Pectin-rich Banana Peel Varieties: A Low-cost Biomass for Pectinase Production by *Aspergillus flavus* in Solid-state Fermentation and its Effect on the Clarification of Orange Juice

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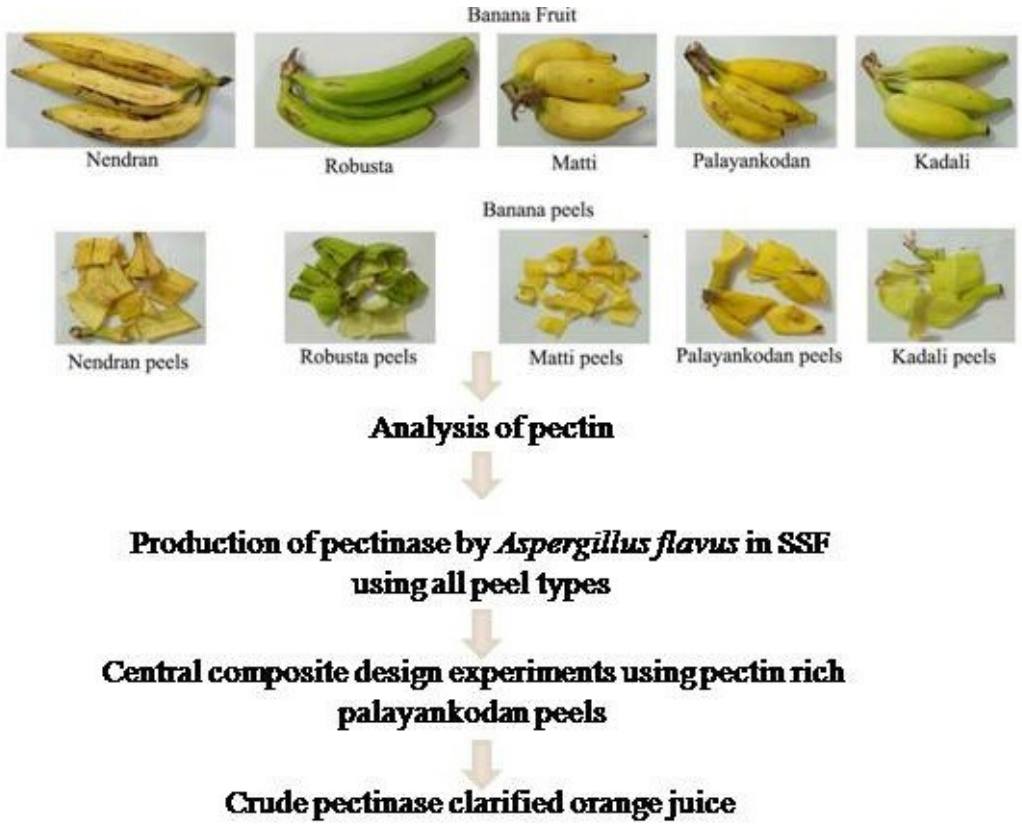
GRAPHICAL ABSTRACT



Pectin-rich Banana Peel Varieties: A Low-cost Biomass for Pectinase Production by *Aspergillus flavus* in Solid-state Fermentation and its Effect on the Clarification of Orange Juice

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A total of five pectin-rich banana peels, including those of two *Musa paradisiaca* cultivars (nendran and kadali) and three *Musa acuminata* varieties (matti, palayankodan, and robusta), were used for pectinase production by *Aspergillus flavus via* solid-state fermentation (SSF). Comparative analysis revealed highest pectin content in the palayankodan peel (18.09 ± 0.29%). The palayankodan peel variety presented 135 ± 2.2 U/gds pectinase activity in SSF using *A. flavus*, whereas robusta peels presented 108 ± 1.9 U/gds pectinase activity in SSF. A one-factor-at-a-time experiment was performed, and the variables moisture, pH, and fermentation period affected enzyme production. A central composite design was used to optimize pectinase production in SSF *via* three prominent variables (moisture content, pH, and fermentation period). The experimental result was statistically significant ($p<0.01$), and twofold enzyme production was achieved. The crude pectinase was extracted from the fermented medium and used as a clarifying agent. The pectinase-treated orange juice presented a decreased turbidity compared to the untreated control. The amount of total sugar and total suspended solids was reduced, whereas the total pH increased. Therefore, the *A. flavus* strain can be utilized for large-scale production of pectinase, which could meet the growing industry demands.

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Keywords: Banana peels; Pectinase; Aspergillus flavus; Orange juice; Clarifying agent

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INTRODUCTION

Microbial pectinases are widely recognized as a heterogeneous group of enzymes capable of hydrolyzing pectin (Abdollahzadeh *et al.* 2020). They have wide applications in several industrial sectors, such as the oil and juice extraction, animal feed preparation, fermentation, wastewater treatment, pharmaceutical, pulp, and paper industries (Sharma *et al.* 2013; Singh *et al*. 2024). Microbial pectinases are produced commercially by various microorganisms because of their potential to use low-cost substrates, reduce production costs, and increase yields *via* either solid-state fermentation or submerged fermentation (Kc *et al.* 2020; Kaul *et al*. 2024). Recently, pectinase production from fungi has attracted increasing attention. Pectinases from the genus *Aspergillus* have been widely used for

commercial preparations of various agroresidues. For example, *Aspergillus niger* was used to optimize endogluconases and exogluconate production from mango waste (Santos *et al.* 2011). In one study, *Aspergillus* NCFT 4269 was used to produce pectinase by utilizing banana peel as a low-cost substrate (Sethi *et al.* 2016). In addition, fungi from the genus *Aspergillus* have the ability to survive, colonize, and produce metabolites on solid substrates (Sandri and daSilveira 2018). Fungi, especially filamentous fungi, prefer solid-state fermentation (SSF) to produce pectinase rather than submerged fermentation because of its potential to improve nutritional properties, reduce contamination, increase enzyme yield, and increase cost-effectiveness (Rojas *et al.* 2011; Demir *et al.* 2012; Santos *et al.* 2015; Alarjani *et al*. 2024).

Lignocellulose (LC) waste is the major resource utilized for the bioconversion of industrially useful products. Banana is an important crop in Asian countries and is widely consumed because of its palatability, nutritional value, digestibility, and cost effectiveness (Arokiyaraj *et al*. 2024). Banana peel is an important source of LC substrate and is generated throughout the year in India; hence, it can be utilized for its possible use as a cheap biomass (Sajish *et al*. 2025). In Asia, banana varieties are cultivated in thousands of hectares and generate tons of industrial waste. Banana peel is one of the major waste products derived from banana fruit. Banana peel comprises cellulose (7 to 10%), hemicelluloses (6 to 9.4%), pectin (10 to 21%), and lignin (6 to 12%) (Emaga *et al.* 2008). It has been recommended as an effective substrate to produce plant cell wall-degrading enzymes under SSF by *Aspergillus terreus* and *Aspergillus niger* (Rehman *et al.* 2014). It has also been used to produce laccase by *Trametes pubescens* (Osma *et al.* 2007) and of alpha-amylase by *Penicillium* spp. and *Bacillus subtilis* (Akkarachaneeyakorn *et al.* 2018). Banana peel is also utilized to produce citric acid from *Aspergillus niger via* koji fermentation (Karthikeyan and Sivakumar 2010), biohydrogen production (Nathoa *et al.* 2014), bioethanol production, and fungal carbohydrase production (Janveja *et al.* 2013). Pectinases are inducible enzymes and microbes utilized pectin rich agricultural residues and improved enzyme production (Shrestha *et al*. 2022; Alfarhan *et al*. 2024).

Statistical tools are useful for optimizing culture conditions to reduce production costs. The optimization process initially involves identifying the suitable variables or factors affecting the production of enzymes, followed by the identification of their optimal culture conditions or nutrient requirements. The one-variable-at-a-time approach has been frequently employed to optimize bioprocesses; however, several limitations have been reported in this traditional approach (Managamuri *et al.* 2016; Marraiki *et al.* 2020). Hence, statistical-based approaches have been recommended for studying the interactions among process variables. The Plackett–Burman design and the two-level factorial design are important statistical tools for identifying variables that have an impact on enzyme production (Al Farraj *et al.* 2020; Shoba *et al*. 2025). These statistical tools allow the elimination of insignificant variables, which reduces the number of total experimental runs. The second step of the optimization process helps to identify the optimum level of variables, predict the optimum response (yield), and validate the response *via* central composite design (CCD) and Box–Behnken design (El-Sheikh *et al.* 2020; Kalaiyarasi *et al.* 2020). Considering the availability of banana fruit peel in Asian countries and the suitability of the substrate for fermentation bioprocesses, this work was performed to utilize low-cost banana fruit peel waste for pectinase production under solid-state fermentation by *Aspergillus flavus*.

