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OPTIMIZATION OF CATALYTIC CONDITIONS FOR β-AMYLASE *IPOMOEA BATATAS* β-AMYLASE

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ABSTRACT
β-Amylase (EC 3.2.1.2) is a crucial enzyme involved in starch degradation and is abundantly present in plant tissues. It plays a vital role in catalyzing the hydrolysis of α-1,4-glycosidic bonds, resulting in the release of maltose units. Sweet potato (Ipomoea batatas) serves as a rich and natural source of this enzyme, making it a practical model for biochemical extraction and analysis. In the present study, β-amylase was extracted from sweet potatoes and its enzymatic activity was evaluated under varying temperatures, substrate concentrations, and enzyme concentrations, while the pH was maintained constant at 4.8 throughout the assay conditions. Enzyme activity assays were performed using soluble starch as the substrate, and maltose production was quantified using colorimetric estimation methods. The primary objective of this study was to determine the optimal temperature and assay conditions required for maximum stability and enzymatic activity of sweet potato β-amylase, which are critical parameters for both laboratory research and industrial utilization. Analysis of the effects of different biochemical factors on reaction rates provided insights into enzyme kinetics and functional adaptability. The results highlight the potential application of plant-derived β-amylase in industries such as food processing, brewing, and starch modification, where controlled starch hydrolysis is essential. Overall, this study contributes to a clear understanding of the enzymatic properties of β-amylase extracted from sweet potatoes and establishes a foundation for optimizing its use in research and industrial processes.

Keywords: *β-amylase, EC 3.2.1.2, sweet potato, enzymatic activity, starch hydrolysis.*

1. INTRODUCTION
β-Amylase (EC 3.2.1.2), also referred to as α-1,4-glucan maltohydrolase, is an exo-type hydrolase enzyme that catalyzes the hydrolysis of *α-1,4-glycosidic* linkages of starch from the non-reducing ends, resulting in the release of maltose units [Lutfunnahar et al., 2022; Chang et al., 1996; Duan et al., 2021]. This enzyme is widely distributed in plants, where it plays a central role in starch degradation during physiological processes such as seed germination and storage root metabolism [Morrison et al., 1993; Obe & Fatoki, 2021]. One of the most prominent plant sources of β-amylase is the sweet potato *(Ipomoea batatas),* in which the enzyme occurs in high concentrations, accounting for approximately 5% of the total soluble protein. The activity of β-amylase is also responsible for the characteristic sweet flavor of cooked sweet potatoes, owing to the conversion of starch into maltose [Morrison et al., 1993; Lutfunnahar et al., 2022; Duan et al., 2021].

Because of its extensive industrial applications in food processing, brewing, and starch modification, characterization of the functional properties of sweet potato β-amylase is of significant importance [Khan et al., 2011; Limegne et al., 2024]. In the present study, β-amylase was extracted from sweet potato and its activity was evaluated under varying enzyme concentrations, substrate concentrations, and temperatures, while maintaining a constant pH of 4.8. Maltose production was quantified using the *3,5-dinitrosalicylic acid* (DNSA) colorimetric method, a widely accepted assay for the estimation of reducing sugars [Chang et al., 1996; Noda et al., 2001]. The primary objective of this study was to determine the optimal conditions required for efficient starch hydrolysis by plant-derived β-amylase.

2. LITERATURE REVIEW
Work on sweet potato *(Ipomoea batatas)* β-amylase spans: (1) extraction/purification and basic properties, (2) effects of variety and environment on activity, (3) use of crude or purified enzyme in processing (brewing, glucose syrups), and (4) immobilization and industrial stabilization strategies.

Core biochemical properties and kinetics
Purified β-amylase from sweet potato tubers shows optimum pH around 5–5.5 and temperature ~50–55°C, and is relatively stable up to ~60°C, closely matching your constant acidic pH and temperature-optimization focus [Chang et al., 1996; Lutfunnahar et al., 2022]. High substrate concentrations of starch can inhibit activity, emphasizing the importance of assaying across a range of starch levels, as in your design (20). Sweet potato β-amylase is highly abundant, forming up to 5% of total protein and a major fraction of soluble protein in roots

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[Morrison et al., 1993]. During cooking, it hydrolyses gelatinized starch from the non-reducing end, generating maltose and reducing texture firmness [Mensah et al., 2016].

