Kinetic characterization of almond β-glucosidase

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Introduction

β-glucosidase (EC 3.2.1.21) are enzymes which catalyze the hydrolysis of O-β-Glycosidase bonds to terminal non-reducing residues in beta-D-glucosides and oligosaccharides, with release of a molecule of glucose. They are involved in several biological functions [1] in bacteria, funghi, plants and mammals alike. Their capacity to catalyze the inverse reaction of transglycosilation [2] makes them quite interesting as an industrial catalyzer [3,4].

The objective of these experiments was to propose a kinetic mechanism for almond β-glucosidase .

Methods

0,9 ml Substrate pNPG	5 min 40 ºC →	0,1ml enzyme	time 40ºC →	1,5 ml NaOH 0,2M

- Standarization of assay conditions

In order to obtain velocities of reaction, we first needed to construct a calibration plot of pNP. After that, assays were run on to detremine optimal enzyme concentration and approximated Km. Then, we checked the linearity of the assay with time.

- Determination of kinetic parameters

Assays were performed in the same conditions previously established and with different substrate conditions. After having obtained initial velocities, macroscopic kinetic parameters were determined.

- Effects of temperature in the cathalysis

Kinetic parameters of the enzyme were determined at different temperatures. Actication energy (Ea) was estimated using an Arrhenius plot. Q10 coefficient was also calculated. Q10=Vmax(T)/Vmax(T-10).

- Inhibition studies

Finally we studied the inhibition of β-glucosidase using different concentrations of glucose (a product of this reaction) and δ- gluconolactone (analogue of the transition state).

