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LIPASE PRODUCTION FROM WASTE FRUIT PEELS FOR BIODIESEL PRODUCTION

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ABSTRACT

Waste pineapple and passion fruit peels have potency as lipase enzyme sources. The combination of both peels with a ratio of 6:4 has the highest enzyme lipase activity after 3 days of fermentation and it has increased to 28.33 ± 0.0 U/ml after purification using the ammonium sulfate precipitation method. The response surface method design experiment was applied to determine the effect of the ratio of molar oil to methanol (1:3 – 1:9), reaction time (1 – 24 hours), and temperature (30 - 60°C). The maximum biodiesel conversion of 82.7% could be achieved using the reaction condition of a ratio molar of 1:9, 13.65 hours reaction time, and a temperature of 46.5°C for enzymatic transesterification of PO. Further, for bio-catalysis biodiesel production from WCO, the maximum conversion of 88% was achieved under reaction conditions of a ratio molar of 1:9, 14 hours reaction time, and a temperature of 48°C.

Keywords: Pineapple, Passion Fruit, Waste Peels, Biodiesel, Lipase.

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INTRODUCTION

Biodiesel has gained attention in recent years as have some advantages such as being biodegradable, less pollutant emission, and non-toxic.[1] Biodiesel produces through a transesterification reaction of lipid/oil and methanol with or without a catalyst.[2,3] Commercial biodiesel production usually uses an alkaline substance as a catalyst due to high catalytic activity generating high yield in a short time.[4] However the homogeneous base catalyst requires neutralization and purification of the product which could increase the biodiesel production cost.[5] Furthermore the wastewater from the purification process has to be treated to avoid environmental damage.[6,7] Recently, heterogeneous substances have been studied and proven could catalyze biodiesel production with high yield.[2,5] The heterogeneous catalyst offers some advantages including being easy to separate, reusable, and comparatively cheap.[4] However some drawbacks of using chemical catalysts were identified such as requiring an excessive volume of methanol to move the reaction toward the biodiesel which has consequences in difficulty to separating the glycerol and catalyst.[8] Currently, the transesterification reaction using enzyme draws high interest for the merits of proceeding esterification/transesterification reaction simultaneously produces biodiesel and glycerol with high yield and purity, tolerates high water and free fatty acid (FFA) content, no soap formation, and simple production processes conducted in mild condition. In addition immobilization of the enzyme could increase the reusability.[9] However the high cost and unstable of immobilized lipase enzyme hinder its wider usage.[4] Therefore finding low-cost lipase for biodiesel production is necessary for enzymatic transesterification reaction. In the past decade, the development of the food processing industry, restaurants, markets, fruit markets, and others has led to an increase in organic solid waste in large quantities such as vegetable pulp and fruit peels.[10,11] Although most of this organic waste has been processed into various products such as biogas[12], the amount that has not been processed and has the potential to pollute the environment remains large.[10] Fruit peel waste is known to be rich in nutrients, easily decomposed, and has high water content which therefore has the potential to be used as a source of lipase enzymes.[11] Several research results proved that after the fermentation process, lipase enzymes could be obtained which have the same catalytic activity as pure lipase enzymes.[10,11,13] The lipase enzyme with an activity of 57.43 U/mL was achieved from the fermentation of a mixture of pomegranate, orange,

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and pineapple peels. This lipase enzyme was then applied as a biocatalyst in biodiesel production from palm oil and gave satisfactory yields.[14] However based on the literature review, no study on the utilization of pineapple and passion fruit peels as lipase enzyme sources have been reported yet. Therefore, this study aimed to use lipase enzymes generated from the fermentation of passion fruit and pineapple peels as biocatalysts for biodiesel production from palm oil (PO) and waste cooking oil (WCO). The response surface method with the Box-Behnken design experiment was used to determine the effects of the ratio molar of PO or WCO to methanol, reaction time and temperature to biodiesel conversion.

EXPERIMENTAL

Material

The pineapple peels were collected from the pineapple canning industry and passion fruit peels from syrup plants in Deli Serdang and Karo, Sumatera Utara – Indonesia, respectively. The chemicals were bought from a local distributor and were used without any pre-treatment.

Isolation Enzyme Lipase from the Fermentation of Pineapple and Passion Fruit Peels

The pineapple and passion fruit peels were washed and crushed to reduce the size. The combination of pineapple and passion fruit peels with a ratio molar of 1:9, 2:8, 3:7, 4:6, 5:5, 6:4, 7:3, 8:2, and 9:1 were made for a total sample of 180 grams in a fermentation bottle. The fermentation was started by adding 20 ml of molasses and 600 ml of distilled water and was incubated at 37°C for 6 days. The fermented products were separated using centrifugation at 4000 rpm for 10 minutes and the supernatant was collected. The activity of enzyme lipase was assayed using the titration method according to the procedure introduced previously.[15,16] Further, the fermented supernatant from the combination of 6:4 which have the highest enzyme activity was purified using the ammonium sulphate precipitation method.[14] The solution was freeze-dried to obtain dry powder for biodiesel production before use as a biocatalyst.