EXPERIMENTAL

Fungi and Culture Conditions

The fungal strain *Aspergillus flavus* (MTCC 277) was used to produce pectinase. The culture strain was obtained from the Institute of Microbial Technology, Pune, India. The strain was cultured on Czapek yeast extract agar (Himedia, Mumbai, India) plates and incubated for 3 days in an incubator at 28± 1 °C. It was further stored at 4 °C and subcultured every 30 days.

Substrate

A total of five banana fruit varieties were collected, and the peels were utilized to produce enzymes. Two *Musa paradisiaca* cultivars, namely, nendran and kadali, and three *Musa acuminata* cultivars, namely, matti, palayankodan, and robusta, were used in this study. All these types of banana fruits were collected from the market and used for analysis. The ripened banana peels were washed and chopped into small pieces (2 cm × 2 cm) (Fig. 1). The peels were dried under sunlight for five days and powdered mechanically. The material was sieved and either used for pretreatment for enzyme production or used directly for pectin content analysis.

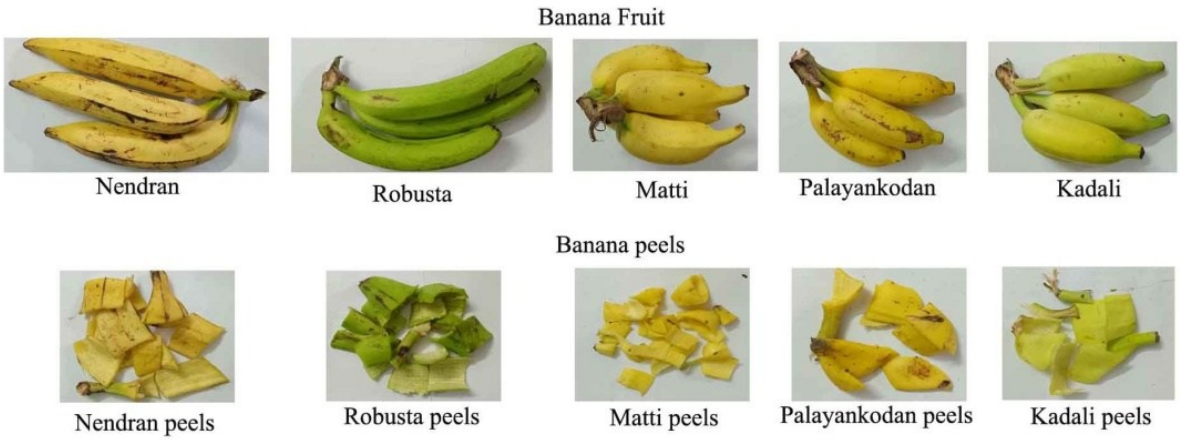


Fig. 1. Banana fruit varieties utilized for the preparation of substrates in solid-state fermentation

Pectin Analysis

The pectin content of banana peels was determined as described previously (Begum *et al.* 2014; Faruque *et al.* 2016), with slight modifications. Approximately 5 g of powdered banana peel was mixed with 50 mL of double distilled water. The pH of the solution was adjusted to 2.2 ± 0.02 using (0.1 N) hydrochloric acid. The mixture was then heated at 90 ± 1 °C for 1 h and cooled to room temperature, after which the precipitate was observed. The mixture was subsequently centrifuged at 5000 × *g* for 10 min and filtered through Whatman No. 1 filter paper. To the filtrate, three volumes of 99% ethanol were added, the precipitate was separated, and the filtrate was dried. The dried filtrate was weighed, and the pectin yield (%) was calculated (Eq. 1):

$$\text{Pectin yield, Ypec (\%)} = \frac{\text{Amount of extracted pectin (g)} \times 100}{\text{Initial amount of dried powdered sample (g)}} \quad (1)$$

Fungal Inoculum Preparation

A total of 50 mL of Sabouraud dextrose broth (SDB) (Himedia, India) was added to a 250-mL Erlenmeyer flask. The mixture was sterilized and cooled, and a loopful of a pure culture of *A. flavus* was inoculated into SDB medium and incubated for 4 days. The mixture was kept on a rotary shaker incubator at 150 rpm until the optimal spore suspension was reached, and it was refrigerated at 4 °C (Sathya *et al*. 2025).

Solid-state Fermentation

The dried banana peels (nendran, kadali, matti, palayankodan, and robusta) were weighed (5 g) and added to a 100-mL Erlenmeyer flask. The banana peel powder was moistened with a salt solution (0.5% NH_4NO_3, 0.02% Na_2SO_4, 0.08% $MgSO_4 \cdot 7H_2O$, 0.4%$ZnSO_4 \cdot 7H_2O$, 0.2% KCl, and 0.1% $Ca(OH)_2$). The initial moisture content of the solid medium was 60%. The substrates were sterilized for 30 min at 121 °C, and the conical flasks were cooled. The flasks were subsequently inoculated with 2% (v/w) spore suspension and incubated for 4 days (Ahmed *et al*. 2021). After 4 days of incubation, the amount of pectinase activity was determined.

Extraction

The fermented culture medium was mixed at a ratio of 1:4 (w/v) with phosphate buffer (pH 5.5) at room temperature. Then, the mixture was placed on a rotary shaker at 150 rpm for 20 min. It was subsequently filtered through Whatman No. 1 filter paper, and the clear supernatant was used for the enzyme assay (Shet *et al*. 2022).

Pectinase Assay

The amount of pectinase (polygalacturonase) activity was evaluated *via* the DNS (3,5-dinitrosalicylic acid) method with slight modifications. A 0.2-mL aliquot of crude enzyme was mixed with 2.8 mL of 1% pectin solution (phosphate buffer, pH 5.5, 0.1 M). The mixture was incubated at 30± 1 °C for 10 min. Then, 2.5 mL of dinitro salicylic acid (DNS) solution was added to stop the enzymatic reaction. To the blank, 0.2 mL of crude enzyme was added. The test tubes were placed in a boiling water bath and diluted with 5 mL of double distilled water. The optical density (OD) of each sample was assayed spectrophotometrically (Shimadzu Corporation, Nakagyo-ku, Kyoto, Japan) at 540 nm against a reagent blank (Miller 1959). α-Galacturonic acid was prepared at various concentrations (0.1 to 1.0 mg/mL) and used as the standard. One unit of pectinase activity was defined as the amount of enzyme that released 1 mL of galacturonic acid per minute under standard assay conditions.

Screening of Variables *via* a One-variable-at-a-time Approach

The SSF was further investigated *via* a one-variable-at-a-time approach. The pectin-rich palayankodan banana peel, which presented the maximum pectinase production, was selected for optimization studies. To optimize the incubation time, the culture was incubated at 28 ± 1 °C for 2 to 5 days. To optimize the moisture content, the moisture content of the culture medium was adjusted with a mineral salt solution ranging from 45 to 65 °C and incubated at 28 ± 1 °C for 5 days. To optimize the pH for enzyme production, a buffer solution (pH 3.5 to 6.0) (0.1 M) was added to the substrate (moisture, 55%) and incubated for five days. Similarly, the optimum incubation temperature was determined by cultivating the fungal strain in banana peel medium (55% moisture, pH 5.0) for five days at various temperatures (25 to 45 °C) in an incubator.

Optimization of Enzyme Production *via* Response Surface Methodology

A central composite design was used to design the experiment *via* three independent variables, namely, fermentation period (A), moisture content (B), and pH (C), and their effects on enzyme activity (response Y). The ranges of the independent variables were selected on the basis of the one-variable-at-a-time approach. The fermentation period (low: 3 days; high: 5 days), moisture content (low: 35%, high: 55%), and pH (low: 3.5; high: 5.5) were selected and analyzed at five different levels (Table 1).

