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ORIGINAL PAPER

Synthesizing Precursors for Functional Food Structured Lipids from Soybean Oil Deodorized Distillates

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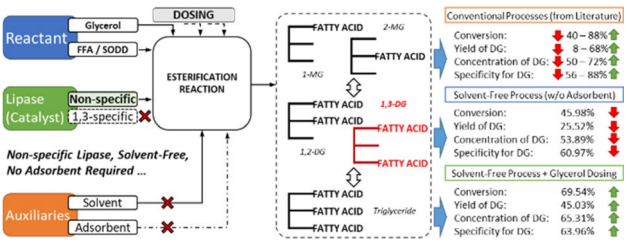
Abstract

A "green" scheme of synthesizing diglycerides as a means of valorizing an oil processing by-product, specifically soybean oil deodorized distillate (SODD), was established. Different glycerol dosing strategies were implemented in the solvent-free lipase catalyzed esterification of free fatty acids with glycerol within molar ratios of 2–4 and temperatures of 40–60 °C. The process responses were compared with non-dosed systems in this study and other similar processes reported in literature. Better 1,3-diglyceride selectivity at comparable yields were observed with glycerol dosed-systems over non-dosed systems. The presence of molecular sieves negatively affected 1,3-diglyceride selectivity, total diglyceride selectivity and yield. Selectivity of 0.91 ± 0.01 g diglycerides/g (mono + triglycerides), free fatty acid conversion of 57.87 ± 3.46%, and yield of 30.59 ± 1.26 g diglyceride/100 g raw material is obtained with SODD at 40 °C, overall free fatty acid to glycerol molar ratio of 2, 4 wt% Novozyme 435 and 48 h. The process scaled better than most solvent-based ones supported with hygroscopic sorbents in terms of reaction mass efficiency.

Graphic Abstract



Keywords Solvent-free · Enzymatic esterification · Glycerol dosing · Selectivity · Soybean oil deodorized distillate · Structured lipid precursors

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Statement of Novelty

A green scheme of precursor synthesis from SODD that improved selectivity and yield without the use of solvents, hydroscopic sorbents & specific lipases was established.

Introduction

Diglycerides (DG) are versatile compounds used as additives for various products and as precursors for more complex compounds. DG, together with monoglycerides (MG), form a group called partial glycerides (PG), which are nonionic but possess both hydrophilic and lipophilic functional groups. Because of this, they function largely as emulsifiers [1] especially in food products, but are also important components of nutraceuticals or functional food [2, 3], pharmaceuticals [4], and cosmetics [5]. When reacted with certain ligands, DG can be converted to structured triglycerides (STG) with unique or important properties such as radical scavenging or anti-oxidative activity with ferulic [6] and caffeic acids [7], weight control activity with D-galactopyranose and pinolenic acid [8], and anti-inflammatory, anti-proliferative & anti-cancer effects with n-3 polyunsaturated fatty acids [9] making them indispensable in human diet and wellness.

The synthesis of DG proceeds through several schemes of reactions allowing for the use of reactants from varied sources including waste products [10]. Among synthesis routes that are commonly investigated are alcoholysis of oils using either ethanol [2] or glycerol [11], and direct esterification of free fatty acids (FFA) with glycerol [12, 13]. Transesterification or interesterification of oils with either fatty acids or other oils/triglycerides (TG) also produce DG as by-products [14–16]. Esterification is desirable among these routes because it produces less by-products at high conversion, requiring minimal purification steps to attain high quality. A number of studies involving the esterification of oleic acid (OA) and glycerol carried out by the same group of researchers focused on the effects of temperature, molar ratio, catalyst type and loading [17], water activity [18], and addition of solvents with varying polarities [19] on 1,3-diolein production, and further using response surface methods to optimize 1,3-diolein yield in the process [13]. These works reported that optimum diolein yield and 1,3-diolein/diolein of about 87% are achieved at ~ 60 °C, ~ 2.5 OA to glycerol molar ratio (OA:G), 7.5 wt% Novozyme 435 and 4.8 wt% t-butanol with respect to OA. Another work involving diolein production using the same reaction system but catalyzed with lipase TL IM, which is *sn*-1,3-specific [20], implemented OA dosing strategies to investigate their influence on yields. It was observed that higher diolein yield was obtained if all OA was fed into the reaction in the beginning rather than adding batch-wisely during reaction.

Addition of solvent minimizes mass transfer problems as the immiscible reactants can be dissolved in it and that product selectivity can be influenced depending on solvent concentration and hydrophobicity [19, 21]. Solvent-free esterification, however, can give better conversions over solvent-based systems in certain cases such as in the enzyme-catalyzed esterification of caprylic acid and glycerol where the conversion reached more than 10% higher as compared to systems using cyclohexane, octane, and dioxane as solvents [22]. This difference is attributed partly on the inhibiting effect of solvent on lipase activity. Also, when exposed to a combination of organic solvent, compounds with surfactant properties, and moderately high temperature, immobilized lipase can be desorbed from its matrix [23] making them less active in succeeding cycles of use. Furthermore, solvents pose some health risks to users [24] apart from contributing to the cost of materials and operational cost in downstream processing.

Solvents also serve to dilute glycerol in the reaction system as high glycerol concentration was reported to be inhibitory towards lipase activity [25, 26]. As dilution by using solvents is not an option in solvent-free esterification of alcohol and fatty acid, a means to avoid this inhibitory effect is to implement alcohol dosing strategies. Dosing of alcohol, e.g. methanol and ethanol, during enzymatic esterification has long been applied in alkyl ester synthesis particularly in biodiesel production to prevent inhibition [27], but has not been exploited in fatty acid and glycerol systems.

In esterification, water is a by-product that influences the equilibrium concentration of the desired product. When in excess, the reaction proceeds in reverse breaking down the partial glycerides and triglycerides that have been formed. However, water is required in some quantity (~ 10 wt%) as some lipases are not as active without them [18, 28]. Regulation of water activity by removal of excess water has been performed through the addition of hygroscopic sorbents such as molecular sieves [29] and silica gel [20]. It is noted that silica gel has been used as an adsorbent for glycerol and the resulting material utilized as a glycerol reservoir to regulate its release in the glycerolysis of TG [30]. It also known to adsorb different classes of lipids at various degrees, more strongly with partial glycerides, making it useful in lipid separation [31]. These could compete with the adsorption of water during solvent-free esterification. Preferably, silica gel is used in combination with solvents such as n-hexane to attain better conversions of the free fatty acids to acyl glycerides [32]. Certain molecular sieves also have the capacity to adsorb fatty acids [33] and other lipids [34]. Instead of hydroscopic sorbents, there were also cases where reactions were carried out at vacuum

conditions for the purpose of removing water from the reacting system [35]. Although this involved no additional material, specialized equipment was necessary to perform the reactions. Auxiliary materials (and/or equipment) may have certain advantages when used or added to the systems above but their presence consequently lowers the reaction mass efficiency of the process, which translates to its reduced "greenness".

A by-product of oil processing and refining that is relevant and potentially a cost-efficient raw material in DG production are fatty acid distillates [36, 37]. Soybean oil deodorizer distillates (SODD), in particular, are in adequate supply and rich in unsaturated fatty acids such as oleic and linoleic acids [38], which make them appropriate in the production of DG or precursors for symmetrical ABA-type (unsaturated-saturated-unsaturated) STG. Such fatty acid distillate has been utilized mainly in the synthesis of alkyl esters [38] applied as biodiesel, and as a source of bioactive compounds including tocopherols [39], steroidal hydrocarbons [40] and squalene [41]. Functional food precursors, therefore, are high-value derivatives that can alternate with biodiesel in making use of its major component, the FFA. There has been a study previously conducted regarding DG production using SODD and *sn*-1,3-specific lipases with Lipozyme RM IM as the preferred enzyme resulting in a product containing 69.9 wt% DG [42]. To achieve higher DG concentration (86.3 wt% DG) in the product, a purification step was required. The ratio of 1,3-DG isomers over 1,2-DG in this product was about 56:44, which is significantly lower than 87:13 that can be obtained in an OA-glycerol system using solvents and a non-specific lipase [13]. This presents a challenge for improving 1,3-DG to 1,2-DG ratios in solvent-free systems, as higher ratios can be achieved with solvents even without employing downstream processing or purification steps.

Considering the cases presented above, it is the objective of this study to explore a minimalist approach to improve process responses, including conversion, product distribution, selectivity, and yield in the production of diglycerides as structured triglyceride precursors from soybean oil deodorizer distillates. Employing OA and glycerol as a model reaction system, glycerol dosing strategies were tested prior to implementing promising schemes to a system using SODD. This is to verify the hypothesis that process responses, most especially product distribution and (regio-)selectivity, can be improved in esterification processes without using solvents, *in-situ* water adsorbents, and *sn*-1,3-specific enzymes.

