USE OF ENZYMES TO PRODUCE SOY-BASED POLYOL

FOR POLYURETHANE

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By

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USE OF ENZYMES TO PRODUCE SOY-BASED POLYOLS FOR POLYURETHANE

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ABSTRACT

Soy-based polyol used in polyurethane applications are sustainable and renewable materials which have been reported to require less energy to produce and to have lower market price. They also have less environmental impact, expand soybean market for soybean farmers and help to reduce consumption of petrochemical-based materials. This project proposes new soy-based polyols produced by enzymatic routes and having good reactivity with isocyanate in polyurethane foam production. Multiple enzymatic routes were evaluated to improve the functionality, specifically, hydroperoxy, epoxy and hydroxy functional groups, of soy-based materials.

Lipoxygenase was applied to soy-based fatty acids and soy-based glycerides. The hydroperoxy groups were created on unsaturated fatty acids when carbon-carbon double bonds were moved and preserved. Lipase B from *Candida antarctica* (Novozyme 435®) was used to epoxidize unsaturated moieties of soybean oil and soy-based oligomers. The soy-based epoxy materials could either be directly used as B-side materials in polyurethane

synthesis, or further derived to other high functionality soy-based polyols. Furthermore, several commercially available lipases were studied to replace unused saturated fatty acid moieties with reactive hydroxy functional groups by the hydrolysis of epoxidized soybean oil and soy-based oligomers.

Enzyme *Candida rugosa* and *Burkholderia cepacia* were found to be selectively remove saturated fatty acids; palmitic and stearic acids, from epoxidized soybean oil. Enzyme *C. rugosa* showed selectivity towards palmitic acid in the hydrolysis of bodied soybean oil.

In addition, high MW soy-based polyols were produced by the cleavage of the epoxidized soybean oil with acid moieties derived from vegetable oils. The high MW acid moieties were synthesized by enzyme reactions including hydrolysis and esterification.

All enzyme reactions proposed in this work were successfully operated at low temperature, less than 70^{o}C. And most of the reaction did not require organic solvent which was occasionally required to preserve enzyme activity. The enzyme technology encourages the development of “green chemistry” and “sustainability” for urethane consumers. The results conclusively demonstrated that selective enzymes can synthesize improved soy-based polyols with high yield and selectivity in manners not possible with conventional chemistry.

CHAPTER 1

INTRODUCTION

1.1. BACKGROUND

Polyols are used to react with isocyanates and form urethane functional groups in polyurethane synthesis. Bio-based polyols are rapidly being developed to replace petrochemical based polyols due to their availability and renewability.

Soy-based polyols are applicable as B-side materials in many polyurethane formulations resulting in comparable polyurethane foams to the foams made from 100% petrochemical-based polyols [1-4]. However, there are some major drawbacks of commercial soy-based polyols:

- To produce the polyols, there are multiple steps of chemical reactions which increase production costs and utilize lots of chemicals and solvents.

- Presenting hydroxy groups are secondary alcohol which has less reactivity compared to primary alcohol [5].

- The polyols contain saturated fatty acid moieties (15% of saturated fatty acids in original soybean oil). These unreactive parts were reported to cause dangling parts after the polymerization and poor polyurethane properties [1, 4].

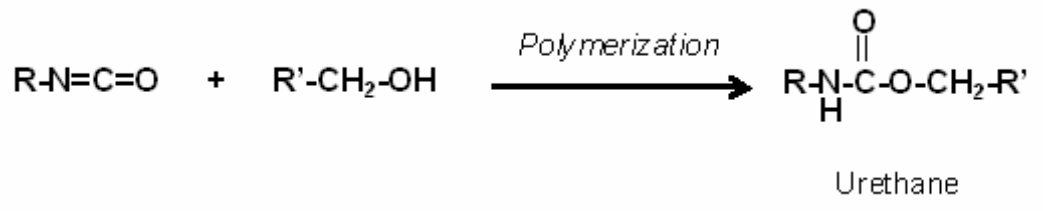
The ultimate goal of this study is to use enzymes to produce improved soy-based polyols replacing petrochemical-based polyols to meet “green chemistry” and “sustainability” goals of urethane consumers.

1.2. POLYURETHANES

The amount of polyurethanes (PUs) produced in the North America was 6.5 billion lbs for 2002; of this amount 5.5 billion lbs were manufactured in the US, which is the world's largest end-user for the materials [6].

PUs in foam form are applied to furniture & bedding, automotive, construction, electrical appliances, recreational equipment, packaging, and miscellaneous products [7]. Among these applications, the PUs can be classified into two major groups; flexible PU foams and rigid PU foams. Flexible PUs are about 62% or higher of the current PUs market and primarily used for cushioning; rigid PUs are used mostly for thermal insulation [8, 9].

PUs are polymers containing urethane functional groups synthesized from the reactions of isocyanates (A-side materials) and polyols (B-side materials). The polyols are compounds containing active hydrogens (typically alcohol or hydroxy functional groups). Because of the large varieties of A-side and B-side materials, PUs could have aliphatic and aromatic hydrocarbons, esters, ethers, amides, urea, and isocyanurate groups [10]. An example of A-side and B-side materials in the polyurethane synthesis is shown by the following typical reaction:



1.3. POLYOLS IN POLYURETHANE SYNTHESIS

In PU synthesis, polyols, or B-side materials, are molecules having at least two isocyanate-reacting moieties [10]. In this study, the isocyanate-reacting groups mostly refer to hydroxy functional groups. There are a number of these polyols available in the market (Figure 1 and Figure 2), namely, polybutadiene, polytetramethylene ether glycol, polypropylene oxide glycol, polybutylene oxide glycol, soy-based polyols, castor oil, etc. Among the commercially available polyols, they can be divided into two groups; petrochemical based-polyols and vegetable oil-based polyols.

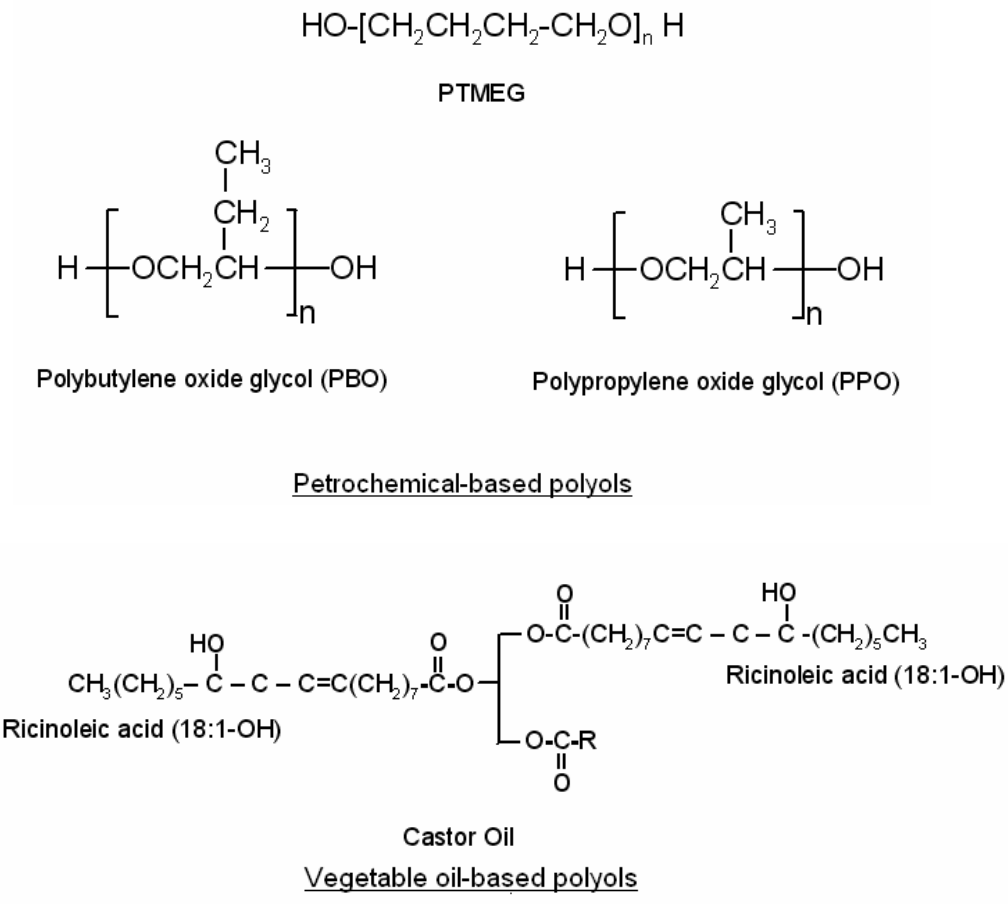


Figure 1. Commercially available polyols.

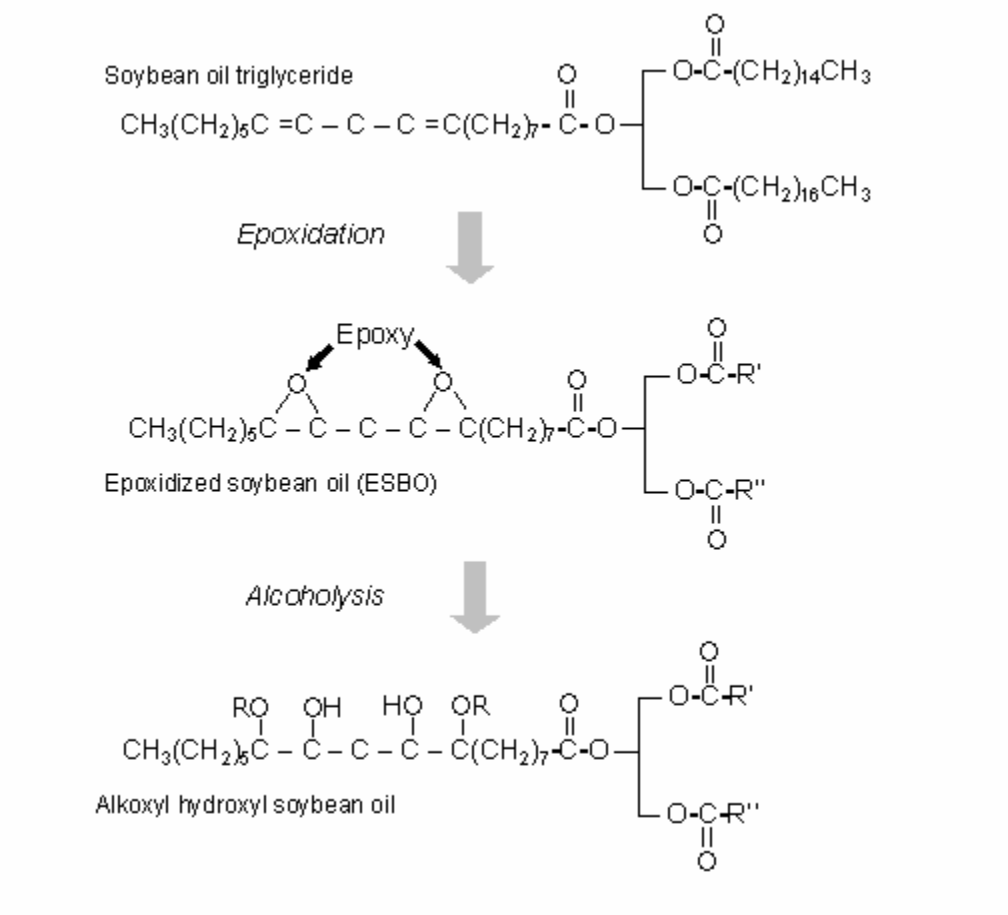


Figure 2. Typically chemistry method to produce alkoxyl hydroxyl soybean oil, an example of commercially available soy-based polyols.

1.3.1. Properties of polyols

There are several physical and chemical properties of polyols; namely, hydroxyl number, acid number, molecular weight and functionality. The properties have significant effects on the characteristic of finished foams. A summery of properties of commercially available polyols used in the different PU applications is presented in Table 1 [10].

Polyol's properties are empirically defined as follows:

Hydroxyl number (mg KOH/g) is the number of milligrams of potassium hydroxide that is equivalent the amount of hydroxy functional group in one gram of sample [10]. The standard testing method of ASTM D4274 (Polyurethane Raw Materials: Determination of Hydroxyl Numbers of Polyols) is usually used to measure the hydroxyl number of polyols used in Polyurethanes.

Hydroxyl equivalent weight is "the number of grams of sample required so that one equivalent weight (17.008 g) of hydroxyl will be present in the sample" [10]. The hydroxyl equivalent weight can be calculated from the hydroxyl number which is expressed in the following equation:

$$\text{Hydroxyl equivalent weight of polyols} = \frac{56.1 \text{ x } 1000}{\text{Hydroxyl number}}$$

Functionality is the number of functional groups reacting to isocyanate per molecule of the functional polyols. The reactive groups could be hydroxy groups, epoxy groups, hydroperoxy groups or amine groups.

Acid number (mg KOH/g) is the number of milligrams of potassium hydroxide required to neutralize the acid present in one gram of a sample of the amount of acid residual material in polyols [10]. The number expressed amount of acidic residue material in polyols, the acid number, is very low, less than 10 (mg KOH/g) for the commercially available polyols.

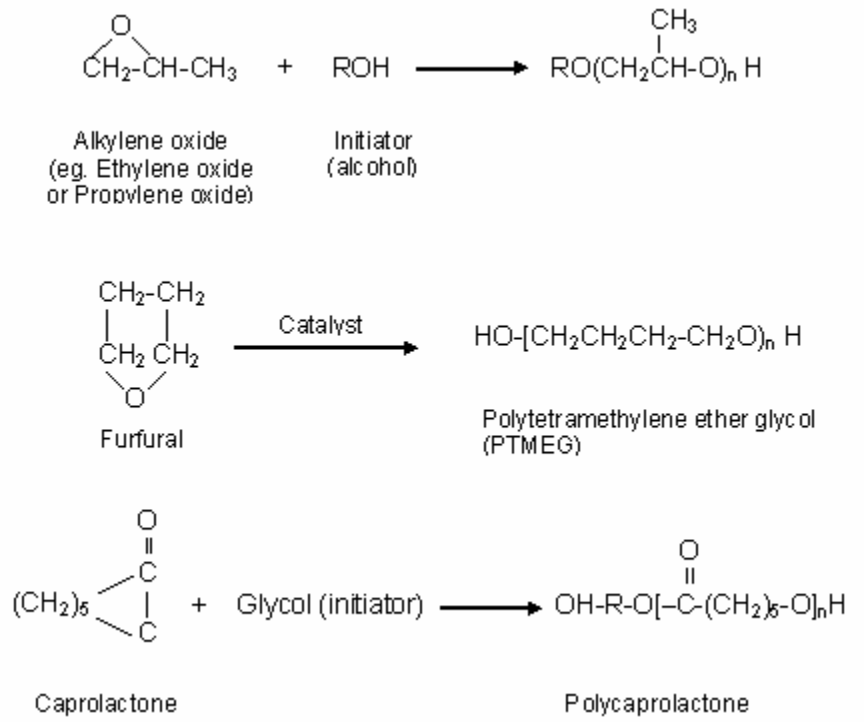
Table 1. Properties of commercially available polyols [10].

Properties	Flexible Foams	Rigid Foams
Molecular weight (Daltons)	1,000-6,500	150-1,000
Functionality	2.0-3.0	3.0-8.0
Hydroxy number (mg KOH/g)	28-160	250-1,000

1.3.2. Petrochemical-based polyols

In 1994, the most consumed polyols for PUs in the US were polyethers (90%) where polyesters (9%) and other specific polyols (1%) also shared the market [10]. There are numbers of categories of commercially available polyols including polyether polyols, polyester polyols and polycarbonates.

Most of widely-used commercial polyols have terminated hydroxy functional groups, or primary alcohol. This is because the primary hydroxy group in polyols is three times more reactive with isocyanate in polyurethane synthesis than secondary hydroxy groups [10]. A few traditional chemical reactions producing petrochemical-based polyols with primary hydroxy groups are displayed by the following reactions:



The petrochemical-based polyol prices are varied and depend directly on the price of petroleum. Recently, polyol prices quickly increase and tend to be more expensive because of global energy crisis. In addition, using the petrochemical based products increases CO_2 production which is claimed to noticeably cause global warming. The alternative products, renewable and sustainable materials, have been importantly developed.

1.3.3. Soy-based polyols

Polyols derived from soybean oil, or soy-based polyols, have been developed and marketable. The soy-based polyols are bio-based products which are inherently sustainable, renewable and biodegradable. Soy-based polyol used in polyurethane (PU) applications are reported to require less energy to

produce and to have lower market price by 20-30% [11]. The bio-based material costs do not depend on the petroleum's price as much as the petrochemical-based polyols.

Commercial soy-based polyols are usually derived from epoxy soybean oil. For example, a widely used soy-based polyols is alkoxyl hydroxyl soybean oil which has triglyceride backbone and is produced from alcoholysis of the epoxidized soybean oil, see Figure 2.

Urethane Soy Systems Company [12] is producing two main groups of the soy-based polyols for PUs. One group is a two functional (R2 family) and the others are three functional (R3 family) polyols. The hydroxyl number are 50-60 and 160-180, respectively. The Urethane Soy Systems polyol products are commercialized under the tradename of Soyol™.

Cognis Oleochemicals [13] are providing Sovermol® polyols to the market that some of their products are plant-based fatty alcohols. Sovermol 1068 is made from soybean oil triglyceride as shown in Figure 2. The produce has a hydroxyl number of about 190 and an equivalent weight of about 280.

Cargill Inc. recently announced a program to develop and commercialize new soy-based polyols [14]. An interesting product from Cargill is "blown soybean oil" used in lubricants, coating and painting applications. Blown soybean oil could be an inexpensive soy-based polyols, but the functionalities produced are not mainly hydroxy groups. In the production of blown soybean oil, oxidation and polymerization randomly takes place with minimal control, and oxygen could be an expensive oxidizing agent.

1.3.4. Polyurethanes from soy-based polyols.

Currently, development of soy-based polyol technology is being encouraged. Among the most active programs in the U.S. academic research programs on the conversion of soybean oil to polymers are the programs of Drs. J. Massingill (Texas State University), R. P. Wool (University of Delaware), Z. Petrović (Pittsburg State University), R. Larock (Iowa State University), and G. Suppes/F. Hsieh (University of Missouri-Columbia). Others (e.g. Dr. G. Wilkes, Virginia Tech. University) specialize in formulating urethanes and less on conversion of soybean oil. Several companies are also performing R&D on soy-based polymers, including Urethane Soy Systems, The Dow Chemical Company, and BioBased Chemicals, LLC.

U.S. Patents 6,399,698, 6,686,435, 6,624,244, 6,573,354, 6,548,609, 6,476,244, 6,465,569, 6,433,121, 6,107,433, 5,932,336, 5,674,802, 5,482,980, 4,220,569, and 4,025,477 are on soy-based polymers and primarily soy-based urethanes.

1.3.5. Use of enzymes to produce soy-based polyols.

None of the existing commercial soy-based polyols are made by enzyme technology. This is the first time that multiple routes of enzyme reactions are studied to have high yield and selectivity in production of soy-based polyols in ways not possible with conventional chemistry. In addition, enzyme reactions are green technology usually consuming less energy and fewer toxic chemicals.

In this work, Chapter 2 describes how lipoxygenase (LOX) attaches hydrogenperoxide functional groups to soy-based materials, including fatty acids and diglyceride derived from soybean oil, and soybean oil triglyceride. The lipase catalyzes the peroxidation reaction of the soy-based materials in buffer solution with the presence of oxygen gas.

Chapter 3 presents commercially available immobilized lipase (Novozyme 435®) to attach epoxy functional groups to soy-based diglyceride, soybean oil triglyceride, bodied soybean oil and blown soybean oil. The epoxidation reaction was operated in (1) a well-mixed reactor and (2) a packed bed reactor when hydrogenperoxide is an oxygen source.

Hydroxy functional groups can be derived from the hydroperoxy and epoxy groups (from Chapter 2 and 3) by chemical methods. However, the hydroperoxy and epoxy also have reactivity with isocyanates and form urethane functional groups. Products from Chapter 2 and 3 could be directly used in the polyurethane synthesis, or further modified to synthesize the better polyols.

Chapter 4 describes use of commercial lipases to hydrolyze epoxidized soybean oil triglyceride where only buffer solution is needed. The epoxidized soybean oil (from Chapter 3) can be hydrolyzed which both selectively removes saturated fatty acids and produces hydroxy groups. Commercially available lipases were investigated with respect to the selectivities toward the saturated fatty acids in the epoxidized soybean oil. The products from the hydrolysis of epoxidized soybean oil have both epoxy and hydroxy functional groups.

The products from Chapter 2, 3 and 4 have high hydroxyl number, high functionality and low molecular weight that are suitable in the synthesis of rigid polyurethanes.

Chapter 5 is similar to Chapter 4. However, the starting material in Chapter 5 is bodied soybean oil which is cheap soy-based oligomers and does not have any epoxy functional groups. Enzyme hydrolysis is applied to cleave off fatty acid, preferably saturated fatty acids, and produce high hydroxy equivalent weight polyols. The hydrolyzed bodied soybean oil has high molecular weight and proper functionality for used in the synthesis of flexible polyurethane foam.

High hydroxy equivalent polyols are also synthesized by the cleavage of acid moieties derived from vegetable oils. High MW acid moieties are produced by two different enzyme routes, hydrolysis and esterification, which are studied in Chapter 6.

The enzyme approaches used in this study are believed, in some applications, to produce better performing polymers. In addition, the reactions are simple and operated at mild operating conditions. Chapter 7 includes a discussion of polyol products produced from different enzyme approaches, a discussion of the advantages of using enzymes, and recommendations for future work.

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CHAPTER 2

SOY-BASED HYDROPEROXY POLYOLS FROM ENZYMATIC PEROXIDATION OF LIPOXYGENASE

2.1. INTRODUCTION

Many studies successfully used soy-based polyols containing hydroxy functional groups as B-side materials in polyurethane synthesis [1-3]. There is no previous work investigating polyurethane foam from hydroperoxy soy-based materials but there are some formulations of polyurethane using the free radical of hydroperoxy compounds as the polymerization initiators [4]. Moreover, hydroperoxy functional groups can be further converted to hydroxy functional groups or epoxy functional groups by chemistry methods [5].

Reactive hydroperoxy octadecanoic acyl moieties can be produced from enzymatic peroxidation of unsaturated fatty acids from vegetable oils by lipoxygenase (LOX) [6-7]. Figure 3 illustrates hydroperoxidation of linoleic acid (C18:2) and linolenic acid (C18:3) by LOX. The enzymatic reaction moves one carbon double bonds and produces a hydroperoxy functional group.

The preferred location of the hydroperoxy group is toward the end of the fatty acid chain (away from carbonyl) which helps reduce the dangling branch in the urethane after polymerization reactions. Therefore, the enzyme reaction was controlled at pH 9.0-10.0 where lipase selectively yields 13-HPODE from linoleic acid or 13-HPOTE from linolenic acid [7].

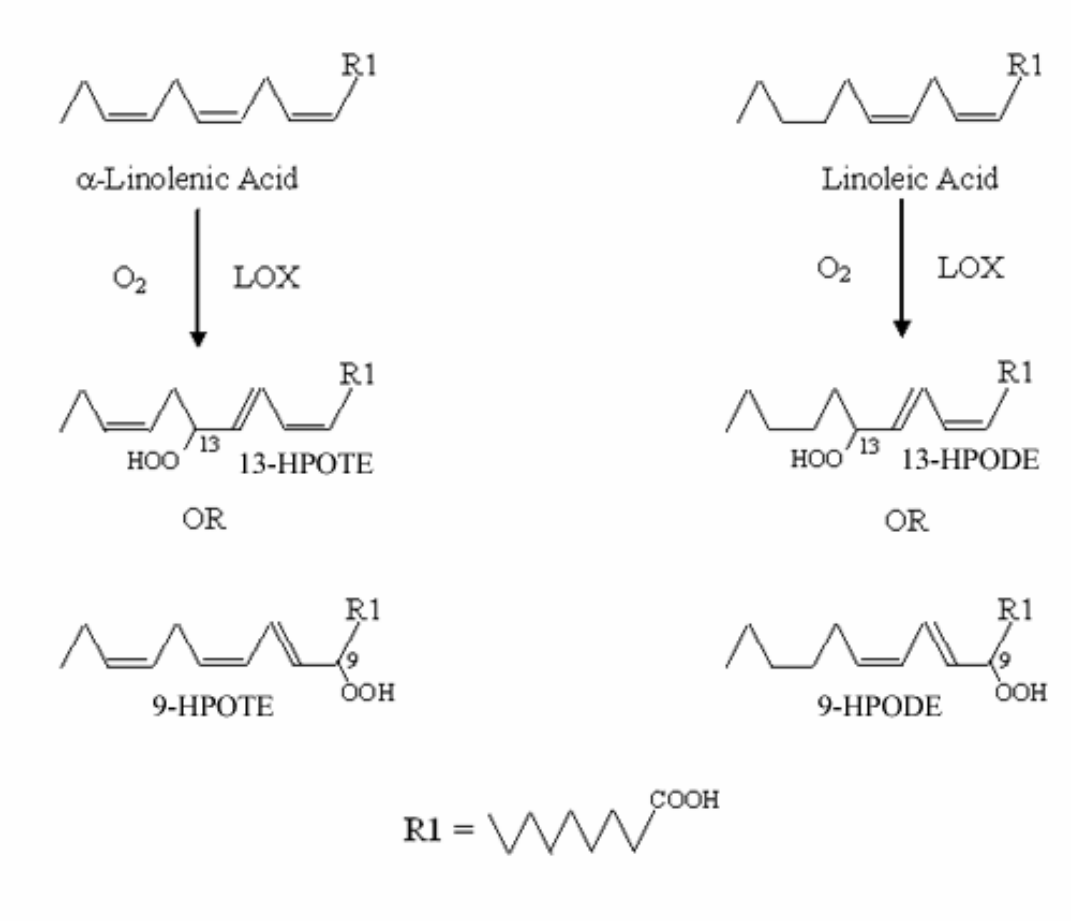


Figure 3. Production of hydroperoxy octadecadienoic acid (HPODE) from linoleic acid and hydroperoxy octadecatrienoic acid (HPOTE) from linolenic acid by LOX.

The production of HPODE from linoleic acid, major fatty acid moiety in soybean oil triglyceride (56%), is the initial step of this study. However, the hydroperoxy fatty acids are not the targeted B-side material in the polymerization of polyurethane. This is because the acid functional groups undesirably consume isocyanates and catalysts, resulting in reduced foaming production rate

[8]. In addition, the low equivalent weight of the fatty acids could create an expensive foaming formulation.

To obtain the higher equivalent weight of hydroperoxy B-side material, diglyceride and triglyceride of soybean oil are starting materials in the enzyme peroxidation. The hydroperoxy products from soy-based glyceride are proposed to be used specifically in the synthesis of rigid polyurethane foam.

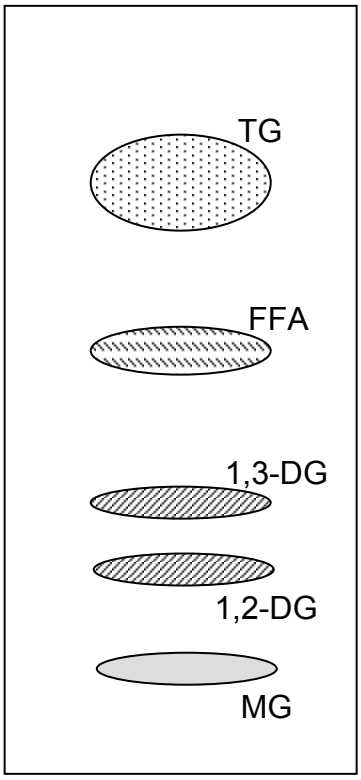
2.2. METHODS

2.2.1. Production of Soy-based diglycerides

Soy-based diglyceride is produced by transesterification between soybean oil and glycerin (2:3 by mole ratio) with 0.5 % of solid NaOH (catalyst) in a closed-well-mixed reactor. The reaction takes place at 120ºC for 24 h.

Transesterified products are analyzed by the simple method of thin layer chromatography (TLC) which is developed in the mixture of hexane/diethyl ether/acetic acid (70/30/1, v/v) [9]. Firstly, a sample is spotted on a silica plate and the TLC plate is developed in the pervious mixture. After that, primuline solution (5 mg primuline in 100 mL of acetone/water, 80/20, v/v) is sprayed on the plate. The plate is dried out at room temperature and it is incubated in the environment of iodine vapor. Sample spots are then visualized.

Relative order of components on TLC are unchanged although R_f values may be slightly different than displayed in Figure 4.



