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ARTICLE

Kinetics of Solvent-Free Lipase-Catalyzed Glycerolysis of Olive Oil in Surfactant System

Alexsandra Valério,[†] Roberta L. Krüger,[†,§] Jorge Ninow,[§] Fernanda C. Corazza,[†] Débora de Oliveira,[†] J. Vladimir Oliveira,[†] and Marcos L. Corazza*[,†,‡]

[†]Department of Food Engineering, URI - Campus de Erechim, Av. Sete de Setembro 1621, Erechim, 99700-000 RS, Brazil, [§]Department of Chemical and Food Engineering, UFSC - Campus de Florianópolis, Caixa Postal 476, 88040-900 SC, Brazil, and [‡]Department of Chemical Engineering, UFPR-Centro Politécnico, Jardim das Américas, Curitiba, 81531-980 PR, Brazil

This work reports experimental data and kinetic modeling of solvent-free glycerolysis of olive oil using a commercial immobilized lipase (Novozym 435) in the presence of Triton X-100 surfactant for the production of monoacylglycerols (MAG) and diacylglycerols (DAG). The experiments were performed in batch mode evaluating the effects of temperature (30−70 °C), enzyme concentration (2.5−18 wt %), Triton X-100 concentration (10−20 wt %), and glycerol to oil molar ratio (3:1, 6:1, and 9:1). Experimental results showed that lipase-catalyzed solvent-free glycerolysis with the addition of Triton X-100 might be a potential alternative route to conventional organic solvent methods, as good conversions were obtained with relatively low enzyme concentrations (9 wt %) in short reaction times (240 min). The glycerolysis and hydrolysis parallel reactions were considered with rate constants estimated by minimizing a maximum likelihood function. A very satisfactory agreement between experimental data and model results was obtained, thus allowing a better understanding of the reaction kinetics.

KEYWORDS: Glycerolysis; kinetic modeling; monoacylglycerols; lipase; surfactant

INTRODUCTION

Monoacylglycerols (MAG) and diacylglycerols (DAG) are nonionic, amphiphilic molecules having both hydrophilic and hydrophobic parts with excellent emulsifying properties (*1*). They are widely used in the food industry, with applications in dairy products, margarines, bakery products, and sauces. In addition, due to their excellent lubricant and plasticizing properties, MAG are also used in textile processing, production of plastics, and formulation of oil for different types of machinery (*2*−*5*).

DAG are naturally occurring minor constituents of edible fats and oils, mainly constituted by triacylglycerols (TAG), and have attracted much attention in recent years due to their several important beneficial properties to human health (*6*, *7*). Moreover, DAG are produced by hydrolysis of triacylglycerols in the intestinal tract, which is catalyzed by pancreatic lipase (*7*, *8*). One should also note that mixtures of mono- and diacylglycerols are important emulsifiers widely used in industrially processed foods (*9*).

With the establishment of worldwide biodiesel government programs, huge amounts of glycerol surplus are expected to occur in the near future, which means an unavoidable driving force for the development of new technologies devoted to the transformation of such byproduct from biodiesel industrial processing.

*Corresponding author (telephone +55-54-35209000; fax +55-54-35209090; e-mail mlcorazza@uricer.edu.br).

Commercial food MAG are manufactured by chemical glycerolysis of fats and oils at high temperatures (220−250 °C) with inorganic alkaline catalysts. Due to the high reaction temperatures involved, dark-colored, burnt-tasting products are formed. Moreover, the chemical catalysis process is energy intensive and provides low yields (30−40%), and the product must be purified by molecular distillation (*4*).

In contrast to the chemical process, the glycerolysis of fats and oils using lipases provides higher MAG yields and excellent product quality. Several glycerolysis systems have been investigated with or without organic solvents, with immobilized or free enzymes, and in microemulsion or other media (*10*). However, in general, MAG yields from enzymatic glycerolysis are low with long reaction times involved (*4*, *5*, *12*).

For the bioconversion of lipophilic compounds, it may be necessary to introduce organic solvents into the reaction system to improve the very limited mutual solubility of glycerol−oil mixtures (*5*). However, the use of organic solvents may produce undesirable physicochemical effects on enzyme molecules, leading to enzyme denaturation, with effects differing depending upon the kinds of organic solvents and enzymes used, and also bring additional separation steps with costs associated with solvent removal (*5*).

An alternative to the use of organic solvents is the use of surfactants to promote larger homogeneity for the system oil/glycerol/enzyme, increasing the interfacial area and enhancing the efficiency of the biocatalyst, mainly for lipases, which are

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characterized as enzymes that act at the interface (*4*, *14*). With the aim of the production of MAG and DAG, lipase-catalyzed glycerolysis experiments have been carried out with free or immobilized lipases (*3*, *5*) in reverse micelle systems and microemulsions (*14*−*17*).

