Kinetic study of liquid lipase-catalyzed glycerolysis from olive oil using Lipozyme TL 100L

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Abstract

Monoacylglycerol (MAG) and diacylglycerol (DAG) are two natural components found in most edible oils and fats. Conventional synthesis of MAG and DAG is usually conducted by glycerolysis of triacylglycerol (TAG) at high temperatures (above 200 °C) in the presence of an alkaline catalyst. In this work, the synthesis of MAG and DAG using enzymatic glycerolysis of olive oil was investigated using Tween 80 as surfactant, *n*-butanol as co-surfactant and the novel lipase in liquid formulation Lipozyme TL 100L as catalyst. Experimental design was used to evaluate the effect of enzyme load and reaction temperature on the feedstock conversion. Enzyme load and system temperature were significant variables in the statistical design and the best condition was found at 35 °C, 7.5 vol% of Lipozyme TL 100 and 2:1 molar ratio (glycerolysis:oil) with conversion of TAG at approximately 98 % after 2 h of process. A mathematical model based on the Ping-Pong Bi-Bi mechanism was used to describe the reaction kinetics. The model adequately described the behavior of the system and can be a useful tool for the design of reactors in larger scales.

Keywords

Monoacylglycerol; diacylglycerol Lipase; Surfactant; Statistical Design; Kinetic Modeling.

Introduction

Monoacylglycerols (MAGs) are esters formed by fatty acids/glycerol largely utilized on industrial demands due its stabilizing, emollient and binding properties. This compound was approved by the Food and Drug Administration (FDA, United States) for use as emulsifiers in food and pharmaceuticals applications for not presenting collateral effects when ingested [1]. Within this same reasoning, diacylglycerols (DAGs) act in the treatment of cholesterol, prevention of obesity and reduction of body fat accumulation, bringing beneficial for reducing diseases related to such health problems.

The synthesis of MAG and DAG is performed in distinct ways. The conventional technique applied industrially occurs by triacylglycerols (TAGs) glycerolysis, usually at high temperatures (above 200 ºC) in the presence of chemical (alkaline) catalyst. Under these conditions, a mixture composed around of 50 wt% of MAG, 40 wt% of DAG and 10 wt% of TAG is obtained [2, 3]. On the other hand, although it is still a scarcely explored topic, researches published recently highlight the potential of the use of lipases as biocatalysts on glycerolysis due to the reaction works at mild reaction conditions, implying a low energy demand of the process and by high selectivity of the enzymes, allowing a higher amount of product synthesis [4, 5].

In the enzymatic glycerolysis, an important point that should be mentioned is the presence of glycerol in the system. Frequently, the glycerol can bound with the enzyme and forming an inactive complex with the glycerol reducing the reaction efficiency once the glycerol is a competitive inhibitor [6]. Thus, ensure the system homogeneity is a crucial factor during the lipase-catalyzed glycerolysis. To aid this point, the use of a surfactant may promote higher homogeneity for the system oil-glycerol-enzyme, increasing the interfacial area, improving the immiscibility between the substrates, providing higher reaction rates and enhancing the biocatalyst efficiency,

63 mainly for lipases which are characterized as acting at the interface [4, 5, 7, 8]. There are two
64 requirements for enzyme glycerolysis to occur: firstly, the oil-glycerol interface is formed; and then
65 the enzyme is adsorbed in that system. Thus, the higher the interface, the higher the amount of
66 enzyme adsorbed, which implies in lower activation energies and, consequently, higher reaction
67 rates [9]. For this, the area of contact between enzymes and substrate is increased with the use of
68 micelles. The micelles provide higher glycerol miscibility on apolar solvents, through the
69 physicochemical characteristics of the micellar system and the interaction with the surface area
70 where TAG break down and form MAG and DAG [10].

71 The majority of studies that address enzymatic glycerolysis to obtain MAG and DAG use
72 immobilized lipases as reaction catalyst [11, 12]. The use of lipases in liquid formulations, as the
73 novel Lipozyme TL 100L recently launched by Novozymes A/S, can be observed in scarce
74 researches [13–15]. These papers evaluated only factors of process such as molar ratio of the
75 system, the source and type of the enzyme activation. Furthermore, kinetic parameters estimation
76 for this process are rare in the literature. However, mathematical modeling and knowledge of
77 reaction kinetics are fundamental in reactors design. After all, modeling is used for the processes
78 analysis to define strategies of operation, optimization and system control. Therefore, it is
79 necessary to use representative, fast computing models with the good mapping of the variables and
80 cause-effect in the processes [16]. Consequently, it is required to know the reaction kinetics to be
81 able to reconcile the complexity of the chosen model and to better represent the reactor with the
82 variables available and proposed [17, 18].

