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Research Article

Production of Chemoenzymatic Catalyzed Monoepoxide Biolubricant: Optimization and Physicochemical Characteristics

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Linoleic acid (LA) is converted to per-carboxylic acid catalyzed by an immobilized lipase from *Candida antarctica* (Novozym 435). This per-carboxylic acid is only intermediate and epoxidized itself in good yields and almost without consecutive reactions. Monoepoxide linoleic acid 9(12)-10(13)-monoepoxy 12(9)-octadecanoic acid (MEOA) was optimized using D-optimal design. At optimum conditions, higher yield% (82.14) and medium oxirane oxygen content (OOC) (4.91%) of MEOA were predicted at 15 μL of H_2O_2, 120 mg of Novozym 435, and 7 h of reaction time. In order to develop better-quality biolubricants, pour point (PP), flash point (FP), viscosity index (VI), and oxidative stability (OT) were determined for LA and MEOA. The results showed that MEOA exhibited good low-temperature behavior with PP of −41°C. FP of MEOA increased to 128°C comparing with 115°C of LA. In a similar fashion, VI for LA was 224 generally several hundred centistokes (cSt) more viscous than MEOA 130.8. The ability of a substance to resist oxidative degradation is another important property for biolubricants. Therefore, LA and MEOA were screened to measure their OT which was observed at 189 and 168°C, respectively.

1. Introduction

Nowadays, people are concerned about green technology that is more environmental friendly and can save our environment. Green technology concept is good for industry which can be realized with sustainable and renewable resources. Epoxidized oils are currently produced by epoxidation of unsaturated plant oils, soybean, linseed oil [1], and *Jatropha curcas* seed oil [2]. Although there are several methods available to epoxidize the double bonds of unsaturated fatty acids, the only method applied on industrial scale is the Prileshajev epoxidation reaction. In this reaction, a peracid from a short-chain fatty acid and hydrogen peroxide under strong acidic conditions is used as the oxidizing agent. The presence of the strong acid in the reaction mixture, however, causes the formation of side products such as vicinal diols, estolides, and other dimmers. Although a carful choice of the peracid and the reaction conditions can help to minimize the epoxides loss, the selectivity of industrial epoxidation of plant oils rarely exceeds 80% [1]. Monoepoxidized oil has gained widely application including functional fluids, fuel additives, polyol replacements, pharmaceutical molecules, reactive diluents in cationic and UV cure applications, surfactants, adhesives, sealants, coatings, and biolubricants [3].

The current used of epoxy biolubricant is costly and harmful to environment. This study will be one of the solutions to obtain cheaper biolubricant to replace the existing epoxy biolubricant. A milder and more selective epoxidation process has been suggested [4], wherein a lipase is used to catalyze the peracid formation. In the lipase-mediated epoxidation of unsaturation fatty acid, the acid itself reacts with hydrogen peroxide to form the peracid, which then epoxidized the double bond. The reaction is therefore often referred to as "self-epoxidation reaction," in spite of the fact that the second step proceeds predominantly via an intermolecular process [5]. Among the various lipases studied so far, Novozym 435, a commercial preparation of *Candida Antarctica* lipase B, has been shown to be the most effective. Most of the investigations have involved dilution of the substrate in organic solvent. Recently, lipase-mediated

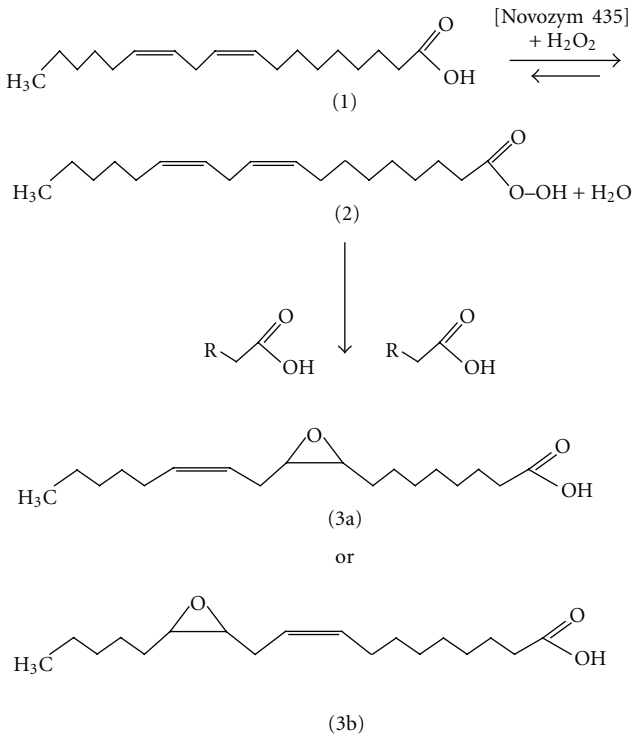

Figure 1: Chemoenzymatic MEOA. Notes: linoleic acid (2); perlinoleic acid (3); 9-10-monoepoxy 12-octadecenoic acid (3a); 12-13-monoepoxy 9-octadecenoic acid (3b).

epoxidation in a solvent-free medium has also been reported [6].

This study focuses on the physicochemical characteristics such as pour point (PP), flash point (FP), viscosity index (VI), and oxidative stability (OT) of the monoepoxidation process, with the aim to determine optimal reaction conditions with regard to reaction efficiency and enzyme stability using D-optimal design. In this paper, producing of MEOA over diepoxide as biolubricants has been studied for improvement of the physicochemical characteristics (i.e., PP, FP, and VI). Linoleic acid (LA; 18 : 2) was used as the model substrate. Figure 1 demonstrates the scheme for the self-epoxidation reaction of LA. Monoepoxidation of LA results in the mixture of two monoepoxides (cis-9, 10-epoxy 12 c-18 : 1 and cis-12, 13 epoxy 9 c- 18 : 1).

