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Integrated Cleaner Biocatalytic Process for Biodiesel Production from Crude Palm Oil Comparing to Refined Palm Oil

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Abstract: An integrated cleaner biocatalyst process was performed for biodiesel production from crude palm oil (CPO) and refined palm oil (RPO). It was evaluated on process efficiency in terms of high purity of biodiesel as well as by-products without purification, less wastewater, less time consuming, and a simple downstream process. A first saponification step was carried out in both f CPO and RPO, a high purity of glycerol (86.25% and 87.5%) was achieved, respectively, while free fatty acids (FFASs) in soap were obtained after hexane extraction. High yields of FFASs were obtained from both CPO and RPO (98.83% and 90.94%). Subsequently, the FFAs were esterified to biodiesel by a biocatalyst of immobilized lipase. The highest biodiesel yields achieved were of 92.14% and 92.58% (CPO and RPO). Remarkably, biodiesel yields obtained from CPO and RPO achieved satisfactory values and the biocatalyst used could be reused for more than 16–17 cycles.

Keywords: Crude Palm Oil (CPO); Refined Palm Oil (RPO); saponification; glycerol; Free Fatty Acid (FFAs); esterification; immobilized lipase; biodiesel

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Citation: Muanruksa, P.; Wongsirichot, P.; Winterburn, J.; Kaewkannetra, P. Integrated Cleaner Biocatalytic Process for Biodiesel Production from Crude Palm Oil Comparing to Refined Palm Oil. *Catalysts* **2021**, *11*, 734. https://doi.org/10.3390/catal11060734

Academic Editor: Francisco Valero

Received: 21 May 2021
Accepted: 14 June 2021
Published: 15 June 2021

1. Introduction

Currently, biodiesel, an alternative biofuel is being considered to replace fossil diesel [1,2]. It can be used as pure 100% biodiesel (B100) or blended with petroleum-based diesel due to its several advantages; biodegradability, excellent lubricity, non-toxicity, cleaner combustion, high colorific value, and production of less engine exhaust gas emissions (CO, CO_2, and SO_x) than petroleum-based diesel [3–6]. Typically, biodiesel can be produced from several oil crops, such as soybean oil, jatropha oil, sunflower oil, coconut oil, palm oil etc. Among these, refined palm oil (RPO) has the lowest price, the highest yield per hectare in Thailand and is widely used as a raw material for biodiesel production in South East Asia [7]. Naturally, there are two kinds of oils obtained from the palm tree; palm oil (PO) and palm kernel oil (PKO). Some physical and chemical methods are required to purify these oils by fractional distillation, leading to different forms of oil, including crude palm oil (CPO), semi-refined palm oil (SRPO), and refined palm oil (RPO). The current palm oil refinery process in Thailand is explained by Dujjanutat et al. [8]. In addition, CPO is also of significant interest to researchers for use as a feedstock for biodiesel production being a non-edible oil with low production cost compared to other plant oils [9,10]. Therefore, both CPO and RPO were chosen to be feedstocks with their good potential for biodiesel production. Traditionally, biodiesel is mostly produced via transesterification of triglycerides (TG) and short chain alcohols, catalyzed by homogeneous alkaline catalysts including NaOH, KOH, CH_3CH_2ONa, etc. The process shows many advantages including high reaction rate, high catalytic efficiency, and low amount of catalyst loading [11,12].

However, the major drawbacks are soap formation, difficult separation, and high impurity biodiesel as well as glycerol. In contrast, the presence of FFAs does not have any effect on the esterification reaction if catalyzed by homogenous acid catalysts such as H_2SO_4, $HClO_4$, HNO_3, etc. In addition, several problems have been encountered for example; long reaction time, low conversion rate, and low biodiesel yield [13,14].

Biodiesel purification is typically carried out in a number of ways, namely by washing with water, hot deionized water, pre-washing, organic solvents, and acidified water [15–18]. These processes are mainly hindered by the wastewater generation. The impurities commonly found in biodiesel can be removed by adsorbents such as magnesium silicate, calcium magnesium silicate, etc. In addition, ion-exchange resin can also be used to obtain pure biodiesel without glycerol contamination. However, the resin reusability is low, presenting a challenge [19]. Impurities including FFAs, inorganic salts, and free ions found in the glycerol are still a major problem for further applications, for example using as feedstocks in the food, pharmaceutical, and cosmetics industries, which require quite high purity glycerol (96–99 wt%.). In addition, glycerol also has beneficial applications for bio-hydrogen, monoacylglycerol (MAG), diacylglycerol (DAG), triacylglycerol (TAG), and other value-added chemicals [20–22]. Recently, it was reported that several methods can be used to purify glycerol such as neutralization, ion exchange/activated carbon adsorption, vacuum distillation, and membrane distillation [23]. In a previous study, Dhabhai et al. reported that the highest purity of glycerol (97.5%) was obtained via serial physicochemical treatment, membrane filtration, and activated carbon treatment [24]. However, saponification of triglycerides (TG) is also reported as being the simplest process to produce high purity glycerol (83–84%) without subsequent additional purification [25]. In addition, López et al. [26] applied a saponification reaction to obtain FFAs from microalgae lipids, these were further used as a substrate for biodiesel production via an esterification reaction catalyzed by lipase Novozyme 435 from *Candida antarctica*. They found that high purity of biodiesel (83 wt %) was obtained within four hours and the biocatalyst could be reused for more than six cycles.

