Vol 1 No 4 December 2020 Pages 133-137 e-ISSN 2708-5694

Journal of Agriculture, Food and Environment (JAFE)

Journal Homepage: http://journal.safebd.org/index.php/jafe

http://doi.org/10.47440/JAFE.2020.1420

Original Article

Use of potato peel waste- a cost effective alternative substrate for the production of industrially useful α-amylase enzyme

F. Islam[1,2], M. A. A. Mamun[1], A. K. Azad[2], S. Yamashoji[3] and M. L. Bari[1*]

[1]Centre for Advanced Research in Sciences, University of Dhaka, Dhaka 1000, Bangladesh
[2]Department of Microbiology, Jagannath University, Dhaka, Bangladesh
[3]Microbial Technology Laboratory, Kobe, Japan

Article History

Received: 16 November 2020

Revised: 07 December 2020

Accepted: 16 December 2020

Published online: 31 December 2020

***Corresponding Author**

M. L. Bari, E-mail: latiful@du.ac.bd

Keywords

α-amylase, *Aspergillus oryzae*, potato peel waste (PPW), submerged fermentation and enhanced nutritional composition.

A B S T R A C T

Potato (*Solanum tuberosum* L.) is the fourth main crop worldwide for human consumption and widely used in food-processing industries, thus generating extremely large amounts of potato peel waste (PPW) as zero value by-product. This study was designed to utilize this PPW as biomaterials for the production of alpha amylase by submerged fermentation using *Aspergillus oryzae*, isolated from commercial white koji. The study results demonstrated that the maximum amylase production (30 U/ml) was achieved in PPW medium supplemented with yeast extract and salt after 72 hrs of incubation at ambient temperature (28 ± 2°C), pH 7.0 and 120 rpm agitation. Extraction of whole amylase from culture filtrate was found suitable with 80% ammonium sulfate saturation and maximum amylase activity of 42.07 U/ml was achieved after desalting. The purified α-amylase was found optimally active and stable up to 50°C.

Introduction

Amylases are a group of enzymes that hydrolyze the starch (Taniguchi and Honnda, 2009) and have various industrial applications including production of sugar syrup, for designing textiles, in laundry industry, in the paper industry for sizing, and in the food industry for preparation of sweet syrups, to increase diastase content of flour, for modification of food for infants, and for the removal of starch in jelly production (Saini *et al*., 2017). To meet the higher demands of these industries, low cost production of amylase is required. Economical bulk production of the enzyme is possible using microorganisms since they are easy to manipulate to obtain enzymes of desired characteristics. α-amylase has been derived from several fungi, yeasts and bacteria. However, the fungal and bacterial enzymes have dominated applications in industrial sectors (de Souza and e Magalhães, 2010).

For industrial conversion of starch into glucose highly active α-amylase is required and fungal strains showed better production of α-amylase than that of bacterial strains (De Souza *et al*., 2010). However, fungal sources are confined mostly to *Aspergillus* and *Penicillium* (Kathiresan and Manivannan, 2006). Among large variety of extracellular enzymes produced by *Aspergillus* species, amylases have the most significant industrial importance (Hernández *et al*., 2008). *Aspergillus oryzae* and *Aspergillus niger* can produce thermo stable α-amylase enzymes that could be used extensively in the industries. Researchers have increased attention to *A. oryzae* because it secretes a vast amount of high value proteins and industrial enzymes, e.g. α-amylase (Jin *et al*., 1998). *A. oryzae* has also been largely used in the production of foods including soy sauce, organic acids such as citric and acetic acids (Kammoun *et al*., 2008).

For economic production of amylase, various agro-industrial residues such as wheat bran (Kaur *et al*., 2003), rice bran (Akpan *et al*., 1999), banana peel (Krishna *et al*., 2012), potato peel (Mushtaq *et al*., 2016), Mustard Oil seed cake (Saxena and Singh, 2011) etc., were reported in the literature. However, the potato peels offer high amount of dietary fiber and also a rich source of various nutrients and bioactive compounds including antioxidants, pigments, vitamins, and minerals (Farvin *et al*., 2012, Pathak *et al*., 2018), the rich nutritive materials were lost within these peels during processing. Current agricultural and

environmental studies focus on advanced techniques for utilizing PPW in food-processing, pharmaceutical, and biosynthesis industries, which increase the value of PPW recycling (Wu 2016). On the other hand, traditionally, PPW is used for producing low-value animal feed and organic fertilizer. The main objective of this study was to produce industrially important amylase enzyme by submerged fermentation using *Aspergillus oryzae* and potato peel waste.

