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Research Article

Production of Pectinase from *Aspergillus* Sp Using Corn Residue and Cocoyam Peel as a Substrate

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Abstract

The growing demand for sustainable and low-cost enzyme production has encouraged the use of agro-residues as alternative substrates for microbial fermentation. This study investigated agricultural spoilt fruits as reservoirs of pectinase-producing fungi and evaluated corn residue and cocoyam peel as potential agro-residues for pectinase production. Spoilt fruits (apple, pear, and pawpaw) were analyzed for fungal load, and the isolates were identified using macroscopic and microscopic characteristics. Proximate composition of the agro-residues was determined to assess their nutritional suitability, while fermentation parameters including time, inoculum size, pH, carbon ratio, and nitrogen concentration were optimized to enhance pectinase yield. Results showed that fungal counts ranged from 3.6×10^4 to 4.6×10^4 CFU/g, with pawpaw supporting the highest load. The isolates included *Aspergillus, Rhizopus, Fusarium, and Penicillium species, with Aspergillus niger* (P2) exhibiting the highest pectinase activity (22 mm clearance zone), while Rhizopus sp. (P1) had the least (9 mm). Proximate analysis revealed that corn residue contained higher protein (11.98%) and carbohydrate (67.95%) contents, whereas cocoyam peel exhibited higher ash (2.04%), lipid (2.37%), and moisture (27.1%) contents. Fermentation studies indicated that pectinase activity increased with time, peaking at 96 h (32.3 U/ml), before declining. Optimum enzyme production was achieved at pH 5.5 (31.4 U/ml), 4% inoculum size (32.6 U/ml), and a 2:2% cocoyam-to-corn residue ratio (38.4 U/ml). Further optimization with nitrogen supplementation showed that 1.0% yeast extract supported the maximum pectinase yield (45.7 U/ml). It is concluded that spoilt fruits are reservoirs of industrially important fungi and that low-cost agro-residues such as corn residue and cocoyam peel can be effectively utilized for enhanced pectinase production under optimized fermentation conditions.

Keywords: *Aspergillus* sp; Cocoyam-Peel; Corn Residue; Pectinase; Production

Introduction and Background

Pectinase refers to a group of enzymes that catalyze the degradation of pectin, a complex polysaccharide present in the middle lamella and primary cell walls of plants [9]. These enzymes are of immense industrial importance, particularly in fruit juice clarification, textile processing, paper and pulp industries, coffee and tea fermentation, and wastewater treatment [13]. Pectinases account for more than 25% of the global food enzyme market and their demand continues to grow due to their diverse industrial applications [6]. Traditionally, pectinases are produced from bacterial and fungal sources, with fungi being preferred because of their extracellular enzyme secretion, high yield, and ability to thrive on inexpensive substrates [11]. Among fungi, *Aspergillus* species, especially *Aspergillus niger*, are the most efficient producers of pectinases, attributed to their robust enzymatic machinery, rapid growth, and adaptability to agro-residue-based fermentation systems [16]. Spoilt fruits are rich natural habitats for *Aspergillus* species and serve as reservoirs for isolating efficient pectinase-producing strains [2]. Agro-industrial residues such as corn residue and cocoyam peel are generated in large quantities and are often discarded as waste, posing environmental challenges. Corn residue is rich in carbohydrates and proteins, while cocoyam peel contains essential minerals, lipids, and high moisture content, making them suitable substrates for microbial fermentation [7]. Utilizing

these agro-residues for enzyme production not only reduces environmental burden but also lowers the cost of industrial enzyme production. The valorization of corn residue and cocoyam peel as substrates for pectinase production thus represents a sustainable and eco-friendly strategy for waste management and enzyme bioprocessing.

Materials and Methods

Sample collection

The Three (3) spoilt fruit samples were collected from Mile 1, Mile 3 and Rumuokoro Market, Rivers State. The spoilt fruit samples were collected using sterile plastic bags. The sample bags were labeled according to the location of the sample collection. The samples were transported in an ice chest to the Microbiology Laboratory, Rivers State University.

Isolation of fungi

One gram of the spoilt fruit sample was placed in 9 ml of sterile normal saline to obtain a spoilt fruit suspension. The suspension was then subjected to 10-fold serial dilution to a dilution of 10^{-4}. Aliquot of 0.1ml of the different dilutions was inoculated on Potato Dextrose Agar (PDA) plate using the spread plate technique. Inoculated plates were incubated at ambient temperature (26-32^{0}C) for 3-5days. After incubation ensuing fungal colonies were characterized and isolated.

Macroscopic and microscopic examination of isolated fungi

The Colonial features of the isolated fungi including top and reverse colour, form and presence/ absence of periphery were noted and recorded. Also, slides of the fungi were prepared for microscopy using lactophenol cotton blue. The slides were viewed under the microscope using 40x objective lens.

Collection of agro-residues as biomass

Yam peels and potato peels were obtained from the Rumuokoro market in Obio-Akpor, Local Government Area of Rivers state. These agro residues were taken to the Microbiology Laboratory, Rivers State University, Nkpolu- Oroworokwu, Port Harcourt, Rivers State. They were dried and pulverized into granules using a mechanical grinder. The processed biomass was packaged, sealed and labelled accordingly.

