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A Kinetic Study of Lipase-Catalyzed Alcoholysis of Palm Kernel Oil

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Abstract

The use of lipases as biocatalysts in interesterification reactions has been the object of growing interest, owing to the importance of esters as emulsifiers, intermediates to produce oleochemicals, and fuel alternatives. We consider in this article a kinetic study of lipase-catalyzed alcoholysis of palm kernel oil, using *n*-hexane as the solvent. In a first step the ester production was maximized by using a Taguchi design, and then an empirical model was built to determine the influence of the process variables. Taking into account the results obtained in the first step, we performed a kinetic study and developed a simple model for this system.

Index Entries: Alcoholysis; lipases; experimental design; vegetable oils; kinetics.

Introduction

Lipases are able to catalyze the hydrolysis, esterification, acidolysis, transesterification, and alcoholysis of a wide variety of substrates and have therefore received much attention as biocatalysts for many processes *(1)*. Examples include the production of high-value specialty fats such as cocoa butter substitute *(2)* and human milk fat substitutes *(3)*, and the production of flavors and fragrances *(4)*. One of the most promising areas of current research is the modification of fats and oils to improve their physical and nutritional properties *(3–6)*.

In this sense, alcoholysis of vegetable oils and animal fats is an important reaction that produces fatty acid alkyl esters that are valuable intermediates in oleochemistry and substitutes for diesel fuel *(7,8)*. Surprisingly, little attention has been focused on the use of lipases for the alcoholysis of triglycerides.

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In a previous study *(9)*, we optimized the lipase-catalyzed ethanolysis of palm and palm kernel oils using Lipozyme IM (*Mucor miehei*) and Novozym 435 (*Candida antarctica*) as catalysts. An experimental design was applied to each system, and the optimal experimental conditions that maximize conversion were established.

However, information regarding the kinetics of the reaction is essential for understanding the reaction mechanism, as well as for a rational design of alcoholysis reactors for future scale up. Taking into account the results obtained previously *(9)*, in this work we have examined the influence of enzyme concentration and oil:ethanol molar ratio on the kinetics of the reaction. The system chosen for this study was palm kernel oil and ethanol using Lipozyme IM as catalyst and *n*-hexane as solvent. Based on a material balance involving four ordinary differential equations, we developed a simple empirical kinetic model with three parameters.

Materials and Methods

Palm kernel oil was used as purchased without any pretreatment. The composition of palm kernel oil was determined by using a gas chromatograph (HP 5890) with a flame ionization detector. The following instrumentation and conditions were used: H_2 as carrier gas, modified polyethylene glycol column (FFAP 2: 25 m × 0.20 mm id × 0.30 μm film), column temperature range of 180–210°C (2°C/min), injector temperature of 250°C, and detector temperature of 280°C. The approximate fatty acid composition in palm kernel oil is 9% capric acid, 47% lauric acid, 15% myristic acid, 9% palmitic acid, and 20% oleic acid. Ethyl alcohol (95%) and *n*-hexane PA were used as substrate and solvent, respectively.

Enzyme

M. miehei (Lipozyme IM) immobilized on a macroporous anion-exchange resin (0.15 U/g and 4% water) was kindly supplied by Novo Nordisk Bioindustrial do Brasil S.A (Araucária, Paraná).

Analytical Methods

Thin-Layer Chromatography

The objective of applying the thin-layer chromatography was to verify the presence of mono-, di-, and triglycerides as well as esters in the oil and reaction products samples. The methodology used in this step was described by Stahl *(10)*.

Using this procedure, we verified that the presence of mono- and diglycerides in the oil and products samples can be neglected. This result was used as a hypothesis in the kinetic study of alcoholysis of palm kernel oil.

