Transesterification of preheated coconut oil catalyzed by Novozym® 435: potential source of biofuel

Soma Chakraborty*, **Magin Benedict F. Ferrer**

Department of Chemistry, Ateneo de Manila University, School of Science and Engineering, Loyola Heights, Quezon City 1108 Philippines

*Author to whom correspondence should be addressed; email: schakraborty@ateneo.edu

ABSTRACT

Coconut oil preheated to 180 and 250°C was subjected to transesterification reaction in the presence of ethanol, catalyzed by Novozym® 435. Preheating did not change the density, viscosity, and composition of the oil. When the reaction was performed at 70°C, approximately 74% of the organic phase rich in fatty acid ester was obtained in 8h of reaction. A decrease in acid value was also observed as the transesterification reaction progressed. The pace of the reaction was much slower when the reaction was performed at room temperature. Approximately 70% organic phase was collected after 7 days of reaction. Novozym® 435 could be recycled 3 times to perform the transesterification reaction. The amount of glycerol produced by Novozym® 435 in the 2nd cycle was comparable to that produced by the fresh beads.

Keywords: Novozym® 435; transesterification; coconut oil; fatty acid ester

INTRODUCTION

The gradual depletion of fossil fuel resources paved the way for the search for alternative fuel sources. One such promising source is biodiesel. It is procured from renewable sources and hence can be considered a source of eco-friendly sustainable energy. It is biodegradable and produces a reduced amount of pollutants such as sulfur oxides, carbon oxides, and nitrogen oxides (Singh et al. 2020).

Since the energy content of biofuel is comparable with conventional fuels, it can be used as a complete replacement for conventional fuel or can be used as a mixture along with a conventional fuel (Tsolakis et al. 2003). Furthermore, no modification of the infrastructure of the storage or delivery system of conventional fuel is required as biodiesel can be pumped, stored, and handled similar to that of conventional fuels (Volpato et al. 2012).

Biodiesel is mono-alkyl esters of long-chain fatty acids produced by transesterification of a triglyceride of lipid. The plant-based lipid feedstock includes vegetable oils (Cerveró et al. 2008; Santori et al 2012; Ramkumar et al 2016), microalgae (Chisti 2007; Sugumar et al. 2020; Najjar et al. 2020; Karthikeyan et al. 2020) and cyanobacteria. (Sarsekeyeva et al. 2015; Uma et al. 2020).

As the oils derived from plants are required for other applications too, their use as the feedstock for biodiesel production will encroach into food, cosmetics, and other industries. Hence, there is a recent drive for exploring waste products such as animal rendering (Adewale et al. 2015; Toldrá-Reig et al. 2020), fish processing waste (Jayasinghe et al. 2012; Cardoso et al, 2019), and used cooking oils (de Araújo et al. 2013; Kawentar et al. 2013; Raqeeb et al. 2015; Pugazhendhi et al. 2020) as the feedstock for biodiesel production.

Disposal of used cooking oil is of concern as it serves as a contaminant for soil and water bodies (Kheang et al, 2006; Hanisah et al, 2013).   Similarly, animal fats with high-fat content pose a threat to aquatic flora and fauna. Thus, conversion of these wastes into commercial-grade biodiesel will not only alleviate the environmental damage but will also serve as sustained feedstock for biodiesel, minimizing the other feedstocks that are in demand to serve other requirements. Moreover, they will be less expensive than the traditional feedstocks as they are procured from discarded materials.

Most commercial biodiesel plants utilize base as the homogeneous catalyst for the transesterification reaction. Moreover, the reactions can be performed at lower temperatures resulting in a high reaction yield in a short time. However, the process suffers from several inherent drawbacks. It demands overall high energy (Singh et al. 2006), multi-step purification of the end product, and treatment of wastewater before disposal or reuse. Similar challenges exist if the base catalyst is replaced by an acid catalyst.

Recently solid heterogeneous catalysts (Issariyakul et al. 2007; Jacobson et al. 2008; Srilatha et al. 2010) are under investigation as they simplify the purification process and do not produce alkaline or acidic wastewater. An enzyme immobilized in a matrix can be considered as one such heterogeneous catalyst. The use of enzymes as the catalyst for transesterification has also been explored due to certain environmental and financial advantages over the conventional chemical method. Studies have revealed that enzymes can catalyze the reactions under mild conditions, generate fewer waste streams, and can convert triglycerides as well as free fatty acids into biodiesel (Christopher et al, 2014; Bajaj et al, 2010; Ghaly et al, 2010; Fjerbaek et al., 2009). Among several biocatalysts, lipases are widely studied as the catalyst for biodiesel production (Arumugam & Ponnusami 2017; Cavalcante et al. 2020; Santaraite et al. 2020).

This study aimed at exploring the potential of Novozym® 435 to catalyze transesterification reaction in preheated coconut oil. Novozym® 435 is the immobilized form of lipase B from *Candida antarctica* immobilized on Lewatit VP OC 1600 resin. Tupufia et al (2013) used lipase for the transesterification of coconut oil and studied the reaction kinetics under various conditions. Results showed that esterification reaction was more predominant and ultrasonication improved the conversion rate. The effect of various parameters such as temperature, amount of catalyst, and oil-to-ethanol ratio on transesterification of coconut oil by Novozym® 435 was investigated by Ribeiro et al (2014). Their study revealed that 80.5% conversion is feasible when the enzyme concentration was 7% and oil:ethanol was 1:10. Though there are some published studies on enzymatic transesterification of coconut oil, the use of preheated coconut oil, simulating the condition of used coconut oil as the feedstock for transesterification has not been explored yet, hence this work.

