ENZYMATIC CHARACTERIZATION OF β-GLUCOSIDASE FROM *Prunus Dulcis*

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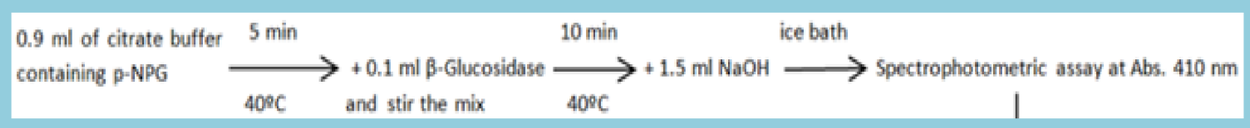
INTRODUCTION

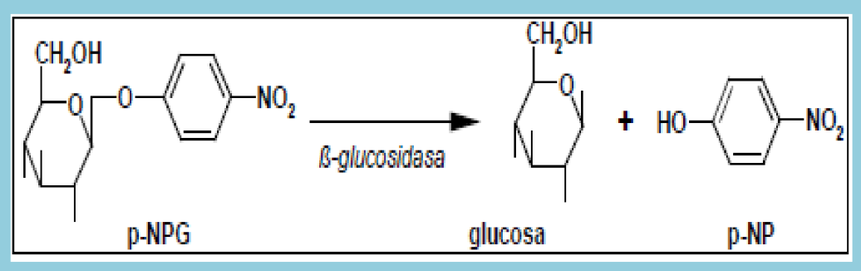
The main objetive is the proposal of a catalytic model for almond β-glucosidase. This experiment has been carried out in four steps: assay conditions standardization, kinetic parameters determination, effect of temperature and inhibition studies by glucose and δ-gluconolactone.

Assay protocol:



MATERIALS AND METHODS

The hydrolysis reaction employed in the study was:



Biologic Material: Commercial solution of β-glucosidase isolated from sweet almond emulsion (*Prunus Dulcis*) (Mr=135 KDa)

Chemicals: The substrate used was p-nitrophenil-β-D-glucoside (pNPG)

Reaction medium:100mM, pH 5.0 sodium citrate buffer

RESULTS

1. Standardization

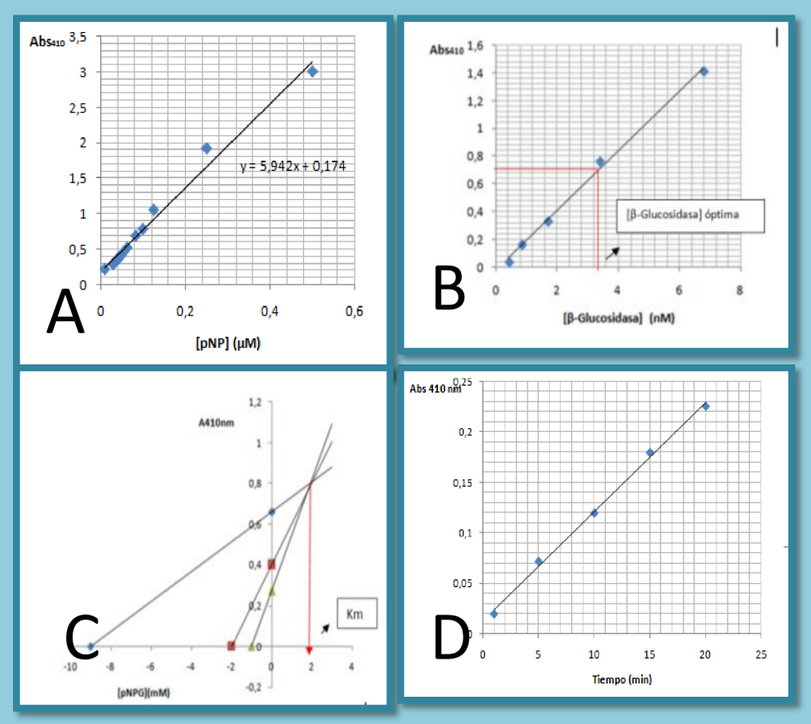


Figure 1. A) Standard curve for pNP. B) Optimum enzyme concentration. C)Eishental-Cornish Bowden plot D) Linearity with time

