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Optimization and Determination of Kinetic Parameters of the Synthesis of 5-Lauryl-hydroxymethylfurfural Catalyzed by Lipases

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Abstract: Hydroxymethylfurfural esters (HMF-esters) have great potential for additive development; for this reason, the goal of this work was to study the optimization of the esterification conversion of HFM and lauric acid using two lipases: the Novozym 435® biocatalyst and immobilized lipase from *Thermomyces lanuginosus* (TL). For the optimization of conversion, a three-level three-factorial Box–Behnken experimental design was used. The models achieved a good fit (R^2 over 90%) for reactions catalyzed with Novozym 435® and immobilized TL lipase. The best conversion, 78.4%, was achieved with immobilized TL lipase using 30 mM HMF, 16 U of biocatalytic activity, and 50 °C. The kinetic parameters without inhibition by the substrate were determined using the Michaelis–Menten mechanism, whereby V_{Max} for both biocatalysts reached the highest values at 50 °C, and the highest enzyme–substrate affinities (low K_m) were reached at temperatures of 30 °C and 40 °C. It can be concluded that immobilized TL lipase has the potential to catalyze this reaction since, under optimal reaction conditions, an 80.6% conversion (value predicted) could be achieved.

Keywords: enzymatic esterification; 5-hydroxymethylfurfural; 5-lauryl-hydroxymethylfurfural; conversion optimization; 5-hydroxymethylfurfural esters; lipases

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1. Introduction

The decrease in fossil resources to produce energy and different chemical compounds of commercial interest has triggered the search for renewable sources for their production, which guarantees a sustainable transition to the manufacture of these products [1–5]. Among these alternatives are residues of lignocellulosic biomass, such as furans, which are the main by-products in the production of bioethanol [6–11]. Among the furans, 5-hydroxymethylfurfural (HMF) is the building block with the greatest potential due to its high likelihood of functionalization. HMF can be produced via the dehydration of sugars (glucose and fructose), and there are different catalytic alternatives for its synthesis [12–16].

HMF can be used to construct many chemicals, such as bioplastics [7–17], liquid fuels [18,19], and esters (HMF-esters) [17,18]. The potential applications of HMF-esters are as fuel-blending agents, additives for bulk chemistry, polymerization monomers, natural surfactants, and fungicides [20–22]. Esterification can also be an alternative to stabilize the HMF molecule, thus making it a workable alternative for storage [17]. HMF-esters can be obtained by chemical [20] and enzymatic synthesis, and enzymatic synthesis is an interesting alternative from an environmental viewpoint [17].

Lipases belong to the group of hydrolases, and their main function in biological systems is the hydrolysis of triglycerides [23]. However, due to their enzyme promiscuity, they can accept a wide variety of substrates and, in the absence of water, catalyze synthetic

reactions [24]. These enzymes have different characteristics and properties depending on the microorganism from which they are isolated [23]. The activity, stability, and selectivity of lipases can be improved by protein engineering and immobilization techniques for the development of robust biocatalysts for industrial applications [25,26].

The esterification of HMF to obtain derivatives has been known for centuries [27]. Different catalytic strategies have been and are currently being developed to improve the conversions and yields of HMF esterification. These strategies consist of the use of acids and bases as catalysts, as well as metal complexes to increase the speed of the reaction [28,29]. In general, the esterification of HMF is a challenge due to its great reactivity since it can be oligomerized and rehydrated to form levulinic acid. The formation of these by-products decreases the yield and selectivity. In this context, biocatalysis offers a sustainable alternative due to its mild operating conditions and its high selectivity [17,30].

Box–Behnken designs (BBDs) are a class of rotatable or nearly rotatable second-order designs based on three-level incomplete factorial designs. These designs can be applied to the optimization of various chemical and physical processes, where the number of experiments is determined according to the requirements of the process. In this context, conversion optimization through its operational variables is an important tool for scaling up a process [31,32]. Due to its potential, this research aims for the first time to optimize the esterification of HMF and lauric acid (Figure 1) in acetone as a reaction medium, using two types of immobilized lipases: lipase B from *Candida antarctica* (Novozym 435®) and *Thermomyces lanuginosus* (TL). The two commercial immobilized lipases were selected to compare their performance in the esterification reaction.