Compositional analysis of the agro-residues

Determination of crude protein

The crude protein content was determined using the Kjehdal method. About 0.5g of agro residues was placed into a clean 250ml conical flask, 15g of digestion catalyst was added and 20ml concentrated sulphuric acid was added. The mixture was heated to digest with the content. The clear solution was then allowed to stand for 30minutes and allowed to cool. After cooling it was made up 100ml with distilled water. About 20ml of the diluted digest was placed in a distillation flask attached to a Liebig condenser connected to a receiver containing 10ml of 2% boric acid indicator. About 40ml of 40% Sodium hydroxide was injected into the digest via a syringe until the digest became strongly alkaline. The mixture was heated to boiling and distilled ammonia gas was condensed into the beaker containing the boric acid. The distillate was titrated with standard 0.1N Hydrochloric acid solution. The volume of hydrochloric acid added to effect this change was recorded as titrate value.

Calculation

%Nitrogen =

%Protein = %Nitrogen x 6.25

Determination of carbohydrate

The carbohydrate content was determined using the Cleg Anthrone method. About 0.1g of the agro residues was placed into a 25ml volumetric flask, 1ml distilled water and 1.3ml of 62% perchloric acid was added and agitated for about 20mins. Then the flask was made up to 25ml mark with distilled water. The solution formed was filtered through a filter paper. Then, 1ml of the filtrate was transferred into a 10ml test tube and diluted to volume with distilled water. 1ml of the working solution was pipette into a clean test tube and 5ml Anthrone reagent was added. Similarly, 1ml distilled water and 5ml Anthrone was mixed in a separate test tube to be used as blank. A standard glucose solution of 0.1ml was also treated with Anthrone reagent. The absorbance of all 3 solutions were read at 630nm. The carbohydrate content, as glucose, was then calculated using the formula below.

$$\% \text{ CHO as glucose} = \frac{25 \times \text{absorbance of sample}}{\text{Absorbance of standard glucose} \times 100}$$

Determination of moisture content

The moisture content was determined using the oven drying method. About one gram (1g) of the agro residues was placed into a clean dried porcelain evaporating dish. The weight of porcelain evaporating dish and sample was noted before drying. The dish was then placed in an oven set at a temperature of 105^{0}C and allowed for six hours. Afterwards, the dish was brought out and placed in a desiccator so as to cool down to room temperature. The cooled dish was then weighed, and the moisture content was calculated as below

$$\% \text{ Moisture} = \frac{\text{Weight of fresh sample} - \text{weight of dried sample}}{\text{Weight of fresh sample}} \times 100$$

Determination of ash

About one gram (1g) of the dried agro residues was placed into porcelain crucible which was previously preheated and weighed. The crucible was placed in a muffled furnace set at a temperature of 630°C for three hours. Afterward, the crucible was brought out and allowed to cool to room temperature, and re-weighed. The ash content was then calculated as below

$$\% \text{ Ash} = \frac{\text{Weight of crucible + Ash sample} - \text{Weight of crucible}}{\text{Weight of sample}} \times \frac{100}{1}$$

Determination of crude fiber

The total fiber content was determined using the [5] method. Two grams (2.0g) agro residue was placed in 500 mL beaker and was hydrolyzed by adding 10mL of 25% sulphuric acid and boiling for about 30 min on a hot plate. The mixture was filtered through a piece of clean white cloth and rinsed with hot distilled water. The residue was boiled again with 20mL of 2.5% sodium hydroxide (NaOH) for 30 min, and was then filtered and rinsed with distilled water. Finally, the residue was collected and transferred into a crucible and was dried in the oven to a constant weight. The weight of the fiber was then calculated using the formula below

$$\text{Crude fiber (\%)} = \frac{\text{Weight of fiber}}{\text{Weight of sample}} \times 100$$

Determination of crude lipids

Take approximately 4- 5g of sample into the thimble and take a note of the sample weight. Place a cotton into the thimblein a way that covers the sample, fold the thimble to enclosed the sample. Take a cellulose thimble (sample holder) and put the sample inside the cellulose thimble. Take the weight of a cleaned and dried flat bottom flask. Set up the soxhlet extraction unit placing the sample in it. Add sufficient amount of n-hexane. After extraction of fat, take out the sample trom the thimble. Place the flask inside the oven to remove moisture and n-hexane. Set the temperature at 110 degree Celsius and dry for 30mins. After 30mins take out the dried flask and cool in desiccator. After cooling, take the weight of flask with fat.

Calculations

$$\text{Crude lipids content (\%)} = \frac{\text{Weight of flask with fat -Weight clean dry flask}}{\text{Weight of sample}} \times 100$$

Screening isolated fungi for pectinase production

Fungal isolates obtained were screened for extracellular pectinase production using a pectin-containing agar medium with the following composition (g L^{-1}): 10.0 pectin, 3.0 $(NH_4)_2HPO_4$, 2.0 KH_2PO_4, 3.0 K_2HPO_4, 0.1 $MgSO_4 \cdot 7H_2O$ and 25.0 agar agar (adjust pH to 5.5–6.0 before sterilization) [15]. The medium was autoclaved at 121°C for 15 minutes, cooled, poured into sterile Petri dishes and allowed to solidify. Pure fungal mycelial plugs (5 mm diameter) or a small pinch of actively growing mycelium were point-inoculated at the centre of each plate. Inoculated plates were incubated at room temperature (28°C) for 3–5 days (or until colonies reached 20–30 mm). After incubation, plates were flooded with Lugol's iodine solution and left for 1 hour, then gently rinsed with deionized water. Isolates expressing pectinase activity exhibited a clear zone of hydrolysis (halo) around the colony, whereas non-producers showed intense staining up to the colony margin [15]. The diameter of the colony (Dc) and the diameter of the colony plus clear zone (Dh) were measured in millimetres using a ruler. Pectinase activity was expressed as:

