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Production and optimization of *Aspergillus niger* glucoamylase using amylopectin from guinea corn starch as the sole carbon source

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Abstract

A Fourteen day experimental study was carried out to determine the day of highest glucoamylase activity using amylopectin from guinea corn starch as the sole carbon source. Two peaks of high activity were observed on the fifth and twelveth days, and were thus mass produced. Specific activities for crude enzymes were found to be 729.45 U/mg and 1046.82 U/mg for day five and twelve harvested enzymes respectively. Ammonium sulphate saturations, 70% and 20%, were found suitable to precipitate proteins with highest glucoamylase activity for day five and twelve harvested enzymes respectively. After ammonium sulphate precipitation and gel filtration, specific activities were found to be 65.98 U/mg and 180.52 U/mg respectively for day five harvested enzyme and 61.51 U/mg and 272.81 U/mg for day twelve harvested enzyme. The pH optimum for day five harvested enzyme were found to be 7.5, 7.5 and 6.0 using tiger nut, cassava and guinea corn starches as substrates respectively, also, the pH optimum for day twelve harvested enzyme were found to be 5.0, 8.5 and 7.0 using tiger nut, cassava and guinea corn starches as substrate, respectively. Optimum temperatures were found to be 50˚C and 45˚C for day five and twelve harvested enzymes, respectively. K_m and V_{max}, of day five harvested enzyme were found to be 770.75 mg/ml and 2500 μmol/min, 158.55 mg/ml and 500 μmol/min and 46.23 mg/ml and 454.53 μmol/min using cassava, guinea corn and tiger nut starches as substrate respectively. K_m and Vmax of day twelve harvested enzyme were found to be 87.1 mg/ml and 384.61 μmol/min, 29.51 mg/ml and 243.90 μmol/min, and 2364 mg/ml and 2500 μmol/min, using cassava, guinea corn and tiger nut starches as substrate respectively.

Keywords: Glucoamylase; Enzyme; *Aspergillus niger* specific activity; Km and Vmax

Introduction

Starch is the plant storage polysaccharide made up of amylose and amylopectin. The amylose component is a polymer of glucose linked by α-1, 4-glucosidic bonds. The amylopectin is a polymer of glucose linked by α-1, 4-glucosidic bonds in the straight chain and α-1, 6 at the branch point. Glucoamylase (1, 4-α- D-glucan glucohydrolases, EC 3.2.1.3) is an exo enzyme of great importance for hydrolysis of starch and other related oligosaccharides and polysaccharides. This enzyme consecutively hydrolyzes 1, 4-alpha-glycosidic linkages from the non-reducing end of starch as well as the 1, 6-alpha-glucosidic linkages of starch yielding, β-D-glucose as the end product (Uma and Nasrin, 2013). In otherwords, Glucoamylase cleaves glucosyl units from the non-reducingend of amylose chain, glycogen and amylopectin linkages (Muhammad *et al*., 2012) , as well as hydrolyzes α -1, 6 linkages, resulting in β- D- glucose as the end-product (Abdalwahab *et al*., 2012). Enzymes of microbial origin are currently becoming increasingly important due to their economic advantages (Damisa *et al*., 2013). Generally, amylases, (that is α- amylases, β-amylases and glucoamylases) can be produced either by submerged fermentation (SmF) or solid state fermentation (SSF) procedures; although, the convectional amylase production is carried out by submerged fermentation (Radha *et al*., 2012). Glucoamylase production from microbial sources especially from *Aspergillus niger* is generally extracellular, and the enzyme can be recovered from culture filtrates (Sarojin *et al*., 2012). However, the extensive utilization of glucoamylase is obtained by using the fungus *Aspergillus niger* in enzyme production. This work is therefore aimed at the production and optimization of glucoamylase from *Aspergillus niger* in a submerged fermentation process using amylopectin from guinea corn starch as the sole carbon source. Glucoamylase is found useful in industries, where starch hydrolysis is required, such as, the food processing industries, textile industries, brewery industries and in fermentation biotechnology.

Materials and methods

Bovine serum albumin (BSA), Sephadex G-100 was purchased from Sigma Chemical Company Limited (USA). Folin–Ciocalteau reagent was purchased from Sigma-Aldrich (USA). Ammonium Sulphate and Tris HCL salt were purchased from British Drug House (BDH) Chemicals Limited (USA). All other chemicals used in this work were of analytical grade and were obtained from reputable sources.

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Collection of plant material

Plant material (guinea corn seed, tiger nut seed and cassava tuber) was purchased from the Ogige market in Nsukka, Enugu state, Nigeria.

Processing of guinea corn and tiger nut starches

The starch from the guinea corn and tiger nut seed was processed using the method described by Agboola *et al*. (1990) with the following modifications. The seeds were sun dried and ground to fine flour. 300 g of the flours weresuspended in 3 L of distilled water for 24 h. The suspended flour was sieved using muslin cloth. The extracted starch was allowed to sediment for 4 h at room temperature. The supernatant was decanted off and the starch washed with 3L of distilled water twice and finally allowed to stand for 4 h. The supernatant was then decanted and the resulting wet starch was sun dried and packaged in an air tight container and stored at room temperature.

