



HAL
open science

Effects of different epoxidation methods of soybean oil on the characteristics of acrylated epoxidized soybean oil-co-poly(methyl methacrylate) copolymer

Pimchanok Saithai, Jérôme Lecomte, Eric Dubreucq, Varaporn Tanrattanakul

► To cite this version:

Pimchanok Saithai, Jérôme Lecomte, Eric Dubreucq, Varaporn Tanrattanakul. Effects of different epoxidation methods of soybean oil on the characteristics of acrylated epoxidized soybean oil-co-poly(methyl methacrylate) copolymer. *Express Polymer Letters*, 2013, 7 (11), pp.910-924. 10.3144/expresspolymlett.2013.89 . hal-01269361

HAL Id: hal-01269361

<https://hal.science/hal-01269361>

Submitted on 29 May 2020

HAL is a multi-disciplinary open access archive for the deposit and dissemination of scientific research documents, whether they are published or not. The documents may come from teaching and research institutions in France or abroad, or from public or private research centers.

L'archive ouverte pluridisciplinaire **HAL**, est destinée au dépôt et à la diffusion de documents scientifiques de niveau recherche, publiés ou non, émanant des établissements d'enseignement et de recherche français ou étrangers, des laboratoires publics ou privés.



Distributed under a Creative Commons Attribution 4.0 International License

Effects of different epoxidation methods of soybean oil on the characteristics of acrylated epoxidized soybean oil-co-poly(methyl methacrylate) copolymer

P. Saithai^{1,2}, J. Lecomte², E. Dubreucq², V. Tanrattanakul^{1*}

¹Bioplastic Research Unit, Department of Materials Science and Technology, Faculty of Science, Prince of Songkla University, 90112 Songkla, Thailand

²Montpellier SupAgro, UMR 1208 IATE, 2 Place Viala, 34060 Montpellier Cedex, France

Received 13 May 2013; accepted in revised form 24 July 2013

Abstract. The effect of the type of epoxidation processes of soybean oil on the characteristics of epoxidized soybean oils (ESOs), acrylated epoxidized soybean oils (AESOs), and acrylated epoxidized soybean oil – poly(methyl methacrylate) copolymers (AESO-co-PMMA) has been investigated. Two epoxidation processes were used: an *in situ* chemical epoxidation using hydrogen peroxide and formic acid, and a chemo-enzymatic epoxidation using 2 enzymes: Novozyme[®] 435 (CALB) and a homemade lipase/acyltransferase (CpLIP2). ESOs containing different numbers of epoxide groups/molecule were synthesized. A commercial ESO (Vikoflex[®] 7170) was employed and it had the highest number of epoxide groups. Acrylation of ESOs was carried out using acrylic acid, and copolymerized with a methyl methacrylate monomer. The chemo-enzymatic epoxidation produced high acid value, particularly from the CpLIP2 (~46–48%) and indicated the formation of epoxidized free fatty acids. In contrast, the ESO synthesized from the chemical epoxidation showed a very low acid value, <0.6%. The AESOs synthesized from the CALB-based ESO and the chemical-based ESO showed a similar number of acrylate groups/molecule while that from the CpLIP2-based ESO showed a very low number of acrylate groups because the carboxylic groups from the epoxidized free fatty acids impeded the acrylation reaction. The lower the number of acrylate groups the lower was the crosslink density, the T_g , and the gel content in the AESO-co-PMMA copolymer.

Keywords: polymer synthesis, tailor-made polymers, biocomposites, thermosetting resins, thermal properties

1. Introduction

In the last two decades bio-based polymers have received increasing attention from the industrial sector and researchers. These polymers or their monomers are derived from renewable resources. They could be a thermoplastic or a thermosetting plastic and they could be biodegradable or non-biodegradable. Plant oil is one of the interesting renewable monomers, particularly soybean oil as it is abundant and cheap. Typically, a triglyceride is the major component in all plant oils and these contain both saturated and unsaturated fatty acids. Their

reactivity depends on the numbers of double bonds (C=C) or the type of fatty acid. Soybean oil has relatively high double bond content but these double bonds are not highly active for typical free radical polymerization. Therefore, the double bonds in the soybean oil have to be converted to more reactive functional groups such as epoxide groups, acrylate groups, hydroxyl groups, and even some bromoacrylated triglycerides, that can be used in the free radical polymerization [1–12]. Epoxidized soybean oil (ESO) has been produced for the last 30 years and is available under various trade names. ESO has

*Corresponding author, e-mail: varaporn.t@psu.ac.th
© BME-PT

been polymerized to form plastic materials such as ESO-co-styrene/divinylbenzene resins [13–18], ESO thermosetting allyl resins [19], a sheet molding compound resin [20], and a hydrogel [21], or even polymer composites such as an organo clay nanocomposite [22], and fiber-reinforced composites [23–25]. According to Hazer and coworkers [26–33], vegetable oils were autooxidized by exposure to sunlight or daylight in air for a given time to produce epoxides, peroxides, peroxides, and hydroperoxides in the molecular chains and were also graft copolymerized by free radical polymerization with other materials such as methyl methacrylate and styrene. Recently, the vegetable oil-based polymers have been prepared by cationic, olefin metathesis and condensation polymerization reactions, including the use of ‘click’ chemistry and carbon dioxide [5, 34–37]. Acrylation of epoxidized vegetable oils is one of the interesting techniques for preparing the vegetable oil-based polymers. The mechanical and thermal properties of acrylated epoxidized soybean oil (AESO) have been reported [3, 5, 8, 20, 23, 38, 39]. It has been established that AESO shows higher mechanical properties than ESO [22, 39]. AESO has been used for copolymerization with other materials such as a vinyl ester resin containing styrene [8–10] and with poly(methyl methacrylate) [38–40]. It was found that the mechanical properties of an AESO-co-PMMA copolymer were higher than those of AESO. Furthermore, the AESO-co-PMMA copolymer is an interesting polymer owing to the high weathering resistance of PMMA. Although ESO- and AESO-based polymers seem to be environmentally friendly materials, the epoxidation process should be improved by using more green chemistry. This is because on an industrial scale, ESO is produced by an *in situ* epoxidation in the presence of a strong acid as a catalyst such as H_2SO_4 and H_3PO_4 . The drawbacks of this method are the corrosion of equipment due to the acidic solution and the product must be neutralized and purified. Moreover, these acids can initiate oxirane ring-opening reactions with water, and lead to the formation of hydroxyl group on the fatty acid backbone and other by-products [41]. To eliminate these problems, enzymes have also been used in the epoxidation process [41]. The enzymes involved in the chemo-enzymatic epoxidation were a peroxygenase and a lipase. Blee and Schuber [42, 43] used the peroxygenase enzyme for epoxidation of mono-

and polyunsaturated fatty acids. Piazza and coworkers [44–46] developed a method for the rapid isolation and immobilization of peroxygenases on membranes, and conducted epoxidation reactions in organic solvents. Lipase B from *Candida antarctica* (CALB) is one of the enzymes used most frequently as a biocatalyst. Lipase enzymes have been shown to produce peroxy acids from hydrogen peroxide and fatty acids by a perhydrolysis reaction. Most of the developments of the lipase-catalyzed chemo-enzymatic epoxidation in plant oils or fatty acids have been studied using a commercial enzyme lipase (Novozyme[®] 435) [41, 47–54]. By using enzymes, the addition of free acids was not required in order to obtain a high conversion, i.e. >80%, and the neutral pH of the reaction mixture was maintained [52]. While ESO has been synthesized by the chemo-enzymatic epoxidation, to the best of our knowledge the conversion of this ESO into AESO has not been reported.

The objectives of the present study were to investigate the effect of the different epoxidation methods on ESO and the effect of the degree of epoxidation (or number of epoxide groups/molecule) in ESO on the characteristics of the ESO, AESO and AESO-co-PMMA copolymer. ESO was synthesized by chemical and chemo-enzymatic epoxidation methods. Peroxy acid produced from formic acid and hydrogen peroxide was used in the chemical epoxidation process. Hydrogen peroxide and lipase were used in the chemo-enzymatic epoxidation. Novozyme[®] 435 and a lipase from *Candida parapsilosis* (CpLIP2) were also employed. CpLIP2 has been shown to catalyze the alcoholysis of various esters in the presence of a large molar excess of water in a biphasic aqueous/lipid reactant medium with hydrolysis of the esters and was also found to be effective for the production of fatty hydroxamic acids in an aqueous medium by an aminolysis of fatty acids [55]. There has been no previous report of the chemo-enzymatic epoxidation of ESO catalyzed by CpLIP2.

2. Experimental

2.1. Materials

Commercialized cooking-grade soybean oil was employed. Commercialized epoxidized soybean oil (Vikoflex[®] 7170) was produced by Arkema Inc. (Philadelphia, PA, USA). Novozyme[®] 435, lipase B from *Candida antarctica* (CALB) immobilized on

macroporous polyacrylate resin beads, was from Sigma-Aldrich Corp. (St. Louis, MO, USA). It has a bead size of 0.3–0.9 mm and its activity is approximately 7,000 PLU/g. An enzyme lipase/acyltransferase CpLIP2 (*Candida parapsilosis*) was produced in our laboratory. Anhydrous sodium sulfate, sulfuric acid, acrylic acid, methyl methacrylate (MMA) and benzoyl peroxide were from Sigma-Aldrich (St. Louis, MO, USA). Hydrogen peroxide, hydroquinone and glacial formic acid were from Merck (Darmstadt, Germany). All chemicals were used as received.