Calculation

Zone of hydrolysis (mm) = Dh – Dc

Hydrolysis index (HI) = Dh / Dc

Fermentation of the agro-residues

Submerged fermentation (SmF) was employed for pectinase production. Four grams of mixtures of corn residue and cocoyam peel in varying ratios (4:0, 3:1, 2:2, 1:3 and 0:4) were introduced into 250 mL Erlenmeyer flasks containing 100 mL of sterile production medium (citrate buffer, pH 5.5) supplemented with mineral salts ($MgSO_4 \cdot 7H_2O$ 0.5 g/L, KH_2PO_4 1.0 g/L, $NaNO_3$ 2.0 g/L, and $CaCl_2$ 0.1 g/L). The medium was sterilized at 121 °C for 15 minutes and allowed to cool. Each flask was inoculated with 1 mL of fungal spore suspension (1×10^6 spores/mL) and incubated at 28 °C on a rotary shaker at 150 rpm for 3–5 days to facilitate enzyme secretion.

Enzyme extraction

At the end of the fermentation period, the culture broth was filtered through Whatman No. 1 filter paper to separate the fungal biomass and residual agro-residues. The filtrate was then centrifuged at 10,000 rpm for 15 minutes at 4 °C, and the clear supernatant obtained was collected as the crude pectinase extract. The enzyme extract was stored at 4 °C until further use for activity assays and characterization.

Partial purification of pectinase

Ammonium sulphate precipitation

The crude pectinase extract was partially purified using stepwise ammonium sulphate precipitation. The enzyme extract was initially brought to 40% saturation by the gradual addition of solid ammonium sulphate with constant stirring at 4 °C. The mixture was left to equilibrate for 2 hours and then centrifuged at 20,000 × g for 15 minutes. The supernatant was further saturated to 70%,

and the resulting precipitate was collected by centrifugation under the same conditions. Finally, the supernatant was adjusted to 90% saturation, and the precipitate obtained was collected. All precipitates were redissolved in 0.05 M citrate buffer (pH 5.0) and dialyzed overnight against the same buffer to remove residual ammonium sulphate. The partially purified enzyme was then used for subsequent assays.

Determination of pectinase activity

Pectinase activity was assayed using the dinitrosalicylic acid (DNS) method for reducing sugar determination. The reaction mixture consisted of 0.5 mL of enzyme extract and 0.5 mL of 1% (w/v) pectin solution prepared in 0.05 M citrate buffer (pH 5.0). The mixture was incubated at 40 °C for 30 minutes, after which the reaction was terminated by adding 1 mL of DNS reagent. The tubes were boiled in a water bath for 5 minutes and cooled to room temperature. The absorbance of the reaction mixture was measured at 540 nm using a UV–visible spectrophotometer. One unit (U) of pectinase activity was defined as the amount of enzyme required to release 1 µmol of galacturonic acid equivalents per minute under the specified assay conditions.

Effect of fermentation time

Fermentation broths were sampled at (24, 48, 72, 96, 120, and 144 hours of incubation. Each sample was centrifuged at 10,000 rpm for 10 minutes, and the clear supernatant was assayed for pectinase activity using the DNS method. Monitoring enzyme activity at different fermentation intervals is essential to determine the phase of maximum enzyme secretion and to establish the optimal harvest time for achieving the highest yield [8,17].

Effect of inoculum size on enzyme production

The effect of inoculum size was studied by varying the volume of fungal spore suspension (1%, 2%, 3%, 4%, 5%, 6%, and 7% v/v) added to the production medium. Flasks were incubated at 28 °C on a shaker at 150 rpm, and enzyme activity was measured.

Effect of pH on enzyme activity

The effect of pH on enzyme activity was determined by preparing reaction mixtures in buffers of varying pH values: citrate buffer (pH 3.5–5.0) and phosphate buffer (pH 5.5–7.0). The mixtures were incubated at 50 °C for 30 minutes. For stability, crude enzyme was pre-incubated at different pH values (3.5–7.0) for 100 minutes, after which residual activity was assayed.

Effect of additional carbon sources on enzyme production

The effect of varying ratios of cocoyam peel and corn residue as carbon sources on pectinase production was studied during fermentation. Different combinations was prepared at 0:4, 1:3, 2:2, 3:1 and 4:0 (% w/v; cocoyam peel: corn residue). Each flask was inoculated with the fungal spore suspension and incubated at 28 °C for 5 days on a rotary shaker (150 rpm). After incubation crude enzyme was extracted and the pectinase activity was determined using the DNS assay method.

