DOI: 10.1016/S1872-2067(15)61040-3 CJC-2015-11-042 编辑润色稿

Kinetic study and kinetic parameters of lipase-catalyzed glycerolysis of sardine oil in a homogeneous medium.

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Abstract

The production of polyunsaturated fatty acids (PUFAs) concentrates by enzymatic catalysis has gained interest due to their stereospecificity and the milder conditions needed compared to the use of inorganic catalysts. The enzymatic glycerolysis of sardine oil by Lipozyme® 435 to get PUFA concentrates in the forms of di- and monoacylglycerols (DAGs, MAGs) in an optimized amount of tert-butanol as the organic solvent was studied. First, mass transfer limitation of the reaction system was analyzed. The effect of different operating variables such as lipase loading, temperature and feed composition was investigated. A semi-empirical kinetic model based on the reversible elementary reactions of glycerolysis and hydrolysis of the glycerides was employed to correlate the experimental kinetic data. A mole ratio glycerol:oil of 3:1 was the optimum, which produced more than 84 wt% of MAG at 50ºC. A comparison with other glycerolysis systems was performed using MAG yield, reaction rate and significance of kinetic parameters.

Keywords: lipase-catalyzed; glycerolysis; tert-butanol; mass transfer; kinetic model.

Received 18 November 2015. Accepted 4 January 2016.

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1. Introduction

Fish oil is rich in omega-3 (n-3) polyunsaturated fatty acids (PUFAs) such as eicosapentaenoic acid (EPA) and docosahexaenoic acid. The health benefits of n-3 fatty acids have been widely established in the literature [1-3]. Among the different types of lipid derivatives containing PUFA concentrates, MAG and DAG has good bioavailability [4, 5]. In addition, MAG or its mixtures with DAG account for 75 % of worldwide emulsifier production [6]. The process currently used in industry to obtain MAG is glycerolysis using an inorganic alkaline catalyst at high temperature (220 - 260ºC). This method has several disadvantages such as it gives a dark color and burnt taste as well as high energy consumption. Furthermore, chemical glycerolysis is not suitable for producing MAG rich in PUFA due to oxidization problems. Enzymatic glycerolysis is an attractive alternative for the production of MAG rich in PUFA since the reaction can be carried out under mild conditions [7] and structured products are obtained.

The immiscibility of the reactants, glycerol and oil leads to mass transfer limitation in the glycerolysis of oils. Different approaches have been used in the literature to improve the contact between the reactants and hence reduce mass transfer limitation. Lipase-catalyzed glycerolysis has been carried out in different reaction media such as organic solvents [8], compressed fluids [9], and ionic liquids [10] in order to improve the mass transfer.

Recently the use of different surfactants to increase the interfacial area [11] and ultrasound irradiation [12] have also been proposed to reduce mass transfer limitation.

This paper is part of a wider project for the optimization of MAG production by enzymatic glycerolysis of sardine oil. First, different tert-alcohols were evaluated as the solvent used to create a homogeneous phase [13]. Tertiary alcohols enhance the enzyme activity and accelerate the reaction rate as compared to the solvent-free system [14]. In a previous work, tert-pentanol was selected as the solvent and the effect of the glycerol:oil mole ratio was evaluated for its effect on kinetic behavior and MAG yield. The glycerolysis product was subsequently fractionated by a two-step molecular distillation to obtain a concentrated product of MAG and DAG rich in PUFA [15]. In this work, a different tertiary alcohol, tert-butanol was used as the solvent. Tert-butanol has been used in different glycerolysis systems of vegetable oils such as olive oil [16, 17], palm oil [18], camellia oil [19] and sunflower oil [8, 20].

The main objective of this work is to present a detailed kinetic study of enzymatic glycerolysis of refined sardine oil in tert-butanol as the solvent catalyzed by a commercial lipase Lipozyme® 435. The amount of tert-butanol added to create a monophasic system has been optimized based on liquid-liquid equilibrium (LLE) data previously determined [13]. This value was compared with the amount of tert-butanol added to other glycerolysis systems. The results in terms of MAG and DAG yields were compared with literature data reported for different type of oils and related to the high activity of the lipase for short and medium chain length fatty acids.

First, the external and internal mass transfer resistances were analyzed in the heterogeneous system of the immobilized lipase. Mass transfer limitation can play an important role in the reaction. However, in most glycerolysis studies reported in the literature, no mass transfer studies were performed.

Mathematical models are needed to predict and optimize the industrial process. However, not many works in the literature deal with the kinetic modeling of glycerolysis. One of the first works was carried out by Moquin et al. [9]. In that work, the kinetics of the non-catalyzed glycerolysis of soybean oil in $SCCO_2$ medium were correlated by a sequence of reversible reactions to take into account the parallel hydrolysis reaction. The same model was used by Valerio et al. [11] in the kinetic study of solvent-free lipase-catalyzed glycerolysis of olive oil by Novozym 435 with Triton X-100 as surfactant. Although glycerolysis and hydrolysis reactions were proposed, no information on the experimental FFA production and rate of change of glycerol were provided and only the TAG, MAG and DAG concentrations were used in the fitting procedure to obtain the kinetic parameters. The mechanism of glycerolysis and hydrolysis of pure POP (1,3-palmitin-2-olein) by *Rhizopus arrhizus* lipase was studied by Tan and Yin [21] by including hydrolysis, esterification and isomerization of MAG and DAG. Cheirsilp et al. [22] proposed a Ping-Pong Bi Bi model that focused on the kinetics of the hydrolysis and esterification steps involved in the glycerolysis of palm oil in an acetone/isooctane mixture (3:1 v/v). Water was dissolved in glycerol (10 % w/v of water added to glycerol) and therefore a large amount of

water was present in the reaction medium. Recently, Voll et al. [17] proposed a kinetic model based on the ordered-sequential Bi Bi mechanism for a lipase-catalyzed glycerolysis system of olive oil in tert-butanol as the solvent. In that work, the reaction products were expressed as total amount of MAG, DAG, TAG and FFA by weight percentage on a solvent-free basis composition. No experimental information on the glycerol concentration rate of change was provided. Fiametti et al. [12] used a similar model to the one proposed by Voll et al. [17] in the glycerolysis of olive oil by ultrasound irradiation. However, the parameters were not provided in the open literature although they could be available upon request to the authors.

In this work, a similar approach to that previously proposed by Moquin et al. [9] was used. The kinetic parameters were compared when possible with previous values reported in the literature. This model was able to consider the concentration of all the compounds involved in the glycerolysis system: TAG, DAG, MAG, FFA, glycerol and water.

2. Experimental

2.1 Materials

Refined sardine oil was provided by Industrias Afines S.L. (Spain) with a water content of 0.19 ± 0.03%. Glycerol was purchased from Sigma Aldrich with a purity of ≥ 99.5% and a water content of 0.18 ± 0.04%. Tert-butanol (TB) was purchased from Merck with a purity of ≥ 99% and a water content of 0.20 ± 0.03%. The products were stored over activated 3 Å molecular sieve to keep them dry. The food grade lipase Lipozyme® 435 from *Candida antarctica* (immobilized on a macroporous hydrophobic acrylic resin) was donated by Novozymes A/S (Bagsvaerd, Denmark). The water content of this lipase was 3.5 ± 0.3% as determined in triplicate by Karl-Fisher titration with a Mitsubishi CA-20 moisture meter. According to Novozymes A/S, the specific activity of the lipase is ≥ 8000 propyl laurate units/g. No additional water was added to the system. Therefore, water present in the reaction medium came only from the reactants.

2.2 Enzymatic Glycerolysis of Sardine Oil

Different vials containing a mixture of sardine oil, glycerol and TB were incubated at different temperatures from 303 to 333 K in a water bath with stirring. Different mole ratios of substrate and enzyme dosage were also studied. The amount of TB added was fixed at a mass ratio of 1.5:1 (TB:substrates) on the basis of previous studies on LLE [13]. At selected time intervals (from five minutes up to eight hours), a sample of the reaction mixture was withdrawn and filtered through a microfilter (0.45 μm, Sartorius RC) to stop the reaction by removing the lipase. All samples were stored at −18 °C prior to analysis.

The reusability of Lipozyme® 435 in this process was tested by recycling the immobilized enzyme in six batches. After each run, the lipase was washed once with TB, and then twice with hexane in order to eliminate the remaining compounds. Afterwards, the lipase was dried at 303 K and stored in a desiccator under vacuum. No significant reduction in enzyme activity was found. In any event, a fresh biocatalyst was used in each run.

Tert-butanol was evaporated under vacuum using a rotary evaporator (Heibolph VV2000) at 333 K. In this way,

TB can be reused by using the molecular sieve to eliminate the water content.

