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Lipase Catalysis for Transesterification Produces Biodiesel Using Coconut Oil as Main Raw Material Source

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Abstract: Transesterification is a chemical method that has been studied to convert fat and oil into biodiesel. Inorganic catalysts often have high catalysis activity and reusability, so they have been predominant for biodiesel production at the industrial scale; however, they often cause severe pollution. Recently, bio-catalytic transesterification has received considerable attention due to its favorable conversion rate and relatively simple processing performance for the production and purification of biodiesel. In this study, two lipases from *Candida rugosa* and *Porcine pancreas* was used to catalyze the transesterification of coconut oil in order to produce biodiesel. The results have shown that the catalysis ability of the lipase from *Candida rugosa* in the transesterification of coconut oil is better than that of *Porcine pancreas*. The use of the lipases from *Candida rugosa* and *Porcine pancreas* reached the oil conversion level of 62.55% and 59.72%, respectively. The better conditions of the enzymes and the reaction were also studied.

Keywords: Transesterification, lipase, Candida rugosa, Porcine pancreas, biodiesel, coconut oil.

1. Introduction

Biodiesel has gained widespread importance in recent years as an alternative, renewable liquid fuel resource and less environmental pollution. Especially, it is used as alternative energy sources for diesel engine. Biodiesel is simple alkyl esters of fatty acid. Those fatty acids are obtained from transformation of triglycerides of vegetable oil or waste oil. The characteristics of vegetable oil or waste oil including high viscosity, low volatility, polyunsaturated chain, free fatty acid are the problems to directly use as diesel [10]. To overcome these problems, three processes are used including pyrolysis, micro-emulsification and transesterification. In transesterification, displacement of alcohol from an ester by another alcohol in the process occurred [10]. The transesterification of oil occurs under extreme high temperature condition and in a long period of time. Many inorganic catalysts are used to catalyze the

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reaction such as alkaline, acid, inorganic heterogeneous, enzymatic catalysts. The use of alkaline or acid catalysts in homogenous catalytic system cause difficulties in process such as removing of catalysts, collection of biodiesel, soap formation [4, 6, 7, 10], etc. The enzymatic catalyst is concerned as the promising solution to overcome those problems [6, 8, 10]. In overall, lipases catalyze the transesterification of triglyceride in gentle condition. They work on several oil sources, simultaneously transform both free fatty acids and triglyceride, and can be easily separated from biodiesel [6, 8, 10]. Many sources of triglyceride were studied to produce biodiesel such as: palm oil, waste oil, sunflower oil, soybean oil, cotton oil, etc. The coconut oil is at the initial stage of research to converse to biodiesel. In this study, the probability to produce biodiesel from Vietnamese coconut oil was tested. The process factors affect the production of diesel from Vietnamese coconut oil were also studied.

2. Materials and Methods

2.1 Materials

Two lipase powder from *Candida rugosa* Type VII - L1754 (LCR) and *Porcine pancreas* Type II - L3126 (LPP) were purchased from Sigma-Aldrich. Olive oil (acid value of 2.45 mgKOH/g, saturated fat 15%, free fatty acid of 1.23%) were supplied by Calofic Vietnam. Coconut oil (acid value of 2.45 mgKOH/g oil; moisture content of 0.47%, 2.1% free fatty acid, iodine value of 5.9 mg KOH/g) were bought from Tinvui Company (Vietnam). Ethanol and other chemicals and solvent were at analytical grade from Sigma-Aldrich.

2.2 Conditions of Enzymatic Transesterification

Screening experiments of the enzymatic transesterification for coconut oil were performed with 8g coconut oil. Each lipase was used to catalyze the reaction at 40°C in 4 hours. The ratio of ethanol to oil was 4:1. The amount of enzyme was 0.2g LPP and 0.12g LCR. The buffer and the stirring speed used in cases of LPP and LCR were 1g of borate buffer pH 8.5 (300rpm) and 1 g of phosphate buffer pH 7.0 (375rpm) respectively. Thin layer chromatography was used to test the biodiesel produced. Mobile phase for thin layer chromatography was hexane:ethylacetate:acid acetic = 6:3:1.

The transesterification reaction was carried out in a 50 mL erlen putted on a magnetic stirrer. 8 g of coconut oil were placed into the erlen, then 0.12 g lipase power was added to catalyse the transesterification reaction at 40°C. The reaction mixture was stirred at a speed of z (rpm), at temperature T (°C), in a duration of t' (h) and the ethanol was slowly added at a speed of 1 drop/sec. The crude biodiesel was the clear layer of reaction mixture and was collected by centrifuging the reaction mixture at 3500 rpm. The initial conditions of transesterrification reaction were 200 rmp (LCR) and 250 rpm (LPP) at the ratio alcohol to oil of 6:1.

