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Production and Characterization of Pectinase from *Aspergillus niger* Using Orange Peels as Carbon Source

Emochone Roy Yohanna[1], Moses Adondua Abah*[1,2], Micheal Abimbola Oladosu[2,3], Okpanachi Nuhu Oyibo[4], Iheanacho Chinedu Christian[5,6], Victoria Chibuihe Nnamdi[7], Wontumi Samuel Odarno[8,9], and Iheakolam Uchenna Caleb[10]

[1]*Department of Biochemistry, Faculty of Biosciencesa, Federal University Wukari, Taraba State, Nigeria*
[2]*ResearchHub Nexus Institute, Nigeria*
[3]*Department of Biochemistry, Faculty of Basic Medical Sciences, University of Lagos, Lagos State, Nigeria*
[4]*Coveup Research Institute, Department of Biochemistry, Faculty of Biological Sciences, University of Nigeria, Nsukka, Enugu State, Nigeria*
[5]*Department of Microbiology, Faculty of Biosciences, Federal University Wukari, Wukari, Taraba State, Nigeria*
[6]*State Key Laboratory of Regional and Urban Ecology, Zhejiang Key Laboratory of Pollution, Control for Port Petrochemical Industry, Ningbo Observation and Research Station, Institute of Urban Environment, Chinese Academy of Sciences, Xiamen 361021, China*
[7]*Department of Chemical Engineering, Roy G. Perry College of Engineering, Prairie View A&M University, USA*
[8]*Department of Food and Agriculture, Ghana Standards Authority, Accra-Ghana*
[9]*Department of Food Science and Technology, Kwame Nkrumah University of Science and Technology, Ghana*
[10]*Department of Microbiology, Faculty of Biological and Physical Sciences, Abia State University, Uturu, Nigeria*

Corresponding author: **Moses Adondua Abah** | E-mail: **m.abah@fuwukari.edu.ng**

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Abstract

Pectinase is a crucial enzyme in the food industry, particularly in juice extraction and clarification. Aspergillus niger is a commonly used fungus for pectinase production. Orange peels, rich in pectin, offer a cost-effective and sustainable carbon source for microbial fermentation. Solid-state fermentation (SSF) and submerged fermentation (SmF) are employed to produce pectinase. Optimizing fermentation conditions and characterizing the enzyme are essential for efficient production.This study aimed at producing and characterizing pectinase from Aspergillus niger using orange peels. A sufficient quantity of orange peels was collected from Wukari New Market, along Wukari-Jalingo Road, Taraba State, and brought to the Biochemistry Laboratory in Federal University Wukari. Taraba State, where they were washed with potable water, minced into small particles, sun-dried, and allowed to decay. 1 g of the decaying sample was weighed aseptically into 9ml of distilled water separately and shaken thoroughly. From this mixture, dilutions were subsequently made up to 10^{-4} and pour plating of 10^{-2}, 10^{-3}, and 10^{-4} were done in sterilised Potato Dextrose Agar (PDA). Streptomycin (100mg/l) was added to the PDA after sterilisation to prevent bacterial growth. Plates were incubated in an inverted position at 30ºC for 7 days. After incubation, the plates were observed for fungal growth. The isolates were examined and identified in the Department of Microbiology, Federal University Wukari, based on colonial and cultural characteristics and morphology of isolate sporing structures. Isolate fungi were identified using a light microscope. Isolates were flooded with iodine-potassium iodide solution for 10min and colonies wereobserved by the appearance of a clear zone around the plates. It was observed that pectinase activitywasoptimumonday3ofthefermentation periodincontrol and both tube 1 and tube 2, while minimum activity recorded on day 1 of fermentation and in the control sample as well as tube 1 and tube 2. pH 5.6 recorded the lowest activity of pectinase which to increase again from pH 6.0 until optimum pH was recorded at 7.0. The peak of pectinase activity was observed at 50 °C following this sharp increase. Pectinase activity was optimum at a substrate concentration 1%. The KmandVmaxvaluesforpectinasewere estimated to be 5.93and2.22,respectively.The study successfully produced pectinase from Aspergillus niger using orange peels as a carbon source, offering a sustainable and eco-friendly approach. This approach reduces waste and adds value to agricultural by-products.