2. Kinetic parameters

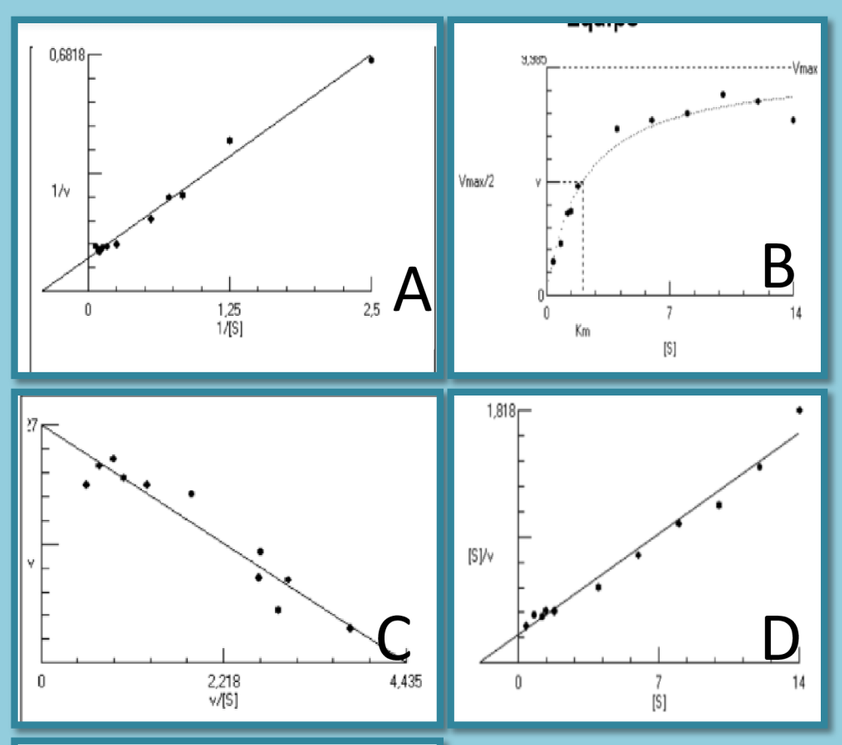


Figure 2. A) Lineaweaver- Burk plot. B) Michaelis-Mentel plot. C) Eadie-Hofstee plot D) Hanes-Woolf plot E) Eishental-Cornish Bowden plot

3. Effect of temperature

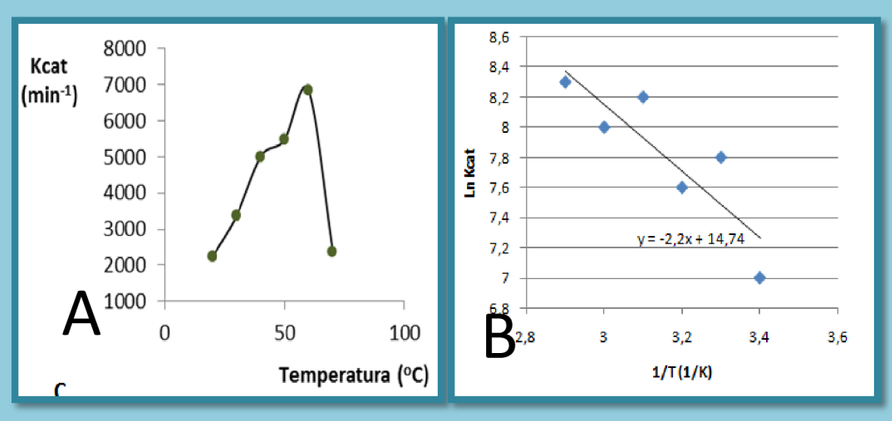


Figure 3. A) Kcat vs temperature. B) Determination of Ea. Ln Kcat vs 1/T (1/K)