Effect of additional nitrogen sources on enzyme production

The effect of nitrogen supplementation was tested by adding yeast extract at different concentrations (0.0%, 0.1%, 0.25%, 0.5%, 0.75%, 1.0%, 1.5%, and 2.0% w/v) to the fermentation medium. Flasks were inoculated and incubated at 28 °C for 5 days under shaking conditions. Enzyme activity was determined using the DNS method.

Statistical analysis

The data obtained from the compositional analysis of the agro residues was analyzed using the One-way ANOVA to ascertain if any significance difference existed in the composition. The data obtained from the time -course study of pectinase activity at p-value <0.05 was ascertained

Results and Discussion

Total fungi count from the agricultural spoilt fruits

The total fungal count from agricultural spoilt fruit samples is presented in Figure 2. From the figure, it can be seen that sample **PP** had the highest fungal population (4.6×10^4 CFU/g), while sample **P** had the least fungal population (3.6×10^4 CFU/g). Sample **A** recorded a moderate fungal count of 4.4×10^4 CFU/g.

Macroscopic and microscopic characteristics of the isolated fungi

The macroscopic and microscopic characteristics of the isolated fungi are presented in Table 1. The fungal flora of the spoilt fruit contained a wide variety of genera which include *Aspergillus, Penicillium, Fusarium* and *Rhizopus* species.

Screening of pectinase activity from the isolated fungi

The screening potential of pectinase activity of the isolated fungi is presented in Figure 3. From the figure, it can be seen that isolate P2 had the highest pectinase activity with a clearance zone size of 22 mm, while isolate P1 recorded the least activity with a clearance zone size of 9 mm. Other isolates such as PP3 (17 mm), A3 (16 mm), and PP1 (15 mm) also exhibited considerable levels of pectinase activity compared to isolates A1 (11 mm) and PP2 (10 mm).

Proximate composition of corn residue and cocoyam peel residues

The proximate composition of the agro-residues is presented in Table 2. The ash content of the Corn Residue and Cocoyam Peel were 1.66 ± 0.0002% and 2.04 ± 0.0002% respectively. The moisture content of the Corn Residue and Cocoyam Peel were 15.29 ± 0.0002% and 27.1 ± 1.9208% respectively. Crude lipid content for Corn Residue and Cocoyam Peel were 1.62 ± 0.001% and 2.37 ± 0.0005% respectively. Carbohydrate contents were 67.95 ± 0.022% and 64.57 ± 2.311% for the Corn Residue and Cocoyam Peel respectively. The crude fibre content of the Corn Residue and Cocoyam Peel were 1.52 ± 0.0005% and 0.88 ± 0.004% respectively. Protein contents were 11.98 ± 0.009% and 3.06 ± 0.001% for the Corn Residue and Cocoyam Peel respectively.

Effect of fermentation time on pectinase production using the agro-residues and fungi

The maximum pectinase production of 32.3 U/ml was obtained at 96 h of fermentation. At 24 h, the enzyme activity was relatively low with a value of 9.1 U/ml. The pectinase production gradually increased with fermentation time, recording 17.4 U/ml and 25.8 U/ml at 48 h and 72 h, respectively. After attaining the peak at 96 h, a decline in enzyme activity was observed with yields of 26.5 U/ml at 120 h and 19.7 U/ml at 144 h of fermentation (Figure 3.3).

Effect of inoculum size on pectinase production using the agro-residues and fungi

The maximum pectinase production of 32.6 U/ml was recorded at an inoculum size of 4% (v/v), while the lowest enzyme activity of 11.3 U/ml was observed at 1% (v/v). As the inoculum size increased from 2% to 3%, the pectinase yield also increased, recording 17.5 U/ml and 24.1 U/ml, respectively. Beyond the optimum point, a gradual decline in enzyme production was observed, with values of 27.2 U/ml, 21.5 U/ml, and 16.1 U/ml at 5%, 6%, and 7% (v/v), respectively (Figure 3.4).

Effect of pH on pectinase production using the agro-residues and the fungi

The effect of pH on pectinase production using the agro-residues and the fungal isolate is presented in Fig. 3.5. From the figure, it can be seen that maximum pectinase activity was obtained at pH 5.5, with the highest production yield of 31.4 U/ml, while the least yield of 7.3 U/ml was recorded at pH 3.5. Pectinase production increased progressively from 11.5 U/ml at pH 4.0 to 17.1 U/ml at pH 4.5, and further to 23.7 U/ml at pH 5.0. Beyond the optimum, a decline in enzyme activity was observed, with yields of 26.3 U/ml, 20.2 U/ml, and 14.6 U/ml recorded at pH 6.0, 6.5, and 7.0, respectively.

Effect of carbon ratio concentration on pectinase production

The maximum pectinase production was observed when the carbon ratio concentration of 2:2% (cocoyam: corn residue) was supplemented in the fermentation medium, with the highest yield of 38.4 U/ml. At a carbon ratio concentration of 3:1%, the enzyme activity was 22.9 U/ml, while 1:3% gave a production yield of 30.3 U/ml. The least pectinase activity of 9.7 U/ml was recorded at 4:0%, whereas 0:4% resulted in a relatively low yield of 14.8 U/ml (Figure 3.6).

