Research article

Lipase-catalyzed solvent-free synthesis of monoglycerides from biodiesel-derived crude glycerol: Optimized using response surface methodology

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A B S T R A C T

The growth of the biodiesel industry has resulted in significant quantity of crude glycerol. It is necessary to explore the synthesis of high-value-added products from crude glycerol. In this study, the enzymatic synthesis of monoglycerides under solvent-free conditions, employing crude glycerol as the primary feedstock, had been investigated. The analysis showed that the highest yield of monoglycerides was obtained after 12 h, and Novozym 435 showed the highest mono-glyceride yield of 18.41 % among the three lipases tested, followed by Lipozyme TL IM and Lipozyme RM IM. Monoglycerides were synthesized from biodiesel-derived crude glycerol using Novozym 435 as the catalyst under solvent-free conditions at different parameters, which were catalyst concentration, substrate molar ratio, and temperature. The yield of monoglycerides was examined in single-factor experiments. Response surface methodology (RSM) was subsequently employed to optimize the synthesis conditions based on the single-factor experimental results. The optimal conditions were at an enzyme concentration of 12.7 wt% and a molar ratio of crude glycerol:oil of 5.7:1 at a reaction temperature of 65.2 °C. The experimental yield of mono-glycerides under the optimal conditions was 28.93 %, which is close to the value predicted from the RSM model (29.02 %).

1. Introduction

Monoglycerides are a class of nonionic surfactants with a lipophilic long-chain alkyl group and two hydrophilic hydroxyl groups. Monoglycerides have emulsifying [1], antistatic [2], lubricating [3], and antibacterial properties [4] and are widely used in various fields, such as food [5], pharmaceuticals [6], cosmetics [7], detergents [8], and chemicals [9]. Currently, the large-scale production of monoglycerides is mainly achieved through chemical methods, including grease hydrolysis, fatty acid esterification, and grease

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glycerolysis [10]. Among them, grease glycerolysis is the most widely used in the industry and has the highest substrate utilization rate. In the chemical method glycerolysis reaction, grease and glycerol are catalyzed by alkali at high temperatures (220–250 °C) and nitrogen as purging gas to provide an inert environment [10]. Although the alkali-catalyzed synthesis of monoglycerides has the advantages of fast reaction speed and high conversion rate, it also faces issues such as high energy consumption, numerous side reactions, equipment corrosion, and waste liquid discharge. In contrast, enzymatic production of monoglycerides under mild conditions (generally below 80 °C) has lower energy consumption, produces no trans fatty acids, and is environmentally friendly, making it an effective alternative strategy with sustainable development significance and industrialization potential that has received widespread attention [11]. Common enzymatic synthesis of monoglycerides reaction systems include the organic solvent system, *trans*-micellar system, solvent-free system, supercritical fluid, ionic liquid, and other reaction systems. Among these, the solvent-free system is a focus of current research since it has the characteristics of a large concentration of reaction substrate and the product is not contaminated by organic solvents [12]. For example, Palacios et al. [13] synthesized polyunsaturated fatty acid-rich monoglycerides using immobilized lipase PS-DI glycerolysis of anchovy oil. The reaction was carried out under solvent-free conditions. The yield and oxidative stability of monoglycerides were optimized using response surface methodology. The optimum reaction conditions were a glycerol/oil ratio of 3/1, 9 wt% Lipase dosage, a stirring rate of 200 rpm, and a reaction time of 4 h, at 45.8 °C, producing a content of 24.8 % of monoglycerides. Similarly, Palacios et al. [14] synthesized monoglycerides by glycerolysis of anchovy oil using Lipozyme RM-IM in a solvent-free system. Monoglycerides yield of 20.34 % was achieved under optimum conditions of 40 °C, a glycerol/oil molar ratio of 2:1, and a 6 wt% enzyme dosage. Microbial oils have also been used as synthetic monoglycerides. Zou et al. [15] examined lipase type, substrate molar ratio, temperature, enzyme addition, and reaction time under solvent-free conditions. The results showed that the monoglyceride content was 15.4 % after 12 h of reaction at 50 °C under 8 wt% de Novozym 435 with a glycerol/oil ratio of 1:1. Fregolente et al. [16] produced monoglycerides from glycerol and soybean oil in a solvent-free system. After 24 h of enzymatic reaction, the monoglyceride content was 21.72 %. The reaction product was further distilled to 80 % monoglycerides and the residue. The residue is rich in diglycerides and triglycerides, which are suitable to replace TG oil in the human diet. While solvent-free enzymatic glycerolysis has utilized various oils to produce monoglycerides, the direct substitution of glycerol with crude glycerol has not been documented. Unrefined crude glycerol presents a cost advantage but may potentially influence the enzymatic reaction. This study investigates the utilization of high-value conversion technology for biodiesel-derived crude glycerol.

Glycerol is the primary raw material used in the synthesis of monoglycerides. Reports indicate that 66 % of global glycerol production is currently sourced from the biodiesel industry [17]. The effective conversion of biodiesel-derived crude glycerol into high-value monoglycerides can reduce the production cost of monoglycerides and improve the sustainability of biodiesel development. In this study, the enzymatic synthesis of monoglycerides under solvent-free conditions was investigated using crude glycerol derived from biodiesel as the raw material. The influence of five factors, including lipase type, reaction time, feeding ratio, catalyst amount, and reaction temperature, on the yield of monoglycerides was analyzed. The results of this study provide a theoretical basis for reducing production costs and improving product quality, which is of great significance for the development of the biodiesel industry. The results of this study can serve as a valuable reference for industries seeking to improve the efficiency and sustainability of their monoglyceride production processes.

2. Materials and methods

2.1. Materials

Crude glycerol (containing 90 % glycerol and 10 % fatty acid methyl ester) was provided by Tangshan Jinlihai Biodiesel Co., Ltd. Novozym 435, Lipozyme TL IM and Lipozyme RM IM were supplied by Novozymes Malaysia Sdn Bhd. Soybean Oil was purchased from Yee Lee Trading Co. Bhd. Monoglyceride (GC) and 2-propanol (HPLC) was purchased from Shanghai Macklin Biochemical Technology Co., Ltd. Glycerol, Chloroform, Perchloric acid, Sodium thiosulfate, Sodium metaperiodate, Sulfuric acid, Hydrochloric acid, Sodium hydroxide, Ethanol, Ethylene glycol, Potassium hydroxide, Potassium iodide, Glacial acetic acid, Starch, Phenolphthalein, Thymolphthalein, Alkali blue 6B, Potassium dichromate, Periodic acid were of analytical grade and were obtained from Sigma-Aldrich.

2.2. Analysis of crude glycerol

The glycerol content was analyzed following the Standard ASTM D7637-10:2021 method. Metal elements in crude glycerol are detected using inductively coupled plasma spectrometry [18]. The assay was done using a PerkinElmer Optima 8000 ICP-OES (USA). Detection program includes 30 metal elements, such as B (249.677 nm), Al (396.153 nm), and Si (251.611 nm), etc. After acid treatment, crude glycerol is introduced into an atomizer within a plasma emission spectrometer and transported by argon gas to the plasma torch, where the metallic elements to be measured were excited and radiate characteristic spectra. The intensity of the spectra is indicative of the concentration of the metallic element in the crude glycerol. The inductively coupled plasma spectrometer was operated under the following conditions: high frequency power of 1.5 kW, carrier gas flow rate of 1.5 L/min, peristaltic pump speed of 120 rpm, and flow rate of 2.0 mL/min. Methanol in crude glycerol was analyzed by a headspace gas chromatography method [19]. GC-7890 with FID detector and DB-1 column (30 m × 0.53 mm × 0.25 μm) was used for the analysis. A 20 mL septum vial was added with 5 g of crude glycerol sample and 50 μL of 2-propanol mixed well and the vial was kept in a water bath at 80 °C for 45 min. Then, 100 μL of gas in the headspace was collected with a gas-tight syringe preheated in an oven at 60 °C and immediately injected into the GC-7890 by manual injection. The samples were injected in non-split mode. Results were calculated based on the area internal standard method. The GC was run under the following conditions: programmed warming (held at 40 °C for 1.2 min, then heated at a

rate of 20 °C per minute up to 50 °C until the end of the run sequence); helium 7.7 mL/min; injector temperature 100 °C; and detector temperature 250 °C.

2.3. Analysis of soybean oil

The acid index and the saponification value of oils and fats were analyzed according to standard ISO 660:2020 method and standard ISO 3657-2020 method, respectively. To determine the acid value, the sample needs to be dissolved in ethanol. Then, thymolphthalein indicator was added, and the acid present in the sample was titrated with an ethanolic solution of potassium hydroxide. For the determination of saponification value, the sample was co-boiled with an ethanolic solution of potassium hydroxide for 2 h. Afterward, alkali blue 6B indicator was added, and the remaining potassium hydroxide was titrated with a standard hydrochloric acid solution. The average molecular weight of soybean oil was determined based on acid value and saponification value [20]. Determination of fatty acid composition of soybean oil by gas chromatography with reference [21]. The assay was done with the instrument of GC-7890. After pre-esterification, soybean oil was mixed well with anhydrous ethanol at 1:5 by volume, and 0.5 μL of the sample was injected into GC-7890. The results were shown according to the area normalization method. The chromatographic conditions were as follows: nitrogen 0.08 MPa; hydrogen 0.075 MPa; column temperature 230 °C; injection temperature 250 °C; detection temperature 250 °C.

2.4. Enzymatic glycerolysis for production of monoglycerides

The reaction was conducted in screw-capped glass tubes (22 mm diameter, 50 mm high, 10 mL volume) in batch mode, as described by Palacios et al. [14]. A molar ratio of 2:1 (calculated based on the pure glycerol content) of crude glycerol and soybean oil was combined in a specific amount, and the reactants were homogeneously mixed. To the reaction substrate mass, 5 wt% of enzyme was added, and the reaction temperature was maintained at 50 °C using a INFORS orbital shaker incubator (speed range 30–550 rpm, temperature range room - 95 °C) at a speed of 180 rpm. The reaction duration was altered at 4, 8, 12, 16, 20, and 24 h. The crude monoglycerides were obtained following the reaction, and the immobilized lipase was separated by centrifugation and filtration at 8000 rpm. The crude monoglycerides were refrigerated at −20 °C for future analysis. The experiment was performed three times under the same conditions.