Microemulsion systems offer many advantages as reaction media for biocatalysis because both hydrophilic and hydrophobic substances can be dissolved in high concentrations. Besides, reverse micelles exhibit relatively ordered structure, are characterized by definite diameter, and provide an enormous interfacial area, which clearly favors lipase-catalyzed reactions. Microemulsions are thermodynamically stable, nanometer size droplets dispersed in an organic phase stabilized by surfactant molecules (*18*). Thus, it seems to be reasonable to consider the use of relatively low-cost surfactants, especially food-grade ones, to conduct enzyme-catalyzed reactions as a promising route to completely eliminate solvent traces from reaction products, bringing many advantages in terms of energy consumption and favorable transport properties.

An understanding of the kinetics of the lipase-catalyzed glycerolysis reaction is of primary importance to determine the optimal operational conditions for this kind of process. Despite the wide application of the products (MAG and DAG) resulting from the lipase-catalyzed glycerolysis, just a few works can be found in the literature regarding the kinetic modeling of such a reaction. In this aspect, the Ping-Pong Bi-Bi model is largely employed in the literature (*19*−*22*) to describe the kinetics of lipase-catalyzed reactions, although other empirical or semiempirical models (*23*, *24*) have been successfully used for enzymatic glycerolysis.

The main objective of the present work is to report experimental data and a semiempirical kinetic modeling for a lipase-catalyzed glycerolysis system. To the best of our knowledge, experimental kinetic data on the solvent-free lipase-catalyzed glycerolysis of olive oil in the presence of Triton X-100 surfactant have not been reported yet. For this purpose, experiments were performed in a batch reactor in the temperature range of 30−70 °C, with Triton X-100 concentration varied from 10 to 20 wt % and enzyme concentration from 2.5 to 18 wt % at glycerol to olive oil molar ratios of 3:1, 6:1, and 9:1.

MATERIALS AND METHODS

Materials. The substrates used in all experiments without any pretreatment were commercial olive oil (Arisco, Brazil) and glycerol (Merck, 99.5%). A commercial immobilized lipase from *Candida antarctica* (Novozym 435) was purchased from Novozymes (Araucária, PR, Brazil). The enzyme was immobilized on a macroporous anionic resin and, according to the manufacturer, the optimum activity is achieved at 70 °C and the initial esterification activity is about 120 U/g using lauric acid and *n*-propanol as substrates (*10*). Triton X-100 (Supelco) was employed as surfactant in the solvent-free glycerolysis of olive oil. Acetonitrile (99.9%) and acetone (99.8%) were purchased from Merck. Authentic standards of 1-(*cis*-9-octadecenoyl)-*rac*-glycerol, glycerol-1,2- and 1,3-dioleate and glyceryl trioleate were purchased from Sigma-Aldrich.

Experimental Procedure. Enzymatic glycerolysis reactions were carried out in a mechanically stirred (IKA-RW 20 digital stirrer) jacketed flask (40 mL) equipped with a sampling pipet and a PT-100 probe (0.1 °C accuracy) for temperature monitoring. The stirring rate was kept constant for all experimental conditions at 600 rpm. Precise amounts of the substrates, enzyme, and Triton X-100 were weighed on a precision scale balance (Ohaus Analytical Standard with 0.0001 g accuracy) and loaded into the reaction vessel. Typically, about 25 g of the substrates (vegetable oil and glycerol) were charged into the reaction vessel, whereas the amounts of enzyme and surfactant were loaded according to pre-established values. At the end of the reaction, the immobilized lipase was removed by vacuum filtration and the products were recovered for further analysis. One important issue when dealing with glycerolysis is to ensure safe sample withdraws of the whole content of the reacting mixture by preventing phase separation, hence allowing samples to be taken from a macroscopic homogeneous one-phase system. For this purpose, a set of preliminary tests were carried out for some experimental conditions with the actual reaction system (glycerol, oil, and Triton X-100 in the presence of the enzyme), performing destructive experiments and comparing with sampling results. In all cases tested, excellent agreement was found, thus ensuring the reliability of the sampling system (*10*, *11*).

Kinetic Study of Enzymatic Glycerolysis. In a previous recent work, Valerio et al. (*25*) determined the optimum conditions for MAG and DAG production in a solvent-free system at 70 °C, a stirring rate of 600 rpm, a glycerol to olive oil molar ratio of 6:1, 16 wt % of Triton X-100 surfactant, and 9 wt % of Novozym 435. From this optimized condition, perturbations were performed around it by varying the temperature, enzyme and surfactant concentrations, and substrates ratio. For this, the kinetic study of the enzymatic glycerolysis was carried out with a batch time of 6 h with samples taken from the bulk reactive system at 30, 45, 60, 120, 180, 240, and 360 min. The reaction kinetic experiments were performed at 30, 50, and 70 °C, with immobilized enzyme concentrations of 2.5, 9, and 18 wt %, Triton X-100 concentrations of 10, 16, and 20 wt % (both enzyme and surfactant based on the total amount of substrate, oil and glycerol), and glycerol to olive oil molar ratios of 3:1, 6:1, and 9:1.