Effect of nitrogen concentration on pectinase production

The production of pectinase with the supplementation of nitrogen concentration (yeast extract) is shown in Figure 3.7. Maximum pectinase activity was observed at 1.0% nitrogen concentration, with a yield of 45.7 U/ml. In the absence of nitrogen supplementation (0.0%), the enzyme activity was very low (6.3 U/ml). As the nitrogen concentration increased, pectinase production gradually improved, recording 15.2 U/ml, 27.8 U/ml, and 36.5 U/ml at 0.1%, 0.25%, and 0.5%, respectively. A further increase to 0.75% gave 41.9 U/ml. Beyond the optimum level, enzyme production declined, with yields of 37.1 U/ml and 28.2 U/ml recorded at 1.5% and 2.0%, respectively.

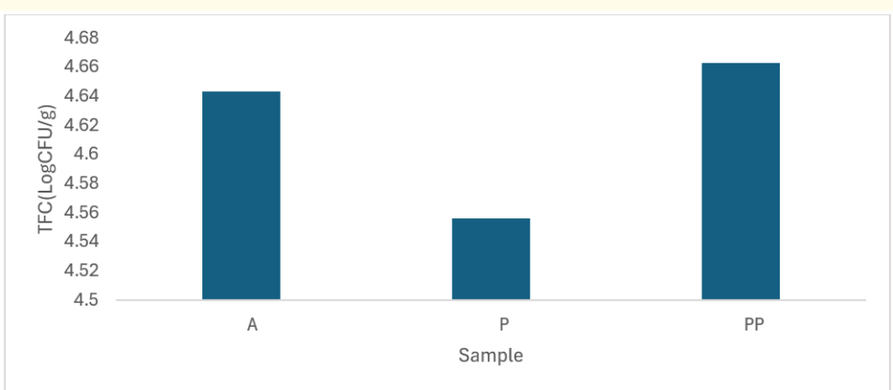

Figure 1: Total Fungi Count from the spoilt fruits.
Key: A= Apple; P = Pear; PP = Paw paw.

Isolate Code	Macroscopic characteristics	Microscopic characteristics	Probable organism
PP2, A1, P1	White cottony growth with yellow reverse	Canoe shaped conidia, with septate hyphae	*Fusarium* sp
PP1, A2	Gray and black fluffy growth with brown reverse.	Aseptate hyphae with round conidia head	*Rhizopus* sp
P2, PP3	Black cottony growth with white radial periphery and brown reverse	Septate hyphae with round conidia head	*Aspergillus niger*
A3	Green lawny growth with white radial periphery.	Septate branching hyphae with chain like conidia	*Penicillium sp*

Table 1: Macroscopic and microscopic characteristics of isolated fungi.

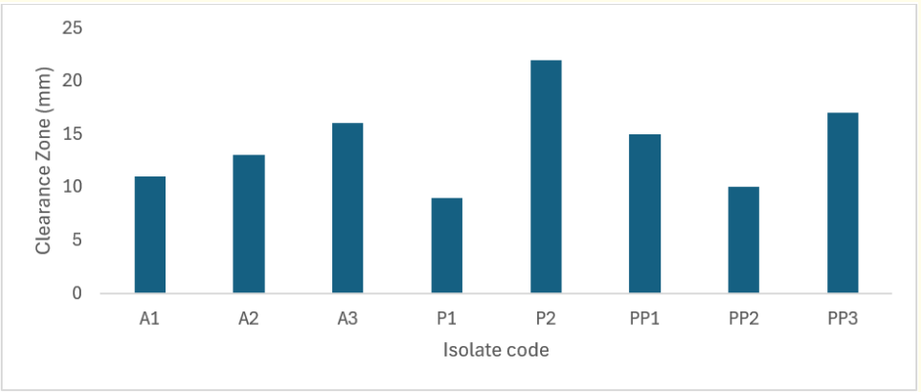

Figure 2: Screening of pectinase activity potential from the isolated fungal.

Table 2: Proximate composition of Corn Residue and Cocoyam peel.

Parameter	Corn Residue	Cocoyam peel	p-value
Ash (%)	1.66 ± 0.0002	2.04 ± 0.0002	0.0001382
Moisture (%)	15.29 ± 0.0002	27.1 ± 1.9208	0.052709
Crude lipid (%)	1.62 ± 0.001	2.37 ± 0.0005	0.001508
Crude fibre (%)	1.52 ± 0.0005	0.88 ± 0.004	0.047098
Crude protein (%)	11.98 ± 0.009	3.06 ± 0.001	0.00497
Carbohydrate (%)	67.95 ± 0.022	64.57 ± 2.311	0.196909

[a] Brown-Forsythe test is significant ($p < .05$), suggesting a violation of the equal variance assumption.