Influence of genotype and environment
Variety and growing conditions strongly affect β-amylase content and activity. Ghanaian varieties differed significantly in β-amylase activity, influencing suitability as enzyme sources for syrup production [Dziedzoave et al., 2010]. East African germplasm shows allelic variation in the Amyβ gene linked to low enzyme activity and firmer cooked roots, underlining genetic control of β-amylase functionality [Morrison et al., 1993]. Recent Cameroonian work also confirms predominance of β- over α-amylase in sweet potato tubers and identifies near-neutral pH optima (5.55–6.55) for total amylase activity [Limegne et al., 2024].

Processing and industrial applications
Crude sweet potato enzyme extracts have been optimized for mashing sorghum; response-surface analysis yielded an extraction temperature of 60°C and pH 5.4–8.9 range suitable for wort production, demonstrating feasibility of directly using plant-derived β-amylase in brewing-type processes [Limegne et al., 2024]. Earlier work identified specific varieties as cost-efficient enzyme sources for glucose syrup production [Dziedzoave et al., 2010]. Purple sweet potato has also been used for glucose syrup, though predominantly via α-amylase and amyloglucosidase, underscoring broader starch-processing relevance of sweet potato enzymes [Pereira et al., 2017]. Immobilized sweet-potato β-amylase on natural matrices (e.g., sodium alginate) shows improved stability and reusability, strengthening its industrial potential [Khan et al., 2011; Tavano et al., 2013; Noda et al., 2001].

Comparison with other β-amylases and stabilization strategies
Microbial β-amylases (e.g., from *Bacillus aryabhattai*) can reach very high specific activities and have pH optima around 6.5 and 50°C, but sweet potato β-amylase still performs competitively in maltose production from starch [Lutfunnahar et al., 2022]. These strategies are conceptually transferable to plant β-amylase.

Although extensive studies have reported the purification, characterization, and application of sweet potato β-amylase, enzymatic optima are often described under variable and non-comparable experimental conditions. Limited work has integrated the effects of temperature, substrate concentration, and enzyme concentration under a single, fixed acidic pH relevant to practical assay conditions. The present study addresses this gap by systematically characterizing sweet potato β-amylase at pH 4.8, evaluating the combined influence of these variables using soluble starch and colorimetric maltose estimation. By establishing consistent operational conditions rather than isolated optima, this work enhances comparability across studies and supports the development of reproducible assays for controlled starch hydrolysis.

3. RESEARCH METHODOLOGY

1. Enzyme Extraction
Fresh, mature sweet potato *(Ipomoea batatas)* tubers were thoroughly washed, peeled, and weighed [Chang et al., 1996; Ramakrishnan & Rathnasamy, 2016; Lutfunnahar et al., 2022]. A 100 mg portion of the tissue was crushed in 5ml of chilled acetate buffer (pH 4.8) under cold conditions to prevent enzyme denaturation[Ramakrishnan & Rathnasamy, 2016; Lutfunnahar et al., 2022]. The homogenate was centrifuged at 3000 rpm for 10 minutes and used the clear supernatant as the crude β-amylase extract for all subsequent assays [Chang et al., 1996; Lutfunnahar et al., 2022].

2. Preparation of Reagents
A 2% (w/v) soluble starch solution was prepared in acetate buffer (pH 4.8) to use as the substrate[Chang et al., 1996; Purwadi et al., 2021; Noda et al., 2001]. The DNSA *(3,5-dinitrosalicylic acid)* reagent was prepared according to the following standard protocols for estimating reducing sugars[Morrison et al., 1993; Obe & Fatoki, 2021]. Acetate buffer (pH 4.8) was used consistently in all experiments to keep a constant pH [Chang et al., 1996; Lutfunnahar et al., 2022].