Keywords: *Aspergillus niger, Orange peels, Pectinase, Fermentation, and Extraction.*

1. Introduction

Pectinases are a group of enzymes that hydrolyze pectic substances, complex polysaccharides abundantly present in the middle lamella and primary cell walls of plants[1]. As biocatalysts, pectinases have gained significant industrial importance due to their role in degrading pectin into simpler molecules such as galacturonic acid and its derivatives.

The demand for pectinase has grown steadily, driven by its diverse applications in fruit juice clarification, textile processing, paper and pulp treatment, waste management, and the extraction of plant-based compounds [2,3]. With the expanding global market for enzyme-based technologies, the quest for cost-effective, sustainable, and high-yield enzyme production systems has become more pressing[4]. Microbial sources, particularly filamentous fungi, offer numerous advantages over plant and animal sources, including ease of cultivation, high enzyme yields, and the ability to utilize inexpensive substrates for fermentation. Among these microorganisms, *Aspergillus niger* stands out as one of the most efficient producers of pectinases[5, 6].

Aspergillus niger, a ubiquitous filamentous fungus, is widely recognized for its Generally Regarded as Safe (GRAS) status and its robust ability to secrete large quantities of extracellular enzymes[7]. The organism's metabolic flexibility enables it to grow on a wide range of agro-industrial wastes, making it an ideal candidate for low-cost enzyme production[8]. Its capability to tolerate acidic conditions and efficiently hydrolyze complex carbon sources further enhances its industrial relevance[9]. Pectinase production by *A. niger* has been extensively described in literature, yet optimization using locally available agricultural residues remains an evolving area of research, especially in developing countries where the availability of commercial substrates may be limited[10]. The use of cost-effective raw materials not only reduces production expenses but also contributes to environmental sustainability by converting waste into valuable products[11].

Orange peels, a major by-product of citrus industries, represent an abundant, underutilized source of pectin and other nutrients suitable for microbial growth[12]. Globally, citrus processing generates millions of tons of peel waste annually, posing significant disposal challenges[13]. In Nigeria and many parts of the world, orange peels are discarded in large quantities despite their high carbohydrate, pectin, and mineral contents that could serve as excellent substrates for fermentation processes[14]. The bioconversion of orange peels into valuable products aligns with the principles of circular bioeconomy, waste minimization, and sustainable industrial practices. Utilizing orange peels as carbon sources for pectinase production, therefore, offers a dual advantage: providing a low-cost substrate for fermentation and reducing environmental pollution associated with citrus waste [15].

The production of pectinase using agro-wastes is influenced by several factors, including pH, temperature, fermentation duration, substrate concentration, and the nature of the microorganism[16]. Solid-state fermentation (SSF) and submerged fermentation (SmF) are two major approaches used for microbial enzyme production, with SSF often regarded as more suitable for fungi and agro-residue utilization. However, submerged fermentation offers better control of process variables and is widely applied for industrial-scale enzyme synthesis[16, 17]. The choice of method significantly affects enzyme yield and characteristics, underscoring the need for optimization and careful selection of process parameters[17]. The composition of orange peels, rich in pectin and simple sugars, supports efficient enzyme induction during fermentation, allowing *A. niger* to secrete pectinase in appreciable quantities.

Characterization of the produced pectinase is a crucial step in determining its potential industrial applications. Enzyme properties such as optimum temperature, optimum pH, stability, substrate specificity, and kinetic parameters define its suitability for specific processes. Industrial pectinases typically operate optimally in acidic environments and at moderate temperatures[18]. Characterizing these parameters helps to evaluate the versatility and efficiency of the enzyme under various processing conditions. Understanding enzyme kinetics also provides insights into the catalytic mechanism and potential modifications needed for enhanced performance19]. Furthermore, partially or fully purified enzymes allow researchers to explore biochemical properties essential for targeted applications[15, 16].

Despite the progress made in enzyme biotechnology, there remains a gap in the utilization of locally sourced agro-wastes for pectinase production, particularly in resource-limited settings where industrial enzymes are often imported at high cost. The adoption of indigenous raw materials such as orange peels could significantly reduce dependence on imported enzymes and promote local enzyme production industries. In addition, research focusing on *Aspergillus niger* strains isolated from local environments could enhance enzyme yield and adaptation to region-specific conditions[17]. Studies integrating microbial fermentation, waste valorization, and enzyme characterization hold immense potential to support food processing industries, agro-based enterprises, and environmental management initiatives[18].

