Research Article

Purification and Characterization of α-Amylase Produced from *Penicillium citrinum*

Mehak Iftikhar[1], Memuna G. Shahid[1], Zohaib Anjum[1], Awais Anjum[1], Ikram-ul-Haq[2], Nazish Mazhar Ali[3] and Reham Shakeel[1]

[1]Department of Botany, Government College University, Lahore, Pakistan; [2]Department of Biotechnology, Government College University, Lahore, Pakistan; [3]Department of Zoology, Government College University, Lahore, Pakistan.

Abstract | This research work was conducted on the Purification and Characterization of α-Amylase enzyme by *Penicillium citrinum* using surface culture fermentation technique. The optimization study was done for α-Amylase enzyme production using fermentation parameters using OFAT (one factor at a time) technique. A variety of parameters, including the impact of incubation temperature, incubation period, pH, and numerous carbon and nitrogen sources. Various concentration of Sucrose, yeast extract, potato peel, KH_2PO_4, $MgSO_4$, $MnSO_4$, $FeSO_4$, $ZnSO_4$ and NaCl were observed on the production of α-Amylase. The results showed that 2.5g sucrose and 5g yeast extract,3.5g potato peel, 2g KH_2PO_4, 1g $MgSO_4$, 1g $MnSO_4$, 0.25g $FeSO_4$, 0.5g $ZnSO_4$ and 1.25g NaCl gave the highest yield of α-Amylase enzyme. The maximum α-Amylase production rate was 1.518±0.1 U/ml for extracellular extract and 2.547±0.1 U/ml for intracellular extract while maintaining the pH of fermentation medium at 5.5 kept at 27 °C with all the optimized culture medium ingredients for 5 days. To partially purify the α-Amylase enzyme, Sodium Sulphate was added to the crude enzyme following solid state fermentation, with constant stirring at room temperature. And after its purification characterization of enzyme was also done. The α-Amylase was checked by varying the temperature, pH and substrate concentration. It was observed that α-Amylase was stable till the 37°C of temperature and gave the maximum activity at pH 5.5. The yield of α-Amylase from *Penicillium citrinum* emerged as a possible source for expanding the sustainable and economical method by which α-Amylase can be utilized in bioremediation, food processing, and other biotechnological applications.

Received | September 25, 2025; **Accepted** | May 18, 2026; **Published** | March 28, 2026
***Correspondence** | Mehak Iftikhar, Department of Botany, Government College University, Lahore, Pakistan; **Email:** mirzamehak968@gmail.com
Citation | Iftikhar, M., M.G. Shahid, Z. Anjum, A. Anjum, I. Haq, N.M. Ali and R. Shakeel. 2026. Purification and characterization of α-amylase produced from *Penicillium citrinum*. *Pakistan Journal of Weed Science Research,* 32(1): 62-68.
DOI | https://dx.doi.org/10.17582/journal.pjwsr/2026/32.1.62.68
Keywords | α-Amylase, Potato peel, OFAT, Purification, Characterization

Introduction

Enzymes are biological catalysts that initiate chemical processes within living organisms. They are typically created by living things found in the different cells in extremely tiny concentrations (approximately 0.1%) (Djekrif-Dakhmouche *et al.*, 2006). A hydrolytic enzyme called α-amylase breaks down α-1, 4-glycosidic bonds in starch and similar polysaccharides to produce glucose, maltotriose, and maltose (Farooq *et al.*, 2019). Due to its wide range of uses in food, textiles, paper, detergents, fermentation, and medicines, it makes for around 25% of the worldwide industrial enzyme market (Kubilay *et al.*, 2010). Because of their high production efficiency, stability, and ease of scaling, microbial α-amylases especially those derived from bacteria and fungi are preferred in industry (Reddy *et al.*, 2003).