Currently, enzymatic esterification of feedstock rich in FFA content is becoming an attractive approach. The key point is minimal pretreatment of low-quality feedstock in an upstream process with a lipase biocatalyst (EC.3.1.1) which shows high potential to promote acidic oil esterification [27]. Furthermore, the enzymatic process presents advantages such as a mild reaction temperature, non-corrosive reaction, high biodiesel purity, simple downstream process, no wastewater from washing steps, and reusable catalyst (if the enzyme is suitably immobilized). Thus, lipase could be applied as a biocatalyst during an esterification reaction to produce biodiesel. There are some doubts which might arise in the use of lipase as a biocatalyst to produce biodiesel such as the high cost, difficulties in downstream recovery, and low alcohol resistance. These problems can be solved by using immobilized lipase on a matrix support. Enzyme entrapment is a simple and cost-effective method which is widely used in industrial applications [28]. During entrapment, the enzyme either reacts with the supporting material or other enzyme molecules through encapsulation within a network of insoluble polymer, such as alginate silica aerogel and celite support sol gel. This is achieved by adding enzyme to a monomer solution before forming a gel, thus trapping the enzyme molecules. After immobilization, the enzyme can move freely within a solid support which allows only substrate and product to pass though and retains the enzyme inside the polymeric network [29,30]. Therefore, this technique was used throughout this study. Alginate is widely used as a supporting material for enzyme immobilization by using the entrapment technique because of the desirable properties; the mild conditions required, high porosity, easy to control porosity and the retention of enzymatic activity. However, fast degradation of the alginate matrix could occur under conditions of high temperature, low pH, and presence of organic solvents [31–33]. In addition, a non-toxic synthetic polymer of polyvinyl alcohol (PVA) provides good properties; high strength and a high ability to stabilize and preserve protein activity. Previously, Martinja et al. [34] reported biodiesel production from palm oil mill effluent using immobilized *Can-*

dida rugosa lipase in PVA-alginate-sulfate beads. Despite low biocatalyst loading (2 g), the esterification reaction was complete within five hours. Accordingly, lipase immobilization on PVA-alginate beads is appropriate to use as biocatalyst for biodiesel production from FFAs in acid oil.

Thus, in this work, an alternative new cleaner biodiesel production process using CPO and RPO as feedstocks was developed and evaluated in terms of wastewater reduction, an easy separation process, a simple purification of biodiesel and glycerol. Saponification of CPO/RPO was first carried out to obtain soap and glycerol. CPO/RPO glycerol was then discharged from the reaction medium. Subsequently, the soap was extracted by hexane to generate FFAs for use as a substrate in subsequent steps. Second, enzymatic esterification of FFAs from CPO/RPO for biodiesel production was optimized by varying the reaction temperature, the MEOH to FFAs ratio, and the agitation rate. Furthermore, the reusability of biocatalyst was also investigated under the optimal condition. The characterization of the fatty acid methyl esters (FAMEs) obtained was also determined.

2. Results

2.1. Fatty Acid Composition of CPO/RPO

FFA content in both CPO and RPO was determined and the results are presented in Table 1. It was revealed that the CPO was mainly composed of oleic acid, palmitoleic acid, stearic acid, and palmitic acid, respectively. Meanwhile, in the case of RPO, the main fatty acids present were oleic acid and palmitoleic acid.

Table 1. Fatty acid composition of crude palm oil (CPO) and refined palm oil (RPO).

Fatty Acid	CPO	RPO
Capric acid (C10:0)	0.07 ± 0.06	0.11 ± 0.03
Lauric acid (C12:0)	0.99 ± 0.03	1.58 ± 0.05
Myristic acid (C14:0)	3.78 ± 0.06	4.74 ± 0.02
Palmitic acid (C16:0)	9.56 ± 0.04	1.55 ± 0.01
Palmitoleic (C16:1)	24.54 ± 0.02	12.50 ± 0.03
Stearic acid (C18:0)	10.49 ± 0.03	1.82 ± 0.04
Oleic acid (C18:1)	45.02 ± 0.05	76.27 ± 0.05
Linoleic acid (C18:2)	4.33 ± 0.02	0.16 ± 0.06
Linolenic acid (C18:3)	0.04 ± 0.01	0.08 ± 0.03
Arachidic acid (C20:0)	0.55 ± 0.03	0.82 ± 0.06
Erucic acid (C22:1)	0.10 ± 0.04	0.37 ± 0.04

2.2. Yield of Fatty Acid and Glycerol Content

High fatty acids yields of 98.83% (RPO) and 90.94% (CPO) were achieved after the extraction process modified from López et al. [26]. Furthermore, high glycerol content from CPO (86.25%) and RPO (87.50%) was also obtained without an additional purification step (See Table 2). It should be noted that the glycerol content obtained in this work was higher than in previous studies in which the reaction was stopped by adding organic solvent [19] or acid pretreatment [35]. Furthermore, the glycerol content obtained from the saponification reaction of CPO/RPO was also lower than in some previous works, who reported various methods such as vacuum distillation [36], sludge-derived activated carbon [37], including a sequential extraction of organic solvents together with discoloration by activated coal [38] and moreover, serial purification steps; physico-chemical treatment, membrane filtration, and activated charcoal adsorption [24], physicochemical and membrane pretreatments [39], ion exchange resins [40]. Other methods used were neutralization, vacuum distillation, adsorption onto activated carbon and adsorption onto a metal filter [41], and a hydro-esterification process [42].

Table 2. Comparative glycerol content obtained from different processing routes.