Glycerol Concentration

The glycerol content evolved during enzymatic alcoholysis was determined using the method described by Coks and van Rede *(11)*. The reaction

Table 1
Range of Variables

Variable	Range[a]
Temperature [*T*] (°C)	40–70°C
Water concentration [*W*] (% w/w)	0–10%
Enzyme concentration [*E*] (% w/w)	5–20%
Oil-ethanol molar ratio [R]	1:3–1:10

[a]Oil = 1 g; solvent = 40 mL.

Table 2
Experimental Design and Conversions Obtained

Experiment	*T* (°C)	[*E*] (% w/w)	[*W*] (% w/w)	R	Conversion (%)
1	40	5	0	1:3	77.4
2	40	5	10	1:10	52.1
3	70	20	0	1:3	28.9
4	70	20	10	1:10	37.7
5	40	20	10	1:3	62.2
6	40	20	0	1:10	34.2
7	70	5	10	1:3	29.8
8	70	5	0	1:10	32.0
9	55	12.5	5	1:6.5	32.9

conversion was calculated by determining the glycerol concentration assuming a maximum glycerol yield at the end of the reaction of 12% of the mass of oil *(12)*, based on its average molecular weight (701.9).

Experimental Procedure and Statistical Analysis

The experiments were performed in stoppered 125-mL Erlenmeyer flasks. Lipase was added to the mixture of oil-ethanol-*n*-hexane (40 mL), and the flasks were agitated at 200 rpm for 6 h in a controlled-temperature shaker. A Taguchi experimental planning with two levels and four variables (temperature, water, and enzyme concentrations and the oil:ethanol molar ratio) was adopted. The variable ranges, as presented in Table 1, were chosen to cover the intervals commonly used *(13)*. The experiments were accomplished randomly, and duplicate runs were conducted for each experimental condition. The standard deviation (SD) was calculated from duplicate runs, resulting in a reproducibility better than 10%. The process conversion was then modeled by an empirical model.

Results and Discussion

Table 2 presents the experimental design as well as the results obtained for the system containing palm kernel oil and Lipozyme IM, which exhibits specificity in the 1,3 positions. Table 2 shows that the highest yield (77.4%)

was achieved at the inferior conditions of temperature, water, and enzyme concentrations and oil:ethanol molar ratio. From an economic point of view, this result is interesting because palm kernel oil, considered a by-product of palm oil production, can be used as a substrate for the production of high-value-added products.

Effect of Process Variables

The influence of temperature, water, and enzyme concentration and the oil:ethanol molar ratio as well as the cross interactions temperature-enzyme concentration and temperature-oil:ethanol molar ratio were investigated. To allow a direct comparison of each variable effect, the independent variables were normalized in the range of –1 to +1, according to Eq. 1:

$$x_i = [2(X_i - X_{min})/(X_{max} - X_{min})] - 1 \quad (1)$$

in which x_i is the normalized value of the variable X at condition i; X_i is the actual value; and X_{min} and X_{max} represent the inferior and superior limit, respectively. The –1 level represents the inferior limit, whereas the +1 level represents the superior limit of each variable.

A statistical modeling technique was used to obtain an empirical model able to reproduce the experimental data. Empirical models were built by assuming that all variable interactions were significant, estimating the parameters related to each variable interaction and main variable effects and discarding the meaningless parameters considering a confidence level of 95%. The objective of using the Student's *t*-test is to evaluate whether the parameters were significantly different from zero. This test takes into account the SD of each parameter according to a well-known procedure available in many textbooks *(14)*. The parameters were estimated through the Maximum Likelihood Method *(15)*. Related to the parameter analysis, it is important to mention that a parameter with a negative value implies in a negative effect of the variable on the process.

Adopting this methodology, the effect of the variables (temperature, enzyme, and water concentrations and oil:ethanol molar ratio) as well as the cross interactions (temperature-enzyme concentration and temperature-oil:ethanol molar ratio) were studied.