2.5. Determination of monoglycerides content

The method for determination of monoglycerides content was analyzed following the Standard ISO 7366:1987 method. To determine the monoglyceride content, the samples were subjected to a three-step separation process using chloroform and acetic acid solutions to remove unreacted free glycerol. Next, perchloric acid and periodate were added to oxidize the monoglycerides in the chloroform solution, followed by the addition of potassium iodide solution. After 30 min of reaction in a dark room, the solution was titrated with a standard sodium thiosulfate solution, and the content of 1-monoglyceride was calculated. The monoglyceride content was calculated by applying the conversion method described by Martin et al. [22].

2.6. One-factor experiment

The enzyme-catalyzed glycerolysis was examined through a single-factor experiment, where the effects of the molar ratio of substrate (2:1, 3:1, 4:1, 5:1, 6:1 for crude glycerol to soybean oil), enzyme concentration (5 wt%, 10 wt%, 15 wt%, 20 wt%, 25 wt% based on total substrate mass), and reaction temperature (30 °C, 40 °C, 50 °C, 60 °C, 70 °C) were evaluated.

2.7. Optimization of reaction conditions using response surface methodology

A Central Composite Design Type (CCD) was used to optimize the reaction conditions through a three-factor, five-level response surface analysis experiment. The study employed Design-Expert 13.0 software to design the experiment, with substrate molar ratio, enzyme concentration, and temperature designated as factors A, B, and C, respectively. Each factor was set to five levels (in coded forms −1.682, −1, 0, 1 and 1.682, respectively). The actual values of each factor were converted to coded values according to the equations below.

Table 1
Factor and levels of response surface method.

Symbol	Factor	Levels				
		−1.682	−1	0	1	1.682
A	Glycerol/Oil ratio	3.32	4	5	6	6.68
B	Enzyme dosage (%)	1.59	5	10	15	18.41
C	Temperature (°C)	43.18	50	60	70	76.82

$$x_i = \frac{X_i - X_{i0}}{\Delta X_i}$$

(1)

where x_i is the independent variable coded value, X_i is the independent variable real value, and X_{i0} is the real value on the center point and ΔX_i is the interval.

The effect of three factors on monoglyceride yield was investigated. A total of 20 experiments were conducted, comprising 14 non-center point trials and 6 center point trials. The response surface experimental factor and level designs are shown in Table 1.

2.8. Statistical analysis

The statistical software package R (version 3.5.3) was employed for conducting the statistical analysis. ANOVA (analysis of variance) was utilized to analyze all experimental data, which were conducted under a completely randomized design (CRD), at a confidence level of $P < 0.05$.

3. Results and discussion

3.1. Physicochemical properties of the biodiesel-derived crude glycerol

Crude glycerol obtained from the biodiesel industry contains numerous impurities, such as methanol, inorganic salts, soap, fatty acids, fatty acid methyl esters, water, etc. The sample used in this test was obtained from the glycerol distillation plant of Tangshan Jinlihai Biodiesel Co., Ltd. The glycerol content was measured to be 90 %, with the remaining 10 % comprising mainly of fatty acid methyl ester, a matter organic non-glycerol (MONG). Fatty acid methyl esters can serve as a raw material for monoglyceride synthesis [23]. Therefore, the presence of fatty acid methyl esters in crude glycerol does not have a detrimental effect on the synthesis of monoglycerides. Crude glycerol may contain inorganic salts and trace amounts of heavy metals that can potentially hinder the activity of lipase or even lead to its inactivation [24]. Thirty elements, including B, were analyzed in the samples using an inductively coupled plasma spectrometer, as illustrated in Fig. 1. The detection wavelengths were specifically chosen based on the target elements for measurement. The results indicated that the crude glycerol samples were devoid of heavy elements such as B, rendering them appropriate as enzymatic reaction substrates.

3.2. Physicochemical properties of the soybean oil

The acidity value of soybean oil was 0.19 mg KOH/g, the saponification index was 190.25 mg KOH/g, and the average molecular weight was 879.3 g/mol. Molecular weight is an important index for evaluating oils. Soybean oil consists mainly of oleic(C18:1) and linoleic acids(C18:2). When soybean oil contains more stearic acid(C18:0), the density and freezing point of the oil also increase [25], as does the molecular weight. The main fatty acid composition of the soybean oil shown in Fig. 2 was: C16:0, 12.7; C18:1, 21.1; C18:2, 55.3; C18:3, 9.3 (w/w%), which is consistent with the composition of soybean oil obtained from natural soybeans [26]. Soybean oil is characterized by its abundance of unsaturated fatty acids, accounting for 85.7 wt% of its composition, as well as its low melting point

| B 249.677 (mg/kg)	Al 396.153 (mg/kg)	Si 251.611 (mg/kg)	Ca 317.933 (mg/kg)	Ti 334.940 (mg/kg)	Cr 267.716 (mg/kg)
ND	ND	ND	ND	ND	ND
Zn 206.200 (mg/kg)	As 188.979 (mg/kg)	Se 196.026 (mg/kg)	Sr 407.771 (mg/kg)	Cd 228.802 (mg/kg)	Sn 189.927 (mg/kg)
ND	ND	ND	ND	ND	ND
Sb 206.836 (mg/kg)	Ba 233.527 (mg/kg)	Pb 220.353 (mg/kg)	Pd 340.458 (mg/kg)	Ni 231.604 (mg/kg)	Cu 327.393 (mg/kg)
ND	ND	ND	ND	ND	ND
Bi 223.061 (mg/kg)	P 178.221 (mg/kg)	K 766.490 (mg/kg)	Na 589.592 (mg/kg)	Co 228.616 (mg/kg)	Li 670.784 (mg/kg)
ND	ND	ND	ND	ND	ND
Mg 285.213 (mg/kg)	Mn 257.610 (mg/kg)	V 290.880 (mg/kg)	Mo 202.031 (mg/kg)	W 207.912 (mg/kg)	Re 197.248 (mg/kg)
ND	ND	ND	ND	ND	ND

Fig. 1. Analysis of metal elements in crude glycerol. The numbers represent the characteristic spectra of the metal elements. ND indicates that no characteristic spectra were detected in the results.

Instrument Type：GC
Detectors：FID
Injector: split flow

Column temperature: programmed temperature rise
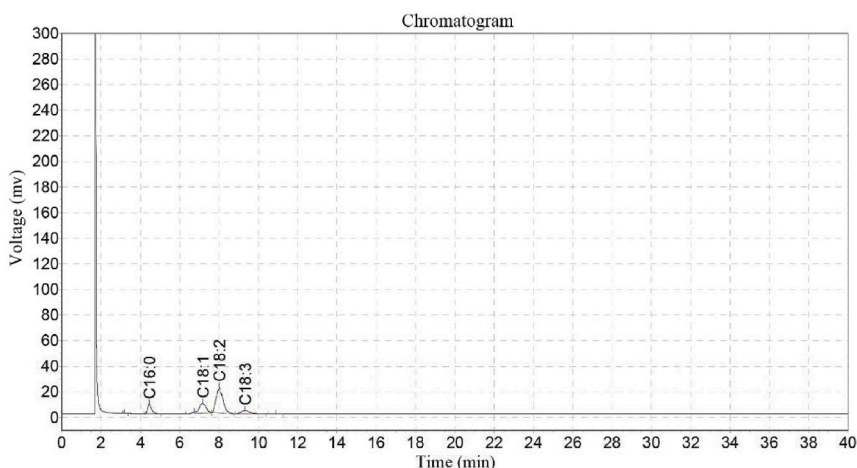
Analysis results table

Peak number	Peak name	Retention time	Peak high	Peak area	Content
1		3. 163	73. 182	557. 250	0. 0656
2	C16:0	4. 438	7607. 542	107663. 102	12. 6760
3		6. 738	1162. 261	11590. 832	1. 3647
4	C18:1	7. 178	7801. 523	179656. 969	21. 1524
5	C18:2	8. 018	20096. 877	469977. 813	55. 3340
6	C18:3	9. 318	2630. 195	78904. 203	9. 2900
7		10. 898	42. 947	996. 900	0. 1174
Total			39414. 527	849347. 068	100. 000

Fig. 2. Gas chromatogram of fatty acid composition of soybean oil.

[26]. The synthesis of monoglycerides from soybean oil resulted in the production of primarily unsaturated monoglycerides. Previous studies have shown that high yields of monoglycerides (>65 %) can be achieved for high melting point fats such as tallow and palm oil using *Pseudomonas* sp. lipase under solvent-free conditions [27]. This is due to the ability of saturated monoglycerides to promote further synthesis of monoglycerides by crystallizing out of the reaction system [28]. However, for corn oil, which is mainly composed of unsaturated monoglycerides, crystallization cannot occur even at low temperatures(5 °C) [27]. The crystallization behavior of monoglycerides in different oils and fats has been studied, with results indicating that the presence of saturated monoglycerides affects the crystallization of oils and fats [29]. Soybean oil, with a lower freezing point than corn oil [26], is unsuitable for obtaining high yields of monoglycerides at low temperatures. In a study by Noureddini et al. [30], it was found that the highest monoglyceride yield from soybean oil was achieved at a reaction temperature of 55 °C. Fregolente et al. [31] screened five lipases for the synthesis of monoglycerides from soybean oil and reported a maximum monoglyceride yield of 32 %.
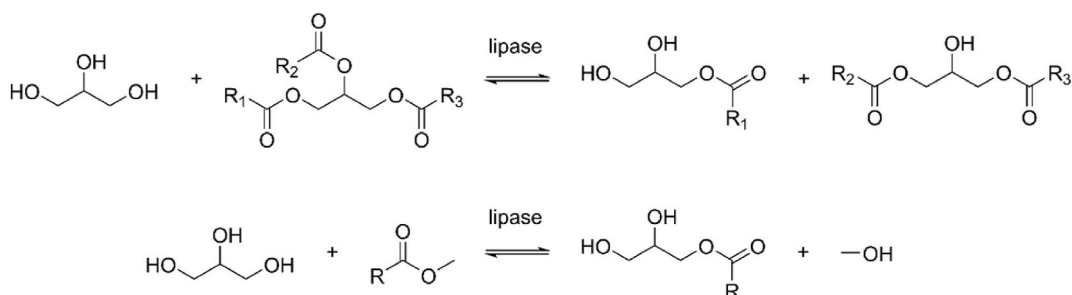
Fig. 3. Synthesis of monoglycerides from crude glycerol. R, R_1, R_2 and R_3 represent same or different fatty acid chains.