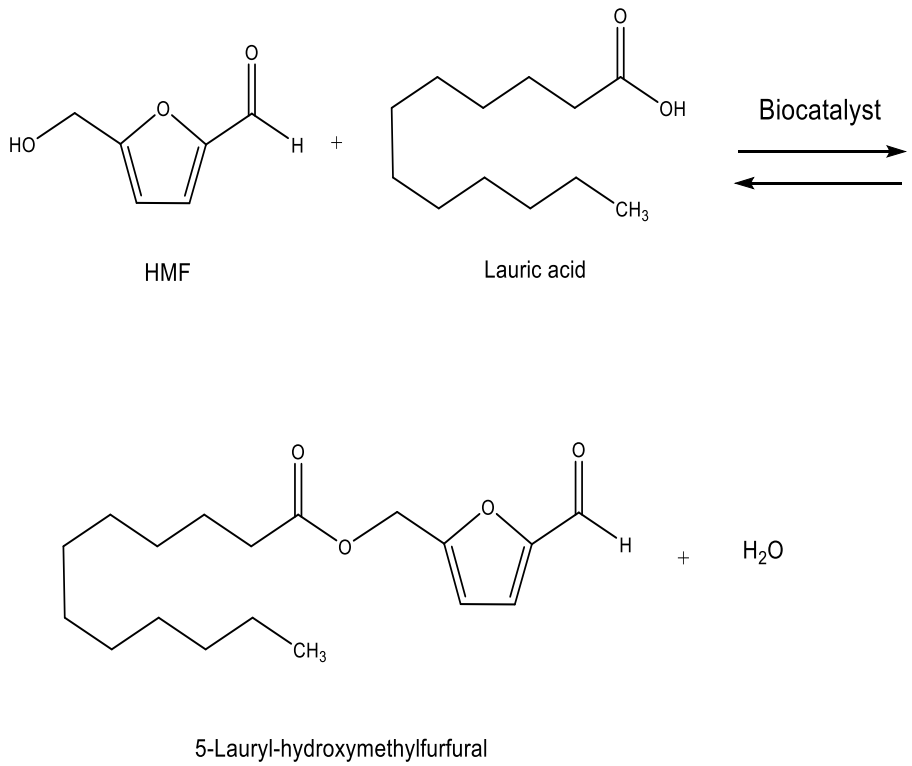

Figure 1. Enzymatic esterification of HMF with lauric acid to produce 5-lauryl-hydroxymethylfurfural.

2. Results and Discussion

2.1. Optimization of Esterification to Produce 5-Lauryl-hydroxymethylfurfural Catalyzed by Novozym 435®

The variables used for conversion optimization were selected in preliminary experimental tests (Figure S1). Figure S1 shows that the highest conversion (77%) was achieved using a 30 mM HMF concentration, followed by esterification, where the HMF concentra-

tion was 10 mM with 73% conversion. These results can be improved by optimizing the operating conditions of the reaction, considering a maximum of 50 °C to prevent solvent (acetone) evaporation, and considering that biocatalysts are thermally stable at that temperature [33]. From the exploratory reactions (Figure S1), the selected variables are the temperature, the HMF concentration, and the activity of the biocatalyst. The concentration of lauric acid was left fixed at 1000 mM (excess substrate) due to the good results obtained in previous investigations [16]. Acetone was used as the reaction medium because it allows the substrates to be solubilized and, at the same time, does not interfere with the reaction. Along with the above, this solvent has been used successfully in works reported in the literature [34–36].

When working with different immobilized lipases, the key is to determine the synthesis activity of each biocatalyst in the esterification of HMF with lauric acid. Table 1 shows that the highest synthesis activity corresponds to the Novozym 435® biocatalyst, and the lowest corresponds to *Rhizomucor miehei lipase* (RM). Due to the insufficient synthesis activity of the RM biocatalyst, Novozym 435® and TL biocatalysts were used in the experimental designs.

Table 1. Synthesis activity of biocatalysts.

Biocatalysts	Activity (U/g)
Novozym 435®	74
Immobilized TL lipase	39

After executing the experimental design using the Statgraphics 19® program, the results were obtained for the reaction catalyzed with Novozym 435®, as detailed in Table S1. The program resulted in a total of thirty experiments with three central points, and the highest conversion of 75% was reached at 65 mM HMF and 16 U of Novozym 435® at 40 °C. This result is 10% lower than that obtained by Krystof et al. [17] (85%), who used 100% lauric acid at 60 °C. The conversion of the esterification of HMF and lauric acid catalyzed by Novozym 435® was fitted by Equation (1), obtained by the optimized experimental design. Using this model, an optimal conversion of 75.7% can be achieved using a temperature of 41 °C, 51 mM HMF, and 10 U of the biocatalyst.

$$\text{Conversion } (\%) = 2.1 + 3.9x_1 + 0.3x_2 - 1.9x_3 - 0.05x_1^2 + 0.03x_1x_2 - 0.002x_1x_3 - 0.004x_2^2 + 0.05x_3^2 \quad (1)$$

where x_1 is the temperature, x_2 is the concentration of HMF, and x_3 is the activity of Novozym 435®.

The analysis of variance (ANOVA) (Table S2) showed a correlation of 93%, while the *p*-values of the variables and their interactions were shown to be significant only for the temperature, concentration, squared temperature interaction, squared concentration, and squared activity with a confidence value of 95% because the *p*-values were greater than 0.05. The interaction that presented the greatest significance (*p*-value = 0.0000) was the squared temperature, the concentration of HMF, the squared concentration of HMF, and squared activity, which agrees with the Pareto diagram in Figure 2, which indicates that the interaction between these variables has the greatest standardized effect. Positive values of the effects indicate that an increase in their levels leads to an increase in the conversion of the reaction. On the contrary, negative values of the effects lead to a decrease in the response (conversion %) when their levels are increased.

