Production and characterization of biodiesel from canola oil through enzymatic transesterification

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Abstract. Biodiesel, a promising type of biofuel, can be produced from various types of renewable feedstocks, ranging from animal fats to plant oil. It is mainly made up of fatty acid alkyl ester compounds due to the transesterification reaction. This work aims to synthesize and characterize biodiesel, known as fatty acid methyl esters, from canola oil using an enzymatic reaction involving immobilized Novozym 435 and *Rhizomucor miehei* (RM IM) lipase enzymes. 4 g of canola oil was added to the reaction mixture consisting of 0.2 g immobilized lipase and 3:1 methanol to oil ratio. First, the enzymatic methanolysis reaction was conducted at the temperature of 35˚C and at agitation rate of 216 rpm for 24 hours. Next, the synthesized biodiesel was characterized using the Gas Chromatography-Mass Spectroscopy (GC/MS) analysis. Based on the analysis results, the main fatty acid methyl esters present in both products were hexadecanoic acid, 9-octadecenoic acid (z)-, 9,12,15-octadecatrienoic acid, (z,z,z)-, and 11,14-eicosadienoic acid. The transesterification of canola oil using both enzymes consistently revealed methyl oleate as the methyl ester with the highest composition, ranging from 67 to 71 %. In conclusion, canola oil was successfully converted into fatty acid methyl ester via the enzymatic transesterification process in this study.

1. Introduction

The presence of biodiesel as an alternative energy source has brought upon significant impacts in the biofuel world. Its selection as one of the most preferred types of biofuel is based primarily on its excellent biodegradability, similar properties to standard diesel, low sulfur content, higher flashpoints, lower toxicity, and most importantly, lower carbon emissions [1]. In addition, it comprises molecules of long-chain fatty acids alkyl esters with a large molecular weight that makes the biodiesel have a higher cetane number, higher heat of combustion, and low NOx emissions [35]. These fatty acids could vary when different oils and fats or triglycerides are used as feedstock in biodiesel production.

Biodiesel is commonly synthesized through the reaction route of transesterification [4]. In this method, the short-chain alcohol consisting of methanol or ethanol is added to the reaction mixture to react with the lipid molecule by consuming basic, acidic, or enzymatic catalysts. Both basic and acidic catalysts could vary in the form of homogenous or heterogenous, while the enzymes would be in the form of free or immobilized ones [5]. Currently, homogenous base-catalyzed transesterification is the most utilized technique for biodiesel production [6-9]. It is immensely applied in the industry because of its rapid reaction with maximum conversion rate, relatively mild reaction condition, and abundance in KOH and NaOH catalysts [2, 10]. However, the base catalyst would produce excessive soaps as a by-product due to its sensitivity towards free fatty acids (FFA), causing catalyst poisoning [5]. As for the

acidic catalyst, it does not undergo saponification due to its insensitivity towards FFA and can catalyze the esterification and transesterification concurrently. However, it still has some drawbacks, including higher alcoholic ratio consumptions, being conducted in harsh conditions, greater water sensitivity, and is highly corrosive to equipment and reactors [3, 10].

As concerns about the feeding of these chemical-typed catalysts issues grow, an emerging technique with high sustainability value is introduced: the enzymatic transesterification process. This biocatalyst improved downstream processing issues in chemical transesterification [5]. Despite their high cost, enzymatic catalysts are more tolerable to low-quality oil with high FFA, have easier product recovery, greater regenerative ability, and require very mild reaction conditions. The most salient is the lesser production of by-products [2,11]. When triglyceride is the main feedstock, lipases act as the primary enzyme for the specific substrate in biodiesel production [12-15]. Lipases, or triacylglycerol ester hydrolases (EC 3.1.1.3), are categorized as carboxylesterases because they can catalyze both the hydrolysis and the formation of long-chain fatty acid alkyl esters in biodiesel transesterification [16].

Moreover, they are extracted from various living organisms, including animals, plants, fungi, and bacteria [2]. The genera *Penicillium*, *Aspergillus*, *Rhizomucor*, and *Geotrichum,* are among the most common fungal species that produce microbial lipases [17]. Lipases are currently available in both immobilized and free forms. On the other hand, the immobilized enzymes are preferred because they are easier to handle, have more control over the reaction process, are biocatalysts that are easier to be manipulated, are more stable, and can be easily separated from products [18].

