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Biotechnological application of biological yeast's invertase: capture and removal of sucrose from fruit juices.

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Abstract
Alarming numbers gathered by diabetes in the recent years motivated our project with the aim to capture and remove naturally occuring sucrose from fruit juices using the immobilized invertase. The structure and activity of the enzyme was evaluated at different pH values, and solutions with pH 3.0 and 4.8 were chosen to test the sucrose capture and removal. At the end, a removal of approximately 4% of the total sucrose per cycle was achieved.

Key words: *Diabetes, invertase, bionanotechnology.*

Introduction

Diabetes, a chronic disease in which the human body is not able to produce or use insulin properly, has been gathering alarming numbers in recent years. In 2017, 425 million people with diabetes in the world were estimated, and the projection for 2045 is for that number is to increase by 48%. It was also stated that half of diabetics remain undiagnosed. Given this scenario, health and research agencies have mobilized to find solutions to fight this disease.[1]

Enzymes are proteins that catalyze chemical reactions with high specificity.[2] Although they require certain conditions, such as pH and temperature, to be able to perform optimally, they are widely used in several industry sectors, like the food industry.[3]

With this in mind, our aim was to develop a nanomaterial using the enzyme beta-fructofuranosidase (invertase) from biological yeast (*Saccharomyces cerevisiae*) to capture and remove sucrose from fruit juices, such as orange juice. In this way, it would be possible to develop low-calorie products suitable for the consumption of people with diabetes or low-calorie intake diets.

Results and Discussion

The invertase from biological yeast was extracted, purified and characterized. The enzyme activity was followed and the converted sucrose quantified by the method described by Timerman[4], based on the colorimetric glucose reaction with 3,5-dinitrosalicylate (DNS), as shown in Image 1-a.

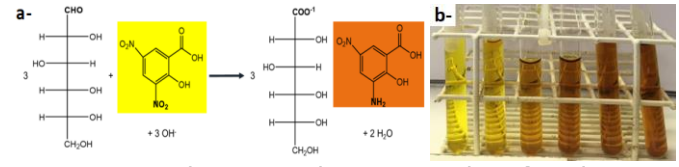

Image 1. a- Glucose and DNS reaction; **b-** Picture of activity assay, glucose concentration increasing from left to right.

As to remove sucrose from the solution, the protonation/deprotonation of the invertase catalytic residues was evaluated at different pH, as shown in Image 2. It was observed that enzyme achieves maximum activity at pH 4.8, while at pH 3.0, 3.5 and 6.0 the enzyme has almost zero activity. Then pH 3.0 was picked for working regimen and to capture the sucrose, since the substrate binding may occur. On the other side, pH 4.8 was picked for the best catalysis results and the products (glucose and fructose) release.

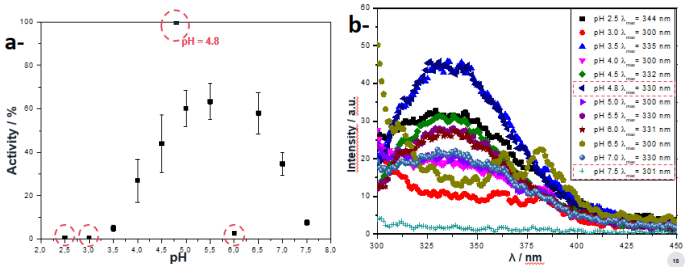

Image 2. a- Activity assay and; **b-** Fluorescence of invertase under various pH conditions.

Finally, after the enzyme was immobilized on the surface of the magnetic nanoparticles, the capture and removal of the sucrose was evaluated. The results are illustrated on Image 3.

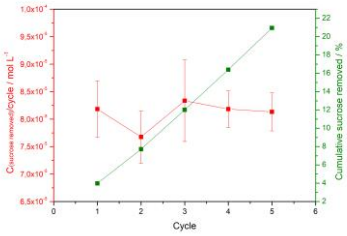

Image 3. Sucrose concentration removed by cycle (mol L^{-1}) and cumulative sucrose removal (%).

Conclusions

Given the structural differences of the enzyme obtained by fluorescence and the activity assay, performed at different pH values, pH 3.0 was picked for the process of capture and removal of the sucrose from the working solution.

The results obtained in the test with the invertase immobilized over the magnetic nanoparticles showed that around 8×10^{-5} mol L^{-1} of sucrose was removed by cycle, or about 4% of the total sucrose from the solution.

Acknowledgement

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[1] http://www.diabetes.org.br
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[3] Gao, S. H.; Zhou, Y.; *et al. Scientific Reports*. **2017**, 7, 44542.
[4] Timerman, A. P. *Protein Purification* **2002**, *pp 29-52.*