83 This work presents experimental results of the kinetics of enzymatic glycerolysis of olive
84 oil in a batch reactor, using Tween 80 as surfactant, *n*-butanol as co-surfactant and the liquid lipase
85 Lipozyme TL 100L, to evaluate the effect of temperature and enzyme concentration in the
86 feedstock conversion. Moreover, a complete mathematical model considering the glycerolysis and

hydrolysis of the raw material to describe the process is also presented.

Materials and Methods

Materials

Refined olive oil (Oliva de Los Andes Premium) with 0.2 wt% of acidity was purchased from a local market and used as feedstock. Free liquid lipase from genetically modified *Thermomyces lanuginosus* microorganism (commercially named as Lipozyme TL 100L) with a nominal enzymatic activity of 100 $LCLU \cdot g^{-1}$ and kindly provided by Novozymes Latin America LTDA (Araucária, Brazil) was used as biocatalyst. Glycerol and *n*-butanol (99.5 % purity, Alphatec, São Paulo, Brazil), Tween 80 PS surfactant (analytical grade, Dynamica, Indaiatuba, Brazil) were the chemicals used in the assays. Standards used in the HPLC analysis (Sigma-Aldrich, São Paulo, Brazil): linoleic acid (97 % purity), oleic acid (> 99 % purity), monopalmitic acid (> 99 % purity), dilinoleic acid (98 % purity), glycerol trioleic (> 99 % purity) and trilinoleic glycerol (98 % purity). Acetonitrile (99.9 % purity, Merck, São Paulo, Brazil) and acetone (99.8 % Merck, São Paulo, Brazil) were used in the chromatographic analysis.

Formation of Microemulsions

Preliminary tests were performed to determine the volumetric ratios of feedstock, water and surfactant, where the follows glycerolysis to oil molar ratio was considered: 1:1; 2:1; 3:1; 1:2 and 1:3, respecting a phase equilibrium diagram. After mixing the components of the microemulsion, it was subjected to constant stirring for 1.5 minutes in a Vortex homogenizer, and then stored at room temperature for 48 hours, according to the procedure described in Pescara [19].

Enzymatic Glycerolysis and Factorial Design

A Central Composite Rotatable Design (CCRD) plot with triplicate at the central point was used to determine the optimum conditions of MAG and DAG synthesis. Data analysis was performed using the software StatSoft Statistica, version 11.0. The effects of temperature and volumetric load of lipase on MAG and DAG yield were evaluated. The reactions were performed in a jacketed glass reactor, under constant stirring by mechanical stirrer (600 rpm) and temperature control. With the reaction parameters set, the enzyme was added and the enzymatic glycerolysis started for a period of 2 hours in pH 7.0. The values of the real and coded variables used in the statistical design are presented in Table 1. After the reaction, the enzyme separation was done by a system composed by a syringe filter, which was transferred to amber flasks brought into a Styrofoam container and then stored into the refrigerator. The filtration process was carried out to purify the sample. After separation, TAG, DAG, and MAG contents were determined by high-performance liquid chromatography.

Table 1. CCRD matrix, with real and coded values used in the reaction of glycerolysis catalyzed by Lipozyme TL 100L and conversions obtained to the TAG.

Asssay	Temperature (°C)	Enzyme Load (vol%)	Conversion (%)
1	30 (-1)	5.0 (-1)	66.61
2	40 (+1)	5.0 (-1)	70.24
3	30 (-1)	10 (+1)	82.76
4	40 (+1)	10 (+1)	86.72
5	28 (-1.41)	7.5 (0)	72.79
6	42 (+1.41)	7.5 (0)	58.28
7	35 (0)	3.97 (-1.41)	67.73
8	35 (0)	11.03 (+1.41)	97.11
9	35 (0)	7.5 (0)	98.12

10	35 (0)	7.5 (0)	98.82
11	35 (0)	7.5 (0)	98.41

126

127 **Sample Analysis**

128 High-performance liquid chromatography (HPLC, Thermo Scientific Ultimate 3000) was
129 used in the quantification of TAG, DAG MAG and FFA according procedure described by Lima
130 et al. [20]. Phenomenex C18 column of 5 μm and 100 Å (250 x 4.6 mm, model 00G-4252-E0),
131 flow rate of 1.0 $mL \cdot min^{-1}$, column temperature of 35 °C and detector temperature of 40 °C were
132 used in the analysis. The mobile phase was acetonitrile:acetone (1:1, $v \cdot v^{-1}$) and the injection volume
133 was 20 μL. The content of reaction products was expressed in terms of the whole amount of MAG,
134 DAG, TAG and FFA as weight percent of the total sample in surfactant-free basis.

