Synthesis and Characterization of Epoxidized Silkworm Pupae Oil and Its Application as Polyvinyl Chloride

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Abstract

This study aimed to prepare epoxidized silkworm pupae oil (ESPO) and investigate their effects on the thermal stability and plasticization of polyvinyl chloride (PVC) films. A chemo-enzymatic method of ESPO was developed in the presence of Lipase SMG1-F278N and H_2O_2 in natural deep eutectic solvents (DESs). Lipase SMG1-F278N could initiate the epoxidation reaction effectively at room temperature with a negligible loss of activities 10 batches. A maximum oxirane value of 6.94% was obtained. The formation of oxirane ring in ESPO was confirmed by FT-IR and ^{13}C NMR spectra. Moreover, ESPO showed a better thermal stability and lower freezing point than epoxidized soybean oil (ESO). It was demonstrated that ESPO had a good frost resistance. In addition, ESPO showed a significantly improved plasticizing effect on flexible polyvinyl chloride (PVC). Compared with ESO, ESPO could increase the tensile elongation at break effectively. A significantly lower migration rate of plasticizer was observed in PVC plasticized with ESPO.

Introduction

In recent decades, epoxidation has been receiving increasing attentions in oil industries as the epoxidized fats and oils could be endowed with desirable toughness used in plasticizers [1]. It has been documented that the epoxidized soybean oil (ESO, 4.6 double bonds per triglyceride chain) enabled the significantly enhanced toughness of petro-based epoxies. Also, these epoxidized oils serve as promising eco-friendly reactive diluents [2]. The epoxidation of numerous oils has been intensively investigated and reported, including mahua oil, karanja oil, cottonseed oil, etc [3-5]. Today, the production of ESO has been successfully industrialized, thereby facilitating the synthesis of valuable plasticizers and polymer stabilizers [6].

It is demonstrated that epoxidized silkworm pupae oils (ESPO) and epoxidized linseed oil (ELO) had better thermal stability and lower coefficient of thermal expansion [7, 8]. Moreover, the epoxidized fats and oils with high levels of linolenic acid exhibited better frost resistance used as plasticizers [9, 10].

Nevertheless, the industrialization of most epoxidized edible oils, including flaxseed oil, safflower oil and rapeseed oil, is greatly limited by high costs [1]. Silkworm pupae oil (SPO), usually extracted from the desilked silkworm pupae, serves as a promising substrate alternative. Almost 450,000 tons of silkworm pupae are reportedly harvested annually in China during the past two decades [3]. Unfortunately, however, tons of silkworm pupae are disposed as industrial wastes after the extraction of nutritional silkworm pupae protein. It is well-documented that silkworm pupae are enriched sources of lipids with a content of ~30% [11]. Specifically, 70~80% of the fatty acid species are composed of oleic acid, linoleic acid and α -linolenic acid (ALA) [12]. Similar unsaturation degrees (iodine value) of SPO with soybean oil are reported in previous studies [13, 14]. Therefore, epoxidation serves as a promising effective way to achieve the high-value application of SPO.

Traditional epoxidation reactions usually entail the stoichiometric use of peracids such as peracetic acid under acidic reaction conditions [15]. These routes are characterized with relatively harsh reaction conditions, low catalytic efficiency and low substrate conversion rates [16]. It is reported that the epoxidation of oils and fats using hydrolases as catalysts represents a promising solution to the above challenges [17]. Nevertheless, the enhancement of robustness of lipase is still a great concern nowadays, especially the resistance to H_2O_2 . Gratifyingly, it was reported that the Lipase SMG1-F278N mutant had higher catalytic activities and better reusability than the wild type Lipase SMG1, especially for the production of propylene glycol monoester [18] and deacidification of oils and fats with high acid values [19]. And it is well-documented that deep eutectic solvents (DESs) enabled the enhanced robustness of lipase in chemoenzymatic epoxidation reactions [17].

In the present study, the chemo-enzymatic epoxidation of SPO catalyzed by Lipase SMG1-F278N was investigated. Furthermore, the thermal stability, cold resistance, mechanical properties and stability of ESPO, ESO and ELO were compared with respect to PVC plasticization.

Materials And Methods

Biological and Chemical Materials

Choline chloride and xylitol were purchased from Aladdin Chemistry Co., Ltd (Shanghai, China). All other reagents of analytical grade were directly used without any further purification. ESO and ELO were purchased from a domestic market.

