Nig. J. Biotech. Vol. 36 (2) : 114–126 (Dec 2019)
ISSN: 0189 1731
Available online at
http://www.ajol.info/index.php/njb/index
and www.biotechsocietynigeria.org
DOI: https://dx.doi.org/10.4314/njb.v36i2.12

NIGERIAN JOURNAL OF BIOTECHNOLOGY

Production, purification, and characterization of α-amylase from *Aspergillus niger, Aspergillus flavus* and *Penicillium expansum* using cassava peels as substrate

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Abstract

Cassava peels are waste generated from cassava processing and are mostly disposed of in Nigeria by dumping indiscriminately in landfills/waste dumps where they remain as huge mounds and constitute nuisance to the environment. This study was carried out using cassava peels as substrate for the production of α – amylase from three fungi (*Aspergillus flavus, Aspergillus niger* and *Penicillium expansum)* selected from twelve microbial isolates obtained from biodegrading cassava peels. The fungi were isolated using cassava peel agar medium and the α – amylase was produced by the solid state fermentation process. The α – amylase was assayed by measuring the decrease in staining power of starch with iodine reagent at 620nm and purified by using Sephadex G-100 and Sephadex C-50. The α – amylase was characterized by examining the effect of temperature, stability at 70°C, pH, substrate concentration, metal ions and EDTA. The results show that the purification fold and specific activity were 95.727, 1.053 Units/mg protein; 81.830, 0.982 Units/mg protein and 85.784, 0.686 Units/mg protein for *A. flavus, A. niger* and *P. expansum* respectively. The optimum temperature and pH were 45°C and 4.5 respectively. It was observed that the α – amylase still retained some activity after heating at 70°C for 35 min. The α – amylase activity increased with increase in substrate concentration and metal ion concentration (Na^+, K^+, Mg^{2+} and Ca^{2+}) but decreased with increase in heavy metal ion concentration (Hg^{2+} and Pb^{2+}) and EDTA.

Key words: Cassava peels, α – amylase, fungi, starch and Sephadex C – 50.
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Introduction

Cassava (*Manihot esculenta* Crantz) is one of the most popular and widely consumed food crop in Nigeria; it is a cornerstone of food security. Nigeria is the world largest producer of cassava (Kayode, 2016). Cassava processing produces large amounts of waste of which the peels constitute a large proportion. Approximately 98 percent of Nigeria's cassava peels annually are wasted due to the constraints associated with drying and the concerns about safety of its use, particularly the hydrocyanide and mycotoxins related food poisoning (ILRI news, 2015). Cassava peels are perishable and are mostly disposed of by dumping indiscriminately in landfill/waste dumps where they remain as huge mounds and constitute nuisance to the environment (Aisien and Ikenebomeh, 2017). Cassava peels are made up of 9.65% starch (Aisien and Ikenebomeh, 2017). Starch is a polymer of glucose linked by the glycosidic bond, amylose and amylopectin are the two types of glucose polymers present in starch. Amylose is a linear polymer consisting of up to 6000 glucose units with α-1,4 glycosidic bonds, while amylopectin consists of short α-1,4 linked to linear chains of 10-60 glucose units and α-1,6 linked to side chains with 15 to 45

glucose units (de Souza and eMagalhaes, 2010). Amylose constitutes 20-25% of the starch molecule and the remaining 75-80% of the starch molecule is amylopectin (Sundarram and Murthy, 2014).

Amylases are important hydrolase enzymes which randomly cleave internal glycosidic linkages in starch molecules and hydrolyze them to yield dextrins and oligosaccharides (Sundarran and Murthy, 2014). The amylase enzymes are of three different types which are the α - amylase, β - amylase and Ɣ – amylase (Sivaramakrishnan et al., 2006).

α – amylase (α – 1, 4 – glucan – 4 - glucanohydrolase) catalyses the initial hydrolysis of starch into shorter oligosaccharides through the cleavage of α – D-(1 -4) glycosidic bonds (Tangphatsornruang et al., 2005). It neither cleaves the terminal glucose residues nor α – 1, 6 – linkages. The end products of α – amylase action are oligosaccharides with varying length of α – configuration and α – limit dextrins which constitute a mixture of maltose, maltotriose and branched oligosaccharides of 6 – 8 glucose units that contain both α – 1, 4 and α – 1, 6 linkages (Whitcomb and Lowe, 2007). In the past, starch was hydrolyzed into glucose by acid hydrolysis, the operating conditions were high acidic concentration and high temperature. These limitations have been overcome by the use of α – amylase. α – amylase have replaced acid hydrolysis in over 75% of starch hydrolyzing processes (Rajunathan and Padhmadas, 2013). α – amylase has wide applications in the brewing, detergent and food industries (Mukesh et al., 2012), textile, clinical, medical, analytical chemistry and pharmaceutical industries, (Gupta et al., 2003).

