

# Enzymatic Epoxidation of Corn Oil by Perstearic Acid

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**Abstract** Epoxidized vegetable oils can be used as renewable biodegradable and non-toxic lubricants, polymer stabilizers, and as intermediates. In this study, as a renewable resource, corn oil rich in oleic and linoleic acids, which was epoxidized using hydrogen peroxide as an oxygen donor and stearic acid as an active oxygen carrier in the presence of Novozym 435. The process was optimized for the enzymatic epoxidation of corn oil with an epoxy oxygen group content of  $5.8 \pm 0.2\%$  and a percentage relative conversion to oxirane of  $85.3 \pm 2.9\%$  under the following conditions: 35 °C, 28% stearic acid load (relative to the weight of corn oil), 2.7:1 mol ratio of  $\text{H}_2\text{O}_2/\text{C}=\text{C}$ -bonds, and 10 h. The influence on the enzymatic epoxidation decreased in the order of stearic acid load > reaction temperature  $\approx$  mole ratio of  $\text{H}_2\text{O}_2/\text{C}=\text{C}$ -bonds > reaction time.

**Keywords** Corn oil · Epoxidation · Immobilized *Candida antarctica* lipase B · Orthogonal design · Stearic acid

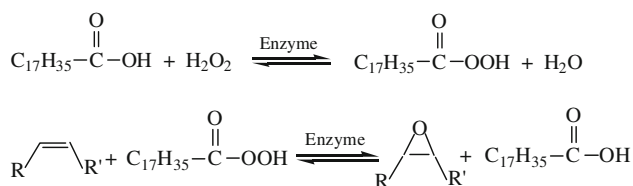
## Introduction

Epoxidized vegetable oils and their derivatives, which are biodegradable, environment-friendly, and renewable

resources, have recently found industrial applications as plasticizers and additives for PVC [1–7]. As a renewable resource, corn oil rich in oleic and linoleic acids, which can be epoxidized with hydrogen peroxide, either in the presence of acetic acid or formic acid, to yield epoxidized corn oil [8]. During this chemical epoxidation, peroxy acids are commonly produced by the reaction of acetic or formic acid and hydrogen peroxide in the presence of strong mineral acids, such as  $\text{H}_2\text{SO}_4$  and  $\text{H}_3\text{PO}_4$  [9, 10], which can cause equipment corrosion and initiate undesirable oxirane ring-opening reactions. Recently, enzymatic epoxidation have been successfully developed [11–15], which is superior to chemical synthesis. However, no reports focused on the enzymatic epoxidation of corn oil and stearic acid as an active oxygen carrier.

In our previous report, it was very interesting that stearic acid was chosen as an active oxygen carrier—it showed a better performance in converting biodiesel double bonds to oxirane groups than traditional oxygen carriers such as, acetic and formic acid [11]. In this study, corn oil was chosen as the raw material for epoxidation. In the presence of immobilized lipase B from *Candida antarctica*, stearic acid was chosen as the active oxygen carrier, and hydrogen peroxide was selected as the oxygen donor. The double bonds of corn oil were epoxidized by perstearic acid previously generated by the reaction of stearic acid and hydrogen peroxide (during this progress, hydrolysis, esterification, and transesterification of triglycerides and perstearic acid also took place). The enzymatic epoxidation mechanism of corn oil is shown in Fig. 1. The effects of reaction time, reaction temperature, load of stearic acid, mole ratio of  $\text{H}_2\text{O}_2$  and double bonds on the enzymatic epoxidation of corn oil were investigated, and the reaction variables were optimized by orthogonal design.

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**Fig. 1** The enzymatic epoxidation mechanism of corn oil in the presence of stearic acid

## Materials and Methods

### Materials

Corn oil (acid value = 0.16 mg/g, iodine value = 114.78 g I<sub>2</sub>/100 g) was purchased from a local supermarket (Zhengzhou, China). The contents of the corn oil determined by GC were oleic acid 33.6 ± 1.0%, linoleic acid 49.0 ± 1.2%, palmitic acid 13.5 ± 0.6%, stearic acid 2.1 ± 0.5%. Hydrogen peroxide (purity = 30%) was purchased from Luoyang Haohua Chemical Co., Ltd (Luoyang, China). Stearic acid (purity >99%; peroxide value = 0.69 mmol/kg) was purchased from Sigma-Aldrich Co. (St. Louis, MO). Novozym 435 (*Candida antarctica* lipase immobilized on polyacrylic resin, 10,000 PLU/g) was from Novozymes A/S (Bagsvaerd, Denmark). All other reagents were of analytical grade.

