



Research Article

## Partial Characterization of $\alpha$ -amylase Produced from *Aspergillus niger* using Potato Peel as Substrate

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### Authors' Contributions

SM performed the experiments. MGS and MI prepared the first draft. MI designed the study. MN did final editing. QS provided research facilities.

### Keywords

$\alpha$ -amylase, Characterization, Potato peel, *Aspergillus niger*.

**Abstract** | In this study, solid state fermentation of potato peels were done by *Aspergillus niger* for the production of  $\alpha$ -amylase. The crude enzyme produced was further characterized. The results showed that enzyme had an optimum pH of 6.0 and stable in range of 4.0-6.0. Optimum temperature of enzyme was 40°C and attained 96% activity at 40°C for 70min. Metal profile showed that  $\alpha$ -amylase activity was enhanced in the presence of Ca<sup>2+</sup>, Cs<sup>1+</sup>, Mn<sup>2+</sup> and Co<sup>2+</sup>, whereas inhibited in the presence of Na<sup>1+</sup>, Mg<sup>2+</sup>, Ag<sup>1+</sup> and Cu<sup>2+</sup>. Enzyme kinetics revealed that crude enzyme exhibit  $K_m$  and  $V_{max}$  of 3.00 mg/ml and 1000.0  $\mu$ M/g using soluble starch as substrate. These results speculated that it could be used for various industrial exploration.

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## Introduction

Alpha amylase (endo-1,4- $\alpha$ -D-glucan glucanohydrolase E.C 3.2.1.1) is an extra cellular enzyme. It produces ultimately a large number of products i.e. maltose and glucose sub units by splitting up 1,4- $\alpha$ -D-glucosidic bonds randomly which are present among consecutive molecules of glucose in straight amylose chain (Saleem and Ebrahim, 2014; Mathew *et al.*, 2016; Kanti, 2016). Alpha amylases are widely used in various industries such as baking, brewing, confectionary, sugar (i.e. fructose and glucose), paper coating, alcohol, pharmaceutical, textile, syrup industries, starch, detergents, digestive aids, and for treatment of sewage (Sundarram and Murthy, 2014;

Elaiyaraja *et al.*, 2016; Veerapagu *et al.*, 2016). Alpha amylases are wide spread in occurrence which can be obtained by different resources e.g. microorganisms, animals and plants etc. However, fungi and bacteria are used for commercial production of amylases (Mathew *et al.*, 2016; Singh *et al.*, 2016), because of a few advantages i.e. reliability, less time and space, low cost required for enzyme production, ease of manipulation and economical bulk production capacity (Khan and Yadav, 2011; Mahmood *et al.*, 2016).

*Aspergillus niger* is a fungus which is prevalent in nature and belongs to genus *Aspergillus*. It is usually found as saprophytes growing on stored grain, dead leaves, and other decaying vegetation. It looks as very dark brown patches or carbon black. *Aspergillus niger*, dominates in solid state fermentation, is a very vital group of microorganisms. It

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possesses some very special properties such as good tolerance to less availability of water and capacity to spread over and to enter inside the solid substrate, therefore, it is extensively grown and used in food industry for making many enzymes such as  $\alpha$ -amylases, amyloglucosidases, cellulases, lactase and acid proteases (Singh *et al.*, 2016; Manpreet *et al.*, 2005). In recent years solid state fermentation (SSF), where the fungus is grown on moist solid substrate, has been utilized increasingly for the production of  $\alpha$ -amylase (Xu *et al.*, 2008) because of numerous benefits e.g. less capital investment, simple technique, marginal end product inhibition, superior and high volumetric productivity, less catabolite repression, low energy requirement, requirement of simple equipment for fermentation, better product recovery and less water output (Singh *et al.*, 2016; Gangadharan *et al.*, 2006).

The cost of  $\alpha$ -amylase production is dependent on the cost of the substrate used during SSF. For the cost effective production of alpha amylases, several researchers considered the use of easily available and inexpensive food and agro wastes such as, wheat bran, potato peel, wheat straw, rice straw, rice husk, and sugarcane bagasse, banana waste and waste of coffee as substrate for  $\alpha$ -amylase production (Murthy *et al.*, 2009; Simair *et al.*, 2017). Potatoes are peeled in various processed food industries and are used for the production of different foodstuffs such as chips, fries and mashed potatoes etc. (Shukla and Kar, 2006; Schieber *et al.*, 2009). The potato peel is considered as waste, discarded and allowed to rot, so it creates many pollution and disposal problems. Therefore, for cost effective production of enzyme, potato peel should be used as a cheap source of substrate because it also include adequate amount of nutrients like protein and carbohydrates, which are essential for the growth of microorganisms (Ajao *et al.*, 2009). The objective of present work is to characterize crude  $\alpha$ -amylase from *Aspergillus niger* using potato peel as substrate.

