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Full Length Research Paper

Partial purification, characterization and hydrolytic activities of amylases from *Bacillus licheniformis* and *Aspergillus niger* cultured on agricultural residues

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α-Amylase and amyloglucosidase produced by amylolytic *Bacillus licheniformis* and *Aspergillus niger* isolated from plantain and yam peels media were partially purified and characterized. Following cultivation of the microbial isolates on the agricultural residue media, crude enzyme solutions were obtained by filtration and centrifugation techniques. Crude α-amylase and amyloglucosidase produced were partially purified with salting-out process, involving ammonium sulphate, after which their pH and thermal characteristics were determined using standard biochemical methods. Partially purified amylases were subsequently employed in hydrolysis of each of starch samples of cassava, maize, sorghum and millet prepared in the laboratory. The results show the specific activity, pH and thermal stabilities of the α-amylase to be 32.75 U, 5.6 to 7.0 and 20 to 60°C, respectively. Partially-purified amyloglucosidase exhibited a specific activity of 2.93 U, pH (4.2 to 5.6) and thermal stabilities (20 to 50°C). Hydrolysates of the four starch samples were also found to be mainly maltose and glucose as revealed on the TLC plates.

Key words: α-Amylase, amyloglucosidase, partial-purification, hydrolysate, starch.

INTRODUCTION

Enzymes known as biocatalysts are favoured over chemical catalysts to improve efficiency of industrial processes. Specificity, lower temperature requirement, ease of application and absence of undesirable bye products are some of the reasons for the preference of the former over the latter (Wiseman, 1985). Plant and animal tissues as well as microbes remain the major sources of enzymes. Many enzymes have found applications in major food processing industries like winery, distillery, bakery, breweries and meat processing industry (Wiseman, 1985; Berry and Patterson, 1990).

Of these enzymes, amylases are known to have an array of applications in the food industry. Industrial production of dextrose powder and dextrose crystals from starch started in 1959. Since then, amylases have been employed in many other industrial processes such as conversion of starch into sugar, dextrin and sugar syrup. These starch hydrolysates can be employed in fermentation processes and as sweeteners in a number of manufactured food products and beverages. Other products of amylases-catalyzed reactions are maltose, high-fructose corn syrup (HFCS), oligosaccharides mixture, maltotetraose syrup, anomalously linked oligosaccharides (Alo mixture). In addition they are used in the removal of starch sizer from textile (desizing), direct fermentation of starch to ethanol, production of high energy foods (Alexander, 1998; Aiyer, 2005). α-Amylase [EC 3.2.1.1] is characterized by its random hydrolysis of α– 1, 4 – glucosidic bonds in amylose and amylopectin molecules of starch while α– 1, 6 – links in amylopectin molecules are resistant to attack. Maltose sugar is usually not attacked while higher molecular weight oligosaccharides of the same homologous series are slowly attacked (Whitaker, 1994; Radley, 2007; DeMoraes, 1999). When it attacks amylose molecule, there is an initial formation of lower molecular weight dextrins and smaller amounts of oligosaccharides including maltose. These

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in turn are further split until nothing remains except small oligosaccharide molecules (maltose and maltotriose). A negligible amount of glucose is produced at this stage. The maltotriose may subsequently be cleared to maltose and glucose, which are ultimate products of exhaustive α-amylase action on amylose (Whitaker, 1994; Radley, 2007; Shaw, 1995). Action of α-amylase on amylopectin follows a pattern similar to that of amylose except that products of complete hydrolysis includes low molecular weight oligosaccharides containing at least one branching point besides maltose and glucose. The appearance of such oligosaccharide in the final hydrolysate is due to the inability of α-amylase to attack α–1, 6– glucosidic bond or the α–1, 4– links in their immediate vicinity (DeMot et al., 1984).

Amyloglucosidase [EC 3.2.1.3], on the other substantially converts starch to glucose. It does this by removing successively glucose units from the non-reducing end of amylose or amylopectin molecules principally by hydrolysis of the α-1, 4 glucosidic bonds. Initial rates of hydrolysis of amylose and amylopectin by pure amyloglucosidase differ considerably. It is faster with amylopectin because of availability of more non-reducing chain ends at the onset (Fleming, 1968; DeMoraes et al., 1999). The main immediate product of hydrolysis is β-glucose. Thermal, pH and kinetics properties of α-amylase, β-amylase and amyloglucosidase derived from microbes have been reported (Pestana and Castillo, 1985; Eke and Oguntimein, 1992; Dobreva et al*.,* 1994; Rama Mohan Reddy et al., 1998).

Search for local sources of these enzymes formed the fulcrum on which this study is based. Adeniran and Abiose (2007, 2009) and Adeniran et al. *(*2008) reported the amylolytic organisms isolated from agricultural residues common in Nigeria. In the present study, we report the partial purification and characterization of α-amylase and amyloglucosidase produced from *Bacillus licheniformis* and *Aspergillus niger* respectively isolated from and later cultured on agricultural residues. This is done with a view to suggest areas where they can find applications.

MATERIALS AND METHODS

Organisms and growth conditions

Bacillus licheniformis and *Aspergillus niger* were selected among the other isolates for their propensity for production of high level of α-amylase and amyloglucosidase. The isolates were kept on Nutrient agar and Potato Dextrose agar slants respectively and stored at 4 ± 1°C in a refrigerator (Akinyosoye et al*.,* 2004)

Production of α-amylase

Submerged fermentation method was used for enzyme production in *B. licheniformis*. Cultivation medium for enzyme production contained 0.5% dry waste powder, 0.5% yeast extract, 0.2% $MgSO_4$, 0.05% K_2HPO_4 and 0.01% $CaCl_2$ (w/v) – modified form of the medium employed by Eke and Oguntimein (1992). Bacterial suspension containing approximately 10^5cfu/ml cell density was inoculated into sterile fermenting medium in all the flasks except control flasks. A 24 h-old bacterial culture was used in each case. The inoculated flasks were incubated for 72 h at 37 ± 1°C in an orbital shaker incubator set at 200 rpm.

Preparation of crude α-amylase

All the flasks were cooled to 4°C after which the solid portion was removed by centrifugation at 12,000 x g for 12 min at 4°C in "Beckman Optima LE-80K" ultracentrifuge. The supernatant served as the crude enzyme (Rani et al., 1994)

Production of fungal amyloglucosidase

Solid state fermentation technique was employed for cultivation and amyloglucosidase production in *Aspergillus niger.* Cultivation medium consisted of 2 g dry wastes, and distilled water 3 g. Dissolved in the distilled water were peptone; 0.2%, ammonium sulphate; 0.3%, potassium dihydrogen phosphate 0.1%, magnesium sulphate heptahydrate; 0.03%, calcium chloride; 0.03% (w/w) (Ali et al., 1989). The ratio of waste to water was according to Ramadas et al*.* (1996) method. The wet medium was then sterilized in an autoclave. After cooling, each flask was inoculated with a spore suspension of approximately 10^5 spores/ml prepared from 7-day-old fungal cultures.

Preparation of crude fungal amyloglucosidase

Following incubation, cultivation waste medium was mixed with distilled water in the ratio of 1:9 (w/v) and agitated at 30°C for 30 min as used by Kuhad and Singh (1993) and Ramadas et al*.* (1996). The mixture was subsequently cooled to 4°C for 1 h before being centrifuged at 5000 x g for 30 min. Enzyme activities were assayed for and expressed per ml of extract.

Partial purification of amylases

The crude extract was thereafter added ammonium sulphate to 15% saturation and left standing for 1 h (Eke and Oguntimein, 1992). Precipitated protein was separated by centrifugation at 5,000 x g at 4°C and discarded. The supernatant was then added ammonium sulphate up to 30% saturation and the precipitate collected by centrifugation at 10,000 x g for 40 min (Yun et al. 2001). The precipitated protein was then scooped into treated dialysis bag in which dialysis against sodium acetate buffer (pH 5.5 and 4.0 for bacterial ∀-amylase and fungal amyloglucosidase, respectively) took place. More enzymes were complexed out of the supernatant by addition of more ammonium sulphate to 65%. The amount of ammonium sulphate added was obtained from the table prepared by Green and Hughes (1955) .The resulting precipitated protein, which was recovered as described above was also scooped into the dialysis bag. Dialysis was carried out overnight until the dialysate was free from ammonium sulphate. Periodically, dialysate was tested with barium chloride (Eke and Oguntimein, 1992). This was repeatedly done until the dialysate was not showing milkiness. Partially purified enzyme was freeze dried and kept sealed in a refrigerator until required (Somiari and Balogh, 1995).

