was undergone a pretreatment process by acid degumming and bleaching. The 20 g of CPO were heated to 80 °C and added with 2 g of phosphoric acid 10% (w/w). The mixture was washed with warm water and centrifuged at 700 rpm for 30 min to separate the water and the impurities from the oil. Then, the oil is heated to 100 °C, and 1% (w/w) of bleaching earth is added to the oil. The mixture was stirred with a magnetic stirrer for 30 min. Then, the Buchner funnel filtration was used to separate the oil from the bleaching earth.

2.2.2 Interesterification of CPO

A 10 ml (± 10 g) of CPO mixed with methyl acetate with in three neck flasks equipped with thermometer and sampling point. The mixture in three neck flask was heated up to 45 °C in an ultrasonic bath water. The 100 mg (1% w/w of CPO) of immobilised lipase was added to the mixture. The reaction was performed for 2 h and 30 min. The sample was centrifuged to separate biodiesel from triacetin and immobilized lipase. The supernatant was then mixed with hexane for Biodiesel analysis using GC MS. The experimental set up is drawn in Figure 1.

2.2.3 Reusability of immobilized lipase

In Stability study for reusability of Immobilized CaLA, after each run of interesterifica-

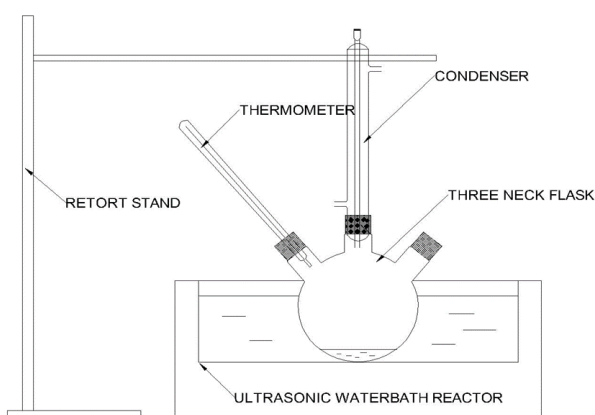


Figure 1. The schematic of interesterification process setup.

tion, biodiesel was centrifuged and the immobilized lipase was extracted, and wash with acetone, prior to next run. The experiment was repeated until fourth time, and the productivity of immobilized lipase was analyzed.

2.2.4 Fatty acid Methyl Ester analysis by GC-MS

The supernatant from centrifuged biodiesel was taken and mixed with hexane homogeneously using vortex. Then, the mixture was inserted into the vial and sent to GC-MS for analysis. The SLB®-5ms Capillary GC Column with helium as carrier gas, was used to analyse the sample. The split ratio was 1:50 with inlet temperature of 250 °C. The Biodiesel (FAME) yield was calculated based total weight of Fatty Acid Methyl Ester (FAME) in product over the weight of Crude Palm Oil (CPO) as shown in equation (3).

$$\%Yield = \frac{\text{Total FAME weight}}{\text{CPO weight}} \times 100\% \quad (3)$$

3. Results and Discussion

3.1 Effect of Pretreatment of CPO for Different Molar Ratio

Experiments were conducted to determine the optimal molar ratio of oil to methyl acetate for both non-pretreatment and pretreatment CPO.

Figure 2 depicts the total FAME Yield from both non-pretreated and pretreated CPO. The interesterification was carried out at 45°C using 180 mg of immobilised CaLA. Biodiesel yields of 51% and 57% were obtained at molar ratios of 1:3 and 1:9 for non-pretreated and pre-

