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STARCH–RICH SUBSTRATE AND ALPHA–AMYLASE SYNTHESIS: NATURAL VERSUS SYNTHETIC APPROACH

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Abstract: A major industrial polysaccharide, starch must be hydrolyzed by α-amylase to yield simpler sugars, which are used extensively in the food, pharmaceutical, textile, and biofuel industries. Synthetic substrates, which are expensive and harmful to the environment, are frequently used in traditional enzyme synthesis. In order to produce α-amylase, this study explores the use of agro-waste materials as economical and environmentally friendly substitutes. On fermentation media enhanced with potato, mango, and banana peels, bacterial and fungal strains *Aspergillus niger* and *Penicillium* were grown. The peels were sun-dried, crushed, and incorporated into a starch-based medium containing peptone, $MgSO_4$, KH_2PO_4, and yeast extract. The media were sterilized, inoculated, and incubated under controlled conditions, with fermentation parameters optimized at three pH levels (6, 7, and 8) and incubation times (12, 24, and 48 hours). Enzyme activity was quantified using the DNS method. Results demonstrated that mango peel was the most effective substrate for enzyme production. Among the tested strains, bacteria exhibited the highest α-amylase activity, with optimum conditions observed at pH 7 and 24 hours of incubation. These findings highlight the potential of fruit peels as sustainable substrates for large-scale α-amylase production, contributing to cost reduction and environmental sustainability in industrial bioprocessing.

Key words: α-Amylase, *Mangifera indica* (mango peel), Agro-waste, *Bacillus licheniformis, Penicillium*

INTRODUCTION

Starch is the second most abundant renewable polysaccharide on Earth after cellulose, and it plays a significant role in the global carbon cycle and Bio-economy. It serves as the primary energy storage in plants and is extensively stored in roots, fruits, seeds, and tubers (Jane, 1995).The structural components of starch include amyloses, which are mainly linear chains of α-(1→4) linked D-glucose units, and amylopectin, which is a highly branching macromolecule with both α-(1→4) and α-(1→6) connections. The proportion of these two substances varies depending on the botanical source; amylopectin typically accounts for 70–80% of starch granules, while amylose makes up 20–30%. The solubility, digestibility, and crystallinity of the starch are all impacted by these structural differences (Bangar *et al*., 2022). In addition to its nutritional value, starch is an essential industrial raw material that is used in food, medicine, textiles, fermentation, adhesives, and paper (Akinfemiwa, *et al*. 2023). Because of its semi-crystalline and granular structure, which makes it difficult to dissolve, starch's direct industrial use is often limited despite its abundance and renewable nature. For efficient use, hydrolysis into smaller molecules, such as glucose and maltose, is required. Despite the use of acid hydrolysis in the past, enzymatic degradation is now favoured due to its efficiency, selectivity, and environmental friendliness (Souza and Magalhães, 2010). The most important enzymes for breaking down starch are amylases. When internal α-(1→4) glycosidic bonds in starch are broken down by the endo-hydrolase α-amylase (EC 3.2.1.1), glucose, maltose, and dextrins are produced. The calcium-dependent metalloenzyme α-amylase is one of the most widely used biocatalysts in industry due to its catalytic adaptability and structural stability (Sivaramakrishnan, *et al.,* 2006). The demand for α-amylase is increasing due to its wide range of applications. In the food industry, it improves dough rheology, speeds up brewing, and raises bread quality (Mobini-Dehkordi and Afzaljavan, 2012). It is used in the textile industry to desize woven fabrics by removing starch-based sizing chemicals (Chi, *et a*l. 2009). In the paper industry, starch viscosity is changed to enhance coating quality, and it aids in the removal of starchy stains in detergents (Mobini-Dehkordi and Afzaljavan, 2012). Furthermore, α-amylase hydrolyses starch into fermentable sugars in order to produce bioethanol, which contributes to the production of sustainable energy (Aiyer, 2005). The enzyme is being researched for potential uses in pharmaceutical formulations in addition to its use as a digestive aid in medications (De Souza and Magalhães, 2010).These extensive industrial uses highlight the need for low-cost, sustainable α-amylase production methods. The importance of substrate optimisation was highlighted by recent research using *Aspergillus Niger*, which demonstrated that lactose and yeast extract as carbon and nitrogen sources, respectively, produced the ideal α-amylase after 72 hours at 30°C and pH 5.5(Ahmad, *et al*. 2019). Consequently, researchers have focused more on agro-industrial waste as possible alternative substrates. Globally, massive volumes of fruit and vegetable waste are generated annually, and if not properly managed, this waste can lead to environmental issues like soil contamination, microbial spoiling, and greenhouse gas emissions. Agro-wastes are great candidates for bioconversion into products with added value because they are frequently rich in proteins, cellulose, hemicellulose, and starch (Perwez and Asheh, 2025). Among agro-residues, fruit and vegetable peels offer unique advantages. Mango (*Mangifera indica*) peels comprise 15-20% of the fruit mass and contain phenolic compounds, pectin, and starch; banana (Musa spp.) peels comprise nearly 35% of the fruit and contain cellulose, starch, and bioactive compounds; and potato (Solanum tuberosum) peels, a byproduct of large-scale food industries, are rich in phenolic compounds and starch (Bhandari and Singhal, 2010). These substrates are widely available, reasonably priced, and environmentally hazardous if discarded, which highlights the two benefits of their value addition for microbial fermentation. Since microorganisms can produce a large number of extracellular enzymes in controlled environments, they are the best systems for producing enzymes. Fungal species such as *Aspergillus niger* and *Penicillium* have long been recognised for their acid-stable α-amylases and high secretion efficiency. On the other hand, bacterial strains particularly those from the genus *Bacillus* are valued for their industrially suitable thermostable enzymes (De Souza and Magalhães, 2010). Solid-state fermentation (SSF), which uses agricultural waste, is a cost-effective and environmentally benign alternative to SmF. SSF mimics the natural habitat of many bacteria, uses less water than liquid fermentation, and often produces higher-quality enzyme titers (Chandrashekhar *et al*. 2012). As a culture medium, SSF uses solid, low-moisture substrates, usually agro-industrial wastes. It has both economic and environmental benefits. For instance, *Bacillus amyloliquefaciens* cultivated on wheat bran showed maximum enzyme activity at 37°C and pH 7.0 following 72 hours of incubation, indicating that optimal production depends on the choice of substrate and fermentation conditions (Pandey, *et al*. 2000). For example, α-amylase activity has been significantly higher in SSF using soybean or wheat bran residues than in conventional SmF systems (Singh, *et al*., 2016). The viability of agro-waste-based SSF for large-scale enzyme synthesis is supported by these findings, especially in environments with limited resources. The current work uses bacterial and fungal (A. niger and Penicillium) strains to examine the potential of mango, banana, and potato peels as natural substrates for α-amylase synthesis.