Enzyme Assay

Methods of Miller (1959) and Adeniran and Abiose (2009) which

involves use of DNSA reagent was employed. Essentially, it involved estimating the amount of reducing sugar produced by the activity of the enzyme on buffered starch. Preparation of buffer and soluble starch substrate was as reported by Demoraes et al. (1999). The substrate for assay was a mixture of 0.5 ml of 0.5% soluble starch, 0.2 ml of sodium acetate buffer (pH 5.5) and 0.3 ml of enzyme extract incubated at 40°C for 30 min in a water bath. The method of Ramadas et al. (1996) was used. The buffer used was 0.02 M sodium acetate (pH 4.0). To 0.4 ml of this was added 0.5 ml of 1% soluble starch. To the mixture was added 0.1 ml of enzyme solution after which the reacting mixture was thoroughly mixed. Incubation was thereafter done for 10 min in a water bath set at 60°C. The resulting reducing sugars in each case were then estimated by Miller method (1959). DNSA (colorimetric) method as used by Miller (1959) was thereafter employed for estimation of reducing sugars produced. 1 ml of DNSA solution was added to the mixture and boiled for 5 min. 4 ml of distilled water was introduced after cooling before absorbance is read at 540 nm in a "Cecil 3041"spectrophotometer. Blank that consisted of 0.3 ml distilled water, 0.5 ml of 0.5% soluble starch, 0.2 ml of buffer received similar treatment. The amount of reducing sugars present in the enzyme in each inoculated flask and in the cultivation medium in uninoculated flask were also determined by adding appropriate amounts of DNSA and distilled water for appropriate corrections (Abiose et al., 1982). One unit of α-amylase activity was the amount of enzyme that produced one micromole of glucose per minute under experimental conditions while one unit of amyloglucosidase activity was the amount of enzyme that liberated one micromole of reducing sugars measured as glucose per minute under experimental conditions.

Estimation of protein contents of crude and partially purified amylase and amyloglucosidase

Protein contents of crude and partially purified bacterial α-amylase and fungal amyloglucosidase was determined by using the method of Lowry et al. (1951) using bovine serum albumin (BSA) as standard. Absorbance of standards and samples were determined at 750 nm in a "Cecil 3041 "spectrophotometer.

Optimal temperature of activity and thermal stability

Effect of temperature on the activities of partially purified bacterial α-amylase and fungal amyloglucosidase were studied between 30 and 80°C using the method of Srivastava (1987). This was carried out by incubation of a mixture of appropriate buffer, enzyme and 1% soluble starch solution at different temperatures. At intervals of 15 min, reaction was stopped and the amount of reducing sugars produced was determined. The thermal stability of partially purified bacterial α-amylase was determined by incubating it in 0.5 M sodium acetate buffer (pH 5.5) at various temperatures (20 to 80°C) without substrate for 2 h. At intervals of 15 min of incubation at 60, 50, 40, 35, and 20°C respectively aliquots of the incubated α-amylase were assayed for residual activity using standard conditions (Eke and Oguntimein, 1992). Fungal amyloglucosidase was incubated in 0.02 M sodium acetate buffer (pH 4.0) after which it was given the same set of treatments as bacterial α-amylase.

Optimal pH of activity and stability

Optimal pH for activity was studied by measuring the activities at various pH values (4.0 to 8.0) and optimum temperature while maintaining other conditions at a constant. The effect of pH on the stability of partially purified α-amylase and amyloglucosidase was carried out by incubating the enzymes without substrate at 20°C for 2 h in a fixed amount of buffer solutions of various pH values (4.2, 5.6, 6.0, 8.0, and 9.4). At 15 min intervals aliquots of 1.0 ml from each buffered enzyme sample was assayed for residual activity at standard conditions (Eke and Oguntimein, 1992). Varying pH was achieved by using different buffers. pH 4 to 6 was attained with (citrate – phosphate buffer 50 mmol/L), and pH 7 to 8 (phosphate buffer 50 mmol/litre), pH 9 to 10 (borate buffer) (William and Chase, 1968).

Extraction of starch samples from cassava, maize, sorhgum, and millet

The methods of Bruisma et al. (1983), Ajibola (1988) and Almazan (1988) shown in Figure 1 were employed to obtain good quality cassava starch from freshly harvested tubers of *Manihot utilissima.* The methods of Watson (1984) and Ji et al. (2004) were employed to obtain starches from maize (*Zea mays* Linn.), sorghum (white variety) (*Sorghum bicolor* L. moerich) and millet (P*ennisetum typhoides* Staff and Hubbard). The grains samples obtained from a local market in Ile-Ife, Nigeria were prepared using the procedure shown in Figure 2.

Identification of hydrolytic products

The types of sugars produced from hydrolysis of 10% soluble starch (of cassava, maize, millet and sorghum) by the amylases were identified with TLC. Bacterial α-amylase (final concentration 32.75 U ml^{-1}) was incubated with 10% of each of soluble starch samples in 0.5M sodium acetate buffer (pH 5.5) for 30 min at 60°C. This was followed by subsequent adjustment of the pH to 4.0 using sodium acetate buffer (pH 4.0) and introduction of fungal amyloglucosidase (2.93 U ml^{-1}) and incubation at 55°C for 30 min. "Eastman Kodak" Chromatogram sheet (pre-coated silica gel adsorbent for thin-layer chromatography was spotted with Sugar standard solutions, 0.5% (w/v) solutions and the hydrolysate while the solvent system was prepared by mixing ethyl acetate, glacial acetic acid, ethanol and distilled water in the ratio of 60:15:15:10. Detection reagent was obtained by mixing naphthoresorcinol (20 mg), ethanol (10 ml of 90% solution), and concentrated sulphuric acid (0.2 ml). Freshly prepared reagent was used for each experiment. The spots were allowed to dry before each plate was introduced into the chromatotank containing the solvent system. Prior saturation of the tank with the solvent system was achieved by lining the inner sides of the tank with filter paper containing the solvent system. The chromatogram sheet was positioned practically upright inside the tank. Development took about one hour. Subsequently, the sheet was removed gently from the tank and allowed to dry for a sufficiently long period of time at room temperature. Freshly prepared detection reagent was then sprayed on the heated plates. Consequent upon this, the sheet was heated at 100°C for 10 min after which defined and distinct coloured spots appeared on the chromatogram sheet against a white background (Rama, 1998).

RESULTS

Purification profile of microbial amylases

The result of the purification profile of the α-amylase and amyloglucosidase is presented in Table 1. Crude culture supernatant of bacterial α-amylase contained about 6.98U of enzyme activity per ml. After ammonium sulphate precipitation and desalting by dialysis, the enzyme preparation contained 32.75U of enzyme activity per ml.

