# KINETIC CHARACTERIZATION OF β-GLUCOSIDASE FROM *Prunus dulcis*, SWEET ALMOND

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## *Abstract*

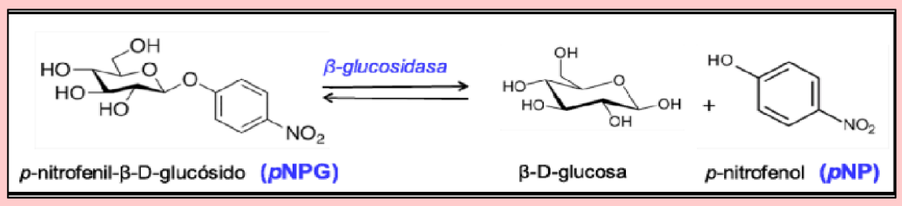
A serie of assays were carried out to propose a kinetic mechanism of the β-Glucosidase of *Prunus dulcis* (EC 3.2.1.21), an enzyme able to catalyse the hydrolysis of the glycosidic bonds to terminal non-reducing residues in β-D-glucosides and oligosaccharides, with release of glucose. It was made by establishing assay conditions standarization, kinetic parameters determination,effect of temperature and inhibition studies by glucose and δ-gluconolactone.

## *Materials and Methods*

**Materials**

- **Biological materials**: Solution of β-Glucosidase of *Prunus dulcis*
- **Chemical reactives**: p-NP, p-NPG, Glucose, δ-Gluconolactone
- **Solution buffer**: Sodium citrate buffer 100 mM pH 5.0

The reaction catalyzed by β-Glucosidase is




**Assay's general protocol**

0,9 ml of citrate buffer containing p-NPG → 5 min, 40º C → + 0,1 ml of β-Glucosidase → time ≠, 40º C → +1,5 ml of NaOH 0,2 M

**Assay's conditions:**

- **Temperature:** 40º C
- **Assay's time:** 10 minutes
- **pH:** 5.0
- **Reactive's concentrations:** Variable depending on the experiment's goal

Temperature and assay's times will be modified when time's standardization and temperature's effects are studied

## *Results*

### Standarization

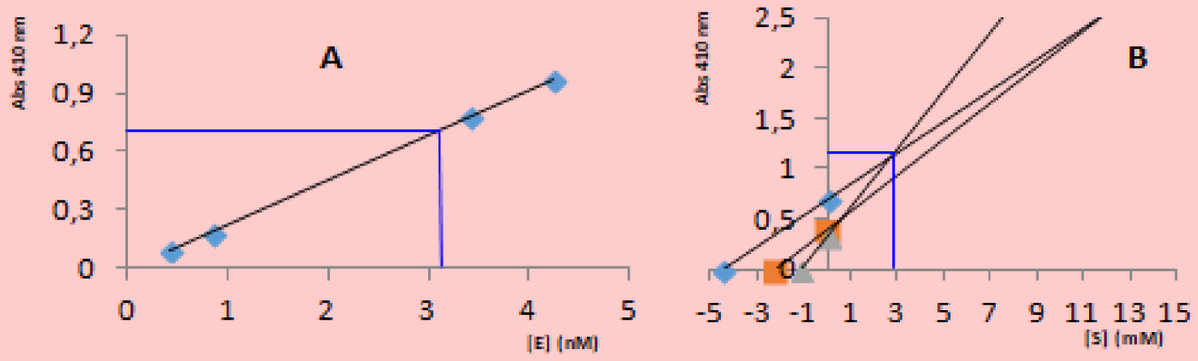



**Figure.1** Standarization experiments **A-** Optime enzyme concentration (3.08 nM) **B-** Aproximated Km (2.8 mM)

### Temperature's effect

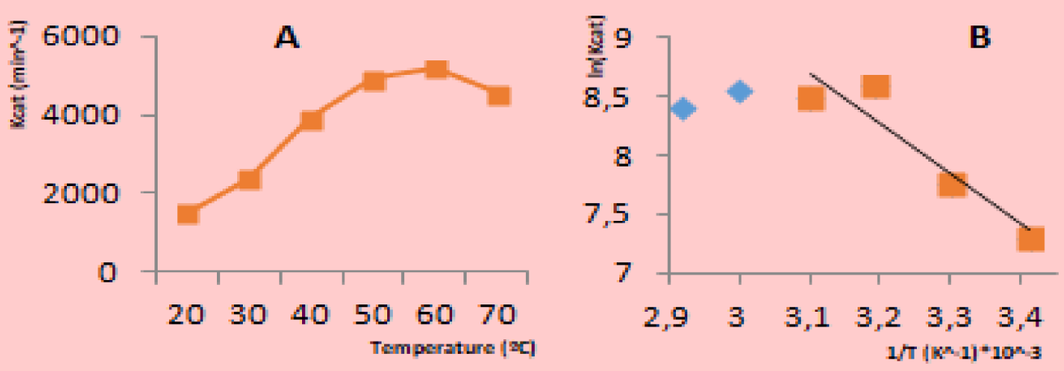



**Figure.2** Temperature assays **A-** Kcat variation with temperature **B-** Ea determination (slope). Ea's value is 35,67 J/mol