2.3 Analysis of the reaction products

The neutral lipid profile (TAG, DAG, MAG and FFA) was analyzed by a normal phase high performance liquid chromatography (NP-HPLC). The chromatographic apparatus consisted of a HPLC system (Agilent 1200) formed by a quaternary pump and an auto-injector. The chromatographic separation of the compounds was carried out at room temperature with a Lichrospher Diol column (5 µm, 4 mm×250 mm) and detection was performed by an evaporative light scattering detector (Agilent 1200 series) at 35 ºC and 0.35 MPa. Gradient elution was achieved by mobile phases A (isooctane) and B (methyl tert-butyl ether:acetic acid = 99.9:0.1, v/v). The method and calibration procedure were previously reported [23]. The regioisomers of DAG and MAG could not be distinguished by the applied analytical procedure. Therefore the total amount of MAG and DAG was reported for the kinetic experiments.

The analysis of the remaining glycerol was performed by a high temperature gas chromatograph (HT-GC) system (HP 6890 Series GC System) equipped with a flame ionization detector (FID), a fused silica capillary column of 30 m × 0.25 mm i.d. coated with a 0.25 µm film thickness of 65% phenyl methylpolisiloxane (65HT) as the stationary phase and an Agilent Technologies 7683B Series automatic injector. The method and calibration procedure were previously reported [13].

2.4 Kinetic modeling

The overall glycerolysis reaction can be described by:

$$TAG + 2\,Gly \rightleftharpoons 3\,MAG \quad [1]$$

However, glycerolysis is believed to follow a two-step reaction. First, one molecule of glycerol reacts with one molecule of TAG to yield one molecule of DAG and another molecule of MAG. The reaction of one molecule of DAG with one molecule of glycerol can also take place to yield two molecules of MAG:

$$TAG + Gly \underset{k_2}{\overset{k_1}{\rightleftharpoons}} DAG + MAG \quad [2]$$

$$DAG + Gly \underset{k_4}{\overset{k_3}{\rightleftharpoons}} 2\,MAG \quad [3]$$

The breakdown of TAG due to reaction with MAG can also occur to produce two molecules of DAG [9]:

$$TAG + MAG \underset{k_6}{\overset{k_5}{\rightleftharpoons}} 2\,DAG \quad [4]$$

Even in the presence of small amounts of water in the glycerolysis reaction medium, unwanted hydrolysis reactions must be considered:

$$TAG + H_2O \underset{k_8}{\overset{k_7}{\rightleftharpoons}} DAG + FFA \quad [5]$$

$$DAG + H_2O \underset{k_{10}}{\overset{k_9}{\rightleftharpoons}} MAG + FFA \quad [6]$$

$$MAG + H_2O \underset{k_{12}}{\overset{k_{11}}{\rightleftharpoons}} GLY + FFA \quad [7]$$

Kinetic models are needed to predict and simulate the reaction. By formulating the mass balance equation for all the species of the reaction system, the concentration profile versus time can be obtained. In this way, the process can be optimized. The rate of change in concentration for each of the reaction components are described by the following differential equations:

$$\frac{d\, n_{TAG}/n_{total}}{dt} = -k_1 x_{TAG} x_{Gly} + k_2 x_{DAG} x_{MAG} - k_5 x_{TAG} x_{MAG} + k_6 (x_{DAG})^2 - k_7 x_{TAG} x_{H_2O} + k_8 x_{DAG} x_{FFA} \quad [8]$$

$$\frac{dn_{DAG}/n_{total}}{dt} = k_1 x_{TAG} x_{Gly} - k_2 x_{DAG} x_{MAG} - k_3 x_{DAG} x_{Gly} + k_4 (x_{MAG})^2 + 2k_5 x_{TAG} x_{MAG} - 2k_6 (x_{DAG})^2 + k_7 x_{TAG} x_{H_2O} - k_8 x_{DAG} x_{FFA} - k_9 x_{DAG} x_{H_2O} + k_{10} x_{MAG} x_{FFA} \quad [9]$$

$$\frac{dn_{MAG}/n_{total}}{dt} = k_1 x_{TAG} x_{Gly} - k_2 x_{DAG} x_{MAG} + 2k_3 x_{DAG} x_{Gly} - 2k_4 (x_{MAG})^2 - k_5 x_{TAG} x_{MAG} + k_6 (x_{DAG})^2 + k_9 x_{DAG} x_{H_2O} - k_{10} x_{MAG} x_{FFA} - k_{11} x_{MAG} x_{H_2O} + k_{12} x_{MAG} x_{FFA} \quad [10]$$

$$\frac{dn_{Gly}/n_{total}}{dt} = -k_1 x_{TAG} x_{Gly} + k_2 x_{DAG} x_{MAG} - k_3 x_{DAG} x_{Gly} + k_4 (x_{MAG})^2 + k_{11} x_{MAG} x_{H_2O} - k_{12} x_{Gly} x_{FFA} \quad [11]$$

$$\frac{dn_{FFA}/n_{total}}{dt} = k_7 x_{TAG} x_{H_2O} - k_8 x_{DAG} x_{FFA} + k_9 x_{DAG} x_{H_2O} - k_{10} x_{MAG} x_{FFA} + k_{11} x_{MAG} x_{H_2O} - k_{12} x_{Gly} x_{FFA} \quad [12]$$

$$\frac{dn_{H_2O}/n_{total}}{dt} = -k_7 x_{TAG} x_{H_2O} + k_8 x_{DAG} x_{FFA} - k_9 x_{DAG} x_{H_2O} + k_{10} x_{MAG} x_{FFA} - k_{11} x_{MAG} x_{H_2O} + k_{12} x_{Gly} x_{FFA} \quad [13]$$

As explained above in the analytical procedure, the stereoisomers of DAG and MAG could not be distinguished and no difference was made between them in the model. The concentrations of the reaction products were expressed on a solvent-free basis. TAG, DAG, MAG, FFA and glycerol concentrations were experimentally determined. The water concentration could not be measured versus reaction time. According to Moquin et al. [9], it is possible to estimate the change in water concentration by subtracting the experimental FFA concentration from the initial water concentration since the formation of one mole FFA requires one mole of

water (Equations 5-7).

The rate constants for the six kinetic equations were obtained by solving the set of differential equations simultaneously. The differential equations were solved numerically with a fourth order Runge-Kutta method and the parameters were optimized by minimizing the following objective function (O.F.):

$$O.\mathrm{F.} = \frac{\sum_{\text{all samples}} \sum_{i=1}^{n} \left(x_{i,exp} - x_{i,calc}\right)^2}{n_{samples}} \cdot 100 \qquad [14]$$

using the simplex Nelder-Mead method. The subscript "i" refers to the different components in the glycerolysis system: TAG, DAG, MAG, FFA, glycerol and water. The subscripts "exp" and "calc" refer to the experimental and calculated mole fraction of the different components for each experimental kinetic data point ($n_{samples}$)

The root mean square deviation (rmsd) was calculated to evaluate the quality of the fitting:

$$rmsd = \sqrt{\frac{\sum_{i=1}^{NOBS} \left(w_i^{exp} - w_i^{calc}\right)^2}{NOBS}} \qquad [15]$$

where NOBS is the total number of kinetic data points for all the kinetic experiments and w_i^{exp} and w_i^{calc} are the experimental and calculated weight fractions for the reaction compounds.

3. Results and discussion

3.1 Mass transfer analysis

External and intraparticle mass transfer resistance can influence the observed reaction rate in heterogeneous catalytic processes such as immobilized lipase biocatalysis. Before the study of the effect of the kinetic variables, the mass transfer rate was analyzed.

Tert-butanol was used as the organic solvent to provide an environment where oil and glycerol can interact since both reactants are completely immiscible. Tert-butanol helps to create a homogeneous phase and also decreases the viscosity of the reaction medium since both reactants are highly viscous, especially glycerol (Table 1). To evaluate the external mass transfer resistance, the glycerolysis reaction was carried out at different stirring speeds, from 120 to 200 rpm, while keeping constant the rest of the reaction conditions. The results are presented in Table 1. From these results, it can be concluded that there was no increase in the initial reaction rate of MAG formation in the speed range studied. This result was expected since external diffusion does not usually control the overall rate unless the stirring speed is very low or the reaction mixture is very viscous [24]. Tert-butanol helps to decrease the viscosity of the reaction medium since its viscosity is 100 time smaller than the viscosity of glycerol (Table 1), resulting in a low external mass transfer resistance and it acts as an inert carrier for the reactants to the active site of the enzyme. Hence, 170 rpm was chosen for all the glycerolysis reactions.

Slow intraparticle diffusion can reduce the overall reaction rate, especially if the reactant molecules are large [25] and have a low mobility in the lipase support. Chesterfield et al. [26] analyzed the relative magnitude of the

external liquid mass transfer resistance to the combined internal resistances (intraparticle diffusion and reaction resistances) in the ethanolysis of waste cooking oil using Novozym 435 by plotting the reciprocal initial reaction rate ($1/r_o$) as a function of inverse lipase loading (1/m). This plot should be a straight line, with a slope proportional to the combined internal resistances, and the intercept is proportional to the interphase mass transfer resistance. Figure 1 illustrates this linear dependence in the glycerolysis of sardine oil. The linear fit proved that the rate controlling step is the combined internal resistances since the intercept can be considered negligible.

