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Effect of Blanching, Ripening and Other Treatments on the Production Characteristics of Pectinolytic Enzymes from Banana Peels By Aspergillus Niger

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Abstract - Three different strains of Apergillus niger isolated from decayed banana peels in Ibadan metropolis, Nigeria depolymerized citrus pectin. The best strain having pectinolytic activity as indicated by the diameter of clear hydrolyzed zone on the medium plates containing commercial citrus pectin as the sole carbon source was selected among the three strains having the largest zone. This isolate was able to produce polygalacturonase and pectin layse enzymes using banana peels (agrowastes) as the sole carbon source. When Solid state fermentation (SSF) and Submerged fermentation (SMF) were carried out with the banana peels as the substrate using the Aspergillus niger with the largest zone, SSF yielded higher level of pectinolytic activity than the SMF. Different treatments of the banana peels used as substrate were carried out by blanching the substrate with cold sodium chloride, treating the banana peels with wood ashes and also allowing the unripe banana to go through ripening stages. Higher yield of pectinases production was obtained when the banana peels were treated compared with when they were not treated at all. Therefore pretreatment of banana peels increases pectinases production.

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Absract - Three different strains of *Apergillus niger* isolated from decayed banana peels in Ibadan metropolis, Nigeria depolymerized citrus pectin. The best strain having pectinolytic activity as indicated by the diameter of clear hydrolyzed zone on the medium plates containing commercial citrus pectin as the sole carbon source was selected among the three strains having the largest zone. This isolate was able to produce polygalacturonase and pectin layse enzymes using banana peels (agrowastes) as the sole carbon source. When Solid state fermentation (SSF) and Submerged fermentation (SMF) were carried out with the banana peels as the substrate using the *Aspergillus niger* with the largest zone, SSF yielded higher level of pectinolytic activity than the SMF. Different treatments of the banana peels used as substrate were carried out by blanching the substrate with cold sodium chloride, treating the banana peels with wood ashes and also allowing the unripe banana to go through ripening stages. Higher yield of pectinases production was obtained when the banana peels were treated compared with when they were not treated at all. Therefore pretreatment of banana peels increases pectinases production.

Keywords : Aspergillus niger, Pectinolytic Activity, Banana peel, Fermentation, Pectinase.

I. Introduction

Enzymes are proteins that catalyze (*i.e.*, increase or decrease the rates of) chemical reactions. (Smith *et al.,* (1997), (Grisham *et al.,* 1999). In enzymatic reactions, the molecules at the beginning of the process are called substrates, and they are converted into different molecules, called the products. Almost all processes in a biological cell need enzymes to occur at significant rates. Since enzymes are selective for their substrates and speed up only a few reactions from among many possibilities, the set of enzymes made in a cell determines which metabolic pathways occur in that cell.

In nature, microorganisms have been endowed with vast potentials. They produce an array of enzymes, which have been exploited commercially over the years. Pectinases are of great significance with tremendous potential to offer to industry (Dayanand *et al.*, 2003.). They are one of the upcoming enzymes of the commercial sector, especially the juice and food industry (kashyap *et al.*, 2001) and in the paper and pulp industry (Beg, Viikari *et al.*, 2001). Pectinolytic enzymes or pectinases are a heterogeneous group of related enzymes that hydrolyze the pectic substances, present mostly in plants. Pectinolytic enzymes are widely distributed in higher plants and microorganisms (Whitaker *et al.*, 1990). They are of prime importance for plants as they help in cell wall extension (Ward *et al.*, 1989) and softening of some plant tissues during maturation and storage (Sakai, 1992, Aguilar *et al.*, 1990).

Pectinases are group of enzymes that attack pectin and depolymerise it by hydrolysis and transelimination as well as by deesterification reactions, which hydrolyses the ester bond between carboxyl and methyl groups of pectin (Ceci and Loranzo, 1998). These enzymes act on pectin, a class of complex polysaccharides found in the cell wall of higher plants and cementing material for the cellulose network (Thakur *et al.*, 1997). Pectinases account for 10% of the global industrial enzymes produced (Stutzenberger, 1992).

These pectinases have wide applications in fruit juice industry and wine industry. In fruit juice industry, it is used for clarification, where reduction in viscosity is caused which ultimately leads to formation of clear juice. They increase the yield of juices by enzymatic liquefaction of pulps; these pectinases also helps in formation of pulpy products by macerating the organized tissue into suspension of intact cells. In wine industry pectinases are mainly used for decreasing astringency by solubilizing anthocyanins without leaching out procyadin polyphenols, and pectinases also increase pigmentation by extracting more anthocyanins (Tucker and Woods, 1991).