135

136 **Kinetic Modeling**

137 The kinetics were determined in three different conditions, according preliminary tests
138 performed in terms of TAG conversion and presented on Table 2, where the effect of the water
139 content in the reaction was evaluated and the formation of MAG, DAG and FFA was monitored.
140 The system temperature, concentration of olive oil and lipase load was fixed for all the experiments
141 at 35 °C, 10 vol% and 7.5 vol% respectively. These amounts utilized were determined through tests
142 of formation and stabilization of the microemulsions. The amount of enzyme and temperature were
143 used based on the results obtained via statistical design.

144

145

146

147 **Table 2.** Reaction conditions applied in the kinetics analysis of the system.

148

Asssay	Glycerol (vol%)	Water (vol%)
1	7.40	5.0
2	7.69	10.0
3	8.00	15.0

149

150 The mathematical approach to perform the modeling presented in this research considered
151 some factors suggested by Choong et al. [21]. These authors tested three mathematical models:
152 ternary model, Ping-Pong Bi-Bi and Ping-Pong-Bi complex for the glycerolysis of palm oil using
153 the enzyme Lipozyme TL IM in a solvent-free system. However, for all models, the hydrolysis
154 reactions were not considered, suggesting that the reactions were conducted under "micro-
155 aqueous" conditions. In this sense, this work presented the Ping-Pong Bi-Bi mathematical model
156 described by the set of equations – Eq. (1) to Eq (6), and also the hydrolysis reactions, characterized
157 by equations Eq (7) to Eq (10).

158 In the kinetic mathematical modeling of the glycerolysis reaction of olive oil, the following
159 elemental reactions were considered:

160

$$TAG + E\ K_1 \rightarrow\ K_{-1} \leftarrow\ E - TAG \quad (1)$$

$$E - TAG\ K_2 \rightarrow\ DAG + E - FFA \quad (2)$$

$$E - FFA\ + GK_3 \rightarrow\ K_{-3} \leftarrow\ E - FFA - G \quad (3)$$

$$E - FFA - GK_4 \rightarrow\ MAG + E \quad (4)$$

$$DAG + E\ K_5 \rightarrow\ K_{-5} \leftarrow\ E - DAG \quad (5)$$

$$E - DAGK_6 \rightarrow\ MAG + E - FFA \quad (6)$$

$$E - TAG\ + WK_7 \rightarrow\ K_{-7} \leftarrow\ DAG + FFA + E \quad (7)$$

$$E - DAG + WK_8 \rightarrow K_{-8} \leftarrow \quad MAG + E + FFA \tag{8}$$

$$MAG + E\, K_9 \rightarrow K_{-9} \leftarrow \quad MAG - E \tag{9}$$

$$E - MAGK_{10} \rightarrow FFA + E + G \tag{10}$$

161

162 From the elementary equations, the following kinetic equations can be written:

163

$$r_1 = -K_1\, C_{TAG}(t)\, C_E(t) + K_{-1}\, C_{TAG-E}(t) \tag{11}$$

$$r_2 = -K_2\, C_{TAG-E}(t) \tag{12}$$

$$r_3 = -K_3\, C_{E-FFA}\,(t)\, C_G(t) + K_{-3}\, C_{E-FFA-G}(t) \tag{13}$$

$$r_4 = -K_4\, C_{E-FFA-G}(t) \tag{14}$$

$$r_5 = -K_5\, C_{DAG}(t) C_E(t) + K_{-5}\, C_{E-DAG}(t) \tag{15}$$

$$r_6 = -K_6\, C_{E-DAG}(t) \tag{16}$$

$$r_7 = -K_7\, C_{E-TAG}\, C_w(t) + K_{-7}\, C_{DAG}(t) C_{FFA}(t) C_E(t) \tag{17}$$

$$r_8 = -K_8\, C_{E-DAG}\, C_w(t) + K_{-7}\, C_{MAG}(t) C_{FFA}(t) C_E(t) \tag{18}$$

$$r_9 = -K_9\, C_{MAG}(t) C_E(t) + K_{-9} C_{E-MAG}(t) \tag{19}$$

$$r_{10} = -K_{10}\, C_{E-MAG}(t) C_E(t) + K_{-10}\, C_{FFA}(t) C_E(t) C_G(t) \tag{20}$$

