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EPOXIDATION SYNTHESIS OF LINOLEIC ACID FOR RENEWABLE ENERGY APPLICATIONS

(Penghasilan Asid Linoleik Berepoksidasi untuk Penggunaan Tenaga yang Diperbaharui)

Bashar Mudhaffar Abdullah[1], Rahimi M. Yusop[2]*, Jumat Salimon[2], Darfizzi Derawi[2], Waled Abdo Ahmed[2]

[1]*Clinical Investigation Centre,*
University Malaya Medical Centre, 13th Floor Main Tower, Lembah Pantai, 59100 Kuala Lumpur, Malaysia
[2]*Oleochemistry Programme, School of Chemical Sciences & Food Technology, Faculty of Science and Technology,*
Universiti Kebangsaan Malaysia, 43600 UKM Bangi, Selangor, Malaysia

**Corresponding author: rahimi@ukm.edu.my*

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Abstract

Monoepoxidation linoleic acid (MEOA) has advantages for industrial applications. MEOA was synthesized using immobilized *Candida antarctica* lipase (Novozym 435®). 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 hours of reaction time. Fourier Transform Infrared Spectroscopy (FTIR) spectra of the MEOA showed monoepoxide group at 820 cm^{-1}. ^{1}H NMR analysis confirmed the monoepoxide group at 2.92 – 3.12 ppm while the monoepoxide signals of ^{13}C NMR appear at 54.59 – 57.29 ppm. LC-MS analysis shows that of MEOA gives m/z at 296.22 as final product. MEOA exhibited good pour point (PP) of -41 ºC. Flash point (FP) of MEOA increased to 128 ºC comparing with 115 ºC of linoleic acid (LA). In a similar fashion, viscosity index (VI) for LA was 224 generally several hundred centistokes (cSt) more viscous than MEOA 130.8. MEOA was screened to measure their oxidative stability (OT) which was observed at 168 ºC. It is evident that increasing 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.

Keywords: linoleic acid, Novozym 435®, self-epoxidation, renewable energy

Abstrak

Mono-pengepoksidaan asid linoleik (MEOA) mempunyai kelebihan untuk aplikasi industri. MEOA telah disintesis menggunakan *Candida Antartika* lipase (Novozym 435 ®) pegun. Pada keadaan yang optimum, peratus hasil MEOA sebanyak 82.1 % dengan kandungan oksigen oksirana (OOC) sebanyak 4.91 % diperolehi dengan menggunakan 15 µL H_2O_2, 120 mg Novozym 435 ® dan 7 jam tindak balas. Analisis ^{1}H NMR mengesahkan kumpulan terepoksida pada 2.92 –3.12 ppm manakala isyarat ^{13}C NMR menunjukkan bacaan pada 54.59 – 57.29 ppm. Analisis LC-MS menunjukkan bahawa MEOA memberikan m/z 296.22 sebagai produk akhir. MEOA menunjukkan takat tuang yang baik pada suhu -41 ºC. Takat kilat MEOA meningkat pada suhu 128 ºC berbanding pada suhu 115ºC oleh asid linoleik (LA). Dalam keadaan yang sama, kebiasaanya VI untuk LA pada 224 beberapa ratus sentistok (cSt) lebih likat daripada MEOA 130.8. MEOA mempunyai kestabilan oksidatif pada suhu 168 ºC. MEOA berpotensi untuk dijadikan sebagai produk pertengahan dalam aplikasi tenaga keterbaharuan.

Kata kunci: asid linoleik, Novozym 435®, pengepoksidaan, tenaga diperbaharui

Introduction

Bio-renewable materials, such as plant oils and unsaturated fatty acid derivatives, have recently received increasing attention as a means of addressing environmental and economic concerns. Epoxidized oils are currently produced by the epoxidation of plant oils such as soybean, linseed oil [1] and *Jatropha curcas* seed oil [2].

