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Lipase-Mediated Preparation of Epoxy Lecithin and Its Evaluation as Plasticizer in Polyester Laminates

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ABSTRACT: A simple chemoenzymatic method was developed for the preparation of epoxy lecithin that contains epoxy oils and phospholipids. The parameters such as lipase concentration, hydrogen peroxide concentration, and time of the reaction were studied. The product was evaluated as a plasticizer in polyester laminates and compared with virgin polyester laminates. The laminates were prepared using various amounts of epoxy lecithin and evaluated for different properties. The epoxy lecithin modified laminates showed good impact strength, tensile, and chemical resistance properties. These laminates were also evaluated for vicat softening point and water absorption. The epoxy lecithin can be used as a plasticizer in polyester laminates. © 2012 Wiley Periodicals, Inc. J. Appl. Polym. Sci. 000: 000–000, 2012

KEYWORDS: epoxy oils; phospholipids; tensile strength; plasticizer; polyester laminates

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INTRODUCTION

Epoxidized soybean oil (ESBO) is a derivative of soybean oil having epoxy functional groups, which is formed by epoxidation of carbon–carbon double bonds in the triglyceride. ESBO usually consists of triglycerides with about 53% diepoxy linoleic acid, 25% epoxy oleic acid, 7% triepoxy linolenic acid, palmitic acid, and stearic acid.[1] In general, epoxy oils are being used as plasticizers, crosslinking agents, stabilizers, and prepolymers. Further, they can be used as intermediates in the synthesis of polyols that are being used in the production of polyurethane, polyester, and plastic resins.[2]

Despite several studies on vegetable oil-based epoxidized products for a variety of industrial applications, no reports were available on preparation and evaluation of epoxidized lecithin, which is an epoxy derivative of lecithin. However, Das and Bhattacharya[3] reported the incorporation of 12-epoxy oleic acid (vernolic acid from *Vernonia anthelmintica* oil) into soybean phospholipids using an immobilized lipase (Lipozyme TL IM) as biocatalyst and sodium methoxide as a chemical catalyst up to 43 and 35%, respectively.

On the other hand, among all the epoxidation processes reported,[4–6] clean and efficient method to epoxidize vegetable oils was either with per carboxylic acids or with organic and inorganic peroxides. However, these methods also use strong mineral acids such as concentrated sulfuric acid as catalyst. The chemoenzymatic methods are simplified approaches unlike chemical epoxidation, as these methods do not involve tedious work up procedures. Further, the by-products such as dihydroxy acids (by epoxide ring-opening with water) and estolides (by epoxide ring-opening with another molecule of carboxylic acid) will not be formed during enzymatic route, which is common in the chemical epoxidation.[5] The only by-product formed during enzymatic epoxidation is small amounts of epoxy-peroxy acid. The chemoenzymatic epoxidation reaction is a selective and environmentally benign alternative to the traditional Prilezhaev epoxidation process,[7,8] currently used in industry to convert unsaturated compounds to the corresponding epoxides. Therefore, the aim of this work was to develop a chemoenzymatic process for epoxidation of soybean lecithin, a by-product obtained during vegetable oil