2.2. Synthesis of epoxidized soybean oil (ESO)

2.2.1. Chemical epoxidation catalyzed by sulfuric acid

A solution of soybean oil (100 g) and glacial formic acid (13.97 g) was heated at a 45–55°C. Sulfuric acid (0.5 mL) was added into the solution. Then, 116.98 g of 30 wt% H₂O₂ solution was added slowly from a dropping funnel and reacted at 45, 50 and 55°C for 1–7 h. The molar ratio of soybean oil: formic acid: hydrogen peroxide was 1:2.64:8.9. The crude product was filtered and washed with distilled water repeatedly until a pH of 7.0 was obtained. The oil phase was dried with anhydrous sodium sulfate then filtered. Finally, the residue (water) was removed using an evaporator at 45–50°C under pressure. The number of epoxide groups per molecule of ESO was calculated from the ¹H-NMR spectrum by using the peak at $\delta = 4.0\text{--}4.4$ ppm for the glycerol backbone and the peak at 0.9 ppm for the methyl group as an internal standard, and using the peak intensity ratio between the peak at 2.8–3.2 and 4.0–4.4 ppm [56]. This number was used to represent the sample code of the ESO. The degree of epoxidation (*DOE*) or percentage of conversion from double bonds to epoxide groups was determined from Equation (1) and the number of starting double bonds of the soybean oil used in the present study was 4.60:

$$DOE = \frac{\text{number of epoxide groups}}{\text{number of starting double bonds}} \cdot 100 \quad (1)$$

We assumed that *DOE* (Degree of epoxidation) of Vikoflex[®] 7170 should be very high, i.e. ~100%. However, there was no data on the starting soybean oil; therefore, it was not possible to determine its *DOE* in the present study. ESOs containing 50 and 75% *DOE* were prepared, and Vikoflex[®] 7170 was a representative of 100% *DOE*.

2.2.2. Chemo-enzymatic epoxidation catalyzed by enzymes

About 18 g of soybean oil, 1.5 g of CALB (~8 wt% of soybean oil), and 9.24 g of 35 wt% hydrogen peroxide solution were mixed together and heated at 55°C in an incubator shaker. The mixture was then cooled and the enzyme was removed by filtration. The final product was purified in a similar way to the chemical epoxidation method.

About 0.5 g of soybean oil was mixed with 1.44 mL of 5 wt% hydrogen peroxide solution in a pH buffer of 5.35 at 30°C. The CpLIP2 content was 30, 90 and 300 U/mL. The mixture was stirred at 300 rpm with a magnetic stirrer. The enzyme was removed and final product was purified.

The number of epoxide groups per molecule of the derived ESOs was calculated from the ¹H-NMR spectrum [56] and the *DOE* was evaluated as described above. In order to compare the different epoxidation methods between the chemical epoxidation and the chemo-enzymatic method, obtaining a similar *DOE* was our concern. As a result, 50 and 75% *DOE* were used.

2.3. Synthesis of acrylated epoxidized soybean oil (AESO)

AESO was prepared from a reaction between ESO and acrylic acid in a similar way to previous reports [39, 40]. About 50 g of ESO were placed in a 250 mL round-bottom flask equipped with a magnetic stirrer and a reflux condenser. Hydroquinone was used as a free radical inhibitor. The molar ratio of ESO: acrylic acid was 1:10. The reaction temperature and time was 110°C and 7 h, respectively. The mixture was cooled to room temperature and diluted with toluene before purifying by washing with distilled water. The final step was dehydration with anhydrous sodium sulfate and the solvent was evaporated using an evaporator. The number of acrylate groups/molecule of the resulting product was determined from the ¹H-NMR spectrum [56]. The number of acrylate groups/molecule and epoxide groups/molecules were assigned in the sample designation of AESO.

2.4. Characterization of ESO and AESO

The ¹H-NMR, ¹³C-NMR and 2D-NMR spectra were recorded qualitatively by a Unity Inova[®] spectrometer (Varian, Germany) at a frequency of 500 MHz using chloroform-d as a solvent. The Fourier trans-

form infrared (FTIR) spectra of the copolymer sheets were recorded by a Bruker® EQUINOX 55 spectrometer (Bruker, Rheinstetten, Germany) from 400 to 4000 cm^{-1} .

The acid value of oil corresponded to its free fatty acid content and can be expressed as the weight% of the most abundant fatty acid of the oil. The titrimetric method was based on the French standard NF T60-204 (December 1985). About 2.0 g of ESO and 40 mL of diethyl ether/ethanol (1:1 v/v) were added followed by adding 3 drops of 20 g/L ethanolic phenolphthalein solution with continuous agitation. This sample solution was titrated with a 0.1 N ethanolic potassium hydroxide solution until the pink color remained stable for at least 10 seconds. The acid value, in weight%, was calculated based on Equation (2):

$$\text{Acid value} [\%] = \frac{(V - V_0) \cdot N \cdot M}{10W} \quad (2)$$

where V was the mL of potassium hydroxide solution used for the sample titration, V_0 was the mL of potassium hydroxide solution used for the blank, N was the titer of the potassium hydroxide solution, M was the molar mass [g/mol] of the most abundant fatty acid in the sample and W was the sample weight [g].

The qualitative analysis of the ESO samples was performed by High-Performance Thin Layer Chromatography (HPTLC) on pre-coated silica gel 60 plates (200 μm thickness, 4–8 μm particle size) from Merck (Darmstadt, Germany). Samples and standards were diluted in a chloroform-methanol mixture (2:1, v/v) at a concentration of 1 mg/mL, except for the standard mixture of monoolein, diolein and triolein (0.33 mg/mL each). The sample and standard solutions were deposited on HPTLC plates in a band-shape of 10 mm width, using an automatic TLC sampler ATS4 by CAMAG® (Muttens, Switzerland). The deposited volumes were the following: soybean oil 2 μL , ESO 4.2 μL , oleic acid (OA) 1 μL , linoleic acid (LA) 1 μL , cis-9,10-epoxystearic acid (ESA) 2 μL , cis-9,10-12,13-diepoxy stearic acid (DESA) 1.3 μL . The development (65 mm migration distance) was performed vertically in an automatic developing chamber ADC2 by CAMAG, with hexane/diethyl ether/acetic acid (70:30:1; v/v/v) as the mobile phase. The plates were visualized after dipping the plate in a solution of phosphoric acid 85%/saturated aqueous copper sulphate

solution/water/methanol (8:10:78:5, v/v/v/v) and heating for 10 minutes at 180°C. The retention factors (R_f) of standard compounds were used for identification of the reaction products. The retention factor (R_f) was defined using Equation (3):

$$R_f = \frac{A}{B} \quad (3)$$

where A was the distance from the deposition line to the center of a spot and B was the distance from the starting point (deposition line) to the solvent front. The more closely the retention factor was close to 1, the more non-polar was the compound. Conversely, the more the retention factor was close to 0, the more polar was the compound.

2.5. Preparation of the AESO-co-PMMA copolymer

A mixture of 50 wt% of MMA and 50 wt% of AESO was well mixed before adding 1 wt% of benzoyl peroxide. The mixture was heated at 70°C for 15 min in a closed container before casting into a glass mold. Then, the mixture was cured at 90°C for 15 min in a thermal oven and at 90°C for 15 min in a vacuum oven sequentially.

2.6. Characterization of the AESO-co-PMMA copolymer

Dynamic mechanical thermal analysis (DMTA) was performed using a Rheometric Scientific® DMTA V (Piscataway, NJ, USA) at a frequency of 1 Hz from –80 to 180°C. The heating rate was 2°C/min in the tension mode had a strain control of 0.01%. The storage modulus (E'), the loss modulus (E'') and the loss tangent ($\tan \delta$) as a function of temperature were recorded. Differential scanning calorimetry (DSC) was carried out on a differential scanning calorimeter (Perkin Elmer® DSC7, Waltham, MA, USA) with a heating rate of 10°C/min from –50 to 100°C in a nitrogen atmosphere. Thermogravimetric analysis (TGA) was performed on a thermogravimetric analyzer (Perkin Elmer® TGA7, Waltham, MA, USA) with a heating rate of 10°C/min in a nitrogen atmosphere from 25 to 1000°C. The temperatures at 5, 10 and 50% of weight loss were determined.

A swelling test was carried out with common solvents for surface coating such as ethanol and water. The copolymer sheets with a sample size of 1 × 2 × 0.2 cm were swollen to an equilibrium state – a

constant weight. The degree of swelling was calculated based on Equation (4):

$$\text{Degree of swelling} [\%] = \frac{W_1 - W_0}{W_0} \cdot 100 \quad (4)$$

where W_0 was the weight of the dried sample before the swelling test and W_1 was the weight of the swollen sample.

The gel content in the copolymer sheet was determined by the insoluble fraction left after treating with tetrahydrofuran (THF) that was a good solvent for AESO and PMMA. The copolymer sheets with the sample size of $1 \times 2 \times 0.2$ cm were immersed in THF at room temperature for 4 days. The insoluble part was removed from the solvent and dried at 60°C until the weight was constant (W_2), after approximately 24 h. The gel content was calculated based on Equation (5) [57, 58]:

$$\text{Gel content} [\%] = \frac{W_2}{W_0} \cdot 100 \quad (5)$$

3. Results and discussion

3.1. Epoxidation of soybean oil

In order to investigate the effect of *DOE* on the AESO-co-PMMA copolymers, three *DOE* values were used: 100, 75 and 50%. It was expected that Vikoflex[®] 7170 has a very high *DOE* ($\sim 100\%$) because the C=C protons were not observed in its $^1\text{H-NMR}$ spectrum. ESO's containing 75 and 50% *DOE* were synthesized by chemical and chemo-enzymatic epoxidations. Owing to the chemical epoxidation, the reaction at 50°C for 3 h provided a 76% *DOE* and the reaction at 45°C for 1 h provided a 52% *DOE*. Their number of epoxide groups per molecule was 3.50 and 2.40, respectively. The structure of the epoxidized triglyceride was confirmed by $^1\text{H-NMR}$, $^{13}\text{C-NMR}$, 2D-NMR and FTIR (not shown here).