Fractionation of the guinea corn starch into amylose and amylopectin

Fractionation of amylose and amylopectin was carried out by following the general procedure of Sobukola and Aboderin (2012). This consists of heating and stirring starch dispersion (0.8%, w/v inwater) in a water bath at 100°C until starch is gelatinized. The starch solution was filtered using filter paper to remove insolubleresidues, and the pH adjusted to 6.3 using phosphate buffer. The solution was stirred in a boiling water bath for 2 h to disperse thestarch molecules. Thereafter, n-Butyl alcohol was added (20%, v/v), and the solution was stirred at 100°C for 1 hr., followed by cooling to room temperature over a period of 24-36 h. Amylose butyl alcohol complex crystals was formed and precipitated during cooling, and was separated by filtration. The amylopectin remaining in the supernatant was recovered by adding excess methyl alcohol.

Processing of cassava tubers for cassava starch

Cassava starch was processed using the method described by Corbishley and Miller (1984) with the following modifications. Freshly harvested cassava tubers were peeled washed clean andgrated. The grated cassava (1.2 kg) was soaked in 4 L of distilled water for 1 h after which it was sieved (3 times) with muslin cloth. The extract allowed to stand for 4 h and the supernatant decanted. The isolated wet starch was sun dried and packaged in plastic airtight container and kept in a cool, dry place.

Isolation and identification of the glucoamylase producing fungi

Glucoamylase producing fungi was isolated and stored according to the method described by Ezugwu *et al*., (2015) and the identification was carried out using the method of Barnett and Hunter (1972).

Fermentation broth for enzyme production

Submerged fermentation (SmF) technique was employed using an Erlenmeyer flask containing 700 ml of sterile cultivation medium optimized for glucoamylase with 2.1g $(NH4)_2SO_3$, 4.2g KH_2PO_4, 0.7g $MgSO_4.7H_2O$, 0.07g $FeSO_4$ and 7g amylopectin from guinea corn starch. From the 700 ml of the sterile cultivation medium optimized for glucoamylase, 50 ml each were poured into fourteen 250 ml cornical flask, labeled day one to day fourteen. These flasks were with aluminum foil and autoclaved at 121°C for 15 minutes to ensure sterility. For inoculation, three days old cultures were used to inoculate the flasks. In every sterile flask, two discs of the respective fungal isolates were added using a cork borer of diameter 10 mm and then plugged properly. The culture medium was incubated for fourteen days (14 days) at room temperature. For each day of harvest, a flask is selected from and mycelia biomass separated by filtration. Each day, the filtrate was analyzed for glucoamylase activity and extracellular protein concentration.

Mass production of the enzyme

After the fourteen days of pilot submerged fermentation (SmF) studies, the day of peak glucoamylase activity was chosen for mass production of enzyme (day five and twelve). The medium for the enzyme production composed of 6.0g $(NH_4)_2SO_3$, 12g KH_2PO_4, 2.0g $MgSO_4.7H_2O$, 0.2g $FeSO_4$ and 17.165g amylopectin from guinea corn starch. Several Erlenmeyer flasks were used to produce two liters of the enzyme. Harvesting was carried out on the day of peak glucoamylase enzyme activity.

Glucoamylase assay

Glucoamylase activity was assayed according to Miller (1959). The colour developed was read by measuring its optical density using a JENWAY 6405 UV/VIS spectrophotometer (Beckman Instruments, Inc. Huston Texas) at 540 nm. One enzyme activity unit (U) was defined as the amount of enzyme required to release one micro mol (μmol) of glucose from the substrate per minute under standard assay condition. The concentration of the released glucose was estimated using a glucose standard curve.

Alpha amylase assay

Amylase activity was determined by the dinitrosalicyclic acid (DNSA) method as described by Bernfeld (1951). The amylase activity was assayed by incubating 0.5 ml of the enzyme with 0.5 ml of 1% w/v starch dissolved in 20 mM sodium phosphate buffer pH 7.0, at 55°C for 60 minutes. The released reducing equivalent (maltose) was determined by the addition of 1 ml of 3, 5- dinitrosalicyclic acid (DNSA), which also stopped the reaction, this was then followed by boiling for 10 minutes, thereafter, 1 ml of tartarate was added to stabilize the colour and the mixture was then allowed to cool and the absorbance read spectrophotometrically at a wavelength of 600 nm against a blank, using JENWAY 6405 UV/VIS spectrophotometer (Beckman Instruments, Inc. Huston Texas). The same procedure was used for the blank except that the blank lacked the enzyme solution. One unit of amylase activity was expressed as the amount of enzyme that releases one micro mole (μmole) of the reducing equivalent (maltose) per minute under assay conditions. Amylase activity was estimated by the amount of reducing equivalent (maltose) released during the hydrolysis of the starch.

