Biodiesel Production by Enzymatic Transesterification of Papaya Seed Oil and Rambutan Seed Oil

C. S. Wong [1], R. Othman [2]

[1, 2] Department of Chemical Engineering, International College (ICOLE), Universiti Kuala Lumpur,
Lot 1988, Vendor City, Taboh Naning, 78000 Alor Gajah, Melaka, Malaysia.
[1] wcsien@unikl.edu.my
[2] rapidah@unikl.edu.my

Abstract— Biodiesel production from vegetable oil has gained attention as an alternative fuel to minimize the usage of fossil fuels and reduce greenhouse gases pollution. In Malaysia, oils from local fruit seeds of papaya and rambutan are potential feedstock for biodiesel production due to their high lipid contents and easily available. In the present study, papaya and rambutan seed oils were extracted via soxhlet apparatus using *n*-hexane and the oil yields were in between 34–40%. The extracted oils were subjected to enzymatic transesterification by the immobilized *Candida rugosa* lipase as a catalyst under room temperature with varies molar ratios of methanol to oil. The highest biodiesel yield for papaya seed oil and rambutan seed oil was found to be 96% and 89% at methanol-to-oil ratios of 6:1 and 8:1, respectively. Results also showed a higher biodiesel yield using lipase immobilized on the magnetic particles as the heterogeneous catalyst compared to the yield obtained using free enzyme as the homogeneous catalyst. The properties of biodiesel such as density, acid value, iodine value and cetane number were analyzed and found to meet the European Standard of Biodiesel. The study shows that papaya and rambutan seed oils have the potential to be used as alternative feedstock for biodiesel production than the full dependence on palm oil in Malaysia.

Keyword - Biodiesel, papaya seed oil, rambutan seed oil, enzymatic transesterification, lipase

I. Introduction

In recent years, it has been on growing importance to explore new energy resources as substitutes to fossil fuels. Biodiesel appears as a recommended energy mainly because biodiesel is renewable, biodegradable and non-toxic, and it significantly reduces toxic and other emissions when burned as a fuel [1, 2].

Biodiesel can be derived from vegetable oil or animal fat. Vegetable oils are becoming attractive as the renewable source for biodiesel production due to its high lipid contents, high availability and low cost. Papaya (*Carica papaya*) and rambutan (*Nephelium lappaceum*) are fruit plants that grow easily in Malaysia. Oil yields from papaya and rambutan seeds were investigated by Puangsri *et al.* [3] and Winayanuwattikun *et al.* [4] to be in between 30–43%. Since they are normally abandoned as wastes, thus the free and high availability of the seeds as the oil source for biodiesel production is worth investigating.

Transesterification is the process applied to produce biodiesel by the reaction of plant oil with an alcohol and having glycerol as a byproduct. Transesterification process requires the presence of catalyst to improve the reaction rate and yield. The most common catalyst used is chemical catalyst, either acid or alkali. However, the chemical transesterification process is not practical and economical at the downstream processing as it needs multi-step purification of end products. On the other hand, enzyme transesterification using lipase is one of the competent and greener methods as it promotes high efficiency and selectivity, low energy consumption, avoids soap formation and low waste amounts [2, 5, 6].

In the present study, we aimed to investigate the potential of papaya and rambutan seed oils as the renewable feedstock for biodiesel production. Enzyme transesterification process using lipase as the catalyst was attempted under different molar ratio of alcohol to oil. The performance of immobilized enzyme was also compared with the free enzyme to study the effect of enzyme immobilization on the biodiesel production.

II. Materials and Methods

A. Materials

Fresh papaya and rambutan fruits were bought from local market. [3-(2-aminoethylamino) propyl] trimethoxy-silane (APTS), methanol, sodium fluoride and tetraethyl orthosilicate (TEOS) were purchased from Merck. Glutaraldehyde, magnetic particles (Fe_3O_4) and *Candida rugosa* lipase were purchased from Sigma-Aldrich.

