Enzymatic Epoxidation of Soybean Oil Using Ionic Liquid as Reaction Media

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Abstract: An ionic liquid (IL) system for the enzymatic epoxidation of soybean oil was studied. The effects of active oxygen carriers (different fatty acids) and ILs ([Bmim]PF₆ and [Bmim]BF₄) on the enzymatic epoxidation were investigated. Response surface methodology (RSM) was used to study and optimize the effects of variables (reaction time, reaction temperature, molar ratio of H₂O₂/C=C-bonds, and molar ratio of fatty acid/C=C-bonds) on the epoxy oxygen group content (EOC) of epoxidized soybean oil (ESO). Results showed that the enzymatic epoxidation of soybean oil can be enhanced using tetradecanoic acid (C₁₃H₂₇COOH) as active oxygen carrier and [Bmim]PF₆ as reaction medium. The optimum EOC of ESO was 5.9 ± 0.3% under the following conditions: reaction temperature 46°C, reaction time 11 h, enzyme load 3% (w/w, relative to the weight of soybean oil), molar ratio of H₂O₂/C=C-bonds 1.8:1, and molar ratio of C₁₃H₂₇COOH/C=C-bonds 0.5:1.

Key words: enzymatic, epoxidation, soybean oil, tetradecanoic acid, ionic liquid

1 INTRODUCTION

During the last few years, owing to the biodegradability of epoxidized soybean oil (ESO) obtained from renewable resources, there has been a growing interest in the use of ESO for the production of a large variety of polymers, such as lubricants, detergents, bioplasticizers, coatings, inks, solvents, and surfactants¹⁻⁶. The traditional methods for epoxy oil preparation include chemical and enzyme catalysis, which involved in inorganic acids used and solvent requirement, such as toluene, benzene, methylene chloride, etc⁷⁻¹¹. In order to develop more green epoxidation methods, green catalyst and reaction media have attracted enormous attention¹²⁻¹⁵.

In the previous reports, enzymatic epoxidation of vegetable oils using toluene as reaction media were developed^{11, 16, 17}. In these reports, relative conversion to oxirane (RCO) of vegetable oils were above 85%. Ionic liquids, as green solvents, owing to their non-volatility, have been used as media in many reactions¹⁸, especially in enzymatic reaction^{12, 19, 20}. However, enzymatic epoxidation of soybean oil using ionic liquid as reaction medium has not been found.

In this study, an ionic liquid (IL) system for the enzymatic epoxidation of soybean oil was investigated. The effect of different fatty acid as active oxygen carrier was also studied. Response surface methodology (RSM) was used to

optimize and better understand the relationship between reaction variables (reaction time, reaction temperature, molar ratio of H₂O₂/C=C-bonds, molar ratio of C₁₃H₂₇COOH/C=C-bonds) and the epoxy oxygen content (EOC) of ESO.

2 EXPERIMENTAL

2.1 Materials

Soybean oil (iodine value = 133.96 gI₂/100 g) was purchased from Shandong Bohai Oil Industry Co., Ltd. GC results of soybean oil were palmitic acid 11.05%, stearic acid 4.54%, oleic acid 22.12%, linoleic acid 54.29%, and linolenic acid 7.99%, respectively. Immobilized lipase Novozym 435 (enzymatic activity 7000 PLU/g) was provided by Novozymes A/S (Bagsvaerd, Denmark). 1-butyl-3-methylimidazolium hexafluorophosphate ([Bmim]PF₆) and 1-butyl-3-methylimidazolium tetrafluoroborate ([Bmim]BF₄) were purchased from Henan Lihua Pharmaceutical Co., Ltd. (Anyang, China). Ethanoic acid was purchased from Tianjin Fengchuan Chemical Reagent Co., Ltd.