Source of Glycerol	Purification Processes	Glycerol Content (%)	References
Saponification of crude palm oil (CPO)	-	86.25	This work
Saponification of refined palm oil (RPO)	-	87.50	This work
Transesterification of rapeseed oil	Stopping and separation by addition of inorganic acid	75	[19]
Commercial crude glycerol	Serial purification steps; physicochemical treatment, membrane filtration and activated charcoal adsorption	97.5	[24]
Transesterification of sunflower oil	Acid pretreatment	75	[35]
Transesterification of palm kernel oil	Vacuum distillation	96.6	[36]
Transesterification of waste used-oil	sludge-derived activated carbon	93	[37]
Transesterification of waste cooking oil	sequential extraction with organic solvents and discoloration with activated coal	99.2	[38]
Commercial crude glycerol	Physicochemical and membrane pretreatments	93.7	[39]
Transesterification of frying oil	Ion-exchange resins	95	[40]
Transesterification of waste cooking oil	Serial purification steps; Neutralization, vacuum distillation, adsorption onto activated carbon, adsorption onto metal filter	78.72	[41]
Hydro-esterification of soybean oil	-	100.00	[42]

Remark: - No purification required.

2.3. Effect of Temperature on Esterification Reaction

Both CPO and RPO biodiesel yields illustrate a significant increase from 30 °C to 40 °C (See Figure 1). A higher reaction temperature could shift the equilibrium to the right-hand side, leading to higher conversion rate because the fast mobility of the reactants led to a greater number of collisions between the reactants than occurs at lower reaction temperatures. This is in agreement with a previous study [43]. On the other hand, biodiesel yields were reduced as reaction temperatures became higher than 40 °C, due to enzyme denaturation at higher temperatures. Similar results were explained by Jambulingam et al. 2019 who studied the effect of temperature on the esterase activity of immobilized lipase and observed that the maximum esterase activity occurred at a temperature of 45 °C. In this study the highest biodiesel yields from CPO (79.67%) and RPO (74.23%) and the lowest FFA content from CPO (2.21 mg KOH/g oil) and RPO (0.20 mg KOH/g oil) were obtained at 40 °C. Therefore, the temperature at 40 °C was further used in subsequent experiments. Similar results were also reported by Tan et al. [44]. Esterification of FFAs in hydrolyzed palm oil using Candida sp. 99–125 lipase as biocatalyst (15 wt%. enzyme loading) led to the highest biodiesel yield (95%) at 40 °C at a total reaction time of 30 h. Previously, biodiesel production from FFAs of CPO via the esterification reaction in the presence of isooctane catalyzed by a commercial enzyme, Novozyme 435, was reported by Talukder et al. [45], with the maximum biodiesel yield (98%) obtained at 40 °C.

2.4. Effect of MEOH to FFA Ratio on Esterification Reaction

The MEOH to FFA ratio was investigated. It was found that biodiesel yields of CPO/RPO continually increased between ratios of 1:1 and 4:1 as shown in Figure 2. Based on stoichiometry, the reaction required one mole of both FFAs and methanol generated FAMEs. As esterification is a reversible reaction, a higher MEOH to FFAs ratio more than 1:1 could drive esterification forward. However, a reduction of biodiesel yield was observed when the ratio used exceeded 4:1. The maximum biodiesel yields of FFAs from CPO (87.36%) and RPO (89.84%) together with the minimum FFAs content of CPO (1.39 mg KOH/g oil) and RPO (0.09 mg KOH/g oil) were reached at a ratio of 4:1. Thus, the ratio of methanol to fatty acid of 4:1 was deemed to be the best ratio for biodiesel production from CPO/RPO. Likewise, in earlier literature [44] which studied biodiesel production from FFAs in hydrolyzed palm oil via esterification catalyzed by Candida sp.99-125 lipase

(15 wt%. enzyme loading). They also found that the highest biodiesel yield (90%) was also obtained when the ratio of MEOH to FFA ratio was at 3:1 with methanol being added twice at different stages of the biodiesel production process. Furthermore, Kareem et al. [46] also reported that the optimal MEOH to FFA ratio for enzymatic biodiesel production from palm oil and palm kernel oil using 5 wt%. enzyme loading was 3:1. Maximum biodiesel yields from palm oil (95%) and palm kernel oil (82.5%) were observed at this molar ratio.