Materials and Methods

Amylase producer

The fungal strain *Aspergillus oryzae* was isolated from commercial white koji preparation and stored on potato dextrose agar (PDA) plates at 4°C and used for this study.

Substrate preparation

The potatoes were bought from local market in Dhaka city, washed with tap water and boiled in an aluminum pot. Then peels were taken from boiled potatoes, washed and allowed to dry in laminar airflow for 1 h, and used as substrate for amylase production. In addition, various parboiled rice (2%) including SonaGuti, GutiSharna, Najirshal, IRRI 28, and IRRI 29 produced in Bangladesh were also used as substrate for amylase production.

Submerged fermentation (SmF)

Fermentation medium (pH 7.0) was composed of 100 ml water, 2.0 g potato peel, 0.5 g yeast extract, 0.1 g K_2HPO_4, 0.05 g $MgSO_4$, and 0.001 g $FeSO_4/7H_2O$, and taken in 500 ml Erlenmeyer flask, and autoclaved at 121°C for 15 min at 15 lbs pressure. After autoclaving, the medium was cooled to room temperature and 1.0 cm×1.0 cm of PDA medium containing *Aspergillus oryzae* culture was added to the fermentation medium. The flask was then placed in shaking incubator (120 rpm) at ambient temperature (28 ± 2°C) for 72 hrs.

Optimization of process parameters

Various process parameters were optimized for maximal enzyme production as follows: incubation period (1-7 days), initial pH (6.5-8.5), substrate concentration (1%, 2% and 3%).

Recovery of enzyme

After the specified incubation period (in each case), the medium was centrifuged at 6,000 rpm for 10 min, supernatant was taken and used as crude enzyme to measure α-amylase activity.

Amylase assay

α-amylase was assayed by estimating liberated reducing sugars employing the 3,5-dinitrosalicylic acid (DNS) method (Miller, 1959). The reaction mixture containing 0.5 ml enzyme extract and 1.0 ml soluble starch solution was incubated for 20 min at 40°C. The reaction was stopped by adding 3.0 ml DNS reagent. Then the mixture was heated in a boiling water bath (90°C) for 15 minutes. After boiling, test tubes containing reaction mixture were cooled under running tap water. Absorbance was taken at 545 nm against the blank in a spectrophotometer. The liberated reducing sugars were estimated by dinitrosalicylic acid (DNS) method and taking glucose as standard (Miller, 1959). The blank contained 0.5 ml distilled water instead of 0.5 ml enzyme extract.

Purification of α-amylase

Precipitation of the α-amylase was performed by adding ammonium sulfate in the enzyme solution following the method described by Wingfield (Wingfield, 2016). Solid $(NH_4)_2SO_4$ was added gradually into every 50 ml enzyme contained in conical flask in order to make it 50, 70, 80, 90 percent saturation and left overnight at 4°C. Then the precipitates were collected by centrifugation at 10,000 rpm for 10 min. The precipitates were dissolved in 5 ml of 0.2M phosphate buffer solution (pH 7). Desalting of dissolved precipitates was done by gel filtration using Sephadex G-25. The column was prepared by adding 20 ml of Tris-HCl buffer (pH 7.5) and 1.0 g of Sephadex G-25. One ml of sample was added slowly into the column. Then 20 ml elution buffer was added into the column and fractions (1.0 ml each) were collected. The enzyme activity and protein concentration (Bradford, 1976) were then assayed with appropriate dilution.

Statistical analysis

All trials were replicated three times and the data presented in the table and figures were the average value ± standard deviation. The significant differences were subjected to analysis of variances using the Microsoft Excel program (Redmond, Washington DC, USA.).