Abbreviations: ESO, epoxidized soybean oil; EOC, epoxy oxygen group content; IL, ionic liquid

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(Tianjin, China). N-octanoic acid and myristate were obtained from Tianjin Guangfu Fine Chemical Industry Research Institute. (Tianjin, China). Decanoic acid and lauric acid were from Chinese Medicine Group Chemical Reagent Co., Ltd. (Tianjin, China). Palmitic acid was from Shanghai Chemical Reagent Split Charging Factory. (Shanghai, China). Oleic acid and stearic acid were from Tianjin Kermel Chemical Reagent Co., Ltd. (Tianjin, China). Hydrogen peroxide (purity = 30%) was purchased from Luoyang Haohua Chemical Co., Ltd (Luoyang, China). All other reagents were analytical grade and used without further purification.

2.2 Reaction procedure

Epoxidation reactions were carried out in 250-ml three-necked round-bottom flasks, and placed in a water bath: 5 g soybean oil was reacted with 0.014 mol fatty acid and 0.15 g immobilized lipase in 5 g ionic liquid at 50°C at a mixing speed of 700 rpm, and 4.5 mL 30% (w/w) hydrogen peroxide was added dropwise during 10 minutes to the reaction mixture. After complete addition of hydrogen peroxide, the reactions were continued further for the desired time duration. These standard conditions were used except when otherwise stated in the text.

Upon completion of the epoxidation reaction, immobilized lipases were removed by filtration. Prior to analysis, the products were washed with distilled water to separate ionic liquid, removed trace water and decomposed traces of unreacted hydrogen peroxide with anhydrous sodium sulfate, and distilled off remaining trace water using a rotary evaporator.

2.3 Response surface design

A three-level-four-factor Box-Behnken design was employed in this study. The variables and their levels selected for the epoxidation of soybean oil were reaction time (6.0 h, 10.0 h, 14.0 h), reaction temperature (35.0°C, 45.0°C, 55.0°C), molar ratio of C₁₃H₂₇COOH/C = C-bonds (0.125:1, 0.5:1, 0.875:1), and molar ratio of H₂O₂/C = C-bonds (0.5:1, 1.5:1, 2.5:1). **Table 1** showed independent factors (X_i), levels, and Box-Behnken design using coded and uncoded parameters.

2.4 Analytical techniques

Epoxy oxygen content (EOC) determination was carried out by the direct method with hydrobromic acid solution in acetic acid²¹. From the oxirane content, the percentage relative conversion to oxirane (RCO) was determined using the following expression:

$$\text{Relative conversion to oxirane (RCO)} = (\text{OO}_{\text{ex}}/\text{OO}_{\text{th}}) \times 100 \quad (1)$$

where OO_{ex} is the content of epoxy oxygen experimentally determined, and OO_{th} is the theoretical maximum epoxy oxygen in 100 g of soybean oil, determined using the

following expression:

$$\text{OO}_{\text{th}} = \{(\text{IV}_0/2A_i)/[100 + (\text{IV}_0/2A_i)A_0]\} \times A_0 \times 100 \quad (2)$$

where A_i (126.9) and A_0 (16.0) are the atomic weights of iodine and oxygen, respectively; and IV_0 is the initial iodine value of soybean oil. The theoretical maximum oxirane oxygen in 100 g of soybean oil (OO_{th}) is 7.79%.

2.5 Statistical analysis

Box-Behnken design for four independent variables was used to obtain the combination of values that optimizes the responses within the region of the three dimensional observation space, which allows one to design a minimum number of experimental runs. The model evaluates the effects of each independent variable on the responses. 3^k factorial tests allow efficient estimation of a second order polynomial regression. The mathematical relationship relating the variables to the responses can be calculated by the quadratic polynomial equation:

$$Y = \beta_0 + \sum_{i=1}^4 \beta_i X_i + \sum_{i=1}^4 \beta_{ii} X_i^2 + \sum_{i=1}^3 \sum_{j=i+1}^4 \beta_{ij} X_i X_j \quad (3)$$

where Y is one of the two responses; X_i and X_j represent the independent variables; β_0 is the constant, β_i the linear term coefficient, β_{ii} the quadratic term coefficient, and β_{ij} the cross term coefficient. The experiments were carried out for analysis using Design Expert. For the present study, a total of 29 tests were necessary to estimate the coefficients.