TG,Tri-glyceride; DG,Di-glyceride;

MG,Mono-glyceride; FFA,Free fatty acid

Figure 4. Migrations of acyl fatty acid and acyl glycerides on TLC plate.

2.2.2. Synthesis of vegetable oil-based hydroperoxy materials by LOX

Soybean lipoxygenase (Type I-B) is purchased from Sigma Aldrich (St. Louis, MO). Linoleic acid (90%) is purchased from City Chemical LLC, (West Heaven, CT). Refined soybean oil (Food Club brand vegetable oil) is purchased from a local grocery store. Commercial soy-based diglyceride (ENOVA^{TM}) is purchased from Archer Daniels Midland Company, ADM (Decatur, IL).

Commercial sodium tetraborate (pH=9.0) is purchased from Sigma Aldrich (St. Louis, MO). Borate buffer prepared in laboratory is also easily made at two concentrations, 0.1 M and 0.2 M.

To prepare 0.2 M of borate buffer (pH ~ 9.2), 200 mmol boric acid and 100 mmol NaOH are combined and brought up to 1 liter with distilled water. For 0.1 M borate buffer (pH ~ 9.2), the 0.2 M buffer is diluted with distilled water.

a) Peroxidation of linoleic acid

The oxidation reactions of linoleic acid to produce HPODE are done in an open system and a closed system. Linoleic acid (90%) and 20 ml of borate buffer are mixed in a cylinder reactor. LOX (1.4 mg) is added into the reaction mixture when the reaction starts.

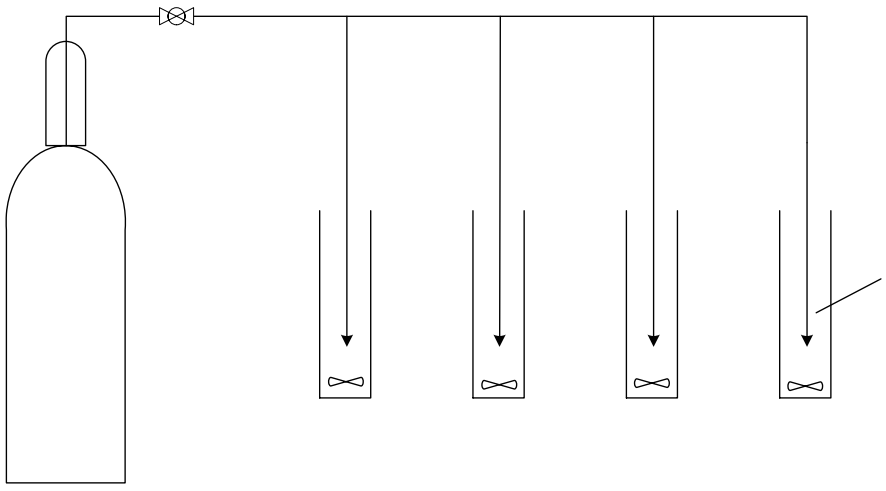


Figure 5. Production of soy-based hydroperoxy materials by LOX in an open reactor (batch operation).

Effects of reaction medium, solvent, substrate concentration and temperature are studied. In the study of temperature effect, the reaction at low temperature is simply carried out in the ice bath where the temperature is varied from 2-7 ^{o}C during the reaction.

Production of HPODE in an open system is illustrated in Figure 5. Oxygen gas is blown over the mixture's surface to avoid foam formation during the reaction. Magnetic stirrer bar is used for making the reaction medium well-mixed and thoroughly saturated with oxygen.

A blank, or control experiment, is also performed concurrently with no enzyme usage. Sampling is done with respect to time to measure hydroperoxide concentration during the reaction.

In closed systems, the reaction is performed in a static pressure reactor. Linoleic acid (1 % wt.), enzyme (4 mg) and 0.2 M borate buffer (8 ml) are mixed in a 10 ml stainless steel reactor. Oxygen gas is loaded in the closed reactor with a pressure of 50 or 100 psi. A sand shaker is set up to hold the reactors and to continuously shake the reactor at room temperature.

b) Peroxidation of diglyceride and triglyceride of soybean oil

The peroxidation of soy-based glycerides is carried out in the open system. There are two sources of soy-based diglycerides in this study, soy-based diglyceride from the transesterifcation between soybean oil and glycerin, and commercial soy-based diglyceride (ENOVA^{TM}).

Glyceride substrate (1% by wt.), either soy-based diglyceride or soybean oil triglyceride, is added in 20 mL of borate buffer (0.1 M) and saturated with oxygen at room temperature (Figure 5). Lipoxygenase (1.4 mg) is added when the reaction starts. Neither surfactant nor solvent is used.

Deoxycolate is used to investigate the effect of the free carboxyl functional group. Sodium deoxycolate (100 mM) is dissolved in 0.1 M borate buffer and the reaction is performed with the commercial diglyceride (1% wt.)

2.2.3. Method of ferrous oxidation in xylenol orange

The ferrous oxidation in xylenol orange, or FOX, method is an analytical method with qualitative and quantitative abilities [10].

Ferrous oxidation in xylenol orange (FOX) is a sensitive method modified to measure hydroperoxide concentration of lipid derivatives [10]. The mechanisms are proposed and shown by the following reactions [11].

Firstly, peroxide oxidizes ferrous ions in the xylenol orange reagent,

$$Fe^{2+} + H_2O_2 \leftrightarrow Fe^{3+} + OH + OH^- \quad (1)$$

After that, with an excess of xylenol orange (XO), most of OH radicals produced from the oxidation of Fe^{2+} react with the XO,

$$XO + OH \leftrightarrow XO{\cdot}OH \quad (2)$$

$$Fe^{2+} + XO{\cdot}OH \leftrightarrow Fe^{3+} + (XO{\cdot}OH^-) \quad (3)$$

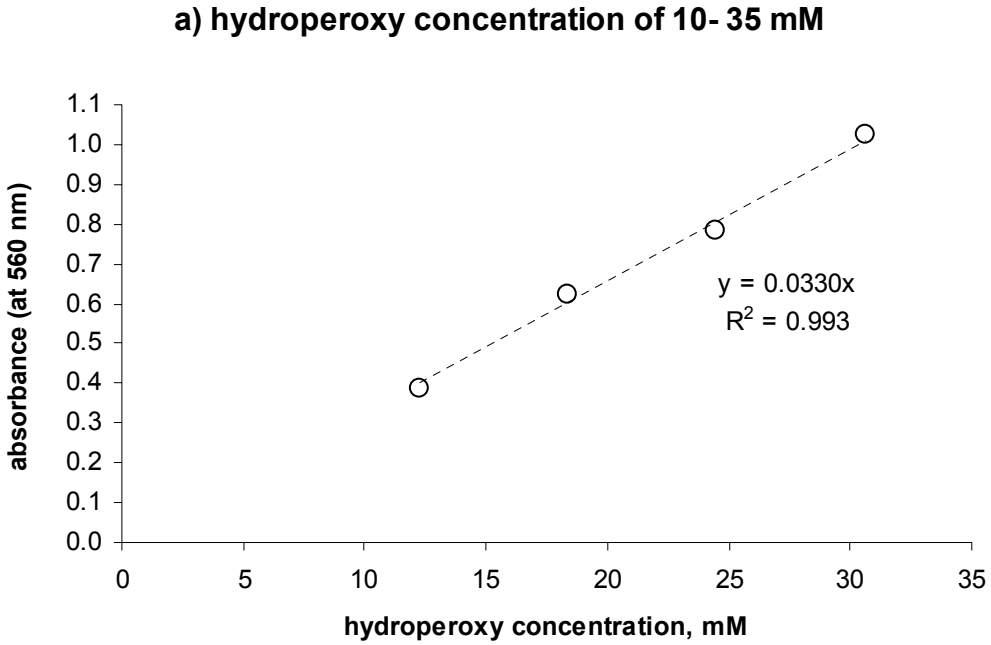
Fe^{3+} forms a chromophore with xylenol orange, which can be quantitatively measured at 560 nm by UV spectrophotometer.

Calibration curves of hydroperoxide are generated by using cumene hydroperoxide as the standard. The FOX assay is a solution containing 100 μM of xylenol orange, 250 μM ammonium ferrous sulfate, 25 mM of H_2SO_4 and 4 μM of the antioxidant butylated hydroxytoluene (BHT). All three compounds are diluted in 90% (V/V) methanol (HPLC grade).

Fresh dye reagent (FOX reagent) is prepared just before use to maintain high sensitivity of the analytical method. The fresh reagent's color is yellow and it turns brown within a day. It is also observed that higher sensitivity can be obtained when sulfuric acid is added in the solution before the xylenol orange; otherwise, the reagent's color will be dark blue.

In order to control absorbance in a range of 0.1-1.0, hydroperoxy samples are diluted with HPLC-methanol before being added into the dye reagent. The mixture is incubated at room temperature (~22 ^{o}C) for 45 min to let the FOX reagent react with hydroproxy functional groups. Ultraviolet spectroscopy (at 560 nm) is used for the analytical method.

Figure 6 shows calibration curves made for samples having hydroperoxiy concentrations from 10 to 35 mM (Figure 6(a)) and from 100 to 500 mM (Figure 6(b)).



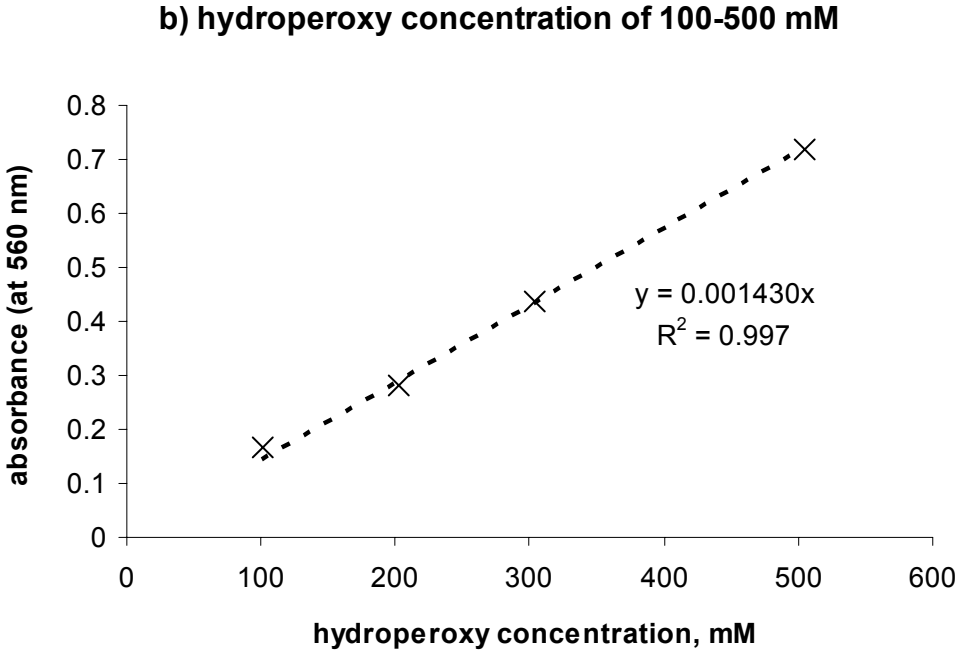


Figure 6. Examples of calibration curves made from FOX method of cumene hydroperoxide.

To control the range of absorbance values, in Figure 6(a), 40 μl of samples are diluted in 0.8 ml methanol, and then 40 μl of the diluted sample is mixed with 160 μl of fresh dye reagent.

Data in Figure 6(b) is created from diluting 40 μl of samples in 2 ml methanol, and then 100 μl of the diluted sample is mixed with 100 μl of fresh dye reagent.

2.3. RESULTS AND DISCUSSION

2.3.1. Production of hydroperoxy decadienoic acid from linoleic acid by LOX

a) Effect of substrate concentration

The effect of substrate concentration to LOX's activity is investigated in an open system and at room temperature. Figure 7 shows the transformation of HPODE from linoleic acid with respect to time when the starting concentration of linoleic acid was varied from 0.3% to 1.5% by wt.

High transformation yields are obtained, >70% (2 h) and >80% (3 h) when the substrate concentration is lower than 1% by wt. The transformation decreases when the substrate concentration is >1.0%.

The results in Figure 7 correspond to Fauconnier and Marlie [12], where it is reported that a reduction of transformation yield occurs when substrate concentration increases. In addition, the reaction yield can not be increased by slowly adding fresh substrate or increasing amount of the lipase.

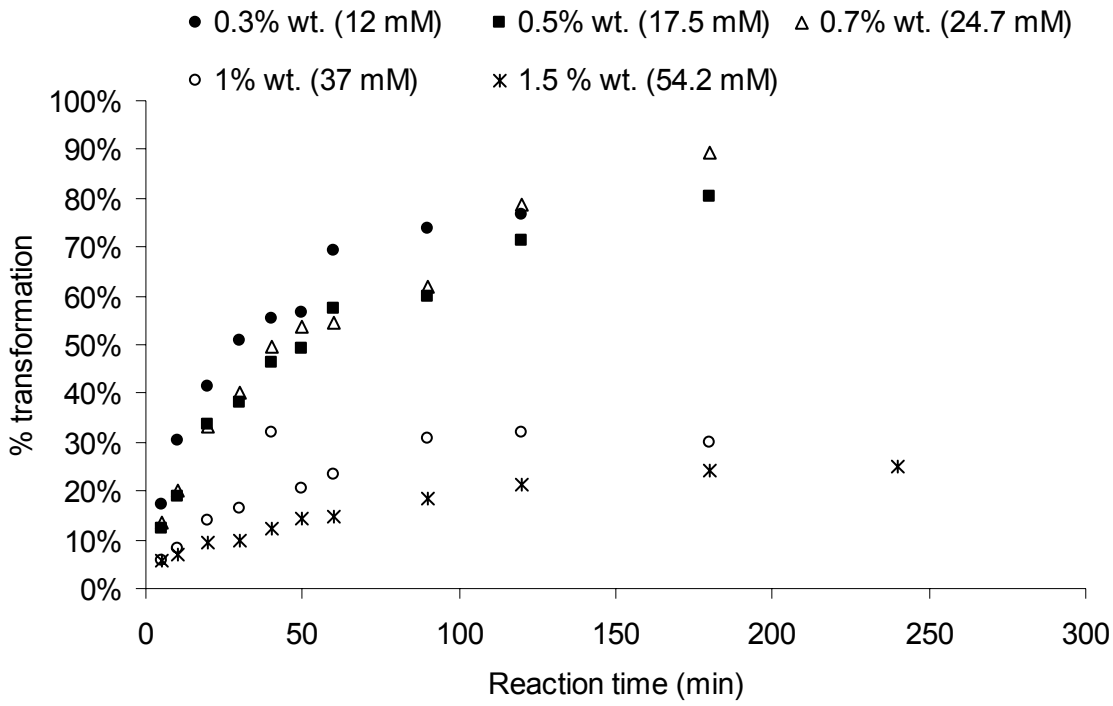


Figure 7. Effect of substrate concentration on peroxidation of linoleic acid (at room temperature and in an open system).

At high substrate concentrations, the aggregation of fatty acids can happen and the enzyme-substrate binding process can be limited. Microfluidizer is later used in the previous study [12] in order to homogenize the fatty acid substrate before the reaction is carried out; consequently, the reaction yield is improved.

b) Effect of solvent

Reaction yields in aqueous buffer are limited when fatty acid is higher than 1% (described previously). Organic solvent is applied to create a biphase

system and improve the distribution of linoleic acid in the buffer phase. Ethanol and isooctane are selected for this study to increase substrate solubility in the aqueous phase.

Figure 8 shows the transformation percentages during the peroxidation reaction of linoleic acid (0.3% wt.) when 0.2 M borate buffer (90%) and ethanol (10%) are applied.

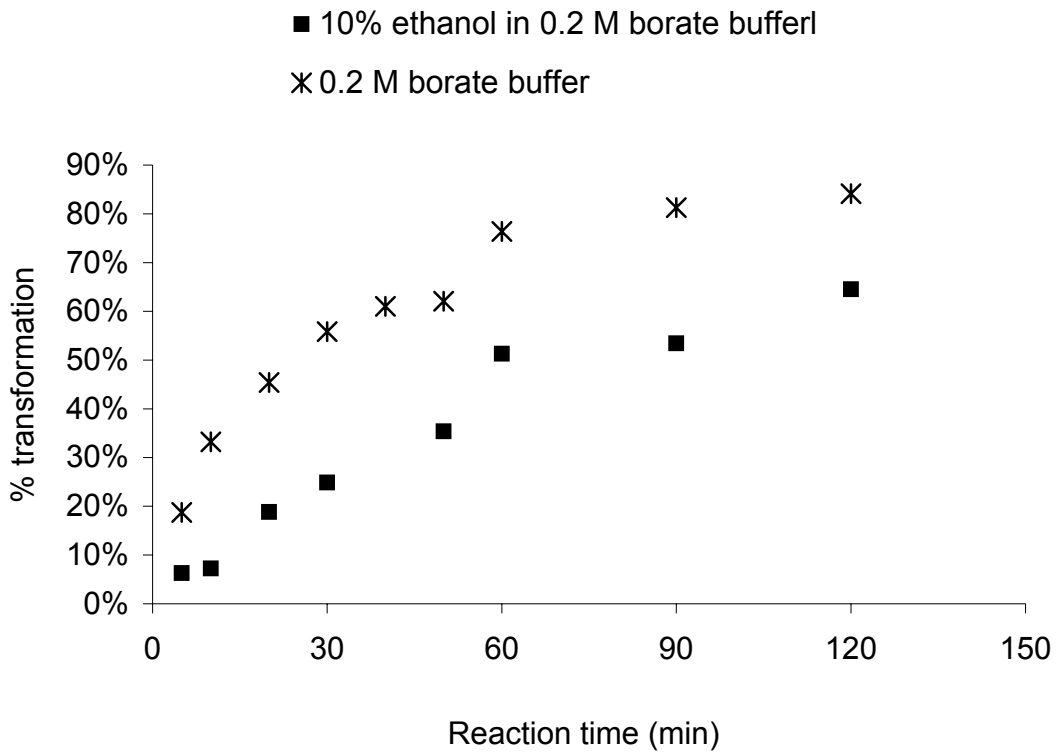


Figure 8. Effect of ethanol on peroxidation of linoleic acid (0.3% wt. substrate, at room temperature and in an open system).

Moreover, the transformation percentages of biphase systems in a combination of iso-octane and 0.2 M borate buffer are reported in Figure 9. Ratio

of 0.2 M borate buffer to iso-ocatane are 8:1 and 16:1 by volume and the starting substrate is 1% by wt.

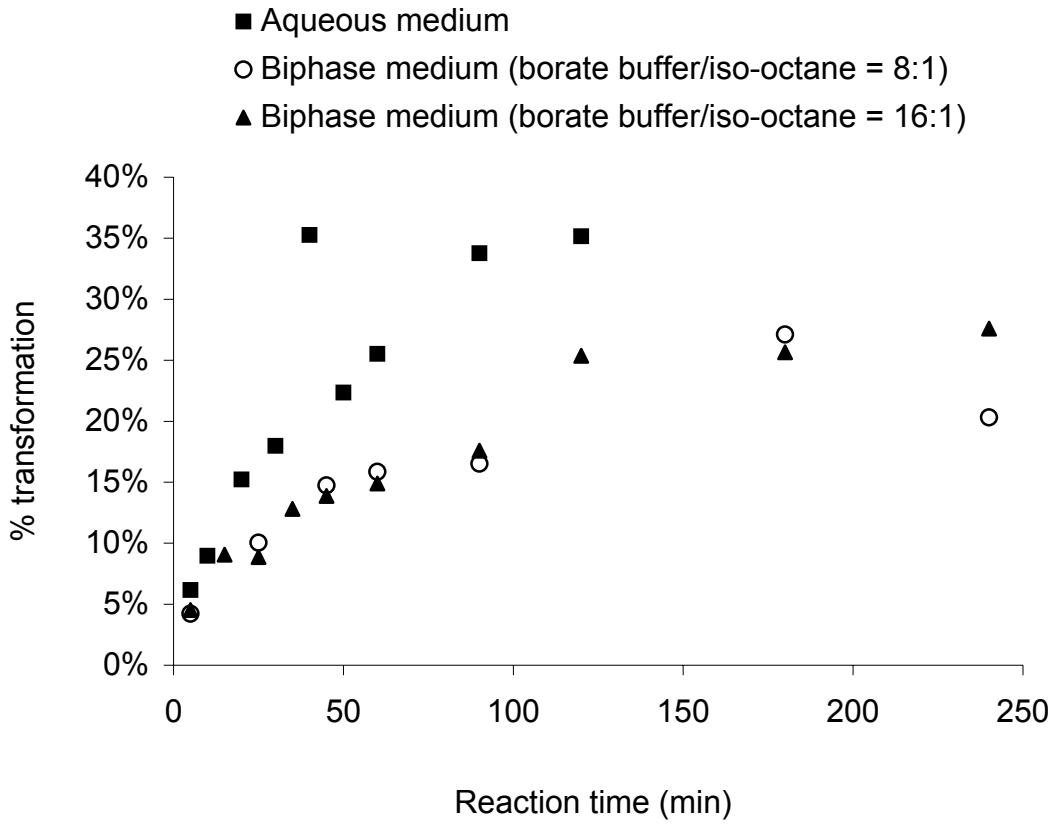


Figure 9. Effect of iso-octane on peroxidation of linoleic acid (1% wt. substrate, at room temperature and in an open system).

It is observed that both ethanol and iso-octane decrease the reaction yields. The solvents possibly alter the enzyme's conformation and active site which cause the reduction of reaction yield. On the other hand, the enzyme and its activity are ruined by the solvent resulting in less reaction conversion.

c) Effect of temperature

Reaction temperature is varied to investigate the effect of temperature on the enzyme's activity.

Figure 10 shows the comparison between the transformation yields of reaction at room temperature and at lower temperature (2-7 ^{o}C). As a result, the conversion at lower temperature is higher than the one under room temperature (22 ^{o}C). The reactions quickly reached the maximum conversion (1 h). However, the experiment is carried out at a very low concentration of the fatty acid substrate.

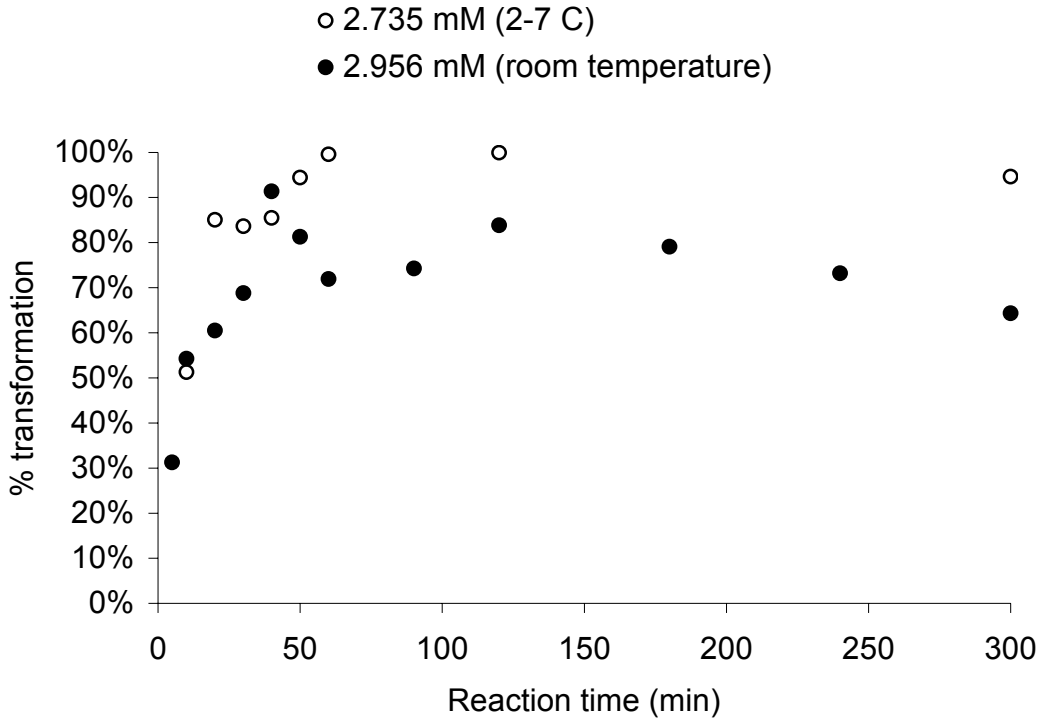


Figure 10. Effect of temperature on peroxidation of linoleic acid (<0.1% wt. substrate in an open system).

Data in Figure 10 and some of the previous graphs also show that the hydroperoxy product concentrations slightly decrease after their maximum yields are obtained. It is possible that hydroperoxy products can be oxidized and degraded by the oxygen gas.

d) Closed reactions in static pressure reactors

Figure 11 displays the reaction yields of the peroxidation of linoleic acid performed in static pressure reactors.

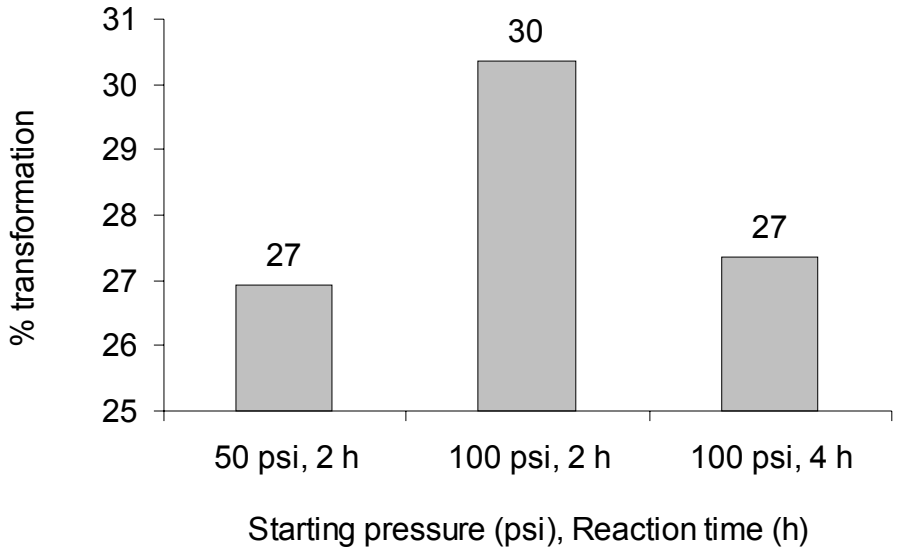


Figure 11. Peroxidation of linoleic acid in a closed system (1% wt. substrate, at room temperature).

The reaction conversions from the closed reactors are similar to results from the open system at room temperature and 1% linoleic acid. However, the

amount of enzyme used in the closed reaction is about 2.9 times the amount used in the open system.

During the reaction, pressure inside the reaction reduced from 50 to 10 psi, and from 100 to 20 psi. The closed system could reduce the consumption of oxygen and foaming problem. However, the static reactors should be further modified to be semi-batch operated where oxygen pressure is controlled.

a) Effect of reaction medium

A good reaction medium should have an appropriate range of pH to keep high enzyme activities. Purchased sodium tetra borate (pH 9.0) and sodium borate buffer (0.2 M, pH 9.2) made in the laboratory are investigated. The product transformations against time are shown in Figure 12.

The reaction in borate buffer (pH 9.2 and 0.2 M) yields higher reaction conversion compared to the purchased buffer (pH 9.0). Although they have different pH values, the difference is very small and the optimum pH for the lipase is known to be higher than 8.0.

However, these two buffers are made from different compounds. The buffer made in the laboratory is a combination of boric acid and sodium hydroxide while the purchase buffer is a combination of sodium tetra borate and hydrochloric acid. The chloride salts could alter enzyme activity.

