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# Immobilized lipase-catalyzed esterification for synthesis of trimethylolpropane triester as a biolubricant

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ABSTRACT

Synthetic oleochemical esters of polyols and fatty acids are biodegradable and possess desirable technical and ecological properties. Trimethylolpropane (TMP) triester has been widely applied as a hydraulic fluid. TMP triester was effectively synthesized by lipase-catalyzed esterification from TMP and high oleic fatty acid from palm oil using an immobilized lipase. The immobilized lipase was prepared with liquid Lipozyme TL 100 L from *Thermomyces lanuginosus* with Duolite A568 as a carrier. The effects of temperature, enzyme loading, vacuum level, and water activity of the enzyme on the synthesis of TMP triester were investigated. The optimum temperature, enzyme loading, and vacuum level were 60 °C, 15% (based on total substrate), and 6.7 kPa, respectively. The optimum water activity range of the enzyme was 0.5–0.9. Under the optimum conditions, the maximum conversion reached up to 95% after 9 h. No significant differences in physical properties were observed between TMP triester from this study and a commercial TMP triester prepared by chemical catalyst.



## 1. Introduction

As pollution and environmental health have become increasingly important public issues, interest in biolubricants has grown because they are biodegradable and environmentally friendly [1]. Because of this trend, biodegradable base stocks have replaced mineral oil base stocks. Vegetable oil based lubricants are biodegradable and have low eco-toxicity compared with mineral oil based lubricants. However, they have several drawbacks, namely low thermal, oxidative, and hydrolytic stabilities and poor low temperature fluidity because of high pour points [2]. Synthetic biolubricants have been developed to overcome these limitations.

Among the synthetic lubricants, synthetic esters of polyols and fatty acids (FA) are considered as environmentally friendly substitutes to mineral oil based lubricants because they have suitable properties for lubricant application. Synthetic esters of polyols and FA generally show good performance at low temperatures, and have high thermo-oxidative stability, high viscosity indices, good antiwear performance, and low evaporation properties [1]. Therefore, these esters are suitable for use as high performance lubricants in industry. Among the polyols, trimethylolpropane (TMP) is commonly used to synthesize TMP triester, because TMP has high performance and moderate price level [3]. TMP triester is an important lubricant and has been widely applied as a hydraulic fluid, crank case lubricant, high temperature grease, and compressor oil [4].

A number of studies have investigated the synthesis of TMP esters via esterification of TMP with free FA or FA methyl esters using a homogeneous or heterogeneous chemical catalyst [3,5–7]. Meanwhile, there have also been several reports on the synthesis of TMP esters by esterification using lipases as the biocatalyst. The lipases most frequently used for synthesis of TMP esters are Novozym 435 from *Candida antarctica*, Lipozyme RM IM from *Rhizomucor miehei*, and *Candida rugosa* lipase. For example, Åkermanet al. [8] achieved 96% conversion using Novozym 435 for esterification of TMP ester from oleic acid in 24 h. In studies of the synthesis of TMP triester by transesterification using *C. rugosa* lipase or Lipozyme RM IM [9,10], conversion of 64% was achieved

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with *C. rugosa* lipase after 24 h and conversion of 90% was achieved with Lipozyme RM IM after 66 h. Overall, Novozym 435 is the most effective lipase considering reaction rate and conversion in the synthesis of TMP triester. However, when the immobilized lipase prepared in this study was used for synthesis of TMP triester, the reaction rate was much faster and the conversion was much higher than with Novozym 435.

The goal of this study was to synthesize TMP triester from TMP and FA using an immobilized lipase. The immobilized lipase was prepared using liquid Lipozyme TL 100 L from *Thermomyces lanuginosus* and Duolite A568 as a carrier. The effects of reaction temperature, enzyme loading, vacuum, and water activity of the enzyme were investigated. The physical properties of TMP triester synthesized in this study were determined and compared with that of a commercial TMP triester prepared by chemical catalyst.

## 2. Materials and methods

### 2.1. Materials

High oleic fatty acid (HOFA) from palm oil was used as the substrate for synthesis of TMP triester. HOFA was donated by ILSHINWELLS (Cheongju, Republic of Korea). The FA compositions of the HOFA were oleic acid (C18:1*n*-9, 80%), linoleic acid (C18:2*n*-6, 11%), palmitic acid (C16:0, 6%), stearic acid (C18:0, 2%) and myristic acid (C14:0, 1%). TMP and commercial TMP triester prepared by chemical catalyst were donated by Ohsung Chemical Ind. Co., Ltd. (Incheon, Republic of Korea). Liquid Lipozyme TL 100 L and Lipozyme TL IM from *T. lanuginosus*, Novozym 435 from *C. antarctica* and Lipozyme RM IM from *R. miehei* were purchased from Novozymes (Seoul, Republic of Korea). Lipase OF from *C. rugosa* was purchased from Meito Sangyo Co., Ltd. (Tokyo, Japan). Lipase PS from *Pseudomonas fluorescens* and Lipase AYS from *C. rugosa* were purchased from Amano Enzymes (Troy, VA, USA). Duolite A568 was purchased from Rohm and Haas (Chauny, France). All of the other chemicals used in this study were of analytical grade, unless otherwise stated.