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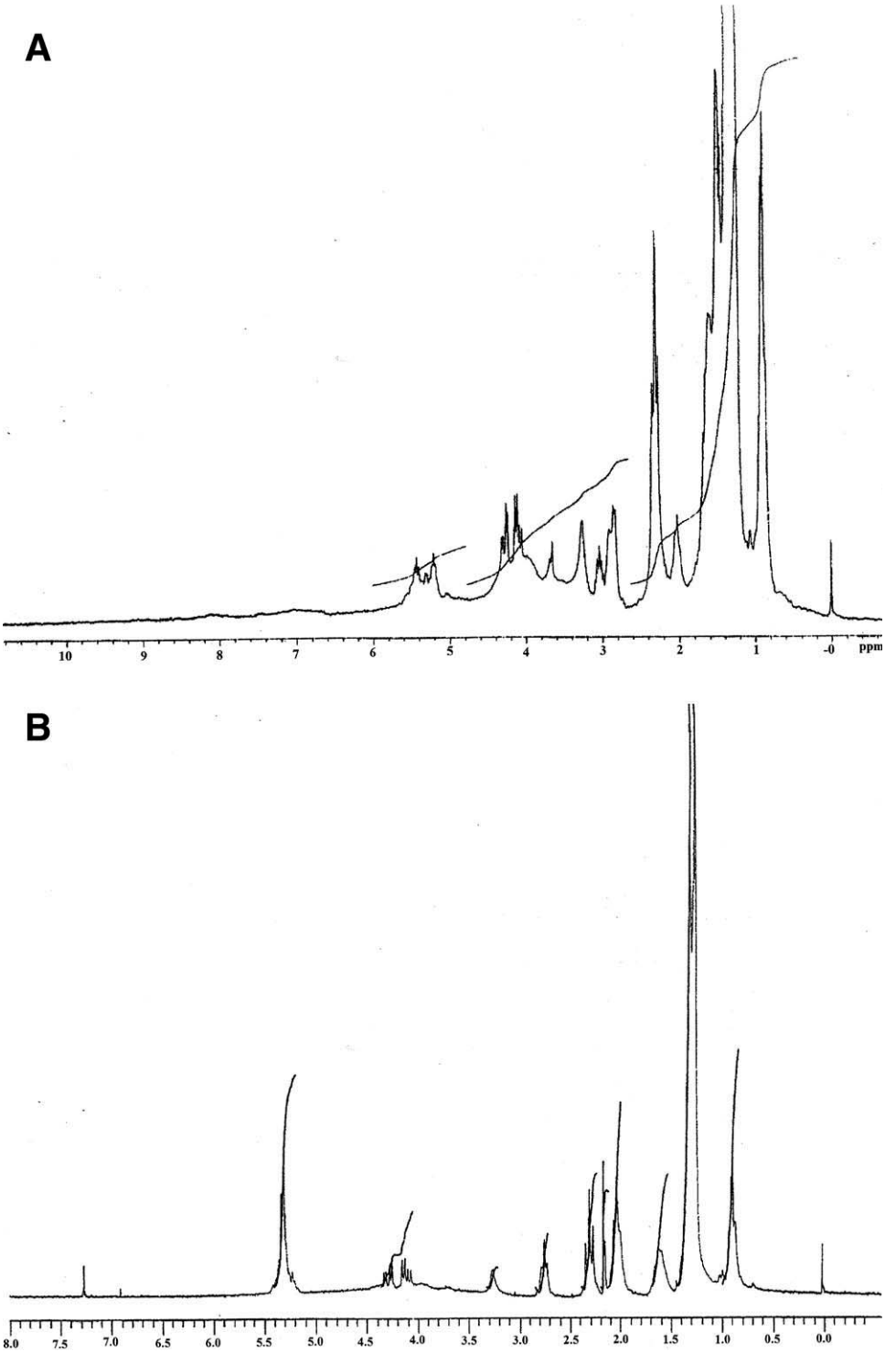

Figure 1. ^{1}H-NMR spectra of epoxidized lecithin (A) and soybean lecithin (B).

refining. The effect of various reaction parameters such as reaction period, lipase dosage, and hydrogen peroxide solution concentration were examined to optimize the epoxidation conditions. Another objective of this study was to use the epoxidized lecithin prepared above as a sustainable plasticizer in polymers.