Table 1. Variables and Their Actual Low and High Values to Produce Pectinase in the Central Composite Design

Factor	Name	Units	Low Actual	High Actual
A	Moisture	%	50	65
B	Fermentation period	(h)	72	120
C	pH		3.5	5.5

For these three independent variables, 20 experimental runs were performed, including six experiments on central points. The experiments were performed in randomized experimental trials, and the experiments were designed *via* Design-Expert (version 8.1) software. The following second-order polynomial equation was applied to fit the experimental data,

$$Y = \beta_0 + \sum \beta_i X_i + \sum \beta_i X_i^2 + \sum \beta_{ii} X_i^2 + \sum \beta_{ij} X_i X_j$$

where X_i=independent variable; X_j=independent variable;Y =response Y (pectinase activity); β_0=constant; and β_i, β_{ii}, and β_{ij}=regression coefficients. Analysis of variance (ANOVA) was performed, the significance of the designed model was analyzed. A p value<0.05 was considered statistically significant. To evaluate the suitability of this model, factors, such as lack of fit (a significant lack of fit is preferred), adequate precision (>4 is preferable), and R^2 values (>0.76 is good), were analyzed. A response surface curve was generated, and an optimum response of the variable was analyzed to study the interactions between the independent variables and pectinase activity.

Juice Clarification Experiment

Orange juice clarification was performed as described previously with slight modifications (Dal Magro *et al.* 2021). Briefly, 250 g of orange fruit (*Citrus* × *sinensis*) was peeled, and the fruit juice was pressed and filtered through cotton gauze. Approximately 100 mL of fruit juice was obtained, and it was equally distributed into five test tubes. Pectinase was added to the fruit juice at various concentrations (2 U/mL to 8 U/mL). Commercial pectinase was added (2 U/mL) (Novozymes, Brazil) to the positive control. Enzymatic treatment was performed for 60 min, and the absorbance of the sample was read at 600 nm *via* an UV–Vis spectrophotometer (Shimadzu Corporation, Nakagyo-ku, Kyoto, Japan).

Determination of Turbidity, Total Suspended Solids, Reducing Sugars, and pH of the Clarified Fruit Juice

The turbidity of the pectinase-treated juice was determined *via* spectrophotometric analysis as described previously by Anderson (2005), with slight modifications. After 60 min of treatment, the sample was centrifuged at 5000 × g for 2 min, and absorbance of the supernatant was analyzed at 600 nm *via* a spectrophotometer (Shimadzu Corporation, Nakagyo-ku, Kyoto, Japan). Juice without any enzyme treatment was considered a control. The total sugar content of the fruit juice was estimated as described previously by Kirk and Sawyer (1991), and the result was expressed in g/100 mL. The juice was filtered through Whatman 4 Qualitative filter paper, and the filtrate was used for the analysis. The pH of the juice was measured *via* a digital pH meter (Hanna, UK). The total soluble solids content was determined *via* a Bellingham refractometer (Xylem Analytics, 82362 Weilheim, Germany), and the results are expressed as percentages.

Statistical Analysis

The mean results were subjected to analysis of variance and comparison of means was performed with the LSD test at $p \leq 0.05$. Different lowercase letters in the same column in the table indicate statistical significance.

RESULTS AND DISCUSSION

Pectin Content

The pectin content in ripe banana peels (nendran, kadali, matti, palayankodan, and robusta) was analyzed, and hydrochloric acid was found to be efficient at extracting pectin from ripe banana peels. The amount of banana peel pectin ranged from 11.31 ± 0.22% to 18.09 ± 0.29% (Table 2). The pectin content was greater in the palayankodan variety (18.09 ± 0.29%) than in the other tested banana varieties. The pectin content of banana peels was consistent with the banana peel contents of other banana peel varieties (Silva *et al.* 2002).

Table 2. Pectin Content of Ripe Banana Peels from Various Banana Varieties

Pectin source	Yield (%)
Kadali	11.31 ± 0.22[a]
Robusta	12.09 ± 0.33[a]
Nendran	14.51 ± 0.13[b]
Matti	15.24 ± 0.15[b]
Palayankodan	18.09 ± 0.29[c]

Different lowercase letters in the same column in the table indicate statistical significance at 5% level (Tukey test $p < 0.05$).

Effect of Substrate on Polygalacturonase Production

The amount of polygalacturonase produced varied with the type of banana peel powder (nendran, kadali, matti, palayankodan, and robusta). These pectin-rich peels were used as the solid substrate to produce polygalacturonase in SSF. Table 3 shows the effects of pectin-rich substrates on polygalacturonase production. The palayankodan variety presented 135 ± 2.2 U/gds enzyme activity, whereas robusta presented 108 ± 1.9 U/gds in the SSF. Solid-state fermentation has several advantages over submerged fermentation, as

solid substrate–microbe interactions are highly effective at achieving high product yields (Al Farraj *et al*. 2020). In the SSF, the water requirement is very low, and product recovery is highly efficient (SavariyarAdimy *et al*. 2024). Agroresidues, such as cantaloupe and watermelon rinds, are utilized to produce polygalacturonase by *Trichoderma* species, and improved production has been reported (Mohamed *et al.* 2013). Zehra *et al.* (2020) used banana peels to produce pectinase and xylanase *via* a coproduction strategy. In addition, Arekemase *et al.* (2020) used banana peel substrates to produce pectinolytic enzymes in submerged fermentation. Doan *et al.* (2021) used pectin-rich substrates, including wheat bran and banana peel, to produce pectinases by *Bacillus amyloliquefaciens*.

Table 3. Polygalacturonase Production by *Aspergillus flavus* in Solid-State Fermentation Using Banana Peels Obtained from Different Banana Varieties

Banana Varieties	Polygalacturonase (U/gds)
Matti	39.1 ± 3.1[a]
Kadali	75.3 ± 1.1[b]
Nendran	87.4 ± 1.1[c]
Robusta	108 ± 1.9[d]
Palayankodan	135 ± 2.2[e]

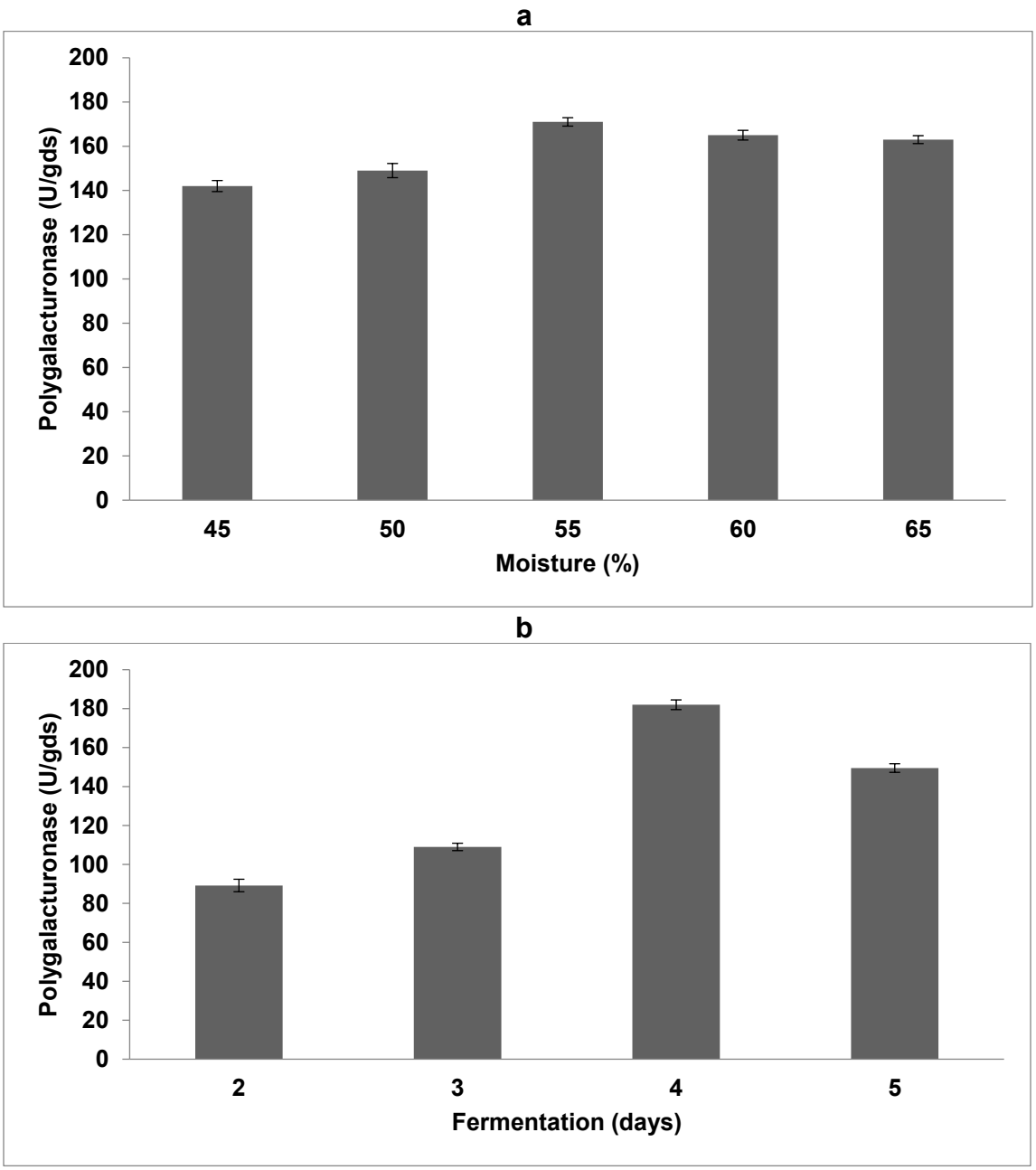
Different lowercase letters in the same column in the table indicate statistical significance at 5% level (Tukey test $p < 0.05$).