METHODS

Materials and Equipment. The reagents used were Minola® Premium coconut cooking oil purchased from a local market, and Novozym® 435 (immobilized, 10%w/w) obtained from Sigma Aldrich. Glycerol was a gift from Chemrez Technologies, Philippines. The fatty acid standards, caprylic acid, and myristic acid were purchased from Merck, lauric acid from SAFC, palmitic acid from Scharlu, and stearic acid from Himedia. Other chemicals were obtained from Alysons' Chemical Enterprises. Novozym® 435 was dried under vacuum in the presence of phosphorus pentoxide (P_2O_5) prior to use.

Gas Chromatography. The composition of the oil samples was determined by Shimadzu GC 2010 Gas Chromatograph with an AOC-20i autoinjector, flame-ionized detector, and He carrier gas. Fatty acid esters of caprylic (C8), capric (C10) lauric (C12), myristic (14), palmitic (C16), and stearic acid (C18) were chosen to study the composition of the oils. These are the fatty acids found in coconut oil along with oleic acid and linoleic acid.

Fourier-Transform Infrared Spectroscopy. FTIR analyses were performed to check the purity and composition of the glycerol and organic phase formed after transesterification reaction using Shimadzu IR Affinity-1 FTIR8400S at a resolution of 4.0 cm^{-1} at 40 scans conducted per sample. Data were processed using Shimadzu IR Solution 1.30.

Preheated Coconut Oil (PCO). Minola coconut cooking oil (100mL) was transferred to a 250-mL beaker and heated at 180 ℃ (PCO180) in a silicone oil bath for 1h under constant stirring. A similar setup was used to heat the cooking oil at 250 ℃ (PCO250).

Characterization of preheated and unheated coconut oil. Acid value (AV) was determined by titrating the oil with ethanolic KOH, using phenolphthalein as the indicator. AV represents the mass of base, KOH, needed to neutralize the fatty acid of the oil. Oil density was measured using the pycnometer and the intrinsic viscosity was measured using an Ubbelohde viscometer with chloroform as the solvent. These measurements were carried out at room temperature.

Reaction at 70℃. Ethanol was used as the acyl acceptor. Ethanol causes less lipase activity inactivation than methyl alcohol (Costa-Silva et al. 2017). Five mL PCO was transferred to a round bottom flask and heated to 70 ℃ under constant stirring. After 10 min, 5 mL ethanol (oil:ethanol =1:8 by mol) and 1.173g of dry Novozym® 435 were transferred to the round bottom flask and were sealed using a rubber septum. The transesterification reaction was allowed to continue for a certain time interval. To terminate the reaction, 15 mL chloroform was added to the product and was vacuum-filtered to remove the enzyme beads. Chloroform was removed from the reaction mixture by rotary evaporation. Once chloroform was removed, the product was transferred to a separatory funnel, 1 mL of glycerol was added, and vigorously shaken to initiate phase separation. The two separated layers were transferred into two separate dry vials and were stored for further characterization.

Reaction at room temperature. The reaction and the workup procedures were similar to that adopted for the reaction at 70 ℃, except that the reaction was performed at room temperature for several days.

Reaction using recycled Novozym® 435. Novozym® 435 beads retrieved after vacuum filtration was washed with chloroform 2-3 times and then vacuum-dried once more in the presence of dry P_2O_5 and then were used to catalyze the transesterification reaction for PCO250. The reaction procedure was the same as that of the transesterification performed using fresh Novozym® 435 beads at 70 ℃.

RESULTS AND DISCUSSION

Comparison of properties and composition of unheated and preheated coconut oil. The Minola Premium coconut oil (100mL) was heated to 180 °C and 250 °C to simulate the cooking and smoking temperatures respectively. Table 1 summarizes the properties of heated and unheated oil.

Table 1. Summary of Physical Properties of Unheated and Preheated Oil[a]

Preheating Temperature	Unheated	180 °C	250 °C
Density (g · mL^{-1})	0.93 ±0.01	0.92 ±0.01	0.92 ±0.01
Viscosity (mL · g^{-1})	1.48 ±0.03	1.50 ±0.05	1.53 ±0.02
AV (mg KOH · g^{-1})	0.12 ±0.01	0.23 ±0.01	0.27 ±0.01

[a] Experiments were performed in triplicate to determine the standard deviation

The density and the viscosity of heated and unheated oil are comparable, which assures that heating coconut oil up to 250 °C does not affect the properties of coconut oil adversely. An increase in AV was observed for the heated samples, indicating that hydrolysis of the oil takes place at a higher temperature, producing FFA.