Results

In this study, α-amylase was produced from *Aspergillus oryzae* by SmF using potato peel waste (PPW) as substrate. Different fermentation conditions were optimized and purification of crude α-amylase and its characterization were done to evaluate industrial potential.

Amylase activity on different substrates

The production of α-amylase by *A. oryzae* in SmF using different kinds of food substrates is shown in Fig 1. Potato peel showed the maximum amylase activity i.e. 10 U/ml among the tested food substances. Though some previous studies also reported the usability of potato peel as potential food waste for amylase production by *Aspergillus* spp. (Mustaq *et al*., 2017; Shahzad *et al*., 2016; Shukla and Kar, 2006), the conditions to enhance the effect of potato peel were tested as below.

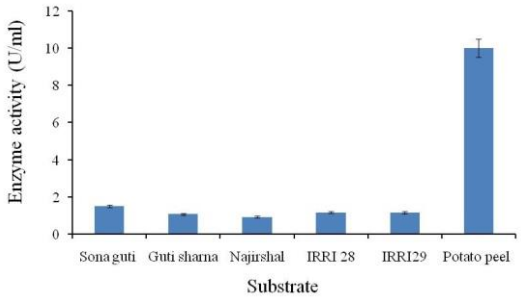

Figure 1. Production of amylase enzyme by *A. oryzae* using different substrates

Selection of suitable medium

For the selection of suitable medium for maximum amylase production in orbital shaker with 120 rpm; at ambient temperature (28±2°C) for 72 hrs, two different medium were tested. Medium 1 contained only substrate (No additional ingredients), and Medium 2 contained the additional ingredients (Yeast extract 5 g, K_2HPO_4 1 g, $MgSO_4$ 0.5 g, and $FeSO_4/7H_2O$ 0.01 g in 1000 ml water). Sampling of each

medium was done at 24 hrs interval. Fig 2 showed that the amylase activity was three fold increased when additional ingredients were added to Medium 1 and the cost of ingredients could be minimized by the increased enzyme activity. Thus, Medium 2 was found to be economically feasible medium for amylase production by *A. oryzae*.

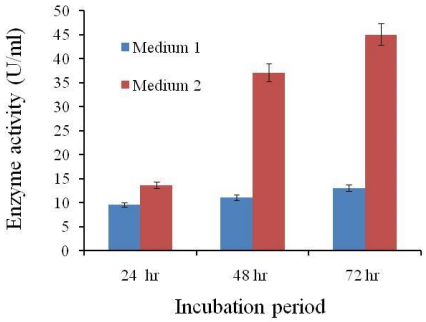

Figure 2. Amylase activity of *A. oryzae* in different media

Effect of substrate concentration on enzyme production

Different amounts of PPW (1%, 2% and 3%, w/v) were used to evaluate the dose-dependent effect on the production of amylase enzyme by *A. oryzae*. Fig 3 (A) showed that maximum enzyme activity (30 U/ml) was found in Medium 2 containing 2% (w/v) PPW as substrate.

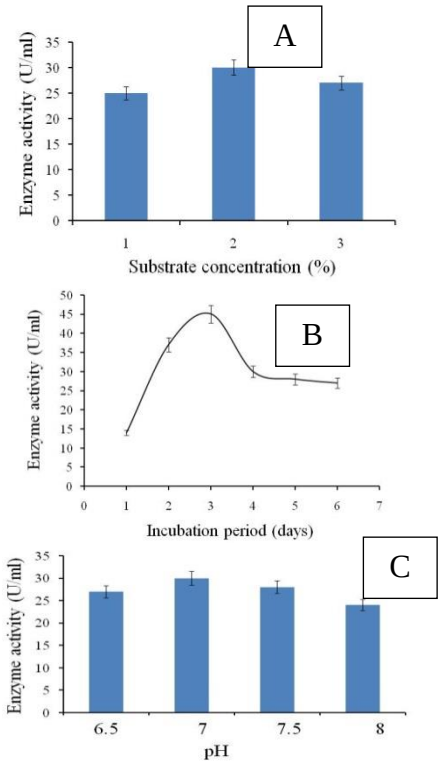

Figure 3. Effect of substrate (potato peel) concentration (A), incubation period (B) and pH (C) on amylase production by *A. oryzae*

