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A study of the optimal conditions for glucoamylases obtained from *Aspergillus niger* using amylopectin from cassava starch as carbon source

Ezugwu, A. L.*, Eze, S. O. O. and Chilaka, F. C.

Department of Biochemistry, University of Nigeria Nsukka, Enugu State, Nigeria.

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A fourteen day pilot study carried out showed that high glucoamylase activities were obtained on the 4 and 11th day of fermentation and the enzymes were harvested on the respective days giving the codes GluAgCSV4 and GluAgCSV11. The optimal pH and optimal temperatures for enzyme activities GluAgCSV4 and GluAgCSV11 were in a range of 6 to 7 and 50 to 55, using cassava, guinea corn and tiger nut starch as substrates, respectively. The enzyme activity (GluAgCSV4) was enhanced by Ca^{2+}, Mn^{2+}, Fe^{2+} and Zn^{2+}. Co^{2+} had inhibitory effect on the enzyme while Pb^{2+} completely inactivated the enzyme. The enzyme activity (GluAgCSV11) was enhanced by Ca^{2+} and Co^{2+}. Zn^{2+}, Fe^{2+} Mn^{2+} and Pb^{2+} completely inactivated the enzyme. The Michaelis constant K_M and maximum velocity V_{max} obtained form Lineweaver-Burk plot of initial velocity data at different substrate concentrations were found to be 90.06 mg/ml and 188.67 µmol/min (using cassava starch as substrate), 173.70 mg/ml and 434.78 µmol/min (using guinea corn starch as substrate) and 28.57 mg/ml and 227.27 µmol/min (using tiger nut starch as substrate), respectively for GluAgCSV4. Also, 271.30 mg/ml and 1000 µmol/min (using cassava starch as substrate, 3093 mg/ml and 10000 µmol/min (using guinea corn starch as substrate) 2625 mg/ml and 10000 µmol/min (using tiger nut starch as substrate), respectively, were obtained for GluAgCSV4.

Keywords: Glucoamylases, *Aspergillus niger*, amylopectin and starch.

INTRODUCTION

Glucoamylases of microbial origin are divided into exo-acting, endo-acting, debranching and cyclodextrin producing enzymes. Glucoamylases hydrolyze α-1, 4 and α -1, 6 linkages and produce glucose as the sole end-product from starch and related polymers (Shenoy et al., 1985; Svensson et al., 2000; Parbat and Singhal, 2011). Beside cellulose, starch is the most abundant carbohydrate in the world (Betiku, 2010; Ozienbge and Onilude, 2011). The primary industrial use of starch is its hydrolysis to sugar syrups that are employed by the food

*Corresponding author. E-mail: arinzelinus.ezugwu@gmail.com. Tel: +2348063743436.

Abbreviations: PDA, Potato dextrose agar; **DNSA,** 3, 5-dinitrosalicyclic acid.

industry to make sweets, drinks, juices and for fermentation into products like citric acid, ethanol, bakers' 'yeast, as well as in paper and textile. The original method of starch hydrolysis known as acid hydrolysis (Kolusheva and Marinova, 2007) requires the use of corrosion resistant materials, gives rise to high colour and salt ash content (after neutralisation), needs more energy for heating and is relatively difficult to control. As a result of these problems, enzymatic hydrolysis becomes a better method for starch hydrolysis since the above mentioned constraints are not peculiar. Starch hydrolysis in industry most times requires a combination of enzymes for complete hydrolysis. There is the need therefore to optimize the necessary conditions for glucoamylase activity to achieve complete starch hydrolysis without necessarily involving multiple enzymes. Starch is a major storage product of economically important crops like cassava, guinea corn and tiger nut. These plants have the potentials to become the principle feedstock for fuel, alcohol and glucose syrup market. This research is aimed at production of glucoamylases from *Aspergillus niger* in a submerged fermentation using amylopectin fractionated from cassava starch as the only carbon source and possibly optimize the conditions for the applications in hydrolysis of cassava, guinea corn and tiger nut starch.

MATERIALS AND METHODS

Materials

Collection of plant samples

The plant materials (cassava tuber, guinea corn, and tiger nut) were obtained at Orba main market, Udenu Local Government, Enugu State, Nigeria.

Processing of cassava and 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 and grated. 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. This was allowed to stand for 4 h and the supernatant decanted. The isolated wet starch was sun dried and packaged in plastic air tight container, labelled and kept in a cool, dry place.

Processing of cereal starch

The starch from cereals 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 were suspended 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 3 L 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 then packaged in an air tight container and stored at room temperature.

Fractionation of 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 in water) in water bath at 100°C until starch is gelatinized. Starch solutions were filtered using filter paper to remove insoluble residues, and the pH adjusted to 6.3 using phosphate buffer. The solution was stirred in a boiling water bath for 2 h to disperse the starch 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.