Protein determination

Protein content of the enzyme was determined by the method of Lowry *et al*., (1951), using Bovine Serum Albumin (BSA) as standard.

Enzyme purification

The crude glucoamylase was purified using following purification techniques; Ammonium Sulphate Precipitation and gel Filtration.

Ammonium sulphate purification

For the crude enzymes, 70% and 20% ammonium sulphate saturation were suitable to precipitate the day five and day twelve enzyme proteins respectively. This was kept at 4°C for 30 h; thereafter it was centrifuged at 4000 rpm for 30 min. The precipitate was collected and re-dissolved in 20 mM acetate buffer pH 5.5. The glucoamylase activity and protein were determined as described.

Gel filtration chromatography

A volume (20 ml) of the precipitated enzyme was introduced into a (75 × 2.0 cm) gel chromatographic column and subjected to gel filtration using sephadex G-100 pre-equilibrated with 0.02 M sodium acetate buffer pH 5.5. Fractions were collected at a flow rate of 5ml/20 min. The protein concentration of each fraction wasmonitored using a JENWAY 6405 UV/VIS spectrophotometer (Beckman Instruments, Inc. Huston Texas) at 280 nm. The Glucoamylase activity of each fraction was assayed as earlier discussed with the active fractions pooled together and stored at -10^{o}C.

Enzyme characterization

The partially purified glucoamylase was characterized with respect to the effect of pH, effect of temperature, effect of substrate concentration and determination of Km and Vmax.

Optimum pH

The optimum pH for enzyme activity was determined using 0.02 M sodium acetate buffer pH 3.5 to 5.5, phosphate buffer pH 6.0 to 7.5 and Tris-HCl buffer pH 8.0 to 10.0 at intervals of 0.5. 0.5 ml of gelatinized starch solution (1%) was equilibrated with 1 ml of the buffers (20 mM) of respective pHs for 5 min at 37°C. 0.5 ml of the enzyme was added and the reaction mixture was mixed properly and allowed to stand for 20 min at 50°C. The glucoamylase activity was assayed as described above using starch as substrate.

Optimum temperature

The optimum temperature for glucoamylase activity was determined by incubating the enzyme with gelatinized starch solution (1%) at 30 to 80°C for 20 min at respective predetermined optimal pHs. Glucoamylase activity was determined by the amount of glucose produced using DNS as described.

Substrate concentration

The effect of substrate concentration on glucoamylase activity was determined by incubating 0.5 ml of enzyme with 10, 20, 30, 40, 50,60, 70, 80, 90, and 100 mg/ml of different starch solutions for 20 min at the respective predetermined optimal pHs and temperatures. The glucoamylase activity was determined using starch from cassava, guinea corn and tiger nut starch.

Results and discussion

Fractionation of the guinea corn starch

Amylopectin from guinea corn starch was used as the carbon source for the production of glucoamylase from *Aspergillus niger*. Table I shows that amylopectin from the guinea corn starch gave a yield of 62.4% at temperature of 100^{o}C and extraction time of 60 min (Sobukola and Aboderin, 2012). Amylopectin yield in starches especially from some cereals have been studied. Mahsa *et al*. (2003) showed that partial fractionation of wheat starch save a yield of 25% amylose and 75% amylopectin. Balole and Legwaila (2006) studied the starch components of guinea corn and found that the guinea corn starch has approximately 70–80% amylopectin content. Lawal *et al*. (2004), in the study on the functional properties of amylopectin and amylose fractions isolated from bambarra groundnut starch, found that the amylose and amylopectin content of the bambarra groundnut starch were

75% and 11% respectively. The high amylopectin content of starch aids starch gelatinization or makes for easy gelatinization of starch (Okporie *et al*., 2014).

Experimental studies

Fig. 1 shows the glucoamylase activities during the pilot study for fourteen (14) days (1-14 days). High glucoamylase activities were obtained on the fifth (5th) day (319.059µ mol/min) and on the twelfth (12th) day (299.5626 µmol/min) of the submerged fermentation. Similar observations were reported by Fabiana *et al*. (2008) in their report; the maximum glucoamylase activity was achieved on day 3 of the submerged fermentation. Also, the decrease in glucoamylase production from day 6 to day 10 and from day 12 to day 14 could be attributed to high glucose concentration in the microorganism. Christiane *et al*. (2011) reported that glucoamylase production by *Aspergillus niger* is repressed due to high glucose concentration in the microorganism, nevertheless, when glucose concentration decreases, the microorganism (*Aspergillus niger*) starts to produce glucoamylase again, to hydrolyze the substrate.

Fig. 1 also shows α -amylase activity during the pilot study of fourteen (14) days (1-14 days). High α-amylase activities were obtained on the fifth (5th) day (103.290 µmol/min) and on the twelfth (12th) day (166.666 µmol/min). Kshipra *et al*. (2011) reported that other amylolytic enzymes particularly α - amylase is most likely to be concomitantly produced with glucoamylase. Fig. 1 also shows protein concentration during the fourteen (14) days (1-14 days) of fermentation. The highest protein concentration was obtained on the fifth (5th) day (13.2826 mg/ml). The protein production observed in this study could be due to protein synthesis during the growth and metabolism of the microorganism.