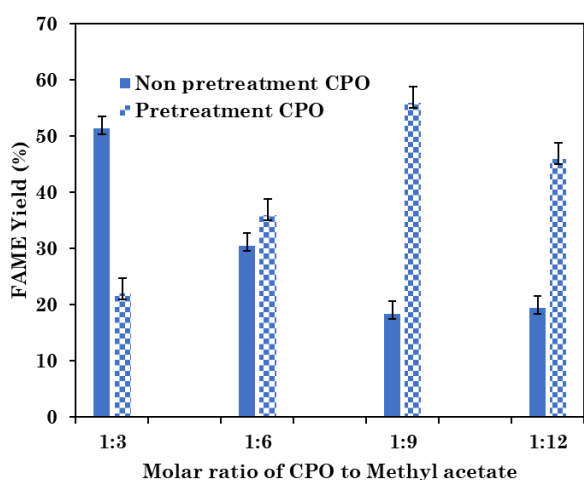


Figure 2. Effect of Molar ratio CPO to methyl acetate for Non pretreated and Pretreated CPO.

treated CPO, respectively. The reaction is unaffected in either case by increasing the concentration of methyl acetate up to molar ratios of 1:6 for non-pretreated oil and 1:12 for pretreated oil. This could be due to excess methyl diluting the oil, reducing the contact of oil and methyl acetate [29]. The findings also showed that the optimal molar ratio for each CPO (pretreated and non-pretreated) is distinct. This difference may exist because non-pretreated CPO naturally contains more phospholipids than pretreated CPO. This phospholipid is widely regarded as a significant impediment in the biodiesel industry. The performance of Immobilised *Candida antarctica* lipase was reported to be negatively affected even with less than 1% phospholipid, implying that successful degumming with complete removal of phospholipid is required [30–32]. The trend result from current study shows similar pattern to previous study from Surendhiran's [33], where the molar ratio increased in a linear fashion from 1:3 to 1:9. When comparing Biodiesel yield from transesterification using alcohol as an acyl acceptor to the current study with methyl acetate, the trend usually results in a lower yield after a molar ratio greater than 1:3, due to alcohol or glycerol inactivating the lipase biocatalyst, as reported by Shimada *et al.* [34]. As a result, based on the highest Biodiesel Yield, subsequent researches were carried out at fixed molar ratio of 1:9 with pretreated CPO.

3.2 Effect of Amount of Immobilized Lipase CaLA

For this investigation, an experiment at Molar ratio 1:9 using pretreated CPO was conducted, to determine the optimal amount of C.

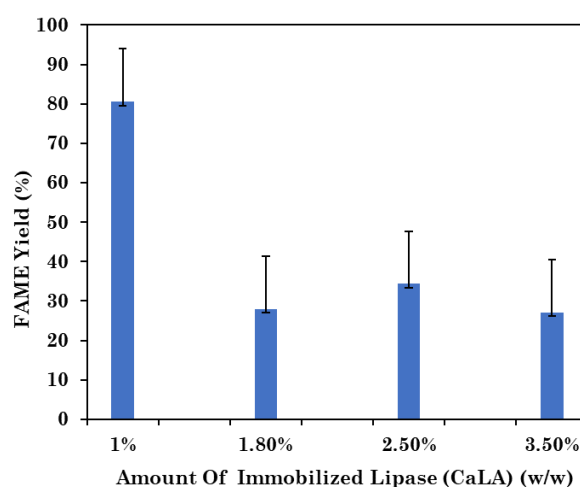


Figure 3. Effect Amount of Immobilized CaLA in Interesterification of pretreated CPO.

antartica lipase A (CaLA) for maximizing biodiesel yield.

Figure 3 depicts the total yield of various amounts of lipase, spanning from 1% (w/v) (100 mg) to 3.5% (w/w) (350 mg). As shown in Table 2, the preponderance of earlier studies on transesterification reported maximum lipase concentrations ranging from 3% to 10% (w/w). Thus, the current study begins below the range and progresses to within the range. The highest Biodiesel yield was achieved at a lipase concentration of 1% w/w with an 80.6% of Biodiesel yield, indicating that the reaction can be catalyzed even at lower enzyme concentrations. When the enzyme dosage exceeded 1% w/w, the yield decreased. This is because the higher the concentration of catalyst, the less effect it has on the reaction. As the amount of enzyme increases, it becomes stationary, which was also observed in study from Yasvanthrajan *et al.* [35]. This could be due to the catalyst clumping together in the reaction media and losing contact with the active sites of the reactant [36]. As a result, 100 mg (1% w/v) of enzyme is the ideal amount.