Analytical Methods. Quantitative analyses of the products were conducted using an HPLC system from Agilent, 1100 series, with refractive index detector. The following instrumentation and conditions were used: Zorbax C_{18} column (4.6 m × 250 mm, 5 μm), flow rate of 1.0 mL/min, column temperature of 35 °C, and detector temperature of 40 °C. The mobile phase was acetonitrile/acetone (1:1, v/v), which was used as a sample-dissolving solvent, and the injection volume was 20 μL (*26*). The quantification of reaction products was carried out using authentic standards of MAG, DAG, and TAG. Calibration curves were built with the following concentrations: 100, 500, 1000, 2000, 5000, 8000, and 10000 ppm. The content of reaction products was expressed in terms of the whole amount of MAG, DAG, and TAG, as weight percent of the total sample in surfactant-free basis. All analyses were replicated at least three times. Before injection, samples were carefully handled with slight warming and gentle agitation to avoid phase separation. On the basis of previous works and experience with the system investigated, experimental uncertainties are estimated to be < 5% in terms of MAG, DAG, and TAG contents (*10*, *11*). The acid value (mg of KOH/g) and water content (wt %) (Karl Fischer titration method, DL 50, Mettler-Toledo) of olive oil were determined as 0.2 and 0.04, respectively. The water content in glycerol samples was monitored during 6 h at ambient conditions, resulting in a maximum value of 3.0 wt %.

Kinetic Modeling. In this work, an attempt to represent the experimental reaction data obtained from solvent-free glycerolysis of olive oil with an immobilized lipase as catalyst in the presence of Triton X-100 surfactant, the following glycerolysis and hydrolysis/esterification equations were adopted (*6*):

$$\mathrm{TAG} + \mathrm{Gly} \underset{k_2}{\overset{k_1}{\rightleftarrows}} \mathrm{MAG} + \mathrm{DAG} \quad (1)$$

$$\mathrm{DAG} + \mathrm{Gly} \underset{k_4}{\overset{k_3}{\rightleftarrows}} 2\mathrm{MAG} \quad (2)$$

$$\mathrm{TAG} + \mathrm{MAG} \underset{k_6}{\overset{k_5}{\rightleftarrows}} 2\mathrm{DAG} \quad (3)$$

$$\mathrm{TAG} + \mathrm{H_2O} \underset{k_8}{\overset{k_7}{\rightleftarrows}} \mathrm{DAG} + \mathrm{FFA} \quad (4)$$

$$\mathrm{DAG} + \mathrm{H_2O} \underset{k_{10}}{\overset{k_9}{\rightleftarrows}} \mathrm{MAG} + \mathrm{FFA} \quad (5)$$

$$\mathrm{MAG} + \mathrm{H_2O} \underset{k_{12}}{\overset{k_{11}}{\rightleftarrows}} \mathrm{Gly} + \mathrm{FFA} \quad (6)$$

FFA denotes the content of free fatty acids, Gly stands for the glycerol content, and H_2O is the water content in the reaction medium.

Considering the steps described in eqs 1−6 as elementary reactions, the reaction rate for each component in the system can be described by the following differential equations:

$$\frac{dTAG}{dt} = -k_1(TAG)(Gly) + k_2(DAG)(MAG) - k_5(TAG)(MAG) + k_6(DAG)^2 - k_7(TAG)(H_2O) + k_8(DAG)(FFA) \quad (7)$$

$$\frac{dDAG}{dt} = k_1(TAG)(Gly) - k_2(DAG)(MAG) - k_3(DAG)(Gly) + k_4(MAG)^2 + 2k_5(TAG)(MAG) - 2k_6(DAG)^2 + k_7(TAG)(H_2O) - k_8(DAG)(FFA) - k_9(DAG)(H_2O) + k_{10}(MAG)(FFA) \quad (8)$$

$$\frac{dMAG}{dt} = k_1(TAG)(Gly) - k_2(DAG)(MAG) + 2k_3(DAG)(Gly) - 2k_4(MAG)^2 - k_5(TAG)(MAG) + k_6(DAG)^2 + k_9(DAG)(H_2O) - k_{10}(MAG)(FFA) - k_{11}(MAG)(H_2O) + k_{12}(Gly)(FFA) \quad (9)$$

