# KINETIC CHARACTERIZATION OF ALMOND ß-GLUCOSIDASE

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## 1.INTRODUCTION

In this assay we will talk about ß-glucosidases and its kinetic mechanism and parameters.

ß-glucosidases (ß-D-glucoside glucohidrolase, EC 3.2.1.21) are enzymes that hydrolyzes the O-ß-glicoside bond of the non-reductor end between two or more glucosides, released ß-D-glucose. It can catalyze transglicosidation's reaction too.

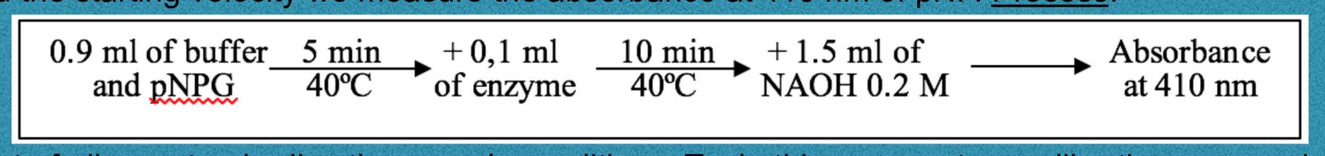
ß-glucosidases are very extended in nature and they are present in a lot of organism. Moreover, glucosidases are used as industrial catalyst and they have biotechnological applications.

This assay is related to ß-glucosidase of sweet almond (*Prunus dulcis*) and the objective is to propose a model of the enzyme's kinetic mechanism.

The experiment realized is an enzymatic assay where assay's appropriate conditions have to be find and the results are the kinetic parameters, the optimal essay temperature, the effects of two inhibitors: glucose and D-glucono-1,5-lactone and the kinetic mechanism of the reaction that is a ping-pong mechanism.

## 2.METHODS

The reaction catalyzed by ß-glucosidase is the transformation of pNPG to pNP and glucose. To find the starting velocity we measure the absorbance at 410 nm of pNP. Process:




First of all, we standardize the assay's conditions. To do this, we create a calibration curve using different concentrations of pNP. We will use this calibration curve to obtain the product quantity, and then, the initial velocity. Secondly, we need to calculate an optimum enzyme concentration at around 0.7 of absorbance. Then, we need to find out the $K_M$ approximate of our substrate in an assay, in order to choose adequate concentrations of substrate for the general experiment. Finally, we evaluate the linearity of the appearance of product with time by running the assay using different times.

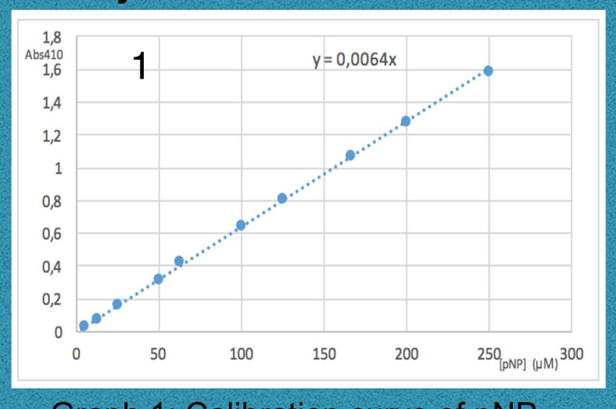
In the general assay, after obtaining and correcting the absorbances, we calculate the initial velocity and the kinetic parameters: $K_M$, Vmax, kcat and catalytic efficiency, using the different representations.

Later, we do a study of the temperature as an environmental factor on the enzymatic activity through the elaboration of an assay at different temperatures to see how the kinetic parameters vary, to determine the activation energy and to calculate $Q_{10}$.