The major Fatty acid methyl ester (FAME)'s composition at amount of lipase 1% w/v is shown in Table 1. Unsaturated Fatty Acid Me-

thyl Ester was majorly found from Biodiesel from this study, with Methyl Oleate and Methyl ester were dominant. Similar major unsaturated methyl ester composition was also reported by Widyawati *et al.* [37].

3.3 Stability Study of Immobilized CaLA in Interesterification

The stability was investigated by reusing the immobilized CaLA in interesterification at a molar ratio of 1:9 in an ultrasonic water bath at 45°C. The experiment was carried out five times reusing the lipase in reaction, and the results is described in Figure 4.

Figure 4 shows that the stability of CaLA was reduced by 92.27% from the first run in interesterification of CPO. This could be because the active site's structure was damaged, resulting in a significant decrease in total yield. Before being reused, the CaLA was washed with acetone to remove any remaining CPO, triacetin, or biodiesel. As a result, it may also be

Table 1. Fatty Acid Methyl Ester composition of biodiesel from pretreated CPO at molar ratio 1:9, amount of CaLA 1% w/w at 45°C.

Methyl Ester Component	Composition (%)
Methyl Oleate	66.67
Methyl Palmitate	23.33
Methyl Linolelaidate	1.67
cis-11,14-Eicosadienoic acid methyl ester	8.33

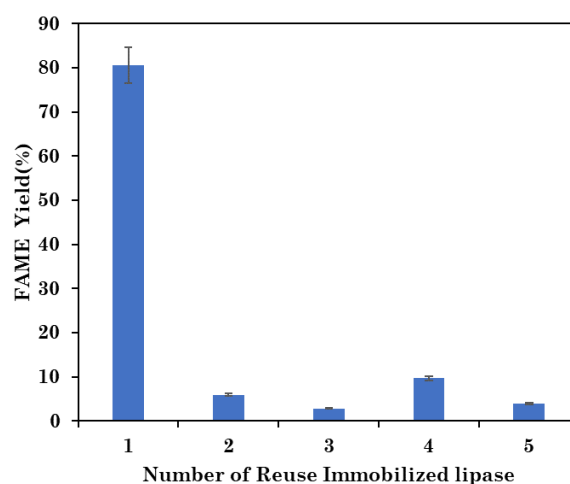


Figure 4. Stability study of Immobilized CaLA in interesterification of pretreated CPO.

Table 2. Yield and reusability of immobilized lipase in transesterification.

No	Immobilized Lipase	Oil	Amount of Lipase	% Yield (1 st run)	Reusability	% Yield after reuse	Ref.
1.	Candida antartica (CaLA)	Crude Palm Oil	1 %w/w	80.92	4 th times	9.7	Current Study
2.	Novozym 435	Waste Cooking Oil	10 %w/v	86.61	4 th times	81.1	[38,39]
3.	Novozym 435 & Lipozyme RMIM	Soybean Oil	5 wt%	57.90	7 th times 2 times	28	[25]
4.	Lipozyme TLIM	Waste Cooking Oil	6 %(w/v)	90.1	7 th times	20	[42]
5.	Pseudomonas cepacia	Waste vegetable oil	3 %w/w	46.3	6 th times	22.39	[43]

the cause of a decrease in catalyst activity, as high solvent concentrations may reduce the interaction between reactant and enzyme [38,39].

A study from Kamal *et al.* [40] also reported that relative activity of the lipase monotonically decreased with increasing concentration of acetone, acetonitrile, and DMF. Showing that lipase is sensitive to acetone. Another factor that can be considered is ultrasound irradiation which changes the structure of enzyme causing it to be deactivated. The structure of enzyme was changing during the reaction added with ultrasound irradiation causing it to deactivate [2]. Thus, other parameters such as acoustic on enzyme stability is suggested to be considered in the future research.