Isolation of glucoamylase producing fungi

Glucoamylase producing fungi were isolated by adopting the method of Martin et al. (2004) as modified by Okoye et al. (2013). Tiger nut starch was fractionated into amylose and amylopectin. The wet amylopectin was left open on shelve to allow micro-organisms to grow on it. A loop of each organism was streaked onto potato dextrose agar PDA under the flame of Bunsen burner. Streaks were made from each side of the plate, marking an initial point, with sterilization of the wire loop after each side has been completed. The plates were thereafter incubated at 35°C till visible colonies were observed. All morphological contrasting colonies were purified by repeated streaking and sub-culturing on separate plates. This process was continued till pure fungal culture was obtained. Pure fungal isolates were maintained on potato dextrose agar (PDA) slopes or slants as stock cultures. PDA media were prepared according to the manufacture's description. In the description, 3.9 g of PDA powder was weighed and added in small volume of distilled water and made up to 100ml. The medium was autoclaved at 121°C, 15 psi for 15 min. It was allowed to cool to about 45°C and then poured into petri dishes and allowed to gel. The plates were then incubated in a B and T Trimline incubator at 37°C for 24 h to check for sterility. Three day old pure cultures were examined. The colour, texture, nature of mycelia or spores and growth patterns were also observed. The three day old pure cultures were used in preparing microscopic slides. A little bit of the mycelia was dropped on the slide and a drop of lactophenol blue was added to it. A cover slip was placed over it and examination was performed under the light microscope at X400 magnification. Identification was carried out by relating features and the micrographs to "Atlas of mycology" by Barnett and Hunter (1972).

Glucoamylase production

Glucoamylase was produced by adopting the method described by Bagheri et al. (2014) with the following modifications. A 250 ml Erlenmeyer flask containing 100 ml of sterile cultivation medium optimized for glucoamylase with 0.3% ammonium sulphate $(NH4)_2SO_3$), potassium dihydrogen phosphate (0.6% KH_2PO_4), magnesium sulphate hepta hydrate 0.1% ($MgSO_4.7H_2O$), 0.01% ferrous sulphate hepta hydrate ($FeSO_4.7H_2O$), 0.1% calcium chloride ($CaCl_2$) and 1%, amylopectin. The flask was stoppered with aluminium foil and autoclaved at 121°C, 15 psi for 15 min. From the PDA slants, fresh plates were prepared and three day 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 was incubated for 7 days at room temperature (30°C). At each day of harvest, flasks were selected from the respective groups and mycelia biomass separated by filtration using filter paper. Each day,

the filtrate was analyzed for glucoamylase activity and extracellular protein concentration till the 14th day of fermentation. After the 14 days pilot SmF studies, 4 and 11th day of fermentation were chosen for mass production.

Glucoamylase assay

Glucoamylase activity was assayed by the method of Parbat and Singhal (2011) with the following modifications. 0.5ml of the enzyme was added into a clean test tube followed by 0.5 ml 1% soluble starch solution in 50 mM acetate buffer (pH 5.5) at 50°C and was allowed to stand for 20 min. 1 ml of 3, 5-dinitrosalicyclic acid (DNSA) reagent was added and boiled for 10 min to stop the reaction. 1 ml of sodium potassium tartarate was added to stabilize the red colour produced. The mixture was then allowed to cool and the glucose released was measured using a JENWAY 6405 UV/VIS spectrophotometer (Beckman/Instruments, Inc., Huston Texas) at 540 nm. Absorbance values were converted to glucose concentrations by extrapolation from the glucose standard curve. Glucoamylase activity unit (U) was expressed as the amount of enzyme releasing one µ mole of glucose equivalent per minute per ml.

α-amylase assay

The α-amylase activity was assayed by adopted the method of Bernfield (1955). The reaction mixture contains 0.5 ml of the enzyme preparation and 0.5 ml of (1% w/v) starch solution in 20mM sodium phosphate buffer (pH 7.0). The reaction mixture was incubated at 55°C for 60 min after which the reaction was stopped by addiction of 1 ml 3, 5-dinitrosalicyclic acid (DNSA) reagent and boiling for 10 min. 1 ml of sodium potassium tartarate was added to stabilize the red colour produced. The mixture was then allowed to cool and the glucose released was measured using a JENWAY 6405 UV/VIS spectrophotometer (Beckman/Instruments, Inc., Huston Texas) at 600 nm. α-amylase activity unit (U) was expressed as the amount of enzyme releasing one µ mole of reducing end groups (maltose) per minute under assay conditions.

Protein determination

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

Partial purification

The crude enzyme preparation was made up to 20 and 70% ammonium sulphate saturation with solid $(NH_4)_2SO_4$ for both GluAgCSV4 and GluAgCSV11, respectively. This was kept at 4°C for 30 h; thereafter it was centrifuged with Cole-palmer VS-13000 micro centrifuge at 4000 rmp 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 above.

Gel filtration chromatography

A volume (20 ml) of the precipitated enzyme was introduced into a (50 × 2.5 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 5 ml/20 min. The protein concentration of each fraction was monitored 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°C

Optimizing glucoamylase activity with respect to pH, temperature, metal ion concentration and substrate concentration

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.

Metal ions concentration

The concentrations, 20, 30, 40 and 50 mM of metal salts ($ZnCL_2$, $CoCl_2$, $MnCl_2$, $FeCl_2$, $PbCl_2$ and $CaCl_2$) were prepared in 20 mM sodium acetate. Each of the reaction mixtures contains 0.5 ml of enzyme solution, 0.5 ml of starch solution (1%) and 1 ml of metal ion solutions (Ca^{2+}, Mg^{2+}, Mn^{2+}, Fe^{2+}, Co^{2+} and Zn^{2+}). The mixtures were incubated for 20 min at the predetermined optimal pHs and temperatures. To study the effect of metal ions on glucoamylase activity/stability, the reaction was carried out with and without metal ions. In all the above experiments, the enzyme activity was calculated as the average of three independent sets of experiments and the standard deviation in all cases was negligible.