## Material and Methods

### Sample collection

In the present study, potato peel was selected as substrate for  $\alpha$ -amylase production. It was collected from Lays, Pepsi-cola International (Pvt) Ltd, Lahore, Pakistan.

### Microorganism

*Aspergillus niger* was obtained from the Microbiology Laboratory, Food & Biotechnology Research Center, Pakistan Council of Scientific & Industrial Research Laboratories Complex, Lahore. The fungus was grown on slants of potato dextrose agar (PDA) for five days before storage, and maintained at 4°C on PDA.

### Inoculum preparation

Five days old PDA slant culture full of fungal spores

were taken and ten ml of sterilized distilled water was added. Under sterilized conditions, spore clusters were broken with the help of an inoculum needle and homogenized suspension of spores were prepared and used as an inoculum source.

### Fermentation technique

Twenty gram of raw potato peel was weighed in 250 ml Erlenmeyer flask and hydrated with 2 ml of salt solution comprising (g/l)  $\text{MgSO}_4$  2,  $\text{KH}_2\text{PO}_4$  10,  $\text{MnSO}_4$  0.5 and NaCl 2. The material was mixed thoroughly, cotton plugged and sterilized at 121°C, 15lb psi for 15 min. After sterilization, the cooled media was inoculated with one milliliter spore suspension of *Aspergillus niger* and incubated at 30°C for 5 days.

### Extraction of crude enzyme

In each of above flasks, 50 ml of citrate buffer (pH 5) was poured and shaken vigorously in a rotary shaker for 1 hour at 200 rpm. Then the fermentation mixture was filtered and centrifugation was taken place for 15 min at 4°C at 8,000 rpm. The supernatant (crude enzyme) was filtered and used to measure activity of crude enzyme.

### Determination of amylase activity

Amylase activity was measured by method as described by Okolo *et al.* (1995). Reaction mixture containing 1 ml of enzyme extract and 1 ml of substrate (i.e. 1% soluble starch solution) was taken in test tube and incubated for 30 min at 50°C. After that reaction was stopped by adding 3ml of DNS reagent and boiled for 10 min. The reaction mixture was allowed to cool at room temperature and absorbance was measured by spectrophotometer at 540nm (Miller, 1959). One unit (IU)  $\alpha$ -amylase activity was defined as the amount of enzyme that releases 1  $\mu\text{g}$  of maltose per minute under the standard reaction conditions.

### Characterization of $\alpha$ -amylase

Characterization of the crude enzyme was conducted by studying the effect of different parameters such as incubation time (10-100 min), incubation temperature (20-100°C), substrate concentration (0.25-2%), pH (4-9) of reaction mixture and metal ions ( $\text{Na}^+$ ,  $\text{Ca}^{2+}$ ,  $\text{Mg}^{2+}$ ,  $\text{Mn}^{2+}$ ,  $\text{Zn}^{2+}$ ,  $\text{Fe}^{2+}$ ,  $\text{Cu}^{2+}$ ,  $\text{Ag}^+$ ,  $\text{Cs}^+$  &  $\text{Co}^{2+}$ ) on  $\alpha$ -amylase activity.

### Statistical analysis

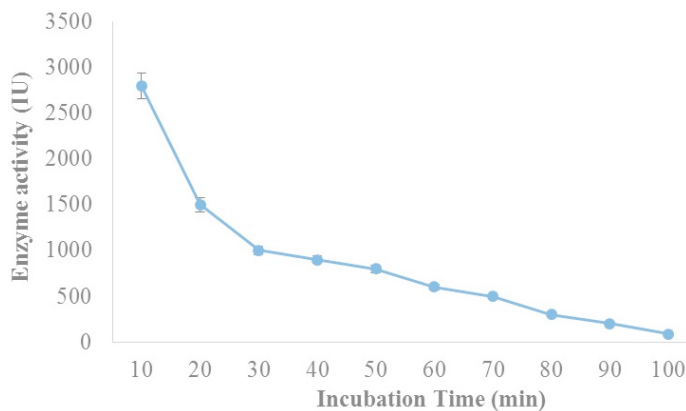
All the data obtained from different experiments were analyzed statistically by SPSS software. ANOVA test was used at  $p < 0.05$  significance level.