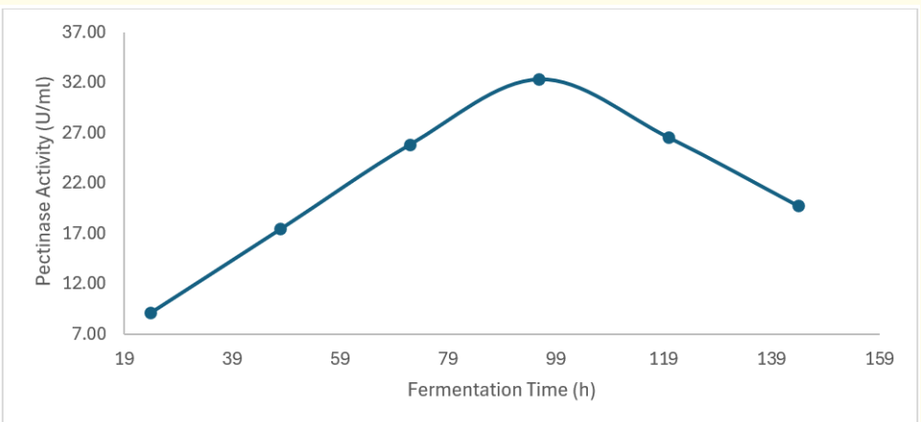

Figure 3: Effect of Fermentation Time on Pectinase Production using the Agro-Residues and Fungi.

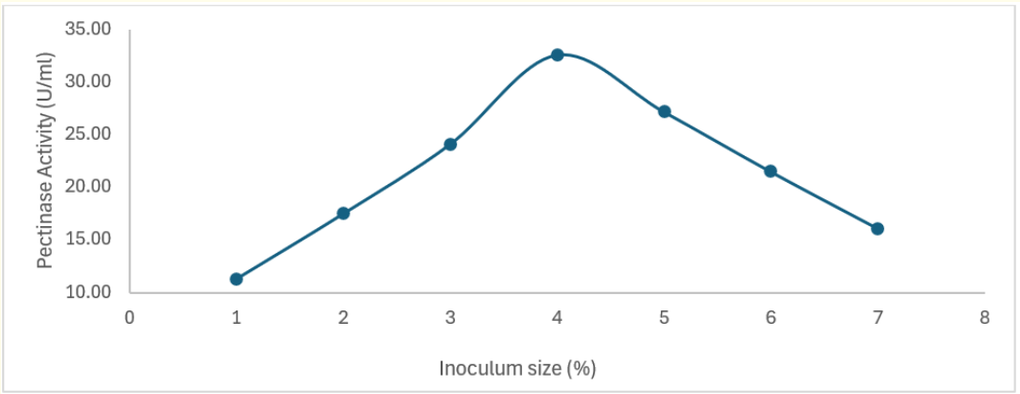

Figure 4: Effect of Inoculum Size on Pectinase Production using the Agro-Residues and Fungi.

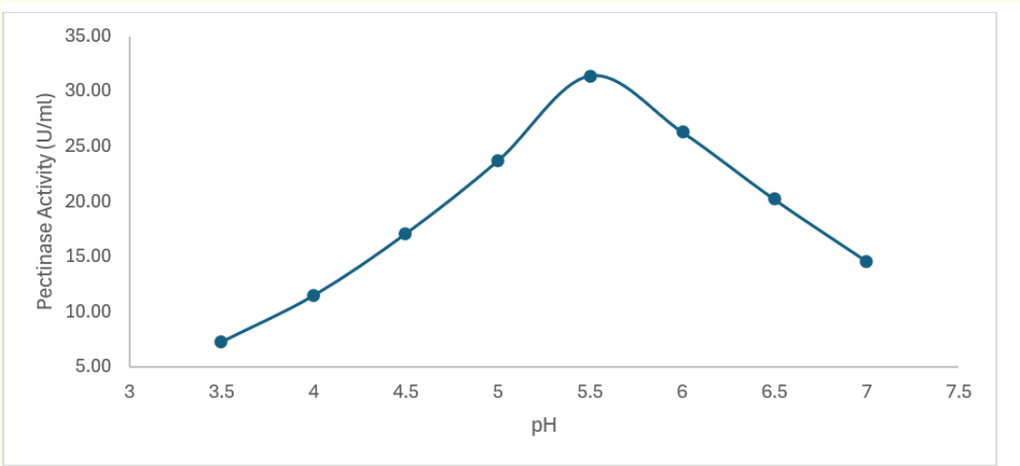

Figure 5: Effect of pH on Pectinase Production using the Agro-Residues and Fungi.

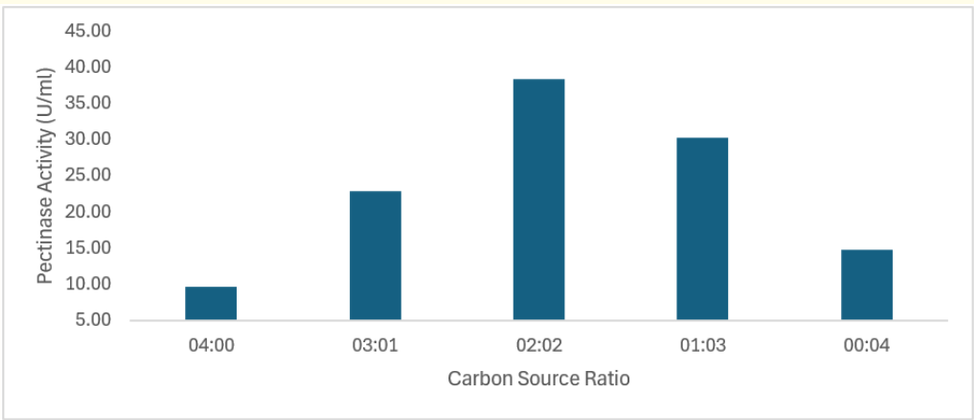

Figure 6: Effect of Carbon Source Ratio on Pectinase Production using the Agro-Residues and Fungi.

