

## Preparation and characterization of epoxidized microbial oil

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**Abstract**—The potential of microbial oil derived from yeasts through fermentation using crops biomass for the formation of plasticizers was investigated. Plasticizers were formed via epoxidation reaction. Five factors of the orthogonal experiment (reaction temperature, time, weight ratio of H<sub>2</sub>O<sub>2</sub>/MO, H<sub>2</sub>SO<sub>4</sub>/MO, and HCOOH/MO) have been used for optimization of parameters. To further enhance the iodine value of microbial oil and increase the epoxy value of the epoxide, an amount of soybean oil was added. The products were characterized by FTIR, <sup>13</sup>C-NMR and <sup>1</sup>H-NMR. Under the optimum condition, the epoxy value and the iodine value of epoxide product were 6.21% and 1.8 g I<sub>2</sub>/100 g with a yield of 91.86% at an oil conversion of 98.43%. This study successfully demonstrated the conversion of crops biomass into value-added chemicals using simple and conventional chemical reactions.

Keywords: Microbial Oil, Plasticizer, Iodine Values, Epoxy Value, Crops Biomass

### INTRODUCTION

Epoxidized oils, with an annual production of about 200000 tons, are one kind of important fine chemical product synthesized by epoxidation reaction. They are frequently used as plasticizers, particularly for polyvinyl chloride (PVC) and serve as a scavenger for hydrochloric acid liberated from PVC when the PVC undergoes heat treatment [1]. Epoxidized oils can improve the elasticity of plastics; hence they play an important role in the manufacture of packing materials. Basically, epoxidized oils are produced from various oils feedstock, such as soybean, palm, rapeseed, sunflower, linseed, rubber seed and castor [2-7]. Obviously, most feedstock for epoxidized oils production come from crops or vegetables. However, using crops or vegetable oils has many disadvantages such as limitation from climate and season, great human being and culture area requirement. More seriously, it raises the issue of “food competition with humans.” Although non-edible oil can be another alternative [8], it still cannot be one stable, low-cost feedstock for epoxidized oils.

Recently, microbial oil (MO) was successfully synthesized from kinds of yeasts through fermentation using crop biomass such as corncob and rice straw [14-16], which contains unsaturated fatty acid and enables the formation of epoxy groups by epoxidation of double bonds. In China, about 116 million tons of corn are produced per year, and more than 20 million tons of corncobs are generated.

Therefore, using corncob hydrolysate for MO production would not only enable the formation of biobased chemicals, but also facili-

tate the development of a meaningful strategy for solving the environmental problems of burning straw. Furthermore, due to their biodegradability, biobased plasticizers will not accumulate in the environment, and this would effectively contribute to the establishment of a “green and sustainable” society.

MO represents a sustainable alternative to vegetable oil and animal fat as feedstock to synthesize fine chemical products, which is due to its advantages such as a short life cycle; less required labor; little limited by location, season, and climate; and easier to scale up without the problem of food competition with humans [9-13]. However, MO contains a relatively lower content of linoleic acid compared with vegetable oil, which means low iodine values and the poor epoxy value of epoxidized microbial oil (EMO). This greatly limits its application in the plasticizer field. To improve the iodine value of MO and the epoxy value of EMO, soybean oil (SBO) with high iodine value can be introduced into MO with a certain concentration. Compared with epoxidized oils production fore pure soybean oil, this technology saves a large amount of soybean oil.

Up to now, the extensive literature references concerning the preparation of MO have been reported, and a few applications of MO were studied. Daniel et al. [17] prepared a plasticizer and surfactant from food-waste and algal biomass-derived lipids; the results showed 0.35 and 0.40 g of plasticizer, and revealed the conversion of food-waste into value-added chemicals by using simple and conventional chemical reactions. However, no detailed research concerning the influence of various experimental conditions on the product was performed; the epoxy value and the iodine value of epoxide products were not measured, which were the essential factors for the epoxides. In this work, we mainly undertook a study of the epoxidation of MO; the effects of some factors, such as reaction time, reaction temperature, ratio of catalyst, formic acid and hydrogen peroxide to MOs were discussed through a series of orthog-

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onal experiments. Finally, SBO was introduced into MO to improve the epoxy value of EMO. By this study, epoxidized oils production could be obtained from new renewable oil sources mainly from crops (corn cob and rice straw, et al.), and this technology thus offers one new industrial crop product, which is used in the fine chemical field.

## EXPERIMENTAL

### 1. Materials

Microbial oil (MO) (Iodine values=62 gI<sub>2</sub>/100 g) was synthesized from oleaginous yeast *Trichosporon coremiiforme* through fermentation with corn cob acid hydrolysate by our Lab. Hydrolysed by dilute sulfuric acid, corn cob could turn into an acid hydrolysate with high sugar concentration (45.7 g·L<sup>-1</sup>) and this hydrolysate could be utilized for microbial oil production without concentration process. After detoxification by overliming and absorption with activated carbon, the corn cob hydrolysate could be used by *T. coremiiforme* efficiently, that a total cell mass of 20.4 g·L<sup>-1</sup> with a lipid content of 37.8% (corresponding to a lipid yield of 7.7 g·L<sup>-1</sup>), and a lipid coefficient of 17.2 could be obtained after cultivation of *T. coremiiforme* on the corn cob hydrolysate for eight days. Soybean oil (SBO) (Iodine values=120 gI<sub>2</sub>/100 g) was freshly pressed oil. These oils' compositions are given in Table 1. The determination was performed in accordance with the international standard PE-EN ISO 5508 (1990). Ethanol 95% (v/v), acetone, ethyl acetate, hydrogen peroxide, carbon tetrachloride, hydrochloric acid, sulfu-

ric acid, sodium hydroxide, sodium bicarbonate, magnesium sulfate, sodium thiosulfate, potassium iodide and bromine were purchased from the Chinese Chemical Company and were of analytical grade. All materials were used without further purification.