Screening of Variables to Produce Polygalacturonase in SFF

In this study, enzyme production was optimized. The optimum moisture content for polygalacturonase production was 55% (v/w), and the yield was 171 ± 1.9 U/gds (Fig. 2a). Moreover, enzyme production was affected at relatively high and low moisture contents. In the current study, polygalacturonase production reached a maximum (182 ± 2.5 U/gds) after 4 days of fermentation (Fig. 2b). The optimum fermentation period varied based on the type of solid medium, moisture content, and type of microorganism. Ketipally *et al.* (2019) used orange peel substrate to produce polygalacturonase, and the maximum production of the enzyme was achieved after 7 days of incubation with *A. nomius* MR103. Barman *et al.* (2015) used banana peel substrate to produce polygalacturonase and achieved a maximum enzyme yield (6.6 U/mL) within three days of culture with *A. niger*. In the current study, the maximum enzyme yield was achieved within four days of fermentation, which was consistent with previous reports (Shanmugavel *et al.* 2018; Nsude *et al.* 2019).Pectinolytic fungi produced the greatest amount of enzyme activity after 6 days of incubation when orange peels were supplemented with 3.0% ammonium sulfate as a nitrogen supplement (Okonji *et al.* 2019), which was greater than that observed in the current study.

The pH is an important factor that influences the production of pectinolytic enzymes and regulates the synthesis of fungal enzymes. In the current study, polygalacturonase production was observed at wide pH ranges of 4.5 to 5.5, and the maximum yield was observed at pH 4.5 (213.8 ± 5.4 U/gds) (Fig. 2c). As reported by Adedayo *et al.* (2021), the initial pH of the culture medium directly influences the fermentation process. The pH of the medium also affects the stability of the culture medium, and the pH of the medium affects membrane permeability and biosynthesis in fungal and bacterial species. The results of the present study were similar to those of Ahmed and Sohail (2020), who reported that pectinolyticenzyme production was high

between pH 3 and 5.5. This result is also supported by that of Manan and Webb (2017), who reported that yeast and fungi prefer acidic pH values for optimal growth and enzyme production. The pH value affects fungal cell proliferation, and the active site is altered, which reduces the enzyme activity of the generated enzymes. Although most of the researchers agreed with the optimum pH obtained in the current study, the enzyme yield varied with manufacturing method, microbial species, and substrate. The effect of incubation temperature on enzyme production was studied, and maximum production was achieved at 30 °C (220.5 ± 3.9 U/gds). At lower initial temperatures, the enzyme activity was lower, and it increased at 30 °C (Fig. 2d).



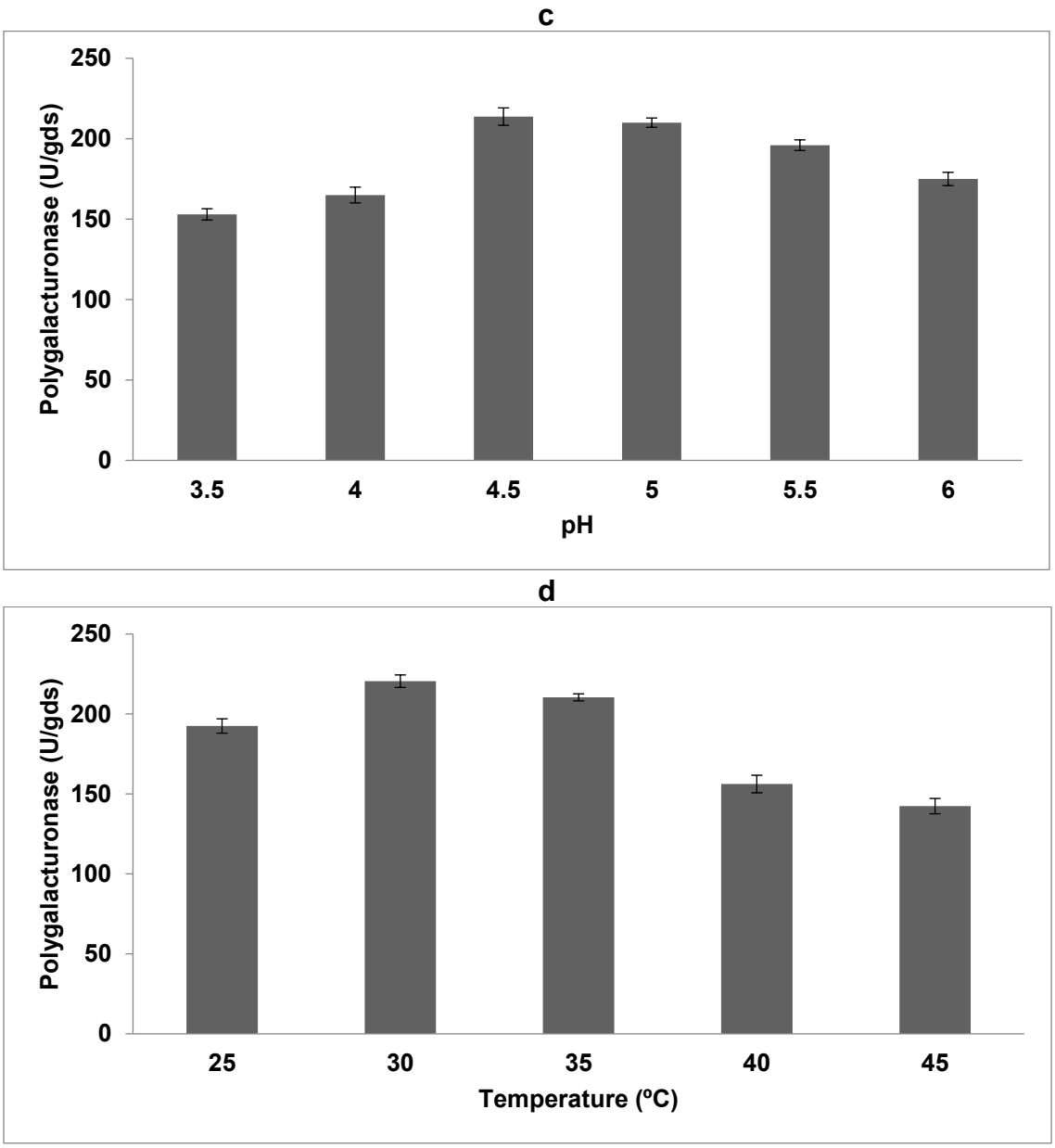


Fig. 2. Effects of moisture (a), fermentation period (b), pH (c), and temperature (d) on polygalacturonase activity during solid-state fermentation with *A. flavus*

The high temperature of the culture medium (>30 °C) was affected enzyme production. The sudden decline in enzyme activity at increasing temperatures may be due to the decreased metabolic function of the organism and the denaturation of the enzyme (Wong *et al.* 2017). In another study, Abdullah *et al.* (2018) reported maximum pectinase production at 30 °C in submerged fermentation using the fungal strain *Aspergillus niger* ABT-5. Moreover, 25 °C was found to be optimal to produce pectinase in *Aspergillus fumigatus* MS16 under SSF (Zehra *et al.* 2020). The optimum temperature for pectinase production observed in the current study was similar to that reported in studies in which *Aspergillus niger* HFD5A-1 (Darah *et al.* 2013), *Aspergillus terreus* NCFT 4692 (Sethi *et al.* 2016), and *Aspergillus fumigates* R6 (Wong *et al.* 2017) were used.