B. Oil Extraction from Plant Seeds

Plant seeds were removed manually from papaya and rambutan fruits. The seeds were dried in an oven at 60ºC for 24 hours. The dried seeds were ground into small particles and extracted using n-hexane for 8 hours in a Soxhlet apparatus. The extracted oil was then undergone evaporation using a rotary evaporator to remove the remaining solvent.

C. Preparation of Coated Magnetic Particles

Two grams of Fe_3O_4 (magnetite) were suspended into 100 ml distilled water. A mixture of 5 ml [3-(2-aminoethylamino) propyl] trimethoxy-silane (APTS), 15 ml of methanol and 5 ml of sodium fluoride (NaF) solution was added and stirred for 10 min. Then 20 ml of tetraethyl orthosilicate (TEOS) were dropped slowly into the flask and stirred vigorously at room temperature for 24 hours. The precipitate was collected, washed three times with ethanol and water, dried in an oven at 313 K for 10 hours [7].

D. Enzyme Immobilization of Candida rugosa Lipase

One hundred millilitres of glutaraldehyde were added to 4.4 g of coated particles and stirred at room temperature. Then, 700 mg of *Candida rugosa* lipase dissolved in 100 ml of phosphate buffer solution (pH 7.0, 0.1 M) was added and stirred for 30 min at room temperature. The mixture was subjected to centrifugation at 3000g for 20 min, and the particles were washed three times with phosphate buffer solution and dried in an oven at 30ºC for 10 hours [8].

E. Enzymatic Transesterification of Plant Seed Oil

Biodiesel was produced by enzymatic transesterification of papaya and rambutan seed oils respectively for 24 hours at room temperature with different methanol/oil ratio ranging from 4:1 to 8:1. With oil basis of 5 g, 10% (w/w) of water was added. The reaction was carried out under a stirring speed of 200 rpm by adding 30% (w/w) of immobilized lipase. At each molar ratio, three steps addition of methanol were performed to avoid lipase denaturation and the starting amounts of methanol and oil used were 0.32 g and 5 g, respectively [9]. After the reaction, the immobilized lipase was separated by a magnetic field and washed with phosphate buffer. The transesterification process was then repeated using free lipase for comparison purpose.

F. Biodiesel Yield and Analysis

Biodiesel yield obtained from the enzymatic transesterification process was calculated based on Eq. (1) [10]:

$$\text{Biodiesel yield (\%)} = \frac{B}{O} \times 100\% \qquad (1)$$

where B = weight of biodiesel produced (g)

O = weight of plant seed oil used (g)

Biodiesel obtained from the enzymatic transesterification was analyzed using gas chromatography (GC) to identify the fatty acid methyl ester contents in the biodiesel. In order to determine the performance of the biodiesel produced, several properties of biodiesel including density, acid value, iodine value and cetane number were analyzed and compared with the European Standard of Biodiesel (EN 14214).

III. RESULTS AND DISCUSSION

A. Plant Seed Oil Content

Soxhlet extraction of papaya seed oil using *n*-hexane managed to obtain a yield of 34.3%, which agreed with the result of 30.7% obtained by Puangsri *et al.* [3]. Winayanuwattikun *et al.* [4] indicated that the range of oil yield from *Carica papaya* was in between 25–48%. Meanwhile, oil yield from rambutan seeds via Soxhlet extraction was found to be 40%, which was in compliance with the range 37–43% reported earlier by Winayanuwattikun *et al.* [4]. Hence, papaya and rambutan seeds are suitable to be the resources of biodiesel due to their high oil content.

B. Effect of Methanol/Oil Ratio on Biodiesel Production

Fig. 1 shows the yields of biodiesel from papaya and rambutan seed oils with different molar ratios of methanol/oil. Results showed an overall increase of biodiesel yield by increasing the methanol to oil molar ratio.