3 RESULTS AND DISCUSSION

3.1 Effect of [Bmim]PF₆ and [Bmim]BF₄ as reaction media

Table 2 shows the effect of different ionic liquids ([Bmim]PF₆ and [Bmim]BF₄) as reaction media on the enzymatic epoxidation of soybean oil. The OO_{ex} ($4.4 \pm 0.2\%$) and RCO (56.4%) using [Bmim]PF₆ as reaction medium were higher than that (OO_{ex} , $2.6 \pm 0.4\%$; RCO, 33.0%) of [Bmim]BF₄, which were lower than that (OO_{ex} , $4.5 \pm 0.3\%$; RCO, 60.7%) using toluene as reaction medium. These results can be attributed to the higher viscosity of ILs than that of toluene and greater hydrophilic of [Bmim]BF₄ than that of [Bmim]PF₆. Therefore, [Bmim]PF₆ was chosen as reaction medium in the next experiments.

3.2 Effect of different fatty acids as oxygen carriers

To investigate the effect of different fatty acids, the epoxidation reactions were performed using different fatty acids as active oxygen carrier, respectively. During the enzymatic epoxidation of soybean oil, Novozym 435 catalyzed the conversion of fatty acids with hydrogen peroxide to peroxy fatty acids. The double bonds of soybean oil were epoxidized by peroxy fatty acids previously formed (**Fig. 1**). Results showed that tetradecanoic acid as active oxygen

Table 1 Response surface design and results of the enzymatic epoxidation of soybean oil affected by reaction time, reaction temperature, substrate ratio, and fatty acid load.

Treatment no.	Reaction Temperature	Reaction Time	Substrate ratio ^a	Fatty acid load ^b	OO _{ex}	RCO
	X ₁ (°C)	X ₂ (h)	X ₃ (mol/mol)	X ₄ (mol/mol)	(%)	(%)
1	0 (45)	1 (14)	-1 (0.5:1)	0 (0.5:1)	3.9 ± 0.3	50.2
2	-1 (35)	0 (10)	0 (1.5:1)	-1 (0.125:1)	1.0 ± 0.1	13.0
3	0 (45)	0 (10)	1 (2.5:1)	-1 (0.125:1)	3.4 ± 0.2	44.0
4	0 (45)	0 (10)	0 (1.5:1)	0 (0.5:1)	5.9 ± 0.3	75.7
5	0 (45)	1 (14)	1 (2.5:1)	0 (0.5:1)	5.4 ± 0.3	69.5
6	1 (55)	-1 (6)	0 (1.5:1)	0 (0.5:1)	4.2 ± 0.5	53.3
7	0 (45)	-1 (6)	-1 (0.5:1)	0 (0.5:1)	3.7 ± 0.1	47.9
8	0 (45)	-1 (6)	0 (1.5:1)	1 (0.875:1)	3.9 ± 0.3	50.1
9	1 (55)	0 (10)	0 (1.5:1)	1 (0.875:1)	3.9 ± 0.5	50.6
10	0 (45)	-1 (6)	0 (1.5:1)	-1 (0.125:1)	1.4 ± 0.4	18.5
11	0 (45)	0 (10)	0 (1.5:1)	0 (0.5:1)	5.7 ± 0.3	73.2
12	0 (45)	0 (10)	0 (1.5:1)	0 (0.5:1)	5.8 ± 0.3	74.5
13	1 (55)	0 (10)	-1 (0.5:1)	0 (0.5:1)	3.9 ± 0.2	49.4
14	-1 (35)	0 (10)	0 (1.5:1)	1 (0.875:1)	3.5 ± 0.5	45.2
15	-1 (35)	0 (10)	1 (2.5:1)	0 (0.5:1)	3.4 ± 0.2	44.2
16	0 (45)	0 (10)	1 (2.5:1)	1 (0.875:1)	4.9 ± 0.3	62.3
17	-1 (35)	1 (14)	0 (1.5:1)	0 (0.5:1)	3.0 ± 0.2	38.3
18	0 (45)	1 (14)	0 (1.5:1)	-1 (0.125:1)	3.3 ± 0.3	42.6
19	-1 (35)	-1 (6)	0 (1.5:1)	0 (0.5:1)	1.9 ± 0.5	24.4
20	1 (55)	0 (10)	1 (2.5:1)	0 (0.5:1)	3.4 ± 0.3	43.9
21	0 (45)	0 (10)	-1 (0.5:1)	1 (0.875:1)	3.8 ± 0.2	49.3
22	1 (55)	0 (10)	0 (1.5:1)	-1 (0.125:1)	1.5 ± 0.3	19.8
23	-1 (35)	0 (10)	-1 (0.5:1)	0 (0.5:1)	2.8 ± 0.4	35.7
24	1 (55)	1 (14)	0 (1.5:1)	0 (0.5:1)	3.5 ± 0.1	44.8
25	0 (45)	0 (10)	-1 (0.5:1)	-1 (0.125:1)	3.3 ± 0.3	42.2
26	0 (45)	0 (10)	0 (1.5:1)	0 (0.5:1)	5.2 ± 0.2	66.8
27	0 (45)	1 (14)	0 (1.5:1)	1 (0.875:1)	5.3 ± 0.1	68.0
28	0 (45)	0 (10)	0 (1.5:1)	0 (0.5:1)	6.0 ± 0.2	77.0
29	0 (45)	-1 (6)	1 (2.5:1)	0 (0.5:1)	4.0 ± 0.3	51.9