The response surfaces of Figure 3 show the effects of the variables' interactions on the reaction's conversion to produce 5-lauryl-hydroxymethylfurfural catalyzed by Novozym 435®. In Figure 3A, the highest conversions (73 to 76%) at 65 mM HMF are achieved at approximately 40 °C and at activities between 12 U and 16 U. Figure 3B shows that the highest conversions (73% to 76%) at 16 U are reached at 40 °C and at a concentration of 65 mM. Figure 3C shows the effect of the activity and concentration on conversion at 40 °C. The 12 U activity corresponds to the yellow response surface, indicating that conversion ranges from 73 to 75%, while 12 to 22 U activities correspond to the dark green response

surface, where conversion is from 71 to 73%, indicating that there is little or no decrease in conversion.

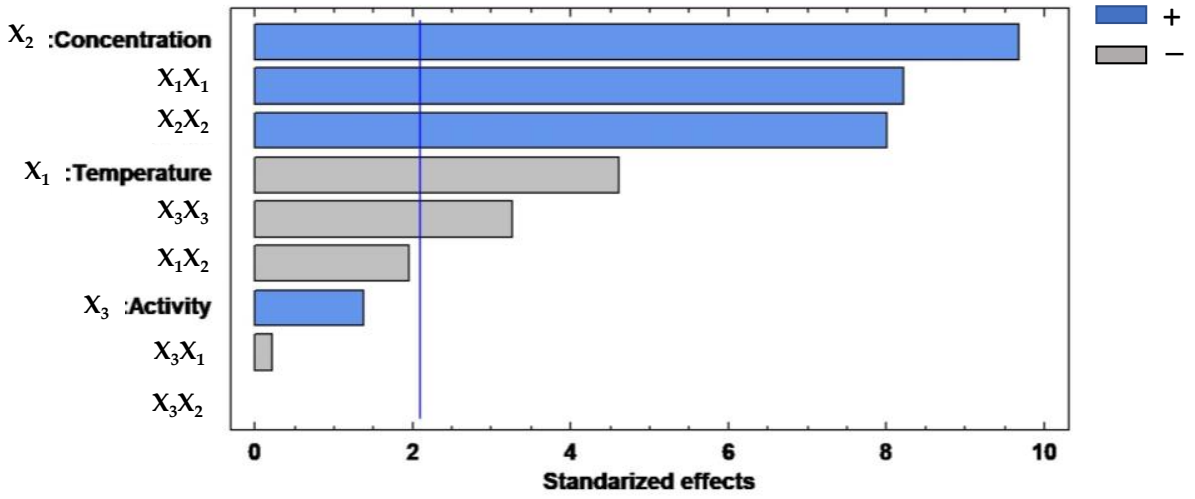

Figure 2. Pareto diagram of the standardized effects of the esterification conversion of HMF and lauric acid in acetone catalyzed by the Novozym 435® biocatalyst, where x_1: temperature; x_2: concentration of HMF; x_3: activity of Novozym 435®.