Optimization of Pectinase Production by Response Surface Methodology

The optimization of culture conditions for the improved production of pectinase by *A. flavus* was performed by analyzing the three most significant media components: pH, fermentation period, and moisture content of the medium. The designed CCD matrix and results are presented in Table 4. The results were subjected to regression analysis, and the regression coefficients of the terms were determined. The significance impacts of the selected bioprocess variables on pectinase activity were analyzed *via* analysis of variance (ANOVA). The PRESS value, R^2 value, adequate precision, and lack of fit value were analyzed, and the model suitability was tested. The lack of fit F-value was >0.05 (0.66), which implies that the lack of fit was not significant and that the insignificant lack of fit was good. The "predicted R^2" value of 0.9527 was very close to the "adjusted R^2" of 0.9776. The adequate precision of the model was >4 (25.456), which indicated an adequate signal of the model. In this model, B, C, AB, BC, A^2, B^2, and C^2 were significant terms (Table 5). Response surface plots were used to study the interactions between the selected independent variables and response (pectinase activity). The influence of the interaction of selected factors (moisture content, incubation time, and pH) on pectinase activity is depicted in response surface plots (Fig. 3). The interaction effects of the initial moisture content of the medium (A) and fermentation period (B) on pectinase activity are presented in Fig. 3a. The 3D graph shows that enzyme activity increased with increasing moisture content up to the optimum level, and decreased enzyme activity was observed at higher moisture levels. In SSF, moisture content is a critical factor that influences the metabolic processes of cells. The low enzyme yield at relatively high moisture levels occurred because the relatively high humidity caused the solid material to form clumps. These clumping particles reduced the interparticle space of the solid medium, which reduced the availability of nutrients to the microorganisms (Bhattacharya *et al*. 2024). In addition, high moisture content affects the porosity of the solid medium, which prevents oxygen transfer and results in low enzyme activity.

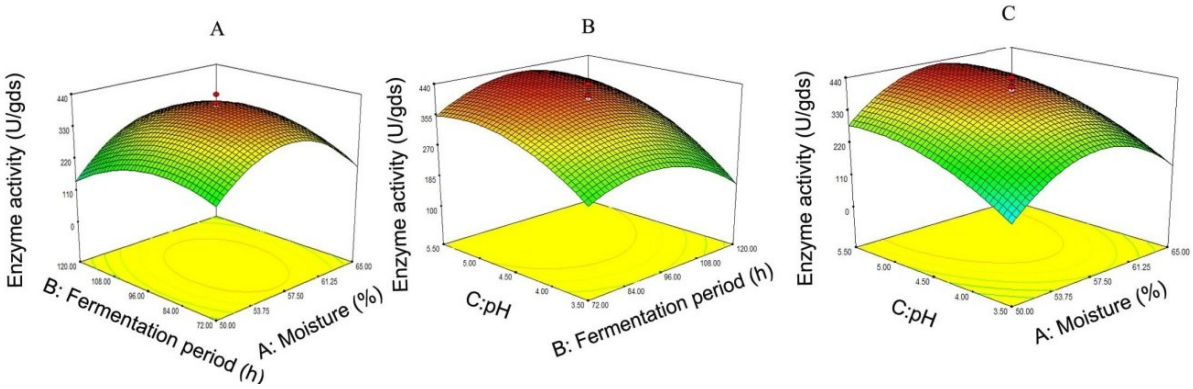


Fig. 3. Response surface plot for the analysis of the interactive effects of variables: (a) interactions between fermentation period and moisture content, (b) interactions between fermentation period and pH, and (c) pH and moisture content

The fermentation period was found to be more significant than that of the moisture content of the medium; as the fermentation period increased, the pectinolytic activity increased. However, as both the fermentation period and moisture content of the medium increased over the optimum period, there was a sharp decline in pectinase activity. This may be due to the viscosity of the particles and the moisture content hindering the active

site of the enzymes. In fermentation bioprocesses, pectinase activity generally decreases above the optimum level (Biz *et al.* 2014), which was similar to the results of the current study. However, in this study, variation in the moisture content of the medium did not significantly affect pectinase activity ($p<0.05$). The interaction effect between pH and fermentation period on pectinase activity is depicted in Fig. 3b. The 3D response surface plot shows that an increase in pH leads to an improvement in enzyme production. However, an increase in incubation time above the optimum level reduced pectinase activity. This could be because the fungal cells may have reached the decline phase within 4 days and exhibited less enzyme production after 4 days. The sharp decline in enzyme biosynthesis at longer incubation times may be due to the depletion of nutrients (Kaur and Gupta 2017). The interaction effect of pH and moisture on pectinase activity is depicted in Fig. 3c. During enzyme bioprocesses, alterations in environmental parameterscause denaturation of the enzymes decrease pectinase activity. Decreased pectinase activity after the optimum fermentation period has been reported previously by various authors (Kaur and Gupta 2017; Oumer and Abate 2018). The optimized culture conditions for analyzing enzyme activity *via* Design-Expert software were 58.7% moisture content, 96 h incubation time, and pH 5.09, with a predicted activity of 428 U/gds. Triplicate experiments were performed to validate the predicted response, and the observed result was 438.3 U/gds. The predicted enzyme activity was very close to the experimental value, which validated the designed experimental model (Qi *et al.* 2009; Roy *et al.* 2018).

Table 4. Central Composite Design to Produce Pectinase in Solid-state Fermentation

No.	Moisture (%)	pH	Fermentation Period (h)	Enzyme Activity (U/gds)
1	57.5	4	108	401.5
2	57.5	4	108	360.3
3	57.5	4	108	398.6
4	50	5	84	1.11
5	50	3	132	225.3
6	70.1	4	108	8.7
7	57.5	4	108	404.2
8	65	5	84	72.4
9	57.5	4	108	409.2
10	57.5	4	108	438.6
11	65	5	132	272.5
12	65	3	84	104.2
13	57.5	2.3	108	227.5
14	57.5	4	67.2	105.8
15	65	3	132	197.18
16	57.5	4	146.4	395.3
17	57.5	5.6	108	165.3
18	50	3	84	94.2
19	44.8	4	108	3.6
20	50	5	132	179.72

Table 5. Analysis of Variance to Produce Pectinase in the Central Composite Design

Source	Sum of Squares	df	Mean Square	F Value	p value Prob> F
Model	438758.1	9	48750.9	93.3	< 0.0001
A-Moisture	1748.47	1	1748.4	3.34	0.0973
B-Fermentation period	2921.83	1	2921.8	5.59	0.0396
C-pH	86943.7	1	86943.7	166.4	< 0.0001
AB	4149.1	1	4149.1	7.94	0.0182
AC	34.569	1	34.56961	0.066	0.8022
BC	2988.80	1	2988.8	5.72	0.0378
A^2	276004.6	1	276004.6	528.33	< 0.0001
B^2	72907.6	1	72907.6	139.56	< 0.0001
C^2	38941.4	1	38941.4	74.54	< 0.0001
Residual	5224.01	10	522.4		
Lack of Fit	2077.1	5	415.4	0.66	0.6702
Pure Error	3146.91	5	629.3		
Cor Total	443982	19			

Fungal Pectinase Reduced the Amount of Suspended Solids, Reducing Sugars, and pH

The total suspended solids in the raw orange juice were 21.2 ± 0.28%, and the total suspended solids were reduced in the pectinase-treated juice. The total percentage of suspended solids decreased with increasing concentrations of pectinase. The decreased sugar content in pectinase-treated juice is caused by the destruction of sugars by microbial enzymatic activity. The reduction in total suspended solids in the enzyme-treated juice results in the breakdown of suspended particles in the fruit juice. The pH of the raw fruit juice was 6.09 ± 0.16, and it increased slowly in the treated juice (Table 6). In contrast, a significant reduction in suspended solids, reducing sugars, and pH was observed in the juice treated with commercial pectinase. The fact that pectinase activity was produced from banana peels as a substrate in *A. flavus* cultivation may have significantly contributed to the efficiency of the orange peel clarification produced with banana peels. The present result is corroborated by a previous report by Barman *et al.* (2015), in which banana peel was used as a medium for pectinase production and used for food processing applications. During fruit juice clarification, nutrient losses are reported in beverages. For example, Haile *et al.* (2022) used pectinase from *Serratia marcescens* for the clarification of apple, lemon, and mango juices and reported a reduced total phenolic content. In the clarified juice, the absorbance value at 600 nm was less than that of the control (orange juice without enzymatic treatment). In this study, the pectinase-treated fruit juice presented decreased turbidity, and the absorbance at 600 nm was low (Fig. 4). In commercial enzyme-treated fruit juice, turbidity was reduced significantly, and no turbidity reduction was observed in the negative control (without enzyme treatment). The application of pectinase in juice clarification was reported previously by Ahmed and Sohail (2020), who achieved 61% orange juice clarification within 180 min of incubation at 30 °C with pectinases from *Geotrichum candidum*AA15. The use of crude enzymes from microbial sources has been

recommended for fruit juice clarification, and the application of crude enzymes could reduce the production cost; this type of application does not require any purification process (Begum *et al.* 2014; Kc *et al.* 2020; Andrade *et al.* 2021).