164

165 Applying the molar balance for each species and considering the productivity based on
166 stoichiometry, was obtained the enzymatic kinetics model for the several species involved in the
167 mechanism of the glycerolysis reaction, described by the Equations (21) to (31):

168

$$\frac{dC_{TAG}}{dt} = r_1 \tag{21}$$

$$\frac{dC_{DAG}}{dt} = -r_2 + r_5 - r_7 \tag{22}$$

$$\frac{dC_{MAG}}{dt} = -r_4 - r_6 - r_8 + r_9 \quad (23)$$

$$\frac{dC_G}{dt} = -r_3 + r_{10} \quad (24)$$

$$\frac{dC_E}{dt} = r_1 - r_4 + r_5 - r_7 - r_8 + r_9 - r_{10} \quad (25)$$

$$\frac{dC_{E-TAG}}{dt} = -r_1 + r_2 + r_7 \quad (26)$$

$$\frac{dC_{E-FFA}}{dt} = -r_2 + r_3 - r_6 \quad (27)$$

$$\frac{dC_{E-FFA-G}}{dt} = -r_3 + r_4 \quad (28)$$

$$\frac{dC_W}{dt} = r_7 + r_8 \quad (29)$$

$$\frac{dC_{FFA}}{dt} = -r_7 - r_8 - r_{10} \quad (30)$$

$$\frac{dC_{MG-E}}{dt} = -r_9 + r_{10} \quad (31)$$

The balance for free enzyme concentration was based on enzyme-substrate complex, where the total enzyme concentration remains constant, represented by the Equation (32):

$$C_E(t=0) = C_E + C_{E-TAG} + C_{E-DAG} - C_{E-MAG} + C_{E-FFA} + C_{E-FFA-G} \quad (32)$$

The initial conditions are given by Equations (33) to (44). All concentration values are expressed in mol·kg^{-1} and were calculated for each component based on the average molecular mass of olive oil, the mass of each element and total mass of oil and water. The enzyme concentration are expressed in g.L^{-1}.

$$C_{TAG}(0) = {C_{TAG,0}}^{exp} \quad (33)$$

$$C_{DAG}(0) = 0 \quad (34)$$

$$C_{MAG}(0) = 0 \quad (35)$$

$$C_{G}(0) = 0 \quad (36)$$

$$C_{E}(0) = {C_{E,0}}^{exp} \quad (37)$$

$$C_{E-TAG}(0) = 0 \quad (38)$$

$$C_{E-FFA}(0) = 0 \quad (39)$$

$$C_{E-FFA-G}(0) = 0 \quad (40)$$

$$C_{W}(0) = {C_{W,0}}^{exp} \quad (41)$$

$$C_{FFA}(0) = 0 \quad (42)$$

$$C_{E-MAG}(0) = 0 \quad (43)$$

$$C_{E-DAG}(0) = 0 \quad (44)$$

Parameter Estimation

The mathematical model parameters (K_1, K_{-1}, K_2, K_3, K_{-3}, K_4, K_5, K_{-5}, K_6, K_7, K_{-7}, K_8, K_{-8}, K_9, K_{-9}, K_{10} e K_{-10}) were estimated from the kinetics of the enzymatic hydrolysis of olive oil (TAG, DAG, MAG and FFA) and minimization of the objective function represented by Equation (32). The simplex Dowhill optimization method developed by Nelder and Mead [22] was used in the pursuit of the objective function minimum.

$$ob = \sum_{j=1}^{nc} \sum_{i=1}^{nd} \left(C_{ji}^{exp} - C_{ji}^{mod} \right)^2 \quad (45)$$

where *nc* is the number of components (TAG, DAG, MAG and FFA); *nd* is the number of experimental data; ${C_{ji}}^{exp}$ is the experimental concentration of component *j* and; ${C_{ji}}^{mod}$ is the

component concentration *j* calculated by the model;

Thus, from the proposed model that consists of the system of Equations (21) to (32) and the particular initial conditions, Equations from (33) to (44) were used to model the behavior of the reaction system and to determine the kinetic parameters of the hydrolysis reactions of olive oil. For the solution of the mathematical model, the numerical method of Rosenbrock [23] was used, using the software Maple®, version 13 (Waterloo Maple Inc., Canada).

