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

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Major : Biochemistry and Biochemical Innovation

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ABSTRACT

Enzymes are highly specific biological proteins that play essential roles in biotechnological production processes. Several products are produced under mild conditions such as in the food, beverage, detergent, textile, and biofuel industries. However, enzymes are sensitive to environmental conditions, which make them difficult to preserve until use due to protein denaturation leading to activity loss. Therefore, improving enzyme stability during storage is key to driving the utilization of enzymes in practical applications. This research aimed to investigate the effects of stabilizing agents on protein content and enzyme activity of the crude enzyme in functional sugar production. Stabilizing agents are commonly used in enzyme formulations, including polyols, sugars, and antioxidants, which significantly maintain protein structure and prevent denaturation. The experiments evaluated enzyme formulations combined with nine stabilizing agents, including glycerol, ascorbic acid, glutathione, mannitol, glutamic acid, sorbitol, sodium benzoate, Tween-20, and sucrose. The enzyme formulations were stored at 4°C for 30 days to assess stability. Protein concentration was determined using the Bradford assay, and enzymatic activity was determined using a commercial functional sugar test kit. The results showed that

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the formulation containing glutathione preserved approximately 92% of the relative protein content, indicating strong structural stabilization. Sucrose retained approximately 94% of the initial protein content, showing effective protection during storage. Meanwhile, glutamic acid and sodium benzoate were particularly effective in maintaining enzymatic activity over prolonged storage. Considering that enzymatic activity is the primary concern for industrial applications, glutamic acid exhibited the most suitable overall stabilizing effect by maintaining high catalytic performance while preserving protein integrity at an acceptable level. These findings demonstrate the potential of glutamic acid to preserve protein concentration and enzymatic activity, supporting its application in the development of stable enzyme formulations for industrial-scale functional sugar production.

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