2. Methodology

2.1. Experimental Procedure. The enzymatic monoepoxidation was carried out using Novozym 435, a commercial catalyst made up of lipase, from *Candida antartica*, immobilized on a polyacrylate resin. 3 Factors (variables) such as hydrogen peroxide (μL, X_1), enzyme (mg, X_2), and ration time (h, X_3) were obtained according to [6]. Table 1 shows different ratios of hydrogen peroxide, enzyme, and reaction time using D-optimal design. In a typical chemoenzymatic monoepoxidation of LA 9(12)-10(13)-monoepoxy 12(9)-octadecanoic acid (MEOA), the LA (1.4 g) was dissolved in 10 mL of toluene, and the lipase was added. After stirring

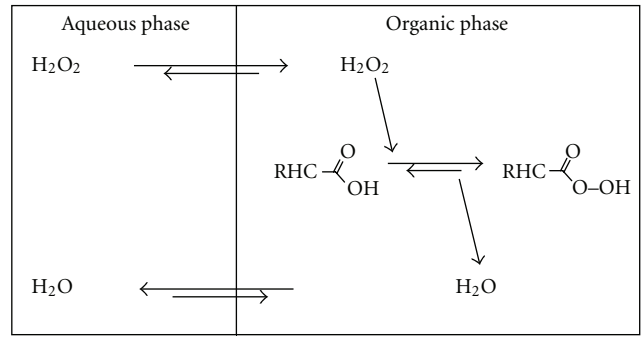

Figure 2: Schematic presentation of the mass transport of hydrogen peroxide and water in an organic-water biphasic system.

Table 1: Independent variables and their levels for D-optimal design of the MEOA reaction.

Independent variables		Variable levels		
		−1	0	+1
H_2O_2 (μL)	X_1	15	17.5	20
Enzyme (mg)	X_2	80	100	120
Time (h)	X_3	6	7	8

for 15 min, 30% H_2O_2 were added, and every 15 min the addition was repeated. Afterwards, the lipase was removed by filtration, the mixture was washed with water to remove excess H_2O_2, and the organic phase was dried over anhydrous sodium sulfate, and solvent was evaporated in a vacuum rotary evaporator.

2.1.1. Yield%. After the laboratory reaction was completed, the actual yield to the theoretical yield was compared to determine the percent yield

$$\frac{\text{actual yield (in grams)}}{\text{theoretical yield (in grams)}} \times 100\% = \text{percent yield.} \quad (1)$$

2.1.2. Oxirane Oxygen Content. OOC% was calculated according to [7].

2.1.3. Iodine Value. Iodine value was determined according to [8].

2.2. Experimental Design and Statistical Analysis. To explore the effect of the operation variables on the response in the region of investigation, a D-optimal design at three levels was performed. Hydrogen peroxide (μL, X_1), amount of enzyme (mg, X_2), and reaction time (h, X_3) were selected as independent variables. The range of values and coded levels of the variables are given in Table 1.

A polynomial equation was used to predict the response as a function of independent variables and their interactions. In this work, the number of independent variables was three,

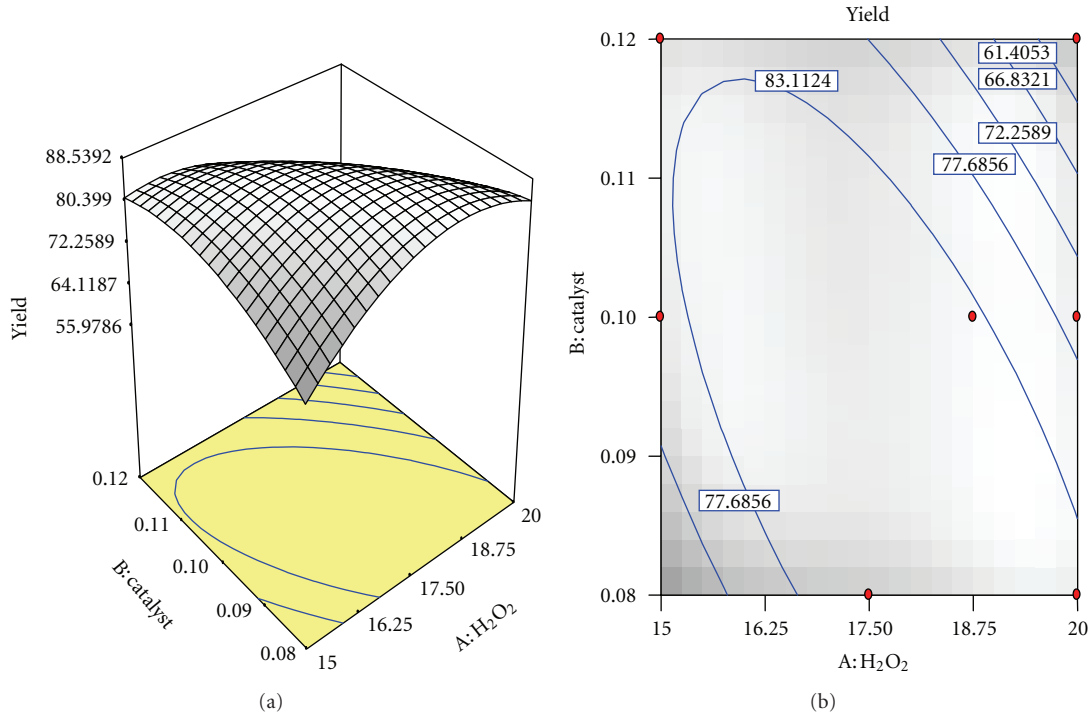

Figure 3: Response surface (a) and contour plots (b) for the effect of the H_2O_2 (X_1, μL) and catalysts Novozym 435 (X_2, mg) on the yield% of MEOA.

and therefore the response for the quadratic polynomials becomes

$$Y = \beta_0 + \sum \beta_i x_i + \sum \beta_{ii} x_i^2 + \sum \sum \beta_{ij} x_i x_j, \quad (2)$$

where β_0; β_i; β_{ii}; β_{ij} are constant, linear, square, and interaction regression coefficient terms, respectively, and x_i and x_j are independent variables. The Minitab software version 14 (Minitab Inc., USA) was used for multiple regression analysis, analysis of variance (ANOVA), and analysis of ridge maximum of data in the response surface regression (RSREG) procedure. The goodness of fit of the model was evaluated by the coefficient of determination R^2 and its statistical significance that was checked by the F-test.

