CHARACTERIZATION OF SWEET ALMOND Β-GLUCOSIDASE.

Raquel Gutiérrez Fernández and Natalia Fernández Matus , Laboratory of Biochemistry and Molecular Biology (UCM) .

1 .INTRODUCTION :

Glucosidases (β-D-glucósido glucohidrolasa, EC 3.2.1.21) catalyze the hydrolysis of the O-glycosidic bond at the non-reducing terminal end of short chain oligosaccharides, disaccharides and aryl alkyl-D-glucosides, releasing D-glucose. It is an enzyme that presents great specificity by subtraction due to the retention of the anomeric carbon during the catalysis. Under certain conditions, the enzyme is also capable of catalyzing the transglycosylation reaction. There are many types of glycosidases from animals, plants, microorganisms that perform different functions. This reaction constitutes a biological process aimed at the modification of small molecules in order to increase their solubility, stability and biological activity [1], which has aroused great interest for their biotechnological applications [2,3].

The main objective of this experiment was the kinetic characterization of the enzyme from the sweet almond, and for this the test was standardized and the characteristics of the enzyme varied depending on the temperature at which it was worked and on the presence or no inhibitors. The substrate that was used was pNPG and the inhibitors were glucose (one of the products of the reaction) and gluconolactone (analog of the transition state of pNPG).

2. METHODS :

First, the trial was standardized. A standard line was created from known concentrations of pNP, the optimal enzyme concentration and the approximate Km were determined. After , the linearity with the time of the appearance of product was checked and experiments were carried out at different temperatures and with the presence of inhibitors to see their behavior.

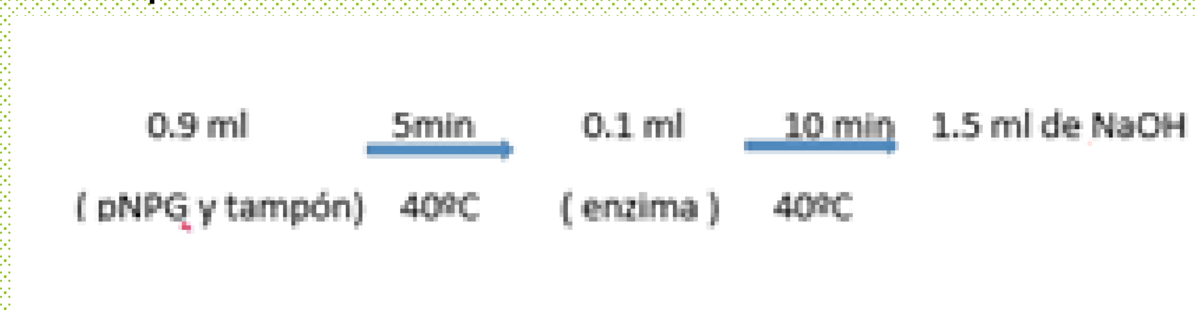

Figure 1 : Protocole used in the study.

3 .RESULTS

OPTIM CONDITIONS

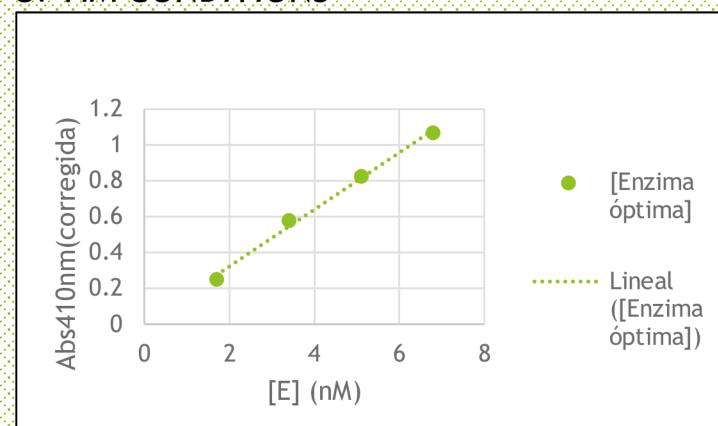

Figure 2 :In order to determine the optimal concentration of the enzyme. [E]= 42nM