Free Lipase G50 (*Penicillium camemberti*) was purchased from Amano Enzyme Products Company Japan (Japan). The wild-type Lipase SMG1 and Lipase SMG1-F278N were produced in our lab according to the method described previously [21]. Lipase SMG1, Lipase SMG1-F278N and Lipase G50 were immobilized onto epoxy resins of ECR 8285 following the procedures of Li et al [18]. The immobilized Lipase SMG1, Lipase SMG1-F278N and Lipase G50 were obtained by filtration and then dried in vacuum at 20 °C for 12 h. The recovered enzymes were stored at 4 °C until use. The esterification activity of immobilized Lipase SMG1, Lipase SMG1-F278N and Lipase G50 were 258, 328 and 423 U/g, respectively.

Extraction of SPO

SPO was extracted by petroleum ether using a Soxhlet extractor. After reflux at 30 °C for 8 h, the solvent was distilled off in a rotary vacuum evaporator (0.09 MPa). Then the obtained silkworm pupae extracts were dried with anhydrous sodium sulfate for 24 h [21].

Preparation of DESs

The DESs consisting of Choline chloride (ChCl, recrystallized from ethanol, filtered, and dried in vacuum) and xylitol (1:1, mol/mol) were mixed and heated at 80 °C in a rotary evaporator for 1 h. The formed

colorless, homogeneous liquids were transferred into a tightly sealed bottle, then placed in a desiccator for further use.

General Procedures for Epoxidation of SPO

Firstly, SPO (4.3 g) and DESs (4.6 g) were added to a conical flask. The mixture was supplemented with 4.6 g of hydrogen peroxide (H_2O_2 : double bonds = 2:1, mol/mol). The epoxidation reaction was initiated by addition of enzymes (20 U/g oil). The reaction medium was mixed for 12 h at 400 rpm. The products were collected at a fixed interval (2 h) and centrifuged for 3 min at 10000 rpm. The excess H_2O_2 was removed by ddH_2O . The immobilized enzymes were recycled after being rinsed with excess ddH_2O and dried in vacuum at 20 °C.

A Scale-up Reaction

A scale-up reaction (~100-fold) was carried out under the optimized conditions. 108 g of SPO, 115 g of DESs and 115 g of hydrogen peroxide (H_2O_2 : double bonds = 2:1, mol/mol) were added to the reaction vessel. After the addition of Lipase SMG1-F278N (20 U/g oil), the epoxidation reaction was catalyzed at 30 °C for 8 h.

Analysis of Fatty Acid Composition by Gas Chromatography

The fatty acid composition was determined as fatty acid methyl esters (FAMES) according to the standard method described in ISO 5509:2000 (E). The FAMES were analyzed by gas chromatography-flame ionization detector (GC-FID) equipped with a CP-Sil 88 capillary column (60 m × 0.25 mm, 0.2 μm film thickness; Dikma Technologies, Beijing, China) [22].

Determination of Physicochemical Indices

The iodine value, oxirane value, acid value, density and moisture of the products were determined by titration according to the American Oil Chemists' Society (AOCS) Official Methods Tg 1a-64, Cd 9-57, Cd 3d-63, Cc 10c-95 and Ac 2-41, respectively.

Characteristics of ESPO

The formation of oxirane ring in ESPO was confirmed by fourier transform infrared spectrometer (FT-IR) and carbon-13 nuclear magnetic resonance (^{13}C NMR) spectra. The FT-IR spectra of the products were recorded on an FT-IR (Thermo Scientific, model Nicolet 6700, Waltham, MA, USA) with a resolution of 4 cm^{-1} . Data were acquired using the FT-IR software (Thermo Scientific, OMNIC series suite, Waltham). The ^1H and ^{13}C NMR spectra of SPO and the derivatives (10~15 mg/mL in CDCl_3) were recorded by a 600 MHz Bruker NMR spectrophotometer.

The thermal degradation of the obtained polymers was determined by thermogravimetric analysis (TGA) using Mettler-Toledo TGA/SDTA 851e. TG analysis was performed using a thermal weightlessness

analyzer (TG209-F3, NETZSCH Co., Germany) in the temperature range of 25-700 °C with a rate of 10 °C/min under N₂ (20 mL/min).

The curing behaviors of epoxidized oils were characterized by a differential scanning calorimeter (DSC) (Netzsch Geratebau, model 204 C, Germany), in the temperature range of -60-80 °C with a rate of 10 °C/min under N₂.

