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Enzymatic Alcoholysis of Palm and Palm Kernel Oils

Optimization by Statistical Methods

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Abstract

The use of lipases as biocatalysts in ester synthesis 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 report the application of lipases in the ethanolysis of palm and palm kernel oils to produce fatty-acid esters, using n-hexane as solvent. In order to maximize ester production, we adopted a Taguchi design and built an empirical model. Using this procedure, we determined the optimal condition for each system and established the influence of process variables in the conversion.

Index Entries: Alcoholysis; lipases; experimental design; vegetable oils; fatty acid esters.

Introduction

Lipases have been extensively used in triglyceride technology, mainly for the biotransformation of oils and fats. Among several important processes for lipid modification are the hydrolysis reactions, synthesis of esters, and transesterification of these materials in the presence of lipases. In these reactions, the triglyceride reacts with a fatty acid (acidolysis), an alcohol (alcoholysis), or another ester (interesterification), resulting in a rearrangement of the triglyceride fatty-acid groups to produce a new triglyceride as a consequence of the competitive hydrolysis and esterification reactions.

Esters obtained from alcohols and fatty acids have many remarkable applications. Those from long-chain acids (12–20 carbon atoms) and short-chain alcohols (3–8 carbon atoms) have been widely employed in food, cosmetics, and pharmaceuticals industries *(1)*. Natural esters such as those from jojoba oil, carnauba wax, and whale oil have been used. However, these oils are expensive, and they are not usually available in large amount.

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Therefore it is desirable to develop methods for the production of such esters using cheaper and more plentiful raw materials *(2).* Among several Brazilian raw materials of interest in the production of high-value added products, palm fruit is one of the most prominent. It is widely used for the production of edible oil and the kernel is used as soap.

Chemical esterification methods use an alcohol and a carboxylic acid in the presence of a mineral acid as catalyst. Sulfuric acid, which is commonly used, leads to the formation of undesirable by-products with a difficult separation step *(3).* Moreover, in this case the starting material is a high-value component (fatty acid). Consequently, we are interested in the alcoholysis reaction using a vegetable oil with low cost and largely produced in Brazil as a raw material for ester synthesis.

This reaction offers several advantages when compared to esterification of fatty acids, mainly owing to the possibility of using vegetable oils as substrates. In this case, a vegetable oil and an alcohol are used as substrates in the glycerol and fatty-acid alkyl esters production. Conventionally, acid and base catalysts have been used *(4,5).* However, the use of acid catalysts usually results in low conversions. The use of base catalysts require the utilization of vegetable oils with low free fatty-acids content (3%). In spite of the high yields obtained (90%) when using pre-treated acid oils, a conversion decrease is observed owing to the soap-removing step. The use of enzymes minimizes this problem, because oils with a high acid content can also be used without a pretreatment and no enzymatic activity loss is observed.

Though direct application of alkyl esters is scarce, they can be used as intermediates in the oleochemical production. Furthermore, the possibility of fractionating makes them useful in the food, cosmetics, and pharmaceuticals industries. Notice that this product can be also used as biodiesel *(6).*

Several researches have reported an alternative method to produce esters through enzymatic reactions using lipases as catalysts *(7–13).* Because biocatalysts have high specific activity and a low impact on the environment, they have become increasingly important for industry. For example, immobilized lipases are used as catalysts for reactions involving biomodification of triglycerides *(14).*

The main objective of our work is the production of ethyl esters from the enzymatic alcoholysis of palm and palm kernel oils, using n-hexane as solvent. Two commercial lipases (Novozym 435 and Lipozyme IM) were compared. The variables in these experiments were temperature, water, and enzyme concentrations in the reaction media and the oil:ethanol molar ratio. An empirical model was built to evaluate the effects of process variables on the conversion and thus to determine the operating conditions that maximize the production of esters for each oil and enzyme combination.