Materials and Methods

Materials

Oleic acid (99%, Showa Chemical Co. Ltd., Japan) and glycerol (≥ 99.5%, JT Baker, USA) were used as model reactants for esterification, with soybean oil deodorized distillate (80% FFA, TTET Union Corporation, Taiwan) as the source of FFA, replacing OA, in the later system or application. In all trials performed, Novozyme 435, a non-specific lipase from *Candida antarctica* immobilized in acrylic resin (10,000 U/g, Novo-Nordisk, Denmark) was used to catalyze the reactions. Ethyl acetate (99.9%, Echo, Taiwan), n-hexane (95%, Tedia High Purity Solvents, USA), ethyl ether (99%, Echo, Taiwan), and formic acid (≥ 98%, Sigma-Aldrich, Germany) were the solvents used in the separation of products. A lipid mix standard consisting of mono-, di- and triolein (1787-1AMP, Sigma-Aldrich), and 1,3-diolein standard (≥ 99% GC, D3627-10MG, Sigma-Aldrich) were used for identification and calibration of components in the analysis of samples.

Esterification of OA or SODD with Glycerol

A 15-ml mixture of OA and glycerol with an OA:G of 0.3–5.7 was prepared in a 50-ml Erlenmeyer flask. This was incubated without cover in an orbital shaker incubator at 40–60 °C and 150 rpm. Lipase of 4 g per 100 g of total reactants was added to start the reaction. Aliquots of 20–25 mg were taken at predetermined times for analysis using gas chromatography. After the reaction, the crude product mixture was separated from the lipase by settling and decantation. It was then stored at –20 °C prior to analysis. For experiments with glycerol dosing, the same protocol was followed except for the initial OA:G which was set around 4, and dosing of glycerol according to different strategies to an overall OA:G of ~ 2. Dosing of additional glycerol was done at 24 h, after the start of the esterification process for single dose (full dose) systems; at 24 h and 36 h for two-dose (half doses) systems. A full dose is equivalent to 750 µl of glycerol. The reaction was terminated 12–24 h after the last addition of glycerol, and the whole process lasted for 48 h. In total, seven sets of conditions involving the model reaction system were investigated, each labeled with ES-T-MRI-DS-MRF, where ES refers to the esterification system undergoing the reaction at temperature T, an initial molar ratio of MRI, added with DS number of glycerol doses, and resulting in an equivalent/overall molar ratio of MRF after all doses of glycerol were added. Four of these were non-dosed systems, namely ES-40 °C-2.0-0-2.0, ES-40 °C-4.4-0-4.4, ES-60 °C-2.0-0-2.0, and ES-60 °C-4.4-0-4.4, which served as points for comparison with three glycerol-dosed systems, specifically ES-40 °C-4.4-1-2.0, ES-40 °C-4.4-2-2.0, and ES-60 °C-4.4-2-2.0. The conditions which resulted in desirable process responses were implemented in the trials involving SODD. The initial free fatty acid to glycerol ratios (FFA:G) of these systems were based on the total oleic and linoleic acid content of the oil distillate. As the effect of the presence of molecular sieves was investigated with

selected SODD systems, each system with the oil distillate was labeled with an extended code, ES-T-MRI-DS-MRF-MS. This has the same meaning as the previous one but with MS referring to the presence of molecular sieve, where zero (0) meant that no molecular sieve was added to the system and one (1) meant that 0.6 g of 4 Å molecular sieves was added per g of SODD. All experiments were performed in duplicates.

Analysis of Free Fatty Acids and Glycerides

An aliquot of 20–25 mg of the product was dissolved in 1 ml of ethyl acetate, homogenized, and then filtered using a 13-mm syringe filter with 0.20-µm PTFE membrane (Acrodisc syringe filters, Pall Corporation, Life Sciences). A 1.5 µl-volume of the filtrate was injected into a gas chromatography unit (Shimadzu GC-2010 Plus) installed with a ZB-5HT Inferno column (15 m × 0.32 mm × 0.1 µm), and equipped with a split injector and FID. It was operated on a program to separate different types of fatty acids and acylglycerides as described in Go et al. [43]. The injector and detector temperatures were set at 370 °C and the initial column temperature at 80 °C. The column temperature was ramped to 370 °C with a total analysis time of 29 min. Nitrogen gas flow in the column was 1.4 ml/min and the injection split ratio was 50. The peaks were identified individually or classified in groups using fatty acid and lipid standards. The peak areas in the chromatograms were then converted to weight percentages using calibration curves established with these standards.

In product analysis, product distribution PD_i (g/100 g), refers to the mass of a component, m_i (g), e.g. residual OA or acylglyceride, that is contained in 100 g of glycerol-free product, m_{GFP} (g).

$$\boldsymbol{PD_i}\left[\frac{\boldsymbol{g}}{100\boldsymbol{g}}\right] = \frac{\boldsymbol{m_i}}{\boldsymbol{m_{GFP}}} \times 100 \tag{1}$$

Glyceride distribution C_i (g/100 g), is the ratio of the mass of a glyceride species, m_i (g) to 100 g of total glycerides in the product, m_{totG} (g).

$$\boldsymbol{C_i}\left[\frac{\boldsymbol{g}}{100\boldsymbol{g}}\right] = \frac{\boldsymbol{m_i}}{\boldsymbol{m_{totG}}} \times 100 \tag{2}$$

To determine the distribution of 1,2- and 1,3-DG, 100 mg of crude glyceride product was dissolved in 0.5 ml of n-hexane, and 10 µl of the resulting mixture was blotted on a TLC Silica gel 60 F254 plate (Merck) previously dried in an oven at 100 °C for 1 h. The separation of components was then carried out in a glass chamber using a pre-mixed solvent composed of 80:20:2 v/v/v of n-hexane/ethyl ether/formic acid. The plate was air-dried and developed using iodine vapor to show the bands of separated glycerides. The bands for 1,2-DG and 1,3-DG were scraped off from the plate and separately placed in vials. Ethyl acetate (1 ml) was used to extract the diglycerides from the scraped material. The mixture was then filtered and the filtrate subjected to GC analysis. The amount of each diglyceride isomer was determined using previously established calibration curves and 1,3-DG concentration (g/100 g total DG) was calculated from the masses of 1,2-DG, $m_{1,2\text{-}DG}$ (g), and 1,3-DG, $m_{1,3\text{-}DG}$ (g), respectively, as follows:

$$1{,}3DG\left[\frac{g}{100g}\right] = \frac{m_{1,3-DG}}{m_{1,2-DG} + m_{1,3-DG}} \times 100 \tag{3}$$

Calculation of Conversion, Selectivity, Yield, and Reaction Mass Efficiency

The moles of each component present in 100 g of glycerol-free product were determined using the weight percentages obtained from chromatograms. OA conversion, ξ_{OA} (%), was then calculated using the following equation:

$$\xi_{OA}[\%] = \left[\frac{n_{MG} + \left(2 \times n_{DG}\right) + \left(3 \times n_{TG}\right)}{n_{OA} + n_{MG} + \left(2 \times n_{DG}\right) + \left(3 \times n_{TG}\right)}\right] \times 100 \tag{4}$$

where n_{OA}, n_{MG}, n_{DG}, and n_{TG} are the moles of residual OA, monoglyceride (MG), diglyceride (DG), and triglyceride (TG) in the product, respectively.

From the OA:G value, the amount of glycerol, n_{GlyOH} (mol), originally used in esterification was determined on the same basis as the other components. Glycerol conversion, ξ_{GlyOH} (%), was then calculated based on the following equation:

$$\xi_{GlyOH}[\%] = \left(\frac{n_{MG} + n_{DG} + n_{TG}}{n_{GlyOH}}\right) \times 100 \tag{5}$$

Diglyceride selectivity, S_{DG} (g/g), is calculated as the ratio of the mass of total diglycerides m_{DG} (g) in the product to the total amount of monoglycerides m_{MG} (g) and triglycerides m_{TG} (g).

$$S_{DG}[g/g] = \frac{m_{DG}}{m_{MG} + m_{TG}} \tag{6}$$

Yield, Y_{DG} (g/100 g), is defined as the mass of total diglycerides m_{DG} (g) in the product that can be obtained from 100 g of the reactants used.

$$Y_{DG}\left[\frac{g}{100g}\right] = \frac{m_{DG}}{m_{OAi} + m_{GlyOHi}} \times 100 \tag{7}$$

where m_{OAi} and m_{GlyOHi} are the total amounts of OA and glycerol used as reactants, respectively.