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 assayed as described above using starch as substrate.

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 starch solution for 20 min at the respective predetermined optimal pHs and temperatures. The glucoamylase activity was assayed as described above using starch from cassava, guinea corn and tiger nut starch as substrate.

RESULTS AND DISCUSSION

In all the above experiments, the results were calculated as the average of three independent sets of experiments and the standard deviation in all cases was negligible. A fourteen day pilot study was carried out to determine the day of highest protein production, α-amylase and glucoamylase activity in submerged fermentation using amylopectin obtained from cassava starch as carbon source. Enzyme activities were assayed using cassava,

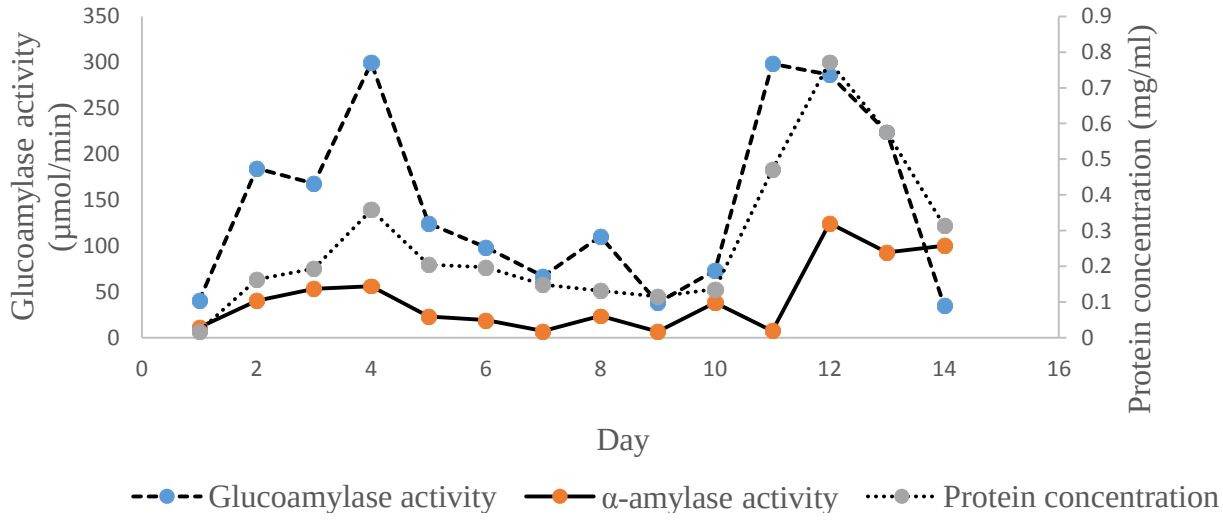

Figure 1. Monitoring the day of highest glucoamylase, α-amylase activity and protein concentration in liquid broth using amylopetin from cassava starch as the only carbon source (cassava starch was used as substrate).

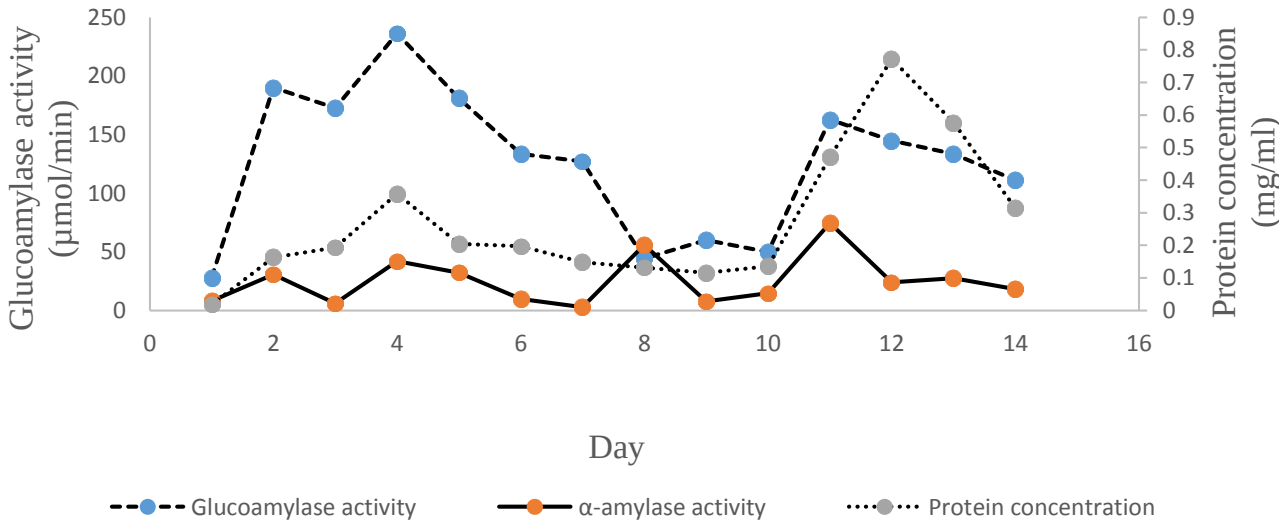

Figure 2. Monitoring the day of highest glucoamylase, α-amylase activity and protein concentration in liquid broth using amylopectin from cassava starch as the only carbon source (tiger nut starch was used as substrate).