$$\frac{dGly}{dt} = -k_1(TAG)(Gly) + k_2(DAG)(MAG) - k_3(DAG)(Gly) + k_4(MAG)^2 + k_{11}(MAG)(H_2O) - k_{12}(Gly)(FFA) \quad (10)$$

$$\frac{dFFA}{dt} = k_7(TAG)(H_2O) - k_8(DAG)(FFA) + k_9(DAG)(H_2O) - k_{10}(MAG)(FFA) + k_{11}(MAG)(H_2O) - k_{12}(Gly)(FFA) \quad (11)$$

$$\frac{dH_2O}{dt} = -k_7(TAG)(H_2O) + k_8(DAG)(FFA) - k_9(DAG)(H_2O) + k_{10}(MAG)(FFA) - k_{11}(MAG)(H_2O) + k_{12}(Gly)(FFA) \quad (12)$$

The kinetic parameters of eqs 7−12 were considered to be proportional to the catalyst concentration (enzyme concentration), according to $k_i = k_i^*[E_t]$, (i = 1, ..., 12), where $[E_t]$ refers to the enzyme concentration. The kinetic parameters, k_{0i} and E_{ai}/R, were estimated with the reparametrized Arrhenius equation (*27*)

$$k_i^* = k_{i,T_{ref}} \exp\left[\frac{-E_{ai}}{R}\left(\frac{1}{T} - \frac{1}{T_{ref}}\right)\right] \quad (13)$$

where

$$k_{i,T_{ref}} = k_{0i} \exp\left(\frac{-E_{ai}}{R}\frac{1}{T}\right) \quad (14)$$

with 333.15 K used as the reference temperature (T_{ref}) and R is the universal gas constant.

Numerical Methods. The parameters of the model (eqs 7−12), k_0 and E_a, were estimated from fitting the experimental data through minimization of the objective function

$$f = \sum_{j}^{NOBS}\sum_{k}^{NCAR}\left[\frac{(C_{jk}^{exptl} - C_{jk}^{calcd})}{\sigma_{jk}}\right]^2 \quad (15)$$

where C_{jk}^{exptl} and C_{jk}^{calcd} refer to experimental and calculated MAG, DAG, and TAG contents (surfactant-free basis), NOBS represents the number of experimental observations (experimental data points), NVAR is the number of variables considered in the fitting procedure (MAG, DAG, and TAG), and σ_{jk} is the standard error (square root of experimental variance) of measured MAG, DAG, and TAG contents, with the following adopted values: 0.5, 0.85, and 1.0 for MAG, DAG, and TAG, respectively. A Matlab 7.0 code was developed and implemented for the parameter estimation step, using the subroutine ode23s (Matlab 7.0 subroutine based on a modified Rosenbrock formula of order 2) (*28*) for solving the differential equations system and the Particle Swarm Optimization algorithm (*29*) for the minimization of the objective function.

Table 1. Estimated Parameters of the Kinetic Model

pre-exponential factor ($g\ mol^{-1}\ min^{-1}$)		energy parameter (K)	
k_{01}	115937.55	$(E_a/R)_1$	3356.44
k_{02}	1.21×10^{-10}	$(E_a/R)_2$	7.01
k_{03}	285.13	$(E_a/R)_3$	5662.52
k_{04}	16.99	$(E_a/R)_4$	3.22×10^{-10}
k_{05}	3729.92	$(E_a/R)_5$	25038.85
k_{06}	2060.15	$(E_a/R)_6$	1378.86
k_{07}	57.70	$(E_a/R)_7$	7557.33
k_{08}	25213.85	$(E_a/R)_8$	1603.70
k_{09}	26.01	$(E_a/R)_9$	1.34×10^{-11}
k_{010}	35.97	$(E_a/R)_{10}$	8354.56
k_{011}	8.65	$(E_a/R)_{11}$	8597.96
k_{012}	82.76	$(E_a/R)_{12}$	9834.91

RESULTS AND DISCUSSION

Determination of Kinetic Parameters. The kinetic constants for the adopted model, shown in **Table 1**, were obtained from a global estimation by regression of all experimental data at 16 wt % fixed content of Triton X-100 surfactant. On the whole, seven experimental data sets, using the three temperature values investigated, comprising 56 experimental data points of MAG, DAG, and TAG contents were used for the kinetic model fitting. The correlation coefficient (R^2) obtained from the fitting procedure was 0.9899, and the values of root-mean-square deviation (rmsd), calculated from eq 16, were: $rmsd_{TAG}$ = 3.11, $rmsd_{DAG}$ = 2.53, and $rmsd_{MAG}$ = 2.81 for TAG, DAG, and MAG contents, respectively.

$$rmsd_k = \sqrt{\frac{\sum_{i=1}^{NOBS}(C_i^{exptl} - C_i^{calcd})^2}{NOBS}} \quad (k \equiv MAG,\ DAG,\ TAG,) \quad (16)$$

where C_i^{exptl} and C_i^{calcd} represent the experimental and calculated content values (wt %) for MAG, DAG, and TAG on a surfactant-free basis.