MATERIALS AND METHODS

Sample collection

Peels from bananas (*Musa spp*.), mangoes (*Mangifera indica*), and potatoes (*Solanum tuberosum*) were selected as potential substrates for the α-amylase production process. To ensure availability and freshness, these substrates were collected from local fruit and vegetable suppliers. Following their collection, the peels were thoroughly cleaned under running tap water and then rinsed with distilled water to remove any remaining contaminants. Following a thorough washing, the peels were arranged evenly on sanitized trays and left to dry naturally in the sun for five consecutive days, or until a steady drop in moisture was observed. This drying stage was essential for fermentation research in order to have a consistent substrate and prevent microbial contamination during storage.

Media Preparation and Source Selection

The experimental design involved the preparation of multiple media to ascertain whether microbial strains and agro-waste substrates were appropriate for producing α-amylase.

Starch Medium

In order to facilitate microbial growth and act as a control, a standard starch medium was made. Peptone, soluble starch, yeast extract, 0.10 gm $MgSO4$, 0.10 gm KH_2PO_4, and 1.0 gm soluble starch, based on 100 ml of distilled water, made up the medium. The medium was put into test tubes and sterile Erlenmeyer flasks and autoclaved for 30 minutes at 121°C and 15 lbs pressure to sterilize it. For 24 hours and 72 hours, respectively, bacterial and fungal cultures were kept in static conditions at 35 °C. After detectable growth was established, the inocula were moved into fermentation flasks to produce enzymes. Following cooling, bacterial strains and fungal strains of *Aspergillus niger* and *Penicillium sp*. were aseptically inoculated.