Although the monoepoxidation of linoleic acid or its methyl ester has been carried out repeatedly in the past, it was only recently that a study of positional selectivity in the monoepoxidation of methyl linoleate was reported [3]. There are several methods to epoxidize the double bonds of unsaturated fatty acids, such as Prileshajev monoepoxidation reaction. In this reaction, a peracid from a short or long chain fatty acid and hydrogen peroxide (H_2O_2) under strong acidic conditions is used as the oxidizing agent. The peracid can either be added to the reaction mixture or is formed in situ, the latter being preferred due to safety reasons. The presence of the strong acid in the reaction mixture, however, causes the formation of side products, such as vicinal diols, estolides and other dimers. Although the careful choice of the peracid and the reaction conditions can help to minimize the epoxide loss, the selectivity of industrial monoepoxidation of unsaturated fatty acids rarely exceeds 80% [4].

The effect of reaction parameters on lipase-mediated chemo-enzymatic monoepoxidation of linoleic acid (LA) has been investigated [4]. H_2O_2 was found to have the most significant effect on the reaction rate and degree of epoxidation. An excess of H_2O_2 with respect to the amount of double bonds was necessary in order to yield total conversion within a short time period, as well as at temperatures above 50 °C to compensate for H_2O_2 decomposition. The reaction rate also increased by increasing the hydrogen peroxide concentration (between 10 and 50 wt-%), albeit at the expense of enzyme inactivation. LA was completely epoxidized when used at a concentration of 0.5-2 M in toluene at 30 °C, while in a solvent-free medium, the reaction was not complete due to the formation of a solid or a highly viscous oily phase, creating mass transfer limitations. Increasing the temperature up to 60 °C also improved the rate of epoxide formation. The monoepoxidation of methyl linoleate was examined using transition metal complexes as catalysts [5]. With a catalytic amount of MTO (4 mol%) and pyridine, methyl linoleate was completely epoxidized by aqueous H_2O_2 within 4 h. Longer reaction times (6 h) were needed with 1 mol% catalyst loading. Manganese tetraphenylporphyrin chloride was found to catalyze the partial epoxidation of methyl linoleate. A monoepoxidized species was obtained as the major product (63%) after 20 hours.

Objective of this study was attempted to improve the efficiency of monoepoxidation under milder conditions that minimize the formation of byproducts. Chemo-enzymatic monoepoxidation uses the immobilized lipase from *Candida Antarctica* (Novozym 435®) to catalyze conversion of fatty acids to peracids with 60% hydrogen peroxide. The fatty acid is then self-epoxidized in an intermolecular reaction. The lipase is remarkably stable under the reaction conditions and can be recovered and reused 15 times without loss of activity. Different lipase catalysts have been studied, of which Novozym 435®, a commercial preparation of *Candida Antarctica* lipase B, has been shown to be the most effective so far. Most of the investigations have involved dilution of the substrate in organic solvent. Recently, lipase mediated monoepoxidation in a solvent-free medium has also been reported [4]. LA, bearing two C=C double bonds, is one of the major components in *Jatropha curcas* seed oil. This study focuses on the impact of various reaction parameters on the monoepoxidation process, with the aim to determine optimal reaction conditions with regard to reaction efficiency and enzyme stability using D-optimal design. LA was used as the model substrate.

Materials and Methods

Experimental procedure

The enzymatic monoepoxidation was carried out using Novozym 435®, a commercial catalyst made up of lipase, from Candida Antarctica, immobilized on a polyacrylate resin [4]. Table 1 shows the different ratios of hydrogen peroxide (H_2O_2), different amounts of enzyme and different reaction times using D-optimal design. Three factors (variables) including hydrogen peroxide (µL, X_1), enzyme (mg, X_2) and reaction time (h, X_3) were performed for 18 experiments at the same experimental conditions.