3.3. Methanol content in crude glycerol before and after monoglycerides synthesis

There are two reactions in the synthesis of monoglycerides using crude glycerol. As shown in Fig. 3, the transesterification reaction between glycerol and soybean oil produces monoglycerides and diglycerides. In addition, fatty acid methyl esters in crude glycerol can also react with glycerol to form monoglycerides and methanol. As shown in Figs. 4 and 5, the crude glycerol sample before the reaction contained almost no methanol, while the crude glycerol sample after the reaction contained significant amounts of methanol. This confirms the reaction of fatty acid methyl esters. It also shows that fatty acid methyl ester, an impurity component in crude glycerol, is effectively utilized as a feedstock and helps to increase the yield of monoglycerides. This direct utilization and conversion technique of crude glycerol is more economical than expensive purification techniques and has great potential [32]. In the scaled-up production of monoglycerides, the methanol produced needs to be taken into consideration. At the end of the monoglyceride synthesis reaction, the methanol content in the remaining glycerol is 0.02 % (2-propanol concentration: 0.8 %, methanol to 2-propanol peak area ratio: 2.63 %). 0.02 % methanol does not affect the reuse of crude glycerol. Since the boiling point of methanol is much lower than that of glycerol, the joint reaction temperature can be considered. The synthesis of monoglycerides while recovering methanol was realized.

### 3.4. Effect of reaction time on the yield of monoglycerides

The influence of reaction time on the yield of monoglycerides using different types of enzymes was studied at a reaction temperature of 50 °C, an oscillator speed of 180 rpm, a substrate molar ratio of 2:1, and enzyme additions of 5 % (Novozym 435, Lipozyme TL IM, and Lipozyme RM IM). The results are presented in Fig. 6. The mass fraction of monoglycerides in the reaction system rose rapidly from 0 to 12 h. After 12 h, the reaction rate decreased, and the mass fraction of monoglycerides increased slowly from 12 to 24 h. This is due to the fact that the reaction is fast because of the high concentration of crude glycerol and soybean oil at the beginning of the reaction, and after a period of time the concentration of the substrate decreases and the reaction slows down until the reaction reached equilibrium. For each of the three different lipases, Novozym 435, Lipozyme TL IM, and Lipozyme RM IM, six groups of data were obtained depending on the reaction time. According to previous studies, the reaction time of solvent-free enzymatic glycerolysis for the synthesis of monoglycerides is basically less than 24 h. Therefore, this study determines the appropriate reaction time over a wide range of time. The experiments were grouped according to reactions of 4 h, 8 h, 12 h, 16 h, 20 h and 24 h. The groups are represented by numbers 1 to 6, respectively. Time was the independent variable and monoglyceride yield was the dependent variable. The data of each group were subjected to the Shapiro-Wilk normality test, homogeneity of variance analysis, and completely

Instrument Type: GC   Detectors: FID   Injector: No split

Column temperature: programmed temperature rise
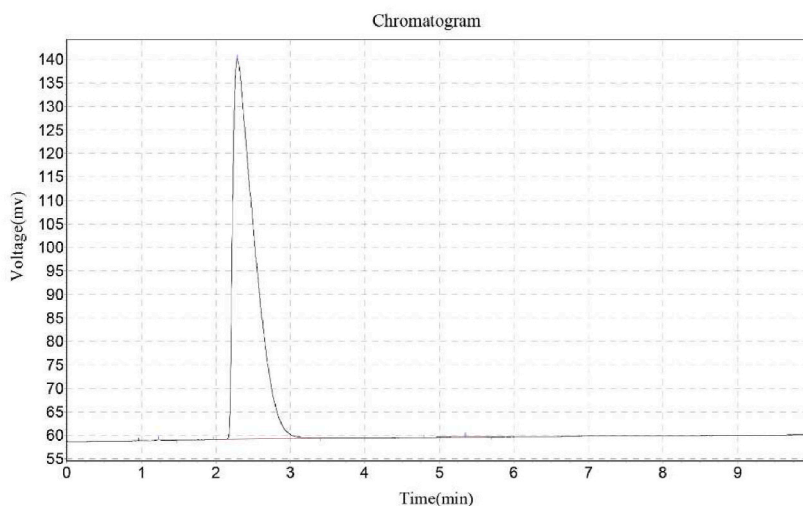
Analysis results table

Peak number	Peak name	Retention time	Peak high	Peak area	Content
1	methanol	1.215	76.066	1627.800	0.1013
2	2-propanol	2.290	80972.898	1606325.750	0.0000
3		5.348	140.193	3988.500	0.0000
Total			81189.157	1611942.050	0.1013

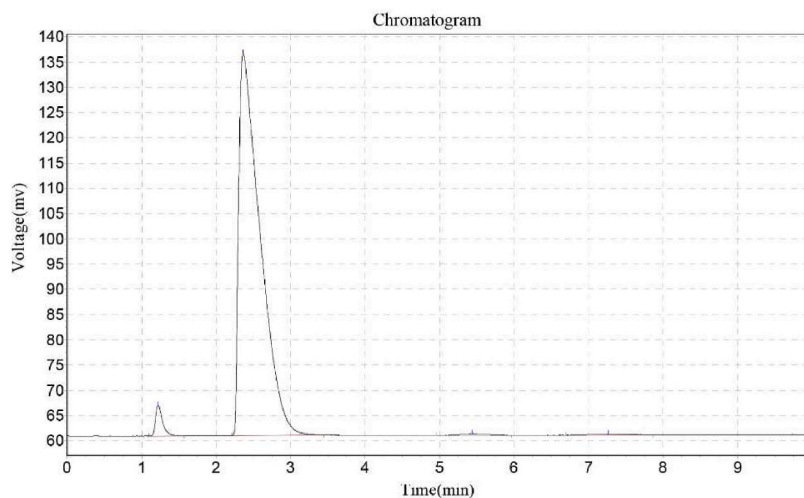**Fig. 4.** Detection of methanol in crude glycerol before synthesizing monoglycerides.

Instrument Type: GC   Detectors: FID   Injector: No split

Column temperature: programmed temperature rise

Analysis results table

Peak number	Peak name	Retention time	Peak high	Peak area	Content
1	methanol	1.223	5963.895	39630.148	2.6304
2	2-propanol	2.365	75637.125	1506600.250	0.0000
3		5.440	245.025	6712.400	0.0000
4		7.265	163.971	6054.800	0.0000
Total			82010.016	1558997.598	2.6304

Fig. 5. Detection of methanol in crude glycerol after synthesizing monoglycerides.
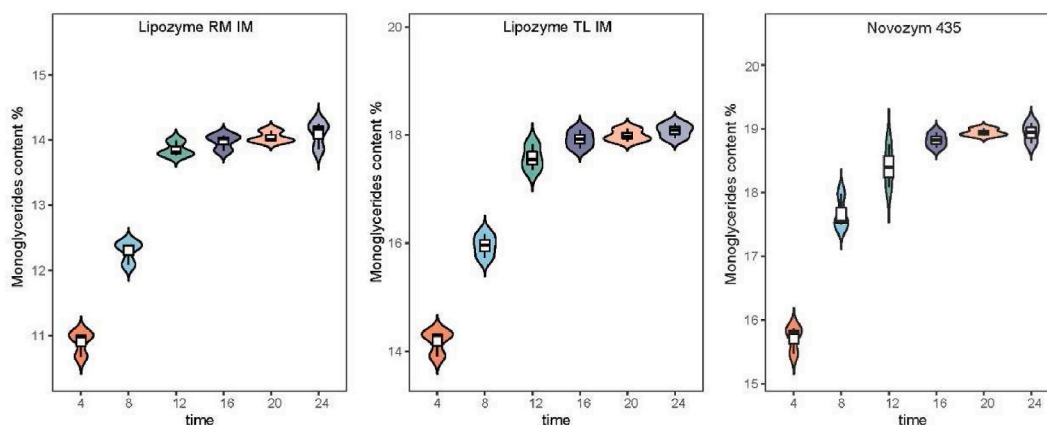

Fig. 6. Effect of reaction time on the yield of monoglycerides (50 °C, 180 rpm, a glycerol/oil molar ratio of 2:1, and 5 wt% enzyme dosage). The three subplots represent, from left to right, the results of reactions with Lipozyme RM IM, Lipozyme TL IM, and Novozym 435 as catalysts. Each subplot consists of six groups of data (groups 4 h, 8 h, 12 h, 16 h, 20 h, and 24 h are represented by orange, cyan, green, purple, beige, and light purple violin plots, respectively). The middle line of the violin plot represents the median, the box represents the interquartile range, the outer line segments of the box represent the maximum and minimum values, and the width of the plot represents the concentration of the data. Each data set consists of the results of three independent parallel experiments (n = 3). Samples from each experiment were obtained on a randomized basis.

randomized ANOVA using R software (version 3.5.3), as shown in Table 2. The results showed that the normality tests of all six data sets were greater than 0.1 under the Lipozyme RM IM catalyst, indicating a normal distribution. The homogeneity of variance was greater than 0.1, indicating equal overall variance of the data in each group. ANOVA $P < 0.05$ indicated that the difference between the mean values of the groups was statistically significant. Comparison between the groups showed that $P > 0.05$ between the groups of 3, 4, 5 and 6. which is not statistically significant. Taking energy consumption into account, the reaction time was determined to be 12 h.

Table 2
Multiple comparisons of monoglyceride yields for 6 durations (One-Way ANOVA).

Enzyme	Group	Reaction duration	MG%	Normality	Homogeneity of variance	ANOVA
Lipozyme RM IM	1	4 h	10.90 ± 0.1882^c	0.1524	P = 0.9003	F = 216.2
	2	8 h	12.29 ± 0.1735^b	0.1101	P > 0.1	P = 2.531e-11
	3	12 h	13.86 ± 0.1172^a	0.3275		P < 0.05
	4	16 h	13.98 ± 0.121^a	0.3976		
	5	20 h	14.05 ± 0.0907^a	0.3172		
	6	24 h	14.09 ± 0.206^a	0.3732		
Lipozyme TL IM	1	4 h	14.18 ± 0.2318^c	0.165	P = 0.925	F = 205.3
	2	8 h	15.95 ± 0.2152^b	0.9231	P > 0.1	P = 3.434e-11
	3	12 h	17.58 ± 0.2411^a	0.8168		P < 0.05
	4	16 h	17.92 ± 0.165^a	0.9666		
	5	20 h	17.99 ± 0.1168^a	0.6678		
	6	24 h	18.08 ± 0.1353^a	0.8777		
Novozym 435	1	4 h	15.71 ± 0.2117^c	0.3631	P = 0.419	F = 104
	2	8 h	17.67 ± 0.2663^b	0.1436	P > 0.1	P = 1.8787e-9
	3	12 h	18.41 ± 0.3404^a	0.9028		P < 0.05
	4	16 h	18.82 ± 0.115^a	0.9521		
	5	20 h	18.94 ± 0.0611^a	0.6369		
	6	24 h	18.94 ± 0.1604^a	0.8624		

Note: Reaction conditions were 50 °C, 180 rpm, a glycerol/oil molar ratio of 2:1, and 5 wt% enzyme dosage. Monoglyceride yield data for the same enzyme are labeled with the same letter to indicate a non-significant difference (P > 0.05), and different letters to indicate a significant difference (P < 0.05).

