Optimization of *Aspergillus niger* α-amylase activity for enhanced Glucose production from Cassava starch

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 30-80^{0}C, substrate concentration 0.3-1.5g/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 from starch as well as accessory products.

Keywords: *Aspergillus niger,* cassava, starch and α-amylase

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 nutrient in addition to its ability to survive drought [1]. Its impressive potential to convert large amount of solar energy into soluble carbohydrates per unit area has earned it elevated placement among other crops with similar potential. With the world cassava production estimated 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 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 almost an unlimited resource material employed in the activities of the food industries where about 54% of the starch produced globally is utilized and the remaining 46% utilized in the non-food industries such as textile,

cosmetics, plastics, adhesive, paper and pharmaceutical industries [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 (150^{o}C-230^{o}C) 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 use 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]. It belongs to the family of endoamylases. Although, α-amylase can be derived from different sources such plants, animal and microorganisms, Microbial sources of this industrial enzyme is adjudged the most ideal owing to its economical bulk production capacity in addition to the facts 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 100^0C), 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 (90-100^0C), 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 the effort sustain uninterrupted supply of raw materials for the pharmaceutical industries and others alike.

MATERIALS AND METHODS

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 obtained by draining boiled small pieces of fresh tuber into a sterile bottle under aseptic condition until use.

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 30 ^{o}C 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.

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.

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.

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 min at 120 rpm. The mixture was filtered through cheese cloth before being centrifuged at 8000 rpm for 15min at 4^{o}C. 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.

Determination of Amylase activity

To a test tube holding 1ml of dissolved cassava starch, 2ml of phosphate buffer was introduced into test tubes after, which 1% NaCl was included. The content was thoroughly mixed before being incubated for 5mins at 37^{o}C 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 37^{o}C. After incubation, 1ml of 2N NaOH was added to the test tube. The reducing sugars liberated were assayed calorimetrically by the addition of 1ml Dinitrosalicyclic acid (DNS) reagent. The contents of the test tube were mixed well and incubated in boiling water bath for 10 minutes. The intensity of the colour developed was read at 520nm. 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 IU per fermented substrates.

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 30-80^{o}C, substrate concentration 0.3-1.5g/l.