The highest yield was at the optimum ratio of 6:1 to convert papaya seed oil into biodiesel. This result agreed with the study done by Freedman *et al.* [11] which stated that most of the vegetable oils behaved similarly and achieved highest biodiesel conversion at a 6:1 molar ratio.

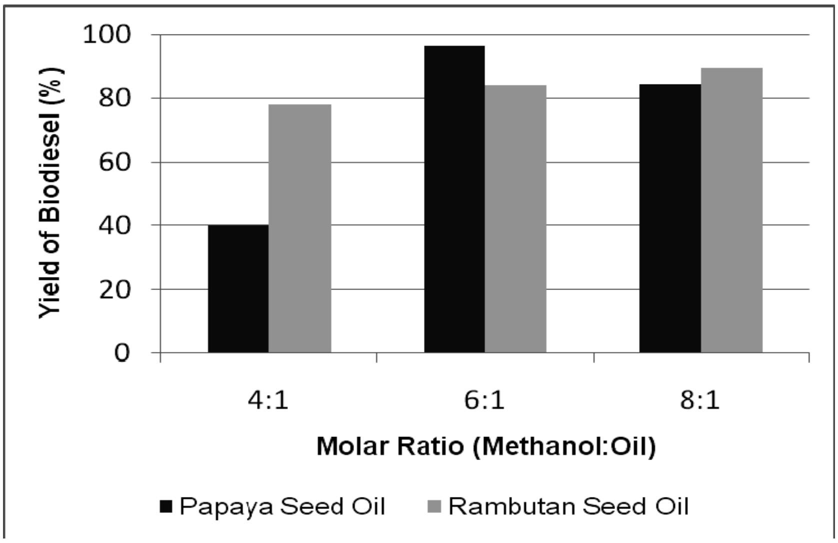

Fig. 1. Biodiesel yield with different molar ratios of methanol to papaya and rambutan seed oils

On the other hand, the highest yield was attained at ratio of 8:1 to convert rambutan seed oil into biodiesel. It was expected as an increase in number of moles of alcohol with respect to the triglycerides (oils) resulted in an increase in the production of fatty acid alkyl esters (biodiesel).

However, decrease of biodiesel yield at a ratio over 8:1 might happen due to the effect of high amount of methanol that could lead to the deactivation of lipase [12]. Therefore, the methanol/oil ratio higher than 8:1 was not investigated in this study.

Watanabe *et al.* [13] also pointed out that owing to the low solubility of methanol in the oil, excessive methanol that exists as drops in the oil might decrease the lipase activity. Thus, in order to prevent the inactivation of lipase, three stepwise addition of methanol was practiced for the transesterification process in this study.

C. Effect of Enzyme Immobilization of Biodiesel Production

The effect of enzyme immobilization was studied by comparing the effectiveness of immobilized lipase and free lipase used in the transesterification process. The result is shown in Fig. 2.