## Results and Discussion

### Effect of incubation period

Alpha amylase activity was performed at different incubation periods (10, 20, 30, 40, 50, 60, 70, 80, 90 &

100 min) and it was observed that enzyme was optimally active (2779.49 U/g) for 10 minute of incubation but subsequently, there was gradual decline in enzyme activity (Figure 1). Similar results were reported by Kanwal *et al.* (2004). Maximum activity for  $\alpha$ -amylase at five minutes incubation of reaction mixture has been reported earlier (Ramachandran *et al.*, 2004; Alva *et al.*, 2007).



**Figure 1: Optimum incubation period for  $\alpha$ -amylase activity (LSD 5%= 92.63).**

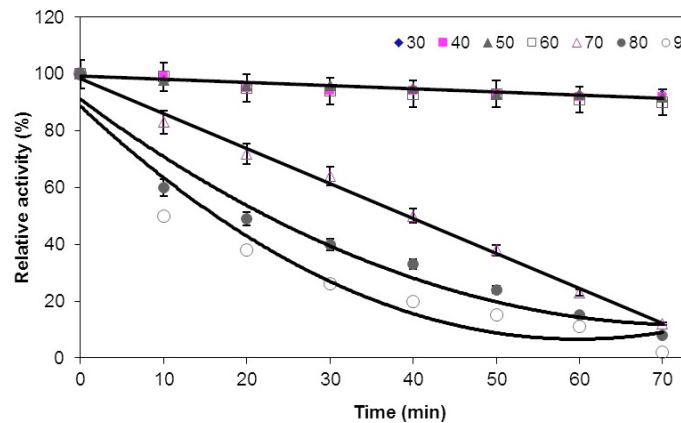
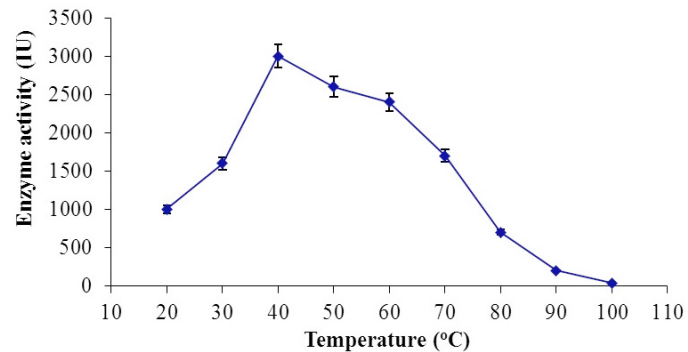
*Effect of temperature on activity and stability*

Effect of different temperatures i.e. 20, 30, 40, 50, 60, 70, 80, 90 & 100°C of reaction mixture on activity of  $\alpha$ -amylase was evaluated. Maximum enzyme activity (2947.08 U/g) was gained at 40°C, whereas, the activity of enzyme showed a declining trend with the increase or decrease in temperature (Figure 2). Thermostability of the enzyme was checked by incubating crude enzyme solution at various temperature for different time period. Results showed that enzyme was stable at temperature 40°C for 70min attaining 96 % enzyme activity. As the temperature gradually increased, the enzyme activity become decreased with the passage of time. Ayansina and Owoseni (2010) showed same results for *Aspergillus flavus*. Maximum  $\alpha$ -amylase activity was reported for *Aspergillus spp.* and *Aspergillus niger* at 45°C (Avwioroko *et al.*, 2015; Wang *et al.*, 2016). Greatest activity for amylase was obtained at incubation temperature of 30°C (Obafemi *et al.*, 2018; Varalakshmi *et al.*, 2009; Nouadri *et al.*, 2010), 50°C (Patel *et al.*, 2005; Mahmood and Rahman, 2008) and even 60°C (Yahya *et al.*, 2016).

