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Optimization of *Aspergillus niger* α-amylase Activity for Enhanced Glucose Production from Cassava Starch

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Authors' contributions

This work was carried out in collaboration among all authors. Author NAO conceptualized the research problem and designed the experimental protocols. Author JCN carried out the experiment. Author MRK sourced the materials for the write-up and also assisted in the laboratory exercise. Author OE edited the work and managed correspondences Author OO performed the graphical interpretation of results generated. All authors read and approved the final manuscript.

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ABSTRACT

The aim of this study was to optimize the hydrolytic activity of *A. niger* α-amylase on cassava starch. Isolation of *Aspergillus niger,* determination of α-amylase activity, α-amylase production and extraction were performed using standard protocols. Parameters such as pH, temperature, substrate concentration were studied using unifactorial approach. pH was varied from 3.6-5.6, temperature 30-80ºC, substrate concentration 0.3-1.5 g/l. In conclusion, for optimal utilization of α-amylase in the production of numerous products of economic significance, the outcome of this work can be relied upon to boost production of glucose and accessory products from starch.

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1. INTRODUCTION

Cassava, commonly known as tapioca, manioc or yucca has been identified as one of the most important food crops in the humid tropics primarily owing to its suitability to conditions of low soil nutrients in addition to its ability to survive drought [1]. Its impressive potential to convert large amount of solar energy into soluble carbohydrate per unit area has earned it, an elevated placement among other crops with similar potential. With the estimation of world cassava production at over 200 million metric tons, cassava is undoubtedly considered a dependable source of starch for diverse industrial products.

Starch, consists of amylose and amylopectin both of which have glucose as monomeric units [2]. Amylose is a linear polymer in which glucose units are linked through the α-1, 4-glycosidic bonds although with about 0.1% of α-1, 6-glycosidic branch points [3]. On the other hand, amylopectin with a far larger proportion of α-1, 6-glycosidic branch points (ca. 4%), also contains α-1, 4-linked glucan chains.

Starch is considered one of the most versatile biomaterials, it is a renewable and an unlimited resource material employed in the activities of the food and the non-food industries where about 54% and 46% respectively [4]. These arrays of industrial applicability of starch are credited to its abundance in nature, affordability, impressive calorific value and inherent physiochemical properties [4].

Enzymatic and acid hydrolytic approaches have been widely explored to convert starch to many value added products such as glucose syrup, maltose syrup, high fructose syrup and maltodextrins which are industrial products of economic significance among others. The acidic hydrolysis which is the older and more traditional method is operational in highly acidic medium of pH 1-2, high temperature (150ºC-230ºC) and high pressure [5]. As a consequence of high thermal and acidic reaction environment that characterise the chemical method of starch hydrolysis, unnecessary by-products which contaminate the end product hydrolysate are formed in addition to corroding processing equipment [6]. More so, the process appears to be totally random and thus is not influenced by the presence of α-1, 6 glycosidic linkages [7].

Aspergillus niger has been the subject of research and industrial processes for several decades. It first acquired practical significance in 1919 when its ability to produce citric acid was industrially exploited [8]. It is a haploid filamentous fungus which is used for waste management and biotransformation. It is one of the microorganisms with notable ability to produce α-amylase, a class of enzymes, with renowned applicability in the food, brewing, textile, detergent and pharmaceutical industries [9].

Amylases are enzymes that break down starch and glycogen [9]. They belong to the family of endoamylases. Although, α-amylase can be derived from plant and animal in addition to microbial sources, Microbial sources of this industrial enzyme is adjudged the most ideal owing to its economical bulk production capacity in addition to the fact that microbes can be easily manipulated to obtain enzymes of desired characteristics [10].

The enzymatic hydrolysis of starch which is characterised by high reaction rate, enhanced resistance of the enzyme to the denaturizing action of solvents, detergents, proteolytic enzymes, is performed under milder conditions of lower temperature (up to 100ºC), normal pressure, pH of medium of about 6.8 [11]. Although often time, enzymatic hydrolysis has been performed with the aid of α-amylase (EC: 3.2.1.1) at temperature (90-100ºC), substrate concentration (20-35%) pH (6-8) etc [11], these parameters usually vary depending on the source of the enzyme [12]. Thus, it is imperative to determine through research the ideal conditions for enhancement of cassava starch hydrolysis using α-amylase derived from *A. Niger* as part of the efforts to sustain uninterrupted supply of raw materials to the pharmaceutical and others allied industries.