3.4 Formation of Triacetin as by Product from Interesterification of CPO

Triacetin is a byproduct of the reaction between triglycerides and methyl acetate that has a higher added value than glycerol [14]. In this study, the formation of triacetin from the interesterification of CPO was analyzed and detected using FTIR. Figure 5 depicts the IR spectra of triacetin resulting from CPO interesterification using CaLA. The dominant spectra were observed to be at $\pm 1741 \text{ cm}^{-1}$ and $\pm 1236 \text{ cm}^{-1}$. Further observation in Figure 6 compares the IR spectra of triacetin derived from interesterification, with commercial Diesel B10 and CPO, to determine which spectrum is the most dominant. It can be seen that

wavelength of $\pm 1741 \text{ cm}^{-1}$ is present in all three spectra. However, wavelength of 1236 cm^{-1} is only present in the IR spectra of triacetin from interesterification of CPO. This verifies the formation of triacetin from the reaction and identifies its characteristic peak. Comparable IR spectra of triacetin ($\pm 12 \text{ cm}^{-1}$) was also reported by Pastres *et al.* [41]

4. Conclusions

In this study, Immobilized *Candida Antartica* Lipase A (CaLA) is successfully conduct enzymatic interesterification with methyl acetate from CPO. The highest yield was obtained when using pretreated CPO in interesterification at a molar ratio of oil to methyl acetate

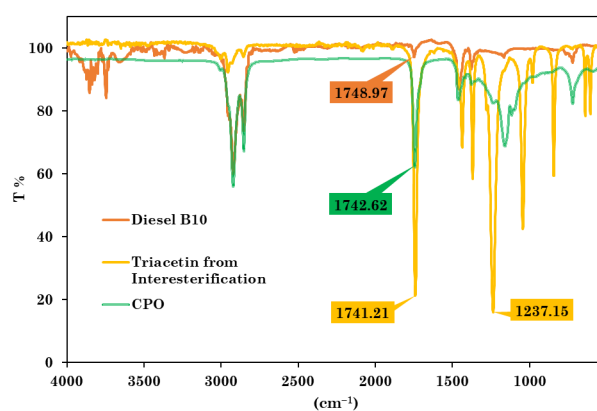


Figure 6. Comparison of IR spectra for commercial diesel B10, triacetin from interesterification and raw CPO (redrawn data).