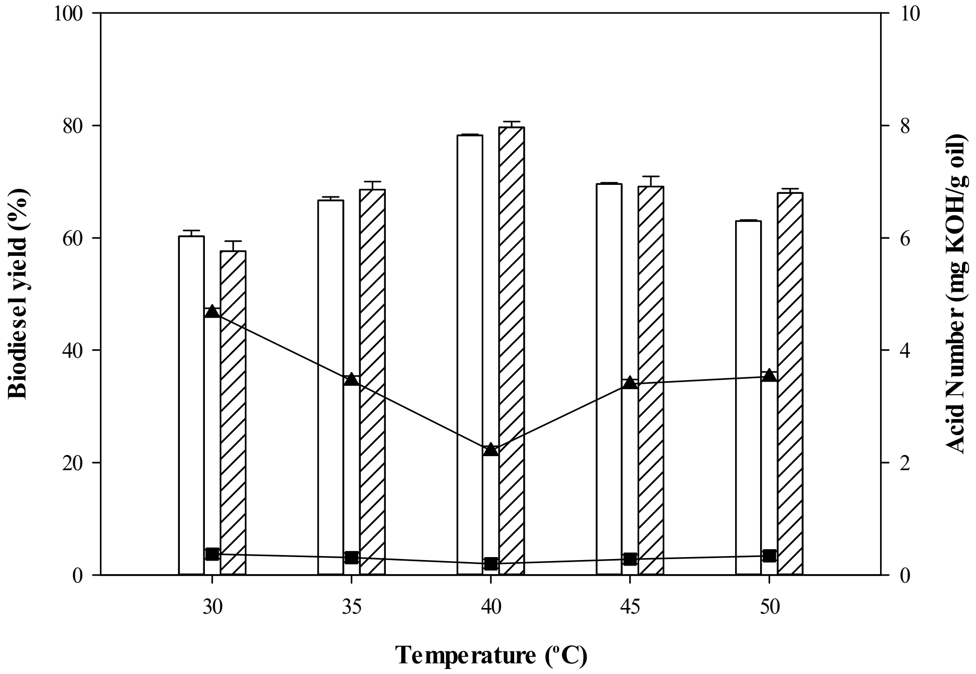

Figure 1. Biodiesel yield (%) as function of temperature (°C) and acid number (mg KOH/g oil) (CPO biodiesel yield RPO biodiesel yield ▲ CPO acid number ■ RPO acid number.

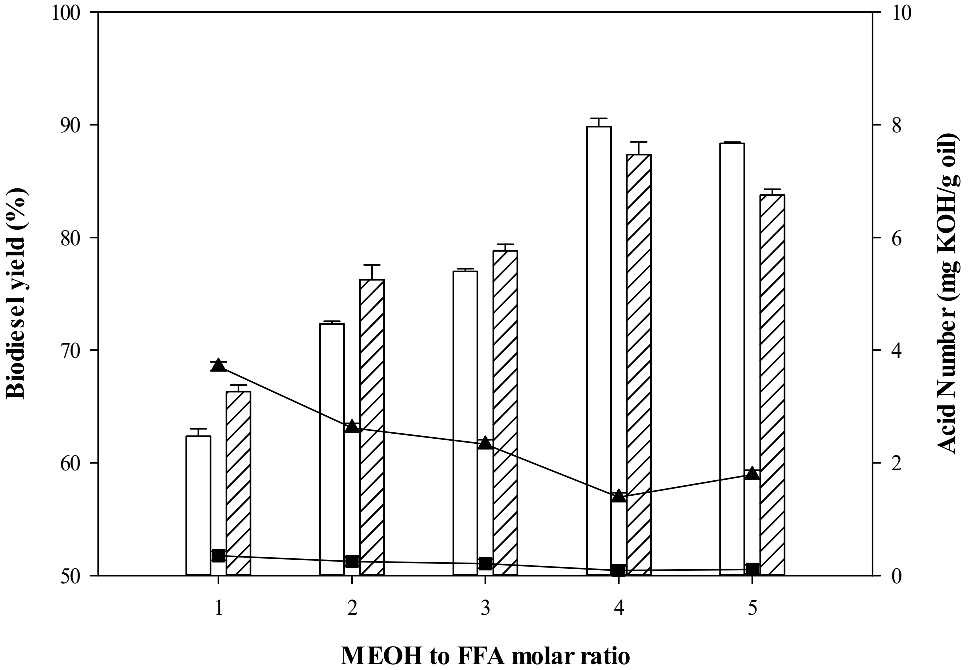

Figure 2. Biodiesel yield (%) as function of MeOH to FFA molar ratio and acid number (mg KOH/g oil) (CPO biodiesel yield RPO biodiesel yield ▲ CPO acid number ■ RPO acid number).

2.5. Effect of Agitation Speed on Esterification Reaction

Third, the influence of agitation speed on biodiesel production was investigated. Both CPO and RPO biodiesel yields presented an upward trend as agitation speed increased from 100 rpm to 250 rpm (See Figure 3). Seemingly, a high agitation speed can promote high efficiency mass transfer of the liquid phases (CPO/RPO and methanol) and also maintain homogeneity leading to effective conversion of reactants in high biodiesel yields [47]. The biodiesel yield was remarkably reduced at agitation speed of 300 rpm, perhaps due to insufficient interaction time between the reactants [43]. The highest biodiesel yields of CPO (92.14%) and RPO (95.28%) and the lowest FFAs content of CPO (0.87 mg KOH/g oil)/RPO (0.25 mg KOH/g oil) were achieved at 250 rpm. Therefore, an agitation speed of 250 rpm was chosen as a suitable agitation speed for esterification of FFAs from CPO/RPO. Similarly, the influence of agitation speed on enzymatic esterification of FFAs in hydrolyzed CPO in the presence of isooctane was previously investigated by Talukder et al. [45]. Their results also revealed that an agitation speed of 250 rpm was the best condition for biodiesel production.

