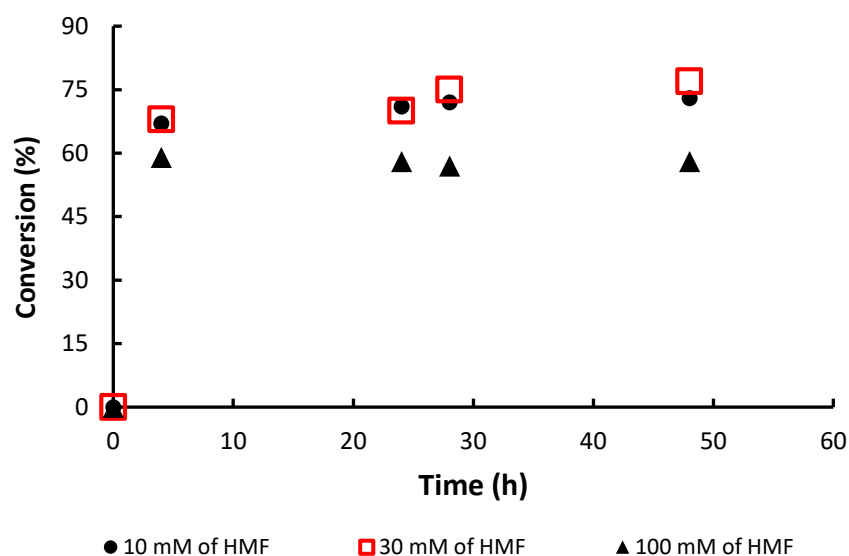


# Supplementary Information

## S1.- Preliminary results of the esterification of HMF with lauric acid catalyzed by lipase

Novozym 435®



**Figure S1.** Esterification of HMF with lauric acid catalyzed by Novozym 435®. Reaction conditions: 1000 mM of lauric acid, 40 °C, 60 mg of Novozym 435®, and 2 mL of reaction volume.

**S2. Optimization of the conversion of the esterification of HMF with lauric acid catalyzed by Novozym 435®**

**Table S1.** Box-Behnken design for surface analysis with Novozym 435®. Reaction conditions: 48 h of reaction and 1000 mM of lauric acid.

| Block | Activity<br>U | Temperature<br>°C | Concentration<br>mM | Conversion<br>(%) |
|-------|---------------|-------------------|---------------------|-------------------|
| 1     | 10            | 50                | 65                  | 72                |
| 1     | 16            | 30                | 30                  | 64                |
| 1     | 22            | 40                | 100                 | 67                |
| 1     | 10            | 40                | 100                 | 66                |
| 1     | 16            | 40                | 65                  | 75                |
| 1     | 22            | 50                | 65                  | 69                |
| 1     | 16            | 40                | 65                  | 70                |
| 1     | 16            | 30                | 100                 | 57                |
| 1     | 10            | 30                | 65                  | 69                |
| 1     | 16            | 40                | 65                  | 70                |
| 1     | 10            | 40                | 30                  | 74                |
| 1     | 22            | 40                | 30                  | 74                |
| 1     | 16            | 50                | 100                 | 63                |
| 1     | 22            | 30                | 65                  | 67                |
| 1     | 16            | 50                | 30                  | 69                |
| 2     | 10            | 50                | 65                  | 71                |
| 2     | 16            | 30                | 30                  | 66                |
| 2     | 22            | 40                | 100                 | 63                |
| 2     | 10            | 40                | 100                 | 65                |
| 2     | 16            | 40                | 65                  | 72                |
| 2     | 22            | 50                | 65                  | 71                |
| 2     | 16            | 40                | 65                  | 74                |
| 2     | 16            | 30                | 100                 | 54                |
| 2     | 10            | 30                | 65                  | 69                |
| 2     | 16            | 40                | 65                  | 73                |
| 2     | 10            | 40                | 30                  | 74                |
| 2     | 22            | 40                | 30                  | 73                |
| 2     | 16            | 50                | 100                 | 62                |
| 2     | 22            | 30                | 65                  | 67                |
| 2     | 16            | 50                | 30                  | 66                |