GC was used to compare the composition of the coconut oil heated to 250 °C with that of unheated oil. A comparison of the chromatograms in Figures 1(a) and (b) reveals that no fatty acid was lost during the heating process. Table 2, which compares the % fatty acid composition of the unheated and preheated coconut oil further shows that no significant change in the composition of the oil was observed before and after heating the oil to 250 °C. Since the fatty acids are not lost during the heating process, the oil already used for cooking purposes can be explored as an alternative feedstock for biodiesel production.
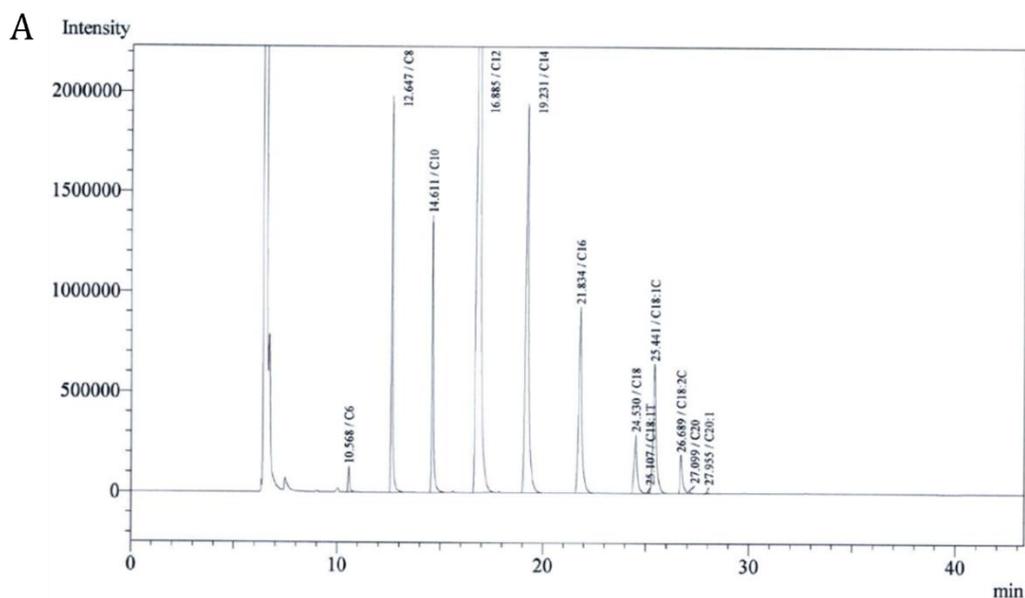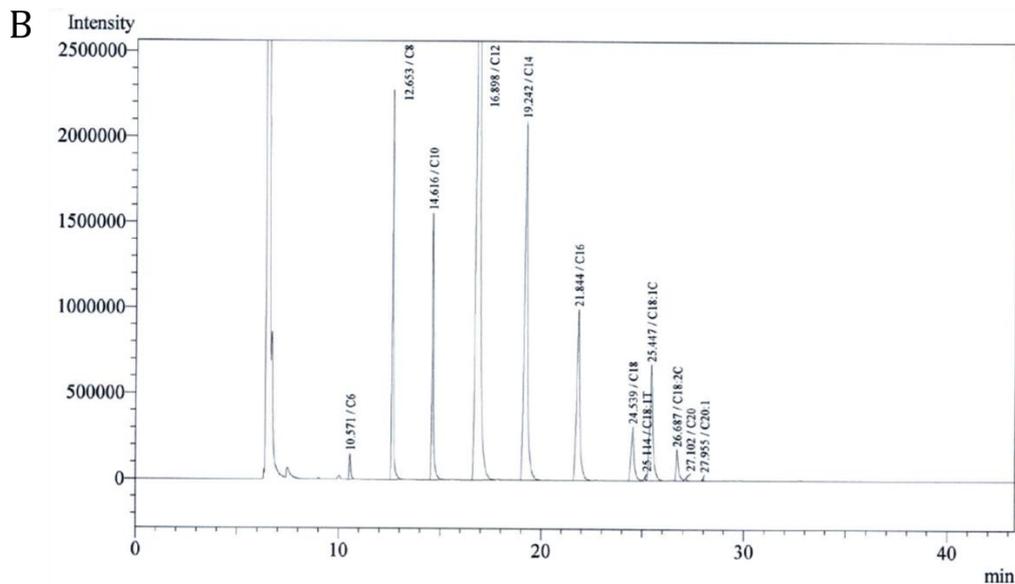
Figure 1. A) Gas chromatogram of unheated coconut oil; B) Gas chromatogram of 250 °C preheated coconut oil.

Table 1. GC profile of unheated and preheated coconut oil before transesterification

	Fatty Acid Composition (%)	
Carbon Chain	**Unheated**	**PCO 250**
C8	8.22	8.36
C12	48.00	48.31
C14	17.82	17.75
C16	8.65	8.60
C18	2.67	2.65

Transesterification reaction in the presence of ethanol. Transesterification of the PCOs with ethanol leads to the formation of glycerol and an organic phase, which consists of a mixture of fatty acid esters. The reaction scheme is shown in Figure 2. Glycerol settles at the bottom layer whereas the organic layer remains on the top in the separatory funnel.
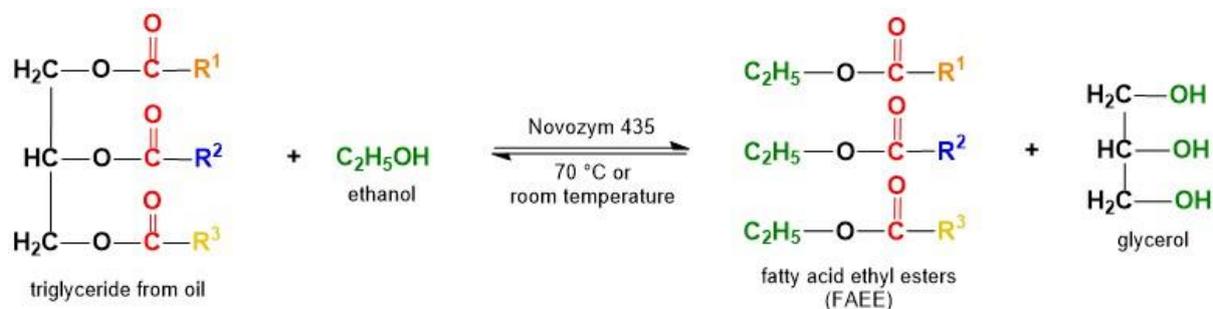

Figure 2 Schematic representation of transesterification reaction of coconut oil and ethanol catalyzed by Novozym® 435.