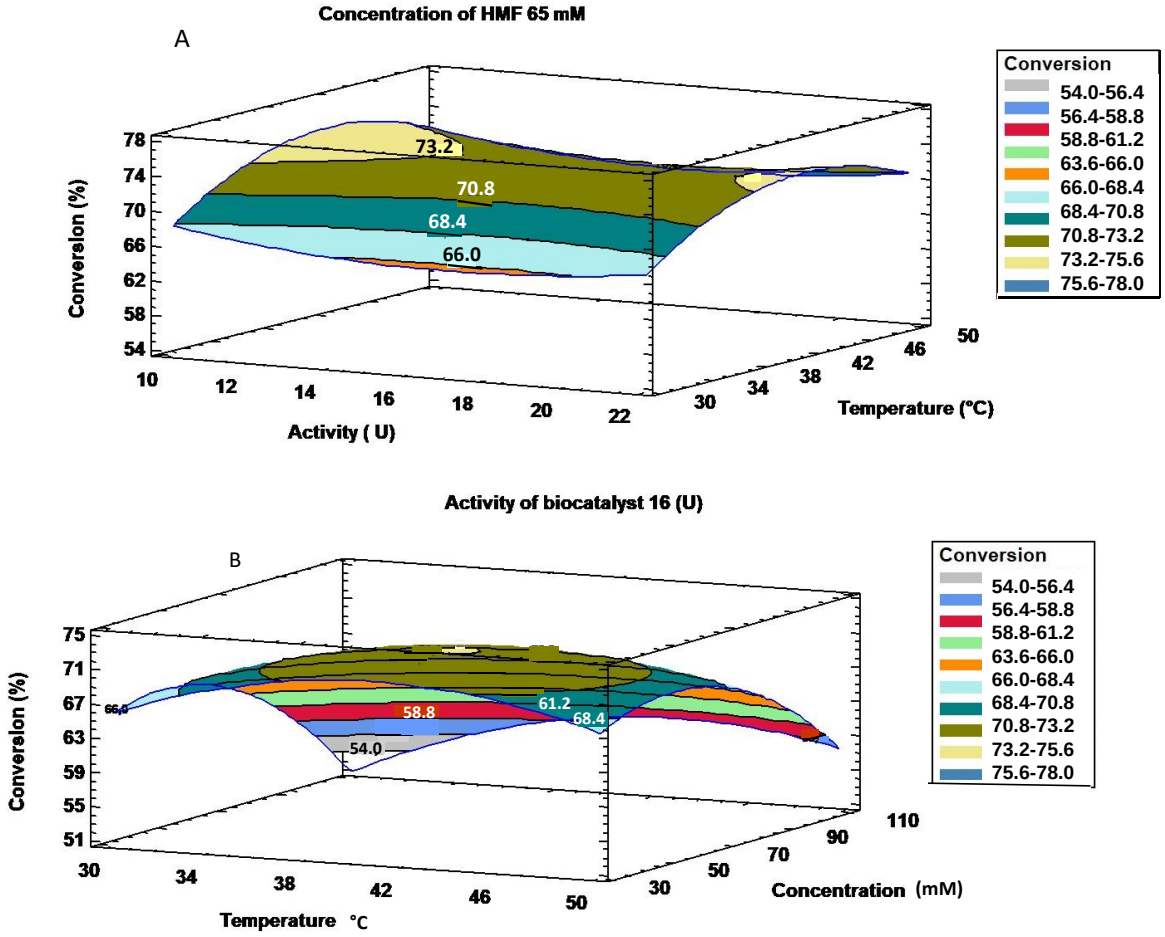

Figure 3. *Cont.*

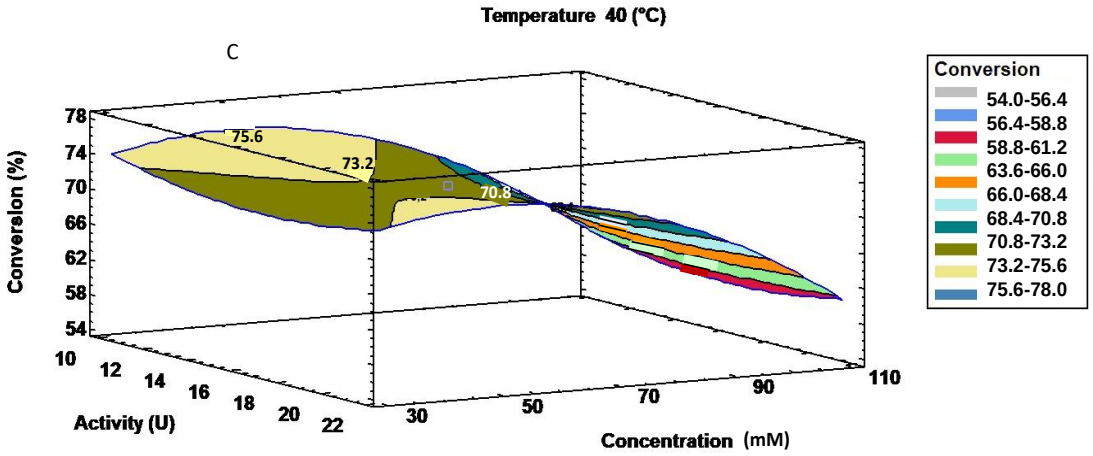

Figure 3. Effects of the variables studied on the conversion of HMF esterification with lauric acid (1000 mM) using Novozym 435® as biocatalyst. (**A**) Response surface for concentration of HMF, (**B**) response surface for the activity of biocatalyst, and (**C**) response surface for temperature.

Table 2 details the conditions and variables that maximize conversion to produce 5-lauryl-hydroxymethylfurfural. As can be seen, the highest conversion (76%) predicted by the Statgraphics 19® program is at the intermediate temperature of the experimental design, 41 °C. The conversion predicted by the Statgraphics 19® program was experimentally evaluated, reaching 75.8% conversion. These results seem to be in contradiction with the Van't Hoff rule modeled by the "Arrhenius equation". However, looking at Table S1, there is one experimental run in which a conversion of 74% is achieved at 40 °C. This is explained by the fact that the temperature influences the conversion, and the concentration of the substrate and the load of the biocatalyst are factors that influence the conversion.

Table 2. Summary of the variables and conditions that maximize the conversion of the reaction to produce 5-lauryl hydroxymethylfurfural catalyzed by Novozym 435® using 1000 mM lauric acid.

Conditions	Values
Highest conversion (75%)	40 °C, 65 mM HMF, and 16 U of Novozym 435®
Most significant variables	Concentration of HMF, temperature, activity of biocatalyst, temperature squared, squared concentration, and squared activity
Optimal value predicted by Statgraphics 19® (experimentally validated in this work)	76% conversion, 40 °C, 65 mM HMF, and 16 U of Novozym 435®

In the literature, works are reported in which lauric acid and sugars are used as substrates, catalyzed by Novozym 435®, to obtain sugar esters with surfactant applications. For example, Vuillemin et al. [37] used Novozym 435® to esterify lauric acid with glucose in MeTHF-3-one as the reaction medium in 79% yield.

2.2. Optimization of Esterification to Produce 5-lauryl-hydroxymethylfurfural Catalyzed by Immobilized TL Lipase

The experimental matrix with the experimental results of esterification conversion to produce 5-lauryl-hydroxymethylfurfural catalyzed by immobilized TL lipase is detailed in Table S3. The highest conversion of 78% was reached at 30 mM HMF and 16 U of biocatalytic activity at 50 °C. Since the TL lipase biocatalyst comes from a thermophilic microorganism, the highest conversion was obtained at 50 °C.

