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*Full Length Research Paper*

# Comparative Evaluation of the Physico-chemical Properties of Chemically and Enzymatically Epoxidised Soybean Oil

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## Abstract

In the present work chemically and enzymatically epoxidized products of soybean oil were synthesized and their properties were compared. Hydrogen peroxide was used as the epoxidizing agent in both types of epoxidation reactions. However, the proportions of H<sub>2</sub>O<sub>2</sub> used were varied in both the cases, the proportions being 1:1 and 1:2 ratios. It was observed that the epoxidation process was strongly influenced by the proportion of hydrogen peroxide. In both the cases higher yield of plasticizer was obtained with higher amount of H<sub>2</sub>O<sub>2</sub>. The synthesized products were characterised in terms of FTIR analysis, acid value, iodine value, oxirane content and viscosity. From the results, the 1:2 enzymatic reactions could be claimed as the process giving better product due to its high yield, comparatively low acid value and lower epoxy ring opening reactions.

**Keywords:** Soybean oil; Enzymatic epoxidation; Chemical epoxidation; Plasticizer; Hydrogen peroxide.

## INTRODUCTION

In recent years there has been an intensive demand in the field of bio-based materials prepared from renewable resources. This has also been an important challenge for the plastic and polymer industries. Polyvinyl chloride and chloride-acetate based polymers are extensively used today for their plasticizing capability, durability and stability [1]. However, the most important problem with the currently used types of plastics and polymers is that the raw materials are derived from non-renewable petroleum resources. Furthermore, the resultant plastic materials are non-biodegradable in

nature. This drawback is of immense concern in terms of environmental safety around the globe. This necessitates the development of bio-based plasticizers from renewable resources. Hence today, the focus is on the development of polymeric materials from vegetable oils, a sustainable resource [2, 3]. Vegetable oil is a readily available and relatively cheap raw material which can be utilized to synthesize different plastics and polymers.

Today, one of the most important epoxidized vegetable oils is epoxidized soybean oil (ESO). The main component of vegetable oil is triglyceride. Soybean oil, a popular vegetable

oil, due to its huge worldwide production, has high double bond content. The double bonds in soybean oil can be easily converted to more stable products by means of introducing functional groups such as epoxide groups, acrylate groups, hydroxyl groups, etc. [4–8]. In soybean oil there is about 15% saturated fatty acids, and about 85% unsaturated fatty acid. The highest unsaturated fatty acid percentage in soybean oil is that of linoleic acid, followed by oleic and palmitic acids [9]. Epoxidation process is a new method by which fatty acids are converted to a three membered epoxide (oxirane) ring as the carbon–carbon double bond reacts with active oxygen.

In the present study an attempt was made to develop epoxidised soybean oil, both by chemical and enzymatic methods. A comparative study between the developed products was performed from all aspects to evaluate the efficiency of the synthesized products. Furthermore, films were developed using the prepared epoxidized soybean oils from both the methods and the film characteristics were also compared.

## MATERIAL AND METHODS

### Materials

Refined soybean oil was purchased from local market. Immobilized *Candida Antarctica* lipase enzyme (NS 435) was used as the catalyst in the enzymatic process which was procured from Novozymes A/S, Bagsvaerd, Denmark. All other reagents were purchased from Merck India Ltd, Mumbai, India.

### Methods

#### Synthesis of epoxidized soybean oil

##### a. Chemical method:

*Product 1 (CM1):* 50 g of refined soybean oil was taken and hydrogen peroxide (H<sub>2</sub>O<sub>2</sub>) in glacial acetic acid was added to it in a proportion of 1:1 (oil:hydrogen peroxide) in the presence of H<sub>2</sub>SO<sub>4</sub> catalyst (3% on the basis of oil). The mixture was slowly stirred while maintaining a temperature range of 50°C to 60°C.