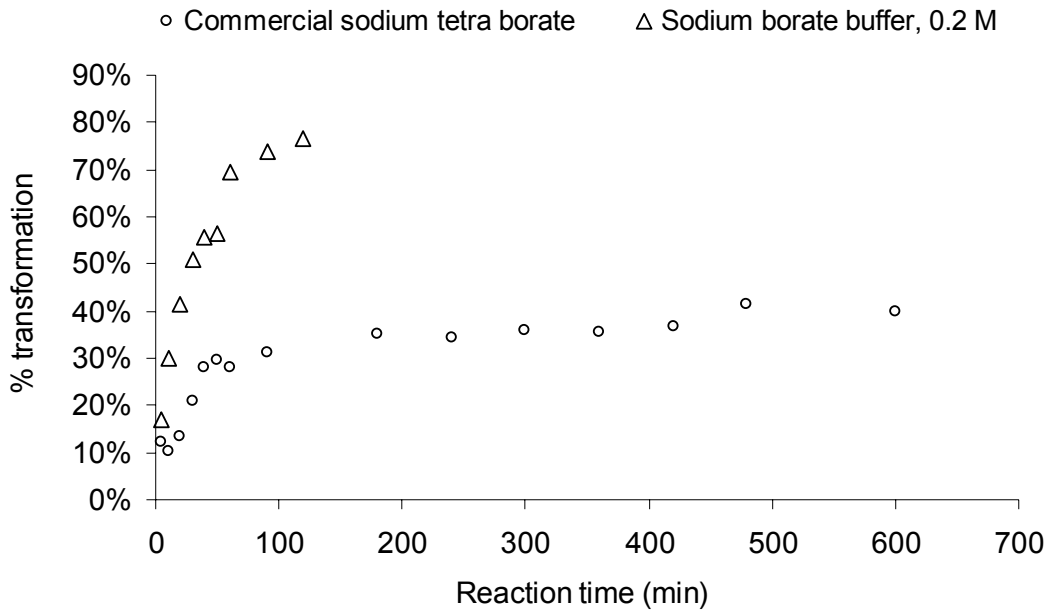


Figure 12. Effect of buffer on peroxidation of linoleic acid (0.3% wt. substrate, at room temperature and in an open system).

The reaction of linoleic acid is also studied in 0.1 M sodium borate buffer which is diluted from the 0.2 M buffer. The reaction performed in the dilute sodium borate buffer (0.1 M) yielded 75% transformation as showed in Figure 13. This is about two times higher the previous reaction performed at 0.2 M sodium borate buffer (Figure 12).

However, the previous studies, effects of temperature, substrate concentration, and solvent, are not re-performed in 0.1 M borate buffer. The production of HDPDE will lead more than one reaction step to produce the final polyol products which have high equivalent weight. Production of soy-based hydroperoxy glycerides are studied in 0.1 M borate buffer and presented in the following section.

2.3.2. Production of soy-based hydroperoxy glycerides by LOX

Soy-based diglyceride from the transesterification reaction is analyzed by TLC chromatography. From observation, the product contains about 80% of diglyceride molecules and it had significant amount of monoglyceride, triglyceride and fatty acid contents.

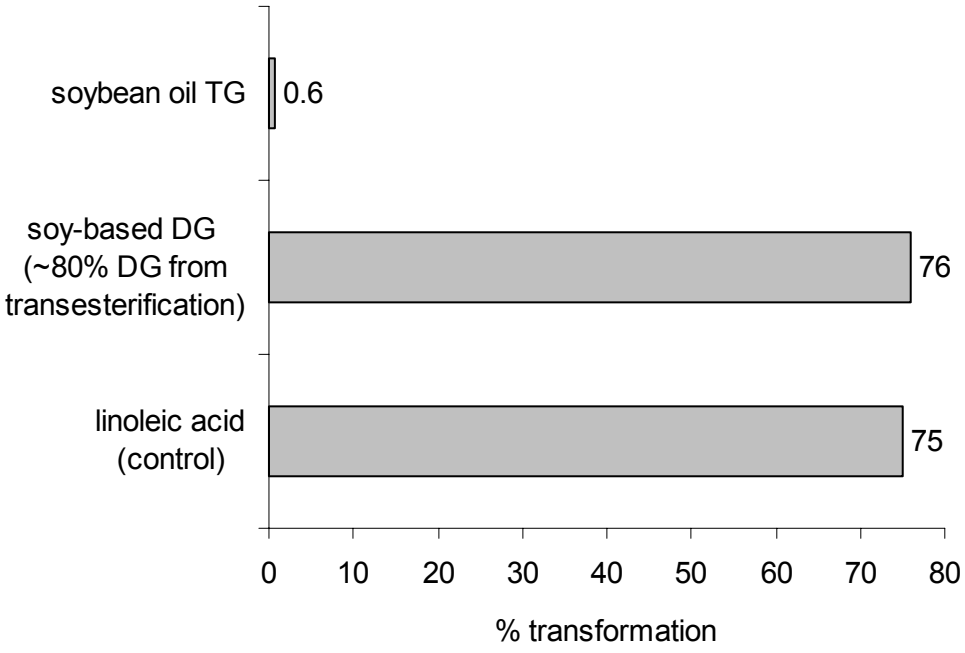


Figure 13. Percent transformation of the peroxidation of unsaturated fatty acid moieties in soy-based glycerides after 2 h (1% wt. substrate in 0.1 M sodium borate buffer, at room temperature and in an open system).

Figure 13 presents percent transformation of the peroxidation of unsaturated fatty acid in 80% soy-based diglyceride and in soybean oil

triglyceride (1% by wt. in 0.1 M borate buffer). And the control experiment is performed with linoleic acid (1% wt.) resulting in about 75% of transformation.

Soy-based diglyceride and soybean oil triglyceride contain about 85% unsaturated fatty acid moieties. After 2 h of the peroxidation, about 75% of the unsaturated fatty acid moieties in the 80% diglyceride are oxidized by LOX and the reaction.

Percent transformation of unsaturated fatty acid moieties in soybean oil triglyceride are very low and produced hydroperoxy triglyceride with less than 0.1 of hydroxy functionality.

The study by Piazza et al. [6] (micro molar scale) showed that relative substrate preference for free LOX was linoleic acid>1-monolinolein>1,3-dilinolein>trilinolein. The data in Figure 13 shows that the conversion of the 80% diglyceride substrate is higher compared to the previous study [6], even though the substrate concentration is a lot higher. This might be because our substrate is not pure diglyceride.

It is possible that hydrophobic groups of fatty acid, monoglyceride and 80% diglyceride could enhance substrate's solubility/dispersion in the buffer resulting in good reaction conversion comparable to the reaction of linoleic acid.

Reaction conversion from the peroxidation of commercial diglyceride is shown in Figure 14. The transformation percentage of the commercial diglyceride is extremely low (<3%) and is not comparable to the value from the previous study [6]. The substrate concentration in this study is higher by 400 times.

From observation, the reaction medium of the 80% diglyceride and the commercial diglyceride look different. The reaction medium of 80% diglyceride made from the transesterification is emulsion and there is foam formation during the reaction. The mixture with the commercial diglyceride is more readily separated into isolatable phases. Also, there is no foam formation; despite gas bubbling through a liquid phase.

a) Effect of free carboxyl group

From the previous results, the reaction conversion is improved when hydrophilic functional groups are present. Free carboxyl group from sodium deoxycolate is reported to improve reaction conversion of the peroxidation of linoleic moieties in the micro molar scale [6].

The effect of free carboxyl group on reaction conversion of the commercial diglyceride is reported in Figure 14 when the starting substrate is 0.6% wt. (21 mM). It found that the reaction conversion slightly increase with the presence of a free carboxylic functional group.

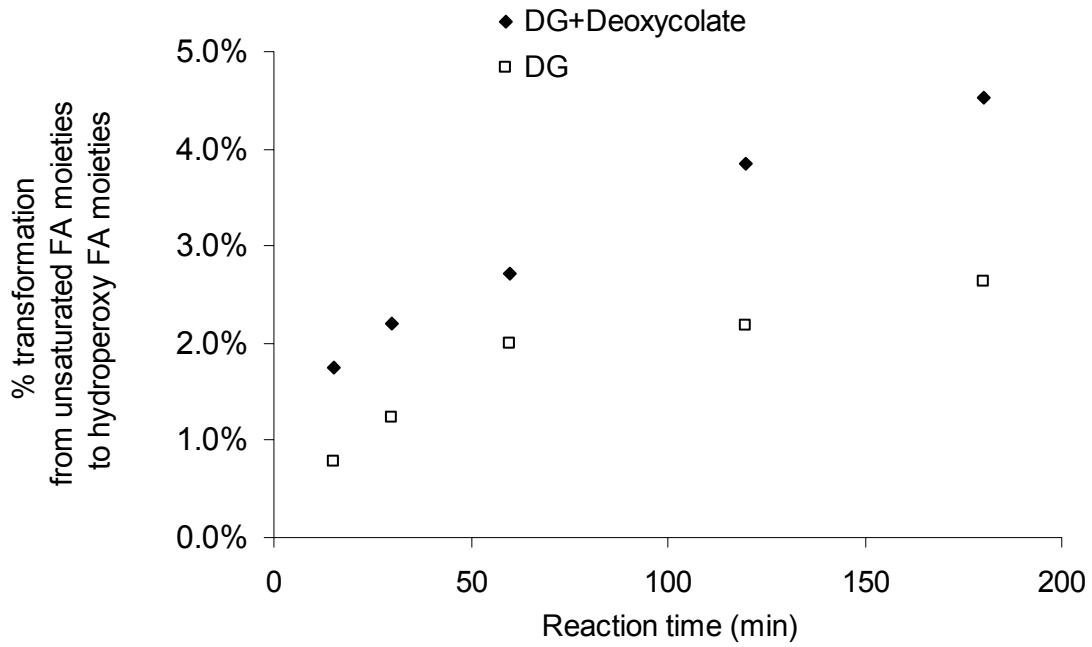


Figure 14. Effect of carboxyl group on percent transformation of the peroxidation of unsaturated fatty acid moieties in commercial soy-based diglyceride (0.6% wt. substrate in 0.1 M sodium borate buffer, at room temperature and in an open system).

2.4. CONCLUSIONS

It might be difficult to commercially develop soy-based hydroperoxy. The reaction conversion is low when substrate concentration (in buffer medium) is approximately greater than 1% by wt.

The aggregation of substrates, when substrate concentration increases, is resolved by using the surfactant and biphase reactions. However, the presence of chemicals and solvent seem to shorten the enzyme's life and activity.

Lipids/water interphase might play an important role as in many reactions involved with enzymes. Fatty acid, monoglyceride and diglyceride have hydrophilic functional groups which might increase the surface area of substrate-water interphase in the emulsion and the enzyme is usually active in the water phase. Percent transformation of triglyceride substrate was very low because the substrate is hydrophobic.

Additionally, the free carboxyl group from the salt additive could improve the reaction conversion by 50% in the study of commercial diglyceride.

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CHAPTER 3

EPOXIDIZED SOY-BASED MATERIALS FROM CHEMO-ENZYMATIC EPOXIDATION

3.1. INTRODUCTION

A well known example of epoxidized soy-based materials is epoxidized soybean oil (Figure 2). Epoxidized soybean oil is a derivative of soybean oil having oxirane (epoxy) functional groups which are produced from the epoxidation of carbon-carbon double bonds in soybean oil.

Epoxidized soybean oils are used as plasticizers, crosslinking agents, stabilizers and pre-polymers. They also are intermediates in the synthesis of soy-based polyols used in polyurethanes, polyesters and plastic resins. Figure 2 illustrates conventional methods to produce epoxidized soybean oil triglycerides from soybean oil triglycerides, and to produce a soy-based polyol from the epoxidized soybean oil [1].

Epoxidized soybean oil can be produced by either a chemical [2] method or an enzymatic method [3-5]. The conventional chemistry method could yield undesirable by-products, low conversion and selectivity, and the reaction is extremely exothermic.

Lipase B from *Candida antarctica* (Novozyme 435®) is immobilized lipase on acrylic resins distributed by Novozymes Inc. Novozyme 435® has been used to epoxidize unsaturated fatty acids moieties derived from plant oils [3-5] in which

carbon-carbon double bonds are oxidized and converted to epoxy functional groups. This enzyme reaction is called chemo-enzymatic epoxidation.

This study investigates chemo-enzymatic epoxidation of soy-based materials including soybean oil triglyceride, soy-based diglyceride, bodied soybean oil and blown soybean oil. The enzyme reaction produces epoxidized soy-based materials that have high functionality and are important materials in the polyol production. The epoxidized soy-based materials are renewable and biodegradable materials making them an attractive alternative to petrochemical based materials. This enzyme reaction also provides a few advantages over the traditional chemistry method which will be described shortly.

3.1.1. Epoxidation of soybean oil triglycerides

Figure 15 describes chemo-enzymatic epoxidation to produce epoxidized soybean oil triglycerides from soybean oil triglycerides [3]. The reaction has some advantages over chemical route:

(1) Instead of using expensive and toxic formic acid, stearic acid or any other plant oil derived fatty acids can be used to form peracid. The peracid will deliver oxygen to oxidize carbon-carbon double bonds in soybean oil triglyceride.

(2) The chemical route in Figure 2 is an extremely exothermic reaction, and usually produces by-products from an epoxy ring opening when an acid and heat present. The enzymatic epoxidation of soybean oil provides high reaction yield and selectivity at room temperature.

(3) The enzyme used (Novozyme 435®) is commercially available in immobilized form which can be recycled under suitable operating conditions.

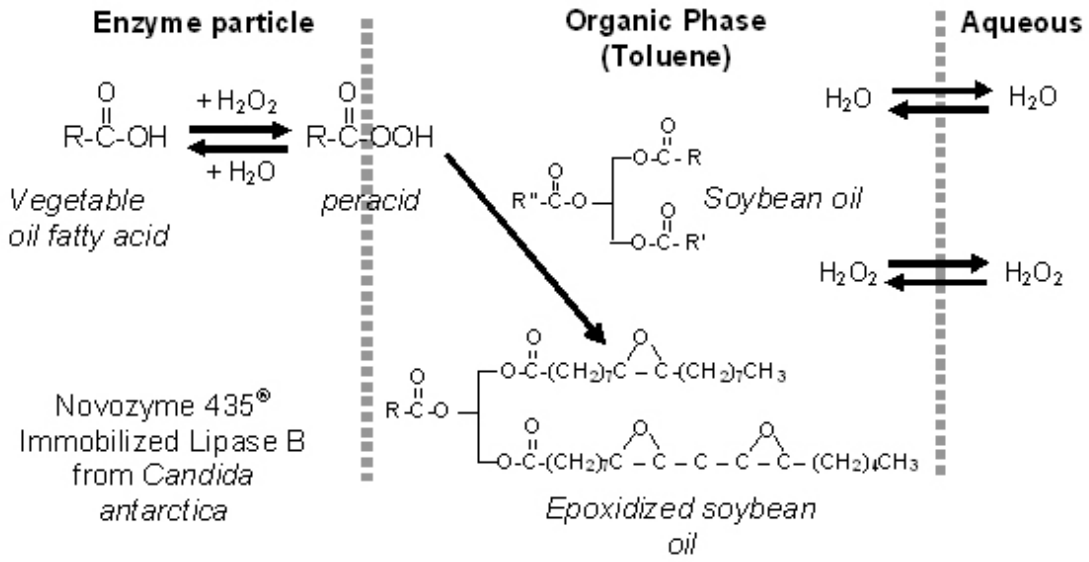

Figure 15. Reaction scheme of the chemo-enzymatic epoxidation of soybean oil by lipase B from *Candida antarctica* (Novozyme 435®).

Two reactor models, well-mixed reactor and packed-bed reactor (PBR), were utilized to produce epoxidized soybean oil by the chemo-enzymatic epoxidation.

3.1.2. Epoxidation of other soy-based materials; blown soybean oil, bodied soybean oil and soy-based diglycerides

Blown and bodied soybean oils are heat polymerization products from soybean oil triglyceride. Blown soybean oil is produced by heat under oxygen or air environment, and bodied soybean oil is produced by heat under nitrogen gas environment. The heat polymerization products, as compared to soybean oil, have higher viscosity indicating high molecular weight, and lower iodine number indicating losses of carbon-carbon double bonds.

These soy-based oligomers can be functionalized. Epoxidation (chemo-enzymatic), alcoholysis or hydrolysis (Chapter 4) produce soy-based polyols having high equivalent weight which is suitable in the applications of flexible polyurethanes.

Products from epoxidation of soy-based diglycerides are soy-based polyols having both hydroxy and epoxy functional groups. The epoxy groups are ready to be converted to hydroxy group and to react with isocyanate as well. However, the epoxidized diglyceride products have low equivalent weight limiting the polyurethane foam formulations.

3.2. METHODS

3.2.1. Chemo-enzymatic epoxidation of soybean oil

Refined soybean oil (food grade) is purchased from a local grocery store. Linoleic acid (90%) is purchased from City Chemical LLC (West Heaven, CT). Stearic acid (>90%) and Novozyme 435® (immobilized lipase B from *Candida*

antarctica on acrylic resin) are purchased from Sigma Aldrich (St. Louis, MO). Hydrogen peroxide solutions (30%) are purchase from Fisher (Houston, TX).

a) Chemo-epoxidation of soybean oil in well-mixed reactor

Soybean oil (5 g), linoleic acid (0.3 g) and toluene (10 ml) are combined in a 125-ml Erlenmeyer flask. Immobilized lipase, Novozyme 435®, (0.53 g) is added to the mixture when the reaction starts. Hydrogenperoxide solution (30%) is added dropwise during the first 5 h of reaction. Three ratios of hydrogenperoxide to C=C double bonds (H_2O_2:C=C) are used; 0.6, 0.8 and 1.0 by mole. The reaction further continued for 24 h in a controlled environment incubator shaker (PSYCROTHERM, New Brunswick, NJ) at room temperature and the speed of 300 rpm.

Water, unreacted hydrogenperoxide and immobilized enzyme are easily removed from the reaction product due to immiscibility of these materials in the oil phase. Fatty acid is removed by saponification method. Either sodium dicarbonate, or sodium carbonate solution (0.5 N) is used to saponify the fatty acid after reaction. After the saponification, fatty acid soap is formed and separated to stay in the water phase. A centrifuge is also used to fasten the phase separation process. Toluene is finally removed from the epoxidized soybean oil before measuring epoxy content.

b) Chemo-epoxidation of soybean oil in packed-bed reactor (PBR)

The PBR design and operation of chemo-enzymatic epoxidation of soybean oil is illustrated in Figure 16. Every 24 h, a small sample (100 μl – 200 μl) in the mixing tank is drawn and reacted with tetramethylammonium hydroxide in methanol to prepare methyl ester derivatives of the epoxided products ready for GC-analysis (see analytical method, section 3.2.3).

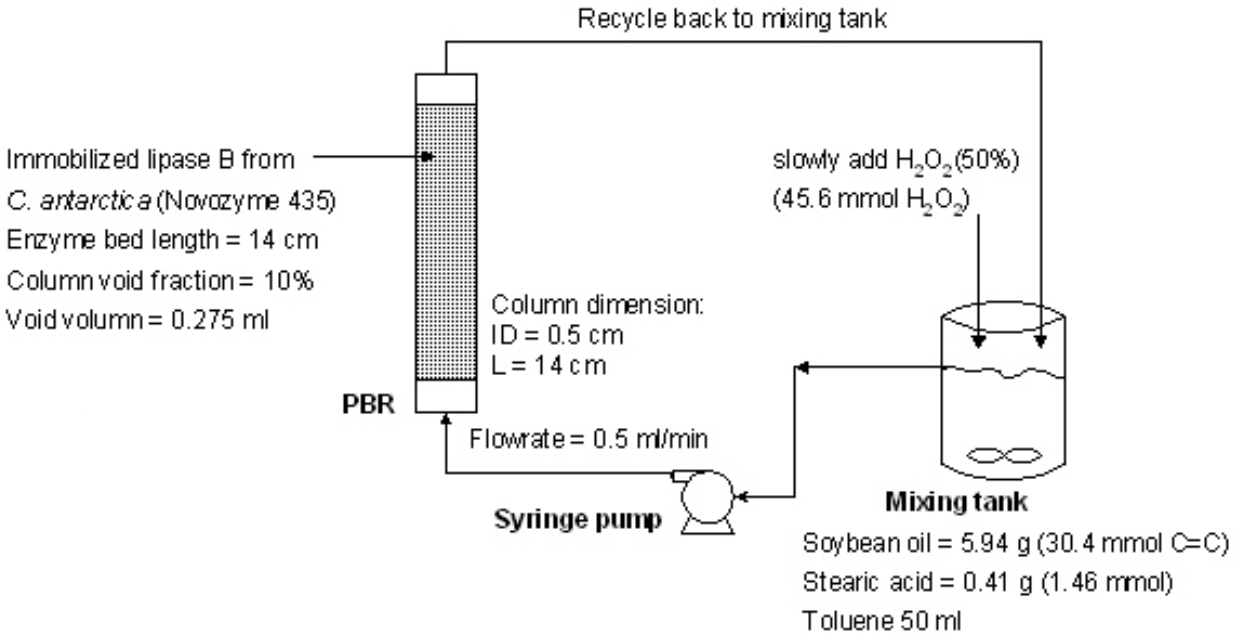

Figure 16. Packed-bed reactor of chemo-enzymatic epoxidation to produce epoxidized soybean oil triglyceride.

3.2.2 Chemo-enzymatic epoxidation of blown soybean oil, bodied soybean oil and soy-based diglycerides

Blown soybean oil (680 Blown SBO Z2-Z4) was a gift from Cargill Inc. (Chicago, IL). Bodied soybean oil is synthesized in the laboratory by heating soybean oil at 330 ^{o}C for 3 h under nitrogen gas environment. Soy-based diglyceride (ENOVA®) is purchased from ADM (Decatur, IL) which is a combination of canola oil and soybean oil. Fatty acid compositions in soybean oil and ENOVA® oil are reported in Table 3.

Table 2. Substrates and enzyme used in the chemo-enzymatic epoxidation of blown soybean oil, bodied soybean oil and soy-based.

Soy-based materials	Iodine no.	Acid	H_2O_2 (30%)	Toluene	Novozyme 435®
Blown SBO 7.3 g	28	Linoleic acid 0.5 g	1.2 ml	-	0.533 g
Bodied SBO 5.2 g	67	Linoleic acid 0.93 g	5.7 ml	20 ml	0.533 g
Soy-based DG (ENOVA®) 7.5 g	70	Linoleic acid 0.68 g	3 ml	50 ml	0.805 g
Soy-based DG (ENOVA®) 7.5 g	70	Formic acid 1.24 g	3 ml	50 ml	0.805 g

Similar to the chemo-enzymatic epoxidation of soybean oil, linoleic acid, hydrogenperoxide (30%) and Novozyme 435® are used in the epoxidation of

blown soybean oil, bodied soybean oil and soy-based diglycerides. However, toluene is not used in the experiment of soy-based diglycerides. These soy-based materials have different degrees of unsaturation and the amount of the starting materials is different as shown in Table 2.

After adding the enzyme in the reaction mixture, hydrogenperoxide (30%) is slowly charged in the first 5 h. Total reaction time is 29 h at room temperature.

3.2.3. Analytical method

a) Titration of epoxy (oxirane oxygen).

After removing toluene from the epoxidized samples, the percent (by weight) of epoxy (oxirane oxygen) functional groups are analyzed by AOCS official methods Cd 9-57. Epoxy functionality of the epoxidized soybean oil (MW~1000) is also calculated by the following equation.

$$\text{Epoxy (oxirane oxygen) functionality} = \frac{(\%\ \text{by weight of epoxy}) \times \text{MW}}{1600}$$

b) Analysis of methyl esters from epoxidized soybean oil

Methylation of glycerides and fatty acids are done with different catalysts before the GC analysis. Diazomethane was used for fatty acid residues and tetramethylammonium hydroxide (TMAH, 25% in methanol) is used for glycerides [6]. Constituents in fatty acid residues and glycerides are analyzed by GC, HP 6890 GC (Wilmington, DE). The column is HP MXT® WAX 70624, capillary 30.0 m x 280 μm x 0.25 μm nominal and the detector is a flame ionization detector

(FID). The injection port temperature is 250 ^{o}C. The temperature program is set from 160 to 220 ^{o}C at 10 ^{o}C/min and held at 220 ^{o}C for 12 min. The carrier gas is H_2 (40 mL/min) and the make up gas is N_2 (35 mL/min). Air flow is 260 ml/min. The split ratio is 75:1.

Table 3. GC-retention times of methyl ester derivatives of fatty acids in soybean oil triglyceride and epoxidized soybean oil triglycerides.

Fatty acid moieties	GC-retention time (min)	% Normalization		
		SBO	ENOVA®	Commerical Epoxidized SBO
Palmitic acid (16:0)	3.45	11.9	2.4	13.2
Stearic acid (18:0)	4.80	4.7	1.5	5
Oleic acid (18:1)	4.95	23.6	38.6	-
Linoleic acid (18:2)	5.29	52.9	48.6	-
Linolenic acid (18:3)	5.75	7	7.5	-
Mono-epoxy fatty acid	8.25	-	-	29.6
Di-epoxy fatty acid	15.50 and 17.28 (two isomers)	-	-	33.5 and 18.7
Tri-epoxy fatty acid (from epoxidized flax seed oil)	17.47, 18.92, 19.27, and 19.79 (four isomers)	-	-	-

GC retention times of monoepoxy acyl moiety and diepoxy acyl moiety are confirmed by epoxy oleate, and epoxy linoleate. GC retention times of triepoxy

acyl moiety are confirmed by epoxy linolenate and epoxidized flax seed oil which contain about 54% of linolenic acid. Oleic acid, linoleic acid, linolenic acid and flax seed oil are individually epoxidized by lipase B from *C. antarctica* (Novozyme 435®) and hydrogen peroxide according to the method by Klass and Warwel [3, 5]. After the epoxidation reaction, the epoxy fatty acids and the epoxidized flax seed oil are methylated by diazomethane and analyzed by GC-FID.

According to the method of GC-FID analysis, GC retention times and compositions of fatty acid moieties in soybean oil, soy-based diglyceride (ENOVA®) and epoxidized soybean oil are reported in Table 3. The fatty acid compositions in the glycerides can be estimated by normalizing their GC-chromatograph.

3.3. RESULTS AND DISCUSSION

3.3.1. Chemo-enzymatic epoxidation of soybean oil

a) Chemo-epoxidation of soybean oil in well-mixed reactor

In the study of the effect of hydroperoxy on the enzyme's activity, the amount of hydrogen peroxide is varied; 0.6, 0.8 and 1.0 of H_2O_2:C=C by mole, which yields an epoxy functionality of 2.8, 3.7, and 4.6 in complete epoxidation. The epoxidation conversion after the reaction is evaluated by the titration of epoxy weight percent and shown in Figure 17.

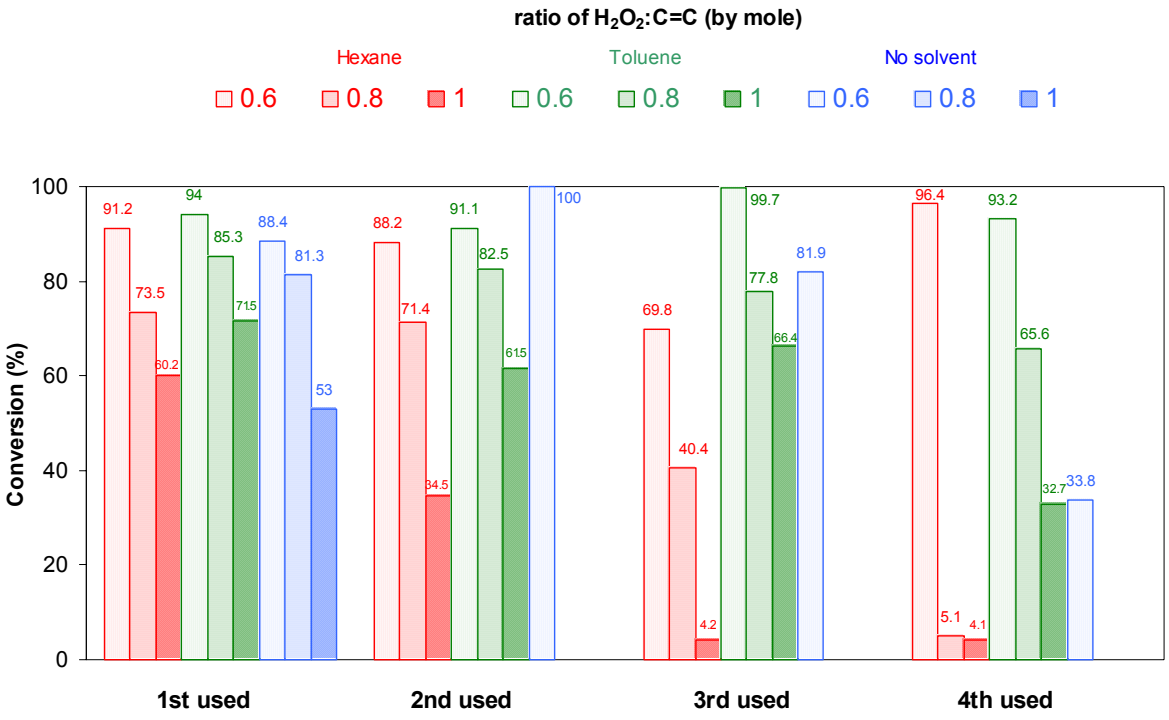

Figure 17. Effect of organic solvent and effect of hydrogen peroxide (H_2O_2) on chemo-enzymatic epoxidation of soybean oil triglyceride by Novozyme 435®.