In general, plasticizers are incorporated into polymer to reduce the modulus, tensile strength, hardness, density, melt viscosity, glass transition temperature, electrostatic chargeability, and volume resistivity of the polymer. Addition of plasticizer increases the flexibility, elongation at break, toughness, dielectric constant, and power factor of a polymer, because of which the

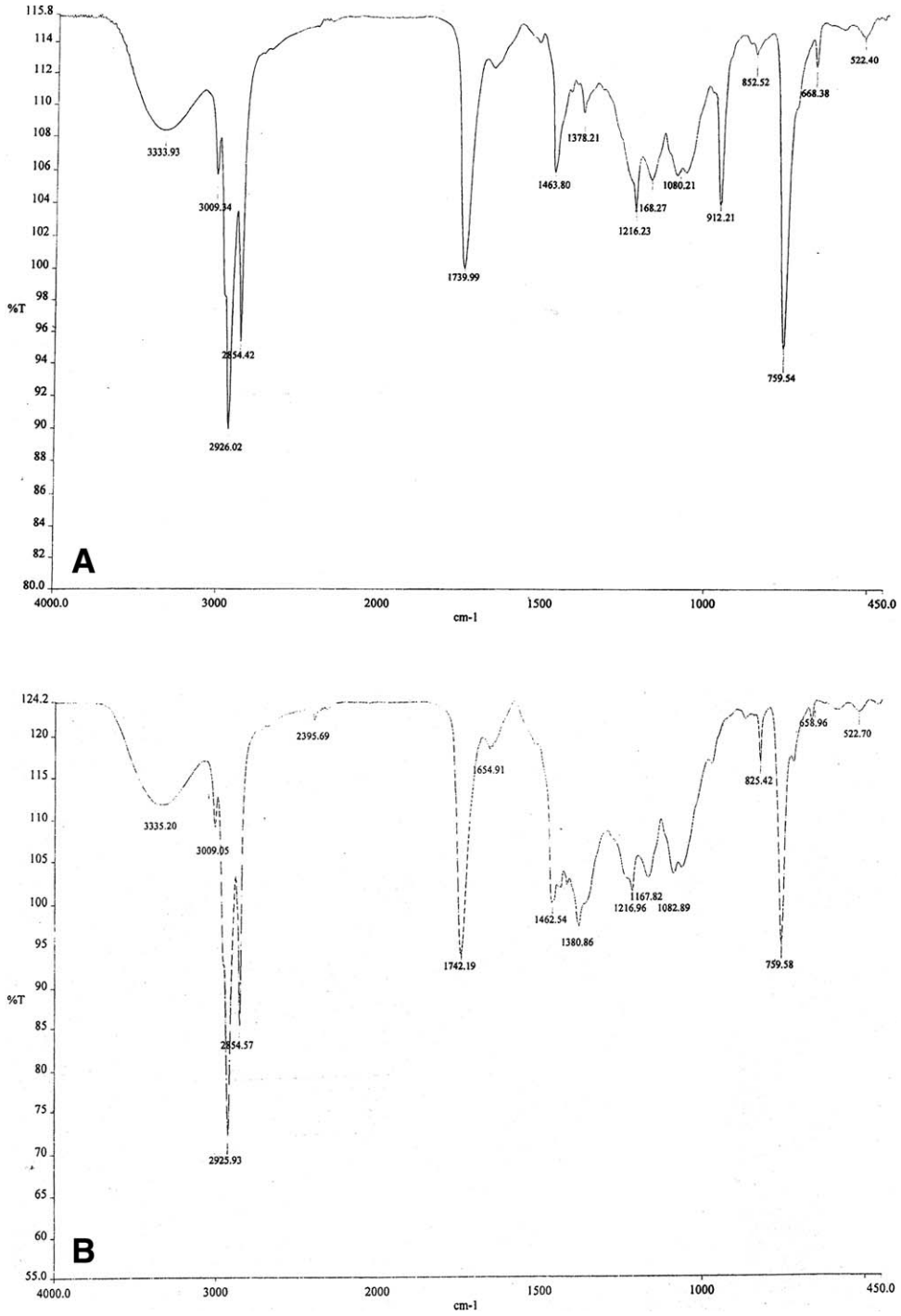

Figure 2. IR spectra of epoxidized lecithin (A) and soybean lecithin (B).

plasticizer industry is growing with a wide range of applications of plastics.[9,10] Therefore, the epoxidized lecithin product prepared in this work was evaluated as plasticizer in polyester laminates preparation. The formulated product was studied for the characteristics such as specific gravity, water absorption, impact strength, vicat softening point (VSP), chemical resistance, and tensile strength.