Effect of incubation period on enzyme production

The rate of production of α-amylase by *A. oryzae* in SmF was shown in Fig 3(B). Enzyme production started after 24 h of incubation at ambient temperature and reached to its maximum level (i.e. 45 U/ml) after 72 h of incubation and then decreased. This decrease might be due to the unavailability of nutrients for large fungal population, and denaturation of the enzyme or due to production of acid by-products (Mahmood *et al*., 2016). Many recent studies also reported that α- amylase yield was maximum at 72 hrs of incubation at room temperature (Ahmed *et al*., 2015; Haq *et al*., 2002; Ramachandran *et al*., 2004; Mahmood *et al*., 2016).

Effect of pH on enzyme production

Effect of medium pH ranging from 6.5-8.0 was done to determine the maximum α- amylase production. Maximum α-amylase production (30 U/ml) was obtained when the medium pH was 7.0, and higher α-amylase production was recorded with pH of the medium ~~was~~ ranging from 6.5 to 8.0 as shown in Fig. 3 (C). Similar experimental results were reported by Ayansina and Owoseni, 2010, who found optimum amylase production with *A. flavus* (isolated from moldy bread) at pH 7.0.

Effect of ammonium sulfate saturation on enzyme precipitation

Ammonium sulfate precipitation was applied as initial step to prepare the crude enzyme extract and the overall view of the precipitation process was shown in Table 1 and the results were shown in Fig 4 (A). The percentage of enzyme extraction was enhanced by increasing the concentration of ammonium sulfate and it was clear from the Fig 4 (A) that the maximum enzyme extraction (920 U) was observed at 80% ammonium sulfate saturation conditions. Below or above this saturation conditions, the amylase activity was lower and thus, 80% ammonium sulfate saturation was found suitable for the extraction of crude amylase from culture filtrate.

Table 1. Total activity and specific activity of enzyme after ammonium sulfate precipitation

Types of Enzyme	Total Activity (U)	Specific Activity (U/mg)
Cell free supernatant	1500	273
50% saturation	10.75	3
70% saturation	125	50
80% saturation	900	349
90% saturation	825	436

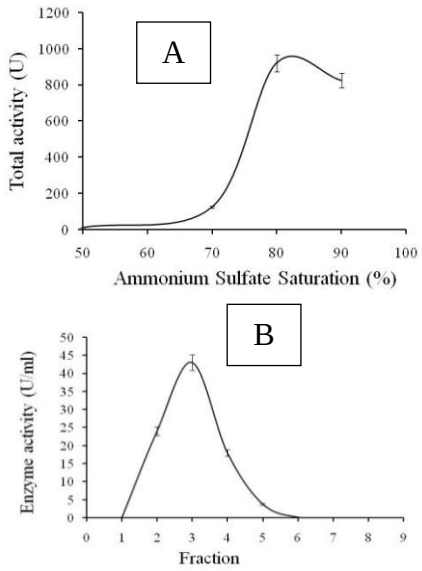

Figure 4. Partial purification of the enzyme produced by *A. oryzae*. (A) Precipitation by Ammonium Sulfate (B) Desalting through Sephadex G25 column
Desalting through Sephadex G-25

Desalting through Sephadex G-25 was applied to remove salt from precipitated amylase and several fractions of crude amylase were achieved through desalting. Fig 4(B) showed that highest enzyme activity (43 U/ml) was found at Fraction 3 and thus was selected for further study.

Effect of temperature on amylase activity and stability

Partially purified α-amylase was assayed at varying temperatures (40°C, 50°C, 55°C and 60°C) to determine the optimum temperature for enzyme activity. The highest amylase activity (64.7 U/ml) was found at 50°C and decreased substantially at 55°C or above temperature, as shown in Fig 5(A). On the other hand, the stability of amylase activity at four different temperatures (25°C, 30°C, 40°C and 50°C) for 60 min was shown in Fig 5 (B). It was observed that holding the enzyme at lower temperature (25°C) showed more stability than that of holding the enzyme at 50°C, which was found 50% less stable.