3. Enzyme Activity Assay (DNSA Method)
β-amylase activity was determined by measuring maltose released from soluble starch using the DNSA colorimetric method [Chang et al., 1996; Noda et al., 2001]. After incubation, the reaction was terminated by adding a DNSA reagent and heating the mixture in a boiling water bath. The developed color was cooled to room temperature and measured absorbance using a colorimeter at 530 nm against a maltose standard curve [Morrison et al., 1993; Obe & Fatoki, 2021].

4. Effect of Enzyme Concentration

The effect of enzyme concentration on β-amylase activity was studied by varying the enzyme extract volume from 0.2 to 1.0 ml while keeping the substrate concentration constant at 2% starch [Chang et al., 1996; Lutfunnahar et al., 2022]. The volume of acetate buffer was adjusted from 2.8 to 2.0 ml to maintain a constant total reaction volume [Chang et al., 1996]. Enzyme activity was measured using the DNSA colorimetric assay [Morrison et al., 1993; Obe & Fatoki, 2021].

5. Effect of Substrate Concentration

To determine the influence of substrate concentration, soluble starch was varied from 0.5% to 2.5% at 0.5% intervals while keeping the enzyme extract volume constant at 1.0 ml [Chang et al., 1996; Purwadi et al., 2021; Noda et al., 2001]. The acetate buffer volume was adjusted from 3.5 to 1.5 ml to maintain a uniform assay volume[Chang et al., 1996]. Maltose formation was quantified colorimetrically at 530 nm [Morrison et al., 1993; Obe & Fatoki, 2021].

6. Effect of Temperature

The optimum temperature for β-amylase activity was evaluated by performing assays at 4°C, 27°C, 37°C, 50°C, and 100°C [Chang et al., 1996; Lutfunnahar et al., 2022; Mensah et al., 2016]. The reaction mixture consisted of 2% starch, 2 ml acetate buffer (pH 4.8), and 1 ml enzyme extract, with all other parameters kept constant [Chang et al., 1996; Lutfunnahar et al., 2022]. Enzyme activity was determined using the DNSA colorimetric method.

7. Effect of Inhibitor (EDTA)

The inhibitory effect of EDTA (Ethylenediaminetetraacetic acid) on β-amylase activity was investigated by keeping the substrate concentration constant at 2% starch and varying EDTA concentrations from 0.2 to 1.0 ml at 0.2 ml intervals [Khan et al., 2011; Das et al., 2017; Noda et al., 2001]. The acetate buffer volume was adjust from 1.8 to 1.0 ml to maintain a constant reaction volume. Enzyme activity measured at 530nm using a colorimeter [Morrison et al., 1993; Obe & Fatoki, 2021].

4. RESULTS

The effect of various experimental parameters on enzyme activity was evaluated by measuring absorbance at 530 nm.