FTIR spectra of the glycerol phase and the organic phase collected after the reaction are given in Figures 3 and 4, respectively. Figure 3 shows a broad peak at 3000-3600 cm^{-1}, for the -OH groups. The C-H stretch peaks at 2886 cm^{-1} and 2936 cm^{-1}, and the C-O stretch at 1416 cm^{-1} indicate the presence of a carbon backbone confirming the production of glycerol. Figure 4 has two strong

peaks at 2855 cm^{-1} and 2924 cm^{-1} indicative of C-H stretching from the multiple C-H bonds of long-chain ethyl esters. C=O and C-O stretching are also present at 1740 and 1178 cm^{-1}, respectively, indicating the presence of the ester group. The presence of these peaks confirms that the transesterification reaction was performed successfully by Novozym® 435.
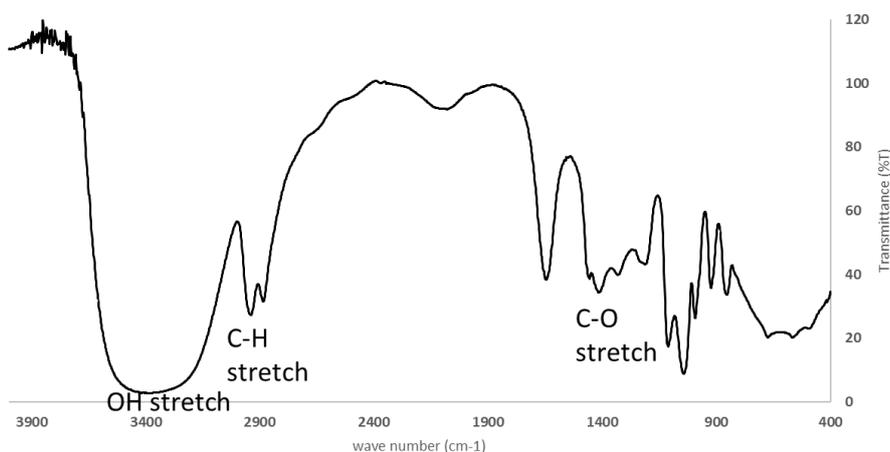

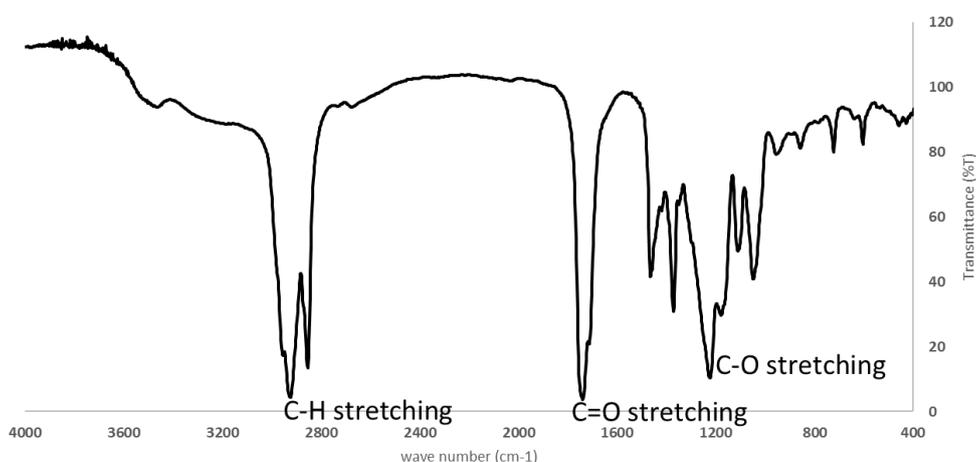Figure 3. FTIR spectrum of the glycerol phase after transesterification

Figure 4. FTIR spectrum of the organic phase after transesterification

Reaction at 70°C. As seen in Figure 5, transesterification reactions when performed at 70 °C, produced a significant amount of fatty acid ester and glycerol, an indication that Novozym® 435 can be explored as an alternative catalyst for biofuel production. The extent of the transesterification reaction was accessed in terms of the amount of glycerol produced, assuming that if the transesterification reaction progresses in the forward direction, more glycerol will be produced as one of the products. Glycerol that was collected after the reaction using a separatory funnel was transferred to a measuring cylinder to determine the volume of glycerol collected, which was plotted as the function of reaction time. The transesterification reaction of coconut oil preheated to 180 °C and 250 °C for 2h produced approximately 1.5mL of glycerol from 5mL of PCO. However, no significant increment in the amount of glycerol was observed when the reaction time was extended beyond 2h. Perhaps the equilibrium was reached within 2h. The amount of glycerol produced was comparable for PCO180 and PCO250. This result suggests that coconut oil heated up to as high as its smoking point can also serve as the feedstock for biofuel production.
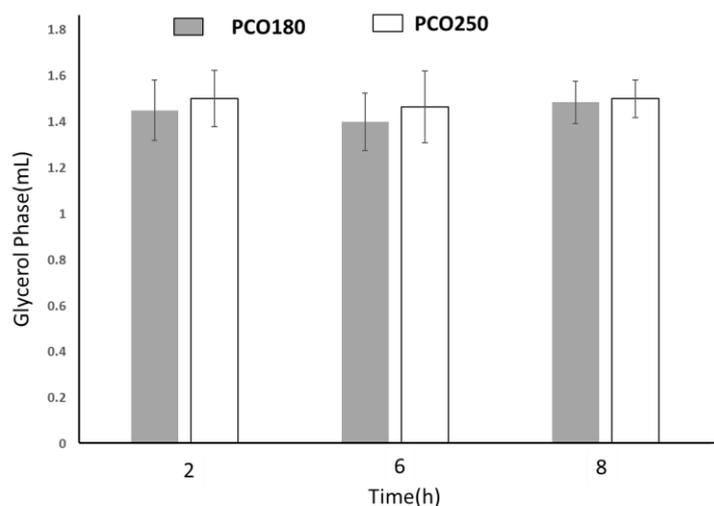
Figure 5. Amount of glycerol from transesterification reaction performed at 70 ˚C.
(Experiments were performed in triplicate to determine the standard deviation.)