The Mechanical Properties of PVC Samples

In the study, the tensile strength and elongation at break of PVC films made of ESPO were investigated to evaluate the comprehensive performance of various epoxy greases according to the American Society for Testing and Materials (ASTM) standards (ASTM D638).

The Thermal Stability and Migration Tests of The PVC Samples

The plastisol was prepared by mixing 50, 60, 70, 80, 90, and 100 phr of plasticizer with 100 phr PVC for 15 min, respectively. Subsequently, the mixture was compounded by double-roller blending rolls (SK-160B, Shanghai Rubber Machinery Factory) at 165 °C for 8 min. The sample was pressed on a flat plate vulcanizing machine (HY-100TA, Shanghai Hengyu Instrument Co., Ltd.) at 170 °C for 6 min to make film with a thickness of 5 mm. The final film was obtained after the temperature of the flat-plate vulcanizing machine dropped to 50 °C.

The thermal stability of PVC samples was tested according to the Congo red method in GB/T 2917.1-2002. PVC samples were cut into 2 mm × 2 mm and placed in test tubes. Then the test tubes were incubated in an oil bath at 170 °C. The time required for color changes of the Congo red test paper represented the thermal stability.

The migration tests were performed according to the following procedures. Firstly, all samples were immersed in *n*-hexane at 50 °C for 2 h. The weight loss was measured gravimetrically. It is considered that the weight of residue extracts present in *n*-hexane is equal to the plasticizer extracted [8].

Secondly, the mass loss of a plastic piece placed between two absorbent films was measured according to ISO 177. Samples were placed on two plates (3 mm thick) of low density polyethylene (LDPE) at 70 °C for 24 h with a circular cylinder (1 mm thick) of PVC plasticized with ESPO in between. The amount of plasticizer migrated was determined according to the weight differences before and after tests [8].

The third migration test was conducted using activated carbon as per ASTM D 1203-94 test method B. A wire cage was used to prevent from direct contact between the plasticized PVC and carbon. The migrated volatile components were measured after the circular cylinder was placed at 100 °C for 24 h.

Statistical Analysis

All experiments were performed in triplicate. The data were presented as mean values $\pm$ standard deviations.

Results And Discussion

Fatty Acid Composition of SPO

The fatty acid composition of SPO is presented in Table 1. ALA was the most abundant fatty acid, accounting for almost 36% of the total fatty acid composition. The total concentration of unsaturated fatty acids was 75.13%, and the iodine value of SPO was 138.7. It was observed that the iodine value of SPO reported herein was consistent with that of soybean oil. Moreover, it was demonstrated that ALA was the primary unsaturated fatty acid. Therefore, SPO has great potentials as an alternative of soybean oil for producing epoxidized products, especially epoxidized ALA type grease in PVC.

Synthesis of ESPO

The superiority of partial glyceride lipase as a catalyst for triglyceride epoxidation has been proved in previous studies [17]. Some side reactions such as hydrolysis could be prevented effectively using partial glyceride lipase. In this study, a mutant of Lipase SMG1 (SMG1-F278N) was selected due to its higher activities and better tolerance to polar solvents [19]. Compared with commercial Lipase G50 and wild-type Lipase SMG1, Lipase SMG1-F278N exhibited higher SPO epoxidation activities (Figure 1). The epoxidation reaction reached to the equilibrium in less time using Lipase SMG1-F278N, with a higher final oxirane value (> 6.9) being observed [23]. Therefore, Lipase SMG1-F278N has superior catalytic activities for epoxidation of animal and vegetable oils.

The previous studies demonstrated that the immobilized SMG1-F278N had a higher temperature tolerance, with the maximal catalytic activities at 30-35 °C [18, 24]. An increasing reaction rate was observed from 20 °C to 30 °C (Figure 1A). However, further increment could not be found after 6 h when the temperature increased to 35 °C. It was reported that the increased temperature and peroxyacid produced both could lead to decreased activities of immobilized lipases [15, 16]. In our study, the low concentration (0-0.5 mol/L) of peroxyacid would not result in a significant loss of enzyme activity. Overall, it could be concluded that high temperatures (> 35 °C) was the main factor leading to the loss of enzyme activities.