2.3. Physicochemical Characteristics

2.3.1. Fourier Transforms Infrared Spectroscopy. Fourier transforms infrared spectroscopy (FTIR) has been carried out according to [8]. FTIR of the products was recorded by using Perkin Elmer Spectrum GX spectrophotometer in the range of 400–4000 cm^{-1} A very thin film of products was covered on NaCl cells (4 mm thickness) and was used for analysis.

2.3.2. Nuclear Magnetic Resonance Spectroscopy. Nuclear magnetic resonance spectroscopy for proton ^1H and ^{13}C NMR has been carried out according to [8]. ^{13}C and ^1H NMR spectra were recorded using a 400 MHz Bruker 300 NMR spectrometer. For these analysis, 20 mg of samples were dissolved in 1 mL of $CDCl_3$ solvent and introduced into the NMR tube.

2.3.3. Low Temperature Operability. The pour point (PP) is defined as the lowest temperature at which a liquid remains pourable (meaning it still behaves as a fluid). This method is routinely used to determine the low-temperature flow properties of fluids. PP values were measured according to [9] using a phase technology analyzer, Model PSA-70S (Hammersmith Gate, Richmond, BC, Canada). Each sample was run in triplicate, and average values rounded to the nearest whole degree are reported. For a greater degree of accuracy, PP measurements were done with a resolution of 1°C instead of the specified 3°C increment. Generally, materials with lower PP exhibit improved fluidity at low temperatures than those with higher PP.

2.3.4. Flash Point Values. The flash point of a volatile liquid is the lowest temperature at which it can vaporize to form an ignitable mixture in air. Flash point determination was run according to the American National Standard Method using a Tag Closed Tester [10]. Each sample was run in triplicate, and the average values rounded to the nearest whole degree are reported.

2.3.5. Viscosity Index Measurements. Viscosity index (VI) is an arbitrary measure for the change of kinematic viscosity with temperature. It is used to characterize the lubricating oil in the automotive industry. Automated multirange viscometer tubes HV M472 obtained from Walter Herzog

Table 2: D-optimal design arrangement and responses for MEOA.

Run no.	H_2O_2 (X_1)	Catalyst[a] (X_2)	Time[b] (X_3)	Y_1, yield (%)	Y_2, OOC (%)	RCO (%)	Y_3, IV (mg/g)	X (%)	SE
1	20	80	8	76.57	6.17	68.40	37.81	76.37	0.89
2	17.5	80	7	88.57	5.48	60.75	58.95	62.53	0.97
3	17.5	100	8	72.14	7.54	83.59	32.22	79.52	1.05
4	20	80	6	72.28	6.4	70.95	40.98	73.95	0.95
5	20	120	7	54.71	5.94	65.85	64.32	59.12	1.11
6	20	120	6	60.28	7.88	87.36	30.87	80.38	1.08
7	15	120	8	73.57	5.48	60.75	53.24	66.16	0.91
8	20	80	7	81.42	5.37	59.53	56.32	64.20	0.92
9	15	100	7	75.68	5.02	55.65	74.64	52.56	1.05
10	18.75	100	7	85.28	5.71	63.30	49.17	68.75	0.92
11	20	100	7	81.14	6.05	67.06	42.72	72.85	0.92
12	15	120	6	70.78	4.57	50.66	76.48	51.39	0.98
13	15	100	6	65.93	3.65	40.46	96.43	38.71	1.00
14	15	120	7	82.14	4.91	54.43	66.65	57.64	0.94
15	17.5	120	8	59.28	6.74	74.72	36.37	76.63	0.97
16	20	100	6	72.85	6.51	72.17	39.76	74.73	0.90
17	15	80	8	77.14	4.34	48.11	83.85	46.71	1.03
18	17.5	100	6	80.85	3.77	41.79	87.09	44.65	0.93

Notes: OOC, oxirane oxygen content; RCO, relative percentage conversion to oxirane; IV, iodine value; X, conversion to double bond; SE, oxirane oxygen selectivity.
[a]Catalyst Novozym 435 (mg).
[b]Monoepoxidation time (h).

(Germany) were used to measure viscosity. Measurements were run in a Temp-Trol (Precision Scientific, Chicago, Ill, USA) viscometer bath set at 40.0 and 100.0°C. The viscosity and viscosity index were calculated using ASTM method [11]. Triplicate measurements were made, and the average values were reported.

2.3.6. Oxidative Stability. Pressurized DSC (PDSC) experiments were accomplished using a DSC 2910 thermal analyzer from TA Instruments (Newcastle, Del, USA). Typically, a 2 μL sample, resulting in a film thickness of <1 mm, was placed in an aluminum pan hermetically sealed with a pinhole lid and oxidized in the presence of dry air (Gateway Airgas, St. Louis, Mo, USA), which was pressurized in the module at a constant pressure of 1378.95 kPa (200 psi). A 10°C min^{-1} heating rate from 50 to 350°C was used during each experiment. The oxidation onset (OT, °C) was calculated from a plot of heat flow (W/g) versus temperature for each experiment. The sample was run in triplicate, and average values rounded to the nearest whole degree are reported.

3. Results and Discussion

In this study, following the reaction experiments, the response surface is approximated by D-optimal design. Hydrogen peroxide is an important reactant for the formation of peracids from fatty acids; hence, the influence of its amount on the monoepoxidation reaction was studied. The addition of H_2O_2 solution to the reaction medium (toluene with LA) leads to the formation of two distinct phases: an organic phase and an aqueous phase. The designs and the responses yield% of MEOA (Y_1), OOC% (Y_2), and IV mg/g (Y_3) are given in Table 2.

The Novozym 435, being adsorbed on a hydrophobic carried, is mainly present in the organic phase, while H_2O_2 will be partitioned in both the aqueous and the organic phases, with the concentration being higher in the aqueous phase (Figure 2) [6]. This was determined performing varying amounts of H_2O_2 (15, 17.5, and 20 μL) which has been added every 15 min, and the addition was repeated 24 times, Novozym 435 (80, 100, and 120 mg), and different times (6, 7, and 8 h). A stoichiometric excess of the required amount of the peroxide was used to compensate for its possible decomposition by light and temperature.