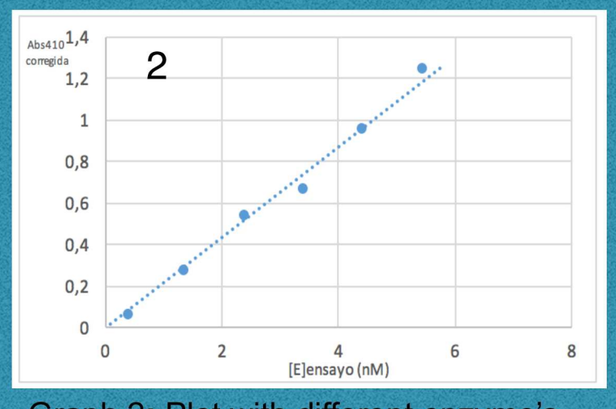
In the end, we examine the inhibitory behavior of glucose, one of the products of the hydrolysis of pNPG, and of D-glucono-1,5-lactone, considered an analogue of the transition state by determining the kinetic parameters in the presence of four increasing concentrations of the inhibitor.

## 3. RESULTS

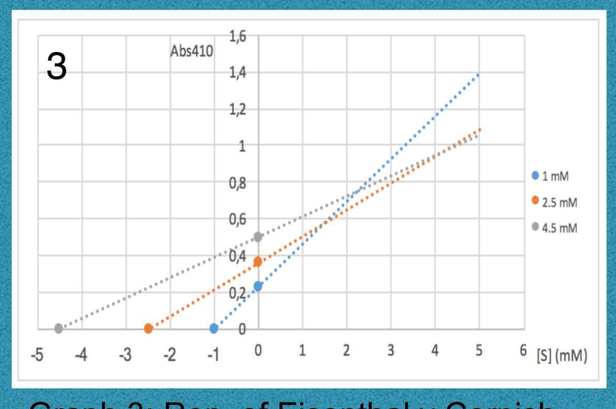
### 3.1 Essay's conditions



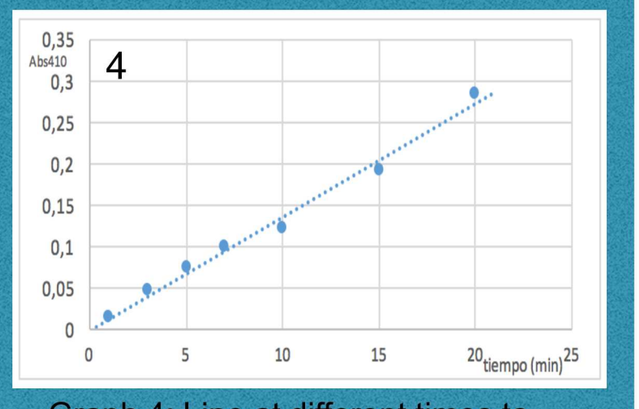

Graph 1: Calibration curve of pNP.




Graph 2: Plot with different enzyme's concentrations.

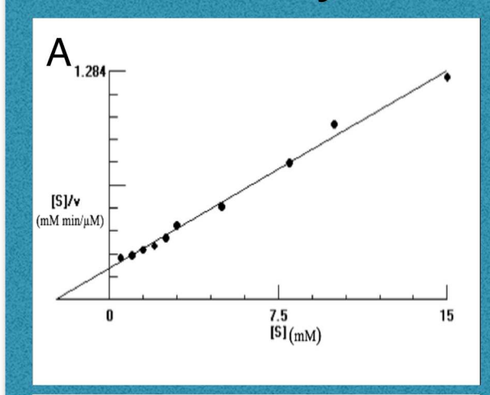


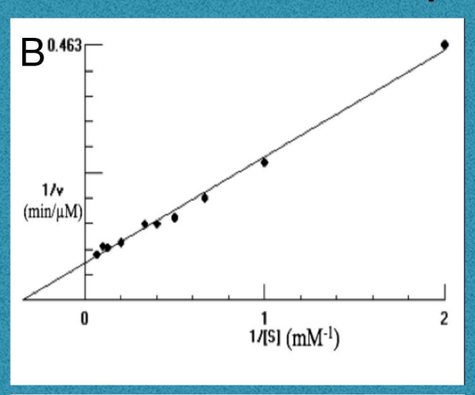

Graph 3: Rep. of Eisenthal y Cornish-Bowden for obtain the approximate $K_M$.