### 2. Preparation of Epoxidized Microbial Oil

MO was partially epoxidized using hydrogen peroxide and formic acid as illustrated in Scheme 1. The procedure used for epoxidation of MO was according to what was previously reported by Y.S. Lu et al. [18]. The relative amount of epoxidation was controlled by adjusting the weight ratios of hydrogen peroxide, sulfuric acid and formic acid. Generally, 50 g of MO, 2-24 g of formic acid (88% in water) and 0.5-2.5 g sulfuric acid were added in a three-neck flask equipped with a mechanical stirrer, reflux condenser, and thermometer. The mixture was stirred in a water bath at 64 °C. Then, 20-40 g of hydrogen peroxide (30%) was added dropwise. After 6 h, 150 mL of ethyl acetate and 100 mL of distilled water were added, resulting in two layers. The organic layer was neutralized to a pH with sodium bicarbonate, and then dried over anhydrous magnesium sulfate and filtered. Finally, the epoxidized microbial oil (EMO) was obtained after vacuum submitted to rotary evaporation at 38 °C for 1 h and dried in a 70 °C drying oven overnight. The obtained products were determined by FTIR, <sup>1</sup>H NMR and <sup>13</sup>C NMR spectroscopy.

### 3. Epoxidation Procedure of MO/SBO Blend Epoxides

Experiments were carried out to optimize the epoxidation route to synthesize the high epoxy value epoxides. Therefore, blends of MO/SBO with weight ratios of 80/20, 70/30, 60/40, 50/50, 40/60 and 30/70 were prepared; epoxidation of the blends was carried out as the same process of EMO. Hereafter, the epoxide was named as E-MO/SBO for short.

### 4. Characterization

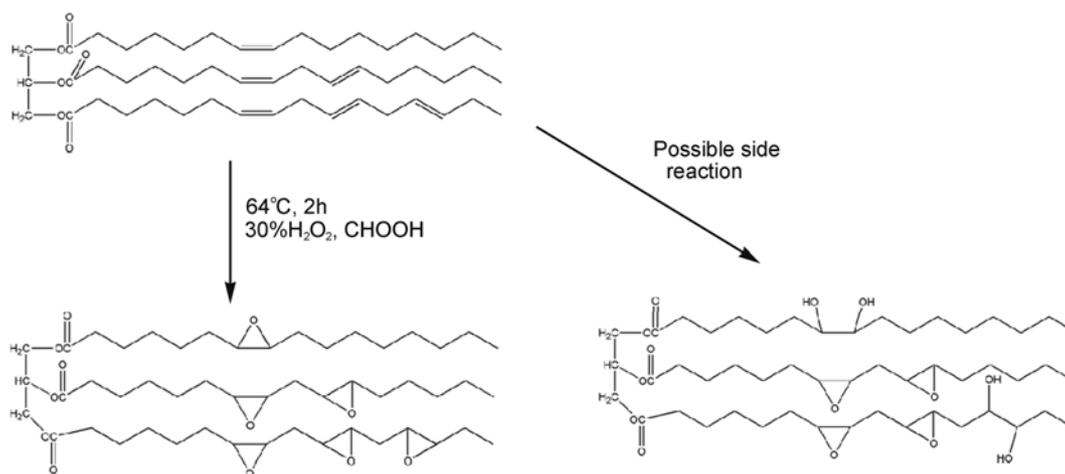
The fatty acids of extracted lipid were converted into fatty acid methyl esters (FAME) by esterification. This involved transesterification of each oil with MeOH to produce the corresponding methyl esters, which could then be analyzed using gas chromatography (GC-7890, Agilent, USA) in an electron impact mode with a HP-INNOWAX polyethylene glycol column (30 m×250 μm×0.25 μm). The column temperature was maintained at 170 °C for 1 min and

Table 1. Fatty acid composition of MO and SBO

Content (%)	C16:0	C18:0	C18:1	C18:2	Others
MO	23.3±0.3	24.9±0.3	40.0±0.3	7.5±0.2	4.3±0.5 <sup>a</sup>
SBO	11.1±0.2	4.0±0.3	23.4±0.3	51.8±0.3	7.3±0.4 <sup>b</sup>

<sup>a</sup>Others were C8:0, C10:0, C12:0, C14:0, C16:1, C18:3, C20:0, C20:1, C20:2, C22:0, and C24:0

<sup>b</sup>Others were C14:0, C16:1, C18:3, C20:0, and C22:0



Scheme 1. Epoxidation reaction schemes of microbial oil.

then increased by  $10\text{ }^{\circ}\text{C min}^{-1}$  up to  $200\text{ }^{\circ}\text{C}$  where it remained for 1 min. After that, it increased to  $230\text{ }^{\circ}\text{C}$  with a temperature gradient of  $3\text{ }^{\circ}\text{C min}^{-1}$  and held for 15 min. Argon was used as the carrier gas at  $1.0\text{ mL min}^{-1}$ , with split ratio was 1 : 10 (v/v). The injector temperature and detector temperature were  $240\text{ }^{\circ}\text{C}$  and  $240\text{ }^{\circ}\text{C}$ , respectively.