**Table S2.** Analysis of variance for conversion from esterification to produce 5-Lauryl-hydroxymethylfurfural catalyzed by Novozym435®.

| Source                                   | Sum of Squares | GI | Middle square | F-Ratio | P-Value |
|--|----------------|----|---------------|---------|---------|
| x <sub>1</sub> : Temperature             | 56.25          | 1  | 56.25         | 21.27   | 0.0002  |
| x <sub>2</sub> : Concentration of HMF    | 248.063        | 1  | 248.063       | 93.78   | 0.0000  |
| x <sub>3</sub> : Activity of Biocatalyst | 5.0625         | 1  | 5.0625        | 1.91    | 0.1826  |
| x <sub>1</sub> x <sub>1</sub>            | 178.513        | 1  | 178.513       | 67.49   | 0.0000  |
| x <sub>1</sub> x <sub>2</sub>            | 10.125         | 1  | 10.125        | 3.83    | 0.0653  |
| x <sub>1</sub> x <sub>3</sub>            | 0.125          | 1  | 0.125         | 0.05    | 0.8302  |
| x <sub>2</sub> x <sub>2</sub>            | 169.551        | 1  | 169.551       | 64.10   | 0.0000  |
| x <sub>2</sub> x <sub>3</sub>            | 0.0            | 1  | 0.0           | 0.00    | 1.0000  |
| x <sub>3</sub> x <sub>3</sub>            | 28.3205        | 1  | 28.3205       | 10.71   | 0.0040  |
| Blocks                                   | 1.2            | 1  | 1.2           | 0.45    | 0.5087  |
| Total error                              | 50.2583        | 19 | 2.64518       |         |         |
| Total (corr.)                            | 746,8          | 29 |               |         |         |

R-squared = 93.2702 %

R-squared (adjusted for g.l.) = 89.7282 %

PRESS = 113,579

R-squared (predicted) = 84.7913 %

Standard error of the est. = 1.6264

Mean absolute error = 1.03833

Durbin-Watson statistic = 2.36734 (P=0.8288)

Residual autocorrelation of Lag 1 = -0.196111

**S3. Optimization of the conversion of the esterification of HMF with lauric acid catalyzed by a lipase TL immobilized**

**Table S3.** Box-Behnken design for surface analysis of the conversion of the esterification to produce 5-Lauryl-hydroxymethylfurfural catalyzed by a TL lipase immobilized. Reaction conditions: 48 h of reaction and 1000 mM of lauric acid.

| Block | Activity<br>U | Temperature<br>°C | Concentration<br>mM | Conversion<br>(%) |
|-------|---------------|-------------------|---------------------|-------------------|
| 1     | 22            | 50                | 65                  | 70.7              |
| 1     | 10            | 40                | 100                 | 56.5              |
| 1     | 22            | 40                | 100                 | 53.3              |
| 1     | 16            | 40                | 65                  | 67                |
| 1     | 16            | 30                | 30                  | 68.6              |
| 1     | 22            | 40                | 30                  | 68                |
| 1     | 16            | 50                | 30                  | 70.6              |
| 1     | 10            | 30                | 65                  | 67.5              |
| 1     | 10            | 50                | 65                  | 59.7              |
| 1     | 22            | 30                | 65                  | 64.5              |
| 1     | 16            | 50                | 100                 | 57.26             |
| 1     | 16            | 40                | 65                  | 68                |
| 1     | 16            | 40                | 65                  | 68                |
| 1     | 10            | 40                | 30                  | 77.7              |
| 1     | 16            | 30                | 100                 | 51                |
| 2     | 22            | 50                | 65                  | 70                |
| 2     | 10            | 40                | 100                 | 58                |
| 2     | 22            | 40                | 100                 | 56                |
| 2     | 16            | 40                | 65                  | 67                |
| 2     | 16            | 30                | 30                  | 68                |
| 2     | 22            | 40                | 30                  | 73                |
| 2     | 16            | 50                | 30                  | 78.4              |
| 2     | 10            | 30                | 65                  | 64                |
| 2     | 10            | 50                | 65                  | 58                |
| 2     | 22            | 30                | 65                  | 60                |
| 2     | 16            | 50                | 100                 | 56                |
| 2     | 16            | 40                | 65                  | 68                |
| 2     | 16            | 40                | 65                  | 69                |
| 2     | 10            | 40                | 30                  | 73                |
| 2     | 16            | 30                | 100                 | 51.2              |