Materials and Methods

Palm and palm kernel oils were used as purchased without any pretreatment. The compositions of palm and palm kernel oils were deter-

Table 1
Range of the Variables

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

mined by using a gas chromatograph (HP 5890) with flame ionization detector. The following instrumentation and conditions were used: H_2 as carrier gas; modified polythylene glicol column (FFAP 2 – 25 m × 0.20 mm i.d. × 0.30 μm film); column temperature, 180–210°C (2°C/min); injector temperature, 250°C; and detector temperature, 280°C. Using this procedure, the approximate fatty-acid composition in palm oil is 40% palmitic acid, 5% stearic acid, 45% oleic acid, and 10% linoleic acid. Palm kernel oil is approx 9% caprilic acid, 47% lauric acid, 15% myristic, 9% palmitic acid, and 20% oleic acid. The oils had 14.5% and 12.3% of free fatty acids for palm and palm kernel oils, respectively. Ethyl alcohol (95%) and n-hexane PA were used as substrate and solvent, respectively.

Enzymes

Two commercial immobilized lipases were kindly supplied by Novo Nordisk Bioindustrial Do Brasil S.A. (Araucária, PR): *Mucor miehei* (Lipozyme IM) immobilized on a macroporous anion exchange resin (0.15 U/g and 4% water) and *Candida antarctica* (Novozym 435) immobilized on a macroporous anionic resin (0.12 U/g and 1.4% water).

Analytical Method

The glycerol content evolved during enzymatic alcoholysis was determined using the method described by Coks and van Rede *(15)*. The reaction conversion was calculated by determining the glycerol concentration assuming a maximum glycerol yield at the end of the reaction of 10% of the mass of oil *(16)*, based on its molecular mass (855.3 and 701.9 for palm and palm kernel oils, respectively).

Experimental Procedure and Statistical Analysis

The experiments were performed in stoppered 125-mL Erlenmeyers 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 2 levels and 4 variables (temperature, water, and enzyme concentration and the oil:ethanol molar ratio) was adopted. The variable ranges, as presented in Table 1, were chosen to cover the intervals commonly used *(17)*. The experimental design is shown in Table 2. The experiments were accomplished randomly, and

Table 2
Taguchi Experimental Planning: Experimental Conditions

Exp.	T (°C)	[E] (%)	[W] (%)	O:EtOH
1	40	5	0	1:3
2	40	5	10	1:10
3	70	20	0	1:3
4	70	20	10	1:10
5	40	20	10	1:3
6	40	20	0	1:10
7	70	5	10	1:3
8	70	5	0	1:10
9	55	12.5	5	1:6.5

duplicate runs were carried out for each experimental condition given a reproducibility better than 10%. The process conversion was then modeled by an empirical model.

Results and Discussion

The experimental results for the two oils using Lipozyme IM, which exhibits specificity in the 1,3 positions, and Novozym 435, a nonspecific lipase, are presented in Table 3. We observed that for both oils higher yields were achieved in the system with Lipozyme IM (52% and 77% for the palm oil and palm kernel oil, respectively). However, the yields for this system were more susceptible to the experimental conditions.

Effects of Process Variables

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

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

where 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, while 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

Table 3
Conversions in the Alcoholysis of Palm and Palm Kernel Oils

	Experimental conditions				Conversion (%)			
					Palm oil		Palm kernel oil	
Exp	T (°C)	[E] (%)	[W] (%)	O:EtOH	Lipozyme	Novozym	Lipozyme	Novozym
1	40	5	0	1:3	37.5	39.0	77.4	34.7
2	40	5	10	1:10	42.3	46.1	52.1	42.1
3	70	20	0	1:3	24.8	42.6	28.9	54.5
4	70	20	10	1:10	34.5	42.3	37.7	35.1
5	40	20	10	1:3	52.3	45.0	62.2	41.5
6	40	20	0	1:10	42.8	37.8	34.2	58.3
7	70	5	10	1:3	16.0	44.0	29.8	35.3
8	70	5	0	1:10	19.1	45.4	32.0	29.2
9	55	12.5	5	1:6.5	41.8	37.6	32.9	33.5