EXPERIMENTAL

Materials and Instruments

Hydrogen peroxide solution (30 and 50% concentrations) and toluene were purchased from M/S Loba Fine Chemicals (Mumbai, India). Novozym 435 [*Candida antarctica* immobilized on macroporous polyacrylate resin beads with an activity of 10,000 propyl laurate units per gram (PLU/g)] was a gift sample from

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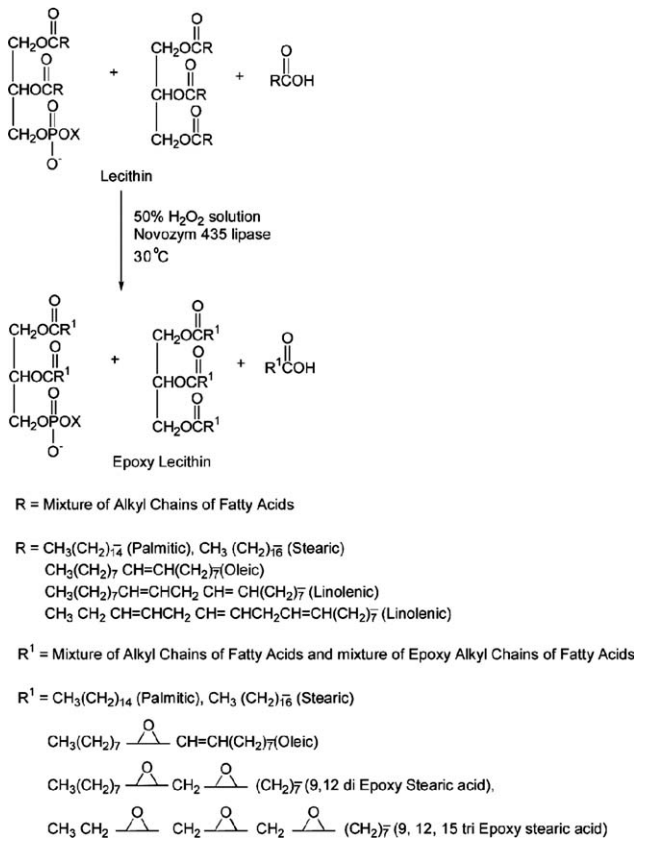


Scheme 1. Chemoenzymatic epoxidation of lecithin.

M/s. Novozymes South Asia (Bangalore, India). Crude soybean lecithin having an acid value (AV) of about 21 was procured from M/s Krithi Industries (Indore, India). Common solvents and reagents were purchased from Spectrochem (Mumbai, India). All the reagents were of analytical grade and were used without further purification. Methrom 665 dosimat was purchased in India.

Typical procedure for Epoxidation of Lecithin

Soybean lecithin (50 g) was taken in toluene (100 mL), which was saturated with both water and hydrogen peroxide, and to this Novozyme 435 lipase (10 g, 20 wt % of substrate) was added. After stirring for 15 min, hydrogen peroxide solution (50%, w/v, 38.4 mL) was added through Methrom 665 dosimat over a period of 8 h at the rate 80 μL/min. Further, stirring was continued for a total period of 96 h at 30°C. After completion of the reaction period, the lipase was separated by filtration from the reaction product using Buckner funnel that was bedded with celite. The filtrate was concentrated on rotary evaporator and dried under reduced pressure to obtain epoxidized product. The reaction conditions were optimized by varying the lipase dosage, hydrogen peroxide concentration, and reaction period. At specific time intervals, samples were collected, and the iodine value (IV) and oxirane value (OV) of the product were determined. At the end of the reaction, the IV was reduced to 25.0 from an initial value of 100, and OV was reached to 3.7 from an initial value of zero. The product was further critically characterized by ^{1}H-NMR and IR spectroscopy.