To evaluate the intraparticle diffusion effect, the lipase Lipozyme® 435 was separated into two fractions by a 400 μm sieve (46 wt % of Lipozyme® 435 particles with ϕ_p > 400 μm). Kinetic experiments were carried out with each of the fractions obtained and compared with the results obtained with unsieved lipase. Figure 2 shows that the initial reaction rate of MAG formation was increased by decreasing the particle size of Lipozyme® 435. This may indicate internal mass transfer limitation for the larger particles, although the same MAG yield was achieved at long reaction time. A significant pore diffusion resistance was also found by Chesterfield et al. [26] in the ethanolysis study with Novozym 435 (technical grade of *Candida antartica*).

The experimental Thiele modulus, φ_{exp}, was calculated to evaluate the intraparticle resistance [27]:

$$\phi_{exp} = \left(\frac{d_p}{6}\right)^2 \frac{r_{exp,substrate}}{D_{eff} C_{substrate,o}} \quad [16]$$

d_p is the mean particle diameter of Lipozyme® 435 (d_p = 383 μm, [26]). The effective diffusivity, D_{eff}, was evaluated using [28]:

$$D_{eff} = \frac{D_{substrate-solvent} \varepsilon_p \sigma}{\tau} \quad [17]$$

where ε_p, τ and σ are Lipozyme® 435 porosity, tortuosity and constriction factor. These values were taken from Chesterfield [26] for Novozym 435 (ε_p = 0.5, τ = 6 and σ = 1). $D_{substrate\text{-}solvent}$ is the molecular diffusivity of the reactants (glycerol and fish oil) in the reaction medium (tert-butanol in this work). It was estimated using the Wilke-Chang equation [29]:

$$D_{substrate-solvent} = \frac{7.4 \cdot 10^{-8} T (M_{substrate} \psi_{solvent})}{\eta_{solvent} V_{substrate}^{0.6}} \quad [18]$$

where $D_{substrate\text{-}solvent}$ is the diffusion coefficient of the substrate in the solvent ($cm^2 \cdot s^{-1}$), $M_{substrate}$ is the molecular weight of the solvent (g/mol), T is the temperature (K), $\eta_{solvent}$ is the viscosity of the solvent, cP, $V_{sustrate}$ is the molar volume of the substrate at its normal boiling temperature, cm^3/mol and ψ the association factor of the solvent (dimensionless, ψ = 1 for non-associated compounds). The parameters values used in the calculation of φ are listed in Table 2. Molar volumes at the normal boiling point were estimated by the Tyn and Calus method [29]:

$$V = 0.285 V_c^{1.048} \quad [19]$$

where V_c is the critical volume in cm^3/mol. V_c for glycerol was 255 cm^3/mol [29]. No data of V_c for fish oil was found in the literature. The corresponding estimated value for triolein (V_c = 3235.65 cm^3/mol) was used [30]. Φ was evaluated for both substrates, glycerol and sardine oil, at 323 K for $r_{exp,glycerol}$ = 0.0173 $mmol \cdot L^{-1} \cdot s^{-1}$, $C_{glycerol,o}$ = 47.5 $mmol \cdot L^{-1}$ $r_{exp,fish\ oil}$ = 0.023 $mmol \cdot L^{-1} \cdot s^{-1}$ $C_{fish\ oil,o}$ = 47.5 $mmol \cdot L^{-1}$.

According to Bailey [31], when Φ is sufficiently large (Φ ≥ 3), diffusion of substrate is slow relative to its consumption. When Φ < 0.3 the limiting rate process is the chemical reaction. Φ for diffusion of glycerol in the reaction medium was found to be $1.9 \cdot 10^{-2}$. However, a value of 0.36 was obtained for the diffusion of fish oil in tert-butanol, probably due to the bigger oil molecules that can lead to more diffusional limitation (Table 2). In any case, the value of Φ was close to the limit of 0.3 and the observed rate can be considered kinetically controlled. Based on the Φ values, the lipase was used in its commercially available size without sieving for further kinetic experiments.

Yang et al. [20] studied the effect of the loading of Novozym 435 on the glycerolysis of sunflower oil. They found that an enzyme loading of more than 10% resulted in only a small increase in MAG yield. Therefore they suggested that 10-15% of enzyme loading was enough to obtain the maximum reaction performance. Moreover, other authors as Valerio et al. and Fiametti et al. [11, 12] have shown that high enzyme concentrations can lead to the formation of aggregates, making the enzyme active site unavailable to the substrates. Based on this and the results shown in Figure 1, further glycerolysis kinetics were performed with 10 wt% of Lipozyme® 435 based on reactant weight.

3.2 Glycerolysis reaction system

The presence of a catalyst is necessary since it has been shown in the literature [16] that under 70ºC the observed reaction rate without a catalyst is nearly zero. Figure 3c shows a typical glycerolysis profile of fish oil at the mole ratio of glycerol:sardine oil of 3:1 at 323 K with 10% of lipase loading in tert-butanol (68 % of tert-butanol). The main reaction product at the above conditions was MAG (around 51 % mole percentage), but DAG and FFA production were also observed although the mole percent was around 3 % for both compounds. The initial water content in the reaction medium was less than 1 % by weight but it was nearly 10 % of the mole content of water in the reaction medium. Therefore FFA production can be observed. TAG consumption was nearly complete with a mole percent at equilibrium conditions lower than 2 %.

3.2.1 Effect of reactant mole ratio

The initial mole reactant ratio (MR) was varied between 1 and 9. Figures 3a-3d show the glycerolysis product profile expressed in mole fraction on a solvent-free basis. The reaction rate of formation of MAG was always higher than that of DAG and FFA. The presence of a solvent, tert-butanol, helped both reactants to diffuse to the active sites of the enzyme and MAG formation was favored. Valerio et al. [11] studied the kinetics of

glycerolysis of olive oil in a surfactant system (with Triton X-100 as surfactant) as an alternative to the use of organic solvents, and found that the DAG initial reaction rate was higher than that of MAG even with an excess of glycerol (MR=9:1). This behavior could be due to mass transfer limitation and can be compared to a situation of low glycerol concentration in the reaction medium.

The optimal MR glycerol:oil must consider the MAG yield as well as the excess of glycerol employed in the glycerolysis reaction. The equilibrium yield of MAG was calculated as:

$$Equilibrium\ MAG\ yield\ (\%) = \frac{Moles\ of\ MAG\ in\ the\ equilibrium}{Initial\ moles\ of\ TAG \cdot 3} \cdot 100 \qquad [20]$$

Figure 4 shows that the MAG equilibrium yield remained practically constant at a MR higher than 5:1. A similar behavior was observed by Chesterfield et al [26] in the ethanolysis of waste cottonseed cooking oil by Novozym 435. These authors proposed the following relationship for the equilibrium yield:

$$Equilibrium\ MAG\ yield\ (\%) = \frac{a}{1+exp\left(\frac{RM_o - RM}{b}\right)} \qquad [21]$$

Non-linear regression was performed by using the Marquardt algorithm (Statgraphics) giving a = 89.285 defined as the limiting normalized MAG equilibrium [26], b = 0.922 and RM_o = 1.35 with r^2 = 0.999. McNeil (1990) also found that the MAG equilibrium yield was independent of the glycerol:oil mole ratio from mole ratio higher than 5:1.

To take into account the excess of glycerol employed, Figure 4 also shows the MAG composition (expressed as mole percentage) on a solvent-free basis and on a solvent and glycerol-free basis. In the lipid basis (no glycerol), on increasing the MR, the MAG content increased sharply from a MR of 1:0 to 3:1 and then the MAG content slightly increased in the lipid fraction. On a solvent-free basis, when glycerol was considered in the global composition, a maximum was observed in the MAG content at a MR of 3:1, due to the excess of glycerol employed that was not consumed.

Table 3 summarizes the glyceride equilibrium composition found in this work, as well as for other glycerolysis systems in the literature that use tert-butanol as solvent and immobilized *Candida antarctica* as the biocatalyst. The results are expressed in weight percentage on a lipid basis since in most studies, the composition was usually expressed this way. Although different lipase loadings were used in Table 3, the data listed in this table corresponded in most cases to equilibrium conditions and the comparison of the MAG yield can be established as valid. Table 3 shows the different results in terms of the MAG and DAG yields at the same initial MR (as will be explained in Section 3.2.2, the effect of temperature on the MAG equilibrium yield was not important). For instance, at the MR glycerol:oil of 4:1, the MAG percentage on a lipid basis ranged from 70% for sunflower oil to 91% for tuna oil. Regarding the type of oil, fish oils gave a higher MAG yield than vegetable oils. According to the shape and properties of the scissile fatty acid binding sites of *Candida antarctica* lipase, in the literature, it has been reported that this lipase has high activity for short and medium chain length fatty acids [32]. Table 4 presents the fatty acid composition of the oils listed in Table 3. It can be observed that fish oils