The chemo-enzymatic epoxidation process catalyzed by the enzyme was studied in terms of the enzyme content and reaction time. Using 8 wt% of CALB for 13.5 h produced an ESO with 72% *DOE* (3.30 epoxide groups/molecule). A lower *DOE* was obtained using milder conditions (5 wt% of CALB for 9 h). The resulting ESO contained 52% *DOE* and 2.40 epoxide groups/molecule.

The present study was a first attempt to use CpLIP2 for the preparation of ESO. Based on a preliminary study in the CpLIP2 system, the *DOE* increased with reaction time. By using 30 U/mL of CpLIP2 for 24 h, the maximum *DOE* was 60%. An increase in the CpLIP2 content and reaction time, i.e., 90 U/mL at 24 h, the *DOE* was 75–78%. A higher content of CpLIP2, i.e. 300 U/mL, did not significantly increase the *DOE*. Therefore, 90 U/mL of CpLIP2 was employed, and 49 and 78% *DOE* were obtained when the reaction time was 4 and 24 h, respectively.

3.2. Characteristics of ESO

The number of epoxide groups/molecule was calculated using the peak at $\delta = 4.0\text{--}4.4$ ppm for the glycerol backbone and the peak at 0.9 ppm for the methyl group as an internal standard and using the intensity ratio between the peak at 2.8–3.2 and 4.0–4.4 ppm [56]. This number is listed in Table 1, and it was used to represent the sample code of the ESO.

The acid value of all ESO products is listed in Table 1. This value depended on the epoxidation system. ESOs derived from the chemical epoxidation process (ESO4.60, ESO3.50, and ESO2.40) had a very low acid value ($<0.6\%$). The CALB-based ESOs (ESO3.30-enz1 and ESO2.40-enz1) showed a higher acid value (3–4%), and the CpLIP2-based ESOs (ESO3.58-enz2 and ESO2.25-enz2) showed the highest value (46–48%). A high acid value indicated a high free fatty acid content. It

Table 1. Sample designation, number of epoxide groups, the *DOE* and acid values of the ESOs

| ESO code | Epoxidation process | No. of epoxide groups/molecule | DOE [%] | Acid value [%] |
|--------------|---------------------|--------------------------------|---------|----------------|
| ESO4.60 | Chemical | 4.60 | –* | 0.12±0.02 |
| ESO3.50 | | 3.50 | 76 | 0.54±0.03 |
| ESO2.40 | | 2.40 | 52 | 0.56±0.06 |
| ESO3.30-enz1 | CALB | 3.30 | 72 | 3.59±0.02 |
| ESO2.40-enz1 | | 2.40 | 52 | 3.24±0.04 |
| ESO3.58-enz2 | CpLIP2 | 3.58 | 78 | 46.46±0.03 |
| ESO2.25-enz2 | | 2.25 | 49 | 48.15±0.03 |

*number of starting double bonds of Vikoflex[®] 7170 was unknown.

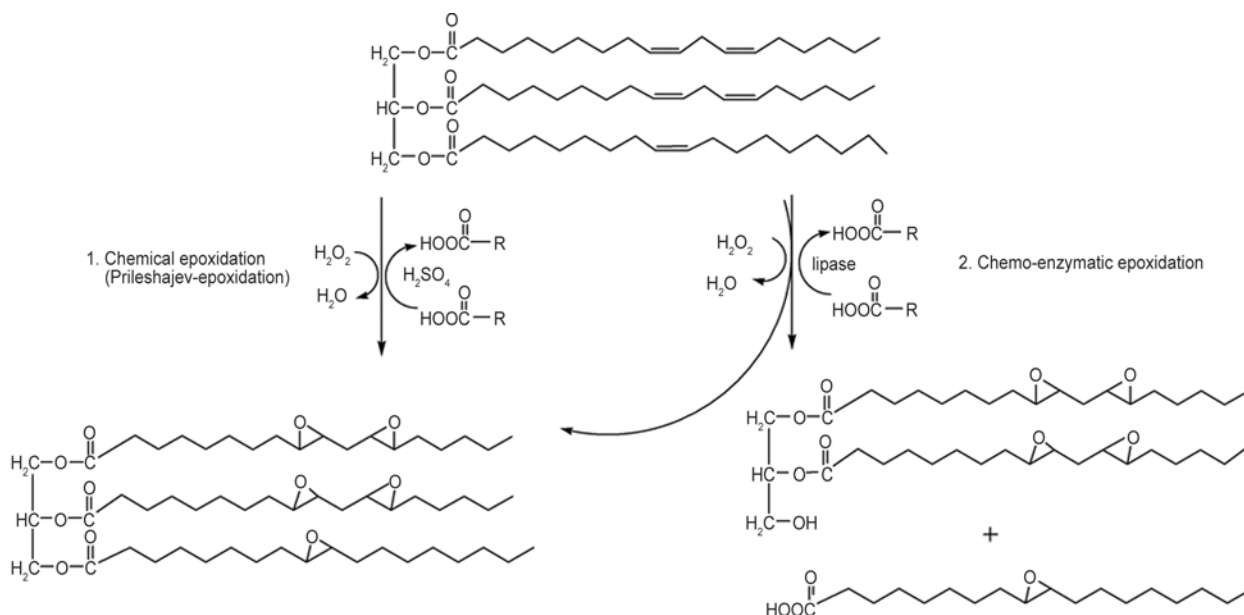


Figure 1. Schematic diagram of the chemical and chemo-enzymatic epoxidation process of soybean oil

was believed that these free fatty acids were epoxidized because the *DOE* or the number of epoxide groups/molecule of the enzyme-based ESO was as high as that of the chemical-based ESO. It was believed that CpLIP2 induced hydrolysis as well as epoxidation to provide the structure of an epoxidized mono-, di- and tri-glycerides including epoxidized free fatty acids as shown in Figure 1. These structures were substantiated by thin layer chromatography (TLC) and NMR/FTIR spectroscopy. A thin layer chromatogram of the 14 samples listed in Table 2 is illustrated in Figure 2. Table 3 details the retention factor (*R_f*) of some epoxidized and non-epoxidized standard molecules separated by chromatography on HPTLC silica gel 60 plates. As depicted in Figure 2, the starting soybean oil

(Track 1) exclusively contained triacylglycerols (TAG) that, owing to their non-polar character, was close to the front of the solvent (*R_f* ≈ 1). Typically, soybean oil consists of 26.6% oleic acid, 52.8% linoleic acid and 5.4% of linolenic acid. The epoxidation of the double bonds of the oleic, linoleic and linolenic acids presented in TAGs increased the

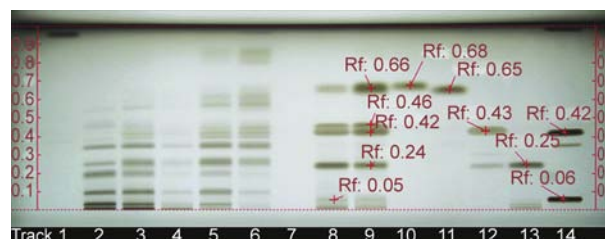


Figure 2. Thin layer chromatogram of samples listed in Table 2

Table 2. Sample ID and characteristics that corresponded to the TLC results shown in Figure 2

| Track | Sample | Concentration [mg/mL] | Band width [mm] | Volume [μL] |
|-------|-----------------------------|-----------------------|-----------------|-------------|
| 1 | Soybean oil | 1 | 10 | 2.0 |
| 2 | ESO4.60 (Vikoflex® 7170) | 1 | 10 | 4.2 |
| 3 | ESO3.50 | 1 | 10 | 4.2 |
| 4 | ESO2.40 | 1 | 10 | 4.2 |
| 5 | ESO3.30-enz1 | 1 | 10 | 4.2 |
| 6 | ESO2.40-enz1 | 1 | 10 | 4.2 |
| 7 | Blank | – | | |
| 8 | ESO3.58-enz2 | 1 | 10 | 4.2 |
| 9 | ESO2.25-enz2 | 1 | 10 | 4.2 |
| 10 | Oleic acid (OA) | 1 | 10 | 1.0 |
| 11 | Linoleic acid (LA) | 1 | 10 | 1.0 |
| 12 | Epoxy stearic acid (ESA) | 1 | 10 | 2.0 |
| 13 | Diepoxy stearic acid (DESA) | 1 | 10 | 1.3 |
| 14 | MO+DO+TO* | 0.33 each | 10 | 10.0 |

*MO: monoolein, DO: diolein, TO: triolein

Table 3. Retention factor of some epoxidized and non-epoxidized standard molecules separated on HPTLC silica gel 60 plates

| Compound type | Reference | Rf |
|------------------------------|----------------------|-----------|
| Fully unsaturated TAG | Triolein | 0.95–1.00 |
| Partially epoxidized TAG | Triolein | 0.80–0.87 |
| Unsaturated fatty acids | Oleic acid | 0.67 |
| | Linoleic acid | 0.65 |
| Fully epoxidized fatty acids | Epoxy stearic acid | 0.43 |
| | Diepoxy stearic acid | 0.24 |
| Diacylglycerol | Diolein | 0.42 |
| Monoacylglycerol | Monoolein | 0.05 |

TAG = triacylglycerols

polarity of the latter. As a result, the greater the number of epoxide groups, the more polar the TAGs. The commercial ESO, ESO4.60, (Track 2) contained neither fully unsaturated TAGs/free fatty acids ($0.63 < R_f < 0.68$) nor partially epoxidized TAGs ($0.80 < R_f < 0.87$). Moreover, its very low acid value (0.12%) meant that it was mainly composed of fully epoxidized TAGs and was probably fully or partially epoxidized di- and monoacylglycerols, and this was confirmed by the absence of DESA ($R_f = 0.24$) and only traces of ESA ($R_f = 0.43$) as shown in Table 3.