α – amylase can be sourced from plants, animals and microbes (Oboh, 2005, Bole et al., 2013, Erdal et al., 2010). The microbial source of α – amylase is preferred to other sources because of its plasticity and vast availability (Shivaji et al., 2013). α – amylase is produced by several bacterial, fungi and genetically modified species of microbes (Sundarram and Murthy, 2014). The *Bacillus* sp is the most widely used source among the bacterial species while among the fungal sources the *Aspergillus* species are mostly used with only few species of *Penicillium*. α – amylases are produced by the solid state fermentation (SSF) and submerge fermentation (SMF) processes (Couto and Sanroman, 2006, Kunamneni et al.,2005). The solid state fermentation process has gained huge interest in recent years due to advantages like yield and high specificity, simple technique, low moisture contents, which prevent bacterial contamination, low capital investment, lower levels of catabolite repression, and better product recovery (Couto and Sanroman, 2006). The SSF is also advantageous in that it uses nutrient rich waste materials which can be easily recycled as substrates (Kunamneni et al., 2005). Sodhi et al (2005) produced α – amylase from *Bacillus* sp using wheat bran, rice bran and corn bran, hence the objective of this study is to produce α – amylase from fungi sources using cassava peels as substrates.

Materials and Methods

Isolation of cassava peel degrading fungi

Sterile cassava peel agar medium (CPAM) was used for the isolation of cassava peel degrading fungi. CPAM was prepared by the addition of 15 g of Agar to 1000 ml of cassava peel extract and sterilized at 120^0C for 15 min. Cassava peel extract was prepared by adding 50 g of ground fresh cassava peels to 1000 ml of distilled water and then filtered through cheese cloth. Serial dilution of 10^{-7} of decomposing cassava peels were used to prepare three plates and incubated aerobically at 28 ± 02^0C for 72 hours. The fungal isolates were sub cultured into sterile malt extract agar plates and further sub cultured into the other sterile malt extract agar plates to obtain pure cultures.

Characterization and identification of fungal isolates

The fungi isolates grown on sterile malt extract agar were identified based on cultural and morphological characteristics using standard fungi identification methods outlined by Barnett and Hunter (1972), Pitt (1979) and Gilman (2001).

Establishment of biodegradability

Fresh cassava peels were washed under running tap water and surface sterilized in 10% v/v sodium hypochlorite for 1hr. (Ikediugwu and Ejale, 1980). The cassava peels in separate sterile petri dish was each inoculated with a 4 day old fungi culture and incubated at 28 ± 2^0C for 7 days. The rate of biodegradability was then examined by measuring the areas degraded by each isolate. This was done in triplicates. Selection of fungal species for extracellular α–

amylase production was done based on the rate of zone of clearance around the colony.

Preparation of inoculums and solid state fermentation

Seventy –two hrs old cultures were used in the preparation of the inoculums, from which a spore suspension with a spore load of approximately 6 x 10^4 spores per ml was made.

Apparently healthy freshly harvested cassava tubers were washed and peeled. The peels were further washed with tap water. A cork borer was used to make cores of cassava peels of 3 mm thickness to expose more surface area for fungal attack. The cassava peel discs were surface sterilized in 3% w/v sodium hypochlorite solution for one hr. The cassava peel discs were afterwards rinsed with six changes of sterile distilled water to remove the residual effect of the sodium hypochlorite. Twenty grams of cassava peel discs were weighed out and transferred into 250ml Erlenmeyer flasks containing 10ml of sterile distilled water for solid state fermentation. Each flask was inoculated with 1ml of the spore suspension and incubated at $28^0C \pm 2^0C$ for 8 days. The experimental set ups were examined daily for fungal growth and degradation of the cassava peel discs.

Assay for α-amylase.

Two grams of cassava peels from the inoculated flasks were homogenized with 30 ml of 2 mM sodium acetate buffer pH 6.8 for 10 min. The homogenate was centrifuged at 6000 x g for 15 min, the supernatant fraction was then incubated for 15 min at 70^0C to inactivate β-amylase. Thereafter, it was cooled to 28 ± 2^0C and used for the assay of α-amylase according to the method of Bidderback (1971) by measuring the decrease in staining power of starch with iodine reagent at 620 nm. α-Amylase activity was expressed as decrease in absorbance min^{-1} ml^{-1} of liquor extract. The reaction mixture contained 2.0 ml of saturated starch solution in a test tube and 1.0 ml of crude enzyme. A starch blank was set up with 2.0 ml of saturated starch solution and 1.0 ml of boiled enzyme. The tubes were incubated at 37^0C for 10 min. and reaction was then stopped by the addition of 1.0 ml of iodine reagent. Absorbance was read at 620 nm using a spectrophotometer against a blank containing 3.0 ml of distilled water and 1.0 ml of iodine reagent. α-amylase activity was expressed as decrease in absorbance in minute/ml of liquor extract.