^{1}H-NMR (200 MHz, $CDCl_3$, δ): 2.9–3.1 (2H, m, epoxy protons). The Figure 1(A,B) illustrates the ^{1}H-NMR spectral data of epoxidized lecithin and lecithin, respectively.

IR (KBr, cm^{-1}): 912 (epoxy ring). The Figure 2(A,B) illustrates the IR spectral data of epoxidized lecithin and lecithin respectively. The process and mechanism are shown in Schemes 1 and 2, respectively.

Characterization

The IV, peroxide value, AV, and OV were determined by AOCS Official Methods Ja 14-91, Ja 8-87, Ja 6-55, and Cd 9-57, respectively.[11] ^{1}H-NMR Spectra were recorded on a Brucker (Wissenbourg, France) ARX 400 Spectrometer (200 MHz) with $CDCl_3$ solvent. IR spectra were recorded on a Perkin Elmer (model: Spectrum BX) FTIR spectrometer using CH_2Cl_2 or KBr.

Preparation of Polyester Laminates

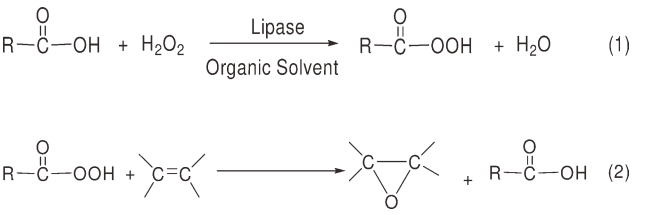
Polyester laminates were prepared using general purpose resin that consists of 67% unsaturated polyester resin and 33% styrene content (M/s.Satyen, Mumbai, India), catalyst (methyl ethyl ketone peroxide), and accelerator (cobalt octoate). The 5% soybean lecithin and 5% epoxy soybean lecithin are used in the preparation of polyester laminate along with virgin laminate (100 g of resin, 1 mL of cobalt accelerator and 1.5 mL of MEKP catalyst). After mixing all the ingredients, the laminate was put into the mold and left it for 24 h. These laminates were post-cured for 3 h at 80°C and tested for various properties. At least 10 samples have been tested, and the average value has been reported here.

Physicomechanical and Chemical Properties

Specific Gravity Test. The specific gravity of laminates shall be expressed as the ratio of the weight of a given volume of the material (at 23°C) to that of an equal volume of water at the same temperature. This was measured according to ASTM procedure[12] using the pycnometer.

Water Absorption Experiment. This method of test covers the procedure for determining the relative rate of water absorption by laminates when completely immersed in water for 1 week. The method is intended to apply to the testing of all types of laminated plastics, including cast, hot-molded and cold-molded, resinous products, and both homogenous and laminated plastics in rod and tube forms and in sheets. Water absorption property was measured according to ASTM procedure.[13]

Impact Strength (Izod). The impact test indicates directly the overall toughness of the material. Toughness is defined as the ability of the material to absorb applied energy under the sudden release of load. Standard test specimens under stipulated conditions were used for notching, mounted for rate of loading.



Scheme 2. Mechanism for chemoenzymatic epoxidation of fatty acids.