*Product 2 (CM2):* 50 g of refined soybean oil was taken and hydrogen peroxide (H<sub>2</sub>O<sub>2</sub>) in

glacial acetic acid was added to it in a proportion of 1:2 (oil:hydrogen peroxide) in the presence of H<sub>2</sub>SO<sub>4</sub> catalyst (3% on the basis of oil). The mixture was slowly stirred while maintaining a temperature range of 50°C to 60°C.

##### b. Enzymatic Method:

*Product 1 (EM1):* 50 g of refined soybean oil was taken and hydrogen peroxide (H<sub>2</sub>O<sub>2</sub>) in glacial acetic acid was added to it in a proportion of 1:1 (oil:hydrogen peroxide) in the presence of lipase enzyme catalyst (5% on the basis of oil). The mixture was slowly stirred while maintaining a temperature range of 40°C to 50°C.

*Product 2 (EM2):* 50 g of refined soybean oil was taken and hydrogen peroxide (H<sub>2</sub>O<sub>2</sub>) in glacial acetic acid was added to it in a proportion of 1:2 (oil:hydrogen peroxide) in the presence of lipase enzyme catalyst (5% on the basis of oil). The mixture was slowly stirred while maintaining a temperature range of 40°C to 50°C.

##### Parameter study

The AOCS Official Methods were employed for determinations of iodine values, acid values and oxirane contents in the oil samples [10].

*Iodine value.* Iodine value of the oil samples was determined following the AOCS Official Method Cd 3a-63. About 5 g of the dry oil was taken into a 500 ml Erlenmeyer flask with glass stopper. To it 25 ml of carbon tetrachloride was added. The content was mixed well. About 25 ml of Wij's solution was added to the mixture. The mixture was swirled well and then flasks were kept in the dark for half an hour. A blank was carried out simultaneously. After standing, 15 ml of potassium iodide solution was added followed by 100 ml of distilled water. The liberated iodine was titrated with standardized sodium thiosulphate solution, using starch as indicator. The blank determination was carried out in a similar manner as the sample but without the oil.

$$\text{Iodine value} = \frac{12.69 \times (B - S) \times N}{W}$$

Where, B = Volume of standard sodium thiosulphate solution required for the blank in ml;

S = Volume of standard sodium thiosulphate solution required for the sample in ml;

N = Strength of the standard sodium thiosulphate solution in normality;

W = Weight of the sample in g.

*Acid value.* Acid value of the oil samples was determined following the AOCS Official Method Te 1a-64.

About 2 g of homogenized oil sample was taken in a 250 ml conical flask and to it 50 ml of freshly neutralized ethyl alcohol was added. The mixture was boiled for about five minutes and titrated while hot against standard sodium hydroxide solution while shaking well during the titration. Phenolphthalein was used as the indicator.

*Oxirane content.* Oxirane content of the oil samples was determined following the AOCS Official Method Cd 1b-87.

About (0.3 – 0.5) g of sample was taken in a 50 ml Erlenmeyer flask. The sample was dissolved in 10 ml of chlorobenzene. It was then titrated with standardized 0.1(N) hydrobromic acid solution (rapidly at first and then slowly) to a blue-green end point that persists for at least 30 seconds.

$$\text{Oxirane Oxygen\%} = \frac{V \times N \times 1.60}{W}$$

Where, V = Volume of standard hydrogen bromide solution in ml;

N = Strength of the standard hydrogen bromide solution in normality;

W = Weight of the sample in g.

Iodine value of the samples from CM1, CM2, EM1 and EM2 withdrawn at 1 hour intervals during the epoxidation reactions, were determined. Acid values of the final products of CM1, CM2, EM1 and EM2 were determined. Oxirane content (%) of the final products of CM1, CM2, EM1 and EM2 were determined. In all the cases data reported was a mean of three consecutive readings.

#### **Determination of Viscosity**

Viscosities of the four different samples (CM1, CM2 and EM1, EM2) were observed using Brookfield viscometer (DV-II+ Pro Viscometer, Brookfield, Brookfield Engineering Labs. Inc. Middleboro, USA) at 100 rpm with S21 spindle

at a temperature of 25°C. Data reported was a mean of three consecutive readings.