guinea corn and tiger nut starch as substrate (Figures 1, 2 and 3). Two major peaks were obtained on day 4 and 11 with activities 299.56 and 298.47 µmol/min, respectively, using cassava starch was used as substrate. Also, using guinea corn starch as substrate, a major peak was obtained on day 4 with glucoamylase activity 236.15 µmol/min, while day 4 exhibited the highest peak with glucoamylase activity (304.30 µmol/min) using tiger nut starch as substrate. Therefore, day 4 and 11 were chosen for mass production of the enzyme. The high increase in glucoamylase activity observed in the study could be due to de-branching activity exhibited by glucoamylase in addition to its α-1, 4-glucosidic activity. Glucoamylase first hydrolyses the amorphous regions before attacking the crystalline regions and both amylose and amylopectin are hydrolysed simultaneously to smaller molecular sizes (Wang and Wang, 2001). The decrease in glucoamylase activity could be due to depletion in the level of branch points in the carbon sources, product inhibition as well as depletion in growth supplement in the broth (Nahar et al., 2008). Alva et al. (2007) reported that the productivity of glucoamylase production is generally subjected to catabolite repression by glucose and other readily

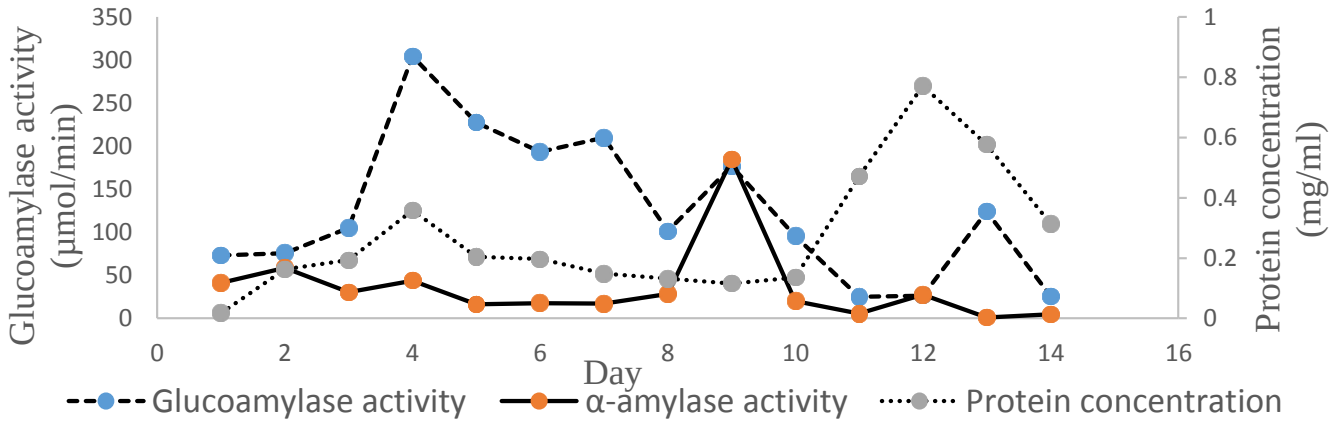

Figure 3. Monitoring the day of highest glucoamylase, α-amylase activity and protein concentration in liquid broth using amylopectin from cassava starch as the only carbon source.

Table 1. Purification table for GluAgCSV4 and GluAgCSV11.

Enzyme	Volume (ml)	Protein (mg/ml)	Total protein	Activity (U)	Total activity	Specific Activity (U/mg)	Purification fold	% Yield
Crude								
GluAgCSV4	1000	0.149	149	193.79	193790	1300.60	1	100
GluAgCSV11	1000	0.132	132	266.58	266580	2019.55	1	100
$(NH_4)_2SO_4$								
GluAgCSV4	350	0.423	148.05	35.29	12351.5	83.43	0.1	6.37
GluAgCSV11	300	0.284	85.20	44.54	13362	156.83	0.1	5.01
Gel filtration								
GluAgCSV4	30	0.286	8.58	53.79	1613.7	188.08	2.3	13.06
GluAgCSV11	30	0.159	4.77	38.99	1169.7	245.22	1.56	8.75

metabolizable substrates.

Ammonium sulphate saturation (20 and 70%) were found suitable to precipitate proteins with highest glucoamylase activity in GluAgCSV4 and GluAgCSV4. After ammonium sulphate precipitation and gel filtration, the specific activities were found to increase from 83.43 to 188.08 U/mg protein with purification fold 2.3 and 156.83 to 245.22 U/mg protein with purification fold 1.56 for GluAgCSV4 and GluAgCSV11, respectively (Table 1). The increase in specific activity could be as a result of exclusion of impurities contributed by ammonium sulphate salt that may have inhibited the enzyme activity.