have the highest content of medium chain length fatty acids as C14:0, C16:0 and C16:1. Based on these results, a relationship between the fatty acid specificity of *Candida antarctica* lipase and MAG yield for the different types of oil was established. From Table 3, it can also be observed that the amount of tert-butanol added to the system was different, ranging from 45 % to 80 %. Tert-butanol helps to create a homogeneous reaction system and avoid mass transfer limitation. Figure 5 shows the binodal curve for the ternary system glycerol + sardine oil + tert-butanol at 303.15 and 323.15 K [13]. In this graph, the initial composition, expressed in weight fraction ($w_{glycerol}$, w_{oil}, $w_{tert\text{-}butanol}$), of the different glycerolysis systems listed in Table 3 were also shown. Although the binodal curves can be different for the oils compared in this work, the miscibility region is expected to be of the same order. From this graph, it can be observed that in most glycerolysis systems, a homogenous phase was obtained by adding enough tert-butanol. That is, the initial glycerolysis composition lies in the one phase region. However, in both glycerolysis studies for olive oil [16, 17], around 45 % weight percentage of tert-butanol was added to system. This amount seems to be not enough to create a homogenous phase. This can explain the low MAG yield obtained in these studies (around 65 %) compared to the other systems. In any case, MAG and DAG formed during the glycerolysis can act as emulsifier to avoid somehow mass transfer limitation. Nonetheless, in these cases, mass transfer limitation probably was present at the beginning of the process. Figure 6 shows the initial reaction rates as a function of initial MR glycerol:oil . It can be observed there was an increase of the initial reaction rate for MAG and glycerol with MR up to 3. At MR larger than 3, a decrease was observed. This could be due to glycerol inhibition of the lipase-catalyzed reaction at a high MR glycerol:oil. The initial reaction rates for TAG consumption and DAG and FFA production continuously decreased on increasing the MR. According to Figures 3 and 6, DAG production is favored by restricting the glycerol amount in the reaction medium. Similar findings were observed in other glycerolysis studies [16]. Krüger et al. [16] reported lower values for the initial reaction rates in the glycerolysis of olive oil at 328 K, 15 wt% of Novozyme and tert-butanol to substrate volume ratio of 1:1 (approximately 45 wt% of tert-butanol, see Table 3). These authors obtained initial reaction rates of 2.136, 1.301 and 1.293 mmol/min at MR of 3:1, 6:1 and 9:1, respectively. The low values obtained by Kruger et al. [16] compared to the values obtained in this work (Figure 6) can be explained by assuming more mass transfer limitation at the beginning of the process due to incomplete miscibility of the reactants (see Figure 5). These authors also reported initial reaction rates for DAG production at the conditions previously detailed of 0.375, 0.221 and 0.208 mmol/min at MR of 3:1, 6:1 and 9:1, respectively. These values are of the same order as the ones obtained in this work (Figure 6). Finally, an optimal mole ratio of 3:1 was chosen taking into account the different effects of the amount of glycerol on the glycerolysis kinetics.

In a previous work, tert-pentanol was used as the organic solvent [15]. In that work, the effect of the glycerol:oil mole ratio on the MAG equilibrium yield and reaction rate was studied at three different values of 1:1, 3:1 and 5:1. A MAG yield up to 90 % was reached at a mole ratio of 3:1, which was slightly higher than the value found

when using tert-butanol as the solvent (84 %). No differences in the MAG yield at a higher mole ratio could be observed for both tert-alcohols. In addition, higher initial reaction rates were observed when using tert-pentanol as the solvent. These findings can be related to the polarity of the solvents (log P_{TB} = 0.35 and log P_{TP} =0.85) and the hydrophobicity of the support of Lipozyme® 435. Due to the higher hydrophobicity of tert-pentanol, the diffusion of reactants to the active site of the enzyme is favored.

Table 5 lists the kinetic parameters (k_1-k_{12}) for the model used in this work and the values of the objective function for the different kinetic experiments. At the different mole ratios studied, the rate constant of the second step, k_3, (DAG to produce MAG) is larger than the first step, k_1, (TAG to DAG). Therefore, the initial breakdown of TAG is slower and it is the rate limiting step. Formation of MAG due to Eq. 3 was found to be negligible (k_6 = 0), as well as hydrolysis of TAG, k_7. From the values of the model parameters, it can be concluded that the esterification rates for glycerol, k_{12}, MAG, k_{10}, and DAG, k_8, with free fatty acids followed the sequence $k_8 < k_{10} < k_{12}$. This result was due to the steric hindrance of these groups [21]. This trend was also observed by Moquin et al. [9] in their kinetic modeling of the glycerolysis of soybean oil in supercritical carbon dioxide medium and Voll et al. [17] using an ordered-sequential Bi Bi mechanism in the glycerolysis of olive oil in tert-butanol. However, Valerio et al. [11] reported the order $k_{10} << k_{12} < k_8$ for a solvent-free Novozym 435 catalyzed glycerolysis of olive oil in a surfactant system. Based on the values of the model parameters (Table 5), the production rate of MAG by esterification of glycerol, k_{12}, is of the same order as the production rate by the hydrolysis reaction of DAG, k_9.

It has been described that the MAG yield is favored at mole ratios larger than the stoichiometric (2:1). Voll et al. [17] proposed that the most obvious hypothesis is that the excess of glycerol can react with DAG to produce 2 moles of MAG (see equations 1-2). This was reflected in the value of the k_3 parameter as a function of mole ratio. k_3 increased sharply from the MR of 1 to 3 and then remained constant, similar to the MAG equilibrium yield dependence on MR (Figure 4). To the contrary, Voll et al. [17] found in their kinetic model that the kinetic parameter for the DAG to MAG step was negligible and attributed the increase in MAG yield with an excess of glycerol to the hydrolysis/esterification steps (equations 4-6). In our study, the kinetic parameter of the hydrolysis of TAG, k_7, was found negligible, although, the parameter for DAG hydrolysis, k_9, and esterification of FFA formed, k_{12}, were considerable.

The continuous lines in Figure 3 are from the model proposed in this work. Good agreement between experimental and calculated product concentrations can be observed.

3.2.2 Effect of temperature

Different kinetic experiments were carried out at different reaction temperatures from 303 to 333 K with an enzyme concentration of 10 wt% (based on substrate weight) and at the previous identified optimal glycerol:sardine oil ratio of 3:1. Figure 7 shows the experimental kinetic data at the different reaction temperatures. The reaction rates of MAG, DAG and FFA formation as well as TAG and glycerol consumption

increased on increasing the reaction temperature (initial reaction rates values at 303, 313, 323 and 333 K for MAG production were 2.69, 4.26, 8.16 and 9.69 mmol·min^{-1}·L^{-1} respectively). An increase of the reaction rate by 3.0 times was obtained from 303 to 323 K. Guo and Xu [10] found an increase by 2.2 times from 303 to 323 K, and Krüger et al. [16] by 1.8 times from 313 to 343 K. An optimal working temperature in the range of 313 – 338 K was reported for Novozyme 435 [11]. From Figure 7, it can be also observed that the reaction temperature has only a slight effect on the equilibrium product concentrations. This behavior with temperature was also observed for other transesterification reactions [16, 33].

Table 6 lists the kinetic parameters (k_1-k_{12}) of the semi-empirical model and the values of the objective function at the different temperatures used in this work. The continuous lines in Figure 7 are from our model. Good agreement can be observed between experimental and calculated product concentrations.

An Arrhenius type dependence of temperature on reaction rate was found:

$$k_i = k_{i,o} exp\left(\frac{E_{act}}{RT}\right) \qquad [22]$$

where $k_{i,o}$ is the preexponential factor, E_{act} the activation energy and R is the gas constant. Table 7 lists the activation energy calculated by fitting the kinetic reaction rate constants of Table 6 to the Arrhenius equation. The most activated steps were steps 1, 2 and 5 (E_{a1}, E_{a2} and E_{a5}) that correspond to TAG consumption and reaction of DAG and MAG. The other reaction steps have small or nearly no dependence on reaction temperature. Only few activation energy data were found in the literature for the different steps in the glycerolysis process and a comparison with literature data is difficult. Guo and Xu [10] reported a single activation energy of 33.20 kJ/mol for MAG formation by glycerolysis of sunflower oil with Novozym 435 in tert-butanol in a temperature range of 298-328 K. This value is of the same order as the highest values listed in Table 7 for the glycerolysis steps. Table 7 also shows the activation energy values reported by Voll et al. [17] using an order sequential Bi-Bi mechanism in the fitting for the glycerolysis of olive oil by Novozym 435 in tert-butanol. As can be clearly observed, a different order of activation energy is obtained for the different steps proposed, especially for the activation energy of the hydrolysis of MAG (step 11) and the reverse esterification of the free fatty acid (step 12). Table 7 also presents the activation energy values obtained by Valerio et al. [11] in the glycerolysis of olive oil by Novozym 435 in a surfactant system. A comparison of the solvent systems (tert-butanol in this case) and surfactant systems is difficult since the kinetic parameters for enzyme behavior depended strongly on the solvent medium [10]. In any case, TAG consumption by reaction with glycerol seems to be temperature dependent in all the cases shown in Table 7. Valerio et al. [11] found that the most sensitive step to temperature was the reaction of TAG with MAG (E_{a5}) with an extremely high activation energy.

