Int.J.Curr.Res.Aca.Rev.2025; 13(12): 17-21

EXCELLENT PUBLISHERS

International Journal of Current Research and Academic Review

ISSN: 2347-3215 (Online) Volume 13 Number 12 (December-2025)

Journal homepage: http://www.ijcrar.com

doi: https://doi.org/10.20546/ijcrar.2025.1312.003

Comparative Analysis of Beta-Amylase Activity in Various Bananas

Gourinanda Suresh, Jiby John Mathew, N. K. Sajeshkumar and Prem Jose Vazhacharickal*

Department of Biotechnology, Mar Augusthinose College, Ramapuram, Kerala, 686576, India

**Corresponding author*

Abstract

This study investigates the beta-amylase enzyme activity extracted from various banana species indigenous to Kerala, India, with a focus on understanding their potential applications in food processing and related industries. Enzyme activity was determined through extraction and measurement in International Units (IU) per gram. Results reveal significant variability in beta-amylase activity among different banana varieties, with Plantain banana (B1) exhibiting the highest activity at 11.57 x 10^{-9} IU, followed by Cavendish banana (B4) at 4.56 x 10^{-9} IU. Conversely, *Musella lasiocarpa* (B3) displayed the lowest activity at 1.32 x 10^{-9} IU. These findings underscore the importance of considering banana variety selection for specific industrial processes requiring efficient starch breakdown. The study contributes to a deeper understanding of the biochemical properties of diverse banana species, offering insights for agricultural practices, food production, and potential avenues for enzyme engineering and genetic modification in crop improvement.

Article Info

Received: 15 October 2025
Accepted: 22 November 2025
Available Online: 20 December 2025

Keywords

Beta-amylase, Enzyme extraction, Starch hydrolysis, Food processing

Introduction

Enzymes are biological catalysts essential for accelerating chemical reactions by lowering the activation energy. One such enzyme, beta-amylase, plays a pivotal role in the hydrolysis of starch into maltose. Beta-amylase is primarily found in bacteria, fungi, and plants, including banana species. Its optimal pH for activity is between 5.2 and 5.4, which is essential for its function in catalyzing the hydrolysis of α-1,4 glycosidic bonds in starch, releasing two glucose units (maltose) at a time.

Banana is one of the most important fruit crops worldwide and is a major source of carbohydrates in many tropical regions, including Kerala, India. The state is home to a variety of banana species, each with its unique characteristics in terms of nutritional content, enzyme activity, and other biochemical properties.

The comparative analysis of enzyme activity, particularly beta-amylase, extracted from various banana species of Kerala can provide insight into the potential differences among these species. Such a study could also have practical applications in the food industry and biotechnology, especially in processes such as brewing, fermentation, and the production of sweeteners.

This project will contribute to a better understanding of the biochemical properties of different banana species and their potential applications in food processing and other industries. Additionally, it may provide valuable information for banana growers and industries that rely on bananas as a raw material.

The outcomes of this study may also lead to further research in enzyme engineering, genetic modification, and crop improvement, which could have broader implications for agriculture and food production.

The objectives of this study were to extract crude enzyme preparations from banana samples, to determine the activity of β-amylase present in these extracts, and to perform a comparative analysis of β-amylase activity among the different samples.

Materials and Methods

Sample collection

Sample collection involved obtaining various bananas, such as Nendra pazham, Njalipoovan, Palayan kodan, Robusta and a Cross banana variety, from local farmeers of Pala Muncipality, Menachil thaluk, Kottayam District, Kerala state, India, and transporting them to the work space.

Sample Coding

Sample Preparation

For the sample preparation, start by taking 10 grams of fresh, washed, and peeled bananas, and cutting them into small pieces. Transfer the banana pieces into a mortar and pestle.

Add 40 ml of sodium phosphate buffer saline to the mortar and homogenize the banana tissue for 3 to 5 minutes until a uniform mixture is achieved.

The resulting homogenized banana and buffer mixture can then be transferred to a suitable container for further analysis such as enzyme extraction or activity determination.

Extraction of crude enzyme

Extraction of crude enzyme was carried out by filtering the homogenized material using muslin cloth. The filtered material was then transferred into centrifuge tubes and centrifuged at 10000 to 12000x g for 20 minutes at a cold temperature.

The resulting supernatant was collected and used as the enzyme source. The enzyme extract was stored at 4°C until further use (Wilson, K., & Walker, J. 2018).