This research, therefore, aims to produce and characterize pectinase from *Aspergillus niger* using orange peels as a carbon source. The study investigates the suitability of orange peels as inexpensive substrates for enzyme induction, evaluates the fermentation conditions necessary for optimal enzyme production, and characterizes the resulting pectinase to determine its biochemical properties. By exploring an eco-friendly, cost-effective production method, this work contributes to the growing body of knowledge on sustainable enzyme biotechnology and provides a scientific basis for industrial utilization of agricultural waste. Additionally, it offers practical relevance to local industries in Nigeria and beyond, promoting sustainable waste management and economic valorization of citrus residues. Ultimately, this study underscores the potential of agro-waste-based enzyme production as a pathway towards achieving environmentally sustainable and economically feasible biotechnological innovation.

2. Materials and Method

2.1 Materials and Reagents Used for the Study

For the purpose of this study, the following materials and reagents were used: sieve, hot plate, beakers, Erlenmeyer flasks, centrifuge, incubator, water bath, dialysis bag, electrophoretic machine, spectrophotometer, petri-dish, 3, 5-dinitrosalicylic acid (DNS), Bovine serum albumin (BSA), Ethanol, Sodium hexametaphosphate, Hydrochloric acid (HCL), distilled water, Acetone, $(NH_4)_2SO_4$; 0.20% K_2HPO_4; 0.02% of $MgSO_4.7H_2O$; 0.10% of nutrient solution (5mg/l $FeSO_4.7H_2O$; 1.6mg/l $MnSO_4.H_2O$; 1.4mg/ml $ZnSO_4.7H_2O$; 2.0mg/l $CoCl_2$), among others.

2.2 Study Area

The research was conducted at the Federal University Wukari, Taraba State, specifically in Biochemistry Laboratory and Microbiology Laboratory between January and April, 2022. Wukari community is the headquarters located in Wukari local Government Area of Taraba State in Nigeria, Taraba Zone, it has an area of 4,308 Km^2 positioned at a latitude of 7°51'0''N and a longitude of 9°47'0''E. The River Donga flows through Wukari Local Government Area. The climate in Wukari is characterized by an average annual temperature of approximately 62°F, which can rise up to 93°F during the dry season. The relative humidity of the area is about 31% to 43%. Spanning roughly 4,268 km^2, the Wukari Local Government Area has a population of about 374,800, based on the 2022 census. The primary occupations in the community include fishing, agriculture, and trade, with the river playing an essential role in these activities [1].

2.2 Collection of Samples (Orange Peels)

A sufficient quantity of orange peels was collected from Wukari New Market, along Wukari-Jalingo Road, Taraba State, and brought to the Biochemistry Laboratory, where they were washed with potable water, minced into small particles, and sun-dried, then allowed to decay.

2.3 Isolation of Fungi

1 g of the decaying sample was weighed aseptically into 9ml of distilled water separately and shaken thoroughly. From this, dilutions were subsequently made up to $10^{-4,}$ and pourplatingof10^{-2}, 10^{-3},and $10^{-4}$was donein sterilised Potato Dextrose Agar (PDA). Streptomycin (100mg/l) was added to the PDA after sterilisation to prevent bacterial growth. Plates were incubated in an inverted position at 30ºC for 7 days. After incubation, the plates were observed for fungal growth[2].

2.4 Morphological Identification

The isolates were examined and identified in the Department of Microbiology, Federal University Wukari, based on colonial and cultural characteristics, and the morphology of isolate sporing structures. The microscopic structure of the isolate was studied using a light microscope [3].

2.5 Screening of Fungi for Pectinase Production

A Potato Dextrose Agar (PDA) plate was used for the screening of pectinase production using the method of [4].Pure cultures were inoculated into sterilized Potato Dextrose Agar medium. The Inoculated plates were incubated at 37°C for 2days. The plates were flooded with iodine- potassium iodide solution for 5–10min and pectinase-producing colonies were detected by the appearance of a clear zone around them. The fungi isolate with the highest value of the clearzone of the hydrolysis of pectinase was selected and stored in the fridge on pectin agar.