Enzyme extraction

Fungal growth in the incubated flasks were noticed at about 48 hours of incubation after which daily analysis for the detection of α – amylase activity commenced. Three flasks, each of *Aspergillus niger, Aspergillus flavus*, and *Penicillium expansum* were analyzed daily. The cassava peel discs in each flask were chilled for 20 min and homogenized with cooled liquid extract solution (1:1 w/v). The extract solution consists of 0.5 M NaCl containing 5 mM of NaN_3 to prevent microbial contamination. The homogenate from each flask was clarified by passing it through glass fiber filter (Whatman Gf/A). Each filtrate was analyzed for α-Amylase. The pH and the protein content of filtrate (crude enzyme) were determined. After the peak of enzyme production was reached few daily analyses were still carried out to confirm the established peak.

Purification of α-Amylase

Ammonium sulphate precipitation

The crude enzyme was precipitated with 90% ammonium sulphate solution at 4^0C for 24hrs. The resulting precipitate was collected by centrifugation at 20,000 x g for 30min. The precipitate was dissolved in 10 ml of citrate phosphate buffer (pH 6.0) and dialyzed against two changes of the same buffer for 24hrs. The protein content of the enzyme was determined by the method of Lowry et al., (1951) sited in Biancarosa et al., (2017).

Further purification using Sephadex G-100 and Sephadex C- 50

The dialyzed enzyme concentrate (10ml) was applied to the Sephadex G-100 column and eluted

with 0.05 M citrate phosphate buffer containing 5 mM of NaN_3 as described by Olutiola and Ayres (1973). Three fractions (5 ml per tube) were collected and the protein content in each was determined by the method of Lowry et al., (1951). These fractions were further analyzed for enzyme activities. The fraction of the enzyme which showed highest enzyme activity after gel filtration were concentrated in a rotary evaporator (Buchi Rota Vapor R) at 28 ± 2^0C. The enzyme concentration was made up to 10ml by adding 0.2 M citrate phosphate buffer (pH 6.0) and applied to the column of Sephadex C – 50. This was then eluted with the same buffer containing 0.2 MKCL. Fractions of 5ml per tube, were collected and their protein content

measured. The enzyme activity was also determined.

Characterization of the purified enzyme

The effect of some physiochemical parameters on the activities of the purified (SP C - 50 fraction) enzyme was examined.

Optimum temperature determination

In order to determine the optimum temperature for α-Amylase activity of the purified enzyme, the reaction mixture was incubated at 5, 10, 15, 20, 25, 30, 35, 40, 45 and 50^0C for 3hrs. Enzyme activity was determined after incubation for each of the above temperature.

Effect of heating at 70^0C

The effect of heat on the stability of the enzyme was determined. Samples of the purified enzyme were heated at 70^0C for different periods of time (0, 5, 10, 15, 20, 25, 30 and 35min respectively). The activity of the heated enzyme was measured by incubating the enzyme substrate mixture at 35^0C for 3hrs.

Optimum pH determination

Optimum pH was determined by preparing substrates with pH ranging from 3.0 to 9.0 citrate

phosphate buffer (0.02 M) was used to prepare substrate of pH 3.0, 3.5, 4.0, 4.5, 5.0, 5.5, 6.0, 6.5 and 7.0 whereas 0.2 M Tris-HCL buffer was used to prepare substrates of pH.7.5, 8.0, 8.5 and 9.0 starch (10%) of pH ranging between 3.0 and 7.0 were employed as substrate for the enzyme assay. The pH of the substrate was adjusted where necessary with 0.1 M HCL and 0.01 M NaOH.

Effect of substrate concentration

In order to determine the concentration of the substrate at which enzyme activity will be highest, the substrate concentration was varied between 2 mg/ml-18 mg/ml.

Determination of the effect of metal ions

The effect of some metal ions at various concentrations on the activity of the enzyme was determined. The substrate was incubated with each test metal ion at 4^0C for 3hrs respectively before being employed in enzyme assays. Different concentration (5 – 40 mg/ml) of Na^+, k^+ Ca^{2+} and Mg^{2+} (sodium chloride, potassium chloride, calcium chloride and magnesium chloride) ions were employed for the investigation. Concentrations of 1 to 10 mg/ml were employed for Hg^{2+} and pb^{2+} (mercury II chloride and lead II chloride) ions.

Effect of Ethylene diamine tetra acetic acid

The effect of ethylene diamine tetra acetic acid at concentrations of 1 to 10 mg/ml on the activity of the enzyme was determined. The substrate was incubated initially with ethylene diamine tetra acetic acid at 4^0C for 3hrs before they were employed in enzyme assay. All the analysis was performed in triplicate. The mean value was taken as the result.

Results and discussion

The following twelve fungi shown in Table 1 were isolated from cassava peels undergoing biodegradation, they were identified based on their cultural and morphological characteristics. They were found to be capable of degrading cassava peels. The order of degradability is *Aspergillus flavus, > Aspergillus niger > Penicillium expansum > Aspergillus tamarri > Penicillium frequentants> Penicillium. crustaceum > Geotrichum candidum > Fusarium oxysporum > Rhizopus oryzae > Trichoderma viride > Mucor hiemalis > Saccharomyces cerevisiae* as shown in Table 1.

