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LIPASE EPOXIDATION OPTIMIZING OF *JATROPHA CURCAS* OIL USING PERLAURIC ACID

R. RAFIEE-MOGHADDAM[a,b,*], J. SALIMON[a], M.D. JELAS HARON[c], H. JAHANGIRIAN[b,d], M. H. SHAH ISMAIL[d] , S. HOSSEINI[d], M. REZAYI[a]

[a]*School of Chemical Sciences and Food Technology, Faculty of Science and Technology, Universiti Kebangsaan Malaysia, 43600 UKM Bangi, Selangor, Malaysia*

[b]*R & D Section of ZargolChemi Productive Co., ZCPC, (3188113984) Eshtehard Industrial Park, Alborz, Iran*

[c]*Chemistry Unit, Centre of Foundation Studies for Agricultural Science, Universiti PutraMalaysia, 43400 UPM Serdang, Selangor, Malaysia*

[d]*Department of Chemical and environmental engineering, Faculty of engineering, Universiti Putra Malaysia, 43400 UPM, Serdang, Selangor, Malaysia*

As a novel renewable resource, *Jatropha curcas* seed oil with an iodine value of 103.57g/100g, and containing almost 44.7% oleic acid, 32.8% linoleic acid as the dominant fatty acids, 13.78% palmitic acid, 6.92% stearic acid and 0.71% palmitoleic acid was epoxidized using hydrogen peroxide as oxygen donor and lauric acid as an active oxygen carrier in the presence of immobilized Candida antarctica Lipase B. In the present work the effects of variables (reaction temperature, enzyme load, the mole ratio of H_2O_2 : C=C bonds, lauric acid load and reaction time) on the oxygen oxirane content (OOC) of epoxidized *Jatropha curcas* were investigated. The process was optimized for the enzymatic epoxidation of Jatropha oil, with the theoretical maximum OOC equal to 6.13%. The OOC of epoxidized Jatropha oil was 5.67% and a percentage relative conversion to oxirane (RCO) was 93.64 % under the following conditions : 50˚C (temperature), 7% enzyme load (relative to the weight of Jatropha of oil), 3.5:1 mol ratio of H_2O_2 : C=C bonds, 23% lauric acid load (relative to the weight of Jatropha oil) and 7.5 h (reaction time).

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Keywords: Jatropha curcas; Enzymatic epoxidation; Lipase; Lauric acid; Oxygen oxirane content

1. Introduction

Epoxidation of vegetable oils is a commercially important reaction. Epoxides are valuable industrial products, specifically epoxidized triglycerides have been used as diluents [1] Coatings, [2] and stabilizers in PVC. The unsaturation of the vegetable oils, which are rich in oleic, linoleic and linolenic acyl groups, can be used to produce epoxides. They are important oleochemicals and have been prepared from linseed, rapeseed, olive, corn, and sunflower, mainly [3].

Epodxides are of great industrial interest and due to their high reactivity of the strained epoxides ring, are known as a good intermediate compound for the production of a variety of derivatives. Polymers, adhesives, resins, monoalcohols, diols, alkoxyalcohols, hydroxyesters, N-hydroxyalkylamides, mercaptoalcohols, aminoalcohols, and hydroxynitriles, could be produced via epoxide ring-opening reactions with suitable reactants [4]. Fatty epoxides can be used directly as plasticizers to improve the flexibility, elasticity and stability of polymers towards heat and UV radiation. Epoxides can also be used as high-temperature lubricants, and the products obtained from ring opening can be employed as low-temperature lubricants [5,6]. Epoxidation of vegetable

* Corresponding author: roshanak.rafiee.@gmail.com

oils consists of adding a single oxygen atom to each unsaturation (carbon-carbon double bond) in the fatty acid chain using percarboxylic acids or organic and inorganic peroxides as oxidizing agents, the original unsaturation is thus converted into an epoxy group. The conversion of unsaturation to epoxy groups can be directly monitored with the oxirane oxygen content, and indirectly with the iodine value.