The Enzymatic Transesterification of PO or WCO

Enzymatic transesterification of PO or WCO with methanol to produce biodiesel was carried out in a screw-capped vial. A total of 0.1 g of lipase enzyme was added to a vial and then added with PO or WCO and methanol at a predetermined ratio and incubated at 100 rpm with temperature variations. After the investigated incubation time reached, 3 mL of n-hexane was added and shaken to form 2 layers. The top layer was taken by decantation and put into a vial and evaporated in an oven. The biodiesel conversion was determined using gas chromatography (GC).

The Statistical Analysis for Optimization of Biodiesel Production

The response surface method (RSM) with Box-Behnken experimental design was used to determine the optimum condition of the enzyme lipase catalyzed the transesterification of PO and WCO to biodiesel using Statistica 13.0 software. The experimental design comprised 3 factors of ratio molar of PO or WCO to methanol, reaction time and temperature at three levels (Table-1) including 12 factorial points and three centre points.

Table-1: Levels of Optimization Conditions

Variables	Symbols	Levels		
		-1	0	1
Ratio molar of PO or WCO to methanol	A	1:3	1:6	1:9
Reaction time (hours)	B	1	12.5	24
Temperature (°C)	C	30	45	60

The relationship between the variables and biodiesel conversion was calculated by the second-order polynomial equation. The optimum biodiesel conversion was determined using the quadratic polynomial equation model as shown in equation 1.

$$Y = \beta_0 + \sum_{I=1}^{1} \beta_I X_i + \sum_{i=1}^{2} \beta_{ii} X_i^2 + \sum_{i=1}^{2} \sum_{j=i=1}^{3} \beta_{ij} X_i X_j \quad (1)$$

Where Y = biodiesel conversion; X_i and X_j are the independent factors; and 0, i, ii and ij are the intercept, linear, quadratic interaction coefficients, respectively. Table-2 presented the biodiesel conversion achieved from the experiment and the prediction conversion calculated based on eqn.-1.

Table-2: The Experimental and Predicted Biodiesel Conversion

Run	A: Ratio Molar	B: Reaction Time	C: Temperature	PO		WCO	
				Conversion	Prediction	Conversion	Prediction
1	3	1	45	65,3	65,189	71,3	72,55
2	9	1	45	75,3	75,686	80,3	79,2
3	3	24	45	70,78	70,394	77,2	78,3
4	9	24	45	78,2	78,311	84,3	83,05
5	6	12,5	45	75,3	76,167	81,2	81,833
6	6	12,5	45	77,4	76,167	82,4	81,833
7	6	12,5	45	75,8	76,167	81,9	81,833
8	3	12,5	30	51,95	51,893	69,2	67,25
9	9	12,5	30	66,2	65,645	72,3	72,7
10	6	1	30	48,78	48,949	55,2	55,9
11	6	24	30	54,1	54,544	64,3	65,15
12	3	12,5	60	67,34	67,895	74,5	74,1
13	9	12,5	60	72,5	72,558	78,1	80,05
14	6	1	60	62,53	62,086	68,3	67,45
15	6	24	60	64,49	64,321	68,5	67,8

RESULTS AND DISCUSSION

The Lipase Activity from Different Peels Composition

The lipase activity produced from pineapple and passion fruit peel waste was determined. The results presented in Fig.-1A showed that the activities increase gradually reaching a maximum of 3.8 ± 0.4 U/ml and 3.53 ± 0.2 U/ml for pineapple and passion fruit peels, respectively at day 5. The different compositions of pineapple and passion fruit peels showed better enzyme activity than a single one. As shown in Fig.- 1B, the enzyme lipase activities rise significantly on day 2 and some combinations detected gradually decrease in the following days except for the combination of 6:4 with the highest enzyme activity observed on day 3 of 7.02 ± 0.1 U/ml. Further, the activity has increased to 28.33 ± 0.0 after purification using the ammonium sulphate precipitation method.

