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# Pectinase Production by *Aspergillus niger* using Pineapple Peel Pectin and Its Application in Coconut Oil Extraction

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**Abstract.** Pectinases, like other industrial enzymes are usually expensive. The use of pineapple peel pectin as substrate is triggered by the large tones of pineapple waste generated in Nigeria. Oil extraction by mechanical/chemical means have associated disadvantages. This research aimed at employing locally produced pectinase for coconut-oil extraction and to compare the yield with commercial pectinase. Fifty grammes of dried pineapple peel powder were employed for pectin production. *Aspergillus niger* isolated from cassava meal was employed to produce pectinase using submerged fermentation for seven days. The activity of pectinase was determined at 24 h interval. The pectinase was partially purified using 3% activated carbon, characterized and employed to extract oil from coconut. The yield of pectin from the pineapple peels was 24.8% after 1 h of extraction time. Highest pectinase activity was observed on day five. Optimum conditions were 40°C, 5.0 and 1% respectively for temperature, pH and substrate concentration. The enzyme was completely inactive after 5 min of heating at 90°C and metal ion (Mg<sup>2+</sup>) stimulated its activity. The mean oil yield from the locally produced pectinase was greater than the commercial pectinase. The pectinase produced from this study enhanced coconut-oil extraction when compared with the mechanical method.

**Keywords:** Pectinase; Pectin; Pineapple peel; Coconut oil; Extraction

## 1. Introduction

Pectinases are enzymes that are capable of hydrolysing pectic substances [1]. They account for more than 25% of food enzymes globally [2]. They are a group of industrially important enzyme [3], having applications in fruit juice industries in-order to obtain a less turbid product and increase fruit juice yield [4] and extraction of coconut oil [5]. Due to high expense involved in the purchase of commercial enzymes, the use of agricultural waste products will help minimize production cost and promote the use of the enzyme in food, leather, feed and textiles industries.

*Aspergillus niger* is frequently used in fungal pectinase production [6]. *Aspergillus niger* can be capitalized on for use in simple cultivation methods to produce pectinases, since it is not a known pathogen and are not known to be antibiotic-producers which are the essential attributes of GRAS (Generally regarded as safe) microorganisms [7]. The application of pectinase from locally made substrate is limited [8]. The cost of commercial enzymes are extremely high, and this is due to the need for refined substrates and patented organisms [9]. Thus, there is the need for cheaper substrates and the use of locally isolated microorganisms.

Pineapple (*Ananas comosus*) peels are examples of agro-wastes abundant in Nigeria which could be put to alternative use as substrates for enzyme production locally through submerged fermentation [10]. Large amount of waste is generated as a result of pineapple processing and utilization. Thus, wealth can be derived from this waste by value addition and products such as pectin, and predominantly pectinases can be easily harnessed. The abundance of this fruit leads to large amount of peels generated. This ends up in the environment contributing immensely to solid waste accumulation and subsequent pollution [11].



The use of N-hexane is predominant in chemical and food industries for oil extraction as it results in high yield. Its high flammability, explosive nature and toxic effects on human health eliciting negative effects on the central nervous system makes it unsafe for both plant and humans [12]. Generally, traditional method of coconut oil extraction do not produce great yield and usually contains increased amounts of moisture, which results in reduced product shelf-life [13]. Extraction processes using enzymes is the modern approach for the extraction of bio-ingredients from plants [14]. This research aimed at maximizing the huge pineapple peel waste generated locally for pectinase production and to employ the pectinase produced for coconut oil extraction as an alternative to the traditional method.

## 2. Materials and Methods

### 2.1 Collection and preparation of Pineapple peels

Pineapple (*Ananas cosmosus*) fruits were sourced locally from an open market in Ogun State, Nigeria. The fruit peels were thoroughly rinsed with sterile distilled water after peeling. The cut peels were oven-dried at 55°C for eight days as described by [15]. The dried fruit peels were finely ground using a mechanical grinder and sealed in polyethylene bags for further use.

### 2.2 Extraction of Pectin

Fifty grammes of ground pineapple peel was weighed into 2.5 L boiling water and pH adjusted to 2.2 ± 0.1 using HCL and 20 g of paper pulp filter aid added. This was boiled at 100°C for 30 min with constant stirring. At the end of 30mins, the filtered residue was washed once with 500 mL boiling water and dried at 60°C overnight [16].