Results

- Assay standarization

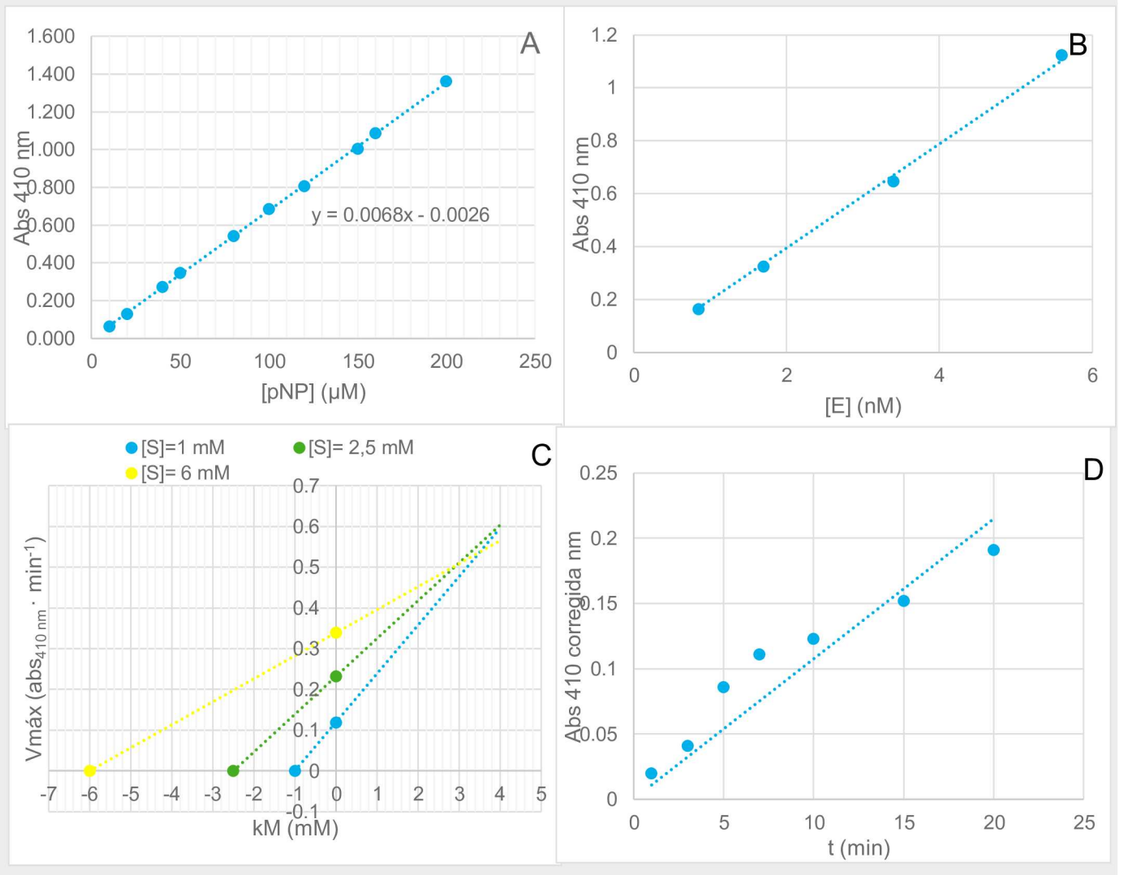


Figure 1. A) pNP calibration plot. B) Optimal [E] (3.5 nM). C) Approximated Km (3.65 mM). D) Linearity with time

Results

-Kinetic parameters

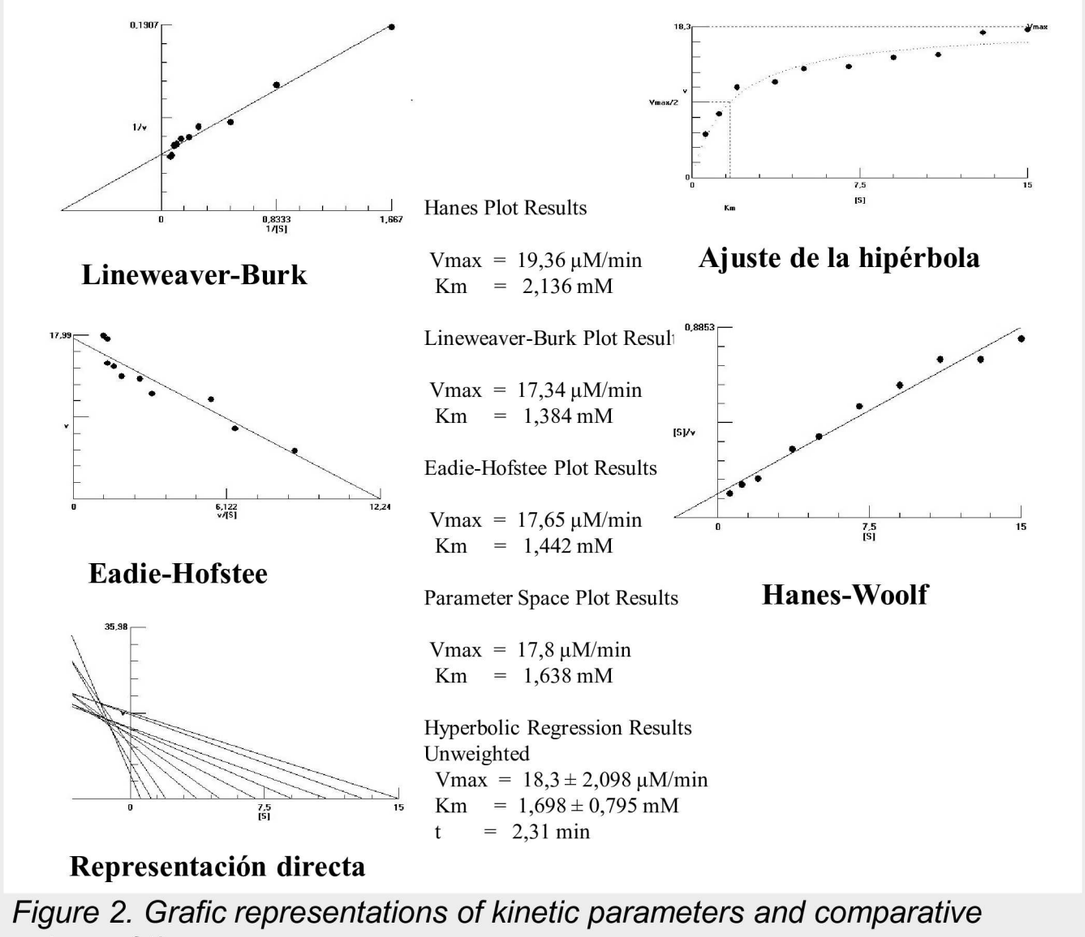
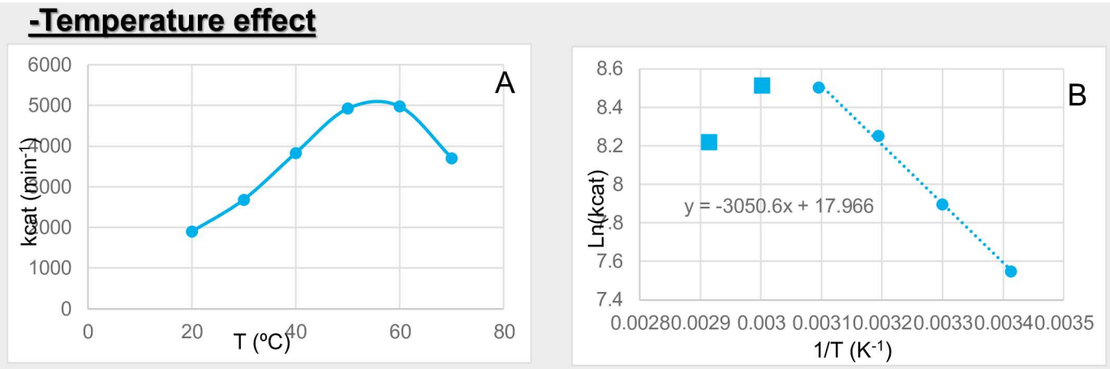