The optimal pH for GluAgCSV4 were 7.5, 7.0 and 6.0 using cassava, guinea corn and tiger nut starch as substrates, respectively while that for GluAgCSV11 were 7.0, 5.0 and 6.0 using cassava, guinea corn and tiger nut starch as substrates (Figures 4 and 5). This suggests that at optimum pH range of 5.0-7.5, cassava, guinea corn and tiger nut starch hydrolysis by GluAgCSV4 and GluAgCSV11 could be very much enhanced. Parbat and Singhal, (2011) reported optimum pH of 5.0 for glucoamylase from *Aspergillus oryzae* using agro industrial products. Similarly, Puri et al. (2013) reported optimum pH of 5.0 for glucoamylase from *A. oryzae*. Rangabhashiyam et al. (2012) reported optimum pH of 5.2 for glucoamylase obtained from *A. niger* using agro waste as carbon source. Nahid et al. (2012) reported acidic pH range of 4.5 to 4.7 for glucoamylase from *A. niger.* Kumar et al. (2013) reported optimum pH 6.0 for glucoamylase from *A. oryzae* using wheat bran as carbon source. Koc and Metin (2010) also reported optimum pH of 6.0 for glucoamylase produced by *Aspergillus flavus*. Zambare (2010) reported optimum pH of 6.0 for glucoamylase from *A. oryzae*. The result of this study has similarity with that of aforementioned authors. The disparity could be as a result of difference substrates used for the enzyme assay. The optimum pH range suggests that, glucoamylases obtained in this study have

Table 2. Optimization table.

Enzyme	Starch source	optimum pH	Optimum Temperature (°C)	K_M (mg/ml)	Vmax (µmol/min)	Activator	Inhibitor
GluAgCSV4	CSV	7.5	55	90.06	188.67	Ca^{2+}, Zn^{2+}, Fe^{2+},	Pb^{2+}, Mn^{+}, Co^{2+},
	GC	7.0		173.7	434.78		
	TN	6.0		28.57	227.27		
GluAgCSV11	CSV	7.0	50	271.30	1000.00	Ca^{2+}, Co^{2+}	Pb^{2+}, Zn^{2+}, Fe^{2+}, Mn^{+}
	GC	5.0		309.3	1000.00		
	TN	6.0		262.50	1000.00		

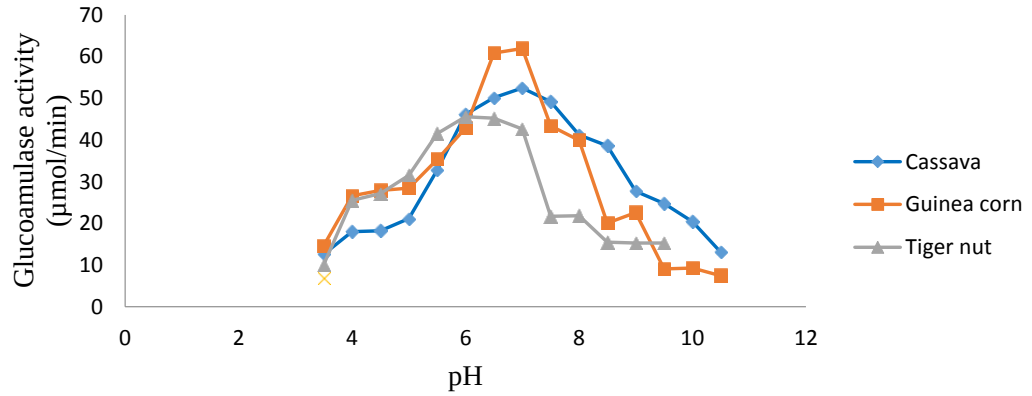

Figure 4. Effect of pH on glucoamylase activity obtained from *Aspergillus niger* on the 4th day of fermentation using amylopectin from cassava starch as carbon source (GluAgCSV4).

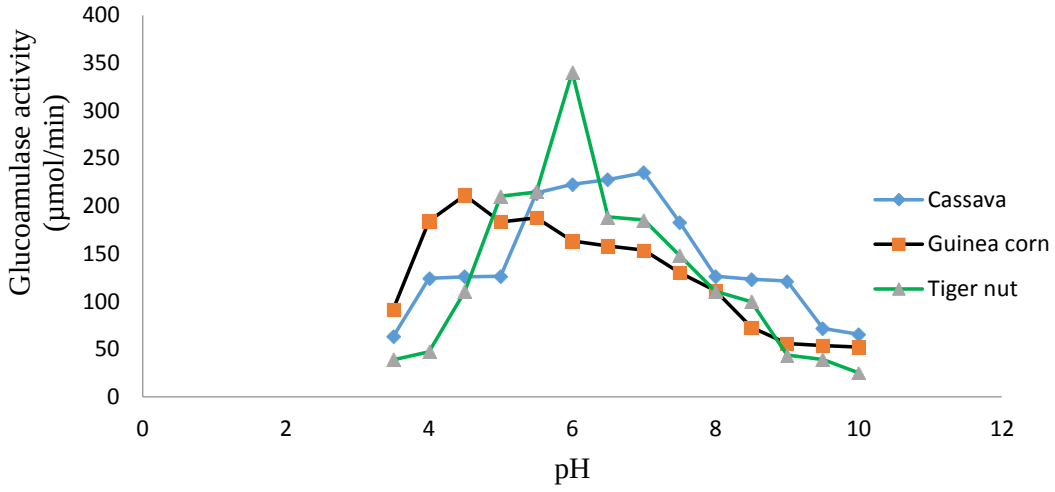

Figure 5. Effect of pH on glucoamylase activity obtained from *Aspergillus niger* on the 11th day of fermentation using amylopectin from cassava starch as carbon source (GluAgCSV11).

wide pH stability which is of industrial importance.