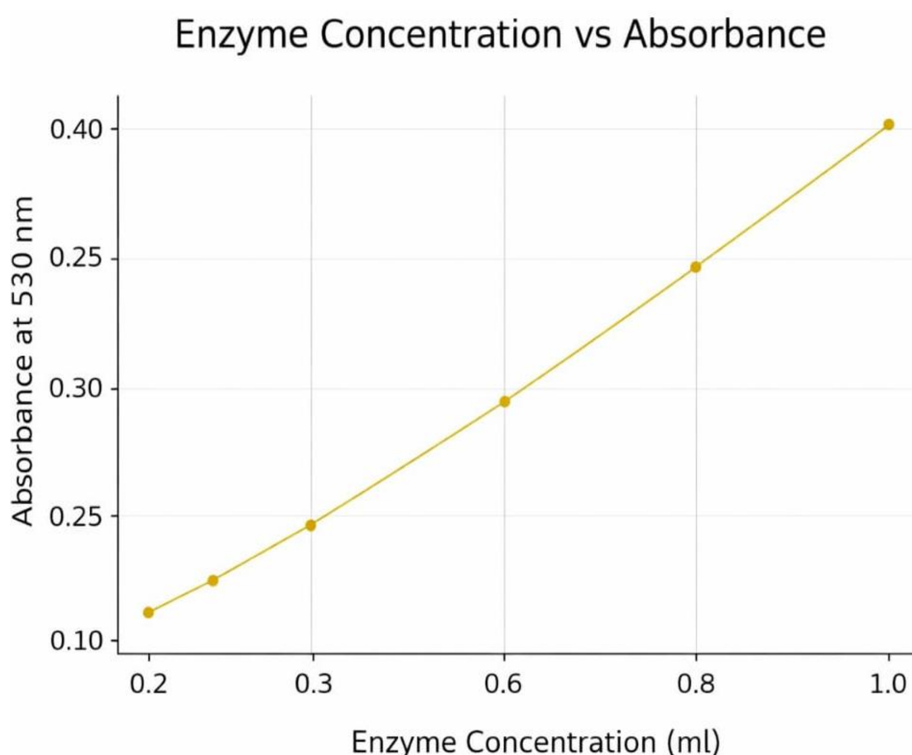

Figure 1. Effect of Enzyme Concentration

The amount of absorbance absorbed increased with increased amounts of enzyme. When the amount of enzyme was increased from 0.2 to 1.0 mL, the amount of absorbance measured increased from 0.08 (A530) to 0.40 (A530). The linear relationship observed shows a direct correlation between enzyme activity and the amount of enzyme present in an experiment.

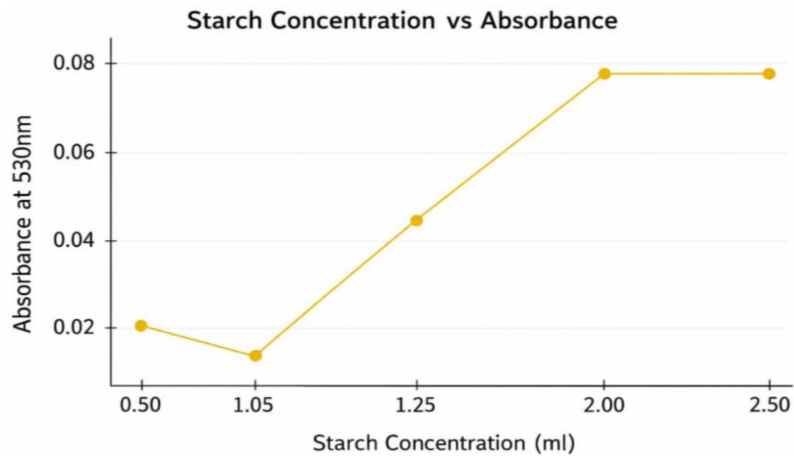

Figure 2. Effect of substrate (starch) concentration

Absorbance at 530 nm increased with increasing starch concentration up to 2.0 mL, after which no further rise was observed, indicating enzyme saturation. The maximum reaction velocity Vmax= 0.067 A530/min, while the substrate constant Km=1.0% (w/v), reflecting the substrate concentration required to achieve half-maximal velocity.

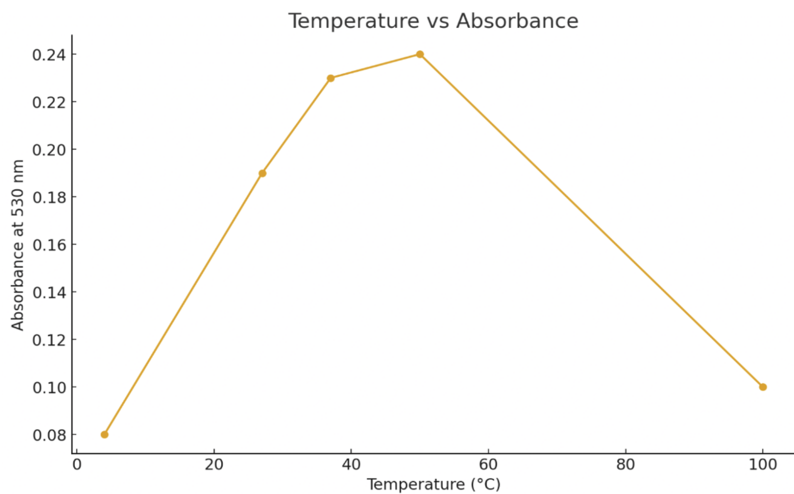

Figure 3. Effect of temperature

Temperature had a significant effect on enzyme activity. The absorbance increased from 0.08 at 4°C to a maximum of 0.24 at 50°C, showing an increase in enzyme activity with an increase in temperature. However, a sudden drop in absorbance was noticed at 100°C, showing denaturation of the enzyme at higher temperatures.