Table 6. Effects of Various Concentrations of Pectinases Extracted from *A. flavus* on Sugar Content, Total Suspended Solid Content and pH

Treatment	Sugar (g/100 mL)	pH	Total Suspended Solids (%)
Raw juice	17.3 ± 0.28[a]	6.09 ± 0.16[a]	21.2 ± 0.28[a]
2 U/mL	16.3 ± 0.11[b]	6.5 ± 0.1[b]	20.3 ± 0.4[b]
4 U/mL	12.5 ± 0.17[c]	6.91 ± 0.22[c]	18.2 ± 0.25[c]
6 U/mL	11.6 ± 0.22[d]	7.02 ± 0.11[c]	17.5 ± 0.11[d]
8 U/mL	10.2 ± 0.31[e]	7.3 ± 0.29[d]	16.9 ± 0.15[e]
Commercial pectinase	9.8 ± 0.42[e]	7.47 ± 0.19[e]	14.9 ± 0.28[f]

Different lowercase letters in the same column in the table indicate statistical significance at 5% level (Tukey test $p < 0.05$).

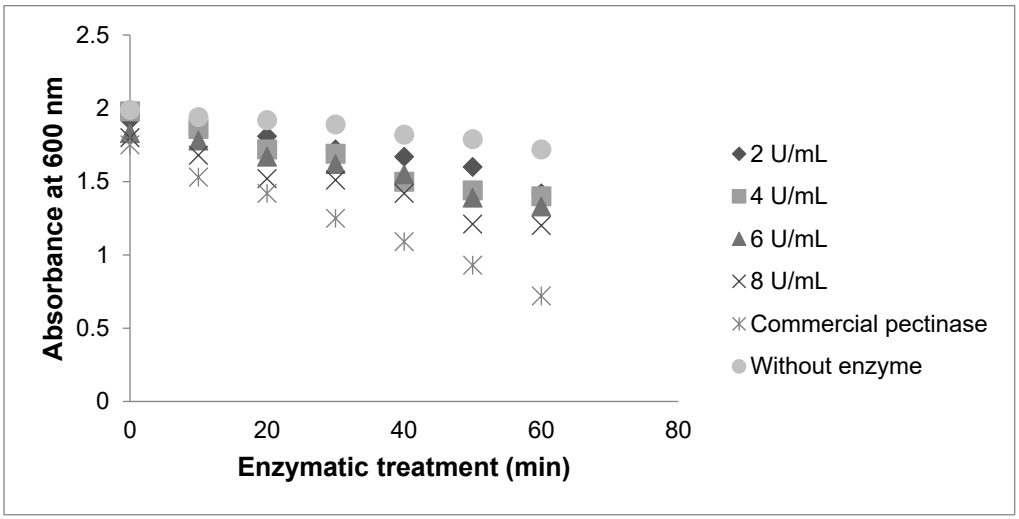


Fig. 4. Effect of *A. flavus* pectinase on orange juice clarification. The crude pectinase was applied at various concentrations (2 U/mL to 8 U/mL) in orange juice and treated for 60 min. As a negative control, the enzyme was not supplemented, and commercial pectinase (8 U/mL) was used as a positive control

CONCLUSIONS

1. Banana fruit peels are good sources of pectin, and these pectin-rich substrates are natural inducers of the production of pectinase in solid-state fermentation by *Aspergillus flavus*. Banana fruit peels are considered as waste and can be utilized for the production of pectinase by microbial fermentation to reduce the production cost.
2. Central composite design improved pectinase production, and the yield was improved over twofold. A limited number of experiments and resources were used due to the application of a statistical approach.

3. The crude pectinase was used as a natural clarifying agent, and it reduced the total suspended solids and sugar level in the orange juice. The efficacy of crude pectinase as a clarifying agent in other fruit juices is another area to explore.

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REFERENCES CITED

Abdollahzadeh, R., Pazhang, M., Najavand, S., Fallahzadeh-Mamaghani, V., and Amani-Ghadim, A. R. (2020). "Screening of pectinase-producing bacteria from farmlands and optimization of enzyme production from selected strain by RSM," *Folia Microbiologica* 65, 705-719. DOI:10.1007/s12223-020-00776-7

Abdullah, R., Jafer, A., Nisar, K., Kaleem, A., Iqtedar, M., Iftikhar, T., Saleem, F., and Naz, S. (2018). "Process optimization for pectinase production by locally isolated fungal strain using submerged fermentation," *Journal of Biosciences* (Online), 1025-1032.

Adedayo, M. R., Mohammed, M. T., Ajiboye, A. E., and Abdulmumini, S. A. (2021). "Pectinolytic activity of *Aspergillus niger* and *Aspergillus flavus* grown on grapefruit (*Citrus parasidis*) peel in solid-state fermentation," *Global Journal of Pure and Applied Sciences* 27(2), 93-105. DOI:10.4314/gjpas.v27i2.2

Ahmed, A., and Sohail, M. (2020). "Characterization of pectinase from *Geotrichum candidum* AA15 and its potential application in orange juice clarification," *Journal of King Saud University-Science* 32(1), 955-961. DOI:10.1016/j.jksus.2019.07.002

Ahmed, T., Rana, M. R., Zzaman, W., Ara, R., and Aziz, M. G. (2021). "Optimization of substrate composition for pectinase production from Satkara (*Citrus macroptera*) peel using *Aspergillus niger*-ATCC 1640 in solid-state fermentation," *Heliyon.* 7(10), article 100166. DOI: 10.1016/j.meafoo.2024.100166

Akkarachaneeyakorn, S., Suwakrai, A., and Pewngam, D. (2018). "Optimization of reducing sugar production from enzymatic hydrolysis of banana peels using response surface methodology," *Songklanakarin Journal of Science and Technol.* 40(1), 1-8.

Alarjani, K. M., Elshikh, M. S., Alghmdi, M. A., Arokiyaraj, S., and Ponnuswamy, V. (2024). "Valorization of date molasses and municipal solid waste for the production of cellulases by *Trichoderma reesei* Al-K1 149 in a tray reactor," *Waste and Biomass Valorization* 15(10), 5833-5842. DOI: 10.1007/s12649-024-02665-3

Alfarhan, A, Rajagopal, R, and Ponnuswamy, V. (2024). "Valorization of unripe banana peel (robusta) powder for the production of tannase using *Bacillus xiamenensis* in submerged fermentation," *BioResources* 19(3), 5396-5412. DOI: 10.15376/biores.19.3.5396-5412

Al Farraj, D. A., Kumar, T. S. J., Vijayaraghavan, P., Elshikh, M. S., Alkufeidy, R. M., Alkubaisi, N. A., and Alshammari, M. K. (2020). "Enhanced production, purification and biochemical characterization of therapeutic potential fibrinolytic enzyme from a new *Bacillus flexus* from marine environment," *Journal of King Saud University-Science* 32(7), 3174-3180. DOI:10.1016/j.jksus.2020.09.004

Andrade, P. M. L., Baptista, L., Bezerra, C. O., Peralta, R. M., Góes-Neto, A., Uetanabaro, A. P. T., and Costa, A. M. D. (2021). "Immobilization and characterization of tannase from *Penicillium rolfsii* CCMB 714 and its efficiency in apple juice clarification," *Journal of Food Measurement and Characterization* 15, 1005-1013. DOI:10.1007/s11694-020-00705-9

Arekemase, O. M., Omotosho, I. O., Agbabiaka, T. O., Ajide-Bamigboye, N. T., Lawal, A. K., Ahmed, T., and Orogu, J. O. (2020). "Optimization of bacteria pectinolytic enzyme production using banana peel as substrate under submerged fermentation," *Science World Journal* 15(1), 56-63.