Table 8 shows the root mean square deviation, rmsd, calculated from Eq. 14 for all the kinetic experiments performed in this work at different temperatures and mole ratios. The low rmsd values obtained for all the products composition, less than 5 wt%, proved that the model fitted the glycerolysis system at the experimental conditions used. Table 8 summarized the rmsd obtained by Voll et al. [17] when correlating kinetic data to an

ordered-sequential Bi Bi mechanism and the values obtained in this work. Similar values of rmsd are obtained for both models.

4. Conclusions

Glycerolysis of sardine oil using Lipozyme® 435 was carried out at different catalyst concentrations, glycerol:oil mole ratios and reaction temperatures. A homogeneous phase was created by adding an optimized amount of tert-butanol based on phase equilibrium calculations. It was shown that external and internal diffusion limitation can be considered negligible and the surface reaction was the rate controlling step. A lipase loading of 10 wt% of unsieved Lipozyme® 435 based on reactant weight was used in all kinetic experiments. A mole ratio of glycerol:oil of 3:1 was the optimum and it produced more than 84 wt% of MAG at 50ºC. Experimental kinetic data were successfully correlated with a kinetic model based on the reversible elementary reactions. TAG consumption by reaction with glycerol and the reverse reaction are the steps more dependent on temperature. This results agrees with those for the TAG consumption in the literature. However, different kinetic parameters for most of the different steps involved in the glycerolysis system can be found in the literature and further studies are needed to be sure of the kinetic parameters of the different steps.

Acknowledgements

Thanks to the Spanish Government through MINECO (CTQ2012-39131-C02-01) and CDTI (Ref. IDI-20111225) for financial support, and to Industrias Afines S.L. for kindly supplying the sardine oil used in this work. AGS acknowledges University of Burgos for a pre-doctoral fellowship.

References

[1] De Deckere E A M, Korver O, Verschuren P M, Katan M B. *Eur J Clin Nutr*, 1998, **52** (10): 749

[2] Kris-Etherton P M, Harris W S, Appel L J. *Circulation*, 2002, **106** (21): 2747

[3] Nichols P D, McManus A, Krail K, Sinclair A J, Miller M. *Nutrients*, 2014, **6** (9): 3727

[4] Hernandez E M. *Lipid Technol*, 2014, **26** (5): 103

[5] Lawson L D, Hughes B G. *Biochem Biophys Res Commun*, 1988, **152** (1): 328

[6] Zhong N, Li L, Xu X, Cheong L, Li B, Hu S, Zhao X. *J Am Oil Chem Soc*, 2009, **86** (8): 783

[7] Bornscheuer U T. *Enzyme Microb Technol*, 1995, **17** (7): 578

[8] Damstrup M L, Jensen T, Sparsø F V, Kiil S Z, Jensen A D, Xu X. *J Am Oil Chem Soc*, 2005, **82** (8): 559

[9] Moquin P H L, Temelli F, King J W, Palcic M M. *J Am Oil Chem Soc*, 2005, **82** (8): 613

[10] Guo Z, Xu X. *Green Chem*, 2006, **8** (1): 54

[11] Valério A, Krüger R L, Ninow J, Corazza F C, De Oliveira D, Vladimir Oliveira J, Corazza M L. *J Agric Food Chem*, 2009, **57** (18): 8350

[12] Fiametti K G, Ustra M K, De Oliveira D, Corazza M L, Furigo Jr A, Vladimir Oliveira J. *Ultrason Sonochem*, 2012, **19** (3): 440

[13] Solaesa Á G, Bucio S L, Sanz M T, Beltrán S, Rebolleda S. *Fluid Phase Equilib*, 2013, **356** (1): 284

[14] Damstrup M L, Abildskov J, Kiil S, Jensen A D, Sparsø F V, Xu X. *J Agric Food Chem*, 2006, **54** (19): 7113

[15] Solaesa Á G, Sanz M T, Falkeborg M, Beltrán S, Guo Z. *Food Chem*, 2016, **190** (1): 960

[16] Krüger R L, Valério A, Balen M, Ninow J L, Oliveira J V, de Oliveira D, Corazza M L. *Eur J Lipid Sci Technol*, 2010, **112** (8): 921

[17] Voll F, Krüger R L, de Castilhos F, Filho L C, Cabral V, Ninow J, Corazza M L. *Biochem Eng J*, 2011, **56** (3): 107

[18] Majid N, Cheirsilp B. *Int J Food Sci Technol*, 2012, **47** (4): 793

[19] Zeng F K, Yang B, Wang Y H, Wang W F, Ning Z X, Li L. *J Am Oil Chem Soc*, 2010, **87** (5): 531

[20] Yang T, Rebsdorf M, Engelrud U, Xu X. *J Agric Food Chem*, 2005, **53** (5): 1475

[21] Tan T, Yin C. *Biochem Eng J*, 2005, **25** (1): 39

[22] Cheirsilp B, Kaewthong W, H-Kittikun A. *Biochem Eng J*, 2007, **35** (1): 71

[23] Solaesa Á G, Bucio S L, Sanz M T, Beltrán S, Rebolleda S. *J Oleo Sci*, 2014, **63** (5): 449

[24] Sanz M T, Murga R, Beltrán S, Cabezas J L, Coca J. *Ind Eng Chem Res*, 2002, **41** (3): 512

[25] Helfferich F G. Ion Exchange. New York: McGraw-Hill, 1962

[26] Chesterfield D M, Rogers P L, Al-Zaini E O, Adesina A A. *Chem Eng J*, 2012, **207-208** (1): 701

[27] Dong H P, Wang Y J, Zheng Y G. *J Mol Catal B Enzym*, 2010, **66** (1-2): 90

[28] Fogler H S. Elements of Chemical Reaction Engineering. Thrid ed. Prentice-Hall International, Inc., 1999

[29] Reid R C, Prausnitz J M, Poling B E. The Properties of Gases & Liquids. Fourth ed. McGraw-Hill Book Company, 1986

[30] Olivares-Carrillo P, Quesada-Medina J, Pérez de los Ríos A, Hernández-Fernández F J. *Chem Eng J*, 2014, **241** (1): 418

[31] Bailey J E, Ollis D F. Biochemical Engineering Fundamentals. 1986

[32] Pleiss J, Fischer M, Schmid R D. *Chem Phys Lipids*, 1998, **93** (1-2): 67

[33] Bucio S L, Solaesa Á G, Sanz M T, Melgosa R, Beltrán S, Sovová H. *J Oleo Sci*, 2015, **64** (4): 431

[34] Sengwa R J, Khatri V, Choudhary S, Sankhla S. *J Mol Liq*, 2010, **154** (2-3): 117

[35] Young F V K, The Chemical & Physical Properties of Crude Fish Oils for Refiners & Hidrogenators, Fish Oil Bulletin, 1986.

[36] Chowdhury F I, Saleh M A. *J Mol Liq*, 2014, **191** (1): 156

[37] Pawongrat R, Xu X, H-Kittikun A. *J Sci Food Agric*, 2008, **88** (2): 256

[38] Pawongrat R, Xu X, H-Kittikun A. *Food Chem*, 2007, **104** (1): 251

Table 1. Initial rate of MAG formation as a function of stirring speed (T = 303.15 K, 2.5 % enzyme loading based on substrate weight, MR = 3:1). Viscosity of reaction compounds.

Orbital speed, rpm	r_o, mmol·L^{-1}·min^{-1}	
120	0.7 ± 0.1	
170	0.8 ± 0.2	
200	0.8 ± 0.1	
	Viscosity, mPa·s	
Compound	303 K	323 K
Glycerol [34]	612	142
Fish oil [35]	60-90*	20-30
Tert-butanol [36]	3.392	1.421

*: value at 298.15 K

Table 2. Thiele modulus and parameter values used in its calculation

Parameter	Value
$V_{glycerol}$	94.82 cm^3·mol^{-1}
$V_{fish\ oil\ =\ triolein}$	1359.20 cm^3·mol^{-1}
$\Phi_{glycerol} = 0.019 \pm 0.004 < 0.3$	$\Phi_{fish\ oil} = 0.35 \pm 0.09 \sim 0.3$

Table 3. Equilibrium composition of glycerolysis reaction found in this work and for other glycerolysis systems found in the literature that use tert-butanol as solvent and immobilized *Candida antarctica* as biocatalyst.

Oil	T, K	% E	MR	% TB	% MAG	% DAG	% TAG	% FFA	Reference
Sardine	323	10	1:1	63	43.0 ± 1.5	25.8 ± 1.9	24.6 ± 1.5	6.6 ± 1.1	This work
			3:1	68	83.3 ± 2.1	6.9 ± 1.1	5.9 ± 1.0	3.8 ± 1.1	
			5:1	68	89.1 ± 1.8	3.7 ± 0.8	3.0 ± 1.0	4.0 ± 1.3	
			9:1	74	92.9 ± 1.5	2.0 ± 0.7	2.4 ± 0.8	2.8 ± 1.1	
Sunflower	323	21	4:1	73	71.3	22.1	0.6	5.2	[8]
Sunflower [a]	313	15	4.5:1	60	70	25	1	4	[20]
Tuna	318	15	4:1	58.6	90.8	2.5	5.5	1.2	[37]
Camelia	323	5	4:1	66	74.1 ± 2.7	24.6 ± 0.1	1.3 ± 0.1	- -[b]	[19]
Olive [a, c]	328	10	6:1	45	67	17	12	4	[16]
	328	2.5	3:1	45	34	15	50	1	
	328	2.5	3:1	80	42	19	36	3	
	343	2.5	9:1	45	53	11	33	3	
	343	2.5	9:1	80	60	14	23	3	
Olive [a]	328	10	6:1	45	~62	~19	~15	~4	[17]

(a) Graphical lecture

(b) No reference to FFA formation

(c) Data at 720 min of reaction time.