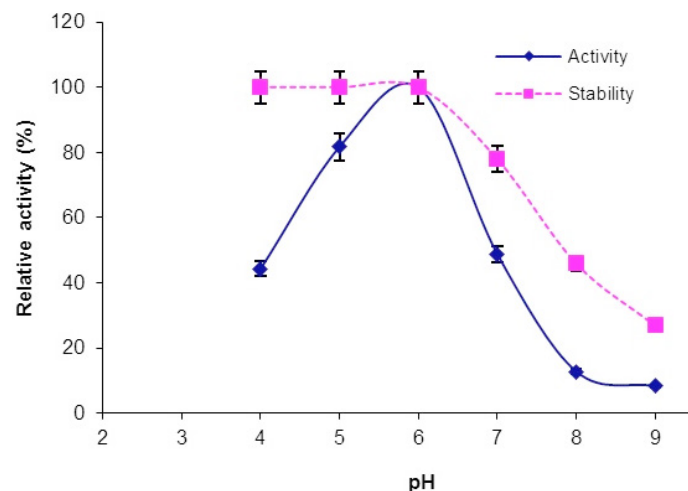
*Effect of pH on activity and stability*

The incubation of enzyme-substrate reaction mixture was carried out at various pH values such as 4, 5, 6, 7, 8 & 9. At pH 6, maximum enzyme activity (2895.02 U/g) was noted and the activity of enzyme was decreased, due to increase or decrease of pH from the optimum value (Figure 3). Enzyme stability was also assessed by pre-incubating enzyme at various pH buffers and enzyme was found stable in pH range of 4-6. Avwioroko *et al.* (2015) reported that *Aspergillus spp.* related with cassava spoilage produced

$\alpha$ -amylase which showed optimum activity within pH range of 4-5. Alpha amylase indicated excellent activity at about pH 6 (Obafemi *et al.*, 2018; Nouadri *et al.*, 2010; El-Safey and Ammar, 2004). It was studied in previous literature that  $\alpha$ -amylase was best active at pH 6.8 (Kanwal *et al.*, 2004), pH 5 (Patel *et al.*, 2005), pH 7 (Tiwari *et al.*, 2007), pH 7 (Wang *et al.*, 2016) and pH 5.6 (Yahya *et al.*, 2016), respectively.



**Figure 2: Optimum incubation temperature for  $\alpha$ -amylase activity (LSD 5%= 125.66).**



**Figure 3: Effect of pH on  $\alpha$ -amylase activity and stability (LSD 5%= 156.06).**

*Kinetics of crude  $\alpha$ -amylase*

Enzyme activity was investigated with different

concentrations of substrate (soluble starch) i.e. 2.5, 5.0, 7.5, 10.0, 12.5, 15.0, 17.5 & 20.0 mg/ml. It was noted that enzyme was optimally active (3014.30 U/g) at 10.0mg/ml soluble starch, subsequently it remained nearly constant (fig 4). Kinetic constants like  $K_m$  and  $V_{max}$  was calculated through Line-Weaver Burk plot. The crude enzyme had  $K_m$  value of 3.00 mg/ml and  $V_{max}$  of 1000.0  $\mu\text{M/g}$  using soluble starch as substrate. Banerjee and Gosh (2017) reported  $K_m$  and  $V_{max}$  of 0.387 mg.ml<sup>-1</sup> and 35.03 U  $\mu\text{l}^{-1}$  min<sup>-1</sup>, for glucoamylase using soluble starch respectively. Crude  $\alpha$ -amylase produced from *Aspergillus* sp. exhibit maximum  $V_{max}$  of 10 U/mg protein and  $K_m$  in range of 0.37 -1.25%w/v (Awwioroko *et al.*, 2015).  $\alpha$ -amylase produced from *Aspergillus oryzae* had  $K_m$  and  $V_{max}$  of 1.4 mg ml<sup>-1</sup> and 37.037 U ml<sup>-1</sup> respectively (Shah *et al.*, 2014).