2.6 Pectinase Production Under Submerged Fermentation

About 10g of solid substrates, 0.1% (0.01 g) (NH4)2NO3, 0.1% $MgSO_4$, and 0.1% $NH_4H_2PO_4$ were weighed into 250ml Erlenmeyer flasks and then autoclaved at 121°C for 20 minutes.

To every sterile flask, one disc of actively growing *Aspergillus niger* from a 4-day agar medium was added using a cork borer of diameter 10 mm, and then plugged properly. The flasks were incubated for 7days at 37°C.at 24hrs interval. A flask was selected, and the mycelia biomass was separated by filtration using a muslin cloth. The mycelia biomass filtered was centrifuged at 12,000rpm for 20 minutes. The resulting supernatant was tested for pectinase activity.

2.7 Polygalacturonase (Pectinase) Assay

Table 1. Polygalacturonase (Pectinase) assay

Reagent	Tube1	Tube2	Blank
1.0%sodiumacetatebuffer(pH 5.5)	1ml	1ml	**1ml**
Enzyme	1ml	1ml	**-**
1%pectin	1ml	1ml	**1ml**
50ºC	50ºC	50ºC	**50ºC**
Time	30min	30min	**30min**
3,5-dinitrosalicylicacidreagent	1.5ml	1.5ml	**1.5ml**
Absorbancereading	**540 nm**	**540 nm**	**540 nm**

2.8 Characterization of Pectinase

2.8.1 Effect of pHon Pectinase Activity

The effect of pH on enzyme activity was tested using sodium acetate buffer (0.1 M; pH range, 4.5–5.0), sodium phosphate buffer (0.1 M; pH range, 6.0–7.0), and Tris-HCl buffer (0.1 M; pH range, 8.0–9.0).Tubes containing0.5 mLof the respective buffersweremixedwith0.5mLof the enzyme. Then, 1 mL of 0.3% (w/υ) pectin solution was added, and all the tubes wereincubated at 30ºC for 10 minutes.

2.8.2 Effect of Temperature on Pectinase Activity

Pectinase activity was investigated at different temperatures ranging from 25–50°C at 5°C intervals in a water bath.

2.8.3 EffectofSubstrateConcentrationonPectinase Activity

The substrate (orange pectin) was prepared in different concentrations ranging from 0.52.0% in different tubes to evaluate substrate concentration on pectinase activity. 1 mL of enzyme suspended in acetate buffer (0.1M, pH 4.2) was mixed with 1 mL of the various substrate concentrations.

2.8.4 Kinetic Parameters of Pectinase

The method reported by [5]wasused in the determination of the kinetic constant andmaximum velocity (Km and Vmax) of pectinase.

2.9 Statistical Analysis

The results observed are the mean of three independent experimental replicates ($n=3), and values are represented as the mean ± standard error.

3. Results

3.1 Pectinase Production Under Submerged Fermentation

Figure 1, shown below, captures the result of pectinase production under submerged fermentation over a period of 4 days. The selected isolates from the primary screening method were subjected to solid-state fermentation in a suitable medium and observed. Pectinase production increased gradually over 24 hours of incubation until optimum pectinase production was achieved. After the optimum incubation period, pectinase production began to decrease.

It was observed that pectinase activity was optimum on day 3 of the fermentation period in the control and both tube 1 and tube 2, while minimum activity was recorded on day 1 of fermentation in the control sample as well as tube 1 and tube 2.

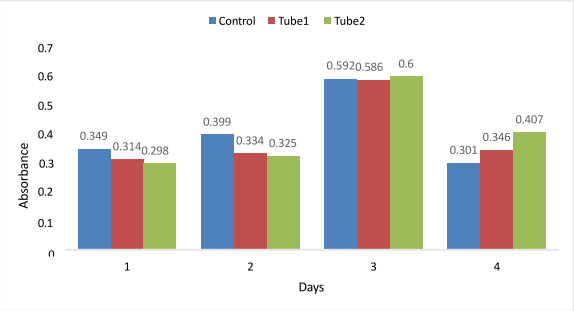

Figure 1. Production of pectinase under submerged fermentationlacturonase (Pectinase) assay

3.2 Effect of pH on Pectinase Activity

The result of the effect of pH on pectinase activityis shown in Figure 2 below. As pH increased from 1 to 4.9, pectinase activity was also seen to increase proportionately. However, a decrease in pectinase activity was observed beyond pH 4.9. pH 5.6 recorded the lowest activity of pectinase,which t hen increased again from pH 6.0 until optimum pH was recorded at 7.0, where pectinase showed its maximum activity. After this, a decrease in pectinase activity was noticed at pH 8.2 when pectinase activity began to rise again.