Scale-up Reaction and Reusability of Immobilized SMG1-F278N

It is generally acknowledged that immobilized lipases could be recycled easily and reused compared with free counterparts. However, the recovery of partial glyceride lipases used for triglyceride epoxidation has not been investigated. In the present study, we explored the reusability of immobilized SMG1-F278N at low temperatures (25 °C and 30 °C) and the scale-up reactions. The results are shown in Figure 2.

After being reused for 10 consecutive batches at 25 °C and 30 °C, the immobilized SMG1-F278N still had 94% and 96% of the original catalytic activities, with oxirane values 6.49 and 6.67 being observed. It was

documented that *Novozyme* 435 retained only 73% of initial activities after being recycled once at 25 °C. The enzyme could not be reused any more due to a significantly decreased activity at > 25 °C [25]. The small amount of peroxyacid (< 0.24%, w/w%) produced in the reaction generally could not lead to enzyme inactivation. The exogenous acids (acetic acid or other acids) added largely inhibited the hydrolysis of oils and fats. Meanwhile, these acids served as effective substrates for production of peroxyacid. However, these exogenous acids could not only lead to decreased stabilities of lipase, but increased the complexity of product separation and purification [26]. This study found that Lipase SMG1-F278N could catalyze the epoxidation of unsaturated fatty acids of SPO effectively without exogenous acids. In addition, the epoxidation reaction reported herein, to a large extent, avoided the hydrolysis of glycerides and improved the enzyme stability. The enzyme could be reused for at least 8 times without significant loss of activities.

The changes of oxirane values of ESPO products are illustrated in Figure 2B. It was found that the scale-up (by 25 times) reaction reached the equilibrium after 6 h with an oxirane value of 6.94%. Overall, this epoxidation reaction could be scaled up with a good reproducibility.

Characterization of ESPO

The physicochemical indices of ESPO were determined. The results are shown in Table 2. Obviously, the acid value, iodine value and heating loss of ESPO were lower than that of ESO and ELO, while the content of epoxy groups of ESPO was higher than ESO. Similar densities were observed between ESO and ELO. These physicochemical parameters indicated that ESPO could serve as a promising alternative of ESO as plastic stabilizers and plasticizers [8, 27]. The acid value of ESPO increased from 0.14 mg/g KOH to 0.47 mg/g KOH after epoxidation. This increment could be explained by the increased levels of free fatty acids produced via hydrolysis of diglycerides. The acid value of ESPO was less than 0.5 mg/g KOH, indicating that the triglycerides were not hydrolyzed.

FT-IR and NMR Analysis

The FTIR spectra of SPO and ESPO are shown in Figure 3. The absorption band at 3009 cm^{-1} was characteristic of =C-H (Figure 3A). The ESPO exhibited an absorption peak at 837 cm^{-1} (Figure 3B), which was attributed to the epoxy group. Moreover, the peak intensity at 3009 cm^{-1} decreased significantly, while the peak intensity at 837 cm^{-1} increased dramatically with increased epoxidation degrees. Therefore, SPO was successfully epoxidized by Lipase SMG1-F278N.

Figure 4 shows the ^{13}C NMR spectrum of the SPO and ESPO. The peaks at δ 172.5-173.5 ppm of SPO and ESPO were attributed to the ester carbonyls in triglycerides. The characteristic peak of CH=CH was observed at δ 127.1 ppm. Obviously, a new peak at δ 31.5 ppm corresponding to the epoxy group was found in the epoxide product. Therefore, it was concluded that the groups were epoxidized by Lipase SMG1-F278N effectively.

Thermal Characterization

It is generally acknowledged that the thermo resistance of epoxy grease *per se* could contribute to the thermal stability of PVC and other plastic products. The thermal degradation kinetics of SPO, ESO, ELO and ESPO are shown in the Figure 5.

As can be seen from Figure 5A, the thermal decomposition temperature of SPO increased from 374 °C to 416.3 °C after epoxidation, which is consistent with the epoxidized vegetable oil reported previously²⁸. Similar thermal decomposition temperatures of ESPO (416.3 °C) and ELO (411.5 °C), could be observed from Figure 5B, which were higher than that of ESO (402.3 °C). Therefore, ESPO could improve the thermal stability of plastic products significantly.

It has been demonstrated that the frost resistance of epoxy grease is closely related to solidification temperatures [9]. In this study, DSC was used to investigate the solidification temperature of ESPO. It was found that the initial solidification temperature of ESPO was -3 °C, which was close to ELO (-5 °C) and lower than ESO (9 °C) (Figure 6). Therefore, ESPO has better frost resistance than ESO as a plasticizer.