Table 1: Biodegradability of cassava peels

Fungi	Degraded distance (mm)
Aspergillus flavus	45.10 ± 2.4
Aspergillus niger	42.70 ± 3.2
Penicillium expansum	37.10 ± 5.0
Aspergillus tamari	33.30 ± 2.8
Penicillium frequentans	26.60 ± 1.9

Penicillium crustaceum	24.40 ± 3.6
Geotrichum candidum	19.80 ± 2.0
Fusarium oxysporum	18.00 ± 1.7
Rhizopus oryzae	15.20 ± 3.5
Trichderma viride	15.00 ± 2.1
Mucor hiemalis	12.90 ± 1.5
Saccharomyces cerevisiae	12.88 ± 1.5

Results are means ± standard deviations of three determinations per microbial isolate.

Three fungi (*Aspergillus flavus, Aspergillus niger* and *Penicillium expansum)* with the highest degraded distance (Table 1) were selected for enzyme studies. These resulted agreed with that of Adeniran and Abiose (2009) who reported that *A. flavus* showed greater potential in the production of α-amylase among the other isolates they tested on agricultural waste.

The crude enzyme produced by the selected fungi inoculated cassava peels exhibited α – amylase

activities. The peak of α-amylase production was recorded at day 4, and this with the associated total protein and pH are presented in Table 2

The α-amylase activity in the crude enzyme from cassava peels inoculated with *Aspergillus flavus, Aspergillus niger and Penicillium expansum* were 0.097 units, 0.088 units and 0.058 units respectively (Table 2). This shows that the α – amylase activity increased in the order *A. flavus> A. niger> P expansum*. This order was also maintained after the purification process. The α –amylase activity after purification was a function of the purification process.

The α-amylase activity for the ammonium sulphate purification process was lower than that of the crude along with the total protein and percentage yield but the specific activity and purification fold were higher, for *A. flavus,* they were 0.082 units, 1.206 mg, 84.536%, 0.068 units/mg protein and 6.181 (Table 3), for *A. niger,* they were 0.073 units, 1.203 mg, 82.955%, 0.066 units/mg protein and 5.500 (Table 4) and that for *P. expansum* were 0.049 units, 0.975 mg, 84.453%, 0.050 units/mg protein and 6.282 respectively (Table 5). The α-amylase activity for the Sephadex G-100 purification process were also lower than that of the ammonium sulphate purification process along with the total protein and percentage yield while the specific activity and purification fold were higher, for *A. flavus,* they were 0.070 units, 0.214 mg, 72.165%, 0327 units/mg protein and 29.737 (Table 3), for *A. niger,* they were 0.062 units, 0.190 mg, 70.455%, 0.326 units/mg protein and 27.170 (Table 4) and that for the *P. expansum* were 0.041 units, 0.165 mg, 70.690%, 0.249 units/mg protein and 31.061 (Table 5). The α-amylase activity for the Sephadex C-50 purification process also followed the same pattern for Sephadex G-100 purification process (Tables 3,4 &5).

Table 2: α-Amylase activity, total protein and pH at the peak of enzyme production

Fungus	Total α-Amylase activity (units)	Total protein (mg)	pH
Aspergillus flavus	0.097	8.68	4.5
Aspergillus niger	0.088	7.280	4.5

Penicillium expansum	0.058	6.990	4.5

The purification profiles for the production of α-amylase from *Aspergillus flavus, Aspergillus niger* and *Penicillium expansum* are presented in Tables 3-5.

Table 3: Purification profile of α-amylase obtained from cassava peels degraded by *Aspergillus flavus*

Fraction	Total α-Amylase activity (units)	Total protein (mg)	Specific activity (unit/mg protein)	Yield %	Purification fold
Crude	0.097	8.680	0.011	100.000	1.000
$(NH_4)_2SO_4$	0.082	1.206	0.068	84.536	6.181
G – 100	0.070	0.214	0.327	72.165	29.737
SP C – 50	0.060	0.057	1.053	61.856	95.727

Table 4: Purification profile of α-amylase obtained from cassava peels degraded by *Aspergillus niger*

Fraction	Total α-Amylase activity (units)	Total protein (mg)	Specific activity (unit/mg protein)	Yield %	Purification fold
Crude	0.088	7.280	0.012	100.000	1.000
$(NH_4)_2SO_4$	0.073	1.203	0.066	82.955	5.500
G – 100	0.062	0.190	0.326	70.455	27.170
SP C – 50	0.053	0.054	0.982	60.227	81.830

Table 5: Purification profile of α-amylase obtained from cassava peels degraded by *Penicillium expansum*

Fraction	Total α-Amylase activity (units)	Total protein (mg)	Specific activity (unit/mg protein)	Yield %	Purification fold
Crude	0.058	0.990	0.008	100.000	1.000
$(NH_4)_2SO_4$	0.049	0.975	0.050	84.453	6.282
G – 100	0.041	0.165	0.249	70.690	31.061

SP C – 50	0.035	0.051	0.686	60.345	85.784

Varalakshim et al., (2009) reported higher α-amylase activity for *A. niger.* Similarly, Shivakumar et al., (2012) also reported higher α-amylase activities and higher values of specific activities for the *Aspergillus* and *Penicillium* species.