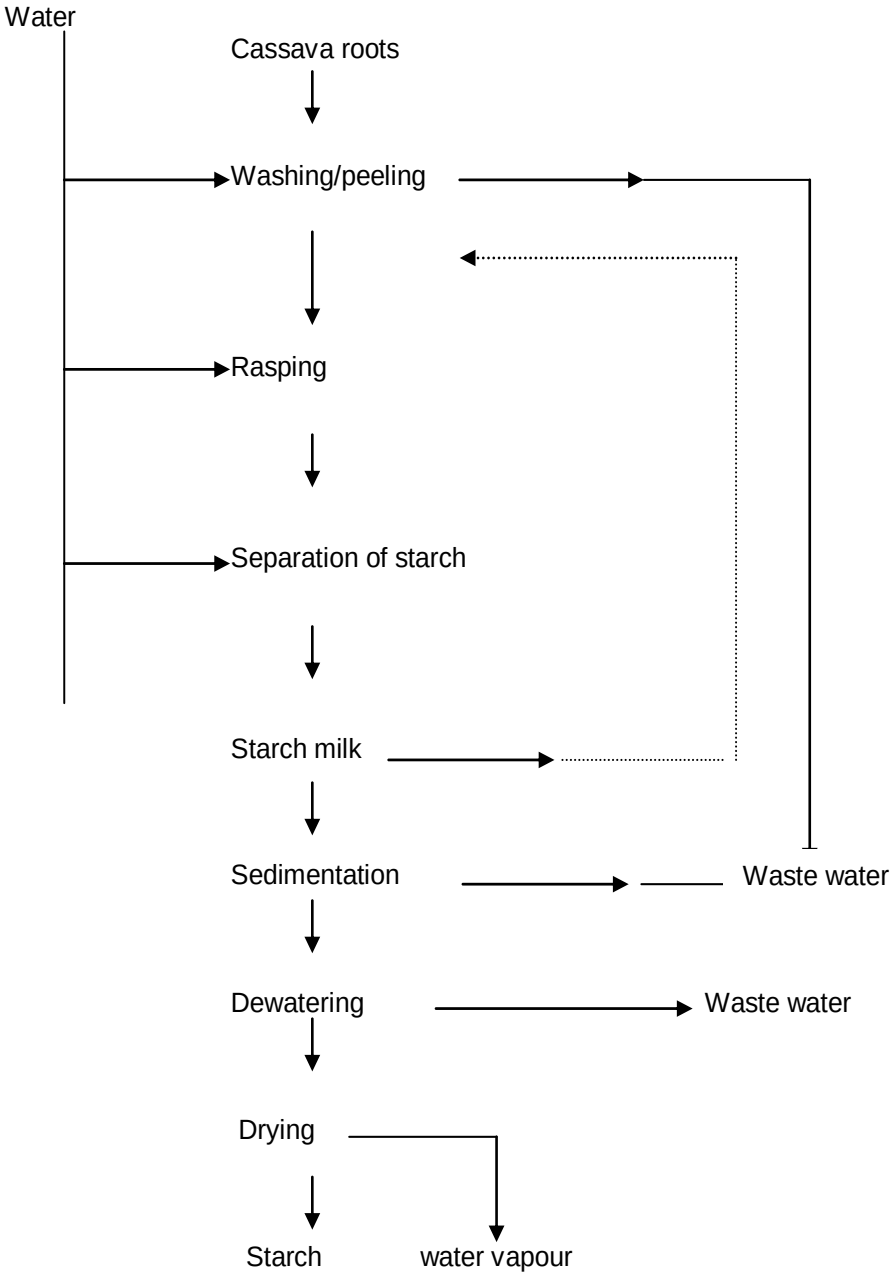

Figure 1. Flow chart for cassava starch extraction. Sources: Bruisma et al. *(*1983), Ajibola (1988) and Almazan (1988).

This achieved a 4.69-fold in purification. Crude culture supernatant of fungal amyloglucosidase exhibited 0.52U of activity per ml while it showed an enzyme activity of 2.93U per ml after ammonium sulphate precipitation and desalting by dialysis. A 5.60-fold purification was attained.

pH stability and activity of bacterial α-amylase

Influence of pH on stability of partially purified α-amylase from *B. licheniformis* is presented in Figure 3. The enzyme maintained much of its stability when incubated at pH 5.6, 6.0, and 7.0 for two hours. At pH 5.6, 1.08% of its activity was lost after 15 min while about 10% was lost after 120 min. A similar trend was observed at pH 6.0 and 7.0. Incubation of the enzyme at pH 4.2 resulted in loss of 18.73, 48.70 and 98% of activity after 15, 60, and 120 min of incubation respectively.

pH activity of partially purified α-amylase from *B. licheniformis* is shown in Figure 3. pH 5.6, 6.0 and 7.0 were observed to favour hydrolysis of starch by the tested partially purified α-amylase as the reaction progressed steadily from 0 to 120th min. Enzyme activity

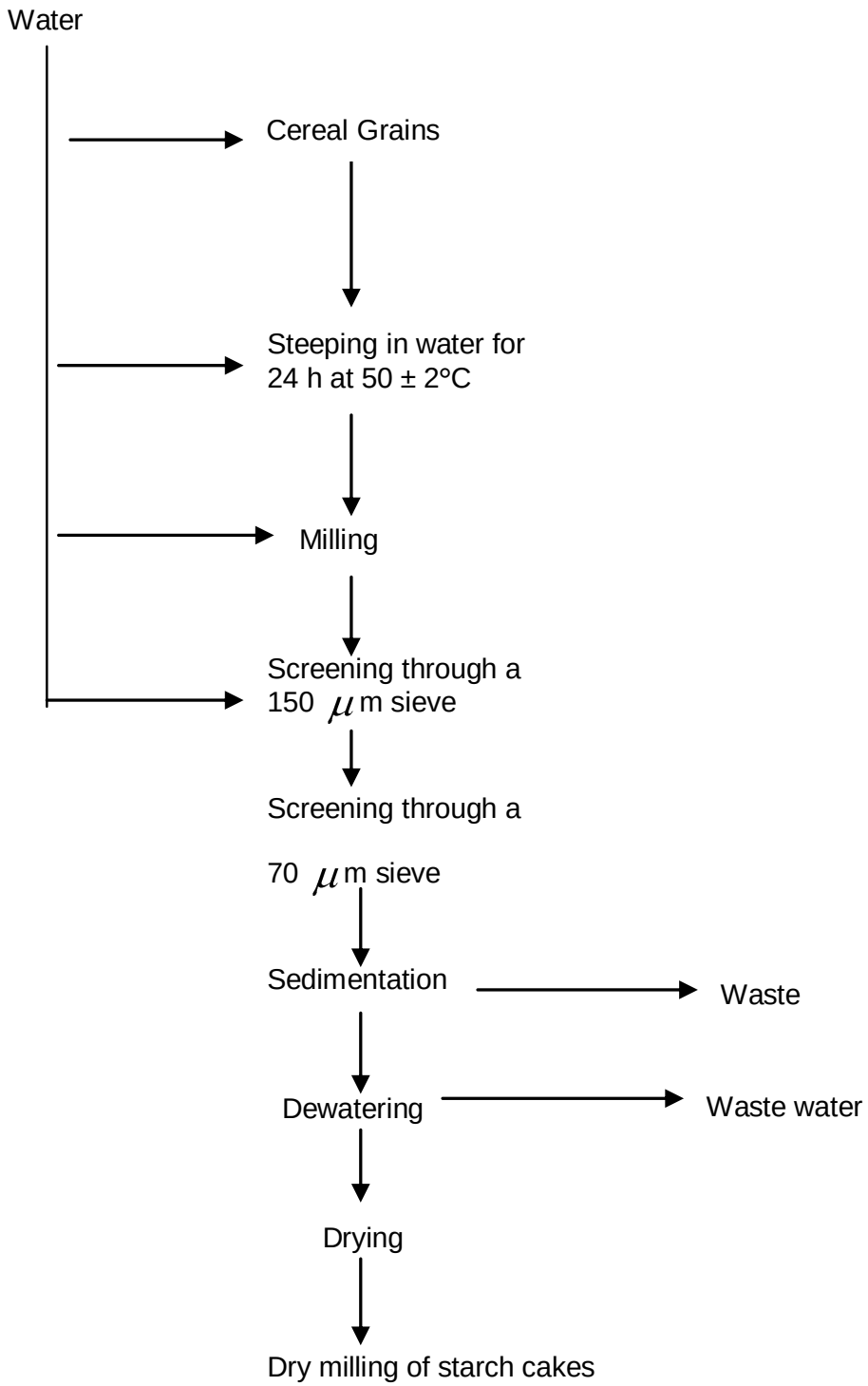

Figure 2. Flow chart for extraction of starches from maize, millet and sorghum grains. Sources: Watson (1984) and Ji et al. (2004).

attained peaks at different times at each of these 3 pH values. At pH 5.6, 6.0 and 7.0, peaks were attained at 105th min, 90 and 105th min, respectively. Enzyme activity also progressed at a similar pattern though at a lesser extent at pH 4.2 and 8.0.

pH stability and activity of fungal amyloglucosidase

pH stability of partially-purified amyloglucosidase from *A. niger* is highlighted in Figures 4 and 5. The enzyme was observed to be relatively stable when exposed to pH 4.2.

Table 1. Purification profile of microbial amylases.