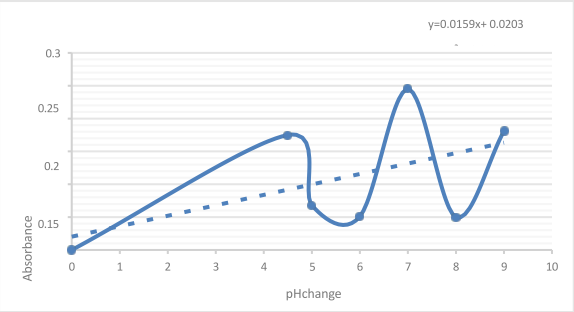

Figure 2. Effect of pH on pectinase activity

3.3 Effect of Temperature on Pectinase Activity

The result of the effect of temperature on pectinase activityis shown in Figure 3 below. It was observed that asthe temperature increased from 0°C to 25 °C, pectinase activity was also seen to increase proportionately. However, a decrease in pectinase activity was observed at 26°C which continued until 30 °C, when pectinase activity began to increase again. At 36 °C, a decrease in pectinase activity was noticed, which persisted until 45 °C;a sharp increase in the activity of pectinase was noticed. The peak of pectinase activity was observed at 50 °C following this sharp increase.

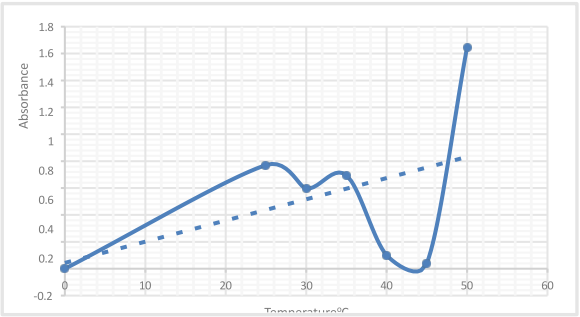

Figure 3. Effect of temperature on spectacular suicide

3.4 Effect of Substrate Concentration on Pectinase Activity

The result of the effect of substrate concentration on pectinase activityis shown in Figure 4 below. It was observed that as substrate concentration increased from 0% to 1%, pectinase activity was also seen to increase proportionately. However, beyond 1% substrate concentration, a stable progression in pectinase activity was noticed inspite of the growing increase in substrate concentration. Pectinase activity was optimum at substrate concentration 1%.

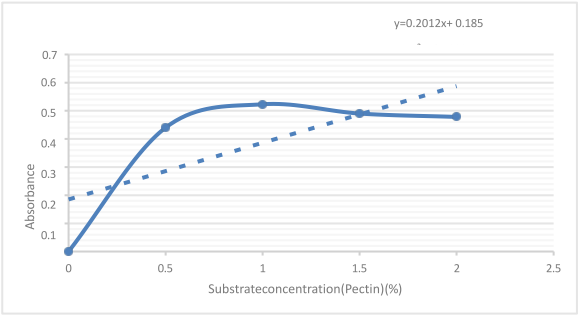

Figure 4. Effect of substrate concentration on pectinase activity

3.5 Kinetic Parameters of Pectinase Activity

From Table 2, which contains the kinetic data for pectinase, and Figure 5, which presents the Lineweaver-Burk plot for pectinase, the Km and Vmax values for pectinase were estimated to be 5.93 and 2.22, respectively.