In a typical chemo-enzymatic monoepoxidation of linoleic acid, 9(12)-10(13)-monoepoxy 12(9)-octadecanoic acid (MEOA), the LA (1.4 g) was dissolved in 10 mL toluene and the lipase was added. After stirring for 15 min, 15-20 µL of 30 % H_2O_2 was added and every 15 min the addition was repeated for 6 – 8 hours. Afterwards the lipase was removed by filtration, the mixture was washed with water to remove excess H_2O_2 and the organic phase was then

dried over anhydrous sodium sulphate; the solvent was evaporated in a vacuum rotary evaporator. The oxirane ring content (OOC %) [6], yield% and iodine value (IV) [7] were measured. The FTIR, ^{1}H, ^{13}C NMR and LC-MS were analyzed and the physicochemical properties of the product were studied [7].

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

Independent variables		**Coded 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

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. H_2O_2 (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 quadratic polynomial regression model was assumed for predicting Y variables. The model proposed for each response of Y was:

$$Y = ß_0 + \sum ß_i x_i + \sum ß_{ii} x_i^2 + \sum \sum ß_{ij} x_i x_j \quad (1)$$

Where $ß_0$; $ß_i$; $ß_{ii}$ and $ß_{ij}$ 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 the analysis of variance (ANOVA). Response surfaces and contour plots were developed using the fitted quadratic polynomial equations obtained from RSREG analysis and holding the independent variables with the least effect on the response at two constant values and changing the levels of the other two variables [8].

Results and Discussion

Reaction and mechanism of the monoepoxidation linoleic acid

Figure 1 demonstrates the scheme for the monoepoxidation reaction of LA (MEOA). MEOA results in a mixture of two monoepoxides (cis-9, 10-epoxy 12c- 18:1 and cis-12, 13 epoxy 9c- 18:1) with yield% of 75.82. H_2O_2 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 show in Figure 2. The Novozym 435®, being adsorbed on a hydrophobic carrier, is mainly present in the organic phase. 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) due to consumption of H_2O_2 for peracid formation in the organic phase.

The ratio of H_2O_2 to H_2O tends to decrease slightly in organic phase. Hence, for the reaction to proceed optimally, it is essential that the transport of the H_2O_2 from the aqueous phase to the organic phase is faster than its utilization in the enzymatic reaction [4].