Purification studies

The partial purification of glucoamylase harvested on day five and day twelve of the submerged fermentation is summarized in Tables IIa and IIb. After ammonium sulphate purification, gel filtration was carried out to purify the glucoamylase. From the elution profile of the gel chromatography on sephadex G-100 (Fig. 2a and 2b), peaks

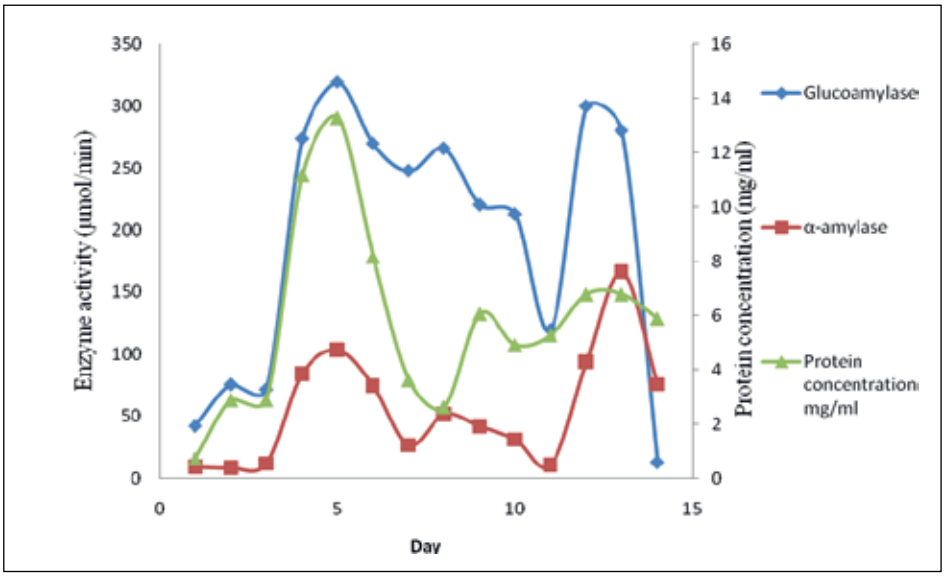


Fig. 1. Progress curve of glucoamylase activity, alpha amylase activity and protein concentration during the fourteen days of fermentation.

Table I. Percentage yield of the amylopectin from guinea corn starch

Weight of the Guinea corn starch (g)	Weight of the dried amylopectin (g)	Percentage yield (%)
20	12.48	62.4

showing glucoamylase activity were obtained, these peaks were pooled together and enzyme activity and protein concentration was determined. In this study, after the gel filtration, glucoamylase activity was 24.19 μmol/min, with a specific activity of 180.52 U/mg as shown in table IIa, and 50.47 μmol/min and 272.81 U/mg as shown in table IIb. The decrease in the enzyme activities after each purification step could be attributed to the removal of impurities such as other proteins which enhances the enzyme activity as the enzyme might have lost some cofactors or ions that it needs for its activity. In a similar manner, decrease in specific activities of the enzymes after ammonium sulphate precipitation may be attributed to the precipitation of other unwanted proteins at different percentages of the ammonium salt during the salting out process. Sangeeta *et al*. (2011) reported similar result during the extraction and purification of glucoamylase and protease produced by *Aspergillus awamori* in single-stage fermentation. Also, the increase in specific activities of the enzymes after gel filtration as shown in tables IIa and IIb, could be ascribed to the fact that for a purification procedure to be successful, the specific activities of the desired enzyme must be greater after the purification procedure than as it was before (Lukong *et al*., 2007).

Table II. (a) Purification table for glucoamylase harvested on day five of the submerged fermentation

Enzyme	Volume (ml)	Protein (mg/ml)	Total protein	Activity (μmol/min)	Total activity	Specific Activity (U/mg)	Purification Fold	% yield
Crude	1000	0.455	455	331.90	331900	729.45	1	100
$(NH_4)_2SO_4$	280	1.662	465.36	109.67	30707.6	65.98	0.10	9.25
Gel filtration	30	0.134	4.02	24.19	725.7	180.52	2.7	2.36

U (Unit) =μmole/min

Table II. (b) Purification table for glucoamylase harvested on day twelve of submerged fermentation

Enzyme	Volume (ml)	Protein (mg/ml)	Total protein	Activity (μmol/min)	Total activity	Specific Activity (U/mg)	Purification Fold	% yield
Crude	1000	0.292	292	305.67	305670	1046.82	1	100
$(NH_4)_2SO_4$	300	1.072	321.6	65.94	19782	61.51	0.1	6.5
Gel filtration	30	0.185	5.55	50.47	1514.1	272.81	4.44	7.7