Table 2. Kinetic data for pectinase

[S]	Vo	1/[S]	1/Vo
0.5	0.18	2	5.56
1	0.32	1	3.13
1.5	0.47	0.67	2.13
2	0.59	0.5	1.7

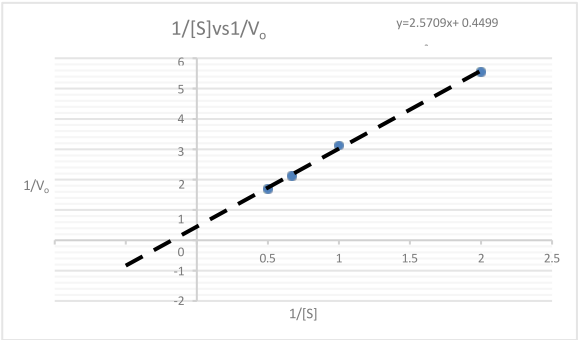

Figure 5. Lineweaver-Burk plot for pectinase Intercept=0.4499,Vmax=2.22

4. Discussion

Pectinase, an enzyme that breaks down pectin in plant cell walls, is widely used in the food, textile, and paper industries[1]. *Aspergillus niger* is a commonly used fungus for pectinase production due to its high yield and GRAS (Generally Recognized as Safe) status[4]. Orange peels, a rich source of pectin, offer a cost-effective and sustainable alternative to commercial pectin[5]. In this study, we produced and characterized pectinase from *Aspergillus niger* using orange peels as a carbon source. The enzyme was produced through solid-state fermentation (SSF) and optimized for pH, temperature, and substrate concentration. The crude enzyme was then characterized for its activity, stability, and kinetic parameters. Our results show promising potential for industrial applications, which will be discussed in detail.

The production and characterization of pectinase from *Aspergillus niger* using orange peels as a carbon source revealed several important trends relating to enzyme synthesis, optimal environmental conditions, and catalytic efficiency. The progressive increase in pectinase production during the submerged fermentation period, as shown in Figure 1, aligns with typical fungal growth dynamics. In this study, pectinase activity increased steadily over the first 24 hours and peaked on day 3 for the control, tube 1, and tube 2 before declining thereafter. This pattern is consistent with reports by [6], who found that *A. niger* reached maximum pectinase production between 48 and 72 hours when grown on citrus waste substrates. Similarly, [7] observed maximum pectinase yields on day 3 of fermentation, attributing the decline beyond this point to nutrient depletion and the accumulation of secondary metabolites that interfere with enzyme synthesis. Thus, the observed peak on day 3 in this study corroborates the widely reported timeframe for optimal pectinase production in *A. niger*.

The decline in enzyme activity after day 3 may be due to feedback inhibition or proteolytic degradation, phenomena frequently noted in fungal fermentations. According to [8], extended fermentation often leads to pH fluctuations and the breakdown of mycelial structures, resulting in reduced enzyme secretion. These explanations support the reduced activity observed on day 4 in the present investigation.

The effect of pH on pectinase activity, as illustrated in Figure 2, showed a complex but interpretable pattern. Enzyme activity increased from pH 1.0 to 4.9, demonstrating the acidophilic nature of fungal pectinases. However, a marked reduction occurred at pH 5.6, followed by a gradual increase to an optimum at pH 7.0. This optimum pH aligns with findings by [9], who reported that crude pectinase extracted from *A. niger* expressed maximum activity at neutral pH. In contrast, some studies have reported optimal pH values in the acidic range (4.0–5.0). For instance, [10] documented an optimum pH of 5.0 for pectinase from *A. niger* grown on mango peels. These differences may arise from the nature of the substrate, fungal strain variability, or purification levels of the enzyme. The observed drop in the activity at pH 8.2 in this study is also supported by [11], who indicated that alkaline conditions tend to destabilize pectinase structure, resulting in decreased catalytic efficiency.

Temperature profiling (Figure 3) showed that pectinase activity increased from 0°C to 25°C, decreased at 26–30°C, and then rose sharply to a maximum at 50°C, with notable fluctuations in between. The highest activity at 50°C agrees with the findings of [12], who reported that *A. niger* pectinase exhibited maximum activity at temperatures between 45°C and 55°C. The temporary decline around 26–30°C may reflect partial temperature-induced conformational changes or mixed populations of isoenzymes. Furthermore, the decrease at 36°C followed by a sharp rise toward 50°C suggests the presence of thermostable pectinase variants, a characteristic previously noted by [13]. According to their work, *A. niger* often produces multiple forms of pectinase, some of which remain active at elevated temperatures. The sensitivity observed at low temperatures (0–10°C) is expected, as enzymatic catalysis becomes inefficient when molecular movement is significantly restricted.