Results and Discussion

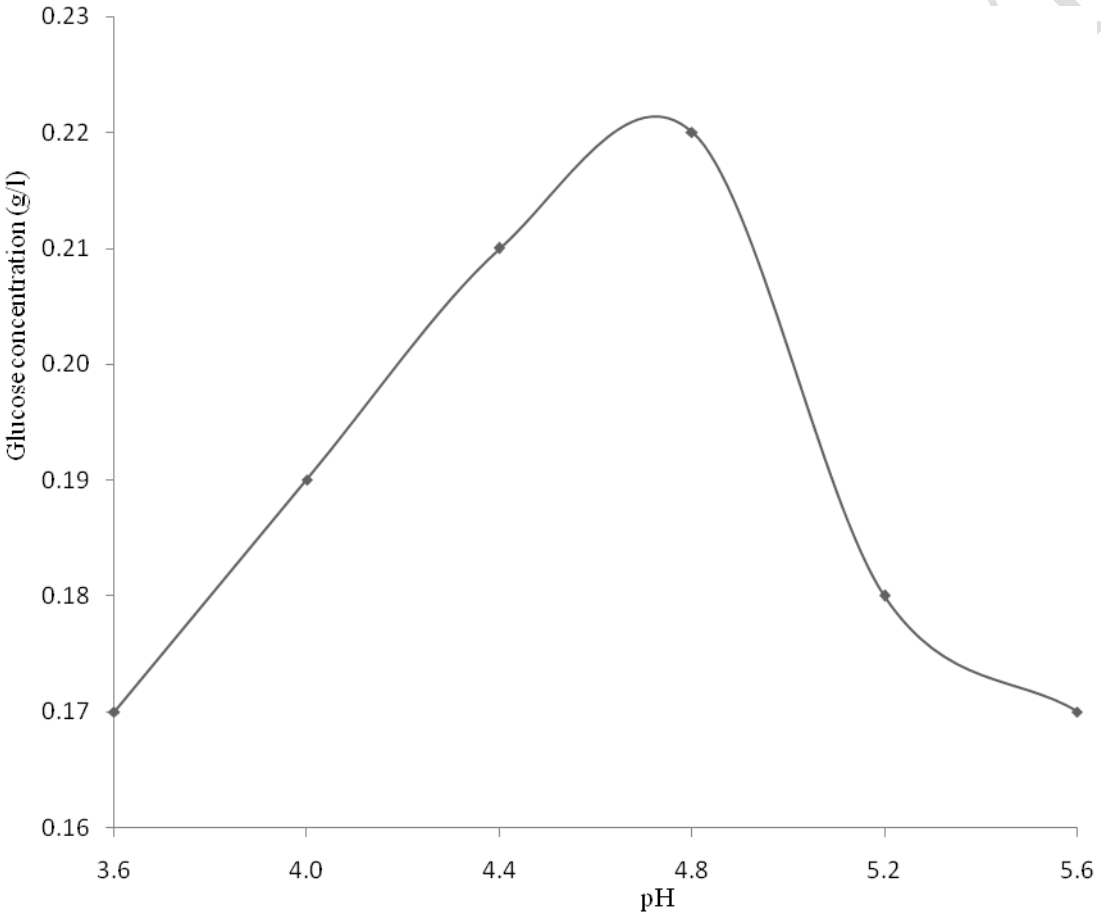

Figure 3: Effect of pH on *A. niger* amylases activity

Hydrolytic activity of *A. niger* α-amylases on cassava starch at varying pH of the reaction medium

Fig 3.0 shows the hydrolytic activity of *A. niger* α-amylases 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 are 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. This finding is in tandem with the report of the Worthington Biochemical Corporation [14] which affirms that extremely high or low pH values generally results in complete loss of activity for most enzymes.

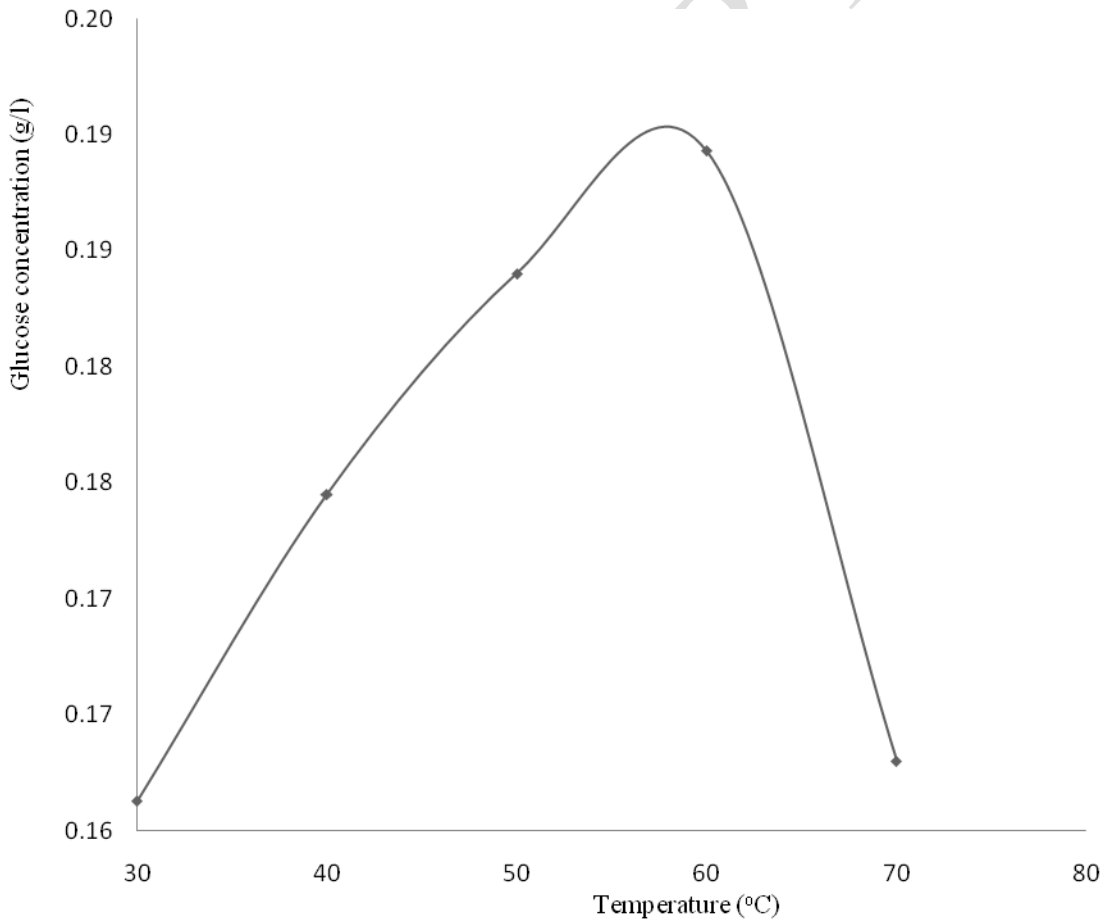

Figure 4: Effect of temperature on *A. niger* amylases activity

Hydrolytic activity of *A. niger* α-amylases on cassava starch at varying temperatures of the reaction medium

Figure 4.0 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^{o}C to 80^{o}C. It was observed that enzyme activity increased progressively with increase in temperature. At 60^{o}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.* [15] which reported appreciable concentration of glucose in enzymatic hydrolysis of starch at reaction temperature of 50^{o}C and 60^{o}C. However, a persistent decline in enzyme activity was observed at temperatures above 60^{o}C. 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*. [16] which has demonstrated that amylase derived from *Heliodiaptomus viduus* lost its catalytic activity at the temperature of 70^{0}C.