U (Unit) =μmole/min

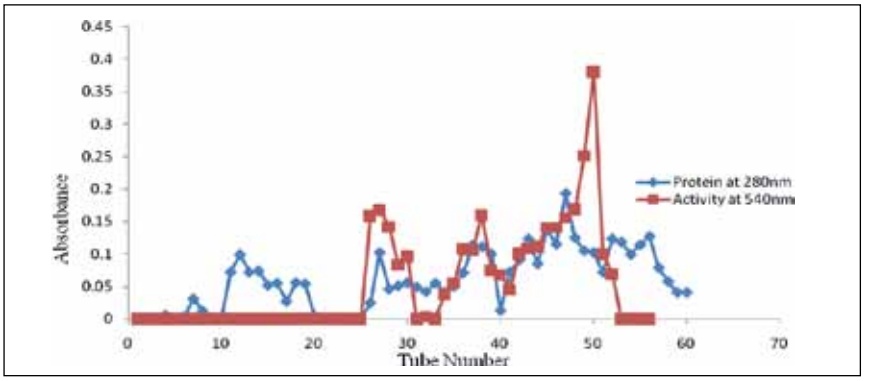


Fig. 2. (a) Elution profile of glucoamylase (harvested on day five of submerged fermentation) during gel filtration on a 120 ml volume of sephadex G-100 packed into a column (75 by 2.0 cm) and equilibrated with 50 mM sodium acetate buffer, pH 5.0. A volume of 5 ml of 60 fractions were collected at an elution rate of 5 ml per 20 min. Glucoamylase activity and protein determination was assayed in each of the fractions collected

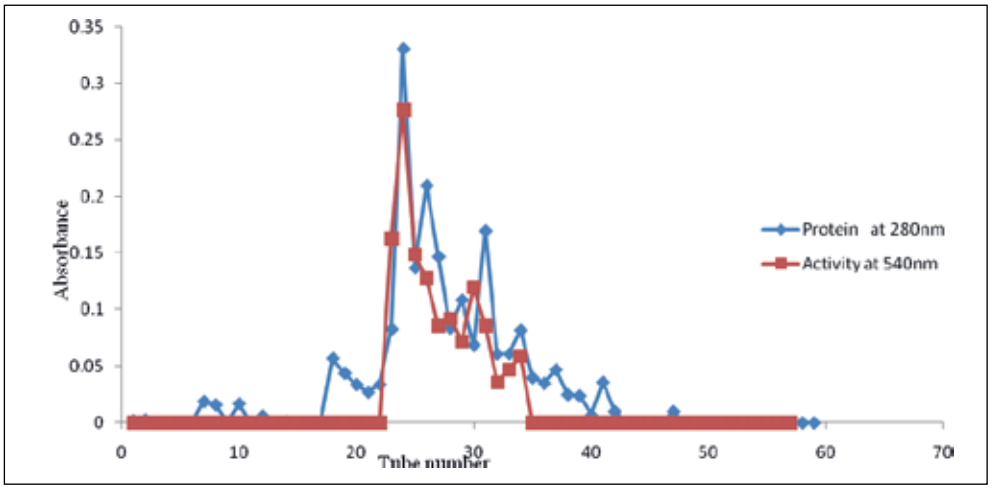
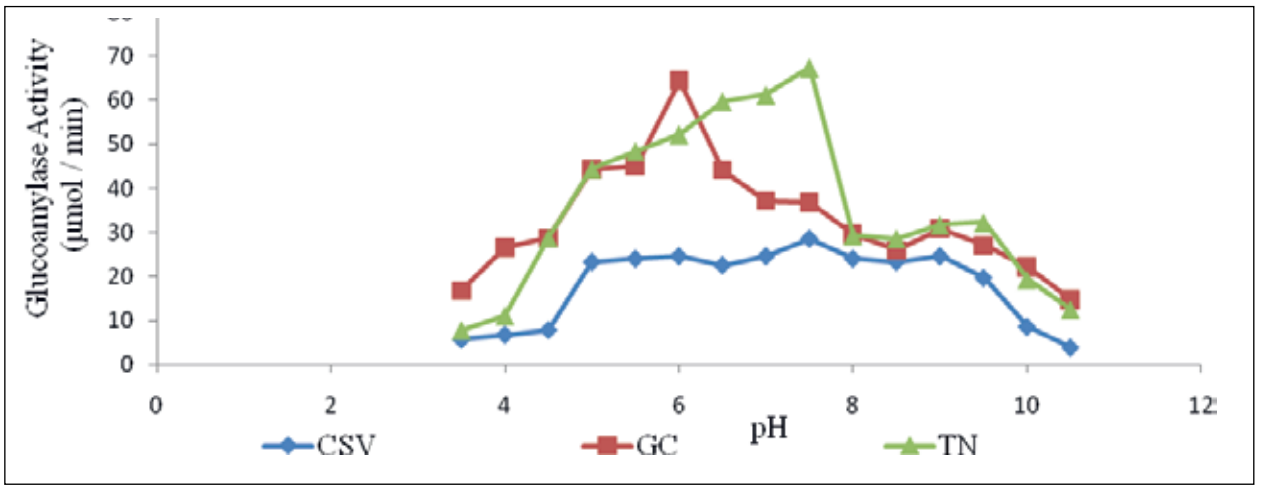