#### **FT-IR Spectroscopy**

FT-IR of the original soybean oil and the final products of CM1, CM2, EM1 and EM2 were recorded on a JASCO FT/IR-6300 type A (Serial no. A014461024), ATR PRO 450-S Spectrophotometer, Version – 2.07.01 [Build 1] in the range 650–4000 cm<sup>-1</sup>. Very thin films of the samples (synthesized products) were applied to demountable liquid cell with luer filling, CaF<sub>2</sub> 0.05 mm. Each spectrum was Fourier-transformed, phase-corrected, and integrated using Sadtler Database.

#### **Film Formation**

About 0.4 g of polyvinyl chloride was added to 30% epoxidized soybean oil. To it 9 ml of THF was added and then stirred for 10 minutes at an ambient temperature. Finally, the mixture was carefully spread over a petridish and the film formed was noted for its texture [11].

#### **Statistical Analysis**

Statistical analysis was performed with one-way analysis of variance (ANOVA). When ANOVA detected significant differences between the means, they were compared using Tukey's test. OriginLab software (OriginLab Corporation, Northampton, UK) was used for statistical analysis. Level of significance was designated as  $P < 0.05$ . Values were expressed as Mean ± SEM.

## **RESULTS AND DISCUSSION**

### **Determination of Iodine Value of Epoxidized Products**

The iodine value of the soybean oil used as raw material was 143.0. From **Table 1** it was observed that in case of 1:1 chemical process (CM1) with increasing reaction time the iodine value dropped rapidly after one hour followed by gradual lowering of the iodine value. In a previous study similar results were obtained where the epoxidation of soybean oil was monitored by the lowering of iodine value [12]. However, after 5 hours the iodine value again increased. Similarly, in case of the 1:1

enzymatic process (EM1) the initial iodine value was found to drop gradually till 5 hours beyond which it was found to increase again. Again in case of 1:2 chemical process (CM2) the initially lowering iodine value showed a reversal after 3 hours. Incidentally for 1:2 enzymatic process (EM2) iodine value decreased very gradually and unlike CM2 here the reversal towards higher iodine value took place only after 6 hours.

Lowering of iodine values indicates oxidation of double bonds in the soybean oil [12]. Hence with time as greater amounts of epoxides are formed the iodine values are found to decrease correspondingly. Here *in-situ* epoxidation takes place using hydrogen peroxide as the oxygen donor and acetic acid as the peroxygen carrier. In case of EM2 it is observed that the lowering of iodine value is a slow process, which is unlikely when comparing with EM1. A possible explanation for this

behaviour is that in case of bio-catalyzed reactions, the enzyme activity is often slowed down when the reaction is carried out at high concentrations of hydrogen peroxide and high temperature [13]. *In situ* epoxidation using hydrogen peroxide is a common technique with commercial application and is of immense importance today [4]. In EM2 twice the amount of hydrogen peroxide was used than that used in EM1. Even in case of EM1 though the lowering of iodine value was greater than CM1, yet the time required was the same in both the cases. The cause of this can be traced back to the same reason as that of application of sufficient amount of hydrogen peroxide at a temperature of up to 50°C. Similar results were obtained previously where the yields of the epoxidized products were obtained at par for chemical and enzymatic processes although higher reaction times were required in case of the enzymatic process [5].