Determination of Enzyme Activity/ Quantization of Maltose

For the quantization of maltose, 8 clean test tubes were meticulously labelled as blank, pure blank, enzyme blank, and T1, T2, T3, T4, T5 for testing. Each tube received approximately 1.0 ml of sodium acetate buffer with a pH of 7.0. Following this, 1.0 ml of starch was added to every test tube and thoroughly mixed. Subsequently, 0.2 ml of enzyme extract was introduced to the designated test tubes. The mixtures were then well-incorporated and left to incubate precisely at 30°C in a water bath for a duration of 10 minutes. After the incubation period, 2 ml of DNS reagent was added to all tubes, ensuring thorough mixing, followed by incubation in a boiling water bath for 5 minutes. A 1 ml portion of Potassium-sodium tartrate reagent was then added to each tube, along with 7 ml of distilled water. Additionally, 0.2 ml of enzyme extract was introduced to the blank. The total assay volume was maintained at 12 ml throughout the procedure. Optical density readings were recorded at 520 nm, allowing for the determination of maltose concentration (Miller, 1959). (Appendix 1)

A standard graph was plotted and the enzyme activity was calculated. One unit of enzyme activity was defined as the amount of enzyme required to liberate 1µmol of sugar per minute under the standard assay conditions and enzyme activity is expressed in terms of micromoles per second on fermented substrates (Appendix 2).

Result and Discussion

The project aimed to extract and determine the activity of beta amylase from various banana varieties. The activity was measured in International Units (IU) per gram. The results obtained are as follows:

The data obtained from the project indicates a significant variation in the activity of beta amylase across different banana varieties. The enzyme's activity levels in different banana varieties may influence their potential uses in food processing and other industries.

Plantain banana (B1) has the highest beta amylase activity at 11.57 x 10^{-9} IU/g. This high activity level suggests a greater potential for plantain bananas in applications involving starch breakdown, such as in brewing, baking, or bioethanol production. Additionally, this result aligns with previous studies that found plantains to have higher enzyme content compared to other bananas.

Cavendish banana (B4) exhibits moderate beta amylase activity at 4.56 x 10^{-9} IU/g, which is lower than plantain but still substantial. As one of the most commercially grown banana varieties worldwide, its moderate enzyme activity can be leveraged in various food processing applications such as starch hydrolysis.

Tissue culture variety (B5) and Musa acuminata (B2) display lower activity levels at 1.76 x 10^{-9} IU/g and 1.68 x 10^{-9} IU/g, respectively. This aligns with previous research on banana tissue culture varieties, which found moderate enzyme activity when compared to other bananas. These varieties may still offer some potential uses in applications requiring enzyme activity, such as in fermentative processes.

Musella lasiocarpa (B3) demonstrates the lowest beta amylase activity at 1.32 x 10^{-9} IU/g. This variety may have limited utility in applications that require high enzyme activity for starch breakdown. However, its unique properties could still hold promise for niche applications.

The results reveal a significant variation in beta-amylase activity among the different banana tubers. Plantain banana (B1) exhibits the highest activity at 11.57 x 10^{-9} IU/g, while Musella lasiocarpa (B3) displays the lowest activity at 1.32 x 10^{-9} IU/g.

Notably, Cavendish banana (B4), one of the most widely cultivated banana varieties globally, shows moderate beta-amylase activity at 4.56 x 10^{-9} IU/g. This finding could be crucial for industries relying on Cavendish bananas for food processing and production.

The results suggest that certain banana varieties, such as Plantain banana (B1), exhibit high beta-amylase activity, indicating their potential suitability for industrial processes requiring efficient starch breakdown.

This could include applications in the production of biofuels, food additives, and pharmaceuticals.

The observed differences in beta-amylase activity among banana varieties may be attributed to a combination of genetic and environmental factors. Genetic variations within the Musa genus and differences in growing conditions, such as soil composition and climate, could influence enzyme expression and activity levels.