Fig. 2. (b) Elution profile of glucoamylase (harvested on day twelve of submerged fermentation.) during gel filtration on a 120 ml volume of sephadex G-100 packed into a column (75 by 2.0 cm) and equilibrated with 50 mM sodium acetate buffer, pH 5.0. A volume of 5 ml of 60 fractions were collected at an elution rate of 5 ml per 20 min. Glucoamylase activity and protein determination was assayed in each of the fractions collected

Characterization of glucoamylase

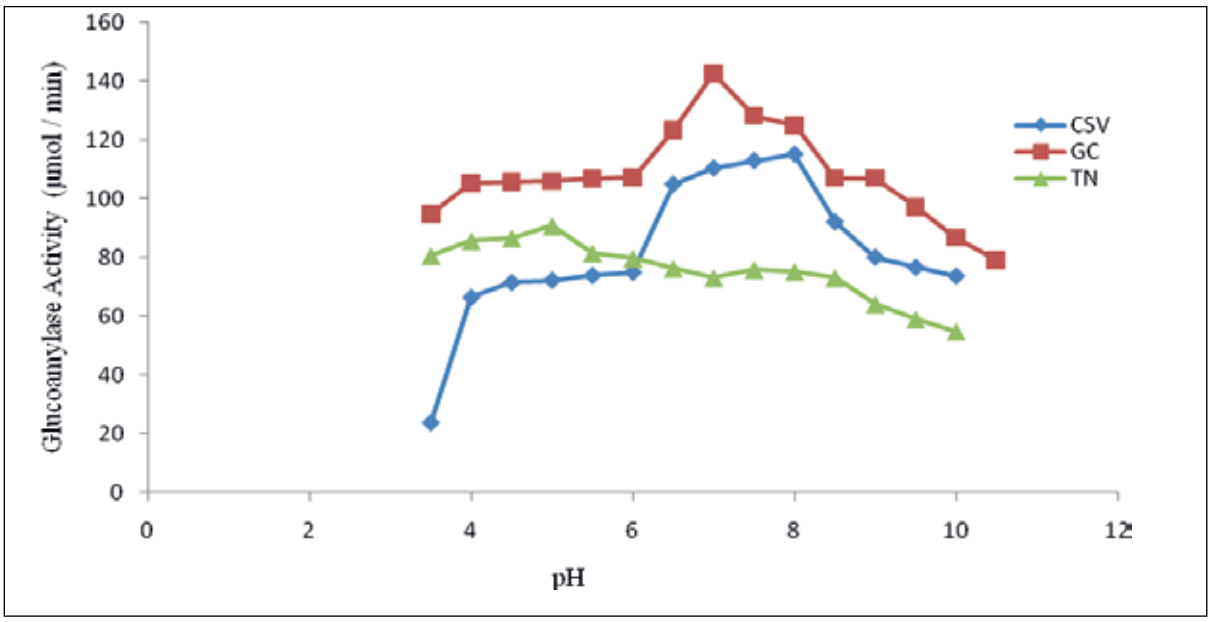
Optimum pH

The effect of pH on the glucoamylase activity is as shown in Fig. 3a and 3b. As the pH increased, the activity of glucoamylase also increased until optimum pH was obtained at 7.5, 7.5 and 6.0 when using tiger nut starch, cassava starch and guinea corn starch as substrates, respectively for the day five harvested enzyme. Similarly optimum pH was obtained at 5.0, 8.5 and 7.0 when using tiger nut starch, cassava starch and guinea corn starch as substrates, respectively, for the day twelve harvested enzyme. James and Lee (1997) reported that the range of glucoamylase pH is between 3.7 and 7.4. Nahar *et al*. (2008) reported that the optimum pH range of glucoamylase varies from 4.5 to 5.5 with stability at pH 7.0. Changes in pH can change the shape of the active site in an enzyme. Extremely high or low pH concentrations usually result in complete loss of enzyme activity due to denaturation (Helms *et al*., 1998).