Filamentous fungi, such as *A. niger* and *A. oryzae*, are key producers of extracellular enzymes, including α-Amylase, under SSF (solid-state fermentation) is due to their capacity to colonize and penetrate solid substrates (Jiby *et al.*, 2016). *Penicillium citrinum* is particularly appealing because to its fast growth, Generally Recognized as Safe (GRAS) status, and ability to release hydrolytic enzymes in acidic environments. The literature on optimal enzyme synthesis, purification, and characterization specifically from *P. citrinum* is still scarce, despite the fact that numerous researches have assessed α-amylase production from fungal species. Their hydrolytic efficiency and tolerance to acidic pH make them ideal for enzyme production (Ertan *et al.*, 2006).

The expanding industrial need for thermostable and pH-tolerant enzymes emphasizes the need to investigate new microbial sources and optimize culture conditions to increase enzyme production and performance. *P. citrinum*, an underutilized but promising species, has the potential to produce high-activity α-amylase for industrial starch breakdown and bioprocessing (Jujjavarapu and Dhagat, 2019; Silaban *et al.*,2020). Optimization of fermentation parameters such as temperature, pH, incubation time, and nutrient composition significantly enhances enzyme yield. Submerged and surface fermentation techniques are widely used, with surface culture involving microbial growth on liquid surfaces followed by enzyme extraction (Jensen *et al.*, 2002). Advanced optimization has improved the production of high-purity α-amylases for industrial and pharmaceutical applications (Elyasi *et al.*, 2020).

In this study, *Penicillium citrinum* was employed for α-amylase production under optimized fermentation conditions. Enzyme characterization included analyses of temperature and incubation effects, and enzyme activity was determined using spectrophotometry. Salt precipitation can help to partially purify the enzyme. Determine the enzyme's activity under a variety of physicochemical conditions. The project aims to provide baseline data to improve fungal α-amylase production and develop cost-effective strategies for industrial enzyme applications (Silaban *et al.*, 2020; Ullah *et al.*, 2025).

Materials and Methods

Sample collection

In this investigation, α-amylase was produced using potato peels as a substrate. They were collected from Lays, Pepsi-Cola International (Pvt) Ltd, located in Lahore, Pakistan.

Obtainment of fungal organism

The fungus species, *Penicillium citrinum*, was obtained from Cell Culture Insight Laboratory, Department of Botany GC University Lahore, Pakistan.

Maintenance of fungal culture

PDA and MEA slants were used to maintain the fungal culture of *Penicillium citrinum*. The media was prepared, sanitized, and placed into aseptic test tubes for solidification.

Inoculation of fungal strains

The *Penicillium citrinum* strain was inoculated on PDA slants, incubated at 25°C for 7 days, and the best-growing slants were kept as parent cultures. PDA slants with excellent growth were chosen for further subculture and experimentation.

Fermentation technique

α-amylase was produced from *Penicillium citrinum* by surface culture fermentation using a modified medium that contained potato peel, sucrose, yeast extract, and different salts, with a pH of 5.5. A spore suspension of *Penicillium citrinum* was aseptically introduced into the medium after it had been autoclaved. For the best enzyme synthesis, the fermentation was incubated for five to seven days at 25°C.

Production of α-amylase enzyme from Penicillium citrinum

Mycelial growth was weighed and collected after five to seven days of incubation. It was then dried and crushed in acetone. The mycelium was centrifuged to extract the intracellular α-amylase, and the leftover media was centrifuged to acquire the extracellular α-amylase.

Determination of α-amylase

Amylase activity was determined using the method described by Kubilay *et al.* (2010). To determine α-amylase activity, a glucose standard curve was created by serially diluting glucose and measuring its optical density at 540 nm. The enzyme activity was measured by incubating the supernatant with starch and DNS solution, then measuring optical density at 540nm (Hiteshi and Gupta, 2014). In fermentation conditions, one unit of -Amylase is produced as one mol of α-amylase (1U/min), which is the unit of enzyme activity U/ml.

Partial purification of α-amylase enzyme

The enzyme was refined using Solid-State Fermentation with sodium sulfate and centrifugation at 10,000 rpm. To assess pure α-amylase absorbance, Sodium citrate buffer (pH 5.5) was used to dissolve the precipitate and Optical density was measured at 540 nm (Prakash *et al.*, 2010).