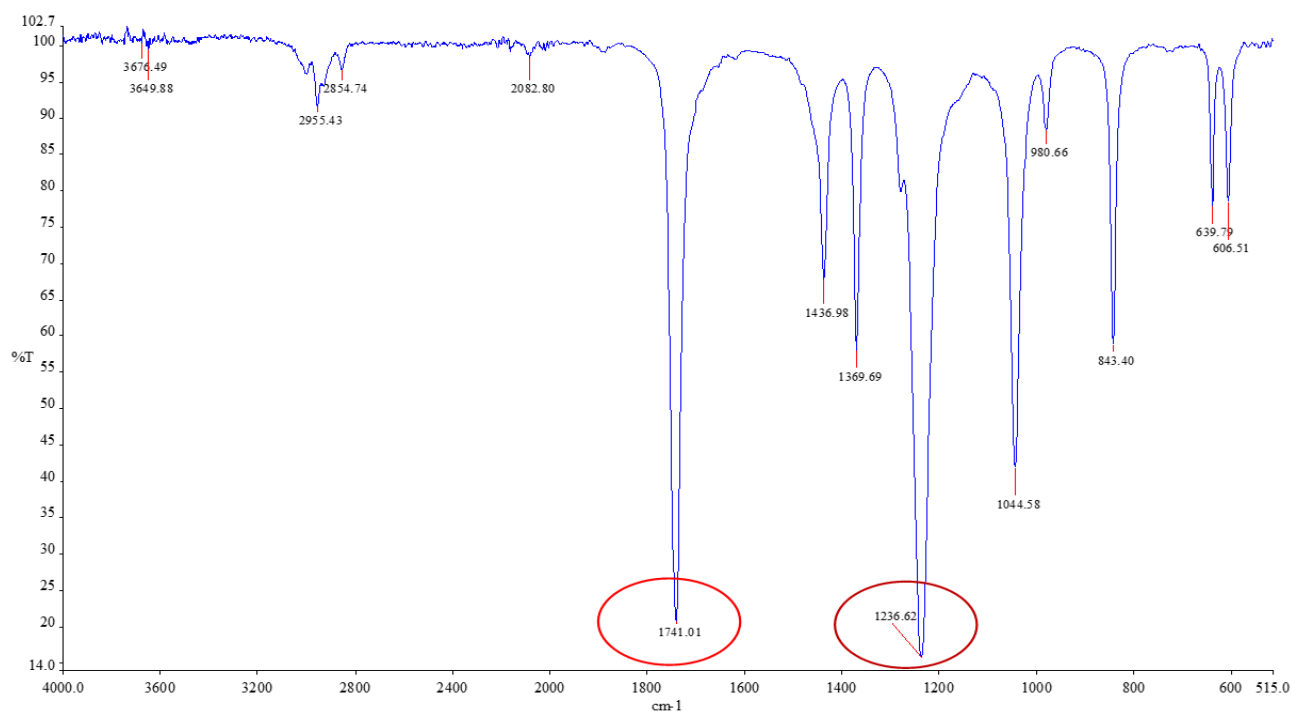


Figure 5. FTIR spectra of product from interesterification of CPO.

greater than the stoichiometric molar ratio. In the study of enzyme dosage, a low amount of immobilized CaLA was found to have more effective activity than a high amount. However, when the stability of immobilized CaLA was tested by reusing the lipase five times, the productivity and biodiesel yield were greatly reduced in the second run. This is most likely due to the use of acetone as a solvent in the washing process of immobilized CaLA after each reaction, which affects the interaction between the enzyme and the reactant. To avoid the destruction of the active site of the lipase, it is recommended to use the proper solvent to remove residue oil or any impurities before reuse.

Acknowledgment

The authors would like to express their gratitude to RMC Universiti Teknologi MARA for financial support provided through grant 600-RMC/SRP-UNNES/5/3 (005/2020). The authors also would like to thanks to Nurhasyimah Binti Muhammad, Dylexia Delaynie and Risal Bin Ishak for FTIR analysis of CPO and Diesel B10.

CRedit Author Statement

Muhammad Zarin Amin Zainal: Investigation, Resources, Data collection, Writing Draft Preparation; Harumi Veny : Conceptualization, Funding acquisition, Methodology, Formal Analysis, Data Curation, Writing Draft Preparation, Editing & Supervision; Fazlena Hamzah: Funding acquisition & Validation. Miradatul Najwa Muhammad Rodhi: Funding acquisition & Validation. Andri Cahyo Kumoro: Review & Funding acquisition. Ratna Dewi Kusumaningtyas: Review & Funding acquisition; Haniif Prasetiawan: Review & Funding acquisition; Dhoni Hartanto: Review & Funding acquisition. All authors have read and agreed to the published version of the manuscript.

