Triacetin hydrolysis by lipase: determination of optimum operational conditions and reaction kinetics

Darja Pečar[1*], Nina Belina[1], Andreja Goršek[1]

[1] *Faculty of Chemistry and Chemical Engineering, University of Maribor, Smetanova 17, 2000 Maribor, Slovenia*

**Corresponding author: darja.pecar@um.si*

Highlights

- Reaction conditions for enzyme hydrolysis of triacetin by lipase were optimized.
- Michaelis-Menten kinetic model was employed.
- Kinetic parameters for hydrolysis of triacetin by lipase were determined.

1. Introduction

Lipases are among the most widely used enzymes due to their high stability and activity at different process conditions and reaction media. They catalyse the hydrolysis of fats and oils [1]. Lipases find applications in various industries, including food, pharmaceutical, fuel, paper, agriculture, and flavour industry. In fact, one of the primary industrial applications of lipases is in the hydrolysis of their natural substrates, triglycerides, typically to obtain free fatty acids or modified oils [2].

Triacetin is an organic molecule, classified as a triglyceride specifically, the triester of glycerol with acetic acid [3]. We were focusing on this molecule because it represents the smallest compound in triacylglycerol family that encompasses all the features of triacylglycerols. It plays various roles, serving as a plant metabolite, a solvent, a fuel additive, an adjuvant, a food additive carrier, a food emulsifier, a food humectant, and an antifungal drug [4]. Selective hydrolysis of triacetin produces multifunctional chiral products. For example, the selective regio-hydrolysis of a single acetyl group of triacetin leads to diacetin, serving as a solvent, plasticizer, and softening agent.

In this study, enzyme-catalyzed triacetin hydrolysis by immobilized lipase was conducted, and the reaction progress was monitored using in-situ FTIR. Reactions were performed at various temperatures, enzyme loadings and buffer *pH* levels. For the kinetic analysis Michaelis-Menten kinetic model was applied.

2. Methods

Reactions with 1 mL of triacetin were conducted at 25, 30 and 35 °C. Various amounts of the enzyme were employed (0.2 g, 0.4 and 0.6 g of lipase). The reactions took place in phosphate buffer with *pH* 6, 7 and 8. Each reaction required 50 mL of a pre-prepared buffer which was placed in the beaker. Once the target temperature was reached, the ReactIR 702L probe was immersed into the buffer. Using IC IR 7.1 data capture program, we configured the spectra to be captured every 2 min. Subsequently, 1 mL of triacetin was added to the beaker. After 30 min, we introduced a catalyst lipase previously immobilized on silica gel particles [1]. Following each experiment, a 3D image of the obtained spectra was generated. The reaction continued for at least 3 h to stabilize the diacetin production curve. The MicroMath's Scientist software was employed for kinetic calculations.

3. Results and discussion

The hydrolysis reactions were initially conducted with a fixed mass of enzyme and at three different temperatures (Figure 1). Subsequently, maintaining a constant temperature, we investigated the impact of different enzyme concentrations on triacetin conversion (Figure 2). Additionally, we studied the influence of phosphate buffer *pH* on reaction progress.

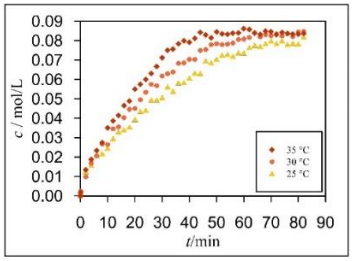

Figure 1. Concentration profiles of diacetin at different temperatures and 0.2 g of lipase.

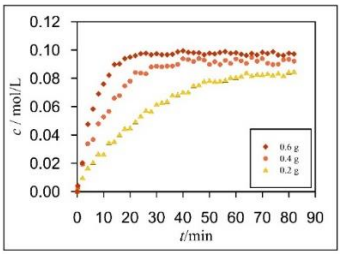

Figure 2. Concentration profiles of diacetin at different amounts of lipase and 25 °C.

The optimum temperature for the hydrolysis reaction of triacetine was found to be 35 °C. Achieving 100 % conversion in the shortest time (25 min) was accomplished by adding 0.6 g of lipase in a buffer with *pH* 8. Maximum conversion was also attained in a buffer with pH 7, albeit requiring a longer reaction time.

Kinetic study was performed using MicroMath's Scientist software. We calculated maximum reaction rate, r_{max} and Michaelis-Menten constant, K_M from the Michaelis-Menten equation. Results for the experiments at fixed temperature and different amounts of enzyme are presented in Table 1. Activation energies for the hydrolysis of triacetin with different amounts of lipase were determined using Arrhenius plot. Results are presented in Table 2.

Table 1. Kinetic parameters for hydrolysis of triacetine by lipase at T = 35 °C and *pH* = 8.

T/°C	m_E/g	r_{max}/mol/L min	k/s^{-1}	K_M/mol/L
35	0.6	30.4	2068.7	200.0
35	0.4	22.5	2298.8	260.9
35	0.2	9.5	1928.8	254.1

Table 2. Activation energies and pre-exponential factors for hydrolysis of triacetine by lipase.

m [g]	E_a [kJ/mol]	k_0 [s^{-1}]
0.6	64.3	$1.4 \cdot 10^{14}$
0.4	109.3	$6.7 \cdot 10^{21}$
0.2	104.2	$7.4 \cdot 10^{20}$

4. Conclusions

In this study, enzyme-catalyzed triacetin hydrolysis was performed, and the reaction progress was monitored using in-situ FTIR. Reactions were conducted at various temperatures, enzyme loadings, and *pH* levels of the phosphate buffer. For the kinetic analysis Michaelis-Menten kinetic model was applied. It was observed that the highest conversion occurred in the reaction carried out at 35 °C, with 0,6 g of lipase and *pH* of 8. The results indicated that the reaction rate increases with *pH*. The progression of the reaction was effectively described by Michaelis-Menten kinetic model.

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Keywords
hydrolysis, triacetin, lipase, kinetics.