Table 2 showed that the yield percentage of MEOA, Y_1, has increased to 82.14%, while OOC%, Y_2, 4.91 and iodine value, Y_3, 66.65 which are considerably compared to the theoretical (OOCt) 9.02% and initial iodine value (IV°) 157.35 mg/g. Subsequent experiments were performed using different amounts of H_2O_2 15, 17.5, and 20 μL for every 1.4 gm of LA in one single step. As seen in Table 2, there was a clear increase in the reaction rate (OOC%) and a decrease (IV mg/g) with increasing H_2O_2 amount. With 15 μL, monoepoxidation was achieved at 7 h using 120 mg Novozym 435, while total epoxidation was observed within 10 h using 30 μL. Increasing the peroxide amount used for the reaction results in increasing peracid formation. In the state of partial epoxidation, the amount of peracids accumulated is not significant (less than 2% of the total) [4], because the chemical reaction in which they are consumed is

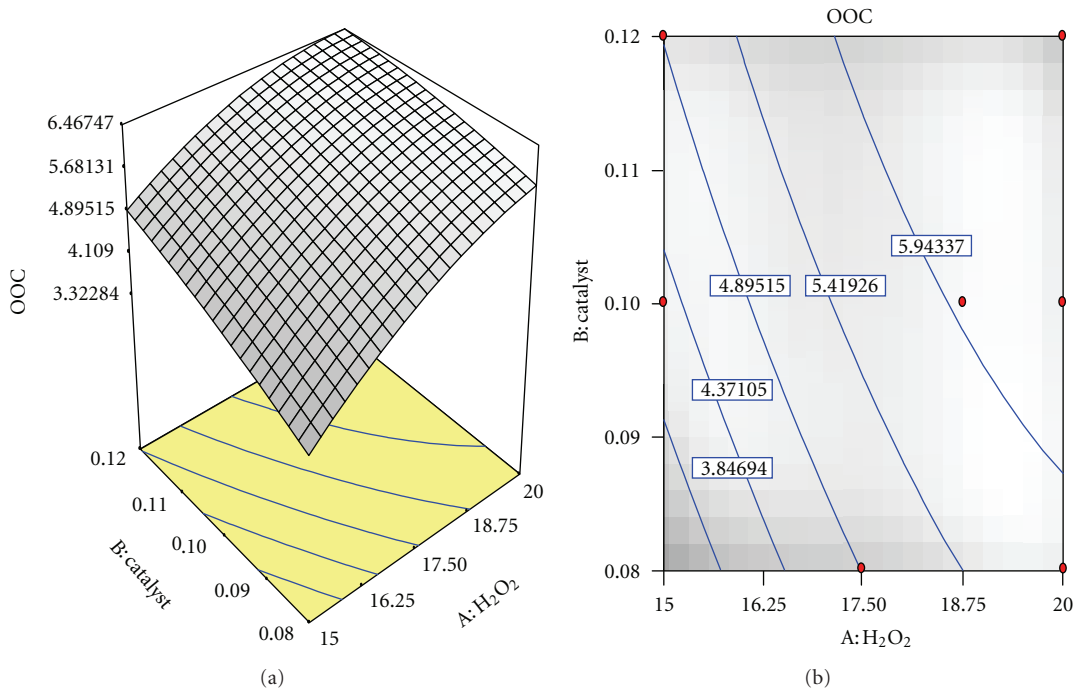

Figure 4: Response surface (a) and contour plots (b) for the effect of the H_2O_2 (X_1, μL) and catalysts Novozym 435 (X_2, mg) on the OOC% of MEOA.

Table 3: Regression coefficients of the predicted quadratic polynomial model for response variables of the yield% of MEOA.

Variables	Coefficients (β), yield% of MEOA (Y_1)	T	P	Notability
Intercept	87.53	8.22	0.0034	***
Linear				
X_1	−2.82	4.91	0.0575	
X_2	−4.50	10.07	0.0131	**
X_3	−0.14	0.010	0.9219	
Quadratic				
X_{11}	−9.06	10.82	0.0110	**
X_{22}	−5.43	5.41	0.0485	**
X_{33}	−9.81	21.03	0.0018	***
Interaction				
X_{12}	−9.74	25.22	0.0010	***
X_{13}	−7.14	12.99	0.0069	***
X_{23}	−7.80	12.83	0.0072	***
R^2	0.90			

Notes: X_1 = amount of H_2O_2; X_2 = catalyst Novozym 435; X_3 = reaction time; $^{**}P < 0.05$; $^{***}P < 0.01$. T: F test value.
See Table 2 for a description of the abbreviations.

Table 4: Regression coefficients of the predicted quadratic polynomial model for response variables of the OOC% for the MEOA.

Variables	Coefficients (β), OOC% (Y_2)	T	P	Notability
Intercept	5.59	2.00	0.1717	
Linear				
X_1	1.00	12.15	0.0082	***
X_2	0.58	3.25	0.1091	
X_3	0.51	2.54	0.1494	
Quadratic				
X_{11}	−0.37	0.36	0.5626	
X_{22}	−0.099	0.036	0.8546	
X_{33}	0.31	0.41	0.5404	
Interaction				
X_{12}	−0.22	0.26	0.6267	
X_{13}	−0.55	1.50	0.2553	
X_{23}	−0.27	0.31	0.5934	
R^2	0.69			

Notes: X_1 = amount of H_2O_2; X_2 = catalyst Novozym 435; X_3 = reaction time; $^{**}P < 0.05$; $^{***}P < 0.01$. T: F test value.
See Table 2 for a description of the abbreviations.

very fast. But once all the double bonds are epoxidized, the remaining peracid is not consumed.