Similarly, the reaction time of Lipozyme TL IM and Novozym 435 was determined as 12 h. In the investigation of enzymatic synthesis of monoglycerides, the attainment of reaction equilibrium time is hindered by numerous factors, such as the type of oil, the species of lipase, and the reaction environment. Of particular importance to the reaction kinetics is the low solubility of the substrate. To overcome this challenge, several methods, such as organic solvents, surfactants, and solid carrier adsorption, have been employed to enhance the interaction between glycerol and the reactants and catalysts, thereby expediting the time necessary to achieve reaction equilibrium. Yang et al. [33] have enzymatically synthesized polyunsaturated fatty acid-rich monoglycerides from sunflower oil, using tert-butanol as a solvent. Remarkably, they achieved a yield of 60–70 % of monoglycerides within 2 h of the reaction, which was over 20 times more efficient than the solvent-free system. Valério et al. [34] optimized the reaction conditions by introducing TritonX-100 at 70 °C, and employing lipase Novozym 100 as the catalyst for 3 h. Palacios et al. [14] performed the reaction by adsorbing glycerol on silica gel, leading to maximum monoglyceride yield after 4 h. However, the aforementioned approach, although significantly reducing the reaction time, escalates the cost of downstream separation. In contrast, the enzymatic synthesis of monoglycerides in a solvent-free system without the addition of any other substances is a sustainable and eco-friendly chemical technique, albeit with a longer reaction time. The present study was conducted in a solvent-free system, requiring a reaction time of 12 h. Comparable results have been reported in previous studies, for instance, a reaction time of 10 h was reported from Yang et al. [35] and a time of 24 h was reported from Fregolente et al. [31].

3.5. Effect of enzyme types on the yield of monoglycerides

Previous findings in Section 3.4 have established that the equilibration time for the three lipases under examination is approximately 12 h. In order to identify the most suitable lipase for catalyzing the synthesis of monoglycerides, we examined the effects of Novozym 435, Lipozyme TL IM, and Lipozyme RM IM on the yield of monoglycerides. The experimental conditions were consistent across all three tests, with a reaction temperature of 50 °C, a reaction time of 12 h, an oscillator speed of 180 rpm, a substrate molar ratio of 2:1, and an enzyme addition of 5 %. As depicted in Table 3, all three enzymes exhibited the ability to enhance the glycerolysis reaction. The reaction outcomes were grouped into three categories based on the catalyst employed, the statistical analyses were done using R software (version 3.5.3) with the Shapiro–Wilk normality test, confirming for homogeneity of variance and analysis of

Table 3
Multiple comparisons of monoglyceride yields by 3 enzymes (One-Way ANOVA).

Enzyme	Reaction conditions	MG%	Normality	Homogeneity of variance	ANOVA
Lipozyme RM IM	50 °C, 180 rpm, a glycerol/oil molar ratio of 2:1, and 5 wt% enzyme dosage,12 h	13.86 ± 0.1172^c	0.3275	P = 0.4505 P > 0.1	F = 281.7 P =
Lipozyme TL IM	50 °C 180 rpm, a glycerol/oil molar ratio of 2:1, and 5 wt% enzyme dosage,12 h	17.6 ± 0.2411^b	0.8168		0.0000011697 P < 0.05
Novozym 435	50 °C, 180 rpm, a glycerol/oil molar ratio of 2:1, and 5 wt% enzyme dosage,12 h	18.41 ± 0.3404^a	0.9028		

Note: Monoglyceride yield data labeled with the same letter indicates a non-significant difference (P > 0.05), while different letters indicate a significant difference (P < 0.05).

variance, revealing a significant variation in catalytic performance among the three enzymes upon comparing the two. The analysis showed that Novozym 435 showed the highest monoglyceride yield among the three lipases tested, followed by Lipozyme TL IM and Lipozyme RM IM. The observed disparities in the outcomes could be attributed to the substrate specificity of the enzyme catalysis [35]. With regards to triglycerides, Lipozyme RM IM exhibits a stringent sn-1,3 position specificity, whereas Lipozyme TL IM displays relatively less sn-1,3 specificity than Lipozyme RM IM [36]. Conversely, Novozym 435 is a non-specific lipase [37]. Under identical conditions, Novozym 435 achieved a yield of 18.41 %, surpassing the yields obtained with Lipozyme RM IM and Lipozyme TL IM by a significant margin. On balance, the Novozym 435 with the best catalytic effect was chosen for the later experimental exploration. Novozym 435, derived from *Candida antarctica* lipase B and immobilized on macroporous acrylic resin through interfacial activation, is a commercially available lipase that has gained widespread recognition for its outstanding catalytic activity and stability in both scientific research and industrial applications [38]. It has been commonly used as a catalyst for glycerolysis of various oils and fats, such as soybean oil [39], sardine oil [40], anchovy oil [14], sunflower oil [35], and olive oil [41]. In the present study, the suitability of Novozym 435 as a catalyst for monoglyceride synthesis was explored in a similar manner. In certain instances, Novozym 435 may not be the preferred choice of lipase for certain applications. For instance, Lipozyme RM IM has been shown to be more efficient than N435 in the glycerolysis of lard [42]. Alternatively, Lipozyme TL IM may be utilized for the enzymatic glycerolysis of palm olein due to its cost-effectiveness [43].

3.6. Effect of substrate ratio on the yield of monoglycerides

The impact of the ratio of crude glycerol to soybean as reaction substrates on the yield of monoglycerides was investigated at a reaction temperature of 50 °C, a reaction time of 12 h, an oscillator speed of 180 rpm, and an enzyme concentration of 5 %. From the results (Fig. 7), the content of monoglycerides increased with an increase in the substrate ratio, reaching a maximum of 22.04 % when the substrate ratio was 5:1. This is because an increase in glycerol can facilitate the conversion of diglycerides and triglycerides to monoglycerides. However, a further increase in the ratio caused the monoglyceride content to decrease. This is because when the glycerol ratio is too high, it can surround the lipase, inhibiting efficient enzyme-substrate contact, which negatively affects the reaction efficiency [44]. Enzymes are hydrophilic macromolecules that can dissolve in glycerol. When the proportion of glycerol in the reaction system is too high, the bound water in the lipase molecule binds to the glycerol molecule, and the glycerol forms a coating on the surface of the enzyme, resulting in the substrate not being able to reach the catalytically active site, thus reducing the substrate conversion rate. The enzymatic glycerolysis process involves complex ping-pong bi-bi mechanisms, where enzymes form complexes with triglycerides, which subsequently lead to the formation of fatty acid-enzyme complexes and diglycerides. In the presence of free glycerol, monoglycerides and enzymes are produced from the fatty acid-enzyme complexes, while diglyceride-enzyme complexes form slowly and can easily dissociate [43]. Augmenting the quantity of glycerol in the substrate can effectively enhance the conversion of fats and oils, and the surplus glycerol aids in the escalation of the monoglyceride yield. A glycerol/oil molar ratio ranging from 3:1~5:1 has been demonstrated to produce high yields of monoglycerides [45]. Valério et al. [34] compared glycerol/olive oil molar ratios of 3:1, 6:1 and 9:1 enzymatic glycerolysis, respectively, and found that excess glycerol in the early stages of the reaction had little effect on the yield of monoglycerides, but was necessary to improve the yield of monoglycerides after a certain period of time.

3.7. Effect of temperature on the yield of monoglycerides

The catalytic reaction of lipase is substantially impacted by temperature [46]. Glycerol and soybean oil exhibit relatively low
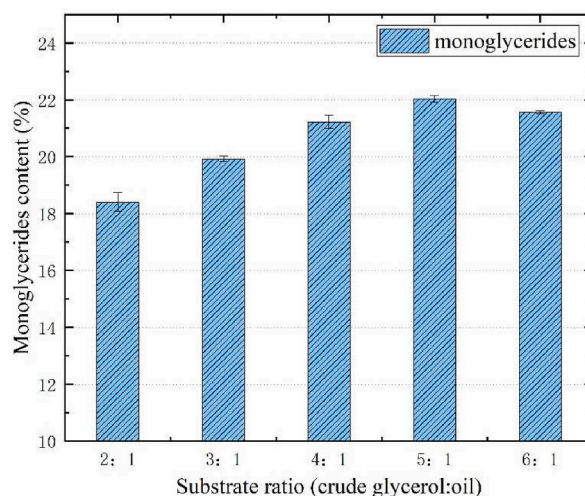
Fig. 7. Effect of substrate ratio on the yield of monoglycerides. Reaction conditions were 50 °C, 180 rpm, and 5 wt% enzyme dosage, 12 h. Error bars represent the standard deviations of three separate measurements (n = 3).

solubility, and elevating the temperature can decrease their viscosity, consequently enhancing their interaction [47]. However, excessively high temperatures may lead to lipase inactivation [48]. The effect of reaction temperature on the yield of monoglycerides was investigated at a substrate molar ratio of 2:1, an oscillator speed of 180 rpm, an enzyme addition of 5 %, and a reaction time of 12 h. As shown in Fig. 8, the content of monoglycerides in the reaction product initially increased and then decreased with an increase in reaction temperature. The maximum yield of monoglycerides, which was 21.3 %, was obtained at a reaction temperature of 60 °C, whereas at 70 °C, the yield decreased to 20.8 %. This is attributed to the excessively high temperature, which may lead to enzyme thermal deactivation. Additionally, Novozym 435 is an immobilized enzyme prepared through interfacial activation. This immobilized enzyme is prone to enzyme shedding caused by surfactant molecules (such as monoglycerides, and diglycerides) and high temperatures, resulting in enzyme deactivation [38]. Novozym 435 exhibits activity over a wide temperature range (20–110 °C), although it is typically employed within the optimal range of 30~60 °C [49]. Raising the temperature enhances the pro-glycerolysis reaction catalyzed by Novozym 435 and reduces the time needed for the reaction to reach equilibrium. An increase in the temperature from 30 to 50 °C led to an augmentation of Novozym 435 activity, which consequently promoted the reaction and increased the yield of monoglycerides within 12 h, as depicted in Fig. 3. During the study of the reaction time at 50 °C (Section 3.3), the yield of monoglycerides continued to increase slowly after 12 h, indicating that the reaction had not yet reached equilibrium. Hence, when the temperature was greater than 50 °C, the yield of monoglycerides obtained a further increase up to 60 °C. This finding is in accordance with previous literature reports, where selection of temperature for the enzymatic glycerolysis reaction under solvent-free conditions typically ranges from 40 to 70 °C. For instance, Fregolente et al. [31] achieved a 32 % yield of monoglycerides at 50 °C, while Tüter et al. [50] obtained a 16 % yield of monoglycerides at 40 °C. Noureddini et al. [30] conducted a study on the enzymatic glycerolysis of soybean oil in the temperature range of 30~70 °C and demonstrated that the maximum yield of monoglycerides was obtained at 55 °C, which was consistent with the residual activity of the *Pseudomonas* sp. lipase used. In addition, Coteron et al. [41] observed that the glycerolysis reaction of olive oil at 75 °C resulted in a threefold increase in the yield of monoglycerides as compared to 50 °C.