References

- [1] Mekhilef, S., Siga, S., Saidur, R. (2011). A review on palm oil biodiesel as a source of renewable fuel. *Renewable and Sustainable Energy Reviews*, 15(4), 1937–1949. DOI: 10.1016/J.RSER.2010.12.012.
- [2] Woittiez, L.S., van Wijk, M.T., Slingerland, M., van Noordwijk, M., Giller, K.E. (2017). Yield gaps in oil palm: A quantitative review of contributing factors. *European Journal of Agronomy*, 83, 57–77. DOI: 10.1016/J.EJA.2016.11.002.
- [3] Ma, F., Hanna, M.A. (1999). Biodiesel production: A review. *Bioresource Technology*, 70(1), 1–15. DOI: 10.1016/S0960-8524(99)00025-5.
- [4] Lee, J.S., Saka, S. (2010). Biodiesel production by heterogeneous catalysts and supercritical technologies. *Bioresource Technology*, 101(19), 7191–7200. DOI: 10.1016/j.biortech.2010.04.071.
- [5] Miao, X., Wu, Q. (2006). Biodiesel production from heterotrophic microalgal oil. *Bioresource Technology*, 97(6), 841–846. DOI: 10.1016/j.biortech.2005.04.008.
- [6] Helwani, Z., Othman, M.R., Aziz, N., Fernando, W.J.N., Kim, J. (2009). Technologies for production of biodiesel focusing on green catalytic techniques: A review. *Fuel Processing Technology*, 90(12), 1502–1514. DOI: 10.1016/j.fuproc.2009.07.016.
- [7] Anastopoulos, G., Zannikou, Y., Stournas, S., Kalligeros, S. (2009). Transesterification of Vegetable Oils with Ethanol and Characterization of the Key Fuel Properties of Ethyl Esters. *Energies*, 2(2), 362–376. DOI: 10.3390/en20200362.
- [8] Chen, J.-W., Wu, W.-T. (2003). Regeneration of immobilized *Candida antarctica* lipase for transesterification. *Journal of Bioscience and Bioengineering*, 95(5), 466–469. DOI: 10.1263/jbb.95.466.
- [9] Maceiras, R., Vega, M., Costa, C., Ramos, P., Márquez, M.C. (2011). Enzyme deactivation during biodiesel production. *Chemical Engineering Journal*, 166(1), 358–361. DOI: 10.1016/J.CEJ.2010.11.007.
- [10] Salis, A., Pinna, M., Monduzzi, M., Solinas, V. (2005). Biodiesel production from triolein and short chain alcohols through biocatalysis. *Journal of Biotechnology*, 119(3), 291–299. DOI: 10.1016/j.jbiotec.2005.04.009.
- [11] Dossat, V., Combes, D., Marty, A. (1999). Continuous enzymatic transesterification of high oleic sunflower oil in a packed bed reactor: Influence of the glycerol production. *Enzyme and Microbial Technology*, 25(3–5), 194–200. DOI: 10.1016/S0141-0229(99)00026-5.
- [12] Casas, A., Ramos, M.J., Pérez, Á. (2011). Kinetics of chemical interesterification of sunflower oil with methyl acetate for biodiesel and triacetin production. *Chemical Engineering Journal*, 171(3), 1324–1332. DOI: 10.1016/j.cej.2011.05.037.
- [13] Ognjanovic, N., Bezbradica, D., Knezevic-Jugovic, Z. (2009). Enzymatic conversion of sunflower oil to biodiesel in a solvent-free system: Process optimization and the immobilized system stability. *Bioresource Technology*, 100(21), 5146–5154. DOI: 10.1016/j.biortech.2009.05.068.