The influence of substrate concentration on pectinase activity (Figure 4) revealed that enzyme activity increased from 0% to 1% pectin concentration and plateaued thereafter, regardless of increasing substrate levels. This observation aligns with the Michaelis–Menten kinetic principle: once the enzyme's active sites become saturated, further increases in substrate concentration do not enhance reaction rates. Similar findings were reported by [14], who observed that pectinase activity from *A. niger* peaked at 1% substrate concentration and stabilized at higher concentrations. The stabilization seen in the present study beyond 1% may indicate substrate inhibition or saturation, both of which have been reported in fungal pectinases.

From Table 2 and the Lineweaver–Burk plot in Figure 5, the estimated Km of 5.93 and Vmax of 2.22 indicate moderate affinity of the pectinase enzyme for its substrate. Comparable Km values have been reported in literature; for example, [15] documented Km values ranging from 3.5 to 6.8 for crude fungal pectinases. The Km in this study suggests efficient substrate binding, while the Vmax reflects the maximum catalytic capacity under the given conditions. The consistency of these values with earlier findings reinforces the suitability of orange peels as a viable carbon source for enzyme production.

Overall, the results of this study reveal that *Aspergillus niger* grown on orange peels can effectively produce pectinase with behavior and characteristics similar to those documented for the organism in other agro-waste-based fermentation systems. The agreement between the present findings and those of previous researchers validates the efficiency of citrus peels as low-cost substrates and emphasizes the robustness of *A. niger* pectinase under varying environmental conditions. The enzyme's stability at neutral pH and relatively high temperatures also underscores its potential for industrial applications such as fruit juice clarification, textile processing, and wastewater treatment.

5. Recommendations

Based on the findings of this study, it is recommended that the use of orange peels and other agro-industrial wastes be encouraged for large-scale enzyme production to reduce waste accumulation and promote cost-effective bioprocessing. Industries involved in fruit juice processing and citrus waste management should collaborate with researchers to explore pilot-scale pectinase production using similar substrates.

Further investigations should focus on optimizing fermentation parameters such as moisture content, inoculum size, aeration, and agitation to maximize enzyme yield and prolong the peak production period observed on day three. Studies on mixed fruit wastes could also be carried out to determine whether combined substrates enhance pectinase synthesis.

Purification and molecular characterization of the enzyme are recommended to better understand its isoforms, structural stability, and potential for genetic improvement. Evaluating storage stability and shelf life will further support industrial utilization.

Industries in food processing, textiles, paper pulping, and wastewater treatment should consider integrating the produced pectinase into their processes due to its optimal activity at moderate temperatures and neutral pH. Finally, government agencies should support waste-to-wealth initiatives by providing incentives and infrastructure to promote sustainable biotechnology and circular economy practices.

Conclusion

This study demonstrated that *Aspergillus niger* efficiently produces pectinase when cultivated on orange peels, confirming the suitability of this agro-industrial waste as a low-cost carbon source. The fermentation profile revealed that pectinase production increased steadily and reached its peak on the third day, after which a gradual decline occurred. This pattern aligns with typical fungal enzyme secretion behavior, where maximum production corresponds to the active growth phase and decreases as nutrients diminish.

Characterization of the enzyme showed that pH, temperature, and substrate concentration significantly influenced pectinase activity. The enzyme exhibited its highest activity at pH 7.0, indicating stability around neutral conditions. Temperature profiling showed optimum activity at 50°C, demonstrating the enzyme's thermostability and potential for applications requiring moderate heat. Substrate concentration analysis revealed a saturation point at 1%, beyond which further increases did not enhance activity, reflecting Michaelis-Menten behavior.

The kinetic parameters (Km = 5.93, Vmax = 2.22) further confirm that the enzyme possesses good affinity for its substrate and satisfactory catalytic efficiency. Overall, this study highlights orange peels as a valuable substrate for cost-effective pectinase production and underscores the potential industrial relevance of *A. niger* pectinase in food processing, textiles, and environmental biotechnology.

Authors' Contributions

The authors of this research have significantly contributed to the study's conception, data collection, and manuscript development. All authors were involved in writing the manuscript or critically reviewing it for its intellectual value. They have reviewed and approved the final version for submission and publication and accept full responsibility for the content and integrity of the work.

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Conflict of Interest

The authors declared that there are no conflicts of interest.

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