FTIR spectra were recorded on a TENSOR27 spectrometer. The frequency range of FTIR was  $4,000\text{--}450\text{ cm}^{-1}$ . Samples of MO, EMO and E-MO/SBO were measured. All tests used the KBr disk technique.  $^1\text{H-NMR}$  and  $^{13}\text{C-NMR}$  were performed using BRUKER VANCE III 400 machine. Five mg MO and EMO were dissolved in 1 mL chloroform-d in a sample vial, respectively. The solution was then transferred into a NMR sample tube with a minimum height of 4 cm prior to NMR analysis.

IR (KBr):  $\nu$  ( $\text{cm}^{-1}$ ): 3008 (w, C=C), 827 (m, epoxy ring).  $^1\text{H-NMR}$  (400 MHz,  $\text{CDCl}_3$ ),  $\delta$  (ppm): 1.5 ( $\text{CH}_2$  adjacent to epoxy ring), 2.0 ( $\text{CH}_2$  adjacent to C=C), 2.9 (epoxy ring H), 5.2 ( $-\text{CH}=\text{CH}-$ ).  $^{13}\text{C-NMR}$  (400 MHz,  $\text{CDCl}_3$ ),  $\delta$  (ppm): 27 ( $\text{CH}_2$  adjacent to epoxy ring), 51, 58 (epoxy ring C), 130 (C=C).

The final products were analyzed for iodine value, epoxy value, conversion and yield. Three samples of each product were tested and the results were arithmetically averaged. The iodine values of MO and MO/SBO blends ( $\text{gI}_2/100\text{g}$ ,  $\text{IV}_{be}$ ) characterized the concentration of carbon-carbon double bonds of MO and MO/SBO blends and were determined using the Wijs method according to ISO 3961. The epoxy value (%), EV was obtained by the direct method with the hydrochloric acid solution in acetone referring to Chinese National Standard GB/T 1677. The iodine values of products ( $\text{g I}_2/100\text{g}$ ,  $\text{IV}_{ac}$ ) revealed the extent of epoxidation reaction and were measured on the basis of Chinese National Standard GB/T 1676. The conversion of microbial oil was calculated using the following expression (1) [19]:

$$C = \frac{\text{IV}_{be} - \text{IV}_{ac}}{\text{IV}_{be}} \cdot 100\% \quad (1)$$

where  $\text{IV}_{be}$  is iodine value of oil sample before epoxidation,  $\text{IV}_{ac}$  is iodine value of oil sample after epoxidation.

From the oxirane content values, the epoxide yield to oxirane was calculated using the following expression (2) [19]:

$$Y = \frac{\text{EV}}{\text{EV}_{max}} \cdot 100\% \quad (2)$$

where EV is the experimentally obtained epoxy value of microbial oil after epoxidation,  $\text{EV}_{max}$  is the theoretically obtainable maximum epoxy value, which was determined from the following expression (3) [20]:

$$\text{EV}_{max} = \left[ \frac{(\text{IV}_{be}/2A_i)}{100 + (\text{IV}_{be}/2A_i)A_0} \right] A_0 \cdot 100 \quad (3)$$

where  $A_i$  (126.9) and  $A_0$  (16.0) are the atomic weights of iodine and oxygen respectively.

## RESULTS AND DISCUSSION

The fatty acid compositions of MO and SBO are shown in Table 1. The mainly accumulated fatty acids were C-16 and C-18 atoms.

Meanwhile, C16:0, C18:1 and C18:2 were the predominantly fatty acids. SBO mainly contained oleic acid (C18:1, 23.4%) and linoleic acid (C18:2, 51.8%), whereas MO contained much less linoleic acid (C18:2, 7.5%) but more oleic acid (C18:1, 40.0%) as well as 24.9% stearic acid (C18:0). Most of the fatty acids in SBO and MO were unsaturated acids. The high content accumulation of the oleic acid (C18:1) and linoleic acid (C18:2) was as a good source of essential fatty acids for chemical conversions, which was due to the higher content double bonds and would obtain the products with higher epoxy values (Scheme 1).

### 1. FTIR Analysis

The epoxidation of MO was evidenced by the peaks of C=C double bonds and epoxy groups before and after the reaction. FTIR spectra of the MO, and the prepared EMO and E-MO/SBO are shown in Fig. 2. In comparison with the spectrum of the MO, the disappearance of C=C group at  $3,008\text{ cm}^{-1}$  was obvious. The final epoxides showed a characteristic signal at  $827\text{ cm}^{-1}$  indicating the presence of epoxy groups. As a result, FTIR could provide infor-

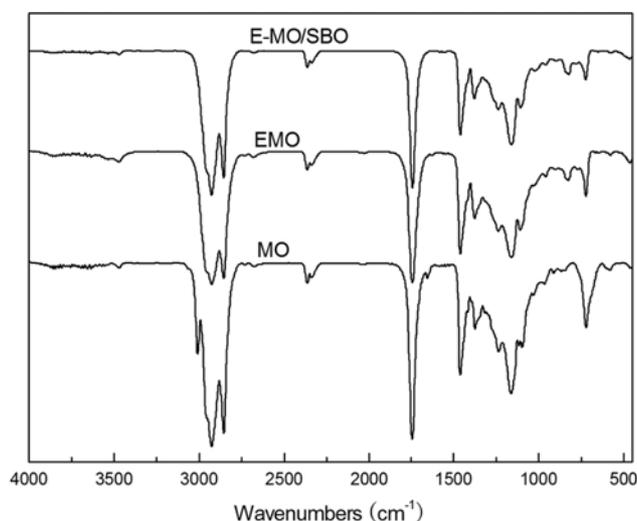


Fig. 1. FTIR spectra of MO, EMO and E-MO/SBO.