Table 3 and Fig. 1 present the results obtained with this procedure. Table 3 shows that temperature, enzyme concentration, and oil:ethanol molar ratio as well as the cross interactions between temperature-enzyme concentration and temperature-oil:ethanol molar ratio had significant effects on the process conversions. The negative effect presented by temperature was an expected result since this enzyme has optimum activity in temperatures around 40°C. In the case of oil:ethanol molar ratio, it was found that ethanol excess may inhibit the enzymatic reaction, being the highest yield achieved at stoichiometric ratio. The negative effect presented by enzyme concentration is interesting from an economical point of view, but this result might be owing to a combination of the variable ranges

Table 3
Regression Results
for System Palm Kernel Oil
and Lipozyme IM[a]

Parameter	SD
a_0, 43.89	1.00
a_1, –12.05	1.12
a_2, –3.23	0.87
a_3, –5.07	1.10
a_4, 1.39	0.94
a_5, 4.69	1.10
a_6, 8.09	1.10

[a] $Y = a_0 + a_1T + a_2E + a_3R + a_4W + a_5TE + a_6TR$. Average absolute deviation, 4.8.

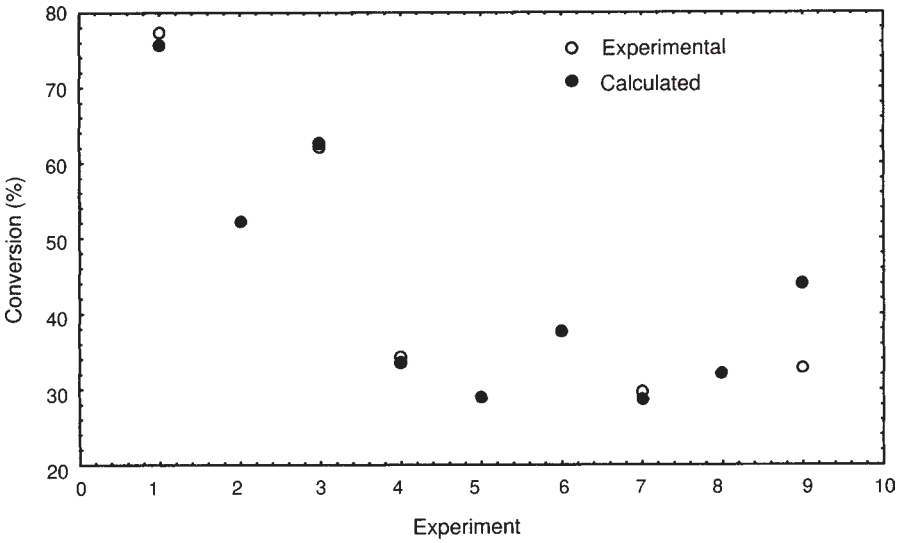

Fig. 1. Experimental and calculated conversions for the system palm kernel oil with Lipozyme IM.

given by experimental design. Figure 1 indicates a good agreement between experimental and calculated conversions and shows that the empirical model is capable of representing the experimental data well.

Kinetic Study

Taking into account the results obtained in the experimental design and statistical analysis, we performed a set of experiments to observe the effect of the enzyme concentration and oil:ethanol molar ratio on the reaction kinetics. These variables were found to have a significant effect on the alcoholysis conversion, as illustrated in Table 3. For this purpose, the other