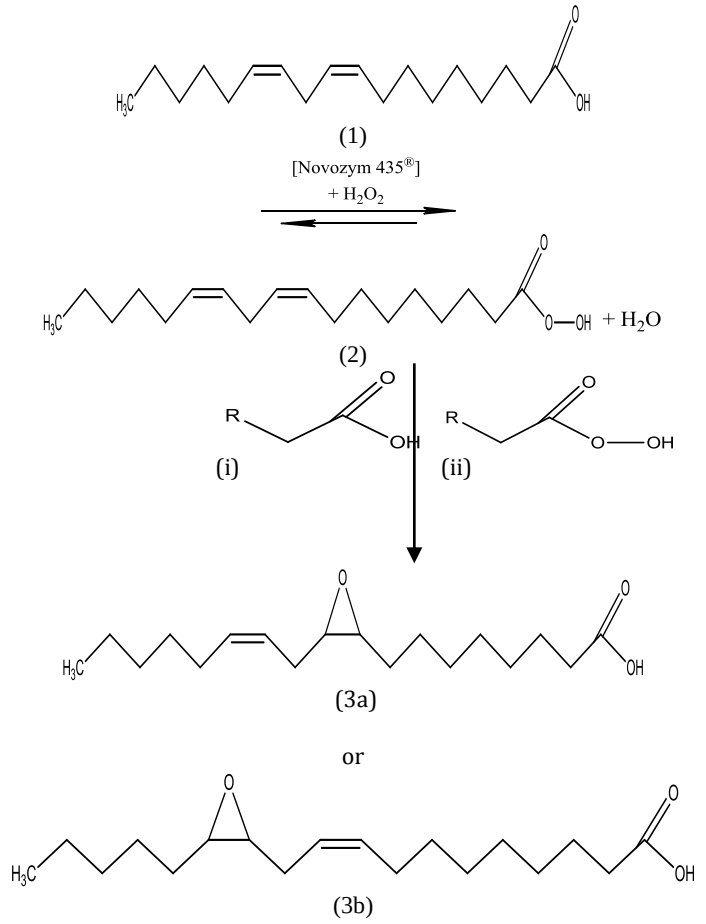

Figure 1. Chemo-enzymatic monoepoxidation of LA

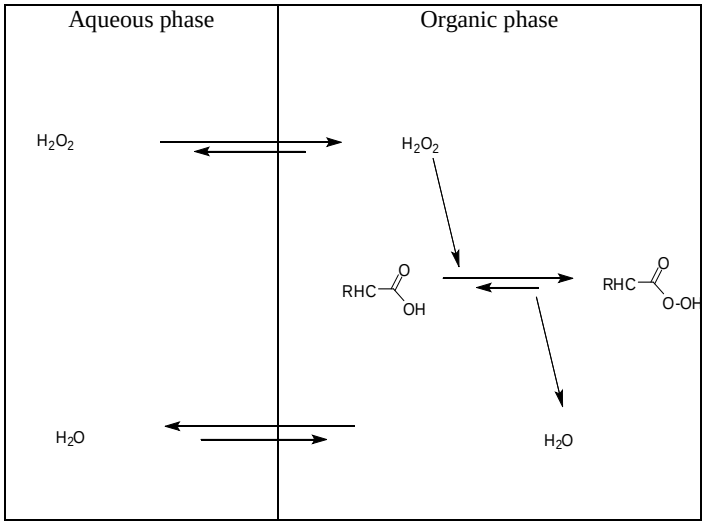

Figure 2. Transport of H_2O_2 and water in an organic-water biphasic system

The reaction synthesis was determined by varying amounts of H_2O_2 (15, 17.5 and 20 µL), which was added every 15 min, the addition was repeated 24 times. The different amounts of Novozym 435® (80, 100 and 120 mg) and different times (6, 7 and 8 hours) also have been used. A stoichiometric excess of the required amount of the H_2O_2 was used to compensate for its possible decomposition by light and temperature. Epoxide contains oxygen as one of the ring atoms. The mechanism of converting the peracid to epoxide involves one-step and the relative stereochemistry of the alkene is maintained in the epoxide [7]. The mechanism is shown schematically in Figure 3.

The double bond acts as the nucleophile with the π-electrons attacking the peracid oxygen. In one-step, the oxygen-oxygen bond breaks and the electrons shift over to form a new π-bond between oxygen and carbon. This causes the π-bond of the existing carbonyl to break and those electrons attack the terminal hydrogen. Finally, the electrons from the O-H bond, which is breaking, add to the alkene carbon to form the three-member ring epoxide [9].

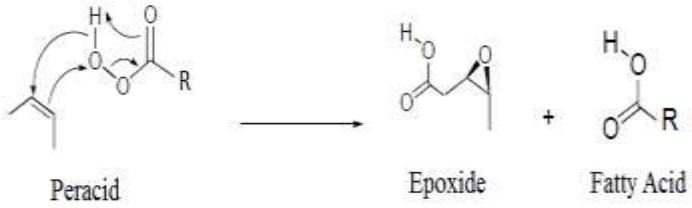

Figure 3. Mechanism of converting peracid to epoxide

Effect of process parameters and statistical analysis

This study demonstrates the application of the proposed response surface methodology (RSM) framework for the optimization of MEOA by using Novozym 435® catalytic monoepoxidation process. Hence, the knowledge about the process is relatively limited, and the design is used to obtain 18 design points within the whole range of three factors for experiments.