### General Procedures for the Enzymatic Epoxidation of Corn Oil

Epoxidation reactions were carried out in 250-ml three-necked round-bottom flasks placed in a water bath: 10 g corn oil was reacted with 2.8 g stearic acid and 0.3 g immobilized lipase in 50 g toluene at 55 °C stirred at a speed of 600 rpm, and 14 g 30% (w/w) hydrogen peroxide was added dropwise to the reaction mixture over a period of 10 min. After the addition of the hydrogen peroxide was complete, the reactions continued further for the desired time duration. These standard conditions were used except when otherwise stated in the text.

Prior to analysis, the products were washed with distilled water, trace water and decomposed traces of unreacted hydrogen peroxide were removed with anhydrous sodium sulfate, and the solvent and remaining trace water were distilled off using a rotary evaporator.

### Orthogonal Design

Orthogonal design can achieve the best combination of parameter levels for optimal performance of a system with only a very limited number of experiments and shorter time. Each combination of experiments selected in an orthogonal design is homodisperse and homogeneous.

**Table 1** Factors and levels for orthogonal design

Variables	Level		
	1	2	3
A, reaction temperature (°C)	35	50	65
B, stearic acid load (relative to the weight of corn oil)	14%	28%	42%
C, mole ratio of H <sub>2</sub> O <sub>2</sub> and double bonds	0.7:1	2.7:1	4.7:1
D, reaction time (h)	8	10	12

Orthogonal design assumes that there are no interactions between the variables, and no optimization equation can be obtained from the results of this design. Epoxidation conditions were optimized by an orthogonal design L<sub>9</sub>(3)<sup>4</sup>. Nine epoxidation reactions were carried out at reaction temperatures of 35, 50, 65 °C, stearic acid loads of 14, 28, 42% (relative to the weight of corn oil), mole ratios of H<sub>2</sub>O<sub>2</sub> and double bonds 0.7:1, 2.7:1, 4.7:1, and reaction times of 8, 10, 12 h on the basis of the single-factor test. Table 1 shows the experimental conditions for the enzymatic epoxidation of corn oil.

### Analytical Techniques

The epoxy oxygen group content (EOC) determination was carried out by the direct method with hydrobromic acid solution in acetic acid [16].

From the oxirane content, the relative conversion to oxirane (RCO) as a percentage was determined using the following formula:

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

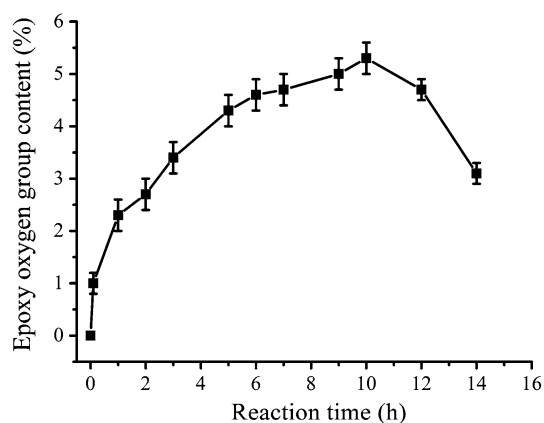
where OO<sub>ex</sub> is the content of oxirane oxygen experimentally determined, and OO<sub>th</sub> is the theoretical maximum oxirane oxygen in 100 g of corn oil, determined using the following expression:

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

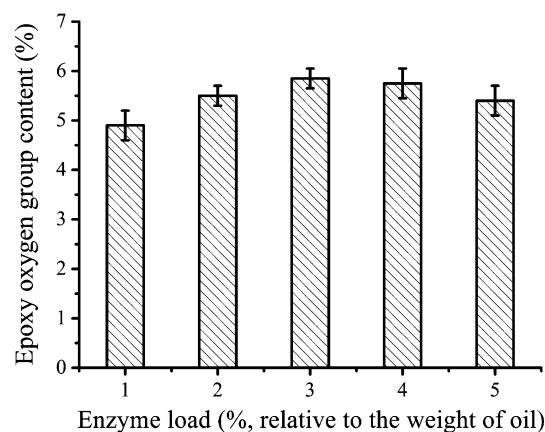
where A<sub>i</sub> (126.9) and A<sub>o</sub> (16.0) are the atomic weights of iodine and oxygen, respectively; and IV<sub>0</sub> is the initial iodine value of corn oil. The theoretical maximum oxirane oxygen content in 100 g of corn oil (OO<sub>th</sub>) is 6.8%.