The quadratic regression coefficient obtained by employing a least squares method technique to predict quadratic polynomial models for the yield% of MEOA (Y_1), OOC% (Y_2), and IV mg/g (Y_3) is given in Tables 3, 4, and 5. For the yield% of MEOA (Y_1), the linear term of Novozym 435 catalyst amount (X_2), quadratic terms of H_2O_2 (X_{11}) and

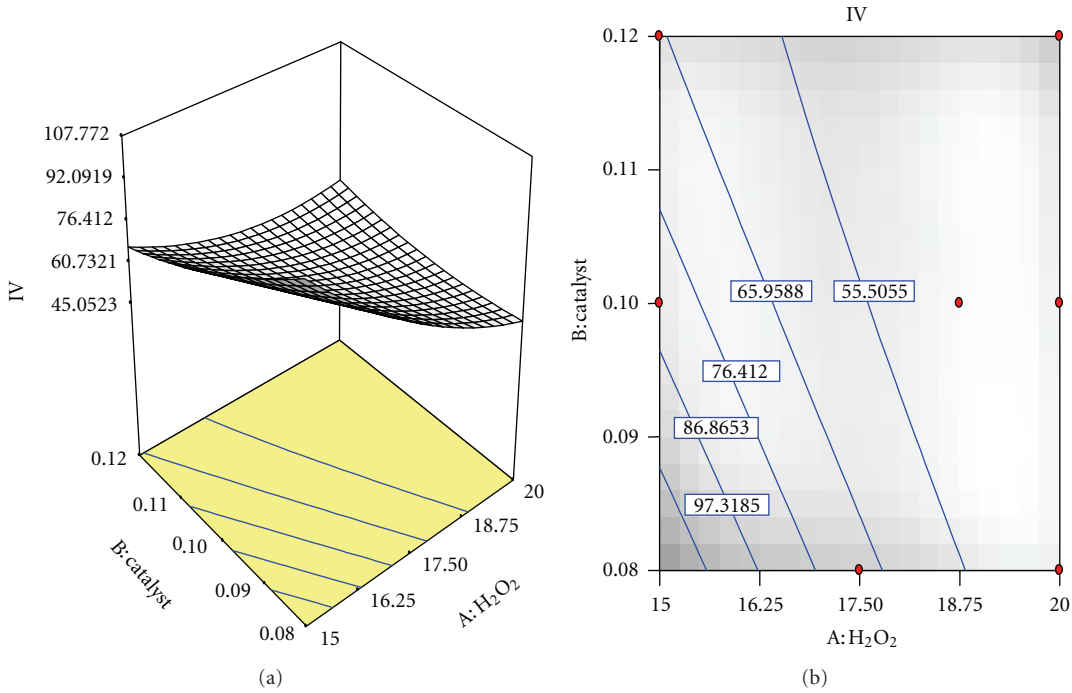

Figure 5: Response surface (a) and contour plots (b) for the effect of the H_2O_2 (X_1, μL) and catalysts Novozym 435 (X_2, mg) on the IV mg/g of MEOA.

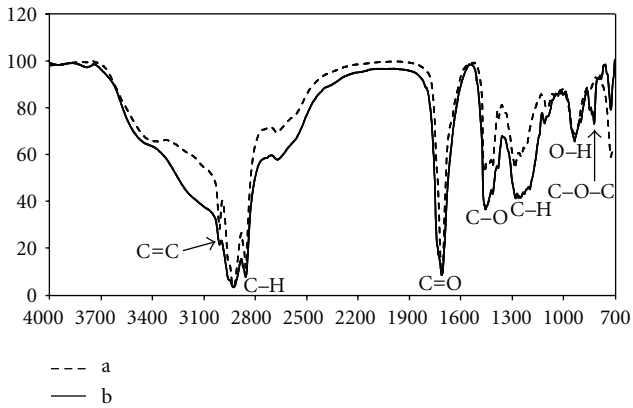

Figure 6: FTIR spectrum of the LA (a) and MEOA (b).

Table 5: Regression coefficients of the predicted quadratic polynomial model for response variables of the IV mg/g for the MEOA.

Variables	Coefficients (β), IV mg/g (Y_3)	T	P	Notability
Intercept	56.24	3.42	0.0489	**
Linear				
X_1	−18.82	21.45	0.0017	***
X_2	−8.91	3.87	0.0849	
X_3	−10.57	5.47	0.0474	**
Quadratic				
X_{11}	8.06	0.84	0.3865	
X_{22}	4.15	0.31	0.5935	
X_{33}	−4.41	0.42	0.5369	
Interaction				
X_{12}	11.59	3.50	0.0985	
X_{13}	10.81	2.92	0.1259	
X_{23}	5.42	0.61	0.4580	
R^2	0.79			

Notes: X_1= amount of H_2O_2; X_2= catalyst Novozym 435; X_3= reaction time; $^{**}P < 0.05$; $^{***}P < 0.01$. T: F test value.
See Table 2 for a description of the abbreviations.

Novozym 435 catalyst amount (X_{22}) were significant ($P < 0.05$). The interaction between H_2O_2 (X_{11}) and Novozym 435 catalyst amount (X_{12}) and the interaction between H_2O_2 (X_{11}), and reaction time (X_{13}) were significant ($P < 0.05$), while its quadratic term of reaction time (X_{33}) was highly significant ($P < 0.01$).

Highly significant ($P < 0.01$) terms of OOC% (Y_2) and IV mg/g (Y_3) for the H_2O_2 (X_1) were linear, while linear term of IV mg/g for the reaction time (X_3) was significant ($P < 0.05$). The coefficients of independent variables (H_2O_2; X_1, catalyst Novozym 435; X_2, and reaction time; X_3) were determined for the quadratic polynomial models Tables 3, 4 and 5.

The lack of fit F-value for all the responses showed that the lack of fit is not significant ($P > 0.05$) relatively to the pure error. This indicates that all the models predicted for the responses were adequate. Regression models for data on responses Y_1, Y_2, and Y_3 were highly significant ($P < 0.01$) with satisfactory R^2. However, R^2 for Y_2 (0.69) was lower although the model was significant. Table 6 summarizes the

Table 6: Analysis of variance (ANOVA) for all the responses of MEOA.

	Source	*Df*	Sum of squares	Mean square	*F* value	*Prob* > *F*	
Y_1	Model	9	1298	144.22	8.22	0.0034	Significant
	Residual	8	140.36	17.55			
	Lack-of-fit	3	144.05	48.02	0.69	0.5763	Not significant
	Pure error	5	3.69	0.738			
Y_2	Model	3	13.62	4.54	6.74	0.0048	Significant
	Residual	14	9.43	0.67			
	Lack-of-fit	3	1.43	0.48	0.66	0.5956	Not significant
	Pure error	11	8.00	0.727			
Y_3	Model	3	4423.70	598.002	8.18	0.002	Significant
	Residual	14	2522.67	180.19			
	Lack-of-fit	3	734.74	244.91	1.51	0.2672	Not significant
	Pure error	11	1787.93	162.539			

Table 7: The main wavelengths in the FTIR functional groups of LA and MEOA.