The conversion optimization for this esterification was fitted by Equation (2). This model can achieve an optimal conversion of 80% at a temperature of 50 °C, 30 mM HMF, and 22 U of biocatalytic activity.

$$\text{Conversion } (\%) = 61.1654 + 1.32807x_1 - 0.00154762x_2 - 1.8945 - 0.0266917x_1^2 - 0.000478571x_1x_2 + 0.0625x_1x_3 - 0.00206667x_2^2 + 0.00267857x_2x_3 - 0.0240046x_3^2 \quad (2)$$

where x_1 is the temperature, x_2 is the concentration of HMF, and x_3 is the activity of immobilized TL lipase.

The analysis of variance (ANOVA) (Table S4) showed a correlation of 89.3%, while the *p*-values of the variables and their interactions were shown to be significant with a confidence value of 95% because the *p*-values were lower than 0.05 for all variables and their interactions. The variables and their interactions that presented the greatest significance (*p*-value = 0.05) were the temperature, the concentration of HMF, temperature squared, the interaction between the temperature and the activity of the biocatalyst, HMF concentration squared, and the interaction between the concentration and the activity of the biocatalyst. These values agree with the Pareto diagram in Figure 4, which indicates that the interactions between these variables have the greatest standardized effects.

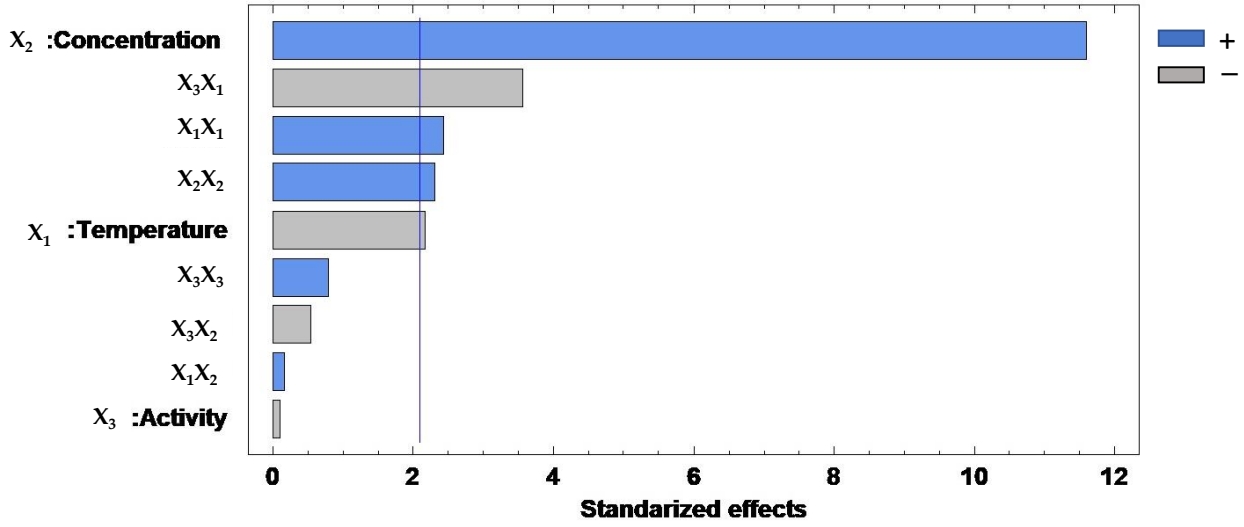

Figure 4. Pareto diagram of the standardized effects of the esterification conversion of HMF and lauric acid in acetone catalyzed by immobilized TL lipase, where x_1: temperature; x_2: concentration of HMF; x_3: activity of biocatalyst.

The response surfaces in Figure 5 detail the effects of the interactions between the variables on the conversion of the esterification of HMF with lauric acid. Figure 5A depicts the effect of the activity and temperature on conversion at a concentration of 65 mM HMF. In this graph, it can be clearly seen that conversion decreased with higher activity. This behavior is because the temperature and substrate concentration also interact simultaneously, contributing to the variation in conversion. Figure 5B shows that when using an activity of 16 U, the highest conversions (73% to 76%) are reached at a temperature of approximately 45 °C and at a concentration of 50 mM HMF. Figure 5C shows that at a temperature of 50 °C, the highest conversions (73% to 76%) are achieved using an enzyme activity of 22 U and a concentration of HMF of 30 mM. Like the behavior with the Novozym 435® biocatalyst, at an intermediate temperature and activity of the biocatalyst, conversions greater than 75% are obtained, while a lower concentration of the substrate (HMF) favors conversion.