The designs and the responses yield percentage of MEOA (Y_1), oxirane oxygen content percentage (OOC, Y_2) and iodine value (IV mg/g, Y_3) are given in Table 2. Following the reaction experiments, the response surface is estimated by D-optimal design. Table 2 shows that the yield% of MEOA of Y_1 increased to (82.14 %) while OOC% of Y_2 (4.91) and IV of Y_3 (77.65) which is considerable compared to the theoretical OOC*t* (9.02) and initial iodine value IV◦ (157.35 mg/g). The conversion percentage of the MEOA is 81.35%. 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 is a clear increase in the reaction rate (OOC percentage) and decrease iodine value (IV mg/g) with increasing H_2O_2 amount. With 15 µL, monoepoxidation was achieved at 7 h using 120 mg Novozym 435®.

The quadratic regression coefficients obtained by employing the least squares method technique to predict quadratic polynomial models for the yield percentage of MEOA (Y_1), OOC percentage (Y_2) and IV mg/g (Y_3) have been explained. For the yield percentage of MEOA (Y_1), the linear term of Novozym 435® catalyst amount (X_2), quadratic terms of H_2O_2 (X_{11}) and 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 terms ($p< 0.01$) of OOC% (Y_2) and IV mg/g (Y_3) for the H_2O_2 (X_1) were linear. The linear term of IV mg/g for the reaction time h (X_3) was significant ($p< 0.05$).

The lack of fit *F*-value for all the responses shows that the lack of fit was not significant ($p> 0.05$) relative 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 (0.90, 0.69 and 0.79, respectively). However, the R^2 for Y_2 (0.69) was lower although the model was significant. Table 3 summarizes the analysis of variance (ANOVA) of all the responses of this study.

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

Run No.	Coded Independent			Responses					
	H_2O_2 (X_1)	Catalyst[a] (X_2)	Time (X_3)	Yield (Y_1, %)	OOC (Y_2, %)	RCO (%)	IV (Y_3, 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	18.7	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	77.65	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)

Table 3. 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	1298.00	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			

These results suggest that linear effect of H_2O_2 is the primary determining factor for MEOA. The H_2O_2 had a very large effect on the results of their monoepoxidation study. The final equations in terms of actual factors are:

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

$$Y_2 = +5.59 +1.00X_1+ 0.58X_2 +0.51X_3 -0.37X_1^2 -0.099X_2^2 +0.31X_3^2 -0.22X_1X_2 -0.55X_1X_3 -0.27X_2X_3 \quad (3)$$

$$Y_3 = +56.24-18.82X_1-8.91X_2-10.57X_3 +8.06X_1^2+4.15X_2^2 4.41X_3^2+11.59X_1X_2+10.81X_1X_3 +5.42X_2X_3 \quad (4)$$

The significant interaction variables in the fitted models were chosen as the axes (amount of H_2O_2; X_1, amount of 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. Figures 4 to 6 are the Design plots for all the responses. In the MEOA, performing the technique using a low amount of H_2O_2 would give the desired OOC% of MEOA, as shown in Figure 5, while IV (Figure 6) was higher at this condition. As shown in Figures 5 and 6, increasing the amount of H_2O_2 led to an increase in the OOC percentage and a reduction of IV. The relationships between the parameters and MEOA were linear or almost linear. High OOC percentage could be obtained by using a high amount of H_2O_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 [4].

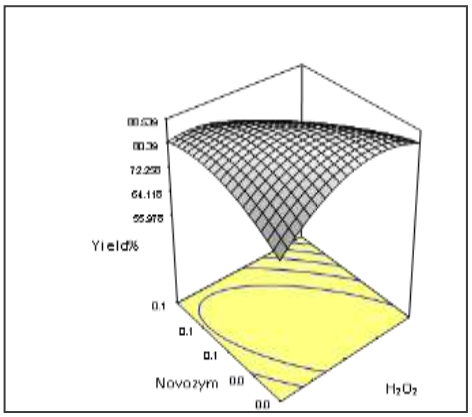

Figure 4. Response surface for the effect of the H_2O_2 (X_1, µL) and catalysts Novozym 435® (X_2, mg) on the yield% of MEOA