Among the widely utilized enzymes, immobilized lipase from *Rhizomucor miehei* (RM IM) and *Candida antarctica* (Novozym 435) is the most utilized in chemical reactions [19-20]. Both of them are immobilized on a microporous resin carrier. For example, it is conveyed in Moreira [21] that the application of Novozym 435 in the esterification process of residual babassu oil (*Orbignya* sp.) was capable of producing around 96.8% conversion of the feedstock into ethyl esters.

In this paper, the feasibility of using Novozym 435 and RM IM in the enzymatic transesterification of canola oil as the triglyceride feedstock was investigated. Canola oil was chosen because it is lower in saturated fat and has the highest content in oleic acid, which is one of the stable fatty acids, as depicted by several works [2, 22-25]. Following that, the synthesized biodiesel from the enzymatic transesterification of both enzymes was characterized by the Gas Chromatography-Mass Spectroscopy (GC/MS) analysis. The types of fatty acid methyl esters obtained were discussed. Thus, the potential of canola oil as a biodiesel feedstock from enzymatic transesterification would be evaluated by the performances of Novozym 435 and RM IM as the enzymes in this study.

2. Materials and methods

2.1 Materials

Immobilized lipase from *Candida antarctica* (Novozym 435) and *Rhizomucor miehei* (RM IM) were supplied by Novozymes Malaysia Sdn Bhd. Fresh canola oil containing an original blend of canola and sunflower oil was purchased from a supermarket in Kuala Lumpur, Malaysia. Methanol with a purity of 99.8% was obtained from Elite Advanced Materials, while other chemicals used were of the reagent or analytical grade.

2.2 Enzymatic transesterification reaction

The reaction was performed in a 50 mL Erlenmeyer flask in an IKA KS 4000 New Incubator Shaker. In the reaction mixture, 4 g of canola oil and methanol 3:1 methanol to oil ratio (w/w) were mixed as substrates. First, Novozym 435 was dried for 24 h at 40˚C to eliminate moisture. Next, 0.2 g of the dried Novozym 435 were added to the mixture. After a reaction time of 24 h, the mixture was centrifuged at 4000 rpm and 4˚C for 25 minutes in a Labnet centrifuge. Finally, the supernatant layer, which contained the fatty acid methyl esters, was subjected to GC/MS analysis. The procedures were then repeated using RM IM as the enzyme.

2.3 . Determination of fatty acid methyl esters composition by GC/MS

The composition of fatty acid methyl esters was determined by GC/MS in an Agilent Technologies chromatograph model 5975 inert XL NetWork GC system. The type of column used was DB-WAX capillary column (30 m x 0.25 mm x 0.25 μm). Next, 1 mL of hexane was added to the samples to extract the fatty acid methyl ester products. First, 1μL of the products were injected using a 1:50 split ratio. Then, the oven temperature was programmed as follows: initial temperature of 40˚C for 2 minutes and increased to 230˚C at 10˚C per minutes for 5 minutes, and finally increased to 250˚C at 3˚C per minutes for 5 minutes. Helium gas acted as the carrier gas with a constant flow rate of 3 mL/ min, and the injector temperature was 250˚C. Next, the composition of fatty acids in the biodiesel product was determined by comparing their mass spectral fragmentation and similar compounds provided in the GC/MS software database (NIST Mass spectral Finder 2.0 Library, NIST/EPA/NIH). Finally, the relative composition of each fatty acid was articulated in terms of the average percentage (%) of individual fatty acids methyl esters concerning the total determined fatty acids [1].

3. Results and discussion

The reaction is based mostly on the Ping Pong Bi Bi mechanism kinetic model in the enzymatic transesterification process. This model assumes that the immobilized enzymes, which in this case, the lipase, are more permittable for the substrates, thus, making the effect of mass transfer negligible. Besides, the formation of di- and monoacylglycerides is not considered since they are intermediate products, making the overall reaction simpler towards the generation of fatty acid methyl ester as the main products [26]. In the context of this reaction, canola oil (CO) as the triglyceride and methanol (M) will act as the substrates. The lipase (L) binds to the first substrate (CO) to form a lipase-canola oil complex (LCO). As a result, the first product, the biodiesel or fatty acid methyl ester (F) and the intermediate lipase (L'), are released. M as the second substrate binds with L' and breakdown of transitory complex (L'M) to free lipase (L) and the second product (G). Equation 1 represents the typical multi-substrate Bi Bi lipase kinetics of the enzymatic transesterification reaction, while equations 2 and 3 describe the reaction mechanism of the Ping Pong Bi Bi kinetic model. The rate constant for each reaction step is denoted by the values k_1 to k_6, respectively.