Reaction mass efficiency, *RME*, is defined as the ratio of the mass of total diglycerides m_{DG} (g) in the product over the total mass of raw materials, Σm_{RMi} (g), used in the reaction mixture including the catalyst m_{cat} (g), solvents m_{sol} (g), sorbents m_{sor} (g), and other reagents.

$$RME\left[\frac{g}{g}\right] = \frac{m_{DG}}{\sum m_{RMi}} = \frac{m_{DG}}{m_{OAi} + m_{GlyOHi} + m_{cat} + m_{sol} + m_{sor} + \ldots} \quad (8)$$

Results and Discussion

Effect of Temperature and Molar Ratio on Conversion, Distribution, and Selectivity

Solvent-free esterification of OA and glycerol was carried out over a wide range of OA:G and different temperatures for 24 h in order to verify their effects on conversion, and more importantly, the distribution of glycerides in the product (Fig. 1). Complete conversion of glycerol (ξ_{GlyOH}) can be achieved at OA:G ≥ 3.2, but OA conversion (ξ_{OA}) can only reach 84% maximally even at the lowest OA:G and the highest temperature investigated. Diglycerides were predominantly produced in all cases except at 60 °C and OA:G ≥ 3.2 where products were largely composed of TG (> 50 g/100 g total glycerides). Specifically, diglyceride distribution (C_{DG}) reached 61.27 ± 0.34 g/100 g of total glycerides at 40 °C and an OA:G of 3.2. Triglyceride distribution (C_{TG}), on the other hand, reached 55.99 ± 2.12 g/100 g of total glycerides at the same OA:G but at a higher temperature of 60 °C. Monoglyceride distribution (C_{MG}) was low to moderate in all cases, not exceeding 31.94 ± 2.22 g/100 g of total glycerides. Diglyceride selectivity (S_{DG}) ranged from 0.42 to 1.58 g/100 g (MG + TG), peaking at the condition where C_{DG} was also maximum. In general, DG, or more broadly PG, tend to be formed at higher concentration when the reaction is carried out at lower temperature and high OA:G. This ostensibly validates the idea that at high OA:G where the fatty acid is considerably in excess over glycerol, the inhibiting effect of glycerol on lipase activity is minimized [21, 22], although some fatty acids can also have either inhibiting or promoting effects [27, 28]. Whichever effect is dominating in the reaction, it is desirable to perform esterification of OA and glycerol at 40 °C and OA:G within 2.0–4.4 considering the results obtained if C_{DG} or S_{DG} were prioritized. This is supported by previously reported works on DG production where the optimum molar ratio was found around 3.5 at 40 °C [20], and between 2 and 2.5 at 60–65 °C [17, 44].

The effects of temperature and time on product distribution and glyceride selectivity were further explored at the upper end of this OA:G range and are illustrated in Fig. 2. Maximum product distribution in terms of total glycerides (PD_{totG}) slightly increased with increasing temperature (Fig. 2a, c, and e), reaching 54.90 ± 3.32 g/100 g glycerol-free product at 40 °C, 59.31 ± 2.14 g/100 g at 50 °C, and 60.97 ± 0.15 g/100 g at 60 °C. This was possible because a higher reaction temperature increases both the rate of reaction and the mutual miscibility of reactants, leading to higher conversions and more products [45]. Also, maximum selectivity for both DG (S_{DG}) and PG (S_{PG}) occurred earlier but within a narrower period as the temperature was increased (Fig. 2b, d, and f). In particular, maximum S_{DG} and S_{PG} were attained in 18–24 h at 40 °C, 10–15 h at 50 °C, and 4–8 h at 60 °C. A longer period of high S_{DG} (or S_{PG}) implies a more flexible process when the intermediate components are the desired products. In all three temperatures, the magnitudes of the S_{DG} and S_{PG} are close and are within the ranges of 1.05–1.30 g DG/g (MG + TG) and 3.30–3.80 g PG/g TG, respectively. Apart from these, the equivalent ξ_{OA} and ξ_{GlyOH} at the stated time ranges also have similar values of 37.69% and 99.98% at 40 °C, 37.88% and 93.51% at 50 °C, and 39.68% and 100% at 60 °C. Similar trends were observed in the esterification of OA and glycerol at initial molar ratios of 2.0–4.0, carried out between 40 and 60 °C but in the presence of 15% Lipozyme TL IM (w/w_{OA}), the solvent toluene, and molecular sieves [20], as well as, in non-catalyzed solvent-free systems at the same molar ratios but at a higher temperature range of 150–200 °C [31]. The peaks of DG selectivity also occurred faster within a narrow time frame as the reaction temperature was increased, although their occurrences were observed at much shorter periods (> 4 h) alongside steep increases in TG because of a higher enzyme loading in the former and higher temperatures in the latter compared to the current study. As the conversion of glycerol was practically complete at maximum S_{DG}, the decrease in selectivity with time after this point is a result of the remaining OA further reacting with DG to form TG according to the established reaction mechanism between OA and glycerol [46]. Because TG is an undesirable product in DG production, the concentration of unreacted OA must be kept low after this stage. In biodiesel production, the stepwise addition of methanol ensured that the conversion of FFA to methyl esters was high or nearly complete such that residual acids were negligible and that the product met certain standards [27, 47]. It was, therefore, hypothesized that glycerol dosing would also be a simple but effective strategy to push the reaction towards higher FFA conversion and, possibly, minimal TG formation. Thus, at the periods when the maximum DG had been achieved, dosing of glycerol was performed to increase ξ_{OA} while still maintaining relatively high instantaneous OA:G.

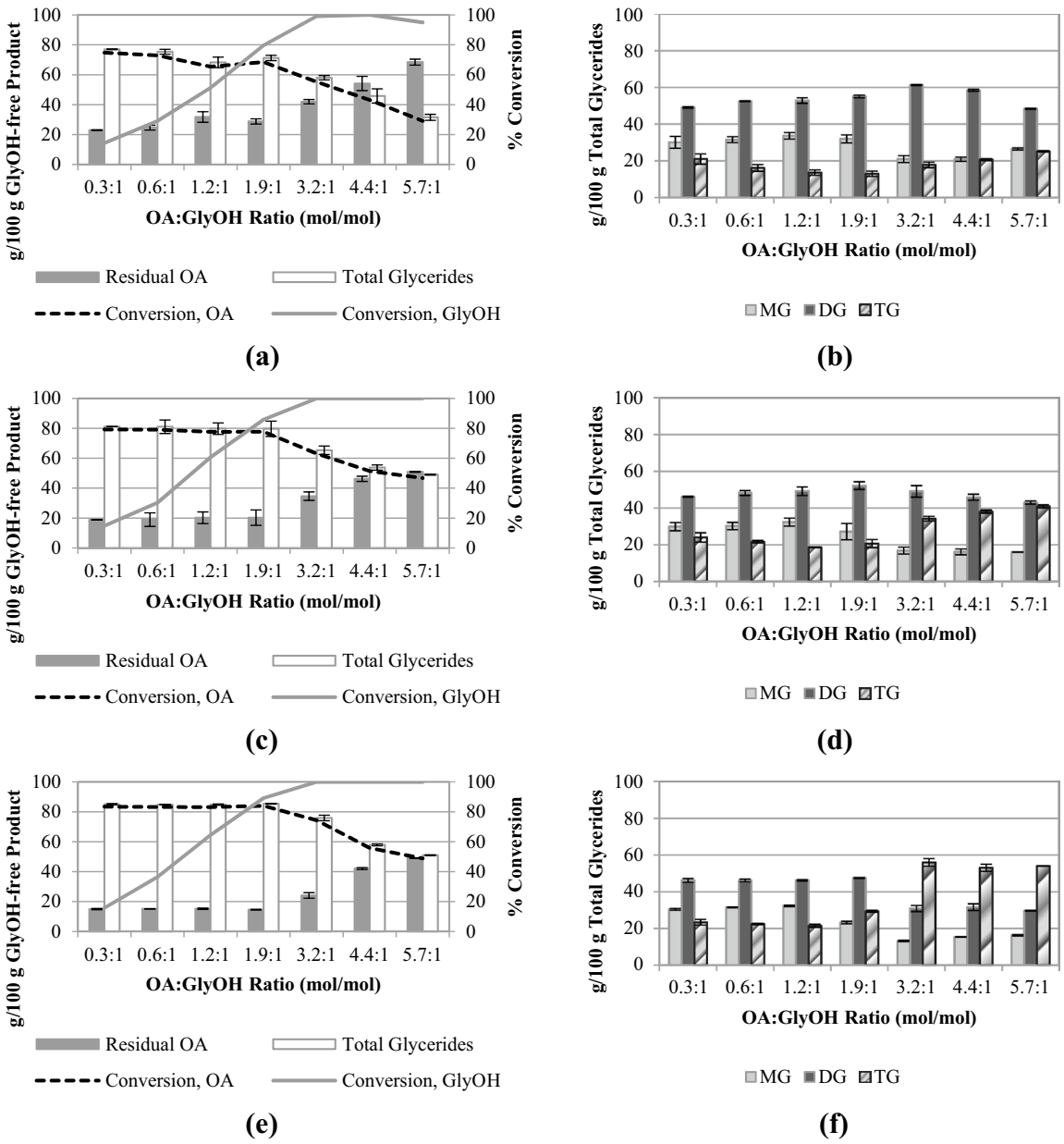

Fig. 1 Product distribution and reactant conversion during esterification of OA and glycerol catalyzed by Novozyme 435 (4 g/100 g reactants) at 40 °C (**a**, **b**), 50 °C (**c**, **d**), and 60 °C (**e**, **f**) for 24 h

Effect of Glycerol Dosing on Conversion, Distribution, Selectivity, and Yield

The effects of glycerol dosing that were initially assumed were confirmed in an experiment, with its results illustrated in Fig. 3. The profiles showed its advantages in the following ways: (a) further reduction in residual OA from 50 g/100 g glycerol-free product in the non-dosed system to approximately 20 g/100 g glycerol-free product in a dosed one, and (b) maintenance of low MG and TG concentrations thereby sustaining high DG selectivity after glycerol addition. Different glycerol dosing strategies were then carried out to determine specific effects on a number of process responses including selectivity and yield, summarized in Table 1.