^a molar ratio of H₂O₂/C=C-bonds; ^b molar ratio of C₁₃H₂₇COOH/C=C-bonds.

carrier showed the best performance than other tested fatty acids. The EOC of ESO using oleic acid as the oxygen carrier was very low. Compared to short carbon chain fatty acids (≤ 10) and saturated fatty acids, long carbon saturated fatty acids can better suppress oxirane-ring opening reaction. These results were different from those previous reports using toluene as reaction medium^{16, 17}, which may be attributed to the differences of reaction media and active oxygen carriers. Compared with traditional oxygen carriers (formic acid, acetic acid, *et al.*), using tetradecano-

ic acid as active oxygen carrier, the formation of acid waste, the epoxy ring opening reactions, and the hydrolysis of soybean oil can be suppressed, which can avoid the formation of undesirable epoxidized di- and mono-glycerides. Therefore, considering the melting points and solubilities of fatty acids, tetradecanoic acid (C₁₃H₂₇COOH) was chosen as the most favorable oxygen carrier for epoxidation of soybean oil using ionic liquid as reaction medium.

Table 2 Effect of different ionic liquids and toluene on the epoxidation of soybean oil. Reaction conditions: 5 g soybean oil, 5 g ionic liquid or toluene, enzyme load 3%, molar ratio of $\text{H}_2\text{O}_2/\text{C}=\text{C}$ -bonds 1.5:1, molar ratio of lauric acid/ $\text{C}=\text{C}$ -bonds 0.5:1, reaction time 10 h, reaction temperature 50°C , mixing speed 700 rpm.

	ILs		Toluene
	[Bmim]PF ₆	[Bmim]BF ₄	
OOex (%)	4.4 ± 0.2	2.6 ± 0.4	4.5 ± 0.3
RCO (%)	56.4	33.0	60.7

Fig. 1 Effect of different fatty acids as active oxygen carriers on the epoxidation of soybean oil. Reaction conditions: 5 g soybean oil, 5 g [Bmim]PF₆, enzyme load 3%, molar ratio of $\text{H}_2\text{O}_2/\text{C}=\text{C}$ -bonds 1.5:1, molar ratio of fatty acid/ $\text{C}=\text{C}$ -bonds 0.5:1, 50°C , and 10 h.