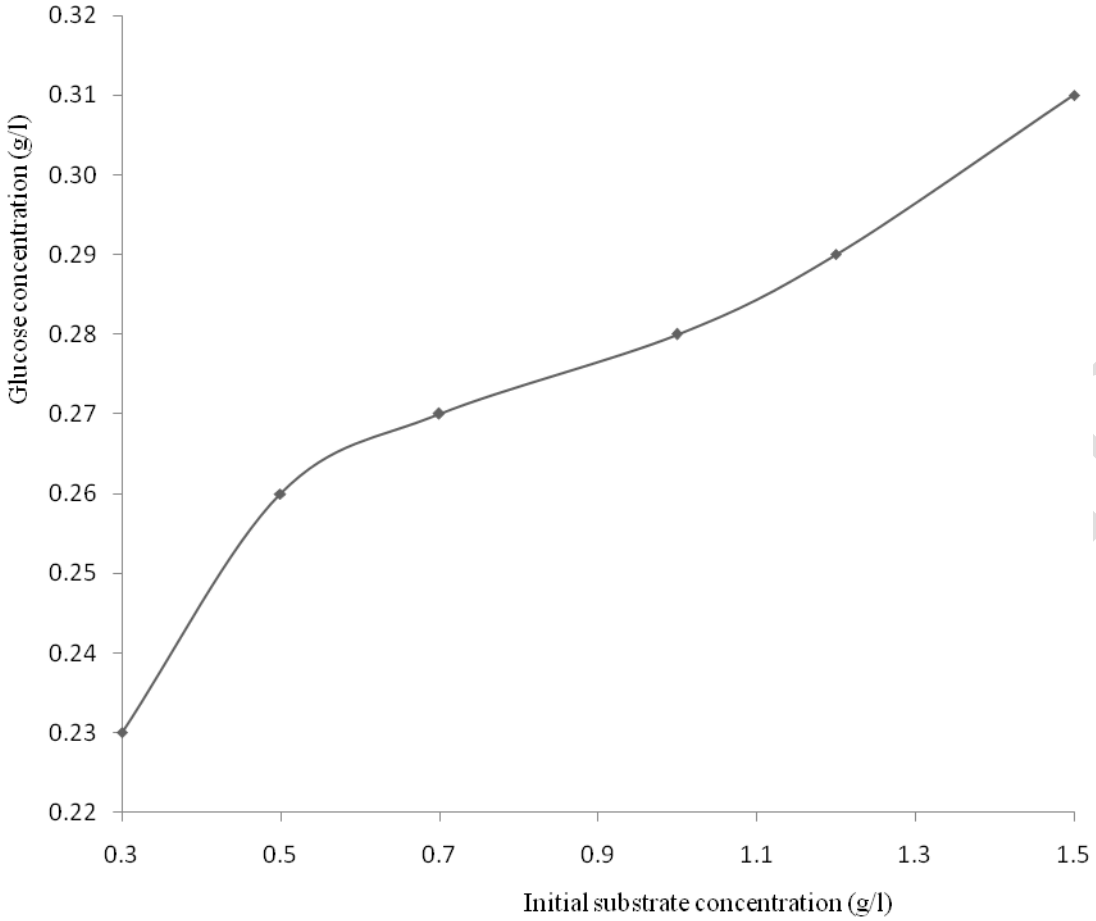

Figure 5: Effect of initial substrate concentration on *A. niger* amylases activity

Hydrolytic activity of *A. niger* α-amylases on cassava starch at varying substrate concentrations of the reaction medium

Figure 5.0 shows the hydrolytic activity of *A. niger* α-amylases 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 increase in glucose concentrations was driven by a concomitant increase in substrate concentration with maximum increase in glucose concentrations (0.31g/l) recorded at the substrate concentration of 1.5g/l. This is in conformity with the report of Worthington Biochemical Corporation [14] 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.

Conclusion

Although α- amylase is known for its impressive starch hydrolysing 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.

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