Purification step	Total enzyme activity (U ml^{-1})	Protein (Mg/ml)	Specific activity (U mg protein $^{-1}$)	Yield (%)	Purification fold
Crude ∀-amylase from *Bacillus licheniformis*	22.06 ± 0.12	3.16 ± 0.2	6.98 ± 0.01	100	1.00
Partially-purified α-amylase from *Bacillus licheniformis*	10.81± 0.12	0.33 ± 0.02	32.75 ± 0.01	45.50	4.69
Crude amyloglucosidase from *Aspergillus niger*	10.02 ± 0.02	19.23 ± 0.13	0.52 ± 0.02	100	1.00
Partially-purified amyloglucosidase from *Aspergillus niger*	5.10 ± 0.10	1.74 ± 0.09	2.93 ± 0.01	31.67	5.60

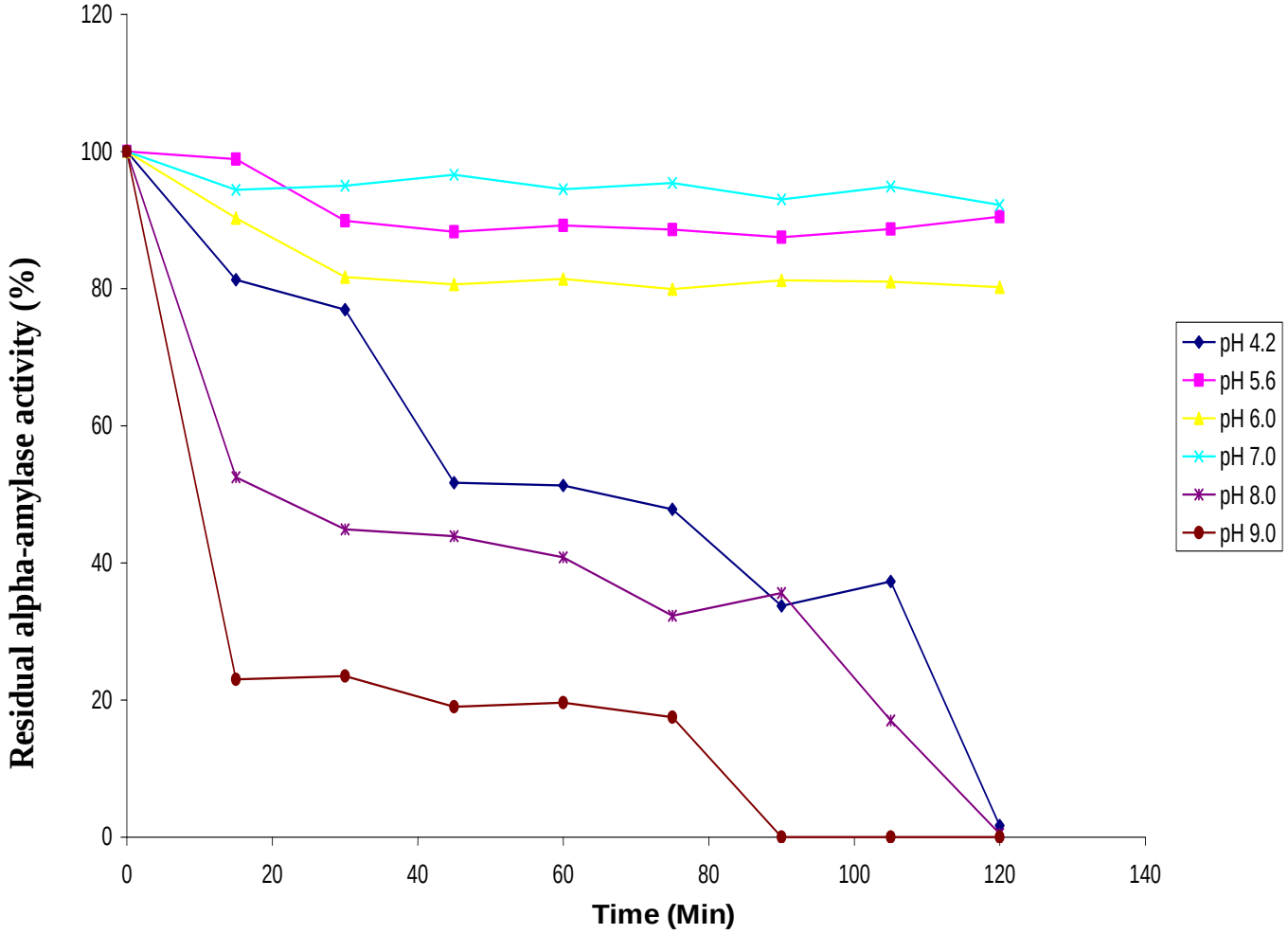

Figure 3. pH stability of partially purified alpha amylase of *Bacillus licheniformis* cultured on plantain peels medium.

After 15 min of incubation, the enzyme lost 27.6% of its activity while it lost 38.9% of its activity after 60 min of incubation. The enzyme retained 56.7% of its activity after 120 min of incubation. At pH 5.6, the enzyme lost 29, 59.5 and 100% of its activity after 15, 60 and 120 min of incubation respectively. At pH 6.0, 7.0, 8.0 and 9.0 much of the enzyme activity was lost after 120 min of incubation.

pH activity of partially-purified amyloglucosidase from *A. niger* is presented in Figure 6. Enzyme activity was observed at pH 4.2, 5.6 and 6.0 while pH 7.0, 8.0 and 9.0 did not favour activity. Peak was attained at 90, 75 and 90th min at pH4.2, 5.6 and 6.0 respectively. After 120 min of hydrolysis, the amount of sugars liberated at pH 4.2,

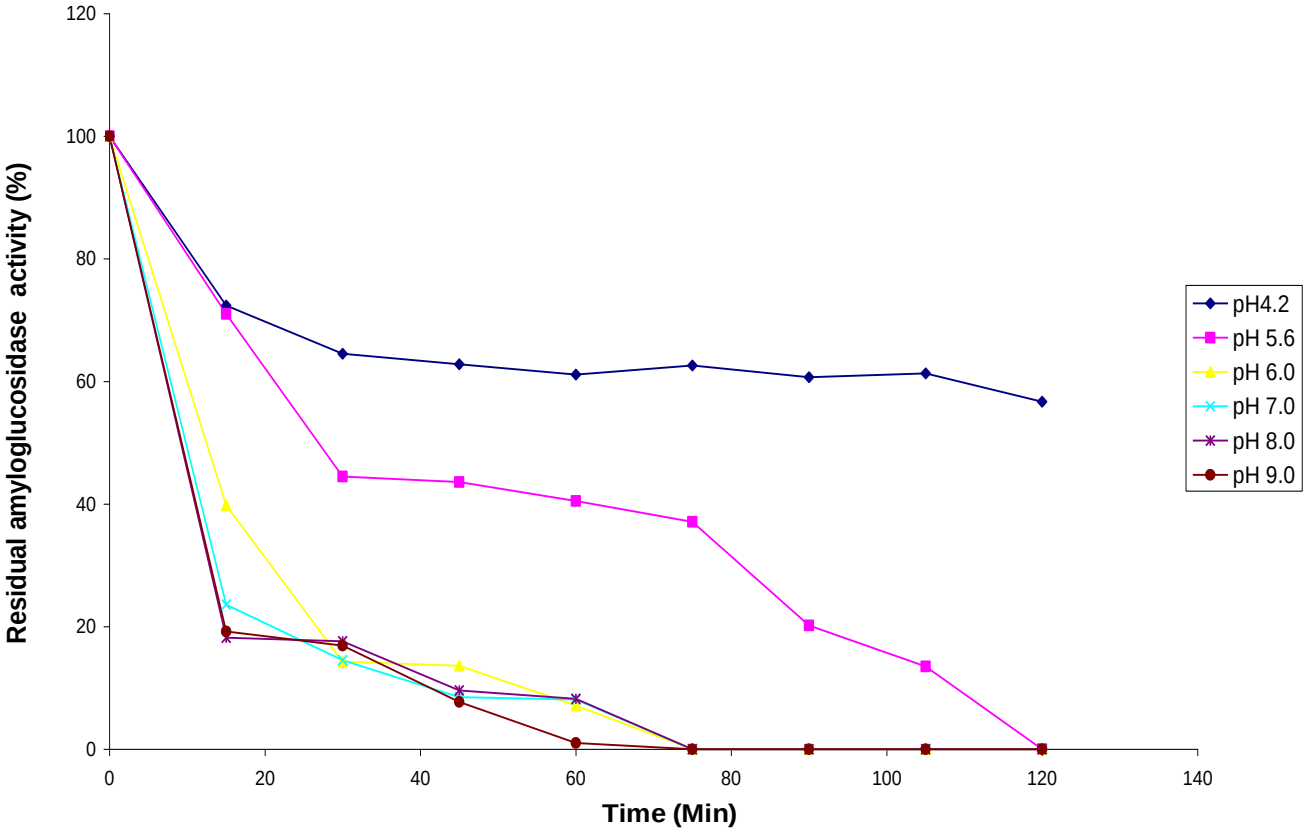

Figure 4. pH stability of partially purified amyloglucosidase of *Aspergillus niger* cultured on yam peels medium.