$$CO + M \overset{L}{\leftrightarrow} F + G \tag{1}$$

$$L + CO \underset{k_2}{\overset{k_1}{\leftrightarrow}} [LCO] \overset{k_3}{\Rightarrow} L' + F \tag{2}$$

$$L' + M \underset{k_5}{\overset{k_4}{\leftrightarrow}} [L'M] \overset{k_6}{\Rightarrow} L + G \tag{3}$$

Figure 1 and Figure 2 depicted the GC/MS chromatography analysis of the methyl esters present in biodiesel products. Based on these, the main fatty acid methyl esters identified in both biodiesel samples obtained from Novozyme 435 and RM IM enzymatic transesterification were hexadecanoic acid, 9-octadecenoic acid (z), 9,12,15-octadecatrienoic acid, (z,z,z)-, and 11,14-Eicosadienoic acid. The retention times for hexadecenoic acid, 9-octadecenoic acid (z), and 9,12,15-octadecatrienoic acid, (z,z,z)-, in biodiesel synthesized from Novozym 435 were 15.77, 17.30, and 18.31 minutes respectively. For RM IM, the retention times for the three methyl esters were 16.09, 17.55, and 18.42 minutes respectively, with 22.44 minutes for 11,14-Eicosadienoic acid. The slight changes in the retention time in the two samples may be caused by the leaking of the septum component in the GC-MS due to the numerous injections from previous samples, thus generating inconsistency in the retention time shift [39].

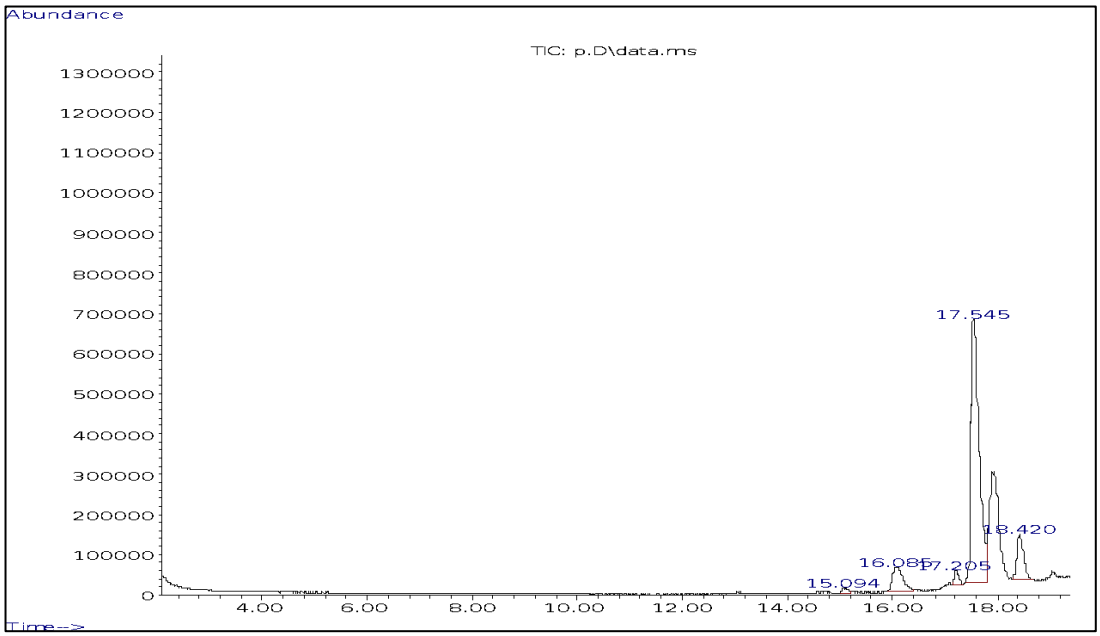

Figure 1. GC/MS analysis of fatty acid methyl ester from Novozyme 435 enzymatic transesterification reaction.