### Kinetic Parameters

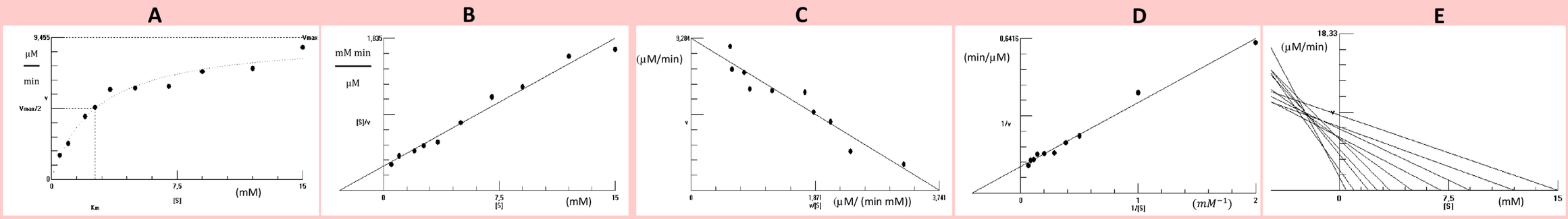



**Figure.3** Plots made with Hyperbolic Regression for different substrate concentrations (data not shown) **A**-Michaelis-Menten's Plot, **B**- Hanes-Wolf's Plot, **C**- Eadie-Hofstee's Plot **D**- Lineweaver-Burk's Plot, **E**- Eisenthal and Cornish-Bowden's Plot.

### Inhibition studies

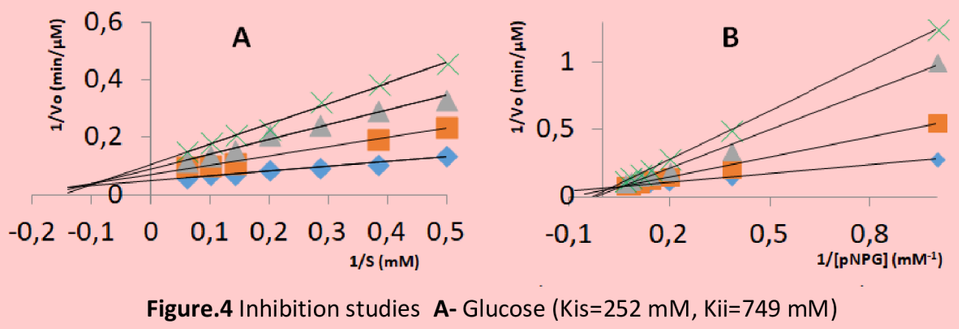



**Figure.4** Inhibition studies **A-** Glucose (Kis=252 mM, Kii=749 mM) **B-** Gluconolactone (Kis=56.5 μM)

## *Conclusion*

The standardized parameters obtained were: [E] = 3.08 nM, Km approximated= 2.3 mM, range of [*p*NPG] = 0.5-15 mM, assay's time= 10 mins and pH 5. Based on them, the kinetic parameters were Km=2.8 mM, Vmax= 9.46 μM/min, Kcat= 3100$min^{-1}$ and Ecat= 1191.39$mM^{-1}min^{-1}$. Temperature's studies showed that the reaction has its maximum Kcat at 60ºC, but we used 40ºC because temperature stabilization studies were not made. Inhibition's studies revealed that δ-gluconolactone is a competitive inhibitor (transition state analog), and glucose is a mixed inhibitor, but we observe that Kis is much lower than Kii wich means that glucose has a higher affinity towards the free enzyme, so we can discard the acompetitive inhibition maintaining glucose as a competitive inhibitor too. Other studies [1] showed that pNP is a mixed inhibitor for the enzyme, therefore we can conclude that the enzyme has a sequential ordered uni-bi mechanism, but considering $H_2O$ as a substrate we can propose another mechanism, a bi-bi ping pong mechanism.


**Figure.5** Cleland scheme for the catalystic mechanism of the β –Glucosidase. (S=pNPG ; P=pNP; G=Glucose ; E=enzyme) **A-** Sequential ordered uni-bi mechanism **B-** Bi-bi ping pong mechanism

## *References*

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2. Bhat, M. K.; Bhat, S., Cellule degrading enzymes and their potential industrial applications. Biotechnology Advances 1997, 15, 583-620.
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