3.3 Effect of independent variables

The effects of the four independent variables on the epoxidation of soybean oil are shown in Fig. 2. With the increasing of reaction temperature ($<45^\circ\text{C}$), the EOC of ESO increased to the maximum EOC of ESO at 45°C (Fig. 2A). However, with further increasing of reaction temperature ($>45^\circ\text{C}$), the EOC of ESO rapidly decreased, which may be attributed to ring-opening of ESO at higher temperatures. The EOC of ESO smoothly increased as the reaction time increased (<10 h), and reached the equilibrium ($5.4 \pm 0.3\%$) at 10 h (Fig. 2B). The EOC of ESO increased as the concentration of enzyme increased in [Bmim]PF₆, and reached the maximum ($5.4 \pm 0.3\%$) at enzyme load 3% (Fig. 2C). Epoxidation reaction could also carry out with no enzyme used, but low EOC ($2.0 \pm 0.5\%$) of ESO was obtained.

In [Bmim]PF₆, tetradecanoic acid and hydrogen peroxide were used as oxygen carrier and oxygen donor. During the epoxidation of soybean oil, tetradecanoic acid was converted to pertetradecanoic acid by the reaction with hydrogen peroxide, then unsaturated double bonds of soybean oil were epoxidized by pertetradecanoic acid (Scheme 1). The concentrations of oxygen carrier (tetradecanoic acid) and oxygen donor (H_2O_2) also showed the significant effect on the EOC of ESO (Fig. 2D, Fig. 2E). The decrease of the EOC of ESO in the excess H_2O_2 may be attributed to an accelerated rate of oxirane ring opening reaction in the presence of excess H_2O_2 .

3.4 Selection of levels of independent variables and experimental design

In this study, reaction time, reaction temperature, substrate ratio (molar ratio of $\text{H}_2\text{O}_2/\text{C}=\text{C}$ -bonds) and fatty acid load (molar ratio of $\text{C}_{13}\text{H}_{27}\text{COOH}/\text{C}=\text{C}$ -bonds) were chosen as independent variables (maximum, 1; minimum, -1 and mean, 0 value for selected independent variables). Therefore, the lower, middle and upper levels of four independent factors were chosen (Table 1).

3.5 Model fitting

RSM was used to evaluate the effects of reaction time, reaction temperature, substrate ratio (molar ratio of $\text{H}_2\text{O}_2/\text{C}=\text{C}$ -bonds), and fatty acid load (molar ratio of $\text{C}_{13}\text{H}_{27}\text{COOH}/\text{C}=\text{C}$ -bonds) on the epoxidation of soybean oil. The results obtained showed in Table 1. Duplicate experiments were carried out at all design points. The results were analyzed employing a multiple regression technique to develop response surface model. Both a linear model and a second-order model were tested, using an F-test at the 95% confidence level. The following two second-order model satisfactorily explained the EOC of ESO with non-significant lack of fit (Table 3). EOC is the predicted value for the EOC of ESO, and X_1 , X_2 , X_3 and X_4 are the coded variables as described in Table 1.

$$\begin{aligned} \text{EOC}(\%) = & 5.72 + 0.40X_1 + 0.44X_2 + 0.26X_3 + 0.94X_4 - \\ & 1.79X_1^2 - 0.85X_2^2 - 0.55X_3^2 - 1.3X_4^2 - \\ & 0.44X_1X_2 - 0.27X_1X_3 - 0.028X_1X_4 + 0.30X_2X_3 \\ & + 0.12X_2X_4 + 0.22X_3X_4 \end{aligned}$$

The computed model *F*-value of 46.28 implied the model was significant. The model also showed statistically insignificant lack of fit (Prob $> F_a$ of 0.1331), and the R^2 of model was 0.9288 (Table 3), which suggested that the analysis using the quadratic regression model to simulate real four-factor and three levels is feasible. The four independent variables, reaction time, reaction temperature, substrate ratio (molar ratio of $\text{H}_2\text{O}_2/\text{C}=\text{C}$ -bonds), and fatty acid load (molar ratio of $\text{C}_{13}\text{H}_{27}\text{COOH}/\text{C}=\text{C}$ -bonds) were the most significant in the process. The influence to the enzy-