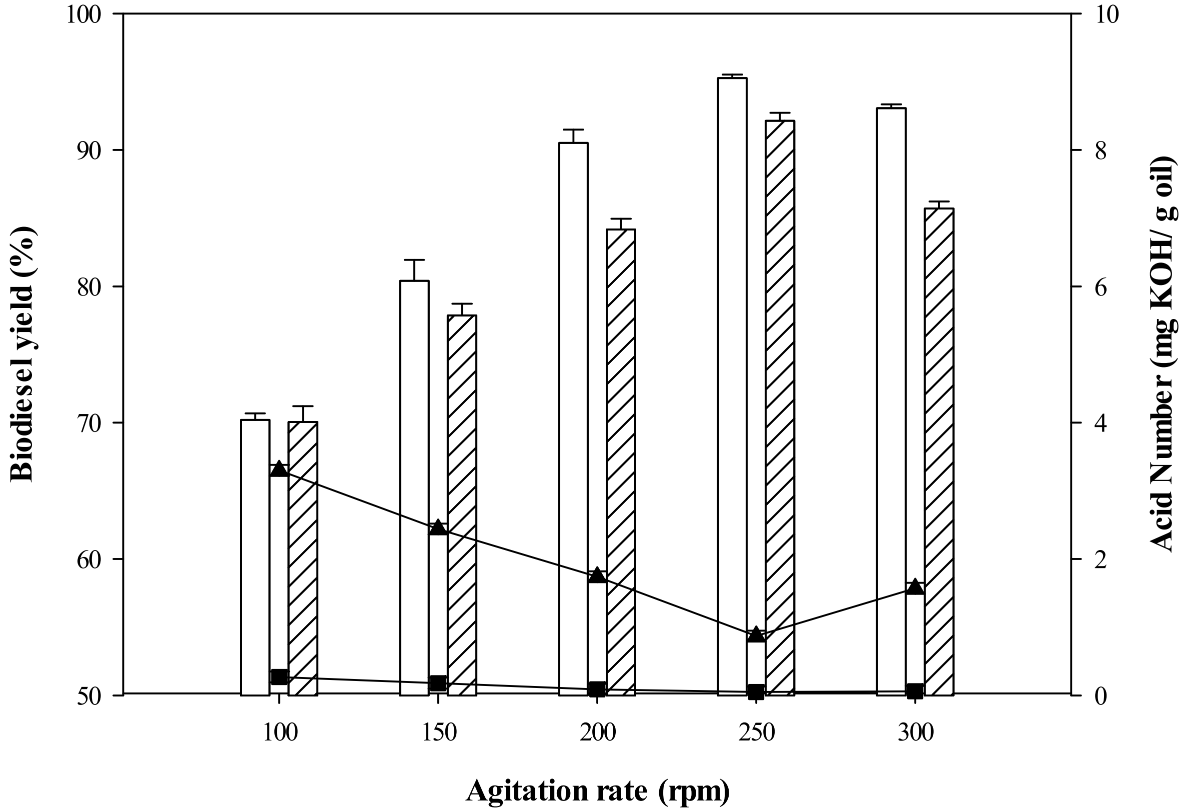

Figure 3. Biodiesel yield (%) as function of agitation rate (rpm) and acid number (mg KOH/g oil) (CPO biodiesel yield RPO biodiesel yield ▲ CPO acid number ■ RPO acid number).

2.6. Reusability and Stability of Immobilized Lipase

The reusability and stability of immobilized alginate-PVA lipase beads in esterification of FFAs obtained from CPO/RPO were carried out under the optimal conditions (40 °C, 4:1 MEOH to FFA ratio and 250 rpm agitation speed for 20 cycles. Remarkably, the immobilized lipase beads gave high biodiesel yields of CPO (92.14%) and RPO (95.28%). It should be noted that the biocatalyst provided high catalytic efficiency as well as high reusability for CPO (16 cycles) and RPO (17 cycles) as shown in Figure 4. Lower catalytic efficiency of biocatalyst was observed after eighteen cycles onwards because lipase lost its stability in methanol. Furthermore, enzyme leakage from the immobilized beads could occur with a high cycle number [48]. Both the CPO and RPO biodiesel yields obtained were in agreement with other works [49–53]. However, considered in terms of reusability and stability, immobilized alginate-PVA lipase *R. oryzae* illustrated higher stability than other biocatalysts when comparing to previous works (See Table 3). Furthermore, the reusability of biocatalyst obtained agreed with other previous works. The immobilized alginate-PVA lipase bead could be reused for over 10–15 cycles with high catalytic efficiency for biodiesel production [14,32]. Therefore, it can be stated that an alternative cleaner process for biodiesel production from CPO/RPO could also enhance catalytic efficiency and stability of immobilized lipase.