- [14] Casas, A., Ruiz, J.R., Ramos, M.J., Pérez, A. (2010). Effects of Triacetin on Biodiesel Quality. *Energy & Fuels*, 24(8), 4481–4489. DOI: 10.1021/ef100406b.
- [15] Lacerda, C.V., Carvalho, M.J.S., Ratton, A.R., Soares, I.P., Borges, L.E.P. (2015). Synthesis of Triacetin and Evaluation on Motor. *Journal of the Brazilian Chemical Society*, 26(8), 1625–1631. DOI: 10.5935/0103-5053.20150133.
- [16] Sheldon, R.A., Wallau, M., Arends, I.W.C.E., Schuchardt, U. (1998). Heterogeneous Catalysts for Liquid-Phase Oxidations: Philosophers' Stones or Trojan Horses?. *Accounts of Chemical Research*, 31(8), 485–493. DOI: 10.1021/ar9700163
- [17] Krajewska, B. (2004). Application of chitin and chitosan-based materials for enzyme immobilizations: a review. *Enzyme and Microbial Technology*, 35(2–3), 126–139. DOI: 10.1016/J.ENZMICTEC.2003.12.013.
- [18] Yeoman, C.J., Han, Y., Dodd, D., Schroeder, C.M., Mackie, R.I., Cann, I.K.O. (2010). Thermostable Enzymes as Biocatalysts in the Biofuel Industry. *Advances in Applied Microbiology*, 70, 1–55. DOI: 10.1016/S0065-2164(10)70001-0.
- [19] Jegannathan, K.R., Abang, S., Poncet, D., Chan, E.S., Ravindra, P. (2008). Production of biodiesel using immobilized lipase - A critical review. *Critical Reviews in Biotechnology*, 28(4), 253–264. DOI: 10.1080/07388550802428392.
- [20] Bernal, C., Illanes, A., Wilson, L. (2014). Heterofunctional hydrophilic-hydrophobic porous silica as support for multipoint covalent immobilization of lipases: Application to lactulose palmitate synthesis. *Langmuir*, 30(12), 3557–3566. DOI: 10.1021/la4047512.
- [21] Mohamad, N.R., Marzuki, N.H.C., Buang, N.A., Huyop, F., Wahab, R.A. (2015). An overview of technologies for immobilization of enzymes and surface analysis techniques for immobilized enzymes. *Biotechnology and Biotechnological Equipment*, 29, 205–220. DOI: 10.1080/13102818.2015.1008192
- [22] Shamel, M.M., Ramachandran, K.B., Hasan, M. (2005). Operational Stability of Lipase Enzyme: Effect of Temperature and Shear. *Developments in Chemical Engineering and Mineral Processing*, 13(5–6), 599–604. DOI: 10.1002/APJ.5500130509.
- [23] Sharma, S., Kanwar, S.S. (2014). Organic solvent tolerant lipases and applications. *The Scientific World Journal*, 2014, 625258. DOI: 10.1155/2014/625258.
- [24] Royon, D., Daz, M., Ellenrieder, G., Locatelli, S. (2007). Enzymatic production of biodiesel from cotton seed oil using t-butanol as a solvent. *Bioresource Technology*, 98(3), 648–653. DOI: 10.1016/j.biortech.2006.02.021.
- [25] Bonrath, W. (2005). Ultrasound supported catalysis. *Ultrasonics Sonochemistry*, 12(1–2), 103–106. DOI: 10.1016/j.ultsonch.2004.03.008.
- [26] Zaks, A., Klibanov, M. (1988). Enzymatic Catalysis in Nonaqueous Solvents. *Journal of Biological Chemistry*, 263(7), 3194–3201. DOI: 10.1016/S0021-9258(18)69054-4.
- [27] Pokhrel, N., Vabbina, P.K., Pala, N. (2016). Sonochemistry: Science and Engineering. *Ultrasonics Sonochemistry*, 29, 104–128. DOI: 10.1016/j.ultsonch.2015.07.023.
- [28] Fomthum, C.J.D., Carrer, M., Houvet, M., Škrbić, T., Graziano, G., Giacometti, A. (2020). Can the roles of polar and non-polar moieties be reversed in non-polar solvents? *Physical Chemistry Chemical Physics*, 22(44), 25848–25858. DOI: 10.1039/D0CP02948C.
- [29] Batistella, L., Lerin, L.A., Brugnere, P., Danielli, A.J., Trentin, C.M., Popielski, A., Treichel, H., Oliveira, J.V., De Oliveira, D. (2012). Ultrasound-assisted lipase-catalyzed transesterification of soybean oil in organic solvent system. *Ultrasonics Sonochemistry*, 19(3), 452–458. DOI: 10.1016/j.ultsonch.2011.11.018.
- [30] Watanabe, Y., Shimada, Y., Sugihara, A., Tominaga, Y. (2002). Conversion of degummed soybean oil to biodiesel fuel with immobilized Candida antarctica lipase. *Journal of Molecular Catalysis - B Enzymatic*, 17(3–5), 151–155. DOI: 10.1016/S1381-1177(02)00022-X.
- [31] Indira, D., Das, B., Bhawsar, H., Moumita, S., Johnson, E.M., Balasubramanian, P., Jayabalan, R. (2018). Investigation on the production of bioethanol from black tea waste biomass in the seawater-based system. *Bioresource Technology Reports*, 4, 209–213. DOI: 10.1016/j.biteb.2018.11.003.
- [32] Van Nieuwenhuyzen, W., Tomás, M.C. (2008). Update on vegetable lecithin and phospholipid technologies. *European Journal of Lipid Science and Technology*, 110(5), 472–486. DOI: 10.1002/EJLT.200800041.
- [33] Surendhiran, D., Sirajunnisa, A.R., Vijay, M. (2015). An alternative method for production of microalgal biodiesel using novel Bacillus lipase. *3 Biotech*, 5(5), 715–725. DOI: 10.1007/s13205-014-0271-4.