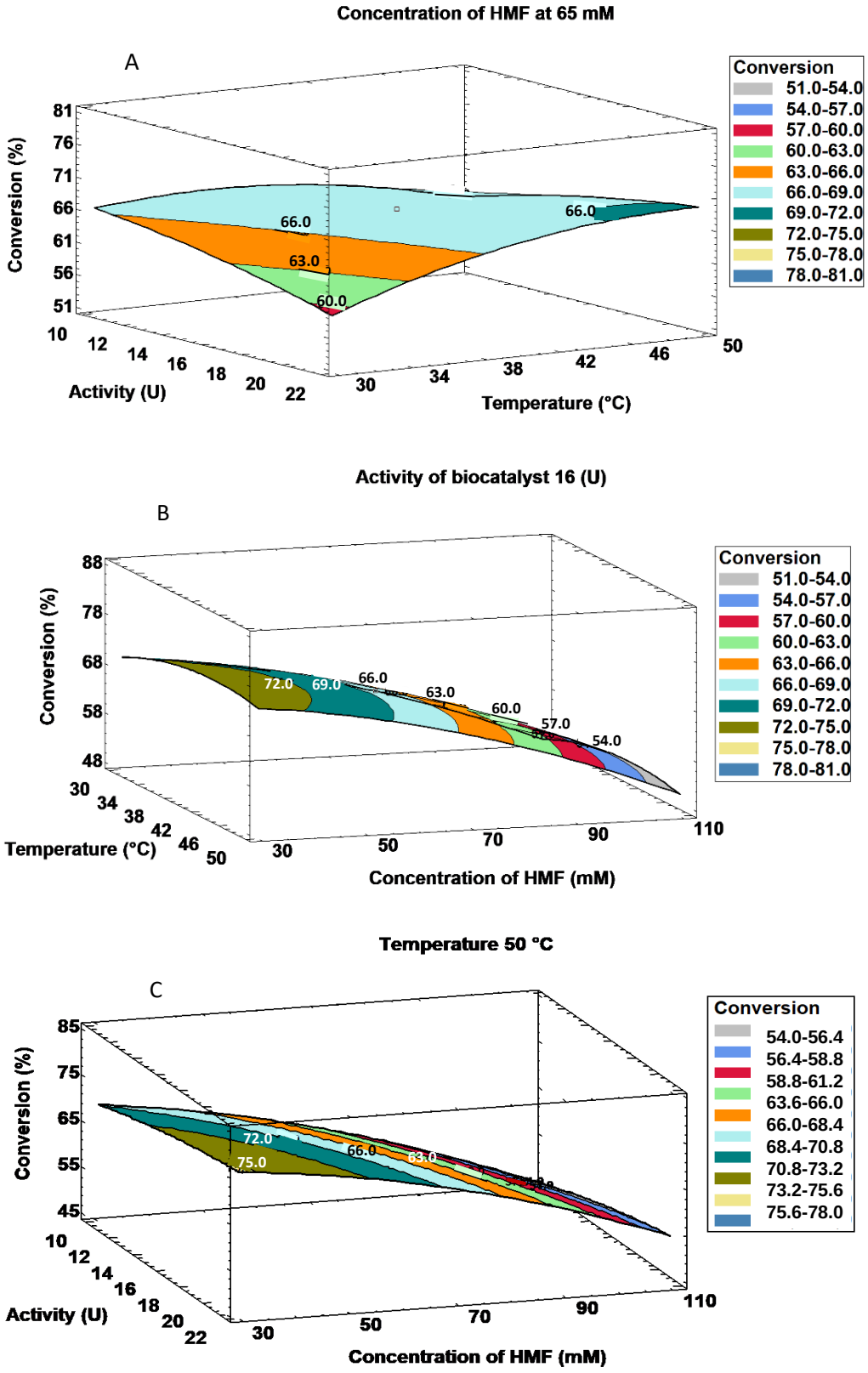

Figure 5. Effect of the variables studied on the conversion of HMF esterification with lauric acid (1000 mM) using immobilized TL lipase. (**A**) Response surface for concentration of HMF, (**B**) response surface for the activity of biocatalyst, and (**C**) response surface for temperature.

Table 3 details the conditions and variables that maximize conversion to produce 5-lauryl-hydroxymethylfurfural catalyzed by immobilized TL lipase. As can be seen, the

highest conversion (80.6%) predicted by the Statgraphics 19® program (and experimentally validated at 80.2%) was achieved using the highest temperature (50 °C), 30 mM HMF, and the highest activity of immobilized TL lipase (22 U).

Table 3. Summary of the variables and conditions that maximize the conversion of the reaction to produce 5-lauryl hydroxymethylfurfural catalyzed by immobilized TL lipase using 1000 mM lauric acid.

Conditions	Values
Highest conversion (78%)	50 °C, 30 mM HMF, and 16 U of biocatalytic activity
Most significant variables	Temperature, concentration of HMF, squared concentration, squared temperature, and activity and temperature interaction.
Optimal value predicted by Statgraphics 19® (experimentally validated in this work)	80.6% of conversion, 30 mM of HMF, 50 °C, and 22 U of biocatalytic activity.

The high conversion obtained with TL lipase agrees with the result of the biocatalyst hydrolysis activity reported by the manufacturer (3000 U/g), which is higher for the Novozym 435® biocatalyst (2000 U/g). However, the synthesis activity is much lower than the hydrolysis activity, as detailed in Table 1. In the experimental data, no enzymatic inactivation was evidenced for either biocatalyst.

An example of the enzymatic esterification of lauric acid and geraniol catalyzed by immobilized TL lipase in hexane with a yield of over 70% was reported in the literature. One aspect to note is that the use of a solvent is essential to achieving high performance due to the solubility of the substrates [38].