Effect of Enzyme Concentration. The effect of enzyme concentration (2.5, 9, and 18 wt %) on MAG and DAG production was evaluated at 70 °C with a 16 wt % Triton X-100 concentration and a glycerol to olive oil molar ratio of 6:1. **Figure 1** shows the experimental data and kinetic modeling results of enzymatic glycerolysis for these conditions, where one can observe the good agreement between experimental and model results for all enzyme concentrations. It can also be noted from this figure that high MAG and DAG contents were reached with a 9 wt % enzyme concentration, and fast initial reaction rates were observed at this condition, resulting in good conversions with relatively short reaction times.

As presented, an increase in enzyme concentration from 2.5 to 9 wt % led to a considerable enhancement in the production of MAG and DAG, especially at short reaction times. Increasing the enzyme concentration from 9 to 18 wt % did not lead to a significant increase in MAG and DAG contents, due probably to the poorer mixing of the reaction and, thereby, occurrence of mass-transfer limitations. This result is in agreement with previous reports, which cite that an increase in lipase loading above a certain amount may not increase the yield, showing that high

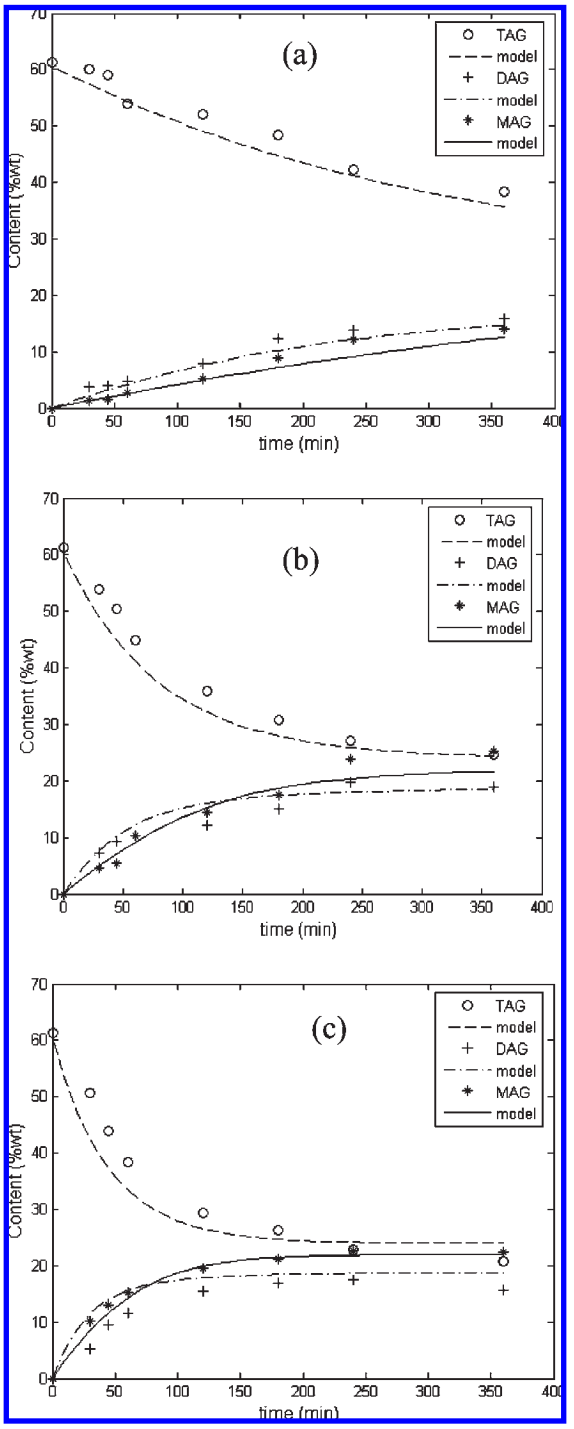

Figure 1. Effect of enzyme concentration on the solvent-free lipase-catalyzed glycerolysis of olive oil in the presence of Triton X-100 surfactant. Enzyme content: (**a**) 2.5 wt %, (**b**) 9 wt %, and (**c**) 18 wt %. Experimental conditions: 70 °C, glycerol to olive oil molar ratio of 6:1, Triton X-100 concentration of 16 wt %, stirring rate of 600 rpm.

enzyme concentrations may lead to the formation of aggregates, thus not making the enzyme active site available to the substrates (*30*, *31*). One should also note from **Figure 1** that, initially, the content of the intermediate reaction product, DAG, is higher than that of MAG but, consistently, as the reaction evolves the content of DAG is progressively surpassed by the final reaction product. Here, it is worth mentioning that after each experimental run, the enzyme activity was measured and no loss of activity was detected in any tested conditions.