Most of amylases are known to be metal ion-dependent enzymes (Deb et al., 2013). The effect of metal ion on glucoamylase activity showed that, GluAgCSV4 activity was enhanced by Ca^{2+}, Mn^{2+}, Fe^{2+} and Zn^{2+} while Co^{2+} had inhibitory effect on the enzyme but Pb^{2+} inactivated the enzyme (Figure 6). Also, GluAgCSV11 activity was enhanced by Ca^{2+} and Co^{2+} however, Zn^{2+}, Fe^{2+}, Mn^{2+}

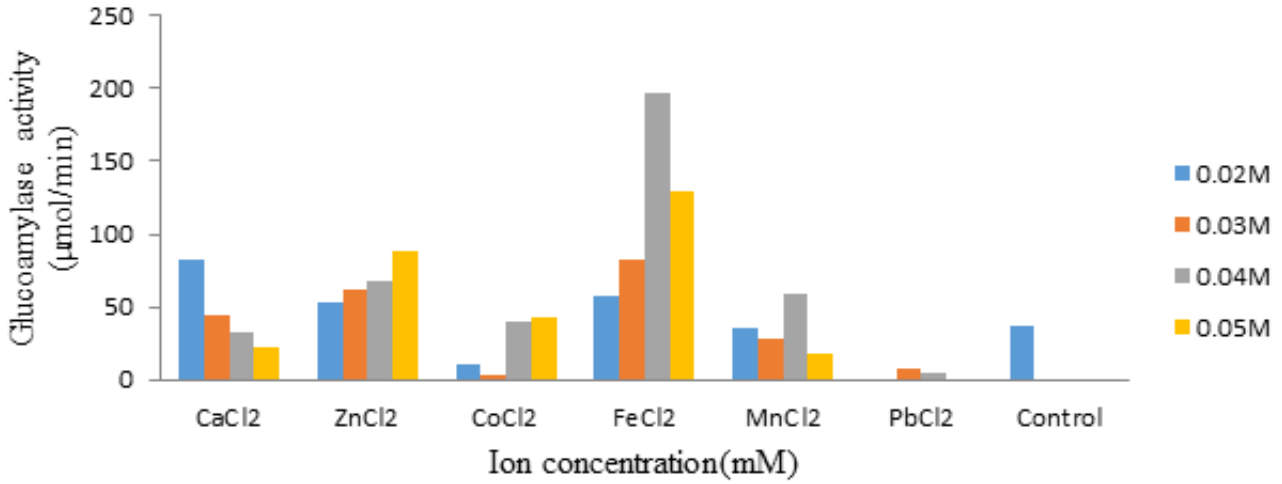

Figure 6. Effect of ion concentration on glucoamylase activity obtained from *Aspergillus niger* on the 4th day of fermentation using amylopectin from cassava starch as carbon source (GluAgCSV4).

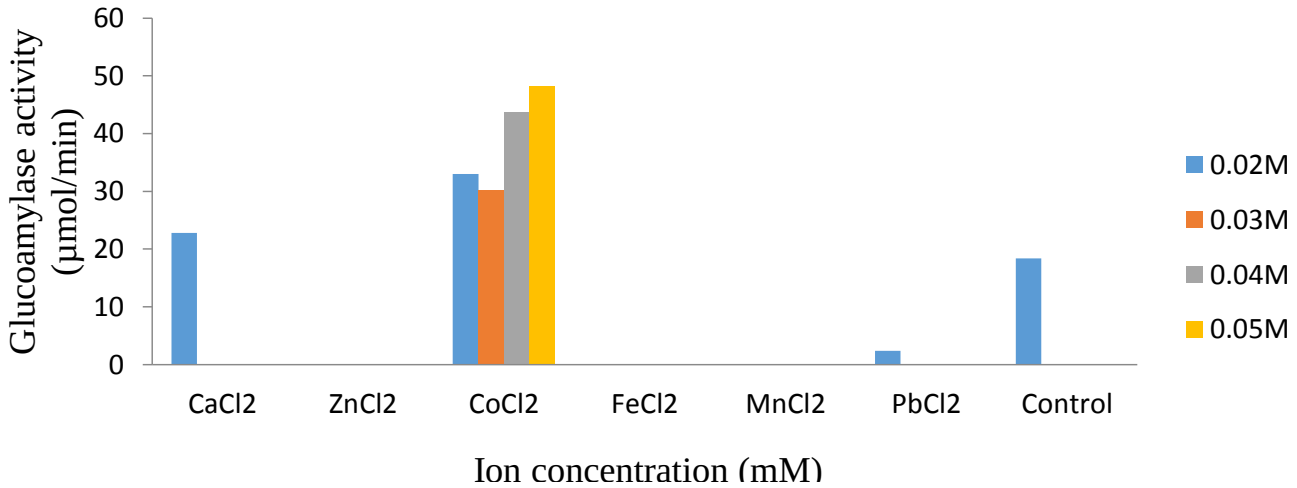

Figure 7. Effect of ion concentration on glucoamylase activity from *Aspergillus niger* on the 11th day of fermentation using amylopectin from cassava starch as carbon source (GluAgCSV11).