**Table S4.** Analysis of variance for conversion from esterification to produce 5-Lauryl-hydroxymethylfurfural catalyzed by a TL lipase immobilized.

| Source                                      | Sum of Squares | GI | Middle square | F-Ratio | P-Value |
|---|----------------|----|---------------|---------|---------|
| x <sub>1</sub> : Temperature                | 41.7962        | 1  | 41.7962       | 4.72    | 0.0426  |
| x <sub>2</sub> : Concentration<br>of HMF    | 1190.94        | 1  | 1190.94       | 134.56  | 0.0000  |
| x <sub>3</sub> : Activity of<br>Biocatalyst | 0.075625       | 1  | 0.075625      | 0.01    | 0.9273  |
| x <sub>1</sub> x <sub>1</sub>               | 52.6113        | 1  | 52.6113       | 5.94    | 0.0248  |
| x <sub>1</sub> x <sub>2</sub>               | 0.22445        | 1  | 0.22445       | 0.03    | 0.8752  |
| x <sub>1</sub> x <sub>3</sub>               | 112.5          | 1  | 112.5         | 12.71   | 0.0021  |
| x <sub>2</sub> x <sub>2</sub>               | 47.3305        | 1  | 47.3305       | 5.35    | 0.0321  |
| x <sub>2</sub> x <sub>3</sub>               | 2.53125        | 1  | 2.53125       | 0.29    | 0.0021  |
| x <sub>3</sub> x <sub>3</sub>               | 5.51471        | 1  | 5.51471       | 0.62    | 0.4396  |
| Blocks                                      | 0.0512533      | 1  | 0.0512533     | 0.01    | 0.9401  |
| Total error                                 | 168.16         | 19 | 8.85054       |         |         |
| Total (corr.)                               | 1610.91        | 29 |               |         |         |

R- squared = 89.5611 %

R- squared (adjusted for g.l.) = 84.067 %

PRESS = 476.79

R- squared (predicted) = 70.4024 porcentaje

Standard error of the est. = 2.97499

Mean absolute error = 1.79653

Durbin-Watson statistic = 2.00971 (P=0.4478)

Residual autocorrelation of Lag 1= -0.013676

#### S4. Determination of kinetic parameters

To determine the kinetic parameters, the Michaelis-Menten mechanism was assumed (Equation S1); 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). The mathematical determination was made using the Lineweaver-Burk inverse method (Equation S2).

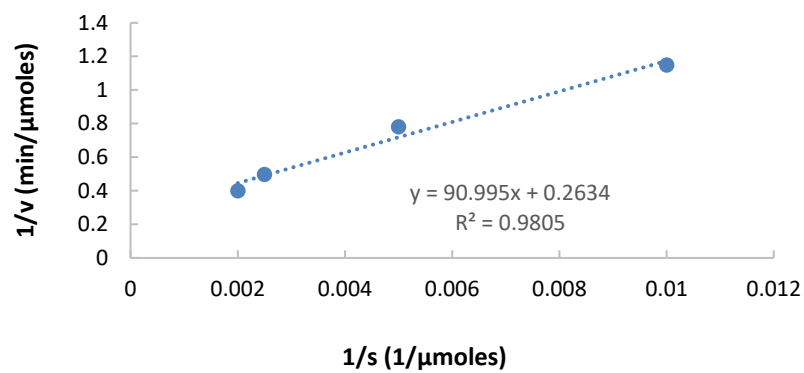
$$v = \frac{V_{\text{Max}} * s}{K_m + s} \quad (\text{S1})$$

$$\frac{1}{v} = \frac{K_m}{V_{\text{Max}}} * \frac{1}{s} + \frac{1}{V_{\text{Max}}} \quad (\text{S2})$$