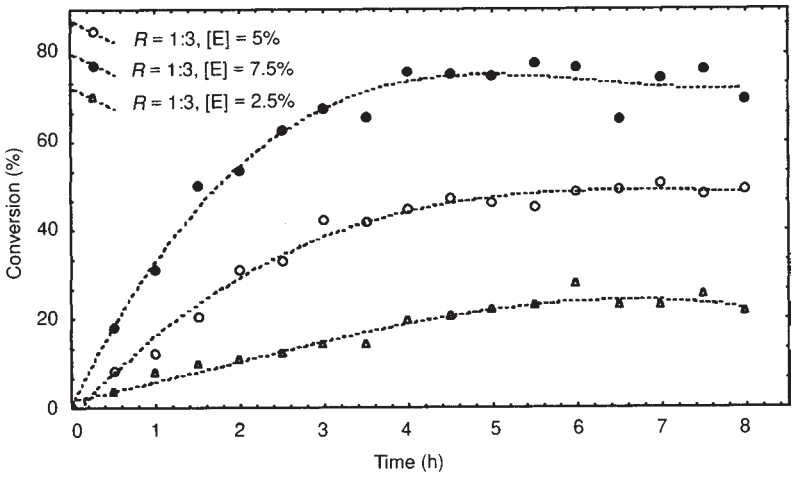

Fig. 2. Influence of enzyme concentration on the kinetics of alcoholysis of palm kernel oil.

variables were maintained at their optimum values (temperature: 40°C; added water concentration: 0% w/w), according to the results of the experimental design.

The effect of immobilized enzyme concentration on the kinetics was observed by varying this variable from 2.5% to 7.5% w/w. Figure 2 presents the results obtained from this step. The enzyme concentration had a positive effect on the kinetics although conclusions can be drawn only from the experimental design for the studied concentrations on that step (5%, 12.5%, and 20% w/w). The isolated effect of the variable on the reaction must be observed through a kinetic study of such a variable, keeping constant all the other variables. In this case, the reaction rate, calculated in the linear range of the curve, expressed in percentage of glycerol/hour, increases linearly with increasing enzyme level (10.05, 20.39, and 30.25 for enzyme concentration of 2.5%, 5%, and 7.5% w/w, respectively); the same behavior was observed for the specific initial rate (4.02, 4.08, and 4.03% glycerol/h·g of lipase, respectively).

The oil:ethanol molar ratio was found to have a significant effect on the alcoholysis of palm kernel oil using Lipozyme IM as catalyst, as also shown in Table 3. These results suggest that this variable has inhibiting effects on the catalytic function of the enzyme. To study these possible effects individually, experiments were conducted maintaining the other variables at a constant concentration while varying the oil:ethanol molar ratio (1:3, 1:5, and 1:7.5). Those results, presented in Fig. 3, indicate that the greatest conversion occurs if the reactants are present in lower amounts. Otherwise, it appears that an excess of ethanol results in a loss of biocatalytic activity. In this case, the reaction rate (5.3, 9.2, and 16.1% glycerol/h for oil:ethanol molar ratio of 1:7.5, 1:5, and 1:3, respectively) also increases linearly with decreasing oil:ethanol molar ratio, corroborating the previous explanation.

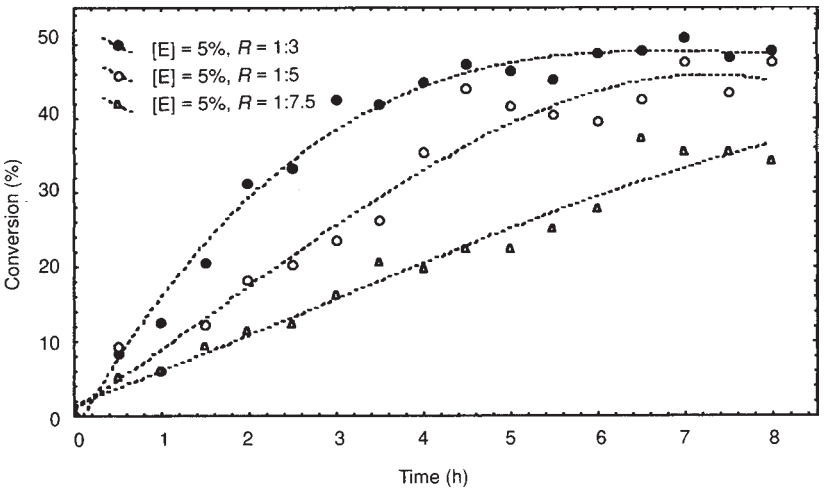

Fig. 3. Influence of oil:ethanol molar ratio on the kinetics of alcoholysis of palm kernel oil.