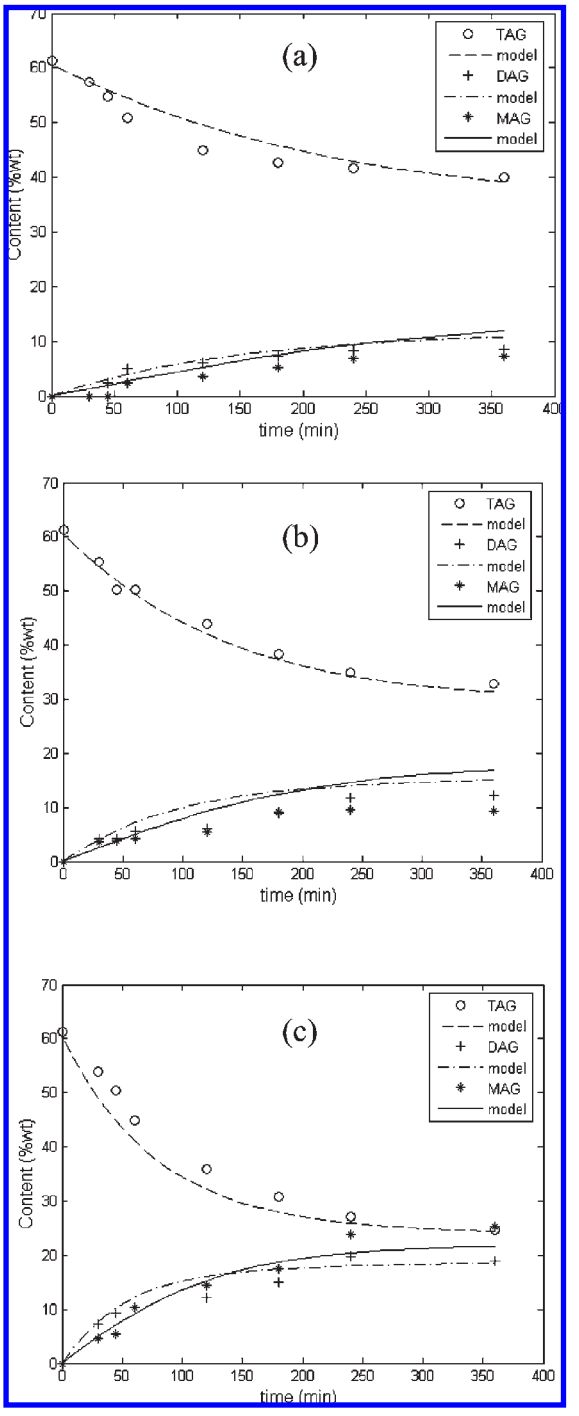

Figure 2. Influence of temperature on the solvent-free lipase-catalyzed glycerolysis of olive oil in the presence of Triton X-100 surfactant at (**a**) 30 °C, (**b**) 50 °C, and (**c**) 70 °C. Experimental conditions: glycerol to olive oil molar ratio of 6:1, enzyme concentration of 9 wt %, Triton X-100 concentration of 16 wt %, and stirring rate of 600 rpm.

Effect of Temperature. To evaluate the effect of temperature on MAG and DAG contents, the glycerol to olive oil molar ratio was fixed at 6:1, enzyme concentration was kept constant at 9 wt %, and the content of Triton X-100 was maintained at 16 wt %. **Figure 2** shows the experimental data and kinetic modeling results; one can observe very slow reaction rates at 30 °C. Conversely, when the temperature was increased from 50 to 70 °C, the reaction became much faster, and higher MAG (22 wt %) and DAG (24 wt %) contents were obtained at 180 and 240 min, respectively.

These results are in agreement with other reports available in the literature. For example, Yang et al. (*32*) evaluated the effect of temperature on the enzymatic glycerolysis of sunflower oil in a solvent-free system constituted by 10 wt % of enzyme (Novozym 435), a glycerol to oil molar ratio of 4.5:1, and 500 rpm of agitation, without surfactant, at 40 and 70 °C. These authors observed that after 24 h of reaction, the conversion of MAG reached 16 wt % at 40 °C, the same value observed with 5 h of reaction at 70 °C (with 8 wt % Novozym 435), a much faster reaction rate but still not attractive from a commercial perspective. Pawongrat et al. (*3*) studied the effect of temperature (30−50 °C) on the MAG production from tuna oil with IM-AK lipase enzyme. They observed that in the temperature range of 30−45 °C, MAG production increased with the temperature.

