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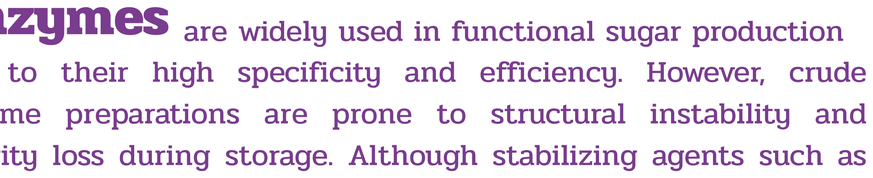
Investigation of Chemical Additives for Improving the Stability of Recombinant Enzymes for Functional Sugar Production

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Abstract Enzyme instability during storage limits its industrial application in functional sugar production. This study evaluated the effects of nine stabilizing agents on protein content and enzymatic activity of crude enzyme formulations stored at 4°C for 30 days. Protein concentration was determined using the Bradford assay, and activity was measured using a commercial functional sugar test kit. Glutathione and sucrose retained over 90% of protein content, while tween-20 and sodium benzoate effectively maintained enzymatic activity. Among the tested agents, sodium benzoate demonstrated the most suitable overall stabilizing performance by preserving catalytic activity and acceptable protein integrity. These findings support its application in industrial enzyme formulation for functional sugar production.

Introduction

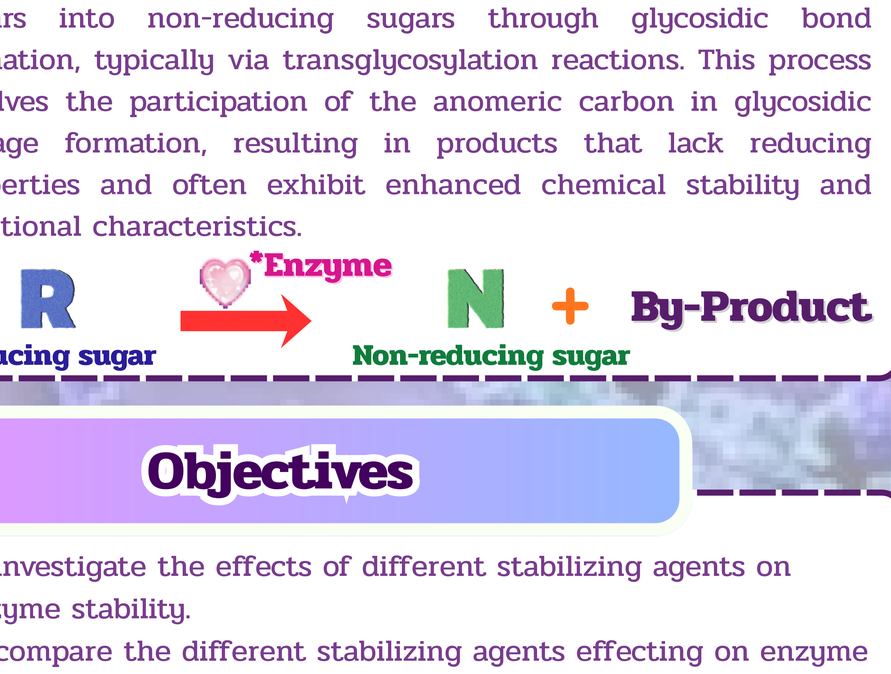
Enzymes are widely used in functional sugar production due to their high specificity and efficiency. However, crude enzyme preparations are prone to structural instability and activity loss during storage. Although stabilizing agents such as polyols, sugars, and amino acids have been applied to improve enzyme stability, comparative evaluations under practical storage conditions remain limited. Therefore, this study investigated the effects of selected stabilizing agents on protein stability and enzymatic activity of crude enzyme formulations. In functional sugar production, enzymatic reactions can convert reducing sugars into non-reducing sugars through glycosidic bond formation, typically via transglycosylation reactions. This process involves the participation of the anomeric carbon in glycosidic linkage formation, resulting in products that lack reducing properties and often exhibit enhanced chemical stability and functional characteristics.



Objectives

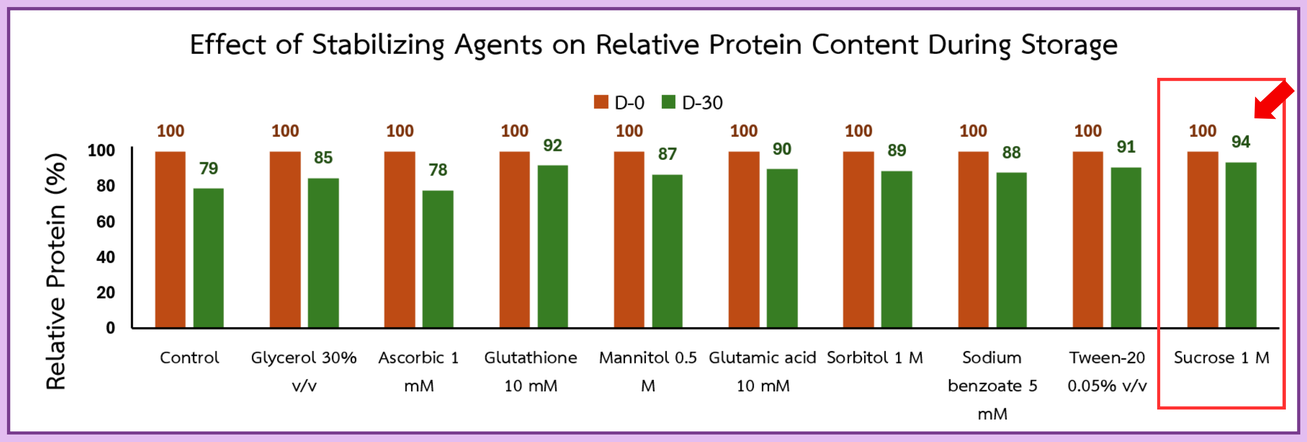
1. To investigate the effects of different stabilizing agents on enzyme stability.
2. To compare the different stabilizing agents effecting on enzyme properties.
3. To evaluate the functional sugar production in terms of industrial feasibility and applicability.