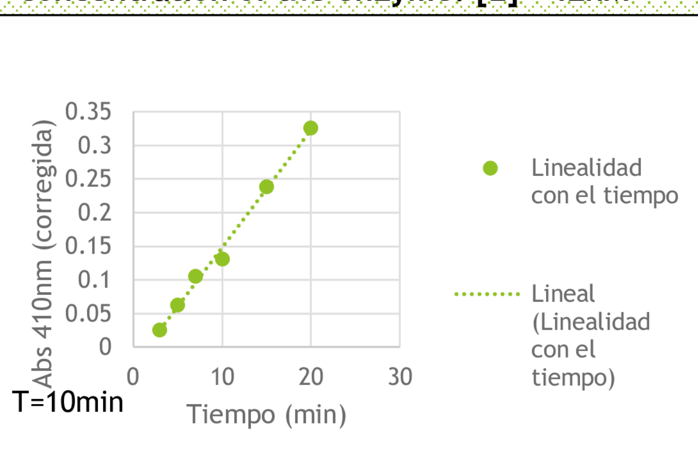

Figure 3:In order to check the linearity of product with the time

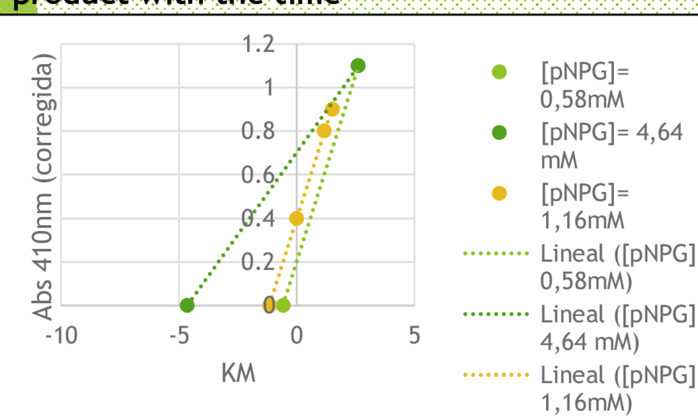

Figure 4 :Cornish-Bowdento determine KM*=2,6

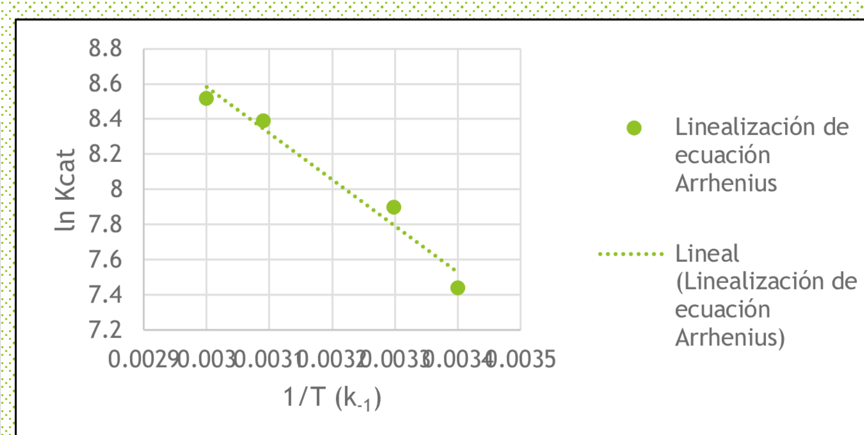

Figure 5 : Arrhenius. In order to determine Ea=34,45

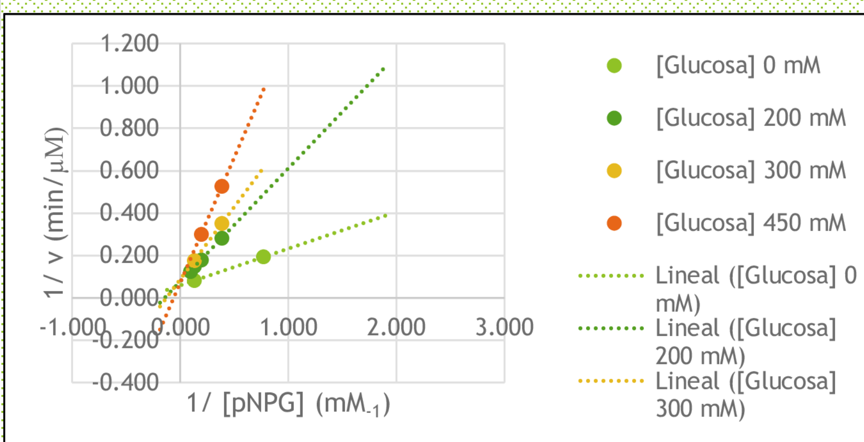

Figure 6: Lineweaver-Burk at different glucose concentrations

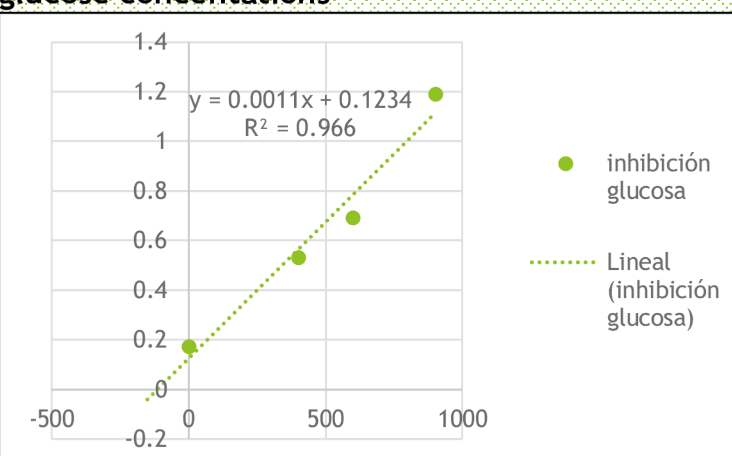

Figure 7 :slope vs inhibitor concentration. Glucose kis=112,18

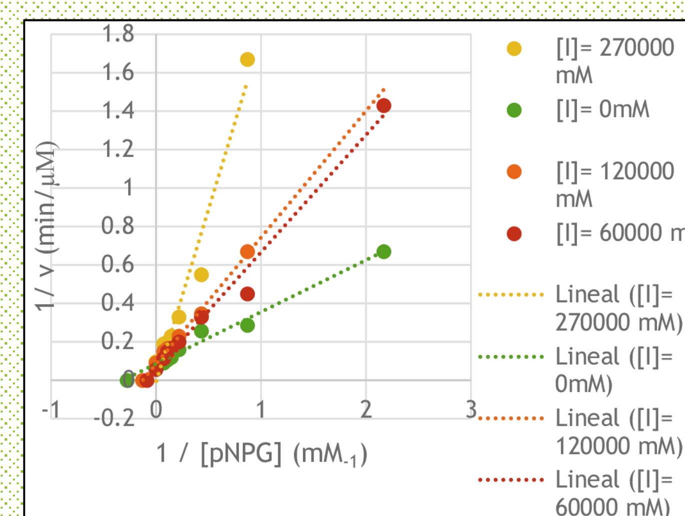

Figure 8:Lineweaver-Burk at different gluconolactone concentrations.

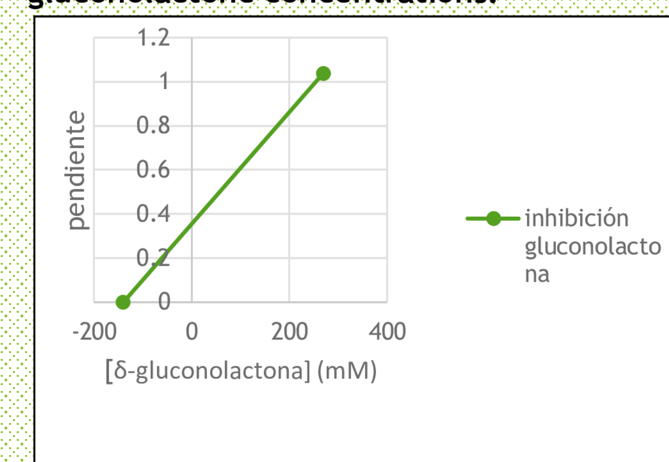

Figure 9 : slope vs inhibitor concentration. Kis=0,139

4 .CONCLUSION

The results show that the temperature at which the enzyme has it´s maximum activity is 55C0. . That means that, the optim temperature is not 55C0 because at that temperature there is a risk that is having part of the enzyme denatured. That´s why the optim temperature is one less than 50C0 so that is enough to be secure that the enzyme is stabilized with time.