Fig. 2 Effect of reaction temperature (A), reaction time (B), enzyme load (C), molar ratio of C₁₃H₂₇COOH/C=C-bonds (D), and molar ratio of H₂O₂/C=C-bonds (E) on the epoxidation of soybean oil in [Bmim]PF₆. (A: enzyme load 3%, molar ratio of H₂O₂/C=C-bonds 1.5:1, molar ratio of C₁₃H₂₇COOH/C=C-bonds 0.5:1, and 10 h; B: enzyme load 3%, molar ratio of H₂O₂/C=C-bonds 1.5:1, molar ratio of C₁₃H₂₇COOH/C=C-bonds 0.5:1, and 45°C; C: molar ratio of H₂O₂/C=C-bonds 1.5:1, molar ratio of C₁₃H₂₇COOH/C=C-bonds 0.5:1, 45°C, and 10 h; D: enzyme load 3%, molar ratio of H₂O₂/C=C-bonds 1.5:1, 45°C, and 10 h; E: enzyme load 3%, molar ratio of C₁₃H₂₇COOH/C=C-bonds 0.5:1, 45°C, and 10 h).

Scheme 1 Enzymatic epoxidation of soybean oil using tetradecanoic acid as oxygen carrier in [Bmim]PF₆.

matic epoxidation of soybean oil decreased in the order of fatty acid load > reaction temperature > reaction time > molar ratio of H₂O₂/C=C-bonds. However, the interactions between the variables was insignificant.

The relationship between responses and interaction of reaction variables can be evaluated by contour plots generated from the predicted model. The maximum EOC (> 5.6%) of ESO appeared in the temperature range of 40.0–50.0°C, reaction time of 9–13 h, fatty acid loads of 0.45:1–0.8:1, and substrate molar ratio of 1.2:1–2.3:1 (Fig. 3). Higher temperature (> 50.0°C) and longer reaction time (> 13 h) resulted in the deactivation of enzyme and ring-opening of the epoxide ring, which lead to the decrease of the EOC of ESO. Enough H₂O₂ and active oxygen carrier tetradecanoic acid (C₁₃H₂₇COOH) were the guarantee for the epoxidation of C=C bonds in soybean oil. But a further in-

creasing of substrate ratio (> 2.2:1) would lead to a decline of the EOC of ESO, which can be attributed to more by-products formed by ring-opening. From the analysis of response surface plots, the interaction between fatty acid load and reaction temperature was more significant than other variables interactions.

3.6 Attaining optimum conditions

Besides the exhibition of the effects of variables on the response, the contour plots also showed several desirable combinations of variables that gave high EOC of ESO, which provided options when practical aspects were considered in real commercialization of the process. According to optimization by the contour plots, a set of conditions were obtained as follows: reaction temperature 46°C, reaction time 11 h, substrate ratio 1.8:1 (molar ratio of H₂O₂/C

Table 3 Regression analysis of variance for response surface quadratic model (ANOVA) pertaining to the predicted EOC of ESO.

Source	Sum of squares	Degree of freedom	Mean square	F value	Prob > F ^a
Model	46.28	14	3.31	13.04	< 0.0001
Reaction time (X_1)	1.89	1	1.89	7.45	0.0163
Reaction temperature (X_2)	2.3	1	2.3	9.06	0.0094
Hydrogen peroxide load (X_3)	0.85	1	0.85	3.35	0.0888
Fatty acid load (X_4)	10.68	1	10.68	42.13	< 0.0001
X_1^2	20.88	1	20.68	82.38	< 0.0002
X_2^2	4.69	1	4.69	18.51	0.0007
X_3^2	1.95	1	1.95	7.68	0.015
X_4^2	12.25	1	12.25	48.33	< 0.0001
X_1X_2	0.76	1	0.76	2.99	0.106
X_1X_3	0.3	1	0.3	1.17	0.2973
X_1X_4	3.03×10^{-3}	1	3.03×10^{-3}	0.012	0.9146
X_2X_3	0.35	1	0.35	1.4	0.2569
X_2X_4	0.058	1	0.058	0.23	0.6409
X_3X_4	0.19	1	0.19	0.75	0.4021
Residual	3.55	14	0.25		
Lack of fit	3.16	10	0.32	3.26	0.1331
Pure Error	0.39	4	0.097		
Cor total	49.83	28			$R^2 = 0.9288$

^a Prob<0.0500 indicates statistical significance; Prob>0.1000 indicates statistical insignificance.