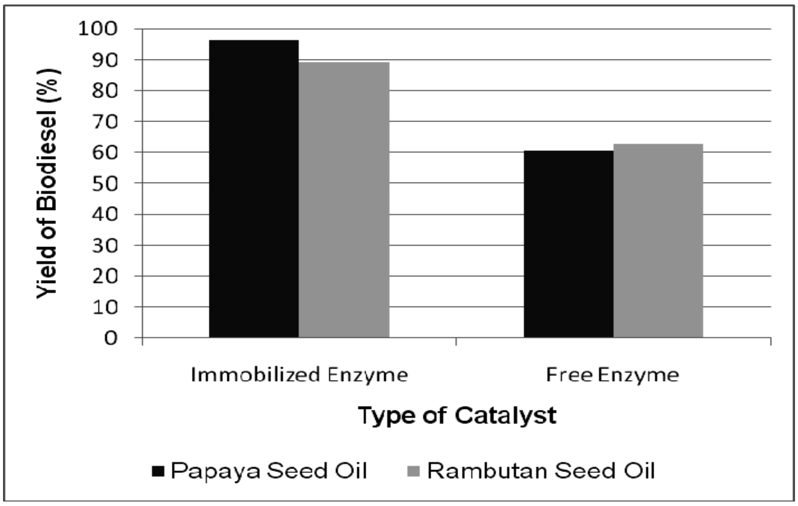

Fig. 2. Comparison of biodiesel yield using immobilized lipase and free lipase

Biodiesel produced using immobilized lipase was proven to be higher (>85%) than using free enzyme (~60%). Enzyme immobilization is an effective method to improve the enzyme stability because the structure of the immobilized enzyme becomes more rigid, preventing the enzyme molecular movement that may lead to enzyme inactivation [14].

Besides, the enzyme that immobilized on the magnetic particles can be easily separated from the reaction medium after the transesterification process for reuse by applying a magnetic field. It offers the benefits in saving both the operating cost and time to produce biodiesel using immobilized enzyme.

D. Biodiesel Properties

The result from GC analysis shows that the major fatty acid of the biodiesel produced from papaya seed oil was linoleic acid (67.4%), followed by palmitic acid (29.5%) and stearic acid (3.1%). It indicated that biodiesel produced from papaya seed oil contains higher amount of unsaturated fatty acid, which is in contrast with the biodiesel produced from rambutan seed oil that contains higher percentage of saturated fatty acid (52.1%). According to Azam *et al.* [15], biodiesel that contains more unsaturated fatty acid tends to remain as liquid at room temperature compared to oil that is easily turned hard due to the higher amount of saturated fatty acids.

Fuel properties of the biodiesel produced such as density, acid value, iodine value and cetane number were analyzed based on EN 14214 test methods and the results are shown in Table I.

TABLE I
Fuel Properties of Biodiesel Produced by Enzymatic Transesterification of Papaya and Rambutan Seed Oils

Properties	Biodiesel from Papaya Seed Oil	Biodiesel from Rambutan Seed Oil	Biodiesel Standard (EN14214) [16]
Density (kg L^{-1})	0.90	0.88	0.86 - 0.90
Acid Value (mg KOH g^{-1})	0.72	0.7	< 0.5
Iodine Value (g I_2 $100g^{-1}$)	112.62	69.21	< 120
Cetane Number	63.75	63.50	> 51

Generally, most of the properties of biodiesel meet the standard except acid value. Acid value indicates the quantity of free fatty acids present in the oil that might cause corrosion in the engine. Since the acid value is higher than the standard requirement, pretreatment towards the papaya and rambutan seed oils is suggested before it proceeds to the enzymatic transesterification.

IV. CONCLUSION

As a conclusion, the present study proved that papaya and rambutan seed oils have the potential to be used as feedstock to produce biodiesel via enzymatic transesterification using lipase. Highest yield of biodiesel was obtained at methanol/oil molar ratio of 6:1–8:1. Biodiesel yield was also found to be higher when the immobilized enzyme was applied for the transesterification process compared to the free enzyme.

The use of immobilized enzyme is important for solving problems concerning the downstream separation of the acid or alkali catalyst from the biodiesel product via chemical transesterification process. This may contribute to the reduction of operating and energy costs in biodiesel production.

The properties of biodiesel produced from papaya seed oil were also analyzed and found to have met the Biodiesel Standard of EN 14214, except that pretreatment on the seed oil is recommended to reduce the acid value of the biodiesel.

ACKNOWLEDGMENT

The authors would like to thank the Institute of Research and Postgraduate Study (IRPS) of Universiti Kuala Lumpur for funding this research through Short Term Research Grant (STRG).