CSV is Cassava Starch. GC is Guinea corn starch. TN is Tiger nut Starch

Fig. 3. (a) Effect of pH on glucoamylase activity for glucoamylaseharvested on day five of submerged fermentation, 20 mm sodium acetate buffer pH 3.5 to 5.5, 20 mM phosphate buffer pH 6.0 to 7.5 and 20 mM Tris-HCl buffer pH 8.0 to 10.0, at intervals of 0.5 was used in the assay



CSV is Cassava Starch. GC is Guinea corn starch. TN is Tiger nut Starch

Fig. 3.(b) Effect of pH on glucoamylase activity for glucoamylase harvested on day twelve of submerged fermentation, 20 mM sodium acetate buffer pH 3.5 to 5.5, 20 mM phosphate buffer pH 6.0 to 7.5 and 20 mM Tris-HCl buffer pH 8.0 to 10.0, at intervals of 0.5 was used in the assay

Optimum temperature

The optimum temperature for glucoamylase harvested on day five was 50°C, with glucoamylase activity of 67.055 µmole/min as shown in Fig. 4a, similarly, the day twelve harvested enzyme had an optimum temperature of 45°C, as shown in Fig. 4b, this was in accordance with Siddhartha *et al*. (2012) who reported that temperature optima for glucoamylases are generally in the range of 45°C to 60°C. Sarojin *et al*. (2012) also reported that optimum glucoamylase activity was obtained at 45°C. All enzymes have an optimal temperature at which reaction rates go fastest without denaturation of the enzyme (Campbell and Reece, 2002).

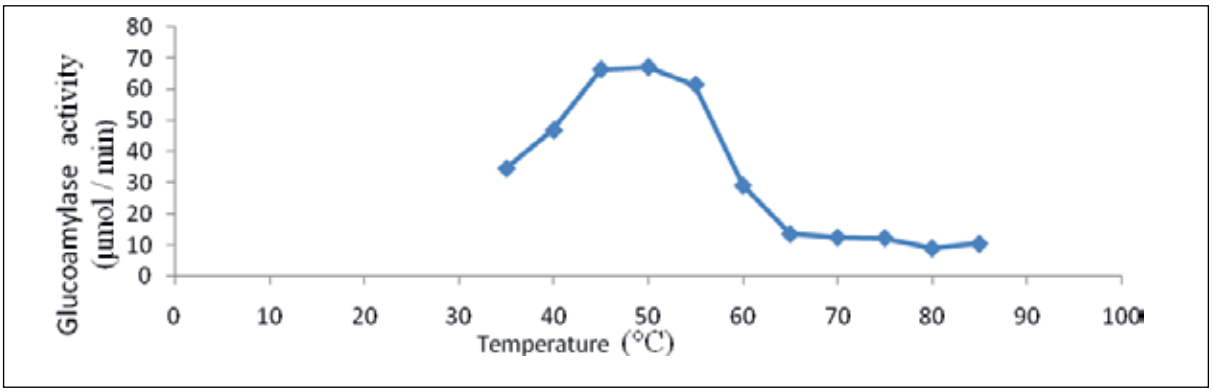


Fig. 4. (a) Effect of temperature on glucoamylase activity forglucoamylase harvested on day five of submerged fermentation. Activity was assayed in 20 mM phosphate buffer pH 7.5 from 30°C to 90°C after incubation for 20 min and coupling with DNS (3, 5-dinitrosalicyclic acid). Optimum temperature of 50 °C was obtained

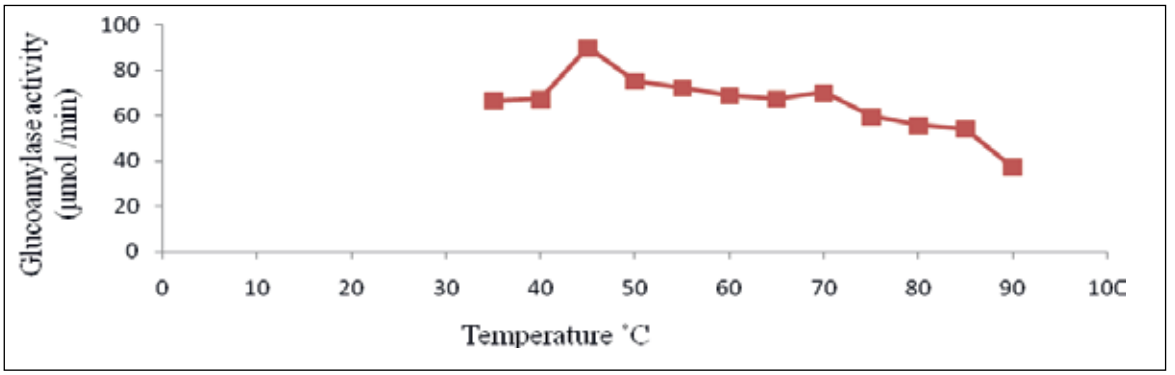


Fig. 4.(b) Effect of temperature on glucoamylase activity forglucoamylase harvested on day twelve of submerged fermentation. Activity was assayed in 20 mM phosphate buffer pH 7.5 from 30°C to 90°C after incubation for 20 min and coupling with DNS (3,5-dinitrosalicyclic acid). Optimum temperature of 45 °C was obtained

Effect of substrate concentration

Fig. 5a summarizes the effect of substrate concentration on glucoamylase activity, for the day five harvested enzyme. The kinetic parameters such as the K_m and V_{max} were evaluated using the Lineweaver-Burk plot of initial velocity data using different concentration of starch (10 mg/ml – 100 mg/ml). They were found to be, 770.75 mg/ml and 2500 μmol/min, 158.55 mg/ml and 500 μmol/min, 46.23 mg/ml and 454.53 μmol/min using cassava starch, guinea corn starch and tiger nut starch as substrates respectively.