Agro-Waste media

To evaluate the efficiency of agro-waste as a substrate, media was created using peel extracts. 150 ml of distilled water were combined with peel powder (0.5 gm), yeast extract (1.5 gm), $MgSO_4$ (0.15 gm) and K_2HPO_4 (0.1 gm) for each substrate (banana, mango, and potato peels). Each medium was separated into three 50 ml flasks and autoclaved for 30 minutes at 121 °C.

Preparation of Inoculum

Different inocula were made for different strains of bacteria and fungi. For each substrate, 0.5 g of peel was added to flasks containing a salt solution (0.06 gm $MgSO_4$, 0.06 gm KH_2PO_4, and 0.6 gm yeast extract diluted in 20 ml distilled water). Each flask contained 30 ml of media, and three test tubes (20 ml each) contained an additional 60 ml. Every piece of media was autoclave sterilized. After chilling, the bacterial and fungal strains *Aspergillus niger* and *Penicillium s*p. were aseptically inoculated using a sterile wire loop. Bacteria were incubated for 24 hours at 35 °C, whereas fungi were incubated for 72 hours. In order to produce enzymes, the generated inocula were then placed into the proper fermentation flasks.

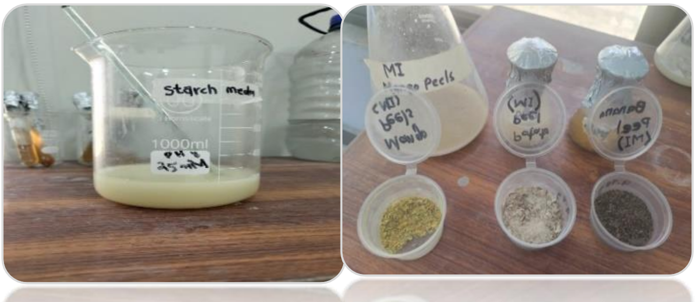

Figure 1 : show the starch media and agro-waste media preparation

Citrate Buffer

To maintain pH stability during fermentation and enzyme assay, citrate buffer (pH 6.60) was made by mixing the appropriate amounts of sodium citrate and citric acid solutions. Prior to incubation, 50 ml of buffer was added to each fermentation flask.

Filtration Process

After the incubation period, the fermentation broth was filtered to remove the solid residues from the crude enzyme extract. A sterile muslin cloth placed over a funnel was used to filter the culture medium before it was transferred into a sanitized beaker. Prior to further analysis, the filtrate also known as the crude cell-free filtrate (CFF) was gathered and kept at 4 °C.

Enzyme assay

For the enzyme assay, 0.1 ml of crude enzyme extract was mixed with 1.0 ml substrate (1% soluble starch in 0.1 M citrate buffer, pH 5.0).The reaction mixture was kept at 50 °C for 15 minutes in a water bath. The reaction was stopped after five minutes and 1.0 ml DNS reagent was added in reaction tube. After chilling, 4.5 ml distilled water was added in tube. The absorbance at 546 nm was measured using a UV-Vis spectrophotometer against reagent blank.

Units of Enzyme

The amount of enzyme that releases 1.0 µmol of reducing sugar from the substrate in a minute at 40 °C from soluble starch.

Microbial Strain Selection

To find out which microbial strain was best at producing α-amylase, comparative experiments were conducted using the bacterial strain (*B. licheniformis*), *Aspergillus niger* and *Penicillium sp*. Equal amounts of agro-waste substrates (5.0 gm peels)were added to fermentation flasks under the same conditions. The crude enzyme extracts were filtered out following a 24-hour and 72-hour incubation period for the bacteria and fungi, respectively. The strain with the highest enzyme activity was selected for additional optimization studies.

Optimization of Fermentation conditions

To boost the yield of α-amylase, optimization tests were conducted with varying pH levels and incubation durations.

Incubation Time for enzyme production

The effect of time on fermentation was evaluated using three different incubation times: 12, 24, and 48 hours. 5.0 gm of mango peels were used as the substrate in 50 ml medium containing yeast extract (1.0 gm) , $MgSO_4$ (0.125 gm) and K_2HPO_4 (0.158gm) for each condition. The sterilized flasks were inoculated and incubated at 30° C. Following sample extraction and filtering at each time interval, the enzyme activity was measured using the DNS method.