REFERENCES

[1] N. N. A. N. Yusuf, S. K. Kamarudin, and Z. Yaakub, “Overview on the current trends in biodiesel production”, Energy Conversion and Management, vol. 52, pp. 2741-2751, 2010.
[2] B. Zhang, Y. Weng, H. Xu, and Z. Mao, “Enzyme immobilization for biodiesel production”, Applied Microbiology Biotechnology, vol. 93, pp. 61-70, 2012.
[3] T. Puangsri, S. M. Abdulkarim, and H. M. Ghazali, “Properties of Carica papaya L. seed oil following extractions using solvents and aqueous enzymatic methods”, Journal of Food Lipids, vol. 12, pp. 62-76, 2004.
[4] P. Winayanuwattikun, C. Kaewpiboon, K. Piriyakananon, S. Tantong, W. Thakernkarnkit, W. Chulalaksananukul, and T. Yongvanich, “Potential plant oil feedstock for lipase catalyzed biodiesel production in Thailand”, Biomass and Bioenergy, vol. 32, pp. 1279-1286, 2008.
[5] S. P. Singh, and D. Singh, “Biodiesel production through the use of different sources and characterization of oils and their esters as the substitute of diesel: a review”, Renewable and Sustainable Energy Reviews, vol. 14, pp. 200-216, 2010.
[6] M. Leca, L. Tcacenco, M. Micuts, and T. Staicu, “Optimization of biodiesel production by transesterification of vegetable oils using lipases”, Romanian Biotechnological Letters, vol. 15, pp. 5618-5630, 2010.
[7] K. J. Dussan, C. A. Cardona, O. H. Giraldo, L. F. Gutiérrez, and V. H. Pérez, “Analysis of a reactive extraction process for biodiesel production using a lipase immobilized on magnetic nanostructures”, Bioresource Technology, vol. 101, pp. 9542-9549. 2010.
[8] L. Zeng, K. Luo, and Y. Gong, “Preparation and characterization of dendritic composite magnetic particles as a novel enzyme immobilization carrier”, Journal of Molecular Catalysis B: Enzymatic, vol. 38, pp. 24-30, 2005.

[9] H. D. Lee, J. M. Kim, H. Y. Shin, S. W. Kang, and S. W. Kim, "Biodiesel production using a mixture of immobilized Rhizopus oryzae and Candida rugosa lipases", Biotechnology and Bioprocess Engineering, vol. 11, pp. 522-525, 2006.
[10] A. Hayyan, M. Z. Alam, M. E. S. Mirghani, N. A. Kabbashi, N. I. N. M. Hakimi, Y. M. Siran, and S. Tahiruddin, "Production of biodiesel from sludge palm oil by esterification process", Journal of Energy and Power Engineering, vol. 26, pp. 485-490, 2010.
[11] B. Freedman, E. H. Pryde, and T. L. Mounts, "Variables affecting the yields of fatty esters from transesterified vegetable oils", Journal of American Oil Chemists' Society, vol. 61, pp. 1638-1643, 1984.
[12] Y. Shimada, Y. Watanabe, A. Sugihara, and Y. Tominaga, "Enzymatic alcoholysis for biodiesel fuel production and application of the reaction to oil processing", Journal of Molecular Catalysis B: Enzymatic, vol. 17, pp. 133-142, 2002.
[13] Y. Watanabe, Y. Shimada, A. Sugihara, and T. Tominaga, "Enzymatic conversion of waste edible oil to biodiesel fuel in a fixed-bed bioreactor", Journal of the American Oil Chemists' Society, vol. 78, pp. 703-707, 2001.
[14] W. Xie, and J. Wang, "Immobilized lipase on magnetic chitosan microspheres for transesterification of soybean oil", Biomass and Bioenergy, vol. 36, pp. 373-380, 2011.
[15] M. M. Azam, A. Waris, and N. M. Nahar, "Prospects and potential of fatty acid methyl esters of some non-traditional seed oils for use as biodiesel in India", Biomass and Bioenergy, vol. 29, pp. 293-302, 2005.
[16] EN14214 Biodiesel Standard, European Committee for Standardization (CEN), 2012.