### 2.3 Isolation and identification of Fungi

*A. niger* was obtained from a 5 day old cassava based staple meal popularly referred to as 'Eba' by cultivation on Potato Dextrose Agar (PDA). Identification of the Genus was based on cultural and microscopic characteristics by using [17]

### 2.4 Chemical Analysis of the Substrate

Two grammes of laboratory produced pectin was weighed into a pre-sterilized bottle (W1) oven dried at 100°C for 2 h. After, two hours it was immediately transferred into a desiccator to cool and re-weighed. Oven drying continued until a constant weight (W2) and percentage moisture was derived using the mathematical expression below; [18]

$$(\%) \text{ Moisture} = \frac{(W1) - (W2)}{(W1)} \times 100 \quad (1)$$

For percentage ash, two grammes of substrate (W1) was placed inside a crucible of known weight with 5 mL HNO<sub>3</sub> and heated slowly until charring began. After charring, sample was transferred into a muffle furnace at 55°C for 6 h and weighed (W2). Percentage ash was calculated using the mathematical expression below; [18].

$$(\%) \text{ Ash} = \frac{(W1) - (W2)}{\text{Weight of sample}} \times 100 \quad (2)$$

### 2.5 Fermentation/Enzyme Production

Seven (250 mL) Erlenmeyer flasks which contained 50 mL of basal medium with nutrient composition as follows; 0.1% NH<sub>4</sub>NO<sub>3</sub>, 0.1% MgSO<sub>4</sub>.7H<sub>2</sub>O, 0.1% NH<sub>4</sub>H<sub>2</sub>PO<sub>4</sub> and 1% ground pineapple peel was autoclaved at 121°C for 20 min. A 10 mm diameter cork borer was used to introduce growing cells into the basal medium and incubated for 7 days at 25 °C. Following every 24 h, a flask was randomly selected and filtrated using a muslin cloth. The cell free filtrates were analysed to determine the activity of the pectinase [19]

### 2.6 Pectinase Assay

A total 0.5 mL of 0.5% laboratory-produced pectin in 0.05 M acetate buffer, pH 5.0 and 0.5 mL of cell-free filtrate was employed. The solution was incubated for 1 h and absorbance was taken at 575

nm. One unit of pectinase activity is the quantity of pectinase in 1mL that liberated the reducing sugar equivalent to 1  $\mu$ g glucose per minute under specified conditions [20]

### *2.7 Partial Purification of Crude Pectinase with Activated Charcoal*

Activated charcoal (3% w/v) was introduced into the crude pectinase (pH 4.5) and incubated at 30°C for 30 min with occasional stirring according to [21]. The mixture was centrifuged at 2500 rpm for 10 min. Pectinase assay was carried out as earlier described [20]

### *2.8 Determination of Protein*

This was determined following standard methods [22]

### *2.9 Characterization of Enzymes*

#### *2.9.1 Effect of Metal-ions on the Activity of Pectinase.*

To evaluate the influence of metal ions, Mg<sup>2+</sup>, Zn<sup>2+</sup>, Fe<sup>3+</sup>, Co<sup>2+</sup> and Cu<sup>2+</sup> (1%) were added separately to the basal medium. Pectinase assay carried out as earlier described [20]

#### *2.9.2 Effect of pH on the Activity of Pectinase.*

This was observed across a pH of 3 - 5.5 using 0.05 M sodium acetate buffer and pH 6.0 using phosphate buffer. A 0.1% substrate was prepared by dissolving 0.1 g laboratory produced-pectin in 100 mL of 0.05 M of the respective buffers. Pectinase assay carried out as earlier described [20].

#### *2.9.3 Effect of Temperature on the Activity of Pectinase.*

This was observed by subjecting the enzyme to varying temperatures between 30-60°C for 1 h and at optimal pH. Pectinase activity was determined by [20].

#### *2.9.4 Effect of Substrate Concentration on Pectinase Activity.*

Different pectin concentrations (0.2% to 3% w/v) in 0.5 M sodium acetate buffer (pH 5.0) were prepared and employed as substrate. Pectinase assay carried out as earlier described [20].

#### *2.9.5 Effect of Time of Heating on Stability of Pectinase Activity.*

Enzyme was subjected to 90°C for varying time intervals (0-30 minutes) respectively. Pectinase assay carried out as earlier described [20].

### *2.10 Collection of Coconut Samples*

Coconut was sourced from a local market in Ogun State, Nigeria. Coconut meal was diced and ground using a blender. This was employed for the oil extraction.