Table 4
Regression Results
for the System Palm Oil and Novozym 435

$$Y = a_0 + a_1T + a_2E + a_3R + a_4H + a_5TE + a_6TR$$

*Average absolute deviation = 5.4

Parameter		Standard deviation
a_0	41.97	1.69
a_1	1.10	1.78
a_2	–0.42	1.78
a_3	–0.41	1.75
a_4	1.50	1.80
a_5	–0.69	1.78
a_6	0.70	1.75

$$*AAD\% = \frac{1}{N}\sum_{i}^{N}\frac{|Y_i^{exp} - Y_i^{calc}|}{Y_i^{exp}} \times 100$$

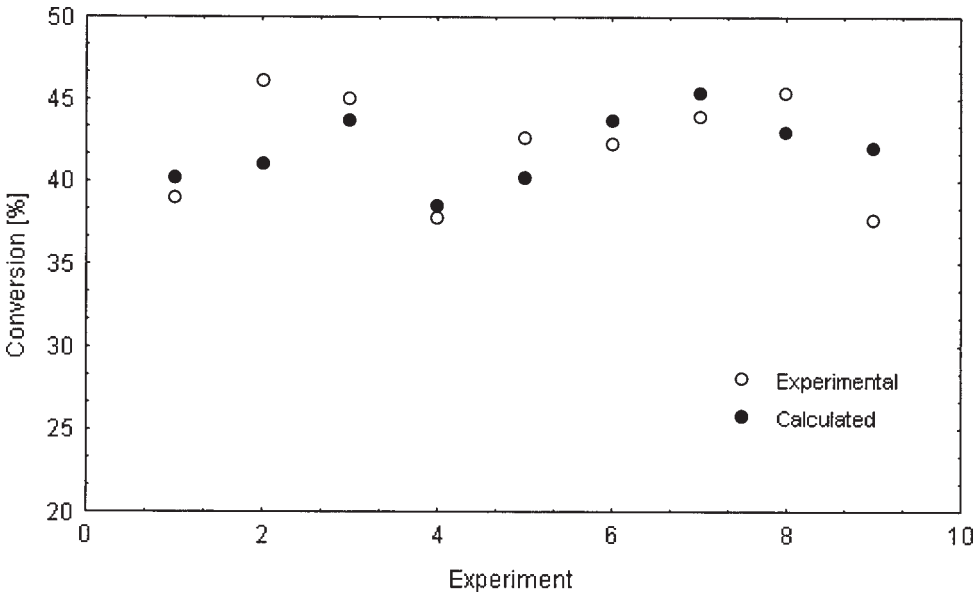

Fig. 1. Experimental and calculated conversions for the system palm oil with Novozym 435.

parameters considering a confidence level of 95%, by using the Student's *t*-test. The parameters were estimated through the Maximum Likelihood Method *(18)*.

Reactions Catalyzed by Novozym 435

According to the Student's *t*-test, no variable is significant for palm-oil conversion within the variables range (Table 4). Figure 1 shows a good fit

Table 5
Regression Results
for the System Palm Kernel Oil
and Novozym 435

$Y = a_0 + a_1T + a_2E + a_3R + a_4H + a_5TE + a_6TR$		
Average absolute deviation = 3.6		
Parameter		Standard deviation
a_0	41.03	0.46
a_1	–3.15	0.41
a_2	6.01	0.56
a_3	–0.09	0.56
a_4	–2.66	0.53
a_5	0.38	0.55
a_6	–6.31	0.55

