KINETIC CHARACTERIZATION OF β-GLUCOSIDASE OF ALMOND

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INTRODUCTION

β-glucosidases(β-D-glycoside glucohydrolases, EC 3.2.1.21) are enzymes that hydrolyze glycosidic bonds of nonreducing terminal glucosyl residues from glycosides and oligosaccharides,releasing β-D-glucose as product(1).These enzymes are very distributed in the nature and also they are involved in different biological functions(2) For example:biomassconverter,production of drinks,food and interest in textile and paper industries.(3)The classification used nowadays is based on the sequence and the folding process; and divides these enzymes in more than 100 families.

The aim of this study is doing a kinetic characterization of the β-glucosidase of almond to determine its kinetic parameters and propose a model for the catalytic mechanism of this enzyme.

MATERIALS AND METHODS

0.9ml buffer 0.1M pH=5 with pNPG → 5 min 40ºC → +0.1ml enzyme → Time 40ºC → +1.5ml NaOH 0.2M → Keep on ice → Absorbance 410nm

1.Standardisation of essay conditions: pNP standard calibration curve,optimal [enzyme], aproximate Km, optimal time of reaction. **2.Determination of kinetics parameters** for pNPG with conditions: 40ºC, optimal [enzyme], optimal time and different pNPG concentrations. **Km, Vmax, Kcat, Ecat.3.Effect of temperature in catalysis**:20º,30º,40º,50º,60º,70ºC. **4.Effect of inhibitors in catalysis:** glucose (product of reaction) and δ-gluconolactone(transition state analogue). **Materials:** β-glucosidase of almond,p-nitrofenol and p-nitrofenil-glucose (pNPG) from [Fluka], citric acid & NaOH from (Panreac).

RESULTS

1.Standardisation of essay conditions:with pNP standard calibration curve (Figure 1) molar extinction coefficient was calculated,ε= 12118.42 $M^{-1}cm^{-1}$. Optimal [enzyme] was obtained interpolating on Figure 2. With Figure 3 is posible to calculate approximate Km, wich is 1.7mM. Figure 4 showed time linearity.

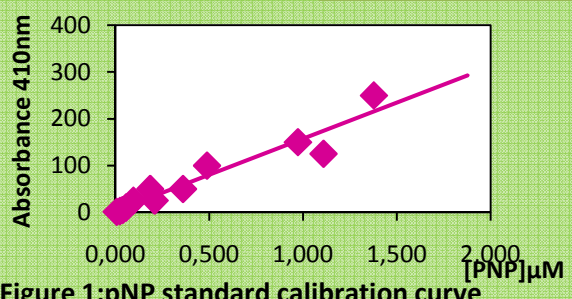

Figure 1:pNP standard calibration curve

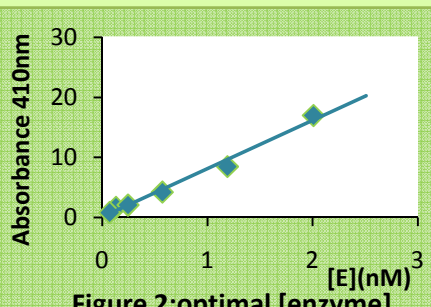

Figure 2:optimal [enzyme]

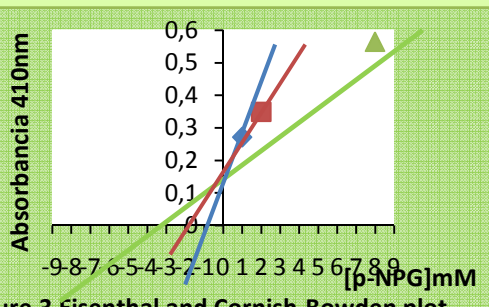

Figure.3 Eisenthal and Cornish-Bowden plot

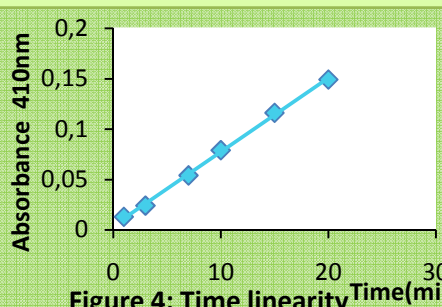

Figure 4: Time linearity

2.Kinetic parameters were calculated by different representations(Figure 5 –9)