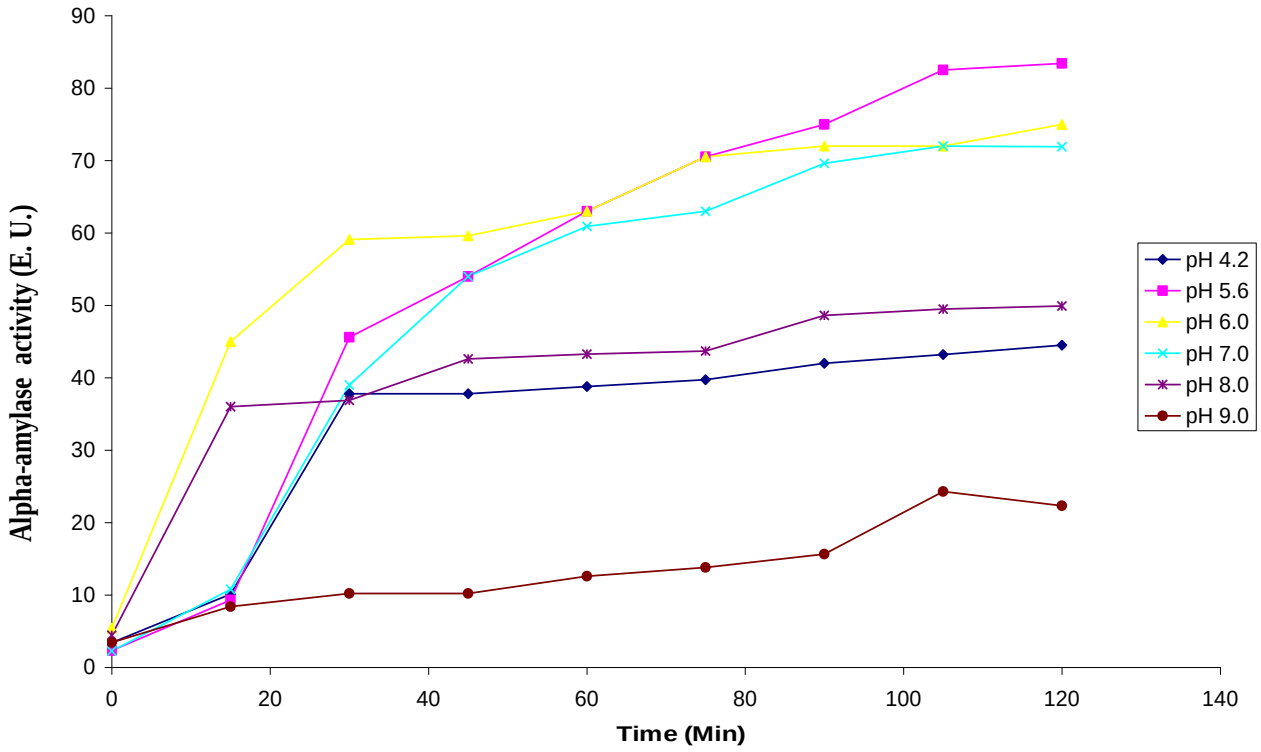

Figure 5. pH activity of partially purified α-amylase of *Bacillus licheniformis* cultured on plantain peels medium.

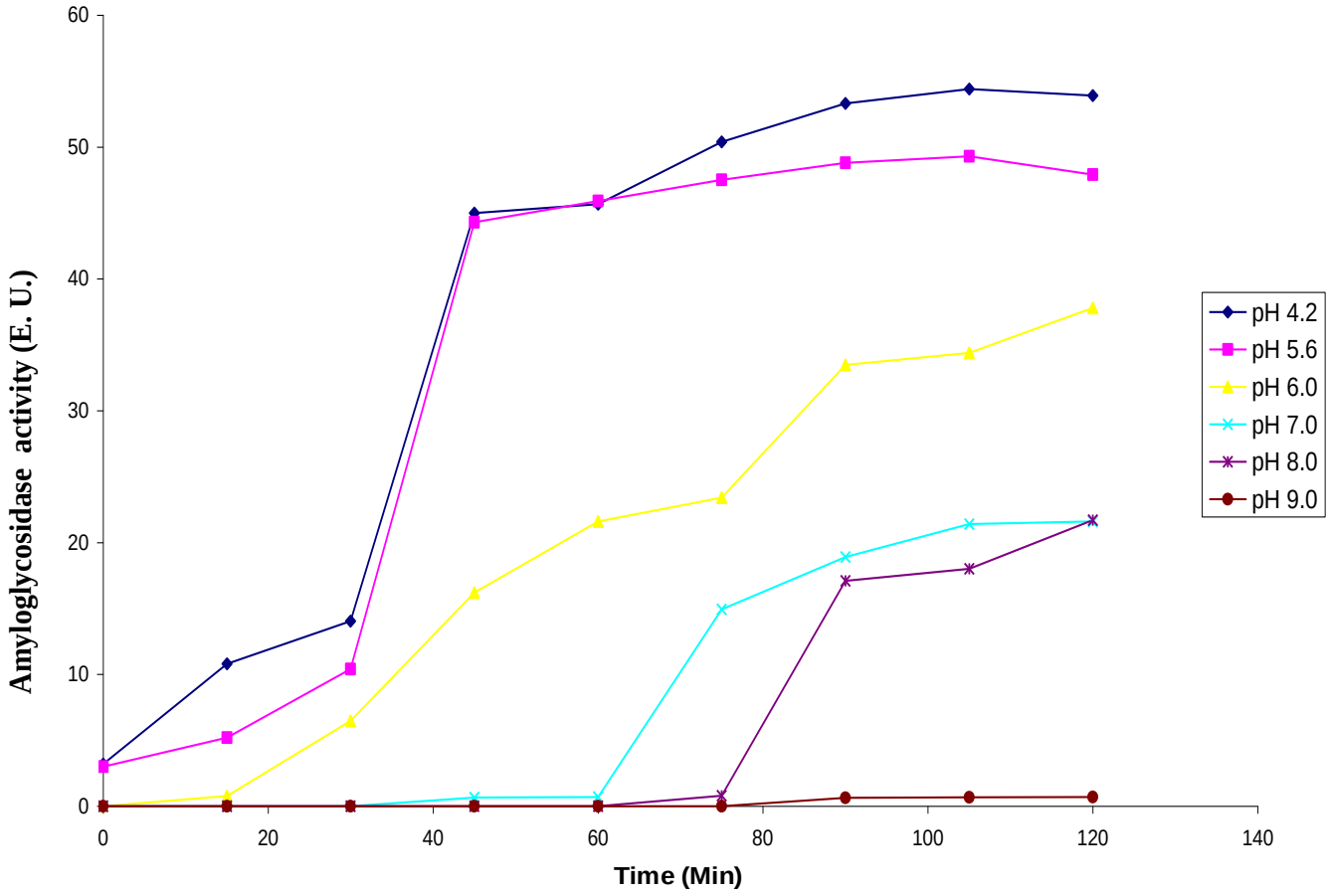

Figure 6. pH activity of partially purified amyloglucosidase of *Aspergillus niger* on yam peels medium.

5.6 and 6.0 showed that the pH values favoured enzyme activity compared to the levels of hydrolysis achieved at pH 7.0, 8.0 and 9.0.

Thermal stability and activity of partially purified bacterial α-amylase

Thermal stability of partially-purified α-amylase from *B. licheniformis* is presented in Figure 7. Much of the enzyme activity was retained at 20 and 35°C of incubation as opposed to that of 40, 50, 60, 70 and 80°C. At 20°C of incubation, 1.70, 15.10 and 18.90% of the enzyme activity was lost after 15, 60 and 120 min of reaction respectively. With 35°C of incubation, 13.40, 14.90 and 29.30% was lost at 15, 60 and 120 min reaction time respectively. At 70°C of incubation, 35.17, 54.09 and more than 80% of the enzyme activity had been lost within 15, 60 and 120 min of reaction respectively. Close to 90% of the enzyme activity was lost after 120 min of incubation at 80°C.