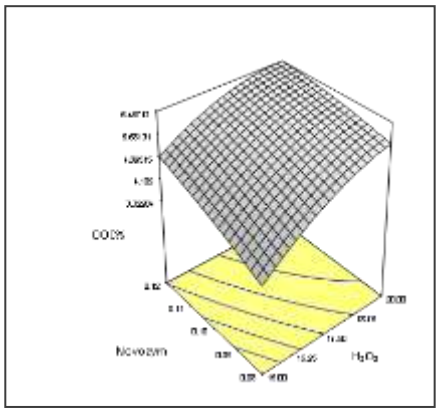

Figure 5. Response surface for the effect of the H_2O_2 (X_1, µL) and catalysts Novozym 435® (X_2, mg) on the OOC% of MEOA

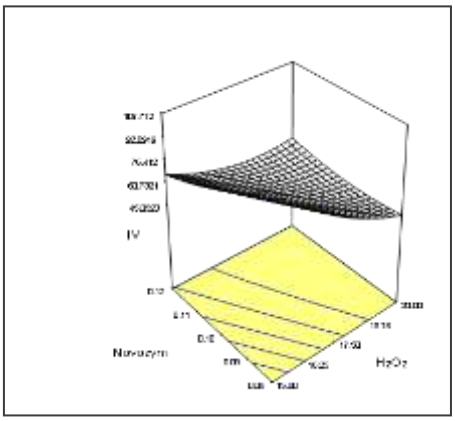

Figure 6. Response surface for the effect of the H_2O_2 (X_1, µL) and catalysts Novozym 435® (X_2, mg) on the IV mg/g of MEOA

The optimum conditions of the experiment to obtain a higher yield percentage of MEOA and medium OOC percentage were predicted at amount of H_2O_2 of 15 µL, catalyst Novozym 435® of 120 mg and 7 h reaction time. At this condition, the yield percentage of MEOA was 82.14%, 4.91% of OOC and 77.65 mg/g of IV. Reconfirmation of the optimal condition experiment was carried out at three replicate. While full epoxidation was observed within 10 h using 30 µL of H_2O_2, increasing the H_2O_2 amount used for the reaction results in the increasing formation of peracid. In the state of MEOA, the amount of peracids accumulated is not significant [10], due to the chemical reaction in which they are consumed is very fast. The reaction could indicate that with a high amount of catalyst, the utilization of H_2O_2 is so fast that the epoxidation of the double bonds does not keep pace with the formation of peracids. However, once all the double bonds are epoxidized, the remaining peracid is not consumed [4]. The observed value was reasonably close to the predicted value, as shown in Figures 7 – 9 respectively.

