KINETIC CHARACTERIZATION OF ALMOND β – GLUCOSIDASE

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1. INTRODUCTION

The ß-glucosidases (EC 3.2.1.21) are the enzymes of the glycosidase family that catalyze the hydrolysis of the O-ß-glycosidic bond, as well as transglycosylation; with retention of the anomeric carbon. They have diverse applications in the industries of the feeding, the elaboration of beverages, the textile, the paper, the biofuels... They are classified according to the different criteria like the specificity of the substrate or the similarities of sequence and folding.

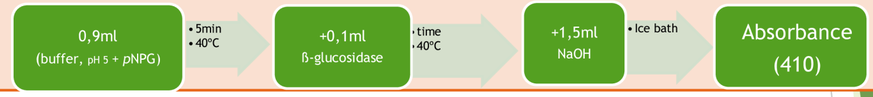
These enzymes are present in abundance in nature: in bacteria and fungi, they are part of a multienzyme complex called cellulase; in plants they perform various functions, (eg: defense mechanism); and in animals, it is related to the metabolism of xenobiotics. In humans, the deficiency of lysosomal acid-glucosidase causes Gaucher's disease. In this study, the ß-glucosidase of Prunus Dulcis was characterized, determining its kinetic parameters for pNPG and carrying out, for this purpose, the previous standardization of the assay. In addition, the effect of temperature and inhibitors (glucose and gluconolactone) for the proposal of a catalytic mechanism was studied.

2. MATERIALS AND METHODS

MATERIALS:
- Biological material: commercial preparation of ß-glucosidase isolated from *Prunus Dulcis* from FLUKA.
-Reactives supplied by FLUKA: p-nitrophenol (pNP), p-nitrophenyl-ß-D-glucoside (pNPG), Glucose and d-gluconolactone.
-Other general use reagents, supplied by PANREAC: NaOH, HCl, citric acid.

METHODS:
Enzymatic assay
An indirect test of appearance of the product was carried out, measuring the appearance of *p*NP in basic medium. The unit of enzymatic activity was defined as the initial rate of reaction, that is, the appearance of product over time. It was expressed as µM concentration of pNP in a reaction volume per minute.

Method scheme:



3. RESULTS

Standardization

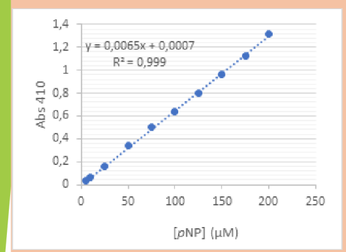


Figure 1: calibration line of *p*NP

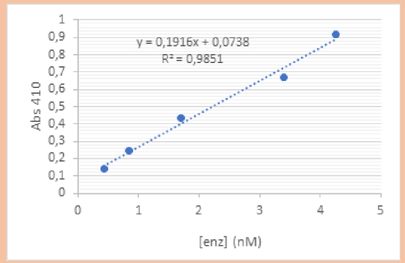


Figure 2: Linearity with enzyme concentration. [E] optimal= 3,2 nM

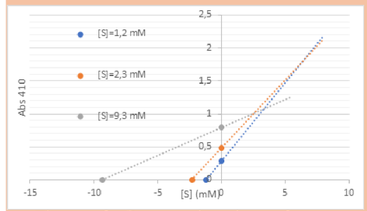


Figure 3: Determination of approximate Km. Km*=3,5mM.

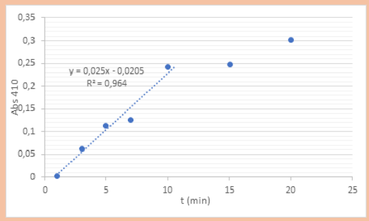


Figure 4: Linearity with time. t optimal= 10min, %S hydrolyzed =5,33

Kinetic parameters

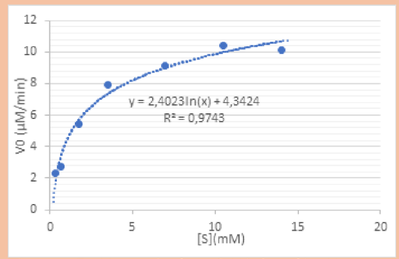


Figure 5. Hyperbola of Michaelis-Menten

Table 1: *Macroscopic kinetic parameters for each representation*

Representation	Km (mM)	Vmáx (µmol/min)
Michaelis-Menten	1,958	11,89
Lineweaver-Burk	1,276	9,876
Eadie-Hofstee	1,835	11,69
Hanes-Woolf	1,514	10,95
Eisenthal y Cornish-Bowden	1,913	11,8

Effect of temperatura

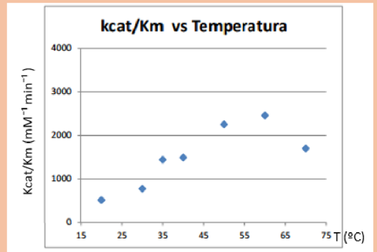


Figure 6: Effect of temperature on the catalytic efficiency. The variations are due to changes in the kcat. Km remains constant. Toptimal=55ºC

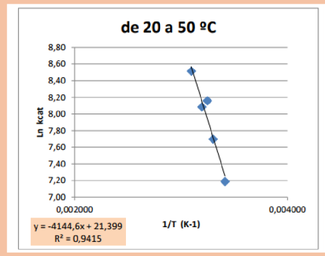


Figure 7: Representation of Arrhenius. Ea=34458 J/mol

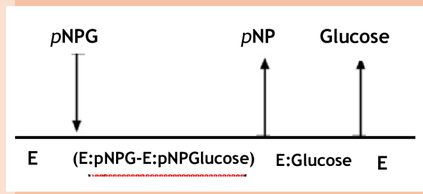


Figure 10: Catalytic mechanism proposed for ß-glucosidase

Inhibitions

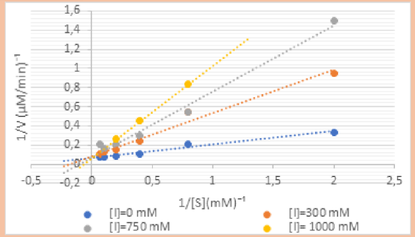


Figure 8: Inhibition of glucose (Kis = 160 mM)

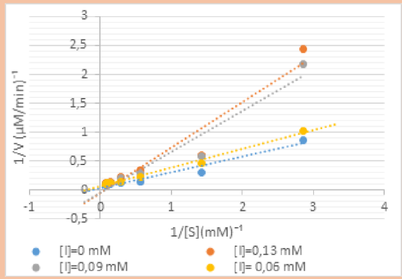


Figure 9: Inhibition of δ-gluconolactona (Kis = 0.08 mM)

4. CONCLUSIONS

In conclusion, the kinetic parameters determined for the ß-glucosidase were: Km = 1,958 mM and Vmax = 11,89 µmol/min, under the following conditions T = 40ºC, pH = 5, t = 10min, [E] = 3,2nM, [S] = (0.2 Km * - 8Km *). The temperature at which maximum activity is reached was 55 ° C. The competitive inhibitions of glucose (Kis = 160 mM) and δ-gluconolactone (Kis = 0.08 mM) indicate that, considering a uni-bi reaction, glucose comes out in second place in an ordered sequential mechanism (fig10) and the δ- Gluconolactone is an analogue of the transition state.

5. REFERENCES

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