Figure 6 reveals that AV decreased as the reaction proceeded. The decline was more pronounced for the oil heated up to 250 ˚C. Most probably instead of directly converting triglycerides to esters, the enzyme first converted them into FFAs, which eventually get converted to FAEEs as the reaction progresses. Hence the acid value decreased with time. However further investigation is required to verify this hypothesis.
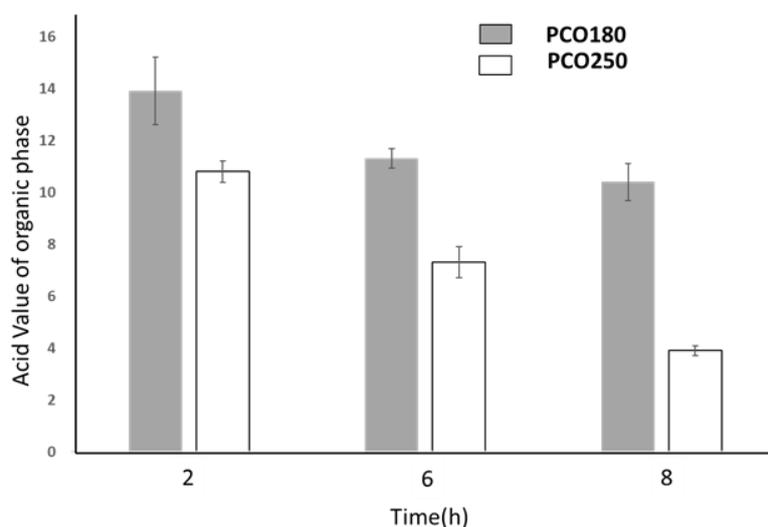

Figure 6. Acid Value (AV) of the organic phase of transesterification reaction performed at 70°C. (Experiments were performed in triplicate to determine the standard deviation.)

The amount of FAEE produced was quantified using Eqns 1 and 2, where the peak area refers to the area corresponding to the peaks of the fatty acid esters in the chromatogram of gas chromatography.

$$Response\ Factor\ (RF_{std}) = \frac{amount\ of\ sample_{std}}{peak\ area_{std}} \tag{1}$$

$$Ethyl\ Ester\ Content = peak\ area_{reaction} * RF_{std} \tag{2}$$

Table 3 tabulates the ethyl ester content of 5 types of FFAE generated from the transesterification of PCO180 and PCO250. The amount of esters produced by PCO180 was more than that produced by PCO250. Furthermore, it was observed for both PCO180 and PCO250 that the amount of FFAE increased when the transesterification reaction was extended from 2h to 4h and decreased when the reaction time was extended to 6h. Probably after 6h, the enzyme engages in a reverse hydrolysis reaction due to the lack of adequate FFAE substrate. Such reverse hydrolysis by Novozym® 435 has been reported in the past (Kobayashi et al. 2000).

Table 3. Ethyl ester content of transesterified samples

Sample	FFAE	Ethyl Ester content (µmol)		
		2h	**4h**	**6h**
PCO180	C8	271.7	262.0	245.0
	C12	593.3	650.0	603.1
	C14	132.6	142.5	129.6
	C16	81.1	90.34	75.5
	C18	92.5	116.0	84.8
PCO250	C8	87.1	92.1	54.6
	C12	183.6	200.9	115.7
	C14	38.0	40.9	23.9
	C16	26.2	28.2	16.5
	C18	41.5	46.8	28.1

Reaction at room temperature. Transesterification reactions were also performed at room temperature to explore the potential of Novozym® 435 to catalyze the reactions with minimal energy input in the form of heat. Reactions were performed at room temperature for 1, 4, and 7 days. Results showed (Figure 7) that Novozym® 435 was able to catalyze the reactions at room temperature. Approximately 1.2 mL of glycerol was produced in 1 day. A marginal increment in its amount was observed when the reaction was performed for 4 days. Prolonged reaction time to produce a significant amount of glycerol suggested that a slower reaction rate at room temperature was observed. Similarly, Figure 8 points out that AV decreases with an increase in reaction time, though the decline is less pronounced than that at 70°C due to a slower reaction rate.
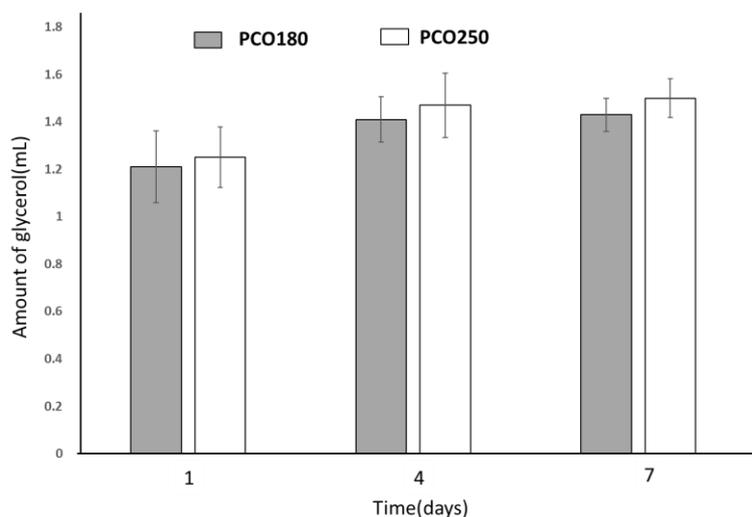
Figure 7. Amount of glycerol from transesterification reaction performed at room temperature. (Experiments were performed in triplicate to determine the standard deviation.)
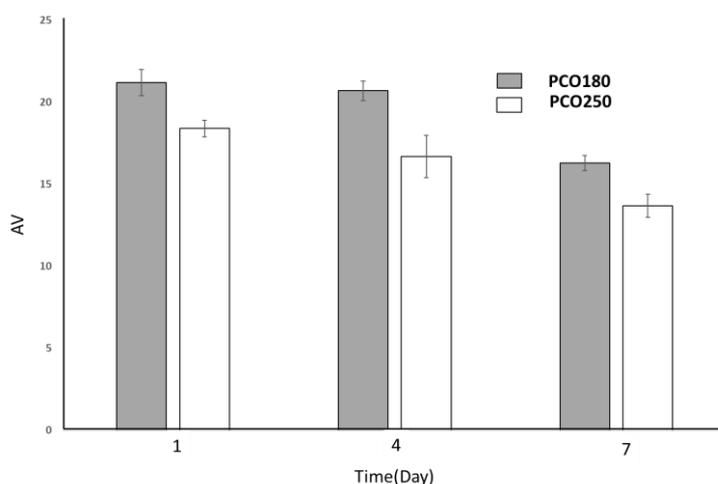