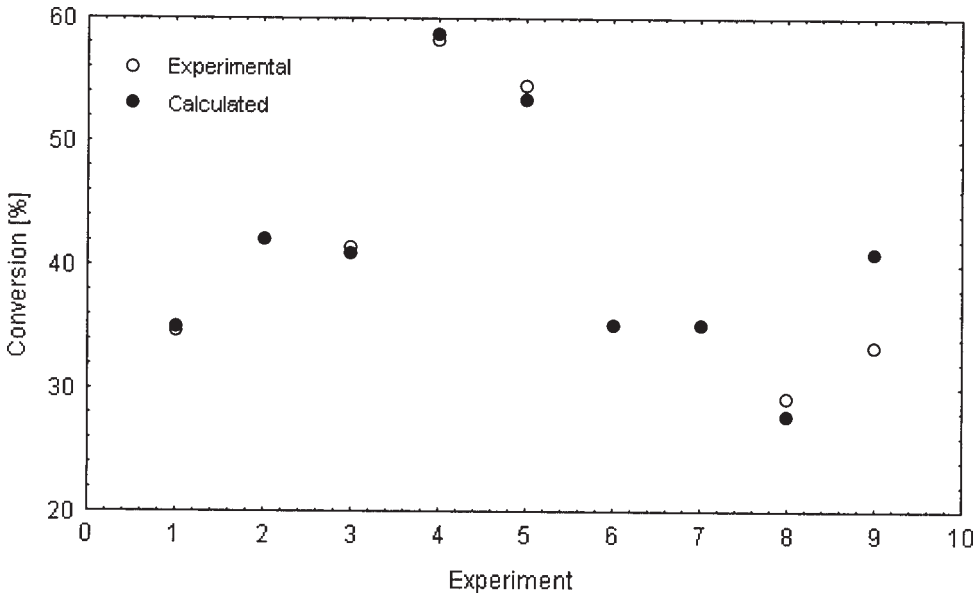

Fig. 2. Experimental and calculated conversions for the system palm kernel oil with Novozym 435.

of model results to the experimental data. The conversion is almost insensitive to variables variation. For the system containing palm kernel oil (Table 5 and Fig. 2), temperature, water, and enzyme concentrations and also the cross-interaction between temperature and oil:ethanol molar ratio significantly affect the process conversion. Though the optimal temperature for this enzyme is about 70°C, a negative effect is observed on the conversion. The added water also presented a negative effect, corroborating that a water excess may change the reaction equilibrium, decreasing the formation of

Table 6
Regression Results
for the System Palm Oil and Lipozyme IM

$Y = a_0 + a_1T + a_2E + a_3R + a_4H + a_5TE + a_6TR$

Average absolute deviation = 5.1

Parameter		Standard deviation
a_0	33.89	1.52
a_1	–10.01	1.56
a_2	4.89	1.53
a_3	0.40	1.15
a_4	2.28	1.46
a_5	0.86	1.55
a_6	2.21	1.60

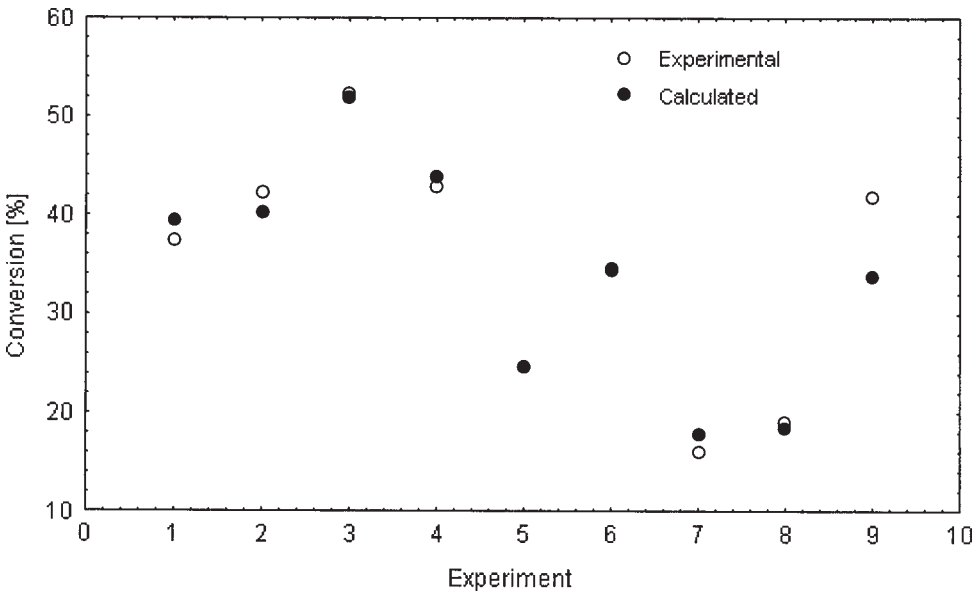

Fig. 3. Experimental and calculated conversions for the system palm oil with Lipozyme IM.