Results and Discussion

Central Composite Rotatable Design (CCRD)

The results for feedstock conversion of the experimental design are presented in Table 1. The conversion of TAG was in the range of 58 % to 98 %. Table 3 presents the results of ANOVA and Figure 1 the Pareto diagram.

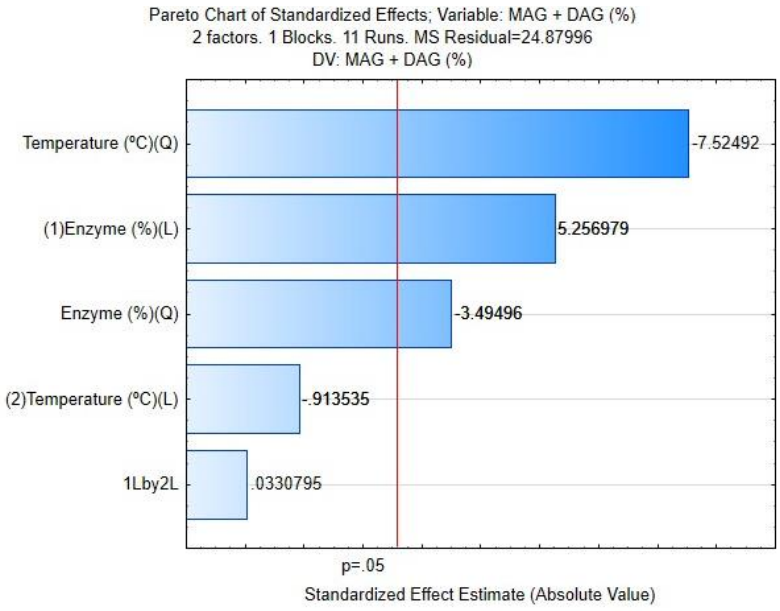

Figure 1. Pareto diagram obtained to the experiments performed.

205 **Table 3.** Analysis of Variance (ANOVA) to the CCRD design.

206

Factor	Sum of Squares	Degree of Freedom	Mean Square	$F_{Calculated}$	*p*-value
(1) Enzyme Load (vol%) (L)	687.58	1	687.58	27.63	0.0033[a]
Enzyme Load (vol%) (Q)	303.90	1	303.90	12.21	0.0174[a]
(2) Temperature (°C) (L)	20.76	1	20.76	0.83	0.4029[b]
Temperature (°C) (Q)	1,408.81	1	1,408.81	56.62	0.0007[a]
1L by 2L	0.03	1	0.03	< 0.01	0.9749[b]
Error	124.40	5	24.88		
Total SS	2,287.70	10			

207 [a] Significant at "Prob > F" less than 0.05;

208 [b] Insignificant at "Prob > F" more than 0.05.

209

Significant variables presented $p < 0.05$. Coefficients with a positive sign indicate a synergic effect whereas negative coefficients indicate an antagonistic effect in the formation of MAG and DAG [24]. Moreover, the results presented show that any variation in the system significantly changes the conversion, statistically stating. This can be explained because an emulsion composes the system, and this emulsion needs balance and stability, which can be affected depending on the compound that is added to the medium. In turn, when using a lipase to promote the catalysis, a special attention must be given to parameter "reaction temperature", since the enzymes are a temperature sensitive protein. This implies that any value below its optimum temperature, the enzymatic activity could be considerably reduced and, mainly, above the optimal temperature, can occurs the protein denaturation and activation loss, which is irreversible, drastically affecting the process yield. Therefore, the knowledge of the "ideal" process conditions is fundamental. Since the process variables are kept in this "ideal" range so that the synthesis of MAG and DAG is maximum, increasing the reaction efficiency [25].

The results presented in the Table 1 and 3, and the Pareto diagram (Figure 1), shows an interactive effect between the variables "enzyme load" and "system temperature" and an independent analysis of the parameters would not be appropriate. Therefore, a contour surface between the parameters considered is necessary to find the region with the best response and thus determine the optimum conditions of the system, where the formation of MAG and DAG is maximum. These results can be observed in the Figure 2 in terms of volumetric concentration of enzyme. The data presented shows that the region of most significant conversion of the triglycerides that composed the feedstock used is close to the central point of the statistical design. In other words, the temperature is around 35 °C and the enzyme load between 7.5 to 9 vol%. According previously discussed, temperatures above 40 °C hampered the raw material conversion since the catalytic capacity of the lipase was negatively affected due these high temperatures. On