Kinetic Modeling

The alcoholysis of palm kernel oil and ethanol, catalyzed by *M. miehei* (Lipozyme IM), can be considered as a homogeneous reaction system, which was modeled by a simplified mechanism that consists of only one step:

$$[T] + 3[A] \leftrightarrow [G] + 3[M] \tag{2}$$

in which $[T]$ = triglyceride molar concentration (mmol/mL); $[A]$ = ethanol molar concentration (mmol/mL); $[G]$ = glycerol molar concentration (mmol/mL); and $[M]$ = ester molar concentration (mmol/mL).

To describe a simple mathematical model for the mentioned alcoholysis reaction system, the following assumptions were adopted and later experimentally verified:

1. The equation is reversible.
2. There are no losses of enzyme activity during the course of the reaction.
3. Formation of mono- and diglycerides was not taken into account. Mono- and diglycerides were not detected under the reaction conditions employed for the kinetic study.
4. Mass transfer limitations in the reaction system were ruled out.

Differential equations in the reaction system can be derived from Eq. 3 as follows:

$$\frac{d[G]}{dt} = k1' [T][A]^3 - k2 [G][M]^3 \tag{3}$$

From the stoichiometry,

$$\frac{d[T]}{dt} = \frac{1}{3}\frac{d[A]}{dt} = -\frac{d[G]}{dt} = -\frac{1}{3}\frac{d[M]}{dt} \tag{4}$$

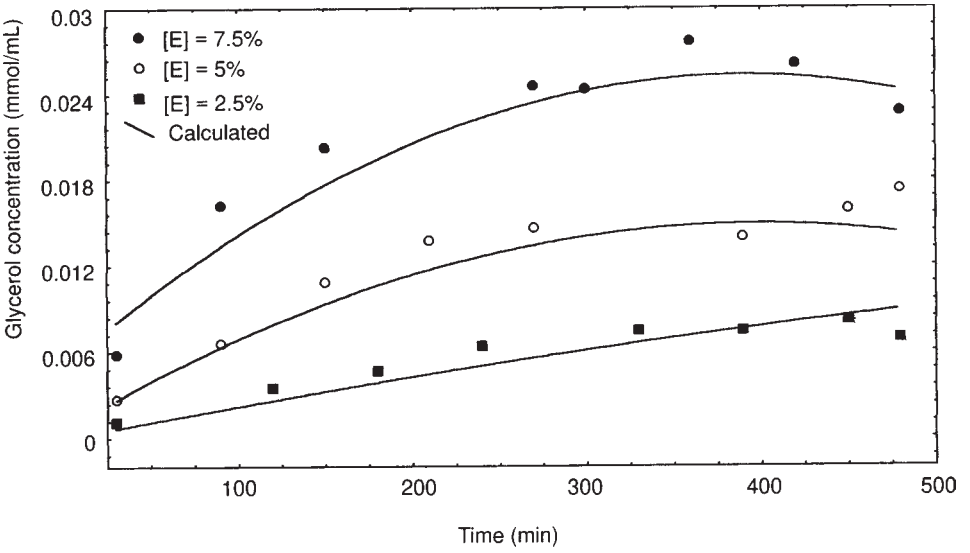

Fig. 4. Experimental vs calculated conversions for the system palm kernel oil and Lipozyme IM, varying the enzyme concentration.

To consider the enzyme concentration and the inhibition caused by an excess of alcohol, the following empirical terms were incorporated into the first parameter ($k1$):

$$k1' = (k1 + k4[E])\,(\exp(k3[A])) \tag{5}$$

in which $[E]$ is the enzyme molar concentration (mmol/mL); and $[A]$ is the alcohol molar concentration.