Figure 8. Acid value (AV) of organic phase of transesterification reaction performed at room temperature. (Experiments were performed in triplicate to determine the standard deviation.)

Recyclability of Novozym® 435. Since Novozym® 435 beads are the immobilized form of the active enzyme *Candida antarctica* lipase B, the beads can be recovered at the end of each reaction. If these recovered beads can catalyze similar reactions, then several batches of transesterification reactions can be performed with the same enzyme beads. To evaluate the recyclability of the biocatalyst, Novozym® 435 beads recovered after the completion of a reaction were dried under a vacuum and then reused for successive sets of reactions. Figure 9 compares the amount of glycerol produced by the catalyst beads when used for the 1st, 2nd, 3rd, and 4th time. Almost the same amount of glycerol was produced after the 1st and 2nd uses, assuring that the catalytic activity of lipase in the beads is still retained after the 1st utilization. However, a drop in the amount of glycerol was observed when the beads were used for the 3rd and 4th time to catalyze the transesterification reaction. Similarly, a decrease in the AV value was also detected after the 2nd use (Figure 10) proving that the catalytic efficacy of Novozym® 435 beads decline after the 2nd cycle. Most likely the glycerol produced during the reaction penetrates the microporous beads and influences the catalytic activity. Such a problem has been reported by Talukder et al (2009). Thus, an efficient washing process is recommended to enhance the catalytic activity of the recycled enzymes.
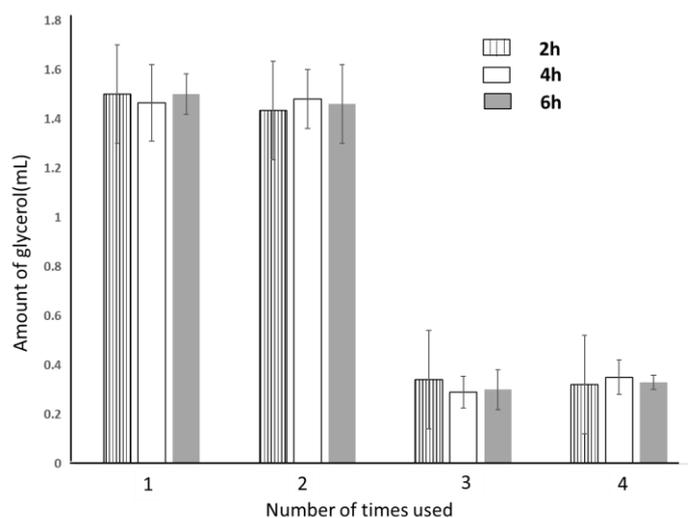
Figure 9. Amount of glycerol produced from transesterification reaction performed at 70°C using fresh and recycled Novozym® 435 beads. (Experiments were performed in triplicate to determine the standard deviation.)
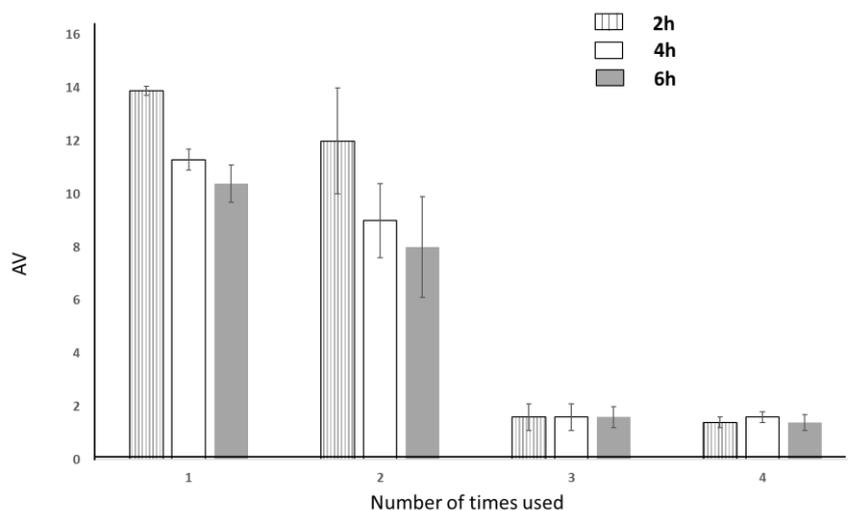

Figure 10. Acid Value (AV) of the organic phase of transesterification reaction performed at 70°C using fresh and recycled Novozym® 435 beads. (Experiments were performed in triplicate to determine the standard deviation.)