Samples of the chemically prepared ESO (Track 3 and 4) having a DOE of 76 and 52% respectively, exhibited a very similar profile to that of Vikoflex[®] 7170 (ESO4.60). The main differences essentially concerned compounds with R_f values > 0.35 , that corresponded to partially epoxidized species and that again was consistent with the epoxidation content of these samples.

Profiles of the samples chemo-enzymatically epoxidized with CALB (Novozyme[®] 435) in track 5 and 6 were similar to the chemically prepared ESO. However, they contained significant amounts of fully unsaturated TAGs, thus revealing the incomplete conversion of soybean oil, but also there were partially epoxidized TAGs and fatty acids (no band exactly matched the fatty acid standards). It is worth mentioning that, despite the high water activity of the reaction medium, the hydrolysis of substrate and products remained limited as was shown by the moderate acid values of both samples (3.2–3.6%). Finally, the composition of samples from the chemo-enzymatic epoxidation with CpLIP2 was radically different from all the others. Soybean oil was completely converted into unsaturated fatty acids ($0.65 < R_f < 0.68$), mono- and di-epoxidized fatty

acids (ESA, $R_f = 0.43$; DESA, $R_f = 0.24$) and most likely mono- and diacylglycerols ($R_f = 0.05$ and $R_f = 0.42$ respectively). These results agreed well with the high acid values of the two samples that ranged from 46 to 48%. Thus, it can be assumed that in the CpLIP2 mediated epoxidation, hydrolysis reactions (substrates and peracids) were faster than the epoxidation itself.

To verify the ESO structure, ¹H-NMR and ¹³C-NMR spectroscopy were used. Figure 3 shows the ¹H-NMR spectra of soybean oil, commercialized ESO and synthesized ESO, and their chemical shift (δ) assignment are listed in Table 4. The presence of an epoxide ring in all the ESO was confirmed with a δ at 2.8–3.2 ppm, epoxy proton (position 4 in the chemical structure). The glyceride backbone in the soybean oil was assigned to the δ at 4.0–4.4 and 5.1, and the δ at 5.2–5.6 ppm (methylene proton) represented the unsaturation or carbon-carbon double bond (C=C) of soybean oil (position 1 in the chemical structure). It was observable that there was no C=C in the commercialized ESO (ESO4.60) whereas the C=C bands continued to appear in the synthe-

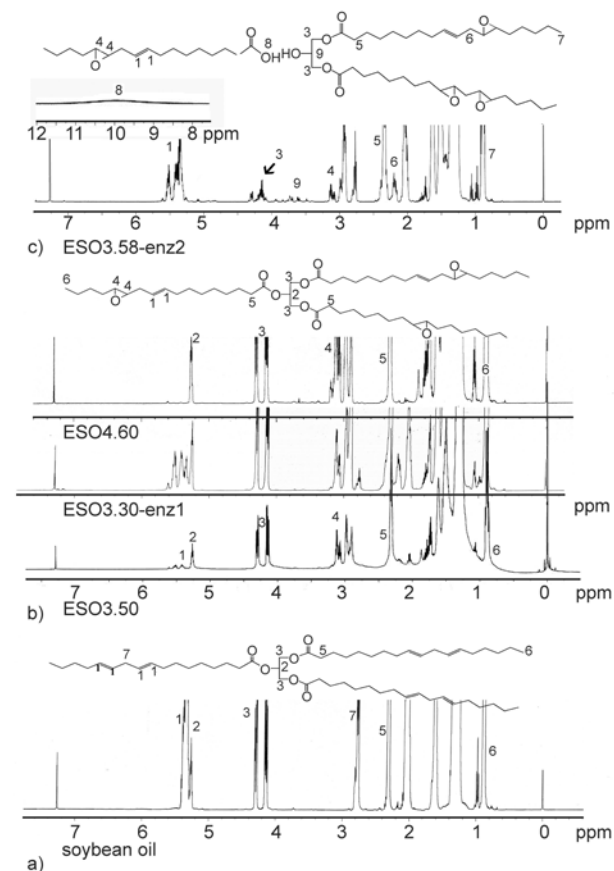
**Figure 3.** ¹H-NMR spectra: (a) soybean oil; (b) ESO4.60, ESO3.30-enz1 and ESO3.50; and (c) ESO3.58-enz2

Table 4. Assignment of the ^1H -NMR spectra of soybean oil, epoxidized soybean oil and epoxidized fatty acids

| Chemical shift, δ [ppm] | Structure | Assignment |
|--------------------------------|-------------------------------------|---|
| 0.88 (triplet) | | Terminal $-\text{CH}_3$ |
| 1.5 (broad singlet) | | β - CH_2 - to epoxy group |
| 1.7 (broad singlet) | | $-\text{CH}_2-$ adjacent to two epoxy group |
| 2.0 (broad singlet) | | $-\text{CH}_2-\text{CH}=\text{CH}-$ |
| 2.0 (broad singlet) | | $-\text{CH}_2-\text{CH}=\text{CH}-$ |
| 2.0 (broad singlet) | | $-\text{CH}_2-\text{CH}=\text{CH}-$ |
| 2.2 (broad singlet) | | $-\text{CH}_2-$ adjacent to one epoxy group and one C=C group |
| 2.8 (broad singlet) | | $-\text{CH}_2-$ adjacent to two C=C group |
| 2.9 | | $-\text{CH}-$ proton of epoxy group |
| 3.1 | | $-\text{CH}-$ proton of epoxy group |
| 3.7 (doublet) | $\text{OH}-\text{CH}_2$ | CH_2 in diglyceride |
| 4.0–4.4 | $\text{CH}_2-\text{CH}-\text{CH}_2$ | $-\text{CH}_2-$ of glycerol backbone |
| 5.1 | $\text{CH}_2-\text{CH}-\text{CH}_2$ | $-\text{CH}-$ of glycerol backbone |
| 5.3 | | $-\text{CH}-$ proton of C=C group |
| 5.4 | | $-\text{CH}-$ proton of C=C group |

sized ESO from both the chemical and chemo-enzymatic epoxidations. The chemical- and CALB-based epoxidation seemed to produce a similar product based on their ^1H -NMR spectra. In contrast, epoxidation with CpLIP2 led to a different chemical structure as shown in Figure 3c. The disappearance of the glyceride protons at 4.2 ppm and the appearance of a methylene attached to a hydroxyl group at 3.7 ppm (position 9) in the diglyceride of ESO catalyzed by CpLIP2 were noticed. The inserted figure showed a small signal at 10 ppm that was assigned to a carboxylic acid (COOH) proton. These results substantiated the assumption that the hydrolysis of triglyceride by CpLIP2 produced the mono-

or di-glyceride that generated the epoxidized free fatty acid in the CpLIP2-based ESO. The ^{13}C -NMR and 2D-NMR results (not shown here) agreed with the results described above. Both the TLC and NMR results indicated that the chemical-based ESO and the CALB-based ESO had epoxidized triglycerides while the CpLIP2-based ESO contained a mixture of epoxidized monoglycerides, epoxidized diglycerides, epoxidized triglycerides, and epoxidized free fatty acids.

Figure 4 shows the FTIR spectra of ESOs. All ESOs showed the $\text{C}=\text{O}$ ester peak of the triglyceride at 1750 cm^{-1} . A unique peak at 1725 cm^{-1} was found only in the CpLIP2-based ESO (ESO3.45-enz2). It

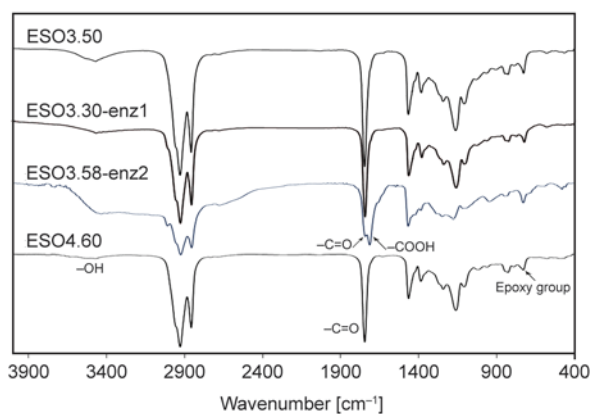


Figure 4. FTIR spectra of ESOs

belongs to the C=O ester peak of the carboxylic acid (COOH). The presence of epoxidized free fatty acid in the CpLIP2-based ESO was again proven by the FTIR result.

3.3. Acrylation of ESO and the characteristics of AESO

The formation of an acrylate group was determined using $^1\text{H-NMR}$. Figure 5 shows the $^1\text{H-NMR}$ spectra of AESOs. The new peaks after acrylation were the peaks at 5.8–6.7 ppm that were assigned to the 3 protons of the acrylate esters. The AESOs also had peaks at 5.3–5.6 ppm ($-\text{CH}=\text{CH}-$) and 3.0–3.2 ppm (epoxide proton) which indicated an incomplete acrylation reaction. The acrylate group in AESO was investigated by FTIR spectroscopy (not shown here). We observed a peak at 1400, 985, 810 cm^{-1} that was attributed to the $\text{CH}=\text{CH}_2$ (acrylate group) and the peak of the residual epoxy groups at 822, 845 cm^{-1} . The number of acrylate groups/molecule of AESO calculated from its $^1\text{H-NMR}$ spectrum [56] is shown in Table 5. The number of acrylate

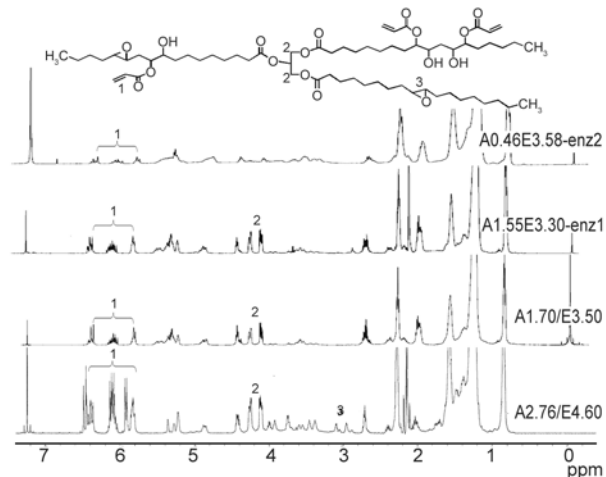


Figure 5. $^1\text{H-NMR}$ spectra of AESOs

groups of the AESO derived from the synthesized ESO was in the range of 0.42–1.70 while that from Vikoflex[®] 7170 was highest (2.76). An incomplete acrylation reaction was also found in the commercialized AESO (Ebecryl[®] 860) that have 3.50 acrylate groups/molecule (not shown here). Based on our knowledge, the complete acrylation reaction of ESO has not been reported. It should be noted that two samples, A1.54/E2.40 and A1.54/E2.40-enz1, were good representatives for comparisons between the chemical epoxidation and chemo-enzymatic epoxidation on the characteristics of the copolymer. They had the same number of epoxide groups and number of acrylate groups.