A group of pectinolytic enzymes known as pectinases hydrolyses pectin. Pectinases are complex hydrolytic enzymes that function as esterases and depolymerases. They include pectinmethyl esterases which catalyse the hydrolysis of methylated carboxylic ester groups in pectin into pectic acid and methanol, pectin lyase which cleave α(1.4)-glycosidic linkages by

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transelimination resulting into galacturonide with a double bond between C-4 and C-5 at the non reducing end ant polygalacturonase which hydrolyse the α(1,4)-glycosidic linkages in homo galacturonans (Call *et al.*, 1985;Delgado *et al.*, 1992; Soares *et al.*, 1999).

Solid state fermentation (SSF) and Submerged fermentation (SmF) are important fermentation methods employed for the production of microbial enzymes (Kavitha *et al.*, 2000; Martin *et al.*, 2000). Microbial growth and product formation usually occur at or near the surface of solid substrate particles with low moisture content; hence SSF appear to be advantageous for microbial enzyme production. The advantages of SSF over the SmF process include higher yield of products (Pandey, 1994), generation of less effluent and requirement of simpler equipment (Bennett, 1998). Reports are very few on the comparison of SmF and SSF for the production of pectinases. The present study involves screening of the *Aspergillus niger* isolated from decayed banana peels for pectinolytic activity using banana peels as fermentation substrate and determining the effect of different treatments of banana peels by blanching it in cold NaCl, treating it with wood ashes and allowing it to undergo ripening stages fot pectinolytic enzymes production.

II. Materials And Method

a) Sample Collection

Banana peels were collected from Ibadan metropolis and were transported to the University of Ibadan Postgraduate Laboratory where they were used as analysis.

b) Isolation And Identification Of Fungal Isolate

Decaying banana peels were collected from fruit selling points within the University of Ibadan and then transported to the laboratory for isolation.

10g of the fungi infected region of the banana peels were weighed into 90ml of sterile distilled water and was shaken properly to obtain the stock solution. Dilutions of 10^{-4} was made and pour plating of 10^{-2} and 10^{-3} was done using sterile Potato Dextrose Agar(PDA) which was sterilized by autoclaving at 121^{0}C for 15mins.Streptomycin was added to the PDA to prevent bacterial contamination. The above dilutions were then plated in duplicates. The inoculated plates were incubated at 25±2^{0}C (room temperature) for 5-7days. Pure cultures were obtained by repeated subculturing on PDA plates and maintained on PDA slants. The isolates were examined and identified in the department of Botany and Microbiology University of Ibadan based on cultural and morphological characteristics of the organism. The microscopic structures of the isolates were studied using microscope. Compendium of soil fungi was also consulted.

c) Screening Of Fungal Isolates For Pectinolytic Activity

The isolates were screened for pectinases producing ability by inoculating them in a sterile medium containing 1% citrus pectin, 0.14% $(NH4)_2SO4$, 0.20% K_2HPO4, 0.02% $MgSO4.7H_20$, 0.1% Nutrient solution (5Mg/L $FeSO4.7H_2O$, 1.6Mg/L MnSO4, 1.4mg/L $ZnSO4.7H_20$, 2.0mg/L $CoCl_2$), 3% agar PH 5.0(Martin *et al.*, 2004).

The medium was sterilized and distributed aseptically on petri dishes. The plates were then inoculated with the organism and incubated for 3-5 days. After incubation plates were stained with iodine solution. Clear zones were formed around the pectinase producing isolates.

d) Quantitative Estimation Of Pectinolytic Activity Of Screened Isolates

Quantitative estimation of pectinolytic activity was done on submerged and solid state fermentation.

e) Submerged Fermentation

The liquid medium containing 0.6%$(NH4)_2SO4$, 0.6%K_2HPO4, 0.6%KH_2PO4, and $MgSO4.7H_20$ 0.01% with 1% pure pectin and 1% dry banana peels as the sole carbon sources and these were added separately to the basal medium. The PH value was adjusted to 5.5 before sterilization at 121^{0}C for 15mins. The pectinolytic isolates identified was used for inoculation using a flamed and cooled cork borer two disc of fungal hyphae from leading edge of actively growing colonies was cut on petri plates with a flamed and cooled needle disc were then transferred to the fermentation medium in sterile flasks and the Erlenmeyer flasks were then plugged properly and incubated for 12 days at room temperature (25±2^{0}C). Aliquots were withdrawn every day 3, 6, 9, and 12 using whatman No.1 filter paper for carrying out polygalacturonase and pectin lyase assays.

f) Preparation Of Substrates For Solid State Fermentation

Fresh banana peels were dried, ground and sieve to obtain smaller substrate particle which provides larger surface area for microbial attack. (Pandey *et al.,* 2002).