CONCLUSIONS

It was observed that the composition and the properties of coconut oil are not compromised when heated up to its smoking point. The study proved that preheated coconut oil, simulating cooking conditions, can undergo transesterification producing a significant amount of fatty acid ester, which is the main component of biofuel. The transesterification reactions were catalyzed by Novozym® 435 in the presence of ethanol. The reactions were feasible at 70°C as well as at room temperature though the rate of reaction was much slower at room temperature. The Novozym® 435 beads could be used efficiently for 2 cycles for the transesterification reaction where a decrease in catalytic efficacy was observed in the 3rd and 4th cycles. The results point to the possibility of exploring used coconut cooking oil as feedstock for biofuel production.

Furthermore, the use of fresh and recycled enzymes as catalysts suggests an eco-friendly process for biofuel production.

ACKNOWLEDGEMENTS

REFERENCES

Adewale P, Dumont M-J, Ngadi M. Recent trends of biodiesel production from animal fat wastes and associated production techniques. Renew Sustain Energy Rev. 2015 May; 45:574-588. https://doi.org/10.1016/j.rser.2015.02.039

Arumugam A, Ponnusami V. Production of biodiesel by enzymatic transesterification of waste sardine oil and evaluation of its engine performance. Heliyon. 2017 Dec; 3(12):e00486. https://doi.org/10.1016/j.heliyon.2017.e00486

Bajaj P, Lohan P, Jha PN, Mehrotra R. Biodiesel production through lipase catalyzed transesterification: An overview. J Mol Catal B Enzym. 2010 Jan; 62(1):9-14. https://doi.org/10.1016/j.molcatb.2009.09.018

Cardoso LdC, de Almeida FNC, Souza GK, Asanom IY, Pereira NC. Synthesis and optimization of ethyl esters from fish oil waste for biodiesel production. Renew Energy. 2019 April; 133:743-748. https://doi.org/10.1016/j.renene.2018.10.081

Cavalcante FTT, Neto FS, de Aguiar Falcao IR, da Silva Souza JE, de Moura Jr. LS, da Silva Sousa P, Rocha TG, de Sousa IG, de Lima Gomes PH, de Souza MCM, dos Santos JCS. Opportunities for improving biodiesel production via lipase catalysis. Fuel. 2021 Mar; 288:119577. https://doi.org/10.1016/j.fuel.2020.119577

Cerveró JM, Coca J, Luque S. Production of biodiesel from vegetable oils , Grasas Y Aceites, 2008 Mar; 59(1):76-83. https://doi.org/10.3989/gya.2008.v59.i1.494

Chisti Y. Biodiesel from microalgae. Biotechnol Adv. 2007 May; 25(3):294-306. https://doi.org/10.1016/j.biotechadv.2007.02.001

Christopher LP, Kumar H, Zambare VP. Enzymatic biodiesel: Challenges and opportunities. Appl Energy. 2014 Apr; 119:497-520. https://doi.org/10.1016/j.apenergy.2014.01.017

Costa-Silva TA, Carvalho AKF, Souza CRF, De Castro HF, Said S, Oliveira WP, Enzymatic transesterification of coconut oil using chitosan-immobilized lipase produced by fluidized-bed system. Energy Fuels. 2017 Nov; 31(11):12209-12216. https://doi.org/10.1021/acs.energyfuels.7b02033

De Araújo CDM, de Andrade CC, E Silva ES, Dupas FA. Biodiesel production from used cooking oil: A review. Renew Sustain Energy Rev. 2013 Nov; 27:445-452. https://doi.org/10.1016/j.rser.2013.06.014

Fjerbaek L , Christensen KV, Norddahl B. A review of the current state of biodiesel production using enzymatic transesterification. Biotechnol Bioeng. 2009 Apr; 102(5):1298-315. https://doi.org/10.1002/bit.22256

Ghaly AE, Dave D, Brooks MS, Budge S. Production of biodiesel by enzymatic transesterification: Review. Am J Biochem Biotechnol. 2010 June; 6(2):54-76. https://doi.org/10.3844/ajbbsp.2010.54.76

Hanisah K, Kumar S, Tajul AY. The management of waste cooking oil: A preliminary survey. Health Environ J. 2013; 4(1):76-81. Available from https://www.researchgate.net/publication/320823701_The_Management_of_Waste_Cooking_Oil_A_Preliminary_Survey

Kawentar WA, Budiman A. Synthesis of biodiesel from second-used cooking oil. Energy Procedia. 2013; 32:190–199. https://doi.org/10.1016/j.egypro.2013.05.025

Kobayashi S, Uyama H, Takamoto T. Lipase-catalyzed degradation of polyesters in organic solvents. A new methodology of polymer recycling using enzyme as catalyst. Biomacromol. 2000 Feb; 1(1):3–5. https://doi.org/10.1021/bm990007c

Issariyakul T, Kulkarni MG, Dalai AK. Bakhshi NN. Production of biodiesel from waste fryer grease using mixed methanol/ethanol system. Fuel Process Technol. 2007 May: 88(5):429-436. https://doi.org/10.1016/j.fuproc.2006.04.007

Jacobson K, Gopinath R, Meher LC, Dalai Ak. Solid acid catalyzed biodiesel production from waste cooking oil, Appl Catal B: Environ. 2008 Dec; 85:86-91. https://doi.org/10.1016/j.apcatb.2008.07.005

Jayasinghe P, Hawboldt K. A review of bio-oils from waste biomass: Focus on fish processing waste. Renew Sustain Energy Rev. 2012 Jan; 16(1):798-821. https://doi.org/10.1016/j.rser.2011.09.005