### Effect of metal ions

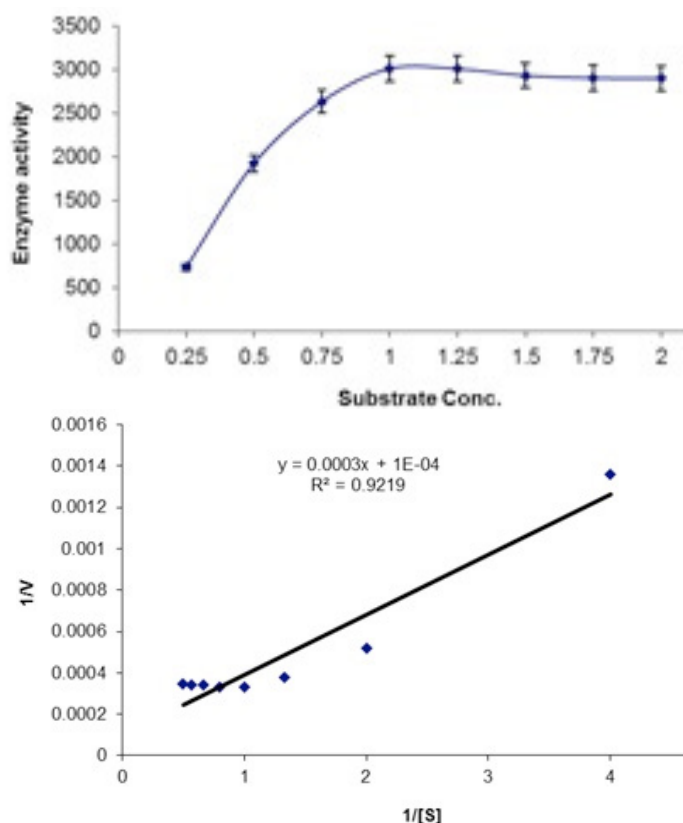
The study of different metal ions exhibited that enzyme activity was enhanced by Ca<sup>2+</sup>, Zn<sup>2+</sup>, Cs<sup>1+</sup>, Co<sup>2+</sup>, Mn<sup>2+</sup> and Fe<sup>2+</sup>, whereas, inhibited by Na<sup>1+</sup>, Mg<sup>2+</sup>, Ag<sup>1+</sup>, Cu<sup>2+</sup> (Figure 5).  $\alpha$ -amylase activity was improved due to Mn<sup>2+</sup>, Ca<sup>2+</sup>, and Fe<sup>2+</sup> ions and inhibited due to Mg<sup>2+</sup>, Na<sup>1+</sup>, Cu<sup>2+</sup>, Hg<sup>2+</sup>, Ag<sup>1+</sup> ions has been observed by many workers (Patel *et al.*, 2005; Tiwari *et al.*, 2007; Varalakshmi *et al.*, 2009; Nouadri *et al.*, 2010). Wang *et al.* (2018) reported that most of the cloned  $\alpha$ -amylase were Ca<sup>2+</sup> independent.  $\alpha$ -amylases produced from *Aspergillus spp.* grown on degrading cassava were slightly stimulated by Mg<sup>2+</sup> and Na<sup>+</sup>, moderately activated by Fe<sup>3+</sup> and Ca<sup>2+</sup>, whereas strongly activated by potassium ion K<sup>+</sup> (Awwioroko *et al.*, 2015).

### Conclusion

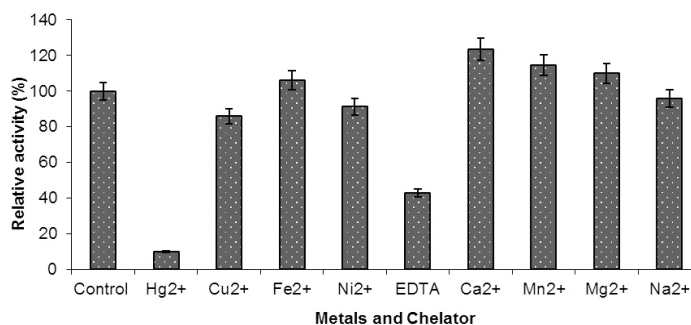
In the current research work, the results presented that potato peel could be used as a good substrate in fermentation media for microbial growth as it contains all the essential nutrients. A significant enzyme activity can be obtained by utilizing potato peel, an agro-residue, as a substrate for SSF. Maximum  $\alpha$ -amylase activity was obtained at pH 6, 40°C substrate concentration 1% incubation period 10 minutes, the enzyme activity was enhanced by Ca<sup>2+</sup>, Cs<sup>1+</sup>, Mn<sup>2+</sup> and Co<sup>2+</sup>, whereas inhibited by Na<sup>1+</sup>, Mg<sup>2+</sup>, Ag<sup>1+</sup> and Cu<sup>2+</sup>.

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**Figure 4:** Effect of substrate concentrations on  $\alpha$ -amylase activity.



**Figure 5:** Effect of different metal ions and chelator on  $\alpha$ -amylase activity.

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