Similarly Fig. 5b summarizes the effect of substrate concentration on glucoamylase activity for the day twelve harvested enzyme,the kinetic parameters such as the $K_m$and V_{max} were evaluated to be 87.1 mg/ml and 384.61 µmol/min, 29.51 mg/ml and 243.90 µmol/min, 2364 mg/ml and 2500 µ mol/min using cassava starch, guinea corn starch and tiger nut starch as substrates respectively.

The kinetic parameters of glucoamylase harvested on day five and glucoamylase harvested on day twelve were obtained using the lineweaver-burk plot derived from the

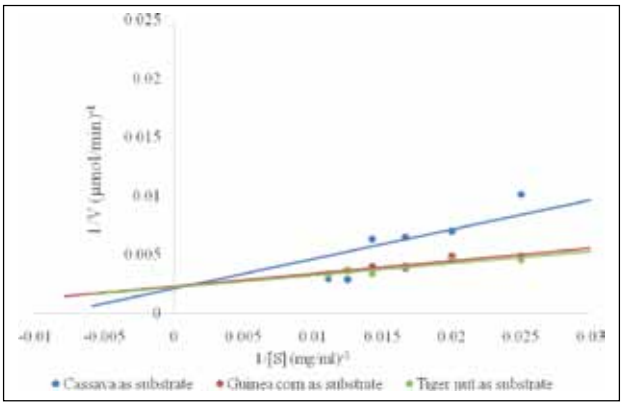


Fig. 5. (a) Line-Weaver Burk plot for glucoamylase harvested on day five of the submerged fermentation using cassava starch, guinea corn starch and tiger nut starch as substrate. The plots were obtained from initial velocities at different substrate concentrations at pH 7.5 (using tiger nut starch and cassava starch) and pH 6.0 (using guinea corn starch) at temperature 50 °C

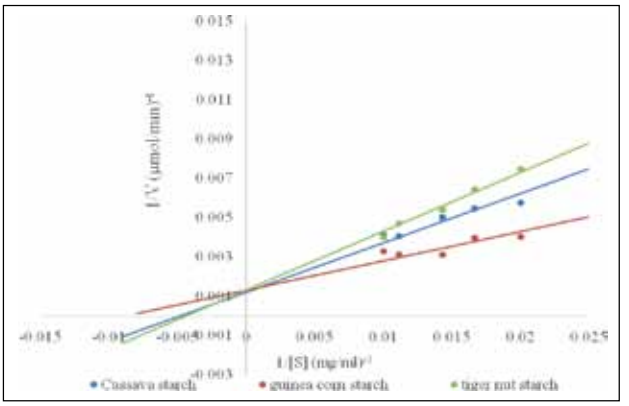


Fig. 5. (b) Line-Weaver Burk plot for glucoamylase harvested on day twelve of the submerged fermentation when cassava starch, guinea corn starch and tiger nut starch were used as substrate. The plots were obtained from initial velocities at different substrate concentrations at pH 5.0, 7.0 and 8.0 using tiger nut starch, guinea corn starch and cassava starch respectively as substrate at temperature 45 °C

Table III. Characterization table for glucoamylase harvested on day five and day twelve

Substrates	Properties							
	pH		Temperature		V_{max} (μmol/min)		K_m (mg / ml)	
	G.A 5	G.A 12	G.A 5	G.A 12	G.A 5	G.A 12	G.A 5	G.A 12
Cassava starch	7.5	8.0	50°C	45°C	2500	384.61	770.75	87.1
Guinea corn starch	6.0	7.0			500	243.90	158.55	29.51
Tiger nut starch	7.5	5.0			454.53	2500	46.23	2364

G.A 5 is glucoamylase harvested on day five while G.A 12 is glucoamylase harvested on day twelve

initial velocities at different substrate concentrations. Table III shows the kinetic properties of both enzymes. The K_m value of an enzyme provides a parameter for comparing enzymes, while the V_{max} or maximum velocity gives information on the turnover number of an enzyme (Anosike, 2001). Christiane *et al*. (2011) reported a Km of 0.34 mg/ml and a Vmax of 160.22 U/ml for glucoamylase isolated from *Aspergillus niger*, Sarojini *et al*., (2012) reported a Km value of 10 g /l and a Vmax of 20 μg/ml/min for glucoamylase isolated from *Aspergillus niger*, from this study, the variations in kinetic parameters of the two enzymes could be attributed to differences in the substrate used.

Conclusion

Results from the kinetic parameters of glucoamylase suggests that the cnzyme isolated from *Aspergillus niger* in a submerged fermentation process, could be useful in starch degradation and in other biotechnological applications where starch is needed. The enzyme can be produced in large quantities from *Aspergillus niger*, when cultured with amylopectin from guinea corn starch.

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