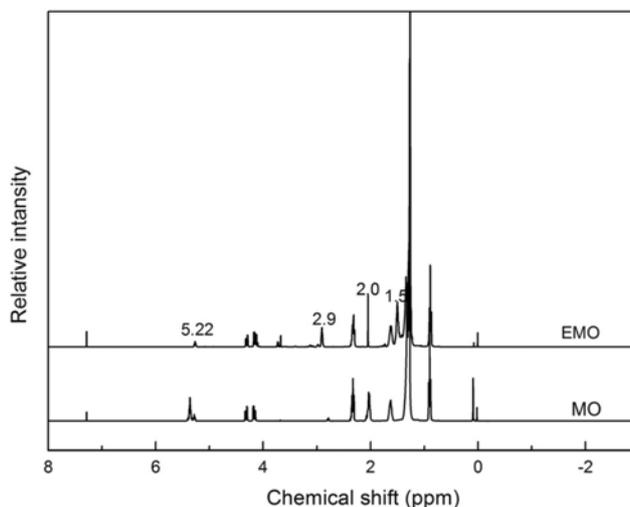


Fig. 2.  $^1\text{H-NMR}$  spectra of MO and EMO.

## 2. $^1\text{H-NMR}$ and $^{13}\text{C-NMR}$ Analysis

$^1\text{H-NMR}$  and  $^{13}\text{C-NMR}$  were used to elucidate the molecular structure of the MO and EMO. Comparison was done between the  $^1\text{H-NMR}$  spectra of MO with EMO (Fig. 3). In the spectra, the C=C and methylene proton adjacent to C=C were detected at 5.22 ppm and 2.0 ppm in MO sample, whereas decrement of rela-

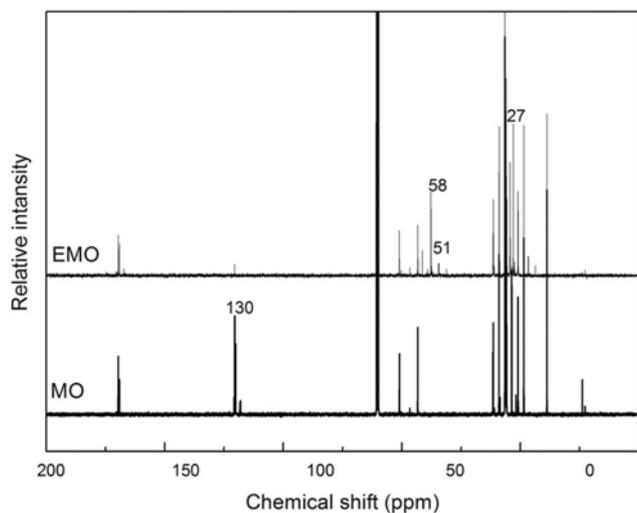


Fig. 3.  $^{13}\text{C-NMR}$  spectrums of MO and EMO.

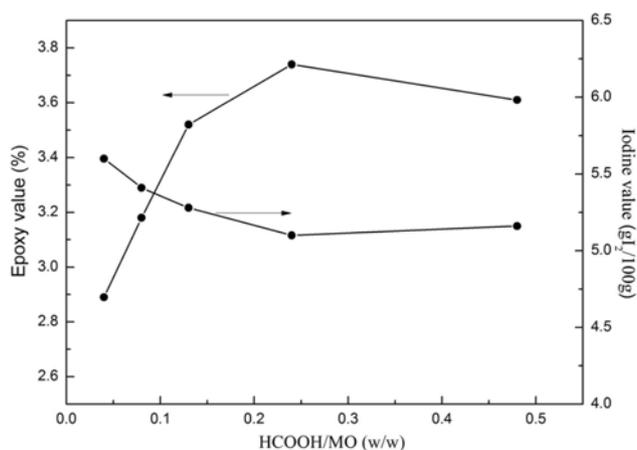


Fig. 4. Effect of HCOOH/MO ratio on the epoxy value and the iodine value of products (reaction temperature=65 °C, reaction time =6 h,  $\text{H}_2\text{O}_2/\text{MO}=0.7 : 1$  (w/w),  $\text{H}_2\text{SO}_4/\text{MO}=0.02 : 1$  (w/w),  $\text{HCOOH}/\text{MO}=0.13 : 1$  (w/w) and mechanical agitation).

tive height of C=C and methylene carbon adjacent to C=C indicated that the C=C had reacted with peroxy acid. However, the reaction was not complete because the weak C=C proton was still detected. Meanwhile, the epoxy ring (2.9 ppm) and methylene proton adjacent to epoxy ring (1.5 ppm) were observed in EMO sample, while no the absorption peak was observed in MO sample.

$^{13}\text{C-NMR}$  spectra of MO and EMO are shown in Fig. 4. For MO, carbon attached to C=C was assigned to peak at 130 ppm, As for EMO, carbon attached to epoxy ring was assigned to peaks at 58 and 51 ppm. Methylene carbon adjacent to epoxy ring was assigned to peak at 27 ppm. The absorption peak of carbon attached to C=C became significantly weaker in the EMO sample, which illustrated that most of the C=C was oxidized; this result was consistent with that of  $^1\text{H-NMR}$ . Consequently, carbon attached to epoxy ring indicated the formation of epoxides resulting from epoxidation reaction, so we obtained the epoxide products.