and Pb^{2+} completely inactivated the enzyme (Figure 7). The optimum pH range obtained suggest that is more likely to readily form nucleophilic hydroxide ions with metals like Ca^{2+}, Co^{2+}, Fe^{2+}, Mn^{2+} and Zn^{2+} by activating a bound water molecule, this stabilizes the negative charges that are formed at the active sites allowing more weak interactions to hold the substrates in proper orientation at the active site of the enzyme there by enhancing the activity. Also, it has been suggested that metal ions probably force amylases to adopt a compact structure, by salting out the hydrophobic residues of the enzyme, thereby inducing resistance to extreme pH and temperatures (Kareem et al., 2014). Since glucoamylases are used together with α-amylase, which requires Ca^{2+} ions in the liquefaction process, stimulation of glucoamylase activity by Ca^{2+}, Co^{2+}, Fe^{2+}, Mn^{2+} and Zn^{2+} ions can make it more suitable for use in industrial starch bioconversion process. The inhibition on glucoamylase activity inhibition may be due to the complexes formed between metals and the enzyme which prevent it from binding to the substrate and form the product (Aziz and Ali, 2012). Koc and Metin, (2010) reported that Mn^{2+}, Ca^{2+} and Co^{2+} activated glucoamylase obtained from *A. flavus* while Fe^{3+}, and Zn^{2+} inhibited the enzyme activity. Jambhulkar (2012) reported that glucoamylase activity from *Rhizopus nigricans* was enhanced by Mn^{2+} while supplementation of K^{+}, Fe^{2+}, Zn^{2+}, Cu^{2+} and Ca^{2+} significantly reduced the glucoamylase activity. Kareem et al. (2014) also, reported that Mn^{2+}, Ca^{2+}, and Fe^{2+} increased activity for glucoamylase obtained from

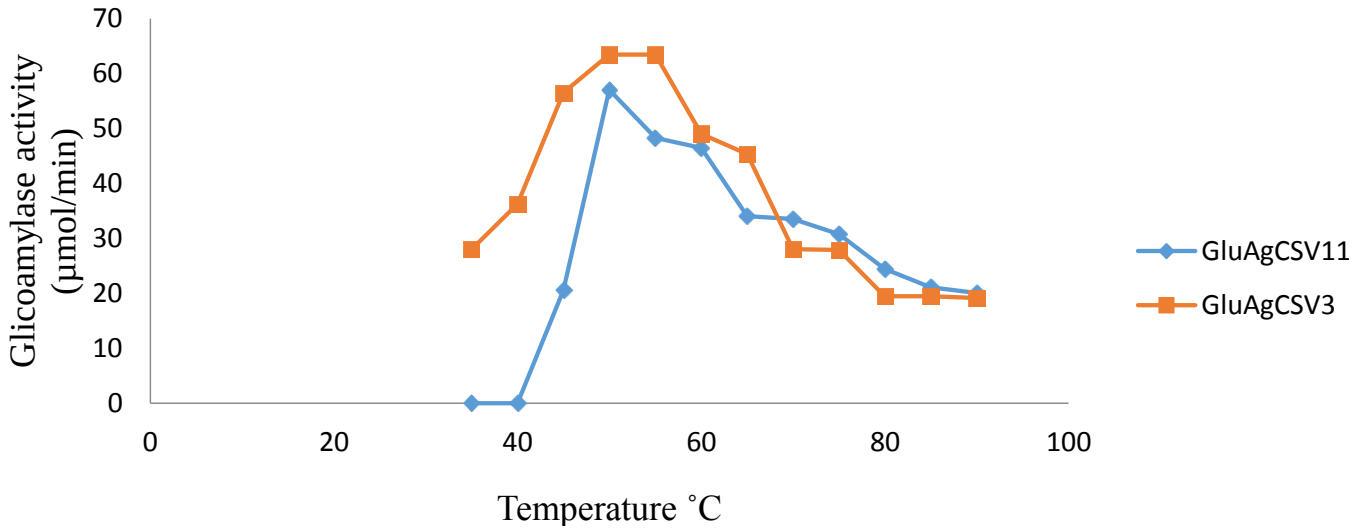

Figure 8. Effect of temperature on glucoamylases (GluAgCSV4 and GluAgCSV11) activity.

Rhizopus oligosporus SK5 mutant. Bagheri et al. (2014) reported that glucoamylase activity was strongly inhibited by Fe^{2+} but Ca^{2+}, Zn^{2+} and Mg^{2+} showed no significant effect on glucoamylase activity.

The optimum temperature for GluAgCSV4 was found to be 55°C while that for GluAgCSV11 was 50°C (Figure 8). Increase in temperature from 35 to 50°C and for the respective enzymes was accompanied by increase in glucoamylase activity beyond which the glucoamylase activity decreased rapidly making 50°C and 50°C the optimal temperatures for both GluAgCSV4 and GluAgCSV11, respectively. These results suggest that GluAgCSV4 and GluAgCSV11 will perform best at 50°C and 55°C in biotechnological applications. The increase in glucoamylase activity as temperature increases may be as a result of change in the enzyme conformation which brings the essential residues to close proximity for catalysis.