**Table 1:** Determination of iodine value of epoxidized products by chemical and enzymatic methods

Time (hours)	Iodine value (Chemical method)		Iodine value (Enzymatic method)	
	CM1	CM2	EM1	EM2
1	140.64±1.44 <sup>a</sup>	21.4±1.23 <sup>b</sup>	32.0±1.07 <sup>c</sup>	32.0±1.13 <sup>c</sup>
2	62.80±1.03 <sup>a</sup>	6.64±0.51 <sup>b</sup>	29.0±1.12 <sup>c</sup>	26.03±0.82 <sup>d</sup>
3	59.17±1.10 <sup>a</sup>	2.3±0.33 <sup>b</sup>	20.15±0.49 <sup>c</sup>	24.41±1.08 <sup>d</sup>
4	44.86±1.11 <sup>a</sup>	5.06±0.62 <sup>b</sup>	19.63±0.84 <sup>c</sup>	22.77±0.95 <sup>d</sup>
5	36.16±1.08 <sup>a</sup>	ND	17.27±0.77 <sup>b</sup>	15.33±0.87 <sup>c</sup>
6	82.57±1.51 <sup>a</sup>	ND	18.12±0.62 <sup>b</sup>	8.66±0.73 <sup>c</sup>
7	ND	ND	ND	16.88±0.56

ND: not determined

Values were expressed as mean±S.E.M, n=3. Means were compared using Tukey's test. Columns not sharing a common superscript were statistically significant (p<0.05).

**Table 2:** Determination of acid value of epoxidized products by chemical (CM1 and CM2) and enzymatic (EM1 and EM2) methods

	Acid value (chemical method)		Acid value (enzymatic method)	
	CM1	CM2	EM1	EM2
<b>Before epoxidation</b>	1.12±0.23 <sup>a</sup>	1.30±0.31 <sup>a</sup>	1.52±0.17 <sup>a</sup>	1.53±0.28 <sup>a</sup>
<b>After epoxidation</b>	16.68±1.31 <sup>b</sup>	19.00±1.51 <sup>b</sup>	12.87±1.47 <sup>b</sup>	17.12±0.89 <sup>b</sup>

Values were expressed as mean±S.E.M, n=3. Means were compared using Tukey's test. Rows not sharing a common superscript were statistically significant (p<0.05).

Another interesting observation was that iodine value increased beyond a certain time for all the four processes. This can be explained by

the possible rupture of the epoxide linkage to the corresponding aldehydes, ketones etc. Due to the increase in the unsaturation content

beyond a certain time, an increase of the iodine value was observed in all the four cases. At high temperatures the decomposition of the epoxy groups was also observed previously for *X. Americana* seed oil, where significant increase of iodine value was also observed [14]. It was also observed that the iodine value for each process varied significantly from the other.

#### **Determination of Acid Value of Epoxidized Products**

Acid value for each process was found to drastically increase after epoxidation (**Table 2**). When acid values of each process before and after epoxidation were compared, it was observed that in case of each process the acid values varied significantly both before and after epoxidation. The increase in acid values after epoxidation can be explained by the fact that during the epoxidation process extensive hydrolysis of the triacyl-, diacyl- and monoacyl-glycerides of soybean oil took place due to the presence of sulphuric acid/lipase enzyme as catalysts in the system. The hydrolysis generated sufficient quantities of free fatty acids in the system resulting in the increment of the acid values. In case of both CM2 and EM2 processes greater extent of hydrolysis took place in comparison to CM1 and EM1 respectively. This could be due to the presence of higher amounts of H<sub>2</sub>O<sub>2</sub> in both the cases which probably promotes the hydrolysis

process. In a previous study, it was observed that the epoxidizing agents induced hydrolysis along with epoxidation during the reactions, hence leading to the variation of acid values accordingly [5].

#### **Determination of Viscosity of Epoxidized Products**

The viscosity of soybean oil at 25°C was 31.0 cP. From **Table 3** it was observed that at 25°C the viscosities of all the products have increased to a great extent. The greater the extent of epoxidation of the oil, the higher will be the viscosity of the final product at a particular temperature. This difference could be due to the increase in the number of epoxy linkages which corresponded to the decrease in the unsaturated bonds of the oil [15]. Due to the formation of epoxy linkages a great degree of hydrogen bonding generated in the products which was responsible for the high viscosity of the epoxidized oil. This bonding was absent in the un-epoxidized oil. Hence viscosity was an important parameter to assess the degree of epoxidation in oil. However, epoxidized soybean oil was found to have a lower viscosity than other epoxidized vegetable oils in a previous study [16]. In the present work the product of CM2 process had significantly high viscosity in comparison to CM1. This was followed by EM2 which in turn had a significantly high viscosity in comparison to EM1.