Thermal activity of partially purified α-amylase from *B. licheniformis* is presented in Figure 8. Exposure to incubation temperatures of 60 and 70°C triggered maximum activity of the amylase. Within 15 min, enzyme activity increased from 3.80 and 3.40 E.U. to 14.33 and 19.80 E.U. respectively at 60 and 70°C respectively. After 60 min of reaction, enzyme activity increased to 45.40 and 45.20 E.U., respectively while further increases to 55.21 and 61.21 E.U. respectively were observed after 120 min of reaction. Treatments with temperatures of 20, 35, 40 and 50°C allowed reaction to proceed at lower rates. Treatment with 80°C triggered fast hydrolysis reaction for the first 45 min. Subsequently, the reaction rate dropped from 34.20 E.U. to 29.60 E.U. from 45th to 60th min of reaction. This low reaction rate was maintained for the last 60 min of the reaction.

Thermal stability and activity of partially purified fungal amyloglucosidase

Thermal stability of partially purified amyloglucosidase from *A. niger* is highlighted in Figure 9. For most part of the reaction time, incubation temperature of 20°C resulted in loss of minimum enzyme activity. After 120 min of incubation, the enzyme maintained about 60% of its activity at 20°C. At 35°C, loss in activity of 32.90, 41.40 and 43.80% was recorded after 15, 60 and 120 min of incubation respectively. At higher temperatures of

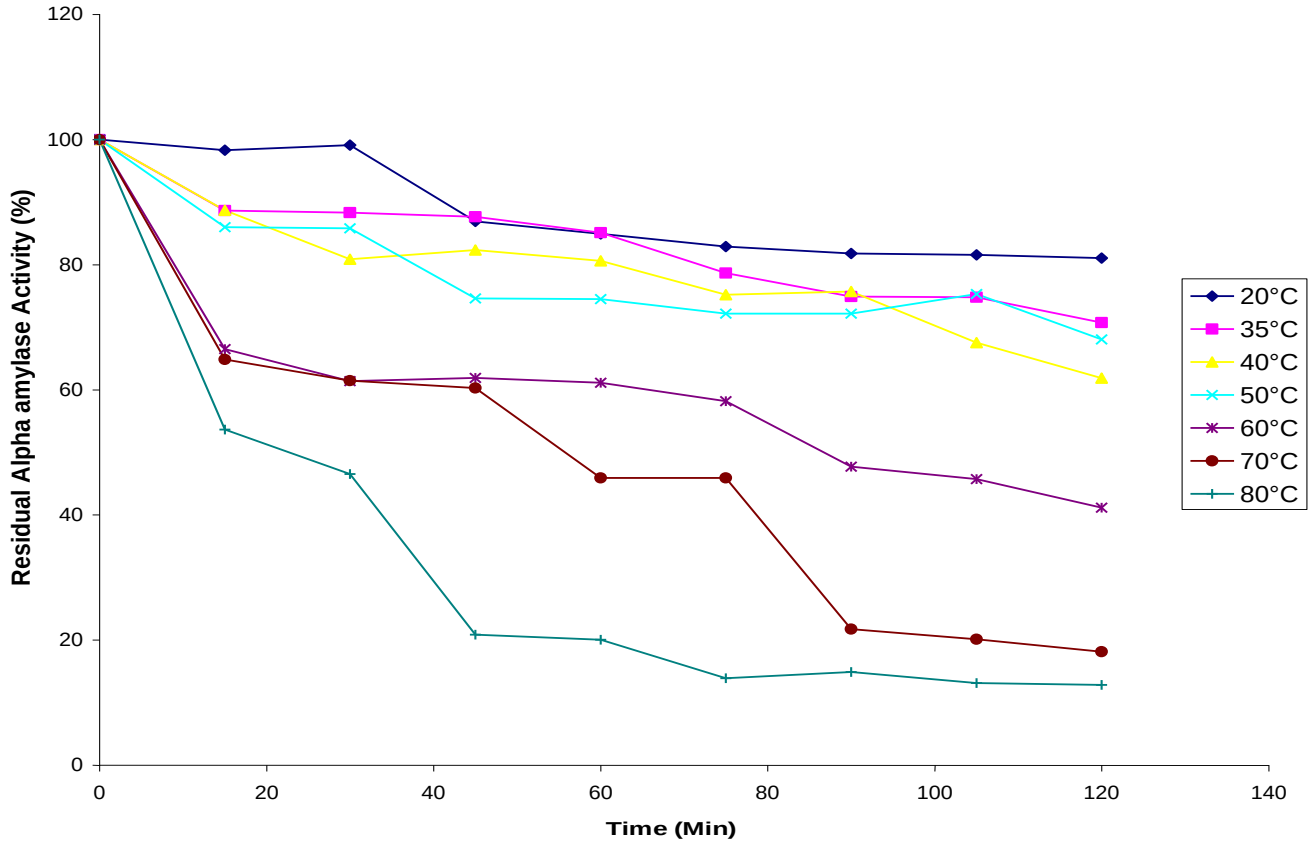

Figure 7. Thermal stability of partially purified alpha amylase of *Bacillus licheniformis* cultured on plantain peels medium.

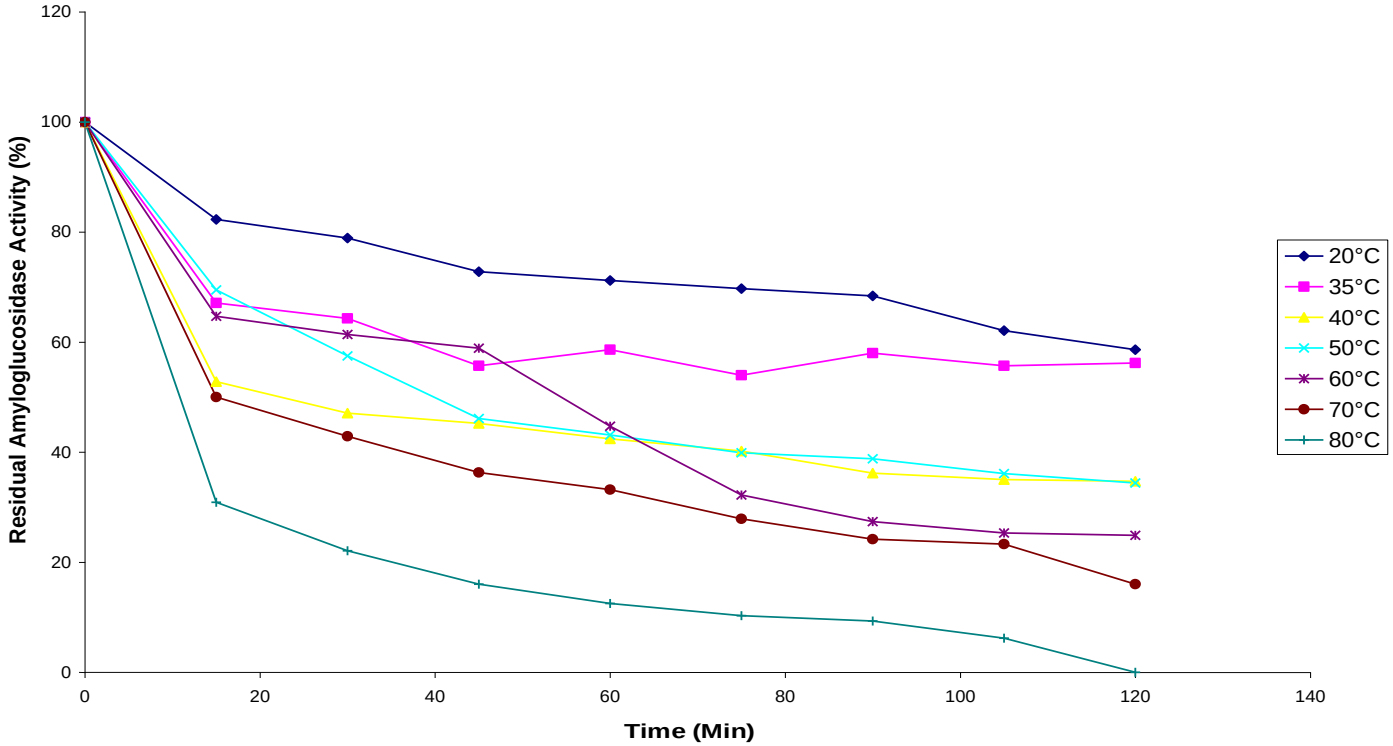

Figure 8. Thermal stability of partially purified amyloglucosidase of *Aspergillus niger* cultured on yam peels medium.