g) Production Of Pectinolytic Enzyme By Solid State Fermentation

Solid state fermentation was carried out using a medium containing 15g of ground dried banana peels and 10ml of the mineral salt solution of 0.6%$(NH4)_2SO4$, 0.6%K_2HPO4, 0.6%KH_2PO4, and $MgSO4.7H_20$ 0.01%. The medium was sterilized at 121^{0}C for 40mins (Martins *et al.*, 2004). One disc of respective fungal hyphae was introduced into 5ml of sterile distilled water, the suspension was shaking using shaker for 10mins for proper dispersion of the spores. From this suspension, 1ml was withdrawn and inoculated into each flask. The flasks were then

incubated at room temperature (25±2^{0}C). For 12days 50ml of sterile distilled water was added on day 3, 6, 9, and 12 and then filtered. The obtained fiterates were used for conducting polygalacturonase and pectin lyase assays.

III. Effect of Different Treatments On Pectinase Production

a) Effect Of Blanching On Pectinase Production

Banana peels were dipped inside 5% NaCl and removed at different time intervals of 5mins, 10mins, 15mins, and 20mins. 15g of the substrate was weighed and 10ml of mineral solution containing 0.6%$(NH4)_2SO4$, 0.6%K_2HPO4, 0.6%KH_2PO4, and $MgSO4.7H_20$ 0.01%. The medium was sterilized at 121^{0}C for 40mins (Martins *et al*., 2004). One disc of respective fungal hyphae was introduced into 5ml of sterile distilled water, the suspension was shaking using shaker for 10mins for proper dispersion of the spores. From this suspension, 1ml was withdrawn and inoculated into each flask. Part of this treated peels were also dried and allowed to be subjected to the fermentation also. The flasks were then incubated at room temperature (25±2^{0}C) for 12days. 50ml of sterile distilled water was added on day 3, 6, 9, and 12 and then filtered. Filtrate was then used as crude enzyme for assay.

b) Effect Of Ripening On Pectinase Production

Banana fingers were monitored for ripening and peels were removed at different days intervals 0, 2, 4,and 6 and both fresh and dry peels were then subjected to SSF by weighing 15g of the substrate and 10ml of mineral solution containing 0.6%$(NH4)_2SO4$, 0.6%K_2HPO4, 0.6%KH_2PO4, and $MgSO4.7H_20$ 0.01%. The medium was sterilized at 121^{0}C for 40mins (Martins *et al*., 2004). One disc of respective fungal hyphae was introduced into 5ml of sterile distilled water, the suspension was shaking using shaker for 10mins for proper dispersion of the spores. From this suspension, 1ml was withdrawn and inoculated into each flask. The flasks were then incubated at room temperature (25±2^{0}C). For 12days 50ml of sterile distilled water was added on day 3, 6, 9, and 12 and then filtered. The filtrates were later used for assays.

c) Effect Of Ashes On Pectinase Production

Banana peels were dipped inside ashes and removed at different time intervals of 5mins, 10mins, 15mins, and 20mins.They were then subjected to SSF by weighing 15g of the substrate and 10ml of mineral solution containing 0.6%$(NH4)_2SO4$, 0.6%K_2HPO4, 0.6%KH_2PO4, and $MgSO4.7H_20$ 0.01%. The medium was sterilized at 121^{0}C for 40mins (Martins *et al*., 2004). One disc of respective fungal hyphae was introduced into 5ml of sterile distilled water, the suspension was shaking using shaker for 10mins for proper dispersion of the spores. From this suspension, 1ml was withdrawn and inoculated into each flask. The flasks were then incubated at room temperature (25±2^{0}C). For 12days 50ml of sterile distilled water was added on day 3, 6, 9, and 12 and then filtered. The filtrates were later used for assays.

d) Polygalacturonase Assay

Polygalacturonase (PG) activity was determined by measuring the release of reducing groups from citrus pectin using the 3,5 dinitrosalicylic acid (DNS) reagent.(Miller, 1959).

The reaction mixture containing 2ml of 1% citrus pectin in 0.2M phosphate citrate buffer P^H (5.5) and 0.5ml of crude enzyme solution was incubated at 40^0 C for 10mins.(Somogyi *et al*., 1945), a modified method. After this 3ml of DNS reagent was added and boiled in water bath for 15mins. After cooling, colour absorbance was read at 540 nm using a spectrophotometer.

e) Pectin Lyase Assay

Pectin lyase activity was determined by measuring the increase in absorbance at 235nm of substrate solution (0.8ml of 1% citrus pectin in 0.2M tris Hcl buffer PH 8.5 hydrolysed by 0.2ml enzyme solution at 40^{0}C (Martin *et al*., 2004).