## 3. Epoxidation Reaction of MO

Here, an orthogonal experimental design method was applied to discuss the ID of the response functions for selecting the optimum epoxidation reaction parameters. The reaction temperature, time, weight ratio of  $\text{H}_2\text{O}_2/\text{MO}$ ,  $\text{H}_2\text{SO}_4/\text{MO}$  and  $\text{HCOOH}/\text{MO}$  were determined as five factors of the orthogonal experiment and each factor had five levels, as shown in Table 2.

The matrix of experiments design and experimentally determined values of response functions (iodine value ( $IV_{av}$ ) of EMO, epoxy value (EV) of EMO, conversion of MO (C), and yield of EMO (Y)) are shown in Table 3. The five ordered degree values (EV) of each factor in the same level were summed, and the corresponding average value  $k_i$  and range R were calculated, respectively, as follows:

$$k_i = \frac{\sum EV_i}{5} \quad (4)$$

$$R = k_{max} - k_{min} \quad (5)$$

where  $k_i$  represents the impact of level to the response function of products ( $i=1, 2, 3, 4, 5$ ). The higher  $k_i$  is, the better response function is.  $k_{max}$  is the maximum among five  $k_i$  values of each factor and  $k_{min}$  is the minimum. R reflects the ID of the factors to response function, and here, ID of the factors is used to measure the influence of various factors on the experimental results. The factor having higher R suggests a stronger impact on response function.

Statistical analysis of the results was carried out after calculation and the results are shown in Table 3. In the example experiment, R was as follows: 0.420, 0.616, 0.300, 0.611 and 0.182, which reflected the ID of the factors were as follow: B>A>C>E>D. Hence, tem-

Table 2. Independent parameters and their ranges at the individual levels

Level i	Temperature (°C)	Weight ratio $\text{H}_2\text{O}_2/\text{MO}$ (g/g)	Weight ratio $\text{HCOOH}/\text{MO}$ (g/g)	Weight ratio $\text{H}_2\text{SO}_4/\text{MO}$ (g/g)	Reaction time (h)
	A	B	C	D	E
1	35	0.4	0.04	0.01	3
2	50	0.5	0.08	0.02	4
3	65	0.6	0.13	0.03	5
4	80	0.7	0.24	0.04	6
5	95	0.8	0.48	0.05	7

**Table 3. Design matrix and experiments results of statistical analysis**

Lp.	A Temp.	B H <sub>2</sub> O <sub>2</sub> /MO	C HCOOH/MO	D H <sub>2</sub> SO <sub>4</sub> /MO	E Time	IV <sub>ac</sub> (g I <sub>2</sub> /100 g)	EV (%)	C (%)	Y (%)
1	1	1	1	1	1	5.5	2.85	91.13	73.08
2	1	2	2	2	2	5.3	3.20	91.45	82.05
3	1	3	3	3	3	5.7	2.95	90.81	75.64
4	1	4	4	4	4	5.5	3.74	91.13	95.90
5	1	5	5	5	5	5.4	3.12	91.29	80.00
6	2	1	2	3	4	5.3	3.41	91.45	87.44
7	2	2	3	4	5	5.2	3.25	91.61	83.33
8	2	3	4	5	1	5.3	3.27	91.45	83.85
9	2	4	5	1	2	5.2	3.24	91.61	83.08
10	2	5	1	2	3	5.5	3.02	91.13	77.44
11	3	1	3	5	2	5.3	3.59	91.45	92.05
12	3	2	4	1	3	5.2	3.74	91.61	95.90
13	3	3	5	2	4	5.4	3.32	91.29	85.13
14	3	4	1	3	5	5.5	3.65	91.13	93.59
15	3	5	2	4	1	5.2	3.64	91.61	93.33
16	4	1	4	2	5	5.1	3.43	91.77	87.95
17	4	2	5	3	1	5.2	3.34	91.61	85.64
18	4	3	1	4	2	5.2	3.55	91.61	91.03
19	4	4	2	5	3	5.3	3.58	91.45	91.79
20	4	5	3	1	4	5.4	3.65	91.29	93.59
21	5	1	5	4	3	5.3	3.26	91.45	83.59
22	5	2	1	5	4	5.5	3.34	91.13	85.64
23	5	3	2	1	5	5.4	3.61	91.29	92.56
24	5	4	3	2	1	5.6	3.38	90.97	86.67
25	5	5	4	3	2	5.5	3.60	91.13	92.31
26	3	5	4	3	4	5.2	3.72	91.61	95.38
27	3	5	4	3	4	5.3	3.69	91.45	94.62
28	3	5	4	3	4	5.3	3.74	91.45	95.90
k <sub>1</sub>	3.168	3.366	3.262	3.344	3.316				
k <sub>2</sub>	3.236	3.410	3.320	3.348	3.426				
k <sub>3</sub>	3.588	3.352	3.302	3.454	3.310				
k <sub>4</sub>	3.510	2.852	3.556	3.380	3.492				
k <sub>5</sub>	3.438	3.468	3.256	3.394	3.412				
R	0.420	0.616	0.300	0.110	0.182				
Best level	A <sub>3</sub>	B <sub>5</sub>	C <sub>4</sub>	D <sub>3</sub>	E <sub>4</sub>				
ID of factors	2	1	3	5	4				
Optimum group	A <sub>3</sub> B <sub>5</sub> C <sub>4</sub> D <sub>3</sub> E <sub>4</sub>								

perature and the weight ratio of H<sub>2</sub>O<sub>2</sub>/MO had a stronger impact on response function; the ratio of H<sub>2</sub>SO<sub>4</sub>/MO did not influence function value in a significant way. From Table 3, k<sub>1</sub>, k<sub>2</sub>, k<sub>3</sub>, k<sub>4</sub> and k<sub>5</sub>, represented the impact of level; the best levels were A<sub>3</sub>, B<sub>5</sub>, C<sub>4</sub>, D<sub>3</sub> and E<sub>4</sub> respectively. Therefore, the optimum reaction factors were A (65 °C), B (0.8:1), C (0.24:1), D (0.03:1), and E (6 h). Under the optimum condition, three groups of experiments were carried out. The results with good repeatability were arithmetically averaged; the epoxy value and the residual iodine value of EMO were 3.72% and 5.3 g I<sub>2</sub>/100 g with a yield of 95.3% at an oil conversion of 91.5%.