Arokiyaraj, S., Elshikh, M. S., Alarjani, K. M., Alghmdi, M. A., and Vijayaraghavan, P. (2024). "Valorization of rubber tree sawdust biomass for the production of laccase-lignin peroxidase and bioconversion of sawdust," *Biomass Conversion and Biorefinery* 2024, 1-4. DOI:10.1007/s13399-024-05322-w

Barman, S., Sit, N., Badwaik, L. S., and Deka, S. C. (2015). "Pectinase production by *Aspergillus niger* using banana (*Musa balbisiana*) peel as substrate and its effect on clarification of banana juice," *Journal of Food Science and Technology* 52, 3579-3589. DOI: 10.1007/s13197-014-1413-8

Begum, R., Aziz, M. G., Uddin, M. B., and Yusof, Y. A. (2014). "Characterization of jackfruit (*Artocarpus heterophyllus*) waste pectin as influenced by various extraction conditions," *Agriculture and Agricultural Science Procedia* 2, 244-251. DOI: 10.1016/j.aaspro.2014.11.035

Bhattacharya, R., Arora, S., and Ghosh, S. (2024). "Bioprocess optimization for food-grade cellulolytic enzyme production from sorghum waste in a novel solid-state fermentation bioreactor for enhanced apple juice clarification," *Journal of Environmental Management* 358, article 120781. DOI: 10.1016/j.jenvman.2024.120781

Biz, A., Farias, F. C., Motter, F. A., de Paula, D. H., Richard, P., Krieger, N., and Mitchell, D. A. (2014). "Pectinase activity determination: An early deceleration in the release of reducing sugars throws a spanner in the works!," *PLoS One* 9(10), article e109529. DOI:10.1371/journal.pone.0109529

Dal Magro, L., Pessoa, J. P. S., Klein, M. P., Fernandez-Lafuente, R., and Rodrigues, R. C. (2021). "Enzymatic clarification of orange juice in continuous bed reactors: Fluidized-bed *versus* packed-bed reactor," *Catalysis Today* 362, 184-191. DOI: 10.1016/j.cattod.2020.02.003

Darah, I., Haritharan, W., and Lim, S. H. (2013). "Involvement of physicochemical parameters on pectinase production by *Aspergillus niger* HFD5A-1," *Journal of Pure and Applied Microbiology* 7, 2541-2549.

Doan, C. T., Chen, C. L., Nguyen, V. B., Tran, T. N., Nguyen, A. D., and Wang, S. L. (2021). "Conversion of pectin-containing byproducts to pectinases by *Bacillus amyloliquefaciens* and its applications on hydrolyzing banana peels for prebiotics production," *Polymers*13(9), article 1483. DOI:10.3390/polym13091483

El-Sheikh, M. A., Rajaselvam, J., Abdel-Salam, E. M., Vijayaraghavan, P., Alatar, A. A., and Biji, G. D. (2020). "*Paecilomyces* sp. ZB is a cell factory for the production of gibberellic acid using a cheap substrate in solid-state fermentation," *Saudi Journal of Biological Sciences* 27(9), 2431-2438. DOI: 10.1016/j.sjbs.2020.06.040

Emaga, T. H., Robert, C., Ronkart, S. N., Wathelet, B., and Paquot, M. (2008). "Dietary fiber components and pectin chemical features of peels during ripening in banana and

plantain varieties," *Bioresource Technology* 99(10), 4346-4354. DOI: 10.1016/j.biortech.2007.08.030

Faruque, M. O., Kamrul, N., Begum, A. A. B., Mazumder, M. A. R., and Uddin, M. B. (2016). "Effect of extraction methods on the characteristics of pectin from mango peel wastes," *International Journal of Agricultural and Food Science* 6(2), 70-75.

Haile, S., Masi, C., and Tafesse, M. (2022). "Isolation and characterization of pectinase-producing bacteria (*Serratia marcescens*) from avocado peel waste for juice clarification," *BMC Microbiology* 22(1), article 145. DOI: 10.1186/s12866-022-02536-8

Janveja, C., Rana, S. S., and Soni, S. K. (2013). "Kitchen waste residues as kitchen waste residues as potential renewable biomass resources for the production of multiple fungal carbohydrases and second generation bioethanol," *Journal of Technology Innovations in Renewable Energy* 2(2), 186-200. DOI: 10.6000/1929-6002.2013.02.02.11

Kalaiyarasi, M., Ahmad, P., and Vijayaraghavan, P. (2020). "Enhanced production antibiotics using green gram husk medium by *Streptomyces s*p. SD1 using response surface methodology," *Journal of King Saud University-Science* 32(3), 2134-2141. DOI: 10.1016/j.jksus.2020.02.014

Karthikeyan, A., and Sivakumar, N. (2010). "Citric acid production by Koji fermentation using banana peel as a novel substrate," *Bioresource Technology* 101(14), 5552-5556. DOI: 10.1016/j.biortech.2010.02.063

Kaul, K., Rajauria, G., and Singh, R. (2024). "Valorization of agro-industrial waste for pectinase production and its influence on circular economy," *Food and Bioproducts Processing* 148, 141-53. DOI: 10.1016/j.fbp.2024.09.008

Kaur, S. J., and Gupta, V. K. (2017). "Production of pectinolytic enzymes pectinase and pectin lyase by *Bacillus subtilis* SAV-21 in solid-state fermentation," *Annals of Microbiology* 67, 333-342. DOI:10.1007/s13213-017-1264-4

Kc, S., Upadhyaya, J., Joshi, D. R., Lekhak, B., Kumar Chaudhary, D., Raj Pant, B., Raj Bajgai, T., Dhital, R., Khanal, S., Koirala, N., *et al.* (2020). "Production, characterization, and industrial application of pectinase enzyme isolated from fungal strains," *Fermentation* 6(2), article 59. DOI: 10.3390/fermentation6020059

Ketipally, R., Kumar, G. K., and Ram, M. R. (2019). "Polygalacturonase production by *Aspergillus nomius* MR103 in solid-state fermentation using agro-industrial wastes," *Journal of Applied and Natural Science* 11(2), 305-310. DOI: 10.31018/jans.v11i2.2039

Kirk, S., and Sawyer, R. (1991). *Pearson's Composition and Analysis of Foods*, Ed. 9, Longman Group Limited, Harlow, UK.

Managamuri, U., Vijayalakshmi, M., Poda, S., Ganduri, V. R. K., and Babu, R. S. (2016). "Optimization of culture conditions by response surface methodology and unstructured kinetic modeling for bioactive metabolite production by *Nocardiopsis litoralis* VSM-8," *3 Biotech* 6, 1-19. DOI: 10.1007/s13205-016-0535-2

Manan, M. A., and Webb, C. (2017). "Design aspects of solid-state fermentation as applied to microbial bioprocessing," *Journal of Applied Biotechnology and Bioengineering* 4(1), 511-532. DOI: 10.15406/jabb.2017.04.00094

Marraiki, N., Vijayaraghavan, P., Elgorban, A. M., Dhas, D. D., Al-Rashed, S., and Yassin, M. T. (2020). "Low cost feedstock for the production of endoglucanase in solid-state fermentation by *Trichoderma hamatum* NGL1 using response surface

methodology and saccharification efficacy," *Journal of King Saud University-Science* 32(2), 1718-1724. DOI:10.1016/j.jksus.2020.01.008

Miller, G. L. (1959). "Use of dinitrosalicylic acid reagent for determination of reducing sugar," *Analytical Chemistry* 31(3), 426-428. DOI: 10.1021/ac60147a030

Mohamed, S. A., Al-Malki, A. L., Khan, J. A., Kabli, S. A., and Al-Garni, S. M. (2013). "Solid-state production of polygalacturonase and xylanase by *Trichoderma* species using cantaloupe and watermelon rinds," *Journal of Microbiology* 51, 605-611. DOI: 10.1007/s12275-013-3016-x

Nathoa, C., Sirisukpoca, U., and Pisutpaisal, N. (2014). "Production of hydrogen and methane from banana peel by two phase anaerobic fermentation," *Energy Procedia* 50, 702-710. DOI:10.1016/j.egypro.2014.06.086

Nsude, C. A., Ujah, I. I., Agbowo, S. S., and Chilaka, F. C. (2019). "Optimization of condition for pectin's production by *Aspergillus niger* in a citrus pectin based submerged fermentation medium," *Annals of Clinical Immunology and Microbiology* 1(1), article 1005.