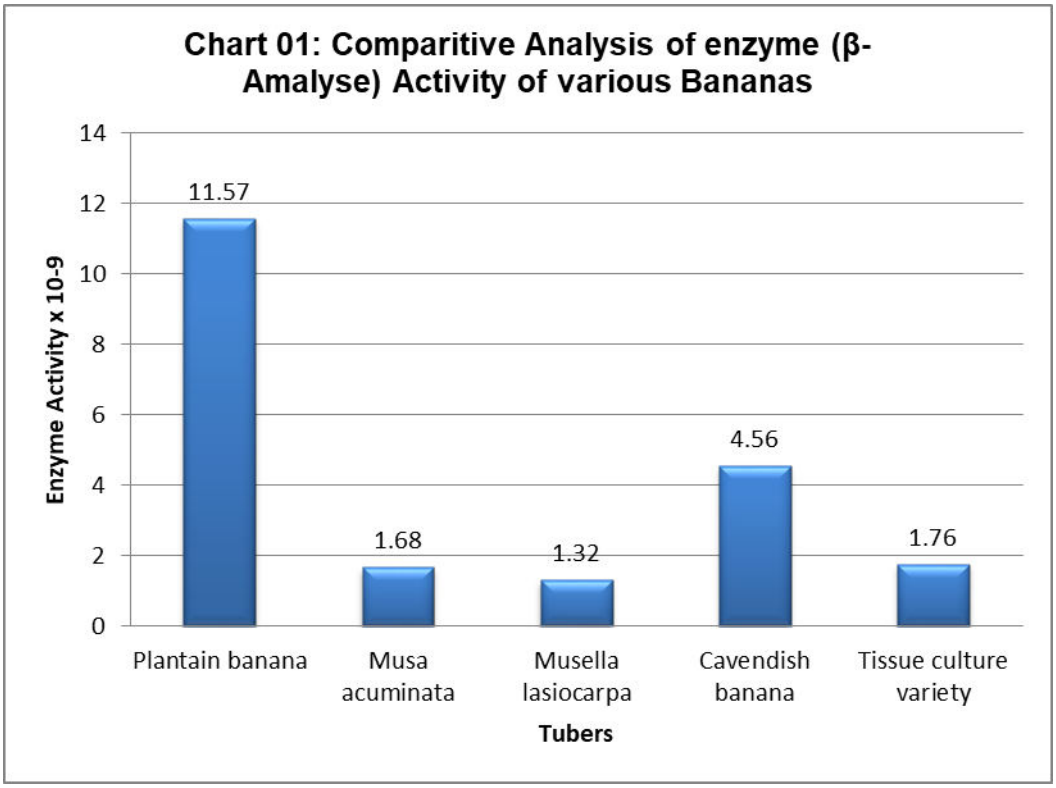
Tissue culture varieties (B5) exhibit moderate beta-amylase activity comparable to Musa acuminata (B2). Given the ease of propagation and potential for genetic manipulation in tissue culture, these varieties could be valuable targets for further research aimed at enhancing enzyme activity through breeding or biotechnological approaches.

Table.1 List of Bananas used for enzyme source

Sl. No.	Scientific name	Common name	Code
1.	*Plantain banana*	Nendra pazham	B1
2.	*Musa acuminata*	Njalipoovan	B2
3.	*Musella lasiocarpa*	Palayan kodan	B3
4.	*Cavendish banana*	Robusta	B4
5.	Tissue culture variety	Cross banana	B5

Table.2 Comparative Analysis of enzyme (β-Amalyse) Activity of various Bananas

Sl. No.	Tuber Name	Code	Activity in IU
1	*Plantain banana*	B1	11.57x10-9
2	*Musa acuminata*	B2	1.68 x10-9
3	*Musella lasiocarpa*	B3	1.32 x10-9
4	*Cavendish banana*	B4	4.56 x10-9
5	*Tissue culture variety*	B5	1.76 x10-9



In conclusion, this project investigates beta-amylase enzyme activity in various banana species native to Kerala, India, with a focus on understanding their potential applications in food processing, brewing, fermentation, and other industries. By comparing the activity levels of beta-amylase extracted from different banana varieties, the project provides valuable insights into their suitability for specific industrial processes.

The study has three main objectives: extracting crude enzyme from the samples (banana), determining beta-amylase enzyme activity, and conducting a comparative analysis of enzyme activity across different banana varieties.

The results revealed significant variations in beta-amylase activity among tested banana species, suggesting potential applications for certain varieties in industries that require efficient starch breakdown. Plantain banana (B1) exhibited the highest activity at 11.57 x 10^{-9} IU, making it a strong candidate for industries such as brewing, baking, and bioethanol production. Cavendish banana showed moderate beta-amylase activity at 4.56 x 10^{-9} IU and remains valuable for starch hydrolysis in the food processing industry.

Tissue culture variety (B5) and Musa acuminata (B2) demonstrated lower beta-amylase activity at 1.76 x 10^{-9} IU and 1.68 x 10^{-9} IU, respectively, suggesting limited potential for applications requiring high levels of starch breakdown. *Musella lasiocarpa* (B3) exhibited the lowest activity at 1.32 x 10^{-9} IU, indicating limited utility in starch breakdown but potential for niche applications.

Variations in beta-amylase activity may be attributed to genetic and environmental factors. Plantain banana's high activity offers potential for industries such as brewing and bioethanol production, while Cavendish's moderate activity aligns with its established role in food processing. Tissue culture and Musa acuminata may still offer value in specific applications. *Musella lasiocarpa*'s low activity suggests limited use in starch breakdown but potential for niche applications.

These findings contribute to a better understanding of banana species' biochemical properties and their potential uses in food processing and biotechnology. Further research could explore genetic and environmental factors influencing enzyme activity and the potential for genetic modification and crop improvement to enhance desired traits.

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How to cite this article:

Gourinanda Suresh, Jiby John Mathew, Sajeshkumar N. K. and Prem Jose Vazhacharickal. 2025. Comparative Analysis of Beta-Amylase Activity in Various Bananas. *Int.J.Curr.Res.Aca.Rev.* 13(12), 17-21. doi: https://doi.org/10.20546/ijcrar.2025.1312.003