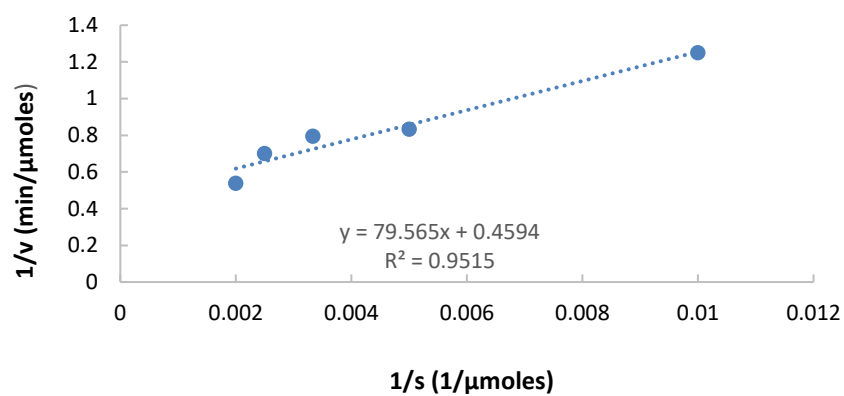
Where  $V_{\text{Max}}$  = maximum reaction rate (mM/min),  $K_m$  =affinity constant (mM),  $s$ = substrate concentration (mM) and  $v$  = enzymatic activity (mM/min).

To determine the kinetic parameters with the Novozym 435<sup>®</sup> biocatalyst, the initial reaction rates were determined at 50 °C, 40 °C, and 30 °C, using a 5 mL reaction medium, 20 mg biocatalyst, and 1000 mM lauric acid. For each temperature, the activities between 20 mM and 100 mM HMF were determined. Then, the kinetic parameters were determined through the graph (1/s) versus (1/v). The activities were determined based on the disappearance of the substrate, so the values are negative. Figure S2 shows the graphs for the calculation of the kinetic parameters at 50 °C, 40 °C and 30 °C.