IV. Results

The present study was carried out in order to determine the effect of blanching, ripening, and other treatments on the production characteristics of pectinolytic enzymes from banana peels by *Aspergillus niger.* It is well reported that *Aspergillus niger* have pectinolytic activity, the isolates was screened for the production of the pectinolytic enzymes.

Figure 1 shows the production of polygalacturonase enzymes by *Aspergillus niger* in both solid state fermentation (SSF) and submerged fermentation (SMF). The fermentation was carried out for 12days and assay of the aliquots was conducted on day 3, 6, 9, 12. For (SSF) the highest yield of polygalacturonase was produced on day 3 of the fermentation and the value ranged from 4.9676-7.5544U/ml. However in SMF the polygalacturonase produced ranged from 4.6265-5.3118U/ml and the highest yield of production was observed on day 9 with 5.3118U/ml. Generally from this study SSF was higher than SMF.

Figure 2 shows the production of pectin lyase enzymes by *Aspergillus niger* in both solid state fermentation (SSF) and submerged fermentation (SMF). For SSF the highest yield of production was observed on day 3 with 22.3214U/ml, while the lowest was seen on day 12 with 19.0178U/ml. For SMF the highest yield of pectin lyase production was observed on day 12 having 18.8393U/ml.

V. Production of Polygalacturonase By Aspergillus Niger

A) Effect Of Blanching On Banana Peels

The polygalacturonase production by *Aspergillus niger* using blanched banana peels (fresh) as substrate in solid state fermentation is shown in figure 3. The polygalacturonase produced ranged from 13.8235-18.5294U/ml in which banana peels blanched for 20mins at day 12 had the highest yield of production while the lowest yield of production was observed at the same 20mins on day 3. However the same quantity of enzyme was produced at 10mins day 12, 15mins day 9 and 20mins day 9 having 17.3529U/ml in all the three.

b) Effect Of Ashing On Banana Peels

The polygalacturonase production by *Aspergillus niger* using banana peels treated with ashes as substrate in solid state fermentation is shown in figure 4. The highest yield of production was 19.8529U/ml observed on day 3 at 15mins while the lowest yield was 13.6765U/ml observed on day 12 at 10mins.However the same quantity of enzyme was produced at 5mins day 3 and 15mins day 9 having 14.7059U/ml of polygalactturonase production.

c) Effect Of Ripening On Banana Peels.

Figure 5 shows the polygalacturonase production by *Aspergillus niger* using banana peels ripened at different days as substrate in solid state fermentation. During the ripening of these banana, the peels were removed at different days intervals as unripe, moderately ripe, ripe and extremely ripe and when SSF were carried out, changes in their polygalacturonase production ranged from 13.9706-29.8529U/ml. The highest yield of production was observed on day 9 when the banana was extremely ripe while the lowest was observed on day 12 when the banana was ripe.

VI. Production of Pectin Lyase By Aspergillus Niger

a) Effect Of Blanching On Banana Peels

The production of pectin lyase by *Aspergillus niger* using blanched banana peels (fresh) as substrate in solid state fermentation is shown in figure 6. The pectin lyase produced ranged from 13.3928-21.9643U/ml in which banana peels blanched for 15mins at day 6 had the highest yield of production while the lowest yield of production was observed at 10mins on day 12. However the same quantity of enzyme was produced at 15mins day 3 and 5mins day 6 having 17.8125U/ml.

b) Effect Of Ashing On Banana Peels

The pectin lyase production by *Aspergillus niger* using banana peels treated with ashes as substrate in solid state fermentation is shown in figure 7. The highest yield of production were observed on day 3 at 5mins and the same day 3 at 15mins with 20.1339U/ml while the lowest yield was 17.0536U/ml observed on that same day 3 at 10mins. However the same quantity of enzyme was produced at 5mins day 9 and 10mins day 9 also having 18.75U/ml of pectin lyase production.

c) Effect Of Ripening On Banana Peels.

Figure 8 shows the pectin lyase production by *Aspergillus niger* using banana peels ripened at different days as substrate in solid state fermentation. During the ripening of these banana, the peels were removed at different days intervals as unripe, moderately ripe, ripe and extremely ripe and when SSF were carried out, changes in their pectin lyase production ranged from 17.7232-20.5804U/ml. The highest yield of production was observed on day 3 when the banana was ripe while the lowest was observed on the same day 3 when the banana was unripe. The same quantity of pectin lyase was produced on day 6 with unripe banana and day 3 with extremely ripe banana with both having 18.3929U/ml.