- [34] Shimada, Y., Watanabe, Y., Samukawa, T., Sugihara, A., Noda, H., Fukuda, H., Tomina-ga, Y. (1999). Conversion of vegetable oil to bi-diesel using immobilized *Candida antarctica* lipase. *Journal of the American Oil Chemists' Society*, 76(7), 789–793. DOI: 10.1007/s11746-999-0067-6.
- [35] Yasvanthrajan, N., Sivakumar, P., Muthuku-mar, K., Murugesan, T., Arunagiri, A. (2021). Production of biodiesel from waste bio-oil through ultrasound assisted transesterifica-tion using immobilized lipase. *Environmental Technology & Innovation*, 21, 101199. DOI: 10.1016/J.ETI.2020.101199.
- [36] Zhao, X., Qi, F., Yuan, C., Du, W., Liu, D. (2015). Lipase-catalyzed process for biodiesel production: Enzyme immobilization, process simulation and optimization. *Renewable and Sustainable Energy Reviews*, 44, 182–197. DOI: 10.1016/j.rser.2014.12.021.
- [37] Widyawati, M., Manurung, R., Afrianto, R. (2014). Synthesis Biodiesel from Palm Oil Through Interesterification Using Imobilized Lipase Enzym as Catalyst: The Effect of Amount of Biocatalyst, Mole Ratio of Reactan, Temperature to Yield. *International Journal of Science and Engineering*, 7(2), 174–177. DOI: 10.12777/ijse.7.2.174-177.
- [38] Gharat, N., Rathod, V.K. (2013). Ultrasound assisted enzyme catalyzed transesterification of waste cooking oil with dimethyl carbonate. *Ultrasonics Sonochemistry*, 20(3), 900–905. DOI: 10.1016/j.ultsonch.2012.10.011.
- [39] Pollardo, A.A., Lee, H.-S., Lee, D., Kim, S., Kim, J. (2018). Solvent effect on the enzymatic production of biodiesel from waste animal fat. *Journal of Cleaner Production*, 185, 382–388. DOI: 10.1016/j.jclepro.2018.02.210.
- [40] Kamal, Z., Yedavalli, P., Deshmukh, M.V., Rao, N.M. (2013). Lipase in aqueous-polar organic solvents: Activity, structure, and stability. *Protein Science*, 22(7), 904–915. DOI: 10.1002/pro.2271.
- [41] Pastres, R., Panzeri, A.L., Visentin, D., Causin, V. (2022). Determination by infrared spectroscopy of triacetin content in diesel: A tool for countering designer fuel frauds. *Talanta Open*, 5, 100109. DOI: 10.1016/j.talo.2022.100109.
- [42] Subhedar, P.B., Gogate, P.R. (2016). Ultra-sound assisted intensification of biodiesel pro-duction using enzymatic interesterification. *Ultrasonics Sonochemistry*, 29, 67–75. DOI: 10.1016/j.ultsonch.2015.09.006.
- [43] Lopresto, C.G., Naccarato, S., Albo, L., De Paola, M.G., Chakraborty, S., Curcio, S., Calabrò, V. (2015). Enzymatic transesterifica-tion of waste vegetable oil to produce bio-diesel. *Ecotoxicology and Environmental Safety*, 121, 229–235. DOI: 10.1016/j.ecoenv.2015.03.028.