Effect of Plasticizer Addition on PVC Mechanical Properties

Some vital processing parameters including curing formulations and mechanical characterization are usually assessed to evaluate the flexibility of PVC.

As shown in Figure 7A, the tensile elongation of ESPO on PVC was higher than that of ESO with the plasticizer content of > 60 phr. The tensile strength of PVC was significantly reduced with the plasticizer content of > 80 phr (Figure 7B). Notably, similar mechanical properties could be found with respect to the PVC sheets plasticized with ESPO and ELO [7]. This observation could be explained by that the majorities of epoxy glycerides in ESPO and ELO were epoxy linolenic acid glycerides [10]. Therefore, ESPO has better plasticizing effects on PVC than ESO.

Thermal Stability and Migration Test of PVC Samples

The results about thermal stability and migration test of PVC samples are shown in Table 3. The results demonstrated that PVCs (80 phr) plasticized with ESPO and ELO showed close thermal stability times of 30 min and 29 min, respectively, which were higher than 26 min of ESO. Therefore, ESPO contributed to better stabilities of PVC than ESO. The emigration tests demonstrated that the migrate rate of ESPO (-0.83 ± 0.02 %) from PVC was significantly lower than ESO (-1.02 ± 0.05 %). Therefore, ESPO serves as a promising alternative of ESO as the raw material for production of plasticizers.

Conclusions

In the present study, an effective chemo-enzymatic route was proposed using Lipase SMG1-F278N as catalysts for ESPO in DESs. The preliminary results showed that a high catalytic efficiency for production of ESPO was observed due to the enhanced robustness of Lipase SMG1-F278N in DESs. Compared with ESO, the ESPO reported in this contribution exhibited better thermal stability and frost resistance. In

addition, a relatively low migration rate of plasticizer was observed in PVC plasticized with ESPO. Overall, ESPO is expected to serve as a promising alternative to ESO used for packaging films.

Declarations

Authors Contributions

Yingrui Ji: experimental activities, Conceptualization, Data curation, Supervision, Formal analysis, Validation, Writing - original draft; Long Xu: Methodology, Writing - review & editing; Qingqing Xu: Investigation; Xuan Liu: Supervision; Sen Lin: Formal analysis; Sentai Liao: experimental activities; Weifei Wang: Resources; Dongming Lan: Funding acquisition

Ethical Approval This article does not contain any studies with human participants or animals performed by any of the authors.

Consent to Participate The authors agreed to participate in this work.

Consent for Publication The authors agreed to publish this work.

Conflict of Interest The authors declare no competing interests.

Finding

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References

1. Zhang, C., Garrison, T. F., Madbouly, S. A., & Kessler, M. R. (2017). Recent advances in vegetable oil-based polymers and their composites. *Prog. Polym. Sci*, 71, 91–143
2. Sahoo, S. K., Khandelwal, V., & Manik, G. (2018). Development of toughened bio-based epoxy with epoxidized linseed oil as reactive diluent and cured with bio-renewable crosslinker. *Polym. Adv. Technol*, 29(1), 565–574
3. Goud, V. V., Patwardhan, A. V., & Pradhan, N. C. (2006). Studies on the epoxidation of mahua oil (*Madhumica indica*) by hydrogen peroxide. *Bioresour. Technol*, 97(12), 1365–1371
4. Joshi, S. M., Gogate, P. R., & Kumar, S. (2018). Intensification of esterification of karanja oil for production of biodiesel using ultrasound assisted approach with optimization using response surface methodology. *Chem. Eng. Process*, 124, 186–198