Wavelength of LA[a]	Wavelength of MEOA[b]	Functional group
3009	3009	C=C bending vibration (aliphatic)
2927, 2855	2927, 2856	C–H stretching vibration (aliphatic)
1719	1711	C=O stretching vibration (carboxylic acid)
1454	1454	C–H scissoring and bending for methylene group
1284	1284	C–O stretching asymmetric (carboxylic acid)
937	934	C–H bending vibration (alkene)
—	820	C–O–C oxirane ring
722	723	C–H group vibration (aliphatic)

Notes: Linoleic acid (a); 9(12)-10(13)-monoepoxy 12(9)-octadecanoic acid (b).

analysis of variance (ANOVA) of all the responses of this study.

These results suggest that linear effect of hydrogen peroxide is the primary determining factors for MEOA. Reference [6] also concluded that this variable had a very large effect on the results of their monoepoxidation study. Final equations in terms of actual factors are:

$$Y_1 = +87.53 - 2.82X_1 - 4.50X_2 - 0.14X_3 - 9.06X_1^2 - 5.43X_2^2 - 9.81X_3^2 - 9.74X_1X_2 - 7.14X_1X_3 - 7.80X_2X_3, \quad (3)$$

$$Y_2 = +5.59 + 1.00X_1 + 0.58X_2 + 0.51X_3 - 0.37X_1^2 - 0.099X_2^2 + 0.31X_3^2 - 0.22X_1X_2 - 0.55X_1X_3 - 0.27X_2X_3, \quad (4)$$

$$Y_3 = +56.24 - 18.82X_1 - 8.91X_2 - 10.57X_3 + 8.06X_1^2 + 4.15X_2^2 4.41X_3^2 + 11.59X_1X_2 + 10.81X_1X_3 + 5.42X_2X_3. \quad (5)$$

RSM is one of the best ways of evaluating the relationships between responses, variables, and interactions that exist. Significant interaction variables in the fitted models (Tables 3 to 5) were chosen as the axes (amount of H_2O_2; X_1, catalyst Novozym 435; X_2 and reaction time; X_3) for the response surface plots. The relationships between independent and dependent variables are shown in the three-dimensional representation as response surfaces. In a contour plot, curves of equal response values are drawn on a plane whose coordinates represent the levels of the independent factors. Each contour represents a specific value for the height of the surface above the plane defined for a combination of the levels of the factors. Therefore, different surface height values enable one to focus attention on the levels of the factors at which changes in the surface height occur [12].

Figures 3 to 5 are the design-expert plots for all the responses. In the MEOA, performing the technique using low amount of H_2O_2 would give the desired OOC% of MEOA as shown in Figure 4, while IV (Figure 5) was higher at this condition. As shown in Figures 4 and 5, the increasing amount of H_2O_2 led to an increase in the OOC% and reduction of IV mg/g. The relationships between the parameters and MEOA were linear or almost linear. High OOC% could be obtained by using high amount of $H2O_2$ at high reaction time. Experimental variables should be carefully controlled in order to recover a medium percentage of MEOA of interest with reasonable yield [6].

Optimum conditions of the experiment to obtain higher yield% of MEOA and medium OOC% were predicted at amount of H_2O_2 μL of 15, catalyst Novozym 435 of 120 mg,

Table 8: The main signals present in ^{13}C NMR functional groups of LA, MEOA, and diepoxide LA.

δ (ppm) LA[a]	δ (ppm) MEOA[b]	δ (ppm) diepoxide LA	Assignment
24.86–29.79	22.69–34.15	22.77–29.44	$-CH_2-$Carbons
—	54.59–57.29	54.61–57.32	(△) epoxide groups
128.27–130.38	124.02–132.89	—	–CH=CH–Olefinic carbons
180.49	179.32	178.79	C=O Carboxylic acid

Notes: Linoleic acid (a); 9(12)-10(13)-monoepoxy 12(9)-octadecanoic acid (b).

Table 9: The main signals present in ^{1}H NMR functional groups of LA, MEOA, and diepoxide LA.

δ (ppm) LA[a]	δ (ppm) MEOA[b]	δ (ppm) diepoxide LA	Assignment
0.88–0.91	0.86–0.88	0.88–0.92	$-CH_3$
1.30–2.77	1.29–2.33	1.34–2.36	$-CH_2$
—	2.92–3.12	2.99–3.13	–CH–O–CH–
5.35–5.36	5.38–5.49	—	–CH=CH–
7.27	7.27	7.27	–COOH

Notes: Linoleic acid (a); 9(12)-10(13)-monoepoxy 12(9)-octadecanoic acid (b).