The effect of temperature on α-amylase activity is shown in Figure 1. The optimum temperature obtained for the α-amylase produced by the above fungi were found to be 45^{0}C. The temperature optima obtained in this study is similar to that reported by Sundarram and Murthy (2014) for α-amylase production by *B. Licheniformis*. Prakasham et al. (2006) also reported an optimum temperature of 45^{0}C for *Penicillium janthinelum* while Thippeswany et al. (2006) reported a temperature optimum of 50^{0}C for *Aspergillus oryzae*. In this study it was observed that the α-amylase activity for the fungi examined increased from 5^{0}C to 45^{0}C after which there was a decline in activity when temperature was increased.

The stability at 70^{0}C of α-amylase produced in this study is shown in Figure 2. It was observed that there was a gradual decrease in the activity of the α-amylase with *P. expansum* recording the highest level of decrease. This was followed by *A. niger* before *A. flavus.* The order of decrease was *P. expansum>A. niger > A. flavus.* This result is in agreement with that reported by Bole et al. (2013), where there were decrease in the α-amylase activity of a *Bacillus sp* when temperature was increased from 10^{0}C to 60^{0}C.

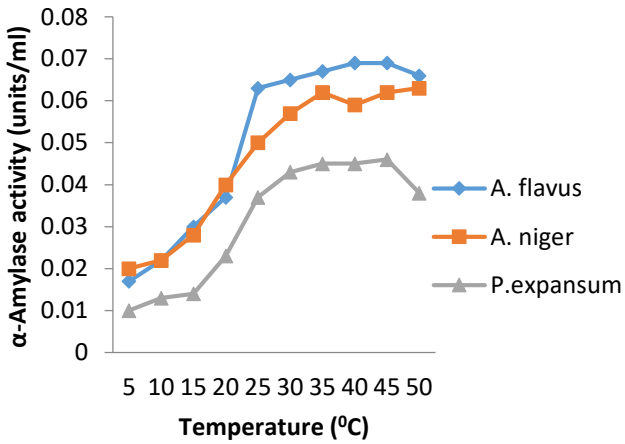

Fig. 1: Effect of temperature (^{0}C) on the activity of purified α-amylase from cassava peels

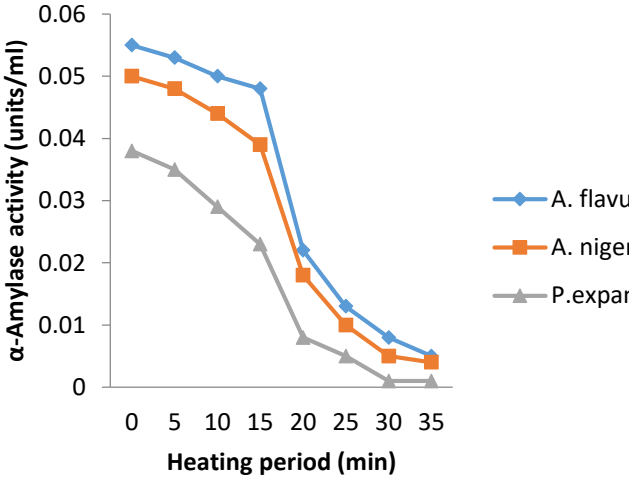

Fig. 2: Effect of heating (70^{0}C) on the activity of purified α-amylase from cassava peels

The effect of pH on the activity of α-amylase is shown in Figures 3. The activity of the α-amylase from A. flavus increased from 0.030 units/ml at a pH of 3.5 to 0.059 units/l at pH 4.5 and thereafter decreased to 0.018 units/ml at pH 7.5. There were similar increases before decreases in α-amylase activity for *A. niger* (increase from 0.035 units/ml at a pH of 3.5 to 0.057 units/ml at PH 4.5 and decreased to 0.019 unit/ml at pH. 7.5) and *P. expansum (*increased from 0.020 units/ml at pH. 3.5 to 0.034 units/ml at pH 4.5 and decreased to 0.008 units/ml at pH. 7.5). The results indicate that the optimum pH for α-amylase activity from the above fungi was 4.5. This result differs from those of de Souza and eMagalhaes (2010), where a pH optimum of 4.95 was reported for *Aspergillus niger* and an optimum pH of 6.0 for *Aspergillus oryzae*.