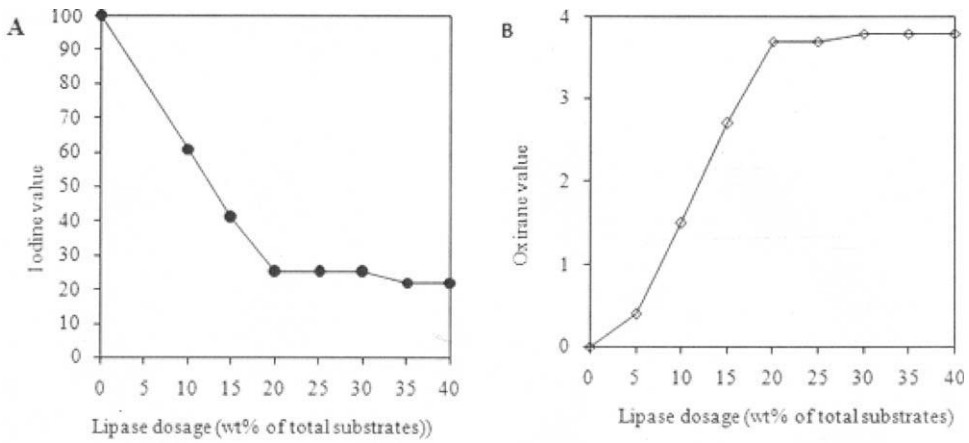


Figure 3. Changes in IV (A) and OV (B) of epoxidized soybean lecithin with respect to lipase dosage.

The higher the impact, the higher will be the toughness. This test was carried out according to ASTM procedure.[14] The measurement was performed using Resil Impactor Junior [Make CEAST S. P. A, Italy; Model P/N 6963.000].

VSP. VSP is temperature at which a flat-ended needle of 1 mm^2 circular cross-section will penetrate the specimen to a depth of 1 mm under a specified load using uniform rate of temperature rise. VSP property of laminated plastics was measured according to ASTM procedure.[15] VSP property was measured on HDT/VSP apparatus (Make: CIPET, Chennai, India; Model: HDT DI01; Serial No. 106).

Chemical Resistance Test. Acetone extraction or chemical changes on the surface of materials such as discoloration staining observed on laminated products. This gives the information about the nature of product surface, susceptibility to chemicals action on surface, and so forth. Acetone extraction process was measured according to ASTM procedure.[16]

Tensile Strength Test. Tensile properties include modulus of elasticity, tensile strength, elongation at break, and energy absorption. Tensile tests may provide the data for research and development, engineering design, quality control, acceptance or rejection under specifications, and for special purposes. Tensile strength of laminates was measured according to ASTM procedure.[17] Tensile strength was measured on Universal Testing Machine (model: AGS-10 KGN, Shimadzu, Japan) with a crosshead speed of 10 mm/min.

RESULTS AND DISCUSSION

Preparation of Epoxy Soybean Lecithin

Soybean lecithin, a by-product of vegetable oil refining was epoxidized using H_2O_2 and Novozym 435 lipase. However, thorough optimization of parameters is required for gaining optimal yield of epoxy lecithin. Hence, in this study, optimization was carried out by varying the reaction parameters such as reaction period, lipase dosage, and hydrogen peroxide solution concentration.

Effect of Lipase Dosage

The effect of lipase dosage on the epoxidation of soybean lecithin was studied using Novozyme 435 by varying the concentration of enzyme from 10 to 30% (wt % of total substrates), while maintaining the reaction time, 96 h, and hydrogen peroxide solution concentration, 38.4 mL, 50%. As the lipase dosage was increased from 10 to 20%, epoxidation reached to maximum (IV reduced to 25.0 from an initial value of 100, OV reached to 3.7 from 0). Further, increase in the dosage of enzyme (i.e., 30%) did not show any significant increase (IV, 23.9; OV, 3.78) as illustrated in Figure 3(A,B), respectively) in the epoxidation. Hence, maximum epoxidation of lecithin was achieved using 20% (wt/wt of total substrates) lipase dosage. Although lipases are well known to catalyze hydrolysis of triglycerides and interesterication, these reactions were not taken into account in the description of the chemoenzymatic epoxidation as comparatively low water content of the organic phase is not favorable for hydrolysis.[18] In addition, the hydrolytic activity toward triglycerides of long-chain fatty acids is low for *Candida antarctica* lipase B; perhaps this might be due to its narrow and deep active site.[19,20] The ^{1}H-NMR of epoxy lecithin showed characteristic epoxy (—C—O—C—) at a chemical shift of 2.9–3.1 ppm, which confirmed the presence of epoxy group in the product and absence of any side products [Figure 1(A)].