2. MATERIALS AND METHODS

2.1 Sample Collection

Cassava starch was purchased from Samaru market Zaria Kaduna State, Nigeria. It was stored in an air tight container until use. White yam water was drained off boiled small pieces of fresh tuber into a sterile bottle where it was stored under aseptic condition until use.

2.2 Isolation of *Aspergillus niger*

A small portion of bread was subjected to a moist condition in dark at room temperature for 2 days. Serial dilution was carried out on the bread sample after which different dilutions were inoculated on potato dextrose agar (PDA) medium. Subsequently, the slants were incubated at 30ºC for 4 days. Fungal cultures were observed on PDA medium. The fungal strain was subjected to lactophenol cotton blue staining for morphology studies. The fungal culture was confirmed as *Aspergillus niger* by studying the morphology and the spore colour.

2.3 Determination of Amylase Activity

The *Aspergillus niger* isolate was tested for amylase production by starch hydrolysis. Following the inoculation of the starch agar medium with the organism and subsequent flooding with iodine solution, the zone of clearance around the microbial growth served as a pointer to the presence of amylase and the fungal isolate was taken for amylase production.

2.4 Enzyme Production

The *Aspergillus niger* was subjected to solid state fermentation in which white yam water was used as the substrate. The substrate occupied about half the entire volume of the bottle. 1% of inoculum was sterilized and inoculated before being incubated at room temperature for six days.

2.5 Enzyme Extraction

Exactly 25 ml of 0.1M phosphate buffer saline (pH 7) was introduced into the inoculated substrate beds and was shaken vigorously in rotary shaker for 15 mins at 120 rpm. The mixture was filtered through cheese cloth before being centrifuged at 8000 rpm for 15 min at 4ºC. The supernatant was filtered through cheese cloth and the filtrate was used as the crude enzyme preparation. α-amylase was assayed by Dinitrosalicyclic acid method.

2.6 Determination of Amylase Activity

To a test tube holding 1 ml of dissolved cassava starch, 2 ml of phosphate buffer was introduced, after which 1% NaCl was included. The content was thoroughly mixed before being incubated for 5 minutes at 37ºC prior to inclusion of crude enzyme into the test tube. The contents of the test tube were mixed well and incubated for another 10 minutes at 37ºC. After incubation, 1 ml of 2N NaOH was added to the test tube. The reducing sugar liberated which was used as an indicator of enzyme activity was assayed calorimetrically by the addition of 1ml Dinitrosalicyclic acid (DNS) reagent.

2.7 Optimization of Process Parameters

The conventional unifactorial approach was relied upon to optimize the investigated parameters which include pH, temperature and substrate concentrations. In this method, all the process parameters were kept constant except the ones under investigation which were varied within a range of values thus; pH 3.6-5.6, temperature 30-80ºC, substrate concentration 0.3-1.5g/l.

3. RESULTS AND DISCUSSION

3.1 Hydrolytic Activity of *A. niger* α-amylases on Cassava Starch at Varying pH of the Reaction Medium

Fig. 1 shows the hydrolytic activity of *A. niger* α-amylase on cassava starch at varying pH of the reaction medium. Enhanced enzyme activity was observed at pH of 4.8 of the reaction medium.

This may be as a result of the fact that the state of ionization of amino acids in the enzyme protein is preserved leading to the protection of the ionic bonds that account for the three dimensional structure of the enzyme and hence enzyme activity evident by the generation of the highest concentration of glucose (0.2188 g/l) after 5 hours of hydrolysis. This result is consistent with the finding of Yabefa [13] which established elevated glucose concentration in enzymatic hydrolysis of starch at pH 4.5. Further increase in pH resulted in a declined enzyme activity. This may be due to the alteration of the state of ionization of amino acids and consequent distortion of the ionic bond that is responsible for the three dimensional structure of α-amylase as well as its activity or changes in the shape or charge properties of the substrate thus impairing the substrate's ability to identify and bind to the active site of the enzyme. This finding is in tandem with the report of the Martinek [15] which affirms that extremely high or low pH values generally results in complete loss of activity for most enzymes.