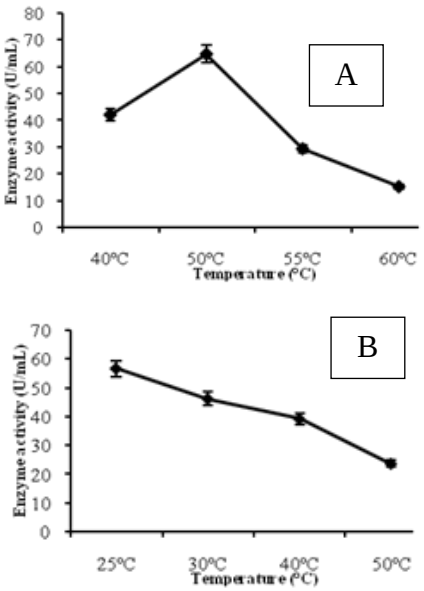

Figure 5. Effect of temperature on (A) Enzyme activity and (B) Enzyme stability

Discussion

Aspergillus oryzae, also known as koji mold, is a filamentous fungus used in Japan to saccharify rice, sweet potato, and barley in the making of alcoholic beverages such as sake and shochu, and also to ferment soybeans for making soy sauce and miso. Among filamentous fungi, *A. oryzae* is known to have prominent potential for the secretory production of various enzymes (Machida *et al.*, 2008). In this study, different types of rice varieties were evaluated for production of amylase enzyme by *A. oryzae* (koji mold) and among the rice varieties used, sonaguti rice varieties showed maximum amylase production (1.5 U/ml), which was 20 times less than that of amylase production using PPW. It was found that the koji mold was shown producing maximum 30 U/ml of amylase enzyme at ambient temperature while the fermentation medium contained 2.0% PPW with additional ingredients at pH 7.0, with agitation 120 rpm. Although, a recently published research where the potato peel was used for production of bioethanol by *Wickerhamia* spp. (Hossain *et al*., 2018), but this is the first study to use PPW as substrate for the production of amylase by *A. oryzae* and the amylase activity was found higher in PPW. Therefore, this study results demonstrated that koji mold can be used for cost effective amylase enzyme production.

Conclusion

In this study 2% potato peel at pH 7.0 after 72 hrs of submerged fermentation at ambient temperature using *A. oryzae*, showed higher amylase production than that of different type of rice. Thus, this study results concluded that potato peel waste could be a cost effective alternative substrate for the production of industrially useful α-amylase enzyme. Further studies on the analysis of nutritional composition of the fermented potato peel will be done to evaluate whether the fermented potato peel possess enhanced nutritional composition and could be useful as nutrient rich animal feed.

References

Akpan I, Bankole MO, AdesemowoA M, Latunde-dada GO (1999). Production of amylase by *A. niger* in a cheap solid medium using rice bran and agricultural materials. Tropical Science 39: 77-79.

Ahmed K, Valeem EE, Haq Q, Mehmood I, Dahot MU (2014). Optimal conditions for the production of industrial enzymes by *Aspergillus niger* using agricultural wastes as sources of carbon. FUUAST J. Biol 4(2): 129-136.

Ayansina AD, Owoseni AA (2010). Studies on amylolytic enzyme synthesized by *Aspergillus flavus* associated with moldy bread. Pakistan Journal of Nutrition 9: 434-437.

Bradford MM (1976). A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. Analytical Biochemistry72 (1–2): 248–254.

De Souza PM, de Oliveira Magalhães P (2010). Application of microbial α-amylase in industry- A review. Brazilian Journal of Microbiology 41(4): 850–861.

Farvin S, Surendraraj A, Jacobsen C (2012). Composition and health benefits of potato peels. In C. Caprara (Ed.), *Potatoes: Production, Consumption and Health Benefits.* Nova Science Publishers. Agriculture Issues and Policies

Gupta R, Gigras P, Mohapatra H, Goswami VK, Chauhan B (2003) Microbial α-amylases: a biotechnological perspective. Process Biochem38:1599–1616.