It is a common sense that temperature presents two important roles in the reacting system. First, an increase in temperature can reduce mixture viscosity, enhance mutual solubility, and improve the diffusion process of substrates, thus reducing mass-transfer limitations and favoring interactions between enzyme particles and substrates. Furthermore, enzymes generally have an "optimal working temperature value", established for each reaction system under evaluation and, in the case of Novozym 435, it is situated in the range of 40−65 °C (*33*−*35*).

It is worth noting from the results obtained that the reparametrized Arrhenius equations (eqs 13 and 14) were capable of successfully describing the temperature dependence of the kinetic constants.

Effect of Glycerol to Olive Oil Molar Ratio. To evaluate the effect of the glycerol to olive oil molar ratio on the glycerolysis reaction, experiments were accomplished at 70 °C with enzyme and Triton X-100 at fixed concentrations of 9 and 16 wt %, respectively. **Figure 3** presents the experimental data and model results for three different values of glycerol to olive oil molar ratio, 3:1, 6:1, and 9:1. From this figure one can observe that MAG and DAG contents were similar up to 180 min for all tested experimental conditions with differences increasing at longer times for a glycerol to oil molar ratio of 6:1.

The stoichiometry of the reaction requires a molar ratio of glycerol to oil of 2:1, but MAG production may be favored by reaction displacement to product formation, increasing the glycerol to oil ratio beyond the stoichiometric value. In the work published by Yang et al. (*32*), the effect of the glycerol to oil molar ratio was investigated in the range of 4.5:1−46:1 for the sunflower oil glycerolysis at 70 °C, 8 wt % of Novozym 435, stirring of 500 rpm, and 5 h of batch time, in a surfactant- and solvent-free system, and the authors obtained 17 wt % of MAG with a glycerol to oil molar ratio of 9:1. These authors argued that the substrate ratio may have two different behaviors in the reaction system: an increase in glycerol amount beyond the stoichiometric value may favor MAG production through reaction displacement to product formation. On the other hand, a rise in glycerol amount, as presented in this work, can affect the system polarity, influencing the system's stability and homogeneity.

Cheirsilp et al. (*23*), when dealing with the glycerolysis of palm olein using immobilized lipase PS, argued that high concentrations of glycerol might lead to an increase in the initial reaction rate of MAG production and also in the final concentration of monoacylglycerols. Coteron et al. (*33*) evaluated the effect of glycerol to oil molar ratio (3:1 and 6:1) on the enzymatic glycerolysis of olive oil without solvents at 75 °C and observed that, after 4 h, both reacting systems reached steady levels of MAG concentration and, after 7 h, the excess of glycerol did not affect the product content. In the present case, the experimental kinetic data show that the excess of glycerol is not so effective at the beginning of the reaction but becomes important after a certain reaction time has elapsed. Again, one should note from **Figure 3** that the employed model was able to satisfactorily describe the kinetics of the glycerolysis reaction.