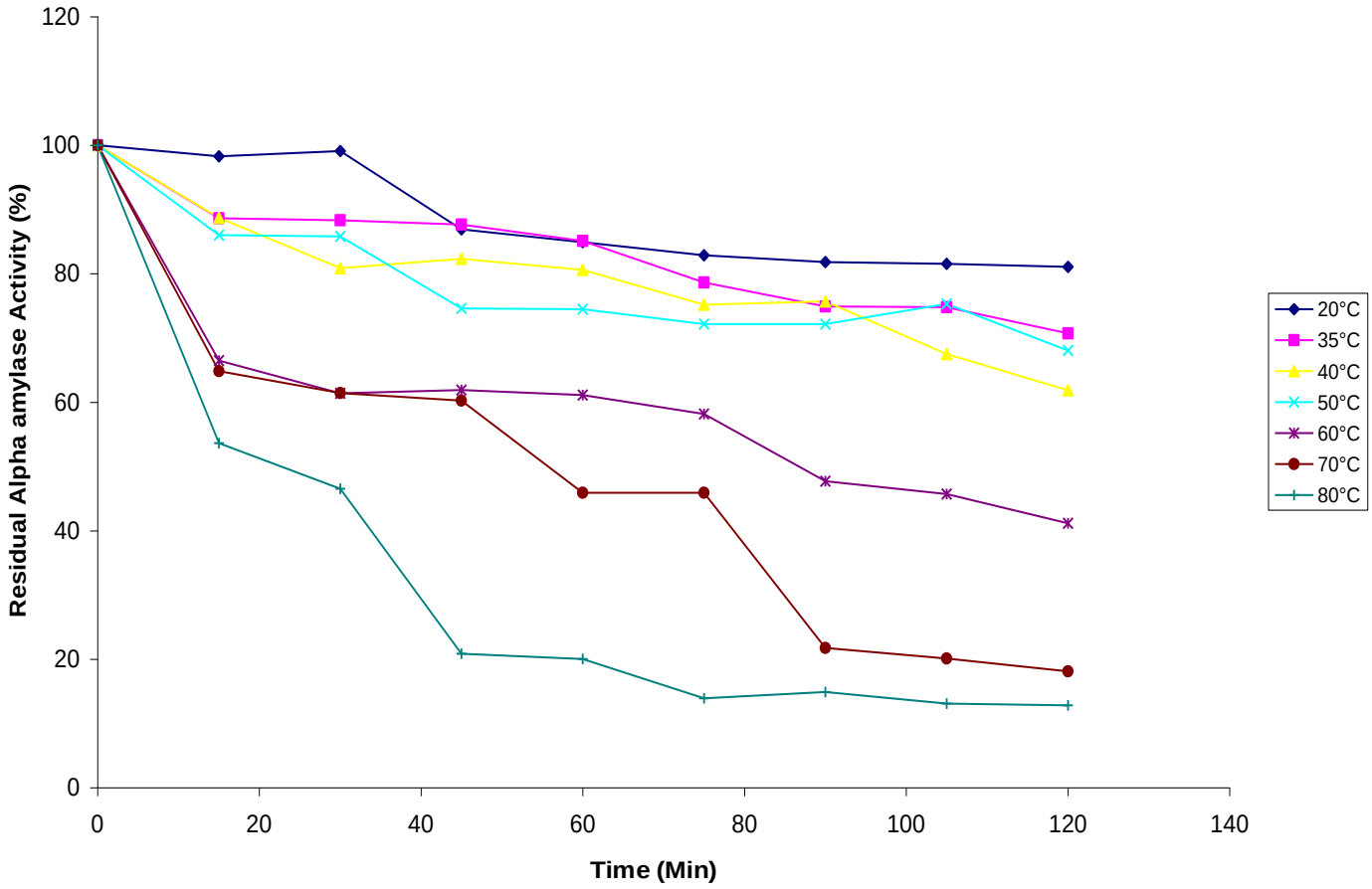

Figure 9. Thermal activity of partially purified α-amylase from *Bacillus licheniformis* cultured on plantain peels medium.

incubation of 60, 70, and 80°C, the loss in enzyme activity was substantial ranging between 60.20 and 100%.

Thermal activity of partially-purified amyloglucosidase from *A. niger* is highlighted in Figure 10. Incubation at temperatures of 50 and 60°C facilitated maximum activity of the enzyme. After 15 min of reaction at 50°C, enzyme activity increased from 13.90 E.U. to 16.89 E.U. while it increased to 27.89 E.U. after 60 min and 43.10 E.U. after 120 min. At 60°C of incubation, the enzyme activity increased from 14.40 to 19.23 E.U within the first 15 min of reaction. Further upsurge in enzyme activity was observed to be 25.90 E.U after 60 min and 42.32 E.U. after 120 min. At incubation temperatures of 20, 35 and 40°C, the enzyme activity was generally low while at 70 and 80°C the enzyme activity increased at initial stage and decreased as the reaction time progressed.

DISCUSSION

pH stability and activity of partially purified bacterial α-amylase

The amylase was most stable at pH 7.0; however, it maintained a moderate stability over a range of pH 5.6 to 7.0. It exhibited optimal activity between pH 5.6 and 6.0. Its activity at pH 7.0 was moderate.

Freer (1983) has elucidated the characteristics of an α-amylase obtained from *Streptococcus bovis* JB1 to include stability at pH 5.5 to 8.5 and optimal pH activity to be between 5.5 and 6.0. Eke and Oguntimein (1992) also reported that a partially purified α-amylase obtained from *Bacillus cereus* showed optimal activity at pH 6.0 to 6.2 while optimal stability between pH 5.7 and 8.0. Lin et al. (1998) also compared the biochemical properties of amylases derived from the genus *Bacillus*. In it, α-amylase derived from *Bacillus licheniformis* possessed an optimal pH of 7.0.

Result obtained from this study is in agreement with most of these submissions. The inference that can be drawn is that if this amylase would be employed in industrial processing the range of pH 5.2 to 7.0 should be maintained. Otherwise the enzyme would not perform satisfactorily. It is equally important to note that the amylase can only be stored within this range of pH. This finding becomes instructive when selecting a carrier for the enzyme as any carrier that will impact pH exceeding the desired range of 5.2 to 7.0 will adversely affect its stability and activity.

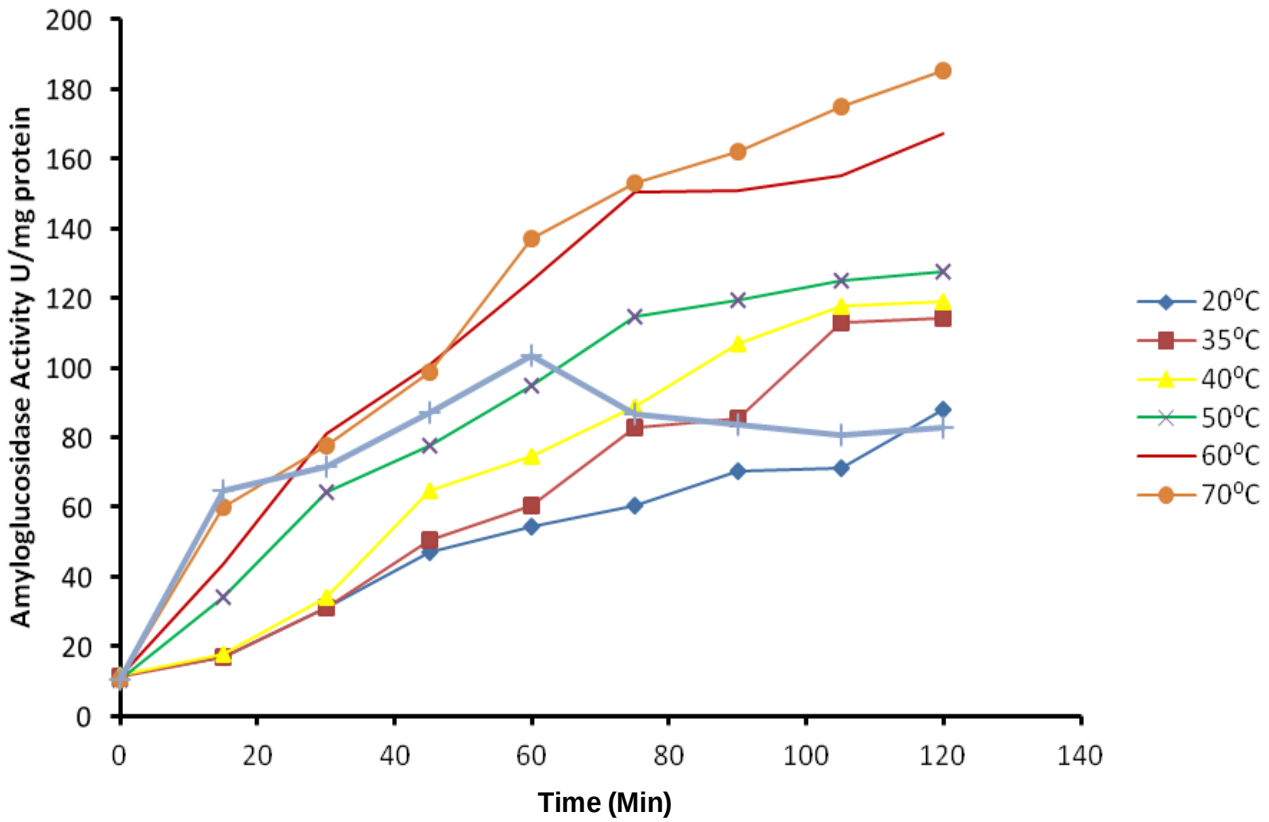

Figure 10. Thermal activity of partially purified amyloglucosidase of *Aspergillus niger* cultured on Yam peels medium.