2.3 Analytical Methods

The component of Vietnamese coconut oil and the product of transesterification reaction were analyzed by gas chromatography with FID detector to qualify the ethyl esters produced. The temperature in the oven was at 210°C, temperature at detector was 250°C.

The ester conversion was calculated in percentage of product weight after centrifugation. The product was analyzed by proton nuclear magnetic resonance spectroscopy (^{1}HNMR) to determine the ethyl ester of biodiesel. The experiments were carried out three times.

3. Results and Discussion

3.1 Effect of the Hydrolysis of Coconut Oil by LCR and LPP

Olive oil is standard substance [11], coconut oil is the main substance for this study with IA = 5.9 mg KOH/g ≈ 2.1% FFA, phosphate buffer pH 7 (LCR), borate buffer pH 9.0 (LPP) and temperature 40°C. The result showed that activity of LCR is better than that of LPP and these activities of LCR and LPP reduce gradually as shown in Figure 1.

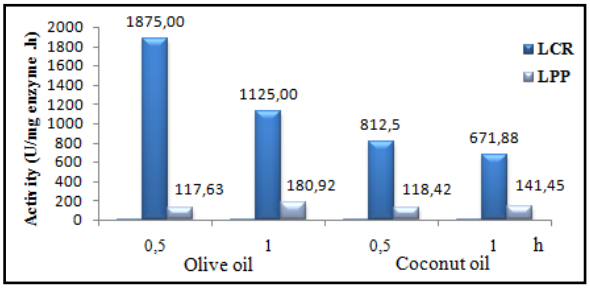
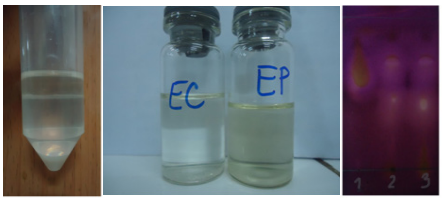


Fig. 1 Effect of the hydrolysis of coconut oil by LCR and LPP

Activity of LCR is better than that of LPP when it is compared by hydrolysis. However, activity for transesterification reaction is different. Throughout the investigation, a series of experiments of every parameter are set up and the better conditions parameters are determined.

3.2 Possibility of biodiesel produced from coconut oil

Screening experiments of the enzymatic transesterification of coconut oil were performed. Figure 1 illustrated the probability of biodiesel production from coconut oil when using lipase as catalyst. The thin layer chromatography showed evident that the coconut oil was transesterified into biodiesel.



(a) (b) (c)

(a) Reaction mixture after centrifugation (3500 rpm, 15 min). The upper layer was biodiesel, lower was enzyme.

(b) Crude biodiesel produced using LPP (EP) and LCR (EC) as catalysts.

(c) Preliminary test:

1: Coconut oil, 2: biodesel produced using LPP as catalyst (lipase from *Porcinepancrease*), 3: biodesel produced using LCR as catalyst (lipase from *Candida rugosa*)

Fig. 2Production of biodiesel from coconut oil.

3.3 Effect of the Ratio of Ethanol to Oil on the Ester Conversion

Under the setting conditions of reaction, mentioned in the 3.2, the ratio of ethanol to oil was studied to achieve the highest ester conversion (%). The output data were shown in Table 1.

Table 1. Effect of the ratio of ethanol 99.7% to coconut oil on the ester conversion

EtOH: oil	$m_{EtOH\ 99.7°}(g)/m_{oil}(8g)$	%CH (LPP)	%CH (LCR)
3:1	1.576	13.19	8.17
4:1	2.101	21.68	15.31
5:1	2.626	24.35	20.45
6:1	3.151	39.62	26.94
7:1	3.676	42.80	32.67
8:1	4.201	44.72	35.73
9:1	4.727	49.46	45.06
10:1	5.252	58.00	51.76
11:1	5.777	62.55	58.07
12:1	6.302	75.85	66.28

**% CH(LPP) and % CH(LCR): the ester conversion of coconut oil under the catalysis of lipase from Porcine pancreas and Candida rugosa.*

The increase of the ratio of ethanol 99.7% to oil resulted in the increase the ester conversion. However, at the ratio from 7:1 to 12:1, the ester of biodiesel was the analysis of product by ^{1}HMNR. Therefore, the ratio of 6:1 was give the highest ester conversion and produced the biodiesel.

3.4 Effect of Temperature on the Ester Conversion

The increase in reaction temperature caused the increase the reaction rate. However, the high temperature in the enzymatic reaction caused denaturation of enzyme, thus the decrease in reaction rate occurred. The optimum temperature of the transesterification was determined by the highest ester conversion, at which the highest reaction rate occurred. In this study, the transeterification reaction was study in temperature range of 30°C to 50°C. The highest ester conversion reached at 35°C for both lipases (Table 2).

