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# Optimization of lipase-catalyzed synthesis of diglycerol monooleate by response surface methodology

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ABSTRACT

The optimization of the lipase-catalyzed esterification of pure diglycerol (3-(2,3-dihydroxypropoxy)propane-1,2-diol) with pure oleic acid to produce pure diglycerol monooleate (E475) which is a non-ionic surfactants, was performed. Six immobilized lipases were tested and the best oleic acid conversion was attained with Novozym 435 from *Candida antarctica* which was selected to optimize the reaction conditions by response surface methodology (RSM). Well-fitting quadratic polynomial regression model for acid conversion was established with regard to temperature (65 °C–75 °C) and catalyst concentration (mass fraction of 1–5%). The two factors investigated positively affected acid conversion, with catalyst concentration having the greatest effect. The regression equation obtained by central composite design of RSM predicted optimal reaction conditions of 77 °C and 5.8%. Under these optimal conditions the model obtained in this work has been tested in scale-up experiment, and the resulting acid conversion was 93.9% with an accuracy of 97.4%. Within the experimental range studied the results model give good agreement with the experimental data.



## 1. Introduction

Polyglycerol esters of fatty acids (PGE, E475) are non-ionic surfactants that have been used during the last years as emulsifiers in food and personal care products [1,2]. More recently, new industrial applications based on PGE have been developed, includes their utilization as antifogging and antistatic additives, lubricants and plasticizers [1,3]. The PGE are an amphipathic molecules with hydrophilic moiety (hydroxyl groups) attached to a hydrophobic backbone (alkyl chain). These two parts provide a compound with interfacial activity and give rise to a wide range of surface chemistry functions.

Glycerol is the main by-product of biodiesel production. As the biodiesel production is increasing exponentially, the crude glycerol generated from the transesterification of oils has also been generated in a large quantity. The valorization of glycerol as by-product increases the economic sustainability of the biodiesel industry. Within this context, among the wide application of glycerol in food, the use of glycerol for the production of non-ionic surfactants like glycerol esters of fatty acids, can be a good way forward to development of surfactants products entirely derived from renewable resource [4,5]. However, glycerol itself is not a good primary constituent of the hydrophilic part of the surfactant and polyglycerols are needed to increase the hydrophilicity and to adjust the hydrophilic-hydrophobic balance of the products. Since the

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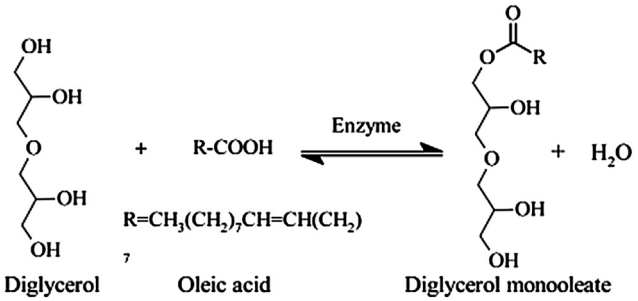


**Scheme 1 – .TIF. Diglycerol monooleate ester synthesis.**

phase behavior of diglycerol and glycerol esters of fatty acid, shown that the presence of strong intramolecular H-bonds within the diglycerol headgroup increases the polar part volume and thus causes a swelling of the lamellar phase [6]. PGE have an unexpectedly low solubility in water; they are of interest as stable carriers for drugs, cosmetics, or specific food ingredients. Among the commercial mixtures of PGE, the main component is diglycerol ester [7] and pure diglycerol esters have been shown to be efficient surfactants [8], thereby creating a demand for more specific synthesis procedures of PGE.

Current processes for production of fatty acid esters of polyglycerols are based on direct esterification of the polyglycerol with fatty acids [9], transesterification of the polyglycerol with a triglyceride or a fatty acid methyl ester [10] or addition polymerization of glycidol to a fatty acid or to a fatty acid monoglyceride [11]. Esterification method is the best suited for the production of designer polyglycerol ester because the desired free fatty acid and polyglycerol can easily be selected prior to polyglycerol ester formation.

Conventional production of polyglycerol esters by esterification commonly involves the use either alkali [9,12] or acid catalysts [10]. But, besides the environmental problem, these catalysts favor side-reactions from the degradation of the fatty acid (oxidation and dimerization) or from the polyglycerol (dehydration into acrolein and oxidation) [13].

A few decades ago, the employment of lipases as biocatalysts for esterification reactions has emerged as a potential route to replace the conventional chemical process [14,15]. The main reason is that they are employed as active and selective catalysts in a variety of reactions, with fewer environmental problems, and in the case of immobilized lipases, they can be reused.

So far, to our knowledge, the use of pure diglycerol to produce diglycerol fatty acid mono-esters has received little attention. The objective of our study was to model the lipase-catalyzed esterification of pure diglycerol with oleic acid to produce pure diglycerol monooleate ester (Scheme 1) in a solvent-free system. Six different commercially available lipases were evaluated for their catalytic activity in the reaction. For selected lipase (Novozym 435), the modeling and optimization of the reaction by RSM was performed with respect to temperature and catalyst concentration as independent variables and acid conversion as a response variable, and then the optimal reaction conditions were proposed.

## 2. Materials and methods

### 2.1. Materials

Diglycerol (purity > 98%) was supplied by Solvay Química S.L. (Spain) and oleic acid (purity > 98%) was supplied by Henkel Iberica. The six immobilized thermostable lipases were provided by Novozymes A/S (Bagsvœrd, Denmark). Table 1 summarizes some of the properties of reported lipases. All solvents used for the chromatographic analysis were of high-performance liquid chromatography (HPLC) grade, supplied by Sigma–Aldrich.

### 2.2. Equipment

Reactions were performed in a solvent-free system using a batch stirred reactor of 500 $cm^3$ volume, under fixed conditions of pressure and temperature. Pressure, stirring speed and temperature controllers were provided. The propeller used was marine-type and the speed was set at 62.8 rad $s^{-1}$. The desired working pressure (7.998 kPa) was maintained by a vacuum pump. This permitted ready elimination of water from the system in range of temperature studied, without significant variations of viscosity of the liquid phase or reaction volume. The reaction temperature was achieved immersing the reactor into a thermostatic bath with an electrical device connected to a PID controller which allows a temperature control of ±0.1 °C.

### 2.3. Lipase-catalyzed esterification

Oleic acid (45 g, 160 μmol) and diglycerol (72 g, 433 μmol) were added to the reactor and the stirring was started. When the desired temperature was reached, the catalyst was added and the vacuum pump was turned on in order to drive the equilibrium towards the diglycerol monooleate ester synthesis.

**Table 1 – Properties of immobilized lipases used in diglycerol monooleate production.**

| Microorganisms | Lipase | Support | Surface area ($dam^2$ $kg^{-1}$) | Pore diameter (nm) |
|---|---|---|---|---|
| *Rhizomucor miehei* | Lipozyme IM-20 | Anion exchange resin | 3.6 | 14.1 |
| | Lipozyme IM | Anion exchange resin | 6.0 | 17.6 |
| | Lipozyme IM-50 | Anion exchange resin | 6.2 | 24.3 |
| *Candida antarctica* | Novozym 435 | Acrylic resin | 2.9 | 29.2 |
| | Novozym SP 435-A | Acrylic resin | 6.7 | 21.0 |
| | Novozym SP 435-L | Acrylic resin | 9.5 | 17.9 |