Characterization of α-amylase enzyme

The effect of temperature, pH, and substrate concentration on α-amylase activity was investigated by incubating the enzyme with varying conditions (temperatures: 25–60°C, pH: 3.5–7, substrate concentrations: 1–50 mM) and measuring activity using common procedures. Each experiment was repeated three times for accuracy.

Statistical analysis

Standard error (SE) was determined in enzyme assays to compare variability among replicates. Mean activity values were plotted against treatment conditions to create graphical representations. Error bars show ±SD. Steel and Torrie's (1996) techniques were used to conduct all statistical analyses.

Results

Production of α-amylase with *Penicillium citrinum*: Table 1 displays the production of α-amylase in a fermentation medium using *Penicillium citrinum*. With extracellular (0.978 U/ml) and intracellular (1.230 U/ml) activity, sample A had the highest α-amylase production, whereas sample B had the lowest with extracellular (0.273 U/ml) and intracellular (0.240 U/ml) activity.

Table 1: *Production of α-amylase with Penicillium citrinum.*

S. No.	Samples	Extracellular α-amylase activity (mg/ml)	Intracellular α-amylase activity (mg/ml)
1	Sample A	0.978 ± 0.004	1.230±0.1
2	Sample B	0.273±0.004	0.240±0.04
3	Sample C	0.281±0.003	0.250±0.01

Effect of pH and temperature on α-amylase production

The maximum activity at various pH levels was in 10 mM sodium acetate buffer at pH 5.5 (0.327 ± 0.005 U/mL), followed by pH 6 (0.271 ± 0.011 U/mL). The optimal amylase activity was found at 37 °C (0.398 ± 0.019 U/mL) and 40 °C (0.382 ± 0.016 U/mL). The standard deviation is indicated by the symbol ±, and each result is the mean of three replicates (Figure 1).