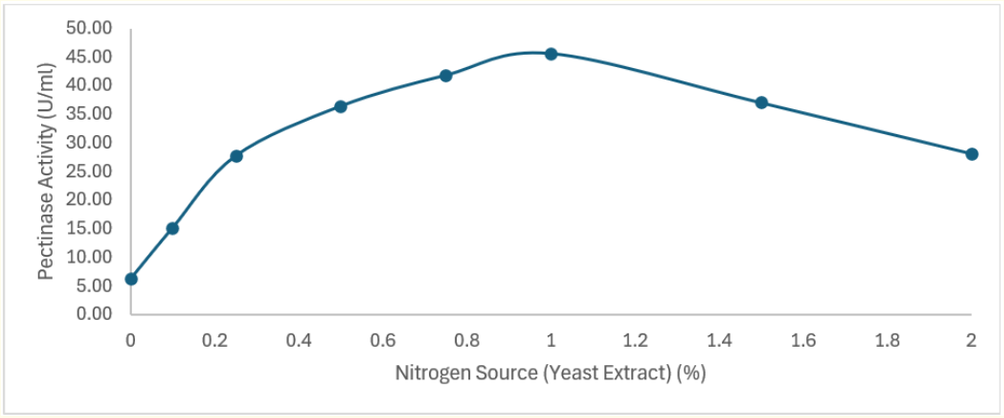

Figure 7: Effect of Nitrogen Source Concentration on the Pectinase Production.

The total fungal count from the agricultural spoilt fruit samples, as shown in Figure 2, revealed variations across the fruits. Pawpaw (PP) exhibited the highest fungal load (**4.6×10^4 CFU/g**), followed by apple (A) with **4.4×10^4 CFU/g**, while pear (P) recorded the lowest (**3.6×10^4 CFU/g**). The relatively higher fungal population in pawpaw may be attributed to its softer pulp structure, high sugar content, and higher moisture level, which create an ideal substrate for fungal colonization and proliferation. This observation agrees with the findings of [2], who reported that fruits with higher sugar and water activity tend to harbor larger fungal loads due to increased susceptibility to microbial invasion. Conversely, the relatively lower fungal count in pear may be due to its firmer texture and lower surface damage, reducing fungal penetration. Nonetheless, the detection of substantial fungal populations across all fruit types indicates that spoilt fruits serve as rich reservoirs of diverse fungal communities, which is consistent with reports by [10] that agricultural wastes, particularly fruits, are hotspots for fungal colonization due to their nutrient richness and decomposable organic matter. The macroscopic and microscopic characterization of the isolates (Table 1) confirmed the presence of **Aspergillus, Penicillium, Fusarium, and Rhizopus species**, which are widely recognized fungal genera in spoiled fruits and organic residues. Aspergillus species were identified macroscopically by black or green colonies with cottony to powdery textures and microscopically by septate hyphae with conidial heads. These features correspond with the descriptions of a ***Aspergillus niger,*** dominant spoilage organism in fruits, as earlier reported by [16]. The prevalence of *Aspergillus* is of both ecological and industrial interest, as it is a prolific producer of extracellular enzymes but also a potential producer of mycotoxins such as aflatoxins and ochratoxins [12]. *Rhizopus* isolates were recognized by their fluffy grayish colonies and aseptate hyphae with round sporangial heads, consistent with ***Rhizopus stolonifer,*** commonly referred to as "black bread mold." Its aggressive hydrolytic activity explains its frequent dominance in fruit spoilage [11]. *Fusarium* species exhibited canoe-shaped conidia and septate hyphae, aligning with [3], who highlighted their wide distribution in plant-derived residues. Their presence is concerning since *Fusarium* can produce mycotoxins such as fumonisins, which are hazardous to human and animal health. *Penicillium* isolates, identified by green colonies with brush-like conidiophores, are well-documented in fruit spoilage and are important for organic matter degradation but also implicated in patulin contamination [6]. The co-occurrence of these genera reflects a complex fungal community structure where species interact synergistically in the degradation of fruit matter, consistent with [2], who emphasized fungal diversity in agro-waste decomposition. The screening for pectinase activity (Figure 3) demonstrated clear differences among isolates. **Isolate P2 (*Aspergillus niger*)** showed the highest clearance zone (**22 mm**), indicating strong extracellular pectinase production, while isolate P1 (*Rhizopus* sp.) had the least activity (**9 mm**). Other isolates, such as PP3 (*Aspergillus* sp.), A3 (*Penicillium* sp.), and PP1 (*Rhizopus* sp.), displayed moderate activities (15–17 mm), suggesting variable enzymatic potentials across species. These variations can be linked to inherent genetic capabilities, metabolic adaptability, and enzyme secretion efficiencies of the different fungi. Similar findings were reported by [13], who noted that *Aspergillus* and *Penicillium* species are prolific producers of pectinases due to their efficient extracellular enzyme systems, whereas *Rhizopus* often demonstrates variable yields depending on growth conditions. The strong activity of *Aspergillus* isolates in this study is consistent with earlier observations that ***A. niger*** is one of the most reliable sources of pectinase for industrial fermentation [9]. The proximate composition of corn residue and cocoyam peel (Table 2) revealed significant differences in their nutritional profiles, with direct implications for fermentation performance. Cocoyam peel exhibited higher **moisture (27.1%), lipid (2.37%)**, and **ash (2.04%)** contents compared to corn residue, suggesting richer mineral and supplementary nutrient composi-