### *2.11 Enzyme-assisted Oil Extraction*

One hundred and twenty grammes of the blended coconut meal was mixed with water (meal/water ratio of 1: 4, w/v). This was allowed to boil and cooled at room temperature. The boiled coconut meal was treatment with enzymes [23]. Each enzyme (commercial, crude and partially purified pectinases) were added at 1% rate and incubated at 37°C, pH 5.9 for about 6 h. This was followed by extraction using the hot-water flotation method. The enzyme-treated coconut meal was transferred into a clean glass beaker. Double quantity of warm water (about 40°C) was then added and stirred. After 1 h of standing, the upper emulsion of the beaker content was transferred into a fresh, clean beaker and washed with fresh warm water. This procedure was repeated twice. The final emulsion was boiled gently to break the emulsion and to allow water evaporation. The oil was decanted into a pre-weighed dish to estimate the oil yield. All extraction was carried out in duplicate for each enzyme

### 3. Results

#### 3.1 Extraction of Pectin

The results of this investigation revealed that pectin extraction yield was 24.8% at pH 2.2, temperature of 60°C and extraction time of 1 h (Table 1).

#### 3.2 Chemical Analysis of the Substrate

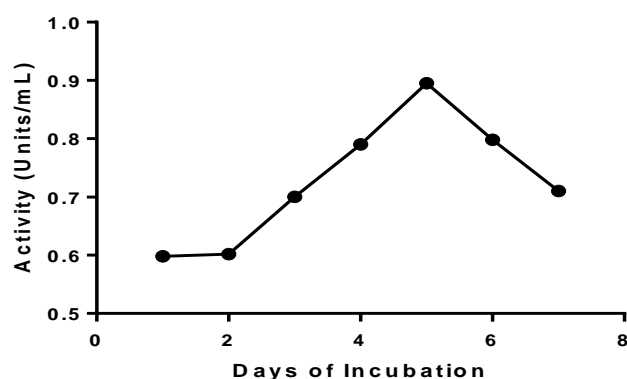
The percentage moisture content was 5.38% while percentage ash content was 1.9% (Table 1).

#### 3.3 Fermentation/Enzyme Production

From this study, enzyme obtained on day five had the highest pectinase activity while day showed the least (Fig. 1).

#### 3.4 Pectinase Assay

Total activity, total protein and specific activity for crude enzyme were determined at 0.885, 0.095 and 9.31 units/mL respectively while the activated carbon fraction was 0.792, 0.080 and 9.90 units/mL respectively (Table 2).



**Figure 1: Activity of pectinase produced by *A. niger* using laboratory pectin as substrate**

**Table 1: Yield and chemical analysis of pineapple pectin substrate**

Sample	Yield (%)	Moisture Content (%)	Ash (%)
Extracted pectin	24.8	5.38	1.9

**Table 2: Purification table of pectinase produced by *A. niger* using laboratory pectin as substrate**

Purification Step	Activity of Enzyme (Units/mL)	Total Protein (Mg/mL)	Specific Activity of Pectinase (Units/mL)	Purification Fold	Yield (%)
Crude Enzyme	0.885	0.095	9.31	1	100
Activated Carbon	0.792	0.080	9.90	1.06	89.5

### 3.5 Characterization of Pectinase

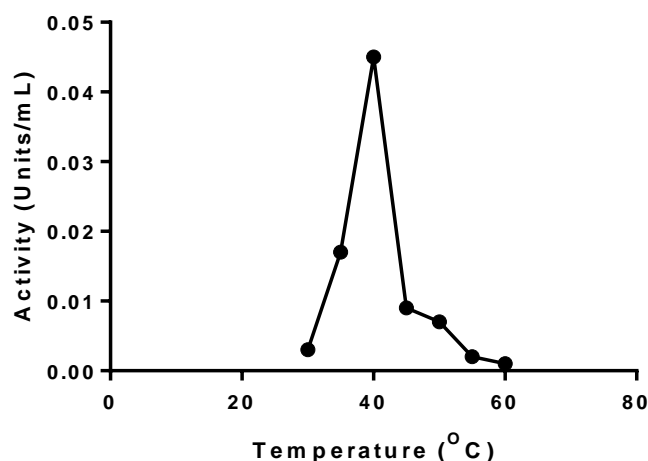
This study revealed that  $Mg^{2+}$  stimulated the activity of pectinase more than the other cations used (Table 3). Incubation temperature greatly altered the activity of pectinase with a continuous rise until an optimum ( $40^{\circ}C$ ) was reached (Fig. 2). There was a continuous increase in the activity of the pectinase until the optimum pH (5) was observed (Fig. 3). The same phenomenon was observed until an optimum substrate concentration (1%) (Fig. 4). The activity of pectinase on exposure to temperature of  $90^{\circ}C$  decreased with time. After 5min, the enzyme lost all its activity and was completely inactivated (Fig. 5).

