Exploration of a Functional Sugar Synthase Activity Assay Method and Evaluation of Stabilizing Agents Effects on Protein Content and Enzyme Activity

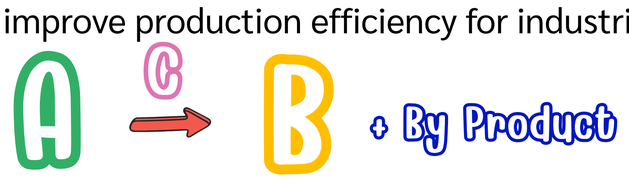
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Abstract

Enzyme stabilization is important for preserving protein structure and catalytic activity. Polyols such as mannitol enhance stability by promoting preferential hydration and reducing protein unfolding and aggregation. This study determined the optimal conditions for functional sugar estimating enzyme activity and evaluated stabilizing agents effect on crude enzyme. The highest activity was obtained at 10% substrate concentration with 30 minutes of incubation. Among five stabilizers tested, Among the five stabilizers tested, 0.5 M mannitol was the most effective stabilizing agent, retaining 66% of protein content, increasing enzymatic activity to 185% relative to the control, and achieving 127% sugar conversion after 18 hours with 30% (w/w) substrate.

Introduction

Enzymes are biological catalysts widely used in the production of functional sugars due to their high specificity and efficiency. Functional sugars have attracted considerable interest because of their health benefits and wide range of industrial applications. In this study, the enzyme catalyzes the conversion of the substrate into a functional sugar product, which is classified as a reducing sugar. However, enzymes are highly sensitive to environmental factors such as temperature and pH, which may affect their structural stability and catalytic performance. Therefore, optimizing enzyme activity assay conditions and evaluating the effects of stabilizing agents are essential to maintain enzyme functionality and improve production efficiency for industrial applications.



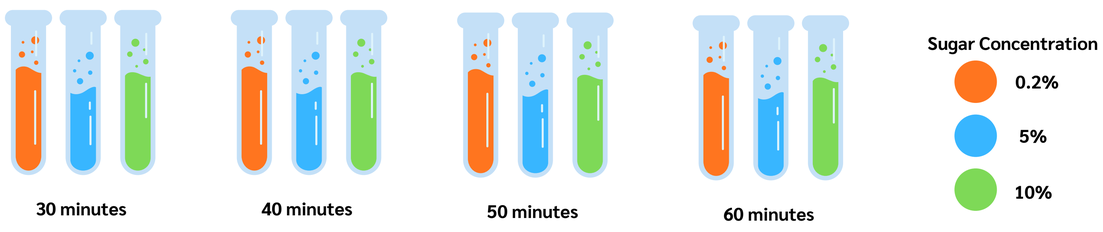
A : Non-reducing sugar B : Reducing sugar C : Enzyme

Objectives

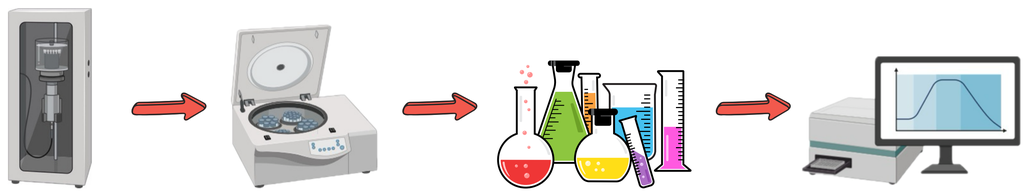
1. To explore the optimal condition to reveal the enzyme activity for functional sugar production
2. To investigate stabilizing agents capable of preserving protein content and enzymatic activity in crude enzyme
3. To compare the effect of different stabilizing agents in maintaining enzyme stability

Methodology

- Explored the optimal condition to reveal the enzyme activity for functional sugar production.



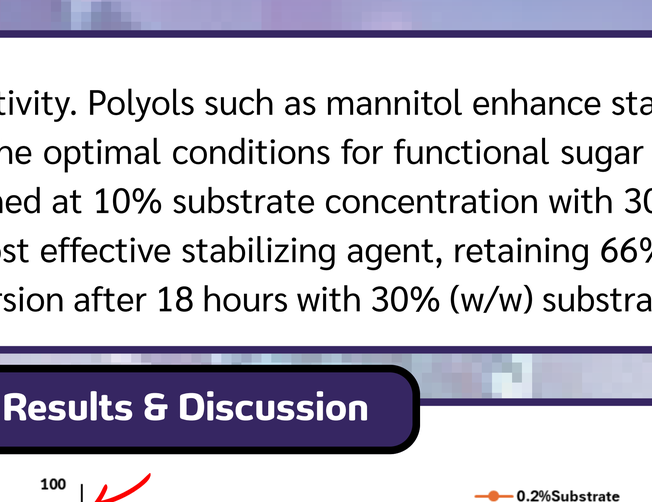
- Investigated stabilizing agents capable of preserving protein content and enzymatic activity in crude enzyme
  - Detection of protein concentrations : Bradford assay
  - Detection of sugar products : DNS Method