Optimization of Enzymatic Transesterification of PO

The fatty acid content of PO was dominated by oleic and palmitic acids at 43.9% and 38.4%, respectively. The optimization of biodiesel production from enzymatic transesterification of PO using lipase enzyme isolated from fermentation of pineapple and passion fruit peels was established based on parameters of ratio molar, reaction time and temperature using the Box-Behnken design experiment. The experimental and predicted biodiesel conversion was presented in Table-2. Hence, a regression model (eqn.-2) derived from equation 1 could be established as presented in Table-1.

$$\text{Biodiesel Conversion (\%)} = 76.2 + (4.6 \text{ x } A) + (1.9 \text{ x } B) + (5.7 \text{ x } C) + (-0.6 \text{ x } AB) + (-2.3 \text{ x } AC) + (-0.8 \text{ x } BC) + (1.6 \text{ x } AA) + (-5.4 \text{ x } BB) + (-13.3 \text{ x } CC) \quad (2)$$

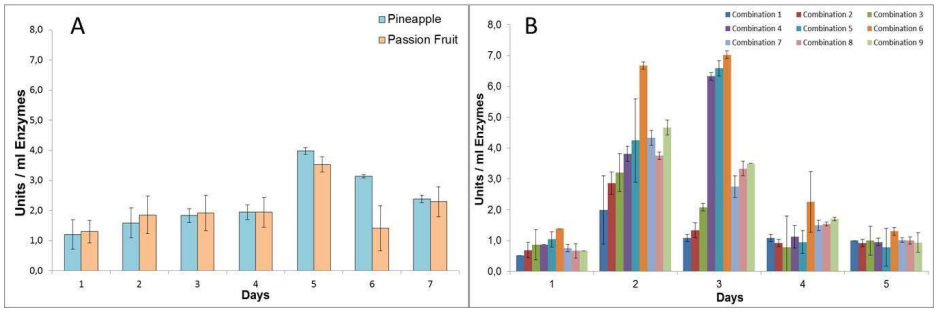

Fig.-1: The Enzyme Activities from the Fermentation of Pineapple and Passion Fruit Peels

The relationship between the predicted and observed biodiesel showed good linearity with a correlation coefficient (R^2) is 99.7%. The model indicates statistically significant as consisting of F- and p-values of 181.76 and 0.00009, respectively. Figure-2 is shown the 3D plot which describes the effect of two

independent variables on biodiesel conversion. Figure-2A showed the interaction effect of reaction time and ratio molar on biodiesel conversion, and it can be detected that increasing ratio molar and reaction time up to a particular value increases it. However, the conversion slowly decreases with the increase in reaction time. A similar graph trend is also observed in Fig.-2B as increasing reaction temperature could decrease the biodiesel conversion after reaching the peak. The influence of reaction time and temperature is shown in Fig.-2C. Enhancing both parameters after the equilibrium state will not enhance the biodiesel conversion. Hence, from this description, the optimum biodiesel conversion of 82.7% was achieved using a ratio molar of 1:9, 13.65 hours reaction time and temperature of 46.5°C.

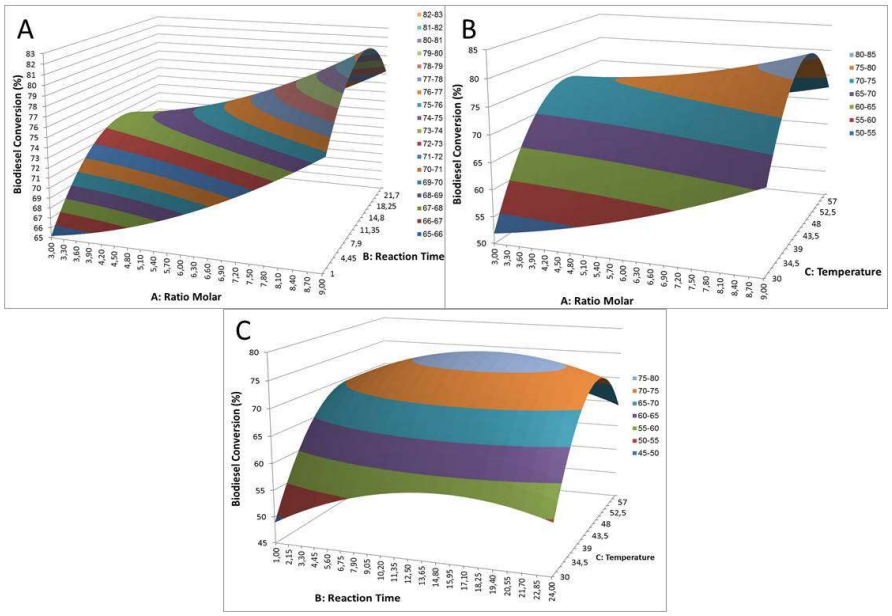

Fig-2: The 3D Graphics Interaction of (A) Ratio Molar and Reaction Time; (B) Ratio Molar and Temperature; and (C) Reaction Time and Temperature on Biodiesel Conversion of the Enzymatic Transesterification of PO.