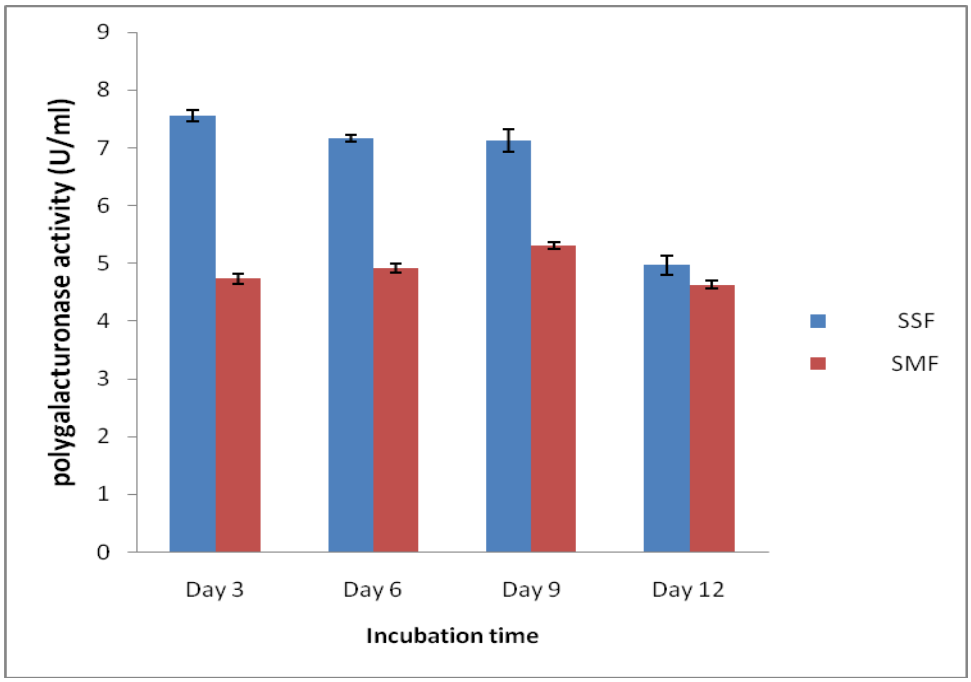

Fig 1 : Production of polygalacturonase by *Aspergillus niger* in solid state fermentation (SSF) and Submerged fermentation (SMF)

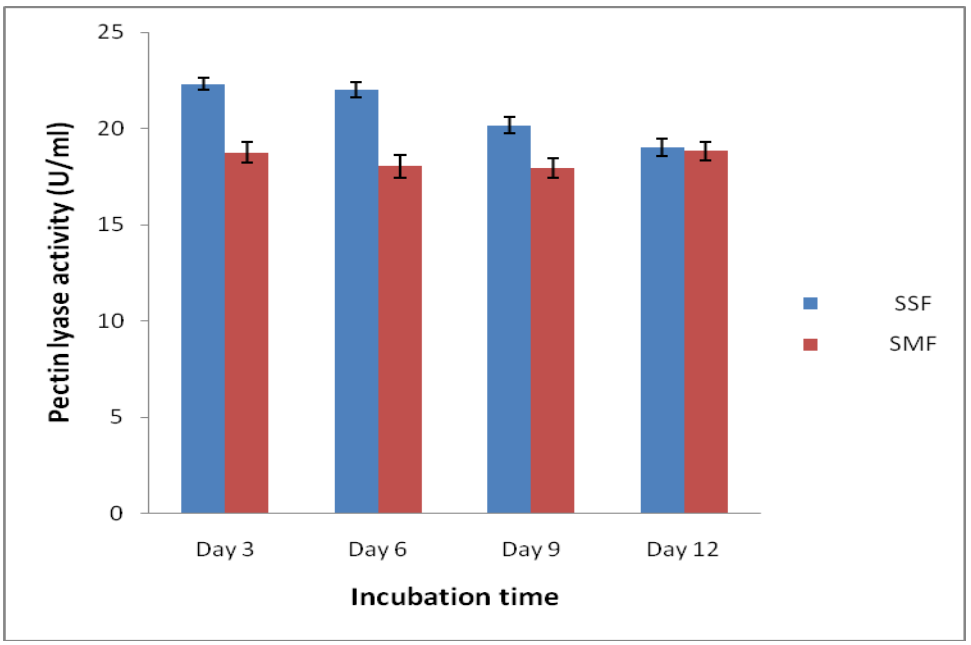

Fig 2 : Production of Pectin lyase by *Aspergillus niger* in solid state fermentation (SSF) and Submerged fermentation (SMF)