Looking at it´s inhibitor constants, it is demostrated that glucose is inhibites less than δ-gluconolactone. This is because δ-gluconolactone behaves as an analogue of transition state so it has an structure really similar to transition state (has more affinity than glucose).It is possible that glucose is the second product in taking off from the active centre. This is because it is a competitive inhibitor (it joins to free enzyme) and when it takes off from the active centre it can inhibite this enzyme (product inhibition). Moreover, changues its conformation from boat conformation to the chair one. For these reasons, we concluded that glucose has a ordered secuential mechanism.

5 . REFERENCES

1.Opassiri, R.; Hua, Y.L.; Wara-Aswapati, O.; Akiyama, T.; Svasti, J.; Esen, A.; Cairns, J.R.K.,β- Glucosidase, exo-β-glucanase and pyridoxine transglucosylase activities of rice BGlu1, Biochemical Journal 2004, 379, 125-131.

2. Thuan, N.H., Sohng, J.K., Recent biotechnological progress in enzymatic synthesis of glycosides. Journal of Industrial Microbiology and Biotechnology 2013. 40, 1329-1356.

3. Mladenoska, I.; Grey, C. E.; Winkelhausen, E.; Kuzmanova, S.; Adlercreutz, P., Competition between transglycosylation and hydrolysis in almond beta-glucosidase-catalyzed conversion of pnitrophenyl-beta-D-glucoside in monophasic water/alcohol mixtures. Biocatalysis and Biotransformation 2007, 25, 382-385.