### 3.6 Coconut Oil Yield

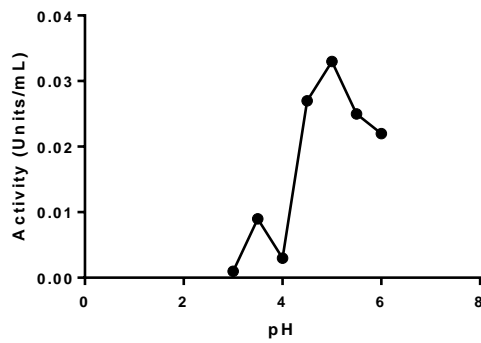
The mean yield of the partially purified pectinase was higher (150 mL) compared to the mean yield of the traditional method (48mL), crude pectinase (122mL) and commercial pectinase (140 mL) (Fig. 6).

**Table 3: Effect of metal ions on pectinase produced by *A. niger* using laboratory pectin as substrate**

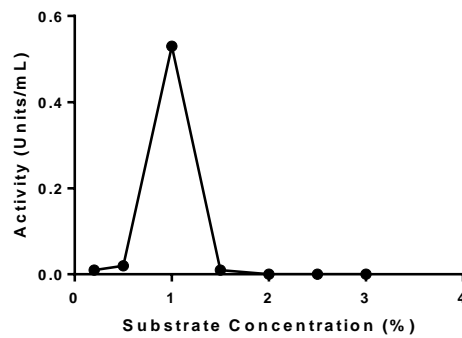
Metal ions	Enzyme Activity (Units/mL)
$Mg^{2+}$	9.100
$Zn^{2+}$	8.890
$Cu^{2+}$	8.370
Co	7.990
$Fe^{2+}$	7.710
Control	2.940



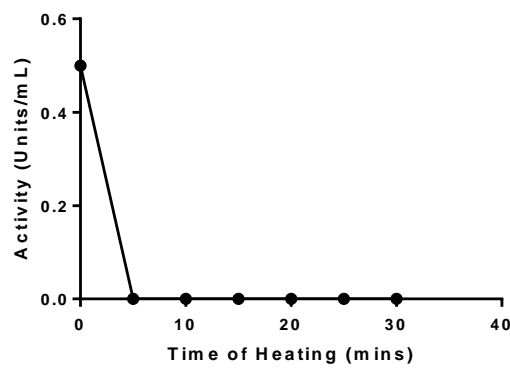
**Figure 2: Effect of temperature on the activity of *A. niger* pectinase produced using laboratory pectin as substrate**



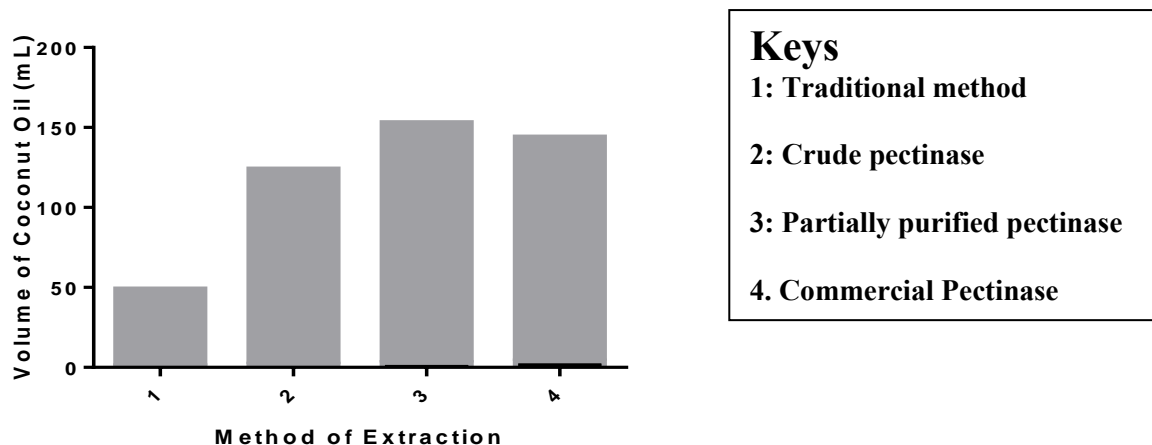
**Figure 3: Effect of pH on the activity of *A. niger* pectinase produced using laboratory pectin as substrate**