The effect of substrate concentration on the activity of α-amylase is shown in Figure 4. The activity of the α-amylase from *A. flavus* increased from 0.020 units/ml to 0.085 unit/ml as the substrate concentration increased from 2 mg/ml to 14 mg/ml. Further increase in substrate concentration from 14 mg/ml to 18 mg/ml resulted in no increase in the α-amylase activity. Similar trends were observed for *A. niger* and *P. expansum.* The order of increase in α-amylase activity with increase in substrate

concentration is *A. flavus* > *A. niger* and *P. expansum*. This result agrees with the report of Aisien and Ikenebomeh (2017), that increase in substrate concentration leads to increase in enzyme activity until a point whereby further increase in substrate concentration will no longer lead to increase in enzyme activity.

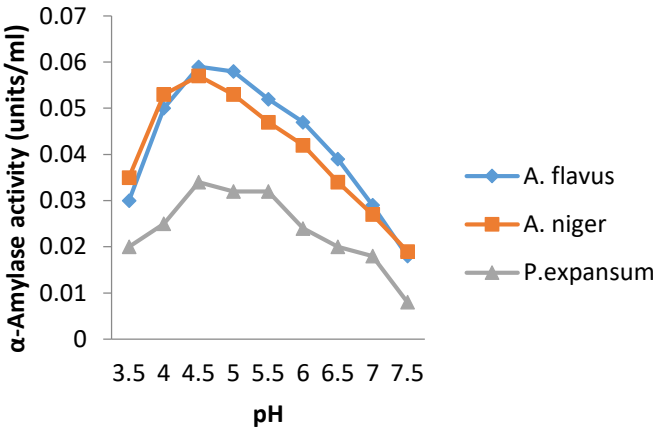

Fig. 3: Effect of pH on the activity of purified α-amylase from cassava peels

Similar explanation was also reported by Sohail et al. (2014), for the amylase of mango mealy bug where enzyme activity was found to increase from 1% to 3% starch substrate, after which further increase in substrate did not lead to increase in enzyme activity.

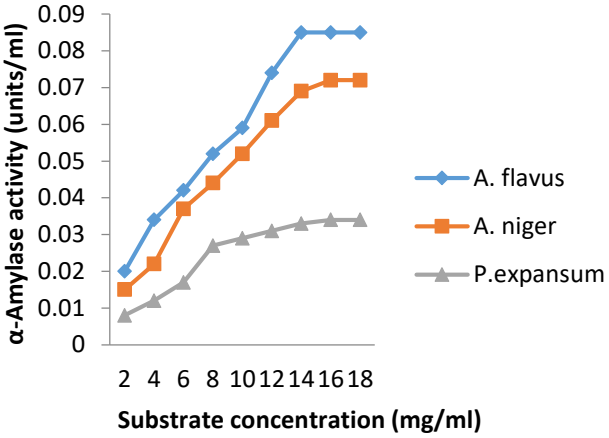

Fig. 4: Effect of substrate concentration on the activity of purified α-amylase from cassava peels

The effect of metal ions (K^+, Na^+, Mg^{2+} and Ca^{2+}) concentration on the activity of the α-amylase are shown in Figures 5 to 8. The results show that increase in metal ions resulted to increase in enzyme activity. When potassium ion concentration was increased from 0 mg/ml to 30 mg/ml there were increases in the α-amylase activity from *A. flavus, A. niger* and *P. expansum* respectively. Further increase in K^+ concentration (35 mg/ml) did not lead to any increase in α-amylase activity of *A. flavus* and *A. niger* but lead to an increase in the α-amylase activity of *P. expansum*. However, the α-amylase activity decreased when 40 mg/ml of K^+ was used in all (*A. flavus, A. niger* and *P. expansum*). Similar trend was observed for Mg^{2+} and the result for both ions shows *A. flavus* > *A. niger* and *P. expansum.* Increase in the concentration of Na^+ and Ca^{2+} showed slight variation in pattern from those of K^+ and Mg^{2+}. However, all the metal ions enhanced the activity of α-amylase irrespective of the source. The order was Ca^{2+} < Na^+ < Mg^{2+} < K^+. These results agreed with those reported by Prakesh et al. (2011), where K^+ moderately increased the enzyme activity to a certain extent and then suppressed. Carvalho et al. (2008), reported that although K^+ did not stimulate the activity of α-amylase from *Bacillus subtilis* no significant inhibition of activity was observed. Saxena and Singh (2011), reported the enhancement of amylase activity in the presence of Na^+, Mg^{2+} and Ca^{2+}. Prakesh et al. (2011), reported that Na^+ had no detectable influence on the activity of amylase. Jha et al. (2013), considered Mg^{2+} to be the best metal ion for enhancing the genus *Aspergillus,* mycelia growth for amylase production, this was followed by Ca^{2+} according to them. Similarly, Sohail et al. (2014), reported the enhancement of amylase activity with Ca^{2+}. All α-amylase bind at least on strongly conserved Ca^{2+} that is required for structural integrity, and for enzymatic activity (Aghajari et al., 2002), hence the ability of Ca^{2+} ion to enhance amylase activity.