Effect of Hydrogen Peroxide Concentration

The effect of hydrogen peroxide concentration was studied using 38.4 mL of H_2O_2 and two different concentrations of hydrogen peroxide (30 and 50%). The extent of epoxidation of lecithin with 30% hydrogen peroxide (IV reduced to 54.3 from an initial value of 100, OV reached to 1.9 from an initial value of 0) was lower compared to the reaction with 50% hydrogen peroxide (IV 25.0 and OV 3.7). Hence 50% H_2O_2 was found to be optimum for effective epoxidation. The addition was continued over a period of 8 h at the rate 80 μL/min until all H_2O_2 (38.4 mL) was added. Even at 50% hydrogen peroxide concentration, no considerable enzyme deactivation was observed. This finding is in well agreement with the past work,[18] where the researchers reported that for only 60% H_2O_2 and at high temperatures, enzyme deactivation was recognized. Further, as the peracid formed in an enzyme-catalyzed reaction will epoxidize double bonds, as seen in Prilezhaev reaction, in the organic phase, but can also decompose at elevated temperatures. Therefore, throughout the study, 30°C temperature was maintained and considered to be the optimum condition.

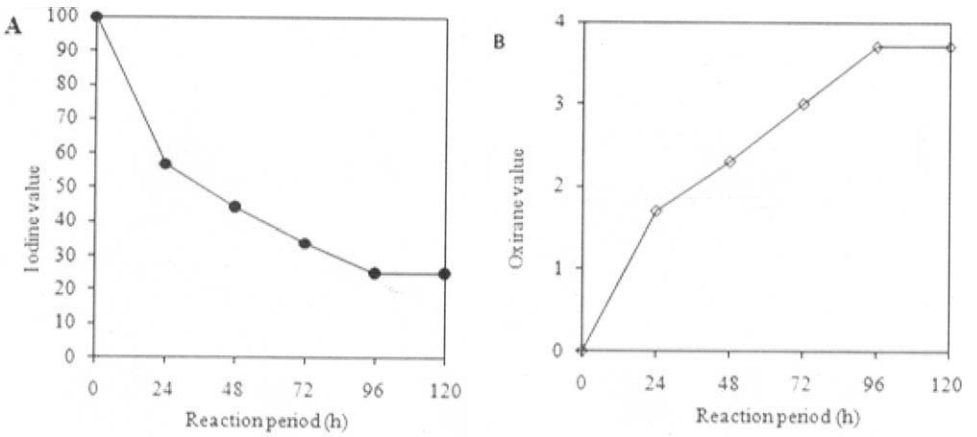


Figure 4. Changes in IV (A) and OV (B) of epoxidized soybean lecithin with respect to reaction period.

Effect of Reaction Period

Using 20% lipase dosage, 50% H_2O_2 solution (38.4 mL), the time course of epoxidation was optimized by varying the reaction period from 24 to 108 h. However, maximum epoxidation was observed at a reaction period of 96 h; this was confirmed based on IV [Figure 4(A)] and OV [Figure 4(B)]. Further prolongation of reaction up to 108 h did not find any significant increase in epoxidation. The optimized conditions for the epoxidation were as follows: lipase dosage, 20%; hydrogen peroxide, 50% solution (38.4 mL); reaction period, 96 h.