= C-bonds), molar ratio of $C_{13}H_{27}COOH/C = C$ -bonds 0.5:1. Under these conditions, the EOC of ESO can attain a maximum $5.9 \pm 0.3\%$, which was well accorded with the predicted result (6.0%). These results indicated that the model developed was considered to be accurate and reliable.

4 CONCLUSION

The enzymatic epoxidation of soybean oil was successfully achieved using ionic liquid [Bmim]PF₆ as reaction medium. Tetradecanoic acid was selected as active oxygen carrier. RSM was used to evaluate the effects of the reaction variables on the epoxidation. Four independent variables (reaction temperature, reaction time, substrate ratio, and tetradecanoic acid load) had significant effect on the epoxidation. The epoxidation conditions were optimized as follows: 5 g soybean oil, 5 g ionic liquid, reaction temperature 46°C, reaction time 11 h, enzyme load 3%, molar ratio of $H_2O_2/C = C$ -bonds 1.8:1, and molar ratio of $C_{13}H_{27}COOH/C = C$ -bonds 0.5:1. Under these conditions, the ECO of ESO was $5.9 \pm 0.3\%$.

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References

- 1) Salimon, J.; Salih, N.; Yousif, E. Biolubricants: Raw materials, chemical modifications and environmental benefits. *Eur. J. Lipid Sci. Technol.* **112**, 519-530 (2010).
- 2) Campanella, A.; Rustoy, E.; Baldessari, A.; Baltanás, M. A. Lubricants from chemically modified vegetable oils. *Bioresour. Technol.* **101**, 245-254 (2010).
- 3) Chua, S. C.; Xu, X.; Guo, Z. Emerging sustainable technology for epoxidation directed toward plant oil-based Plasticizers. *Process Biochem.* **47**, 1439-1451 (2012).
- 4) Gerbase, A. E.; Petzhold, C. L.; Costa, A. P. O. Dynamic Mechanical and Thermal Behavior of Epoxy Resins Based on Soybean Oil. *J. Am. Oil Chem. Soc.* **79**, 797-802 (2002).

Fig. 3 Response contour plots between two variables for the epoxidation of soybean oil in [Bmim]PF₆: (A) reaction temperature and time combined with molar ratio of C₁₃H₂₇COOH/C=C-bonds and substrate ratio fixed at 0.5:1 and 1.5:1, respectively; (B) substrate ratio and reaction temperature with molar ratio of C₁₃H₂₇COOH/C=C-bonds 0.5:1 for 10 h; (C) molar ratios of C₁₃H₂₇COOH/C=C-bonds and reaction temperature with molar ratio of H₂O₂/C=C-bonds 1.5:1 for 10 h; (D) molar ratio of H₂O₂/C=C-bonds and reaction time with molar ratio of C₁₃H₂₇COOH/C=C-bonds 0.5:1 at 60°C; (E) molar ratio of C₁₃H₂₇COOH/C=C-bonds and reaction time with molar ratio of H₂O₂/C=C-bonds 1.5:1 at 60°C; (F) molar ratio of C₁₃H₂₇COOH/C=C-bonds and substrate ratio at 45°C for 10 h.