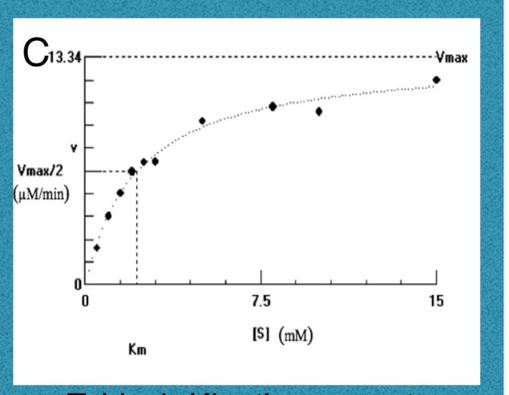


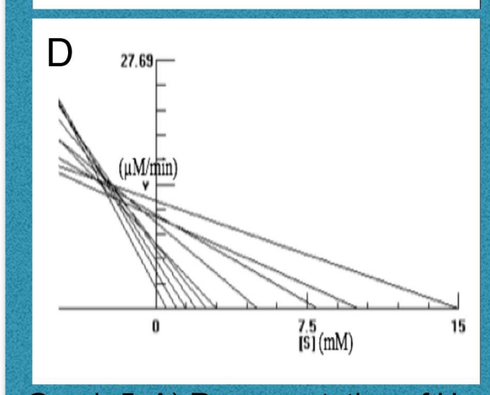

Graph 4: Line at different times to verify the lineality with time of the product.

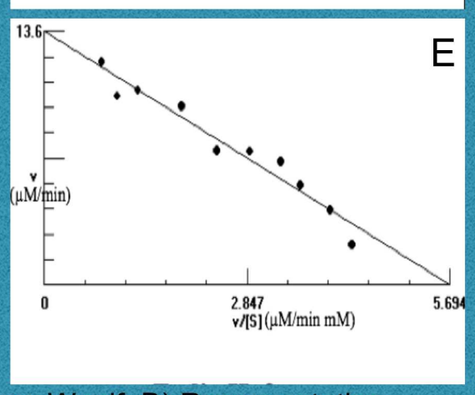
### 3.2 General essay and determination of the kinetic parameters



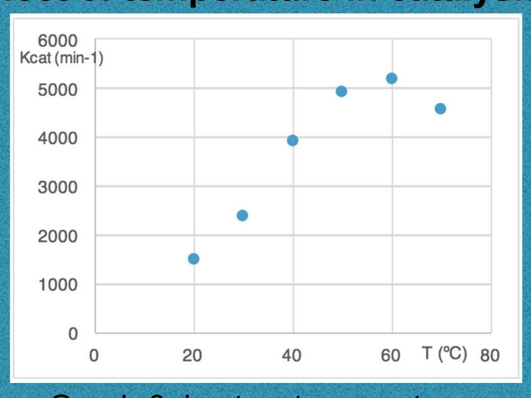




Graph 5: A) Representation of Hanes-Woolf, B) Representation of Lineweaver-Burk, C) Representation of Michaelis-Menten, D) Representation of Eisenthal y Cornish-Bowden and E) Representation of Eadie-Hofste

Table 1: Kinetic parameters of each representation

| | A | B | C | D | E |
|---|---|---|---|---|---|
| Vmax (M/min) | 15,01 | 13,53 | 13,6 | 13,9 | 13,3 |
| Km (mM) | 2,9 | 2,37 | 2,4 | 2,43 | 2,24 |
| Kcat ($min^{-1}$) | 4415 | 3979 | 4000 | 4074 | 3924 |
| EC ($min^{-1}$ $mM^{-1}$) | 1524 | 1676 | 1675 | 1680 | 1753 |