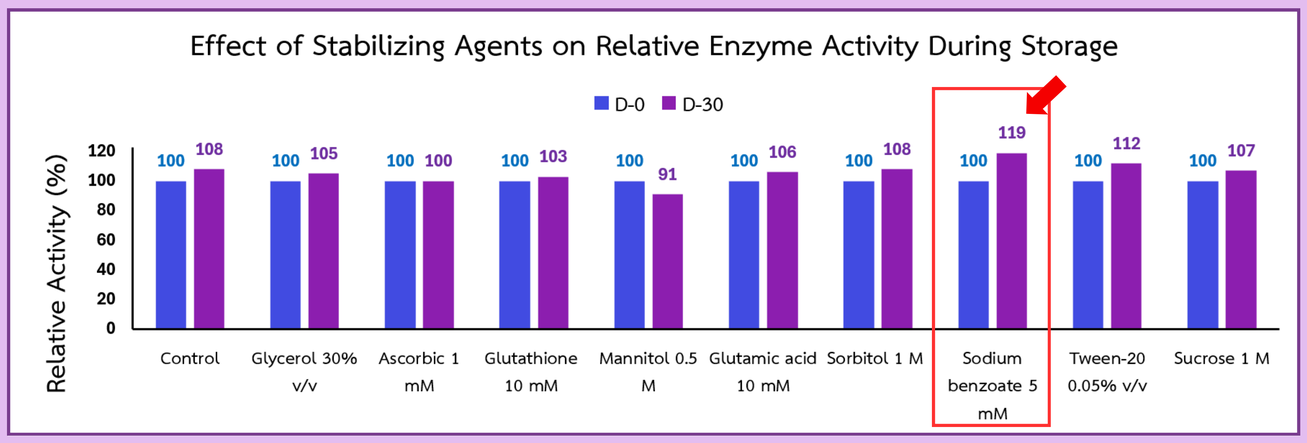
Methodology



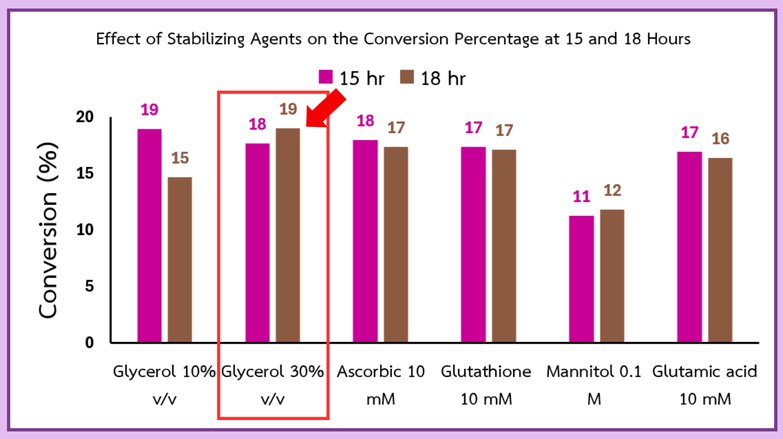
1. Protein concentration was determined using the Bradford assay.
2. Enzymatic activity was determined using a commercial functional sugar test kit.
3. Functional sugar production was run for 15 and 18 hours of reaction time.

Results & Discussion





**Note: Initial readings at Day 0 were influenced by incomplete homogenization. The values reached a stable state as the experiment progressed through Day 30.*



- Sucrose 1 M best preserved protein content (94%) The remaining protein was detected in 94% of relative protein concentration as kept at 1 M of sucrose, while enzyme was kept in 5 mM of sodium benzoate, the highest enzyme activity as 119% was shown after 30 days of storage time.
- Glycerol at 30% (v/v) provided the best results after 18 hours of the functional sugar production.

Conclusion

The results demonstrated that different stabilizing agents exert distinct protective effects during storage. Sucrose 1 M showed the highest ability to preserve relative protein content (94%), indicating strong structural stabilization. Meanwhile, sodium benzoate 5 mM exhibited the greatest enhancement in relative enzyme activity (119%) after 30 days of storage time. These findings suggest that sucrose is more effective in maintaining protein structural integrity, whereas sodium benzoate plays a significant role in preserving or enhancing enzymatic activity.

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