- 5) Hwang, H.; Adhvaryu, A.; Erhan, S. Z. Preparation and Properties of Lubricant Basestocks from Epoxidized Soybean Oil and 2-Ethylhexanol. *J. Am. Oil Chem. Soc.* **80**, 811-815 (2003).
- 6) Orellana-Coca, C.; Billakanti, J. M.; Mattiasson, B.; Hatti-Kaul, R. Lipase mediated simultaneous esterification and epoxidation of oleic acid for the production of alkylepoxystearates. *J. Mol. Catal. B: Enzym.* **44**, 133-137 (2007).
- 7) Hilker, I.; Bothe, D.; Prüss, J.; Warnecke, H. J. Chemo-enzymatic epoxidation of unsaturated plant oils. *Chem. Eng. Sci.* **56**, 427-432 (2001).
- 8) Jiang, P.; Chen, M.; Dong, Y.; Lu, Y.; Ye, X.; Zhang, W. Novel Two-Phase Catalysis with Organometallic Compounds for Epoxidation of Vegetable Oils by Hydrogen Peroxide. *J. Am. Oil Chem. Soc.* **87**, 83-91 (2010).
- 9) Dinda, S.; Patwardhan, A. V.; Goud, V. V.; Pradhan, N. C. Epoxidation of cottonseed oil by aqueous hydrogen peroxide catalysed by liquid inorganic acids. *Biore-sour. Technol.* **99**, 3737-3744 (2008).
- 10) Okieimen, F. E.; Bakare, O. I.; Okieimen, C. O. Studies on the epoxidation of rubber seed oil. *Ind. Crops Prod.* **15**, 139-144 (2002).
- 11) Klaas, M. R.; Warwel, S. Complete and partial epoxidation of plant oils by lipase-catalyzed perhydrolysis. *Ind. Crops Prod.* **9**, 125-132 (1999).
- 12) Silva, W. S. D.; Lapis, A. A. M.; Suarez, P. A. Z.; Neto, B. A. D. Enzyme-mediated epoxidation of methyl oleate

- supported by imidazolium-based ionic liquids. *J. Mol. Catal. B: Enzym.* **68**, 98-103 (2011).
- 13) Behr, A.; Tenhumberg, N.; Wintzer, A. An efficient reaction protocol for the ruthenium-catalysed epoxidation of methyl oleate. *Eur. J. Lipid Sci. Technol.* **114**, 905-910 (2012).
 - 14) Orellana-Coca, C.; Camocho, S.; Adlercreutz, D.; Mattiasson, B.; Hatti-Kaul, R. Chemo-enzymatic epoxidation of linoleic acid: Parameters influencing the reaction. *Eur. J. Lipid Sci. Technol.* **107**, 864-870 (2005).
 - 15) Goud, V. V.; Patwardhan, A. V.; Dinda, S.; Pradhan, N. C. Epoxidation of karanja (*Pongamia glabra*) oil catalysed by acidic ion exchange resin. *Eur. J. Lipid Sci. Technol.* **109**, 575-584 (2007).
 - 16) Sun, S.; Ke, X.; Cui, L.; Yang, G.; Bi, Y.; Song, F.; Xu, X. Enzymatic epoxidation of *Sapindus mukorossi* seed oil by perstearic acid optimized using response surface methodology. *Ind. Crops Prod.* **33**, 676-682 (2011).
 - 17) Lu, H.; Sun, S.; Bi, Y.; Yang, G.; Ma, R.; Yang, H. Enzymatic epoxidation of soybean oil methyl esters in the presence of free fatty acids. *Eur. J. Lipid Sci. Technol.* **112**, 1101-1105 (2010).
 - 18) Sheldon, R. Catalytic reactions in ionic liquids. *Chem. Commun.* **23**, 2399-2407 (2001).
 - 19) Moniruzzaman, M.; Nakashima, K.; Kamiya, N.; Goto, M. Recent advances of enzymatic reactions in ionic liquids. *Biochem. Eng. J.* **48**, 295-314 (2010).
 - 20) Sureshkumar, M.; Lee, C. K. Biocatalytic reactions in hydrophobic ionic liquids. *J. Mol. Catal. B: Enzym.* **60**, 1-12 (2009).
 - 21) Paquot, C.; Hautfenne, A. Standard Methods for the Analysis of Oils, Fats and Derivatives, 7th ed., Blackwell Scientific Publications, Oxford, pp 118-119 (1987).
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