The effects of the parameters (reaction temperature, time, weight ratio of H<sub>2</sub>O<sub>2</sub>/MO, H<sub>2</sub>SO<sub>4</sub>/MO and HCOOH/MO) on IV<sub>ac</sub> and EV were investigated, and the results are shown in Figs. 4-8. The charts showed the influence of one independent parameter on the levels resulted from the experimental design, while the remaining independent parameters were established at a certain level.

Fig. 4 presents the effect of the weight ratio of HCOOH/MO on the epoxy values and the iodine values of products; the lowest iodine value was obtained at higher weight ratio of HCOOH/MO, as well as the highest epoxy value was achieved. With a further increasing HCOOH content, the epoxy value was decreased, whereas

the iodine value increased. Generally, epoxidation was mostly associated with side reactions, such as hydroxylation, oxidation, oxygenation, and dimer formation [21]. Faria-Machado et al. [22] reported side products and the presence of hydroxyl groups when epoxidation was performed using  $H_2O_2$  and acetic at  $60^\circ C$  for 6 h. Therefore, we inferred that the reaction environment contained water, catalyst, and organic acid in our studies, which maybe caused decomposition of the epoxy group hydrolysis (Scheme 1). We found when the weight ratio of  $HCOOH/MO$  was above 0.24, the higher  $HCOOH$  content, the easier for the side reaction to be happen.

Fig. 5 illustrates the effect related with reaction time. It shows that the epoxy value was increased first and then decreased with the reaction time increasing from 3 to 7 h, while the iodine value had opposite results during the reaction. When the reaction time was too short, conversion of double bonds to oxirane was incomplete, resulting in lower epoxy value. However, when the reaction time was too long, the ring-opening reaction of the oxirane was

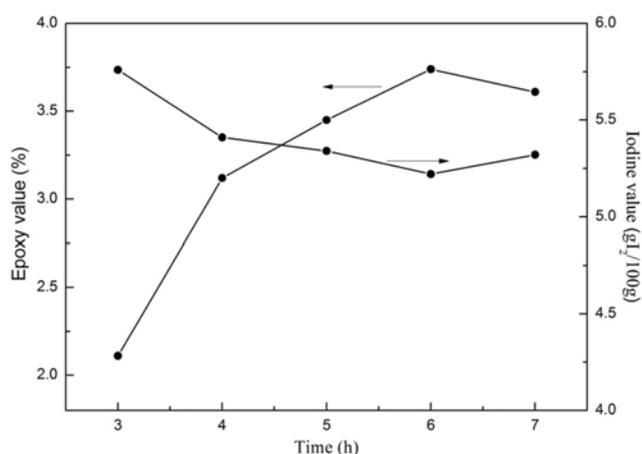


Fig. 5. Effect of reaction time on the epoxy value and the iodine value of products (reaction temperature= $65^\circ C$ ,  $H_2O_2/MO=0.7:1$  (w/w),  $H_2SO_4/MO=0.02:1$  (w/w),  $HCOOH/MO=0.13:1$  (w/w) and mechanical agitation).

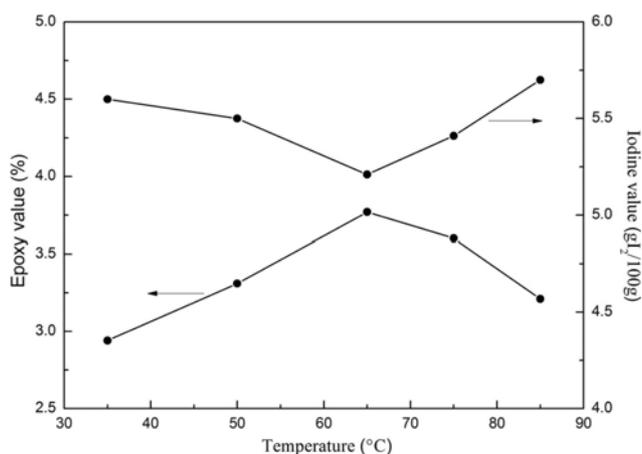


Fig. 6. Effect of reaction temperature on the epoxy value and the iodine value of products (reaction time=6 h,  $H_2O_2/SBO=0.7:1$  (w/w),  $H_2SO_4/MO=0.02:1$  (w/w),  $HCOOH/MO=0.13:1$  (w/w) and mechanical agitation).

dominated in the system, leading to lower epoxy value as well. As expected, the iodine value showed a great reduction compared with that of MO, which indicated a little increment in C=C bond formations in the products, and MO were successfully epoxidized. Furthermore, the reaction was basically completed after 6 h and, thus, the overall reaction time can be reduced with considerable impact on process economy.

The results in Fig. 6 obviously show that the optimum of the epoxy value and the iodine value was obtained at  $65^\circ C$ . With a further increase in temperature, the epoxy value was increased, whereas the residual iodine value decreased up to 5.3 and then increased dramatically. The reason could be that the epoxidation reaction was carried out incompletely at low temperature, while high temperature led to accelerate dissociation of  $H_2O_2$ , thus could reduce efficiency of  $H_2O_2$ , leading to lower epoxy value. The moderate reaction conditions used herein resulted in a good yield of products and  $>90\%$  of the initial unsaturated C=C groups were converted. This finding was in agreement with that of Akintayo et al. [23] who found an almost complete conversion.