Table 4. Composition of medium chain length fatty acids in the oils used in the glycerolysis systems listed in Table 3.

Oil	Medium chain length fatty acids (%)			Reference
	C14:0	C16:0	C16:1	
Sardine	12.4 ± 0.4	22.8 ± 0.2	12.5 ± 0.1	This work
Tuna	4.2	30.6	4.7	[38]
Sunflower	0.1	6.7	0.2	[8]
Olive	0.1 – 1.2	7.0 – 16.0	-	[16, 17]
Camellia	-	8.2	-	[19]

Table 5. Calculated kinetic parameters at different glycerol:oil mole ratios (T = 323.15 K, 10 wt% Lipozyme® 435 based on substrate weight). Objective function and root mean square deviation (wt %) for the glycerolysis products.

Model	Mole ratio			
parameter	1:1	3:1	5:1	9:1
k_1	0.0350	0.0264	0.0263	0.0285
k_2	0.0023	0.0248	0.0292	0.0276
k_3	0.7638	0.9167	0.9411	0.9411
k_4	0.0749	0.0400	0.0348	0.0338
k_5	0.0108	0.0096	0.0047	--
k_6	--	--	--	--
k_7	--	--	--	--
k_8	0.0711	0.0046	--	--
k_9	1.8168	1.9017	1.9022	1.9019
k_{10}	0.4052	0.0234	0.0202	0.0209
k_{11}	0.5853	0.8911	0.8942	0.8983
k_{12}	1.9714	1.8052	1.8033	1.8008
O.F.	0.0019	0.0013	0.0009	0.0012
Root mean squared deviation (wt %)				
TAG	4.7	3.4	5.6	4.6
DAG	1.0	0.9	0.4	0.2
MAG	3.8	2.7	5.8	5.6
FFA	1.1	0.7	0.5	0.3
Glycerol	0.4	1.3	1.7	2.2
Water	0.2	0.3	0.1	0.3

Table 6. Calculated kinetic parameters at different reaction temperatures (MR = 3:1, 10 wt% Lipozyme® 435 based on substrate weight). Objective function and root mean square deviation (wt %) for the glycerolysis products.

Model	Reaction Temperature, K			
parameter	303	313	323	333
k_1	0.0104	0.0164	0.0264	0.0357
k_2	0.0106	0.0201	0.0248	0.0494

k_3	0.9132	0.9132	0.9167	0.9194
k_4	0.0380	0.0395	0.0400	0.0436
k_5	0.0072	0.0114	0.0096	0.0174
k_6	--	--	--	--
k_7	--	--	--	--
k_8	0.0041	0.0049	0.0046	0.0049
k_9	1.9020	1.9017	1.9017	1.9021
k_{10}	0.0207	0.0223	0.0234	0.0241
k_{11}	0.8967	0.8923	0.8911	0.8970
k_{12}	1.8017	1.8041	1.8052	1.8069
O.F.	0.0027	0.0021	0.0013	0.0030
Root mean squared deviation (wt %)				
TAG	4.7	4.7	3.4	5.5
DAG	0.6	0.7	0.9	0.6
MAG	2.8	3.1	2.7	4.7
FFA	0.4	0.6	0.7	0.7
Glycerol	1.9	2.3	1.3	1.7
Water	0.4	0.2	0.3	0.3

Table 7. Activation energy, kJ/mol, for the different steps in some glycerolysis systems

Step	Ea,i (this work)	Ea,i [17]*	Ea,i [11]**
1	35.18	18.30	27.91
2	40.12	$5.36 \cdot 10^{-5}$	0.06
3	0.20	--	47.08
4	3.52	$1.097 \cdot 10^{-4}$	$2.68 \cdot 10^{-12}$
5	20.64	$8.397 \cdot 10^{-4}$	208.17
6	--	0.35	11.46
7	--	15.55	62.83
8	4.10	--	13.33
9	0.001	2.33	$1.11 \cdot 10^{-13}$
10	4.20	--	69.46
11	0.009	45.46	71.48

12	0.08	15.77	81.77

(*) Solvent = tert-butanol

(**) Surfactant system

Table 8. Root mean square deviation (wt%) for glycerolysis products obtained with our model equations (1-6)

TAG	DAG	MAG	FFA	Glycerol	Water	Reference
4.74	0.63	4.02	0.60	1.65	0.25	This work
4.19	2.73	3.58	1.04	--	--	[17]

(--) data not reported

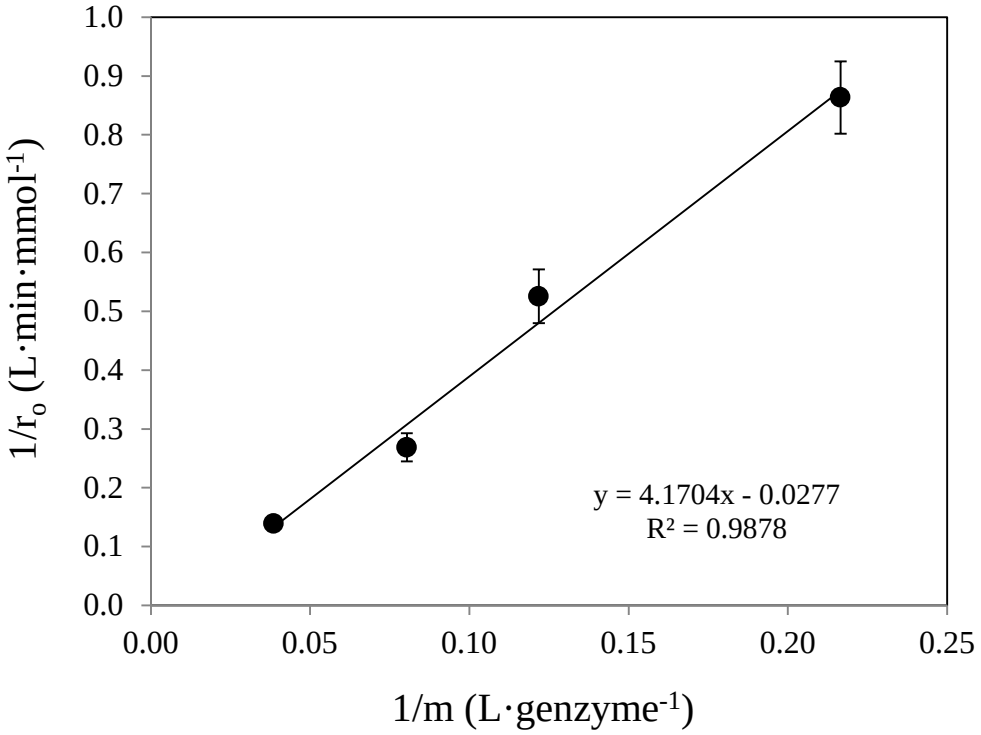


Figure 1. Effect of catalyst loading on initial reaction rate of MAG formation (T = 323 K, MR = 3:1).