Generally, there are four methods for the production of epoxides from olefinic molecules: (1) the unsaturated oils react with a percaboxylic acid usually peracetic and performic acid that formed in situ using hydrogen peroxide, in the presence a strong mineral acids like concentrated H_2SO_4 and H_3PO_4 as catalyst, and in the case of long chain fatty acids, these acids serve even as solvent [7-9]. This method is known as Prileshajev method of epoxidation which are carried out for plant oils such as Soya bean on an industrial scale. (2) With organic and inorganic peroxides [10], (3) with halohydrins for the epoxidation of double bonds with electron-deficient double bonds - an environmentally unfriendly process, and (4) using molecular oxygen [11], that is just effectual for simple molecules and it is not an effective method for epoxidation vegetable oils because leads to degradation oil to smaller compounds. However this method is cheaper and greener. According to the above in order to efficient epoxidation of vegetable oils, percarboxylic acids, organic and inorganic peroxides could be used as reagents for cleaner processes, and utilizing heterogeneous catalysts instead of traditional homogeneous ones are recommended [12,13]. Many kinds of catalysts such as methyltrioxorhenium [14], ammonium molybdate, ion exchange resins [15], phase transfer catalysts such as quaternary ammonium tetrakis (diperoxotungsto) phosphates, and crown ethers have been investigated in order to improving the selectivity and increasing the conversion of epoxidation. The potential of Lipases in the oleochemistry industry has been shown lately and Lipase-catalyzed epoxidation has been developed relatively recently. They appear to provide a new, clean and very promising technique for epoxidation of unsaturated compounds especially vegetable oils [16, 17]. Warwel et al. [18] produced epoxidized vegetable oils and reported extremely high yields and catalyst reuses up to 15 times. Heterogeneous catalysts based on immobilized Lipases have shown high converting unsaturated fatty acids and their methyl esters to percarboxylic acids, utilizing H_2O_2 as oxidant. In Lipase –mediated epoxidation, reaction carries out in neutral pH and has a high selectivity / specificity with little or no by-product formation [19, 20] therefore products have high conversion. Furthermore enzyme is stable and can be reused, so these factors are important from economically point of view [21]. Due to these advantages mentioned above, enzymatic reaction is preferred to chemical catalyst reaction. Among the various Lipases studied so far, Novozym 435® (Candida antarctica lipase on polyacrilic resin), is the most outstanding, effective and convenient Lipase for epoxidation of various fatty substrates. A research group from Novo Nordisk A/S (Copenhagen, Denmark) described chemo-enzymatic epoxidation process for the first time [22]. Chemo-enzymatic epoxidation of Soyabean oil, Sunflower oil and some other plants oil are also reported before [19, 21].

In this study, Jatropha oil was chosen as the raw material for epoxidation. Jatropha is a genus of over 170 plants from the Euphorbiceae family native to Central America and Caribbean, but commonly found across most of the tropical and subtropical regions of the world. This plant grows quickly and well in rain-fed and marginalized land, survives in porous lands, stony soil but not in compact soil. Moreover this plant can reach the age of 40-50 years. Jatropha curcas seed oil with iodine value of 103.57 contains of toxic compounds, which make it non-edible and thus does not compete with any food crop, as a result this oil is considered a suitable source, and preferred to chemical modifications for many industrial products especially in biodiesel and biolubricant fields. The objective of this work was to optimize reaction parameters for the enzymatic epoxidation reaction of Jatropha curcas seed oil. Epoxidation reaction was carried out in the presence of immobilized Lipase B from Candida antarctica, lauric acid was chosen as the active oxygen carrier, and hydrogen peroxide was selected as the oxygen donor. The double bonds of Jatropha oil were epoxidized by perlauric acid previously generated by the reaction of lauric acid and hydrogen peroxide. Jatropha oil is looked up on as one of the most appropriate renewable alternative sources of biodiesel and biolubricant fields in terms of availability and cost [23]. In the present work the effects of reaction time, reaction temperature, load of lauric acid, the mole ratio of H_2O_2 and double bonds on the enzymatic epoxidation of Jatropha oil were investigated.

2. Experimental Section

2.1. Material and equipment

Malaysian *Jatropha curcas* seeds were obtained from UKM experimental plot and itsoil was gained by soxhlet extraction method, hydrogen peroxide (30%) were purchased from Univar and J. T. Baker, immobilized Lipase (Nonozyme 435) used was the product from Novo Nordisk (Denmark), lauric acid (purity >99%; peroxide value = 0.69 mmol/kg) was purchased from Sigma–Aldrich Co. All other reagents were of analytical grade and used without further purification. A GC analysis was performed on Shimadzu gas chromatograph equipped with flame ionization detector and capillary column (30 m x 0.25 mm x 0.25 J.1m films). HPLC analysis was performed using a Water model 1515 equipped with ELS detector and spherisorb C18 column (250 mm × 4.8 mm × 3 mm), which was used for analysis of TAG. Fourier transform infrared (FTIR) spectra were recorded on a Perkin Elmer GX FTIR Spectrophotometer (USA). Characterization test was carried out by using carbon and proton nuclear magnetic resonance (^{1}HNMR and ^{13}CNMR) (Bruker AV-III-600 FTNMR 600 MHZ spectrometer with a cry probe).