Without glycerol dosing, increases in conversion were achieved by performing the reactions at higher temperature as can be seen with ES-40 °C-2.0-0-2.0 against ES-60 °C-2.0-0-2.0, or ES-40 °C-4.4-0-4.4 against ES-60 °C-4.4-0-4.4. It is already well-established that temperature affects rates of reaction generally in a positive direction, i.e. increasing the temperature increases the reaction rate. Also, the catalyst influences the range at which

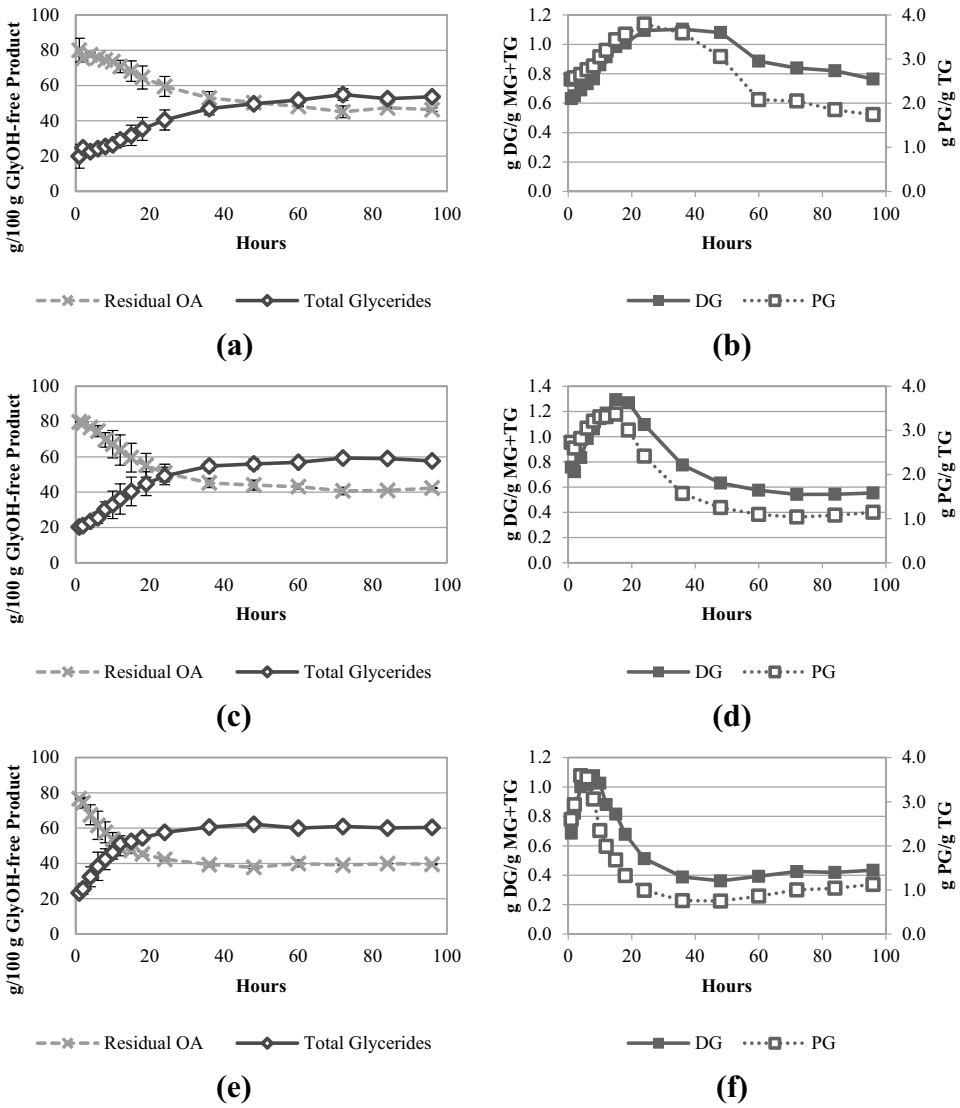

Fig. 2 Product distribution and glyceride selectivity during esterification of OA and glycerol at an initial OA:G of 4.4, catalyzed by Novozyme 435 (4 g/100 g reactants) carried out under 40 °C (**a**, **b**), 50 °C (**c**, **d**), and 60 °C (**e**, **f**)

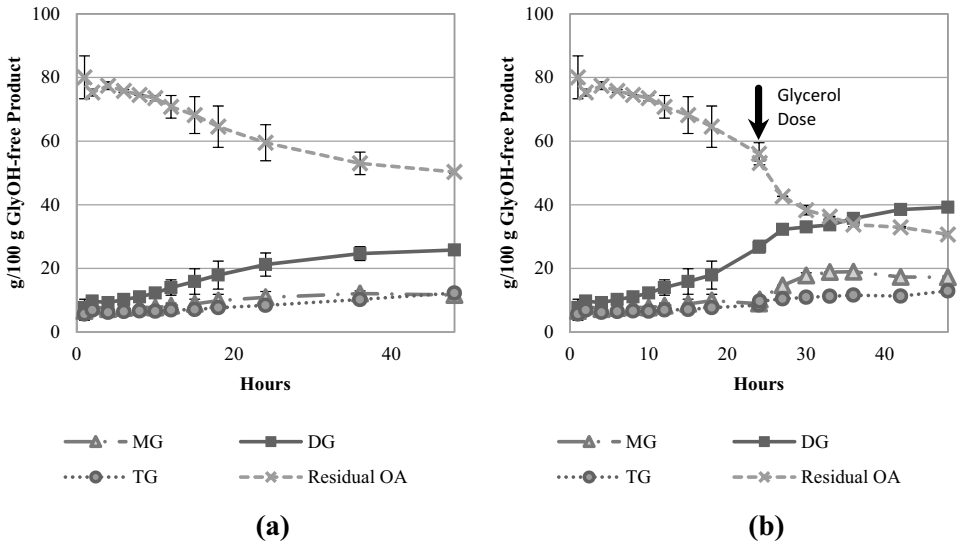

Fig. 3 Esterification of oleic acid and glycerol at an initial OA:G of 4.4 catalyzed by 4 g Novozyme 435/100 g reactants carried out under 40 °C **a** without glycerol dosing, ES-40 °C-4.4-0-4.4 and **b** with 750-µL glycerol dose at 24 h, ES-40 °C-4.4-1-2.0

Table 1 Effect of different strategies on conversion, distribution, selectivity, and yield

Strategy (ES-T-MRI-DS-MRF)[a]	Conversion (ξ_{OA}), %	Glyceride Distribution, g/100 g Total Glycerides			DG Selectivity (S_{DG}), g DG/g (MG + TG)	DG Yield (Y_{DG}), g DG/100 g RM
		C_{MG}	C_{DG}	C_{TG}		
ES-40 °C-2.0-0-2.0	74.47 ± 0.55	28.95 ± 0.04	56.30 ± 0.20	14.75 ± 0.24	1.29 ± 0.01	40.68 ± 0.10
ES-40 °C-4.4-0-4.4	45.98 ± 0.09	21.28 ± 0.60	53.89 ± 0.38	24.83 ± 0.98	1.17 ± 0.02	25.52 ± 0.15
ES-60 °C-2.0-0-2.0	86.82 ± 0.00	15.37 ± 0.00	54.84 ± 0.02	29.79 ± 0.02	1.21 ± 0.00	45.01 ± 0.03
ES-60 °C-4.4-0-4.4	58.08 ± 0.77	14.90 ± 0.40	29.33 ± 0.10	55.76 ± 0.50	0.42 ± 0.00	16.95 ± 0.15
ES-40 °C-4.4-1-2.0	66.44 ± 0.50	25.14 ± 0.38	56.90 ± 0.99	17.97 ± 0.61	1.32 ± 0.05	37.80 ± 0.41
ES-40 °C-4.4-2-2.0	69.54 ± 0.64	16.39 ± 0.08	65.31 ± 0.16	18.30 ± 0.08	1.88 ± 0.01	45.03 ± 0.48
ES-60 °C-4.4-2-2.0	87.62 ± 0.14	14.34 ± 0.18	54.16 ± 0.18	31.49 ± 0.36	1.18 ± 0.01	45.64 ± 0.09

