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EFFECT OF MOLAR WEIGHT OF CARBOXYLIC ACIDS ON THE ENZYMATIC ESTERIFICATION OF GLYCEROL

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ABSTRACT

Glycerol is a by-product in biodiesel synthesis, and its current market condition allows the possibility to transform into value-added compounds. In this work, the enzymatic esterification between glycerol and carboxylic acids of different molar weights was studied to obtain glycerides of industrial relevance. Therefore, eight different carboxylic acids were evaluated: formic, acetic, levulinic, caprylic, capric, lauric, stearic, and oleic. Immobilized lipase from *Candida Antarctica* was employed as a catalyst. Solvent-free reactions were carried out at 65 °C, 450 rpm, at a molar ratio of glycerol: carboxylic acid of 1:1 and a 1,6% enzyme concentration (based on reagents weight). Conversion of carboxylic acids was followed with time by titration. Under these conditions, acids from octanoic to oleic, which initially formed biphasic systems with glycerol, showed high conversions (68%-80%) and initial reaction rates in the same magnitude order. On the other hand, no enzymatic catalysis was observed with formic, acetic, and levulinic acids. Formic acid exhibited a higher rate and 58% of conversion without catalyst. However, for acetic and levulinic acids, conversion was low in uncatalyzed reactions. Then, for these acids, toluene was added as a reaction solvent. As a result, conversions and initial rates increased for these lighter acids, indicating the need for a non-polar media or a biphasic character to activate the enzyme.

Keywords: *Glycerol, Glycerides, Lipase, Direct Esterification.*

1. INTRODUCTION

In the last years, ecological requirements produced an accelerated growth of the biofuel industry. The biodiesel industry produces glycerol as a by-product representing 10% of the total mass. Thus, it is generated in large amounts. This causes a low price of glycerol and a favorable scenery to propose its transformation in higher-value compounds (Zheng, 2008).

One option is the direct esterification between glycerol (GLY) and carboxylic acids (CA) to produce glycerides. This reaction generates mono, di, and triglycerides with different applications such as emulsifiers, surfactants, plasticizers, among others. Currently, they are obtained through triglyceride glycerolysis. However, one advantage of direct esterification

lies in selecting type and number of carboxylic acid units to achieve specific applications.

This reaction can be biocatalyzed by lipases with the advantages of mild temperature conditions (Stergiou, 2013) and versatility in front of many substrates and media reactions. Most enzymatic syntheses are performed in the presence of organic non-polar solvents, although solvent-free systems are technically feasible (Freitas, 2007). Furthermore, reactions in aqueous and low-water media are described (Silvestini and Cianci, 2020). The lipase source and the solvent influence were widely studied on glycerol esterification, especially with fatty acids (Kristensen *et al.*, 2005; Dutta Banik *et al.*, 2016). Jassen *et al.* (1993) studied the effect of molecular weight in the thermodynamic equilibrium of esterification of glycerol and carboxylic acids in

range C6-C18 with a Lipase from *Chrombacterium viscosum*. They found that equilibrium constants are similar in all cases.

In this work, eight different carboxylic acids were used to evaluate the influence of molar weight and unsaturations in equilibrium conversion and initial rate values. A lipase B from *Candida antarctica* (CaLB1) adsorbed on dry hydrophobic acrylic beads was used as a biocatalyst. The acids used were: formic (C1), acetic (C2), levulinic (C5), caprylic (C8), capric (C10), lauric (C12), stearic (C18), and oleic (C18).

2. MATERIALS AND METHODS

2.1. Materials

The enzyme used was a Lipase from *Candida antarctica* (lipase B adsorbed on acrylic beads. Activity: 10000 PLU / g. IMMCALB-T1-350) supplied by ChiralVision (the Netherlands). All chemicals were of analytical grade; levulinic acid, caprylic acid, capric acid (>98% purity, Sigma-Aldrich, USA), anhydrous glycerol 98%, formic acid 85%, ethyl ether, toluene, KOH (Cicarelli, Argentina), lauric acid 99% (Serquim, Argentina), stearic acid 65%, oleic acid 77% (Pura Química, Argentina) and acetic acid (Biopack, Argentina).

2.2. Esterification Reaction

Reactions were carried out in a three-necked glass reactor immersed in a thermostatic bath. Agitation was provided through a mechanical stirrer at 450 rpm. The temperature was registered using a "K" type thermocouple submerged in the liquid system, and a lateral position was kept free for sampling. Experiments were carried out at a 1:1 glycerol:acid molar ratio, and the initial reaction volume was 30 mL. The mixture was incubated at 65 °C for 4 hours. The lipase concentration was 1,6% of the reagent weight. When solvent was used, 15 mL of reagents were prepared with the same molar ratio and lipase concentration, and 15 mL of toluene was added.

In order to measure the carboxylic acid conversion, samples were taken in time intervals with an automatic pipette. These aliquots were diluted in 15 mL of an ethanol/ethyl ether 50:50 (v/v) mixture and titrated with KOH solution in ethanol using phenolphthalein as the endpoint indicator.

To calculate the initial reaction rate, the conversion was plotted against time; then, a non-linear regression was used to fit the curve and the derivative at time zero was determined. Finally, the rate was expressed as a function of the enzyme

weight and the initial concentration of acid.