It should also be noted that the acidity of lauric acid does not cause a loss of enzymatic activity since Krystof et al. [17] carried out esterification using lauric acid as a substrate and reaction medium at the same time, obtaining yields greater than 80%.

2.3. Determination of the Kinetic Parameters of the Esterification Reaction

The determination of the apparent kinetic parameters (for immobilized enzymes) was carried out by measuring the initial enzymatic activities at different substrate concentrations and temperatures (Figures S2 and S3). In this case, a Michaelis–Menten mechanism was used because lauric acid is present in very large excess, and the biocatalyst seems to be saturated with lauric acid (therefore, it is pseudo-zero-order with respect to lauric acid). In addition, no inhibition was found in this system using the initial reaction rate.

As can be seen in Table 4, the maximum reaction rates for both biocatalysts were reached at 50 °C because increasing the temperature leads to more frequent collisions between molecules and a substantial decrease in the viscosity, which reduces mass-transfer limitations. As the temperature increases, the intensified diffusion of substrates occurs, which results in high reaction rates.

Table 4. Kinetic parameters of immobilized TL lipase and the Novozym 435® biocatalyst in esterification to obtain 5-lauryl-hydroxymethylfurfural.

	Novozym 435®		Immobilized TL Lipase	
Temperature (°C)	**K_m (mM)**	**V_{Max} (mM/min)**	**K_m (mM)**	**V_{Max} (mM/min)**
50	69.2	0.76	461.5	0.5
40	34	0.43	123.5	0.18
30	45	0.46	103.1	0.18

With respect to the affinity constants, the lower the numerical value, the higher they are. In this case, the lower the V_{Max} , the higher the K_m. The V_{Max} of immobilized TL lipase at 50 °C is almost 3 times higher than that obtained at 40 °C, while with the Novozym 435® biocatalyst, the V_{max} at 50 °C is 1.7 times higher than the V_{max} obtained at 40 °C. The behavior of immobilized TL lipase is consistent with its thermophilic nature.

If V_{Max} is compared with conversions obtained through statistical optimization, agreement can be observed with respect to immobilized TL lipase since the optimal conversion conditions were at 50 °C. Regarding the Novozym 435® biocatalyst, V_{Max} was reached at 50 °C, while conversion optimization yielded an optimal value at 40 °C. This behavior may be due to longer reaction times, and phenomena such as the inactivation of the biocatalyst at high temperatures may occur.

3. Materials and Methods

3.1. Chemicals

5-Hydroxymethylfurfural (HMF) and lipases from TL and RM immobilized on Immobead 150 were purchased from Sigma Aldrich. Immobilized lipase B from *Candida antarctica* (Novozym 435®) was kindly donated by Blumos S.A (Santiago, Chile). The enzymatic activities reported by the manufacturers in the tributyrin hydrolysis reaction are 3000 U/g for immobilized TL lipase and 2000 U/g for Novozym 435®.

Lauric acid at 99% (Acros Organics, Geel, Belgium) was purchased from SAGU Ltd.a, Santiago, Chile. Acetone, formic acid, and HPLC-grade methanol were purchased from Merck S.A, Santiago, Chile.

3.2. Biocatalyst Synthesis Activity Assays

The activities of immobilized lipases were determined for the esterification of 5-hydroxymethylfurfural (HMF) and lauric acid. One activity unit (U) is defined as the amount of biocatalyst required to produce 1 µmol of HMF-esters per min under standard assay conditions. In a typical experiment, the reaction mixture containing 0.3 mmol HMF, 5 mmol lauric acid, 0.2 g of immobilized lipases, and 5 mL of acetone was incubated in a shaking incubator (40 °C, 168 rpm) to react for 6 h. Over the time course of the reaction, 100 µL aliquots were withdrawn from the system at different time intervals for HPLC analysis.

3.3. High-Performance Liquid Chromatography (HPLC) Analysis

The decrease in HMF concentration was quantified by HPLC (JASCO LC- 4000 with a diode array detector) with a GL Sciences C18 HPLC column (100 mm × 4.6 mm I.D) at 285 nm under the following conditions. The mobile phase was composed of solutions A and B (where A = water/formic acid (99.5/0.5) and B = methanol (100)) at a volume ratio of A/B = 85/15 for 5 min, then A/B = 40/60 for 7 min, and finally A/B = 85/15 for 10 min. The flow rate was 0.6 mL/min at 35 °C, and the retention time for HMF was 4.0 min.

3.4. Enzymatic Esterification of HMF with Lauric Acid in a Batch Reactor

The enzymatic esterification of HMF with lauric acid to produce 5-lauryl-hydroxymethylfurfural in a batch operation was carried out within a sealed reactor at 165 rpm and 40 °C. Before the reactions, blanks were made without the presence of the biocatalyst, which showed that the reaction did not occur. The reaction media were prepared by mixing HMF (100 mM) with lauric acid (1000 mM) in 5 mL of acetone for 48 h. The reaction was started by adding 150 mg of Novozym 435®, and reaction samples were drawn at different times to analyze the reaction products. The conversion was calculated with the following equation.

$$\text{Conversion } (\%) = \left[\frac{n_{so} - n_s}{n_{so}}\right] \times 100 \quad (3)$$

where n_{so} = the amount of substrate S at the start of the reaction (mol), and n_s = the amount of substrate s at the end of the reaction (mol).