3.8. Effect of enzyme concentration on the yield of monoglycerides

Lipase Novozym 435 served as the catalyst for the reaction, and the amount of enzyme directly impacted the reaction rate. The effect of enzyme concentration on the yield of monoglycerides was studied at a reaction temperature of 50 °C, a reaction time of 12 h, an oscillator speed of 180 rpm, and a substrate molar ratio of 2:1. The findings, as presented in Fig. 9, demonstrate a direct correlation between the amount of lipase added and the monoglyceride content, which can be attributed to the role of enzyme quantity in promoting the reaction when the catalyst is insufficient. Specifically, a 23.3 % yield was achieved with a lipase concentration of 10 % relative to the substrate mass. It is noteworthy that the percentage content of reaction products remained relatively stable when the amount of enzyme exceeded 10 %. Typically, augmenting the enzyme concentration results in enhanced reaction conversion, albeit only up to a certain threshold, beyond which enzyme amount fails to considerably advance the reaction [51]. In contrast, an excessive enzyme concentration can trigger enzyme aggregation, precluding the active site of the enzyme from acting on the substrate, and culminating in enzyme failure [52]. This is consistent with findings from other studies. Fregolente et al. [31] found in their study on enzymatic glycerolysis of soybean oil that the yield of monoglycerides was higher under 10 % enzyme loading compared to 5 % enzyme loading. In another study, Palacios et al. [14] used response surface methodology to optimize the glycerolysis conditions of anchovy oil with a 6 % enzyme load. In a separate study, Naik et al. [53] chose a 15 % enzyme load for glycerolysis of sunflower oil, and Valerio et al. [54] found a high yield of monoglycerides at a 9 % enzyme concentration.
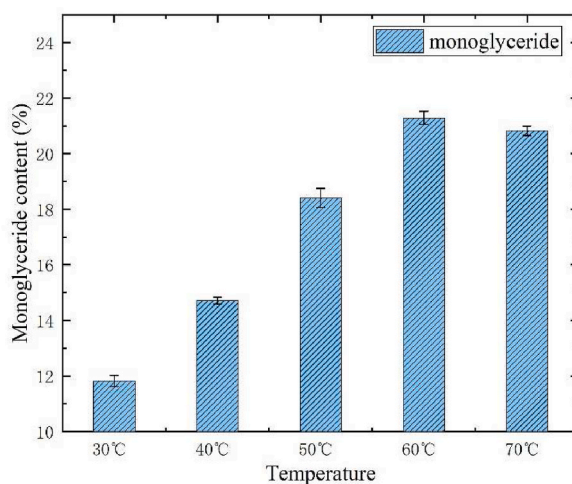

Fig. 8. Effect of temperature on the yield of monoglycerides. Reaction conditions were 180 rpm, a glycerol/oil molar ratio of 2:1, and 5 wt% enzyme dosage,12 h. Error bars represent the standard deviations of three separate measurements (n = 3).
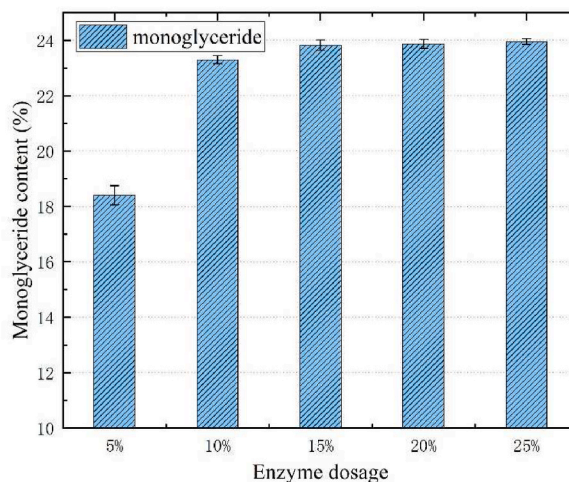
Fig. 9. Effect of enzyme concentration on the yield of monoglycerides. Reaction conditions were 50 °C, 180 rpm, a glycerol/oil molar ratio of 2:1, and 12 h. Error bars represent the standard deviations of three separate measurements (n = 3).

3.9. Response surface methodology for optimizing the synthesis of monoglycerides

The glycerolysis of soybean oil using Novozym 435 under solvent-free conditions is a complex process. This process involves six intermediate reaction steps [43], and the yield of the final product monoglycerides can be influenced by various reaction conditions. Therefore, the molar ratio of the substrate, the reaction temperature, and the amount of enzyme were selected as variables of interest. In the previous single-factor experiments, conditions with the highest yield of monoglycerides (substrate molar ratio of 5:1, reaction temperature of 60 °C, and enzyme concentration of 10 %) were obtained. Based on the results of these experiments, response surface methodology was employed to further explore the interactions among the reaction parameters and their effects on the yield of monoglycerides. The response surface experiments were designed using Design-Expert 13.0 software, following the Central Composite Design Type. The experimental designs and test results are reported in Table 4.

The Design-Expert 13.0 software can be used to analyze and process the experimental data, from which the model equation and variance results can be obtained. As shown in Table 5, the simulated regression yielded a P-value of less than 0.0001, indicating that the model is meaningful. Moreover, the out-of-fit term had a P-value of 0.8354, which is not significant, suggesting that the model fits well with the experiment and that the out-of-fit is negligible. This model can, therefore, be utilized for the analysis and prediction of enzyme-catalyzed synthesis of monoglycerides. The model-corrected coefficient of determination (Adj R^2) was 0.9982, indicating that only 0.2 % of the monoglyceride content could not be explained by these models. The correlation coefficient (R^2) was 99.9 %,

Table 4
Response surface experimental results for glycerolysis using Novozym 435.

	Factors			Response
Trial	Substrate molar ratio (Glycerol/Oil)	Enzyme dosage (%, w/w)	Reaction temperature (°C)	MG (%)
1	6	15	50	26.01
2	5	10	60	28.12
3	5	10	60	27.86
4	4	15	70	27.13
5	6	5	50	21.56
6	5	10	76.8179	27.79
7	4	5	50	21.23
8	5	10	60	27.95
9	5	18.409	60	25.17
10	6	15	70	28.33
11	5	10	60	28.13
12	5	10	43.1821	24.31
13	5	10	60	28.23
14	3.31821	10	60	26.42
15	4	5	70	23.32
16	4	15	50	25.42
17	5	1.59104	60	18.06
18	5	10	60	27.91
19	6	5	70	24.32
20	6.68179	10	60	27.75

Note: %, (w/w) based on the total oil.

Table 5
Analysis of variance for the quadratic model of enzymatic glycerolysis.

Source	Sum of Squares	df	Mean Square	F-value	F-critical	p-value	
Model	156.07	9	17.34	1154.97	3.02	<0.0001	significant
A	2.10	1	2.10	139.94	4.96	<0.0001	[b]
B	59.13	1	59.13	3938.39	4.96	<0.0001	[b]
C	15.89	1	15.89	1058.54	4.96	<0.0001	[b]
AB	0.0265	1	0.0265	1.76	4.96	0.2139	
AC	0.2048	1	0.2048	13.64	4.96	0.0042	[b]
BC	0.0840	1	0.0840	5.60	4.96	0.0395	[a]
A²	1.75	1	1.75	116.57	4.96	<0.0001	[b]
B²	75.08	1	75.08	5000.30	4.96	<0.0001	[b]
C²	7.36	1	7.36	489.91	4.96	<0.0001	[b]
Residual	0.1501	10	0.0150				
Lack of Fit	0.0424	5	0.0085	0.3937		0.8354	not significant
Pure Error	0.1077	5	0.0215				
Total	156.22	19					
R² = 0.999			Adjusted R² = 0.9982			C.V.% = 0.4758	

Note.

[a] $P < 0.05$ significant.

[b] $P < 0.01$ highly significant. "A" represents the molar ratio of glycerol to soybean oil, "B" represents enzyme dosage, "C" represents the reaction temperature. F-critical of $\alpha = 0.05$.

demonstrating a good fit between the experimental and predicted values of monoglyceride content in this model, and a significant linear relationship between the independent variables and the response values. A ratio greater than 4 is desirable. The ratio value of 118.609 indicates an adequate signal.

The significance results of the model indicate that the primary terms A, B, and C were highly significant, while the secondary terms A^2, B^2, and C^2 were also highly significant. The interaction term AC was highly significant, whereas the interaction term BC was significant, and the interaction term AB was not significant. This suggests that the change in response values by the three factors is not a general linear relationship.

The response surface plots and contour plots of the effects of three factors, substrate molar ratio, enzyme dosage, reaction temperature and their interactions on the yield of monoglycerides are shown in Figs. 7–9.

Fig. 10 shows that, when the enzyme concentration is held constant, the monoglyceride content increases slowly as the molar ratio of substrate varies from 4:1 to 6:1. However, when the substrate molar ratio is held constant, the monoglyceride content increases rapidly with increasing enzyme dosage. At low molar ratios, the monoglyceride content first increases and then slightly decreases with increasing enzyme dosage due to excess enzyme relative to the substrate. Increasing the molar ratio of the substrate is known to promote the glycerolysis reaction and result in a higher yield of monoglycerides [53]. However, excessive glycerol can lead to a decrease in yield. This is because an increase in glycerol concentration results in an increase in viscosity, which in turn hinders the mass transfer of reactants [54]. Moreover, the enzyme's bound water preferentially binds to glycerol, leading to the wrapping of the enzyme in excess glycerol. This reduces the enzyme's contact with the lipid, resulting in a decrease in enzyme activity and a consequent decrease in yield [55]. It was observed that this trend is less pronounced at high molar ratios. This suggests that there is an interactive effect of molar ratio and enzyme concentration on monoglyceride content, but it is not significant.