3.2 Hydrolytic Activity of *A. niger* α-amylases on Cassava Starch at Varying Temperatures of the Reaction Medium

Fig. 2 shows the hydrolytic activity of *A. niger* α-amylase on cassava starch at varying temperatures of the reaction medium. The temperature of the reaction medium was varied from 30°C to 80°C. It was observed that enzyme activity increased progressively with increase in temperature. At 60°C, optimal activity of the enzyme was observed as this coincided with enhanced production of glucose recorded at 0.1893 g/l after 5 hours of hydrolysis. This observation is in tandem with the results of Baskar et al. [13] which reported appreciable concentration of glucose in enzymatic hydrolysis of starch at reaction temperature of 50°C and 60°C. However, a persistent decline in enzyme activity was observed at temperatures above 60ºC. This could be attributed to loss of the three dimensional structure of the enzyme to denaturation resulting from high temperature. This result is consistent with the finding of Tapan, et al. [14] which has demonstrated that amylase derived from *Heliodiaptomus viduus* lost its catalytic activity at the temperature of 70ºC.

3.3 Hydrolytic Activity of *A. niger* α-amylases on Cassava Starch at Varying Substrate Concentrations of the Reaction Medium

Fig. 3 shows the hydrolytic activity of *A. niger* α-amylase on cassava starch at varying substrate concentrations. The investigation was carried out at varying substrate (cassava starch) concentrations ranging from 0.3 to 1.5 g/l. Observations made established that increased enzyme activity was driven by a concomitant increase in substrate concentration with maximum increase in glucose concentration (0.31 g/l) recorded at the substrate concentration of 1.5 g/l. This result is in conformity with the report of Martinek [15] which confirms that increasing substrate concentration brings about a gradual increase in enzyme activity until the maximum concentration is attained during which further increase in substrate concentration will not increase enzyme activity.