The number of acrylate groups represents the degree of acrylation. Typically, the acrylation reaction has a first-order dependence on the epoxide concentration of oil, pure triglyceride and fatty acid methyl ester. It seemed that the effect of the number of epoxide groups (or *DOE*) on the number of acrylate groups appeared only in the AESOs derived from the chemical-based ESO, i.e., the number of acrylate groups decreased from 1.70 to 1.54 when the number of epoxide groups decreased from 3.50 to 2.40 (Table 5). In contrast, the acid value played an important role on the acrylation of the enzyme-based ESOs for which the numbers of their acrylate groups did not change significantly although their numbers of epoxide groups were different. The CALB-based ESOs had 2 epoxide contents i.e., 3.30 (ESO3.30-enz1) and 2.40 (ESO2.40-enz1). After acrylation, the derived AESOs showed a similar number of acrylate groups, i.e., 1.55 (A1.55/E3.30-enz1) and 1.54 (A1.54/E2.40-enz1). This behavior was more clearly observed in the CpLIP2-based AESOs. Their starting ESO had a significantly different epoxide content, i.e., 3.58 (ESO3.58-enz2) and 2.25 (ESO2.25-enz2), but the derived AESOs had a similar number of acrylate groups, i.e.

Table 5. Sample designation and number of acrylate groups of the AESO

| AESO code | No. of acrylate groups/molecule in AESO | No. of epoxide groups/molecule in ESO |
|------------------|---|---------------------------------------|
| A2.76/E4.60 | 2.76 | 4.60 |
| A1.70/E3.50 | 1.70 | 3.50 |
| A1.54/E2.40 | 1.54 | 2.40 |
| A1.55/E3.30-enz1 | 1.55 | 3.30 |
| A1.54/E2.40-enz1 | 1.54 | 2.40 |
| A0.46/E3.58-enz2 | 0.46 | 3.58 |
| A0.42/E2.25-enz2 | 0.42 | 2.25 |

0.42–0.46. This was because the epoxide group reacted with the carboxylic group of the epoxidized free fatty acid by an addition reaction [59, 60]. This indicated that the presence of epoxidized free fatty acid was very important for further modifications of ESO. The number of epoxide groups/molecule may not be a key factor for the synthesis of AESO by using the enzymatic ESO. The free fatty acid formed during the chemo-enzymatic epoxidation was able to be epoxidized and that was a reason for the high number of epoxide groups found in the ESO3.58-enz2. The occurrence of the carboxylic groups in the epoxidized free fatty acids did not favor the attraction of acrylic acid. Therefore, their number of acrylate groups was very low, i.e., 0.46.

3.4. Characteristics of the AESO-co-PMMA copolymer

The thermal stability of the AESO-co-PMMA copolymer was determined in terms of the thermal degradation temperature (T_d) at a selected weight loss such as 5, 10 and 50%. TGA thermograms of the copolymers are demonstrated in Figure 6 and their T_d are listed in Table 6. Sample designations of the copolymers followed the name of the AESO which included the number of acrylate groups and the number of epoxide groups. For example, Co-A2.76/E4.60 and Co-A1.55/E3.30-enz1 was synthesized from A2.76/E4.60 and A1.55/E3.30-enz1, respectively. PMMA had the lowest thermal stability because of its linear molecular structure. The network structure produced a much higher thermal stability of the copolymers, except for Co-A0.46/E3.58-enz2 that showed a slightly higher thermal stability than PMMA due to its low crosslinking. Excluding Co-A0.46/E3.58-enz2, there was no sig-

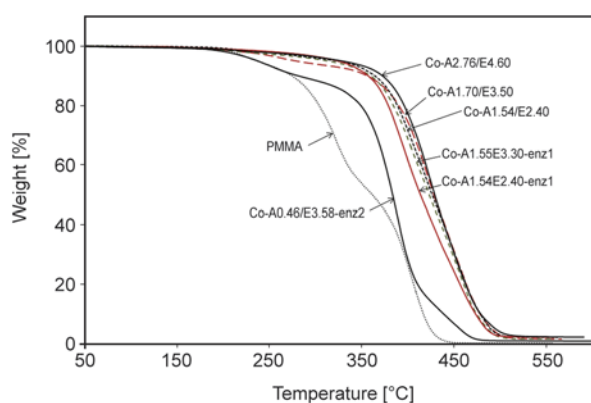


Figure 6. TGA thermograms of PMMA and AESO-co-PMMA copolymers

Table 6. Glass transition temperature (T_g) and thermal degradation temperature (T_d) of the PMMA and AESO-c-PMMA copolymer

| Sample | T_g [°C] | | T_d [°C] at weight loss of | | |
|---------------------|------------|--------|------------------------------|-----|-----|
| | DMTA | DSC | 5% | 10% | 50% |
| PMMA | 94 | – | 236 | 274 | 360 |
| Co-A2.76/E4.60 | 40 | 3.92 | 340 | 368 | 425 |
| Co-A1.70/E3.50 | 14 | –15.96 | 278 | 355 | 427 |
| Co-A1.55/E3.30-enz1 | –1 | –23.54 | 324 | 364 | 426 |
| Co-A1.54/E2.40 | –9 | –26.13 | 313 | 359 | 422 |
| Co-A1.54/E2.40-enz1 | –8 | –24.12 | 324 | 356 | 413 |
| Co-A0.46/E3.58-enz2 | N/A | N/A | 236 | 280 | 383 |

N/A: not applicable (no data)

nificant difference in the thermal stability among these copolymers. The high acid value and the very low acrylate groups in the CpLIP2-based AESO did not promote copolymerization with MMA. Consequently, the Co-A0.46/E3.58-enz2 was very soft and weak, and it was unable to copolymerize with A0.42/E2.25-enz2.

The glass transition temperature (T_g) of the copolymers was examined by DMTA and DSC. The $\tan \delta$ as a function of the temperature of the copolymers is shown in Figure 7. The temperature at the maximum $\tan \delta$ was the α transition temperature and equivalent to T_g but a different value was obtained by the DSC due to the different testing conditions. The Co-A0.46/E3.58-enz2 was unable to form a continuous sheet for DMTA testing. All copolymers and PMMA showed a very broad peak and their T_g is listed in Table 6. The T_g of PMMA was 94°C while the T_g of the copolymers were in the range of –9 to 40°C. The copolymers showed a lower T_g than PMMA although they were a thermosetting plastic. This is because the flexible and relatively short chains of the triglyceride in the copolymers

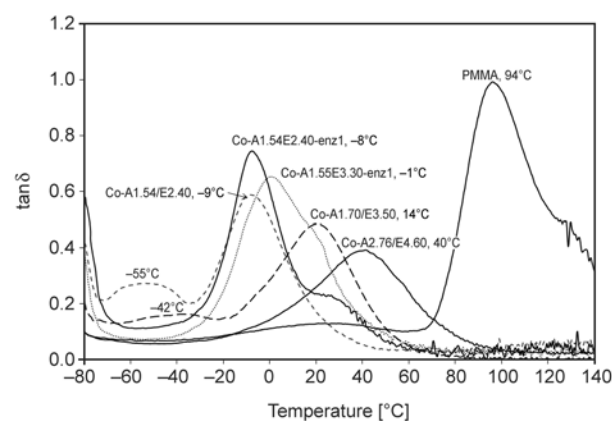


Figure 7. $\tan \delta$ as a function of the temperature of PMMA and AESO-co-PMMA copolymers

contributed to the lower T_g . The T_g also depended on the number of acrylate groups. More acrylate groups caused more crosslinking and produced a higher T_g . The single T_g indicated that a random copolymer has been derived. The Co-A1.70/E3.50 and the Co-A1.54/E2.40 showed a sub- T_g or β transition temperature while the CALB-based copolymers showed a shoulder. These may be due to phase segregation of the un-crosslinked structure or some homopolymer of PMMA, so a further study is required. The T_g of the copolymers obtained from DSC is listed in Table 6. It agreed with the results from the DMTA.

The degree of swelling in water and ethanol of the copolymers are tabulated in Table 7. The copolymers provided good stability in water (1–4%) whereas a lower stability in ethanol (15–18%) was observed. This is because ethanol is less polar than water; therefore, ethanol could penetrate into the non-polar chains of the copolymers more than water. Generally speaking, there were only slight differences in the degree of swelling among those copolymers.