The decrease in the activity could be as a result of thermal denaturation at high temperature. Jebor et al. (2014) reported optimum temperature of 40°C for glucoamylase from *A. niger.* Sarojini et al. (2012) also used *A. niger* and reported optimum temperature of 45°C. Deshmukh et al. (2011) reported optimum incubation temperature of 40°C for glucoamylase obtained from sorghum. Cereia et al. (2000) reported temperature optimum of 60°C for glucoamylase obtained from *Scytalidium thermophilum*. El-Gendy (2012) reported optimum temperature range of 50 to 60°C for glucoamylase obtained from marine endophytic *Aspergillus* sp. JAN-25 under optimized solid-state fermentation conditions on agro residues. Koc and Metin (2010) also reported optimum temperature of 60°C for glucoamylase obtained from *A. flavus*. Norouzian et al. (2006) reported optimum temperature of 70°C for glucoamylse obtained from *A. niger.*

The Michealis constant (Km) and maximum velocity (Vmax) obtained from the Lineweaver-Burk plot of initial velocity data at different substrate concentrations were 90.06 mg/ml;188.67 µmol/min, 173.7 mg/ml; 434.78 µmol/min, and 28.57 mg/ml; 227.27 µmol/min for GluAgCSV4 using cassava, guinea corn and tiger nut starch as substrates while the Michaelis constant for GluAgCSV11were 271.30, 309.30 and 262.50 mg/ml using cassava, guinea corn and tiger nut starch as substrates with the same maximum velocity of 1000 µmol/min (Table 2). This suggests that GluAgCSV11 could be of better industrial use since it has very high affinity for the 3 different substrates than GluAgCSV4 and as such has the chance of becoming the better amylase for industrial application than GluAgCSV4 (Figure 9 and 10). Jebor et al. (2014) worked with glucoamylase from *A. niger* and reported K_M and V_{max} of 2.8 mM, 9.8 mM/min, respectively. The results of this study show that glucoamylases obtained from this research have high affinity for their respective substrates, especially starch obtained from underutilized plant (tiger nut).

Conclusion

The results of the study suggest that the glucoamylases obtained from *Aspergillus niger* using amylopectin as carbon source possess the properties to solve biotechnological applications. Also, GluAgCSV11 could be of better industrial use since it has very high affinity for the 3 different substrates than GluAgCSV4 and as such has the chance of becoming the best amylase for local starch fermentation industry than GluAgCSV4

Conflict of interests

The authors did not declare any conflict of interest.

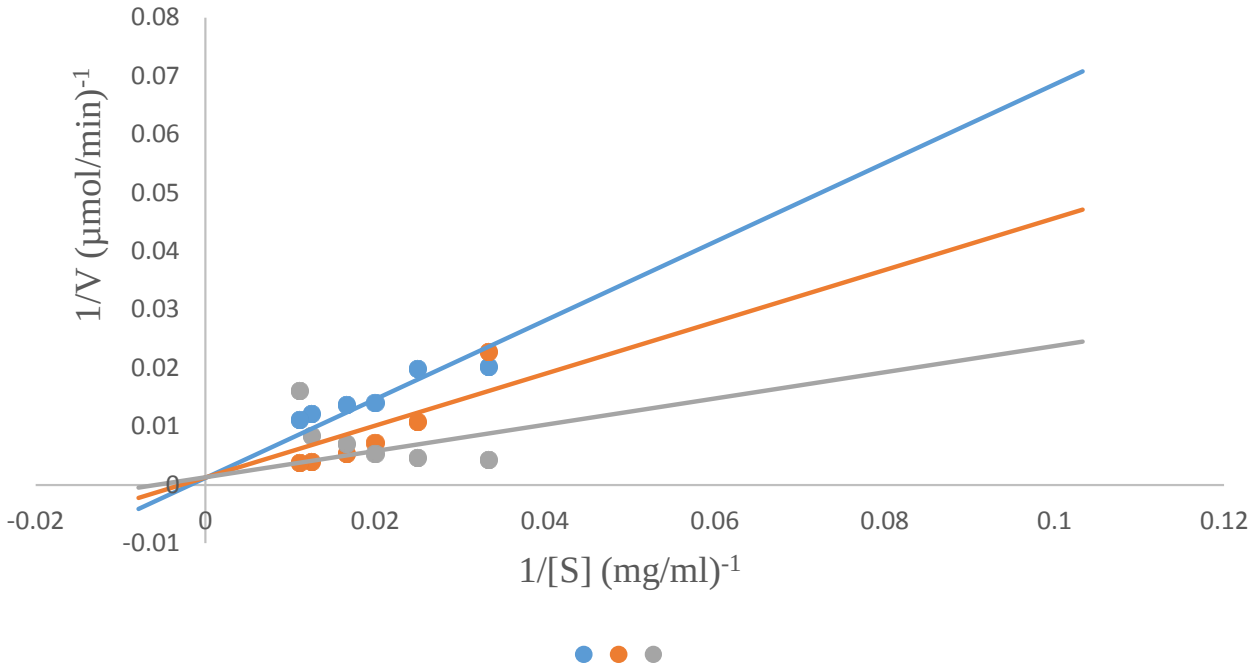

Figure 10. LineWeaver-Burk plot for (GluAgCSV11) using Cassava, guinea corn starch and tiger nut starch as substrate.

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