Optimization of Enzymatic Transesterification of WCO

Lauric acid (35.2%) is the main fatty acid contained in WCO followed by oleic acid (14.7%). The 15 experimental conditions generated from the Box-Behnken design experiment were conducted to establish the optimum condition for enzymatic transesterification of WCO to biodiesel. The biodiesel conversion for each experiment is presented in Table-2 while the prediction conversion was obtained from the regression eqn-3.

$$\text{Biodiesel Conversion (\%)} = 81.8 + (2.9 \text{ x A}) + (2.4 \text{ x B}) + (3.6 \text{ x C}) + (-0{,}5 \text{ x AB}) + (0.1 \text{ x AC}) + (-2.2 \text{ x BC}) + (2.9 \text{ x AA}) + (-6.5 \text{ x BB}) + (-11.3 \text{ x CC}) \quad (3)$$

The analysis of variance indicates that the predictability of the model is at a 98.2% confidence interval. A p-value of 0.0008 depicts that the model is statistically significant. Figure-3A shows the 3D plot for the interaction effect between the ratio of molar and reaction time toward biodiesel conversion. It was observed for all ratio molar that increasing reaction time could increase the biodiesel conversion. Conversely, increasing reaction time could decrease biodiesel conversion. The interaction between the ratio of molar and temperature also shows a similar graph pattern as presented in Fig.-3B. Response surface plots in Fig.- 3C show that biodiesel conversion has a positive interaction with reaction time and temperature. The maximum biodiesel conversion was achieved in the central point of both factors and increasing the point resulted in a gradual decrease. The optimal levels of process variables for enzymatic transesterification of WCO are a ratio molar of 1:9, a reaction time of 14 hours and a temperature of 48°C. The predicted biodiesel conversion under this condition was 88%.

CONCLUSION

Lipase enzyme obtained from the fermentation combination of pineapple and passion fruit peels had an activity of 7.02 ± 0.1 U/ml which had increased to 28.33 ± 0.0 U/ml after purification using ammonium

sulphate precipitation method. The enzyme was used as a biocatalyst in the transesterification of PO and WCO to biodiesel. The Box-Behnken method identifies the significant effect of variables on biodiesel conversion for both PO and WCO.

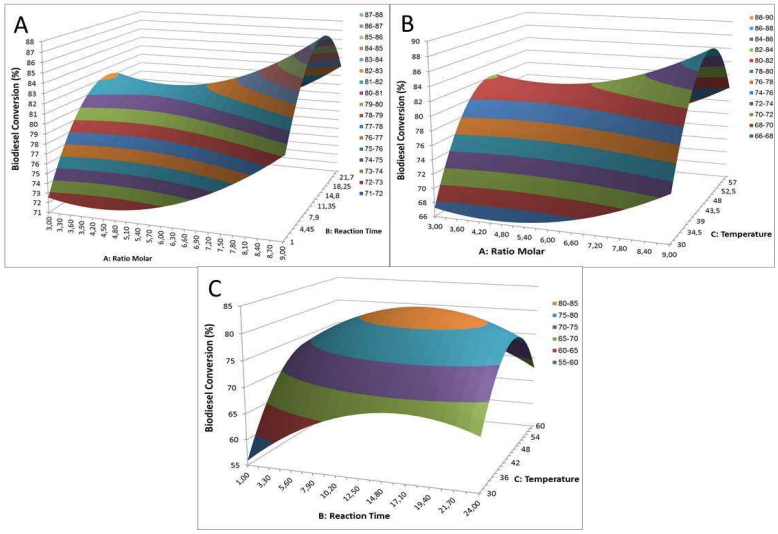

Fig.-3: Response Surface Graphics of (A) Ratio Molar and Reaction Time; (B) Ratio Molar and Temperature; and (C) Reaction Time and Temperature on Biodiesel Conversion of the Enzymatic Transesterification of WCO

The maximum conversion of 82.7% and 88% could be achieved using a reaction condition of ratio molar of 1:9, 13.65 hours reaction time and temperature of 46.5°C and a reaction condition of ratio molar of 1:9, a reaction time of 14 hours and temperature of 48°C for PO and WCO, respectively.

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CONFLICT OF INTERESTS

The authors declare that there is no conflict of interest.

AUTHOR CONTRIBUTIONS

All the authors contributed significantly to this manuscript, participated in reviewing/editing and approved the final draft for publication. The research profile of the authors can be verified from their ORCID ids, given below:

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