KINETIC OF THE ENZYMATIC HYDROLYSIS OF OLIVE OIL USING A PH-STAT TITRATION WORKSTATION

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Introduction. The mechanism of the enzymatic hydrolysis of oils is well known and studied. The common method to follows the extent of the reaction is taken samples of the mixture during time and manually titrated (Gamez *et al*.,2003). Using the pH-Stat, we accurately followed the kinetic reaction in a long period of time.
The objective of this work was to determine the kinetic parameters for the enzymatic hydrolysis of olive oil.

Methods. Lipase F-AP15 from *Rhizopus oryzae* (Amano Enzyme Inc.) was evaluated at different concentration of enzyme (0.25 and 0.75 %w/w of oil) and temperature (25℃ and 40℃). All experiments were run on a computer electronic TitraLab 856 (Radiometer Analytical). A celstir jacketed reactor (50 mL) was connected to a recirculation bath to maintain the temperature. The mixture of reaction was prepared mixing 10 g of olive oil (Sigma-Aldrich) and 18 ml of 0.1 M phosphate buffer, pH 7. Lipase was dissolved in 3 ml of buffer. Titrating solution 0.1 M KOH was placed in the burette of the equipment an continued feeded to the reaction mixtures as required to maintain a pH=7.0, with a PID algorithm program. Experiments were monitored every two minutes during 24 hours of reaction. A pseudo first order model (eq.1) of deactivation (Noriega, J., 2010) was used to fit the extent of the kinetic data, and the integral method of Michaelis-Menten equation (eq.2) was used to determine the Km, Vmax, kcat, catalytic efficency, values.

$$H\% = 1 - \exp\left[\frac{k_0}{k_D}\left(e^{-k_D t} - 1\right)\right] \qquad \text{eq.1}$$

$$\frac{[FAA]}{t} = V_m - K_m \frac{\ln\left(\frac{[FAA]}{[S]_0 - [FAA]} + 1\right)}{t} \qquad \text{eq.2}$$

Results. The pseudo first order model of deactivation fit the extent of reaction very well (R^2>0.98) for all kinetic study (Fig 1).It can be noted that the initial rate constant k_0 is higher when at 25℃, and 0.75% of enzyme load, but when increased temperature, this was decreased.

Table 1. Kinetic parameters for the hydrolysis of olive oil with lipase F-AP15

Exp	V_{max} (mol/s)	k_m (mol/l)	k_0 (mol/mol h)	k_D (1/h)	k_{cat} (s^{-1})	k_m/k_{cat} (mol/l s)
1	500	515.25	0.02873	0.096	2.5 x 10^7	4.8 x 10^4
2	400	401.04	0.0659	0.240	2 x 10^7	5 x 10^4
3	10000	20093	0.0106	0.246	5.01 x 10^8	2.5 x 10^4

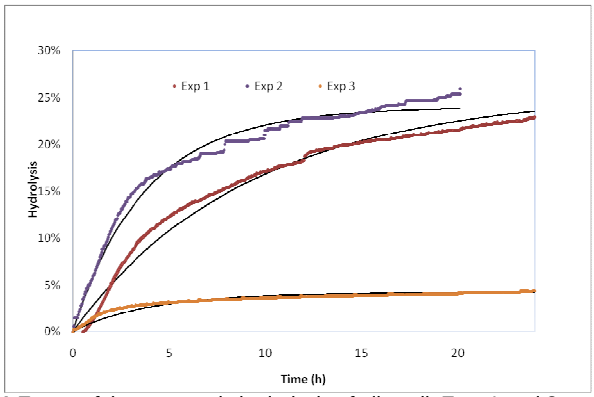

Fig.1 Extent of the enzymatic hydrolysis of olive oil**.** Exp. 1 and 2 were at 25℃ with 0.25% and 0.75%w/w. Exp3 was 40℃ and 0.7 5%w/w oil.

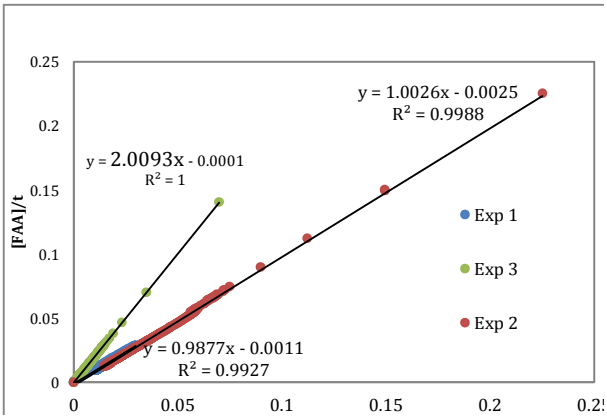

Fig. 2 Integral method for each experiments that is used to determinate the rate constant k_m and the max reaction rate

The explanation for this case is that this enzyme optimally works at 30℃ (Ben Salah, A, 1994). The lipases sho ws higher catalytic efficiency (K_M/k_{cat}) at 25℃, no matter the concentration used.

Conclusions. The pH-Stat and the models applied permitted an accurately way to determine the kinetic parameters of hydrolysis of oils. The results of this work will be used in a future graduate project about a design a CSTR to hydrolysis of vegetal oil.

References

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