Ea= 18.28 KJ/mol
Q10 (30/40) = 0.85
Q10 (40/50)= 1.9

4. Inhibition studies

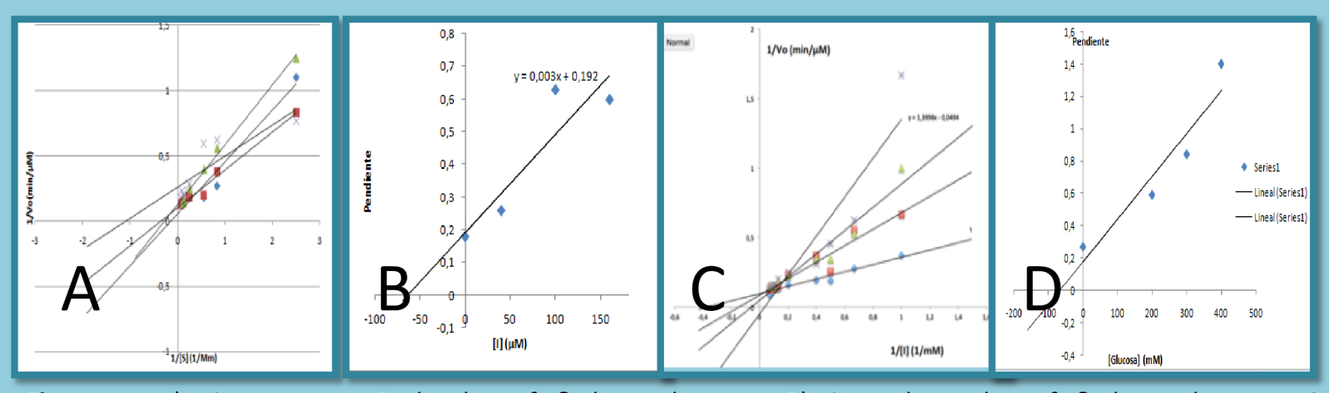
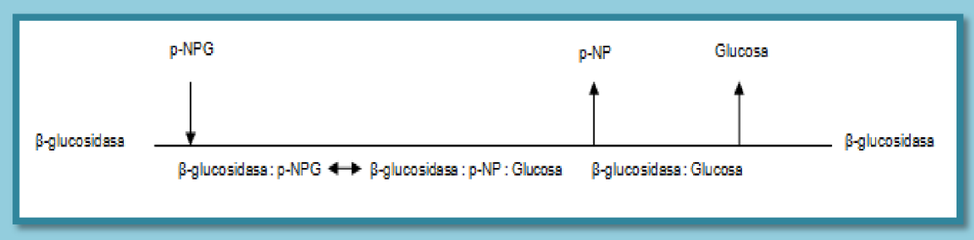


Figure 4. A) Lineaweaver- Burk plot of δ-gluconolactone. B) Secondary plot of δ-gluconolactone. C)Lineaweaver- Burk plot of glucose. D) Secondary plot of glucose

Cleland scheme



Conclusion

The standardized parameters obtained were: [E]=3.15 nM, Km*= 1.8 mM, range of [pNPG]= 0.4-12 mM, time of assay= 10 min and pH 5. The kinetic parameters were: Vmax= 10 µM/min, Km= 2.1 mM, kcat= 3170 min^{-1} and catalytic efficiency= 1538 1/ mM min. The optimum temperature was 50ºC.Both glucose and δ-gluconolactone are competitive inhibitors whose Kic were 60 mM and 0.06 mM respectively. For this reason δ-gluconolactone (transition state analog) is better inhibitor than glucose (last product)