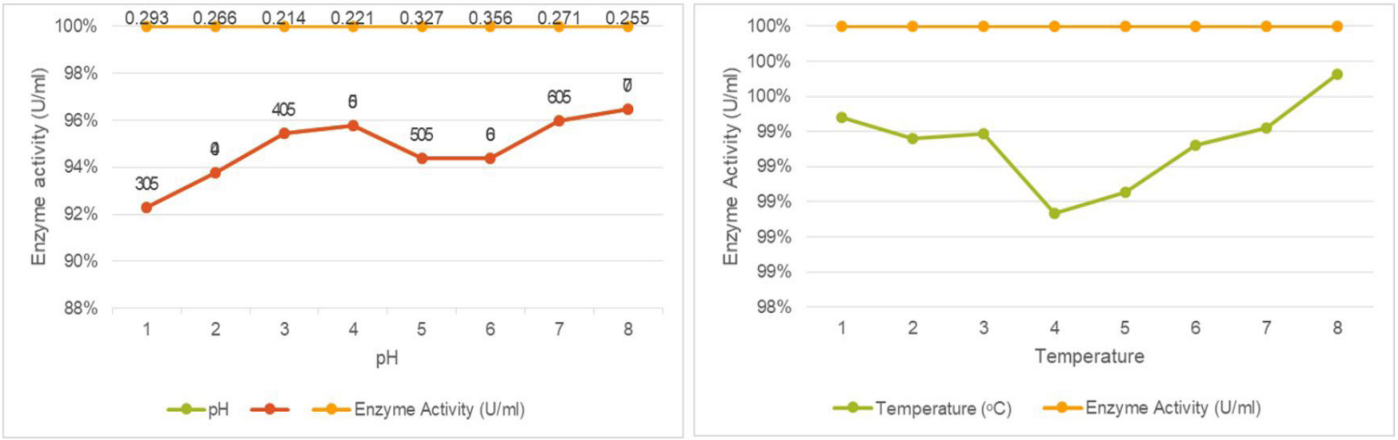

Figure 1: *Effect of pH and temperature on α-amylase production.*

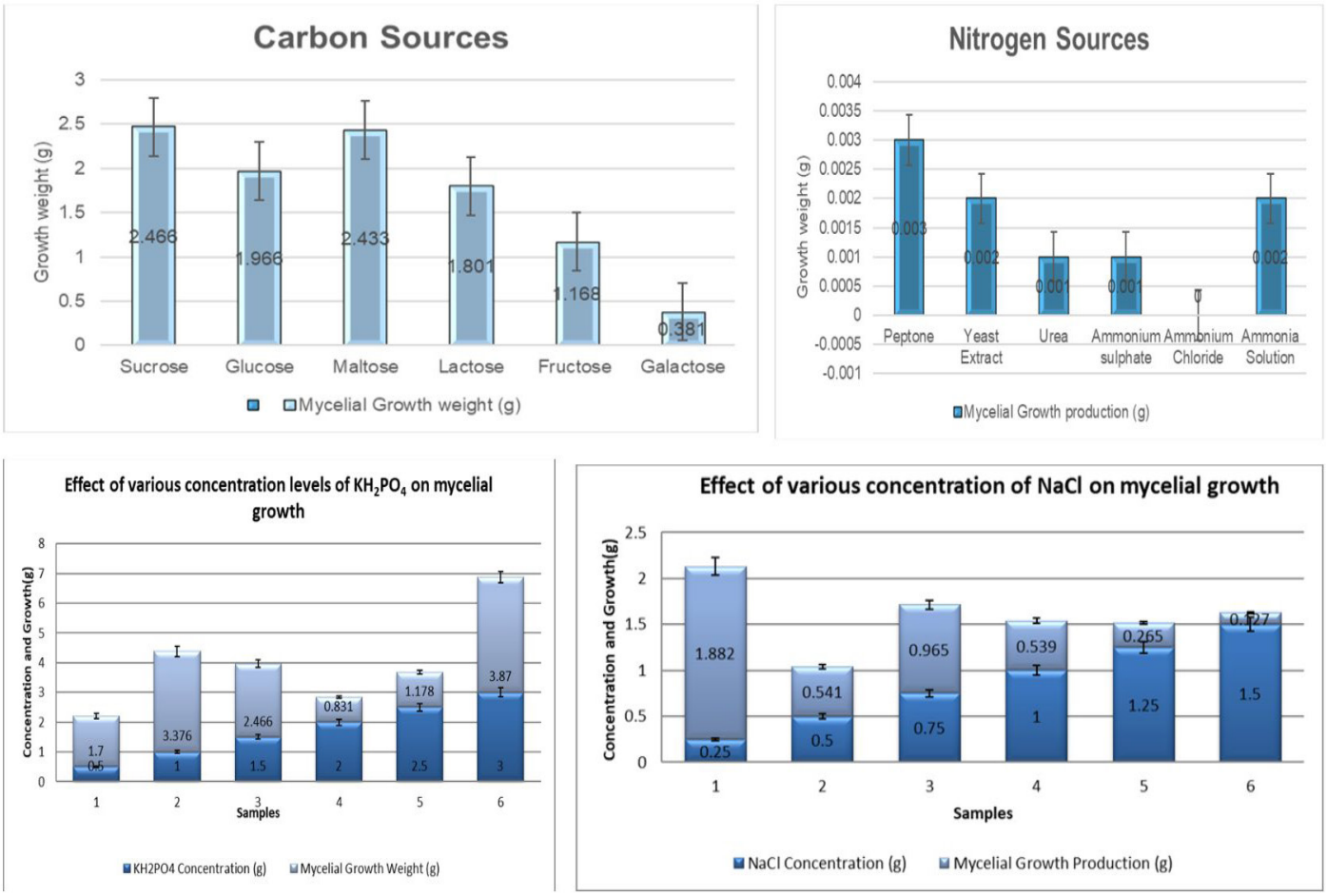

Figure 2: *Effect of carbon, Nitrogen sources and inorganic salts α-amylase production.*

Effect of carbon, nitrogen sources and inorganic salts on α-amylase enzyme production

Maximum production of α-amylase was observed with sucrose (1.518±0.1 U/ml extracellular) and fructose (2.547±0.1 U/ml intracellular) as carbon sources, peptone (0.420±0.004 U/ml extracellular, 0.301±0.001 U/ml intracellular) as a nitrogen source, and 2g KH_2PO_4 (0.372±0.002 U/ml extracellular) and 3g KH_2PO_4 (0.372±0.002 U/ml extracellular) and 1.190±0.004 U/ml intracellular) as phosphate sources, and 1.5g NaCl (0.340±0.003 U/ml extracellular) and 0.75g NaCl (0.585±0.004 U/ml intracellular) as sodium sources, the highest α-amylase production was observed Figure 2.