[a]Total process time is 48 h; Code (ES-T-MRI-DS-MRF) refers to the following: Esterification System (ES) with the reaction carried out at T—reaction temperature, MRI—initial molar ratio, DS—number of glycerol doses implemented, and MRF—overall molar ratio after all glycerol doses have been added; a single-dose system is added with a full dose of glycerol at 24 h, and a two-dose system is added with equal doses at 24 h and 36 h, all to an overall molar ratio of 2.0

optimum activities are observed. Novozyme 435, which is used in this study, often exhibits significant activities within the range of 40–90 °C [48], and reached maximum activities close to 60 °C in solvent-free systems [49]. At a fixed reaction temperature of 40 °C, dosing with glycerol resulted in a significant increase in OA conversion for both ES-40 °C-4.4-1-2.0 and ES-40 °C-4.4-2-2.0 when compared to the non-dosed system ES-40 °C-4.4-0-4.4, but were lower when compared to ES-40 °C-2.0-0-2.0. The increase in conversion of the dosed systems relative to ES-40 °C-4.4-0-4.4 was expected because, upon dosing, more glycerol became available for the residual fatty acids to react, especially when at 24 h of reaction, ξ_{GlyOH} was already at 100% (Fig. 1a). The difference in conversion of dosed systems when compared to ES-40 °C-2.0-0-2.0 can be explained along the same line of reasoning, although in this particular case, more glycerol was already available right at the beginning of the process for system ES-40 °C-2.0-0-2.0 than for the glycerol-dosed systems. Implementing both dosing and increase in temperature, specifically with ES-60 °C-4.4-2-2.0, yielded the highest conversion among the strategies, which supported the assumption that glycerol dosing consistently improves conversion at different temperatures. Fatty acid dosing, however, did not result in a better process response expressed in terms of DG yield as reported in the synthesis of 1,3-diolein catalyzed by Lipozyme TL IM with t-butanol as solvent [20]. In such case, it was observed that the yield decreased when the dosing frequency was increased at lesser amounts per dose, and that the best result was obtained when all of the fatty acid was added at the beginning of the reaction. No explanation was offered as to why this occurred in the said study, but apparently, OA dosing gave an opposite effect compared to glycerol dosing. As higher conversions do not always translate to higher concentration of the desired product when pertaining to glyceride synthesis, other indicators were also considered such as yield, distribution, or selectivity in order to determine the best conditions to be adopted.

Diglyceride distribution (C_{DG}) or selectivity (S_{DG}) becomes a critical factor to consider when complete separation between the total glycerides and residual reactants can be achieved. Selectivity (S_{DG}) follows the same trend as C_{DG} because it is essentially the same response but with the effects of C_{MG} and C_{TG} incorporated into one value. In this perspective, the system with the highest C_{DG} or S_{DG} will result in higher product yields in terms of the desired component. Dosing had a positive influence on C_{DG} as can be observed in all glycerol-dosed systems at 40 °C. They had consistently larger C_{DG} values when compared against both non-dosed systems. It also appeared that the frequency of dosing, i.e. more doses at correspondingly lower amounts (ES-40 °C-4.0-2-2.0 versus ES-40 °C-4.4-1-2.0), increases the same response. With more glycerol introduced at first dose in ES-40 °C-4.4-1-2.0 than in ES-40 °C-4.4-2-2.0, there is a tendency to form more partial glycerides particularly MG than TG. The second dose of glycerol in ES-40 °C-4.4-2-2.0 resulted in the same overall OA:G for both systems, which could have allowed glycerolysis of DG and TG to occur in order for their compositions to be the same at equilibrium. But because glycerolysis is a slower process than esterification, as implied in a separate study comparing dicapryl glycerol production via esterification of caprylic acid and glycerol against the glycerolysis of TG containing tricaprylin [22], ES-40 °C-4.4-2-2.0 ended up being richer in DG and TG when compared to ES-40 °C-4.4-1-2.0 after 48 h. However, temperature had a negative effect as it lowered C_{DG} when increased from 40 to 60 °C (ES-40 °C-4.4-2-2.0 versus ES-60 °C-4.4-2-2.0). This is owing to the fact that at higher temperature the formation of TG, which is an undesirable product in DG synthesis, increased as shown in Fig. 1f. This occurrence has been consistently observed

in several studies on DG production based on esterification processes [31, 49]. The DG yield (Y_{DG}) is another response that is important to consider as it gives a more practical basis of process efficiency. Because it is influenced by conversion and selectivity, the observed trends with dosing can differ from the trends of both responses when taken separately. In this case, dosing generally improved yields at both temperatures investigated, with selectivity contributing largely for the increase at 40 °C whereas conversion played a larger role at 60 °C (Table 1). The implementation of dosing at high temperature resulted in the highest Y_{DG} which suggests that this combination is best for DG production when the type and amounts of other glycerides is not a critical matter.

Overall, glycerol dosing is an effective means of improving process responses, with effects on the selectivity for diglycerides that are the most consistent. Among all schemes implemented, ES-40 °C-4.4-2-2.0 is evidently the most appropriate one to explore further or apply to other systems as it had collectively the best results upon considering different process responses.

Esterification of SODD with Glycerol

Soybean oil deodorizer distillates (SODD) contain FFA in substantial concentrations ranging from 28% [50] to as much as 85.5% [51], with > 75% unsaturated fatty acids with respect to the total FFA [38, 51]. The higher the FFA concentration the more suited it is for conversion to DG [52]. In this study, the SODD collected comprised of 80.07 ± 0.34 wt% FFA mainly oleic and linoleic acids, 14.89 ± 0.41 wt% TG, 5.04 ± 0.06 wt% unsaponifiable matter mostly tocopherols, and traces of MG and DG. Following the conditions of ES-40 °C-4.4-2-2.0 and the reference system ES-40 °C-2.0-0-2.0, esterification was performed between SODD and glycerol, labelled ES-40 °C-4.4-2-2.0-0 and ES-40 °C-2.0-0-2.0-0 respectively, and the results listed in Table 2, together with one response added, which is the 1,3-DG concentration with respect to the total DG.

The values obtained for all process responses were lower with SODD than those achieved with the corresponding systems involving OA and glycerol. This is possibly influenced by the presence of other components already in SODD prior to esterification, most notably TG, which altered the effective concentrations of the reactants and consequently, the distribution of glycerides as well as the selectivity at the end of the process. In a previous study [52], pretreatment by enzymatic hydrolysis of mixed deodorized distillates prior to lipase-catalyzed esterification of the product with glycerol eliminated the interference of acyl glycerides and improved DG concentration from 46.0 to 66.8 wt%. The trend in conversion remained the same as with the model system, slightly decreasing with glycerol dosing, but apparently there is no significant difference between ES-40 °C-4.4-2-2.0-0 and ES-40 °C-2.0-0-2.0-0 when looking at other process output variables. However, glycerol dosing has drawn out an increase in the selectivity for 1,3-diglycerides. This constituted an improvement in the process even though other product characteristics such as glyceride distribution and yield remained practically the same. To verify further whether this observation was not an artifact but a result of dosing, a lower initial FFA:G was implemented, still within 2.0–4.0, plus dosing with glycerol to a final FFA:G of 2.0 (ES-40 °C-3.2-1-2.0-0). The conversion increased when compared to both ES-40 °C-4.4-2-2.0-0 and ES-40 °C-2.0-0-2.0-0, but DG yield and selectivity decreased as C_{MG} increased at the expense of both DG and

Table 2 DG production using SODD and glycerol with modified dosing strategies

Strategy (ES-T-MRI-DS-MRF-MS)[a]	FFA Conversion (%)	Glyceride Distribution (g/100 g Total Glycerides)			Selectivity, S_{DG} (g DG/g MG + TG)	1,3-DG (g/100 g total DG)	DG Yield (g/100 g RM)
		C_{MG}	C_{DG}	C_{TG}			
ES-40 °C-2.0-0-2.0-0	62.44 ± 2.82	23.67 ± 2.91	47.86 ± 0.09	28.47 ± 3.00	0.92 ± 0.00	60.97	32.41 ± 1.14
ES-40 °C-4.4-2-2.0-0	57.87 ± 3.46	23.70 ± 3.75	47.67 ± 0.24	28.63 ± 3.51	0.91 ± 0.01	63.96	30.59 ± 1.26
Effect of the presence of molecular sieves							
ES-40 °C-3.2-1-2.0-0	64.89 ± 2.07	29.05 ± 0.20	44.18 ± 0.21	26.78 ± 0.02	0.79 ± 0.01	63.35	30.02 ± 0.83
ES-40 °C-3.2-1-2.0-1	65.03 ± 0.83	21.32 ± 0.85	39.00 ± 0.35	39.69 ± 0.50	0.64 ± 0.01	59.26	26.11 ± 0.46