**Table 3:** Determination of viscosities of epoxidized products by chemical (CM1 and CM2) and enzymatic (EM1 and EM2) methods at 25.0°C

Viscosity in cP (chemical method)		Viscosity in cP (enzymatic method)	
CM1	CM2	EM1	EM2
66.6±1.22 <sup>a</sup>	81.1±1.58 <sup>b</sup>	70.3±1.61 <sup>c</sup>	75.1±1.07 <sup>d</sup>

Values were expressed as mean±S.E.M, n=3. Means were compared using Tukey's test. Columns not sharing a common superscript were statistically significant (p<0.05).

#### **Determination of Oxirane Oxygen of Epoxidized Products**

Epoxy content or the oxirane oxygen content is the most important property for epoxidized oils. From **Table 4**, it was observed

that the highest epoxy content among the four types of epoxidized soybean oil was for CM2 followed by EM2. Epoxy content of CM1 was the lowest. From the data it was observed that fully epoxidized soybean oil could not be

achieved, which may be due to some side reactions like epoxy ring opening or ring rupture. It was also observed that in case of enzymatic reaction the extent of epoxidation could be suitably controlled by restraining the amount of H<sub>2</sub>O<sub>2</sub> used. Hence at lower hydrogen peroxide content significantly higher epoxidation of the soybean oil was observed as in case of enzymatic epoxidation (EM1) in comparison to

the chemically epoxidized method (CM1). The reverse was the case for chemical epoxidation method. Here, with increasing proportion of H<sub>2</sub>O<sub>2</sub> greater yield of epoxidized oil was obtained for CM2, than that for EM2. It was observed in a previous study that the oxirane ring content increased with time and increasing proportion of hydrogen peroxide [17].

**Table 4:** Determination of oxirane oxygen of epoxidized products by chemical (CM1 and CM2) and enzymatic (EM1 and EM2) methods

Oxirane oxygen (%) (chemical method)		Oxirane oxygen (%) (enzymatic method)	
CM1	CM2	EM1	EM2
67.72±1.62 <sup>a</sup>	79.0±1.58 <sup>b</sup>	74.36±1.91 <sup>c</sup>	76.1±1.37 <sup>d</sup>

Values are expressed as mean±S.E.M, n=3. Means were compared using Tukey's test. Columns not sharing a common superscript are statistically significant ( $p < 0.05$ ).

### FT-IR Spectroscopic study

The characteristic peak near 3000 cm<sup>-1</sup> for soybean oil (**Figure 1A**) can be attributed to C–H stretching of soybean oil unsaturations. This peak was found to decrease considerably for CM2 (**Figure 1C**) and almost disappear for EM2 (**Figure 1E**) in comparison to CM1 (**Figure 1B**) and EM1 (**Figure 1D**). The peaks at 2850 cm<sup>-1</sup> for CM1, CM2, EM1 and EM2 may be attributed to the C–O–C stretching of the epoxide linkage. The disappearance of the peak at 3010 cm<sup>-1</sup>, of EM2, which was generated due to the C–H stretching of non-conjugated unsaturation, clearly indicated towards the utilization of the C=C bond to form epoxide linkage. The disappearance of peaks at 800 – 850 cm<sup>-1</sup> of soybean oil from the epoxidised products indicated towards the formation of epoxide linkage. It indicated that the C=C bonds were converted either partially or completely into epoxidised products.