Effect of metal ions on α-amylase production

Metal ion concentrations ($MgSO_4$, $MnSO_4$, $ZnSO_4$, and $FeSO_4$) had varying effects on α-Amylase production. The highest extracellular and intracellular α-Amylase activities were observed at 1g of $MgSO_4$, 1g of $MnSO_4$, 0.5g of $ZnSO_4$, and 0.25g of $FeSO_4$, while the lowest α-Amylase activities were observed at 0.25g of $MgSO_4$, 1.5g of $MnSO_4$, 1.5g of $ZnSO_4$ Figure 3.

Partial purification of α-amylase

The partial purification of α-Amylase is displayed in Table 2, where sample D yielded the highest yield (1.270±0.002 U/ml) and sample C yielded the lowest yield (0.138±0.002U/ml). The mean±standard deviation of three replicates is represented by each value.

Table 2: *Partial purification of α-amylase.*

S. No.	Samples	Purified α-amylase enzyme
1	Sample A	0.553±0.002
2	Sample B	0.355± 0.001
3	Sample C	0.138±0.002
4	Sample D	1.270±0.002

Discussion

Amylases are enzymes that hydrolyze starch molecules to produce glucose-based polymers. They are widely employed in sectors such as medicines, food processing, and fermentation. *Penicillium citrinum* produces α-amylase efficiently during surface culture fermentation, supporting previous

reports that filamentous fungi are reliable sources of hydrolytic enzymes due to their ability to penetrate solid substrates and secrete high levels of extracellular enzymes. Kathiresan and Manivannan (2006) published similar findings, demonstrating significant amylolytic activity in *Penicillium* species isolated from varied biological settings. Our findings further show that *P. citrinum* can be successfully grown on agro-industrial waste materials like potato peels, making the procedure both cost-effective and environmentally friendly.

Penicillium citrinum was used to purify and characterize α-amylase using surface culture fermentation. Table 1 shows the maximum levels of α-amylase production and mycelial development. This study's ideal pH for enzyme activity was 5.5, which is consistent with research by Saranraj and Stella (2013) and Gupta *et al.* (2003), which found that fungal α-amylases usually show their highest catalytic efficiency in acidic to slightly acidic conditions. The enzyme retained half of its activity at 60°C after 15 minutes, was most active at 40°C, and continued to be active between 40 °C and 70°C. These results align with those of Ogbonna *et al.* (2018) (Figure 1).

Initially, different carbon sources (sucrose, glucose, fructose, maltose, and galactose) were investigated in the fermentation medium to determine how they affected α-amylase production and mycelial growth. Sucrose (2.5g) was discovered to be the best carbon source for maximizing enzyme yield (Figure 2). Faiq *et al.* (2025) found that sucrose concentration in fermentation media plays a crucial role in producing α-amylase. Similarly, fructose increased intracellular enzyme synthesis, implying that intracellular and extracellular amylases follow different regulatory processes. The minimal reaction to organic nitrogen sources is consistent with the findings of Sun *et al.* (2010), who discovered that nitrogen supplies have a stronger influence on fungal biomass than enzyme induction. This could explain why peptone stimulated moderate enzyme production but did not significantly increase synthesis.

Furthermore, the study investigated the impact of organic nitrogen sources on fungal growth and enzyme synthesis. The study found that organic nitrogen did not significantly increase α-amylase output (Figure 2). Sun *et al.* (2010) found that nitrogen supplies can boost fungal growth but may not directly contribute to α-amylase synthesis.