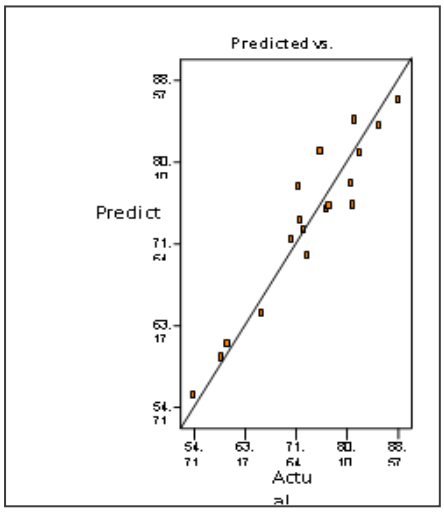

Figure 7. Predicated vs. actual plot of Y_1

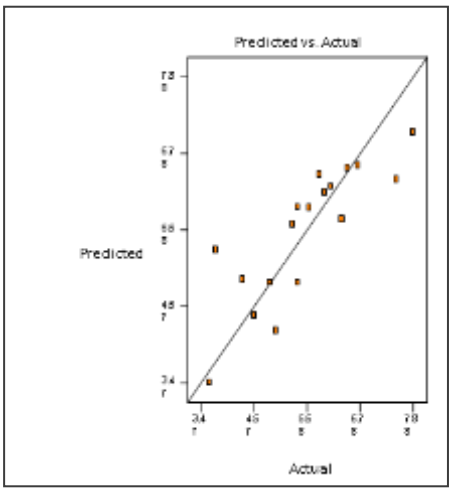

Figure 8. Predicated vs. actual plot of Y_2

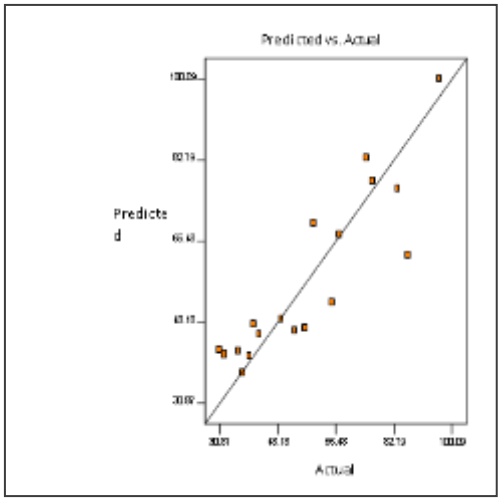

Figure 9. Predicated vs. actual plot of Y_3

FTIR analysis of MEOA

In order to prove the presence of the oxirane ring of MEOA, the final product was tested by FTIR. The oxirane ring of MEOA can be detected at 820 cm^{-1} [9]. 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} [11]. Peaks at 2927 – 2856 cm^{-1} indicate the CH_2 and CH_3 scissoring of LA and MEOA. FTIR spectrum also shows absorption bands at 722, 723 cm^{-1} for (C-H) group vibration.

^{1}H and ^{13}C NMR Analysis of MEOA

The ^{1}H NMR spectroscopy shows the main signal assignments in LA, MEOA and di-epoxide LA. 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. The distinguishable groups are the protons of the terminal methyl of the fatty acid chain. The signals at 0.88 – 0.86 ppm indicate the methylene group ($-CH_3$) of LA, which also appear in MEOA 0.86 – 0.88 ppm and di-epoxide LA 0.88 – 0.92 ppm next to the terminal methyl ($-CH_2$) at 1.30 – 2.06 ppm of LA, 1.29 – 2.03 of MEOA and 1.34 – 1.75 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 while they disappeared in di-epoxide LA [12]. The signal at 7.27 ppm referred to the solvent D_2O for LA, MEOA and di-epoxide LA [7].

The ^{13}C spectroscopy shows the main signals assignment of the LA. 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 refers to the unsaturated carbon atoms (olefinic carbons); 24.86 – 29.79 ppm due to methylene carbon atoms in fatty acid moieties of LA [13]. The results confirm the oxirane ring of MEOA 54.59 – 57.29 ppm and di-epoxide LA at about 54.61 – 57.32 ppm. Indeed, it appears 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, [5], while they had disappeared in the di-epoxide LA.

The ^{13}C-NMR spectra indicate the existence of the carbonyl group (carboxylic acid) in their structure MEOA 179.32 ppm and di-epoxide 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 [14]. The signals at 76.91 – 77.23 ppm refer to the solvent D_2O for LA, MEOA and di-epoxide LA [6].

Physicochemical Characteristics

This approach is used to study the low temperature flow behavior of fatty acids by monoepoxide ring. MEOA increased the pour point (PP) to 15 °C significantly comparing with LA at -2 °C. It can be assumed that the presence of the oxirane ring has a tendency to form crystalline structures that make it semi solid at low temperature through uniform stacking of the 'bend' epoxy ring [9]. Flash point (FP) is another important factor in determining how well oil will behave as a potential biolubricant. FP 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. FP refers to both flammable oils and combustible oils. Although there are various international standards for defining each, most agree that oils with a flash point of less than 43°C are flammable, while those having a FP above this temperature are combustible [7]. The FP of MEOA, which increased to 128 °C compared with 115 °C of LA, which 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 that 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 the viscosity of a biolubricant changes with variations in temperature. 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, the decreased viscosity index (VI) of 130.8 of MEOA is the result of less double bonds [7].