2.2. Reaction procedure

Epoxidation reactions were carried out in 250-mL three-necked round-bottom flasks equipped with a condenser, an electric heating mantle, a digital temperature controller and thermometer in a water bath. The central neck of the flask was connected to the reflux condenser and the thermometer was set through one of the side necks in order to record the temperature of the reaction mixture. A calculated amount of Jatropha oil, 8.72g (10 mmol) was placed in the flask with lauric acid, 2g (10mmol) and immobilized lipase, 872mg in toluene, 25mL at 50°C and 16.21 g hydrogen peroxide (30% w/w) was added drop wise to the reaction mixture at a rate such that the hydrogen peroxide addition was completed within an hour. After the complete addition of hydrogen peroxide, the reaction was continued for the desired time with rapid stirring 900 rpm. The rapid stirring was maintained throughout the experiments so that a fine dispersion of oil was achieved. Upon completion of the epoxidation reaction, immobilized biocatalysts were removed by filtration and the filtrate was held in a separator funnel, the lower layer (aqueous layer) was discarded. Prior to analysis, the organic layer was washed three times with 150 ml of 5% sodium bicarbonate solution to remove free fatty acids and then washed three times with 150 ml of distilled water. The organic layer was dried overnight with anhydrous sodium sulfate in order to remove trace water and decomposed traces of unreacted hydrogen peroxide. The sodium sulfate was removed by filtration under vacuum with filter paper, and the solvent and remaining trace water were distilling off using a rotary evaporator.

The sample was then analyzed for Oxygen oxirane content (OOC_{ex}) value. Oxygen oxirane content (OOC_{ex}) value was determined by direct method using the hydrobromic acid solution in glacial acetic acid (AOCS Cd 9-57). The conversion of unsaturation to epoxy groups was determined using the following equation:

$$\% \text{ conversion} = OOC_{ex} / OOC_{th} \times 100$$

Where $OOC_{ex}$is the experimentally determined and $OOC_{th}$is the theoretical maximum predicted of OOC in 100 g of *Jatropha Curcas* oil, determined using the following expression:

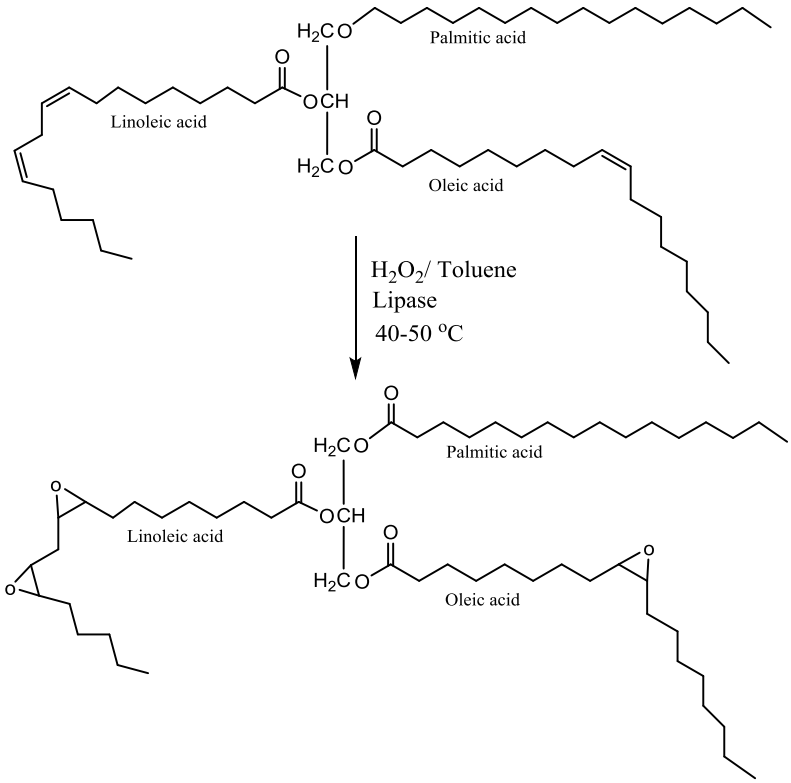
$$OOC_{th} = \{IV_0/2Ai /100 + (IV_0/2A_i)\, A_0\} \times A_0 \times 100$$