Karthikeyan S, Periyasamy M, Prathima A. Biodiesel from microalgae: Environmental aspects. Mater Today: Proc. 2020; 33:3664-3667. https://doi.org/10.1016/j.matpr.2020.05.779

Kheang LS, May CY, Foon CS, Ngan MA. Recovery and conversion of palm olein-derived used frying oil to methyl esters for biodiesel. J Oil Palm Res. 2006 June; 18:247–252. Available from https://www.researchgate.net/publication/260639865_Recovery_and_conversion_of_palm_olein-derived_used_frying_oil_to_methyl_esters_for_biodiesel

Najjar YSH, Abu-Shamleh A. Harvesting of microalgae by centrifugation for biodiesel production: A review. Algal Res. 2020 Oct; 51:102046. https://doi.org/10.1016/j.algal.2020.102046

Pugazhendhi A, Alagumalai A, Mathimani T, Atabani AE. Optimization, kinetic and thermodynamic studies on sustainable biodiesel production from waste cooking oil: An Indian perspective. Fuel. 2020 Aug; 273:117725. https://doi.org/10.1016/j.fuel.2020.117725

Ramkumar S, Kirubakaran V. Biodiesel from vegetable oil as alternate fuel for C.I engine and feasibility study of thermal cracking: A critical review. Energ Convers Manage. 2016 June; 118:155-169. https://doi.org/10.1016/j.enconman.2016.03.071

Raqeeb MA, Bhargavi R. Biodiesel production from waste cooking oil. J Chem Pharm Res. 2015; 7(12):670-681.   https://www.jocpr.com/articles/biodiesel-production-from-waste-cooking-oil.pdf

Ribeiro LMO, Silva AE, Silva MCS, Almeida RMRG. A Study on ethanolysis and methanolysis of coconut oil for enzymatically catalyzed production of biodiesel. J Sustain Bioenergy Syst. 2014 Dec; 4: 215-224. https://doi.org/10.4236/jsbs.2014.44020

Santori G, Di Nicola G, Moglie M, Polonara F. A review analyzing the industrial biodiesel production practice starting from vegetable oil refining. Appl Energy. 2012 April; 92:109-132. https://doi.org/10.1016/j.apenergy.2011.10.031

Santaraite M, Sendzikiene E, Makareviciene V, Kazancev K. Biodiesel production by lipase-catalyzed in situ transesterification of rapeseed oil containing a high free fatty acid content with ethanol in diesel fuel media. Energies 2020 May; 13:2588. https://doi.org/10.3390/en13102588

Sarsekeyeva F, Zayadan BK, Usserbaeva A, Bedbenov VS, Sinetova MA,   Los DA. Cyanofuels: biofuels from cyanobacteria. Reality and perspectives. Photosynth Res 2015 Feb; 125: 329–340. https://doi.org/10.1007/s11120-015-0103-3

Singh D, Sharma D, Soni SL, Sharma S, Sharma PK, Jhalani A. A review on feedstocks, production processes, and yield for different generations of biodiesel. Fuel 2020 Feb; 15:116553. https://doi.org/10.1016/j.fuel.2019.116553

Singh A, He B, Thompson J, Van Gerpen J. Process optimization of biodiesel production using alkaline catalysts. Appl Eng Agric. 2006; 22(4):597-600. https://doi.org/10.13031/2013.21213

Srilatha K, Issariyakul T, Lingaiah N, Prasad PSS, Kozinski J, Dalai AK. Efficient esterification and transesterification of used cooking oil using 12-tungstophosphoric acid (TPA)/Nb2O5 catalyst. Energy Fuels 2010 Feb; 24(9):4748-4755. https://doi.org/10.1021/ef901307w

Sugumar R, Rajesh R, Kannan P, Venkatesan P. Biodiesel production from the biomass of *Dunaliella salina* green microalgae using organic solvent. Mater Today: Proc. 2020; 33(7):3260-3264. https://doi.org/10.1016/j.matpr.2020.04.652

Talukder MMR, Wu JC, Nguyen TBV, Fen NM, Melissa YLS. Novozym 435 for production of biodiesel from unrefined palm oil: Comparison of methanolysis methods. J Mol Catal B Enzym. 2009 Oct; 60(3-4):106-112. https://doi.org/10.1016/j.molcatb.2009.04.004

Toldrá-Reig F, Mora L, Toldrá F. Trends in biodiesel production from animal. Waste Appl Sci. 2020 May;10:3644. https://doi.org/10.3390/app10103644

Tsolakis A, Megaritis A, Wyszynski ML. Application of exhaust gas fuel Reforming in compression ignition engines fueled by diesel and biodiesel fuel mixtures. Energy Fuels. 2003 Sept; 17(6):1464–1473. https://doi.org/10.1021/ef0300693

Tupufia SC, Jeon YJ, Marquis C, Adesina AA, Rogers PL. Enzymatic conversion of coconut oil for biodiesel production. Fuel Process Technol. 2013 Feb; 106:721-726. https://doi.org/10.1016/j.fuproc.2012.10.007

Uma VS, Gnanasekaran D, Lakshmanan U,   Dharmar P. Survey and isolation of marine cyanobacteria from eastern coast of India as a biodiesel feedstock. Biocatal Agric Biotechnol. 2020 Mar; 24:101541. https://doi.org/10.1016/j.bcab.2020.101541

Volpato CES, Conde AP, Barbosa JA, Salvador N. Performance of cycle diesel engine using biodiesel of olive oil. Agric Eng Ciênc Agrotec. 2012 June; 36(3):348-353. https://doi.org/10.1590/S1413-70542012000300011