It is evident from Fig. 11 that the yield of monoglycerides becomes larger with increasing temperature at higher substrate molar ratios. Similarly, the yield of monoglycerides became larger with increasing molar ratio of substrates at higher reaction temperatures. The reaction temperature was found to decrease the viscosity of glycerol, which was measured as 142 cSt at 50 °C for pure glycerol, and decreased to 50.6 cSt at 70 °C, a 2.8-fold decrease [56]. This increase in temperature led to significant improvement in the fluidity of the reaction system, resulting in a faster reaction rate and higher product content. The highest point of monoglyceride content was observed in the region above 60 °C and above the molar ratio of 5:1, indicating a significant interaction between temperature and substrate molar ratio.

Fig. 12 illustrates the effect of temperature and enzyme concentration on monoglyceride content. When the enzyme concentration is 5 %, the monoglyceride content increases with rising temperature. At lower temperatures, the monoglyceride yield initially increases but later decreases, whereas at higher temperatures, the yield increases with increasing of enzyme dosage. Enzyme-substrate interaction is known to be more active at higher temperatures, leading to an increase in reaction rate [57]. However, beyond a certain temperature, protein thermal denaturation occurs, leading to a sharp decline in reaction rate [58]. Novozym 435, which is capable of catalyzing at 110 °C [49], does not undergo denaturation at 50–70 °C and significantly enhances the yield of monoglyceride. It is evident that the interaction between enzyme dosage and temperature has a significant effect on the monoglyceride content.

Response surface experimental regression analysis was performed using Design-Expert 13.0 software. The analysis yielded response values and regression equations for each factor (reaction temperature, enzyme, and substrate molar ratio) by fitting the analysis results.

$MG\% = -23.97669 + 2.80223A + 2.30762B + 0.905689C + 0.0115AB + 0.016AC - 0.00205BC - 0.348498A^2 - 0.091297B^2 - 0.007144C^2$ where "A" represents the molar ratio of crude glycerol to soybean oil (in the range of 4 and 6), "B" represents enzyme dosage (in the range of 5 % and 15 %), "C" represents the reaction temperature (in the range of 50 °C and 70 °C). Based on the regression
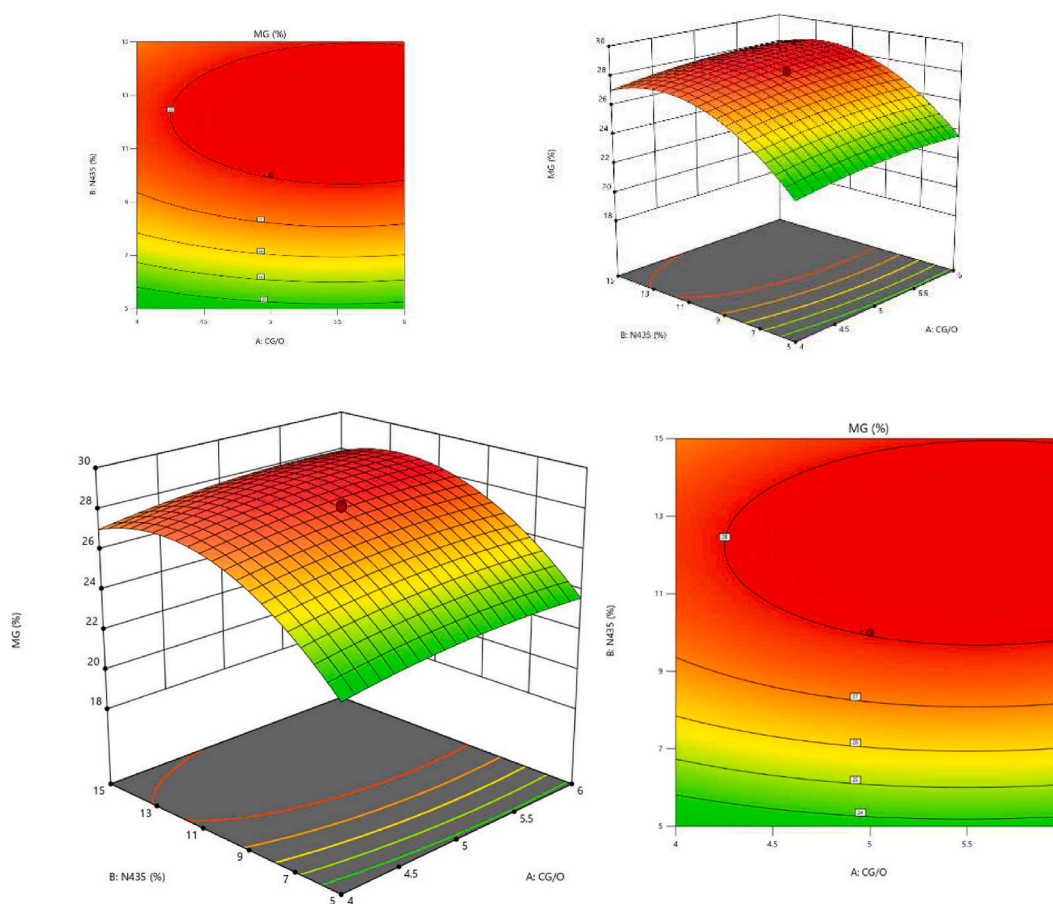
Fig. 10. Effects of substrate ratio, enzyme concentration and their interactions on monoglycerides content and contour.

equation, the maximum value of MG was predicted to be 29.021 %. The optimum reaction conditions were A = 5.689, B = 12.655 %, and C = 65.247 °C. Further experiments were conducted under these optimal conditions to verify the experimental results of 28.93 %. The difference of 0.091 % between the software prediction and the actual result suggests that the model is accurate and reliable.

4. Economic and environmental assessment

The preparation of monoglycerides by enzymatic glycerolysis under solvent-free conditions is a green and environmentally friendly synthetic method that is sustainable. However, due to the high cost of enzymes, there are still many challenges to be faced to reach the level of large-scale production. Facing the challenges, this study makes efforts in several aspects. Firstly, monoglycerides were successfully synthesized from crude glycerol and soybean oil with a yield of 28.98 %, which is similar to the results reported in the literature (pure glycerol and soybean oil) [59]. In addition, the fatty acid methyl esters in crude glycerol were effectively utilized instead of being removed as impurities. This strategy of direct utilization of crude glycerol has great potential. For example, in 2020, the price of glycerol (industrial grade) in the United States was 660 euros per ton, while the price of 80 % crude glycerol was less than 280 euros per ton [60]. Secondly, solvent-free systems produce essentially no waste. The residue of the reaction product after extraction of the monoglycerides is simple in composition. This product is rich in diglycerides and triglycerides and can be used as fat or as a raw material for the synthesis of monoglycerides [16]. In addition, the conditions of enzymatic catalyzed reaction are very important. In this study, after the selection of enzymes and optimization of conditions, the reaction yield was increased, the reaction time was shortened to 12 h, and the energy consumption was reduced. This work has significant environmental benefits. Biodiesel-derived glycerol belongs to the bulk bio-based materials, and its utilization is divided into purification and direct utilization. Glycerol purification is costly and puts pressure on the environment, while direct application is developing rapidly, and various technologies have been applied to industrial production [61]. The solvent-free enzymatic synthesis of crude glycerol into monoglycerides not only avoids the environmental pollution caused by organic solvents but also the impact of crude glycerol on the environment. In conclusion, this study on the high-value conversion of crude glycerol provides a new method for the green synthesis of monoglycerides.
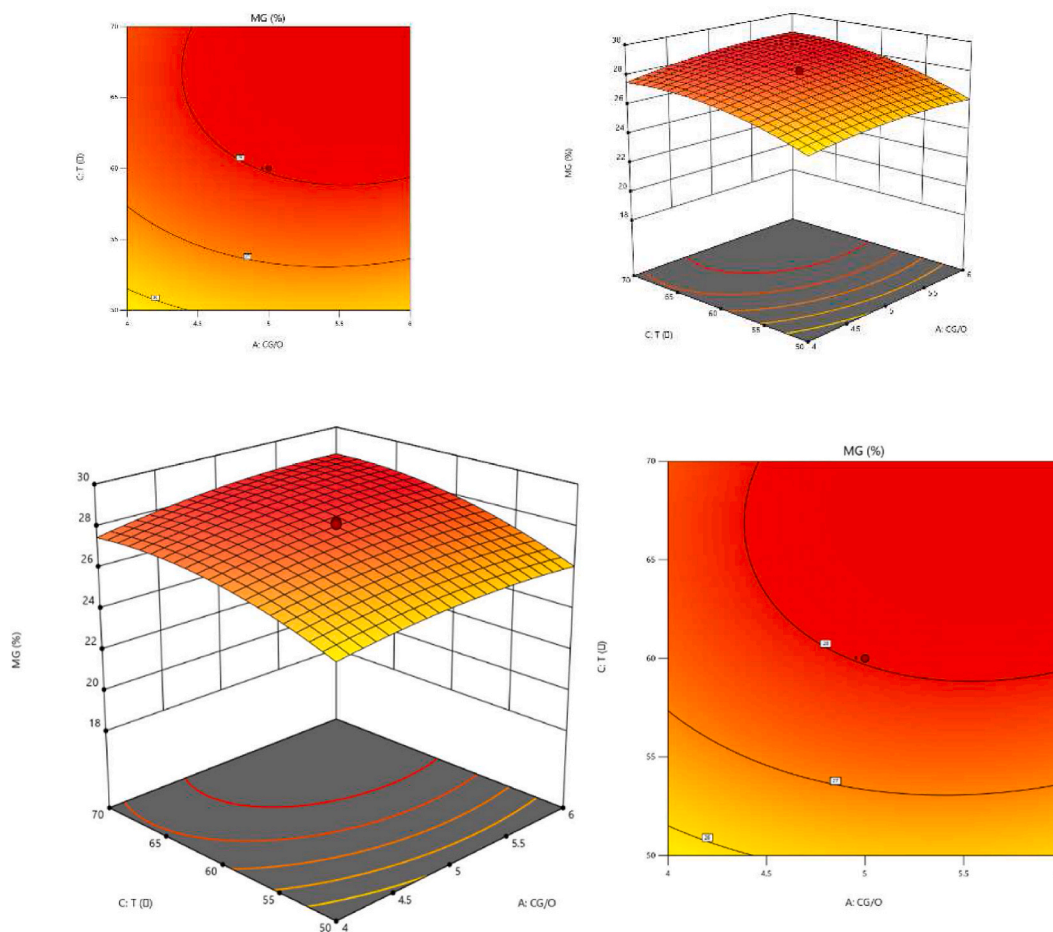
Fig. 11. Effects of substrate ratio, temperature and their interactions on monoglycerides content and contour.