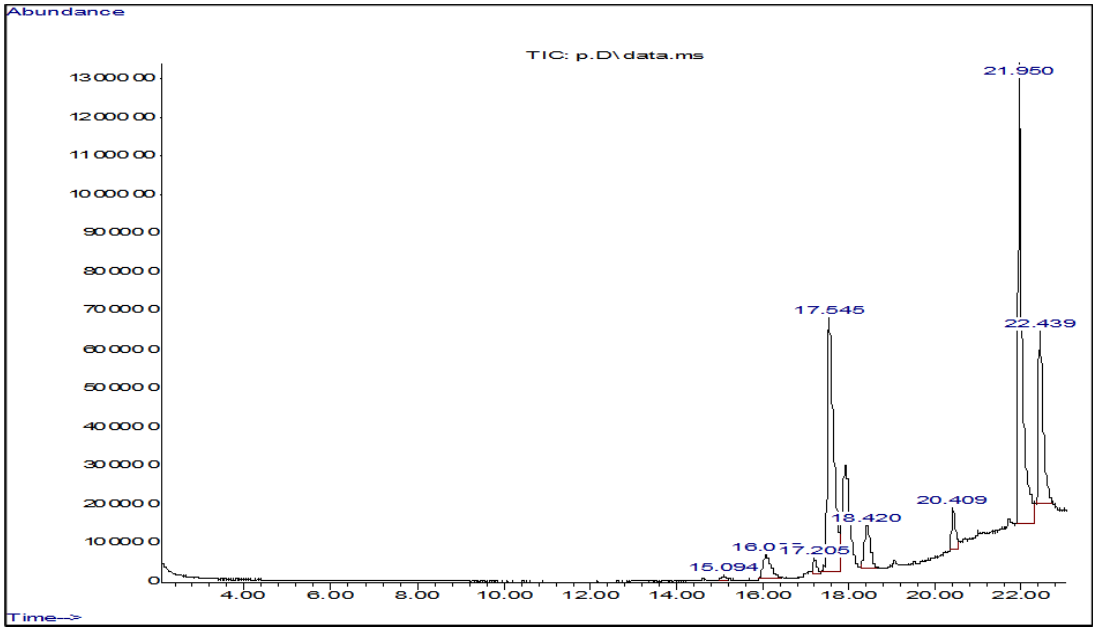

Figure 2. GC/MS analysis of fatty acid methyl ester from RM IM enzymatic transesterification reaction.

Generally, the methyl ester profile obtained in this study was comparable to the most common fatty acid methyl esters found from the transesterification of canola oil, sunflower oil, and soybean oil, as depicted in Table 1. They primarily consisted of saturated (C16:0), monounsaturated (C18:1), and polyunsaturated methyl esters (C18:3 and C20:2).

Table 1. Comparison of fatty acid methyl ester composition of the biodiesel from canola oil with sunflower oil and soybean oil

Type of fatty acid methyl esters	Formula	Common name	Molecular weight	Greenseed Canola oil (%) [24],	Sunflower oil (%) [27]	Canola oil (%) [28]	Sunflower oil (%) [29]	Soybean oil (%) [30]	Canola oil (%) This study	Canola oil (%) This study
				Potassium hydroxide as catalyst	Sodium methoxide as catalyst	Sodium hydroxide as catalyst	Sodium hydroxide as catalyst	Hydrobromic acid as catalyst	RM IM as catalyst	Novozym as catalyst
Hexadecanoic acid, C16:0	$C_{17}H_{34}O_2$	Methyl palmitate	270.45	4.36	4.50	4.60	6.73	8.95	4.01	12.70
9-octadecenoic acid (z)-, C18:1	$C_{19}H_{36}O_2$	Methyl oleate	296.50	60.92	82.0	64.30	31.28	22.95	71.86	67.39
9,12,15-octadecatrienoic acid, (z,z,z)-, C18:3	$C_{19}H_{32}O_2$	Methyl linolenate	292.50	6.79	0.20	7.60	Less than 0.3	5.95	4.67	13.61
11,14-Eicosadienoic acid, C20:2	$C_{21}H_{38}O_2$	Methyl cis,cis-11,14-eicosadienoate	322.50	-	0.10	-	0.14	-	18.69	-