From Figure 17, commercially available immobilized lipase B from *C. antarctica* (Novozyme 435®) is an effective biocatalyst in the epoxidation of soybean oil triglyceride. The reaction could yield over 90% conversion and the lipase is also reusable with high activity under some operating conditions.

In hexane or toluene, the lipase's activity is well maintained after four reuses when less hydrogenperoxide is used (0.6 mole ratio of H_2O_2:C=C). From the presented data, the hydroperoxide solution could reduce the enzyme's activity and shorten the enzyme's life indicated by the decrease of reaction

conversion after three uses when the higher amount of hydrogenperoxide is used.

The organic solvents could preserve the enzyme's activity. At 0.8 and 1.0 mole ratios of H_2O_2:C=C and without any solvent, the enzyme did not yield any significant conversion after two uses.

Toluene and hexane gave comparable results until the second use of the enzyme. After that, toluene could yield the higher conversion and it means that toluene preserves the enzyme's activity better than hexane. However, at the lower H_2O_2 concentration (0.6 H_2O_2:C=C by mole), the reaction conversion in hexane and toluene are always comparable with use of the recycled enzyme.

To epoxidize unsaturated oil by Novozyme 435®, all previous studies [3-5] use toluene. This might be because the log P value of toluene is suitable for the enzyme and the reaction. A study reported that toluene is a good solvent helping to stabilize Novozyme 435® due to the appropriate log P values of the solvent [7].

The definition of log P, or octanol-water partition coefficient, from Baum [8] is that "the ratio of a chemical's molar concentration in the 1-octanol phase to its molar concentration in the water phase of an octano-water system at equilibrium". Appropriate values of log P will allow good mass transfer between organic solvent and water phase in the chemo-enzymatic epoxidation and high reaction conversion can be obtained.

Warwel and Klass [5] found that the lipase's activity is still preserved after 15 uses in the epoxidation of fatty acids; however high amounts of toluene are used in their study. It could be concluded that high amount of solvent is

necessary to preserve the lipase's reactivity to produce high functionality of epoxidized soybean oil with use of the recycled immobilized lipase.

b) Chemo-epoxidation of soybean oil in packed-bed reactor (PBR)

According to the operation of PBR producing epoxidized soybean oil in Figure 16, a sample is taken every 24 h for 72 h. GC-FID analysis is used to determine the percentage of each fatty acid methyl ester. It is found that the maximum disappearance of unsaturated fatty acids occurs after 48 h and is reported by Figure 18.

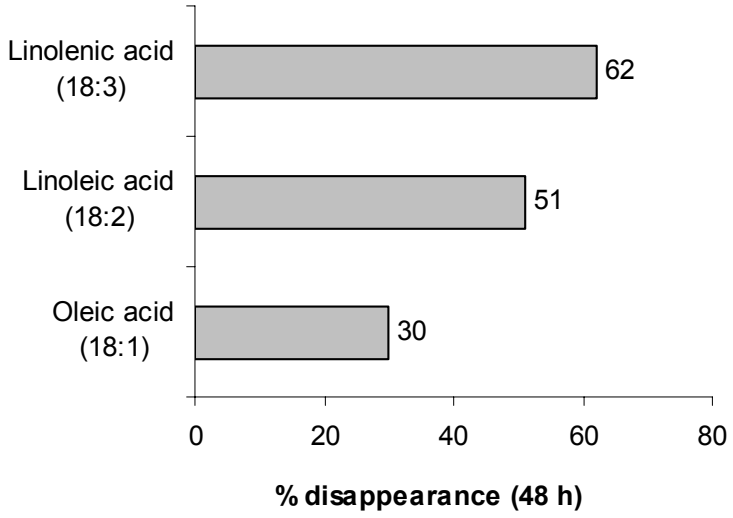

Figure 18. Percent of disappearance of unsaturated fatty acid moieties in soybean oil triglyceride after 48 h of chemo-enzymatic epoxidation by PBR of Novozyme 435®.

Among unsaturated fatty acids in soybean oil, the disappearing percentage of the linolenic acid (18:3) is highest followed by the linoleic acid (18:2) and the oleic acid (18:1), respectively. However, the reaction yields produced from PBR are not as high as those produced from the well-mixed reactor. In addition, the epoxy fatty acid moieties are predominately mono-epoxy stearic acids, analyzed by GC-FID analysis. Hydrophilicity of the enzyme's support might cause poor mass transfer resulting in low epoxidation yield.

The enzyme's support (anion exchange resin) interacts with water and H_2O_2 molecules resulting in poor mass transfer between enzyme and organic solvent (substrate) phase. From the observation, water phase is not recycled back to the mixing tank. High concentration of H_2O_2 in the packed-bed could also increase the rate of the enzyme's deactivation.

To increase epoxidation yield by PBR operation, a proper surfactant could be used to create a reverse micelle system and not deactivate the enzyme.

3.3.2. Chemo-enzymatic epoxidation of blown soybean oil, bodied soybean oil and soy-based diglycerides

Soybean oil triglyceride has iodine number of 120 and 85% of unsaturated fatty acid. Unfortunately, mole of C=C bonds can not be accurately estimated from the iodine number. Percent conversions of the chemo-enzymatic epoxidation of blown soybean oil and bodied soybean oil are not presented, but epoxy contents (% wt.) of the epoxy products are reported.

The epoxy content of epoxidized blown soybean oil, epoxidized bodied soybean oil and epoxidized soy-based diglycerides are evaluated by the titration method and are reported in Figure 19. For a reference, the complete epoxidized soybean oil has 6.8-7.0% epoxy content (by wt).

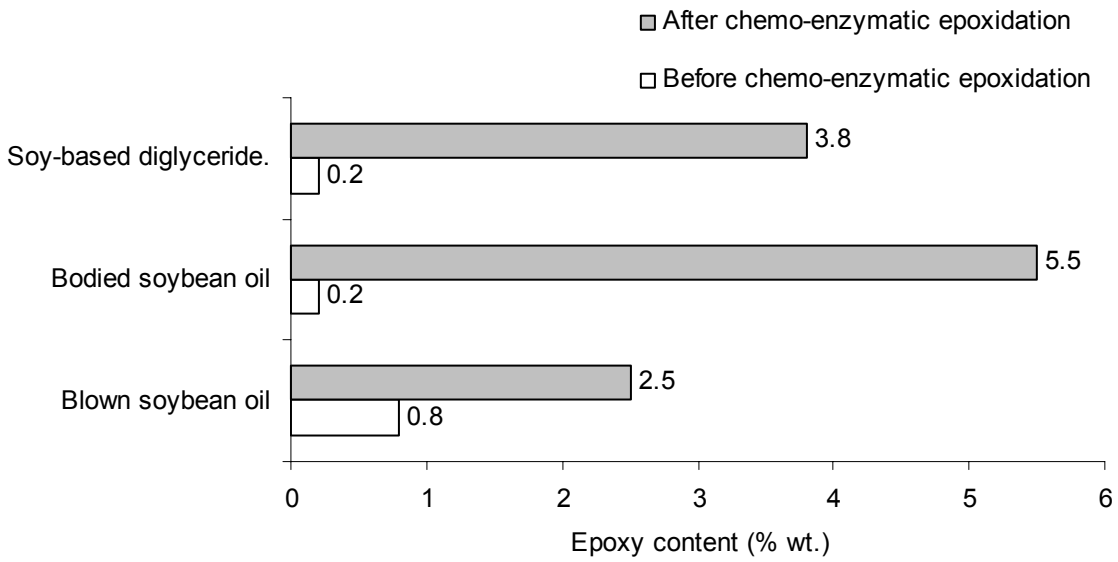

Figure 19. Epoxy content (% wt.) of the products from chemo-enzymatic epoxidation of soy-based materials.

Originally, epoxy content in blown soybean oil is a little higher than in the other soy-based materials (Figure 19). This is because blown soybean oil is the oxidized product from heat and oxygen gas. Blown soybean oil could have either epoxy or peroxy functional groups detected by the titration method. The production of bodied soybean oil was performed under N_2 gas environment where any oxidizing functional group should not be produced.

Iodine number of bodied soybean oil and soy-based diglyceride are comparable (Table 2) which is about 55-58% of the iodine number of soybean oil triglyceride. However, the epoxidation product of bodied soybean oil is about 5.5% epoxy content and of soy-based diglyceride is about 3.8% epoxy content.

Low reaction yield of the epoxidation of soy-based diglyceride is limited by the amount of hydrogen peroxide used which is 0.5:1 of H_2O_2:C=C (by mole). From GC-analysis of ENOVA® oil (Table 3), the substrate has C=C functionality of 3.4, which could be converted to 9% of epoxy content if the complete epoxidation is achieved.

As a result, the reaction conversion of the epoxidation of soy-based diglyceride under the described condition is about 76%. The reaction conversion is not changed when linoleic acid is replaced with formic acid which is a common acid used in chemical route of the epoxidation.

Blown soybean oil has less degree of unsaturation indicated by low iodine number. The epoxidized blown soybean oil, which is produced under the described conditions, contained 2.5% epoxy content.

3.4. CONCLUSIONS

Novozyme 435® is an effective catalyst in the chemo-enzymatic epoxidation of soybean oil and soy-based materials having C=C double bonds. The reaction can be well performed at ambient conditions and the lipase is reusable with high reactivity under proper conditions. Reaction conversion and yield are also high with this biocatalyst.

However, the lipase is sensitive to hydrogenperoxide which could ruin the lipase's activity and life. Toluene is found to be an excellent solvent for the reaction and necessary if the high epoxy functionality product and lipase's reusability are required.

Moreover, with the presenting operating conditions, the well-mixed reactor yields a higher conversion than packed-bed reactor (PBR). It is possible that mass transfer is limited in PBR. Commercialization of the PBR of chemo-enzymatic epoxidation, with a reverse micelle system, should be further studied.

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CHAPTER 4

SOY-BASED POLYOLS FROM SELECTIVE HYDROLYSIS OF COMMERCIAL LIPASES AND EFFECTS OF EPOXY GROUP ON THE ENZYMATIC HYDROLYSIS

4.1. INTRODUCTION

This is the first published account of the usage of enzyme hydrolysis to produce soy-based polyols. The enzyme hydrolysis is “green” chemistry method which is simple and reserves energy consumption. This enzyme method yields hydroxy groups which are the most preferable functional group for the soy-based polyols.

Epoxidized soybean oil is a derivative of soybean oil having oxirane (epoxy) functional groups which are products from epoxidation of carbon-carbon double bonds in soybean oil. Applications of the epoxidized soybean oil were given in Chapter 3.

Epoxidized soybean oil triglycerides (Figure 20) can be produced by either chemical or enzymatic oxidation of soybean oil triglycerides [1-2]. Soybean oil typically has about 85% of unsaturated fatty acids and about 15% of saturated fatty acids approximately including 11% of palmitic acid and 4% of stearic acid [3]. The saturated fatty acids cannot be oxidized because they lack carbon-carbon double bonds. These saturated moieties are unreactive, form branches

when polymerized and are reported to cause poor properties in the final product of polyurethane synthesis [4-6].

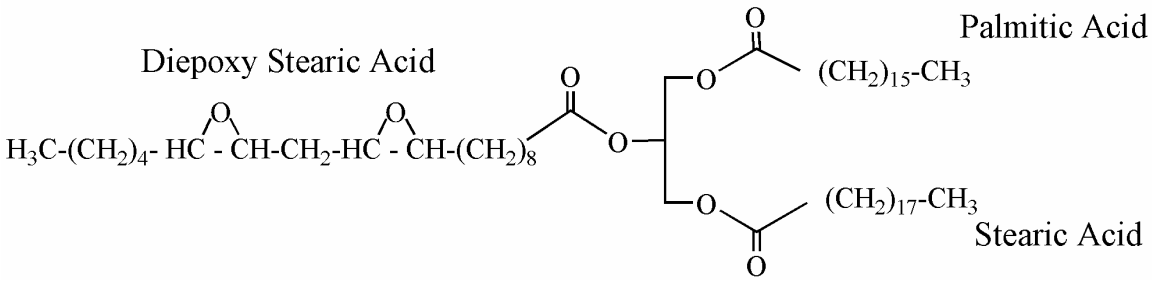

Figure 20. An example of an epoxy acyl moiety presenting in epoxidized soybean oil triglycerides.

Lipase, or esterase, is a renewable biocatalyst broadly employed in the transformation of lipids, especially in the production of structured lipids which need its high stereoselectivity and regioselectivity. Generally, selectivity of lipases depends on enzyme structure, substrate structure, factors affecting binding of the enzyme to the substrate and other factors influencing the enzyme activity [7].

Researchers have reported lipase selectivity for the hydrolysis of castor oil [8-9] and the impact of epoxy groups of trivernolin on enzymes [10]. However, there is no previous work reporting the effects of epoxy groups on lipase selectivity with the reaction of epoxidized soybean oil. This investigation of lipase hydrolysis selectivity will be widely useful in the preparation of an epoxidized

soybean oil that is low in saturated acid content and has high hydroxy number, which yields a better product for plastic and polymer reactions.

The mechanism of lipase binding of substrates also involves substrate configuration and conformation. Most lipases have been determined to be 1,3-regioselective enzymes in the hydrolysis and esterificaiton of lipids. There are only a few lipases reported to be non-selective enzymes or 2-regioselective enzymes [11-12].

The regioselective composition of natural oils provides a valuable composition control to which results are compared when evaluating free fatty acid compositons. In soybean oil, more than 99% of total saturated fatty acids are located at 1- or 3-position in the triglyceride molecule [13, 3]. Partial hydrolysis combined with a free fatty acid composition high in saturated fatty acids can be used as part of an argument for regioselective hydrolysis.

In this study, soybean oil triglycerides and epoxidized soybean oil triglycerides were hydrolyzed by eight lipases and their selectivities to saturated fatty acids were determined. Lipase selectivity was determined by comparing the composition of hydrolyzed fatty acids to triglyceride substrates after 5-50% hydrolysis.

The objectives are to better understand the impact of epoxy groups on this discrimination and to find an enzyme which has high selectivity toward saturated fatty acids in the hydrolysis of epoxidized soybean oil. The preferred enzyme will not only selectively cleave off saturated fatty acids from epoxidized soybean oil triglyceride but will also replace the saturated unused parts with highly reactive

and useful primary hydroxy groups. The primary hydroxy group in polyols is three times more reactive with isocyanate in polyurethane synthesis than secondary hydroxy groups [14]. Commercially available epoxidized soybean oil triglycerides, containing palmitic acid (13.2%), stearic acid (5.0%), monoepoxy stearic acid (29.4%), and diepoxy stearic acid (52.4%), was the substrate with soybean oil used in control studies.

In addition, preliminary studies of preparation of immobilized enzymes are performed. The immobilized enzymes are tested in both well-mixed reactor and packed bed reactor in the hydrolysis of epoxidized soybean oil.

4.2. METHODS

4.2.1. Materials

Lipases from *C. rugosa* (Lipase AY "Amano"), *B. cepacia* (Lipase PS "Amano"), *Pseudomonas* sp. (Cholesterol esterase, "Amano" 2), *P. roquefortii* (Lipase R "Amano"), *P. camembertii* (Lipase G "Amano"), *A. niger* (Lipase A "Amano"), *M. javanicus* (Lipase M "Amano") and immobilized lipase from *B. cepacia* were gifts from Amano Enzyme USA, Elgin, IL. And lipase *R. miehei* was purchased from Sigma-Aldrich, St. Louis, MO.

Epoxidized soybean oil (VIKOFLEX7170) was purchased from ATOFINA Chemicals Inc, Philadelphia, PA. Refined soybean oil (Food Club brand vegetable oil) was purchased from a local grocery store. Diazald, Tetramethylammonium Hydroxide (TMAH, 25% in methanol), Oleic acid (90%), Linolenic acid (99%), Hydrogen Peroxide and Novozyme 435® (lipase B from

Candida antarctica) were purchased from Sigma Aldrich, St. Louis, MO. Linoleic acid (90%) was purchased from City Chemical LLC, West Heaven, CT. Flax seed oil was purchased from Jedwards International, Inc., Quincy, MA. Methanol, Diethyl ether, Potassium bicarbonate and Sulfuric acid were from Fisher, Houston, TX.

4.2.2 Hydrolysis of soybean oil and epoxidized soybean oil

The enzymes obtained from Amano Enzyme Inc. were studied at their optimum pH and temperature as recommended in the product specification sheets and the reactions with *R. miehei* lipase were operated at 45^{o}C and pH 7.0. Table 4 shows operating conditions, and enzyme activity as reported from the enzyme suppliers.

Two grams of soybean oil, or epoxidized soybean oil, and two grams of buffer solution were mixed in a 125-mL Erlenmeyer flask. The reactions were performed in a controlled environment incubator shaker (PSYCROTHERM, New Brunswick, NJ) at the speed of 300 rpm. For a reaction at given pH, temperature and time, three replications and one control (substrate + buffer, and without enzyme) were carried out concurrently. The enzyme unit was 67.5 units per gram of substrate. The reaction was stopped by adding 20 mL of a mixture of methanol and diethyl ether (80:20).

Table 4. Operating pH and temperature for enzyme hydrolysis screening test.

Lipase	pH	Temperature (^{o}C)	Activity, (units/gram)
C. rugosa	7.0	45	≥ 30,000
B. cepacia	7.0	50	≥ 30,000
Pseudomonas sp.	7.0	35	≥ 10,000
P. camembertii	5.0	30	≥ 50,000
P. roquefortii	7.0	40	≥ 10,000
A. niger	6.0	45	12,000-15,000
M. javanicus	7.0	40	≥ 10,000
R. miehei	7.0	45	≥ 20,000

Only in the limit of zero hydrolysis will the true, fundamental selectivity of the hydrolysis be revealed in a single concentration profile. Conversion data at 100% hydrolysis will not reveal information on selectivity. Reaction times of this investigation were selected to provide about 15% conversion since soybean oil contains about 15% saturated fatty acids. Actual conversions are reported in the results and typically varied from 5-20%.

4.2.3. Fatty acids/glycerides recovery

After stopping the reaction, 80 mL of 0.5 M potassium bicarbonate and 15 mL of diethyl ether were added into the reaction product (glyceride-fatty acid mixtures). The mixture was placed in a separatory funnel. The glyceride portion

(oil phase) was separated from the free fatty acid soap which was in the lower water phase. Free fatty acid soap residues were recovered from the water phase by acidification with sulfuric acid and then by solvent extraction with diethyl ether. Eventually, the diethyl ether in both glycerides and acid residue was evaporated at 45 ^{o}C.

4.2.4. Immobilization of lipases

Immobilized enzymes from *C. rugosa* and *B. cepacia* are commercially available but they are too expensive for commercialization approaches. Many different methods are published to immobilize the enzymes.

In adsorption immobilization, enzymes will have physical interactions (charge effect) with the support after adsorbing enzymes on support's surface. It is the easiest immobilization method and minimizes to chance enzyme's structures, but interaction between enzyme and support is weak and sensitive to process conditions.

Due to the availability of support materials in the laboratory, silica based materials and an ion exchange resin are selected to perform preliminary experiment. For lipase *C. rugosa* (isoelectric point, pI = 4.3) in pH 7.0 buffer solution (both in immobilization and hydrolysis process), anion exchange resin is preferred rather than cation exchange resin. The lipase tends to have negative charges and interacts with the anion exchanger at the neutral pH.

Commercial glass beads are soaked up in HCl solution for a couple hours to remove silicon on the surface and are later neutralized by distilled water. All kinds of support materials are dried overnight in the oven before used.

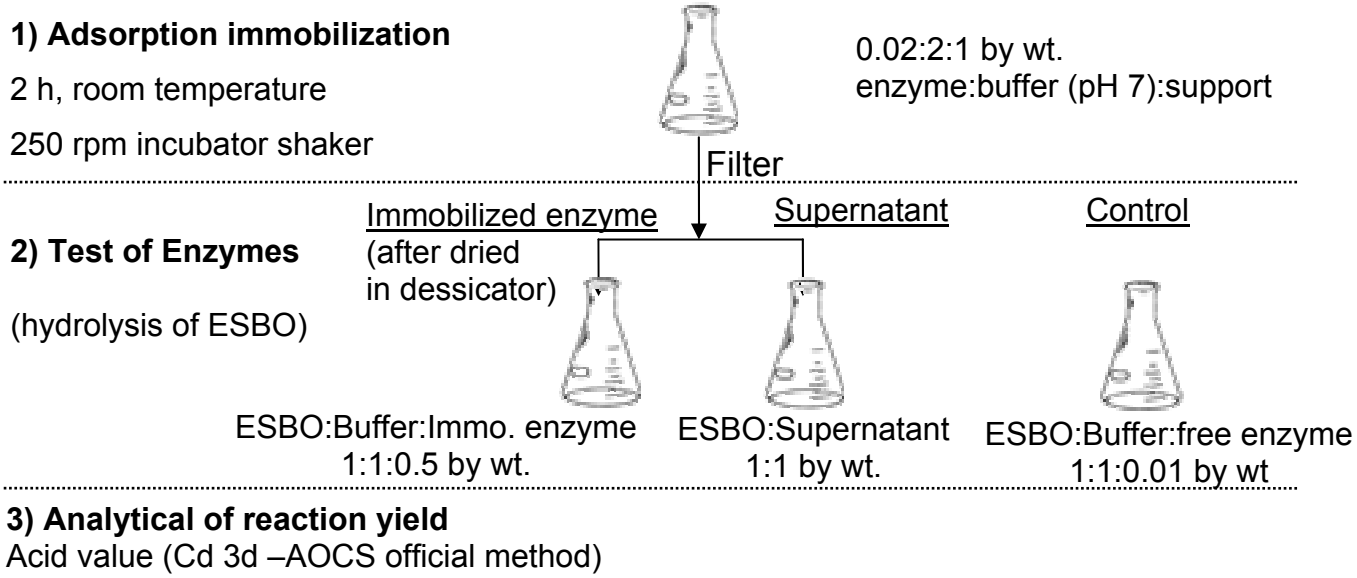

Figure 21. Adsorption immobilization of enzyme *C. rugosa* on different support materials.

Enzyme *C. rugosa* has suspended on the available carriers. To immobilize enzyme, free enzyme in phosphate buffer (pH 7.0) was mixed with dry supports under controlled conditions. Immobilized enzymes were later filtrated and dry in desiccator containing silica gel before used. The immobilized enzymes and supernatant were tested in hydrolysis reactions of epoxidized soybean oil where

free enzyme was used as the control. Material and methods of adsorption immobilization and the test results are shown in Figure 21.

Moreover, another common method of enzyme immobilization was investigated. Covalent immobilization is a method that enzyme has strong covalent bonding to the support. Enzyme has more stability to pH changing but the bonding process could alter enzyme activity or binding site.

To bind *C. rugosa* enzyme to silica supports, γ-APTES (3-aminopropyl trimethoxysilane) was first used to apply amino groups on the supports. After that, glutaraldehyde was applied as a bifunctional spacer and covalently bind enzyme to the amino groups.

Furthermore, PBR apparatus of selective hydrolysis of epoxidized soybean oil was set up as similar as the one in Figure 16. For selective hydrolysis, the mixing tank contained 50% by weight of epoxidized soybean oil in water which was charged to the PBR with a flowrate of 1 ml/min. The enzyme packed bed was 6 cm long of commercially immobilized lipase from *B. cepacia.*

4.2.5. Analysis of reaction products

a) Analysis of methyl esters from epoxidized soybean oil

Methylation of glycerides and fatty acids were done with different catalysts before the GC analysis. Diazomethane was used for fatty acid residues and tetramethylammonium hydroxide (TMAH, 25% in methanol) was used for glycerides [15]. Constituents in fatty acid residues and glycerides were analyzed by GC, HP 6890 GC (Wilmington, DE). The column was HP MXT®WAX 70624,

capillary 30.0 m x 280 μm. x 0.25 μm nominal and the detector was a flame ionization detector (FID). The injection port temperature was 250 ^{o}C. The temperature program was set from 160 to 220 ^{o}C at 10 ^{o}C/min and hold at 220 ^{o}C for 12 min. Carrier gases was H_2 (40 mL/min) and make up gas was N_2 (35 mL/min). Air flow was 260 ml/min. The split ratio was 75:1.

GC retention times of monoepoxy acyl moiety and diepoxy acyl moiety were confirmed by epoxy oleate, and epoxy linoleate while the GC retention time of triepoxy acyl moiety was confirmed by epoxy linolenate and epoxy flax seed oil which contains about 54% of linolenic acid. Oleic acid, linoleic acid, linolenic acid and flax see oil were individually epoxidized by lipase B from *C. antarctica* (Novozyme 435®) and hydrogen peroxide [1, 2]. After the epoxidation reaction, the epoxy fatty acids and the epoxy flax seed oil were methylated by diazomethane and analyzed by GC-FID.

b) Analysis of methyl ester from soybean oil

For the reaction products produced from the reaction of soybean oil, sodium methoxide was used to catalyze the methylation of glycerides and sulfuric acid was used for the free fatty acid residues. GC analysis was similar to that used for epoxy derivatives.

4.3. RESULTS AND DISCUSSION

4.3.1. Enzymatic hydrolysis of soybean oil by free lipases

Figure 22 presents hydrolysis conversions and compositions of the glyceride phase after product workup. Lipase from *C. rugosa* gave the highest reaction yield (24 h) that was about 25% hydrolysis. *A. niger* lipase was the second most active lipase at 12.5% hydrolysis. Lipases from *M. javanicus* and *B. cepacia* produced about 7% and 10% of hydrolysis while lipases from *R. miehie*, *P. roquefortii* and *Pseudomonas* sp. yielded less than 5% hydrolysis.

The lipase from *P. camembertii* was ineffective for this reaction. This corresponds with the previous studies reporting that the enzyme is an ineffective catalyst for the hydrolysis of triglycerides and shows hydrolysis activity for monoglycerides and diglycerides [12, 16].

4.3.2. Enzymatic hydrolysis of epoxidized soybean oil by free lipases

Figure 23 shows hydrolysis conversions and constituents in the glyceride phase and fatty acid phase after the enzyme hydrolysis of epoxidized soybean oil. Although soybean oil contains about 7% of linolenic acid, neither linolenic acid nor the triepoxy acyl moiety exists in the commercially available epoxidized soybean oil.

This study investigated the effects of the epoxy groups on the lipase reaction and lipase selectivity because the presenting data can not explain lipase regioselectvity and stereoselectivity. Diepoxy stearic acids derived from both linoleic acid (fully epoxidized) and linolenic acid (not fully epoxidized) are

combined and reported as one data point. Analogously, both non-fully epoxidated linoleic acid and non-fully linolenic epoxidated acid on mono epoxidated acid, and fully epoxidated oleic acid, are reported as the monoepoxy acyl moiety.

Lipases from *C. rugosa*, *B. cepecia* and *M. javanicus* were efficient biocatalysts resulting in about 20% conversion by *C. rugosa* lipase, higher than 40% conversion by *B. cepecia* lipase, and about 15% by *M. javanicus* lipase (2 h). Lipases from *A. niger* slowly catalyzed the reaction and yielded 20% (24 h). Lipases from *R. miehei*, *P. roquefortii*, and *Pseudomonas* sp. yielded about 3-5% hydrolysis (2 h).

Similarly to the reactions with soybean oil, *P. camembertii* was ineffective toward hydrolyzing epoxidized triglycerides.