### 2.2. Enzyme immobilization

Immobilization of enzyme was performed as described in our previous study [11]. The enzyme solution was prepared by mixing liquid Lipozyme TL 100 L (120 mL) with sodium phosphate buffer (30 mL, 50 mM, pH 7.0). The enzyme solution (150 mL) was added to a flask containing Duolite A568 (15 g), which acted as a carrier. This mixture was shaken at 250 rpm and incubated at 30 °C for 17 h in an orbital shaker. The carrier was then separated from the enzyme solution by filtration and immediately washed with buffer solution (150 mL) to remove unbound enzyme. The carrier with the immobilized enzyme was dried overnight at room temperature and then dried in a vacuum oven for 12 h at 40 °C. The immobilized enzymes were stored at 4 °C before use.

### 2.3. Equilibration of water activity

The immobilized enzyme was pre-equilibrated in individual sealed containers with saturated salt solutions of known water activity. The salts used were LiCl ($a_w = 0.11$), $MgCl_2$ ($a_w = 0.33$), $Mg(NO_3)_2$($a_w = 0.53$), NaCl ($a_w = 0.75$), $K_2CO_4$($a_w = 0.97$). The equilibration process was carried out at 25 °C for over 24 h.

### 2.4. Lipase-catalyzed esterification

Lipase-catalyzed esterification of TMP with FA were carried out in a 50-mL water-jacketed glass vessel. The scheme of the reaction was shown in Scheme 1. TMP (0.4 g, 3.1 mmol) and FA (2.6 g, 9.2 mmol) were placed in a reactor preheated to the desired temperature using a water circulator. The reaction was initiated by adding enzyme to the substrate mixture with stirring at 250 rpm under vacuum. The vacuum level was controlled with a micrometering valve (Swagelok, Solon, OH, USA) and monitored using a digital vacuum gauge (Teledyne, Thousand Oaks, CA, USA). Samples (100 μL) were withdrawn from the reaction mixture at appropriate intervals and dissolved in chloroform. Individual sample was then filtered through a 0.45 μm nylon microfilter (Pall Corporation, Port Washington, NY, USA). The samples were analyzed by gas chromatography. All experiments were conducted in triplicate.

### 2.5. Product analysis

The conversion in the reaction mixture was determined by dissolving samples (10 μL) obtained under various reaction conditions in chloroform (1 mL). A gas chromatograph (model 3800; Varian Inc., Palo Alto, CA, USA) equipped with a DB-1ht column (15 m × 0.25 mm i.d.; J&W Scientific, Folsom, CA, USA) and a flame ionization detector was used for the analysis. The column temperature was held at 150 °C for 2 min, increased to 370 °C at a rate of 25 °C/min and then held at 370 °C for 5 min. Helium at a flow rate of 1.5 mL/min was used as the carrier gas and the split ratio was 1:50. The injector and detector temperatures were set at 340 and 350 °C, respectively.

The conversion to TMP triester was calculated using the following equation:

$$\text{Conversion}(\%) = \frac{TMP\ triester}{FA + TMP\ monoester + TMP\ diester + TMP\ triester} \times 100 \quad (1)$$

### 2.6. Determination of the physical properties

To determine the physical properties of the synthesized TMP triester, a large-scale version of the lipase-catalyzed esterification was conducted under the optimum conditions. After the reaction, the final product was separated from the enzyme by filtration.

The viscosity and viscosity index were determined according to the ASTM D445 and ASTM D2270 methods, respectively. The viscosity was calculated based on the time taken for the fluid to flow through a glass capillary tube (Cannon-Fenske Routine viscometer, Cannon Instrument Co., State College, PA). The kinetic viscosity was obtained as the product of this time and the tube constant. The viscosity index was calculated taking into account the product viscosities at 40 and 100 °C. The pour point and cloud point were measured using a Tanaka Mini-Pour/Cloud Point Tester (Model MPC-102 S, Tanaka Scientific Ltd., Tokyo, Japan) according to the ASTM D2500 and ASTM D6749 methods, respectively. The color was determined using a colorimeter (PFX195, The Tintometer Ltd,