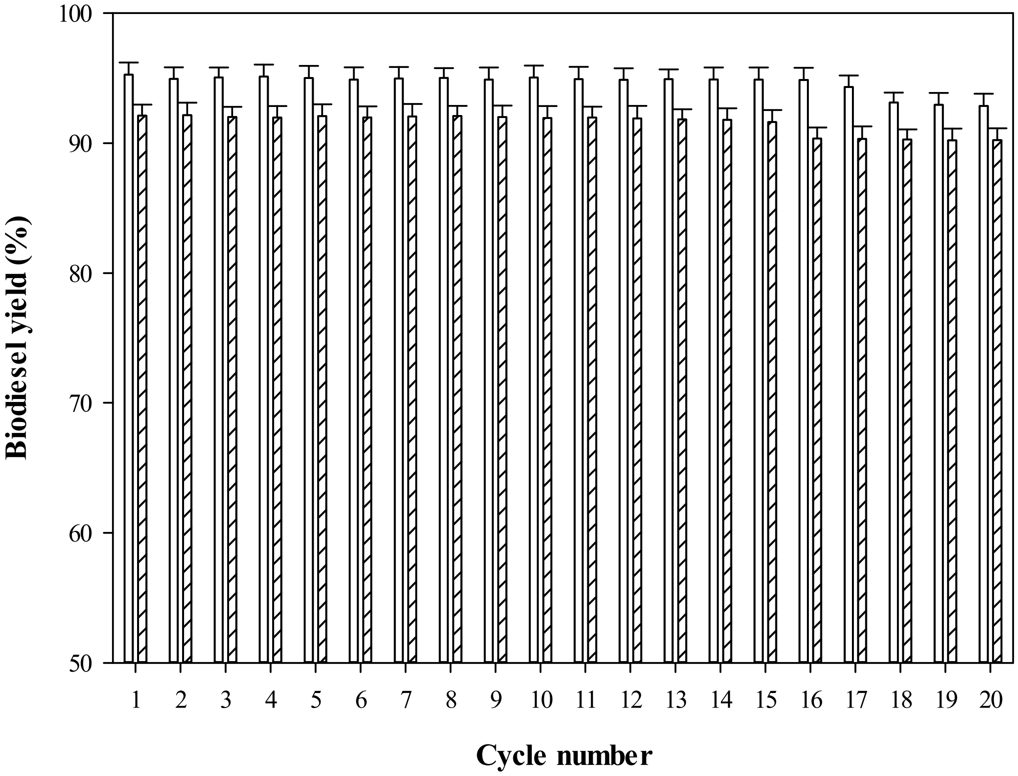

Figure 4. Biodiesel yield (%) as a function of cycle number for testing reusability and stability of immobilized lipase during esterification reaction (CPO biodiesel yield RPO biodiesel yield).

Table 3. Comparative biodiesel yield obtained from CPO and RPO via different processes.

Raw Material	Processes	Biodiesel Yield (%)	Reusability (Cycles)	Reference
CPO	Fatty acid extraction/esterification catalyzed by immobilized *R. oryzae* lipase	92.14	16	This work
RPO	Fatty acid extraction/esterification catalyzed by immobilized *R. oryzae* lipase	95.28	17	This work
CPO	Esterification catalyzed by Perchloric acid	88	-	[49]
CPO	Esterification catalyzed by choline chloride based deep eutectic solvent/alkaline trans-esterification	92	3	[50]
RPO	Trans- esterification catalyzed by immobilized *Thermomyces lanuginosus* lipase on Fe_3O_4	97	5	[51]
RPO	Trans- esterification catalyzed by sulfonated graphene catalyst	98	4	[52]
RPO	Trans-esterification catalyzed by fermented solid containing *Burkholderia contaminans* lipase	90	5	[53]

2.7. Composition of Biodiesel

The composition of CPO/RPO biodiesel was analyzed by the GC-MS method. For CPO, the biodiesel obtained mainly consisted of methyl esters of oleic acid (45.02%), palmitoleic acid (24.54%), stearic acid (10.49%), and palmitic acid (9.56%), respectively. Meanwhile, RPO biodiesel was found to contain mostly oleic and palmitoleic acid methyl ester at 76.27% and 12.50% (See Figure 5). The proportion of monounsaturated fatty acid was higher than that of saturated fatty acid found in both CPO/RPO biodiesel, for example palmitoleic acid (C16:1) and oleic acid (C18:1). This improved the biodiesel quality in terms of low viscosity, good oxidative stability, and good cold flow properties [2]. In addition, the reduction of solidification problems at low temperature could also be addressed [54]. However, the saturated fatty acid or palmitic acid (C16:0) and stearic acid (C18:0) assisted in obtaining a high cetane number and led to the formation of less nitrogen oxides (NOx) [54–56].

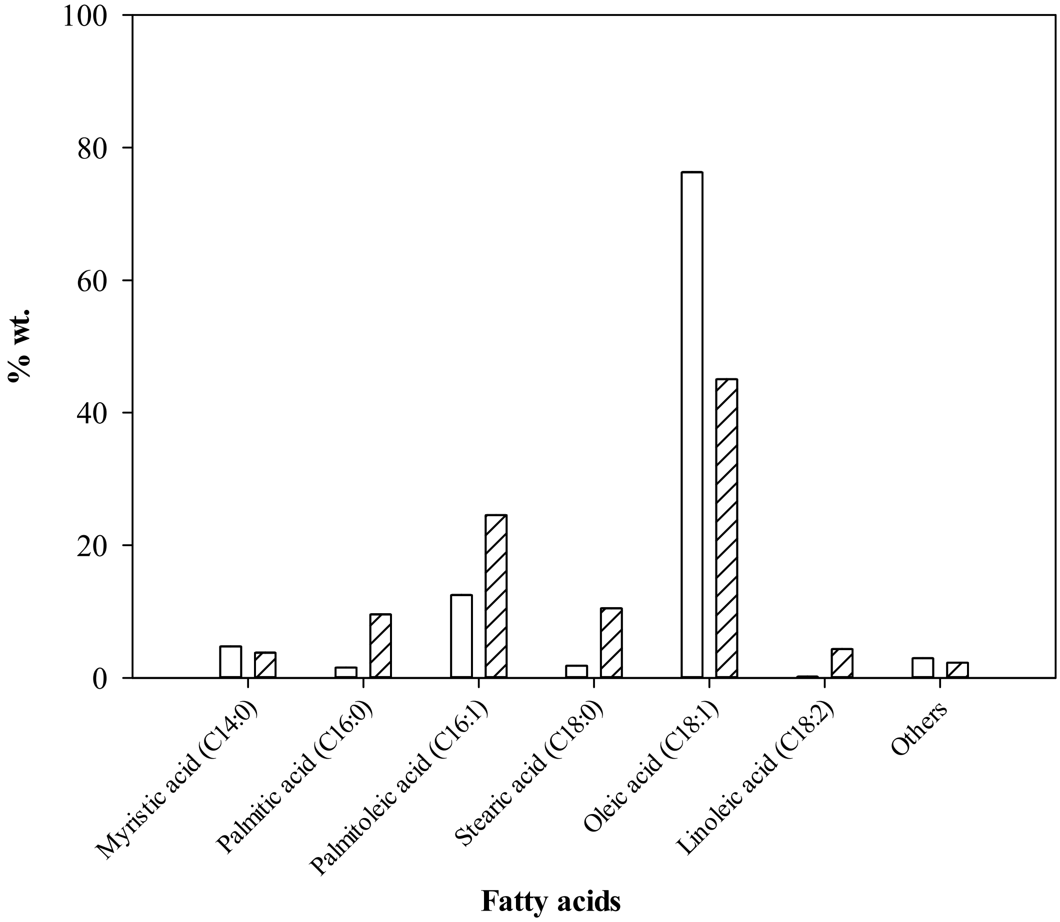

Figure 5. Comparison of fatty acid methyl esters (FAME) found in CPO/RPO biodiesel as a function of weight (%) (CPO FAME, RPO FAME).