Tetrahydrofuran (THF) is a good solvent of PMMA and AESO. To compare the crosslink density among these copolymers, the solubility in THF was investigated and reported as gel content (Table 7). The higher gel content indicated a higher density of crosslinks. The Co-A2.76/E4.60 had the highest gel content owing to it having the highest number of acrylate groups whereas the A0.46/E3.58-enz2 had the lowest gel content. The chemo-enzymatic epoxidation produced the lower gel content because of the formation of epoxidized free fatty acids that was attributed to a reduction in the molecules that could be crosslinked (epoxidized triglycerides). This effect was obvious in the A0.46/E3.58-enz2 with its starting ESO having a very high acid value and a high number of epoxide groups. On the other hand, the gel contents of the CALB-based copolymers

were slightly lower than those of the chemical-based copolymers. Comparing the Co-A1.54/E2.40 with the Co-A1.54/E2.40-enz1, their physical properties were in the same range. Their T_g was -26 and -24°C , and their α transition temperature was -9 and -8°C , respectively. Furthermore, their degree of swelling was also similar. This behavior implied that the small acid value in the CALB-based ESO ($\sim 3\%$) did not affect the copolymerization. However, the presence of the free fatty acid in the CALB-based ESO did influence the degree of acrylation and copolymerization.

Finally, we proposed models of the molecular structure of the copolymers which depended on the epoxidation method as shown in Figure 8. Figure 8a shows the molecular structure of the AESO-co-PMMA copolymer derived from the chemical epoxidation. By using chemo-enzymatic epoxidation, the molecular structure varied based on the epoxidized products. The epoxidized triglycerides provided the network structure as shown in Figure 8a. The epoxidized diglycerides with or without the free fatty acids would generate the network (Figure 8b). The epoxidized free fatty acids may lead to the crosslinked or linear structure that depended on the number of acrylate groups per fatty acid chain (Figure 8c and 8d).

4. Conclusions

The present work has provided a systematic study on the effect of the epoxidation process of soybean oil on the characteristics of the epoxidized soybean oil, acrylated epoxidized soybean oil, and acrylated epoxidized soybean oil-co-poly(methyl methacrylate) copolymer. Two different epoxidation processes were carried out: a chemical and chemo-enzymatic epoxidation. Novozyme[®] 435, lipase B from *Candida antarctica* (CALB) and an enzyme lipase/acyltransferase CpLIP2 (*Candida parapsilosis*) were employed. The epoxidation process had a strong influence on the resulting product. The CpLIP2-based epoxidation also generated free fatty acids, monoglycerides and diglycerides due to the hydrolysis reaction and subsequently these became epoxidized products, as indicated by a very high acid value and the $^1\text{H-NMR}$ and FTIR spectra and TLC chromatograms. The dependence of the acrylation reaction on the epoxide content or degree of epoxidation was observed only in the chemical-based epoxidized soybean oil. The presence of the epoxi-

Table 7. The degree of swelling and gel content of the AESO-co-PMMA copolymer

| Sample | Degree of swelling [%] | | Gel content [%] (THF) |
|---------------------|------------------------|-------|--------------------------|
| | Ethanol | Water | |
| Co-A2.76/E4.60 | 15.39 | 3.79 | 96.68 |
| Co-A1.70/E3.50 | 16.89 | 3.58 | 92.27 |
| Co-A1.55/E3.30-enz1 | 14.70 | 1.19 | 90.46 |
| Co-A1.54/E2.40 | 17.08 | 0.98 | 91.50 |
| Co-A1.54/E2.40-enz1 | 17.67 | 0.62 | 89.99 |
| Co-A0.46/E3.58-enz2 | – | – | 65.58 |

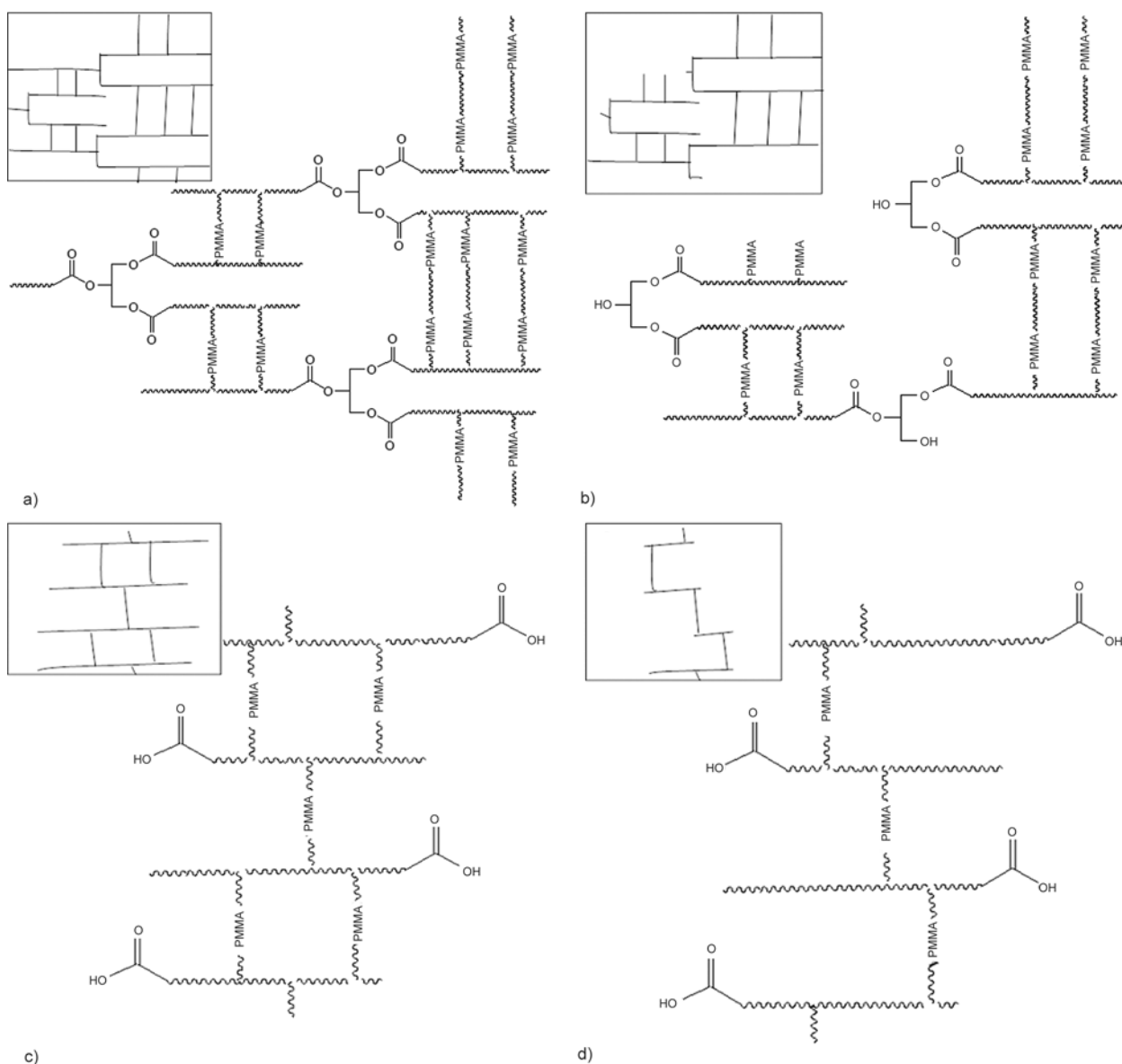


Figure 8. Molecular structure modeling of the AESO-co-PMMA copolymer derived from (a) the epoxidized triglyceride; (b) the epoxidized diglyceride with or without the free fatty acid; and (c–d) the epoxidized free fatty acid

dized free fatty acids in the enzymatic ESOs played a major role on the acrylation reaction. The higher the acid value in the ESO, the lower were the numbers of acrylate groups in the AESO obtained. The presence of carboxylic groups impeded the acrylation reaction. CpLIP2 was not suitable for synthesis of ESO under the present conditions. Although the small acid value in the CALB-based ESO did not affect the copolymers' properties, but it strongly affected the number of acrylate groups of the AESO.

Acknowledgements

The authors would like to acknowledge the financial support from the Research Development and Engineering (RD&E) fund through The National Nanotechnology Center (NANOTEC) Thailand (P-10-11333) to Prince of Songkla University. Thanks to Dr. Brian Hodgson for assistance with the English.

References

- [1] Sinadinović-Fišer S., Janković M., Petrović Z. S.: Kinetics of *in situ* epoxidation of soybean oil in bulk catalyzed by ion exchange resin. *Journal of the American Oil Chemists' Society*, **78**, 725–731 (2001). DOI: [10.1007/s11746-001-0333-9](https://doi.org/10.1007/s11746-001-0333-9)