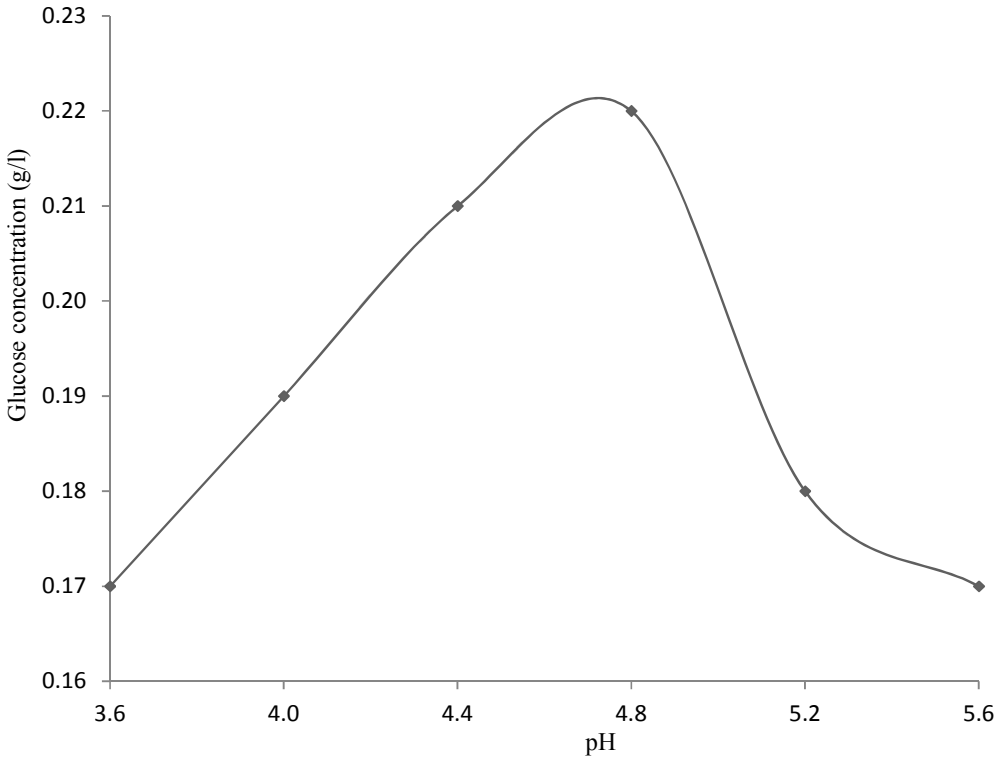

Fig. 1. Effect of pH on *A. niger* amylase activity

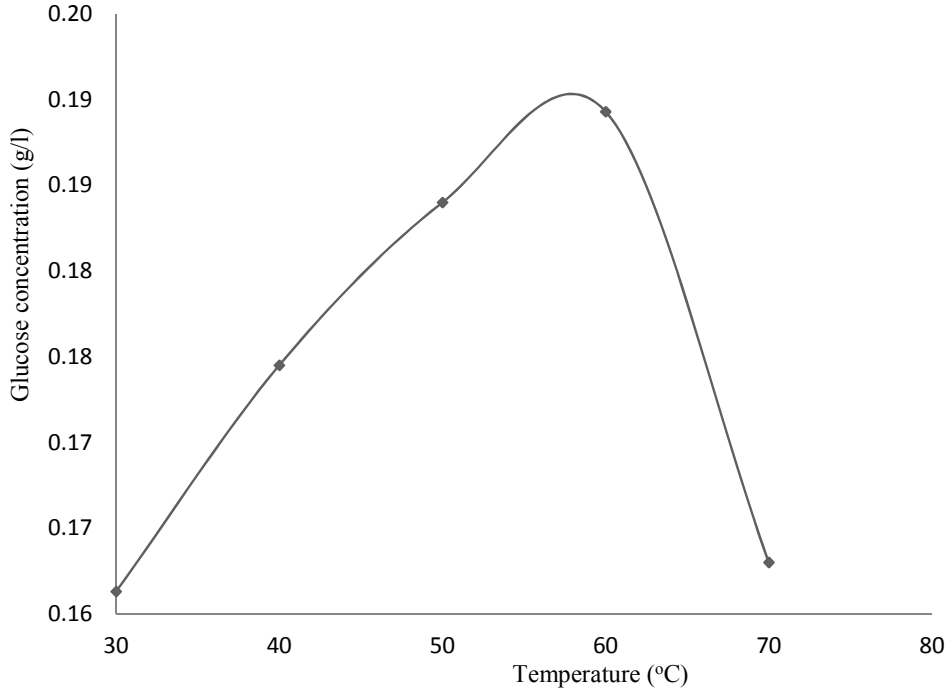

Fig. 2. Effect of temperature on *A. niger* amylase activity

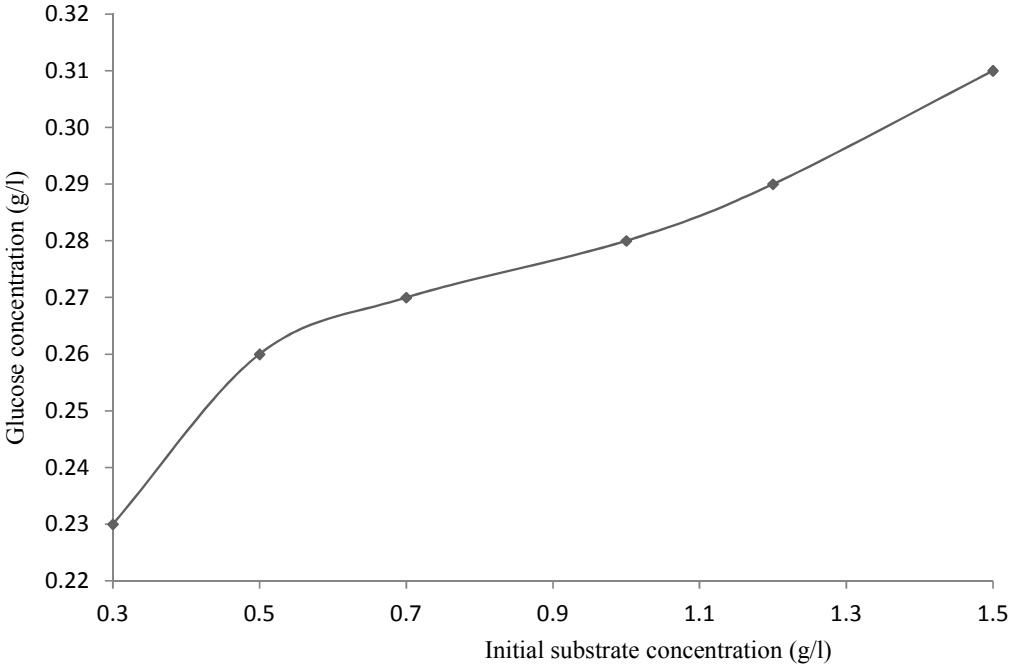

Fig. 3. Effect of initial substrate concentration on *A. niger* amylase activity

4. CONCLUSION

Although α- amylase is known for its impressive starch hydrolyzing potential, the conditions required to optimize its activity strictly relies on its source. Thus, this research has armed operators of enzyme based industries with tangible information required to boost productivity and profits.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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