[a]Total process time is 48 h; Code (ER-T-MRI-DS-MRF-MS) refers to the following: Esterification System (ES) with the reaction carried out at T—reaction temperature, MRI—initial molar ratio, DS—number of glycerol doses implemented, MRF—final/overall molar ratio, and MS—presence of molecular sieves where 0 has no molecular sieves (activated at 175 °C under vacuum for 24 h prior to their use) & 1 has 0.6 g of 4 Å molecular sieves per 1 g of SODD; a single-dose system is added with a full dose of glycerol at 24 h, and a two-dose system is added with equal doses at 24 h and 36 h to an overall molar ratio of 2.0

TG (Table 2), which are consistent with the result between ES-40 °C-4.4-1-2.0 and ES-40 °C-4.4-2-2.0 (Table 1) involving the model reactants. More importantly, the amount of 1,3-DG relative to the total DG remained higher than the non-dosed system but closer and incrementally lower than that of ES-40 °C-4.4-2-2.0-0 having had closer initial and overall molar ratios. With higher conversion of the fatty acids in ES-40 °C-3.2-1-2.0-0, more water is expected to be produced which could have increased the rate of hydrolysis that resulted in higher MG concentration as compared to ES-40 °C-4.4-2-2.0-0. To validate this assumption, trials based on ES-40 °C-3.2-1-2.0-0 conditions were run with molecular sieves (ES-40 °C-3.2-1-2.0-1) as a means of *in-situ* water removal. From the values obtained for C_{DG}, S_{DG}, and Y_{DG}, the presence of these hygroscopic sorbents had a negative impact as the reaction pushed towards higher TG formation. More importantly, the 1,3-DG decreased relative to the total DG formed. Apart from these, the product obtained had a darker hue when compared to the system without molecular sieves. From the GC chromatograms, it was seen that some peaks of the unsaponifiable matter, specifically tocopherols, disappeared, which implied that they may have been oxidized in the presence of molecular sieves. In any case, these showed that employing molecular sieves also have detrimental effects in solvent-free DG production especially when using SODD. In fact, the use of molecular sieves may not be necessary as reported in a study involving the production of high-purity DG via solvent-free esterification of OA and glycerol catalyzed by a *Rhizopus oryzae* lipase immobilized in magnetic nanoparticles [53], as there was no substantial difference in the concentration of 1,3-DG between the systems with and without this water-adsorbing material.

Comparison with Related Esterification-Based DG Production Systems

The production of DG based on the esterification of OA and glycerol had been studied in several occasions (Table 3), but employing different reaction strategies. Simply comparing the product quality and typical process performance as obtained from the strategy employed in this study, either with OA or SODD, the values fall within the ranges previously reported in literature. Among the works presented, the conversion, distribution, and selectivity of the product from ES-40 °C-4.4-2-2.0 resemble that of the reaction system involving *Candida* sp. 99–125 lipase as catalyst and cyclodextrin as a stabilizer [28]. The two systems were

Table 3 Comparison with lipase-catalyzed systems for DG production reported in literature

Temp (°C)/ Time (h)	Reactants FFA:G, (mol/mol)	Catalyst (wt%, specificity)	Auxiliary Materials/Special Conditions (wt%)	ξ_{FFA} (%)	S_{DG} (g/g)	PD_{DG}/C_{DG} (g/100 g)	Y_{DG} (g/100 g)	*RME* (g/g)	References
40/4	OA & GlyOH 1:5	*Candida* sp. 99–125 lipase 10, non-sp	cyclodextrin, 1.5:1[a] H_2O, 10	65.9	1.80	44.2/64.3 (54.3)[b]	18.2	0.14	Table 1 [28]
30/24	OA & GlyOH 1:10	*Candida rugosa* lipase 4.0, non-sp	n-hexane, 80 H_2O, 6	76.9	1.01	39.6/50.1	8.7	0.05	Figure 3 [29]
60/7	OA & GlyOH 2.5:1	Novozyme 435 9, non-sp	t-butanol, 53 molecular sieves, 57	88.0	3.54	69.6/78.0	68.4	0.31	Figure 5 [17]
40/3	OA & GlyOH 3.5:1	Lipozyme TL IM 18, sp	toluene, 80 H_2O, 0.33[c] molecular sieves, 61	40.4	2.12	29.6/68.2	29.1	0.11	Figure 9 [20]
65/2	OA & GlyOH 2:1	Lipozyme RM IM 6, sp	0.01 Mpa vacuum	79.6 (87.7) [c]	2.64	59.1/72.6	55.9	0.53	Figure 5 [44]
40/48	OA & GlyOH 2:1[d]	Novozyme 435 4, non-sp	–	69.5	1.88	46.9/65.3	45.0	0.43	This study
40/48	OA + LA (SODD) & GlyOH 2:1[d]	Novozyme 435 4, non-sp	–	57.9	0.91	30.6/47.7	30.6	0.29	This study

Data were calculated based on available information in figures/tables from respective references; all wt% are relative to the initial mass of reactants, S_{DG} is in g DG/g (MG + TG), PD_{DG} in g DG/100 g glycerol-free product, C_{DG} in g DG/100 g total glycerides, Y_{DG} in g DG/100 g total weight of reactants, and *RME* in g DG/g total raw materials; [a]cyclodextrin/lipase mass ratio, [b]reported as is in reference, [c]water activity, [d]overall FFA:G

both catalyzed by a non-specific lipase and carried out at 40 °C. There is a large difference in terms of Y_{DG}, however, mainly because the latter used excess glycerol (OA:G of 1:5) resulting in a low yield despite of the higher enzyme loading involved. This is consistent with another study which used a much lower OA:G of 1:10, also catalyzed with a non-specific lipase from *Candida rugosa* at 30 °C, that brought in Y_{DG} of a much lower value [29]. On the other hand, better conversion, distribution, selectivity, and DG yield were achieved with a system using higher OA:G (2.5:1), t-butanol as solvent, molecular sieves for water removal, and Novozyme 435, which contains a non-specific lipase from *Candida antarctica* [17]. All these supported the previous results presented in Fig. 1, that led to the decision of performing the esterification reactions at higher OA:G. The product from ES-40 °C-4.4-2-2.0-0, which utilized SODD as the FFA source, had a more unique set of response values, because unlike the others, SODD contained mixed fatty acids and other components.

The use of *sn*-1,3-specific lipases generally yielded better DG selectivity as those obtained with lipozyme TL IM [20] and lipozyme RM IM [44]. The former, which worked with high OA:G (3.5:1), molecular sieves, and toluene as solvent resulted in an S_{DG} of 2.12, whereas the latter with an OA:G of 2:1 but carried out under vacuum produced an S_{DG} of 2.64. The experiments performed at the conditions implemented in this study with the use of lipase RM IM supported these results as higher S_{DG} of 1.49 g DG/g (MG + TG) against 1.17 g DG/g (MG + TG) with Novozyme 435 was achieved while practically having similar total glycerides formed at 40 °C (54.90 ± 3.32 g total glycerides/100 g glycerol-free product for Novozyme 435 against 50.58 ± 1.18 g/100 g for Lipase RM IM). Unfortunately, lipase RM IM was poor in stability and lost its catalytic activity after the first cycle. Thus, the robustness of Novozyme 435 over lipase RM IM, and more importantly, many other lipases [54] makes Novozyme 435 attractive to use despite its non-specific property. In fact, its non-specific character allows for further esterification of the DG with another fatty acid to produce designer lipids without the addition of another type of catalyst. Moreover, the specificity of Novozyme 435 can be manipulated through the use of certain solvents, as was reported when various types of solvents were investigated in the synthesis of 1,3-diolein by esterification [19], and also to a significant extent through glycerol dosing as shown in this study.

The best characteristic of the process presented in this study is its "greenness" as it is solvent-free and the strategy involved in improving process responses is based on a reactant feeding scheme instead of the use of solvents or hygroscopic sorbents. This can be seen from its reactant mass efficiency value (*RME*) of 0.43. The other system which gave a better *RME* value is also solvent-free but used Lipozyme RM IM and carried out the operation under vacuum at 65 °C [33]. By itself, manipulation of operating conditions, in this case pressure, instead of changing reactant mixture composition is a generally a green means of manipulating process output. However, reactors designed to operate under vacuum conditions are cost-intensive as it would require accessories for pressure control apart from the fact that materials of construction should also withstand such extreme condition.

The weakest aspect of the process investigated in this study is the process time. This is due to the time intervals of glycerol dosing involved. This can be addressed through a reactor design with features compatible with the implementation of this strategy, such as in a plug flow reactor, where after achieving steady state, this will become a minor issue. It is also possible to shorten process time through an increase in enzyme loading as can be noted with other processes reported, except for the work of Yesiloglu and Kilic [29], where employed enzyme loadings are 1.5–4.5 times higher than the one implemented in the present study. This made it possible for them to reach maximum S_{DG} within shorter reaction times.

Overall, the process currently investigated had generated products with qualities that are within those reported in literature but excel in terms of its simple raw material composition and flexible strategy for manipulating conversion, distribution, and selectivity. Without solvents, there is no need for a solvent recovery step and the health issues related to the consumption of products contaminated with these substances are avoided. Furthermore, special accessories required in reactors to regulate pressure such as those employing vacuum conditions could potentially be avoided.