For all the epoxidized soybean oils the C=O stretching of esters was observed for triglyceride at 1740 - 1750 cm<sup>-1</sup>, which was however less pronounced for the 1:2 enzymatic products. It appeared due to the C=O ester peak of the carboxylic acid group of triglycerides. The other peaks at 2100 - 2150 cm<sup>-1</sup> can be attributed to the stretching of the

remaining C=C unsaturated bonds, which may also have been generated due to epoxy ring opening.

### Film formation

On critical observation of the film formed by the developed plasticizers it was evident that the product of 1:2 enzymatic process (EM2) formed a film that was very uniform, with minimal pores. It formed a continuous film without any breakage and was wrinkle-free. Moreover, it did not show any rupture when a shear stress was applied on it. Similar was the case with 1:2 chemical epoxidation process (CM2). But the films produced by 1:1 enzymatic process (EM1) and 1:1 chemical process (CM1), were not uniform, continuous, and were intercepted by intermittent wrinkles.

The study showed that the addition of the four synthesized plasticizers to the polymer enhanced the thickness and stability of the generated films. Furthermore, an increase in the flexibility of the film was also noted in all the four cases.

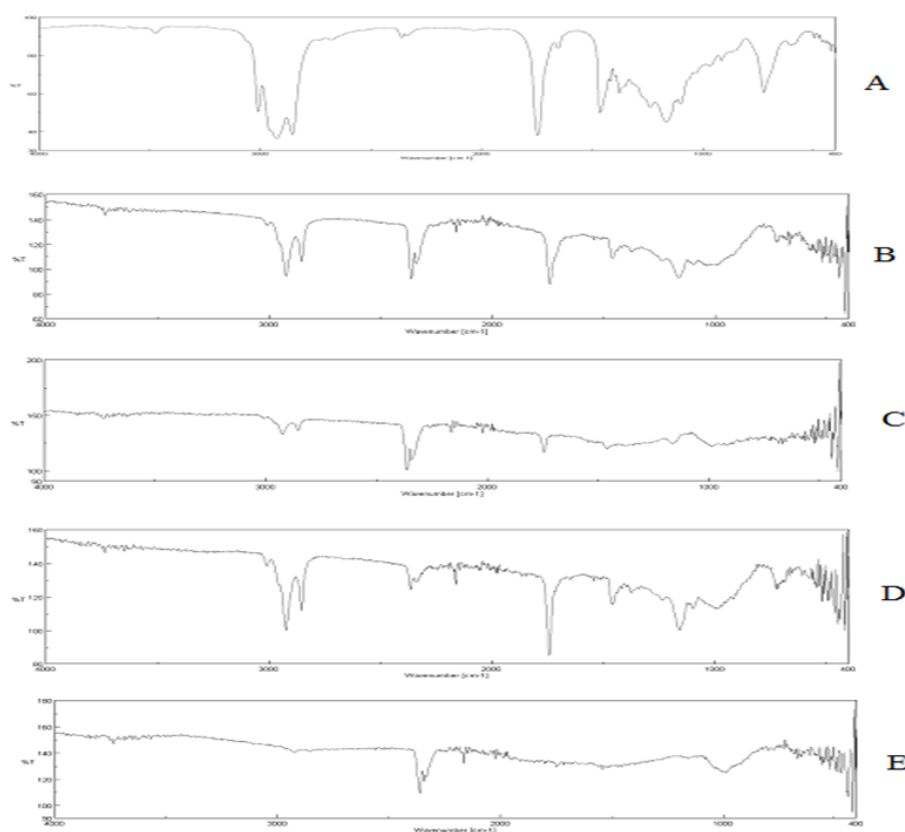


Figure 1

**Figure 1:** FTIR spectra of (A) Original soybean oil; (B) 1:1 Chemically epoxidized product (CM1); (C) 1:2 Chemically epoxidized product (CM2); (D) 1:1 Enzymatically epoxidized product (EM1); (E) 1:2 Enzymatically epoxidized product (EM2)