Table 1 portrays the comparison of the fatty acid methyl ester compositions of the canola oil with sunflower oil and soybean oil in biodiesel production by different researchers. Based on the results in Table 1, even though the canola oil was used as the raw material for both enzymatic transesterification in this study, there were slight variations in the fatty acid methyl ester compositions in each sample depicted in Figures 1 and 2. However, methyl oleate remained the highest amount of fatty acid methyl ester detected in both samples and acted as the main composition of canola oil biodiesel. The main monounsaturated fatty acid in the biodiesel feedstock is oleic acid, accounting for around 55 % of canola oil [2]. The result is consistent with the finding by Mohamad [36], in which the methyl oleate made up the majority of methyl esters composition of the biodiesel synthesized from vegetable palm oil with the percentage of 42.40%. In terms of methyl oleate's significance in biodiesel as biofuel in vehicles, the higher methyl oleate compound in the biodiesel would also result in better engine component performance with better ignition properties than the other methyl ester types [31]. Besides, other work reported that adding oleate compound into biofuel would enhance biodiesel's low-temperature performance and oxidation stability while providing higher economic value [32].

Although canola oil was used as the feedstock in this study, the types of methyl ester obtained from the enzymatic transesterification processes differed slightly from those reported using the same feedstock. The biodiesel sample synthesized from RM IM catalyzed transesterification contained 11,14-Eicosadienoic acid as the polyunsaturated methyl ester in approximately 19 %. This type of fatty acid methyl ester is usually a trace component in sunflower oil [27,29]. It is to note that the fresh canola oil used as feedstock in this work was reported to contain a mixture of sunflower oil, which could explain the biodiesel's fatty acid methyl esters composition obtained. Therefore, it could be deduced that the variation in methyl esters could be attributed to the different compositions of the canola oil feedstock used in this work and the literature.

As for methyl palmitate and methyl linolenate, they are commonly found in the biodiesel synthesized from almost all feedstocks since palmitic acid and linolenic acid are among the main fatty acids that make up the triglyceride feedstocks [2]. These fatty acids possess high nutritional values in the edible oils, especially linolenic acid, which acts as a monounsaturated fatty acid. As it is easily oxidized, this linolenic compound contributes significantly to the quality of biodiesel [34]. Two different types of lipase enzyme used in the reaction were most likely responsible for the inconsistency in the fatty acid methyl ester composition in both biodiesel samples obtained in this work. Despite the fact that they are both lipases, they are extracted from two different biological species. Lipase selectivity is also affected by the number of carbon atoms and the degree of unsaturation of fatty acid molecules [33]. As a result, the number of methyl esters produced by Novozym 435 enzymatic action is not the same as RM IM's. It also showed that each type of lipase enzyme is different in its transesterification activities. Since the enzymatic activity of RM IM is more active with more accessible active sites than in Novozym 435, it thus produced better results with higher methyl oleate composition and the additional presence of 11,14-Eicosadienoic acid in the biodiesel. Besides, the higher polarity of the support system in RM IM, which is the weak anion-exchange resins, with the acyl migration effect makes it easier to esterify the triglyceride molecules in the oil [37].

Nevertheless, the mechanism of the enzyme-substrate action is still too complex to be annotated due to the presence of several polyunsaturated fatty acid mixtures in the oil [37]. The enzyme's nature also brings about the expected differences in enzyme activity, specificity, and stability, allowing the transesterification activity to gain variations in results [38]. For example, it was reported by Corrêa [33] that Novozym 435 produced 83.5% conversion of soybean oil deodorizer distillate (SODD) in the esterification process with ethanol. In contrast, Lipozym RM IM only produced 59.1% conversion of SODD in the same reaction conditions.

4. Conclusions

In this work, canola oil was found to be a viable triglyceride feedstock for the enzymatic transesterification for biodiesel production. The highest fatty acid methyl esters in both biodiesel samples were determined. Methyl oleate, with the relative composition of 71.86 and 67.39% of total fatty acid methyl esters for both RM IM and Novozym 435, catalyzed transesterification, respectively, were found to be consistent with the findings from previous literature. The application of different enzymes as catalysts in the transesterification process was also proven to produce different types of methyl esters from the same feedstock due to the different selectivity of each enzyme.

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