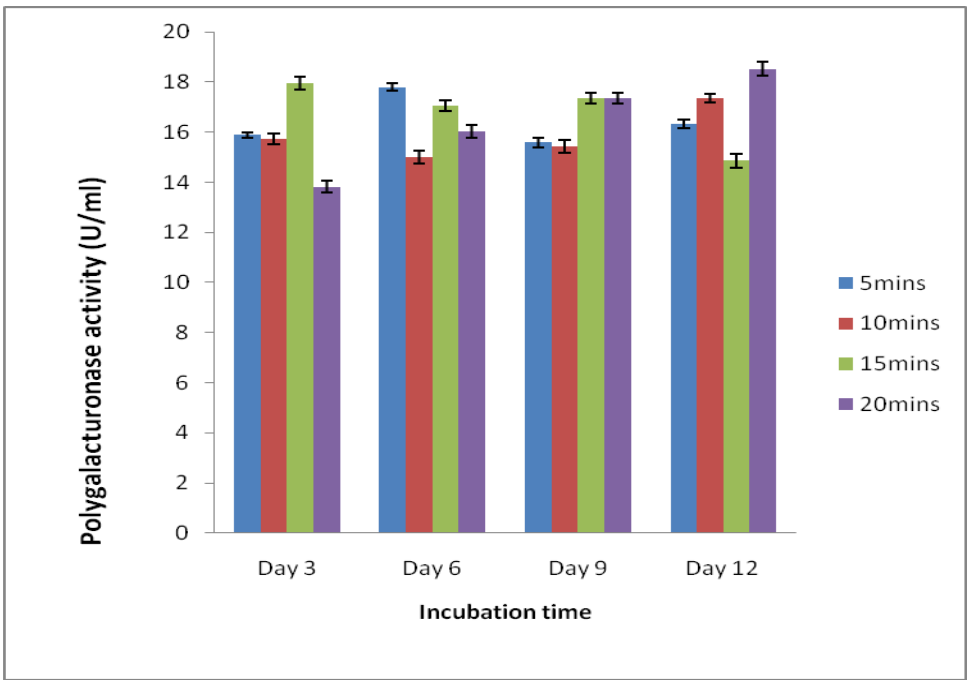

Fig 3 : Production of by polygalacturonase by *Aspergillus niger* using blanched banana peels as substrate in solid state fermentation (fresh).

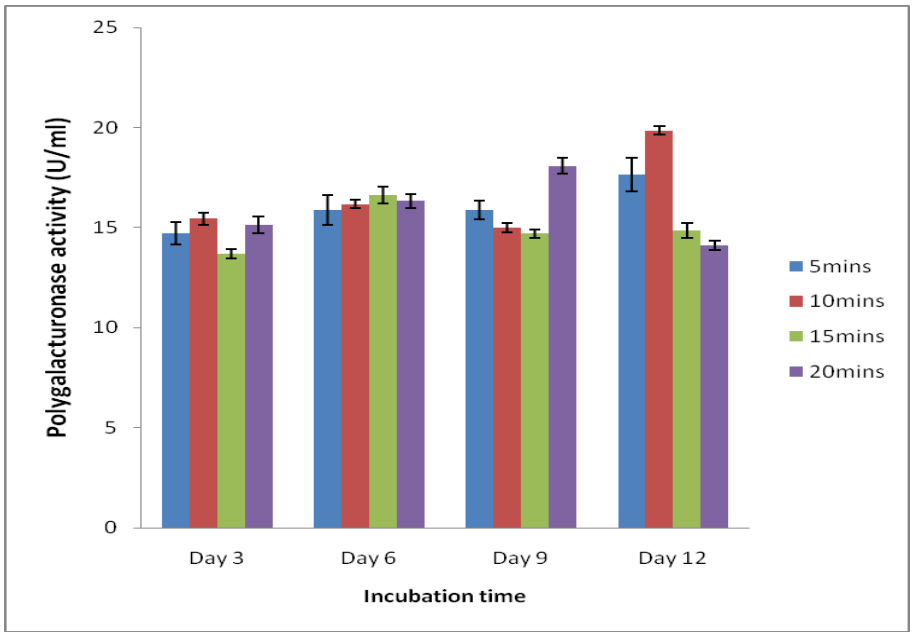

Fig. 4 : Production of polygalacturonase by *Aspergillus niger* using banana peels treated with ashes as substrate in solid state fermentation