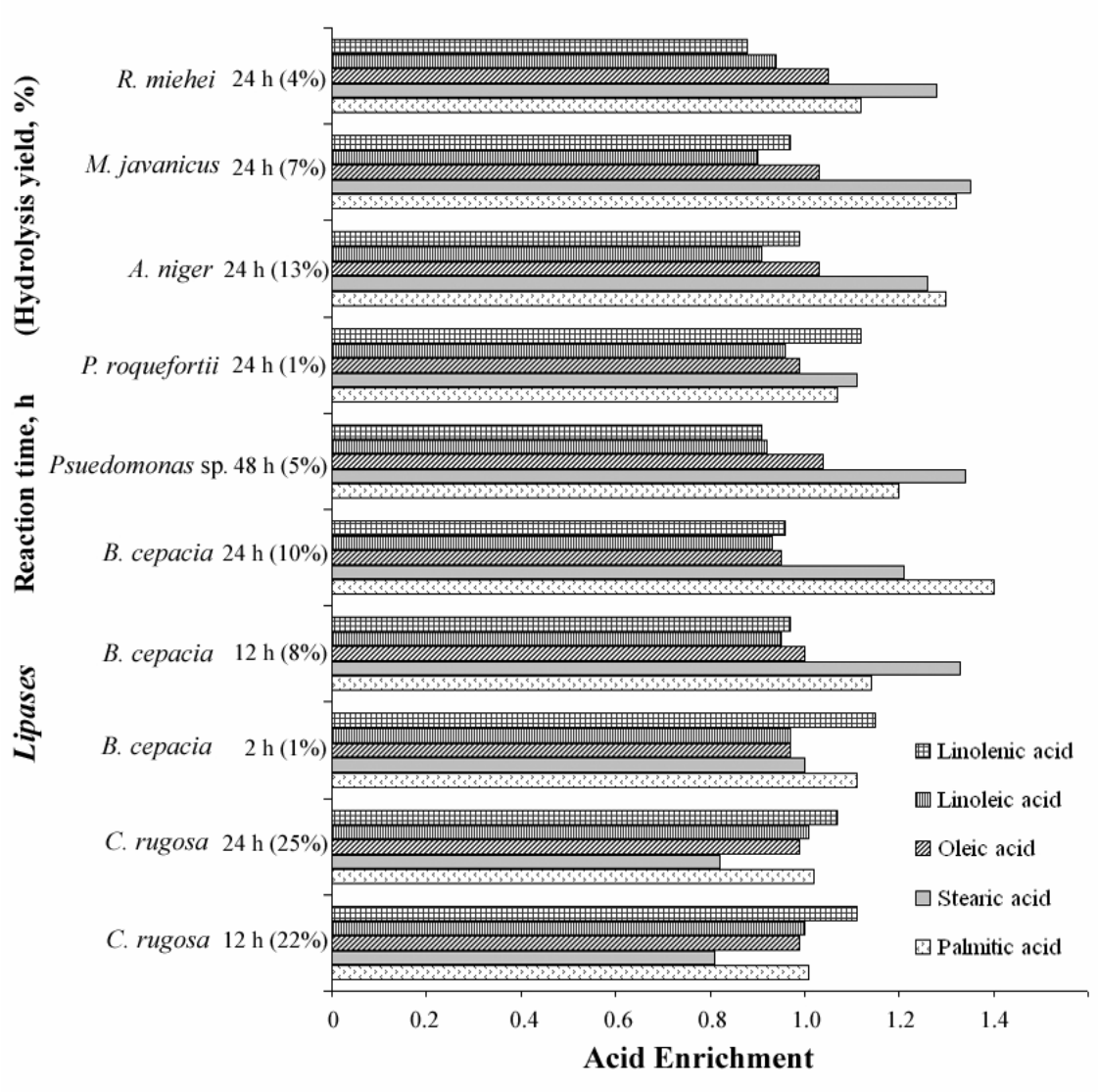

Figure 22. Enrichment numbers in fatty acid residues after hydrolysis of soybean oil.

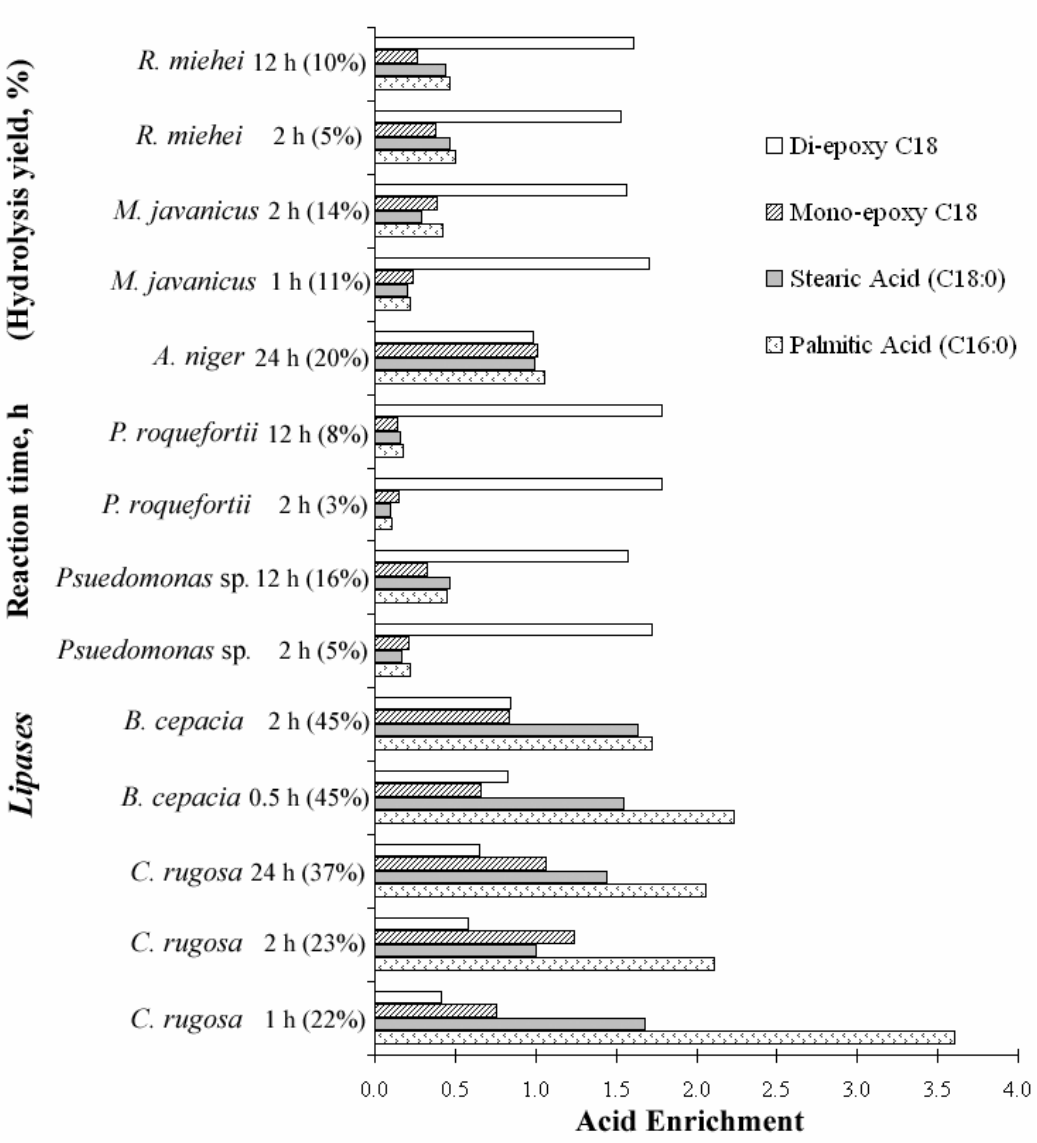

Figure 23. Enrichment numbers in fatty acid residues after hydrolysis of epoxidized soybean oil.

4.3.3. Enzymatic hydrolysis of epoxidized soybean oil by immobilized enzymes

According to the immobilization method in Figure 21, immobilization of enzyme *C. rugosa* on silica gel gave the most effective catalyst. After 2 h of the

immobilization, there was significant amount of active enzymes in supernatant indicated by high hydrolysis yield by the supernatant.

Adsorption time was increased to 24 h; however, both immobilized enzyme and supernatant from 24 h of stirring time gave poor reaction yields for all support materials. At the longer time of the stirring process, enzyme could be washed out from the supports or deactivated by shear forced from rigorously mixing action. On the other hand, 2 h mixing time was possibly the optimum but support's surface can not adsorb all enzymes due to excess enzyme loading.

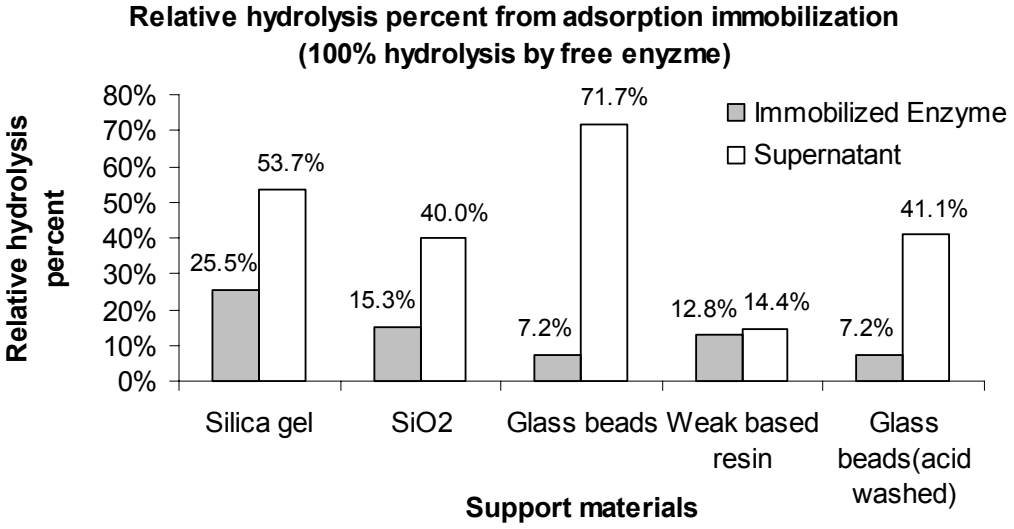

Figure 24. Yield from hydrolysis reaction performed (step 3 in Figure 21). Reporting hydrolysis percent relative to the control experiment of free enzyme.

Regarding to the current data, very low performance of immobilized enzyme from covalent binding can not be explained. Materials and methods of

covalent immobilization could be further developed and investigated to achieve the better performance of immobilized enzyme as successfully done in literatures [17].

Compared to covalent immobilization, adsorption method can engage more active enzyme on silica-based support materials. Immobilized enzyme of *C. rugosa* on Silica gel by adsorption also showed selectivity against palmitic acid, as same as the free enzyme's selectivity in Figure 23. The results conclusively demonstrated that enzyme from *C. rugosa* can be suspended by the easy method of adsorption immobilization without changing of the preferred selectivity.

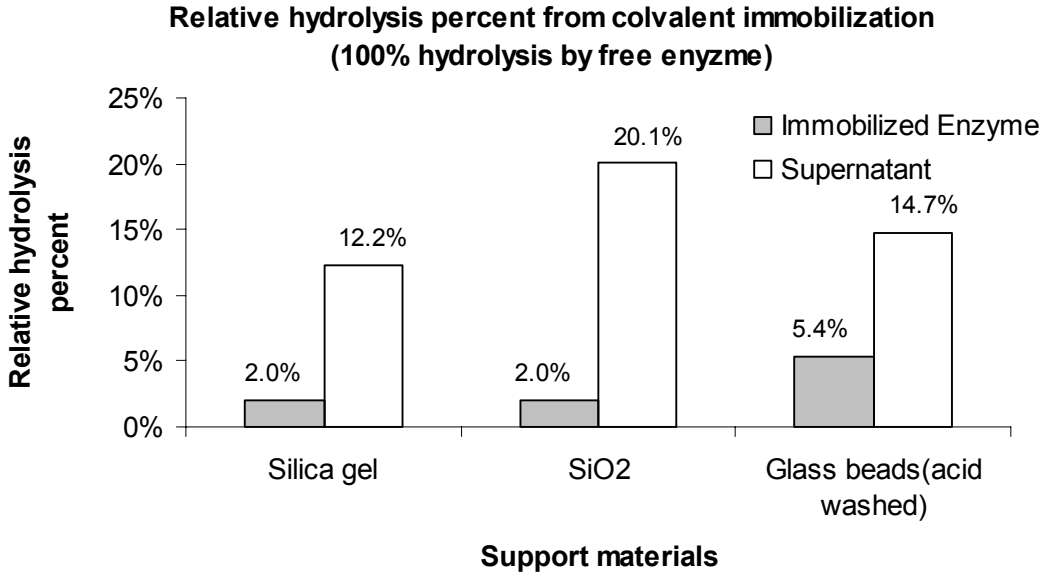

Figure 25. Yield from hydrolysis reaction from covalent immobilization. Expressing hydrolysis percent relative to the control experiment of free enzyme

The hydrolysis reaction yielded about 80% after 48 h at room temperature. It is positive that selective hydrolysis of epoxidized soybean oil does not require any surfactant, even though enzyme's support is hydrophilic. It might be due to the hydrophilic epoxy groups in substrate tending to increase interface area between lipids and water. The emulsifying of epoxidized soybean oil was observed during the experiment. Lipid-water mixtures during and after the hydrolysis of epoxidized soybean oil were cloudy while mixtures with soybean oil were less cloudy and more-readily separated into isolatable phases.

4.3.4. Lipase selectivity of soybean oil triglycerides

After product workup, the enrichment number of each acyl moiety in fatty acid phase was calculated in order to investigate enzyme selectivity. The following equation defines the enrichment number:

$$\text{Enrichment number of acyl moiety 'A' in fatty acid residue} = \frac{(\%\text{normalization of 'A' in fatty acid phase})}{(\%\text{normalization of 'A' in triglyceride substrate})}$$

where A is palmitic acid, stearic acid, or other acyl moieties

Total of every component's signal is 100 in percent normalization

The higher the enrichment number, the higher the enzyme selectivity toward hydrolyzing a particular acyl moiety. Figure 22 and Figure 23 how

enrichment numbers from the reactions of soybean oil triglyceride and of epoxidized soybean oil triglyceride, respectively.

In view of experimental error and the need for a threshold selectivity to realize practical usefulness, a deviation of 15% was identified as a threshold value to identify a significant selectivity. Based on this criteria and the data of Figure 22, *C. rugosa* lipase discriminated against stearic acid while it has been known to be a non-selectvie enzyme in previous work [18]. Lipases from *B. cepacia, Pseudomonas* sp., *A. niger*, *M. javanicus* and *R. miehei* promoted hydrolysis with higher enrichment numbers of saturated fatty acids than those of unsaturated fatty acids. Lipases of *P. roquefortii* seemed to show non-selective ability (24 h); however, its hydrolysis yield was too low to conclude that the enzyme was non-selective. In addition, some previous literature determined lipase from *P. roquefortii* to be a 1,3-positional and short-chain-specific lipase [12, 16].

In enzymatic modification of triglycerides, most of enzymes are 1,3-selective lipases [11]. A lot of publications have determined that all of the enzymes used in this study, except lipase from *C. rugosa*, are 1,3-regioselective [11-12, 16,18].

Soybean oil has <20% of saturated fatty acids where 99% of them are located at 1,3-position. It is possible that lipases either showed saturated fatty acid selectivity or 1,3-selectivity. It cannot be determined based on the available information. The present results suggest that the selectivity is dependent upon

the reaction conditions and/or degree of saturation in a manner more complicated than simple 1,3-regioselectivity.

4.3.5. Lipase selectivity of epoxidized soybean oil triglycerides

Figure 23 compares enrichment numbers from the hydrolysis of epoxidized soybean oil. Lipase from *B. cepacia* selectively cleaves off saturated fatty acids, both palmitic and stearic acids, when hydrolysis rates of the saturated fatty acids are 2 times faster than those of epoxy fatty acids (0.5 h). The rate of hydrolysis of palmitic acid was initially shown to be higher than that of stearic acid; however, they were similar at longer reaction times (2 h). There was no difference between rates of hydrolyzing the monoepoxy acyl moiety, and the diepoxy acyl moiety by *B.cepacia* lipase (similar enrichment numbers).

C. rugosa lipase selectively hydrolyzed palmitic and stearic acids from epoxidized soybean oil triglycerides with a greater selectivity to palmitic acid than stearic acid. The selective cleavage of palmitic acid was prominent at conversions up to the highest measured conversions of 37%. The greatest selectivity to cleaving palmitic acid was at lower conversions which is consistent with the higher concentrations of bound palmitic acid at the onset of hydrolysis.

Figure 23 showed that epoxidized soybean oil has about 52% of diepoxy acyl moieties being the prominent bound acyl moieties in epoxidized soybean oil. However, *C. rugosa* slowly hydrolyzed diepoxy acyl moieties as indicated by its lowest enrichment number in the acid residues.

The lipase from *C. rugosa* was previously reported to selectively cleave short chain fatty acids and to be a non-selective enzyme depending on substrates and reactions [9, 12]. The enzyme itself likes straight chain fatty acid rather than bulky substrates due to its tunnel-like active site [19]. Shorter fatty acids tend to be straighter than longer fatty acid chains, and saturated fatty acids do not bend like unsaturated fatty acids bending at carbon double bonds. This might cause lipase *C. rugosa* to show the most selectivity to palmitic acid. *C. rugosa* lipase showed a higher number of palmitic acid enrichment with the hydrolysis of epoxidized soybean oil than that of the soybean oil (Figure 22 and Figure 23). The enrichment numbers in excess of 3.5 (2 h) were the highest observed--it is possible that the intrinsic selectivity of *C. rugosa* lipase to short chain fatty acids was ultimately limited by thermodynamic equilibrium and non-enzymatic acyl moieties migration [18].

Lipases from *P. roquefortii*, *M. javanicus*, *R. miehei* and *Psudomonas* sp. favorably hydrolyzed diepoxy acyl moieties which on the surface appear to possibly be contrary to the previously reported 1,3-regioselective. However, these lipases could have selectivity toward diepoxy acyl moieties at position 1 or 3 in epoxidized soybean oil. These lipases discriminated against the hydrolysis of palmitic acid, stearic acid and monoepoxy stearic acid. Lipase from *P. roquefortii* catalyzed the hydrolysis reaction of diepoxy acyl moieties about 10-17 times faster than the others.

A. niger lipases showed almost identical enrichment numbers (about 1.0) for all acyl moieties (Figure 22). From these results, *A. niger* enzyme can be

either non-selective or 1,3-regioselective in the hydrolysis of epoxidized soybean oil, according to <19% of saturated fatty acids at position 1 or 3 and complicated thermodynamic equilibrium including non-enzymatic acyl migration [18].

4.3.6. Effect of epoxy functional group on hydrolysis conversions

Rates of hydrolysis significantly increased in the reaction of epoxidized soybean oil relative to soybean oil (see Figure 22 and Figure 23). The reaction conversion increased from 25% to 37% (24 h) by *C. rugosa* lipase. The hydrolysis of epoxidized soybean oil by *B. cepacia* lipase results in a 45 % conversion (2 h) while the reaction with soybean oil yielded only 1% (2 h). Figure 26 shows the comparison between the reaction of soybean oil and of epoxidized soybean oil with some lipases. It is likely due to the emulsifying characteristics of the epoxy group which tends to increase the interface area between lipids and water.

The emulsifying nature of epoxidized soybean oil was confirmed by observations. Lipid-water mixtures during and after the hydrolysis of epoxidized soybean oil were cloudy while mixtures with soybean oil were less cloudy and more-readily separated into isolatable phases.

Epoxy acyl moieties both increased hydrolysis rates and altered lipase selectivities. It is possible that 1,3-regioselective lipases became highly selective to diepoxy acyl moieties at the 1 and 3 positions, and in such an instance, the present study would not contradict previous conclusions on 1,3-regioselectivity. One explanation for the selectivity trends exhibited by certain enzymes and not

by others is the role of water in the immediate surroundings of the enzyme molecule toward promoting reaction.

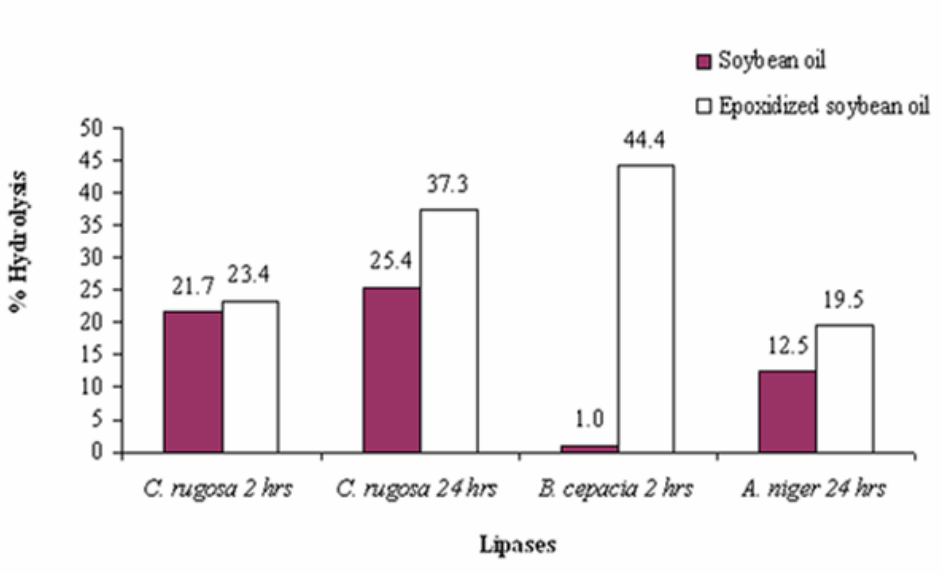

Figure 26. Effects of the presence of epoxy acyl moieties of soybean oil triglycerides.

4.4. CONCLUSION

The presence of epoxy moieties in epoxidized soybean oil was observed to increase the lipid-water interface and enhance the rates of enzyme hydrolysis reactions. The epoxy functional group also affected the enzyme selectivity. Based on this screening of eight lipases, selective hydrolysis can be more-readily attained in epoxidized soybean oil than normal soybean oil.

Partial hydrolysis of epoxidized soybean oil triglycerides by lipases from *B. cepacia* and *C. rugosa* could selectively replace saturated fatty acid moieties with hydroxy functional groups which are expected to be primary alcohol (high reactivity). The selectively hydrolyzed product, epoxidized soybean oil based

material, has higher functionality in terms of a higher percentage of epoxy and higher hydroxy number, which is believed in some applications, to produce better performing polymers by decreasing the number of non-functional fatty acid branches on the polymer structure.

The reaction is very simple and operated at mild operating conditions where only water is needed as a reagent and no surfactant and solvent are required.

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CHAPTER 5

HYDROLYSIS OF BODIED SOYBEAN OIL BY COMMERCIAL ENZYMES TO PRODUCE NEW SOY-BASED POLYOLS

5.1. INTRODUCTION

Commercial polyols are polyesters, polyethers, castor oil, and soy-based polyols [1]. Among these commercial polyols, renewable-based polyols are used to replace petrochemical-based polyols to meet “green chemistry” and “sustainability” goals of urethane consumers.

The largest non-functional branches on soy-based polyols are saturated fatty acid branches. Soybean oil triglyceride has about 15% of saturated fatty acid moieties which are 11% of palmitic (16:0) and 4% of stearic (18:0) fatty acid moieties. These saturated fatty acids reduce the reactivity of soy-based polyols and affect the final foam properties [2]. Consequently, commercial soy-based polyols are mixed typically with petrochemical-based polyols to attain acceptable foam properties [3].

Primary alcohols on polyols are usually three times more reactive with isocyanate than secondary alcohols [4]. Typical soy-based polyols have secondary alcohols derived from epoxy ring opening reaction near the middle of the fatty acid chain. Petrović et al. [2] proposed improving canola-based and soy-based polyols from ozonolysis reaction to produce primary hydroxy groups at the carbon-ended of fatty acid moieties (away from carbonyl). However, this can lead

to the creation of a mixture of 4 to 8 carbon byproducts that can be a problem in waste disposal or purification to create marketable materials.

Partial hydrolysis by 1,3-region-specific lipase produces and could improve soy-based polyols when the enzyme reaction yields primary alcohols. Moreover, about 99% of the saturated fatty acid moieties in soybean oil triglycerides are located at the 1,3-positions [5]. The enzyme hydrolysis could replace the undesirable saturated fatty acid moieties with the primary alcohol in soy-based materials. A disadvantage of hydrolysis of soybean oil triglycerides is that the final product will have low molecular weights of about 550, and if this is the only means of imparting B-side reactivity, the product will be prominently zero or one-functional.

Heat polymerization or heat bodying is an easy and economical method to create soy-based oligomers as starting materials that can be subsequently functionalized. The bodying reactions include reaction of the carbon double bonds as shown in Figure 27. Diel-alder reaction takes place after conjugated linoleic acid (C18:2) or linolenic acid (C18:3) in soybean oil is formed by heat. The reaction was explained elsewhere [6].

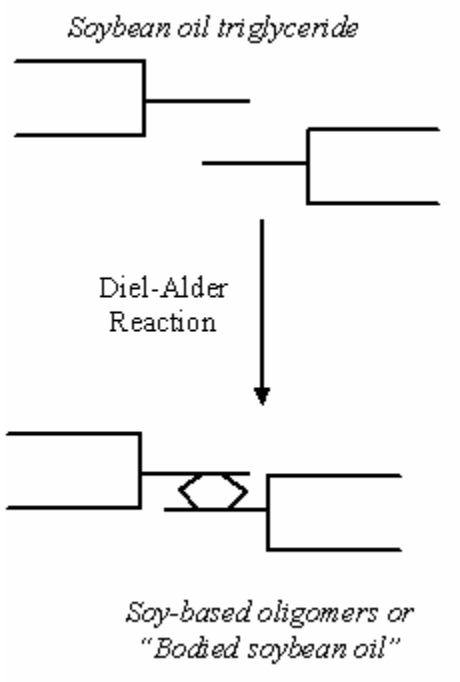

Figure 27. Heat polymerization of soybean oil to produce "bodied soybean oil".

Most of the unsaturated fatty acid moieties in soybean oil triglyceride have secondary carbonyl groups [5]. During the bodying of soybean oil triglyceride, the molecule crosslinking most likely occurs at the 2-position fatty acid moieties where the 1,3-regionspecific lipases, or most of the lipases, fortunately have poor reactivity. A bodied soybean oil molecule that is enzyme hydrolyzed to remove some of saturated fatty acids (Figure 28) will tend to have multiple primary hydroxy moieties in the polyol and will tend to engage more of the mass of the soy-based polyols in the polyurethane foam structures. In addition, the high

reactivity of primary hydroxy groups could improve the formulation, for instance, it could reduce catalyst loading, or additive loading in the foam formulations.

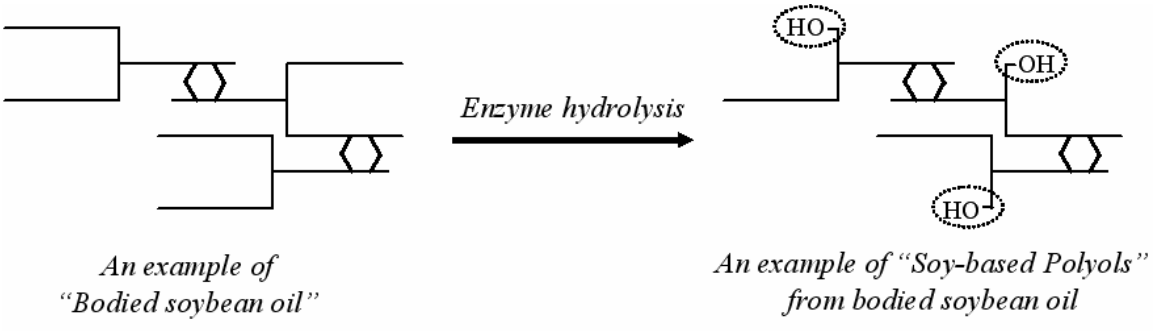

Figure 28. Examples of soy-based polyols from bodied soybean oil by selective hydrolysis of lipases.

This paper proposes new soy-based polyols derived from bodied soybean oil by enzyme hydrolysis. Several commercial enzymes are investigated for removing saturated fatty acids from the bodied soybean oil. The desired polyols are soy-based oligomers having hydroxy numbers suitable for use in polyurethane foam formulations and primary alcohols that are more-reactive.

5.2. MATERIALS AND METHODS

5.2.1. Materials

Lipases from *Candida rugosa* (Lipase AY "Amano"), *Burkholderia cepacia* (Lipase PS "Amano"), *Penicillium roquefortii* (Lipase R "Amano"), *Aspergillus niger* (Lipase A "Amano"), and *Mucor javanicus* (Lipase M "Amano") were

contributed by Amano Enzyme USA (Elgin, IL, USA) and lipase *Rhizomucor miehei* was purchased from Sigma-Aldrich (St. Louis, MO, USA). Food-grade refined soybean oil was obtained from a local grocery store.

5.2.2. Synthesis of bodied soybean oil

Bodied soybean oil was produced by heating soybean oil at 330^{o}C for 45 min under nitrogen gas environment. The heating process was done in 1-liter Parr reactor with volatile matters being removed during the reaction by a nitrogen purge. After 45 min, the bodying process increased viscosity by 23% and reduced the iodine number by 45% with viscosity and iodine values for the bodied soybean oil of 0.67 cm^2s^{-1} and 80, respectively. Molecular weight distribution was investigated by GPC.

5.2.3. Hydrolysis reaction by commercial lipases

Bodied soybean oil was partially hydrolyzed by commercial lipases without any surfactant or any organic solvent. Bodied soybean oil (15 g), phosphate buffer at pH 7.0 (15 g) and lipase (70 mg) were placed in a 125-ml flask and the reaction conditions were controlled by an incubator shaker (Psycrotherm, New Brunswick, NJ, USA) at 45^{o}C and 300 rpm. Three replications and one control (substrate + buffer, and without enzyme) were carried out concurrently.