5. Dinda, S., Patwardhan, A. V., Goud, V. V., & Pradhan, N. C. (2008). Epoxidation of cottonseed oil by aqueous hydrogen peroxide catalysed by liquid inorganic acids. *Bioresour. Technol*, 99(9), 3737–3744
6. Klaas, M. R., & Warwel, S. (1999). Complete and partial epoxidation of plant oils by lipase-catalyzed perhydrolysis. *Ind. Crop. Prod*, 9(2), 125–132
7. Xia, Y., & Larock, R. C. (2010). Vegetable oil-based polymeric materials: synthesis, properties, and applications. *Green Chemistry*. 12(11).
8. Fenollar, O., Garcia-Sanoguera, D., Sanchez-Nacher, L., Lopez, J., & Balart, R. (2010). Effect of the epoxidized linseed oil concentration as natural plasticizer in vinyl plastisols. *J. Mater. Sci*, 45(16), 4406–4413
9. Fenollar, O., Sanchez-Nacher, L., Garcia-Sanoguera, D., López, J., & Balart, R. (2009). The effect of the curing time and temperature on final properties of flexible PVC with an epoxidized fatty acid ester as natural-based plasticizer. *J. Mater. Sci*, 44(14), 3702–3711
10. Milchert, E., Smagowicz, A., & Lewandowski, G. (2010). Optimization of the reaction parameters of epoxidation of rapeseed oil with peracetic acid. *J. Chem. Technol. Biot*, 85(8), 1099–1107
11. Wei, Z., Liao, A., Zhang, H., Liu, J., & Jiang, S. (2009). Optimization of supercritical carbon dioxide extraction of silkworm pupal oil applying the response surface methodology. *Bioresour. Technol*, 100(18), 4214–4219
12. Wang, W., Xu, L., Zou, Y., Pang, D., Shi, W., Mu, L. ... Wang, Y. (2019). Comprehensive Identification of Principal Lipid Classes and Tocochromanols in Silkworm (*Antheraea pernyi* and *Bombyx mori*) Pupae Oils. *Eur. J. Lipid Sci. Technol*. 122(2).
13. Jiang, Y. (2013). Separation of Polyunsaturated Fatty Acids from Silkworm Oil with Urea Adduction. *The. Food. Ind*, 34(5), 35–37
14. Allauddin, S., Somisetti, V., Ravinder, T., Rao, B., Narayan, R., & Raju, K. (2016). One-pot synthesis and physicochemical properties of high functionality soy polyols and their polyurethane-Urea coatings. *Ind. Crop. Prod*, 85, 361–371
15. Orellana-Coca, C., Camocho, S., Adlercreutz, D., Mattiasson, B., & Rajni, H-K. (2005). Chemo-enzymatic epoxidation of linoleic acid: Parameters influencing the reaction. *Eur. J. Lipid Sci. Technol*, 107(12), 864–870
16. Quadrosjr, J. V. D., & Giudici, R. (2016). Epoxidation of soybean oil at maximum heat removal and single addition of all reactants. *Chem. Eng. Processing*, 100, 87–93
17. Zhou, P., Wang, X., Zeng, C., Wang, W., Yang, B., & Hollmann, F. (2017). Deep Eutectic Solvents Enable More Robust Chemoenzymatic Epoxidation Reactions. *ChemCatChem*, 9(6), 934–936
18. Li, X., Li, D., Wang, W., Durrani, R., & Yang, B. (2016). Immobilization of SMG1-F278N lipase onto a novel epoxy resin: Characterization and its application in synthesis of partial glycerides. *J. Mol. Catal. B-Enzym*, 133, 154–160
19. Li, D., Wang, W., Durrani, R., Li, X., Yang, B., & Wang, Y. (2016). Simplified Enzymatic Upgrading of High-Acid Rice Bran Oil Using Ethanol as a Novel Acyl Acceptor. *J. Agric. Food. Chem*, 64(35), 6730–

20. Xu, T., Lu, L., Hou, S., Xu, J., Yang, B., & Wang, Y. (2012). Crystal structure of a mono- and diacylglycerol lipase from *Malassezia globosa* reveals a novel lid conformation and insights into the substrate specificity. *J. Struct. Biol*, 178(3), 363–369
21. Hu, B., Li, C., Zhang, Z., Zhao, Q., Zhu, Y., & Su, Z. (2017). Microwave-assisted extraction of silkworm pupal oil and evaluation of its fatty acid composition, physicochemical properties and antioxidant activities. *Food. Chem*, 231, 348–355
22. Wang, Y., Mai, Q., Qin, X., Yang, B., & Wang, Z. (2010). Establishment of an evaluation model for human milk fat substitutes. *J. Agric. Food. Chem*, 58(1), 642–649
23. Wang, X., Tang, Q., Grzegorz, M. P., Yang, B., & Wang, Y. (2015). A mechanistic study into the epoxidation of carboxylic acid and alkene in a mono, di-acylglycerol lipase. *Biochem. Biophys. Res. Commun*, 460(2), 392–396
24. Wang, W., Xu, Y., Qin, X., Lan, D., Yang, B., & Wang, Y. (2014). Immobilization of lipase SMG1 and its application in synthesis of partial glycerides. *Eur. J. Lipid Sci. Technol*, 116(8), 1063–1069
25. Vlček, T., & Petrović, Z. S. (2006). Optimization of the chemoenzymatic epoxidation of soybean oil. *J. Am. Oil Chem. Soc*, 83(3), 247–252
26. Dinda, S., Goud, V. V., Patwardhan, A. V., & Pradhan, N. C. (2011). Selective epoxidation of natural triglycerides using acidic ion exchange resin as catalyst. *Asia-Pac J Chem Eng*, 6(6), 870–878
27. Eiichi, K. N., Kanako, Y., Mitsuyoshi, N., Kazuo, M., & Tsuyoshi, M. (2002). Lipid Profiles and Oxidative Stability of Silkworm Pupal Oil. *J. Oleo. Sci*, 51(11), 681–690
28. Kim, J. R., & Sharma, S. (2012). The development and comparison of bio-thermoset plastics from epoxidized plant oils. *Ind. Crop. Prod*, 36(1), 485–499