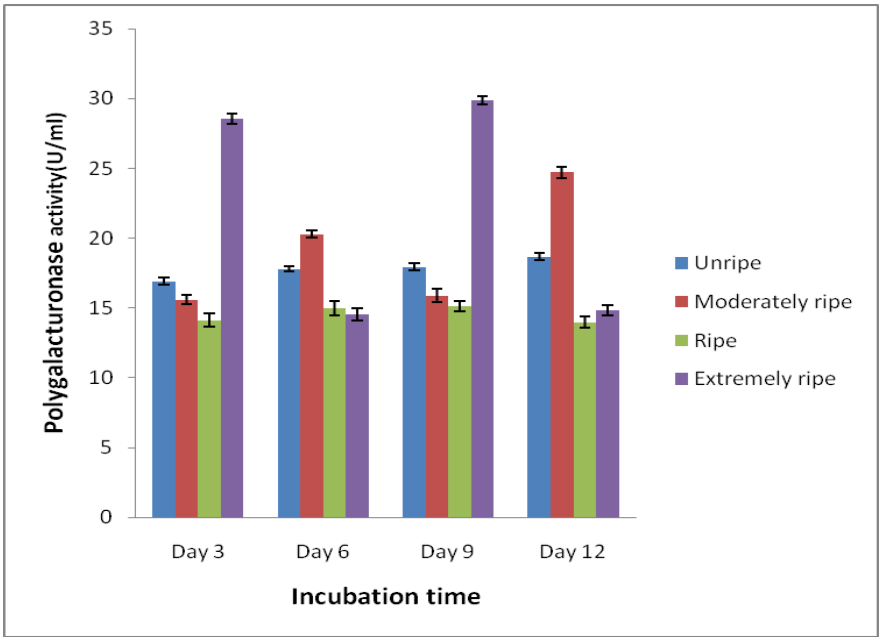

Fig. 5 : Production of polygalacturonase by *Aspergillus niger* using ripening banana peels as substrate in solid state fermentation (fresh)

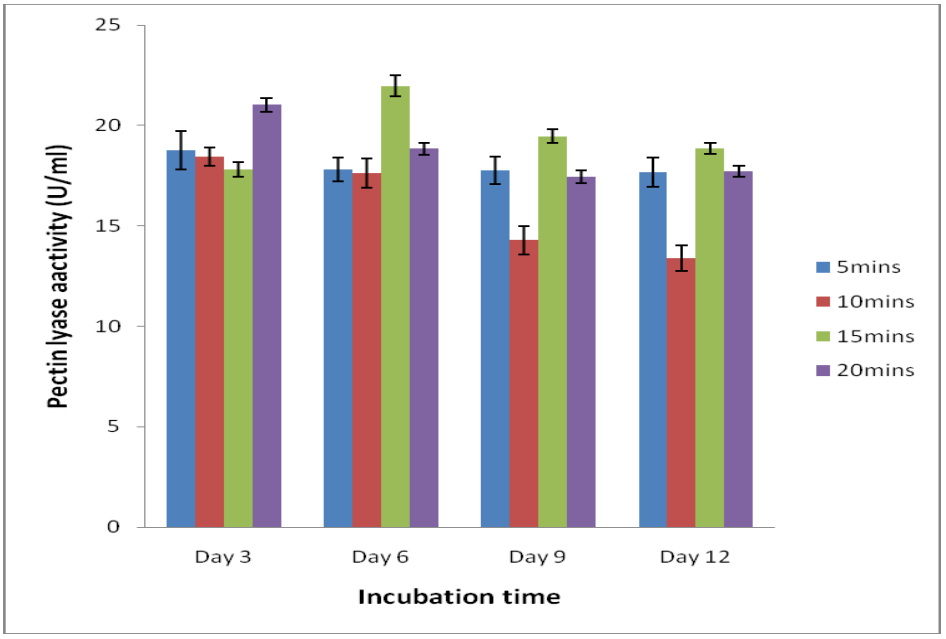

Fig. 6 : Production of Pectin lyase by *Aspergillus niger* using blanched banana peels as substrate in solid state fermentation (fresh)

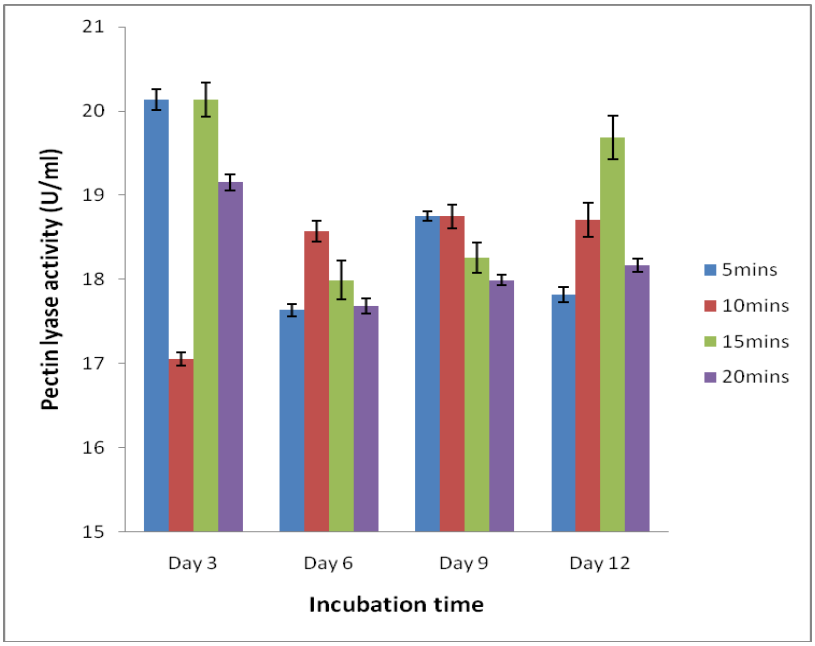

Fig.7 : Production of Pectin lyase by *Aspergillus niger* using banana peels treated with ashes as substrate in solid state fermentation