234 the other hand, temperatures below 30 °C were not sufficient to activate the catalyst, also resulting
235 in low yields. Lower values of temperatures tend to cause a decrease of enzymatic activity, whereas
236 larger values can cause potential protein denaturation and in both, reduction of the activity occurs
237 [26, 27]. Regarding the biocatalyst load, as expected, an increase in the lipase concentration used
238 in the medium returned an increase in the observed conversions. However, it is worth noting that
239 there was little difference in the TAG conversions when the lipase load increased from 7.5 vol%
240 to 10 vol%, making it more economically viable to adopt the value of 7.5 vol% of Lipozyme TL
241 100 as ideal. Is not difficult observe in situations when an excess of enzyme is used a formation of
242 agglomerates that resulting in a mass transfer resistance and consequently decreasing the
243 conversion. Thus, the best conditions obtained from the results presented is 35 °C, 7.5 vol% of
244 Lipozyme TL 100, a molar ratio of 2:1 (glycerolysis:oil) at 600 rpm rate and 2 h of reaction,
245 resulting in an average conversion of 98.5 %.

246 The results found for the best conditions are in agreement with the study published by
247 Raizer [28]. This study synthesized DAG by enzymatic glycerolysis from sunflower oil by
248 ultrasound assistance and used a CCRD with temperature and amount of enzyme as independent
249 variables. Through ANOVA and Pareto Diagram, the author concluded that the most significant
250 variable was the enzyme load.

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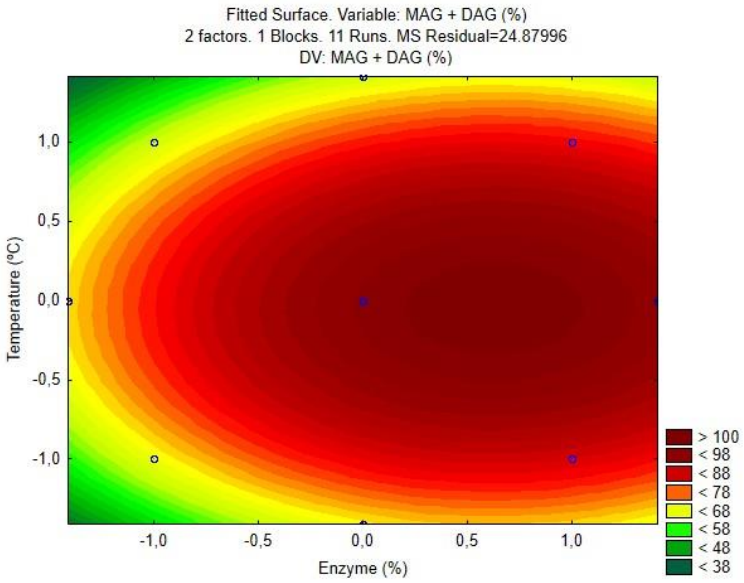

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253 **Figure 2.** Contour surface for TAG conversion varying temperature and biocatalyst load.

254

255 **Mathematical Modeling**

256 The kinetic constants were initially estimated using 7.5 vol% of the Lipozyme TL 100
257 enzyme and assuming the hypothesis of having a reaction coefficient as an independent factor in
258 the medium. Such parameter is important since the use of excess enzyme may result in enzyme
259 aggregation, which ends up exposing the active site of the enzyme to substrate molecules and thus,
260 the model would not be efficient and would have to reformulate the conditions of analysis. The
261 values of the estimated parameters obtained to the model are presented in Table 4. The results of
262 the simulations are presented in Figures 3 to 5. It is possible to observe that the proposed model
263 appropriately represented the kinetics of glycerolysis of olive oil.

264

265 **Table 4.** Estimated kinetic reaction constants.

266

Parameter	K_1 ($L \cdot mol^{-1} \cdot min^{-1}$)	K_{-1} (min^{-1})	K_2 (min^{-1})	K_3 ($L \cdot mol^{-1} \cdot min^{-1}$)	K_{-3} (min^{-1})	K_4 (min^{-1})
Estimated Value	13.76	181.99	53.27	$1.108 \cdot 10^4$	$8.179 \cdot 10^3$	$2.725 \cdot 10^3$
Parameter	K_5 ($L \cdot mol^{-1} \cdot min^{-1}$)	K_{-5} (min^{-1})	K_6 (min^{-1})	K_7 ($L \cdot mol^{-1} \cdot min^{-1}$)	K_{-7} ($L^2 \cdot g^{-1} \cdot mol^{-1} \cdot min^{-1}$)	K_8 ($L \cdot mol^{-1} \cdot min^{-1}$)
Estimated Value	3.47	90.21	7.33	37.44	1.83	1.90
Parameter	K_{-8} ($L^2 \cdot g^{-1} \cdot mol^{-1} \cdot min^{-1}$)	K_9 ($L \cdot mol^{-1} \cdot min^{-1}$)	K_{-9} ($L^2 \cdot g^{-1} mol^{-1} \cdot min^{-1}$)	K_{10} ($L \cdot mol^{-}1 \cdot min^{-1}$)	K_{-10} ($L^2 \cdot g^{-1} \cdot mol^{-1} \cdot min^{-1}$)	
Estimated Value	0.50	23.49	145.92	65.26	7.04	