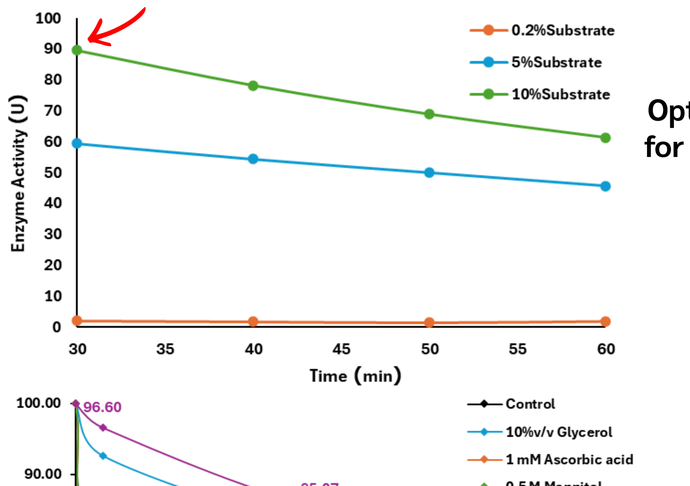
Results & Discussion



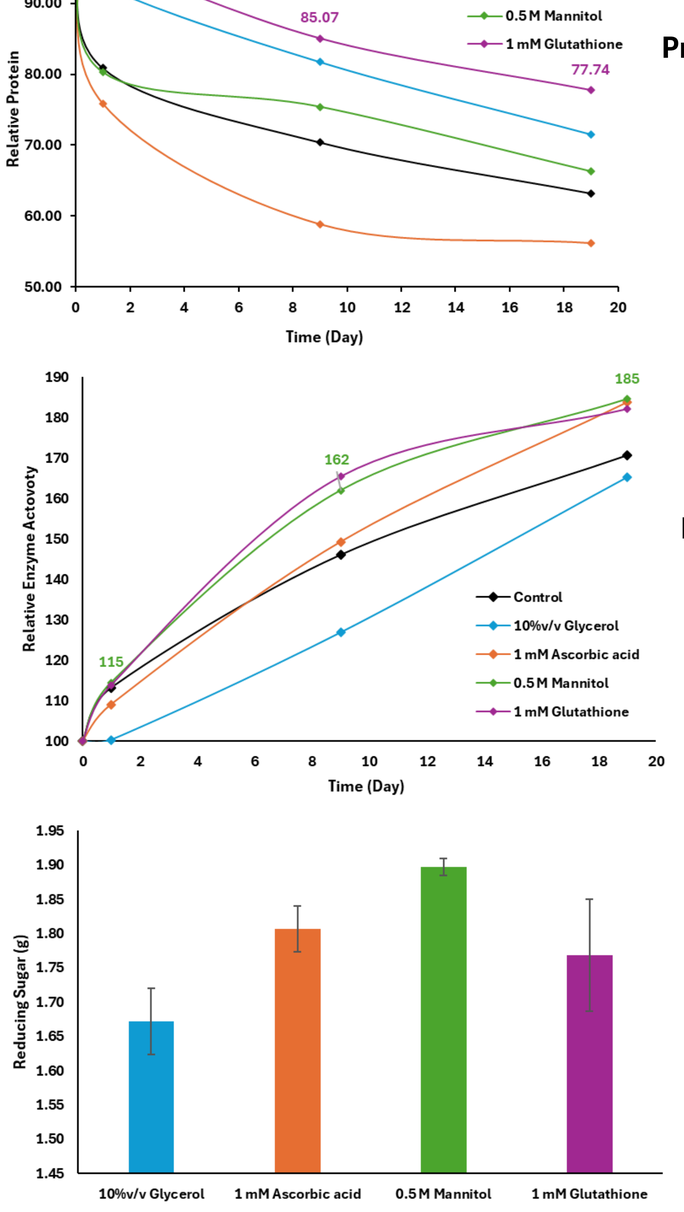
Optimization of Assay Conditions for Functional Sugar Synthesizing Enzyme



Protein Content in Crude Enzyme After 19 Days of Storage with Different Stabilizers



Residual Enzyme Activity After 19 Days of Storage Time with Different Stabilizers



Functional Sugar Production Yields After 18 hours of Incubation Time

Conclusion

- The optimal conditions for enzyme activity were 10% substrate concentration and an incubation time of 30 minutes.
- Protein stability was best maintained by 1 mM glutathione about 77.74%
- Enzyme activity was highest with 0.5 M mannitol about 185%
- Sugar conversion at 18 hours showed that 0.5 M mannitol resulted in the highest enzyme activity about 127%.

Acknowledgement

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