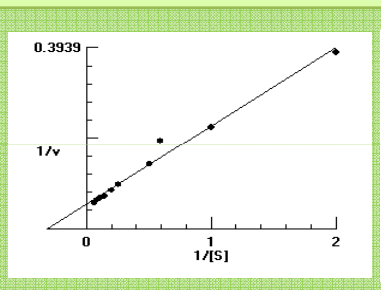

Figure 5: Lineweaver-Burk

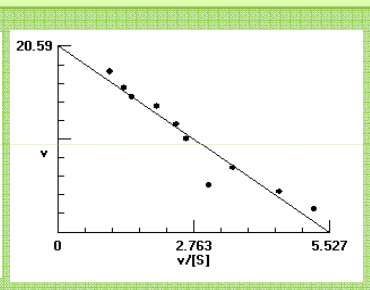

Figure 6:Eadie-Hofstee

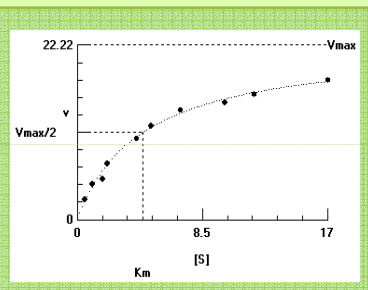

Figure 7: Michaelis-Menten

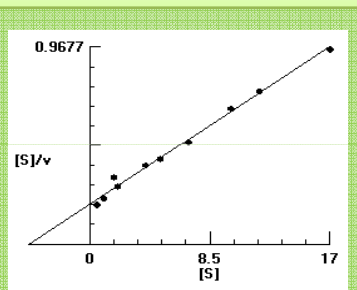

Figure 8:Hanes-Woolf

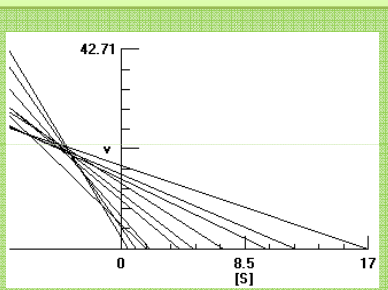

Figure 9: Parameter space

Table:1 Kinetic parameters

Km (mM)= 4.456	
Vmáx (µM/min)= 22.22	
Kcat (min-1)= 4114.81	
Kcat/Km (min-1 Mm-1)= 923.43	

3.Kinetic parameters for each **temperature** were used for represent Figure 10.

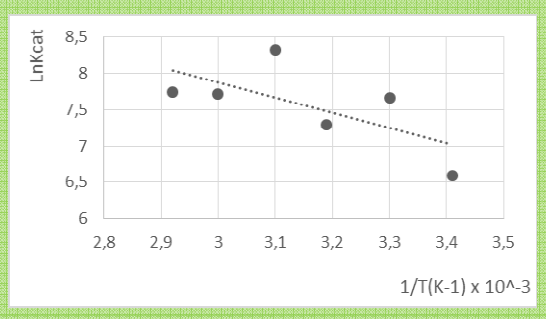

Figure 10: Linearization of Arrhenius equation
Ea=19.47KJ/molK ; Q10=0,7

4.Inhibitors' behaviour was determined in presence of different concentration of each inhibitor. Figure 11 and 12 showed that both were competitive inhibitors. Their KEIs were calculated in Figure 13 and 14.

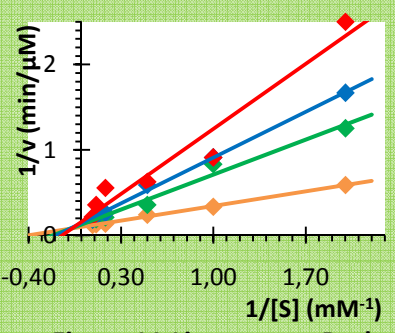

Figure 11:Lineweaver-Burk for glucose.

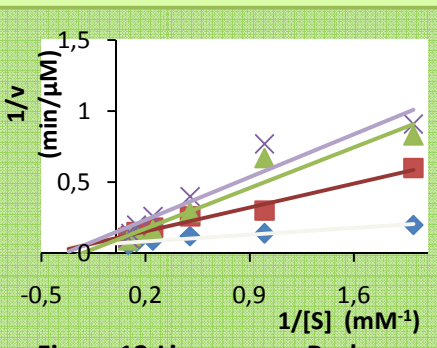

Figure 12:Lineweaver-Burk for δ-gluconolactone

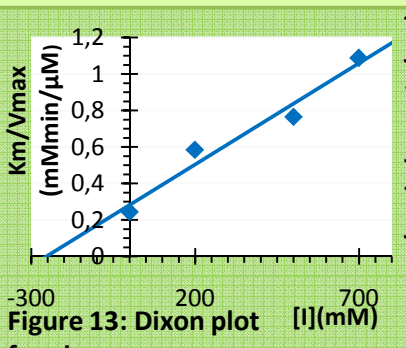

Figure 13: Dixon plot for glucose

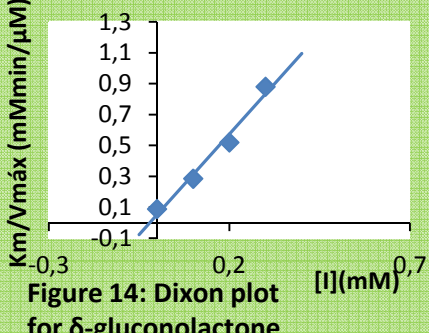

Figure 14: Dixon plot for δ-gluconolactone

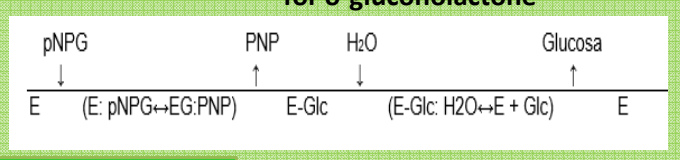

Figure 15 :Cleland Scheme

CONCLUSION

The conditions of assay established for the β-glucosidase are: an optimal concentration of enzyme of 5.4nM,10 minutes of time of assay.The optimum temperature of catalysis,fluctuates between 40ºC and 50ºC since the enzyme at 60ºC starts losing stability and activity. The glucose and the δ-gluconolactone show a competitive inhibition,with KEI_s of 200mM and 0.03mM respectively.So that,theδ-gluconolactone is a better inhibitor. A crypto ping-pong mechanism was proposed to this reaction,where PNP is the first product and the second is glucose(Figure:15)

BIBLIOGRAPHY

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(3) Bhat, M. K.; Bhat, S., Cellulose degrading enzymes and their potential industrial applications. Biotechnology Advances 1997