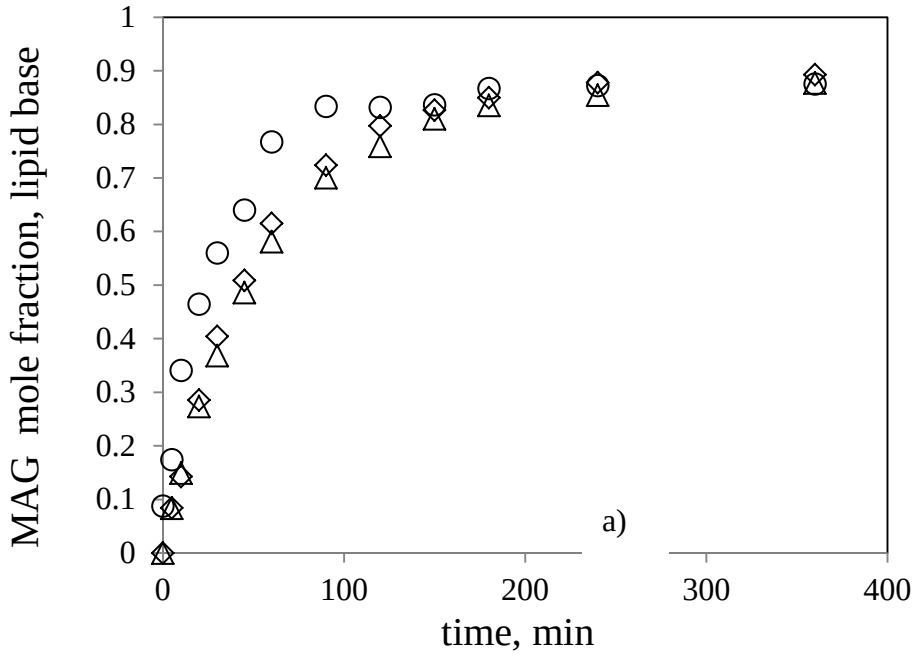
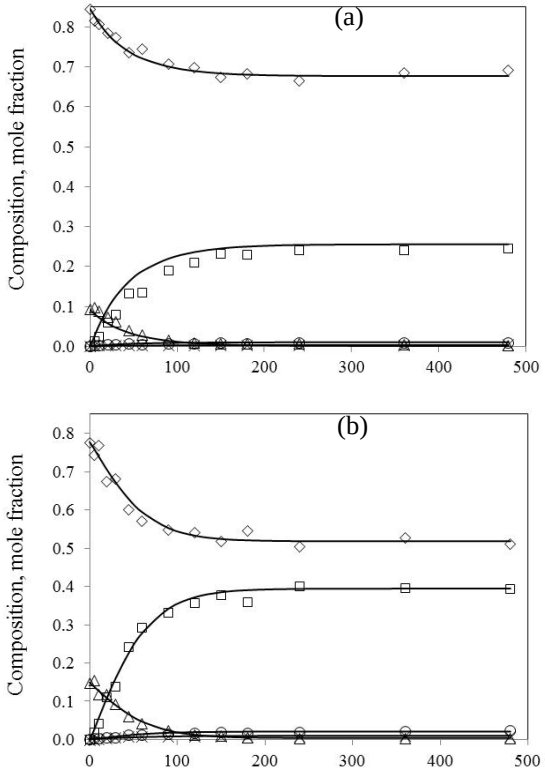


Figure 2. Effect of particle size (○) d_p < 400 µm; (◇) unsieved lipase; (△) d_p > 400 µm on MAG formation reaction: T = 323 K, 5 wt % Lipozyme® 435 loading, MR =3:1. Standard uncertainty u (mole fraction) = 0.02.



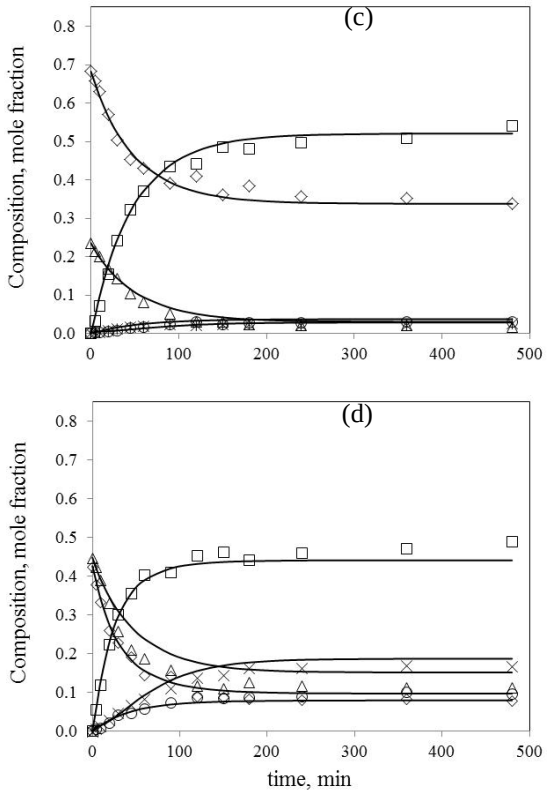


Figure 3. Time course of the glycerolysis reaction at different mole ratios (MR): (a) 9:1, (b) 5:1, (c) 3:1 (d) 1:1; 323 K, 10 wt % Lipozyme® 435 loading; □ MAG ○ FFA ∆ TAG ◇ glycerol × DAG. Continuous lines are for the model in this work. Standard uncertainty u (mole fraction) = 0.02.

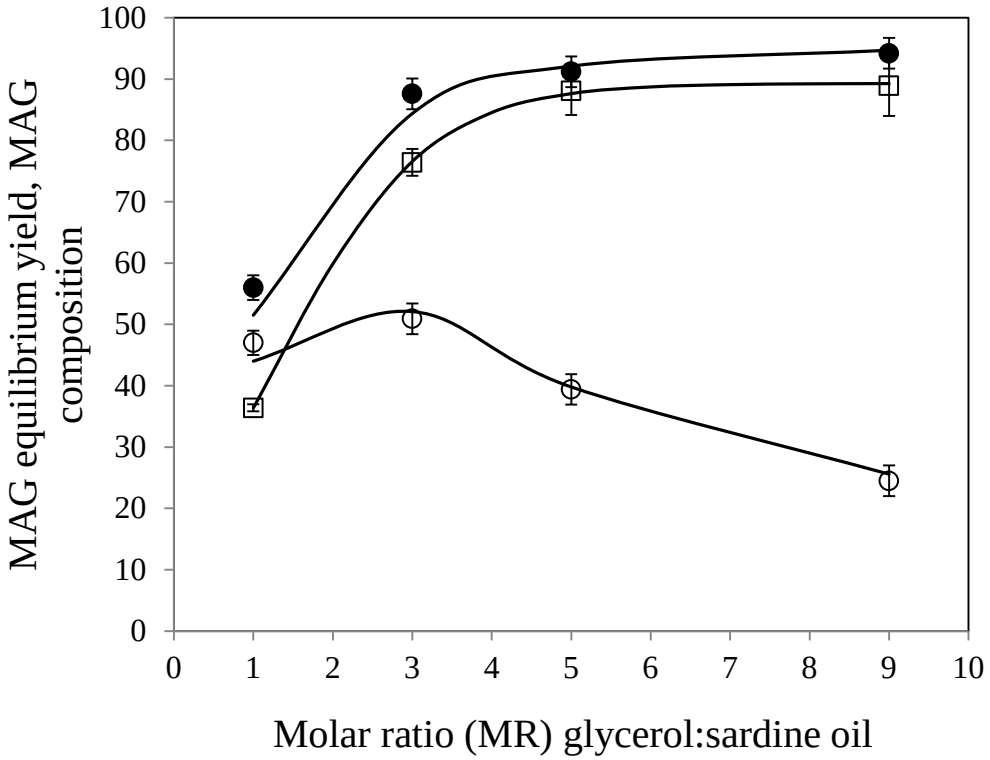


Figure 4. MAG equilibrium yield (□) as a function of initial mole ratio (MR) glycerol:oil. The continuous line is for Eq. 20. MAG composition as mole percentage on a solvent and glycerol free-basis (●) and on a solvent free-basis (○). Continuous lines are the equilibrium composition obtained with the model in this work.

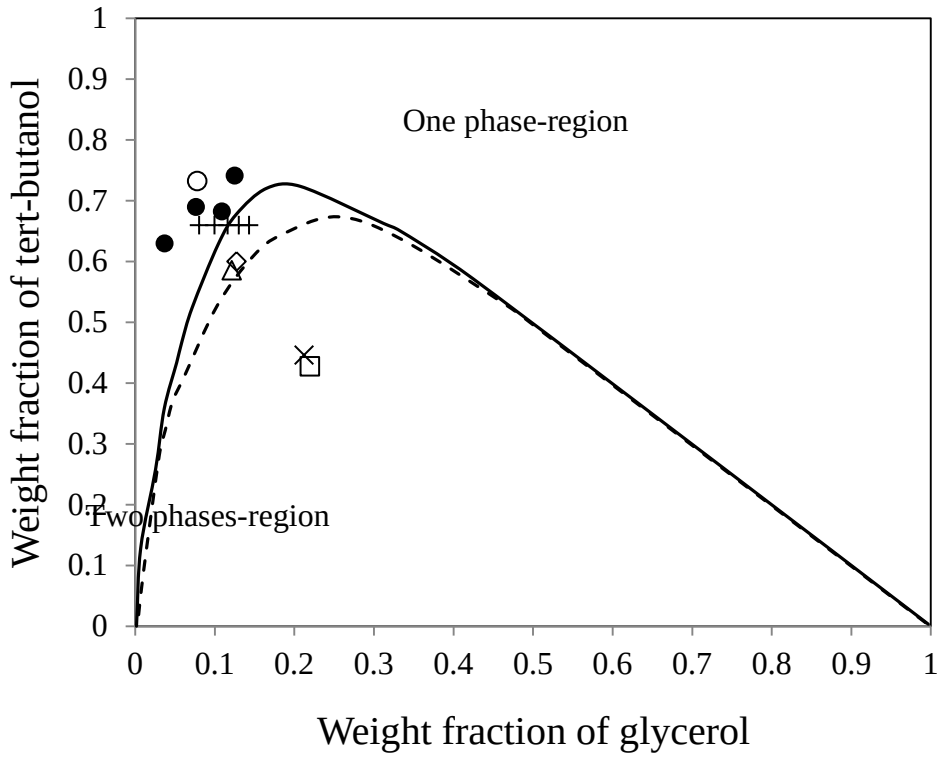


Figure 5. Binodal curve of the ternary system glycerol + fish oil + tert-butanol at 303.15 K (–) and 323.15 K (---). Initial composition of glycerolysis reaction in tert-butanol medium: ● this work, ○ Sunflower oil [8], ◇ Sunflower oil [20], Δ Tuna oil [37], + Camellia oil [19], □ Olive oil [17], × Olive oil [16].