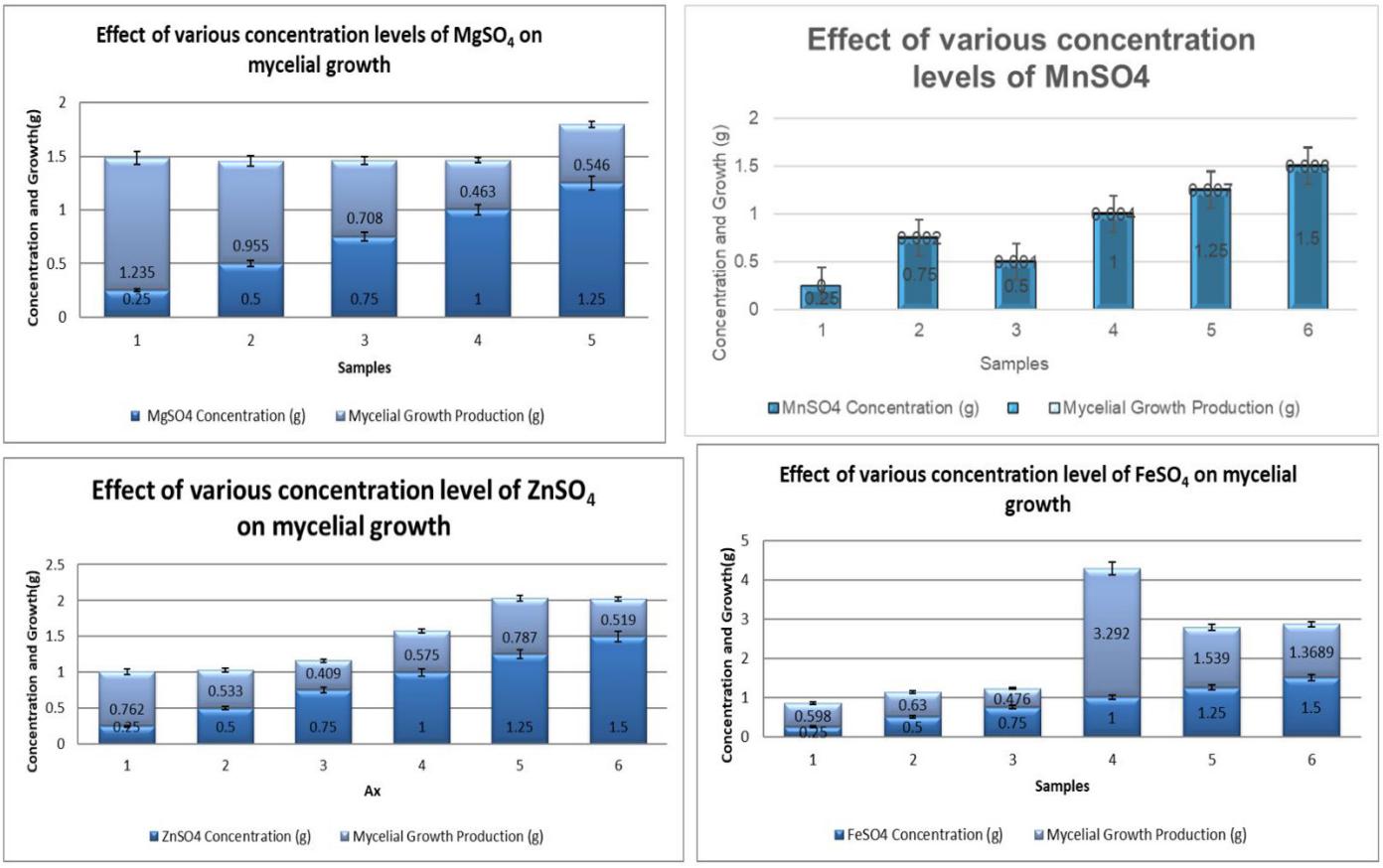

Figure 3: *Effect of metal ions on α-amylase production.*

Inorganic salts (KH_2PO_4) and cofactors ($MgSO_4$, $MnSO_4$, $ZnSO_4$, NaCl, and $FeSO_4$) were investigated further. The enzyme production was maximum with 3g KH_2PO_4 (Figure 2), 1.25g $ZnSO_4$, and 0.25g $MgSO_4$ (Figure 3). Inorganic salts were also important for enzyme control. In our work, KH_2PO_4, a key buffering and metabolic salt, dramatically boosted α-amylase production. This is similar with previous findings by Kammoun *et al*. (2008), who showed that phosphate enhances fungal metabolism and enzyme biosynthesis. The highest α-amylase yield was found at 1g $MnSO_4$ and 1g $FeSO_4$ (Figure 3). $MnSO_4$ had the least effect in increasing enzyme synthesis compared to other salts. Sindhu (2005) found that using an adjusted medium with $MgSO_4$ and $FeSO_4$, which function as cofactors during fungal fermentation, increased α-amylase production considerably.