Table 2. Effect of temperature on the ester conversion

t (°C)	30	35	40	45	50
% CH(LPP)	34.87	36.23	29.89	29.89	26.54
% CH(LCR)	28.52	31.17	30.57	25.54	23.65

**% CH(LPP) and % CH(LCR): ester conversion of coconut oil under the catalysis of lipase from Porcine pancreas and Candida rugosa.*

The ester conversion increased when the temperature was increasing from 30°C to 35°C. The higher temperature caused the decrease of ester conversion in both case of lipases. This might due to the unfolding of enzyme structures which affected the active site of enzymes. The output data also showed that the lipase from *Porcine pancreas* had higher activity than the lipase from *Candida rugosa*.

3.5 Effect of pH on the Ester Conversion

Each lipase was catalytically active at a certain range of pH. The activities of enzyme were variable depended on pH. The ionic strength of buffer might cause the change in structure of enzyme and thus leaded to irreversible denaturation of enzyme. Overall, in this study, *Candida rugosa* lipase gave better ester conversion. The lipase from *Cadidarugosa*catalysed the transesterification of coconut oil at the alkaline conditions while the lipase from Porcine pancrease worked at neutral pH 7.0 (Table 3).

Table 3. Effect of pH on ester conversion

pH	% CH(LPP)	pH	% CH(LCR)
7.5	36.30	6.5	26.40
8.0	37.15	7.0	31.43
8.5	37.23	7.5	29.68
9.0	38.27	8.0	29.45

At the pH differ from optimum pH, symmetry distortions of the prosthetic group occurred. The alkaline or acid environment also caused the change of electrostatic charges on the enzyme's active site or the ionization of dissociable substrate.

3.6 Effect of Ethanol Concentration on the Ester Conversion

Ethanol caused the precipitation of enzyme. However when enzymes combined with substrates to form enzyme – substrate complex at the initial period before adding ethanol, the precipitation of enzyme in the complex was not occurred. An appropriate amount of ethanol induced the combination of the enzyme-substrate complex with ethanol in the second part of reaction, and then released the enzyme. In this study, the ethanol solution was tested to reach the highest ester conversion.

Table 4. Effect of the amount of ethanol on the ester conversion

EtOH(°)	% CH(LPP)	% CH(LCR)
99.7	33.96	27.15
98.0	38.77	31.53
96.0	35.25	27.58
94.0	35.81	25.85
92.0	36.01	24.22
90.0	33.96	23.43

Solution of 98% EtOH was suitable for transesterification reaction under the catalysis of lipase from *Porcinepancrease* and *Candida Rugosa* (Table 4) The ester conversion reached the highest level.

3.7 Effect of Stirring Speed on the Ester Conversion

The stirring enhanced the contact and combination of enzymes and coconut oil resulted in the effectively transesterification. However the high stirring speed caused the reduction of catalytic activity of enzyme. The stirring speed for tranesterification reaction in the presence of LPP and LCR was carried out in range of 150 rpm to 300 rpm. The ester conversion was highest at the speed of 200rpm for LPP (39.11%) and at 250 rpm for LCR case (33.17%) (Table 5).

Table 5. Effect of stirring speed on the ester conversion

Stirring speed (rpm)	% CH(LPP)	% CH(LCR)
150	33.03	28.37
200	39.11	28.26
250	33.52	33.17
300	34.36	25.34
400	31.48	25.81

3.8 Effect of Buffer on the Ester Conversion

The solvent hydrophilicity affected the enzyme flexibility and thus influenced the reaction rate. The appropriate amount of buffer ensured a necessary water layer on the enzyme surface to catalyze the reaction [2]. The buffer ensured the pH and the ionic strength of the reaction mixture. In addition, the lipase activity depended on the interfacial area [1, 2, 5]. The amount of buffer added in the reaction mixture was studied. The transesterification of coconut oil in the presence of LPP gave the highest ester conversion when adding 2.0g buffer. In case of LCR, the appropriate amount of buffer was 2.5g.

Table 6. Effect of amount of buffer on the ester conversion

m (g)	0.5	1.0	1.5	2.0	2.5	3.0
%CH(LPP)	29.12	37.27	42.18	48.73	0.00	0.00
%CH(LCR)	31.23	34.78	38.93	47.12	50.18	0.00

3.9 Effect of Coconut oil Content on the Ester Conversion

According to Michaelis and Menten, the affinity of enzyme to substrate raises with the increase of enzyme concentration and thus the reaction rate. When increase the substrate concentration, the reaction rate increases until reach the maximum value and no longer depends on the substrate concentration. In the presence of Porcine pancreas lipase, the amount of coconut oi of 8,5g was gave the highest ester conversion. The optimum amount of coconut oil in case of *Candida rugosa* lipase was 8,0g.