tion. Corn residue, however, had higher **protein (11.98%)** and **carbohydrate (67.95%)**, making it more suitable as a primary carbon and nitrogen source for microbial growth. This finding aligns with [7], who emphasized that cereal residues are rich in carbohydrates that support microbial enzyme biosynthesis, while tuber residues like cocoyam contribute valuable minerals and moisture to enhance microbial metabolism. The relatively low crude fiber contents (1.52% in corn residue and 0.88% in cocoyam peel) suggest minimal lignocellulosic recalcitrance, reducing the need for extensive pretreatment. Collectively, the complementary nutrient composition of the residues highlights their suitability as combined substrates for fungal pectinase production. The effect of fermentation time on pectinase production (Figure 3) showed that enzyme activity increased progressively up to **96 h,** with a maximum yield of **32.3 U/ml,** after which activity declined. This trend indicates that pectinase synthesis is growth-associated, with maximum production occurring during the exponential to early stationary growth phases. The decline beyond 96 h can be attributed to nutrient depletion, accumulation of inhibitory metabolites, or proteolytic degradation of the enzyme. Similar observations were reported by [1], who noted that fungal pectinase production peaks between 72–120 h depending on substrate type and fungal strain. Inoculum size (Figure 4) had a significant impact on pectinase yield, with the optimum at **4% (32.6 U/ml).** Lower inoculum sizes (1–2%) resulted in reduced activity due to insufficient fungal biomass, while higher inoculum sizes (>5%) led to declines, likely due to nutrient competition and oxygen limitation at high cell densities. This agrees with the report of [14], who emphasized that an optimum inoculum size balances rapid microbial establishment and efficient substrate utilization without inducing metabolic stress. The effect of pH on pectinase production (Figure 5) showed that **pH 5.5** was optimal (**31.4 U/ml**), while more acidic (3.5) or neutral (7.0) conditions resulted in lower activities (7.3 U/ml and 14.6 U/ml, respectively). This demonstrates that the fungal isolate favors slightly acidic conditions for maximum enzyme secretion, consistent with [9], who observed that most fungal pectinases exhibit optimal activity within pH 4.5–6.0. Deviation from this range likely alters enzyme conformation and reduces catalytic efficiency. The effect of carbon ratio supplementation (Figure 6) revealed that a **balanced 2:2% cocoyam–corn residue ratio** produced the highest pectinase yield (**38.4 U/ml**). This suggests a synergistic effect of combining both substrates, where corn residue provided abundant carbohydrates while cocoyam contributed essential minerals and moisture. Single-substrate ratios (4:0% and 0:4%) resulted in significantly lower yields, indicating the importance of balanced nutrient diversity for optimal enzyme induction. This finding is consistent with studies by [10], who reported that mixed agro-residues often enhance microbial enzyme production compared to single substrates. Nitrogen concentration (Figure 7) showed that the maximum pectinase yield (**45.7 U/ml**) was achieved at **1.0% yeast extract**, with declines at higher levels. This demonstrates the critical role of balanced nitrogen availability, as insufficient nitrogen limits enzyme synthesis, while excessive levels suppress extracellular enzyme secretion through catabolite repression. Similar trends were reported by [4], who found that moderate nitrogen supplementation promotes fungal enzyme yields by balancing growth and secondary metabolite production.

Conclusion

This study has demonstrated that agricultural spoilt fruits harbor diverse fungal communities with significant enzymatic potential. Enumeration revealed that pawpaw (PP) supported the highest fungal load (4.6×10^4 CFU/g), while pear (P) had the lowest (3.6×10^4 CFU/g), reflecting fruit-specific differences in nutrient composition and susceptibility to microbial colonization. The isolated fungi were identified as *Aspergillus, Rhizopus, Fusarium*, and *Penicillium* species, all of which are widely reported in fruit spoilage and recognized as prolific producers of extracellular enzymes. Screening revealed that *Aspergillus niger* (P2) was the most efficient pectinase producer, exhibiting the highest clearance zone (22 mm), while *Rhizopus* sp. (P1) recorded the least activity (9 mm), underscoring the strain-dependent variability in enzymatic capabilities. Proximate analysis of corn residue and cocoyam peel confirmed their suitability as low-cost agro-residues for fermentation. Corn residue was rich in protein (11.98%) and carbohydrates (67.95%), while cocoyam peel had higher moisture (27.1%), ash (2.04%), and lipid (2.37%) contents, highlighting their complementary roles as balanced fermentation substrates. Fermentation parameters revealed that pectinase production was significantly influenced by environmental and nutritional factors. Maximum yield (45.7 U/ml) was obtained under optimal conditions of 96 h fermentation time, 4% inoculum size, pH 5.5, 2:2% cocoyam-to-corn residue carbon ratio, and 1.0% yeast extract nitrogen supplementation. Beyond these conditions, enzyme activity declined, indicating the importance of fine-tuning process parameters.

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