esters. As expected, the enzyme concentration within the range studied presented a positive effect on the conversion. Besides the isolated effects of these variables, cross-interactions should also be taken into account, because cross-interaction between temperature and oil:ethanol molar ratio had a negative effect on the conversion. Concerning the different results obtained for the two oils, one can conclude that oil composition, oil saturation level, and also physical-chemical properties may interfere with enzymatic catalysis.

Table 7
Regression Results
for the System Palm Kernel Oil
and Lipozyme IM

$Y = a_0 + a_1T + a_2E + a_3R + a_4H + a_5TE + a_6TR$		
Average absolute deviation = 4.8		
Parameter		Standard deviation
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

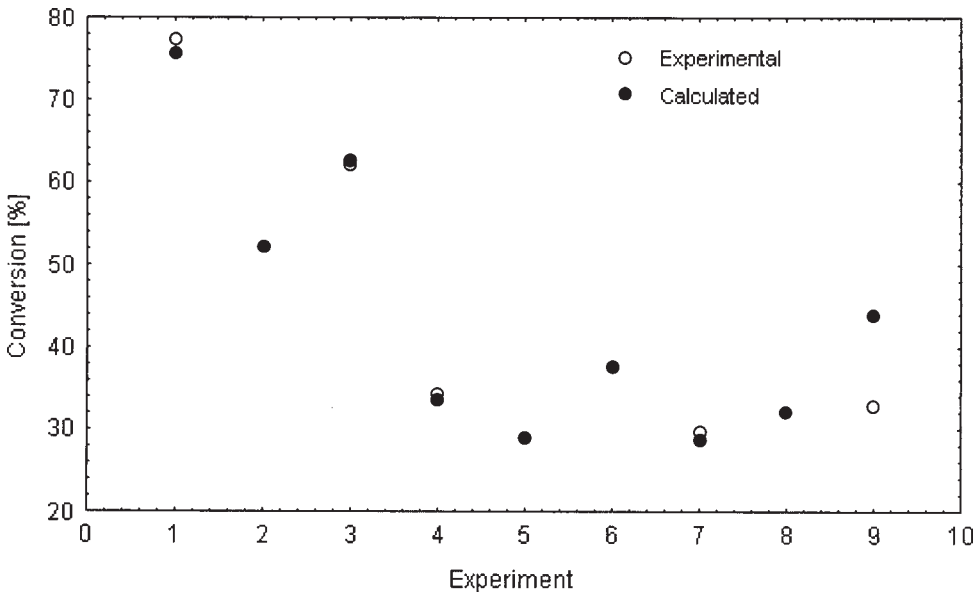

Fig. 4. Experimental and calculated conversions for the system palm kernel oil with Lipozyme IM.

Reactions Catalyzed by Lipozyme IM

The variable temperature had a pronounced effect on reaction conversion for palm and palm kernel oils; however, its cross-interaction with enzyme concentration and oil:ethanol molar ratio has a positive effect (Tables 6 and 7). For the system containing palm oil (Table 6 and Fig. 3), all other variables presented a positive effect on conversion, with the enzyme concentration the most significant. For the system containing palm kernel oil, Table 7 and Fig. 4 show that oil:ethanol ratio had a strong influence on the conversion and that ethanol excess may inhibit the enzymatic reaction.

Conclusions

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

The results obtained using Novozym 435 presented lower conversions when compared to the systems using Lipozyme IM.

According to this study the greatest conversion, 77.45%, was achieved for the system palm kernel oil and Lipozyme IM, at the inferior conditions of temperature, water, enzyme concentration, and oil:ethanol molar ratio. From an economic viewpoint, this result may be important because cheap raw materials can be used for the production of high-value added products.

Acknowledgments

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