Three reaction times were used: 1.5, 3, and 24 h. After the desired reaction times, the reaction products were left at room temperature to cool, and then washed and analyzed. The reaction conditions produced 15% to 50% of

hydrolysis and the isolated polyols was typically about 50% by weight of the bodied soybean oil.

5.2.4. Analytical methods

a) Recovery of hydrolyzed fatty acids and ester glycerols

After the reaction, 45 ml of Na_2CO_3 (0.5 M) and 90 ml of diethyl ether were placed and mixed together with reaction product in a separatory funnel. The mixture was left overnight before high speed centrifuge was applied to help separate fatty acid soap from the ether phase. Ester glycerides were in the ether phase (upper portion), whereas liberated fatty acids (free fatty acid soaps) were in the water phase (lower portion). Free fatty acids were recovered by acidification with HCl (conc.) and then solvent extraction by diethyl ether. Finally, diethyl ether in both the ether glycerides and hydrolyzed fatty acids was removed at 50 ^{o}C in an oven.

Washing studies were also performed with polyol product and $NaHCO_3$ (aqueous 0.5 M, pH 8.0)

b) GC-FID analysis

GC-FID analysis of esters derived from soybean oil and bodied soybean oil was performed to investigate the compositions of the fatty acid moieties in the oils.

After evaporating the solvent, ester glycerides and hydrolyzed fatty acid were esterified with n-butanol and H_2SO_4 catalyst. To make butyl esters, a 4.5 ml

vial contained 100 mg of sample was filled with HPLC grade n-butanol (until full) and 1 drop of H_2SO_4 (conc.) was added. The reaction was allowed to take place at 70^oC for 24 h. The butyl ester products were analyzed by GC, HP 6890 GC (Wilmington, DE). The column was HP MXTWAX 70624, capillary 30.0 m x 280 m. x 0.25 m nominal and the detector was flame ionization (FID). The injection port temperature was 250 ^oC. The temperature program was set from 160 to 220 ^oC at 10 ^oC/min and hold at 220 ^oC for 12 min. Carrier gases was H_2 (40 ml/min). Make up gas was N_2 (35 ml/min). Air flow was 260 ml/min. The split ratio was 75:1.

c) Acid number, saponification number, percent of hydrolysis and hydroxy number

Acid numbers (mg KOH/g sample) of all reaction products were evaluated according to the AOCS official method (AOCS Te 1a-64, 1997). Saponification numbers of soybean oil and bodied soybean oil are the same, 190 (mg KOH/g sample), evaluated by the AOCS official method (AOCS Tl 1a-64)

During the hydrolysis, when lipases attack one mole of carbonyl group, one mole of fatty acids is released and one of hydroxy functional group is promoted. Accordingly, the percent of hydrolysis is defined by the following equation.

% Hydrolysis

$$= \frac{\text{Acid number of reaction product before product workup (mg KOH/g)}}{190\ (\text{mg KOH/g})} \times 100$$

Furthermore, by the definition of enzyme hydrolysis, hydroxy numbers of soy-based polyols were approximately equal to the acid numbers of the reaction products before product workup.

d) Acid enrichment number

After the product workup, saturated fatty acids, both in the ester glycerides phases and the acid residue phases, were quantitatively and qualitatively analyzed to perform mass balance and assure consistency of the results. Percentages of saturated fatty acids in the acid residue were compared to their percentages in the bodied soybean oil and reported in term of acid enrichment number (AEN). The following equation defines the enrichment number:

Enrichment of acyl-fatty acid ‘A’ in acid residue

$$= \frac{\text{Percentage of 'A' in acid residue}}{\text{Percentage of 'A' in triglyceride substrate}}$$

where A is palmitic acid or stearic acid

Preferred enzyme is the one giving the acid enrichment numbers higher than 1.0 for the saturated fatty acids resulting in less saturated fatty acids in the glyceride phase (polyol product) and the presence of primary alcohol.

e) Gel permeation chromatography (GPC)

GPC was used to investigate molecular weight (MW) distribution chromatograph. Dry sample (50 mg) was dissolved in 5 g of tetrahydrofuran (THF, Chromasol® from Sigma-Aldrich, St. Louis, MO, USA) and 500 μl of the sample solution was transferred to a 1-ml vial. A standard curve, a plot of MW against retention time, was generated from the GPC retention times of polyethylene glycols (MW = 3800, 1600, 1500, and 600), soybean oil, and linoleic acid.

The HPLC, Hewlett Packard series 1100 with degasser, quaternary pump and autosampler was used. Data acquisition, program control, and analysis were set and done by the HP Chemstation software version 06.01 (Palo, Alto, CA, USA). The detector was a light scattering detection, Altech 500 ELSD (Deerfield, IL, USA). Two Viscogel-columns, I-MBLMW-3078 from Viscotek (Houston, TX, USA) were connected in series. The mobile phase was THF with a flowrate of 0.5 ml/min at a pressure of 16.2 psig. Drift tube temperature for ELSD was 60^{o}C. Compressed air was the nebulization gas with a flowrate of 3.0 l/min at a pressure of 7.5 psig. Sample injected volume was set at 25 μl and the needle injector was cleaned by THF after each sample injection. Analyzing time was 40 min per sample.

5.3. RESULTS AND DISCUSSION

5.3.1. Esterification for GC-FID analysis

Methanol is usually used in the esterification of glycerides and of fatty acids before analysis by GC. However, ester glycerides and fatty acids from bodied soybean oil were difficult to esterify with methanol, or even with ethanol. Immiscible oil in the solvent was visible when methanol or ethanol was used. The longer acyl alcohols, n-propanol as well as n-butanol were found to esterify completely the bodied soybean oil and its hydrolyzed products. Homogeneous liquid product was obtained with n-propanol or n-butanol so that n-butanol was used in this study.

5.3.2. Recovery of liberated fatty acids and ester glycerides

Two reactions were performed to hydrolyze bodied soybean oil with enzyme *C . rugosa* (1.8 mg enzyme/gram oil). After the reaction reached 40% hydrolysis (acid number about 76 mg KOH/g), the products were washed with different base solutions; Na_2CO_3 (0.5 M, pH 11.0) and $NaHCO_3$ (0.5 M, pH 8.0), to remove fatty acids. Table 5 shows acid numbers of hydrolysis products washed by Na_2CO_3 and $NaHCO_3$ solutions (0.5 M).

Two-phase separation was observed with Na_2CO_3 solution (0.5 M, pH 11.0) and the base solution efficiently washed most of the fatty acid soaps out from the ester glyceride phase. The $NaHCO_3$ solution (0.5 M, pH 8.0) did not remove all the fatty acid due to its lower pH. Hayes et al. [7] explained that the

base solution with a pH range of 11.0 to 13.0 effectively saponified and removed most of the fatty acid in the ester glyceride phase.

Table 5. Effect of pH on fatty acid removal after enzyme hydrolysis.

Reactor no.	Base solution to form fatty acid soaps	After treated by based solution	
		Acid number	% acid removal
1	0.5 M Na_2CO_3 (pH 11.0)	10	87%
2	0.5 M $NaHCO_3$ (pH 9.0)	75	1%

5.3.3. Enzyme hydrolysis and the significance in hydrolyzing saturated fatty acids

The percent hydrolysis (Table 6) is defined by the acid number of hydrolyzed product. Saturated fatty acid content in the bodied soybean oil is about 17% which is a little higher than in original soybean oil triglycerides (15%). If 17% of saturated fatty acids is targeted to be cleaved off, the percent hydrolysis will be about 32%. Hydrolysis of 32% was obtained after less than 24 h of reaction by all enzymes except lipase from *A. niger*. Hydrolysis rate by enzyme *A. niger* was slower than by the others.

Acid enrichment numbers of saturated fatty acids in the acid residue phase were calculated and reported in Table 6. The acid enrichment numbers that 1 represent that the enzyme having significance in hydrolyzing the saturated fatty acids. Accordingly, percent of saturated fatty acid in the hydrolyzed

glyceride (polyols product) phase will be less than in the bodied soybean oil, and preferable primary alcohol should be present.

Lipases from *B. cepacia*, *A. niger*, *M. javanicus*, and *R. miehei* showed acid enrichment number higher than 1 for palmitic acid and stearic acid in the acid residue phase for some conditions. Most enzymes tend to be 1,3-regioselective and these four enzymes are known as 1,3-regionselective enzymes. *C. rugosa* lipase is reported to be non-selective lipase [8-10]; however, this does not follow the observations summarized in Table 6.

Lipase from *C. rugosa* showed significance in the hydrolysis of palmitic acid, the shortest fatty acid moiety present in soybean oil. This is possibly due to its tunnel-like binding site which prefers to bind straight fatty acid rather than to bind bulky substrates [11]. Larger fatty acids or fatty acid moieties derived from heat bodying process tend to bend and be bulky, so that palmitic acid is probably easier and faster to fit in the lipase *C. rugosa*'s active site.

Soybean oil contains about 85% of unsaturated fatty acids and about 15% of saturated fatty acids. Ninety-nine percent of the saturated fatty acids are at the 1,3-positions and about 50% of the 1,3-positoins are occupied by unsaturated fatty acids. Furthermore, fatty acid moieties at the 2-positions are able to migrate during hydrolysis reactions [12]. A high percent of hydrolysis will lead to reduced acid enrichment numbers for the saturated fatty acids.

Table 6. Hydrolysis (%) and acid enrichment numbers of saturated fatty acids in the acid residue phase after the hydrolysis of bodied soybean oil.

Enzyme	1.5 h			3 h			24 h		
	AEN	Hydrolysis (%)	OH-number (mgKOH/g)	AEN	Hydrolysis (%)	OH-number (mgKOH/g)	AEN	Hydrolysis (%)	OH-number (mgKOH/g)
C. rugosa	1.6 ± 0.1			1.4 ± 0.2			1.3 ± 0.2		
(C16:0)									
(C18:0)	1.1 ± 0.1			0.9 ± 0.1			0.9 ± 0.1		
	-	22	~ 42	-	27	~ 51	-	42	~ 80
B. cepacia	1.1 ± <0.1			1.2 ± 0.1			1.2 ± 0.1		
(C16:0)									
(C18:0)	1.0 ± <0.1			1.1 ± 0.1			1.1 ± 0.1		
	-	24	~ 46	-	35	~ 67	-	44	~ 84
A. niger	1.2 ± <0.1			1.1 ± 0.1			1.0 ± 0.1		
(C16:0)									
(C18:0)	1.1 ± <0.1			1.0 ± 0.1			1.0 ± 0.1		
	-	15	~ 29	-	15	~ 29	-	23	~ 44
M. javanicus	1.1 ± 0.1			1.1 ± 0.1			1.1 ± <0.1		
(C16:0)									
(C18:0)	1.0 ± 0.1			1.1 ± 0.1			1.0 ± <0.1		
	-	17	~ 32	-	21	~ 40	-	43	~ 82
R. miehei	-			1.1± 0.1			1.1 ± 0.1		
(C16:0)									
(C18:0)	-			1.0± <0.1			1.1 ± 0.1		
	-	-	-	-	29	~ 55	-	39	~ 74

AEN = acid enrichment number in acid residue portion

Hydrolysis (%) = percent hydrolysis

OH-number = estimated OH-number

The hydrolysis of bodied soybean oil by enzyme *P. roquefortii* (1.5 h, 3 h and 24 h) and *R. miehei* (1.5 h) were also performed. Their results are not reported here because their fatty acid soaps could not be separated from the polyols during the product workup. The solutions were cloudy and jelly-like after washing with the base solution.

5.3.4. Acid and hydroxy numbers

Typically, commercial soy-based polyols have hydroxy numbers greater than 50 (mg KOH/g) and acid number less than 8 (mg KOH/g). High acid numbers in polyols will delay reaction times for polyurethane synthesis, and the carboxylic groups will undesirably consume isocyanates, forming unstable anhydrides [1]. Acidity will also consume catalyst.

Hydroxy numbers reported in Table 6 were equal to acid number of the hydrolyzed product (before product workup) because one mole of hydroxy is formed when one mole of acid is hydrolyzed. A few polyol products were tested their hydroxy numbers by the standard method of hydroxy number titration (ASTM D4274, 2005). The reported hydroxy numbers in Table 6 were comparable to the numbers from the titration method.

Soy-based polyols having hydroxy number of > 40 (mg KOH/g) can be produced by some enzyme hydrolysis after short reaction time (Table 6). Lipases *C. rugosa* and *B. cepacia* can quickly produce polyols having the hydroxy number of > 40 (mg KOH/g) after 1.5 h of the hydrolysis. The polyols which have hydroxy number > 40 (mg KOH/g), were also produced by lipases *M. javanicus*

and *R. miehei* after 3 h, and by lipase *A. niger* after 24 h of the reaction. However, commercial soy-based polyols usually have the minimum hydroxy number higher than 50 (mg KOH/g) which can be obtained by the longer reaction time.

Even though a carbonyl group is hydrolyzed, that fatty acid moiety might not be released from ester glycerides because it could be bounded to other fatty acid moieties (from bodying reaction) attached to other ester glyceride molecules. Higher functionality can be obtained by increasing the amount of enzyme used or extending reaction time at the expense of removing unsaturated fatty acids or cross-linked fatty acids. As a result, the polyols would have high acid number with bound carboxylic acid moieties and lower average molecular weights.

In addition, the product workup was found to be more difficult when the reaction time was longer. The product was emulsion after the saponification and the separation process took longer. Mutiple times of product workup were applied to the product having high acid number to remove most of the hydrolyzed acids and reduce acid number.

5.3.5. Molecular weight distribution

The GC-chromatographs of fatty acid butyl esters of soybean oil and bodied soybean oil are presented in Figure 29. Bodied soybean oil was not fully polymerized after 45 min of heat bodying process. The linolenic acids (C18:3) were converted completely after 30 min of the heating process, but some of

linoleic acid (C18:2) and oleic acid (C18:1) remained in the bodied soybean oil. Erhan et al. [13] also found the rapid reaction of linolenic acid moiety in the bodying of soybean oil.

The GC-chromatograph of bodied soybean oil revealed new fatty acid peaks next to the oleic acid and linoleic acid peaks. These new peaks could not be fully resolved by extending the heating time and decreasing the heating rate. These new peaks could be conjugates and isomers of linoleic acid and oleic acid.

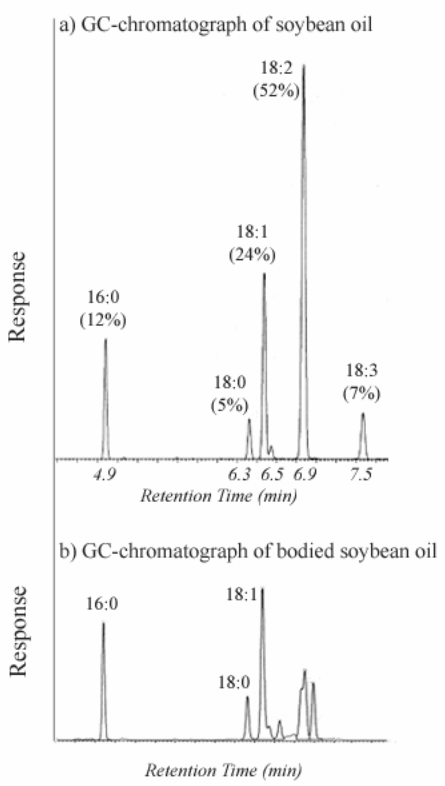

Figure 29. GC retention times of acid moieties in soybean oil and bodied soybean oil (bodying 45 mins at 330 ^{o}C).

Non-fully polymerized bodied soybean oil was also confirmed by GPC analysis. The GPC- chromatograph of bodied soybean oil showed that the bodied soybean oil contains one, two, and three units of soybean oil triglyceride (TAG) after 45 min of the bodying.

With heat only in the bodying process, soybean oil possibly polymerizes by a chain-growth mechanism rather than a step growth mechanism. This leads to a bimodal distribution in the final product. The bodying process is currently being improved to achieve proper MWs of bodied soybean oil with uniform molecular structures. Heterogeneous catalysts are being evaluated as a means to both reduce reaction temperatures and promote step-growth mechanisms.

GPC analysis of two of the polyol products produced from the hydrolysis of bodied soybean oil by lipase *C. rugosa*, before and after product workup are given in Figure 30 and Figure 31.

The GPC triglyceride peak (retention time = 30.2 min) of bodied soybean oil was significantly decreased after the hydrolysis by lipase *C. rugosa* after 24 h. Considering Figure 30(a) and Figure 31(a), the lipase preferably attacked ester bonds of triglyceride molecules and produced more normal fatty acid (MW~300, retention time = 33.7 min) during the 1.5 h to 24 h of reaction. This can be explained by the tunnel-like binding site of lipase *C. rugosa*, which prefers to bind straight chains rather than bulky fatty moieties. However, high molecular weight derivatives as revealed by GPC analysis of the acid residue phase (Figure 30(c) and Figure 31(c)) indicate that *C. rugosa* lipase liberated both normal and cross-linked fatty acids from the bodied soybean oil.

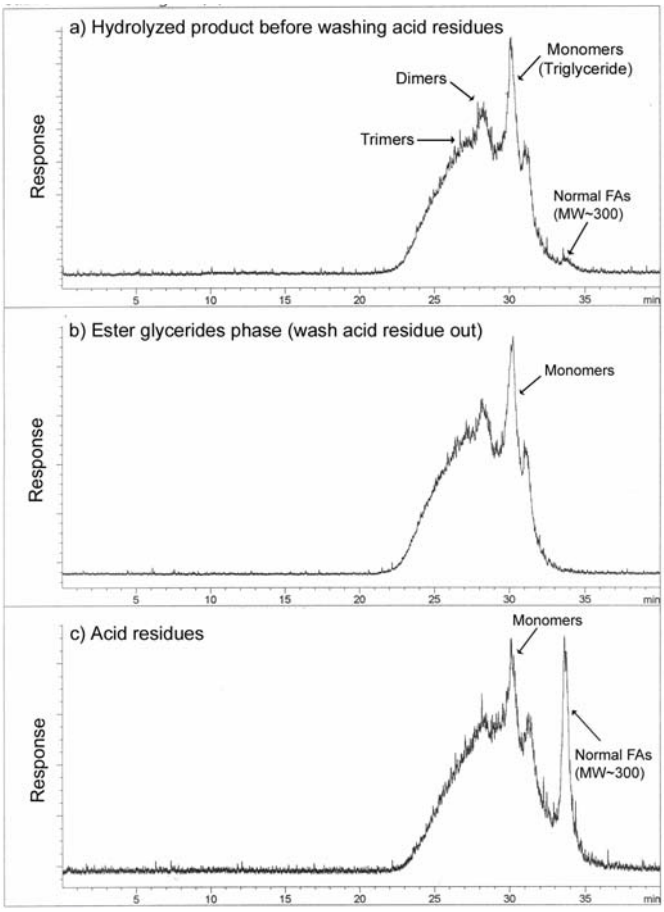

Figure 30. GPC analysis of selective hydrolysis of bodied soybean oil by lipase *C. rugosa* after 1.5 h; before product workup (a) and after product workup (b) and (c).

The polyols produced from the hydrolysis of bodied soybean oil by *C. rugosa* lipase, as determined by Figure 30(b) Figure 31(b), were derived from one, two, and three unit oligomers of soybean oil triglycerides.

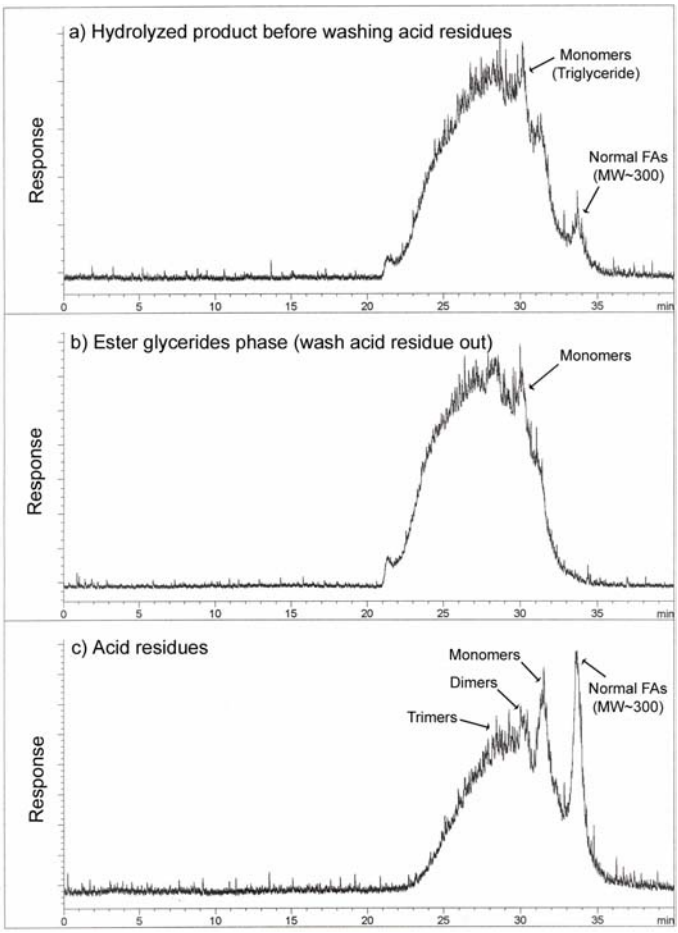

Figure 31. GPC analysis of selective hydrolysis of bodied soybean oil by lipase *C. rugosa* after 24 h; before product workup (a) and after product workup (b) and (c).

5.4. CONCLUSIONS

Bodied soybean oil was successfully functionalized by enzyme hydrolysis to produce soy-based polyols with primary functionality and molecular weights higher than traditional soy-based polyols. Enzymes from *C. rugosa* significantly

liberated palmitic acid, the shortest fatty acid moiety in bodied soybean oil, because of its tunnel-like binding site. The lipase is recommended to use in the hydrolysis of bodied soybean oil to produce soy-based polyols.

The simple hydrolysis of bodied soybean oil by lipase *C. rugosa* produced soy-based polyols having hydroxy number of about 50 (mg KOH/g), after 3 h. The hydroxy number is comparable to the number of some commercially petrochemical based polyols.

Furthermore, the product from the green or enzyme process have lower saturated fatty acids contents and reactive primary alcohols—both of which are difficult to attain using soybean oil as a feedstock.

5.5. REFERENCES

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CHAPTER 6

PREPARATION OF HIGH HYDROXYL EQUIVALENT WEIGHT POLYOLS FROM VEGETABLE OIL USED IN POLYURETHANES

6.1. INTRODUCTION

Markets for vegetable oil based polyols in the polyurethane are growing due to the economic, environment and availability advantages. Castor oil triglyceride and soy-based polyols are commercially available and currently used in some polyurethane formulations [1-4]. Castor oil and a typical commercial soy-based polyol (Alkoxyl hydroxyl soybean oil), which are triglyceride backbone polyols, are depicted in Figure 32(a) and (b).

To produce triglyceride-backbone polymers or pre-polymers, oxirane opening of epoxidized soybean oil (ESBO) with water, alcohol, amine and carboxylic acid have been studied [5]. The products are used in the fields of polymer synthesis, lubricants and detergents [5].

In the polyurethane applications, water and alcohol have been used to open the epoxy rings of epoxidized soybean oil and produce commercial soy-based polyols, such as the alkoxyl hydroxyl soybean oil in Figure 32(b). However, the final polyols have low hydroxyl (OH) equivalent weights that limit applicability. If the vegetable based-polyols had high OH equivalent weight, the sustainable materials could be more engaged in the polymer structure replacing utilization of non-renewable materials for urethane consumers.

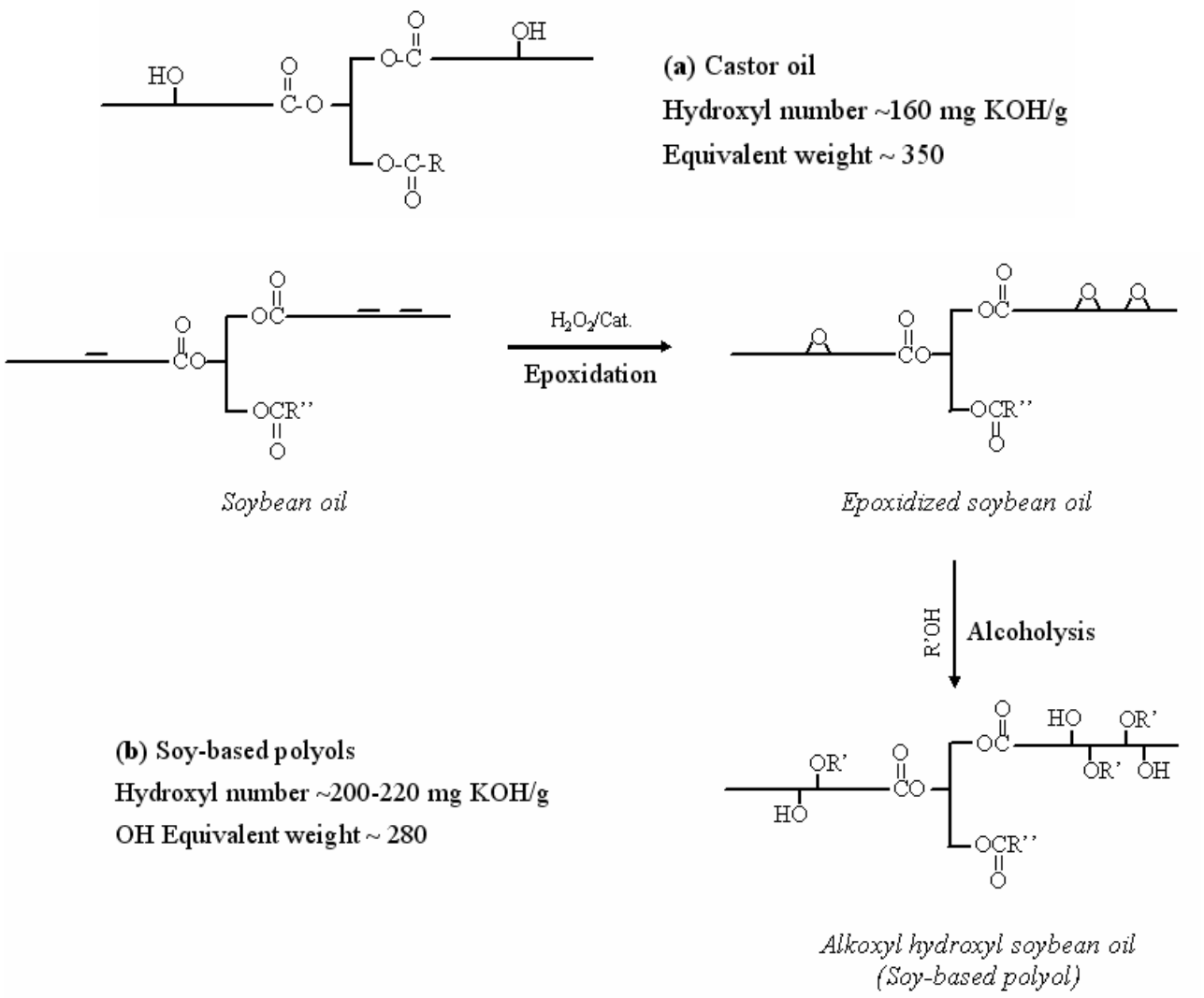


Figure 32. Castor oil (a) and a typically commercial product of soy-based polyols (b) or alkoxyl hydroxyl soybean oil.

To produce the high OH equivalent weight, high MW acid moieties are synthesized followed by reaction-addition to the epoxy (oxirane) rings of epoxidized soybean oil. The process is simple where ESBO is combined and

reacted with the acid moieties at 170 ^{o}C. No solvents or catalyst are necessary [5] which is illustrated by Figure 33. The acid moieties could be normal fatty acids, hydrolyzed bodied soybean oil (Figure 34), and fatty acid estolides (Figure 35).