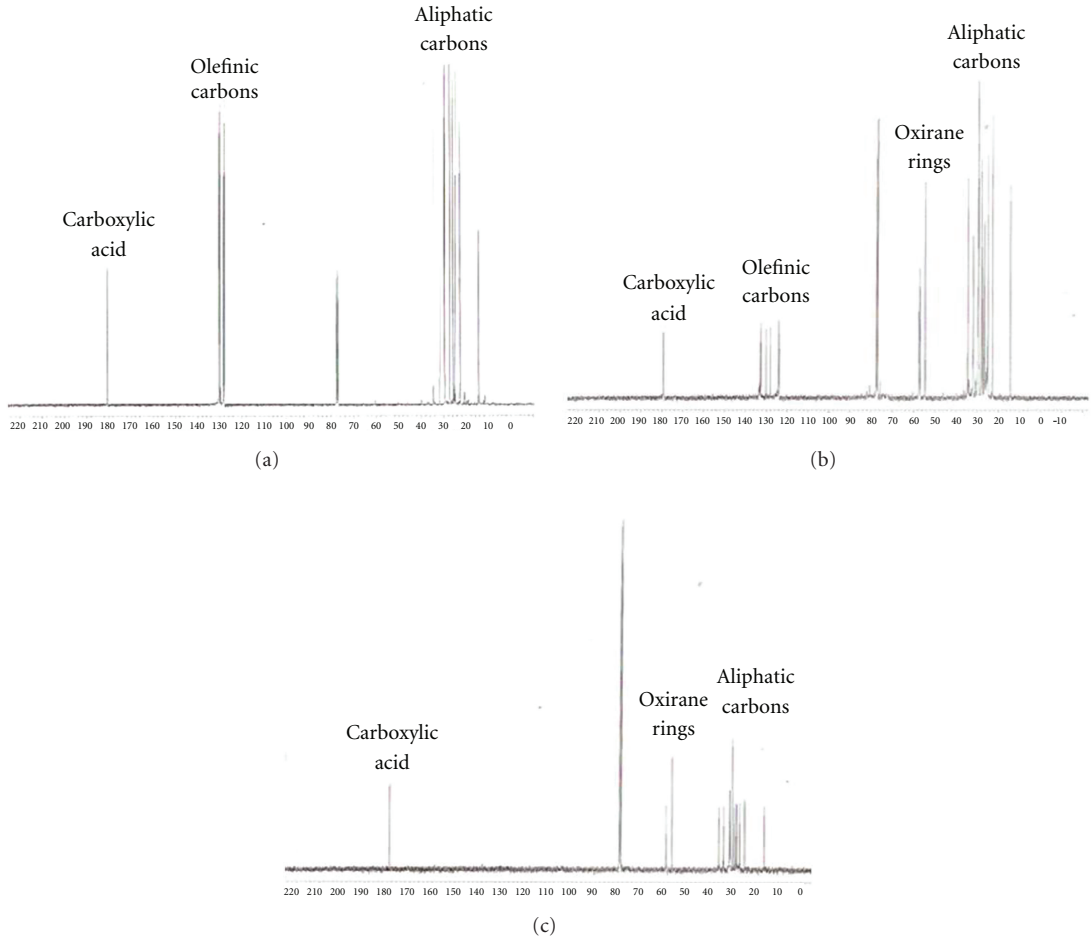

Figure 7: ^{13}C NMR spectrum of LA (a), MEOA, (b) and diepoxide LA (c).

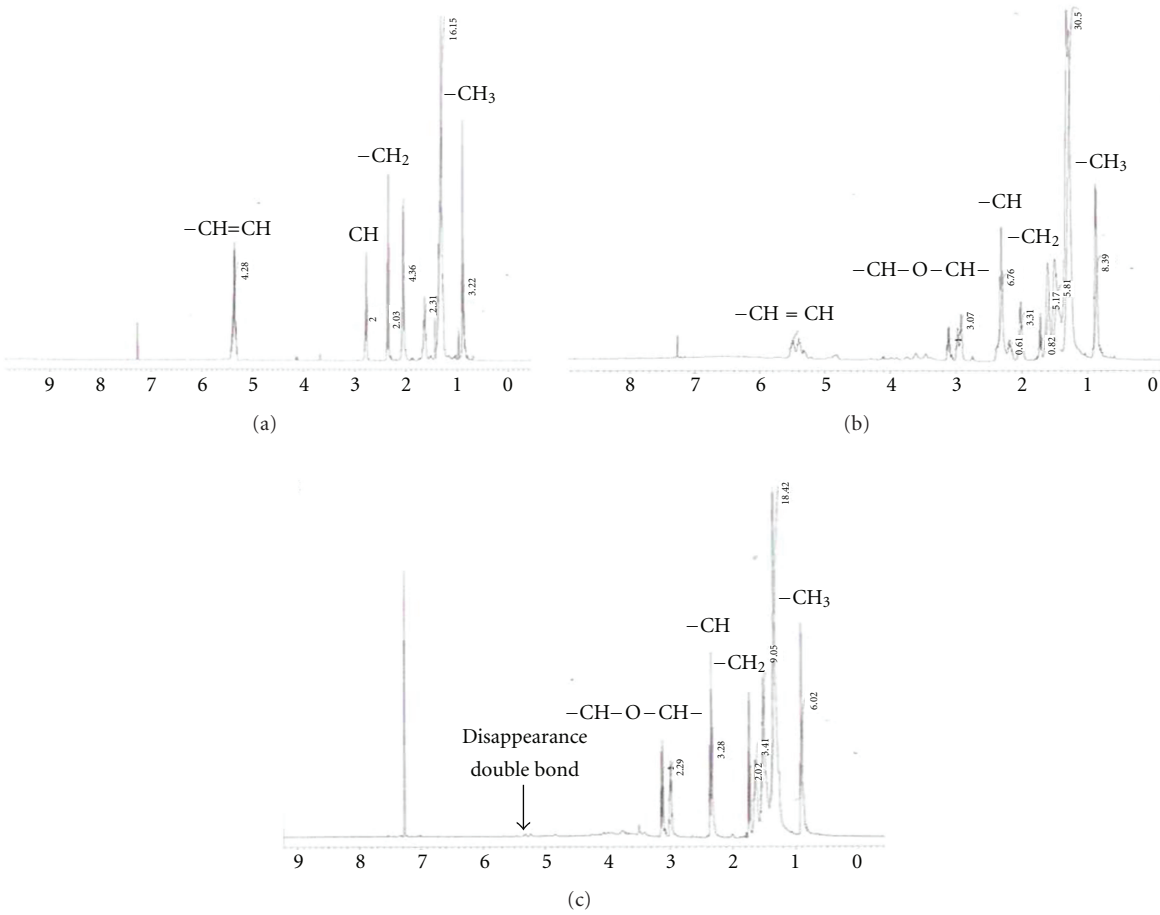

Figure 8: ^{1}H NMR spectrum of LA (a), MEOA (b) and di-epoxide LA (c).

and 7 h of reaction time. At this condition, the yield% of MEOA was 82.14%, 4.91% of OOC, and 66.65 mg/g of IV.

In order to prove the present oxirane ring of MEOA, final product was tested by FTIR. The comparison between LA (a) and MEOA (b), FTIR spectra is shown in Figure 6. The main peaks and their assignment to functional groups are given in Table 7. Oxirane ring of MEOA can be detected at wavenumber 820 cm^{-1}.