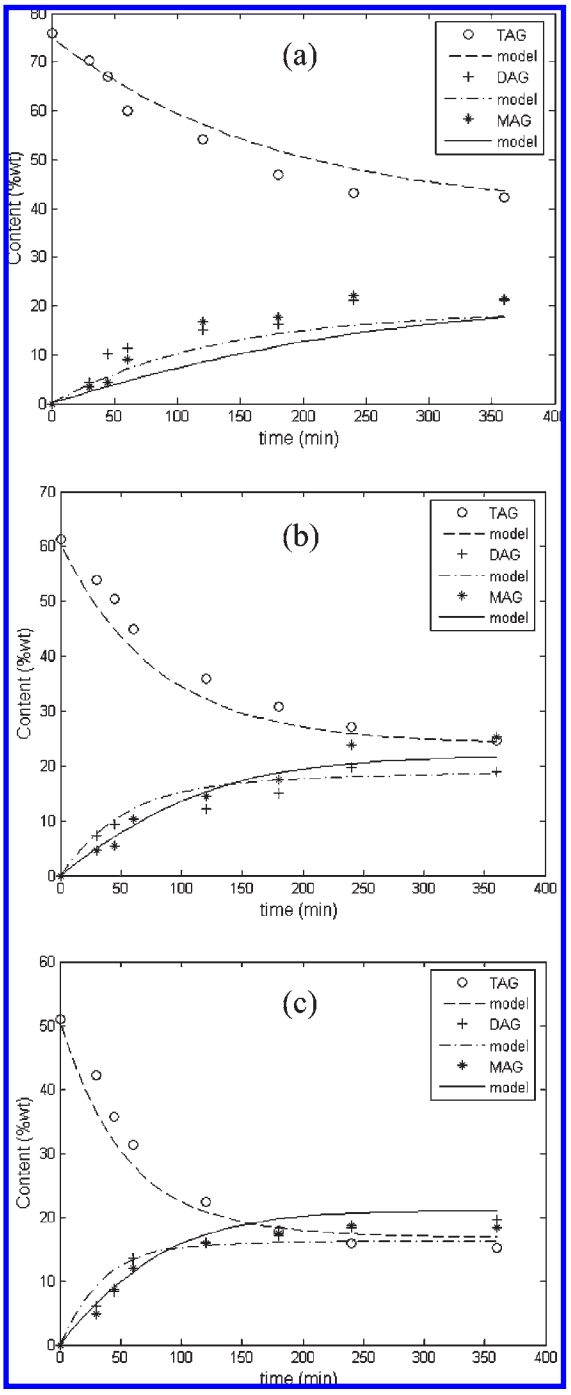

Figure 3. Kinetics of lipase-catalyzed glycerolysis of olive oil in the presence of Triton X-100 surfactant at various glycerol to olive oil molar ratios: (**a**) 3:1; (**b**) 6:1; (**c**) 9:1. Experimental conditions: 70 °C, enzyme concentration of 9 wt %, Triton X-100 concentration of 16 wt %, and stirring rate of 600 rpm.

Effect of Triton X-100 Surfactant Concentration. The surfactant plays an important role in the microemulsion formation, providing a high interfacial area for the enzyme action. The effect of the surfactant was evaluated by adopting the values of 10, 16, and 20 wt % for Triton X-100 concentration, at 70 °C, with 9 wt % of immobilized enzyme and a glycerol to olive oil molar ratio of 6:1. Experimental data and model results are presented in **Figure 4**,

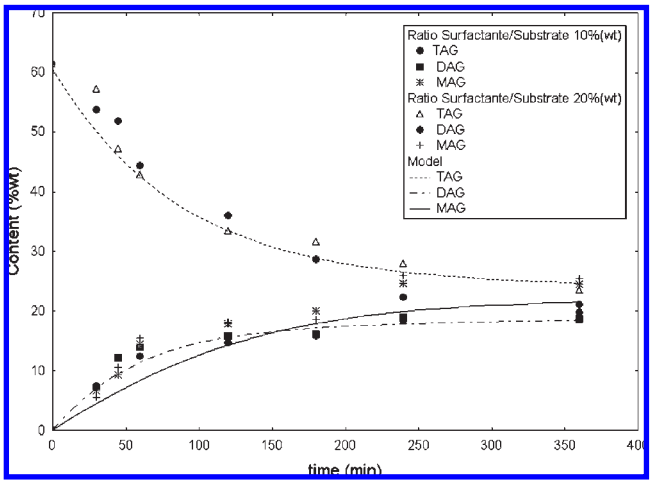

Figure 4. Effect of surfactant concentration on the lipase-catalyzed glycerolysis of olive oil in the presence of Triton X-100 surfactant: 10 and 20 wt % surfactant contents. Experimental conditions: 70 °C, enzyme concentration of 9 wt %, glycerol to olive oil molar ratio of 6:1, and stirring rate of 600 rpm.

which shows the MAG, DAG, and TAG content profiles at different surfactant concentrations.

From these results it can be inferred that the surfactant concentration did not promote a great difference in the course of MAG and DAG reaction yield, possibly due to the concentration range adopted. This fact was also confirmed by the good agreement of the predicted results from the kinetic model, because the lowest and highest values of surfactant contents were not used in the parameter estimation. In both cases, after 360 min of reaction, about 24 wt % of MAG and 18 wt % of DAG contents were reached. Stamatis et al. (*14*) in a review paper cited that enzymes (particularly lipases) could act in the micellar interface, interacting with the hydrophilic or hydrophobic parts of the substrates, corroborating the importance of the surfactant presence in the reaction medium. According to the authors, lipases are capable of interacting with the hydrophilic or hydrophobic parts of the substrates, which means that water-in-oil microemulsions (reverse micelles) represent a unique microheterogeneous system for enzymatic reactions.

Conclusions. In this work, a kinetic modeling approach together with experimental data of the solvent-free lipase-catalyzed glycerolysis of olive oil with surfactant is presented. Reaction kinetics was investigated by varying the temperature, enzyme, and surfactant concentrations and using different glycerol to oil molar ratios. Appreciable contents of MAG and DAG (>35 wt %) were achieved at mild temperatures (around 50 °C), with relatively low enzyme concentrations (9 wt %) in short reaction times (240 min). The kinetics approach showed that a very satisfactory agreement between experimental data and model results was obtained, thus allowing a better understanding of the reaction kinetics, pointing to a promising route to be further investigated and improved for use in process optimization.

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