Conclusion

Solvent-free lipase-catalyzed esterification of OA and glycerol performed at high OA:G with glycerol dosing led to improved conversion, selectivity, distribution and yield for diglycerides. The best strategy at a fixed lipase loading of 4 wt% was by using an OA:G of 4.4, 40 °C, 50/50 glycerol dosing in a total reaction time of 48 h, which resulted in ξ_{OA} of 69.54%, S_{DG} of 1.88 g DG/(g MG + TG), C_{DG} of 65.31 g DG/g total glycerides, and Y_{DG} of 45.03 g PG/100 g RM. When applied with SODD, the corresponding process response values were obtained: ξ_{OA} of 57.87%, S_{DG} of 0.91 g DG/(g MG + TG), C_{DG} of 47.67 g DG/g total glycerides, and Y_{DG} of 30.59 g PG/100 g RM. Additionally, dosing increased the 1,3-DG concentration to 63.96 g/100 g DG. Glycerol dosing is a "green" strategy of manipulating output variables, as it does so without using other raw materials apart from those necessary for the reaction to occur.

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Compliance with Ethical Standards

Conflict of interest The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

References

1. Lauridsen, J.B.: Food emulsifiers: surface activity, edibility, manufacture, composition, and application. J. Am. Oil Chem. Soc. **53**, 400–407 (1976). https://doi.org/10.1007/BF02605731
2. Zhang, Y., Wang, X., Zou, S., Xie, D., Jin, Q., Wang, X.: Synthesis of 2-docosahexaenoylglycerol by enzymatic ethanolysis. Bioresour. Technol. **251**, 334–340 (2018). https://doi.org/10.1016/j.biortech.2017.12.025
3. Zheng, M.-M., Huang, Q., Huang, F., Guo, P., Xiang, X., Deng, Q., Li, W., Wan, C., Zheng, C.: Production of novel "functional oil" rich in diglycerides and phytosterol esters with "one-pot" enzymatic transesterification. J. Agric. Food Chem. **62**, 5142–5148 (2014). https://doi.org/10.1021/jf500744n
4. Khaddaj-Mallat, R., Morin, C., Rousseau, É.: Novel n-3 PUFA monoacylglycerides of pharmacological and medicinal interest: anti-inflammatory and anti-proliferative effects. Eur. J. Pharmacol. **792**, 70–77 (2016). https://doi.org/10.1016/j.ejphar.2016.10.038
5. Sun, S., Hou, X., Hu, B.: Synthesis of glyceryl monocaffeate using ionic liquids as catalysts. J. Mol. Liq. **248**, 643–650 (2017). https://doi.org/10.1016/j.molliq.2017.10.102
6. Sun, S., Song, F., Bi, Y., Yang, G., Liu, W.: Solvent-free enzymatic transesterification of ethyl ferulate and monostearin: optimized by response surface methodology. J. Biotechnol. **164**, 340–345 (2013). https://doi.org/10.1016/j.jbiotec.2013.01.013
7. Sun, S., Hu, B.: Enzymatic preparation of novel caffeoyl structured lipids using monoacylglycerols as caffeoyl acceptor and transesterification mechanism. Biochem. Eng. J. **124**, 78–87 (2017). https://doi.org/10.1016/j.bej.2017.05.002
8. Kim, J., Chung, M., Choi, H.-D., Choi, I., Kim, B.H.: Enzymatic synthesis of structured monogalactosyldiacylglycerols enriched in pinolenic acid. J. Agric. Food Chem. **66**, 8079–8085 (2018). https://doi.org/10.1021/acs.jafc.8b02599
9. Morin, C., Rousseau, É., Fortin, S.: Anti-proliferative effects of a new docosapentaenoic acid monoacylglyceride in colorectal carcinoma cells. Prostaglandins Leukot. Essent. Fat. Acids. **89**, 203–213 (2013). https://doi.org/10.1016/j.plefa.2013.07.004
10. Mostafa, N.A., Maher, A., Abdelmoez, W.: Production of mono-, di-, and triglycerides from waste fatty acids through esterification with glycerol. Adv. Biosci. Biotechnol. **04**, 900–907 (2013). https://doi.org/10.4236/abb.2013.49118
11. Ferretti, C.A., Spotti, M.L., Di Cosimo, J.I.: Diglyceride-rich oils from glycerolysis of edible vegetable oils. Catal. Today. **302**, 233–241 (2018). https://doi.org/10.1016/j.cattod.2017.04.008
12. Singh, D., Patidar, P., Ganesh, A., Mahajani, S.: Esterification of oleic acid with glycerol in the presence of supported zinc oxide as catalyst. Ind. Eng. Chem. Res. **52**, 14776–14786 (2013). https://doi.org/10.1021/ie401636v
13. Duan, Z.-Q., Du, W., Liu, D.-H.: Rational synthesis of 1,3-diolein by enzymatic esterification. J. Biotechnol. **159**, 44–49 (2012). https://doi.org/10.1016/j.jbiotec.2012.02.006
14. Devi, B.L.A.P., Zhang, H., Damstrup, M.L., Guo, Z., Zhang, L., Lue, B.-M., Xu, X.: Enzymatic synthesis of designer lipids. Oléagineux, Corps gras, Lipides. **15**, 189–195 (2008). https://doi.org/10.1051/ocl.2008.0194
15. Oliveira, P.D., Rodrigues, A.M.C., Bezerra, C.V., Silva, L.H.M.: Chemical interesterification of blends with palm stearin and patawa oil. Food Chem. **215**, 369–376 (2017). https://doi.org/10.1016/j.foodchem.2016.07.165
16. Jiménez, M.J., Esteban, L., Robles, A., Hita, E., González, P.A., Muñío, M.M., Molina, E.: Production of triacylglycerols rich in palmitic acid at sn-2 position by lipase-catalyzed acidolysis. Biochem. Eng. J. **51**, 172–179 (2010). https://doi.org/10.1016/j.bej.2010.06.015
17. Duan, Z.-Q., Du, W., Liu, D.-H.: Novozym 435-catalyzed 1,3-diacylglycerol preparation via esterification in t-butanol system. Process Biochem. **45**, 1923–1927 (2010a). https://doi.org/10.1016/j.procbio.2010.03.007
18. Duan, Z.-Q., Du, W., Liu, D.-H.: The pronounced effect of water activity on the positional selectivity of Novozym 435 during 1,3-diolein synthesis by esterification. Catal. Commun. **11**, 356–358 (2010b). https://doi.org/10.1016/j.catcom.2009.10.030
19. Duan, Z.-Q., Du, W., Liu, D.-H.: The solvent influence on the positional selectivity of Novozym 435 during 1,3-diolein synthesis by esterification. Bioresour. Technol. **101**, 2568–2571 (2010c). https://doi.org/10.1016/j.biortech.2009.11.087
20. Wang, Z., Du, W., Dai, L., Liu, D.: Study on Lipozyme TL IM-catalyzed esterification of oleic acid and glycerol for 1,3-diolein preparation. J. Mol. Catal. B Enzym. **127**, 11–17 (2016). https://doi.org/10.1016/j.molcatb.2016.01.010
21. Duan, Z.-Q., Du, W., Liu, D.-H.: The mechanism of solvent effect on the positional selectivity of *Candida antarctica* lipase B during 1,3-diolein synthesis by esterification. Bioresour. Technol. **102**, 11048–11050 (2011). https://doi.org/10.1016/j.biortech.2011.09.003
22. Hu, D., Chen, J., Xia, Y.: A comparative study on production of middle chain diacylglycerol through enzymatic esterification and glycerolysis. J. Ind. Eng. Chem. **19**, 1457–1463 (2013). https://doi.org/10.1016/j.jiec.2013.01.009
23. Ortiz, C., Ferreira, M.L., Barbosa, O., dos Santos, J.C.S., Rodrigues, R.C., Berenguer-Murcia, Á., Briand, L.E., Fernandez-Lafuente, R.: Novozym 435: the "perfect" lipase immobilized biocatalyst? Catal. Sci. Technol. **9**, 2380–2420 (2019). https://doi.org/10.1039/C9CY00415G
24. Brautbar, N., Williams, J., II.: Industrial solvents and liver toxicity: risk assessment, risk factors and mechanisms. Int. J. Hyg. Environ. Heal. **205**, 479–491 (2002)
25. Bélafi-Bakó, K., Kovács, F., Gubicza, L., Hancsók, J.: Enzymatic biodiesel production from sunflower oil by *Candida antarctica* lipase in a solvent-free system. Biocatal. Biotransformation. **20**, 437–439 (2002). https://doi.org/10.1080/10242420210000040855
26. Lu, J., Deng, L., Nie, K., Wang, F., Tan, T.: Stability of immobilized *Candida* sp. 99–125 lipase for biodiesel production. Chem. Eng. Technol. **35**, 2120–2124 (2012). https://doi.org/10.1002/ceat.201200254