For the carboxylic acid carbonyl functional groups (C=O), FTIR spectrum showed absorption bands of LA and MEOA at 1719 and 1711 cm^{-1}, respectively, while stretching vibration peak of C=C can be detected at wavenumber 3009 cm^{-1} [13], and peaks at 2927–2856 cm^{-1} indicated the CH_2 and CH_3 scissoring of LA and MEOA based on Figures 6(a) and 6(b). FTIR spectrum also showed absorption bands at 722, 723 for (C-H) group.

Figure 7(a) indicates the ^{13}C NMR spectrum of LA. The ^{13}C spectroscopy shows the main signals assignment of the LA as shown in Table 8(a). The signals at 180.49 ppm refer to the carbon atom of the carbonyl group (carboxylic acid). The signals at 128.27–130.38 ppm refer to the unsaturated carbon atoms (olefinic carbons); 24.86–29.79 ppm due to methylene carbon atoms in fatty acid moieties of LA [8].

Figures 7(b) and 7(c) can be confirmed by the oxirane ring of MEOA 54.59–57.29 ppm and diepoxide LA at about 54.61–57.32 ppm. Indeed, it appeared that the signals were present in the MEOA, as four peaks of roughly equal intensity (132.89, 132.72, 130.15, and 124.02 ppm) were observed in the alkenic carbon region in the ^{13}C NMR spectrum Figure 7(b), while they disappeared in the diepoxide LA (Figure 7(c)) [14]. The ^{13}C-NMR spectra indicate the existence of carbonyl group (carboxylic acid) in their structure MEOA 179.32 ppm and diepoxide LA at about 178.79 ppm. The other distinctive signals were aliphatic carbons MEOA at about 22.69–29.38 ppm and di-epoxide LA at about 22.77–29.44 ppm, which are common for these types of compounds [15].

^{1}H NMR spectroscopy shows the main signals assignments in LA, MEOA and di-epoxide LA as shown in Table 9. The ^{1}H NMR spectra for the products show some of the key features for a typical (-CH-O-CH-) at about 2.92–3.12 ppm of MEOA, and about 2.99–3.13 ppm of di-epoxide LA (Figure 8(b)). The distinguishable groups are the protons of the terminal methyl of the fatty acid chain. The signals at 0.88–0.86 ppm referred to the methylene group (-CH_3) of LA Figure 8(a) which also appear in MEOA 0.86–0.88 ppm and di-epoxide LA 0.88–0.92 ppm (Figures 8(b) and 8(c)) next to

Table 10: Physicochemical properties of LA and MEOA.

Properties	LA[a]	MEOA[b]
Pour point (°C)	−2	−41
Flash point (°C)	115	128
Viscosity index (°C)	224	130.8
Oxidative stability OT (°C)	189	168

Notes: Linoleic acid (a); 9(12)-10(13)-monoepoxy 12(9)-octadecanoic acid (b).

the terminal methyl (-CH_2) at 1.30–2.77 ppm of LA, 1.29–2.33 of MEOA, and 1.34–2.36 ppm of di-epoxide LA.

However, the methane proton signals (-CH=CH-) were shifted upfield at about 5.35–5.36 ppm of LA and 5.38–5.49 ppm of MEOA [16], while they disappeared in di-epoxide LA. Another distinctive feature is the hydroxyl proton (-COOH) of the carboxylic acid at about 7.27 ppm.

This approach is used here to improve the low-temperature flow behavior of fatty acids by monoepoxide ring. MEOA improves the PP at −41°C significantly comparing with LA at −2°C. Monoepoxidation to produce MEOA was the most effective decreasing the PP to −41°C (Table 10). It can be assumed that the presence of oxirane ring on the fatty acid creates a steric barrier around the individual molecules and inhibits crystallization, resulting in lower PP [17].

Flash point is another important factor in determining how well oil will behave as a potential biolubricant. The flash point is often used as a descriptive characteristic of oil fuel, and it is also used to describe oils that are not normally used as fuels. Flash point refers to both flammable oils and combustible oils. There are various international standards for defining each, but most agree that oils with a flash point less than 43°C are flammable, while those having a flash point above this temperature are combustible [18]. Table 10 has shown the improvement in flash point of MEOA which increased to 128°C comparing with 115°C of LA that means the result agrees with the various international standards.

The efficiency of the biolubricant in reducing friction and wear is greatly influenced by its viscosity. Generally, the least viscous biolubricant which still forces the two moving surfaces apart is desired. If the biolubricant is too viscous, it will require a large amount of energy to move; if it is too thin, the surfaces will rub and friction will increase. The viscosity index highlights how a biolubricants viscosity changes with variations in temperature. Many biolubricant applications require performing across a wide range of conditions, for example, in an engine. Automotive biolubricants must reduce friction between engine components when it is started from cold (relative to engine operating temperatures) as well as when it is running (up to 200°C). The best oils (with the highest VI) will not vary much in viscosity over such a temperature range and therefore will perform well throughout. In MEOA, decreased viscosity index (VI) to 130.8 of MEOA is the result of less double bond (Table 10).

The ability of a substance to resist oxidative degradation is another important characteristic of biolubricants. Therefore, MEOA was screened to measure their oxidation stability using PDSC through determination of OT. PDSC is an effective method for measuring oxidation stability of oleochemicals in an accelerated mode [19]. The OT is the temperature at which a rapid increase in the rate of oxidation is observed at a constant, high pressure (200 psi). A high OT would suggest high oxidation stability of the material. OT was calculated from a plot of heat flow (W/g) versus temperature that was generated by the sample upon degradation and by definition. In this chapter, oxirane ring of MEOA did not show improvement in the oxidation stability of OT for MEOA at 168°C comparing with LA at 189°C (Table 10).

4. Conclusion

RSM and 3-factor D-optimal design were employed for optimization of MEOA. From the present study, it is evident that hydrogen peroxide is the most critical parameter influencing the chemoenzymatic monoepoxidation reaction. Increase in the hydrogen peroxide amount has a strong effect on the reaction kinetics; however, a large excess of hydrogen peroxide results in accumulation of peracid in the final product. Based on the results obtained, MEOA had a positive influence on the low-temperature properties PP and FP.

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