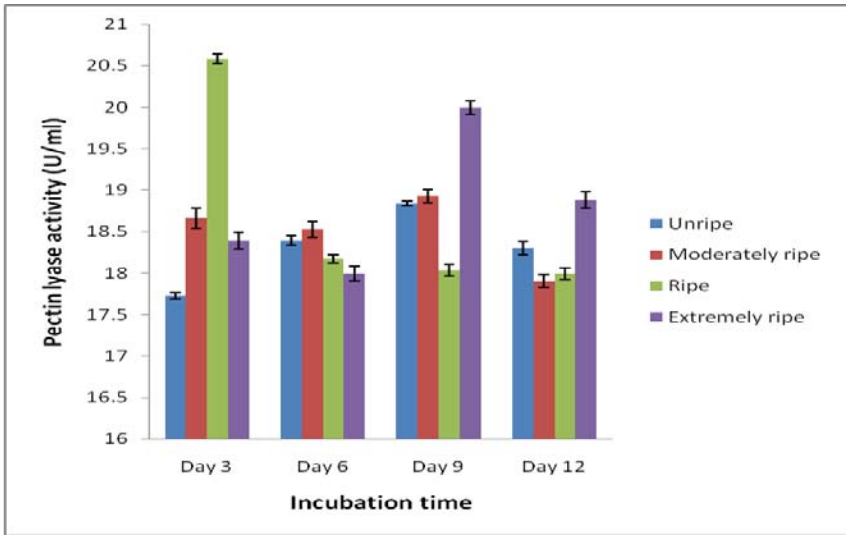

Fig.8 : Production of Pectin lyase by *Aspergillus niger* using ripening banana peels as substrate in solid state fermentation (fresh)

VII. Disscussion And Conclusion

Three different Aspergillus niger were isolated from banana peels in Ibadan metropolis as strain A-C. The best pectinolytic activity based on screening method was given by strain B. The present study shows that the strain of *A. niger* produce pectinases which hydrolyze pectins. *Aspergillus niger* isolated was reported to produce cellulases (Chinedu *et al*., 2008 a; Nwodo-Chinedu *et al*., 2007a) and xylanases (Chinedu *et al*., 2008b; Okafor *et al*., 2007a, b). Thus, this fungus produce the full complement of enzymes required for the hydrolysis of pectinolytic biomass. This explains why the fungi thrive on waste plant matter and are capable of utilizing such waste materials as carbon sources in their culture media (Nwodo-Chinedu *et al*., 2007 b). Pectinase production from Solid state fermentation (SSF) culture of this organism was significantly higher than that obtained by submerged fermentation (SmF). The higher level of pectinase activity by SSF is observed in polygalacturonase and pectin lyase. Several workers proposed the use of SSF for pectinase production, using different solid agricultural and agro-industrial residues as substrates such as wheat bran banana peels and soy bran (Castilho *et al*., 1999, 2000; Singh *et al*., 1999). The present result clearly supports the use of SSF over SmF for pectinases production by filamentous fungi.

Lignocellulosic waste of Banana plant left over otherwise for natural degradation in field was effectively used as component in the medium for the production of enzymes (Baig *et al*., 2003). Subsequently these enzymes produced on the medium containing banana agro waste can be further implicated in the saccharification of the same agro waste. Many researchers have studied the effect of agrowaste pre-treatment by alkali or steam (Okeke and Obi, 1994; Kirk and Farrel, 1987; Durand *et al*., 1984; Waldron and Eveleigh, 1986; Ekhlund *et al*., 1990)

In conclusion, *Aspergillus niger* isolated from banana peels from Ibadan metropolis, Nigeria is a pectinolytic fungi. Banana peels had been identified as a suitable low-cost substrate for pectinase production by the strains of *A. niger* . Higher levels of pectinase activity were obtained by SSF compared to SmF. The use of banana peels for pectinase production will not only reduce the production costs of the enzyme but also help decrease pollution-load due to the agro-industrial waste. Banana peels offer a good medium for the production of pectinase and *Aspergillus niger* can be used for large scale production of pectinase using banana peels and when they were subjected to different treatments pectinase production was higher than when the peels were not treated at all.

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