- [2] Petrović Z. S., Zlatanić A., Lava C. C., Sinadinović-Fišer S.: Epoxidation of soybean oil in toluene with peroxyacetic and peroxyformic acids – Kinetics and side reactions. *European Journal of Lipid Science and Technology*, **104**, 293–299 (2002).
DOI: [10.1002/1438-9312\(200205\)104:5<293::AID-EJLT293>3.0.CO;2-W](https://doi.org/10.1002/1438-9312(200205)104:5<293::AID-EJLT293>3.0.CO;2-W)
- [3] La Scala J., Wool R. P.: Property analysis of triglyceride-based thermosets. *Polymer*, **46**, 61–69 (2005).
DOI: [10.1016/j.polymer.2004.11.002](https://doi.org/10.1016/j.polymer.2004.11.002)
- [4] Esen H., Küsefoğlu S., Wool R.: Photolytic and free-radical polymerization of monomethyl maleate esters of epoxidized plant oil triglycerides. *Journal of Applied Polymer Science*, **103**, 626–633 (2007).
DOI: [10.1002/app.25155](https://doi.org/10.1002/app.25155)
- [5] Bonnaillie L. M., Wool R. P.: Thermosetting foam with a high bio-based content from acrylated epoxidized soybean oil and carbon dioxide. *Journal of Applied Polymer Science*, **105**, 1042–1052 (2007).
DOI: [10.1002/app.26182](https://doi.org/10.1002/app.26182)
- [6] Biresaw G., Liu Z. S., Erhan S. Z.: Investigation of the surface properties of polymeric soaps obtained by ring-opening polymerization of epoxidized soybean oil. *Journal of Applied Polymer Science*, **108**, 1976–1985 (2008).
DOI: [10.1002/app.27784](https://doi.org/10.1002/app.27784)
- [7] Liu A., Erhan S. Z.: Ring-opening polymerization of epoxidized soybean oil. *Journal of the American Oil Chemists' Society*, **87**, 437–444 (2010).
DOI: [10.1007/s11746-009-1514-0](https://doi.org/10.1007/s11746-009-1514-0)
- [8] Grishchuk S., Karger-Kocsis J.: Hybrid thermosets from vinyl ester resin and acrylated epoxidized soybean oil (AESO). *Express Polymer Letters*, **5**, 2–11 (2011).
DOI: [10.3144/expresspolymlett.2011.2](https://doi.org/10.3144/expresspolymlett.2011.2)
- [9] Grishchuk S., Leanza R., Kirchner P., Karger-Kocsis J.: 'Greening' of unsaturated polyester resin based bulk molding compound with acrylated epoxidized soybean and linseed oils: Effect of urethane hybridization. *Journal of Reinforced Plastics and Composites*, **30**, 1455–1466 (2011).
DOI: [10.1177/0731684411421541](https://doi.org/10.1177/0731684411421541)
- [10] Grishchuk S., Karger-Kocsis J.: Modification of vinyl ester and vinyl ester–urethane resin-based bulk molding compounds (BMC) with acrylated epoxidized soybean and linseed oils. *Journal of Materials Science*, **47**, 3391–3399 (2012).
DOI: [10.1007/s10853-011-6186-0](https://doi.org/10.1007/s10853-011-6186-0)
- [11] Guo A., Cho Y., Petrović Z. S.: Structure and properties of halogenated and nonhalogenated soy-based polyols. *Journal of Polymer Science Part A: Polymer Chemistry*, **38**, 3900–3910 (2000).
DOI: [10.1002/1099-0518\(20001101\)38:21<3900::AID-POLA70>3.0.CO;2-E](https://doi.org/10.1002/1099-0518(20001101)38:21<3900::AID-POLA70>3.0.CO;2-E)
- [12] Eren T., Küsefoğlu S. H.: Synthesis and polymerization of the bromoacrylated plant oil triglycerides to rigid, flame-retardant polymers. *Journal of Applied Polymer Science*, **91**, 2700–2710 (2004).
DOI: [10.1002/app.13471](https://doi.org/10.1002/app.13471)
- [13] Li F., Hanson M. V., Larock R. C.: Soybean oil–divinylbenzene thermosetting polymers: Synthesis, structure, properties and their relationships. *Polymer*, **42**, 1567–1579 (2001).
DOI: [10.1016/S0032-3861\(00\)00546-2](https://doi.org/10.1016/S0032-3861(00)00546-2)
- [14] Li F., Larock R. C.: New soybean oil–styrene–divinylbenzene thermosetting copolymers. I. Synthesis and characterization. *Journal of Applied Polymer Science*, **80**, 658–670 (2001).
DOI: [10.1002/1097-4628\(20010425\)80:4<658::AID-APP1142>3.0.CO;2-D](https://doi.org/10.1002/1097-4628(20010425)80:4<658::AID-APP1142>3.0.CO;2-D)
- [15] Li F., Larock R. C.: New soybean oil–styrene–divinylbenzene thermosetting copolymers. II. Dynamic mechanical properties. *Journal of Polymer Science Part B: Polymer Physics*, **38**, 2721–2738 (2000).
DOI: [10.1002/1099-0488\(20001101\)38:21<2721::AID-POLB30>3.0.CO;2-D](https://doi.org/10.1002/1099-0488(20001101)38:21<2721::AID-POLB30>3.0.CO;2-D)
- [16] Li F., Larock R. C.: New soybean oil–styrene–divinylbenzene thermosetting copolymers. III. Tensile stress–strain behavior. *Journal of Polymer Science Part B: Polymer Physics*, **39**, 60–77 (2001).
DOI: [10.1002/1099-0488\(20010101\)39:1<60::AID-POLB60>3.0.CO;2-K](https://doi.org/10.1002/1099-0488(20010101)39:1<60::AID-POLB60>3.0.CO;2-K)
- [17] Li F., Larock R. C.: New soybean oil–styrene–divinylbenzene thermosetting copolymers. IV. Good damping properties. *Polymers for Advanced Technology*, **13**, 436–449 (2002).
DOI: [10.1002/pat.206](https://doi.org/10.1002/pat.206)
- [18] Li F., Larock R. C.: New soybean oil–styrene–divinylbenzene thermosetting copolymers. V. Shape memory effect. *Journal of Applied Polymer Science*, **84**, 1533–1543 (2002).
DOI: [10.1002/app.10493](https://doi.org/10.1002/app.10493)
- [19] Luo Q., Liu M., Xu Y., Ionescu M., Petrović Z. S.: Thermosetting allyl resins derived from soybean oil. *Macromolecules*, **11**, 7149–7157 (2011).
DOI: [10.1021/ma201366e](https://doi.org/10.1021/ma201366e)
- [20] Lu J., Khot S., Wool R. P.: New sheet molding compound resins from soybean oil. I. Synthesis and characterization. *Polymer*, **46**, 71–80 (2005).
DOI: [10.1016/j.polymer.2004.10.060](https://doi.org/10.1016/j.polymer.2004.10.060)
- [21] Xu J., Liu Z., Erhan S. Z.: Viscoelastic properties of a biological hydrogel produced from soybean oil. *Journal of the American Oil Chemists' Society*, **85**, 285–290 (2008).
DOI: [10.1007/s11746-008-1193-2](https://doi.org/10.1007/s11746-008-1193-2)
- [22] Tanrattanakul V., Saithai P.: Mechanical properties of bioplastics and bioplastic–organoclay nanocomposites prepared from epoxidized soybean oil with different epoxide contents. *Journal of Applied Polymer Science*, **114**, 3057–3067 (2009).
DOI: [10.1002/app.30842](https://doi.org/10.1002/app.30842)