However, the reason is not clear for incomplete epoxidation; as there are several factors, namely, reaction system, enzyme efficiency, and temperature, hydrogen peroxide concentration still to be extensively studied. This is because of complex reaction system that consists of an aqueous phase containing the hydrogen peroxide, an organic phase containing toluene as solvent, triglycerides, fatty acids, phospholipids, and immobilized enzyme as solid phase. The fatty components are considered to be insoluble in the aqueous phase, whereas water and hydrogen peroxide are exchanged between the two liquid phases. Further, the mechanism for lipase-catalyzed epoxidation of oils was already reported by Hilker et al.[18] The same mechanism could be suggested to epoxidation of lecithin, as it has similar structural features compared to oils. The reaction takes place in two steps: (1) the peroxy oxygen from hydrogen peroxide is delivered to form peroxy fatty acids from free fatty acids present in lecithin and (2) the peroxy fatty acids deliver their peroxy oxygen to the double bonds of fatty acids present in lecithin (Scheme 2) to prepare epoxy lecithin.

Evaluation of Epoxy Lecithin as Plasticizer in Polyester Laminates

The prepared laminate samples were tested for tensile strength, VSP, impact strength, chemical resistance, and so forth. The sample prepared with epoxy soybean lecithin exhibited good impact strength and flexibility at lower concentrations compared to soybean lecithin. The results (Table I) revealed that epoxy soybean lecithin can be used as plasticizer in these polyester laminates.

The evaluation studies further indicated that specific gravity of epoxy lecithin-polyester (1.247) sample was similar to that of soybean lecithin-polyester (1.24) and slightly varied from virgin polyester (1.251). Water absorption of 5% epoxy lecithin-polyester sample (0.49%) and soybean lecithin (0.344%) was slightly higher compared to virgin sample (0.27%). Impact strength (Izod, J/mm) of these laminates indicated that the strength was improved in epoxy laminate (0.0267) compared to virgin (0.0257) and soybean lecithin (0.0145) based laminates. This improvement was a positive sign for absorption of sudden

Table I. Comparison of Physicomechanical, Chemical Properties of Virgin Polyester Sample with Other Lecithin-Polyester Samples

	Sample		
Characteristic	Virgin polyester	5% soybean lecithin[a]	5% epoxy lecithin[a]
Specific gravity	1.251	1.24	1.247
Water absorption (%)	0.27	0.344	0.49
Impact strength (J/mm)	0.0257	0.0145	0.0267
VSP (°C)	77	68	67
Chemical resistance (Acetone/½ h)	No change	No change	No change
T_g (°C)	135.7	134.0	134.7
Tensile strength (N/mm^2)	19.92	19.94	19.83
Elongation (%)	5.1	3.78	9.06

[a]5% lecithin was added to the polyester sample to check the plasticizer properties.

release of energy, and hence, this can be used for the application of helmets, wind shields, and so forth, where high impact strength is required. The drop in VSP was due to increase in flexibility of epoxy lecithin-polyester (67°C) when compared to virgin polyester (77°C) material. Chemical resistance property remained same for both virgin polyester and epoxy lecithin-polyester samples. This indicated that there was no change in chemical resistance of virgin sample even after addition of epoxy lecithin. It was observed that there was a minor variation in tensile strength (N/mm^2) along with an improvement in elongation in epoxy lecithin laminates. It was also a positive sign for plasticizer properties, which indicated that brittleness has reduced in this material, and it became soft compared to other two materials. This can be considered as a phenomenal change for application point of view. Elongation of epoxy lecithin-polyester sample (9.06%) was higher compared to virgin polyester (5.1%) and soybean lecithin (3.78%).

CONCLUSIONS

In this study, a simple chemoenzymatic approach was developed for the preparation of epoxy-lecithin from commercial soybean lecithin. The influence of several parameters such as reaction period, lipase dosage, and hydrogen peroxide concentration on epoxidation were examined to optimize the reaction conditions. Further, the product was evaluated to be the plasticizer in polyester laminates by performing the experiments such as specific gravity, water absorption, impact strength, VSP, chemical resistance, and tensile strength. Except the enhancement in water absorption property, all the other physicomechanical and chemical properties were quite positive for epoxy lecithin-polyester laminate sample compared to virgin and soy bean lecithin-polyester samples in view of plasticizer applications.

ACKNOWLEDGMENT

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