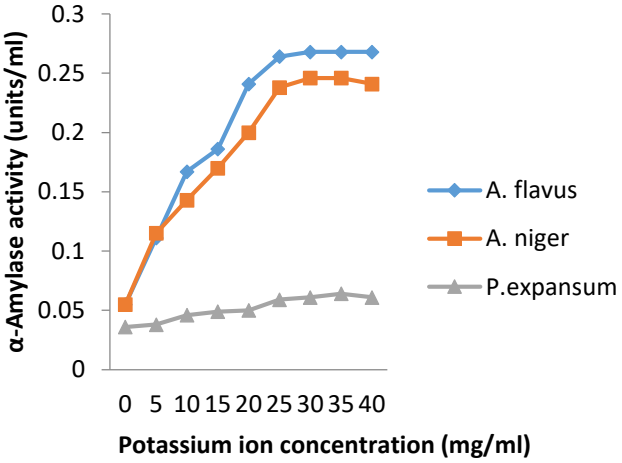

Fig. 5: Potassium ion (K^+) on the activity of purified α-amylase from cassava peels

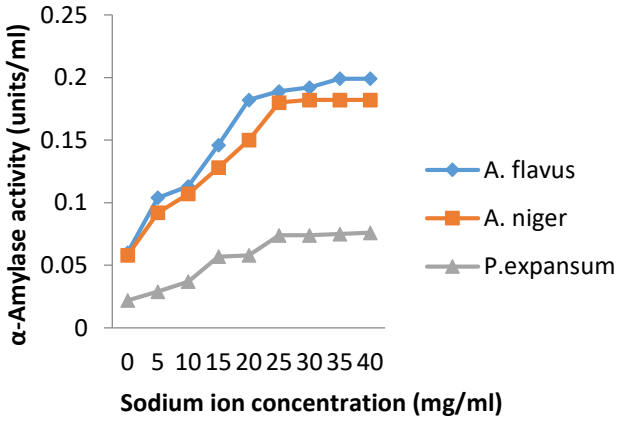

Fig.6: Sodium ion (Na^+) on the activity of purified α-amylase from cassava peels

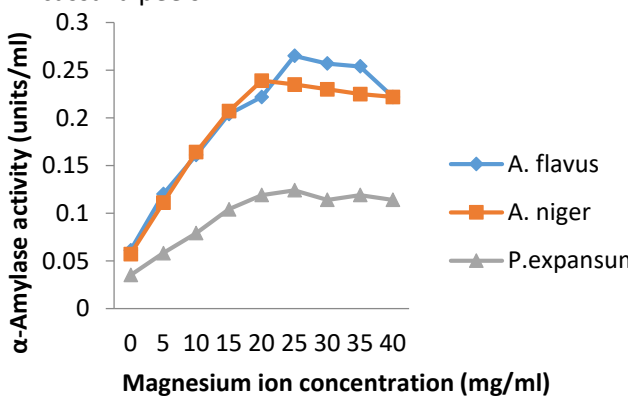

Fig. 7: Effect of Magnesium ion (Mg^{2+}) on the activity of purified α-amylase from cassava peels

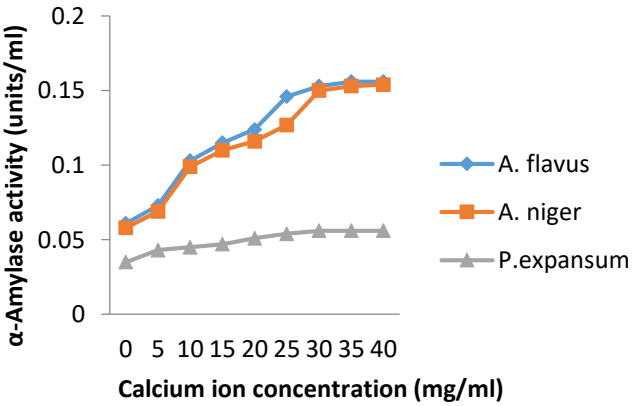

Fig. 8: Effect of Calcium ion (Ca^{2+}) on the activity of purified α-amylase from cassava peels

The effect of heavy metal ions (Hg^{2+} and Pb^{2+}) concentration on the activity of α-amylase are shown in Figures 9 – 10. The α-amylase activity from *P. expansum* drastically decreased from 0.033 units/ml to 0.002 units/ml as the Hg^{2+} concentration increase from 0 mg/ml to 4 mg/ml, further increase in Hg^{2+} concentration from 4 mg/ml to 10 mg/ml resulted in the loss of enzyme activity. Similar drastic decrease in activity was observed for the α-amylase from *A. Flavus* and *A. niger,* but the -amylase became deactivated when 8 mg/ml of Hg^{2+}concentration was applied. Lead ion (Pb^{2+}) was also found to gradually decrease the activity of the α-amylase irrespective of the source. However, it was observed that increasing the concentration of the lead ion from 0 mg/ml to 10 mg/ml did not result in α-amylase deactivation but only resulted in decrease in activity. In general, the effect of heavy metals (Hg^{2+} and Pb^{2+}) on the activity of α-amylase was inhibitory at all concentrations (Figures 9 and 10). This report is in agreement with that of Sohail et al (2014) who reported the inhibition of amylase activity by Pb^{2+} and Hg^{2+}.