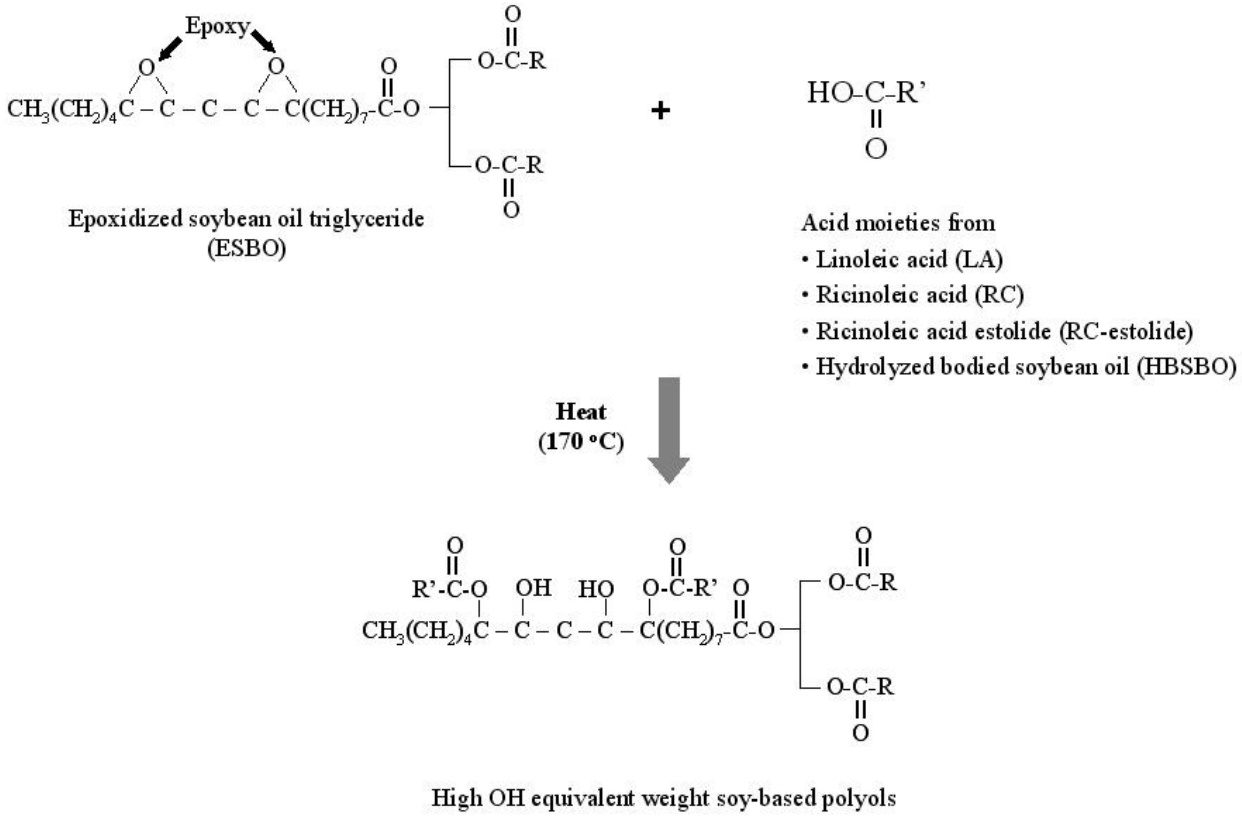


Figure 33. Cleavage of epoxy functional groups of ESBO by acid moieties producing high equivalent weight polyols.

The reaction-addition to ESBO by linoleic acid (LA), which is the predominant fatty acid in soybean oil, was performed as a control experiment.

Ricinoleic acid (RC) was used to add alcohol moiety and potentially create a competitive, higher equivalent weight polyol [1-2].

In addition, the epoxy rings were reacted with the acid estolides having the average MW of higher than 700. The acid estolides are made from enzymatic esterification of ricinoleic acid which is the green chemistry method and yields high selectivity.

High MW acid of hydrolyzed bodied soybean oil (HBSBO) was also added to the epoxy functional group. The hydrolyzed bodied soybean oil was synthesized by a two-step process consisting of heat bodying (heat polymerization) of soybean oil followed by enzyme hydrolysis of the bodied soybean oil (BSBO) illustrated by Figure 34. Presence of reactive primary alcohol from the enzyme hydrolysis is a possibly advantage of the polyol made from HBSBO and ESBO.

Properties of the final polyols; namely, acid number, hydroxyl number, hydroxyl equivalent weight, epoxy content and viscosity, were determined to compare these new vegetable based polyols with a commercially available soy-based polyols.

The objective of this study was to investigate environmental-friendly method to produce high OH equivalent weight polyols from vegetable oils. The product was targeted to be comparable to, or better than the commercially available soy-based polyols served as a B-side material in the preparation of polyurethanes

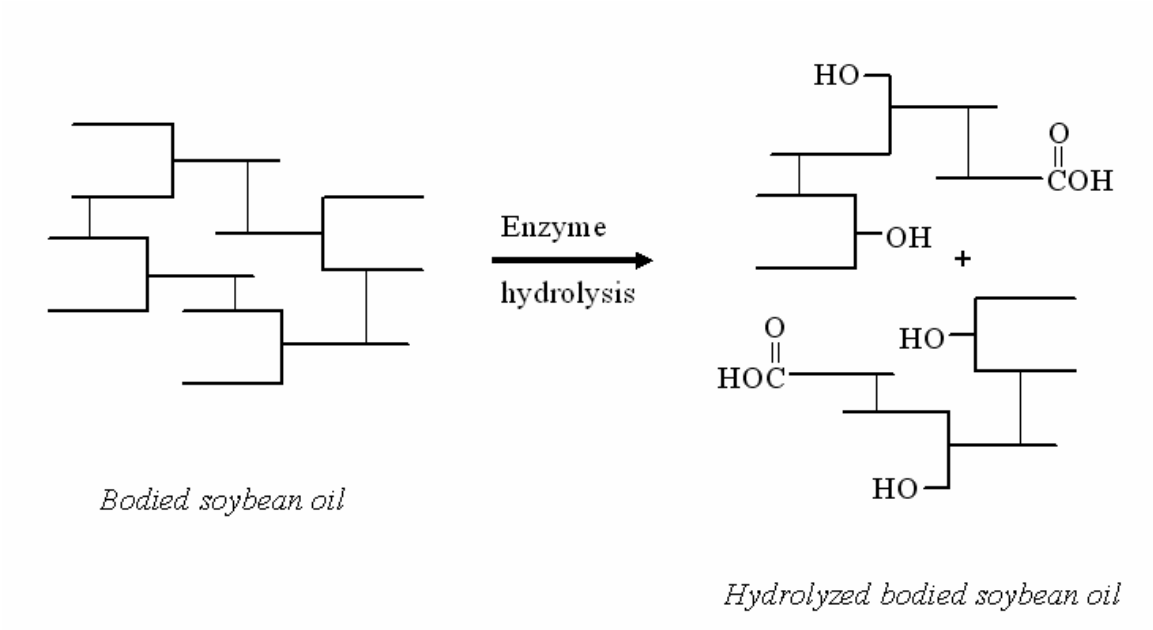


Figure 34. Bodied soybean oil (BSBO) and an example of hydrolyzed bodied soybean oil (HBSBO).

6.2. MATERIALS AND METHODS

6.2.1. Materials

Castor oil is purchased from Alnor Oil Company (Valley Stream, NY). Soybean oil (food grade) is obtained from a local grocery store. Epoxidized soybean oil (Vikoflex® 7170) is purchased from Atofina Chemicals (Philadelphia, PA). Ricinoleic acid (technical grade) is purchased from Arro Corporation (Hodgkins, IL). Enzyme *Candida rugosa* (lipase Amano "AYS") is a gift from Amano Enzyme Inc. USA (Elgin, IL). Immobilized lipase B from *C. antarctica* (Novozyme 435®), lipase *R. miehei* and anthraquinone catalyst (90%) are obtained from Sigma Aldrich (St. Louis, MO).

6.2.2. Determination of acid number and acid equivalent weight

Acid numbers of dry samples are evaluated according to the method of acid value, AOCS Te 1a-64.

The equivalent weight of Ricinoleic acid estolides, which are produced from the enzyme esterification, can be determined by the following equation:

$$\text{Acid equivalent weight} = \frac{56.1 \times 1000}{\text{Acid number (mg KOH/g)}}$$

Acid numbers (mg KOH/g sample) are one of the typical properties reported in commercial polyol specification. The numbers are normally less than 10 (mg KOH/g). The acid number should be kept low because the reaction between carboxylic group and catalyst or isocyanate is not preferable and causes slow foaming reaction rate [10].

6.2.3. Determination of hydroxyl number and hydroxyl equivalent weight

Hydroxyl number is one of the most important properties of polyols indicating numbers of hydroxy functional group per gram of dry polyols. The number is evaluated according to the determination of hydroxyl numbers of polyols, ASTM 4274-05.

Hydroxyl equivalent weight is defined as “number of grams of sample required so that one gram equivalent weight of hydroxyl (17.008) will be present in the sample” [6]. Polyols’ equivalent weight can be determined by the following equation:

$$\text{Hydroxyl equivalent weight} = \frac{56.1 \times 1000}{\text{Hydroxyl number (mg KOH/g)}}$$

The hydroxyl equivalent weight is also defined as the ratio of molecular weight to hydroxyl functionality.

6.2.4. Determination of viscosity

The viscosity of the polyols is measured by Stress & Strain control Rheometer, Rheostress-RS1000 (HAAKE by Thermo, Waltham, MA). The viscosity test is performed at 22 ± 0.5 ^{o}C

6.2.5. Determination of epoxy content

The epoxy content of a dry sample is analyzed by an official method, AOCS Cd 9-57, oxirane oxygen. The number indicates unreacted epoxy functional groups after the reaction of epoxy opening.

6.2.6. Synthesis of ricinoleic acid estolides

To produce ricinoleic acid (RC) estolides, lipase from *C. rugosa* and immobilized lipase B from *C. antarctica* (Novozyme 435®) are used in the esterification without any organic solvent. The esterification takes place at temperatures of 40 ^{o}C and 60 ^{o}C, and at pressures of 1 atm (open system) and 0.63 atm. The reaction scheme can be explained by Figure 35. Vacuum pressure (0.63 atm) was applied to remove water, an esterification product, and prevent the reversible reaction from taking place.

Purchased nricinoleic acid had an acid number of 142 (mg KOH/g) which can be converted to the acid equivalent weight of about 395. Acid numbers of ricinoleic acid decreased when the fatty acid was kept at room temperature (22 ^{o}C) due to slowly condensation polymerization [7]. To maintain the acid number of the hydroxy fatty acids, all samples were kept in the refrigerator (below 5 ^{o}C).

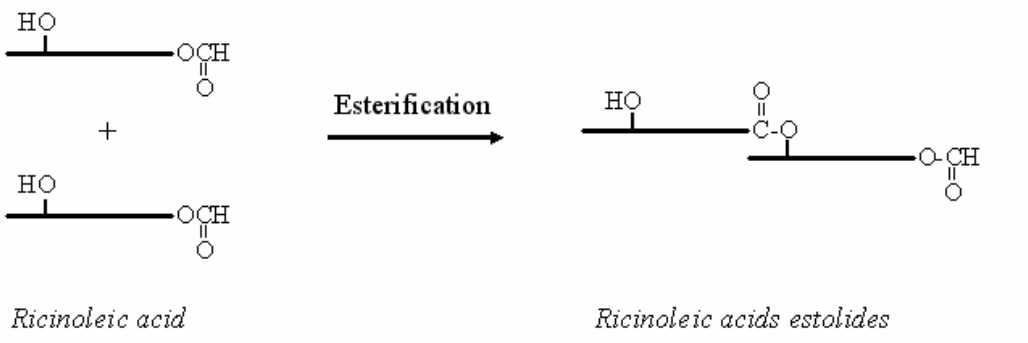


Figure 35. Preparation of RC estolides by enzyme esterification.

To start the esterification, enzyme *C. rugosa* (0.6 g) or Novozyme 435 (1 g) is combined with 15 g of ricinoleic acid fatty acid in a 125-erlenmeyer flask and the operation mode is well-mixed batch. Three reactions are performed concurrently and the standard deviation is calculated.

After the reaction is performed (usually after 120 h), the immobilized enzyme is removed from the reaction product by centrifuge. Acetone is used to

wash Novozyme 435® and the immobilized enzyme is reused for the next reaction after evaporating acetone at 60 °C.

Novozyme 435® is reused to investigate the enzyme's life time. The lipase is washed with acetone and dried after every reaction before recycled.

6.2.7. Synthesis of bodied soybean oil and hydrolyzed bodied soybean oil

BSBO is produced by heating soybean oil with 2.5% of anthraquinone catalyst at 260°C for 6 h. The bodying process is done in 1-liter Parr reactor with volatile matters being removed during the reaction by a venting channel. The solid catalyst is reusable and is removed from the BSBO by centrifuge. After 6 h, the bodying process increases the viscosity by 5.5 times and reduces the iodine number by 25% with viscosity and iodine values for the bodied soybean oil of 313 mPa·s (at 22 °C) and 90, respectively. The heat bodying process and mechanism are reported by Erhan et al. [8-9].

BSBO is partially hydrolyzed by commercial lipases without any surfactant or organic solvent. The bodied soybean oil, phosphate buffer pH = 7.0 (0.7 g/g oil), and *R. miehie* lipase (6.6 μl/g oil) are combined and mixed before the reaction starts. The hydrolysis takes place at 45°C and 1 atm in a well mixed reactor for 3 days.

After the reaction, water and enzyme are separated from the oil phase by centrifuging (4000 rpm, 30 min). The product HBSBO has an acid number of about 83 (mg KOH/g). Examples of BSBO and HBSBO are displayed in Figure

34. The HBSBO has acid functional groups with high MW and are furthered used to open the epoxy ring of EBSO.

6.2.8. Preparation of polyols from reaction-addition to epoxidized soybean oil by linoleic acid and ricinoleic acid

Linoleic acid (LA) and ricinoleic acid (RC) are used to open oxirane rings of epoxidized soybean oil (ESBO). To perform the reaction, ESBO and LA (acid number = 190 mg KOH/g), or ESBO and RC (acid number = 142 mg KOH/g) are combined and reacted at 170 ^{o}C, atmospheric pressure. The reaction is simply performed in a well-mixed batch reactor. Three ratios of epoxy functional group to acid functional group are used; 1:1, 1:0.8 and 1:0.5 by mole. Samples are collected with respect to time to measure acid number and epoxy content for the kinetic studies.

6.2.9. Preparation of from reaction-addition to epoxidized soybean oil by ricinoleic acid estolides

Polyols with the higher hydroxyl equivalent weight are made by the cleavage of epoxy rings with fatty acid estolides. The fatty acid estolides are yielded from enzyme esterification of RC which was previously described. The RC estolide with acid number of 79 (produced under 60 ^{o}C, 1 atm, 120 h by Novozyme 435®) is combined with ESBO when the ratio of epoxy to acid is 1:0.66 by mole. The reaction takes place in a batch well-mixed reactor at 170 ^{o}C and 1 atm until the acid number of polyols product is less than 10 (mg KOH/g).

6.2.10. Preparation of polyols from reaction-addition to epoxidized soybean oil by hydrolyzed bodied soybean oil

HBSBO (acid number = 83 mg KOH/g) is produced from the enzyme hydrolysis of BSBO which was previously described. The HBSBO and ESBO are combined with the ratio of epoxy per acid of 1:0.66. The reaction takes place at 170 ^{o}C and 1 atm until the acid number is less than 10 (mg KOH/g). Acid number and epoxy content are determined against time.

6.3. RESULTS AND DISCUSSION

6.3.1. Synthesis of RC estolides

Acid equivalent weights of ricinoleic acid estolides synthesized by enzyme tranesterification are shown in Figure 36. The higher the reaction conversion is, the higher the acid equivalent weight is or the higher the average MW.

From Figure 36, enzyme *C. rugosa* yielded the higher acid equivalent weight of RC estolides at 40 ^{o}C. For enzyme *C. rugosa*, the vacuum condition did not significantly improve the reaction conversion. The acid equivalent weight reduced 50% at 60^{o}C—the enzyme *C. rugosa* could be deactivated at the higher temperature (60 ^{o}C) reducing its activity and the reaction conversion.

Novozyme 435® yielded a comparable reaction conversion at 60 ^{o}C, and 40^{o}C (vacuum pressure). The reaction conversion was increased about 45% at 40 ^{o}C by the vacuum pressure. Water could be removed from the reaction medium at higher temperature and vacuum resulting in the higher reaction

conversion. Additionally, Novozyme 435® is a thermostable lipase which can be used at high temperature without deactivation.

RC estolides produced from recycled the immobilized lipase, Novozyme 435®, are presented in Figure. 37.

Novozyme 435® yields the comparable reaction conversion at 60 °C, and 40°C (vacuum pressure). The reaction conversion is increased about 45% at 40 °C by the vacuum pressure. Water could be removed from the reaction medium at higher temperature and vacuum resulting in the higher reaction conversion. Additionally, Novozyme 435® is a thermostable lipase which can be used at high temperature without deactivation.

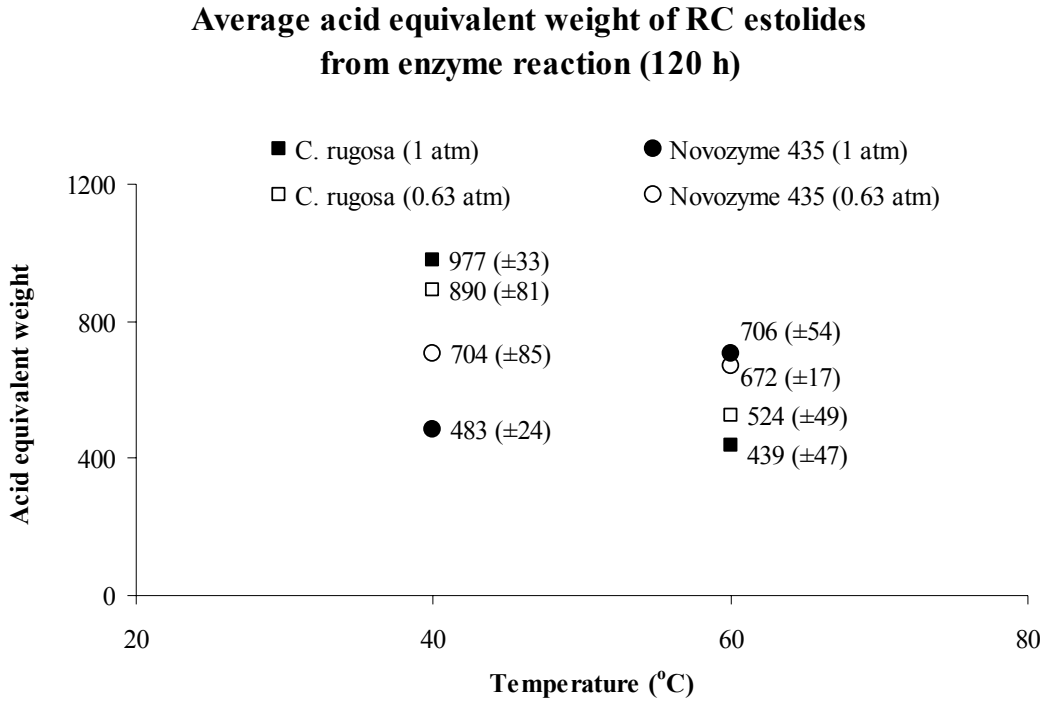


Figure 36. Acid equivalent weight of RC estolides from enzyme esterification (120 h).

RC estolides produced from the recycled immobilized lipase, Novozyme 435®, are presented in Figure 37. The immobilized enzyme, Novozyme 435®, is recycled and used at 1 atm (60 ^{o}C and 70 ^{o}C), as a result (Figure 37), the enzyme's activity and reactivity are still good after 7 times of recycling with a batch well-mixed operation (60-70 ^{o}C).

The average equivalent weight of RC-estolide, (60 ^{o}C, 1 atm, 120 h) is 706 ± 54 (Figure 36). Four data from Figure 37, at the same reaction conditions, are found to have a deviation of 2% to 12% from the average value. Repeatable data is not performed for the enzyme recycling.

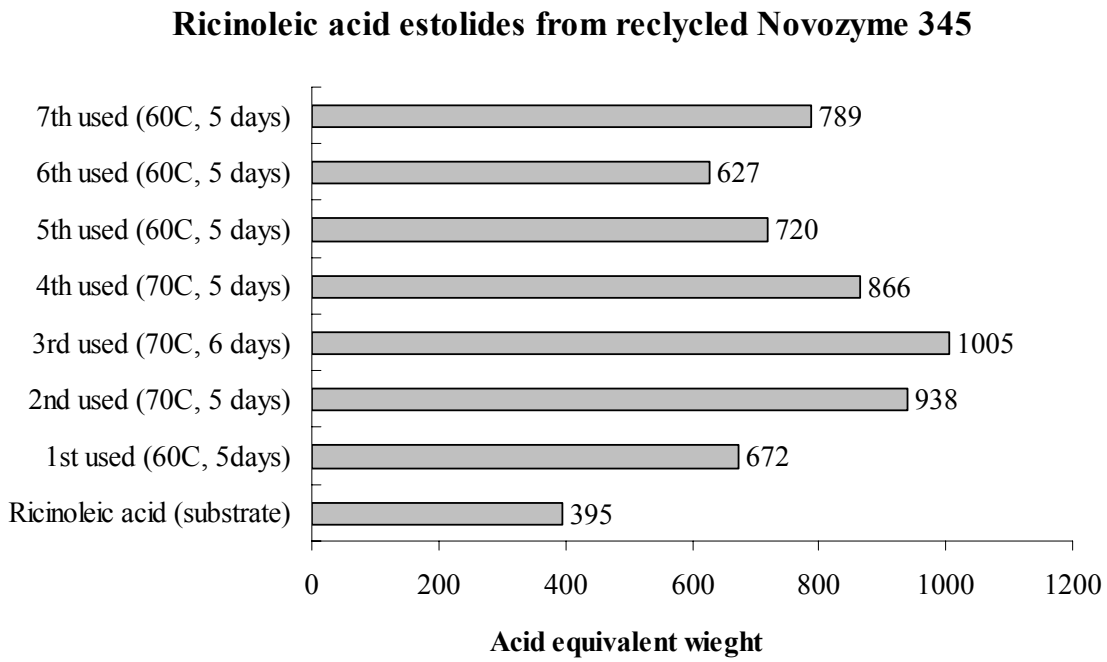
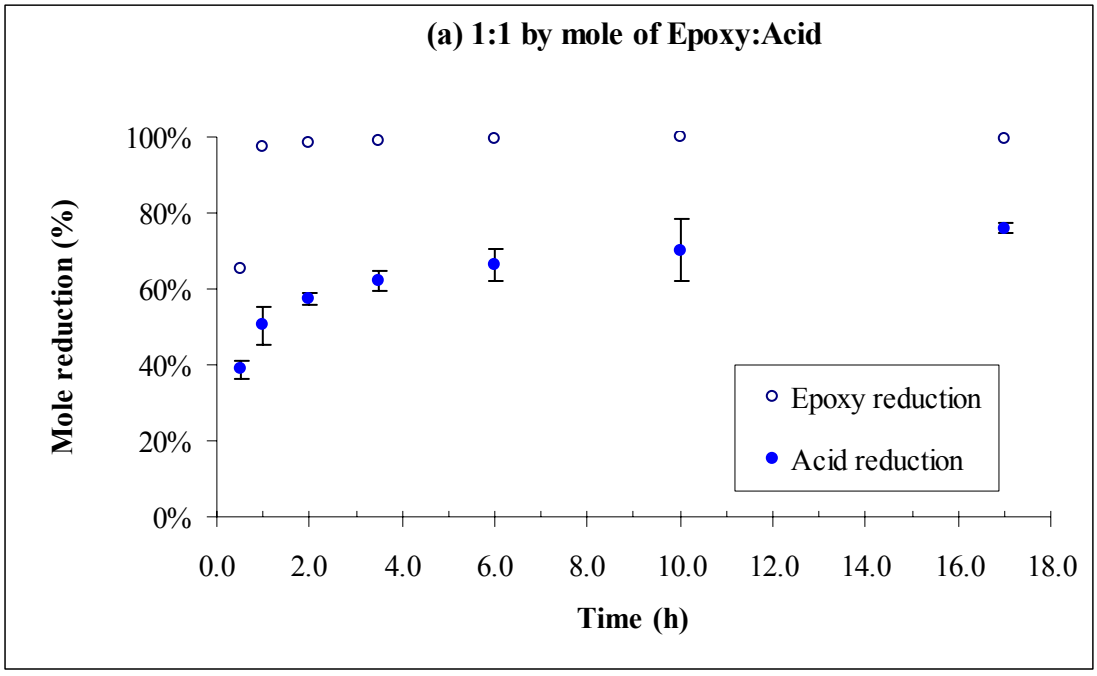


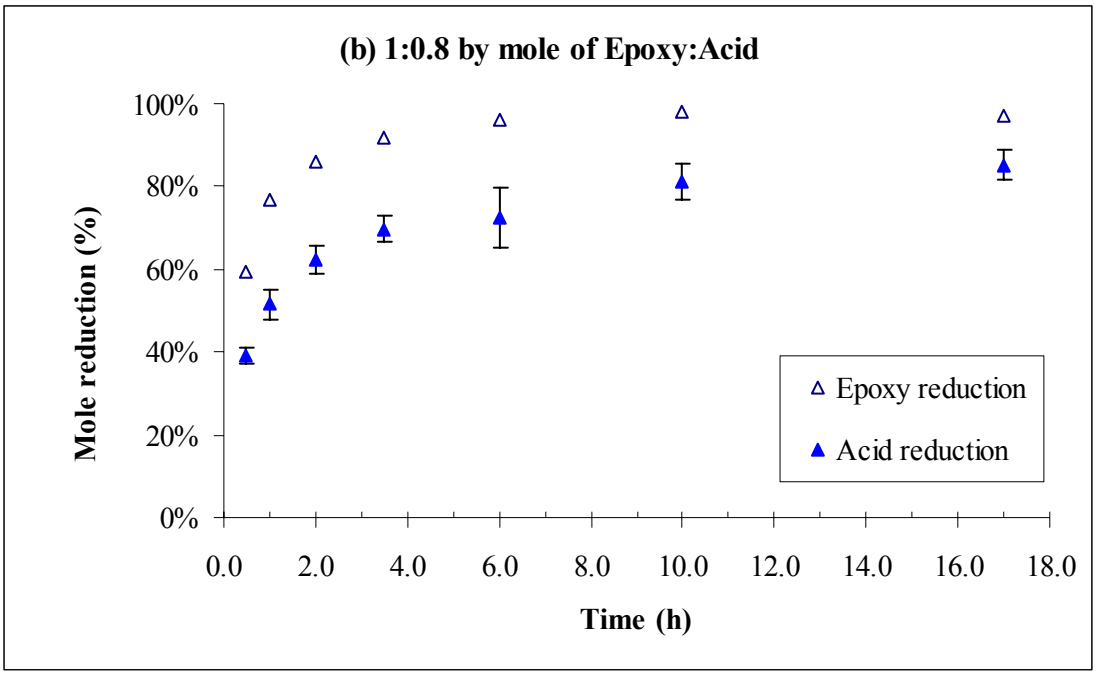
Figure 37. Ricinoleic acid estolides produced from recycled Novozyme 435®.

Although the highest conversion is yielded by enzyme *C. rugosa*, Novozyme 435® is recommended regarding the production cost. The lipase is immobilized (on acrylic resin) which is recyclable and durable. Also, a longer reaction time or higher temperature could yield a higher conversion of this reaction by Novozyme 435®.

6.3.2. Reaction-addition to epoxidized soybean oil by linoleic acid

Moles of acid and of epoxy groups during the reaction are calculated from the epoxy content and the acid number by the standard testing methods, AOCS Cd 9-57 and AOCS Te 1a-64, respectively. The disappearance rates of epoxy and acid groups during the batch reaction between ESBO and LA are shown in Figure 38(a), (b) and (c). The standard deviation for the acid disappearance is shown as bars. The standard deviation of the epoxy degradation is less than 5% for all the data.





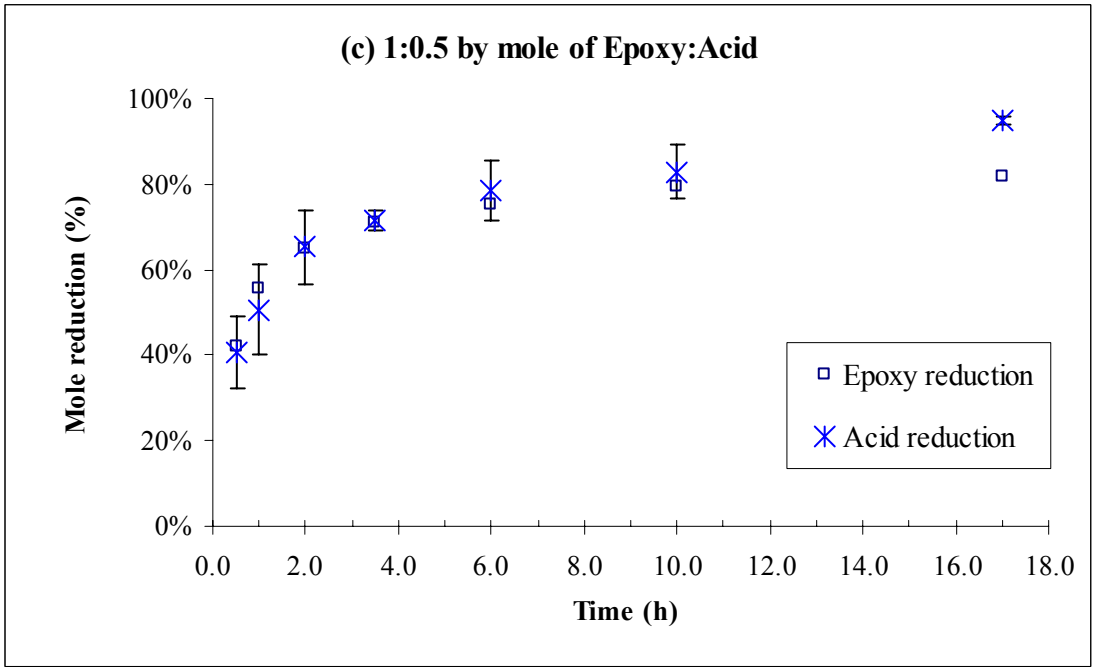


Figure 38. Disappearance rates of epoxy and acid groups during the reaction between ESBO and LA (170 ^{o}C) where three values of mole ratio of epoxy to acid groups were used; 1:1, 1:0.8 and 1:0.5.