Figure 2. Grafic representations of kinetic parameters and comparative values of them.

-Temperature effect



- *Figure 3. A) Effect of temperature on kcat. B) Arrhenius plot. Ea= 25.35 kJ/mol. Q10 =1.43*

-Inhibition studies

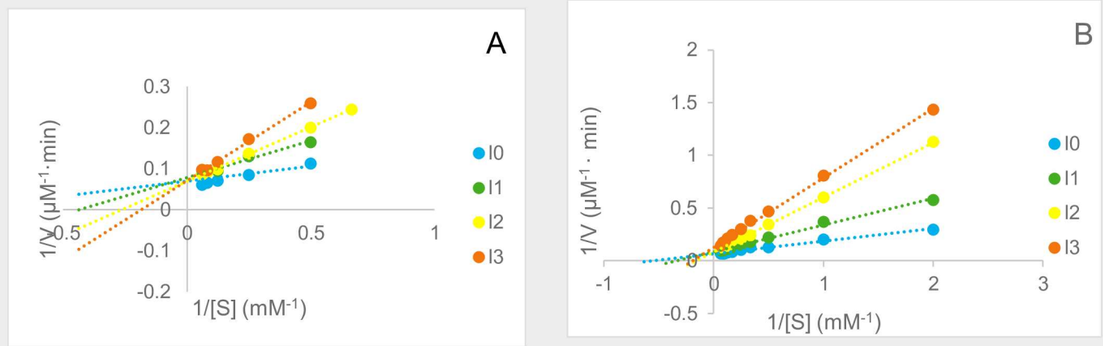


Figure 4. A) Inhibition by δ-gluconolactone (Kis= 0.06 mM). B) Inhibition by glucose (Kis= 205 mM).

Conclussions

In conclusion, these experiments were held in quite reasonable conditions according to the obtained experimental values for the kinetic parameters, which only differ slightly from other studies [5,6,7], as does the incubation temperature of 40ºC. Attending to the inhibition study (a competitive type), and since the ultimate goal of these experiments was to propose a kinetic mechanism for almond β-glucosidase, we concluded this mechanism to be a uni-bi ping-pong type as showed in *figure 5.*

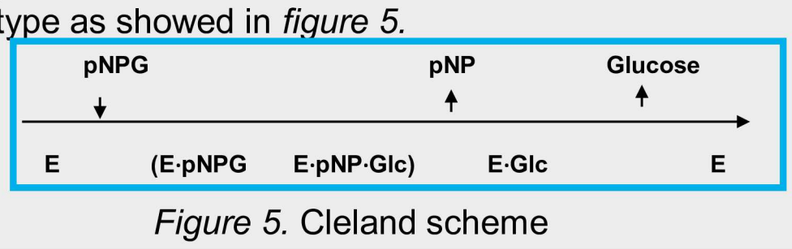


Figure 5. Cleland scheme

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