- [23] Khot S. N., Lanscala J. L., Can E., Morye S. S., Williams G. I., Palmese G. R., Kusefoglou S. H., Wool R. P.: Development and application of triglyceride-based polymers and composites. *Journal of Applied Polymer Science*, **82**, 703–723 (2001).
DOI: [10.1002/app.1897](https://doi.org/10.1002/app.1897)
- [24] Liu Z. S., Erhran S. Z., Xu J., Calvert P. D.: Development of soybean oil-based composites by solid free-form fabrication method: Epoxidized soybean oil with bis or polyalkyleneamine curing agents system. *Journal of Applied Polymer Science*, **85**, 2100–2107 (2002).
DOI: [10.1002/app.10763](https://doi.org/10.1002/app.10763)
- [25] Liu Z. S., Erhran S. Z., Akin D. E., Barton F. E.: ‘Green’ composites from renewable resources: preparation of epoxidized soybean oil and flax fiber composites. *Journal of Agricultural and Food Chemistry*, **54**, 2134–2137 (2006).
DOI: [10.1021/jf0526745](https://doi.org/10.1021/jf0526745)
- [26] Çakmaklı B., Hazer B., Tekin I. O., Kizgut S., Koksall M., Menciloglu Y.: Synthesis and characterization of polymeric linseed oil grafted methyl methacrylate or styrene. *Macromolecular Bioscience*, **4**, 649–655 (2004).
DOI: [10.1002/mabi.200300117](https://doi.org/10.1002/mabi.200300117)
- [27] Çakmaklı B., Hazer B., Tekin I. O., Cömert F. B.: Synthesis and characterization of polymeric soybean oil-g-methyl methacrylate (and *n*-butyl methacrylate) graft copolymers: Biocompatibility and bacterial adhesion. *Biomacromolecules*, **6**, 1750–1758 (2005).
DOI: [10.1021/bm050063f](https://doi.org/10.1021/bm050063f)
- [28] Çakmaklı B., Hazer B., Tekin I. O., Açıkgöz S., Can M.: Polymeric linoleic acid–polyolefin conjugates: Cell adhesion and biocompatibility. *Journal of American Oil Chemists' Society*, **84**, 73–81 (2007).
DOI: [10.1007/s11746-006-1011-7](https://doi.org/10.1007/s11746-006-1011-7)
- [29] Alli A., Hazer B.: Poly(*N*-isopropylacrylamide) thermoresponsive cross-linked conjugates containing polymeric soybean oil and/or polypropylene glycol. *European Polymer Journal*, **44**, 1701–1713 (2008).
DOI: [10.1016/j.eurpolymj.2008.04.004](https://doi.org/10.1016/j.eurpolymj.2008.04.004)
- [30] Keleş E., Hazer B.: Autooxidized polyunsaturated oils/oily acids: Post-it applications and reactions with Fe(III) and adhesion properties. *Macromolecular Symposia*, **269**, 154–160 (2008).
DOI: [10.1002/masy.200850919](https://doi.org/10.1002/masy.200850919)
- [31] Hazer D. B., Hazer B., Kaymaz F.: Synthesis of microbial elastomers based on soybean oily acids. *Biocompatibility studies*. *Biomedical Materials*, **4**, 035011/1–035011/9 (2009).
DOI: [10.1088/1748-6041/4/3/035011](https://doi.org/10.1088/1748-6041/4/3/035011)
- [32] Hazer B., Hazer D. B., Çoban B.: Synthesis of microbial elastomers based on soybean oil. Autoxidation kinetics, thermal and mechanical properties. *Journal of Polymer Research*, **17**, 567–577 (2010).
DOI: [10.1007/s10965-009-9345-0](https://doi.org/10.1007/s10965-009-9345-0)
- [33] Acar M., Çoban S., Hazer B.: Novel water soluble soya oil polymer from oxidized soya oil polymer and diethanol amine. *Journal of Macromolecular Science Part A: Pure and Applied Chemistry*, **50**, 287–296 (2013).
DOI: [10.1080/10601325.2013.755443](https://doi.org/10.1080/10601325.2013.755443)
- [34] Xia Y., Larock R. C.: Vegetable oil-based polymeric materials: Synthesis, properties, and applications. *Green Chemistry*, **12**, 1893–1909 (2010).
DOI: [10.1039/C0GC00264J](https://doi.org/10.1039/C0GC00264J)
- [35] Hong J., Luo Q., Wan X., Petrović Z. S., Shah B. K.: Biopolymers from vegetable oils via catalyst- and solvent-free ‘click’ chemistry: Effects of cross-linking density. *Biomacromolecules*, **13**, 261–266 (2012).
DOI: [10.1021/bm201554x](https://doi.org/10.1021/bm201554x)
- [36] Liu Z., Doll K. M., Holser R. A.: Boron trifluoride catalyzed ring-opening polymerization of epoxidized soybean oil in liquid carbon dioxide. *Green Chemistry*, **11**, 1774–1780 (2009).
DOI: [10.1039/B915742P](https://doi.org/10.1039/B915742P)
- [37] Liu Z., Shurma B. K., Erhan S. Z.: From oligomers to molecular giants of soybean oil in supercritical carbon dioxide medium: 1. Preparation of polymers with lower molecular weight from soybean oil. *Biomacromolecules*, **8**, 233–239 (2007).
DOI: [10.1021/bm060496y](https://doi.org/10.1021/bm060496y)
- [38] Li Y., Fu L., Lai S., Cai X., Yang L.: Synthesis and characterization of cast resin based on different saturation epoxidized soybean oil. *European Journal of Lipid Science and Technology*, **112**, 511–516 (2010).
DOI: [10.1002/ejlt.200900191](https://doi.org/10.1002/ejlt.200900191)
- [39] Saithai P., Tanrattanakul V., Chinpa W., Kaewtathip K., Dubreucq E.: Synthesis and characterization of triglyceride-based copolymer from soybean oil. *Materials Science Forum*, **695**, 320–323 (2011).
DOI: [10.4028/www.scientific.net/MSF.695.320](https://doi.org/10.4028/www.scientific.net/MSF.695.320)
- [40] Saithai P., Lecomte J., Dubreucq E., Tanrattanakul V.: Bioplastic nanocomposite prepared from acrylated epoxidized soybean oil-PMMA copolymer and nanotitanium dioxide. *Advanced Science Letters*, **19**, 862–865 (2013).
DOI: [10.1166/asl.2013.4811](https://doi.org/10.1166/asl.2013.4811)
- [41] gen. Klaas M. R., Warwel S.: Complete and partial epoxidation of plant oils by lipase-catalyzed perhydrolysis. *Industrial Crops and Products*, **9**, 125–132 (1999).
DOI: [10.1016/S0926-6690\(98\)00023-5](https://doi.org/10.1016/S0926-6690(98)00023-5)
- [42] Blée E., Schuber F.: Efficient epoxidation of unsaturated fatty acids by a hydroperoxide-dependent oxygenase. *Journal of Biological Chemistry*, **265**, 12887–12894 (1990).
- [43] Blée E.: Biosynthesis of phytooxylipins: The peroxygenase pathway. *European Journal of Lipid Science and Technology: Lipid/Fett*, **100**, 121–127 (1998).
DOI: [10.1002/\(SICI\)1521-4133\(19985\)100:4/5<121::AID-LJPI121>3.0.CO;2-4](https://doi.org/10.1002/(SICI)1521-4133(19985)100:4/5<121::AID-LJPI121>3.0.CO;2-4)

- [44] Piazza G. J., Foglia T. A., Nuñez A.: Optimizing reaction parameters for the enzymatic synthesis of epoxidized oleic acid with oat seed peroxygenase. *Journal of the American Oil Chemists' Society*, **78**, 589–592 (2001).
DOI: [10.1007/s11746-001-0309-9](https://doi.org/10.1007/s11746-001-0309-9)
- [45] Piazza G. J., Nuñez A., Foglia T. A.: Epoxidation of carbon-carbon double bond with membrane bound peroxygenase. U.S. Patent 6485949, USA (2002).
- [46] Piazza G. J., Nuñez A., Foglia T. A.: Epoxidation of fatty acids, fatty methyl esters, and alkenes by immobilized oat seed peroxygenase. *Journal of Molecular Catalysis B: Enzymatic*, **21**, 143–151 (2003).
DOI: [10.1016/S1381-1177\(02\)00122-4](https://doi.org/10.1016/S1381-1177(02)00122-4)
- [47] Hilker I., Bothe D., Prüss J., Warnecke H-J.: Chemo-enzymatic epoxidation of unsaturated plant oils. *Chemical Engineering Science*, **56**, 427–432 (2001).
DOI: [10.1016/S0009-2509\(00\)00245-1](https://doi.org/10.1016/S0009-2509(00)00245-1)
- [48] Yadav G. D., Devi K. M.: A kinetic model for the enzyme-catalyzed self-epoxidation of oleic acid. *Journal of the American Oil Chemists' Society*, **78**, 347–351 (2001).
DOI: [10.1007/s11746-001-0267-2](https://doi.org/10.1007/s11746-001-0267-2)
- [49] Orellana-Coca C., Billakanti J. M., Mattiasson B., Hatti-Kaul R.: Lipase mediated simultaneous esterification and epoxidation of oleic acid for the production of alkylepoxystearates. *Journal of Molecular Catalysis B: Enzymatic*, **44**, 133–137 (2007).
DOI: [10.1016/j.molcatb.2006.09.002](https://doi.org/10.1016/j.molcatb.2006.09.002)
- [50] Orellana-Coca C., Camocho S., Adlercreutz, D., Mahiasson B., Hatti-Kaul, R.: Chemo-enzymatic epoxidation of linoleic acid: Parameters influencing the reaction. *European Journal of Lipid Science and Technology*, **107**, 864–870 (2005).
DOI: [10.1002/ejlt.200500253](https://doi.org/10.1002/ejlt.200500253)
- [51] Orellana-Coca C., Törnvall U., Adlercreutz D., Mattiasson R., Hatti-Kaul R.: Chemo-enzymatic epoxidation of oleic acid and methyl oleate in solvent-free medium. *Biocatalysis and Biotransformation*, **23**, 431–437 (2005).
DOI: [10.1080/10242420500389488](https://doi.org/10.1080/10242420500389488)
- [52] Vlček T., Petrović Z. S.: Optimization of the chemoenzymatic epoxidation of soybean oil. *Journal of the American Oil Chemists' Society*, **83**, 247–252 (2006).
DOI: [10.1007/s11746-006-1200-4](https://doi.org/10.1007/s11746-006-1200-4)
- [53] Tiran C., Lecomte J., Dubreucq E., Villeneuve P.: Chemo-enzymatic epoxidation of fatty compounds – Focus on processes involving a lipase-catalyzed perhydrolysis step. *Dossier OCL*, **15**, 179–183 (2008).
DOI: [10.1684/ocl.2008.0191](https://doi.org/10.1684/ocl.2008.0191)
- [56] de Cassia S. Schneider R., Lara L. R. S., Bitencourt T. B., da Graça Nascimento M., dos Santos Nunes M. R.: Chemo-enzymatic epoxidation of sunflower oil methyl esters. *Journal of the Brazilian Chemical Society*, **20**, 1473–1477 (2009).
DOI: [10.1590/S0103-50532009000800013](https://doi.org/10.1590/S0103-50532009000800013)
- [55] Neugnot V., Moulin G., Dubreucq E., Bigey F.: The lipase/acyltransferase from *Candida parapsilosis*. *European Journal of Biochemistry*, **269**, 1734–1745 (2002).
DOI: [10.1046/j.1432-1327.2002.02828.x](https://doi.org/10.1046/j.1432-1327.2002.02828.x)
- [56] Campanella A., La Scala J. J., Wool R. P.: Fatty acid-based comonomers as styrene replacements in soybean and castor oil-based thermosetting polymers. *Journal of Applied Polymer Science*, **119**, 1000–1010 (2011).
DOI: [10.1002/app.32810](https://doi.org/10.1002/app.32810)
- [57] Sangermano M., Malucelli G., Amerio E., Bongiovanni R., Priola A., Gianni A. D., Voit B., Rizza G.: Preparation and characterization of nanostructured TiO₂/epoxy polymeric films. *Macromolecular Materials and Engineering*, **291**, 517–523 (2006).
DOI: [10.1002/mame.200500420](https://doi.org/10.1002/mame.200500420)
- [58] Zhang H., Qi R., Tong M., Su M., Huang M.: *In situ* solvothermal synthesis and characterization of transparent epoxy/TiO₂ nanocomposites. *Journal of Applied Polymer Science*, **125**, 1152–1160 (2012).
DOI: [10.1002/app.34699](https://doi.org/10.1002/app.34699)
- [59] La Scala J., Wool R. P.: The effect of fatty acid composition on the acrylation kinetics of epoxidized triacylglycerols. *Journal of the American Oil Chemists' Society*, **79**, 59–63 (2002).
DOI: [10.1007/s11746-002-0435-4](https://doi.org/10.1007/s11746-002-0435-4)
- [60] La Scala J. J., Sand J. M., Orlicki J. A., Robinette E. J., Palmese G. R.: Fatty acid-based monomers as styrene replacements for liquid molding resins. *Polymer*, **45**, 7729–7737 (2004).
DOI: [10.1016/j.polymer.2004.08.056](https://doi.org/10.1016/j.polymer.2004.08.056)