3. Materials and Methods

3.1. Feedstocks and Chemicals

Different grades of palm oil; crude palm oil (CPO) containing acid value of about 11.02 and saponification value (211.25) together with refined palm oil (RPO) were used throughout this study. The CPO sample was kindly provided by Esarn Palm Oil (ESP) Co., Ltd. Sakon Nakorn province, North-East Thailand. Prior to use, samples of CPO were pretreated by heating at 80 °C and filtering with a cotton sheet. Meanwhile, samples of RPO with acid value 0.92 and saponification value 217.06 were collected from a local supermarket near KhonKaen University, KhonKaen, Thailand. All chemical substances used such as calcium chloride ($CaCl_2$), potassium hydroxide (KOH), methanol, hydrochloric acid (HCl), and polyvinyl alcohol were analytical grade. The solid form of alginate was food grade. Only an enzyme lipase powder of *Rhizopus oryzae* was purchased from Sigma Co., Ltd., Bangkok, central area of Thailand.

3.2. Separation of Free Fatty Acid and Glycerol

To obtain FFAs, 100 mL of CPO or RPO was separately added to a 250 mL-Erlenmeyer flask and then hexane was added to carry out the extraction. The method used was modified from a previous work [26]. The flow diagram of the process is shown in Figure 6. First, CPO and RPO were saponified by 5 M potassium hydroxide (KOH) solution. The reaction mixture consisted of 100 mL of either CPO or RPO and 5 M KOH. The saponification reaction was performed at 60 °C, 250 rpm, for about 30 min. After that, it was left at room temperature. The by-product of glycerol was separated from the reaction medium, with the glycerol bottom layer settling out from the top soap layer. Subsequently, water (30% v/v) was added and 37% HCl was used to adjust the pH down to 1. Second, FFAs were extracted with 45 mL hexane with shaking at 250 rpm for 30 min. Then, both the hydro-alcoholic and hexanic phases were separated using a separating funnel. Finally, hexane was eliminated by evaporation and the FFA solution was washed in triplicate before being used as a substrate in further experiments. The FFA solution was analyzed as described in Section 3.3. The glycerol content was determined using the iodometric titration method [57].

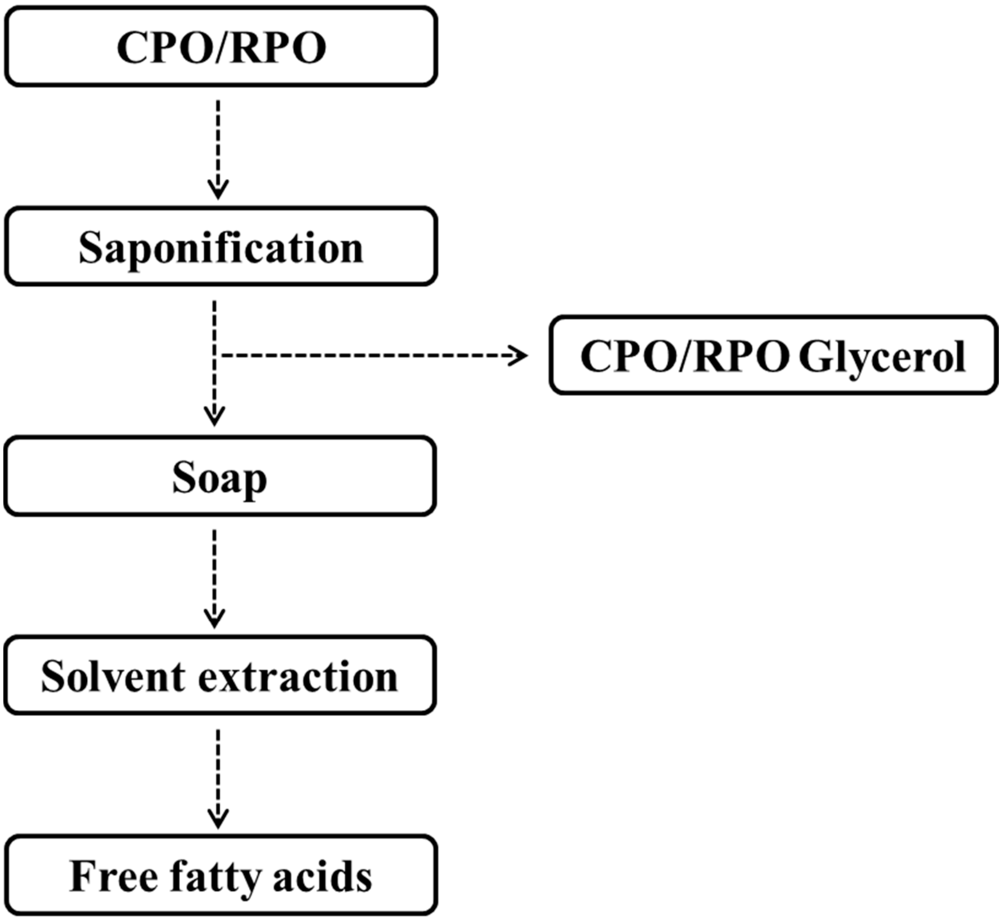

Figure 6. Flow diagram of free fatty acids (FFAs) extracted from crude palm oil (CPO) and refined palm oil (RPO).