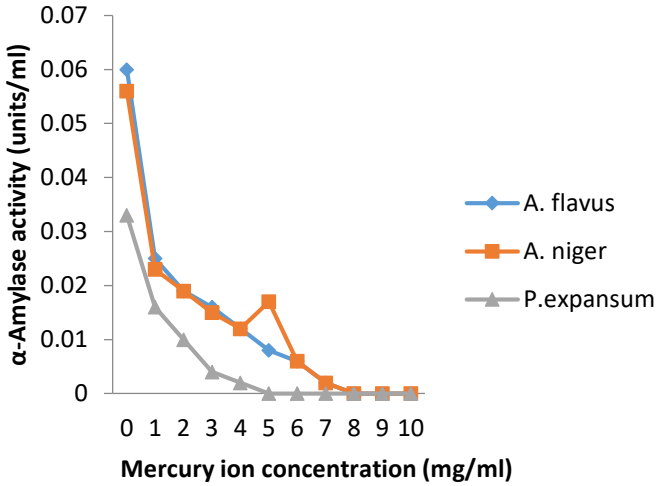

Fig. 9: Effect of Mercury ion (Hg^{2+}) on the activity of purified α-amylase from cassava peels

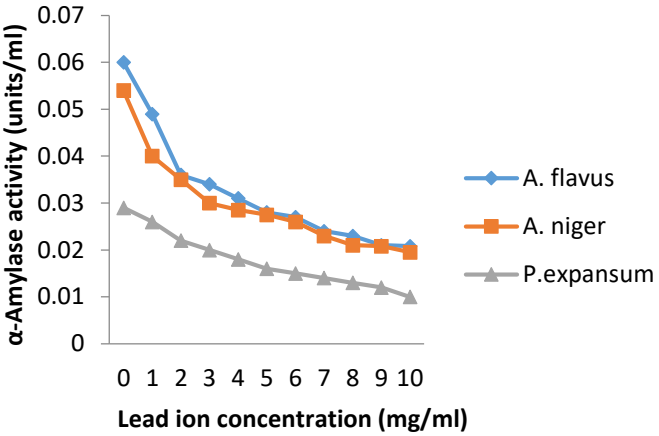

Fig. 10: Effect of Lead ion (Pb^{2+}) on the activity of purified α-amylase from cassava peels

Figure 11 shows the effect of EDTA on the activity of α-amylase. It was observed that the activity of α-amylase from *A. flavus, A. niger* and *P. expansum* were inhibited by all concentrations of EDTA used. This report also agrees with that of Fossi et al. (2011), where EDTA acted as an inhibitor to the α-amylase from *Lactobacillus fermentum* (04BBA19). Goyal et al. (2005) also reported that EDTA inhibited the activity of α-amylase from a *Bacillus* sp. Prakash et al. (2011) reported that EDTA had no detectable influence on the activity of α-amylase from soybean.

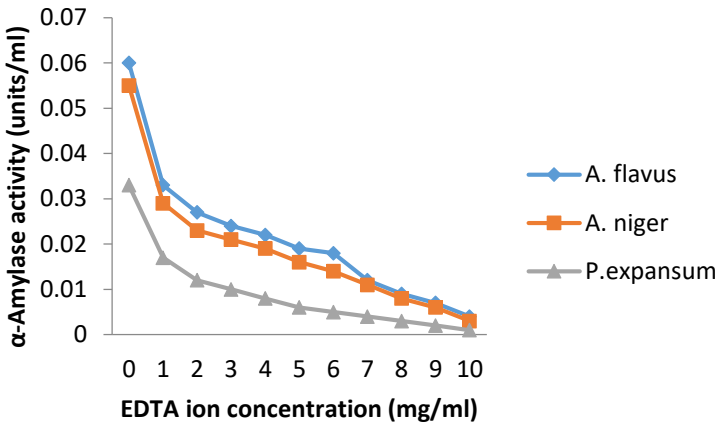

Fig. 11: Effect of EDTA on the activity of purified α-amylase from cassava peels

Conclusion

Alpha-amylase, an important industrial enzyme can be produced by using cassava peels a biodegradable waste as substrate, thereby reducing its cost of production. The fungi (*A. flavus, A. niger* and *P. expansum*) used in this study for the production of α-amylase were isolated from cassava peels undergoing biodegradation, this further proves that α-amylase can be produced at a very low cost.

Acknowledgment

We wish to acknowledge Prof. F. A. Aisien of the Chemical Engineering Department, University of Benin for his immerse contributions towards the successful completion of this work.

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