5. Conclusion

In this study, monoglycerides were synthesized from biodiesel-derived crude glycerol using lipase-catalyzed glycerolysis under solvent-free conditions. The enzyme-catalyzed monoglyceride synthesis process was optimized using single-factor tests, Central Composite Design Type, and response surface analysis. The optimal process conditions were determined as follows: a reaction time of 12 h, a reaction temperature of 65.2 °C, the use of N435 enzyme, an enzyme dosage of 12.7 wt%, and a crude glycerol to soybean oil ratio of 5.7:1 (mol/mol). The theoretical and actual yields of monoglycerides were 29.021 % and 28.93 %, respectively, under these conditions, and the model exhibited a satisfactory predictive capacity. The optimized process led to a 10.52 % increase in the yield of monoglycerides compared to the previous experimental results. Although the yield of monoglycerides in this study was not high, the solvent-free products have potential applications for food, feed, and daily necessities industries. In addition, this technique of directly utilizing crude glycerol has significant cost advantages and environmental benefits, and is promising for the sustainable development of the biodiesel industry.

Data availability

The authors declare that the data supporting the results of this study are available in the paper, and requests for more detailed data should be addressed to the corresponding author with the consent of all authors.
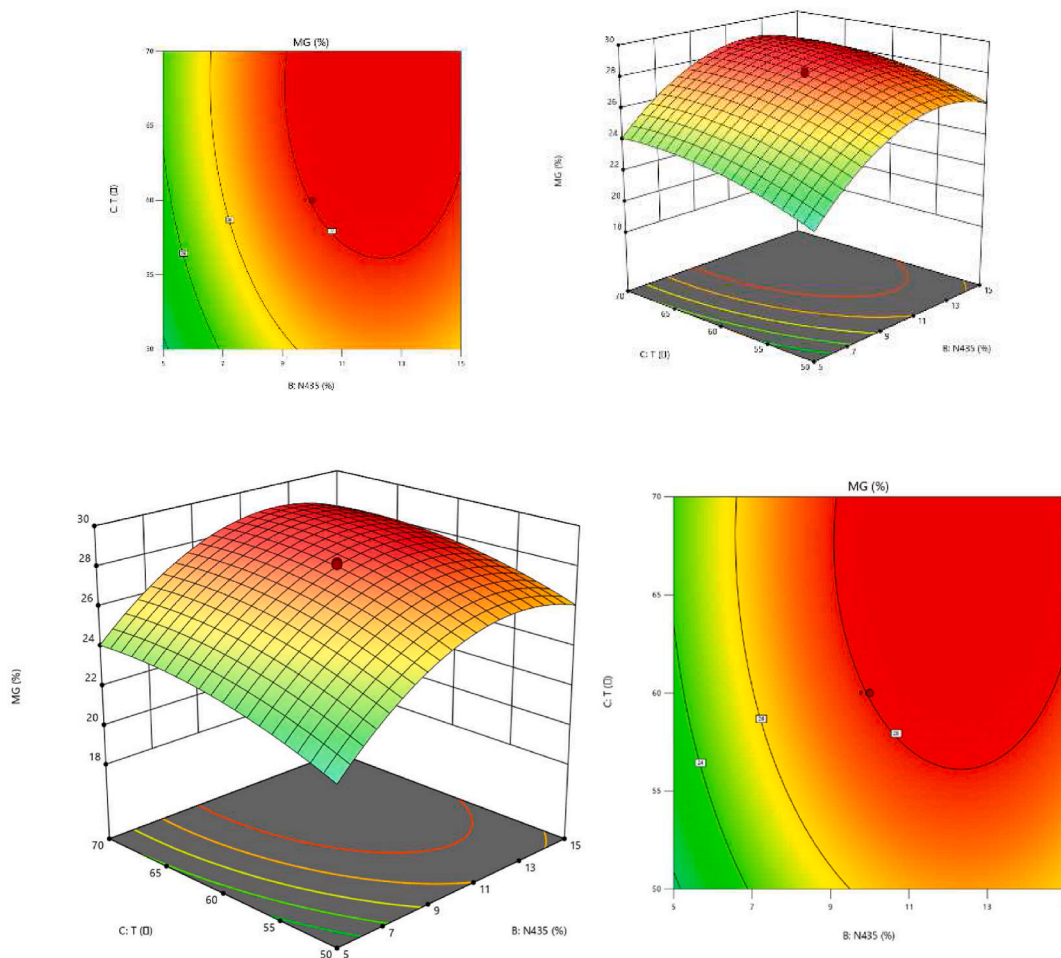
Funding

Fig. 12. Effects of enzyme concentration, temperature and their interactions on monoglycerides content and contour.

CRediT authorship contribution statement

Hong Wang: Writing – original draft, Visualization, Methodology, Funding acquisition, Formal analysis, Data curation. **HongPeng Li:** Writing – original draft, Visualization, Methodology, Formal analysis, Data curation. **Chee Keong Lee:** Writing – review & editing, Supervision, Resources, Formal analysis, Conceptualization. **Noreen Suliani Mat Nanyan:** Writing – review & editing, Sharif Nafiu Usman, Writing – review & editing. **Guan Seng Tay:** Writing – review & editing, Supervision, Resources, Project administration, Investigation, Conceptualization.

Declaration of competing interest

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests:Hong Wang reports financial support was provided by Ministry of Science and Technology of the People's Republic of China. If there are other authors, they declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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References

[1] A. Ghazalah, M. Abd-Elsamee, M. Ibrahim, S.S. Abdelgayed, M. Abdelkader, D. Gonzalez-Sanchez, A. Wealleans, Effects of a combination of lysolecithin, synthetic emulsifier, and monoglycerides on growth performance, intestinal morphology, and selected carcass traits in broilers fed low-energy diets, Animals 11 (11) (2021) 3037.

[2] T. Miyazawa, M. Itaya, G.C. Burdeos, K. Nakagawa, T. Miyazawa, A critical review of the use of surfactant-coated nanoparticles in nanomedicine and food nanotechnology, Int. J. Nanomed. 16 (2021) 3937.

[3] X. Zhao, D. Li, H. Zhu, J. Ma, Y. An, Advanced developments in environmentally friendly lubricants for water-based drilling fluid: a review, RSC Adv. 12 (35) (2022) 22853–22868.

[4] F.O. Nitbani, J. Jumina, Monoglycerides as an antifungal agent. Apolipoproteins, Triglycerides and Cholesterol, 2020.

[5] M. Pang, L. Cao, S. Kang, S. Jiang, L. Cao, Controlled release of flavor substances from sesame-oil-based oleogels prepared using biological waxes or monoglycerides, Foods 10 (8) (2021) 1828.

[6] J.Y.B. Tan, B.K. Yoon, N.-J. Cho, J. Lovrić, M. Jug, J.A. Jackman, Lipid Nanoparticle technology for delivering biologically active fatty acids and monoglycerides, Int. J. Mol. Sci. 22 (18) (2021) 9664.

[7] R. Martinez, C. Rosado, M.V.R. Velasco, S.C.d.S. Lannes, A.R. Baby, Main features and applications of organogels in cosmetics, Int. J. Cosmet. Sci. 41 (2) (2019) 109–117.

[8] N. Gooran, B.K. Yoon, J.A. Jackman, Supported lipid bilayer platform for characterizing the membrane-disruptive behaviors of triton X-100 and potential detergent replacements, Int. J. Mol. Sci. 23 (2) (2022) 869.

[9] R.L. Quirino, K. Monroe, C.H. Fleischer III, E. Biswas, M.R. Kessler, Thermosetting polymers from renewable sources, Polym. Int. 70 (2) (2021) 167–180.

[10] M.S. Alvarez Serafini, G.M. Tonetto, Synthesis of glycerides of fatty acids by inorganic solid catalysts: a review, ChemBioEng Rev. 9 (1) (2022) 110–123.

[11] R.N. Vilas Boas, H.F. de Castro, A review of synthesis of esters with aromatic, emulsifying, and lubricant properties by biotransformation using lipases, Biotechnol. Bioeng. 119 (3) (2022) 725–742.

[12] Y. Satyawali, L. Cauwenberghs, M. Maesen, W. Dejonghe, Lipase catalyzed solvent free synthesis of monoacylglycerols in various reaction systems and coupling reaction with pervaporation for in situ water removal, Chem. Eng. Process 166 (2021) 108475.

[13] D. Palacios, N. Ortega, N. Rubio-Rodríguez, M.D. Busto, Lipase-catalyzed glycerolysis of anchovy oil in a solvent-free system: simultaneous optimization of monoacylglycerol synthesis and end-product oxidative stability, Food Chem. 271 (2019) 372–379.

[14] D. Palacios, M.D. Busto, S.M. Albillos, N. Ortega, Synthesis and oxidative stability of monoacylglycerols containing polyunsaturated fatty acids by enzymatic glycerolysis in a solvent-free system, LWT 154 (2022) 112600.

[15] X. Zou, K. Nadege, I. Ninette, S. Wen, S. Wu, X. Jiang, H. Zhang, Q. Jin, X. Wang, Preparation of docosahexaenoic acid-rich diacylglycerol-rich oil by lipase-catalyzed glycerolysis of microbial oil from schizochytrium sp. in a solvent-free system, J. Am. Oil Chem. Soc. 97 (3) (2020) 263–270.

[16] P.B.L. Fregolente, G.M.F. Pinto, M.R. Wolf-Maciel, R.M. Filho, Monoglyceride and diglyceride production through lipase-catalyzed glycerolysis and molecular distillation, Appl. Biochem. Biotechnol. 160 (2010) 1879–1887.

[17] J. Kaur, A.K. Sarma, M.K. Jha, P. Gera, Valorisation of crude glycerol to value-added products: perspectives of process technology, economics and environmental issues, Biotechnology Reports 27 (2020) e00487.

[18] M. Thompson, Handbook of Inductively Coupled Plasma Spectrometry, Springer Science & Business Media, 2012.