The influence of the weight ratio between MO and  $H_2O_2$  (1 : 0.6, 1 : 0.7, 1 : 0.8 and 1 : 0.9) on the epoxy value and the iodine value is enumerated in Fig. 7. With the increasing of  $H_2O_2/MO$  ratio, the epoxy value was increased; and the iodine value was decreased. When the weight ratio increased further to 0.9, only minor difference in the final epoxy value was observed. The epoxy value and the iodine value were 3.78% and 5.4 I<sub>2</sub>/100 g, which was more acceptable for the reaction.

The effect of the weight ratio between MO and sulfuric acid is summarized in Fig. 8. From the results, we could see that no obvious effect of the iodine value was detected. The epoxy value varied in 3.38%-3.74%, and increased up to 0.04 of  $H_2SO_4/MO$  ratio and then decreased. This was attributed to the ring-opening side reaction at high sulfuric acid content. Moreover, it could result in dissociation of epoxides and generate fatty acids, which was able to reduce the quality of the product.

#### 4. Epoxidation Reaction of MO/SBO

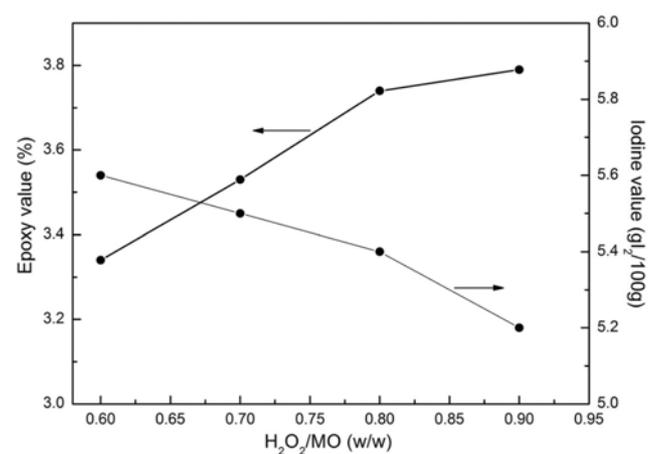


Fig. 7. Effect of  $H_2O_2/MO$  ratio on the epoxy value and the iodine value of products (reaction time=6 h, reaction temperature= $65^\circ C$ ,  $H_2SO_4/MO=0.02:1$  (w/w),  $HCOOH/MO=0.13:1$  (w/w) and mechanical agitation).

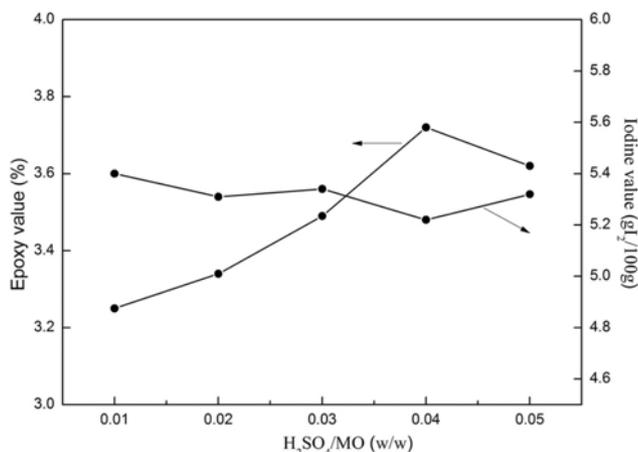


Fig. 8. Effect of H<sub>2</sub>SO<sub>4</sub>/MO ratio on the epoxy value and the iodine value of products (reaction time=6 h, reaction temperature=65 °C, H<sub>2</sub>O<sub>2</sub>/MO=0.8:1 (w/w), HCOOH/MO=0.13:1 (w/w) and mechanical agitation).

Table 4. Effect of SBO/MO ratio on the response functions (IV<sub>be</sub>, IV<sub>ae</sub>, EV, C and Y)

MO/SBO (w/w)	IV <sub>be</sub> (g I <sub>2</sub> /100 g)	IV <sub>ae</sub> (g I <sub>2</sub> /100 g)	EV (%)	C (%)	Y (%)
100/0	62	5.3	3.72	91.50	95.30
90/10	65	5.5	3.72	91.54	94.42
80/20	77	5.4	4.20	92.86	90.71
70/30	87	3.2	4.37	96.32	84.04
60/40	95	2.0	4.95	97.89	87.61
50/50	97	1.8	5.06	98.14	87.85
40/60	109	1.6	6.08	98.53	94.56
30/70	115	1.8	6.21	98.43	91.86

Compared with commercialized high-quality plasticizers, EMO had low epoxy values under the optimal parameters of the epoxidation reaction, which limited its application in the plasticizer fields (Table 3). To improve the iodine value of MO and the epoxy value of EMO, soybean oil (SBO) was introduced into MO. As shown in Table 4, we could clearly see that the iodine value (IV<sub>be</sub>) of MO was increased with the introduction of SBO. Meanwhile, the effect of the SBO/MO ratio on response functions is listed in Table 4. The EV and C of all functions with the exception of IV<sub>ae</sub> and Y were increased with the increasing SBO content; the best EV was exerted by the 30/70 specimens. The epoxy value of epoxide could reach 6.21% with a yield of 98.43% at an oil conversion of 98.53%, which indicated that E-MO/SBO could be used as plasticizer to improve the elasticity of plastics. So, blending MO and SBO to improve the epoxy value of EMO was accepted as an effective and economical route generally.