Penicillium citrinum α-amylase was purified in a single step using ammonium sulfate precipitation (Table 2). Similar purification patterns have been reported for *Aspergillus* and *Penicillium* enzymes (Sazzad and Sabita, 2008), demonstrating the efficacy of this technique for downstream processing. Although single-step precipitation produced partially purified enzyme, the activity levels imply that additional purification stages, such as ion-exchange chromatography, could improve enzyme purity for industrial uses.

Conclusions

Penicillium citrinum has the ability to manufacture more α-amylase by surface culture fermentation, whereas fungi and bacteria also produce the enzyme. Optimizing fungal strains and fermentation conditions can increase yield, with temperature and incubation time having a substantial impact on enzyme activity and stability. The study recommends the OFAT method as a realistic way to increase α-Amylase production. Purification was restricted to ammonium sulfate; enzyme kinetics and industrial-scale parameters were not investigated. Future studies should include chromatographic purification, kinetic profiling, and bioreactor optimization.

Novelty Statement

The present work describes the first detailed purification and biochemical characterization of alpha-amylase from Penicillium citrinum showing its unique kinetic and physicochemical properties with promising industrial applicability

Author's Contribution

Mehak Iftikhar: Orignal idea, experimental work, Analysis.
Memuna G. Shahid: Research Supervisor, editor, analysis, experimentataion.
Zohaib Anjum: Analysis, experimental work.
Awais Anjum and Reham Shakeel: Analysis.
Ikram-ul-Haq: Editor, statistical analysis.
Nazish Mazhar Ali: Editor.

Generative AI and AI assisted technology statement
The authors declare that no generative AI and AI assisted technology was used in the creation of this manuscript.

Conflict of interest
The authors have declared no conflict of interest.

References

Djekrif-Dakhmouche, S.Z., Meraihi, Z. and Bennamoun, L., 2006. Application of a statistical design to the optimization of culture medium for -amylase production by *Aspergillus niger* ATCC 16404 grown on orange waste powder. J. Food Process Eng., 73: 190–197. https://doi.org/10.1016/j.jfoodeng.2005.01.021

Elyasi, F., Ahmadi, B., Yari, Y., Khosroshahi, A. and Dilmaghani, A., 2020. Microbial alpha-amylase production: Progress, challenges and perspectives. Adv. Pharm. Bull., 10(3): 350–358. https://doi.org/10.34172/apb.2020.043.

Ertan, F., Yagar, H. and Balkan, B., 2006. Some properties of free and immobilized αamylase from *Penicillium griseofulvum* by Solid State Fermentation.Prep.Biochem.Biotech.,36:81-91. https://doi.org/10.1080/10826060500388553

Faiq, M., Rauf, A., Qayash, M., Ikram, M., Jabbar, K., Khan, G., Barin, T., Khan, W., Ullah, T., Ullah, M., Khan, I., Bibi, F. and Munir, M., 2025. Alternate substrates for the growth and yield of oyster mushroom (*Pleurotus ostreatus*). Pak. J. Weed Sci. Res., 31(1): 66-73.