At the 1:1 mole ratio (Figure 38(a)), all epoxy functional groups substantially disappeared after 2 h which was faster than the acid functional groups. With the equal mole ratio, the comparable disappearance rates of the both functional groups are desirable with lower by-product production. The higher disappearance rate of epoxy functional groups suggests side-reactions, other than reaction addition of acid groups to epoxy groups, also occur.

In addition to the carboxylic acid reaction with epoxy, hydroxy groups which are produced from the reaction-addition of epoxy group by acid group, can also react with the epoxy. The reaction between the epoxy and hydroxy groups is displayed in Figure 39. Therefore, an excess amount of epoxy functional group is required to eliminate the acid number of the final polyols.

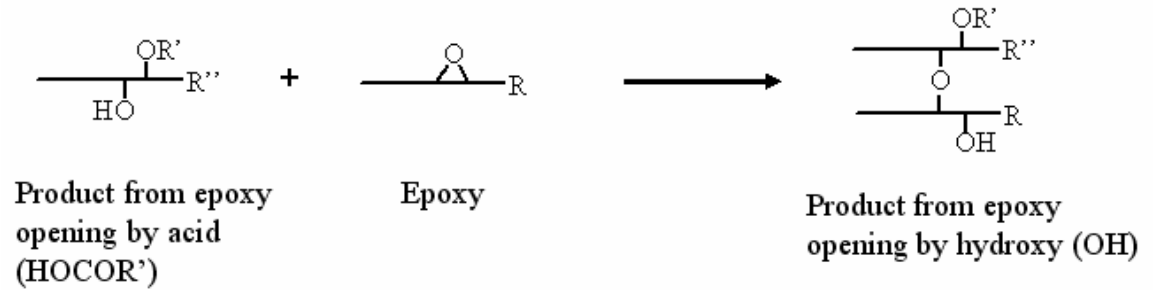


Figure 39. Possible reaction of epoxy and hydroxy groups after the acid groups react.

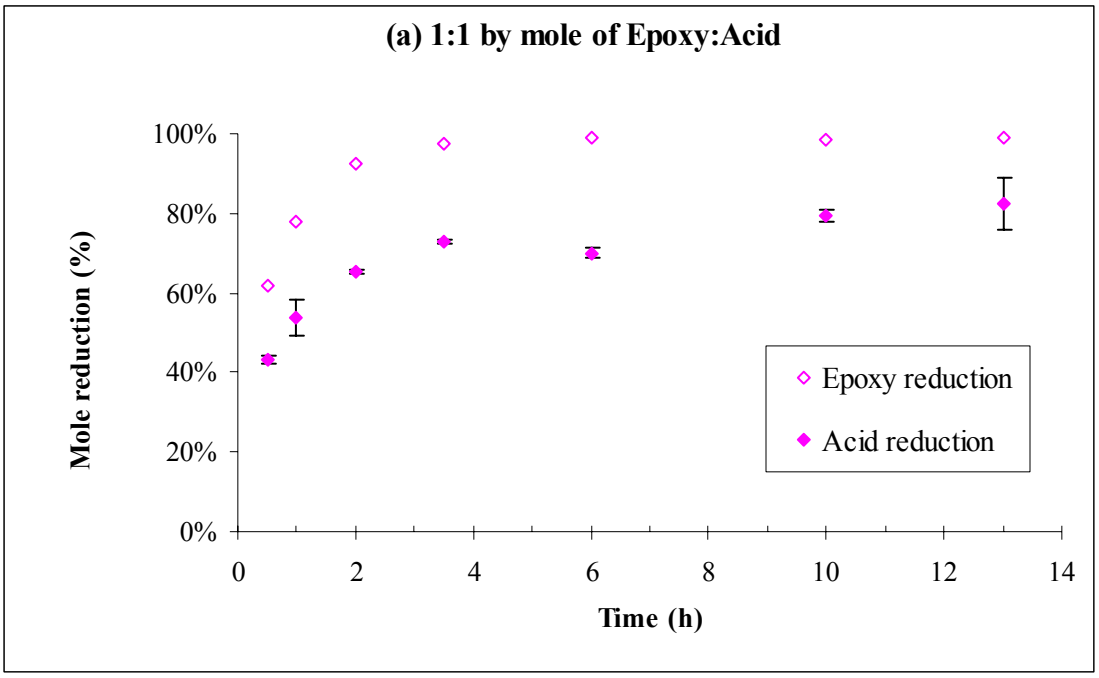
The disappearance rates of the epoxy and acid groups were more similar with the 1:0.8 mole ratio and were comparable with the 1:0.5 mole ratio. The excess epoxy groups were necessary to react with most of the acid groups.

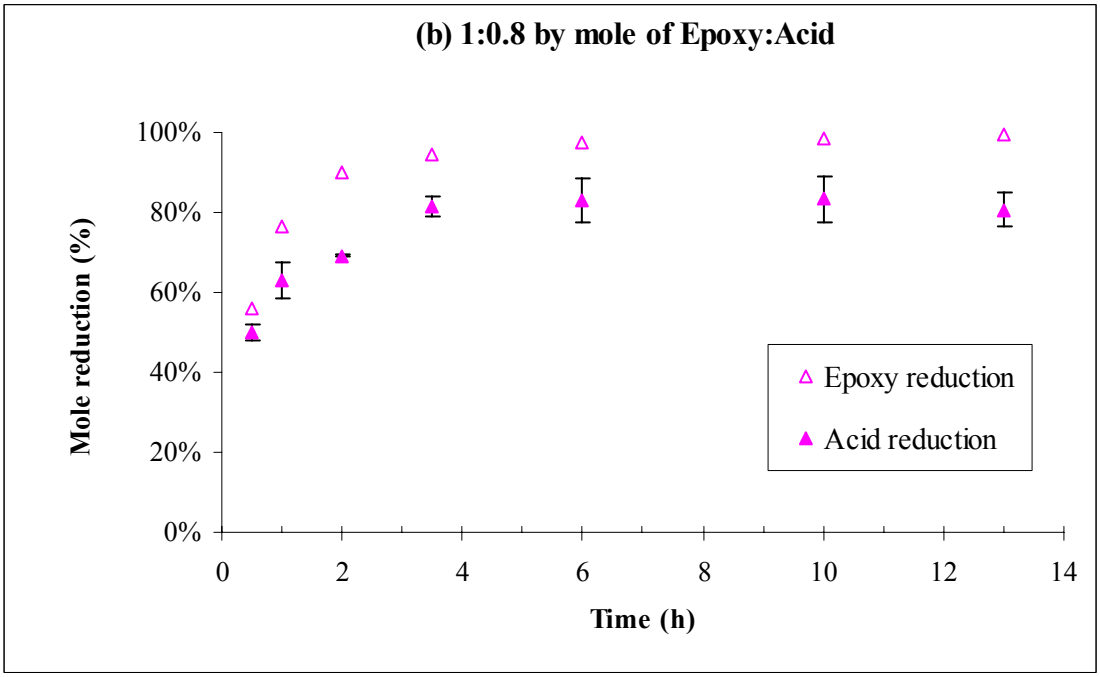
Residual acidity is not desirable in the final polyols. Properties of polyols produced from the reaction-addition of epoxidized soybean oil by linoleic acid are shown in Table 7.

Polyols made from ESBO and LA could contain a significant amount of non-functional moieties which will not react with the isocyanate and compromise the polyurethane's properties [1-2]. This disadvantage was improved by replacing LA with RC which are below presented and discussed.

6.3.3. Reaction-addition to epoxidized soybean oil by ricinoleic acid

The kinetic data of reactions between ESBO and RC are shown in Figure 40 (a), (b) and (c) with mole ratios of epoxy to acid of 1:1, 1:0.8 and 1:0.5. The standard deviation of acid disappearance rates are also shown in the graph. The standard deviations of epoxy disappearance rates are not displayed but they are less than 5%, 10% and 2%, for the data of mole ratios of 1:1, 1:0.8 and 1:0.5, respectively.


(a) 1:1 by mole of Epoxy:Acid
Mole reduction (%)
100%
80%
60%
40%
20%
0%
0
2
4
6
8
10
12
14
Time (h)
Epoxy reduction
Acid reduction


(b) 1:0.8 by mole of Epoxy:Acid
Mole reduction (%)
100%
80%
60%
40%
20%
0%
0
2
4
6
8
10
12
14
Time (h)
Epoxy reduction
Acid reduction

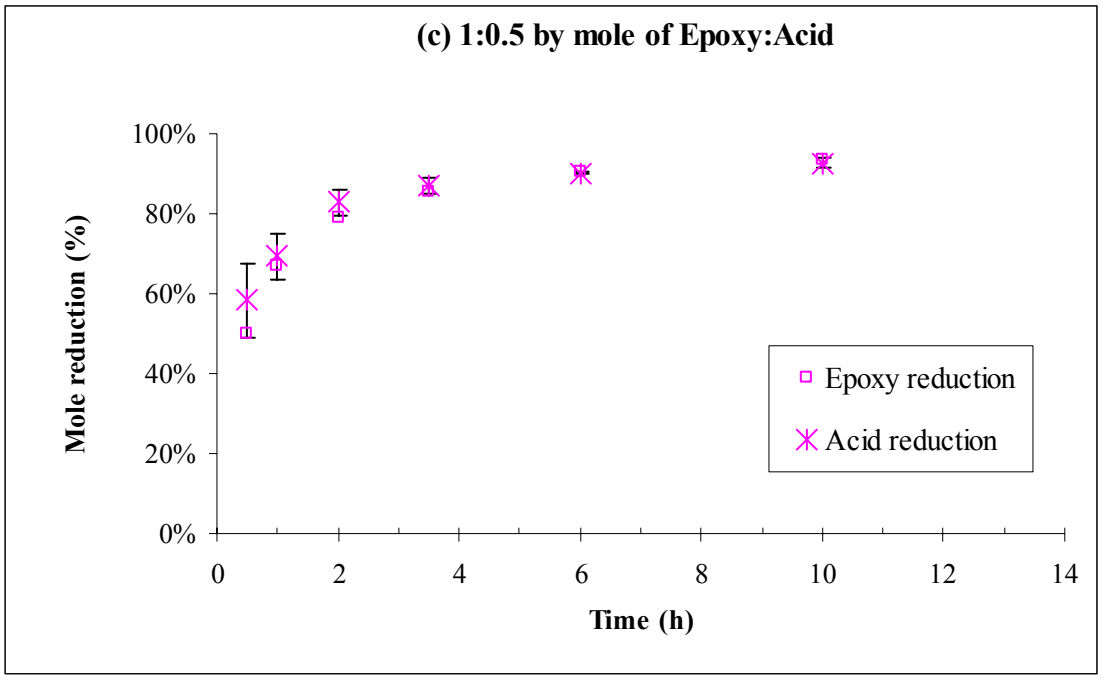


Figure 40. Disappearance rates of epoxy and acid groups during the reaction between ESBO and RC (170 ^{o}C) where three values of mole ratio of epoxy to acid groups are used; 1:1, 1:0.8 and 1:0.5.

With the ratio of 1:1 (Figure 40(a)) and 1:0.8 (Figure 40(b)), the differences between disappearance rates of epoxy and acid are similar and the epoxy groups react faster than the acid groups. The epoxy could also react with hydroxy functional groups created from the epoxy opening reaction which is shown in Figure 39. However, the hydroxy group on RC did not seem to have a faster rate of the epoxy disappearance, when compared with the reaction of ESBO and LA.

The disappearance rates of both of the functional groups are not different with the 1:0.5 ratio (Figure 40(c)). Properties of the final polyols made from ESBO and RC are displayed in Table 7.

6.3.4. Reaction-addition to epoxidized soybean oil by ricinoleic acid estolides

High MW of the ricinoleic acid estolides (equivalent weight = 710) could increase the hydroxyl equivalent weight of final polyols. Disappearance rates of epoxy and acid functional groups are shown in Figure 41 when the mole ratio of epoxy to acid is 1:0.66.

The error bars in Figure 41 represent the standard deviation of the acid disappearance rate where three duplicate reactions are performed. For the epoxy disappearance rate, the calculated standard deviation is less than 7% for the first two data points and less than 4% for the last three data points.

Rates of acid and epoxy appearance are found to be identical when the ESBO and RC estolide reacts at the mole ratio of 1:0.66. Furthermore, the polyol made from RC estolide and ESBO has low acid number (less than 10 mg KOH/g) after only 6 h. However, the acid number of RC estolide was considerably lower than the acid numbers of LA and RC. The final polyol's properties are presented in Table 7.

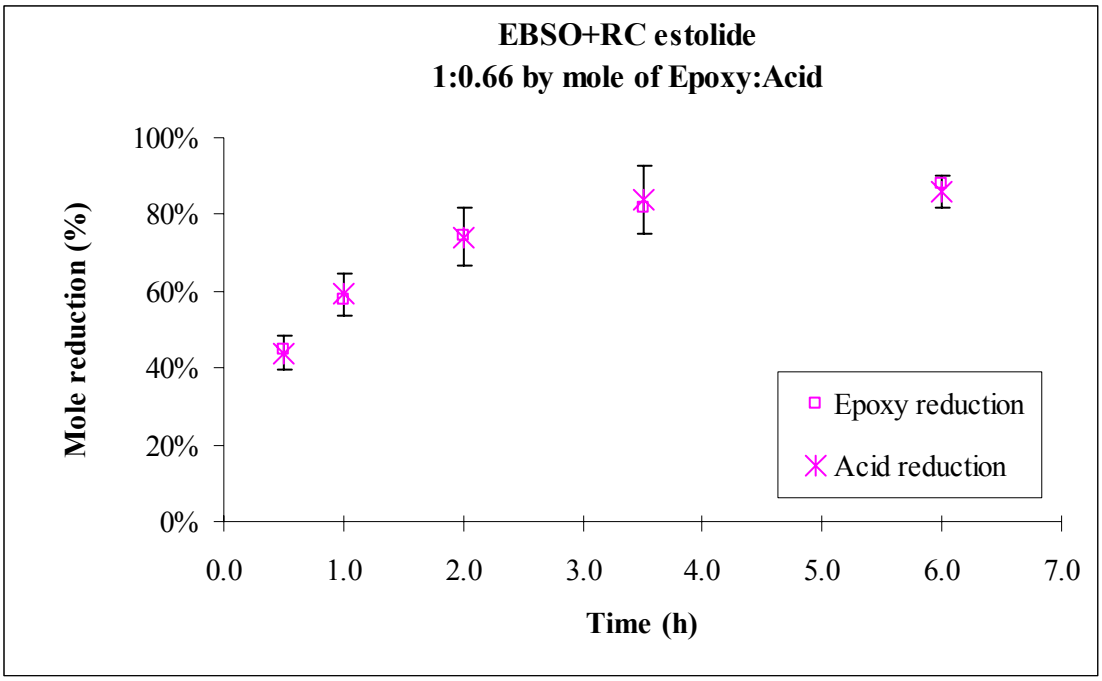


Figure 41. Disappearance rates of epoxy and acid groups during the reaction between ESBO and RC estolide (170 ^{o}C) with the mole ratio of the epoxy to acid of 1:0.66.

6.3.5. Reaction-addition to epoxidized soybean oil by hydrolyzed bodied soybean oil

The hydrolyzed bodied soybean oil has an acid number of 83 (mg KOH/g) which is similar to the RC estolide used in the previous reaction. The same mole ratio of epoxy to acid is used (1:0.66). Kinetic data of the reaction of ESBO and HBSBO is shown in Figure 42.

The standard deviation of the disappearance rate of epoxy functional group is not displayed in Figure 42 which is found to be less than 2% for every data point.

Disappearance rate of epoxy functional group was a little higher than that of acid functional group during the first few hours of reaction. After 6 h, the polyol products have a low acid number (10 mg KOH/g) and the polyol's characteristics are compared to the other polyols as summarized in Table 7.

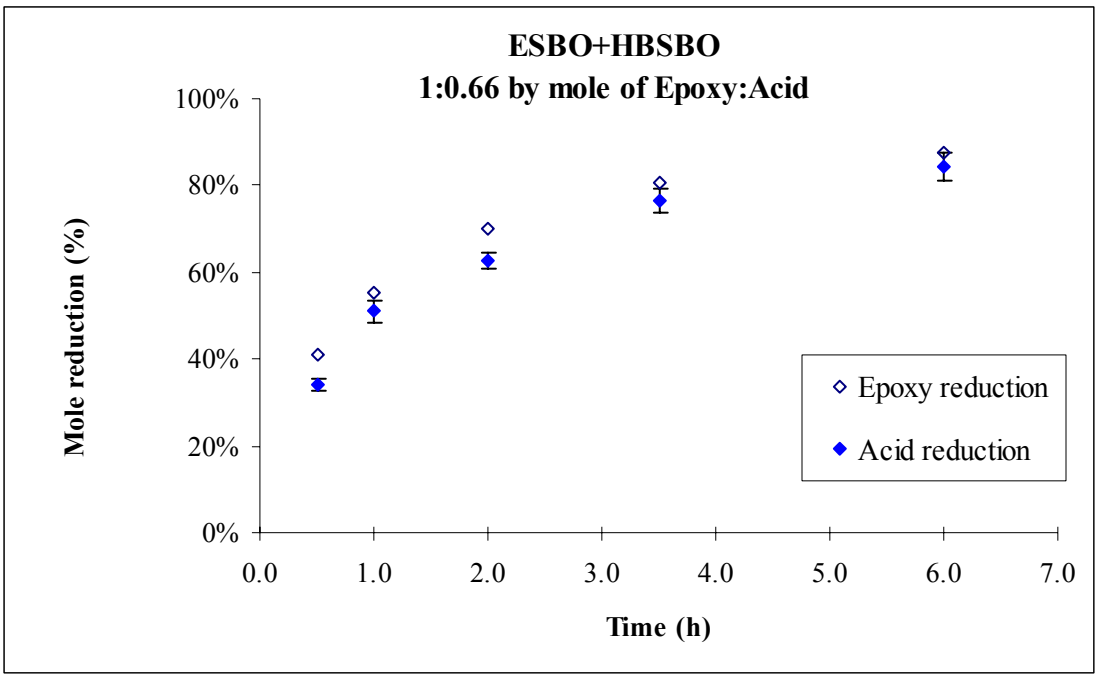


Figure 42. Disappearance rates of epoxy and acid groups during the reaction between ESBO and HBSBO (170 ^{o}C) with the mole ratio epoxy to acid of 1:0.66.

Initially, the enzyme hydrolysis is simply used to produce HBSBO where both hydroxy and carboxylic acid functional groups are created. The polyols produced from the HBSBO have an exceptional advantage over the other polyols. They contain primary alcohols (from the enzyme hydrolysis) which have

high reactivity and could be very useful in reducing catalyst loading in the polyurethane synthesis.

Enzyme *R. miehei* is selected to be used in the enzyme hydrolysis of HBSBO. The lipase has been known to be a 1,3-specific lipase which prefers to create primary alcohol during the reaction.

6.3.6. Properties of high OH equivalent weight soy-based polyols

Typical properties reported for the commercial polyols are acid number, hydroxyl number, OH equivalent weight, MW, functionality and viscosity. The apparent MW of soy-based polyols analyzed by gel permeation chromatography (GPC) is found to be higher than their real value due to their bulky molecular structure [8]. The relative MW of soy-based polyols can be easily observed by their viscosity and OH numbers. The higher viscosity, the higher the MW. However, the OH and epoxy functional groups also increase the polyols' viscosity.

Table 7. Properties of polyols made from ESBO and vegetable oil based acid moieties.

Acid moieties (acid number)	Ratio of epoxy to acid functional groups (by mole)	Reaction time (h)	Properties of polyols				
			Acid number (mg KOH/g)	Hydroxyl number (mg KOH/g)	OH equivalent weight	Epoxy content (% by wt.)	Viscosity (22°C) (mPa s)
LA (190)	1:1	17	25	76	740	<0.1	1400
	1:0.8	17	14	107	520	<0.1	2540
	1:0.5	17	4	112	500	0.8	2860
RC (142)	1:1	13	16	159	350	<0.1	9420
	1:0.8	13	16	163	340	<0.1	8620
	1:0.5	10	5	152	370	0.2	7670
RC estolide (79)	1:0.66	6	8	109	520	0.3	5290
HBSBO (83)	1:0.66	6	10	82	680	0.3	3000
Alkoxyl hydroxyl soybean oil (Sovermol® 1068)* *A commercial product and product's properties by Cognis Oleochemicals		-	0-3.9	180-205	270-310	-	3000-6000 (at 20 °C)

Properties of high equivalent weight soy-based polyols produced from ESBO are shown in Table 7. Properties of a commercially available soy-based polyol, Sovermol® 1068 (alkoxyl hydroxyl soybean oil), are also shown in the same table.

Normally, the acid numbers of commercially available polyols are lower than 10 (mg KOH/g). An excess amount of epoxy group is needed to reduce the polyols' acid number because the possible side reactions could also take place (Figure 39).

LA and RC have high acid numbers (190 and 142 mg KOH/g, respectively) which require about 50% excess amount of mole epoxy (1:0.5 mole ratio) in the reaction of epoxy cleavage to produce the polyols with low acid number. The excess amount of epoxy for the reactions of RC estolide and of HBSBO and EBSO is about 34% (1:0.66 mole ratio) to produce the low acid number products where the acid number RC estolide and HBSBO are 79 and 83 (mg KOH/g), respectively. A higher excess amount of epoxy group is required when the acid moiety has high acid number to reduce the acid number of the final polyol product.

The epoxy content in Table 7 indicates unreacted epoxy functional groups which are from the excess ESBO. The unreacted epoxy groups are actually useful for reacting with isocyanate and forming oxazolidone [6] which is an urethane group. The epoxy functional groups also affect to the polyols' hydroxyl number. An epoxy group yields double hydroxyl number compared to a hydroxy

group where ESBO has about 2 times of the hydroxyl number (about 400) compared to the alkoxyl hydroxyl SBO

As might be expected, with the 50% excess epoxy, the epoxy content of the final polyols made from RC and ESBO is less than the one made from LA and ESBO, even though the RC has a lower acid number. This is because RC has a hydroxy group which could also react with the epoxy groups.

In addition, the reaction of hydroxy groups and epoxy groups could create the higher MW of the final polyols which increases the polyols' OH equivalent weight when hydroxy groups on a molecule react with epoxy groups on the other molecule (Figure 39). However, the high amounts epoxy utilized could cause higher production cost due to the expensive ESBO.

Considering polyols in Table 7 with the acid number less than 10, the produced vegetable oil based polyols have OH numbers of 82-152 (mg KOH/g) and OH equivalent weight of 370-680. The commercially available alkoxyl methoxyl soybean oil, which has triglyceride backbone, has a higher OH number and a lower equivalent weight.

The Hydroxyl numbers of the polyols produced from RC and ESBO did not increase much compare to the commercial product. It is because RC originally has an OH functional group which increases the polyols' OH number and functionality. However, the product would contain less non-functional moiety.

The viscosity of the products from ESBO and LA increased when the starting amount of ESBO increases. This was probably because the higher reaction conversion between LA and ESBO (low acid number and epoxy content)

resulting in thelarger molecular structure. The highest viscosity product from ESBO and LA also contained the highest epoxy content which could significantly affect to the viscosity.

Polyols made from ESBO and RC had very high viscosity, 2 or 3 times higher compared to commercial polyols of similar molecular weight. This is probably because the hydroxy functional groups on RC moieties increase the viscosity. With a different ratio of epoxy to acid, the products have similar OH number and epoxy content. However, they have different viscosity.

6.4. CONCLUSIONS

Reaction-addition to epoxidized soybean oil by vegetable oil based acids is a straight-forward and environmental-friendly process. Solvents and catalysts are not necessary.

Vegetable oil based polyols were successfully produced with acid numbers less than 10 (mg KOH/g), hydroxy numbers of 82-152 mg KOH/g, and OH equivalent weights of 370-680. These OH equivalent weights are higher than alkoxyl hydroxyl soybean oil. The polyols with high OH equivalent weight would engage higher mass of renewable material in the final polymer products. However, the viscosity of these polyols might be very high since the products have bulky molecular structure while most of petrochemical based polyols have linear molecular structure.

Enzymes effectively produce ricinoleic acid estolides and hydrolyze bodied soybean oil at mild operating conditions. Water is the only reagent

required for thy hydrolysis. The results conclusively demonstrate that enzymes can synthesize improved soy-based polyols in manners not possible with conventional chemistry; for instances producing primary alcohol which could usefully reduce catalyst loading in the polyurethane synthesis and performing at mild condition without any solvent or toxic catalyst.

The enzyme reaction is even more economical when the immobilized enzyme is used. Recycled. Novozyme 435® was still active after used for 7 times of batch operation of preparation of ricinoleic acid at 60^{o}C (1 atm).

6.5. REFERENCES

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CHAPTER 7

CONCLUSIONS AND RECOMMENDATIONS

Soy-based polyols are sustainable and renewable materials used in the polymer synthesis. These bio-based materials have been developed and used for decades. They are more interesting recently due to global energy crisis and pollution concerns.

Although uses of soy-based polyols in polyurethane is not new, soy-based polyols from enzyme catalysis have not been reported anywhere. Soy-based polyols from the enzyme reactions have some properties superior to the ones from chemical routes. Due to the high selectivity of the biocatalyst, high reactivity primary hydroxy groups can be yielded. The polymer synthesis can be more effective and economical if the polyols contain the high reactivity functionality.

Multiple enzyme reactions are applied to soybean oil triglyceride, soy-based diglyceride, soy-based fatty acids and soy-based oligomers to increase functionality and produce soy-based polyols. Three different reactive functionalities are produced by proposed enzyme reactions in this work. Hydroperoxide groups are produced by the peroxidation of lipoxygenase. Epoxy groups are produced by the epoxidation of lipase B from *Candida antarctica*. And hydroxy groups are yielded by the hydrolysis of lipases in which a couple of lipases are found to selectively replace undesirable moieties with primary hydroxy groups.

Enzyme esterification is also studied to improve the soy-based polyol products. The reaction increases MW of soy-based polyols in high selectivity creating high equivalent weight polyols. The high equivalent weight products create new applications of soy-based polyols and increase mass of the renewable materials engaged in polyurethane.

Most of the enzyme reactions evaluated in this work do not require any organic solvent. Toluene is used in the epoxidation of soy-based materials to preserve the life of the enzyme which is immobilized and reused. Solvent does not participate in the reaction and can be recycled.

Apparently, enzymes are expensive and sensitive catalysts. Immobilizations of enzymes contribute to preserve enzymes' activity and reuse the biocatalysts. Novozyme 435® is immobilized lipase B from *C. antarctica* which is commercially available and widely used in many applications including epoxidation and esterification. The immobilized lipase is found to be recyclable for several times under proper operating conditions.

Packed bed reactor (PBR) is a reactor design which is generally applied to the enzyme reactions with the reusable immobilized enzyme. The immobilized enzymes are kept and the reactor generates high conversion per reactor volume. The PBR operation is believed to reduce production cost and is currently used in many commercial processes.

Preliminary studies of PBRs with immobilized enzymes are performed for hydrolysis and epoxidation, which are described in Chapter 3 and 4. However, there are some flaws including mass transfer problems and low immobilization

yields in the catalyst preparation. Future work should focus on eliminating these flaws.

The results conclusively demonstrate that the proposed enzyme technology can synthesize improved soy-based polyols in manners not possible with conventional chemistry. In addition, the processes are energy saving and environmental friendly technologies.

The optimization of reactor design and operation, with respect to commercialization, are recommended for future work. If effective enzymes are immobilized and reused in PBRs operating at high yield and selectivity, the enzyme reactions will be more economical. Thus, the enzyme technology can produce highly competitive soy-based products in the polyols market.

VITA

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