### CONCLUSIONS

A series of EMOs were prepared through epoxidation reaction. The test results revealed that the optimal epoxy value and the iodine value were 3.74% and 5.4 g I<sub>2</sub>/100 g, respectively. The study showed

that the iodine value of MO was increased with the introduction of SBO; when the content of SBO was above 60%, the epoxy value of epoxide could reach 6%; the iodine value was less than 2.0%. Under the optimum condition, the epoxy value and the residual iodine value of E-MO/SBO were 6.21% and 1.8 g I<sub>2</sub>/100 g with a yield of 91.86% at an oil conversion of 98.43%. Ultimately, it was shown that using simple and conventional chemical reactions, MO could be utilized in the production of value-added chemicals.

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### NOMENCLATURE

EMO	: epoxidized microbial oil
MO	: microbial oil
SBO	: soybean oil
FAME	: fatty acid methyl esters
E-MO/SBO	: epoxidized microbial oil/soybean oil
FTIR	: fourier transform infrared spectroscopy
IV <sub>ae</sub>	: iodine value of microbial oil before epoxidation
IV <sub>be</sub>	: iodine value of microbial oil after epoxidation
EV	: epoxy value
EV <sub>max</sub>	: theoretically maximum epoxy value
C	: conversion of microbial oil
Y	: epoxide yield
A <sub>i</sub>	: atomic weights of iodine
A <sub>0</sub>	: atomic weights of oxygen

### REFERENCES

1. E. A. J. Al-Mulla, A. H. Suhail and S. A. Aowda, *Ind. Crop. Prod.*, **33**, 23 (2011).
2. A. K. R. Somidi, R. V. Sharma and A. K. Dalai, *Ind. Eng. Chem. Res.*, **53**, 18668 (2014).
3. P. J. Roumanet, F. Lafleche, N. Jarroux, Y. Raoul, S. Claude and P. Guégan, *Eur. Polym. J.*, **49**, 813 (2013).
4. X. F. Chen, C. Huang, X. Y. Yang, L. Xiong, X. D. Chen and L. L. Ma, *Bioresour. Technol.*, **143**, 18 (2013).
5. S. G. Tan, Z. Ahmad and W. S. Chow, *Ind. Crop. Prod.*, **43**, 378 (2013).
6. A. A. Abdelmalik, A. P. Abbott, J. C. Fothergill, S. Dodd and R. C. Harris, *Ind. Crop. Prod.*, **33**, 32 (2011).
7. E. Sharmin, S. M. Ashraf and S. Ahmad, *Int. J. Biol. Macromol.*, **40**, 407 (2007).
8. S. Ahmad, S. M. Ashraf, F. Naqvi, S. Yadav and F. Zafar, *J. Macromol. Sci. A.*, **43**, 1409 (2006).
9. M. J. Jiménez Callejón, A. Robles Medina, M. D. Macías Sánchez,

- E. Hita Peña, L. Esteban Cerdán, P.A. González Moreno and E. Molina Grima, *Bioresour. Technol.*, **169**, 198 (2014).
10. P.J. McGinn, K.E. Dickinson, K. Park, C.G. Whitney, S.P. MacQuarrie, F.J. Black, J.C. Frigon, S.R. Guiot and S.J.B. O'Leary, *Algal Res.*, **1**, 155 (2012).
11. M. Onay, C. Sonmez, H.A. Oktem and A.M. Yucel, *Bioresour. Technol.*, **169**, 62 (2014).
12. J.S. Poli, M.A. da Silva, E.P. Siqueira, V.M. Pasa, C.A. Rosa and P. Valente, *Bioresour. Technol.*, **161**, 320 (2014).
13. C.E. Reis, J. Zhang and B. Hu, *Appl. Biochem. Biotechnol.*, **174**, 411 (2014).
14. A.S.A.A. Hazmi, M.A. Min, C.S. Luqman, Z.M. Mek and H. Mohd, *Ind. Crop. Prod.*, **50**, 563 (2013).
15. C. Huang, X.F. Chen, X.Y. Yang, L. Xiong, X.Q. Lin, J. Yang, B. Wang and X.D. Chen, *Appl. Biochem. Biotechnol.*, **172**, 2197 (2014).
16. C. Huang, X.X. Cui, H. Wu and W.Y. Lou, *Int. J. Green. Energy*, **11**, 787 (2014).
17. D. Pleissner, K.Y. Lau, C. Zhang and C.S.K. Lin, *ChemSusChem*, **8**, 1686 (2014).
18. Y.S. Lu and R.C. Larock, *Biomacromolecules*, **9**, 3332 (2008).
19. M. Eugeniusz, S. Anna and L. Grzegorz, *J. Chem. Technol. Biotechnol.*, **85**, 1099 (2010).
20. S.P. Dinda, V.G. Anand, V.P. Valbhav and C. Narayan, *Bioresour. Technol.*, **99**, 3737 (2008).
21. Z.S. Petrović, A. Zlatanić, C.C. Lava and S. Sinadinović-Fiser, *Eur. J. Lipid Sci. Technol.*, **104**, 293 (2002).
22. A.F. Faria-Machado, M.A. da Silva, M.G. Adeodato Vieira and M.M. Beppu, *J. Appl. Polym. Sci.*, **127**, 3543 (2013).
23. E.T. Akintayo, T. Ziegler and A. Onipede, *Bull. Chem. Soc. Ethiop.*, **20**, 75 (2006).