Effect of pH on enzyme production

The pH of the fermentation medium was adjusted to 6.0, 7.0, and 8.0 in order to examine the impact of pH on enzyme production. A digital pH meter that had been calibrated was used and pH was adjusted using 1.0% HCl and 1.0% NaOH solutions. Standard conditions were followed during the fermentation process, and enzyme activity was measured.

RESULTS AND DISCUSSION

The potential carbon sources for α-amylase synthesis under uniform fermentation conditions were evaluated for three agro-waste substrates: banana, potato, and mango peels. The enzyme activity of these was highest in mango peels (825.17 U/ml/min), followed by banana peels (414.88 U/ml/min) and potato peels (521.10 U/ml/min). Because of their high content of simple sugar and carbohydrates and their less fibrous structure, which promotes microbial hydrolysis and nutrient availability, mango peels perform better than other materials. Suman et al. [19] reported similar results, emphasising fruit peels as efficient substrates for microbial enzyme synthesis because of their biodegradability and sugar-rich composition.

Effect of Incubation Time on enzyme production

Using mango peel as the substrate, enzyme activity was measured at 12, 24 and 48 hour intervals. The highest activity, 825.17 U/mL/min, was recorded at 24 hours, while yields were lower at 12 hours (73.34 U/mL/min) and 48 hours (18.52 U/mL/min). This pattern is consistent with the kinetics of microbial fermentation, which demonstrates that the late exponential phase is when the greatest amount of enzyme secretion occurs, followed by a decrease due to proteolytic degradation and nutrient depletion (Gupta *et al*. 2003). *Bacillus subtilis* reached its peak α-amylase activity 24 hours into submerged fermentation (Patel *et al*. 2005).

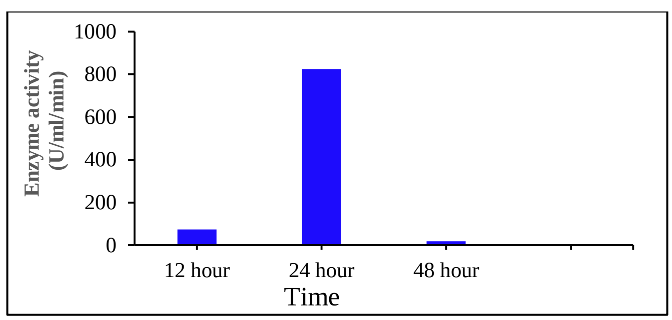

Figure 2. Effect of time on enzyme production

Effect of pH on enzyme production

Three pH values (6.0, 7.0, and 8.0) were used to further optimize the pH effect. Enzyme production was found to be 8699 and 7578 U/ml/min at pH 6.0 and 8.0, respectively, while the highest activity (9950 U/ml/min) was obtained at pH 7.0. These findings are in line with previous research that indicated that Bacillus species produced the most α-amylase at a pH of 7.0 (Riaz *et a*l. 2009: Singh *et al.* 2016). Furthermore, Qader *et al*. (2006) emphasized that the pH range of 6.5 to 7.0 is usually the best for maximum yields of enzyme from bacterial fermentation on fruit-based substrates.

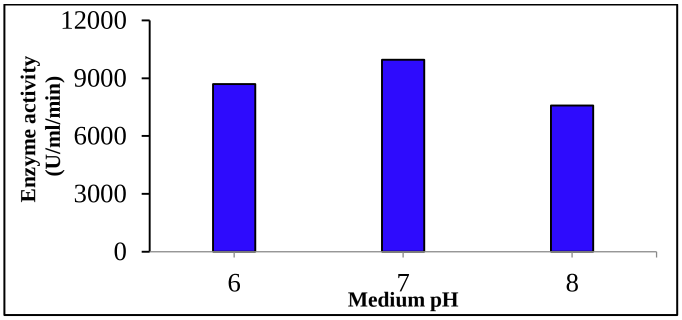

Figure 3. Effect of pH on enzyme production

CONCLUSIONS

This work highlights the potential of agricultural waste, particularly mango peels, as an economical, sustainable, and efficient substrate for microbial fermentation-based α-amylase production. Among the substrates examined, mango peel exhibited the highest enzyme activity due to its high carbohydrate content and favourable biodegradability. *Bacillus licheniformis* out performed *Aspergillus niger* and *Penicillium sp*. in comparative strain studies, achieving the highest α-amylase activity at pH 7.0 and after 24 hours of incubation. These findings showed that bacterial systems, in particular, are more effective enzyme producers in the environments studied. The results demonstrated that the potential of green biotechnological techniques demonstrated that how to convert agro-industrial waste into beneficial bio-products.