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 the Jatropha oil. The theoretical maximum oxirane oxygen content in 100 g of *Jatropha curcas* oil is 6.13%. In order to determine the optimal condition for the epoxidation Jatropha oil four factors were studied. The variables selected for the epoxidation of *Jatropha curcas* oil were reaction time (2.30, 5.0, 7.0, and 10.0 h), reaction temperature (40.0, 50.0, 60.0, and 70.0°C), enzyme load (3.00%, 5.00%, 7.00%, and 10%) relative to the weight of oil), and substrate ratio (2.5:1, 3:1, 3.5:1, 4:1, 4.5:1, mole ratio of H_2O_2 : C = C bonds). In this study lauric acid was chosen as the active oxygen carrier instead of stearic acid that has been

shown by some recent studies as oxygen carrier [24]. The double bonds of Jatrophaoil were epoxidized by perlauric acid previously generated by the reaction of lauric acid and hydrogen peroxide. Perlauric acid is generated in presence of Lipase as shown in scheme 1.

Scheme1. Generation of perlauric acid

Next step is including attack of C=C to peracid that generated before in situ. Scheme 2 demonstrates the chemo-enzymatic epoxidation reaction of Jatropha oil structure.



Scheme 2. Epoxidation reaction of Jatropha oil

3. Results and Discussion

3.1. Characterization of Jatropha oiland Epoxidized Jatropha oil

Jatropha seed oil can produce about 40-60% oil. Oleic and linoleic acids are the dominate fatty acids which are unsaturated. Gas chromatography, flame ionization detector, (GC-FID) analysis of Jatropha oil showed that oleic acid has the highest percentage of composition of 42.8% followed by linoleic acid with 36.2% (this study). Thus, Jatropha seed oil can be classified as oleic–linoleic oil compared to others vegetable oils, Jatropha oil seed has highest oleic contain than palm oil, palm kernel, sunflower, coconut and soybean oil. Fatty acid composition of Jatropha oil and some other plant are given in table 1. According to this table *Jatropha curcas* oil in this study contained unsaturated fatty acid about 79.29%.

Table 1. Fatty acid composition of Jatrhopa oil and some other plant oils

	***J. curcas* oil**	**Palm kernel oil**	**Sunflower oil**	**Soyabean oil**	**Palm oil**
Oleic acid	42.8	15.4	21.1	23.4	39.2
Linoleic acid	36.2	2.4	66.2	53.2	10.1
Palmtic acid	13.8	8.4	–	11.0	44.
Stearic acid	6.9	2.4	4.5	4.0	4.5
Palmitoliec acid	0.7	-	-	-	-

The major fatty acids in Jatropha seed oil were the oleic and linoleic acid. Thus, Jatropha seed oil can be classified as oleic–linoleic oil. The oil content of Jatropha kernel was determined at 63.16% that was found higher than linseed, soybean, and palm kernel which is 33.33%, 8.35% and 44.6%, respectively, [25]. The high iodine value and oxidative stability showed that the seed oil upholds the good qualities of plant oil and semi-drying oil purposes [26]. Furthermore results of TAG (triacylglcerols) composition of Jatropha oil by HPLC based on reversed phase analysis shows there are twelve TAGs in the oil composition. The major peaks were PLL (palmitic-linoleic-linoleic) with 21.64% followed by OOL (oleic- oleic-linoleic) with 18.33% and OLL (oleic-linoleic-linoleic) 17.36%. From industrial point of view triacylglcerols profile (TAGs) studies are important.

Functional groups of Jatropha oil and epoxy ring in epoxidized oil were detected by FTIR, ^{1}HNMR and ^{13}CNMR analysis as following details.

3.1.1. Jatropha oil FTIR, ^{1}HNMR and ^{13}CNMR

Peak at 3007 cm^{-1} indicates the double bond C=C, and peaks at 2924, 2854 cm^{-1} refer to CH_2 and CH_3. Absorption bond at 1745 cm-1 belongs to ester carbonyl function groups (C=O). For ^{1}HNMR analysis, signals at 4.14-4.14 ppm and 4.26-4.27 ppm are adjacent to methylene groups of glycerol CH_2-O-CO- (α H) and CH-O-CO- (β H) respectively. The peak at 5.32-5.37 ppm refers to diallylic methylne. Moreover signals at 0.85-0.87 ppm and 1.24-1.29 ppm indicate -CH_3 and -CH_2 groups. For ^{13}CNMR analysis, signals at 62.20 ppm indicate C_1 or C_3 of glycerol, 69.00 belongs to C_2 of glycerol. The peaks at 172.95-173.35 ppm relates to the carbonyl carbon (ester).