Table 7. Effect of coconut oil content on the ester conversion

m (g)	%CH(LPP)	%CH(LCR)
7.0	51.42	52.60
7.5	52.12	52.96
8.0	56.69	56.83
8.5	58.62	56.26
9.0	56.75	56.78

Therefore, the amount of coconut oil was chosen to set reaction condition for the next experiments.

3.10 Effect of Enzyme Loading on the Ester Conversion

The important point of enzyme catalysis is un-happened reverse reaction compared to chemical catalysis. In case of an excess of substrate was added in the reaction mixture, the reaction rate depended on enzyme load. In this study, the experiments on enzyme load were carried out to determine the enzyme amount needed to reach the maximum ester conversion. The enzyme load was studied in range of 0.10 g to 0.3g.

Table 8. Effect of enzyme loading on the ester conversion

m (g)	0.10	0.12	0.15	0.20	0.25	0.30
%CH(LPP)	41.09	-	46.50	59.64	59.42	58.50
%CH(LCR)	44.56	56.01	57.69	56.83	56.26	56.78

The lipase from *Candida rugosa* showed the effective catalysis at 0.15g of enzyme.The lipase from *Porcine pancrea*s catalysed the transesterification of coconut oil at higher amount of enzyme to reach equal ester conversion.

3.11 Effect of Reaction Time on the Ester Conversion

The duration of the transesterification reaction may affect the ester conversion of substrate and the stability and activity of enzyme [3]. The reaction time of transesterification reaction may be extended in hours for inorganic and enzymatic calatysts [9]. In this study, the catalysis of lipase was studied in five duration of reaction time. In the case of lipase from *Porcinepancrease*, the ester conversion reach the maximum after 5 hours. The ester conversion decreased when extending the reaction time. The lipase from *Candida rugosa*catalysed the transesterification of coconut oil and the ester conversion was highest after 6 hours of reaction.

Table 9. Effect of reaction time on the ester conversion

t (h)	4	5	6	7	8
%CH(LPP)	58.54	62.55	57.04	58.08	56.14
%CH(LCR)	56.69	57.77	59.72	58.21	58.29

Ability to maintain activity in reaction media without protection is low, so enzyme will be lost activity gradually in short time. Therefore, immobilization of enzyme may increase the ester conversion. Iso et al. carried out the transesterification of rum oil/triolein and propanol, using lipase from immobilized Pseudomonas fluorescens in reaction time of 10 hours resulted in the conversion of 90% [9].

3.12 Analyzing Biodiesel Product

With the better catalyzing conditions of both LPP and LCR are the higher ester indexes, and the color of product is also stronger at better condition as Figure 3.

(a) (b)

(a) Crude biodiesel produced using LPP as catalyst.

(b) Crude biodiesel produced using LCR as catalyst.

Fig. 3 Color of products from coconut oil's transesterification.

Result also showed that some of ethyl esters of appropriate acid are mainly produced such as octan-, decan-, dodecan-, tetradecan-oic acid, as shown in Table 10.

Table 10. Components of products

Ethyl ester	LCR (%)	LPP (%)
Octanoic acid, ethyl ester	0.335	0.119
Decanoic acid, ethyl ester	0.140	0.056
Dodecanoic acid, ethyl ester	0.268	0.181
Tetradecanoic acid, ethyl ester	0.028	0.070

Comparing components of products to those of coconut oil indicated that these free lipase enzymes are able to catalyze to convert esters of saturated acids and medium number of carbon's acids to ethyl ester appropriately in investigated conditions, as shown in Table 11.

Table 11. Components of coconut oil was analyzed by GC/MS

Acid	Number of carbon	% acid	% ester	Acid	Number of carbon	% acid	% ester
Caproic	6:00	0.7	0.78	Palmitic	16:00	7.5	7.87
Caprylic	8:00	8.7	9.47	Stearic	18:00	2.6	2.72
Capric	10:00	6.9	7.41	Oleic	18:01	4.2	4.39
Lauric	12:00	50.8	54.02	Linoleic	18:02	0.6	0.63
Myristic	14:00	17.8	18.79	Linolenic	18:03	0.0	0.00

4. Conclusion

The present study demonstrates that free lipase enzymes are able to catalyze the transesterification of coconut oil with ethanol to produce ethyl esters and use method of measurement on ester index to identify product. The lipase from *Candida rugosa* and from *Porcine pancreas* showed the catalytic activities to transesterify coconut oil to produce biodiesel at vary ester conversion. Vietnamese coconut oil performed as a promising material for biodiesel production. The ester conversion at the optimum conditions studied was 62.55% (LCR), 59.72% (LPP). The lipase from *Candida rugosa* showed the better catalytic activity than lipase from *Porcine pancreas*. Vietnam has a large source of low price coconut oil. Therefore, the production of biodiesel from coconut oil, waste oil using lipase as catalyst, need to be investigated.

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