Conflict of interest

Authors declare no conflict of interest.

REFERENCES

Ahmad, M. A., U. Isah, I. A. Raubilu, S. I. Muhammad, and D. Ibrahim (2019). An overview of the enzyme: Amylase and its industrial potentials. *Bayero J. Pure Appl. Sci*., 12, 352-358.

Aiyer, P. V. (2005). Amylases and their applications. *Afr. J. Biotechnol*., 3.1525-1529.

Akinfemiwa, O., M. Zubair, & T. Muniraj, (2023) Amylase. In Stat Pearls. Stat Pearls Publishing.

Bangar, S. P., A. O. Ashogbon, A. Singh, V. Chaudhary and W. S. Whiteside, (2022) Enzymatic modification of starch: A green approach for starch applications. *Carbohydr. Polym*, 287, 119265

Bhandari, S. and R. S. Singhal (2010). Mango peel as substrate for production of extra cellular polygalacturonase from Aspergillus fumigatus. *Indian J. Hort*., 67, 140-143.

Chandrashekhar U., R. I. Kallur and B. B. Kaliwal(2012). Production of α-amylase using banana waste by Bacillus subtilis under solid state fermentation. Eur. J. Exp. Biol., 2, 1044-1052.

Chi, Z., Z. Chi, G. Liu, F. Wang, L. Ju and T. Zhang, (2009). Saccharomycopsis fibuligera and its applications in biotechnology. *Biotechnol. Adv*., 27, 423-431.

De -Souza, P. M. and P. O. Magalhães, (2010) Application of microbial α-amylase in industry-A review. *Braz J Microbiol*., 41, 850-861.

De Souza, P. M. and P.D. O. Magalhães (2010). Application of microbial α-amylase in industry-A review. *Braz J Microbiol*., 41, 850-861.

Gupta, R., P. Gigras, H. Mohapatra, V. K. Goswami, and B. Chauhan(2003). Microbial α-amylases: A biotechnological perspective. *Process Biochem*, 38, 1599-1616.

Jane, J. (1995) Starch Properties, Modifications, and Applications. *J. Macromol. Sci., Part A: Pure Appl. Chem* Part A, 32, 751–757.

Mobini-Dehkordi M., and F. Afzaljavan (2012). Application of alpha-amylase in biotechnology. *J. Biol. Today, World*, 1, 39-50.

Pandey, A., C. R. Soccol, and D. Mitchell, (2000). New developments in solid-state fermentation: I—Bioprocesses and products. *Process Biochem*., 35, 1153–1169.

Patel, S. A., S. N. Naik, and R. M. Kothari (2005). Enhanced production of α-amylase by Bacillus subtilis using submerged fermentation. *J. Appl. Microbiol*. 99, 1354–1362

Perwez, M. and S. A. Asheh (2025).Valorization of agro-industrial waste through solid-state fermentation: Mini review. *Biotechnol. Rep*., 45, e00873.

Qader S.A.U., S. Bano, A. Aman, N. Syed and A. Azhar (2006). Enhanced production and extracellular activity of commercially important amylolytic enzyme by a newly isolated strain of Bacillus. sp. AS-1. *Turk. J. Biochem*. 3, 135-140.

Riaz, A., S.A.U. Qader, A. Anwar and S. Iqbal (2009). Immobilization of a thermostable α-amylase on calcium alginate beads from Bacillus subtilis KIBGE-HAR. *Aust. J. Basic Appl. Sci.* 3, 2883-2887.

Singh, R., M. Kumar, A. Mittal, and P. K Mehta, (2016). Microbial enzymes: Industrial progress in 21st century. *3 Biotech*, 6, 174. doi: 10.1007/s13205-016-0485-8

Sivaramakrishnan, S., D. Gangadharan, K. M. Nampoothiri, C. R. Soccol, and A. Pandey (2006) α-Amylases from Microbial Sources-An Overview on Recent Developments. Food Technol. Biotechnol. 44, 173-184.

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