### 3.3 Effect of temperature in catalysis

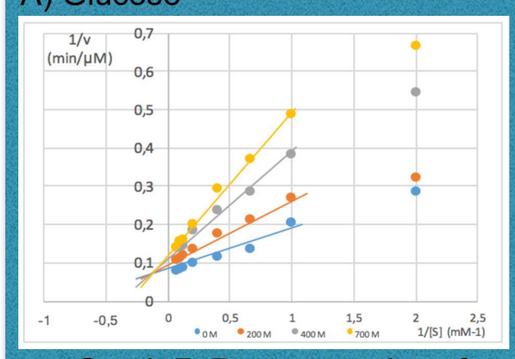



Graph 6: kcat vs temperature

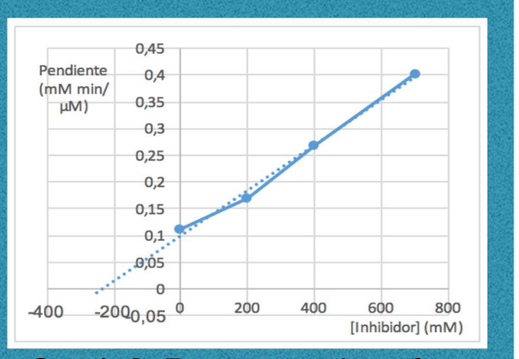
Ea is 26.55 kJ/mol and $Q_{10}$ is 1.64.

### 3.4 Studies of reversible inhibition for ß-glucosidase

A) Glucose

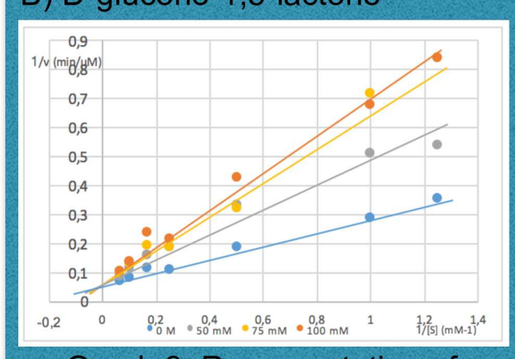



Graph 7: Representation of Lineweaver-Burk.

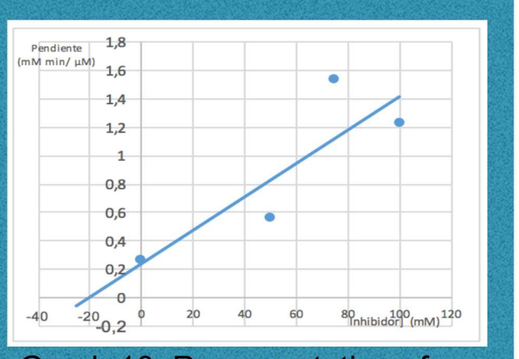



Graph 8: Representation of Dixon. Kis=250 mM

B) D-glucono-1,5-lactone



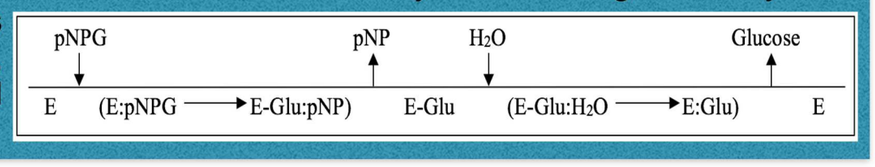

Graph 9: Representation of Lineweaver-Burk.




Graph 10: Representation of Dixon.Kis=20 mM

## 4. CONCLUSION

In first place, we have obtained the appropriate conditions for this assay: 3.4 nM as enzyme's concentration because it is the concentration closer to 0.7 of Abs410; 0.5 to 15 mM as interval of substrate's concentration because 0.5 mM (lowest concentration) is lineal with time, so linearity at the highest concentrations is guaranteed; and ten minutes of time of incubation. Then, we calculated the kinetic parameters of the reaction: $K_M$ is 2.24 mM (very near to the approximate $K_M$ calculated in the experiment, 2.3 mM), Vmax is 13.34 M/min, kcat is 3924 $min^{-1}$ and catalytic efficiency is 1753 $min^{-1}$ $mM^{-1}$. The optimal essay temperature is 50ºC to prevent denaturalization although 60ºC is the one that gets the most activity; and the activation energy of the reaction is 26.55 kJ/mol. Glucose is a competitive inhibitor of the enzyme, showing low levels of inhibition (Kis=250 mM) because it is a product of the reaction and practice a negative feedback effect over ß-glucosidase. And D-glucono-1,5-lactone acts as a competitive inhibitor too, but with highest levels of inhibition (Kis=20 mM) due to it is an analogue of the transition state and the enzyme shows higher affinity for it. In the end, the kinetic mechanism of the reaction is proposed as a ping-pong mechanism where pNPG binds first, pNP is released as first product, water binds as a second substrate and glucose releases as a second product.




## 5. REFERENCES

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