### Statistical Analyses

All experiments were performed at least in triplicate. Results were expressed as means ± SEM. For orthogonal array design analysis of experiments, a two-way analysis of variance (ANOVA) was used. Statistical significance was considered at *p* < 0.05.



**Fig. 2** Effects of reaction time on the epoxidation of corn oil. Reaction conditions: 10 g corn oil, 2.8 g stearic acid, 0.3 g immobilized lipase, reaction temperature 60 °C, mixing speed 600 rpm, and 14 g 30% hydrogen peroxide



**Fig. 3** Effects of enzyme load on the epoxidation of corn oil. Reaction conditions: 10 g corn oil, 2.8 g stearic acid, required quantity immobilized lipase, 10 h, reaction temperature 50 °C, mixing speed 600 rpm, and 14 g 30% hydrogen peroxide

## Results and Discussions

### Effects of Reaction Time

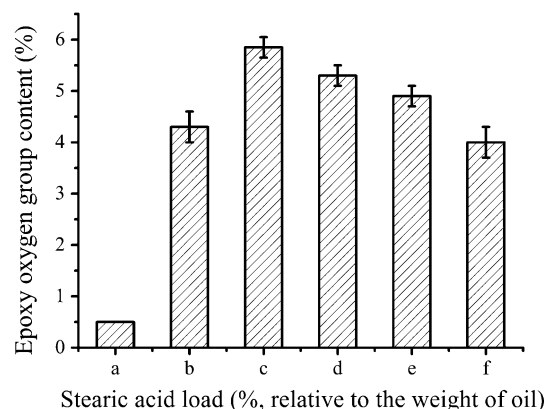
With increasing reaction time (<10 h), the EOC of epoxidized corn oil smoothly increased, and reached a maximum ( $5.3 \pm 0.3\%$ ) at 10 h (Fig. 2). These results were attributed to lower acidity and the active oxygen carrier of stearic acid and the small amount of by-product water formed at the beginning of the enzymatic epoxidation. However, with further increases in reaction time (>10 h), the EOC of epoxidized corn oil rapidly decreased, which was ascribed to more by-product water being formed and the undesirable oxirane-ring opening reactions of epoxidized corn oil.

### Effects of Enzyme Load

An increase in enzyme load from 1% (w/w, relative to the weight of corn oil) to 3% resulted in a linear increase in the EOC of epoxidized corn oil. The results suggested that the external transfer limitation was essentially eliminated in the reaction system, which is in agreement with previous studies [17, 18]. The EOC of epoxidized corn oil appeared to decrease, but the change was not significant at high levels of enzyme load (>4%) (Fig. 3). The mixing speeds between 400 and 850 rpm had only a minor effect on the EOC of epoxidized corn oil, which indicated that an internal mass transfer limitation should account for low initial reaction rates, which corresponds to previous report [19].

### Effects of Stearic Acid Load

The EOC of 0% stearic acid load was lower than 1%, which was ascribed to the fact that corn oil was hydrolyzed



**Fig. 4** Effects of stearic acid load on the epoxidation of corn oil. a (0%), b (14%), c (28%), d (42%), e (56%), f (70%). Reaction conditions: 10 g corn oil, the required quantity stearic acid, 0.3 g immobilized lipase, 10 h, reaction temperature 50 °C, mixing speed 600 rpm, and 14 g 30% hydrogen peroxide

by lipase, and the resultant free fatty acids from the hydrolysis of corn oil were epoxidized by  $H_2O_2$ . Similar perhydrolysis mechanism of oils can also be found in a previous report [13]. An increase in the stearic acid load (<28%, relative to the weight of oil) was accompanied by an increase in the EOC of epoxidized corn oil (Fig. 4). These results were ascribed to the effect of the active oxygen carrier and a suppression of corn oil hydrolysis in order to avoid the formation of undesirable epoxidized di- and monoglycerides, which showed a better performance than traditional oxygen carriers such as acetic and formic acid. Similar results can also be found in previous reports [11, 20]. However, with further increase in the stearic acid load (>28%), the EOC of epoxidized corn oil declined. This can be explained by the enzymatic epoxidation mechanism of corn oil in the presence of stearic acid