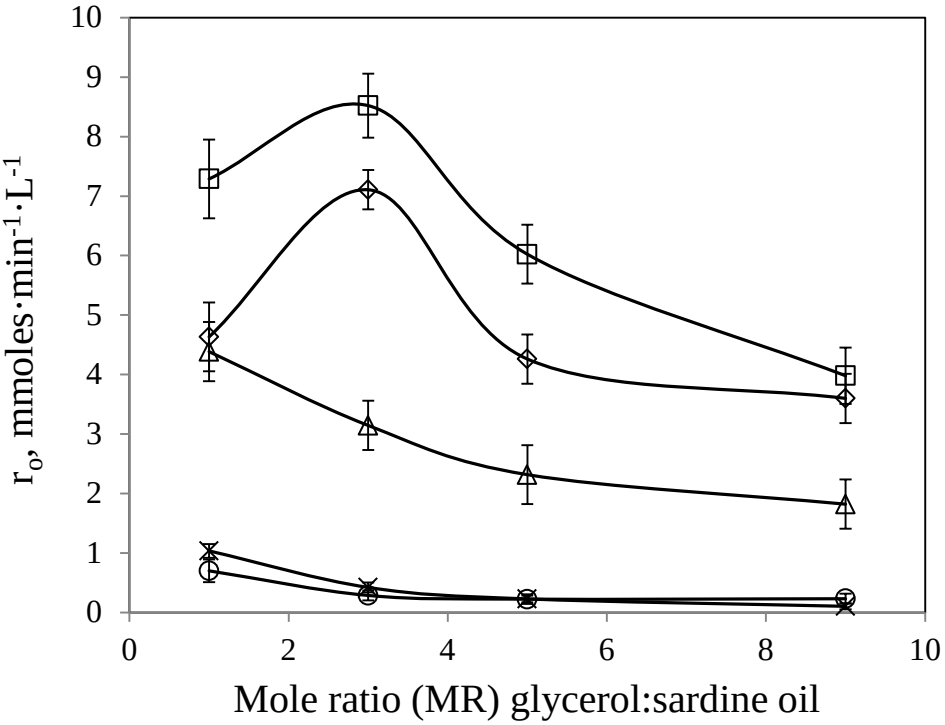
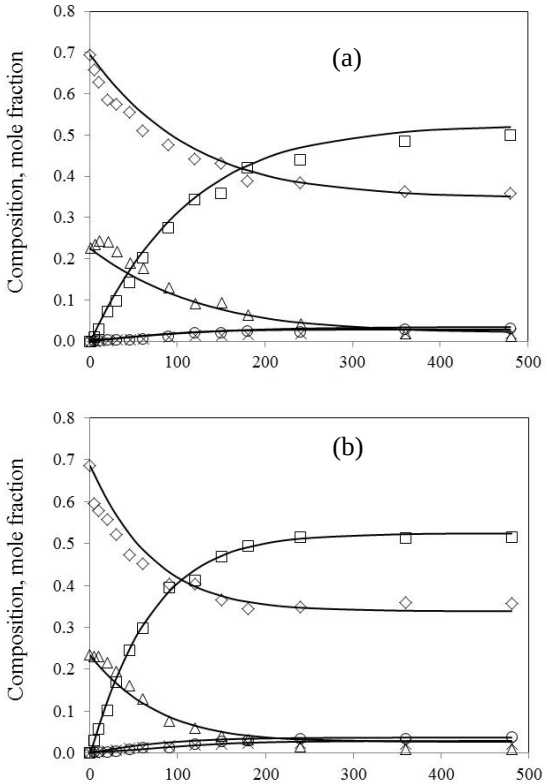


Figure 6. Initial reaction rate as a function of initial mole ratio glycerol:oil (323 K, 10 wt % Lipozyme® 435 loading): □ MAG ○ FFA ∆ TAG ◇ glycerol × DAG.



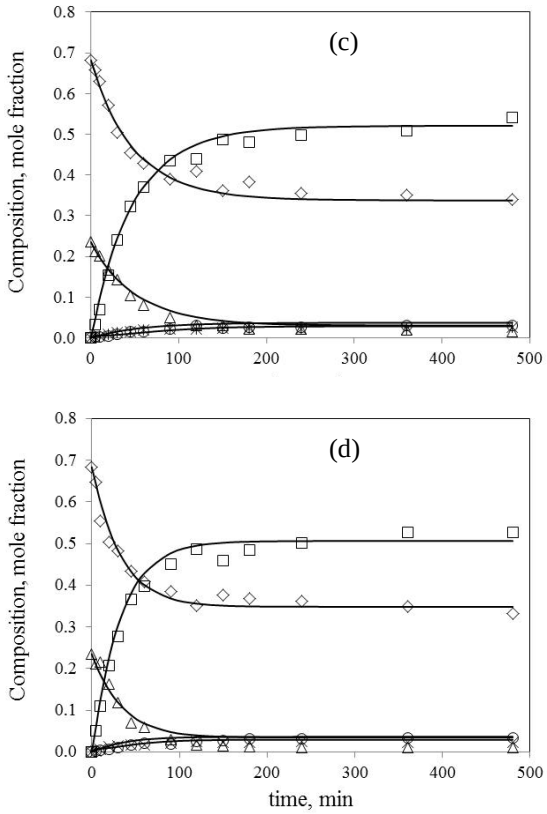


Figure 7. Time course for the glycerolysis reaction at different temperatures: (a) 303 K, (b) 313 K, (c) 323 K, (d) 333 K; 10 wt % Lipozyme® 435 loading, MR =3:1; □ MAG ○ FFA ∆ TAG ◇ glycerol × DAG. Continuous lines are for the model in this work. Standard uncertainty u (mole fraction) = 0.02.

Kinetic study of lipase-catalyzed glycerolysis of sardine oil in a homogeneous media. Comparison of glycerolysis kinetic parameters.

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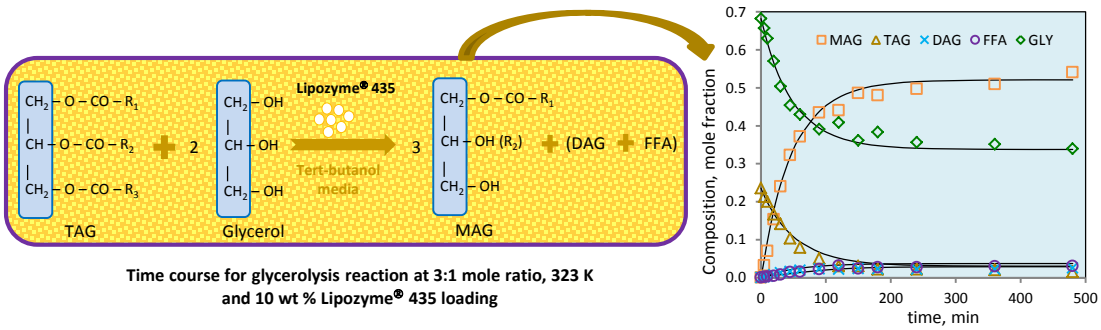
This work presents a detailed kinetic study of enzymatic glycerolysis of sardine oil catalyzed by the commercial lipase Lipozyme® 435. Glycerolysis is carried out in tert-butanol to create a homogeneous system avoiding mass transfer limitations.



Kinetic study of lipase-catalyzed glycerolysis of sardine oil in a homogeneous media.Comparison of

glycerolysis kinetic parameters 均相介质中脂肪酶催化沙丁鱼油甘油解反应动力学研究：甘油解动力学参数的比较

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摘要：多不饱和脂肪酸(PUFAs)的生产多集中在酶催化，与无机催化相比，酶催化具有定向性和更温和的反应条件，因而酶催化制取多不饱和脂肪酸的路线在最近几年引起人们较大的兴趣。本文以优化量的叔丁醇为有机溶剂，研究了脂肪酶 Lipozyme® 435 催化沙丁鱼油甘油解反应生成 PUFA，后者多以甘油一酯或二酯(DAGs, MAGs)的形式存在。首先分析了反应系统的传质影响，考察了脂肪酶载量，温度和进料浓度等因素的影响，采用一个基于甘油酯甘油解和水解这对可逆基元反应的半经验动力学模型，成功地关联了实验的动力学数据。结果表明，50 ^{o}C 甘油与油的摩尔比为 3:1 时最优，生成的 MAG 可达 84wt%以上。本文还考察了其他的甘油解体系，并在 MAG 收率，反应速率和动力学参数意义等方面进行了比较。

关键词：脂肪酶催化；甘油解反应；叔丁醇；传质；动力学模型

收稿日期: 2015-11-18. 接受日期：2016-01-04.

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