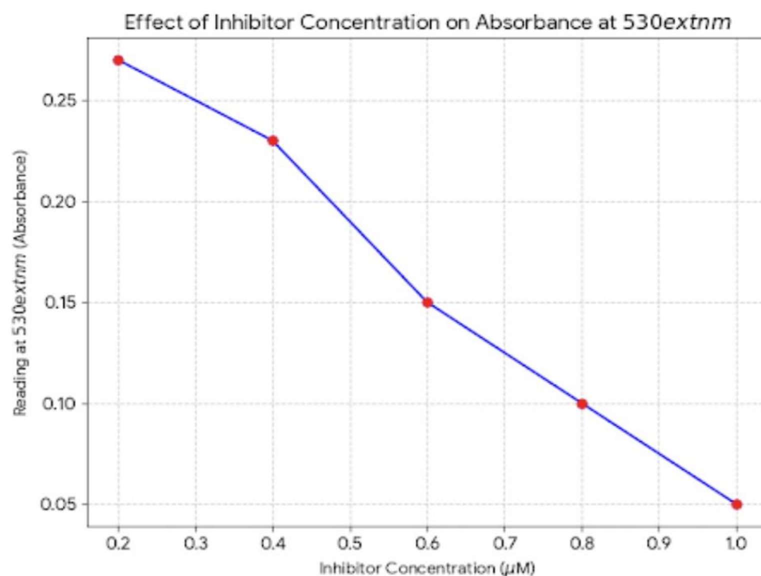

Figure 4. Effect of the inhibitor concentration

When the absorbance was measured against concentrations, an inverse relationship was obtained. At an absorbance of 0.27, the inhibitor concentration was 0.2 µM, while at 1.0 µM of the inhibitor, the absorbance was 0.05. This shows that higher concentrations of the inhibitor have more significant levels of the enzyme's activity.

5. DISCUSSION

The present study shows how enzyme concentration, substrate concentration, temperature, and inhibitor concentration affect β-amylase activity. When enzyme concentration increased from 0.2 to 1.0 mL, absorbance also increased. This indicates a direct link between enzyme amount and reaction rate under substrate-excess conditions. Increasing starch concentration raised absorbance up to 2%. After that point, no further increase was seen, confirming that the enzyme was saturated. The calculated Vmax of 0.067 A530/min, represents the highest reaction speed. The Km value of 1.0 shows the substrate concentration needed for half-maximal activity, indicating a moderate binding strength between the enzyme and substrate. Kinetic parameters were determined using Michaelis- Menten kinetics. Temperature had a major impact on enzyme activity. The highest absorbance occurred at 50°C, suggesting these conditions are optimal for catalysis. The sharp drop in activity at 100°C shows that the enzyme denatured due to high heat. There was a negative relationship between inhibitor concentration and enzyme activity. Increasing the inhibitor concentration caused a significant drop in absorbance, confirming that β-amylase activity was effectively inhibited.Overall, the findings align with traditional enzyme kinetics and support the use of the DNSA method for analyzing enzyme activity.

6. CONCLUSION

In conclusion, the above study clearly establishes the relationship between enzyme activity and other experimental parameters like enzyme concentration, substrate concentration, temperature, and inhibitor concentration. The activity of the enzyme increased with the rise in enzyme and substrate concentration to reach an optimum point, beyond which saturation occurred, establishing the maximum velocity of the reaction (Vmax). Temperature had a significant effect on the activity of the enzyme, where the maximum absorbance was recorded at the optimum temperature, while higher temperatures resulted in lower activity due to the denaturation of the enzyme. However, the increase in inhibitor concentration resulted in a substantial decrease in the activity of the enzyme, thus establishing its inhibitory role.

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