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Biochemical Characterisation of Protease from Pulp and Peel of Unripe Malaysian Bananas (*Musa acuminata*)

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Structured Abstract

Background: Proteases are enzymes of considerable importance in food, pharmaceutical, and biotechnological industries due to their ability to catalyse protein hydrolysis. Most commercially available proteases are obtained from microbial and animal sources, which are often associated with high production costs, limited stability under varying conditions, and ethical concerns. Consequently, plant-based proteases have attracted increasing interest as sustainable and economical alternatives. Unripe bananas (*Musa acuminata*), which are widely cultivated in Malaysia, are potential sources of proteolytic enzymes, particularly in their pulp and peel tissues. Therefore, this study aims to characterise the biochemical properties of protease extracted from the pulp and peel of unripe Malaysian bananas.

Methods: Protease was extracted from banana pulp and peel using buffer extraction, followed by partial purification via ammonium sulphate precipitation. Protease activity was determined using the casein digestion assay, while total protein content was measured using the Kjeldahl method. The specific activity of protease was calculated to evaluate enzyme purity. Biochemical characterisation was carried out by determining the optimum pH and temperature. All analyses were conducted in triplicate, and statistical tests were used to identify significant differences between pulp and peel protease extracts.

Results: Protease activity was successfully detected in both pulp and peel extracts of unripe Musa acuminata. Overall, peel extracts exhibited higher protease activity and specific activity compared to pulp extracts. The highest protease activity was observed in banana peel, with values reaching up to 393.61 CDU/mg, while pulp extracts showed comparatively lower activity, with values around 298.21–342.42 CDU/mg. Specific activity increased following partial purification, indicating effective enrichment of active protease fractions. The protease demonstrated optimum activity at mildly acidic to neutral pH and moderate temperatures.

Conclusion: Unripe Malaysian bananas, particularly the peel, are promising sources of plant-based protease with favourable biochemical characteristics. The utilisation of banana peels as an enzyme source adds value to agricultural by-products and supports sustainable enzyme production for potential food and biotechnological applications.

Keywords: Protease, *Musa acuminata*, Banana Pulp, Banana Peel, Enzyme Characterisation

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