3.5. Conversion Optimization

Exploratory experimental tests were carried out to find the optimal conditions for the enzymatic synthesis of 5-lauryl-hydroxymethylfurfural (Figure S1). According to the preliminary results, the determining variables that increase conversion are the concentration of HMF, the activity of the biocatalyst, and the temperature. To reduce the number of experiments and determine the statistical relevance of the effects of the factors under study, as well as to evaluate the interaction between them, the Box–Behnken experimental design (BBD) was used, which is more efficient than the central composite design and the full three-level factorial [39]. The Box–Behnken experimental design (BBD) was developed using the Statgraphics 19® program to obtain a response surface with three central points per block with three experimental factors (temperature (°C), activity of biocatalyst (U), and concentration of HMF (mM)) and the conversion of the reaction as a response variable. The ranges and levels of the independent experimental variables are detailed in Table 5. The same experimental design was used for catalyzing the esterification with immobilized TL lipase.

Table 5. Independent experimental variables for the optimization of conversion.

Factors	Units	Levels		
		−	**0**	**+**
x_1: Temperature	°C	30	40	50
x_2: Concentration of HMF	mM	30	65	100
x_3: Activity of biocatalyst	U	10	16	22

Additionally, as a part of the results, an analysis of variance (ANOVA) was performed, and a Pareto chart was obtained to analyze the standardized effects of the variables involved.

The conversion results obtained from Table 1 using the three biocatalysts were fitted to the second-order polynomial equation:

$$Y = \beta_0 + \sum_{i=1}^{k} \beta_i x_i + \sum_{i=1}^{k} \beta_{ii} x_i^2 + \sum_{i>j}^{k} \beta_{ij} x_i x_j \quad (4)$$

where Y represents the predicted conversion (%) of the reaction, x_i and x_j represent independent variables ($i \neq j$; i and j range from 1 to k), and k is the number of independent parameters (k = 3). β_0, β_i, $\beta_{ii,}$ and β_{ij} are regression coefficients for the intercept, linearity, quadratic, and interactive terms, respectively.

3.6. Determination of Kinetic Parameters

For the determination of the kinetic parameters, the Lineweaver–Burk approach was used to obtain insight into the mechanism and reaction kinetics. In this particular case, since lauric acid is present in very large excess, the biocatalyst seems to be saturated with lauric acid (therefore, it is pseudo-zero-order with respect to lauric acid). In addition, as stated above, no inhibition was detected in the system. Thus, the rate expression can be simplified to the Michaelis–Menten model (Equation (5)). The kinetic parameters were determined using initial enzymatic activities at 30 °C, 40 °C, and 50 °C for the two biocatalysts. More details can be found in Supplementary Materials S4.

$$v = \frac{V_{Max} \times s}{K_m + s} \quad (5)$$

where V_{Max} = maximum reaction rate (mM/min), K_m = affinity constant (mM), s = substrate concentration (mM), and v = enzymatic activity (mM/min).

4. Conclusions

In this work, the application of the response surface methodology and Box–Behnken design from the viewpoint of the conversion of esterification to produce 5-lauryl-hydroxymethylfurfural catalyzed by immobilized lipases was discussed. In the esterification catalyzed by Novozym 435®, the predicted optimal conversion (76%) would be reached at 40 °C, 65 mM HMF, and 16 U of biocatalytic activity (the value predicted by the program). On the other hand, using immobilized TL lipase, the optimal conversion of 80.6% would be reached (the value predicted by the program) at a temperature of 50 °C, 30 mM HMF, and 22 U of biocatalytic activity. The mathematical models fit the experimental data obtained with reactions catalyzed by Novozym 435® and immobilized TL lipase. The effects of the independent variables and their interactions were significant in the esterification catalyzed by Novozym 435® and immobilized TL lipase. The determination of the kinetic parameters according to the Michaelis–Menten mechanism showed that the highest V_{Max} values were reached at 50 °C, and the highest enzyme–substrate affinities (low K_m) were reached at temperatures of 30 °C and 40 °C. Finally, according to the conditions present in this study and from the viewpoint of the biocatalyst, immobilized TL lipase proved to be the most appropriate candidate for the synthesis of 5-lauryl-hydroxymethylfurfural. The results predicted in this research were experimentally validated.

Supplementary Materials: The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/catal13010019/s1. Figure S1: Esterification of HMF with lauric acid catalyzed by Novozym 435®; Figure S2: Determination of kinetic parameters with Novozym 435®; Figure S3: Determination of kinetic parameters with TL lipase immobilized; Table S1: Box–Behnken design for surface analysis with Novozym 435®; Table S2: Analysis of variance for conversion from esterification to produce 5-lauryl-hydroxymethylfurfural catalyzed by Novozym 435®; Table S3: Box–Behnken design for surface analysis of the conversion of the esterification to produce 5-lauryl-hydroxymethylfurfural catalyzed by immobilized TL lipase; Table S4: Analysis of variance for conversion from esterification to produce 5-lauryl-hydroxymethylfurfural catalyzed by immobilized TL lipase.

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