Tables

Table 1. Fatty acid composition of SPO

Fatty acids	Abbreviation	Concentration (wt%)	Ref (Eiichi et al., 2002)	Ref (Hu et al., 2017)	Ref (Liu et al., 2015)
Palmitic acid	C16:0	19.62 ± 0.47	24.90	23.18 ± 0.52	19.50 ± 0.97
Palmitoleic acid	<i>cis</i> -9-C16:1	1.39 ± 0.12	0.80	1.07 ± 0.09	ND
Stearic acid	C18:0	4.73 ± 0.51	5.40	4.69 ± 0.17	7.05 ± 0.35
Oleic acid	<i>cis</i> -9-C18:1	29.72 ± 0.61	24.30	28.32 ± 0.63	31.16 ± 1.56
Linoleic acid	<i>cis</i> -9,12-C18:2	7.96 ± 0.23	6.30	3.88 ± 0.13	7.55 ± 0.38
Linolenic acid	<i>cis</i> -9,12,15-C18:3	36.07 ± 0.64	36.00	38.25 ± 0.75	34.73 ± 1.74
Others		0.51 ± 0.03	0.20	0.59 ± 0.09	ND

Table 2. Physico-chemical indices of modified SPO

parameters	SPO	ESPO	ESO	ELO	Detection method
Acid value (mg KOH/g)	0.14 ± 0.02	0.47 ± 0.05	0.51 ± 0.03	1.02 ± 0.06	AOCS Cd 3d-63
Iodine value (g I ₂ /100g)	138.7	4.31 ± 0.23	5.01 ± 0.38	4.47 ± 0.25	AOCS Tg 1a-64
Oxirane value (%)	0.00	6.93 ± 0.37	5.74 ± 0.45	9.01 ± 0.43	AOCS Cd 9-57
Water solubility	Non soluble	Non soluble	Non soluble	Non soluble	
Density at 20 °C (g/cm ³)	0.93 ± 0.02	0.99 ± 0.01	0.99 ± 0.01	1.03 ± 0.02	AOCS Cc 10c-95
Heating loss (%)	0.12 ± 0.01	0.19 ± 0.01	0.21 ± 0.02	0.34 ± 0.03	AOCS Ac 2-41

Table 3. Thermal stability and migration test

Samples	Thermal stability time (min)	<i>n</i> -hexane (%)	Migration (%)	Active carbon (%)
ESO-PVC	26 ± 1.6	0.19 ± 0.03	-1.02 ± 0.05	0.18 ± 0.02
ELO-PVC	29 ± 1.9	0.11 ± 0.04	-0.81 ± 0.03	0.13 ± 0.01
ESPO-PVC	30 ± 1.3	0.12 ± 0.02	-0.83 ± 0.02	0.14 ± 0.01

Figures

Figure 1

Effects of temperature on epoxidation activities of different enzymes

Figure 2

Reaction batch and reaction scale on the epoxidation reaction

Figure 3

FTIR spectra of SPO before epoxidation (blue) after epoxidation (red)

Figure 4

^{13}C NMR spectra of SPO (A) before epoxidation (B) after epoxidation

Figure 5

TGA thermographs of the SPO, ESPO, ESO and ELO

Figure 6

The heat flow analysis of epoxidized oils, the curing agent by DSC

Figure 7

Effects of plasticizer content on mechanical properties