3.1.2. Epoxidized Jatropha oil FTIR, ^{1}HNMR and ^{13}CNMR

The formation of epoxy groups was confirmed by disappearance the double bonds (3007 cm^{-1}) and appearance doublet at 824 and 843 cm^{-1} (epoxy ring) in the FTIR spectrum of product. For ^{1}HNMR analysis, the peak at 2.90-3.12 ppm indicates oxirane ring while, the methane proton signals (-CH=CH-) at 5.32-5.37 ppm were disappeared. The signals at 4.15-4.17 and 4.29 ppm referred to proton α and β in glycerol structure. For ^{13}CNMR signal at 57.48 ppm indicates the presence of oxirane ring and signals at 128.00 -130.08 ppm (-C=C-) were disappeared.

3.2. Effect of molar ratio of substrate

The first step was focused on the determination of the hydrogen peroxide content that is important mainly for lowering the cost by reducing the amount of unreacted peroxide at the end of the reaction. The best results were obtained at 3.5:1 molar ratio of hydrogen peroxide: double bonds.

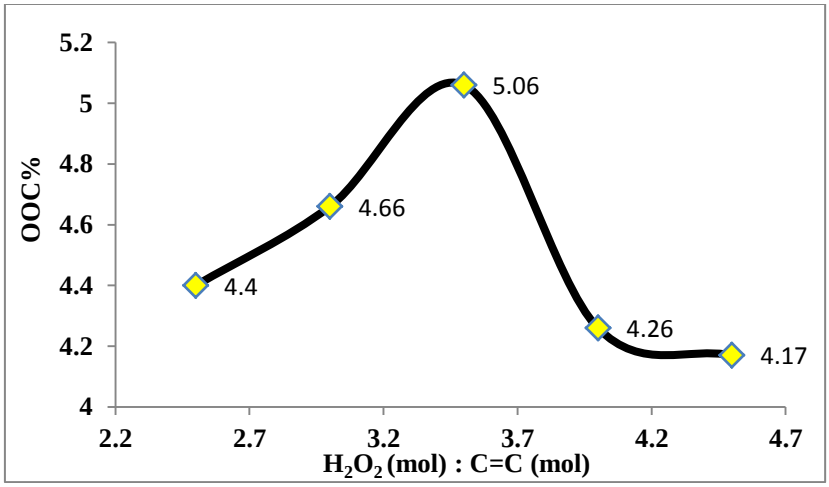


Fig.1. Effect of mol ratio: Reaction condition: 10% enzyme load (relative to the weight of Jatropha oil),temperature50◦C, 23% lauric acid (relative to the weight of Jatropha oil), 10 h reaction time.

Fig. 1 shows the effect of varying substrate ratio (mole ratio of H_2O_2 : C=C bonds) on the epoxidation reaction of Jatropha oil. The increasing of substrate ratio of 2.5:1 to 4.5:1 could increase the OOC (oxygen oxirane content) of epoxidized Jatropha oil, but a further increasing of substrate ratio (>3.5:1) would lead to a decline of the OOC of epoxidized Jatropha oil. This one may be due to derangement in mass transfer of product because of increasing hydrogen peroxide concentration in the media. Furthermore in lower substrate (<2.5:1) by increasing the molar ratio of hydrogen peroxide, pH of the reaction system decreased, and the Lewis acidity of the biocatalyst center increased, which can lead to significant hydrolysis and increase the formation rate of 1,2-diols of Jatropha oil [24, 27]. However, in high concentration of peroxide (>4.5:1) seems to deactivate Novozyme 435 [24, 19] and the epoxidation system could generate by products. This phenomenon was similar to that reported by Sun et al and Jiang et al [24,27]. This amount of substrate ratio (mole ratio of H_2O_2: C= C-bonds) was found to be optimal and was used as a constant parameter in further experiments. The results are summarized in Table 2.

Table 2. Effect of mol ratio on epoxidation reaction of Jatropha oil.Reaction condition : 10% enzyme load (relative to the weight of Jatropha oil), temperature 50◦C, 23% lauric acid (relative to the weight of Jatropha oil), 10 h reaction time.

Sample	H_2O_2 (g)	H_2O_2:C= C	OOC%	RCO%
Exp.1	10.13	2.5:1	4.40	71.78
Exp.2	12.16	3:1	4.66	76.01
Exp.3	14.18	3.5:1	5.06	82.54
Exp.4	16.21	4:1	4.26	69.49
Exp.5	18.23	4.5:1	4.17	68.03

3.3. Effect of temperature

The effect of temperature on the course of epoxidation of Jatropha oil was investigated at four different temperatures, namely 40, 50, 60 and 70° C. The changes in the OOC at four different temperatures are presented in Table 3. As expected, higher temperatures favored the epoxidation reaction, and a maximum 85% conversion was obtained at 50°C after a reaction time of 10 h. raising reaction temperature causes increased the epoxidation of Jatropha oil.