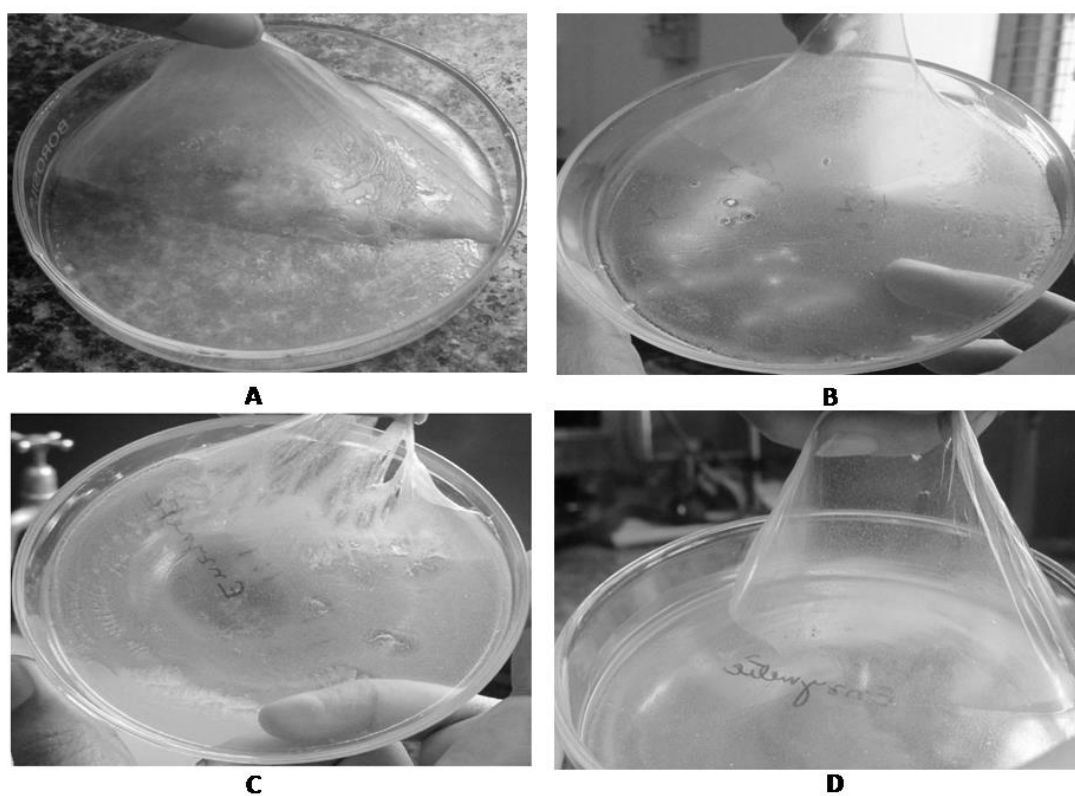


Figure 2

**Figure 2:** Film formation by of (A) 1:1 Chemically epoxidized product (CM1); (B) 1:2 Chemically epoxidized product (CM2); (C) 1:1 Enzymatically epoxidized product (EM1); (D) 1:2 Enzymatically epoxidized product (EM2)

## CONCLUSION

In the present work a detailed study was conducted on the effect of the chemical and enzymatic epoxidation processes on soybean oil. It was observed that the epoxidation process, in either case was, strongly influenced by the proportion of epoxidizing agent used. In both the cases higher yield of plasticizer was obtained when greater amount of H<sub>2</sub>O<sub>2</sub> was used. However, such processes also generated large amounts of free fatty acids due to the subsequent hydrolysis reactions. Also possible side reaction of the epoxy ring opening could be detected by the FTIR analysis. In conclusion it can be said that of the two processes the 1:2 enzymatic reactions could be short-listed as the suitable process for synthesis of plasticizers due to its high yield, comparatively low acid value and lower epoxy ring opening reactions. In future other vegetable oils and epoxidizing agents can be used to develop bio-degradable plasticizers.

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## Conflict of interest

The authors have declared no conflict of interest.

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