Haq I, Abdullah R, Ashraf H, Shah AH (2002).Isolation and screening of fungi for the biosynthesis of α-amylase. Biotechnology 1(2): 61-66.

Hernández MS, Rodríguez MR, Guerra NP, Rosés RP (2006). Amylase production by *Aspergillus niger* in submerged cultivation on two wastes from food industries. J Food Process Eng.73:93–100.

Hossain T, Miah AB, Mahmud SA, Mahin AA (2018). Enhanced Bioethanol Production from Potato Peel Waste Via Consolidated Bioprocessing with Statistically Optimized Medium. Appl Biochem Biotechnol. 186(2):425-442. doi: 10.1007/s12010-018-2747-x

Ul Haq I, Abdullah R, Ashraf H, Shah Ah (2002). Isolation and Screening of Fungi for the Biosynthesis of Alpha Amylase. Biotechnology 1: 61-66.

Jin B, van Leeuwen HJ, Patel B, Yu Q (1998).Utilization of starch processing wastewater for production of microbial biomass protein and fungal α-amylase by *Aspergillus oryzae*. Bioresour. Techno l66:201–206.

Kammoun R, Naili B, Bejar S (2008). Application of a statistical design to the optimization of parameters and

culture medium for alpha-amylase production by *Aspergillus oryzae* CBS 819.72 grown on gruel (wheat grinding by-product). Bioresour Technol 99:5602–5609.

Kathiresan K, Manivannan S (2006). α-Amylase production by *Penicillium fellutanum* isolated from mangrove rhizosphere soil. Afr. J. Biotechnol 5:829–832.

Kaur P, Grewal H, Kocher GS (2003). Production of α-amylase by *Aspergillus niger* using wheat bran in submerged and solid state fermentations. *Indian Journal of Microbiology* 43: 143-145.

Krishna PR, Srivastava A, Ramaswamy N, Suprasanna P, D'souza SF (2012). Banana peel as substrate for α-amylase production using *Aspergillus niger* NCIM 616 and process optimization. Indian Journal of Biotechnology 11: 314-319.

Machida M, Osamu Yamada, Gomi K (2008). Genomics of *Aspergillus oryzae*: Learning from the History of Koji Mold and Exploration of Its Future. DNA Res15(4): 173–183.

Mahmood S, Shahid MG, Nadeem M, Irfan M, Syed Q (2016). Production and optimization of α-amylase from *Aspergillus niger* using potato peel as substrate. Pak. J. Biotechnol 13 (2): 101-109.

Miller GL (1959). Use of Dinitrosalicylic Acid Reagent for Determination of Reducing Sugar. Journal of Analytical Chemistry 31: 426-428.

Mushtaq Q, Irfan M, Tabssum F & Qazi JI (2017). Potato peels: A potential food waste for amylase production, Journal of food process engineering, 40:e12512.

Ramachandran S, Patel AK, Nampoothiri KM, Francis F, Nagy V, Szakacs G, Pandey A (2004).Coconut oil cake - a potential raw material for the production of α-amylase. Bioresource Technol. 93(2): 169-174.

Pathak PD, Mandavgane SA, Puranik NM, Jambhulkar SJ, Kulkarni BD (2018). Valorization of potato peel: a biorefinery approach. Critical Reviews in Biotechnology 38 (2): 218-230. DOI: 10.1080/07388551 .2017.1331337

Rajagopalan G, Krishnan C (2008). Alpha-amylase production from catabolite de repressed *Bacillus subtilis* KCC103 utilizing sugarcane bagasse hydrolysate. Bioresour Technol 99: 3044–3050.

Reddy NS, Nimmagadda A, Rao KRSS (2003).An overview of the microbial α-amylase family. Afr. J. Biotechnol 2:645–648.

Saxena R, Singh R (2011). Amylase production by solid-state fermentation of agro-industrial wastes using *Bacillus* sp. Braz. J. Microbiol 42 (4): 1334-1342.

Wingfield PT (2001). Protein precipitation using ammonium sulfate. Curr Protoc Protein Sci. Appendix 3: Appendix–3F. doi:10.1002/0471140864.psa03fs13.