**Figure 4: Effect of substrate concentration on the activity of *A. niger* pectinase produced using laboratory pectin as substrate**



**Figure 5: Effect of heat (90°C) on pectinase produced by *A. niger* using laboratory pectin as substrate**



**Figure 6: Mean Coconut Oil Yield from different extraction methods**

#### 4. Discussion

The study report pectinase production by *A. niger* isolated from 5-day old cassava meal. Filamentous fungi have been associated with fermented cassava product e.g lafun [24] and with the spoilage of Cassava finished product [25]. Several studies previously documented the production of pectinase *Aspergillus niger* when various substrates were utilized [26; 27; 28; 29].

The laboratory produced pectin from pineapple peels as substrate yielded 24.8% of pectin at pH 2.2, 60°C and after 1 h. Pectin extraction from pineapple peels have been documented by [30] with a minimal extraction yield of 13.781%. [31] also reported pectin extraction from pineapple and banana fruits.

Higher activity of the enzyme was noted on day 5. This was contrary to the report of [32], where they reported optimal pectinase activity on day 4. [33] also documented highest pectinase activity by *Aspergillus niger* isolated from corn cob on the 5th day incubation. This is in line with the result of this research. Several factors e.g length of fermentation, composition of basal medium, type of fermenter may contribute to the activity of the enzyme. In their report, [5] observed a higher pectinase activity when agro wastes used as pectin source were complemented with additional sources of carbon and nitrogen. The pectinase activity after submerged fermentation was found to be stimulated with  $Mg^{2+}$ . This result compares favourably with the result of [34] where the stimulation of pectinase activity with  $Mg^{2+}$  and  $Ca^{2+}$  was reported. [35] also reported optimum pectinase activity using  $Mg^{2+}$ .

Purification with activated charcoal resulted in 89.5% yield compared with [36] which reported a pectinase yield of 37% using activated charcoal. Purification of enzymes using activated charcoal has been reported to be more effective when compared to conventional methods using ammonium sulphate precipitation as the latter often results in protein denaturation due to conformational changes while gel filtration technique may be more expensive in developing countries [21].

Incubation temperature greatly altered the activity of the pectinase. Optimum activity of pectinase occurred at 40°C. [37] has documented 35°C as the optimal temperature for pectinase produced by *Aspergillus niger* which differs from the results in this study. [38] reported optimum temperature for pectinase activity at 50°C. Also, [33] reported the optimum temperature for pectinase activity at 50°C. Optimum pH 5.0 was obtained for *A. niger* pectinase. Although [39], reported optimum pH 4.0 for pectinase produced by *Aspergillus niger* BC23 isolated from *Irvingiaga bonensis* (African mango) fruit. Also, [38] reported optimum pH 3.5 while 6.8 was noted in the pectinase by *Aspergillus niger*



from soil samples of chittoor district, Andhra Pradesh, South India [7]. Changes in pH can change the shape of the active site in an enzyme. Variations in pH may result in enzyme denaturation by significantly altering the structure of the enzyme's active-site [40; 41]. One percent substrate concentration showed the highest activity in this study. This contradicts the findings of [42] that reported an optimum substrate concentration of 2% for pectinase production by *Trichoderma viride*. An increase in amount of substrate results in improved substrate-product interactions, which eventually leads to formation of more products [40]. Pectinase from this study remained active at 60°C but lost its activity after five minutes of heating at 90°C. This correlates with the findings of [10] where pectinase was stable at 60°C but became completely inactive after five minutes of heating at 90°C. However, [43] reported 72% loss in pectinase activity after heating at 80°C for 15 min. When applied to the extraction of coconut oil, the crude, partially purified laboratory pectinase and commercial pectinase resulted in 52.1%, 62.5% and 58.3% respectively compared the traditional method which yielded 20.8%. Enzyme-assisted oil extraction gave a yield of 65.74% as reported by [44].

## 5. Conclusion

The partially purified pectinase produced resulted in greater oil output compared to the traditional method and even commercial pectinase. Thus, locally produced pectinase may serve as a suitable alternative to the use of commercial pectinase for oil extraction. This will have great impact on the overall cost of coconut oil extraction.

## 6. Acknowledgments

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