Nevertheless, when the temperature exceeded 50◦C, the OOC of epoxidized Jatropha oil declined. At higher reaction temperatures (> 50◦C), the OOC of epoxidized Jatropha oil decreased, which was attributable to loss of enzyme activity and ring opening of the epoxides ring [28], which in turns indicates that increasing the reaction temperature above 50◦C had a negative effect on the enzyme activity mainly due to make a more open conformation in enzyme structure, may be increasing the accessibility to sensitive amino acids in the inner regions of the enzyme, and consequently decreasing its catalytic activity [29]. As shown in figure 2 the maximum OOC (> 5%) of epoxidized Jatropha oil appeared in the temperature of 50◦C. A reaction temperature of 50°C gave approximately 82% conversion to oxirane after a reaction time of 10 h. A temperature of 50°C was, therefore considered optimal for the epoxidation reaction and was used as a constant parameter in further experiments in our investigation.

Table 3. Effect of temperature on epoxidation reaction of Jatropha oil. Reaction condition: 3.5:1 mol ratio of H_2O_2:C=C bonds, 10% enzyme load (relative to the weight of Jatropha oil), 23% lauric acid (relative to the weight of Jatropha oil), 10 h reaction time.

Sample	Temperature °C	OOC%	RCO%
Exp.1	40	4.42	72.10
EXP.2	45	5.03	82.22
Exp.3	50	5.25	85.69
Exp.4	60	4.21	68.68
Exp.5	70	3.32	54.16

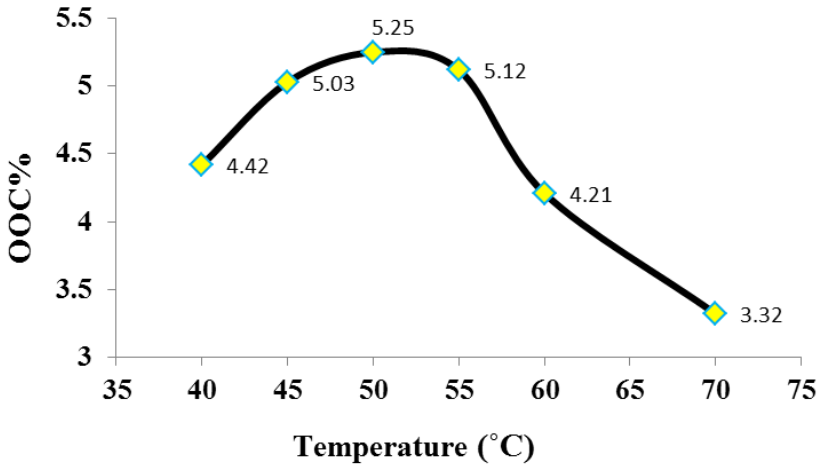


Fig. 2. Effect of temperature: Reaction condition:3.5:1 mol ratio of H_2O_2:C=C bonds, 10% enzyme load (relative to the weight of Jatropha oil), 23% lauric acid (relative to the weight of Jatropha oil), 10 h reaction time.

3.4. Effect of enzyme load

The effect of the catalyst loading was investigated in the range of 3-10 wt% relative to the weight of Jaropha oil. Figure 3 shows the results obtained for the catalyst loading in that range. As can be seen in Figure 3 by increasing the amount of enzyme, the OOC of epoxidized Jatropha oil is increased. This result was ascribed to increasing the total active volume and the total surface area of the catalyst which consequently leads to enhance the formation rate of perluric acid. In the other word by increasing the active species present (perluric acid) in turns enhance the epoxidation conversion.