267

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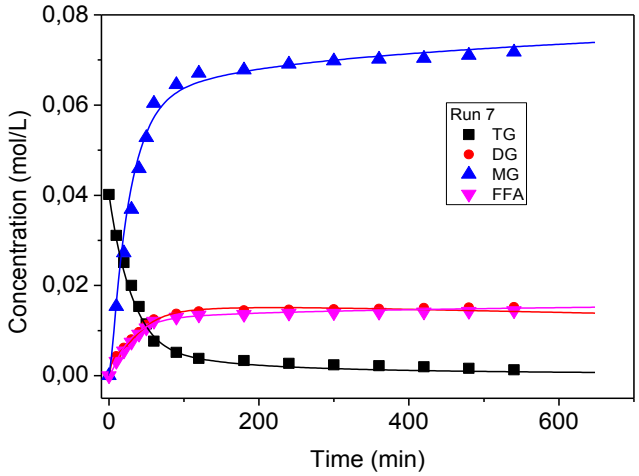

Figure 3. Kinetics of glycerolysis of olive oil. Reaction conditions: 35 °C, 7.4 % of glycerol, 10 vol% of olive oil, 5 vol% of water and 7.5vol % enzyme.

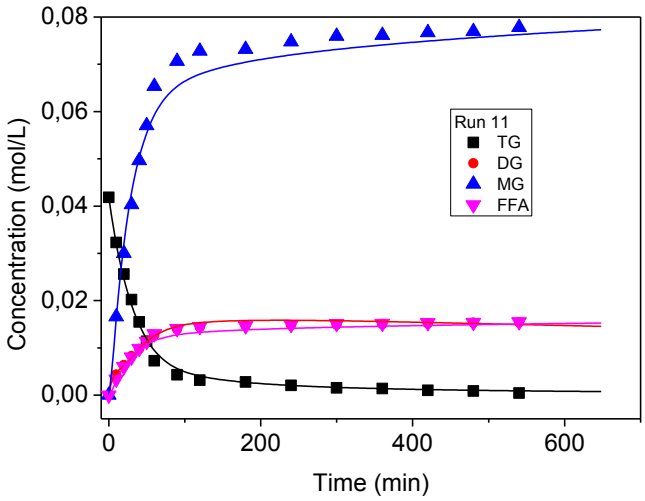

Figure 4. Kinetics of glycerolysis of olive oil. Reaction conditions: 35 °C, 7.69 % of glycerol, 10 vol% of olive oil, 10 vol% of water and 7.5 vol% of enzyme.

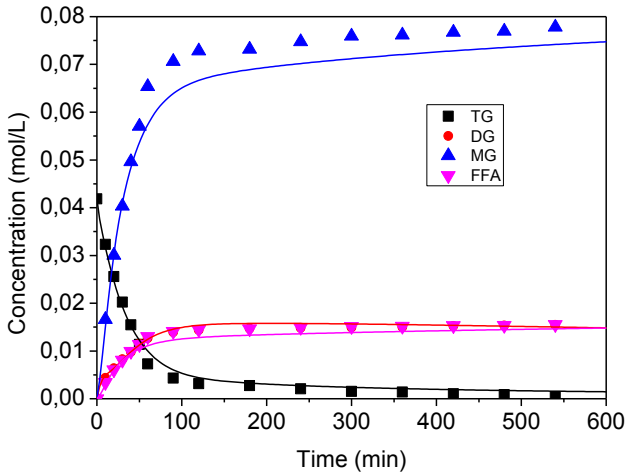

Figure 5. Kinetics of glycerolysis of olive oil. Reaction conditions: 35 °C, 8.0 % of glycerol, 10 vol% of olive oil, 15 of vol% of water and 7.5 vol % of enzyme.