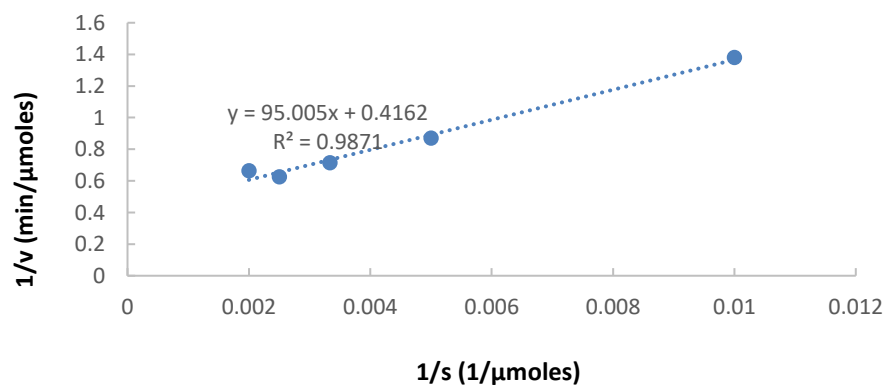
### Kinetic parameters at 50°C



### Kinetic parameters at 40°C



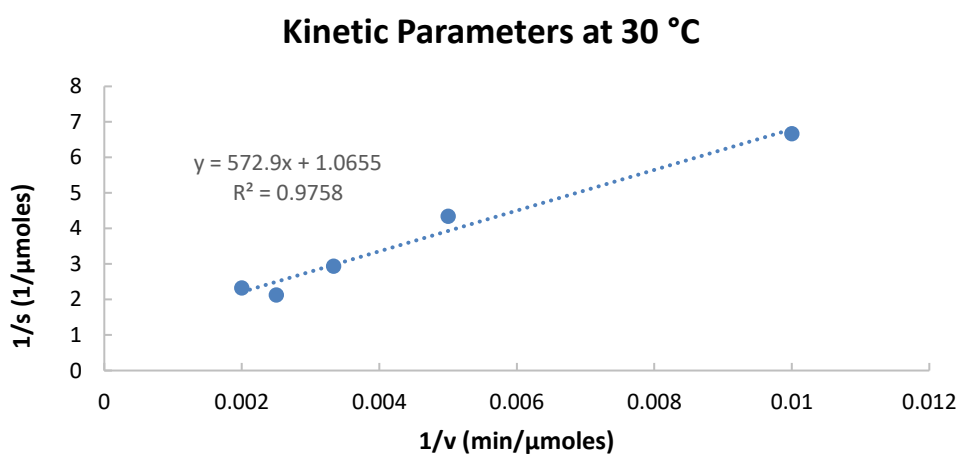
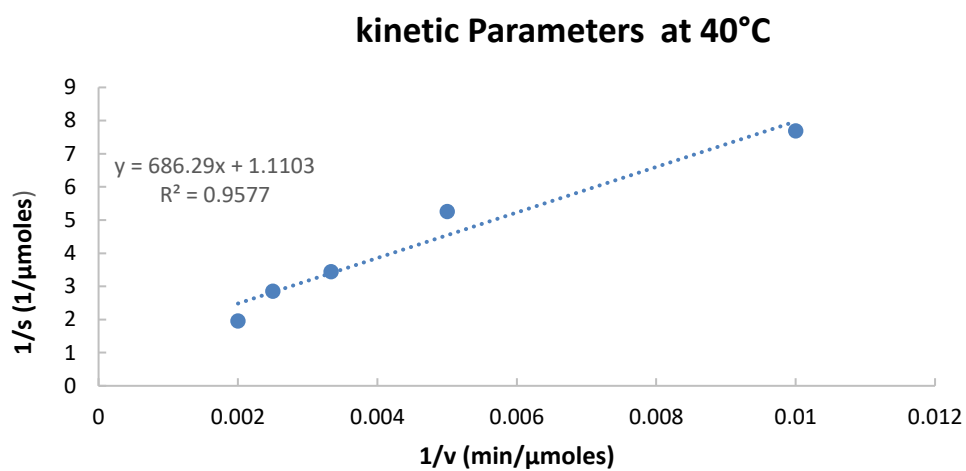
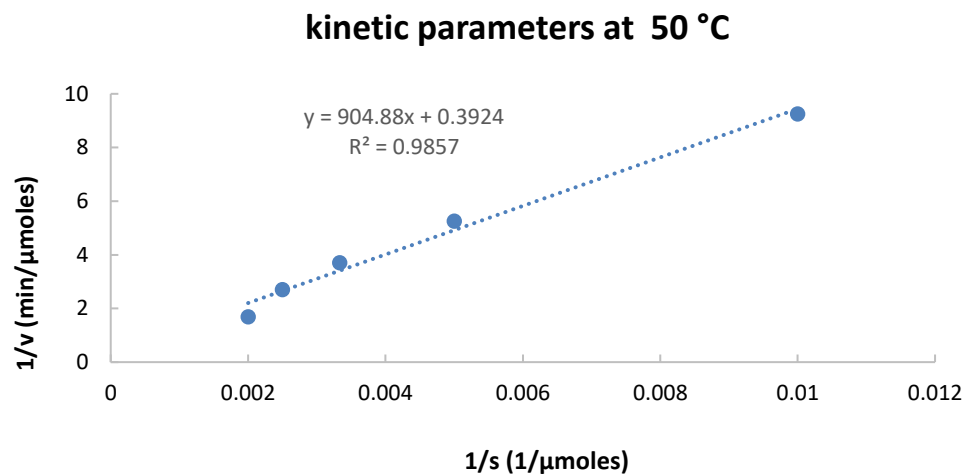
### Kinetic parameters at 30°C



**Figure S2.** Determination of kinetic parameters with Novozym 435<sup>®</sup>.

To determine the kinetic parameters with the TL lipase immobilized, the initial reaction rates were determined at 50 °C, 40 °C, and 30 °C, using a 5 mL reaction medium, 410 mg biocatalyst, and 1000 mM lauric acid. For each temperature, the activities between 20 mM and 100 mM HMF were determined. Then, the kinetic parameters were determined through the graph  $(1/s)$  versus  $(1/v)$ . The activities were determined based on the disappearance of the substrate, so the values are negative. Figure S3 shows the graphs for the calculation of the kinetic parameters at 50 °C, 40 °C and 30 °C.





**Figure S3.** Determination of kinetic parameters with TL lipase immobilized.