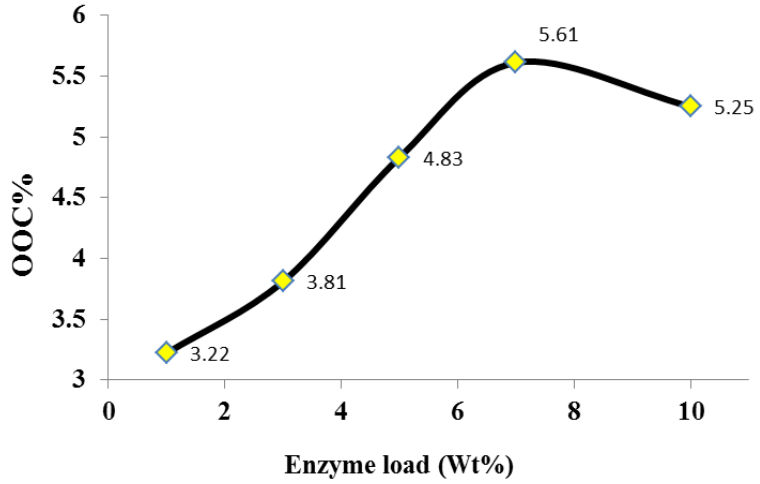


Fig. 3. Effect of enzyme load: Reaction condition 3.5:1 mol ratio of H_2O_2:C=C bonds, temperature 50◦ C, 23% lauric acid (relative to the weight of Jatropha oil), 10 h reaction time.

However, the use of higher enzyme load (>7%) gave problems due to the external transfer limitation that was essentially eliminated in the reaction system. These results are in agreement with studies of Sun et al. [24]. During reaction time tested, maximum OOC can be achieved at enzyme loads 7% based on initial weight of oil. Amount of OOC and RCO in different enzyme load are summarized in Table 4.

Table 4. Effect of enzyme load on epoxidation reaction of Jatropha oil. Reaction condition : 3.5:1 mol ratio of H_2O_2:C=C bonds, temperature 50˚ C, 23% lauric acid (relative to the weight of Jatropha oil), 10 h reaction time, temperature 50˚ C.

Sample	Enzyme load (Based on weight of oil)	OOC%	RCO%
Exp.1	1%	3.22	52.53
Exp.2	3%	3.81	62.15
Exp.3	5%	4.83	78.79
Exp.4	7%	5.61	91.52
Exp.5	10%	5.25	85.69

3.5. Effect of the amount of lauric acid

In the next step, the different concentrations ranging of lauric acid from (17–29%) based on the weight of oil were studied to showing the influence of FFA concentration on conversion. An increase in the amount of lauric acid to 23% based on the weight of oil caused to increase the OOC of epoxidized Jatropha oil. With further increasing the amount of lauric acid (23–29%), OOC of epoxidized oil almost constant at the same level. Figure 4 shows the changes of OOC amount in different concentration of lauric acid. As can be seen without using any fatty acid the conversion was approximately 70%.

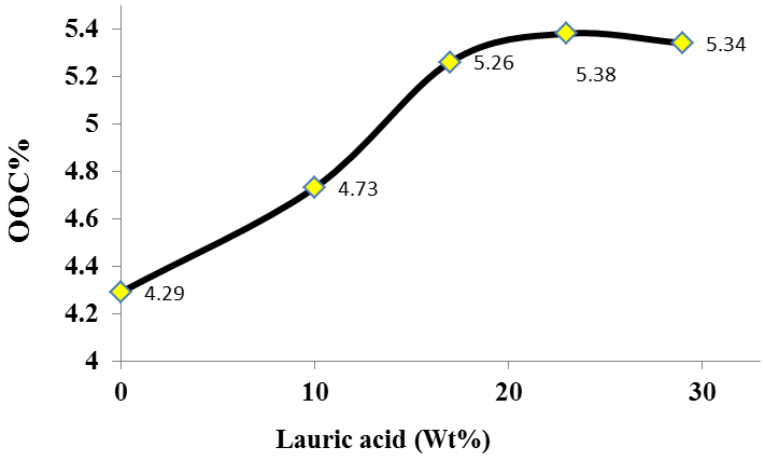


Fig. 4. Effect of lauric acid load: Reaction condition : 3.5:1 mol ratio of H_2O_2:C=C bonds, 23% lauric acid (relative to the weight of Jatropha oil),10 h reaction time, temperature 50◦ C.

Also the influence of stearic acid was studied in epoxidation reaction which was reported in some investigations but lauric acid showed better performance. These results suggested that using lauric acid as an active oxygen carrier showed more performance than traditional oxygen carriers, such as, acetic and formic acid and even stearic acid which was reported before [24]. This phenomenon described by this reason which mechanisms in epoxidation reaction are based on transfer the oxygen atom to double bond and this one occurs in presence of lauric acid which changes to perlauric acid in H_2O_2 media, so it acts as an oxygen carrier. In the other part of this study in order to find the influence of presence FFA from original oil, on epoxidation reaction, 5% mole of Jatropha oil fatty acids added to reaction media and OOC was determined after 18, 24, 42, 54, 66 hours in room temperature. The results showed there is no significant difference in conversion percentage between reaction with or without FFA and, as mentioned above the conversion was approximately 70% in epoxidation reaction without FFA. These results are, however, agreement with the result of Tomás Vlcek and Zoran S. Petrovic [19] and contrary to the result of Klass and Warwel [30]. Table 5 shows the OOC in different amounts of lauric acid in epoxidation reaction.