pH stability and activity of partially purified fungal amyloglucosidase

The enzyme was relatively stable at pH 4.2 though it lost about 43.30% of its activity during the duration of the study. It showed optimal activity at pH 4.2 and 5.6.

Gomes et al. (2005) reported a wide range of pH (5.0 to 9.0) over which glucoamylase produced from *Aspergillus flavus,* maintained stability. In the same work, gluco-amylase from *Thermomyces lanuginosus* showed stability between pH 7.0 and 8.0. Fossi et al. (2005) also submits that a thermostable amylase from ascomycetes yeast strain exhibited stability within a wide range of pH3.0 and 8.0 after incubation at 30°C for 30 min.

The amyloglucosidase tested in this study showed a specific pH of stability. This is at variance with the findings of Gomes et al. (2005) and Fossi et al. (2005).

Gomes et al. (2005) also found optimal pH of activity of the glucoamylase from *Aspergillus flavus* to be 4.0 while that of *Thermomyces lanuginosus* was observed to be 4.5.

The result obtained in this study for optimal pH of activity which was between 4.2 and 5.6 is in consonance with previous findings. Application of the enzyme to industrial setting requires close monitoring since it cannot function over a wide range of pH. The need to apply a buffer system cannot be over emphasized. During storage of the enzyme, this should also be given consideration.

Thermal stability and activity of partially purified bacterial α-amylase

The enzyme was observed to be stable between 20 and 35°C, partially stable at between 40 and 60 °C and not stable above 60°C. Eke and Oguntimein (1992) reported on the thermal stability of a partially-purified amylase from *Bacillus cereus* which was put at about 35°C. The study of Lin et al. (1998) showed the raw-starch-degrading amylase to be thermally stable at 60°C and partially stable at 70 and 80°C.

The result obtained in this study agrees with the findings of Lin et al., (2005). In the course of this study, the optimal temperature of activity of the enzyme was found to be between 60 and 70°C. Eke and Oguntimein (1992) found that α-amylase from *B. cereus* was 50°C. Lin et al. (1998) also reported 60 and 70°C for amylase from *Bacillus* sp TS-23. The α-amylase used in this study exhibited similar properties with that of Lin et al. (1998). This amylase has the advantage of application in processes where relatively high temperatures are required for starch hydrolysis otherwise it would have

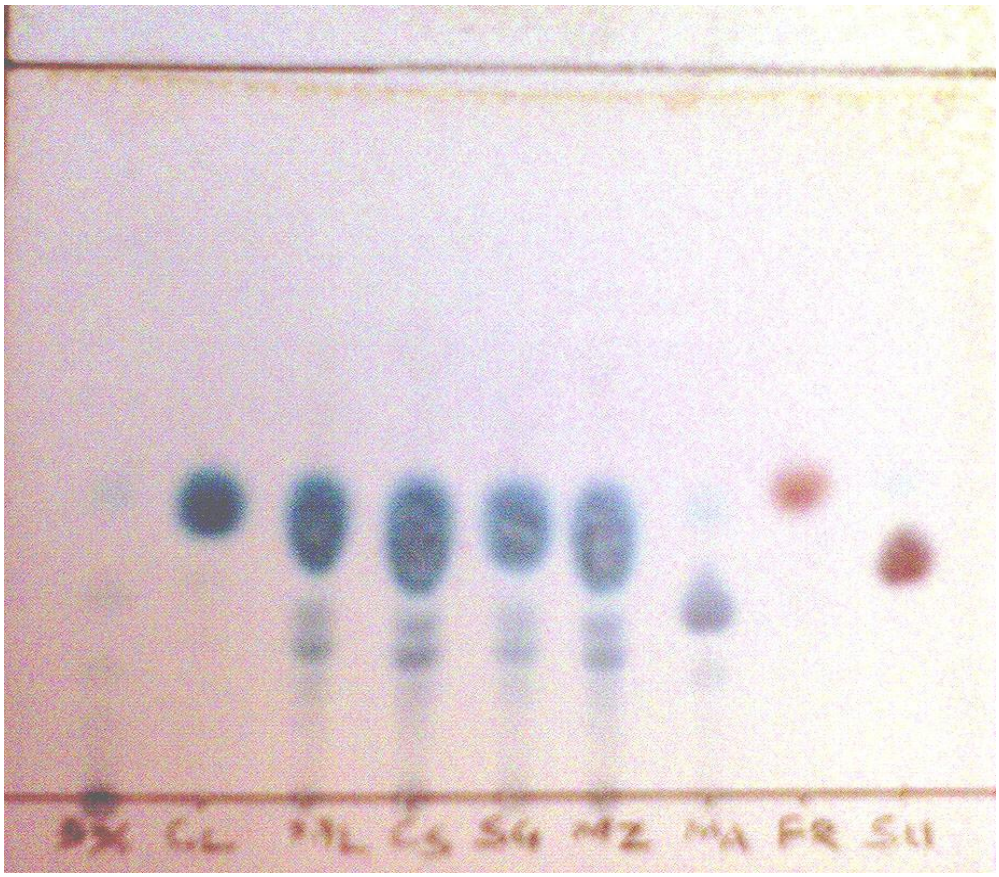

Figure 11. Chromatoplate showing starch hydrolysates and sugar standards. DX = Dextrin, GL = glucose, ML = millet starch hydrolysate, Cs = cassava starch hydrolysate, SG = sorghum starch hydrolysate, Mz = maize starch hydrolysate, MA = maltose, FR = fructose, and Su = sucrose.

been denatured before the 1-4 glycosidic bonds in amylose and amylopectin molecules of the starch are attacked by the enzyme.

Thermal stability and activity of partially purified fungal amyloglucosidase

Relatively, the enzyme was stable over 20 and 35°C.The enzyme retained about 56% its activity over 120 min at these temperatures. More of its potency (about 65%) was lost between 40 and 50°C. About 25% of its activity was retained after exposure to 60°C.

Fossi et al. (2005) found amylase from actinomycetes yeast strain to be thermally stable at 20 to 70°C. In the report of Gomes et al. (2005), glucoamylase produced from *Aspergillus flavus* and *Thermomyces lanuginosus* exhibited partial stability (80% residual activity) and enhanced activity (120% residual activity) respectively at 60°C after 1 h. There is no much variation compared to what was obtained in this study except that the enzyme was relatively stable over a relatively lower temperature condition.

Optimal thermal activity of the amyloglucosidase used in this study was exhibited between 50 and 60°C. Gomes et al. (2005) reported optimal thermal activity of 65 and 70°C for glucoamylase derived from *A. flavus* and *Thermomyces lanuginosus* respectively. This study has revealed a glucoamylase or amyloglucosidase of a lower thermal optimal activity.

Glucoamylase or amyloglucosidase are mostly applied to starch hydrolysis at lower temperatures than liquefying α-amylase to starches. The temperature at which this enzyme exhibited activity is such that may allow its suitability in industrial setting.

Thin-layer chromatography of hydrolytic products of cassava, maize, millet and sorghum starches

Result of thin layer chromatography analysis showed glucose and maltose to be the predominant sugars in the starch hydrolysates. R_f values of syrups of cassava, maize, millet and sorghum starches were found to be similar to that of glucose and maltose but were different from those of dextrin, fructose and sucrose as seen in Figure 11. However, this does not completely rule out presence of other sugars, which could be present in

quantities that could not be detected by thin layer chromatography.

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