**Table 2** Analysis of  $L_9(3)^4$  test results

No.	A	B	C	D	EOC (%)	ROC (%)
1	1	1	1	1	2.4 ± 0.3	35.3 ± 4.4
2	1	2	2	2	5.8 ± 0.2	85.3 ± 2.9
3	1	3	3	3	5.4 ± 0.3	79.4 ± 4.4
4	2	1	2	3	3.2 ± 0.3	47.1 ± 4.4
5	2	2	3	1	5.7 ± 0.2	83.8 ± 2.9
6	2	3	1	2	4.9 ± 0.3	72.1 ± 4.4
7	3	1	3	2	2.6 ± 0.3	38.2 ± 4.4
8	3	2	1	3	4.2 ± 0.2	61.8 ± 2.9
9	3	3	2	1	4.7 ± 0.2	69.1 ± 2.9
$K_1$	13.6 <sup>a</sup>	8.2	11.5	12.8		
$K_2$	13.8	15.7	13.7	13.3		
$K_3$	11.5	15.0	13.7	12.8		
$k_1$	4.5 <sup>b</sup>	2.7	3.8	4.3		
$k_2$	4.6	5.2	4.6	4.4		
$k_3$	3.8	5.0	4.6	4.3		
$R$	0.8 <sup>c</sup>	2.5	0.8	0.1		

<sup>a</sup>  $K_i^A = \sum$  the amount of EOC at  $A_i$

<sup>b</sup>  $k_i^A = K_i^A/3$

<sup>c</sup>  $R_i^A = \max\{k_i^A\} - \min\{k_i^A\}$

(Fig. 1). The formation of perstearic acid from the reaction of stearic acid and hydrogen peroxide is a reversible reaction. Higher concentrations of stearic acid can lead to an increase in the equilibrium concentration of  $H_2O$  in the reaction mixture, which results in an increase in the level of ring-opening of the oxirane ring and a decrease in the EOC of the epoxidized corn oil.

#### Optimization of Enzymatic Epoxidation of Corn Oil

Optimization of the enzymatic epoxidation variables (reaction temperature, stearic acid load, mole ratio of  $H_2O_2$  and double bonds and reaction time) were examined using an orthogonal design (Table 2).

Table 2 indicated that the maximum EOC of epoxidized corn oil was  $5.8 \pm 0.2\%$  (ROC  $85.3 \pm 2.9\%$ ), and the minimal EOC was  $2.4 \pm 0.3\%$  (ROC  $35.3 \pm 4.4\%$ ). A further orthogonal analysis was warranted, and the  $K$ ,  $k$  and  $R$  values were calculated and listed in Table 2. The stearic acid load was found to be the most important influence on the epoxidation according to the  $R$  value. The influence on the EOC of epoxidized corn oil decreased in the order: stearic acid load > reaction temperature  $\approx$  mole ratio of  $H_2O_2/C=C$ -bonds > reaction time.

Increasing reaction temperatures (<50 °C) favored the formation of perstearic acid and a more rapid epoxidation rate, which was attributed to the acceleration of diffusion and intrinsic enhancement of enzyme activity. But, the EOC of epoxidized corn oil decreased at higher reaction

temperatures (>50 °C), which was ascribed to loss of enzyme activity and a higher rate of hydrolysis (oxirane cleavage) of the epoxidized corn oil.

According to the  $K$  value, the optimal epoxidation variables were  $A_2B_2C_2D_2$  (50 °C, 28% stearic acid load, 2.7:1 mol ratio of  $H_2O_2$  and double bonds, and 10 h). Under these optimum conditions, an EOC of  $5.8 \pm 0.2\%$  of epoxidized corn oil was obtained, which was similar to that of treatment 2 (35 °C, 28% stearic acid load, 2.7:1 mol ratio of  $H_2O_2$  and double bonds, and 10 h). In order to reduce the cost of production and maintain the activity of enzyme, we selected the optimum technology as follows: 35 °C, 28% stearic acid load, and 2.7:1 mol ratio of  $H_2O_2/C=C$ -bonds, and 10 h. Under these conditions, the EOC and RCO of epoxidized corn oil were  $5.8 \pm 0.2\%$  and  $85.3 \pm 2.9\%$ , respectively, which are higher than that (EOC 5.0%; RCO 73.5%) of a previous report [8].

#### Conclusion

The epoxidation of corn oil was successfully achieved using stearic acid as an active oxygen carrier and Novozym 435 from *Candida antarctica* B as a biocatalyst. Stearic acid as an active oxygen carrier demonstrated a good performance in the enzymatic epoxidation. The influence of reaction variables on the enzymatic epoxidation decreased in the order of stearic acid load > reaction temperature  $\approx$  mole ratio of  $H_2O_2/C=C$ -bonds > reaction time. The EOC ( $5.8 \pm 0.2\%$ ) and RCO ( $85.3 \pm 2.9\%$ ) of epoxidized corn oil were obtained under optimized conditions: 35 °C, 28% stearic acid load, 2.7:1 mol ratio of  $H_2O_2/C=C$ -bonds, and 10.0 h.

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