3.3. Determination of Recovery FFAs

The FFA content was determined in terms of acid number. First, 5 g of oil sample was dissolved in 50 mL of alcohol–ether solution. Then, it was titrated with KOH (0.1 M) using phenolphthalein as an indicator. The end point was indicated by a stable pink color appearance. Acid number (mg KOH/g oil) was calculated as shown in Equation (1). The amount of FFAs obtained from CPO/RPO was determined as increasing acid number. The percentage of recovery FFAs was calculated using Equation (2).

$$\text{Acid number (mg KOH/g oil)} = \frac{\text{V} \times \text{N} \times 56.1}{\text{W}} \tag{1}$$

where V = volume of KOH consumed (ml); N = normality of KOH; W = sample weight (g)

$$\text{Recovery FFAs (\%)} = \frac{\text{AN2} - \text{AN1}}{\text{AN2}} \times 100 \tag{2}$$

where AN1 = acid number of oil sample at initial time; AN2 = acid number of oil sample after extraction process

3.4. Preparation of Immobilized Alginate and PVA Lipase

The immobilized lipase beads were prepared by mixing 5% (*w*/*v*) lipase solution in pH 7.0 phosphate buffer (10 mL) and 90 mL of matrix solution of polyvinyl alcohol (PVA) and alginate (ratio of 1:1). Then, it was dropped into 0.1M $CaCl_2$ solution onto a magnetic stirrer. The immobilized beads were incubated in $CaCl_2$ at 4 °C for 24 h and the obtained beads were washed in fresh water for 30 min. Finally, the biocatalyst of immobilized alginate-PVA beads was then kept at 4 °C for biodiesel production.

3.5. Biodiesel Production

Biodiesel production via esterification reaction of FFAs from CPO/RPO was carried out in a 250 mL-Erlenmeyer flask with a working volume of 50 mL of FFA solution mixed with methanol together with 2% *w*/*v* biocatalyst. After incubation, the immobilized beads were separated from reaction mixture by filtration to stop the reaction. The final reaction mixture was further analyzed for biodiesel yield as described in Section 3.3. Experiments were carried out to optimize variables; temperatures (30–50 °C), ratios of MEOH to FFAs (1:1–5:1), and 100–300 rpm agitation rate. All sample reactions were carried out in triplicate to give an understanding of reproducibility and results obtained are all shown as a mean value ± standard deviation. The acid numbers in oil samples were analyzed using the standard method of biodiesel (ASTM D 6751). The percentage of biodiesel yield was determined as a reduction of acid number in oil samples at the initial time and after the reaction time as shown in Equation (3). Biodiesel or fatty acid methyl esters (FAMEs) were analyzed in both CPO/RPO biodiesel in accordance with European Standard method (EN 14103:2003) by Gas Chromatography–Mass Spectroscopy (GC-MS) as described in our previous studies [58].

$$\text{Biodiesel yield (\%)} = \frac{\text{AN1} - \text{AN2}}{\text{AN1}} \times 100 \tag{3}$$

where AN1 = acid number of oil sample at initial time; AN2 = acid number of oil sample after the reaction

4. Conclusions

Chemical fatty acid extraction was successful in chemical fatty acid extraction coupling with an enzymatic esterification to clearly improve biodiesel production from CPO and RPO. Under the optimal conditions, high biodiesel yields of 92.14% (CPO) and 95.28% (RPO) were achieved. Furthermore, the immobilized biocatalyst used, possesses high reusability and stability up to 16–17 cycles. Moreover, high glycerol content was also obtained without any additional purification processes. This new route for cleaner biodiesel production presents an alternative approach for biodiesel production from both refined and unrefined vegetable oils with several advantages; low cost production, low water demand, and together with a simple downstream process.

Author Contributions: This article was performed as collaborative research between P.K. and J.W. as well as P.M and P.W. who are assistant researchers. For P.K.; the work included: conceptualization, methodology, resources, formal analysis, data curation, visualization, and writing—original draft preparation, P.M., P.W. planned and performed the experiments and prepared the software. Meanwhile, J.W., carried out the validation and investigation. In addition, P.K. performed the main supervision, project administration, funding acquisition, review, and corrections. All authors have read and agreed to the published version of the manuscript.

Funding: This research was co-funded by Royal Golden Jubilee (RGJ) PhD Programme (Contract no. PHD/0018/2557) and Newton Fund Institutional Links (IL) 2019/2020.

Acknowledgments: All authors would like to sincerely and gratefully acknowledge all sponsors: The Royal Golden Jubilee (RGJ) Ph.D. Programme (Contract no. PHD/0018/2557), Bangkok, Thailand and Newton Fund for Institutional Links (IL 2019/2020), the British Council, London, UK for for mainly funding research support, collaborative research, travel bursa-ry and PhD researcher exchange. In addition, the Centre for Alternative Energy Research and Development (AERD), Faculty of Engineering, Khon Kaen University, Khon Kaen, Thailand and are also thanked for the matching fund and travel bursary.

Conflicts of Interest: The authors declare no conflict of interest.

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