3. RESULTS AND DISCUSSION:

Figure 1. shows the results for the conversion of the eight acids. Under these conditions, the upper molecular weight acids (from C8 to C18:1) reached equilibrium due to the activity of the enzyme. In previous work, reactions with the same molar ratio and without catalysis at 100 °C did not extend conversion above 20%, except for C1 and C2 (**Table 1**). Therefore, blank reactions were carried out for C1 and C2 at 65 °C to verify the lipase activity in these cases, and the blank curves were similar to reaction curves (**Figure 2.**)

Figure 1. Acid conversion vs. time. C₁(●) C₂(■) C₅(●) C₈(▲) C₁₀(▼) C₁₂(◆) C₁₈(◀) C_{18:1}(▶)

Table 1. Acid conversion for enzymatic and blank reactions at 65°C and 100°C after 4 h.

Acid	X _{1,6%} CaLB - 65°C	X _{Blank-} 100°C	X _{Blank-} 65°C
C1	0,59	0,55	0,59
C2	0,10	0,60	0,10
C5	0,11	0,16	
C8	0,69	0,11	
C10	0,75	0,20	
C12	0,80	0,18	
C18	0,73	-	
C18:1	0,68	-	

These results indicate that the enzyme did not catalyze reactions with acids from C1 to C5. This behavior corresponds to the number of phases at the beginning of the reaction. The

systems were biphasic when increasing the molecular weight of the acids due to the increment of the hydrophobicity, starting from C8. Therefore, the enzyme showed high activity in two-phase systems with non-polar substrates, but the activity was low or negligible in polar systems, probably due to the hydrophobicity of the natural lipase substrates. Smaller acids had a polar behavior and higher acidity, negatively affecting the enzyme activity. Furthermore, some authors compared different solvents concluding that less polar media increases lipase activity whereas hydrophilic solvents interfere with the interactions between the substrate and the active site of the enzyme (Dutta Banik *et al.*, 2016; Li *et al.*, 2010). The reactions with C2 and C5 were carried out to test these hypotheses, adding toluene as a non-polar solvent. **Figure 3.** shows that conversion increased from 11% to 34% for C5 and from 10% to 24% for C2.

Figure 2. Effect of enzyme catalysis in light acid conversion at 65°C

C1 blank (■) C2 blank (●) C1 (▲) C2 (▼)

For the analysis three groups of acids could be identified: light acids: C1, C2, and C5; medium acids: C8, C10, and C12; and heavy acids: C18 and C18:1. **Figure 4.** shows the initial reaction rate of all of them.

C1 exhibited the highest initial rate, but the enzyme did not affect the reaction, as mentioned before. Then, in this case, the rate is the effect of its higher reactivity as a reagent and not as a substrate of the enzyme. C2 showed the same initial rate with and without solvent, and C8 only exceeded it. However, the conversion was much lower than the rest of the acids. The initial reaction

rate with toluene was higher for C5 than without solvent. Nevertheless, it was the lowest except for the unsaturated acid. C5 is the only acid with a second functional group; this keto group may interact with the active site causing an inhibition (Dutta Banik *et al.*, 2016).

Figure 3. Influence of solvent in acid conversion

C5 toluene (■) C2 toluene (●) C5 (▲) C2 (▼)

Figure 4. Initial rate in enzyme catalysis reaction. C2-T and C5-T reactions with toluene.

In the medium acids group, it was observed that the higher the molecular weight, the lower the reaction rate, but the final conversion values showed the opposite behavior (**Table 1**). C18 disrupted this tendency because it exhibited a higher rate and lower conversion in comparison to C12. Nevertheless, the conversion was near to the latter. Regarding the enzyme activity over saturated and unsaturated acids, it was observed that the presence of a double bond reduces the

initial rate, probably due to steric hindrance.

8. Zheng, Y., Chen, X., Shen, Y., *Chemical Reviews*, **2008**, 110(3), p. 1807.

4. CONCLUSIONS:

A better understanding of the performance of enzymatic esterification reactions between different carboxylic acids and glycerol was achieved. Systems without solvent were used for acids from C1 to C18:1. The enzymatic catalysis did not work in single-phase systems with high polarity. However, this behavior changed when a non-polar solvent increased the conversion and reaction rate for C1, C2, and C5 acids. On the other hand, the less polar acids which initially form biphasic systems with glycerol showed acceptable conversions and similar reaction rates. These results indicate the need for a non-polar media or a biphasic character to activate the enzyme at the reaction conditions studied in this work.

5. REFERENCES:

1. Dutta Banik, S., Nordblad, M., Woodley, J. M., Peters, G. H., *ACS Catalysis*, **2016**, 6(10), pp. 6350–6361.
2. Freitas, L., Perez, V. H., Santos, J. C., de Castro, H. F., *Journal of the Brazilian Chemical Society*, **2007**, 18(7), pp. 1360–1366.
3. Janssen, A. E. M., Van der Padt, A. and Riet, K. V. t., *Biotechnology and Bioengineering*, **1993**, 42(8), pp. 953–962.
4. Kristensen, J. B., Xu, X. and Mu, H., *JAOCS*, **2005**, 82(5), pp. 329–334.
5. Li, C. Tan, T., Zhang, H., Feng, W. and, *Journal of Biological Chemistry*, **2010**, 285(37), pp. 28434–28441.
6. Silvestrini, L., Cianci, M., *International Journal of Biological Macromolecules*. Elsevier B.V., **2020**, 158, pp. 358–363.
7. Stergiou, P. Y., Foukis, A., Filippou, M., Koukouritak, M., Parapouli, M., Theodorou, L. G., Hatziloukas, E., Afendra, A., Pandey, A., Papamichael, E. M., *Biotechnology Advances*, **2013**, 31(8), pp. 1846–1859.