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 the determination of OT.

PDSC is an effective method for measuring oxidation stability of oleochemicals in an accelerated mode [15]. 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 study, the OT of MEOA at 168°C was higher compared to LA at 159°C, indicating that the epoxy function either reduces the abstractibility of α-hydrogens or quenches the oxidation process [16].

Liquid Chromatography Mass Spectroscopy for the MEOA

Formed upon partial LA and MEOA were confirmed the liquid chromatography mass spectroscopy detection (LC-MS). The ion source was operated in the negative mode to produce $[M - H]^-$ precursor ions, which are prone to charge-remote fragmentation [4]. LC-MS analysis shows that of LA and MEOA gives m/z as 280.2335 and 296.2278 (Figure 10). LC-MS spectra obtained upon charge-remote fragmentation are usually straightforward to interpret and have been widely used for structural determination of fatty acids and related ester compounds [17].

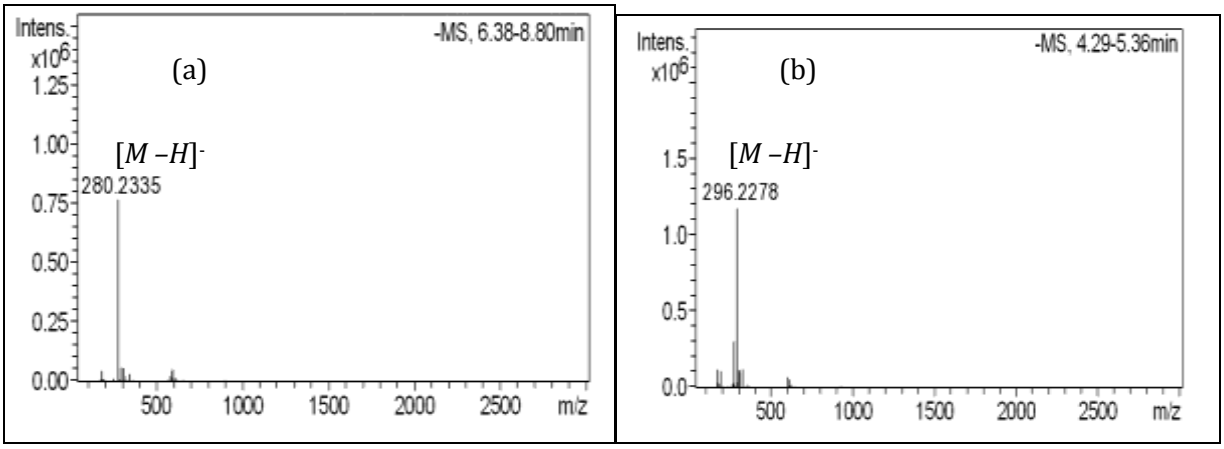

Figure 10. LC-MS analysis of LA (a) and MEOA (b)

Conclusion

It is evident that H_2O_2 is the most critical parameter influencing the chemo-enzymatic monoepoxidation reaction. Increasing the H_2O_2 amount has a strong effect on the reaction kinetics; however, a large excess of H_2O_2 results in the accumulation of peracid in the final product. At optimum conditions, a higher yield percentage of 82.14 and medium oxirane oxygen content OOC percentage of 4.91 and IV of 77.65 mg/g were predicted at 15 μL of H_2O_2, 120 mg of Novozym 435, and 7 h of reaction time. The conversion percentage of the MEOA is 81.35%. The MEOA exhibited high PP of 15°C. In a similar fashion, VI for LA was 224 generally several hundred centistokes more viscous than that MEOA (130.8).

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