The differential equations were solved using the software Maple V providing the time profile of glycerol for each condition studied. The kinetic parameters were estimated by using the Maximum Likelihood Method *(15)*. In a first step, the parameters ($k1$, $k2$, and $k3$) were estimated considering only the variation in the alcohol molar concentration and, afterward, the variation in the enzyme concentration by estimating $k1$, $k2$, and $k4$ parameters. The results obtained showed that, when varying the enzyme concentration, the $k4$ parameter had no significant influence on the reaction rate. The main objective of this preliminary study was to evaluate the isolated effect of the enzyme concentration and oil:ethanol molar ratio. Thus, a global fitting was performed keeping the $k4$ parameter constant and estimating $k1$, $k2$, and $k3$, resulting in the following values: $k1 = 0.890$ mL^3/mmol^3·min; $k2 = 2.5812 \times 10^{-8}$ mL^3/mmol^3·min; $k3 = 16{,}5542.76$ mL/mmol; and $k4 = 2.649$ mL^4/mmol^4·min.

By using the F-test *(15)* which compares the model and experimental variances, one can conclude that the model and experimental values are equivalent considering a confidence level of 95%. Based on these results and through Figs. 4 and 5, we found that this simple model was capable of

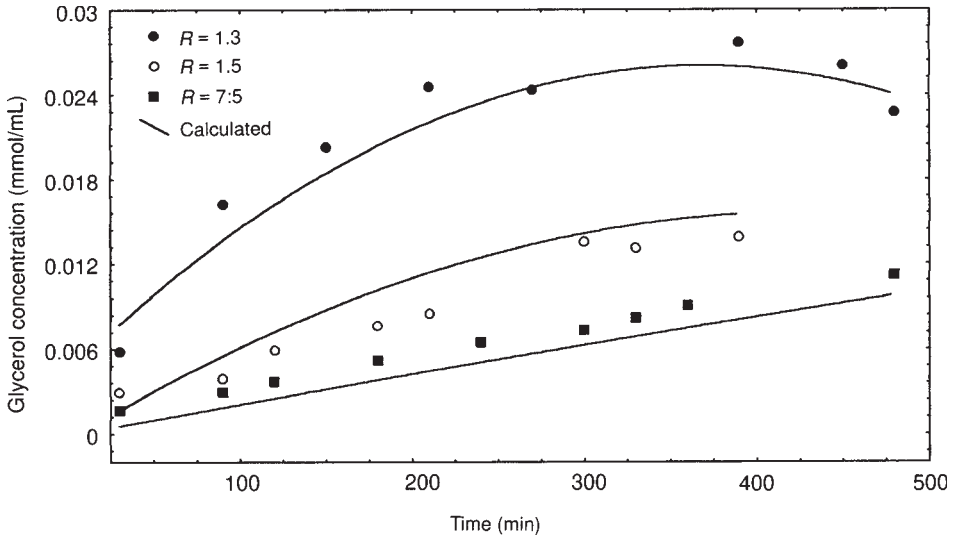

Fig. 5. Experimental vs calculated conversions for the system palm kernel oil and Lipozyme IM, varying the oil:ethanol molar ratio.

reproducing the experimental data in all ranges of experimental variables studied.

Conclusion

The use of an experimental design for the production of esters from enzymatic reactions of vegetable oils proved to be a rational means of investigating the influence of process variables on the conversion. Empirical models were built to represent experimental data and allow the determination of process variables that maximize the conversion.

Using the information obtained in this step, a kinetic study was performed varying the enzyme concentration and oil:ethanol molar ratio and observing the effects of these variables on the reaction kinetics.

An empirical model was then built to describe the kinetic results compared to those obtained experimentally. In spite of being a simple model with only four parameters, a global estimation was performed for all experimental conditions, leading to good agreement with the experimental data.

From this study we can conclude that the enzyme concentration has a positive effect on the reaction kinetics, showing that an increase in this variable causes an increase in the process conversion. With respect to the oil:ethanol molar, we found that it has a negative effect, probably owing to an inhibiting effect caused by excess alcohol.

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