Experimental kinetics data show that the concentration of TAG had a significant reduction between 0 and 120 minutes, indicating that the amount of enzyme used was adequate to promote the reaction. In the same way, it is verified that the higher production of MAG and DAG was in this period of time since such products are formed from the decomposition of the TAG. This was also observed by Corzo-Martínez et al. [29] and Fiametti [30] that the higher formation of MAG and DAG occurred within the first 120 minutes of reaction. It was also noted that the formation of MAG was much higher than that of DAG, but both stabilized at similar times, which indicates another sign that the amount of enzyme was sufficient in the process.

This model of curves is characteristic in TAG hydrolysis reactions, even with different matrices. In the work of Solaesa et al. [25], the authors performed enzymatic glycerolysis tests on several systems with sardine oil as a source of TAG. As such, they analyzed levels of MAG and DAG in micellar systems and catalyzed by the enzyme Lipozyme 435. Comparing all the kinetic curves of the TAG, MAG, DAG and FFA compounds, it is seen that there is the same pattern of

294 formation, with more considerable amounts of MAG formation, followed by DAG and, finally,
295 FFA.

296 When analyzing the water fraction in the reaction medium, it did not present significant
297 differences, since the limiting factors for the process were the amount of enzyme and temperature
298 of the system. According studies conducted by Irimescu et. al [31] and Yang et. al [32]
299 microemulsions with several enzymes were tested, among them lipases, immobilized or not, and
300 achieved significant TAG conversion rates, and the amount of water was also crucial for the
301 stabilization of the system micellar. On the other hand, in the work of Moya-Ramírez et al. [33],
302 the authors obtained MAG and DAG from used frying oil and the reaction of glycerolysis with the
303 use of several enzymes was strongly dependent on the amount of water inside the micellar system
304 and the conditions with higher enzyme concentrations, better results were obtained. However, the
305 conversion range of triglycerides was in the range of 30 % inferior, relatively low when compared
306 to the present study. This may have occurred due to the raw material used by the authors (used
307 frying oil) contains several impurities and contaminants due to the breakage and oxidation that the
308 oil had undergone.

309 Another critical parameter in the glycerolysis reaction is the formation of a stable micellar
310 system. The high conversions of consumption of triglycerides show that this happened in the cases
311 studied. All the results obtained can be tested, for example, in the automotive lubricant industries,
312 as an alternative emulsifier between base oil and performance additives.

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Conclusions

In this work, the enzymatic glycerolysis reaction of olive oil was investigated using glycerolysis catalyzed by the commercial liquid enzyme Lypozyme TL 100L. The results of the experimental design showed that the amount of enzyme used and the temperature of the system were significant variables in the conversion of triglyceride. The experimental condition of 35 °C, 7.5 vol% of Lipozyme TL 100 enzyme, 2:1 molar ratio (glycerolysis:oil) and reaction time of 2 hours was the highest conversion of triglycerides (98.45 %). The mathematical model that considers the reaction mechanism of Ping Pong Bi Bi type and hydrolysis reactions proved to be efficient to describe reaction kinetics and can be an essential tool for reactor projects that operate at larger scales.

Declarations

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

- Consent to Participate: All authors have consented to participate in the paper.

- Consent to Publish: All authors have consented to publish the paper.

- Authors Contributions:

George F. Finco: experimental data collection

Karina G. Fiametti: laboratory assays execution

Edson A. da Silva: experimental design and modeling

Fernando Palú: experimental data execution

João H. C. Wancura: manuscript writing and discussion

Alexsandra Valério: research planning

J. Vladimir Oliveira: overall research planning, writing the manuscript

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List of Figure Captions

Figure 1. Pareto diagram obtained to the experiments performed.

Figure 2. Contour surface for TAG conversion varying temperature and biocatalyst load.

Figure 3. Kinetics of glycerolysis of olive oil. Reaction conditions: 35 °C, 7.4 vol% of glycerol, 10 vol% of olive oil, 5 vol% of water and 7.5vol % enzyme.

Figure 4. Kinetics of glycerolysis of olive oil. Reaction conditions: 35 °C, 7.69 vol% of glycerol, 10 vol% of olive oil, 10 vol% of water and 7.5 vol% of enzyme.

Figure 5. Kinetics of glycerolysis of olive oil. Reaction conditions: 35 °C, 8.0 vol% of glycerol, 10 vol% of olive oil, 15 of vol% of water and 7.5 vol % of enzyme.

List of Table Captions