Farooq, M.A., Ali, S., Hassan, A., Tahir, H.M., Mumtaz, S. and Mumtaz, S., 2019. Biosynthesis and industrial applications of α-amylase: A review. Arch Microbiol., 203(4): 1281–1292.

https://doi.org/10.1007/s00203-020-02128-y

Gupta, R., Gigras, P., Mohapatra, H., Goswami, V.K. and Chauhan, B., 2003. Microbial α-amylases: A biotechnological perspective. Process Biochem., 38: 159-616. https://doi.org/10.1016/S0032-9592(03)00053-0

Hiteshi, K. and Gupta, R., 2014. Thermal adaptation of α-amylases: A review. Extremophiles, 18(6): 937-944. https://doi.org/10.1007/s00792-014-0674-5

Jensen, B., Nebelong, P., Olsen, J. and Reeslev, M., 2002. Enzyme production in continuous cultivation by the thermophilic fungus, *Thermomyces lanuginosus*. Biotechnol. Lett., 24: 41-45. https://doi.org/10.1023/A:1013805232462

Jiby, J.M., Prem, J.V., Sajeshkuma, N.K. and Anjaly, A., 2016. Amylase Production by *Aspergillus niger* through submerged fermentation using starchy food products as substrate. Int. J. Herb. Med., 4(6): 34-40.

Jujjavarapu, S.E. and Dhagat, S., 2019. Evolutionary trends in industrial production of α-amylase. Recent Pat. Biotechnol., 13(1): 4–18. https://doi.org/10.2174/2211550107666180816093436

Kammoun, R., Naili, B. and 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). Biol. Res. Technol., 99: 5602-5609. https://doi.org/10.1016/j.biortech.2007.10.045

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

Kubilay, M., Öznur, K., Burcu, B.A.Z. and Halil, B.H., 2010. Purification and characterization of α-amylase produced by *Penicillium citrinum* HBF62. Afr. J. Biotechnol., 9: 7692-7701.

Ogbonna, C.N., Nnaji, O.B. and Chioke, O.J., 2018. Isolation of amylase and cellulase producing fungi from decaying tubers and optimization of their enzyme production in solid and submerged cultures. IJBFS., 6: 9-17.

Prakash, O. and Jaiswal, N., 2010. α-amylase: An ideal representative of thermostable enzymes. Appl. Biochem. Biotechnol., 160(8): 2401–2414. https://doi.org/10.1007/s12010-009-8735-4

Reddy, N.S., Nimmagadda, A. and Sambasiva, R.K.R.S., 2003. An overview of the microbial -amylase family. Afr. J. Biotechnol., 2: 645-648. https://doi.org/10.5897/AJB2003.000-1119

Saranraj, P. and Stella, D., 2013. Fungal amylase: A review. Int. J. Microbiol. Res. Rev., 4: 203- 211.

Sazzad, M. and Sabita, R.R., 2008. Production and partial characterization of extracellular α-amylases by *Trichoderma viride*. Br. J. Med., 25: 99- 103. https://doi.org/10.3329/bjm.v25i2.4870

Silaban, S., Marika, D.B. and Simorangkir, M., 2020. Isolation and characterization of amylase-producing amylolytic bacteria from rice soil samples. J. Phys. Conf. Ser., 1485(1): 012006. https://doi.org/10.1088/1742-6596/1485/1/012006

Sindhu, R., 2005. Isolation, purification and characterization of α-amylase from *Penicillium janthinellum*. Ph. D. Thesis School of Biosciences, Mahatma Gandhi University solid state fermentation by the thermophilic fungus *Thermomyces lanuginosus*. J. Biosci. Bioeng., 100: 168-171. https://doi.org/10.1263/jbb.100.168

Steel, R.G.D., Torrie, J.H., 1996. Principles and procedures of statistics: A biometrical approach 3rd ed. McGraw-Hill, 1996.

Sun, H., Zhao, P., Ge, X., Xia, Y., Hao, Z., Liu, J. and Peng, M., 2010. Recent advances in microbial raw starch degrading enzymes. Appl. Biochem. Biotechnol., 160: 988-1003. https://doi.org/10.1007/s12010-009-8579-y

Ullah, S., Saeed, M., Sohail, M., Ali,S., Aziz, L., Noreen, A., Subhan, G., Khan, I. and Shakir, L., 2025. Indigenous knowledge of medicinal plants used by the tribal communities of Hasham Valley, District Dir Lower, Khyber Pakhtunkhwa Pakistan. Pak. J. Weed Sci. Res., 31(1): 37-55.