[19] C. Nazato, A.d.C. Romero, A.L. Abdalla, Determination of methanol residues in crude glycerol for animal feed by gas chromatography, Sci. Agric. 76 (2019) 527–531.

[20] R.K. Campos de Carvalho, F.d.S. Ortega, A.d.A. Morandim-Giannetti, Alkyd resin synthesis by enzymatic alcoholysis, Iran. Polym. J. (Engl. Ed.) 28 (9) (2019) 747–757.

[21] D. Firestone, W. Horwitz, IUPAC gas chromatographic method for determination of fatty acid composition: collaborative study, J. Assoc. Off. Anal. Chem. 62 (4) (2020) 709–721.

[22] J.B. Martin, The equilibrium between symmetrical and unsymmetrical monoglycerides and determination of total monoglycerides, J. Am. Chem. Soc. 75 (22) (1953) 5483–5486.

[23] P. Kalita, B. Basumatary, P. Saikia, B. Das, S. Basumatary, Biodiesel as renewable biofuel produced via enzyme-based catalyzed transesterification, Energy Nexus (2022) 100087.

[24] A. Chatzifragkou, S. Papanikolaou, Effect of impurities in biodiesel-derived waste glycerol on the performance and feasibility of biotechnological processes, Appl. Microbiol. Biotechnol. 95 (1) (2012) 13–27.

[25] E. Ike, The study of viscosity-temperature dependence and activation energy for palm oil and soybean oil, Global J. Pure Appl. Sci. 25 (2) (2019) 209–217.

[26] F. Gunstone, The Chemistry of Oils and Fats: Sources, Composition, Properties and Uses, John Wiley & Sons, 2009.

[27] G.P. McNeill, S. Shimizu, T. Yamane, High-yield enzymatic glycerolysis of fats and oils, J. Am. Oil Chem. Soc. 68 (1991) 1–5.

[28] G.P. McNeill, T. Yamane, Further improvements in the yield of monoglycerides during enzymatic glycerolysis of fats and oils, J. Am. Oil Chem. Soc. 68 (1991) 6–10.

[29] A. Alfutimie, N. Al-Janabi, R. Curtis, G.J.T. Tiddy, The Effect of monoglycerides on the crystallisation of triglyceride, Colloids Surf. A Physicochem. Eng. Asp. 494 (2016) 170–179.

[30] H. Noureddini, S. Harmeier, Enzymatic glycerolysis of soybean oil, J. Am. Oil Chem. Soc. 75 (10) (1998) 1359–1365.

[31] P.B.L. Fregolente, L.V. Fregolente, G.M.F. Pinto, B.C. Batistella, M.R. Wolf-Maciel, R.M. Filho, Monoglycerides and diglycerides synthesis in a solvent-free system by lipase-catalyzed glycerolysis. Biotechnology for Fuels and Chemicals: Proceedings of the Twenty-Ninth Symposium on Biotechnology for Fuels and Chemicals Held April 29–May 2, 2007, Springer, Denver, Colorado, 2008, pp. 285–292.

[32] H. Wang, H. Li, C.K. Lee, N. Suliani, M. Nanyan, G.S. Tay, A systematic review on utilization of biodiesel-derived crude glycerol in sustainable polymers preparation, Int. J. Biol. Macromol. (2024) 129536.

[33] T. Yang, M. Rebsdorf, U. Engelrud, X. Xu, Enzymatic production of monoacylglycerols containing polyunsaturated fatty acids through an efficient glycerolysis system, J. Agric. Food Chem. 53 (5) (2005) 1475–1481.

[34] A. Valério, R.L. Krüger, J. Ninow, F.C. Corazza, D. de Oliveira, J.V. Oliveira, M.L. Corazza, Kinetics of solvent-free lipase-catalyzed glycerolysis of olive oil in surfactant system, J. Agric. Food Chem. 57 (18) (2009) 8350–8356.

[35] T. Yang, M. Rebsdorf, U. Engelrud, X. Xu, Monoacylglycerol synthesis via enzymatic glycerolysis using a simple and efficient reaction system, J. Food Lipids 12 (4) (2005) 299–312.

[36] H. Yang, Y. Mu, H. Chen, C. Su, T. Yang, Z. Xiu, Sn-1,3-specific interesterification of soybean oil with medium-chain triacylglycerol catalyzed by Lipozyme TL IM, Chin. J. Chem. Eng. 22 (9) (2014) 1016–1020.

[37] E. Hernández-Martín, C. Otero, Different enzyme requirements for the synthesis of biodiesel: Novozym® 435 and Lipozyme® TL IM, Bioresour. Technol. 99 (2) (2008) 277–286.

[38] C. Ortiz, M.L. Ferreira, O. Barbosa, J.C. dos Santos, R.C. Rodrigues, Á. Berenguer-Murcia, L.E. Briand, R. Fernandez-Lafuente, Novozym 435: the "perfect" lipase immobilized biocatalyst? Catal. Sci. Technol. 9 (10) (2019) 2380–2420.

[39] N. Zhang, X. Yang, J. Fu, Q. Chen, Z. Song, Y. Wang, Production of diacylglycerol-enriched oil by glycerolysis of soybean oil using a bubble column reactor in a solvent-free system, J. Oleo Sci. 65 (3) (2016) 207–216.

[40] Á.G. Solaesa, M.T. Sanz, R. Melgosa, S. Beltrán, Substrates emulsification process to improve lipase-catalyzed sardine oil glycerolysis in different systems. Evaluation of lipid oxidation of the reaction products, Food Res. Int. 100 (2017) 572–578.

[41] A. Coteron, M. Martinez, J. Aracil, Reactions of olive oil and glycerol over immobilized lipases, J. Am. Oil Chem. Soc. 75 (5) (1998) 657–660.

[42] X. Diao, H. Guan, B. Kong, X. Zhao, Preparation of diacylglycerol from lard by enzymatic glycerolysis and its compositional characteristics, Korean Journal for Food Science of Animal Resources 37 (6) (2017) 813.

[43] T.S.Y. Choong, C.M. Yeoh, E.-T. Phuah, W.-L. Siew, Y.-Y. Lee, T.-K. Tang, L. Chuah Abdullah, Kinetic study of lipase-catalyzed glycerolysis of palm olein using Lipozyme TLIM in solvent-free system, PLoS One 13 (2) (2018) e0192375.

[44] M.K. Naik, S. Naik, S. Mohanty, Enzymatic glycerolysis for conversion of sunflower oil to food based emulsifiers, Catal. Today 237 (2014) 145–149.

[45] N. Zhong, L. Li, X. Xu, L.Z. Cheong, Z. Xu, B. Li, High yield of monoacylglycerols production through low-temperature chemical and enzymatic glycerolysis, Eur. J. Lipid Sci. Technol. 115 (6) (2013) 684–690.

[46] A.L. Bose, D. Bhattacharjee, D. Goswami, Process parameters influence product yield and kinetic parameters in lipase catalysis, ChemBioEng Rev. 11 (2) (2024) 178–196.

[47] A.G. Ferreira, A.P. Egas, I.M. Fonseca, A.C. Costa, D.C. Abreu, L.Q. Lobo, The viscosity of glycerol, J. Chem. Therm. 113 (2017) 162–182.

[48] A.R. Ismail, H. Kashtoh, K.-H. Baek, Temperature-resistant and solvent-tolerant lipases as industrial biocatalysts: biotechnological approaches and applications, Int. J. Biol. Macromol. 187 (2021) 127–142.

[49] A.M. Testera, M. Santos, A. Girotti, F.J. Arias, J.M. Báñez, M. Alonso, J.C. Rodríguez-Cabello, A novel lipase-catalyzed method for preparing ELR-based bioconjugates, Int. J. Biol. Macromol. 121 (2019) 752–759.

[50] M. Tuter, L. Dandik, H.A.e. Aksoy, Solvent-free glycerolysis of sunflower oil and anchovy oil catalyzed by a 1, 3-specific lipase, Biotechnol. Lett. 20 (1998) 291–294.

[51] S.A. Korma, X. Zou, A.H. Ali, S.M. Abed, Q. Jin, X. Wang, Preparation of structured lipids enriched with medium- and long-chain triacylglycerols by enzymatic interesterification for infant formula, Food Bioprod. Process. 107 (2018) 121–130.

[52] Y. Zhang, X. Wang, D. Xie, S. Zou, Q. Jin, X. Wang, Synthesis and concentration of 2-monoacylglycerols rich in polyunsaturated fatty acids, Food Chem. 250 (2018) 60–66.

[53] M.K. Naik, S.N. Naik, S. Mohanty, Enzymatic glycerolysis for conversion of sunflower oil to food based emulsifiers, Catal. Today 237 (2014) 145–149.

[54] A. Valerio, R.L. Kruger, J. Ninow, F.C. Corazza, D. de Oliveira, J.V. Oliveira, M.L. Corazza, Kinetics of solvent-free lipase-catalyzed glycerolysis of olive oil in surfactant system, J. Agric. Food Chem. 57 (18) (2009) 8350–8356.

[55] L.-Z. Cheong, C.-P. Tan, K. Long, M.S.A. Yusoff, N. Arifin, S.-K. Lo, O.-M. Lai, Production of a diacylglycerol-enriched palm olein using lipase-catalyzed partial hydrolysis: optimization using response surface methodology, Food Chem. 105 (4) (2007) 1614–1622.

[56] J.B. Segur, H.E. Oberstar, Viscosity of glycerol and its aqueous solutions, Ind. Eng. Chem. 43 (9) (1951) 2117–2120.

[57] A.L. Paiva, V.M. Balcao, F.X. Malcata, Kinetics and mechanisms of reactions catalyzed by immobilized lipases, Enzym. Microb. Technol. 27 (3–5) (2000) 187–204.

[58] H. Wang, H. Li, C.K. Lee, N.S. Mat Nanyan, G.S. Tay, Recent advances in the enzymatic synthesis of polyester, Polymers 14 (23) (2022) 5059.

[59] J. Zheng, Y. Liang, J. Li, S. Lin, Q. Zhang, K. Zuo, N. Zhong, X. Xu, Enzymatic preparation of mono- and diacylglycerols: a review, Grain & Oil Science and Technology 6 (4) (2023) 185–205.

[60] T. Attarbachi, M.D. Kingsley, V. Spallina, New trends on crude glycerol purification: a review, Fuel 340 (2023) 127485.

[61] H. Wang, H. Li, C.K. Lee, N.S. Mat Nanyan, G.S. Tay, A systematic review on utilization of biodiesel-derived crude glycerol in sustainable polymers preparation, Int. J. Biol. Macromol. 261 (2024) 129536.