27. Lai, C.C., Zullaikah, S., Vali, S.R., Ju, Y.H.: Lipase-catalyzed production of biodiesel from rice bran oil. J. Chem. Technol. Biotechnol. **80**, 331–337 (2005). https://doi.org/10.1002/jctb.1208
28. Zhao, Y., Liu, J., Deng, L., Wang, F., Tan, T.: Optimization of *Candida* sp. 99–125 lipase catalyzed esterification for synthesis of monoglyceride and diglyceride in solvent-free system. J. Mol. Catal. B Enzym. **72**, 157–162 (2011). https://doi.org/10.1016/j.molcatb.2011.05.014
29. Yesiloglu, Y., Kilic, I.: Lipase-catalyzed esterification of glycerol and oleic acid. J. Am. Oil Chem. Soc. **81**, 281–284 (2004). https://doi.org/10.1007/s11746-004-0896-5
30. Yeoh, C.M., Choong, T.S.Y., Abdullah, L.C., Yunus, R., Siew, W.L.: Influence of silica gel in production of diacylglycerol via enzymatic glycerolysis of palm olein. Eur. J. Lipid Sci. Technol. **111**, 599–606 (2009). https://doi.org/10.1002/ejlt.200800265
31. Ting, R.R., Agapay, R., Angkawijaya, A.E., Tran Nguyen, P.L., Truong, C.T., Ju, Y.: Diglyceride production via noncatalyzed esterification of glycerol and oleic acid. Asia-Pacific J. Chem. Eng. **14**, 1–11 (2019). https://doi.org/10.1002/apj.2383
32. Castillo, E., Dossat, V., Marty, A., Stéphane Condoret, J., Combes, D.: The role of silica gel in lipase-catalyzed esterification reactions of high-polar substrates. JAOCS J. Am. Oil Chem. Soc. **74**, 77–85 (1997). https://doi.org/10.1007/s11746-997-0148-3
33. Üstün, G.: Separation of fatty acid methyl esters from tall oil by selective adsorption. JAOCS J. Am. Oil Chem. Soc. **73**, 203–210 (1996). https://doi.org/10.1007/BF02523896
34. Chandrasekar, G., Vinu, A., Murugesan, V., Hartmann, M.: Adsorption of vitamin E on mesoporous silica molecular sieves. In: Raj, S. (ed.) Materials research bulletin, pp. 1169–1176. Elsevier, Amsterdam (2005)
35. Rosu, R., Yasui, M., Iwasaki, Y., Yamane, T.: Enzymatic synthesis of symmetrical 1,3-diacylglycerols by direct esterification of glycerol in solvent-free system. JAOCS J. Am. Oil Chem. Soc. **76**, 839–843 (1999). https://doi.org/10.1007/s11746-999-0074-7
36. Chongkhong, S., Tongurai, C., Chetpattananondh, P., Bunyakan, C.: Biodiesel production by esterification of palm fatty acid distillate. Biomass Bioenergy **31**, 563–568 (2007). https://doi.org/10.1016/j.biombioe.2007.03.001
37. Chongkhong, S., Tongurai, C., Chetpattananondh, P.: Continuous esterification for biodiesel production from palm fatty acid distillate using economical process. Renew. Energy **34**, 1059–1063 (2009). https://doi.org/10.1016/j.renene.2008.07.008
38. D'Amato Villardi, H.G., Leal, M.F., De Azevedo Andrade, P.H., Pessoa, F.L.P., Salgado, A.M., De Oliveira, A.R.G.: Catalytic and non-catalytic esterification of soybean oil deodorizer distillate by ethanol: kinetic modelling. Chem. Eng. Trans. **57**, 1999–2004 (2017). https://doi.org/10.3303/CET1757334
39. Shimada, Y., Nakai, S., Suenaga, M., Sugihara, A., Kitano, M., Tominaga, Y.: Facile purification of tocopherols from soybean oil deodorizer distillate in high yield using lipase. JAOCS J. Am. Oil Chem. Soc. **77**, 1009–1013 (2000). https://doi.org/10.1007/s11746-000-0160-z
40. Kasim, N.S., Gunawan, S., Ju, Y.H.: Isolation and identification of steroidal hydrocarbons in soybean oil deodorizer distillate. Food Chem. **117**, 15–19 (2009). https://doi.org/10.1016/j.foodchem.2009.03.066
41. Gunawan, S., Kasim, N.S., Ju, Y.H.: Separation and purification of squalene from soybean oil deodorizer distillate. Sep. Purif. Technol. **60**, 128–135 (2008). https://doi.org/10.1016/j.seppur.2007.08.001
42. Lo, S.K., Baharin, B.S., Tan, C.P., Lai, O.M.: Lipase-catalysed production and chemical composition of diacylglycerols from soybean oil deodoriser distillate. Eur. J. Lipid Sci. Technol. **106**, 218–224 (2004). https://doi.org/10.1002/ejlt.200300888
43. Go, A.W., Sutanto, S., Ismadji, S., Ju, Y.H.: Catalyst free production of partial glycerides: acetone as solvent. RSC Adv. **5**, 30833–30840 (2015). https://doi.org/10.1039/c5ra03249k
44. Meng, Z., Lu, S., Geng, W., Huang, J., Wang, X., Liu, Y.: Preliminary study on acyl incorporation and migration in the production of 1,3-diacylglycerol by immobilized lipozyme RM IM-catalyzed esterification. Food Sci. Technol. Res. **20**, 175–182 (2014). https://doi.org/10.3136/fstr.20.175
45. de Gomes, S.M., Santos, M.R.D., Salviano, A.B., Mendonça, F.G., Menezes, I.R.S., Jurisch, M., Rodrigues, G.D., Augusti, R., Martins, P.S., Lago, R.M.: Biphasic reaction of glycerol and oleic acid: Byproducts formation and phase transfer autocatalytic effect. Catal. Today. **344**, 227–233 (2020). https://doi.org/10.1016/j.cattod.2019.02.011
46. Kong, P.S., Pérès, Y., Wan Daud, W.M.A., Cognet, P., Aroua, M.K.: Esterification of glycerol with oleic acid over hydrophobic zirconia-silica acid catalyst and commercial acid catalyst: optimization and influence of catalyst acidity. Front. Chem. **7**, 1–11 (2019). https://doi.org/10.3389/fchem.2019.00205
47. Andrade-Tacca, C.A., Chang, C.C., Chen, Y.H., Ji, D.R., Wang, Y.Y., Yen, Y.Q., Chang, C.Y.: Reduction of FFA in jatropha curcas oil via sequential direct-ultrasonic irradiation and dosage of methanol/sulfuric acid catalyst mixture on esterification process. Energy Convers. Manag. **88**, 1078–1085 (2014). https://doi.org/10.1016/j.enconman.2014.09.020
48. Huang, Z., Cao, Z., Guo, Z., Chen, L., Wang, Z., Sui, X., Jiang, L.: Lipase catalysis of α-linolenic acid-rich medium- and long-chain triacylglycerols from perilla oil and medium-chain triacylglycerols with reduced by-products. J. Sci. Food Agric. **100**, 4565–4574 (2020). https://doi.org/10.1002/jsfa.10515
49. Abd Razak, N.N., Abd Razak, N.N., Pérès, Y., Gew, L.T., Cognet, P., Aroua, M.K., Aroua, M.K.: Effect of reaction medium mixture on the lipase catalyzed synthesis of diacylglycerol. Ind. Eng. Chem. Res. **59**, 9869–9881 (2020). https://doi.org/10.1021/acs.iecr.0c00298
50. Wang, L., Du, W., Liu, D., Li, L., Dai, N.: Lipase-catalyzed biodiesel production from soybean oil deodorizer distillate with absorbent present in tert-butanol system. J. Mol. Catal. B Enzym. **43**, 29–32 (2006). https://doi.org/10.1016/j.molcatb.2006.03.005
51. Gangopadhyay, S., Nandi, S., Ghosh, S.: Biooxidation of fatty acid distillates to dibasic acids by a mutant of *Candida tropicalis*. J. Oleo Sci. **56**, 13–17 (2007). https://doi.org/10.5650/jos.56.13
52. Tangkam, K., Weber, N., Wiege, B.: Solvent-free lipase-catalyzed preparation of diglycerides from co-products of vegetable oil refining. Grasas Aceites **59**, 245–253 (2008). https://doi.org/10.3989/gya.2008.v59.i3.515
53. Zhao, J.F., Tao-Wang, Lin, J.P., Yang, L.R., Wu, M.B.: Preparation of high-purity 1,3-diacylglycerol using performance-enhanced lipase immobilized on nanosized magnetite particles. Biotechnol. Bioprocess Eng. **24**, 326–336 (2019). https://doi.org/10.1007/s12257-018-0458-3
54. Encinar, J.M., González, J.F., Sánchez, N., Nogales-Delgado, S.: Sunflower oil transesterification with methanol using immobilized lipase enzymes. Bioprocess Biosyst. Eng. **42**, 157–166 (2019). https://doi.org/10.1007/s00449-018-2023-z