Table 5. Effect of lauric acid load on epoxidation reaction of Jatropha oil Reaction condition : 3.5:1 mol ratio of H_2O_2:C=C bonds, 23% lauric acid (relative to the weight of Jatropha oil), 10 h reaction time, temperature 50° C.

Sample	Lauric acid (Based on weight of oil)	OOC%	RCO%
Exp.1	0%	4.29	69.98
Exp.2	10%	4.73	77.16
Exp.3	17%	5.26	85.81
Exp.4	23%	5.38	87.77
Exp.529%	5.34	87.11	

3.6. Effect of the amount of reaction time

Raising the reaction temperature is beneficial for increasing the epoxidation of Jatropha oil. As can be seen in figure 5 with increasing reaction time till 7.5 h, the amount of OOC increased, and reached a maximum at 7.5 h.

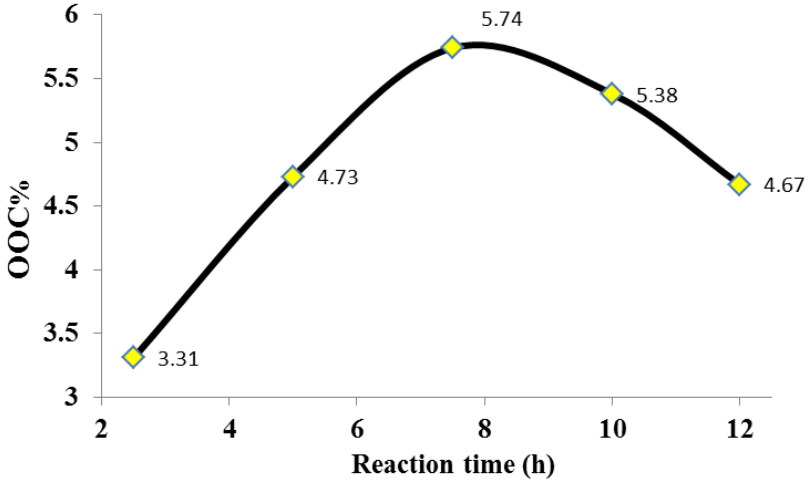


Fig. 5. :Effect of reaction time: Reaction condition : 3.5:1 mol ratio of H_2O_2:C=C bonds, 7% enzyme load (relative to the weight of Jatropha oil),23% lauric acid (relative to the weight of Jatropha oil), 50◦ C

With further increases in reaction time the OOC of epoxidized Jatropha oil decreased which was ascribed to more by-products, water being formed and the undesirable oxirane-ring opening reactions. Similar behavior was reported by S. Sun et. al. [24]. Results are given in Table 6.

Table 6. Effect of reaction time on epoxidation reaction of Jatropha oil. Reaction condition : 3.5:1 mol ratio of H_2O_2:C=C bonds, 7% enzyme load (relative to the weight of Jatropha oil), 23% lauric acid (relative to the weight of Jatropha oil), 50°C.

Sample	Time (h)	OOC%	RCO%
Exp.1	2.5	3.31	54.00
Exp.2	5	4.73	77.16
Exp.3	7.5	5.74	93.64
Exp.4	10	5.38	87.77

4. Conclusions

The epoxidation of *Jatropha curcas* seed oil was achieved using lauric acid as active oxygen carrier and Novozym 435 from Candida antarctica B as a biocatalyst. These results are important for the chemical industry and present the possibility for the production of oil derivatives through a cleaner technology. Using lipase as the catalyst causes reduction in the use of undesirable chemical reagents. The main advantage of the enzymatic catalyst is high selectivity and the elimination of ring-opening reactions. Lauric acid as an active oxygen carrier showed a good performance in the enzymatic epoxidation of Jatropha oil. The influence of reaction variables on the enzymatic epoxidation decreased in the order of, reaction temperature, lauric acid load, mole ratio of H_2O_2 : C=C-bonds, reaction time. The OOC 5.74% and RCO 93.64% of epoxidized *Jatropha curcas* oil were obtained under optimized conditions: 50° C, 7% enzyme load, 23% lauric acid load (relative to the weight of Jatropha oil) , 3.5:1 mole ratio of H_2O_2 : C=C bonds and 7.5 h reaction time.

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