Okonji, R. E., Itakorode, B. O., Ovumedia, J. O., and Adedeji, O. S. (2019). "Purification and biochemical characterization of pectinase produced by *Aspergillus fumigatus* isolated from soil of decomposing plant materials," *Journal of Applied Biology and Biotechnology* 7(3), 1-8. DOI: 10.7324/JABB.2019.70301

Osma, J. F., Herrera, J. L. T., and Couto, S. R. (2007). "Banana skin: A novel waste for laccase production by *Trametes pubescens* under solid-state conditions. Application to synthetic dye decolouration," *Dyes and Pigments* 75(1), 32-37. DOI: 10.1016/j.dyepig.2006.05.021

Oumer, O. J., and Abate, D. (2018). "Comparative studies of pectinase production by *Bacillus subtilis* strain Btk 27 in submerged and solid-state fermentations," *BioMed Research International* 2018(1), article 1514795. DOI: 10.1155/2018/1514795

Qi, B., Chen, X., Shen, F., Su, Y., and Wan, Y. (2009). "Optimization of enzymatic hydrolysis of wheat straw pretreated by alkaline peroxide using response surface methodology," *Industrial and Engineering Chemistry Research* 48(15), 7346-7353. DOI: 10.1021/Ie801686

Rehman, S., Aslam, H., Ahmad, A., Khan, S. A., and Sohail, M. (2014). "Production of plant cell wall degrading enzymes by monoculture and coculture of *Aspergillus niger* and *Aspergillus terreus* under SSF of banana peels," *Brazilian Journal of Microbiology* 45, 1485-1492. DOI:10.1590/S1517-83822014000400045

Rojas, N. L., Ortiz, G. E., Baruque, D. J., Cavalitto, S. F., and Ghiringhelli, P. D. (2011). "Production of heterologous polygalacturonase I from *Aspergillus kawachii* in *Saccharomyces cerevisiae* in batch and fed-batch cultures," *Journal of Industrial Microbiology and Biotechnology* 38(9), 1437-1447. DOI:10.1007/s10295-010-0929-9

Roy, M., Kumar, R., Ramteke, A., and Sit, N. (2018). "Identification of lipase producing fungus isolated from dairy waste contaminated soil and optimization of culture conditions for lipase production by the isolated fungus," *The Journal of Microbiology, Biotechnology and Food Sciences* 8(1), article 698. DOI: 10.15414/jmbfs.2018.8.1.698-704

Sajish, S., Tomar, G. S., Singh, S., and Kaushik, R. (2025). "A low-cost and sustainable approach to microbial pectinase production from fruit processing wastes: From peel to profit," *Environmental Technology*. 2025, article 55498. DOI: 10.1080/09593330.2025.2555498

Sandri, I. G., and Silveira, M. M. D. (2018). "Production and application of pectinases from *Aspergillus niger* obtained in solid-state cultivation," *Beverages* 4(3), article 48. DOI: 10.3390/beverages4030048

Santos, T. C. D., Cavalcanti, I. S., Bonomo, R. C. F., Santana, N. B., and Franco, M. (2011). "Optimization of productions of cellulolytic enzymes by *Aspergillus niger* using residue of mango a substrate," *Ciência Rural*41, 2210-2216. DOI: 10.1590/S0103-84782011005000145

Santos, T. C. D., Diniz, G. A., Brito, A. R. D., Pires, A. J. V., and Franco, M. (2015). "Effect of solid-state fermentation on nutritional content and evaluation of degradability in cactus pear," *Revista Caatinga* 28(03), 248-254. DOI: 10.1590/1983-21252015v28n328rc

Sathya, T. A., Alarjani, K. M., Elshikh, M. S., Flanetraj, S. R., and Ponnuswamy, V. (2025). "Co-composting of green leaves and kitchen waste: Characterization of organic amendments, microbial activity and analysis of defense enzymes in plants," *Biomass Conversion and Biorefinery* 15(17), 24331-24342. DOI: 10.1007/s13399-024-05608-z

SavariyarAdimy, P. S., Elshikh, M. S., Ali, M. A., and Biji, G. D. (2024). "Bioconversion of Agro-residues to make extracellular polysaccharides in solid state fermentation via *Trichoderma hamatum* using response surface methodology: Antioxidant and a-glucosidase inhibitor activity," *BioResources* 19(4). DOI: 10.15376/biores.19.4.8368-8387

Sethi, B. K., Nanda, P. K., and Sahoo, S. (2016). "Enhanced production of pectinase by *Aspergillusterreus* NCFT 4269.10 using banana peels as substrate," *3 Biotech* 6, 1-15. DOI: 10.1007/s13205-015-0353-y

Shanmugavel, M., Vasantharaj, S., Yazhmozhi, A., Bhavsar, P., Aswin, P., Felshia, C., Mani, U., Ranganathan, B., and Gnanamani, A. (2018). "A study on pectinases from *Aspergillus tamarii*: Toward greener approach for cotton bioscouring and phytopigments processing," *Biocatalysis and Agricultural Biotechnology* 15, 295-303. DOI: 10.1016/j.bcab.2018.06.013

Sharma, N., Rathore, M., and Sharma, M. (2013). "Microbial pectinase: Sources, characterization and applications," *Reviews in Environmental Science and BioTechnology* 12(1), 45-60. DOI: 10.1007/s11157-012-9276-9

Shet, A. R., Muhsinah, A. B., Alsayari, A., Achappa, S., Desai, S. V., Mahnashi, M. H., Muddapur, U. M., Shaikh, I. A., Mannasaheb, B. A., and Khan, A. A. (2022). "Media optimization by response surface methodology for the enhanced production of acidic extracellular pectinase by the indigenously isolated novel strain *Aspergillus cervinus* ARS2 using solid-state fermentation," *Fermentation* 8(10), article 485. DOI: 10.3390/fermentation8100485

Shoba, P., Alarjani, K. M., Elshikh, M. S., and Vijayaraghavan, P. (2025). "Bioprocess optimization of *Penicillium oxalicum* SM03 for the production of cellulases on freshwater alga *Salvinia molesta* biomass in solid-state fermentation," *Biomass Conversion and Biorefinery* 15(6), 8239-8251. DOI: 10.1007/s13399-024-05974-8

Shrestha, S., Chio, C., Khatiwada, J. R., Kognou, A. L., and Qin, W. (2022). "Formulation of the agro-waste mixture for multi-enzyme (pectinase, xylanase, and cellulase) production by mixture design method exploiting *Streptomyces* sp.", *Bioresource Technology Reports* 19, article 101142. DOI: 10.1016/j.biteb.2022.101142

Silva, D., Martins, E. D. S., Silva, R. D., and Gomes, E. (2002). "Pectinase production by *Penicillium viridicatum* RFC3 by solid state fermentation using agricultural wastes and agro-industrial by-products," *Brazilian Journal of Microbiology* 33, 318-324. DOI: 10.1590/S1517-83822002000400008

Singh, B., Soni, S. K., Mathur, P., and Garg, N. (2024). "Microbial multienzyme *viz*., pectinase, cellulase and amylase production using fruit and vegetable waste as substrate—A review," *Applied Microbiology* 4(3), 1232-1246. DOI: 10.3390/applmicrobiol4030084

Wong, L. Y., Saad, W. Z., Mohamad, R., and Tahir, P. M. (2017). "Optimization of cultural conditions for polygalacturonase production by a newly isolated *Aspergillus fumigatus* R6 capable of retting kenaf," *Industrial Crops and Products* 97, 175-183. DOI: 10.1016/j.indcrop.2016.12.019

Zehra, M., Syed, M. N., and Sohail, M. (2020). "Banana peels: A promising substrate for the coproduction of pectinase and xylanase from MS16," *Polish Journal of Microbiology* 69(1), 19-26. DOI: 10.33073/pjm-2020-002

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